LabWorks APPNOTE

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A Comparison of Techniques for Sampling of Plant Volatiles in Four Plant Varieties

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Keywords

GERSTEL LabWorks Platform, Plant Volatiles, Metabolites, Air Sampling, TF-SPME, GERSTEL Twister®, GC-MS

Abstract

In this study, volatiles were collected from the headspace around plants for subsequent determination by GC/MS. Passive air sampling was performed using Thin Film Solid Phase Microextraction (TF-SPME) devices coated with divinylbenzene/polydimethylsiloxane (DVB/PDMS) as well as with the GERSTEL Twister® coated with PDMS. Active air sampling was performed onto thermal desorption tubes filled with PDMS foam and Tenax® TA, respectively. Violet star petunias, oakleaf hydrangeas, citronella, and lemon thyme plants were used for this study. Overall, passive sampling with the TF-SPME device covered a broad range of plant volatiles and with lower detection limits compared to the other techniques but required a longer sampling time.

Introduction

Thousands of plant varieties exist, and each of them produces and contains thousands of chemical compounds that make up a diverse and unique plant volatile profile. These volatile organic compounds (VOCs) are comprised mainly of terpenoids, fatty acids, aromatics, and amino acid derivatives [1]. Plant volatile determination is widely practiced. Specifically, plant metabolomics are gaining traction as metabolites and their processes provide vital information regarding phenotypes. Plant volatiles and/or metabolites serve as markers and indicators, for example, in breeding performed to optimize plants for greener production and food sustainability, postharvest protection, increased crop yields, and consumer acceptance. VOCs found in all plant types can attract pollinators, induce allelopathic effects, and promote the growth and development of the plant itself while also inhibiting pathogens [2,3]. Plant volatile determination is therefore of interest to plant breeders and producers, flavor and fragrance producers, phytologists, and even apiarists.

Active sampling with sorbent traps used in tandem with air sampling devices or dynamic headspace (DHS) sampling in a chamber are all established techniques used for extracting plant volatiles [4]. However, these techniques have some disadvantages such as requiring the plant to be taken from its natural environment, requiring optimization of sorbent material, and the purchase of additional equipment (flow meters and sampling pumps). Passive sampling of volatiles in the field has been demonstrated using the GERSTEL Twister [5,6,7]. This technique offers simplicity compared to the previous methods used for plant volatiles.

The GERSTEL Twister is based on PDMS sorption. To expand the polarity range of plant volatiles extracted, alternative or complementary forms of passive sampling can be used, such as Thin Film Solid Phase Microextraction (TF-SPME). TF-SPME devices are a 20 x 4.8 mm carbon mesh coated with PDMS and impregnated with a material such as Carboxen, Divinylbenzene (DVB) or Hydrophilic Lipophilic Balanced (HLB) particles. The addition of particles enhances the selectivity of these devices for both headspace and immersion sampling. This results in lower detection limits for the more polar volatiles.

The challenge with this type of analysis is in choosing an appropriate sampling technique that will yield an accurate representation of the plant's volatile makeup. In this work, active air sampling with Tenax® TA and PDMS foam sorbent tubes, and passive air sampling with PDMS/DVB TF-SPME devices and the GERSTEL Twister® were compared.

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TF-SPME devices are effective in terms of extraction efficiency and selectivity of plant volatiles, but they also require longer sampling times, sometimes overnight. The TF-SPME devices allow for sampling in the field, are available with three different sorbents to achieve the optimal sampling of plant volatile varieties, and can be used in combination with the GERSTEL Twister® to achieve lower detection limits.

For active air sampling, sorbent type, sampling rate, sampling volume, temperature, and humidity must all be considered when optimizing a method.

Experimental

Instrumentation

GERSTEL LabWorks Platform, and Agilent 8890 GC with 5977B Inert plus MSD.

Analysis Conditions

LabWorks Platform	
TF-SPME	PDMS/DVB
TD Tubes	PDMS Foam, Tenax TA
TDU 2	Splitless 40 °C (0.50 min); 720 °C/min; 260 °C (5 min)
CIS 4	Split Vent Flow: 50 mL/min Purge Flow to Split Vent 20 mL/min at 0.01 min -75° C (0.20 min) ; 12° C/sec, 270° C (5 min)
Agilent 8890 GC	
Pneumatics	He; Pi = 7.1 psi Constant flow = 1.0 mL/min
Column	30 m Rxi-5Sil MS (Restek) d_i = 0.25 mm, d_f = 0.25 µm
Oven	40 °C (2 min); 15 °C/min; 280 °C (2 min)
Agilent 5977B MSD	
Scan Parameters	Full scan, 40-350 amu
Solvent Delay	1 min

Sample Preparation

A citronella plant, lemon thyme, and violet star petunias were purchased from a local store. Oakleaf hydrangeas in bloom were obtained from a nearby neighborhood. Plant volatiles from the violet star petunia were actively sampled using thermal desorption tubes filled with PDMS foam and Tenax® TA, respectively. The oakleaf hydrangea was actively sampled with only a PDMS foam filled thermal desorption tube. Thermal desorption tubes were placed directly in the pistil of both flower types. Whole air was sampled onto the tubes at 50 mL/min flow rate for 2 hours resulting in a total volume of 6.0 L. A general air sampling method was used for this study, but was not fully optimized.

A PDMS/DVB TF-SPME device was placed in a preconditioned tea strainer and each plant's headspace passively sampled overnight for approximately 14 hours. The same was done using the GERSTEL Twister® for the violet star petunias and the citronella plant. The TF-SPME devices and GERSTEL Twisters were subsequently removed from the respective plants, rinsed with water, dried with a Kimwipe®, and placed in a TDU tube for thermal desorption. The TF-SPME devices and GERSTEL Twisters were placed in separate, empty thermal desorption tubes on a TDU tray along with the PDMS foam and Tenax TA filled tubes for sample introduction to the GC/MS.

Sample Introduction

Samples were desorbed in splitless mode under a 50 mL/min helium flow at 260 °C for 5 minutes. Analytes were cold trapped in the CIS 4 inlet at -75 °C on a glass bead filled liner. When desorption was complete, analytes were transferred to the GC column in split mode (20:1) by heating the inlet to 270 °C for 5 minutes.

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Results and Discussion

Figure 1 shows the stacked view of the total ion chromatograms (TICs) of plant volatiles extracted from violet star petunias by TF-SPME (top) and the GERSTEL Twister® (bottom). Each sampling technique provided a slightly different chromatographic profile, and relevant peaks are labeled in Figure 1. While both techniques sorbed a variety of compounds, including straight chain alkanes, terpenes, fatty acids, aldehydes, and alcohols, the TF-SPME device was able to capture a wider range of compounds. The PDMS phase captured VVOCs and VOCs with great affinity, whereas the DVB phase enhances the capture of these compounds, along with semivolatile organic compounds (SVOCs). For example, the TF-SPME device showed enhanced response for vanillin, trans-isoeugenol, and benzophenone.

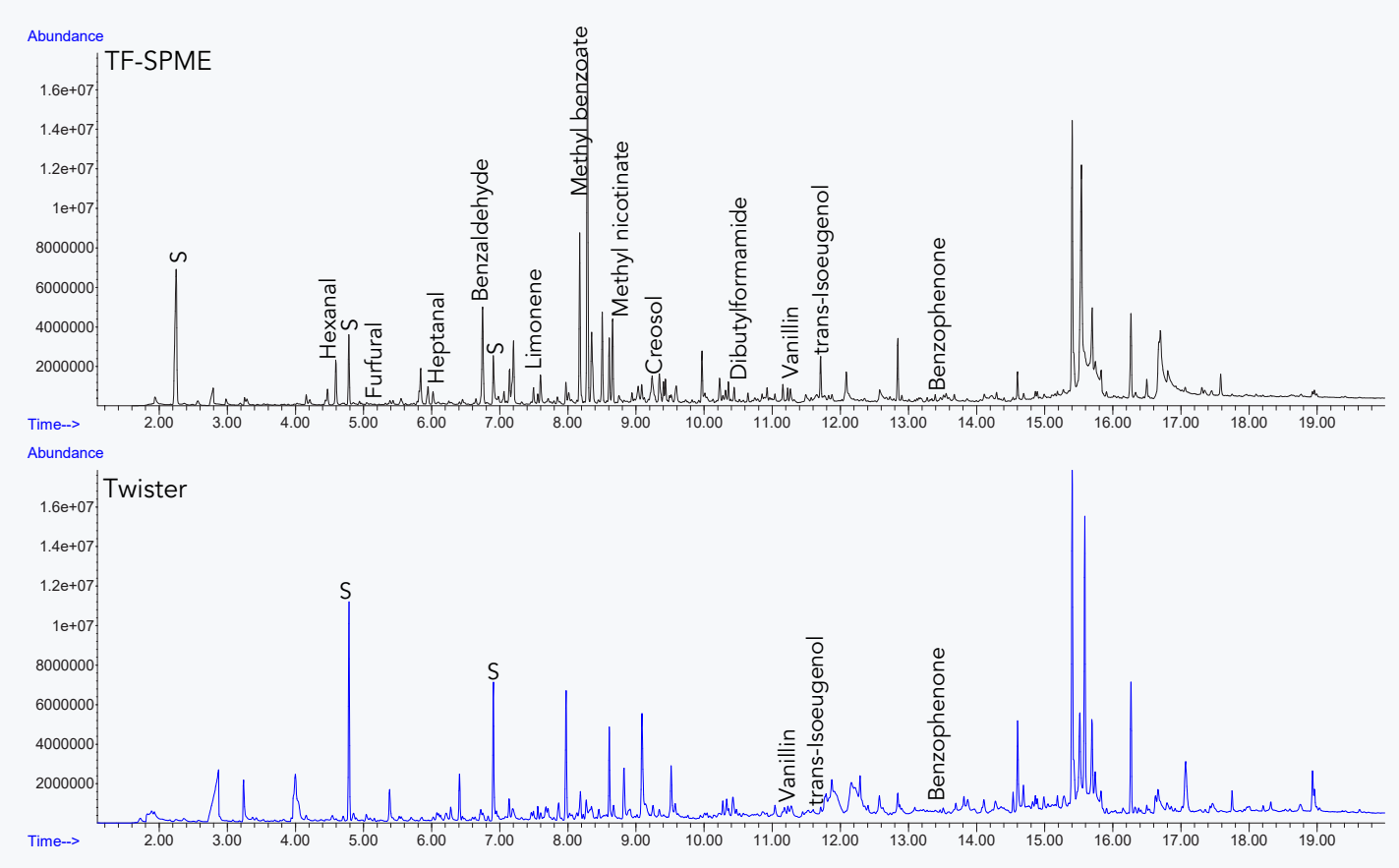

Figure 1: Stacked view of TICs obtained after extracting plant volatiles from violet star petunias using PDMS/DVB TF-SPME (top) and the GERSTEL Twister® (bottom). Siloxane peaks are labeled S.

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Figure 2 shows the TICs of plant volatiles captured from violet star petunias by active air sampling onto thermal desorption tubes filled with Tenax® TA (top) and PDMS foam (bottom). Similar plant volatiles extracted from both techniques are labeled in Figure 2. Compounds like beta-myrcene and creosol were captured using Tenax® TA. Compounds like furanmethanol, levoglucosenone, camphor, and linalyl acetate are seen in the chromatogram for the PDMS foam tube. This shows that different sorbent types capture different compounds and that multiple sorbents should be evaluated as part of the method development process. For this sample type, Tenax® TA provided the best results.

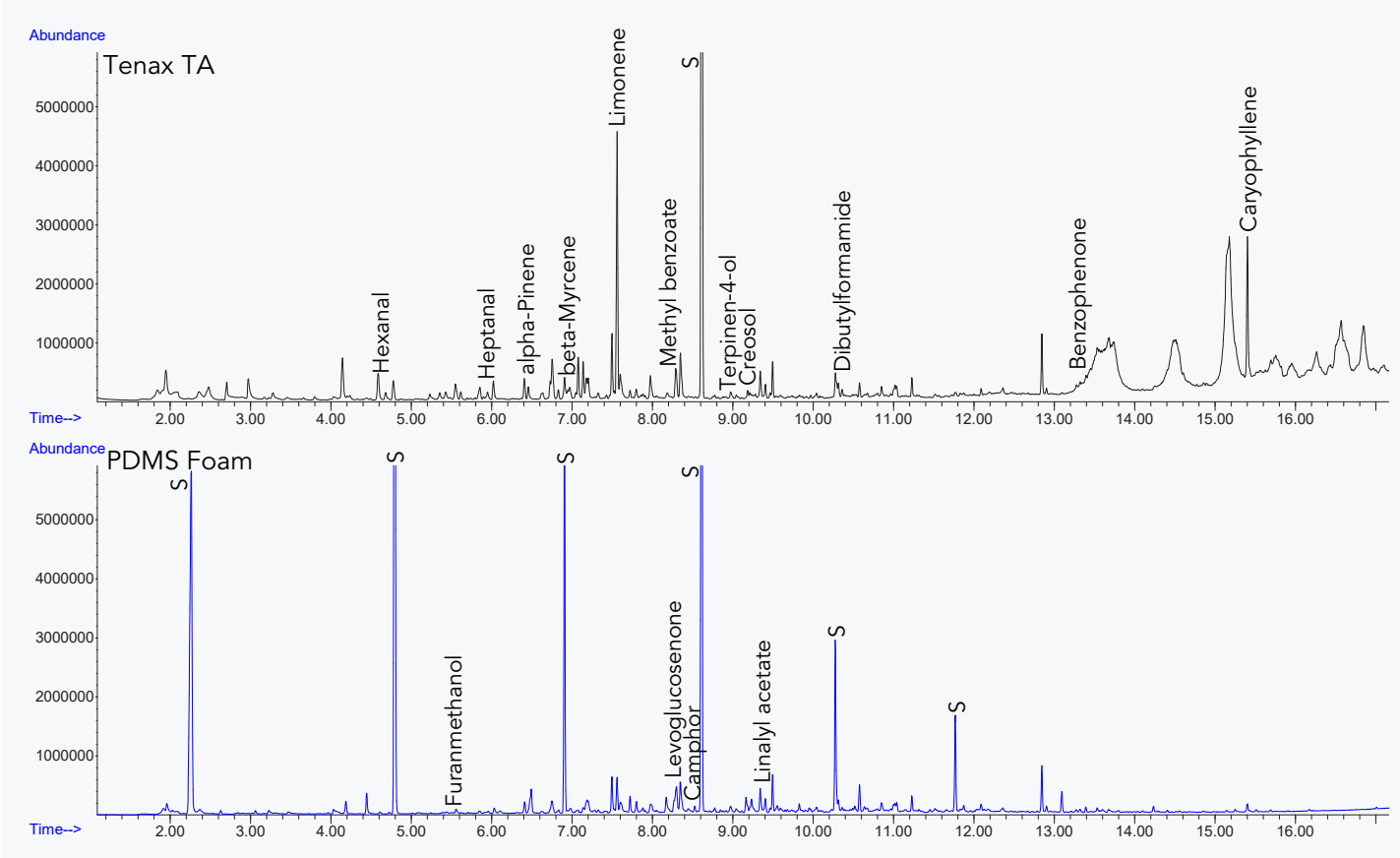

Figure 2: Stacked view of TICs obtained after extracting plant volatiles from violet star petunias using Tenax® TA (top) and PDMS foam (bottom). Siloxane peaks are labeled S.

Comparing Figure 1 to Figure 2, several similar compounds were identified, but differences in the compounds found were also observed. This is due both to differences in sampling time and to the time of day the sampling occurred. Plants emit certain volatiles during the day to attract pollinators, while at night these volatiles are minimized through an endogenous circadian rhythm [1], roughly the equivalent of a sleep–wake cycle that repeats every 24 hours. This is relevant to the study as the TF-SPME and GERSTEL Twisters were employed overnight, whereas the active sampling was done for a 2 hour period in the morning hours.

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For the oakleaf hydrangeas, plant volatile determination from overnight passive sampling with the TF-SPME device and 2 hours of active sampling with PDMS foam in the morning were obtained. Figure 3 shows the TICs of plant volatiles obtained from the oakleaf hydrangeas by TF-SPME (top), and PDMS foam (bottom). The TF-SPME device extracted more compounds than PDMS foam and with better extraction efficiencies. However, some compounds extracted with the PDMS foam were not found with TF-SPME. The comparison demonstrates the value of using both approaches for sampling plant emissions as they are clearly complementary.

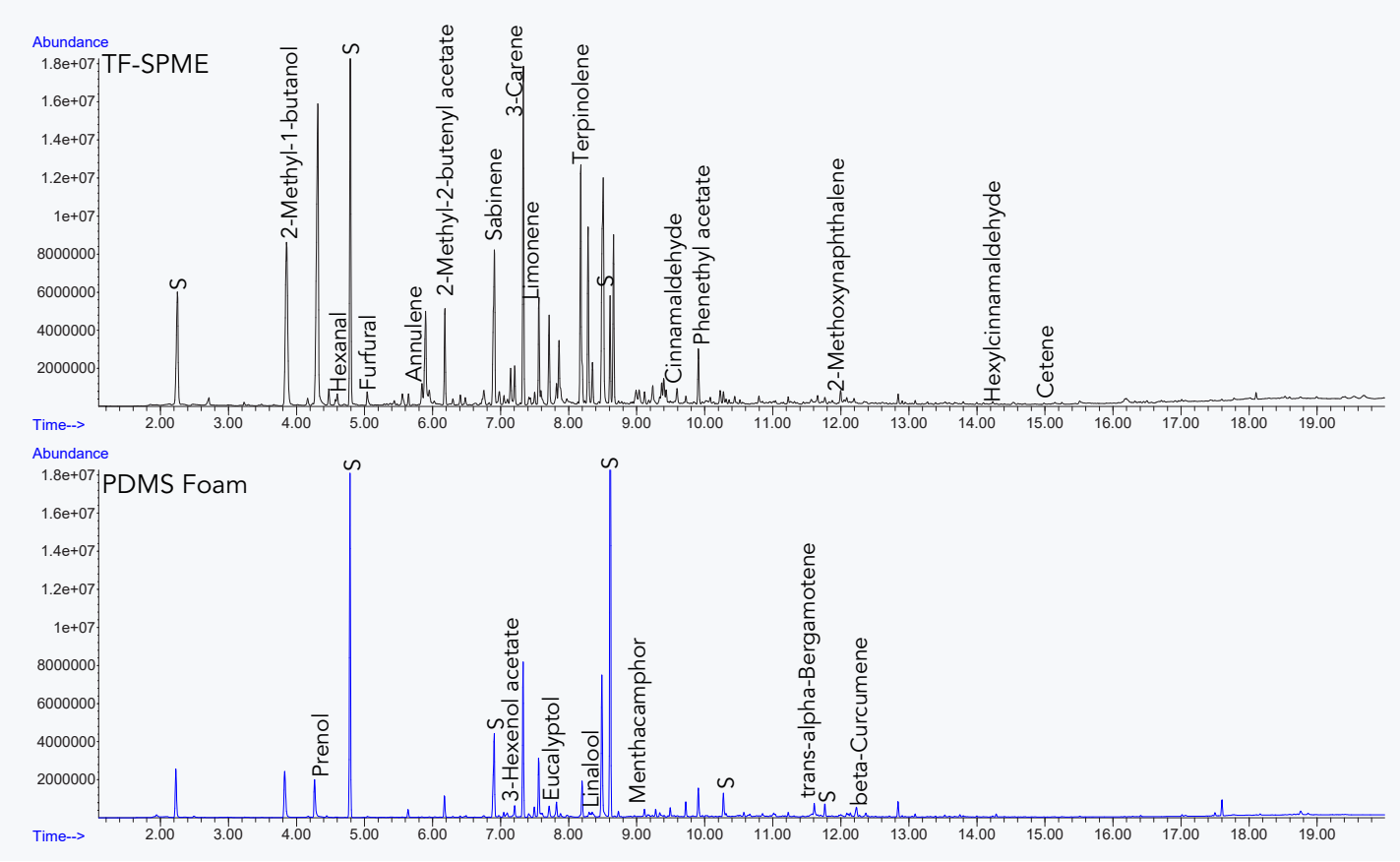

Figure 3: Stacked view of TICs obtained after extracting plant volatiles from oakleaf hydrangeas using PDMS/DVB TF-SPME (top) and PDMS foam (bottom). Siloxane peaks are labeled S.

As also seen in the results from the violet star petunias, the increase in the number of compounds found with the TF-SPME device is likely due to the increased sampling time, sorbent phase, and variation in plant volatile emissions throughout the day.

The violet star petunias and oakleaf hydrangeas have similar plant volatile profiles including several aldehydes, terpenes and straight chain alkanes. However, they also exhibit key differences: Phenylethyl alcohol, eucalyptol, and cetene are seen only in the chromatogram for the oakleaf hydrangeas. When time of day is taken into consideration, the PDMS foam sorbent was able to extract eucalyptol from the oakleaf hydrangeas where the TF-SPME device did not. This is important because research has shown that eucalyptol attracts bees thus indicating how and why plant volatile emissions vary throughout the day [8].

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For the citronella plant, overnight sampling with the TF-SPME device and GERSTEL Twister® was conducted. Figure 4 shows the stacked view of the TICs of plant volatiles captured from the citronella plant by TF-SPME (top) and the GERSTEL Twister® (bottom). As can be seen, considerably higher extraction efficiencies are achieved with the TF-SPME device for the majority of compounds identified. The GERSTEL Twister® was able to extract key citronella plant VOCs like limonene, citronellol, citronellal, and geraniol. The best approach for sampling would be to use both devices simultaneously to capture the widest range of VOCs and to achieve the best detection limits.

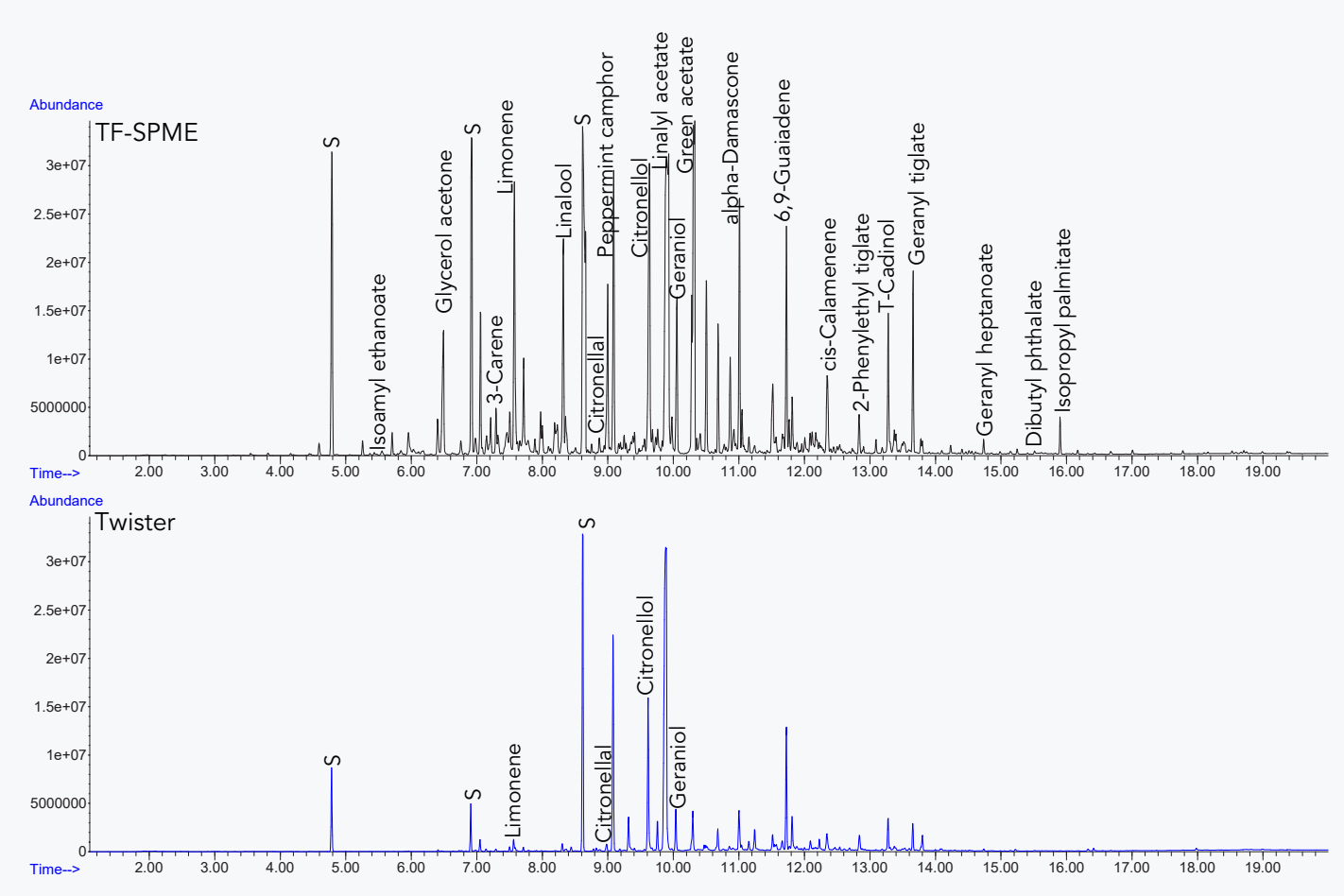

Figure 4: Stacked view of TICs obtained after extracting plant volatiles from a citronella plant using PDMS/DVB TF-SPME (top) and the GERSTEL Twister® (bottom). Siloxane peaks are labeled S.

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For lemon thyme, overnight passive sampling with the TF-SPME device was performed. Figure 5 shows the TIC of plant volatiles captured from lemon thyme by TF-SPME. A wide range of compounds were identified including monoterpenoids, such as geraniol and thymol, monoterpene aldehydes, such as citral, alcohols, and plant metabolites unique to the lemon thyme plant.

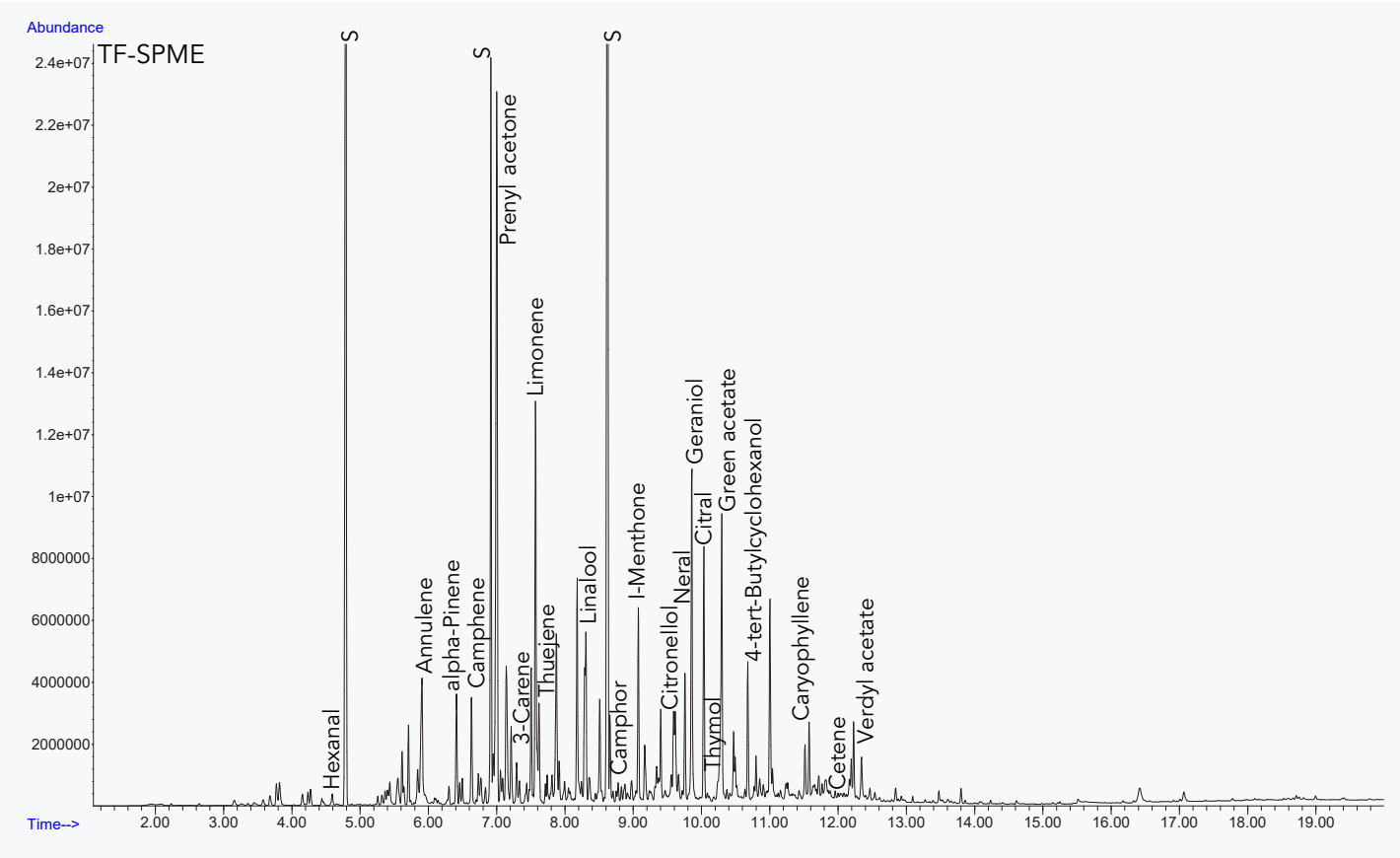

Figure 5: TIC obtained after extracting plant volatiles from lemon thyme using PDMS/DVB TF-SPME. Siloxane peaks are labeled S.

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Conclusions

This work highlights various sampling techniques that can be employed to determine a variety of plant volatiles from different sources. Several factors will affect the chromatographic profile obtained from a sampling technique. These include sampling mode (passive or active), sampling time, sorbent material, time of day, and environmental conditions. The method or combination of methods chosen ultimately depends on the specific analysis goals thus requiring careful consideration and method development.

Passive sampling with DVB/PDMS TF-SPME consistently provided the best chromatographic profiles for violet star petunias, oakleaf hydrangeas, citronella and lemon thyme plants. TF-SPME can be used for field sampling of plant volatiles without removing a plant from its original state. Its only disadvantage is the need for longer sampling times. The GERSTEL Twister® can be used in conjunction with TF-SPME to provide enhanced extraction recovery and overall sensitivity.

Active air sampling is also used to capture plant VOCs and may provide additional information relative to passive sampling. In this study, Tenax® TA provided better results than PDMS foam filled thermal desorption tubes. When developing the active air sampling method, sorbent material, sampling time, and the sampling flow rate need to be considered.

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