Indian Journal of Agricultural Sciences **84** (1): 158–60, January 2014/Short Communications

Sex pheromone of citrus trunk borer (*Anoplophora versteegi*) (Coleoptera : Cerambycidae) - A new finding

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Received: 7 February 2012; Revised accepted: 9 December 2013

Key words : Citrus trunk borer, Electroentennogram, Gas Chromatograph, Olfactometer, Pheromone, Saturated hydrocarbons.

Citrus trunk borer, *Anoplophora versteegi* Ritsema (Coleoptera: Cerambycidae) is the most destructive insect pest of citrus in the entire north eastern region of India (Sachan and Gangwar 1982) and is a predominant species responsible for citrus decline. The adults of trunk borer appears in the third week of March to second week of April in the state of Meghalaya (Saikia *et al.* 2011). Cryptic nature of citrus trunk borer grub makes it difficult to control as grubs are found within the trunk of living trees. The damaged trees eventually die within a year of severe infestation. Thakur and Shylesha (1996) found 60-80 per cent damage to *Khasi* mandarin plants due to the citrus trunk borer in Meghalaya. The lack of information on the role of semiochemicals in reproduction hinders development of effective detection and management strategies for this serious pest of *Khasi* mandarin. Considering the severity of the pest, an experiment was conducted to study the presence of pheromonal compounds on adult citrus trunk borer.

The laboratory experiment was conducted in the Division of Entomology, ICAR Research Complex for NEH Region, Umiam during 2010 to study the response of male beetle to different odour sources, viz. live unmated female, dead female, live male and heads, thoraxes, abdomens, elytra and ovipositors of freeze-killed females. The odour source was kept on one arm of a plastic olfactometer (80 × 19.5 × 10 cm length × width × height and attached with two 17.75 cm long arms) and other arm was kept blank and an active male was released at the opposite end of olfactometer. At the opposite end of olfactometer with a central opening which was attached to a vacuum dust collector that sucks air through the olfactometer (3.2 m/s at exit). The data on male beetles reaching either at treated or blank arm for each odour source was recorded. The observations on non arrival of male beetle at either arm of olfactometer within 15 minutes of release were also recorded as not responded. For each odour source, the trial was replicated twenty five times. The vacuum dust collector attached to the olfactometer was run for five minutes without any oudour source at its arm before starting an event to clear the air inside the olfactometer.

In dual choice olfactometric bioassay, the highest number of male beetles (84.21%) were found to be attracted towards the female abdomen ($\chi^2 = 8.89$, $\beta<0.005$) which was followed by live unmated female ($\chi^2 = 6.25$, $\beta<0.05$) and dead female ($\chi^2 = 5.40$, $\beta<0.05$) (Table 1). Male beetles showed least response (28.57%) towards male ($\chi^2=1.29$, $\beta>0.05$). The response of male beetles to female head, thorax, elytra, ovipositor and control (hexane) were 42.86 ($\chi^2 = 0.14$, $\beta>0.05$), 62.50 ($\chi^2 = 0.50$, $\beta>0.05$), 70.00 ($\chi^2 = 1.60$, $\beta>0.05$), 69.23 ($\chi^2 = 1.92$, $\beta>0.05$) and 60.00 per cent ($\chi^2 = 0.20$, $\beta>0.05$), respectively. Laboratory bioassays confirmed that female *A. versteegi* produced a pheromone that attracted males and the pheromone persisted after females were killed and that pheromone was associated with the abdomen. Cervantes *et al.* (2006) observed that the males of *Prionus californicus* Motschulsky (Coleoptera : Cerambycidae) were strongly attracted to live females and to carcasses of freshly killed females in olfactometer bioassays. Their findings demonstrated that the female longhorned beetle (*P. californicus*) produced a volatile pheromone from the ovipositor that attracted males over a distance. Spikes *et al.* (2010) also observed that males of hard wood stump borer, *Mallodon dasystomus* (Say) (Coleoptera: Cerambycidae) attempted to mate with solvent-washed dead females treated with as little as 0.15 ± 0.03 female equivalents of conspecific

Part of Ph D thesis submitted by the first author

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Table 1 Response of male beetle to different odour sources

Treatments	No. of males responded	No. of males at		Chi-square χ^2	p
		Treated end	Open end		
Live unmated female	16 (64)	13 (81.25)	3 (18.75)	6.25	0.012
Dead female	15 (60)	12 (80.00)	3(20.00)	5.40	0.02
Head	7 (28)	3 (42.86)	4(57.14)	0.14	0.71
Thorax	8 (32)	5 (62.50)	3(37.50)	0.50	0.48
Abdomen	19 (76)	16 (84.21)	3(15.79)	8.89	0.003
Elytra	10 (40)	7 (70.00)	3(30.00)	1.60	0.21
Ovipositor	13 (52)	9 (69.23)	4(30.77)	1.92	0.17
Male	7 (28)	2 (28.57)	5(71.43)	1.29	0.26
Hexane	5 (20)	3 (60.00)	2(40.00)	0.20	0.65

Values in parenthesis are the percentage of total number of responded male beetles to a particular treatment and percentage of responded male beetle at treated and open end of olfactrometer

cuticular extracts, confirming that compounds on the cuticle of females are essential for mate recognition. However, Sugeno *et al.* (2006) observed that *Gastrophysa atrocyanea* (Coleoptera: Chrysomelidae) males attempted to mate with dummy treated with the female equivalent of the female elytra extracts. Male beetles of citrus trunk borer showed least response toward male which indicated that males did not produce any attractive volatile compound.

Studies on electrophysiological response of citrus trunk borer conducted at Division of Entomology, IARI, New Delhi during 2010 showed that the responses of male antennae to five different concentrations of female body wash extracts in hexane did not differ significantly from control honey solution (Table 2). EAG response of male antennae to the abdominal extract of female beetles was the highest for the concentration 10%, which differed significantly from other concentrations and control. The female abdominal extract at 0.001% concentration recorded the lowest response to male antennae (0.108 ± 0.048) and was on par with the 0.01% female abdominal extract and control. EAG response of *A. versteegi* male antennae to abdominal extract of female beetles was higher as compared to female's body wash, which confirmed the presence of pheromone on abdomen of female beetles. Liendo *et al.* (2005) reported that the presence of male sex pheromone in *S. breve* and female antennae showed a very strong deflection of EAG only when stimulated by odours of male head, prothorax and pterothorax with abdomen.

Table 2 Response of male antenna to different concentrations of body wash and abdominal extract of female beetles

Concentrations	Response of male antenna (–mV ± SD)	
	Female body wash	Female abdomen
0.001%	0.102 ± 0.049[a]	0.108 ± 0.048[a]
0.01%	0.124 ± 0.028[a]	0.131 ± 0.043[ab]
0.1%	0.130 ± 0.033[a]	0.519 ± 0.325[b]
1%	0.135 ± 0.016[a]	0.777 ± 0.377[c]
10%	0.140 ± 0.036[a]	1.032 ± 0.348[d]
Honey solution (10%)	0.123 ± 0.018[a]	0.119 ± 0.042[ab]
SEd	0.014	0.112
CD (P=0.05)	NS	0.224

Hydrocarbons C_{12} to C_{30} were detected on male and female beetle body wash and female abdomen extract of citrus trunk borer in gas chromatography of pheromonal

Table 3 Gas chromatographic analysis of body wash of adult *Anoplophora versteegi*

Saturated hydrocarbons	Female bodywash (ppm/5 female)	Female abdomen (ppm/5 female)	Male body wash (ppm/5 male)
Decane	ND	ND	ND
Undecane	ND	ND	ND
Dodecane	ND	53.43	ND
Tridecane	3.03	18.31	7.29
Tetradecane	ND	5797.51	1.65
Pentadecane	3.09	ND	65.32
Hexadecane	0.95	1576.45	3.84
Heptadecane	2.32	14.50	ND
Octadecane	0.65	178.13	ND
Nonadecane	8.88	54.59	101.87
Eicosane	10.73	972.46	60.99
Henicosane	5.38	360.75	144.84
Docosane	0.99	0.66	35.00
Tricosane	1.57	0.55	2.81
Tetracosane	ND	ND	0.85
Pentacosane	6.52	589.93	27.45
Hexacosane	54.99	609.37	121.61
Heptacosane	25.33	46.57	17.22
Octacosane	10.47	238.08	99.73
Nonacosane	39.03	206.68	77.51
Tricotane	27.38	35.87	130.74

ND, Not detected

compounds at Division of Entomology, IARI, New Delhi during 2010. Tridecane, pentadecane, hexadecane, nonadecane, eicosane, henicosane, docosane, tricosane, pentacosane, hexacosane, heptacosane, octacosane, nonacosane and tricosane were common in body wash of both sexes with different proportions (Table 3). Seventeen hydrocarbons were detected on abdominal extract of adult females, out of which, concentrations of tetradecane, hexadecane, eicosane, hexacosane and pentacosane were much higher in comparison to the male body wash, which might have played an important role for the attraction of male beetles. Fosbroke and Gottschalk (2001), Parker and Andrus (2002) reported that several cuticular hydrocarbons from adult females elicit mating behaviour in males. Allison *et al.* (2004) mentioned that the cuticular hydrocarbons elicited pre-copulatory or copulatory behaviour in *Psacothea hilaris* and *Xylotrechus colonus* (F.). Heptadecane, octadecane and dodecane were present in female beetles whereas these were absent in male body wash, might have also contributed for the attraction of male beetles. Spikes *et al.* (2010) reported that the cuticular hydrocarbon profiles of hard wood stump borer females (*Mallodon dasystomus*) contained 13 compounds that were not present in profiles of males.

SUMMARY

The highest numbers of male citrus trunk borer beetle (84.21%) were attracted toward the female abdomen in olfactometric bioassay. The higher EAG response of male antennae to abdominal extract of female beetles confirmed the presence of pheromone on abdominal part of female beetles. Fourteen hydrocarbons were common in body wash of both sexes. The concentrations of tetradecane, hexadecane, eicosane, hexacosane and pentacosane on abdominal extract of adult female were much higher in comparison to the male body wash, which played an important role for the attraction of male beetles. Heptadecane, octadecane and dodecane present in female beetles was absent in male. These studies confirmed the presence of the sex pheromone in the female abdomen.

ACKNOWLEDGEMENTS

The authors are thankful to the Director, ICAR Research Complex for NEH Region, Umiam for providing the facilities for carrying the experiment and Dr R D Gautam, Professor, Division of Entomology, IARI, New Delhi for providing the facilities for carrying out the EAG and GLC work.

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