Effect of *Aspidiotus rigidus* infestation on the volatile chemical profile of the host plant *Garcinia mangostana*

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Summary   Plants respond to stress or damage by releasing volatile compounds, primarily for defense purposes. These volatiles function as signals for different interactions of the plant with its environment. In this study, the volatile chemical profile of healthy *Garcinia mangostana* L. (mangosteen) leaves was compared against leaves infested with the scale insect, *Aspidiotus rigidus* Reyne (Hemiptera: Diaspididae) through solid phase microextraction (SPME) coupled with gas chromatography- mass spectrometry (GC-MS). Analyses revealed that leaves emit the terpene kaur-16-ene in response to *A. rigidus* infestation. Kaur-16-ene is a precursor of gibberellin, a plant hormone for growth and development. The results suggest that the emission of kaur-16-ene in infested *G. mangostana* may play a role relevant in increasing the resistance of the plant towards infestations by herbivores.

Additional keywords: diterpenes, plant physiology, scale insect, semiochemistry

Introduction

Plants adopt various mechanisms to deter and counteract herbivore - induced damage. The emission of volatile chemicals upon attack is one of the effective ways plants deal with herbivory. These herbivore - induced plant volatiles (HIPV) serve various purposes such as a warning to surrounding plants of similar species, attract predators of the herbivore (De Moraes *et al.*,1998), and increase the fitness and vitality of the HIPV emitting plant (Schuman *et al.*, 2012). The identification of HIPVs is not only important for understanding complex plant - insect dynamic interactions, but it can also pave the way for the development of effective and sustainable pest control measures (Shrivastava *et al.,* 2010; Dicke *et al.,* 1990). Regarding the

chemical profile of HIPVs, the green leaf volatiles (GLV) which are released by the plants as soon as they are attacked or wounded, are composed mainly of alcohols, aldehydes, and esters. In addition, the volatile signals that are commonly released for defense include secondary metabolites produced from different pathways like lipoxygenase (LOX), shikimic acid, and terpenoids (Dudareva *et al.,* 2006).

Recently, a massive infestation on plantations of coconut, *Cocos nucifera* (L.) (Arecaceae), was observed in the Philippines by the coconut scale insect, *Aspidiotus rigidus* Reyne (Hemiptera: Diaspididae). The infestation caused a great decline in the coconut palms of the area, affecting around 50-70% of the coconut farms in 2013 (Watson *et al*., 2015). Another economically important host of the scale is mangosteen, *Garcinia mangostana* L. (Clusiaceae). It is considered a priority crop in the Philippines wherein 13,352 metric tons of this fruit was produced during the 2007 – 2011 period (Bureau of Agricultural Statistics, 2012).

Aspidiotus rigidus settles on the lower surface of the leaf, blocking the stomata and preventing the plant to photosynthesize which may even result to death (Watson *et al*., 2015). Little is known about the inter-

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action of *A. rigidus* with the host plant and information about scale insect - induced volatile compounds is scarce. Considering that scale insects in general pose a serious threat to agricultural productivity (Miller *et al.,* 2005), understanding their interaction with the host plant is of paramount importance. In this study, the effect of *A. rigidus* infestation on the volatile chemical profile of the host plant *G. mangostana* was investigated. *Aspidiotus rigidus* is the only scale species infesting mangosteen (Watson *et al.,* 2015) and hence its selection as host plant in the experiments eliminates the possibility for infestation of the plant tissue by other similar scale insect species, e.g. *Aspidiotus desctructor* Signoret (Hemiptera: Diaspididae), infesting coconut. The results presented are expected to contribute to the understanding of the pest - host plant interaction, which may be useful in devising strategies to mitigate the damage caused by *A. rigidus*.

Materials and Methods

Leaf Sample Collection

Healthy and *A. rigidus* - infested mangosteen leaves were collected from De La Salle University - Science and Technology Complex in Laguna, Philippines from January to May 2016. Mature leaves weighing 1.5g to 4.5g were used for the analysis. For the infested leaves, leaves with similar degrees of infestation were harvested on the basis of visual estimation of the presence of scales on the underside of the leaves (number of scales). The collected leaves were acclimatized for 1 hour prior to analysis.

Extraction of Volatiles

To extract the volatiles, a Supelco 0.1 mm Polydimethylsiloxane Solid Phase Microextraction (SPME) fiber was used. The SPME fiber was baked prior to use at 250°C for 30 minutes following manufacturer's instructions. A 500 mL Pyrex® Erlenmeyer Flask covered with aluminum foil and parafilm was used as a headspace chamber. The flask was cleaned with technical grade acetone and oven baked at 100°C for 1 hour before analysis. Once cooled, one mangosteen leaf sample was placed inside. This was heated to 30°C to 40°C while the fiber was exposed for 25 minutes. Heating facilitates efficient extraction of the volatile compounds (Silva *et al.,* 2017). After extraction, the fiber was directly injected to the GCMS. Two blank runs were also conducted to ensure the integrity of the yielded data. The first blank involved running the GCMS using the programmed temperature without SPME injection. This was done to make sure that the column used for the testing is clean. The second blank involved the exposure of the SPME fiber to the oven baked flasks which did not contain the leaf samples. All analyses were done in eight replicates.

Chromatographic and Spectroscopic Analysis

Gas Chromatography coupled to Mass Spectrometry (GC-MS) analysis was performed on Agilent Technologies 7890A GC System and Agilent Technologies 5977A MSD. A HP-5 MS ultra-inlet capillary column (30 m x 250 mm x 0.25 mm) was used. The injection temperature was set to 250°C and operated on splitless mode. The oven was held at 50°C for 5 minutes then programmed at 10°C/10 minutes until the final temperature of 200°C. Helium was used as the carrier gas with constant flow of 1 mL/min. 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.

Statistical Analysis

The Mann – Whitney U test for nonparametric analysis was conducted using the software Statistica, on the basis of the com-

pound peak area. All analyses were done at a 5% significance level.

Results and Discussion

The results of the GC-MS analysis on the healthy and infested leaves are presented in Figure 1. Peak identifications were limited to compounds that exhibited match factors greater than 800, which signifies a high degree of confidence in the identification (Hubschmann, 2015; Stein, 1999). The compounds emitted by infested or healthy mangosteen leaves are summarized in Table 1. The profile of volatile compounds emitted by mangosteen leaves infested with *A. rigidus* was different to the one emitted by healthy leaves. Both quantitative and qualitative differences were found.
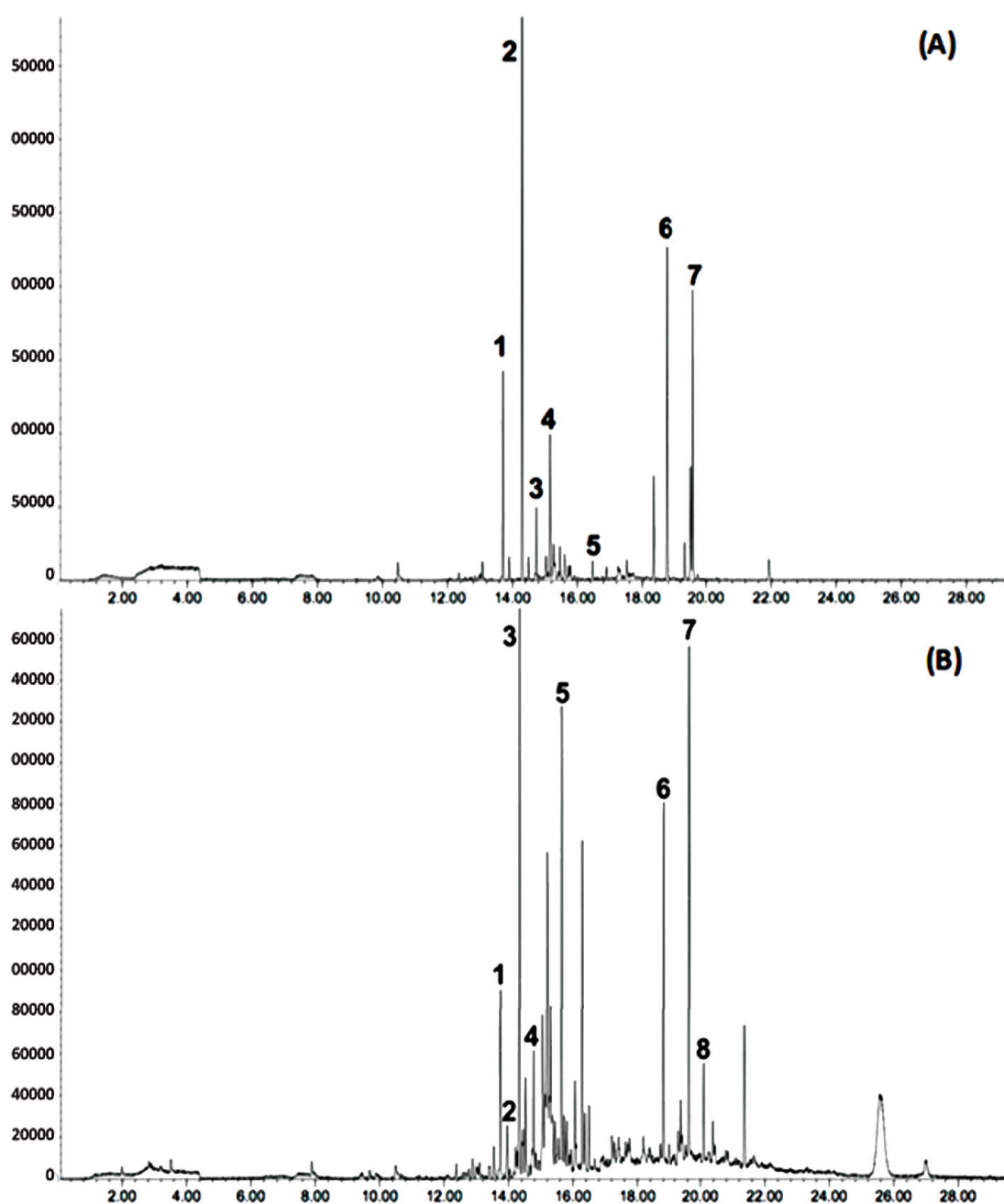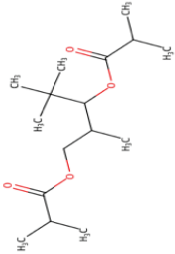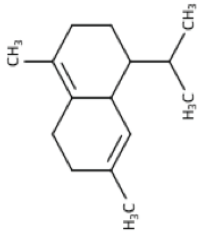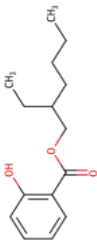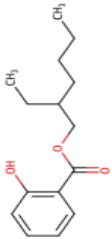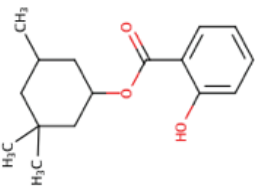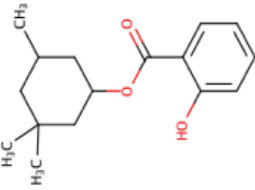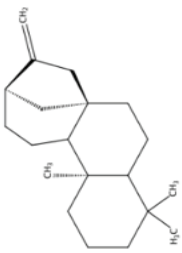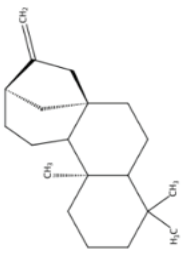

Figure 1. Chromatograms of healthy leaves of *Garcinia mangostana* (A) and infested leaves by *Aspidiotus rigidus* (B).

Table 1. Retention time and match factors of volatile compounds from healthy leaves of *Garcinia mangostana* (A) and infested leaves by *Aspidiotus rigidus* (B).

	Healthy Mangosteen Leaves (A)					Infested Mangosteen Leaves (B)			
Label	Retention Time (min)	Identity of Compound	Match Factor	Structure	Label	Retention Time (min)	Identity of Compound	Match Factor	Structure
1	13.712	alfa-copaene	943		1	13.74	alfa-copaene	922	
2	14.296	caryophyllene	947		2	13.952	cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1a,2ß,4ß)]-	885	
3	14.742	humulene	906		3	14.324	caryophyllene	935	
4	15.166	naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-,[2R-(2a,4aa,8aß]]-	907		4	14.524	bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-yl-3-pentenyl)-	930	

No.	RT	Compound	Match	Structure
5	15.634	naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	915	
6	18.804	2-ethylhexyl salicylate	865	
7	19.634	Homosalate	919	
8	20.109	kaur-16-ene	809	

No.	RT	Compound	Match	Structure
5	16.465	propanoic acid, 2-methyl-1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	825	
6	18.759	2-ethylhexyl salicylate	878	
7	19.589	homosalate	928	

Caryophyllene, which was a common compound in healthy and infested leaves is a sesquiterpene found in oils produced by plants (Pinho-da-Silva *et al.,* 2012). β-caryophyllene is one of the most common volatile compounds secreted by plants that has the ability to limit the growth and development of insects infesting plants (Huang *et al.,* 2011). In addition, β-caryophyllene can attract naturally – occurring parasitoids of the phytophagous insects (Wang *et al.,* 2015).

The volatile compounds cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1a,2ß,4ß)], bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl), and kaur-16-ene-were emitted only by mangosteen leaves infested by *A. rigidus*. Notably, kaur-16-ene emission was significantly elevated in infested *vs* healthy leaves (Mann – Whitney U test, p-value = 0.000939). The emission of kaur-16-ene in infested leaves suggests that it is the physiological response of the plant to the infestation of *A. rigidus* which may play a role in the compensation of the plant to the damage.

Kaur-16-ene has been reported to be emitted by coniferous trees in Japan (Matsunaga *et al.,* 2012) but its role in herbivore – induced signaling is not known. Kaur-16-ene is recognized to be a precursor to the hormone gibberellic acid (GA) which affects the growth and development of plants (MacMillan 1997). GA helps the plants to compensate the damage caused by certain herbivores (Bari and Jones, 2009). Gibberellins, in general, function as promoters and regulators of growth and development (Otsuka *et al.,* 2004). They also alter plant metabolism and induce physiological responses in plants (e.g. invertase activity and photosynthetic rate) favoring growth under stress (Iqbal *et al.,* 2011). The increased fitness and vitality as a consequence of stress is widespread among plants, and is considered to be a form of adaptive plasticity (Anurag, 1998). Some examples of plants that can exhibit this form of defense adaptation include radish (Anurag, 1999) and tobacco (Kessler and Baldwin, 2004).

In conclusion, the infestation of the scale insect *A. rigidus* on the *G. mangostana* leads to the emission of the terpene kaur-16-ene, which possibly suggests the induction of hormone precursor compounds that will compensate for the damage. Overall, the results presented are expected to contribute to our understanding on the chemical ecological interaction of *A. rigidus* with the host plant and its natural enemies. Future research direction will aim at analyzing the potential of the HIPV kaur-16-ene to attract known parasitoids of *A. rigidus*.

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Επίδραση της προσβολής από το κοκκοειδές *Aspidiotus rigidus* στο χημικό προφίλ των πτητικών ουσιών του φυτού ξενιστή *Garcinia mangostana*

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Περίληψη   Τα φυτά ανταποκρίνονται στο στρες ή στις βλάβες απελευθερώνοντας πτητικές ενώσεις, κυρίως για αμυντικούς σκοπούς. Αυτές οι πτητικές ενώσεις λειτουργούν ως σήματα για διαφορετικές αλληλεπιδράσεις του φυτού με το περιβάλλον του. Σε αυτή τη μελέτη, το χημικό προφίλ πτητικών ουσιών από υγιή φύλλα του φυτού *Garcinia mangostana* L. συγκρίθηκε με τα φύλλα που είχαν προσβληθεί από το κοκκοειδές *Aspidiotus rigidus* Reyne (Hemiptera: Diaspididae) με τη μέθοδο της μικροεκχύλισης σε στερεή φάση (SPME) σε συνδυασμό με αέρια χρωματογραφία- φασματομετρία μάζας (GC-MS).

Οι αναλύσεις έδειξαν ότι τα φύλλα εκλύουν το τερπένιο kaur-16-ene σε απόκριση της προσβολής από το *A. rigidus.* Το kaur-16-ene είναι πρόδρομος της ορμόνης ανάπτυξης γιβερελλίνη. Τα αποτελέσματα υποδεικνύουν ότι η εκπομπή kaur-16-ene σε προσβεβλημένα φυτά *G. mangostana* μπορεί να παίζει ένα ρόλο στην αύξηση της ανθεκτικότητας του φυτού έναντι των προσβολών από φυτοφάγα έντομα.