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Composition of the Volatiles from Intact and Mechanically Pierced Tea Aphid–Tea Shoot Complexes and Their Attraction to Natural Enemies of the Tea Aphid

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The volatile components from intact tea shoots (ITS), obtained by air entrainment, were identified by their mass spectra and retention times and confirmed by comparison with standard samples. They are *E*-2-hexenal, ocimene, *Z*-3-hexenyl acetate, *Z*-3-hexen-1-ol, butanoic acid-3-hexenyl ester, linalool, 1-octanol, geraniol, and indole. Volatiles from mechanically pierced tea shoots (MPTS) were identified as *E*-2-hexenal, ocimene, *Z*-3-hexen-1-ol, butanoic acid-3-hexenyl ester, linalool, geraniol, indole, *E*-2-hexenoic acid, *Z*-3-hexenyl formate, methyl salicylate, and benzyl alcohol, and volatiles from tea aphid−tea shoot complexes (TATSC) were identified as *E*-2-hexenal, ocimene, *Z*-3-hexenyl acetate, *Z*-3-hexen-1-ol, linalool, geraniol, indole, benzaldehyde, and *E*-2-hexenoic acid. *Z*-3-Hexen-1-ol is the main component in the three different types of volatiles, and the amount of benzaldehyde in TATSC volatiles is very ample. The attraction of the volatiles from ITS, MPTS, and TATSC, and the nine components of TATSC volatiles to the natural enemies, the coccinellid, *Coccinella septempunctata*, the parasite, *Aphidius* sp., and the lacewing, *Chrysopa sinica*, were determined by electroantennogram (EAG) and the wind tunnel bioassay. TATSC volatiles and benzaldehyde elicited much larger EAG responses and stronger upwind flight and arresting behavior from each natural enemy in the wind tunnel than other infochemicals.

KEYWORDS: Intact tea shoots; mechanically pierced tea shoots; tea aphid-tea shoot complexes; volatiles; benzaldehyde; electroantennogram; wind tunnel; *Toxoptera aurantii*; *Coccinella septempunctata*; *Aphidius* sp.; *Chrysopa sinica*

INTRODUCTION

Tea is one of the major drinks throughout the world, and the tea plant, *Camellia sinensis* (L.) O. Kuntze, is an important crop in China, Japan, India, Kenya, etc. The high grade teas are processed using the young shoots of the plants. Generally, the intact fresh shoots give off the attractive faint scents. However, if the plants are injured mechanically or damaged by pests, the scents emitted by the fresh shoots can smell different from the original ones. Therefore, it is inferred that the composition of the volatiles from the shoots has changed, an area of research about which little is understood so far.

Tea aphid, *Toxoptera aurantii* (Boyer) (Homoptera: Aphididae), pierces and sucks the shoot sap and seriously occurs in tea gardens in some countries that produce a great amount of merchant tea (*1*−*3*). In Chinese gardens, the aphid reproduces 20 ∼ 30 generations, while the tea plants sprout four or five times a year and are always colonized by the population of tea aphid (*4*). Insecticides are usually not applied to control the pests, to avoid residues in the commercial tea, especially in Chinese spring tea. Therefore, the pest can seriously reduce tea output and lower tea quality. However, in China, larvae and adults of the ladybeetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), are voracious predators of tea aphids; in the laboratory an adult can consume 100−120 aphids per day, and the population of *C. septempunctata* closely follows the population of tea aphid. Larvae and adults of the lacewing, *Chrysopa sinica* Tjeder (Neuroptera: Chrysopidae), also prey on tea aphids. The lacewings largely occur in May, and from September to October, and an adult can consume 70−90 tea aphids per day under laboratory conditions. The aphid parasite, *Aphidius* sp. (Hymenoptera: Braconidae: Aphidiinae), also attacks both nymphal and adult aphids. The average percentage of parasitism was around 10% in May to June, and about 15% in September to October (*4*). Some studies have shown that specific blends of odors produced by herbivore-injured plants as well as those released by the pests themselves are often attractive to certain insect predators and parasitoids (*5*, *6*). If the infochemicals were isolated and synthesized, we may use

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them to mediate natural enemies controling the aphids. Some attempts have been made to use synomones and kairomones in the manipulation of natural enemies to suppress pests (*7*, *8*). However, much less is known about the chemical communications among insects in tea gardens.

In the current study, we investigated the aroma composition of intact tea shoots and mechanically pierced tea shoots, determining also the relationship of the released volatiles to the flavor/aroma profile of tea aphid−tea shoot complexes. This report also addresses the chemical attraction of natural parasitoids of tea aphids.

MATERIALS AND METHODS

Preparation of Analysis of Volatiles. Intact tea shoots (ITS) and tea aphid−tea shoot complexes (TATSC) were used to collect the volatiles immediately after being gathered from the tea garden. Each tea shoot used was around 7-cm long and had one bud and three leaves, weighing approximately 0.7 g, in which one tea aphid−tea shoot complex carries about 100 tea aphids. After the intact tea shoots were collected from the tea garden, each was pierced by a no. 5 insect needle 100 times, and these act as mechanically pierced tea shoots (MPTS).

ITS, MPTS, and TATSC (200 g) were placed in a 20-L glass vessel with an inlet and an outlet (*9*) and were replaced at intervals of 12 h, respectively. Dried and purified air was guided through the inlet at 250−300 mL/min for 48 h. A tenax TA column with 150 mg of absorbent (Supelco Co., U. S.) activated under 275 °C with nitrogen for 4 h was attached to the outlet. Volatiles were desorbed by eluting the tenax traps with 1 mL of freshly distilled hexane, and 20 μL of 100 ppm decanoic acid ethyl ester was used as the internal standard. The resulting extract was concentrated to about 20 μL under a stream of nitrogen, 2 μL of which was immediately injected into a GC-MS. The gas chromatograph was the HP-5890A model, fitted with a PEG-20 M quartz capillary column (30 m, 0.2 mm i.d.). The mass spectrometer was the HP-5972 MSD model. The GC oven was maintained at 50 °C for 5 min and then programmed at 2 °C/min to 250 °C. Identifications were confirmed with retention time and peak enhancement on co-injection with authentic commercial samples. Referring to the above method, another 1 mL of eluting solution of the volatiles from the same weight of ITS, MPTS, and TATSC, collected by air entrainment, was prepared for behavioral bioassays.

Insects. The natural enemies were all collected from tea gardens. *Chrysopa sinica* and *Coccinella septempunctata* were reared on tea aphids in 500-mL glass containers, and were starved for 24 h before use in behavior tests. The parasitoid, *Aphidius* sp., was reared on colonies of tea aphids. In preparation for the tests, female parasitoids that had emerged from mummies isolated in glass vials were mated within 24 h of emergence and were not allowed to lay eggs or to have any experience with host-related odors. They were also starved for 24 h before being used in behavior tests. Each individual natural enemy was tested only once in the wind tunnel bioassays.

Electrophysiology. Electroantennogram (EAG) recordings from each species of natural enemies were made by Ag−AgCl glass electrodes filled with Kaiseling solution. Odor presentation was similar to that in previous studies (*10*, *11*). The antenna was excised and penetrated with the indifferent electrode. The tip of the antenna was cut and placed inside the recording electrode. The signals were passed through an amplifier (Syntech CS-05), displayed on an oscilloscope, and stored in a computer using Syntech Software. Compounds were tested against 15 individual antennae. The delivery system carried a filter paper in a disposable glass Pasteur pipet (15-cm long), the tip of which was inserted about 3 mm into a small hole in the wall of a steel tube (15 mm diameter, 15 cm long) directed over the antennal preparation. Twenty microliters of standard solutions of the test compounds were applied to filter paper strips (5 × 60 mm), and solvent was allowed to evaporate for 30 s before the paper strip was inserted into the glass pipet. An air stimulus controller (model CS-05b, Syntech) was used for air and odor delivery with a constant flow (120 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 Pasteur pipet into the main air flow for 0.5 s. Intervals of at least 2 min were maintained between stimulations.

Odor sources included (1) air entrainment extracts of ITS, MPTS, and TATSC; and (2) nine components from the volatiles of TATSC, whose concentration was 10^{-4} g/mL, and hexane was solvent.

Wind Tunnel Bioassays. Bioassays were carried out in a wind tunnel, 90-cm long, 30-cm high, and 30-cm wide, as designed in ref *12*. The tunnel was divided into two sections, the first one being close to an electric fan that pulled air into the tunnel. The air speed in the tunnel was fixed at 40 cm/s, and an illumination of 3400 lux was obtained from fluorescent lamp located at 40 cm above the tunnel. In the second, the dispenser, an ampule filled with 1 mL of the odor source solution, was hung at 15 cm from ground, and it was closed by a gauze web to get rid of the influence of the visual sense as described by ref *13*. Downwind 40 cm was a releasing platform 15 cm high, on which a glass tube containing a natural enemy, being ringent on both ends, was placed. Its nozzle was oriented toward the odor. The natural enemy was released one by one.

The odor sources included: (1) air entraiment extracts from ITS, MPTS, and TATSC, respectively; (2) benzaldehyde, *Z*-2-hexenal, and geraniol, whose concentration is 100 μg/μL, and the solvent is liquid paraffin, as well as hexane. Each treatment (chemical blend) was replicated 20 times. One treatment was completed each day, and then the tunnel was thoroughly washed before starting a new treatment. The following types of behavior were recorded: flying upwind and landing at the source. The number of responding insects was analyzed using the χ^2 test (*14*).

RESULTS

Analysis of Volatiles of Various Tea Shoots. Figure 1 A, **B**, and **C** show the GC-MS analysis of air entrainment samples from ITS, MPTS, and TATSC, and the identity of compounds present, respectively. Green leaf volatiles, *Z*-3-hexen-1-ol, *E*-2-hexenal, and *Z*-3-hexenyl acetate, are the main components of ITS volatiles.

Butanoic acid, 3-hexenyl ester, and 1-octanol were little reported in the merchant teas. 3,7-Dimethyl-1,3,6-octatriene is first reported in the fresh tea leaves in Chinese tea volatiles research (*15*). The amount of benzaldehyde in TATSC volatiles is very ample. Their relative contents are shown in **Figure 2**.

Electrophysiology. EAG responses from each natural enemy elicited by TATSC were the largest, and next were those by benzaldehyde as well as *E*-2-hexenal. EAG responses of *C. septempunctata* and *C. sinica* to the tested odors were larger than those of *Aphidius* sp. (**Figure 3**).

Wind Tunnel Bioassays. TATSC and benzaldehyde intensely evoked upwind flying and landing of each natural enemy. Both infochemicals elicited a stronger attraction than those induced by the other odors. The effect of geraniol was poor. *C. sinica*, *Aphidius* sp., and *C. septempunctata* still strongly responded to TATSC, benzaldehyde, and *E*-2-hexenal (**Table 1**).

DISCUSSION

Aroma is one of the important characteristics of tea. In particular, high grade teas possess the delicious perfume of which people are fond. During tea processing, the fresh shoots are twisted, and the leaf cells are destroyed. The substrates directly contact enzymes and produce a great deal of the aromatic elements, for example, linalool, geranoil, indole, nerolidol, terpineol, etc. Therefore, the processed tea shoots can usually release the pleasing fragrances (*15*−*18*). However, if the processing is inappropriate, the tea shoots can give off distasteful odors (*19*, 20). In this work, tea shoots were pierced with insect needles, and these shoots produced volatiles different from those of ITS. This process only imitates tea aphids piercing

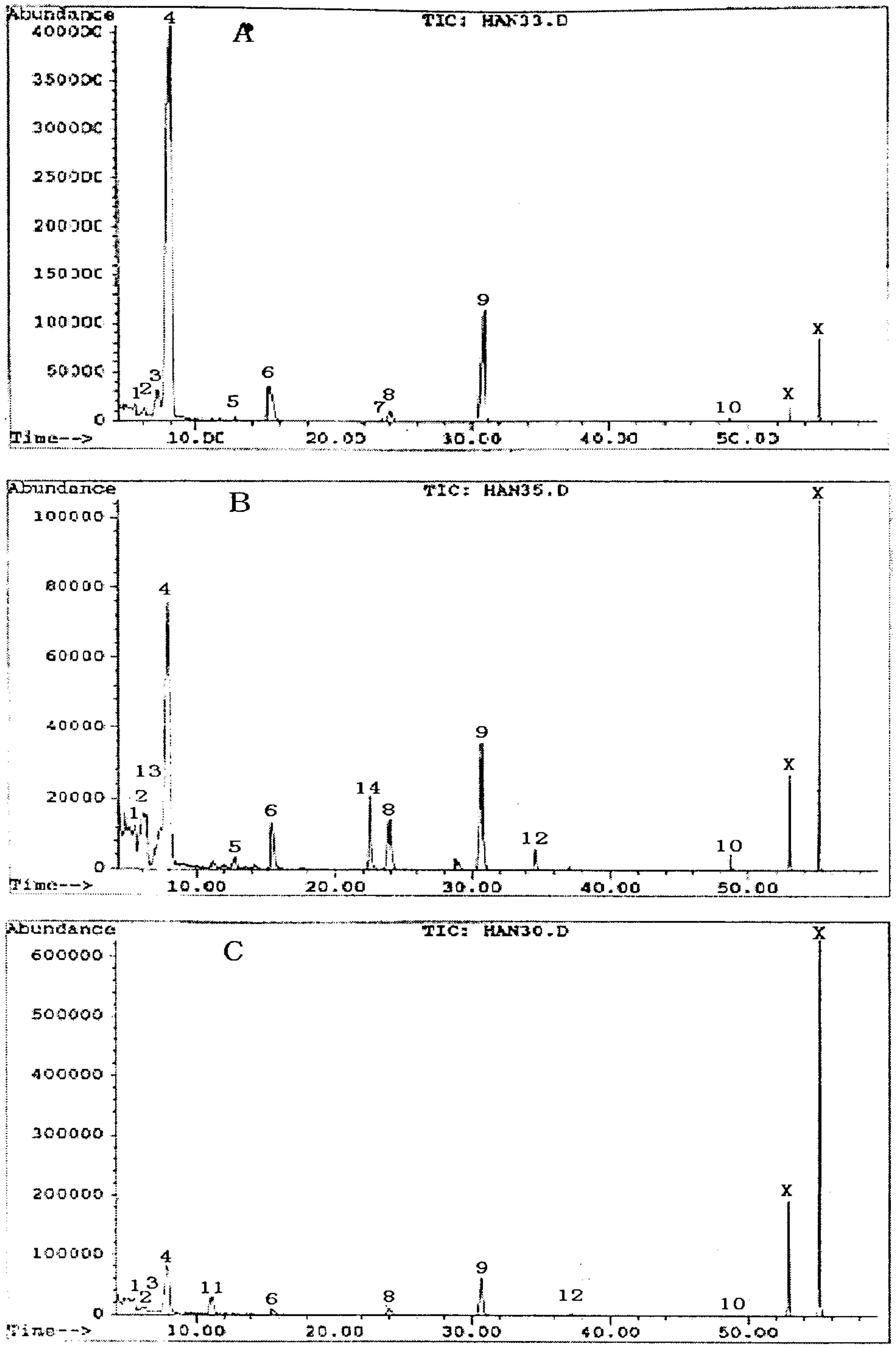

Figure 1. Trace from the gas chromatographic run of air entrainments from intact tea shoots (A), mechanically pierced tea shoots (B), and tea aphid–tea shoot complexes (C). 1, *E*-2-hexenal; 2, ocimene; 3, *Z*-3-hexenyl acetate; 4, *Z*-3-hexen-1-ol; 5, butanoic acid, 3-hexenyl ester; 6, linalool; 7, 1-octanol; 8, decanoic acid, ethyl ester; 9, geraniol; 10, indole; 11, benzaldehyde; 12, *E*-2-hexenoic acid; 13, *Z*-3-hexenyl formate; 14, methyl salicylate; 15, benzyl alcohol.

fresh shoots. We think if the tissues of tea shoots are mechanically pierced, different volatile components may be produced than if pierced by tea aphids. When tea aphids pierce the shoots, the tissue of tea shoots may be mechanically injured. On the other hand, the saliva of aphids contains many kinds of enzymes (*21*). The enzymes can eliminate plant tissues and alter plant metabolism (*22*). Under both mechanical and biochemical actions, the host plants, after being injured by herbivores, often release a specific odor or a change may occur in the relative ratios of many volatile compounds, which could attract predators and parasitic insects (*23*−*26*). The content of benzaldehyde and *E*-hexenal from TATSC is much higher than those in ITS and MPTS; furthermore, both have a stronger attraction, and the relative ratios of the components in TATSC are also divergent from those in ITS and MPTS. We think several natural enemies exploit benzaldehyde, *E*-2-hexenal, and TATSC volatiles as long-range cues (*27*) to locate and approach tea aphids.

Benzaldehyde exists in tea leaves in the form of cyanogenic glycosides, i.e., prunasin, which can be hydrolyzed into benzaldehyde, hydrocyanic acid, and glucose by *β*-D-glucosidase (*28*). The large amounts of benzaldehyde detected in the volatiles entrained from the tea aphid−tea shoot complexes possibly

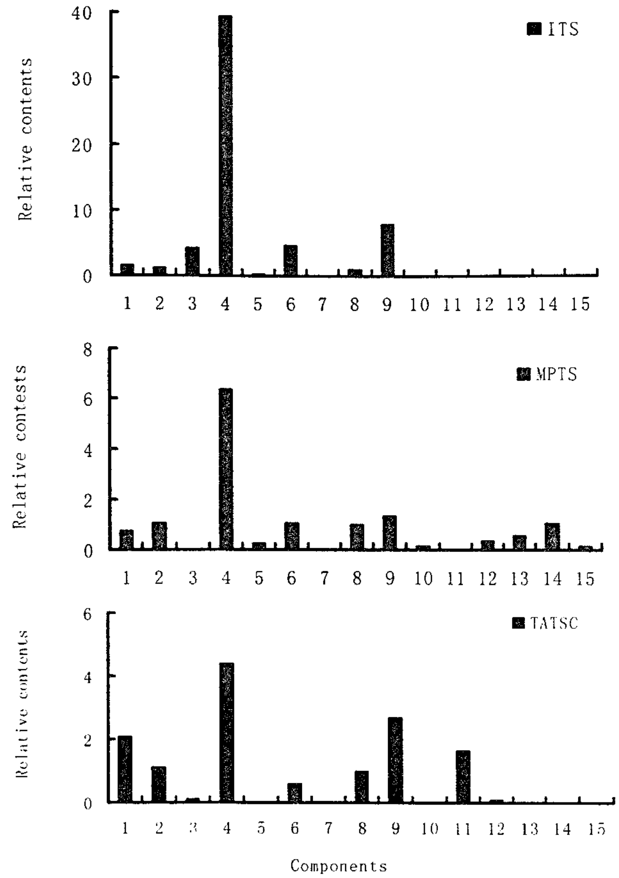

Figure 2. Relative contents of various components in volatiles from ITS, MPTS, and TATSC. 1, *E*-2-hexenal; 2, ocimene; 3, *Z*-3-hexenyl acetate; 4, *Z*-3-hexen-1-ol; 5, butanoic acid, 3-hexenyl ester; 6, linalool; 7, 1-octanol; 8, decanoic acid, ethyl ester; 9, geraniol; 10, indole; 11, benzaldehyde; 12, *E*-2-hexenoic acid; 13, *Z*-3-hexenyl formate; 14, methyl salicylate; 15, benzyl alcohol.

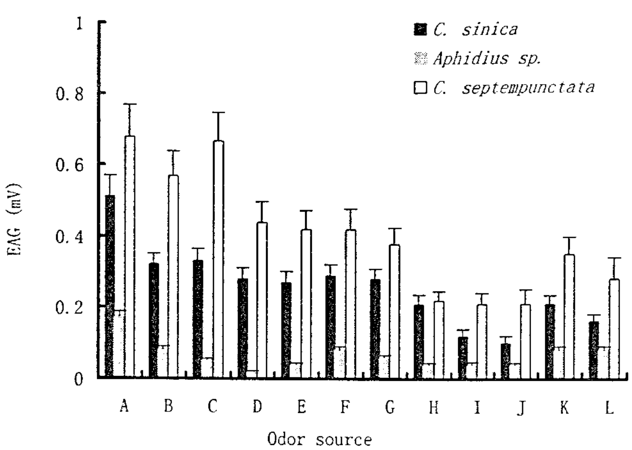

Figure 3. Electroantennogram responses of three kinds of natural enemies to various odors. A, TATSC; B, benzaldehyde; C, *E*-2-hexenal; D, *Z*-3-hexenyl acetate; E, ocimene; F, MPTS; G, ITS; H, *Z*-3-hexen-1-ol; I, linalool; J, geraniol; K, indole; L, *E*-2-hexenoic acid.

resulted from enzymes present in the saliva of the aphids. Cyanogenic compounds are widely distributed in the plant kingdom (*29*). These are usually stabilized by glycosylation giving rise to the cyanogenic glycosides. If a plant is injured, the glycosides are hydrolyzed by glycosidases, and the resulting cyanogenic compounds decompose under the influence of pH or nitrilase activity to a carbonyl component and HCN (*30*). This carbonyl component is an aldehyde or ketone, e.g., benzaldehyde in the case of prunasin (*31*). We consider it a tea aphid-induced component emitted by TATSC. While the green leaf odors are ubiquitous throughout the plant world, some may be triggered by mechanical damage in this work, but they elicit less EAG responses and arresting behavior in wind tunnel than aldehydes do. The efficacy of benzaldehyde and *E*-2-hexenal as well as TATSC volatiles preparations in alluring natural enemies in tea gardens should be further investigated.

Table 1. Percentage of Three Natural Enemies to Several Odors in Wind Tunnel[a]

	percentage					
	C. sinica		*Aphidius* sp.		*C. septempunctata*	
compound	flying	landing	flying	landing	flying	landing
TATSC	70**	50*	70**	45*	70**	50*
benzaldehyde	65**	45*	60**	45*	65**	50*
E-2-hexenal	60**	40*	55**	45*	60**	40*
MPTS	55**	20	60**	15	60**	15
ITS	55**	20	55**	15	55**	15
geraniol	15	5	20	5	15	0
hexane	5	0	5	0	5	0

[a] Asterisks indicate differences (χ^2 test) from hexane: $*P \leq 0.05$; $**P \leq 0.01$.

Our understanding of the communication mechanisms between pests and predatory insects is poor (*32*). So far, there are a few relative reports. The lacewing, *Chrysopa cognata*, showed strong EAG responses to sex pheromones of aphids and significantly arresting effects to lures of the sex pheromones in fields (*8*). The Green lacewing, *Chrysoperla carnea*, exposed intense responses to semiochemicals released from their prey and host plant; even the EAG responses of the lacewings to extracts of corn leaf may differ between males and females (*33*). In the current study, *Chrysopa sinica* also displayed the strong EAG responses and attraction to the synomones from herbivored-induced volatiles; however, we did not distinguish males and females lacewings in the tests. The volatiles from aphid, *Rhopalosiphum padi* (L.), infested barley plants, *Hordeum vulgare*, and from previously aphid-infested and uninfested plants, collected by conventional polymer entrainment techniques and eluted by diethyl ether, were used as odor sources in olfactometer bioassays. The seven-spotted ladybird, *Coccinella septempunctata* L. (Coleoptera, Coccinellidae), responded positively to volatiles from aphid-infested plants and from previously aphid-infested plants but not to volatiles from uninfested plants (*26*), which are similar to those results we found.

It has been shown that chemical signals may be emitted from the whole plant, i.e., also the unharmed parts (*34*). But in this work, the used control tea shoots were picked up from tea plants whose whole plants were never damaged by tea aphids; they did not show similar effects.

On the sensory evaluation, people are extremely interested in the aroma of normal tea, especially in the perfume of high grade teas, and pay little attention to the unpleasing odors from herbivored tea, whose concentration is low. So little is known about the chemical analysis of the odors from herbivored tea. However, generally, the blend of alcohols, aldehydes, esters, and acids, etc. composes tea aroma, in which alcohols, esters, and so on seem to contribute the aromatic characteristics of the high quality tea, whereas acids and aldehydes contribute to the unpleasant smell (*19*, *20*, *35*, *36*). In this study, we determined the acid and the large amount of aldehydes in the volatiles from TATSC, which may be responsible for the unpleasing scents of pest-injured tea. During the tests, we used the absorbing method, which was considered to be superior to the simultaneous distillation extraction technique (SDE) (*37*).

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