Japanese wax scale inducing persimmon trees to change their volatiles so as to attract the natural enemies

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Abstract: Japanese wax scale, *Ceroplastes japonicus* Green (Hemiptera: Coccoidea: Coccidae), is an important pest of persimmon, *Diospyros kaki,* in north China. In this paper, the effect of damage by *C. japonicus* on the emission of volatiles from the persimmon canopy and its effect on the attraction of three predators, namely the two lady-beetle, *Chilocorus kuwanae* Silvestri and *Harmonia axyridis* Pallas and a green lacewing, *Chrysopa sinica* Tiedet, were evaluated. The volatiles were collected from twigs and leaves on sample trees in the forest by using a headspace volatile trapping instrument. Twigs and leaves were sampled from 3 classes of damage by the Japanese wax scale: i) severe damage, ii) light damage and iii) almost no damage. Volatile compounds were analyzed by using TCT-GC-MS equipment. A glass Y-tube olfactometer was used to test the attractive activity of the volatiles emitted from the damaged twigs and leaves to the natural enemies. The results showed that the damaged twigs and leaves attracted significantly greater numbers of individuals of the three predators than the undamaged leaves in a proportion of 74.17%, 66.67% and 66.67% for *Chilocorus* kuwanae, *Harmonia axyridis* and *Chrysopa sinica* respectively. The attraction strength varied with season; the most significant attraction effect being displayed in the autumn. The diurnal rhythm also effected the attraction effect, the most significant attraction being detected at midnight and at noon. In total, 60 compounds were detected from the volatile samples from the damaged persimmon trees, including 17 terpenoids (62.68%) and 6 alcoholic compounds (7.52%). Six compounds were selected for bioassay trials, of which two, a-pinene and linalool, showed a remarkable attractive effect to the predator *Harmonia axyridis*.

Key words: *Ceroplastes japonicus* Green, persimmon, plant volatiles, natural enemies.

Introduction

Japanese wax scale, *Ceroplastes japonicus* Green (Hemiptera: Coccoidea: Coccidae) is a destructive pest in many forests, fruit orchards and ornamental plants in China. More than 150 species of host plant have been recorded. Forests of persimmon, *Diospyros kaki* L., and jujube, *Ziziphus jujube* Mill, are so heavily damaged by this scale in north China that fruit loss is about 70% (Xie, 1998). The wax scale is hard to control using chemical pesticides due to the thick protective wax test secreted by the scale. Although some parasitoids and predators exist in the fields and forests, their role in controlling scale populations is limited. A time delay in the build-up of the populations of natural enemies in relation to the scale infestation and the population fluctuations in the field are two main constraints in their role as biological controllers.

In recent years, more and more evidence has been provided that the food chains existing in the tri-trophic levels of the host plants- herbivores- natural enemies are linked in great degree by semiochemicals. For example, the researches of Vinson (1986), Turlings *et al.* (1991, 1995, 1998), Dick *et al.* (1990, 1993), Vet *et al.* (1992), Reed *et al.* (1995), Ngisong *et al.* (1996), Takeshi Shimoda *et al.* (1997), Le Ru *et al.* (1999), Xu Ning *et al.* (1999), Han Chen (2000) have all reported on the role of the volatiles from infested plants as indirect defences to attract the natural enemies. These discoveries are significant for a deeper understanding of pest bio-control and the co-evolution of the herbivores, their host plants and natural enemies. However, only a few scale insects have been used in these research studies. For example, Nadel and van Alphen (1987) showed that the parasitoid *Apoanagyrus lopezi* (Hymenoptera: Encyrtidae) of the cassava mealybug *Phenacoccus manihoti* Matile-Ferrero could be attracted by cassava, *Manihoti esculenta*, damaged by the mealybug. After that, Souissi *et al*. (1998, 1999) studied the olfactory responses and behavioral responses of *A. lopezi* to the plant, mealybug, and plant-mealybug complexes. Xie *et al*. (2004) reported that the fresh twigs and leaves of Bunge prickly-ash trees, *Zanthoxylum bungeanus* damaged by a mealybug, *Phenacoccus azaleae,* showed an attractive effect on the ladybeetle, *Harmonia axyridis*. At the same time, they discovered that the composition of the damaged host plant changed.

In recent years, we have conducted studies on the role of semiochemicals in the tritrophic systems of persimmon tree and jujube tree - Japanese wax scale, *Ceroplastes japonicus* - natural enemies and its relevance for biological control (Yang, 2006). In this paper, we evaluate the effect of *C. japonicus* infestation on induced host plant emission of volatiles and their relevance in attracting the natural enemies, *Chrysopa sinica* Tiedet (Neuroptera: Chrysopidae), and *Harmonia axyridis* Pallas and *Chilocorus kuwanae* Silvestri (Coleoptera: Coccinellidae).

Materials and methods

The forest conditions and sampling period

Three persimmon forest stands located at Wanrong County of Shanxi Province of northern China, differing in damage level (undamaged, slightly damaged and heavily damaged) were used. The persimmon trees were about 50 years old and about 10m high, with crowns 8~10m wide. The trees were similar in the three stands. Each stands was separated by about 1000m distance. In the slightly damaged forest, scale populations were on average 6 scales/per leaf and 12 scales/10cm length of 1~2 years old twigs. In the heavily damaged forest, scale populations were more than 16 scales/leaf and more than 30 scales/10cm length of twig. The sampling period was from May to October in 2004, 2005 and 2006.

Predator's response to different odour sources

Samples of twigs and leaves were collected from each damage category on 15 trees. Samples were collected according to the four directions around the tree canopy and from three heights. In total 24 twig samples were taken per tree. The three predators were collected in the adult stages in the forest. The fresh samples of twigs and leaves and the predatory insects were taken to the laboratory as quickly as possible.

To test the tropism response of the three predators to the damaged and undamaged twigs and leaves, a glass Y-tube olfactometer system was used. The twigs and leaves from the damaged sample trees used as test odours were set in a round odour source box that connected with one arm (A arm) of the Y-tube and the twigs and leaves from either the undamaged sample trees or the blank control were set in another odour source box, connected to another arm (B arm). The target predatory insects were introduced from the open end of the straight arm (C arm) of the Y-tube. The tropism response and behaviour of the predators were observed and recorded. Six specimens of each species were tested individually. To test for the possible contribution of the test and body of the wax scale to the attraction activity of the three predators, the tests were repeated after removing the bodies of the wax scale from the damaged twigs and leaves before the test.

To test whether the response of predators varied at different times of day, we conducted tropism tests for all three predators at four times during one day, between 1:00~3:00, 7:00~9:00, 13:00~15:00 and 19:00~21:00, respectively. These tests were conducted in September. The plant materials used in the tests were all collected at the same time of the day for each of the tests.

Based on the composition of the volatiles from the damaged host trees, we selected six compounds that could be bought in the market to test the tropism response of *Harmonia axyridis*. The six compounds were: α-pinene, Linalool, 1-Octanol, 1-Octane, Hexanal and Naphthalene,2-methyl, and these were tested at four concentrations from 10^{-3}~10^{-6} g/mL against a blank control.

Collection of the volatiles and analysis

For collecting the volatiles of both the damaged and undamaged persimmon trees, a special headspace-Tenax-GR volatile trapping instrument was used in the sample forest. The volatile collection lasted one hour each time. During the collection process, the volatiles released by the sample twigs and leaves were kept in a special sample tube by adsorption of the solvent that was put in the tube beforehand. These tubes were carried to laboratory and their composition determined and analysed by applying the Thermal desadsorption - Cold Trap- Gas Chromatograph/Mass Spectrometry (TCT-GC/MS). Five samples per class of damage were used.

Results

Response of the predators to wax-scale damaged and undamaged persimmon trees

When the target predators were introduced into the straight tube of the Y-tube olfactometer, they first crawled around and swung their antennae to search the odour source. Some individuals crawled into the arm A that lead to the test odour source, others went towards the arm B, with the control odour source, while still others made little response at all. Some even crawled into the tube with the control odour source for a while, and then returned back to the tube leading to the test odour source. The numbers of the predators that effectively entered each arm or which made no choice, i.e., remained in the straight tube, were recorded.

The three predators differed significantly in their response the odour source of the highly damaged twigs and leaves of persimmon in comparison with the blank control (Table 1). In a comparison between the heavily damaged and slightly damaged twigs and leaves, all three predators were significantly more likely to enter the odour sources of the heavily damaged twigs and leaves. However, the three predators did not show significant differences in their response in a comparison between the slight damaged and the undamaged plant materials.

Table 1. Comparison of the tropic responses of three natural enemies to twigs and leaves of persimmon with three different levels of wax scale infestation.

Odour Source	*Chrysopa sinica*			*Harmonia axyridis*			*Chilocorus kuwanae*		
	Total No.	**Tendency percent**	***t*-Test**	**Total No.**	**Tendency percent**	***t*-Test**	**Total No.**	**Tendency percent**	***t*-Test**
Hd	91	**62.19**	$P<0.01$	79	**65.83**	$P<0.01$	90	**75.00**	$P<0.01$
Blank	53	38.81		31	25.83		25	20.83	
Sd	73	51.05	$P>0.05$	52	43.33	$P>0.05$	48	40.00	$P>0.05$
Ud	70	48.95		49	40.83		44	36.67	
Hd	90	**62.07**	$P<0.01$	70	**58.33**	$P<0.01$	81	**67.50**	$P<0.01$
Sd	55	37.93		40	33.33		33	27.50	

Notes: Hd: heavily damaged persimmon twigs and leaves; Sd: slightly damaged persimmon twigs and leaves. Ud: undamaged persimmon twigs and leaves; blank: without any odour materials.

Daily rhythm of response of the three predators

The three predators displayed a similar diurnal rhythm in their attraction response (Table 2). The strongest tropic response appeared at two periods of day, early morning (1:00~3:00) and early afternoon (13:00~15:00). In contrast, no significant difference to the two odours sources was observed at 7:00~9:00 in the morning and 19:00~21:00 in the evening (Table 2).

Damaged plant materials were still significantly more attractive to the three predators compared to the undamaged ones both at 3 and 6 hours after the scale insects had been removed from the plants (Table 3). Yet, it can be seen that the difference in response between the groups decreased with time, being higher at 3 than at 6 hours post removal. On the other hand, the bodies of the wax scales also attracted significantly more of all three predators in comparison with the undamaged tree (Table 3).

Table 2. Comparison of the tropic responses of three natural enemies to twigs and leaves of persimmon with three different levels of wax scale infestation at four different times of day.

Odour Source and Time	*Chrysopa sinica*			*Harmonia axyridis*			*Chilocorus kuwanae*		
	Total No.	Tendency percent	*t*-Test	Total No.	Tendency percent	*t*-Test	Total No.	Tendency percent	*t*-Test
Hd-a	106	**67.95**	*P* < 0.01	78	**65.00**	*P* < 0.01	84	**70.00**	*P* < 0.01
Ud-a	50	32.05		29	24.17		28	23.33	
Hd-b	90	50.56	*P* > 0.05	52	43.33	*P* > 0 05	56	46.67	*P* > 0.05
Ud-b	88	49.44		49	40.83		49	40.83	
Hd-c	122	**66.67**	*P* < 0.01	80	**66.67**	*P* < 0.01	89	**74.17**	*P* < 0.01
Ud-c	61	33.33		32	26.67		24	20.00	
Hd-d	82	50.93	*P* > 0.05	55	45.83	*P* > 0 05	52	43.33	*P* > 0.05
Ud-d	79	49.07		52	43.33		46	38.33	

Hd: heavily damaged persimmon twigs and leaves; Sd: slightly damaged persimmon twigs and leaves. Ud: undamaged persimmon twigs and leaves; blank: without any odor materials. The following letters refer to the time of day when the tests were conducted: a- 1:00~3:00. b- 7:00~9:00. c- 13:00~15:00. d- 19:00~21:00.

Table 3. Comparison of the tropic response of three predators to wax scale damaged twigs and leaves with wax scale bodies and damaged twigs and leaves with the wax scale bodies removed.

Odour Source and Time	*Chrysopa sinica*			*Harmonia axyridis*			*Chilocorus kuwanae*		
	Total No.	Tendency percent	*t*-Test	Total No.	Tendency percent	*t*-Test	Total No.	Tendency percent	*t*-Test
Hd-3	81	**61.84**	*P* < 0.01	62	**51.67**	*P* < 0.05	75	**62.50**	*P* < 0.01
Ud	50	38.16		49	40.83		40	33.33	
Hd-6	82	56.55	*P*< 0.05	59	49.17	*P*< 0.05	64	53.33	*P*< 0.05
Ud	63	43.45		52	43.33		48	40.00	
WB	75	**58.59**	*P* < 0.01	63	**52.50**	*P* < 0.01	71	**59.17**	*P* < 0.01
Ud	53	41.41		45	37.50		40	33.33	

WB- wax scale body only, Ud- undamaged leaves and twigs, Hd-3 and Hd-6 - heavily damaged twigs and leaves 3 and 6 hours respectively after the scale bodies had been removed.

Composition of the volatiles emissions

By comparing the components of the volatiles from the damaged persimmon tree with that from the undamaged one, we found that the persimmon trees were induced to change their volatile compositions by the wax scale attack (Table 4). Some components were detected only from the damaged samples, while other components changed their relative abundance.

About 60 components were detected in the volatiles. The terpenoid compounds might be the most important volatiles produced by the damaged tree to attract the natural enemies. Of these, four terpenoid components were produced only from the damages samples: Camphene, Bicyclo[3,1,1]hept-2-ene,2,6,6-trimethyl-, Ocimene and 2,6-Dimethyl-1,3,5,7-octatetraene (E). At the same time, six terpenoid components increased in their relative abundance to differing degrees: 4[10]-Thujene, Bicyclo[3,1,1]heptane,6,6-dimethyl-2-methylene-(1S), D-Limonene, Hexa-hydro-farnesol, Bicyclo[2,2,1]heptan-2-one,1,7,7-trimethyl-[S] and Farnesane. Another five terpenoid components did not change in their abundance in the volatiles of the damaged trees.

Alcohols was the second most important components. Two, namely 3-Hexen-1-ol and 1-Decanol, were newly synthesized and another four alcohol compounds (1-Octanol, 1-Hexadecanol, 2-methyl-, 1-Undecanol and 1-Pentadecanol) increased in their abundance in the volatiles from the damaged trees. A third group of the compounds in the volatiles from the damaged trees was esters, including 1-Butanol, 3-methyl-, acetate, Dibutyl phthalate and Tricyclo[4.2.2.0.2.5]deca-7,9-diene-7,8-dicarboxylic acid, 3-cyano-, dimethyl ester. Their abundance increased after the host tree was attacked by the wax scale. Long chain saturated and unsaturated hydrocarbons comprised the fourth group of compounds including 23 compounds. The relative contents of these substances did not vary obviously between the undamaged and damaged trees. The fifth group detected from the volatiles was one ketone, acetone, three aldehyde compounds including hexanal, nonanal and dodecanal, and one acid, acetic acid.

Response of Harmonia axyridis *to six chosen compounds*

The results (Table 5) showed that α-pinene, Linalool and Hexanal at concentrations of 10^{-4} and 10^{-5} g/mL displayed significant attraction to *H. axyridis*. In addition, a significant attraction was also found for α-pinene at a concentration of 10^{-5} g/mL that was not observed for Linalool and Hexanal. The other three compounds (Napthalene, 2-methyl, 1-Octane and 1-Octanol) showed no visible attraction at any concentration. On the other hand, 1-Octanol gave the opposite result at concentrations of 10^{-3}g/mL and 10^{-4}g/mL, suggesting repellency to the lady beetle. This test confirmed that some compositions of the volatiles of the damaged twigs and leaves were able to attract the natural enemy but at specific concentrations.

Table 4. Differences in the composition of the volatiles from undamaged and wax scale damaged persimmon trees. Only those volatiles showing difference between damaged and undamaged leaves are included.

Compound	Molecular formula	Relative molecular weight	Relative content (%)	
			Un-damaged	Damaged
I. Terpenoid compound				
1. 3-Thujene	$C_{10}H_{16}$	136	3.41	3.08
2. 1R-Pinene	$C_{10}H_{16}$	136	15.42	9.53
3. Camphene	$C_{10}H_{16}$	136	—	0.18
4. Bicyclo[3,1,1]hept-2-ene,2,6,6-trimethyl-	$C_{10}H_{16}$	136	—	3.46
5. D-Limonene	$C_{10}H_{16}$	136	2.92	3.36
6. Ocimene	$C_{10}H_{16}$	136	—	9.26
7. Terpinene	$C_{10}H_{16}$	136	0.32	0.17
8. 2,6-Dimethyl-1,3,5,7-octatetraene,(E)	$C_{10}H_{14}$	134	—	0.19
9. Bicyclo[2,2,1]heptan-2-one,1,7,7-trimethyl-,[S]	$C_{10}H_{16}O$	152	0.38	0.79
10. Caryophyllene (1)	$C_{15}H_{24}$	204	32.91	20.10
11. Caryophyllene (2)	$C_{15}H_{24}$	204	7.75	5.53
II. Alcohol compound				
1. 3-Hexen-1-ol	$C_6H_{12}O$	100	—	0.28
2. 1-Octanol	$C_8H_{18}O$	130	0.26	0.34
3. 1-Decanol	$C_{10}H_{22}O$	158	—	1.51
4. 1-Undecanol	$C_{11}H_{24}O$	172	0.28	2.70
5. 1-Pentadecanol	$C_{15}H_{32}O$	228	0.51	1.97
III. Ester compound				
1. 1-Butanol, 3-methyl-, acetate	$C_7H_{14}O_2$	130	0.29	1.62
2. Tricyclo[4.2.2.0.2.5]deca-7,9-diene-7,8-dicarboxylic acid, 3-cyano-, dimethyl ester	$C_{15}H_{15}NO_4$	273	0.52	0.80
IV. Hydrocarbon compound				
1. Nonane	C_9H_{20}	128	0.30	1.06
2. Tridecane, 4-methyl-	$C_{14}H_{30}$	198	—	0.26
3. Nonadecane	$C_{19}H_{40}$	268	—	0.33
V. Ketone, aldehyde and acid compound				
1. Acetone	C_3H_6O	58	1.30	0.68
2. Hexanal	$C_6H_{12}O$	100	0.30	0.61
3. Nonanal	$C_9H_{18}O$	142	4.17	2.32
4. Acetic acid	$C_2H_4O_2$	60	0.54	0.89

Table 5. Taxis choice of lady beetle, *Harmonia axyridis*, to the six compounds.

Concen-tration (g/Ml)	Test lady beetles	Taxis of lady beetles	Hexanal		Linalool		α-pinene		Naphthalene, 2-methyl-		1-Octane		1-Octanol	
			R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
		NC	10	11	8	9	10	9	13	10	9	10	1	2
		NV	10	9	9	9	10	10	7	7	11	10	18	17
10^{-3}	20	NR	0	0	3	2	0	1	0	3	0	0	1	1
		AP%	0	10	-5	0	0	-5	30	15	-10	0	-85	-75
		t-Test	P > 0.05		P > 0.05		P > 0.05		P > 0.05		P > 0.05		P < 0.01	
		NC	13	12	16	17	12	13	11	12	9	11	2	2
		NV	7	7	4	2	6	3	9	8	8	8	16	16
10^{-4}	20	NR	0	1	0	1	2	4	0	0	3	1	2	2
		AP%	30	25	60	75	30	50	10	20	5	15	-70	-70
		t-Test	P < 0 01		P < 0 01		P < 0 05		P > 0 05		P > 0.05		P < 0.01	
		NC	12	14	9	10	14	15	10	9	9	8	8	9
		NV	5	6	6	6	4	5	10	10	9	9	8	8
10^{-5}	20	NR	3	0	5	4	2	0	0	1	2	3	4	3
		AP%	35	40	15	20	50	50	0	-5	0	-5	0	5
		t-Test	P < 0 05		P < 0 05		P < 0 01		P > 0 05		P > 0 05		P > 0 05	
		NC	11	12	10	11	13	12	9	10	7	7	10	9
		NV	9	8	8	6	4	6	9	8	8	6	8	9
10^{-6}	20	NR	0	0	2	3	3	2	2	2	5	7	2	2
		AP%	10	20	10	25	45	30	0	10	-5	5	10	0
		t-Test	P > 0.05		P > 0.05		P < 0.05		P > 0.05		P > 0.05		P > 0.05	

R1-Repetition 1; R2-Repetition 2; NC-Number of individuals entered into the arm with the compound; NV: Number of individuals entered into the vacant arm; NR: Number of individuals with no response; AP: Attracted percentage.

Discussion and conclusion

These results provide evidence that the attack of the Japanese wax scale, *Ceroplastes japonicus* on persimmon trees can stimulate host plant defence by inducing the production of volatile compounds different from undamaged trees. Damaged persimmon trees might release some special volatiles that serve as signals to attract natural enemies. Similar results have been found in other tri-trophic systems, for example, with the parasitoid *Apoanagyrus lopezi* (Hymenoptera: Encyrtidae) which is attracted to the host plant, cassava damaged by the mealybug, *Phenacoccus manihoti* (Nadel & Van Alphen, 1987). Also the parasitoid *Diaeretiella rapae* M'Intosh (Hymenoptera: Aphidiidae) responded to odour of plants, aphids, and plant-aphid complexes (Reed *et al*., 1995). The tropism tests of the three predators used here proved that only the most heavily damaged trees could give a visible response by the predators. This phenomenon indicates a switch effect in the host plant to release the volatiles to attract the natural enemies. A similar switch effect was noticed in the study on *Aphis craccivora* Koch inducing *Vicia faba* leaves to attract its natural enemy (Wang et al., 1994). This switch effect may be useful to determine the pest population control threshold.

Of the four time periods tested, the damaged twigs and leaves showed attraction activity only just after midnight and just after noon. This may be related to the host plant's physiology and to the temperature, humidity and atmosphere. The real reason needs to be studied in the future.

After removing the scale body from the damaged twigs and leaves, the volatiles emitted by the damaged twigs and leaves were still attractive to the predators. However, the volatiles released by the wax test and body of the wax scale were also attractive to the predators. Therefore, we may conclude that the volatiles from both the damaged persimmon trees and from the wax scale itself attracted the natural enemies. These two treatments might act together synergistically. In previous studies, it has been reported that natural enemy insects showed stronger tropic responses to the complex of the infested host plant and herbivores, even when they preferred the volatiles of the infested host plant (Turlings *et al*., 1990; 1991; 1995; Souissi *et al*., 1998; 1999).

By analysing the chemical compositions of the volatiles of the heavily damaged and undamaged trees, big differences were found in several compounds between the two groups. However, it is absolutely necessary to study the function of the chemical compounds in the volatiles. Some may be attractive to the natural enemies, while others might be toxic compounds or deterrents against the Japanese wax scale to prevent the wax scale feeding again on this particular host plant. Of the six compounds used to test their attractive activity to the ladybeetle, *Harmonia axyridis*, we showed that three were highly attractive to the predator but only at specific concentrations. These findings may have practical applications in the use of semiochemicals to enhance biological control. Other studies are still needed to apply these semiochemicals in simulated biological control conditions.

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