Field Evaluation of a Binary Sex Pheromone for Sweetpotato Vine Borer (Lepidoptera: Crambidae) in Hawaii

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Abstract

The sweetpotato vine borer, Omphisa anastomosalis (Guenée), is a primarily Asian pest of sweetpotato, Ipomoea batatas L. Damage by O. anastomosalis infestation can cause root yield losses of 30%–50%. A binary sex pheromone for O. anastomosalis, consisting of Type I [(10E,14E)-10,14-hexadecadienal (E10,E14-16:Ald)] and Type II [(3Z,6Z,9Z)-3,6,9-docosatriene (Z3,Z6,Z9-22:H)] components, was identified in Vietnam from extracts of female pheromone glands. A structurally similar Type II compound [(3Z,6Z,9Z)-3,6,9-docosatriene (Z3,Z6,Z9-22:Hi), not recovered from female pheromone glands, was also found to synergize the attractiveness of the Type I component. Additional field work has been needed to determine whether these synergistic enhancements of attractiveness also occur in other parts of the geographical distribution of this moth species. Herein, results of studies are reported which document that both Z3,Z6,Z9-23:H and Z3,Z6,Z9-22:H also synergistically enhance male response to E10,E14-16:Ald in Hawaii sweetpotato field populations. Trap catch tends to be enhanced with increase of dose and lower Type I:Type II ratios. Among the compound doses and ratios tested, trap catch increased with the addition of the Type II component by over 13 times relative to traps baited with the Type I component alone, which significantly enhanced sweetpotato vine borer detection. Using a 2.0 mg: 4.0 mg Type I: Type II loading, there was continued catch over 12 wk, during which time the Type I component weathered at a faster rate than the Type II component. This binary sex pheromone seems to have promise for both monitoring and suppression of field populations of O. anastomosalis throughout its geographical range.

Key words: sweetpotato, sex pheromone, (10E,14E)-10,14-hexadecadienal, (3Z,6Z,9Z)-3,6,9-docosatriene

The sweetpotato vine borer, Omphisa anastomosalis (Guenée), is a primarily Asian pest of sweetpotato, Ipomoea batatas L., having been reported in Bangladesh, Burma, Cambodia, China, Indonesia, Japan, Laos, Malaysia, New Guinea, Philippines, Sri Lanka, Taiwan, Thailand, and Vietnam. It is also present in Hawaii and the Pacific (Zimmerman 1958, Das and Islam 1985, Talekar and Pollard 1991). Talekar and Pollard (1991) report it to be second in economic importance to sweetpotato weevils (Cylas spp., Coleoptera: Brentidae) and Euscepes postfasciatus [Fairmaire] (Coleoptera: Curculionidae) in areas where these species coexist. Ho (1970) and Talekar and Cheng (1987) have reported that O. anastomosalis infestation has resulted in yield losses of 30%–50%. Although applications of chemical insecticides have been used in attempts to control this pest, control can be difficult because immature stages are sheltered from contact with pesticides by feeding within the sweetpotato vines (Talekar and Pollard 1991). Research at the Asian Vegetable Research and Development Center (Talekar and Pollard 1991, Talekar et al. 1992) demonstrated that females release a sex pheromone to which males are attracted. Wakamura et al. (2010) identified an electroantennogram (EAG) – active compound, (10E,14E)-10,14-hexadecadienal (E10,E14-16:Ald, Type I), in extracts of abdominal tips of O. anastomosalis virgin females. Male response to this compound, though, was subsequently found to be synergized by the addition of (3Z,6Z,9Z)-3,6,9-tricosatriene (Z3,Z6,Z9-23:H, Type II), also present in the pheromone glands of female O. anastomosalis in Vietnam. The degree of synergistic enhancement of attraction was shown to vary with the ratio of the Type I: Type II components. Additionally, it was shown that males showed limited specificity to the chain length of the Type II component, with male attraction numerically (but not statistically) higher when (3Z,6Z,9Z)-3,6,9-docosatriene (Z3,Z6,Z9-22:H) was used rather than Z3,Z6,Z9-23:H as the Type II component (Yan et al. 2014). Although Yan et al. (2014) did not report that Z3,Z6,Z9-22:H was present in abdominal tips of O. anastomosalis virgin females, a subsequent paper by Vang et al. 

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(2018) with a different crambid species (the eggplant fruit borer, Lecinodes orbonalis Guénée [Coleoptera: Crambidae]) reported that Z3,6,Z9-22:H was recovered as an EAG-active component of an extract of the abdomen and thorax. A comparable extract from O. anastomosalis has not been made and tested for EAG activity, so it is not known whether Z3,6,Z9-22:H may also be present in an abdomen + thorax extract from O. anastomosalis virgin females. Such an occurrence would give a biological basis for the synergism with Z3,6,Z9-22:H, rather than just ascribing it to limited specificity by O. anastomosalis males to the chain length of the Type II compound.

Yan et al. (2014) indicated that evaluation of the binary sex pheromone in Japan and other countries is important to assess whether all O. anastomosalis populations utilize Z3,6,Z9-23:H in mating communication. Herein, we document the presence of both Type I and Type II (Z3,6,Z9-23:H) pheromone components in pheromone glands of females in Hawaii O. anastomosalis populations and report on the results of field studies with sweetpotato vine borer populations in Hawaii assessing the relative effectiveness of the Type I pheromone component alone versus several doses and ratios of the binary sex pheromone. We, also, assess whether the addition of Z3,6,Z9-22:H produces synergistic enhancement of the attractiveness of the Type I component in Hawaii as it has been reported to do so in Vietnam. In addition, using the percentage of weeks where moths were caught in traps of each treatment, we calculate the relative effectiveness of each treatment in detecting the presence of O. anastomosalis field populations. Finally, we also report on the persistence of the two components over a 12-wk weathering period in sweetpotato fields in Hawaii.

Materials and Methods

Chemicals

(10E,14E)-10,14-hexadecadienal (E10,E14-16:Ald) (94.2% purity, gas chromatography with flame-ionization detection [GC-FID]) was obtained from Shin-Etsu Chemical Co., Ltd., Tokyo, Japan. It should be noted that several other companies tried to synthesize this compound for our use, but were not successful, showing that it may be a challenging compound to synthesize. (3Z,6Z,9Z)-3,6,9-tricosatriene (Z3,6,Z9-23:H) and (3Z,6Z,9Z)-3,6,9-docosatriene (Z3,6,Z9-22:H) (~95% purity for each, GC-FID) were obtained from Pherobank BV, Duurstede, the Netherlands. The internal standards (ISTD) (Z)-13-octadecenal and hecicosane were obtained from Bedoukian (Danbury, CT) and Sigma-Aldrich (St. Louis, MO), respectively.

Insects and Gland Extraction

Infested cut sweetpotato roots were collected from February–May 2014 from harvested sweetpotato fields in the vicinity of Pepeekeo on Hawaii Island, Hawaii, and held to permit caterpillar maturation. Recovered pupae, separated by gender identification characteristics (Talekar et al. 1992), were placed, by sex, in two separate padded containers, and sent to the USDA-ARS National Center for Agricultural Utilization Research (NCAUR; Peoria, IL), where they were placed in individual screened emerging vials and kept in an incubator at 27°C with a relative humidity of about 50%. Light was provided by eight 40-W fluorescent tubes set about 0.5 m above and behind the vials, and the daily light cycle was a photoperiod of 17:7 (LD) h. Emerging moths had access to drops of 5% honey-water solution. Two- to three-day old virgin female moths were immobilized by cooling and gland tissues were exposed by a gentle push on the abdomen, tissues were cut off with a razor blade and placed in vials containing 500-µl HPLC-grade petroleum ether (boiling point 36°C; Fisher Scientific, Waltham, MA) and a few crystals of anhydrous sodium sulfate. Gland tissues were extracted for 20–60 min at room temperature (RT), after which the solvent extract was removed and filtered through a small plug of glass wool to remove scales and other particulates. Extracts were stored at −20°C and prior to gas chromatography-electroantennographic detections (GC-EAD) and gas chromatography-mass spectrometry (GC-MS) analysis extracts were concentrated (approx. 2–6 female equivalents/µl under a gentle stream of nitrogen).

Lure Formulation and Weathering Residue Analysis

Grey rubber septa (West Pharmaceuticals Services, Exton, PA) were used as dispensers for the moth pheromone compounds in field experiments and septa weathering analysis. Septa were loaded with 200 µl of a petroleum ether solution with the desired concentration of compounds. After loading the chemicals, all septa were aired in a hood until the solvent had evaporated and then stored individually in heat-sealed pouches at −20°C until shipment to Hawaii. For reference, volatile collections (RT, airflow at 200 ml/min) made by methods described by Soroka et al. (2005) of freshly dosed septa with 2.5 mg E10,E14-16:Ald showed an average release rate of 31.7 ng/h (n = 3) measured during a 3-d collection period. Three of the four weathered septa for each age were randomly selected for analysis. Septa were each cut into 16 pieces and placed into scintillation vials to which 5 ml of petroleum ether and ISTDs were added. Z13:18:Ald was used as the ISTD for the Type I compound, whereas hecicosane was used as the ISTD for the Type II compound. The scintillation vials were then placed in a shaker overnight at RT and 100 rpm, following which extracts were removed, diluted, and analyzed by GC-FID.

Instrumentation

Gland extract were analyzed by GC-FID and coupled GC-MS. Samples were injected in splitless mode using a Hewlett Packard 6890 GC, interfaced to a Hewlett Packard 5973 mass selective detector (electron impact, 70 eV). For gland extracts and chemical comparison analysis, a 30-m DB-5 capillary column (0.25-mm ID, 0.25-µm film thickness, J&W Scientific, Folsom, CA) was used. The temperature program was 50°C for 1 min, then rising to 280°C at 10°C/min and holding for 5 min at 280°C. The temperature of the inlet was 250°C, and the transfer line temperature was 280°C. The Wiley MS library (Wiley 2005) was installed on the data system. For the analysis of the weathered extracted septa, a 30-m Stabilwax capillary column (0.32-mm ID, 0.25-µm film thickness, Restek, Bellefonte, PA) was used. The temperature program was 100°C for 1 min, then rising to 240°C at 5°C/min and holding for 2 min at 240°C. The temperature of the inlet was 255°C, and FID detector temperature was 255°C. Due to extracted septa compounds unrelated to the Type I, Type II, and ISTDs, which interfered with the residue analysis on the DB-5 capillary column, the Stabilwax column was chosen for the residue analysis. All of the GC analyses used He as carrier gas at constant pressure (41.4 KPa).

Electrophysiology

Coupled GC-EAD were made by methods and equipment generally described by Cossé and Bartelt (2000). GC-EAD connections were made by inserting a glass-pipette silver-grounding electrode (WPI Inc., Sarasota, FL) into the back of an excised moth head. A second glass-pipette silver-grounding probe was connected to the cut tip of one of the male antennae. Both electrodes were filled with a Beadle-Ephrussi (Ephrussi and Beadle 1936) saline solution. Electrodes were placed into position using battery-powered piezo-drive micromanipulators.
Study Sites

Three pheromone trials were conducted in sweetpotato fields in Hawaii, two replicate ‘dose trials’ and a ‘ratio trial’. The first dose trial and the ratio trial were conducted in two nearby recently harvested sweetpotato fields. In the process of recovering marketable sweetpotato roots, culled roots were left by the grower throughout the fields. Over time, there was growth of new sweetpotato vines along with a diverse array of weeds. Roots left on the surface of the ground were infested by the resident sweetpotato vine borer population. The access road to these fields was located about 4.7 km south southwest of Hononu, Hawaii Island, HI. The field used for the first dose test was located at: Universal Transverse Mercator [UTM] grid: Easting 0274893, Northing 2196651, Zone 05 Q, 394 m elevation (Site 1). The field used for the ratio test trial was located at: UTM grid (USGS 2001): Easting 0274952, Northing 2196719, Zone 05 Q, 383 m elevation (Site 2). The repeat dose test was conducted in a fully vegetated (preharvest) sweetpotato field located near Papaikou, Hawaii Island, HI, at: UTM grid: Easting 0276361, Northing 2198186, Zone 05 Q, 396 m elevation (Site 3). The field used for the weathering trial was located near the sites for the first dose test and the ratio test, at: UTM grid: Easting 0275171, Northing 2196741, Zone 05 Q, 369 m elevation (Site 4). In order to determine environmental parameters that might affect pheromone weathering rates and sweetpotato vine borer adult activity, rainfall, temperature, relative humidity, and wind speed were recorded over the course of each trial using a Wireless Vantage Pro2 weather station (Davis Instruments, Hayward, CA) positioned in or near the sweetpotato field where the trial was conducted.

Bioassay 1: Dose Test – Harvested Field

In this test, relative male sweetpotato vine borer trap response was tested among traps with one of two doses of Type I compound and with traps treated with a 1:2 ratio of Type I: Type II compounds but with two different dose levels. For each dose level involving both Type I and Type II compounds, there were two treatments, one where 3Z,6Z,9Z-22:H was used as the Type I compound and one where 3Z,6Z,9Z-22:H was used. All lures were placed in plastic baskets and hung down from the top center of Delta traps (Great Lakes IPM Inc., Vestaburg, MI). Type I and Type II components were held in separate baskets. A sticky insert card was placed at the bottom of the traps to capture attracted males. The traps were hung on plastic fence post (DARE Products, Inc. Battle Creek, MI) with the bottom of the triangular trap positioned 0.5 m above the ground. Traps were first deployed on 25 September 2017. The following treatments were used:

1) One septum loaded with 1.0 mg of E10E14-16:Ald;
2) One septum loaded with 2.0 mg of E10E14-16:Ald;
3) One septum loaded with 1.0 mg of E10E14-16:Ald and one septum loaded with 2.0 mg of Z3,Z6,Z9-23:H;
4) One septum loaded with 1.0 mg of E10E14-16:Ald and one septum loaded with 2.0 mg of Z3,Z6,Z9-22:H;
5) One septum loaded with 2.0 mg of E10E14-16:Ald and two septa each loaded with 2.0 mg of Z3,Z6,Z9-23:H;
6) One septum loaded with 2.0 mg of E10E14-16:Ald and two septa each loaded with 2.0 mg of Z3,Z6,Z9-22:H.

Three traps of each treatment were included in the trial and the trial was set out in a randomized complete block design with a 10 × 10 m spacing. In the first week of deployment, a trap baited with a 1- to 3-d-old live virgin female was included in each block. All traps were serviced daily over the first 4 d. When traps were serviced, any dead females, or females older than 4-d, were replaced with new live virgin females (1- to 3-d old).

After the first week, traps were serviced weekly with trap location within each block rerandomized each week after trap servicing. Weekly trap servicing was terminated, after 8 wk, on 21 November 2017.

Bioassay 2: Dose Test – Vegetated Field

This trial was a repeat of Bioassay 1: Dose Test – Harvested Field but conducted in an ‘in-crop’ sweetpotato field ~5.1 km from the site of dose test 1, and no live female treatment was included in the first week of this trial. Traps were initially deployed on 9 January 2018, set out at 0.25 m above the sweetpotato vegetation with bioassay logistics as described above for Bioassay 1. This test was terminated after 8 wk on 6 March 2018.

Bioassay 3: Ratio Test – Harvested Field

In this test, male sweetpotato vine borer trap response was tested among traps treated with Type I lure alone versus traps with several different ratios of Type I: Type II components. Bioassay logistics were as described above for Bioassay 1, but the traps were first deployed on 11 September 2017. The following treatments were tested:

1) One septum loaded with 1.5 mg of E10E14-16:Ald (Type I);
2) One septum loaded with 1.5 mg of E10E14-16:Ald plus two septa each loaded with 1.5 mg of Z3,Z6,Z9-22:H (structurally similar to the Type II component) (3.0 mg total: a 1:2 ratio);
3) One septum loaded with 1.5 mg of E10E14-16:Ald plus five septa each loaded with 1.5 mg of Z3,Z6,Z9-22:H (7.5 mg total: a 1:5 ratio);
4) One septum loaded with 1.5 mg of E10E14-16:Ald plus five septa each loaded with 1.5 mg of Z3,Z6,Z9-23:H (Type II) (7.5 mg total: a 1:5 ratio);
5) One septum loaded with 1.5 mg of E10E14-16:Ald plus ten septa each loaded with 1.5 mg of Z3,Z6,Z9-22:H (15.0 mg total: a 1:10 ratio).

Three traps of each treatment were included in the trial and the trial was set out in a randomized complete block design with a 10 × 10 m spacing. Traps were initially deployed on 11 September 2017. In the first week of deployment, a trap baited with a 1- to 3-d-old live virgin female was included in each block with new moths replacing older moths as detailed above for Bioassay 1. All traps were serviced daily over the first 4 d. After the first week, traps were serviced weekly with trap location within each block rerandomized each week after trap servicing. Weekly trap servicing was terminated, after 8 wk, on 7 November 2017.

Bioassay 4: Weathering Test

In order to determine the duration of attractiveness of the binary sex pheromone system, a 12-wk weathering trial was conducted. This
trial was conducted in a vegetated (preharvest) sweetpotato field in the Honouma, Hawaii Island, area [Site 4] near the fields used in Bioassays 1 and 3. For this test, separate septa treated with 2.0-mg Type I lure and 4.0-mg Type II lure (Z3,Z6,Z9-23:Hi) were set out in separate plastic baskets suspended from the top center of Delta traps (without sticky inserts) and set out in the sweetpotato field on 6 February 2018 (12-wk traps), 27 February (9-wk traps), 20 March (6-wk traps), and 10 April (3-wk traps). One set of septa (0-wk septa), formulated at the same time as the other septa, was placed in a −20°C freezer at the Otis laboratory. Week 3, 6, 9, and 12 septa were all held in a −80°C freezer at the Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center (DKI-PBARC) until the morning of their field deployment. Four replicate traps of each weathering age were set out in a randomized block design, with traps adjusted to 0.25 m above the sweetpotato vegetation and set out 12 m apart in rows and 12 m apart between rows. When the 6-wk weathering traps were set out, all traps were set out in a new section of the field (12 m from the previous section) and sticky insert cards were added to each trap. Sticky insert cards were retrieved after 1 wk. When the 3-wk weathering traps were set out (at week 9 of the weathering trial), all traps were set out in a new section of the field (12 m from the previous section) and sticky insert cards were again added to each trap. Sticky insert cards were retrieved and replaced with new sticky insert cards after 1 wk, with traps again moved to a new section of the field. Sticky insert card retrieval and replacement and trap grid movement was repeated on weeks 10 and 11 of the weathering trial, with the weathering trial terminated after 12 wk. Upon the completion of the 12-wk weathering period, all septa were brought back to the DKI-PBARC laboratory and placed in separate heat-sealed pouch envelopes and labeled with trap number and weathering week. All septa were placed in a −80°C freezer until they were sent on ice to the USDA APHIS PPQ S&T Otis lab (Buzzards Bay, MA). Upon receiving the package, the septa were held in a −20°C freezer until the residual content of the Type I and Type II lure components were determined.

Calculation of Relative Sweetpotato Vine Borer Detection Effectiveness

As an indication of the relative effectiveness of different lure formulations in detecting the presence of adult sweetpotato vine borer, the percentage of occasions, over the first 8 wk of each replicate of each treatment where one or more male sweetpotato vine borers were captured, was calculated for Bioassays 1–3. As an example, if in a given trap, male sweetpotato vine borer adults were caught on six of eight service weeks, that trap would have a 75% detection percentage. This does not indicate what percentage of male moths in the field were caught but does provide a comparison among treatments of how often male moths were caught.

Statistical Analyses

For Tests 1–3, analysis of variance (ANOVA) was conducted on square root transformed total male catches over the first 8 wk of the trial, with Tukey’s HSD used for mean separation. In the presentation of results, numbers of males captured per trap per week followed by the same letter are not significantly different at the α = 0.05 level. Also, for Tests 1–3, as an indication of the relative effectiveness of different lure formulations in detecting the presence of sweetpotato vine borer, ANOVA was conducted on arcsine transformed percentage of occasions over the first 8 wk of the trial where one or more male sweetpotato vine borers were captured. Tukey’s HSD was, again, used for mean separation. For Tests 1 and 3 (where a virgin female treatment was included on week 1), ANOVA was also conducted on square root transformed daily trap catches over the first 4 d of the trial and for the accumulated trap catch over the first 4 d, with Tukey’s HSD used for mean separation (SAS Institute Inc. 2013). For the weathering trial, no analysis was done with the 1 wk catch data starting after 6 wk of weathering because only one of the 12 traps caught sweetpotato vine borer adults. A best fit exponential decay curve was determined based on square root transformed trap catch data, with the decay curve graphed based on untransformed trap catch data. Using untransformed residue values, best-fit equations were identified for Type I and Type II compounds, separately, to describe the change in chemical residue over time. Square root transformations of catch data and arcsine transformations of percentage data were used to improve the normality of the data for analysis by ANOVA (Sokal and Rohlf 1981).

Results

Gland Extraction and Electrophysiology

GC-MS analysis of extract made from 5 to 12 virgin female gland (n = 4) confirmed the presence of E10,E14-16:Ald and Z3,Z6,Z9-23:Hi as reported earlier by Yan et al. (2014). Mass spectra and GC retention times of the two compounds match those of the authenticated synthetic samples. A representative GC-EAD analysis is presented in Fig. 1. Male antennae (n = 9) consistently responded to the presence of the E10,E14-16:Ald in the gland extract, whereas responses to the natural Z3,Z6,Z9-23:Hi was less frequent (four out of nine antennae). No repeatable additional antennal responses were observed.

Study Sites

Average weekly rainfall, average temperature, average relative humidity, and average wind speed for each trial are presented in Table 1. Over the course of the studies reported here, average temperatures ranged from 19.3–22.2°C. Even in winter months (e.g., 19.33 ± 0.05°C in Dose test 2 – Vegetated Field [09 January–06 March 2018]) temperatures and rainfall are adequate for sweetpotato production, making sweetpotato a year-round crop in Hawaii.

Bioassay 1: Dose Test – Harvested Field

Over the course of the first week where a virgin female treatment was included, there was a significant difference in catch among treatments on day 1 (F = 4.94; df = 6, 14; P = 0.0065) and on day 3 (F = 8.36; df = 6, 14; P = 0.0006) (see Table 2). Average catch with live virgin female baited traps was numerically greater than catch in all other treatments on Days 1 (13.0) and 3 (1.67). It was, also, significantly greater than in all other treatments except the 2:4 treatment with Z3,Z6,Z9-22:Hi (2.67) (day 1), and significantly greater than in all other treatments except the 1:2 treatment with Z3,Z6,Z9-23:Hi (1.00) (day 3). There was no significant difference in catch among treatments on either day 2 or day 4. Based on total catch over the first four days, catch with the live virgin female baited trap was significantly greater than catch in all other treatments except the 2:4 treatment with Z3,Z6,Z9-22:Hi. Based on catch over 8 wk (with no inclusion of virgin female trap results), there was a significant difference in male catch among treatments (F = 8.94; df = 5, 12; P = 0.0010). Three out of four of the treatments with both Type I and Type II components had significantly greater catch than the treatments with only the Type I component. The 1.0-mg Type I component + the 2.0-mg Type II component (23:Hi) was numerically, but not significantly, greater than the treatments with only a Type I component. Trap catch
Fig. 1. Simultaneously recorded GC-FID and GC-EAD from a male *O. anastomosalis* antenna responding (arrows) to the presence of E10,E14-16:Ald (A) and Z3,Z6,Z9-23:H (B) in a female *O. anastomosalis* gland extract (~5 female equivalents).

**Table 1.** Average (±SEM) temperature, percentage relative humidity, wind speed, and weekly rain at sites used for the Dose Tests (1 and 2), the Ratio Test, and the Weathering Test.

| Test                                | Dates                        | Temp (°C) | % Relative Humidity | Wind Speed (m/s) | Average 4-Day or Weekly Rain (mm) |
|-------------------------------------|------------------------------|-----------|---------------------|------------------|-----------------------------------|
| 1. Dose Test 1 – Harvested Field    | Days 1–4 25 Sept. to 29 Sept. 2017 | 23.0 ± 0.26 | 75.1 ± 0.51         | 2.95 ± 0.08      | 16.9 ± 0.02                       |
|                                     | 8 wk 27 Sept. to 21 Nov. 2017 | 21.6 ± 0.06 | 81.6 ± 0.11         | 2.25 ± 0.03      | 48.7 ± 6.60                       |
| 2. Dose Test 2 – Vegetated Field    | 8 wk 09 Jan. to 06 Mar. 2018  | 19.33 ± 0.05 | 87.3 ± 0.09         | 3.71 ± 0.03      | 112.2 ± 0.01                       |
| 3. Ratio Test – Harvested Field     | Days 1–4 11 Sept. to 15 Sept. 2017 | 22.3 ± 0.23 | 81.1 ± 0.51         | 2.48 ± 0.06      | 91.0 ± 0.03                       |
|                                     | 8 wk 12 Sept. to 07 Nov. 2017 | 22.2 ± 0.06 | 80.1 ± 0.12         | 2.66 ± 0.03      | 50.3 ± 6.60                       |
| 4. Weathering Test – Vegetated Field| 12 wk 06 Feb. – 01 May 2018 | 19.59 ± 0.15 | 92.1 ± 0.20         | 2.11 ± 0.07      | 135.0 ± 51.9                      |

Data are presented separately for the first four days of the Dose Test – Harvested Field and the Ratio Test where a live virgin female treatment was included.

**Table 2.** Dose Test – Harvested Field

| Compound (mg) | Males captured |
|---------------|----------------|
|               | Day 1 | Days 1–4 | /trap/8 wk | 8 Wk |
| E10,E14-16:Ald | 0b    | 0b       | 2.67 ± 0.67b | 8 |
| Z3,Z6,Z9-22:H | 0b    | 0b       | 2.67 ± 1.67b | 8 |
| Z3,Z6,Z9-23:H | 2b    | 6b       | 18.00 ± 5.77ab | 54 |
| Ratio         | 5b    | 6b       | 24.67 ± 4.06a | 74 |
| 1:0           | 2b    | 4b       | 28.33 ± 3.84a | 85 |
| 2:0           | 8ab   | 18ab     | 34.67 ± 13.78a | 104 |
| Virgin Female | 39a   | 50a      |

Catches of *O. anastomosalis* males in traps, baited with different ratios of E10,E14-16:Ald to Z3,Z6,Z9-23:H or Z3,Z6,Z9-22:H, deployed in a postharvest sweetpotato field near Honomo, Hawaii Island, Hawaii. The test was conducted from 25 Sept. 2017 to 21 Nov. 2017 with weekly trap servicing and three replicates per treatment. Numbers of males captured per trap per week followed by the same letter are not significantly different at the α = 0.05 level. Numbers listed in the ‘8-wk Total’ column are the total number of males caught in each treatment over the full 8 wk of trapping. Three replicate traps with live virgin females were deployed within the trap array RCB design during the first week. Total male catch on day 1 and over the first 4 d is presented for all treatments including the virgin female-baited traps. Statistical results presented are based on average catch of the three traps rather than on the total catch presented here.
There was a significant difference in male catch among treatments. Bioassay 2: Dose Test – Vegetated Field enhancement by addition of a Type II component ranged from 6.75 to 13 times greater than catch with only the Type I component. There was no difference in catch in traps baited with 1.0 versus 2.0 mg of the Type I component. Catch in traps baited with 2.0 mg:4.0 mg Type I: Type II doses were numerically, but not statistically, higher than catch in traps baited with 1.0 mg: 2.0 mg Type I: Type II doses. Average catch with the 22:H versus 23:H Type II components was comparable (see Table 3). In terms of detection of sweetpotato vine borer, there was a significant difference in detection among treatments ($F = 6.27; df = 5, 12; P = 0.0044$). All four treatments that included both Type I and Type II components had higher relative detection percentages than the treatments with only the Type I component, but only the 2.0 mg:4.0 mg (22:H) treatment had significantly higher relative detection percentage than either the 1.0 mg or 2.0 mg Type I only treatments. Numerically, average relative detection percentage declined in the order of 2.0 mg:4.0 mg (22:H) > 2.0 mg:4.0 mg (23:H) > 1.0 mg:2.0 mg (22:H) > 1.0 mg:2.0 mg (23:H) > Type I only (1.0 mg) > Type I only (2.0 mg) (91.7% > 87.5% > 83.3% > 79.2% > 33.3% > 20.8%).

Table 3. Dose Test – Vegetated Field

| Compound (mg) | Males captured |
|---------------|---------------|
| E10,E14-16:Ald Z3,Z6,Z9-22:H | Z3,Z6,Z9-23:H | Ratio | /trap/8 wk | Total |
| 1.0 | 0.0 | 0.0 | 1:0 | 4.67 ± 3.18b | 14 |
| 2.0 | 0.0 | 0.0 | 1:0 | 5.67 ± 0.67b | 17 |
| 1.0 | 0.0 | 2.0 | 1:2 | 17.67 ± 0.33a | 53 |
| 1.0 | 2.0 | 0.0 | 1:2 | 18.33 ± 3.93a | 55 |
| 2.0 | 0.0 | 4.0 | 2:4 | 22.67 ± 2.96a | 68 |
| 2.0 | 4.0 | 0.0 | 2:4 | 18.67 ± 1.86a | 56 |

Table 4. Ratio Test – Harvested Field

| Compound (mg) | Males captured |
|---------------|---------------|
| E10,E14-16:Ald Z3,Z6,Z9-22:H | Z3,Z6,Z9-23:H | Ratio | Day 1 | Days 1–4 | /trap/8 wk | $\$ Wk |
| 1.5 | 0.0 | 0.0 | 1:5:0 | 0b | 1a | 2.67 ± 1.20b | 8 |
| 1.5 | 3.0 | 0.0 | 1:2 | 2ab | 6a | 22.00 ± 3.06a | 66 |
| 1.5 | 7.5 | 0.0 | 1:5 | 0b | 4a | 31.33 ± 4.81a | 94 |
| 1.5 | 0.0 | 7.5 | 1:5 | 1ab | 5a | 32.67 ± 0.88a | 98 |
| 1.5 | 15.0 | 0.0 | 1:10 | 0b | 5a | 29.00 ± 0.58a | 87 |

Virgin female 6a 6a

Catches of $O. anastomosalis$ males in traps, baited with different ratios of E10,E14-16:Ald to Z3,Z6,Z9-23:H or Z3,Z6,Z9-22:H, deployed in a postharvest sweetpotato field near Honomu, Hawaii Island, Hawaii. The test was conducted from 11 Sept., 2017 to 6 Nov., 2017 with weekly trap servicing and three replicates per treatment. Numbers of males captured per trap per week followed by the same letter are not significantly different at the $ \alpha = 0.05$ level. Numbers listed in the “8 Week Total” column are the total number of males caught in each treatment over the full eight weeks of trapping. Three replicate traps with live virgin females were deployed within the trap array RCB design during the first week. Total male catch on day 1 and over the first 4 d is presented for all treatments including the virgin female-baited traps. Statistical results presented are based on average catch of the three traps rather than on the total catch presented here.

3.29 to 4.00 times greater than catch with only the Type I component. There was no significant difference in catch in traps baited with 1.0 versus 2.0 mg of the Type I component. Catch in traps baited with 2.0 mg:4.0 mg Type I: Type II doses was comparable to catch in traps baited with 1.0 mg: 2.0 mg Type I: Type II doses. Catch enhancement with the 22:H versus 23:H Type II components was comparable (see Table 3). In terms of detection of sweetpotato vine borer, there was a significant difference in relative detection among treatments ($F = 7.52; df = 5, 12; P = 0.0021$). All four treatments that included both Type I and Type II components had higher relative detection percentages than the treatments with only the Type I component, but only the 1.0 mg:2.0 mg (22:H) treatment had significantly higher relative detection percentage than either the 1.0 mg or 2.0 mg Type I only treatments. Numerically, average relative detection percentage declined in the order of 1.0 mg:2.0 mg (22:H) > 1.0 mg:2.0 mg (23:H) > 2.0 mg:4.0 mg (23:H) > 2.0 mg:4.0 mg (22:H) > Type I only (1.0 mg) > Type I only (2.0 mg) (83.3% > 79.2% > 75.0% > 66.7% > 41.7% > 20.8%).

Bioassay 3: Ratio Test – Harvested Field

Over the course of the first week where a virgin female treatment was included, there was a significant difference in catch among treatments on day 1 ($F = 3.22; df = 5, 11; P = 0.049$). Average catch with live virgin female baited traps (3.0) was numerically greater than catch in all other treatments. It was, also, statistically greater than catch in the 1:5 ratio trap having Z3,Z6,Z9-23:H, the 1:10 ratio...
trap and the Type I only trap (all with 0.0 catch). It was, though, not significantly greater than the 1:2 ratio trap (0.67) and the 1:5 ratio trap with Z3,26,Z9-22:H (0.33). No moths were caught in live virgin female traps on days 2–4. Based on total catch over the first four days, there was no significant difference in average trap catch among treatments ($F = 0.57; df = 5, 12; P = 0.72$). Based on catch over eight weeks (with no inclusion of virgin female trap results), there was a significant difference in male catch among treatments ($F = 34.24; df = 4, 10; P < 0.0001$). All treatments with both Type I and Type II components had significantly greater catch than the treatment with only the Type I component, but there was no significant difference among any of the treatments with both Type I and Type II components. Trap catch enhancement by addition of a Type II component ranged from 8.25 to 12.25 times greater than catch with only the Type I component, with lower Type I: Type II ratios having numerically (though not significantly) higher catches. Catch enhancement with the 22:H versus 23:H Type II components was comparable (see Table 4). In terms of relative detection of sweetpotato vine borer, there was a significant difference in relative detection among treatments ($F = 12.47; df = 4, 10; P = 0.0007$). All four treatments that included both Type I and Type II components had significantly higher relative detection percentages than the treatment with only the Type I component. There was, though, no statistical difference in relative detection percentages among the four treatments that had both the Type I and Type II components, but, numerically, average relative detection percentage declined in the order of 1:10 > 1:5 (22:H) > 1:2 > 1:5 (23:H) (100.0% > 95.8% > 91.7% = 91.7%). Average percentage detection for the Type I only treatment was only 20.8%.

**Bioassay 4: Weathering Test—Chemical Residue in Septa**

Decrease in the residue of the Type I and Type II components in the septa over time is shown in Fig. 2. Change in the Type I component residue over time was fit to the following quadratic equation: chemical concentration (mg) = 0.0051 (week)$^2$ − 0.1649 (week) + 1.9325 ($R^2 = 0.990$). Change in the Type II component residue over time was fit to the following linear equation: chemical concentration (mg) = −0.1113 (week) + 3.7129 ($R^2 = 0.904$). The Type I component concentration in the septa declined from 1.93 mg at week 0 to 0.69 mg at week 12, a 64.4% decrease (the residue being 35.6% of the original dose). The Type II component concentration in the septa declined from 3.71 mg at week 0 to 2.38 mg, at week 12, a 36.0% decrease (the residue being 64.0% of the original dose). As the Type I and Type II pheromone components have volatilized over time, the Type I:Type II ratio has decreased. What began as a close to 1:2 ratio (1:1.92) ended as 1:3.45 ratio after 12 wk. The progressive lowering of the ratio has calculated exponential decay curve has shown the decrease (the residue being 64.0% of the original dose). As the Type I and Type II pheromone components have volatilized over time, the Type I:Type II ratio has decreased. What began as a close to 1:2 ratio (1:1.92) ended as 1:3.45 ratio after 12 wk. The progressive lowering of the ratio would tend to enhance the attractiveness of the blend, providing a counter-effect to the reduction in attraction expected by the decreased doses of the two components.

**Bioassay 4: Weathering Test—Sweetpotato Vine Borer Catch**

Average weekly moth catch and a calculated moth catch exponential decay curve are presented in Fig. 3. The calculated exponential decay curve, based on square root transformed trap catch data, was not statistically significant (ANOVA results: $F = 0.363; df = 1,10; P = 0.56$). The calculated exponential decay curve, based on untransformed trap catch data, was: trap catch = 0.621exp(−0.28 × [no. of weeks]). Based on this exponential decay curve, catch at week 12 was 73.7% of catch at week 1. Clearly, though, because of the lack of statistical significance of the calculated decay curve, a repeat weathering trial is needed to better document change in lure response over time, preferably conducted in a field with a higher sweetpotato vine borer population.

**Discussion**

Results of our trials show that Hawaii populations of the sweetpotato vine borer utilize Z3,26,Z9-23:H in mating communication as had earlier been demonstrated for field populations in Vietnam (Yan et al. 2014). Inclusion of the Type II component with the Type I component enhanced trap catch by as much as 13 times over catch in traps baited by the Type I component only. Lures with both Type I and Type II components also significantly enhanced sweetpotato vine borer detection capability relative to traps with only the Type I lure. In all three bioassays, both Z3,26,Z9-22:H and Z3,26,Z9-23:H synergistically enhanced the attractiveness of the Type I component.
enhancement of trap catch by the addition of the Type II compound in the vegetated field compared with 6.75- to 13-fold enhancement of trap catch in the harvested field. We think, though, that this difference in trap catch enhancement may just be a chance higher catch in the traps baited with the Type I only lure rather than a substantive difference in trapping effectiveness.

As reported by Yan et al. (2014), Z3,Z6,Z9-23:H has been reported to be a sex pheromone component in four other crambid species (Coleoptera: Crambidae): the Alpinia stem borer (Conogethes pluto (Butler); El-Sayed et al. 2013), the yellow peach moth (Conogethes punctiferalis (Gueneé); Xiao et al. 2012), the red banded mango caterpillar (Deanolis sublimathalis Snellen; Gibb et al. 2007), and the tomato fruit borer (Neoleucinodes elegantalis (Gueneé); Cabrera et al. 2001, Jaffe et al. 2007). Vang et al. (2018) have recently reported a sixth crambid species, L. orbomalis Gueneé, for which both Z3,Z6,Z9-23:H and Z3,Z6,Z9-22:H were found to be sex pheromone components. Field work with the tomato fruit borer moth shows promise for the use of a binary sex pheromone system involving Z3,Z6,Z9-23:H for population field suppression through mass trapping. With N. elegantalis, a selected proportion of the two components (different than found in the blend produced by females) has been found to be very efficient for mass trapping of male N. elegantalis in the field. Mass trapping with the selected blend has significantly reduced in-field damage of tomatoes and is used to control N. elegantalis infestations in tomato plantations in Colombia, Brazil, Ecuador, and Venezuela (Jaffe et al. 2007).

As noted by Yan et al. (2014), the enhanced attractiveness of the Type I + Type II lure, together with the duration of its attractiveness, commend its use as a sweetpotato vine borer population monitoring tool and also provide a tool that could be used to improve understanding of sweetpotato vine borer ecology, including improved understanding of habitation areas. We have made some preliminary use of this lure to look at spatial distribution of sweetpotato vine borer field populations and the influence of trap height on catch. Presentation of these results, however, is beyond the scope of this paper and is reported elsewhere (Mc.G.T. and C.D.S., unpublished data).

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