Roman Kaiser

Scent of the Vanishing Flora

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Verlag Helvetica Chimica Acta · Zürich

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CH-8600 Dübendorf
Switzerland

Published jointly by
VHCA, Verlag Helvetica Chimica Acta, Zürich (Switzerland)
WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim (Federal Republic of Germany)

Editorial Directors: Dr. M. Volkan Kisakürek, Thomas Kolitzus
Production Manager: Bernhard Rügemer
Cover Concept: Hans Siegwart
Graphic Design: Jürg Riedweg

A contribution to the
2010 International Year of Biodiversity
from *Givaudan*
and *Verlag Helvetica Chimica Acta*

Library of Congress Card No. applied for
A CIP catalogue record for this book is available from the British Library

Die Deutsche Bibliothek – CIP-Cataloguing-in-Publication-Data
A catalogue record for this publication is available from Die Deutsche Bibliothek

ISBN-10 3-906390-64-0
ISBN-13 978-3-906390-64-2

Printed on acid-free paper.

Printing: Konrad Triltsch, Print und Digitale Medien, D-97199 Ochsenfurt-Hohestadt, Germany
Printed in Germany

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1. Introduction to the *'Scent of the Vanishing Flora'*

At the *XVI International Botanical Congress*, held from August 1–7, 1999, in St. Louis, Missouri, *Peter H. Raven*[1] delivered one of his many important talks which was entitled ***'Plants in Peril: What Should We Do?'*** and he started as follows:

'*Plants provide, directly or indirectly, all of our food; most of our medicine; a large portion of the chemical substances that we use; or our shelter, and of our clothing. They protect the topsoil, insure the quality of the water we drink; determine local climates; and ultimately provide food and shelter for all other living beings. They are beautiful and diverse, and nourish our souls as well as our bodies. Yet, we are destroying them in a frightening rate, so that as many as* ***100,000*** *of the estimated total* ***300,000 species*** *may be gone or on the way to extinction by the middle of the next century. If we do not take action now, by the end of the century, we may have destroyed two-thirds of the plant species we currently use and enjoy.*

Why are we doing this? *Are we mad, or do we just not understand the enormity of the crime that we are perpetrating on our children, their children, and* ***all human beings who will live in the future?*** *By taking concrete steps in the near future, we could prevent the extinction of the majority of these plant species.* ***We must commit to these steps, starting here, starting now.***'

Let us change to the most recent time, more than ten years later, to the time I was doing the final work on the manuscript for this book. Under the News Headlines '*Moving from Words to Action*', we could read: '*12 January 2010, Berlin (Germany). The International Year of Biodiversity was launched yesterday by the German Chancellor Angela Merkel. Germany will hold the Presidency of the Convention on Biological Diversity until October when Japan will take over. Chancellor Merkel urged the world to increase efforts for biodiversity and said 'The question of preserving biological diversity is on the same scale as climate protection', and* ***'we need a sea change. Here, now, immediately – not some time in the future.'***' Nearly the same words as expressed in 1999 by *Peter H. Raven*, the noble defender of the plant kingdom[2], but since then it has become so much worse.

In 1995, I had the fortune that the beautiful and unique book by *Dugald Stermer* '*Vanishing Flora*'[3] found its way into my hands shortly after its publication. It comprises artistically outstanding and scrupulously accurate illustrations of 74 threatened or even extinct species from around the world

Fig. 1.1.1. *Franklinia alatamaha* BARTRAM EX MARSH., *the so-called Lost Franklinia, one of the 74 art crafts in Dugald Stermer's book 'Vanishing Flora'*[3]. *A photo of this wonderful North American Theaceae, which is extinct in nature, can be seen in Fig. 2.1.1.*

with corresponding essays, and he attempts in his strongly worded introduction to make people aware that not only the animal but also the plant kingdom is in peril – that, globally, at least 10% of all species are in imminent danger of extinction. Today, the estimates are at least three times higher…

Since my early studies with scented orchids[4], I was aware that many of them need protection, and in my ongoing search for new scent molecules and scent concepts in nature, I encountered again and again endangered plants emitting beautiful, or sometimes also repulsive, scents and, after having seen *Dugald Stermer*'s book, I felt the desire to complement it with '*The Scent of the Vanishing Flora*'. Therefore, I am grateful for having the permission to reproduce two of these 74 illustrations of threatened species in the introduction of this second, complementary book covering the '*Vanishing Flora*'. The first art craft (*Fig. 1.1.1*) shows the most famous discovery of American botanists *John* and *William Bartram*, the exquisite *Franklinia alatamaha*, which has not been encountered in nature since 1803. This stunning and at-

Fig. 1.1.2. *Gladiolus watermeyeri* L. Bolus *(Iridaceae) is a second example from Dugald Stermer's book 'Vanishing Flora'*[3]*. A photo of this rare endemic species taken at the natural habitat near Nieuwoudtville is shown as Fig. 2.6.1.*

tractively scented Theaceae, which can still be seen at the Historic Bartram's Garden in Philadelphia, will be discussed as the first species in this book (see *Sect. 2.1*). The second art craft (*Fig. 1.1.2*) shows the equally beautiful *Gladiolus watermeyeri* which I had the privilege to study at its natural habitat in the Nieuwoudtville region of South Africa (see *Sect. 2.6*).

I am grateful to the General Management of *Givaudan* for having supported this project which allowed me to work, after many years of 'pre-studies', for ten years intensively on this topic, and to write this book covering 267 endangered scented plant species from all over the world.

1.1. Extinction of Species and Monitoring Endangered Species

Facts about the Extinction of Species

As recently summarized by '*IUCN Red List*'[5], the world is, and always has been, in a state of flux. Over hundreds of millions of years, continents have broken apart, oceans appeared, and mountains formed and worn away. With geological change come changes in living things: species, populations, and whole lineages disappear, and new ones emerge. Extinction is, therefore, a natural process. According to the fossil record, no species has yet proved immortal; as few as 2–4% of the species that have ever lived are believed to have survived today[5]. The rest is extinct, the vast majority having disappeared long before the emergence of humans.

However, the rapid loss of species we are facing today is estimated by experts to be between 1,000 and 10,000 times higher than the rate (so-called 'background' rate) that prevailed over the past 60 million years. Throughout most of geological history, new species evolved faster than existing species disappeared, thus continuously increasing the planet's biodiversity. Now, evolution is falling behind. In contrast to the five mass-extinction events in the geological history, the current extinction phenomenon is one for which a single species – ours – appears to be almost exclusively responsible. This new expected mass-extinction event is often referred as the *Sixth Great Extinction*[6] and might affect mankind as much as global warming. No doubt, both phenomena have to be considered simultaneously if our goal is to survive.

The greatest threat to the world's living creatures is the degradation and destruction of habitat, affecting nine out of ten threatened species[6]. Thus, habitat loss and degradation affect 89% of all threatened birds, 83% of mammals, and 91% of threatened plants[5]. Humans have transformed nearly half of the planet's ice-free land, with serious effects on the rest of nature. We have made agricultural fields out of prairies, savannahs, including related biotopes and forests. Each year, the earth's forest cover shrinks by 16 million hectares, with by far most of the loss occurring in tropical forests, which host 80 to 90% of all species of land plants, fungi, and animals. Ecologically rich wetlands have been reduced to half over the past century, and other freshwater and terrestrial ecosystems have been degraded by pollution. Deserts have expanded to occupy previously vegetated areas, accelerated in some cases by overgrazing of domesticated animals[6].

According to recent survey of some 1100 animal and plant species, between 15 and 37% of them could be wiped out by 2050 through the influence of climate change[7], an additional negative aspect not included in earlier estimates. Yet, the actual loss may be greater because of the complexity of natural systems. The extinction of key species could have knock-on effects, causing further extinctions.

In 2002, an often cited article by *Nigel C.A. Pitman* and *Peter M. Jørgensen* entitled '*Estimating the Size of the World's Threatened Flora*' appeared in *Science*[8] from which I should cite their summary: '*The most commonly cited figure for the fraction of the global flora threatened with extinction – 13% – is known to be seriously underestimated, because it does not include a reliable tally of species at risk in the tropical latitudes where most of the world's plants grow. Here we estimate the missing tropical data from global patterns of plant endemism. The result suggest that as many as half of world's plant species may qualify as threatened with extinction under the World Conservation Union (IUCN) classification scheme.*'

This estimate seems to correspond well to the latest number, 8457, of threatened plant species given in the *IUCN Red List 2007*[5]; this is *ca.* 2% of the world's described plants. As only 4% of the world's described plants have been evaluated yet, the true percentage of threatened plant species is correspondingly much higher.

Monitoring Endangered Species

Widely recognized as the most authoritative and comprehensive assessment of the global conservation status of plants and animals is the ***IUCN Red List of Threatened Species*** (also known as the ***IUCN Red List*** or ***Red Data List***), which is also thought to guide conservation efforts at all levels around the world[9]. The **I**nternational **U**nion for the **C**onservation of **N**ature and Natural Resources (**IUCN**) is the world's main authority taking care of this enormous task.

Species are classified in nine groups, defined by criteria such as rate of decline, population size, area of geographic distribution, and degree of population and distribution fragmentation:

- **Extinct (EX)** – No individual remaining.
- **Extinct in the Wild (EW)** – Known only to survive in captivity, or as a naturalized population outside its historic rang.
- **Critically Endangered (CR)** – Extremely high risk of extinction in the wild.
- **Endangered (EN)** – High risk of extinction in the wild.
- **Vulnerable (VU)** – High risk of endangerment in the wild.
- **Lower Risk (LR)** – This category is at lower risk if it was evaluated and did not fit into CR, EN or VU groupings. Organism in this category can be subdivided into three subcategories:

Conservation Dependent (LR/cd) – Depends on conservation to keep the species out of any of the categories above.

Near Threatened (LR/nt) – are not conservation dependent, but might be close to or approaching vulnerable status.

Least Concern (LR/lc) – do not fall into either cd or nt but need observation.

- **Data Deficient (DD)** – Not enough data to make an assessment of risk of extinction.
- **Not Evaluated (NE)** – Has not yet been evaluated against the IUCN criteria.

When discussing the *IUCN Red List*, the official term '*Threatened*' is a grouping of the three categories: Critically Endangered, Endangered, and Vulnerable. As already mentioned above, only 4% of the world's described plants have been evaluated yet by IUCN, and therefore, the term 'threatened' should be used also for 'endangered' species not yet covered by IUCN. In such cases, I always used this term after consulting expert opinions. In fact, only two plant groups, *i.e.*, cycads[10] and conifers[11], have been comprehensively assessed to date. I would like to illustrate this with the example of *Abeliophyllum distichum* endemic to an exceedingly small area in Korea. In 1998, a review of the plant's conservation status found that '*it is close to extinction and qualifies for the IUCN Category of 'Critically Endangered', indicating a high risk of extinction in the near future*'; but as of 2009 it has not yet been formally assessed for the *IUCN Red List*. This species is described in more detail in *Sect. 2.2*.

Another term often used in this context is *CITES*, which concerns trade of endangered species across international borders. The **C**onvention on **I**nternational **T**rade in **E**ndangered **S**pecies of Wild Fauna and Flora (**CITES**), also known as the *Washington Convention*, is an international agreement between governments, which drafted the results of a resolution adopted in 1973 at a meeting of IUCN. Its aim is to ensure that international trade of specimens of wild animals and plants does not threaten their survival, and it is in accordance with varying degrees of protection of more than 33,000 species of animals and plants. Today, CITES has received the ratification of *ca.* 180 nations.

The species that are protected by CITES are collected in three lists, known as Appendices. **Appendix I** contains the most threatened species, supposedly those animals and plants that will definitely become extinct, unless enormous efforts are made to save them. **Appendix II** lists species or groups of organism where trade is being monitored. These species are not necessarily threatened but might become so if excessive trade of wild individuals occurs. **Appendix III** is for species threatened within particular countries but are not threatened globally or over their entire range.

1.2. Investigating the Scent of the Vanishing Flora

By the 1970s, methods of instrumental analysis, particularly capillary gas chromatography (GC) and mass spectrometry (MS), had reached such a high level of sensitivity, thanks to modern electronics, that also the analytical investigation of micro-samples could be envisaged. This prompted looking for methods that would allow trapping the scents emitted by living flower/plants in a quality as perceived by the human nose. These requirements were not met by destructive isolation methods such as micro-extraction/distillation, which might influence the original scent. The method of choice was instead specified as a close-to-nature trapping technique that, for the first time, would enable the analytical investigation of rare and endangered species in a non-destructive way. Such a method was soon developed, the emitted scent being trapped on a small amount of suitable adsorbent porous polymer, such as *Porapak* or *Tenax*, or charcoal, followed by solvent extraction.

In the mid 1970s, we implemented a long-term research program at *Givaudan* with the aim of investigating and, in promising cases, subsequently synthetically reconstituting such original and attractive scents that are not available as commercial essential oils or related products. The method has since proved to be effective, and, over the past ten years, has been especially adapted to field experiments conducted under extreme conditions such as those in rain forests.

To collect the flower scent of, *e.g.*, *Pachira insignis*, a fascinating Bombacaceae native to the neotropics, a single flower is inserted into a glass vessel of adapted size and shape without damaging the flower (*Fig. 1.2.1*). The scented air surrounding the flower is then drawn through the adsorption trap by means of a battery-operated pump over a period of 30 min to 2 h (30 ml/min), depending on the intensity of the scent. The adsorption trap, containing 2–5 mg of adsorbent, in this case *Porapak Super Q*, is placed as close as possible to the scent source within the glass vessel. While air and moisture pass these micro-traps unhindered, the scent is adsorbed and accumulates to amounts of 1–200 µg during the collection time. For flowers or plant parts with very complex shapes, it is more practical to simply isolate the scent source from the environment to the extent possible with a suitably shaped object, *e.g.*, a glass funnel. The adsorption trap is then cantered as near as possible to the position where the scent release is judged to be maximum. Afterwards, the adsorbed scent is eluted with an adequate amount of suitable solvent, usually 20–60 µg of hexane/acetone 10:1, directly into a micro-ampoule, which is then sealed and kept cool until the return to the lab-

Fig. 1.2.1. *Trapping scent samples in the field (Pachira insignis, a Bombacaceae native to Amazonia)*

oratory. Finally, the samples thus obtained are investigated by a combination of capillary gas chromatography and mass spectrometry (GC/MS), and complementary methods.

The flower frequently has such a shape that the micro-trap can be introduced directly into the flower without using a glass vessel, as sometimes practiced with so-called 'SPME (Solid-Phase Micro-Extraction) fibers' by other researchers. However, both the 'SPME-fiber' method, which we have been using many years for routine measurements, and the headspace trapping techniques with subsequent thermo-adsorption have the fatal disadvantage that only one injection into the GC/MS system per experiment is possible, allowing only a rough overview analysis. Working in the fragrance industry requires one to be totally scent-oriented, which means that at least a second and third injection are needed for the so-called 'GC-sniffing', in order to recognize trace constituents often of decisive olfactory importance. Thanks to the enormous advances achieved in instrumental analysis, even NMR spectroscopy can be involved today in the structure elucidation of new scent components of such micro-samples, provided 1–5 μg can be isolated *via* preparative capillary GC. In practice, this is only realistic with samples obtained by trapping and subsequent solvent elution.

In a very similar way, fragrant molecules can also be adsorbed by all types of aqueous solutions – such as fruit juices, plant saps, or environmental fluids – by so-called SPE (Solid-Phase Extraction) methods[12,13]. The aqueous solution (40–80 ml) is passed through a small amount of a porous polymer capable to adsorb the aroma compounds, while the more polar components as hydroxy acids/carboxylic acids or sugars pass unhindered. Subsequently, the aroma part is recovered by elution with an adequate solvent, and the obtained elute is investigated as already described for the headspace samples.

Furthermore, the complementary investigation of a micro-extract of the fragrant plant tissue in question – if available at all for this purpose – is always helpful, since it facilitates the estimation of the quantitative data and allows a better insight into the less volatile part of the natural scent. Such micro-extracts may be obtained either by extracting the flowers/plants with highly purified solvents such as hexane in the classical way or with liquid CO_2 in modern computer-controlled, highly convenient laboratory systems.

By applying these methods over the past 30 years, we have investigated more than 2500 flower, plant, fruit, wood, and herb scents out of a selection of *ca.* 9000 species of scented plants evaluated during this time. Publications on, *e.g.*, the scent of orchids[4,14] and of cacti[15], on new or uncommon volatile compounds among the most diverse floral scents[16,17], on scents found in rain forests[18], and, more generally, on a great variety of meaningful scents around the world[19] provide partial overviews of these investigations.

As illustrated in another publication[20], our approach to trapping, investigating, and reconstructing natural scents is designed, however, to study not only well-defined flower, herb, fruit, and wood scents, but it can also be applied to the investigation of entire olfactory 'scenarios', perceived in a certain environment as, *e.g.*, in a 'Maquis' biotope at the Ligurian coast.

Contributions published by *Mookherjee et al.*[21], *Joulain*[22], *Brunke et al.*[23], *Nakamura*[24], *Surburg et al.*[25], and others show that other groups in the fragrance industry probably began to address the topic of scent trapping at more or less the same time. Related and complementary methods have been reviewed by *Bicchi* and *Joulain*[26], *Kaiser*[27], *Dobson*[28], and *Knudsen et al.*[29].

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2. Illustrating the Scent of the Vanishing Flora

2.1. No More Existing in Nature

'When the last individual of a race or a living thing breathes no more, another heaven and another earth must pass before such a one can be again.'
William Beebe (1877 – 1962)

Franklinia alatamaha. The exquisite *Franklinia alatamaha* tree (*Fig. 2.1.1*), which develops in late summer day after day fresh creamy-white, attractively scented flowers, and in fall sticking scarlet foliage, is the most famous discovery of American botanists *John* and *William Bartram*. Father and son discovered this small tree of the Theaceae growing along the Altamaha River in Georgia on October 1, 1765, and saved it later from extinction by propagating its seed at their Philadelphia Garden, now America's oldest botanical garden. They named it *Franklinia alatamaha* in honor of *John Bartram*'s great friend, *Benjamin Franklin*. This tree was never again seen in the wild after 1803, but because of the *Bartram*'s Franklinias does still exist in cultivation.

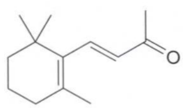
Franklinia alatamaha is apparently extinct in the wild, without known reasons for its vanishing. Prior to 1900, the Franklinia was extremely rare in gardens, and could easily have lost to the world altogether. According to *Joel Fry*, the curator of Historic Bartram's Garden in Philadelphia[1], there is little record of significant effort to ensure its survival after 1850, when the last *Bartram* heirs were forced to sell the family botanic garden. The current resurgence of the Franklinia is largely due to luck and the natural tenacity of the plant itself. Today, it is flourishing in cultivation and according to a Census initiated by Historic Bartram's Garden, around 2000 trees were reported.

As a member of the Tea family or Theaceae, *Franklinia alatamaha* exhibits a strong resemblance in both leaves and flowers to the Asian Camellias, especially to *Camellia sinensis*, the famous tea bush, and its flower scent contains, as I know now, compounds often found in the genus *Camellia*. However, the 18th century American botanists *Bartram* and *Marshall*[1] describe it as scented '*with the fragrance of the China Orange*' (orange blossom) which is not so close anymore.

Fig. 2.1.1. *Franklinia alatamaha* BARTRAM EX MARSH., *a wonderful North American Theaceae no more seen in nature since 1803*

No wonder that I wanted to study the Franklinia as an icon species for the project '*The Scent of the Vanishing Flora*' and, therefore, I contacted in early July 2008 *Joel Fry*, who was very positive about this request. After careful monitoring the development of the blooming, he recommended August 12 as the optimal day. *Joel* was a great host and gave the best support in every regard for which I would like to express my sincere thanks. It was even a sunny day, and under optimal conditions I could follow the scent development from early morning until the late afternoon.

The scent appeared the strongest and most equilibrated between 9 a.m. and 1 p.m., but, quite astonishingly, the individual flowers showed remarkable differences. The first one was characterized by pleasant henna-, freesia-, and tea flower-related main note, in the second methyl benzoate (**4**) and methyl salicylate (**5**) came somewhat to the top, and in the third one the spiciness of eugenol (**6**) became clearly recognizable reminiscent of a *Daphne* or *Dianthus* species with spicy aromatic-floral scent. Reevaluating these flowers 10 min later, the same sequence of olfactory notes in the opposite (!) order as in the first evaluation became apparent! The analytical investigation of the trapped headspace samples revealed that the aromatic/spicy compounds mentioned above are embedded in a matrix dominated by carotenoid-derived compounds as β-ionone (**1**), dihydro-β-ionone (**2**), and α-ionone (**3**) which tend to a quick adaptation, provoking after a while a temporary anosmia. The henna-, freesia-, and tea flower-related notes typical for the first sniff and mainly due to the ionones **1**–**3** and their interactions with **4** to **6** make, therefore, room to the 'antagonists', methyl benzoate (**4**) and methyl salicylate (**5**), and finally eugenol (**6**), nearly as individual notes; a reminder that individual scent components in a mixture may influence each other in various ways, and that reliable scent descriptions may be sometimes a difficult task. Among *ca.* 2500 fragrant plant species we investigated so far in our laboratory, several representatives contain the compounds **1**–**6** but no one containing these compounds in a comparable quantitative ratio.



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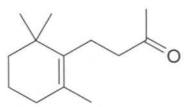

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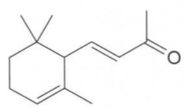

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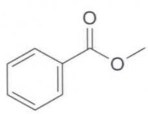

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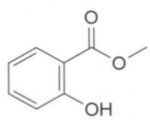

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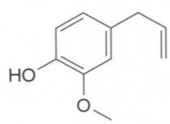

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