Less is More? Basil Growth and Flowering under Below-Recommended Nitrogen Fertilization Rates[1]

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Abstract

Basil (*Ocimum basilicum* L.) is an herb commonly used as a flavoring additive for food. Its cultivation requires the selection of an adequate fertilization level that results in the greatest crop yield. This study evaluated the effects of four sub-recommended nitrogen fertilization levels [0, 21, 48 and 91 ppm N (mg·L^{-1} nitrogen)] on basil (cv. 'Dark Opal') growth, flower production, time of flowering, and leaf and stem N and C contents. The nitrogen fertilization level closest to the optimal rate (91 ppm N) produced plants with greater canopy weights, and more plants flowered than at the lower fertilization levels. However, plants fertilized at 21 ppm N were 14.7% taller and had about the same number of fully expanded leaves and branches, but with more flower clusters, than plants fertilized at 91 ppm N at 56 days after the initiation of the fertilization treatments. Growers may benefit from a lower-than-recommended nitrogen fertilization level (21 ppm N), which produced plants with more flowering clusters per flower head and with just as many leaves as higher nitrogen fertilization levels.

Index words: *Ocimum basilicum*, greenhouse, vegetable, herb.

Chemicals used in this study: calcium nitrate; ammonium nitrate; urea; P_2O_5 (liquid phosphorus solution); potassium acetate (ACE 29 0-0-29); dolomitic lime; minor elements solution (energy turf and ornamental micronutrients).

Species used in this study: basil, *Ocimum basilicum* L., cv. 'Dark Opal'.

Significance to the Horticulture Industry

Greenhouse production of vegetables and herbs is becoming an increasingly prominent and profitable segment of the green industry. Basil is one of the most commonly produced herbs. Efficient production of basil seedlings and finished plants requires careful balance of nutrition to achieve enhanced growth and plant mass, while reducing leaching or runoff of excess fertilizer. We documented that plants fertilized at closest-to-optimal rate (91 ppm N) produced numerically greater canopy weights, and more plants flowered earlier than other fertilization rates (0, 21 and 48 ppm N). Plants fertilized at about 20% of the optimal rate (21 ppm N) grew taller, had similar numbers of leaves and branches, and had more flower clusters than plants fertilized at 91 ppm N at the end of the treatment. Results of this study suggest that 'Dark Opal' basil can be grown optimally and efficiently in a greenhouse at nitrogen fertilization levels that are as low as 21 and 48 ppm N. By reducing the amount of nitrogen, growers would avoid the added expense of applying more-than-sufficient nitrogen fertilizer. This would decrease fertilizer input costs and increase overall profit. Growers aiming to cultivate basil with more leaves per plant and earlier flowering for maximum seed production may consider fertilizing basil with a nitrogen level within or closest to the recommended range. However, if growers choose to fertilize a basil crop with a lower nitrogen fertilization rate, they would still produce leafy plants, plus these plants will have more flowering nodes per flower head, providing for more seed produced per plant.

Introduction

Basil is used for culinary, medicinal and cosmetic products, though it is most commonly consumed as an herb in food (Hamasaki et al. 1994, Putievsky and Galambosi 1999, Roghaye et al. 2012, Sifola and Barbieri 2006). Leaves and seeds are the main products, where leaves are sold in fresh or dry form for cooking and seed are collected for sale on agricultural commodity markets (Hamasaki et al. 1994, Putievsky and Galambosi 1999, Roghaye et al. 2012). There are many cultivars of basil, varying in color and scent, and some are prized as ornamentals (Putievsky and Galambosi 1999).

Native to central Asia, basil is well adapted to warm climates that provide plenty of light (Hamasaki et al. 1994, Putievsky and Galambosi 1999). Although basil can grow in soils with pH levels from 4.3 to 8.2, the optimum is 6.0 to 7.5 (Hamasaki et al. 1994, Puteivsky and Galambosi 1999). Basil is sensitive to water stress and requires adequate moisture for development (Putievsky and Galambosi 1999). Depending on cultivar, basil plants typically reach 50 to 60 cm in height (Sharafzadeh et al. 2011). About 30 to 35 days after planting, the leaves from basil plants are ready for harvest (Hamasaki et al. 1994).

Nitrogen fertilization is one of the most important factors that influences plant quality and abundance (Erisman 2011). The recommended fertilization rate for greenhouse-grown basil is 100 to 150 ppm N per irrigation, and the sources of nitrogen (N), potassium (K) and phosphorus (P) should be balanced (Hamasaki et al. 1994, Hamrick 2003, Putievsky and Galambosi 1999). Higher nitrogen fertilization levels produce greater biomass, more leaves, and higher leaf N content (Bufalo et al. 2015, Roghaye et al. 2012, Sifola and Barbieri 2006), whereas lower fertilization levels increase essential oil production in basil (Zheljazkov et al. 2008).

As the use of manufactured fertilizer has increased over the last several decades, nutrient pollution of water bodies and groundwater has become an ever-increasing problem globally (Carpenter et al. 1998, Constantin et al. 2010). In the United States, farming is the most significant anthropogenic activity contributing to nitrogen pollution (Ribaudo 2011, Ribaudo et al. 2011). Researchers from the United States Department

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of Agriculture, Economic Research Service (USDA-ERS) documented that, of total emissions, agriculturally derived nitrogen sources contributed 73% of the nitrous oxide, 84% of the ammonium and 54% of the nitrate emissions (Ribaudo 2011, Ribaudo et al. 2011 and references therein). Nutrients from manufactured fertilizers and manures can leach into groundwater and enter surface water through runoff (Lehmann and Schroth 2003). Nitrogen and phosphorus are the most significant nutrient sources leading to eutrophication of water bodies (Carpenter et al. 1998, EPA 2016a). In an effort to reduce nutrient pollution, the Environmental Protection Agency (EPA) develops standards for environmental quality (EPA 2016b) and the USDA established the Environmental Quality Incentives Program (EQIP) (USDA 2014). Both agencies encourage agricultural operators to actively manage and improve their fertilizer application methods, apply fertilizers at a time when crops would benefit the most, and apply only the amount of nutrients needed for optimal crop production and yield (EPA 2016c, Ribaudo 2011, Ribaudo et al. 2011).

The goal of this study was to identify a nitrogen fertilization level below that typically recommended (100 to 150 ppm N) that would produce leafy basil plants of suitable size with higher canopy weights and more flower clusters. The effects of four sub-recommended or near-recommended levels of nitrogen fertilization (0, 21, 48, and 91 ppm N) were evaluated on basil growth and development, flowering, and plant tissue N and C contents. The results of this study will help to establish a practical and economical fertilization program for the production of basil in greenhouses for fresh markets.

Materials and Methods

The study was conducted from May to August, 2014, in a ventilated and naturally lighted greenhouse at Clemson University's Pee Dee Research and Education Center (PDREC), Florence, SC. Temperature and relative humidity were recorded with data loggers (HOBO UX 100 Temp/RH 3.5% Data Logger; Onset Computer Corporation, Bourne, MA). The ambient temperature and relative humidity (means ± standard error) from July 8 to August 22 were 28.0 ± 0.1 C (82 F) (95% CI = 28.3–28.5 C) and 79.4 ± 0.2% (95% CI = 79.1–79.8%), respectively.

Potted plants from the four nitrogen fertilization levels were randomly selected for determining pH of the leachate on June 21 (n = 32), June 25 (n = 24), June 27 (n = 27), July 19 (n = 8), July 23 (n = 12), and July 30 (n = 12) with a calibrated pH meter (pH 100, ExStik; Extech Instruments Corporation, Nahua, NH). The average pH across all nitrogen levels were 6.31 ± 0.05 (P = 0.4348) on June 21, 5.93 ± 0.07 (P = 0.8359) on June 25, 5.55 ± 0.07 (P = 0.0052) on June 27, 6.51 ± 0.07 (P = 0.1048) on July 19, 6.67 ± 0.10 (P = 0.0043) on July 23, and 6.77 ± 0.15 (P = 0.4571) on 30 July 30 and did not differ significantly among N treatments. Pots receiving the higher nitrogen fertilization levels tended to have a lower pH. The average pH across all measurement dates and nitrogen levels was 6.15 ± 0.05 (95% CI = 6.05–6.25).

Basil seeds (cv. 'Dark Opal', Ferry-Morse Seed Co., Fulton, KY) were sown on May 16 in two germination trays (Landmark Plastic Corporation, Akron, OH) filled with moist Fafard Super Fine Germinating Mix (SunGro Horticulture, Agawam, MA). The seedlings emerged on May 2. Each tray of basil seedlings received 200 ml (6.8 fl oz) of fertilizer solution at 48 ppm N every two days and was irrigated with tap water as needed. The amount of fertilizer solution at each application was increased to 400 ml (13.6 fl oz) per tray on June 3 and 800 ml (27.4 fl oz) on June 11. The nitrogen fertilizer solution was composed of calcium nitrate (15% nitrogen; Fisher Scientific, Waltham, MA), ammonium nitrate (34% nitrogen, Certified ACS, Fisher Scientific), urea (46% nitrogen, Potash Corporation of Saskatchewan, Saskatoon, Canada), P_2O_5 (40 ppm P of Liquid Phosphorus Solution 0-30-0; Growth Products, Inc., White Plains, NY) and potassium acetate (77 ppm K of ACE 29 0-0-29, Custom Agronomics, Inc., Palm City, FL).

On May 29, seedlings were transplanted individually to 72-cell trays (Landmark Plastic Corporation, Akron, OH) filled with a uniform mixture of 75% peat moss (Pro-Moss Sphagnum Peat Moss, Premier Tech Horticulture, Ltd, Quakertown, PA), 25% sand (Golf Agronomics, Lugoff, SC) and dolomitic lime [3.7 kg·m^{-3} (6.2 lb·yd^{-3}) of peat moss-sand mixture]. Dolomitic lime was added to the peat moss-sand mixture to raise the pH of the substrate (Boodley and Sheldrake 1982, Shaughnessy 1999). The substrate was heat sterilized before use in an electric soil sterilizer (Pro-Grow Model SS-60R, Pro-Grow Supply Corp., Brookfield, WI) at 82 C (180 F) for 24 hours.

On June 20, 20 seedlings were selected and assigned randomly to four groups of five plants each using a random sequence generator (RANDOM.ORG, Randomness and Integrity Services Ltd. 2015). The seedlings were harvested to determine fresh and dry canopy and root weights, and aboveground heights before application of the fertilization treatments. Fresh canopies and roots were weighed separately, placed in paper bags, dried in a drying oven at 60 C (140 F) for 24 hours, and weighed again to determine dry weights. No significant differences were found among the four groups for seedling height (P = 0.4950), canopy fresh weight (P = 0.9701), canopy dry weight (P = 0.9910), root fresh weight (P = 0.3625), and root dry weight (P = 0.4324). These seedlings had an average aboveground height of 13.53 ± 0.32 cm, canopy fresh weight of 0.73 ± 0.03 g, canopy dry weight of 0.10 g, fresh root weight of 0.19 ± 0.06 g, and dry root weight of 0.03 g. The remaining seedlings were transplanted individually to standard 6" pots [10 cm (4 in) height and 15 cm (6 in) diameter] filled with sterilized substrate. Saucers [3.2 cm (1.3 in) tall and 20.3 cm (8 in) diameter)] were placed under the pots to collect leachate.

Water sources for the fertilizer solutions and daily irrigation were located in the laboratory and greenhouse, respectively. One water sample [500 ml (0.5 qt)] from each location was collected mid-day on May 28 in sterilized plastic bottles, and sent to the Agricultural Services Laboratory (ASL; Clemson, SC) to analyze for pH, mineral and minor element contents. Analyses showed that the pH of both water sources was 7.3, with low amounts of minerals and minor elements.

Two samples of the sterilized substrate (0.9 kg each) were also collected on May 28 in plastic bags, and sent to the ASL for analysis of pH level, mineral contents, electrical conductivity and total dissolved solids. Analysis reported that both samples had the same pH (5.6 to 5.7), and similar constitutions of total dissolved solids, electrical conductivity, and mineral content.

On June 21, 10 potted plants were assigned randomly to each of four nitrogen fertilization levels, and each plant received 100 ml (3.4 fl oz) of its assigned nitrogen solution daily for one week. After the first week, the plants were given 200 ml (6.8 fl oz) of their respective fertilizer solutions 3 times a week for the duration of the study. The four nitrogen fertilization treatments were 0, 21, 48, and 91 ppm N. The fertilizer solutions of different nitrogen concentrations were prepared with the nitrogen, phosphorus, and potassium sources used for the seedling fertilizer solution. The amounts of calcium nitrate, ammonium nitrate and urea were increased proportionally to achieve the desired nitrogen fertilization rates while the concentrations of potassium and phosphorus remained constant across the different fertilization rates. A minor elements solution (Energy Turf and Ornamental Micronutrients; Vereens Turf Center, Longs, SC) was diluted, and 5 ml (0.17 fl oz) of the diluted solution was provided to each plant weekly. Each 5 ml of diluted minor elements solution contained 1.639×10^{-4}g magnesium, 3.278×10^{-6}g boron, 4.917×10^{-4}g iron, 6.557×10^{-4}g manganese, and 3.278×10^{-6}g zinc. Wooden dowels were placed in the pots and the plants were secured upright by tying them loosely to the dowels.

Plant heights, numbers of fully expanded leaves, numbers of branches, stem widths, and the presence of flowering heads per plant were measured on July 14 (24 days after the start of nitrogen treatments), July 21 (31 days), July 28 (38 days), and August 11 (52 days). The widest points of the plant canopies were recorded for plant widths taken on July 14 (n = 35), July 21 (n = 20), and August 11 (n = 39). Fully expanded leaves were defined as leaves that had reached at least 2.5 cm (1 in) in length and 1.9 cm (0.75 in) at the widest. Terminals with at least two fully expanded leaves were considered branches. Stem widths were measured 2.5 cm above the substrate surface. Senesced leaves that had fallen off plants during the course of the study were harvested and recorded. On August 15 (56 days after nitrogen treatments), the numbers of flower/bud clusters per flowering head were recorded for all plants.

Five plants from each nitrogen level were selected randomly and harvested on August 17. The canopy fresh weights, root fresh weights and root lengths were measured immediately after harvest. Each plant was divided into three regions based on its height: bottom (0 to 30% plant height), middle (30 to 60%) and top (60 to 90%). For each plant, two leaves were taken from one node in the middle plant height section and two leaves from a node in the top section. Leaves were not collected from the bottom region because some leaves in the bottom region had begun senescence, and not every plant had a bottom section with fully expanded leaves. Surface areas of the leaves were measured using an LI-3100 Leaf Area Meter (LI-COR, Lincoln, NE). Two stem sections [2.5 cm (1 in) in length with nodes in the middle] were also taken from each of the middle and top plant height regions of each plant. The leaves and stem sections were placed in a drying oven with the remaining plant materials and dried at 60 C (140 F) for 72 hours, after which canopy and root dry weights were measured.

From each plant, all leaves collected for surface area measurements were combined, placed in a mortar and ground to a fine powder. For each plant, 0.11 g (0.04 oz) of ground leaf sample was placed in a micro-centrifuge tube and submitted to the ASL to determine N and C content. There were three leaf samples from each of the nitrogen treatment levels. The same was done for stem sections collected from each plant.

Data on plant heights, numbers of fully expanded leaves, numbers of branches, stem widths, weights of senesced leaves, leaf surface area measurements (middle plant height region), root dry weights and root lengths did not meet the assumption of normal distribution by Shapiro-Wilk test (PROC UNIVARIATE, SAS Institute 2011, SAS Version 9.3. SAS Institute Inc., Cary, NC). Subsequent transformations were not successful in normalizing data distribution. Therefore, the ranks of plant heights, numbers of fully expanded leaves, numbers of branches, stem widths, weights of senesced leaves, leaf surface area measurements (middle plant height region), root dry weights and root lengths were used as data for statistical analyses. All data were analyzed with one-way analysis of variance (ANOVA) to detect significant differences among the nitrogen fertilization treatments (JMP 11, SAS Institute 2013, JMP Version 11, SAS Institute Inc., Cary, NC.) at $\alpha = 0.05$. Means were separated with Student's t-test when significant differences were detected by ANOVA.

Results and Discussion

Basil plants fertilized at 21 ppm N were the numerically tallest at all assessment dates, but did not differ significantly from plants fertilized at 48 and 91 ppm N at 38 and 52 days after initiation of treatment (DAT) (Table 1). Plants fertilized at 91 ppm N had the greatest plant widths of all treated plants throughout the experiment. Unfertilized plants had the fewest fully expanded leaves at all measurement dates, whereas fertilized plants had similar numbers of leaves regardless of the nitrogen concentrations. Fertilized and unfertilized plants had similar numbers of fully expanded leaves at the end of the experiment. Fertilized plants had consistently more branches and greater stem width than the unfertilized plants, with plants fertilized at 91 ppm N having the greatest number of branches and stem width. At 52 DAT, plants fertilized at 48 ppm N were 20.9% taller, had 5.8% more leaves, 245% more branches and 12.5% larger stems than the unfertilized plants, whereas those fertilized with 21 or 91 ppm N were similar in size to those fertilized at 48 ppm N.

Plants fertilized at 91 ppm N had numerically greater canopy fresh weights, canopy dry weights, and surface areas of leaves from the middle height region than other fertilized and unfertilized plants (Table 2). However, the average canopy dry weights of plants fertilized at 21 and 48 ppm N were not significantly different from those of plants fertilized at 91 ppm. The surface areas of leaves collected from the medium height region of plants fertilized at 21 and 48 ppm N were slightly but not significantly greater from those collected from unfertilized plants. The weights of senesced leaves and surface areas of leaves from the top height region were not significantly different among the fertilization levels.

In this study, the growth responses of plants fertilized at 48 ppm N appeared to straddle between those of the lower sub-recommended nitrogen level (21 ppm N) and the near-recommended nitrogen level (91 ppm N). Plants fertilized at 48 ppm N were similar to those fertilized at 21 ppm N in every plant growth parameter measured in this study. However, plants fertilized at 48 ppm N were also similar in the numbers of branches, stem widths and leaf surface areas as those fertilized at 91 ppm N, while the same parameters of plants fertilized at 21 ppm N were significantly lower than plants fertilized at 91 ppm N. The results suggest that lower sub-recommended nitrogen fertilization rates could produce

Table 1. Mean (± standard error) canopy height, canopy width, numbers of fully expanded leaves, numbers of branches, stem widths, and plant widths of basil plants at 24, 31, 38 and 52 days after initiation of fertilizer treatments. Means within a column followed by the same letters are not significantly different by Student's t test.

Plant parameters	Nitrogen fertilization level (ppm)	Days after start of nitrogen treatments			
		24	31	38	52
Canopy height (cm)	0	31.7 ± 0.76c	37.9 ± 1.41c	42.7 ± 2.16b	48.0 ± 2.50b
	21	35.7 ± 0.45a	45.6 ± 1.15a	51.2 ± 2.17a	64.1 ± 1.54a
	48	33.7 ± 0.51b	43.2 ± 1.02ab	49.5 ± 1.71a	58.0 ± 1.84a
	91	33.6 ± 0.66b	42.3 ± 0.60b	47.9 ± 0.88a	55.9 ± 1.41a
	P value	0.0001	< 0.0001	0.0125	< 0.0001
Canopy width (cm)	0	16.6 ± 0.31c	17.6 ± 0.43c	—	18.1 ± 0.27d
	21	18.3 ± 0.24b	19.0 ± 0.23b	—	19.3 ± 0.54c
	48	18.7 ± 0.47ab	19.4 ± 0.64b	—	20.5 ± 0.32b
	91	19.5 ± 0.42a	20.5 ± 0.44a	—	21.5 ± 0.36a
	P value	< 0.0001	0.0043	—	< 0.0001
Numbers of fully expanded leaves	0	19.8 ± 0.95b	18.6 ± 1.44b	18.0 ± 1.26b	17.2 ± 0.97
	21	21.0 ± 0.91ab	22.7 ± 1.08a	21.7 ± 1.34ab	19.5 ± 0.79
	48	22.8 ± 0.51a	22.3 ± 1.10a	20.8 ± 1.00ab	18.2 ± 1.62
	91	22.3 ± 0.62a	24.3 ± 0.68a	22.9 ± 0.79a	18.2 ± 1.49
	P value	0.0247	0.0089	0.0294	0.6330
Numbers of branches	0	1.5 ± 0.58c	2.4 ± 0.87c	2.5 ± 0.86c	4.0 ± 1.51b
	21	6.7 ± 1.02ab	9.9 ± 1.05b	11.9 ± 1.50b	14.3 ± 1.13a
	48	4.9 ± 1.02b	10.0 ± 1.33b	14.0 ± 0.96ab	13.8 ± 1.75a
	91	8.5 ± 1.31a	14.3 ± 1.26a	17.2 ± 1.03a	16.7 ± 1.17a
	P value	0.0002	< 0.0001	< 0.0001	< 0.0001
Stem width (cm)	0	0.38 ± 0.02	0.38 ± 0.02c	0.40 ± 0.02c	0.40 ± 0.01b
	21	0.37 ± 0.02	0.40 ± 0.02bc	0.41 ± 0.01bc	0.41 ± 0.02b
	48	0.39 ± 0.02	0.44 ± 0.01ab	0.43 ± 0.01ab	0.45 ± 0.01a
	91	0.39 ± 0.02	0.45 ± 0.02a	0.44 ± 0.01a	0.46 ± 0.02a
	P value	0.7172	0.0074	0.0085	0.0036

basil plants with attributes and yields close to those of plants fertilized with a near-recommended rate.

Other studies have reported similar responses of basil plants to moderate fertilization rates. Sifola and Barbieri (2006) found that the highest nitrogen fertilization level [300 kg·ha^{-1} 270 lb·A^{-1}]) led to significantly greater aboveground fresh biomass and fresh leaf biomass of basil plants of three cultivars than two lower nitrogen fertilization levels [0 and 100 kg·ha^{-1} (0 and 90 lb·A^{-1})), whereas no significant differences in plant heights and numbers of leaves per plant were found between plants fertilized at 100 and 300 kg·ha^{-1} N. Fresh and dry weights of basil plants (cv. 'Genovese') fertilized at 75 kg·ha^{-1} N, 50 kg·ha^{-1} P and 50 kg·ha^{-1} K (the moderate fertilization level) were the greatest but not significantly different from those of plants fertilized at the highest fertilization level (100 kg·ha^{-1} N, 75 kg·ha^{-1} P and 75 kg·ha^{-1} K) (Roghaye et al. 2012). Nguyen and Niembyer (2008) reported that basil plants fertilized with moderate nitrogen levels (0.5 and 1.0 mM N) were generally taller and larger than those fertilized at low and high nitrogen levels (0.1 mM N and 5.0 mM N).

These studies demonstrated that high fertilization levels did not always produce the best yield in basil. In addition, these studies also suggested that basil cultivars responded differently to nitrogen availability. Nguyen and Niembyer (2008) reported that 'Sweet Thai' and 'Genovese' basil plants could not survive fertilization at 0.1 mM and 5.0 mM N, respectively. Nguyen and Niembyer (2008) also showed that 'Dark Opal' basil was better able to grow and withstand extreme nitrogen levels, both low and high, compared to 'Sweet Thai' and 'Genovese' cultivars. Extremes in nitrogen fertilization (low nitrogen and high nitrogen) are both detrimental to plant growth, and some cultivars are more

Table 2. Canopy measurements (means ± standard error) of basil plants harvested at 58 days after initiation of fertilization treatments. Means within a column followed by the same letters are not significantly different by Student's t test.

Nitrogen fertilization levels (ppm)	Canopy fresh weight (g)	Canopy dry weight (g)	Weight of senesced leaves (g)	Leaf surface area (cm^2) — middle region[z]	Leaf surface area (cm^2) — top region[y]
0	13.61 ± 3.97c	2.95 ± 0.73b	0.10	11.39 ± 1.63b	8.25 ± 1.57
21	26.42 ± 0.85b	5.10 ± 0.28a	0.11 ± 0.02	11.77 ± 1.59b	11.04 ± 0.61
48	25.42 ± 1.53b	4.52 ± 0.37a	0.17 ± 0.04	14.83 ± 1.03ab	12.58 ± 0.39
91	35.18 ± 1.74a	5.78 ± 0.28a	0.18 ± 0.03	16.72 ± 1.42a	11.54 ± 1.58
P value	< 0.0001	0.0029	0.1653	0.0341	0.0735

[z]Middle Region of a plant include canopy at 30–60% of the canopy height.
[y]Top Region of a plant include canopy at 60–90% of the canopy height.

Table 3. **Root weights and lengths (means ± standard error) of basils harvested at 58 days after the start of nitrogen treatments. Means within a column followed by the same letters are not significantly different by Student's t test.**

Nitrogen fertilization levels (ppm)	Root fresh weight (g)	Root dry weight (g)	Root length (cm)
0	7.32 ± 0.66b	0.75 ± 0.06	22.67 ± 1.79ab
21	14.74 ± 0.74a	1.48 ± 0.08	23.24 ± 2.42a
48	12.01 ± 2.19a	1.13 ± 0.22	17.59 ± 1.09bc
91	12.32 ± 1.75a	1.48 ± 0.28	17.27 ± 1.30c
P value	0.0199	0.0669	0.0424

sensitive to nitrogen availability than others (Nguyen and Niembyer 2008).

Unfertilized plants and plants fertilized at 21 ppm N had significantly longer roots than those fertilized at 91 ppm N (Table 3). Fertilized plants had significantly greater root fresh weights, and a trend for greater root dry weights than the unfertilized plants. Plants fertilized with low levels of nitrogen typically produce longer roots than plants fertilized with adequate or excessive nitrogen (Gao et al. 2015, Hodge 2004, Honěk 1991, van Vuuren et al. 1996). Greater root growth may allow plants grown in conditions of lower nitrogen availability to locate nutrients in the soil more efficiently (Forde and Lorenzo 2001, Hodge 2004).

Plants fertilized at 91 ppm N had significantly higher N content in plant tissues than unfertilized plants, with 3.15% for leaves (P = 0.0002) and 3.97% for stem sections (P < 0.0001) (Fig. 1A and 1C). Leaf N content of plants fertilized at 21 and 48 ppm N (0.82% and 1.37%, respectively) were similar to that in the unfertilized plants (0.65%). Stem N content of plants fertilized at 91 g $N \cdot L^{-1}$ and 48 ppm N (2.64%) were higher than those of plants fertilized at 0 and 21 ppm N (0.48% and 0.23%, respectively).

Leaves of unfertilized and fertilized plants did not differ significantly in average C content (P = 0.8812, average = 39.2%) (Fig. 1B). Stems from plants fertilized at 21 ppm N had significantly higher C content (43.63%) than stems from plants fertilized at 91 ppm N (42.14%C), but C content of stems from plants fertilized at 21 and 48 ppm N (42.60% C) were not significantly different from each other (P = 0.0166) (Fig. 1D).

We found that the N content of 'Dark Opal' basil plant tissues increased with increasing nitrogen fertilization level

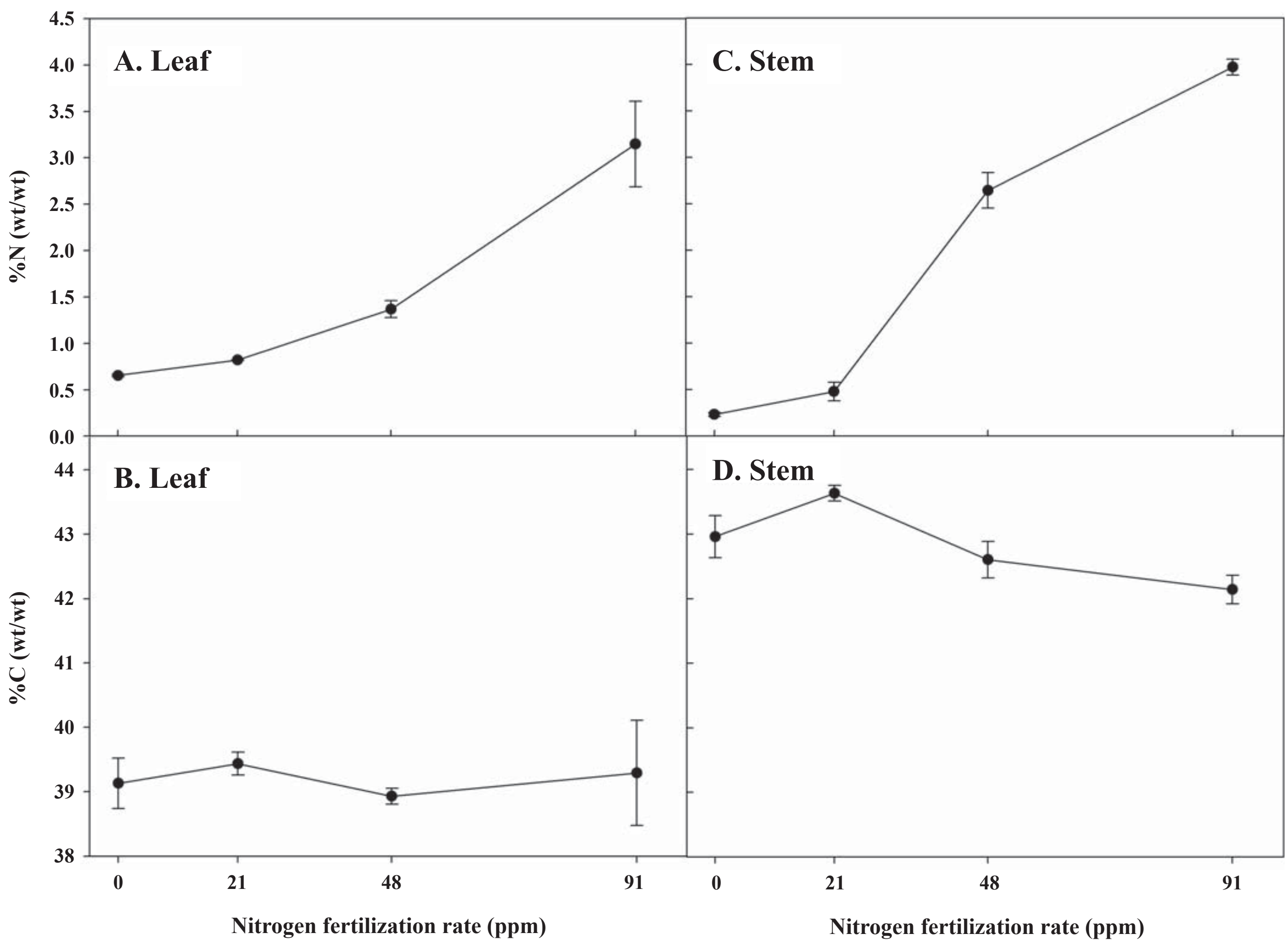

Fig. 1. **Nitrogen and carbon contents (%) of basil leaves and stem sections from different nitrogen fertilization treatments. A) %N in leaves; B) %C in leaves; C) %N in stems; D) %C in stems.**

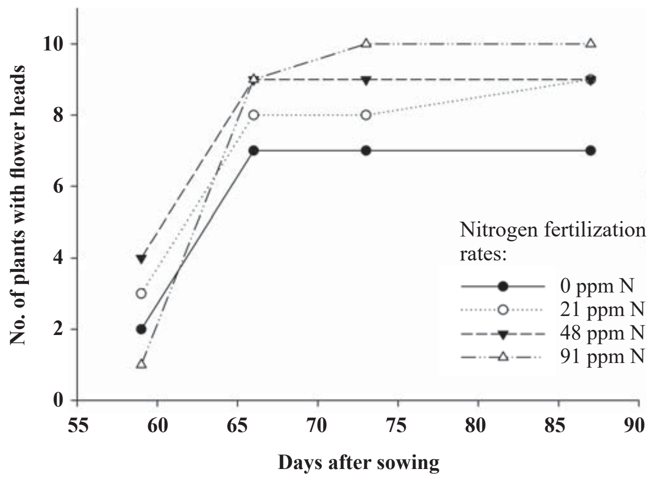

Fig. 2. Numbers of basil plants with flowering heads for each nitrogen level treatment at 24, 31, 38 and 52 days after initiation of treatments. There were 10 plants fertilized with each nitrogen treatment level.

(Fig. 1A and 1C). Similarly, a high nitrogen level (250 kg·ha^{-1} N) produced 'Genovese' basil plants with high tissue nitrogen content (Bufalo et al. 2015). In this study, the average C content of leaves did not differ significantly among the nitrogen levels, but the average C content of stem sections from 21 ppm N fertilized plants was the numerically greatest (Fig. 1B and 1D). Plant stems contain carbon-based structural compounds, such as cellulose and lignin (Raven et al. 2005). In the absence of additional nitrogen, plants fertilized with 21 ppm N might have allocated the available carbon for assimilation into new stem tissue, thereby elongating the main-stem and enhancing plant height.

There are no known previous studies that have analyzed for the production of flower heads and flower clusters in basil plants fertilized with different levels of nitrogen. In this study, a similar percentage of plants from all treatments developed flowering heads by 31 DAT ($P = 0.6192$) (Fig. 2). However, a greater percentage of fertilized plants developed flower heads at 39 and 52 DAT than the unfertilized plants, and that sub-recommended fertilization rates resulted in fewer plants producing flower heads in basil than those fertilized with 91 ppm N (Fig. 2). All plants fertilized at 91 ppm N had developed flower heads by 38 DAT, whereas 90% of plants fertilized at 21 and 48 ppm N and 70% of unfertilized plants developed flower heads by 52 DAT.

Plants fertilized at the four nitrogen levels did not differ significantly in numbers of flower/bud clusters per flowering head at 56 DAT ($P = 0.3549$). Plants fertilized at 21 ppm N had on average 9.5 ± 2.7 flower clusters per flowering head, whereas those fertilized at 48 and 91 ppm N had 4.2 ± 1.6 and 2.6 ± 0.6 clusters per flower head respectively, and unfertilized plants had 4.0 ± 2.1 flower clusters per flower head.

Hirose et al. (2005) suggested that environments with limited nutrient availability favor annual plants with high reproductive nitrogen use efficiency (RNUE). In this study, plants from the 21 ppm N treatment level had a low supply of nitrogen, which led to the plants being faced with a trade-off between vegetative growth and development of reproductive structures. We may infer that plants from the 21 ppm N treatment experienced a higher RNUE, in terms of flower cluster development, when compared to basil plants from the other nitrogen treatments. In contrast, plants from the highest nitrogen treatment (91 ppm N) were able to use available nitrogen to maintain canopy growth and direct some of the resource to reproduction. Plants from the 91 ppm N treatment had the lowest average number of flower clusters per head, but had the greatest canopy widths, numbers of branches, stem widths and canopy fresh and dry weights than plants from the lower nitrogen treatments. In greenhouse vegetable production, high nitrogen fertilization leads to vegetative proliferation and growth with a reduction in root development, fruit production and fruit palatability (Yasuor et al. 2013).

We did not analyze for the numbers of seed or quality of the seed produced by plants from the different nitrogen treatments. Therefore, we are not able to determine which of the four nitrogen levels would produce the greatest number and quality of seeds. However, because plants fertilized at 21 ppm N had, on average, the most flower clusters per head, these plants might also have had the potential of producing more seed than plants fertilized at 91 ppm N. However, plants from the high nitrogen levels might have been able to use the available nitrogen to produce higher quality seeds. Future studies may explore the influence of various fertilization rates on the quality and quantity of basil seeds.

We did not investigate the effects of nitrogen fertilization on the amount of essential oils in 'Dark Opal' basil plants. Basil plants fertilized at high nitrogen levels are known to produce lower amounts of total phenolic content, phenolic compounds and antioxidants than at lower nitrogen treatments (Nguyen and Niembyer 2008). Zheljazkov et al. (2008) also reported higher essential oil yields between 50 and 60 kg·ha^{-1} N. Under the carbon/nutrient balance hypothesis, plants receiving the low nitrogen fertilization treatment would produce more carbon-based compounds and metabolites (e.g. phenolic compounds) because of the limited availability of nitrogen (Nguyen and Niembyer 2008). Based on these studies, we may infer that the essential oil yield per unit of plant tissue would not have differed significantly between 48 and 91 ppm N treatments because the numbers of fully expanded leaves, leaf surface areas, and canopy dry weights did not differ significantly across nitrogen treatments. We also may infer that the total phenolic and antioxidant contents in basil plants fertilized with the lower nitrogen levels (21 and 48 ppm N) might have been higher than those from plants fertilized with 91 ppm N.

Literature Cited

Boodley, J.W. and R. Sheldrake. 1982. Cornell peat-lite mixes for commercial plant growing. A Cornell Cooperative Extension Publication, Information Bulletin 43. http://www.greenhouse.cornell.edu/crops/factsheets/peatlite.pdf. Accessed April 19, 2016.

Bufalo, J., C.L. Cantrell, T. Astatkie, V.D. Zheljazkov, A. Gawde, and C.S.F. Boaro. 2015. Organic versus conventional fertilization effects on sweet basil (*Ocimum basilicum* L.) growth in a greenhouse system. Ind. Crops Prod. 74:249–254.

Carpenter, S.R., N.F. Caraco, D.L. Correll, R.W. Howarth, A.N. Sharpley, and V.H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecol. Appl. 8:559–568.

Constantin, J, B. Mary, F. Laurent, G. Aubrion, A. Fontaine, P. Kerveillant, and N. Beaudoin. 2010. Effects of catch crops, no till and reduced nitrogen fertilization on nitrogen leaching and balance in three long-term experiments. Agric. Ecosyst. Environ. 135:268–278.

Erisman, J.W. 2011. The new global nitrogen cycle, p. 5–15. *In*: J.C. Polacco and C.D. Todd (Eds.). Ecological Aspects of Nitrogen Metabolism in Plants. John Wiley and Sons, Inc., West Sussex, UK.

Forde, B. and H. Lorenzo. 2001. The nutritional control of root development. Plant Soil 232:51–68.

Gao, K., F. Chen, L. Yuan, F. Zhang, and G. Mi. 2015. A comprehensive analysis of root morphological changes and nitrogen allocation in maize in response to low nitrogen stress. Plant Cell Environ. 38:740–750.

Hamasaki, R.T., H.R. Valenzuela, D.M. Tsuda, and J.Y. Uchida. 1994. Fresh basil production guidelines for Hawai'i. Research Extension Series 154 – Hawaii Institute of Tropical Agriculture and Human Resources. http://www.ctahr.hawaii.edu/oc/freepubs/pdf/RES-154.pdf. Accessed April 19, 2016.

Hamrick, D. 2003. Ball RedBook: Crop Production. Volume 2, 17th Edition. Ball Publishing; West Chicago, IL. 736 pp.

Hirose T., T. Kinugasa, and Y. Shitaka. 2005. Time of flowering, costs of reproduction, and reproductive output in annuals, p. 155–184. *In*: E. G. Reekie and F. A. Bazzaz (Eds.). Reproductive Allocation in Plants. Elesevier, Inc., Philadelphia, PA.

Hodge, A. 2004. The plastic plant: Root responses to heterogeneous supplies of nutrients. New Phytol. 162:9–24.

Honěk, A. 1991. Environment stress, plant quality and abundance of cereal aphid (Hom., Aphididae) on winter wheat. J. Appl. Entomol. 112:65–70.

Lehmann, J. and G. Schroth. 2003. Chapter 7: Nutrient leaching, p. 151–166. *In*: G. Schroth and F.L. Sinclair (Eds.). Trees, Crops and Soil Fertility. CABI Publishing, England.

Nguyen, P.M. and E.D. Niembyer. 2008. Effects of nitrogen fertilization on the phenolic composition and antioxidant properties of basil (*Ocimum basilicum* L.). J. Agric. Food Chem. 56:8685–8691.

Putievsky, E. and B. Galambosi. 1999. Production systems of sweet basil, p. 37–63. *In*: R. Hiltunen and Y. Holm (Eds.). Basil: the Genus *Ocimum*. Hardwood Academic Publishers, Australia.

RANDOM.ORG©. 2015. Randomness and Integrity Services Ltd., Dublin, Ireland, 1998–2016. https://www.random.org/. Accessed May 16, 2014.

Raven, P.H., R.F. Evert, and S.E. Eichhorn. 2005. The molecular composition of plant cells p. 15–34. *In*: Biology of Plants. W.H. Freeman and Company, New York, NY.

Ribaudo, M. 2011. Reducing agriculture's nitrogen footprint: Are new policy approaches needed? U.S. Department of Agriculture, Economic Research Service. http://www.ers.usda.gov/amber-waves/2011-september/nitrogen-footprint.aspx#.VsYkN_krLIV. Accessed April 19, 2016.

Ribaudo, M., J. Delgado, L. Hansen, M. Livingston, R. Mosheim, and J. Williamson. 2011. Nitrogen in agricultural systems: Implications for conservation policy. ERR – 127. U. S. Department of Agriculture, Economic Research Services. Washington, DC. 89 pp.

Roghaye, A.M., S. Zolfaghar, M. Vahid, O. Mohsen, and S.A. Ghaffar. 2012. Effect of graded levels of NPK on herb, oil yield and oil composition of basil (*Ocimum basilicum* L.). Global J. Res. Med. Plants Indigenous Med. 1:258–264.

Sharafzadeh, S., M. Esmaeili, and A.H. Mohammadi. 2011. Interaction effects of nitrogen, phosphorus, and potassium on growth essential oil and total phenolic content of sweet basil. Adv. Environ. Biol. 5:1285–1289.

Shaughnessy, D. 1999. Indoor plants — soil mixes. Clemson University Cooperative Extension, HGIC 1456. http://www.clemson.edu/extension/hgic/plants/pdf/hgic1456.pdf. Accessed 19 April 2016.

Sifola, M.I. and G. Barbieri. 2006. Growth, yield and essential oil content of three cultivars of basil grown under different levels of nitrogen in the field. Sci. Hort. 108:408–413.

U.S. Environmental Protection Agency (EPA). 2016a. Nutrient pollution: The effects: Environment. http://www.epa.gov/nutrientpollution/effects-environment. Accessed 19 April 2016.

U.S. Environmental Protection Agency (EPA). 2016b. Nutrient policy data: What EPA is doing. http://www.epa.gov/nutrient-policy-data/what-epa-doing. Accessed 19 April 2016.

U.S. Environmental Protection Agency (EPA). 2016c. Nutrient pollution: The sources and solutions: Agriculture. http://www.epa.gov/nutrientpollution/sources-and-solutions-agriculture. Accessed 19 April 2016.

U.S. Department of Agriculture (USDA). 2014. EQIP: Environmental Quality Incentives Program, 2014 EQIP Farm Bill Fact Sheet. http://www.nrcs.usda.gov/wps/portal/nrcs/main/national/programs/financial/eqip/. Accessed 19 April 2016.

van Vuuren, M.M.I., D. Robinson, and B.S. Griffiths. 1996. Nutrient inflow and root proliferation during the exploitation of a temporally and spatially discrete source of nitrogen in soil. Plant Soil 178:185–192.

Yasuor, H., A. Ben-Gal, U. Yermiyahu, E. Beit-Yannai, and S. Cohen. 2013. Nitrogen management of greenhouse pepper production: Agronomic, nutritional, and environmental implications. HortScience 48:1241–1249.

Zheljazkov, V.D., C.L. Cantrell, M.W. Ebelhar, D.E. Rowe, and C. Coker. 2008. Productivity, oil content, and oil composition of sweet basil as a function of nitrogen and sulfur fertilization. HortScience 43:1415–1422.