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Nitrogen Form Alters Sweet Basil Growth and Essential Oil Content and Composition

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Abstract. **Sweet basil (*Ocimum basilicum* L.) plants were grown, until flower buds became visible, in a peat-lite mix and watered daily with a complete nutrient solution with 10 mM N as either NO_3^- or NH_4^+. Ammonium decreased plant height and stem plus petiole dry weight. Leaf blade dry weight was not affected by N form. However, the essential oil content was decreased by 28% with NH_4^+, thereby decreasing the essential oil yield per plant. Although NH_4^+ decreased the content (nl·g^{-1} leaf blade dry weight) of linalool and eugenol, their percentage was not altered. Therefore, the changes in total yield of these individual constituents was simply a reflection of less total extractable essential oil. The total amount of the other major constituents in sweet basil, 1,8-cineole, methyl chavicol, and total sesquiterpenes was not affected significantly. While N form did not alter the percentage of monoterpenes and aromatic polypropanoides, NH_4^+-N increased the total sesquiterpene percentage. Nitrogen form altered the essential oil content and composition of sweet basil and, therefore, should be considered in nutritional studies with aromatic plants.**

The major inorganic forms of N absorbed by plants are NO_3^- and NH_4^+. Both forms of N can be present naturally in the soil solution, NH_4^+ from decay of organic matter and NO_3^- from nitrification of NH_4^+. While both forms of N can also be applied, NH_4^+-N is less costly and, therefore, the form most often applied. Ammonium is rapidly nitrified to NO_3^- under most soil conditions (Haynes, 1986a). However, there are intervals of time in which the NH_4^+ form of N can constitute the major portion of N available to the plant. These periods of high NH_4^+ availability are associated with: 1) fertilizer application techniques such as banding of Cl^- containing NH_4^+-N fertilizers (Roseberg et al., 1986), side dressing or banding urea or other NH_4^+-N fertilizers, and use of nitrification inhibitors (Hageman, 1984); and 2) soil conditions such as acid soils, including acid peat-muck soils (Haynes, 1986b), low soil temperatures (Haynes, 1986a), and poor aeration (Haynes, 1986a; Wells and Turner, 1984), the latter two reducing the rate of nitrification. Since many herbs, including sweet basil, can be cut several times over the growing season, they often are side-dressed with NH_4^+-N fertilizers.

Ammonium is assimilated as it is absorbed or produced from reduction of NO_3^-. Unlike NO_3^-, which can be stored and is only reduced if sufficient carbohydrates for assimilation are available, for NH_4^+, plants generally prevent toxic accumulation of free NH_4^+ by assimilation (Haynes, 1986b). Since the detoxification of NH_4^+ places an immediate demand on the plant for carbon skeletons (Givan, 1979), we hypothesized that carbon skeletons for secondary metabolite production may be reduced and, therefore, alter both the content and composition of secondary metabolites in sweet basil.

Seeds of sweet basil were sown in 15-cm standard pots filled with 1.5 liters of a peat-lite mix. The experiment was carried out under greenhouse conditions (day maximum, 35C; night minimum, 18C) from early April through mid-May. The seedlings were thinned to one plant per pot. Subsequent to full cotyledon expansion, the seedlings were watered daily with a nutrient solution made up as follows (in mM): 3 $Ca(NO_3)_2$ and 4 KNO_3 for NO_3^--N, [5 $(NH_4)_2SO_4$, 3 $CaCl_2$, and 2 K_2SO_4 for NH_4^+-N] plus 1 KH_2PO_4, 2 $MgSO_4$, and micronutrients (mg·liter^{-1}): Fe as $FeSO_4$ (2.5) and DTPA (2.5), B as H_3BO_3 (0.5), Mn as $MnSO_4$ (1.0), Zn as $ZnSO_4$ (0.05), Cu as $CuSO_4$ (0.02), and Mo as $(NH_4)_6Mo_7O_{24}$ (0.01). Because the soil solution pH increases or decreases, respectively, with absorption of NO_3^- and NH_4^+ from solution, the NO_3^--N solution was adjusted to pH 5.5 and the NH_4^+-N solution to pH 6.5. This procedure, plus the buffer capacity of the peat-lite mix, precluded the pH from falling below 5.5 or rising above 6.5.

Twenty basil plants were included in each of the two N form treatments (NO_3^--N and NH_4^+-N). Plants were arranged in a randomized complete block design with two blocks and 10 replicates of each N form treatment within a block. Plant weight and height measurements were recorded on individual plants, but the essential oils were distilled from the leaf blades of 10 plants bulked from each treatment. When the basil plants reached the

Table 1. Influence of N form on sweet basil weight and height.

Nitrogen form	Leaf blade wt (g)	Stem plus petiole wt (g)	Stem ht (cm)
NO_3-N	5.37	2.89	39.6
NH_4-N	5.10	2.05	32.5
Significance	NS	**	**

NS,**Nonsignificant or significant F test at $P = 0.01$, respectively.

Table 2. Influence of N form on the essential oil content of sweet basil.

Nitrogen form	Total essential oil content (µl·g^{-1} leaf blade dry wt)[z]	Content of the major essential oil constituents (nl·g^{-1} leaf blade dry wt)				
		1,8-Cineole	Linalool	Methyl chavicol	Eugenol	Sesquiterpenes
NO_3-N	4.38	338	1700	1820	4.73	202
NH_4-N	3.14	224	1300	1280	1.57	273
Significance	***	NS	*	NS	**	NS

[z]To convert µl·g^{-1} to percent, multiply essential oil content by 0.1.
NS,*,**,***Nonsignificant or significant F test at $P = 0.1$, 0.05, or 0.01, respectively.

Table 3. Influence of N form on the major essential oil constituents in sweet basil.

Nitrogen form	Essential oil composition (% total essential oil)[z]				
	1,8-Cineole	Linalool	Methyl chavicol	Eugenol	Sesquiterpenes
NO_3-N	7.73	38.7	41.6	0.11	4.61
NH_4-N	7.05	41.0	41.2	0.05	8.66
Significance	NS	NS	NS	NS	*

[z]Data arcsin square root of percent transformation for statistical analysis, actual mean percentages presented.
NS,*Nonsignificant or significant F test at $P = 0.10$, respectively.

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seven-leaf stage (flower buds just visible), the experiment was terminated. Plant height was measured from the cotyledonary node to the shoot apex. The stem was severed from the root system at the cotyledonary node and leaf blades separated from petioles. The leaf blades and the stem plus petioles were dried in a forced-air oven at 40C so that no volatiles were lost, and dry weight was recorded. Essential oil analysis was performed on the leaf blade tissue only.

Distillation. Leaf blades from 10 plants were bulked (≈50 g dry weight), crushed into small pieces, and placed into a 1000-ml round-bottomed boiling flask with 500 ml of distilled, deionized water. The essential oil was extracted by hydrodistillation with a modified clevenger trap (ASTA, 1968). The distillation period was 1 hr and the essential oil content was determined on a volume per gram of leaf blade dry weight basis. The essential oil samples were stored in sealed silica vials at 2C in the dark.

Gas chromatography. Essential oil samples from each of the distillations were analyzed separately and the relative peak area for individual constituents averaged (unpublished data). Identification of essential oil constituents was based on retention time and the relative percentage determined with a Varian 3700 gas chromatograph equipped with FID and a Varian electronic 4270 integrator. A fused silica capillary column (12 m × 0.22 mm, i.d.) with a OV 101 (Varian, polydimethylsiloxane) bonded phase was used. Direct injection of 0.5 μl of essential oil samples with N_2 as a carrier gas (100:1 split vent ratio) and an oven temperature held isothermal at 80C for 2 min and then programmed to increase at 3C per min to 180C gave complete elution of all peaks (sensitivity 10^{-10}). The injector and detector temperatures were 180C and 300C, respectively.

Leaf blade dry weight was not affected by N form (Table 1). However, both plant height and stem plus petiole weight were reduced by NH_4^+. We observed that the reduction in plant height was associated with shorter internodes. Because NH_4^+ can increase water stress due to a decrease in root hydraulic conductivity (Adler et al., 1987), the NH_4^+ plants may have undergone mild water stress that could have decreased cell elongation.

Ammonium decreased the essential oil content ($\mu l \cdot g^{-1}$ leaf blade dry weight) by 28% (Table 2). Since leaf blade dry weight was not affected by N form, the essential oil yield per plant was decreased by NH_4^+. In poppies, although morphine content per unit capsule or upper stalk dry weight was not affected by N form, NH_4^+ decreased total morphine yield because capsule and upper stalk dry weight were decreased (Costes et al., 1976). In mint, even though the percentage of essential oil in the leaves increased with NH_4^+, the essential oil content per plant was higher with NO_3^- because NH_4^+ decreased plant growth (Singh and Singh, 1978). This difference in effect of N form on growth between the poppy and mint studies and our study on sweet basil may be attributed to the use of sand compared to a peat-lite mix, respectively. Magalhaes and Wilcox (1984b) observed with tomatoes that NH_4^+-N decreased growth when the medium was sand even when the pH was controlled, but not in peat.

Although NH_4^+ decreased the content of linalool and eugenol (Table 2), their percentage was not altered (Table 3). Therefore, the changes in total yield of these individual constituents was simply a reflection of less total extractable essential oil. The total amount of the other major constituents in sweet basil, 1,8-cineole, methyl chavicol, and total sesquiterpenes was not significantly affected (Table 2). While N form did not alter the percentage of monoterpenes and aromatic polypropanoides, NH_4^+ increased the total sesquiterpene percentage (Table 3).

This study does not provide the definitive response of sweet basil to N form because that response is environmentally dependent (Adler et al., 1987; Magalhaes and Wilcox, 1984a). It does demonstrate, however, that N form can alter the essential oil content and composition of sweet basil and, therefore, should be considered in nutritional studies with aromatic plants.

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