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Sex attractant formulations and traps for the grape moth Eupoecilia ambiguella Hb.

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The effect of the dodecyl acetate and *(E)*-9-dodecenyl acetate content on the attractiveness of *(Z)*-9-dodecenyl acetate formulations to grape moth, *Eupoecilia ambiguella* HB., males has been studied. Dodecyl acetate is a synergist; dispensers containing attractant and synergist in a 1:5 ratio outcaught the virgin females nearly three-fold. *(E)*-9-dodecenyl acetate was found to be an inhibitor with no apparent synergistic action. An effective and inexpensive trap made from a current milk carton material and an impregnation procedure for mass-production of pheromone dispensers are described.

Sex attraction in the grape moth *Eupoecilia ambiguella* HB. has been a subject of extensive studies by GÖTZ (1939, 1941). Chemical analysis of female extracts and electroantennogram studies with male moths led to the identification of *(Z)*-9-dodecenyl acetate as a major component of the sex pheromone. This compound is attractive to male moths, although less so than virgin females (ARN *et al.*, 1976). Investigations to improve attractancy have been conducted in two directions, 1) a more detailed analysis of the female secretion and 2) an evaluation of analogs as secondary components by field screening. This report deals with effects of *(E)*-9-dodecenyl acetate as secondary components on trap catches, evaluation of various dispensers, and efficacies of various old and new traps.

MATERIALS AND METHODS

Chemicals

The chemicals used in the first experiments were kindly supplied by Dr. W. ROELOFS of Cornell University and Drs. J. TETTE and C. HENRICK of Zoecon Corporation; later samples were purchased from Farchan Chemicals, now a division of Chemical Samples Co., Columbus, Ohio (U.S.A.). Dodecyl acetate was prepared from dodecyl alcohol (Fluka AG, Buchs). Purification of geometric isomers was accomplished on a 12x250 mm column packed with Partisil 10 containing 20% $AgNO_3$ (Chrompack B.V., Middelburg, The Netherlands), following the procedure by HEATH *et al.* (1975) but using toluene instead of benzene as a solvent and removing traces of eluted silver nitrate by passing the eluate through a column of NaCl. Isomeric purity was determined on glass capillary columns with Ucon 50 HB 5100 or Silar 10 C as liquid phases, kindly furnished by Dr. H.R. BUSER at the Wädenswil Research Station.

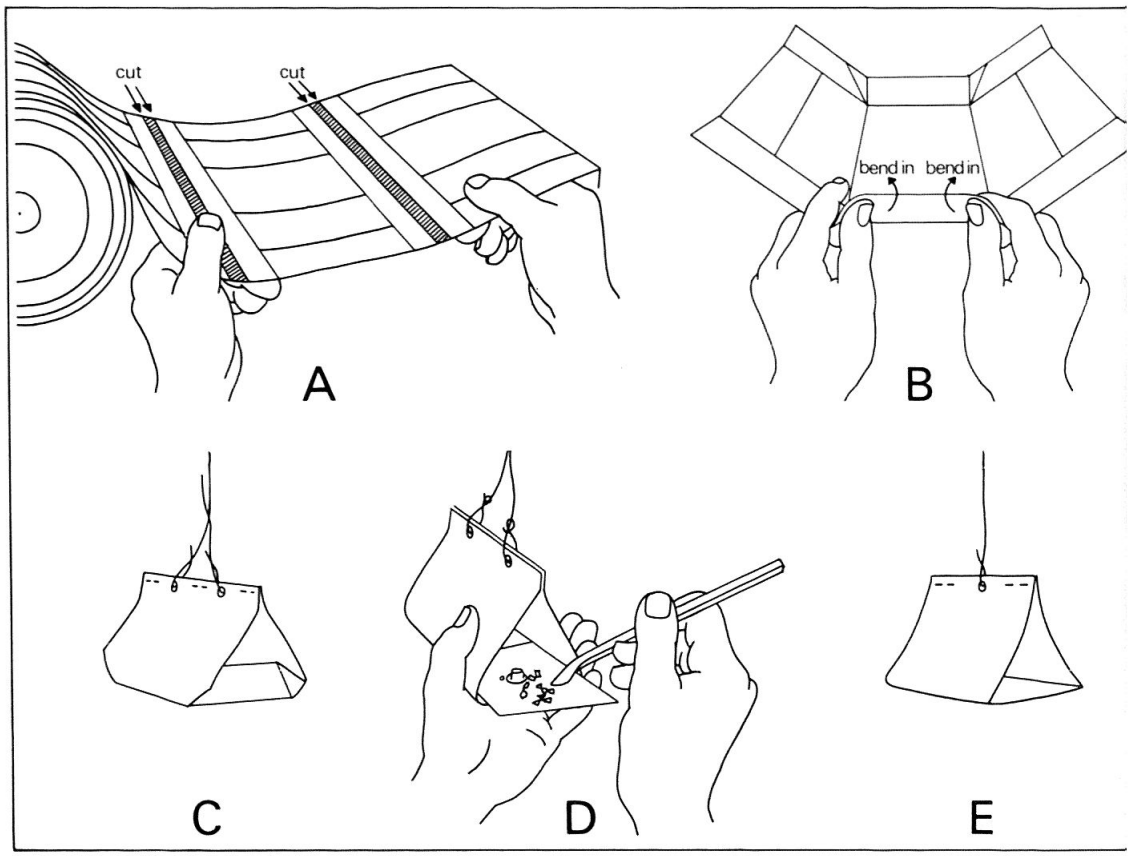

Fig.1: The Tetra Trap and its preparation from milk carton paper.

Attractant dispensers

The polyethylene capsule was charged by placing the chemicals, neat or in solution, in the cavity of a 10 mm o.d. tube closure (Polyäthylenstopfen No. 2.1449, Semadeni AG, Bern), and inserting an 8.5 mm closure (No. 2.1446). The mini cap consisted of only one polyethylene closure of 7 mm o.d. (No. 2.1443) which was impregnated with the attractant in the following way. The desired number of caps were covered with methanol in a beaker and the chemical was added under magnetic stirring. Then twice the volume of water was added and stirring continued until cloudiness disappeared (1 h or overnight). This procedure is in essence a solvent partition in which the nonpolar acetates are quantitatively absorbed by the polyethylene. The sameness of the caps assures a uniform loading.

The rubber caps were serum bottle caps (Tellergummikappen) No. 90142 from Auer, Bittmann & Soulié AG, Zürich. The chemical was placed in the cavity neat or in solution and allowed to be taken up by the rubber.

Traps

Sectar and Pherocon 1C traps were purchased from Zoecon Corporation, Palo Alto, California. The Montedison trap (ARSURA *et al.*, 1977) was kindly supplied by Dr. P. PICCARDI of Montedison S.p.A. Novara, Italy. The OILB codling moth trap (GRANGES & BAGGIOLINI, 1971) was made from a cylindrical polyethylene container of 135 mm length and 95 mm diameter by cutting out a

half circle opening from the screw-on lid and the bottom. Unless the traps were supplied with adhesive, Bird Tanglefoot (now Tangle Trap, the Tanglefoot Company, Grand Rapids, Michigan, U.S.A.) was used.

An inexpensive disposable trap which we call the Tetra Trap was made from plastic-coated paper used for 1 liter Tetra Brik milk cartons. Normally the paper made for packing pasteurized milk was used with the brown side out, but some tests also involved aluminium-coated Tetra Brik paper. The paper was purchased in rolls 322 mm wide from Tetra Pak AG, Zürich. The trap shown in fig. 1 (C) with a base surface of 166x96 mm was made from one section of paper (A) bending it along some of the pre-scored lines. Flaps are formed by a diagonal crease at each corner (B). The trap is stapled together at the top (C). The flap can be opened repeatedly to inspect the catch (D). We also tested an open version made from a 15 cm section of paper (E).

Design of trapping experiments

Trapping of native *E. ambiguella* was done in vineyards in various locations in Switzerland. For experiments at Wädenswil, male moths from a laboratory culture were released in the vineyard. No effort was made to distinguish between wild and released moths caught, but populations of wild moths were known to be low in that vineyard.

Traps to be compared were hung into the foliage at 1.0 to 1.5 m height and 1.8 to 3 m apart in one or two parallel rows. Sets of replicates were spaced evenly over the area available (15–100 m apart) except in the Wädenswil vineyard where they were placed adjacent to one another. Relative positions of traps were systematically changed between replicates. Catches recorded were transformed to log (x+1) and subjected to two-way analysis of variance and DUNCAN's multiple range test. Numbers given in the tables followed by the same letter are not significantly different at the 95% probability level.

Table 1: Grape moth catches with *(Z)*-9-dodecenyl acetate containing various proportions of *E* isomer.

TEST NUMBER	1	2	3		4	
LOCATION	Andelfingen	Wädenswil	Maienfeld		Uerikon	
YEAR/MOTH FLIGHT	1974/2	1975	1977/1	1977/2	1977/1	1977/2
TRAP	Sectar	Pherocon	Pherocon		Pherocon	
DISPENSER	rubber	rubber	polyethylene		polyethylene[2]	
DOSAGE (mg)	0.01	0.01	1		1	
NO. REPLICATES	8	5	6		6	
E isomer content:						
0.1 %			34 g	90 i	19 lm	72 o
0.2 %	51 a	87 ef	33 g	109 i	21 l	3 p
0.5 %[1]		154 e	30 g	16 k	14 lm	0 p
1 %	58 a	136 e	15 h	4 k	5 mn	0 p
2 %	22 b	133 e	9 h	2 k	6 mn	1 p
3 %	12 bc	155 e				
4 %	5 cd	93 ef				
5 %	0 d		6 h	1 k	3 n	0 p
6 %	2 d	51 f				
8 %	0 d					

[1] 0.6 % in test 2. [2] 1 mg dodecyl acetate (mini cap) added for 2nd flight.

Table 2: Grape moth catches with *(Z)*-9-dodecenyl acetate of varying isomeric purity, with or without addition of dodecyl acetate or *(Z)*-9-dodecen-1-ol, and with virgin females.

TEST NUMBER	5	6	7	8	9
LOCATION	Wädenswil	Perroy	Perroy	Yvorne	Yvorne
YEAR/MOTH FLIGHT	1975	1976/1	1976/2	1977/1	1977/2
TRAP	Pherocon	Pherocon	Pherocon	Pherocon	Pherocon
DISPENSER	polyethylene[2]	polyethylene	polyethylene	mini	polyethylene
NO. REPLICATES	6	6	6	30	30
amount per dispenser:					
1 mg Z9-12Ac (2% E)	8 c	234 fg			
+ 0.01 mg 12Ac	2 c				
+ 0.2 mg 12Ac	3 c				
+ 5 mg 12Ac	46 b	1353 d	297 hi		
same, separate caps			222 ik		
0.17 mg Z9-12Ac (2% E)			67 k		
+ 0.83 mg 12Ac			143 ik		
1 mg Z9-12Ac (0.2% E[1])		381 ef			
+ 5 mg 12Ac			472 h		824 o
0.17 mg)9-12Ac (0.1%E)					
+ 0.83 mg 12Ac, mini cap				329 m	
1 mg Z9-12Ac (2% E), rubber		416 e			
+ 0.1 mg Z9-12OH		159 g			
5 mg 12Ac			6 l		
virgin females	119 a			118 n	294 p

[1]0.1 % E in test 8. [2]unless indicated

RESULTS

(E)-9-dodecenyl acetate as an inhibitor

In previous tests it was found that the *E* isomer is a strong inhibitor when present at concentrations of a few percent and above. However, no conclusion could be reached if, like in several other lepidoptera, a small amount of geometrical isomer is required for attraction of *E. ambiguella*.

Table 1 lists the results of trapping experiments in which the *E* isomer was added in increasing proportions to purified *(Z)*-9-dodecenyl acetate. In tests 1 and 2 using a load of 10 µg of attractant per cap, inhibition by the *E* isomer became significant at 2 and 6%, respectively. In tests 3 and 4 using a load of 1 mg, inhibition during the first flight started at 1% *E*. During the second flight inhibition started at even lower proportions of *E* isomer. This may not be due to a different response of the two generations of the insect, however, but to isomerization occurring in the caps left in the field for both flights. Test 12 (table 3) shows that performance of a polyethylene capsule aged during one flight is very low compared to a fresh one. Since results of some tests indicated that inhibition by the *E* isomer may be less pronounced in the presence of dodecyl acetate, a mini cap containing 1 mg dodecyl acetate was added to each trap in test 4 during the second flight. However, catches in this case were inhibited at an even lower level of *E* isomer than in test 3 where no dodecyl acetate was present.

Other comparisons of non-purified (2% *E*) with and purified (0.1–0.2% *E*) *(Z)*-9-dodecenyl acetate, alone and in combination with dodecyl acetate, are included

in tests no. 6, 7 and 10–12 (tables 2 and 3). Only in one case (test 12), the 2% *E* material made significantly lower catches than the purified product. It is conceivable that the sensitivity towards the inhibitor varies with ecotypes or generations of the insect. The questions of threshold concentration for inhibition and the effect of dodecyl acetate on it could thus not be fully answered from these tests; however, where differences were found, the highest catches were always obtained with samples containing the smallest amount of *(E)*-9-dodecenyl acetate.

Dodecyl acetate as a synergist

Dodecyl acetate was tested as an additive because it is a synergistic pheromone component in many lepidoptera. Test 5 with released males (table 2) shows that while small amounts of dodecyl acetate appear to have no effect, a large excess increases catches drastically. This synergism was confirmed in test 6 with a free-living population. As shown in test 7, the high catch in the presence of dodecyl acetate was not a result of dilution of *(Z)*-9-dodecenyl acetate by the additive. The same high catch was obtained when the two chemicals were dispensed from different capsules and lowering the dose of the monoene led to a decrease of catch. Dodecyl acetate alone was unattractive. In test 5 with a product containing 2% *E* isomer, the 1:5 mixture of attractant and synergist was less attractive than the live females, but when made using the purified monoene, the mixture outcaught the live females nearly three-fold in both polyethylene and mini caps at two different loads (tests 8 and 9).

(Z)-9-dodecenyl alcohol was tested as an additive in test 6. This compound was found to be inhibitory at the level of 10%.

Dispenser tests

Because of the advantages of the impregnation procedure in the preparation of large numbers of dispensers in one batch, efforts were made to prepare a

Table 3: Grape moth catches with various dispenser formulations containing *(Z)*-9-dodecenyl acetate and dodecyl acetate.

TEST NUMBER			10	11	12
LOCATION			Schinznach	Yvorne	Yvorne
YEAR/MOTH FLIGHT			1977/2	1977/1	1977/2
TRAP			Pherocon	Pherocon	Pherocon
NO. REPLICATES			6	7	7
amount per dispenser: Z9-12Ac	12Ac	dispenser type:			
1 mg (2% E)	5 mg	polyethylene	51 ab	190 cd	81 h
1 mg (0.1% E)	5 mg	polyethylene	110 a	173 c	484 g
same, carried over from first flight					14 i
0.17 mg (0.1% E)	0.83 mg	mini	38 b	52 f	
0.33 mg (0.1% E)	1.67 mg	mini	61 ab	109 def	
0.83 mg (0.1% E)	4.17 mg	mini	51 ab	111 de	
2 mg (0.1% E)	6 mg	impregn. 10 mm			85 h
2 mg (0.1% E)	2 mg	mini			76 h

dispenser equal in performance to the standard polyethylene capsule by this method. The upper limit for loading the 7 mm mini cap was found to be ca. 5 mg of chemical, so other sizes of caps and component ratios were tried. As seen in table 3, all formulations tested showed good attractancy. However, none was consistently as good as the polyethylene capsule. The mini cap containing 1 mg of the 1:5 mixture of *(Z)*-9-dodecenyl and dodecyl acetates has nevertheless proved useful for insect dispersal and other studies where highest attractancy is not of prime importance.

Evaluation of traps

Results of trap comparisons are shown in table 4. Test 13 shows that while the OILB, Montedison and Pherocon traps were about equal in performance, the open version of the Tetra Trap was considerably less attractive. The aluminized outside surface apparently did not deter insects from entering the trap. Since it was assumed that the low performance of the open Tetra trap was due to the fact the insects could escape without touching the sticker, the cap was placed directly on the adhesive for test 14. In this case trap performed equally well as the other three. A drastic improvement, however, was obtained by adding the flaps to the Tetra trap. This trap outcaught by far all others traps tested.

Table 4:
Grape moth catches with various traps.

TEST NUMBER	13	14
LOCATION	Maienfeld	Maienfeld
YEAR/MOTH FLIGHT	1977/1	1977/2
DISPENSER	mini cap[1]	mini cap[1]
NO. REPLICATES	6	6
OILB codling moth trap	132 a	198 b
Pherocon 1C	116 a	119 c
Montedison	196 a	227 bc
Tetra Trap open (brown)		
dispenser suspended	24 b	
dispenser on adhesive		118 bc
Tetra Trap open (aluminium)		
dispenser suspended	46 b	
Tetra Trap with flaps		459 a

[1] containing 0.17 mg Z9-12Ac (0.1% E) and 0.83 mg 12Ac

DISCUSSION

The present experiments show that *(E)*-9-dodecenyl acetate is a strong inhibitor of grape moth attraction to the sex pheromone component *(Z)*-9-dodecenyl acetate. The inhibitory effect was in some cases apparent at concentrations as low as 0.2%; in others it was not significant even at 4% *E* isomer. A requirement of the *E* isomer for best attraction was in no case found. It can be recommended to use *(Z)*-9-dodecenyl acetate of highest isomeric purity for *E. ambiguella* trapping.

The results also show that dodecyl acetete is a strong synergist. Dispensers containing this compound and *(Z)*-9-dodecenyl acetate in a 5:1 ratio caught nearly

three times as many moths as did the live females. No effort has yet been made to determine optimum concentration of the synergist.

Based on these results the formulations used for monitoring grape moth flight contains a 1:5 mixture of highly purified *(Z)*-9-dodecenyl acetate and dodecyl acetate. Comparative tests with this formulation (type E) are described in an accompanying paper (ARN *et al.*, 1979).

Loading of dispensers by impregnation in aqueous methanol is a convenient way of mass preparing polyethylene caps. The minicap caught fewer grape moth males than did the polyethylene capsule, perhaps due to the smaller surface resulting in a lower release rate. In similar tests with the nun moth using disparlure (N. MAKSYMOW, pers. comm.) and with the plum fruit moth using *(Z)*-8-dodecenyl acetate (E. MANI, pers. comm.), the two dispensers were about equal in performance.

Of the traps tested, the two commercial types performed similarly well as the cylindrical OILB codling moth trap. The Tetra trap with flaps which is described in this paper caught more grape moths than all other traps tested. Preliminary results show that this trap can be used for various tortricids such as the codling moth or the plum fruit moth, or for noctuids such as *Mamestra brassicae* L. or *Agrotis segetum* SCHIFF. Main advantages of the trap are that it can be shipped flat and assembled in little time in the field, that the material is inexpensive and obtainable in many countries.

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