Journal of Medicinally Active Plants

Volume 9
Issue 1 *Issue 1*

3-31-2020

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Manuscript received: March 18, 2020

Keywords: *Nepeta cataria* L. var. *citriodora,* citronellol, geraniol, caryophyllene oxide, (E)-caryophyllene

ABSTRACT

The chemical profiles of essential oil-bearing plants can greatly vary as a response to ecological and seasonal changes. For specialty crops such as catnip (*Nepeta cataria* L.) and lemon catnip, (*Nepeta cataria* L. var. *citriodora*), optimizing these conditions based on field performance evaluations and chemical analyses of aromatic profiles can provide a benchmark for future harvest timelines. In this study, we describe the field performance of five lemon-scented *N. cataria* selections and one commercial lemon catnip line, based on biomass, essential oil yield and essential oil composition, while determining the effects of harvesting time on plant performance and chemical composition. The essential oil was extracted via hydro-distillation and analyzed via gas chromatography–mass spectrometry (GC-MS). The four compounds in highest concentration present in the assessed genotypes were citronellol, geraniol, (E)-caryophyllene and caryophyllene oxide. Biomass, essential oil yield and chemical composition were significantly affected by harvesting times in all genotypes. Essential oils from catnip and lemon catnip cultivars have been used commercially as a source of natural insect repellents. Understanding how ecological and genetic factors affect the secondary metabolism of these crops is a fundamental step for product standardization and commercialization.

INTRODUCTION

Lemon catnip, *Nepeta cataria* L. var. *citriodora*, differs from the common catnip (*Nepeta cataria*) in its chemical composition due to the presence of lemon notes within its essential oil. Catnip species belong to the Lamiaceae family and sources point the origin of these species to different region in Eastern and Southern Europe, Southeast and Central Asia, North Africa and even North America (Duda et al., 2015; Ibrahim et al., 2017; Reichert et al., 2016; Said-Al et al., 2018; USDA, 2019; Zhu et al., 2009). The terpene content of the common catnip plant, *Nepeta cataria*, is largely dominated by the iridoid monoterpenes known as nepetalactones (Regnier et al., 1967). In addition to inducing a euphoric-like state in cats, the volatile nepetalactones are efficient at repelling mosquitoes and other insect and arthropod pests generating significant interest in their applications in natural insect repellent formulations. When in sufficient concentrations, these compounds could provide a safe alternative to the standard repellent DEET (N,N-Diethyl-3-methylbenzamide) (Reichert et al., 2019; Waller et al., 1969).

In contrast, the lemon catnip variety produces extremely low, often undetectable amounts of nepetalactone (Said-Al et al., 2018). Klimek et al. (2000) determined the major essential oil components in lemon catnip to be nerol, geraniol and citral; while, Said-Al et al. (2018) reported nerol, citronellal, neral and caryophyllene oxide to be the main compounds. Other authors report the monoterpene citronellol as one of the major compounds in the lemon varieties (Wesołowska et al., 2011). Citronellol imparts a sweet lemon scent and can be found in many aromatic plant species including *Cymbopogon nardus* (citronella), *Melissa officinalis* (lemon balm), and *Pelargonium graveolens* (rose geranium) (Debboun et al., 2014). It is a common fragrance ingredient used in cosmetic products, and it too exhibits repellency against mosquitoes and ticks (Debboun et al., 2014). Geraniol is characterized by a rose-like fragrance that is used in perfumery and can be used in candles or aerosol products to deliver spatial repellency against mosquitoes (Debboun et al., 2014). Citral is another compound commonly found in this catnip variety that provides a lemon-citrus odor due to its component mixture of neral and geranial (Martins et al., 2017). Citral is also present in lemon basil (*Ocimum basilicum* var. *citriodorum; and O.×citriodorum*) (Morales and Simon, 1977; Simon et al. 1999), as well as in *Cymbopogon citratus* (lemongrass) and *Verbena officinalis* (verbena) and exhibits repellency against several mosquito species including *Aedes albopictus* and *Aedes aegypti* (Debboun et al., 2014; Martins et al., 2017).

While these compounds are naturally and abundantly present in lemon catnip, measures can be taken to optimize their yield within the essential oils during harvesting and postharvest handling. The objective of this study was to describe the field performance of five lemon-scented *N. cataria* selections and one commercial lemon catnip based on biomass, essential oil yield and essential oil composition, while determining the effects of harvesting time on plant performance and chemical composition. Evaluating the field performance of essential oil-bearing plants can improve postharvest processing and handling leading to superior quality and greater standardization for the production of distilled essential oils. Our objectives were to understand the diversity of aroma volatiles and ascertain whether lemon scented catnip genotypes could have the appropriate chemistry to be used as natural plant sources of insect repellent compounds.

MATERIALS AND METHODS

Genotypes and seedling production. Commercial lemon catnip (*Nepeta cataria* var. *citriodora*) seeds were purchased from Richters Herbs (Goodwood, Ontario, Canada). In addition, five lemon-scented catnips (CN3, CN5, CN6, CL1 and CL2) were assessed. These are *Nepeta cataria* genotypes from the Rutgers University and New Jersey Agricultural Experiment Station germplasm collection and breeding program that were selected for the absence or production of trace amounts of nepetalactone for comparative genetics aiming to understand nepetalactone inheritance. These lines are characterized by being completely devoid or producing little amounts (less than 1%) of nepetalactone and by a distinct 'lemony' aroma, more or less pronounced depending on the line. The selfed progeny of CN3, CN5, CN6, CL1 and CL2 as well as the commercial lemon catnip seeds were sown in polypropylene 128-cell plug trays filled with commercial soil media and kept under greenhouse conditions, with daily watering until reaching transplanting size (15-20 cm) at 45 days after sowing.

Growth conditions, harvesting and post-harvesting. During Spring 2017 (late may), the seedlings were transplanted to field at the New Jersey Agricultural Experiment Station Clifford E. & Melda Snyder Research Farm, Pittstown, NJ. The soil was disc plowed and fertilized with 1009 kg/ha of 15-15-15 N-P-K and, subsequently, beds were mechanically raised, followed by the placement of drip irrigation system and 0.032mm plastic mulch. Plants were spaced 0.61m apart within the rows and the rows were spaced 2.74 cm apart from each other. During the period of the experiment, no phytosanitary measures were necessary to control pests and diseases, while weeds were controlled manually.

Since catnip is a perennial crop, the same plants were harvested three times for the evaluation of biomass, essential oil content and chemical composition: 1) 90 days after transplanting (July 30, 2017); 2) 1st regrowth, 60 days after the first harvest (September 29, 2017); and 3) 2nd regrowth (June 28, 2018). The plants were cut back 10cm above ground level and then allowed to grow again for the second and third harvests. The harvesting dates were determined by the time when the plants were in full flowering stage. After harvesting, plants shoot biomass was dried to constant weight at 37°C in a walk-in forced air Powell Tobacco dryer (MarCo Manufacturing Company LLC, Bennettsville, SC) and weighed for determination of dry mass.

Essential oil extraction and chemical analysis. Essential oil was extracted by the hydrodistillation method, using a Clevenger-type apparatus as described by Juliani et al. (2008). Samples of 100g of grounded dried catnip shoot biomass was placed in 2 Lr round bottom glass flasks, and 1 L of deionized water was subsequently added. The flasks containing the water and plant material were then placed over heating mantles for 2 hours and the essential oils were condensed and collected in in a Clevenger type receiver trap. The amount of essential oil collected from the samples was weighed and expressed as a percentage of dry shoot mass. Yield of individual compounds were calculated considering the relative abundance (% peak area obtained by GC) and the essential oil yield, and were expressed as mg of individual compound per plant.

For the chemical analysis, 10 µL of essential oil was added to 1.5ml of Chromatographic grade methyl tert-butyl ether (MTBE) and dried over anhydrous Na_2SO_4. The supernatant was transferred to sealed vials, remaining refrigerated at 4°C until subsequent analysis.

The essential oils were analyzed by gas chromatography coupled to mass spectrometry (GC-MS). Compound separation was performed on a Shimadzu 2010 Plus gas chromatograph equipped with an AOC-6000 auto-sampler. Injection conditions were the same as described by Reichert et al. (2019). Identification of compounds was performed by comparison of MS spectra to that in mass spectral libraries (NIST05.lib, NIST05s.lib, W10N14.lib and W10N14R.lib) and validated by association of calculated retention indices (based on a C8-C20 alkane series) with published retention indices of essential oil constituents (Adams, 2017).

Statistical analysis. The experiment consisted of a split-plot in time scheme (6 genotypes as plots and 3 harvesting times as subplots) with a randomized complete block design, comprising 3 replications and 8 plants per experimental unit. Analysis of variance (ANOVA) assumptions were visually accessed and, when deemed appropriate, data was submitted to ANOVA. When significant differences were observed, the means were compared by the Scott-Knott test ($p=\leq 0.01$). The analyses were performed using the statistical software Assistant 7.7 (Silva and Azevedo, 2016).

RESULTS AND DISCUSSION

Lemon catnip and lemon-scented catnip genotypes significantly differed in essential oil yield and composition, with significant changes also as a function of harvest time, especially in the lemon-scented selections CN5, CN6 and CL1.

Biomass production per plant did not vary significantly among the genotypes across different harvests. Only when the three harvest averages were considered for each genotype, statistically significant differences were observed for this variable, with CN5 showing the lowest biomass yield (average of 95.69 g of dry shoot mass) when compared to the other lines, which did not differ from each other (Table 1).

Essential oil contents (% of dry shoot mass) presented an interaction effect between genotypes and harvesting time. Commercial lemon catnip reached the highest content at the third harvest (June 2018, 0.75%) and had superior results compared to those observed for the catnip selections in all three harvests. At harvests 1 and 2 (July 2017 and September 2017), the catnip selections did not differ from each other. However, at the third harvest (June 2018), CL1 and CL2 showed higher essential oil contents when compared to the other selections (Table 1).

As expected, essential oil yield (g per plant) followed the same pattern of essential oil content, with the commercial lemon catnip (CIT) showing higher yields at the third harvest and outperforming the catnip selections at all harvests, with a total yield of 1.35 g per plant considering the sum of three harvests. CL1 and CL2 showed higher yields at harvest 3 (0.16 and 0.14 g per plant, respectively) when compared to CN3, CN5 and CN6 (Table 1). The lower essential oil yields observed in the lemon-scented selections in comparison to the commercial lemon catnip may be related to the fact that these lines were nepetalactone-producing lines that were then selected to produce no to low amounts of nepetalactone. Since nepetalactones, in many cases, can comprise the majority of the total essential oil on a relative basis (Reichert et al., 2016; 2019; Vukovic et al., 2016), the selective removal of such compounds could have significantly reduced the total essential oil content.

The chemical composition of the essential oils across all the assessed catnips indicated 25 aroma volatiles in total, with 22 identified and 3 remaining unknown, and of all the essential oil constituents, four were found in greatest concentration: citronellol, geraniol, (E)-caryophyllene and caryophyllene oxide (Table 2). These results for *N. cataria* var. *citriodora* are in agreement with prior observations (Klimek et al., 2000; Said-Al et al., 2018; Wesołowska et al., 2011).

The commercial lemon catnip genotype showed a stable chemical profile, with citronellol (30.7-36.9% of total peak area) and geraniol (20.5-32.3%) as major compounds in all three harvests. Citral (neral + geranial) contents varied from 5.2% in the first harvest to 15.1% in the second harvest in this line. Interestingly, relatively high amounts of *Z,E*- and *E,Z*-nepetalactones were detected at harvest 3 of commercial *N. cataria* var. *citriodora* (16.8 and 11.1%, respectively), with smaller percentages in previous harvests, although never devoid of these monoterpenes as seen in the selections CN6 and CL1. CN3, CN5 and CL2 genotypes presented either trace amounts or a maximum of 1.9% of nepetalactones in their essential oil composition (CN3 at harvest 3) (Table 2).

Although not reaching more than 3.2% of total essential oil composition in commercial lemon catnip and selections CN6 and CL1, (E)-caryophyllene was the most abundant compound in CN3 harvested in September 2017 and June 2018 (54.7 and 50.1%, of total oil composition, respectively) and the second most abundant for the same line harvested in July 2017 (41.1%), after caryophyllene oxide. (E)-caryophyllene and caryophyllene oxide were the two major compounds of CN3, CN5 and CL2 essential oils in all three harvests, in some cases comprising more than 85% of total oil composition. Caryophyllene oxide was the single major compound in the essential oils of CN5 and CL2 in all three harvests and the main component of CN6 and CL1 in harvests 1 and 2, comprising 66% of CL1 essential oil (harvest 2). Interestingly, for both CN6 and CL1 plants harvested in June 2018, a significantly change in chemical profile was observed, with percentages of caryophyllene oxide reduced to no more than 30.2% and citronellol as the main essential oil component.

Citronellol is a monoterpene present in the essential oils of several medicinal plants with has many pharmacological activities and low toxicological activity (Santos et al., 2019). Citronellol contents were higher in the commercial lemon catnip in harvests 1 and 2 when compared to the other studied lines. At harvest three, significant increases in the percentage of citronellol were observed in CN6 and CL2, which, at that time, did not differ from commercial *N. cataria* var. *citriodora.* CN3 produced only negligible amounts of citronellol and was not considered for statistical analysis. From the citronellol-producing lines, CL2 presented the overall lowest contents of the monoterpene, especially in harvests 1 and 3, not differing from CR5 in harvest 2. In terms of yield, commercial lemon catnip also presented the best results in all three harvests, up to 300.5 mg in harvest 3. The lemon-scented selections did not differ from each other on citronellal yield at harvests 1 and 2. CL1 produced statistically superior results for citronellol yield at harvest 3 when compared to the CN5, CN6, and CL2, although inferior to commercial lemon catnip (Table 3).

Monitoring of citronellol fluctuations in catnip essential oils is of special importance considering the current use of this plant as an insect repellent. Although the repellency of *N. cataria* is attributed to the presence of nepetalactones (Reichert et al., 2019), the presence of other potentially repellent components in the essential oil could possibly boost the bioactivity and contribute to improved effectiveness. Citronellol is widely known as an arthropod repellent, with proven efficacy against ticks, mites and mosquitoes (Ferreira et al., 2017; Salman and Erbas, 2014; Tabari et al., 2017), among others.

Another monoterpene alcohol with great potential arthropod-repellent activity is geraniol (Müller et al., 2009). Geraniol was identified in negligible amounts in the essential oil of the selections CN3 and CL2 (Table 2), which were not considered for statistical analysis. However, the monoterpene was considerably abundant in the essential oil of commercial lemon-scented catnip in all three harvests and in the essential oils of CN6 and CL1 at harvest 3. An overall increase in geranial content was observed for the lemon-scented selections at harvest 3, even for CN5, which reached amounts up to 10.9% as compared to 0.1% in harvest 2 (Table 4). As for geraniol yield, commercial *N. cataria* var. *citriodora* was superior to the other genotypes in all three harvests, and had a peak yield in harvest three, with 237.95 mg of geraniol per plant. Geraniol yields were not different among CN5, CN6 and CL1 for harvests 1 and 2. At harvest 3, CN5 presented the lowest yield (2.04 mg per plant) and CN6 and CL1 did not differ from each other (Table 4).

Sesquiterpenes were also among the identified aromatic volatile constituents mainly in the lemon-scented selections CN3, CN5 and CL2 and were more abundant on plants harvested in July and September 2017 as compared to essential oils from June 2018. Although humulene epoxide and α-humulene are important compounds and were frequently found in these genotypes, the main sesquiterpenes identified were (E)-caryophyllene and caryophyllene oxide in all three harvests (Table 2).

At harvest 1 and 3, CN3 and CL2 had the highest (E)-caryophyllene contents, followed by CN5, which was, in turn, statistically higher concentrations to the selections CN6 and CL1 and the commercial lemon catnip. At harvest 2, CN3 had a higher (E)-caryophyllene content than CL2, which produced significantly higher contents than the other studied genotypes. As per yield in grams per plant, CL2 had the best results in harvests 1 and 3 (19.77 and 57.08 mg per plant, respectively), while CN3 produced the highest amount at harvest 2, 46.48 mg per plant (Table 5). (E)-caryophyllene is a commercially important natural bicyclic sesquiterpene, commonly identified in the essential oils of numerous spices and food plants, having several biological activities such as anti-inflammatory, antibiotic, antioxidant, anti-carcinogenic and local anesthetic (Pant et al., 2014). Research also showed this sesquiterpene to inhibit the growth of the tobacco budworm (*Heliothis virescens* F.) when administered to the insect's diet (Gunasena et al., 1988), suggesting a chemical defense role of this compound against herbivory.

The (E)-caryophyllene oxidation derivative, caryophyllene oxide was also identified in the oils of commercial lemon catnip and lemon-scented selections (Table 2, 6). Both (E)-caryophyllene and caryophyllene oxide have strong wooden odor and are two natural substances approved as flavorings by the Food and Drug Administration (FDA) and by the European Food Safety Authority (EFSA) (Fidyt et al., 2016).

In the three harvests, caryophyllene oxide contents were higher in the essential oils from lemon-scented selections than in commercial *N. cataria* var. *citriodora.* At harvest 1, lemon-scented lines did not differ from each other regarding caryophyllene oxide contents in the essential oil. CN5, CN6 and CL1 had the highest contents at harvest 2 and, for plants harvested in June 2018, CN3, CN5 AND CL2 showed higher accumulation. For all genotypes, except CL2, caryophyllene oxide contents significantly were lower in June 2018 when compared to previous harvests (Table 6). As for caryophyllene oxide yields, commercial lemon catnip and selection CL2 were higher to the other genotypes at harvest one (28.53 and 30.05 mg per plant, respectively). CL2 was also higher accumulator than other genotypes, including commercial lemon catnip, at harvest 3, producing 63.77 mg per plant. Genotypes harvested in September 2017 (harvest 2) did not differ statistically among themselves (average of 21.74 mg per plant) (Table 6).

Similarly to the previously discussed compounds, caryophyllene oxide has also been reported as a mosquito repellent (Omolo et al., 2004; Silva et al., 2008) and, additionally, has been described as an efficient ant repellent (Hubert and Wiemer, 1985). Thus, the accumulation of this compound in the essential oil of *N. cataria* can be a strategic advantage for commercial purposes as would be the high content of citronellol and geraniol. Research has shown caryophyllene oxide as one of the main chemical compounds overexpressed when plants interact with herbivores (Delphia et al., 2007; Troncoso et al., 2011), suggesting the function of this compound as part of plants chemical defense and emphasizing the influence ecological factors can exert on plant secondary metabolism. In addition to biotic interactions, caryophyllene oxide contents in plant tissues are also known to be strongly affected by temperature, precipitation and Zn, Cu, Fe and Mn levels in the soil (Duarte et al., 2010).

In the present study, the changes observed across harvests in the same lines suggest that plant ecological factors along with growth stages may play a major role on catnip and lemon catnip chemical composition. Understanding how each of these factors and their interaction with the genotype component influence the aromatic profile of *N. cataria* and *N. cataria* var. *citriodora* consists of a strategic approach to predict and manipulate the production of metabolites of interest and to meet the standardization required to expand the market of these specialty crops for pest control formulations and other industries.

Table 1. Biomass, essential oil content and essential oil yield of catnip *(N. cataria*) and lemon catnip (*N. cataria* var. *citriodora*) genotypes harvested at different times.

Harvest	*Genotypes* CIT	CN3	CN5	CN6	CL1	CL2	*Mean*
				Plant Biomass (g per plant)			
July 2017	93.75	106.25	87.08	114.58	129.17	129.17	110.0ns
Sep 2017	83.33	133.33	100.0	120.83	87.91	87.50	102.15
June 2018	131.95	139.58	100.0	129.17	133.50	150.0	130.70
Mean	103.01 A	125.39 A	95.69 B	121.53 A	116.86 A	122.22 A	
				Essential oil content (% of dry shoots biomass)			
July 2017	0.23 bA	0.02 aB	0.04 aB	0.04 aB	0.02 bB	0.04 aB	0.07
Sep 2017	0.18 bA	0.06 aB	0.04 aB	0.02 aB	0.03 bB	0.06 aB	0.06
June 2018	0.75 aA	0.03 aC	0.05 aC	0.06 aC	0.12 aB	0.09aB	0.18
Mean	0.38	0.04	0.04	0.04	0.06	0.06	
				Essential oil yield (g per plant)			
July 2017	0.22 bA	0.02 aB	0.04 aB	0.04 aB	0.02 bB	0.06 aB	0.07
Sep 2017	0.15 bA	0.08 aB	0.03 aB	0.02 aB	0.03 bB	0.05 aB	0.06
June 2018	0.98 aA	0.04 aC	0.05 aC	0.08 aC	0.16 aB	0.14 aB	0.24
Mean	0.45	0.05	0.04	0.05	0.07	0.08	

Means followed by different letters (uppercase for genotypes and lowercase for harvesting times) are significantly different according to the Scott-Knott test (p=≤0.01). When interaction between genotypes and harvesting times was significant, isolated means comparisons were not performed. ns: non-significant. CIT: Commercial *N. cataria* var. *citriodora.* CN3, CN5, CN6, CL1 and CL2 are lemon-scented catnip genotypes from Rutgers-NJAES germplasm.

Table 2. Chemical constituents and relative percentages of total chromatogram area of essential oils from different genotypes of catnip (*Nepeta cataria* L.) and lemon catnip (*Nepeta cataria* var. *citriodora*) harvested at different times.

			July 2017						September 2017						June 2018					
			CIT	CN3	CN5	CN6	CL1	CL2	CIT	CN3	CN5	CN6	CL1	CL2	CIT	CN3	CN5	CN6	CL1	CL2
RT	RI	Compound	% Area																	
8.32	984	β-pinene	-	-	-	-	-	-	-	-	-	-	-	-		-	-	2.2±0.1	-	-
9.46	1099	Linalool	1.2±0.1	-	-	T	10.2±6.8	T	T	-	-	-	-	-	-	-	-	0.9±0.1	T	-
9.58	1110	(Z)-Rose oxide	0.5±0.5	-	-	-	-	-	0.9±0.8	-	-	-	-	-	T	-	-	-	-	-
9.92	1154	Nerol oxide	1.0±0.1	-	-	-	-	-	3.2±0.7	-	-	1.1±1.0	T	-	1.5±0.1	-	1.0±0.3	2.2±0.3	1.7±0.5	-
10.37	1204	Myrtenal	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-
10.47	1224	Citronellol	36.9±0.6	0.8±0.7	5.5±4.8	16.9±2.0	13.7±7.1	T	36.8±2.3	-	T	15.9±1.0	11.5±2.2	0.6±0.5	30.7±0.7	-	17.5±1.0	32.6±1.5	34.2±0.7	T
10.63	1239	Neral	2.5±0.1	-	T	T	1.1±0.9	-	6.8±0.6	-	-	2.4±0.3	1.1±0.5	-	4.3±0.2	-	3.8±0.3	4.8±0.2	3.4±0.1	-
10.70	1250	Geraniol	32.3±0.8	-	2.4±2.1	11.7±1.6	8.2±5.1	-	20.5±0.6	-	T	3.5±0.5	2.4±0.7	-	24.3±0.7	-	10.9±0.3	22.8±0.2	20.2±0.4	-
10.84	1268	Geranial	2.7±0.4	-	-	0.9±0.8	0.5±0.9	-	8.3±0.7	-	-	3.6±0.1	1.9±0.1	-	4.4±0.5	T	4.6±0.2	5.8±0.4	5.1±0.5	-
11.63	1377	(Z,E)-nepetalactone	-	-	-	-	-	-	1.0±1.8	T	T	-	-	0.6±0.5	16.8±1.2	1.9±0.3	-	-	-	-
11.84	1408	(E,Z)-nepetalactone	7.9±0.8	-	-	-	-	-	0.8±0.8	-	-	-	-	0.8±0.2	11.1±0.5	-	-	-	-	-
11.94	1421	(Z)-caryophyllene	-	T	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-
12.04	1442	(E)-caryophyllene	2.8±0.4	41.1±1.3	19.6±14.2	5.4±1.2	3.2±0.6	36.2±4.9	1.8±0.3	54.7±2.7	31.6±12.2	T	T	43.4±3.5	3.2±0.2	50.1±2.1	15.6±0.3	2.7±0.3	2.0±0.2	42.1±0.5
12.12	1456	(E)-β-farnesene	-	-	-	-	-	-	-	0.7±0.3	-	-	-	1.9±03	-	0.7±0.1	-	-	-	-
12.28	1476	α-humulene	-	3.9±0.2	0.6±0.5	-	-	2.9±0.2	-	4.1±0.6	1.5±0.2	-	-	4.2±0.4	T	4.9±0.4	1.3±0.1	-	-	4.0±0.3
12.35	1491	(E)-β-ionone	-	T	-	-	-	T	-	T	T	-	-	-	-	-	-	-	-	0.7±0.2
12.90	1581	Unknown 1	-	T	2.4±1.8	T	-	-	-	T	T	-	-	-	-	0.7±0.1	1.0±0.2	-	-	0.5±0.3
13.11	1607	Caryophyllene oxide	11.8±07	46.4±1.0	52.8±18.6	48.4±2.4	42.1±6.0	50.9±3.2	18.8±1.7	35.5±0.1	58.7±9.0	64.2±3.0	66.0±3.9	43.6±3.9	3.6±0.2	36.9±3.3	42.4±2.4	24.9±1.1	30.2±1.4	46.3±1.0
13.19	1629	2-*epi*-β-cedren-3-one	-	0.8±0.3	-	-	-	T	-	T	-	-	-	0.9±0.1	-	0.5±0.1	-	-	-	0.7±0.2
13.27	1641	Humulene epoxide II	T	3.4±0.3	2.3±2.0	2.7±0.4	2.5±0.8	2.6±0.3	0.6±0.5	1.7±0.5	1.2±0.8	0.6±1.1	0.8±0.8	2.8±0.2	-	2.5±2.1	2.1±0.1	1.1±0.1	1.4±0.2	3.3±0.3
13.59	1667	Aromadendrene epoxide	-	-	-	1.1±1.2	3.7±4.9	T	-	-	T	-	-	-	-	-	-	-	-	-
14.39	1840	Hexahydrofarnesyl acetone	-	0.9±0.3	T	0.7±0.6	1.0±1.6	3.4±0.3	-	1.9±0.4	5.1±1.9	2.6±0.2	5.6±2.0	0.7±0.2	-	-	-	-	-	1.1±0.1
14.98	1955	n-Hexadecanoic acid	-	1.6±1.4	9.3±16.1	-	-	2.7±1.1	-	T	0.8±0.7	-	-	0.6±0.5	-	1.4±0..1	-	-	-	0.8±0.1
15.59	2071	Unknown 2	-	-	3.1±2.7	7.5±1.0	9.2±4.7	-	-	-	-	3.6±1.0	7.5±1.1	-	-	-	-	-	0.8±0.1	-
15.80	2112	Unknown 3	-	-	1.3±1.1	4.1±0.7	4.5±2.1	-	-	-	-	2.6±2.3	3.0±2.5	-	-	-	-	-	0.5±0.5	-
		Total identified peaks	100.0	99.72	92.58	87.95	86.26	100.0	100.0	99.82	99.89	93.83	89.46	100.0	100.0	100.0	99.03	100.0	98.64	99.46

Data are the mean of three replicates ± SD. CIT: Commercial *N. cataria* var. *citriodora.* CN3, CN5, CN6, CL1 and CL2 are lemon-scented catnip genotypes from Rutgers-NJAES germplasm. T: trace amounts (less than 0.5%). RT: retention time. RI: retention indexes experimentally calculated using homologue series of n-alkanes (C8-C20) on a non-polar HP5-MS column.

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Table 3. Citronellol content (peak area percentage) and yield of catnip *(N. cataria*) and lemon catnip (*N. cataria* var. *citriodora*) genotypes harvested at different times.

	Genotypes						
Harvest	CIT	CN3*	CN5	CN6	CL1	CL2	Mean
	Citronellol content (% of essential oil)						
July 2017	36.87 aA	-	5.50 bC	16.93 bB	13.73 bB	0.46 aD	12.39
Sep 2017	36.80 aA	-	<0.01 bD	15.88 bB	11.51 bC	0.55 aD	10.79
June 2018	30.67 bA	-	17.45 aB	32.58 aA	34.24 aA	0.43 aC	19.23
Mean	37.78	-	7.65	21.79	19.83	0.48	
	Citronellol yield (mg per plant)						
July 2017	81.14 bA	-	1.73 aB	6.72 aB	3.08 bB	0.27 aB	15.52
Sep 2017	56.31 bA	-	<0.1 bB	3.55 aB	2.91 bB	0.23 aB	10.49
June 2018	300.52 aA	-	8.52 aC	24.38 aC	55.66 aB	0.61 aC	64.95
Mean	145.99	-	3.42	11.55	20.55	0.36	

Means followed by different letters (uppercase for genotypes and lowercase for harvesting times) are significantly different according to the Scott-Knott test (p=≤0.01). When interaction between genotypes and harvesting times was significant, isolated means comparisons were not performed. *CN3 genotype removed from citronellol statistical analyses due to one or more combinations having values equal to zero for all three repetitions, therefore not meeting the assumptions for analysis of variance (both normality and variance homogeneity). CIT: Commercial *N. cataria* var. *citriodora.* CN3, CN5, CN6, CL1 and CL2 are lemon-scented catnip genotypes from Rutgers-NJAES germplasm.

Table 4. Geraniol content (peak area percentage) and yield of catnip *(N. cataria*) and lemon catnip (*N. cataria* var. *citriodora*) genotypes harvested at different times.

	Genotypes						
Harvest	CIT	CN3*	CN5	CN6	CL1	CL2*	Mean
	Geraniol content (% of essential oil)						
July 2017	32.43 aA	-	2.41 bD	11.17 bB	8.20 bC	-	9.02
Sep 2017	20.50 cA	-	0.10 cC	3.51 cB	2.39 cB	-	4.42
June 2018	24.30 bA	-	10.90 aC	22.76 aA	20.19 aB	-	13.02
Mean	25.71	-	4.47	12.48	10.26	-	
	Geraniol yield (mg per plant)						
July 2017	71.33 bA	-	0.77 aB	4.41 aB	1.81 bB	-	13.05
Sep 2017	30.84 cA	-	0.05 aB	0.73 aB	0.60 bB	-	5.37
June 2018	237.95 aA	-	5.32 aC	17.11aB	32.79 aB	-	48.86
Mean	113.37	-	2.04	7.42	11.74	-	

Means followed by different letters (uppercase for genotypes and lowercase for harvesting times) are significantly different according to the Scott-Knott test (p=≤0.01). When interaction between genotypes and harvesting times was significant, isolated means comparisons were not performed. *CN3 and CL2 genotypes removed from geraniol statistical analyses due to one or more combinations having values equal to zero for all three repetitions, therefore not meeting the assumptions for analysis of variance (both normality and variance homogeneity). CIT: Commercial *N. cataria* var. *citriodora.* CN3, CN5, CN6, CL1 and CL2 are lemon-scented catnip genotypes from Rutgers-NJAES germplasm.

Table 5. (E)-caryophyllene content (peak area percentage) and yield of catnip *(N. cataria*) and lemon catnip (*N. cataria* var. *citriodora*) genotypes harvested at different times.

	Genotypes						
Harvest	CIT	CN3	CN5	CN6	CL1	CL2	Mean
	(E)-caryophyllene content (% of essential oil)						
July 2017	2.87 aC	41.06 bA	19.58 bB	5.37 aC	3.23 aC	36.21 aA	18.05
Sep 2017	1.83 aD	54.68 aA	31.62 aC	<0.01 aD	<0.01 aD	43.4 aB	21.92
June 2018	3.17 aC	50.11 aA	15.60 bB	2.65 aC	2.02 aC	42.12 aA	19.28
Mean	2.62	48.61	22.26	2.67	1.75	40.58	
	(*E)-caryophyllene yield (mg per plant)*						
July 2017	6.50 bB	8.77 cB	8.25 aB	2.12 aB	0.81 aB	19.77 bA	7.70
Sep 2017	2.84 bC	46.48 aA	12.17 aB	<0.1 aC	<0.1 aC	21.94 bB	13.90
June 2018	31.34 aB	21.94 bC	7.61 aD	2.00 aD	3.28 aD	57.08 aA	20.71
Mean	13.56	25.73	9.34	1.37	1.36	33.26	

Means followed by different letters (uppercase for genotypes and lowercase for harvesting times) are significantly different according to the Scott-Knott test (p=≤0.01). When interaction between genotypes and harvesting times was significant, isolated means comparisons were not performed. CIT: Commercial *N. cataria* var. *citriodora.* CN3, CN5, CN6, CL1 and CL2 are lemon-scented catnip genotypes from Rutgers-NJAES germplasm.

Table 6. Caryophyllene oxide content (peak area percentage) and yield of catnip *(N. cataria*) and lemon catnip (*N. cataria* var. *citriodora*) genotypes harvested at different times.

	Genotypes						
Harvest	CIT	CN3	CN5	CN6	CL1	CL2	Mean
	Caryophyllene oxide content (% of essential oil)						
July 2017	11.77 aB	46.37 aA	52.89 aA	48.38 bA	42.11 bA	50.93 aA	42.07
Sep 2017	18.77 aC	35.47 bB	58.72 aA	64.18 aA	65.99 aA	43.62 aB	47.79
June 2018	3.57 bC	36.92 bA	42.38 bA	24.87 cB	30.23 cB	46.34 aA	30.72
Mean	11.37	39.59	51.33	45.81	46.11	46.97	
	Caryophyllene oxide yield (mg per plant)						
July 2017	25.59 aA	9.89 bB	18.81 aB	19.15 aB	10.40 bB	30.05 bA	18.98
Sep 2017	28.53 aA	30.12 aA	19.12 aA	14.29 aA	16.91 bA	21.47 bA	21.74
June 2018	35.17 aC	16.17 bD	20.69 aD	18.69 aD	49.58 aB	63.77 aA	34.01
Mean	29.76	18.73	19.54	17.38	25.63	38.43	

Means followed by different letters (uppercase for genotypes and lowercase for harvesting times) are significantly different according to the Scott-Knott test (p=≤0.01). When interaction between genotypes and harvesting times was significant, isolated means comparisons were not performed. CIT: Commercial *N. cataria* var. *citriodora.* CN3, CN5, CN6, CL1 and CL2 are lemon-scented catnip genotypes from Rutgers-NJAES germplasm.

ACKNOWLEDGMENTS

This study was funded by the New Jersey Agriculture Experiment Station and HATCH project NJ12158 and the Deployed Warfighter Protection (DWFP) Program in support of the research project, War Fighter Protection from Arthropods Utilizing Naturally Sourced Repellents (Grant W911QY1910007). We also thank the Rutgers IFNH Center for Food Systems

Sustainability, and Dr. Rodolfo Juliani, Adam Morgan, Joe Florentine and Ed Dager and for their assistance and support. We also recognize and thank the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) (DOC_PLENO/ proc. nº 88881.129327/2016-01) for providing a Graduate Student Fellowship to the senior author, as this work is part of his dissertation studies.

REFERENCES

Adams, R.P. 2017. *Identification of Essential Oil Components by Gas Chromatography/ Mass Spectrometry*. Allured Publ. Corp., Carol Stream, IL. 809 p.

Debboun, M., Frances, S. P., and Strickman, D. 2014. *Insect repellents handbook*. CRC Press., Boca Raton, FL. 409 p.

Delphia, C.M., Mescher, M.C., and Moraes, C.M. 2007. Induction of plant volatiles by herbivores with different feeding habits and the effects of induced defenses on host-plant selection by thrips. Journal of Chemical Ecology 33:997-1012.

Duarte, A.R., Naves, R.R., Santos, S.C., Seraphin, J.C., and Ferri, P.H. 2010. Genetic and environmental influence on essential oil composition of *Eugenia dysenterica*. Journal of the Brazilian Chemical Society 21: 1459-1467.

Duda, S.C., Mărghitaş, L.A, Dezmirean, D.S., Bobiş, O., and Duda, M.M. 2015. *Nepeta cataria*: medicinal plant of interest in phytotherapy and bee keeping. Hop and Medicinal Plants 23:34-38.

Ferreira, L.L., Filho, J.G., Mascarin, G.M., León, A.A.P., and Borges, L.M.F. 2017. *In vitro* repellency of DEET and β-citronellol against the ticks *Rhipicephalus sanguineus* sensu lato and *Amblyomma sculptum*. Veterinary Parasitology 239: 42-45.

Fidyt, K., Fiedorowicz, A., Strządała, L., and Szumny, A. 2016. Β-caryophyllene and β-caryophyllene oxide- natural compounds of anticancer and analgesic properties. Cancer Medicine 5: 3007-3017.

Gunasena, G.H., Vinson, S.B., Williams, H.J., and Stipanovic, R.D. 1988. Effects of caryophyllene, caryophyllene oxide, and their interaction with gossypol on the growth and development of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae). Journal of Economic Entomology 81: 93-97.

Hubert, T.D., and Wiemer, D.F. 1985. Ant-repellent terpenoids from *Melampodium divaricatum*. Phytochemistry 24: 1197-1198.

Ibrahim, M.E., El-Sawi, S.A., and Ibrahim, F.M. 2017. *Nepeta cataria* L, one of the promising aromatic plants in Egypt: seed germination, growth and essential oil production. Journal of Materials and Environmental Science 8:1990-1995.

Juliani, H.R., J.E. Simon, C. Quansah, E. Asare, R. Akromah, D. Acquaye, J. Asante-Dartey, M.L.K. Mensah, T.C. Fleischer, R. Dickson, K. Annan and Mensah, A.Y. 2008. Chemical diversity of *Lippia multiflora* from West Africa. Journal of Essential Oil Research 20:49-55.

Klimek, B., Majda, T., Góra, J., and Patora, J. 2000. Investigation of the essential oil from lemon catnip (*Nepeta cataria* L. var *citriodora*) in comparison to the oil from lemon balm (*Melissa officinalis* L.). Herba Polonica 46: 226-234.

Martins, H.B., Selis, N.D.N., Nascimento, F. S., Carvalho, S.P.D., Gusmão, L.D.O., Nascimento, J.D.S., Brito, A.K.P., Souza, S.I.D., Oliveira, M.V.D., Timenetsky, J., and Yatsuda, R. 2017. Anti-inflammatory activity of the essential oil citral in experimental infection with *staphylococcus aureus* in a model air pouch. Evidence-Based Complementary and Alternative Medicine 2017: 1-10.

Morales, M.R. and J.E. Simon. 1977. 'Sweet Dani': A new culinary and ornamental lemon basil. Cultivar release in HortScience 32(1):148-149.

Müller, G.C., Junnila, A., Butler, J., Kravchenko, V.D., Revay, E.E., Weiss, R.W., and Schlein, Y. 2009. Efficacy of the botanical repellents geraniol, linalool, and citronella against mosquitoes. Journal of Vector Ecology 34: 2-8.

Omolo, M.O., Okinyo, D., Ndiege, I.O., Lwande, W., and Hassanali, A. 2004. Repellency of essential oils of some Kenyan plants against *Anopheles gambiae*. Phytochemistry 65: 2797-2802.

Pant, A., Saikia, S.K., Shukla, V., Asthana, J., Akhoon, B.A., and Pandey, R. 2014. Beta-caryophyllene modulates expression of stress response genes and mediates longevity in *Caenorhabditis elegans*. Experimental Gerontology 57: 81-95.

Regnier, F.E., Eeisenbraun, E.J., and Waller, G.R. 1967. Nepetalactone and epinepetalactone from *Nepeta cataria* L. Phytochemistry 6: 1271-1280.

Reichert, W., Ejercito, J., Guda, T., Dong, X., Wu, Q., Ray, A., and Simon, J.E. 2019. Repellency Assessment of *Nepeta cataria* Essential Oils and Isolated Nepetalactones on *Aedes aegypti*. Scientific Reports, 9: 1-9.

Reichert, W., Park, H.C., Juliani, H.R., Simon, J.E. 2016. 'CR9': A New Highly Aromatic Catnip *Nepeta cataria* L. Cultivar Rich in Z, E-Nepetalactone. HortScience 51: 588-591.

Said-Al Ahl, H., Naguib, N.Y., Hussein, M.S. 2018. Evaluation growth and essential oil content of catmint and lemon catnip plants as new cultivated medicinal plants in Egypt. Annals of Agricultural Sciences 63: 201-205.

Salman, S.Y., and Erbaş, S. 2014. Contact and repellency effects of *Rosa damascena* Mill. essential oil and its two major constituents against *Tetranychus urticae* Koch (Acari: Tetranychidae). Türk. Entomol Derg 38: 365-376.

Santos, P.L., Matos, J.P.S., Picot, L., Almeida, J.R., Quintans, J.S., and Quintans-Júnior, L.J. 2019. Citronellol, a monoterpene alcohol with promising pharmacological activities-A systematic review. Food and Chemical Toxicology 123: 459-469.

Silva, F.D.A.E., and Azevedo, C.D. 2016. The Assistat Software Version 7.7 and its use in the analysis of experimental data. African Journal of Agricultural Research 11: 3733-3740.

Silva, W.J., Dória, G.A.A., Maia, R.T., Nunes, R. S., Carvalho, G.A., Blank, A.F., and Cavalcanti, S.C.H. 2008. Effects of essential oils on *Aedes aegypti* larvae: alternatives to environmentally safe insecticides. Bioresource Technology 99: 3251-3255.

Simon, J.E., M.R. Morales, W.B. Phippen, R.F. Vieira and Hao, Z. 1999. Basil: A source or aroma compounds and a popular culinary and ornamental herb, pp.499-505. In: J. Janick (ed). *Perspectives on New Crops and New Uses*. ASHS Press, Alexandria, VA.

Tabari, M.A., Youssefi, M.R., Esfandiari, A., and Benelli, G. 2017. Toxicity of β-citronellol, geraniol and linalool from *Pelargonium roseum* essential oil against the West Nile and filariasis vector *Culex pipiens* (Diptera: Culicidae). Research in Veterinary Science 114: 36-40.

Troncoso, C., Becerra, J., Bittner, M., Perez, C., Saez, K., Sanchez-Olate, M., and Rios, D. 2011. Chemical defense responses in *Eucalyptus globulus* (Labill) plants. Journal of the Chilean Chemical Society 56: 768-770.

United States Department of Agriculture (USDA). 2019. *Nepeta cataria* L. Catnip. Accessed August 17, 2019 at https://plants.usda.gov/core/profile?symbol=NECA2#

Vukovic, N., Vukic, M., Djelic, G., Hutkova, J., and Kacaniova, M. 2016. Chemical Composition and Antibacterial Activity of Essential Oils of Various Plant Organs of Wild Growing *Nepeta cataria* from Serbia. Journal of Essential Oil Bearing Plants 19: 1404–1412.

Waller, G.R., Price., G.H. and Mitchell, E.D. 1969. Feline attractant, cis, trans-nepetalactone: Metabolism in the domestic cat. Science: 164: 1281–1282.

Wesołowska, A., Jadczak, D., and Grzeszczuk, M. 2011. GC-MS analysis of lemon catnip (*Nepeta cataria* L. var. *citriodora* Balbis) essential oil. Acta Chromatographica 23: 169-180.

Zhu, J.J., Zeng, X.P., Berkebile, D., Du, H.J., Tong, Y., and Qian, K. 2009. Efficacy and safety of catnip (*Nepeta cataria*) as a novel filth fly repellent. Medical and Veterinary Entomology 23: 209-216.