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Preliminary comparison of vetiver root essential oils from cleansed (bacteria- and fungus-free) versus non-cleansed (normal) vetiver plants

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Abstract

The 'Sunshine' cultivar of vetiver (*Vetiveria zizanioides* (L.) Nash, =*Chrysopogon zizanioides* (L.) Roberty) was subjected to meristem tissue culture in order to produce plants that were bacteria- and fungi-free. Tissue cultured ("cleansed" or phytosanitary) vetiver was grown for five months in pots of sterilized soil, and the oil compared to non-cleansed (normal) vetiver plants grown in the same conditions except using pots of unsterilized soil. The steam distilled (24 h) oil of the roots from tissue cultured (cleansed) vetiver yielded 0.02% clear oil compared to a 0.35% yield of light yellow oil for the normal vetiver plants, a 17-fold smaller yield. GC/MS analyses of the oils revealed that the non-cleansed (normal) vetiver had the typical vetiver oil profile, whereas the tissue cultured (cleansed) vetiver produced large amounts of C_{19}–C_{29} alkanes plus several alkanols along with typical vetiver oil compounds, but lacked presumed fungal metabolites such as β-funebrene, prezizaene, α-amorphene, and β-vetispirene. An unidentified biotic factor (apparently bacteria or fungi) appears to enhance the oil production in normal vetiver by both increasing yield and by the generation of signature oil compounds. These preliminary results of endogenous microbial

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transformations of plant chemistry may have broader physiological implications, especially among monocotyledons (including cereals).

Keywords: Vetiver; *Vetiveria zizanioides*; *Chrysopogon zizanioides*; Poaceae; Essential oils; Mycorrhiza; Bacteria; Biotransformation

1. Introduction

Vetiver grass (*Vetiveria zizanioides* (L.) Nash, syn. *Chrysopogon zizanioides* (L.) Roberty), the roots of which produce an important essential oil, has been utilized in many parts of the world for soil and water management. Hedges of the non-seeding vetivers provide an effective living dam against erosion (NRC, 1993), and this technique is now in use in more than 100 countries. The origin of the non-seeding vetiver is not known. However, *V. zizanioides* seems to have originated in the area from India to Vietnam, and its fragrant roots, from which is extracted the essential ''Oil of Vetiver'', have been used for centuries for mats and perfumes (NRC, 1993).

Adams and Dafforn (1998) examined 121 accessions of pantropical vetiver and found that 86% appeared to be a single clone (no variation in the DNA examined). That clone was named 'Sunshine' (after a collection site in Sunshine, LA, USA). Included in that analysis were plants from Haiti and Reunion that clustered with the 'Sunshine' group, indicating that the vetiver cultivars used for commercial essential oil production are 'Sunshine' or very similar cultivars. This work was expanded by Adams et al. (1998) to include the closely related genera, *Chrysopogon* and *Sorghum*. Based on an overlap of genetic and morphological data, Veldkamp (1999) combined *Vetiveria* and *Chrysopogon* under *Chrysopogon*. Although this has led to the recognition of *Chrysopogon zizanioides* (L.) Roberty as a proper classification for *Vetiveria zizanioides* (L.) Nash, in this paper we will continue to use both names for clarity. Analysis of additional collections in Thailand cultivars from Bangkok (Adams et al., 1999) revealed that 'Sunshine' and its allied cultivars form the bulk of the vegetatively propagated cultivars in the world. Adams et al. (2003) reported on the growth and oils of 13 distinct DNA types of the 'Sunshine' group grown in test plots in Florida, Nepal and Portugal. No single DNA type (cultigen) was found to be superior in all plots. The oil yields (g/g root dry wt.) were highest in Portugal, followed by Nepal, then Florida. However, yields of oil per plant (g/plant) were much higher in Nepal (1.79 g), followed by Florida (1.23 g), then Portugal (0.85 g). The oil composition varied slightly by strains and by plots.

Weyerstahl et al. (1996, 1997, 2000a,b,c) have exhaustively examined vetiver oil from Haiti. He states (Weyerstahl et al., 2000c) that the composition is so complex (most GC peaks contained 2–4 components) that general, routine analyses of vetiver oils are probably not possible. Weyerstahl et al. (2000c) also notes that vetiver oil reminds him of agarwood oil that is obtained from fungus infected trees of *Aquilaria*, which contain constituents with eremophilane, eudesmane, spir-

ovetivane, guaiane and 2-*epi*-prezizaane skeletons. These sesquiterpene families are also present in vetiver oil. Vetiver has been reported (Wong, 2003) to contain arbuscular mycorrhizal fungi (AMF). The *endo*-mycorrhiza could well be producing biotransformations of the vetiver oil. In addition, Viano et al. (1991) and Bertea and Camusso (2002) report intracellular bacteria in association with essential oil cells in vetiver root (glands). It is also possible that bacteria could be making biotransformations of the essential oil.

The purpose of this study was to conduct preliminary research to compare the essential oils of plants cleansed (i.e., that do not contain internal bacteria or fungi) versus non-cleansed, wild type plants with their normally associated internal microorganisms. To examine these effects, 'Sunshine' vetiver, obtained from tissue culture (so it had no internal bacteria or fungi) was grown in sterilized soil alongside non-cleansed (normal) 'Sunshine' plants grown in non-sterile soil. The roots were harvested, the oil extracted and analyzed. This paper reports on the comparison of these oils.

2. Materials and methods

A portion of a single 'Sunshine' vetiver plant from our test plot in Florida (Adams et al., 2003) was removed and subjected to meristem tissue culture. Fungi- and bacteria-free 'Sunshine' vetiver plantlets were generated using the shoot apical meristem culture method (Smith, 2000). From these plantlets, one individual produced roots and in May, 2003, this cleansed, rooted plantlet was transferred to a 10 l pot containing heat-sterilized potting soil. Three additional (non-cleansed) plantlets were taken from the original vetiver and re-planted in non-sterilized potting soil in 10 l pots. All plants were grown in the same soil in pots, outside, in Waco, TX, USA under ambient conditions, with supplemental watering as needed, and not fertilized. After the summer growing season (153 days, until mid-October), each plant was removed from its pot. The roots were separated from the culm and washed to remove the soil. Root portions were examined by microscopy to determine the presence or absence of AMF and for internal bacteria. The balance of the roots was used for steam distillation on the same day as harvested.

The roots were steam distilled for 24 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen, and the samples stored at $-20\ ^{\circ}C$ until analyzed. The extracted roots were oven dried (48 h, 100 $^{\circ}C$) for dry weight basis determination of oil yields.

The essential oils were analyzed on a Hewlett–Packard 5972 MSD, directly coupled to a HP5980 gas chromatograph. EI mass spectra were collected at 70 eV ionization voltage over the mass range m/z 41–425. Oil samples of 0.1 μl (5% concentration) were injected and split 1/10. Analytical conditions: Column: J & W DB-5, (0.26 mm × 30 m, 0.25 μm film thickness); carrier gas: Helium at 1 ml/min; injector temperature 220 $^{\circ}C$; split ratio: 10:1; oven programming: initial temperature: 60 $^{\circ}C$, gradient 3 $^{\circ}C$/min., final temperature: 246 $^{\circ}C$. The percentages of each compound are TIC (total ion count) values. Identifications were made by library

searches of our volatile oil library (Adams, 2001), coupled with retention time data of reference compounds.

3. Results and discussion

A major difference between the oils of the cleansed plant and the non-cleansed, normal, vetiver was the very low oil yield from the cleansed (meristem tissue cultured) plant. Oil production is thought to reach a maximum after two growing seasons (Adams et al., 2003). Due to time constraints, it was necessary to harvest these plants after a single growing season. Even so, note the very low yield (0.02%) from the tissue cultured plant (Table 1), versus 0.29–0.40% in non-tissue cultured plants. It appears that some essential oil production stimulant factor is absent in the tissue cultured plant, because all the plants were grown in the same kind of soil and under the same conditions.

In addition, analyses of the oil compositions revealed (Table 2) that the oil from the tissue cultured (cleansed) plant is not very similar to "normal" vetiver oil (Table 2). Notice the large number of alkanes and alkanols present. Of particular interest are the C_{19}–C_{29} alkanes, which are completely absent in the vetiver oils from the non-cleansed (normal) plants (Table 2). On the other hand, the tissue cultured vetiver does not produce β-funebrene, prezizaene, α-amorphene, or β-vetispirene, which are found in the non-cleansed (normal) vetiver oils (Table 2). Weyerstahl et al. (2000c) considered these families of compounds as products of fungal metabolism. However, in the present study, there was very little or no AMF found in the roots of any plants in either treatment.

A comparison is shown in Table 3 of key vetiver oil components from the cleansed and non-cleansed plants from Florida grown 'Sunshine' (non-sterile environment) and from commercial vetiver oils from Haiti. The concentrations of the key components in the oil of the non-cleansed plants are quite similar to the Florida 'Sunshine' and Haitian oils (Table 3). Interestingly, vetivenic acid is present in a high concentration in plants grown in the plot in Florida (8.4%, Table 3), but generally absent in oils from other sources (Adams et al., 2003). In contrast, the oil profile of the cleansed plant has smaller concentrations of the key components (Table 3), due to the presence of large amounts of alkanes and

Table 1
Comparison of oil yields (oven dry weight basis) for various treatments of vetiver plants of 'Sunshine' genotype

Accession	Yield (%)	Treatment
10075	0.02	Tissue cultured (cleansed), grown in heat-sterilized potting soil: bacteria- and mycorrhiza-free (no AMF)
10076	0.40	Non-cleansed plant, grown in non-sterile potting soil (no AMF)
10077	0.35	Non-cleansed plant, grown in non-sterile potting soil (no AMF)
10078	0.29	Non-cleansed plant, grown in non-sterile potting soil (6% AMF colonization)

Table 2
Composition of the essential oils of five month old, cleansed (no bacteria, no mycorrhiza) and non-cleansed vetiver plants, each grown in pots, outside in ambient conditions in Waco, TX, USA

HI	Compound	Tissue culture plant	Normal (non-cleansed) plants		
1087	*o*-Guaiacol	0.2	–	–	–
1201	Decanal	t	–	–	–
1309	*p*-Vinyl guaicol	1.0	–	–	–
1372	Longicyclene	–	–	0.1	–
1388	α-Duprezianene	–	–	0.1	–
1390	β-Elemene	–	–	0.1	–
1407	(*Z*)-Isoeugenol	t	–	–	–
1414	β-Funebrene	–	–	0.2	–
1442	6,9-Guaiadene	–	–	0.1	–
1449	(*E*)-Isoeugenol	6.4	2.0	1.9	2.1
1450	Prezizaene	–	0.1	0.1	0.1
1453	Khusimene	0.4	0.9	1.3	0.6
1484	α-Amorphene	–	1.1	2.5	1.2
1495	β-Vetispirene	–	1.2	2.3	1.1
1500	Pentadecane	0.5	–	–	–
1504	1-Dodecanamide, *N*,*N*-dimethyl- [a]	1.6	–	–	–
1512	δ-Amorphene	–	0.9	2.0	0.9
1533	γ-Vetivene	–	0.5	0.2	0.2
1548	Elemol	–	0.4	0.5	0.5
1554	β-Vetivenene [a]	–	2.5	3.7	2.0
1600	Hexadecane	1.6	–	–	–
1621	Sesquiterpene alcohol (M222)	1.6	3.5	2.4	4.5
1653	α-cadinol	0.2	2.0	4.1	4.6
1669	*epi*-Zizanone	2.4	–	–	–
1671	Tetradecanol	0.4	–	–	–
1700	Heptadecane	1.4	–	–	–
1700	Eudesm-7(11)-en-4-ol	–	0.1	0.1	0.8
1715	Nootkatol	1.3	0.9	0.8	1.8
1730	Vetiselinenol	1.4	2.4	2.5	3.0
1742	Khusimol	11.5	24.6	13.1	16.5
1793	(*E*)-Isovalencenol	4.2	9.2	8.9	11.8
1795	Sesquiterpene alcohol (M220)	1.4	2.8	2.9	3.7
1800	Octadecane	0.8	–	–	–
1803	14-Hydroxy-δ-cadinene	0.8	1.6	2.1	2.4
1823	β-Vetivone	1.1	2.1	2.4	2.7
1830	Sesquiterpene ketone (M218)	0.6	1.3	1.9	1.8
1842	α-Vetivone	0.9	1.9	1.5	2.0
1900	Nonadecane	0.5	–	–	–
1960	Hexadecanoic acid	1.7	–	–	–
2000	Eicosane	0.2	–	–	–
2057	Manool	0.2	–	–	–
2100	Heneicosane	0.6	–	–	–
2200	Docosane	2.4	–	–	–
2300	Tricosane	4.3	–	–	–
2400	Tetracosane	5.2	–	–	–
2500	Pentacosane	4.9	–	–	–

(continued on next page)

Table 2 (*continued*)

HI	Compound	Tissue culture plant	Normal (non-cleansed) plants		
2600	Hexacosane	3.8	–	–	–
2700	Heptacosane	2.4	–	–	–
2800	Octacosane	1.4	–	–	–
2900	Nonacosane	0.7	–	–	–
	% Total identified	66.4	54.4	50.6	54.3

HI, Harbourn Index or arithmetic index (Douglas, 1969).
[a] Tentatively identified.

alkanols. These alkanes and alkanols in the oil from the cleansed plant reduce the concentrations of the typical vetiver oil components.

It appears that the pathway for the formation of the key vetiver oil compounds remained relatively balanced in the cleansed plants, but the key components were just very reduced in percentages due to the additional production of alkanes and alkanols. Thus, although many diagnostic "Oil of Vetiver" compounds are present in all profiles, they are much reduced in the oil from the uncleansed plant, which also contains many anomalous components. Notably, the oil produced by tissue cultured (cleansed) vetiver has a very different aroma from normal (non-cleansed) vetiver oils.

This preliminary study has shown that a vetiver cleansed of bacteria and fungi (presumed to normally be associated with field grown vetiver) produced only trace amounts of oil and a strikingly different composition compared to the oils from non-cleansed vetiver plants. Curiously, AMF were missing or very rare in the non-cleansed plants (unlike numerous reports of normal field vetiver), which heightens our interest in the as-yet-unidentified intracellular bacteria. In the future, these unusual results will be investigated by the use of larger, replicated samples and direct comparisons between AMF inoculated versus non-inoculated plants, and by

Table 3
Comparison of the major compounds of five month old, pot-grown cleansed and non-cleansed 'Sunshine' vetiver plants versus 'Sunshine' plants from our Florida field plot (2 years growth) and commercial Haitian "Oil of Vetiver" samples

Source	HI1622	Vetsol	Khsmol	Isovol	HI1795	β-Vet	Veta	HI1830	α-Vet
Cleansed plant	1.6	1.4	11.5	4.2	1.4	1.1	0.0	0.6	0.9
Non-cleansed plants	3.5	2.6	18.1	10.0	3.1	2.4	0.0	1.7	1.8
Florida field-grown	2.3	3.6	20.2	16.5	3.3	4.0	8.4	2.1	5.1
Haiti, Berje oil	1.7	4.2	17.1	11.7	2.2	3.0	1.2	1.3	4.4
Haiti, Texarome oil	1.4	5.2	22.9	16.1	2.5	4.8	–	0.8	6.6

Vetsol, vetiselinenol; khsmol, khusimol; isovol, (*E*) isovalencenol; β-vet, β-vetivone; veta, vetivenic acid; α-vet, α-vetivone.

longer term bacterial inoculation studies, coupled with microbial isolation and identification.

Little research has been conducted in an attempt to untangle the relative quantitative and qualitative contributions of microorganisms to the tableau of secondary compounds formed in plants, and their associative effects on physiology. Vetiver grass, because of its distinct clonal diversity, wide edaphic distribution, and growing importance to the poor in marginal environments, may serve as a useful model for understanding microbial interactions in other plants, especially monocotyledons (including the cereal grasses).

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