Identification of the chemical mediating attraction of *Holotrichia consanguinea* beetles to its most preferred host tree

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Abstract

Behavioural study of whitegrub beetles of *Holotrichia consanguinea* showed *Azadirachta indica*, *Prosopis cinerarium* and *Ziziphus jujubeas* its preferred host trees. Hexane was identified as the best solvent for extracting attractant chemicals from the host tree leaves and *A indica* and *P cinerarium* as the most preferred host trees for *H consanguinea*. Chemical volatiles were isolated from the two most preferred host trees of *H consanguinea* by trapping in Tenax followed by elution in hexane and also by non-destructive extraction in hexane. Bioassay in wind tunnel showed that GLVs trapped in Tenax attracted more beetles than the extracts and that *Azadirachta indica* (Neem) appeared better source of attractant chemical (kairomone) than *Prosopis cinnerarium* (Khejri). Fractionated of green leaf volatiles by a reverse phase HPLC using gradient mobile phase of acetonitrile and water showed presence of a number of chemical components. Chemical component(s) present in fractions eluted between RT 1-2 min from green leaf volatiles of *A indica* and *P cinnerarium* were most active in attracting the beetles. The chemical present in the most active fraction of GLV attracting the beetles was identified as isomers of hexenyl acetate by GC-MS.

Key words

Kairomone, Hexenyl acetate, Naphthelene, Scarab beetles, Aggregation pheromone

Introduction

H. consanguisnea is the most serious scarab pest in India particularly in the states of Rajasthan, Gujarat, Haryana, Punjab, U.P. and Bihar. A pheromone of this species has been isolated and its chemical structure determined (Leal *et al.* 1996). This pheromone in combination with insecticides has proved to be effective in management of this whitegrub species. However, there is scope for improvement in the management methodology by using kairomones alone or in combination with pheromone. Kairomones, being a type of aggregation pheromone (Guerin *et al.* 1986), can play a very important role in the management of insect pests especially because they can act against more than one species of the same genus. Using leaf extracts, the species *Azadirachta indica* and *Prosopis cinnerarium* have been identified as the best sources of kairomones for *H. consanguinea* (Yadav and Yadava 1999). It is difficult to purify and identify active kairomone component using total leaf extract, hence there was a need to isolate green leaf volatiles to minimize the impurities of chemicals other than kairomone. This experiment was, therefore, undertaken to develop a method for isolation of volatile semiochemicals from the leaves of *Azadirachta indica*and *Propopis cinnerarium* by trapping them in an absorbent such as Tenax (Bartlef *et al* 1993, Allard and Thomas 1999) and non destructive extraction (Moore 2000), so that they have minimum impurities of compounds other than the kairomone and the bioassay of different fractions becomes easy and less time consuming. As a first step, the most preferred host tree for *H. consanguisnea* and the best solvent for isolation of kairomone were identified by making leaf extracts from different host trees and evaluating their attractiveness in the field.

Methods

Leaf extracts and their response in the field

Leaves (40 g) of each sample were ground and extracted in different solvents viz water, methanol:chloroform (2:1 v/v) and hexane. The leaves were taken from middle peripheral area of the plant canopy. The plants wee 10-12 years old and the experiment consisted of three replications. The

extracts were filtered through Whatman filter paper 40. Cotton plugs soaked in extracts were placed in traps fixed at 10 meter distance in field for half an hour from 8 pm and the number of *H. consanguinea* beetles trapped was counted. The candidate extracts were replicated in randomized block design. Capture data were collected daily for at least three consecutive days and, after inspection, the traps were randomized.

Isolation of green leaf volatiles

Tenax column washed with HPLC grade hexane was air dried and activated in oven at 120?C for two hours. Fresh green leaves of *A indica* or *P cinerarium* were packed in a 'sample column' (a glass column 30 cm long x 6 cm dia). Air drawn through a silica column (8.0 cm x 2.5 cm) and charcoal column (10.0 cm x 1.5 cm dia) at a flow rates of 50 ml/min was passed through the sample column, which was connected to a clean and activated Tenex column (10 cm x 5 mm dia). The experiment was run for 24 hrs at 27?C to trap the green leaf volatiles in Tenex that were eluted by HPLC grade hexane in a total volume of 5 ml. The experiment run without any sample in the sample column acted as control. For non-destructive extraction, 5 g leaves of *A indica* or *P cinerarium* were completely submerged in hexane in 30 ml capped glass tubes for overnight at 27?C. The solvent was decanted, passed through a charcoal column (10 cm x 5 mm dia) to remove coloured materials and concentrated to 2 ml under a stream of nitrogen.

Fractionation of GLVs

Green leaf volatiles of *A indica* and *P cinerarium* were fractionated and collected at 1 ml/min by reverse phase HPLC at 230 nm (Bruce *et al.*1999) using Lichrospher 100 RP-18 column and continuous gradient of acetonitrile and water as mobile phase in the following order 40: 60, 60: 40, 80: 20, 100: 0. The chemical nature of the active component of kairomone present in the fraction showing maximum activity was identified by GC-MS at the Animal Research Institute, Department of Primary Industries, Brisbane, Australia.

Bioassay:

Response to kairomone preparations was observed in a self-designed wind tunnel (180 cm L x 15 cm W x 15 cm H), taking 10 beetles (male and female) of *H. consanguinea* from 8 PM onwards each time. For bioassay, 200 µl of the kairomone extract/fraction was baited onto sponge piece (7 cm x 4 cm) that was suspended at one end of the tunnel. The beetles were placed in the centre of the tunnel and the air was blown through the sponge piece with the help of battery operated small fan. Number of beetles reaching the sponge piece was counted after 5 min. The other end of the tunnel was kept open The sponge baited with hexane served as control.

Results

Evaluation of leaf extracts in the field showed that extracts in hexane attracted the lowest number of *H. consanguinea* beetles for all host plants (Table 1). Out of three host plants, *A indica* leaf extracts attracted the highest number of beetles followed by those of *P cinerarium.* Leaf extracts of *Ziziphus jujubeas* did not attract any significant number of beetles. Subsequently, *A indica* and *P cinerarium* leaves were used for isolation of GLVs in hexane. There was no attraction to the blank control or to leaf extracts in water. It was interesting to observe that host plant leaf extracts attracted more *H. consanguinea* female beetles than male beetles. Sex pheromone, on the other hand attract only one sex; usually male beetles.

As shown in Table 2 bioassays in the wind tunnel showed that green leaf volatiles of *A indica* were more effective in attracting *H. consanguinea* beetles (47%) than those of *P cinerarium* (43%). Leaf volatiles isolated by non-destructive extraction also showed similar results. Extracts from *A indica* leaves attracted more beetles (42%) than extracts of *P cinerarium* leaves (40%). Both the methods yielded consistent results for two consecutive years and GLVs trapped in Tenax attracted more beetles than the leaf extracts. Blank controls attracted only 10-12 percent beetles.

HPLC profile of released volatiles from leaves was different than those from non-destructive leaf extracts. Non-destructive leaf extracts yielded many more chemical components than trapped GLVs. Green leaf volatiles of *A indica* (AHT) yielded 9 peaks while those of *P cinerarium* (PHT) yielded 10 peaks. Bioassays of major fractions of GLVs from the two host plants showed that the *A indica* GLV fraction eluted at 1.64 min and *P cinerarium* GLVs fraction eluted at 1.56 min had the maximum kairomone activity (Table 3). However, the *A indica* GLV fraction AHT-1 attracted more beetles (64%) than the *P cinerarium* GLV fraction PHT-1 (34 %). The other two fractions of *A indica* leaf volatiles AHT-2 and AHT-3 attracted only 40% and 10% beetles, respectively. The other three fractions from *P cinerarium* GLVs PHT-2, PHT-3 and PHT-9 attracted 30%, 16% and 20% beetles, respectively.

GC-MS analysis of the two fractions showing maximum kairomone activity showed the presence of naphthalene in both the fractions but it is unlikely to be from a natural source and is likely to have entered the trapping system from the laboratory system. However, the GLV fraction of *A indica* had two green leaf volatiles but in very low levels as compared to naphthalene. They were isomers of hexenyl acetate and seem to be biologically active attractants for *H. consanguinea* beetles. However it will be important to see if profiles of GLVs are different in situ than the detached leaves. In an earlier study the profile of released volatiles has been found different from that of broad bean (Nicky *et al* 1999). Hexenyl acetate has been reported to be one of the chemicals mediating attraction of scarab beetle *Anomala octiescostata* to dandelion, *Taraxacum officinale* (Leal *et al*., 1994) and also as main GLV in *Betula pendula* at the time when bark beetles search in flight for host trees (Zhang *et al*. 1999)

Table 1. Attraction of *Holotrichia consanguinea* beetles towards extracts of host plants leaves in field.

Host plants	Solvent used for extraction	Number of beetles collected		
		Male	Female	Total
Azadirachta indica	Water	0	0	0
	Methanol : Chloroform	2.75	5.75	8.50
	Hexane	4.25	6.50	10.75
	Control	0	0	0
Prosopis cinnerarium	Water	0	0	0
	Methanol : Chloroform	2.50	3.50	5.0
	Hexane	4.00	4.50	8.50
	Control	0	0	0
Ziziphus jujubeas	Water	0	0	0

Methanol : Chloroform	0.50	0.75	1.25
Hexane	1.25	0.50	1.75
Control	0	0	0

Data are average of 4 replications on different days. Ratio of methanol:chloroform was 2:1 v/v

Table 2. Bioassay of green leaf volatiles for *Holotrichia consanguinea* beetles in wind tunnel.

Holotrichia consanguinea beetles	Number of beetles out of 10 reaching kairomone source at 5 min with					
	Non destructive extract of leaves in hexane			Green leaf volatiles trapped in Tenax		
	Control	A indica	P cinnerarium	Control	A indica	P cinnerarium
Male	0.5	2.0	2.0	0.6	2.3	2.3
Female	0.5	2.2	2.0	0.6	2.4	2.0
Total	1.0	4.2	4.0	1.2	4.7	4.3
Percent of total	10.0	42.0	40.0	12.0	47.0	43.0

Data are average of 10 replications on different days.

Table 3. Bioassay of different fractions of green leaf volatiles from *Azadirachta indica* and *Prosopis cinnerarium* for *Holotrichia consanguinea* in wind tunnel.

Holotrichia consanguinea beetles	Number of beetles out of 10 reaching GLV fractions								
	Control-1 (acetonitrile	Control -2 (water)	AHT 1	AHT 2	AHT 3	PHT 1	PHT 2	PHT 3	PHT 9
Total	1.0	0.6	6.4	3.0	1.0	3.4	3.0	1.6	2.0
Per cent	10.0	6.0	64.0	30.0	10.0	34.0	30.0	16.0	20.0

Data are average of 10 replications. AHT and PHT stands for GLV from *Azadirachta indica* and *Prosopis cinnerarium*, respectively trapped in Tenax (T) and eluted with hexane (H) and number indicates fractions.

Conclusion

The study showed that semiochemicals from two most preferred host trees of *Holotrichia consanguinea* can be trapped in tenax and eluted in hexane. GC-MS analysis of these semiochemicals showed presence of naphthalene in both the cases. It is unlikely to be from a natural source and is likely to have entered the trapping system from the laboratory system. However, the semiochemicals of *A indica* had two green leaf volatiles although in very low levels as compared to naphthalene. They were isomers of hexenyl acetate and seem to be biologically active attractants for *H. consanguinea* beetles. However it will be important to see if profiles of GLVs are different in situ than the detached leaves.

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