

# Selectivity mechanism of *Anoplophora glabripennis* on four different species of maples

Fengjuan ZHANG<sup>1,2</sup>, Youju JIN (✉)<sup>1</sup>, Huajun CHEN<sup>1</sup>, Xiaoying WU<sup>1</sup>

<sup>1</sup> College of Plant Sciences, Beijing Forestry University, Beijing 100083, China

<sup>2</sup> Department of Life Science, Hebei Normal University of Science & Technology, Changli 066600, China

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**Abstract** *Anoplophora glabripennis* (Motsch.) is a wood-boring beetle that is native to China. For a long time, it caused great losses in the economy and ecology of northwest China. Attractants are often used to control insects. The volatiles emitted from the host plant play an important role for insects in finding their target. To explore the mechanism of selectivity to different host plants, the response of *Anoplophora glabripennis* to four different host plants was investigated, which included *Acer negundo* L., *Acer mono* Maxim., *Acer truncatum* Bunge. and *Acer platanoides* L., and the compounds in the profiles of volatiles were identified from these species. The olfactory responses of *Anoplophora glabripennis* to the odors of different plants showed preference for certain host plants: *Acer negundo*, *Acer mono* and *Acer truncatum*. The attraction of *Acer negundo* and *Acer mono* was significantly different ( $p < 0.05$ ). The attraction of *Acer negundo* to the insects was stronger than to *Acer mono*. *Acer platanoides* neither attracted nor repelled the insects. Compounds in the profiles of volatiles from the above four species were identified and quantified by gas chromatography-mass spectrometry (GC-MS) equipped with a CP-4020 thermodesorption and cold trap (TCT) device. The constituents of volatiles and the relative concentrations were different in the four host species. The amount of ketones, alcohols and aldehydes in the four plants showed the same order: *Acer negundo* < *Acer mono* < *Acer truncatum* < *Acer platanoides*, while that of alkanes and esters was quite different: *Acer negundo* L. > *Acer mono* Maxim. > *Acer truncatum* Bunge. > *Acer platanoides* L.. 1-penten-3-ol, ocimene and trans-Geranylacetone were repellent to *Anoplophora glabripennis*. 1-penten-3-ol and trans-gerranylacetone were identified in *Acer platanoides*, and Ocimene was the most

attractive to *Anoplophora glabripennis* among these species. The extent of feeding damage caused by *Anoplophora glabripennis* differed among four species. The sequences was *Acer negundo* > *Acer mono* > *Acer truncatum* > *Acer platanoides*. The epidermal hairs of the four host plants revealed that the extent of damage was related to the physical characteristics of the host plants.

**Keywords** host plants, host selection, volatiles, *Anoplophora glabripennis*, olfactory response

## 1 Introduction

Applying semiochemicals is one of the effective means to monitor and control *Anoplophora glabripennis*. Volatiles released by host plants play an important role for pests in finding the targets (Zhu et al., 1999; Kessler and Baldwin, 2001; Nowak et al., 2001; Haack, 2003; Luo et al., 2003; Frédéric et al., 2004). Volatile compounds can affect the behavior of the herbivores. Studying on these chemicals and the mechanism not only find out the chemical relationship between the insects and the host plants but also provide the new thought and technological means. *Anoplophora glabripennis* (Motschulsky) is a wood-boring beetle that feeds on the trees such as *Ulmus pumila* L., *Acer truncatum* Bunge. and *Acer negundo* L.. It was reported that 18 genera were damaged by *Anoplophora glabripennis* and great economic losses had been made (Keena, 2002; Smith and Bancroft, 2002; Morewood et al., 2004). The volatiles emitted from the host plant play an important role for insects in finding their targets. To explore the mechanism of selectivity to different host plants, the response of *Anoplophora glabripennis* to four different host plants was investigated, which were *Acer negundo* L., *Acer mono* Maxim., *Acer truncatum* Bunge. and *Acer platanoides* L., and the compounds in the profiles of volatiles were identified from these species.

## 2 Materials and methods

### 2.1 Materials

The five-year-old plants of *Acer negundo* L., *Acer mono* Maxim., *Acer truncatum* Bunge. and *Acer platanoides* L. were used for these studies. The adults of *Anoplophora glabripennis* (Motsch.) were provided by Langfang forest protection.

### 2.2 Collection and analysis of headspace volatiles

The leaves and twigs were placed in plastic bags (Reynolds plastic bags, turkey size 19 in  $\times$  23<sup>1/2</sup>, Reynolds Metals Company, USA). The air in the bag was drawn out in a short time. The incoming air was filtered through activated carbon and GDX-101 for half an hour to allow accumulation of volatiles. Subsequently, the headspace volatiles from the treatments and air controls were simultaneously collected on Tenax GR packed in a glass tube (16 cm  $\times$  0.3 cm ID) for 40 min with an airflow rate of approximately 100 mL  $\cdot$  min<sup>-1</sup>. Each material was repeated for three times.

Volatiles were identified and quantified by gas chromatography-mass spectrometry (GC-MS) (Trace 2000-Voyager, Finnigan, Thermo-Quest). The instrument was equipped with a CP-4020 thermoacoustic computed tomography (TCT) device (Chrompack, The Netherlands), a split/splitless injector and a capillary column (CP-Sil 5 CB low bleed/MS30 m  $\times$  0.32 mm ID, film thickness of 1  $\mu$ m). The pressure of the carrier gas (helium) was adjusted to 6 kPa. The mass spectrometry (MS) condition was EI of 70 eV, full scan ( $m/z$  from 10 to 400) with a scanning rate at 0.4 sec  $\cdot$  scan<sup>-1</sup>. Volatiles were desorbed from the Tenax GR by heating in the thermal desorption device (Chrompack, the Netherlands) at 250°C for 10 min, and cryo-focused in a cold trap at -100°C, then quickly heated to 200°C for 1 min to transfer the volatile compounds into the GC-MS (Trace 2000-Voyager, Finnigan, Thermo-Quest) using the following temperature program: initial temperature 20°C; 10°C  $\cdot$  min<sup>-1</sup> to 250°C, hold 10 min, total time 39 min. The compounds were identified by searching the National Institute of Standard and Technology (NIST) library in the Xcalibur data system (Finnigan), and by comparison of RI (retention index). Quantification of volatiles was determined by the relative content of the area of the peaks.

### 2.3 Y-tube olfactometer experiments

The binary *Anoplophora glabripennis* choice comparisons were conducted in a closed system Y-tube olfactometer (Takabayashi and Dicke, 1991; Li et al., 2003). In this system, the air flows over a charcoal filter and then over the two odor sources, through the arms of the Y, and into the base of the Y where they mixed. The insect was placed at the base of the Y and given 50 min to walk upwind towards the odor sources in

each arm. The airflow was 4 L  $\cdot$  min<sup>-1</sup> in each arm. The position of the odor sources was switched after five predators made a choice. Three of the comparisons were performed with independent sets of plants.

### 2.4 Feeding experiments

The same growth status of branch in four kinds of host plants was selected for the experiments. The leaves and twigs of the maple were hitched in wire netting. Seven male insects (*Anoplophora glabripennis*) were placed into the wire netting at eight in the evening and removed at eight in the next morning. The area of the damaged-branch was measured.

### 2.5 Observation of the epidermis hairs of the hosts

The epidermis hairs of the host plant leaves were observed by polish expression method. Collodion was laid on the middle of the leaves' epidermis and torn off after a few minutes. The collodion was placed in the piece of glass and the epidermis hairs could be watched by optics microscope.

### 2.6 Statistical analysis

Data were analyzed with Statistical Package for the Social Science (SPSS) software. Data were subjected to Levene's test for homogeneity of variances, and analyzed with ANOVA (analysis of variance). Mean separations were accomplished with a Duncan test at  $P < 0.05$ .

## 3 Results and analysis

### 3.1 Olfactory response of *Anoplophora glabripennis* to volatile compounds from the different hosts

The behaviors of *Anoplophora glabripennis* to volatiles from the different hosts were listed in Table 1. The four maples had different attractions to the insect. The attractions of *Acer negundo* and *Acer mono* were significantly different ( $p < 0.05$ ). The attraction of *Acer negundo* to the insect was stronger than that of *Acer mono*. *Acer truncatum* attracted the insect but the attraction was not significant, *Acer platanoides* neither attracted nor repelled the insect.

### 3.2 Volatiles from the different hosts of *Anoplophora glabripennis*

Volatiles released from the host plants included alkylates, esters, aldehydes, alcohols, terpenoids and ketones (Table 2). The results showed that different plants had different chemical fingerprints. The main volatile compounds released from *Acer negundo* were esters, aldehydes and alkylates. The compounds of esters were (E)-3-hexenyl acetate, acetic acid pentyl ester, ethyl acetate, (E)-4-hexenyl acetate and acetic

**Table 1** Behavior response of *Anoplophora glabripennis* to different host plants

Species	Group 1		Group 2		Group 3		Average	
	Attracting arm	Control arm	Attracting arm	Control arm	Attracting arm	Control arm	Attracting arm	Control arm
<i>Acer nnegundo</i>	9	2	8	2	6	1	7.67	1.67
<i>Acer mono</i>	6	1	9	2	4	0	6.33	1.00
<i>Acer truncatum</i>	1	1	4	3	6	3	3.67	2.33
<i>Acer platanoides</i>	4	3	5	7	4	3	4.33	4.33

Note: the data show the frequency and times of *Anoplophora glabripennis* entering into the trap.

**Table 2** Main chemical composites of volatiles from host trees of *Anoplophora glabripennis* adults (%)

Compounds	<i>Acer nnegundo</i>	<i>Acer mono</i>	<i>Acer truncatum</i>	<i>Acer platanoides</i>
<b>Alkylations</b>	<b>7.41</b>	<b>2.02</b>	<b>1.06</b>	<b>0.33</b>
Pentane,2-Methyl-	0.13 ± 0.07	1.18 ± 0.01		
Hexane	1.66 ± 1.63	0.33 ± 0.43	0.58 ± 0.32	0.12 ± 0.01
Methylcyclopentane	0.05 ± 0.05			
2-Nitrobutane		0.02 ± 0.02		
Heptane	0.20 ± 0.07			0.09 ± 0.05
2-Methylheptane				
1-Nitrobutane		0.05 ± 0.04		
Hexane,2,4-dimethyl-	0.69 ± 0.25		0.08 ± 0.07	0.02 ± 0.02
Bicyclo[3,10]hexane,4-methyl-1-(1-methylethyl)-	4.66 ± 0.61			
1,4-Methylcyclohexane				0.02 ± 0.00
Octane	0.08 ± 0.05			
Decanae				0.01 ± 0.00
Decane2,6,7-trimethyl-				
Cyclohexane,2-ethyl-1,1-dimethyl-3-methylene-		0.40 ± 0.03	0.40 ± 0.02	0.06 ± 0.07
<b>Esters</b>	<b>79.26</b>	<b>72.1</b>	<b>62.08</b>	<b>57.87</b>
Ethyl acetate	2.24 ± 1.97	0.31 ± 0.19	3.26 ± 0.33	0.66 ± 0.26
Hexenyl butyrate		0.06 ± 0.02		0.10 ± 0.10
(E) 4-Hexenyl acetate	0.21 ± 0.20			0.04 ± 0.01
Acetic acid, pentyl ester	0.11 ± 0.06			
Methyl-3-hexenate				0.05 ± 0.05
(E) 3-Hexen-1-ol,acetat-	54.81 ± 13.49	65.3 ± 0.5	58.37 ± 8.11	56.87 ± 12.98
3-cyclohexenyl acetate				0.06 ± 0.01
Acetic acid, hexyl ester	21.85 ± 26.95		0.31 ± 0.19	
4-Hexenyl butyrate		5.46 ± 3.60		
Trans-3-Hexenyl butyrate	0.04 ± 0.03		0.07 ± 0.06	
Methyl Salicylate -		0.15 ± 0.13	0.06 ± 0.08	0.06 ± 0.03
Isovaleric acid cis-3-hexeny ester		0.48 ± 0.13		
3-Hexenyl pentanoate				0.01 ± 0.00
Bornyl acetate				0.01 ± 0.01
<b>Aldehydes</b>	<b>9.47</b>	<b>10.15</b>	<b>11.72</b>	<b>14.07</b>
2-Propanal,2-methyl-				0.01 ± 0.01
2-Propenal-2-methyl-		0.06 ± 0.01		0.02 ± 0.01
Pentanal			0.03 ± 0.00	0.02 ± 0.01
E-2-Pentenal				0.01 ± 0.03
2-Methyl-2-butenal				0.02 ± 0.01
Pentanal,3-methyl-	0.63 ± 0.31	0.51 ± 0.18	0.02 ± 0.00	
4-Butenal,2-methyl-				7.73 ± 0.74
2-Hexenal	0.09 ± 0.03	0.39 ± 0.49	0.56 ± 0.59	3.45 ± 1.58
Heptanal		0.05 ± 0.01	0.35 ± 0.23	0.10 ± 0.00
E,E-2,4-Hexenal				0.32 ± 0.15
Benzaldehyde		0.34 ± 0.18	0.16 ± 0.13	0.01 ± 0.00
(E)-2-Octenal			0.05 ± 0.00	
(Z)-2-Decenal		0.05 ± 0.03		
E-2-Nonenal		0.05 ± 0.00	0.24 ± 0.17	
Nonanal	4.19 ± 3.44	4.72 ± 2.39	5.05 ± 1.00	1.02 ± 0.11
E-2-Decenal		0.94 ± 0.68		
Decanal	4.56 ± 3.80	0.22 ± 0.15	4.59 ± 0.12	1.13 ± 0.40
Undecanal		2.83 ± 1.64	0.68 ± 0.46	0.04 ± 0.00
<b>Alcohols</b>	<b>0</b>	<b>8.46</b>	<b>11.82</b>	<b>21.68</b>
1-Propanol				0.03 ± 0.02
1-Propanol,2-methyl-				0.02 ± 0.01

(Continued)

Compounds	<i>Acer negundo</i>	<i>Acer mono</i>	<i>Acer truncatum</i>	<i>Acer platanoides</i>
(E)-1-penten-3-ol				0.12 ± 0.03
Z-2-Hexenol		8.17 ± 11.0	11.31 ± 2.63	20.46 ± 15.56
Pentanol,3-methyl-			0.11 ± 0.15	
Hexanol				0.83 ± 0.73
Ethylhexanol			0.18 ± 0.01	0.04 ± 0.04
Trans-2-Nonene				0.06 ± 0.05
Trans-2-Decenol		0.30 ± 0.02	0.22 ± 0.17	0.13 ± 0.02
<b>Terpenoids</b>	<b>3.2</b>	<b>6.55</b>	<b>12.49</b>	<b>2.33</b>
α-Pinene	1.11 ± 0.31	4.89 ± 4.72	9.67 ± 1.97	1.77 ± 0.01
Camphene			0.20 ± 0.11	
β-Pinene	1.55 ± 0.97	0.25 ± 0.06	1.33 ± 0.60	0.47 ± 0.21
ρ-Metha-1,4(8)-diene	0.16 ± 0.08			0.01 ± 0.01
3-Carene			0.05 ± 0.01	0.09 ± 0.08
Thujene		0.57 ± 0.49		
α-Terpinene	0.38 ± 0.28	0.06 ± 0.06		
Limonene		0.63 ± 0.11	0.36 ± 0.31	
Phellandrene		0.19 ± 0.09	0.25 ± 0.05	
Ocimene		0.04 ± 0.00	0.54 ± 0.28	
β-Terpinene		0.19 ± 0.13		
Caryophyllene		0.31 ± 0.02		
<b>Ketones</b>	<b>0</b>	<b>0.03</b>	<b>0.15</b>	<b>3.7</b>
2,3-Butanone				0.05 ± 0.02
3-Pentanone				0.11 ± 0.06
2-Heptanone6-methyl-		0.03 ± 0.18	0.06 ± 0.03	
2(5H)-Furanone,5-ethyl-				3.21 ± 0.36
Acetophenone			0.09 ± 0.01	
Isophoron				0.02 ± 0.01
Trans-geranylacetone				0.32 ± 0.06
<b>Others</b>	<b>0.05</b>	<b>0.54</b>	<b>0.38</b>	<b>0.03</b>
Allyl methyl ether	0.05 ± 0.04			
Hexanoic acid			0.12 ± 0.10	
Cyclohexane, isothiocyanato-		0.54 ± 0.24	0.26 ± 0.19	0.03 ± 0.00

Note: Data mean ± SD.

acid pentyl ester. The main volatile compounds released from *Acer mono* were esters (e.g. (E)-3-hexenyl acetate, 4-Hexenyl butyrate) and aldehydes (e.g. nonanal, E-2-decenal, dacenal and 2-hexenal). The main volatiles of *Acer truncatum* were esters ((E)-3-hexenyl acetate, ethyl acetate), terpenoid (e.g. α-pinene, β-pinene, ocimene and limonene) and alcohol (e.g. Z-2-hexenol, ethylhexanol and trans-2-decenol). The main kinds of volatiles released from *Acer platanoides* were esters (e.g. (E)-3-hexenyl acetate, alcohols (e.g. Z-2-hexenol) and aldehydes (e.g. 2-methyl-2-butenal, 2-hexenal, dacenal and nonanal). Hexane, ethyl acetate, 2-hexenal, α-pinene, β-pinene, (E)-3-hexenyl acetate, dacenal and nonanal were the common volatiles among the four maples.

The volatiles released from the plant played the important roles in the process of *Anoplophora glabripennis* to search the host. Li et al. (2003) found dacenal, nonanal, (E)-3-hexenyl acetate, 2-hexenal significantly attracted *Anoplophora glabripennis*, respectively. Ocimene, (E)-1-penten-3-ol, and trans-geranylacetone significantly repelled the insect, respectively. Ethyl acetate, (E)-2-hexenal, α-pinene, β-pinene and (E)-3-hexen-1-ol, acetate were detected in *Acer negundo*, and ocimene, (E)-1-penten-3-ol and trans-geranylacetone were not detected. Ethyl acetate, 2-hexenal, (E)-3-hexenyl acetate, dacenal, nonanal, and ocimene were

among the volatiles of *Acer mono*, and (E)-1-penten-3-ol and trans-geranylacetone were not detected. Among the volatiles of *Acer truncatum* ethyl acetate, 2-hexenal, (E)-3-hexen-1-ol, acetate, dacenal, nonanal, and ocimene were detected, while (E)-1-penten-3-ol and trans-geranylacetone were not detected. Among the volatiles of *Acer platanoides* ethyl acetate, 2-hexenal, (E)-3-hexenyl acetate, dacenal, nonanal, (E)-1-penten-3-ol and trans-geranylacetone were detected, while ocimene was not detected. The constituents of volatiles and the relative concentrations were different in the four host species. The amount of ketones, alcohols and aldehydes in the four plants showed the same order: *Acer negundo* < *Acer mono* < *Acer truncatum* < *Acer platanoides*, while that of alkanes and esters was quite different: *Acer negundo* L. > *Acer mono* Maxim. > *Acer truncatum* Bunge. > *Acer platanoides* L.. The results showed that the different constituents of volatiles released from the different hosts maybe lead to the different attraction to the insect. The concentration of the volatile also played an important role for the herbivore in finding the host (Schneider, 1986; Visser and Yan, 1986; Visser, 1995). It was found that the concentration of the volatile was different in the different host, which led to the four maples having different attraction.

### 3.3 Damage extent of four maples feeded by *Anoplophora glabripennis*

The extent of damage by *Anoplophora glabripennis* to the four maples were different (Table 3). The most serious damage was *Acer negundo*, and *Acer mono* and *Acer truncatum* were less. The least was *Acer platanoides*.

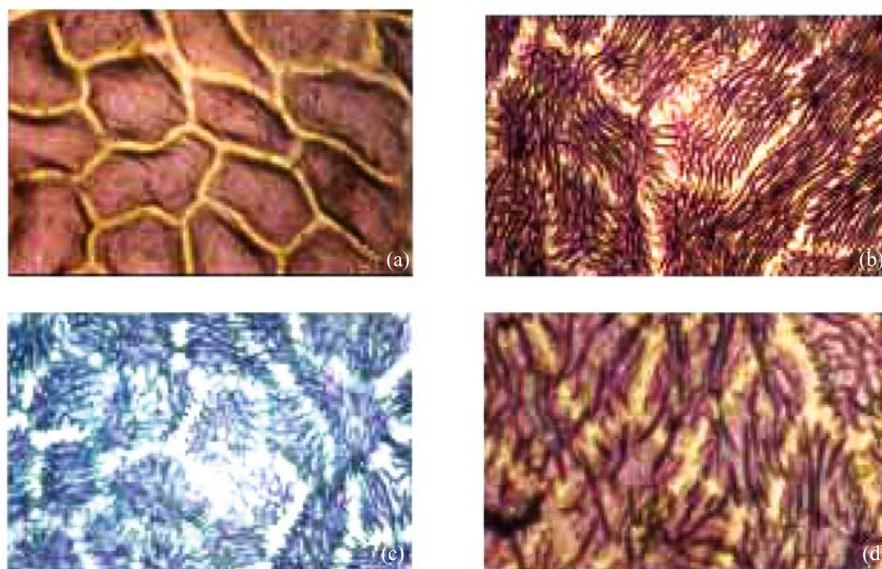
**Table 3** Comparison of the damaged area of the four plants by *Anoplophora glabripennis*

Species	Feeding sites		
	Percentage of the damaged stem	Percentage of the damaged petiole	Percentage of the damaged leaves
<i>Acer negundo</i>	80	80	30
<i>Acer mono</i>	30	30	20
<i>Acer truncatum</i>	20	20	20
<i>Acer platanoides</i>	–	–	15

Note: “–” means no damage.

### 3.4 Physical characteristic of the host plants

The epidermal hairs of the four host plants were examined. The results revealed that *Acer negundo* had few hairs, while the others had quite a lot of epidermal hairs, which may be one of the reasons that the damage of *Acer negundo* was less than that of *Acer mono* (Fig. 1). It was concluded that the extent of damage may be related to the physical characteristics of the host plants. The insect-resistant tree had more epidermal hairs than insect-susceptible tree had (Wei and Li, 1995).



**Fig. 1** Leaf epiderm hairs of four different maples. (a) *Ashleaf maple*; (b) *Acer mono*; (c) *Acer truncatum*; (d) *Norway maple*

## 4 Discussion

### 4.1 Different host plants had different constituents of volatiles

The test of GC-MS showed that the constituents of volatiles emitted from four host plants and the relative content of each compound were different in every host plant. Alcohols and ketones were not found in the volatiles of *Acer platanoides* (Table 2). The amount order of ketones, alcohols and aldehydes in the four plants showed as follows: *Acer negundo* < *Acer mono* < *Acer truncatum* < *Acer platanoides*, which agreed with the order of the insect-resist ability. While the amount order of alkanes and esters was quite opposite: *Acer negundo* > *Acer mono* > *Acer truncatum* > *Acer platanoides*.

### 4.2 Extent of attraction to *Anoplophora glabripennis* differed among different host plants

Among the four maples the attraction of *Acer negundo* was the best, the second was *Acer mono*, and the attraction of volatiles emitted from *Acer negundo* and *Acer mono* was significantly different ( $p < 0.05$ ), which accorded with the study of Li et al. (Li et al., 2002a; 2002b). The constituents of volatiles and relative concentrations were different in the four host species, which lead to the different attraction to *Anoplophora glabripennis*. First, trans-2-hexenol was detected in the volatiles of *Acer mono*, *Acer truncatum*, and *Acer platanoides*. Ocimene and geranylacetone were detected in *Acer mono* and *Acer truncatum*, and (E)-1-penten-3-ol was identified in only *Acer platanoides*. As a matter of fact, many researchers had proved that trans-2-hexenol, ocimene,

germanylacetone, (E)-1-penten-3-ol were significantly repellent to *Anoplophora glabripennis*, which was probably one reason that *Acer negundo* was very repellent to *Anoplophora glabripennis*. In addition, the relative amount of volatiles was related to tropism behavior of *Anoplophora glabripennis*. Alcohols and ketones were not found in *Acer platanoides*, and the relative content of aldehydes was lower than those in other three species, the relative content of alkanes and esters was higher than that of *Acer mono*, *Acer truncatum*, and *Acer platanoides*. The contents of alcohols, aldehydes and ketones in *Acer mono* were higher than those in *Acer negundo*, but were lower than those in *Acer truncatum* and *Acer platanoides*. It was supposed that those above may lead to the attraction of *Acer mono* to *Anoplophora glabripennis* better than that of *Acer truncatum* and *Acer platanoides* (Li et al., 2002a).

4.3 It was the complicated process for the herbivore to search, distinguish and accept the host

The volatile compounds are the major contributors to the characteristic leaf odor for various classes of plants, and they are also utilized as attractants by the insects. The results showed that *Acer negundo* and *Acer mono* attracted *Anoplophora glabripennis* significantly, but the extent of damage of them was different. One of the main reasons was the difference in the physical characteristics of the two plants. For example, *Acer negundo* had few epidermis hairs, while *Acer mono* had lots of hairs. Previous study showed that sometimes the physical defense was more than chemical defense; for example, epidermis hairs, wax and bark were not suit for the insect to eat and to lay eggs. The structures may change the sense of taste of the insect, and then influence its behavior and growth. Besides, the water content and chemical constituents of host were concerned to the sense of taste of the insect. High water content prevents the larva from entering the tree (Hanks et al., 1991; 1995; Hanks, 1999). Tannin and phenolic acids were the effective chemicals in the tree that were closely concerned with the insect-resistance (Auger et al., 1991; Hulme, 1995; Eom et al., 1998; Hirota and Shuichiro, 1996). The insect's growth depended on the nutrition ingredients of the plant (Van den Boom et al., 2003; Li et al., 2005). Yang thought that the resistant plant had low contents of reducing sugar, protein, amino acid and unsaturated fatty acids. High content of cellulose, lignin and residues did not help the insect to eat (Yang et al., 1992). High content of terpenoid promoted the insect-resistance of the plant (Nerg et al., 2004). The results showed that the content of terpenoid was more in *Acer mono* than that in *Acer negundo*, which may be one of the reasons that the extent of *Acer mono* was lower than that of *Acer negundo*. At the same time, the content of terpenoid in *Acer platanoides* was lowest, but the extent of damage was the lightest. The reason should be further studied. To sum up, the damage of *Anoplophora glabripennis* to the host resulted from the combination of many factors. The selective process of the insect to the plant was in connection with not only the classes of the plant (Hensley et al., 1991;

Wen et al., 1998; Kuser et al., 2001; Li et al., 2003), but also environment factors. When the plant's insect-resistance is going to be studied further, many factors should be taken into account.

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