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Pheromone analogues with ether structure of (Z)-11-hexadecenil acetate

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Abstract:

Ethers 1 and 2 were synthetised and were tested on *Mamestra brassicae* L. (Lepidoptera: Noctuidae) male moths in comparison with 11Z-16Ac, the sexual pheromone of the insect.

The biological tests of 1 (EAG, olfactometer and field) have shown no attractivity of 1, but no inhibition effect.

The preexposure tests with 1000 ug of 2 have demenstrate the same ability as of 1000 ug 11Z-16Ac to suppress the mating of the *M. brasscae* preexposed males. These results open the possibility of using the ether analogue 2 in the mating disruption technique for plant protection.

Rezumat

Analogi feromonali cu structură eterică, pentru (Z)-11-hexadecenil acetat.

Doi compuşi eterici (1 şi 2) au fost sintetizaţi şi testaţi pe masculii de *Mamestra brassicae* L. (Lepidoptera: Noctuidae), comparativ cu Z-11-hexadecenil acetat, componentul principal al feromonului sexual al acestei specii. Testarea biologică a compusului 1 (prin Electroantenografie, olfactometrie şi în câmp cu metoda capcanelor) nu a pus în evidenţă un fenomen de atractivitate, dar nici efect de inhibiţie în răspunsul masculilor. In testul de preexpunere a masculilor la doza de 1000 ug a compusului 2 a pus în evidenţă o aceeaşi capacitate de a întrerupe împerecherea dintre sexe, similar cu doza de 1000 ug a compusului principal al feromonului sexual (11Z-16Ac). Rezultatele obţinute oferă posibilitatea folosirii analogului eteric 2 în tehnica de întrerupere a împerecherii adulţilor, ca modalitate practică de combatere directă a dăunătorilor pentru protecţia plantelor.

Keywords: Sex pheromone analogues, olfactometer test, EAG technique, preexposure test, field trap test, lepidoptera, 11Z-hexadecen-1-yl acetate, 1-acetoxy-decyl-amyl-ether, 1-acetoxy-decyl-buthyl-eter, *Mamestra brassicae*.

Sex pheromones analogues are considered the structural analogues compounds which mimics their biological activity.

The main biological activity of the sex pheromone in the nature consist in the attraction of the opposite sex insect for mating.

By permeating the air with a higher amount of synthetic sex pheromone, the laboratory and field tests have shown the ability of the same compounds to disrupt the mating. The males are prevent finding the females. This technique, called mating disruption, has been demonstrated to be effective against several species of lepidoptera and registered for use in plant protection around the world.

The mating disruption can be achieved with sex attractants, inhibitors, synergists, analogues or

other substances (ARN 1992; BENGTSSON et al. 1994; STAN et al. 1996).

A drastic decrease of the biological activity by the following changes in the sex pheromone molecule was established: the chain length, the hydrocarbon site, the unsaturation position, the optical or geometrical isomerism.

Material and method

Chemical synthesis

In the synthesis of the pheromone analogues with etheric structure we try to keep the important site for the biological activity of he pheromone molecules: chain length, hydrocarbon site, acetate group (Fig. 1). The changes were done at the double bound structure, one of the sp^2 hybridised Carbon was replaced with an etheric Oxygen. An electronic similitude of the molecules can be considered, by replacing the π electrons from the double bond with the p electrons from Oxygen.

The chemical structure of 11Z-16Ac and of the synthesised sex pheromone etheric analogues, compounds **1** and **2**, are presented in Fig. 1 and Table 1.

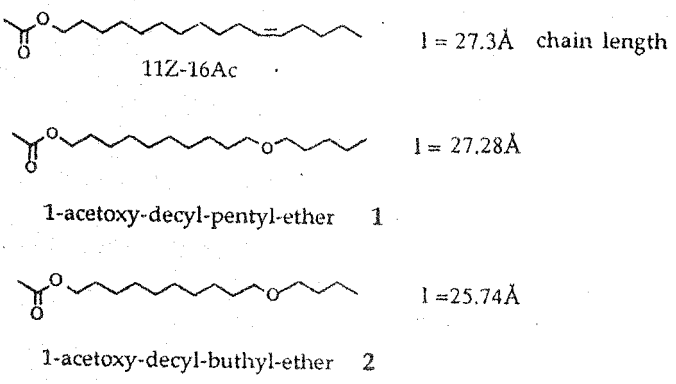

Fig. 1. The chemical structure of the tested pheromone and etheric analogues **1** and **2**

The tested chemicals were prepared in the Laboratory of Natural Products from the Institute of Chemistry, Cluj-Napoca (POP et al. 1981). The substances were purified on SiO_2 column and the chemical purity of the tested compounds was determined by GC-MS and was over 98%.

The key step in the etheric sex pheromone analogues synthesis is the phase transfer catalysis reaction (STARKS & LIOTTA 1978):

$$R\text{-}X + R'OH \xrightarrow[NaOH\ aq.50\%]{catalyst} R\text{-}O\text{-}R'$$

phase transfer catalyst : Bu_4NBr, 18-crown-6 ether

The ether acetate analogues are obtained by acetylation with AcCl/AcOH or Ac_2O/Py.

Biological tests

The biological test were performed at the Biological Research Institute from Cluj-Napoca.

Insects. 2-3 days old male moths, reared on artificial diet M-33 (STAN 1993a) were used. The insects were sexed as pupae, kept in termostated cabinets at 23°C and brought at least 2 hours earlier in the experimental laboratory to allow adjustment to temperature and photoperiod conditions. The mating tests were performed in termostated laboratory, at 20°C and the light intensity was 0.4 lucs.

Preexposure and mating tests. These experiments were performed in olfactometer and in Berzelius glasses, respectively. *Mamestra brassicae* male noths were preexposed for 8-10 hours to the analogue **2** (100 and 1000 ug) or 1000 ug 11Z-16Ac. Flight and behaviour was recorded in olfactometer (STAN 1993b) as response to calling females.

In a second experiment, the preexposed *M. brassicae* male moths to analogue **2** or to 11Z11-16Ac (as in the previous experiment) were introduced, with virgine female of 1-2 days, in Berzelius glasses for 48 hours. The female abdomens were cut and the spermatophores were counted. As a blank unexposed male moths from the same generation were used as control.

Table 1

Phisico-chemical data on the synthetised and tested sex pheromone analogues with ether structure.

Ether pheromone analogues	Physico-chemical data
$AcO\text{-}C_{10}H_{20}\text{-}O\text{-}C_5H_{11}$ $C_{17}H_{34}O_3$ M=286 1	$nD^{18} = 1.4530$ MS = 287, 286, 285, 271, 226, 243, 215, 199, 155, 137 (100%), 125, 111, 109, 99, 97, 85, 83, 81, 71, 69, 67, 57, 55,53, 43, 41, 39.
$AcO\text{-}C_{10}H_{20}\text{-}O\text{-}C_4H_9$ $C_{16}H_{32}O_3$ M=272 2	$nD^{18} = 1.4510$ MS = 273, 271, 229, 215, 212, 199, 185, 169, 157, 155, 137, 123, 109, 111, 97, 95, 81, 83, 69, 61, 57 (100%), 55.

Olfactometric tests. We used an original olfactometer, "type cages" (TOMESCU et al. 1980) but cages were replaced with two glass cylinders (40 cm diameter, 50 cm the length) (Fig. 2) connected by a glass tube (3.5 cm diameter and 15 cm length) with inverse cone (1 cm diameter) (STAN 1991, 1993b).

Testing time was 10 minutes. Before each experiemnt a test was run, to check the possible contamination. The testing substance was put on a filter paper.

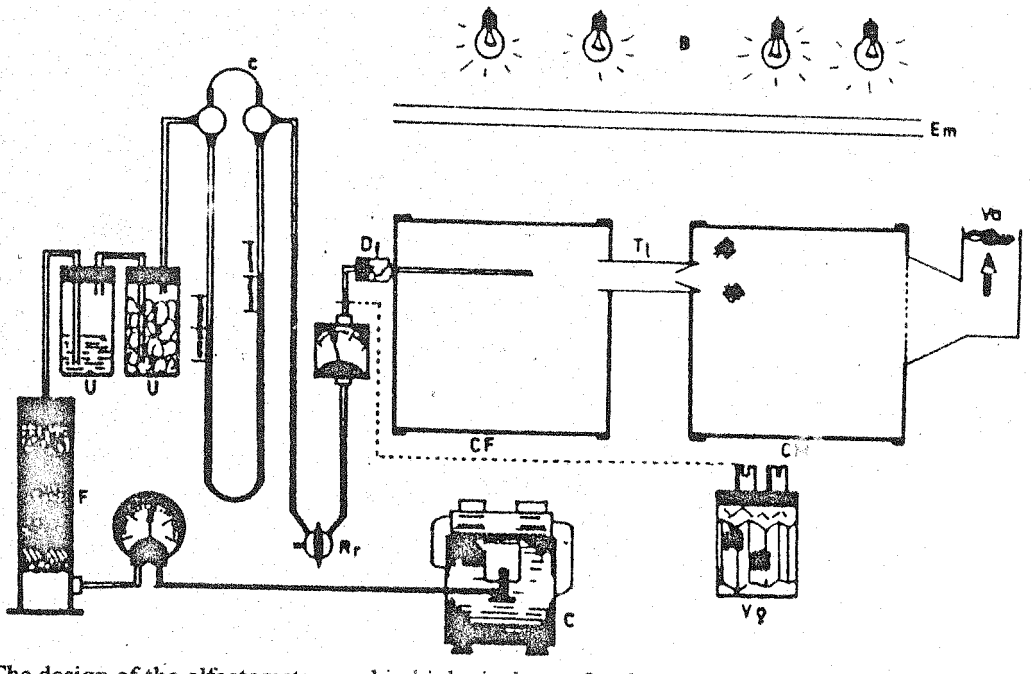

Fig. 2. The design of the olfactometer used in biological tests for the study of *Mamestra brassicae* male moths response to sex pheromone and its analogues under laboratory conditions (details in STAN, 1995).

The positive answer was considered for the males moving towards the source (female, pheromone or analogue) and cross the connection tube (**R1**), showing their ability to "smell", to lock on and touch down the source, by typical mating sequence behaviour (**R2**). From the five parameters of a typical male response to female odour or equivalent synthetic attractant or analogue, we have recorded **R2** which consist in the number of males showing the answer characterised by activation,

take off, lock on and passing through the connection tube to the cage containing the source (STAN 1991, 1993, 1996c). Each experiment was run with 50 insects in 4-5 repetition. For check the attractivity of the analogue 1 in each experiment 70-80 male moths, in 8-10 repetitions, were used. Unexposed male moths, from the same generation, were used as control.

EAG test. The EAG measurements were conducted on the complete equipment (micro manipulator, amplifier and Volcraft oscilloscope) purchased from Syntech, Netherlands in the Institute of Zoology, Bulgarian Academy of Sciences. The used method was similar to that described by ROELOFS et al. (1971). 11Z-16Ac and the analogue 1 were tested in two dosages and two repetition on 15 *M. brassicae* male antennae. Duncan's New Multiple Range Test (P=0,05) was used for statistics.

Field tests. The trapping test was conducted in both, a cabbage culture and a forest during the second flight season in Cluj area. Trap tests were carried out in 1985 and 1990 with the analogue 1 and with 11Z-16Ac. The method of randomised block design was used. The modified Montedison traps (30 x 32 cm) which had been reported to be effective for catching Noctuidae species (STAN et al. 1987; STAN 1996c) were placed 1.0 m above the soil surface in cabbage culture and 1,5-1,8 m in forest and were separated by at 25 m distance. Two plots were selected in every ecosystem and two randomized groups of traps were used per plot. A distance of at least 75 m between each trap groups and a distance of at least 100 m between plots was selected. The traps were inspected daily and each trap was moved at random to different positions. One observation constituted one replicate.

Prior to analysis of variance by Duncan'sNMRT, field data were transformed to log (x+1).

Results

Preexposure and mating tests. In olfactometer, the response behaviour to calling females of the preexposed males (to 1000 ug analogue 2 or to 11Z-16Ac) was diminished with 52% and 62%, respectively (Fig. 3).

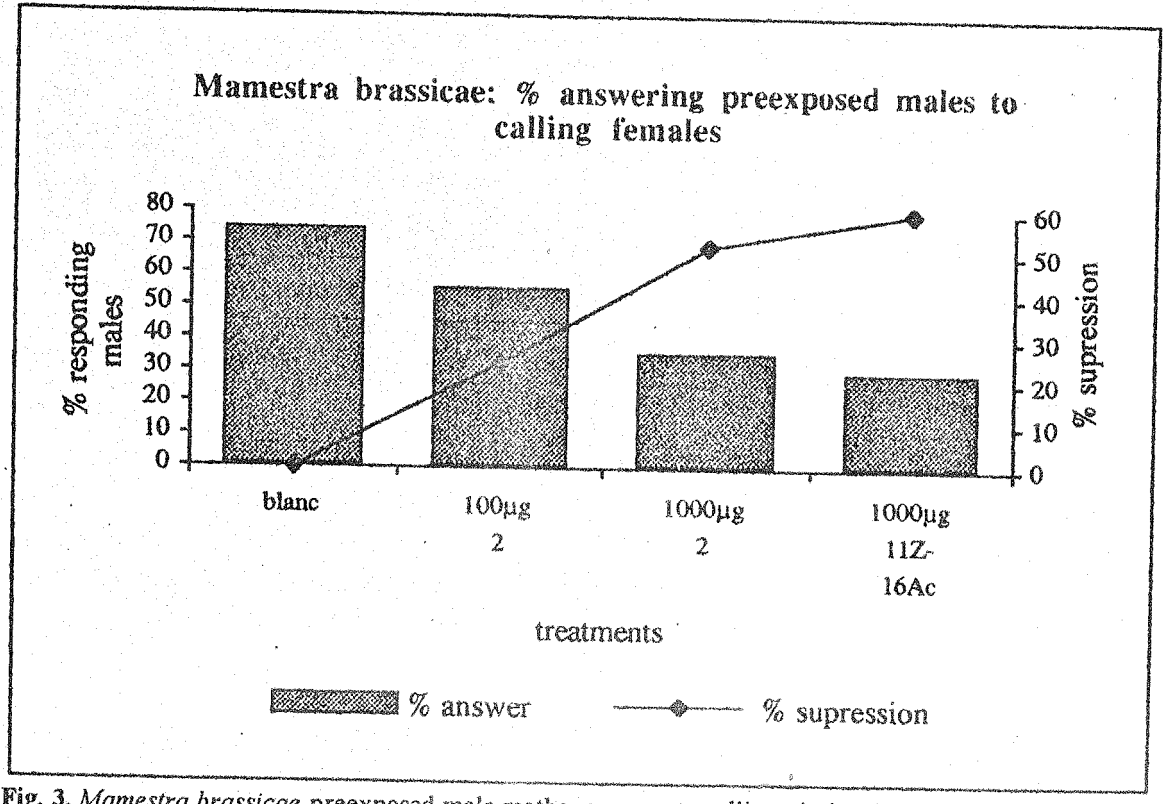

Fig. 3. *Mamestra brassicae* preexposed male moths response to calling virgine females in olfactometer under laboratory conditions.

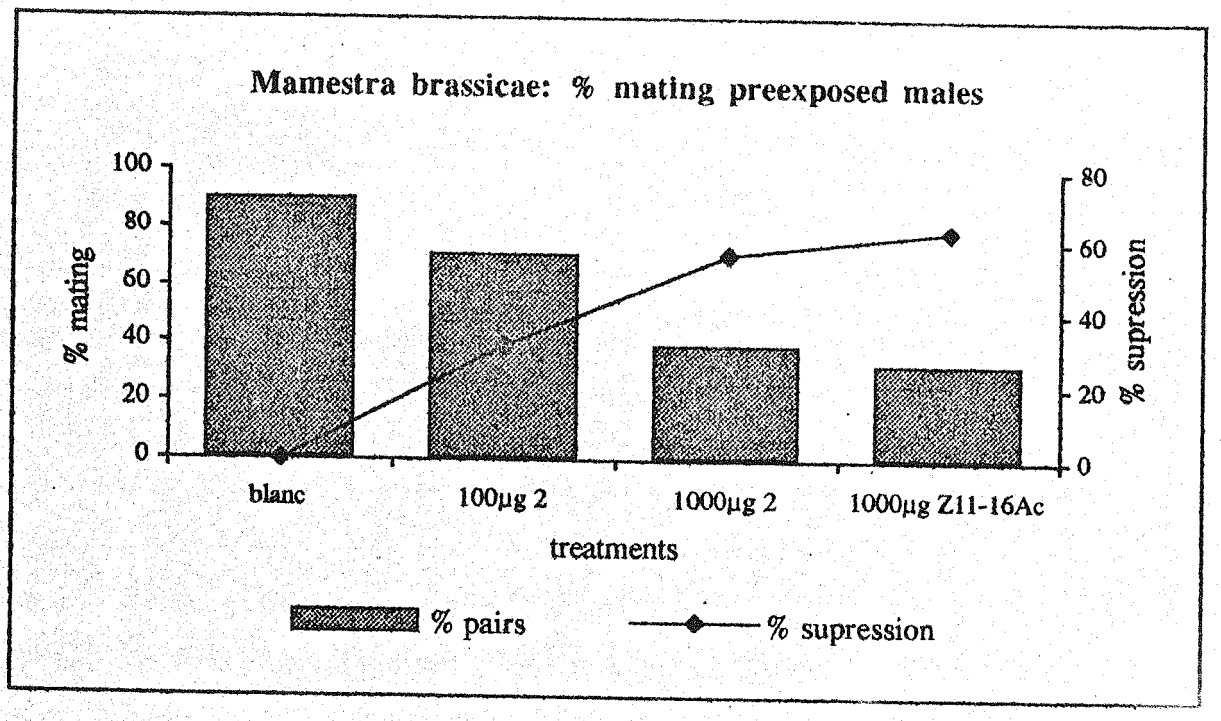

Fig. 4. Mating suppression of the preexposed *Mamestra brassicae* male moths to analogue 2 and 11Z-16Ac, in the mating test under laboratory conditions.

The results în the mating test have shown a diminution of the mating with 63.1% after a preesposure to 1000 ug of 11Z-16Ac and 56% at the male moths preexposed to 1000 ug of analogue 2 (Fig. 4).

Olfactometric test. Data presented în Table 2 show that the analogue 1 is not attractive. However, we have observed an activation of the males, what demonstrate an interaction between the insects antenna and released analogue, but no orientation of the males towards the source could be observed. In a mixture (19:1) with the sex pheromone (11Z-16Ac), the analogue 1 had a synergistic effect, increasing the number of males who touch the source. A higher ratio of analogue in the mixture decreased the attractivity.

It is important to notice that no inhibition effcet was recorded of the ether **1**.

Table 2

The response behaviour of *Mamestra brassicae* male moths to 11Z-16Ac and to its etheric analogue 1, in olfactometer test under laboratory conditions.

Compozition	Amount	Response (%)	
		R2	R1
Females	4	72.56	65.00
11Z-16Ac	3 ug	90.42	76.24
1	3 ug	0	0
1	10 ug	0	0
11Z-16Ac + 1 (19:1)	3 ug	98.16	97.28
11Z-16Ac + 1 (9:1)	3 ug	68.48	67.44
11Z-16Ac + 1 (8:2)	3 ug	76.15	63.24

EAG test. The response of *Mamestra brassicae* antennae to analogue 1 in a dosage of 10 ug is almost as high as to the control while the 100 ug of the same compound elicited significantly higher response. However, the response is significantly lower than that elicited by 11Z-16Ac, in both 10 and 100 ug dosages (Table 3).

Table 3

EAG response of *Mamestra brassicae* male antennae at two dosages of 11Z-16Ac ant to its atheric analogue, 1.

Compound	Dose (ug)	Mean answer* (% to control)
11Z-16Ac	10	160.67[b]
Analogue 1	10	99.54[d]
11Z-16Ac	100	220.28[a]
Analogue 1	100	132.60[c]

* - Response followed by different letter are significantly different; Duncan'sNMRT; P=0,05.

Field test. The male response behaviour in pheromone traps from both ecosystems (cabbage culture and forest) shown that the analogue 1 was not attractive (Table 4, 5). In 1985 the greatest number of males were caught in traps with 11Z-16Ac and the attractivity of different mixtures of 11z-16Ac and analogue 1 decrease with the diminuation of 11Z-16Ac amount (Table 4). No response reaction was noticed for mixture between 16:Ac and 1.

Table 4

Studies of the attractivity of 11Z-16Ac ant its etheric analogue 1, at diffrent ratio mixture of them in field tests with pheromone traps in a cabbage culture. FH Someşeni, 20.VII-13.VIII. 1985.

Variant	Dose (mg)	x ♂♂/trap/day*	
		I	II
11Z-16Ac	4	0.84[a]	2.21[a]
Analogue 1	4	0[b]	0[b]
11Z-16Ac + 1	3 + 1	0.12[c]	0.24[c]
11Z-16Ac + 1	3 + 1	0.02[b]	0.04[b]
16:Ac + 1	8 + 0.8	0[b]	0[b]
16:Ac + 1	4 + 0.4	0[b]	0[b]

* - I, II - represent data for the two plots; The mean values in the same column followed by the same letter are not significantly different (Duncan'sNMRT; P=0,05).

Data from 1990 are presented in Table 5. The number of *M. brassicae* male moths was significantly greater in the natural ecosystem (forest). These results have been confirmed our other field capture data for *M. brassicae* (STAN et al. 1987; STAN et al. 1994). A constant greater population level which was registered yearly depended by mating and flight behaviours and by favorable environmental conditions. The greatest number of males was caught in traps with 11Z-16Ac 4 mg, in the both ecosystems. Other data were similary with data obtained in 1985.

Table 5

Number male moths of *Mamestra brassicae* caught in pheromonal traps baited with 11Z-16Ac in different dose and different mixtures between 11Z-16Ac and analogue 1. Cluj area, 28.VII-15.VIII, 1990. A - deciduous forest, SDE Floreşti; B - cabbage culture, SDE Floreşti.

Variant	Dose (mg)	x ♂♂/trap/day*	
		A	B
11Z-16Ac	0.5	2.11^a	0.16^a
11Z-16Ac	1.0	2.04^a	0.12^a
11Z-16Ac	2.0	6.18^b	0.08^b
11Z-16Ac	3.0	4.26^c	0.14^a
11Z-16Ac	4.0	7.22^d	$0,10^a$
Analogue 1	4.0	0^e	0^b
11Z-16Ac + 1	3 + 1	4.26^c	0.06^b
11Z-16Ac + 1	1 + 3	0.28^e	0.04^b
11Z-16Ac + 1	1 + 1	$2,18^a$	0.08^b

* The mean values in the same column followed by the same letter are not significantly different (Duncan'sNMRT; P=0,05).

In pheromonal traps baited with 11Z-16Ac were catched male moths for any other Noctuidae species and for these species a similar behaviour was observed (Table 6).

Discussion

The literature mention the synthesis and biological tests of many sex pheromone mimics: halogenated compounds (SUBCHEV et al. 1989; PRESTWICH et al. 1990; BENGTSSON et al. 1990a; CAMPS et al. 1984, 1990; JÖNSSON et al. 1991;

LINN et al. 1992; WENQUI et al. 1993; LUCAS et al. 1994; KLUN et al. 1994), analogues with Carbon chain modification (CAMPS et al. 1988; BENGTSSON et al. 1990b; HOSKOVEC et al. 1993, 1994), analogues with changed functional group (MITCHELL et al. 1978; SUBCHEV et al. 1989; CAMPS et al. 1990; HOSKOVEC et al. 1993), Sulf containing analogues (CAMPS et al. 1990), oxime analogues (MARTIN & WEBER 1994). The analogues with an important attractivity towards lepidopteran insects are mentioned in the List of Sex Pheromones (ARN et al. 1996). The chlorinated compound 11Cl-(E,E)-8,10-dodecadien-1-pl has the same attractivity for *Cydia pomonella* males (Tortricidae) as the sex pheromone (E,E)-8,10-dodecadien-1-ol. The difluorinated compounds 10,11-difluoro- and 8,9-difluoro-(E,E)- and 8,9,10,11-tetrafluoro 8,10-dodecadien-1-ol are highly attractive for the males of the same species (LUCAS et al. 1994). The compound 11,11-difluoro-(Z)-9-dodecen-1-yl acetate is attractive for *Eupoecilia ambiguella* and *Cnephasia incertana* males.

Table 6

The number of male moths of different Noctuidae species captured in pheromonal traps baited with 11Z-16Ac single and in mixture with analogue 1. Cluj area, FH Someşeni, 1990 (vegetable cultures).

Species	x ♂♂/trap/day from different variants and dosage				
	11Z-16Ac	Analog.1	11Z-16Ac + analog. 1		
	4 mg	4 mg	3+1 mg	1+3 mg	1+1 mg
M. brassicae	1.04	0	0.62	0.06	0.12
D. trifolii	8.22	0	11.52	4.26	0
O. plecta	2.64	0	0	0	0
L. suasa	0.38	0	0	0	0
L. w-latinum	2.58	0	0	0	0
A. monoglypha	0.60	0	0	0	0

The pentafluorinated compound 11,11,11,12,12-pentafluoro-(Z)-9-dodecen-1-yl acetate attracts the *Cucullia lucifuga* (Noctuidae, Cucullinae) and *Axylia putris* (Noctuidae, Noctuinae) males (BENGTSSON et al. 1990a). *Grapholitha molesta* males are attracted by 7,7-difluoro-(Z)-8-dodecen-1-il acetate.

Sulf containing mimics, oximes, chain branched isomers, optical or geometrical isomers have a low attractivity, some are inhibitors.

The sex pheromone analogues with ether structure were prepared in good yields, simple reaction conditions, reduced number of reaction steps, simple purification methods, low costs by phase transfer catalisis. These characteristics are highlighted in comparison with the sex pheromone synthesis.

The preliminary biological tests have demonstrated that the insects are not indifferent to the ether analogues structures. The laboratory tests with compounds 1 show no attractivity of the compound for *mamestra brassicae* male moths but also no inhibitory effect. The insects are activated but they are not able to find the pheromone plume. An important proof of the interaction between the insects and analogues is the 60% diminution of the mating after the males preexposure to 1000 ug of compound 2.

Although the 11Z-16Ac is a main pheromonal compound in many lepidopteran species and its attractivity has been evidenced in our capture tests in the field (STAN & POP 1992) or in desorientation (STAN et al. 1996), we intend to extend our experiences in the field to see if it is possible to use the ether sex pheromone analogues for mating disruption. A disruption with a non-attractive compound could have a practical advantage.

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