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Volatile chemical profile of the feeding hosts of the coconut scale insect, *Aspidiotus rigidus* Reyne

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Abstract

The coconut industry represents a major agricultural industry, especially in tropical countries. Coconut - derived products have found widespread applications, and are commercially valuable. One of the major insect pests is the coconut scale insect, *Aspidiotus rigidus* Reyne. This has recently infested significant portions of coconut plantations in the Philippines. Effective pest control management strategies are therefore needed in order to address the current infestation, and prevent future occurrences. One effective method is the use of kairomones, semiochemicals that attract the pest to their hosts. However, information about the semiochemicals related to *A. rigidus* still remains largely unknown. In this study, new feeding hosts for *A. rigidus* were identified, and their volatile chemical profiles based from healthy and non-infested leaves were obtained through gas-chromatography mass spectrometry. The volatile profiles of some of the feeding hosts coconut, mangosteen, areca, and licuala showed the presence of a common compound, propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl) propyl ester. This compound may play a role in the host detection and feeding behavior of *A. rigidus*. The results presented in this study are potentially useful in deepening our understanding about the behavior of *A. rigidus*, which will be beneficial in the development of effective kairomone-based pest control strategy.

Introduction

The coconut (*Cocos nucifera)* is a high-value crop and represents a major industry in tropical countries, especially the Philippines. In 2013, 3.55 million hectares of land were devoted to coconut plantations which account for 26% of agricultural land (Philippine Coconut Authority, 2014). The Philippine coconut industry contributes 1.14% to the gross national product (GNP) and accounts for 59% in world coconut exports. In 2009, the Philippine coconut industry was under threat due to the infestation brought about by the coconut scale insect *Aspidiotus rigidus* (Watson et al., 2015). A 60% decrease in total revenues or 200 million pesos worth of loss from the coconut industry was observed in 2013 due to the rampant infestation (Philippine Coconut Authority, 2015). *Aspidiotus rigidus* is a new pest in the

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Philippines and is characterized by having a very tough cuticle. This morphological trait enables the differentiation between a similar coconut scale insect, the *A. destructor* which has been present in the country since 1905 (Cockerell, 1905). *Aspidiotus rigidus* damages coconut trees by encrusting itself at the lower leaf surfaces resulting to stomatal blockage. This stops photosynthesis thereby turning leaves into yellow. Infested trees produce coconuts with less meat and sour water, and eventually die within six months. Predators of *A. rigidus* include predatory beetles *Chilocorus nigrita* Fabricius, *Chilocorus melas* Weise and *Telsimia nitida* Chapin (Watson et al., 2015). On the other hand, parasitoids of *A. rigidus* also include *Comperiella unifasciata* (Reyne, 1947) and the recently discovered Philippine *C. calauanica* (Hymenoptera: Encrytidae) (Almarinez et al., 2015). The characteristically tough cuticle of *A. rigidus* deters other predators that normally prey on *A. destructor* (Reyne, 1948). A possible reason for the rampant multiplication of *A. rigidus* in the Philippines is because the humidity in the country is an ideal environment for them to thrive (Watson et al., 2015).

The losses of the coconut industry are projected to reach billions of pesos if the infestations are left unmanaged. Therefore, efforts to arrest, control and prevent further infestation are necessary to save a crucial national industry and source of livelihood. Current strategies of curbing infestation include cultural control, and the application of pesticides. However, cultural control is not efficient in controlling infestations whereas the application of pesticides poses health and environmental risks. One of the effective pest control management approaches is the utilization of semiochemicals, specifically kairomones, which are volatile phytochemicals that facilitate interaction between the plant and the insect in a way that is detrimental to the plant (Nordlund and Lewis, 1976). Pests through olfactory recognition locate their plant host through kairomones (Bruce et al., 2005). Therefore, kairomones can be used as baits for traps, and can be a reliable form of pest control management. This strategy has been successfully applied for various pests such as wood-boring beetles (Sweeney et al., 2014), codling moth (Landolt et al., 2014), flower thrips (El-Sayed et al., 2014), among others. Identification of alternate feeding hosts in addition to the determination of the authentic volatile phytochemical profile of the feeding host plant are therefore crucial prerequisites in developing a reliable kairomone-based method for pest control management. However, information on the alternative feeding hosts for *A. rigidus* are scarce, in addition to their authentic volatile phytochemical profile. Aside from coconut, what is known is that mangosteen (*Garcinia mangostana*) is a differential host for *A. rigidus* (Watson et al., 2015). Thus, this study aims to identify alternative feeding hosts of *A. rigidus* from the palm family and determine their volatile phytochemical profile. The study focused on common members of the palm family, which are easily accessible. The results obtained are expected to be beneficial in the development of kairomone-based pest control management strategy for this pest.

Materials and Methods

Leaf samples

Leaf samples used for analysis were coconut *(Cocos nucifera* Linnaeus*)* taken from the secondary forest of De La Salle University - Science and Technology Complex in Laguna, mangosteen *(Garcinia mangostana* Linnaeus*)* from Tiaong, Quezon Province, licuala *(Licuala grandis* Wendi*)*, and areca *(Areca catechu Burm. f.)* from the campus of De La Salle University, Manila.

Infestation studies

A no-choice test was conducted to determine establishment of *A. rigidus* on selected palm species, namely *C. nucifera, L. grandis,* and *A. catechu.* Five seedlings per species were placed in hamper cages. A leaf on each seedling was infected with *A. rigidus* crawlers by attaching a 1 in^2 segment cut from scale-infested coconut leaflets. One week post-infection, the leaf was checked for the presence of first instar nymphs in the white cap stage. For standardized counting, twenty nymphs were kept alive on the leaf while the remaining nymphs were killed by pricking and scraping with a dissecting needle. Monitoring and counting of nymphs that remained alive were done weekly for four weeks. A separate but similar no-choice test was done using *G. mangostana* as host, with establishment having been monitored on thirty nymphs until maturity at around 46 to 50 days. Percent establishment was computed using the Equation 1:

$$\% \text{ establishment } = \left[\frac{n_i - n_f}{n_i}\right] \times 100$$

where n_i = initial number of live scales
n_f = final number of live scales

Single-factor Analysis of Variance (ANOVA) was done to compare mean % establishment across species, with post hoc Scheffe Test to determine which pair(s) of species exhibited significant statistical difference. The statistical analyses were done using STATISTICA 8.0.

Extraction of volatiles

The flasks utilized were rinsed with technical grade acetone and were baked overnight in the oven. Prior to use, the flasks were heated at 30–40°C for 30 minutes. Once cooled, one mature, healthy and non-infested leaf sample was placed inside a covered 500 mL Erlenmeyer flask. The fiber was then exposed for 20 minutes at 30–40°C (Silva et al., 2017). Volatiles were extracted using a manual Solid Phase Micro Extraction (SPME) holder with Supelco 100 µm Polydimethylsiloxane (PDMS) (Meyer et al., 2003). After extraction, the fiber was injected directly into the GCMS for analysis. To ensure optimized desorption of the volatile chemicals and to avoid degradation of the SPME fiber, a Shimadzu 0.75 mm ID SPME Liner p/n 221–75196 glass inlet

liner was inserted in the Shimadzu GCMS system. Blank samples and analysis were consistently conducted. Two types of blanks were employed, wherein the first blank involves running the GCMS at the programmed settings to check the integrity of the column. The second blank involves SPME fiber exposure to an empty Erlenmeyer flask, and the exposed SPME fiber was subjected to GCMS. All analyses were repeated at least in triplicates.

Chromatographic and spectroscopic analysis

Gas chromatography coupled to mass spectrometry was used to perform the headspace analysis. The GCMS from Agilent Technologies 7890A GC System and Agilent Technologies 5977A MSD were used for profiling while the Shimadzu GCMS - QP2010 Ultra Gas Chromatography - Mass Spectrometer and Shimadzu GC for Mass Spec GC - 2010 Plus for validation of the identity of compounds. Both GCMS systems use an HP-5 MS ultra inlet capillary column (30 m × 250 mm × 0.25 mm). The injection temperature was set to 250^{o}C and operated on splitless mode. The oven was held at 50^{o}C for 5 minutes then programmed at 10^{o}C/10 minutes until the final temperature of 200^{o}C. Helium was used as the carrier gas with constant flow of 1 mL/min for Agilent System and 1.22 mL/min for Shimadzu System. Detection was performed in Electron Impact (EI) mode. Spectra acquisition was performed in scanning mode (mass range m/z 50–550). Chromatograms and spectra were recorded by means of GC/MSD ChemStation Software and MassHunter Workstation with MSD Chemstation DA Software (Agilent Technologies). The identity of the compound was determined by National Institute of Standards and Technology (NIST) Mass Spectral Library 2.0 and Wiley Registry of Mass Spectral Data, 11th edition. All analyses were repeated at least in triplicates.

Results and Discussion

Semiochemicals are natural chemicals emitted by organisms that mediate their interaction with the environment (Nordlund and Lewis, 1976). Semiochemical - based approaches in pest control management is an effective way to address infestation of agriculturally important crops. The use of semiochemicals offers the advantage of specificity, safety, efficacy, among others (Nordlund et. al., 1981). Pheromones and kairomones are the usual semiochemicals utilized for pest control management where they can be used to control and monitor the pest population. *Aspidiotus rigidus* reproduces asexually, which may limit the efficacy, if any, of pheromones (Reyne, 1948). It is thus ideal to focus on searching for kairomones for *A. rigidus*, since kairomones are odors that are responsible for food host attraction. A crucial prerequisite in kairomone searching involves identification of feeding hosts, and obtaining their authentic volatile chemical profile. Common members of the palm family were evaluated for their ability as alternative feeding hosts for *A. rigidus*. *A. catechu* and *L. grandis* were found to be effective feeding hosts, and exhibited % establishment values comparable with known hosts, coconut and mangosteen (Table 1).

The volatile chemical profiles of the non-infested leaves from the four feeding hosts of *A. rigidus* were then obtained through GC-MS. It is hypothesized that these plant hosts in which *A. rigidus* is attracted to might emit a common compound. This common compound might be the specific chemical attractant which *A. rigidus* detects and responds to. Thus, cross-comparison of the gas chromatograms of the four feeding hosts was conducted, and revealed the presence of a common compound that was eluted at around 16 minutes (Fig. 1).

The compound was identified as propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester (Fig. 2).

The identification was based on the match factor between the mass spectrum of the compound and the library. A match factor of > 800 signifies a high degree of confidence in the compound identification (Stein, 1999; Hübschmann, 2015). It is noteworthy that the identified compound was the only common compound observed from the analyzed feeding hosts of *A. rigidus*. Moreover, the known feeding host mangosteeen, which does not belong to the palm family, also emits this compound. The discovery of this common compound in the healthy leaves of the feeding hosts may thus give rise in the development of lures and baits useful in the pest population management of *A. rigidus.*

Semiochemicals can be classified according to their chemical structures and functional groups. According to the Pherobase (El-Sayed, 2014), most semiochemicals have the following functional groups: esters, hydrocarbons, ketones, alcohols, amines, aldehydes and carboxylic acids. With esters, carboxylic esters and acetate esters are the most common. The volatile compound propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester was also detected in the study of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) by Alfaro et. al (2011) as a possible sex pheromone but was not confirmed by field tests.

In summary, this study was able to identify new alternative feeding hosts for the coconut scale insect, *A. rigidus*. These new hosts from the palm family, Areca and Licuala exhibited percent establishment values similar to known hosts, coconut and mangosteen. Cross comparison of the volatile chemical profile of these four plants species revealed the presence of a single, common compound. The compound was identified as propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester, which may play a role in the host detection and feeding behavior of *A. rigidus*. The results presented in this study are expected to deepen our understanding about the behavior of *A. rigidus*, which will be beneficial in the development of effective kairomone-based pest control strategy for inclusion in the Integrated Pest Management for *A. rigidus*.

Table 1 Percent establishment values of *Aspidiotus rigidus*. There was no significant difference in the establishment values of the insect in the different feeding hosts.

Leaf Sample	% Establishment Value
Cocos nucifera	95.79 ± 4.21
Licuala grandis	92.00 ± 5.15
Areca catechu	97.89 ± 6.86
Garcinia mangostana	98.33 ± 1.05

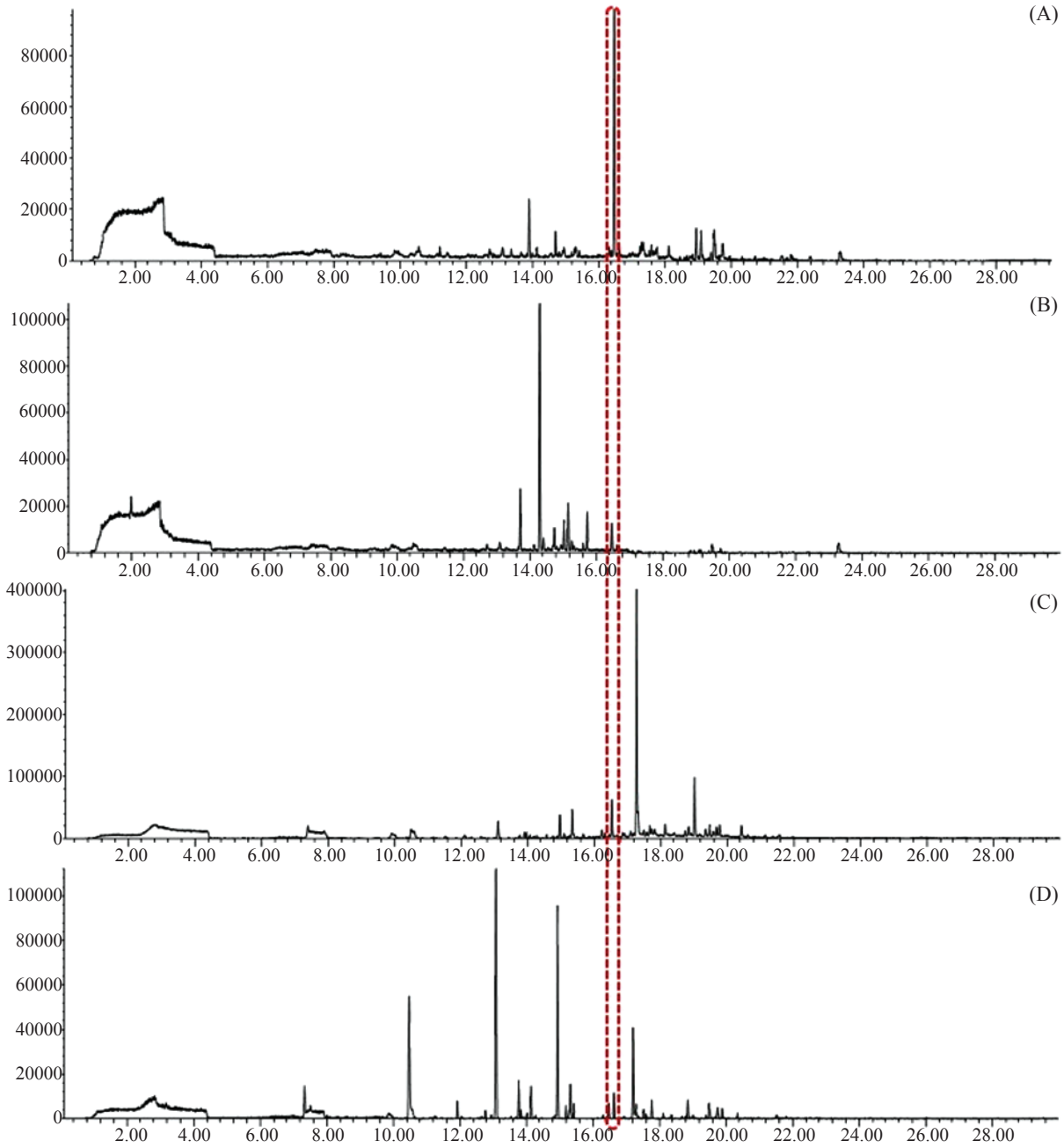

Fig. 1 Overlayed gas chromatogram of the feeding hosts of *Aspidiotus rigidus*. The common compound eluted at around 16 minutes suggests it may have a role in the host recognition of the insect: (A) *Cocos nucifera*: (B) *Garcinia mangostana:* (C) *Areca catechu:* (D) *Licuala grandis*

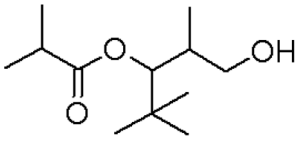

Fig. 2 Chemical structure of the common compound detected from the four feeding hosts of *Aspidiotus rigidus*

Table 2 Match factor in the identification of the common compound emitted by the feeding hosts. A match factor greater than 800 is considered reliable.

Leaf Sample	Match Factor for Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester
Cocos nucifera	900
Licuala grandis	850
Areca catechu	910
Garcinia mangostana	880

Conflict of Interest

The authors declare that there are no conflicts of interest.

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