Do conspecific herbivores track resource depletion through host phenology-specific HIPVs?

P. D. Kamala Jayanthi¹,* Anjana Subramoniam¹, P. Saravan Kumar¹, B. R. Jayanthimala¹ and A. Rekha²

¹Division of Entomology and Nematology, and ²Division of Fruit Crops, ICAR-Indian Institute of Horticultural Research, Hessaraghatta Lake PO, Bengaluru 560 089, India

Conspecific herbivores use herbivore-induced plant volatiles (HIPVs) as cues while selecting an optimal site for oviposition. This is to ascertain the availability of nourishment for their progeny so that they get the best chance at survival. In the present study, phytophagous eulophid seed-borer Anselmella kerrichi was significantly (time spent: \(F_{3,30} = 13.12, P < 0.0001\); number of entries: \(F_{3,30} = 4.21, P = 0.02\)) attracted to HIPVs from immature fruits of Java plum, Syzygium cumini (time spent: 4.77 ± 0.40 min; number of entries: 2.27 ± 0.24) when also given the choice of mature fruits (time spent: 1.76 ± 0.32 min; number of entries: 1.46 ± 0.16), indicating that herbivores can assess resource depletion from host phenology-specific HIPVs. The chemical cues like \(\alpha\)-pinene, \(\beta\)-pinene, (z)-ocimene, undecane, 3,7-dimethyl decane, neo-\(\alpha\)-ocimene, allo-ocimene, ethyl benzoate, 2,6,11-trimethyldodecane, \(\alpha\)-copaene and \(\beta\)-caryophyllene, which are present in immature fruit volatiles elicited antennal response in a GC-EAD analysis. Olfactometer analyses with the synthetic compounds also revealed that A. kerrichi was significantly attracted to these cues and the synthetic blend composed of the above compounds proved to be much more efficient in attracting female wasps when compared to a natural blend. Field evaluations using the synthetic blend showed that it could attract a significant number of A. kerrichi, indicating the scope of using this blend of synthetic HIPVs as a sustainable IPM tool.

Keywords: Anselmella kerrichi, herbivore-induced plant volatiles, host phenology, resource depletion, Syzygium cumini.

Plants upon being attacked by herbivores emit a number of distinct volatiles often known as herbivore-induced plant volatiles (HIPVs) that have multiple functions at different trophic levels¹⁻⁵. From the plant’s perspective, these HIPVs act as communication signals to attract natural enemies and alert various parts of same plant or neighbouring plants, thereby serving as a direct and indirect plant defence⁶. The role of HIPVs was studied in detail against a handful of insect pests, from the point of view of herbivores. However, the ecological function of HIPVs to conspecifics was found to be variable depending upon the herbivore species, thus making the community interactions quite complex. This emphasizes the need to understand and study the utilization of HIPVs by conspecifics on a case-by-case basis. Conspecific herbivores, particularly moths, are often repelled by the HIPVs produced by larval feeding as observed in Trichoplusia ni (Hübner)⁷, Heliotis virescens (Fabricius)⁸, Spodoptera frugiperda (J.E. Smith)⁹, Manduca quinquemaculata (Haworth) and Manduca sexta (Linnaeus)⁸. On the contrary, HIPVs also serve as attractive cues to conspecifics as noticed in Colorado potato beetle, Leptinotarsa decemlineata (Say)¹⁰, Mamestra brassicae (Linnaeus), Spodoptera littoralis (Boisduval)¹¹, leaf beetle, Oreina cacalvae (Schrank)¹², Japanese beetles Popillia japonica Newman¹³ and thrips, Scirtothrips dorsalis (Hood)⁵.

The herbivores that display attraction or repulsion to HIPVs produced by conspecifics must have their own interests. Herbivores which exhibit attraction have their interests in host location¹²⁻⁵, better fitness cost to progeny¹⁰,¹²,¹⁴, compromised host defence¹⁵, easy access to cohorts and mates¹⁰,¹², avoidance of natural enemies due to clustering¹⁶ and attracting hyperparasitoids¹⁷⁻¹⁹. The herbivores which exhibit repulsion may also have their own advantages like reduction in competition for resources² to avoid attack of natural enemies³,⁴,⁵ in order to avoid poor nutrition status of the host plant due to induced defences¹⁰.

Thus, the varied behavioural responses of herbivores to HIPVs produced by conspecifics not only remain confusing, but also demand a case-by-case analysis as mentioned earlier. Host–plant phenology additionally performs a significant role in influencing the decision of herbivores in rejecting or accepting a host with conspecifics, as host phenology precisely defines the availability of food resources to their progeny. Particularly among short-lived herbivores whose progeny are mostly internal feeders, and cannot relocate on depletion of food resources, it becomes all the more important for the gravid females to

*For correspondence. (e-mail: jainsect@gmail.com)
synchronize their egg-laying with the appropriate stage of host phenology that maximizes the progeny fitness\textsuperscript{21}. *Anselmella kerrichi* (Narayanan, Subba Rao & Patel), a short-lived phytophagous, eulophid wasp attacks immature fruits of jamun (Java plum), *Syzygium cumini* Skeels (family Myrtaceae), causing extensive damage\textsuperscript{22}. Upon oviposition in immature fruits, the emerging grubs tunnel down and reach the seed kernel. They feed on the seed tissue and develop as well as pupate within it. The emerging adult wasps burrow out of the fruit through its epidermis, making numerous exit holes on the surface, thereby affecting the fruit quality\textsuperscript{22}. The present study was conducted to understand the response of jamun seed borer, *A. kerrichi* to HIPVs produced from different host fruit phenological stages damaged by conspecifics.

**Materials and methods**

**Insect collection**

The eulophid wasp-infested fruits were collected from the field in ICAR-Indian Institute of Horticultural Research, Bengaluru (13.1348°N, 77.4960°E) during March 2019 and placed in plastic containers (15 × 12 × 20 cm) for wasp emergence. The emerged insects were allowed to mate and collected in separate plastic vials (5 × 2 × 2 cm) for performing both olfactometer assays as well as coupled gas chromatography-electroantennodetection (GC-EAD) studies. The male and female insects were differentiated on the basis of abdomen size.

**Chemicals**

Authentic chemical standards (>95% purity) of α-pinene (97%), β-pinene (97%), (z)-ocimene (>90%), undecane (>99%), allo-ocimene (80% technical grade), ethyl benzoate (>99%) and β-caryophyllene (>80%) were purchased from Sigma Aldrich. The remaining chemicals for which no standards are commercially available were identified through NIST spectral library 14.

**Air entrainment collection**

Headspace samples of wasp-infested immature and mature jamun fruits (cv. Dhupdal, *n* = 25) were collected by air entrainment method according to the procedure described earlier\textsuperscript{23}. The fruit traits namely size, colour, softness of berries were considered to assess fruit maturity\textsuperscript{22}. Both the immature (small green and hard berries) and mature (large, purple soft berries) bunches infested by wasps were detached from the tree using secateurs, placed in polythene covers with minimal disturbance and brought to the laboratory for collection of volatiles. Fruit bunches were placed inside a round-bottomed glass container (10 cm height; 8 cm diameter) covered with a glass lid (with inlet and outlet ports on top) with flanges to seal it airtight. Purified air was pushed and pulled through the inlet and outlet ports respectively, according to the procedure described earlier\textsuperscript{23}. The volatiles were entrained for 24 h into the odour cartridges made up of adsorbent, Porapak Q (50 mg, 60/80 mesh; Supelco, Sigma, India). The cartridges were eluted into the glass vials using 800 µ redistilled diethyl ether; this served as the test sample of fruit volatiles. An internal standard of 500 ng/µl of *n*-pentadecane (99% Pure, Sigma-Aldrich, India) was added to the extract for chemical quantification\textsuperscript{24}. Volatile samples were stored in a freezer (−20°C) until further use.

**Olfactometer bioassays**

Single- and dual-choice olfactometer bioassays were carried out to measure the behavioural response of *A. kerrichi* females to headspace samples of immature and mature fruit volatiles as well as synthetic standards and synthetic volatile blends, according to the procedure described by Kamala Jayanthi *et al.*\textsuperscript{23} using a Perspex four-arm olfactometer (120 mm diameter)\textsuperscript{25}. Data on the time spent and number of entries made by *A. kerrichi* female into each arm were recorded using OLFA software (F. Nazzi, Udine, Italy) across the assays. Initially single-choice assays were performed, where the insects were tested with immature and mature jamun volatiles individually against solvent control (*n* = 12). Later a dual-choice assay was performed, where both the fruit volatiles were offered to the female wasps along with solvent control to identify the most preferred sample. Secondly, the response of insects to synthetic compounds (identified from the most preferred sample) that elicited antennal response during GC-EAD was tested individually against solvent control (*n* = 12). Among these compounds only the ones that are commercially available were tested in the olfactometer bioassays. Thirdly, a dual-choice assay was performed to compare the synthetic blend (that contained GC-EAD responded and identified compounds at the same concentration and ratio as the headspace sample) with the natural headspace sample (*n* = 12). The data of single-choice assays were subjected to paired *t*-test and those of dual-choice assays were subjected to one-way ANOVA with Bonferroni multiple comparisons using GraphPad Prism (ver. 7.03).

**Coupled gas chromatography-electroantennodetection**

The GC-EAD recordings were made according to the procedure described earlier\textsuperscript{23}. The insects were starved for 2 h before conducting the experiment and the head of a mated female *A. kerrichi* (*n* = 6) anaesthetized by chilling.
was separated from the body with a microscalpel and placed between the indifferent and the recording electrodes. The Porapak Q elutes collected in a solvent (DEE, diethyl ether, Merck, 99.97%) were analysed on a GC system (Agilent 7890B GC) equipped with a flame ionization detector (FID) and a non-polar HP-5 column bonded phase fused silica capillary column (30 m length $\times$ 0.250 mm diameter $\times$ 0.25 $\mu$m thickness). The thermal programme was set initially at an oven temperature of 60°C for 1 min, and then ramped at 15°C/min to 240°C held for 2 min at pressure 8.2995 psi on split-less mode (40 ml/min). Injector and detector temperatures were 250°C and 300°C respectively. Nitrogen was used as the carrier gas at constant flow of 1 ml/min.

For coupled GC-EAD analysis, the effluent from GC column was split into two parts in the ratio 1 : 1, and each part was simultaneously directed to the GC detector and the antennal preparation through a heated (250°C) transfer line. The electrode was connected to a high impedance DC amplifier (IDAC-4; Syntech). Compounds eluting from the GC column were delivered to the antenna through a glass tube (12 $\times$ 0.8 cm), carried by a humidified and purified supplemental airflow at 200 ml/min. The antennal and FID signals were simultaneously recorded and analysed using a customized software package (Syntech, Germany). Peaks eluting from the GC column were considered to be active if they elicited EAG activity in three or more of the six coupled runs carried out.

***Gas-chromatography coupled mass spectrometry analysis***

Chemical composition of Porapak Q elutes was analysed using GC-MS (Agilent 7890B GC) system equipped with mass spectrophotometry (Agilent 5977 MSD). A capillary column, (HP-5 MS UI) of 30 m length, 0.250 mm diameter and 0.25 $\mu$m film thickness (Agilent J&W) was used to examine the samples. The oven temperature was set as described previously for the GC system. MS was in full scan mode (70 eV) and amu ranged from 40 to 450. One microlitre of the sample was injected in split-less mode (40 ml/min) with injection temperature at 270°C. Helium was used as the carrier gas at a flow rate of 1 ml/min. Individual volatile compounds were identified by comparing the GC retention time, Kovats index calculated using homologous series of n-alkanes (C7 to C30 procured from Sigma-Aldrich, India) as standard and comparing the MS spectra with NIST 14 spectral library. Identified EAD compounds were authenticated by co-injecting standard synthetic compounds along with samples. The relative abundance of each compound was calculated based on the internal standard (n-pentadecane) within the sample. A heat map was plotted for comparing the GC-MS-identified compounds using GraphPad Prism software (ver. 7.0).

***Studies with synthetic HIPVs***

The pure-grade chemicals of seven compounds that are found significantly attractive to *A. kerrichi* in the olfactometer assays, namely $\alpha$-pinene, $\beta$-pinene, (z)-ocimene, undecane, allo-ocimene, ethyl benzoate and $\beta$-caryophyllene were made into a synthetic blend similar to natural headspace sample concentration as identified through GC-MS. The synthetic blend of all authentic standards, ethyl benzoate (5.44 $\mu$g/ml), $\beta$-pinene (47.50 $\mu$g/ml), allo-ocimene (83.24 $\mu$g/ml), undecane (196.30 $\mu$g/ml), (z)-ocimene (23.40 $\mu$g/ml) and $\alpha$-pinene (76.5 $\mu$g/ml) was prepared with hexane (99.9%, Sigma-Aldrich, USA). The blend was used in bioassays as well as in *A. kerrichi* field-trapping studies. Also, 250 $\mu$l of synthetic blend filled into 50-gauge PE (polyethylene) sachets of dimensions 24 cm$^2$ served as lures for field studies.

***Field trapping***

To test the attraction of the synthetic lure, the PE sachets prepared were stuck to white A4-sized sticky traps (29 cm $\times$ 11 cm). These traps were randomly hung on the branches of jamun trees at 2 m above the ground. Data on the numbers of *A. kerrichi* trapped were collected daily over a week. The white sticky traps without PE sachets served as control and were placed on separate trees. The experiment was replicated thrice and data were subjected to a non-parametric Mann–Whitney *U* test (unpaired; $\alpha = 0.05$) using GraphPad Prism (ver. 7.0).

***Results***

In the present study, the gravid female euplophid wasps, did not exhibit any preference to spend time ($P = 0.12$) in the region treated with mature fruit HIPVs (1.79 $\pm$ 0.17 min) compared to the control (2.40 $\pm$ 0.24). However, they made a significantly greater number of entries ($P = 0.02$) into the arm (2.75 $\pm$ 0.28) treated with mature fruit HIPVs compared to the control arm (1.89 $\pm$ 0.25). The female wasps spent significantly more time (4.68 $\pm$ 0.41 min in the treated arm; 2.45 $\pm$ 0.88 min in control arm; $P = 0.03$) and made significantly more entries (2.58 $\pm$ 0.19 in the treatment arm; 1.75 $\pm$ 0.14 in control arm; $P = 0.002$) into the treatment arm containing immature fruit HIPVs over the control (Figure 1).

In the dual-choice assay, when both the immature and mature fruit HIPVs were presented, the female *A. kerrichi* wasps spent significantly more time ($F_{3,40} = 13.12$, $P < 0.0001$) in the region treated with immature fruit HIPVs (4.77 $\pm$ 0.40 min) over mature fruit HIPVs (1.76 $\pm$ 0.32 min) and the control regions (control 1; 1.87 $\pm$ 0.45 min; control 2: 2.03 $\pm$ 0.42 min). Similar trend was observed for the number of entries made by female wasps, where a significantly greater number of
The main volatile components: monoterpenes, sesquiterpenes, oxygenated monoterpenes and cyclic terpenes were predominantly found in both immature and mature jamun fruit volatiles. Compounds like 3,7-nonatriene, 4,8-dimethyl-3E (38.6 µg/ml) and phenol group containing compounds like methylguaiacol (81.9 µg/ml) were exclusively found in immature fruit volatiles and completely absent in mature fruit volatiles. Aldehydes such as decanal (4.4 µg/ml) and n-heptanal (4.26 µg/ml) were present only in mature fruit volatiles, while ethyl-benzaldehyde (5.93 µg/ml) and cis-5-methyl-2-isopropyl-2-hexene-1-al (1.23 µg/ml) were present only in immature fruit volatiles and not present in mature fruit volatiles. The esters/ acids such as ethyl benzoate, methyl salicylate, 1,2,4-benzenetricarboxylic acid, and 1,2-dimethyl ester were found significantly (P = 0.05) in higher concentrations (342.9, 79.7 and 1.31 µg/ml) in mature fruit volatiles compared to the immature fruit volatiles (5.44, 5.5 and 0.31 µg/ml). Compounds such as benzoic acid, 4-ethoxy- ethyl ester (6.73 µg/ml), acetic acid, oxo ((1-phenylethyl) amino)-, hydrazide (1.90 µg/ml) were exclusively present in mature fruit volatiles and completely absent in immature fruit volatiles, whereas ethyl p-ethoxybenzoate (27.05 µg/ml) was found only in immature fruit volatiles and completely absent in mature fruit volatiles.

O-decylhydroxylamine (3.50 µg/ml), an amine group-containing compound and 3-ethylacetophenone (6.61 µg/ml) were found only in immature fruit volatiles and completely absent in mature fruit volatiles. Similarly, acetophenones like p-acetylbenzene (8.70 µg/ml) were found only in mature fruit volatiles and not in immature fruit volatiles.

The compounds of alkane group like n-undecane (192.7, 196.3 µg/ml) and 2,6,11-trimethyldecane (39.50, 72.10 µg/ml) were predominantly found in both mature and immature fruit volatiles respectively. Compounds like 2,4,6-trimethyldecane (27.2 µg/ml), 2-methyltetradecane (25.90 µg/ml), 2-methyltetradecane (33.80 µg/ml), 2,6,10-trimethyltetradecane (23.70 µg/ml) and 7,7-diethylheptadecane (1.50 µg/ml) were exclusively found in mature fruit volatiles and completely absent in immature ones. Compounds like 2,5-dimethyl-2-undecene (9.98 µg/ml), 3,7-dimethyl decane (198.95 µg/ml), n-tetradecane (3.75 µg/ml), n-cetane (43.83 µg/ml), phytane (9.63 µg/ml) and 2,6,10,15-tetramethylheptadecane

Figure 1. Behavioural response of female Anselmella kerrichi to volatiles collected from infested mature (a, b) and immature fruits (c, d) of jamun. Syzygium cumini (n = 12). a. Time spent by the wasps did not differ significantly between the mature fruit volatiles and control (P = NS). b. Wasps made a significantly greater number of entries into mature fruit volatiles-treated region over control (P = 0.02). c. Time spent by the wasps differed significantly between immature fruit volatiles and control (P = 0.03). d. Wasps made a significantly greater number of entries into the immature fruit volatiles-treated region over control (P = 0.002).

Figure 2. Dual-choice assay showing the behavioural response of A. kerrichi to volatiles collected from infested immature and mature fruits of jamun. a. Wasps spent significantly more time (P < 0.0001). b. Wasps made significantly greater number of entries in immature fruit volatiles treated region compared to other treatments (P = 0.02).

Table 1. log-transformed data of the main volatile compounds present in the volatile profile emissions (Figure 3) (Supplementary Table 1). The main volatile components: monoterpenes, namely α-pinene (76.53 µg/ml), 37.3 µg/ml), β-pinene (47.5 µg/ml), 33.2 µg/ml) and allo-oicimene (83.24 µg/ml), 7.6 µg/ml) were found to vary significantly (P = 0.05) between the immature and mature jamun fruit volatiles respectively. Compounds like 3,7-octadiene-2,6-diol, 2,6-dimethyl (10.56 µg/ml), 3-carene (15.62 µg/ml), neo-allo-oicimene (42.41 µg/ml), α-pyronene (8.58 µg/ml) and elixene (10.27 µg/ml) were exclusively found in immature fruit volatiles and completely absent in mature fruit volatiles, while compounds such as β-myrcene (24.2 µg/ml), limonene (5.21 µg/ml), trans-β-oicimene (52.1 µg/ml), linalool oxide (12.2 µg/ml), trans-linalool oxide (furanoid; 10.8 µg/ml) and caryophyllene oxide (8.60 µg/ml) were found only in mature fruit volatiles and completely absent in immature fruit volatiles.

The acyclic monoterpenoid, 1,3,7-nonatriene, 4,8-dimethyl-3E (38.6 µg/ml) and phenol group containing compounds like methylguaiacol (81.9 µg/ml) were exclusively found in immature fruit volatiles and completely absent in mature fruit volatiles. Aldehydes such as decanal (4.4 µg/ml) and n-heptanal (4.26 µg/ml) were present only in mature fruit volatiles, while ethyl-benzaldehyde (5.93 µg/ml) and cis-5-methyl-2-isopropyl-2-hexene-1-al (1.23 µg/ml) were present only in immature fruit volatiles and not present in mature fruit volatiles. The esters/ acids such as ethyl benzoate, methyl salicylate, 1,2,4-benzenetricarboxylic acid, and 1,2-dimethyl ester were found significantly (P = 0.05) in higher concentrations (342.9, 79.7 and 1.31 µg/ml) in mature fruit volatiles compared to the immature fruit volatiles (5.44, 5.5 and 0.31 µg/ml). Compounds such as benzoic acid, 4-ethoxy- ethyl ester (6.73 µg/ml), acetic acid, oxo ((1-phenylethyl) amino)-, hydrazide (1.90 µg/ml) were exclusively present in mature fruit volatiles and completely absent in immature fruit volatiles, whereas ethyl p-ethoxybenzoate (27.05 µg/ml) was found only in immature fruit volatiles and completely absent in mature fruit volatiles.
Alloocimene, alloocimene, and caryophyllene in the immature fruit HIPVs (Figure 4).

The four arm olfactometer bioassays with individual A. kerrichi antenna revealed eleven electrophysiologically active compounds, viz. α-pinene, β-pinene, (z)-ocimene, undecane, 3,7-dimethyl decane, neo-alloocimene, allo-ocimene, ethyl benzoate, 2,6,11-trimethyldecane, α-copaene, β-caryophyllene in the immature fruit HIPVs (Figure 3).

Coupled GC-EAD using female A. kerrichi antenna revealed eleven electrophysiologically active compounds, viz. α-pinene, β-pinene, (z)-ocimene, undecane, 3,7-dimethyl decane, neo-alloocimene, allo-ocimene, ethyl benzoate, 2,6,11-trimethyldecane, α-copaene, β-caryophyllene in the immature fruit HIPVs (Figure 3).

The four-arm olfactometer bioassays with individual synthetic compounds revealed that the female wasps spent significantly more time in the treated regions of synthetic compounds, namely ethyl benzoate, α-pinene, β-pinene, (z)-ocimene, undecane, allo-ocimene and ethyl benzoate. Similar trend was noticed with the number of entries made by female wasps into the arm treated with synthetic compounds over respective control arms. Female wasps did not show any preference for β-caryophyllene.

**Table 1.** Response of female *Anselmella kerrichi* to electrophysiologically active compounds (10 μl) tested individually in a four-arm olfactometer bioassay (n = 12). Time spent and entries made by the female wasps into the treated regions were compared

<table>
<thead>
<tr>
<th>Compound</th>
<th>Time spent (min)</th>
<th>Entries (number)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment</td>
<td>Control</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>6.52 ± 0.43</td>
<td>1.06 ± 0.21</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>5.44 ± 0.40</td>
<td>1.51 ± 0.13</td>
</tr>
<tr>
<td>(z)-Ocimene</td>
<td>3.45 ± 0.40</td>
<td>2.06 ± 0.17</td>
</tr>
<tr>
<td>Undecane</td>
<td>5.93 ± 0.68</td>
<td>1.33 ± 0.27</td>
</tr>
<tr>
<td>Allo-ocimene</td>
<td>4.56 ± 0.69</td>
<td>2.16 ± 0.80</td>
</tr>
<tr>
<td>Ethyl benzoate</td>
<td>5.24 ± 0.89</td>
<td>1.39 ± 0.30</td>
</tr>
<tr>
<td>β-Caryophyllene</td>
<td>2.02 ± 0.34</td>
<td>2.55 ± 0.24</td>
</tr>
</tbody>
</table>

(0.92 μg/ml) were only found in immature fruit volatiles and completely absent in mature fruit volatiles. Ketone group-containing compound 1-adamantyl methyl ketone (3.55 μg/ml) was found to be present only in immature fruit volatiles and not in mature fruit volatiles. Whereas alkyl benzene, p-cymene (5.80 μg/ml) was found only in mature fruit volatiles and absent in immature fruit volatiles (Figure 3).

**Figure 3.** Heat map showing differences in the volatile emissions of infested mature and immature jamun fruits.
either for time spent or entries made into the treated regions over control (Table 1). Due to the non-availability of compounds 3,7-dimethyl decane, neo-alloocimene and 2,6,11-trimethyldodecane, olfactometer bioassays for these were not carried out.

The gravid female eulophid wasps spent significantly more time ($P < 0.0001$) in the synthetic blend treated region (6.03 ± 1.31 min) compared to the control (1.57 ± 0.41). The number of entries was also more in the treated region (2.6 ± 0.78 min) compared to the control (1.2 ± 0.39). In the dual-choice assay, when both the blends (synthetic and natural) were given, the female wasps spent significantly more time ($F_{3,36} = 57.43, P < 0.0001$) in the synthetic blend-treated region (6.69 ± 0.24 min) over natural blend (3.23 ± 0.53 min) and the control regions (control 1: 1.81 ± 0.33 min; control 2: 0.66 ± 0.18 min). Similar trend was observed for the number of entries made by female wasps, where a significantly greater number of entries ($F_{3,36} = 4.38; P = 0.01$) was noticed into the synthetic blend-treated region (2.81 ± 0.25)
RESEARCH ARTICLES

compared to the regions treated with natural blend (2.01 ± 0.33) and the controls (control 1: 1.70 ± 0.52; control 2: 1.00 ± 0.26) (Figure 5).

Field evaluations revealed a significant number of captures in the traps that contained synthetic blend as the lure (treatment = 2.86 ± 0.73; control = 0.05 ± 0.05; Mann–Whitney U = 73.50; P < 0.0001) over control.

Discussion

Plant-produced HIPVs (when attacked by herbivores) are known to have multiple biological functions in herbivore–plant interactions involving conspecific/heterospecific herbivores, natural enemies, neighbouring plants and different parts within the damaged plant27. Therefore, HIPVs are considered to trigger multi-trophic interactions and also aid trophic relationships, supporting the fitness and survival of members representing diverse guilds27.

The response of conspecific herbivores to HIPVs remains confusing because of their contradictory behavioural responses to these volatiles. Earlier studies mention that the ovipositing herbivores prefer plants that do not emit HIPVs1. This might be due to the fact that early detection and avoidance of damaged plants by conspecifics have numerous benefits such as avoiding intra- or interspecific competitors28; heightened defence response in attacked plants29, higher parasitization and predation30. The HIPVs also attract conspecific herbivores for similar benefits like progeny fitness14, compromised plant defences15, avoiding natural enemies16 and finding mating partners10,12. Therefore, the role of HIPVs in the case of conspecific herbivores is puzzling as they have opposed roles like attraction3, and avoidance31, as the case may be, in the evolutionary context of their progeny fitness.

Egg-laying choice of female is always a trade-off between the immediate risk by natural enemies and availability of food resources32. The latter often becomes more important for egg-laying females as depletion of food resources impacts the survival of their progeny adversely when the mobility of immature stages is limited. In the present study, female wasps were attracted to HIPVs released from the immature fruits over mature fruits in single- and dual-choice bioassays. This may be due to the fact that female wasps can assess the quality of oviposition site in terms of food availability for their progeny. The gravid A. kerrichi wasps usually oviposit into the greenish immature fruits of S. cuminii. The emerging grubs of A. kerrichi tunnel through the pulp and reach the seed kernels. They feed exclusively on the seed kernel tissue forming a gall-like shell case and pupate within the seed33. The mature, ripe fruits of S. cuminii are deep purple in colour and cannot be retained on the tree for long (http://agritech.tnau.ac.in/horticulture/horti_fruits_jamun.html). Thus, the female A. kerrichi would have sensed the unsuitable phenological stage of the host fruit through HIPVs of the mature fruits and would have avoided them as the eggs laid by wasps will have a better chance at reaching adulthood in immature fruits and the wasp grubs will have time to develop and close into adults. Similar behaviour was observed in Pieris brassicae, where gravid females were observed to assess resource depletion in terms of future quantity and/or quality potential of food plants to their progeny while making their egg-laying choice33.

The heat-map analysis of HIPVs released from immature and mature fruit stages of S. cuminii differed significantly in their volatile composition both qualitatively and quantitatively, indicating these differences might be perceived by the gravid female wasps while choosing their egg-laying sites. The synthetic GC-EAD active compounds, namely ethyl benzoate, α-pinene, β-pinene, (Z)-ocimene, undecane, allo-ocimene, and ethyl benzoate could attract female wasps in olfactometer bioassays establishing their role in oviposition site selection of A. kerrichi. Further, the synthetic blend prepared from these active compounds could successfully attract the female wasps both in olfactometer assays as well as on the field.

Conclusions

The present study emphasizes that herbivores might assess the nutritional quality of an oviposition site based on HIPVs to reduce the risk of offspring losses. These HIPVs not only aid the herbivores regarding the compromised defences of host plant (=weak host) due to conspecifics, but also help the gravid females to track the

Figure 5. Single-choice assay showing the behavioural response of A. kerrichi to synthetic blend of infested immature jamun fruits. (a) Wasps spent significantly more time (P < 0.0001) and (b) made significantly greater number of entries into regions treated with synthetic blend compared to other treatments (P = 0.01). c, d, Dual-choice assay showing the behavioural response of A. kerrichi to headspace sample collected from infested immature fruits of S. cuminii and its synthetic blend.
resource depletion thereby impacting their oviposition site selection. The attraction of female A. kerrichi to synthetic HIPVs in the laboratory and field conditions ascertains the scope of using them as IPM tools for sustainable management of this phytophagous eupholid seed-borer.


ACKNOWLEDGEMENTS. We thank the Director, ICAR-Indian Institute of Horticultural Research, Bengaluru for providing research facilities. Technical support from T. S. Rajanna and J. Sagar is acknowledged. Financial support from the Indian Council of Agricultural Research, New Delhi through a National Fellow Project is acknowledged.

Received 16 January 2021; revised accepted 11 May 2021
doi: 10.18520/cs/v121/i2/286-293