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Analysis of essential oils derived from lavender plants produced through tissue culture, and their potential uses for repelling insects

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Abstract

Several species of the genus *Lavandula* are grown for the production of valuable essential oils. In an attempt to improve essential oil yield and composition, we regenerated over 600 plants through tissue culture in the presence of the mutagen ethyl methanesulfonate (EMS) (Falk et al., 2008; Desautels et al., 2009). Here we report the results of analyzing the essential oil from these plants. Most notably, several lavandin (*L.* x *intermedia*) lines produced larger quantities of oil with a desirable linalool to camphor ratio. In addition to their potential use in agriculture, these plants produce novel scientific tools for studying essential oil production. Insight into essential oil production could lead to the development of improved plants. It should be noted that oil yield and composition can be significantly affected by plant age and developmental stage. As a result, our preliminary results must be repeated over several years for consistency.

Introduction

Essential oils from *Lavandula* spp. are widely used in the cosmetic and alternative medicine industries. The most highly valued lavender oils have a high linalool to camphor ratio, and are obtained from species of *L. angustifolia* (English lavender). While *L. angustifolia* oils have a preferable terpenoid profile for certain applications (for example in aromatherapy), oil yields from this species are relatively low. One of the most successful strategies to improve lavender oil yields has been through the production of lavender hybrids, known as lavandins (*L.* x *intermedia*). While lavandin cultivars, including Provence and Grosso, produce greater oil yields, their oil is generally considered to be of lower quality due to the high camphor content. Hence, one of the goals of lavender growers is to grow high yielding cultivars that produce oils with a preferable terpene profile.

(2010), pp 1-12.

Chemical mutagenesis is a common strategy used to develop new varieties of plants, and ethyl methanesulfonate (EMS) is among the most common mutagenic agents for carrying out plant mutagenesis experiments. EMS is known for inducing nucleic acid point mutations and chromosome deletions (Alcantara et al., 1996), and has been successfully used to develop male sterility (van der Veen et al., 1968) and herbicide resistance (Jander et al., 2003) in *Arabidopsis*, in addition to introducing a myriad of functional and physiological mutations (see Watanabe et al., 2007). We have previously reported on the production of several hundred EMS-treated lavender plants through plant tissue culture (Falk et al. 2008; Desautels et al., 2009). These plants are being maintained in a field site in Kelowna, BC, under natural growing condition. Here we report the analysis (in terms of yield and composition) of the essential oil from 229 of these plants.

Certain terpenoid essential oil constituents are commonly used to repel flying insect pests in the home as well as protect harvested grains and legumes from insect herbivores. For example, female diamondback moths (*Plutella xylostella*) were shown to be repelled by the volatile oil of *Chrysanthemum morifolium* (Liu et al., 2005). Tobacco hornworm caterpillars (*Manduca sexta*) were shown to be repelled by transgenic tobacco lines that emit high amounts of the common terpenoid isoprene (Laothawornkitkul et al., 2008). In another experiment, *Arabidopsis* plants genetically engineered to emit (*E*)-β-farnesene were shown to repel green peach aphid (*Myzus persicae*) (Beale et al., 2006). Thus, the potency of plant volatiles as natural products to repel insect herbivores is becoming increasingly realized. In this context, we evaluated the effects of lavandin (cv Provence) essential oil as a fruit fly repellant. More specifically, two chemotypes of lavandin oils were tested, including a normal (unchanged) chemotype and a chemotype in which linalool acetate – a major constituent of lavandin essential oils - was virtually eliminated (Desautels et al., 2009). Both lavandin oils tested effectively repelled fruit flies.

Materials and methods

Plant material and mutagenesis

EMS treatment of plants and their transfer to field has been previously reported (Falk et al. 2008; Desautels et al., 2009). Flower tissue from fully matured plants were harvested in August, 2009, and were stored in a freezer at -80 °C until analyzed.

Evaluation of essential oil content by GCMS

Essential oil was extracted from 5-10 grams of floral tissue using a simultaneous steam distillation / solvent extraction procedure previously described (Falk et al., 2008). For quantification purposes, 1 mg of menthol was used as an internal standard. Extracted oil samples were diluted in pentane 100-fold, and analyzed using a Varian GC 3800 gas chromatographer coupled to a Saturn 2200 Ion Trap (Varian Inc.) mass detector, equipped with a 30 m x 0.25 mm capillary column covered with an acid-modified polyethylene glycol film (0.25 μm) (ECTM 1000, Alltech). The oven temperature was initiated at 40 °C (held for 3 min), increased to 170 °C at 7 °C/min, and then to 230 °C at the rate of 30 °C/min. The flow rate for helium (the carrier gas) was set to 1 ml/min. The primary lavender EO constituents borneol, 1,8-cineole, camphor, linalool, linalyl acetate were identified by comparison of their mass spectra to their respective mass spectra cataloged by the National Institute of Standards and Technology (NIST). Percent of total essential oil content was calculated for each compound by dividing the area of the peak corresponding to each specific compound by the sum of all peaks. Reported values represent the mean for two GC run replicates.

Fly food experiments

Fruit fly (*Drosophila melanogaster*) was generously provided by Dr Mark Rheault (UBC Okanagan). Flies were starved for at least four hours and subsequently exposed to fly food (Ashburner, 1989) containing different concentrations of "EO-1" (high in linalool; no linalyl acetate) and "EO-2" (high in linalool and linalyl acetate). Flies were observed over the course of two hours to monitor feeding habits with regards to food containing lavender oil and food without lavender oil.

Results and discussion

EMS treated lavender

Essential oils were extracted from one *L. angustifolia* cultivar (cv Lady) and two lavandin cultivars (cv Provence and cv Grosso). A total of 233 lavender plants (189 of cv Provence, 18 of cv Grosso, and 26 of cv Lady) were analyzed. As expected, the wild-type lavandin cultivars produced typically higher oil yields than cv Lady (eg. 31.2 mg/g in cv Grosso fresh tissue compared to 9.2 mg/g in cv Lady fresh tissue).

One of the primary objectives of this study was to use EMS to develop cv Provence, cv Grosso, and cv Lady plants with high overall yield, characteristic of lavandin cultivars, in addition to a high linalool to camphor ratio, characteristic of *L. angustifolia* cultivars. Tables 1 and 2 shows the top five EMS treated cv Provence and cv Grosso plants in terms of linalool to camphor ratio, respectively, while Tables 3 and 4 shows the top five EMS treated cv Provence and cv Grosso plants in terms of total oil yield, respectively. To focus only on high-yielding cv Provence plants of the 189 assayed, only plants with a minimum oil yield of 30.3 mg/g (average cv Provence oil yield plus one standard deviation) are shown in Table 1. Since cv Lady plants typically have negligible camphor content, Table 5 shows the top five EMS treated cv Lady plants in terms of oil yield, only.

These results show that while EMS treatment of cv Grosso resulted in plants with high linalool to camphor ratio, these EMS treated plants did not produce particularly high oil yields. Conversely, some of the highest oil yielding plants of EMS treated cv Provence were also among the best in terms of linalool to camphor ratio. For example, n-3 appears in both Table 1 and Table 3. Another EMS treated cv Provence plant, n-4 (Table 1), displayed preferable characteristics, as it had the best linalool to camphor profile of all lavandin plants in our study, but was also high in terms of oil yield (35.7 mg/g).

EMS treatment of cv Lady also resulted in the production of high yielding plants that maintained a preferable linalool to camphor ratio. Given that cv Lady plants typically yield 9.2 mg/g fresh tissue, treating these lavenders with EMS resulted in many plants with much improved oil yields (see Table 5). Plant e-45, for example, produced over three times more essential oil than a typical cv Lady plant, while maintaining a 3.6 linalool to camphor ratio. It should be noted that EO yield and composition are heavily influenced by time of harvest and other environmentsal factors. These preliminary results must be repeated to ensure accuracy. However, these results illustrate the potential applications of EMS to develop preferable lavender phenotypes.

Effects of lavender oil on fruit fly feeding preferences

In addition to developing lavender plants with high yields and linalool to camphor ratios, EMS treatment of lavender resulted in a number of plants with singularly high concentrations of linalool and low levels of linalyl acetate (EO-2), as well as plants with high concentrations of both linalool and linalyl acetate (EO-1). To determine the impact of these lavender oils on the feeding preferences of fruit flies, we conducted experiments with using fly food laced with various concentrations of these two different lavender oil types, along with control fly food preparations lacking lavender oil. Fruit flies preferred fly food without any lavender essential oil, as fly food preparations with oil concentrations as low as 0.1 % was enough to significantly repel flies (Fig. 1) for at least two hours. However, fruit flies did not prefer between fly food preparations containing either "EO-1" or "EO-2" (Table 6). Furthermore, increasing lavender oil concentrations of the fly food to 1% did not result in significant reduction in the number of feeding fruit flies (data not shown). The observation that higher concentrations of essential oil did not result in a corresponding decrease in the number of feeding fruit flies may be a result of saturation of olfactory receptors in a similar manner that was described by Peana et al. (2002). These results show that small amounts of lavandin essential oil can significantly repel fruit flies, and that lavandin oil high in either linalool (EO-1), or linalool and linalyl acetate (EO-2) is sufficient for repelling fruit flies.

These experiments represent efforts to use EMS to produce lavender plants with enhanced essential oil outputs. Furthermore, the results illustrate the potency of lavender oil as a fruit fly repellant. These investigations are important for developing an understanding of lavender oil production and reveal the potential roles for essential oil as a herbivore repellant. Further research is currently underway to identify and characterize the genes responsible for shifts in lavender oil metabolism observed in these EMS treated plants.

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Table 1. The top five EMS treated cv Provence plants ranked by linalool to camphor ratio (minimum total oil yield of 30.3 mg/g).

Plant Name	Cineole (mg/g)	Camphor (mg/g)	Linalool (mg/g)	Linalyl Acetate (mg/g)	Borneol (mg/g)	Oil Yeild (mg/g)	Linalool : Camphor Ratio
n-4	8.9	1.4	15.9	1.1	8.4	35.7	11.7
h-20	3.4	2.7	13.4	10.3	1.0	30.8	5.0
i-3	3.3	3.6	15.5	10.9	0.0	33.3	4.3
n-3	3.5	4.6	19.8	10.6	1.7	40.2	4.3
h-18	3.4	3.6	15.5	10.7	0.0	33.2	4.2

Table 2. The top five EMS treated cv Grosso plants ranked by linalool to camphor ratio.

Plant Name	Cineole (mg/g)	Camphor (mg/g)	Linalool (mg/g)	Linalyl Acetate (mg/g)	Borneol (mg/g)	Oil Yeild (mg/g)	Linalool : Camphor Ratio
e-26	4.5	0.4	7.4	0.3	4.3	16.8	17.9
p-13	4.6	0.5	8.5	0.4	4.1	18.0	16.1
p-14	4.0	0.4	6.9	0.4	4.3	16.0	16.1
p-12	4.2	0.5	8.6	0.4	4.0	17.8	15.9
p-7	6.0	0.7	9.5	0.6	5.5	22.2	14.6

Table 3. The top five EMS treated cv Provence plants ranked by oil yield.

Plant Name	Cineole (mg/g)	Camphor (mg/g)	Linalool (mg/g)	Linalyl Acetate (mg/g)	Borneol (mg/g)	Oil Yeild (mg/g)	Linalool : Camphor Ratio
k-11	3.9	4.8	16.0	14.7	1.6	41.0	3.3
n-3	3.5	4.6	19.8	10.6	1.7	40.2	4.3
k-3	3.1	4.6	17.5	12.3	1.7	39.2	3.8
l-2	3.8	4.5	16.5	12.6	1.6	39.0	3.6
k-20	4.5	4.7	15.8	12.3	1.6	38.9	3.4

Table 4. The top five EMS treated cv Grosso plants ranked by oil yield.

Plant Name	Cineole (mg/g)	Camphor (mg/g)	Linalool (mg/g)	Linalyl Acetate (mg/g)	Borneol (mg/g)	Oil Yeild (mg/g)	Linalool : Camphor Ratio
p-15	2.9	4.3	15.4	9.6	1.6	33.8	3.6
e-23	3.4	3.5	13.5	8.8	1.3	30.6	3.8
p-11	3.4	3.4	12.5	8.5	1.2	29.1	3.6
e-27	3.4	3.3	12.5	7.8	1.3	28.4	3.7
e-28	2.5	3.2	12.5	7.6	1.2	27.0	4.0

Table 5. The top five EMS treated cv Lady plants ranked oil yield.

Plant Name	Cineole (mg/g)	Camphor (mg/g)	Linalool (mg/g)	Linalyl Acetate (mg/g)	Borneol (mg/g)	Oil Yeild (mg/g)	Linalool : Camphor Ratio
e-45	2.8	3.4	12.5	10.6	1.3	30.6	3.6
g-11	2.6	3.4	12.1	7.9	1.2	27.3	3.6
e-49	5.0	0.5	6.1	0.4	4.4	16.4	13.3
d-49	5.3	0.0	5.6	0.4	4.1	15.4	n/a
e-44	0.4	0.0	5.8	5.2	0.2	11.7	215.5

Table 6. Comparison of fly feeding preferences using fly food laced with two different lavender essential oils. Representative sample using fly food with 0.1% of each '1 Peak' and '2 Peak' lavender oil shown.

	Number of flies on feeding stage	
Time (h)	Food with 0.1 % 1 Peak Oil concentration	Food with 0.1 % 2 Peak Oil concentration
0.00	0	0
0.25	4	7
0.50	10	12
0.75	15	19
1.00	5	10
1.25	3	7
1.50	13	8
1.75	10	8
2.00	12	9

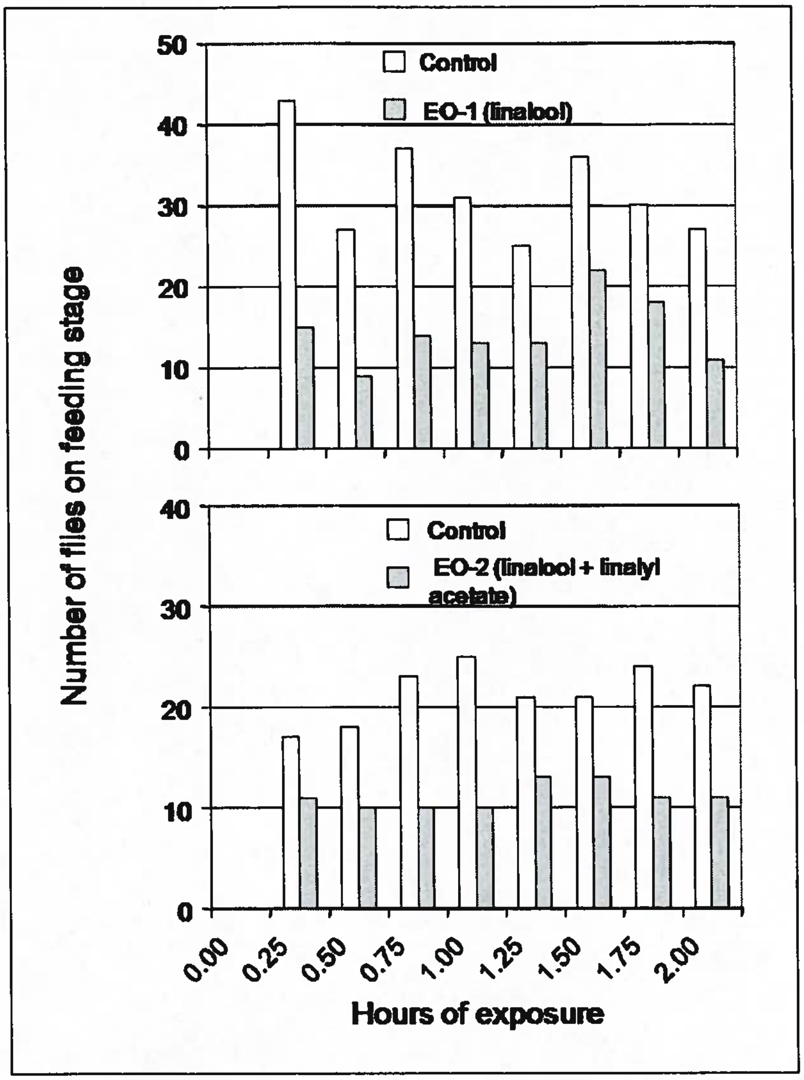

Fig 1. Comparison of fly feeding preference with and without lavender essential oil. Representative sample using fly food with 0.1% of each lavender oil shown.