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Effect of storage of pheromone lures for *Amyelois transitella*: field performance and compound ratios

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The navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), is an important pest of California tree nut crops (almond, pistachio, and walnut) worth > US $6 billion per year in unprocessed form (Burks and Higbee 2015). The sex pheromone of this species is important for control by mating disruption (Higbee and Burks 2008), and potentially important as a monitoring tool (Burks and Higbee 2015). The fact that a sex pheromone lure was not available for this pest until long after such lures had been developed for most other important moth pests of tree crops was probably due, in part, to the unusual complexity of the sex pheromone of this species (Leal et al. 2005).

An important component of the sex pheromone, (*Z,Z*)-11,13-hexadecadienal (Z11,Z13-16:Ald) was discovered over 35 years ago (Coffelt et al. 1979). Subsequent studies identified additional attractive components (Leal et al. 2005; Kanno et al. 2010; Kuenen et al. 2010). In addition to the previously mentioned aldehyde, other components in an attractive blend include (*Z,Z*)-11,13-Hexadecadienol (Z11,Z13-16:OH), (*Z,E*)-11,13-Hexadecadienol (Z11,E13-16:OH), and (*Z,Z,Z,Z,Z*)-3,6,9,12,15-Tricosapentaene (C_{23} Pentaene). These 4 components attract optimally in the laboratory at the respective ratio of 100:100:5:5 (Kanno et al. 2010; Kuenen et al. 2010). In the flight tunnel, Z11,Z13-16:Ald alone is insufficient to produce source contact, and the presence of C_{23} pentaene is absolutely necessary to obtain source contact.

The necessity to present compounds with very different volatility complicated initial efforts to produce a practical lure (Higbee et al. 2014). These difficulties were overcome, however, and a lure was commercially introduced in 2013 (NOW Biolure, Suterra LLC, Bend, Oregon, USA) (Higbee et al. 2014; Burks and Higbee 2015). A second manufacturer introduced 2 additional pheromone lures in 2015. A technical flyer for these products (http://www.trece.com/PDF/Pherocon_Navel%20Orangeworm_flyer.pdf) suggests that 1 (NOW L2L, Trécé, Adair, Oklahoma, USA) was intended to be similarly attractive to the existing pheromone lure, whereas the other (NOW L2H) was intended to be more attractive. Given the complexity and expense of the navel orangeworm pheromone lure compared to lures for other species, it seemed prudent to examine the effect of end-user storage of lures from previous years as well as compare effectiveness of lures between manufacturers. Here we report the results of a field trial comparing the 2015 Trécé lures with newly acquired 2015 Suterra LLC lures, and lures acquired from Suterra LLC in 2013 and 2014 and stored in a domestic freezer at –20 °C. When the data from this trial revealed the unexpected result that NOW Biolure kept in storage from previous years was more attractive than current-year NOW Biolure, analysis by gas chromatography and mass spectrometry (CG-MS) was used to gain insight on the reason for this finding.

The field experiment was conducted in a 28 ha block of almond, *Prunus dulcis* Mill. (D.A. Web) (Rosaceae) located at 39.6556°N, 121.8377°W. Traps were placed in the orchard on 1 Mar 2015, and data were collected until 25 Jun 2015. Data were collected weekly, and lures and liners were changed every 4 wk (i.e., 26 Mar, 23 Apr, and 21 May), so 4 sets of lures were examined for each of the 5 lure types and 4 physical replicates. The 4-wk lure interval was conservatively short of the manufacturers' recommended change intervals of > 5 wk. Trees were 7.6 m high and planted offset in north-south rows, 5.1 m wide, with 6.1 m between trees within rows. Treatments were randomly assigned to 20 positions for the length of the study; 2 per row in an off-set pattern, such that traps were 130 m apart within rows and the nearest neighboring trap was 75–90 m away. Association of lure type and age with the cumulative number of males captured for each 4-week period was analyzed by a series of 1-way ANOVAs, 1 per each of the four 4-week periods between lure changes. No transformation was used because the Kolmogorov-Smirnov test revealed no significant departure from a Gaussian distribution, and because the observed frequency distribution was generally symmetrical around the mean (SAS Inst. 2015). The Tukey procedure was used to adjust for multiple comparisons. In addition, regression of cumulative trap count on lure age was examined for NOW Biolure for each of these 4 periods.

Volatiles from the lures were collected on a 95 cm length of a DB-1 gas chromatography capillary column (0.53 mm ID, 1.5 µm film thickness; Agilent Technologies, Inc., Santa Clara, California, USA) by methods of Kuenen and Hicks (2015). Volatile collections were made about 2 mm from the lure membrane with an air flow of 15 ml per m at room temperature (24–25 °C). The lures were placed at the bottom of a 400 ml Pyrex flask capped with aluminum foil from which the DB-1 column was inserted down through a small hole in the foil cap. Volatile collections from the Biolure were made for 3 h and the pheromone was subsequently eluted from the column with 35 µl of hexane into glass vials to which 10 ng of the internal standard were added. The eluate was diluted to approximately half of the original volume, and 1 µl was analyzed on GC-MS. Five lures each were examined from 2015 and 2013, and 3 lures were examined for 2014. The analyses were performed in Jun 2015. *Z*11-tetradecenyl acetate (*Z*11-14:Ac) (Bedoukian Research Inc., Danbury, Connecticut, USA) was used as an internal standard. A stock solution of the internal standard was prepared gravimetrically in HPLC grade hexane (Fisher Scientific, Pittsburgh, Pennsylvania) at a concentration of 100 and 10 ng/µl. All compounds and solvents were > 95% pure by GC-MS. Analyses were performed on a Hewlett Packard model 6890 gas chromatograph equipped with a cool on-column injector and a 5975C MSD detector in EI mode (Agilent Technologies, Santa

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Clara, California, USA), using a 30 M, 0.32 mm ID, 0.25 µm film thickness Stabilwax®-DA analytical column (Restek Corp, Bellefonte, Pennsylvania, USA). Carrier gas (UHP He) was held at a constant flow (1.5 ml per min). Analyses of pheromone components were determined with select ion monitoring scanning the 7 most abundant ions for each component as follows in order of most abundance: *Z*11,*Z*13-16:Ald and isomers (*m/z* 67.1, 82.1, 95.1, 55.1, 41.1, 109.1, 123.1); *Z*11,*Z*13-16:OH and isomers (*m/z* 67.1, 82.1, 95.1, 55.1, 41.1, 109.1, 121.1); C_{23} pentaene (*m/z* 79.1, 91.1, 67.1, 41.1, 105.1, 55.1, 119.1). The ions (*m/z* 82.1, 68.1, 55.1, 43.1, 96.1, 109.1, 194.2) were monitored for the internal standard Z11-14:Ac. Integration of peaks was used to estimate the amount of the compounds recovered. Changes in the amount and ratio of pheromone components were examined using Pearson correlation analysis. Based on findings from this correlation analysis, ANOVA was used to compare the ratio of C_{23} pentaene:Z11,Z13-16:Ald in emissions between NOW Biolure acquired in different years. The limit of detection for C_{23} pentaene by this method is ≤ 0.01 ng, and the amount observed in all collections in this analysis exceeded this limit by at least 70-fold.

There were significant differences among lure categories in the number of males captured during the first 3 lure periods between lure changes, but not for the final set of lures examined (Fig. 1). The most consistent trend in these first 3 sets of lures examined is a positive linear relationship between the time of NOW Biolure in end-user storage and the number of males captured, as indicated by significant r^2 values (r^2 and *P* values were 0.44, 0.0178; 0.75, 0.0002; and 0.63, 0.0021, respectively). Differences between mean males captured by the 2015 lures were not significant (Fig. 1). However, the performance of the Trécé L-2H lure relative to the 2013 NOW Biolure declined in the 3rd interval (Fig. 1c) compared to the first 2 (Fig. 1a, b). Most of the males captured in traps between 21 May and 25 Jun were captured in the final week of this period. The degree-day accumulation and the seasonal abundance pattern suggests that those males emerged from eggs laid in 2015, whereas males trapped with the first 3 sets of lures were from the generation overwintering from 2014. That may be relevant to differences in patterns of response to lures evident between the first 3 sets of lures (Figs. 1a-c) and the 4th (Fig. 1d).

Analysis of the GC-MS data revealed no significant correlation ($P > 0.05$) between lure age and the overall emission rate, or the ratio of most specific components. The single exception was a significant correlation ($r^2 = 0.77$, $P < 0.0001$) between time in end-user storage and C_{23} pentaene as a proportion of overall emission. Emission of the C_{23} pentaene as a percentage of Z11,Z13-16:Ald differed significantly between NOW Biolure acquired in 2013, 2014, and 2015 ($F_{2,10} = 23.03$, $P = 0.0002$) (Fig. 2).

These data imply increasing field efficacy of lures with an increase of the ratio of C_{23} pentaene:Z11,Z13-16:Ald over the range of 1 to 3%. In contrast, a wind tunnel study found increased efficacy with the addition of 1.5% C_{23} pentaene to Z11,Z13-16:Ald, but no further improvement with an increase to 5% C_{23} pentaene (Kanno et al. 2010). However, that study did not examine the effect of different proportions of C_{23} pentaene as part of a 4-component blend, nor did it examine effects in the field. The increase in the emission ratio of C_{23} pentaene:Z11,Z13-16:Ald may be due to differential effects of storage at –20 °C to stability of components. Effects of this storage on physical characteristics of release membranes could also be a factor. This study examined lures held in a domestic freezer, which undergoes freeze-thaw cycles as part of a defrost feature intended for consumer convenience. We knew the time of the lures in end-user storage, but not the date of manufacture. It should also be noted that, prior to sale, lures are presumably held in commercial-grade cold storage facilities in which fluctuations in temperature are carefully avoided.

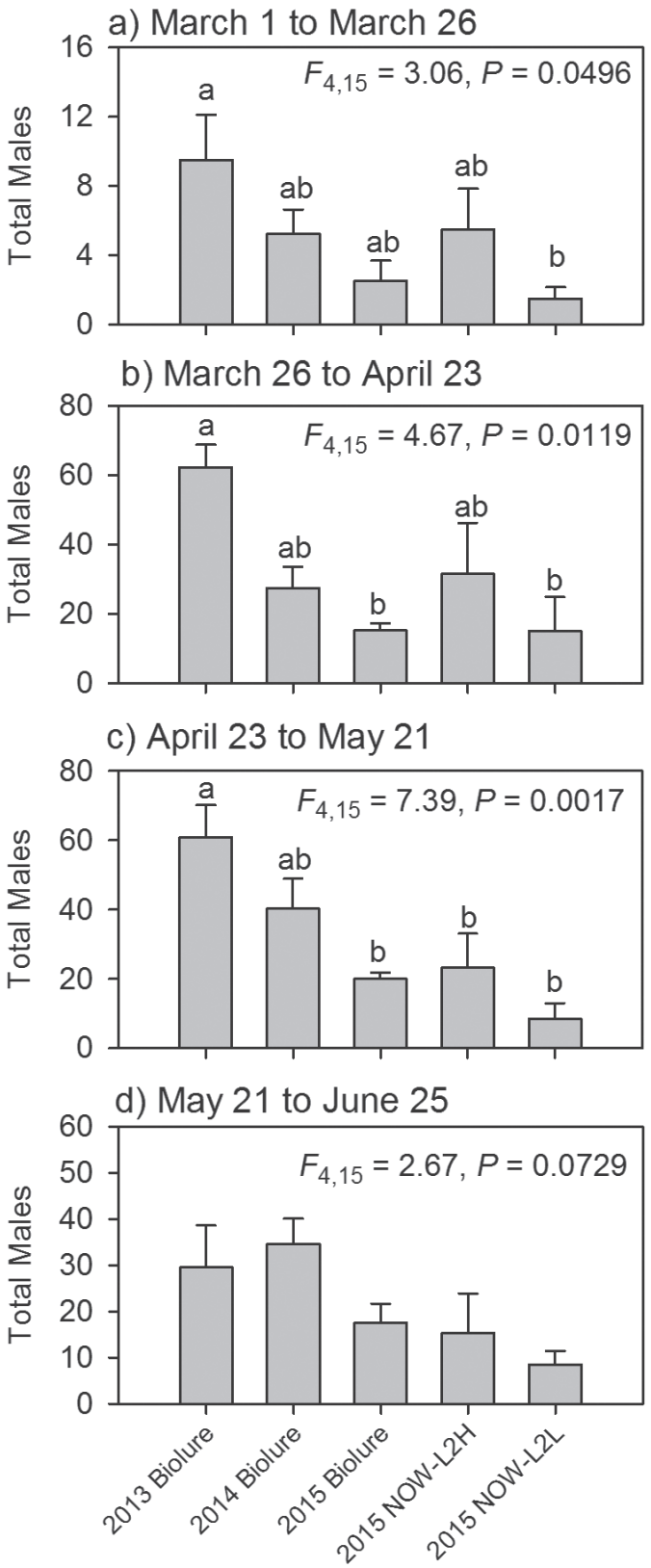

Fig. 1. Cumulative total of males (mean and SE, n = 4) captured by Suterra NOW Biolure acquired in 2013, 2014, or 2015, or by Trécé NOW-L2L or NOW-L2H during 4-week periods between lure change. Sets of lures were tested during Mar through Jun of 2015 as follows: (a) 1 Mar to 26 Mar; (b) 26 Mar to 23 Apr; (c) 23 Apr to 21 May; and (d) 21 May to 25 Jun. Means with different superscripts are significantly different (ANOVA, experiment-wise $P < 0.05$).

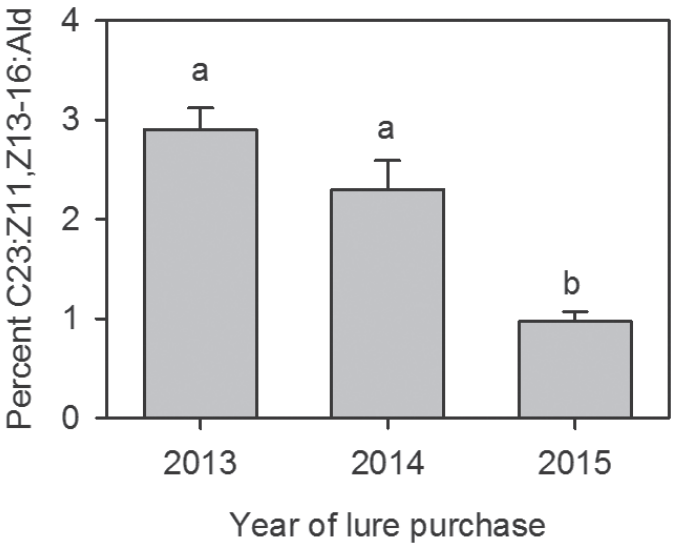

Fig. 2. Effect of time of storage on emission of C_{23} pentaene by NOW Biolure. Emission rate of pheromone components was evaluated by GC-MS in Jun 2015 for lures purchased prior to the field season in 2013, 2014, and 2015. Lures were stored at –20 °C prior to analysis. The rate of pentaene emission is expressed as a percentage of the rate of emission of Z11,Z13-16:Ald. Means with different letters are significantly different (ANOVA, $P < 0.05$).

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Summary

Experiments during the flight of the overwintering generation of navel orangeworm revealed that Suterra NOW Biolure pheromone lures held in storage at –20 °C increased significantly in field effectiveness with time in storage over a period of 0 to 2 years. This increase in field effectiveness coincided with an increase in emission of (*Z,Z,Z,Z,Z*)-3,6,9,12,15-tricosapentaene as a proportion of (*Z,Z*)-11,13-hexadecadienal. These observations indicate that users should not presume equal effectiveness when lures are held over from the previous year, and suggest that manufacturers could improve lure effectiveness by increasing the proportion of (*Z,Z,Z,Z,Z*)-3,6,9,12,15-tricosapentaene in the lure emission.

Sumario

Investigaciones durante el vuelo de *Amyelois transitella* que emergieron de larvas nacidas en el año pasado revelaron que los cebos de feromonas, “Suterra NOW Biolure”, atrajeron significativamente más machos sobre un periodo desde 0 hasta 2 años con temperatura de –20 °C. También incrementó significativamente la proporción de (*Z,Z,Z,Z,Z*)-3,6,9,12,15-tricosapentaene entre los compuestos emitidos por los cebos guardados en este tiempo. Estas observaciones indican que los que usan estos cebos no deben de suponer que la atracción es igual entre cebos nuevos y los conservados desde el año pasado, y también sugieren que los fabricantes podría hacer más eficaz los cebos con un aumento de la proporción de (*Z,Z,Z,Z,Z*)-3,6,9,12,15-tricosapentaene entre los compuestos emitidos.

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