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Effect of Application Date on Absorption of ^{15}Nitrogen by Highbush Blueberry

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Abstract. **Rates of absorption of ^{15}N-enriched ammonium sulfate by young 'Bluecrop' highbush blueberries (*Vaccinium corymbosum* L.) were compared following applications on six dates between late April and September. Ammonium sulfate solutions containing 2.1 g N (10.2 atom % ^{15}N) were dripped directly into the root zone of single bushes. Soil covers and irrigation were used to maintain similar soil moisture conditions during treatment periods. Treated bushes from each application date were excavated after 2 weeks of exposure and separated into roots, stems, and current season's growth (new shoots, leaves, fruit). Tissues were dried, weighed, and analyzed for ^{15}N and ^{14}N by mass spectrometry. Soils were also analyzed for labeled and nonlabeled N. Bushes treated in late May, June, and July absorbed a greater percentage of applied N (6% to 9%) than bushes treated in April, August, or September (1% to 3%). Absorption of N appeared to be affected more by the demand of the plants than soil N availability. Plants absorbed N most efficiently during active growth between late bloom and fruit maturity.**

Efficient use of fertilizer N in crop production can reduce production costs and minimize the detrimental effects of N movement into surface water or groundwater. Highbush blueberries are adapted to coarse-textured soils where the water table is relatively close to the soil surface. Although these conditions appear conducive to N leaching, the effect of blueberry production on water quality has not been studied extensively.

Researchers have described how blueberry growth and yield are affected by N rates (Bailey et al., 1966; Ballinger et al., 1963; Cummings, 1978; Eck, 1977) and forms (Cain, 1952; Merhaut and Darnell, 1995; Peterson et al., 1988; Sugiyama and Ishigaki 1994; Townsend, 1967). In Michigan, N recommendations for blueberries include 70 kg·ha^{-1} applied each spring at budbreak as either urea or ammonium sulfate (Hanson and Hancock, 1996). Split applications (half at budbreak, half at petalfall) resulted in higher yields than single applications (Hanson and Retamales, 1992) and are suggested on sandy soils. Growers have used leaf analysis since the 1960s to adjust seasonal N rates based on plant N status (Hanson and Hancock, 1996; Kenworthy 1979).

Blueberries may recover relatively low percentages of soil-applied N, depending on environmental conditions and cultural practices. Mature bushes treated with labeled urea at budbreak recovered only 32% of applied N by the following fall (Retamales and Hanson, 1989). The remaining N was still in the root zone (15%) or not accounted for (53%). It is not clear why N recovery was low in this study. However, NH_4^+ and NO_3^- levels increased below the root zone 2 to 3 months after N applications in another study (Retamales and Hanson, 1990), suggesting that leaching losses were partly responsible. Nitrogen use efficiency (NUE) measured in other perennial fruit crops ranged from 20% to 50%, depending on the species and experimental conditions (Weinbaum et al., 1992). In contrast, uptake efficiency of wheat in the field may be >70%, with an additional 20% potentially accessible in the soil (Powlson et al., 1986).

The seasonal pattern of N accumulation by plants reflects their demand for N and can be useful in timing fertilization for greatest NUE (Weinbaum et al., 1992). However, the seasonal pattern of N uptake by blueberry plants has not been documented. Weinbaum et al. (1978) studied uptake throughout the season in potted plum trees and found that trees absorbed N efficiently only when leaves were present between the initiation of shoot elongation and the onset of dormancy in the fall. Trees absorbed the greatest amount of N when their leaf mass was greatest. Nitrogen uptake was independent of temperature within a limited range during the growing period. Nitrogen uptake efficiency was thought to be reduced when fruit were maturing, possibly because carbohydrates were diverted from the roots, limiting the energy available for nitrate uptake.

Since there are indications that N uptake may occur most rapidly when biomass accumulation is greatest (Weinbaum et al., 1978), growth patterns may reflect N demand. Growth patterns have been described for individual components of blueberry plants, such as shoots (Gough et al., 1976; Gough et al., 1978), fruit (Young, 1952), and roots (Abbott and Gough, 1987), but patterns of whole-plant dry matter accumulation have not been reported. Abbott and Gough (1987) found that shoots grew most rapidly before fruit maturity, but growth continued through September. In contrast, root growth rate was moderate during bloom and greatest after fruit maturation.

Nitrogen demand is complicated in perennial plants such as blueberries because N absorbed one year may be retained in the plant and used during subsequent seasons. There is a need for a greater understanding of N cycling and dynamics in blueberry bushes. Birkhold and Darnell (1993) found that N stored in potted 2-year-old rabbiteye blueberry plants supplied 90% of the N in reproductive tissues at anthesis and 50% as late as fruit maturity. Under field conditions, only 6% of the N content of mature bushes at the end of the season was derived from fertilizer applied that spring (Retamales and Hanson, 1989). Since N is retained in bushes for use during subsequent seasons, increasing plant N reserves late in the season may benefit bushes during the following year. Perhaps late season fertilization would benefit plants by contributing N to this storage pool. Late summer applications contributed more N to apricot blossoms the following spring than applications earlier in the summer (Weinbaum et al., 1980).

The fact that NUE is low when blueberries are fertilized at budbreak (Retamales and Hanson, 1989) suggests that this timing

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does not match plant demand. The purpose of this experiment was to compare the NUE of highbush blueberry at different times during the growing season and describe the seasonal pattern of dry matter and N accumulation by blueberry bushes.

Materials and Methods

A 1990 planting of 'Bluecrop' at the Southwest Michigan Research and Extension Center, Benton Harbor, was used. Soil was a Selfridge sandy loam (loamy, mixed, mesic Aquic, Arenic, Hapludalfs) (Bowman, 1986). The pH in 1989 was 6.2, but sulfur was added in 1990 and 1991 to acidify the soil to a pH of 4.8 (1993). The soil had a cation-exchange capacity (CEC) of .96 meq·kg^{-1}, and contained 16% clay, 17% silt, and 67% sand.

Treatments consisted of 10 g of 10.2 atom % ^{15}N ammonium sulfate (Isotec Inc., Miamisburg, Ohio) applied to individual plants (N equivalent to 5.7 kg·ha^{-1} at 2717 plants/ha) on one of six dates in 1993: 23 Apr. (budscale separation); 25 May (just before petalfall); 24 June (first blue fruit); 23 July (just after fruit harvest); 24 Aug. (shoot growth cessation); or 23 Sept. (fall leaf coloration). Treated plants remained in contact with the labeled N for 14 d and were then harvested for analysis.

The soil in the circular 0.237-m^2 area beneath each plant was irrigated to field capacity just before treatment with labeled N. To accomplish this, a 55-cm-diameter, 10-cm-tall collar was placed around each plant and pushed about 3 cm into the soil. Water was added to a depth of 2.5 cm above the soil surface and allowed to percolate into the soil. Only 1.25 cm was added before the late-August treatment due to rain received the previous night.

The labeled N was dissolved in 2 L of water and dripped directly into the root zone using four 500-mL intravenous bottles suspended above each plant. Each bottle was attached to a 6-mm-diameter plastic tube inserted 15 cm into the soil on a 20-cm square configuration around each plant. The flow rate from each bottle was adjusted with clamps so as not to exceed the rate of percolation. In an attempt to maintain similar soil N concentrations throughout the experiment, plants to receive labeled N treatments at later dates received maintenance N applications of 14.8 kg·ha^{-1} as nonlabeled granular ammonium sulfate monthly.

Efforts were made to maintain similar soil water potentials during each 2-week treatment period. Plastic raincovers were placed beneath the foliage of treated plants to prevent rain from falling on treated soil areas during the 2-week treatment periods. Raincovers had an A-frame shape to allow air flow beneath them and avoid heat buildup. Tensiometers were placed beneath the rain shelters to monitor soil water potential, and irrigation was applied when necessary to maintain a matric potential of <20 kPa during the treatment periods. Additional water was required only after the first week during the fourth treatment period.

Soil temperatures under raincovers and noncovered soils were measured using bimetal thermometers (Weston Electric Inst., Newark, N.J.). Three measurements in covered and noncovered soils were taken at 2- or 4-week intervals at 12.5 cm deep. Average daily precipitation and temperatures were recorded through the season.

Soil sampling and analysis. Soil was collected at the end of the 2-week treatment period by first removing a 30-cm-wide × 20-cm-deep soil column underneath the plant. Next, soil was screened through 12-mm square mesh hardware cloth to separate the roots from the soil. The screened soil was then mixed thoroughly and sampled. Samples were held at 2 °C for up to 2 d and then dried in a forced-air drier at 40 °C for 3 to 5 d, ground, and sifted again through a 2-mm screen (Custom Laboratory Equip. Inc., Orange City Fla.).

Inorganic N (NH_4^+ and NO_3^-) was extracted by agitating 20 g of soil in 100 mL 1 N KCl at 150 revolutions/min on a solution agitator (New Brunswick Scientific Co., Edison N.J.) for 45 min. Suspensions were passed through Whatman no. 5 filter paper that had previously been rinsed with deionized water and dried. Total ammonium and nitrate were determined with a flow-injection analyzer using Quikchem methods 12-107-06-1-A and 12-107-04-1-F, respectively (Lachat Instr., Milwaukee, Wis.).

After ammonium and nitrate concentrations were measured, a sequential diffusion technique was carried out (Brooks et al., 1989) to separate the ammonium and nitrate fractions. The ^{15}N enrichment of the separate fractions was determined by mass spectrometry (Europa Scientific Tracer Mass) according to methods of Harris and Paul (1989). The equation from Cabrera and Kissel (1989) was used to compute the percentage of N originating from fertilizer (NFF):

$$NFF = [(A - B)/(C - B)] \times 100$$

where A = atom %^{15}N of sample, B = atom % ^{15}N ambient, and C = atom % ^{15}N fertilizer (10.2%).

Plant sampling and analysis. Whole plants were removed 2 weeks after ^{15}N applications and partitioned into roots, stems, and current season's growth (shoots, leaves, and fruit). Soil was excavated at the base of the plant 1.0 m in diameter and 0.3 m deep. The soil was sifted through a 12-mm mesh screen to collect root tissues. Roots were rinsed thoroughly with tap water. Fresh mass of all tissues was recorded and dry mass was measured after forced-air drying at 40 °C for 5 d. Tissues were ground with a Wiley mill to pass through a 40-mesh screen, mixed thoroughly, and reground in a smaller mill with the same mesh. Total N and ^{15}N/^{14}N ratios of plant tissues were measured by mass spectrometry. Total N uptake and fertilizer uptake were calculated. The equation used for determining fertilizer content of plant material is the same as that indicated for soil N data.

Flowers and fruit on plants harvested at the end of the first three treatment periods were included with the new growth fractions. The first ripe berries were present at the conclusion of treatment period 3 (8 July). All mature and immature berries were harvested on 19 July from plants to be treated in periods 4, 5, and 6. Fruit dry mass per bush was determined, and these masses were added to the new growth fractions when the bushes were later excavated and partitioned. Six randomly selected fruit samples were analyzed for total N, and the total N content of the berries from the last three treatment periods was estimated by multiplying the fruit dry mass by the mean berry N concentration.

Experiment design and analysis. A randomized complete-block design with six single plant replicates was used. Factors were replication (block) and application time. Plants were selected from three rows and blocked according to size. At least one buffer plant separated treated plants. Plant and soil samples were statistically analyzed by two-way analysis of variance (M-Stat Statistical Package, Michigan State Univ.). Significant differences between means were determined with an LSD (5%).

Results

Treatment date significantly affected the quantity of fertilizer N absorbed by plants (Table 1). Bushes exposed to fertilizer for 2 weeks in late May, June, or July absorbed significantly more fertilizer N (146 to 194 mg, or 7% to 9% of that applied) than bushes treated in late April, August, and September (14 to 62 mg, 1% to 3% of applied).

Treatment date had similar effects on the amount of fertilizer N

in individual plant parts, such as roots, stems, and new growth (Table 1). Treatment in May, June, and July resulted in the greatest amount of fertilizer N in the new growth (68 to 81 mg) and stems one year or older (26 to 28 mg). Roots contained significantly more fertilizer N following treatment in late July (98 mg) than following treatment at any other date. Plants treated in April, August, and September tended to retain greater percentages of the fertilizer N in the roots (65% to 71%), whereas those treated in May, June and July partitioned more of their fertilizer N to aboveground parts (50% to 73%).

Total plant N was lowest in early May, increased until early August, then remained constant through August and September (Table 1). Plants accumulated N most rapidly between 8 July and 6 Aug. Changes in the total N content of the individual plant parts during the season generally reflected the pattern of N accumulation in the whole plant. The total N content of current season's growth (leaves, new stems, fruit) increased during May and June, then remained relatively constant from July to September. Fruit were removed from all plants on 19 July, between the third and fourth treatment periods. Fruit from six bushes contained an average of 250 mg/bush of N. The total N content of stems older than 1 year did not change in May or June but increased in July and September. Root tissue N content increased in July but remained constant at other times.

Plants accumulated dry mass gradually in May and June and most rapidly in July and early August. There was little change in dry mass between early August and October (fruit mass for the last three treatments was included). Patterns of dry matter accumulation in individual plant parts were similar to that of the whole plant.

Total inorganic soil N (fertilizer-derived plus native) concentrations at the end of each treatment period were not significantly different (data not shown). Nitrogen levels ranged from a low of 18.7 $\mu g \cdot g^{-1}$ soil in September to a high of 43.5 mg in June.

Total inorganic fertilizer-derived N in the root-zone soil was highest at the end of the first period in May (36.4 $\mu g \cdot g^{-1}$) and lowest in September (2.8 µg) (Table 2). Total fertilizer-derived N levels generally declined as the season progressed.

Fertilizer-derived ammonium concentrations expressed as N were highest following the first treatment period (31.6 $\mu g \cdot g^{-1}$) and lowest following the last three treatment periods (1.4 to 5.8 µg) (Table 2). Fertilizer-derived nitrate levels were similar after each treatment period, except after period 5, during which there was relatively high rainfall (Table 2).

Rainfall totalled 3.7, 5.9, 3.9, 6.9, 10.7, and 6.6 cm during treatment periods 1 to 6, respectively. Average soil temperatures at a depth of 15 cm were lower under raincovers (14.5 °C) than in uncovered soil (15.2 °C). Soil temperatures under the covers were 12, 14, 21, 17, 14, and 11 °C at the end of the six respective treatment periods.

Discussion

Application timing greatly affected the amount of fertilizer N absorbed by blueberry plants. Differences may reflect the demand by the plant and/or soil N availability. During most of the treatment periods in this experiment, plant N uptake appeared to be influenced primarily by plant N demand and growth.

Low fertilizer uptake in April (period 1) appeared to reflect low

Table 1. Fertilizer-derived N, total N, and dry mass of 'Bluecrop' blueberry bushes treated on different dates with ^{15}N-labeled ammonium sulfate and harvested 2 weeks later.

Treatment period	Application date	Sampling date	New growth[z]	Stems[y]	Roots	Whole plant
			Fertilizer-derived N (mg)			
1	23 Apr.	7 May	3 b[x]	1 c	10 c	14 b
2	25 May	8 June	81 a	26 a	39 bc	146 a
3	24 June	8 July	80 a	24 a	55 b	159 a
4	23 July	6 Aug.	68 a	28 a	98 a	194 a
5	24 Aug.	7 Sept.	13 b	5 bc	33 bc	51 b
6	23 Sept.	7 Oct.	9 b	12 b	41 bc	62 b
LSD (0.05)			26.6	9.1	37.6	60.0
			Total N (mg)			
1	23 Apr	7 May	170 c	420 cd	960 b	1550 c
2	25 May	8 June	740 b	370 d	830 b	1940 bc
3	24 June	8 July	1140 a	370 d	810 b	2320 b
4	23 July	6 Aug	1430 a	540 b	1490 a	3560 a
5	24 Aug	7 Sept	1360 a	500 bc	1380 a	3210 a
6	23 Sept	7 Oct	1100 a	760 a	1520 a	3330 a
LSD (0.05)			382	109	398	750
			Dry mass (g)			
1	23 Apr	7 May	5 b	68 d	79 b	152 c
2	25 May	8 June	35 b	69 d	93 b	198 bc
3	24 June	8 July	76 a	78 cd	90 b	244 b
4	23 July	6 Aug	102 a	102 bc	148 a	405 a
5	24 Aug	7 Sept	106 a	113 ab	160 a	411 a
6	23 Sept	7 Oct	104 a	141 a	165 a	441 a
LSD (0.05)			37	32	34	78

[z]Current season's shoots, leaves, and fruit.
[y]Aboveground canes >1 year old.
[x]Mean separation within columns by LSD, $P = 0.05$.

Table 2. Fertilizer-derived and native nitrate and ammonium N concentrations in the soil ($\mu g \cdot g^{-1}$) in the root zone 2 weeks after applications of ^{15}N-labeled ammonium sulfate on different dates.

Treatment	Application	Sampling	Fertilizer-derived N			Native N		
period	date	date	NH_4	NO_3	NH_4+NO_3	NH_4	NO_3	NH_4+NO_3
1	23 Apr.	1 May	31.6 a[z]	4.8 ab	36.4 a	5.1 c	0.8 c	5.9 c
2	25 May	8 June	18.9 b	7.2 a	26.1 ab	14.5 a	2.9 c	17.4 ab
3	24 June	8 July	10.4 bc	5.5 a	14.9 bc	8.9 bc	5.8 b	14.7 b
4	23 July	6 Aug.	4.2 c	6.3 a	10.5 cd	10.6 ab	11.5 a	22.1 a
5	24 Aug.	7 Sept.	1.4 c	1.4 b	2.8 d	8.3 bc	7.7 b	15.9 b
6	23 Sept.	7 Oct.	5.8 c	7.7 a	13.5 cd	7.4 bc	5.8 b	13.2 b
LSD (0.05)			9.2	3.7	11.3	4.8	2.7	5.9

[z]Means separated within columns by LSD, $P = 0.05$.

plant demand. Buds were breaking during this time, but, since shoots and leaves had not emerged, transpiration was likely inadequate to move soil water and N to the roots. Plant dry matter accumulation was likely minimal during these 2 weeks, so plants may also have had little demand for N at this time. Soil N did not appear to be limiting, since fertilizer-derived N in the soil at the end of this treatment period was higher than after other periods (Table 2). Also, 84% of the inorganic fertilizer N was in the ammonium form, which is absorbed more rapidly by blueberries than the nitrate form (Merhaut and Darnell, 1995; Peterson et al., 1988). Absorption of labeled fertilizer N can be reduced by high native soil N levels because labeled N concentrations are effectively diluted within the larger pool of native N. This did not appear to occur during period 1, since native ammonium and nitrate levels were low at this time (Table 2).

Absorption of fertilizer-derived N was greatest from late May to early August during periods 2 to 4 (Table 1). Plants were between early petalfall and postfruit harvest during these periods and were rapidly accumulating dry matter and total N in stems, new growth, and roots (Table 1). High fertilizer N absorption occurred in spite of the fact that fertilizer-derived ammonium and nitrate levels in the soil were declining from earlier levels, in actual concentrations and relative to native ammonium and nitrate levels. Greater plant uptake may have contributed to the decline in soil N concentrations. Efficient uptake of fertilizer N at these times appears to reflect the high demand by bushes.

Low fertilizer N uptake during late August to early September (Table 1) apparently resulted from low soil N availability rather than low plant demand. Soil fertilizer N concentrations (ammonium and nitrate) were lower at the end of this treatment period than after any other period (Table 2). The fertilizer N to native N ratio in the soil was also extremely low. As a result, fertilizer N was diluted within the native N pool. The low levels of fertilizer N in the soil and limited absorption by bushes may have resulted from the high rainfall during this treatment period (10.7 cm) compared to the other periods (3.7 to 6.9 cm). Rain may have leached fertilizer N from the root zone, whereas native N levels were replenished by mineralization and remained relatively high. The low absorption of fertilizer N during this period likely resulted from the unusually high rainfall during this study and may not accurately reflect plant demand.

Fertilizer N uptake was also low during the final treatment period in September (Table 1). This likely reflected the low plant demand associated with the onset of dormancy. Leaves were senescing during this period, and plant dry mass was no longer increasing. Total plant N also did not change from levels during the previous treatment period. Uptake was not limited by soil fertilizer N since concentrations were similar to those observed in the middle of the season (Table 2) when absorption was maximum. Soil fertilizer N levels were also high relative to native N levels, so fertilizer N uptake was not reduced due to dilution in the soil.

The relative proportions of ammonium and nitrate in the soil 2 weeks after applications gives some indication of nitrification rates. Nitrification appeared somewhat slower early in the season, since only 16% and 29% of the fertilizer-derived N was in the nitrate form at the end of periods 1 and 2, respectively. Nitrification rates may have been higher later in the season, since 34% to 55% of fertilizer N was in the nitrate form after treatment periods 3 to 6. Nitrification may be undesirable in blueberry soils since nitrate is more prone to leaching and absorbed less readily by blueberries.

Absorption of fertilizer N by blueberry bushes was generally proportional to growth rates. Efficient absorption occurred only after shoots and leaves had begun active growth, and absorption decreased as growth ceased late in the season. This overall pattern is consistent with findings in plum (Weinbaum et al., 1978) and grapevines (Conradie, 1986). Results indicate that blueberries have a high N demand and absorption capacity from late bloom (late May) until after fruit harvest (mid-August). Fertilization practices that maintain sufficient N in the root zone during this 2- to 3-month period would likely optimize NUE. Granular fertilizers may need to be applied slightly earlier to allow time for granules to dissolve and N to move into the soil. A second study on the same soil indicated that fertilizer increased N levels in the root zone for only 6 to 8 weeks after application (Throop, 1995). Multiple applications may be necessary to maintain sufficient soil N levels throughout the period of high demand. Single fertilizer applications may not provide for efficient N use; but, if this is practiced, the optimum timing may be after bloom, rather than at budbreak or earlier.

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