Scientia Horticulturae 182 (2015) 73–76

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Diurnal effects on spearmint oil yields and composition

Jennifer Bufalo[a,b], Valtcho D. Zheljazkov[c,*], Charles L. Cantrell[a], Tess Astatkie[d], Lyn Ciampa[c], Ekaterina Jeliazkova[c]

[a] U.S. Department of Agriculture, Agricultural Research Service, Natural Products, Utilization Research Unit, Thad Cochran Center, University, Mississippi 38677, USA
[b] Department of Botany, Institute of Biosciences (IB), UNESP—Univ. Estadual Paulista, PO Box 510, Botucatu 18618-970, SP, Brazil
[c] University of Wyoming, Department of Plant Sciences and Sheridan Research and Extension Center, 663 Wyarno Road, Sheridan, WY 82801, USA
[d] Dalhousie University, Faculty of Agriculture, 50 Pictou Road, PO Box 550, Truro, NS, Canada B2N 5E3

ARTICLE INFO

Article history:
Received 24 September 2014
Received in revised form
10 November 2014
Accepted 18 November 2014
Available online 24 December 2014

Keywords:
Mentha spicata L.
Diurnal changes
Essential oil yield
Essential oil composition
Carvone

ABSTRACT

'Native' spearmint (*Mentha spicata* L.) is one of the two spearmint species grown commercially in the United States and other countries for essential oil production. The two major constituents of spearmint oil are carvone and limonene. It is not known if the essential oil yield (content) and composition of spearmint oil are affected by diurnal variation, and when it would be the best time for harvesting flowering spearmints within a 24 h period. Therefore, the objective of this study was to evaluate the effect of diurnal variation on yield and composition of spearmint 'Native' essential oil for the environmental conditions of Northern Wyoming, at elevation 1170 m above the sea level. The experiment was conducted on a 3-year old well established spearmint plantation. The harvest times were every 2 h within a 24-h period: 7:00 AM, 9:00 AM, 11:00AM, 1:00 PM, 3:00 PM, 5:00 PM, 7:00 PM, 9:00 PM, 11:00 PM, 1:00 AM, 3:00 AM, and 5:00 AM. Essential oil yield varied from 0.96 to 1.47 g of oil per 100 g of dry herbage; the maximum oil yield was obtained at 9:00 AM and the minimum at 7:00 PM. The concentration of carvone in the oil varied from 44.1% (at 1:00 PM) to 66.4% (at 9:00 PM) of the total oil. However, the yield of carvone (a function of oil yield and carvone concentration in the oil) was the highest at 3:00 AM and the lowest at 1:00 PM. The concentration of limonene (10.7–15.8% of the oil) was the highest at 7:00 PM and the lowest at 1:00 PM, whereas the yield of limonene was the highest at 9:00 PM. For best essential oil yields, flowering spearmint should be harvested at around 9:00 AM. However, to obtain oil with high carvone concentration, spearmint should be harvested at 9:00 PM. Harvests at 1:00 PM would result in spearmint oil with low concentrations of both carvone and limonene, and hence, should be avoided.

1. Introduction

Mentha remains an economically important genus of the Lamiaceae family with about 19 species and 13 natural hybrids. One the most common species for cultivation is *Mentha spicata* L. (Lawrence, 2006; Kumar et al., 2011). This species is cultivated commercially for use in teas and for essential oil production, which has economic importance in perfumery, confectionary and pharmaceutical preparations and specialty chemical production (Telci et al., 2010). Commonly called spearmint, the variety 'Native' is widely grown in the United States and in other countries (Bienvenu et al., 1999; Lawrence, 2006).

Species of the genus *Mentha* contain different compounds in their essential oils. Specific constituents such as pulegone, menthone, and carvone have antimicrobial, insecticidal and genotoxic activities (Sivropoulou et al., 1995; Franzios et al., 1997; Sokovic et al., 2009; Kanatt et al., 2008). In *M. spicata*, the major compounds are carvone (Jirovetz et al., 2002), limonene, 1,8-cineole (eucalyptol) (Maffei et al., 1986; Kokkini et al., 1995; Zheljazkov et al., 2010a). Other chemotypes of *M. spicata* have been reported with major constituents such as piperitone-oxide (32–46% of the oil) and 1,8 cineole (10–41% of the oil, depending on location) (Cook et al., 2007), or with major oil constituent piperitenone epoxide (66–82% of the oil) (Misra et al., 1989). However, the commercial spearmint varieties for essential oil production belong to the carvone chemotype. Spearmint whole essential oil and the individual oil constituent carvone have been shown to have antimicrobial

* Corresponding author. Tel.: +1 307 7372415.
E-mail addresses: jenniferbufalo@yahoo.com.br (J. Bufalo), vjeliazk@uwyo.edu, valtcho.pubs@gmail.com (V.D. Zheljazkov), Charles.Cantrell@ARS.USDA.GOV (C.L. Cantrell), Astatkie@dal.ca (T. Astatkie), okayknow@gmail.com (L. Ciampa), ejeliazk@uwyo.edu (E. Jeliazkova).

http://dx.doi.org/10.1016/j.scienta.2014.11.018
0304-4238/

properties (de Carvalho and da Fonseca, 2006; Kanatt et al., 2008; Sokovic et al., 2009).

Although the biosynthesis of secondary metabolites is controlled by genetic processes, it is also strongly affected by climatic conditions such as light, temperature, irrigation, soil and nutrition as well as the season and the time plant material is harvested (Lawrence, 2006; Baghalian et al., 2008; Viuda-Martos et al., 2008; Shanjani et al., 2010; Zheljazkov et al., 2010b; Butkiene and Mockute, 2011).

Previous studies found significant impact of diurnal changes on essential oil yield and composition on a number of crops such as basil (*Ocimum gratissimum* L.) (De Vasconcelos Silva et al., 1999), *Pelargonium* sp. (Rao et al., 2001), oil-bearing rose (*Rosa damascena* Mill.) (Kumar et al., 2013), dill (*Anethum graveolens* L.) (Zlatev, 1977), lavender (*Lavandula angustifolia* Mill.) (Hassiotis et al., 2010), coriander (*Coriandrum sativum* L.) Ramezani et al., 2009b), savory (*Satureja* spp.) (Genova, 1980), and clary sage (*Salvia sclarea* L.) (Shevchenko, 1973; Tsvetkov and Balinova-Tsvetkova, 1976). Basil, lavender, savory, and clary sage are from the same plant family as spearmint. However, diurnal changes in spearmint essential oil yield and composition are not known. Therefore, the objective of this study was to evaluate the effect of diurnal variation on yield and composition, of spearmint 'Native' essential oil.

2. Materials and methods

2.1. Plant materials and growth conditions

Cultivar 'Native' of spearmint (*M. spicata* L.) was used as a model plant in this study. The field trial was established in 2011 on irrigated area using certified virus-free 'Native' spearmint transplants at the Sheridan Research and Extension Center Experimental Fields, at 44 45.686′N and −106 55.479′W, elevation 1170 m above sea level. Land preparation, weed control, transplanting, fertilization, and irrigation were performed as described earlier (Zheljazkov et al., 2012, 2013). Due to the relatively dry climate and the remoteness to commercial mint plantations, no pests and diseases were observed. This study was conducted in 2013 on a well-established 3-year spearmint plantation.

Briefly, the weed control was conducted with Sinbar (Terbacil 80% WP) (DuPont, Wilmington, DE) at the rate of 2 kg/ha, applied in early spring. Plants were grown on raised beds, irrigated with low-pressure drip tape, calculated to distribute 2.5 cm water/week (0.2 mm, emitters spaced at 30 cm, 1703 cm^3/min/30.5 m). Nitrogen in the form of ammonium nitrate at 180 kg N/ha was surface applied 2 weeks after transplanting.

2.2. Harvesting and drying

All samples were obtained within a 24-h period on 24 and 25th of July, 2013. Spearmint was at flowering stage at the time of harvest, to ensure best essential oil content and composition. Spearmint plants were harvested every 2 h: 7:00 AM, 9:00 AM, 11:00 AM, 1:00 PM, 3:00 PM, 5:00 PM, 7:00 PM, 9:00 PM, 11:00 PM, 1:00 AM, 3:00 AM, and 5:00 AM, each harvest in 3 replicates, resulting in 36 spearmint samples. Each spearmint biomass sample was 600 g of fresh weight. In addition, 1 kg fresh biomass samples were taken at each sampling time for moisture content determination (all in 3 replicates). The fresh spearmint samples for oil content and composition were dried in a well-ventilated barn at shade, whereas the 1 kg samples for moisture determination were dried in a dryer at 65 °C until constant weight.

2.3. Essential oil distillation

All 36 spearmint biomass samples for oil content and composition were extracted via steam distillation in 2-L steam distillation units for 60 min (Gawde et al., 2009; Zheljazkov et al., 2010a,b). The beginning of each distillation was measured when the first drop of essential oil was out of the condenser and in the separator. At the end of the each distillation, the power was turned off; the oil and the water were decanted from the separator into glass vials. The oil was separated from the water, the oil weight measured on an analytical scale, and kept in a freezer at −14 °C until the analyses. The essential oil content (yield) was calculated as grams of oil per 100 g of fresh herbage (corrected for moisture content, using the difference between the fresh and dried weight of the biomass samples).

2.4. Gas chromatography—FID quantification

All spearmint oil samples were analyzed by GC–FID on a Varian CP-3800 GC equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm, with a film thickness of 0.25 μm) operated using the following conditions: injector temperature, 240 °C; column temperature, 60–120 at 3 °C/min, then held at 240 °C at 20 °C/min for 5 min; carrier gas, He; injection volume, 1 μL (split on FID, split ratio 50:1); FID temperature was 300 °C. Commercial standards *R*-(−)-carvone (Sigma–Aldrich, St. Louis, MO), and (*R*)-(+)-limonene (Fluka, Switzerland) were used for quantitative analysis of spearmint oil constituents. Quantitative analysis of essential oil was previously described by Zheljazkov et al. (2010a) and is briefly described below. With four concentration points, an external standard least squares regression for quantification was used. Each standard was used to formulate a separate calibration curve using FID response data. Linearity was imposed by using response factors (RF) and regression coefficients independently. Response factors (RF) were calculated using the equation RF = DR/C, where DR was the detector response in peak area (PA) and C is the concentration of the analyzed substance. The chromatograms of each of the essential oil samples from all harvests and replicates were compared to the chromatograms from standards. Target peaks were confirmed by retention time. Confirmed integrated peaks were used to determine the percentage of each chemical constituent in the essential oil. The RF of the target chemical constituent was used to determine the percentage of that constituent in each essential oil sample using the equation (PA/RF/C) × 100 = % (peak area/response factor/concentration).

2.5. Statistical analyses

The effect of harvest time (12 levels: 7:00 AM, 9:00 AM, 11:00 AM, 1:00 PM, 3:00 PM, 5:00 PM, 7:00 PM, 9:00 PM, 11:00 PM, 1:00 AM, 3:00 AM and 5:00 AM) on moisture (%), oil yield (oil content g/100 g dry weight, adjusted to 80% moisture), concentration (%) and yield (mg/100 g dried material) of carvone and limonene was determined using ANOVA analysis of a Completely Randomized Design. The analysis was completed using the Mixed Procedure of SAS (SAS, 2010), and the validity of model assumptions was verified by examining the residuals as described in Montgomery (2013). Since the effect of time was either marginally significant (*p*-value between 0.05 and 0.1; concentration and yield of limonene) or significant (*p*-value less than 0.05; the other four responses), multiple means comparison of time was completed using the LSD Multiple Means Comparison method at the 1% level of significance to protect the Type I experiment wise error rate from over inflation.