Mechanism of Adult Action and Mating in *Anoplophora glabripennis* (Motsch.)*

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Abstract. We studied the mating behavior of adult *Anoplophora glabripennis* (Motsch.) including search patterns, decision-making, responses, and tropisms of male and female beetles to body odors and odor residues. The results demonstrated that contact olfactory recognition was the leading factor in the mate-searching process. In general, mating started when the male was attracted to the female by visual stimuli. The female sex pheromone is a contact pheromone, and hence, must be detected directly by males using chemical sensors on antennae, labial palpi, and maxillary palpi.

KEY WORDS *Anoplophora glabripennis*, mating, action mechanism, pheromone

Anoplophora glabripennis (Motsch.) is a wood-boring pest of various broad-leaved trees including poplar, willow, elm, and maple. This insect, although native to China, is a destructive pest primarily of reforestation and protective forests in northern China. The mating behavior of adults is documented [1, 2], however, studies on the behavioral mechanisms of mating are lacking. We conducted a series of laboratory experiments to determine (1) the responses of adults to body odor, (2) the existence of a female sex pheromone, and (3) the function of the female sex pheromone in mating behavior. The results of this study increase our understanding of *A. glabripennis* reproduction and may prove useful for the development of management strategies for this pest.

Materials and Methods

Tropism Responses of Adult to Body Odor. AY-tube olfactmeter was used for this test. The speed of airflow inside the tube was adjusted to about 10 liter/min. Fast-frozen male and female beetles were placed in the material chamber within the tube to serve as bait. Laboratory-reared even-aged adults were allowed to climb up the tube within the olfactometer to determine the response and tropism of male and female towards body odor. The door of the insect chamber was then closed and the odor selected by each test adult was recorded within 10 min.

Extraction of Male and Female Pheromones. Insect pheromones were extracted with hexane or ethyl acetate. The bodies or wings of certain aged, fast-frozen male or female beetles were immersed in solvent in a sealed tube or flask and stirred for 30 – 40 s. Insect bodies or wings were then removed from the solution and anhydrous Na_2SO_4 was added. After stirring for several minutes, the solution was stored in a cooling chamber in still for 24 hr. Na_2SO_4 and other solid impurities were filtered away before the solution was condensed through depressive distillation at 30ºC. The extract was refrigerated for later use.

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Contact Responses of Adults to Body Odor Residue. (1) Directional responses of adult *A. glabripennis* to odor residue were tested in the Y-tube. The upper surface of the treatment tunnel was spread with the hexane extract from one male or female; an equal volume of hexane alone was used for the control. To allow for complete evaporation of the solvent (24 hrs), male or female beetles of known age were introduced into the Y-tube and their responses were recorded. (2) The response of male to female pheromone was tested using a glass rob (7mm in diameter and 30 mm in length) inside a clear plastic rearing chamber (12 cm in diameter and 5 cm in height). The extract from one female was applied to the glass rod. After evaporation of the solvent (24 hr), male *A. glabripennis* were introduced into the chamber with the glass rod and their responses were recorded.

Contact Responses of Male Antennae to Female Pheromone. The response of male antennae to female pheromone was tested by direct contact. Hexane extract from one female was dripped onto the surface of a 3 cm long glass rod (7mm in diameter). After evaporation of the solvent (24 hr), we placed male antennae in contact with the glass rob containing the female extract and recorded the antennal responses.

Results and Discussion

Tropism Response of Adult to Body Odor. There was no significant difference between male and female beetles responses to body odors from the same or opposite sex using the Y-tube olfactometer (Table 1). This indicates that mating behavior is not stimulated by adult body odor from a distance. Therefore, search and recognition of by adult *A. glabripennis* may be visual and through contact olfaction.

Table 1. The tropism of adult *A. glabripennis* to body odor in Y-tube olfactometer

Sex	Food supplement	Age (d)	No. of insects tested	Odor source[a]		No. of insects responded		χ^2 value[b]
				A	B	A	B	
	P. opera	5	29	Female body	Filter paper	16	13	0.14
Male	*P. opera*	4 ~ 15	32	Female body	Filter paper	17	15	0.03
	P. opera	5	30	Male body	Filter paper	17	13	0.30
	P. opera	8 ~ 20	40	Male body	Filter paper	21	19	0.03
	P. opera	5	21	Female body	Filter paper	6	15	3.05
Female	*P. opera*	6 ~ 15	40	Female body	Filter paper	18	22	0.23
	P. opera	5	29	Male body	Filter paper	17	12	0.55
	P. opera	7 ~ 18	41	Male body	Filter paper	19	22	0.10

[a.] Female beetles were field-collected (n=20) and males beetles were laboratory-reared (8-d old); both were quick frozen at – 40ºC.

[b.] $\chi^2_{0.05(1)} = 3.84$.

Contact Response of Adults to Body Odor Residue.

The Tropism of Adult to Body Odor Residue. Laboratory observations showed that adult *A. glabripennis* frequently touch the floor with their labial and maxillary palpi when walking. After stimulation by certain chemicals [3], adults immediately stop walking and respond by increased sensory activity that includes trembling palpi, rubbing palpi across the tarsi on the same side, cleaning of antennae with fore and middle legs, and turning around.

We discovered highly significant sensory perception by male and female *A. glabripennis* to individual odor residues (Table 2). (1) Male *A. glabripennis* sensed female odor residue through mouthparts palpi and showed significant tropism. Some individuals even expressed mating intention by bending their abdomen. This indicated that mating behavior of male *A. glabripennis* can be induced by female pheromone through direct contact with the chemical sensors located on labial palpi and maxillary palpi. (2) Male *A. glabripennis* responded with significant avoidance to same-sex odor residue. (3) Female *A. glabripennis* tended to avoid odor residue from both males and females. Results of this study demonstrated that contact response is an important means of adult by *A. glabripennis.*

Table 2. Tropism of adult *A. glabripennis* to body odor residue through direct contact

Sex	No. of insects tested	Age (d)	Odor source[a]		No. of insects responded		χ^2 value[b]
			A	B	A	B	
	21	10 ~ 12	Female extract	Solvent	17	4	6.86
	23	9 ~ 11	Female extract	Solvent	17	6	4.35
	25	10 ~ 11	Female extract	Solvent	23	2	16.00
Male	30	8 ~ 15	Male extract	Solvent	7	23	7.50
	30	9 ~ 17	Male extract	Solvent	7	23	7.50
	30	12 ~ 17	Male extract	Solvent	6	24	9.63
	30	8 ~ 14	Solvent	Solvent	16	14	0.03
	23	10 ~ 12	Female extract	Solvent	2	21	14.09
	25	10 ~ 13	Female extract	Solvent	3	22	12.96
	33	10 ~ 13	Female extract	Solvent	3	30	20.48
Female	30	5 ~ 13	Male extract	Solvent	9	21	4.03
	30	11 ~ 15	Male extract	Solvent	7	23	7.50
	30	5 ~ 14	Male extract	Solvent	5	25	12.03
	27	12 ~ 13	Solvent	Solvent	16	11	0.59

[a.] Insect extract came from the whole insect by using hexane as solvent.
[b.] $\chi^2_{0.05(1)} = 3.84$. $\chi^2_{0.01(1)} = 6.64$.

Response of Males to Glass Rod Coated with Female Pheromone. (1) We extracted a contact sex pheromone using ethyl acetate or hexane as the solvent. (2) Using our assay, we determined 16.7% of males became sexually excited after contacting the pheromone-coated glass rod with their labial and maxillary palpi. Reports of mating behavior in the field suggest visual stimuli are also involved because mating generally starts after a male sees a female and walks swiftly toward it [4]. This, together with our lab results, suggests the importance of both vision and contact in the mating behavior of *A. glabripennis*. (3) The female sex pheromone evaporated relatively slowly, and the coated glass rod continued to induce male sexual behaviors after 50 d at room temperature.

Contact Response of Male Antennae to Female Pheromone. We found males were very sensitive to direct antennal contact with the female pheromone, expressed immediate excitement of males including searching, chasing, walking, and bending their abdomens toward the coated glass rod (Table 3 and Fig.1). No mating responses were observed when antennae were very close to or prior to contact with the glass rod. Stimulation of male *A. glabripennis* sexual behavior was only observed after direct antennal contact with the glass rod. Laboratory experiments showed that male *A. glabripennis* were sexually excited and induced to mate through direct contact of chemical sensors with female pheromone. These observations demonstrated that the female pheromone of *A. glabripennis* is a contact pheromone.

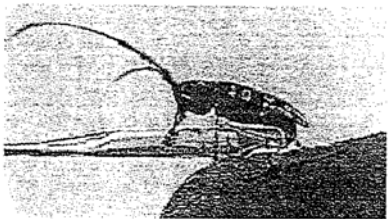

Fig. 1. Male *A. glabripennis* bends its abdomen to the glass rob coated with female pheromone

Table 3. Contact response of male *A. glabripennis* antennae to female pheromone

Pheromone concentration (female quantum)	No. of males tested	No. of males expressed excitement	Rate of male excited (%)	No. of males with bending abdomen	Rate of male bent (%)
1	40	9	22.5	4	10.0
2	40	6	15.0	6	15.0

Conclusions

In summary, we found that (1) contact olfactory responses are important during the mate-searching process of adult *A. glabripennis* and (2) female *A. glabripennis* produce a contact pheromone, detected by males through direct contact with sensors on labial and maxillary palp.

Mating can be divided into the follow three stages: (1) a male approaches a female living in the same habitat through normal activities, or follows the odor residue trail of female; (2) the male, following visual stimuli or other activities such as direct body contact, then approaches, chases, and ends up on top of the female; and (3) the female contact pheromone causes male *A. glabripennis* to grab the female, bend its abdomen, and mate by insertion of its genitalia.

References Cited

Li, W., and C. Wu. [eds.] 1993. Integrated pest management of poplar longhorned beetles. Forestry Publishing House of China, Beijing, China. pp. 62-66.

Li, X., and K. Zhang. 1991. The chemical sensors of *Anoplophora nobilis* Ganglbauer on labial palpi and maxillary palpi. Insect Knowledge 6: 357-358.

Zhang, B., and Q. Xu. 1991. Behavioral study on *Anoplophora glabripennis*. Bulletin of Forest Insects and Diseases. 4: 14-17.

Zhang, K., X. Li, and J. Zhou. 1988. Reproductive behavior of *Anoplophora nobilis* Ganglbauer. Journal of Northwest Agriculture University. 16 (suppl.): 78-81.

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