Commercial Lure Comparison for Monitoring of *Cryptophlebia* spp. (Lepidoptera: Tortricidae) Associated with Macadamia in Hawai'i

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Abstract. Cryptophlebia ombrodelta and C. illepida (Lepidoptera: Tortricidae) are two key pests of macadamia in Hawai'i. Current options for monitoring and management of these pests are limited. Male Cryptophlebia moth activity can be reliably monitored using sex pheromone lures, however current information on the relative attractiveness of commercial lures is lacking. Thus, we compared four commercially manufactured pheromone lures for attraction to the two Cryptophlebia species, relative to non-baited control. Two different trap colors were also assessed. The study was conducted at two commercial macadamia orchards located in Kea'au and Kapa'au, Hawai'i from November 2021 to January 2022 using delta traps. Traps were deployed along the border of the orchard at each study location and serviced weekly. At both sites, 58–94% of captured moths were male C. ombrodelta, while only 1–28% were male C. illepida. In Kea'au, the PHEROCON MNB lure was most attractive to C. ombrodelta, followed by the PHEROCON OFM Combo Dual lure, while C. ombrodelta captures in the other lures did not vary from that of the non-baited control. Similarly, in Kapa'au, C. ombrodelta was most attracted to PHEROCON MNB lure and captures in other lures were equal to that of the control. In Kea'au, C. illepida numbers were highest in traps baited with PHEROCON OFM L2 and OFM Combo Dual lures, followed by PHEROCON and Alpha Scents' MNB lures. Trap color did not affect the capture rates of Cryptophlebia. Results suggest that commercially available lures can be used to effectively monitor these moth pests. Implications of the results to the development of research approaches to study Cryptophlebia ecology and management strategies are also discussed.

Key words: koa seedworm, litchi fruit moth, sex pheromone, trapping

Macadamia nut, *Macadamia integrifolia* (Maiden & Betche), is an important agricultural commodity in Hawai'i with an average of ~\$51 M USD in farm gate value over the past five years (USDA NASS 2020). In 2020, a total of 6,800 harvested hectares across the state produced 18.5 M kgs of net, wet-in-shell nuts (USDA NASS 2020). Among the serious threats to macadamia production in Hawai'i are losses due to infestations by *Cryptophlebia* (Lepidoptera: Tortricidae) moths. In the 2019–2020 growing season, 18.6 % of the harvested nuts were culled at the processing facilities primarily due to *Cryptophlebia* attacks, equating to \sim \$2.9 M USD worth of losses (USDA NASS 2020).

The two species of Cryptophlebia moth pests associated with macadamia nut production in Hawai'i are C. ombrodelta (Lower) and C. illepida (Butler) (Jones 2002). Cryptophlebia ombrodelta (common names: macadamia nut borer and litchi fruit moth) is an invasive species from Australia that was first detected in Hawai'i in the 1950s (Jones 2002). Cryptophlebia illepida (common name: koa seedworm) is a native species first reported on macadamia in the early 1900s (Namba 1957). These species overlap in their host plant range in Hawai'i (Zimmerman 1978), and their larval stage is morphologically similar, hence they have been collectively referred to as koa seedworm in Hawai'i (Jones 2002). The adult stages of the moths can be separated from each other based on morphological characteristics (Zimmerman 1978, Jones 2002, Gilligan and Epstein 2014).

The injuries caused by Cryptophlebia spp. on macadamia nuts are characterized by the entry holes on the husk often associated with frass or masticated tissue (Jones 1994a). Females prefer to oviposit on developing nuts with a diameter of 20-28 mm (Jones 1994b). The immature larva bores into the nut ~4 days after oviposition and subsequently feeds internally until pupation (Jones et al. 1997). If attacks occur after shell hardening, larval feeding damage occurs primarily on the husk of the developing green nuts (Jones and Caprio 1992). Consequently, their attacks result in premature nut drop while harvested nuts with Cryptophlebia infestations are culled (Jones 1994a). Currently, monitoring and management options for these moth pests are limited and outdated (Kawate and Tarutani 2006).

Although complete pheromone components for male C. ombrodelta and C. illepida are still not reported, adult males from both species have been found to be attracted to pheromone lure blends similar to the oriental fruit moth (Grapholita molesta Busck, Lepidoptera: Tortricidae) pheromone blends, with the most attractive blend being 93% (Z)-8dodecenyl acetate, 4% (E)-8-dodecenyl acetate, 1% (Z)-8-dodecenol, and 2% (Z)-7-dedecenyl acetate (Chang 1995). Earlier trapping studies of C. ombrodelta in Australia tested commercial lures containing (Z)-8-dodecenyl acetate and dodecyl alcohol which proved to be reliable in monitoring orchard moth populations despite overall low male moth captures (Sinclair and Sinclair 1980). McQuate and Follett (2006) used a sex pheromone-based sprayable product to suppress Cryptophlebia moth activity, which resulted in decreased moth captures in treated orchards, but no effect on oviposition or injury.

Although pheromone-baited trapping has shown to be a viable option for monitoring Cryptophlebia moths, this strategy has not been extensively adopted by growers in Hawai'i in recent years. This could be attributed to the lack of updated research on these moth pests, as well as limited information on currently available commercial lures and their relative effectiveness. Therefore, in this study, we selected four different commercial lures that contain some of known putative pheromone components of C. ombrodelta and C. illepida and compared their attractiveness to C. ombrodelta and C. illepida in the field. In addition, two different trap colors were compared to help further refine trapping approaches for these pests.

Materials and Methods

The study was conducted at two commercial orchards on Hawai'i island from the week of 22 November 2021 to the week of 17 January 2022. One study site was located at a 29-ha conventionally managed orchard block in Kea'au (19°39'14.02"N, 155° 2'2.23"W), consisting of 49-yr old macadamia trees (var. 246, 344, 508, 660). The other study site was at a 12-ha organically managed orchard block in Kapa'au (20°13'16.18"N, 155°47'13.88"W) planted with 35-yr old macadamia trees (var. 246, 344, 508). Both orchard sites are bordered by a row of mature cook pines, Araucaria columnaris (G. Forst.) separating them from other macadamia planted blocks. These sites were chosen due to anecdotal reports of Cryptophlebia infestations by orchard managers and owners. None of these sites were being managed for Cryptophlebia spp. infestations.

PHEROCON VI Delta traps (Trécé Inc., Adair, OK) with replaceable sticky cardstock liners were used for this study and compared in two different colors, white and orange. The four commercial lures were: (1) macadamia nut borer (MNB) lure manufactured by Alpha Scents, Inc. (Canby, OR), (2) PHEROCON oriental fruit moth (OFM) L2, (3) PHEROCON OFM Combo Dual, and (4) PHEROCON MNB lure (Trécé, Inc., Adair, Oklahoma). All these lures contain (Z)-8-dodecen-1yl acetate (Z8-12:Ac) as the main active ingredient. PHEROCON OFM L2 and MNB lures include other components, such as (E)-8-dodecenyl acetate (E8-12-Ac) and (Z)-8-dodecenyl alcohol (Z8-12:OH), while the PHEROCON OFM Combo Dual lure contains the codling moth (Cydia pomonella L) pheromone, (E,E)-8,10-dodecadienol, and plant derived semiochemicals, terpinyl acetate and acetic acid (Walgenbach et al. 2021). Commercial lures were compared against captures in non-baited control traps and both colors were used in the control.

A randomized complete block design

with four blocks was established at each study site, and treatments were randomly assigned in each block with a total of four replicates for each lure and trap color combinations. The traps were deployed, 15 m apart, along the exterior border of an orchard block and hung on the lower tree canopies ~1.5 m from the ground. Traps were serviced weekly by replacing the sticky liners from each trap, and lure and trap treatments were re-randomized weekly within each block. Lures were replaced at a 4-wk interval. Captured moths were identified as C. illepida or C. ombrodelta by examining their morphological features (Zimmerman 1978, Jones 2002, Gilligan and Epstein 2014).

Response variables including captured non-Cryptophlebia moths were summarized per study location (Table 1); however, only male Cryptophlebia moth and non-Cryptophlebia moth data were subjected to further statistical comparative analysis. Numbers of female Cryptophlebia moths captured were too low to warrant additional comparative statistical analysis. A repeated measures two-way ANOVA using mixed model analysis was conducted with color and lure treatments as fixed factors, date as a repeated factor, and block as a blocking factor, to examine the interaction between trap color and lure treatments, and differences in numbers of captured moths among the treatments. Whenever applicable, a t-test or Tukey's HSD was run for mean comparisons between the two colors and among the lure treatments, respectively. All analyses were conducted separately for each study site using JMP Pro version 16 (SAS Institute Inc., Cary, NC, USA).

Results

At both sites, more male C. ombrodelta moths were captured in Kea'au and Kapa'au comprising 58% and 94% of

Table 1. Relative numbers of moths captured at two macadamia nut orchards on Hawai'i island from November 2021 to January 2022 using commercially manufactured sex pheromone lures.

	C. ombrodelta		C. illepida		Other	Total
Site	Male	Female	Male	Female	Moths	Moths
Kea'au	1181 (58.0%)	24 (1.2%)	570 (28.0%)	46 (2.3%)	214 (10.5%)	2035
Kapa'au	1273 (93.5%)	12 (0.9%)	15 (1.1%)	6 (0.4%)	56 (4.1%)	1362

the total captures, respectively (Table 1). While only 28% and 1% of the total moths trapped in Kea'au and Kapa'au, respectively, were *C. illepida* male moths. Overall captures of female *Cryptophlebia* moths at both sites were less than 3%. Non-*Cryptophlebia* moths constituted 11% in Kea'au and only 4% of the total captures in Kapa'au.

At both sites, there was no interaction between lure treatments and trap colors on male *C. ombrodelta* (Kea'au: $F_{4,81} = 1.41, P = 0.238$, Kapa'au: $F_{4,172} = 1.53, P = 0.194$) or *C. illepida* captures in Kea'au (Kea'au: $F_{4,197} = 2.30, P = 0.059$). Captures of *C. illepida* in Kapa'au were too low (Table 1) to warrant statistical comparisons.

Trap color did not influence captures of *C. ombrodelta* at both sites (Keeau: $F_{1,81} = 0.06$, P = 0.807, Kapa'au: $F_{1,172} = 1.60$, P = 0.207) and *C. illepida* captures in Kea'au ($F_{1,197} = 2.12$, P = 0.147). However, in Kea'au, significantly more non-*Cryptophlebia* moths were captured in the orange traps regardless of lure type ($F_{1,227} = 13.97$, P = 0.0002).

Captures of *C. ombrodelta* varied significantly among the lure treatments at both sites (Kea'au: $F_{4,81} = 91.52$, P < 0.0001, Kapa'au: $F_{1,172} = 41.47$, P < 0.0001, Figure 1). In Kea'au, significantly more *C. ombrodelta* moths were captured in traps baited with the PHEROCON MNB lure compared to the other lure types, while those captured by PHEROCON OFM L2 and Alpha Scents' MNB lures were not significantly different than the

control (Figure 1A). Traps baited with the PHEROCON OFM Combo Dual lure captured significantly fewer moths than those containing the PHEROCON MNB lure, but higher than in the other treatments (Figure 1A). Similarly in Kapa'au, captures of *C. ombrodelta* in delta traps varied significantly among the different lure types with the highest numbers observed when using the PHEROCON MNB lure in comparison to all other treatments (Figure 1B).

For *C. illepida* captures in Kea'au, significantly greater numbers of moths were observed in traps baited with PHEROCON OFM L2 and OFM Combo Dual lures compared with the two commercial MNB lures ($F_{4,197} = 27.98$, P < 0.0001, Figure 2). *Cryptophlebia illepida* moths were captured significantly more frequently in traps with the two commercial MNB lures than in the non-baited control (Figure 2).

Discussion

This study was initiated to provide updated information necessary for improved monitoring and management of two moth pests of macadamia in Hawai'i. Most of the substantial basic and applied research conducted on *Cryptophlebia* pests in Hawai'i occurred in the late 1980s through early 2000s (Jones 2002, Kawate and Tarutani 2006). Given the numerous changes in the macadamia nut production system throughout the years, including but not limited to, pest



Figure 1. Mean \pm SEM number of male *C. ombrodelta* moths captured in traps, baited with different commercially manufactured lures, deployed in Kea'au (A) and Kapa'au (B) from November 2021 to January 2022.



Figure 2. Mean \pm SEM number of male *C. illepida* moths captured in traps, baited with different commercially manufactured lures, deployed in Kea'au from November 2021 to January 2022.

complex and management practices (Jones 2002, Kawate and Tarutani 2006, Gutierrez-Coarite et al. 2017) and climatic changes (Elison Timm et al. 2011, Lauer et al. 2013), updated studies are needed. Jones (2002) noted that in the 1990s, the majority (~80%) of Cryptophlebia moths in macadamia orchards in Hawai'i were C. illepida; however, the method of determining this number was not specified. This estimate could be partly based on pheromone trapping as demonstrated by Jones and Carpio (1992) reporting that C. illepida were more abundant than C. ombrodelta throughout most of the year as monitored by traps baited with OFM pheromones manufactured by Trécé, Inc. In contrast, we found consistently more C. ombrodelta than C. illepida at our study sites using pheromone-baited traps baited with OFM and MNB lures. Our current findings are also in agreement with the preliminary results from an ongoing study investigating the seasonal trends of Cryptophlebia moth activity using pheromone lures in commercial orchards from May to October, showing C. ombrodelta as the most abundant species (Acebes-Doria and de Rocquigny, unpublished). However, the relative abundance of moth captures in our studies may not directly correlate with relative moth population abundance in the field as trap captures may depend upon each moth species' relative attraction to the components within each commercial lure. To help address this matter, studies are being initiated to identity Cryptophlebia larvae found infesting nuts in the field using molecular techniques to confirm the population abundance differences between the two species. In addition, since the two sites in our study were not being managed for Cryptophlebia spp. infestations (e.g., no insecticidal applications), management efforts could be ruled out as a factor for the relative species abundance. The relative

field population abundance between the two moth species is relevant, especially when developing targeted management practices for these pests. From a practical standpoint, identifying the most abundant moth species in the system dictates the right pheromone lure to use for monitoring, and which pheromone blend to use for future pheromone-based behavioral strategies against moth pests such as mating disruption (Carde and Minks 1995) and attract and kill (Charmillot et al. 2000, Huang et al. 2015).

For monitoring purposes, the PHEROCON MNB lure was the most effective for attracting C. ombrodelta as opposed to the other lures, while the PHEROCON OFM L2 and OFM Combo Dual lures were equally attractive to C. illepida along with the MNB lures. These findings support previous studies conducted by Chang (1995) demonstrating that C. ombrodelta were highly attracted to 100% Z8-12:Ac, the main component of the PHEROCON MNB lure, and that C. illepida males were mostly attracted to the OFM pheromone blend than to other blends. Jones and Carpio (1992) also found that C. illepida numbers were greater than C. ombrodelta in traps baited with OFM pheromone blend. On the other hand, the Alpha Scents' MNB lure, which also primarily contains Z8-12:Ac, did not prove to be attractive to C. ombrodelta and C. illepida in this study. Gas chromatography analysis of the Alpha Scents' MNB lures from the same batch tested in the field showed presence of Z8-12:Ac, so the low attraction for this lure may be due to other factors, which may include presence of other ingredients, differences in concentrations and/or release rates.

Since the lures are based on sex pheromone components produced primarily by female moths to attract male moths, most of the captured moths in our study are males, with a small percentage of captured females. This included captures in traps baited with the PHEROCON OFM Combo Dual lure which contains plant derived semiochemicals that are attractive to males and females of OFM and codling moth. OFM and codling moths are known to utilize pome fruit for feeding and reproduction in temperate areas (van der Geest and Evenhuis 1991) while Cryptophlebia moths utilize tropical tree fruit for food and oviposition (Jones 2002). Given the different host range of Cryptophlebia compared to OFM and codling moth, it is likely that Cryptophlebia moths respond better to volatile cues associated with its host plants. Using a sex pheromone in combination with plant-derived volatiles was shown to enhance trap captures of codling moth (Knight et al. 2005) and has since become utilized in codling moth mating disruption programs. Future studies to determine plant-derived volatiles attractive to Crytophlebia moths would aid in the refinement of the monitoring and management strategies for these pests.

Studies on other Tortricid moths have shown that trap colors influence capture rates of target species (Knight and Fisher 2006, Myers et al. 2009, Barros-Parada et al. 2013). In our study, the two colors, orange and white, did not affect the capture rates of Cryptophlebia moths, regardless of lure association. On the other hand, more non-Cryptophlebia moths were captured in orange traps compared with white traps. Myers et al. (2009) found that trap colors also did not influence captures of target Tortricid pests including obliquebanded leafroller, Chorisfoneura rosaceana (Harris), tufted apple bud moth, Platynota idaeusalis (Walker), codling moth and OFM. However, captures of non-target insects (flies and bees) were higher in blue and white colored traps, therefore the authors suggested to use

yellow, red, or orange delta traps. In our study, other insects were captured in both traps including small wasps and flies but not in numbers that would cause concerns or difficulty in trap servicing, thus we did not count them individually. From a practical perspective for monitoring of *Cryptophlebia* moths, utilizing white delta traps would ensure more targeted captures and less non-target captures of other moths.

In summary, this study showed relative attraction rates of different commercial lure formulations to two Cryptophlebia moth species that are problematic in macadamia nut production in Hawai'i. The findings provided updated information on the population distribution of these species and offered practical information on available options for monitoring these pests, which will help inform management decisions when needed. Results from this study will also be useful in future studies on Cryptophlebia behavior and ecology including an ongoing experiment investigating the spatiotemporal patterns of Cryptophlebia mating activity in commercial macadamia orchards. Results will also be valuable in investigating future behaviorally based management strategies such as mating disruption and attract-and-kill.

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