Synthesis of female sex pheromone of Red Hairy Caterpillar, Amsacta albistriga Walker (Lepidoptera: Arctiidae) and its evaluation

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Research Article

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Abstract

The present studies were done for identifying and evaluating the female sex pheromone of red hairy caterpillar; *Amsacta albistriga*. Four pheromone components were identified from female *A. albistriga* as Octa decanal, (Z, Z)-9,12-Octa decadienal, (Z, Z, Z)-9,12,15-Octa decatrienal and (Z,Z,Z)-3,6,9-Heneicosatriene and were synthesized at IICT, Hyderabad into 4 blends. These blends were tested in laboratory conditions against mated and unmated male *A. albistriga*. Studies revealed that slow olfactory receptor potentials could be recorded from an isolated antenna positioned between two glass capillary microelectrodes connected to an amplifier and a recording instrument. The mated and unmated male *A. albistriga* were responded differently for EAG studies and antennal response was observed at 10 milli volts for mated insects and > 20 milli volts for unmated one. Among all the four blends, blend 3 was effective in capturing the male *A. albistriga* in preliminary lab and field studies. These studies are useful for developing integrated management strategy with pheromone lures and traps in red hairy caterpillar management.

Introduction

Red hairy caterpillar, *Amsacta albistriga* is a major lepidopteran, polyphagous insect pest attacking several crop plants and weeds. Groundnut is the major crop infested with *A. albistriga* under different agro-ecological regions of India. In southern states of India it is observed in dryland tracts of Andhra Pradesh, Tamilnadu, Telangana where groundnut grown as rainfed crop. It is an endemic pest in different parts of Andhra Pradesh, severely ravaging the groundnut crop from 20 days to 65 days crop stage. The emergence of the moths coincided with the pre-monsoon and early monsoon showers reaching the moisture saturation upto 15-30cm soil depth in endemic pockets of Andhra Pradesh. the adult female lays eggs in batches to a maximum of 1000 per female, which might be hatched in 3-5 days. The first instar larvae usually found in batches, later moved to adjacent plants and causing foliar damage. The crop damage is very severe during dryspell period in monsoon and larval stage would be continued due to inability of the larvae to enter into soil pupation under moisture stress condition. Entire vegetation eaten away by the caterpillars and leaving stems and stalks. In severe conditions, 100 per cent defoliation was noticed in outbreak years. Due to hairy nature of the larvae, it is difficult to manage with chemical control measures. Among the other control practices, pheromone technique is an important one, that can helps in monitoring of adult emergence and maximum crop protection through mass trapping as, a viable technique without any negative impact on beneficial organisms, suitable in avoiding environmental and health hazards caused by poisonous chemical insecticides.

Lepidopteran female sex pheromones have been identified from more
than 670 species (Ando *et al*. 2004, El-Sayed *et al.,* 2005). Insect pheromones are essential components of monitoring and management tools against devastating pests of agricultural crops. Mating disruption, mass trapping, attract-and-kill, and push-pull are some of the direct pest control strategies that depend on the use of pheromones. Keeping the importance of this, the chemical constituents of female sex pheromone were identified in collaboration with IICT and NBAIR as well as and synthesized and

evaluated against *A. albistriga* in groundnut crop grown in dryland tracts nearby RARS, Tirupati during 2016 – 2018.

Methods And Materials

Insect rearing and Pheromone Extraction:

The test insect *A.albistriga* egg masses, larval populations were collected from endemic regions viz., Piler, Kalikiri, Vayalpadu mandals of Chittoor district during 2015-2016, 2016-2017 and 2017-2018. Field collected egg masses and larvae of red hairy caterpillar, *A. albistriga* were reared till pupation. About 100 cocoons were sexed, and kept in cages at room temperature around 28 – 30^{O}C. After eclosion, the pheromone glands of 2- or 3-d-old virgin females, which had shown calling behavior were excised and immersed in hexane (50µl/g and) for 15 min. The crude extract was used for structural analysis of pheromone components after filtration.

Identification and synthesis of pheromone components:

The four pheromone components of *A. albistriga viz.*, Octa decanal (1.0), (Z,Z)-9,12-Octa decadienal(1.0), (Z,Z,Z)-9,12,15-Octa decatrienal (11.5) and (Z,Z,Z)-3,6,9-Heneicosa triene (24.0) were identified at IICT, Hyderabad.

4 pheromone components viz., Octa decanal, (Z9,Z12) -Octa decadienal (1.0), (Z9,Z12,Z15)- Octa decatrienal, (Z3,Z6,Z9)- Heneicosatriene (24.0) were synthesized and prepared 4 blends in different combinations i.e.

Blend 1: 1, 2, 3 & 4 compounds

Blend2 : 1, 2 & 3 compounds

Blend3 : 1, 1 & 4 compounds

Blend4 : 1, 3 & 4 compounds

These blends were further made into plastic and silica dispensers in different colours for testing in Electro antennogram (EAG), wind tunnel and olfactometer.

Gas Chromatography Electroantennography (GC-EAG) studies:

EAG preparation: Live *A. albistriga* adults were used in Electro-antennogram preparation so as to maximize their useful life and such EAG preparations could last for at least one working day without significant deteoration. EAG preparations made with male *A. albistriga* moths. The moth antennae were laid out in a V form with the ventral surface uppermost and held in place with a series of U-shaped wires (0.2mm dia) made from the lengths of the copper taken from the screening in coaxial cables.

Electroantennography was performed by removing the antenna from adults of male red hairy caterpillar and inserted two chlorided silver wires for contact onto the two ends and amplifying the voltage between them while applying an odour puff to see a deflection as well by leaving the animal intact and inserting a ground silver wire.

In this technique, the compounds partitioned in chromatographic columns were analyzed on an EAD, which consists of two electrodes (one for recording, and the other one for reference) connected to a recently excised insect olfactory organ antenna), head, or even the whole body from alive or test individuals. When active pheromone components came in touch with the biological receptor; *i.e.,* antenna an electric potential difference is produced as the compound passes through with a signal commensurate to the concentration and response factor that is produced in the insect, and for compounds with no stimulating effect, the electric potential remains without remarkable alterations. The electric potential difference produces a current that is measured by an electrode, and then an electroantennogram is generated (Knolhoff, *et al.*, 2014).

Gas Chromatography- Electroantennographic Detection (GC-EAD): Modern high resolution capillary gas chromatography (GC) is a powerful technique for the separation of small amounts of individual components in complex mixtures. However, the physico-chemical detectors used to monitor the eluting fractions are not selective to specific components of bio-logical activity. However, a highly selective and sensitive detection apparatus is present on the antenna of many insects, and this detector system is used in the EAG bioassay. Combining the separation power of the GC with the EAG technique fully utilizes the analytical capabilities of these two techniques. The insect antenna as a GC detector the effluent from the column has to be directed to the antennal preparation. In practice this is realized by splitting the column effluent into two branches: one leading to the standard detector (usually an FID), the other one leading to the antennal preparation outside the GC oven via a suitable transfer line. To prevent condensation of fractions in the transfer line, the compounds heated up to the maximum temperature of the applied GC program. After leaving the heated transfer line the effluent is mixed in a constant flow of filtered and humidified air, which is directed to the antenna as in a standard EAG arrangement.

During the GC run the antenna is continuously exposed to the eluting fractions; which shows the response to fractions containing compounds, which activate the receptor cells on the antenna. The signal from the antenna is monitored and recorded simultaneously with the signal from the FID, and both signals are synchronized in time.

During continuous EAD recording in GC-EAD measurement the antenna is exposed to stimuli eluting as ‘peaks’ in the chromatogram, and the signal from the antenna is recorded continuously. Without automatic base line control the signal from the antennae would run out of the display window after some time; Therefore, the automatic base line control is very useful in GC-EAD recording. However, the baseline correction should not be too strong: there should be enough time to follow the elution of active ‘peaks’. The effectiveness of the automatic baseline control can be adjusted by changing the Time Constant (T.C.) of the control system. The higher the value of the selected Time Constant (T.C.), the more time is

needed to return to zero after a certain deflection of the signal. The Time Constant is the time required to reduce the original deflection of a signal by 63% of this deflection. For EAG signals evoked during application of a short puff, a good value would be a time constant of 1 - 3 seconds. However, for recording of GC-EAD signals from the effluent of a gas chromatograph (GC-EAD recording), a Time Constant of 5 - 10 seconds is usually an appropriate setting.

Development of proper dispenser and design of a suitable trap for monitoring and mass trapping of *A. albistriga*

The four Pheromone components of female *A. albistriga* adults synthesized at IICT, Hyderabad which were made into 4 blends and they were impregnated into plastic and silica septa in different colors like red, blue, cream (light yellow) and white (Fig.2).

Olfactometer

Olfactometers are devices built to present odor stimuli in a standardized computer-controlled manner with determined air flow, odor concentration, odor duration, onset, and offset. Sometimes even humidity, temperature, and other features can be controlled. Stimuli are typically embedded in a constant flow of odorless, humidified air of controlled temperature in order to avoid simultaneous mechanical or thermal stimulation. Further, it is also possible to present odor at a specific moment depending on physiological measures, such as respiratory rhythm; for example, odor releasing may occur just during inhalation (Fig.3a & 3b).

Results And Discussion

Biology studies

Adults of red hairy caterpillar; *A albristriga* emerged from soil soon after the onset of south west monsoon during last week of June and July months. The mass emergence of adults is well synchronized with rains received on 20^{th} (21.2mm), 22^{nd} (58.3mm) and 29^{th} June (58.3mm). During July highest rainfall of 71.2mm was received on 29^{th} July, 2016. The adults emerged were collected and reared in the laboratory for studying the biology and reproductive behavior. The adults mate immediately after their emergence.

Laboratory multiplication of Red hairy caterpillar *A. albistriga* from egg to adult stage indicated that, an egg period of 4-5 days with a fecundity of 697 to 1316 eggs / female, larval duration of 19-21 days with six larval instars, pupal period of 20 days in soil under ambient soil moisture conditions, and adult emergence with the soaking of soil upto 15cm depth. First generation was noticed during June month with the onset of monsoon and second generation in September with continuous rain period during August and September months. Only 45-50 per cent adult emergence was noticed in second generation. Whereas the previous reports revealed only one generation of red hairy caterpillar. Kalaisekar (2014) also studied the bionomics of red hairy caterpillar; *A. albistriga*. Singh and Singh (1990) conducted the

developmental studies of *Amsacta moorei* in the laboratory at 26-31.5°C and 55-80% RH on soybean. The arctiid completed only 1 generation, and development took 35.45±4.25 days from egg to adult emergence (Table.1 & Fig).

Table 1

Biology of red hairy caterpillar reared on groundnut during 2016

S. No	Stage	Duration (days)
	Oviposition period	1-5
1	Fecundity	697 to 1316 eggs/ female
2	Egg period	4-5
	Larval period	
3	First instar	3-5
4	Second	3-4
5	Third	4
6	Fourth	4
7	Fifth	4
8	Sixth	5
9	Pupa	20
10	Adult	3-5

The synthesized pheromone components of female red hairy caterpillar *A. albistriga* was used for antennal response of male *A. albistriga* through Olfactometer and EAG studies at IICT.

The antennal response of male *A. albistriga* was observed at > 10milli volts. The mated and unmated male *A. albistriga* were tested for EAG studies and the difference of antennal response was observed at 10 milli volts for mated *A. albistriga* and >20 milli volts for unmated one (Fig5 a & 5b.).

Evaluation of the synthesized pheromones:

Preliminary lab studies indicated that, among the four blends tested, blend 3 was found effective in attracting more male *A. albistriga* moths. The dispenser made of silica was comparatively effective for capturing the *A. albistriga* adults.

Preliminary field demonstrations were conducted at farmer's fields of Piler, Kalikiri, mandals of Chittoor district during *kharif*, 2016 and 2017 by installing pheromone traps in groundnut crop from June to September months (Fig.7).

Conclusion

The four female sex pheromone components of red hairy caterpillar; *Amsacta albistriga* were identified as Octa decanal, (Z, Z)-9,12-Octa decadienal, (Z, Z, Z)-9,12,15-Octa decatrienal and (Z,Z,Z)-3,6,9-Heneicosatriene and were synthesized at IICT, Hyderabad into 4 blends at different concentrations (025, 0.50, 1.0, 2.0%). These blends were tested in fields of groundnut crop with different blends and dispensers. Blend 3 was effective in silica dispenser for capturing more *A. albistriga* male moths.

Declarations

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CONFLICT OF INTEREST:

The authors have no competing interests as defined by Springer, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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Figures

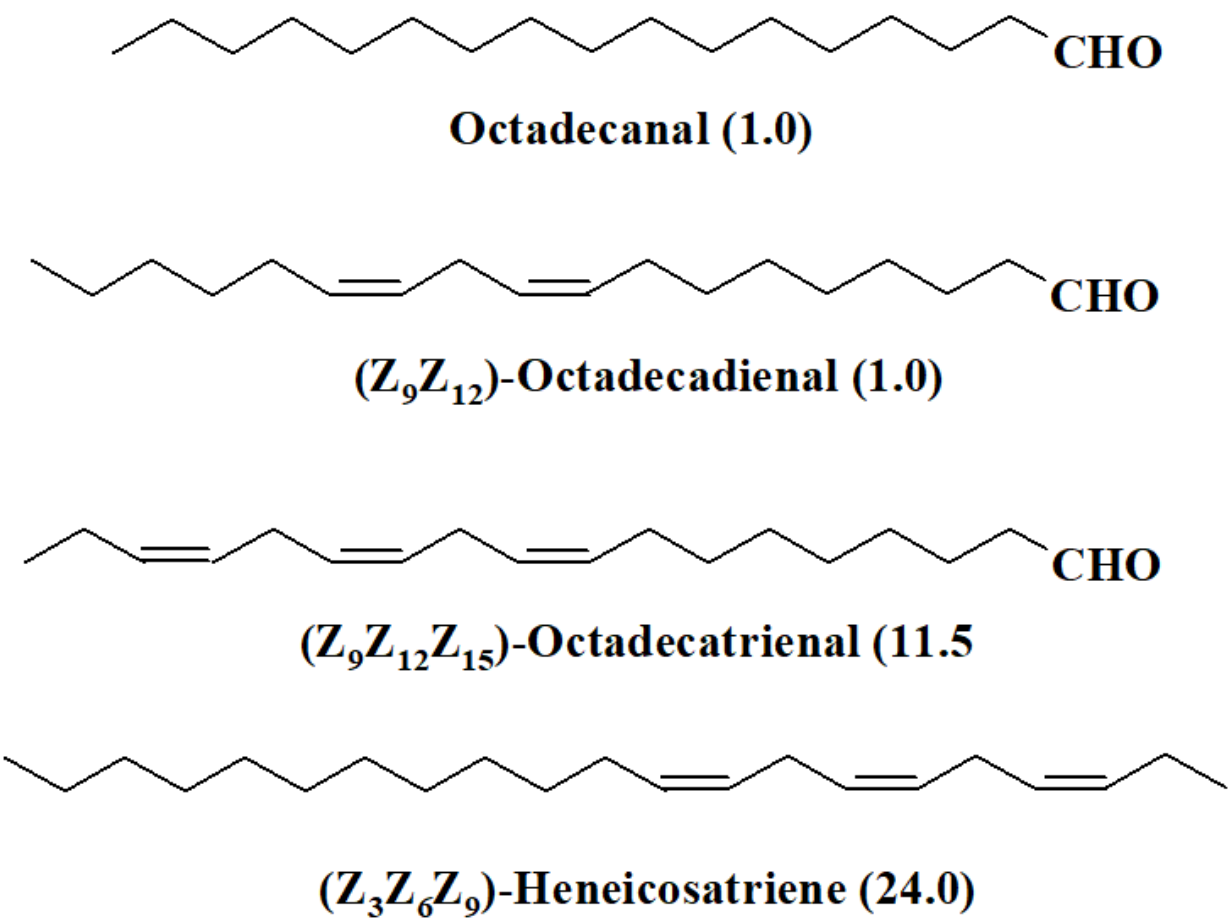

Figure 1

Schematic diagram of the chemical constituents of female red hairy caterpillar pheromone; *Amsacta albistriga*

Figure 2

Pheromone lures

Figure 3

a: Olfactometer:

b: Antennal electrode Holder:

Adult *A. albristriga*

Eggs

Larvae (Caterpillars)

Severely infested groundnut crop

Larva entering into soil for pupation

Pupae

Figure 4

Life stages of *A.albistriga*

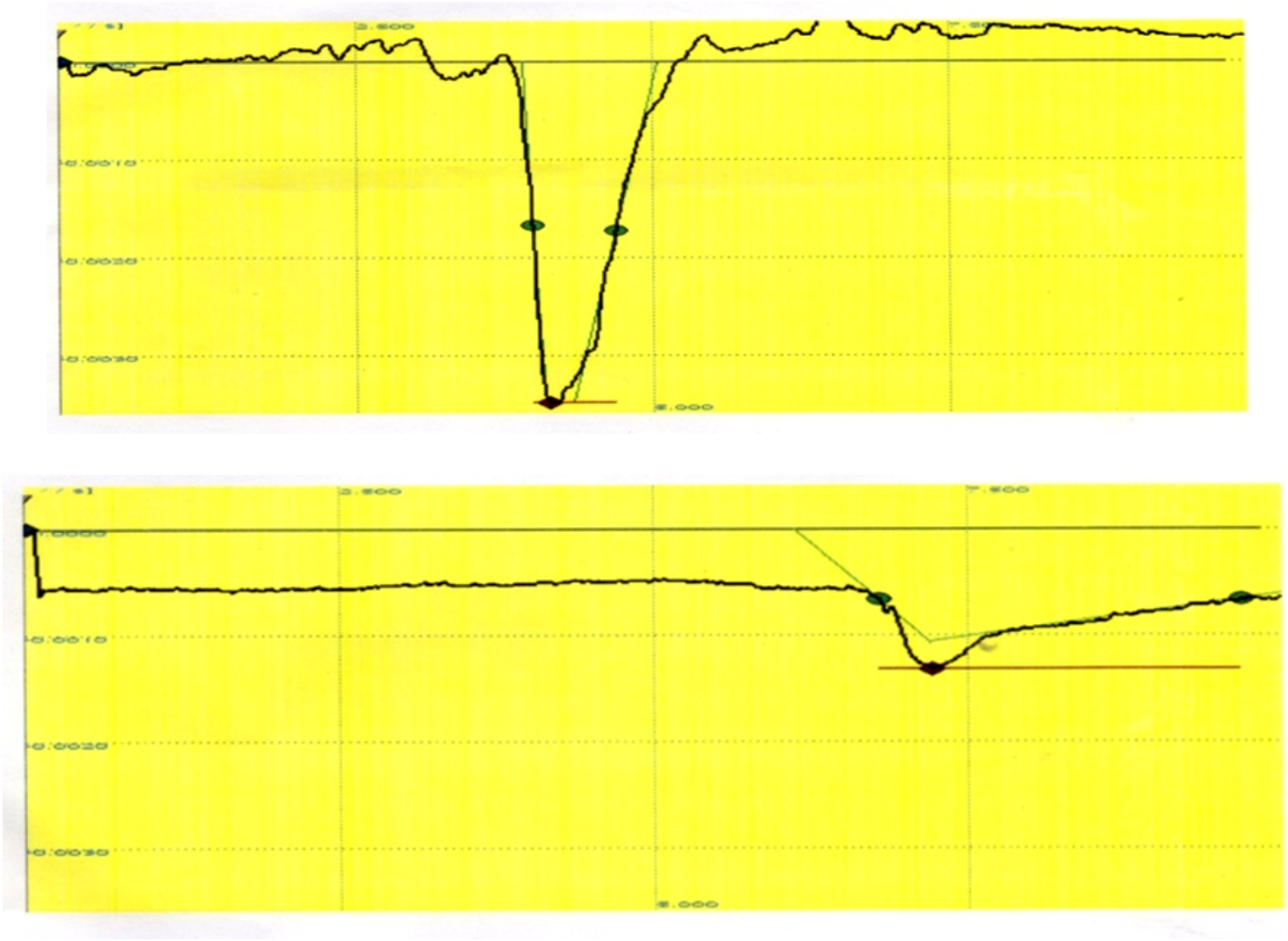

Figure 5

a: EAG response of male *Amsacta albistriga* (unmated) to 0.25% synthetic pheromone blend

b: EAG response of male *Amsacta albistriga* (mated) to 0.25% synthetic pheromone blend

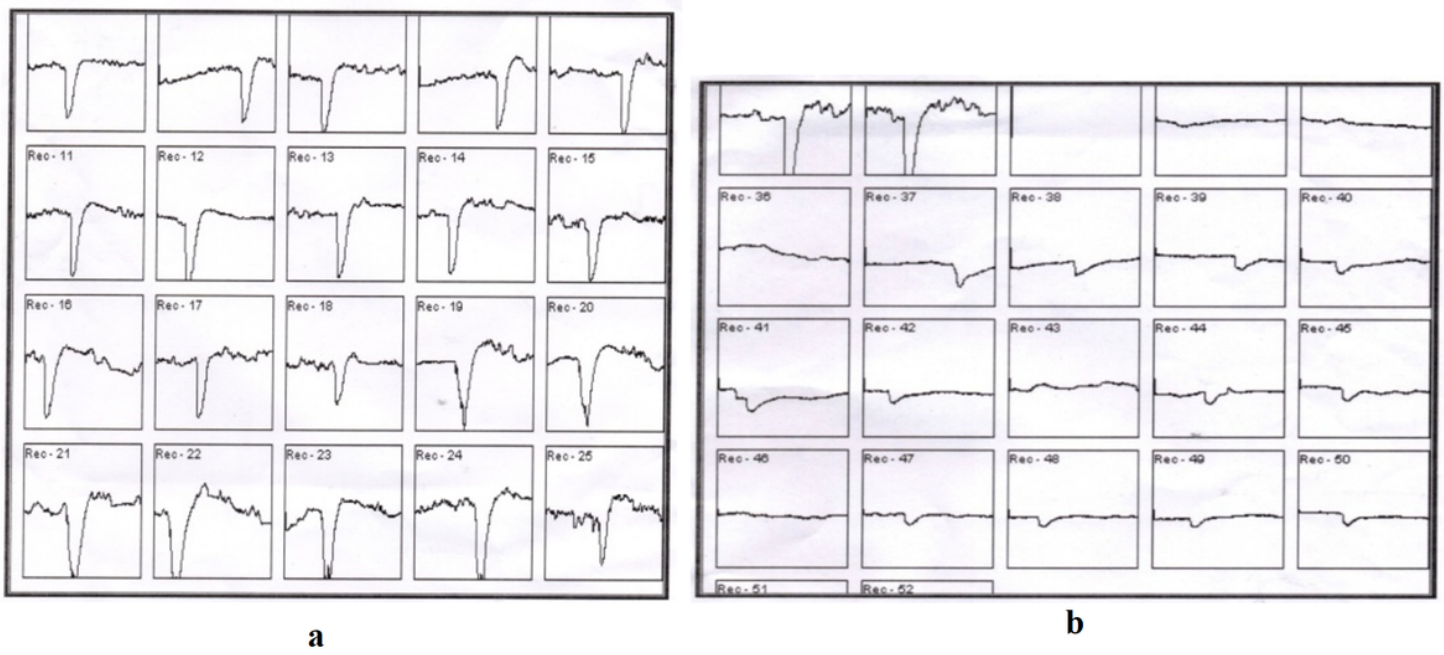

Figure 6

a: Antennal response of *A. albistriga* (unmated male) to pheromone blend-4

b: Antennal response of *A. albistriga* (mated male) to pheromone blend-4

Figure 7

Adult captured in sleeve trap with pheromone lures