Spodoptera littoralisの雄誘引剤としての合成性フェロモンのイスラエルにおける野外試験

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著者	玉木, 佳男 Kehat, M Greenberg, S
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Field Evaluation of the Synthetic Sex Pheromone, as an Attractant for Males of the Cotton Leafworm, *Spodoptera littoralis* (BOISD.), in Israel

Moshe KEHAT and Shaul GREENBERG

Division of Entomology, A. R. O., The Volcani Center, Bet Dagan, Israel

Yoshio TAMAKI

Division of Entomology, National Institute of Agricultural Sciences, Nishigahara, Tokyo 114, *Japan*

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Different formulations of the synthetic female sex pheromone of *Spodoptera littoralis* were evaluated in the field. A trap baited with 1 mg of *cis*-9, *trans*-11-tetradecadienyl acetate (72% active isomer) alone adsorbed in a rubber septum was equivalent in effectiveness to a trap baited with virgin females for at least 77 days. The purified attractant (92% active isomer), however, was significantly less efficient in attractiveness than the partially purified synthetic attractant (72% active isomer). The addition of a small amount of *cis*-9, *trans*-12-tetradecadienyl acetate to the purified attractant strongly enhanced the attraction. However, *cis*-9, *trans*-12-tetradecadienyl acetate had no synergistic effect when added to the partially purified attractant. The addition of increased amounts of *cis*-9, *trans*-12-tetradecadienty acetate markedly reduced the catch of male moths. The combination of 4.8-mg attractant (97% purity) with 12-mg antioxidant adsorbed on a filter paper was somewhat less efficient in attractiveness than virgin females.

INTRODUCTION

The cotton leafworm *Spodoptera littoralis* (BOISD.) is a well known pest on cotton plants and various vegetable crops in Israel (AVIDOV and HARPAZ, 1969). Efficiency and persistence of a synthetic pheromone are important factors for establishing a pheromone-based pest-monitoring system that would provide a sound basis for control measures of this pest.

The female sex pheromone of *S. littoralis* has recently been isolated and synthesized, and *cis*-9, *trans*-11-tetradecadienyl acetate (*cis*-9, *trans*-11-TDDA) was identified as its major component (NESBITT et al., 1973; TAMAKI et al., 1973; TAMAKI and YUSHIMA, 1974b). However, whereas admixtures with three other compounds which were proposed as pheromonal components by NESBITT et al. (1973) showed no advantage (CAMPION et al., 1974; NEUMARK et al., 1974) a synergistic pheromonal component has been identified as *cis*-9, *trans*-12-tetradecadienyl acetate (*cis*-9, *trans*-12-TDDA) (TAMAKI et al., 1973; TAMAKI and YUSHIMA, 1974b). The use of an antioxidant was also reported to be synergistic to the attractant (NEUMARK et al., 1974).

As a result, different formulations of synthetic sex pheromone have been suggested for use: the attractant only (CAMPION et al., 1974), a combination of the attractant

with an antioxidant (NEUMARK et al., 1974), and a mixture of the attractant with cis-9, trans-12-TDDA (for which the name 'litlure' was proposed) (TAMAKI and YUSHIMA, 1974a; YUSHIMA et al., 1974). Furthermore, different dispensers (polyethene bottles, filter papers or rubber septa) and loadings (1000 μg-5000 μg) have been proposed (CAMPION et al., 1974; NEUMARK et al., 1974; YUSHIMA et al., 1974). Also, while the effectiveness (as compared with virgin females) of the synthetic pheromone was thoroughly studied for the formulation presented by CAMPION et al. (1974), only preliminary results were reported for the mixture of the attractant with *cis*-9-, *trans*-12-TDDA (KEHAT et al., 1975), and none for the combination of the attractant with an antioxidant (NEUMARK et al., 1974). All these facts indicated the need for further investigation.

This study, which was carried out in Israel during 1974–1975, presents the results of field experiments aiming at evaluating persistence and effectiveness of different formulations of the synthetic sex pheromone of *S. littoralis*.

MATERIALS AND METHODS

Compounds. The synthetic lot of cis-9, trans-11-TDDA (component A) contained 72% of the active isomer, and that of cis-9, trans-12-TDDA (component B) contained 79% of the active isomer. These materials are the products of Takeda Chemical Ind., Japan. The synthetic compounds were dissolved in hexane, applied to rubber septa (Zoecon, or Takeda Chemical Products, Japan, Code No. 'Ao') at different ratios of A : B (10 : 1, 20 : 1, 100 : 1, 1000 : 1, 10,000 : 1, 1 : 0) and then placed in traps (1 mg of mixture per trap) in the field. The efficiency of purified (92% active isomer) component A alone or in combinations with purified (97% active isomer) component B (A : B= 1: 0, 20 : 1, 100 : 1) was also tested. An automatic preparative gas chromatograph (Perkin-Elmer, model F-21) equipped with a 5-m column of 15% PEGA was used for the purification. The combinations of the attractant with an antioxidant dissolved on filter papers (Whatman No. 1) were kindly supplied by Dr. S. NEUMARK and I. TEICH, Ministry of Agriculture, Yafo, Israel. The attractant, *cis*-9, *trans*-11-TDDA was at least 97–98% geometrically pure (supplied by Dr. M. JACOBSON, Biologically Active Natural Products Laboratory, Beltsville, Md., U.S.A.). The antioxidant used was *N*-octyl-*N'*-phenyl-*p*-phenylenediamine (UOP688). The combinations tested contained 16 μg or 4800 μg of attractant with 12-mg antioxidant.

Virgin females. Virgin females of *S. littoralis* used in virgin female traps (one female per trap) were obtained from a stock culture of the insect maintained on artificial diet[1] modified from the simple medium of SHOREY and HALE (1965). Two-day-old virgin females were used throughout the tests.

Traps. The traps used consisted of a plastic container (17-cm diam.) filled with a dilute detergent solution to prevent escape of the males which had been attracted. A protective wooden roof was fixed 5–7 cm above the container and a screen cage, containing a virgin female or a synthetic pheromone, was hung from the center of the roof above the surface of the water. The trap was fixed at the same height as that of the cotton plants. Moths attracted to the pheromone source were drowned in the water, collected and counted. For further details of the trap design, see KEHAT et al. (1975).

[1] Composition of diet: kidney beans, 330 g; alfalfa pellets, 110 g; Torula yeast, 68 g; ascorbic acid 7 g; methyl *p*-hydroxybenzoate, 8 g; chloramphenicol, 2 g; agar 24 g; water, 1400 cc.

Field test procedures. Field tests were conducted in a cotton field at Givat Brenner, Israel, during Aug.—Sept. 1974 and May—Oct. 1975. Adjacent traps were located at least 50 m apart. Five or six traps were used for each of the formulations tested. The number of males attracted to each trap on each night was recorded on the following morning.

RESULTS

A trap baited with 1 mg of *cis*-9, *trans*-11-TDDA (72% purity) alone (component A) adsorbed on a rubber septum was equivalent in effectiveness to a trap baited with a virgin female, for at least 77 days (Fig. 1; Table 1, series a). The addition of small amounts of *cis*-9, *trans*-12-TDDA —79% purity (component B) to component A (A : B= 100 : 1, 1000 : 1, 10,000 : 1) did not inhibit or enhance the attraction (Table 1, series b). The addition of increased amounts of component B to component A (A : B=10 : 1, 20 : 1) markedly reduced the catch of male moths, the level of inhibition induced by component B was related to the amount of this substance in the mixture (Fig. 2; Table 1, series c).

A trap baited with 1 mg of purified (92% active isomer) component A alone adsorbed on a rubber septum was significantly less efficient in attractiveness to a trap baited with the more easily prepared 72% component A. The addition of a small amount of component B to a purified component A (A : B=100 : 1) strongly enhanced the attraction and the mixture was as efficient in attractiveness as the 72% component A alone. The addition of an increased amount of component B to the purified component A

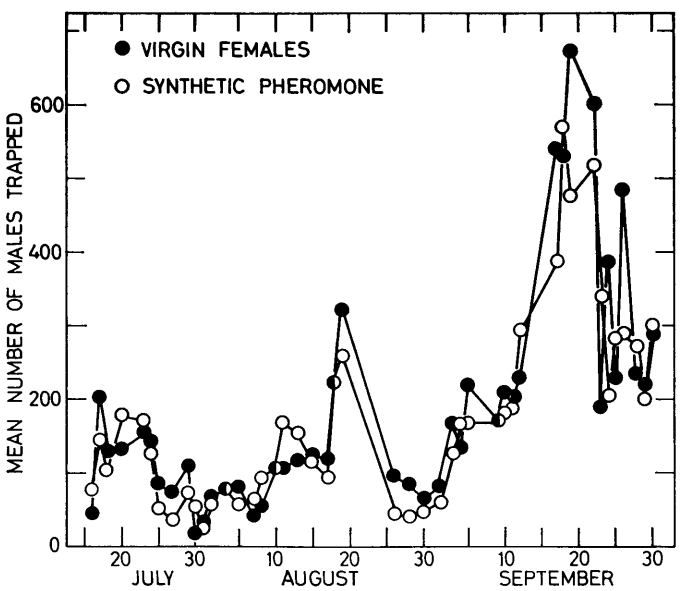

Fig. 1. Mean number of males trapped daily in traps baited with 1 mg of *cis*-9, *trans*-11-TDDA (72% purity) adsorbed on rubber septa and in traps baited with a two-day-old virgin female.

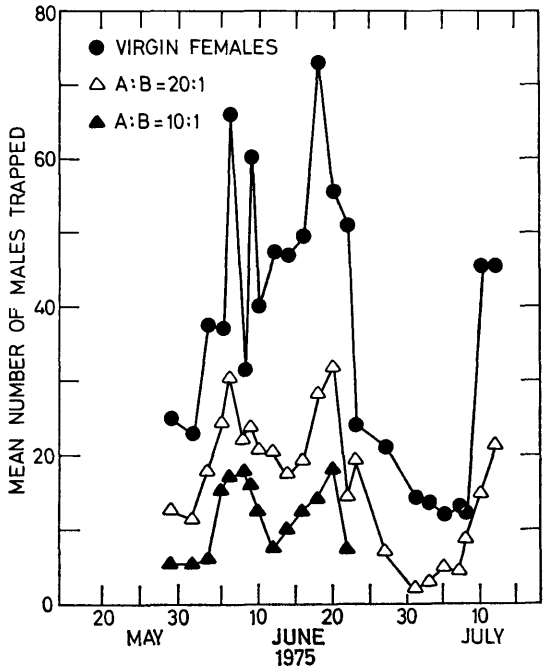

Fig. 2. Mean number of males trapped daily in traps baited with a mixture of *cis*-9, *trans*-11-TDDA (72%) and *cis*-9, *trans*-12-TDDA (79%) at the ratios of 20 : 1 and 10 : 1, and in traps baited with a virgin female.

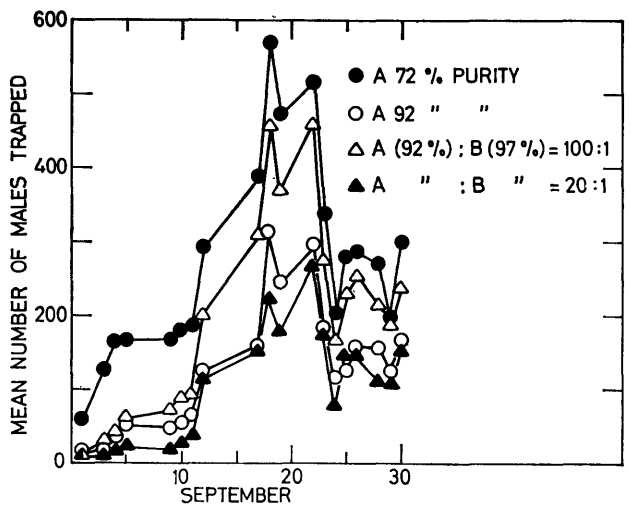

Fig. 3. Mean number of males trapped daily in traps baited with (1) *cis*-9, *trans*-11-TDDA (72% purity); (2) *cis*-9, *trans*-11-TDDA (92% purity); (3) a mixture at the ratio 100 : 1 of *cis*-9, *trans*-11-TDDA (92% purity): *cis*-9, *trans*-12-TDDA (97% purity); (4) a mixture at the ratio 20 : 1 of *cis*-9, *trans*-11-TDDA (92% purity): *cis*-9, *trans*-12-TDDA (97% purity).

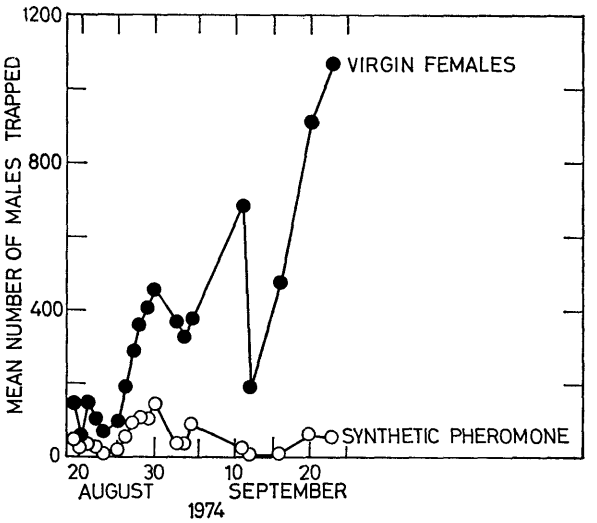

Fig. 4. Mean number of males trapped daily in traps baited with a combination of 16 μg of *cis*-9, *trans*-11-TDDA (97% purity) and 12-mg antioxidant adsorbed on filter paper, and in traps baited with a virgin female.

TABLE 1. MEAN NUMBER OF MALES CAPTURED IN TRAPS BAITED WITH DIFFERENT FORMULATIONS OF SYNTHETIC PHEROMONE AND IN TRAPS BAITED WITH A VIRGIN FEMALE (ONE FEMALE PER TRAP)

Series	Formulation tested	Experimental period	No. of observation in days	Mean number of males[4] captured per trap	
				total	per day
a	A[1](72% purity)	16.VII.75	45	7858.9a	174.6a
	Virgin female	−30.IX.75		8636.3a	191.9a
b	A[1](72% purity)	16.VII.75 −19.VIII.75	23	2527.6a	109.9a
	A[1](72%) : B[3](79%) = 100 : 1			2491.9a	108.3a
	〃 = 1000 : 1			1904.8a	82.8a
	〃 = 10000 : 1			2611.0a	113.5a
	Virgin female			2579.8a	112.2a
c	A[1](72%) : B[3](79%) = 10 : 1	29.V.75 −22.VI.75	14	166.6a	11.9a
	〃 = 20 : 1			297.7b	21.3b
	Virgin female			642.6c	45.9c
d	A[1](72% purity)	12.IX.75 −30.IX.75	12	4140.8b	345.0b
	A[1](92% purity)			2192.2a	182.7a
	A[1](92%) : B[3](97%) = 100 : 1			3364.5b	280.4b
	〃 = 20 : 1			1934.6a	161.2a
e	Antioxidant + 16 μg A[2]	19.VIII.74 −4.IX.74	14	837.2a	59.8a
	Virgin female			3402.0b	243.0b
f	Antioxidant + 4800 A[2]	6.V.75 −23.VII.75	42	538.4a	12.8a
	Virgin female			1626.4b	38.7b

[1] *cis*-9, *trans*-11-TDDA (made in Japan) adsorbed on a rubber septum.

[2] *cis*-9, *trans*-11-TDDA (made in U.S.A.) adsorbed on filter paper.

[3] *cis*-9, *trans*-12-TDDA (made in Japan) adsorbed on a rubber septum.

[4] Figures followed by different letters differ significantly at $p < 0.05$.

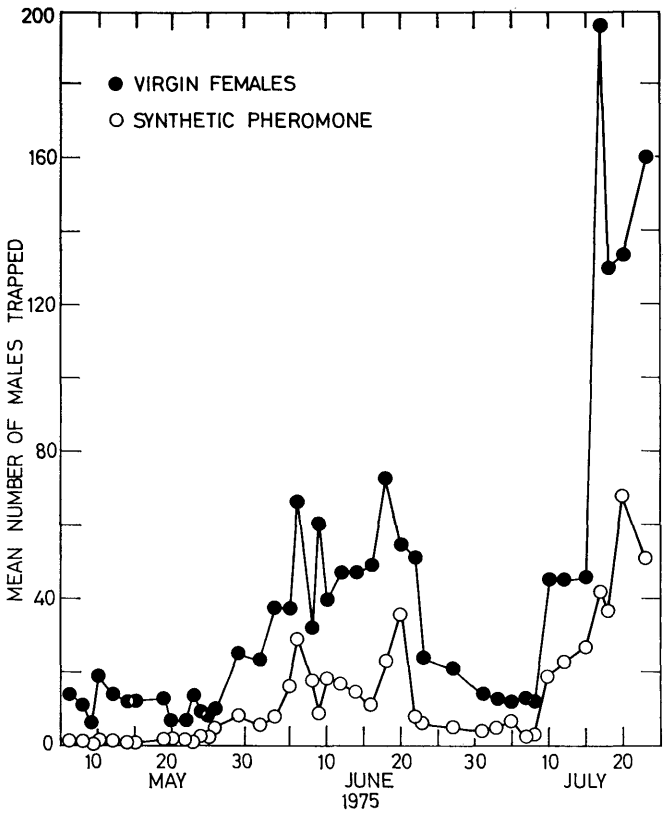

Fig. 5. Mean number of males trapped daily in traps baited with a combination of 4800μg of *cis*-9, *trans*-11-TDDA (97% purity) and 12-mg antioxidant adsorbed in filter paper, and in traps baited with a virgin female.

(A : B=20 : 1) did not inhibit or enhance the attraction (Fig. 3; Table 1, series d).

A trap baited with a combination of 16 μg of attractant (97–98% purity) with 12 mg of antioxidant adsorbed on a filter paper was four times less effective than a trap baited with a virgin female, and remained attractive for only 17–19 days (Fig. 4; Table 1, series e). Increasing the amount of attractant in the combination up to 4800 μg extended its attractive period (to at least 80 days); efficiency in attracting males, however, was significantly lower than that recorded for a virgin female (Fig. 5, Table 1, series f).

DISCUSSION

Cis-9, *trans*-11-TDDA was independently identified as the major component of the sex pheromone of *S. littoralis* (NESBITT et al., 1973; TAMAKI et al., 1973; TAMAKI and YUSHIMA, 1974a). However, whereas no synergistic activity was reported (CAMPION et al., 1974; NEUMARK et al., 1974) for any of the other compounds identified by NESBITT et al. (1973) as pheromonal components, a synergistic pheromonal component was identi-

fied by TAMAKI et al. (1973) and by TAMAKI and YUSHIMA (1974a) as *cis*-9, *trans*-12-TDDA. Thus, *cis*-9, *trans*-11-TDDA (component A) and *cis*-9, *trans*-12-TDDA (component B), the same compounds as the female sex pheromone of *S. litura* (TAMAKI et al., 1973; TAMAKI and YUSHIMA, 1974a; YUSHIMA et al., 1974), have been identified as major components also of the female sex pheromone of *S. littoralis*.

Results of this study confirm the synergistic activity of component B when in combination with component A. However, this synergistic activity is detectable only when the purified attractant (92% active isomer) is used, and not the partially purified synthetic attractant (72% active isomer). The partially purified synthetic attractant alone is equivalent in effectiveness to a virgin female; its attractiveness is not enhanced by adding small amounts of component B; it is significantly more efficient than the highly purified attractant; and as effective as the mixture of the purified components. These facts suggest the presence of trace amounts of additional synergistic components (or perhaps the presence of trace amounts of component B itself) in the partially purified synthetic attractant. Gas-chromatographic and mass-spectrometric analyses of the synthetic lot of component A revealed that 0.3–1.1% (relative to *cis*-9, *trans*-11-TDDA) of 9,12-TDDA is contained as an impurity (TAMAKI, unpublished). CAMPION et al. (1974), however, using the NESBITT et al. (1973) formulation, have reported that the 100% isomer is much more attractive than the 70% material. He concluded, therefore, that only *cis*-9, *trans*-11-TDDA is necessary to attract male moths of *S. littoralis*, in contrast to the present results. This seems to indicate that in the case of synthetic pheromones even minor differences in synthesis procedures, or in the purity of the materials used, are of great importance in determining effectiveness.

At the ratios of A : B=10 : 1 or 20 : 1, component B strongly inhibits the response of *S. litoiralis* males to their own pheromone. Similar results have been reported elsewhere (KEHAT et al., 1975). An inhibitory effect by a part of the pheromone complex of the *S. littoralis* female was reported (CAMPION et al., 1974) also for *cis*-9-tetradecenyl acetate, one of the pheromonal components identified by NESBITT et al. (1974). Such an inhibition, either by component B or by *cis*-9-tetradecenyl acetate, may serve to indicate mated status in female moths and may have use as a pest control agent by utilizing 'inhibition' as opposed to a 'confusion' technique.

No difference was reported in the optimum ratio of component A to component B for the two allopatric sibling species *S. littoralis* and *S. litura* (TAMAKI et al., 1973; TAMAKI and YUSHIMA, 1974b). However, the present results indicate that the optimal ratio of components A : B in *S. littoralis* differs from that of *S. litura*. Lower levels of component B are required for *S. littoralis* than for *S. litura*. These facts indicate differences in the sex pheromonal complex between *S. littoralis* and *S. litura*.

Different formulations of synthetic pheromones of *S. littoralis* have been suggested for use (CAMPION et al., 1974; NEUMARK et al., 1974). The synthetic pheromone 'litlure' (the mixture of components A and B at ratios of 10 : 1 or 20 : 1) which is in common use in Japan for monitoring *S. litura* populations (TAMAKI, 1974; YUSHIMA et al., 1974), is somewhat inadequate for monitoring *S. littoralis* populations. The combination of the attractant with an antioxidant, as recommended by NEUMARK et al. (1974), is also insufficiently effective to give reliable detection of *S. littoralis* population levels. The formulation proposed by CAMPION (1974), who had used the attractant only (the 70% material dispensed in polyethene bottles) without any synergistic or stabilizing components, is similar in efficiency but inferior in persistence to the one described in this

study: the use of attractant only (the 72% active isomer) adsorbed on a rubber septum. The formulation of 1 mg of component A alone (the 72% isomer), and the mixture of purified component A, with component B at the ratio A : B=100 : 1 adsorbed on a rubber septum, were found in this study to be very effective for monitoring *S. littoralis* populations and are recommended for use.

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