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Peppermint Productivity and Oil Composition as a Function of Nitrogen, Growth Stage, and Harvest Time

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ABSTRACT

The commercial production of peppermint (*Mentha* × *piperita* L.) is concentrated in more northern latitudes worldwide (north of the 41st parallel), including the United States. This 2-yr field study in Mississippi evaluated the effect of N (0, 80, and 160 kg/ha), growth stage (bud formation and flowering), and harvest time or cut (first cut in mid-July, second cut beginning of October) on peppermint yields, oil content, and composition. Biomass and oil yields were higher from the first cut than from the second. Overall, N increased biomass and oil yields. Contrary to literature reports that peppermint requires long days north of the 41st parallel to reach flowering, peppermint in Mississippi (at 34°43′22″ N lat) did reach flowering. The average oil yields at bud formation and at flowering were 165 and 122 kg/ha, respectively, and were greater than the average peppermint essential oil yields for the United States in 2008. Generally, (–)-menthol concentration in the oil from the 2007 harvest was lower than in the oil from the 2008 harvest. The average (–)-menthol concentration in the oil from the fertilized plots harvested at flowering in 2008 was 43 to 46%, but (–)-menthol in the other treatments was below 37%. Our results suggest the first harvest in Mississippi should be delayed until the end of July to promote conversion of (–)-menthone to (–)-menthol. Peppermint could provide two harvests per growing season under the Mississippi climate, with oil yields and composition similar to those from other peppermint production regions.

Peppermint is grown for commercial production of peppermint essential oil, for production of peppermint dry leaves, or for the fresh herb market (Lawrence, 2006; Mustjatse, 1985; Topalov, 1989). Peppermint essential oil is a major aromatic agent used extensively in chewing gum, toothpaste, mouth washes, pharmaceuticals, and confectionary and aromatherapy products (Lawrence, 2006; Topalov, 1989; Mint Industry Research Council, 2009). Commercial production of peppermint essential oil is concentrated in the northwestern United States, where long days and cooler nights promote monoterpene synthesis and accumulation (Burbott and Loomis, 1967; Lawrence, 2006). Current understanding is that large commercial production of peppermint may not be successful south of the 40th to 41st parallel (Johnson, 2001) because of the shorter days (<15 h) in the summer and the inability of peppermint to form flowers under short days (Langston and Leopold, 1954; Burbott and Loomis, 1967). However, the U.S. essential oil industry has been looking to expand mint production areas in the South due to the decline of peppermint production areas in Idaho and the northwestern United States due to expanding corn acreage. The peppermint essential oil production in the United States decreased 19.4% from 3.1 million kg in 2007 to 2.5 million kg in 2008 (National Agricultural Statistics Service, 2009). However, there is no prior research on peppermint productivity, essential oil content, and composition in the southeastern United States. The objective of this study was to evaluate the effect of N (0, 80, and 160 kg/ha), growth stage (bud formation and flowering), and cut (first cut in mid-July, and the second cut beginning of October) on peppermint biomass yield, oil content, and oil composition.

MATERIALS AND METHODS

Plant Material and Field Experiments

Peppermint 'Black Mitcham', the traditional cultivar used in commercial peppermint plantations in the United States, was used in this study. Peppermint is a vegetatively propagated natural hybrid of *M. aquatica* × *M. spicata*, a sterile allohexaploid with $2n = 72$ (Tucker, 1992). The plant material (virus free and purchased from Summit Plant Laboratories, Ft. Collins, CO) was transplanted into the field at the North Mississippi Research and Extension Center at Verona, MS (34°43′22″ N and –88°43′22″ W) during the first week of May, 2007. The 2008 peppermint was reestablished naturally from the overwintering rhizomes. The experimental design was a two-factor factorial of N and growth stage in four blocks. Individual field plots were 6.1 m long and contained 40 plants spaced every 30 cm set in two rows and 30 cm apart on raised beds (15 cm high and 75 cm wide). Drip irrigation tubing was placed at the 5-cm depth in the soil between rows at the time of bed formation. Plants were watered as necessary to maintain soil moisture of approximately 75 to 80% field capacity.

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Abbreviations: GC–MS, gas chromatography–mass spectrophotometer analyses; OM, organic matter.

Table 1. Soil characteristics from soil samples taken at the end of the second growing season in 2008 from plots assigned to the three nutrient treatments and the two growth stages.

Stage	N-rate appl.	OM†	pH	NO_3–N	P	K	Ca	Mg	Zn	S	Na
	kg/ha	%		kg/ha							
Bud	0	1.38	6.20	3.4	137	102	2216	107	2.2	222	117
Bud	80	1.29	5.60	3.4	141	120	2165	106	2.2	208	129
Bud	160	1.59	5.37	3.4	140	92	2011	107	2.3	257	145
Flowering	0	1.15	6.30	3.4	128	76	1899	95	2.0	185	102
Flowering	80	1.42	6.03	3.4	159	66	1892	100	2.2	229	118
Flowering	160	1.71	5.57	3.4	156	95	1888	106	2.2	276	128

† OM, organic matter.

The soil was Quitman sandy loam (fine-loamy, siliceous, semiactive, thermic, Aquic Paleudult). Selected soil properties measured at the end of the 2008 experiment are provided in Table 1. Before land preparation, the nutrient level of the soil was analyzed using the Lancaster soil test method (Cox, 2001), and the concentration of available nutrients was measured with inductively coupled argon plasma spectrometer (ICAP) (PerkinElmer, Norwalk, CT). Phosphorus (79 kg/ha) and K (103 kg/ha) fertilizers were applied based on soil test recommendations and before land preparation.

Experimental treatment combinations consisted of three N application rates: 0, 80, and 160 kg/ha, and two growth stages: bud formation and flowering. The responses were measured repeatedly (first cut in mid-July and second cut at the beginning of October). Ammonium nitrate fertilizer was applied in 80 kg N/ha increments; the first increment was applied before planting, and the subsequent increment of 80 kg/ha N was applied after the first harvest (Cut 1). Weeds were controlled with a preplant application of the herbicide Sinbar [Terbacil (3-tertbutyl-5-chloro-6-methyluracil)] at 2 kg/ha. Plants were harvested by hand at floral initiation (bud formation) and at flowering by cutting the plants approximately 10 cm above the soil surface. Fresh weights were taken immediately on harvest, and dry weights were recorded after the plants were air-dried to uniform weight.

Essential Oil Extraction and Gas Chromatography-Mass Spectrophotometer Analyses

The essential oil was extracted from representative subsamples (leaves, stems, and flowers) randomly taken from each plot by steam distillation for 1 h, on a modified Clevenger collector apparatus using a 2.0-L distillation system (Furnis et al., 1989). The essential oil from each plot sample was weighed, and the oil yield was calculated as the weight (g) of oil per weight (g) of peppermint tissue.

Chemical standards and peppermint oil samples were analyzed by gas chromatography–mass spectrophotometer analyses (GC–MS) on a Varian (Palo Alto, CA) CP-3800 GC coupled to a Varian Saturn 2000 MS/MS. The GC–MS methods for analysis and conditions are identical to those previously described by Zheljazkov et al. (2008). Briefly, the GC had a DB-5 fused silica capillary column (30 m × 0.25 mm, with film thickness of 0.25 mm) under the following T program: injector temperature, 240°C, column temperature 60 to 240°C at 3°C/min, then held at 240°C for 5 min; carrier gas, He; injection volume, 1 mL (splitless). The MS mass had a prescan ionization time of 100 microseconds, an ion trap temperature of 150°C, manifold temperature of 60°C, and a transfer line temperature of 170°C.

Quantitative Analysis

Eucalyptol, (–)-menthol, (–)-menthone, and (+)-menthofuran are the major constituents of peppermint oil and for that reason they were chosen to be quantified. Eucalyptol, (–)-menthol, (–)-menthone, and (+)-menthofuran GC grade standards were purchased from Fluka (Buchs, Switzerland). All four analytes were used to formulate separate calibration curves. All calculations were performed by generation of standard curves within Varian's (Palo Alto, CA) Saturn GC/MS Workstation software package v. 6.40. The chromatograms of each of the oils from the field experiments were compared with the standard injections. The target peaks were confirmed by both retention time and mass spectral data. Confirmed integrated peaks were then used to determine the percentage of each chemical constituent in the essential oil.

Statistical Analysis

The experiment was conducted in 2007 and in 2008, and the responses were measured repeatedly at two harvests (Cut 1 and Cut 2), with a third N Rate (160 kg/ha) applied just after the first cut. The data were analyzed as repeated measures in a two-factor factorial design with eight blocks using combinations of the four blocks and the 2 yr as the blocks. The ANOVA was completed using the Mixed Procedure of SAS (SAS Institute, 2003), and further multiple means comparison was completed for significant ($P < 0.05$) and marginally significant ($0.05 < P < 0.1$) effects by comparing the least squares means of the corresponding treatment combinations using the lsmeans statement of Proc Mixed with pdiff option to produce P values for all pair-wise differences. Letter groupings were generated using a 0.05 level of significance. For each response, the validity of model assumptions on the error terms was verified by examining the residuals as described in Montgomery (2009). Correlation analysis was also conducted to explore relationships between the soil characteristics listed in Table 1 and the nine response variables for Cut 1 and Cut 2 at bud formation and flowering stages in 2008.

RESULTS

Growth stage had a significant effect on oil content, (–)-menthol concentration, and on eucalyptol concentration and yield regardless of N and harvest time, whereas the effect of N treatment was significant with respect to yields (on a per-area basis) of eucalyptol, (–)-menthone, and (–)-menthol, irrespective of growth stage and harvest time (Table 2). The main effect of cut (harvest time) was significant only on oil content and the yield of eucalyptol (Table 2). The interaction of stage and cut was significant with respect to fresh herbage and oil yields, the concentrations of (–)-menthone and (+)-menthofuran in the oil, and the yields of (–)-menthone and (–)-menthol. The interaction effect

Table 2. ANOVA *P* values for the main and interaction effects of growth stage (Stage), N treatment (N Trt) and cut on fresh herbage yield (FrHY), % oil fresh herbage (OilFH), oil yield (OilY), % eucalyptol concentration (EucC), % (–)-menthone concentration (M-oneC), % (–)-menthol concentration (M-olC), % (+)-menthofuran concentration (M-furC), eucalyptol yield (EucY), (–)-menthone yield (M-oneY), (–)-menthol yield (M-olY) and (+)-menthofuran yield (M-furY) at Verona, MS.

SV	FrHY	OilFH	OilY	EucC	M-oneC	M-olC	M-furC	EucY	M-oneY	M-olY	M-furY
Block	0.090	0.215	0.105	0.001	0.001	0.002	0.001	0.001	0.719	0.001	0.001
Stage	0.027	0.002†	0.001	0.001	0.046	0.012	0.503	0.001	0.055	0.051	0.015
N Trt	0.001	0.724	0.001	0.291	0.408	0.413	0.424	0.068	0.001	0.001	0.024
Stage × N Trt	0.538	0.978	0.711	0.696	0.496	0.634	0.531	0.969	0.964	0.672	0.477
Cut	0.001	0.001	0.001	0.001	0.001	0.104	0.001	0.009	0.001	0.004	0.257
Stage × Cut	0.001	0.323	0.004	0.492	0.001	0.120	0.012	0.685	0.033	0.031	0.001
Trt × Cut	0.022	0.629	0.019	0.023	0.464	0.394	0.761	0.704	0.657	0.405	0.027
Stage × Trt × Cut	0.979	0.381	0.279	0.507	0.315	0.318	0.947	0.526	0.920	0.501	0.036

† Significant effects that need multiple means comparison are underlined.

Table 3. Means together with letter groupings of fresh herbage yield (FrHY), oil yield (OilY), (–)-menthone concentration (M-oneC), (+)-menthofuran concentration (M-furC), (–)-menthone yield (M-oneY), and (–)-menthol yield (M-olY) for the four treatment combinations of stage and cut at Verona, MS.

Stage	Cut	FrHY	OilY	M-oneC	M-furC	M-oneY	M-olY
		kg/ha		%		kg/ha	
Bud formation	Cut 1	37,080 a†	85.4 a	17.37 a	5.23 c	12.74 a	26.3 a
Bud formation	Cut 2	28,112 b	79.6 ab	6.96 c	12.42 a	5.68 c	24.9 a
Flowering	Cut 1	37,706 a	72.6 b	12.49 b	7.28 bc	8.50 b	26.2 a
Flowering	Cut 2	19,384 c	49.5 c	13.73 b	8.83 b	6.08 bc	17.7 b

† Means followed by the same letter are not significantly different at the 0.05 level.

Table 4. Means together with letter groupings of fresh herbage yield (FrHY), oil yield (OilY), and eucalyptol concentration (EucC) for the five treatment combinations of N treatment and cut at Verona, MS.

N Trt	Cut	FrHY	OilY	EucC
kg/ha		kg/ha		%
0	Cut 1	29,334 b†	57.8 b	5.72 a
0	Cut 2	18,557 d	50.1 b	4.71 cd
80	Cut 1	39,794 a	81.1 a	5.28 b
80	Cut 2	23,282 c	60.0 b	4.90 c
160	Cut 2	29,405 b	83.7 a	4.48 d

† Means followed by the same letter are not significantly different at the 0.05 level.

of N treatment and cut was significant on the yields of fresh herbage and oil, and on the concentration of eucalyptol, whereas the three-way stage × N treatment × cut interaction effect was significant on the yield of (+)-menthofuran (Table 2).

As expected, the fresh herbage yields were greater from the first cut than from the second cut, irrespective of stage (growth stage), (Table 3). The highest oil yields were obtained from the first cut at bud formation, and the lowest from the second cut at flowering due to the relatively short time for regrowth (Table 3). The average oil yield over the 2-yr period from peppermint harvested at bud formation was 165 kg/ha, whereas the oil yield from peppermint harvested at flowering was 122 kg/ha. These yields were higher than the average peppermint oil yields for the United States in 2008, which were reported to be 103 kg/ha (National Agricultural Statistics Service, 2009). However, oil yields in this study were calculated from relatively small plots and under optimized irrigation and weed control regimes, which might not be the case with many commercial peppermint operations. Still, the calculated oil yields in this study were obtained using relatively low N application rates, whereas commercial peppermint growers are often applying higher N rates (R. Lundy, Mint Industry Research Council, 2009, personal communication). (–)-Menthone concentration and yields were the highest, whereas (+)-menthofuran concentration was the lowest in the first cut at bud formation (Table 3). (–)-Menthol yields were lowest from the second cut at flowering. The above results suggest the possibility for presence of other factors (other than growth stage or cut) affecting peppermint oil composition.

The essential oil yields were greater from the first cut fertilized with 80 kg N/ha, and from the second cut fertilized with 160 kg N/ha and lower from the other N treatments (Table 4). The overall oil content, and the concentration and yield of eucalyptol of peppermint were higher at bud formation than at flowering (Table 5). However, (and as expected) the concentration of (–)-menthol was higher when the peppermint was harvested at flowering. In 2008, the average (–)-menthol concentration in the oil from the fertilized plots harvested at flowering was 43 to 46%, but (–)-menthol in the other treatments was below 37%. In 2007, (–)-menthol concentration in the oil was below 31%. The oil content of peppermint from the second cut was greater while the eucalyptol yield was greater from the first cut (Table 5).

The yields of eucalyptol, (–)-menthone, and (–)-menthol were greater in the higher N rates compared with the unfertilized

Table 5. Means together with letter groupings of oil fresh herbage (OilFH), eucalyptol concentration (EucC), (–)-menthol concentration (M-olC) and eucalyptol yield (EucY) for the two stages and the two cuts at Verona, MS.

Stage	OilFH	EucC	M-olC	EucY	Cut	OilFH	EucY
	%			kg/ha		%	kg/ha
Bud formation	0.26 a†	5.36 a	30.9 b	4.28 a	Cut 1	0.21 b	3.90 a
Flowering	0.22 b	4.66 b	35.1 a	2.84 b	Cut 2	0.27 a	3.20 b

† Means followed by the same letter are not significantly different at the 0.05 level.

Table 6. Means together with letter groupings of eucalyptol yield (EucY), (–)-menthone yield (M-oneY), and (–)-menthol yield (M-olY) for the three N treatments at Verona, MS.

N treatment	EucY	M-oneY	M-olY
	kg/ha		
0	3.09 b†	6.24 b	17.2 c
8	3.58 ab	9.05 a	22.8 b
160	4.03 a	9.69 a	32.0 a

† Means followed by the same letter are not significantly different at the 0.05 level.

control (Table 6). The yield of (+)-menthofuran in peppermint harvested at bud formation was greater in the fertilized second cut and lower in the first cut from the unfertilized treatments (Fig. 1). However, when peppermint was harvested at flowering stage, (+)-menthofuran yield from the first cut was significantly greater from the 80 kg N/ha fertilization regime than from the unfertilized control. The yield from the second cut was the highest at the 160 kg N/ha rate, and increasing N from 0 to 80 kg N/ha did not result in increased yield (Fig. 1).

There were many significant correlations between soil properties and the measured plant responses (Table 7). Fresh herbage and oil yields from the second cut were positively correlated with soil organic matter (OM). Oil yields were also positively correlated with soil available Mg but negatively with soil pH. Eucalyptol concentration was negatively correlated with soil available K. (–)-Menthol concentration in the oil from the first cuts was positively correlated with OM, but negatively correlated with soil pH. (–)-Menthol concentration from the second cut at flowering was negatively correlated with soil available K. In most instances, the (–)-menthol yields as per area basis was positively correlated with soil OM and negatively correlated with soil pH. The above results suggest that high OM and slightly acidic pH (compared with more acidic) would improve the yields of fresh herbage, essential oil and (–)-menthol, and would increase (–)-menthol concentration in the essential oil. As N application rates increased, soil pH decreased (Table 1), indicating a possible need for lime application.

DISCUSSION

Generally, our results agree with previous reports on peppermint response to increased N application rates in other parts of the world (Mitchell and Farris, 1996; Jeliazkova et al., 1999; Zheljazkov and Margina, 1996; Zheljazkov, 1998) or in the northwestern United States (Mitchell and Farris, 1996). For example, Mitchell and Farris (1996) obtained the highest yields of peppermint at N application rate of 280 kg/ha, and Jeliazkova et al. (1999) observed the highest biomass yields at N at 306 or even up to 530 kg/ha.

The lower yields of the second cuts are a function of shorter vegetation period (from mid-July until beginning of October, compared with the April until July period), higher temperatures during early plant growth and development of the second cut, shorter days in the second half of the season, and increased plant density resulting in totally different crop canopy architecture (Zheljazkov, 1998). The increased plant density of the regrowth after the first cut results in decreased branching and a different ratio of old, mature to young, or immature leaves. Old leaves contain more (–)-menthol

Table 7. Correlations between soil characteristics (soil) and fresh herbage yield (FrHY), oil fresh herbage (OilFH), oil yield (OilY), eucalyptol concentration (EucC), % (–)-menthone concentration (M-oneC), (–)-menthol concentration (M-olC), (+)-menthofuran concentration (M-furC), eucalyptol yield (EucY), (–)-menthone yield (M-oneY), (–)-menthol yield (M-olY) and (+)-menthofuran yield (M-furY) measured at Verona, MS. Only correlations significantly different from zero at the 0.05 level are shown.

Stage	Cut	Soil	FrHY	OilFH	OilY	EucC	M-oneC	M-olC	M-furC	EucY	M-oneY	M-olY	M-furY
Flower	1	OM†	–‡	–0.75	–	–	–	0.89	0.71	–	–	–	–
Flower	2	OM	0.65	–	0.67	–	–	–	–	0.67	–	0.65	–
Bud	1	OM	–	–	–	–	–	0.81	–	0.70	–	0.80	0.68
Bud	2	OM	0.72	–	0.82	–	–	–	–	0.75	0.67	0.77	0.67
Flower	1	pH	–	0.73	–	–	–	–0.83	-0.71	–	–	–	–
Bud	1	pH	–	–	–	–	–	–0.67	–	–0.65	–	–0.73	–
Bud	2	pH	–	–	–0.72	–	–	–	–	–0.66	–	–0.68	–0.68

† OM, organic matter.

‡ A dash indicates an insignificant correlation.

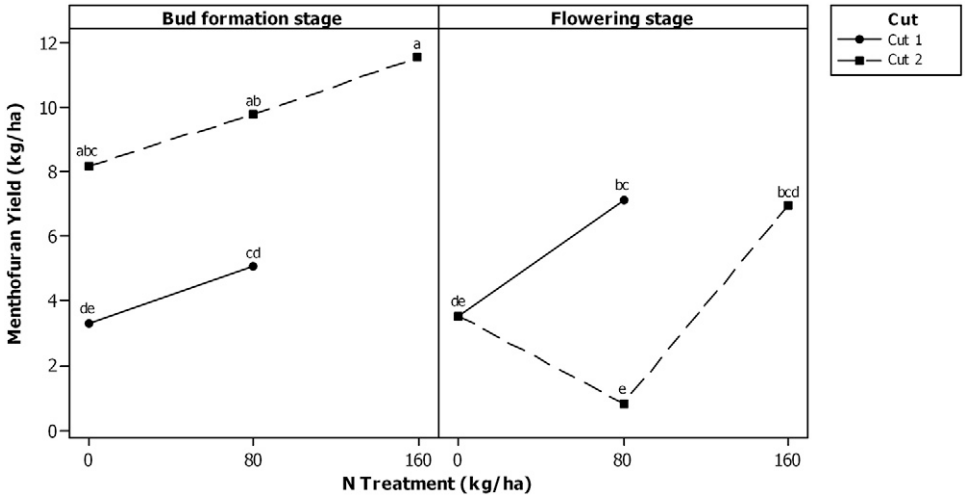

Fig. 1. Interaction plot of (+)-Menthofuran yield (kg/ha) versus N rate for the two cuts and the two phases (bud form and flowering) at the Verona, MS, location. Means sharing the same letter are not significantly different at the 0.05 level of significance.

(Topalov, 1989), hence, it is expected that (–)-menthol concentration in the first cut should be greater than in the second cut, as was the case in this study. Our results confirm prior reports on significant effects of harvesting date on (–)-menthol (Chalchat and Michet, 1997; Rohloff et al., 2005). (–)-Menthone and (+)-menthofuran concentrations in this study were different between the two cuts, confirming previous reports (Chalchat and Michet, 1997; Chalchat et al., 1997; Court et al., 1993; Topalov and Zheljazkov, 1991). Higher (–)-menthone concentration in the plants harvested at bud formation compared with plants harvested at flowering suggest a relatively early harvest (Burbott and Loomis, 1969; Court et al., 1993; Clark and Menary, 2006; Chalchat et al., 1997; Mustjatse, 1985; Topalov and Zheljazkov, 1991). Presumably, if the harvest was delayed for a week or two, most of the (–)-menthone would have been converted into (–)-menthol, thus increasing the overall quality of the essential oil. Prior research with Mitcham type peppermint in central France indicated an increase in (–)-menthol concentration from 20% in early July to as high as 50% in mid-August (Chalchat and Michet, 1997). However, caution is advised when comparing the results from this study with literature data; in earlier reports, (–)-menthol was reported based on GC flame ionization detector integration data as an area percentage, which is relative data, whereas we did an absolute quantification of (–)-menthol and the other oil constituents in this study by generating separate calibration curves for each analyte. Also, highest oil yields in northern California (at 40.5° N lat) were obtained at harvesting dates 29 July and 13 August (Marcum and Hanson, 2006). (–)-Menthone is a precursor for (–)-menthol, and (+)-menthofuran is a side product of (–)-menthone biosynthesis from (+)-pulegone (Burbott and Loomis, 1967; Mahmoud and Croteau, 2003; Rios-Estepa et al., 2008). Hence, the higher concentration of (+)-menthofuran in the second cut at bud formation is due to the fact that (–)-menthone has been spent, whereas the lower concentration of (+)-menthofuran in the second cut at the same stage is due to a higher concentration of (–)-menthone, which would have been converted into (–)-menthol and (+)-menthofuran. The measured responses indicated the presence of other factors that modify peppermint productivity and oil composition. Further research is needed to identify and quantify the effect of these other factors.

Our results suggest peppermint can be grown under the climate of Mississippi and provides two cuts (harvests) per growing season. Overall, N application increased biomass and oil yields. The oil yields obtained in this study were comparable or more often greater than the average peppermint oil yields for the United States in 2008. Our results suggest the first cut under the Mississippi climate should be delayed until the end of July to promote greater accumulation of (–)-menthol and decreased concentration of (–)-menthone in the essential oil.

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