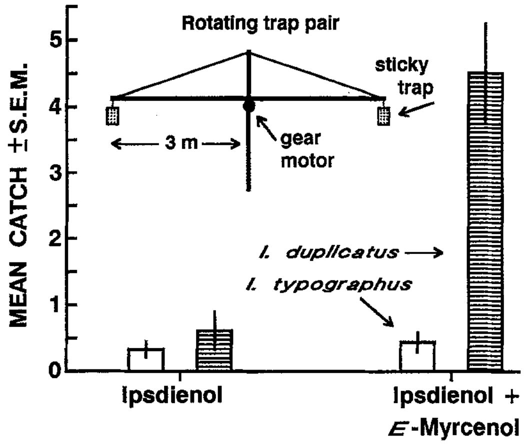

Figure 2. Catch of *Ips duplicatus* and *I. typographus* on a pair of slowly rotating sticky traps (30 cm × 30 cm diameter) baited with racemic ipsdienol and ipsdienol plus *E*-myrcenol (n = 7, Värmland, Sweden, 23 May – 16 June 1989; n = 11, Ås, Norway, 6–13 June 1990). Release rates for ipsdienol and *E*-myrcenol were each about 100–200 ng/min (equivalent to natural rates from at least 50 males). The sex ratio of *I. duplicatus* caught on the most attractive bait was 1.5 females per male (1.0–2.4, 95% binomial confidence interval).

insects with respect to trap position, will allow more reliable discrimination among behaviours elicited by semiochemical blends than current methods permit.

1 Wood, D. L., A. Rev. Ent. *27* (1982) 411.
2 Byers, J. A., Experientia *45* (1989) 271.
3 Silverstein, R. M., Rodin, J. O., and Wood, D. L., Science *154* (1966) 509.
4 Vité, J. P., Bakke, A., and Renwick, J. A. A., Can Ent. *104* (1972) 1967.
5 Bakke, A., Frøyen, P., and Skattebøl, L., Naturwissenschaften *64* (1977) 98.
6 Bakke, A., Norw. J. Ent. *22* (1975) 67.
7 Schlyter, F., Birgersson, G., Byers, J. A., Löfqvist, J., and Bergström, G., J. chem. Ecol. *13* (1987) 701.
8 Lanne, B. S., Schlyter, F., Byers, J. A., Löfqvist, J., Leufvén, A., Bergström, G., Van Der Pers, J. N. C., Unelius, R., Baeckström, P., and Norin, T., J. chem. Ecol. *13* (1987) 1045.
9 Birgersson, G., Schlyter, F., Bergström, G., and Löfqvist, J., J. chem. Ecol. *10* (1984) 1029.
10 Büchi, G., and Wüest, H., Helv. chim. Acta *50* (1967) 2440.
11 Payne, T. L., Coster, J. E., Richerson, J. V., Hart, E. R., Hedden, R. L., and Edson, L. J., J. Georgia ent Soc. *13* (1978) 85.
12 Hopping, G. R., Can. Ent. *97* (1963) 422.
13 Francke, W., Bartels, J., Schmutzenhofer, H., Kohnle, U., and Vité, J. P., Z. Naturforsch. *43* (1988) 958.
14 Gries, G., Pierce, H. D. Jr, Lindgren, B. S., and Borden, J. H., J. econ. Ent. *81* (1988) 1715.
15 Miller, D. R., Gries, G., and Borden, J. H., Can. Ent. *122* (1990) 401.

0014-4754/90/11-12/1209-03$1.50 + 0.20/0

Fluorine-substituted pheromone components affect the behavior of the grape berry moth

M. Bengtsson[a,*], St. Rauscher[a], H. Arn[a,+], W.-C. Sun[b] and G. D. Prestwich[b,+]

[a]Swiss Federal Research Station, CH-8820 Wädenswil (Switzerland), and [b]Department of Chemistry, State University of New York, Stony Brook (New York 11794-3400, USA)
Received 14 May 1990; accepted 26 June 1990

Summary. Partially fluorinated analogs of (*Z*)-9-dodecenyl acetate, the major pheromone component of the grape berry moth *Eupoecilia ambiguella*, produced a variety of different behaviors in wind tunnel and field trapping experiments. A difluoro analog was a potent attractant, the trifluoromethyl analog had little attractancy, and the pentafluoroethyl analog was a potent synergist for the natural pheromone.
Key words. Pheromone analog; moth behavior; *Eupoecilia ambiguella*; grape berry moth; hydrophobic sites; receptor binding; fluorination.

The tortricid moth *Eupoecilia ambiguella* (Lepidoptera, Tortricidae) is a major insect pest of vineyards in Europe[2]. Recent studies have focused on identification of the complete sex pheromone produced by the female moth as well as optimization of the blend used in field trapping experiments, including the synergism of trap catches by dodecyl acetate and octadecyl acetate[3]. This pheromone trapping system allows examination of the effects of altering the hydrophobicity of the *n*-alkyl terminus of a pheromone component on moth behavior. Replacement of hydrogen atoms by fluorine atoms in biological molecules causes only a small steric perturbation but leads to major changes in hydrophobicity, flexibility, and polarity of the hydrocarbon chain[4,5]. In this study, we systematically replaced the two methylene hydrogens, the three methyl hydrogens, or all five ethyl hydrogens by fluorine atoms. Unexpectedly varied behavioral responses to three fluorinated analogs of (*Z*)-9-dodecenyl acetate (*Z*9-12:Ac) (**1**), the major component of the *E. ambiguella* pheromone blend[6], were observed. First, the 11,11-difluoro analog (**4**) was equipotent to *Z*9-12:Ac. Second, the 11,11,12,12,12-pentafluoroethyl analog (**2**) was essentially inactive alone but synergized trap catches when tested with *Z*9-12:Ac. Finally, the

12,12,12-trifluoromethyl analog (**3**) was inactive alone as a Z9-12:Ac mimic, inhibited trap catches of *E. ambiguella* when mixed with Z9-12:Ac, and attracted males of several moth species which normally respond to a Z9-14:Ac-containing sex pheromone.

Fluorinated pheromone analogs **2-4** (fig. 1) were synthesized and purified to $>99\%$ chemical and geometric purity[7]. Headspace analysis by capillary gas chromatography (CGC) and CGC retention times indicated that the volatilities and polarities of the fluorinated materials were different from those of **1**. The difluoro analog **4** was less volatile than the parent compound, while the trifluoromethyl analog **3** and the pentafluoroethyl analog **2** were more volatile. The CGC retention times on polar OV-240-OH column were: **1**, 20 min; **2**, 20.2 min; **3**, 30.6 min; **4**, 31.4 min; CGC on nonpolar SE-54 column: **1**, 19.9 min; **2**, 17.2 min; **3**, 18.9 min; **4**, 20.3 min. The headspace analysis on filter paper containing equal amounts of the four components showed a ratio in the vapor of **1**:**2**:**3**:**4** = 1:7:1.4:0.5.

Initially, the analogs were tested with *E. ambiguella* males at doses of 0.010–20 µg on filter paper in a wind tunnel and compared with Z9–12:Ac (**1**), or as an additional control, with calling female moths. As indicated in figure 2, the 11,11-difluoro-Z9–12:Ac (**4**) was as effective as virgin females and often more effective than Z9–12:Ac in eliciting flight activation, take-off, upwind flight, and landing. In addition, the apparent dosage range for maximal response was higher by an order or magnitude, possibly as a result of its lower vapor pressure. It appeared that sensory adaptation was not occurring with the difluoro analog **4** even at very high vapor concentrations. In contrast, the 11,11,12,12,12-pentafluoro-Z9–12:Ac (**2**) was weakly active, evoking activation and take-off, but with surprisingly low net attraction to the source. Moreover, males took significantly longer to respond to the pentafluoroethyl analog **2**. The mean times to take-off were: 36 ± 27.7 s for pentafluoroethyl analog **2**, 22.7 ± 17.2 s for Z9-12:Ac (**1**), and 24.2 ± 19.9 s for 11,11-difluoro-Z9–12:Ac (**4**). The value for the pentafluoroethyl analog is significantly different ($p = 0.05$) from the other two values as indicated by Kruskal-Wallis test followed by Scheffe's F-test. Finally, the 12,12,12-trifluoro-Z9–12:Ac (**3**) showed very weak activation and no moths completed flights in response to this material.

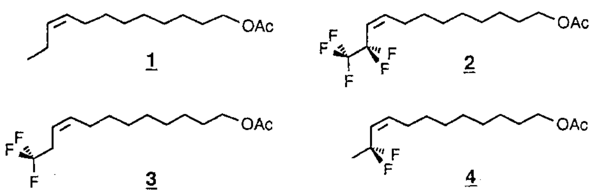

Figure 1. Structures of (Z)-9-dodecenyl acetate and three fluorinated analogs.

Field trapping was conducted with Z9–12:Ac and with the three fluorinated analogs in a vineyard in Maienfeld, Switzerland while moths were actively flying in May and June 1989. Five dosages ranging from 5 to 2000 µg were applied to rubber septa placed in Tetra traps[3], with 10 replicates per trap spaced 2 m apart and 20 m between replicate groups. Trap catches after 4 weeks are illustrated in figure 3.

Consistent with the wind tunnel results, the 11,11-difluoro-Z9–12:Ac showed attractancy indistinguishable from the non-fluorinated compound. At high doses, males of a second tortricid (*Cnephasia incertana* Tr.) were also trapped in significant numbers. Mimicry was not always observed; this difluoro analog could not substitute for Z9–12:Ac in a 1:1 blend of Z9–12:Ac and E9–12-Ac as a field attractant for another tortricid (*Enarmonia formosana*) which was active locally during the field trials (data not shown).

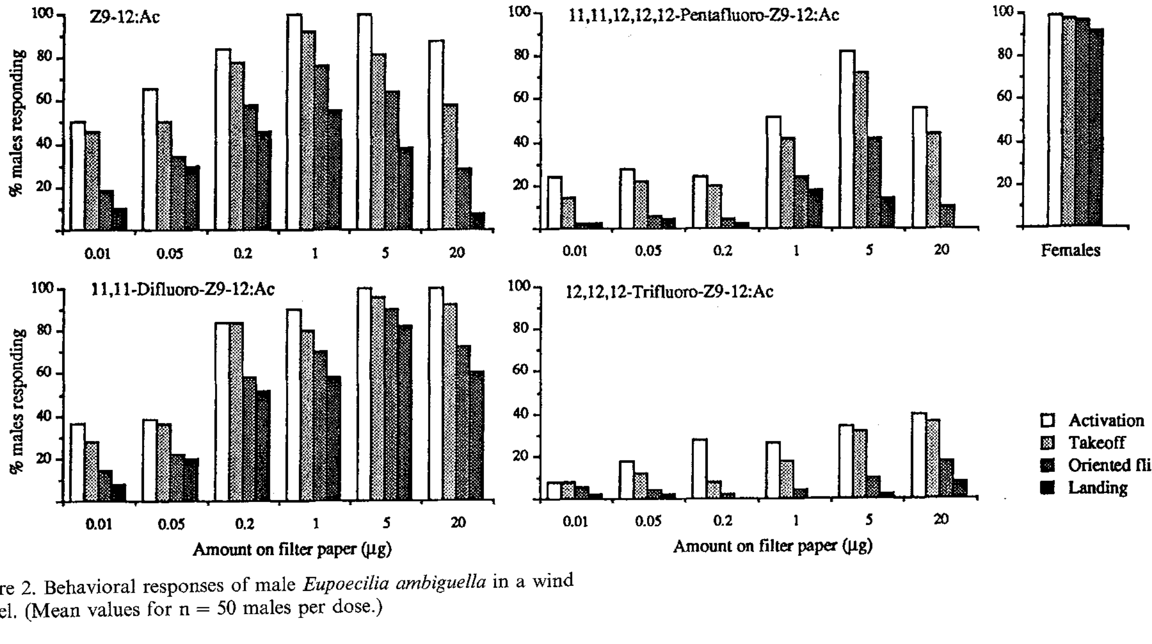

Figure 2. Behavioral responses of male *Eupoecilia ambiguella* in a wind tunnel. (Mean values for n = 50 males per dose.)

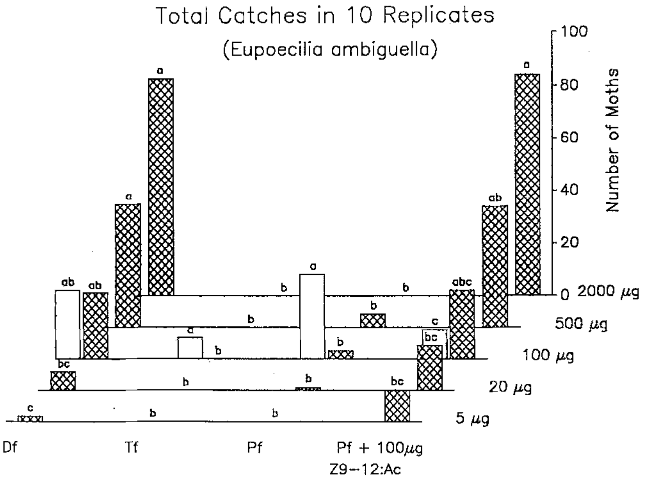

Figure 3. Field trapping of *Eupoecilia ambiguella* with fluorinated analogs of Z9–12:Ac. Key: Df, difluoro analog **4**; Tf, trifluoromethyl analog **3**; Pf, pentafluoroethyl analog **2**. Values (hatched bars) are for total catch in ten replicates; different letters indicate values significantly different at the 95% confidence level (Tukey-Kramer method). For comparison, a check dose (ten replicate traps) of 100 µg Z9–12:Ac (open bars) was run with each dose-response series. The total catches and significance levels for these check traps are: Df series, 26ab; Tf series, 8a; Pf series, 32a; Pf + Z9–12:Ac series, 11c.

The pentafluoroethyl analog **2**, as predicted from wind tunnel results, showed no attraction in the field for any of the tortricid species. Similarly, the trifluoromethyl analog **3** failed to attract the tortricid males. To our surprise, however, significant catches of two noctuids (*Axylia putris*[8] and *Cucullia lucifuga*[9]) occurred with the trifluoromethyl analog **3**. These noctuids normally respond to Z9–14:Ac or blends containing this compound. In contrast, the trifluoromethyl analog **3** failed to substitute for Z9–14:Ac in trapping *Adoxophyes orana*, which responds to a 1:9 ratio of Z11-14:Ac and Z9–14-Ac (data not shown).

Equally unexpected was the observation that the pentafluoroethyl analog **2** synergistically increased trap catches of *E. ambiguella* and of one or more *Cnephasia species* (Tortricidae). Perhaps this can be explained by the increased pre-flight activity caused by the pentafluoroethyl analog **2**; with more moths 'activated' in the field, a greater number were able to follow the Z9–12:Ac signal to its source. The inactive trifluoromethyl analog **3** did not show this synergistic activity; indeed, at the 2000 µg level, it significantly inhibited trap catches of *E. ambiguella*.

The introduction of multiple fluorine atoms into the hydrophobic chain of a pheromone thus appears to have multiple and complex effects on moth behavior. The simplistic notion that fluorine acts as a hydrogen isostere to disrupt hydrophobic interactions[10] does not fully account for the variety of effects observed. Fluorination at the allylic position seems to enhance or at least preserve pheromonal activity in selected species. For example, single cells respond to a difluoromethylene analog of the pheromone of the turnip moth *Agrotis segetum*, 7,7-difluoro-Z5–10:Ac (Sun, W.-C., Prestwich, G. D., Liljefors, T., Bengtsson, M. and Hansson, B. S., unpublished results). Recently, electrophysiological and flight responses of the oriental fruit moth *Grapholita molesta* to 7,7-difluoro-Z8–12:Ac were reported[11].

Fluorination of the terminal methyl group destroys activity, clearly demonstrating the importance of this hydrophobic interaction in pheromone-receptor interactions[12]. The complete replacement of the alkyl chain by a perfluoroalkyl chain results in a pheromonally-active material with a higher vapor pressure and apparently lower receptor affinity[5]. However, judicious replacement of allylic methylene hydrogens with fluorine gives highly attractive analogs[7,11]. Fluorinated pheromone analogs can provide a new window into the molecular basis of pheromone action[13,14].

* Present address: Organic Chemistry 3, The Chemical Center, University of Lund, P.O. Box 124, S-221 00 Lund, Sweden.
\+ Authors for correspondence.

1 G. D. P. and W.-C. S. thank the Herman Frasch Foundation for financial support and Dr I. Ujváry for helpful discussions. We are grateful to Professor W. Sauter (Swiss Federal Institute of Technology, Zürich), for moth identifications.

2 Bovey, P., in: Entomologie Appliquée à l'Agriculture, 2/1, p. 456. Ed. A. S. Balachovsky. Masson et Cie., Paris 1966.
3 Arn, H., Rauscher, S., Buser, H.-R., and Guerin, P. M., J. chem. Ecol. *12* (1986) 1417.
4 Prestwich, G. D., Pestic. Sci. *37* (1986) 430.
5 Prestwich, G. D., Sun, W.-C., Mayer, M. S., and Dickens, J. C., J. chem. Ecol. *16* (1990) 1761.
6 Arn, H., Rauscher, H. S., Buser, H.-R., and Roelofs, W. L., Z. Naturforsch. *31c* (1976) 499.
7 Sun, W.-C., and Prestwich, G. D., Tetrahedron Lett. *32* (1990) 801.
8 Szöcs, G., Toth, M., and Novak, L., Z. ang. Ent. *91* (1981) 272.
9 Frérot, B., Chambon, J. P., and Cocquempot, C., Ann. Soc. Ent. fr. *23* (1987) 151.
10 Turberg, M. P., and Brady, J. E., J. Am. chem. Soc. *110* (1988) 7797.
11 Masnyk, M., Fried, J., and Roelofs, W. L., Tetrahedron Lett. *30* (1989) 3243.
12 Liljefors, T., Bengtsson, M., and Hansson, B. S., J. chem. Ecol. *13* (1987) 2023.
13 Vogt, R. G., in: Pheromone Biochemistry, p. 385. Eds G. D. Prestwich and G. J. Blomquist. Academic Press, New York 1987.
14 Prestwich, G. D., in: Advances in the Chemistry of Insect Control, p. 235. Ed. L. Crombie. Royal Society of Chemistry, Cambridge, England 1990.

0014-4754/90/11-12/1211-03$1.50 + 0.20/0