

## REVIEW

# The Sex Pheromone of the Lima Bean Pod Borer *Etiella zinckenella* (Treitschke)

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### Abstract

The lima bean pod borer *Etiella zinckenella* (Lepidoptera: Pyralidae) is a severe agricultural pest attacking various leguminous crops in many areas of the world. Insecticidal control is ineffective because larvae feed inside the pods of legumes and so cannot readily be reached by sprays after egg hatch; therefore, monitoring by pheromone-baited traps would assist decisions on when to spray insecticides in a more effective and efficient manner. Four components have been found in the female pheromone glands of this species: tetradecyl acetate, (*Z*)-9-tetradecenyl acetate (Z9-14:Ac), (*Z*)-11-tetradecenyl acetate (Z11-14:Ac), and (*E*)-11-tetradecenyl acetate. The binary blend of Z9-14:Ac and Z11-14:Ac attracts many males in Europe and North Africa but it attracts almost no males in East Asia or Australia, suggesting possible geographic variation in the pheromone communication system of this species. A recent study reexamined the sex pheromone of *E. zinckenella* in Japanese populations and discovered another essential component, (*E*)-9-dodecenyl acetate. Here, we review studies of the sex pheromone and its geographic differences in *E. zinckenella* and discuss the potential use of pheromones for monitoring this cosmopolitan pest of legumes.

**Discipline:** Insect pest

**Additional key words:** monitoring, lima bean pod borer moth, *Etiella behrii*, *Etiella hobsoni*, soybean, geographic variation

## Introduction

Lepidoptera are one of the largest groups of insects, comprising approximately 150,000 described species, about 94% of which are moths<sup>5</sup>. Although moths are highly divergent in morphology and biology, most species use a common reproduction system that involves long-distance attraction of males with volatile chemicals produced by females, i.e., sex pheromones<sup>2</sup>.

A number of studies have analyzed and identified pheromone chemicals in several hundred moths and revealed that most moth pheromones are composed of multi-component blends of even-numbered 10- to 18-carbon long-chain acetates, alcohols, and aldehydes, typically with one or two positions of unsaturation along the chain<sup>1,29</sup>. Although the compounds in a pheromone blend are often shared with other species, particular compositions and their blend ratios can provide a species-specific signal<sup>23</sup>. Thus, the pheromone blend emitted by a female moth generally constitutes a unique signal to attract conspecific males. Such a signal

can avoid mistakes in mating among different species<sup>2,3,13,19</sup>. This signal specificity is one of the fundamental concepts to have emerged from numerous early field studies of sex pheromones<sup>11</sup>.

Pheromone signals should not deviate from the median of the population due to decreased ability to attract a mate. Consequently, the traits of female pheromone production and male pheromone response are supposed to be coordinately tuned to the population norm under stabilizing selection<sup>13</sup>. Yet in spite of such a species-specific, conservative feature, considerable variability in pheromones has occasionally been found within and between populations of the same species<sup>12</sup>. The nature of variation in pheromone communication systems is critical for the evolution of these systems and for their role in mate-finding and speciation in moths<sup>12,13</sup>. In addition, this variation often causes problems in the use of pheromones in pest management<sup>21</sup>.

Recently, we reported geographic differences between the sex pheromone of the lima bean pod borer *Etiella zinckenella* (Treitschke) (Lepidoptera: Pyralidae: Phycitinae) and that of other populations<sup>22</sup>. This moth is a severe agricul-

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tural pest attacking various leguminous crops in many areas of the world<sup>16,18,28</sup>. Here, we review the sex pheromone of *E. zinckenella* and mention its possible use for monitoring.

### Distribution and host plants of *Etiella zinckenella*

*E. zinckenella* is a pantropical species, widespread in Nearctic, Neotropical, Ethiopian, Oriental, and southern Palearctic regions, and in the northern part of Australia and on some Pacific islands<sup>15,28</sup>. Despite worldwide distribution with much variation in size and color, there is no evidence of local populations differing in morphology, so it seems likely that *E. zinckenella* has spread rapidly in recent times, probably with the assistance of human activity<sup>28</sup>. This moth has strong flight ability and can migrate long distances, which would have facilitated dispersal<sup>20,28</sup>.

The genus *Etiella* Zeller includes seven species, the other six of which are distributed mainly in Australasian or Oriental regions<sup>28</sup>. Because *E. scitivittalis* (Walker), with more primitive morphological characters, is known at present only in eastern Australia, it is considered that the genus is Australasian in origin and that *E. zinckenella* has been particularly successful, spreading rapidly throughout tropical and temperate countries<sup>28</sup>. In Japan, the border of the distribution of *E. zinckenella* seems to be the annual average isotherm of 11–12°C, and population densities are higher where the annual average temperature is >14°C<sup>14,16</sup>.

Larvae of all species of *Etiella* feed on the seeds in the pods of leguminous plants<sup>28</sup>. *E. zinckenella* is found on 21 genera and 30 species of legumes<sup>15</sup>, but its preference for host plants appears to differ among local populations. For example, it severely damages soybean (*Glycine max*) in East and Southeast Asia and in Europe but does not in North America<sup>15,16</sup>, where it is considered one of the most serious pests of lima bean (*Phaseolus lunatus*)<sup>18</sup>. Yellow lupin (*Lupinus luteus*), crotalaria (*Crotalaria juncea*), pea (*Pisum sativum*), and *Robinia pseudacacia* are other major crops infested by *E. zinckenella*<sup>15,16</sup>. It is noteworthy that *E. zinckenella* had long been recognized as a pest of soybean in Indonesia<sup>4,17</sup>, but further investigations in East Java revealed the predominant species to be *E. behrii* (Zeller)<sup>9,30</sup>. In addition,

*E. hobsoni* (Butler) is abundant in soybean fields in West and Central Java<sup>17</sup>. These species could be confused with each other owing to their similarity in external characters<sup>9,17</sup>.

### Sex pheromone of *Etiella zinckenella*

Females of *E. zinckenella* begin releasing pheromones several hours after sunset, adopting a calling posture typical of pyralid moths, with the wings slightly elevated along the dorsum and the abdomen bent sharply above the normal body position<sup>8,10</sup>. While holding this posture, the females expose the abdominal terminal segments, which contain pheromone glands<sup>10</sup>.

Four compounds eliciting electroantennographic responses in males have been identified from the female pheromone-gland extracts of Hungarian and Egyptian populations of *E. zinckenella*: tetradecyl acetate (14:Ac), (*Z*)-9-tetradecenyl acetate (Z9-14:Ac), (*Z*)-11-tetradecenyl acetate (Z11-14:Ac), and (*E*)-11-tetradecenyl acetate (E11-14:Ac)<sup>24</sup>. Synthetic mixtures of these components successfully attracted males in fields in both Hungary and Egypt<sup>24</sup>. Subsequently, an optimal blend of a binary mixture of Z9-14:Ac and Z11-14:Ac in a 3:100 ratio was confirmed in Europe and North Africa<sup>26</sup>. Moreover, the addition of E11-14:Ac at >10% had an adverse effect on attraction, although the addition of 14:Ac had no influence<sup>26</sup>. These results lead to the conclusion that Z9-14:Ac and Z11-14:Ac are the key sex pheromone components of *E. zinckenella* (Table 1).

At present, female sex pheromones of *Etiella* species other than *E. zinckenella* have been studied only in *E. behrii*<sup>27</sup>. In contrast to the *E. zinckenella* pheromone, which is composed of tetradecyl/tetradecenyl acetates, the sex pheromone of *E. behrii* is a mixture of tetradecenyl acetates and dodecyl/dodecenyl acetates: Z11-14:Ac, E11-14:Ac, (*E*)-9-dodecenyl acetate (E9-12:Ac), and dodecyl acetate<sup>27</sup>. Maximum attraction was obtained at the blend ratio of 90:6.3:10:0.7, respectively<sup>27</sup> (Table 1). Because *E. zinckenella* and *E. behrii* sometimes co-occur in Japan<sup>30</sup> and Southeast Asia to Australia<sup>28</sup>, the sex pheromones would play an important role in species recognition in mating<sup>9</sup>.

**Table 1. Optimal blend of pheromone components (µg) for attracting males of *Etiella zinckenella* and *E. behrii***

	Z9-14:Ac	Z11-14:Ac	E11-14:Ac	E9-12:Ac	12:Ac
<i>E. zinckenella</i>					
Hungary	30	1000	-- <sup>a</sup>	Nt <sup>c</sup>	Nt
Japan	120	360	- <sup>b</sup>	3	Nt
<i>E. behrii</i>					
Indonesia	--	9	0.63	1	0.07

a: Negative effect on attraction.

b: No apparent effect on attraction.

c: Not tested.

Differences in the compositions and the blend ratios of the pheromone components may be key factors in discriminating between the species<sup>9,27</sup>. Alternatively, the concentrations of pheromones emitted by females may elicit species-specific responses in males. The amount of pheromone components extracted from virgin females was small in *E. behrii* (<0.2 ng/female) but relatively large in *E. zinckenella* (>2 ng of Z11-14:Ac)<sup>22,27</sup> (Table 1). Similarly, the pheromone dose for maximum male attraction is very small in *E. behrii* (~10 µg/rubber septum) compared with that in *E. zinckenella* (~1000 µg/septum)<sup>22,27</sup> (Table 1).

Male *E. zinckenella* possess a pair of brush organs between the meso- and meta-thorax, which are extruded in courtship<sup>7,10</sup>. These brushes have a porous surface and a mesh-like internal structure, suggesting that they disseminate male scent<sup>7</sup>. One component of the male scent is ammonia<sup>7</sup>, which is considered a kind of pheromone produced by males in courtship, although its behavioral function has not been conclusively demonstrated.

### Geographic differences in the sex pheromone of *Etiella zinckenella*

As described above, the binary blend of Z9-14:Ac and Z11-14:Ac attracted many males of *E. zinckenella* in Europe, North Africa, and India<sup>24,25,26</sup>. However, pheromone traps baited with mixtures of the four tetradecyl/tetradecenyl acetates captured no males in East Asia or Australia<sup>25,26</sup>, or in Japan<sup>22</sup>, suggesting possible geographic differences in the pheromonal communication system. This evidence encouraged us to examine the sex pheromone of *E. zinckenella* in Japanese populations.

To begin, we investigated pheromone gland extracts of female moths collected in Japan and searched for other possible pheromone components. Using gas chromatography – mass spectrometry, we discovered a very small amount of a dodecenyl acetate in addition to the four tetradecyl/tetradecenyl acetates previously identified<sup>22</sup>. From the retention times, the compound was identified as E9-12:Ac<sup>22</sup>. The proportion of E9-12:Ac was less than 0.6% that of Z11-14:Ac, the most abundant component found in the extract<sup>22</sup>. Moreover, the addition of a small amount of synthetic E9-12:Ac significantly increased male attraction to the mixtures of the tetradecyl/tetradecenyl acetates compared to virgin females in the field<sup>22</sup>. From these results, we conclude that E9-12:Ac is a sex pheromone component of the *E. zinckenella* population in Japan (Table 1).

It is possible that E9-12:Ac is used in the pheromonal communication system of *E. zinckenella* in other populations, especially where mixtures of the tetradecenyl acetates are much less attractive than virgin females, such as in Taiwan<sup>26</sup>. Alternatively, *E. behrii* as well as *E. zinckenella* may occur in Taiwan. A higher content of E9-12:Ac is

likely to decrease the response of male *E. zinckenella* to the pheromone source<sup>22</sup>. As E9-12:Ac is a common pheromone component of *E. zinckenella* and *E. behrii* and the amount of E9-12:Ac in the pheromone is minor in *E. zinckenella* but the second greatest in *E. behrii*, the ratio of E9-12:Ac would be important for conspecific mate attraction in *E. zinckenella* and *E. behrii*<sup>22</sup>.

Data on the geographic difference in pheromone communication of *E. zinckenella* are limited, and further studies are necessary to elucidate why the binary blend of Z9-14:Ac and Z11-14:Ac failed to attract males in the eastern circum-Pacific region and why the additional E9-12:Ac is indispensable in the Japanese populations. Taxonomic and systematic reinvestigation of each population would also be essential because some members of *Etiella* spp. may be confused with *E. zinckenella* due to similarity in morphology, as described above<sup>9,17</sup>.

### The potential of monitoring using pheromone traps for management of *Etiella zinckenella*

Monitoring the occurrence of pests to predict damage in crops would assist decisions on when to spray insecticides in a more effective and efficient manner<sup>21</sup>. Especially in *Etiella* borers, insecticidal control is ineffective because the larvae feed inside the pods of legumes and so cannot readily be reached by sprays after egg hatch. Traps baited with pheromones would be useful for monitoring, unlike other sampling methods that can be very time consuming and require technical expertise<sup>6,21</sup>.

Pheromone traps can usually provide reliable results for monitoring the occurrence of specific species. As mentioned above, different *E. zinckenella* populations prefer different host plants<sup>15,16</sup>, and *E. behrii* and *E. hobsoni* occur sympatrically and have likely been confused in several regions<sup>9,17,30</sup>. Thus, species-specific monitoring by pheromone traps would help the identification and evaluation of the dominant species in each crop at each locality. Studies of the sex pheromone of other *Etiella* spp. should be undertaken in the future.

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