Novel Techniques for Capturing Scents in the Rainforest Canopy: the Final Frontier

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Science and perfumery have been inextricably linked from the beginning of commercial perfumery. Perfumery was founded upon extracts of natural products, but as the demand for fragrance increased it was necessary to find ways of supplementing them. The successful synthesis of aroma chemicals started in the 19th century. In 1833 Dumas synthesized camphor, and in 1837 Liebig and Wohler synthesized benzaldehyde. However, progress was relatively slow as the rapidly growing science of organic chemistry was first focused on synthesizing dyes for the textile industry. The pace of change increased rapidly in 1875 and 1876 with two major discoveries of synthetic routes to coumarin by Perkins and vanillin by Triemann and Reimer. These aroma chemicals are credited with the establishment of the synthetic perfume chemical industry. The Grasse perfume industry, whose roots were firmly embedded in naturals, ignored synthetic aroma chemicals, and its dominance as the leading perfume center was gradually lost. The impact of synthetic chemistry on the perfumery industry was clearly recognized by Louis Roure,[1] one of the leaders and creators of the fragrance industry. He pointed out that an error had been committed by Grasse in having neglected the vigorous application of chemical research to the study of flowers to identify and synthesize aroma chemicals.

Development of Headspace Technology

As the use by perfumers of synthetic aroma chemicals grew and became accepted practice, the perfumers' pallette expanded with evolving synthetic chemistry techniques. These new aroma chemicals brought a richness of new scents to the perfumer and laid the basis for modern perfumery. Synthetic chemicals, however, do not replace naturals but supplement and complement them. As the industry became larger and more sophisticated, perfumers again looked to nature for further inspiration. The perfumers challenged the fragrance scientists to provide the scents of plants and flowers not available in commercial quantities. This challenge was met in the 1970's by a technique referred to as headspace analysis that utilized the development of advanced analytical techniques such as gas chromatography and mass spectrometry (GC/MS). These methods made it possible to analyze accurately the scent molecules surrounding the flowers. The headspace technique is described in detail elsewhere.[2] It consists of enclosing the scent emitter in a suitably shaped glass vessel. The scented air is then drawn though a trap, containing an adsorption material such as Tenax[a], by a pump for between half an hour and two hours. The adsorbed scent is then extracted by a small amount of solvent and analyzed by a GC/MS. The technique has been used for more than 20 years to study a wide variety of natural scents, Givaudan Roure, for example, has investigated over 800 flowers, plants and fruits.[3,4,5]

In the 1980's Givaudan Roure expanded this technique to recreate the complex olfactory scent of a location, rather than a single flower or plant. Aromascapes[b] of a variety of scenes, such as the Sequoia forest, Arcadia and the Ligurian coast were undertaken.[6]

ScentTrek[c] Technology

Creativity is a tireless task master; it constantly demands feeding with ideas, discontinuities that inspire new themes. To meet this challenge, Givaudan Roure in 1993 innovated the concept of biodiversity prospecting for scent and aroma chemicals. Biodiversity prospecting was first done by the pharmaceutical industry to find new drugs from rainforests. These forests cover about 6% of the earth's land and are home to around 60% of its plants and animals. It is a cornucopia of plants and flowers with a great diversity of scents. It also provides an abundant source of unknown plants that potentially may yield new and exciting molecules for the fragrance industry. The rainforests are disappearing

[a] Tenax, Supelco Inc., Bellefonte, PA
[b] Aromascapes, Givaudan Roure, Teaneck, NJ
[c] ScentTrek, Givaudan Roure, Teaneck, NJ

at a great rate and, without biodiversity prospecting, many scents and aroma molecules may never be discovered.

In 1994 we set off on a voyage of discovery amongst a profusion of tropical flowers and plants in their natural habitat; the rainforests of Costa Rica. The collection of a flower's scent requires its peak scenting period to be known to the gatherer, because a flower's scent production is cyclical. The scent is the plants' method of attracting pollinators, and, as it requires energy for the biosynthetic process, it gears its production to the presence of its pollinators. This biorhythm is controlled by an internal biological clock that is activated by external things such as heat and light. Because both the quality and quantity of the scent peaks when pollinators are present, a knowledge of a plant's biorhythm is fundamental to reproducing the best scent. In conventional headspace technology, the perfumer or scientist can sit with the flower and determine by smell when the collection should take place. This process is impractical in the rainforest. To make biodiversity prospecting in difficult-to-access rainforests, the ScentTrek technology[7,8] was developed. The technology is built around a portable computer-controlled system that is set up in the rainforest and left to follow a plant's biorhythm throughout the day and night. The equipment gathers the scent of a flower at hourly intervals for analysis. The peak olfactive moment is defined as the maximum scent emission. The detailed analysis at this point is used to reconstitute the fragrance. The ScentTrek technology allowed us to escape the confines and artificiality of the greenhouse, shifting our research into rainforests to meet our perfumers' aspirations for new and exciting scents. The technology allowed us to break new ground and go where no perfumers had gone before.

GreenSky Canopy: the Final Frontier

Although our expeditions to rainforests in Costa Rica, Indonesia and Malaysia fulfilled our creative expectations, we were constantly frustrated during these outings by the discovery of flower petals on the forest floor or tantalizing wafts of fragrance. We identified their sources high above us in the canopy. However, this green sky, towering 150–300 feet above us, with its abundance of flowers that are visible through binoculars, was inaccessible. Our only access to canopy flowers was in light gaps, the spaces created by falling trees, which sometimes contained younger flowering plants and trees.

Our voyage of discovery could not be limited to the serendipitous finding of light gaps. We knew we must find ways to operate ScentTrek in this final frontier. The canopy is home to millions of plants and creatures. It is estimated that eight out of 10 species in the canopy remain undiscovered. It is the last and greatest ecological frontier. W.C. Beebe,[9] in 1917, had this same desire to explore the final frontier. He reflected that the canopy presents another continent to be discovered, not upon the floor but one to two hundred feet above it, where a rich harvest awaits anyone who can overcome the obstacles to reach the summit of the jungle trees. The importance to botanical research of exploring this tapestry of plants in the final frontier high above our heads has also been recently reviewed by E.O. Wilson.[10]

The structure of the canopy is complex. The crown of the imposing tropical trees is a radiating web of slender branches, some with flowers at their tips. Interwoven amongst the treetops are other canopy plants. The most fascinating of these are the epiphytes. Held aloft by the trees, these plants are abundant. It is estimated[11] that a tenth of all flowering plants in the tropics have taken to epiphytic life. Epiphytes do not have roots that reach the ground. They must gather nutrients and moisture hundreds of feet above the forest floor. They do this from little pockets of humus caught up in the intertwined branches and also by gathering nutrients from the moist air. Epiphytes are often referred to as "air plants." Most epiphytic species exist only in the canopy. In addition to these epiphytes, a myriad of other plants, such as climbing plants like vines crisscross the canopy. Vines belong to twice as many families of flowering plants as epiphytes. Without doubt the canopy presents the greatest biodiversity on earth.

This unexplored botanical continent is a researcher's paradise and, although lagging behind the techniques developed to explore that other remote continent—the seabed, progress in exploration methods has been made over the last decade. M.W. Moffet[12] briefly describes the techniques that canopy researchers are innovating to reach this final frontier. Some of the techniques described are:

- Methods adapted from rock climbing techniques[13]
- Cranes and sophisticated cantilever devices[14]
- Towers like erector sets[15]
- A "canopy raft": an inflated platform transported by a hot air balloon that is lowered onto the section of canopy to be researched[16]

GreenSky Technology

Regardless of the method of ascent used to reach the green sky, our initial survey of the canopy indicated that new techniques are required to maximize the collection of scent. The interwoven fine lace of the treetops is delicate. Classical headspace and ScentTrek equipment are too heavy and are not suitable for suspension in such a fragile network. The new GreenSky[d] technology was developed to allow us to collect both day and night scenting flowers in this fragile canopy.

The method for collecting day-scenting flowers in the canopy was inspired by a collaborative project Givaudan Roure has with Scott Mori of the New York Botanical Garden. Mori is studying what aroma chemicals attract certain species of pollinators. Discussing the collection of scents, he drew our attention to the Euglossine bee. The male Euglossine bee not only collects nectar from the

[d] GreenSky, Givaudan Roure, Teaneck, NJ

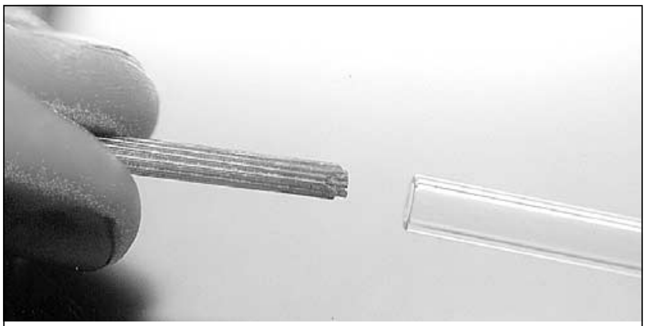

Figure 1. Zenith Trap as it fits into a thermal-desorber tube

flowers it visits, but also collects its perfume. The bee collects the fragrance with its foretarsal brushes and transfers it to its hind tibia for storage. The males use the fragrance as a pheremone.[17] To mimic the Euglossine Bee's 'dip and collect' technique, was our design principle; a collection trap that could reach gently into a flower and rapidly extract the perfume.

Zenith Trap

To meet this specification we defined that the technique must be capable of collecting sufficient amounts of each of the individual components of the flower's scent within a contact time of five minutes or less. This indicated that a dynamic system was needed rather than one that relies on convective transport of scent molecules to the adsorption site.

The collector we designed consists of a bundle of fused silica capillary tubes whose inner surfaces are coated with different substrates with various degrees of absorption capacity for aroma chemicals of different polarities. This collector we termed the Zenith Trap.[e]

The coatings, length and pumping rates were optimized for rapid quantitative collection of aroma chemicals. The size of the Zenith Trap is such that it fits into a thermal-desorber tube (Figure 1).

The Zenith Trap is attached to a small pump via a flexible tube so that the flower's scent is drawn through the capillary bundle. The Zenith Trap and the connecting tube are housed within a semi-rigid tube so that the capillary bundle can be withdrawn during the locating operation. This protects the capillary bundle from breakage.

The new collection device is attached to an extendable aluminum rod so the capillary bundle can be extended out to reach flowers at the extremity of branches. Once the flower is reached, the Zenith Trap, like the Euglossine bee's foretarsal brushes, is inserted gently into the flower and the scented air is drawn slowly through it by a pump to adsorb the aroma chemicals.

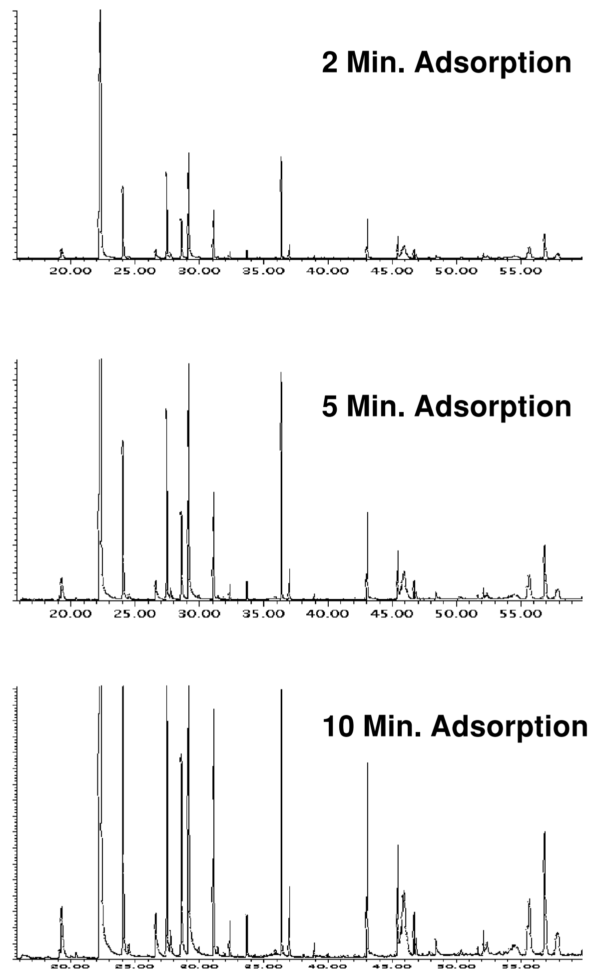

Figure 2. Analysis of blue hyacinth volatiles gathered by Zenith Trap

A blue hyacinth was used as a fragrance source for our commissioning trials. The analysis of the volatiles adsorbed on the Zenith Trap was carried out by placing the trap in a Gerstel TDS-2 thermal desorption system[f], coupled with a Gerstel CIS 4 cooled injection system[g] incorporated into a Hewlett Packard 6890 gas chromatograph[h] equipped with a Hewlett Packard 5973 mass selective detector[i]. The GC profile of the scent molecules collected by the Zenith Trap as a function collection time is shown in Figure 2. It can be seen that even after two minutes, sufficient fragrance molecules are collected with this new technique to be able to reconstitute the scent of the flower.

Comparison with Current Collection Systems

In order to validate the new methods we have compared it to the two methods used today to study the scent of flowers.

Comparison with conventional headspace: The chemical composition of the blue hyacinth collected for 30 min by conventional headspace using a Tenax trap is compared to the composition obtained after five min collection

[e] Zenith Trap, Givaudan Roure, Teaneck, NJ
[f] Gerstel TDS-2, Gerstel Inc., Baltimore, MD
[g] Gerstel CIS 4, Gerstel Inc., Baltimore, MD
[h] Hewlett Packard 6890, Hewlett Packard Corp., Palo Alto, CA
[i] Hewlett Packard 5973, Hewlett Packard Corp., Palo Alto, CA

time in the new Zenith Trap in Table 1. The result shows that after five min collection the chemical profile of the scent collected with the Zenith Trap is similar to that of conventional headspace using Tenax.

Comparison with solid-phase micro extraction (SPME): SPME is a solid-phase adsorber coated onto a fused-silica fiber developed as a capture system for organic molecules.[18] Several types of solid phases exist. The ones we examined are shown in Table 2.

The SPME fiber was placed within 1cm of the flower and left exposed for 30 min. The Zenith Trap was also positioned within 1cm of the flower and exposed for five min. The main components of the blue hyacinth scent, collected by the Zenith Trap and SPME are shown in Table 3.

The SPME after a collection time of 30 min does not appear to be as efficient as the Zenith Trap after five minutes. SPME has been used for determining the aroma chemicals of flowers. However, the collection time quoted was 30- 60 min,[19] which may explain why SPME appears not to collect all the components.

Collection of Scent from Night-Pollinated Flowers

To collect the scent of night-pollinated flowers, we designed a miniaturized version of our ScentTrek collection device. The collection manifold was redesigned to accept subminiature valves. The electronics and computer were integrated with a new miniaturized pump and flow controller. These changes reduced both the weight from 20 lb to below 2 lb as well as the size so that it was easier to carry and manipulate in the canopy. In addition, a lightweight plastic collection chamber was used to replace heavy glassware and a raft system was used to distribute the weight (Figure 3).

The principle of the collection is the same as the original "timeslicer" in that the computer activates a pump that draws a scent through a Tenax trap for two hours and then switches the collection to a second trap for a further two-hour collection. Thus, 12 two-hour slices of the plant's scenting period can be collected. The peak of fragrance emission and the chemical composition during this period can be determined by GC/MS analysis.

The new equipment was tested in a ScentTrek in a rainforest at Sau, in the center of French Guiana. The biorhythm of a night-blooming flower, which we called evening glory, is shown in Figure 4. The background curve is the total volatiles and the bars represent the three major components.

The peak olfactive moment occurs between 12 and 4am. This is when maximum pollinator activity would occur. This new lightweight portable "timeslicer" extends our exploration capability to both daytime and night-pollinated flowers. It can be set up in the canopy to follow a flower's biorhythm overnight. Its position is identified via a global positioning system for later retrieval.

Summary

GreenSky technology provides us with unique methods to collect the scent of flowers in the rainforest canopy. The Zenith Trap can be extended out to day-pollinated flowers to acquire the scent molecules. The miniaturized "timeslicer" is used to capture the scent of the night-pollinated flowers at the peak of their biorhythm. These techniques allow us to follow Louis Roure's advice: to apply vigorously scientific research to the scent of flowers. It allows us to explore the greatest biodiversity on earth, the canopy of the rainforest and to provide new scents and aroma chemicals for our perfumers into the next millennium.

'The knowledge of nature as it is—not as we imagine it to be—constitutes true science.'
—Paracelsus

References:

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Table 1. Comparison of percent composition for blue hyacinth

Component	Components (%) Zenith	Headspace
Benzaldehyde	1.2	2.3
p-Cymene	0.2	0.2
Limonene	0.2	1.2
Benzyl alcohol	1.5	2.5
Phenylacetaldehyde	1.0	1.1
trans Ocimene	1.0	7.2
Phenylethanol	25.5	22.2
Benzyl acetate	13.7	20.1
Aldehyde C-10	0.6	0.5
Phenylpropyl alcohol	1.4	0.7
Phenylethyl acetate	2.4	7.7
Cinnamic aldehyde	4.6	2.9
Cinnamic alcohol	16.0	9.0
Methyl 2-methoxybenzoate	0.7	0.8
Eugenol	0.6	0.8
1,2,4 Trimethoxy benzene	11.6	7.2
Methyl eugenol	0.6	0.9
Cinnamyl acetate	0.5	0.2
α Farnesene	6.3	5.1
Benzyl benzoate	9.3	6.3
Phenylethyl benzoate	1.1	1.1
	100.0	**100.0**

Table 2. Solid-phase micro-extraction fibers (SPME)

Supelco SPME Fibers Coating	Polarity	Catalog Number
Polydimethylsiloxane (PDMS) 100 um	Non Polar	5-7300
Carboxen/Polydimethylsiloxane 75 um	Slightly Polar	5-7318
Polyarcrylate (PA) 85 um	Mid Polar	5-7304
Carbowax/divenylbenzene CW/DVB 65um	Polar	5-7312

1. Y. R. Navares and G Mazur, Natural Perfume Materials, Reinhold Publishing Corporation (1947) p137.
2. R. Kaiser, Trapping, Investigation and Reconsitution of Flower Scents, Ch. 7 in Perfumers: Art Science and Technology, P.M. Muller and D Lamparsky eds, London and New York, Elsevier Applied Science (1991) pp 213-250.
3. R. Kaiser, The Scent of Orchids, Amsterdam, Elsevier (1993)
4. R Kaiser, An Introduction to the Scent of Cacti, Flav Frag J., 10, (1995) pp 153-164.
5. R Kaiser, New or uncommon Volatile Compounds, in vol 2, Flavours, Fragrance and Essential Oils, Istanbul, Turkey, m15-19 October 1995, Istanbul, AREP Publ (1995) pp 135- 168.
6. R. Kaiser, Environmental Scents at the Ligurian Coast, Flav Frag J, 22, (1997) pp 7-18
7. T. McGee and K. Purzycki, see M Roubi, C&EN, Capturing the Essence of a Pleasant Scent, Feb. (1997) pp 34-35.
8. T. McGee and K Purzycki see S Dorman, Givaudan Roure's ScentTrek Searches for Novel Scents, Spray Technology and Marketing Feb. (1997) pp 32-35.
9. W.C. Beebe (W.C. Beebe, G I Hartley and P G Howes, Tropical Wild Life in British Guiana, New York, New York Zoological Society (1917)
10. E. O. Wilson, Rainforest Canopy: The High Frontier, National Geographic 180, (1991) pp 78-107.
11. A. Gentry and C Dodson Diversity and Biogeography of Neotropical Vascular Epiphytes" Annals of Missouri Botanical Garden, 74, 205-233, 1987)
12. M. W. Moffet The High Frontier, Exploring The Tropical Rainforest Canopy, Harvard University Press, Cambridge, USA (1993)

Figure 3. Miniature ScentTrek device for night-pollinated flowers

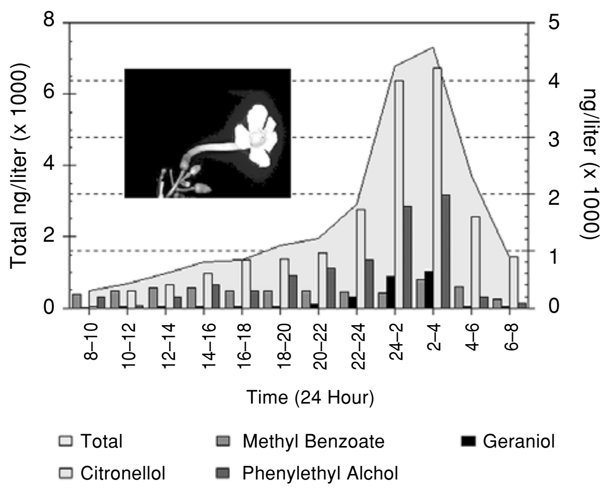

Figure 4. Analysis of evening glory collected by ScentTrek

Table 3. Comparison of SPME and Zenith Trap

Flower		Blue Hyacinth				
Ret. Time	**Component**	**PDMS %**	**Carboxen/PD %**	**PA %**	**CW/DVB %**	**Zenith %**
14.85	Benzaldehyde	0	0	0.1	0	1.2
18.78	Limonene	0	0	0.04	0	0.2
19.15	Benzyl alcohol	1.4	0	2.0	0.3	0.2
19.28	Phenylacetaldehyde	2.8	0	2.3	2.1	1.5
20.38	trans Ocimene	11	0	0.1	6.8	1.0
22.30	Phenylethanol	20.7	0.9	43.8	60.6	1.0
24.05	Benzyl acetate	20.1	0.6	8.0	5.9	25.5
25.96	Aldehyde C-10	0	0.8	0.2	0.2	13.7
26.58	Phenylpropyl alcohol	1.4	0	1.5	0.6	0.6
27.46	Phenylethyl acetate	5.1	0.8	7.5	4.6	1.4
27.76	Cinnamic aldehyde	0	0	0.9	2.7	2.4
28.60	Indole	0.9	0.2	4.9	1.8	4.6
29.17	Cinnamic alcohol	3.2	0	11.1	3.6	16.0
29.96	Me-2-methoxybenzoate	2.7	0	0.1	0.1	0.7
31.11	1,2,4-Trimethoxy benzene	3.9	0.5	4.3	2.8	0.6
32.27	Methyl eugenol	0.8	0.1	0.5	0.4	11.7
33.68	Cinnamyl acetate	0.5	0	0.7	0.1	0.6
36.38	α Farnesene	18	88.2	6.8	6.1	0.5
43.06	Benzyl benzoate	5.7	4.4	3.0	1.1	6.3
45.44	Phenylethyl benzoate	2	3.6	2.1	0.4	9.3

13. D. R. Perry and J. Williams, The Tropical Rainforest Canopy: A Method Providing Total Access, Biotropica, 13 (1981) pp 283-285.
14. G. G .Parker, A. P. Smith and K. P. Hogan Access to the Upper Forest Canopy with a Large Tower Crane, Bioscience, 42 (1992) pp 664-70.
15. J. C. Luval see M. W. Moffet, ch 4 Gardens in the Sky, in The High Frontier, Exploring the Rainforest Canopy, Harvard University Press, Cambridge USA (1993) pp 65-81.
16. F. Hallé, A Raft Atop the Rainforest, National Geographic 178 (1990) pp 129-38.
17. J.D. Ackerman, Geographic and Seasonal Variation in Fragrance Choice and Preference of Male Euglossine Bees, Biotropica, 21 (1989) pp 340-347.
18. C.L. Arthur and J Pawlinszn, “Solid Pashe Micro Extraction with Thermal Desorbtion Using Fused Optical Silica Fibers. J Anal. Chem., 62, (1990) 2145-2148.
19. B. D. Mookherjee, S. M. Patel, R. W. Trenkle and R. A. Wilson, A Novel Technology to Study the Emission of Fragrance from the Skin, Perf. & Flav., 23 (1998) pp 1 - 11.

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