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How rainfall, relative humidity and temperature influence volatile emissions from apple trees in situ

Armelle Vallat, Hainan Gu, Silvia Dorn *

Institute of Plant Sciences, Applied Entomology, Swiss Federal Institute of Technology (ETH), CH-8092 Zurich, Switzerland

Abstract

Headspace volatiles from apple-bearing twigs were collected in the field with a Radiello sampler during three different diurnal periods over the complete fruit growing season. Analyses by thermal desorption–GC–MS identified a total of 62 compounds in changing quantities, including the terpenoids α-pinene, camphene, β-pinene, limonene, β-caryophyllene and (E,E)-α-farnesene, the aldehydes (E)-2-hexenal, benzaldehyde and nonanal, and the alcohol (Z)-3-hexen-1-ol. The variations in emission of these plant odours were statistically related to temperature, humidity and rainfall in the field. Remarkably, rainfall had a significant positive influence on changes in volatile release during all three diurnal periods, and further factors of significance were temperature and relative humidity around noon, relative humidity in the late afternoon, and temperature and relative humidity during the night. Rainfall was associated consistently with an increase in the late afternoon in terpene and aldehyde volatiles with a known repellent effect on the codling moth, one of the key pests of apple fruit. During the summer of 2003, a season characterized by below-average rainfall, some postulated effects of drought on trees were tested by establishing correlations with rainfall. Emissions of the wood terpenes α-pinene, β-pinene and limonene were negatively correlated with rainfall. Another monoterpene, camphene, was only detected in this summer but not in the previous years, and its emissions were negatively correlated with rainfall, further supporting the theory that drought can result in higher formation of secondary metabolites. Finally, the two green leaf volatiles (E)-2-hexenal and (Z)-3-hexen-1-ol were negatively correlated with rainfall, coinciding well with the expectation that water deficit stress increases activity of lipoxygenase. To our knowledge, this work represents the first empirical study concerning the influence of abiotic factors on volatile emissions from apple trees in situ.
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Keywords: Malus domestica Borkh.; Lepidoptera Tortricidae; Cydia pomonella; Apple tree; Abiotic factors; Temperature; Relative humidity; Rainfall; Volatiles; Plant stress

1. Introduction

Chemical changes in plants in response to changing environmental conditions have intrigued experts in chemistry, ecology and food science. Variation in emitted plant odours can be triggered by a changing biotic environment such as herbivore feeding (reviewed by Karban and Baldwin, 1997; Dicke and van Loon, 2000; Mattiacci et al., 2000), or by varying abiotic factors such as temperature and humidity (Takabayashi et al., 1994; Gouinguené and Turlings, 2002). Altered chemical emissions can influence interactions with organisms in the environment of the plant, resulting for example in a modified susceptibility to insects and pathogens (Hildebrand, 1989). Changes in the composition of secondary metabolites can in the case of an agricultural crop such as apple, have a significant influence on the flavour (Schmitz-Eiberger et al., 2003). While variations due to biotic factors, in particular induction

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* Corresponding author. Tel.: +41 44 632 3921; fax: +41 44 632 1171.
E-mail address: silvia.dorn@ipw.agrl.ethz.ch (S. Dorn).

of volatile release by herbivore insects, have been studied intensively (e.g. Boevé et al., 1996; Hern and Dorn, 2001), relatively little information is available on how abiotic conditions affect odour emissions.

Abiotic conditions and subsequent volatile releases from plants may be subject to dramatic changes during a diurnal cycle (Loughrin et al., 1994). Temperature and humidity were shown to influence the composition of released blends significantly, even in perennial plants. In the pine, *Pinus halepensis* L., monoterpene emission rate increased with temperature at different humidities, while temperature had only an impact at high humidity in the oak, *Quercus ilex* L. (Llusià and Peñuelas, 1999). These experiments were carried out with potted plants under standardized indoor conditions, similarly as those with the slash pine, *Pinus elliottii* Engelm., where monoterpene emissions increased again with temperature, and light had no direct effect (Tingey et al., 1980).

Water deficit stress is known as a further key abiotic factor influencing plant metabolism profoundly. It alters carbon allocation between roots and shoots (Teskey et al., 1987) and changes nutrient uptake ratios and nutrient circulation (Schulze, 1991), with major implications on volatile emissions and ecology (Takabayashi et al., 1994). In potted apple plants grown under largely controlled conditions in the greenhouse, water deficit stress increased the emission of volatiles with a chain length of n-C_6-carbons (Ebel et al., 1995).

Laboratory and greenhouse studies are ideal frames for factorial experiments, but they provide incomplete information with respect to the complex situations and interactions found in the field. Potted plants, for example, have a limited soil volume surrounding the rhizosphere, hence even experimental designs to study water deficit stress require relatively frequent irrigation to avoid complete desiccation of the plants. Furthermore, rainfall cannot be optimally simulated by soil irrigation, as the leaves remain dry instead of being wetted by drops of water falling on them over a certain period of time. Finally, the response of plants in the field may be subject to combined influences of several environmental variables, including temperature, relative humidity and rainfall.

This study aims at addressing this gap in knowledge by investigating the influence of the abiotic factors, temperature, relative humidity, and rainfall on the emission of volatiles from the apple tree (*Malus domestica* Borkh.) in situ. The volatile profiles from the headspace of fruit-bearing twigs with leaves will be analysed in total, with special emphasis on the terpenes and aldehydes which are important constituents of apple volatiles (Takabayashi et al., 1994; Hern and Dorn, 2002) and can have a behavioural effect on leptidopteran herbivore insects of the apple tree. One of the most serious pests of apple worldwide, the codling moth *Cydia pomonella* L. (Lepidoptera, Tortricidae)

(Dorn et al., 1999), utilises odours from apple trees in search for oviposition sites (Yan et al., 1999; Hern and Dorn, 2002). The moth flies towards the host tree in the evening while no movements are observed at noon (Bovey, 1966). Mated females are attracted to the dosages of some terpenes as they are emitted from healthy apples, including (E,E)-α-farnesene (Hern and Dorn, 1999), limonene and β-caryophyllene, while they are repelled by β-pinene and the aldehydes nonanal and benzaldehyde (Vallat and Dorn, 2005). In the field in late August, emissions from ripening apple fruit were found to be neither attractant nor repellent for approaching female codling moths (Hern and Dorn, 2002). All these findings together indicate that changing compositions of volatile blends emitted from the apple plant might result in contrasting behavioural responses of this herbivore pest insect, and that the influence of abiotic conditions might play an important role in this ecological context.

Starting from volatile collections carried out over the complete growing season and during three different diurnal periods, statistical methods were applied to evaluate the effect of humidity, temperature and rainfall on the profiles of volatiles and on components known for their bioactivity on the codling moth. To our knowledge, this work represents the first empirical study concerning the influence of abiotic factors on volatile emission from apple trees under field conditions.

2. Results and discussion

2.1. Seasonal changes in volatile emissions

Overall, 62 volatile compounds were identified in the headspace of apple fruit-bearing twigs with leaves, among them 9 terpenoids and 10 aldehydes (Tables 1 and 2). The 10 compounds printed in bold are discussed in more detail below; all of them are apple constituents and most of them are behaviourally effective on the codling moth. Volatile emissions in situ changed significantly over the growing season 2003 (ANOVA repeated measures: $F = 147.202$, $P < 0.0001$). Emissions from three individual apple trees used for volatile collection did not differ significantly (ANOVA repeated measures: $F = 0.865$, $P > 0.05$). Furthermore, no significant interaction was found between individual apple trees and the sampling dates (ANOVA repeated measures: $F = 0.788$, $P > 0.05$).

The marked seasonal dynamics of volatile emission observed in summer 2003, with two maxima of overall emissions measured in early June and mid August, coincide well with the results of a previous study that focused on apple fruit only and that was carried out in summer 1998 (Hern and Dorn, 2003). Altered biotic conditions such as infestation by codling moth larvae

Table 1
Total volatile compounds detected in the headspace of apple bearing-twig with leaves (period 3.30 p.m. to 7.30 p.m.) over the growing season, mean peak area ± SE (mV min × 10³, n = 15)

Compounds	RT[a] (min)	Petal fall stage 29 April	Fruitlet Ø[b], 15 mm 22 May	Fruit Ø, 24 mm 5 June	Fruit Ø, 35 mm 19 June	Fruit Ø, 45 mm 2 July	Fruit Ø, 55 mm 15 July	Fruit Ø, 60 mm 29 July	Fruit Ø, 68 mm 12 August	Fruit Ø, 72 mm 26 August
Terpenoids										
α-Pinene*[,c,d,e,h]	7.76	667 ± 185	1099 ± 241	2916 ± 421	2391 ± 182	3484 ± 199	591 ± 44	2744 ± 253	2333 ± 383	1350 ± 464
Camphene*[,c,d,e]	8.26	266 ± 74	158 ± 47	162 ± 33	153 ± 44	137 ± 30	16 ± 28	71 ± 11	77 ± 12	nd
β-Pinene*[,c,d,e]	9.23	298 ± 59	291 ± 66	920 ± 157	728 ± 52	944 ± 81	192 ± 32	586 ± 55	619 ± 80	311 ± 131
Δ³-Carene*[,c,d,e]	10.45	nd	nd	12383 ± 3867	7605 ± 1904	7313 ± 826	2911 ± 288	5428 ± 1291	4106 ± 932	nd
Limonene*[,c,d,e,h]	11.08	1875 ± 367	707 ± 162	1148 ± 144	796 ± 80	1330 ± 193	138 ± 32	521 ± 108	447 ± 71	2239 ± 702
Ocimene*	13.22	779 ± 321	223 ± 70	1234 ± 257	1116 ± 289	1352 ± 271	136 ± 27	261 ± 54	423 ± 66	nd
β-Caryophyllene*[,c,d, e]	22.40	14906 ± 3442	1075 ± 272	12275 ± 1794	13649 ± 1479	11703 ± 2070	4999 ± 367	3988 ± 681	9378 ± 2264	840 ± 197
(E,E)-α-Farnesene*[,d,e]	22.80	14793 ± 2147	1975 ± 680	9616 ± 2018	9540 ± 972	7092 ± 1372	3353 ± 410	2668 ± 410	3834 ± 979	853 ± 203
Spathulenol*[,c]	24.40	5341 ± 2176	717 ± 225	3212 ± 509	3682 ± 489	3228 ± 591	1457 ± 201	1193 ± 229	1761 ± 501	1509 ± 203
Aldehydes										
Hexanal*[,c,d]	3.90	2409 ± 560	887 ± 276	1732 ± 377	1911 ± 281	2418 ± 530	1337 ± 154	1870 ± 497	2468 ± 435	1582 ± 412
(E)-2-hexenal*[,c,d]	4.90	1362 ± 179	214 ± 54	618 ± 201	842 ± 355	964 ± 148	354 ± 68	315 ± 77	501 ± 119	640 ± 127
Heptanal*[,c,d]	6.70	2302 ± 336	635 ± 137	1584 ± 185	1667 ± 219	1769 ± 206	1033 ± 162	857 ± 106	1205 ± 160	1195 ± 439
Benzaldehyde*[,e]	8.60	4040 ± 1237	1060 ± 140	1440 ± 191	1201 ± 211	1148 ± 191	609 ± 54	766 ± 186	684 ± 74	592 ± 310
Octanal[d]	10.22	2011 ± 366	1236 ± 411	4286 ± 465	4296 ± 562	4474 ± 471	1577 ± 153	1643 ± 129	2384 ± 571	nd
Nonanal*[,d,e]	13.73	7783 ± 930	2373 ± 436	17229 ± 2558	21682 ± 2715	22031 ± 2258	6997 ± 238	6445 ± 867	10669 ± 2045	266 ± 70
Decanal*[,d,e]	16.95	7525 ± 959	1478 ± 420	9222 ± 1220	14020 ± 1838	13501 ± 2511	7871 ± 268	5625 ± 509	10009 ± 1828	1289 ± 581
4-Methoxybenzaldehyde	18.38	496 ± 133	177 ± 46	573 ± 76	433 ± 39	1081 ± 196	nd	122 ± 29	164 ± 40	nd
Undecanal	20.00	3938 ± 530	471 ± 145	2258 ± 382	3377 ± 459	4400 ± 710	1899 ± 192	1461 ± 277	2553 ± 709	nd
3,5-di-tbut-4-hydroxybenz.[†,i]	31.60	1070 ± 295	nd	1094 ± 353	1024 ± 157	1170 ± 227	614 ± 40	386 ± 61	589 ± 108	nd
Alcohols/phenols										
(Z)-3-hexen-1-ol*[,c,e]	5.30	4569 ± 2658	657 ± 114	580 ± 76	967 ± 241	822 ± 126	499 ± 58	647 ± 106	968 ± 213	857 ± 280
Phenol*[,f,g,h]	9.55	1385 ± 163	584 ± 76	869 ± 177	871 ± 143	873 ± 77	391 ± 36	458 ± 76	507 ± 67	331 ± 102
2-Ethylhexan-1-ol*[,g,h]	11.18	11604 ± 1898	1748 ± 447	1961 ± 342	2494 ± 477	2597 ± 249	1230 ± 276	2636 ± 777	2308 ± 473	nd
Benzyl alcohol[e]	11.30	3302 ± 616	nd	nd	nd	nd	nd	nd	nd	nd
m-tert-Butyl phenol[i]	19.65	3887 ± 599	1109 ± 418	2080 ± 347	2735 ± 564	2353 ± 301	1088 ± 251	711 ± 231	782 ± 253	133 ± 35
Butylated hydroxytoluene[i]	25.60	6099 ± 881	767 ± 192	3543 ± 573	4718 ± 512	2992 ± 379	1400 ± 254	1042 ± 221	2601 ± 953	nd
Ether-alcohols										
2-Butoxyethanol[h]	6.90	324 ± 98	405 ± 140	766 ± 85	76 ± 42	58 ± 33	32 ± 55	nd	nd	nd
1(2-Butoxyethoxy)-ethanol[i]	16.50	2583 ± 546	441 ± 78	1762 ± 315	1141 ± 181	1044 ± 67	166 ± 32	103 ± 21	247 ± 54	nd
2-Phenoxyethanol[i]	17.40	2803 ± 501	1006 ± 236	2694 ± 599	2071 ± 477	1737 ± 252	316 ± 45	306 ± 47	221 ± 60	nd
Ketones										
6-Methyl-5-hepten-2-one*	9.70	2305 ± 596	812 ± 165	1287 ± 198	1742 ± 179	1728 ± 102	592 ± 25	401 ± 35	770 ± 134	784 ± 291
Acetophenone*[,d,h]	12.30	1917 ± 319	468 ± 44	665 ± 90	612 ± 105	688 ± 72	189 ± 37	289 ± 33	236 ± 36	nd
Benzophenone*[,d,e]	28.40	2549 ± 756	749 ± 225	4457 ± 347	5666 ± 1099	3391 ± 195	2041 ± 174	1425 ± 342	2637 ± 858	1103 ± 298
Acids										
2-Ethylhexanoic acid*[,i]	14.63	354 ± 80	50 ± 52	409 ± 101	2269 ± 693	1696 ± 326	438 ± 37	nd	879 ± 269	nd
Octanoic acid*[,e,h]	16.10	1415 ± 247	316 ± 75	657 ± 104	1817 ± 283	1267 ± 334	359 ± 44	270 ± 81	nd	nd
Nonanoic acid[d]	19.00	nd	nd	nd	nd	nd	nd	212 ± 41	1455 ± 362	nd
Esters										

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	RT[a]									
Butyl acetate[*,d,e,h]	4.20	339 ± 91	119 ± 50	207 ± 69	186 ± 25	227 ± 56	143 ± 15	152 ± 25	77 ± 11	136 ± 40
Butyl laurate[*,d]	32.04	nd	nd	61 ± 66	643 ± 83	253 ± 52	70 ± 46	nd	65 ± 93	nd
Isopropyl myristate[e,h]	32.88	759 ± 337	207 ± 47	108 ± 17	243 ± 86	144 ± 46	308 ± 33	118 ± 39	113 ± 30	nd
Butyl myristate[d,e]	36.18	579 ± 224	124 ± 29	131 ± 20	232 ± 53	104 ± 27	nd	nd	nd	nd
Isopropyl palmitate[e,h]	36.95	2095 ± 532	125 ± 65	220 ± 41	347 ± 81	381 ± 105	142 ± 32	60 ± 66	84 ± 58	nd
Alkanes										
4-Methyloctane	5.50	465 ± 78	205 ± 42	696 ± 116	519 ± 78	1112 ± 131	nd	705 ± 102	147 ± 26	484 ± 148
n-Undecane	13.63	3045 ± 1081	488 ± 95	2536 ± 211	2192 ± 294	2062 ± 226	386 ± 37	434 ± 97	905 ± 183	2438 ± 851
n-Dodecane[e]	16.85	7984 ± 1285	700 ± 189	5961 ± 793	5955 ± 869	4942 ± 411	1570 ± 167	1220 ± 202	2543 ± 666	nd
n-Tridecane[d]	19.83	8478 ± 1777	521 ± 141	6001 ± 638	6439 ± 766	3957 ± 339	1880 ± 232	1604 ± 269	3989 ± 127	274 ± 108
n-Tetradecane[d]	22.65	12427 ± 3344	1025 ± 148	9061 ± 1396	10226 ± 1598	7592 ± 1332	3436 ± 329	2575 ± 365	5910 ± 1222	1076 ± 234
n-Eicosane[*,c,e]	36.45	832 ± 168	459 ± 169	294 ± 38	543 ± 99	123 ± 42	333 ± 35	232 ± 69	197 ± 33	nd
Alkenes										
Hexene	3.75	1097 ± 326	245 ± 102	544 ± 57	556 ± 76	825 ± 92	388 ± 57	821 ± 265	757 ± 200	555 ± 204
Unknown compound	12.60	334 ± 76	191 ± 44	799 ± 188	918 ± 167	745 ± 69	277 ± 38	263 ± 38	329 ± 36	nd
Unknown compound	15.93	nd	nd	nd	nd	nd	nd	351 ± 52	567 ± 109	nd
1-Dodecene[e]	16.57	1771 ± 291	468 ± 109	1623 ± 211	1692 ± 250	1509 ± 197	375 ± 40	294 ± 50	523 ± 113	nd
Other										
Diphenylamine[i]	28.20	5562 ± 1594	485 ± 174	2381 ± 595	2302 ± 609	1890 ± 355	1153 ± 225	875 ± 302	1149 ± 296	2107 ± 660
Benzothiazole[g]	17.40	nd	nd	nd	nd	1393 ± 242	406 ± 63	293 ± 70	488 ± 77	nd
Aromatic hydrocarbons										
Ethylbenzene[*,g]	5.40	996 ± 159	1509 ± 315	398 ± 72	296 ± 26	1541 ± 275	298 ± 46	264 ± 31	224 ± 29	503 ± 134
p-Xylene[*,g,h]	5.65	2744 ± 364	3251 ± 595	1083 ± 212	646 ± 68	1905 ± 175	718 ± 28	948 ± 202	630 ± 108	1185 ± 359
Styrene[g]	6.30	2711 ± 570	393 ± 82	1327 ± 353	443 ± 117	425 ± 54	307 ± 58	335 ± 95	266 ± 67	563 ± 211
m-Xylene[*,g,h]	6.40	1557 ± 222	1327 ± 288	871 ± 148	516 ± 46	1168 ± 164	392 ± 41	565 ± 57	423 ± 51	nd
Propylbenzene[*,g]	8.45	543 ± 118	363 ± 65	296 ± 43	188 ± 45	355 ± 22	80 ± 15	183 ± 40	114 ± 23	84 ± 26
3-Ethyltoluene[*,g]	8.70	1741 ± 286	1876 ± 333	1174 ± 232	631 ± 88	2172 ± 242	2986 ± 49	961 ± 146	571 ± 135	nd
1,3,5-Trimethylbenzene[*,g]	8.95	930 ± 123	657 ± 128	730 ± 160	433 ± 59	1005 ± 114	98 ± 17	nd	278 ± 42	nd
2-Ethyltoluene[*,g]	9.35	853 ± 129	481 ± 97	415 ± 71	318 ± 30	660 ± 59	105 ± 16	244 ± 27	193 ± 35	nd
1,2,4-Trimethylbenzene[*,g]	9.80	3342 ± 713	2468 ± 216	3913 ± 382	1825 ± 428	3793 ± 199	484 ± 52	1291 ± 260	1054 ± 205	nd
1,2,3-Trimethylbenzene[*,g]	10.80	670 ± 103	478 ± 53	752 ± 139	564 ± 81	1018 ± 105	142 ± 33	306 ± 67	303 ± 57	218 ± 54
Naphthalene	16.15	2428 ± 602	544 ± 126	2597 ± 231	1658 ± 237	2098 ± 181	595 ± 37	453 ± 86	1462 ± 593	nd

In bold: compounds discussed in the text.

nd = not detected.

[a] RT retention time.

[b] Ø diameter of apple fruit.

Origins of volatiles according to literature reports:

[c] Apple leaf.

[d] Apple fruit.

[e] Flower of apple tree (Dimick and Hoskin, 1983; Maarse and Visscher, 1989; Altenburger and Matile, 1990; Loughrin et al., 1990; Omata et al., 1990; Buchbauer et al., 1993; Lopez et al., 1998; Raguso and Pellmyr, 1998; Young et al., 1999; Rapparini et al., 2001; Schiestl and Marion-Poll, 2002).

[f] Plant origin reported from different plant species (Barron and Etiévant, 1990; Suárez et al., 1993; Mattheis et al., 1997; Vernin et al., 1998; Ibáñez et al., 1999; Picone et al., 2002; Pino et al., 2002).

[g] Air pollutants (e.g. Brown et al., 1994; Grosjean et al., 1998; Galiulin et al., 2002).

[h] Liquid carriers used in pesticides (Anonymous, 1995; Nabors et al., 2003).

[i] Environmental contaminants (pers. comm. R. Kaiser and F. Jüttner).

[*] Definitively identified.

[†] 3,5-di-tert butyl-4-hydroxybenzaldehyde[f].

Table 2
Terpenes, aldehydes and alcohols/phenols detected in the headspace of apple bearing-twig with leaves (period 7.30 p.m. to 7.30 a.m.) over the growing season, mean peak area ± SE (mV min × 10³, n = 15)

Compounds	RT[a] (min)	Petal fall stage 29 April	Fruitlet Ø [b] 15 mm 22 May	Fruit Ø, 24 mm 5 June	Fruit Ø, 35 mm 19 June	Fruit Ø, 45 mm 2 July	Fruit Ø, 55 mm 15 July	Fruit Ø, 60 mm 29 July	Fruit Ø, 68 mm 12 August	Fruit Ø, 72 mm 26 August
Terpenoids										
α-Pinene[*,c,d,e,h]	7.76	575 ± 91	3173 ± 172	2373 ± 314	1871 ± 235	2498 ± 495	4207 ± 230	1879 ± 126	1812 ± 343	942 ± 201
Camphene[*,c,d,e]	8.26	242 ± 65	276 ± 59	182 ± 31	144 ± 39	149 ± 118	211 ± 33	73 ± 18	145 ± 32	nd
β-Pinene[*,c,d,e]	9.23	271 ± 49	790 ± 149	809 ± 69	502 ± 157	548 ± 30	1128 ± 53	447 ± 28	608 ± 72	185 ± 77
Δ³-Carene[*,c,d,e]	10.45	nd	nd	9843 ± 2278	6015 ± 762	4676 ± 279	7688 ± 2166	3762 ± 703	3904 ± 998	nd
Limonene[*,c,d,e,h]	11.08	1170 ± 197	2058 ± 242	946 ± 173	535 ± 74	735 ± 37	1766 ± 104	351 ± 73	312 ± 37	1065 ± 321
Ocimene[*]	13.22	779 ± 267	644 ± 103	1088 ± 235	789 ± 215	967 ± 177	1132 ± 114	209 ± 61	321 ± 40	nd
β-Caryophyllene[*,c,d,e]	22.40	4718 ± 431	3293 ± 304	8583 ± 641	7302 ± 1017	4972 ± 332	14182 ± 1621	2172 ± 267	4313 ± 939	448 ± 86
(E,E)-α-Farnesene[*,d,e]	22.80	7153 ± 777	5747 ± 537	8405 ± 420	6742 ± 1029	5568 ± 1103	9179 ± 1210	1722 ± 409	2740 ± 485	395 ± 157
Spathulenol[*,c]	24.40	2528 ± 182	2020 ± 192	3475 ± 208	2801 ± 396	2632 ± 642	4141 ± 565	772 ± 196	1185 ± 263	636 ± 175
Aldehydes										
Hexanal[*,c,d]	3.90	1947 ± 343	1951 ± 452	2521 ± 349	1573 ± 241	1597 ± 300	2327 ± 267	920 ± 201	1683 ± 236	995 ± 171
(E)-2-hexenal[*,c,d]	4.90	879 ± 253	694 ± 212	867 ± 114	699 ± 176	678 ± 203	1066 ± 163	183 ± 36	297 ± 53	262 ± 119
Heptanal[*,c,d]	6.70	1407 ± 172	1340 ± 135	1796 ± 92	1383 ± 291	1302 ± 292	1969 ± 90	634 ± 52	1267 ± 140	682 ± 68
Benzaldehyde[*,e]	8.60	3397 ± 1264	1375 ± 211	1708 ± 334	983 ± 178	1088 ± 160	1337 ± 129	654 ± 49	972 ± 172	503 ± 108
Octanal[d]	10.22	1316 ± 148	2445 ± 220	4258 ± 502	3255 ± 386	2514 ± 221	4154 ± 192	1116 ± 98	2052 ± 196	nd
Nonanal[*,d,e]	13.73	4607 ± 538	7619 ± 624	16700 ± 1591	17391 ± 2403	15070 ± 3332	21406 ± 1468	4286 ± 451	8777 ± 1394	87 ± 31
Decanal[d,e]	16.95	3547 ± 357	3838 ± 235	9536 ± 1303	9773 ± 1531	10194 ± 2748	18772 ± 2023	3612 ± 434	6000 ± 663	619 ± 126
4-Methoxybenzaldehyde	18.38	265 ± 66	207 ± 50	604 ± 112	320 ± 42	167 ± 18	nd	87 ± 19	140 ± 18	nd
Undecanal	20.00	1832 ± 243	1485 ± 486	2742 ± 128	2809 ± 375	2352 ± 332	5871 ± 646	1040 ± 168	1539 ± 269	nd
3,5-di-*t*but-4-hydroxybenz.[†,i]	31.60	nd	nd	844 ± 215	908 ± 160	567 ± 70	1612 ± 230	226 ± 59	528 ± 88	nd
Alcohols/phenols										
(Z)-3-hexen-1-ol[*,c,e]	5.30	12208 ± 5409	881 ± 195	507 ± 41	968 ± 181	453 ± 29	1607 ± 751	395 ± 45	607 ± 110	440 ± 182
Phenol[*,f,g,h]	9.55	976 ± 219	1026 ± 188	785 ± 89	784 ± 93	598 ± 61	796 ± 59	315 ± 48	383 ± 73	120 ± 59
2-Ethylhexan-1-ol[*,g,h]	11.18	2778 ± 1054	4527 ± 379	3300 ± 735	1990 ± 249	2928 ± 546	832 ± 121	1901 ± 555	2335 ± 535	nd
Benzene methanol[e]	11.30	439 ± 87	nd	nd	nd	nd	nd	nd	nd	nd
m-tert-Butyl phenol[i]	19.65	2225 ± 900	2172 ± 444	2221 ± 266	2190 ± 408	1407 ± 265	1841 ± 224	424 ± 104	556 ± 154	164 ± 72
Butylated hydroxytoluene[i]	25.60	2460 ± 728	2503 ± 270	4135 ± 352	3416 ± 672	2045 ± 342	2999 ± 339	732 ± 227	1377 ± 428	nd

In bold: compounds discussed in the text.

nd = not detected.

[a] RT retention time.

[b] Ø diameter of apple fruit.

Origins of volatiles according to literature reports:

[c] Apple leaf.

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[i] Environmental contaminants (pers. comm. R. Kaiser and F. Jüttner).

[*] Definitively identified.

[†] 3,5-di-*tert* butyl-4-hydroxybenzaldehyde[i].

are known to be associated with increased emissions of distinct volatile components (Landolt et al., 2000; Hern and Dorn, 2001, 2002), but the directions of change under altered abiotic conditions remained to be elucidated.

2.2. Effect of abiotic factors on overall emission of volatiles

The influence of changing abiotic conditions on the release of odours from apple trees in the field was assessed using three diurnal periods of sampling (11.30 a.m. to 3.30 p.m., 3.30 p.m. to 7.30 p.m., and 7.30 p.m. to 7.30 a.m.) focusing on the plant compounds printed in bold (Table 1). Effects of two key abiotic factors, i.e. temperature (T, °C) and relative humidity (RH, %), on the volatile emissions were examined by stepwise multiple regression. The relationship of volatile emission with rainfall (RF, mm) was analysed based on the daily mean value of precipitation. During the first time period, i.e. around noon, all climatic factors, daily rainfall, temperature, and relative humidity had significant effects on the volatile emissions, with the relationship of the mean peak area (MP) with the three climatic factors fitting the regression equation: MP = $-206262542.238 + 6695047.734T + 552760.474$RF + 1611342.263RH (ANOVA: $F = 39.965$; $P < 0.0001$). During the second time period covering late afternoon, variation in emitted plant odours was related to daily rainfall and relative humidity. The relationship of the mean peak area with these two climatic factors fit the multiple regression equation: MP = $39055144.992 + 396987.358$RF $- 420587.824$RH (ANOVA: $F = 60.630$; $P < 0.0001$). During the third time period, i.e. over night, the volatile emissions related to changes in daily rainfall, temperature and relative humidity, with the regression equation being described as: MP = $-61402139.964 + 3239346.896T + 303294.358$RF $+ 417034.574$RH (ANOVA: $F = 32.340$; $P < 0.0001$).

Daily rainfall significantly influenced the emission of volatiles from fruit-bearing twig with leaves in the field. Remarkably, the direction of change was an increase in emission. Similarly, release of flavour components in *Camellia sinensis* (L.) and *Olea europaea* (L.) were positively influenced by rainfall under natural conditions (Owuor, 1992; Gálan et al., 2004).

In addition to these climatic factors, the application of pesticides might also have an influence on the composition of volatiles emitted by the tree. The study orchard was managed properly to yield healthy fruit, similar to the orchards used for related studies on plant volatile emissions (Boevé et al., 1996; Hern and Dorn, 2003). The possible abiotic influence of plant protection agents on the volatile blend released by the plant could be subject to an independent study.

2.3. Effect of abiotic factors on terpene emissions

Emissions of the three terpenes which attract codling moth in the dosage ranges measured in our study, (E,E)-α-farnesene, β-caryophyllene and limonene, were negatively correlated to temperature and relative humidity during the late afternoons. The relationship between these terpenes and the two abiotic factors can be described by the following regression equations: (E,E)-α-farnesene MP = $40831767.292 - 783004.289T - 347390.983$RH (ANOVA: $F = 32.077$, $P < 0.0001$); β-caryophyllene MP = $35034738.946 - 526831.204T - 319017.389$RH (ANOVA: $F = 26.198$, $P < 0.0001$); limonene MP = $7365641.878 - 150242.554T - 58008.595$RH (ANOVA: $F = 34.211$, $P < 0.0001$). Hence, the low relative humidity of ambient air registered for late afternoons, together with the relatively high temperatures, are expected to favour release of these three compounds. During the time period from 7.30 p.m. to 7.30 a.m., temperature was correlated with emissions of all three compounds, as shown in the following three equations (E,E)-α-farnesene MP = $703174.073 + 286138.896T$ (ANOVA: $F = 12.961$, $P = 0.0004$); β-caryophyllene MP = $-7709673.807 + 822657.519T$ (ANOVA: $F = 95.490$, $P < 0.0001$) and limonene MP = $1632505.343 - 39645.104T$ (ANOVA: $F = 5.838$, $P = 0.017$). These relationships indicate that high temperatures during the night positively influence the release of (E,E)-α-farnesene and β-caryophyllene, but not of limonene. To our knowledge, this is the first study to analyse such correlations, as a previous investigation focusing on emissions of terpenes such as farnesene, caryophyllene and limonene could not measure ambient air humidity (Rosenfeld et al., 2002).

Emissions of the terpene which repels codling moth in the dosage range measured in our study, β-pinene, were positively correlated with temperature and daily rainfall over the growing season for the time period covering late afternoon, as described by the regression equation: MP = $200948.427 + 9776.572T + 3544.703$RF (ANOVA: $F = 8.170$, $P = 0.0005$). Similarly, emissions were positively correlated with these two abiotic factors, as well as with relative humidity, during the period from 7.30 p.m. to 7.30 a.m., as reflected by the equation MP = $-2499218.638 + 69145.950T + 3180.868$RF + 25260.079RH (ANOVA: $F = 27.268$, $P < 0.0001$). Total monoterpene emissions, including α- and β-pinene, from potted *P. halepensis* L. and *Q. ilex* L. in a climatic chamber were higher at 35 °C than at 15 °C, and an influence of relative humidity was found in the second case (Llusià and Peñuelas, 2000). Greenhouse investigations on α-pinene, a volatile of *Q. ilex*, indicated a 3-fold increase in emission with an increase in temperature from 20 to 30 °C (Loreto et al., 1996), however, data on relative humidity are not available.

In conclusion, the direction in change of release of the discussed bioactive mono- and sesquiterpenes

cannot be generalized, as increase or decrease of emission was subject to compound-dependent daily changes. These daily changes might be related to the photophase (Loughrin et al., 1994). In the light of these findings, it is difficult to extrapolate from results of laboratory and controlled greenhouse studies to the total field complexity.

When considering all factors, the emissions of (E,E)-α-farnesene, β-caryophyllene, limonene, and β-pinene from apple trees always depend on temperature, sometimes also on the interactions between temperature and relative humidity, and in the case of β-pinene also on daily rainfall. Since these volatiles, at the amounts detected over the growing season, have a behavioural effect on *C. pomonella* females (Hern and Dorn, 1999; A. Vallat and S. Dorn, 2005), our findings suggest that marked changes in abiotic conditions could eventually have impact on this species and possibly other insects. In the case of the codling moth, however, a robust behaviour was noted towards (E,E)-α-farnesene, as response did not change unless the terpene dosage was altered by an order of several magnitudes (Hern and Dorn, 1999). Indeed, the mean peak area of (E,E)-α-farnesene between the beginning of July to mid August had an amplitude of ±30% only in the variation of emission (MP = 5204572 ± 1675014 mV min).

The inclusion of rainfall into our study appears to have contributed considerably to the understanding of variation in emissions of wood terpenes, aldehydes (see below) and total volatiles. A recent field study on terpene emission from *P. halepensis*, *Q. ilex* and five other Mediterranean woody plant species measured temperature and relative humidity as abiotic parameters (Llusià and Peñuelas, 2000). The authors came to the conclusion that each of these factors alone is insufficient for understanding changes in emissions. The documented emissions did not fully follow temperature, relative humidity, or the physiological parameters measured, suggesting that a combination of these factors or other factors such as long-term drought exposure could be involved in the emission control (Llusià and Peñuelas, 2000).

2.4. Effect of abiotic factors on aldehyde emissions

Emissions of the aldehydes nonanal and benzaldehyde, which repel codling moths in the dosage ranges measured in our study, were positively related to daily rainfall. The relationships with temperature and relative humidity, however, were positive for nonanal and negative for benzaldehyde, as described by the regression equations: for the late afternoon period, nonanal MP = −28973104.934 + 1008485.069T + 162701.421RF + 221897.741RH (ANOVA: F = 13.303, P < 0.0001) and benzaldehyde MP = 8311561.603 − 172336.510T + 16837.991RF − 70176.236RH (ANOVA: F = 50.744, P < 0.0001); during night, nonanal MP = −68900436.949 + 2184573.681T + 127453.813RF + 550536.518RH (ANOVA: F = 70.176, P < 0.0001) and benzaldehyde MP = 7057077.567 − 80017.676T + 14912.635RF − 63654.119RH (ANOVA: F = 41.403, P < 0.0001).

Behavioural response of the codling moth to rapidly changing abiotic conditions might be balanced, to a certain degree, by their opposite relationships with these two aldehydes. While increasing temperature and relative humidity favoured the release of nonanal, the same conditions lowered the release of benzaldehyde. Daily rainfall, however, was consistently associated with an increase in all three volatiles with a known repellent effect on females, i.e. nonanal, benzaldehyde and β-pinene. This finding seems to support our hypothesis that female codling moths are behaviourally adapted to the conditions that are suboptimal for oviposition, e.g. when the plant tissue is wet.

2.5. Verification of effect of water deficit stress in the field

Due to the extremely dry and warm weather conditions in Switzerland in summer 2003 (Fallot, 2003), there was water deficit stress in the non-irrigated trial orchard. These exceptional field conditions are well suitable for verifying some postulated effects of drought on trees.

The concentration of α-pinene significantly increased in Scots Pine (*Pinus sylvestris* L.) under severe drought (Turtola et al., 2000), suggesting that drought stress could alter emissions of wood terpenes also in apple trees. In fact, a significant negative correlation was found between rainfall and emissions of α-pinene in the current study (Spearman's non-parametric: r = −0.565, P < 0.0001). The lower the precipitation was, the higher the release of this volatile wood terpene. In addition, further significant correlations were determined for two other monoterpenes, β-pinene (r = −0.499, P = 0.0001) and limonene (r = −0.919, P < 0.0001).

Water deficit was found to retard the growth of conifers, and it was postulated that the carbon fixed in photosynthesis could be used for the formation of secondary metabolites (Turtola et al., 2000, and literature cited therein). Interestingly, the monoterpene camphene was detected in the headspace of apple trees throughout the dry season 2003, being negatively correlated to rainfall (Spearman's non-parametric: r = −0.263, P = 0.0430), while it was not detected in the pilot study in summer 2002 (A. Vallat and S. Dorn, 2005). Our results support the theory that drought can result in higher formation of secondary metabolites.

Potted apple trees, subject to severe water stress, emitted 5–310 times higher levels of green leaf volatiles

(consisting of C_6-alcohols, aldehydes and esters) (Ebel et al., 1995). In the current field study, the two green leaf volatiles, (E)-2-hexenal and (Z)-3-hexen-1-ol, were significantly negatively correlated to rainfall (Spearman's non-parametric: $r = -0.221$, $P = 0.0245$ and $r = -0.558$, $P < 0.0001$, respectively). Thus, the water deficit in apple trees implicated an increase in the released amounts of (E)-2-hexenal and (Z)-3-hexen-1-ol. Green leaf volatiles are produced by the lipoxygenase pathway (LOX, EC 1.13.11.12) (Siedow, 1991), and water deficit stress increases the enzymatic activity of lipoxygenase (Hildebrand, 1989).

In conclusion, the drought-caused water deficit in apple trees results in significant changes in volatile emissions in situ, which can be reversed by rainfall.

3. Experimental

3.1. Study site and plant material

Apple trees of the cultivar Golden Delicious were used to collect the volatile organic compounds. The dwarf apple trees in a commercial orchard (Zurich, Switzerland) at an altitude of 520 m were kept under hail nets and cultivated with integrated production regime, and subjected to pesticide treatments. The study site within the city limits might have led to a certain amount of air pollutants in the headspace of the trees sampled, but respective compounds were not relevant for the purpose of this study (Table 1, footnote). Disease management comprised applications of dithianon (quinone; 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 moths 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. Hence, most pesticides were applied during the first part of the season, and liquid formulations, the carrier of which might result in volatile emissions (Table 1, footnote), were not used after July anymore, a fact which may underly the seasonal fluctuations noted for p-xylene and m-xylene (Table 1). Anthropogenic influences clearly contribute to the chemical environment of the codling moth in a modern agroecosystem.

3.2. Sampling

Plant samples were collected throughout the season from the same three trees growing adjacent to each other to keep variation due to sampling from various plants minimal (Hern and Dorn, 2003). Samples were randomly collected from the middle part of the canopy. For volatile collection, five individual samples were taken from each of the three trees per sampling date. They consisted of a twig with one fruit and 6 ± 2 leaves, and were visually inspected to ensure that no apparent herbivore or disease damage was present.

3.3. Volatile collection – diffusive sampling

Headspace volatiles were collected in situ in the apple orchard by means of radial diffusive sampling (Radiello model 3310, Rupprecht & Patashnick Co., Albany). The collection took place during three sampling periods: 11.30 a.m. to 3.30 p.m., 3.30 p.m. to 7.30 p.m., 7.30 p.m. to 7.30 a.m. Nine samplings were performed between the end of April and the end of August on the following days: April 29–30, May 22–23, June 5–6, June 19–20, July 2–3, July 15–16, July 29–30, August 12–13, and August 26–27 (Fig. 1). Sixty samples were collected at every sampling date. For collecting the volatile, a Radiello's sampler was positioned horizontally inside a plastic bag (Nalophan, Kalle GmbH, Wiesbaden, Germany), that was then tied securely around the fruit-bearing twig with leaves. The adsorbent cartridge, containing the Tenax-TA polymer, was placed inside the yellow diffusive body of the Radiello and was screwed onto a supporting plate attached near the fruit-bearing twig with leaves. At the end of the collecting time the cartridges were inserted into a sorbent tube (Markes Int. Ltd™, Pontyclun, UK) and analyzed by thermal desorption–GC–MS.

3.4. Thermal desorption–GC–MS

Samples were analyzed using thermal desorption (Unity, Markes Int. Ltd™, Pontyclun, UK) connected to a GC–MS instrument (GC 6890 mass selective, MS detector 5973; Hewlett Packard Company, Palo Alto, CA). The analysis details were as follows: pre-column (5 m × 0.25 mm; HP-Retention Gap); column (30 m × 0.25 mm I.D.; 0.25 μm; phase EC™-5; 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. The thermal desorption details were as follows: tube desorption 5 min, 300 °C and cold trap (4 cm-Tenax-TA + 2 cm-Carbopack™ B; mesh size 60–80; Supelco, Bellefonte, PA) was held at −10 °C throughout the tube desorption process then heated at a rate of 60 °C/min to 300 °C; cold trap desorption time
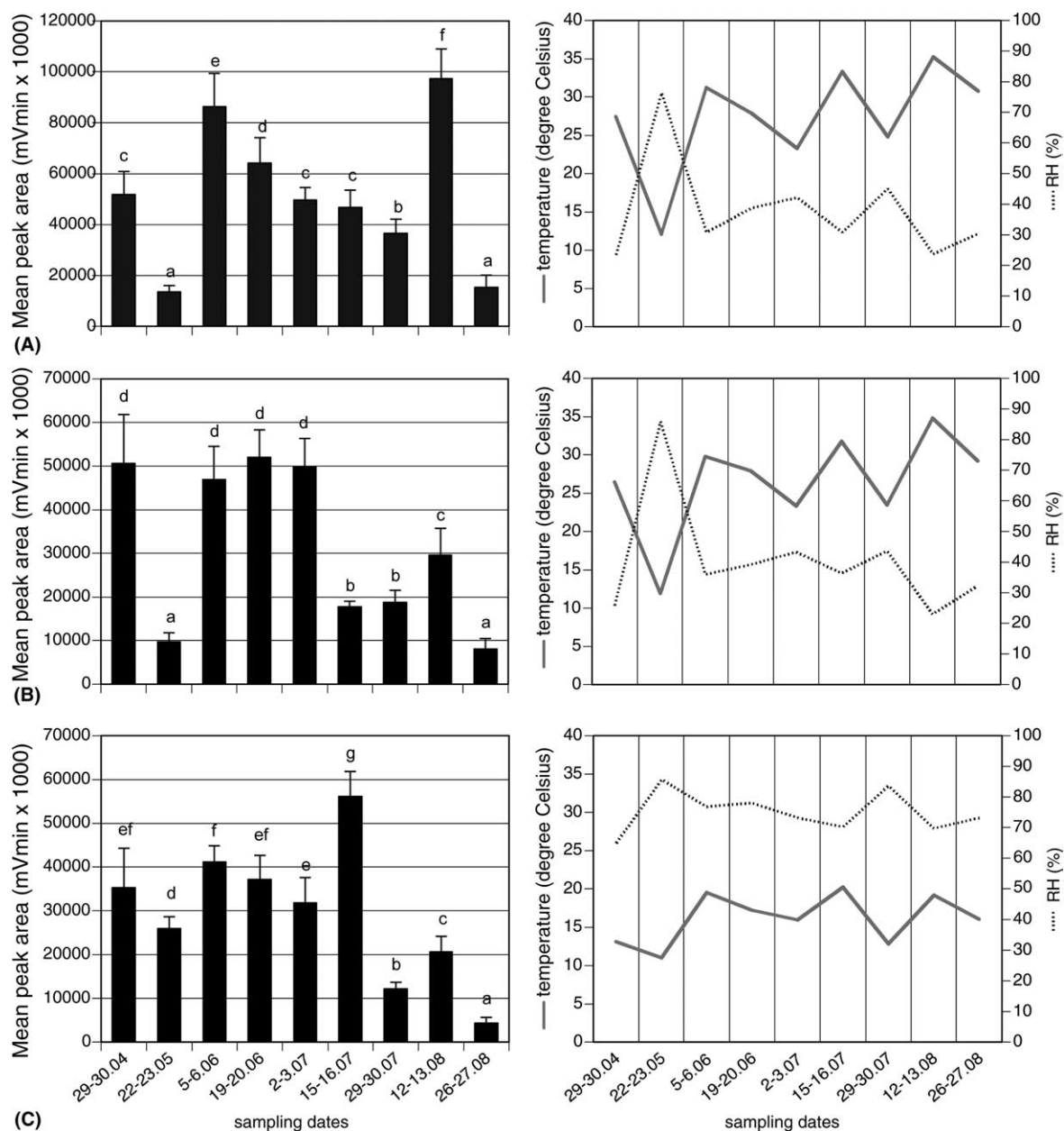
Fig. 1. Variation in volatile emissions, measured as the mean peak area of the compounds printed in bold (Table 1) detected by TD–GC–MS, from samples of Golden Delicious apple trees over the growing season of 2003 (left column), along with variations of mean temperature and mean relative humidity (RH) (right column). Collections during three sampling periods presented separately, (A) 11.30 a.m. to 3.30 p.m.; (B) 3.30 p.m. to 7.30 p.m.; (C) 7.30 p.m. to 7.30 a.m. Each mean (±SE) was calculated from 60 replicates. Letters above bars indicate significant differences among mean peak areas for different sampling dates (Scheffe's post hoc test, applied separately to A, B, C; $P < 0.05$).

3 min. MS conditions: quadrupole and source temperatures were 150 and 230 °C, respectively, using full scan mode.

As the internal standard, hexyl benzene (50 ng/μl) diluted in hexane was added to each analysis. 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 of the compounds marked with an asteriks including all compounds printed in bold (Tables 1 and 2) were compared with standard compounds which

were purchased from chemical suppliers or obtained from other laboratories.

3.5. Climatic data

A weather station (PC weather station, Conrad Electronic, Germany) was installed in the orchard to record ambient temperature (°C) and relative humidity (%). Recordings were obtained at 1 m above ground every 10 min. Mean values for each sampling period were calculated as the arithmetic average of these recordings. Rel-

ative humidity was higher during the night; in contrast, temperature was higher around noon and late afternoon (Fig. 1). Data on daily precipitation (mm) were obtained from a nearby weather station of the National Meteorological Station in Zurich. Rainfall was unusually low from June to the end of August 2003, totalling to 250 mm as compared to 344, 435, 414, 439 mm recorded during the preceding 4 years. Rainfall at the sampling dates was as follows: 63 mm on April 29–30; 26 mm on May 22–23; 42 mm on June 5–6; 0 mm on June 19–20; 65.5 mm on July 2–3; 10 mm on July 15–16; 4.5 mm on July 29–30; 0 mm on August 12–13; 0 mm on August 26–27. The orchard was not irrigated.

3.6. Data analysis

Variation in volatile emissions from the three individual apple trees in relation to the different sampling dates was analyzed using ANOVA with repeated measures. Stepwise multiple regressions were applied to determine the effects of climatic factors on volatile emissions over the growing season. Spearman's nonparametric correlation was calculated to examine the possible relationship between variation in volatile emissions and water deficit stress on apple trees. All analyses were performed using the software SPSS 11 for Mac OS X.

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