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Attraction of *Euwallacea* nr. *fornicatus* (Coleoptera: Curculionidae: Scolytinae) to lures containing quercivorol

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Ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) have recently emerged as important pests that vector diseases to food crops and ornamental plants. *Euwallacea* nr. *fornicatus* (Eichhoff) is an exotic ambrosia beetle that cultivates the fungal *Fusarium* spp. (Hypocreales: Nectriaceae) pathogens on avocados and ornamental plants in south Florida (Kasson et al. 2013). A morphologically similar *Euwallacea* sp. is causing severe dieback in avocado and other hosts in California and Israel (Eskalen et al. 2012; Mendel et al. 2012). Populations of *E.* nr. *fornicatus* were first detected in a commercial avocado orchard in south Florida during 2012 (Carrillo et al. 2012). Subsequent surveys revealed the presence of low numbers of this beetle in 7 additional avocado orchards, but *Fusarium* dieback has not been observed yet in Florida avocados. A laboratory colony of *E.* nr. *fornicatus* is maintained at the University of Florida–Tropical Research and Education Center (TREC) on a sawdust-based ambrosia beetle diet (Castrillo et al. 2011), amended with avocado sawdust. Kasson et al. (2013) identified 2 ambrosia fusaria (*Ambrosia fusarium* AF-6 and AF-8) cultivated by *E.* nr. *fornicatus* in Florida.

A better understanding of the potential damage that this species can cause to avocados and other plants is needed. The objective of this research was to test potential attractants to trap *E.* nr. *fornicatus* in south Florida. Effective attractants can be used to establish a trapping program to study the seasonality and flight behavior of this beetle. Traps can be utilized as an early warning system to indicate the presence of the disease vectors, and as a timing tool for the application of pesticides. Traps may have a useful role as a mass trapping tool if a strong attractant is available. An efficient trapping system may become part of an integrated management solution for *Fusarium* dieback in avocados and ornamental plants.

Two field trials testing potential attractants to trap *Euwallacea* spp. were conducted in south Florida. Trial 1 was designed to evaluate commercially available (Synergy Semiochemicals Inc., Burnaby, British Columbia, Canada) lures delivered in bubble caps as attractants for *Euwallacea* nr. *fornicatus*. Blocks of 7 multi-funnel (8 funnels) Lindgren traps were deployed in 11 avocado orchards in south Florida between Mar and Jun 2014. Traps were suspended 1.5 m above the ground on galvanized electrical conduit stands spaced at least 20 m apart and at least 20 m away from any avocado tree. Traps used "wet" collection cups filled with commercial propylene glycol solution. Six lure combinations and one no-lure control treatment were tested: 1) Ultra High Release Ethanol pouches (UHR), 2) UHR + Cubeb oil, 3) UHR + Conophtorin, 4) UHR + Quercivorol, 5) UHR + Chalcogram, 6) UHR + Conophtorin + Quercivorol + Chalcogram, and 7) a no-lure control. In order to minimize position effects, trap positions were moved in a systematic way so each trap position had each lure treatment for 1 wk. All lures are formulated (Synergy Semichemicals Inc.) to last 90 d so they were not changed during the experiment, which ran for 7 wk. Insects were collected weekly via pouring the collection cup fluid through a fine (200 µ mesh) paint strainer. The insects retained on the filters were transferred to the Tropical Fruit Entomology Laboratory (TREC, Homestead, Florida, USA) for identification.

Trial 2 was designed to compare the attractiveness of the most attractive lure from trial 1 with the avocado sawdust-based diet used to rear *E.* nr. *fornicatus*, and the beetle diet inoculated with beetle fungal symbionts. Blocks of 4 multi-funnel Lindgren traps were deployed in 14 avocado orchards in south Florida known to have *E.* nr. *fornicatus* infestations. Trap positions were moved systematically and serviced weekly during 4 wk as described above. Lure treatments included: 1) 25 mL of *E.* nr. *fornicatus* diet (provided by M. Cooperband, USDA APHIS). Diet was inoculated with the beetle's fungal symbionts 3 wk prior to trapping. To inoculate the diet with the symbionts, an *E.* nr. *fornicatus* female was placed in a vial with 25 mL of diet, using handling techniques described by Castrillo et al. (2011). After 5 d, the female was removed from the diet by using a sterile paint brush, but her fungal associates remained in the diet. All beetles and fungi were obtained from the *E.* nr. *fornicatus* colony maintained under sterile conditions at TREC. The fungal associates were allowed to grow on the diet for 2 more weeks (22 ± 1 °C, 80% RH, and complete darkness), before deployment in field traps. The diet plug from a vial containing diet and fungus was placed in a mesh bag and hung in the traps as lures. The diet + fungus lures were replaced every week. Additional treatments included 2) 25 mL of *E.* nr. *fornicatus* diet without fungus replaced every week and kept at the same conditions described before; 3) UHR + Quercivorol lures; and 4) no-lure control.

Data analysis: Traps with volatile attractant treatments and controls were deployed in a randomized complete block design (11 and 14 replicates for trials 1 and 2, respectively), with sites (avocado orchards) considered as blocks. Due to variance heterogeneity and non-normali-

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ty of data, differences in adult *E.* nr. *fornicatus* captures in traps baited with the different lure treatments were determined using Kruskal-Wallis and Wilcoxon pairwise rank sum tests (SAS Institute 2012).

Trial 1. The greatest number of *E.* nr. *fornicatus* females were captured with the UHR + Quercivorol lure combination, followed by the combination of 4 lures combined together (UHR + Conophtorin + Quercivorol + Chalcogram), UHR + Cubeb oil, UHR alone, and UHR + Conophtorin. Captures of *E.* nr. *fornicatus* in unbaited (no-lure) control and UHR + Chalchogram baited traps were significantly lower than in UHR + Quercivorol traps (χ^2 = 12.29; *P* = 0.002; *n* = 70) (Fig. 1).

Trial 2. The greatest number of *E.* nr. *fornicatus* females was captured with the UHR + Quercivorol lure combination, followed by the diet + fungus attractant. However, the mean number of beetles captured using the diet + fungus attractant was not statistically different from the no-lure control. The diet alone and the no-lure control treatments captured significantly fewer *E.* nr. *fornicatus* females than UHR + Quercivorol (χ^2 = 13.19; *P* = 0.004; *n* = 112) (Fig. 2).

The Quercivorol + UHR treatment was the most powerful attractant for *E.* nr. *fornicatus* in the 2 field trials. Although UHR is a standard lure for ambrosia beetles, UHR alone did not increase the captures relative to the unbaited control suggesting that the Quercivorol lure was the more attractive constituent for *E.* nr. *fornicatus*. Quercivorol, (1S,4R)-p-menth-2-en-1-ol, is the only pheromone isolated from any species of ambrosia beetles (Kashiwagi et al. 2006). This compound is considered a sexual aggregation pheromone originally isolated from boring frass of unmated males of the ambrosia beetle *Platypus quercivorus* Murayama (Coleoptera: Curculionidae: Platypodinae) (Kashiwagi et al. 2006; Tokoro et al. 2007). However, it is important to note that the lures containing quercivorol (# 3250, Synergy Semiochemicals Inc.) contained all 4 enantiomers of the compound as well as lower amounts of several other related compounds that could potentially affect attraction (A. Cossé, unpublished), so we are unable at this time to say definitively which of these specific components was responsible for the observed attraction. Also, the existence of a sexual pheromone in *E.* nr. *fornicatus* is unlikely because of the type of reproduction of these beetles. Males of *E.* nr. *fornicatus*, like in other Xyleborini, are flightless and spend most of their lives inside their natal galleries where they mate with sibling or parental females. Females mate prior to dispersal and host-seeking, making long-range attraction between sexes unnecessary and unlikely. Further studies to understand which specific lure components were attractive to *E.* nr. *fornicatus* and why are warranted. Quercivorol +UHR was attractive to *E.* nr. *fornicatus* in Florida where populations of this beetle are low (Carrillo, pers. observ.). The attractiveness of quercivorol to other *Euwallacea* spp. should be tested in conditions where population levels are higher like California and Israel.

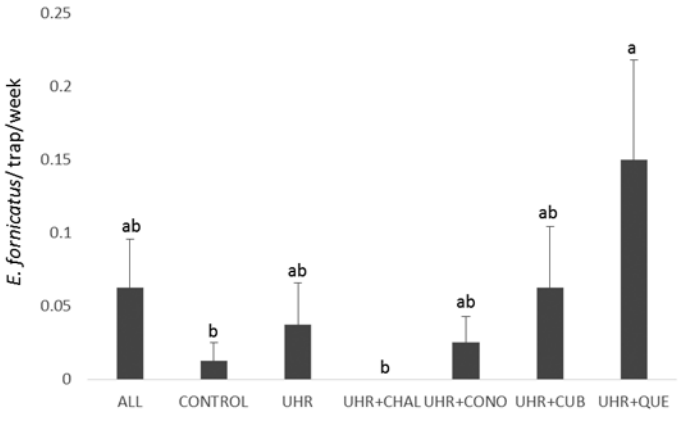

Fig. 1. Commercial lure testing for attractiveness to *Euwallacea* nr. *fornicatus* in 11 avocado orchards. Different letters above each column represent significant differences (*P* < 0.01).

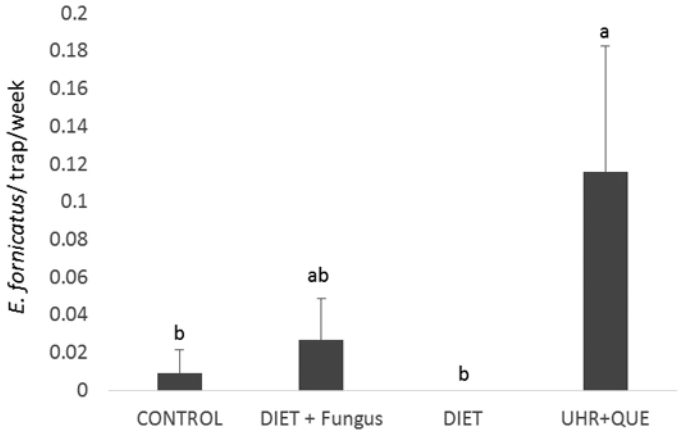

Fig. 2. Attractiveness of quercivorol + UHR, an avocado sawdust-based ambrosia beetle diet, and the beetle diet inoculated with beetle fungal symbionts. Different letters above each column represent significant differences (*P* < 0.01).

No attraction of *E.* nr. *fornicatus* to its fugal associates or the avocado saw dust diet was observed in this study. However, there is no assurance that the exposed treatments were not contaminated by other microorganisms when exposed to field conditions. In order to get a more accurate idea of the attractiveness of the fungal symbionts, the fungal volatiles must be identified, synthesized, and released in a controlled manner.

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Summary

Euwallacea nr. *fornicatus* (Eichhoff) (Coleoptera: Curculionidae: Scolytinae) is an exotic ambrosia beetle that vectors fungal *Fusarium* spp. to avocados. Two field trials testing potential attractants to trap *Euwallacea* spp. were conducted in south Florida. Quercivorol + Ultra High Release Ethanol (URH) was the most powerful attractant for *E.* nr. *fornicatus* in the 2 field trials. In Florida, the populations of *E.* nr. *fornicatus* are small. The attractiveness of quercivorol lures to other *Euwallacea* spp. should be tested in conditions where population levels are greater as in California and Israel.

Key Words: avocado; ambrosia beetle; attractant; quercivorol

Sumario

Euwallacea nr. *fornicatus* (Eichhoff) (Coleoptera: Curculionidae: Scolytinae) es un escarabajo ambrosial exótico que transmite hongos del genero *Fusarium* spp. a árboles de aguacate. Se realizaron dos experimentos de campo para probar el potencial de atrayentes para *Euwallacea* spp. en el sur de Florida. Quercivorol + Etanol en alta taza de liberación (URH) atrajo más *E.* nr. *fornicatus* en los dos experimentos. En Florida las poblaciones de *E.* nr. *fornicatus* son bajas. La atracción de quercivorol a otras especies de *Euwallacea* spp. debe ser probada

en lugares donde las poblaciones de estos insectos son más altas como California e Israel.

Palabras Clave: Aguacate; escarabajos ambrosiales; atrayentes; quercivorol

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