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Changes in Volatile Emissions from Apple Trees and Associated Response of Adult Female Codling Moths over the Fruit-Growing Season

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Odors in the headspace of apple trees were characterized by in situ volatile collections in the orchard. Sixty-two compounds were quantitatively identified with thermal desorption−gas chromatography−mass spectrometry over the complete fruit-growing season. Overall quantities in the headspace of fruit-bearing twig were highest at petal fall and at the beginning of June and August. Interestingly, the latter two periods coincide with the flight maxima of the codling moth, *Cydia pomonella*, one of the principal pest insects of apple fruit worldwide. Dual-choice bioassays with mated adult female moths in a Y-tube olfactometer showed that the blend of plant-derived volatiles repelled this key pest of apple at petal fall and attracted it from July to mid-August. Single-component analysis indicated that benzaldehyde and butyl acetate might contribute to the observed repellent effect, but the constituents accounting for the attractant effect mid-season remain to be further elucidated. The attractant effect clearly originates from the apple fruit and not from the twig with leaves, as bioassays demonstrated conclusively.

KEYWORDS: Volatile emissions; thermal desorption; GC-MS; codling moth; *Cydia pomonella*; Y-tube olfactometer; apple; seasonal dynamics

INTRODUCTION

There is growing evidence that attraction of moths to their host plants is largely guided by volatile phytochemicals, which are perceived by specialized chemoreceptor neurons on the antenna (*1*). However, the knowledge of the mechanisms guiding insects to their host in the field is limited. Previous work (*2*) monitored the volatile emissions from apple (*Malus domestica* Borkh.) in situ over the season with a solid-phase microextraction technique. Although the absolute quantities of the compounds identified in the blend were not determined, the results indicate temporal changes in volatile emissions from apple fruit, which may have an effect on the orientation of associated fruit moths.

One of the most serious pests of the apple worldwide is the codling moth, *Cydia pomonella* L. (Lepidoptera, Tortricidae) (*3*). Mated female moths deposit their eggs on the leaves near a growing apple or, later in the season, directly onto the fruit (*4*), and neonate larvae penetrate the fruit, causing considerable damage. Codling moth populations are predominantly sedentary; however, a small proportion of the population has the capacity for long flights (*3, 5*), but it is unknown yet at which time of the season they leave the orchard to undertake interhabitat flights. It has been suggested that changing volatile emissions from the apple tree, for example, following the infestation by a herbivore insect, might contribute to or even trigger the dislocation of a proportion of the population (*6*).

The response of the codling moth to volatile emissions from the apple tree has been reported for few phenological plant stages using electroantennography (*7*). Some compounds from branches with leaves and green fruit, for example, β-caryophyllene and (*E*,*E*)-α-farnesene, elicited an antennal response in the codling moth (*7, 8*). However, antennal response, which measures peripheral sensory perception, does not allow for a direct conclusion on subsequent behavioral activity of the insect. Indeed, both butyl hexanoate and methyl salicylate elicited antennal response in the codling moth (*7, 9*), but in bioassays a contrasting behavior was observed as butyl hexanoate turned out to be an attractant, whereas methyl salicylate is a repellent (*10*; A. Hern and S. Dorn, unpublished results). Knowledge of the behavioral effects of apple volatiles is currently limited to a few points in time over the season. Surprisingly, volatiles from healthy apples were neither attractant nor repellent for mated female codling moths in late August (*11*), whereas the moths were attracted to branches with green apples earlier in the season (*12*). However, still unknown is the effect of volatile emissions from the apple tree on codling moth behavior throughout the season, as is the absolute quantification of the volatiles emitted.

We chose to address this gap by performing volatile collections in situ and subsequent analyses and by conducting parallel laboratory bioassays on the response of the codling moth to fresh plant material. A radial diffuse (or passive) sampling

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system was used, followed by thermal desorption. The radial diffusive sampling shows little sensitivity to relative humidity, detects low concentrations, and is extremely portable for field work (e.g., ref *13*). The radial diffusive sampling is frequently employed in microbiological experiments (e.g., ref *14*), as well as for the environmental monitoring of air quality and for toxic compound analyses (e.g., refs *15* and *16*). Thermal desorption, on the other hand, offers the advantage of one-step elution and direct injection of trapped volatiles, eliminating the steps of solvent elution, storage, and concentration, during which trapped volatiles may be lost.

The goal of this study was to characterize headspace volatiles from the apple tree over the whole season in a quantitative manner and to elucidate the response of codling moth females to volatiles released from fruit-bearing twigs. To gain even more in-depth insight to the host plant−herbivore relationship, additional bioassays were conducted, separately for fruit and for twigs with leaves, in the second part of the season, and selected single compounds were tested for behavioral effects on the codling moth.

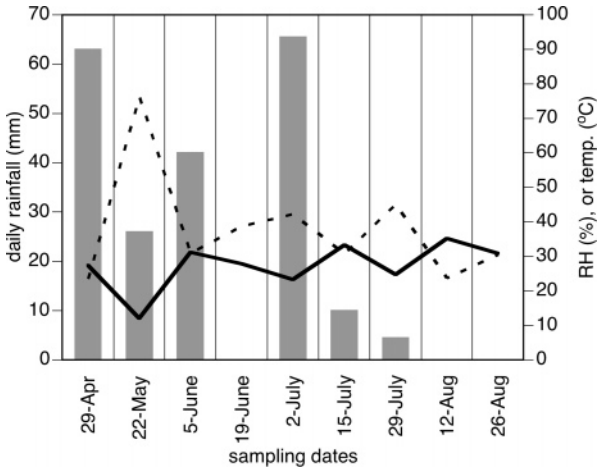

Figure 1. Climatic conditions over the growing season: total rainfall (mm) per 24 h at the sampling dates (bars); means of relative humidity (%) (dotted line) and of temperature (°C) (solid line) between 11.30 a.m. and 3.30 p.m. at the sampling dates.

MATERIALS AND METHODS

Insect. *C. pomonella* pupae were purchased from a commercial breeding station, which reared them on artificial diet containing water, agar, maize grits, wheat flakes, brewer's yeast, and preservatives (*6*). On arrival the pupae were placed in a climatic chamber at 25 °C with a light-18−dark-6 photoperiod and 65% relative humidity. Males and females were paired after emergence in a plastic box (30 × 10 × 10 cm) with access to water and kept under the same environmental conditions as the pupae.

Study Site and Plant Material. Apple trees of the cultivar Golden Delicious were used to collect the volatile organic compounds and for picking the plant material for the bioassays. The dwarf apple trees in a commercial orchard (Zurich, Switzerland) at an altitude of 520 m were kept under hail nets, cultivated with an integrated production regime, and subjected to pesticide treatments. The study site within the city limits as well as the plant protectants used might have led to a certain amount of contaminants in the headspace of the trees sampled, but environmental contaminants belong to the chemical environment of the codling moth in modern agroecosystems. Disease management comprised applications of dithianon (quinine; Delan WG; WG 70 g/100 g; 300, 800, and 400 L/ha) in April and May, difenoconazole (triazole; Slick; EC 250 g/L; 300 L/ha) in May, captan (phthalimide; Captan S WG; WG 80 g/100 g; 400 L/ha) in May and July, trifloxystrobin (strobilurin; Flint; WG 50 g/100 g; 400 L/ha) in June and July, folpet (phthalimide; Folpet 80; WP 80 g/100 g; 400 L/ha), and dichlofluanid [sulfamide; Euparen-Cu; WP (30 g/100 g, 15 g/100 g); 400 L/ha] in August. Protection against aphids was achieved with thiacloprid (pyridylmethylamine; Alanto; SC 480 g/L; 400 L/ha) at the beginning of May and against codling moth and other Lepidoptera with fenoxycarb (carbamate; Insegar DG; WG 25 g/100 g; 400 L/ha), chlorpyrifos-methyl (organophosphate; Reldan 40; EC 400 g/L; 400 l/ha), and diflubenzuron (benzoyl urea; Dimilin SC; SC 480 g/L; 400 L/ha) at the beginning of June and July.

Sampling. Plant samples were taken throughout the season from the same three trees growing adjacent to each other to keep variation due to sampling from various plants minimal (*2*). Samples were randomly collected from the middle part of the canopy. For volatile collection, five individual samples were taken from each of the trees per sampling date. Each sample consisted of a fruit-bearing twig with 6 ± 2 leaves, addressed as "fruit−twig−leaves complex". For each bioassay, two samples were picked from two of the three trees randomly. Throughout the season, sampling was made of the fruit−twig−leaves complex. In the second part of the season, additional samples were taken that represent the separate components of this complex, namely, the fruit and the twig with leaves. The latter was addressed as the "twig−leaves complex". The samples selected were visually inspected to ensure that no apparent herbivore or disease damage was present. Golden Delicious apple harvest began in mid-September in 2003.

Volatile Collection. Headspace volatiles were collected in situ in the apple orchard by means of radial diffusive sampling (Radiello model 3310, Rupprecht & Patashnick Co., Albany, NY). The collection took place from 11:30 a.m. to 3:30 p.m. at the following nine sampling dates between the end of April and the end of August: April 29, May 22, June 5, June 19, July 2, July 15, July 29, August 12, and August 26.

The sampler was positioned horizontally inside a plastic bag (Nalophan, Kalle GmbH, Wiesbaden, Germany) securely tied around the fruit−twig−leaves complex. The adsorbent cartridge, containing Tenax-TA polymer, was inserted into the yellow diffusive body of the Radiello's sampler, which was then screwed to the supporting plate hanging near the fruit−twig−leaves complex. At the end of the collecting time the cartridge was inserted into a sorbent tube (Markes Int. Ltd., Pontyclun, U.K.) and analyzed by thermal desorption−gas chromatography−mass spectrometry (TD-GC-MS). Tenax-TA is considered to be adsorbent, showing the overall best properties for sampling complex mixtures of volatiles. It is known as an adsorbent for sampling low amounts of organic compounds in air and to be inert, thermally stable, and hydrophobic, with high storage stability and a low background (e.g., Sunesson et al., 1995).

Climatic Data. A weather station (PC weather station, Conrad Electronic, Berlin, Germany) was installed in the orchard under the hail net to record ambient temperature and air humidity. Recordings from 1 m above ground were obtained every 10 min. The mean values for the daily block hour of sampling were calculated as the arithmetic average of these recordings. Rainfall data were obtained from a nearby weather station in Zurich of the National Meteorological Station, "MeteoSwiss". Except for at the very beginning of the trial period, temperature was high and air humidity low at the sampling dates (**Figure 1**). Precipitation was extremely low from mid June to the end of August 2003, amounting to a total of 250 mm only. Hence, these abiotic environmental conditions were relatively stable over a long period of time.

Volatile Analyses. Samples were analyzed using a Hewlett-Packard GC-MS instrument (GC 6890 mass selective, MS detector 5973). Analyses were carried out using a thermal desorption system (Unity, Markes Int. Ltd.) in which the desorbed headspace volatiles were transferred to the GC column without use of a solvent. Prior to sampling, the sorbent tubes were thermally conditioned for 45 min at 320 °C followed by 30 min at 335 °C with a flow of helium through the trap (flow rate = 60 mL/min at ambient temperature). The analysis details were as follows: precolumn HP-Retention Gap (uncoated, deactivated), 5 m × 0.25 mm (Hewlett-Packard Co., Palo Alto, CA); column ECONO-CAP, phase EC-5, 30 m in length; 0.25 mm diameter; 0.25 μm film thickness (Alltech Associates, Inc., Deerfield, IL). The oven

temperature was programmed from 50 °C with a 5 min hold, then ramped at a rate of 5 °C/min to the final temperature of 300 °C (total run time = 65 min). The thermal desorption details were as follows: prepurge time, 3 min, split on; tube desorption, 5 min, 300 °C, split off. The cold trap, packed with a 4 cm bed of Tenax-TA (mesh size 60−80, Supelco, Bellefonte, PA) and a 2 cm bed of Carbopack B (mesh size 60−80, Supelco), was held at −10 °C throughout the tube desorption process and then heated at a rate of 60 °C/min to 300 °C; the total cold trap desorption time was 3 min. The mass selective detector was operated in scan mode. The MS quadrupole and MS source temperatures were 150 and 230 °C, respectively. As the internal standard, hexyl benzene (50 ng/μL) diluted in hexane was added to each analysis by injecting it directly onto the polymer. Components of the volatile blends were identified by comparison of their mass spectra with those in the NIST98 and our own library of phytochemical compounds. In addition, retention times were compared with standard compounds, which were purchased from chemical suppliers or obtained from other laboratories. The concentration of each compound was calculated from the peak area obtained by TD-GC-MS.

Behavioral Trials. The codling moths were offered a dual choice in an all-glass Y-tube olfactometer (for details, see ref *6*). An odor source (or a respective control) was placed in each of the two chambers of 12 cm length and 10 cm diameter. These chambers were provided with a green gauze separating the sample from the moth. The chambers were each connected to one of the two 20 cm long branches of the Y-tube olfactometer, which converged into a 20 cm long common arm. The glass tube had an overall diameter of 2.5 cm with ground glass joints. Moistened, activated-charcoal-filtered air entered each arm of the Y-tube olfactometer at 750 ± 5 mL/min. Mated females (2−4 days old) were used for the bioassays. Prior to each set of tests, moths were allowed to acclimatize for 1 h. The female moths were used only once and were not exposed to odor sources prior to the bioassay. Moths were placed individually at the entrance of the common arm. The position of the moth was recorded after 10 min. A response was recorded if the female moths entered the test arm (plant sample or chemical compound as odor source) or the control arm (no odor source provided or solvent as the control in bioassays with chemical compounds). No choice was noted if the moth remained in the common arm. Tests were conducted at scotophase condition with room temperature ranging between 23 and 24 °C. A 60 W red light bulb allowed for behavioral observation.

Odors tested comprised plant material and chemical compounds. Plant material samples were freshly picked between 2:00 and 3:00 p.m. on the day of the bioassays. For transportation to the laboratory, they were placed in a plastic bag with moistened cotton. The fruit−twig−leaves complex consisted of an apple randomly picked on one of the three apple trees and a twig cut from the tip of a branch. The cut in the stalk of the apple fruit and in the twig were sealed with Parafilm after collection. The twig had 6 ± 2 leaves and was 10−15 cm long. Sixty-five female moths were tested with the fruit−twig−leaves complex, the apple fruit alone, or twig−leaves complex against no odor source as control. For each experiment, two samples of each plant material were used.

Chemical compounds were diluted with solvent to 50 μL volume and placed on an 11 mm diameter silicon/Teflon septum (Supelco). The septum was then inserted into the odor chamber of the Y-tube olfactometer. Fifty microliters of solvent was placed in the other chamber as control. Fresh chemicals and new septa were used for each moth. The behavior of 50 mated female *C. pomonella* was recorded for each of the chemical compounds listed below.

Chemicals. The concentrations of the selected synthetic chemical compounds tested in the bioassay reflect the ratios of these compounds averaged over all sampling dates in July and August. Compounds were selected on the basis of availability and expected chance for bioactivity. The chemicals tested comprised butyl acetate (purity ≥ 99.7%, Fluka, Buchs, Switzerland; 40 mg/L), decanal (purity ≥ 99%, Sigma, Buchs, Switzerland; 40 mg/L), nonanal (purity ≥ 95%, Fluka; 75 mg/L), benzaldehyde (purity ≥ 99.5%, Fluka; 25 mg/L), 2-ethyl-1-hexanol (purity ≥ 99.5%, Fluka; 100 mg/L), β-caryophyllene (purity ≥ 80%, Aldrich, Buchs, Switzerland; 70 mg/L), limonene (purity ≥ 97%, Aldrich; 60 mg/L), α-pinene (purity ≥ 98%, Aldrich; 15 mg/L), β-pinene (purity ≥ 98.5%, Fluka; 5 mg/L), and (*E*)-2-hexenal (purity ≥ 97%, Fluka; 15 mg/L), which were diluted in hexane (purity > 99%, Fluka) prior to the bioassay (*6*).

Data Analysis. The choice response of *C. pomonella* moths to the fruit−twig−leaves complex or to the control consisting of no odor source was analyzed using a one-sample χ^2-square test (*17*). The χ^2-square test was also used for bioassays with the single compounds. Variation in volatile emissions from three individual apple trees in relation to different sampling dates was analyzed using ANOVA with repeated measures.

RESULTS

Volatile Analyses. A total of 62 compounds were identified from the headspace of fruit-bearing twigs of Golden Delicious apple trees during the growing season 2003. They comprised 9 terpenoids, 10 aldehydes, 6 alcohols/phenols, 10 hydrocarbons (alkanes and alkenes), 3 carboxylic acids, 5 esters, 3 ketones, 3 ether alcohols, 11 aromatic compounds, which are benzene derivatives, diphenylamine, and benzothiazole (**Table 1**). Volatile emission changed significantly over the growing season (ANOVA repeated measures: $F = 147.202$, $P < 0.0001$). Volatiles collected from three individual apple trees used in this study, however, did not differ significantly (ANOVA repeated measures: $F = 0.865$, $P > 0.05$). Furthermore, no significant interaction was found between the three individual apple trees and the sampling dates (ANOVA repeated measures: $F = 0.788$, $P > 0.05$). All of the presented compounds were apparent at two or more sampling dates and 30 compounds even throughout the entire sampling period (**Table 1**). As a synopsis of the quantitative releases, the total mean peak area at a given sampling date was summed over all compounds (**Figure 2**). A total of three maxima of emissions was reached at distinct times in the season: the first maximum coincided with the petal fall stage. The second maximum was reached early in June and the third one in mid-August. Quantities of volatiles were lowest at the end of May, the end of July, and the end of August. A large number of compounds were no longer detectable at this last sampling date, in contrast to the previous dates with minimum emissions (**Table 1**).

Behavioral Trials with Plant Volatile Blends. Bioactivity of volatiles emitted from freshly collected twigs with fruit and leaves on young mated codling moth females changed markedly with progressing season (**Figure 3**). Volatiles emitted from the twig with a green apple fruitlet (diameter = 15 mm) at the end of May were avoided by the moths ($\chi^2 = 6.125$, $P < 0.05$). During the subsequent early growth stages of the apple fruit, odors from the fruit−twig−leaves complex were neither attractant nor repellent. Starting in July until mid-August, the volatile blend was significantly attractant (sampling dates July 1−3, $\chi^2 = 10$, $P < 0.01$; July 17−19, $\chi^2 = 21.951$, $P < 0.001$; July 29−31, $\chi^2 = 54.857$, $P < 0.001$; Aug 13−14, $\chi^2 = 49.209$, $P < 0.001$). After this period, no significant preference for the test odor was noted.

During the period from mid-July to the end of September, bioassays were performed not only with fruit in combination with twig and leaves (**Figure 3**) but also for the separate components (**Figures 4** and **5**). Volatiles from apple fruit were significantly attractant midseason (sampling dates July 18−19, $\chi^2 = 21.951$, $P < 0.001$; Aug 5−6, $\chi^2 = 16.488$, $P < 0.001$; Aug 19−21, $\chi^2 = 21.951$, $P < 0.001$) but not afterward. Interestingly, volatiles from the twig−leaves complex were significantly avoided during the period in which the apple fruits were attractant (July 18−19, $\chi^2 = 18.270$, $P < 0.001$; Aug 4−5, $\chi^2 = 25.714$, $P < 0.001$; Aug 20−21, $\chi^2 = 8.308$, $P < 0.01$), but again no preference of codling moth females was noted afterward.

Table 1. Time Course of Emission of All Compounds Detected by TD-GC-MS over the Growing Season, Concentration ± SE (mg/L, $n = 15$)

		petal fall stage	fruitlet	fruit						
compound[a]	RT,[b] min	April 29	Ø[c] 15 mm, May 22	Ø 24 mm, June 5	Ø 35 mm, June 19	Ø 45 mm, July 2	Ø 55 mm, July 15	Ø 60 mm, July 29	Ø 68 mm, Aug 12	Ø 72 mm, Aug 26
alkanes										
4-methyloctane	5.50	5.3 ± 0.6	1.9 ± 0.4	18.9 ± 1.0	8.3 ± 0.9	11.0 ± 0.9	0.4 ± 0.1	15.6 ± 1.5	10.6 ± 0.8	8.7 ± 2.7
n-undecane	13.63	11.6 ± 1.9	2.8 ± 1.0	23.0 ± 3.5	13.0 ± 2.6	9.5 ± 1.0	5.6 ± 1.0	3.9 ± 0.8	13.8 ± 0.8	12.9 ± 3.4
n-dodecane[e]	16.85	31.3 ± 4.8	4.0 ± 1.1	57.0 ± 17.6	31.9 ± 3.7	18.8 ± 2.0	23.8 ± 2.5	11.2 ± 2.1	40.0 ± 6.8	nd[d]
n-tridecane[d]	19.83	29.7 ± 6.2	3.5 ± 0.9	54.0 ± 15.0	37.1 ± 9.0	17.6 ± 2.0	37.2 ± 7.0	13.4 ± 2.9	54.8 ± 8.3	3.9 ± 1.6
n-tetradecane[d]	22.65	49.5 ± 11.1	6.1 ± 1.3	71.2 ± 14.7	50.5 ± 13.2	26.4 ± 2.4	56.9 ± 4.8	22.8 ± 5.7	97.7 ± 26.3	7.6 ± 2.2
n-eicosane*[,1,c,e]	36.45	4.8 ± 1.2	1.3 ± 0.3	2.1 ± 0.4	4.3 ± 0.9	0.7 ± 0.1	0.5 ± 0.1	1.3 ± 0.4	1.0 ± 0.3	nd
alkenes										
hexene	3.75	3.9 ± 0.9	1.0 ± 0.2	2.7 ± 0.4	4.5 ± 0.6	3.0 ± 0.4	4.9 ± 0.7	3.8 ± 1.2	4.0 ± 1.6	2.8 ± 0.9
unknown compound	12.60	1.3 ± 0.3	1.1 ± 0.3	7.8 ± 1.3	5.3 ± 1.0	4.3 ± 0.7	2.5 ± 0.4	2.9 ± 0.8	4.1 ± 0.7	nd
unknown compound	15.93	nd	nd	nd	nd	nd	nd	3.0 ± 0.6	10.6 ± 1.6	nd
1-dodecene[e]	16.57	8.8 ± 1.3	2.6 ± 0.6	14.5 ± 2.2	9.6 ± 0.9	5.9 ± 0.6	4.3 ± 0.8	2.7 ± 0.4	8.2 ± 1.3	nd
other										
diphenylamine[i]	28.20	22.6 ± 6.0	2.7 ± 0.9	16.1 ± 3.4	13.4 ± 2.9	7.0 ± 1.3	13.3 ± 1.7	6.2 ± 1.9	19.1 ± 2.9	13.3 ± 5.7
benzothiazole[g]	17.40	nd	nd	nd	nd	6.9 ± 0.6	3.7 ± 0.8	2.4 ± 0.5	8.8 ± 1.5	nd
alcohols/phenols										
(*Z*)-3-hexen-1-ol*[,1,c,e]	5.30	26.6 ± 9.7	2.5 ± 0.4	5.7 ± 1.0	4.6 ± 0.4	3.2 ± 0.4	2.7 ± 0.2	4.8 ± 1.5	7.1 ± 1.1	8.9 ± 3.2
phenol*[,1,f,g,h]	9.55	9.9 ± 0.8	4.2 ± 0.9	5.9 ± 0.9	4.4 ± 0.6	4.3 ± 0.5	2.5 ± 0.2	4.1 ± 0.1	5.0 ± 1.0	2.5 ± 0.8
2-ethyl-1-hexanol*[,1,g,h]	11.18	116.0 ± 12.3	13.2 ± 1.6	22.7 ± 11.7	24.6 ± 3.1	13.6 ± 1.3	12.1 ± 3.2	43.3 ± 7.5	17.9 ± 2.6	nd
benzyl alcohol[e]	11.30	10.5 ± 1.8	0.2 ± 0.2	nd	nd	nd	nd	nd	nd	nd
m-tertbutyl phenol[i]	19.65	17.5 ± 2.8	4.6 ± 1.4	17.1 ± 3.0	14.5 ± 3.0	8.9 ± 1.1	8.5 ± 1.7	4.7 ± 1.1	12.1 ± 2.1	0.9 ± 0.2
butylated hydroxytoluene[i]	25.60	27.0 ± 5.4	4.5 ± 1.4	44.4 ± 8.4	23.7 ± 3.9	12.1 ± 1.1	13.7 ± 2.4	8.8 ± 1.3	45.8 ± 9.4	nd
ether alcohols										
2-butoxyethanol[h]	6.90	1.7 ± 0.3	2.2 ± 0.7	1.2 ± 0.4	0.9 ± 0.1	0.8 ± 0.1	nd	0.6 ± 0.2	nd	nd
1(2-butoxyethoxy)-ethanol[i]	16.50	11.4 ± 2.6	3.3 ± 0.7	17.5 ± 3.7	9.1 ± 1.1	5.5 ± 0.7	0.9 ± 0.2	1.4 ± 0.4	6.2 ± 1.1	nd
2-phenoxyethanol[i]	17.40	16.6 ± 2.5	6.4 ± 1.1	13.4 ± 2.0	17.1 ± 4.0	8.5 ± 1.7	3.7 ± 1.0	2.5 ± 0.5	8.9 ± 1.5	nd
ketones										
6-methyl-5-hepten-2-one*[,3]	9.70	8.5 ± 1.2	5.5 ± 1.1	12.9 ± 1.0	9.1 ± 0.8	8.9 ± 0.4	5.3 ± 0.6	7.1 ± 0.8	9.9 ± 1.2	9.0 ± 2.3
acetophenone*[,1,d,h]	12.30	11.8 ± 1.7	3.3 ± 0.4	8.8 ± 1.2	4.9 ± 0.7	4.9 ± 0.6	1.7 ± 0.2	2.8 ± 0.4	4.7 ± 0.6	nd
benzophenone*[,1,d,e]	28.40	9.5 ± 2.9	4.3 ± 1.5	34.1 ± 7.2	28.4 ± 2.8	15.2 ± 3.5	23.9 ± 3.3	11.7 ± 3.1	39.4 ± 10.5	5.1 ± 1.7
aldehydes										
hexanal*[,3,c,d]	3.90	12.7 ± 2.9	5.5 ± 1.1	12.1 ± 1.9	9.9 ± 1.3	7.8 ± 1.8	13.0 ± 2.3	11.1 ± 4.3	11.5 ± 1.5	8.7 ± 1.7
(*E*)-2-hexenal*[,1,c,d]	4.90	6.6 ± 1.1	1.1 ± 0.3	4.5 ± 1.2	4.3 ± 1.6	4.0 ± 0.6	4.5 ± 1.1	2.4 ± 0.5	26.7 ± 5.2	4.5 ± 1.4
heptanal*[,3,c,d]	6.70	11.7 ± 1.2	4.1 ± 0.8	15.2 ± 1.0	10.5 ± 1.2	8.6 ± 0.9	7.8 ± 0.9	6.7 ± 0.6	11.6 ± 1.3	6.7 ± 1.9
benzaldehyde*[,1,e]	8.60	23.8 ± 4.0	7.5 ± 1.1	12.5 ± 1.2	7.9 ± 1.1	7.9 ± 0.8	4.7 ± 0.7	6.3 ± 0.4	7.5 ± 0.9	10.2 ± 3.0
octanal[d]	10.22	8.6 ± 1.1	5.8 ± 1.2	55.5 ± 5.9	27.9 ± 3.8	23.1 ± 1.2	15.2 ± 2.0	15.0 ± 1.9	29.0 ± 2.6	nd
nonanal*[,1,d,e]	13.73	35.4 ± 3.7	16.3 ± 4.1	149.9 ± 19.1	120.2 ± 11.7	99.8 ± 8.8	71.9 ± 9.1	51.4 ± 9.1	143.3 ± 13.2	1.2 ± 0.4
decanal*[,2,d,e]	16.95	34.4 ± 4.4	9.0 ± 1.4	51.8 ± 11.8	74.6 ± 7.7	64.6 ± 6.7	79.4 ± 8.0	40.0 ± 6.2	108.7 ± 16.0	5.9 ± 2.8
4-methoxybenzaldehyde	18.38	2.4 ± 0.6	1.1 ± 0.3	4.7 ± 0.8	4.2 ± 0.9	1.9 ± 0.1	nd	1.3 ± 0.4	1.8 ± 0.4	nd
undecanal	20.00	19.2 ± 2.4	3.4 ± 0.8	17.6 ± 1.5	16.6 ± 2.9	18.2 ± 1.3	20.3 ± 1.6	11.7 ± 2.0	39.1 ± 7.1	nd
3,5-di-*tert*-butyl-4-hydroxy-benzaldehyde[i]	31.60	5.4 ± 2.2	nd	6.6 ± 2.2	7.5 ± 1.0	4.1 ± 0.9	8.2 ± 1.6	2.1 ± 0.2	11.6 ± 2.5	nd
acids										
2-ethylhexanoic acid*[,3,i]	14.63	2.0 ± 0.4	nd	21.5 ± 1.6	10.7 ± 1.1	5.9 ± 1.3	6.1 ± 1.8	4.7 ± 0.9	7.0 ± 1.2	nd
octanoic acid*[,3,e,h]	16.10	7.0 ± 2.4	2.1 ± 0.5	6.9 ± 1.2	10.8 ± 1.1	7.0 ± 0.5	4.2 ± 0.8	2.1 ± 0.2	nd	nd
nonanoic acid[d]	19.00	nd	nd	nd	nd	nd	nd	1.1 ± 0.3	14.7 ± 1.9	nd
esters										
butyl acetate*[,1,d,e,h]	4.20	2.8 ± 0.7	0.6 ± 0.2	2.0 ± 0.3	1.2 ± 0.3	1.1 ± 0.2	0.5 ± 0.1	1.7 ± 0.3	1.5 ± 0.1	1.3 ± 0.4
butyl laurate*[,1,d]	32.04	nd	nd	nd	2.7 ± 0.3	1.2 ± 0.1	0.3 ± 0.2	nd	0.6 ± 0.3	nd
isopropyl myristate[h]	32.88	10.0 ± 2.1	nd	2.5 ± 0.3	2.3 ± 0.9	1.1 ± 0.3	1.8 ± 0.5	0.4 ± 0.3	0.7 ± 0.3	nd
butyl myristate	36.18	2.2 ± 1.0	0.5 ± 0.2	0.5 ± 0.1	2.2 ± 0.5	0.4 ± 0.1	nd	0.3 ± 0.2	0.4 ± 0.3	nd
isopropyl palmitate	36.95	3.6 ± 0.6	0.7 ± 0.6	0.8 ± 0.2	14.8 ± 0.5	0.4 ± 0.1	2.2 ± 0.2	0.8 ± 0.3	1.1 ± 0.3	nd
terpenoids										
α-pinene*[,3,c,d,e,h]	7.76	4.7 ± 0.6	7.7 ± 0.5	29.4 ± 2.7	17.3 ± 1.8	20.1 ± 1.1	6.5 ± 1.3	29.1 ± 1.6	42.7 ± 4.0	10.9 ± 3.5
camphene*[,3,c,d,e]	8.26	1.0 ± 0.3	0.8 ± 0.2	1.4 ± 0.1	0.9 ± 0.2	0.7 ± 0.1	0.4 ± 0.1	0.7 ± 0.1	1.3 ± 0.2	nd
β-pinene*[,1,c,d,e]	9.23	2.0 ± 0.6	2.2 ± 0.4	9.7 ± 0.7	4.9 ± 0.7	5.3 ± 0.7	1.8 ± 0.2	6.2 ± 0.7	11.3 ± 0.8	2.5 ± 0.8
Δ^3-carene*[,3,c,d,e]	10.45	nd	nd	82.8 ± 22.3	52.0 ± 9.7	43.8 ± 5.8	16.1 ± 2.3	44.2 ± 13.4	80.1 ± 17.1	nd
limonene*[,3,c,d,e,h]	11.08	12.4 ± 2.1	4.9 ± 1.1	17.2 ± 1.3	7.4 ± 1.1	9.0 ± 0.6	1.7 ± 0.2	5.9 ± 1.3	14.6 ± 1.3	17.8 ± 6.8
ocimene*[,4]	13.22	2.3 ± 0.6	1.3 ± 0.2	13.1 ± 2.9	8.0 ± 2.0	6.9 ± 0.6	2.1 ± 0.3	3.9 ± 0.7	9.6 ± 1.3	nd
β-caryophyllene*[,3,c,d,e]	22.40	50.5 ± 12.2	5.7 ± 1.1	90.7 ± 17.1	72.8 ± 14.7	44.0 ± 5.5	73.7 ± 12.9	34.7 ± 6.7	123.4 ± 17.1	5.8 ± 1.1
(*E*,*E*)-α-farnesene*[,4,d,e]	22.80	67.8 ± 7.0	11.3 ± 2.4	64.1 ± 14.1	46.2 ± 11.3	27.5 ± 3.2	40.7 ± 4.5	21.6 ± 3.1	56.2 ± 9.0	6.0 ± 2.8
spathulenol*[,4,c]	24.40	17.6 ± 8.4	4.1 ± 1.0	25.3 ± 5.6	19.5 ± 4.1	12.3 ± 1.4	16.7 ± 1.7	9.3 ± 1.6	22.6 ± 2.9	7.8 ± 2.4
aromatics										
ethylbenzene*[,1,g]	5.40	14.1 ± 1.5	11.3 ± 1.9	17.2 ± 3.0	10.6 ± 1.3	12.6 ± 1.8	2.2 ± 0.3	8.7 ± 0.5	11.1 ± 0.6	9.1 ± 2.7
p-xylene*[,1,g,h]	5.65	36.9 ± 3.0	38.5 ± 7.3	43.8 ± 4.9	10.0 ± 1.7	36.1 ± 2.2	5.7 ± 0.6	33.5 ± 2.7	44.4 ± 3.7	23.9 ± 6.6
styrene[g]	6.30	44.8 ± 4.7	3.8 ± 0.6	9.3 ± 1.2	4.3 ± 0.8	4.8 ± 0.7	2.8 ± 0.9	3.6 ± 0.2	2.8 ± 0.3	10.4 ± 0.3
m-xylene*[,1,g,h]	6.40	15.0 ± 1.0	11.6 ± 0.8	21.4 ± 1.4	4.7 ± 0.2	15.6 ± 0.9	3.3 ± 0.3	13.5 ± 1.1	19.7 ± 2.0	nd
propylbenzene*[,1,g]	8.45	4.6 ± 1.0	3.1 ± 0.6	5.9 ± 0.4	2.0 ± 0.2	4.5 ± 0.5	1.0 ± 0.2	3.3 ± 0.2	6.1 ± 0.4	0.8 ± 0.2
3-ethyltoluene*[,1,g]	8.70	15.7 ± 1.1	13.2 ± 1.0	32.0 ± 1.5	9.2 ± 1.9	22.7 ± 1.6	3.7 ± 1.0	15.8 ± 2.0	32.5 ± 2.8	nd
1,3,5-trimethylbenzene*[,1,g]	8.95	7.3 ± 1.0	4.6 ± 0.5	12.5 ± 1.1	4.7 ± 0.7	8.1 ± 0.7	1.6 ± 0.2	0.3 ± 0.2	1.0 ± 0.2	nd
2-ethyltoluene*[,1,g]	9.35	6.4 ± 0.5	3.6 ± 0.6	8.0 ± 0.6	3.2 ± 0.5	5.5 ± 0.4	1.3 ± 0.2	3.8 ± 0.5	8.3 ± 1.4	nd
1,2,4-trimethylbenzene*[,1,g]	9.80	23.1 ± 2.5	17.5 ± 1.7	57.3 ± 3.9	26.2 ± 4.1	31.1 ± 1.5	5.7 ± 0.8	17.8 ± 2.9	48.3 ± 4.7	nd
1,2,3-trimethylbenzene*[,3,g]	10.80	4.3 ± 0.4	3.6 ± 0.7	10.9 ± 0.9	4.9 ± 0.8	6.4 ± 0.5	1.9 ± 0.4	3.8 ± 0.7	9.8 ± 0.9	2.0 ± 0.7
naphthalene	16.15	13.9 ± 1.7	3.5 ± 0.8	21.2 ± 2.0	16.8 ± 2.0	12.1 ± 2.4	13.1 ± 2.3	5.9 ± 2.7	25.0 ± 2.4	nd

[a] *, definitively identified. Source of standards: [1], Fluka; [2], Sigma; [3], Aldrich; [4], from Dr. R. Kaiser (Givaudan-Roure, Switzerland). Origins of volatiles according to literature reports: [c], apple leaf; [d], apple fruit; [e], flower of apple tree (*18*, *19*, *21*, *33–38*); [f], plant origin reported from different plant species (*39–42*); [i], environmental contaminants (personal communication from R. Kaiser and F. Jüttner) including [g], air pollutants (e.g., refs *47–49*), and [h], liquid carriers used in pesticides (*46*, *47*). [b] Retention time. [c] Diameter of apple fruit. [d] Not detected.

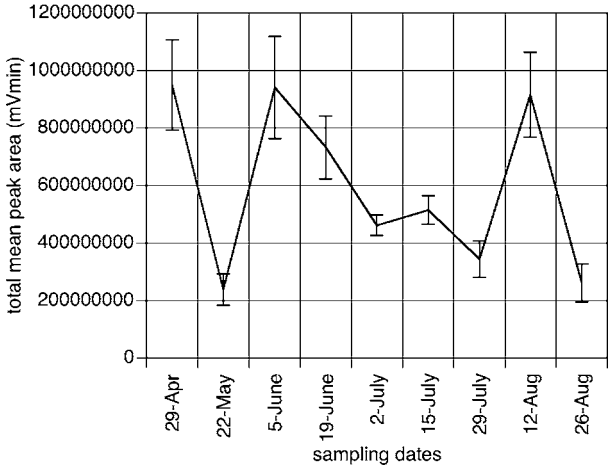

Figure 2. Total mean peak area of the sum of all compounds detected over the growing season of 2003, during daytime between 11.30 a.m. and 3.30 p.m.; $N = 60$ samples for each sampling date.

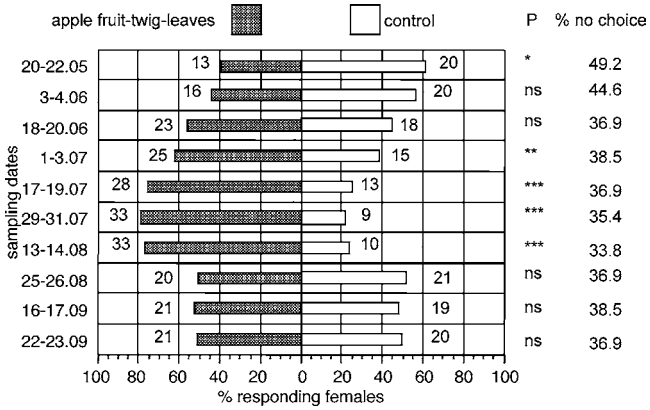

Figure 3. Response of mated female *C. pomonella* to apple fruit–twig–leaves complex (control = no odor source) in a Y-tube olfactometer over the growing season. Numbers refer to the number of *C. pomonella* choosing an odor source. $N = 65$ females per treatment. χ^2 test: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; ns = $P \geq 0.05$.

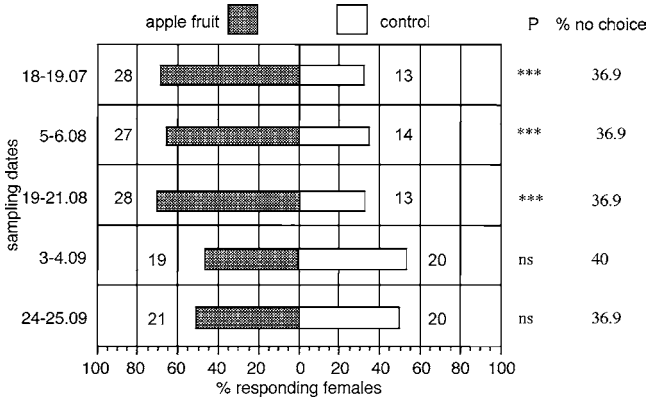

Figure 4. Response of mated female *C. pomonella* to apple fruit (control = no odor source) in a Y-tube olfactometer over the growing season. Numbers refer to the number of *C. pomonella* choosing an odor source. $N = 65$ females per treatment. χ^2 test: *** = $P < 0.001$; ns = $P \geq 0.05$.

Behavioral Trials with Synthetic Compounds. The behavioral response of mated female codling moths to selected chemicals from different groups was tested in bioassays (**Figure 6**). A significant attraction was recorded for β-caryophyllene ($\chi^2 = 4.172$, $P < 0.05$) and limonene ($\chi^2 = 6.533$, $P < 0.05$).

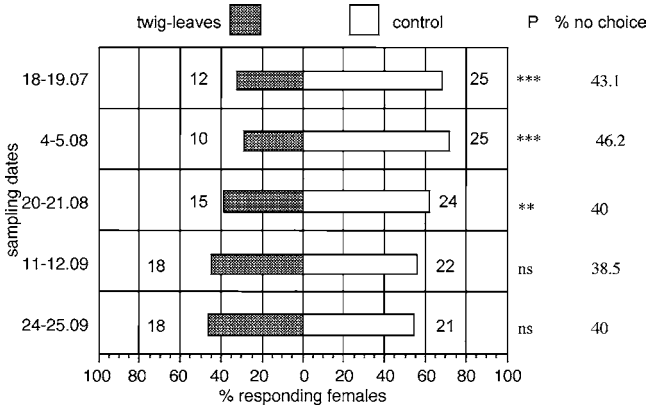

Figure 5. Response of mated female *C. pomonella* to twig–leaves complex (control = no odor source) in a Y-tube olfactometer over the growing season. Numbers refer to the number of *C. pomonella* choosing an odor source. $N = 65$ females per treatment. χ^2 test: ** = $P < 0.01$; *** = $P < 0.001$; ns = $P \geq 0.05$.

Compounds with a repellent activity at the tested dosage comprised butyl acetate ($\chi^2 = 15.207$, $P < 0.001$), nonanal ($\chi^2 = 10.8$, $P < 0.01$), benzaldehyde ($\chi^2 = 10.125$, $P < 0.01$), and β-pinene ($\chi^2 = 10.125$, $P < 0.01$). Decanal, 2-ethyl-1-hexanol, α-pinene, and (*E*)-2-hexenal were neither repellent nor attractant for codling moths at the dosage tested.

DISCUSSION

Seasonal Dynamics of Volatile Emissions Eliciting Different Responses in Codling Moth. The temporal dynamics of volatiles in the headspace of apple trees were characterized, for the first time in a quantitative manner, over the complete apple-growing season and were put into relation with the behavioral response of one of its principal pests, the codling moth. The progressive phenological stages of the apple tree emit blends of volatiles subject to marked change, both in qualitative composition and in quantities released. The codling moth responded to these changing chemical signals as it was repelled at the petal fall stage, attracted midsummer, and neither repelled nor attracted in late August in laboratory bioassays.

The petal fall period, when fruit growth starts, was characterized by high amounts of volatiles collected. Twenty-five compounds were detected, and three of them, (*Z*)-3-hexen-1-ol, 2-ethyl-1-hexanol, and benzaldehyde, reached their seasonal maximum at this early phenological stage. Benzyl alcohol was detected only on the first two sampling dates, and it is known to be associated with the flowering period (*7*). Four early-season compounds are, to our knowledge, reported for the first time from apple trees: butyl myristate, isopropyl myristate, isopropyl palmitate, and 2-butoxyethanol. We postulate that these compounds are of flower origin as their quantities decrease continuously after bloom, as described for benzaldehyde and butyl acetate for which flower origin has been shown (*18*, *19*). Benzaldehyde and butyl acetate exhibited a strong repellent effect on codling moth females, indicating that these two flower compounds might be involved in the repellent effect of the tree early in the season. The seasonal flight period of the codling moth starts only after petal fall in Switzerland, whereas flight in warmer regions may begin as early as during the flowering period (*20*), suggesting that the tree might then benefit from these chemical signals. Characteristic for early-season emissions are, in addition to benzyl alcohol, benzaldehyde and butyl acetate (*7*) and also monoterpenes (*21*). In our study, limonene, β-caryophyllene, and (*E*,*E*)-α-farnesene prevailed quantitatively.

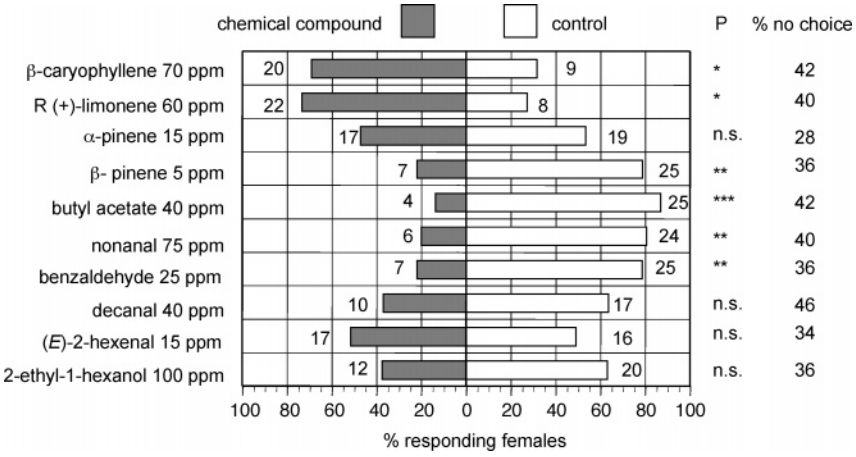

Figure 6. Response of mated female *C. pomonella* to chemical compounds diluted in hexane (50 μL). The same amount (50 μL) of hexane was used as control odor source. Numbers refer to the number of *C. pomonella* choosing an odor source. $N = 50$ females per treatment. χ^2 test: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; ns = $P \geq 0.05$.

However, Δ^3-carene was not detected before June, in contrast to the study by Rapparini et al. (*21*), who collected the volatiles not at petal fall but during bloom and from a different apple cultivar.

In late May, twigs with fruitlet and leaves emitted very low amounts of volatiles, further substantiating the semiquantitative data from a different year where overall volatile emission was low at this period (*2*). The blend emitted in late May did not elicit any choice behavior in the codling moth in the laboratory. Monoterpenoids identified in the plant material include limonene and camphene, coinciding with Rapparini et al. (*21*). β-Pinene was identified throughout the season in our study in contrast to that of Rapparini et al. (*21*), who failed to detect this monoterpene after blooming, possibly due to the different methodology used.

By early June, when the green fruits had reached a size of ~25 mm, volatiles in the headspace of the fruit-bearing twig reached a second maximum, coinciding with the peak in the flight of the first codling moth generation (*22*). Interestingly, the volatiles from twigs collected at this time did not yet significantly attract female codling moths, as such an effect was only observed 4 weeks later when the fruit had reached a size of ~45 mm. The early period when the olfactory cues from the host plant do not yet attract moths might be used by a proportion of the population to leave the orchard in search of new habitats (*3*, *23*).

During July, the amount of volatiles collected in the headspace of the fruit−twig−leaves complex remained relatively constant, whereas a further seasonal maximum was confirmed (*2*) for the first half of August when fruit are ~65 mm in size. This peak coincides with the main flight of the second generation of codling moths (*22*). Codling moths were attracted in the bioassay to the fruit-bearing twigs from July to mid August. During this period, leaves account for a sizable proportion of the biomass, but previous studies were limited to fruit emissions (*2*, *7*) or to the analysis of monoterpenes only (*21*) and are thus not directly comparable. Current data allow only for a limited interpretation of the attractant effect of host-plant odors midsummer.

β-Caryophyllene, which was attractant in our bioassay, reached its maximum emission in the first half of August. Indeed, codling moths showed in the bioassay a clear preference for the fruit-bearing twig with leaves during this period, when given a choice with a blank. We assume that additional host-plant-derived volatile compounds were involved in the long-lasting female attraction, which was observed over a month and a half. Limonene and (*E*,*E*)-α-farnesene (*2*; and discussion below) might have contributed to this effect. The positive response of the moths shows that the repellent effect of nonanal and β-pinene was overcome in the total blend. Similarly, volatiles from mature fruit containing a repellent and an attractant component were no longer repellent but behaviorally ineffective (*24*). In many instances, a mixture of several compounds may determine a moth's response, as recently reported for peach-tree-derived volatiles, which attract the oriental fruit moth, *Cydia molesta* (Busck) (*25*).

A sharp decrease in the amount of volatiles collected in the headspace of fruit-bearing twigs followed the end of August. The preference of female moths for volatiles from the freshly picked plant material declined as well, leaving the host plant neither attractant nor repellent for this pest. Thirty-two compounds were no longer detected at the end of August 2003 in the headspace of the apple cultivar Golden Delicious, particularly esters, aromatic compounds, ether alcohols, acids, and the two terpenoids Δ^3-carene and ocimene. Limonene, for which female attraction was demonstrated in our bioassay, reached its maximum at the end of August, but was obviously without any significant influence on the resulting bioactivity of the blend. Studies from previous summers using different apple cultivars and volatile collection systems detected a large number of esters in the cultivars Red Delicious and Red Astrachan (*26*), found the terpenoid ocimene in the cultivars Bohnäpfel and Discovery (*7*, *11*), and identified Δ^3-carene in the cultivar Earligold (*21*). These marked differences might be due in part to the volatile collection system used and, to a larger degree, to the exceptionally high drought stress of the apple trees in the late summer of 2003 in Switzerland. The Tenax adsorbent resin used (*27*) and the low-oxygen atmosphere in the plastic bag used in the current volatile collection system (*28*) might have contributed to the low level of esters detected. Furthermore, it is known that water deficiency can alter the volatile emission of apple trees substantially (*29*).

Our findings on the seasonal dynamics of headspace volatiles and the different responses of the codling moth in the bioassay strongly indicate that reports on volatile releases and insect response should specify the growth stage of the fruits and the abiotic ambient conditions prevailing. To our knowledge, this is the first study to demonstrate the marked changes in insect behavior during apple development. For subsequent studies aimed at establishing closer relationships between volatile

emissions in situ from infested versus healthy apple and bioactivity on adult insects, it is suggested that either a portable bioassay system for field use be developed or samples of cut plant material be chemically analyzed in parallel to the analyses of field-collected volatiles and the laboratory bioassay. Some quantitative, but not qualitative, differences were noted between in situ and ex situ volatile collections of spruce seed cones (*30*); however, similar amounts of most compounds were found when these two treatments were compared in the case of Fraser fir (*31*). Hence, it is possible but not certain that there are some deviations in the volatiles from intact and cut plant material. Available single-compound bioassays with constituents of the volatile blend of apple revealed a relatively robust behavior of the codling moth over a wide dosage range of the same compound (*6*, *24*), suggesting that quantitative differences in the volatile composition will not easily reverse the response of the codling moth. In deciduous tropical trees, volatile emissions from plant material in situ and ex situ (cut) did not differ beyond the general variability between samples, and the olfactory attraction of associated insects (pollinators) remained at the a constantly high level (R. Kaiser, personal communication).

Behavioral Response of *C. pomonella* to Host Tree Volatiles. Depending on the region, the seasonal flight period of the codling moth may start as early as during bloom, but females are not known to be attracted to apple flowers (*7*). Our bioassay even showed a repellent effect of volatiles during petal fall. A clear positive response of codling moth females to branches with apples was noted when the green fruits had reached a diameter of ~45 mm at the beginning of July. In previous no-choice wind tunnel bioassays, ovipositing females were attracted to branches with green apples 3 weeks after bloom, but the proportion of females attracted remained low, that is, 18%, despite the fact that both olfactory and visual cues were provided (*7*). Olfactory female attraction to 20 cm long branches with several small green apples and leaves in a Y-tube olfactometer has been reported (*12*), but the growth stage of the fruits was not further specified.

However, surprising results were obtained in the present study when apple fruits and twig−leaves were tested separately. Between mid-July and mid-August, volatiles from the twig−leaves complex were repellent to female codling moth, whereas those from apple fruits were clearly attractant. Hence, the female attraction by the complete twig with leaves and apple fruit reported above must be due to the volatiles emitted by the fruit. This conclusion is in good agreement with the field observation that oviposition of the codling moth directly onto the fruit is frequent during the late part of the season, that is, during the flight of the second generation of the codling moth (*4*). Further investigation in future years should reveal to what degree the marked repellent effect of leaves during the dry summer 2003 was related to water stress of the apple tree.

Toward the end of the fruit-growing season, the behavioral effect of the apple fruit and of the twig with leaves on codling moth females waned, similarly as for the combined system comprising apples, twigs, and leaves. A recent study (*11*) also reported no behavioral effect on codling moth females of volatiles from healthy apple fruits picked in August.

Biological Effect of (*E*,*E*)-α-Farnesene. (*E*,*E*)-α-Farnesene is one of the few compounds for which behavioral effects on adult codling moth have been widely established. At low dosages, this terpene attracts mated females (*6*, *9*) and stimulates oviposition (*32*), but these effects wane with increasing dosage. In olfactometer bioassays, a negative dose−response to this compound was demonstrated, with very high dosages, mimicking herbivore-induced volatile emissions from infested apples, being repellent (*6*).

Quantitative analysis indicated an (*E*,*E*)-α-farnesene emission from apple trees of between 6 and 70 ng/μL in this study. This concentration range corresponds to ~1.5−35 ng/L of air in the plastic bag used for volatile collection. The lower end of this range coincides with the range of calculated atmospheric concentration of α-farnesene in the olfactometer air (2−22 ng/L) that resulted in attraction of codling moth females in the bioassays of Hern and Dorn (*6*). The maximum value was found in our study in plant samples from the fruit−twig−leaves complex, which were behaviorally ineffective on the codling moth. We conclude that (*E*,*E*)-α-farnesene in our study was present at dosages which are either attractant or ineffective but not at those dosages which are repellent.

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