Synergistic Effect of Corn Leaf Volatile Odor Substances on Sex Pheromone of Asian Corn Borer, *Ostrinia furnacalis* (Guenée)

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Abstract. After proposing a new total synthetic route for pheromone of *Ostrinia furnacalis* (Guenée) in a high overall yields, short synthetic, and cheap starting materials way, we used the method of electroantennogram (EAG) to detect the synergistic effects of five common corn leaf volatiles, which include benzaldehyde, phenylacetaldehyde, cis-3-hexenol, trans-2-hexenol and corn oil, on sex pheromones of Asian corn borer. The result showed that the five substances can significantly increase the activity of Asian corn borer sex pheromone and have a synergistic effect, but there was no significant difference between each other.

1. Introduction

Corn is the second largest crop production in China, while the Asia Corn Borer (ACB), *Ostrinia furnacalis* (Guenée), is the commonest and the most harmful pest of maize in the most of corn growing areas in China, it can lead to a serious reduction in corn yield (6-9 million tons per year on average), and cause a serious economic loss in agricultural production. [1-2] Farmers used to prevent ACB by using chemical pesticide, however, it may result in resistance, environmental pollution, and the elimination of natural enemies. [3] Therefore, it is important to vigorously develop some new control methods of ACB.

With the new concept of Integrated Pest Management (IPM) put forward, nowadays the environmentally friendly combination of agricultural control, chemical control, physical control and biocontrol are used to prevent ACB. Thereinto, biocontrol, especially the pheromone mass trapping mating disrupting technique, is widely used due to the less pollution, more safety, less damage to natural enemies, more difficult to drug resistance, and greater selectivity.

The chemical structure of ACB sex pheromone is the mixture of (E) and (Z)-12-tetradecenyl acetates. [4-5] In prior studies, scientists have discovered several ways to synthesize ACB sex pheromone, but these methods have some disadvantages such as lower overall yields, longer synthetic routes, and more expensive starting materials [6], which has been overcome by our previous research that described a new [13C+1C] strategy to synthesize the ACB sex pheromone. [7]

Antenna is a significant part of the insect's sensory system, which located on the insect's head with a large number of chemoreceptors that can form complexes with plant volatiles, causing cell depolarization and generate action potentials. The sum of these action potentials is the antenna potential.
Electroantennogram (EAG) is an electrophysiological research method widely used in entomological research that can be used to detect the chemical signal response of insect antennae to volatile in plants, which is invented by Schneider [8].

Plant volatile odor substances refer to a variety of micro-concentration volatile secondary biomass released from the leaves, flowers, fruits and other surfaces of plants. Studies have shown that plant volatile odorants can act synergistically with insect pheromones, which means host plant volatile odorants can significantly increase the behavioral responses of insect pheromones to insects. [9-11]

Herein, we used EAG to study whether it exists a synergistic effect between the volatile odorants such as benzaldehyde, phenylacetaldehyde, cis-3-hexenol, trans-2-hexenol and corn oil, which are common in several corn fields, n-hexane as the solvent (Table 1), with ACB sex pheromone. In order to increase the reactivity of sex pheromone to male moths, as well as enhance the trapping effect of the pheromone mass trapping technique.

| Test Compound            | Purity | Manufacturer     |
|--------------------------|--------|------------------|
| benzaldehyde             | 98%    | Energy Chemical  |
| phenylacetaldehyde       | 99%    | Energy Chemical  |
| cis-3-hexenol            | 98%    | Aladdin          |
| trans-2-hexenol          | 98%    | Aladdin          |
| corn oil                 | 95%    | Aladdin          |
| n-hexane                 | 98%    | J&K Scientific Ltd. |

### 2. Experimental

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#### 2.1. Chemicals

With concentration of 1μg/μL n-hexane solution of Asian corn borer sex pheromone at 73:27 cis-trans-isomer ratio as the foundation, add 1μg, of benzaldehyde, phenylacetaldehyde, cis-3-hexenol, trans-2-hexenol, and corn oil respectively, which are plant volatile odor substances commonly found in corn fields, were configured as a stimulatory compound (1μg sex pheromone+1μg odorant)/μg in n-hexane solution.

#### 2.2. Electroantennogram tests

Placing two days old male Asian corn borer in ice tubes and frozen for about 2 minutes to perform sedation. Then the antennae were cut from the root with an ophthalmic surgical scissors, cut off the end of the antennae, and it was connected to the electrodes with conducting gel. The filter strip (length 5cm, width 0.5cm) was folded into a V type, and a 1μL irritant solution was pipetted evenly over the filter paper strip. The filter strip is placed in Pasteur pipette as a source of odor after the solvent is substantially volatilized, n-hexane solvent was used as a blank control, used 100μg of sex pheromone as a standard stimulus. The Pasteur pipettes containing the sample were numbered and connected to an airflow stimulation control device of Electroantennography, the nozzle was aligned with the antenna with the distance of 1cm. The test was performed when the potential curve on the oscilloscope approaches a straight line, then recorded the antennae potential data. Test stimulations were performed by giving puffs of air (500mL/min) for 0.5s at 30s intervals, while the continuous gas flow was 1200 mL/min, each concentration was stimulated twice (30s for intervals). The average value was taken as the antennae potential response. For each set of experiments, 6 antennae were repeated.

The above-mentioned method was used to determine the activity of five mixed substances on the antennae of male Asian corn borer. The synergistic effects of benzaldehyde, phenylacetaldehyde, cis-3-hexenol, trans-2-hexenal, and corn oil on sex pheromones of Asian corn borer were investigated.
2.3. Statistical analysis
EAG data was obtained from each group of 6 antennae. EAG response was obtained by measuring the difference between the EAG waveform peak value and the baseline (the response to the solvent control). The data was analyzed by PASW Statistics 18.0 for analysis of variance (ANOVA) and LSD multiple comparison analysis method.

3. Results and Discussion
In order to study whether it exists a synergistic effect between the volatile odorants which are common in several corn fields with ACB sex pheromone, herein the EAG technique was used, and the results are shown in Figure 1.

![Fig. 1. Electroantennogram responses [mV ± SE (standard error)] of ACB male moths to binary blends of the ratio 73: 27 (Z:E) in different doses. Male moths treated with 1 μL of n-hexane were used as control. Bars with the same letter are not significantly different (ANOVA and LSD, p < 0.05).](image)

The result showed that, after adding 1μg of plant odorants into n-hexane solution of Asian corn borer sex pheromone, the antennal response of the male Asian corn borer was significantly enhanced, which was significantly higher than value of control. There was a significant difference in the activity of the five mixed sex pheromones and control, but there was no significant difference among the five mixed sex pheromones, indicating that 1μg of benzaldehyde, phenylacetaldehyde, cis-3-hexenol, trans-2-hexenal, and corn oil all had synergistic effects on the sex pheromones of Asian corn borer. There was no significant difference in synergism between the five substances at a dose of 1μg, slightly stronger with cis-3-hexenol, and slightly weaker with corn oil.

4. Conclusions
In conclusion, we used Electroantennogram technique to study whether it exists a synergistic effect between several common volatile odorants such as benzaldehyde, phenylacetaldehyde, cis-3-hexenol, trans-2-hexenal, and corn oil, n-hexane as the solvent with ACB sex pheromone. The result showed that there was a significant difference in the activity of the five mixed sex pheromones and control. It is convinced that the five substances can significantly increase the activity of ACB sex pheromone and have a synergistic effect. There was no significant difference in synergism between the five substances at a dose of 1μg, slightly stronger with cis-3-hexenol, and slightly weaker with corn oil. This study shows that plant volatile odor substances have the potential to develop sex pheromone synergists and have research value.
Acknowledgments
Financial support of this research provided by Science and Technology Planning Project of Jilin Province (20140204022NY, 20160414015GH, and 20180201012NY) is greatly acknowledged. We thanks Fan Zhang and Su Wang from Beijing Academy of Agricultural and Forestry Sciences, Beijing, for providing EAG systems and *O. furnacalis* pupae.

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