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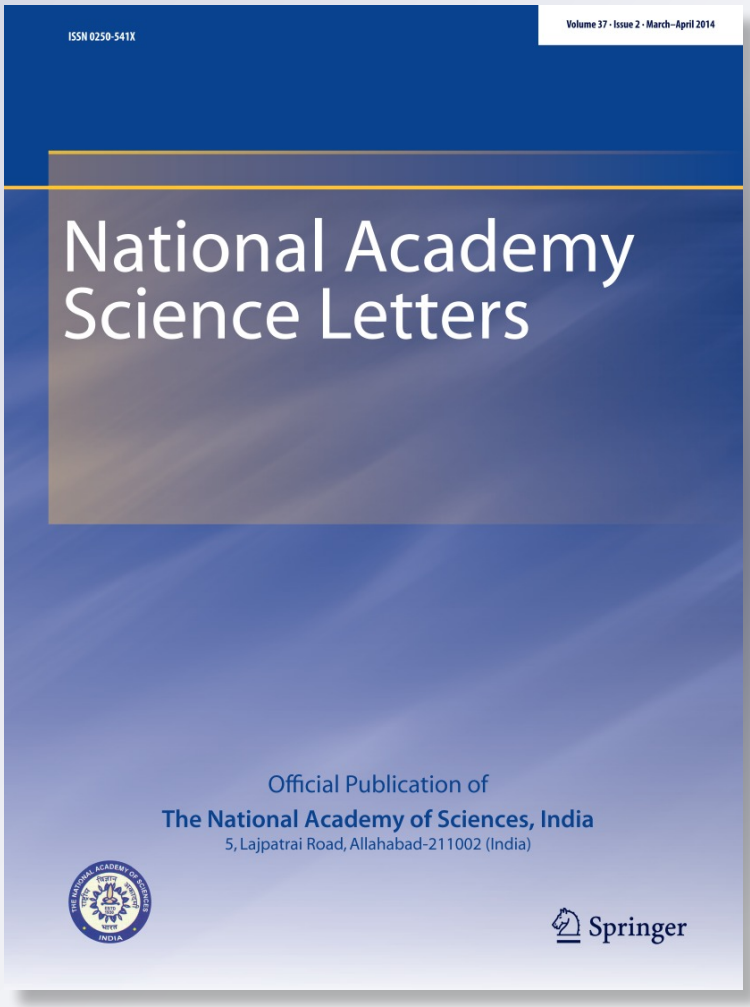

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RESEARCH ARTICLE

The Terpenoids Released by *Persea bombycina* Due to Feeding by *Antheraea assama* Westwood

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Abstract The green leaf volatiles (GLV) are constitutively emitted by the plants, but their release is reported to be increased or decreased by herbivory which might be a component of the plant's defense strategy against herbivory. Such defense response of plant may be exploited for enhancing herbivore fitness and required to be identified. The present investigation was carried out to identify the terpenoid compounds released due to feeding by *Antheraea assama* Westwood. For the purpose the leaves of *Persea bombycina* were fed to fifth instar larvae of *A. assama* under 'in situ' conditions for 6–144 h durations. Analysis of volatiles trapped in synthetic adsorbant from fed leaves, mechanically damaged leaves and undamaged leaves revealed that due to feeding by *A. assama* larvae, the *P. bombycina* produces three blends of terpene compounds: (1) GLV released as constitutive defence (terpinene), (2) volatiles released by continuous feeding (*R*(−)-α-phellandrene, γ-carene, terpinene-4-ol, α-humulene and β-caryophyllene) and (3) volatiles released on systemic induction (*para*-cymene, D-limonene, nerol, eugenol, citral, β-citronellol and farnesene).

Keywords Volatile · Feeding · Mechanical damage

Introduction

In recent years there has been increasing evidence suggesting the role of induced plant defenses serving insect-plant intertrophic interactions. Plants like corn, cotton, lima bean, tobacco and apple are reported to release volatile organic compounds in response to herbivore attack [1–4]. These volatile compounds released systemically are different in quality and quantity from those released constitutively as baseline green leaf volatiles (GLV) by plants. Monoterpenoids and sesquiterpenoids on induction are synthesized by isoprenoid pathways [5, 6]. It is more than a decade that terpenoids were shown to be important semiochemicals in insect plant interactions and their role has been illustrated recently in plant–plant interactions too [7]. Such chemicals are reported as attractant for predators and parasites and are therefore considered to enhance plant fitness against herbivory. The same chemicals may be utilized to enhance the fitness of herbivore by using them to lure and kill their parasitoids. Such an endeavour bears importance only if the herbivore is a beneficial organism and needs protection.

Antheraea assama Westwood (Lepidoptera: Saturniidae) is a silk producing polyphagous insect but has a restricted diet breadth. The insect is prone to attack of parasitoids and pathogens which limit their productivity. In the present investigation, we have analyzed the terpenoid compounds emanated by leaves of *Persea bombycina* due to feeding by *A. assama.* This is the first report that examines the terpenoid compounds released by host plant due to feeding by a beneficial insect.

Materials and Methods

Plants

The saplings of *P. bombycina* were grown in the garden of Department of Life Sciences, Dibrugarh University, Assam, India. All the plants were 3–4 years old.

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Insects

The larvae of *A. assama* hatching from disease-free eggs obtained from the Government Sericulture Farm were cultured on leaves of its primary host plant, *P. bombycina*, grown in the botanical garden within the campus of the Department of Life Sciences, Dibrugarh University, Assam, India.

Collection of Volatile

Leaves of 3–4 years old *P. bombycina* plant were fed to fifth instar larvae under both 'in situ' (on plants grown in the botanical garden of the Department of Life Sciences) and 'ex situ' (on plucked twigs) conditions for different durations of 6–144 h (the period of study was determined based on preliminary works showing systemic emission on herbivory). The volatiles of leaves were trapped in synthetic adsorbent Porapak Q at interval of different hours from adjacent 'feeding free' parts of fed plant, undamaged part of mechanically damaged plants and leaves of undamaged plants [8] (Fig. 1). Mechanical damage was done by punching the leaves at regular intervals to match with the feeding pattern of the larvae.

GC and GC–MS Conditions

The volatiles collected were subjected to GC/GC–MS analysis. Following injection at 2,20 °C, column temperature was maintained at 40 °C for 3 min and then programmed at 5 °C/min to 220 °C, 5 min at 220 °C, at 10 °C/min to 250 °C and 5 min at 250 °C, FID temperature was 250 °C. Data were collected and analyzed with Varian Galaxy software. Experimental samples were co-eluted with external standard compounds (Sigma-Aldrich) for identification and quantification. Three replications were maintained for each. Selected samples were analyzed by GC (Varian) and GC coupled to mass spectrometer (Agilent). Confirmations of identities were based on retention time of external standard in GC and mass spectra obtained from GC–MS.

Statistical Analysis

Data were analyzed in SPSS 17. As volatile emission was very low, nonparametric Kruskal–Wallis test was done to determine significance in variations of volatile emission at different time interval. Results were judged at 95 % confidence level ($p \leq 0.05$).

Results and Discussion

The analysis of the volatiles emitted by undamaged, mechanically damaged and fed leaves of *P. bombycina* by *A. assama* larvae revealed variations in pattern of release of terpenoid compounds under different leaf conditions and are presented below:

Emission of Chemical on Mechanical Damage

During our study, the volatiles collected from undamaged leaves of *P. bombycina* was found to contain several terpenoid compounds namely 1-*R*-α- pinene, camphene, *para*-cymene, D-limonene, nerol, citral, terpinene, eugenol, γ-carene, *R*(−)-α-phellandrene, β-caryophyllene, β-citronellol, terpinene-4-ol, α-humulene, and farnesene at low level (Fig. 2). But after mechanical damage, these volatile chemicals were released in a significantly greater quantity (Fig. 2). Such increased volatile release may be due to rupturing of cells caused by mechanical damage [9] and their emissions are expected to cease with cessation of damage [3].

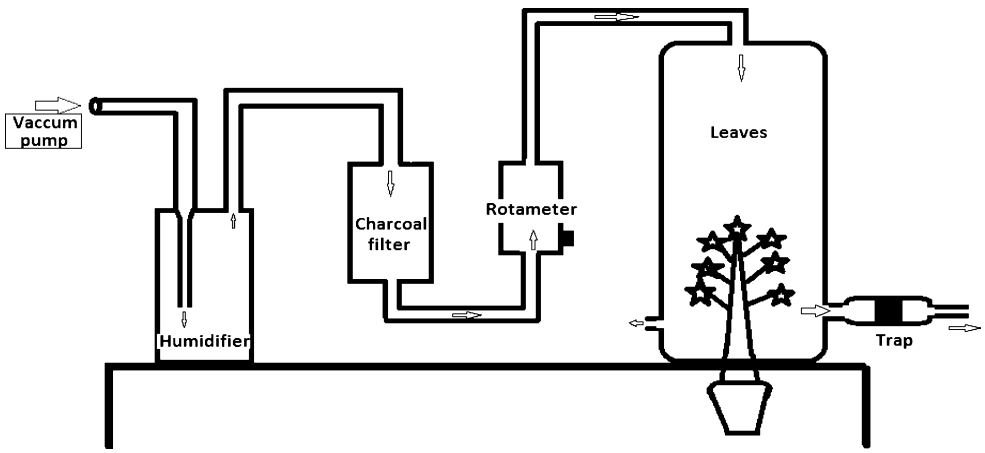


Fig. 1 Trapping of volatile organic compounds

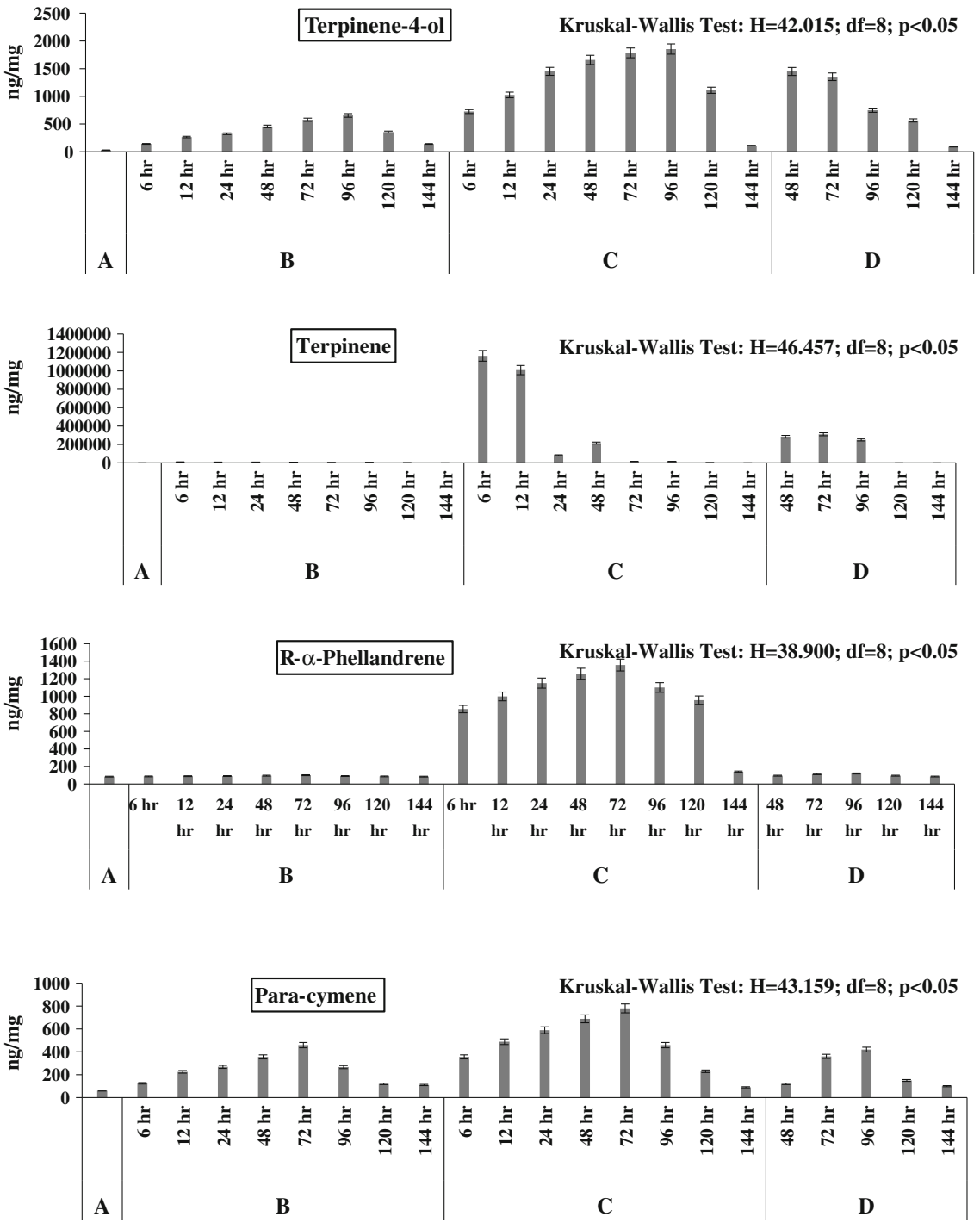
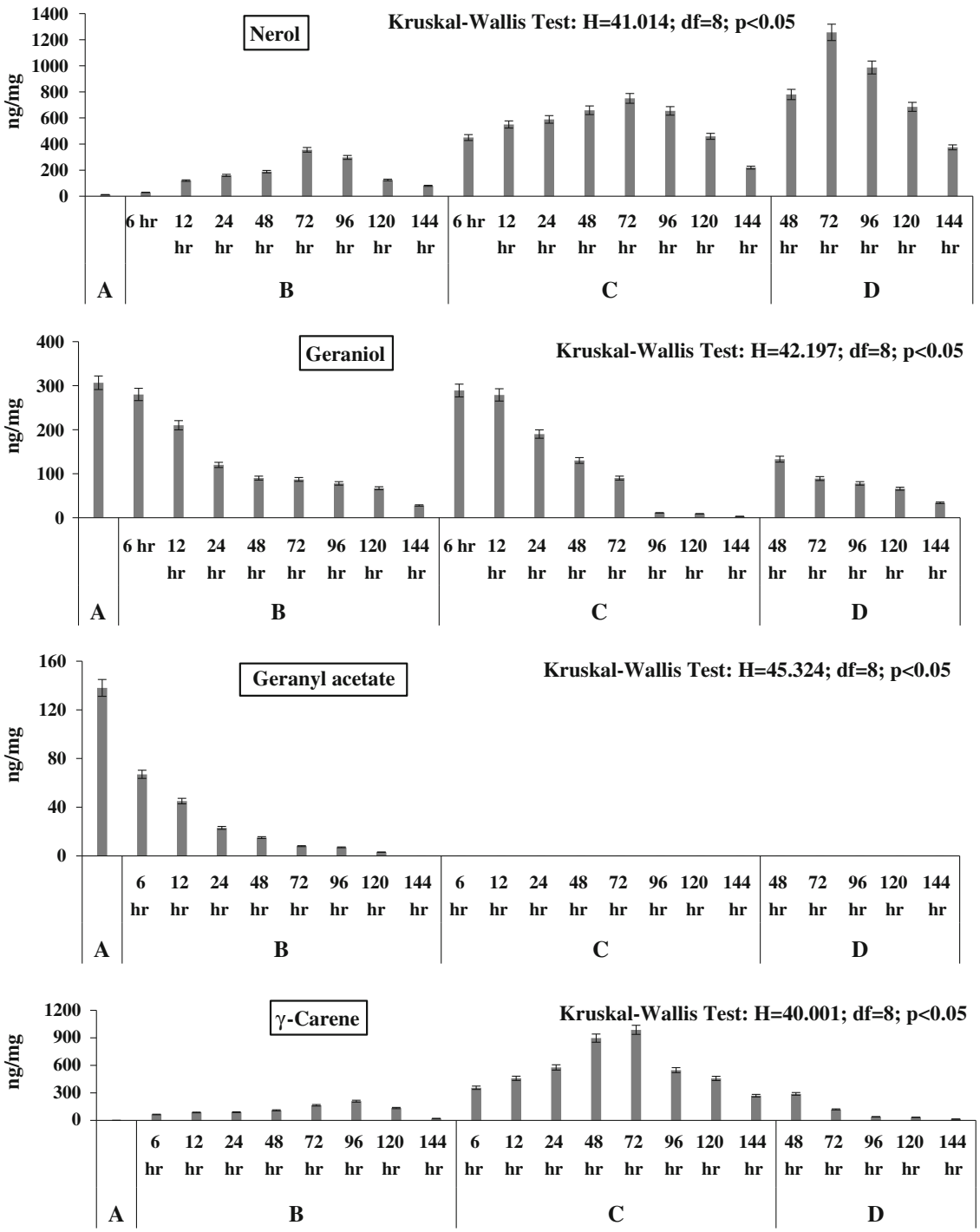


Fig. 2 Pattern of release of terpenoid compounds by leaves of *P. bombycina* under different conditions. (A= undamaged, B= mechanically damaged, C= continuous feeding and D= larvae removed after 24hrs of continuous feeding)

Emission of Volatile Chemicals Under 'In Situ' Condition

Among the acyclic monoterpenes and its ester, geraniol and geranyl acetate were found only in the undamaged leaves of *P. bombycina* at high amount but their concentrations decreased later on both after mechanical damage and feeding. Therefore they are the natural GLV of *P. bombycina* and their release is not induced by feeding of *A. assama.* Probably these chemicals are not associated with defence mechanism of *P. bombycina.*

Under 'in situ' condition as shown in Fig. 2, terpinene was emitted initially at higher amount after feeding by *A. assama* but their level decreased with time. Release of such



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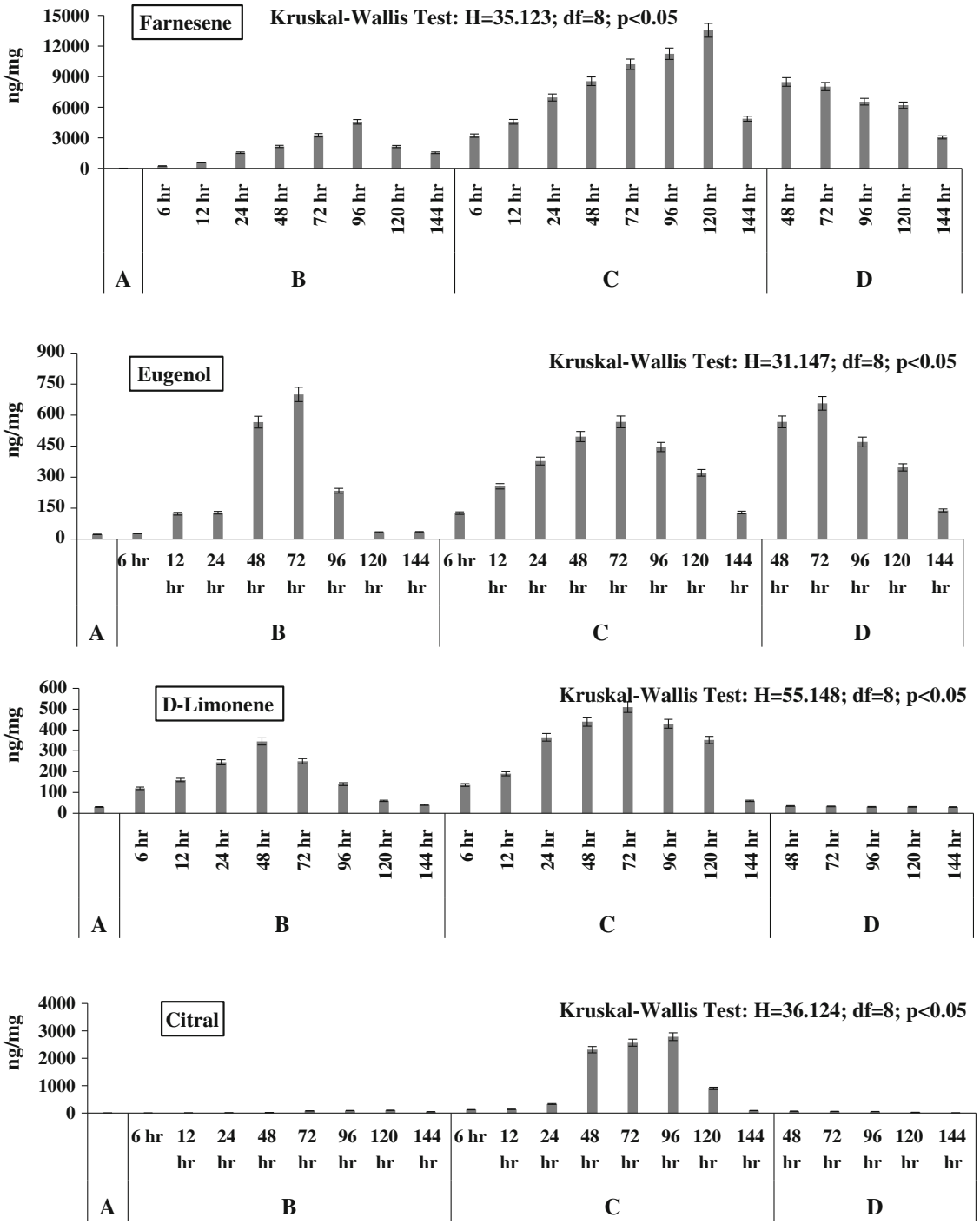
chemicals may not be considered to be associated with feeding or release may be from already synthesized and stored pool. Similarly in cotton plants some terpenoid compounds were reported to be stored in special glands as constitutive defense and later emitted on herbivore attack [10].

Other compounds like 1-*R*-α-pinene was emitted transiently. Nerol, the isomer of geraniol however was released consistently due to feeding. Geraniol is normally reported to be insect attractant while nerol acts as repellent. In undamaged *P. bombycina* leaf the level of geraniol was 306.62 ng/mg and geranyl acetate was 138 ng/mg which



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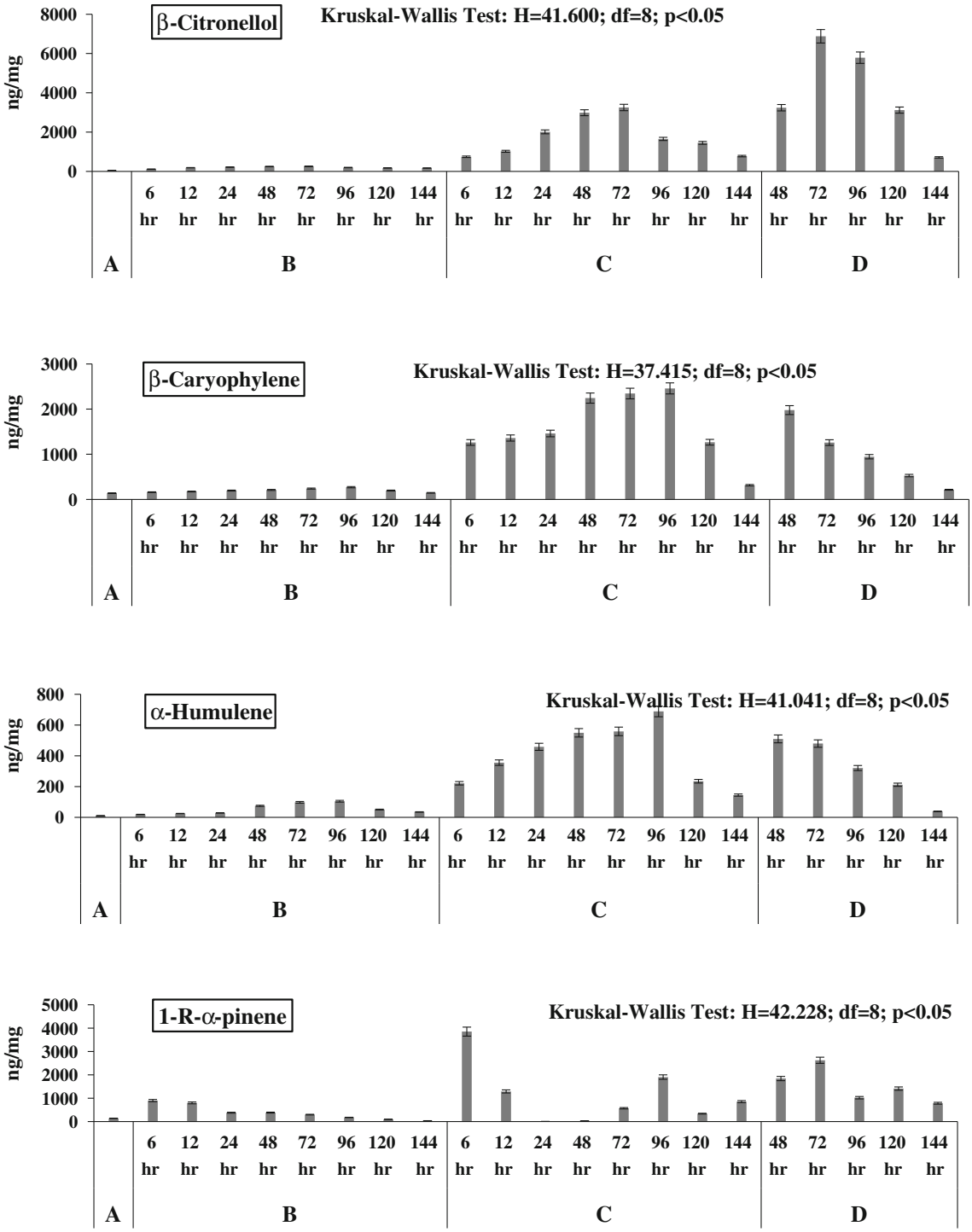
decreased later. But in case of nerol in undamaged leaf it was 12.19 ng/mg which increased after feeding up to the level of 1,256 ng/mg. This might indicate isomerisation of geraniol to nerol or new synthesis of nerol. Similarly with nerol the other acyclic oxygenated monoterpene β-citronellol and citral, cyclic oxygenated monoterpene namely eugenol and cyclic hydrocarbon monoterpenes namely D-limonene and *para*-cymene were also released consistently from 6 to 144 h. Similarly the sesquiterpene farnesene was also released consistently. Consistent release might indicate their de-novo synthesis induced by caterpillar feeding. Release of GLV may be systemically induced due to



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feeding by herbivores and they might serve for plant's indirect defense [11].

Terpenoids in general are synthesized from isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate (DMAPP), which are derived in cytosol, by the mevalonic acid (MVA) pathway [12], and in plastids by the methyl-erythritol-phosphate (MEP) pathway [13–15]. Certain induced terpenes are synthesized from terpene alcohols through oxidative bond cleavage [16]. As in our study some of the terpenoids were consistently present and some occurred transiently at different time intervals, they might be synthesized in different pathways [17]. This

might indicate induction or suppression of different steps in the synthesis of terpenoids by feeding of *A. assama.* In a study made in maize different terpenoids were reported to show different peak hours for release and the release pattern also varied with the type of cultivar [8].

Quantification of total amount of the consistently released volatiles showed that the amount of acyclic oxygenated monoterpene compounds nerol, β-citronellol, and citral ranged from 1,155.19 to 1,739.89 ng/mg. The amount of release of cyclic monoterpenes (oxygenated and hydrocarbon) eugenol, D-limonene and *para*-cymene ranged from 310 to 569.85 ng/mg. Consistent but considerably low amount of release in case of cyclic monoterpene (D-limonene) after continuous feeding might suggest a slow rate of their de-novo synthesis. Terpenes like citral occurred lately in high level at 48–96 h. Similarly, in cotton plant, systemic release of volatile was reported to take 3–4 days [10].

Emissions of Volatile Chemicals After Larvae Were Removed from the Plant

Emission of *R*(−)-α-phellandrene, γ-carene, terpinene-4-ol, α-humulene, β-caryophyllene, 1-*R*-α-pinene, *para*-cymene, nerol, eugenol, β-citronellol and farnesene were released in high amount when the larvae were continuously feeding, but their concentrations reduced or stopped after larvae were removed from the plant (Fig. 2). Emission of terpinene, geraniol, terpinene-4-ol, α-humulene, γ-carene and β-caryophyllene were continuously released till 72–96 h post treatment after which their emission stopped or decreased. D-limonene, citral and *R*(−)-α-phellandrene ceased shortly after the larvae were removed. While continued emission might indicate induction of certain metabolic pathway, cessation after removal of larvae might indicate withdrawal of induction.

A comparative analysis of the results suggest that due to feeding by *A. assama* larvae, the *P. bombycina* produces three blends of terpene compounds: (1) GLV released as constitutive defence (terpinene), (2) volatiles released by continuous feeding (*R*(−)-α-phellandrene, γ-carene, terpinene-4-ol, α-humulene and β-caryophyllene) and (3) volatiles released on systemic induction (*para*-cymene, D-limonene, nerol, eugenol, citral, β-citronellol and farnesene).

Over a long time, these terpenoids are known as semiochemicals in insect-host plant interactions. There has been a co-evolution in plant defense and insect attack strategy, and various modes of plant fitness against herbivory and the fitness of herbivore are evolved. *A. assama* Westwood is a silk producing polyphagous insect. Further study on role of these terpenoid compounds on multitrophic interactions circumscribing *A. assama* and *P. bombycina* will be helpful in highlighting further if any co-evolutionary strategy have been used by the herbivore and the plant.

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