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# Disruption of *Darna pallivitta* (Lepidoptera: Limacodidae) by Conventional and Mobile Pheromone Deployment

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**ABSTRACT.** Identification of the *Darna pallivitta* (Moore) pheromone component n-butyl (*E*)-7,9-decadienoate (*E*7,9-10:COOn-Bu) has made it possible to investigate communication disruption to control this lepidopteran pest. Conventional communication disruption trials showed marked decreases in the mean number of male moths captured in *E*7,9-10:COOnBu-treated fields compared with control fields. For traps baited with *E*7,9-10:COOnBu, percent disruptions were 94.4% and 92.1% for septa (1 g pheromone/ha, 1-wk trial duration) and spirals (6 g pheromone/ha, 8-wk trial duration) respectively. For traps baited with virgin female moths, percent disruption was 73.3% using septa disruptors (1 g pheromone/ha, 1-wk trial duration). Mobile communication disruption using *Bactrocera cucurbitae* (Coquillett) as carriers for *E*7,9-10:COOn-Bu was evaluated in the following three areas: fly survivorship, attraction of male moths to treated flies, and moth disruption in a small-scale field trial. Topical application of *E*7,9-10:COOnBu showed no significant decrease in survivorship at 50 and 80 μg/fly. However, decreased survivorship was observed at 100 μg/fly and linear regression showed *E*7,9-10:COOnBu dose was significantly correlated with *B. cucurbitae* survivorship. Traps containing honey–pheromone-fed flies attracted and caught *D. pallivitta* over a 1-wk period, demonstrating the attractiveness of the carrier. Releasing *E*7,9-10:COOnBu-fed *B. cucurbitae* (∼2 g pheromone/ha, 1-wk trial duration) resulted in significantly reduced trap catches in treatment fields compared with control fields on the first 2 d of the field trial. Percent disruptions were 84.7% (day 1) and 56.0% (day 2). These results suggest that both conventional communication disruption and mobile communication disruption have potential to control *D. pallivitta*.

**Key Words**: melon fly, n-butyl (*E*)-7,9-decadienoate, sex pheromone, invasive species, communication disruption

The nettle caterpillar moth, *Darna pallivitta* (Moore) (Lepidoptera: Limacodidae), is an important invasive pest in the Hawaiian Islands with impacts on both agriculture and public health. Nettle caterpillars defoliate ornamental nursery stock, damage a number of agricultural crops (including coffee and macadamia), and have been reported to feed on over 45 plant species in 22 families (Conant et al. 2002). As suggested by their common name, *D. pallivitta* caterpillars have urticating hairs that can cause painful stings and skin inflammations. While the nettle moth is a problem for homeowners because of the direct plant damage and the injuries that occur during yard work, nursery producers must also comply with regulations on plant movement due to the quarantine status of *D. pallivitta*. To help mitigate the negative impacts of *D. pallivitta*, the Hawai'i Department of Agriculture began releasing a Eulophid parasite, *Aroplectrus dimerus* Lin, in mid-2010 (Bautista et al. 2014). The results of these releases have been encouraging, and it is hoped that the parasite will help to reduce the moth populations. Monitoring of moth populations has been aided by the identification of a sex pheromone lure, n-butyl (*E*)-7,9-decadienoate (*E*7,9-10:COOnBu), for *D. pallivitta* (Siderhurst et al. 2007). The pheromone lure has been used in trapping grids on several islands to monitor and delimit moth populations and to help document declines in *D. pallivitta* associated with releases of *A. dimerus* (N. Reimer, personal communication).

Although moth sex pheromone lures can be used as monitoring tools, they also have potential to directly reduce pest moth populations when used in pheromone-based control strategies, such as mass trapping or mating disruption. Mass trapping involves deploying a large number of pheromone traps in an area to capture male moths before they can mate with females. If enough mates are removed in this way, the pest population will decline to a point where economic damage is reduced (El-Sayed et al. 2006). Mating disruption uses synthetic sex pheromones to interfere with the ability of an insect to locate a mate (Sarfraz et al. 2006). With lepidopteran pests, the most commonly used strategy is to release the female sex pheromone which masks the pheromone plumes produced by females or causes false trail following in male moths or both (Suckling 2000). Either mechanism can potentially lead to reductions in the moth population due to the reduced probability of male moths successfully locating and mating with females (Bartell 1982).

A more recently proposed control method involving pheromones, termed mobile mating disruption (MMD), involves the use of a carrier species to disrupt normal mating communication in a target pest species. The first investigation of this cross species disruption was studied with melon fly, [*Bactrocera cucurbitae* (Coquillett)] and oriental fruit fly, [*B. dorsalis* (Hendel)] using the male specific lures for both species (Suckling et al. 2007). Although a reduction in mating was observed in large field cages, mating in neither species was completely inhibited. Sex pheromones are often more powerful behavioral triggers than other semiochemicals and were therefore the subject of a more recent MMD study using Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), as carriers for the light brown apple moth, *Epiphyas postvittana* (Walker), pheromones (*E*)-11-tetradecenyl acetate and (*E*,*E*)-9,11-tetradecadien-1-yl acetate (Suckling et al. 2011). Pheromone-treated *C. capitata* flies used in this MMD field study were prepared by spraying flies with an aqueous suspension of microencapsulated pheromone. Results showed percent disruptions of ≥90% during the first 24 h, which fell off during the following several days. Suckling et al. (2011) suggest that this novel method of pheromone dispersal may provide advantages over conventional dispersal methods such as offering a more socially acceptable approach to spreading an active agent to control invasive insects in urban environments and other areas that are geographically inaccessible for conventional pheromone mating disruption applications (Suckling et al. 2011).

Apart from establishing a proof-of-concept for MMD, Suckling et al. (2011) provided a framework for continued study of the control technique. In particular, they suggest the following stages be addressed when experimenting with new carrier/pest systems: 1) "do no harm" to the carrier organism; 2) sustained attraction and a scalable application method and 3) orientation disruption in the field. In addition to the three areas of this framework, we were also interested to assess the amount and longevity of pheromone carried by treated "carrier" insects. In this study, we return to using *B. cucurbitae* as used by Suckling et al. (2007). In addition to having been used in a previous MMD study, melon flies have the advantage of being a large tephritid, and they were easily obtained from stocks mass-reared at the USDA ARS Pacific Basin Agricultural Research Center.

The objectives of the presented research were to evaluate the use of communication disruption techniques to control *D. pallivitta* using both conventional disruptors (rubber septa or plastic spiral matrix releasing *E*7,9-10:COOnBu) and mobile disruption (*E*7,9-10:COOnBu carried and released by *B. cucurbitae*). Conventional disruption techniques were tested in separate experiments with septa and spiral disruptors in easily accessible fields dominated by tall grasses. Mobile disruption experiments were conducted to assess mortality induced by application of *E*7,9-10:COOnBu to adult *B. cucurbitae*, to assess strength and longevity of attraction of *D. pallivitta* to *E*7,9-10:COOnBu-treated *B. cucurbitae*, and to assess mobile disruption under field conditions.

## Materials and Methods

**Insects.** Female *D. pallivitta* moths used in experiment 2 were collected as caterpillars from grasses on the University of Hawai'i, Manoa, Waiakea Research Station, Hilo, HI (GPS coordinates: Zone 5, 281751 E, 2173285 N). Caterpillars were reared on cut Hawai'ian ti [*Cordyline fruticosa* (L.) (Agavaceae)] and sorghum [*Sorghum* spec. (Poaceae)] leaves in plastic rectangular containers (Rubbermaid, Fairlawn, OH). Cocoons were removed and placed in individual covered plastic cups. Following eclosion, moths were sorted by sex and held in different chambers. Both cocoons and moths were held at ∼24°C on a photoperiod of 12:12 (L:D) h. Male *D. pallivitta* used to determine the sensitivity of olfactory perception of *E*7,9-10:COOnBu were live captured in one-way bucket traps baited with *E*7,9-10:COOnBu. Traps were made from large plastic buckets (∼20 cm by 20 cm) with four equidistant holes (2.5 cm in diameter), 3 cm from the top, into which was fitted a 4-ml clear plastic vial with a 12-mm hole in the center. Attracted male moths pass through the "one-way" vial hole, become trapped, and are collected in the morning.

Adult *B. cucurbitae* flies used in experiments 4–6 were obtained as pupae from the mass-rearing unit at the USDA ARS Pacific Basin Agricultural Research Center, Mass-Rearing Facility in Honolulu, HI. Larvae were reared on a standard wheat, sugar, yeast diet (Tanaka et al. 1969). For experiment 6, pupae were shipped by air to Hilo, HI. Before treatment in experiments 4–5, pupae were placed into 30 cm by 30 cm by 30 cm cubical aluminum screen cages containing sugar, water, and hydrolyzed protein. Flies held in the laboratory were kept at 24°C, 60–80% relative humidity, and a photoperiod of 12:12 (L:D) h. Prior to testing, flies were immobilized at 5°C and separated into groups of either 50 males or females and were then held at room temperature (24°C) for at least 1 h before use.

**Instrumentation.** Three different gas chromatographs (GC) were used in this study. *E*7,9-10:COOnBu residues on *B. cucurbitae* were analyzed by either GC-mass spectroscopy (GC/MS) or GC-flame ionization detector (GC/FID). GC/MS analyses were performed on a Agilent Technologies 6890N GC (Palo Alto, CA) interfaced to a Hewlett-Packard 5973 mass selective detector equipped with an HP-5MS column (30 m by 0.25 mm ID, 0.25 µm film thickness). The temperature program used was 60–240°C at 10°C/min with a 1-min start delay and the injector temperature set at 250°C using helium as a carrier gas (1.1 ml/min). GC/FID analyses were performed on an Agilent Technologies 6890 GC programmed to match the conditions of the GC/MS and equipped with an HP-5 column (30 m by 0.25 mm ID, 0.25 µm film thickness).

Male *D. pallivitta* antennal responses to *E*7,9-10:COOnBu were recorded using a GC-electroantennogram detector (GC/EAD) system. This instrument consisted of an Agilent Technologies 6890 GC coupled to a Syntech EAD system (Hilversum, The Netherlands). The GC was equipped with an HP-5 column (30 m by 0.25 mm ID, 0.25 µm film thickness) with helium as carrier gas (2.3 ml/min) and makeup gas (10 ml/min), which were combined with a Y-type connector. A Graphpack-3D/2 flow splitter was attached to the base of the connector, and the effluent was split 1:1 between the FID and the EAD via a heated transfer line (250°C). The injector, in splitless mode, and FID were held at 250 and 275°C, respectively. The oven temperature program began at 60°C for 1 min and then ramped at 10°C/min to 240°C. Whole moth heads or excised antennae were secured with electrode gel (Spectra 360; Parker Laboratories, Inc., Fairfield, NJ) between the parallel metal paddle electrodes of a Syntech EAG probe antenna holder (5-mm separation between paddles). Humidified air was passed over the antennal prep and acted as a carrier for effluent from the EAD transfer line. The signal generated by the EAD was passed through a Syntech NL 1200 high-impedance amplifier and analyzed with the FID signal using Syntech GC-EAD2000 software, version 2.5.

**Synthetic Pheromone Lures.** *E*7,9-10:COOnBu was synthesized by Pacific Agriscience, Singapore, as per Siderhurst et al. (2007). The material was analyzed by GC-FID and shown to be >95% pure with the major impurity being the *Z* isomer. $^{1}$H-NMR and GC/MS of the Pacific Agriscience material were consistent with Siderhurst et al. (2007). Red rubber septa were loaded with two amounts of *E*7,9-10:COOnBu, 250 µg for trap lures or 2.5 mg for disruptors (experiments 1 and 2). White plastic polymer spirals loaded with 20 mg of *E*7,9-10:COOnBu were prepared by Scentry Biologicals, Inc., Billings, MT, for use as disruptors (experiment 3).

**Conventional Communication Disruption (Experiments 1–3).** Experiments to assess the ability of synthetic of *E*7,9-10:COOnBu lures to disrupt the *D. pallivitta* communication system were conducted at four separate field sites on the University of Hawai'i, Manoa, Waiakea Research Station, Hilo, HI. All sites were fields of tall grass, predominantly California grass [*Brachiaria mutica* (Forsk.) Stapf] and Pangola grass (*Digitaria eriantha* Steud.), both averaging ∼1 m in height, bordered by forested areas, predominantly *Psidium cattleianum* Sabine.

Experiment 1, conducted 28 June 2007 to 13 July 2007, assessed the effect of 2.5 mg *E*7,9-10:COOnBu disruptors on captures of male *D. pallivitta* in traps baited with 250 µg *E*7,9-10:COOnBu lures using a crossover-repeated measures design. In each of the four fields (GPS coordinates: Zone 5, 281753 E, 2173386 N, 281694 E, 2173536 N, 282048 E, 2173993 N, and 282198 E, 2173842 N), four Jackson traps (delta type trap; sticky surface area ∼130 cm$^2$; Better World Manufacturing, Fresno, CA), baited with 250 µg of *E*7,9-10:COOnBu, were suspended at a height of 1 m on poles. The baited Jackson traps formed the corners of a 10 by 10 m square in the center of the experimental area. In two of the fields, 40 2.5-mg *E*7,9-10:COOnBu rubber septa disruptors were attached at a height of 1 m to poles spread evenly through a 30.5 by 30.5 m (∼0.1 ha) area surrounding the four Jackson traps. The sticky trap inserts were replaced daily, and the number of moths captured was recorded for days 1–7. The control and *E*7,9-10:COOnBu-disrupted fields were switched at the end of 1 wk. Each field was considered a replicate, with traps sampling within each field (4 replicates, 2 treatment, 16 traps, 112 subreplicates per treatment).

Experiment 2, conducted 3 August 2007 to 16 August 2007, directly evaluated mating disruption by assessing the effect of 2.5 mg *E*7,9-10:COOnBu disruptors on captures of male *D. pallivitta* in traps baited with caged female *D. pallivitta.* In two fields (GPS coordinates: Zone 5, 281694 E, 2173536 N, and 282048 E, 2173993 N), four Jackson traps, baited with live, 2–3-d-old, virgin female moths confined in 3 by 3 cm

conical screen cages containing a cotton wick loaded with sugar syrup, were suspended at a height of 1 m on poles forming a 10 by 10 m square in the center of the experimental area. Disruptors were placed in one of the fields as described in experiment 1. The control and *E*7,9-10:COOnBu-disrupted fields were not rotated for this experiment, as results from experiment 1 showed little variation between fields, and so as to avoid residual in the treated fields (depressed populations from treatment in week 1 carrying over into week 2 controls). The experiment included 2 replicates, 2 treatments, 8 traps, and 24 subreplicates per treatment. The sticky trap inserts were replaced daily, female moths were replaced on the third day, and the number of moths captured was recorded for days 1–6.

Experiment 3, conducted 19 August 2009 to 15 October 2009, assessed the effect of 15 mg *E*7,9-10:COOnBu spiral disruptors on captures of male *D. pallivitta* in traps baited with 250 µg *E*7,9-10:COOnBu lures. In two fields (GPS coordinates: Zone 5, 281694 E, 2173536 N, and 282048 E, 2173993 N), four Jackson traps, baited with 250 µg of *E*7,9-10:COOnBu, were suspended at a height of 1 m on poles forming a 10 by 10 m square in the center of the experimental area. In addition to the baited Jackson traps, four blank Jackson traps were suspended at 1 m from poles setup in a second 10 by 10 m square (sticky insert but no lure, negative controls). In one of the fields, 40 15-mg *E*7,9-10:COOnBu plastic polymer spiral disruptors were attached at a height of 1 m to poles spread evenly through a 30.5 by 30.5 m (∼0.1 ha) area surrounding the eight Jackson traps. The sticky trap inserts were replaced every 1–7 d (usually at 3–4-d intervals), and the number of moths captured was recorded. The 250 µg *E*7,9-10:COOnBu septa lures were replaced in the 5th week of the experiment, 18 September 2009. This study aimed to assess both effectiveness and longevity of disruption so control and *E*7,9-10:COOnBu-disrupted fields were not rotated. The experiment was unreplicated due to resource constraints (2 treatments, 16 traps, 32 subreplicates per treatment).

**E7,9-10:COOnBu-Induced Mortality in B. cucurbitae, "do no harm" (Experiments 4).** Experiment 4, conducted 31 March 2008 to 26 May 2007, assessed mortality of *B. cucurbitae* caused by direct application of *E*7,9-10:COOnBu dissolved in acetone. Three-day-old male melon flies were immobilized at 5°C and placed ventral side down in Petri dishes on ice. The flies were topically treated on the dorsal side of the thorax with 1 µl of either a *E*7,9-10:COOnBu solution (50, 80, or 100 µg/µl) in acetone or acetone alone (vehicle, treated control) using a 50 µl Hamilton syringe. Flies were kept in ∼1 liter soda cup "cages" with screened lids and given agar and sugar. Five cages containing 20 flies each were used for each treatment dose (20 flies per replicate, 5 replicates per treatment). Five cages containing 20 male flies each were held as an untreated control. Dead flies were counted and removed after 1, 3, and 7 d, with the number of surviving flies calculated for each day.

An additional test assessed mortality in field cages. Three groups of 100 flies each were treated with either 80 µg/ml of pheromone (treatment), 1 µl acetone (vehicle control), or nothing (control) and placed in 3 by 3 by 2.5 m screen cages. Temperature, humidity, and light were monitored and recorded in laboratory and field cage holding areas. Dead flies were counted and removed after 1, 3, and 7 d, with the number of surviving flies calculated for each day. This mortality bioassay was repeated five times (100 flies per replicate, 15 replicates per treatment).

**Attraction of D. pallivitta to E7,9-10:COOnBu-Treated B. cucurbitae (Experiment 5).** Experiment 5, conducted 18 June 2009 to 2 July 2009, assessed the strength and longevity of attraction of *D. pallivitta* females to *E*7,9-10:COOnBu-treated *B. cucurbitae*. Groups of 20 *B. cucurbitae* pupae were held in 500 ml plastic containers (11.5 cm in diameter and 7.5 cm in height). Flies were allowed to emerge and given water and 0.1 g of 1, 5, or 10% (wt/wt) pheromone to honey solution. After 4–5 d, individual flies were placed in a screened cone cage (4.5 cm in diameter, 5 cm in height) with a 1-cm wet cotton wick. Cone cages were placed in the middle of the sticky insert of a large plastic delta sticky trap (sticky surface area ∼380 $cm^2$, Scentry Biologicals, Inc., Billings, MT).

Treatments tested included traps baited with individual melon flies fed 1, 5, or 10% *E*7,9-10:COOnBu in honey and traps baited with 250 µg of *E*7,9-10:COOnBu on a rubber septa (positive control). Traps were deployed in two field locations: University of Hawai'i, Manoa, Waiakea Research Station, Hilo, HI (GPS coordinates, UTM: 281695 E, 2173404 N), and United States Pacific Basin Agricultural Research Center, USDA-ARS, Hilo, HI (GPS coordinates, 280593 E, 2179480 N). Three traps per treatment were placed 20 m apart in a random design and rotated on each service day. Traps were serviced at 1, 4, 5, 6, and 7 d with captured moths removed and counted (2 sites, 12 traps, 60 replicates per treatment).

**Analysis of E7,9-10:COOnBu on Treated B. cucurbitae (Experiment 6).** Experiment 6 was conducted using a series of analyses to quantify the amount of *E*7,9-10:COOnBu on treated *B. cucurbitae*. To assess pheromone extraction efficiency, melon flies treated either topically or injected with *E*7,9-10:COOnBu were extracted with 2 ml of hexane, which was subsequently concentrated and quantified by GC/MS. Because of matrix interference, pheromone concentrations in GC/MS traces were determined by external calibration using the extracted ion chromatograph command in Agilent Chemstation software (Agilent Technologies, Santa Clara, CA). The four ions used for quantification, *m*/*z* 67, 108, 150, and 224, had linear standard curves ($R^2 \geq 0.995$) in the concentration range 1 pg–100 ng. *B. cucurbitae* fed 1, 5, or 10% *E*7,9-10:COOnBu in honey (from experiment 5) were analyzed in the same manner, immediately after being removed from their feeding chambers.

In addition to measuring initial pheromone loadings for melon flies fed 1, 5, or 10% *E*7,9-10:COOnBu in honey, an attempt was made to quantify the pheromone released from treated *B. cucurbitae* using headspace trapping. To accomplish this, groups of 10 treated flies (fed 5% pheromone in honey) were placed in a glass aeration chamber (12 cm by 3 cm) equipped with inlet and outlet ports. The chamber was flushed with ambient air flowing at 20–25 ml/min and exiting through the outlet port to a Porapak Type Q (50–80 mesh) glass column (9.5 cm, 4 mm ID). A small cotton wick wetted with water was also placed in the aeration chamber. The effluent was collected overnight and extracted with 2 ml of hexane. In a separate test, 25 treated melon flies were placed in a half-pint glass canning jar fitted to inlet and outlet ports allowing for a Porapak Q column to collect the effluent as previously described. Ethyl nonanoate was added to Porapak Q extracts as an internal standard before concentration and analysis by GC/FID.

A final instrument analysis was conducted comparing the sensitivity of the *D. pallivitta* antenna (GC/EAD) to that of the MS and FID. The dilution series used to construct standard curves (1 fg–100 ng) was run on the GC/EAD and minimum detection limits were recorded.

**Mobile Communication Disruption (Experiment 7).** Experiment 7, conducted 18 June 2009 to 2 July 2009, assessed the effect of released *E*7,9-10:COOnBu-treated *B. cucurbitae* on captures of male *D. pallivitta* in traps baited with 250 µg *E*7,9-10:COOnBu lures. Sterile *B. cucurbitae* pupae (100 ml, irradiated at 100 Gys [2 dbe]) were placed in modified action packers (49 cm by 31 cm by 39 cm, Rubbermaid, Atlanta, GA) with screened windows. Following eclosure, flies were given water, sugar, and protein. When the flies were 5-d old, the sugar and protein were removed and the flies were fed 1 g of ∼5% *E*7,9-10:COOnBu in honey (wt/wt, ∼50 mg pheromone) for 2 d before being released in the field. Four Jackson traps containing 250 µg *E*7,9-10:COOnBu lures and 4 blank Jackson traps were placed 5 m apart in the center of two 0.1 hectare macadamia nut tree plots (GPS coordinates, UTM: Zone 5, 281627 E, 2173036 N, and 282254 E, 2173762 N). To begin the experiment, *E*7,9-10:COOnBu-treated *B. cucurbitae* were released in the treatment field (∼2 g pheromone/ha), and untreated *B. cucurbitae* were released in the control field, both from modified action packers. Empty action packers were removed from the field immediately following the fly releases to prevent

attraction to residual *E*7,9-10:COOnBu. Jackson traps were serviced at 1, 2, 3, and 7 d after the *B. cucurbitae* release. The control and *E*7,9-10:COOnBu-disrupted fields were not rotated for this experiment, so as to avoid residual in the treatment field. The experiment included 2 replicates, 2 treatments, 8 traps, and 32 subreplicates per treatment.

**Data Analysis.** All trap catches were pooled and converted to moths captured per field per day. Moth catches from experiments 1 and 7 were log transformed to equalize variance before analysis (untransformed data shown in Table 1 and Fig. 1). Numbers of male *D. pallivitta* captured in experiments 1, 2, and 7 were analyzed using multivariate analysis of variance (MANOVA) for repeated measures (SAS 2012). Experiments 2 and 3 were not replicated and were therefore analyzed using an intraclass correlation, calculated using the maximum likelihood estimates of variance between and within fields receiving the same treatments (Perrett 2006, Perrett and Higgins 2006, Austin and Wilcox 2012). Experiments 1–3 were conducted in the same fields, so an estimate of the intraclass correlation coefficient of 0.26 was calculated from the replicated data of experiment 1. Using adjusted standard errors, pairwise comparisons were made using *t*-tests (SAS 2000). Unbaited control traps in experiments 3 and 7 did not capture any *D. pallivitta* and so were excluded from further analysis. The total (experiments 1–3 and 7), daily (experiments 1 and 7), and weekly (experiment 3) mean number of males caught per field per day were compared. Daily (experiments 1 and 7) and weekly (experiment 3) percentage disruptions were calculated using the formula (1 − [mean of treatment/ mean of control field catch]) * 100 and differences were analyzed using Fisher's exact test because of the small sample sizes. Differences in percent disruption between time intervals were compared in 2 by 2 contingency tables with multiple hypothesis testing error measured by false discovery rate using the False Discovery Rate Tool (Microsoft, www.microsoft.com). Percent survivorship of *B. cucurbitae* after treatment (experiment 4) and mean number of male *D. pallivitta* captured in traps baited with *E*7,9-10:COOnBu-treated *B. cucurbitae* (experiment 5) were analyzed by analysis of variance followed by comparison of means using Tukey's HSD studentized range test (SAS 2012).<AQ15/> Additionally, *B. cucurbitae* survivorship (experiment 4) was plotted against *E*7,9-10:COOnBu and a linear regression analysis was performed. All analyses of significance were made at the $P < 0.05$ level unless otherwise stated.

## Results

All conventional communication disruption trials (experiments 1–3) showed marked decreases in the number of male *D. pallivitta* captured in *E*7,9-10:COOnBu-treated fields compared with control fields (Fig. 1; Table 1). For traps baited with *E*7,9-10:COOnBu (experiments 1 and 3), the total percent disruptions were 94.4% and 92.1% for septa and spirals respectively, with time interval disruption ranges of 90.5–98.2% (daily) and 62.5–98.6% (weekly), respectively. Mean daily captures of male *D. pallivitta* in *E*7,9-10:COOnBu-treated fields were lower than the corresponding control fields for all days tested (Fig. 1).

For experiment 1, repeated measures MANOVA showed a significant decrease in the mean numbers of moths per field in *E*7,9-10:COOnBu-treated fields ($P = 0.0003$), with marginally insignificant effects of time ($P = 0.052$) and time by field ($P = 0.059$). Additionally, there were no significant differences in daily percent disruptions throughout the 7-d period (Fig. 1).

During the first week of experiment 2, in which traps were baited with female *D. pallivitta*, total male *D. pallivitta* trap captures were 77 and 20 for the control and *E*7,9-10:COOnBu-treated fields respectively, while in the second week, they fell to 9 and 3, respectively. Repeated measure analysis showed no differences between mean daily captures of male *D. pallivitta* in control or *E*7,9-10:COOnBu-treated fields ($P = 0.46$), possibly due to the precipitous fall in the moth population during the second week of this experiment. However, when week 1 was analyzed as an unreplicated experiment, using subsamples (moths per trap per d) and adjusted estimates of errors, the mean number of moths caught in the treatment field was lower than in the control (*t*-test, $P = 0.0026$). The percent disruption for traps baited with female *D. pallivitta* was 73.3%.

Experiment 3 showed lower weekly moth catches in the *E*7,9-10:COOnBu spiral-treated field when compared with the control field (*t*-test using adjusted errors, $P < 0.0001$)(Fig. 1). In contrast to experiment 1, daily percent disruptions did vary by week in experiment 3 ($P < 0.05$, $Q < 0.05$). Weeks 1 and 4–7 showed the highest percent disruption, while there is a marked reduction in disruption in week 8 (Fig. 1).

Results of experiment 4 laboratory tests showed significantly lower survivorship in only the *B. cucurbitae* treated with the 100 µg *E*7,9-10:COOnBu dose compared with other *E*7,9-10:COOnBu doses and controls (Table 2). Linear regression analyses of *B. cucurbitae* survivorship against *E*7,9-10:COOnBu dose showed significant correlations at each time interval (day 1: $y = -0.35(8)\ x + 103(5)$, $R^2 = 0.4396$, $F = 18.04$, $P < 0.01$, day 3: $y = -0.39(8)\ x + 103(5)$, $R^2 = 0.4991$, $F = 22.92$, $P < 0.01$, day 7: $y = -0.42(8)\ x + 103(5)$, $R^2 = 0.5475$, $F = 27.83$, $P < 0.01$) and for all days combined (days 1, 3, and 7: $y = -0.38(5)\ x + 103(3)$, $R^2 = 0.4934$, $F = 71.09$, $P < 0.01$). No differences in mean percent survivorship were observed between treatment groups in the experiment 4 cage tests (Table 2).

Results of experiment 5 tests showed that individual melon flies fed 1, 5, and 10% *E*7,9-10:COOnBu and honey mixture were attractive to male *D. pallivitta* for up to 7 d in the field (Table 3). There were no differences in the mean number of nettle moths caught in traps baited with *B. cucurbitae* fed different concentrations of *E*7,9-10:COOnBu (Table 3). However, trap captures for individual flies fed *E*7,9-10:COOnBu were lower than the positive control (250 µg *E*7,9-10:COOnBu on a rubber septa) at all time intervals (Table 3). Several observations were noted during the experiment. First, flies were observed directly feeding on the pheromone/honey mixture after eclosure but were sometime also observed falling into and crawling out of the mixture. This makes it difficult to determine how much pheromone/honey is being ingested and how much is simply adhering to the body of the fly. Additionally, it was observed that flies never consumed all of the provided pheromone/honey mixture. Finally, flies normally lived for several days after being confined in the trap, but usually all flies were dead by the 4th day of the trial period (7 d).

Results of experiment 6 (GC analyses) showed appreciable levels of *E*7,9-10:COOnBu on treated melon flies. Concentrations of pheromone

**Table 1. Communication disruption of *D. pallivitta* field tests (experiments 1–3 and 7)[a]**

| | Experimental setup | | Experimental results | | |
|---|---|---|---|---|---|
| Experiment no. | Trap lure | Disruptor | Treatment (moths/field/d ± SE) | Control (moths/field/d ± SE) | % Disruption |
| 1 | E7,9-10:COOnBu on septa | E7,9-10:COOnBu on septa | 1.0 ± 0.2 a | 17 ± 2 b | 94.4 |
| 2 | Female *D. pallivitta* | E7,9-10:COOnBu on septa | 2.0 ± 0.4 a | 7 ± 2 b | 73.3 |
| 3 | E7,9-10:COOnBu on septa | E7,9-10:COOnBu on spiral | 0.7 ± 0.2 a | 9.2 ± 0.8 b | 92.1 |
| 7 | E7,9-10:COOnBu on septa | E7,9-10:COOnBu on melon fly | 18 ± 4 a | 38 ± 5 b | 52.1 |

[a] Means followed by different letters within each experiment are significantly different at $P < 0.05$; by *t*-test (experiment 1), by *t*-test using subsamples, and adjusted estimates of errors (experiments 2, 3, and 7).

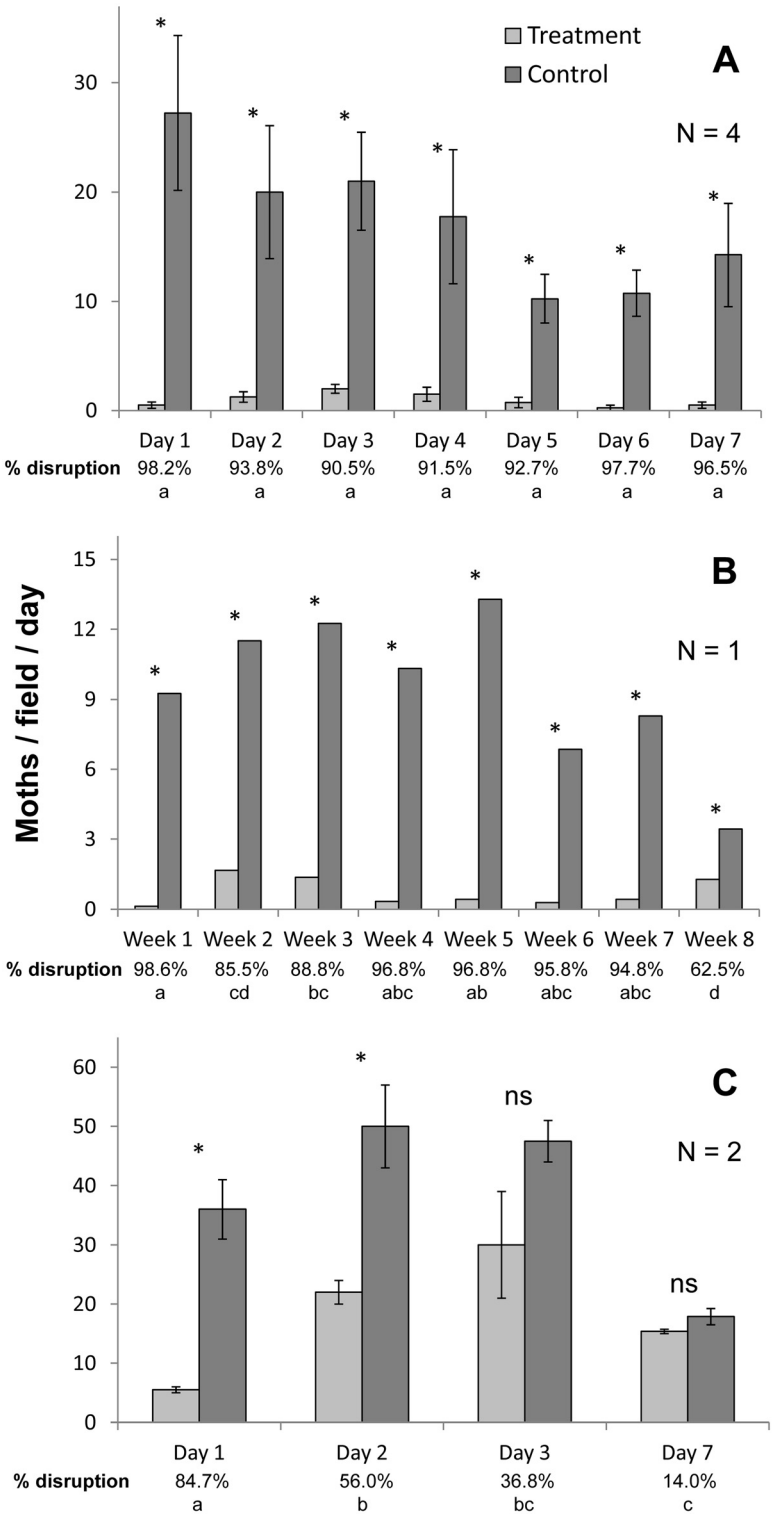


**Fig. 1.** Field trials of *D. pallivitta* communication disruption over time. (A) Disruption by 2.5 mg *E*7,9-10:COOnBu rubber septa disruptors (experiment 1), (B) by 15 mg *E*7,9-10:COOnBu spiral disruptors (experiment 3), and (C) by released *E*7,9-10:COOnBu-treated *B. cucurbitae* (experiment 7). Bars represent numbers (mean ± SE) of males captured in Jackson traps baited with 250 μg of n-butyl (*E*)-7,9-decadienoate per field per day. Treatment and control trap captures significantly different (A and C compared with *t*-tests, B compared subsamples with *t*-tests using adjusted estimates of errors) at a given time interval are marked ($P < 0.05$). Percent disruptions are given for each time interval with different letters below percentages representing significantly different ($P < 0.05$) between time intervals by 2 by 2 contingency tables with multiple hypothesis testing error measured by false discovery rate.

**Table 2. Survivorship of adult *B. cucurbitae* after *E*7,9-10:COOnBu application (experiment 4)[a]**

| | | % Survivorship | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Laboratory test | | | | | Cage test | | |
| | *N* | 50 μg | 80 μg | 100 μg | Acetone | Control | 80 μg | Acetone | Control |
| Day 1 | 5 | 95 ± 2 a | 87 ± 5 a | $(5 \pm 1) \times 10^2$ b | 99.0 ± 0.3 a | 98.8 ± 0.4 a | 30 ± 8 a | 44 ± 7 a | 43 ± 6 a |
| Day 3 | 5 | 95 ± 2 a | 85 ± 5 a | $(5 \pm 1) \times 10^2$ b | 98.0 ± 0.3 a | 98.6 ± 0.5 a | 11 ± 4 a | 8 ± 3 a | 16 ± 4 a |
| Day 7 | 5 | 94 ± 2 a | 83 ± 5 a | $(4 \pm 1) \times 10^2$ b | 97.6 ± 0.5 a | 98.4 ± 0.6 a | 2 ± 1 a | 4 ± 3 a | 2 ± 1 a |

[a] Means followed by different letters within each day are significantly different at $P < 0.05$; Tukey's HSD.

**Table 3. Attraction of *D. pallivitta* males to traps baited with a single, caged, *E*7,9-10:COOnBu-treated male *B. cucurbitae* (experiment 5)[a]**

| | | Moths/trap/d ± SE | | | | |
|---|---|---|---|---|---|---|
| Trap lure | *N* | Day 1 | Day 4 | Day 5 | Day 6 | Day 7 |
| *B. cucurbitae* fed 1% *E*7,9-10:COOnBu | 12 | 3 ± 1 a | 2.2 ± 0.7 a | 1.8 ± 0.9 a | 1.8 ± 0.8 a | 1.8 ± 0.8 a |
| *B. cucurbitae* fed 5% *E*7,9-10:COOnBu | 12 | 4 ± 1 a | 2.6 ± 0.8 a | 3 ± 1 a | 1.2 ± 0.4 a | 3 ± 1 a |
| *B. cucurbitae* fed 10% *E*7,9-10:COOnBu | 12 | 3 ± 1 a | 1.2 ± 0.3 a | 3 ± 2 a | 1.9 ± 0.7 a | 1.6 ± 0.8 a |
| *E*7,9-10:COOnBu on septa (250 μg) | 12 | 11 ± 2 b | 6 ± 1 b | 17 ± 3 b | 11 ± 2 b | 17 ± 3 b |

[a] Means followed by different letters within each day are significantly different at $P < 0.05$; Tukey's HSD.

for topically treated *B. cucurbitae* were determined to be $(4 \pm 3) \times 10^2$ ng per fly and 7 ± 2 μg per fly for 100 ng and 10 μg loading rates, respectively. Melon flies injected with 10 μg *E*7,9-10:COOnBu were found to contain 8 ± 2 μg of pheromone per fly. Concentrations of pheromone for *E*7,9-10:COOnBu/honey-fed *B. cucurbitae* were 0.03 ± 0.02 μg per fly (1% *E*7,9-10:COOnBu in honey), 0.03 ± 0.01 μg per fly (5%), and 0.2 ± 0.1 per fly μg (10%). Headspace analysis of melon flies fed 5% pheromone in honey showed daily releases of 0.011 ± 0.004 μg per fly during the first 24 h trapping period, 0.2 ± 0.2 ng per fly for the second 24 h period, and 0.3 ± 0.3 ng per fly for the third, with no *E*7,9-10:COOnBu detected in headspace collections after 72 h. Melon fly survivorship was near 98.5% during the first 24-h collection period, fell to 46.2% after 48 h, and no flies survived to 96 h. Detection limits determined for EAD and FID were 1 pg and 10 ng, respectively. Detection limits for MS correlated with *E*7,9-10:COOnBu fragment intensity: *m*/*z* 67 (1 pg), 108 (100 pg), 150 (100 pg), and 224 (1 ng).

Results of experiment 7 MMD field tests showed a significant reduction in moth captures per field with released *D. pallivitta*-fed melon flies compared with the control field on the first 2 d of the field trial (day 1, $P = 0.026$, day 2, $P = 0.061$) (Fig. 1). Percent disruption was significantly higher on day 1 (84.7%) than on any other day. Percent disruption on day 2 (56.0%) was also significantly higher than disruption on day 7. Inspection of modified action packers after melon fly releases showed little or no residual pheromone/honey mixture, suggesting that it had been almost completely consumed by or adhered to the flies.

## Discussion

Field trial results show that high levels of communication disruption of *D. pallivitta* can be achieved with conventional pheromone treatment using *E*7,9-10:COOnBu. To our knowledge, experiment 2 is the first demonstration of premating communication disruption in the moth family Limacodidae. Percent disruptions were more pronounced with traps baited with *E*7,9-10:COOnBu (experiments 1 and 3) than with traps baited with live female moths (experiment 2) although these results were likely affected by population fluctuations during experiment 2. Percent disruptions for experiments 1 and 3 did not decrease with time over the intervals tested, with the exception of week 8 trap captures in experiment 3. Therefore, rubber septa at the given loading and disruptor density effectively disrupted *D. pallivitta* for at least 7 d, while spiral provided high level disruption for up 7 wk. Although the percentage of female mating was not directly addressed in this study, premating communication disruption is likely correlated with mating suppression. While testing to establish direct effects on mating the population control, and to assess *E*7,9-10:COOnBu mating disruption on a larger scale are needed, this study demonstrates the potential for conventional communication disruption *D. pallivitta* to control pest populations.

Assessment of mobile communication disruption using the three-step framework suggested by Suckling et al. (2011), 1) "do no harm" to the carrier organism, 2) sustained attraction and a scalable application method, and 3) orientation disruption in the field, showed promising results but also left some unanswered questions about the feasibility and effectiveness of the technique for *D. pallivitta* control. Although the "do no harm" trials (experiment 4) only showed significantly lower survivorship at the 100 μg dose, the linear regression analysis suggests that *E*7,9-10:COOnBu does have some toxic effects on the carrier organism, *B. cucurbitae*. This agrees well with the demonstration by Suckling et al. (2011) that treating various insects with topical application of moth pheromone can be done without severe impact on the treated insect up to a dose of 40 μg per carrier. However, it is not currently well understood how carrier survivorship affects MMD effectiveness in the field. It is possible that carrier organisms must only remain mobile for a limited amount of time until they disperse from the release site. After dispersal, it may actually be desirably to have carrier organisms become immobile to decrease pheromone dilution in a given area by movement out of the target area. Additionally, disruption may not be predicated on the carrier organism being alive as traps baited with pheromone-treated *B. cucurbitae* captured male *D. pallivitta* up to a week after deployment in the field, at which time all melon flies contained within the trap were dead. Analysis of carrier survivorship is further complicated by the fact that "do no harm" experiments were conducted with topically applied *E*7,9-10:COOnBu, while other experiments were carried out with pheromone/honey-fed flies. Because of difficulties associated with knowing the exact amount of pheromone consumed or stuck to flies using the pheromone/honey feeding treatment method, topical application was used to insure precise and accurate pheromone

application. All topical doses were designed to be "high end," and flies in subsequent experiments with *E*7,9-10:COOnBu in honey were treated at lower rates, maximum 50–500 ng per fly for experiments 5 and 6 and 30 ng per fly for experiment 7. Typical flies in experiments 5–7 would be expected to have three orders of magnitude less pheromone than the lowest dose topically applied and are therefore likely to experience inconsequential *E*7,9-10:COOnBu-induced mortality.

Pheromone/honey-fed flies were used for experiments 5–7 as a "scalable application method" in preference to topical application of *E*7,9-10:COOnBu, which was considered to be impractical for treatment of larger numbers of flies. Pheromone/honey mixture has the advantage over Suckling et al. (2011) of not requiring microencapsulation formulation or special equipment for spraying. "Sustained attraction" was demonstrated through the field trials using individual pheromone-fed melon flies (experiment 5). Trap catches with treated *B. cucurbitae* were at least half that of the *E*7,9-10:COOnBu septa lure, which reflects the large difference in pheromone presented, approximately three orders of magnitude. No negative controls were included in experiment 5 as previous work showed very low captures with unbaited traps ($0.04 \pm 0.02$ moth per trap per day, $n = 118$) with *D. pallivitta* present (Siderhurst et al. 2007). Interestingly, traps with treated melon flies caught moths over a 7-d period even though headspace analyses were unable to detect *E*7,9-10:COOnBu after 72 h. However, as shown by the differences in detector responses (experiment 6), male *D. pallivitta* can at least detect *E*7,9-10:COOnBu at the detection limit of the instruments used in this study, and behavioral data (experiment 5) suggests they might be much more sensitive.

GC/MS analyses of topically treated and pheromone-injected *B. cucurbitae* showed values slightly below applied/injected amount, suggesting an under estimation of the *E*7,9-10:COOnBu on flies due to systematic error(s). However, this under estimation is unlikely to explain the difference in the amounts of pheromone found in both solvent extractions (~30–160 ng per fly) and headspace collections ($11 \pm 4$ ng per fly) of *E*7,9-10:COOnBu-fed melon flies, and the maximum possible loading rate (50–500 ng per fly, if all pheromone was eaten/adhered to flies). Although it is possible that flies are metabolizing ingested pheromone, it seems more likely that loading rates are lower than maximal due to flies not consuming (or becoming adhered with) the pheromone/honey mixture. Because of this observation, the amount of presented pheromone/honey mixture was lowered for the MMD field trial (experiment 7).

"Orientation disruption in the field" was demonstrated by the decreased captures of male *D. pallivitta* in fields treated with *E*7,9-10:COOnBu-fed *B. cucurbitae*. However, the disruptive effect was less pronounced than with conventional pheromone treatments (experiments 1 and 3) and it diminished more quickly over time. This dissipation over time may be due to the dispersal of the pheromone-fed flies and/or loss of pheromone from the flies bodies. Although the experiments were not designed for direct comparisons between conventional and MMD pheromone control strategies, and notably different field sites were used and different pheromone rates were used, the highest percent disruption with MMD was numerically lower for all daily/weekly disruptions throughout experiment 1 and for the first 7 wk of experiment 3. This despite the fact that the amount of pheromone released per treatment area was twice that of experiment 1. Additionally, the disruptive effect observed during this study was smaller than that observed by Suckling et al. (2011), who used *C. capitata* as a carrier to disrupt *E. postvittana*. This is somewhat surprising as the amount of pheromone released on treated flies was substantially lower, 60 mg/ha, for the Suckling et al. (2011) study. The previous study also used a larger experimental field area, 1–4 ha, and more release sites, the cumulative effect of which may have helped to insure more even carrier dispersion and lowered the probability that flies would move out of the treatment area. Despite these shortcomings, this constitutes only the second instance of a moth species susceptible to mobile communication disruption.

Increasingly, state agencies are being looked at for their quick response to control or eradicate new invasive species. Particularly in Hawai'i, the use of classical biological control for insects has been highly effective from a long term perspective. However, the extended time necessary for demonstrating the lack of adverse effects on nontargets and the subsequent approval processes can allow for further expansion of invasive species at a time when suppression and containment is critical. Mobile communication disruption allows the utilization of identified pheromones with sterile insect carriers, especially in areas where insects are already being mass reared for release as part of an existing control program. In particular, programs rearing sterile tephritids currently exist in Hawai'i, California, and Florida and might produce flies for use as pheromone carriers as needed to combat outbreaks of invasive insects. This concept also potentially increases the range of dispersal and ecosystems available to mobile insects like flies that may not be available by hand or aerial release.

The greatest use of mobile communication disruption may not be as a standalone control technique but when used in concert with other techniques. Bloem and Carpenter (2001) reported the use of sterile insect technique (SIT) in combination with other suppression methods, including mating disruption, in projects around the world. Judd and Gardiner (2005) reported the difficulty of relying on one technique (SIT) to control codling moth, *Cydia pomonella* (L.), and their subsequent successful efforts to include mating disruption, and tree-banding and sanitation, particularly in organic orchards where pesticides could not be used for integrated pest management. In many cases, each technique may have strengths and limitations, but the combination of techniques may increase the overall effectiveness. Additionally, it may be possible to piggyback mobile communication disruption releases on existing SIT releases if it can be shown that semiochemical treatment does not interfere with SIT effectiveness.

Both conventional and mobile communication disruption offer promise for controlling pest populations of *D. pallivitta*. For conventional disruption, we would suggest that further research be conducted on larger-scale, longer-term population suppression in a working agricultural area such as a commercial floriculture operation. For mobile communication disruption, the research questions are more numerous and the usefulness of the technique less certain. Pressing topics include better understanding carrier movements and vegetation preferences on mobile communication disruption effectiveness, optimal semiochemical loading of carrier organisms, behavioral observations of target species toward carrier organisms, and perhaps most importantly improving semiochemical longevity and persistence under field conditions. It is hoped that by better understanding these issues, mobile communication disruption can become more efficient, cost effective, and better matched to situations in which it can provide pest insect control.

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