A Method to Detect Baseline Emission and Plant Damage Induced Volatile Emission in a Greenhouse

R. Jansen¹, J.W. Hofstee¹, F. Verstappen², H. Bouwmeester², M. Posthumus³ and E. van Henten¹,⁴

¹Wageningen University, Farm Technology Group, P.O. Box 17, 6700 AA, Wageningen, The Netherlands
²Plant Research International, P.O. Box 14, 6700 AA, Wageningen, The Netherlands
³Wageningen University, Laboratory of Organic Chemistry, P.O. Box 8026, 6700 EG, Wageningen, The Netherlands
⁴Wageningen UR Greenhouse Horticulture, P.O. Box 16, Wageningen, The Netherlands

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Abstract

The objective of this research was to ascertain if 1) baseline emission and 2) damage induced emission of volatile plant substances could be detected under greenhouse conditions. A laboratory method was validated for analysing the air in a semi-closed greenhouse with 44 m² floor area. This greenhouse, with a volume of 270 m³, was climate controlled and light was supplied with assimilation lamps. Sixty tomato plants (Lycopersicon esculentum Mill cv. Moneymaker) were grown in this greenhouse. These plants were artificially damaged on a weekly interval by stroking the stems. Continuous flow pumps were used to purge the air surrounding the plants through tubes containing an adsorbent. This sampling step was performed before and directly after damage of the plants. After sampling, the tubes were transferred to the lab for analysis. The analysis of volatile compounds was performed using a high-throughput gas chromatography-mass spectrometry system. The method enabled the detection of baseline level emission and the emission of volatiles released after artificially damaging the tomato plants during a 6 weeks growing period. Most dominant compounds for baseline emission were the monoterpenes β-phellandrene, 2-carene, limonene, α-phellandrene and α-pinene. Directly after damage, these compounds showed an increase of up to 100 times compared to baseline level emission. With these results, we prove that it is possible to detect baseline- and plant damage induced volatile emission in a greenhouse. This area of research is promising but more research needs to be done to determine whether it is possible to detect plant damage due to pests and pathogens using volatile sensing.

INTRODUCTION

Early detection and location of plant damage due to pests and pathogens is a major challenge in commercial greenhouse cultivation. It allows the crop manager to perform site-specific actions instead of full field treatment. This will reduce the use of pesticides. Previous laboratory experiments have revealed that sensing volatiles released by the damaged plants might offer a powerful technique to monitor the status of greenhouse crops.

Such laboratory experiments that confirm the change of volatile substances released after damage are not new (e.g. Heiden et al., 2003). A common method used in such studies is the use of dynamic sampling to concentrate the volatiles of interest and thereafter gas chromatography coupled to mass spectrometry for analysis. However, the validation of such method to detect plant induced volatiles in a greenhouse has not been practiced until now. The objective of this research was to ascertain if 1) baseline emission and 2) damage induced emission of volatile plant substances could be detected under greenhouse conditions. The primary research question related to this objective is whether dynamic sampling and gas chromatographic - mass spectrometric analysis allows the identification of plant induced volatiles in a greenhouse. Additionally we checked whether this method allows the monitoring of plant damage induced volatiles.
MATERIALS AND METHODS

Plant Material
Seeds of tomato plants (*Lycopersicon esculentum* Mill) of the cultivar Moneymaker were germinated in a standard greenhouse at 20°C and 50% relative humidity. When plants were about 7 weeks of age, 60 plants were transferred to a semi-closed greenhouse. At that age the individual plants were about 80 cm tall. In this semi-closed greenhouse the plants remained until the age of 12 weeks. During this time period of 6 weeks, on Wednesday, plants were winded, and flowers were pollinated using a vibrating device. During the time period of 6 weeks, on Thursday, plants were artificially damaged by stroking the full length of the stem of each individual plant using a stainless steel bar. Also the length of 3 randomly selected plants was measured during the time period of 6 weeks, on Thursday, to estimate the growth of the plants in the semi-closed greenhouse. We used 3 independent replicate studies for this paper. The first study was from February – March, the second from April – May and the third from June – July.

Greenhouse
The semi-closed greenhouse used for the experiments has been described by Körner et al. (2007). In short, a closed greenhouse with 44 m² floor area was used. The total volume of this greenhouse including basement was 270 m³. The greenhouse was sealed to minimize ventilation (~ 0.7 mol of air per second). Electrical heating and direct mechanical cooling situated in the basement controlled temperature and humidity. The temperature was set at 22°C during day and 16°C during the night. Assimilation lamps were installed. These lamps turned on when radiation outside the greenhouse dropped below 150 W/m² and turned off when the radiation outside increased over 250 W/m². The relative humidity inside the greenhouse was maintained to about 70% during the day and 90% during night. Temperature and relative humidity of the air in the greenhouse was measured with dry and wet bulb platinum resistance temperature detectors. A third sensor measured temperature and relative humidity for climate control purposes. Pure CO₂ was injected into the greenhouse proportionally to the difference between measured CO₂ concentration and the CO₂ set point using an infrared gas analyser and a mass-flow controller to maintain a CO₂ concentration of 420 ppm throughout the experiments.

Air Sampling for Volatile Analysis
Continuous flow pumps were used to purge 6 liters of air surrounding the plants through stainless steel tubes (Markes international Ltd, UK) containing 200 mg of tenax-TA 20/35 (Grace- Alltech, Breda, The Netherlands). Air was sucked through these tubes at 300 ml/min during 60 minutes. This sampling step was performed before and directly after damage of the plants at a fixed starting time point i.e. 13.00 h. The air was sampled at 3 locations within the greenhouse to provide insight into the spatial distribution of volatile substances inside the greenhouse. These 3 sampling points were located in the left-rear, centre and right-front location of the greenhouse at a height of 1 m. After sampling, the tubes were immediately capped and transferred to the lab for analysis.

Gas Chromatography – Mass Spectrometry
The analysis of volatile compounds was performed using a high-throughput gas chromatography / mass spectrometry system (GC-MS). Before analyses the tubes were dry-purged with helium at ambient temperature with a flow of 100 ml/min for 10 minutes to remove unwanted water. The high-throughput headspace analysis method was developed on a Trace GC Ultra™ (Thermo Electron Corporation, Auston, TX USA) equipped with a Trace DSQ quadrupole mass spectrometer (Thermo Electron Corporation, Auston, TX USA). Samples were transferred from the trap using a thermal desorption system (TDS) at 250°C for 5 min (Ultra-TD™, Markes international LTD, UK). Analytes were then transferred to an electronically-cooled focusing trap at -5°C (Unity™, Markes international LTD, UK). Analytes were transferred to the column by
heating the cold trap to 250°C at approximately 40°C/sec. The GC was held at the initial temperature of 40°C for 3.5 min followed by a linear thermal gradient of 10°C/min to 280°C and held for 2.5 min resulting in an overall runtime of 25 min. A multi-use autosampler was used for the automatic desorption of the standard tubes (UltrA™, Markes international Ltd, UK). The mass scan range was set from 45 to 450 amu (atomic mass unit) at a scan rate of 5077 amu/sec. The column flow was approximately 1 ml/min (Restek Rtx-5 MS, 30 m x 0.25 mm i.d. x 1 μm film thickness). The TC-20 Multi-tube conditioning unit (Markes international Ltd, UK) was used for cleaning the tubes in between the measurements at 310°C for 40 min. Compounds were identified by comparing the mass spectra with mass spectra libraries i.e. Wiley mass spectral library, NIST library and the Wageningen Mass spectral library. Corresponding peak areas were then determined using the XCalibur version 2.0, software (ThermoFinnigan). In some cases the peaks for different compounds were not fully separated. In such case we used a fragment in one compound that was not abundant in the other to calculate peak areas. To confirm identifications we cross-checked the Kovats retention index (KI) values with the KI-reference guide (Adams, 2001). To calculate the KI we used the retention times obtained from an alkane-mixture (Sigma, St. Louis), analyzed under the same GC-MS conditions as were used for our samples.

RESULTS AND DISCUSSION

Per samples, we identified up to 17 compounds that are well known to be released from tomato (Kant et al., 2004; Deng et al., 2005). These compounds and their chemical classes are given in Table 1.

The main noise in the chromatographic profiles resulted from the analytical system itself such as siloxanes, and background compounds in the greenhouse air such as toluene, benzene and naphthalene. In contrast to toluene and benzene, naphthalene has –to the best of our knowledge- never been described in literature as plant emission. Naphthalene was always present at almost constant concentration in both empty and cropped greenhouse. The response for naphthalene was in good agreement with the weekly calibrations (R²=0.7). This good agreement and the nearly constant concentration indicates that the sample collection and thermal desorption procedures were quantitative. Using specific mass to charge ratios (m/z) we could focus on plant specific compounds such as terpenes. The 5 most dominant compounds in baseline emissions were β-phellandrene, 2-carene, limonene, α-phellandrene and α-pinene. This was independent on the age of the plants. An example of some typical chromatographic data is presented in Figure 1.

Spatial Distribution

The signal representing 2-carene was selected to calculate the relative difference in-between the three locations. This signal was selected because 2-carene maintained a large signal to noise ratio (s/n) throughout the experiments. Although the s/n representing β-phellandrene was about 3 times larger, this compound turned out to be inappropriate because this signal reached saturation level of the ms several times. The relative difference in-between the three locations was on average 7.5% for 2-carene (n=26). This 7.5% relative error in-between the 3 locations is below the summed measurement error of the GC/MS (5%) + sampling procedure (5%). Therefore the average of these three locations is used in the remainder of this paper.

Baseline Emission

Emissions before damage can be seen as baseline emission from plants. It can be expected that baseline emissions correlates closely to leaf area. Leaf area is difficult to measure in a non-invasive way. Therefore we measured the length of 3 individual plants throughout the experiments. The increase in length turned out to be closely related to the baseline emissions of all compounds (Fig. 2).
**Damage Induced Emission**

After stroking the stems, the emission of most compounds increased at least 10 times. Tomato plants possess several types of trichomes including glandular trichomes that are present in high density on leaves, petioles, and stems and that contain terpenes (Snyder and Carter, 1985). As a result of these strokes, it was observed that trichomes located on the stems were damaged. All dominant terpenes detected in the greenhouse were recently described after analysing of trichome content of tomato (Schie, 2007). Therefore we believe that damage of these trichomes is the most important mechanism resulting in the increases of most terpenes. Interestingly, no substantial increase was observed for the compounds methyl salicylate (MeSA), \((E,E)-4,8,12\text{-trimethyl-1,3,7,11-tridecatetraene}\) (TMTT) and \(\alpha\)-copaene. The concentration of these three compounds remained almost constant after damage. This difference suggests a different mechanism including synthesis and subsequent emission. In Table 2 the relative increase (damage induced emission / baseline emission) of some prominent compounds are listed.

From Table 2, it can be seen that large variations of up to 80% occurred in-between the replicate studies. Assuming that stroking the plants is a well reproducible treatment in terms of damage an explanation would be related to plant level. Probably the number and content of trichomes play an important role in the increase. Fundamental biological processes leading to trichome contents influences this increase after damage. As these biological processes are partly determined by light it might be true that the differences in the quantity and quality of light –due to different times of the year- between the replicates could explain this variation. In addition to the large variation in-between the replicate studies, Table 2 shows a general trend for the individual compounds, that is a constant relative increase per week. This constant relative increase was different for each compound and was generally larger for the more dominant compounds. In contrast to the constant relative increase, the absolute increase each week is different for most compounds. This difference can be seen in Figure 3.

The difference in absolute increase per week is probably due to the increase in length and therefore an increase in the total number of trichomes per stem. As each week the total length of the stem was stroked this result fits to the absolute increase per week throughout the growing period. In addition to this, a strong relation existed between the emissions of the individual compounds. This relation was independent of plant age and was not affected by the damage.

The close relation between most compounds suggests a same emission mechanism for these compounds. This mechanism is to a large extent related to diffusion out of the trichomes in case of undamaged plants and ‘leakage’ out of trichomes after damage.

Two compounds detected in trace amount inside the greenhouse were methyl salicylate (MeSA) and TMTT. These compounds are sometimes described as main compound for baseline level emission of tomato (Kant et al., 2004) while in other research these compound are almost or completely absent in the blend of control tomato (Deng et al., 2005). A reason for this difference is probably the sampling method. In research in which the chamber is continuously flushed with clean air, MeSA and TMTT dominates (e.g. Kant et al., 2004) whereas experiments performed in closed chamber do show the trichome related terpenes as main compounds (Deng et al., 2005). The reason for this difference might be accumulation of trichome related monoterpenes in closed chambers. Sequential sampling in combination with evaporating standards could provide insight into the chemical lifetime of compounds inside the greenhouse. Nevertheless, in all previously described work there was an increase of MeSA and/or TMTT after biotic stresses such as herbivore and pathogen infection of tomato (Farag and Pare, 2002; Deng et al., 2004). Therefore analysing the emission of MeSA and/or TMTT in greenhouse might offer an interesting technique to detect biotic stress.

Detection of a sudden increase of terpenes due to damage of trichomes seems interesting because necrotic pathogens and several insect species damage trichomes. On the other hand, emission of terpenes caused by damage of trichomes seems not suitable as such damage also occurs due to plant jostling by greenhouse workers doing routine
activities such as de-leafing, stem winding and harvesting. Emissions related to cell wall degradation such as \((Z)-3\text{-hexen-1-ol}\) seems to be more interesting in that aspect. Although this product is a general product from breakdown of cell-membranes, such airborne signal might offer a sensitive indicator of increased risk for pest or pathogen outbreak in a greenhouse.

**CONCLUSIONS**

The proposed method enabled the detection of baseline level emission and the emission of volatiles released after artificially damage of the tomato plants during the 6 weeks growing period in the greenhouse. Most dominant compounds for baseline emission were the monoterpenes \(\beta\)-phellandrene, 2-carene, limonene, \(\alpha\)-phellandrene and \(\alpha\)-pinene. Directly after damage, these compounds showed an increase of up to 100 times compared to baseline level emission. With these results, we prove that it is possible to detect baseline emission and plant damage induced volatiles in a greenhouse using dynamic sampling and gas chromatographic – mass spectrometric analysis. This area of research is promising but more research needs to be done to determine whether it is possible to detect plant damage due to pests and pathogens using volatile sensing.

**Literature Cited**


## Tables

Table 1. Chemical compounds detected in the greenhouse. Molecular weight (MW), base peak (BP) and retention time (Rt) are given for these compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>MW</th>
<th>BP</th>
<th>Rt</th>
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<tr>
<td>α-terpinene</td>
<td>C10H16</td>
<td>136</td>
<td>121</td>
<td>13.59</td>
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<td>β-phellandrene</td>
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<td>136</td>
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<td>C10H16</td>
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<td>93</td>
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<td>Limonene</td>
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<td>β-pinene</td>
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<td>136</td>
<td>93</td>
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<tr>
<td>γ-terpinene</td>
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<td>136</td>
<td>93</td>
<td>14.32</td>
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<td>Methyl salicylate</td>
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<tr>
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<td>TMTT</td>
<td>C16H26</td>
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<td>69</td>
<td>21.40</td>
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### Monoterpenes

<table>
<thead>
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<th>Compound</th>
<th>Plant age [weeks]</th>
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<tbody>
<tr>
<td>β-phellandrene</td>
<td>0.8 (0.5)</td>
</tr>
<tr>
<td>2-carene</td>
<td>0.8 (0.5)</td>
</tr>
<tr>
<td>α-copaene</td>
<td>0.8 (0.5)</td>
</tr>
<tr>
<td>α-pinene</td>
<td>0.8 (0.5)</td>
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</tbody>
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### Sesquiterpenes

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<th>Compound</th>
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<tr>
<td>β-phellandrene</td>
<td>0.8 (0.5)</td>
</tr>
<tr>
<td>2-carene</td>
<td>0.8 (0.5)</td>
</tr>
<tr>
<td>α-copaene</td>
<td>0.8 (0.5)</td>
</tr>
<tr>
<td>α-pinene</td>
<td>0.8 (0.5)</td>
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</tbody>
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### Phenol

<table>
<thead>
<tr>
<th>Compound</th>
<th>Plant age [weeks]</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-phellandrene</td>
<td>0.8 (0.5)</td>
</tr>
<tr>
<td>2-carene</td>
<td>0.8 (0.5)</td>
</tr>
<tr>
<td>α-copaene</td>
<td>0.8 (0.5)</td>
</tr>
<tr>
<td>α-pinene</td>
<td>0.8 (0.5)</td>
</tr>
</tbody>
</table>

Table 2. Relative increase (damage induced emission / baseline emission) of 4 prominent compounds from tomato in the greenhouse after stroking the stems of 60 plants. Average and standard deviation are presented for the three independent replicates.

<table>
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<tr>
<th>Compound</th>
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<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
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<tr>
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<td>50.6</td>
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<td>2-carene</td>
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<tr>
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<td>0.8</td>
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<td>1.7</td>
<td>1.2</td>
<td>1.3</td>
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<tr>
<td>α-pinene</td>
<td>17.2</td>
<td>12.6</td>
<td>11.4</td>
<td>25.0</td>
<td>16.4</td>
<td>30.0</td>
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</tbody>
</table>
Figures

Fig. 1. Typical chromatographic profiles for samples taken from the air surrounding 60 tomato plants of 8 weeks old in a semi-closed greenhouse. A) Total ion current (TIC) chromatogram; B) selective ion current (SIC) chromatogram for a reduced part of the chromatographic profile (retention time: 10.00 – 15.00 min) using m/z 93 as representative for terpene emissions. Note the different ranges on the y-axis. 1 = toluene, 2 = siloxane, 3 = 2-carene, 4 = β-phellandrene, 5 = decanal, 6 = unidentified impurity, 7 = α-pinene, 8 = α-phellandrene, 9 = limonene.

Fig. 2. Baseline level emissions of β-phellandrene and α-copaene (grey bars); average and standard deviation are presented for the three independent replicates. On the second y-axis is the typical increase in length of tomato plants during an experiment (dots); average and standard deviation is for 3 randomly selected plants.
Fig. 3. Emission of some typical compounds in the greenhouse before (white bars) and after damage (grey bars) of plants. Average and standard deviation are presented for the 3 independent replicates. Note the different y-axis.