Electrophysiological and behavioural responses of sweetpotato weevil, *Cylas formicarius* to green leaf volatiles and terpenoids

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The key to modifying the behaviour of sweetpotato weevil (SPW) *Cylas formicarius* Fab. lies in understanding the olfactory receptor system and its response to various host plant volatiles produced by sweetpotato. Green leaf volatiles and aromatic terpenoids have been used to study the olfactory receptor depolarization, recovery time from action potential and the rate of response in male and female *C. formicarius* using electroantennography. Among the 12 compounds tested, *cis*-3-hexen-1-ol elicited consistently high action potentials in both genders, indicative of hyperpolarization of olfactory receptors. Highest axonal action potentials were elicited by α-humulene, sabinene, (–)-*trans*-caryophyllene, *cis*-3-hexen-1-ol and *cis*-3-hexenal from female antennal receptors compared to those of males ($P < 0.01$). The rate of response was found to be higher in females than male weevils to each of the compounds. *Cis*-3-hexen-1-ol which produced highest amplitude (depolarization), was observed to have attracted the least number of weevils. *Cis*-3-hexen-1-ol, *cis*-3-hexenal and α-humulene attracted much fewer females in dual choice olfactometer tests, indicating that these compounds act as repellents to both male and female SPWs. Except ocimene, ylang-ylang oil and *cis*-3-hexenyl acetate, all the nine compounds acted as repellents to SPW in olfactometer bioassays.

Keywords: *Cylas formicarius*, electroantennography, olfactometry, semiochemicals, sweetpotato.

THE sweetpotato weevil (SPW), *Cylas formicarius* Fab. (Coleoptera: Curculionidae), is the most important economic pest in the sweetpotato growing countries. It causes damage in both field and storage, and encourages secondary infestation by many storage pests. In India, SPW damage to the crop ranges from 5% to 65%, depending on the geographic location. Several bitter-tasting and toxic furanoterpenoids are synthesized by the storage roots in response to feeding by SPW larvae, which renders the product unfit for consumption[1]. Farmers manage the pest unsuccessfully, as they are often dependent on only cultural methods of control, and resist using any synthetic pesticides, as every part of the sweetpotato plant is used for different purposes – the vines as cattle feed and the storage roots for human consumption. Often the weevils are noticed in the canopy above-ground and infest the vines; much damage is found in the storage roots below the ground. Because of the cryptic nature of the SPW, it is essential to control the pest targeting both above- and below-ground populations. In general, integrated pest management strategies are more successful for above-ground insects, for which several tactics have been developed, often ignoring the below-ground/soil insect pests, emphasizing the need to develop management tactics for such cryptic pests.

Semiochemicals have been successfully used in the management of a number of insect pest species. Sex pheromones for SPWs are used for monitoring and controlling them, but they attract only male weevils, allowing female weevils to continue to damage or oviposit on the crop. Female weevils can inflict significant loss to storage roots, as they are attracted more towards storage roots than the males[2–4]. Attracting or repelling the pest using behaviour-altering chemicals is often adopted when the product is of high value and pesticide sprays are not acceptable. Sweet potato (*Ipomoea batatas* (L.) Lam (Convulvulacea) plants produce different types of compounds with activity towards SPW[2,3,5], and the degree to which plants attract SPWs is known to vary among varieties[2,4,5] and species of *Ipomoea* L.[6]. A total of 33 compounds were identified from storage roots and aerial parts of sweetpotato, including 23 terpenes[3]. The sesquiterpene volatile fraction acted as a repellent to female SPW and three oxygenated monoterpenes acted as attractants[3]. Decades after the discovery of many of the behaviourally active compounds to *C. formicarius* from its host plant, there is no information available on the practical use of such compounds in SPW management, especially those of the repellants or female attractants, in spite of availability of modern electrophysiologically sensitive instrumentation.

Electrophysiology coupled with olfactometer studies opened up multifarious directions to understand the ecological function of the volatile compounds[7] that led to the identification of many behaviourally active compounds, including the sex pheromone of SPW[8]. The electroantennogram (EAG)[9], registering responses to wide varieties of odourants, has been successfully used for pheromone identification in the past and it is now used widely for identification of host-odour volatiles important to behaviour such as attraction[10,11]. The use of insect antenna as an odour detector could be one of the most sensitive techniques for the detection of volatile compounds, especially when they are present at low concentrations[12]. The insect antenna consists of a number of olfactory receptors[13,14] which respond differently to compounds[15,16] with differences in amplitude (action potential or depolarization), recovery time and sensitivity[15]. The variations in

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sensitivities of antennal olfactory receptors help understand the differential responses of *C. formicarius* using the EAG system. A better understanding of the female olfactory reception would help identify behaviour-modifying host plant volatiles for the female SPW. Thus, the present study is designed to elucidate the complexities in the olfactory reception in both male and female SPWs.

Sweetpotato storage roots (tubers) were collected from experimental plots maintained at the Regional Centre, Central Tuber Crops Research Institute (ICAR), Bhubaneswar and put in plastic cylindrical boxes (14 cm base diameter, 19 cm height, with the top covered with cotton cloth) for emergence of SPWs. The newly emerged weevils were provided with 1% honey solution every alternate day and maintained at 28°C in BOD incubator (model no. YORCO YSI-440, York Scientific Industries Pvt Ltd, New Delhi). Healthy and active weevils, starved for 48 h, were used for EAG or Y-tube olfactometer bioassays.

Twelve compounds (Sigma Aldrich, USA) were used to study the olfactory responses from antennal receptors and *Y*-tube olfactometer bioassays. Among these, the first group consisted of eight single pure compounds (α-humulene, α-gurjunene, methyl jasmonate, sabinene, (–)-*trans*-caryophyllene, *cis*-3-hexen-1-ol, *cis*-3-hexenal and *cis*-3-hexenyl acetate), and the second group consisted of four mixtures of compounds or geometric isomers (geraniol, ocimene, citral, ylang-ylang oil). The test compounds were selected as these were reported to occur in various parts of sweetpotato[3,15]. Ylang-ylang oil was used since ylangene was not available commercially.

The EAG method[9] is an electrophysiological method which registers the olfactory responses of antennal receptors of insects to volatile compounds. The use of insect antennae as an odour detector is one of the most sensitive techniques for the detection of volatile compounds. In our study, the antennal responses of adults of both genders to the 12 compounds were investigated using the standard EAG method[4]. Each of the test compounds was dissolved in *n*-hexane at a concentration of 0.1% (v/v). The olfactory stimuli were prepared by applying 50 µl of solution onto filter paper (Whatman no. 1) strips, 5 cm × 0.5 cm in size. The solvent was allowed to evaporate for 1 min before placing the impregnated filter paper in a glass Pasteur pipette (6 cm long). The narrow terminal of the pipette was inserted into a hole (2 mm diameter, 150 mm upstream from the outlet) on the wall of the mixing tube (8 mm diameter) of the EAG where constant humidified air was delivered over the insect antenna at 50 ml/min. The mixing tube was kept at a distance of 5 mm from the antennal arrangement. By injecting a puff of purified air for 0.2 sec and using a stimulus controller (model CS-55, Syntech® Ltd, Germany), odour stimulation was administered. By puffing compounds to the insect, the EAG signal was recorded only after the instrument was stabilized and a baseline signal was obtained. Two consecutive stimuli were recorded in a time gap of 1 min in order to provide time for recovery of antennal olfactory responsiveness from the previous signal. The EAG response was amplified, recorded, analysed and stored by the EAG system (Syntech®, Germany), which was linked to a computer system (Windows XP) with IDAC-2 (data acquisition interface board) using EAG Pro software. The maximum amplitude of depolarization elicited by the antennal response of insects to volatiles was registered as absolute EAG responses. *Cis*-3-hexen-1-ol, which elicited the highest EAG response, was used as control and also to ascertain the life of antenna during the entire process. This process was repeated four times for each compound.

A dual-choice olfactometer bioassay was used to test the responsiveness of SPW to different compounds. The olfactometer consisted of a *Y*-shaped glass tube (borosilicate glass), with all the three cross-sectional terminals attached to small glass lids separately. These glass lids had chambers categorized as treatment, control and main chambers. The former two were attached to the top two arms of the *Y*-tube while the latter was at the basal end. Each of the three arms of the *Y*-tube was 20 cm long and 30 mm diameter. Next 50 µl each of chemical (0.1% v/v) and control solutions (*n*-hexane) was impregnated onto separate filter papers (Whatman no. 1, 6 cm × 6 mm), allowed to evaporate and then placed in the removable glass lid of the treatment and control chambers respectively. Thirty, 10-day-old weevils were released one after another and their choice was recorded. Weevils found in any arm just after 1 cm of the junction point were regarded as having a choice to that arm (either towards the compound or the control). The experiment was conducted in dark and a black cloth was spread below the *Y*-tube to prevent weevils from getting disturbed with visual cues. The *Y*-tube was rotated by 180° once to prevent the directional influence. The experiment was repeated four times for each compound and sex. The *Y*-tube was cleaned and rinsed with hexane after every experiment.

To find whether there is any significant difference between the responses of male and female weevils to the compounds, the data were analysed with two-sample or two-tailed Student's *t*-test ($P \leq 0.01, 0.05$) to compare the response between genders. Analysis of variance (ANOVA) was also performed to know the differential response of male and female *C. formicarius* to different compounds. Means were separated using Duncan's Multiple Range Test (DMRT). The chi-square (χ^2) test was conducted to determine the choice of the weevils moving towards or away from the compound under the assumption of independence.

Insects have developed a highly receptive olfactory system that perceives many volatile compounds which guide them to their host plants or to suitable habitat for mating or oviposition[17–20]. To understand the preference of SPW towards its host plant, it is necessary to

Table 1. Behavioural response (%) of sweetpotato weevil *C. formicarius* (Fab.) that moves towards standard (single) compounds in a dual-choice olfactometer

Standard compound	Response (%) of weevils			
	Male	Female	$t_{6;0.05}$, $t_{6;0.01}$*	χ^2 value (*P*-value)
α-Humulene	19.17b	6.67ab	6.89**	8.335 (0.004)
α-Gurjunene	5.00a	7.50ab	–1.25**	5.45 (0.0195)
Methyl jasmonate	25.00b	24.17c	0.12^{NS}	0.022 (0.881)
Sabinene	5.00a	5.83a	-0.36^{NS}	0.08 (0.776)
(–)-*Trans*-caryophyllene	5.83a	16.67bc	–2.73*	7.053 (0.008)
Cis-3-hexen-1-ol	5.00a	5.83a	-0.76^{NS}	0.08 (0.776)
Cis-3-hexenal	6.67a	10.00ab	-1.42^{NS}	0.87 (0.35)
Cis-3-hexenyl acetate	5.83a	35.84d	–4.59**	4.50 (0.034)
$F_{7,21;0.05}$, $F_{7,21;0.01}$#	$7.22^{\#\#}$	$10.00^{\#\#}$		

Values in the column not followed by the same letters are significantly different ($P \leq 0.05$) by Duncan's Multiple Range Test (DMRT).

*$t_{6;0.05}$, $t_{6;0.01}$ table value: Probability, $P = 0.01 = 3.707$ denoted by **, $P = 0.05 = 2.447$ denoted by *, NS, non-significant.

#$F_{7,21;0.05}$, $F_{7,21;0.01}$ table value: Probability, $P = 0.01 = 3.64$ denoted by ##, $P = 0.05 = 2.49$ denoted by #, NS, Non-significant. χ^2 table value: Probability $P = 0.01 = 6.64$, $P = 0.05 = 3.84$.

Table 2. Behavioural response (%) of *C. formicarius* Fab. that moves towards standard (mixture) compounds in a dual-choice olfactometer

Standard compound	Response (%) of weevils			
	Male	Female	$t_{6;0.05}$, $t_{6;0.01}$*	χ^2 value (*P*-value)
Geraniol	4.99a	13.33a	–3.78**	5.004 (0.025)
Citral	8.33a	8.34a	0.87^{NS}	4.75 (0.029)
Ocimene	4.17a	30.83b	–7.65**	11.88 (0.0005)
Ylang-ylang oil	19.17b	41.67c	–3.28*	14.35 (0.0002)
$F_{3,9;0.05;}F_{3,9;0.01}$#	$15.45^{\#\#}$	$24.53^{\#\#}$		

Values in the column not followed by the same letters are significantly different ($P \leq 0.05$) by DMRT.

*$t_{6;0.05}$, $t_{6;0.01}$ table value: Probability, $P = 0.01 = 3.707$ denoted by **, $P = 0.05 = 2.447$ denoted by *; NS, non-significant.

#$F_{3,9;0.05}$, $F_{3,9;0.01}$ table value: Probability, $P = 0.01 = 6.99$ denoted by ##, $P = 0.05 = 3.86$ denoted by #; NS, Non-significant.

χ^2 table value: Probability, $P = 0.01 = 6.64$, $P = 0.05 = 3.84$.

understand its olfactory behaviour towards the compounds produced by the sweetpotato plant. The dual-choice olfactometer bioassay studies showed that the female weevils respond more to the first (Table 1) and second groups (Table 2), whereas greater number of weevils moved away from all the 12 compounds. In general, these 12 compounds attracted males to the order of 5–25%, whereas in females the response was 5.83–1.67%. The response of females to terpenoids compared to green leaf volatiles was higher than that of males[4,21–25]. Of the several compounds tested in the first group, α-humulene had attracted male weevils, thrice the number of females ($t_{6;\,0.01} = 6.89$) followed by methyl jasmonate; whereas a significantly higher number of female weevils was found to be attracted to α-gurjunene, (–)-*trans*-caryophyllene and *cis*-3-hexenyl acetate than males ($P < 0.05$). Males were least attracted to *cis*-3-hexen-1-ol, sabinene and α-gurjunene and females to sabinene, suggesting that these compounds may contain repellent activity against *C. formicarius* (Table 1).

Likewise, when tested with the second group of compounds, in general, all the four compounds, viz. geraniol, citral, ocimene, ylang-ylang oil attracted more female weevils than the males. Inversely, the low percentage indicates repellency of the compounds. However, a significantly higher number of females was attracted to geraniol, ocimene and ylang-ylang oil ($P < 0.05$). Both male (19.17%) and female (41.67%) weevils showed highest response to ylang-ylang oil compared to the remaining second array compounds. Ocimene and citral have the least response to male (4.17%) and female (8.34%) weevils respectively, compared to rest of the compounds (Table 2). There was a significant difference between expected and observed values of percentage response of weevils towards different compounds, indicating that the movement of weevils either towards the compounds or away from them, is not by chance, but by preference (χ^2 test, $N = 30$, $P < 0.05$; Tables 1 and 2). The ability of both genders to detect plant volatiles is probably due to their similar habitat, which requires the

use of the same clues to locate host-plants for survival and reproduction[16]. Less attraction of females to α-humulene, α-gurjunene, sabinene, *cis*-3-hexen-1-ol and citral shows that these compounds may be acting as repellents[26]. Female SPWs were attracted to methyl jasmonate, (–)-*trans*-caryophyllene, *cis*-3-hexenyl acetate, geraniol, ocimene and ylang-ylang oil, but to a lesser degree, indicating a relatively high level of binding site-specificity for these compounds[27]. Geraniol and (*E*)-citral, the geometrical isomers of nerol and (*Z*)-citral, also attracted female SPWs. The female was repelled by α-humulene, α-gurjunene and ylangene at a concentration range 0.1–10 nl/10 µl hexane[3]. The sesquiterpene volatile fraction, comprising these three compounds, was released from storage roots[3]. Volatiles, i.e. the mixture of compounds considered in the second array, were found more effective than the single volatile chemicals in the first array[28].

The higher depolarization in female antennae is possibly because of highly receptive olfactory system that is sensitive to plant volatiles[21]. Several studies have measured EAG responses to single, known odourants under field conditions[29–34], which help measure the relative concentrations and fluctuations of known semiochemical components in ambient air on site in real time. Among the 12 compounds, the highest receptor amplitude was recorded with *cis*-3-hexen-1-ol in both sexes of *C. formicarius* (males 3.37 mV and females 6.01 mV), which is indicative of hyperpolarization of olfactory receptors, when stimulated with *cis*-3-hexen-1-ol[15], whereas the lowest amplitude (0.16 mV) was in males to (–)-*trans*-caryophyllene and in females (0.38 mV) to methyl jasmonate (Figure 1 *a*). A significantly greater amplitude was observed to be elicited from female antennal receptors to α-humulene, sabinene, (–)-*trans*-caryophyllene, *cis*-3-hexen-1-ol and *cis*-3-hexenal compared to males ($P < 0.01$). The action potential (amplitude) of both male and female SPWs was significantly different across each compound ($P < 0.01$). Out of the four compounds in the second group, significantly highest amplitude was observed in the EAG peaks of ylang-ylang oil in both male (1.29 mV) and female (2.63 mV) weevils ($P < 0.01$), while the significantly lowest amplitude was measured to be 0.38 mV in the response of males to ocimene and 1.26 mV in the response of females to geraniol (Figure 1 *a*). The alcohol property of this compound possibly caused the artificial hyperpolarization.

Among the first group of compounds, α-humulene and α-gurjunene elicited EAG response for the longest time duration ($F_{7,14;\,0.05} = 3.59$) from male weevils, and *cis*-3-hexen-1-ol and *cis*-3-hexenal from the female weevils ($F_{7,14;\,0.01} = 1.94$; Figure 1 *b*). EAG duration in male weevils was noted to be significantly higher than that of female weevils after exposure to *cis*-3-hexenyl acetate, while the reverse case was found in methyl jasmonate. It is observed that different EAG shapes occur and the responses to methyl jasmonate recover faster than those to other chemicals. The shape corresponds to the affinity of the chemical for the receptor sites. Kaissling[35] proposed that recovery is related to compound-specific velocities of an early inactivation. The EAG responses of female SPW to components of ylang-ylang oil are characterized by slower return to the baseline than responses to other chemicals. Among the second array of compounds, both male (4.33 sec) and female (2.31 sec) weevils responded significantly for the longest duration to ylang-ylang oil. Males responded for longer duration than females, indicating that male weevils recover much slower than females. The rate of response elicited by female antennal receptors was found to be the highest for *cis*-3-hexen-1-ol and lowest for methyl jasmonate. Among the first group of compounds, the rate of EAG signal was recorded to be significantly higher in females than males, except for methyl jasmonate (Figure 1 *c*). Among the second group of compounds, significantly highest rate of EAG response from male weevils was found for ylang-ylang oil (0.35 mV/sec) and in case of female weevils the same was noted for citral (1.45 mV/sec).

The observed EAG responses depend on sensitivity of the olfactory receptors on the antenna; and relative comparison can be made between odourants[15]. In general, greater amplitude of the antennal receptors was observed in female weevils than males. Female SPWs could detect specific compounds, even if they were present and/or emitted in minute quantities[5]. In this study, greater response in females in low or moderate doses, elicited by volatiles from aromatic compounds, sesquiterpenoids and monoterpenoids, showed that these plant volatiles are important olfactory cues in host-plant location for female insects. The receptors on antennae of male weevils require more time to recover, when exposed to the sesquiterpenoids under study (α-humulene, α-gurjunene); and the same is for green leaf volatiles (*cis*-3-hexen-1-ol and *cis*-3-hexenal) in case of females. It appears that the activity of the insect olfactory system is related to molecular structures of behaviour-modifying chemicals. Stimulation with alcohols results in higher EAG responses than does stimulation with aldehydes[15]. This has been observed in our experiment with *cis*-3-hexen-1-ol and *cis*-3-hexenal. The effectiveness or response (amplitude) appears to be optimal with the hydroxyl group, indicating that the change in the functional group at the C-chain terminal reduces the response[15,36].

Considering the result of the structurally related compounds, it can be interpreted that in case of male weevils, there is an inverse relationship of action potential (amplitude) with behavioural responses in olfactometry. This indicates that less attraction of male weevils (i.e. more repellency) in olfactometry bioassay shows higher EAG response. The results also indicate that in females, action potential decreases through alcohol to aldehyde and finally to acetate, which is also in the same order of increase in the percentage of attractiveness. The compounds which

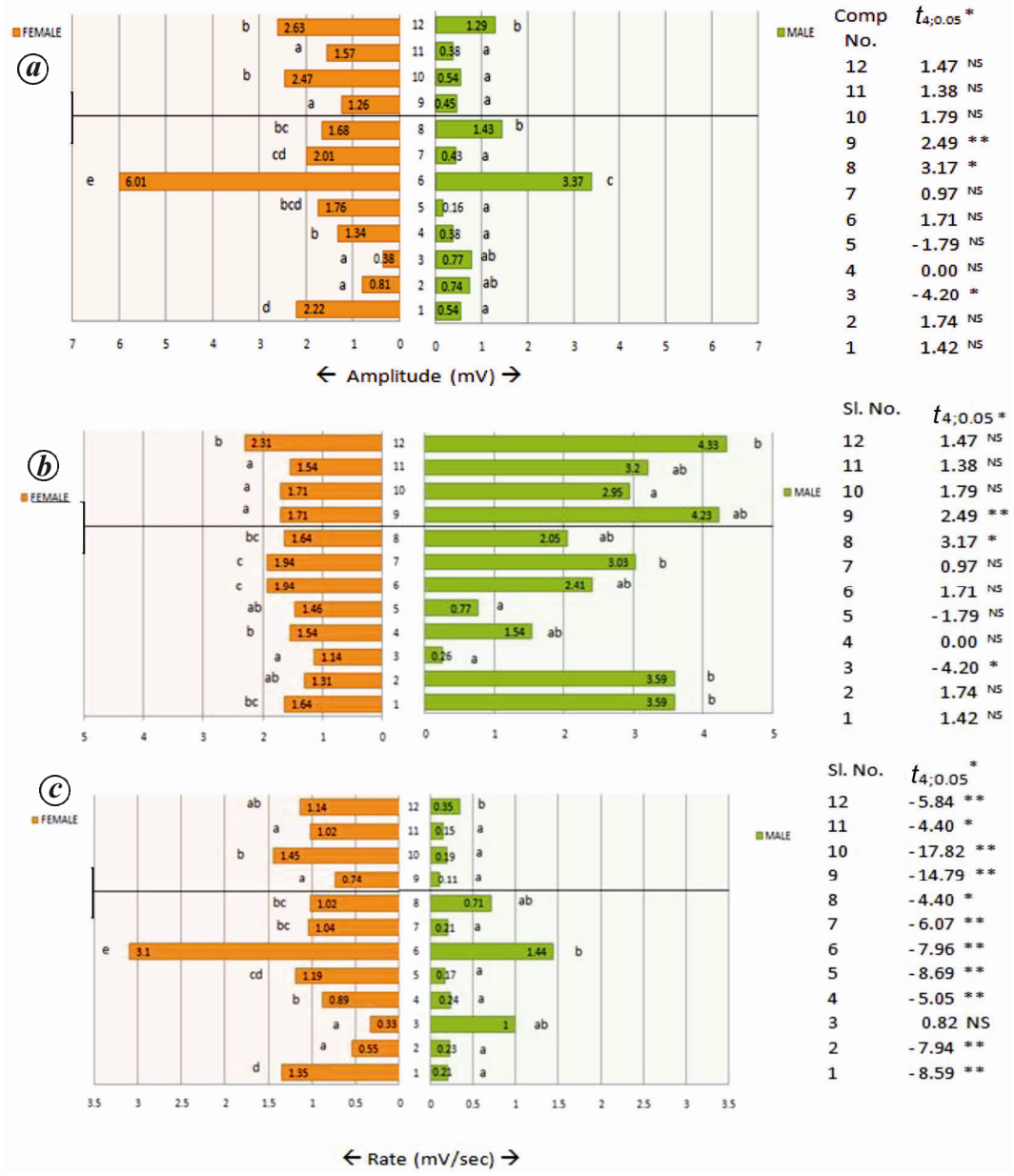

Figure 1. ***a***, Mean EAG amplitude of the response of female and male *Cylas formicarius* to volatile compounds (1, α-Humulene; 2, α-Gurjunene; 3, Methyl jasmonate; 4, Sabinene; 5, (–)-*Trans*-caryophyllene; 6, *Cis*-3-hexen-1-ol; 7, *Cis*-3-hexenal; 8, *Cis*-3-hexenyl acetate; 9, Geraniol; 10, Citral; 11, Ocimene, and 12, Ylang-ylang oil), $F_{7,14;\,0.05}$, $F_{7,14;\,0.01}$ $^{\#}$Calculated value: 14.6$^{\#\#}$ for male sweet potato weevil (SPW) and 121.98$^{\#\#}$ for female SPW in response to first array compounds, $F_{3,6;\,0.05}$, $F_{3,6;\,0.01}$. $^{\$}$Calculated value: 58.88$^{\$\$}$ for male SPW and 15.15$^{\$\$}$ for female SPW in response to second array compounds. ***b***, Mean EAG recovery time of the response of female and male *C. formicarius* to volatile compounds, $F_{7,14;\,0.05}$, $F_{7,14;\,0.01}$ $^{\#}$Calculated value: 2.86$^{\#}$ for male SPW and 6.1$^{\#\#}$ for female SPW in response to first array compounds, $F_{3,6;\,0.05}$, $F_{3,6;\,0.01}$ $^{\$}$Calculated value: 2.55$^{\$\$}$ for male SPW and 25.69$^{\$\$}$ for female SPW in response to second array compounds. ***c***, Mean EAG action potential rate for the response of female and male *C. formicarius* to volatile compounds, $F_{7,14;\,0.05}$, $F_{7,14;\,0.01}$$^{\#}$. Calculated value: 1.60^{NS} for 78.27$^{\#\#}$ male SPW and for female SPW in response to first array compounds, $F_{3,6;\,0.05}$, $F_{3,6;\,0.01}$. $^{\$}$Calculated value: 7.38$^{\$}$ for male SPW and 4.94$^{\$}$ for female SPW in response to second array compounds. *a–c* values not followed by the same letters are significantly different ($P \leq 0.05$) by Duncan's Multiple Range Test. *$t_{4;0.05}$, $t_{4;0.01}$ table value: Probability, $P = 0.01 = 4.604$ denoted by **, $P = 0.05 = 2.776$ denoted by *; NS, Nonsignificant. $^{\#}F_{7,14;0.05}$, $F_{7,14;0.01}$ table value: Probability, $P = 0.01 = 4.28$ denoted by $^{\#\#}$, $P = 0.05 = 2.76$ denoted by $^{\#}$; NS, Nonsignificant. $F_{3,6;0.05}$, $F_{3,6;0.01}$ table value: Probability, $P = 0.01 = 9.78$ denoted by $^{\$\$}$, $P = 0.05 = 4.76$ denoted by $^{\$}$; NS, Nonsignificant.

elicited greater depolarization from resting potential in females were sabinene, (–)-*trans*-caryophyllene, *cis*-3-hexenal and *cis*-3-hexen-1-ol, ultimately exciting the membranes for each single puff leading to high recovery time. The highest action potential rate indicates that these compounds act precisely at the receptor level, leading to

continuous signal transduction among individual receptors. This makes the olfactory receptors of the weevils vulnerable to continuous exposure to the behaviour-modifying chemicals, allowing them either to move away from the compounds, or the females decide not to visit the chamber containing these compounds. Conversely, the two terpenoids, α-humulene and α-gurjunene, which elicited less response in males compared to females, displayed longer recovery time to attain resting potential. This suggests that these compounds have adverse effects on the preference of males to a higher extent than that of females, exposing them to open up their receptors for longer duration resulting in higher time for signal communication to the central nervous system. The present study demonstrates that different odour compounds can be distinguished by comparing the relative EAG responses of the antennae from the two genders. Further studies on the basic concepts of differential response to behaviour-modifying chemicals suggest the mechanism of the difference in perception of chemicals by male and female insects to their advantage.

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Distribution and conservation status of the caenophidian snake, *Xylophis captaini* Gower & Winkler, 2007 in the Western Ghats, India

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We update the distribution of the little known Captain's Wood Snake (*Xylophis captaini*) in the Western Ghats, based on new observations and collation of the literature. The Maximum Entropy (MaxEnt) algorithm was used to predict the distribution of the species, which showed potential sites south of 10°N and elevations between 50 and 1000 m amsl. *Xylophis captaini* is listed as 'Least Concern' under IUCN criteria, and we suggest the possible elevation of its status to the 'Near-Threatened' category on account of its narrow distributional range and general lack of data on its ecology. The present study highlights the utility of niche models in assessing the distribution of cryptic and little known species in biodiversity-rich areas such as the Western Ghats.

Keywords: Agasthyamalai Biosphere Reserve, Captain's Wood Snake, ecological niche modelling, endemic species.

THE Western Ghats is one of the 34 global biodiversity hotspots[1]. This mountain range has a rich snake fauna with several endemics, but data on their distribution range are mostly scanty. The genus *Xylophis* Beddome, 1878 is endemic to the Western Ghats and currently three species are known. Gower and Winkler described *Xylophis captaini* and provided details on the distribution of this species and its congeners[2]; *X. perroteti* and *X. stenorhynchus* based on examination of specimens deposited in various museums. More recently, *X. captaini* has been reported from Ponmudi Hills, Agasthyamalai Biosphere Reserve (ABR) of Kerala and the Ambadi Estate in Tamil Nadu[3,4].

Spatial distributions of species and the factors that regulate them are prerequisites for developing conservation plans. With the support of new algorithms to analyse spatial databases, one can predict the 'ecological niches', or at least the broad-scale spatial aspects of species based on observed occurrences. BIOCLIM[5], DOMAIN[6], Genetic Algorithm for Rule-Set Prediction (GARP)[7], Ecological Niche Factor Analysis (ENFA)[8] and Maximum Entropy (MaxEnt)[9] are a few widely used ecological niche modelling approaches. Each algorithm has its merits and limitations in predicting species distribution[10].

Xylophis captaini was suggested to be categorized as 'Least Concern' under IUCN criteria, assuming that its occurrence in a variety of habitats (agricultural fields, plantations and natural forests)[2], which was also supported by a recent assessment[11]. In this study, we update the distribution of this little known caenophidian snake based on collation from the literature[2–4] and new observations in ABR. Furthermore, notes on the habitat, extent of its distribution across elevation and comments on the conservation status of the species are discussed.

We surveyed along the southwestern slopes of the ABR from March 2012 to December 2013 using time constrained visual encounter survey[12]. Upon locating *X. captaini* (Figure 1), geo-coordinates (using a Garmin 12 Channel GPS), elevation and forest type were recorded. Snout-vent and tail length were measured using flexible twine and a metal scale (accuracy: 1 mm). Colour, number of ventrals and subcaudal scales were noted in the field and snakes were released at the site of encounter. All individuals were identified based on the taxonomic characteristics provided by Gower and Winkler[2].

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