"Essential oils, asthma, plant gases and thunderstorms: A prospective study of human respiratory response to ambient biogenic volatile organic compounds (BVOCs)"

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To determine what plant-related substances might be in ambient air samples, stamens from flowers that commonly bloom in the coastal urban areas of SE Queensland were examined, and components identified. Initially flowers from *Callistemon viminalis* were hexane extracted to develop the methodology and to discover if volatile compounds were present in the extracts and could therefore be analysed by GCMS (Gas Chromatography Mass Spectroscopy) using an HP1 column.

Samples of *Callistemon viminalis, Eucalyptus (Eucalyptus ficifolia X E. ptychocarpa)* "Summer Beauty", *Melaleuca leucadendra*, and *Melaleuca quinquenervia* were examined using GCMS analysis of Solid Phase Micro-Extraction (SPME) samples. Named samples, gathered with permission from Redcliffe City Council, from verge plantings were confirmed by botanical staff at the Queensland Herbarium, Brisbane. Stamens cut from freshly harvested flowers were wrapped in aluminium foil packages and placed in insulated containers, cooled by dry ice to delay spoilage, for the 18-hour journey to the laboratory in Tasmania.

Upon arrival, the headspaces of stamens from harvested flowers were sampled by unwrapping the foil packages and placing them in a zip lock plastic bag, which were then sealed and allowed to equilibrate at room temperature for 3 hours. The headspace was then sampled by piercing the bag with a SPME needle fitted with a 100µm polydimethylsiloxane coated filament. This was then protracted for 10 minutes of absorption at 20°C. The needle was then retracted, removed from the bag and desorbed into the GC injection port at 240°C for GCMS analysis using a 25m, 0.52µm, HP1 column at 15psi with a temperature program of 60 °C (for 2min), rising at 6°C/min to 240°C. (This description of GCMS analysis was provided by the scientific officer at the University of Tasmania, contracted by Griffith University to complete the analysis.)

Thunderstorm floral sample (Grevillea)

Figure A: Thunderstorm floral sampling

An opportunistic capture of emissions from a flower, before and after a storm, involved the wrapping of *Grevillea* 'Robyn Gordon' flower still on the branch (Figure A). From a nursery, (parentage is *Grevillea banksii* × *Grevillea bipinnatifida*, on tag) this popular variety was a three-year-old shrub. Identity was confirmed by staff at the Queensland Herbarium.

This floral study explores the relationship between storms and volatile emissions. Volatiles from live attached flowers were sampled by GCMS desorption of Tenax attached to a SKC Universal Sample Pump. At one end, Tygon tubing held the Tenax tube, and the other end was attached to the pump. The flower was enclosed snugly, but not crushed, to exclude as much air as possible to concentrate the floral emissions. It was wrapped in aluminium foil and sampled with Tenax TA 20/35 Mesh 100mg tube inserted into an air space in the foil 'bag' created with the foil edges pressed together to hold it in place. The pump ran for 10 minutes with a reading of 3 litres per minute with the Tenax tube in place in the foil 'bag'.

One sample was taken as a storm gathered strength; there were light wind gusts and light rain at 2.45 pm on 8/5/2001 at Flaxton, 76km north of the air sampling site. Thirty minutes later, after the storm had passed, another 10-minute sample was taken. During the 'storm' there was little rain but considerable rumbling of thunder. The aim was to capture floral emissions as the storm built up, and then, following the storm, determine if there was a difference in quality and/or quantity of emissions. From decision to collect, to collection, was only 20 minutes. Collection was unplanned and opportunistic, as equipment was available. After sampling, the tube was removed, wrapped in three layers of foil and twisted at each end and placed in a screw-capped glass tube. The tubes were packed in a plastic container and sent by air express to the laboratory in Tasmania, taking two days.

Cut flower analysis

Terpene content of likely influential flowers was determined prior to trapping of BVOCs in ambient air, to determine 'target' compounds. Flower sampling results from cut flowers SPME (solid phase micro extraction) and GCMS (gas chromatography mass spectroscopy) analysis where relative amounts of terpenes were H= high levels, M= medium, and L= Low:

- *Melaleuca quinquenervia* (abundantly naturally occurring cream bottlebrush in the wetlands and coastal NSW and Queensland): caryophyllene (H), alpha pinene (H), beta pinene (M), 1,8 cineole (H), limonene (M), alpha terpinene (L), terpinene 4-ol (M), alpha terpineol (H), globulol (M), unresolved hydrocarbon (L).

- *Eucalyptus* "Summer Beauty" (red variety commonly planted as specimen tree in gardens): caryophyllene (H), beta phellandrene, bicyclogermacrene, humulene, bornyl acetate (H), alpha pinene (H), beta pinene (M), 1,8 cineole (M), limonene (M), alpha terpinene (M), terpinene 4-ol (M), methyl eugenol (M), globulol (L), unresolved hydrocarbon (M).

- *Melaleuca leucadendra* (common weeping cream bottlebrush - extensively planted as a street tree): caryophyllene (H), alpha pinene (M), beta pinene (L), 1,8 cineole (M), limonene (L), alpha terpinene (L), terpinene 4-ol (M), alpha terpineol (M), methyl eugenol (H), globulol (L), unresolved hydrocarbon (M).

- *Callistemon viminalis* (common weeping red bottlebrush - planted extensively along freeways, streets and in gardens): caryophyllene (H), alpha pinene (M), beta pinene (L), 1,8 cineole (H), limonene (L), alpha terpinene (L), terpinene 4-ol (M), alpha terpineol (H), globulol (L), bornyl acetate(L), unresolved hydrocarbon (L).

Floral emissions before and after a thunderstorm

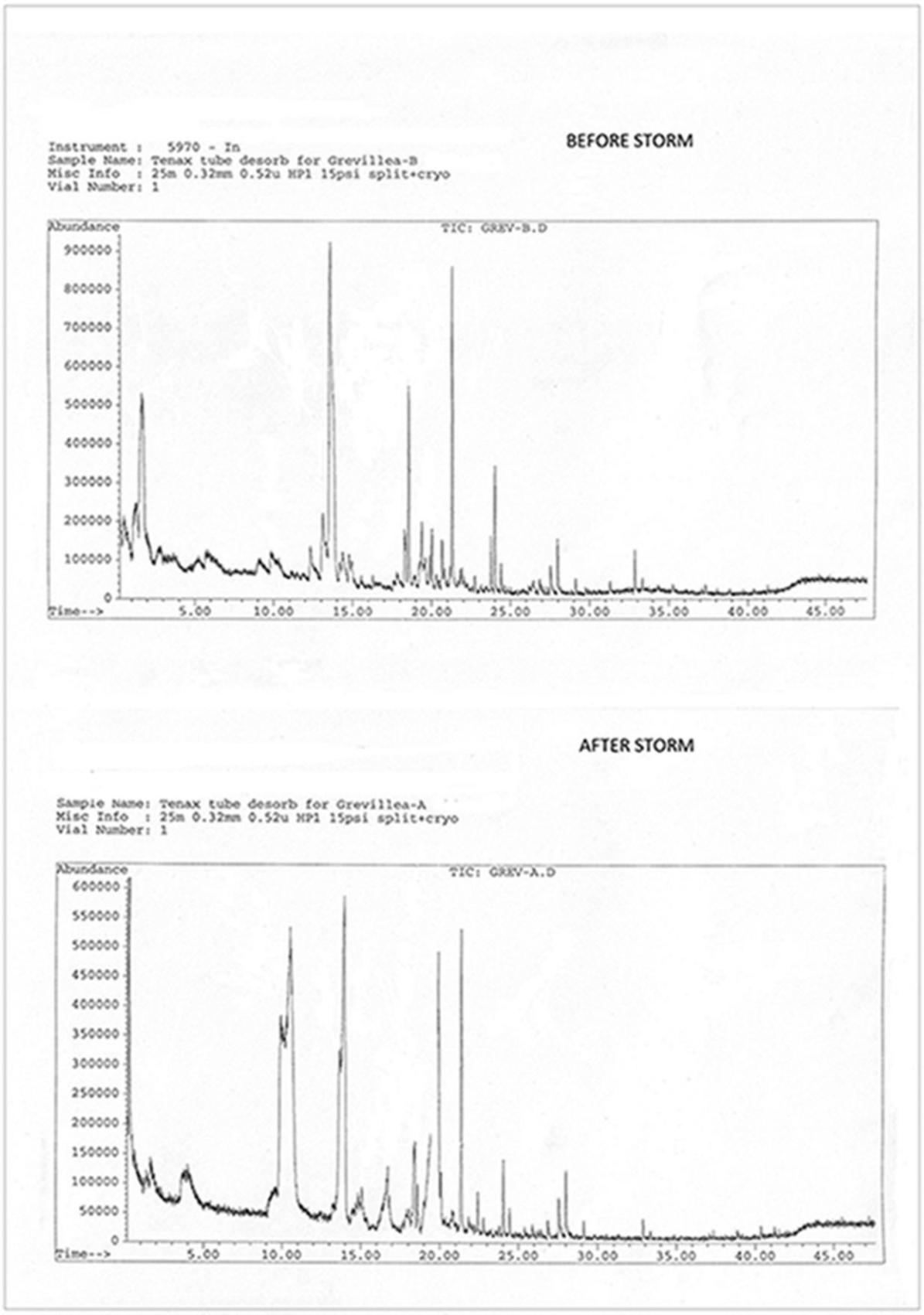
Emissions from *Grevillea* 'Robyn Gordon' were captured before and after a storm. GCMS output graphs of both sets of emissions are shown in Figure B. The graphs show increased linalool, hexenal and hexanoic acid emissions before the storm, compared to after the storm.

Figure B's Y axes scales are different (maximum before storm is 900,000; after is 600,000); there are increased linalool, benzaldehyde, hexenal and hexanoic acid emissions before the storm, compared to after. Component are shown in Table A.

Table A. Floral emissions before and after a storm

Grevillea 'Robyn Gordon'	Before storm	After storm
Linalool	M-H	-
Cis beta-ocimene	L	L-M
Benzaldehyde	L-M	-
Hexenal	H	L
Hexanoic acid	H	-
Alpha-phellandrene	-	L
Sabinene	-	T
Camphor	-	T

Figure B. GCMS output BVOCs from Grevillea before and after a thunderstorm



Air sampling

Air was sampled from 7am to 7am just as participants might be taking their morning medication, having just completed their respiratory diary. Symptom ratings referred to the air sample just collected for the immediately previous 24 hours. Sampling took place three times per week, always once on Sunday. Each sample was at least 48 hours after the previous one; some were 72 hours apart. Participants were unaware of the sampling schedule and chemical targets in the air samples; the researcher was unaware of the air sample results until months after collection, and respiratory diary completion.

Air sampling procedure

Air samples were collected via a Tenax tube inserted into Tygon tubing connected to an SKC Universal Sample Pump located under cover. Tenax TA 20/35 Mesh 100mg tubes were used (SKC 226-123). The Tygon tubing with Tenax attached was then taped with duct tape to the outside wall of the shed, under cover. The tubing was taped without kinks that might otherwise impede flow to the pump.

Since sampling was estimated to occur over a temperature range of 4˚C to 38˚C, allowance was made for 'breakthrough' of terpenes, as gaseous volume expands with increased temperature. Trial sampling enabled the limits to be set to avoid 'breakthrough' in hot weather. To avoid this, the sample level was set very low at only 550ml per minute i.e. approximately one twentieth of the human rate of breathing in air. Pilot testing with several grades of Tenax and a range of pump rates and sampling times occurred before this rate was determined as suitable. The pump was calibrated before it was set for each sample with a 5% tolerance deemed acceptable.

After samples were disconnected from the Tygon tubing, they were wrapped in aluminum foil twice and placed in a glass vial with a metallic lined screw cap to prevent absorption of any plastics into the sample. They were kept at room temperature until the end of the week when the week's batch was sent together by air for analysis to the laboratory in Tasmania.

GCMS analysis of Tenax air samples

Analysis was performed by desorption into a Hewlett Packard 5890 Series 2 gas chromatograph equipped with a cryotron, a split injection system, an HP-1 cross linked methyl silicone gum column (30m x 0.32mm with 0.54μm film thickness) and linked to a Hewlett Packard 5970 Mass Spectrometer. Desorption of the Tenax tubes was performed by replacing the normal injector liners with them, in the injector chamber when the temperature had fallen below the desorption temperature of the type of chemicals being screened for (<100°C). Carrier gas was Helium @ 3 ml/min., with a head pressure of 10 psi. The Oven temperature program was 10°C for 4 min, then 8°C/min to 280°C where it was held for 10 minutes.

Identification of compounds from the chromatograms was made with reference to the NITS MS library database. No attempt to quantify the relative abundance of the various compounds identified was made beyond a very general classification of the ion counts for each of those compounds into VVH (very, very high), VH (very high), VH-H (very high to high), H (high), H-M (high to medium), M (medium), M-L (medium to low), L (low), L-T (low to trace), T (trace) and zero.

Estimation of total ion count of volatile compounds

Trace = 0-20,000
Low = 20,000-100,000
Medium = 100,000-500,000
High = 500,000-250,0000
Very high = > 250,0000
(This description of GCMS analysis of air samples and total ion count was provided by the scientific officer at the University of Tasmania under contract to Griffith University.)

Control measure: air sampling

Tenax tubes were sent to Tasmania for analysis in weekly batches. A control tube was added to each batch. It was never exposed to ambient air, and never taken out of its capped glass tube. When the air sample tubes were opened and desorbed, the control tube was treated the same and checked that it was free of terpenes or other contaminants. If the control tube was contaminated, the week's samples were discarded. This occurred twice in the 20 weeks of sampling and was due to a very minor contamination thought to have occurred from the laboratory where the tubes had been kept briefly during the analysis. The tubes were discarded.
Control tube selection was random; it was chosen from each new batch that returned desorbed from the laboratory in Tasmania. Multiple tubes were purchased new and reused after desorption five times each. The control tube served as a measure of the effectiveness of the desorption process.

Selecting focus components of air sampling

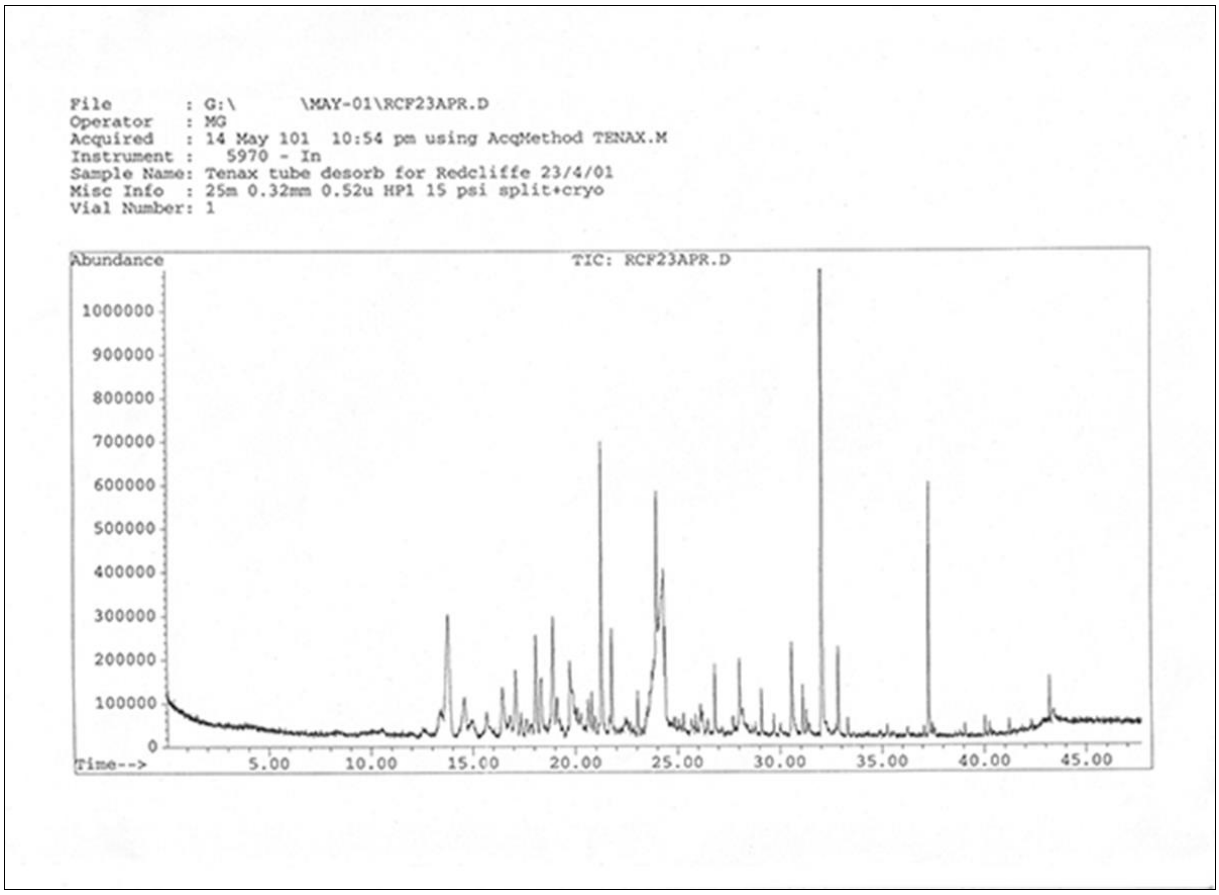
The focus compounds of air sampling were those identified in cut flowers and pilot air samples. The approximation to gases from living flowers is acknowledged, with loss of precision due to cutting and transport time; transport in a foam box containing dry ice, to maintain as much freshness as possible, minimized this. To contain project costs, only frequently appearing compounds were selected for identification and statistical analyses: alpha pinene, beta pinene, 1,8 cineole, camphor, linalyl acetate, linalool, limonene and benzaldehyde and benzoic acid. Influential variables may not have been measured in this first attempt to identify them, and the selective and limited nature of the investigation is acknowledged. There are likely to be many, many more not tracked here.

BVOC exploration

Exploratory principal components analysis (PCA) was applied to the BVOCs after suitability of data was assessed. The Kaiser Meyer-Olkin coefficient was .776 exceeding the recommended .6 and Bartlett's Test of Sphericity was highly significant at p<.0001, so suitability for factor analysis was established. PCA revealed two orthogonal components with eigenvalues exceeding 1, explaining 61% (component 1) and 12% (component 2) of the variance (totalling 72%) of same day measures. Correlation between components was .04.
Loadings on Component 1 were: camphor .90, limonene .89, beta pinene and linalool both .88, 1,8 cineole .87, alpha pinene .86 and linalyl acetate .85. Loading on Component 2 were: benzoic acid .78 and benzaldehyde .61.

To investigate influence from plants flowering in only one season, and others in several, and relative contribution of ambient chemicals, a seasonal comparison using univariate general linear regression models was employed. Too few air sample data points prevented analysis of complex multivariate effects. The Figure C shows a typical chromatogram of an air sample from the project.

Figure C. Example of GCMS output of an autumn air sample



Air spora collection

Fungal taxa selected for quantification were: *Cladosporium, Alternaria* and 'other fungi'. Pollens counted were Myrtaceae (any genera), *Pinus* (pine), Poaceae (grasses), *Acacia* (wattle), *Casuarina* (she-oak, or Australian pine), Asteraceae (daisy family) and 'other pollen'. Air spora were sampled with a 7-Day Volumetric trap from Burkhard Manufacturing Company, UK. The orifice was situated two metres above ground level to approximate head height. Collection tapes were changed weekly and they were divided into daily strips and mounted on glass slides, stained with Calberla's solution and examined under a light microscope. Counts were made using a 40X objective (light microscope 10x eyepiece) viewing a strip 100 microns wide from the middle of the slide in a lengthwise transverse. Daily levels representing mid-morning to mid-morning were recorded. Missing air spora information from 11 days was due to equipment failure.

Air pollutants

Air pollutants were monitored at Deception Bay Environmental Protection Authority (EPA) of Queensland site. EPA recorded levels for nitrogen monoxide (NO) and nitrogen dioxide (NO_2), particles less than 10 microns (PM_{10}), and ozone (O_3) were accessed.

Meteorological variables

Meteorological variables were measured from Brisbane Airport weather station (about 21 km from the sampling site): daily means for atmospheric pressure, mean temperature, and wind speed; precipitation, thunder (heard/not heard), and relative humidity at 9am.