Ethylene production and cell wall formation in mesophyll protoplasts

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Summary. The regulation of ethylene synthesis, in relation to the presence of a new cell wall, has been investigated for *Nicotiana sylvestris* leaf protoplasts. It is clear that the production of ethylene is not controlled by the new wall, which has no action on the ethylene formation. The addition of dichlorobenzonitrile to cultivated protoplasts causes the inhibition of wall formation, without any other apparent deleterious effects.

The enzymes responsible for ethylene production have not yet been localized. It has been suggested that they are located in the plasmalemma[1] and that the wall could be essential for their activity[2]. In fact, it was observed that protoplasts freshly isolated from ethylene-producing tissues had lost this capability, but when cultivated they produced ethylene again, simultaneously with the new wall formation[1]. The loss of ethylene synthesis was also shown to depend, at least in part, on the toxicity of the hydrolytic wall-digesting enzymes used to obtain protoplasts[3]. Consequently, it was difficult to draw conclusions about whether the inability of protoplasts to produce ethylene was due to the activity of hydrolytic enzymes or to the absence of the cell wall.

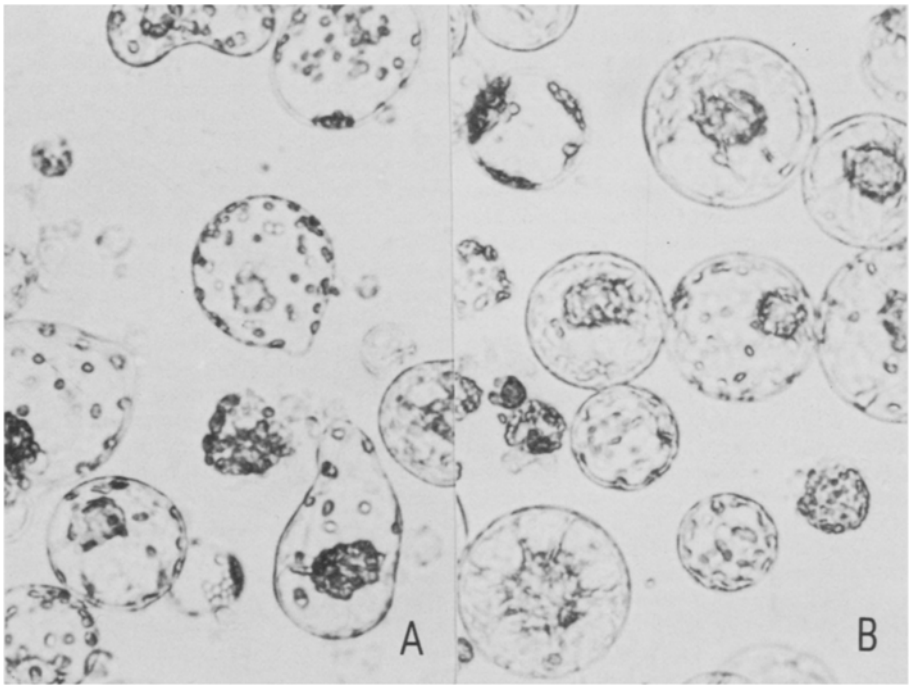
Protoplasts, analyzed in the present study, were isolated from *Nicotiana sylvestris* mesophyll cells and cultivated. A part of these protoplasts were allowed to deposit a new cell wall while a second part was not. The ability of each protoplast population to produce ethylene was compared and discussed.

The conditions for growth of the *N. sylvestris* plants and the procedure for protoplast isolation from mesophyll cells have been previously described[4], and so has the ethylene analysis[5]. Fresh protoplasts were washed 3 times in 6% aqueous sorbitol, to remove hydrolytic enzymes and then collected and cultivated (dark, 23 °C) in small vials (15 ml) with 10^5 protoplasts per vial. Protoplast control medium was that of Murashige and Skoog[6] × 1.5 with 3.2 g/l sorbitol, 1.35 g/l $CaCl_2 2H_2O$ and 0.2 mg/l 2,4-D, 0.6 mg/l NAA, 0.8 mg/l KIN (initial pH 5.6). 6 mg/l dichlorobenzonitrile (DCB) were added to this medium to inhibit wall synthesis. The vials were sealed with rubber septa (neoprene) previously sterilized in Na hypochloride (hot sterilisation caused the septa to release ethylene). Every 24 h a 1-ml samples of the vial atmosphere was withdrawn and replaced by 1 ml sterile air. Ethylene was determined by gas chromatography and the presence of the wall was detected using calcofluor white fluorescence[7].

Deformations from an initial spherical form were visible in many *N. sylvestris* protoplasts after a 24-h incubation. These changes have to be related to the presence of new wall material, detected by fluorescence. Fibrils were also observed after 24 h by scanning microscopy (unpublished data). With the increasing time of culture the deformations

Table 1. Ethylene production by mesophyll protoplasts cultivated in media with or without dichlorobenzonitrile (DCB)

Duration of culture (h)	Ethylene production (ppb per 10^5 protoplasts, ± SD)	
	Without DCB (control)	With DCB
24	423 ± 39	430 ± 24
48	747 ± 27	809 ± 34
72	1116 ± 54	1077 ± 36
144	1716 ± 65	1516 ± 216



Mesophyll protoplasts cultivated (6 days) in a control medium (A) and in a medium containing DCB (B). × 440.

and the fluorescence increased. In contrast, DCB-treated protoplasts remained spherical and no fluorescence could be noted. But except for their form, they apparently behaved like non-treated protoplasts and after 5–7 days of culture, divisions occurred (fig., A and B).

Similar observations concerning DCB effects on protoplasts have previously been reported, the absence of the wall being proved by electron microscopy[8]. As for ethylene production, no differences were obtained between control and DCB-treated protoplasts, i.e. between protoplasts with or without a new wall. Ethylene formation was quite linear during the first 3 days, then decreased until the 6 th day (table 1). DCB itself did not interfere with ethylene metabolism, as could be concluded from the data reported in table 2, for which tissues were tested instead of protoplasts.

In conclusion, ethylene production by protoplasts seems not to be linked to the synthesis of a new wall, which cannot regulate ethylene production at least for protoplasts.

Table 2. Ethylene production by mesophyll tissues cultivated in media with or without dichlorobenzonitrile (DCB)

Duration of culture (h)	Ethylene production (ppb × 10^3 per g of fresh weigt, ± SD) Without DBC (control)	With DBC
24	3.5±0.4	3.7±0.4
48	9.3±1.1	8.7±1.3
72	17.7±2.9	17.9±3.0

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Trace co-attractants in synthetic sex lures for 22 noctuid moths

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Summary. Trace components contributed significantly to the potency of synthetic sex attractant lures for males of many species of Noctuidae. Improved synthetic blends for 12 moths including *Euxoa ochrogaster* and *Trichoplusia ni*, and new lure blends for 10 moths are described. In every case the trace constituents were structural analogs of the main lure components.

Sex attractants for lepidoptera are recognized as blends rather than single biologically active chemicals, noctuid moth pheromones affording many examples of multi-component systems[1]. Lure blends which involve trace components were demonstrated in the sex pheromones of the tobacco budworm *Heliothis virescens* and the corn earworm *H. zea*[2]. More recently, trace co-attractants have been reported in sex pheromones of the armyworm *Pseudaletia unipuncta*[3,4] and the red-backed cutworm *Euxoa ochrogaster*[5–7]. We now report new or improved synthetic sex attractant blends, containing trace co-attractants, for males of 22 noctuid species.

Materials and methods. High-purity (> 99%) chemicals were used, synthesized by recognized methods. Assays by capillary gas chromatography[7] showed that, to the detection limit of 0.01%, each compound was free of geometrical isomers, positional isomers, homologs and analog impurities. Replicated tests were carried out using double-cone traps[8] which required moths to land prior to trap entry and thus provided some measurement of attraction at close range in the later stages of the male response sequence[9]. Red rubber septa (5×9 mm) were employed as blend dispensers, lure materials being impregnated into the septa via dilute solutions in hexane.

Trace co-attractants were mostly demonstrated by 2-stage field screening experiments. In the 1st stage a synthetic compound or blend known to lure target species to traps was admixed with 10% of each of about 30 analogs including *(Z)*-5-decenyl acetate and the primary alcohols, acetates and aldehydes based on the alkenyl groups *(Z)*-5-dodecenyl, *(Z)*-7-dodecenyl, *(Z)*-9-dodecenyl, *(Z)*-5-tetradecenyl, *(Z)*-7-tetradecenyl, *(Z)*-9-tetradecenyl, *(Z)*-11-tetradecenyl, *(Z)*-7-hexadecenyl, *(Z)*-9-hexadecenyl and *(Z)*-11-hexadecenyl. This array constitutes the main range of biologically active monoolefins known in noctuid sex attractants[10,11]. Those analogs which eliminated or greatly suppressed trapping were re-tested at 1% and 0.1% levels of admixture in the 2nd stage of the screening. The rationale for initial testing at 10% levels was limitation of the number of chemicals for subsequent tests to those which inhibited trapping when present in substantial amounts in the lure. Such compounds, inhibitory at 10%, might be true trapping inhibitors which suppress trapping at all levels of addition to a lure, or might be trace co-attractants producing trapping suppression only at super-optimal levels[12,13]. In this way many candidate trace co-attractants could be identified, inhibitory effects at 10% being easily observed among a large number of non-inhibiting treatments. In subsequent trapping tests where the inhibitors were added in trace amounts (0.1–1%) to the lures, those associated with trapping significantly above controls were considered to be trace co-attractants and were then subjected to further replicated trapping tests to determine the most effective blend ratios. Trace effects in a few species were discovered by chance during examination of other target moths. In every instance ancillary dose/capture experiments were performed to ensure that blend ratios were optimized at near-optimal blend release rates. Species having optimal blend ratios smaller than 100:1 were not considered to qualify as 'trace' systems and are not included in the tables. Standard abbreviations for attractant chemicals are used in the tables and below: thus Z7-12:Ac = *(Z)*-7-dodecenyl acetate, Z9-14:Ald = *(Z)*-9-tetradecenal, Z11-16:OH = *(Z)*-11-hexadecenol, and so forth.

Results and discussion. Trace co-attractant systems were defined and field proven for 22 noctuids representing 5 sub-families, showing that such systems are general and widespread in the Noctuidae. Typically, inclusion of the