that conditioning volleys in the contralateral aortic nerve delay and diminish facilitation of the testing response. The testing depressor response starts to increase at an interval of 32 msec and reaches its maximum of 188% at 128 msec. At that point the depressor response is rapidly decreased and starting from an interval of 1024 msec the response is diminished below the control value. At intervals between 16 and 128 msec differences between mean sizes of depressor responses conditioned by volleys in the same and in the contralateral aortic nerve are statistically significant.

In several preparations the strength of the conditioning pulses was decreased to excite only myelinated aortic afferents. These volleys applied to the same or contralateral aortic nerve (open and filled squares of fig. 2) did not affect the size of the testing response which did not deviate significantly from the control in either testing interval.

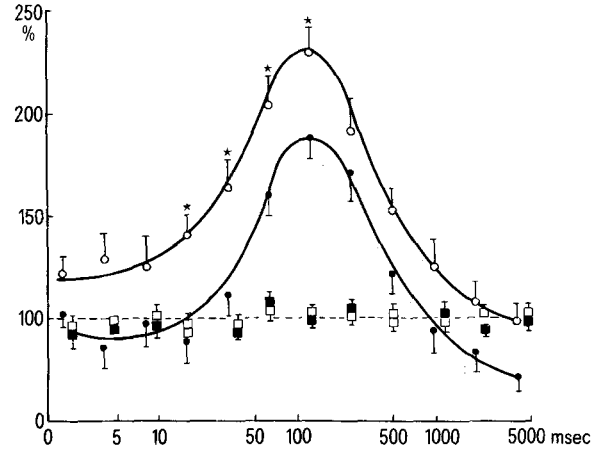

Figure 2. Changes in the size of depressor responses produced by conditioning volleys in the aortic nerves drawn using a semilogarithmic scale. The testing depressor responses were evoked by single volleys exciting C fibers in the aortic nerve. They were preceded by identical volleys in the same aortic nerve (○—○) and in contralateral aortic nerve (●—●). Note the lack of effects of conditioning volleys activating A fibers in the same (□—□) and in the contralateral aortic nerve (■—■). Abscissa, intervals between conditioning and testing volleys in msec. Ordinate, the size of the testing response expressed in percentages of the control response not preceded by conditioning volleys and taken as 100. The intervals between conditioning and testing volleys were in geometric series (2, 4, 8 ... 4096 msec). Different testing intervals were studied in random sequence. Each point represents the mean ± SEM of 12 experiments. *Significantly different ($p < 0.05$) from depressor responses when the conditioning volley was applied to the contralateral aortic nerve.

Recent studies have shown that there is synergistic interaction between blood pressure responses to repetitive stimulation of A and C fibers in the aortic nerve[3]. The lack of effect of conditioning volleys in A fibers indicates that facilitation of depressor responses seen in our experiments is solely related to activation of C fibers. We have obtained a smaller percentage of rabbits responding by depressor responses to volleys activating C fibers than has been reported by other authors[2]. This divergence, which is of a quantitative character, may be explained by different experimental methods. The time course of facilitation of depressor responses evoked by single conditioning volleys in the same aortic nerve is about 40 times shorter and in the contralateral nerve about 180 times shorter than when the interaction of responses produced by repetitive stimulations is studied[1]. It is, however, similar to the time course of changes in spinal polysynaptic responses conditioned by single volleys and of changes in the sympathetic reflex discharge preceded by single shocks in a somatic nerve[4-6]. This comparison gives an idea of the extent of excitation of the vasomotor mechanisms by single volleys. Taking into account the latencies of evoked potentials in the brain stem which were produced there by stimulation of the aortic nerve and the fact that bulbospinal pathway responsible for blood pressure falls is very slow[7-9], our data suggest that single volleys in C fibers excite relatively small number of neurones and thus evoke a very restricted activation of the vasomotor mechanisms. Evoked potentials recorded from the brain stem by stimulation of low-threshold baroreceptor afferents show stronger interaction with potentials produced by ipsilateral than by contralateral baroreceptor fibers[8]. The smaller facilitatory effect of volleys in the contralateral aortic nerve confirms these results and extends their validity to interactions between volleys in non-myelinated aortic afferents.

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Spruce budworm: Effects of different blends of sex pheromone components on disruption of male attraction

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Summary. Disruption of attraction of male *Choristoneura fumiferana* to the natural sex pheromone in an atmosphere permeated by blends of the 2 pheromone components is greatest with ratios of the 2 components close to the natural blend.

Evidence has recently been presented by Schmidt et al.[1] showing that the degree of disruption of male *Choristoneura fumiferana* (Clem.) in atmosphere permeated by blends of the 2 pheromone isomers, (E)- and (Z)-11-tetradecenal (TDAL), is independent of the (E):(Z) ratio. This is in contrast to earlier results with *Argyrotaenia velutinana*[2] and we have therefore carried out further experiments on disruption of *C. fumiferana* with different isomer ratios.

The experimental design was similar to that used by Schmidt et al.[1] and consisted of recording the number of male spruce budworm captured in a sticky trap baited with the sex pheromone, and comparing this catch with catches in other traps baited with sex pheromone which were surrounded by dispensers emitting various blends of the 2 pheromone components. The reduction in catch is then a measure of the disruption of male orientation. The objective was to determine if the degree of disruption was the same with all ratios of the (E)- and (Z)-11-TDAL or, if not, which ratio gave the greatest disruption.

The traps used were Pherocon 1CP or 1C in which the bottom half of the trap is lined with sticky material and the top is non-sticky, with the lure suspended from it. The pheromone blends were formulated in polyvinylchloride pellets (PVC)[3] which have been shown to emit the pheromone relatively constantly over periods of several weeks[4].

Two experiments were carried out. In each, 6 plots were laid out to accommodate the 5 blends of (E) and (Z) being tested and the check treatment in which PVC containing no -11-TDAL was deployed. The plots were at least 100 m apart to avoid interference between treatments. In each plot the central trap was baited either with 2 virgin females or with a PVC lure containing synthetic pheromone, a 96:4 blend of (E):(Z) TDAL (fulure)[5]. This central trap was surrounded by 6 pieces of PVC in which the pheromone blend being tested was incorporated. Each of these PVC dispensers was covered by the top half of a Pherocon 1C trap; since this contained no sticky material, any male insects attracted were free to come and go. These dispensers were spaced evenly around the central trap in a circle, with radii varying from 5 m to 20 m on different days.

In both experiments the procedure was as follows. On day 1 around mid-day, the test chemicals were deployed around the central traps at the specified distance. Checks were carried out at intervals during the afternoon and evening, to ensure that the sticky bottoms of the central traps were changed before they became saturated with males. At the end of the observations for the day, all traps and dispensers were removed and the plots were left without treatment for 1-2 days. This allowed male moths, which may have been attracted into a plot but not captured, to redistribute themselves throughout the stand. The experiment was then repeated, with a different sequence of both central traps and peripheral traps in each plot. In the 1st experiment (1979) 2 parallel tests were carried out, one in which the central traps were baited with 2 virgin females, the other in which they were baited with PVC pellets containing 3% fulure by weight. The 5 blends tested were 96%, 40%, 6.5%, 5.5%, and ca. 0.8% (Z)[6], and 1 plot contained PVC pellets with no -11-TDAL as a check. 3 different spacings of the test chemicals were assayed. In the 2nd experiment (1980) the 5 blends assayed were 100%, 35%, 14%, 7% and 1% (Z)[6]. No females were used in the central traps but 3 concentrations of fulure, 0.3, 0.03 and 0.0003% by weight, were used as lures.

Results are shown in figures 1 and 2. Since, in both experiments, the total number of moths captured varied from day to day, the trap catches in the treated plots for each day are given as a percentage of the catches in the check plots. It is evident that the degree of disruption does vary with the different ratios of the pheromone components, and is highest with the higher proportions of the (E)-isomer. This was confirmed by linear regression analysis of each day's data which gave F ratios significant at $p=0.001$ for the slope of the line in each case[7]. Since there were no significant differences attributable to the distance of dispensers from the central trap (fig. 1) or to concentrations of fulure in the central trap (fig. 2), the data were pooled for each of the 3 graphs. 2nd-order curves of the form $Y=a+b\ \ln(X+1)+c\ [\ln(X+1)]^2$ were fitted to each set of data (where Y = percent catch, and X = percent (Z)-isomer). In each case the χ^2 term was significant, implying that there is an upward inflection to the curve at the low percentages of the (Z)-isomer. Both these regressions and visual inspection of figures 1 and 2 suggest that maximum disruption occurs with (E:Z) blends close to the 96:4 ratio of the natural pheromone.

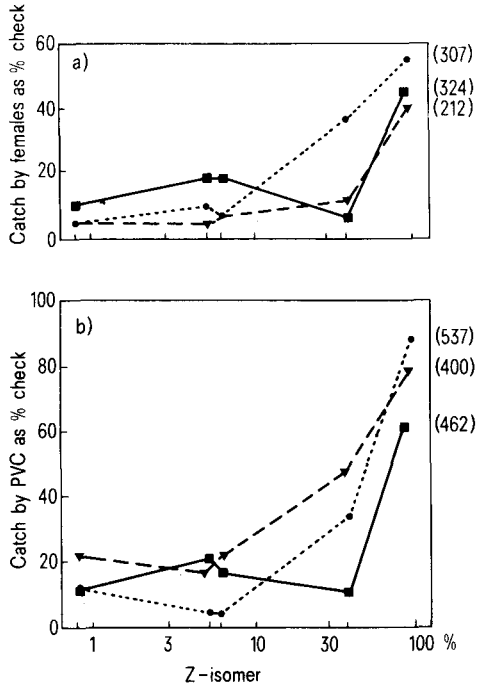

Figure 1. Catches of male spruce budworm moths in traps surrounded by dispensers emitting various blends of (E)- and (Z)-11-tetradecenal. Catches are expressed as percentages in check traps (surrounded by blank dispensers). *a* Centre trap baited with 2 virgin female moths, *b* with synthetic attractant. (——, dispensers spaced 5 m from center; – – –, 10 m; ..., 20 m; numbers in parentheses denote total catches in check traps.)

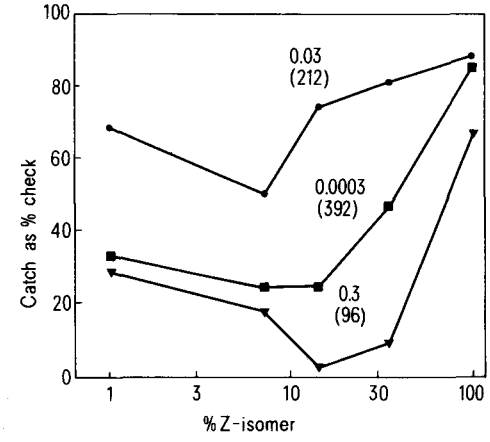

Figure 2. Catches of male spruce budworm moths in traps surrounded by dispensers 10 m away emitting various blends of (E)- and (Z)-11-tetradecenal. Catches are expressed as percentages in check traps (surrounded by blank dispensers). All center traps baited with synthetic attractant at 3 different concentrations in PVC by weight 0.3, 0.03, and 0.0003%. (Numbers in parentheses denote total catches in check traps.)

These results, which are at odds with those of Schmidt et al.[1], are of importance for developing the correct strategy for disrupting mating behavior. As Schmidt et al. point out[1], there would be distinct advantages from the technical and commercial viewpoints if there was wide latitude in the range of blends required to disrupt mating. The fact that this is not so implies that a precise blend (specifically a 96:4 ratio of (E:Z)-11-TDAL) should be used to disrupt mating to ensure maximum effectiveness.

It must be remembered, however, that disruption of any of the many steps in the mating procedure of the spruce budworm could result in mating failure. Orientation of males to females is only one such step; other steps may be affected differently by other blends.

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6 The (E)- and (Z)-TDALs were obtained from ChemSampCo, Columbus, Ohio, and were further purified by argentation column chromatography on Hi-Flosil-Ag (20% $AgNo_3$), 60/200 mesh, eluting with 9:1 pentane: ether. Following formulation, (E):(Z) ratios in the PVC were determined by extracting PVC pellets for 6 h in hexane and analyzing the solvent by GLC. The chemical analyses and purifications were carried out by Ms Linda MacDonald of the Forest Pest Management Institute, Sault Ste. Marie, Ont., to whom I would like to express my thanks.
7 Analyses of the data were done with the help of Dr J. Régnière of the Great Lakes Forest Research Centre, Sault Ste. Marie, Ont. whom I would also like to thank.

The secret of truffles: A steroidal pheromone?

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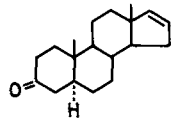
Summary. The steroid 5α-androst-16-en-3α-ol has a pronounced musk-like scent. It is a major constituent of the pheromone of the boar. It occurs also in axillary sweat of men but is devoid of androgenic activity. The presence of this steroid has been demonstrated in truffles *(Tuber melanosporum)* both by radioimmunoassay and by gas chromatography-mass spectrometry in quantities of 40-60 ng/g fresh material. This offers an explanation for the ability of pigs to detect truffles growing as deep as 1 m under ground.

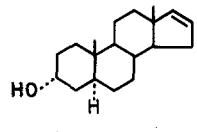
Some C_{19}-Δ16 steroids which have no androgenic activity exhibit a very peculiar smell[1-3] (fig.). The compounds are synthesized in the testes of the boar, are transfered to the salivary glands from which they are secreted during the premating behaviour. This scent, emanating from the saliva foam, is smelt by the sow and prompts her standing reflex. Thus Δ16-steroids, mainly 5α-androst-16-en-3α-ol are male sex pheromones in the pig[3,4]. Δ16-Steroids have also been detected in humans: 5α-androst-16-en-3-one and 5α-androst-16-en-3α-ol are synthesized by the testes and secreted with the axillary sweat in men[5-7]. 5α-Androst-16-en-3α-ol was found in the urine of women[8,9]. A possible pheromonal action of 5α-androst-16-en-3α-ol might be concluded from the studies of Kirk-Smith et al.[10]; judging the sexual attractiveness of photographs of normally dressed women the volunteers gave higher grades while smelling 5α-androst-16-en-3α-ol. The occurrence of Δ16-steroids is not confined to the animal kingdom. Celery and parsnip contain about 8 ng/g plant of 5α-androst-16-en-3-one[11]. Being aware of the ability of pigs to pinpoint the location of truffles (Tuber melanosporum), growing as deep as 1 m under ground, we extended our search for Δ16-steroids to this valuable fungus.

In a pilot study 1 g of canned truffles (from the Périgord, France) were homogenized in 3 ml of water and extracted with methylene chloride/ethylacetate (1/1). Aliquots of this crude extract were analyzed with our RIA system for 5α-androst-16-en-3-one, which cross-reacts with 5α-androst-16-en-3α-ol to 8%[12]. Further aliquots were subjected to TLC (silica gel, benzene/acetone = 85/15) in parallel to tritiated standards. After elution and subsequent radioimmunological measurement only 5α-androst-16-en-3α-ol but not 5α-androst-16-en-3-one was detected in a concentration (corrected for losses on TLC) which is in good agreement to the value of 25 ng/g measured in the crude extract (table).

For a 2nd study 4 g of fresh white truffles (Italian origin, purchased in a gourmet restaurant) and 4 g of black truffles from the Périgord (kindly provided by Mr Flourey) were



5α-androst-16-en-3-one (urine smell).



5α-androst-16-en-3α-ol (musk smell).

Odoriferous Δ16-steroids.

5α-androst-16-en-3α-ol in truffles (ng/g): comparative measurements by RIA and GC-MS

Sample	RIA*	GC-MS
Canned truffles	26.3	-
Fresh black truffles	59.0	42.1
Fresh white truffles	61.6	58.6

* Corrected for cross reactivity. All values corrected for procedural losses.