Attractive plant volatiles as a control method against apple fruit moth (*Argyresthia conjugella* Zell.)?

Title German

Gunnhild Jaastad[1], Geir K. Knudsen[2], Sverre Kobro[2], Anna-Carin Bäckman[3], Peter Witzgall[3] and Marie Bengtsson[3]

Abstract

Apple fruit moth, *Argyresthia conjugella* Zell. (Lepidoptera: Argyresthiidae), is the most important pest of apples in Scandinavia. In years when its primary host, rowan (*Sorbus aucuparia* L.), has little or no berries for egglaying, female *A. conjugella* fly into apple orchards to lay their eggs. In some years the entire apple crop can be destroyed. Volatiles from apples and rowan have been collected and identified. In GC-EAD tests females have responded to several compounds found in both rowan and apple. Some of these compounds were used in field trapping tests during 2002, and a mixture of two compounds trapped significantly more females and males compared to control traps. However, field trapping results from 2003 indicate that the two-compound blend seem to trap insects too late in the season to prevent egglaying in apples. Several new compounds were also tested in 2003, and some of these gave promising results. The results will be discussed in relation to use attractive plant volatiles as a control method against *A. conjugella* females.

Keywords: apple fruit moth, Argyresthia conjugella, plant volatiles, egglaying

Introduction:

Apple fruit moth (*Argyresthia conjugella* Zell.) is a serious pest of apple in Fennoscandia (Ahlberg, 1927). Flowering and fruitsetting of rowan is highly variable (Kobro et al., 2003) and in years when its primary host, rowan (*Sorbus aucuparia* L.) has little or no berries, the apple fruit moth invades apple orchards and the female moth oviposit on apples (Ahlberg, 1927; Schøyen, 1913). The egg hatches and the larvae bore into the developing fruit. The entire apple crop can be destroyed.

There is growing evidence that host-finding in moths is largly guided by secondary plant metabolites (Dethier, 1982; Schoonhoven et al., 1998). Gravid females make the critical host choice prior to and during oviposition, since newly hatched larvae cannot migrate over long distances.

Apple and rowan belong to the same subfamily Pomoidea (Rosaceae), and related plants resemble each other also with respect to secondary metobolism (Berenbaum and Seigeler, 1992). The objective of this study was to (1) compare volatiles emitted from apples and rowan, (2) investigate the composition of volatile compounds emitted from these two host plants and the response on *A. conjugella* females, (3) investigate the attraction of females to different volatile compounds in field trapping tests and (4) investigate the possibility of using attractive plant volatiles as a control method against *A. conjugella*.

Material and Methods:

Insects

[1] The Norwegian Crop Research Institute, Ullensvang Research Centre, N-5781 Lofthus, Norway, [2] The Norwegian Crop Research Institute, Plant protection Centre, Høgskolevn. 7, N-1432 Ås, Norway, [3] Departement of Crop Science, Swedish University of Agricultural Sciences, S-230 53 Alnarp, Sweden

Last-instar larvae of *A. conjugella* were collected from rowan berries in Southern Norway, and were hibernated outdoor. When adults emerged from mid-June to mid-July, they were sexed daily and kept in Plexiglass cages until the trials.

Collection of volatiles

Volatiles were collected from rowan and apples at the optimum phenological stage (after egglaying were expected to start (Kobro, 1988)) by putting freshly cut branches into a 2000-ml Erlenmeyer jar in the laboratory. Charcoal-filtered air was pulled through the jar at 150 ml/min, and over a Porapak Q (75 mg, 80/100 mesh) (Alltech, Deerfield, IL) for 6 to 12 hr. Filters were extracted with 300 µl of redestilled hexane. Glassware was heated to 350℃ during 10 hr before use. After sample collection, the sample volumes were reduced under a stream of nitrogen to a volume of 20 µl. Samples were sealed in glass capillary tubes and stored at -19℃.

Identification of volatiles

Chemical identification of compounds in filter extracts was done on a Hewlett Packard 5970 B GC-MS system, with electron impact ionization (70 eV), interfaced with a Hewlett Packard 5890 GC. Helium was used as carrier gas, on a 30 m x 0.25 mm DB-Wax column (J&W Scientific, Folsom, CA, USA), programmed from 40℃ (hold 2 min) at 8℃/ min to 230℃ (hold 5 min). Compounds were identified by comparison of mass spectra earlier reported from Rosaceae, or available in the computer library. Some identifications were confirmed by comparison of GC retention times and spectra with those of known synthetic and purified authentic reference compounds. Detection level was *ca*. 0.5 ng.

Response of *A. conjugella* to volatiles

Field collections were also analysed by GC coupled to electroantennographic detection (GC-EAD; Arn et al., 1975), using a Hewlett Packard 6890 GC with a HP-INNOWax column (30 m x 0.25 mm ID), programmed from 40℃ (hold 2 min) at 7℃/min to 230℃, and at 10°/min to 240℃ (hold 5 min). EAD recordings were done with excised apple fruit moth male (n = 5) and female antennae (n = 7) suspended between two saline-filled wells. Antennal signals were amplified and recorded using GC-EAD software (Syntech, Hilversum, The Netherlands). One arm of the split column led into a glass tube (Ø 8 mm), with a charcoal-filtered and humidified air stream (0.5 l/min), the other to the flame ionization detector (FID). Split ratio between FID and EAD was approximately 1:1. Apple fruit moth antennae were 0.5 cm from the end of this glass tube, 30 cm from the EAD-outlet of the GC.

Attraction of *A. conjugella* to volatiles in field trapping tests

Compounds that elicited a reaction in the GC-EAD test was tried out in several field trapping tests in 2002 and 2003. Compounds were applied on red rubber septa and mounted in tetra traps either singly or in combination with several other compounds. Traps were placed in rowan, each combination and replicate was tested for at least one week. The combination that trapped most females in 2002 was tried out as a control method in 4 organic orchards in 2003. Tetra traps with a two-compound blend on red rubber septa was placed along the border of each orchard. Four orchards about 100 m apart from each of the organic orchards (experimental) was used as control orchards. In addition, the same combination of plant volatile compounds was placed in a gradient from forest to apples during the entire season 2003.

Results:

Several volatile compounds emitted from rowan and apples were found to be identical, and many of these compounds elicited a reaction in female antennae (Figure 1).

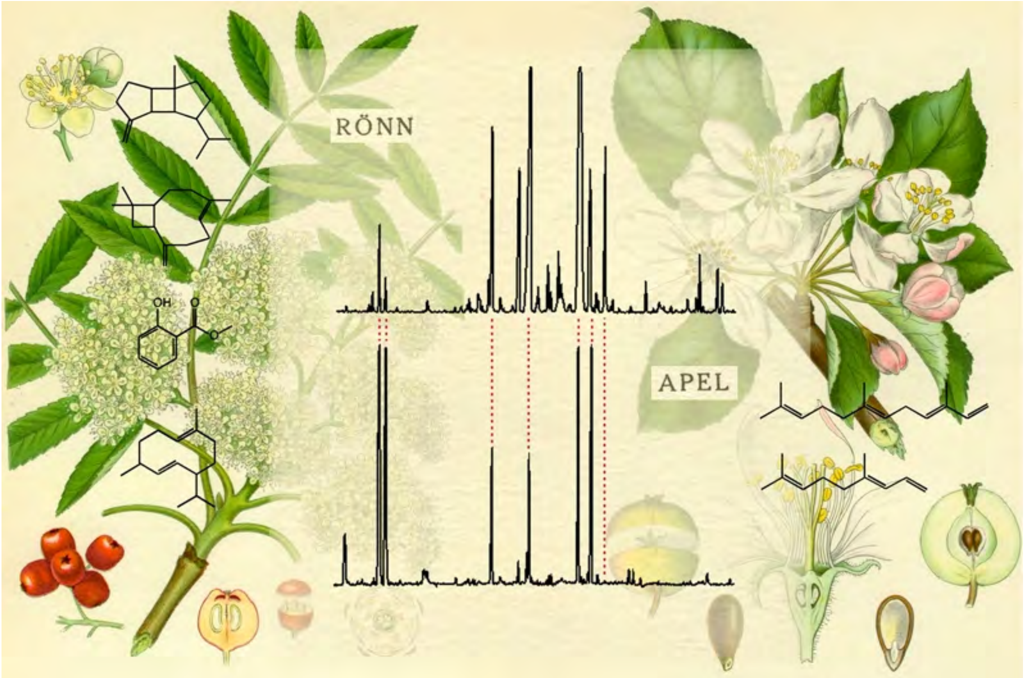

Figure 1. Preliminary results from GC-MS and GC-EAD experiments with volatiles emitted from apples and rowan. Dotted line indicates female response to the compound in GC-EAD.

In 2002 several of the compounds that elicited a reaction in female antennae in the GC-EAD were tested in field trapping tests, from which results from one test is shown here (Figure 2). The other compounds tested did not catch as many females or males as 2-phenyl ethanol and anethol in combination.

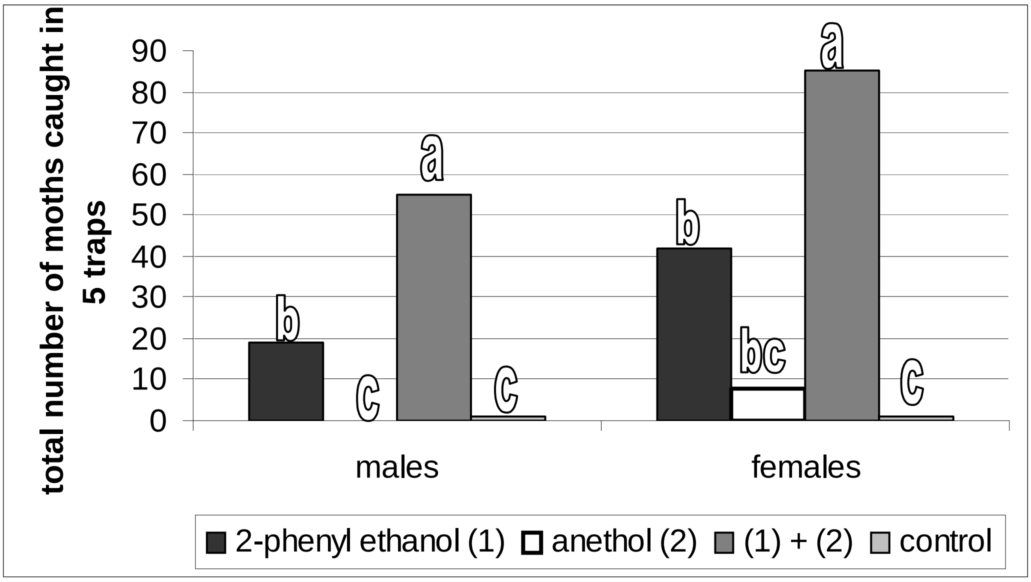

Figure 2. Total number of females and males caught in traps baited with plant volatiles placed in rowan during week 30 in 2002 (n = 5). Different letters indicate significantly different means within one sex ($p < 0.05$, Tukey's test).

In 2003, attack in apples was expected to be high according to the prognosis (Edland, 1978). However, only in one of the four experimental (organic) orchards attack in apples was found and similarly in only one of the control orchards (Table 1). Very few apple fruit moths were caught in plant volatile traps and pheromone traps (Table 1).

Table 1. Mean number of *A. conjugella* caught in pheromone (n = 4) and plant volatile traps (n = 36) placed in four organic orchards in Norway during the season 2003. The attack of *A. conjugella* larvae in apples (% attack in 200 apples) is also shown.

Orchard number	Trap type	Week number 26	27	28	29	30	31	32	Attack Exp.	Control
1	Pheromone	.	1.5	1.25	0	0	0	0	0.05	0
	Plantvolatile	.	0	0.03	0	0	0.03	0		
2	Pheromone	6	2.25	2	1.25	0.5	0	.	0	0.05
	Plantvolatile	0.03	0.2	0.03	0.14	0	0.03	.		
3	Pheromone	3	3	.	.	2.25	.	.	0	0
	Plantvolatile	0.06	0.14	.	.	0.14	.	.		
4	Pheromone	.	1.5	0.5	0.5	0	.	.	0	0
	Plantvolatile	.	0.11	0	0	0	.	.		

In the trial where tetra traps with plant volatiles were placed in a gradient from forest to orchard, most females were caught in traps placed in rowan with berries compared to rowan without berries. No females were caught in traps placed apples (Figure 3). The number of females caught increased after week 27.

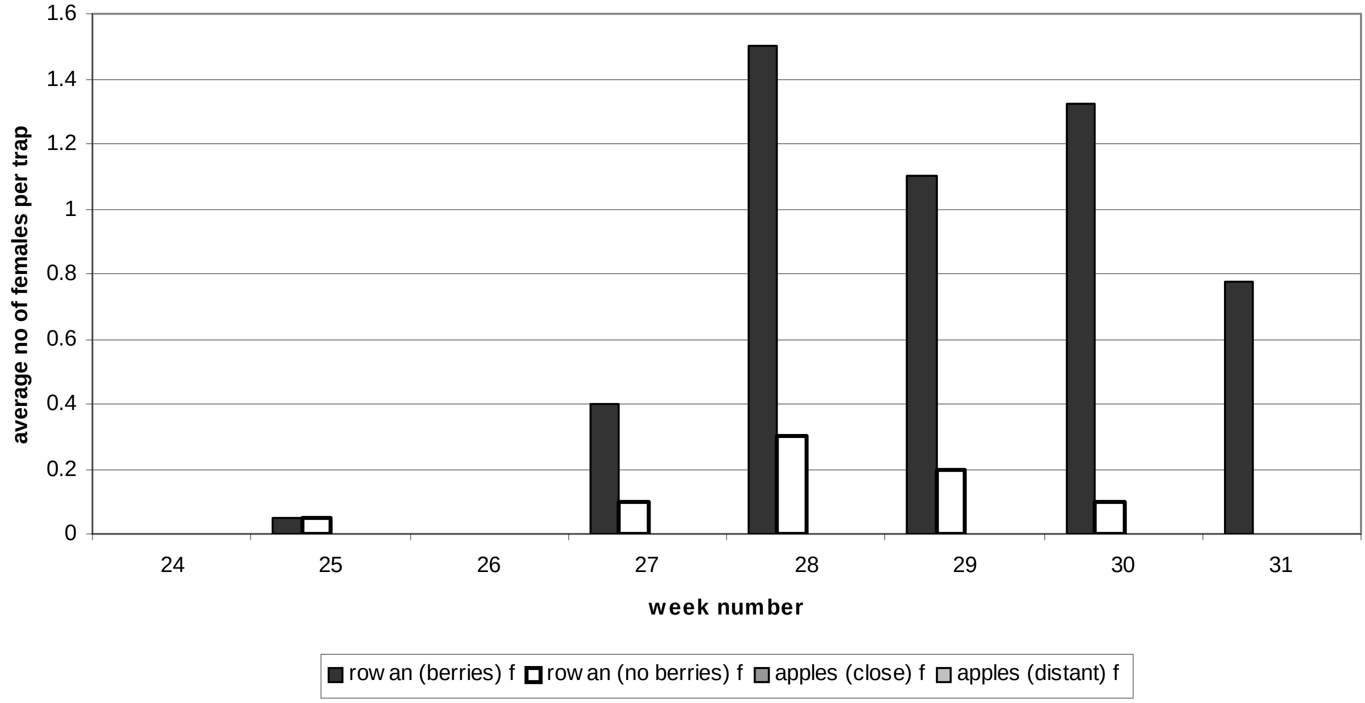

Figure 3. Average number of females caught in traps baited with a blend of 2-phenyl ethanol and anethol placed in a gradient from forest to apple orchard during the season 2003 (n = 10).

In field trapping tests with different volatile compounds in 2003, several new compounds identified from rowan attracted *A. conjugella* females.

Discussion:

Apples and rowan resemble each other with respect to volatiles that are attractive to the apple fruit moth. Some of these volatiles are attractive to both male and female *A. conjugella*. The two-component blend of 2-phenyl ethanol and anethol caught significantly more males and females compared to control traps, and it caught more females than males. Despite the very small popula-

tion of apple fruit moth in 2003, as indicated by low catches in pheromone traps and little damage on apples, the plant volatile traps attracted females in rowan. Figure 3 show that the attraction is time related. According to Kobro (1988) females start egglaying at a summation of approximately 320 daydegrees after full blossom on rowan. In 2003 this was in week 25, and results from both experiments with the two-compound blend in 2003 show that the attraction of females increase in week 27. Thus, the two-compound blend might attract females too late to prevent egg laying.
Figure 2 and 3 also show that the two-compound blend attracts females in the presence of natural volatiles emitted from rowan. As apple fruit moth has rowan as its primary host, and only attack apples in years when there is a lack of egglaying places in rowan, attractive volatiles might be a control method against this pest. The volatiles used in such a strategy must be more attractive than the natural volatiles emitted from apples. Unpublished data from 2003 indicate that several new compounds are attractive to apple fruit moth females. The addition of one or more of these compounds to the two-compound blend might increase the attractivity of the trap and make it more useful as a control method.

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