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 (E7,9:10: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 E7,9:10:COOnBu-treated fields compared with control fields. For traps baited with E7,9:10: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* (Coquillet) as carriers for E7,9:10:COOnBu was evaluated in the following three areas: fly survivorship, attraction of male moths to treated fields, and moth disruption in a small-scale field trial. Topical application of E7,9:10: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 E7,9:10: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 E7,9:10: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 because of the potential risk of plant movement due to the quarantine status of *D. pallivitta*. To help mitigate the negative impacts of *D. pallivitta*, the Hawaii Department of Agriculture began releasing a Eulophid parasite, *Aroplectrus dimerus* Hendel, 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 (E7,9:10: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* (Coquillet) 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 \( E7,9\text{-}10\text{:COOnBu} \)) and mobile disruption \( (E7,9\text{-}10\text{:COOnBu} \) 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 \( E7,9\text{-}10\text{:COOnBu} \) to adult \( B. cucurbitae \), and to assess strength and longevity of attraction of \( D. pallivitta \) to \( E7,9\text{-}10\text{:COOnBu} \)-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‘i\’an 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 \( -24^\circ\text{C} \) on a photoperiod of 12:12 (L:D) h. Male \( D. pallivitta \) used to determine the sensitivity of olfactory perception of \( E7,9\text{-}10\text{:COOnBu} \) were live captured in one-way bucket traps baited with \( E7,9\text{-}10\text{:COOnBu} \). Traps were made from large plastic buckets (\( \sim 20 \) cm diameter) 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. \( E7,9\text{-}10\text{:COOnBu} \) 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 \( \mu \text{m} \) 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 \( \mu \text{m} \) film thickness).

Male \( D. pallivitta \) antennal responses to \( E7,9\text{-}10\text{:COOnBu} \) 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 \( \mu \text{m} \) 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.** \( E7,9\text{-}10\text{:COOnBu} \) 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. \( ^1\text{H}-\text{NMR} \) and GC/MS of the Pacific Agriscience material were consistent with Siderhurst et al. (2007). Red rubber septa were loaded with two amounts of \( E7,9\text{-}10\text{:COOnBu} \), 250 \( \mu \text{g} \) for trap lures or 2.5 mg for disruptors (experiments 1 and 2). White plastic polymer spirals loaded with mixing \( E7,9\text{-}10\text{:COOnBu} \) 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 \( E7,9\text{-}10\text{:COOnBu} \) 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 \( \sim 1 \) 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 \( E7,9\text{-}10\text{:COOnBu} \) disruptors on captures of male \( D. pallivitta \) in traps baited with 250 \( \mu \text{g} \) \( E7,9\text{-}10\text{:COOnBu} \) 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 \( \sim 130 \) \( \text{cm}^2 \); Better World Manufacturing, Fresno, CA), baited with 250 \( \mu \text{g} \) of \( E7,9\text{-}10\text{:COOnBu} \), 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 \( E7,9\text{-}10\text{:COOnBu} \) rubber septa disruptors were attached at a height of 1 m to poles spread evenly along 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 \( E7,9\text{-}10\text{:COOnBu} \)-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 \( E7,9\text{-}10\text{:COOnBu} \) 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 $E7,9\text{-}10\text{C}_7\text{OOnBu}$-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 $E7,9\text{-}10\text{C}_7\text{OOnBu}$ spiral disruptors on captures of male $D.\ pallivitta$ in traps baited with 250 μg $E7,9\text{-}10\text{C}_7\text{OOnBu}$ 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 $E7,9\text{-}10\text{C}_7\text{OOnBu}$, 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 $E7,9\text{-}10\text{C}_7\text{OOnBu}$ 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 $E7,9\text{-}10\text{C}_7\text{OOnBu}$ 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 $E7,9\text{-}10\text{C}_7\text{OOnBu}$-disrupted fields were not rotated. The experiment was unreplicated due to resource constraints (2 treatments, 16 traps, 32 subreplicates per treatment).

$E7,9\text{-}10\text{C}_7\text{OOnBu}$-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 $E7,9\text{-}10\text{C}_7\text{OOnBu}$ 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 $E7,9\text{-}10\text{C}_7\text{OOnBu}$ 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 $E7,9\text{-}10\text{C}_7\text{OOnBu}$-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 $E7,9\text{-}10\text{C}_7\text{OOnBu}$-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 cm², Scentry Biologicals, Inc., Billings, MT).

Treatments tested included traps baited with individual melon flies fed 1, 5, or 10% $E7,9\text{-}10\text{C}_7\text{OOnBu}$ in honey and traps baited with 250 μg of $E7,9\text{-}10\text{C}_7\text{OOnBu}$ on a rubber septa (positive control). Traps were deployed in two field locations: University of Hawai‘i, Mānoa, Wai‘akea 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, 2174980 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 $E7,9\text{-}10\text{C}_7\text{OOnBu}$ on Treated $B.\ cucurbitae$ (Experiment 6). Experiment 6 was conducted using a series of analyses to quantify the amount of $E7,9\text{-}10\text{C}_7\text{OOnBu}$ on treated $B.\ cucurbitae$. To assess pheromone extraction efficiency, melon flies treated either topically or injected with $E7,9\text{-}10\text{C}_7\text{OOnBu}$ 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 ($R^2\geq0.995$) in the concentration range 1 pg–100 ng. $B.\ cucurbitae$ fed 1, 5, or 10% $E7,9\text{-}10\text{C}_7\text{OOnBu}$ 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% $E7,9\text{-}10\text{C}_7\text{OOnBu}$ 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 $E7,9\text{-}10\text{C}_7\text{OOnBu}$-treated $B.\ cucurbitae$ on captures of male $D.\ pallivitta$ in traps baited with 250 μg $E7,9\text{-}10\text{C}_7\text{OOnBu}$ lures. Sterile $B.\ cucurbitae$ pupae (100 ml, irradiated at 100 Gy [2 dbe]) were placed in modified action packers (49 cm by 31 cm by 39 cm, Rubbermaid, Atlanta, GA) with screened windows. Following eclosion, 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% $E7,9\text{-}10\text{C}_7\text{OOnBu}$ in honey (wt/wt, ~50 mg pheromone) for 2 d before being released in the field. Four Jackson traps containing 250 μg $E7,9\text{-}10\text{C}_7\text{OOnBu}$ 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, $E7,9\text{-}10\text{C}_7\text{OOnBu}$-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 E7,9-10:COOnBu. Jackson traps were serviced at 1, 2, 3, and 7 d after the B. cucurbitae release. The control and E7,9-10: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 (experiments 1 and 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 (SAS). The total percent disruptions were 94.4% and 92.1% for septa and spirals respectively, with time interval ranges of 90.5–98.2% (daily) and 62.5–98.6% (weekly), respectively. Mean daily captures of male D. pallivitta in E7,9-10:COOnBu-treated fields were lower than the positive control (E7,9-10: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 E7,9-10:COOnBu (Table 3). However, trap captures for individual flies fed E7,9-10:COOnBu were lower than the positive control (250 μg E7,9-10:COOnBu) 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 exposure 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 E7,9-10:COOnBu on treated melon flies. Concentrations of pheromone

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

| Experimental setup | Experimental results |
|--------------------|----------------------|
| **Treatment**      | **Control**          | **% Disruption** |
| **Experiment no.** | **Trap lure**        | **Disruptor**   | **(moths/field/d ± SE)** | **(moths/field/d ± SE)** |
| 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 |
| 4                   | E7,9-10:COOnBu on septa | E7,9-10:COOnBu on melon fly | 18 ± 4 a | 38 ± 5 b | 52.1 |

* 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:*COO*Bu rubber septa disruptors (experiment 1), (B) by 15 mg *E*7,9-10:*COO*Bu spiral disruptors (experiment 3), and (C) by released *E*7,9-10:*COO*Bu-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.
for topically treated B. cucurbitae were determined to be \((4 \pm 3) \times 10^3\) ng per fly and \(7 \pm 2\) µg per fly for 100 ng and 10 µg loading rates, respectively. Melon flies injected with 10 µg E7,9-10:COOnBu were found to contain \(8 \pm 2\) µg of pheromone per fly. Concentrations of pheromone for E7,9-10:COOnBu-treated B. cucurbitae were \(0.03 \pm 0.02\) µg per fly (1% E7,9-10:COOnBu in honey), \(0.03 \pm 0.01\) µg per fly (5%), and \(0.2 \pm 0.1\) per fly µg (10%). Headspace analysis of melon flies fed 5% pheromone in honey showed daily releases of \(0.011 \pm 0.004\) µg per fly during the first 24 h trapping period, \(0.2 \pm 0.2\) ng per fly for the second 24 h period, and \(0.3 \pm 0.3\) ng per fly for the third, with no E7,9-10: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, 46.2% after 48 h, and no flies survived to 72 h. Melon fly survivorship was near 98.5% during the first 24 h trapping period, \(0.2 \pm 0.2\) per fly for the second 24 h period, and \(0.3 \pm 0.3\) per fly for the third, with no E7,9-10: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 E7,9-10: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 E7,9-10: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 E7,9-10: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 6.
application. All topical doses were designed to be “high end,” and flies in subsequent experiments with E7,9-10: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 E7,9-10:COOnBu-induced mortality.

Pheromone/honey-fed flies were used for experiments 5–7 as a “scalable application method” in preference to topical application of E7,9-10: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 melan flies (experiment 5). Trap catches with treated B. cucurbitae were at least half that of the E7,9-10:COOnBu seata 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 ± 0.02 moth per trap per day, n = 118) with D. pallivittaa (Siderhurst et al. 2007). Interestingly, traps with treated melan flies caught moths over a 7–21d period even though headspace analyses were unable to detect E7,9-10:COOnBu after 72 h. However, as shown by the differences in detector responses (experiment 6), male D. pallivittaa at least detect E7,9-10: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 E7,9-10: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 ± 4 ng per fly) of E7,9-10:COOnBu-fed melan 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. pallivittaa in fields treated with E7,9-10:COOnBu-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 non-targets 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. Bloom 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. pallivittaa. 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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References Cited

Austin, C. N., and W. F. Wilcox. 2012. Effects of sunlight exposure on grapevine powdery mildew development. Phytopathology 102: 857–866.

Bartell, R. J. 1982. Mechanisms of communication disruption by pheromone in the control of Lepidoptera: a review. Physiol. Entomol. 7: 353–364.

Bautista, R. C., J. A. Yelemar, P. Conant, D. K. Arakaki, and N. Reimer. 2014. Taming a stinging caterpillar in Hawaii with a parasitic wasp. Biocontrol News and Information 35: 9N.

Bloom, S., and J. E. Carpenter. 2001. Evaluation of population suppression by irradiated lepidoptera and their progeny. Fla. Entomol. 84: 165–171.

Conant, P., A. H. Hara, W. T. Nagamine, C. M. Kishimoto, and R. A. Heu. 2002. Nettle caterpillar Darna pallivittaa Moore (Lepidoptera: Limacodidae). New pest advisory No. 01-03. State of Hawai‘i, Department of Agriculture, Honolulu, HI.
El-Sayed, A. M., D. M. Suckling, C. H. Wearing, and J. A. Byers. 2006. Potential of mass trapping for long-term pest management and eradication of invasive species. J. Econ. Entomol. 99: 1550–1564.

Judd, G.J.R., and M.G.T., Gardiner. 2005. Towards eradication of codling moth in British Columbia by complimentary actions of mating disruption, tree banding and sterile insect technique: five-year study in organic orchards. Crop Prot. 24: 718–733.

Perrett, J. J. 2006. A method for analyzing unreplicated experiments using information on the intraclass correlation coefficient. J. Mod. Appl. Stat. Methods 5: 432–442.

Perrett, J. J., and J. J. Higgins. 2006. A method for analyzing unreplicated agricultural experiments. Crop Sci. 46: 2482–2485.

Sarfraz, R. M., M. Evenden, A. Keddie, and L. Dosdall. 2006. Pheromone-mediated mating disruption: a powerful tool in insect pest management. Outlooks Pest Manage. 17: 36–45.

SAS. 2000. The SAS system for Windows [V8]. SAS Institute Inc, Cary, NC.

SAS. 2012. JMP [V10.0.0]. SAS Institute Inc, Cary, NC.

Siderhurst, M. S., E. B. Jang, A. H. Hara, and P. Conant. 2007. n-Butyl (E)-7,9-decadienoate: sex pheromone component of the nettle caterpillar, Darna pallivitta. Entomol. Exp. Appl. 125: 63–69.

Suckling, D. M. 2000. Issues affecting the use of pheromones and other semiochemicals in orchards. Crop Prot. 19: 677–683.

Suckling, D. M., E. B. Jang, L. A. Carvalho, J. T. Nagata, E. L. Schneider, and A. M. El-Sayed. 2007. Can ménage-à-trois be used for controlling insects? J. Chem. Ecol. 33: 1494–1504.

Tanaka, N., L. F. Steiner, K. Ohinata, and R. Okamoto. 1969. Low-cost larval rearing medium for mass production of Oriental and Mediterranean fruit flies. J. Econ. Entomol. 62: 967–968.

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