无翅茶蚜对茶树挥发物的触角电生理和行为反应

韩宝瑜,韩宝红

(农业部茶叶化学工程重点开放实验室,中国农业科学院茶叶研究所,杭州 310008)

摘要:分别使用昆虫触角电位仪(EAG)和四臂嗅觉仪,测定了无翅茶蚜 *Toxoptera aurantii* Boyer 对 14 种茶树挥发性化合物、14 种挥发物中"绿叶气味"组成的混合物(GLV)、14 种挥发物的混合物(ACB)、以及新鲜嫩叶、芽、嫩茎、成叶和茶蚜为害嫩叶(ADYL)的 EAG 反应和行为反应。ACB 引出最大的 EAG 反应值,茶树挥发物主要组分 *Z*-3-己烯-1-醇、*E*-2-己烯醛、n-己醇、水杨酸甲酯和苯甲醇也引起较大的 EAG 反应值。4 种正常茶梢的器官也引出较大的 EAG 反应,以嫩叶最强、依次为芽、嫩茎和成叶。有趣的是 ADYL 引出弱的负的 EAG 值。用嗅觉仪进行的生物测定表明,嫩叶以及主要的茶梢挥发性成分乙酸-*Z*-3-己烯酯、水杨酸甲酯、*E*-2-己烯-1-醇和 *Z*-3-己烯-1-醇等也具有较强引诱活性。研究显示无翅茶蚜可能利用茶梢挥发物作为利它素而寻觅适宜的取食场所,如茶树嫩梢。

关键词:无翅茶蚜;茶梢挥发物;触角电生理;行为反应;引诱

文章编号:1000-0933(2007)11-4485-06 **中图分类号**:Q968 **文献标识码**:A

EAG and behavioral responses of the wingless tea aphid *Toxoptera aurantii* (Homoptera: Aphididae) to tea plant volatiles

HAN Bao-Yu, HAN Bao-Hong

Key Laboratory of Tea Chemical Engineering of Ministry of Agriculture, Tea Research Institute of Chinese Academy of Agricultural Sciences, Hangzhou 310008, *China*

***Acta Ecologica Sinica*, 2007, 27(1): 4485 ~ 4490.**

Abstract: Electrophysiological and behavioral responses of the apterous tea aphid, *Toxoptera aurantii* Boyer, to fourteen synthetic volatiles identified from tea shoots, and their partial (GLV mixture) and full (ACB) blends, as well as fresh young tea leaves, buds, tender stems, adult tea leaves and tea aphid-damaged young leaves (ADYL), were studied by using an electroantennography (EAG) and a four-arm olfactometer. The full blend elicited the largest EAG responses. Major volatile components from the tea shoots, *Z*-3-hexen-1-ol, *E*-2-hexenal, n-hexanol, methyl salicylate and benzylalcohol, were strongly EAG active. All the tea tissue materials tested also elicited significant EAG responses, with the young tea leaf being the strongest, followed by bud, tender stem and adult tea leaf. Surprisingly, ADYL elicited a weakly negative EAG response. In the olfactory assays, the fresh and tender tea leaves, as well as the individual major volatile components from the tender

基金项目:中国农科院杰出人才基金资助项目(2002-382);浙江省重点资助项目(2004C22040);浙江省青年人才培养专项资助项目(R304451);中国农科院院长基金课题资助项目(2005-42)

收稿日期:2007-01-22;修订日期:2007-08-09

作者简介:韩宝瑜(1963 ~),男,安徽人,博士,研究员,从事昆虫化学生态学、茶树病虫防治和茶叶香气研究. E-mail: han-insect@263.net

致谢:Thank Prof. Chen Zong-Mao for his supervision, and Dr. Qing-He Zhang from the USA for his excellent suggestion.

Foundation item: The project was financially supported by the Grant from the Research Foundation for Outstanding Talents of CAAS (No. 2002-382); the Key Project of Zhejiang Province (No. 2004C22040); the Grant from youth talent trainning project of Natural Science Foundation of Zhejiang Province (No. R304451); a grant from Natural Science Foundation of CAAS (No. 2005-42)

Received date: 2007-01-22; **Accepted date**: 2007-08-09

Biography: HAN Bao-Yu, Ph D., Professor, mainly engaged in chemical ecology of insects, tea plant disease and pest control and tea aroma. E-mail: han-insect@263.net

shoots (EAG-active), e. g. *Z*-3-hexenyl acetate, methyl salicylate, *E*-2-hexen-1-ol, *Z*-3-hexen-1-ol, etc. were all attractive. This result might indicate that the apteral tea aphids may use tea shoot volatiles as kairomone to find their optimal feeding sites, i. e. fresh tender tea shoots.

Key Words: wingless tea aphid; tea shoot volatiles; electrophysiology; behaviour responses; attraction

Tea plants, *Camellia sinensis* (L.) O. Kuntze, are grown throughout the Central and Southern China. High quality teas are mostly processed from the tender shoots of the tea plants. The tea aphid, *Toxoptera aurantii* (Boyer) (Homoptera: Aphididae), pierces and sucks the sap of tender tea shoots and excretes honeydew onto the tea leaves, and reduces the photosynthesis of the tea leaves [1]. This aphid could sometimes cause severe tea shoot damages, and result in significant loss in tea yield and deterioration in tea quality, since no chemical control measures, such as spraying the insecticides, are allowed due to the residue considerations. In the tea garden system, the tea aphid is a non-host alternating species. Under long day-time conditions these aphids reproduce virginoparae, while the short day-time in fall induces the formation of the sexual forms. During the tea growing season, the aphids live in the mode of parthenogenesis and produce only the female individuals, and most of them are wingless. Generally, the number of the aphids per tea shoot ranges from a couples of dozens to several hundreds. If the number exceeds three hundreds or so, a batch of winged aphids will appear and fly away.

The apterous virginoparae of the vetch aphid, *Megoura viciae* Buckton (Homoptera: Aphididae) perceives both the general host plant volatiles and the more-specific components associated with the odour blends of non-host plant species [2]. And the further study, both at the sensory and behavioural level, may explore the possibilities of plant odour manipulation [2]. Plant volatiles play a very important role in host selection of some aphid species, including the winged tea aphid [1,3-5]. The objectives of this study were to determine if the tea shoot volatiles are also perceived by (electrophysiologically) and attractive to (behaviourall) the wingless tea aphid.

1 Materials and methods

1.1 Electrophysiological test

Electroantennogram (EAG) recordings from the wingless tea aphids were made using Ag-AgCl glass capillary electrodes filled with Ringer's solution. The antenna of tested adult aphid was excised and inserted into the reference electrode. The tip of the antenna was cut and placed inside the recording electrode. Odor presentation was similar to that in the previous studies [1,6]. Twenty μl of standard solution of each test compound was applied onto filter paper strips (5 mm × 60 mm), and the solvent was allowed to evaporate for 30 s before the paper strip was inserted into the glass Pasteur pipette (5 cm long). The tip of the pipette was inserted about 3 mm into a small hole on the wall of a steel tube (5 mm diameter × 15 cm long) directed over the antennal preparation. An air stimulus controller (model CS-05b, Syntech) was used for air and odor delivery with a constant flow (20 ml/min) of charcoal-filtered and humidified air passed over the antenna through the open end of the steel tube positioned 15 mm from the antenna. During odor stimulation, 20 ml/min of air was applied through the pipette into the main airflow for 0.5 s. Intervals of 2 min were maintained between stimulations. Before the normal test, we selected a few of the tested aphids to conduct preliminary test. If the *Z*-3-hexen-1-ol at concentration of 10^{-6} g/ml could elicit strong EAG responses by the pre-tested aphids, whereas the liquid paraffin elicited no responses, this batch of tested aphids were considered as in good condition. Then, each compound was tested against 15 individual aphid antennae. The signals were passed through an amplifier (Syntech CS-05), displayed on an oscilloscope and stored in a computer using Syntech software.

The tested odors were (i) the main components from the volatiles of the tea shoots [1,7,8]: *Z*-3-hexen-1-ol, *E*-2-

hexenal, *Z*-3-hexenyl acetate, 2-penten-1-ol, 1-penten-3-ol, n-hexanol, n-pentanol, linalool, geraniol, methyl salicylate, benzaldehyde, benzoic acid, benzylalcohol and indole (all chromatographically pure), each diluted in paraffin at concentration of 10^{-2}, 10^{-4}, and 10^{-6} g/ml; (2) the full blend, composed of all the odour components listed above (ACB) at an equal ratio, at concentrations of 10^{-2}, 10^{-4}, and 10^{-6} g/ml; (3) a mixture of green leaf volatiles (GLV), composed of *Z*-3-hexen-1-ol, *E*-2-hexenal, *Z*-3-hexenyl acetate, 2-penten-1-ol, 1-penten-3-ol, n-hexanol and n-pentanol (at an equal ratio), at concentrations of 10^{-2}, 10^{-4}, and 10^{-6} g/ml; (4) natural materials (40 mg each): the 1st leaf below the tea buds (L), tea bud, tender stem, and the 4th leaf below the tea bud (4L), and the 1st leaf below the tea bud damaged by five wingless aphids for 48 h, i. e. tea aphid-damaged young leaves (ADYL).

1.2 Four-arm olfactometer bioassays

1.2.1 Insects

The tested aphids were taken from a colony started with one virginopara in 2000. Aphids in the colony were reared continuously on the tea seedlings in the experimental tea gardens of Tea Research Institute of Chinese Academy of Agricultural Sciences.

1.2.2 Four-arm olfactometer and test protocol

The four-arm olfactometer used was modified according to Pettersson and made from clear plexiglass [9–11]. The length of the square edges was 10 cm, and the height of central test chamber was 1 cm. Each arm was connected to a glass jar containing either an odor source or an empty control jar, humidified jar, charcoal filter and flow meter. Before the test, one open ampoule filled with 1 ml of the odor source solution and 3 open ampoules with paraffin were placed into the odor source jar and 3 control jars, respectively. A pump drew air through the central opening of the olfactometer, and the airflow of each arm was same and maintained at 110 ml/min.

Each compound or tea shoot material was tested against 20 wingless aphids, which were introduced into the olfactometer once. Before aphids were introduced, air was drawn in the olfactometer for 10 s. After aphids were introduced, numbers of aphids situated at each arm (three control areas and one odor area) and "A" zone at 3, 6 and 9 min were recorded. The "A" zone in the central area of olfactometer facing air drawing gob. The tests were repeated six times.

After each test, the olfactometer was washed with pure alcohol and dried. Treatments for each arm were re-arranged after each test in a rotation fashion. Odor source jars and control jars were cleaned with potassium dichromate solution after each test.

The bioassays were conducted during the day-time between 9:00 and 14:00, at 21－24℃, and 80 % relative humidity.

1.2.3 Tested odor sources

The tested odors were *Z*-3-hexenyl acetate, methyl salicylate, *E*-2-haxenal, *Z*-3 hexen-1-ol, geraniol, n-hexanol, benzylalcohol, linalool, 1-penten-3-ol, and benzyl alcohol (with their purities > 98%), each diluted in hexane at concentration of 10^{-4} g/ml, as well as 0.7 g of fresh and tender tea shoots (TS).

1.2.4 Statistical analysis

Averages of the aphid members from three control areas were taken as CK values. A Duncan's test was conducted to separate means among different treatments such as odour sources, CK control and the "A" zones.

2 Results

2.1 Electrophysiological responses

2.1.1 Response profile from volatiles

EAG responses of the aphid antennae to the tested chemicals and the natural materials ranged from 0 to 300 μV, with most of responses being between 50 and 150 μV (Fig. 1). At the dosages tested, the EAG responses decreased as increase of the odor dosage (Fig. 2). At high dosage (10^{-2} g/ml), chemical #1, #2, #15 and #16 seemed to elicit higher EAG responses than others, while #8 (geraniol) and #11 (benzoic acid) were almost inactive. At dosage of 10^{-4} g/ml, chemical # 15, the full blend, showed the strongest EAG activity, followed by #1 (E-2-hexenal), #9 (MS) and #13 (indole). Interestingly, #8 and #11 elicited decent EAG activities at lower concentrations. At 10^{-6} g/ml level, #16 (Z-3-hexen-1-ol) showed the strongest EAG, followed by #1, #12 and #15. #8 and #11 were as active as at 10^{-4} g/ml. In any cases, liquid paraffin elicited no obvious EAG responses.

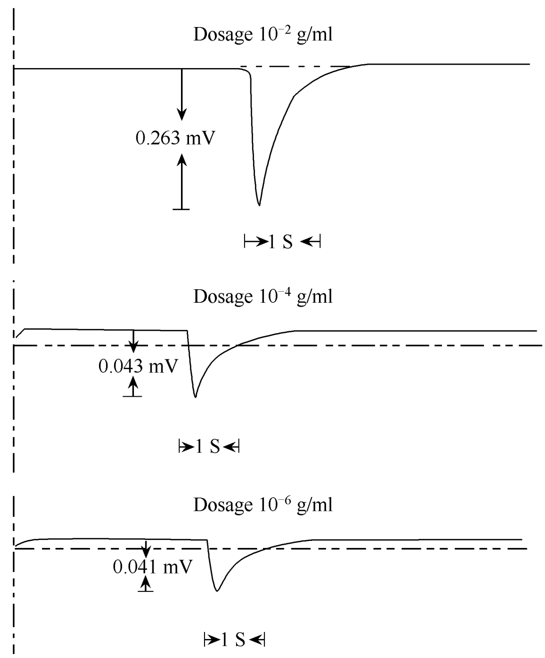

Fig. 1 Representative EAGs from apterous adult aphids to *Z*-3-hexen-1-ol at 10^{-6}, 10^{-4}, and 10^{-2} g/ml

2.1.2 EAGs elicited by tea shoot tissues

EAG responses to natural materials (L, bud, tender stem, 4L and ADYL) were recorded as follows (mean ± SD): (34.0 ± 18.0), (24.0 ± 16.0), (20.0 ± 15.0), (07.0 ± 12.0) μV, and (-10.0 ± 3.0) μV, respectively. Surprisingly, ADYL elicited a weakly negative EAG activity.

2.2 Attraction of wingless tea aphids to tea plant volatiles in an olfactometer

The differences in the numbers of aphids among CK, odor area and "A" zone were significant ($p < 0.01$), indicating a clear attraction to the tested odors by the apterous aphids (Table1). Furthermore, the differences in the attraction of wingless aphids to the various odors were also significant. The fresh and tender tea leaf, *Z*-3 -hexenyl acetate, methyl salicylate and *E*-2-haxenal, etc. were more attractive than others (Table 2).

Table 1 Difference among the aphid number in CK, odor area and "A" zone

Area	Average
Odor area	24.7 A
"A" zone	11.2 B
CK area	8.0 B

Notes: Duncan's test is used. Different letters in the same column are for significant level of $p < 0.01$

Table 2 Difference in numbers of wingless tea aphids dispersing in odor area, CK and "A" Zone

Odor resource		Odor area	CK	A Zone (Non-response)
fresh and tender tea leaf	a	32.0 ± 0.1	6.9 ± 2.1	7.2 ± 2.9
Z-3-hexenyl acetate	a	31.0 ± 0.7	6. 3 ± 1.6	10.0 ± 2.8
Methyl salicylate	ab	28.4 ± 1.9	5.8 ± 2.3	4.0 ± 2.5
E-2-haxenal	ab	28.0 ± 2.5	7.1 ± 1.5	10.8 ± 4.1
Z-3-hexen-1-ol	ab	27.6 ± 2.1	8.5 ± 2.2	6.8 ± 2.0
Geraniol	b	26.0 ± 0.7	7.9 ± 2.6	10.2 ± 1.6
n - hexanol	b	25.6 ± 3.2	8.5 ± 2.8	8.8 ± 0.5
Benzylalcohol	c	21.4 ± 2.6	9.8 ± 2.9	9.2 ± 3.7
Linalool	c	20.8 ± 2.5	8.1 ± 1.2	14.8 ± 3 .1
1-penten-3-ol	cd	17.8 ± 2.5	8.9 ± 1.6	15.6 ± 4.7
Benzyl alcohol	d	13.8 ± 2.3	10.2 ± 2.0	15.6 ± 3.6

Notes: Different letters in the second column indicate statistical significance of $p < 0.05$

3 Discussion

Significant EAG responses to plant volatiles by both apterous and alate virginoparae of several aphid species have been reported [2,12]. The adult wingless and winged virginoparae of *Aphis fabae* Scop. showed a similar peripheral perception to all the tested thirteen of the plant volatiles with an exception to allyl isothiocyanate [3]. Furthermore, some of the EAG active compounds found in the current study, such as the unsaturated and saturated C_6 aldehydes and alcohols (*E*-2-hexenal, *Z*-3-hexen-1-ol and hexanol) were also antennally active in other aphids [2,4,12,13]. Methyl salicylate showed obvious EAG responses as well by *Sitobion avenae* (F.) and *Rhopalosiphum padi* (L.) [4]. Benzylalcohol was EAG active on *T. aurantii* Boyer, but its EAG activity has not been reported in other aphid species yet. *Z*-3-hexen-1-ol, *E*-2-hexenal, methyl salicylate and benzylalcohol are major volatile components released from fresh tea shoots [8]. Our previous and current results clearly indicated that tea shoots/leaves and volatiles identified from these natural materials not only elicited strong EAG responses but also showed significant attraction to both the apterous and the alate tea aphids [1].

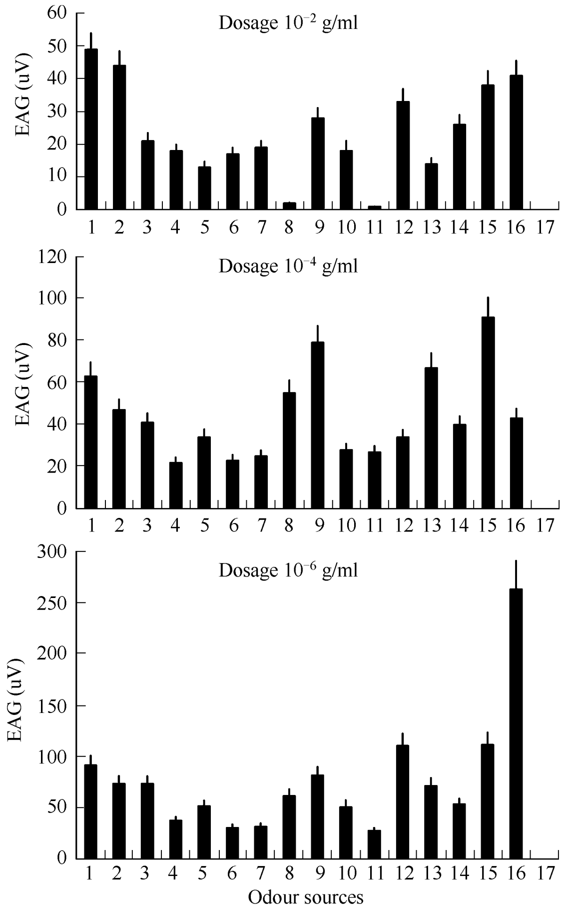

Fig. 2 EAG responses of apterous virginoparae of the tea aphid to an array of synthetic tea shoot volatiles and paraffin

1, *E*-2-hexenal; 2, n-hexanol; 3, 2-penten-1-ol; 4, n-pentanol; 5, *Z*-3-hexenyl acetate; 6, 1-penten-3-ol; 7, linalool; 8, geraniol; 9, methyl salicylate; 10, Benzaldehyde; 11, benzoic acid; 12, benzylalcohol; 13, indole; 14, green leaf volatiles; 15, all component blend; 16, *Z*-3-hexen-1-ol; 17, liquid paraffin

On the other hand, alate and apterous aphids showed different antennal sensitivities toward their host plant volatiles. Antennae of both alatae and apterae of *Sitobion avenae* (Fabr.) responded to alcohols containing six to seven carbon atoms, green leaf volatiles and benzaldehyde; however, alatae showed stronger EAG responses than did the apterae [5]. Similar differences in EAG responses to hexanol or *Z*-3-hexen-1-ol were also found between alatae and apterae of *Nasonovia ribis-nigris* (Mosley) [6]. Higher antennal sensitivities by the alatae to specific volatile cues or to a set of common volatile cues in a given proportion would be beneficial for their in-flight host-finding efficacy [3]. The same was also true for the tea aphid [1]; however, apterae of the tea aphids not only showed constant EAG activities, but were also significantly attractive to the volatiles from the tea shoots. This might be one the reasons that apterous of the tea aphid prefer to feed on fresh tea shoots.

Unlike the other published EAG data, the EAG responses of the tea aphid in our study decreased as increase of the odor dosages at range of 10^{-6}—10^{-2} g/ml, which might indicate an unique EAG response characteristic of the tea aphid. Further study might uncover the optimal dosages for EAG response by this aphid species by decreasing the dosage.

Herbivore induced plant volatiles (HIPVs) by aphids or other insects might repel different species of aphids [3,17]. In the present study, volatiles from the tea leaves badly damaged by the apterous aphids elicited negative EAG responses. It is not known if the negative EAG values might indicate any potentially negative behavioral effect on the aphids. However, it is possible that some of HIPVs might play a role in regulating the spacing behavior of the aphids, or further inducing the formation of winged tea aphids for dispersion or migration to other tea shoots.

References:

[1] Han B Y, Zhou C S, Cui L. Attraction of winged tea aphids to tea plant volatiles measured by olfactometry and electroantennography. Trop. Agric. (Trinidad), 2005, 82 (2): 138－142.

[2] Visser J H, Piron P G M. Olfactory antennal responses to plant volatiles in apterous virginoparae of the vetch aphid *Megoura viciae*. Entomol. Exp. Appl., 1995, 77: 37－46.

[3] Quiroz A, Pettersson J, Pickett J A, Wadhams L J, Niemeyer H M. Semiochemicals mediating spacing behavior of bird cherry-oat aphid, *Rhopalosiphum padi*, feeding on cereals. J. Chem. Ecol., 1997, 23: 2599－2607.

[4] Quiroz A, Niemeyer H M. Olfactometer-assessed responses of aphid *Rhopalosiphum padi* to wheat and oat volatiles. J. Chem. Ecol., 1998, 24 (): 113－124.

[5] Russell G B, Faundez E H, Niemeyer H M. Selection of *Nothofagus* host trees by the aphids *Neuquenaphis staryi* and *Neuquenaphis edwardsi*. J. Chem. Ecol., 2004, 30: 2231－2241.

[6] Park K C, Elias D, Donato B, Hardie J. Electroantennogram and behavioural responses of different forms of the bird cherry-oat aphid, *Rhopalosiphum padi*, to sex pheromone and a plant volatile. J. Insect Physiol., 2000, 46: 597－604.

[7] Han BY, Chen Z M. Composition of the volatiles from intact and tea aphid-damaged tea shoots and their allurement to several natural enemies of the tea aphid. J. Appl. Ent., 2002, 126: 497－500.

[8] Li M J. Alcohols in the volatiles of tea. In: CHEN Z M ed. Chinese Tea Big Dictionary. Beijing: Chinese Light Industry Press, 2000. 352.

[9] Pettersson J. Studies on *Rhopalosiphum padi* (L.). I. Laboratory studies on olfactometric responses to the winter host *Prunus padus* L. Lantbrukhoegsk. Ann., 1970, 36: 381－399.

[10] Hou Z Y, Yan F S, Chen X. Olfactory responses of *Lysiphlebia japonica* to volatile chemicals and fresh leaves of the host plants of cotton aphids in olfactometer. Entomologia Sinica, 1996, 3: 49－57.

[11] Hou Z Y, Chen X, Zhang Y, Guo B A, Yan F S. EAG and orientation tests on the parasitoid *Lysiphlebia japonica* (Hym., Aphidiidae) to volatile chemicals extracted from host plants of cotton aphid *Aphis gossypii* (Hom., Aphidae). J. Appl. Ent., 1997, 121: 495－500.

[12] Visser J H, Piron P G M, Hardie J. The aphids' peripheral perception of plant volatiles. Entomol. Exp. Appl, 1996, 80: 35－38.

[13] Hardie J, Visser J H, Piron P G M. Peripheral odour perception by adult aphid forms with the same genotype but different host-plant preference. J. Insect Physiol., 1995, 41: 91－97.

[14] Liu Y, Chen J L, Ni H X. Electroantennogram responses of *Sitobion avenae* and *Rhopalosiphum padi* to wheat plant volatiles. Acta Entomologia Sinica, 2003, 46: 679－683.

[15] Yan F S, Visser J H. Electroantennogram responses of the cereal aphid *Sitobion avenae* to plant volatile components. Proceedings of 5th International Symposium on Insect-Plant Relationships. Pudoc, Wageningen, 1982. 387－388.

[16] van Giessen W A, Fescemyer H W, Burrows P M, Peterson J K, Barnett O W. Quantification of electroantennogram responses of the primary rhinaria of *Acyrtosiphum pisum* (Harris) to $C_4－C_8$ primary alcohola and aldehydes. J. Chem. Ecol., 1994, 20: 909－927.

[17] Bernasconi M L, Turlings T C J, Ambrosetti L, Bassetti P, Dorn S. Herbivore-induced emissions of maize volatiles repel the corn leaf aphid, *Rhopalosiphum maidis*. Entomol. Exp. Appl., 1998, 87: 133－142.

参考文献：

[8] 李名君. 茶叶挥发性醇类物质. 见：陈宗懋主编：中国茶叶大辞典. 北京：轻工业出版社，2000. 352.

[14] 刘勇，陈巨莲，倪汉祥. 麦长管蚜和禾谷缢管蚜对小麦挥发物的触角电位反应. 昆虫学报，2003，46 (6)：679～683.