JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Biosynthetic Origin of Geosmin in Red Beets (*Beta vulgaris* L.)

GUIPING LU,*,† CHARLES G. EDWARDS,† JOHN K. FELLMAN,‡ D. SCOTT MATTINSON,‡ AND JOHN NAVAZIO§

Department of Food Science and Human Nutrition, Washington State University, Pullman, Washington 99164-6376; Department of Horticulture and Landscape Architecture, Washington State University, Pullman, Washington 99164-6414; and Alf Christianson Seed Company, Mt. Vernon, Washington 98273

Geosmin provides the characteristic but sometimes undesirable "earthy" flavor to red table beets. To date, it is not known whether geosmin is a byproduct of beet metabolism or synthesized by soil-borne microorganisms and taken up by the beets during maturation. Analysis of mature beet roots revealed that peels contained 6 times the amount of geosmin compared to the bodies and cores. Sterilized beet seeds were aseptically grown in a basal medium prior to analysis for the presence of geosmin. Using a headspace solid-phase microextraction (HSPME) method, the relative recovery of geosmin from beet seedling extracts was 72.0 ± 4.2% with (−)-menthone as the internal standard. The presence of geosmin in aseptically grown beet seedlings was confirmed by gas chromatography−mass spectrometry using authentic geosmin as the standard. During aseptic growth, the concentration of geosmin in seedlings remained constant for up to 5 months but increased at 6 months. Geosmin added to the growth medium was not absorbed by the seedlings. These studies support the conclusion that red beets are capable of endogenous synthesis of geosmin.

KEYWORDS: **Geosmin; red beets (*Beta vulgaris* L.); biosynthetic origin; headspace solid-phase microextraction (HSPME)**

INTRODUCTION

A major odor compound found in soil (*1*), geosmin [*trans*-1,10-dimethyl-*trans*-(9)-decalol] is often associated with "earthy" or "musty" odors in water, fish, dry beans, canned mushrooms, red beets, and even wine (*2*−*8*). At concentrations lower than 5.8 μg/L, geosmin can impart a characteristic "beet" flavor to cooked beet juice, whereas higher concentrations are described as being "earthy" (*9*). Geosmin is a known metabolite of several microorganisms including actinomycetes (*10*, *11*), blue-green algae (*11*−*14*), certain fungi (*15*, *16*), and the myxobacterium *Nanocystis exedens* (*17*).

To date, the biosynthetic origin of geosmin in red beets has not been determined. Because many soil-borne microorganisms produce geosmin, it is possible that red beets absorb this flavor compound during maturation. Alternatively, geosmin may be a byproduct of microorganisms associated with beet roots or through the metabolism of red beets themselves. In an earlier attempt to identify the origin of geosmin, Tyler (*18*) grew beets in sterile sand and then analyzed the roots for geosmin. Although geosmin was detected in the beet roots, firm conclusions could not be made because the beet seeds were not sterilized before germination and the beets were not grown under completely aseptic conditions. The experiments of Tyler (*18*) were also limited by the analytical method because large amounts of beet roots were required (1 kg) and it was very difficult to aseptically grow this weight of beets.

Recently, a headspace solid-phase microextraction (HSPME) method was developed that requires only 5 g of tissue for analysis of geosmin (*19*). Using this method, the biosynthetic origin of geosmin in red beets was investigated by aseptically growing selected cultivars of red beets.

MATERIALS AND METHODS

Chemicals. Standard geosmin (minimum 98%) was obtained from Sigma (St. Louis, MO). (−)-Menthone (95% purity) was purchased from Aldrich (Milwaukee, WI), whereas NaCl (crystals, reagent, 99.9% purity) and methanol (HPLC grade) were acquired from J. T. Baker (Phillipsburg, NJ). Difco plate count agar (PCA) and actinomycete isolation agar (AIA) were obtained from Fisher Scientific (Pittsburgh, PA). Murashige and Skoog basal salts (MSBS) were purchased from PhytoTechnology Laboratories (Shawnee Mission, KS).

Growth Conditions for Beets. Seeds of cultivars Round Red, Detroit Dark Red, Chioggia, and Crosby Green Top were provided by the Alf Christianson Seed Co. (Mt. Vernon, WA).

After removal of the seed coat of Round Red, Detroit Dark Red, and Chioggia, seeds were surface sterilized by sequential soaks in (a) 75% v/v ethanol for 1 min, (b) 25% v/v Clorox regular bleach + 0.25% w/v Tween 80 for 25 min, and (c) sterile distilled water for 2 min (three times) performed in a laminar flow hood. Six to eight seeds were then inoculated into individual 11.4 × 14 cm Phytacon culture vessels

* Address correspondence to this author at 416 W. P. Garrigus Building, Department of Animal Sciences, University of Kentucky, Lexington, KY 40546-0215 [e-mail gpl@uky.edu; telephone (859) 257-5985; fax (859) 257-7537].
† Department of Food Science and Human Nutrition.
‡ Department of Horticulture and Landscape Architecture.
§ Alf Christianson Seed Co.

10.1021/jf020905r CCC: $25.00 © 2003 American Chemical Society
Published on Web 01/14/2003

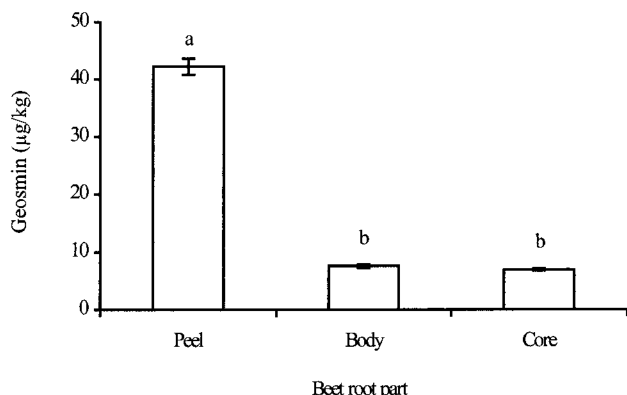

Figure 1. Distribution of geosmin within mature beet roots of cv. Crosby Green Top. Means with different letters are significantly different ($p \leq 0.05$). Error bars indicate one standard deviation.

(Sigma-Aldrich, St. Louis, MO) containing ~100 mL of MSBS medium solidified with 1% agar. The beets were grown under fluorescent light for a 16 h photoperiod at ambient temperature. After 2.5 months, Round Red beet seedlings in 15 vessels were transferred into individual vessels that contained sterile MSBS medium and geosmin (2.5 μg/mL). Round Red seedlings were harvested after 4, 5, and 6 months of growth, whereas seedlings grown with geosmin were harvested after 5 months only. Detroit Dark Red and Chioggia beet seedlings were harvested after 10 days and 4 months of growth.

In addition to the aseptically grown beets, Round Red seeds were germinated in 10.2 × 10.2 cm plastic pots containing potting soil (Soil Conditioners, Zillah, WA). The mix contained 60% v/v peat moss and 37.5% v/v pumice with the remainder (2.5%) being a nutrient mixture of agricultural lime (58.4%), triple phosphate (14.6%), calcium nitrate (14.6%), potassium−magnesium (10.9%), gypsum (0.73%), and iron sulfate (0.73%). These beets were grown under the same conditions as the aseptically grown seedlings and watered with tap water. After 3 months of growth, these plants were harvested for determination of the relative recovery of geosmin from beet seedlings.

Crosby Green Top and Chioggia beets were grown in 16.5 × 17.8 cm plastic pots containing potting soil in a greenhouse to obtain roots. Growing conditions were a 14 h photoperiod at 21 °C in the daytime and 13 °C at night. After 10 days of growth, plants were thinned to one per pot. The plants were irrigated with tap water daily and twice with 100 mL of Hoagland's no. 2 solution during the experiment. After 4 months of growth, the roots were harvested for geosmin analysis and determination of microbial growth.

Determination of Microbial Contamination. Aseptically grown seedlings were removed from the growing vessels in a laminar flow hood. The hypocotyl of each seedling was inoculated into PCA and AIA media in Petri plates and incubated at 30 °C for 1 week. The remaining seedling parts were stored in a refrigerator and analyzed for geosmin after the seedling was tested to be sterile.

To examine if there was microbial growth inside beet roots, two greenhouse-grown roots of Chioggia were surface sterilized with the same sterilization procedure as for seeds, except that 30% v/v Clorox regular bleach was used. The roots were then cut into approximately 60 mm × 50 mm × 5 mm blocks in a laminar flow hood with a sterile knife, inoculated into PCA and AIA media, and incubated at 30 °C for 1 week.

Analysis for Geosmin. Because the matrix of beet seedlings is different from that of beet roots, the relative recovery of geosmin from seedlings was determined using (−)-menthone as the internal standard. A calibration curve for calculation of the relative recovery of geosmin from seedlings was prepared by extracting the headspace of each 40 mL vial sealed by a cap equipped with a PTFE/silicone septum (Supelco, Bellefonte, PA). Each vial contained 15 g of deionized water, 5.60 g of NaCl, and selected amounts of geosmin to yield concentrations ranging from 0.753 to 10.8 ng/mL. The internal standard, (−)-menthone, was added to yield a concentration of 5.82 ng/mL. The beet plants grown in the potting soil under fluorescent light for a 16 h photoperiod at ambient temperature were used as the matrix for spike of geosmin to determine the relative recovery of geosmin from seedlings. The seedlings were washed with distilled water and blotted dry with paper towels. Seedling samples were prepared in the same way as root samples (*19*) and spiked with a known amount of geosmin (6.50 μg/kg of plant tissue) and (−)-menthone (17.5 μg/kg of plant tissue), the same amount of (−)-menthone used to prepare the calibration curve for calculation of relative recovery of geosmin from seedlings. Samples either spiked with (−)-menthone (17.5 μg/kg) or unspiked with either (−)-menthone or geosmin were used as controls. To determine the geosmin concentration in aseptically grown beet seedlings, samples were spiked with (−)-menthone (17.5 μg/kg). Extraction of geosmin from seedling samples was described by Lu et al. (*19*).

The peels, bodies, and cores of greenhouse-grown Crosby Green Top roots were analyzed using the HSPME method (*19*) to determine the distribution of geosmin in beet roots. The peels (~1 mm thick) of five representative roots were peeled and combined for analysis, similar to the cores (cylinder of 2.15 cm in diameter). The remaining root portions represented "bodies", primarily parenchyma tissue.

Although gas chromatographic analysis of geosmin was described previously (*19*), mass spectrometry was accomplished using a Hewlett-Packard 5890II/5970 GC−MSD equipped with a 60 m × 0.25 mm i.d., 0.25 μm film thickness, DB-1 column (J&W Scientific Inc., Folsom, CA). Chromatographic temperatures were as follows: 35 °C for 5 min, increased to 50 °C at 2 °C/min, increased to 200 °C at 5 °C/min, and held for 5 min, for a total run time of 47.5 min. Transfer line and ion source temperatures were held at 250 °C. The linear velocity of He carrier gas was 30 cm/s. Mass spectra were obtained by electron ionization at 70 eV and recorded with an HP 59970C ChemStation. Initial identification of geosmin was made by matching the spectrum of the geosmin peak against the Wiley/NBS library. Confirmation of identification was made by comparison of the retention time and spectrum of the geosmin peak with those of standard geosmin.

Statistical Analysis. All analyses were performed in triplicate with the data analyzed by Tukey's pairwise comparison using SAS (SAS Institute Inc., Cary, NC) at $p \leq 0.05$ defined as a significant difference.

RESULTS AND DISCUSSION

Relative Recovery of Geosmin from Beet Seedlings. The calibration curve for calculation of the relative recovery of geosmin from seedlings was prepared. The HSPME using polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibers yielded an excellent linear response for the calibration curve (R^2 = 0.999). The correlation equation was the following: weight ratio of geosmin/menthone = 0.602 × area ratio of geosmin/menthone − 0.0541.

To mimic the matrix of aseptically grown seedlings, the beet plants grown in potting soils under the same conditions as aseptically grown seedlings were used to determine the relative recovery of geosmin. The seedling samples spiked with (−)-menthone and the unspiked samples were used to subtract the amount of geosmin and (−)-menthone originally in the samples because there was a tiny peak with the same retention time as (−)-menthone in the extract using the HSPME method (*19*). When standard geosmin was spiked to seedling samples to yield a concentration of 6.50 μg/kg of plant tissue, the relative recovery of geosmin was 72.0 ± 4.2%. Compared to the relative recovery of geosmin from beet roots (99.2 ± 7.4%) (*19*), the recovery of geosmin from seedlings was lower. Geosmin and (−)-menthone may have different volatilities in roots and seedlings because their matrices are not the same. Thus, different relative recoveries of geosmin from the two matrices were expected.

Origin of Geosmin in Red Beets. The geosmin concentrations in the peels, bodies, and cores of Crosby Green Top roots were measured to determine the distribution of the flavor compound in roots (**Figure 1**). The geosmin concentration in the peel tissue was ~6 times that found in the bodies and cores, whereas there was no significant difference between the bodies

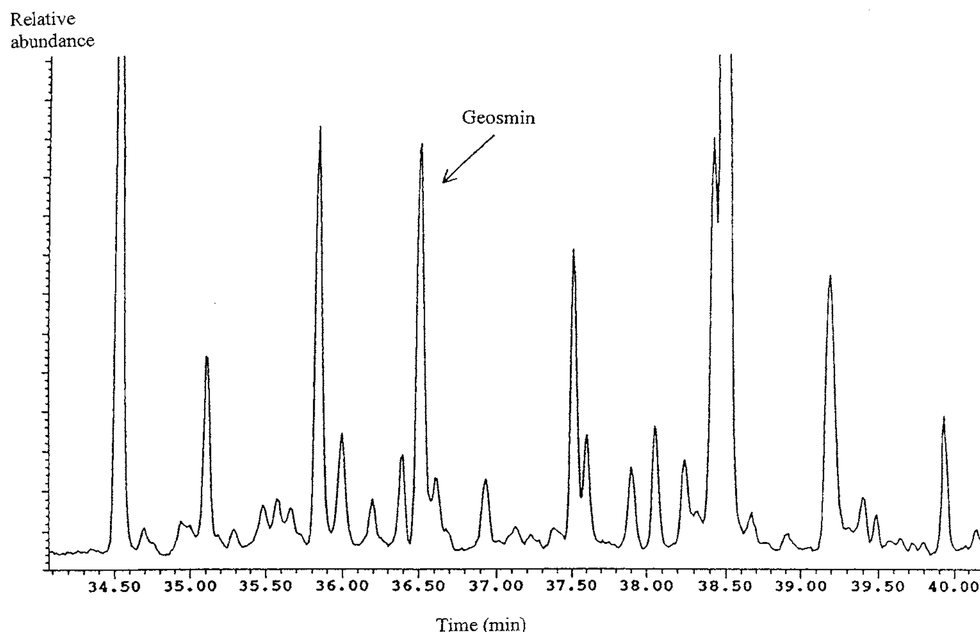

Figure 2. GC-MS chromatogram of an extract from cv. Round Red beet seedlings aseptically grown for 4 months.

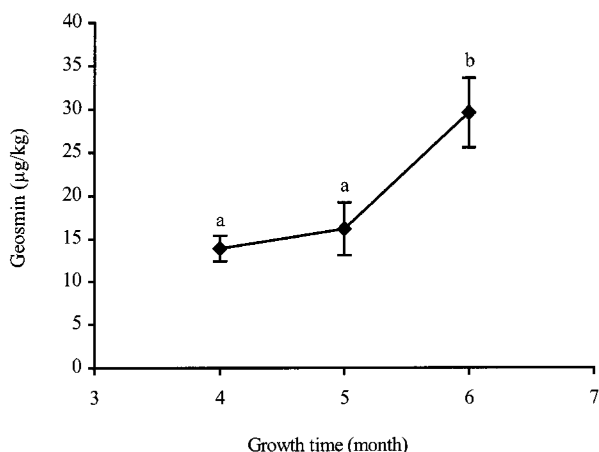

Figure 3. Concentration of geosmin in cv. Round Red beet seedlings aseptically grown for 4, 5, or 6 months. Means with different letters are significantly different ($p \leq 0.05$). Error bars indicate one standard deviation.

and cores. These results were consistent with those of Tyler et al. (*20*). The evidence that the peels of beet roots contained a much higher geosmin concentration than the bodies and cores suggested that geosmin in beets may be absorbed by the surface of roots from soil and then transported to the bodies and cores. This hypothesis is supported by evidence that 1 and 8.7 μg of geosmin/kg were detected in garden soil and cropland soil (*1*, *21*), respectively.

To determine if the plants absorb geosmin from an external source, geosmin was added to the MSBS growth medium for beet seedlings. The concentration of geosmin (2.5 μg/mL of medium) added to the medium was considerably higher than that found in garden soil and cropland soil (*1*, *21*). However, no significant difference was found in geosmin concentration between Round Red seedlings grown in the MSBS medium amended with geosmin and those grown in medium with no added geosmin (data not shown). Thus, aseptically grown seedlings were unable to assimilate geosmin from an external source.

Besides the external source, the geosmin in beets may be produced by microorganisms inside beet roots. Thus, greenhouse-grown Chioggia roots were cut into thin layers and inoculated to PCA and AIA media to examine the presence of microorganisms inside beet roots. All of the Chioggia roots demonstrated microbial growth in both PCA and AIA media 3 days after incubation at 30 °C. There was more microbial growth in outer tissues compared to inner sections of roots. In addition, the root centers were contaminated by microorganisms. Lutman (*22*) also observed the presence of actinomycete filaments in the intercellular region of beet roots by photomicrography. Thus, it is possible that geosmin in red beets is produced by microorganisms inside the beet roots. However, it is unknown if the species of actinomycetes inside the roots are endophytes and capable of synthesis of geosmin or not. Whereas certain nutrient and environmental conditions are required to produce geosmin for the cultures of actinomycetes isolated (*23*−*25*). The source of geosmin from the microorganisms inside beets is further unclear by the evidence that actinomycete filaments were observed not only in beet roots but also in potatoes, carrots, parsnips, and turnip roots (*22*), yet beet roots are the only root vegetable known to contain geosmin. Therefore, more evidence is needed to clarify the possible source of geosmin from the microorganisms inside beet roots.

To further ascertain the origin of geosmin in red beets, beets were aseptically grown in plastic vessels containing MSBS medium to exclude the microbial source of geosmin. Beet seedlings grown in the plastic vessels were tested for microbial contamination before analysis to make sure all of the seedlings used for geosmin analysis were sterile. Only two contaminated seedlings (discarded) were identified using the PCA medium assay. The geosmin peak was first identified by GC−FID in sterile seedlings and later confirmed by GC−MS (**Figure 2**). The peak identified with an arrow was confirmed as geosmin by comparison of the peak spectrum with that of authentic geosmin. The data conclusively show that geosmin was present in the absence of any microorganisms normally associated with beet tissue and imply that beet plants are capable of the synthesis of geosmin.

Change in the geosmin concentration in aseptically grown seedlings of Round Red was determined during growth (**Figure 3**). Here, there was no significant difference between 4- and 5-month-old seedlings. However, the concentration of the

compound in the 6-month-old seedlings was significantly higher than that in 4- and 5-month-old seedlings. One possible explanation for the data is that geosmin may be synthesized and stored primarily in beet roots. Because beet seedlings aseptically grown after 4 months developed primary and secondary roots, geosmin was detected in all of the seedlings grown for >4 months. The production of geosmin in beet seedlings increased at 6 months of growth probably because the primary roots of 6-month-old seedlings were larger than those of 4- and 5-month-old seedlings.

The geosmin concentration in aseptically grown seedlings was also different among beet cultivars. Chioggia (135 ± 30.6 μg/kg of plant tissue) had significantly higher geosmin than Detroit Dark Red (65.2 ± 25.9 μg/kg of plant tissue). Chioggia seedlings developed larger primary roots and more secondary roots than Detroit Dark Red. Thus, geosmin production in seedlings varied among beet cultivars.

ABBREVIATIONS USED

AIA, actinomycete isolation agar; HSPME, headspace solid-phase microextraction; MSBS, Murashige and Skoog basal salts; PCA, plate count agar; PDMS/DVB, polydimethylsiloxane/divinylbenzene.

LITERATURE CITED

(1) Buttery, R. G.; Garibaldi, J. A. Geosmin and methylisoborneol in garden soil. *J. Agric. Food Chem.* **1976**, *24*, 1246−1247.

(2) Murray, K. E.; Bannister, P. A.; Buttery, R. G. Geosmin: an important volatile constituent of beetroot (*Beta vulgaris*). *Chem. Ind. (London)* **1975**, *15*, 973−974.

(3) Acree, T. E.; Lee, C. Y.; Butts, R. M.; Barnard, J. Geosmin, the earthy component of table beet odor. *J. Agric. Food Chem.* **1976**, *24*, 430−431.

(4) Buttery, R. G.; Guadagni, D. G.; Ling, L. C. Geosmin, a musty off-flavor of drybeans. *J. Agric. Food Chem*. **1976**, *24*, 419−420.

(5) Darriet, P.; Pons, M.; Lamy, S.; Dubourdieu, D. Identification and quantification of geosmin, an earthy odorant contaminating wines. *J. Agric. Food Chem*. **2000**, *48*, 4835−4838.

(6) Lloyd, S. W.; Grimm, C. C. Analysis of 2-methylisoborneol and geosmin in catfish by microwave distillation-solid-phase microextraction*. J. Agric. Food Chem*. **1999**, *47*, 164−169.

(7) Maga, J. A. Musty/earthy aromas. *Food Rev. Int.* **1987**, *3*, 269−284.

(8) Watson, S. B.; Brownlee, B.; Satchwill, T.; Hargesheimer, E. E. Quantitative analysis of trace levels of geosmin and MIB in source and drinking water using headspace SPME. *Water Res.* **2000**, *34*, 2818−2828.

(9) Tyler, L. D.; Acree, T. E.; Smith, N. L. Sensory evaluation of geosmin in juice made from cooked beets. *J. Food Sci.* **1979**, *44*, 79−81.

(10) Gerber, N. N. Volatile substances from actinomycetes: their role in the odor pollution of water. *CRC Crit. Rev. Microbiol.* **1979**, *7*, 191−214.

(11) Medsker, L. L.; Jenkins, D.; Thomas, J. F. Odorous compounds in natural waters: an earthy-smelling compound associated with blue-green algae and actinomycetes. *Environ. Sci. Technol*. **1968**, *2*, 461−464.

(12) Naes, H.; Utkilen, H. C.; Post, A. F. Geosmin production in the cyanobacterium *Oscillatoria brevis. Arch. Microbiol.* **1989**, *151*, 407−410.

(13) Safferman, R. S.; Rosen, A. A.; Mashni, C. I.; Morris, M. E. Earthy-smelling substance from a blue-green alga. *Environ. Sci. Technol.* **1967**, *1*, 429−430.

(14) Tabachek, J.-A. L.; Yurkowski, M. Isolation and identification of blue-green algae producing muddy odor metabolites, geosmin, and 2-methylisoborneol, in saline lakes in Manitoba. *J. Fish. Res. Board Can.* **1976**, *33*, 25−35.

(15) Gerber, N. N. Volatile substances from actinomycetes: their role in the odor pollution of water. *Water Sci. Technol.* **1983**, *15*, 115−125.

(16) Mattheis, J. P.; Roberts, R. G. Identification of geosmin as a volatile metabolite of *Penicillium expansum*. *Appl. Environ. Microbiol.* **1992**, *58*, 3170−3172.

(17) Trowitzsch, W.; Witte, L.; Reichenbach, H. Geosmin from earthy smelling cultures of *Nannocystis exedens* (Myxobacterales). *FEMS Microbiol. Lett.* **1981**, *12*, 257−260.

(18) Tyler, L. D. The importance and origin of earthy odor components in beets. Ph.D. dissertation, Cornell University, Ithaca, NY, 1979.

(19) Lu, G.; Edwards, C. G.; Fellman, J. K.; Mattinson, D. S.; Navazio, J. Quantitative determination of geosmin in red beets (*Beta vulgaris* L.) using headspace solid-phase microextraction. *J. Agric. Food Chem.* **2002**, 1021−1025.

(20) Tyler, L. D.; Acree, T. E.; Becker, R. F.; Nelson, R. R.; Butts, R. M. Effect of maturity, cultivar, field history, and the operations of peeling and coring on the geosmin content of *Beta vulgaris*. *J. Agric. Food Chem*. **1978**, *26*, 1466−1469.

(21) Stahl, P. D.; Parkin, T. B. Purge-and-trap extraction of geosmin and 2-methylisoborneol from soil. *Soil Sci. Soc. Am. J.* **1994**, *58*, 1163−1167.

(22) Lutman, B. F. *Actinomycetes in Various Parts of the Potato and Other Plants*; Agricultural Experiment Station Bulletin 522; University of Vermont and State Agricultural College: Burlington, VT, April 1945.

(23) Blevins, W. T.; Schrader, K. K.; Saadoun, I. Comparative physiology of geosmin production by *Streptomyces halstedii* and *Anabaena* sp. *Water Sci. Technol*. **1995**, *31*, 127−133.

(24) Dionigi, C. P.; Greene, D. A.; Millie, D. F.; Johnsen, P. B. Mixed function oxidase inhibitors affect production of the off-flavor microbial metabolite geosmin. *Pestic. Biochem. Physiol.* **1990**, *38*, 76−80.

(25) Dionigi, C. P.; Ingram, D. A. Effects of temperature and oxygen concentration on geosmin production by *Streptomyces tendae* and *Penicillium expansum. J. Agric. Food Chem.* **1994**, *42*, 143−145.

Received for review August 23, 2002. Revised manuscript received November 21, 2002. Accepted November 25, 2002. We are grateful to Alf Christianson Seed Co. (Mt. Vernon, WA) and the Robert MacDonald Vegetable Seed Memorial Fund for assistance and financial support.

JF020905R