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Nitrate Esters: Novel Sex Pheromone Components of the Cotton Leafperforator, *Bucculatrix thurberiella* Busck. (Lepidoptera: Lyonetiidae)

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Abstract. (Z)-9-Tetradecenyl nitrate and (Z)-8-tridecenyl nitrate have been identified in volatiles from virgin female cotton leafperforator moths, *Bucculatrix thurberiella*, and shown to elicit electroantennogram responses from male moths. A 100:2 blend of the synthetic compounds is highly attractive to male moths in the field, and these compounds are proposed to be components of the female sex pheromone.

The cotton leafperforator, *Bucculatrix thurberiella* Busck. (Lepidoptera: Lyonetiidae), is a pest specific to cotton in the New World from Peru to the southern USA. It is a chronic pest in Central and South America, but of only sporadic, localised importance in the USA where its appearance is often associated with overuse of insecticides against other pests[1]. The female moths were reported to produce a sex pheromone[2], and identification of this was undertaken as part of a project to improve methods for control of the main pests of cotton in Peru.

B. thurberiella larvae and pupae were collected in Peru and Arizona, and reared through several generations on cotton plants in insectaries in the UK. Fifth instar larvae were sexed by the presence or absence of male gonads visible through the cuticle, and the sexes kept separately until emergence. Volatiles were collected from virgin female moths during the whole of the dark period on the first and second nights after emergence[3]. These were analysed by capillary gas chromatography (GC) linked directly to electroantennographic (EAG) recording[5] from a male *B. thurberiella* moth using non-polar and polar GC columns[6] temperature programmed to observe compounds eluting between octyl and eicosyl acetates covering the range of typical Lepidopterous sex pheromone components. Two EAG responses were observed at Kovat's Indices (KI) 1697 and 1797 on the non-polar column and KI 2100 and 2204 on the polar column, indicating the presence of two pheromone components (I) and (II) respectively. The second EAG response in each case coincided with a GC peak representing approximately 0.5 ng per female per night, but no significant peak could be reliably assigned to the first response.

Analysis by GC linked to low resolution mass spectrometry (MS)[7] in EI mode showed the peak at the retention time of the second EAG response (II) had a mass spectrum similar to those of unsaturated alcohols and aldehydes typical of Lepidopterous pheromones with the addition of an ion at m/z 46 (Fig. 1a). Single ion monitoring showed the presence of a compound with this unusual ion at the retention time of the first response (I) on both columns at approximately 2% of the major component. In these GC-MS analyses the presence of (Z)-9-tetradecenol at KI 1648 and 2220 on non-polar and polar columns respectively was confirmed by comparison of its GC retention times and mass spectrum with those of all the positional and geometric tetradecenol isomers[8]. (Z)-9-Tetradecenol was not observed when cold, on-column injection was used instead of splitless injection, suggesting it to be a thermal breakdown product of the pheromone component (II). Further evidence for this was provided by the *iso*butane CI mass spectrum of (II) which was identical to that of (Z)-9-tetradecenol with highest mass ion at m/z 213 (M + 1).

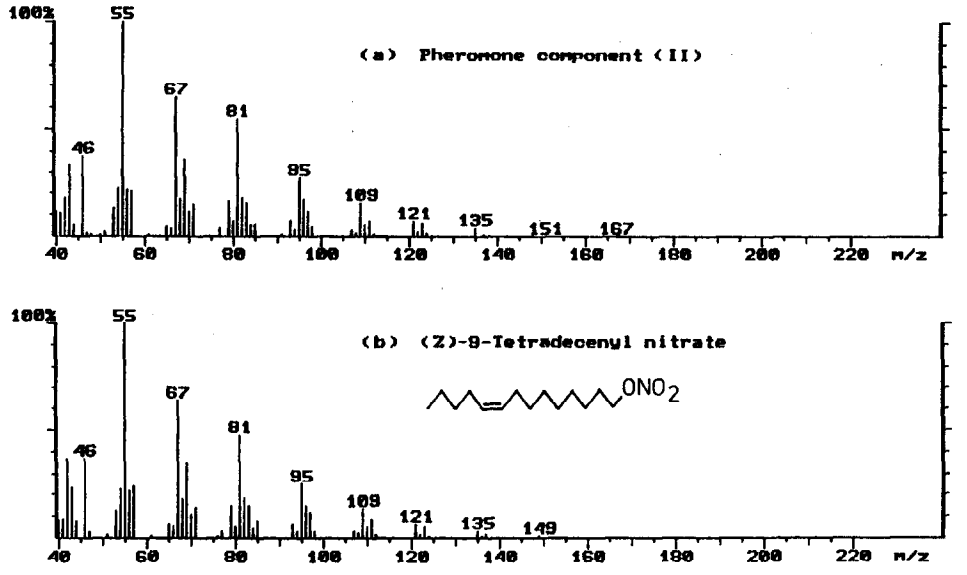

Fig 1. EI Mass Spectra of (a) Pheromone Component (II), and (b) (Z)-9-Tetradecenyl Nitrate

Table 1. GC Retention Data[13] for Pheromone Components and Synthetic Nitrate Esters

Compound	CP Sil 5 (KI)	CP Wax 57 (KI)	Compound	CP Sil 5 (KI)	CP Wax 57 (KI)
Component (I)	1692	2097	Component (II)	1797	2204
13:ONO_2	1711	2063	14:ONO_2	1817	2165
E7-13:ONO_2	1690	2087	E8-14:ONO_2	1794	2193
Z7-13:ONO_2	1686	2090	Z8-14:ONO_2	1791	2196
E8-13:ONO_2	1693	2092	E9-14:ONO_2	1799	2198
Z8-13:ONO_2	1692	2098	Z9-14:ONO_2	1797	2204
E9-13:ONO_2	1698	2095	E10-14:ONO_2	1803	2201
Z9-13:ONO_2	1701	2204	Z10-14:ONO_2	1805	2213
peak width at half height	1.5	1.6	peak width at half height	1.9	1.9

Analysis by GC linked to high resolution mass spectrometry[9] showed the EI mass spectrum of (II) to have an ion at m/z 45.9931 corresponding to NO_2 (m/z 45.9929), typical of nitrate esters[10].

Aliphatic nitrate esters were synthesised by reaction of the corresponding alcohols with a 1:1 mixture of nitric acid and acetic anhydride at 0°C followed by aqueous workup, flash chromatography to remove traces (< 5%) of the corresponding acetate and kugelrohr distillation. Conversion of saturated and acetylenic alcohols to the corresponding nitrate esters was virtually quantitative, but reaction of olefinic alcohols was extremely capricious, often giving a very poor yield and generally giving extensive geometric isomerisation of the double bond, presumably due to the presence of traces of nitrous acid. Although the geometric isomers could be separated by chromatography on a silver-loaded ion exchange resin[11], (Z)-8-tridecenyl nitrate and

(*Z*)-9-tetradecenyl nitrate were subsequently prepared in good and reliable yield by nitration of the corresponding acetylenic alcohols and hydrogenation of the resulting nitrate in petroleum spirit over Lindlar catalyst at 0°C. The products contained less than 2% of the *E* isomers by GC analysis.

These nitrate esters were characterised by IR and NMR spectroscopy[12] as well as MS, and showed significant (10-30%) decomposition to the corresponding alcohols during GC analysis with splitless injection. Consideration of the relative retention times of the saturated nitrate esters and the presence of (*Z*)-9-tetradecenol in analyses of the natural pheromone suggested the major pheromone component (II) to be (*Z*)-9-tetradecenyl nitrate (Z9-14:ONO_2). The GC retention times of this isomer as well as those of the *E* isomer and the *Z* and *E* isomers of 8- and 10-tetradecenyl nitrate were compared with those of the natural pheromone component (II) on non-polar and polar GC columns under conditions giving optimal separations of these isomers[13], and only the (*Z*)-9- isomer had the same retention times as the natural pheromone component on both phases (Table 1) and an identical mass spectrum (Fig. 1b). The *Z* and *E* isomers of 7-, 8- and 9-tridecenyl nitrate were also synthesised, and comparison of retention times showed that only the (*Z*)-8- isomer (Z8-13:ONO_2) had similar retention times to the natural pheromone component (I) on both phases (Table 1).

In order to confirm that these compounds were genuinely produced by the live insects and not produced by reaction on the trap adsorbent or by decomposition of dead insects, for example, nanogram quantities of synthetic unsaturated hydrocarbons, acetates, aldehydes and alcohols were entrained under identical conditions as above. No nitrates were obtained and a similar result was obtained on entraining volatiles from dead insects. Collections were also made from virgin females using Porapak Q (100 mg) as trap adsorbent, and the same nitrate esters were collected as with the activated charcoal filters.

Table 2. Catches of Male *B. thurberiella* Moths in Pheromone Traps[15]

Pheromone components (µg)			Detransformed mean catch/night	
Z9-14:ONO_2	Z8-13:ONO_2	Z9-13:ONO_2	polythene vial	rubber septum
1. Arizona, USA (4 replicates; 5 periods)				
1000*	0	0	25.8 b	26.9 b
1000*	20*	0	584.8 a	528.0 a
1000*	0	20*	21.5 b	20.4 b
unbaited			5.8 c	
2. Arizona, USA (6 replicates, 1 night)				
1000*	20*	0	865.1 a	
virgin female moth			39.1 b	
virgin male moth			2.1 c	
unbaited trap			22.3 b	
3. Piura, Peru (10 replicates, 30 nights)				
1000	20	0	9.1	
1000*	20*	0	12.6	

* compounds contain approx 50% of the *E* isomer

EAG responses to 0.1 ng of Z9-14:ONO_2, Z8-13:ONO_2 and Z9-13:ONO_2 were 0.30, 0.24 and 0.00 mV above blank respectively[14].

Initial field tests[15] were carried out in Arizona, USA. Z9-14:ONO_2 alone was weakly attractive to *B. thurberiella* male moths, and this attractiveness was greatly increased by addition of 2% of Z8-13:ONO_2. Addition of 2% of Z9-13:ONO_2 had no effect (Table 2). The mixture of Z9-14:ONO_2 with 2% of Z8-13:ONO_2 was significantly more attractive than a virgin female moth (Table 2). These tests were carried out with material prepared by direct nitration of the corresponding olefinic alcohol and contained approximately 50% of the corresponding *E* isomers. Experiments carried out in Peru confirmed that the blend of isomerically pure components (< 0.1% *E*) was not significantly different in attractiveness (Table 2).

The laboratory and field results indicate that Z9-14:ONO_2 and Z8-13:ONO_2 are components of the female sex pheromone of *B. thurberiella*. Such nitrate esters are extremely rare as natural products[16]. (*E*)-1-Nitro-1-pentadecene was reported as a termite defensive compound[17], but this is the first time such compounds have been found as sex pheromone components in Lepidoptera.

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References and Notes

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2. Rejesus, R.S. & Reynolds, H.T. *Journal of Economic Entomology* **1970,** *63*, 961-964.
3. Charcoal-filtered air (2 litre/min) was drawn over groups of ten virgin female moths in a silanised glass vessel (12 cm x 4 cm); volatiles were trapped on a microcharcoal filter (5 mg)[4], and were removed with dichloromethane (3 x 10 µl).
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6. Fused silica capillary GC columns (25 m x 0.32 mm i.d.) coated with non-polar CP Sil5 CB (methylsilicone) or polar CP Wax 57 CB (Carbowax equivalent); oven temperature 70°C for 2 min, then programmed at 20°C/min to 100°C then at 4°C/min to 230°C; helium carrier gas at 0.4 kg/cm^2; splitless injection at 200°C.
7. Finnigan ITD 700 in EI or CI (*iso*butane) mode; trap 220°C, open-split interface 200°C; GC columns as above.
8. Cork, A., Murlis J. & Meganasa, T. *J. Chemical Ecology* **1989**, *15*, 1349-1364; Horiike, M. & Hirano, C. *Agricultural & Biological Chemistry* **1982**, *46*, 2667-2672.
9. Finnigan MAT 90; fused silica capillary column (20m x 0.25 mm i.d.) coated with OV 1, oven temperature 90°C for 1 min then programmed at 15°C/min to 220°C, splitless injection.
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11. Houx, N.W.H., Voerman, S. & Jongen, W.M.F. *J. Chromatography* **1974**, *96*, 25-32.
12. Data for Z9-14:ONO_2: bp 96°C/0.005 mm; IR (film) 1630, 1277, 860 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) **δ** 0.90 (bt, J=7.5, 3H), 1.2-1.5 (m, 14H), 1.72 (dt, J=7.5, 2H), 1.9-2.1 (m, 4H), 4.45 (t, J=7.5, 2H, $\underline{CH_2}$-ONO_2), 5.3-5.45 (m, 2H); ^{13}C NMR (62.9 MHz, $CDCl_3$) **δ** 14.06, 22.43, 25.71, 26.85, 27.01, 27.23, 29.18 (2C), 29.34, 29.76, 32.05, 73.53 ($\underline{C}H_2$-ONO_2), 129.81, 130.08.
13. GC-MS: CP Sil 5 CB, oven temperature 70°C for 2 min, then at 20°C/min to 100°C, then at 1°C/min to 220°C; CP Wax 57 CB, oven temperature 70°C for 2 min then at 20°C/min to 120°C, then at 1°C/min to 220°C; retention data in Kovat's Indices (KI) relative to retention times of saturated hydrocarbons; peak width at half height indicates resolution.
14. Responses to 0.1 ng applied to inner wall of Pasteur pipette and exposed to EAG preparation in 3-sec pulse of nitrogen (500 ml/min); means of three replicates.
15. Field tests used sticky delta traps with polythene vials or rubber septa as pheromone dispensers; treatments were replicated as indicated; in expt. 1 treatments in each replicate were rerandomised on five occasions; mean catches per night in each replicate were transformed to log(x+1) for analysis of variance; means followed by different letters in one expt. are significantly different at the 5% level of probability.
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