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EFFECT OF THE TIMING OF NITROGEN APPLICATION ON SOIL NITROGEN AND NITROGEN USE EFFICIENCY OF VITIS LABRUSCA IN A SHORT-SEASON REGION

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By

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ABSTRACT

EFFECT OF THE TIMING OF NITROGEN APPLICATION ON SOIL NITROGEN AND NITROGEN USE EFFICIENCY OF VITIS LABRUSCA IN A SHORT-SEASON REGION

By

Randall J. Vos

Nitrogen (N) is applied to vineyards from prior to budbreak to post-harvest, depending on the region. The goals of this work were to determine the how the time of N application affects soil N levels and fertilizer N recovery by *Vitis labrusca* L. grapevines in Michigan, a short-season region. Labeled ammonium nitrate ($^{15}NH_4{}^{15}NO_3$) was applied to the soil beneath the vines at a rate of 68 kg N/Ha at different times between budbreak and six weeks after bloom. Soil was sampled after the fertilizer applications to follow the inorganic N dynamics. The vines and soil were excavated at the end of the growing season to quantify fertilizer N recovery. By the end of the season, grapevines contained less than 20% of the N applied. Vines fertilized at budbreak generally contained less fertilizer N and allocated a greater fraction of the fertilizer N to the fruit and leaves. Vines fertilized later in the season allocated more of the absorbed fertilizer N and total N from all sources, to the roots. At the end of the season, more fertilizer N remained in the soil from the later applications. High levels of inorganic N were maintained the longest in the soil, following the N applications at bloom. Based on these findings, applications of N to vineyards in short-season regions are recommended to be between bloom and six weeks after bloom, due to a higher recovery of fertilizer N in the vines and more fertilizer N remaining in the soil than the budbreak applications.

DEDICATION

I would like to thank my friends and family back home and elsewhere for their continued sustenance during my time at MSU. I would like to specifically thank the people at MSU whom have made my time here more than just getting a degree, especially my officemate Marlene Ayala. I would also like to thank the community that I grew to be a part of, people who still practice true hospitality: Mauricio Cañoles, Marcus Duck, Janelle Glady, Eric Hanson, Karen Maguylo, Adriana Nikoloudi, Ron Perry, Dario Steffanelli, Costanza Zavalloni, Roberto Zoppolo, etc.....

"No good tree bears bad fruit, nor does a bad tree bear good fruit. Each tree is recognized by its own fruit. People do not pick figs from thorn bushes, or grapes from briars. The good man brings good things out of the good stored up in his heart, and the evil man brings evil things out of the evil in his heart. For out of the overflow of his heart his mouth speaks" Luke 6:43-45 (NIV)

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PREFACE

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REVIEW OF LITERATURE

INTRODUCTION

Nitrogen (N) is an essential nutrient for plant growth and is the most frequently applied nutrient to vineyards. Nitrogen is a component of amino acids, chlorophyll, and lecithins in plants. The N requirement for grapevines is generally lower than that for other crops (Christensen et al. 1978). Nitrogen fertilization can increase vineyard yields (Ahmedullah and Roberts 1991; Bell and Robson 1999; Kliewer et al. 1991; Partridge and Veatch 1931) and compensates for N removed from the vineyard through crop harvest (Williams 1987).

Nitrogen from fertilizer is either taken up by the plants, incorporated into soil organic matter, or is lost from agricultural systems via leaching, erosion, volatilization, and denitrification. Application rates of N in fruit production are usually much higher than the total amount removed by the crop. Nitrogen use efficiency (NUE) is a term used to quantify fertilizer N uptake by plants. NUE is the amount of fertilizer N taken up by the plant, divided by the total amount of N that was applied (Weinbaum et al. 1992).

Studies using fertilizers enriched with ^{15}N as a tracer have shown that agricultural crops absorb only a portion of applied N. The stable isotope of N, ^{15}N, has eight neutrons compared to the more commonly found ^{14}N which has seven neutrons. Natural abundance levels of stable N isotopes are 0.37% ^{15}N and 99.63% of ^{14}N. The different quantities of each N isotope can be measured by mass spectrometry (Dawson and Brooks 2001). Studies in vineyards indicate that vines recover from 7 (non-irrigated vineyards) to 42% (irrigated vineyards) of the N fertilizer during the season in which it was applied, depending on the time of application, irrigation method, and climate (Hanson and Howell 1995; Williams 1991). Other woody fruit crops, such as blueberries and citrus, recovered 35 to 61% of applied N (Dasberg 1987; Retamales et al. 1989). More efficient recovery of N fertilizer has been reported for annual crops (corn and barley) with up to 43 to 85% NUE (Mahli et al. 1995; Reddy and Reddy 1993; Tran and Giroux 1998).

These studies indicate that grapevines exhibit a lower NUE than other crops. Therefore, there is a need to determine the most efficient time of N applications to vineyards to increase the NUE of grapevines for economic as well as environmental reasons.

GLOBAL ENVIRONMENTAL IMPACT FROM NITROGEN FERTILIZATION

The use of industrially fixed N is increasing in agriculture. In 2001, agriculture worldwide used 83 million tons of N produced by industrial fixation via the Haber-Bosch process. Sixty-one million tons of industrially fixed N were used in agriculture in 1961 (Jenkinson 2001). Anthropocentric activities such as N fertilization in agriculture, have created a situation where N can be viewed as either a nutrient or a pollutant, depending on its location and form.

Contamination of Water:

Nitrogen is commonly lost from soil via the leaching of nitrate (NO_3); ultimately NO_3 can end up in both ground- and surface water. Excess NO_3 in drinking water, which commonly originates from groundwater, can cause adverse health complications in humans such as methemoglobinemia. Nitrogen in combination with high levels of phosphorus can also cause eutrophication of surface water (Newbould 1989).

Atmospheric Pollution:

Nitrogen accumulates in the atmosphere in four major forms: NO, NO_2, N_2O, and NH_3. Contributions of N_2O and NH_3 to the atmosphere can be traced to agriculture. Agricultural soils contribute 10 million tons of NH_3 gas per year and release 2.1 million tons of N_2O into the atmosphere each year, which is a third of the total N_2O released due to human activity. Whereas greenhouse gases such as NH_3 are only in the atmosphere for a matter of hours or days, the half-life of N_2O can be as long as 130 years. Therefore, the persistence of N_2O in the atmosphere is cause for concern because of its lasting impact on the atmosphere (Jenkinson 2001).

SOIL AND NITROGEN DYNAMICS

Most of the N in soil is in organic forms. The availability of N independent of fertilization (native N) is closely related to the amount of organic matter in the soil. Mineralization is the process of the conversion of organic N into inorganic forms. The inorganic forms of N, ammonium (NH_4) and nitrate (NO_3), are available for uptake by plants and NO_3 is very mobile in the soil. Nitrogen in soil organic matter is mineralized at a rate of 1.5 to 3.5% per year (Brady and Weil 1996).

Nitrification is the conversion of NH_4 to NO_3 via microbial oxidation. Ammonium-based N fertilizers must be converted to NO_3 for uptake by most plants; therefore, nitrification is often an important aspect of plant NUE. The rate of nitrification in temperate soils is maximal at 25 to 35°C, and is nearly negligible below 10°C (Brady and Weil 1996; Prasad and Power 1997).

NITROGEN IN VINEYARDS

Contamination of Water by Viticultural Nitrogen Fertilization:

German vineyards have been cited as a contributor of NO_3 to groundwater (Schaller 1991). Groundwater NO_3 concentrations from viticultural areas in Germany were 8 to 30 times higher than those from forested areas. In addition, surface water flowing out of the vineyards had 50-75% higher nitrate concentrations than water upstream of the vineyards (Schaller 1991).

Mineralization of Organic forms of Nitrogen in Vineyards:

Fertilizer N applied to vineyards was most effective at increasing productivity of the vines in shallow soils with low organic matter (Partridge and Veatch 1931). Partridge and Veatch (1931) concluded that the amount of soil organic matter had a major impact on vine vigor and productivity. Mineralization rates for native N in vineyards can range from 2.9 to 3.1% (Schaller 1991).

Estimates in German vineyards (Schaller 1991) report that mineralization of native N in tilled (non-vegetative row middles) vineyards can supply the total N needs of a grapevine throughout the growing season. In vineyards with a vineyard floor of grass vegetation, a shortage of native N for the grapevines is most likely to occur shortly after budbreak due to the additional N demand by the grass vegetation (Schaller 1991).

Fate of Fertilizer Nitrogen in Vineyards:

Hajrasuliha et al. (1998) followed the fate of ^{15}N enriched ammonium ($(NH_4)_2SO_4$) and nitrate (KNO_3) fertilizers applied to 3-year-old, trickle-irrigated 'Thompson Seedless' (*Vitis vinifera*) grapevines. Vines were fertilized with 50 kg N/Ha in late April, and destructively harvested at fruit harvest in late September. At vine harvest, NO_3 treated vines contained 22% of the fertilizer N; 13% was soil organic N; and 66% remained as soil inorganic N. The NH_4 applications resulted in similar recoveries of the fertilizer N in the vines (24%), while 19% was bound as soil organic N, and 48% remained as soil inorganic N. The majority of the fertilizer N that was incorporated into organic forms of soil N was in the top 60 cm of the soil. The NH_4 and NO_3 fertilizers had leached to a depth of 150 cm or more than 240 cm, respectively. It is presumed that much of the inorganic N in the NO_3 form would likely leach below the rooting zone during the subsequent winter and spring.

GRAPEVINE ROOT GROWTH

Grapevine roots are an important tissue in the uptake, storage, and translocation of N within the vine (Bates et al. 2002; Conradie 1980). The bulk of nutrient and water uptake by vines occurs at the tips of actively growing feeder roots, called the absorption zone. The absorption zone does not have the corky outer layer, which is present on older roots (Winkler et al. 1974). The most dynamic root turnover during the season occurs in fine roots (feeder roots) (Bates et al. 2002). The feeder root system may go through multiple cycles of growth, senescence, and re-growth during a growing season (Winkler et al. 1974). Conradie (1980) observed that active N accumulation after fruit harvest coincides with increased root growth in South Africa. Therefore these studies suggest that N uptake by grapevines may coincide with periods of active root growth.

The number of actively growing root tips fluctuates throughout the growing season. Van Zyl (1988) showed that the most active period of root tip proliferation in mature *V. vinifera* 'Columbard' on the 99R rootstock in South Africa, occurred during bloom, for a month following fruit harvest, and to a lesser extent during veraison. At these times, vines had 2 to 15 times more actively growing root tips than during the rest of the season.

Seasonal changes in root growth and N content also occurred in 3-year-old 'Concord' vines in a short-season region in New York (Bates et al. 2002). Dry matter accumulation for fine roots (<2 mm) was the most dynamic. Peak periods of fine root growth occurred from late spring dormancy to budbreak, 32 days post-bloom to veraison, and from fruit harvest to leaf fall. Fine root dry matter increased 5 fold from April (bud swell) to the beginning of November (dormancy).

NITROGEN FERTILIZATION PRACTICES IN VINEYARDS

Fertilization with N is recommended in vineyards to stimulate increased production. Recommendations for fertilizing mature vineyards in Michigan are 57 kg N/Ha, between budbreak and bloom (Hanson 1996). Fertilization recommendations for mature vineyards in New York and Pennsylvania also suggest N applications between budbreak and bloom (Bates 2001). Recommendations from other short-season regions are N applications 30 days before the commencement of vine growth in the spring (Cahoon et al. 1991; Funt et al. 1997). Winkler et al. (1974) recommends that fertilizer N be in the rooting zone when vine growth begins in the spring. The University of California Cooperative Extension, Tulare County recommends N fertilization to vineyards at a rate of 28 to 57 kg N/Ha applied between after budbreak to fruit set, or after fruit harvest (Peacock et al. 1996).

Fertilization practices are adjusted by observing vine growth. Excessive N fertilization can cause negative impacts (such as shading) on the vine due to excessive growth (Hanson 1996; Peacock et al. 1996; Smart 1991).

EFFECTS OF NITROGEN FERTILIZATION ON PLANT PHYSIOLOGY

Cold Hardiness:

There is evidence that low N levels enhance the cold hardiness of plants. Rapidly growing shoots of conifers were more susceptible to cold temperature injury than shoots that were growing slowly (Alden and Herman 1971). Late season N applications may induce plant growth late in the season and impede natural cold acclimation and shoot maturity. Fall applications of N to apple orchards can decrease cold hardiness (Pellett and Carter 1981). High levels of soil fertility have been correlated with an increase in frost injury to some grapevines (Alden and Herman 1971).

However, Wample et al. (1991) reviewed the literature on the interactions of N and cold hardiness in grapevines, and concluded that N nutrition has only a small impact on cold hardiness, and that factors such as rootstocks, sun exposure, cropload, and climate affect cold hardiness more directly than N management. Wample (1993) reported that N fertilization from the beginning of the growing season until fruit harvest will have little affect on reducing the cold hardiness of 'Riesling' grapevines.

Fruit Quality:

There is a general belief that N fertilization of grapevines reduces some of the quality aspects of fruit (sugar, anthocyanins, fruit color, etc.), but the experiments that have been performed to investigate this relationship often have variable results (Smart 1991).

The timing of N applications to grapevines can have variable effects on fruit quality. Christensen et al. (1994) investigated the responses of grapevines to N applied at different times in the growing season. Treatments consisted of no N, or N applied to vines either at budbreak, fruit set, veraison, or post-harvest. Four *V. vinifera* cultivars were studied ('Barbara', 'Chenin blanc', 'French Colombard', and 'Grenache'). Nitrogen applied to vines at veraison had small negative impacts on the fruit quality of 'Chenin blanc' and 'Grenache'. The negative effects on the fruit included a slightly reduced yield, higher incidence of fruit rot, lower titratable acidity, and higher pH. The authors concluded that fertilizing with N at times other than veraison would slightly increase fruit quality in N sensitive cultivars.

Dukes et al. (1991) concluded that fruit quality, as measured by titratable acidity, pH, and soluble solids concentration, of *V. vinifera* 'Sauvignon blanc' was not affected by the time of urea application. However fruit from vines fertilized between fruit set and fruit harvest had significantly higher total N concentration (TNC) in both the free-run and cold-settled juice than those fertilized at budbreak or post harvest. A decrease in fermentation time to dryness was correlated with an increase in TNC of the cold-settled juice. The authors concluded that higher TNC, which can be affected by the timing of N application, reduced fermentation time and improved wine quality. Goldspink and Gordon (1991) obtained comparable results by increasing TNC with N applications between fruit set to veraison.

NITROGEN DYNAMICS IN THE GRAPVINE

There is evidence that growth of the vine early in the growing season is substantially dependent on N reserves in the vine (Conradie 1980; Conradie 1986; Lohnertz 1991; Wermelinger 1991; Williams 1991). Mobilization of N from perennial (woody) tissues of the vine prior to bloom is independent of N levels in soil (Lohnertz 1991; Wermelinger 1991). Later in the growing season the main source of N for growth is from soil N, but N reserves from woody tissue (roots and trunk) can be mobilized when soil N levels are insufficient (Wermelinger 1991; Williams 1991).

Woody tissues act as a buffer in the N dynamics within the grapevine. Lohnertz (1991) described the change of N content within the woody parts of 25 year-old *V. vinifera* 'Riesling'. Between leaf unfolding and veraison, N in the woody tissues was released at rates of approximately 20-150 g N/Ha/day. From veraison until fruit harvest, woody tissue accumulated N at rates of 30-200 g N/Ha/day. It could not be determined what portion of this increase of N in the woody tissues was due to mobilization from the leaves or to additional uptake of N from the soil.

Bates et al. (2002) excavated 3 year-old 'Concord' vines in New York on eight dates from dormancy in the spring to leaf fall. When the vines were dormant in the spring, the roots contained 75% of the total N in the vines. From budbreak to 2 weeks prior to bloom, N accumulated in thin and fine roots. From bloom to the end of rapid shoot growth, there was a net loss of N from the entire root system and large accumulation of N in the shoots and fruit. Substantial accumulation of N in the root tissue occurred after fruit harvest.

Studies with potted vines in South Africa have shown that 20-30% of N needed for the growth of annual tissues from budbreak to the end of bloom comes from mobilized N from perennial vine structures (Conradie 1980; 1986). Similar results were observed in field studies in Germany and California, where 20 to 40% of N used for growth from budbreak to bloom, was mobilized from perennial structures (Lohnertz 1991; Schaller 1989 cited in Wermelinger 1991; Williams 1991).

Conradie (1991) observed that potted vines began active root uptake of nutrients began when soil temperatures reached 10°C, and concluded that if there was a delay in the soil temperature reaching 10°C early in the season, more N reserves than normal would be mobilized from the perennial structures of the vine to accommodate early season growth.

NITROGEN UPTAKE AND ACCUMULATION BY GRAPEVINES

Grapevines take up soil N predominantly in the nitrate form (Roubelakis-Angelakis and Kliewer. 1992; Winkler et al. 1974). The determination of the stages of vine development in which N is most rapidly accumulated in the vine will help to identify optimum times for N fertilization.

Hanson and Howell (1995) destructively harvested mature *V. labrusca* 'Concord' vines in Michigan to quantify the N accumulation throughout the growing season. The vines were fertilized at budbreak with 90 kg N/Ha and harvested at 2- to 4-week intervals. The pattern of N accumulation paralleled the pattern of dry-matter accumulation. The rate of N accumulation was highest during the period from 2 weeks after budbreak to fruit harvest, except for a brief decline during veraison.

A similar study in Germany (Lohnertz 1991), with 25-year-old *V. vinifera* 'Riesling' vines, showed comparable results. The time of most rapid N uptake started 2 weeks prior to bloom and lasted until the pea-size-berry stage (4 weeks prior to veraison). During this period, N absorption rates for the vines ranged from approximately 600 to 1700 g N/ha/day. Absorption rates were 50 g N/Ha/day from budbreak to 2 weeks prior to bloom, and 200 g N/Ha/day from the pea-size-berry stage to veraison. During the first 2 weeks after veraison, N was absorbed at a rate of approximately 800 g N/Ha/day.

Lohnertz concluded that fruit quality can be influenced by N uptake during veraison due to the large uptake of N at that time, and that vines continued to absorb N until vegetative growth stopped.

Potted vines of 'Chenin blanc' *V. vinifera* absorbed N most rapidly from bloom until veraison (Conradie 1980). Later studies by Conradie (1986; 1991) indicated that rapid N uptake occurred after bloom through fruit harvest. A Hoagland solution containing ^{15}N labeled KNO_3 was applied to the vines at different phenological stages and was leached out of the pots at the end of those stages. The rate of absorption by individual vines was 210 mg N/vine/week, from budbreak until the end of rapid shoot growth. The most rapid uptake occurred at rates of 450 and 366 mg N/vine/week, from the end of rapid shoot growth to veraison and from veraison to fruit harvest, respectively. Vines absorbed 138 mg N/vine/week after fruit harvest (Conradie 1986).

Williams (1991) excavated whole 5 year-old, unfertilized, *V. vinifera* 'Thompson Seedless' vines over a 2-year period. Whole vines were sampled beginning at 3 months before budbreak until fruit harvest. The N accumulation pattern varied each year. In the first year, high N accumulation rates (approximately 480 g N/Ha/day) occurred for 130 days following budbreak. The accumulation rate was constant during this period except for a low uptake during bloom. In the first year there was no accumulation of N from 130 days after budbreak until fruit harvest. In the second season the accumulation rate was very consistent (approximately 430 g N/Ha/day) for the entire 180 days between budbreak and fruit harvest. The authors did not speculate on reasons for the differences between the years, but they may reflect yearly weather variations.

Some general patterns of N accumulation emerge from these studies. Nitrogen accumulation parallels dry matter accumulation in grapevines (Conradie 1980; Hanson and Howell 1995; Wermelinger 1991). During active shoot and fruit growth, grapevines require more N (Peacock 1982). The period of greatest N uptake throughout the various viticultural regions generally occurs from bloom to veraison.

FERTILILZER NITROGEN USE EFFICIENCY IN GRAPEVINES

Several factors influence the optimum time of N application to maximize N uptake in grapevines. Equally important factors are the rate of N uptake, duration of the uptake, and climatic conditions such as precipitation and temperature, also affect N fertilizer uptake.

Conradie (1986) reported that the stage from the end of rapid shoot growth to veraison accounted for 19% of the total N taken up by the vines during the growing season. Nitrogen absorbed during the post-harvest phenological stage accounted for 27% of the total N accumulated in the vines. In other studies the post-harvest period of N absorption accounted for as much as 34% of the total N accumulated during the growing season (Conradie 1980; 1991). Therefore, Conradie (1980; 1986; 1991) suggests that post-harvest applications of N would be used efficiently by grapevines in South Africa. Conradie (1986) concluded that the uptake of N during post-harvest is dependent upon the presence of a functional leaf canopy. Therefore, the longer the canopy is retained the more N can be absorbed after fruit harvest.

In California, N fertilizers applied later in the season were absorbed by vines more efficiently, than those applied in the spring. Peacock et al. (1989), applied $(^{15}NH_4)_2SO_4$ at a rate of 112 kg N/Ha to mature, furrow irrigated 'Thompson Seedless' vines. The applications were made in two vineyards, at three different times: early March (Budbreak), early July, and late September (post-harvest). The most efficient time of application in the one vineyard was September, while the most efficient time at the second site was July. The authors attributed the difference between the two vineyards to a low soil pH at the second site, which would delay nitrification, making the September application less available for uptake prior to leaf senescence. The authors suggest that post-harvest applications be made at least 3 to 4 weeks prior to leaf fall, and that NO_3 fertilizers should be used because they can be taken up directly by the vines. March applications of N fertilizers were prone to leaching and denitrification at both sites. Conradie (1980) also attributed a lower NUE in spring applications in South Africa to leaching. The Peacock et al. (1989) study provides only a recovery of fertilizer N for a sampling of the vegetative tissues and not the entire vine.

Williams (1991) found that with drip-irrigated 6 year-old 'Thompson Seedless' vines in California, a single fertilizer application resulted in higher NUE than small, repeated applications. Vines that had previously never been fertilized were fertilized with ^{15}N labeled potassium nitrate. One application of 25 g N/vine when berries were 8 mm (mid-May) resulted in 42% accumulation of applied N in the vine. Ten applications of 2.76 g N/vine, beginning at around budbreak and continuing at 2-week intervals, resulted in 34% recovery.

Most of the N viticultural studies discussed have been associated with long-season viticultural regions. In Michigan, a short-season viticultural region, Hanson and Howell (1995) applied double ^{15}N labeled NH_4NO_3 (50 kg N/Ha) to mature vines of the French-American hybrid 'Seyval blanc'. Vines were either treated with fertilizer during budbreak or bloom, and were destructively harvested when the leaves began to senesce. Vines fertilized at budbreak absorbed 7.1% of the applied N, while those fertilized at bloom absorbed 10.6% of the N. There was no statistical difference between the amount of fertilizer N in the vines from the two application times and the application times in this study were only 45 days apart. A wider range of application times of N in a short-season climate has not been investigated.

Substantial N accumulation in the vine after fruit harvest is only a significant factor in long-season regions. For example, in South Africa there can be a 3-month post-harvest period of active canopy before leaf senescence begins (Conradie 1980; 1991). In short season regions, leaf senescence frequently occurs soon after harvest, therefore post-harvest applications of N may not be as effective because a functioning leaf canopy is absent (Bates et al. 2002; Hanson and Howell 1995; Lohnertz 1991). Effectiveness of post-harvest applications of N in long-season areas may also be reduced with late ripening varieties (Conradie 1991).

SUMMARY

Efficient N fertilization of vineyards involves identifying the stage of vine growth when N uptake is most rapid, quantifying the duration of the uptake, investigating soil-fertilizer interactions, and correlating climatic conditions such as precipitation and temperature with N uptake. Evidence from long-season regions supports the value and efficiency of grapevine utilization of post harvest applications of N. This is likely not feasible in short-season areas, where leaves senesce during or shortly after fruit harvest. Information on NUE and the timing of N fertilization in short-season regions is limited.

Research using ^{15}N labeled fertilizers has been done primarily on *V. vinifera* and to a lesser extent on French-American hybrids.

No field studies have been conducted to compare the NUE of different times of N application in the short-season viticultural regions such as Michigan and New York for *V. labrusca*, the predominant grape species grown there. A wide range of times of N application for grapes has not been thoroughly investigated for any species of grapes in short-season regions. Furthermore, although the efficiency of the uptake of N fertilizers by grapevines has been documented in several situations (Conradie 1980; 1986; 1991; Hanson and Howell 1995; Williams 1991), the fate of the N fertilizer that was not absorbed by grapevines has not been well documented, especially in short-season regions. This is an important issue because the majority of applied N is not taken up by vines. The fertilizer N not used by the grapevines will either be incorporated in soil organic matter, remain in inorganic forms, or be volatilized. This portion of the fertilizer N may eventually become spatially inaccessible to the grapevines and therefore have the potential to become an environmental contaminant.

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EFFECT OF THE TIMING OF NITROGEN APPLICATION ON SOIL NITROGEN AND NITROGEN USE EFFICIENCY OF VITIS LABRUSCA IN A SHORT-SEASON REGION

INTRODUCTION

Nitrogen (N) accumulation and movement in woody plants is a dynamic process that is difficult to quantify because N accumulates and is retained in the tissues of perennial plants over multiple seasons. The pattern of N accumulation in grapevines during the growing season has been studied in many viticultural regions (Conradie 1980; Hanson and Howell 1995; Lohnertz 1991; Williams 1991). Nitrogen is the most commonly applied fertilizer amendment to vineyards (Christensen et al. 1978), yet little is known about the optimal timing of field applications, especially in short-season areas. Fertilization recommendations for vineyards range from pre-budbreak applications (Cahoon et al. 1991; Funt et al. 1997; Winkler et al. 1974); applications from budbreak to bloom (Bates 2001; Hanson 1996; Wolf and Poling 1995); or any time after budbreak until fruit set, or a post-harvest application in Sept (Peacock et al. 1996). Regardless of the region, the most commonly recommended time for N application to vineyards is at budbreak.

The patterns of N accumulation by *Vitis labrusca* L. 'Concord' have been described in the short-season regions of Michigan (Hanson and Howell 1995) and New York (Bates et al. 2002). The most rapid accumulation of N by the grapevines in the study by Hanson and Howell (1995) was from 2 weeks prior to bloom until fruit harvest. Sequential destructive vine harvests by Bates et al. (2002) showed that 85% of the total N accumulated by the 3 year-old 'Concord' vines in the growing season occurred from bloom to veraison. A similar pattern of N accumulation was observed in mature *V. vinifera* 'Riesling' in Germany (Lohnertz 1991). Conradie (1986) found that the most rapid absorption of N by potted vines of 'Chenin blanc' occurred from the end of rapid shoot growth until fruit harvest.

Labeled ^{15}N fertilizers are useful to investigate N use efficiency (NUE), which is the percentage of applied fertilizer N absorbed by grapevines. NUE of grapevines has varied considerably among ^{15}N field studies. Hanson and Howell (1995) found that 'Seyval blanc' vines absorbed 7% of N applied at budbreak and 11% of N applied at bloom. Williams (1991) reported a higher NUE for N applied to irrigated 'Thompson Seedless' vines in California. May applications of N to vines that were furrow or drip irrigated resulted in a NUE of 14% and 42%, respectively.

The optimum time for N application may vary with region. South African studies (Conradie 1980; 1991) indicate that post-harvest applications of N are efficient, because up to 34% of the total N absorbed by 'Chenin blanc' occurred after fruit harvest. A study in California (Peacock et al. 1989) reported that vines utilized July and September (post-harvest) applications of N more efficiently than March (budbreak) applications. Late season or post-harvest applications of N are assumed to be less effective in short-season regions, where there is often a short period of active canopy following fruit harvest (Bates et al. 2002, Hanson and Howell 1995; Lohnertz 1991).

Vineyard fertilization practices have been linked to increased levels of nitrate in surface and groundwater in Germany (Schaller 1991). This is presumably due to the low NUE of grapevines, which results in a large portion of the fertilizer N being left in the soil. The mobility of fertilizer N in vineyards has been investigated by tracing the fate of

N fertilizers applied in the spring to drip irrigated 'Thompson Seedless' vines in California (Hajrasuliha et al. 1998). By late September 57% of the applied N was still in inorganic forms of N in the soil and 16% was organic N. Throughout the course of the growing season, there was considerable movement of fertilizer N through the soil profile and fertilizer N was found at the deepest sampling depth (2.4 m) when the vines were excavated.

The majority of the N research with grapevines has been done in long-season regions with *V. vinifera* varieties. The application of this information to short-season viticultural regions may be limited because of the critical difference in the duration of active vine canopy after fruit harvest. Few studies have addressed these issues in short-season regions or have looked into the N dynamics of *V. labrusca*, which is the predominantly grown species in these areas. There has also been little investigation on the fate of the substantial portion of fertilizer N that is not used by grapevines or how the time of N application impacts the persistence of the fertilizer N in the soil. The following studies were performed with *V. labrusca* to investigate how the time of N application in a short-season region, Michigan, affects NUE of the grapevines and the fate of fertilizer N that is not used by the vines.

MATERIALS AND METHODS

These studies were conducted in 1990 plantings of *Vitis labrusca* L. 'Concord' and 'Niagara' located at the Southwest Michigan Research and Extension Center, Benton Harbor, Michigan. Vines were spaced at 3.0 m between rows and 2.1 m between vines (1555 vines/Ha) in a Spinks loamy fine sand. The grapevines were trained to the Hudson River umbrella system, and were annually balance pruned to retain 44 nodes/kg of cane prunings, up to a maximum of 65 nodes per vine.

Study 1: 'Niagara'. In 2001, 24 *V. labrusca* 'Niagara' vines were selected and arranged in a randomized complete block design, and were blocked with 6 replications according to vine pruning weights. The treatments consisted of applications of 124.4 g of double-labeled $^{15}NH_4{}^{15}NO_3$ (43.5 g N/vine or 67.6 kg N/Ha) at 15 atom percent ^{15}N. Single vines were treated at one of four times: budbreak (30 April); bloom (8 June); 3 weeks post-bloom (3WPB) (30 June); and six weeks post-bloom (6WPB) (18 July). The fertilizer was dissolved in 10 L water and applied evenly with a backpack sprayer to a 3.0 by 2.1 m area surrounding the vine. The vineyard was irrigated with 2.5 cm of water following each fertilizer application and at 2-week intervals there after. Between fertilizer and irrigation applications, the vineyard floor was covered with clear plastic to exclude rainfall. A one-vine minimum buffer was maintained between all treated vines. Vines buffering the treated vine were not fertilized during 2001.

Fruit was harvested and weighed on 11 Sept. Above ground vine tissues were removed prior to leaf senescence on 27 Sept. Roots were removed with a backhoe on 1 and 3 Oct by excavating the 3.0 by 2.1 m area of the vine space to a depth of 45 cm. The soil was sifted through a 1 by 1 cm wire screen to remove the roots. Roots were rinsed with water to remove attached soil. Vine tissues were separated into five categories: fruit; leaves; shoots; trunk (non-root woody tissue from above and below ground); and roots.

The vegetative tissues were shredded in a brush chipper, immediately weighed, and the chipper was cleaned between samples. A representative sample of ~10% of the total fresh weight was dried at 37°C in a forced air oven until there was no change in mass. Dry root, shoot, and trunk samples were ground separately in a Wiley Mill to pass through a 2 mm screen. Leaf samples and the previously ground root, shoot, and trunk samples were then ground separately in a Wiley Mill to pass through a 0.3 mm screen. Berry samples were taken randomly from the harvested clusters, weighed, freeze dried, and re-weighed to determine dry matter, and then ground in a small coffee grinder.

Soil samples were taken from each of the treated vine spaces during the root excavations at depths of 0 to 45 cm and 45 to 90 cm. Each sample was ground with a mortar and pestle and a Kjeldahl digestion was performed on each sample to determine soil N content.

Total N and ^{15}N content in the tissues and soils were determined with a Europa Scientific Tracer Mass (Crewe, England) mass spectrometer. Natural abundance of ^{15}N for vine tissues and soil was determined via mass spectrometry on leaf and soil samples from untreated areas of the vineyard (0.368 and 0.386, respectively). The amount of tissue and soil N that was derived from the fertilizer was calculated using the mass spectrometry values and natural abundance of ^{15}N, according to Cabrera and Kessel (1989). The partitioning of fertilizer N within the grapevines was calculated by dividing the fertilizer N in the various vine tissues by the total amount of the fertilizer N absorbed by the vines.

Bulk density measurements were obtained by weighing dried soil cores taken to a depth of 90 cm at 6 sites within the vineyard. Soil volume of the vine space at a given depth was calculated by multiplying the vine space area (2.1 by 3.0 m) by the sampling depth. Soil mass of the vine space for a given sample depth was calculated by multiplying the soil volume by the soil bulk density (1.5 g dry soil/cm^3). Soil N levels within the vine space were calculated by dividing the soil mass of the vine space by the sample weight, and then multiplying by the total N and fertilizer N levels from the soil samples. Grapevine dry matter was calculated by multiplying the percent dry matter of the samples, by the total fresh weight of the vine tissue. Nitrogen levels in the vine tissues were calculated by multiplying the percent N of the mass spectrometry samples, by the dry weight of the vine tissues. Conversions of the soil and vine N levels to a Ha basis were made by multiplying the single vine data by the planting density of 1555 vines/Ha.

Due to questions raised about the effect of plastic mulch on soil temperatures and vine nutrient uptake in Study 1, thermocouples were buried in 2002 at depths of 5, 15, and 45 cm at four sites in the 'Niagara' vineyard. Two sites were covered with a 3 by 3 m section of 5 mil clear plastic mulch. The other two sites were left uncovered. Temperature measurements were recorded at ~1430 hr throughout the 2002 growing season.

Study 2: 'Concord'. This study was conducted in 2002 in a block of frost irrigated *V. labrusca* 'Concord', because the 'Niagara' vineyard sustained frost damage.

Vines of equal vigor and bud survival were selected and arranged in a completely randomized design. Treatments consisted of a N application at either budbreak (14 May), bloom (19 June), or 6WPB (29 July). Each treatment was replicated on 7 vines in a completely randomized design. Groundcover in and directly around the treated vine space was killed prior to budbreak with glyphosate herbicide, and sprayed periodically during the season to maintain a vegetation-free vine space. Fertilizer application materials, rates, and methods were the same as in Study 1, except that the fertilizer was enriched to 9% atom ^{15}N and 43.8 g of actual N was applied per vine (68.1 kg N/Ha). Vines bordering the treated vines were not fertilized during the 2002. Plastic was not placed over the soil. Immediately following each fertilizer application, 2.5 cm of irrigation was applied with solid-set sprinklers. Additional irrigation was applied in the season during a drought period on 22 July, and on 19 and 21 May to protect developing buds during freezing temperatures.

Fruit and above ground vine tissues were harvested on 24 Sept and 1 Oct, respectively. Vine tissues were harvested, segregated, and sub-sampled as in Study 1. Roots were excavated on 8 and 10 October. Half of the vine spaces of three vines were excavated to a depth of 90 cm in order to compare the root recovery in the top 0.45 m of soil to that of 45 to 90 cm. Vine tissues in Study 2 were first weighed, shredded, and then sampled and processed as in Study 1.

Leaves were harvested from three shoots per vine from three of the grapevines adjacent to three of the treated vines (one from each treatment) to quantify the amount of fertilizer N that was taken up by the bordering vines. The bordering vines that were sampled were not adjacent to any other treated vines in the plot. The amount of leaf N derived from the fertilizer for the buffering vines was calculated the same as the treated vines.

Soil samples were taken at depths of 0 to 60 cm and 6 to 90 cm when the roots were excavated. These samples were processed and analyzed as in Study 1 except the mass spectrometry was done at the UC Davis Stable Isotopes Facility. Only three replications of the 60 to 90 cm soil samples were analyzed for ^{15}N content.

Soil was collected at 1, 15, 29, and 43 days after each fertilizer treatment by removing four 1.8 cm diameter cores per plot, representing four depths: 0 to 15.3 cm; 15.3 to 30.5 cm; 30.5 to 45.8 cm; and 45.8 to 61.0 cm. Sampling of the soil profile was accomplished by pushing the soil probe to a depth of 61 cm and then dividing the soil core into quarters. Soil was dried at 37°C and inorganic N in the soil samples was quantified by extracting 15 g of soil with 75 ml of 1N KCL. The soil-KCL solution was agitated at 180 revolutions/minute for 45 minutes. The soil solution was filtered with Whatman #2 filter paper that had been previously soaked in de-ionized water and dried. All soil extracts and Kjeldahl digestions were analyzed at the Soils Testing Laboratory of Michigan State University.

Experimental Analysis. The experimental model in Study 1 was a randomized complete block design with 6 replications. Both Study 1 and 2 were analyzed using SAS statistical analysis software (Cary, North Carolina). A two-factor analysis of variance was used to analyze Study 1 with time of N application being the treatment, and pruning weights being the blocking factor. Study 2 was a completely randomized design with 7 replications, analyzed with a one factor analyses of variance, with the time of N application being the treatment. When treatment effects were significant at P 0.05, means were separated by a Fisher's Least Significant Differences (LSD) test at P= 0.05.

RESULTS

Study 1: 'Niagara'. Cool, cloudy weather during bloom in 2001 limited the fruit set and yield from the 'Niagara' vines. Vine yields were extremely variable and averaged 1.6 t/Ha across all plots. Time of N application had no effect on the total dry matter of the grapevines (P=0.30) (Figure 1). Root dry matter (P=0.0034) was higher in the 3WPB and 6WPB treatments than in the earlier two treatments. Trunk dry matter (P=0.057) and fruit dry matter (Table 1) were affected by the time of N application at only a 90% confidence level.

The total N content of the grapevines was not affected by the time of N application (P=0.46). Total N in the roots was higher in the 6WPB treated vines than the Budbreak and Bloom treatments (P= 0.012). The total N in the fruit (P=0.060) was affected by the treatments at only a 90% confidence level (Table 1).

Vines recovered the equivalent of 10.6, 11.8, 12.1, and 12.6 kg/Ha of fertilizer N when fertilized at budbreak, bloom, 3WPB, and 6WPB, respectively (Figure 1). Although these data might suggest a trend of increasing NUE with the later application time, the data were extremely variable and NUE was not significantly affected by the time of N application (P=0.58). Linear regression analysis using days after the Budbreak treatment as the independent variable and total fertilizer N in the vines as the dependent variable, was also not significant (P=0.15). The NUE for the study ranged from 15.7% (Budbreak) to 18.6% (6WPB). When looking at specific tissues within the vines, there was a significantly higher amount of fertilizer N in the root tissue (P=0.0018) of the 6WPB treated vines than the other treatments. The fertilizer N content of the fruit

(P=0.015) was significantly higher in the vines of the Budbreak treatment than with the later N applications.

The partitioning of fertilizer N into vine parts, expressed as a percentage of total vine fertilizer N, was also affected by the time of application (Figure 2). A higher proportion of fertilizer N was partitioned to the fruit of vines fertilized at budbreak (P=0.016) than any other treatments. The partitioning of fertilizer N to the leaves was higher in the Budbreak and Bloom treatments than 6WPB (P=0.0085). Roots from the 6WPB treated vines had a higher percentage of fertilizer N (P=0.0029) partitioned to them than did the other treatments.

At the end of the season more fertilizer N remained in the top 45 cm soil from the 3WPB treatment than the Budbreak and Bloom treatments (Table 2). Timing of N application did not affect fertilizer N levels in the 45 to 90 cm soil depth. The total amount of fertilizer N remaining in the 0 to 90 cm soil profile in Oct was 29, 35, 44, and 39% of the amount applied at budbreak, bloom, 3WPB, and 6WPB, respectively.

Soil temperatures were consistently higher in the plots that were covered with plastic. The mean soil temperatures for the plotted dates were 5.4, 2.6, and 1.7°C higher under the plastic than the non-plastic covered soil, for the respective 5, 15, and 45 cm depths (Figure 3).

Study 2: 'Concord'. The 'Concord' vines in 2002 produced more typical yields (overall mean 11.7 t/Ha). The dry matter content of the whole vines or individual vine tissues were not significantly affected by the time of N application (Figure 4), except for the root dry matter (P=0.074), which was only affected by the treatments to a 90%

confidence level. Excavation of roots to a depth of 90 cm showed that 94.7% ± 2.5 of the roots that were recovered were in the top 45 cm of soil.

The total N content of roots was higher in the vines treated at bloom than those treated at budbreak (P=0.041). Total N of the fruit was affected by the time of N fertilizer application at a 90% confidence level (Table 3).

Vines recovered more fertilizer N when applied at bloom (11.4 kg/Ha) and 6WPB (10.1 kg/Ha), that when fertilized at budbreak (6.7 kg/Ha) (Figure 4). NUE was significantly higher in the Bloom and 6WPB treatments (16.7 and 14.8%) than in the Budbreak treatment (9.8%) (P   0.0001). Fruit contained more fertilizer N when the vines were treated at budbreak and bloom as compared to the 6WPB treatment (P=0.023). Budbreak applications resulted in significantly less fertilizer N in the roots than Bloom or 6WPB applications (P   0.0001). The amount of fertilizer N in the leaves was only significantly affected by the treatments to a 90% confidence level (P=0.074). The N treatments did not significantly affect juice soluble solids, pH, or titrable acid levels of the fruit (overall means: 16.5 Brix, P=0.81; 3.5 pH, P=0.68; 0.35 TA, P=0.55).

The percentage of fertilizer N partitioned to the fruit of the vines was highest in the Budbreak treatment and lowest in the 6WPB treatment (P   0.0001) (Figure 5). Leaves from the vines treated at budbreak had a higher proportion of fertilizer N than those from the other treatments (P=0.0037). Fertilizer N was partitioned more to roots in the Bloom and 6WPB treatments than the Budbreak treatment (P=0.0009).

The vines adjacent to the treated vines absorbed fertilizer N. The N from fertilizer in the leaves of the buffer vines was 8, 9, and 36% of the average ^{15}N fertilizer enrichment of the leaves from all the treated vines, for the vines straight across the row, diagonally across the row, and in the same row adjacent to the treated vines, respectively (Figure 6).

Later N applications resulted in higher fertilizer N levels remaining in the soil in Oct (Table 4). Fertilizer N levels in the 0 to 60 cm depth of soil were highest in the 6WPB treatment and lowest in the Budbreak treatment. Fertilizer N levels in the 60 to 90 cm depth of soil were low enough to possibly impair the ability of mass spectrometry to precisely quantify N and ^{15}N levels, although there still appears to be more fertilizer N in the 6WPB treatment than the Budbreak treatment. In Oct, the sampled soil profile contained 13, 35, and 61% of the fertilizer N applied at budbreak, bloom, and 6WPB, respectively.

Inorganic levels of N were variable in the soil samples following the fertilizer applications (Figure 7). The day after fertilization there was a high proportion of ammonium N to nitrate N in the soil of all the treatments, as compared to the later sampling dates. In the Budbreak and 6WPB treatments, inorganic levels of N rapidly declined 2 weeks after the fertilizer application. High levels of inorganic N were measured the longest in the vineyard soil of the vines that were fertilized at bloom.

Vines treated at budbreak received more precipitation during the 15 days after N application (9.5 cm), than the Bloom (3.3 cm) and 6WPB (6.2 cm) treatments (Figure 8). Forty-three days after each of the fertilizer applications the vines treated at budbreak, bloom, and 6WPB had received 19.3, 14.0, and 18.0 cm of precipitation, respectively.

Table 1: Effect of time of nitrogen (N) application on dry matter, fertilizer N, and total N in the fruit of individual 'Niagara' vines from Study 1 in 2001.

Time of N	g/vine		
Application	Dry Matter	Fertilizer N	Total N
Budbreak[z]	440	0.45 a[y]	4.4
Bloom	130	0.16 b	1.7
3WPB	150	0.10 b	1.6
6WPB	100	0.07 b	1.3
P-value	0.057	0.016	0.060

[z]Budbreak= 30 April; Bloom= 8 June; 3WPB= 30 June; 6WPB= 18 July

[y]means within columns followed by a different letter are signifcantly different by LSD (P=0.05).

Table 2: Effect of time of nitrogen (N) application (67.6 kg N/Ha) on the amount of fertilizer N remaining in the soil in Oct, for Study 1 'Niagara' 2001

Time of N Application	**Fertilizer N (kg/Ha)**	
	0-45 cm	**45-90 cm**
Budbreak[z]	11.2 b[y]	8.4
Bloom	11.5 b	12.1
3WPB	21.1 a	8.5
6WPB	15.0 ab	11.7
p-value	0.02	0.75

[z]Budbreak= 30 April; Bloom= 8 June; 3WPB= 30 June; 6WPB= 18 July

[y]means within columns followed by a different letter are signifcantly different by LSD (P=0.05).

Table 3: Effect of time of nitrogen (N) application on dry matter, fertilizer N, and total N in the fruit of individual 'Concord' vines from Study 2 in 2002.

Time of N	g/vine		
Application	Dry Matter	Fertilizer N	Total N
Budbreak[z]	1730	0.69 a[x]	14.9
Bloom	1550	0.71 a	11.8
6WPB	1250	0.36 b	10.3
P-value	0.137	0.023	0.077

[z]Budbreak= 14 May; Bloom= 19 June; 3WPB= 29 July

[y] means within columns followed by a different letter are signifcantly different by LSD (P=0.05).

Table 4: Effect of time of nitrogen (N) application (68.1 kg N/Ha) on the amount of fertilizer N remaining in the soil in Oct, for Study 2 'Concord' 2002

Time of N Application	Fertilizer N (kg/Ha)	
	0-60 cm	60-90 cm
Budbreak^z	6.4 c^y	2.4 b^y
Bloom	20.1 b	3.8 ab
6WPB	32.8 a	8.6 a
p-value	0.0007	0.046

^{z}Budbreak= 14 May; Bloom= 19 June; 3WPB= 29 July

^{y}means within columns followed by a different letter are signifcantly different by LSD (P=0.05).

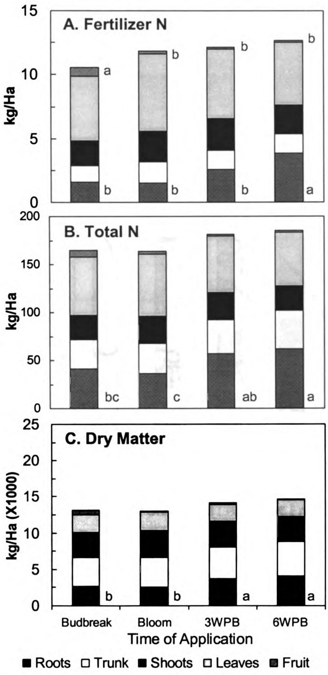

Figure 1: Effect of time of N application to 'Niagara' vines in 2001, Study 1, on (A) fertilizer N absorbed by the vines, (B) total N in the vines, and (C) dry matter of vines at the end of the season. Lower case letters represent differences (LSD P=0.05) in the same vine organs. Budbreak= 30 April; Bloom= 8 June; 3WPB= 30 June; 6WPB= 18 July.

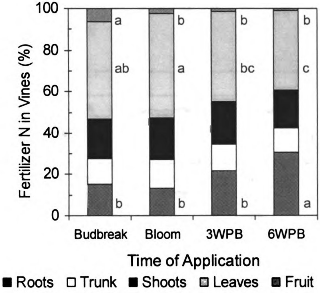

Figure 2: Effect of time of N application on the partitioning of fertilizer N in 'Niagara' grapevines at the end of the season in 2001, Study 1. Letters represent separation of treatment means of the same vine organs (LSD P=0.05). Budbreak= 30 April; Bloom= 8 June; 3WPB= 30 June; 6WPB= 18 July.

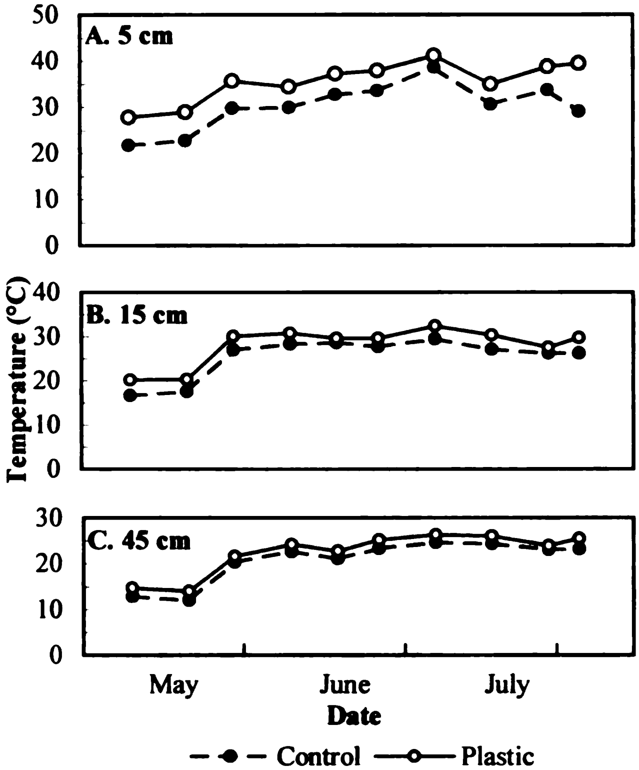

Figure 3: A comparison of soil temperatures at depths of (A) 5, (B) 15, and (C) 45 cm at ~1430 hr under existing sod groundcover (control) or 5 mil plastic installed on 06 May. Data points represent the mean of 2 observations

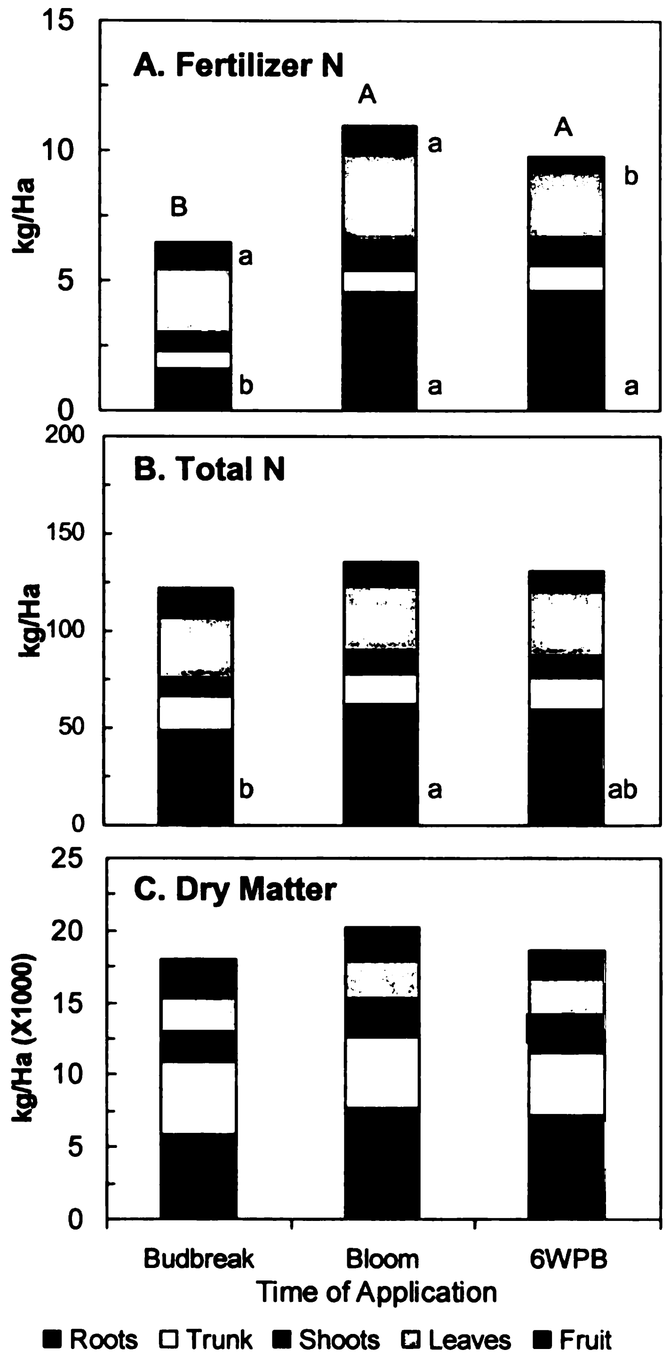

Figure 4: Effect of time of N application to 'Concord' vines in 2002, Study 2, on (A) fertilizer N absorbed by the vines, (B) total N in the vines, and (C) dry matter of vines at the end of the season. Upper case letters and lower case letters represent differences (LSD P=0.05) in the whole vine and vine organs, respectively. Budbreak= 14 May; Bloom= 19 June; 6WPB= 29 July.

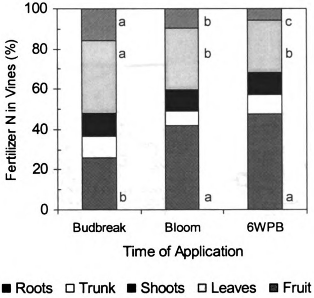

Figure 5: Effect of time of N application on the partitioning of fertilizer N in 'Concord' grapevines at the end of the season in 2002, Study 2. Letters represent separation of treatment means of the same vine organs (LSD P=0.05). Budbreak= 14 May; Bloom= 19 June; 6WPB= 29 July.

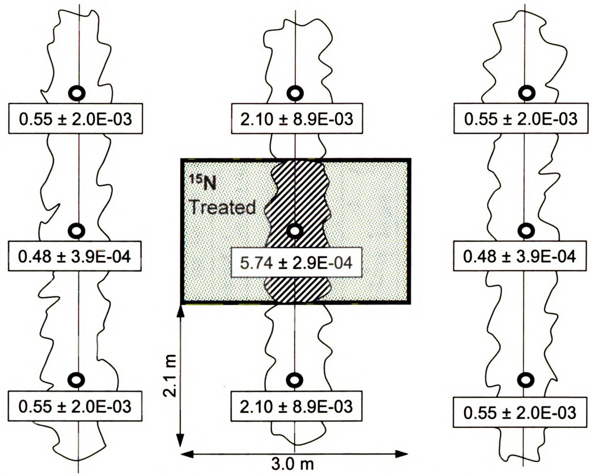

Figure 6: Schematic of a portion of the vineyard plot, showing a treated vine and adjacent vines in the 'Concord' 2002, Study 2. Shading represents the treated and excavated vineyard floor and vine. Values represent the percent of nitrogen in the leaves that is from labeled fertilizer. Values of the vines adjacent to the treated vine represent the means of three vines and standard errors. The treated vine values represent the mean and standard error of all the treated vines.

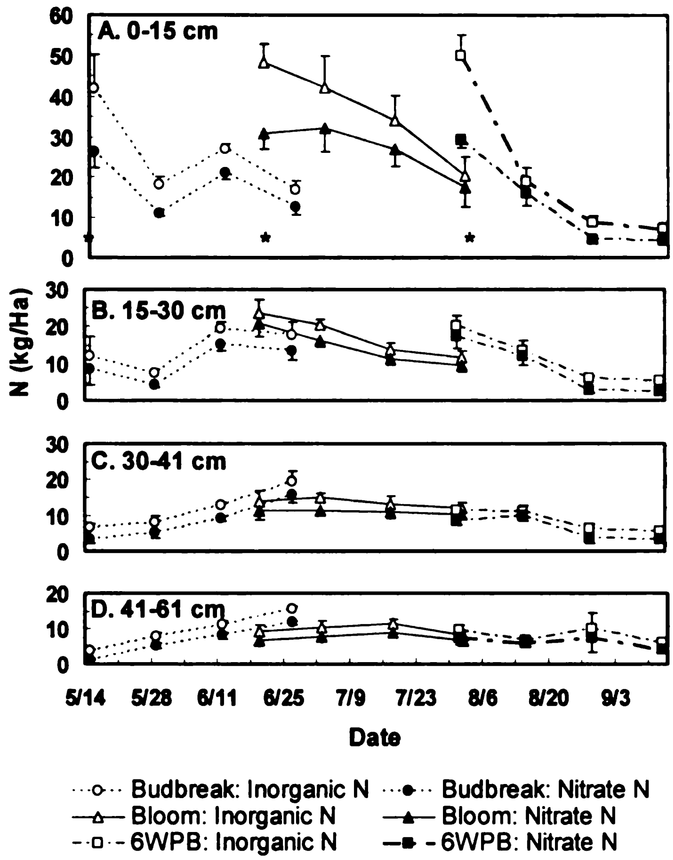

Figure 7: Inorganic N (NH_4 and NO_3) and nitrate N at 1, 15, 29, and 43 days following each fertilizer application in the soil profile at (A) 0-15.3 cm (B) 15.3-30.5 cm (C) 30.5-40.8 cm (D) 40.8-61.0 cm in vineyard soil of 'Concord' in 2002, Study 2. Bars represent standard error and * denotes the time of fertilizer application at Budbreak= 14 May; Bloom= 19 June; 6WPB= 29 July, respectively.

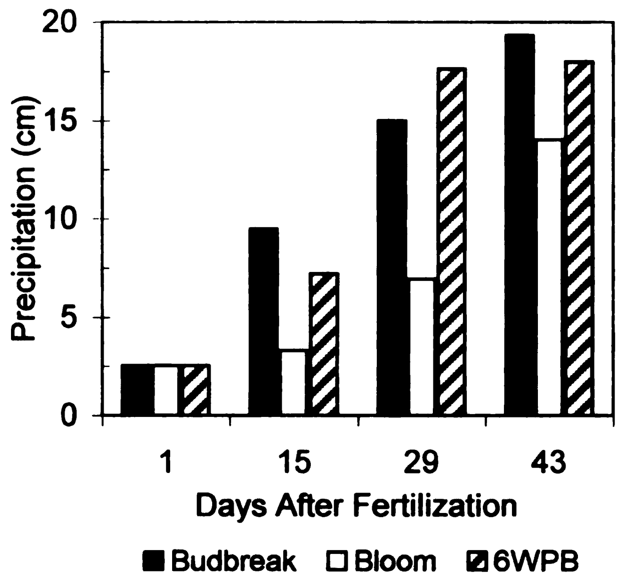

Figure 8: Accumulated rainfall and irrigation for each fertilizer treatment on the soil sampling dates following each fertilizer application in 2002 during Study 2 at the Southwest Michigan Research and Extension Center, Benton Harbor, MI. Budbreak= 14 May; Bloom= 19 June; 6WPB=29 July.

DISCUSSION

There was a trend for N applications later than budbreak to be taken up by the vines more efficiently, this was most evident in Study 2 with 'Concord'. This general trend was also seen in Study 1 with 'Niagara' vines, but was not significant due to a large variability of the vines. More efficient absorption of N applications later than budbreak has been shown in other studies. Hanson and Howell (1995) found that applications of N at budbreak to 'Seyval blanc' had a 7% NUE while vines treated at bloom had a 11% NUE, though the difference in NUE was not significant. Peacock et al. (1989) concluded that in California vineyards, budbreak N applications were not absorbed by vines as efficiently as July and Sept (post-harvest) applications.

There are several reasons why N applied at bloom and post-bloom may be taken up by vines more effectively than N applied at budbreak. Budbreak applications of N in some regions are more prone to leaching due to precipitation patterns (Conradie 1980; Peacock et al. 1991). Twenty to 40% of N used for grapevine growth from budbreak to bloom is mobilized from perennial vine structures (Conradie 1980; 1986; Lohnertz 1991; Schaller 1989 cited in Wermelinger 1991; Williams 1991). Mobilization of N from perennial structures of the vine prior to bloom is independent of soil N levels (Lohnertz 1991; Wermelinger 1991). Low soil temperatures early in the growing season can impair the uptake of N by woody plants (Dong et al. 2001). Conradie (1991) reported that grapevine root absorption of nutrients was initiated when soil temperatures reached 10°C.

The documented N accumulation pattern for 'Concord' in short-season viticultural regions (Bates et al. 2002; Hanson and Howell 1995) supports the findings that applications of N from bloom to 6WPB will be used by the vines more efficiently than budbreak applications. According to Bates et al. (2002), 85% of the N absorbed in the season occurred from bloom to veraison. Hanson and Howell (1995) found over 80% of N absorption occurred between bloom and fruit maturity.

Plastic mulch in the vineyard raised soil temperatures prematurely in the growing season (Figure 3). At early stages of growth, apple trees at the same phenological stage absorbed significantly more N when the rooting zone was at a higher temperature (Dong et al. 2001). High soil temperatures under the plastic may have stimulated N uptake to begin earlier in the season in Study 1 than normal, and may have increased uptake of fertilizer applied at budbreak. This effect may have lessened the effect of the time of N application on the NUE of the grapevines in Study 1. The impact of plastic mulch, in addition to cultivar and weather differences, may have caused the lack of large differences in NUE between the treatments in Study 1 as compared to the treatment differences within Study 2.

Inorganic N levels drop off rapidly following N applications (Figure 7). The inorganic N levels of the soil and precipitation accumulation (Figure 8) following the fertilizer applications do not accurately explain the differences in vine and soil recovery of fertilizer N. Lohnertz (1991) reported that vine N status can not be predicted by soil inorganic N levels. High levels of inorganic N were recorded following the N application at bloom, but fertilizer N recovery in the vines was not higher than the 6WPB applications, where inorganic N levels dropped of rapidly after the fertilizer application (Figure 7). Inorganic N levels drop off rapidly after fertilization at 6WPB and budbreak, but when N applications were applied at 6WPB, fertilizer N was recovered by vines more efficiently than at budbreak. Thus the low NUE of N applied at budbreak is most likely due to limited ability of the vines to absorb N at budbreak and not solely because of low inorganic N levels or by precipitation following the fertilizer application.

Fruit from the vines fertilized at budbreak contained more fertilizer N than the 6WPB treatments for both Study 1 and 2 (Figures 1 and 4). The percent of the fertilizer N partitioned to the fruit also followed this pattern (Figures 2 and 5). The higher allocation of fertilizer N to the fruit from the early N applications could be related to a need for N in the growth of clusters early in the growing season. Conradie (1986) reported that the major sink of N absorbed by the vines from the end of bloom until the end of rapid shoot growth was the fruit clusters. Hanson and Howell (1995) reported no difference between partitioning of fertilizer N to fruit from N applied at budbreak and bloom.

The percent of absorbed fertilizer N partitioned to the leaves was lower with the later application times in both Study 1 and 2 (Figures 2 and 5). A similar pattern was reported by Conradie (1991) where at fruit harvest, a higher proportion of spring absorbed N (30%) was partitioned to leaves than summer absorbed N (22%). Data from Hanson and Howell (1995) do not correspond to this trend, where applications of N at bloom resulted in more fertilizer N in the leaves than budbreak applications to 'Seyval blanc'.

When N was applied at budbreak there was less fertilizer N in the roots, than with the 6WPB applications (Figures 1 and 4). The percent of fertilizer N absorbed, that was partitioned to the roots was also lower for the N applied at budbreak than for N applied 6WPB (Figures 2 and 5). These trends did not occur in the study on 'Seyval blanc' by Hanson and Howell (1995). Conradie (1986) concluded that late N applications (post-harvest) in the long-season region of South Africa resulted in preferential allocation of the fertilizer N to perennial storage structures of the grapevines. Data from this study suggest that the storage of N reserves begins earlier in the season in short-season regions.

Overall these data indicate that N applied early in the growing season in Michigan to *V. labrusca* is allocated towards annual vine tissues (leaves and fruit), whereas the N from the later applications is preferentially allocated to roots of the grapevines, which may increase N reserves within the vine.

When N was applied later in the season, there was a higher amount of total N in the roots, which is likely due to increased root growth from the vines that had N applied to them later in the season (Figures 1 and 4). In Study 1, there were significantly higher amounts of root dry matter in the 3WPB and 6WPB treatments than the earlier treatments (Figure 1). There was a similar trend for lower root dry matter in the Budbreak treated vines in Study 2, but it was only significant to a 90% confidence level (P=0.074). Similarly, less root dry matter was previously reported (Hanson and Howell 1995) for vines that received N at budbreak than those fertilized at bloom. According to Conradie (1986), applications of N that lead to more N in the perennial structures of the vine are more important that those that allocate N to the annual structures because of the large impact these have on N reserves that influence the following season's growth. It is unknown what affects increased amounts of N and dry matter accumulation in the roots of the vines in these studies will have on long term vine growth.

Later applications of N are likely to have more of the fertilizer N in the soil because the fertilizer N had less time to leach from the sampled soil profile by the time the vines were excavated in Oct (Tables 2 and 4). However there are other possible explanations for the difference in retention of fertilizer N in the soil. Higher amounts of fertilizer remaining at the end of the season from later applications could be attributed to higher immobilization of fertilizer N. The low levels of soil inorganic N (Figure 7) six weeks after the 6WPB fertilizer application (mid-Sept) suggests that the large amount of fertilizer N remaining in the soil in the 6WPB treatment at vine excavation (early Oct) would likely be in organic forms. Considerable amounts of N fertilizer applied to citrus orchards can be quickly integrated with soil organic matter (Weinbaum 1992). It is unknown if the amount of fertilizer N remaining in the soil in Oct can be correlated to soil N levels in the subsequent growing season. The soil data from Study 2 are assumed to be more representative of a normal growing season and terms of soil temperatures and rainfall events, than with Study 1.

At the sampling times in Sept and Oct, the 'Niagara' vines and soil (to a 90 cm depth) in Study 1 contained 44.7, 52.4, 61.9, and 58.2% (30.2, 35.4, 41.7, and 39.3 kg N/Ha) of the applied fertilizer N for the Budbreak, Bloom, 3WPB, and 6WPB treatments, respectively. 'Concord' vines and soil in Study 2 contained 22.8, 52.8, and 75.7% of the applied fertilizer N (15.5, 35.3, 51.5 kg N/Ha) for the Budbreak, Bloom, and 6WPB treatments, respectively.

Hajrasuliha et al. (1998) reported high recoveries (90 to 100%) of spring applied ^{15}N fertilizer in the above ground portions of the grapevines and soil in late Sept. This higher recovery rate compared to that of Study 1 and 2 could be attributed to deeper soil sampling (2.4 m deep) and the method used to apply N (fertigation). Fertilizer N applied as nitrate had mostly leached to a depth between 1.2 and 1.5 m. Twelve to 19% of the applied fertilizer N, was converted to organic N and the majority of it was located in the top 60 cm of soil. The data from this experiment suggest that much of the N unaccounted for in our studies may have been leached below the 90 cm sampling depth. The relative proportion of organic to inorganic forms of fertilizer N at the vine harvest in our studies is unknown. However, it would be reasonable to expect that a substantial portion of the fertilizer N in the shallow section of soil in Oct was in organic forms of N.

Vines adjacent to the treated vines in Study 2 recovered considerable amounts of the fertilizer N (Figure 6). The vines adjacent to the treated vines were not fertilized, and because they had less available N in their vine space they may have absorbed more fertilizer N from the ^{15}N treated vineyard floor. The high levels of fertilizer N adjacent to the treated vines were comparable to the recovery of fertilizer N by adjacent vines in Stroehlein et al. (1990). In previous viticulture ^{15}N fertilization experiments investigating NUE of entire field-grown vines (Hajrasuliha et al. 1998; Hanson and Howell 1995; Williams 1991), there was no sampling of the vines adjacent to the treated vines.

If the values of the fertilizer recovery of all the adjacent vines were factored into the total NUE of the fertilizer application (Figure 6), the NUE on a vineyard basis could be double. This indicates that it is possible to determine relative differences of efficiency between times of N application by only excavating the treated vines. However, such estimates are insufficient to determine total NUE of field N applications by sampling only the treated vines, due to the extensive, spreading grapevine root systems (Perry et al.1990; Winkler et al. 1974). By sampling only the vines directly fertilized with labeled fertilizer, there is a low estimation of total vineyard recovery of fertilizer N because adjacent vine uptake of fertilizer N is not taken into consideration.

It appeared that more roots were located in the soil of the vegetation free strip beneath the vines, than in the vegetative row middles. The majority of the roots in Study 2 were in the top 45 cm of soil, with only a small portion (5%) recovered from 45 to 90 cm depth of soil. Although vines were excavated only to 45 cm in Study 1, few roots were observed penetrating below 45 cm. A high proportion of roots of 'Concord' vines, was also found in the top 45 cm of soil by Perry et al. (1983). Approximately 75% of the root dry matter, sampled to a 75 cm soil depth, was found in the in top 45 cm. Therefore, fertilizer N that leached below 45 cm would not have been accessible to the roots of the vines in our studies, since most of them were in the top 45 cm of soil.

Although NUE values for this study were low (9.8 to 18.6% of applied N), they were higher than those in an earlier study in Michigan, in which 'Seyval blanc' vines recovered 7.1 to 10.6% of applied N (Hanson and Howell 1995). Low values in the previous study may have resulted from competition for N from the sod vineyard floor, and the fact that the vines were smaller (6.4 kg dry matter/vine) than those in our studies (10.4 kg). Williams (1991) reported a similar NUE of 14% for N applies in May to furrow irrigated vineyards, but 42% recovery for drip irrigated vines. The higher NUE of the drip-irrigated vines is likely attributed to fertilizing in a more targeted manner to the root zone.

There was little to no fruit in Study 1 versus an average crop in Study 2, this may have affected fertilizer N uptake. It is unknown whether the crop level of grapevines affects N uptake, but our data suggest it is unlikely since the mean NUE efficiency of Study 1 (17.3%) was higher than that of Study 2 (13.9%). Another difference between the studies was that more root dry matter was found in the 'Concord' vines in Study 2 than with the 'Niagara' vines in Study 1 (Figure 1 and 3). Reasons for this are unknown, but cultivar and vineyard site could have affected the amount of root dry matter. Large differences in the rooting patterns of different grape cultivars have been previously reported (Perry et al. 1983).

Nitrogen application rates in mature commercial vineyards in Michigan range from 0 to 227 kg N/Ha (Tom Zabadal, personal communication). NUE of N applications is considered to be inversely related to the rate of N application (Weinbaum et al. 1992). At higher rates of fertilization, more total fertilizer N could be absorbed by the vines, but the NUE would likely decrease with increasing rates.

The majority of the N in the vines in both studies was not from fertilizer N applied during the season in which they were excavated. Fertilizer N represented 6.4 to 7.2% of total vine N in the 2001 study with 'Niagara' vines, and only 3.6 to 5.4% for 'Concord' vines in 2002. Hanson and Howell (1995) report that vigorous, mature 'Concord' vines in Michigan absorbed 57 g N/vine (77 kg N/Ha) in a growing season. This means that the majority of the N accumulated in the vine during a growing season is not from fertilizer N applied in that season, because the grapevines in our studies only recovered the equivalent of 6.7 to 12.6 kg N/Ha of the 68 kg N/Ha fertilizer application. This indicates that native N provided the majority of N absorbed by the vines in our studies.

CONCLUSIONS

These studies indicate that *V. labrusca* grapevines recover a relatively small portion of applied fertilizer N (9.8 to 18.6%) under Michigan growing conditions. Ammonium nitrate applications to 'Concord' vines at budbreak were not used as efficiently by the vines as applications at bloom or six weeks after bloom, therefore vineyard uptake of fertilizer N may be enhanced by applying N from bloom until six weeks post-bloom instead of at budbreak. This trend also occurred in 'Niagara', but the N uptake pattern and movement of fertilizer N through the soil may have been affected by the use of plastic mulch

Both Study 1 and 2 resulted in very consistent patterns of fertilizer N partitioning patterns within the vines, in spite of both cultivar and seasonal differences between the two studies. *V. labrusca* vines fertilized at budbreak allocated significantly more of the fertilizer N to the reproductive organs (fruit) and leaves, than later applications of N. Later times of application resulted in more fertilizer N being allocated to the roots of 'Concord' and 'Niagara' grapevines. Whereas N applied to *V. labrusca* grapevines early in the growing season is preferentially allocated to the growth of annual tissues (leaves and fruit), fertilization later in the season (6WPB) resulted in more fertilizer N and more total N from all sources being allocated to perennial vine structures (roots).

The majority of the fertilizer N (82-90%) that was applied to the vines at a rate of 68 kg N/Ha, was not used by the treated vines in that growing season. Root distribution data indicate that much of the fertilizer N below the 45 cm depth would not be accessible to the vines in this study. There were notable treatment differences in the amount of fertilizer N that remained in the soil. Generally the later applications had more fertilizer

N remaining in the soil when the vines were excavated. It is unknown exactly how much of the fertilizer N in the treated vine spaces was incorporated into soil organic matter and could be potentially available to vines in subsequent growing seasons. Therefore an advantage of the Bloom and 6WPB N applications might not only be a higher NUE than the budbreak applications, but also a higher retention of fertilizer N in the soil.

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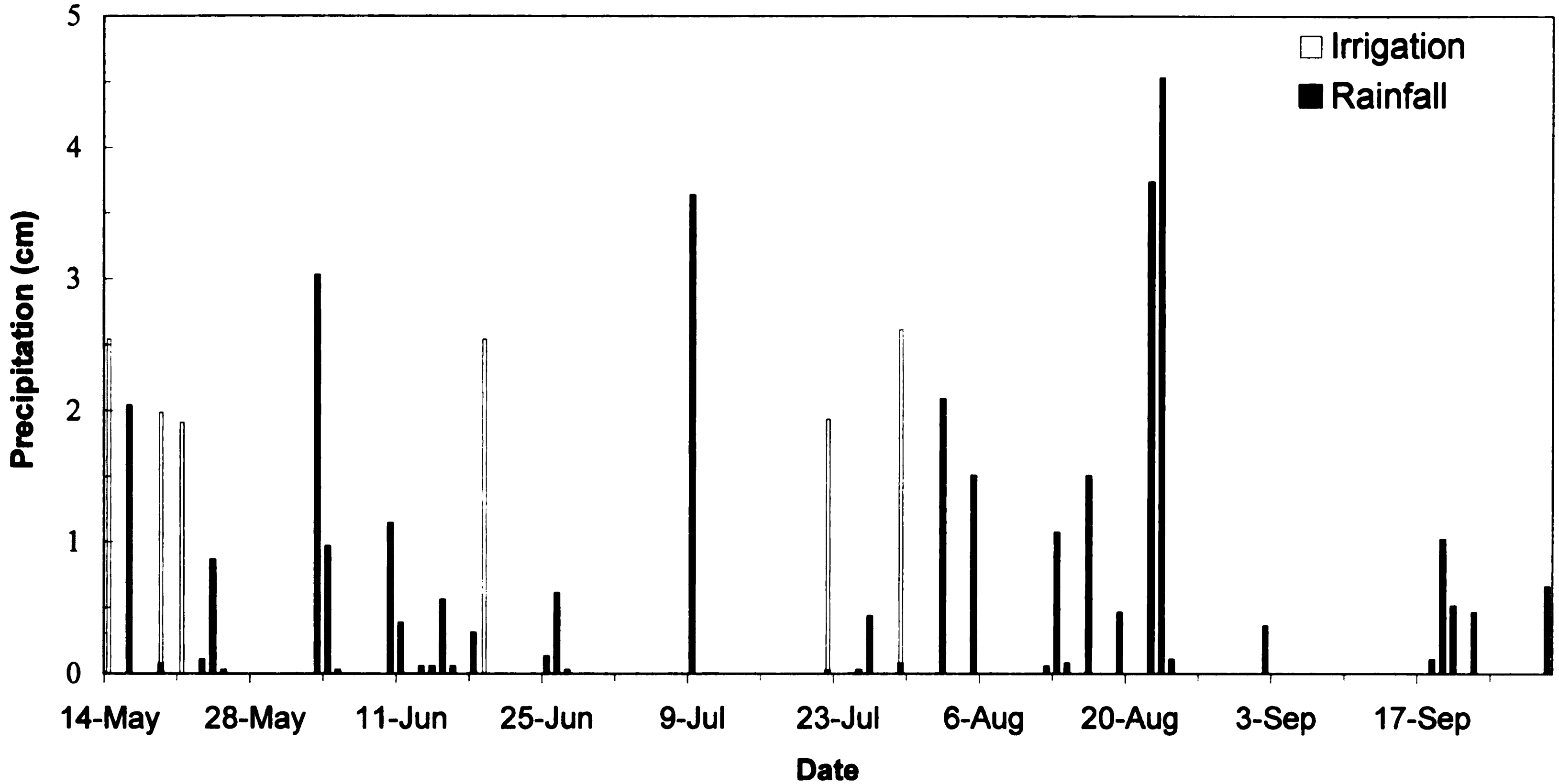
APPENDICES



Appendix 1: Rainfall and irrigation events during Study 2, 2002 at the Southwest Michigan Research and Extension Center, Benton Harbor, MI. Fertilizer applications were on 14 May (Budbreak), 19 June (Bloom), and 29 July (six weeks after bloom).

Time of N Application	Fertilizer Derived N g/vine (% of total in vine) Niagara 2001					
	Roots	Trunk	Shoots	Leaves	Fruit	Total
Budbreak	1.02 (2.3)	0.83 (1.9)	1.26 (2.9)	3.24 (7.4)	0.45 (1.0)	6.81 (15.5)
Bloom	0.99 (2.3)	1.04 (2.4)	1.58 (3.6)	3.84 (8.8)	0.16 (0.4)	7.60 (17.4)
3WPB	1.64 (3.7)	0.99 (2.3)	1.60 (3.6)	3.49 (8.0)	0.10 (0.2)	7.81 (17.8)
6WPB	2.47 (5.6)	0.97 (2.2)	1.45 (3.3)	3.17 (7.2)	0.07 (0.2)	8.13 (18.6)

applied 43.8 g N/vine

Time of N Application	Total N g/vine (% of total N/vine) Niagara 2001					
	Roots	Trunk	Shoots	Leaves	Fruit	Total
Budbreak	26.7 (25.3)	19.3 (18.3)	16.6 (16.7)	38.8 (36.7)	4.3 (4.0)	105.7 (100)
Bloom	23.2 (22.1)	20.1 (19.2)	18.6 (17.7)	41.5 (39.5)	1.7 (1.6)	105.1 (100)
3WPB	36.6 (31.3)	22.4 (19.1)	18.7 (16.0)	37.6 (32.2)	1.6 (1.3)	116.9 (100)
6WPB	40.0 (33.5)	25.4 (21.3)	16.8 (14.1)	35.8 (30.0)	1.3 (1.1)	119.4 (100)

Time of N Application	Dry Mass kg/vine (% of total dry matter/vine) Niagara 2001					
	Roots	Trunk	Shoots	Leaves	Fruit	Total
Budbreak	1.74 (20.6)	2.51 (29.7))	2.24 (26.5)	1.51 (17.9)	0.44 (5.2)	8.44 (100)
Bloom	1.65 (19.6)	2.58 (30.6)	2.44 (29.0)	1.60 (19.1)	0.13 (1.6)	8.40 (100)
3WPB	2.34 (25.7)	2.83 (30.9)	2.32 (25.4)	1.49 (16.4)	0.15 (1.6)	9.13 (100)
6WPB	2.62 (27.8)	3.08 (32.6)	2.19 (23.2)	1.43 (15.2)	0.10 (1.1)	9.43 (100)

Appendix 2: Effect of time of N application to 'Niagara' vines in 2001, Study 1, on the fertilizer N, total N, and dry matter within the vines at the end of the season. Budbreak=30 April; Bloom= 8 June; 3WPB= 30 June; 6WPB= 18 July.

Time of N Application	Fertilizer Derived N g/vine (% of applied) Concord 2002					
	Roots	Trunk	Shoots	Leaves	Fruit	Total
Budbreak	1.09 (2.5)	0.44 (1.0)	0.51 (1.2)	1.6 (3.7)	0.69 (1.6)	4.32 (9.9)
Bloom	3.04 (7.0)	0.56 (1.3)	0.76 (1.7)	2.23 (5.1)	0.71 (1.6)	7.30 (16.8)
6WPB	3.09 (7.1)	0.63 (1.4)	0.73 (1.7)	1.70 (3.9)	0.36 (0.8)	6.51 (15.0)

applied 43.5 g N/vine

Time of N Application	Total N g/vine (% of total N/vine) Concord 2002					
	Roots	Trunk	Shoots	Leaves	Fruit	Total
Budbreak	48.9 (40.2)	17.1 (14.1)	10.4 (8.6)	30.4 (25.0)	14.9 (12.2)	121.5 (100)
Bloom	62.5 (46.2)	15.4 (11.4)	12.2 (9.0)	33.3 (24.6)	11.8 (8.7)	135.3 (100)
6WPB	59.7 (45.8)	16.2 (12.4)	12.0 (9.2)	32.1 (24.6)	10.3 (7.9)	130.2 (100)

Time of N Application	Dry Mass kg/vine (% of total dry matter/vine) Concord 2002					
	Roots	Trunk	Shoots	Leaves	Fruit	Total
Budbreak	3.82 (33.0)	3.16 (27.3)	1.41 (12.2)	1.46 (12.6)	1.73 (14.9)	11.58 (100)
Bloom	4.97 (38.1)	3.17 (24.3)	1.73 (13.3)	1.61 (12.3)	1.55 (11.8)	13.03 (100)
6WPB	4.63 (38.7)	2.81 (23.5)	1.68 (14.0)	1.59 (13.3)	1.25 (10.5)	11.96 (100)

Appendix 3: Effect of time of N application to 'Concord' vines in 2002, Study 2, on the fertilizer N, total N, and dry matter within the vines at the end of the season. Budbreak= 14 May; Bloom= 19 June; 6WPB= 29 July.