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NITROGEN USE EFFICIENCY AND SOIL NITRIFICATION RATES IN HIGHBUSH BLUEBERRIES (*Vaccinium corymbosum* L.)

By

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ABSTRACT

NITROGEN USE EFFICIENCY AND SOIL NITRIFICATION RATES IN HIGHBUSH BLUEBERRY (Vaccinium corymbosum L.)

By

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Seasonal nitrogen demand of young highbush blueberries in the field was compared by applying N fertilizer on six dates between late April and September. Ammonium sulfate solutions (10.2 atom % 15N) were dripped into the root zone of single bushes. Bushes were excavated after two weeks of exposure and analyzed for N. Bushes treated in late May, June and July absorbed a greater percentage of applied N (7-9%) than bushes treated in April, August or September (1-3%). N uptake was generally dictated by plant demand rather than by soil N concentrations or availability.

Also, fertilizer N uptake by blueberries was compared with and without soil applied dicyandiamide (DCD), a nitrification inhibitor. DCD did not effect plant fertilizer uptake. Soil nitrate content was significantly lower in DCD treated soils for 2 weeks following application, but ammonium levels were not affected.

Dedicated to my son Calen, who
shares a fascination for plants.

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TABLE OF CONTENTS

LIST OF FIGURES

Chapter I

Chapter II

LIST OF TABLES

Chapter II

INTRODUCTION

There is a need to optimize nitrogen use efficiency (NUE) in crop production. NUE is defined as the amount of nitrogen (N) recovered by the plant divided by the amount of fertilizer N applied (Weinbaum, 1992). This is a useful term because it includes environmental factors that effect N plant recovery, not simply efficiency of uptake by the plant.

Movement of N from agricultural soils and subsequent contamination of non-target areas can present a risk to health. Loss of N also results in an increase of fertilization and energy costs (Keeney, 1982; Stevenson, 1982).

This research was conducted to assess blueberry N demand through the season. A knowledge of plant uptake through the season is necessary to make appropriately timed N applications and optimize NUE (Weinbaum, 1992). Also, effectiveness of the nitrification inhibitor dicyandiamide (DCD) was tested. It may be desirable to prevent nitrification in blueberry soils to avoid leaching losses and to enhance plant N uptake.

Experiments are presented in sections, each of which is in the format of the Journal of the American Society for Horticultural Science.

A general literature review, which follows, will provide a detailed summary on the subject of nitrogen usage in agriculture and in blueberry systems specifically.

LITERATURE REVIEW

Potential for N Contamination From Blueberry Crops, Health and Environmental Risks

Adverse health and environmental effects caused by increased levels of N in well water include a correlation with increased incidence of methemoglobinemia (blue baby syndrome) in infants. Methemoglobinemia can occur when Fe in hemoglobin is oxidized by nitrate from drinking water resulting in methemoglobin (NRC, 1978).

In addition, contamination by N forms can lead to increased cancer rates caused by nitrosamine produced in the stomach after consumption of nitrate, respiratory illness caused by peroxyacyl nitrates, alkyl nitrates, NO_3^- aerosols, NO_2^- and HNO_3 vapor in the atmosphere (NRC, 1978). Also, HNO_3 increases acidity of rain, NO_2^- from denitrification can lead to depletion of stratospheric ozone and excess to non-crop plants can lead to eutrophication of waters and excess plant growth elsewhere (Keeney, 1982).

In general, N levels in ground water have risen with increased use of N fertilizer (Keeney, 1982). However, there has been little effort to assess the extent of N contamination resulting from N applications in blueberry

fields. In Western lower Michigan, where most blueberry farming is carried out in the State, Baum (1990) found that 11.7% of 883 wells tested contained nitrate in excess of the maximum permissible contamination level for drinking water according to EPA guidelines. Baum found nitrate levels in wells near blueberry plantations were lower than those in comparable rural sites. This was attributed to the relatively low amounts of N used and dilution from high water infiltration through porous soils. Baum observed that wells are vulnerable to contamination near typical blueberry sites where highly permeable soils are present (Baum, 1990). It should be noted that the authors study focused on herbicide leaching and the nitrate study was not rigorous (Baum, Personal Communication). For this reason, results from the study may therefore be in question.

Loss of N from the target site also represents an economic loss since N is no longer available for the growth of crop plants. Ammonium sulfate applications represent nearly 5% of the costs in an established blueberry operation (Kelsey and Thomas, 1993). Urea is a less expensive N source that is also used. Costs will undoubtedly increase with greater human population and resulting demand for N which is needed for increased food production (Keeney, 1982).

General Blueberry Crop Characteristics and Culture

The roots of the blueberry (Family: Ericaceae, Vaccinium sp.) tend to be fibrous, lacking in root hairs and

grow shallow. Depth of rooting is often limited by existence of a heavy B horizon (Eck, 1988). The water table in typical Michigan blueberry areas tends to be shallow, further limiting root penetration. Blueberries generally require porous soils to thrive (Eck, 1988). Ideal pH is 4.0 to 5.0. In blueberry cropping situations, N is the only nutrient that is usually required each year. Deficiencies of other nutrients are less common. Recommendations in Michigan call for applications of from 17 to 75 kg N/ha (depending on bush age and size) applied as ammonium sulfate or urea just before or during bud break (Hanson and Hancock, 1986). Split applications are recommended on coarser soils so that half is applied at bud break and half at petal fall (Hancock and Hanson, 1986). Split applications on coarse soils improved yields over single applications (Hanson and Retamales, 1992; Hancock and Hanson, 1986). Fertilization based on soil and leaf analysis has been practiced since 1964 (Kenworthy, 1979; Kenworthy et al.,1985; Hancock and Hanson, 1986).

The ammonium form of N is most efficiently used by blueberries (Hancock and Hanson, 1986; Cain, 1952, Peterson et al., 1988; Townsend, 1968; Sugiyama and Ishigaki, 1994) but tends to acidify the soil during the nitrification process when two H+ ions are produced for each ammonium ion that is oxidized to a nitrite ion (Faurie et al., 1990). Urea is less acidifying than ammonium sulfate because of an initial localized increase in pH following hydrolysis.

Higher uptake efficiency for ammonium over nitrate forms and the physiological dynamics involved are still not well understood although low nitrate reductase activity found in blueberry plants may explain this relative inefficiency in utilizing nitrate (Townsend 1970; Havill, Lee and Stewart, 1974; Dirr et al., 1971; Sugiyama and Ishigaki, 1994).

Gough et al. (1987) found that a temperature range between 14-18C corresponded with most active unsuberized root and shoot growth in blueberry. An initial peak was seen in both shoot and root growth at fruit set, with shoots showing a greater rate of growth than roots. Bloom to fruit maturity coincided with a decrease in both shoot and root growth. After removal of fruit, root and shoot growth rate peaked again with roots exhibiting a faster rate of growth than shoots. Gough (1987) characterized root and shoot growth as being positively correlated rather than competitive.

Kender (1967) found plant growth rate was higher at 30C than 18C in lowbush blueberry. Significant differences in shoot length were seen at both temperatures under daylengths of 10 and 16 hours although growth was retarded in the plants held at 18C. Flower bud initiation tended to occur under long nights and vegetative growth was encouraged under the shorter night breaks. Hall and Ludwig (1961) made similar observations on highbush blueberries where daylengths of 8, 10, 12, 14 and 16 hours were used at

temperatures of 16 and 20C. Growth rate slowed at the lower temperature. In addition, the authors saw a tendency for flower bud initiation in short days (<12h), although two of seven clones tested produced flower buds under 16 hour days. Vegetative growth increased with longer day length. Cooler temperatures resulted in less growth at given daylengths.

Dynamics and Potential for Nitrification, Denitrification and Leaching in Blueberry Cropping.

Dynamics of Nitrification

In the soil, decomposing proteins, inorganic N and other nitrogenous substances that originate from organic matter and chemical inputs can be oxidated by chemo-autotrophic and heterotrophic organisms. Metabolic transformations of ammonia, nitrite and organic N can lead to production of nitrate. This phenomenon is referred to as nitrification (Schmidt, 1982). Faurie (1990) estimates that 75% of inorganic N may be nitrified in cultivated soils although rate of nitrification tends to drop with lowered pH.

Primary nitrifiers belong to two general groups of chemolithic, autotrophic soil bacteria in the family Nitrobacteraceae: ammonia and nitrite oxidizers (Prosser, 1989). All identified members of Nitrobacteraceae are gram-negative but exhibit a wide range of morphologies.

It appears that various strains evolved the ability to utilize ammonium or nitrite independently (Prosser, 1989). Ammonia oxidizers include the genera Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus and Nitrovibrio. Identified nitrite oxidizers include the genera Nitrobacter, Nitrococcus, Nitrospira and Nitrospina. Nitrosomonas spp. and Nitrobacter spp.. These are the only genera that have been studied in detail (Prosser, 1989).

Many of these organisms are capable of reversing the process of nitrification and can produce nitrite, ammonia, nitric and nitrous oxides and nitrogen gas (Prosser, 1989). Energy is produced exclusively through utilization of carbon dioxide, water and ammonia or nitrite substrates and are thus, by definition, autotrophic.

Ammonia and nitrite often provide minimal energy for growth since 80-95% of this energy is required for reduction equivalence and fixation of carbon dioxide (Kelly, 1978; Glover, 1985). Nitrifiers may adapt to low N levels by increasing the amount of cell membrane needed for intake. In addition, inhibition can occur with high levels of substrate, notably ammonium (Prosser, 1989). Thus, in general, peak efficiency of nitrifiers occurs in an apparently narrow range of N concentrations.

Many heterotrophic nitrite, ammonium and amino nitrogen oxidizers have been identified (Focht and Verstraete, 1976). Stroo et al. (1986) identified a nitrate forming, acid

tolerant fungus named Absidia cylindrospora, that produced nitrate in the presence of soil in culture media to pH 3.2.

The nitrification rate of heterotrophic organisms is thought to be 1,000 to 10,000 times lower than that of autotrophs (Focht and Verstraete, 1976). The population of heterotrophs that can be supported by available soil resources is estimated to be 100 to 1000 times smaller than that of autotrophs under similar conditions and it is unlikely that heterotrophs derive significant amounts of energy from the process of nitrification (Focht and Verstraete, 1976). The C/N ratio is of much greater importance since rate of metabolism in heterotrophs is primarily limited by carbon and less by N content.

Much has been published implicating heterotrophs as the main agents of nitrification at low pH (<4.5) (Focht and Verstraete, 1976). Evidence included low populations of autotrophs, nitrate formation correlated with carbon content or additions of organic N from peptone which may be utilized by heterotrophs, and inhibition with various forms of ammonium (Focht and Verstraete, 1976). However, the identification of a truly acidophilic strain of Nitrobacter (Hankinson and Schmidt, 1988) and the apparent ability for autotrophic adaptation to low pH provides evidence for autotrophic activity at low pH (Bhuiya and Walker, 1976; Hankinson and Schmidt, 1984; Walker and Wickramasinghe, 1978; Martikainen and Nurmiah-Lassila, 1984).

Studying the effects of pH on nitrifiers is difficult. Often results may be an artifact of the medium used and adaptations by organisms. For example, Allison (1989) found N.europaea active in sand culture to pH 5.7. The same strain was not active in liquid medium below pH 7.0.

Various mechanisms for autotrophic adaptation to low pH have been cited. Molina (1985) described a mechanism whereby clumping of ammonia oxidizers around soil micro-aggregates resulted in a neutralized site. Hankinson and Schmidt (1985) and Overrein (1967) theorized that heterotrophs mineralized organic nitrogen and this release of ammonia would then raise pH in a soil microsite followed by a reduction in pH from nitrifying autotrophs. Studies where inhibitors were used to differentiate autotrophic activity showed that autotrophs were indeed nitrifying at low pH. Further assay showed that these organisms were not acidophilic, i.e. could not thrive under acid conditions in liquid medium (Prosser, 1989). It appeared that there was a coupling of heterotrophic mineralizers which tended to raise pH, and autotrophic nitrifiers that lowered pH. It is thought that close proximity modified the microsite to the advantage of both types of organisms (Prosser, 1989).

Specific nitrifying autotrophs that operate at pH 4.0 to 5.0 have been identified in forest and tea plantation soils. These include Nitrosolobus, Nitrosomonas, Nitrospira and Nitrovibrio. (Bhuiya and Walker, 1976; Hankinson and

Schmidt, 1984; Walker and Wickramasinghe, 1978; Martikainen and Nurmiah-Lassila, 1984). None of these nitrifiers were found to be truly acidophilic in lab cultures. Hankinson and Schmidt (1988) however, isolated a strain of Nitrobacter that oxidized nitrite at pH as low as 3.5 in pure culture.

Management practices may increase potential for nitrification. Eaton and Patriquin (1988) observed a greater capacity for nitrification where lowbush blueberry field soils had been previously fertilized with ammonium compared to those that had not been fertilized. It is generally agreed that nitrifier populations increase rapidly upon moderate addition of ammonium in soil (Hadas et al., 1986; Sabey et al., 1959; Walker, 1975).

Hadas et al. (1986) studied nitrification rates in profiles of several soils that were either cultivated or left undisturbed. Nitrification rates were much higher in cultivated and fertilized soils than in undisturbed control soils.

A gradual decrease in nitrification rate and a delay of nitrification onset occurred with increased depth in fertilized soils. The decrease in nitrification rate with depth was more dramatic in undisturbed soils. Either a decrease in pH or HCO concentrations greatly decreased the maximum rate of nitrification (Hadas et al., 1986).

Nitrification Inhibition

Nitrification was reduced by slight decreases in pH, especially in lower pH ranges (Wickramasinghe et al., 1985). Salt additions such as potassium chloride and N-P-K fertilizers reduced nitrification (Strayer et al., 1981, Wickramasinghe et al., 1985; Hendrickson et al., 1978). Nitrification is reduced by higher ammonium applications (Dancer et al., 1973; Lange and Elliot; 1991), larger urea granule size, lower temperatures and incorporation of fertilizers (Sudhakara and Prasad, 1985; Yadvinder-Singh and Beauchamp, 1987; Hendrickson et al., 1978). Species and quantity of native nitrifier populations also play a crucial role in nitrification rates (Keeney, 1978).

Various pesticides inhibit nitrification in both ammonia and nitrite oxidizing autotrophs (Winely and San Clemente, 1971; Nishihara, 1962). Organic matter composition, CEC and pH may also effect rate of nitrification (Keeney, 1978).

Characteristics of nitrification inhibitors that are desirable for agriculture include an ability to block oxidation of ammonia but not phytotoxic nitrite, do not otherwise harm beneficial or non-target flora or fauna, a longevity of at least several weeks following fertilizer application, and are inexpensive (Hauck, 1980). Benefits of reduced nitrification include less loss due to leaching and potentially greater plant availability. Use of nitrification

inhibitors may increase the possibility for gaseous loss of N (Prakasa Rao and Puttanna, 1987; Martin et al., 1993). Use of an inhibitor that would maintain the ammonium form would be ideal for blueberry culture since ammonium is utilized more efficiently than nitrate (Townsend, 1970; Havill et al., 1974; Dirr et al., 1971; Sugiyama and Ishigaki, 1994).

There are at least 12 inhibitors that are used commercially or in research. Nitrapyrin (2-Chloro-6-(trichloromethyl) pyridine) is the most commonly used inhibitor in the United States and is effective in inhibiting certain autotrophs but not heterotrophs (Hauck, 1980). Nitrapyrin acts by inhibiting cytochrome oxidase involved in oxidation of ammonia in Nitrosomonas and is effective in a pH range of 3.6 and higher (Kreitinger, 1985; Wickramasinghe, 1985). Optimal pH for inhibition with nitrapyrin is in the neutral to alkaline pH range (Alexander, 1965; Focht and Verstraete, 1977).

The nitrification inhibiting effect of dicyandiamide (DCD) has been known for many years, although its mode of action is not well understood. Dicyandiamide sulfate inhibits cytochrome oxidase in intact cells or extracts of Nitrosomonas europea (Nuti et al., 1975) and blocks oxidation of ammonium to nitrite (Vilsmeier, 1981).

DCD is decomposed through enzymatic reaction or surface catalysis with iron oxides where water is added to DCD to

form guanylic urea. Deamination and decarboxylation through microbial metabolism causes formation of guanidine. Further degradation results in urea. Further hydrolysis occurs with resulting ammonium and carbon dioxide products and thus serves as an N fertilizer after decomposition (Vilsmeier, 1981). Breakdown of DCD is strongly temperature dependant. This is likely a result of temperature effects on microbial activity (Vilsmeier, 1981). Although the effectiveness of DCD under field conditions varies, it is generally less effective than nitrapyrin in reducing nitrification (Martin et al., 1993; Wickramasinghe et al., 1985). DCD was found to be ineffective in a neutral Highfield soil (Wickramasinghe et al., 1985). Prakasa Rao and Puttanna (1987) found that nitrification was inhibited on a sandy loam soil at high rates (15 to 20 ppm) of DCD.

DCD greatly increased volatilization losses of N when surface applied with urea. Others have reported problems with increased ammonia volatilization losses and resulting lowered yields with use of DCD and other inhibitors particularly with use of urea (Bundy and Bremner 1974, Cornforth and Chesney 1971, Rodgers 1983, Clay et al.1990, Fox and Bandel 1989). Inhibitors should be incorporated into soil to reduce gaseous losses (Rodgers 1983).

Another dynamic that limits the apparent effectiveness of inhibitors and resulting plant uptake occurs when nitrogen rates are in excess of plant requirements and

therefore no differences in yield are discerned. Martin et al. (1993) found potato yield response to DCD in sandy, irrigated soils insignificant except at a moderate level of fertilization and a DCD rate of 11.6 kg/ha. Conditions conducive to leaching may also have made DCD effects more noticeable.

Vilsmeier (1991a) found decreased yield in potted wheat with DCD applications. Total N uptake was equal in DCD treated and untreated plants but yield was lower in DCD treated plants. The author theorized that N was displaced in the plant by unaltered (metabolically inactive) DCD.

To summarize, factors that seem to favor the effective use of DCD include application with moderate to lower temperatures, lower pH, use with moderate fertilization, proper rate of DCD application, soil incorporation and avoidance of excessive irrigation.

Denitrification

Denitrification is the biochemical reduction of nitrate or nitrite to gaseous nitrogen either as molecular N or an oxide of N (Foth, 1988). Denitrification occurs under anaerobic conditions and may be confined to microsites in the soil (Focht and Verstraete, 1976). Christenson (1985) identified 15 species of bacteria responsible for gaseous N production, the most commonly identified genera being *Alcaligenes* and *Pseudomonas*. Denitrifying bacteria that

operate at low pH include Pseudomonas spp. and Bacillus spp (Christenson, 1985; Wickramasinghe, 1985). In peat soils, increased pH increases denitrification (Firestone, 1982).

There appear to be two biochemical pathways for nitrate reduction utilizing either assimilatory or dissimilatory nitrate reductase. Assimilatory nitrate reductases are soluble enzymes which can be inhibited by chlorate but not oxygen. Dissimilatory nitrate reductases are particle bound and competitively inhibited by azide and oxygen. Both reductases have the same proteins but are controlled for different activity through cellular regulation (Focht and Verstraete, 1976). Denitrification begins with nitrate reduction and results in dinitrogen as represented by the following (Cox and Payne, 1973):

$$NO_3^- > NO_2^- > NO > N_2O > N_2$$

Rate of denitrification is limited by the C/N ratio of the soil. Beyond limitations of carbonaceous substrate, rate of denitrification is thought to follow a zero or first order kinetic model depending on the carbon and nitrate concentration. Maximum rate of denitrification and loss of N in a desert soil saturated with glucose and nitrate was found to be 1500 ug N/ml soil/day (Focht and Verstraete 1976). Optimal C/N ratio for oxidation of carbon and reduction of nitrate is thought to be between 2 and 3 in

waste water (Dawson and Murphy, 1973).

Wetting and drying cycles have a major effect upon all microbial processes. Nitrification and denitrification rates have been shown to increase drastically after wetting of air dried soils (Birch, 1958). Losses of organic N have also been found to be greatest under severe wetting and drying cycles (Cawse and Sheldon, 1972).

Denitrifiers are active in a range between 15 to 75C although optimal temperatures are 25 to 35C (Buswell et.al., 1954). Campbell et al. (1971) found fluctuating ammonium levels in sterile soils with varied temperatures which suggests a non-biological mineralization process.

Volatilization

Volatilization of N as ammonia can be a significant source of loss when ammonium fertilizers are used (Rodgers, 1983; Stevenson, 1982; Nelson, 1982). Calcareous soils with a low CEC and thus low ammonium adsorption ability favor volatilization (Nelson, 1982). Similarly, pH of the soil solution can determine the rate of gaseous loss because of the effect H^+ concentration has on ammonium and ammonia equilibria, although this effect can be modified by soil texture and CEC (Nelson, 1982). Additions of ammonium sources can modify the pH in localized areas and also influence the rate of loss (Nelson, 1982). Volatilization can be reduced through incorporation of N into the soil (Rodgers, 1983; Nelson, 1982).

Leaching

Factors that influence rate of leaching of N include permeability and porosity of soil, water flow, chemical form of N applied, availability of oxygen, and cation exchange capacity of the soil.

Although ammonium tends to be adsorbed to soil particles, nitrate is an anion that does not adsorb to negatively charged soil particles and is easily leached. For this reason nitrification is a concern. In many soils nitrate leaching is the primary cause of nitrogen loss (Eaton, 1987).

From a study carried out in a commercial blueberry field Retamales and Hanson (1990) reported a general decline in topsoil NH_4^+ and NO_3^- and an increase in subsoil NH_4^+ and NO_3^- 2-3 months after fertilization which was probably due to leaching. Potential for surface and ground water contamination can be high where chemical inputs are easily leached. Coarse, porous soils which are common to blueberry croplands may contribute to leaching problems.

Campbell et al., (1993) found increased leaching of N below the root zone with increased N and water inputs as a result of ground cover management in wheat cropping. Also, It was suggested that low N rates resulted in poor tillering of wheat and less transpiration which in turn resulted in lower plant recovery and greater leaching of N. Wheat recovery of N is generally higher than in perennials

(Powlson et al., 1986; Weinbaum, 1992) thus, one might expect to see greater losses under blueberry with similar inputs. Several models exist to predict leaching potential at a given site. One model called NLEAP used by Campbell et al. (1993) uses daily precipitation, pan evaporation and mean monthly air temperature as variables. Crop management inputs, including amount of fertilizer applied and expected yields were based on experimental data. The model predicted actual trends except where N dynamics were modified by high water inputs and high and low N rates as described in the previous paragraph (Campbell et al., 1993).

Immobilization, Mineralization and Use of Labelled Nitrogen

Immobilization usually refers to transformations of N that result in forms unavailable to plants. Immobilization occurs through incorporation of N into the microbial biomass with resultant resistance to further availability for plant use and degradation (Bartholomew, 1965; Jenkinson et al., 1985). Substantial percentages of applied N may be incorporated into the biomass within 1 day of application of ammonium sulfate (McGill et al.,1975) and instances of near complete immobilization of labelled N have occurred within one week after application (Kelley and Stevenson, 1985).

When labelled N is immobilized, remineralized 15N may be very dilute as a result of mixing with a large natural N reserve in the soil biomass. In effect, a 65 kg/ha application of N may be diluted by a 3000 kg organic N

reserve during the immobilization phase (Bartholomew, 1965), although this probably presents an over-simplified view of mineralization.

Mineralization rate may be governed by the type of carbon and N reserve pool into which the N has been incorporated. Reserve pools can be referred to based on N availability: a) a debris pool which may release inorganic N easily, b) an active pool consisting of microbial cells and metabolites, c) a medium turnover pool which may store N for decades to centuries, and d) a stable pool which releases mineral N most slowly. N stability, in the case of medium and stable pools, is thought to be induced through physical and chemical transformations which restrict access to microbial enzymes and uptake and availability in general (Strickland et al., 1992).

Between one to ten percent of the organic N reserves in a soil may be mineralized within a year (Bremner, 1965; Scarsbrook, 1965). Immobilization and mineralization dynamics may make it necessary to use short treatment periods to avoid confounding results in plant N uptake experiments.

It is important to maintain a relatively constant level in the $^{15}N/^{14}N$ ratio when using labelled N for studying plant assimilation efficiency. Uptake efficiency may appear to be greater if the $^{15}N/^{14}N$ ratio in soil is greater. This may occur through depletion of the existing pool of inorganic N

in the soil. Conversely, uptake efficiency may appear be low with a comparatively lower $^{15}N/^{14}N$ ratio.

A similar consideration when carrying out fertilizer efficiency studies is the phenomenon whereby N is immobilized or mineralized from soil with the addition of inorganic N. This effect has been termed the "added nitrogen interaction" (ANI) as described by Jenkinson et al. (1985). Generally an increase in soil inorganic N in addition to the applied N has been reported (Azam et al., 1993). Addition of salts, particularly ammonium salts, have been shown to increase mineralization as a result of death of microorganisms. Nitrate additions seem to have no such effect (Jenkinson et al.,1985).

Apparent ANIs can be minimized where treatment periods are short, plants are already established and fertilizer N is separated from inorganic soil N in time or space (Jenkinson et al., 1985). Similarly, if fertilizer N concentrations are well above the natural inorganic pool, ANIs may be minimized. High organic matter content can also increase the possibility for ANI by virtue of greater capacity for mineralization (Jenkinson, 1985). Thus, it is important to consider the effect of ANI's in N uptake studies before drawing conclusions.

It is important to consider mineralization and immobilization potentials of a given soil when determining application rates. There may be considerable native

inorganic N released from organic matter that may fulfill plant requirements (Greenham, 1976; Bremner, 1965; Scarsbrook, 1965). Conversely, immobilization of N may occur at an unusually high rate, for example with the addition of sawdust or other mulch, as is commonly practiced in blueberries.

Nitrogen Uptake and Utilization by Perennial Plants

Weinbaum et al. (1978) found a highly significant correlation between the presence of leaves and efficiency of N uptake in prune trees. Trees with the greatest leaf mass also had the highest fertilizer N content. Nitrogen uptake efficiency was thought to be reduced during the period of fruit maturation because carbohydrates were diverted from the roots limiting energy dependant uptake of nitrate. Soil temperature was a minor factor in plant regulation of N uptake by the plant.

The authors also found the largest percentage of N in the roots during bud swell. During rapid shoot growth the largest percentage of N was translocated to the shoots with only 18% in the roots at that time (Weinbaum et al., 1978).

Weinbaum et al. (1992) advised against dormant season fertilization because of likelihood of leaching and/or denitrification and inability of the plant to take up N prior to the period of leaf expansion and rapid shoot growth. Timing of applications should be based on demand by

the plant (Weinbaum et al., 1978; 1992)

Weinbaum (1981) also stressed the importance of prompt irrigation after dry surface fertilization for movement of fertilizer to the root zone and to ensure availability during periods of high N demand. This would depend on the type of carrier used.

Chuntanaparb and Cummings (1980) found a pattern similar to that described by Weinbaum et al. (1978) regarding seasonal variation of leaf N. Concentrations of N were highest early in the season then dropped near midsummer where levels either elevated slightly, in the case of apple grape and peach, or stabilized and continued to fall at a lower rate in mid-August, in the case of blueberry, when presumably leaf senescence was initiated.

Nitrogen recovery by blueberry is relatively low with standard granular fertilizer surface applications. Retamales and Hanson (1989) followed the fate of urea applied to mature blueberry bushes. Plant NUE was 32.4%. Fifteen percent was still within the soil root zone. The remainder was leached, volatilized or incorporated in soil organic matter. This is a typical rate of uptake for perennial fruit plants. Generally N uptake efficiency ranges from 25% to 50% (Weinbaum, 1992). By way of contrast, N recovery in wheat ranged from 51% to 68% the first year and increased to over 90% after 4 years (Powlson et al., 1986).

Dramatic increases in NUE using drip fertigation have

been demonstrated (Weinbaum, 1992). Uptake efficiency was increased 2 to 3 times over previous surface applications when drip fertigation was used in cherry and grape respectively (Weinbaum, 1992).

Proper timing of fertilizer applications may increase N use by plants and avoid waste and possible harmful effects caused by movement of N away from the plant. Retamales and Hanson (1992) found higher blueberry yields using split applications at bud break and at petal fall compared to single applications at bud break.

Timing of fertilization should be based on plant demand (Weinbaum, 1992). Retamales and Hanson (1989) reported that fertilizer N was the primary form of N utilized in the leaf. This may be indicative of the importance of available soil N during leaf growth.

Growth may be also be supported by N absorbed the previous season. Birkhold and Darnell (1993) studied the use of stored N in 2 year old, potted rabbiteye blueberry and found that plant stored N was the primary source of nitrogen in reproductive tissue up to fruit maturity when 50% of the total N was still from the previous year. This suggests that maintaining adequate plant and soil N levels late in the season may be important.

Summary

Factors that may influence the availability of N to blueberries have been discussed. A summary of these interacting variables include: a) Plant N demand, b) Microbial, physical and chemical activity in the soil, c) Timing of N applications, d) Climate and irrigation regime e) Soil characteristics such as texture, CEC and organic matter content, f) Soil management practices such as tillage and use of mulch.

An attempt was made to understand N dynamics under conditions similar to those found in blueberry production. However, relatively few published studies deal specifically with fate of N in blueberry culture. This may be because blueberry production represents a small portion of overall agricultural production and resources for research and public interest in general are commensurate. No field studies have been carried out to follow N demand in highbush blueberries through the growing season. A detailed description of N uptake in blueberries might be useful in determining fertilization timing and thereby increase the efficiency of N usage. For this reason one of the following experiments was carried out to measure the rate of N uptake in field grown blueberries throughout the season as a means to gain insight into the proper timing of N application.

Also, plant N and soil nitrate and ammonium balances as influenced by presence of the nitrification inhibitor dicyandiamide (DCD) were studied. Nitrification of ammonium sources of fertilizer is a phenomenon that may affect blueberry growth directly since ammonium is utilized more efficiently by the plant than nitrate. Also, nitrate is not adsorbed to soil particles and is therefore likely to leach, unlike ammonium.

These issues concerning plant and soil N status are of practical importance to the grower in optimizing the efficiency of fertilizer usage for economical production of high quality fruit. Likewise, it is important to understand soil N dynamics in blueberry production when considering the broader issue of environmental nitrate contamination.

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CHAPTER 1

Effect of Application Date on ^{15}N Uptake Efficiency of Highbush Blueberry

ABSTRACT

Absorption of ^{15}N-enriched ammonium sulfate by young highbush blueberries (Vaccinium corymbosum L. var. "Bluecrop") was compared following applications on six dates between late April and September. Ammonium sulfate solutions containing 2.1 grams 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. Bushes were excavated after two weeks of exposure and separated into roots, stems and current seasons growth (new shoots, leaves, fruit). Tissues were dried, weighed and analyzed for ^{15}N and ^{14}N by mass spectrometry. Soil was also analyzed for fertilizer and total N. Bushes treated in late May, June and July absorbed a greater percentage of applied N (6-9%) than bushes treated in April, August or September (1-3%). Generally Plants absorbed N in proportion to their demand rather than as a result of soil nitrogen availability.

INTRODUCTION

Proper timing of fertilizer applications may increase nitrogen (N) usage by plants, reduce waste, and minimize the detrimental effects of N movement into non-target areas. Highbush blueberries are adapted to porous soils with shallow water tables. These conditions are also highly suited to leaching and movement of applied N. Retamales and Hanson (1989) reported a general decline in fertilizer NH_4^+ and NO_3^- within the root zone of mature blueberries and an increase in levels below the root zone two to three months after fertilization, which was probably due to leaching.

Blueberries may recover relatively low percentages of added N. Retamales (1988) reported that only 32.4% of labelled urea N applied at bud break went into mature bushes. The remainder was either still within the root zone (15%), leached or volatilized. This figure is consistent with results in other studies or woody fruit crops where uptake efficiency was measured. Uptake efficiency for perennial fruit crops ranges from 20% to 50% depending on physiological and environmental factors (Weinbaum, 1992). In contrast, wheat may have an uptake efficiency of over 90% in the field (Powlson et al., 1986).

The effect of N form on growth and yield of blueberries has been studied in detail (Bailey et.al., 1966; Townsend, 1970; Havill et.al., 1974; Dirr, 1971; Bishop et al., 1971; Sugiyama and Ishigaki 1994). Also, seasonal fertilizer application rates based on leaf N content and soil analysis have been used on a voluntary basis since 1964 (Kenworthy 1979; Kenworthy et.al., 1985; Hancock and Hanson, 1986). However, the pattern of N uptake and actual demand by blueberry plants through the growing season has not been documented. An understanding of plant demand is important in improving overall NUE of a given crop (Weinbaum, 1992).

There is a need for a greater understanding of N partitioning and dynamics in blueberry plants. Birkhold and Darnell (1993) studied the use of stored N in potted 2 year old rabbiteye blueberry and found that reserves in the plant supplied most of the N required for early growth, and reserves accounted for 50% of the total N in reproductive tissue even as late as fruit maturity. This suggests that there may be a need to maintain certain N levels within the plant late in the season. Without detailed information regarding N uptake through the season, it is difficult to predict if late season fertilization would contribute to plant N storage.

Weinbaum et al. (1978) studied uptake throughout the season in potted plum trees. The authors found a highly significant correlation between the presence of leaves and

efficiency of nitrogen uptake. In effect, trees with the greatest leaf mass absorbed more fertilizer N. Nitrogen uptake efficiency was thought to be reduced during the period of fruit maturation, possibly because carbohydrates were diverted from the roots, which limited energy dependant uptake of nitrate.

The authors also found the largest percentage of N in the roots during bud swell. Nitrogen uptake efficiency was highest from shoot elongation until fall and the onset of dormancy. During rapid shoot growth the largest percentage of N was translocated. Nitrogen uptake was independent of temperature (Weinbaum et al., 1978) within a limited range during the growing period. Since there are indications that N uptake is a function of biomass accumulation (Weinbaum et al., 1978), it may be useful to make inferences of N uptake based on growth patterns. Gough et al. (1987) found root and shoot growth in highbush blueberries was negatively correlated with fruit growth. Root and shoot growth were greatest before and after fruit maturation. Although shoot and root growth were positively correlated, shoots expanded more prior to fruit set with roots expanding more after fruit set. This might indicate that bushes were absorbing N from the period of shoot growth and at least through fruit maturity.

Kender (1967) found plant growth rate in lowbush blueberry was higher at 30C than 18C with greater shoot

length under both temperature regimes with greater daylength. In Michigan, one might expect growth rates and therefore uptake rates corresponding with similar seasonal temperatures i.e. with growth at a maximum in late June to July.

Findings by Birkhold and Darnell (1993) and Retamales and Hanson (1989) concerning storage N and fate of applied N in blueberry and Gough et al. (1987) and Kender (1967) regarding biomass accumulation give indications that fertilizing practices may not be matching plant demand since applications are generally prescribed just prior to bud break and the most rapid growth appears to be later.

Physical properties of a given fertilizer would also dictate application timing. For example, granular fertilizer is more dependant upon environmental conditions such as water infiltration than is liquid fertilizer to reach the root zone. Hanson and Retamales (1992) saw increased yield with split applications of N over single applications. This may be a result of maintaining more constant and higher levels of N through the season.

The purpose of this experiment was to describe the rate of fertilizer N uptake by highbush blueberry on a monthly basis throughout the growing season. A knowledge of plant N demand and uptake rate is necessary in determining the appropriate timing for N fertilization.

MATERIALS AND METHODS

A 1990 planting of "Bluecrop" at the Southwest Michigan Research and Extension Center, Benton Harbor, Michigan was used. Soil was a Selfridge sandy loam (loamy, mixed, mesic Aquic, Arenic, Hapludalfs) (Soil Conservation Service, 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). CEC was measured at 9.6 meq/100 grams of soil. Clay content was 16%, silt content was 17% and the remainder was sand.

Treatments included 10 g. of 10.2 atom % ^{15}N ammonium sulfate (Isotec Inc., Miamisburg, Ohio) applied in 1993 on either 23 April (bud scale separation), 25 May (late full bloom), 24 June (fruit expansion), 23 July (post fruit removal), 24 August (shoot growth cessation) or 23 September (dormancy onset). The duration of the treatment periods lasted for 14 days after each treatment date, after which, respective treatment replicates were harvested in their entirety.

An effort was made to maintain uniform soil water potentials throughout the experiment. The soil in the circular .237 sq. meter area beneath each plant was irrigated to field capacity prior to treatment with labelled

ammonium sulfate. To accomplish this, a 55 cm diameter, 10 cm tall collar was sunk into the soil approximately 3 cm at the base of each plant to maintain control of soil water content. With one exception, 2.5 cm of water was added and allowed to percolate into the soil. The late August treatment received only 1.25 cm of water to adjust for 4.5 cm of rain received the previous night. Tensiometers were used to monitor osmotic potential through the treatment period.

Labelled ammonium sulfate was dissolved in 2 liters of water and applied using four 500 ml intravenous bottles that were attached to four 6mm plastic tubes inserted 15 cm beneath the soil surface, directly into the root zone. Tubes were placed on a 20 cm square configuration around each plant. Fertilizer drip rate did not exceed rate of percolation. All six plant replicates were harvested 2 weeks after treatment for each respective treatment period. Plants to be treated at later dates received identical rates of non-labelled granular ammonium sulfate on a monthly basis to maintain consistent soil N concentrations throughout the experiment.

To further control soil water, plastic rain covers were placed beneath the foliage of treated plants to prevent rain from falling on treated soil areas during the two week treatment periods. Thus, an attempt was made to restrict water inputs after the initial applications. Rain covers had

an "A-frame" shape to allow air flow beneath them and avoid heat build-up. Foliage was not covered. Soil water potential was monitored with tensiometers and irrigation was applied when necessary to maintain an osmotic potential of less than 20 kpa throughout the treatment periods. Additional water was required only after the first week during the fourth treatment period.

Soil Sampling and Analysis. Soil was collected at the end of the two week treatment period by first removing a 30 cm wide by 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 subsampled in the field. Soil samples were finally dried in a forced air drier at 40 C, 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 grams of soil in 100 ml 1N KCl at 150 RPM on a solution agitator (New Brunswick Scientific Co., Edison NJ) for 45 minutes. Suspensions were passed through Whatman #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, WI).

After ammonium and nitrate concentrations were measured in each sample, a sequential diffusion technique was carried out (Brooks, 1989; 1992) to separate the ammonium and nitrate fractions. The ^{15}N enrichment of the separate fractions were 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:

$$((A - B)/(C-B))*100$$

A = Enrichment of sample (^{15}N atom %)
B = Ambient enrichment (natural atom % ^{15}N)
C = Enrichment of fertilizer (10.2 atom % ^{15}N)

Soil temperatures under rain covers and non-covered soils were measured using bi-metal thermometers (Weston Elec Inst., Newark, NJ). Three measurements in covered and non-covered soils were taken at 2 or 4 week intervals at 12.5 cm depth. Average daily precipitation and temperatures were recorded through the season.

Plant Sampling and Analysis. Whole plants were removed two weeks after ^{15}N applications and partitioned into roots, stems and current seasons growth (shoots, leaves and fruit). Soil was excavated at the base of the plant one meter in diameter and 30 cm in depth. The soil was sifted through a 12 mm mesh screen to collect root tissues. Roots were rinsed thoroughly with tap water. Fresh weights of all tissues were recorded and dry weights measured after forced air drying at

40C for 5 days. 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 soils in the proceeding section.

Experiment Design and Analysis. A randomized complete block design with six single plant replicates was used. 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 University). Significant differences between means were determined with an LSD (5%).

RESULTS

Fertilizer N Uptake Efficiency. Differences in whole plant uptake of fertilizer N due to treatment date were highly significant. Bushes exposed to fertilizer for 2 weeks in late May, June or July absorbed a significantly greater percentage of fertilizer N (7 to 9%) than bushes treated in late April, August and September (1-3%) (Figure 1).

Fertilizer N Partitioning. Treatment date also affected the fertilizer N content of the root, stem and new growth (shoots, leaves and fruit) partitions. The following comparisons indicate significant fertilizer uptake trends within these partitions (LSD, 5%).

New growth tissue contained the greatest amount of fertilizer N (0.07 to 0.08 grams) during late May, June and July treatment periods (Figure 1) and least in April, August and September. Stem tissue also contained the highest fertilizer N levels (0.02 to 0.03 grams) following treatments in late May, June and July. The fertilizer N content of roots was highest during the late July treatment period, just after fruit removal (Figure 1), and statistically lower at other times.

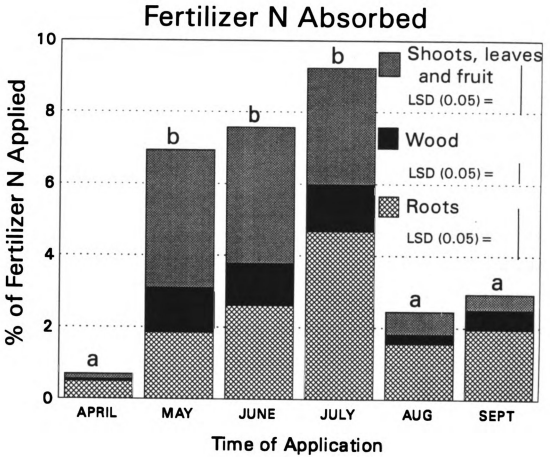

Figure 1. Plant Fertilizer N uptake over a two week treatment period. Letters indicate significant differences in total plant fertilizer N (LSD, 5% level).

Total Plant Nitrogen . Total plant N was lowest in late April, increased until late July, then remained constant through August and September (Figure 2). Plants accumulated N most rapidly between the harvest dates of 8 July and 6 August (June and July treatments).

Highly significant differences in total N accumulation were observed within root, stem and new growth (shoots, leaves and fruit) partitions relative to treatment date. The following comparisons indicate significant differences within these partitions (LSD, 5%).

Total N content of current seasons growth (leaves, new stems, fruit) increased gradually until harvest of the June treatment (1.14 grams), then remained relatively constant until the September treatment harvest when it dropped to an intermediate level (1.098 grams).

Stem tissue total N varied slightly through the season although there were two periods of increase between June and July (0.53 grams N) and August and September (0.76 grams N).

Root tissue N content increased significantly, relative to other periods, only between the June and July treatment harvest dates. N content remained constant thereafter. Mean N content of roots increased from 0.81 grams to 1.49 grams between June and July harvests.

Fruit was removed from all plants on 19 July, 3 days before the fourth treatment period began. Fruit from 6 bushes contained an average of 0.25 g of N/bush. This was

included in the total N computations for the last three treatments (Figure 2).

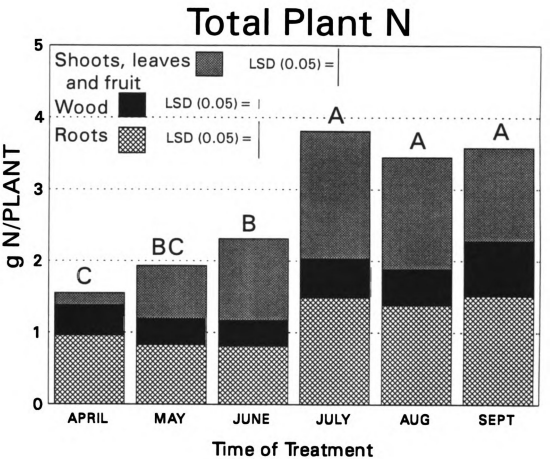

Figure 2. Total nitrogen content (fertilizer-derived plus native) of plant parts. Letters indicate significant differences between total plant N with all parts combined (LSD, 5% level).

Plant Dry Weight. Plant dry weight increased gradually between the April and June harvest dates, and most rapidly between June and July harvest dates. Dry weight did not change between August and October (Fruit weight for the last three treatments was included). Shoot and leaf weights increased most rapidly in June, July and August, whereas root weights increased mostly during July. Stem growth occurred mostly after July (Figure 3).

Soil Nitrogen. Total inorganic soil N at the end of each treatment period did not vary significantly (Figure 4) although fertilizer N, which was included as a component of total N, did varied significantly (Figure 4). Total inorganic N concentrations in the surface 20 cm of soil was greatest in May and June (80 to 90 kg/ha) and lowest in September (Figure 4).

Total inorganic soil fertilizer N was highest in May, and decreased progressively to the lowest level in September, the next to last sampling date. Levels in October were statistically similar to those in August (Figure 4).

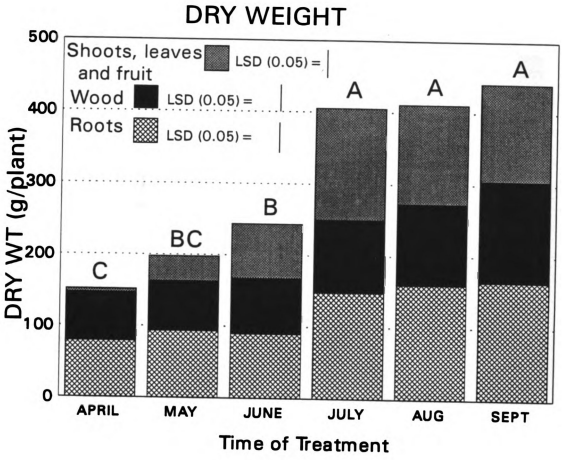

Figure 3. Monthly changes in dry weight of indicated plant parts. Letters indicate significant differences between total weight means (LSD, 5% level).

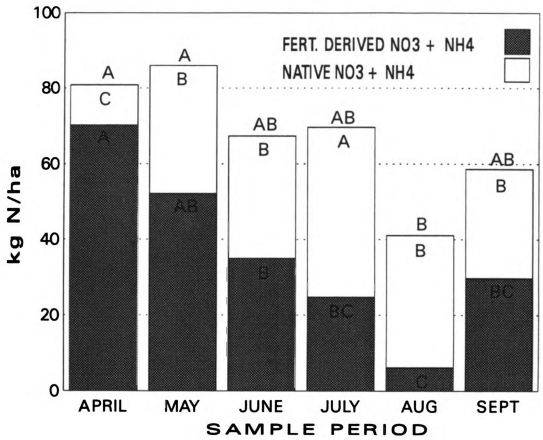

Figure 4. Native and fertilizer derived inorganic N (NO_3^- plus NH_4^+) in the top 20 cm of soil. Letters indicate significant differences (LSD, 5% level) in fertilizer-derived, native and total NO_3^- plus NH_4^+ between dates.

Fertilizer ammonium remaining in the soil was greatest following the first treatment period, decreased from May to August then remained relatively constant (Figure 5). Fertilizer derived nitrate levels were similar after each treatment period although they dropped temporarily in September during a period of relatively high rainfall (Figure 6).

Soil Temperature. Average season temperatures under rain covers (14.5C) was lower than temperatures of uncovered soils measured (15.2C) at a depth of 15 cm.

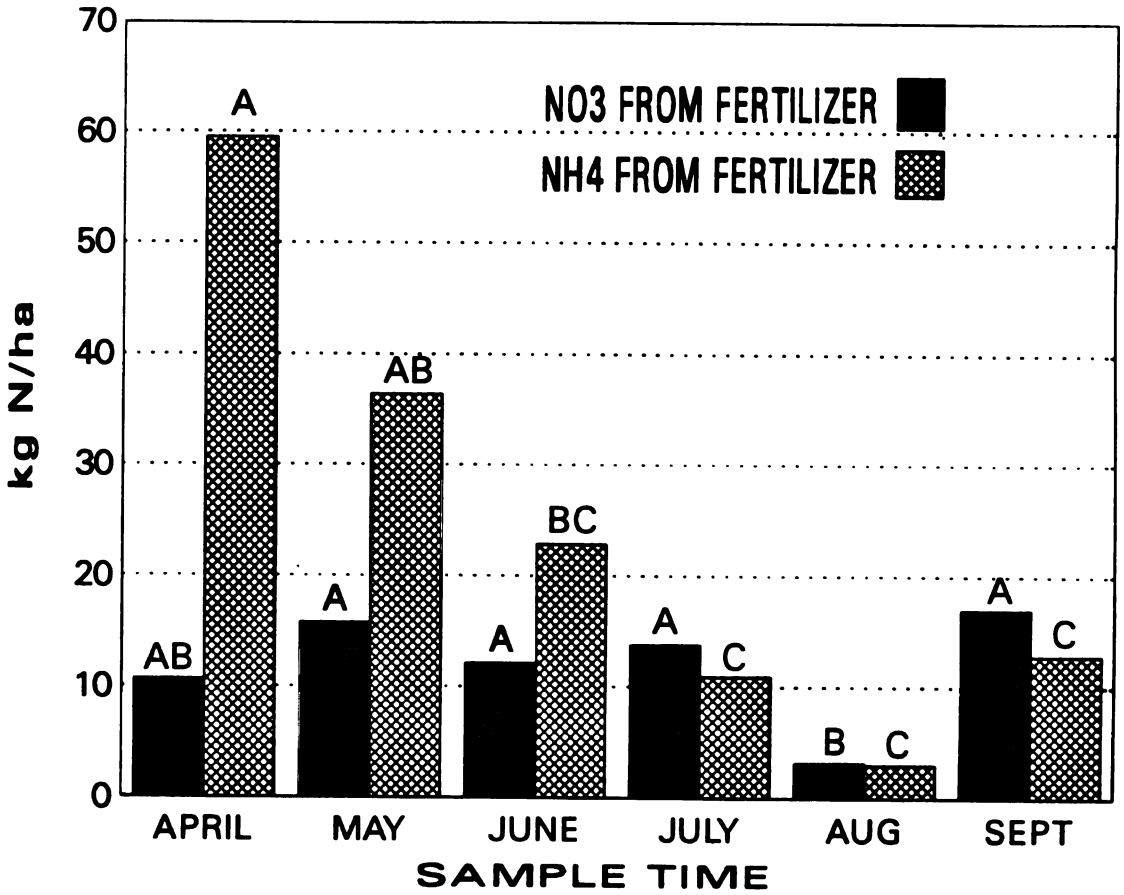

Figure 5. Fertilizer derived nitrate and ammonium remaining in top 20 cm soil after a 2 week treatment period. Letters indicate significant differences between means (LSD, 5% level) within N forms.

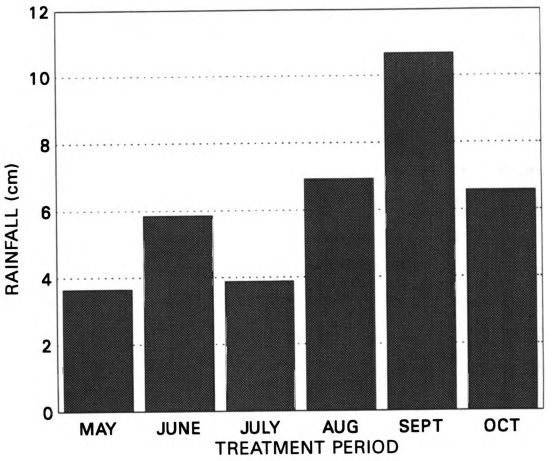

Figure 6. Total rainfall accumulation during the six two-week treatment periods.

DISCUSSION

Fertilizer application timing greatly effected the amount of fertilizer N absorbed by blueberry plants. Uptake rate may reflect the demand by the plant and/or soil N availability. Two important aspects related to soil N availability may affect plant fertilizer N content. First, uptake may be affected by total labelled N in the soil. One would expect decreased ^{15}N uptake with decreasing total amounts below that required by plants. Second, the ratio of fertilizer N to native N can effect uptake of labelled N. Plants do not discriminate between ^{15}N and ^{14}N. Thus, if the ratio of fertilizer N to native N is low (i.e. fertilizer N is diluted by greater native N) fertilizer N uptake rate can appear low despite high rates of uptake. In this experiment, plant N uptake appeared to be influenced primarily by plant N demand and growth.

Low fertilizer N uptake in April appeared to reflect low plant N demand. Other workers have found high levels of fertilizer N in new growth or that uptake corresponded primarily with new growth (Weinbaum, 1978; Birkhold and Darnell, 1993; Retamales and Hanson, 1990). Low biomass accumulation occurred in April (Figure 3) during apparent

low N demand (Figure 1) and therefore seems consistent with the hypothesis that uptake reflects growth rate. Low accumulation occurred despite the presence of high levels of fertilizer N in the soil (Figure 4).

Plant fertilizer N content increased dramatically during the periods starting in May (Figure 1) when an increase in the biomass of new growth only was observed (Figure 3). Fertilizer N uptake was sustained through the July treatment period. During the July period four days after fruit removal, uptake was similar to that found in May and June, however root fertilizer content was significantly higher than at any other time (Figure 1). This presumably reflected a significant increase in root biomass (Figure 3) and associated N demands.

Also, during the May, June and July treatment periods, plant fertilizer N concentrations remained high even though soil N concentrations gradually decreased through these periods (Figure 4). During these periods, plant uptake may have contributed to the decline in soil fertilizer N. Also, the ratio of fertilizer N to native N decreased as uptake remained high from the May through July treatment periods (Figures 1 and 4). Decreasing N label in the soil would result in a decrease in apparent plant fertilizer uptake since the labelled nitrogen pool would be diluted, not an increase as was observed. Thus, it appears that high plant uptake is occurring as a result of plant N demand rather

than as might occur if soil content and availability dictated plant uptake.

Low fertilizer N uptake during the August treatment (Figure 1) may have resulted from low soil N availability rather than low plant demand. High rainfall during this period (Figure 6) may have leached fertilizer from the root zone. Low soil fertilizer N may have resulted in reduced apparent plant uptake especially considering the presence of an exceptionally low soil fertilizer to native N ratio (Figure 4). Leaching may have removed fertilizer while leaving relatively constant levels of native N available through mineralization. It seems likely that efforts to measure plant demand were confounded by this loss of soil fertilizer N during the fifth treatment period.

The low fertilizer uptake during the final treatment period in September appeared to reflect low demand by bushes (Figure 1). Soil fertilizer N to ambient N ratio and total soil N levels were similar to those found on the initial four sampling dates (Figure 4) suggesting that soil N availability was not limiting. Therefore, low fertilizer N uptake during the last treatment period was probably the result of low plant demand associated with onset of dormancy rather than low soil N availability.

This overall pattern of higher N uptake during the period of active growth early in the growing season is consistent with findings in plum (Weinbaum and Muraoka,

1978), almond (Weinbaum and Muraoka, 1984) and apple (Millard and Neilson, 1988). Results presented here indicate that fertilization at bud break (approximately April 20 in 1993) as currently recommended would have been premature since the most active N uptake did not begin until one month later. However, optimum timing may also depend on the type of fertilizer carrier used. Granular N-fertilizers require water (rain or irrigation) to dissolve and move N into the root zone. Thus, application prior to plant need, depending on N infiltration rate, seems important. It is advisable to irrigate soon after application (Weinbaum 1981) to avoid gaseous losses through volitilization or denitrification.

If liquid fertilizers are used, or if fertigation is available, a more appropriate developmental period for application would appear to be during full bloom. Birkhold and Darnell (1993) also found increased dependance on available soil N at this period, which was concurrent with onset of vegetative growth.

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CHAPTER 2

Effects of Dicyandiamide on Plant N Utilization and Soil Nitrification

ABSTRACT

Nitrification rates and N recovery by 3 year-old highbush blueberry (Vaccinium corymbosum L. cv."Bluecrop") were compared following field applications of ammonium sulfate with and without the nitrification inhibitor dicyandiamide (DCD) on a soil with pH 4.8. Ammonium sulfate solutions containing 7.9 grams N (10.2 atom % ^{15}N), with or without 0.6 g DCD, were applied to the soil surface beneath bushes. Nitrate concentrations were significantly lower in the DCD treated soils in the first 2 weeks following application.

Uptake of fertilizer-N by highbush blueberry plants was observed by collecting fruit during the growing season and excavating and partitioning plants at the end of the season. Tissues were dried, ground and analyzed for ^{15}N enrichment by mass spectrometry. No significant differences due to treatment were observed in plants.

INTRODUCTION

Efficient use of N fertilizer is desirable in order to minimize adverse effects on water quality and minimize fertilizer costs. The dynamics and fate of N fertilizers need to be understood in different cropping systems in order to optimize N use efficiency. Highbush blueberries are most adapted to course textured, low pH soils. Water tables are often perched and shallow. Plants are typically shallow rooted and generally have low nutrient requirements (Eck, 1988). Often only N applications are required annually (Hanson and Hancock, 1987). This combination of low plant N demand, course soil texture and shallow water table make blueberry systems vulnerable to N loss. In fact, recovery of surface applied urea-N in blueberry has been shown to be only 32% in mature bushes over the course of a season (Retamales and Hanson, 1990). Also, fertilization has resulted in increased soil N levels below the root zone (Retamales and Hanson, 1989), suggesting that leaching losses of N may occur.

Blueberries differ from most crop plants in utilizing ammonium-N more efficiently than nitrate N (Cain, 1952; Townsend, 1970; Dirr, 1971; Havill et.al., 1974; Sugiyama

and Ishigaki, 1994). In order to optimize uptake, it may be desirable to maintain ammonium N within the blueberry root zone when plant demand is high. Nitrification is the biological oxidation of ammonium to nitrate. This is accomplished by soil microbes and is effected by type of nitrifier species, soil pH, temperature, water and other substrates present. The rate of nitrification is typically low in low pH soils (Allison and Prosser, 1993; Hankinson and Schmidt, 1988).

Hankinson and Schmidt (1988) identified an acidophilic strain of Nitrobacter and there is evidence of high pH microsites that allow nitrification to occur (Allison and Prosser, 1993) at low pH. The chemical form of N and its proximity to plant roots affects availability and plant utilization.

Ammonium N may also be desirable because it is generally resistant to leaching. Ammonium adsorbs to soil particles, whereas the negatively charged nitrate ion leaches easily because it does not adsorb to soil particles. In many soils nitrate leaching is the primary mode of N loss (Eaton, 1987). Thus, it would seem particularly advantagous to minimize nitrification and maintain N in the ammonium form in blueberry soils.

Dicyandiamide (DCD) inhibits nitrification, although the mode of action is not fully understood. Dicyandiamide sulfate inhibits cytochrome oxidase in intact cells or

extracts of Nitrosomonas europea (Nuti et al., 1975) thus blocking oxidation of ammonium to nitrite (Vilsmeier, 1981).

DCD is decomposed to ammonium (Vilsmeier, 1981), which can serve as an N source for plants. Although the environmental factors affecting DCD breakdown are not well characterized (Vilsmeier, 1981), temperature has an effect, possibly through its influence on microbial activity. The effectiveness of DCD varies. Generally, DCD is less effective than nitrapyrin in reducing nitrification (Wickramasinghe et al., 1985, Martin et. al., 1993), possibly because it is more prone to degradation and volatilization.

Prakasa Rao and Puttanna (1987) found that DCD inhibited nitrification on a sandy loam soil at high rates (15 to 20 ppm), but greatly increased volatilization losses of ammonia with surface applied urea. Others have reported increased ammonia volatilization losses that resulted in lower yields when DCD or other inhibitors were applied to the soil surface particularly when used with urea (Bundy and Bremner, 1974; Cornforth and Chesney, 1971; Rodgers, 1983; Clay et al.,1990). Soil incorporation of inhibitors may reduce gaseous losses (Rodgers, 1983). Vilsmeier (1991) found decreased yield in wheat which was thought to be a result of plant uptake of unreduced DCD. Total N was the same for DCD treated and untreated plants although yields were thought to be reduced due to N displacement by

metabolically inactive DCD. DCD may have been taken up by mass flow.

Martin et al. (1993) found potato yields were increased by DCD on a sandy, irrigated soil when moderate N rates with higher DCD rates (11.2 kg/ha) were used, whereas higher fertilization rates and/or lower DCD rates (5.6 kg/ha) did not improve yields. With high rates of N, plant yield was the same possibly because sufficient N was present regardless of leaching losses.

The purpose of this experiment was to determine if DCD would inhibit nitrification and increase plant fertilizer uptake by blueberries. We were also interested in describing the general dynamics and fate of fertilizer N applied to a low pH blueberry soil.

METHODS AND MATERIALS

The study was conducted on a 1990 planting of "Bluecrop" at the Southwest Michigan Research and Extension Center, Benton Harbor, Michigan. The soil was a Selfridge sandy loam with an adjusted pH of 4.8. Original pH was 6.2 (1989) and was acidified with sulfur in 1990 and 1991. CEC was 9.6 meq/100 grams of soil. Clay content was 18%, silt content was 15% and the remainder was sand. Bulk density averaged 1.6 grams per cubic centimeter.

A one liter solution containing 37.5 grams of 10 atom% ^{15}N ammonium sulfate (Isotec Inc., Miamisburg, OH) with 0.6 grams of DCD (5.6 kg/ha Conklin Company, Inc.) was sprayed evenly onto a 1.11 sq. meter area beneath six plants (equivalent rate; 71 kg N/ha). An equal amount of labelled ammonium sulfate was applied without DCD to 6 control plants. Treatments were applied on 1 May, 1993. Treated areas were kept free of weeds for the duration of the experiment.

Soil Sampling/Analysis. Soil samples were collected from each plot on 1 May, 7 May, 14 May, 18 June, 12 August and 7 September using a 2.5 cm diameter auger. Each sample was a composite of five cores collected throughout each plot. Soil was sampled from three depths: 0-12.5 cm, 12.5-25 cm and 25-

37.5 cm. Soil was dried in a forced air drier at 45 C, ground and sifted through a 2 mm screen (Custom Laboratory Equip.Inc, Orange City, Fla.).

Soil NO_3^- and NH_4^+ were extracted by agitating 20 grams of dry soil in 100 ml 1N KCl at 150 RPM on a solution agitator (New Brunswick Scientific Co., Edison NJ) for 45 minutes. Suspensions were then funnel filtered through #5 Whatman paper that had previously been rinsed with deionized water and dried. Total ammonium and nitrate concentrations in extracts 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, WI).

In order to determine the amount of fertilizer derived nitrate and ammonium, the $^{15}N/^{14}N$ ratios of the ammonium and nitrate fractions were measured separately. A diffusion technique was used to separate the ammonium from the nitrate (Brooks 1989; 1992). The $^{15}N/^{14}N$ ratio of each fraction was determined using mass spectrometry (Harris and Paul 1989).

Total soil N (organic plus inorganic N) of samples collected after seven days was determined by mass spectrometry. Soil samples were prepared by tumbling 15 gram subsamples in jars containing two inch steel rods for fine grinding. 40 to 50 mg samples were then tin encapsulated and analyzed directly. This information was necessary to evaluate the amount of fertilizer N that was immobilized and not extractable with KCl.

The equation from Cabrera and Kissel (1989) for computing percentage of fertilizer derived N was:

$$((A - B)/(C-B))*100$$

Where: A = Enrichment of sample (^{15}N atom %)
B = Ambient enrichment (natural atom % ^{15}N)
C = Enrichment of fertilizer (10.2 atom % ^{15}N)

Bulk density was measured using a standard cylinder core sampling procedure. Intact soil cores of a known volume were taken at the three sample depths, dried and weighed. Bulk density measurements were required in order to report ammonium and nitrate quantities on a kilogram per hectare basis for each depth.

Plant Sampling/Analysis. Plants were removed 7 October, 1993 and separated into roots, stems (older than 1 year) and current seasons growth (new shoots, fruit and leaves). Fruit was also harvested upon maturity on 19 July. To recover all plant roots, soil was excavated at the base of plants in an area one meter in diameter and 30 cm in depth. Roots were removed by sifting the soil through a 1.25 cm screen. Roots were later washed over a 0.5 cm screen to remove residual soil. Tissue fresh weights were determined and dry weights recorded after forced air drying. Plant material was ground twice to pass through a 40 mesh screen. The total N concentrations and $^{14}N/^{15}N$ ratio were determined by mass spectrometry (Europa Scientific Tracer Mass; Harris and Paul, 1989). The equation for determining fertilizer content is the same as that used for soil indicated in the

proceeding section.

Experiment Design. A randomized completely blocked design was used with 6 single plant replicates. Bushes were selected from three rows and blocked according to size. At least one buffer plant separated treated plants. Plant parameters were analyzed by a two-way analysis of variance (M-Stat Statistical Package, Michigan State University).

Soil samples were analyzed in a randomized completely blocked design with treatments (control, DCD) as the main plot, and time of sampling and depth of sample as split plots. All 6 sample dates were included in the original analysis. However, since variation was high in the sixth sampling due to very low residual fertilizer and presumably variable loss, only 5 sample dates were combined for analyzed.

RESULTS

Soil N dynamics. The addition of DCD reduced fertilizer derived NO_3^- levels in the top 12.5 cm of soil 7 and 14 days after treatment (Figure 7). Fertilizer derived NO_3^- concentrations in the control plots peaked on the third sampling date after 25 days, whereas concentrations in DCD treated soils were highest on the fourth sampling date after 49 days.

DCD had no effect on fertilizer derived NO_3^- levels in soils sampled at 12.5-25 or 25-37.5 cm depths (Figure 7). Also, NO_3^- concentrations decreased with depth of sampling. In general, fertilizer derived NO_3^- peaks in lower layers appeared at the 49 day sampling date then remained relatively constant (Figure 7).

Total NO_3^- (fertilizer plus native) levels were not effected by treatments at any sampling depth or date, so data were combined (Figure 8). Total NO_3^- remained relatively constant through time. Total NO_3^- in the top layer increased gradually to a maximum 145 days after applications, then declined slightly by the end of the season. Total NO_3^- levels in the middle and bottom sample layers increased through the final sampling date (Figure 8).

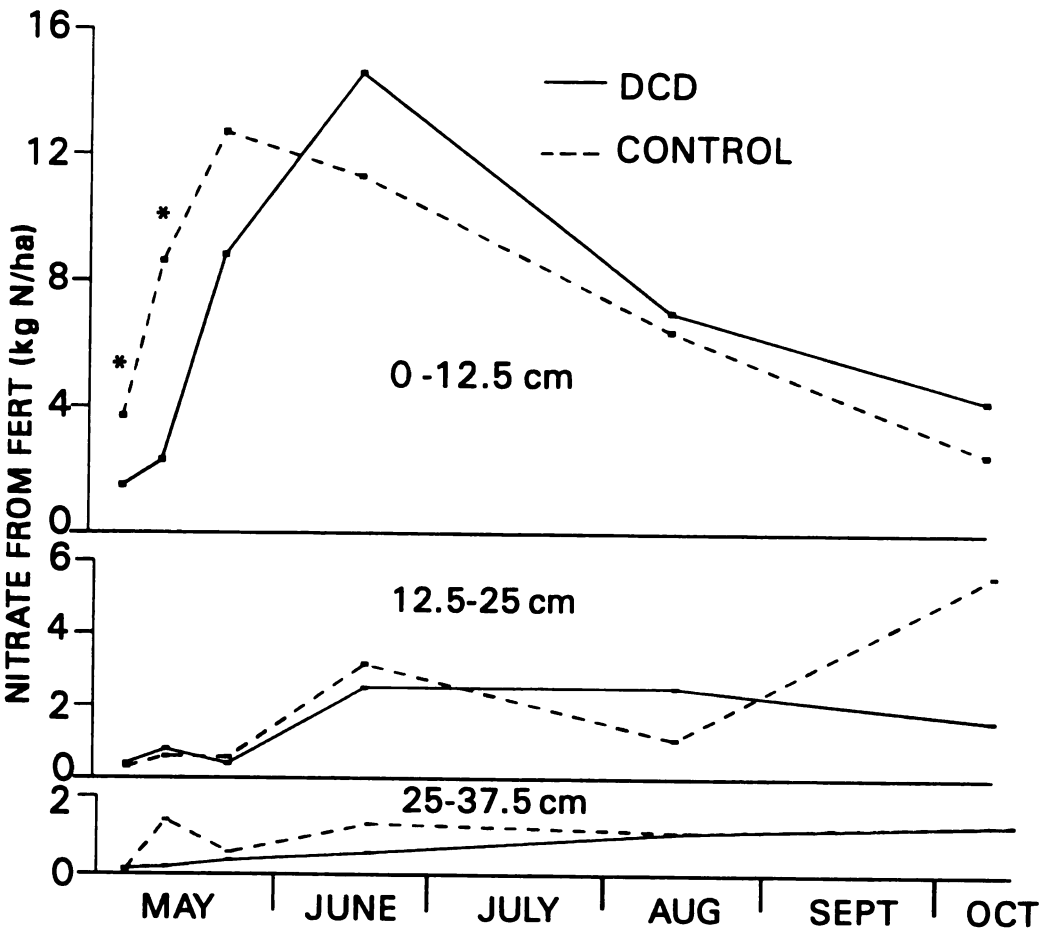

Figure 7. Changes in fertilizer derived nitrate levels with depth following application on 1 May. * indicates significant differences (P = 0.05) between treatments on a given sampling date.

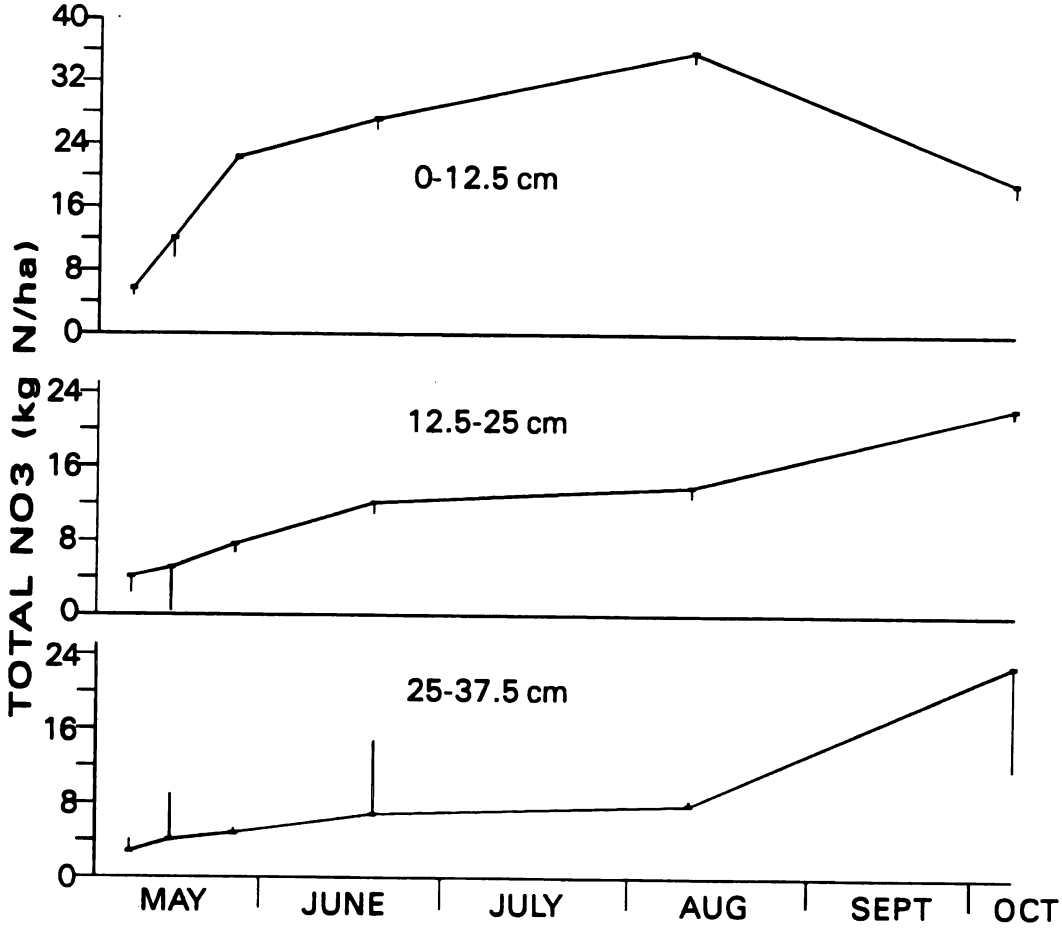

Figure 8. Changes in total soil nitrate levels (fertilizer derived plus native) following ammonium sulfate applications. Data are means on control and DCD treatments. Vertical bars represent one standard error.

Treatments had no effects on fertilizer derived or total NH_4^+ concentrations, so data from DCD treated and non-treated control soils were combined for comparison.

The concentration of fertilizer-derived NH_4^+ in the top layer fluctuated between 40 and 25 kg N/ha during the first 25 days after application (Figure 9), then decreased to less than 1 kg N/ha after 100 days.

Fertilizer derived NH_4^+ in the middle and lower soil layers followed similar patterns but concentrations were much lower or negligible (Figure 9). Total NH_4^+ levels (fertilizer derived plus native) dropped after 49 days then levelled off for the remainder of the experiment near 22 kg N/ha. Lower layers increased then remained steady near 17 and 11 kg N/ha for middle and bottom sample layers respectively (Figure 10).

Total inorganic N. Total inorganic N concentrations were not significantly affected by treatment, therefore data from DCD treated and control plots were combined (Figure 11). Total inorganic N (native plus fertilizer-derived) in the surface 12.5 cm of soil varied through the season between 85 and 43 kg N/ha, whereas middle and bottom layers were lower in the season near 15 and 10 kg N/ha, then increased late in the season to 49 and 43 kg N/ha respectively.

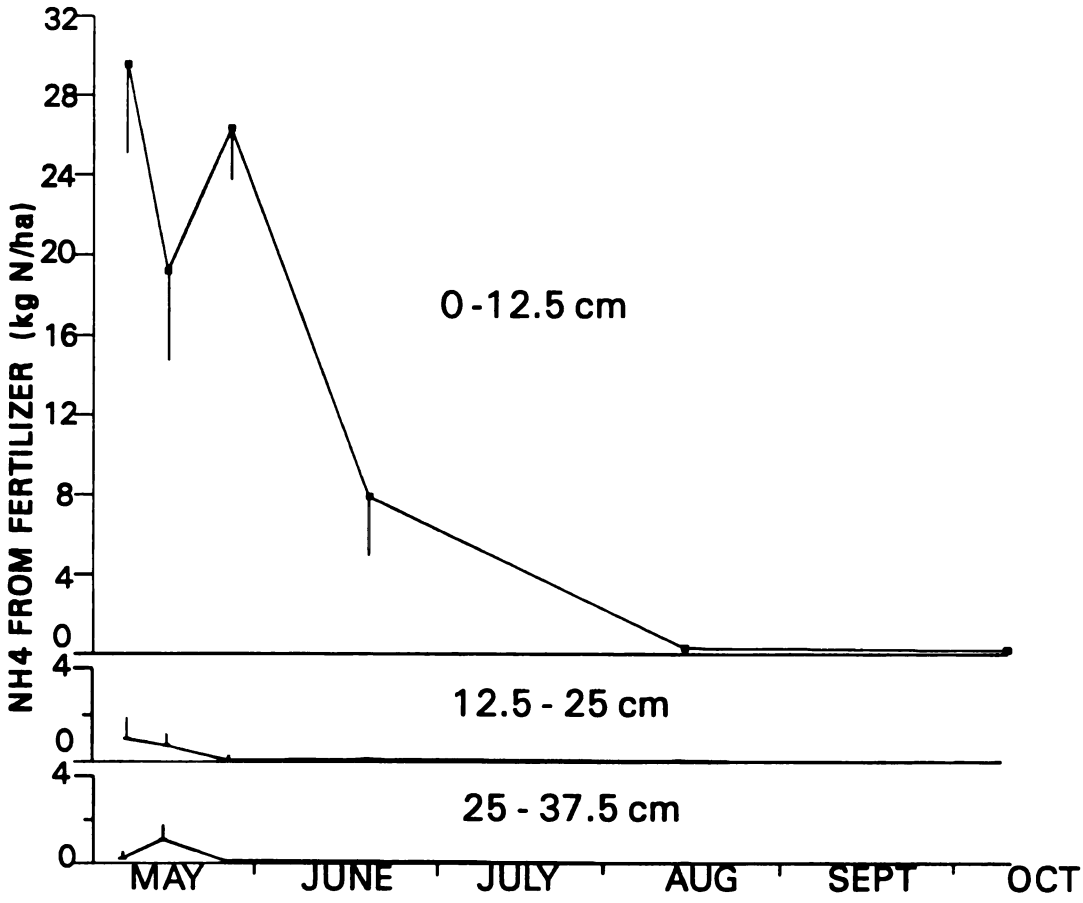

Figure 9. Changes in fertilizer derived ammonium levels with depth following ammonium sulfate applications on 1 May. Data are means of control and DCD treatments. Vertical bars represent one standard error.

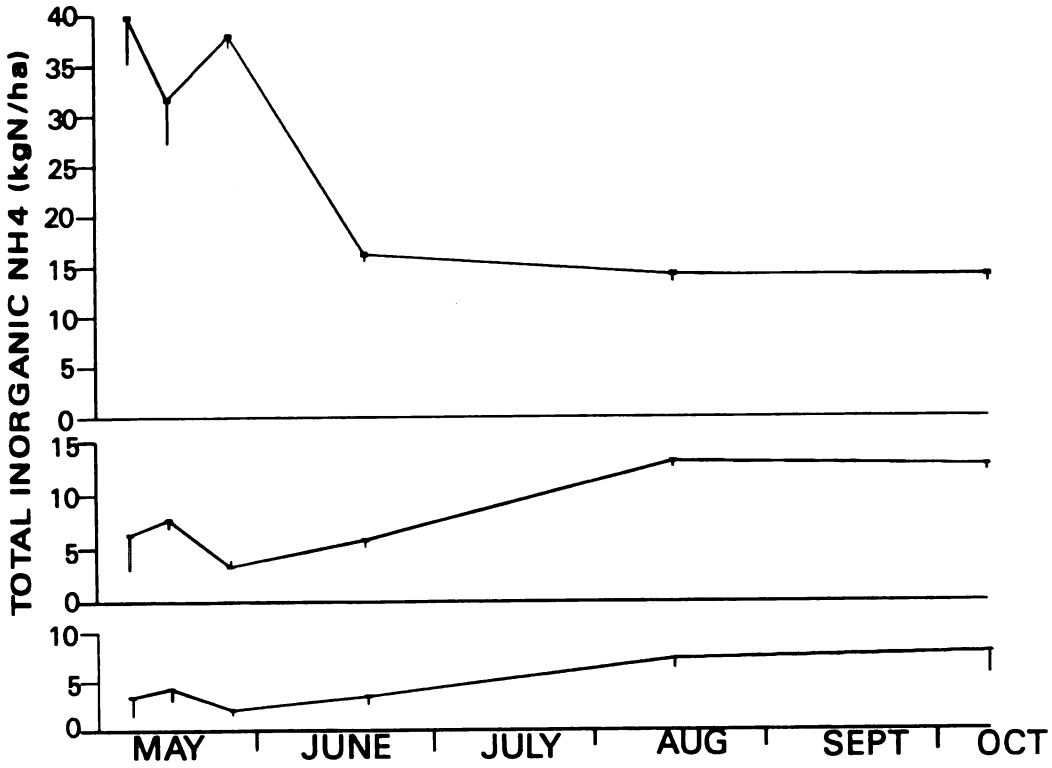

Figure 10. Changes in total ammonium (fertilizer-derived plus native) with depth following ammonium sulfate applications. Data are means on control and DCD treatments. Vertical bars represent one standard error.

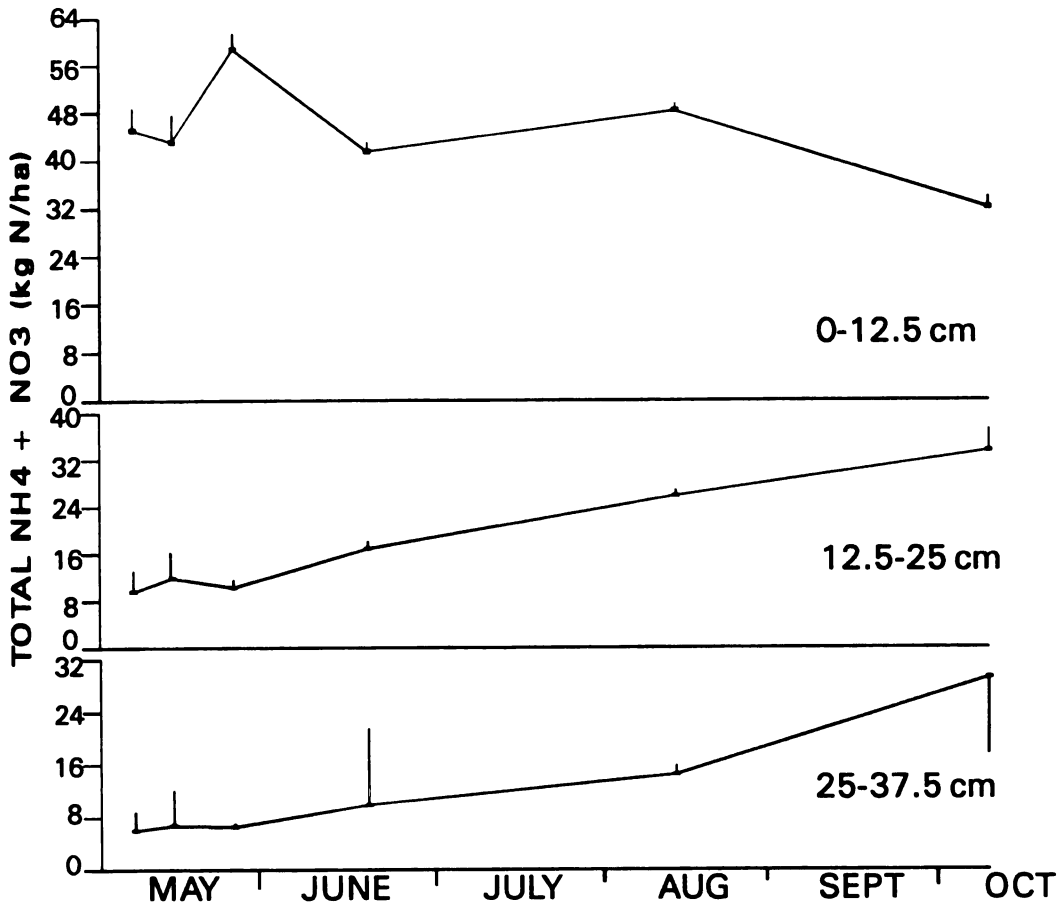

Figure 11. Changes in total inorganic N (NO_3^- plus NH_4^+) with depth following ammonium sulfate applications on 1 May. Data are means on control and DCD treatments. Vertical bars represent one standard error.

After treatment, total available N in the three sampled profiles combined was never lower than 87 Kg N/ha despite rapid loss of fertilizer, however one week prior to fertilization samples contained an average total of only 11 KgN/ha.

Immobilized N. Immobilized labelled nitrogen which was not extractable with 1N KCl averaged 11% in both DCD treated and untreated soils.

Plant N uptake. Plant fertilizer N concentration, total N and dry weight in the whole plant and within plant partitions were not effected by treatment with DCD (Table 1). Coefficient of variation of total plant fertilizer N was 97%.

Table 1. The effect of DCD on fertilizer N, Dry weight and total N in various plant parts of highbush blueberry bushes.

Treatment	Fruit	Stem	Root	New Shoot/Leaves	Total
		Fertilizer N (g)			
Control	0.037	0.074	0.095	0.12	0.33
DCD	0.014	0.03	0.06	0.05	0.16
		Dry Wt (g)			
Control	42	76	82	54	255
DCD	36	63	86	44	229
		Total N (g)			
Control	0.21	0.50	0.76	2.1	3.6
DCD	0.19	0.39	0.78	1.9	3.3

DISCUSSION

Addition of DCD resulted in lower fertilizer-derived nitrate in top soil surface layers sampled seven and fourteen days after treatment (Figure 7). This indicates that DCD as used in this experiment was relatively ineffective in inhibiting nitrification. The limited period of effectiveness of DCD may have been a function of the low concentrations (5.6 kg DCD/ha) applied. Martin et al. (1993) found no differences in yield in potato using a similar rate but recorded significant yield increases with a rate of 11.2 kg DCD/ha. Amendments were incorporated into the soil. Soil ammonium and nitrate balances were not reported by the authors however nitrification inhibitors generally have a more obvious effect on soil dynamics of N than on crop yield.

DCD may also have been ineffective if DCD had been decomposed too quickly. N products of DCD do not inhibit nitrification. Soil temperature is positively correlated with rate of DCD degradation (Vilsmeier, 1981). Vilsmeier found that 90% of applied DCD was degraded at 25C within 35 days. In the present experiment, soil temperatures at 10 cm were above 25C within the first two weeks of application. One might expect more extreme temperatures near the surface

of the soil since temperatures are buffered by soil. DCD was surface applied in this experiment, therefore it is possible that DCD was exposed to temperatures in excess of those recorded. The specific role of elevated temperature in increasing DCD degradation was not detailed by Vilsmeier, although it is well known that rate of biological metabolism is closely linked to temperature and could conceivably cause faster breakdown. Rate of degradation of DCD is also positively correlated with increasing organic matter and ferrous iron hydroxides (Reddy, 1964; Amberg and Vilsmeier, 1979). Ferrous iron hydroxide content was not assessed.

Changes in concentrations of fertilizer derived ammonium and nitrate (Figures 7, 9) indicate that nitrification occurred readily in this soil. Significant nitrification occurred after just seven days in control soils. Levels of fertilizer ammonium dropped 75% to 13.5 kg N/ha by 49 days after treatment. Fertilizer nitrate increased to 21 kg N/ha during this time, primarily in the top sample layer. There was an increase of fertilizer nitrate in middle and lower sample layers until day 49 which remained relatively stable thereafter.

Loss of fertilizer N likely occurred through leaching and other unidentified mechanisms (figure 12). Generally constant levels of fertilizer nitrate were observed in lower soil layers after day 49 from application (figures 7, 12), and additional nitrate may have leached below this zone.

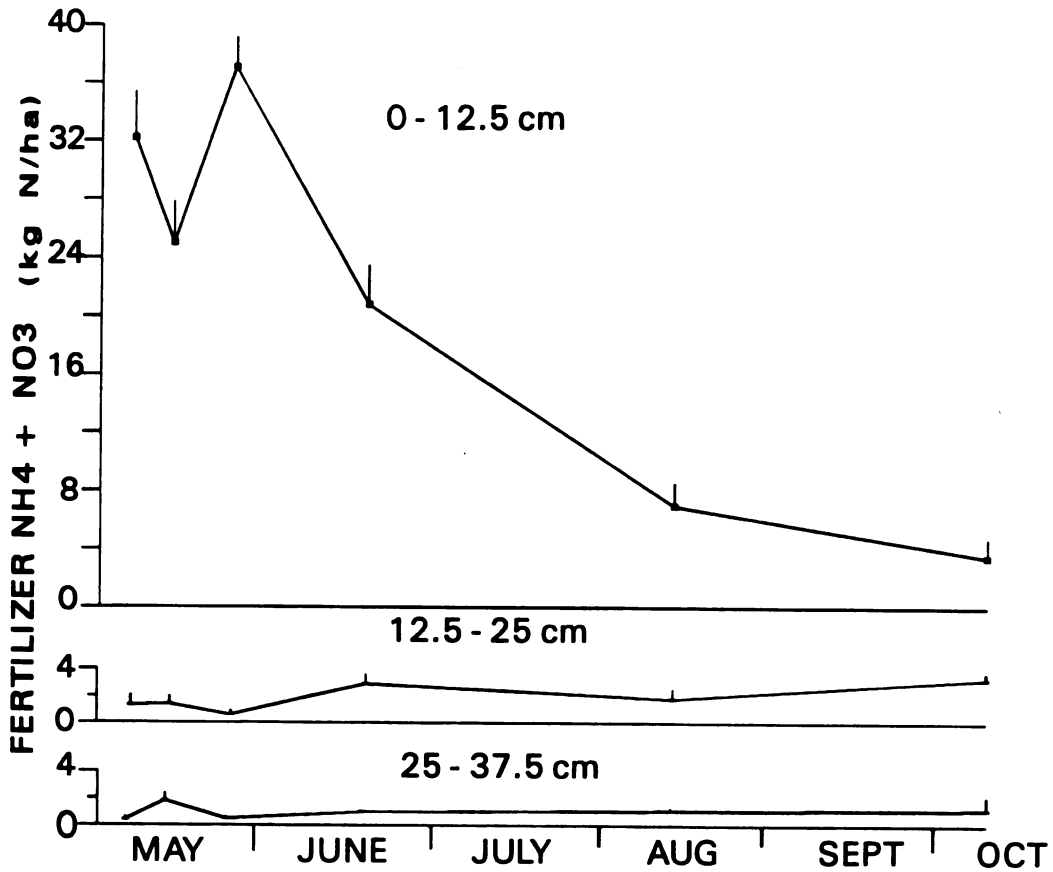

Figure 12. Changes in total inorganic (NO_3^- plus NH_4^+) fertilizer derived N with depth following ammonium sulfate applications on 1 May. Data are means on control and DCD treatments. Vertical bars represent one standard error.

Losses through denitrification may have been low considering the low pH and generally well drained soils present at the site. Eaton and Patriquin (1989) found low rates of denitrification in lowbush blueberry soils that had been saturated in the lab and concluded that denitrification results in only small losses of N in blueberry soils under normal field conditions.

One week after application, soil fertilizer N levels (nitrate and ammonium) totalled 47 kg N/ha. This was 24 kg N/ha less than the 71 kg N/ha originally applied (Figure 12). It was found that some soil fixation occurred. Approximately 11% of total fertilizer N was not extracted with KCl in this experiment. Kowalenko (1978) found that 59% of field applied ammonium was immediately fixed in a Bainsville clay loam. Roughly 33% of this was tightly fixed and could not be extracted with KCl. In addition, in preliminary tests of the diffusion technique (Brooks et al., 1989) used to recover nitrate and ammonium N, recovery was found to average 95% of the total N content. Thus, approximately 14 kg N/ha was not accounted for.

Volatilization of ammonia may have resulted in substantial N loss. Cornforth and Chesney (1971) found losses of 22 kg N/ha after 28 days due to volatilization where ammonium sulfate was surface applied with a nitrification inhibitor to a field soil of pH 5.6. Use of a nitrification inhibitor may have increased volatile losses

by delaying conversion to nitrate. Liquid surface application, sufficient air flow and high humidity all contribute to volatilization of ammonia (Nelson 1982) and were characteristic of procedures and conditions present at the experiment site. Gaseous losses were not measured and total amounts of N in soil were not significantly different between treatments, therefore, it is impossible to assess the extent of gaseous N loss as a result of DCD treatment in this experiment.

Plant N uptake varied considerably (Table 1). This variation probably reflected differences in plant vigor resulting from an irregular clay pan in the experimental area. The extent of the clay pan was recognized after the study was initiated. Uneven drainage was observed later in the season and several plants exhibited various degrees of chlorosis.

Although differences were not significant, N uptake was generally lower in plants grown in DCD treated soils. There were few differences in soil N fractions as a result of DCD treatment, therefore, it seems unlikely that there would be any effect on plant uptake. During the first two weeks when concentrations of soil nitrate were significantly different between treatments, plant uptake was found to be low (Chapter 1, Figure 1). Also, a delay in nitrification was observed for only a two week period. This would seem to be too short in duration to affect N uptake by plants. On

the contrary, this factor would more likely result in less uptake in the controls, which had higher soil nitrate levels in the first two weeks.

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SUMMARY AND CONCLUSIONS

The seasonal demand of crop plants for N needs to be understood in order to conserve fertilizer N and avoid pollution of surface and/or ground water. These results indicate that the greatest N uptake by blueberries was associated with vigorous growth in May June and July. Fertilization at bud break as currently recommended would appear to be premature since significant N uptake did not begin until one month later. However, optimum application timing may also depend on the type of fertilizer used. Granular fertilizers may require some time to dissolve and move into the root zone. Where granular fertilizer is used, somewhat earlier application may be appropriate because of this delay. For this reason also, it is advisable to irrigate soon after application to avoid gaseous losses.

The results of the experiment assessing DCD as a nitrification inhibitor were not conclusive. The primary reason was that soil heterogeneity contributed to greater variability in plant vigor and N uptake was probably affected. Soil nitrification rates were probably also affected due to variable physical effects and possibly, related microbial interactions.

Secondly, DCD rates were low relative to those used in other studies. Higher rates may have been more effective in inhibiting nitrification and may have increased N uptake and/or yield. Also, DCD may degrade too quickly to be effective. Workers have had inconsistent results with DCD and nitrapyrin, often because degradation of the inhibitor occurs relatively soon after application.

A final weakness of the DCD study related to the procedure involving surface application of DCD and fertilizer N. Volatilization may have resulted in N losses that confounded results. Decreased yields or greater soil losses of N have been observed with use of DCD as a result of volatilization. Thus, it is still unclear whether a nitrification inhibitor will increase nitrogen use efficiency in blueberry with present practices. Indeed, DCD use may be problematic in blueberry systems because of the tendency for loss through volatilization with surface application. Fertilizers are generally surface applied in blueberries. Soil injection with a nitrification inhibitor may prove to be more useful in increasing efficiency of nitrogen inputs.

Fertilizer nitrogen was found to be readily nitrified and losses through leaching, volatilization and/or soil fixation were significant. At present, the most efficient means for reduction of N loss appear to be through properly timed applications applied at a rate based on plant demand.

Avoidance of N leaching is important. N application methods including drip fertigation, soil incorporation and use of split applications are effective methods that increase N use efficiency in other perennial crops.

APPENDIX ONE

Blueberry Nitrogen Uptake as Influenced by Photoperiod

INTRODUCTION

Blueberry nitrogen use efficiency decreased abruptly with onset of leaf senescence in the Fall (Chapter 1). Hall et al.,(1962; 1963) found greater vegetative growth in plants exposed to 16 hour daylengths compared to those exposed to less than 12 hours. Plants receiving 12 hour daylengths initiated more flower primordia. This investigation was carried out to see if shortening photoperiod was an environmental stimulus for onset of dormancy and reduced fertilizer nitrogen uptake.

MATERIALS AND METHODS

Two gallon potted highbush blueberries (var. Bluecrop) were situated at the Horticulture Teaching and Research Center at Michigan State University in East Lansing, Mich. during Summer 1994. Two light treatment regimes were imposed: Naturally decreasing daylength and natural daylength augmented with 2 to 10 umoles/m^2/sec of incandescent light for a total constant daylength of 16 hours. Treatments were separated by a double layer of black 4 mil polyethylene sheeting 1.7 meters high by 3 meters long.

Labelled fertilizer was applied on three dates: 19 July, 18 August and 19 September. 16 plants per date (eight per light regime) were fertilized with one gram of 10.2 atom % ^{15}N ammonium sulfate and harvested seven days later. Soil samples were collected at harvest. Liquid fertilizer (containing 21-12-12) was applied throughout the three month duration of the experiment. Soils were covered during treatment. Soil and tissue analysis was carried out as in chapters one and two. Plant and soil samples were statistically analyzed by factorial analysis of variance (M-Stat Statistical Package, Michigan State University). Eight plants for each light regime and 16 plants per date were utilized although the light treatment was not replicated. The experimental design consisted of a completely randomized design with date as a split plot on light treatment, although light treatment was not replicated.

RESULTS AND DISCUSSION

No significant differences in fertilizer N uptake due to light treatments were observed. It was observed however, that fertilizer N uptake increased through the season as biomass accumulation slowed during leaf senescence (Table 2). Senescence was also indicated by an increase in percentage of "black tips" or aborted shoot apexes. Soil analysis revealed that an increase in plant fertilizer N was probably a result of greater loss of native inorganic N later in the season and the relative increase in the

proportion of fertilizer N in the soil as the experiment progressed (Table 2). An attempt was made to adjust for varying enrichment but results were inconclusive. Thus, greater plant fertilizer N uptake was a result of greater label enrichment in the soil, not increased plant demand. Confounding results associated with varying label enrichment in this experiment make obvious the need to maintain control of nutrient concentrations of the soil when using similar methods for investigating plant uptake.

No significant differences were observed in either total plant nitrogen or biomass as a result of treatment or in respect to date of fertilization.

Table 2. Average plant and soil nitrogen contents from combined means of natural and augmented light treatments. Significant differences were among combined means of fertilizer N only.

	Plant (g)			Soil (ppm)	
Date	Total N	Fert N		Total Inorg.N	Total Fert N
19 July	0.86	0.018	C[z]	41	6.64
18 August	1.22	0.030	B	24	5.22
19 Sept	1.33	0.06	A	15	5.92
Significance	NS	*		NS	NS

[z]Mean separation within the column by LSD at $P \leq 0.05$.
* Significant at $P \leq 0.05$.