Pergamon

Bioorganic & Medicinal Chemistry, Vol. 4, No. 3, pp 495-497, 1996

0968-0896/96 $15.00+0.00

S0968-0896(96)00031-4

Optimization of Blends of Synthetic Pheromone Components for Trapping Male Limabean Pod Borers (*Etiella zinckenella* Tr.) (Lepidoptera: Phycitidae): Preliminary Evidence on Geographical Differences

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Abstract—From the five previously identified pheromone components of the limabean pod borer (*Etiella zinckenella* Tr.) (Lepidoptera: Phycitidae), the 100:3 mixture of (*Z*)-11-tetradecenyl acetate and (*Z*)-9-tetradecenyl acetate was necessary for maximal attraction of males into traps in tests performed in Hungary. The addition of (*E*)-11-tetradecenyl acetate in percentages higher than 10–30% generally had an adverse effect on catches, while the addition of the other two compounds present in pheromone extracts had no influence on catches. In contrast to the results in Hungary, none of the traps baited with combinations of the above compounds captured any moths in tests performed in Taiwan, suggesting possible geographical differences in pheromonal response of European and Eastern Asian populations of *E. zinckenella*. Copyright © 1996 Elsevier Science Ltd

Introduction

The limabean pod borer, *E. zinckenella* Tr. (Lepidoptera, Phycitidae) is an important agricultural pest attacking various Leguminosae and Fabaceae in many agricultural areas of the world.[1] Some years ago we elucidated the chemical structures of several components of the female sex pheromone; notably, the presence of (*Z*)-11-tetradecenyl acetate (*Z*11–14Ac; abbreviation of compounds' names according to ref. 2), (*Z*)-9-tetradecenyl acetate (*Z*9–14Ac), (*E*)-11-tetradecenyl acetate (*E*11–14Ac), tetradecyl acetate (S14Ac) and (*Z*)-11-hexadecenyl acetate (*Z*11–16Ac) in the approximate ratios 100:22–35:13–4:39–40:40–243 in populations from Hungary and Egypt.[3] However, due to unusually low population levels at the time of the study, the relative importance of these components in attracting male moths into traps has not been studied in detail. In this paper we report on results of optimization tests performed with various mixtures of the identified pheromone components. Preliminary evidence that pheromonal variation occurs in geographically distinct populations of *E. zinckenella* is also presented.

Results and Discussion

Previous experience showed that from the components present in pheromone extracts, traps baited with *Z*11–14Ac regularly captured low numbers of male *E. zinckenella*, while *Z*9–14Ac evoked largest responses from male antennae in electroantennography (EAG) and combined GC–EAD.[3] In the first test of the present study, where binary mixtures of the above two compounds were tested, the best captures were observed in traps baited with 100:3 or 10:1 mixtures of *Z*11–14Ac and *Z*9–14Ac (Fig. 1).

The third pheromone component, *E*11–14Ac, evoked medium responses from male antennae in EAG and GC–EAD.[3] In the second test of the present study, however, addition of varying amounts of *E*11–14Ac to the binary mixture of *Z*11–14Ac and *Z*9–14Ac did not increase catches (Table 1). In fact, addition of higher than 10–30% of *E*11–14Ac resulted in decreased catches.

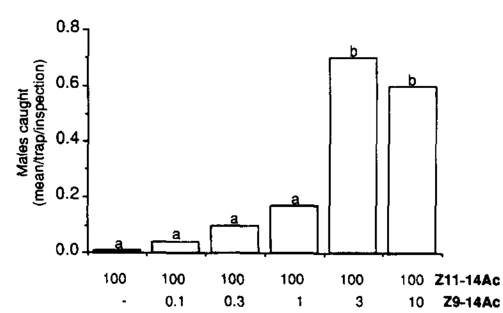

Figure 1. Catches of male *E. zinckenella* in traps baited with different combinations of *Z*11–14Ac and *Z*9–14Ac in Hungary (11 June–18 July 1991, Érd-Elviramajor, Pest county; columns with same letter are not significantly different at $p=5\%$ by ANOVA followed by Duncan's NMRT).

Key words: *Etiella zinckenella*, (*Z*)-11-tetradecenyl acetate, (*Z*)-9-tetradecenyl acetate, geographical difference, pheromone.

Table 1. Catches of male *E. zinckenella* in traps baited with different combinations of *Z*11–14Ac, *Z*9–14Ac and *E*11–14Ac in Hungary (Test 1: 11 July–21 Aug. 1991 Érd-Elviramajor, Pest county; Test 2: 5–9 July 1993, Tura, Heves county. Means with same letter within one column are not significantly different at $p=5\%$ by ANOVA followed by Duncan's NMRT)

Composition of baits (µg)			Males caught (mean/trap/inspection)	
*Z*11-14Ac	*Z*9-14Ac	*E*11-14Ac	Test 1	Test 2
100	—	—	Not tested	4.56a
100	3	—	2.14c	19.13d
100	3	1	1.48bc	16.40cd
100	3	3	1.38bc	18.00d
100	3	10	1.18bc	13.45c
100	3	30	0.65ab	8.70b
100	3	100	0.35a	Not tested

Table 2. The effect of the addition of *Z*11–16Ac or S14Ac on catches of male *E. zinckenella* in traps baited with a 100:3 blend of *Z*11–14Ac and *Z*9–14Ac in Hungary (29 July–17 Aug 1993, Tura, Heves county; for significance see Table 1)

Composition of baits (µg)				Males caught (mean/trap/inspection)
*Z*11-14Ac	*Z*9-14Ac	*Z*11-16Ac	S14Ac	
100	3	—	—	3.5a
100	3	30	—	4.8a
100	3	100	—	2.9a
100	3	300	—	3.5a
100	3	—	3	3.7a
100	3	—	10	4.0a
100	3	—	30	4.3a

Within the dose range 10–300 µg of the binary 100:3 blend of *Z*11–14Ac/*Z*9–14Ac, there was a linear response in insects captured (Fig. 2). Addition of 3% of *E*11–14Ac to 100–10 µg dose levels of the binary mixture did not influence captures. However, binary mixtures at the higher doses of 300 and 1000 µg caught better than corresponding mixtures containing *E*11–14Ac as a third component (Fig. 2).

We conclude that *E*11–14Ac, although present in the pheromone extract,[3] is not necessary for maximal attraction of male *E. zinckenella* in the field.

The presence of both S14Ac and *Z*11–16Ac in the pheromone extract has been indicated.[3] In a final trial of the present study, the addition of *Z*11–16Ac or S14Ac to the optimal 100:3 *Z*11–14Ac:*Z*9–14Ac mixture was tested. Neither *Z*11–16Ac nor S14Ac influenced catches recorded by the 100:3 mixture of *Z*11–14Ac and *Z*9–14Ac at any of the ratios tested (Table 2).

Based on the results of the above tests we conclude that from the components identified from the female-produced sex pheromone of *E. zinckenella*,[3] the binary mixture of *Z*11–14Ac and *Z*9–14Ac is best suited for attracting males. Geometrical purity of *Z*11–14Ac samples used for making up the baits is of practical importance, since the presence of the *trans* isomer may have adverse effects on activity.

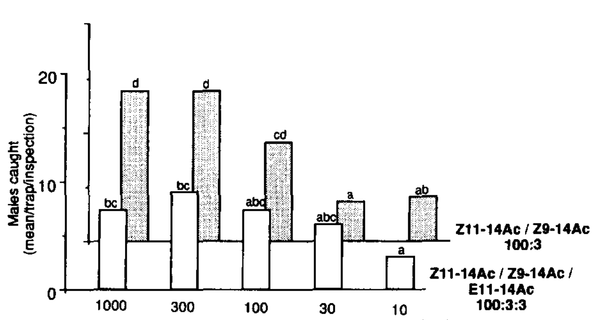

Figure 2. Effect of the addition of *E*11–14Ac on catches of male *E. zinckenella* in traps baited with different dosages of a 100:3 blend of *Z*11–14Ac and *Z*9–14Ac in Hungary (12–26 July 1991, Tura, Heves county; columns with same letter are not significantly different at $p=5\%$ by ANOVA followed by Duncan's NMRT).

The above conclusion was further corroborated in a subtraction test, where at both sites in Hungary the baits containing both *Z*11–14Ac and *Z*9–14Ac caught the highest numbers (Table 3). At Site I with lower population density, the addition of *E*11–14Ac resulted in significantly lower activity, while there was no difference between the two baits at Site II, where the population density was evidently higher. It is possible that at Site II, with higher insect population pressure, the sticky trap containing the standard binary mixture plus *E*11–14Ac was already catching insects to the maximum limit of its trapping capacity. The absence of *E*11–14Ac from the standard binary mixture, as at Site I, could have caught more insects, but the limited trapping capacity of the sticky surface prevented this. We did not conduct any direct study on the trapping capacity of the traps used in our study.

In contrast to the above results, it surprised us that the same test performed in Taiwan did not show any combinations being active, although traps baited with

Table 3. Catches of male *E. zinckenella* in traps baited with different combinations of *Z*11–14Ac, *Z*9–14Ac and *E*11–14Ac in Hungary and in Taiwan (Hungary I: 20 June–14 July 1991 Érd-Elviramajor, Pest county; Hungary II: 5 July–4 Aug. 1993, Tura, Heves county; Taiwan: 14–23 Nov. 1994, Shanhua, Tainan; female-baited traps were baited with four 2–4 day-old females; for significance see Table 1)

Composition of baits (µg)			Males caught (mean/trap/inspection)		
*Z*11-14Ac	*Z*9-14Ac	*E*11-14Ac	Hungary I	Hungary II	Taiwan
100	3	100	1.20a	15.10a	0.17b
100	3	—	3.40b	15.63a	0.17b
100	—	10	0.00	1.87b	0.67b
—	3	10	0.00	0.03a	0.00
—	3	—	0.03c	0.00	0.17b
100	—	—	0.23c	3.20b	0.50b
Female-baited			Not tested	Not tested	11.83a

virgin females and operated parallely did catch large numbers of male *E. zinckenella* (Table 3). Traps baited with similar combinations of synthetic compounds failed to catch *E. zinckenella* in preliminary tests in Japan as well (H. Sugie, pers. comm.).

The above preliminary results strongly suggest the occurrence of pheromonal differences between populations in Europe and Eastern Asia in *E. zinckenella*. A more detailed study on geographical variation in response to the pheromone is underway.

Experimental

Trapping tests in Hungary were conducted in stands of *Robinia pseudo-acacia* L. trees (which is a major host plant of the species in the region). Tests in Taiwan were conducted in soybean, the major host plant of the species in Asia. Trappings were conducted by using triangular traps with removable sticky inserts made from polyethylene sheets.[4] Traps were suspended 1.5 m above the ground from the branches of trees (Hungary), or at a height of 1.0 m from wooden poles (Taiwan). Traps containing different lures were set up in rectangular blocks. The distance of traps within a block was 4–5 m. The distance between blocks ranged from 100 to 1000 m (Hungary) or was 20 m (Taiwan). Traps were moved one position forward within a block at each occasion when the traps were inspected. At the same time, the number of captured males was recorded and sticky inserts changed to new ones. Traps were inspected each second day (Hungary) or each day (Taiwan).

Capture data were transformed to $\sqrt{x+0.5}$ and were analysed for significance by ANOVA followed by Duncan's New Multiple Range Test. Statistical analyses were performed by the software packages StatView® v4.01 and SuperAnova® v1.11 (Abacus Concepts Inc., Berkeley, USA).

Synthetic samples were obtained from S. Voerman (Wageningen, The Netherlands) and were >99% pure both chemically and isomerically by GC (S. Voerman, pers. comm.).

Dispensers for the tests were prepared in the Budapest laboratory by using pieces of rubber tubing (Taurus, Budapest, Hungary; No. MSZ 9691/6; extracted three times in boiling ethanol for 10 min, then also three times in methylene chloride overnight, prior to usage). For making up the baits the required amounts of compounds were administered to the surface of the dispensers in hexane solutions. Loaded dispensers were wrapped singly in pieces of aluminum foil and were airmailed to Taiwan, or stored at −65 °C until use.

(*Received* 31 *August* 1995; *accepted* 14 *September* 1995)

Acknowledgements

This publication is partially based on work sponsored by a grant from the Hungarian Academy of Science OTKA T 017693.

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