Short-range attraction and oviposition stimulant of a biocontrol agent, *Galerucella placida* Baly (Coleoptera: Chrysomelidae) toward weed leaf surface waxes

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Research Paper

Abstract

Two Polygonaceae weeds, *Rumex dentatus* L. and *Polygonum glabrum* Willd. are abundant in wheat- and rice-fields, respectively, in India. *Galerucella placida* Baly (Coleoptera: Chrysomelidae) is a biocontrol agent of these two weeds. The importance of long-chain alkanes and free fatty acids present in leaf surface waxes of these weeds was assessed as short-range attractant and ovipositional stimulant in *G. placida* females. Extraction, TLC, GC-MS and GC-FID analyses demonstrated 19 *n*-alkanes from *n*-C_{14} to *n*-C_{35} and 14 free fatty acids from C12:0 to C22:0 in leaf surface waxes. Hentriacontane was predominant among alkanes in both weeds, while oleic acid and docosanoic acid were predominant among free fatty acids in *R. dentatus* and *P. glabrum*, respectively. Females of *G. placida* were attracted toward one leaf equivalent surface wax of both weeds against the control solvent (petroleum ether) in a short Y-tube olfactometer bioassay. But, the insect could not differentiate between one leaf equivalent surface wax of *R. dentatus* and *P. glabrum*, indicating that both weed leaves were equally attractive in females. A synthetic blend of either 2.44, 35.57 and 23.58 µg ml^{-1} of octadecane, heptacosane and nonacosane, respectively, resembling the amounts present in one leaf equivalent surface wax of *R. dentatus* or 4.08, 19.54 and 23.58 µg ml^{-1} of octadecane, palmitoleic acid and docosanoic acid, respectively, resembling the amounts present in one leaf equivalent surface wax of *P. glabrum* acted as short-range attractant and ovipositional stimulant in *G. placida*. These results could be a basis for host plant specificity of the biocontrol agent.

Introduction

Despite considerable effort, weed invasion is a major threat to both the crop production and natural ecosystems. Kanatas *et al.* (2020) estimated that weeds generally cause 5, 10 and 25% yield loss in developed, less developed and least developed countries, respectively. The genus *Polygonum* (Polygonaceae) consists of ca. 250 species, which is commonly known as 'knotweed' or 'smartweed', while the genus *Rumex* is commonly designated as 'dock' or 'sorrel' and is comprised of ca. 200 species (Muller, 1981; Sambamurty, 2005). *Rumex dentatus* L., is a major weed of wheat in India and Pakistan, while *Polygonum glabrum* Willd., is considered as a significant weed of rice in India (Anjum and Bajwa, 2010; Singh *et al.*, 2015; Koner *et al.*, 2019). In India, *P. glabrum* is abundant throughout the year, while the emergence and development of *R. dentatus* is analogous to that of wheat and mustard in India and Pakistan. *Rumex dentatus* is abundant in Europe, Asia, Mediterranean region and Egypt, while *P. glabrum* is present in India, Sri Lanka, Nepal, Afghanistan, South Japan, Malaysia, South China and Africa (Hadjichambis *et al.*, 2008; Elzaawely and Tawata, 2012; Al-Sherif *et al.*, 2018). Wheat is one of the major cereal crops in India and is cultivated ca. 29.8 million ha area in India (Gupta *et al.*, 2013), while rice is grown ca. 44 million ha area in India. Both weeds, *P. glabrum* and *R. dentatus* impede production of rice and wheat, respectively. Herbicides are applied by growers to manage these weeds (Abbas *et al.*, 2009; Naseer-ud-Din *et al.*, 2011). Application of herbicides to control weeds in crop-fields may harm and reduce the biocontrol agents consuming the weeds, and at the same time, weeds may be resistant to herbicides. So, the use of native biocontrol agents to control weeds will be cost effective and will not leave any chemical residues in environment (Culliney, 2005).

The biological control of weeds through insect herbivores is one of the methods to control weeds without disturbing the crop. Larvae and adults of *Lema praeusta* (Fab.) gregariously consume leaves of two Commelinaceae rice-field weeds, *Commelina benghalensis* L. and *Murdannia nudiflora* (L.) Brenan in India (Das *et al.*, 2019a). The chrysomelid, *Altica cyanea* Weber is considered as a biocontrol agent of Onagraceae weeds – *Ludwigia adscendens* (L.) Hara, *L. parviflora* Roxb. and *L. octovalvis* (Jacq.) Raven in India (Mitra *et al.*, 2021). Both

larvae and adults of *Galerucella placida* Baly (Coleoptera: Chrysomelidae) feed on leaves of *P. glabrum* and *R. dentatus* (Koner *et al.*, 2019). Larvae of *G. placida* voraciously consume leaves for 10.55 ± 0.08 and 12.28 ± 0.09 days through three instars to complete their larval development on *R. dentatus* and *P. glabrum*, respectively (Koner *et al.*, 2019). After pupation (3–4 days), newly emerged females feed for 44.77 ± 0.16 and 41.12 ± 0.57 days on leaves of *R. dentatus* and *P. glabrum*, respectively (Koner *et al.*, 2019). This insect is abundant in Bangladesh, Nepal, Sri Lanka, Myanmar (Burma), Thailand, Laos, Vietnam, Sumatra and Java (Kimoto, 1989; Hnatiuk, 1990; Beenen, 1998). *Galerucella placida* has been recorded to feed on *Persicaria hydropiper* (synonym *Polygonum hydropiper*) in Australia (Reid, 2001), while the insect has been reported to feed on *Polygonum perfoliatum* L., *P. lapathifolium* L. and *P. hydropiper* L. (Polygonaceae) in China (Ding *et al.*, 2004). In addition, the insect feeds on *P. orientale* L. in India (Malik *et al.*, 2018). The insect, *G. placida* is perfectly harmless on crop plants such as rice, wheat, mustard and potato as *R. dentatus* and *P. glabrum* grow in association with these crop plants. An intense competition between *R. dentatus* and wheat occurs when weed density is exceeded ca. 20 plants m^{-2}, and in extreme condition, i.e., 30 *R. dentatus* plants m^{-2} result 80% yield loss of wheat (Waheed *et al.*, 2017). Rice faces maximum competition with *P. glabrum* weeds in its early growth stage and when the crop growth is 2–3% in the early establishment stage, the weeds already attain 20–30% growth (Deka and Barua, 2015). The aqueous extracts and residues of *Polygonum* species inhibit germination and seedling growth of rice (Abbas *et al.*, 2016).

The leaf surface is the site where the first physical contact with the biocontrol agent, *G. placida* occurs, and if the leaf surface is suitable then females lay eggs on the abaxial surface of the leaves, implicating that leaf surface wax compounds might act as semiochemicals to stimulate the females to lay eggs. Therefore, an understanding of the insect egg laying behavior, and identification and quantification of the leaf surface wax compounds accountable for such a behavior could help to monitor the biocontrol agent in biocontrol program (Padovan *et al.*, 2010; Smith and Beck, 2013; Wheeler and Schaffner, 2013). The leaf surface wax profile such as long-chain alkanes, free fatty acids, alcohols, aldehydes, esters and acetates differ among plant species (Jetter *et al.*, 2000; Mitra *et al.*, 2017). After reaching close range to the host plant, long-chain alkanes and free fatty acids, which are major components of leaf surface wax, can serve as short-range attractant and oviposition stimulant of an insect herbivore (Müller and Hilker, 2001; Müller, 2006; Manosalva *et al.*, 2011; Mitra *et al.*, 2020). Five *n*-alkanes, hexacosane, heptacosane, octacosane, nonacosane and tritriacontane present in the surface wax of corn leaves serve as ovipositional stimulant in *Ostrinia nubilalis* (Hübner) (Udayagiri and Mason, 1997). Different blends consisting of *n*-alkanes and fatty acids present in the surface waxes of young, mature and senescent leaves of *P. orientale* served as short-range attractant in *G. placida* (Malik and Barik, 2015; Malik *et al.*, 2017). Therefore, it is of considerable interest to study whether leaf surface waxes of both weeds, *R. dentatus* and *P. glabrum* could serve as short-range attractant and oviposition stimulant of their biocontrol agent, *G. placida*.

The aims of the present study are (i) to identify the composition, and quantify the amounts of *n*-alkanes and free fatty acids present in the leaf surface waxes of *R. dentatus* and *P. glabrum*, (ii) to observe whether the leaf surface waxes of both *R. dentatus* and *P. glabrum* serve as short-range attractant and

ovipositional stimulant in *G. placida*, (iii) to find the role of individual synthetic compounds, and blends of synthetic alkanes and fatty acids resembling the leaf surface waxes of both weeds in *G. placida* through a short Y-tube olfactometer, and (iv) to observe whether the most attractive synthetic blends comparable to the leaf surface waxes of both weeds could serve as ovipositional stimulant in *G. placida*. If the long-chain *n*-alkanes and free fatty acids present in the leaf surface waxes of both weeds are used by the insect in short-range host finding and oviposition, then this approach will contribute to the evaluation of host plant specificity of the prospective biological control agent.

Materials and methods

Insects

Light traps were used to collect adults of *G. placida* from *P. orientale* weeds growing in fields neighboring to the University of Burdwan (23°16′ N & 87°54′ E), Burdwan, West Bengal, India and reared on the same leaves at 21°C, $65 \pm 5\%$ relative humidity (RH) and 12L : 12D photoperiod in a biological oxygen demand incubator. A moist piece of cotton was wrapped around the petiole of *P. orientale* leaves followed by encasing an aluminum foil to prevent moisture loss from leaves, and new leaves were supplied daily by replacing the preceding one. Newly emerged F_2 males and females started to mate after 1–2 days. Mated females of 4–6 days old (3–4 days after initial mating) were used for olfactometer and ovipositional bioassays.

Plant materials

Two to three weeks old mature leaves of *R. dentatus* and *P. glabrum* were collected from wheat- and rice-fields, respectively, adjoining to this university during December, 2018–January, 2019. Collected leaves were immediately washed with distilled water and dried by paper toweling.

Extraction of leaf surface waxes

Seventy-five grams leaves each of *R. dentatus* and *P. glabrum* were separately collected for five times in the morning at 8 a.m., and the gum arabic method of Jetter and Schäffer (2001) was employed to isolate leaf surface waxes from an intact leaf. Before use of gum arabic (Sigma Aldrich, Germany), the contaminants of gum arabic were removed by soxhlet extraction with hot chloroform. An aqueous solution of ca. 0.1 ml [50% (w/w)] gum arabic was put on per cm^2 of leaf surface on the adaxial and abaxial surfaces of each leaf by a small paintbrush. After drying for 1 h, a thin whitish adhesive layer was removed from each leaf by forceps, keeping the leaves as undamaged and intact (without an injury in the epidermal and mesophyll tissue). The gum arabic fractions of adaxial and abaxial leaf surfaces were separately collected for each leaf followed by put together to get the crude surface waxes and extracted with water and chloroform (v/v). After vigorous agitation and phase separation, the organic solution was removed, and the solvent was evaporated under reduced pressure. The isolation of leaf surface waxes using gum arabic method was repeated five times from five different batches of 75 g leaves of either *R. dentatus* or *P. glabrum*. Each dried crude extract obtained from a batch of 75 g leaves of either *R. dentatus* or *P. glabrum* was then dissolved in 30 ml chloroform and equally

divided into three crude fractions [each 10 ml fraction was ca. 25 g leaves; number of leaves for each 25 g of *R. dentatus* and *P. glabrum* were 15 ± 1 and 35 ± 1 (mean ± standard error; five replicates of each type of leaf), respectively]. The first, second and third fractions (each fraction was corresponding to ca. 25 g leaves) of each crude extract obtained from 75 g leaves of either *R. dentatus* or *P. glabrum* were utilized for (i) identification and quantification of alkanes, (ii) identification and quantification of free fatty acids and (iii) bioassays, respectively.

Identification and quantification of alkanes

Alkanes were identified and quantified according the protocol of Mobarak *et al.* (2020). The first fraction of each crude extract (equivalent to ca. 25 g of leaves) was fractioned by thin layer chromatography (TLC) on silica gel G (Sigma St. Louis, MO, USA) layers (thickness 0.5 mm) with carbon tetrachloride as the mobile phase. A faint yellowish band appeared on the TLC plate, and the plate was air-dried under laboratory conditions. The single hydrocarbon band appeared in each TLC plate was scrapped from the plate and then extracted with chloroform. Five separate crude extracts obtained from five different batches of leaves of either *R. dentatus* or *P. glabrum* were used to isolate five purified alkane samples for either *R. dentatus* or *P. glabrum*. Each crude extract obtained from a batch of leaves was used to isolate a purified alkane sample, and five separate alkane samples for either *R. dentatus* or *P. glabrum* leaves were prepared for gas chromatography-mass spectrometry (GC-MS) and GC- flame ionization detector (GC-FID) for identification and quantification, respectively. Half portion of each sample was used for identification by GC-MS and the remainder for quantification of alkane compounds by GC-FID.

For identification of alkanes, the extracts were analyzed with a Clarus 690 GC coupled to a SQ8C Mass Selective Detector using a SE-30 column (Agilent, USA; length: 30 m × 0.32 mm × 0.25-μm film thickness). The oven temperature program was initially 170°C held for 1 min, then raised at 4°C min^{-1} to 300°C and finally held for 15 min (Sarkar *et al.*, 2014). Helium was the carrier gas. The flow rate of helium was 1 ml min^{-1}. The MS parameters were 280°C at the interface, ionization energy 70 eV, scan speed ca. 5 scans/sec and scanned over the mass range of 40–600 mass units. The identity of compounds was confirmed by injections of mixture of synthetic *n*-alkanes (*n*-C$_{14}$ to *n*-C$_{35}$). Alkanes were verified by comparison of the diagnostic ions and GC retention times with those of respective authentic standards.

For quantification of compounds, five separate extracts obtained from five different batches of leaves of either *R. dentatus* or *P. glabrum* were analyzed by a Techcomp Gas Chromatograph (Em Macau, Rua De Pequim, Nos. 202A-246, Centro Financeiro F7, Hong Kong) model 7900 fitted with a SE-30 capillary column (Agilent, USA; length: 30 m × 0.32 mm × 0.25-μm film thickness) and a flame ionization detector which was run under the same temperature conditions as mentioned in GC-MS analysis. The carrier gas was nitrogen with a flow rate of 18.5 ml min^{-1}. The volume of the sample injected was 1 μl with a split ratio of 1:5. The peaks were identified by comparing retention times with those of standard *n*-alkanes from *n*-C$_{14}$ to *n*-C$_{35}$, and the area of each peak was converted into quantity of *n*-alkanes based on internal standard heneicosane (*n*-C$_{21}$). All *n*-alkanes (>99% purity) between *n*-C$_{14}$ and *n*-C$_{35}$ were purchased from Sigma Aldrich, Steinheim, Germany.

Identification and quantification of free fatty acids

Free fatty acids were identified and quantified according to the protocol of Mitra *et al.* (2020). The second fraction of each crude extract obtained from either *R. dentatus* or *P. glabrum* leaves (equivalent to ca. 25 g of leaves) was mixed with diethyl ether and filtered through Whatman No. 41 filter paper. The extract was purified by TLC on silica gel G layers (thickness 0.5 mm) with *n*-butanol: acetic acid: water (4:1:5; this mixture was shaken and water was separated from this mixture by a separating funnel and discarded) as the mobile phase (Das *et al.*, 2019*b*). The band was eluted from the silica gel layer with diethyl ether, and diethyl ether was removed under reduced pressure to get purified free fatty acids. The purified free fatty acids were esterified with 3 ml BF$_3$-methanol followed by warming for 5 min in a hot water bath at 50–60°C, and cooled. Hexane (30 ml) was added to this mixture followed by washing with saturated NaCl twice in a separating funnel. The aqueous layer of each sample was discarded and the hexane fraction was passed through 50 g anhydrous Na$_2$SO$_4$ twice. One portion of each esterified sample (hexane fraction) was used for GC-MS and another for GC-FID. Five separate crude extracts obtained from five different batches of leaves of either *R. dentatus* or *P. glabrum* leaves were used to separate free fatty acids samples followed by esterification, and in this way, five separate samples for either *R. dentatus* or *P. glabrum* leaves were prepared.

One portion of the esterified fatty acids was analyzed with a Clarus 690 GC coupled to a SQ8C Mass Selective Detector with a SE-30 column (Agilent, USA; length: 30 m × 0.32 mm × 0.25-μm film thickness). The oven temperature program was initially held at 160 °C for 2 min, then raised at the rate of 3°C min^{-1} to 220°C and finally held at 220°C for 18 min (Mukherjee *et al.*, 2014; Sarkar and Barik, 2015). Helium was the carrier gas. The flow rate of helium was 1 ml min^{-1}. The MS temperature parameter was 280°C at the interface, ionization energy 70 eV, scan speed ca. 5 scans/sec, and scanned over the mass range of 40–600 mass units. Fatty acids were verified by comparing the diagnostic ions and GC retention times with those of respective standard esterified fatty acids [methyl laurate (C12:0), methyl tridecanoate (C13:0), methyl tetradecanoate (C14:0), methyl pentadecanoate (C15:0), methyl palmitate (C16:0), methyl palmitoleate (C16:1), methyl stearate (C18:0), methyl oleate (C18:1), methyl linoleate (C18:2), methyl linolenate (C18:3), methyl nonadecanoate (C19:0), methyl arachidate (C20:0), methyl heneicosanoate (C21:0) and methyl docosanoate (C22:0)]. All standard esterified fatty acids (fatty acid methyl esters) were purchased from Sigma-Aldrich, Steinheim, Germany.

The remaining portion of esterified fatty acids (five separate samples obtained from five different batches of leaves of either *R. dentatus* or *P. glabrum*) were analyzed using a Techcomp Gas Chromatograph model 7900 fitted with a SE-30 capillary column (Agilent, USA; length: 30 m × 0.32 mm × 0.25-μm film thickness) and a flame ionization detector which was run under the same temperature conditions as described for GC-MS analysis. The injector port temperature was 280°C. The carrier gas was nitrogen with a flow rate of 20 ml min^{-1} (Mukherjee *et al.*, 2014; Sarkar and Barik, 2015). The volume of the sample injected was 1 μl with a split ratio of 1:5. The peaks were identified by comparing retention times with those of standard esterified fatty acids. The amount of individual free fatty acids was computed from the GC peak areas and the areas of all peaks were converted into quantities of fatty acids based on reference standard

methyl tricosanoate (C23:0). Solvents (*n*-hexane, chloroform, *n*-butanol, acetic acid and methanol) used were of analytical grade and purchased from E. Merck (Mumbai, India).

Olfactometer bioassays

Prior to use in olfactometer bioassays, mated *G. placida* females (4–6 days old) were provided with water but without food for 6 h. Females were used in bioassays as they employ olfactory cues both for feeding and egg laying. A Y-tube olfactometer (internal radius of 0.6 cm, length of stem or each arm is 5 cm, two lateral arms at an angle of 45°) was used to measure the attractiveness of *G. placida* (Supplementary fig. 1). A glass-made micro kit adapter was attached with the end of each arm of the Y followed by attachment of a glass vial (1 cm radius × 3 cm long) with the micro kit adapter. Each adapter had two entrances – one air inlet tube for approaching air into the glass vial and another one as outlet tube connecting the glass vial to an arm of the olfactometer. A piece (2×2 cm^2) of Whatman No. 41 filter paper moistened with 1 ml of the test compounds was placed in one glass vial, while the other glass vial contained a filter paper of same size moistened with 1 ml of the control solvent (petroleum ether). Charcoal-filtered air was pushed into the system at 200 ml min^{-1}. All the connections between different parts of the set-up consisted of Teflon tubing.

The olfactometer bioassays were performed in the laboratory at $21 \pm 1°C$, $70 \pm 5\%$ RH and light intensity 150 lux. To test any intrinsic bias of *G. placida* within the Y-tube olfactometer, Whatman No. 41 filter papers without test stimuli were provided in the glass vials (1 cm radius × 3 cm long) at the commencement of the experiment. Females did not display directional bias in the Y-tube olfactometer. In preliminary assays, females did not show attraction to the control solvent (petroleum ether). One ml of the test sample and the control solvent were applied to the separate filter paper pieces followed by evaporation of the control solvent in open space under laboratory condition, and these filter papers were placed into the glass vials prior to release of the first insect into the olfactometer for each experiment. One adult *G. placida* female was placed into the porous glass vial (1 cm radius × 3 cm long), which was then attached with the stem of olfactometer and exposed to a particular odor, consisting of 1 ml of the control solvent (petroleum ether) in one glass vial and 1 ml of the test sample (leaf surface waxes, individual synthetic alkanes and fatty acids or synthetic blends consisting of alkanes and fatty acids) in another glass vial. The behavior of each female was observed for 2 min such as wandering in the Y-tube. The choice of each female was recorded when a female reached at the end of one arm followed by removal of the female from the Y-tube, and the choice of female was recorded as a positive (showed attraction toward test samples) or negative (did not show attraction toward test samples) response, respectively. A female was rejected when the female did not make a choice within 2 min (non-responding, i.e., the insect was in the stem of Y-tube or did not exhibit any movement until the end of observation period), and was substituted by a new one (Mukherjee *et al.*, 2015; Sarkar *et al.*, 2015). Each experiment with one test sample was performed until 60 naïve females had responded (each insect was tested once throughout olfactory bioassays). After testing five insects, the olfactometer set-up was washed with petroleum ether followed by acetone and the position of two arms was systematically changed in order to avoid positional bias.

Dual choice bioassays with female G. placida toward crude surface waxes

The behavioral responses of females toward one leaf equivalent surface wax (crude extract) of either *R. dentatus* or *P. glabrum* were tested against the control solvent (petroleum ether) (Supplementary tables 1 and 2). Further, the behavioral responses of females toward one leaf equivalent surface wax (crude extract) of *R. dentatus* and *P. glabrum* were tested against each other (Supplementary table 1).

Dual choice bioassays with female G. placida toward synthetic compounds or blends

The behavioral responses of females toward individual synthetic compounds (alkanes and fatty acids) resembling the amounts present in one leaf equivalent surface wax of *R. dentatus* or *P. glabrum* were tested against the control solvent to observe a response of the insect toward individual compounds. Individual synthetic compounds were dissolved in 1 ml petroleum ether and were tested against 1 ml control solvent (Supplementary tables 3a and 3b). Females showed behavioral responses to nine individual compounds (hexadecane, octadecane, tetracosane, pentacosane, heptacosane, nonacosane, lauric acid, palmitoleic acid and nonadecanoic acid) resembling the amounts present in one leaf equivalent surface wax of *R. dentatus*. Females exhibited behavioral responses to six individual compounds (octadecane, heptacosane, lauric acid, palmitoleic acid, nonadecanoic acid and docosanoic acid) resembling the amounts present in one leaf equivalent surface wax of *P. glabrum*. The insect did not indicate any response (the insect remained in the stem of the olfactometer) to the rest of identified individual alkane and fatty acid compounds resembling the amounts present in one leaf equivalent surface wax of *R. dentatus* and *P. glabrum*. The standard synthetic *n*-alkanes and fatty acids identified in this study were all purchased from Sigma Aldrich, Steinheim, Germany.

The insect showed behavioral response to those individual synthetic compounds (the amounts present in one leaf equivalent surface wax) were combined comparable to one leaf equivalent surface wax of either *R. dentatus* or *P. glabrum* and were tested against the control solvent. Further, the insect showed attraction to those individual synthetic compounds (comparable to the amounts present in one leaf equivalent surface wax) were combined resembling one leaf equivalent surface wax of either *R. dentatus* or *P. glabrum* [*R. dentatus* : 2.44 µg octadecane + 35.57 µg heptacosane + 23.58 µg nonacosane were dissolved in 1 ml petroleum ether, hereafter will be denoted as RD blend 3; *P. glabrum*: 4.08 µg octadecane + 19.54 µg palmitoleic acid + 23.58 µg docosanoic acid were dissolved in 1 ml petroleum ether, hereafter will be denoted as PG blend 3] and were tested against the control solvent.

The behavioral responses of female *G. placida* toward one leaf equivalent surface wax of *R. dentatus* were tested against nine individual compounds (hexadecane, octadecane, tetracosane, pentacosane, heptacosane, nonacosane, lauric acid, palmitoleic acid and nonadecanoic acid) or synthetic blends (a synthetic blend of above nine compounds or a synthetic blend of three compounds, i.e., RD blend 3) comparable to the amounts present in one leaf equivalent surface wax of *R. dentatus* to observe the role of individual synthetic compounds or synthetic blends against one leaf equivalent surface wax of *R. dentatus*. Similarly, the behavioral responses of female *G. placida* toward one leaf equivalent surface wax extracted from *P. glabrum* were tested

against six individual compounds (octadecane, heptacosane, lauric acid, palmitoleic acid, nonadecanoic acid and docosanoic acid) or synthetic blends (a synthetic blend of above six compounds or a synthetic blend of three compounds, i.e., PG blend 3) comparable to the amounts present in one leaf equivalent surface wax of *P. glabrum* to observe the role of individual synthetic compounds or synthetic blends against one leaf equivalent surface wax of *P. glabrum*. A synthetic blend of three compounds resembling one leaf equivalent surface wax of *R. dentatus* (RD blend 3) was tested against a synthetic blend of three compounds resembling one leaf equivalent surface wax of *P. glabrum* (PG blend 3) to observe whether females preferred a particular synthetic blend.

Dose responses of female G. placida *toward synthetic compounds against the control solvent*

The insect displayed attraction toward five individual compounds (octadecane, heptacosane, nonacosane, palmitoleic acid and docosanoic acid) resembling the amounts present in one leaf equivalent surface wax of both weeds. So, dose responses of female *G. placida* toward these five compounds were tested at different doses (octadecane : 1.5, 3, 6, 12 and 24 µg were separately dissolved in 1 ml petroleum ether, respectively; heptacosane: 15, 30, 60 and 120 µg were separately dissolved in 1 ml petroleum ether, respectively; nonacosane: 15, 30 and 60 µg were separately dissolved in 1 ml petroleum ether, respectively; palmitoleic acid: 5, 10, 20 and 40 µg were separately dissolved in 1 ml petroleum ether, respectively; docosanoic acid: 20, 40 and 80 µg were separately dissolved in 1 ml petroleum ether, respectively).

Oviposition assay

Three-day-old mated females were employed for oviposition assays. For oviposition assay, 10 square glass chambers (15×15 cm^2) were used, and coarse grade emery papers were placed along the sides of each glass chamber to avoid egg laying on the wall as well as on the floor of glass chamber. During oviposition assay, females were provided a cotton piece moistened with sucrose solution in a Petri dish (3 cm diameter). Primarily, females did not show any bias for egg laying on the filter paper (Whatman No. 41, 2×2 cm^2) moistened with the control solvent (petroleum ether). One ml of the test sample and the control solvent were applied on two separate filter paper pieces and permitted to evaporate the solvent in open space followed by placing of these filter papers in two round Petri dishes (each Petri dish 3 cm diameter). One filter paper with the test sample in a Petri dish and the other filter paper with the control solvent in another Petri dish were positioned with a gap of 8 cm inside a glass chamber (15×15 cm^2), and a mated female was released in the experimental glass chamber. Ten mated females were separately tested for each experiment excluding the number of insects that did not show egg laying behavior. Each mated female was observed at 24 h interval after releasing in a square glass chamber (15×15 cm^2), and when the mated females laid eggs for the first time then the mated female was discarded and eggs were counted.

Oviposition assays with female G. placida *toward crude surface waxes*

A single *R. dentatus* leaf vs a single dewaxed *R. dentatus* leaf, and a single *P. glabrum* leaf vs a single *P. glabrum* dewaxed leaf (for dewaxing of leaves, surface waxes were removed by gum arabic method) were tested to find whether leaf surface waxes of both weeds stimulated females to lay eggs (Supplementary table 4). Further, a single *R. dentatus* and *P. glabrum* leaf was tested against each other to find whether a single leaf of a particular weed was preferred by females to lay more eggs (Supplementary table 4). One leaf equivalent surface wax of *R. dentatus* or *P. glabrum* was tested against the control solvent to observe whether crude leaf surface waxes of both weeds stimulated females to lay eggs (Supplementary table 4). In addition, one leaf equivalent surface wax of *R. dentatus* and *P. glabrum* was tested against each other to find whether leaf surface wax of a particular weed was preferred by females to lay more eggs (Supplementary table 4).

Oviposition assays of G. placida *females toward synthetic compounds and blends*

Three individual synthetic compounds, octadecane, heptacosane and nonacosane (as *G. placida* showed significant attraction responses toward these three individual compounds in olfactometer bioassays) at similar amounts present in one leaf equivalent surface wax of *R. dentatus* were tested against the control solvent to observe whether these three compounds individually stimulated females to lay eggs. Similarly, three individual synthetic compounds, octadecane, palmitoleic acid and docosanoic acid (as *G. placida* showed significant attraction responses toward these three individual compounds in olfactometer bioassays) at similar amounts present in one leaf equivalent surface wax of *P. glabrum* were tested against the control solvent to observe whether individual compounds could stimulate females to lay eggs.

RD blend 3 or PG blend 3 was tested against the control solvent to find whether synthetic blends at similar amounts present in one leaf equivalent surface wax of *R. dentatus* or *P. glabrum* stimulated females to lay eggs (Supplementary table 4). One leaf equivalent surface wax of *R. dentatus* vs RD blend 3, and one leaf equivalent surface wax of *P. glabrum* vs PG blend 3 were conducted to find whether synthetic blends and leaf surface waxes of both weeds were equally stimulated females to lay eggs (Supplementary table 4). RD blend 3 was tested against PG blend 3 to find whether a particular synthetic blend stimulated females to lay more eggs (Supplementary table 4).

Statistical analyses

Student's *t* test was applied on the data of total amounts of alkanes and free fatty acids, and amounts of individual alkanes and free fatty acids present in *R. dentatus* and *P. glabrum* leaf surface waxes (Zar, 1999). The Principal component analysis (PCA) was performed to show differences between the chemical composition of *R. dentatus* and *P. glabrum* using individual alkanes and free fatty acids as variables (XLSTAT version 13). The data recorded on olfactometer bioassays and oviposition assays of *G. placida* to the test samples were analyzed based on the null hypothesis that the probability of scores for the test compound(s) or control solvent is equal to 50%, i.e., by a Chi-square test (H_0: $P = 50\%$) (Karmakar *et al.*, 2020; Mitra *et al.*, 2020). Insects that did not respond by choosing either arm of the olfactometer were not included in the analyses.

Results

Leaf surface waxes

Total amount of crude surface waxes from two types of weed leaves were homogeneously distributed as revealed by Levene's
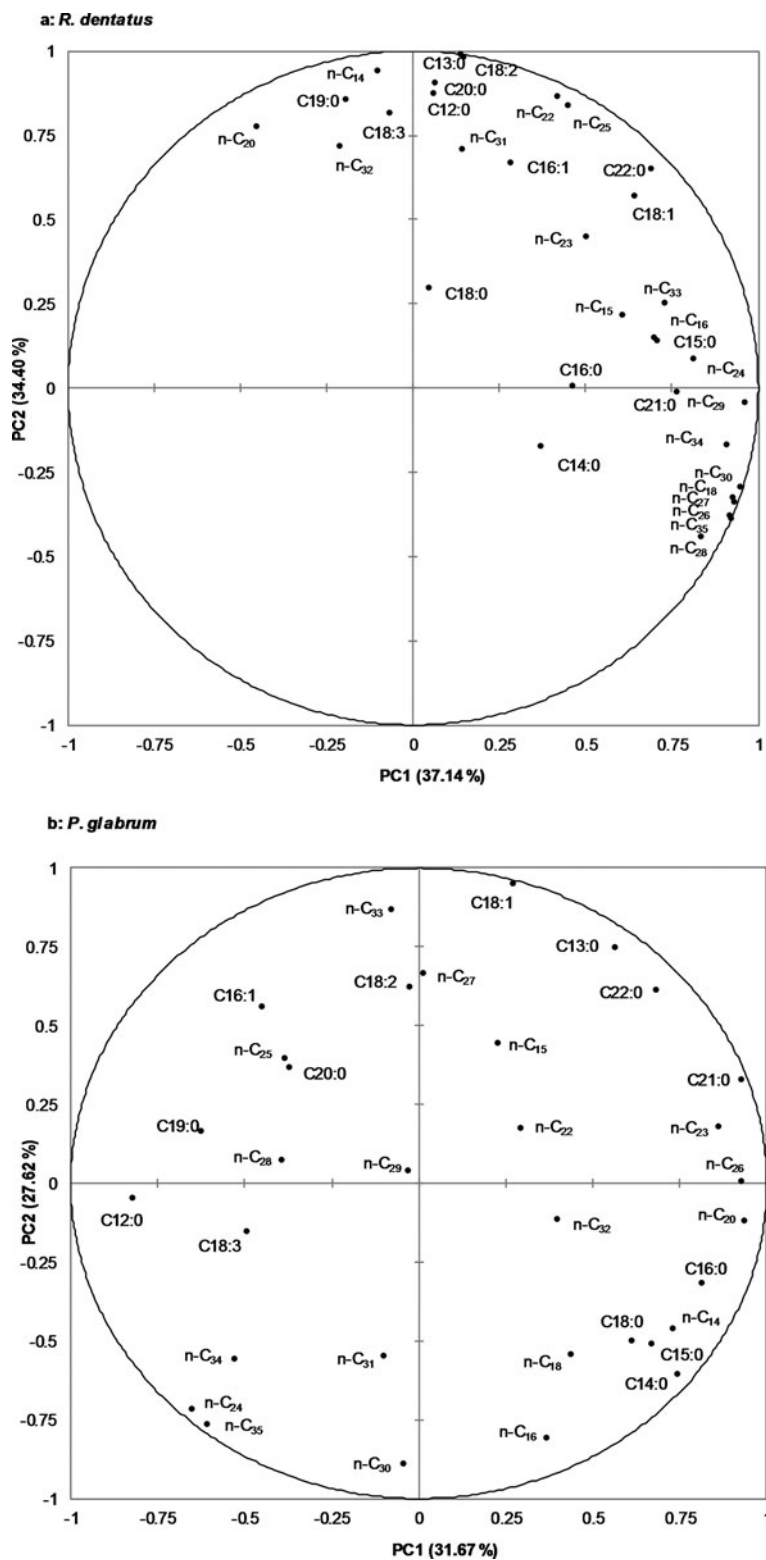
Figure 1. Principal component analysis (PCA) of *n*-alkanes (*n*-C_{14} to *n*-C_{35}) and free fatty acids (C12:0 to C22:0) from leaf surface waxes of two weeds, **a:** *Rumex dentatus*, **b:** *Polygonum glabrum*.

test (W) of homogeneity of variance ($W = 0.370$; $df = 8$; $P = 0.560$). Total amounts of crude leaf surface waxes were higher in *R. dentatus* (48.30 ± 1.30 mg, mean ± SE) compared to *P. glabrum* (43.14 ± 1.55 mg, mean ± SE) ($t = -2.547$; $df = 8$; $P < 0.05$). In *R. dentatus*, alkanes and free fatty acids represented for 29.33 ± 1.33 and 4.24 ± 0.25 mg (mean ± SE), respectively; whereas in *P. glabrum*, alkanes and free fatty acids accounted for 23.97 ± 0.89

and 5.34 ± 0.15 mg (mean ± SE), respectively, with the balance consisting of unidentified surface wax compounds.

For *R. dentatus*, the first two principal components, PC1 and PC2, represented for 37.14% and 34.40% of the total variance, respectively (fig. 1a); whereas for *P. glabrum*, PC1 and PC2, represented for 31.67% and 27.62% of the total variance, respectively (fig. 1b). Except for pentadecane (*n*-C_{15}), octadecane (*n*-C_{18}),

Table 1. Composition of alkanes (μg/25 g leaf) in *Polygonum glabrum* and *Rumex dentatus* leaves.

Alkanes	Amount (μg) (Mean ± SE, $N = 5$)		t	df	P value
	P. glabrum	*R. dentatus*			
Tetradecane (n-C_{14})	7.21 ± 0.59	1837.92 ± 118.86	−15.402	8	0.0001
Pentadecane (n-C_{15})	3794.51 ± 206.31	4248.14 ± 237.50	−1.442	8	0.187
Hexadecane (n-C_{16})	130.40 ± 7.80	230.37 ± 18.21	−5.047	8	0.001
Octadecane (n-C_{18})	142.96 ± 11.90	36.67 ± 3.92	8.482	8	0.0001
Eicosane (n-C_{20})	230.11 ± 27.69	2917.96 ± 240.65	−11.096	8	0.0001
Docosane (n-C_{22})	3157.15 ± 205.10	2805.08 ± 148.39	1.391	8	0.202
Tricosane (n-C_{23})	2602.41 ± 213.47	1853.40 ± 94.23	3.21	8	0.012
Tetracosane (n-C_{24})	130.66 ± 7.65	140.86 ± 8.85	−0.872	8	0.408
Pentacosane (n-C_{25})	1419.28 ± 75.00	1105.86 ± 88.92	2.694	8	0.027
Hexacosane (n-C_{26})	82.21 ± 4.26	76.50 ± 8.69	0.59	8	0.572
Heptacosane (n-C_{27})	618.74 ± 31.57	533.54 ± 33.38	1.854	8	0.101
Octacosane (n-C_{28})	55.74 ± 5.41	136.02 ± 10.59	−6.752	8	0.0001
Nonacosane (n-C_{29})	322.44 ± 16.95	353.71 ± 19.86	−1.198	8	0.265
Triacontane (n-C_{30})	61.98 ± 4.41	106.98 ± 8.06	−4.90	8	0.001
Hentriacontane (n-C_{31})	5564.72 ± 314.55	5824.64 ± 316.87	−0.582	8	0.577
Dotriacontane (n-C_{32})	59.09 ± 4.66	68.79 ± 5.86	−1.296	8	0.231
Tritriacontane (n-C_{33})	5332.88 ± 372.64	5758.37 ± 292.23	−0.898	8	0.395
Tetratriacontane (n-C_{34})	44.30 ± 5.31	194.97 ± 16.75	−8.572	8	0.0001
Pentatriacontane (n-C_{35})	104.28 ± 13.31	608.41 ± 53.24	−9.187	8	0.0001
Total	23,861.07 ± 887.86	28,838.18 ± 1332.47	−3.108	8	0.014

docosane (n-C_{22}), tricosane (n-C_{23}), tridecanoic acid (C13:0), tetradecanoic acid (C14:0), oleic acid (C18:1), nonadecanoic acid (C19:0) and docosanoic acid (C22:0), where no differences in terms of their relative abundance were observed in biplots between *R. dentatus* and *P. glabrum*, rest of the alkanes and free fatty acids were different in terms of their relative abundances between *R. dentatus* and *P. glabrum* (fig. 1a, b).

Alkanes in leaf surface waxes

Total amount of alkanes was higher in the leaf surface waxes of *R. dentatus* compared to *P. glabrum* ($t = −3.320$; $df = 8$; $P < 0.05$). The identified n-alkanes and unidentified branched-chain alkanes in the leaf surface waxes of *R. dentatus* represented for 28.84 ± 1.33 and 0.49 ± 0.02 mg (mean ± SE), respectively. In *P. glabrum*, identified n-alkanes and unidentified branched-chain alkanes accounted for 23.86 ± 0.89 and 0.11 ± 0.01 mg (mean ± SE), respectively. Nineteen n-alkanes in the series of n-C_{14} to n-C_{35} were detected in the leaf surface waxes of both weeds (table 1, Supplementary fig. 2). Heptadecane, nonadecane and heneicosane were absent between n-C_{14} and n-C_{35} alkanes in the leaf surface waxes of both weeds. The amounts of n-C_{15}, n-C_{22}, n-C_{24}, n-C_{26}, n-C_{27}, n-C_{29}, n-C_{31}, n-C_{32} and n-C_{33} did not significantly differ between *R. dentatus* and *P. glabrum* leaf surface waxes. The amounts of alkanes, n-C_{14}, n-C_{16}, n-C_{20}, n-C_{28}, n-C_{30}, n-C_{34} and n-C_{35} were higher in the leaf surface waxes of *R. dentatus* compared to *P. glabrum*, while n-C_{18}, n-C_{23} and n-C_{25} were higher in the leaf surface waxes of *P. glabrum* compared to *R. dentatus* (table 1). Hentriacontane

(n-C_{31}) was predominant followed by tritriacontane (n-C_{33}) in the leaf surface waxes of both weeds; whereas n-C_{18} and n-C_{14} were the least abundant in the leaf surface waxes of *R. dentatus* and *P. glabrum*, respectively.

Free fatty acids in leaf surface waxes

Total amount of free fatty acids was higher in the leaf surface waxes of *P. glabrum* compared to *R. dentatus* ($t = 3.790$; $df = 8$; $P = 0.0053$). Fourteen free fatty acids from C12:0 to C22:0 were detected in the leaf surface waxes of both weeds (table 2, Supplementary fig. 3). Docosanoic acid (C22:0) and oleic acid (C18:1) predominated among all free fatty acids present in *P. glabrum* and *R. dentatus*, respectively; whereas stearic acid (C18:0) and linolenic acid (C18:3) were the least abundant in *P. glabrum* and *R. dentatus*, respectively (table 2). The amounts of eight free fatty acids, C12:0, C13:0, C16:1, C18:3, C18:2, C19:0, C20:0 and C22:0 were significantly ($P < 0.05$) higher in *P. glabrum* compared to *R. dentatus*; whereas the amounts of five free fatty acids, C14:0, C15:0, C16:0, C18:1 and C18:0 were significantly higher in *R. dentatus* compared to *P. glabrum*. There was no significant difference in the amount of C21:0 fatty acid between *P. glabrum* and *R. dentatus* (table 2).

Olfactometer bioassays with G. placida females toward crude leaf surface waxes

Females showed attraction toward one leaf equivalent surface wax of *R. dentatus* ($\chi^2 = 15$, $df = 1$, $P = 0.0001$) or *P. glabrum*

Table 2. Composition of free fatty acids (μg/25 g leaf) in *Polygonum glabrum* and *Rumex dentatus* leaves.

	Amount (μg) (Mean ± SE, $N = 5$)				
Fatty acids	*P. glabrum*	*R. dentatus*	t	df	*P* value
Lauric acid (C12:0)	207.81 ± 22.25	78.15 ± 8.72	5.427	8	0.001
Tridecanoic acid (C13:0)	764.66 ± 43.52	628.07 ± 33.89	2.476	8	0.0383
Tetradecanoic acid (C14:0)	153.54 ± 18.49	695.40 ± 65.07	−8.010	8	0.0001
Pentadecanoic acid (C15:0)	139.13 ± 18.33	233.02 ± 15.75	−3.885	8	0.0046
Palmitoleic acid (C16:1)	683.74 ± 57.17	84.17 ± 9.90	10.334	8	0.0001
Palmitic acid (C16:0)	210.79 ± 11.85	539.39 ± 39.91	−7.893	8	0.0001
Linolenic acid (C18:3)	88.89 ± 9.40	59.69 ± 8.46	2.309	8	0.0497
Linoleic acid (C18:2)	470.23 ± 29.93	77.42 ± 9.04	12.566	8	0.0001
Oleic acid (C18:1)	643.36 ± 32.07	794.98 ± 44.73	−2.754	8	0.0249
Stearic acid (C18:0)	60.90 ± 8.43	218.33 ± 22.61	−6.525	8	0.0002
Nonadecanoic acid (C19:0)	218.00 ± 9.05	156.00 ± 16.93	3.229	8	0.0121
Arachidic acid (C20:0)	431.14 ± 31.49	132.04 ± 10.54	9.008	8	0.0001
Heneicosanoic acid (C21:0)	442.47 ± 26.19	400.04 ± 29.86	1.068	8	0.3166
Docosanoic acid (C22:0)	825.39 ± 44.70	143.41 ± 11.53	14.772	8	0.0001
Total	5340.04 ± 146.98	4240.10 ± 250.24	3.790	8	0.0053
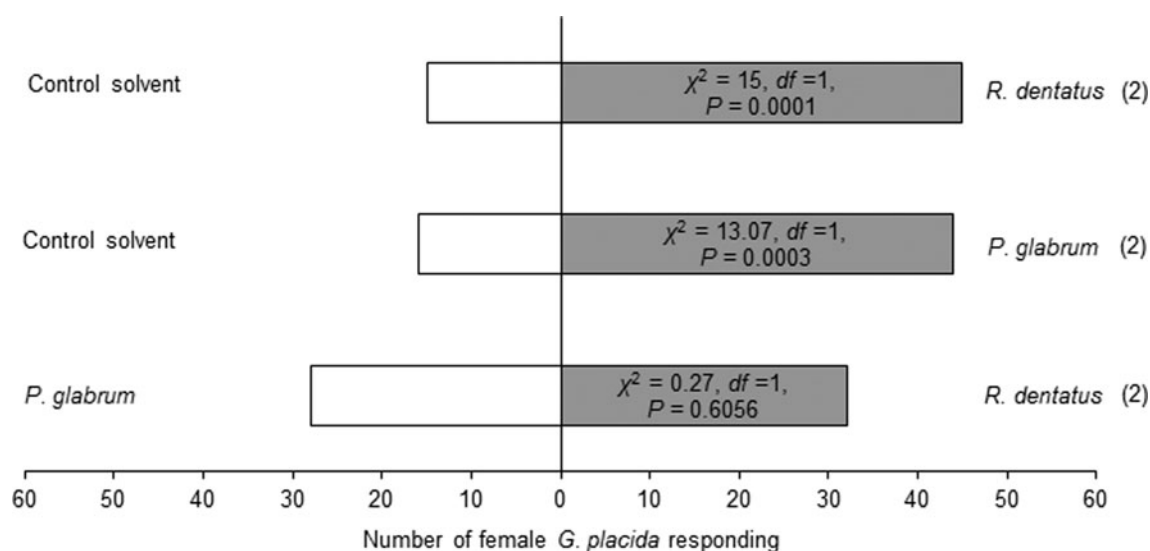
Figure 2. Behavioral responses of *Galerucella placida* females toward one leaf equivalent surface wax of *Rumex dentatus* and *Polygonum glabrum* against the control solvent (petroleum ether), and *R. dentatus* vs. *P. glabrum* in the Y-tube olfactometer bioassay. Each bioassay was performed until 60 naïve females had responded. Numbers within parentheses are the number of insects that did not respond to either treatment.

($\chi^2 = 13.07$, $df = 1$, $P = 0.0003$) compared to the control solvent (fig. 2). But, females could not discriminate between one leaf equivalent surface wax of *R. dentatus* and *P. glabrum* ($\chi^2 = 0.27$, $df = 1$, $P = 0.6056$) (fig. 2).

Olfactometer bioassays with G. placida *females toward synthetic compounds or blends*

Among the identified 19 alkanes and 14 free fatty acids present in the leaf surface waxes of *R. dentatus*, females showed responses toward nine individual synthetic compounds (hexadecane,

octadecane, tetracosane, pentacosane, heptacosane, nonacosane, lauric acid, palmitoleic acid and nonadecanoic acid) at similar amounts present in one leaf equivalent surface wax of *R. dentatus* compared to the control solvent (table 3). Females showed attraction toward a synthetic blend of above nine compounds at similar amounts present in one leaf equivalent surface wax of *R. dentatus* compared to the control solvent ($\chi^2 = 13.07$, $df = 1$, $P = 0.0003$). Among nine individual compounds, females showed significant attraction responses toward three compounds, octadecane ($\chi^2 = 4.27$, $df = 1$, $P = 0.0389$) or heptacosane ($\chi^2 = 5.40$, $df = 1$, $P = 0.0201$) or nonacosane ($\chi^2 = 6.67$, $df = 1$, $P = 0.0098$) at similar

Table 3. Behavioral responses of *Galerucella placida* females toward individual synthetic compounds or synthetic blends at similar amounts present in one leaf equivalent wax of *Rumex dentatus* or *Polygonum glabrum* vs the control solvent (petroleum ether) in the Y-tube olfactometer bioassay (N = 60 in each bioassay).

Comparison		Insects responded		Non-responders	χ^2 (df = 1)	P values
T1	T2	T1	T2			
Synthetic compounds resembling one leaf equivalent surface wax of *R. dentatus* (μg ml^{-1})	Control solvent					
a. Hexadecane (15.36)		32	28	4	0.27	0.6056
b. Octadecane (2.44)		38	22	3	4.27	0.0389
c. Tetracosane (9.39)		32	28	5	0.27	0.6056
d. Pentacosane (73.72)		36	24	4	2.40	0.1213
e. Heptacosane (35.57)		39	21	3	5.40	0.0201
f. Nonacosane (23.58)		40	20	2	6.67	0.0098
g. Lauric acid (5.21)		36	24	4	2.40	0.1213
h. Palmitoleic acid (5.61)		32	28	5	0.27	0.6056
i. Nonadecanoic acid (10.40)		34	26	5	1.07	0.3017
a + b + c + d + e + f + g + h + i		44	16	2	13.07	0.0003
b + e + f (RD blend 3)		43	17	2	11.27	0.0008
Synthetic compounds resembling one leaf equivalent surface wax of *P. glabrum* (μg ml^{-1})						
b. Octadecane (4.08)		39	21	3	5.40	0.0201
e. Heptacosane (17.68)		35	25	4	1.67	0.1967
g. Lauric acid (5.94)		37	23	4	3.27	0.0707
h. Palmitoleic acid (19.54)		39	21	3	5.40	0.0201
i. Nonadecanoic acid (6.23)		32	28	5	0.27	0.6056
j. Docosanoic acid (23.58)		38	22	5	4.27	0.0389
b + e + g + h + i + j		43	17	2	11.27	0.0008
b + h + j (PG blend 3)		42	18	2	9.60	0.0019

amounts present in one leaf equivalent surface wax of *R. dentatus* compared to the control solvent (table 3). The insect showed significant attraction responses toward a synthetic blend of above three compounds, i.e., RD blend 3 (octadecane, heptacosane and nonacosane) ($\chi^2 = 11.27$, *df* = 1, *P* = 0.0008) at similar amounts present in one leaf equivalent surface wax of *R. dentatus* compared to the control solvent (table 3).

Among a total of 33 compounds (19 alkanes and 14 free fatty acids) present in the leaf surface waxes of *P. glabrum*, females displayed responses to six individual synthetic compounds (octadecane, heptacosane, lauric acid, palmitoleic acid, nonadecanoic acid and docosanoic acid) at similar amounts present in one leaf equivalent surface wax of *P. glabrum* compared to the control solvent (table 3). Females showed attraction toward a synthetic blend of above six compounds at similar amounts present in one leaf equivalent surface wax of *P. glabrum* compared to the control solvent ($\chi^2 = 11.27$, *df* = 1, *P* = 0.0008) (table 3). Among six individual compounds, females showed significant attraction responses toward three compounds, octadecane ($\chi^2 = 5.40$, *df* = 1, *P* = 0.0201) or palmitoleic acid ($\chi^2 = 5.40$, *df* = 1, *P* = 0.0201) or docosanoic acid ($\chi^2 = 4.27$, *df* = 1, *P* = 0.0389) at similar amounts present in one leaf equivalent surface wax of *P. glabrum* compared to the control solvent (table 3). The insect showed significant attraction responses
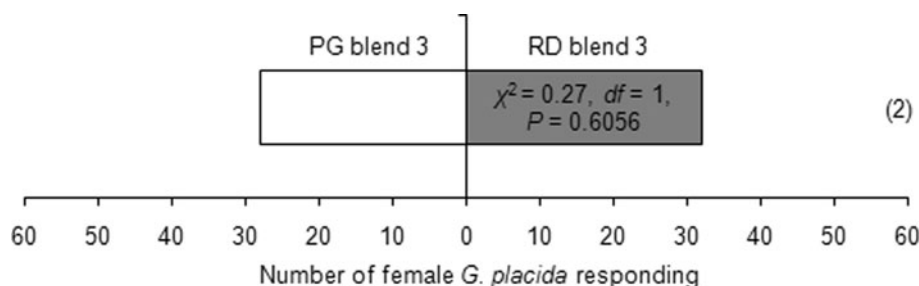
toward a synthetic blend of above three compounds, i.e., PG blend 3 (octadecane, palmitoleic acid and docosanoic acid) ($\chi^2 = 9.60$, *df* = 1, *P* = 0.0019) at similar amounts present in one leaf equivalent surface wax of *P. glabrum* compared to the control solvent (table 3).

Females were attracted toward one leaf equivalent surface wax of *R. dentatus* when tested against individual hexadecane or octadecane or tetracosane or pentacosane or lauric acid or palmitoleic acid or nonadecanoic acid at similar amounts present in one leaf equivalent surface wax of *R. dentatus* (table 4). But, females could not distinguish between one leaf equivalent surface wax of *R. dentatus* and individual heptacosane or nonacosane at similar amounts present in one leaf equivalent surface wax of *R. dentatus* (table 4). Females could not differentiate between one leaf equivalent surface wax of *R. dentatus* and a synthetic blend of nine compounds (hexadecane, octadecane, tetracosane, pentacosane, heptacosane, nonacosane, lauric acid, palmitoleic acid and nonadecanoic acid) ($\chi^2 = 0.07$, *df* = 1, *P* = 0.7963) or RD blend 3 (octadecane, heptacosane and nonacosane) ($\chi^2 = 0.27$, *df* = 1, *P* = 0.6056) (table 4).

Females showed attraction toward one leaf equivalent surface wax of *P. glabrum* compared to individual heptacosane or lauric acid or nonadecanoic acid at similar amounts present in one leaf equivalent surface wax of *P. glabrum* (table 4). But, females could not differentiate between one leaf equivalent surface wax

Table 4. Behavioral responses of *Galerucella placida* females to one leaf equivalent wax of *Rumex dentatus* and *Polygonum glabrum* vs individual synthetic compounds or synthetic blends at similar amounts present in one leaf equivalent wax of *R. dentatus* and *P. glabrum* in the Y-tube olfactometer bioassay (*N* = 60 in each bioassay).

Comparison		Insects responded		Non-responders	χ^2 (*df* = 1)	*P* values
T1	T2	T1	T2			
One leaf equivalent surface wax of *R. dentatus*	Synthetic compounds or blends resembling one leaf equivalent surface wax of *R. dentatus* (µg ml^{-1})					
	a. Hexadecane (15.36)	44	16	1	13.07	0.0003
	b. Octadecane (2.44)	38	22	3	4.27	0.0389
	c. Tetracosane (9.39)	44	16	2	13.07	0.0003
	d. Pentacosane (73.72)	39	21	3	5.40	0.0201
	e. Heptacosane (35.57)	37	23	4	3.27	0.0707
	f. Nonacosane (23.58)	36	24	3	2.40	0.1213
	g. Lauric acid (5.21)	39	21	2	5.40	0.0201
	h. Palmitoleic acid (5.61)	44	16	1	13.07	0.0003
	i. Nonadecanoic acid (10.40)	41	19	2	8.07	0.0045
	a + b + c + d + e + f + g + h + i	31	29	1	0.07	0.7963
	b + e + f (RD blend 3)	32	28	2	0.27	0.6056
One leaf equivalent surface wax of *P. glabrum*	Synthetic compounds or blends resembling one leaf equivalent surface wax of *P. glabrum* (µg ml^{-1})					
	b. Octadecane (4.08)	36	24	3	2.40	0.1213
	e. Heptacosane (17.68)	40	20	2	6.67	0.0098
	g. Lauric acid (5.94)	38	22	3	4.27	0.0389
	h. Palmitoleic acid (19.54)	36	24	3	2.40	0.1213
	i. Nonadecanoic acid (6.23)	42	18	1	9.60	0.0019
	j. Docosanoic acid (23.58)	37	23	4	3.27	0.0707
	b + e + g + h + i + j	31	29	1	0.07	0.7963
	b + h + j (PG blend 3)	32	28	1	0.27	0.6056

Figure 3. Behavioral responses of *Galerucella placida* females toward RD (*Rumex dentatus*) blend 3 (2.44, 35.57 and 23.58 µg ml^{-1} of octadecane, heptacosane and nonacosane, respectively) against PG (*Polygonum glabrum*) blend 3 (4.08, 19.54 and 23.58 µg ml^{-1} of octadecane, palmitoleic acid and docosanoic acid, respectively) in the Y-tube olfactometer bioassay. Each bioassay was performed until 60 naïve females had responded. Numbers within parentheses are the number of insects that did not respond to either treatment.

of *P. glabrum* and individual octadecane or palmitoleic acid or docosanoic acid at similar amounts present in one leaf equivalent surface wax of *P. glabrum* (table 4). Females could not discriminate between one leaf equivalent surface wax of *P. glabrum* and a synthetic blend of six compounds (octadecane, heptacosane, lauric acid, palmitoleic acid, nonadecanoic acid and docosanoic acid) ($\chi^2 = 0.07$, *df* = 1, *P* = 0.7963) or PG blend 3 (octadecane, palmitoleic acid and docosanoic acid) ($\chi^2 = 0.27$, *df* = 1, *P* = 0.6056) (table 4). Females also could not distinguish between RD blend 3 and PG blend 3 ($\chi^2 = 0.27$, *df* = 1, *P* = 0.6056) (fig. 3).

In dose response bioassays, females started to show positive responses toward octadecane at 3 µg ml^{-1} ($\chi^2 = 4.27$, *df* = 1, *P* = 0.0389) and showed the highest positive responses at 24 µg ml^{-1} ($\chi^2 = 21.60$, *df* = 1, *P* < 0.0001) (table 5). Females started to display positive responses toward heptacosane at 30 µg ml^{-1} ($\chi^2 = 4.27$, *df* = 1, *P* = 0.0389) and showed the highest positive responses at 120 µg ml^{-1} ($\chi^2 = 21.60$, *df* = 1, *P* < 0.0001) (table 5). Females started to show positive responses toward nonacosane at 30 µg ml^{-1} ($\chi^2 = 8.07$, *df* = 1, *P* = 0.0045) and showed the highest positive responses at 60 µg ml^{-1} ($\chi^2 = 19.27$, *df* = 1,

Table 5. Responses of *Galerucella placida* females toward individual synthetic compound (T1) vs the control solvent (T2) (petroleum ether) in the Y-tube olfactometer bioassay ($N = 60$ in each concentration bioassay).

Synthetic compounds	Concentration ($\mu g\ ml^{-1}$)	Insects responded T1	T2	Non-responders	χ^2 ($df = 1$)	P values
Octadecane (*n*-C_{18})	1.5	36	24	3	2.40	0.1213
	3	38	22	2	4.27	0.0389
	6	40	20	2	6.67	0.0098
	12	44	16	1	13.07	0.0003
	24	48	12	0	21.60	<0.0001
Heptacosane (*n*-C_{27})	15	33	27	4	0.60	0.4386
	30	38	22	3	4.27	0.0389
	60	43	17	2	11.27	0.0008
	120	48	12	1	21.60	<0.0001
Nonacosane (*n*-C_{29})	15	35	25	4	1.67	0.1967
	30	41	19	2	8.07	0.0045
	60	47	13	1	19.27	<0.0001
Palmitoleic acid (C16:1)	5	31	29	4	0.07	0.7963
	10	36	24	3	2.40	0.1213
	20	39	21	2	5.40	0.0201
	40	49	11	0	24.07	<0.0001
Docosanoic acid (C22:0)	20	36	24	3	2.40	0.1213
	40	41	19	2	8.07	0.0045
	80	47	13	1	19.27	<0.0001

$P < 0.0001$). Females started to exhibit positive responses toward palmitoleic acid at 20 $\mu g\ ml^{-1}$ ($\chi^2 = 5.40$, $df = 1$, $P = 0.0201$) and showed the highest positive responses at 40 $\mu g\ ml^{-1}$ ($\chi^2 = 24.07$, $df = 1$, $P < 0.0001$) (table 5). Females started to display positive responses toward docosanoic acid at 40 $\mu g\ ml^{-1}$ ($\chi^2 = 8.07$, $df = 1$, $P = 0.0045$) and showed the highest positive responses at 80 $\mu g\ ml^{-1}$ ($\chi^2 = 19.27$, $df = 1$, $P < 0.0001$) (table 5).

Oviposition assays with G. placida *females toward leaf surface waxes*

Females laid significantly more eggs on the intact leaf of each weed than the dewaxed leaf of same weed (*R. dentatus*: $\chi^2 = 246.41$, $df = 1$, $P < 0.0001$; *P. glabrum*: $\chi^2 = 122.61$, $df = 1$, $P < 0.0001$) (table 6). Females could not distinguish between *R. dentatus* and *P. glabrum* leaves for egg laying ($\chi^2 = 1.05$, $df = 1$, $P = 0.3049$) (table 6). Females laid significantly more eggs on one leaf equivalent surface wax of *R. dentatus* ($\chi^2 = 143.15$, $df = 1$, $P < 0.0001$) or *P. glabrum* ($\chi^2 = 63.41$, $df = 1$, $P < 0.0001$) compared to the control solvent (table 6). Females could not distinguish between one leaf equivalent surface wax of *R. dentatus* and *P. glabrum* for egg laying ($\chi^2 = 3.71$, $df = 1$, $P = 0.0541$) (table 6).

Oviposition assays with G. placida *females toward synthetic compounds or blends*

Individual synthetic octadecane ($\chi^2 = 0.10$, $df = 1$, $P = 0.7532$) or heptacosane ($\chi^2 = 3.34$, $df = 1$, $P = 0.0677$) or nonacosane

($\chi^2 = 2.66$, $df = 1$, $P = 0.1028$) at similar amounts present in one leaf equivalent surface wax of *R. dentatus* could not significantly stimulate females to lay more eggs compared to the control solvent (Supplementary table 5). Similarly, individual synthetic octadecane ($\chi^2 = 3.41$, $df = 1$, $P = 0.0647$) or palmitoleic acid ($\chi^2 = 2.19$, $df = 1$, $P = 0.1390$) or docosanoic acid ($\chi^2 = 0.17$, $df = 1$, $P = 0.6799$) at similar amounts present in one leaf equivalent surface wax of *P. glabrum* could not significantly stimulate females to lay more eggs compared to the control solvent (Supplementary table 5).

Females laid significantly more eggs on RD blend 3 ($\chi^2 = 131.28$, $df = 1$, $P < 0.0001$) or PG blend 3 ($\chi^2 = 21.08$, $df = 1$, $P < 0.0001$) compared to the control solvent (table 6). Females could not distinguish between one leaf equivalent surface wax of *R. dentatus* and RD blend 3 for egg laying ($\chi^2 = 0.01$, $df = 1$, $P = 0.9156$) (table 6). Females could not discriminate between one leaf equivalent surface wax of *P. glabrum* and PG blend 3 for egg laying ($\chi^2 = 3.28$, $df = 1$, $P = 0.07$) (table 6). Females also could not discriminate for egg laying between RD blend 3 and PG blend 3 ($\chi^2 = 1.45$, $df = 1$, $P = 0.2286$) (table 6).

Discussion

The chemical characteristics of plant leaf surface waxes influence the acceptance or rejection behavior of an insect herbivore. The suitability of a host plant by a monophagous or oligophagous beetle is dependent on the chemical recognition of a plant as the beetle feeds and develops only on one or limited plants within one

Table 6. Oviposition assays of *Galerucella placida* females toward leaf surface waxes of *Rumex dentatus* or *Polygonum glabrum* plants and synthetic blends at similar amounts present in one leaf equivalent surface wax of *R. dentatus* or *P. glabrum* ($N = 10$ in each bioassay, number of eggs laid by insects were used for χ^2 test).

Comparison		No. of insects laid eggs		Non-responders	Eggs laid		χ^2	P values
		T1	T2		T1	T2	($df = 1$)	
A single leaf of *R. dentatus* (T1) vs. Dewaxed *R. dentatus* leaf (T2)		9	1	1	343	37	246.41	<0.0001[‡]
A single leaf of *P. glabrum* (T1) vs. Dewaxed *P. glabrum* leaf (T2)		8	2	2	296	81	122.61	<0.0001[‡]
A single leaf of *R. dentatus* (T1) vs. A single leaf of *P. glabrum* (T2)		5	5	1	200	180	1.05	0.3049[n.s.]
One leaf equivalent surface wax of *R. dentatus* (T1) vs. Control solvent (petroleum ether) (T2)		8	2	2	304	72	143.15	<0.0001[‡]
One leaf equivalent surface wax of *P. glabrum* (T1) vs. Control solvent (T2)		7	3	1	264	110	63.41	<0.0001[‡]
One leaf equivalent surface wax of *R. dentatus* (T1) vs. One leaf equivalent surface wax of *P. glabrum* (T2)		6	4	2	203	166	3.71	0.0541[n.s.]
A synthetic blend of three compounds resembling one leaf equivalent surface wax of *R. dentatus*[a] (T1) vs.	Control solvent (T2)	8	2	1	290	72	131.28	<0.0001[‡]
A synthetic blend of three compounds resembling one leaf equivalent surface wax of *P. glabrum*[b] (T1) vs.	Control solvent (T2)	6	4	1	223	136	21.08	<0.0001[‡]
One leaf equivalent surface wax of *R. dentatus* (T1) vs.	A synthetic blend of three compounds resembling one leaf equivalent surface wax of *R. dentatus*[a] (T2)	5	5	1	179	177	0.01	0.9156[n.s.]
One leaf equivalent surface wax of *P. glabrum* (T1) vs.	A synthetic blend of three compounds resembling one leaf equivalent surface wax of *P. glabrum*[b] (T2)	6	4	1	193	159	3.28	0.07[n.s.]
A synthetic blend of three compounds resembling one leaf equivalent surface wax of *R. dentatus*[a] (T1) vs.	A synthetic blend of three compounds resembling one leaf equivalent surface wax of *P. glabrum*[b] (T2)	6	4	1	194	171	1.45	0.2286[n.s.]

[a]Indicates RD blend 3: a synthetic blend of three compounds (2.44 µg octadecane + 35.57 µg heptacosane + 23.58 µg nonacosane) at similar amounts present in one leaf equivalent surface wax of *R. dentatus*.
[b]Indicates PG blend 3: a synthetic blend of three compounds (4.08 µg octadecane + 19.54 µg palmitoleic acid + 23.58 µg docosanoic acid) at similar amounts present in one leaf equivalent surface wax of *P. glabrum*.
[‡]Indicates P value is significant.
[n.s.]Indicates P value is not significant.

plant family. The current research demonstrated that *G. placida* employs leaf surface waxes of Polygonaceae weeds, *R. dentatus* and *P. glabrum* for short-range attraction and oviposition stimulation. Insects employ long-range volatile organic compounds as olfactory cues to find a host, and after coming close range of the host, insects also employ leaf surface wax compounds as short-range cues for oviposition (Mitra *et al.*, 2017; Das *et al.*, 2019*b*). Leaf surface waxes from the rice-field weed, *Ludwigia octovalvis* (Jacq.) Raven are used by the biocontrol agent, *Altica cyanea* Weber (Coleoptera: Chrysomelidae) as short-range attractant and oviposition stimulant (Mitra *et al.*, 2017). Therefore, this study is in agreement with the hypothesis that the leaf surface wax compounds could serve as short-range attractant and oviposition stimulant (Eigenbrode and Espelie, 1995; Mitra *et al.*, 2017; Das *et al.*, 2019*b*, Mitra *et al.*, 2020; Mobarak *et al.*, 2020).

The present study revealed that long-chain alkanes (*R. dentatus* – 60.72%, *P. glabrum* – 55.57%) and free fatty acids (*R. dentatus* – 8.78%, *P. glabrum* – 12.38%) are the major constituents of leaf surface waxes of both weed species. In the surface waxes of *P. orientale* (Polygonaceae) mature leaves, 19 *n*-alkanes from n-C_{15} to n-C_{33} and 15 free fatty acids from C12:0 to C22:0 were identified, and the predominant alkanes and free fatty acids were nonacosane and palmitic acid, respectively (Malik and Barik, 2015; Malik *et al.*, 2017). In the current investigation, hentriacontane (*n*-C_{31}) was the predominant alkane, while docosanoic acid (C22:0) and oleic acid (C18:1) were the most abundant free fatty acids in leaf surface waxes of *P. glabrum* and *R. dentatus*, respectively. A number of studies demonstrated that different long-chain alkanes and free fatty acids are predominant in the leaf surface waxes of diverse plant species (Tomasi *et al.*, 2018; Macel *et al.*, 2020; Weber and Schwark, 2020).

A body of research indicates that *n*-alkanes and free fatty acids present in leaf surface waxes play an important role in plant−insect interactions such as short-range attractant (Manosalva *et al.*, 2011; Mukherjee *et al.*, 2013, 2014; Sarkar *et al.*, 2013; Karmakar *et al.*, 2016; Mitra *et al.*, 2020; Mobarak *et al.*, 2020) and oviposition stimulant (Udayagiri and Mason, 1997; Parr *et al.*, 1998; Grant *et al.*, 2000; Li and Ishikawa, 2006; Das *et al.*, 2019*b*). In this study, olfactometer bioassay results revealed that females of *G. placida* showed attraction toward one leaf equivalent surface wax of *R. dentatus* and *P. glabrum* weeds against the control solvent (petroleum ether). But, the insect could not make a distinction between one leaf equivalent surface wax of *R. dentatus* and *P. glabrum*, indicating that one leaf equivalent surface wax of both weeds equally attracted the insect. A synthetic blend of three compounds (octadecane, heptacosane and nonacosane) comparable to the amounts present in one leaf equivalent surface wax of *R. dentatus* or another synthetic blend of three compounds (octadecane, palmitoleic acid and docosanoic acid) comparable to the amounts present in one leaf equivalent surface wax of *P. glabrum* acted as short-range attractant in olfactometer bioassay and stimulated oviposition in *G. placida*, indicative of that *G. placida* females could identify *R. dentatus* and *P. glabrum* host plants for oviposition by different surface wax profiles. Some of these compounds present in leaf surface waxes of plants might be ubiquitous in leaf surface wax of diverse plants, but the precise qualitative combination and amounts of compounds differ between plants by which an insect can discriminate between surface waxes of host plants and non-host plants (Mitra *et al.*, 2017). Attraction of insects could fade away when the amounts of key compounds were changed (Bruce *et al.*, 2005; Najar-Rodriguez

et al., 2010; Bruce and Pickett, 2011). Similarly, olfactory receptor neurons of an insect register fluxes and modulate behavioral responses by tuning the detection of ecologically relevant odors (Riffell *et al.*, 2009*a*, *b*; Galizia and Rössler, 2010). This observation suggests that the amount of compounds present in one leaf equivalent surface wax of *R. dentatus* and *P. glabrum* becomes vital components, which act as olfactory cues for oviposition in *G. placida* (Bruce *et al.*, 2005; Bruce and Pickett, 2011).

The term 'biotype' refers an infraspecific group of organisms that are not morphologically discernible, but differing by a biological function (Eastop, 1973). In the current investigation, we observed that larvae and adults of *G. placida* feed on *R. dentatus*, *P. glabrum* and *P. orientale* belonging to Polygonaceae in adjoining areas of the University of Burdwan and different districts of West Bengal, India. However, we did not observe the insect to feed on other crop plants such as rice, wheat, potato, mustard and maize as *R. dentatus* and *P. glabrum* grow in association with these crops in crop fields, indicating that *G. placida* has a particular biotype in this region. In a previous study, Malik and Barik (2015) demonstrated that a synthetic blend of 3.59, 7.89, 44.82, 9.91, 32.31, 18.33 and 15.88 µg of lauric, myristic, pentadecanoic, palmitoleic, heptadecanoic, nonadecanoic and docosanoic acids comparable to the amounts present in two leaf (mature) equivalent surface waxes of *P. orientale* showed attraction of *G. placida*; whereas in another study, a synthetic blend of 1.60, 0.86, 11.90, 17.79, 1.82, 1.54, 12.06, 6.60, 6.55 and 25.45 µg of n-C_{16}, n-C_{17}, n-C_{18}, n-C_{20}, n-C_{21}, n-C_{23}, n-C_{24}, n-C_{26}, n-C_{28} and n-C_{31}, respectively, comparable to the amounts present in two leaf (mature) equivalent surface waxes of *P. orientale* exhibited attraction of the *G. placida* (Malik *et al.*, 2017). The effectiveness of alkanes or free fatty acids present in two mature leaf equivalent surface waxes of *P. orientale* was separately tested as short-range attractant in *G. placida*, but these two studies did not consider to observe the effectiveness of alkanes and free fatty acids together comparable to the amounts present in one leaf equivalent surface wax of *P. orientale* toward *G. placida*. This is the explanation for a higher number of alkanes and fatty acids as well as their increased amounts are required to cause the short-range attraction of *G. placida* toward leaf surface waxes of *P. orientale*. Further, the effectiveness of alkanes and free fatty acids present in leaf surface waxes of *P. orientale* as oviposition stimulant in *G. placida* was not considered. However, the current research provides a complete knowledge on the effectiveness of alkanes and free fatty acids present in one leaf equivalent surface wax of both weeds, *R. dentatus* and *P. glabrum*, which could serve as short-range attractant and oviposition stimulant in *G. placida*.

This study summarizes that females of *G. placida* were attracted toward a synthetic blend of either 2.44, 35.57 and 23.58 µg ml⁻¹ of octadecane, heptacosane and nonacosane, respectively, resembling the amounts present in one leaf equivalent surface wax of *R. dentatus* or 4.08, 19.54 and 23.58 µg ml⁻¹ of octadecane, palmitoleic acid and docosanoic acid, respectively, resembling the amounts present in one leaf equivalent surface wax of *P. glabrum*, which could serve as close-range olfactory cues both for host recognition and acceptance process as well as for oviposition in *G. placida* females. This information could be employed to effectively screen surface wax profile of other non-target plants for their susceptibility toward *G. placida*. Further investigations on behavioral responses of *G. placida* females toward leaf surface waxes of other plants in the ecological perspective should be a topic for future research.

References

Abbas G, Ali MA, Abbas Z, Aslam M and Akram M (2009) Impact of different herbicides on broadleaf weeds and yield of wheat. *Pakistan Journal of Weed Science Research* **15**, 1–10.

Abbas T, Tanveer A, Khaliq A and Safdar ME (2016) Comparative allelopathic potential of native and invasive weeds in rice ecosystem. *Pakistan Journal of Weed Science Research* **22**, 269–283.

Al-Sherif EA, Ismael MA, Karam MA and Elfayoumi HH (2018) Weed flora of Fayoum (Egypt), one of the oldest agricultural regions in the world. *Planta Daninha* **36**, e0181719990.

Anjum T and Bajwa R (2010) Competition losses caused by *Rumex dentatus* L. and *Chenopodium album* L. in wheat (*Triticum aestivum* L.). *Philippine Agricultural Scientist* **93**, 365–368.

Beenen R (1998) *Galerucella placida* a proper species from South East Asia (Coleoptera: Chrysomelidae: Galerucinae). *Serangga* **31**, 107–110.

Bruce TJA and Pickett JA (2011) Perception of plant volatile blends by herbivorous insects – finding the right mix. *Phytochemistry* **72**, 1605–1611.

Bruce TJA, Wadhams LJ and Woodcock CM (2005) Insect host location: a volatile situation. *Trends in Plant Science* **10**, 269–274.

Culliney TW (2005) Benefits of classical biological control for managing invasive plants. *Critical Reviews in Plant Sciences* **24**, 131–150.

Das S, Koner A and Barik A (2019a) Biology and life history of *Lema praeusta* (Fab.) (Coleoptera: Chrysomelidae), a biocontrol agent of two Commelinaceae weeds, *Commelina benghalensis* and *Murdannia nudiflora*. *Bulletin of Entomological Research* **109**, 463–471.

Das S, Koner A and Barik A (2019b) A beetle biocontrol agent of rice-field weeds recognizes its host plants by surface wax long-chain alkanes and free fatty acids. *Chemoecology* **29**, 155–170.

Deka J and Barua IC (2015) Problem weeds and their management in the North-East Himalayas. *Indian Journal of Weed Science* **47**, 296–305.

Ding J, Fu W, Reardon R, Wu Y and Zhang G (2004) Exploratory survey in China for potential insect biocontrol agents of mile-a-minute weed, *Polygonum perfoliatum* L., in Eastern USA. *Biological Control* **30**, 487–495.

Eastop VF (1973) Biotypes of aphids. *Bulletin of the Entomological Society of New Zealand* **2**, 40–51.

Eigenbrode SD and Espelie KE (1995) Effects of plant epicuticular lipids on insect herbivores. *Annual Review of Entomology* **40**, 171–194.

Elzaawely AA and Tawata S (2012) Antioxidant capacity and phenolic content of *Rumex dentatus* L. grown in Egypt. *Journal of Crop Science & Biotechnology* **15**, 59–64.

Galizia CG and Rössler W (2010) Parallel olfactory systems in insects: anatomy and function. *Annual Review of Entomology* **55**, 399–420.

Grant GG, Zhao B and Langevin D (2000) Oviposition response of spruce budworm (Lepidoptera: Tortricidae) to aliphatic carboxylic acids. *Environmental Entomology* **29**, 164–170.

Gupta A, Verma A, Chhokar RS, Sheoran S, Sendhil R and Sharma I (2013) Annual Report 2012–2013. Karnal, Haryana, India. Directorate of Wheat Research (Indian Council of Agricultural Research).

Hadjichambis ACH, Paraskeva-Hadjichambi D, Della A, Giusti ME, De Pasquale C, Lenzarini C, Censorii E, Gonzales-Tejero MR, Sanchez-Rojas CP, Ramiro-Gutierrez JM, Skoula M, Johnson C, Sarpaki A, Hmamouchi M, Jorhi S, El-Demerdash M, El-Zayat M and Pieroni A (2008) Wild and semi-domesticated food plant consumption in seven circum-Mediterranean areas. *International Journal of Food Sciences and Nutrition* **59**, 383–414.

Hnatiuk RJ (1990) *Australian Flora and Fauna Series Number 11: Census of Australian Vascular Plants*. Canberra: Australian Government Publishing Service.

Jetter R and Schäffer S (2001) Chemical composition of the *Prunus laurocerasus* leaf surface. Dynamic changes of the epicuticular wax film during leaf development. *Plant Physiology* **126**, 1725–1737.

Jetter R, Schäffer S and Riederer M (2000) Leaf cuticular waxes are arranged in chemically and mechanically distinct layers: evidence from *Prunus laurocerasus* L. *Plant, Cell & Environment* **23**, 619–628.

Kanatas P, Travlos IS, Gazoulis I, Tataridas A, Tsekoura A and Antonopoulos N (2020) Benefits and limitations of decision support systems (DSS) with a special emphasis on weeds. *Agronomy* **10**, 548.

Karmakar A, Malik U and Barik A (2016) Effects of leaf epicuticular wax compounds from *Solena amplexicaulis* (Lam.) Gandhi on olfactory responses of a generalist insect herbivore. *Allelopathy Journal* **37**, 253–272.

Karmakar A, Mitra P, Koner A, Das S and Barik A (2020) Fruit volatiles of creeping cucumber (*Solena amplexicaulis*) attract a generalist insect herbivore. *Journal of Chemical Ecology* **46**, 275–287.

Kimoto S (1989) Chrysomelidae (Coleoptera) of Thailand, Cambodia, Laos and Vietnam. IV. Galerucinae. *Esakia* **27**, 1–241.

Koner A, Debnath R and Barik A (2019) Age-stage, two-sex life table and food utilization efficiencies of *Galerucella placida* Baly (Coleoptera: Chrysomelidae) on two Polygonaceae weeds. *Journal of Asia-Pacific Entomology* **22**, 1136–1144.

Li G and Ishikawa Y (2006) Leaf epicuticular wax chemicals of the Japanese knotweed *Fallopia japonica* as oviposition stimulants for *Ostrinia latipennis*. *Journal of Chemical Ecology* **32**, 595–604.

Macel M, Visschers IGS, Peters JL, van Dam NM and de Graaf RM (2020) High concentrations of very long chain leaf wax alkanes of thrips susceptible pepper accessions (*Capsicum* spp). *Journal of Chemical Ecology* **46**, 1082–1089.

Malik U and Barik A (2015) Free fatty acids from the weed, *Polygonum orientale* leaves for attraction of the potential biocontrol agent, *Galerucella placida* (Coleoptera: Chrysomelidae). *Biocontrol Science & Technology* **25**, 593–607.

Malik U, Mitra S and Barik A (2017) Attraction of the biocontrol agent, *Galerucella placida* Baly (Coleoptera: Chrysomelidae) to the leaf surface alkanes of the weed, *Polygonum orientale* L. *Allelopathy Journal* **40**, 103–116.

Malik U, Das S and Barik A (2018) Biology of *Galerucella placida* Baly (Coleoptera: Chrysomelidae) on the rice-field weed *Polygonum orientale* L. (Polygonaceae). *Proceedings of the Zoological Society* **71**, 257–264.

Manosalva L, Pardo F, Perich F, Mutis A, Parra L, Ortega F, Isaacs R and Quiroz A (2011) Behavioral responses of clover root borer to long-chain fatty acids from young red clover (*Trifolium pratense*) roots. *Environmental Entomology* **40**, 399–404.

Mitra S, Sarkar N and Barik A (2017) Long-chain alkanes and fatty acids from *Ludwigia octovalvis* weed leaf surface waxes as short-range attractant and ovipositional stimulant to *Altica cyanea* (Weber) (Coleoptera: Chrysomelidae). *Bulletin of Entomological Research* **107**, 391–400.

Mitra P, Das S and Barik A (2020) Leaf waxes from *Lathyrus sativus*: short-range attractant and stimulant for nymph laying in a viviparous insect. *Chemoecology* **30**, 117–129.

Mitra S, Mobarak SH and Barik A (2021) Age-stage, two-sex life table of the biocontrol agent, *Altica cyanea* on three *Ludwigia* species. *Biologia* **76**, 101–112.

Mobarak SH, Koner A, Mitra S, Mitra P and Barik A (2020) The importance of leaf surface wax as short-range attractant and oviposition stimulant in a generalist Lepidoptera. *Journal of Applied Entomology* **144**, 616–631.

Mukherjee A, Sarkar N and Barik A (2013) Alkanes in flower surface waxes of *Momordica cochinchinensis* influence attraction to *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae). *Neotropical Entomology* **42**, 366–371.

Mukherjee A, Sarkar N and Barik A (2014) Long-chain free fatty acids from *Momordica cochinchinensis* leaves as attractants to its insect pest, *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae). *Journal of Asia-Pacific Entomology* **17**, 229–234.

Mukherjee A, Sarkar N and Barik A (2015) *Momordica cochinchinensis* (Cucurbitaceae) leaf volatiles: semiochemicals for host location by the insect

pest, *Aulacophora foveicollis* (Coleoptera: Chrysomelidae). *Chemoecology* **25**, 93–104.

Muller J (1981) Fossil pollen records of extant angiosperms. *The Botanical Review* **47**, 1–142.

Müller C (2006) Plant–insect interactions on cuticular surfaces. In Riederer M and Müller C (eds), *Biology of the Plant Cuticle*. Oxford: Blackwell Publishing, pp. 398–422.

Müller C and Hilker M (2001) Host finding and oviposition behavior in a chrysomelid specialist–the importance of host plant surface waxes. *Journal of Chemical Ecology* **27**, 985–994.

Najar-Rodriguez AJ, Galizia CG, Stierle J and Dorn S (2010) Behavioural and neurophysiological responses of an insect to changing ratios of constituents in host plant-derived volatile mixtures. *The Journal of Experimental Biology* **213**, 3388–3397.

Naseer-ud-Din GM, Shehzad MA and Nasrullah HM (2011) Efficacy of various pre and post-emergence herbicides to control weeds in wheat. *Pakistan Journal of Agricultural Sciences* **48**, 185–190.

Padovan A, Keszei A, Köllner TG, Degenhardt J and Foley WJ (2010) The molecular basis of host plant selection in *Melaleuca quinquenervia* by a successful biological control agent. *Phytochemistry* **71**, 1237–1244.

Parr MJ, Tran BMD, Simmonds MSJ, Kite GC and Credland PF (1998) Influence of some fatty acids on oviposition by the bruchid beetle, *Callosobruchus maculatus*. *Journal of Chemical Ecology* **24**, 1577–1593.

Reid CAM (2001) *Galerucella placida* Baly in Australia (Coleoptera: Chrysomelidae: Galerucinae). *Australian Journal of Entomology* **40**, 331–334.

Riffell JA, Lei H, Christensen TA and Hildebrand JG (2009a) Characterization and coding of behaviorally significant odor mixtures. *Current Biology* **19**, 335–340.

Riffell JA, Lei H and Hildebrand JG (2009b) Neural correlates of behavior in the moth *Manduca sexta* in response to complex odors. *Proceedings of the National Academy of Sciences of the USA* **106**, 19219–19226.

Sambamurty AVSS (2005) *Taxonomy of Angiosperms*. New Delhi, India: I.K. International Pvt. Ltd.

Sarkar N and Barik A (2015) Free fatty acids from *Momordica charantia* L. flower surface waxes influencing attraction of *Epilachna dodecastigma* (Wied.)

(Coleoptera: Coccinellidae). *International Journal of Pest Management* **61**, 47–53.

Sarkar N, Mukherjee A and Barik A (2013) Long-chain alkanes: allelochemicals for host location by the insect pest, *Epilachna dodecastigma* (Coleoptera: Coccinellidae). *Applied Entomology & Zoology* **48**, 171–179.

Sarkar N, Malik U and Barik A (2014) *n*-alkanes in epicuticular waxes of *Vigna unguiculata* (L.) Walp. leaves. *Acta Botanica Gallica* **161**, 373–377.

Sarkar N, Mukherjee A and Barik A (2015) Attraction of *Epilachna dodecastigma* (Coleoptera: Coccinellidae) to *Momordica charantia* (Cucurbitaceae) leaf volatiles. *The Canadian Entomologist* **147**, 169–180.

Singh V, Gupta S, Singh H and Raghubanshi AS (2015) Ecophysiological characteristics of five weeds and a wheat crop in the Indo-Gangetic Plains, India. *Weed Biology & Management* **15**, 102–112.

Smith L and Beck JJ (2013) Effect of mechanical damage on emission of volatile organic compounds from plant leaves and implications for evaluation of host plant specificity of prospective biological control agents of weeds. *Biocontrol Science & Technology* **23**, 880–907.

Tomasi P, Dyer JM, Jenks MA and Abdel-Haleem H (2018) Characterization of leaf cuticular wax classes and constituents in a spring *Camelina sativa* diversity panel. *Industrial Crops & Products* **112**, 247–251.

Udayagiri S and Mason CE (1997) Epicuticular wax chemicals in Zea mays influence oviposition in *Ostrinia nubilalis*. *Journal of Chemical Ecology* **23**, 1675–1687.

Waheed Z, Usman K and Ali I (2017) Response of wheat to varying densities of *Rumex dentatus* under irrigated condition of Dera Ismail Khan, Pakistan. *Sarhad Journal of Agriculture* **33**, 1–7.

Weber J and Schwark L (2020) Epicuticular wax lipid composition of endemic European *Betula* species in a simulated ontogenetic/diagenetic continuum and its application to chemotaxonomy and paleobotany. *Science of the Total Environment* **730**, 138324.

Wheeler GS and Schaffner U (2013) Improved understanding of weed biological control safety and impact with chemical ecology: a review. *Invasive Plant Science & Management* **6**, 16–29.

Zar JH (1999) *Biostatistical Analysis*. New Jersey: Prentice-Hall.