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Epicuticular wax chemicals of *Lablab purpureus* subsp. *bengalensis* influence short-range attraction and oviposition responses in *Aphis craccivora* and *Aphis gossypii*

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Research Paper

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Abstract

Lablab purpureus subsp. *bengalensis* (Jacq.) Verdc. is an important legume of India and Africa. Both aphids, *Aphis craccivora* Koch and *A. gossypii* Glover (Hemiptera: Aphididae), are important herbivorous pests of this legume crop. These viviparous females lay nymphs on the leaf surface of this legume plant. Therefore, it is of considerable interest to study whether leaf surface wax chemicals (long-chain alkanes and free fatty acids) of this legume plant served as short-range attractants and oviposition stimulants in both females to lay nymphs. Twenty-one *n*-alkanes from *n*-C_{12} to *n*-C_{35} and 11 free fatty acids from C12:0 to C22:0 were identified in leaf surface waxes. Nonacosane and nonadecanoic acid were the most abundant among *n*-alkanes and free fatty acids, respectively. Both females were attracted towards one leaf equivalent surface wax against the control solvent (petroleum ether) in short Y-tube olfactometer bioassays. A synthetic blend of tetradecane, pentadecane, tetracosane, tridecanoic acid, tetradecanoic acid, and heneicosanoic acid comparable to one leaf equivalent surface wax served as short-range attractants and oviposition stimulants in *A. craccivora*; whereas a synthetic blend of tetradecane, hexadecane, docosane, nonadecanoic acid, and arachidic acid comparable to one leaf equivalent surface wax acted as short-range attractants and oviposition stimulants in *A. gossypii*. These results can provide the basis for efficient pest management strategies of *A. craccivora* and *A. gossypii* against *L. purpureus* subsp. *bengalensis* using host plant leaf surface wax compounds. Further, SEM studies of antennae and forelegs of both aphids were conducted to observe sensilla structures, which help in chemoreception.

Introduction

Lablab purpureus (L.) Sweet (Fabaceae), commonly known as lablab bean, field bean, hyacinth bean, and dolichos bean, is an ancient legume grown in Indian subcontinents, southeast Asia, and Africa (Maass *et al.*, 2005, 2010; Ram Bahadur *et al.*, 2016; Amkul *et al.*, 2021; Letting *et al.*, 2021). The species is extremely diverse, which includes one wild subspecies *uncinatus* Verdc. and two cultivated subspecies, subsp. *purpureus* and subsp. *bengalensis* (Jacq.) Verdc. (Verdcourt, 1970). Lablab is mainly grown as a vegetable crop, and pods and mature seeds are consumed for high sources of proteins and carbohydrates, while young leaves are consumed as vegetable for minerals and vitamins (Amkul *et al.*, 2021). Dry mature seeds possess high amounts of carbohydrates (60%) and proteins (25%) (Hossain *et al.*, 2016), and essential amino acids (leucine and lysine) (Deka and Sarkar, 1990; Kala *et al.*, 2010). Low lipid content (1.2%) is recorded from dry lablab seeds, but it is found to possess essential fatty acids such as linoleic acid (omega-6) and alpha-linoleic acid (omega-3) (Kala *et al.*, 2010). Further, lablab leaves serve as moderate sources of protein (15–40%) depending on the stages of maturity (Murphy and Colucci, 1999). Lablab is considered a drought-tolerant crop as the plant develops tap root up to 2 m and tuber-like roots, which can grow again when the proper environment is available (Amkul *et al.*, 2021).

Aphis craccivora Koch and *A. gossypii* Glover (Hemiptera: Aphididae) are polyphagous pests globally. *Aphis craccivora*, commonly known as cowpea aphid, prefers plants of Leguminosae but is found on 50 host plants of 19 families (Brady and White, 2013); whereas *A. gossypii*, commonly known as cotton-melon aphid, feeds on 320 plant species belonging to 46 families (Blackman and Eastop, 1985). Both aphids cause direct damage by extracting cell sap from plant leaves and also serve as a vector of plant viruses. These two aphids are considered major pests of lablab (Singh *et al.*, 2014, 2016; Mondal *et al.*, 2017; Ahmed *et al.*, 2019; Choudhary *et al.*, 2020). These aphids prefer to stay underside of the leaves, and upper leaf surfaces and flower parts are their secondary choice. During severe infestation, both aphids cover the entire host plant. Heavy infestation by these aphids causes wrinkling of leaves,

which results in the flower reduction, abnormal fruit growth and shape, and yield losses of up to 26–39% (Ahmed *et al*., 2019).

After arriving close range of the host plant, the first physical contact occurs when an insect touches the leaf surface (Schoonhoven *et al*., 2005; Fernández *et al*., 2019). Therefore, a leaf surface comprised of wax chemicals plays an important role in the host acceptance process in herbivorous insects. So, an evaluation of leaf surface wax chemicals serving as oviposition stimulants in insect pests of economic importance is important due to their possible use in manipulation of the insect behaviour in crop field. Hence, the identification of leaf surface wax chemicals serving as short-range attractants and oviposition stimulants to an insect pest of economic importance may aid in developing traps baited with leaf surface wax chemicals as a part of integrated pest management (IPM) strategy, which will be eco-friendly because synthetic insecticides are hazardous to the environment.

Extracts of leaf surface wax mainly comprise of long-chain alkanes, free fatty acids, esters, aldehydes, and primary and secondary alcohols, which differ between plant species (Mukherjee and Barik, 2016; Mamrutha *et al*., 2017; Tomasi *et al*., 2018; Mitra *et al*., 2020a; Mobarak *et al*., 2020). Surface wax contains long-chain alkanes and free fatty acids which can serve as short-range attractants and oviposition stimulants in herbivorous insects (Eigenbrode and Espelie, 1995; Müller and Hilker, 2001; Schoonhoven *et al*., 2005; Müller, 2006; Manosalva *et al*., 2011; Mitra *et al*., 2017, 2020a; Das *et al*., 2019). Five long-chain alkanes such as hexacosane, heptacosane, octacosane, nonacosane, and tritriacontane are present in the epicuticular wax of corn (*Zea mays* L.) leaves and stimulate oviposition in *Ostrinia nubilalis* (Hübner) (Udayagiri and Mason, 1997). Long-chain *n*-alkanes and free fatty acids present in the surface waxes of the Japanese knotweed *Fallopia japonica* (Houtt.) Ronse Decr. served as oviposition stimulants in *O. nubilalis* (Li and Ishikawa, 2006). Leaf surface waxes of sugarcane and maize consist of alkanes and other chemicals that stimulate oviposition in *O. furnacalis* (Jiang *et al*., 2015). Mobarak *et al*. (2020) elucidated that long-chain alkanes and free fatty acids present in the leaf surface waxes of green gram [*Vigna radiata* (L.) R. Wilczek] act as short-range attractants and stimulated oviposition in *Spilosoma obliqua* Walker. To date, no study has demonstrated the role of leaf surface waxes of lablab in *A. craccivora* and *A. gossypii*.

The purpose of the study was to (i) observe whether leaf surface waxes of lablab act as short-range attractants and oviposition stimulants in viviparous apterous *A. craccivora* and *A. gossypii*, (ii) observe the behavioural responses of *A. craccivora* and *A. gossypii* through Y-tube olfactometer bioassays towards the individual synthetic *n*-alkanes and fatty acids followed by blends comparable to the amounts present in one leaf equivalent surface wax of lablab, (iii) investigate whether leaf surface waxes and most attractive synthetic blends (*n*-alkanes and free fatty acids) comparable to the amounts present in one leaf equivalent surface wax of lablab stimulate oviposition in *A. craccivora* and *A. gossypii*, and (iv) study the morphology of chemosensory structures present on the antennae and foreleg of apterous *A. craccivora* and *A. gossypii*.

Materials and methods

Insects

Adults of *A. craccivora* and *A. gossypii* were collected from cowpea plant [*Vigna unguiculata* (L.) Walp] and eggplant (*Solanum melongena* L.), respectively, and maintained on the same leaves from which they were collected. They were reared at 22 ± 1°C, 65 ± 10% relative humidity (RH) and 12L:12D photoperiod in a biological oxygen demand (BOD) incubator. A moist piece of cotton was attached to the cut end of the petiole of each leaf and this was wrapped with aluminium foil to prevent water loss from the leaf, and fresh leaves were provided at 24 h intervals by replacing the previous ones. The second generation *A. craccivora* and *A. gossypii* were also fed on leaves of cowpea and eggplant, respectively. Adult F2 viviparous females (2–3 days old) of both aphids were used for olfactory bioassays and viviparity assays.

Plant materials

Seeds of lablab (local race) were germinated on moistened filter papers. Each seed with cotyledon was planted in the field of Crop Research Farm, University of Burdwan (23°16′N, 87°54′E) and grown in natural conditions in the winter season (November 2021–March 2022) under a photoperiod of 11L:13D at 18–25°C. Mature leaves (6–7-weeks old plants) were collected for extraction of surface waxes, and leaves were washed with deionised water followed by paper towelling.

Extraction of leaf surface waxes

Seventy-five grams leaves of lablab were separately collected five times (5 × 75 g) at morning 8 a.m., and leaf surface waxes from an intact leaf were isolated by the gum arabic method (Jetter and Schäffer, 2001). The contaminants of gum arabic (Roth, Karlsruhe, Germany) were separated by soxhlet extraction with hot chloroform, and this gum arabic was used. An aqueous solution of ca. 0.1 ml [50% (w/w)] gum arabic was put on the adaxial and abaxial surface of each leaf (per cm^2 of leaf surface) by a small paintbrush. After drying for 1 h, a thin whitish adhesive layer was isolated from each leaf by forceps, keeping the leaves as undamaged and intact (without damaging in the epidermal and mesophyll tissue). The gum arabic fractions of adaxial and abaxial leaf surfaces were collected for each leaf and extracted with water/chloroform. After vigorous agitation and phase separation, the organic solution was removed and the solvent was evaporated under reduced pressure.

Each dried crude extract obtained from 75 g leaves was then dissolved in 30 ml chloroform and divided into three equal crude fractions [each 10 ml crude fraction was equivalent to 25 g of leaves; 25 g leaves contain 27 ± 1 (mean ± standard error) leaves]. One mg nonadecane (n-C_{19}) was added as an internal standard to the second fraction of each crude extract for the identification and quantification of alkanes, while tricosanoic acid (C23:0, 1 mg) was added to the third fraction of each crude extract for identification and quantification of free fatty acids. Each fraction of crude extract was filtered through Whatman No. 41 filter paper and evaporated to dryness at room temperature. The first, second, and third fractions were used for (i) olfactometer and viviparity bioassays, (ii) identification and quantification of alkanes, and (iii) identification and quantification of free fatty acids, respectively.

Olfactometer bioassays

A Y-tube was used to perform the bioassays of gravid apterous viviparous *A. craccivora* and *A. gossypii* females, similar to that used by Mitra *et al*. (2020a) (Supplementary fig. 1). The glass Y-tube olfactometer (1 cm inner diameter) is comprised of a

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5-cm base tube and two 5-cm branching arms that had 45° inside angle leading from the base tube. The arms at the top of glass Y were attached with two glass-made adapters that were attached to 3 cm glass vials (1 cm diameter), each containing a piece (2×2 cm^2) of Whatman No. 41 filter paper. Charcoal-filtered air (70 ml min^{-1}) was pushed through an inlet tube of each adapter, so the purified air could enter into the glass vial and another one as an outlet tube, which connected the glass vial to an arm of the olfactometer. All components of the set-up were joined by Teflon tubing.

All bioassays were performed in the laboratory between 09:00 and 16:00 h at 21 ± 1°C, 65 ± 10% RH and 150 lx light intensity. Adult females (2–3 days old) of *A. craccivora* and *A. gossypii* were starved for at least 3 h prior to bioassays. Glass vial with clean filter paper and filter paper moistened with 1 ml petroleum ether in another glass vial served as control. In preliminary bioassays, the behavioural response of females to the control solvent (petroleum ether) was neutral. One millilitre of the test sample and the control solvent were applied to separate filter paper pieces and allowed to evaporate the solvent, and these filter papers were introduced into the glass vials before the first insect was released into the olfactometer, for each experiment tested as a sample against control. Individual females were released into a porous glass vial (1 cm diameter × 3 cm long) through an aspirator, which was then attached to the base tube of the glass Y and exposed to a particular odour, 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 comprised of alkanes and fatty acids compounds) in another glass vial. Each insect was given 3 min to respond to the treatment, and the choice for the left or right arm of the olfactometer was noted when the insect reached the end of one of the Y-tube arms, and the choice of the insect was recorded as a positive (showed attraction to test samples) or negative (did not show attraction to test samples) response, respectively, and subsequently, the female was discarded. On the rare occasion, a female failed to make a choice within 3 min or remained in the base tube of the glass Y, it was removed from the Y-tube and recorded as no response (Mitra *et al.*, 2020b; Debnath *et al.*, 2021). For each treatment, 60 females were tested excluding the number of females who did not respond. Each female was tested once. After every five insects, all parts of the olfactometer set-up were cleaned with petroleum ether followed by acetone, left to dry, and subsequently, the odour sources were switched between left and right arms to avoid positional biases.

Dual choice bioassays with viviparous A. craccivora *and* A. gossypii *females towards crude surface waxes*

Bioassay 1: Behavioural responses of *A. craccivora* or *A. gossypii* females towards one leaf equivalent surface wax (crude extract: 766 µg) of *L. purpureus* subsp. *bengalensis* were tested against the control solvent (petroleum ether) to observe whether leaf surface waxes attracted *A. craccivora* or *A. gossypii*.

Viviparity assays

Glass-made I-tube (length of I-tube: 10 cm and internal diameter: 1 cm, in the middle of I-tube a hole with 0.3 cm diameter where an aphid was released) having attached with glass vials (1 cm diameter × 5 cm long) were used for viviparity bioassays (Supplementary fig. 2; Mitra *et al.*, 2020a). A test sample (1 ml) and the control solvent (1 ml) were applied to separate filter paper pieces and allowed to evaporate the solvent, and these filter papers were separately placed in two glass vials (1 cm diameter × 5 cm long). We observed that females did not lay nymph on the filter paper or filter paper moistened with the control solvent (petroleum ether). Ten viviparous females of *A. craccivora* or *A. gossypii* were separately tested for each experiment apart from the insects that did not react. Each female was observed continuously for 6 h after releasing in an I-tube, and when a female laid nymph for the first time, nymphs were counted and this female was discarded. If a female did not lay nymphs within 6 h, it was also discarded.

Viviparity assays of A. craccivora *and* A. gossypii *females with leaf surface waxes*

Viviparity assay 1: A single leaf of *L. purpureus* subsp. *bengalensis* was tested against the dewaxed leaf to observe whether leaf surface waxes stimulated *A. craccivora* and *A. gossypii* females to lay nymphs.

Viviparity assay 2: One leaf equivalent surface wax (crude wax) from *L. purpureus* subsp. *bengalensis* vs the control solvent (petroleum ether) was tested to observe whether crude leaf surface waxes stimulated females of *A. craccivora* and *A. gossypii* to lay nymphs.

Identification and quantification of alkanes

Alkanes were identified and quantified according to the protocol of Mitra *et al.* (2020a). The second 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 that appeared in each TLC plate was eluted from the silica gel layer with chloroform. A total of five purified alkane samples were prepared for gas chromatography-mass spectrometry (GC-MS) and GC-flame ionisation detection (FID) for identification and quantification, respectively. Half a 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 samples were analysed with Clarus 690 GC coupled to an 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 MS parameters were 280°C at the interface, ionisation energy 70 eV, scan speed 5 scans s^{-1} and scanned over the mass range 40–600 mass units. The identity of the compounds was confirmed by injections of a mixture of synthetic *n*-alkanes (*n*-C_{12} 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 samples were analysed by a Techcomp GC (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 ionisation detector which was run under same temperature conditions as mentioned

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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_{12} to *n*-C_{35}, and the areas of each peak were converted into quantities of *n*-alkanes based on internal standard nonadecane (*n*-C_{19}) and internal response factor (IRF). All *n*-alkanes (≥99% purity) between *n*-C_{12} and *n*-C_{35} were purchased from Sigma-Aldrich.

Identification and quantification of free fatty acids

Free fatty acids were identified and quantified according to the protocol of Mitra *et al.* (2020a). The third fraction of each crude extract of lablab leaves (equivalent to ca. 25 g of leaves) were 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). The band was eluted from the silica gel layer with diethyl ether after which it 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_2SO_4 twice. One portion of each esterified sample (hexane fraction) was used for GC-MS and another for GC-FID. The extraction of free fatty acids from each crude extract was separately repeated five times followed by esterification, and a total of five samples were prepared.

One portion of the esterified fatty acids was analysed 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 (Malik and Barik, 2016). Helium was the carrier gas. The MS temperature parameter was 280°C at the interface, ionisation energy 70 eV, scan speed 5 scans s^{-1}, and scanned over the mass range 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 palmitoleate (C16:1), methyl heptadecanoate (C17:0), methyl stearate (C18:0), 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, ≥99% purity) were purchased from Sigma-Aldrich, Germany.

The remaining portion of the esterified fatty acids (five separate samples) were analysed 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 ionisation detector, which was run under 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} (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 each peak were converted into quantities of fatty acids based on reference standard methyl tricosanoate (1 mg) and IRF. All solvents used were of analytical grade and purchased from E. Merck (Mumbai, India).

Dual choice bioassays with viviparous A. craccivora and A. gossypii females towards individual synthetic compounds and synthetic blends

Bioassay 2: Behavioural responses of females towards individual synthetic compounds (21 *n*-alkanes and 11 free fatty acids), comparable to the amounts of individual compounds present in one leaf equivalent surface wax of lablab dissolved in 1 ml petroleum ether and tested against 1 ml control solvent to observe the role of individual compounds in *A. craccivora* and *A. gossypii* females (Supplementary table 1A, B).

The insect showed behavioural 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 lablab and were tested against the control solvent (Supplementary table 1A, B). Further, the insect showed attraction to those individual synthetic compounds (comparable to the amounts present in one leaf equivalent surface wax) that were combined resembling one leaf equivalent surface wax of lablab [for *A. craccivora*: 16.95 µg tetradecane + 4.66 µg pentadecane + 19.38 µg tetracosane + 5.98 µg tridecanoic acid + 6.13 µg tetradecanoic acid + 9.40 µg heneicosanoic acid were dissolved in 1 ml petroleum ether, hereafter this blend will be denoted as AC blend 6; for *A. gossypii*: 16.95 µg tetradecane + 39.87 µg hexadecane + 12.93 µg docosane + 18.82 µg nonadecanoic acid + 5.06 µg arachidic acid were dissolved in 1 ml petroleum ether, hereafter this blend will be denoted as AG blend 5] were assayed against the solvent control.

Bioassay 3: One leaf equivalent surface wax of lablab was tested against individual synthetic compounds or synthetic blends comparable to the amounts present in one leaf equivalent surface wax of lablab to observe whether crude surface wax and synthetic blends were equally attractive to the test insects.

Bioassay 4: Dose–response bioassays of *A. craccivora* and *A. gossypii* females towards individual synthetic compounds were tested to observe the lowest and highest doses where the female responded initially and showed the highest ($P < 0.0001$) attraction. Dose–response bioassays of *A. craccivora* females towards six individual compounds were tested at different doses against the control solvent (tetradecane: 10, 20, and 40 µg ml^{-1} petroleum ether; pentadecane: 2.5, 5, and 10 µg ml^{-1} petroleum ether; tetracosane: 10, 20, and 40 µg ml^{-1} petroleum ether; tridecanoic acid or heneicosanoic acid: 4, 8, and 16 µg ml^{-1} petroleum ether; and tetradecanoic acid: 3, 6, and 12 µg ml^{-1} petroleum ether) as females showed attraction towards six individual compounds. In addition, dose–response bioassays of *A. gossypii* females towards five individual compounds were tested at different doses against the control solvent (tetradecane or docosane: 10, 20, and 40 µg ml^{-1} petroleum ether; hexadecane: 25, 50, and 100 µg ml^{-1} petroleum ether; nonadecanoic acid: 15, 30, and 60 µg ml^{-1} petroleum ether; and arachidic acid: 2, 4, and 8 µg ml^{-1} petroleum ether) as females showed attraction towards five individual compounds.

Viviparity assays of A. craccivora and A. gossypii females with individual synthetic compounds and synthetic blends

Viviparity assay 3: Individual synthetic compounds (21 *n*-alkanes and 11 free fatty acids), comparable to the amounts present in one leaf equivalent leaf surface wax of lablab, were tested against the

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control solvent for either *A. craccivora* or *A. gossypii* to observe the effects of individual synthetic compounds on nymph laying by both females (Supplementary table 1A, B). AC blend 6 (*A. craccivora* showed the highest attraction in the Y-tube olfactometer bioassay against solvent controls) was tested against the control solvent to observe whether synthetic blend resembling one leaf equivalent surface wax of lablab stimulated *A. craccivora* females to lay nymphs. Further, AG blend 5 (*A. gossypii* showed the highest attraction in the Y-tube olfactometer bioassay against solvent controls) vs the control solvent were conducted to observe whether synthetic blends resembling one leaf equivalent surface wax of lablab stimulated *A. gossypii* females to lay nymphs.

Viviparity assay 4: One leaf equivalent surface wax of lablab vs AC blend 6 were tested against *A. craccivora*, and one leaf equivalent surface wax of lablab vs AG blend 5 was tested against *A. gossypii*. These experiments were performed to observe whether crude surface waxes and synthetic blends were equally acting as stimulants in both females to lay nymphs.

Scanning electron microscopy (SEM) studies

Adult viviparous females (3 days old) of *A. craccivora* and *A. gossypii* were anaesthetized by chloroform and preserved in 70% ethanol. The entire antennae and foreleg of *A. craccivora* and *A. gossypii* were dissected under a stereoscopic microscope (Labomed, Luxeo 2S Stereo Microscope). The antennae or foreleg of each aphid was passed through graded alcohol (50, 70, 90, and 100% alcohol, for each grade 15 min) to dehydrate the sample. Each sample was mounted on aluminium stubs with double-sided adhesive carbon tape and sputter-coated with gold in a Quorum SC 7620 sputter coater (Quorum Technologies Ltd., England, UK) to obtain a layer of 25 nm thick. Each sample was imaged with a Zeiss (Sigma 300) field emission scanning electron microscope (SEM) at 5 kV voltage with a secondary electron detector. Microphotographs of possible chemosensory structures present in antennae and foreleg of each aphid were obtained.

Statistical analyses

Data recorded on Y-tube olfactometer bioassays and viviparity assays by *A. craccivora* and *A. gossypii* females towards test samples were analysed 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: $P = 50\%$) (Debnath *et al.*, 2021; Mobarak *et al.*, 2022). Insects that remained in the common arm of the olfactometer were excluded from the analyses.

Results

Olfactometer bioassays with viviparous A. craccivora *and* A. gossypii *females towards crude surface waxes*

Bioassay 1: Females of *A. craccivora* and *A. gossypii* showed attraction towards one leaf equivalent surface wax of lablab against solvent controls (*A. craccivora*: $\chi^2 = 19.27$, $df = 1$, $P < 0.0001$; *A. gossypii*: $\chi^2 = 13.07$, $df = 1$, $P = 0.0003$) (fig. 1A, B).

Viviparity assays with A. craccivora *and* A. gossypii *females towards crude surface waxes*

Viviparity assay 1: Females of *A. craccivora* and *A. gossypii* significantly laid more nymphs on the intact leaf of lablab compared to the dewaxed leaf (*A. craccivora*: $\chi^2 = 28.49$, $df = 1$, $P < 0.0001$; *A. gossypii*: $\chi^2 = 27.94$, $df = 1$, $P < 0.0001$). This observation suggested that the leaf surface wax stimulated both viviparous aphids to lay nymphs (table 1).

Viviparity assay 2: The crude leaf surface wax of lablab significantly influenced *A. craccivora* and *A. gossypii* females to lay more nymphs when tested against filter papers containing the control solvent (*A. craccivora*: $\chi^2 = 27.65$, $df = 1$, $P < 0.0001$; *A. gossypii*: $\chi^2 = 23.17$, $df = 1$, $P < 0.0001$). This observation suggested that the crude leaf surface wax chemicals stimulated both female

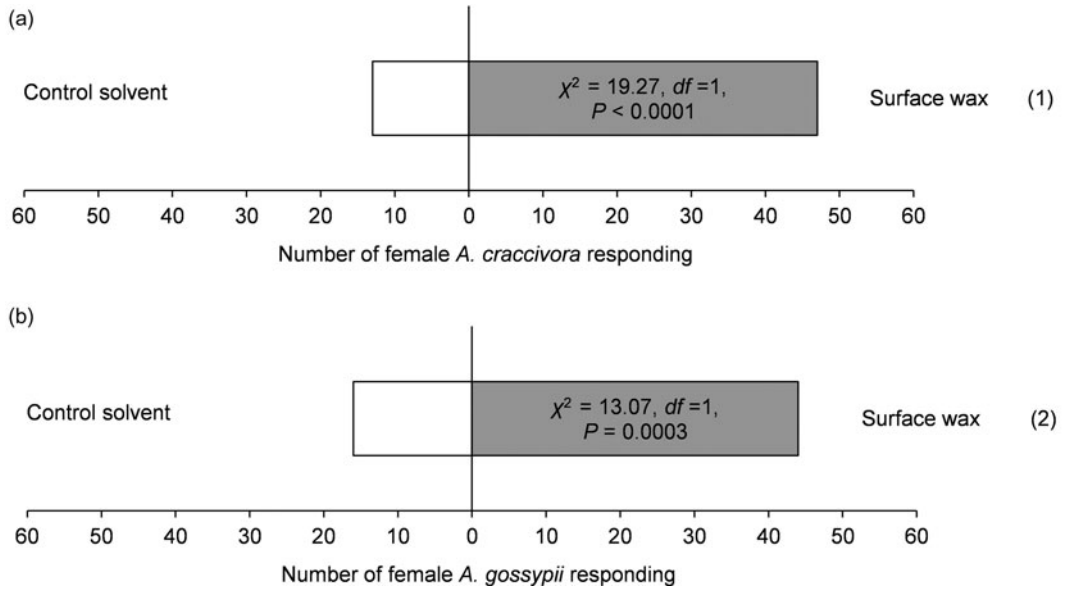

Figure 1. Behavioural responses of viviparous *Aphis craccivora* (A) and *Aphis gossypii* (B) females towards one leaf equivalent surface wax of *Lablab purpureus* subsp. *bengalensis* against solvent controls (petroleum ether) in the Y-tube olfactometer bioassay. Numbers in brackets are the number of insects that did not respond to either treatment.

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Table 1. Viviparity assays of *Aphis craccivora* and *A. gossypii* females towards *Lablab purpureus* subsp. *bengalensis* (*N* = 10 in each bioassay)

Comparison		No. of insects laid nymphs			Nymphs laid			
T1	T2	T1	T2	Non-responders	T1	T2	χ^2 (*df* = 1)	*P*-values
Viviparity assays of *A. craccivora*								
A single leaf	Dewaxed leaf	9	1	1	50	9	28.49	0.0001
One leaf equivalent crude surface wax	Control solvent (petroleum ether)	8	2	1	47	8	27.65	0.0001
Tetradecane (16.95 µg)	Control solvent	7	3	2	44	10	21.40	0.0001
Pentadecane (4.66 µg)	Control solvent	6	4	2	39	14	11.79	0.0005
Tetracosane (19.38 µg)	Control solvent	8	2	2	44	8	24.92	0.0001
Tridecanoic acid (5.98 µg)	Control solvent	7	3	2	43	10	20.54	0.0001
Tetradecanoic acid (6.13 µg)	Control solvent	6	4	2	38	13	12.25	0.0005
Heneicosanoic acid (9.40 µg)	Control solvent	7	3	2	45	9	24.00	0.0001
AC blend 6*	Control solvent	8	2	2	47	9	25.79	0.0001
One leaf equivalent crude surface wax	AC blend 6	5	5	1	29	26	0.16	0.6892
Viviparity assays of *A. gossypii*								
A single leaf	Dewaxed leaf	9	1	1	43	6	27.94	0.0001
One leaf equivalent crude surface wax	Control solvent (petroleum ether)	8	2	1	40	7	23.17	0.0001
Tetradecane (16.95 µg)	Control solvent	7	3	2	30	10	10.00	0.0016
Hexadecane (39.87 µg)	Control solvent	7	3	2	30	11	8.80	0.003
Docosane (12.93 µg)	Control solvent	7	3	2	36	7	19.56	0.0001
Nonadecanoic acid (18.82 µg)	Control solvent	6	4	3	31	11	9.52	0.002
Arachidic acid (5.06 µg)	Control solvent	6	4	3	32	11	10.26	0.0014
AG blend 5**	Control solvent	8	2	1	35	8	16.95	0.0001
One leaf equivalent crude surface wax	AG blend 5	5	5	2	25	19	0.82	0.3652

*AC blend 6: 16.95 µg tetradecane + 4.66 µg pentadecane + 19.38 µg tetracosane + 5.98 µg tridecanoic acid + 6.13 µg tetradecanoic acid + 9.40 µg heneicosanoic acid were dissolved in 1 ml petroleum ether.
**AG blend 5: 16.95 µg tetradecane + 39.87 µg hexadecane + 12.93 µg docosane + 18.82 µg nonadecanoic acid + 5.06 µg arachidic acid were dissolved in 1 ml petroleum ether.

aphids to lay more nymphs compared to solvent controls (table 1).

Leaf surface wax composition in lablab

Among the total amount of leaf surface waxes (20.69 ± 0.39 mg), alkanes and free fatty acids accounted for 11.95 ± 0.42 and 2.40 ± 0.14 mg (mean ± SE), respectively, with the remaining consisting of unidentified surface wax compounds. The identified *n*-alkanes in leaf surface waxes accounted for 11.30 ± 0.04 mg (mean ± SE), while 0.65 ± 0.04 mg (mean ± SE) was unidentified branched-chain alkanes (table 2). Twenty-one *n*-alkanes from *n*-C_{12} to *n*-C_{35} were detected in the leaf surface waxes (table 2; Supplementary fig. 3). Nonacosane (*n*-C_{29}) and heneicosane (*n*-C_{21}) were the predominant and least abundant alkanes in leaf surface waxes, respectively. Eleven free fatty acids from C12:0 to C22:0 were detected in leaf surface waxes (table 3; Supplementary fig. 4). Nonadecanoid acid (C19:0) was the most abundant while stearic acid (C18:0) was the least abundant among free fatty acids in leaf surface waxes (table 3).

Olfactometer bioassays with viviparous A. craccivora *and* A. gossypii *females*

Bioassay 2: Among all identified 21 *n*-alkanes and 11 free fatty acids present in the leaf surface wax of lablab, *A. craccivora* females showed responses to 14 individual synthetic compounds (tetradecane, pentadecane, octadecane, docosane, tetracosane, pentacosane, heptacosane, dotriacontane, tritriacontane, tridecanoic acid, tetradecanoic acid, linolenic acid, nonadecanoic acid, and heneicosanoic acid) comparable to the amounts present in one leaf equivalent surface wax of lablab against solvent controls (table 4). Females of *A. craccivora* were attracted towards a synthetic blend of above 14 compounds against solvent controls ($\chi^2 = 19.27$, $df = 1$, $P < 0.0001$) (table 4). *Aphis craccivora* females showed attraction towards six individual compounds [tetradecane or tetradecanoic acid ($\chi^2 = 5.40$, $df = 1$, $P = 0.0201$), pentadecane ($\chi^2 = 4.27$, $df = 1$, $P = 0.0389$), tetracosane or tridecanoic acid or heneicosanoic acid ($\chi^2 = 6.67$, $df = 1$, $P = 0.0098$)] or AC blend 6 ($\chi^2 = 13.07$, $df = 1$, $P = 0.0003$) compared to the solvent controls (table 4).

Among all identified 21 *n*-alkanes and 11 free fatty acids present in the leaf surface wax of lablab, *A. gossypii* females showed

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Table 2. Composition of alkanes (μg/25 g leaf) (Mean ± SE, $N = 5$) in the leaf surface waxes of *Lablab purpureus* subsp. *bengalensis*

Alkane	Amount (μg)
Dodecane (*n*-C_{12})	123.42 ± 10.30
Tetradecane (*n*-C_{14})	457.73 ± 44.34
Pentadecane (*n*-C_{15})	125.93 ± 9.80
Hexadecane (*n*-C_{16})	1076.53 ± 104.73
Octadecane (*n*-C_{18})	691.05 ± 51.97
Eicosane (*n*-C_{20})	662.01 ± 45.14
Heneicosane (*n*-C_{21})	58.82 ± 6.66
Docosane (*n*-C_{22})	349.12 ± 31.50
Tricosane (*n*-C_{23})	387.80 ± 25.93
Tetracosane (*n*-C_{24})	523.31 ± 32.39
Pentacosane (*n*-C_{25})	989.73 ± 68.69
Hexacosane (*n*-C_{26})	596.62 ± 51.98
Heptacosane (*n*-C_{27})	697.04 ± 43.58
Octacosane (*n*-C_{28})	402.21 ± 32.39
Nonacosane (*n*-C_{29})	1501.34 ± 76.05
Triacontane (*n*-C_{30})	228.95 ± 19.46
Hentriacontane (*n*-C_{31})	724.26 ± 51.62
Dotriacontane (*n*-C_{32})	540.45 ± 53.75
Tritriacontane (*n*-C_{33})	617.64 ± 51.34
Tetratriacontane (*n*-C_{34})	300.08 ± 25.61
Pentatriacontane (*n*-C_{35})	246.11 ± 22.26
Total	11,300.16 ± 400.55

responses to 11 individual synthetic compounds (tetradecane, hexadecane, docosane, pentacosane, heptacosane, nonacosane, triacontane, pentatriacontane, tetradecanoic acid, nonadecanoic acid, and arachidic acid) comparable to the amounts present in one leaf equivalent surface wax of lablab against solvent controls (table 5). Females of *A. gossypii* were attracted towards a synthetic blend of above 11 compounds against solvent controls ($\chi^2 = 13.07$, $df = 1$, $P = 0.0003$) (table 5). *Aphis gossypii* females showed attraction towards five individual compounds [tetradecane or docosane or nonadecanoic acid ($\chi^2 = 4.27$, $df = 1$, $P = 0.0389$) and hexadecane or arachidic acid ($\chi^2 = 5.40$, $df = 1$, $P = 0.0201$)] or AG blend 5 ($\chi^2 = 8.07$, $df = 1$, $P = 0.0045$) compared to the solvent controls (table 5).

Table 3. Composition of free fatty acids (μg/25 g leaf) (Mean ± SE, $N = 5$) in the leaf surface waxes of *Lablab purpureus* subsp. *bengalensis*

Fatty acid	Amount (μg)
Lauric acid (C12:0)	165.74 ± 15.72
Tridecanoic acid (C13:0)	161.59 ± 15.61
Tetradecanoic acid (C14:0)	165.47 ± 16.69
Palmitoleic acid (C16:1)	92.66 ± 8.59
Heptadecanoic acid (C17:0)	271.29 ± 27.29
Linolenic acid (C18:3)	482.50 ± 43.44
Stearic acid (C18:0)	63.55 ± 6.03
Nonadecanoic acid (C19:0)	508.04 ± 50.72
Arachidic acid (C20:0)	136.58 ± 10.41
Heneicosanoic acid (C21:0)	253.82 ± 25.82
Docosanoic acid (C22:0)	101.75 ± 9.87
Total	2402.98 ± 136.55

Bioassay 3: *Aphis craccivora* females could not differentiate between one leaf equivalent surface wax of lablab and a synthetic blend of 14 compounds (tetradecane, pentadecane, octadecane, docosane, tetracosane, pentacosane, heptacosane, dotriacontane, tritriacontane, tridecanoic acid, tetradecanoic acid, linolenic acid, nonadecanoic acid, and heneicosanoic acid) comparable to the amounts present in one leaf equivalent surface wax of lablab ($\chi^2 = 0.07$, $df = 1$, $P = 0.7963$) or AC blend 6 ($\chi^2 = 0.27$, $df = 1$, $P = 0.6056$) (table 6).

Aphis gossypii females could not discriminate between one leaf equivalent surface wax of lablab and a synthetic blend of 11 compounds (tetradecane, hexadecane, docosane, pentacosane, heptacosane, nonacosane, triacontane, pentatriacontane, tetradecanoic acid, nonadecanoic acid, and arachidic acid) comparable to the amounts present in one leaf equivalent surface wax of lablab ($\chi^2 = 0.27$, $df = 1$, $P = 0.6056$) or AG blend 5 ($\chi^2 = 0.60$, $df = 1$, $P = 0.4386$) (table 7).

Bioassay 4: In dose–response bioassays, *A. craccivora* females started to exhibit attraction towards tetradecane or tetracosane at 20 μg ml^{-1} petroleum ether and exhibited the highest attraction at 40 μg ml^{-1} petroleum ether (Supplementary table 2). Females were attracted towards pentadecane at 5 μg ml^{-1} petroleum ether and revealed the highest attraction at 10 μg ml^{-1} petroleum ether (Supplementary table 2). Females started to express attraction towards tridecanoic acid or heneicosanoic acid at 8 μg ml^{-1} petroleum ether and expressed the highest attraction at 16 μg ml^{-1} petroleum ether (Supplementary table 2). Females of *A. craccivora* began to indicate attraction towards tetradecanoic acid at 6 μg ml^{-1} petroleum ether and showed the highest attraction at 12 μg ml^{-1} petroleum ether (Supplementary table 2).

Aphis gossypii females started to show attraction towards tetradecane or docosane at 20 μg ml^{-1} petroleum ether and showed the highest attraction at 40 μg ml^{-1} petroleum ether (Supplementary table 3). Females of *A. gossypii* were attracted towards hexadecane at 50 μg ml^{-1} petroleum ether and displayed the highest attraction at 100 μg ml^{-1} petroleum ether (Supplementary table 3). Females started to exhibit attraction towards nonadecanoic acid at 30 μg ml^{-1} petroleum ether and exhibited the highest attraction at 60 μg ml^{-1} petroleum ether (Supplementary table 3). Females started to display attraction towards arachidic acid at 4 μg ml^{-1} petroleum ether and displayed the highest attraction at 8 μg ml^{-1} petroleum ether (Supplementary table 3).

Viviparity assays with A. craccivora *and* A. gossypii *females*

Viviparity assay 3: Among all identified 21 *n*-alkanes and 11 free fatty acids present in lablab leaf surface waxes, females of *A. craccivora* significantly laid nymphs on 6 individual synthetic compounds (tetradecane, pentadecane, tetracosane, tridecanoic acid, tetradecanoic acid, and heneicosanoic acid) that were attractive to the aphid in Y-tube olfactometer bioassays (table 1; Supplementary table 4). Females of *A. craccivora* significantly laid nymphs on tetradecane ($\chi^2 = 21.40$, $df = 1$, $P < 0.0001$) or pentadecane ($\chi^2 = 11.79$, $df = 1$, $P = 0.0005$) or tetracosane ($\chi^2 = 24.92$, $df = 1$, $P < 0.0001$) or tridecanoic acid ($\chi^2 = 20.54$, $df = 1$,

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Table 4. Behavioural responses of *Aphis craccivora* viviparous females towards individual synthetic compounds or synthetic blends comparable to the amounts present in one leaf equivalent surface wax of *Lablab purpureus* subsp. *bengalensis* vs solvent controls (petroleum ether) ($N = 60$ in each bioassay)

Comparison		Insects responded				
T1	T2	T1	T2	Non-responders	χ^2 ($df = 1$)	*P*-values
Synthetic compounds comparable to one leaf equivalent surface wax (μg ml^{-1})	Control solvent					
a. Tetradecane (16.95)		39	21	3	5.40	0.0201
b. Pentadecane (4.66)		38	22	4	4.27	0.0389
d. Octadecane (25.59)		37	23	5	3.27	0.0707
e. Docosane (12.93)		35	25	4	1.67	0.1967
f. Tetracosane (19.38)		40	20	3	6.67	0.0098
g. Pentacosane (36.66)		36	24	5	2.40	0.1213
h. Heptacosane (25.82)		37	23	4	3.27	0.0707
k. Dotriacontane (20.02)		36	24	4	2.40	0.1213
l. Tritriacontane (22.88)		37	23	3	3.27	0.0707
n. Tridecanoic acid (5.98)		40	20	2	6.67	0.0098
o. Tetradecanoic acid (6.13)		39	21	3	5.40	0.0201
p. Linolenic acid (17.87)		33	27	5	0.60	0.4386
q. Nonadecanoic acid (18.82)		33	27	6	0.60	0.4386
s. Heneicosanoic acid (9.40)		40	20	2	6.67	0.0098
a + b + d + e + f + g + h + k + l + n + o + p + q + s		47	13	1	19.27	0.0001
a + b + f + n + o + s (AC blend 6)		44	16	2	13.07	0.0003

Table 5. Behavioural responses of *Aphis gossypii* viviparous females towards individual synthetic compounds or synthetic blends comparable to the amounts present in one leaf equivalent surface wax of *Lablab purpureus* subsp. *bengalensis* vs solvent controls (petroleum ether) ($N = 60$ in each bioassay)

Comparison		Insects responded				
T1	T2	T1	T2	Non-responders	χ^2 ($df = 1$)	*P*-values
Synthetic compounds comparable to one leaf equivalent surface wax (μg ml^{-1})	Control solvent					
a. Tetradecane (16.95)		38	22	3	4.27	0.0389
c. Hexadecane (39.87)		39	21	2	5.40	0.0201
e. Docosane (12.93)		38	22	3	4.27	0.0389
g. Pentacosane (36.66)		35	25	4	1.67	0.1967
h. Heptacosane (25.82)		33	27	5	0.60	0.4386
i. Nonacosane (55.61)		37	23	3	3.27	0.0707
j. Triacontane (8.48)		33	27	5	0.60	0.4386
m. Pentatriacontane (9.12)		37	23	3	3.27	0.0707
o. Tetradecanoic acid (6.13)		34	26	4	1.07	0.3017
q. Nonadecanoic acid (18.82)		38	22	3	4.27	0.0389
r. Arachidic acid (5.06)		39	21	2	5.40	0.0201
a + c + e + g + h + i + j + m + o + q + r		44	16	1	13.07	0.0003
a + c + e + q + r (AG blend 5)		41	19	2	8.07	0.0045

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Table 6. Behavioural responses of *Aphis craccivora* females towards one leaf equivalent surface wax of *Lablab purpureus* subsp. *bengalensis* vs individual synthetic compounds or synthetic blends comparable to the amounts present in one leaf equivalent surface wax of *L. purpureus* subsp. *bengalensis* ($N = 60$ in each bioassay)

Comparison		Insects responded				
T1	T2	T1	T2	Non-responders	χ^2 ($df = 1$)	*P*-values
One leaf equivalent surface wax	Synthetic compounds comparable to one leaf equivalent surface wax ($\mu g\,ml^{-1}$)					
	a. Tetradecane (16.95)	38	22	2	4.27	0.0389
	b. Pentadecane (4.66)	39	21	2	5.40	0.0201
	d. Octadecane (25.59)	42	18	1	9.60	0.0019
	e. Docosane (12.93)	44	16	1	13.07	0.0003
	f. Tetracosane (19.38)	37	23	3	3.27	0.0707
	g. Pentacosane (36.66)	43	17	1	11.27	0.0008
	h. Heptacosane (25.82)	41	19	2	8.07	0.0045
	k. Dotriacontane (20.02)	43	17	1	11.27	0.0008
	l. Tritriacontane (22.88)	41	19	2	8.07	0.0045
	n. Tridecanoic acid (5.98)	37	23	3	3.27	0.0707
	o. Tetradecanoic acid (6.13)	38	22	3	4.27	0.0389
	p. Linolenic acid (17.87)	45	15	1	15.00	0.0001
	q. Nonadecanoic acid (18.82)	45	15	1	15.00	0.0001
	s. Heneicosanoic acid (9.40)	37	23	3	3.27	0.0707
	a + b + d + e + f + g + h + k + l + n + o + p + q + s	31	29	1	0.07	0.7963
	a + b + f + n + o + s (AC blend 6)	32	28	2	0.27	0.6056

Table 7. Behavioural responses of *Aphis gossypii* females towards one leaf equivalent surface wax of *Lablab purpureus* subsp. *bengalensis* vs individual synthetic compounds or synthetic blends comparable to the amounts present in one leaf equivalent surface wax of *L. purpureus* subsp. *bengalensis* ($N = 60$ in each bioassay)

Comparison		Insects responded				
T1	T2	T1	T2	Non-responders	χ^2 ($df = 1$)	*P*-values
One leaf equivalent surface wax	Synthetic compounds comparable to one leaf equivalent surface wax (μg ml^{-1})					
	a. Tetradecane (16.95)	36	24	2	2.40	0.1213
	c. Hexadecane (39.87)	35	25	2	1.67	0.1967
	e. Docosane (12.93)	36	24	2	2.40	0.1213
	g. Pentacosane (36.66)	38	22	1	4.27	0.0389
	h. Heptacosane (25.82)	39	21	1	5.40	0.0201
	i. Nonacosane (55.61)	38	22	2	4.27	0.0389
	j. Triacontane (8.48)	39	21	1	5.40	0.0201
	m. Pentatriacontane (9.12)	38	22	2	4.27	0.0389
	o. Tetradecanoic acid (6.13)	38	22	1	4.27	0.0389
	q. Nonadecanoic acid (18.82)	36	24	3	2.40	0.1213
	r. Arachidic acid (5.06)	35	25	2	1.67	0.1967
	a + c + e + g + h + i + j + m + o + q + r	32	28	1	0.27	0.6056
	a + c + e + q + r (AG blend 5)	33	27	2	0.60	0.4386

$P < 0.0001$) or tetradecanoic acid ($\chi^2 = 12.25$, $df = 1$, $P = 0.0005$) or heneicosanoic acid ($\chi^2 = 24$, $df = 1$, $P < 0.0001$), or AC blend 6 ($\chi^2 = 25.79$, $df = 1$, $P < 0.0001$) comparable to one leaf equivalent surface wax of lablab against the control solvent (table 1).

Among all identified 21 *n*-alkanes and 11 free fatty acids present in lablab leaf surface waxes, *A. gossypii* females significantly laid nymphs on 5 individual synthetic compounds (tetradecane, hexadecane, docosane, nonadecanoic acid, and arachidic acid) that were attractive to the aphid in Y-tube olfactometer bioassays (table 1; Supplementary table 4). Females of *A. gossypii* significantly laid nymphs on tetradecane ($\chi^2 = 10$, $df = 1$, $P = 0.0016$) or hexadecane ($\chi^2 = 8.80$, $df = 1$, $P = 0.003$) or docosane ($\chi^2 = 19.56$, $df = 1$, $P < 0.0001$) or nonadecanoic acid ($\chi^2 = 9.52$, $df = 1$, $P = 0.002$) or arachidic acid ($\chi^2 = 10.26$, $df = 1$, $P = 0.0014$), or AG blend 5 ($\chi^2 = 16.95$, $df = 1$, $P < 0.0001$) comparable to one leaf equivalent surface wax of lablab against the control solvent (table 1). These observations revealed that synthetic blends comparable to the leaf surface wax of lablab stimulated females of *A. craccivora* and *A. gossypii* to lay nymphs.

Viviparity assay 4: *Aphis craccivora* females did not reveal a difference for nymph laying between one leaf equivalent surface wax of lablab and AC blend 6 ($\chi^2 = 0.16$, $df = 1$, $P = 0.6892$) (table 1). Females of *A. gossypii* did not exhibit a preference for nymph laying between one leaf equivalent surface wax of lablab and AG blend 5 ($\chi^2 = 0.82$, $df = 1$, $P = 0.3652$) (table 1).

SEM of antennae and foreleg

SEM observations of viviparous females of *A. craccivora* and *A. gossypii* showed the differences of antennae and forelegs both in the morphology and sensilla distribution (figs 2 and 3; Supplementary figs 5 and 6). On the antennae of both *A. craccivora* and *A. gossypii*, different types of sensilla were observed depending on the segment (figs 2 and 3). Type II trichoid sensilla were present on the antenna tip of the 6th segment and along the process terminalis on the same segment of both aphids (figs 2 and 3). Type II trichoid sensilla present on the antenna tip looked like short hairs with a blunt tip showing fissure-like structures and grooves. Type I trichoid sensilla were visible from the base of the antenna to the 6th segment, which was characterised by a grooved surface and a swollen tip with porous structures. Primary rhinaria were observed on the 5th and 6th antennal segments (figs 2 and 3). A placoid sensillum was located in the distal end of the 5th segment; whereas on the 6th segment, one large placoid sensillum, two smaller ones, and type I and type II coeloconic sensilla were observed, which were surrounded by cuticular fringes (figs 2 and 3). In the legs of both *A. craccivora* and *A. gossypii*, numerous trichoid sensilla were present, which were uniform in size, shape, and distribution. SEM images of the foreleg revealed the insertion of the sensillum basal portion in a cuticular extension on the leg (Supplementary figs 5 and 6).

Discussion

Responses of herbivorous insects towards plant leaf surfaces are a crucial step in finding a suitable host and acceptance of insects (Schoonhoven *et al.*, 2005). So, it is of considerable interest to understand how aphids use plant leaf surface wax compounds as oviposition stimulants to lay nymphs as aphids cause major economic losses in agricultural crops. An aphid's ability to discriminate between host plants and non-host plants by chemicals of epicuticular waxes suggests that sensory cues emanating from the epicuticular wax chemicals play an essential role in host acceptance (Fernández *et al.*, 2019). The olfactometer bioassays of *A. craccivora* and *A. gossypii* suggested that leaf surface waxes from lablab significantly attracted both aphids from short-range for nymph laying. The oviposition assays indicated that females

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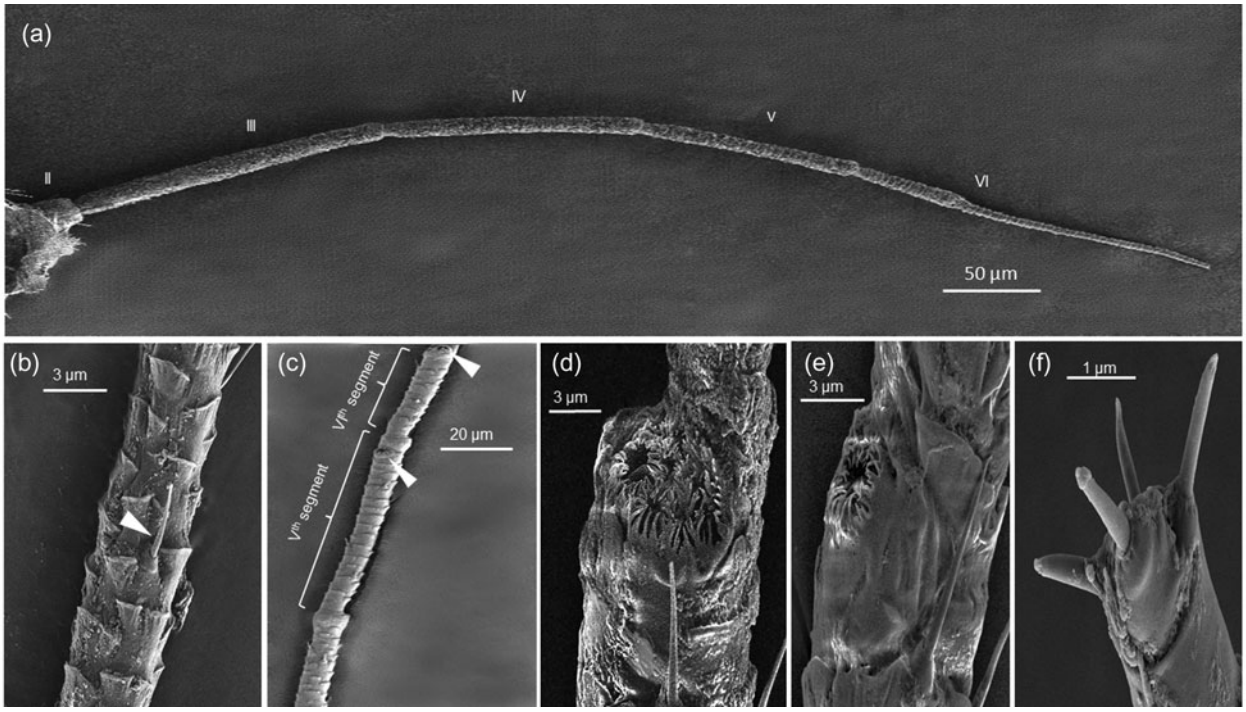

Figure 2. SEM of antennae on apterous *Aphis craccivora*: (a) antennae general view, (b) 3rd antennal segment showing type I trichoid sensilla; (c) view of primary rhinaria on 5th and 6th antennal segment; (d) details of primary rhinaria on 6th antennal segment; (e) details of primary rhinaria on 5th antennal segment; and (f) type II trichoid sensilla located on the terminal part of the antennae.

of *A. craccivora* and *A. gossypii* showed a preference to lay nymphs on intact leaf containing epicuticular wax in comparison to dewaxed leaf. In addition, this was confirmed by the result that both females of *A. craccivora* and *A. gossypii* preferred to lay nymphs on filter papers containing crude epicuticular wax. We identified different types of chemosensilla from the antennae

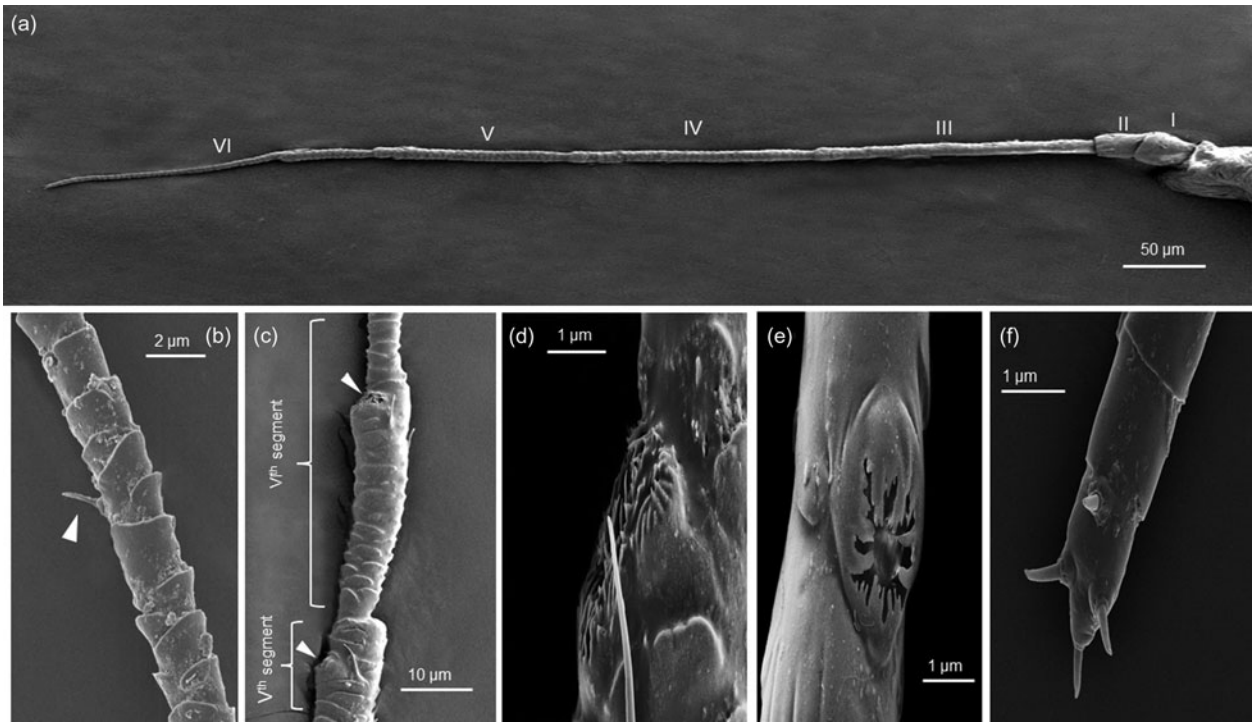

Figure 3. SEM of antennae on apterous *Aphis gossypii*: (a) antennae general view, (b) 3rd antennal segment showing type I trichoid sensilla; (c) view of primary rhinaria on 5th and 6th antennal segment; (d) details of primary rhinaria on 6th antennal segment; (e) details of primary rhinaria on 5th antennal segment; and (f) type II trichoid sensilla located on the terminal part of the antennae.

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and forelegs of both *A. craccivora* and *A. gossypii* to observe the sensory organs associated with insect olfaction and oviposition stimulants. The short Y-tube olfactometer bioassays and viviparity assay experiments are necessary to decide whether the clear preference by viviparous females is due to attraction or oviposition-stimulatory effects.

Here, *n*-alkanes from *n*-C_{12} to *n*-C_{35}, and free fatty acids from C12:0 to C22:0 represented the alkane and free fatty acid profiles in the leaf surface waxes of lablab. The current study indicated that alkanes and free fatty acids accounted for 57.76 and 11.60% of total surface wax chemicals in lablab, respectively. Several studies indicate that alkanes were the most abundant in the leaf surface waxes of various plants such as *Lathyrus sativus* L. (46.95 and 62.68% in BIO L 212 Ratan and Nirma B-1 cultivars, respectively) (Mitra *et al.*, 2020a), *V. radiata* (60.20, 59.38, and 55.25% in PDM 54, PUSA BAISAKAHI, and SAMRAT cultivars, respectively) (Mobarak *et al.*, 2020) and *Trichosanthes anguina* L. (60.61, 62.67, and 58.60% in MNSR-1, Baruipur Long, and Polo No. 1 cultivars, respectively) (Debnath *et al.*, 2021). Similar to the current study, free fatty acids represented for leaf surface waxes of *L. sativus* (20.51 and 8.08% in BIO L 212 Ratan and Nirma B-1 cultivars, respectively) (Mitra *et al.*, 2020a), *V. radiata* (10.56, 9.34, and 14.01% in PDM 54, PUSA BAISAKAHI, and SAMRAT cultivars, respectively) (Mobarak *et al.*, 2020), and *T. anguina* (13.30, 10.56, and 12.70% in MNSR-1, Baruipur Long, and Polo No. 1 cultivars, respectively) (Debnath *et al.*, 2021). A number of studies demonstrated that *n*-alkanes from C_{15} to C_{36} and free fatty acids from C12:0 to C21:0 were common chemicals in the leaf surface waxes of various plants (Li and Ishikawa, 2006; Das *et al.*, 2019; Mitra *et al.*, 2020a; Mobarak *et al.*, 2020). Twenty *n*-alkanes from *n*-C_{15} to *n*-C_{36} and 13 free fatty acids from C12:0 to C21:0 were detected in leaf surface waxes of three green gram cultivars (PDM 54, PUSA BAISAKHI, and SAMRAT) (Mobarak *et al.*, 2020). Further, 18 *n*-alkanes from *n*-C_{15} to *n*-C_{36} and 14 free fatty acids from C12:0 to C22:0 were identified in the leaf surface waxes of two cultivars [BIO L 212 Ratan and Nirmal B-1] of *L. sativus* plants (Mitra *et al.*, 2020a). Different alkanes and free fatty acids were predominant in the leaf surface waxes of various plant species (Piasentier *et al.*, 2000; Dodoš *et al.*, 2015; Karmakar *et al.*, 2016). Nonacosane was the most abundant alkane in the leaf surface wax of *Argemone mexicana* L. (Bhattacharjee *et al.*, 2010). Nonacosane and hexadecanoic acid were the most abundant among *n*-alkanes and free fatty acids in *Fallopia japonica* (Houtt.) Ronse Decr., respectively (Li and Ishikawa, 2006). Heneicosanoic acid and palmitoleic acid were the most abundant free fatty acids in the leaf surface waxes of PDM 54, and PUSA BAISAKHI and SAMRAT cultivars of green gram plants, respectively (Mobarak *et al.*, 2020). Heptadecane and stearic acid were the most abundant among alkanes and free fatty acids in three cultivars of *T. anguina* plants, respectively (Debnath *et al.*, 2021). However, nonacosane and nonadecanoic acid were the most abundant among *n*-alkanes and free fatty acids in lablab, respectively. This study is in agreement with the hypothesis that variations in the compositions of leaf surface wax compounds might happen among plant species (Piasentier *et al.*, 2000; Dodoš *et al.*, 2015; Wang *et al.*, 2015; Das *et al.*, 2019; Koner *et al.*, 2022).

After coming close range of the host plants, leaf surfaces wax chemicals such as long-chain alkanes and free fatty acids serve a vital role in plant-insect interactions such as short-range attractants (Phelan *et al.*, 1991; Manosalva *et al.*, 2011; Sarkar *et al.*, 2013; Malik and Barik, 2015; Mukherjee *et al.*, 2015; Karmakar *et al.*, 2016; Malik *et al.*, 2017) and oviposition stimulants (Udayagiri and Mason, 1997; Parr *et al.*, 1998; Grant *et al.*, 2000; Li and Ishikawa, 2006; Mitra *et al.*, 2017, 2020a, 2020b; Das *et al.*, 2019; Debnath *et al.*, 2021). This study also reveals that females of *A. craccivora* and *A. gossypii* employ leaf surface wax chemicals such as alkanes and free fatty acids as olfactory cues, which stimulated females to lay nymphs on the leaves of lablab. Females of *A. craccivora* and *A. gossypii* used in this study were reared on cowpea plants and eggplant, respectively, with no prior contact with chemicals of lablab leaf surface wax, therefore discarding the possibility of associative learning by previous experience. However, females of *A. craccivora* laid nymphs on a synthetic blend of six compounds (tetradecane, pentadecane, tetracosane, tridecanoic acid, tetradecanoic acid, and heneicosanoic acid) comparable to the amounts present in one leaf equivalent surface wax of lablab; whereas *A. gossypii* females laid nymphs on a synthetic blend of five compounds (tetradecane, hexadecane, docosane, nondecanoic acid, and arachidic acid) comparable to the amounts present in one leaf equivalent surface wax of lablab, suggesting that the viviparous *A. craccivora* and *A. gossypii* females could recognise leaves of lablab mainly by both the qualitative (by discrete chemical compounds) and quantitative (by a precise amount of compounds) as contact cues to lay nymphs. A synthetic blend of either pentadecane, tridecanoic acid, and linoleic acid resembling one leaf equivalent wax of BIO L 212 Ratan cultivar or pentadecane, docosane, pentacosane, heptacosane, tritriacontane, and linoleic acid resembling one leaf equivalent wax of Nirmal B-1 cultivar of *L. sativus* served as short-range attractants and oviposition stimulants in *A. craccivora* females (Mitra *et al.*, 2020a). A comparison of the leaf surface wax chemicals of lablab and *L. sativus* suggested that long-chain alkanes and free fatty acids are common in both plant species but the specific combination and amounts of these compounds were different in both plant species, indicating *A. craccivora* females employ distinct chemicals for oviposition stimulants. A synthetic blend of either octadecane, heptacosane, and nonacosane comparable to one leaf equivalent surface wax of *Rumex dentatus* L. or octadecane, palmitoleic acid, and docosanoic acid comparable to one leaf equivalent surface wax of *Polygonum glabrum* Willd. served as short-range attractants and ovipositional stimulants in *Galerucella placida* Baly (Koner *et al.*, 2022). Further, a blend of heptadecane, eicosane, hexacosane, and stearic acid, a blend of hexacosane and stearic acid, and a blend of pentadecane and stearic acid comparable to amounts present in one leaf equivalent surface wax of MNSR-1, Baruipur Long, and Polo No.1 cultivars of *T. anguina*, respectively, served as short-range attractants and stimulated females of *Diaphania indica* (Saunders) to lay eggs (Debnath *et al.*, 2021). The current study suggested that most insects could respond to the precise amount of compounds though olfactory sensilla of antenna and other body parts (leg, mouthparts, and cauda) as the host plant acceptance process to lay eggs or nymphs, and the olfactory signals for egg or nymph laying behaviour by the females could fade away when the absolute amount of crucial compounds was substituted (Udayagiri and Mason, 1997; Parr *et al.*, 1998; Grant *et al.*, 2000; Li and Ishikawa, 2006; Mitra *et al.*, 2017; Mitra *et al.*, 2020a, 2020b).

This study summarises that the leaf surface wax chemicals of lablab influenced females of *A. craccivora* and *A. gossypii* to lay nymphs. Females of *A. craccivora* were attracted towards a synthetic blend of 16.95 µg tetradecane, 4.66 µg pentadecane, 19.38 µg tetracosane, 5.98 µg tridecanoic acid, 6.13 µg tetradecanoic

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acid, and 9.40 µg heneicosanoic acid; whereas *A. gossypii* females showed attraction towards a synthetic blend of 16.95 µg tetradecane, 39.87 µg hexadecane, 12.93 µg docosane, 18.82 µg nonadecanoic acid, and 5.06 µg arachidic acid resembling one leaf equivalent surface wax of lablab. This information could be employed in genetic engineering programs to develop plant genotypes that are resistant or less preferred to *A. craccivora* and *A. gossypii* (Eigenbrode and Espelie, 1995). In addition, once volatile organic compounds (VOCs) from *L. purpureus* subsp. *bengalensis* plants causing long-range attraction of *A. craccivora* and *A. gossypii* have been identified, then the above-mentioned synthetic blends along with the VOCs of leaves could be used as lures to develop baited traps in the IPM program. Bioassays in a greenhouse to assess the responses of *A. craccivora* and *A. gossypii* females towards the above two synthetic blends are required to authenticate the results obtained in this study.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0007485323000445.

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Author contributions. A. B. designed experiments. S. K. and S. D. performed bioassays. S. K. and S. D. did chemical analyses. S. K. and S. D. analysed data. S. K. made the figures. A. B. wrote the manuscript. All authors edited the manuscript and approved the final version of the manuscript.

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Competing interests. The authors declare no conflict of interest.

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