Caryophyllene quantification in headspace extracts

1 Samples

The samples were injected on an Agilent 7693A Automatic Liquid Sampler (Agilent Technologies, Santa Clara, USA) in splitless mode and the peaks at the retention time of the authentic standard (-)-b-caryophyllene were integrated on an Agilent 7890A gas chromatograph equipped with an Agilent DB-WAXetr (extended temperature range) column of 30 m × 0.25 mm × 0.25 μm coupled with a flame ionization detector (FID) at 250 °C. The oven program for the 7890A was 60 °C for 2 min, 30 °C/min to 180 °C, 5 °C/min to 230 °C, 20 °C/min to 245 °C (15 min).

Each sample was a mixture of five rounds of 22 h odour collection, extracted with 2 ml of hexane (in total 5 × 2 = 10 ml). Plant material was replaced each round. Ten μl of each sample was mixed with 10 μl of n-hexane containing the injection standard. The mixture was reduced to 3 μl under a gentle stream of N_2 and injected, after which the ratio of β-caryophyllene in each sample was calculated to a constant contamination peak always present in the solvent at retention time 6.99 on this column.

2 Results

Carob moth showed a clear EAD response to the authentic β-caryophyllene (Fig. S5.1) and the retention time of the active peak also matched on the DB-wax column used to quantify the amount of β-caryophyllene in the extracts. The amount of β-caryophyllene relative to the solvent peak at retention time 6.99, was significantly different among different plant materials tested ($F = 264.11$, d.f. = 4, 10; $P < 0.0001$; see table S1 for details). Unripe pomegranate and pomegranate flowers contained relatively high amounts of the compound whereas the extracts of pistachio and ripe pomegranate, whether cracked or uncracked, contained low amounts of β-caryophyllene (Figure S5).

3 Statistical analysis

The script for GLM analysis for caryophyllene levels in Pomegranate samples (Caryophyllene_Statistic.R.txt) can be found in S0/Data and scripts.