The Yield and Composition of Dill Essential Oil in Relation to N Application, Season of Cultivation and Stage of Harvest

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Abstract

The effect of nitrogen (N) fertilization, season of cultivation and stage of harvest on the growth, foliar essential oil composition and yield of dill (*Anethum graveolens* L. cv. Ducat) was studied in two consecutive crops. Seeds were sown in October (autumn-winter crop) and January (spring crop) and the plants transplanted to a substrate of peat and perlite (1:1 v/v) 30 and 39 days later, respectively. Nitrogen (NH_4NO_3) was applied weekly at four levels (50, 150, 300, 450ppm) in a completely randomized design. The plants were harvested at 158 (autumn-winter crop) and 83 (spring crop) days after sowing. The plant foliage was weighed and the essential oils were isolated by hydro-distillation and analyzed by GC/MS. The foliage fresh weight per plant progressively increased with increasing N up to 450 ppm in the autumn/winter, but was maximal at 300 ppm N in the spring. The essential oil concentration within the foliage was low and was unaffected by N application in the autumn/winter, but was significantly higher at 300 ppm N than at other N levels in the spring. The main components of the foliar essential oil were α-phellandrene, β-phellandrene, dill ether, α-pinene, β-pinene, α-thujene, myrcene, and π-cymene. In both crops α-phellandrene was the principal constituent. In the spring the concentration of all the essential oil constituents identified (except π-cymene) was highest at 300 ppm N, whereas in the autumn/winter crop the concentrations of α-phellandrene, β-phellandrene and dill ether were also higher at 300 ppm N, but the other constituents were not affected by higher N. It is concluded that for the autumn/winter crop 450 ppm N is optimal for biomass and foliar oil yield (biomass x oil concentration) whereas in the spring 300 ppm N is recommended.

INTRODUCTION

Dill (*Anethum graveolens* L.) is an annual aromatic herb of the Apiaceae (Umbelliferae) family, grown widely throughout Europe, America and Asia for use as a fresh herb and for the production of essential oil, which is extensively used by the food industry for flavoring foods and beverages (Clark and Menary, 1984).

Essential oils (also known as volatile or ethereal oils, or simply as the oil of the plant from which they are extracted) are concentrated, hydrophobic liquids that contain volatile aroma compounds. The oil is "essential" in the sense that it carries a scent or essence that is distinctive of the plant (Sellar, 2001). The leaves of dill contain 0.05–0.35% essential oil the concentration of which increases during wilting (Melchior and Kastner, 1974). The main constituent of dill foliage essential oil is phellandrene (47% α-phellandrene and 9.4% β-phellandrene just prior to bud formation), but decreasing during

flowering (Huopalahti and Linko, 1983). Similarly, the foliage content of limonene, pinene and dill ether changes with plant maturation (Callan et al., 2007). In contrast, dill fruit and seeds have an essential oil content of 3-4% the principal constituent of which is carvone (11-13 mg g^{-1}) (Bailer et al., 2001).

Wander and Bouwmeester (1998) reported that dill biomass, seed and carvone yield increased with increasing nitrogen (N) fertilization. Moreover, Singh et al. (1987) concluded that the oil content of the whole plant was higher at the time of seed filling and related to N application. In the present paper, we extend this work by examining the effect of growth factors (N-fertilization, season of cultivation and stage of harvest) on the yield and composition of essential oil derived from the foliage.

MATERIALS AND METHODS

Plant material and cultivation

The experiments were carried out at the Agricultural University of Athens. Seeds of dill (*Anethum graveolens* L. cv. Ducat) were sown in trays containing commercial peat compost (Klasmann-Deilmann KTS 2) in October 2007 (crop 1) and January 2008 (crop 2) and placed in an unheated greenhouse. At the stage of 3-4 true leaves (30 and 39 days after sowing respectively) the plants were transplanted to 11 L pots containing a mixture of peat and perlite (1:1 v/v) enriched with 150g potassium monophosphate, 40g potassium sulphate, 20g magnesium sulphate, 10g trace elements (Nutrileaf) and 300g marble per m^3. Each pot contained two plants. The pots were spaced at 50 x 50 cm in an unheated, plastic-covered greenhouse. Nitrogen (NH_4NO_3) was applied weekly during irrigation at four levels (50, 150, 300 and 450 ppm) from one week after transplantation. Each N level consisted of four replicates of 10 plants in fully randomized blocks. The duration of the crop was 158 (autumn) and 83 (spring) days from sowing, with harvest being performed just prior to flowering. At harvest, plants were cut 1cm above the soil and any old, senesced leaves were removed. Plant height, leaf number and foliage fresh weight were recorded.

Extraction and analysis

Immediately after harvest, leaves were placed in sealed, airtight plastic food-bags and stored at -20°C until essential oil extraction. Essential oil yield was measured with the use of hydro-distillation using a Clevenger apparatus (Petropoulos et al., 2009). Samples of frozen leaves (100g) were boiled in distilled water in 1000 ml round flasks heated by thermo-mantles (Barnstead Electrothermal EMV 1000), for 3 hours. The organic phase (containing the essential oil) was put into 30 ml glass bottles, sealed with parafilm and stored at -20°C until analyzed. Before gas chromatography analysis the essential oil was separated from organic phase with the use of a 50 ml extraction funnel and diethyl ether as the extractant.

Essential oils were analyzed by gas chromatography (GC) using a Hewlett Packard 5890 II gas chromatograph equipped with a FID detector and a HP-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 μm). Injector and detector temperatures were set at 220°C and 290°C, respectively. The column temperature was initially kept at 50° C for 5 min, then gradually increased to 220°C at a rate of 3°C/min and maintained for 5 min. The flow rate of helium was 1 ml/min. Quantitative data were obtained electronically from FID area percent data without the use of correction factors.

Each extraction was replicated three times and the compound percentages are the means of the three replicates.

Gas Chromatography/Mass Spectrometry (GC/MS) analysis was performed under the same conditions as GC (column, oven temperature, flow rate of the carrier gas) using a Hewlett Packard 5890 II GC equipped with a Hewlett Packard 5972 mass selective detector in the electron impact mode (70 eV). Injector and MS transfer line temperatures were set at 220°C and 290°C, respectively. Tentative identification of the compounds was based on the comparison of their relative retention time and mass spectra with those of pure standards and the NBS75K library data of the GC/MS system and literature data (Adams, 2001).

Statistical analysis

The results were subjected to analysis of variance (ANOVA) and means compared by the application of Duncan's multiple range test using the statistical package Statgraphics plus 5.1.

RESULTS AND DISCUSSION

Plant biomass

The foliage fresh weight per plant progressively increased with increasing nitrogen application in the autumn (Table 1), whereas in the spring the highest yield was recorded at 300 ppm N. When calculated on an area basis, the highest yield (1556 kg ha^{-1}) was observed at 450 ppm N in the autumn/winter crop, while the highest yield of the spring crop was 1197 kg ha^{-1} at 300 ppm N (Table 1). Except at the lowest N level (50 ppm) foliage yield was higher in the autumn/winter than in the spring, reflecting the longer growth period of the former.

Foliar oil concentration

The essential oil concentration within the foliage was low (less than 0.3 ml 100 g fresh $weight^{-1}$, i.e. less than 20ml ha^{-1}). Oil concentration was not affected by N concentration in the autumn/winter, but was significantly higher at 300ppm N in the spring crop. At 450 ppm N, oil yield was significantly higher in the autumn/winter than in the spring (Table 2).

The composition of the foliar oil

The principal constituents of the foliar essential oil were α-phellandrene, β-phellandrene, dill ether and π-cymene, followed by α-pinene, β-pinene, α-thujene and myrcene in smaller quantities (Tables 3 and 4). In both crops, α-phellandrene was the principal constituent, with the highest concentration (133-168 mg 100g fresh $weight^{-1}$) being recorded at 300 ppm N. The concentration of β-phellandrene was also high at 300 ppm N (11-15 mg 100g fresh $weight^{-1}$), as was that of dill ether (17-20 mg 100g fresh $weight^{-1}$). No effect of N application on the concentrations of the other essential oil components was observed in the autumn/winter crop (Table 3), but in the spring the concentrations of all components were higher at 300 ppm N, except π-cymene (Table 4).

Discussion

Our results indicate that the biomass of dill (cv. Ducat) increased with N application, as reported for cv. Budakalaszi cultivated for seed oil (Hornok, 1980; 1983)

and for an unstated dill cultivar grown hydroponically (Udagawa, 1995). The efficacy of N application, however, varied with season, a higher N level being indicated for the autumn/winter than for the spring (Table 1).

An effect of season was also observed on oil yield. Although the oil yield of dill foliage was low, it was nevertheless higher during the autumn/winter than in the spring at 450 ppm N. In the spring the oil concentration was significantly higher at 300 ppm N than at the other N levels applied, whereas in the autumn/winter no effect of N level on oil concentration was found (Table 2). Elsewhere, the oil concentration of the foliage of hydroponically grown dill was found to decrease at a nutrient level of 3.6 mS cm^{-1} (Udagawa, 1995), whereas that of the seed was apparently not affected by N application (Hornok, 1980).

The composition of the foliar oil was affected by N application to a greater degree in the spring (Table 3) than in the autumn/winter (Table 4), indicating a significant influence of season. Udagawa (1995) reported that both the concentration of α-phellandrene and the total leaf oil concentration of dill plants grown hydroponically decreased when the nutrient concentration rose from 2.4 to 3.6 mS cm^{-1}; however apart from cis-3-hexenol (the concentration of which was unaffected by nutrient level) no other compounds were identified in that study. Although we did not detect carvone in the foliar oil of dill cv. Dukat, it has been detected in other studies (Strehle et al., 2006), but only at flowering (Huopalahti and Linko, 1983). Carvone is the principal component of the essential oil of dill seed and the relative concentration varies with the stage of flowering and seed development (Callan et al., 2006). In their experiments, Callan et al. (2007) harvested plants after flower formation; however, when harvest was carried out prior to flowering (as in our experiments) α-phellandrene was the major constituent of the oil and carvone was not present.

CONCLUSION

Based on the nitrogen rates used in this study, it is concluded that for a long-cycle autumn/winter crop (158 days) 450 ppm N is optimal for biomass and foliar oil yield (biomass x oil concentration) whereas in the shorter spring crop (83 days duration) 300 ppm N is optimum.

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Table 1: The effect of nitrogen fertilization on the fresh weight of foliage biomass.

Crop	N	Weight of foliage biomass	
	(ppm)	g plant^{-1}	kg ha^{-1}
Autumn-Winter	50	58.19 d (b)	484.92 d (b)
	150	133.39 c (a)	1111.58 c (a)
	300	162.48 b (a)	1354.00 b (a)
	450	186.73 a (a)	1556.08 a (a)
Spring	50	102.51 b (a)	854.25 b (a)
	150	110.12 b (b)	917.67 b (b)
	300	143.74 a (b)	1197.83 a (b)
	450	111.69 b (b)	930.75 b (b)

Means for each crop within each column followed by the same letter are not significantly different at p=0.05. Means for each N level followed by a different letter in parenthesis are significantly different (p=0.05) between the two crops (autumn-winter and spring)

Table 2: The effect of nitrogen fertilization on dill foliar oil yield.

	ml oil 100 g fresh weight^{-1}		ml oil ha^{-1}	
N (ppm)	Autumn-winter crop	Spring crop	Autumn-winter crop	Spring crop
50	nd	0.13 b	nd	9.86 b
150	0.21 a	0.14 b	15.93 a	10.62 b
300	0.20 a	0.19 a	15.17 a	14.41 a
450	0.26 a *	0.11 b *	19.72 a *	8.35 b *

Means within each column followed by the same letter are not significantly different at p=0.05. Means followed by an asterisk (*) differ significantly (p=0.05) between the two crops (autumn-winter and spring). Nd = not determined.

Table 3: The principal constituents of dill herb oil (mg 100 g fresh weight^{-1}) of the autumn-winter crop in relation to N application.

			N application rate			
A/A	Oil composition	R.T.	50 ppm	150 ppm	300 ppm	450 ppm
1	α- thujene	8.1	nd	0.14 a	0.19 a	0.12 a
2	α - pinene	8.37	nd	1.14 a	1.99 a	0.99 a
3	β-pinene	9.9	nd	0.04 a	0.05 a	0.03 a
4	myrcene	10.4	nd	0.61 a	0.57 a	0.50 a
5	α phellandrene	11.5	nd	21.14 b	168.29 a	0.45 c
6	π-cymene	11.9	nd	19.85 b	5.86 c	26.95 a
7	β-phellandrene	12.3	nd	4.42 a	15.07 a	2.87 a
8	dill ether	19.5	nd	8.11 a	20.26 a	6.17 a

Means within each row followed by the same letter are not significantly different at *P*=0.05. nd = not determined. R.T. = retention time.

Table 4: The principal constituents of dill herb oil (mg 100 g fresh weight^{-1}) of the spring crop in relation to N application.

			N application rate			
A/A	Oil composition	R.T.	50 ppm	150 ppm	300 ppm	450 ppm
1	α- thujene	8.1	0.06 b	0.08 b	0.16 a	0.05 b
2	α - pinene	8.37	0.44 b	0.56 b	1.49 a	0.22 b
3	β-pinene	9.9	0.022 b	0.03 b	0.06 a	0 c
4	myrcene	10.4	0.3 b	0.29 b	0.60 a	0.16 b
5	α-phellandrene	11.5	32.96 bc	49.00 b	133.04 a	12.49 c
6	π-cymene	11.9	0.48 a	4.32 a	0.07 a	0.37 a
7	β-phellandrene	12.3	2.66 b	1.89 b	11.27 a	1.19 b
8	dill ether	19.5	4.77 b	6.77 b	17.64 a	2.35 c

Means within each row followed by the same letter are not significantly different at *P*=0.05. nd = not determined. R.T. = retention time.