THE RELATIONSHIP BETWEEN MINERAL NUTRITION AND LATE-SEASON BUNCH STEM NECROSIS OF CABERNET SAUVIGNON (*VITIS VINIFERA* L.) GRAPEVINES

by

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(Abstract)

Late-season Bunch Stem Necrosis (BSN) is observed as a necrosis of the cluster stem (rachis) that leads to shriveling of berries on the affected portion of the cluster. Field experiments were conducted over three years at two vineyards in northern Virginia to examine relationships between specific nutrients and the incidence of BSN of Cabernet Sauvignon grapevines. Nutrients, used alone or in combination, included nitrogen, magnesium, and calcium. During the 1997 and 1998 seasons at Leesburg vineyard, applications of nitrogen, magnesium, and calcium produced little change in bloom-time petiole mineral concentration. Fertilizer treatments appeared to have no effect on BSN incidence, but the incidence of BSN was low ($\leq 1\%$) in the control plots each year. During the 1996 season at Winchester vineyard, bloom-time leaf petiole and véraison rachis nitrogen concentration of unfertilized (control) vines were 0.80% and 1.16%, respectively. The corresponding control BSN incidence was 41% at harvest time. Application of nitrogen fertilizer at 112 kg/ha actual nitrogen increased bloom-time leaf petiole and véraison cluster stem nitrogen concentration to 1.85% and 2.18%, respectively. The corresponding BSN incidence was reduced to 14% at harvest time. BSN symptoms were not as pronounced during the 1997 season; however, all treatments, including the control plots, had elevated nitrogen levels in 1997. During the 1998 season, bloom-time leaf petiole and véraison rachis nitrogen concentration of unfertilized vines were 0.88% and 0.98%, respectively. The corresponding BSN incidence was 23% at

harvest time. Application of nitrogen fertilizer again increased bloom-time leaf petiole and véraison rachis nitrogen concentration to 1.18% and 1.34%, respectively. Corresponding BSN was reduced to 3% at harvest time. Magnesium and calcium had no impact on BSN incidence; however, BSN symptoms were reduced when either was combined with nitrogen fertilizer. The relationship between mineral nutrition and BSN incidence at Leesburg was inconclusive. The BSN of Cabernet Sauvignon at Winchester was, however, positively associated with depressed bloom-time petiole total nitrogen concentrations. Véraison rachis analysis consistently revealed an increase in nitrogen concentration due to application of nitrogen fertilizer. Véraison tissue analysis may be a good diagnostic tool of vine nitrogen status. Magnesium and calcium appeared not to be involved in the disorder. The results illustrate that BSN-prone vineyards should be individually examined for nutrient imbalance or other stresses that may be contributing to BSN.

DEDICATION

I dedicate this thesis to my loving parents Bonnie and Dean Capps. Their support and sacrifices over the years have allowed for the achievement of many of my goals.

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CHAPTER ONE

INTRODUCTION

Late-season bunch stem necrosis (BSN) is a physiological disorder of the bunch stem (rachis) of grapevines (Brendel *et al.*, 1983). The disorder may appear any time during the early stages of berry ripening (véraison). The BSN symptoms include dark, necrotic lesions on the rachis or individual pedicels that may spread and eventually girdle the affected part of the cluster rachis (Morrison and Iodi, 1990; Stellwaag-Kittler, 1983). Berries distal to a lesion cease normal development, and the unripe berries either abscise or remain on the cluster in a withered condition. Frequently only the cluster tip or a shoulder is affected, while the rest of the cluster develops normally. Symptomatic and non-symptomatic clusters may be borne on the same vine.

Low temperatures during bloom were inversely related to the incidence of BSN in Switzerland (Theiler and Muller, 1986). However, in Australia, Holzapfel and Coombe (1995) reported that cool temperatures during the 20 days prior to bloom, and/or during the week of véraison, promoted BSN, while temperatures during flowering had no bearing on symptom expression. Precipitation and relative humidity have also been implicated with the occurrence of BSN. Rainfall prior to or during véraison has been associated with BSN incidence in Germany (Redl, 1987) and Australia (Holzapfel and Coombe, 1995). Grapevines grown under high humidity had a greater incidence of BSN compared to grapevines grown under low humidity (Jordan, 1985).

There are conflicting reports regarding the association of essential nutrients and the incidence of BSN. A high ratio of potassium to magnesium and/or calcium in affected tissue, and the application of calcium and/or magnesium fertilizers effectively reduced the incidence of BSN in Europe (Boselli *et al.*, 1983; Brendel *et al.*, 1983; Hartmeir and Grill, 1965; Haub 1986; Lauber and Koblet, 1967). In California, BSN was not reduced by applications of calcium and magnesium. An increase in the incidence of BSN was reported with applications of nitrogen fertilizer (Christensen and Boggero, 1985).

Similarly confusing results were observed in Virginia vineyards surveyed in 1995. One of the vineyards surveyed suggested that BSN incidence of Cabernet Sauvignon was associated with a deficient tissue concentration of nitrogen. This association was not universal in all vineyards surveyed; therefore, there was a need to examine several nutrients. Experiments were initiated in 1996 to determine the effect of nitrogen, calcium, and magnesium on BSN incidence in two Cabernet Sauvignon vineyards in Virginia.

BSN has been correlated with numerous factors; however, no universal cause and effect relationships have been demonstrated. The purpose of this study was to determine if mineral nutrition was associated with BSN of Cabernet Sauvignon under Virginia growing conditions. Cabernet Sauvignon is an important cultivar in Virginia and is frequently affected by BSN. One objective of this study was to determine if there was a direct association of tissue nitrogen concentration with the incidence of BSN. Another objective was to explore the possibility that either Mg or Ca were involved in the disorder. Changes in the vine tissue concentration of nitrogen, calcium, and magnesium in Cabernet Sauvignon grapevines was attempted by the application of fertilizer.

CHAPTER TWO
REVIEW OF LITERATURE

Physiological disorders of flower and fruit clusters of grapevines occur world-wide. Symptoms vary with respect to specific tissues affected and by phenological stage of organ development. Those differences have led to the description of at least two distinct disorders:

a). Late-season Bunch Stem Necrosis (BSN): Rachis tissue affected; symptoms may occur any time after véraison; synonyms include "rachis necrosis", "waterberry", "shanking", "stiellähme", "dessèchement de la rafle", "palo negro", and "disseccamento del rachide".

b). Inflorescence Necrosis (IN): Pedicels and flowers affected, symptoms occur at or before bloom; synonym is "early bunch stem necrosis" (EBSN).

I use the expression Bunch Stem Necrosis in this thesis to describe the physiological disorder affecting the rachis and pedicels of clusters during the early stages of véraison causing berries distal to a lesion to cease normal development.

Symptoms: The first stage of BSN appears as small dark lesions on the rachis and/or pedicels of grape clusters (Stellwagg-Kittler, 1983). Theiler (1970) reported that these lesions form around the stomata and destroy the guard cells and subsidiary cells. Polyphenols are oxidized in the affected cells and cell walls producing visible necrotic areas. The necrotic stomatal region may spread and affect collenchyma and parenchyma tissues of the peduncle and, at a severe stage, the phloem tissue (Brendel *et al.*, 1983). Christensen and Boggero (1985) described this stage as 1-3 mm diameter brown or black spots that become necrotic, sunken, and increase in size affecting more area of the cluster stem. Stellwaag-Kittler (1983) reported that the small necrotic islands of varying shape and size were harmless but when they surround the cluster stem they led to death. A

necrotic lesion may increase in size and girdle the rachis, causing desiccation of the rachis distal to the lesion, which either abscises or remains on the cluster in a dry condition (Morrison and Iodi, 1990). Cluster shoulders and or tips are frequently symptomatic, while the rest of the cluster develops normally. In some cases, BSN may affect only the pedicels of a cluster causing a few symptomatic berries scattered throughout an otherwise healthy cluster. Symptomatic and non-symptomatic clusters frequently appear on the same vine.

The necrotic areas of the rachis interrupt the normal flow of sugars and other translocates to the cluster. The BSN-affected berries are dull in appearance, soft, and lack normal sugar, color, and flavor (Bioletti, 1923). Morrison and Iodi (1990) reported that symptomatic berries had retarded sugar and potassium accumulation, continued accumulation of calcium and tartaric acid, and delayed or reduced berry growth, compared to berries on non-symptomatic clusters. Morrison and Iodi's 1990 observations were consistent with a previous report (Ureta *et al.*, 1981) of BSN berry composition. The higher titratable acidity of BSN-affected berries appears to be due to the higher concentration of tartaric acid (Morrison and Iodi, 1990; Ureta *et al.*, 1981). In normally developing berries, tartrate accumulation stops at véraison and concentration declines as berry growth continues (Saito and Kasai, 1968). The higher concentration of tartaric acid in BSN-affected berries is primarily due to slower berry expansion (Morrison and Iodi, 1990). The appearance of breaks in xylem vessels of the peripheral vascular bundles in normally developing berries coincides with the onset of ripening. Xylem water flow appears to cease due to these breaks (Düring *et al.*, 1987). The influx of calcium into fruit takes place nearly exclusively in the xylem (Marschner, 1995). The continued influx of calcium into BSN berries after véraison suggests that the breakage in xylem vessels does not occur in fruit affected by BSN (Morrison and Iodi, 1990). However, Düring and Lang (1993) indicated that the failure of proper xylem development in BSN-affected clusters close to rachis nodes reduced hydraulic conductance. Düring and Lang (1993) suggested that xylem water flow past these rachis nodes reduced calcium transport.

Cultivars and Nomenclature: The list of cultivars that reportedly express BSN is lengthy (Fregoni and Scienza, 1970). BSN nomenclature is descriptive of the rachis symptoms. Hence, the names "shanking" (New Zealand) (Jordan, 1985), "stiellähme" (Germany) (Stellwaag-Kittler, 1983), "dessèchement de la rafle" (France) (Ureta *et al.*, 1981), "palo negro" (Chile) (Ruiz and Moyano, 1993), "disseccamento del rachide" (Italy) (Fregoni and Scienza, 1972), and "rachis necrosis" (Canada) (Cline, 1987) are all found in the literature. In California, "waterberry" refers to the watery, soft, and flabby appearance of BSN affected berries [L. P. Christensen, personal communication, 1999]. Winkler *et al.* (1974) described two conditions of waterberry. In the first condition, affected berries were mainly confined to the tips of clusters. Bioletti (1923) attributed this condition to overcropping, which prevented proper nourishment and complete development of the affected berries. The most common cause of under-nourishment of berries is overcropping (Winkler *et al.*, 1974). In the second condition, affected berries were scattered thoughout the cluster. Kasimatis (1957) reported that this latter condition was most prevalent in thinned, vigorous vines carrying crops well within their capacity. The first symptoms of waterberry were necrotic spots on individual berry pedicels and the occurrence of flaccid berries scattered throughout the cluster (Kasimatis, 1957).

Environment: The cause of bunch stem necrosis is uncertain. Because no pathogen has been linked to BSN, research has focused on possible environmental factors but inconsistencies exist. In New Zealand, during the 1983-1984 season, four-year old Italia vines were grown in pots under controlled environmental conditions, either at high (80%) or low (40%) relative humidity maintained from flowering to harvest (Jordan, 1985). Other environmental conditions were the same for both treatments. Vines in the higher humidity had a greater incidence of BSN (77%) compared to the lower humidity (34%) (Jordan, 1985). The authors reported the results without statistical analysis and did not indicate whether the results were repeatable. A common feature of BSN is the annual variability of expression (Haystead *et al.* 1988; Holzapfel and Coombe, 1995), which suggests an environmental mediation of symptoms.

Studies in German and Swiss vineyards have shown an inverse relationship between average maximum day temperatures during the time of flowering and the occurrence of BSN (Brechbuhler, 1987; Gysi, 1983; Theiler and Müller, 1986, 1987). In a long-term study (1976-1984), Theiler and Müller (1986) correlated the frequency of BSN in the cultivar Müller-Thurgau with the mean temperature and the amount of precipitation during five periods of grapevine development. Theiler and Müller (1986) reported that only during flowering was there a significant correlation between climatic factors and the occurrence of BSN. Conversely, Redl (1987), working in Austria, found no correlation between climatic factors during flowering and the occurrence of BSN in the cultivar Grüner Veltliner.

In a three-year study (1989-1992), Holzapfel and Coombe (1995) found no relationship between temperature and/or rainfall during the *flowering period* and the occurrence of BSN in Cabernet Sauvignon grapevines in Australia. However, an inverse relationship between lower temperatures during the 20 days *before flowering* and *during véraison* with BSN incidence was observed. During the 1989-1990 growing season (26% BSN), the average daily mean temperature *before flowering* and *during véraison* was lower compared to the same time intervals during the 1990-1991 growing season with 3% BSN incidence (Holzapfel and Coombe, 1995). Holzapfel and Coombe (1995) reported a relationship of a high incidence of BSN with rainfall during the time of véraison in the 1989-1990 season. The relationship was not supported due to the lack of rainfall during the two weeks straddling véraison in the 1991-1992 growing season, a season with an intermediate occurrence of BSN (11%). The differences in these studies suggest factors other than environmental conditions are involved with the occurrence of BSN.

Mineral nutrition: BSN has occasionally been associated with calcium or magnesium deficiency (Pearson and Goheen, 1988) and has been included in a group of physiological disorders caused by an incorrect metabolism of calcium, such as bitter pit of apples and blossom end rot of tomatoes and peppers (Boselli and Fregonia, 1986). French, German, Italian, and Swiss research suggested an imbalance of potassium (K), calcium (Ca), and/or magnesium (Mg) in the rachis and leaf tissue of grapevines led to BSN (Boselli *et*

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al., 1983; Brendel *et al.*, 1983; Fabre *et al.*, 1983; Leonhardt, 1987). Those findings were not, however, consistent with research in Austria and California.

 Brechbuhler (1975) reported that the ratio of K to Mg and/or Ca (K/ Mg + Ca) increased in petiole and rachis tissue up to véraison and then dropped. Initial symptoms of BSN corresponded with that drop. The BSN-prone cultivars (e.g. Gewürztraminer) had higher K/ Mg + Ca ratios in rachis tissue than did less susceptible cultivars (e.g. Sylvaner) (Brechbuhler, 1975). Leonhardt (1987) recommended maintaining a 2:1 ratio of K to Mg. However, tissue analysis of both rachis and leaves showed no significant correlation between those cations in the cultivars Riesling and Grüner Veltliner in Austria (Redl, 1983). In California, a lower K/ Mg + Ca ratio was reported in BSN symptomatic rachis tissue compared to non-symptomatic tissue of Thompson Seedless (Christensen and Boggero, 1985; Christensen *et al.*, 1991). In Australia, Holzapfel and Coombe (1996) found the comparison of mineral concentrations in Cabernet Sauvignon rachis tissue and BSN incidence to be "inconsistent".

Potassium is a monovalent cation and has a high rate of uptake by plant tissue (Marschner, 1995; Mengel and Kirkby, 1987). Potassium plays a key role in plant water relations, to activate certain enzymes, and for protein synthesis (Marschner, 1995). Potassium is highly mobile in plant xylem and phloem tissue. Calcium is a divalent cation and its rate of uptake can be depressed by an abundance of potassium and magnesium (Mengel and Kirkby, 1987). Calcium is needed for cell wall formation, development of proteins, activation of some enzymes, carbohydrate transport and it plays a role in N metabolism (Marschner, 1995). Calcium transport is principally acropetal in the xylem transpiration stream. Magnesium is a divalent cation and its rate of uptake can be reduced by other cations such as K, NH_4, Ca, and Mg (Marschner, 1995; Mengel and Kirkby, 1987). Magnesium is an activator of several enzymes that catalyze carbohydrate metabolism. It is also involved in regulation of cellular pH, and has structural and regulatory roles in the synthesis of proteins (Marschner, 1995; Mengel and Kirkby, 1987). The most familiar function of Mg is its role as the central atom of the chlorophyll molecule, essential for photosynthesis (Christensen *et al.*, 1978; Marschner, 1995;

Mengel and Kirkby, 1987). In contrast to Ca, Mg is highly mobile in phloem and can be remobilized from older plant tissue to actively growing tissue (Mengel and Kirkby, 1987).

Calcium and magnesium: Because Ca and Mg have such important structural roles, and because BSN has been reduced with foliar applications of these divalent cations, it is generally accepted in France, Germany, Greece, Italy, and Switzerland that a deficiency of Ca and/or Mg is associated with BSN (Brendel *et al.*, 1983; Boselli *et al.*, 1983; Boselli and Fregonia, 1986; Bübl, 1985; Cocucci *et al.*, 1988; Fabre *et al.*, 1983; Haub, 1986; Rumbos, 1989). The most commonly used mineral solutions are calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), magnesium oxide (MgO), and magnesium sulfate ($MgSO_4$ or Epsom Salt) (Beetz and Bauer, 1983; Boselli and Fregonia, 1986; Bübl, 1985; Fabre *et al.*, 1983; Haub, 1986; Lauber and Koblet, 1967; Rumbos, 1989). Lauber and Koblet (1967) reported that four applications of $CaCl_2$ or $MgCl_2$ (0.75% concentration), starting at véraison, had no effect on the incidence of BSN of the cultivar Blauburgunder (Pinot noir). However, the application of $CaCl_2$ plus $MgCl_2$ on Riesling x Sylvaner was 90% effective in reducing the incidence of BSN (Lauber and Koblet, 1967).   Similar results were reported using a 0.5% solution of $CaCl_2$ and $MgCl_2$ (Koblet *et al.*, 1969). Some scorching of the leaves was observed in the Riesling x Sylvaner and, to a lesser extent, with Pinot noir by those treatments.

In an experiment on Riesling grapevines from 1978-1980, foliar applications of $MgSO_4$ were applied at different times and rates in an attempt to control BSN (Beetz and Baur, 1983). In 1978, a 5% concentration of $MgSO_4$ was applied five times starting shortly before bloom until véraison with 93% control. The same concentration applied once before véraison and once at véraison resulted in 91% control (Beetz and Baur, 1983). Similar results using $MgSO_4$ or Mg-base fertilizer like Wuxal-Magnesia (78% MgO and 1% N and trace elements) and Fertilon Combi (9% MgO and chelated trace elements) were obtained by Bübl (1985), Fabre *et al.* (1983), Haub (1986), Jürgens and Becker (1987), and Leonhardt (1987). However, the efficacy of the treatments varied by location, cultivar, and year (Haub, 1986; Koblet *et al.*, 1969; Lauber and Koblet, 1967;

Rumbos, 1989). Magnesium compounds were more effective than Ca compounds in reducing BSN in Germany (Haub, 1986). Boselli and Fregonia (1986) obtained similar results with the cultivar Croatina in Italy. Thorough wetting of the grape cluster with the divalent cations is recommend.

Attempts to control BSN with the application of fertilizers containing Ca and Mg has also been investigated in North America. In Canada from 1979-1985, BSN of Canada Muscat was reduced with soil applications of dolomitic lime and foliar applications of $CaCl_2$ and/or $MgSO_4$ and BSN of Himrod, was reduced (Cline, 1987). Foliar application of $CaCl_2$ was least effective. Cline (1987) reported that treatment effects on petiole and rachis composition were not consistent but the high K content of the petioles suggested an imbalance of K with Ca and Mg may explain the effect of the treatments and may be partially responsible for BSN. The author did not, however, include corresponding tissue analysis data, nor were data presented statistically analyzed. In New Zealand, five applications, between berry set and véraison, of $MgSO_4$ (3% solution) on green-house-grown Italia grapevines reduced the BSN incidence to 17% compared with a 65% incidence of unsprayed bunches (Jordan, 1985), but without tissue or statistical analysis.

Holzapfel and Coombe (1994) evaluated the efficacy of Mg sprays for BSN control of green-house-grown Flame Seedless and field grown Cabernet Sauvignon in South Australia. Two applications of 2% $MgSO_4$, applied at the start of véraison, reduced BSN levels from 49% (control) to 25% on Flame Seedless. The reduction was even greater (16% BSN) when a total of 4 applications were made. The Cabernet Sauvignon experiment was conducted for three growing seasons (1990-1992) with a significant reduction of BSN observed only in the 1990 season (Holzapfel and Coombe, 1994). The data were not, however, presented in a way to determine the effect by treatment. The finding of sub-optimal levels of manganese (Mn) in petiole and bunch stem tissue of Cabernet Sauvignon treatment vines, resulted in an additional experiment in the 1993 season including $MnSO_4$ in the treatments and other trace minerals (Holzapfel and Coombe, 1994). Holzapfel and Coombe (1994) reported that these experiments showed Mg sprays slightly reduced BSN and the addition of Mn and other trace elements had no

effect. The authors did not, however, report corresponding tissue analysis and BSN incidence results.

Nitrogen: Application of nitrogen fertilizers has occasionally increased the occurrence of BSN (Christensen and Boggero, 1985; Cooper *et al.*, 1987; Gysi, 1983; Redl and Weindlmayr, 1983; Ruiz and Moyano, 1993). Nitrogen (N) is translocated in the xylem and is needed to build compounds essential for plant growth and development, including amino acids, proteins, enzymes, and nucleic acids (Marschner, 1995; Mengel and Kirkby, 1987). The pigment in green chlorophyll and anthocyanins in fruit require N. Nitrate (NO_3^-) and ammonium (NH_4^+) are the forms of nitrogen that are taken up and metabolized by plants (Marschner, 1995; Mengel and Kirkby, 1987). Nitrate can be translocated unaltered in the xylem but almost all of the NH_4^+ is assimilated in the root tissue and redistributed as amino acids (Mengel and Kirkby, 1987). Excessive levels of N can cause poor fruit set and reduce carbohydrate storage (Christensen, 1978).

In California, Christensen and Boggero (1985) studied the effect of soil application treatments of Ca, Mg, N, phosphorous (P), or N and P fertilizers on BSN. The work was done over three years (1980-1982), at three locations with Thompson Seedless grapevines. Total N petiole tissue concentrations were only significantly increased at one location, but petiole tissue NO_3^- levels were significantly higher in all locations due to treatments containing nitrogen (Christensen and Boggero, 1985). The authors presented bloom-time petiole analysis as a three-year mean and not by individual year. Christensen and Boggero (1985) reported BSN incidence was significantly increased in the N and/or N plus P plots at two locations in one out of the three years (1980) when compared to the control. The only year of BSN data presented by the authors was for 1980, which showed a significant increase in BSN at one site due to applications of N and/or N plus P. The application of N or N plus P increased BSN level from 20% (control) to 40% and 36% respectively (Christensen and Boggero, 1985). In Switzerland, Gysi (1983) reported that N increased the incidence BSN of Riesling X Sylvaner in two out of eight years. In Chile, Cooper *et al.* (1987) also reported an increase in the incidence of BSN of Sultanine (Thompson Seedless) with applications of N fertilizer.

A second study, in conjunction with the California study previously discussed, N, P, or N plus P soil treatments were tested at two locations. Each location contained areas with a history of either low or high BSN incidence of Thompson Seedless (Christensen and Boggero, 1985). In 1981 and 1982, fertilizer treatments were established in each low and high area location. In both the low and high incidence areas at both locations, bloom-time petiole analysis showed total tissue N concentration and NO_3^- concentration in most of the treatments that received N were significantly higher compared to the controls (Christensen and Boggero, 1985). Again, bloom-time petiole analysis was shown as a mean of both years and the 1982-rachis analysis results that were reported were not statistically analyzed. BSN was significantly increased in N treatments in only one location (low incidence area) in both years (Christensen and Boggero, 1985). A composite rachis analysis (both studies, seven locations) for the N only and control treatments showed that BSN symptomatic-clusters had significantly higher N and NH_4^+ levels in rachises and was closely related to BSN incidence (Christensen and Boggero, 1985). Calcium and Mg on the other hand, had no bearing on BSN incidence. Total N levels above 1.5% and NH_4^+ levels above 3000 ppm in the rachis were associated with BSN development in California (Christensen and Boggero, 1985; Christensen *et al.*, 1991). However, total mean N concentration (both studies, seven locations) of BSN symptomatic rachis tissue of the control treatment was not significantly different from non-symptomatic rachis tissue of the N only treatment 2.25% and 2.02% respectively. Austrian researchers Redl and Weindlmayr (1983) also reported similar results of higher N levels in BSN-symptomatic rachis tissue compared to non-symptomatic rachis tissue. NH_4^+ concentration was generally higher in the rachis tissue of BSN-affected clusters compared to healthy rachis tissue in Chile, but in some cases the concentrations of NH_4^+ did not differ between affected and healthy tissue (Ruiz and Moyano, 1993).

Swiss researchers Keller and Koblet (1995) induced BSN by placing the rachis of excised clusters of Müller-Thurgau grapevines in various solutions (0-10 mM) of phosphinothricin (PPT). The assimilation of NO_3^- and the fixation of molecular N_2 give rise to ammonia (NH_3) and for its assimilation three enzymes are important: glutamate dehydrogenase (GDH), glutanine synthetase (GS), and glutamine synthase (GOGAT)

(Mengel and Kirkby, 1987). Some inhibitors of the GS/GOGAT pathway can increase the concentration of NH_4^+ in grapevine leaves, flowers, fruit and pedicels (Gu *et al.* 1991). Ammonia accumulates in tissues treated with PPT due to the selective inhibition of glutamine synthetase (GS) by PPT, leading to a constriction in photosynthetic activity and senescence of tissue (Keller and Koblet, 1995). Keller and Koblet (1995) suggested that GS is present and NH_4^+ is assimilated in all organs of the grape cluster at any stage of development because PPT induced BSN there is an indirect implication of NH_4^+ accumulation in the development of BSN. The authors did not, however, perform corresponding tissue analysis. Keller and Koblet (1995) proposed that NH_4^+ buildup was a secondary effect related to senescence of the rachis tissue due to carbon starvation in the vine. Keller and Koblet (1995) suggested the hypothesis of carbon starvation being associated with BSN in a previous study of carbon starvation and Inflorescence Necrosis (Keller and Koblet, 1994).

In California, Chang and Kliewer (1991) studied the effect of NO_3^- and NH_4^+ applications rates on the development of BSN and tissue composition using two-year old green-house-grown Chardonnay, Pinot noir, and Cabernet Sauvignon. Vines that received the NH_4^+ treatments showed symptoms of BSN shortly after véraison. The BSN incidence increased with increasing rate of NH_4^+ (Chang and Kliewer, 1991). Chardonnay and Cabernet Sauvignon vines that received NO_3^- along with Ca did not show typical BSN symptoms but Pinot noir vines receiving the same treatments did show some degree of BSN (Chang and Kliewer, 1991). Conversely to Christensen and Boggero (1985, 1991), Chang and Kliewer (1991) reported that Pinot noir vines that received NO_3^- (99% BSN) accumulated little NH_4^+ in the petiole and rachis tissue. Vines that received NO_3^- without Ca and all vines that received NH_4^+ had lower levels of calcium in both petiole and rachis tissue (Chang and Kliewer, 1991). These findings suggest that Ca deficiency or perhaps the ratio of Ca to other nutrients is associated with symptoms typical of BSN (Chang and Kliewer, 1991). Similar to these results, in Australia, rachis analysis of Cabernet Sauvignon grapevines in three locations over three years found no correlation between NH_4^+ tissue level and BSN incidence (Holzapfel and Coombe, 1998; Coombe, 1998). However, no notable involvement of calcium was indicated in a parallel study of minerals

in relation to BSN on the same vines used in the NH_4^+ experiment (Holzapfel and Coombe, 1996; 1998).

Polyamines: It has been suggested that the metabolism of agmatine resulting in release of NH_4^+ and putrescine may induce BSN (Christensen *et al.*, 1991; Coombe, 1998; Holzapfel and Coombe, 1998; Rafael *et al.*, 1998). Agmatine is formed by the decarboxylation of arginine (Smith, 1984). Agmatine can be converted to carbamylputrescine, which is hydrolyzed to putrescine and carbamic acid (Mengel and Kirkby, 1987; Smith, 1984). These reactions are promoted under stress conditions (Smith, 1988). The polyamine putrescine occurs ubiquitously in plants (Smith, 1984; 1985). Stress factors known to cause accumulation of putrescine in plants encompass K and Mg deficiency, osmotic shock and desiccation, cold injury, sulfur dioxide pollution, cadmium, and excess NH_4^+ (Smith, 1985; 1988). Smith (1984; 1985) suggested that the common factor which may relate K and Mg deficiencies and NH_4^+ excess is the response to soil acidification. A function of putrescine may be to maintain ionic balance and control pH in the plant (Smith, 1985).

In Chile, Rafael *et al.* (1998) analyzed BSN symptomatic bunches and non-symptomatic bunches of Flame Seedless and Beauty Seedless. In both cultivars, putrescine levels were higher in the BSN symptomatic clusters compared to the non-symptomatic clusters and the level of putrescine significantly increased from the proximal end of the cluster to the terminal end which was not seen in the non-symptomatic clusters (Rafael *et al.*, 1998). Potassium levels were lower in the BSN affected clusters compared to the healthy cluster (Rafael *et al.*, 1998). Potassium levels were not presented. The Rafael *et al.* (1998) paper was originally published in the Agricultura Técnica and was translated from Spanish and the editor then condensed the results of the paper, thus limiting the interpretation. Perfusion of agmatine at 50 or 100 mM into Cabernet Sauvignon grape peduncles induced 33% and 67% BSN, respectively, compared to no BSN in the control (Holzapfel and Coombe, 1998). Agmatine significantly increased the concentration of released NH_4^+. Free NH_4^+ slightly increased at the 25 and 50 mM agmatine doses and was 17 times greater in the 100mM treatment, compared to the control (Holzapfel and

Coombe, 1998). Abscisic acid (ABA) increased from 1.48 to 4.28 $\mu g/g$ dry weight of rachis when the agmatine dose was increased from 0 to 100mM (Holzapfel and Coombe, 1998).

Phytohormones: The endogenous phytohormones gibberellic acid (GA_3) and abscisic acid (ABA) have been implicated in BSN (Baldacchino *et al.*, 1987a; Beetz and Bauer, 1983; Haub, 1983; Theiler and Coombe, 1985). In Germany, a single application 100-ppm GA_3 to Riesling grapevines shortly before véraison was 89-99% effective in controlling BSN in 1978 and 53-69% effective in 1979 (Beetz and Bauer, 1983). Haub (1983) reported similar results with a single application of GA_3 shortly before véraison. The negative effect of reduced bud burst and fruit set in the following year made GA_3 use impractical (Haub, 1983). In France, Baldacchino *et al.* (1987a) found higher ABA concentrations in BSN-symptomatic rachises compared to non-symptomatic rachises of Cabernet Sauvignon grapevines. BSN was induced in Cabernet Sauvignon grapevines when 10 nM of ABA was injected into the sap stream of the rachis shortly before or at véraison (Baldacchino *et al.*, 1987b). In Australia, similar results were reported with Cabernet Sauvignon (Holzapfel and Coombe, 1997). BSN affected rachises had a three-fold higher concentration of ABA on average compared to healthy rachises (Holzapfel and Coombe, 1997). The concentration of ABA varied from site to site and year to year, thus no correlation was evident between ABA concentrations in the rachis and the incidence of BSN (Holzapfel and Coombe, 1997). Holzapfel and Coombe (1998) were also able to induce BSN of Cabernet Sauvignon grapevines by perfusing solutions of ABA into individual peduncles. A weak correlation between extracted ABA and incidence of BSN was observed but there were also several cases where ABA levels were elevated with no corresponding increase of BSN incidence (Holzapfel and Coombe, 1998). The results do not exclude an ABA connection with BSN but higher levels of ABA in symptomatic tissue may reflect the presence of necrotic tissue (Holzapfel and Coombe, 1997; 1998).

CHAPTER THREE
ROLE OF MINERAL NUTRIENTS ON BUNCH STEM NECROSIS OF CABERNET SAUVIGNON IN VIRGINIA

Introduction

Late-season bunch stem necrosis (BSN) is a physiological disorder of the bunch stem (rachis) of grapevines (Brendel *et al.*, 1983). BSN nomenclature is descriptive of the rachis symptoms. Hence, the names waterberry (California) (Christensen and Boggero, 1985), shanking (New Zealand) (Jordan, 1985), stiellähme (Germany) (Stellwaag-Kittler, 1983), dessèchement de la rafle (France) (Ureta *et al.*, 1981), palo negro (Chile) (Ruiz and Moyano, 1993), disseccamento del rachide (Italy) (Fregoni and Scienza, 1972), and rachis necrosis (Canada) (Cline, 1987) are used in the literature.

BSN may appear any time after the beginning of berry ripening (véraison). The first symptoms appear as small dark lesions on the rachis and/or pedicels of grape clusters (Stellwagg-Kittler, 1983). Christensen and Boggero (1985) described this stage as 1-3 mm diameter brown or black spots that become necrotic and sunken. Lesions may expand to girdle the rachis, leading to desiccation of the rachis distal to the lesion. Affected portions of clusters either abscise or remain on the cluster in a dry condition (Ureta *et al.*, 1981). Cluster shoulders and/or tips are frequently symptomatic, while the remainder of the cluster develops normally. BSN affected berries are soft and dull in appearance. Morrison and Iodi (1990) reported that symptomatic berries exhibited retarded sugar and potassium accumulation, continued accumulation of calcium and tartaric acid, and delayed or reduced berry growth, compared to berries on non-symptomatic clusters.

The cause of BSN is uncertain. Because no pathogen has been linked to BSN, research has focused on environmental, hormonal, and/or nutritional imbalances. A common

feature of BSN is the annual variability of expression (Haystead *et al.* 1988 and

Holzapfel and Coombe, 1995), which suggest an environmental mediation of symptoms.

Environmental factors, such as high humidity, temperature extremes, and precipitation

during different phenological stages of grapevine development have been reported to be

associated with BSN (Jordan, 1985; Redl, 1987; Theiler and Müller, 1986; Holzapfel and

Coombe, 1995). The differences in these studies suggest factors other than

environmental conditions are involved with the occurrence of BSN.

There are conflicting reports regarding the association of essential nutrients and the

incidence of BSN. A high ratio of potassium (K) to magnesium (Mg) and/or calcium

(Ca) in affected tissue has been associated with BSN (Brechbuhler, 1975). The

application of Mg and/or Ca fertilizers effectively reduced the incidence of BSN in

Europe (Boselli *et al.*, 1983; Brendel *et al.*, 1983; Hartmeir and Grill, 1965; Haub 1986;

Lauber and Koblet, 1967). In apparent contrast, BSN was not reduced by applications of

Ca and Mg in California, where an increase in the incidence of BSN was reported with

applications of nitrogen (N) fertilizer (Christensen and Boggero, 1985). Total N and

ammonium (NH_4^+) levels were higher in BSN-symptomatic rachis tissue compared to

non-symptomatic rachis tissue (Christensen and Boggero, 1985; Ruiz and Moyano,

1993). In some cases, concentrations of NH_4^+ did not differ between affected and healthy

tissue (Ruiz and Moyano, 1993). In Australia, rachis analysis of Cabernet Sauvignon

grapevines found no correlation between NH_4^+ tissue level and BSN incidence (Holzapfel

and Coombe, 1998).

Similarly confusing results were observed in Virginia vineyards surveyed in 1995. One

of the vineyards surveyed suggested that BSN incidence of Cabernet Sauvignon was

associated with a deficient tissue concentration of nitrogen. This association was not

universal in all vineyards surveyed, and hence the need to examine several nutrients.

Experiments were initiated in 1996 to determine the effect of nitrogen, calcium, and

magnesium on BSN incidence in two Cabernet Sauvignon vineyards in Virginia.

BSN has been correlated with numerous factors. However, no universal cause and effect

relationships have been demonstrated. The purpose of this study was to determine if

mineral nutrition was associated with BSN of Cabernet Sauvignon under Virginia's humid growing conditions. Cabernet Sauvignon is an important grape cultivar in Virginia and is frequently affected by BSN. One objective of this study was to determine if there was a direct association of tissue nitrogen concentration with the incidence of BSN. Another objective was to explore the possibility that either Mg or Ca were involved in the disorder.

Materials and Methods

Vineyards: Ten year old, non-irrigated Cabernet Sauvignon vines were used at Leesburg, Virginia (39° 5' N) during the 1997 and 1998 season. Grapevines at Leesburg vineyard were spaced 3.0 m apart with two vines per 3.0 m plot. Vineyard rows were 3.7 m apart and oriented approximately north/south. Vines were trained to a Casarsa, vertical shoot positioned training system, and were unilateral cordon-trained, spur-pruned, and shoots were vertically positioned upright. Additionally, Cabernet Sauvignon vines (7 years old in 1996) at Winchester, Virginia (39°12' N) were used during the 1996, 1997 and 1998 seasons. Grapevines at Winchester vineyard were spaced 2.1 m apart with three vines per 6.4 m plot. Vineyard rows were 3.7 m apart and oriented north/south. Vines were trained to an open-lyre, divided canopy training system, and were cordon-trained, spur-pruned, and shoots were vertically positioned upright.

Treatment and experimental design: Fertilizer treatments were applied to two-vine plots at Leesburg vineyard and three-vine plots at Winchester vineyard, each replicated five times in a completely randomized design. To standardize canopy area and crop level, each treatment plot at both vineyards was shoot thinned to 15 shoots per meter of cordon prior to bloom. Crop levels at both vineyards were established 30 days after bloom. Vine shoot length at Leesburg and Winchester vineyard was maintained at 17 nodes by shoot trimming. Vines at Leesburg vineyard were shoot trimmed once in July of each growing season. Vines at Winchester vineyard were shoot trimmed twice, early-June and late-July, during each growing season.

Four fertilizer treatments were applied at Leesburg and Winchester vineyards using the same treatments, which were repeated each year of the experiment.

Fertilizer treatments:

T_1- Control (no fertilizer)

T_2- Ammonium nitrate (NH_4NO_3) 128 kg/ha actual N (split soil application of actual N, 28 kg/ha budbreak, 56 kg/ha bloom, and 28 kg/ha 30-days after bloom).

T_3- Magnesium sulfate ($MgSO_4$) 280 kg/ha + calcium chloride ($CaCl_2$) 94 kg/ha (split soil application of $MgSO_4$, 140 kg/ha budbreak, 140 kg/ha bloom; seven foliar applications of $CaCl_2$ starting at five nodes applied every two weeks, 13.42 kg/ha $CaCl_2$ per application).

T_4- NH_4NO_3 + $MgSO_4$ + $CaCl_2$ (combination of T_2 and T_3).

Ammonium nitrate and $MgSO_4$ were applied in the row under treatment vines and incorporated into the soil. A backpack sprayer was used to apply the $CaCl_2$. Entire vine canopy and clusters of $CaCl_2$ vines were sprayed till runoff. At Winchester vineyard $CaCl_2$ was not part of the treatment in 1996.

Soil analysis: To determine soil nutrient status, soil samples were collected at a depth 0-20 cm and from 20-40 cm at Leesburg and Winchester vineyard sites, 1997 and 1996 respectively, prior to application of the first fertilizer treatment. Three soil samples at each depth were collected at both vineyards, not specific for treatment. Soil samples consisted of 15 probes per sample depth and were representative of each vineyard site. Soil samples by treatment plot were collected near completion of the experiment in 1998 at both vineyard sites. Soil samples consisted of ten probes, two from under the trellis for each treatment rep. Soil samples were processed by a commercial testing laboratory (A & L Eastern Agricultural Laboratories, Inc., Richmond, Virginia 23237).

Tissue analysis: Plant tissue samples were collected every year, at each vineyard, for each treatment plot at bloom and repeated at véraison. Samples consisted of 80 leaf petioles per treatment plot. Leaves opposite flower clusters were sampled at bloom and again at véraison from mid-shoot leaves. Leaf petioles were separated from blades and placed in paper bags. Additional tissue samples were collected at the Winchester

vineyard and consisted of 20 rachises from each treatment plot at bloom and véraison (berries removed). Rachis samples collected did not have visible symptoms of BSN even if BSN was present on other clusters. During the 1996 season at Winchester vineyard, bloom-time leaf petiole samples and véraison rachis samples were the only tissue samples collected. Tissues were promptly dried at 90° C for 24 hour prior to shipping. Spectrum Analytic Inc., (Washington C.H., Ohio 43160), performed standard tissue analyses. Leaf petiole tissue analysis results by treatment for both vineyards were compared to standard petiole nutrient sufficiency ranges of *Vitis vinifera* grapevines for Virginia, Oregon, and British Columbia (Appendix A).

Canopy descriptors: Point quadrat analysis (PQA) (Smart and Robinson, 1991) was conducted on all treatment plots approximately 60 days after budbreak to determine canopy characteristics. At this point the canopy had developed and shoots had been hedged. A thin metal rod was inserted horizontally through the fruiting zone of the vine canopy at equal intervals. Treatment plots at the Leesburg and Winchester vineyards received 10 and 42 probes per treatment plot, respectively. The contact of the probe was recorded as either fruit cluster, leaf, or canopy gap. Leaf layer number and percent exposed fruit were calculated from the data for each treatment. Leaf layer number equaled the total number of leaf contacts divided by the number of insertions. Percent exposed fruit equaled the number of exterior fruit divided by the total number of contacted clusters multiplied by 100. Photosynthetic photon flux (PPF) measurements were made at both vineyards to determine canopy light characteristics. Light measurements were performed prior to véraison with a 1.0-meter line quantum sensor (model LI-191SB, LI-COR, Inc. Lincoln, NE 68504) with a photometer (LI-COR model 185B). Light measures were made between 1100 and 1600 hours EDT on a clear day at both vineyards during July. The sensor was inserted into the canopy parallel to the row, in the center of the fruiting zone. Three readings per meter of canopy for each treatment plot were obtained in the 1997 Leesburg measurements: vertical upright, 45° left of vertical, and 45° right of vertical. One reading per meter of canopy for each treatment plot was obtained in the 1996 and 1997 Winchester measurements: 45° east for the east canopy and 45° west for the west canopy. Readings were then averaged to obtain a

single PPF reading for each vineyard treatment plot. An additional ambient PPF measurement was determined by taking a maximal PPF reading above the canopy for each treatment plot. The ratio of each interior reading to the ambient PPF provided a percentage of available photosynthetically active radiation (PAR) that penetrated the canopy.

Single leaf photosynthesis measurements were made at Leesburg vineyard in 1998 and at Winchester vineyard in 1997 and 1998. Net photosynthesis was measured using a portable infrared gas analyzer [ADC LCA2, The Analytical Development Company (ADC). Hoddesdon, England EN11 OAQ] with a leaf chamber. Treatment plots at Leesburg vineyard and Winchester Vineyard received four and twelve measurements, respectively, between 900 and 1600 hours at ambient light levels between 1600 and 1900 μmol$\bullet$m$^{-2}\bullet$s^{-1}. Measurements were taken on healthy, well-exposed leaves at approximately the sixth node.

Assimilation rate (Assim) was determined using the formula adapted from Long (1982):

$$Assim = \frac{F}{A}(\Delta CO_2)\left(\frac{1-Xe}{1-Xo}\right)$$

$$F = 0.3 \times 0.0446428\left(\frac{273.15}{273.15 + Xs}\right)$$

A = area of leaf chamber (0.000625 m^2).

ΔCO_2 = change in CO_2 concentration of air passing through the leaf chamber (ppm $\times$ volume $\times$ 10^{-6}).

$$Xe = Xs \times \left(\frac{RHleaf}{100}\right)$$

$$Xo = Xs \times \left(\frac{RHnoleaf}{100}\right)$$

Xs = Temperature variable for the saturation mole fraction of water vapor.

RHleaf = Relative humidity with leaf.

RHnoleaf = Relative humidity no leaf.

Percent bunch stem necrosis: Each treatment plot was rated for BSN incidence every two weeks at both vineyards. BSN rating started at véraison and continued through harvest. Fifty clusters at Leesburg vineyard and one hundred clusters at Winchester vineyard, chosen at random for each treatment plot, were visually rated and percent BSN was calculated for each plot.

Components of yield and berry chemistry: Berry samples were collected at both vineyards from every treatment plot at each BSN rating. Berry samples did not include BSN-affected berries. Berry sampling was done to ensure that treatments were not simply delaying the onset of BSN. Fifty berries were randomly sampled, and percent soluble solids concentration, pH, and berry weight were determined. Percent soluble solids concentration was determined with a temperature compensating, hand-held refractometer (Model 10430, Reichert Scientific Instrument, Buffalo, NY, 14240). A portable pH meter was used to determine pH (Model AP5, Fisher Scientific, Denver Instrument Co. Denver, CO, 80004). At harvest, fruit from each vine was harvested and weighed. Other yield components included clusters per vine, cluster weight, and berry weight. Cane pruning weights were collected after each growing season. Crop load (fruit weight per vine/pruning weight per vine) was determined for each treatment.

Statistical analysis: All dependent variables measured in the course of the study were subjected to one-way analysis of variance to determine the significance of the various fertilizer treatments. Where significant F-tests occurred, the treatment means were separated using appropriate means separation techniques (e. g., Duncan's MRT). Multiple measures within a treatment replicate, such as the point quadrat analysis and canopy light data, were averaged by treatment replicate prior to ANOVA (SAS Institute, 1990). All statistical analysis was performed using SAS-PC (ver. 6.12) (SAS Institute, 1990) software.

Results

Effect of fertilizer on BSN of Cabernet Sauvignon, Leesburg, Virginia 1997-1998:

Soil analysis: Soil was a yellowish-brown loam over red silty clay weathered mainly from sandstone and limestone. The soil surface (A horizon) was removed prior to the installation of the vineyard. Soil samples collected on 7 May 1997 prior to establishment of the fertilizer treatments indicated the soil to be moderately fertile with a pH of 7.0 at 0-20 cm and 5.8 at 20-40 cm respectively (Appendix B). Soil samples collected by treatment on 28 July 1998 near completion of the experiment were similar among treatments for mineral concentrations (Appendix C). Soil pH decreased in the fertilizer treatments with the greatest reduction occurring in N treatment plots (Appendix C).

Petiole analysis: Fertilizer treatments had no statistically significant ($P \leq .05$) effect on bloom-time leaf petioles' (collected 20 June 1997) total N, K, Mg or Ca concentration, or on K/(Mg + Ca) ratio (Table 3.1). Véraison leaf petioles collected on September 21 revealed no difference for N, Mg, or Ca concentrations; however, the concentration of K, as well as the K/(Mg + Ca) ratio, were significantly higher in the Mg + Ca plots than the control plots (Table 3.1). Bloom-time leaf petioles collected 3 June 1998 again revealed that total N and Ca concentrations were not different (Table 3.2). Mg concentration in the N plots was significantly higher than the control plots (Table 3.2). Potassium concentration as well as the K/(Mg + Ca) ratio were higher with the Mg + Ca treatment compared to the control (Table 3.2). Véraison leaf petioles collect on 11 August indicated as well, that K concentration was significantly higher and Mg concentration lower in the Mg + Ca plots (Table 3.2). The K/(Ca + Mg) ratio was significantly higher in the Mg + Ca plots compared to the control plots (Table 3.2). Complete bloom-time and véraison leaf petiole elemental analysis for 1997 and 1998 are presented in Appendix D and E, respectively.

Canopy descriptor: Based on point quadrat analysis (PQA) measurements in 1997, uniform canopy density occurred, where canopies averaged 2.6 leaf layers, 42 % exposed

Table 3.1. Bloom-time and véraison leaf petiole elemental composition of Cabernet Sauvignon, Leesburg 1997.

| | Mean nutrient concentration (percent dry weight) of petioles[z] | | | | | | | | | |
| | N | | K | | Mg | | Ca | | K/(Mg + Ca) | |
Treatments	Bloom	Véraison	Bloom	Véraison	Bloom	Véraison	Bloom	Véraison	Bloom	Véraison
Control	1.04 b	1.08	2.44	2.28 b	0.59	0.96	2.00	1.52	0.98	0.95 b
N only	1.20 a	1.06	2.02	2.73 b	0.61	1.18	1.74	1.44	0.92	1.21 b
Mg + Ca	1.08 ab	1.10	2.88	5.40 a	0.48	0.47	1.70	1.54	1.32	2.75 a
N + Mg + Ca	1.06 b	1.10	2.28	2.28 b	0.59	1.04	2.28	1.66	0.82	1.18 b

[z]Elemental concentrations are means of five separate samples. Means within columns followed by the same letter, or by no letter, are not significantly different at $P \leq .05$, using Duncan's multiple range test.

Table 3.2. Bloom-time and véraison leaf petiole elemental composition of Cabernet Sauvignon, Leesburg 1998.

| | Mean nutrient concentration (percent dry weight) of petioles[z] | | | | | | | | | |
| | N | | K | | Mg | | Ca | | K/(Mg + Ca) | |
Treatments	Bloom	Véraison	Bloom	Véraison	Bloom	Véraison	Bloom	Véraison	Bloom	Véraison
Control	1.01	0.73	1.66 b	1.62 b	0.53 bc	1.36 a	1.94	1.78	0.72 b	0.52 b
N only	0.97	0.74	1.16 b	1.59 b	0.71 a	1.80 a	1.83	1.66	0.48 b	0.53 b
Mg + Ca	0.88	0.67	2.50 a	4.12 a	0.40 c	0.77 b	1.72	1.80	1.22 a	1.73 a
N + Mg + Ca	0.99	0.77	1.39 b	2.14 b	0.61 ab	1.41 a	1.92	1.90	0.58 b	0.69 b

[z]Elemental concentrations are means of five separate samples. Means within columns followed by the same letter, or by no letter, are not significantly different at $P \leq .05$, using Duncan's multiple range test.

fruit, and 2 % gaps in the fruiting zone (Appendix F).   Light or photosynthetic photon flux (PPF) measurement showed no significant treatment difference (Appendix H). Uniform canopy density was observed in 1998 as well, where canopies averaged 3.0 leaf layers, 38 % exposed fruit, and 1 % gaps in the fruiting zone (Appendix H). Net photosynthesis of healthy leaves did not differ significantly among treatments in1998 (Appendix F).

Components of yield and fruit chemistry: No significant difference occurred in either year for cluster weight, fruit weight per vine, pruning weight, or crop load (Appendix G). Berry weight, soluble solids concentration, and pH did not significantly differ in either year, with the exception that berry pH was increased by all treatments relative to the control (Table 3.3).

Percent bunch stem necrosis: BSN incidence at harvest (2 October) was low and did not differ among plots in 1997 (Table 3.3). Similarly, BSN at harvest in 1998 was not affected by treatment (Table 3.3).

Table 3. 3. Berry weight, soluble solids concentration (SSC), pH, and percent bunch stem necrosis (BSN) of Cabernet Sauvignon at harvest, Leesburg, 1997 and 1998.

	Berry wt. (g)		SSC (%)		pH		BSN[z] (%)	
Treatment	1997	1998	1997	1998	1997	1998	1997	1998
Control	1.3	1.4	20.8	23.9	3.38	3.42 b	1	0
N only	1.4	1.4	20.6	22.8	3.50	3.51 a	2	10
Mg + Ca	1.4	1.4	20.9	23.4	3.67	3.56 a	1	0
N + Mg + Ca	1.3	1.4	20.4	22.9	3.50	3.56 a	5	0

[z]Percent BSN the mean of five, 50-cluster counts.

Berry weight, soluble solids concentration, and pH are means of five separate 50-berry samples, means followed by the same letter within a column, or by no letter, are not significantly different at $P \leq .05$, using Duncan's multiple range test.

Effect of fertilizer on BSN of Cabernet Sauvignon, Winchester Virginia 1996-1998

Soil analysis: Soil was a dark brown loam over yellowish-red silty clay weathered mainly from sandstone and limestone. Soil samples collected on 7 April 1996, prior to establishment of fertilizer treatments, indicated the soil to be moderately fertile with a pH of 6.4 at 0-20 cm and 6.7 at 20-40 cm (Appendix H). Soil samples collected by treatment near completion of the experiment revealed that soil Mg concentration was increased in the two treatments that received Mg fertilizer (Appendix I). Soil pH was decreased in all treatment plots, relative to control.

Tissue analysis: Bloom-time leaf petioles collected on 17 June 1996 showed total N concentration in the N plots and N + Mg plots was significantly increased by applications of nitrogen compared to the control plots (Table 3.4). No significant difference was revealed for K and Mg concentration or the K/(Mg + Ca) ratio (Table 3.4). Véraison rachis samples collected on 18 August were also significantly higher in total N concentration in the N plots and N + Mg plots compared to the control plots (Table 3.4). Significantly higher concentrations of Mg and Ca was revealed in the Mg plots compared to the control plots, but no significant difference was revealed for the K/(Mg + Ca) ratio (Table 3.4).

Bloom-time leaf petioles collected on 23 June 1997 indicated that total N concentration in the N plots was significantly higher compared to the control plots (Table 3.5). Magnesium was significantly higher in all fertilizer plots compared to the control plots, but no significant difference was revealed for the K/(Mg + Ca) ratio (Table 3.5). Véraison leaf petiole samples collected on 19 August showed significantly higher total N concentration in the N plots and Mg + Ca plots compared to the control plots (Table 3.6). Leaf petiole K, Mg, and Ca concentrations were significantly higher in all fertilizer treatment plots compared to control plots, but no significant difference occurred for the K/(Mg + Ca) ratio (Table 3.6). Véraison rachis analysis revealed a similar relationship of mineral concentrations as the véraison petiole results, but the K/(Mg + Ca) ratio was

significantly higher in all fertilizer treatment plots compared to the control plots (Table 3.7).

Bloom-time leaf petioles collected 8 June 1998 revealed total N concentration in the N plots and N + Mg plots was significantly increased by applications of N fertilizer compared to the control plots (Table 3.5). Potassium and Ca concentrations in the N plots were significantly higher compared to the control plots (Table 3.5). Bloom-time petiole Mg and Ca concentrations were significantly increased in the Mg + Ca and N + Mg + Ca plots by applications of Mg and Ca fertilizers compared to the control plots (Table 3.5). However, the K/(Mg + Ca) ratio was not affected by any treatment (Table 3.5). Bloom-time rachis total N concentration in the N and N + Mg + Ca plots was also significantly higher compared to the control plots (Appendix K). Rachis K concentration was significantly higher in all fertilizer plots compared to the control, but only the K/(Mg + Ca) ratio was significantly higher in the N plots compared to the control (Appendix K).

Leaf petioles collected at véraison on 10 August 1998 indicated that total N concentration was significantly higher in the N and N + Mg + Ca plots compared to the control plots (Table 3.6). Magnesium concentration was significantly increased in the Mg + Ca plots and N + Mg + Ca plots compared to the control plots but no statistically significant difference in Ca concentration and K/(Mg + Ca) ratio was indicated (Table 3.6). Véraison rachis analysis indicated total N concentration in the N and N + Mg + Ca plots was significantly increased due to N fertilizer compared to the control (Table 3.7). Rachis K concentration was significantly higher in the N and N + Mg + Ca plots, but only the N plots K/(Mg + Ca) ratio significantly differed from the control plots (Table 3.7). Complete tissue elemental analyses from 1996-1998 at Winchester vineyard are presented in Appendices J-M.

Canopy descriptors: Point qaudrat analysis (PQA) measurements in 1996 demonstrated a thin uniform canopy, where canopies averaged 0.9 leaf layers, 62 % exposed fruit, and 14 % gaps in the fruiting zone (Appendix N). Light measurements of the N and

Table 3.4. Bloom-time leaf petiole and véraison cluster stem (rachis) elemental composition of Cabernet Sauvignon, Winchester 1996.

| | Mean nutrient concentration (percent dry weight) of bloom-time petioles and véraison cluster rachis[z] | | | | | | | | | |
| | N | | K | | Mg | | Ca | | K/(Mg + Ca) | |
Treatments	Bloom	Véraison	Bloom	Véraison	Bloom	Véraison	Bloom	Véraison	Bloom	Véraison
Control	0.80 b	1.16 b	5.09	4.71	0.37	0.17 b	1.97 a	0.86 b	2.2	4.6
N only	1.85 a	2.18 a	4.60	4.38	0.46	0.18 b	1.84 a	0.77 b	2.2	4.7
Mg[y]	0.93 b	1.02 b	4.82	4.74	0.37	0.23 a	1.85 a	0.98 a	2.0	4.0
N + Mg[y]	1.66 a	1.84 a	4.95	4.28	0.48	0.20 b	1.61 b	0.79 b	2.4	4.4

[z]Elemental concentrations are means of five separate samples. Means within columns followed by the same letter, or by no letter, are not significantly different at $P \leq .05$, using Duncan's multiple range test.

[y]Calcium was not included in the fertilizer treatments in 1996.

Table 3.5. Bloom-time leaf petiole elemental composition of Cabernet Sauvignon, Winchester 1997 and 1998.

| | Mean nutrient concentration (percent dry weight) of petioles[z] | | | | | | | | | |
| | N | | K | | Mg | | Ca | | K/(Mg + Ca) | |
Treatments	1997	1998	1997	1998	1997	1998	1997	1998	1997	1998
Control	1.32 b	0.88 b	2.38	3.16 b	0.23 b	0.25 b	1.38	1.42 b	1.7	1.9
N only	1.85 a	1.18 a	1.86	3.72 a	0.26 ab	0.30 ab	1.30	1.80 a	1.7	1.8
Mg + Ca	1.34 b	0.89 b	2.88	3.16 b	0.27 a	0.33 a	1.40	1.78 a	1.5	1.5
N + Mg + Ca	1.56 ab	1.09 a	2.88	3.58 ab	0.28 a	0.35 a	1.42	1.72 a	1.2	1.8

[z]Elemental concentrations are means of five separate samples. Means within columns followed by the same letter, or by no letter, are not significantly different at $P \leq .05$, using Duncan's multiple range test.

Table 3.6. Véraison leaf petiole elemental composition of Cabernet Sauvignon, Winchester 1997 and 1998.

	Mean nutrient concentration (percent dry weight) of petioles[z]									
	N		K		Mg		Ca		K/(Mg + Ca)	
Treatments	1997	1998	1997	1998	1997	1998	1997	1998	1997	1998
Control	1.00 b	0.78 b	1.34 b	5.68 b	0.19 b	0.39 b	0.68 b	1.60	2.2	2.9
N only	1.08 a	0.90 a	6.54 a	6.58 a	0.44 a	0.47 ab	1.66 a	1.80	3.2	2.9
Mg + Ca	1.08 a	0.80 b	6.22 a	5.74 b	0.42 a	0.50 a	1.60 a	1.98	3.1	2.3
N + Mg + Ca	1.05 ab	0.88 a	6.10 a	6.34 ab	0.38 a	0.56 a	1.70 a	1.84	3.0	2.7

[z]Elemental concentrations are means of five separate samples. Means followed by the same letter within a column, or by no letter, are not significantly different at P ≤ .05, using Duncan's multiple range test.

Table 3.7. Véraison cluster stem (rachis) elemental composition of Cabernet Sauvignon, Winchester 1997 and 1998.

	Mean nutrient concentration (percent dry weight) of rachis[z]									
	N		K		Mg		Ca		K/(Mg + Ca)	
Treatments	1997	1998	1997	1998	1997	1998	1997	1998	1997	1998
Control	0.98 b	1.04 b	1.30 b	4.32 c	0.17 b	0.28	0.34 b	1.14	2.6 c	3.1 b
N only	1.34 a	1.40 a	5.00 a	5.22 a	0.23 a	0.23	0.91 a	1.00	4.4 a	4.4 a
Mg + Ca	1.01 b	1.04 b	4.56 a	4.70 cb	0.27 a	0.29	1.01 a	1.16	3.6 b	3.3 b
N + Mg + Ca	1.26 a	1.26 a	4.64 a	5.16 ab	0.26 a	0.25	1.02 a	1.11	3.7 ab	3.9 ab

[z]Elemental concentrations are means of five separate samples. Means followed by the same letter within a column, or by no letter, are not significantly different at $P \leq .05$, using Duncan's multiple range test.

N + Mg + Ca plots showed a slight reduction of PPF (shading) in the fruit zone compared to control plots (Appendix W). A thin uniform canopy was observed in 1997 as well, where canopies averaged 2.1 leaf layers, 60 % exposed fruit, and 6% gaps in the fruiting zone (Appendix N). Light measurements demonstrated slight shading in the fruit zone of all fertilizer treatments compared to the control (Appendix O). Treatment vines that received N fertilizer appeared to have larger leaves and a deeper green coloration. Net assimilation rates were significantly greater in treatments that received N fertilizer compared to the control and Mg + Ca plots (Appendix O). Thin uniform canopy was also demonstrated in 1998 (Appendix N). Net photosynthetic rate was significantly greater in the N and N + Mg + Ca plots and significantly lower in the Mg + Ca plots compared to the control plots (Appendix O).

Components of yield: No significant difference was observed at harvest (26 October) in 1996 for berry weight and soluble solids concentration (Table 3.8). The N plots berry pH was significantly higher compared to control plots. (Table 3.8). Pruning weight was significantly greater in the N + Mg plots compared to the control (Appendix P). Cluster weight of all fertilizer treatments was significantly greater compared to the control. However, no significant difference was revealed for crop per vine and crop load in 1996 (Appendix P). Treatment plots harvested on 23 August 1997 indicated berry weight was significantly greater in the N and Mg + Ca plots compared to the control plots (Table 3.8). No significant difference was observed for soluble solids concentration, but berry pH was significantly higher in the N and N + Mg + Ca plots compared to the control plots (Table 3.8). Pruning weight per vine was significantly increased in all fertilizer treatments compared to the control (Appendix P). Nitrogen application increased cluster weight, but no significant difference was revealed for crop per vine and crop load in 1997 (Appendix P). At harvest 6 October 1998, no significant difference was observed for berry weight (Table 3.7). Soluble solids concentration was significantly reduce in all fertilizer treatment plots compared to the control (Table 3.7). Berry pH was significantly higher in the N plots compared to the control plots (Table 3.7). Pruning weight per vine increased in the N treatments. Crop per vine was similar between treatments, but crop

load was significantly greater in the control compared to the fertilizer treatments (Appendix P).

Percent bunch stem necrosis: BSN symptoms were first observed on 29 August 1996 at a relatively low fruit soluble solids concentration (15.5%). BSN incidence increased quickly as fruit ripened to18.5%, after which little increase in BSN incidence occurred (Figure 3.1). At harvest, BSN was significantly lower in the treatments that received N fertilizer compared to those that did not (Table 3.8). This relationship was observed at each BSN rating (Figure 3.1). The BSN incidence of the Mg plots was not significantly different from that of the control plots.

BSN incidence was low for all treatment plots in 1997 (Table 3.8). However, BSN was significantly lower at harvest in 1997 in all fertilizer treatments compared to the control. BSN symptoms were observed on 26 August 1998 at a soluble solids concentration of 17.0% and little increase in BSN incidence occurred after 20% soluble solids concentration (Figure 3.2). BSN at harvest was significantly lower in the N and N + Mg +Ca plots compared to the control (Table 3.8). BSN incidence in the Mg + Ca plots was not significantly different from the control plots at harvest on any BSN rating date (Table 3.8; Figure 3.2). The relationship of significantly lower BSN incidences in the N plots and N + Mg plots compared to the control plots at harvest was observed at each BSN rating in 1998(Figure 3.2).

Meteorological data: Meteorological data were gathered (1 April – 31 October) during each year of the study at Winchester. The 1998 season accumulated the greatest number of growing degrees units 4006 (Figure 3.3). Growing degree units were less in the 1997 season (3099) the coolest year (Figure 3.3). Precipitation was the greatest in the 1996 season, which also received the greatest amount of precipitation from week 1 through 16 (April-July) (Figure 3.4). The 1997 season experienced the least precipitation during the entire season (Figure 3.4).

Table 3.8. Berry weight, soluble solids concentration (SSC), pH, and bunch stem necrosis (BSN) of Cabernet Sauvignon at harvest, Winchester 1996, 1997 and 1998.

Treatments	Berry weight (g)			SSC %			pH			BSN[z] %		
	1996	1997	1998	1996	1997	1998	1996	1997	1998	1996	1997	1998
Control	1.5	1.6 b	1.4 b	19.9	22.0	22.7 a	3.71 b	3.61 b	3.61	41 a	7 a	23 a
N only	1.5	1.7 a	1.4 b	19.5	21.7	21.2 c	3.84 a	3.82 a	3.82	14 b	3 b	3 b
Mg + Ca[y]	1.5	1.6 b	1.4 b	19.9	21.8	22.1 b	3.76 b	3.70 ab	3.70	39 a	2 b	17 a
N + Mg + Ca[y]	1.5	1.6 b	1.5 a	19.7	21.9	22.1 b	3.85 a	3.73 ab	3.73	9 b	2 b	3 b

[z]Percent BSN are means of five 100-cluster counts, means followed by the same letter within a column, or by no letter, are not significantly different at $P \leq .05$ level, using Duncan's multiple range test.

Berry weight, soluble solids concentration, and pH are means of five 50-berry samples, means followed by the same letter within a column, or by no letter, are not significantly different at $P \leq .05$ level, using Duncan's multiple range test.

[y]Calcium was not included in the fertilizer treatments in 1996.
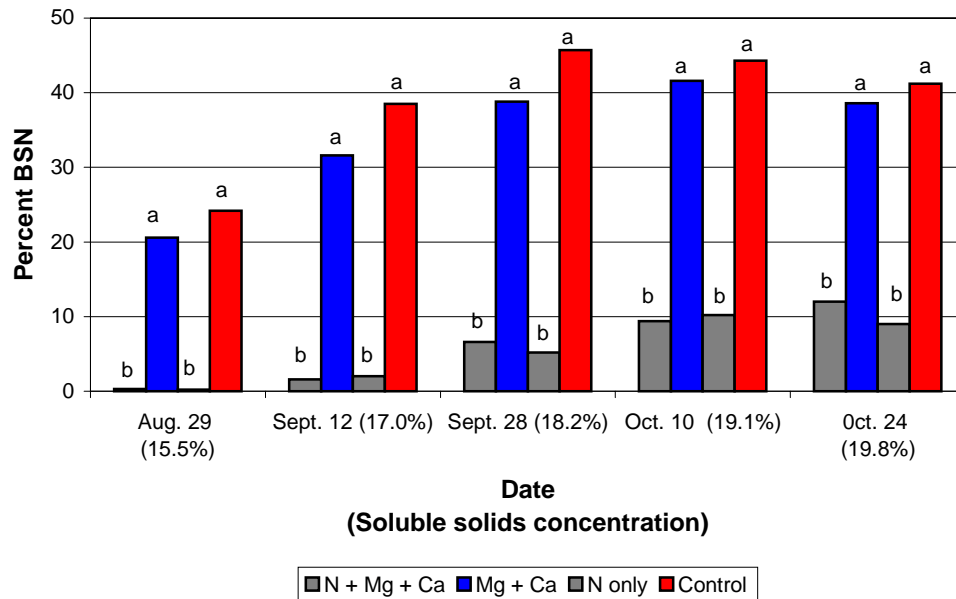
Figure 3.1. Sampling date and corresponding percent bunch stem necrosis (BSN) and soluble solids concentration of Cabernet Sauvignon grapevines at Winchester during 1996. Bars indicated by the same letter are not significantly different ($P \leq$ .05), using Duncan's multiple range test. Soluble solids concentration are means of all fertilizer treatments.

Figure 3.2. **Percent bunch stem necrosis (BSN) and soluble solids concentration of Cabernet Sauvignon grapevines at Winchester during 1998. Bars indicated by the same letter are not significantly different ($P \leq .05$), using Duncan's multiple range test. Soluble solids concentration are means of all fertilizer treatments.**

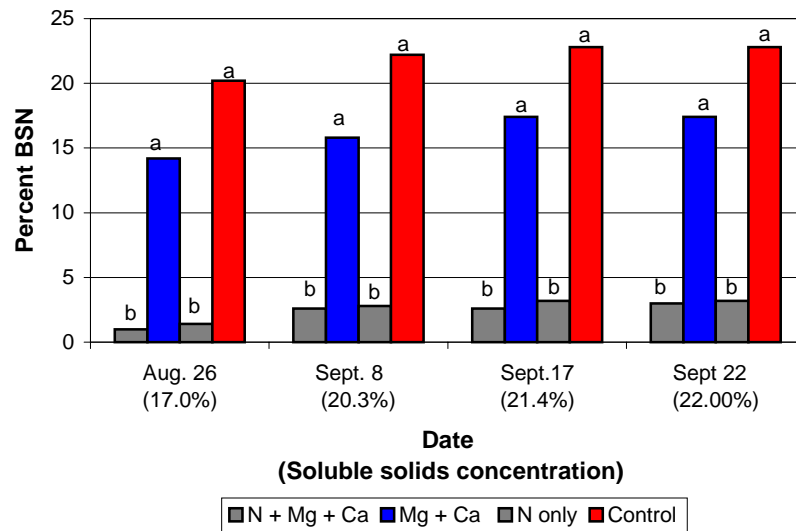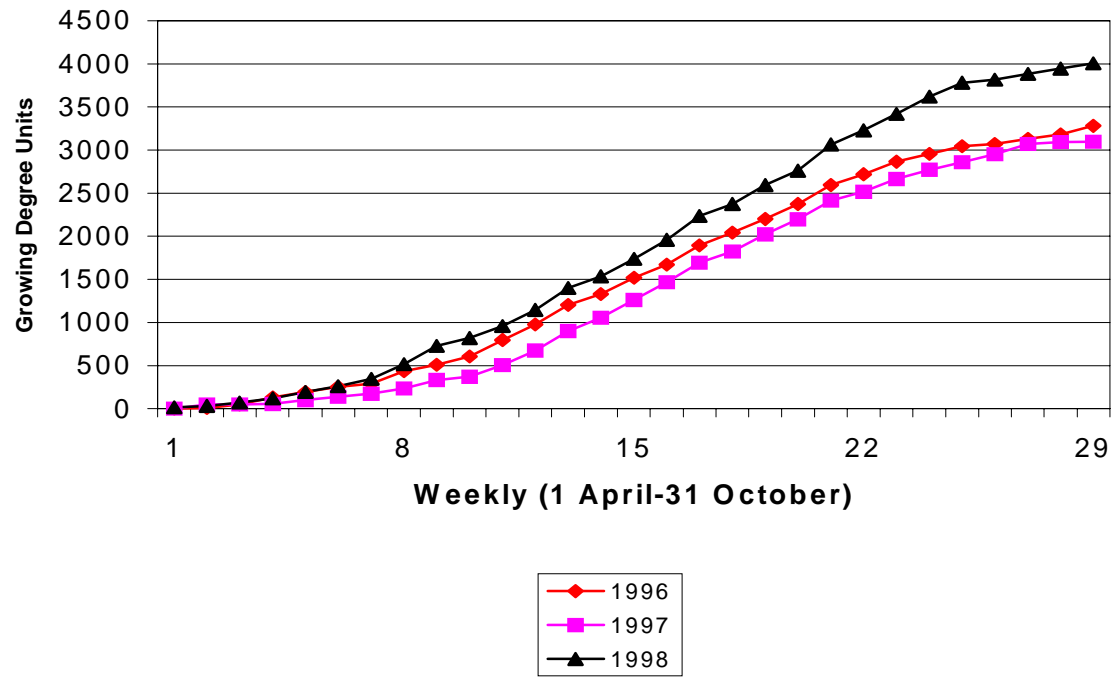**Figure 3.3. Accumulated growing degree units (50° F base) at Winchester vineyard from 1 April – 31 October 1996-1998.**
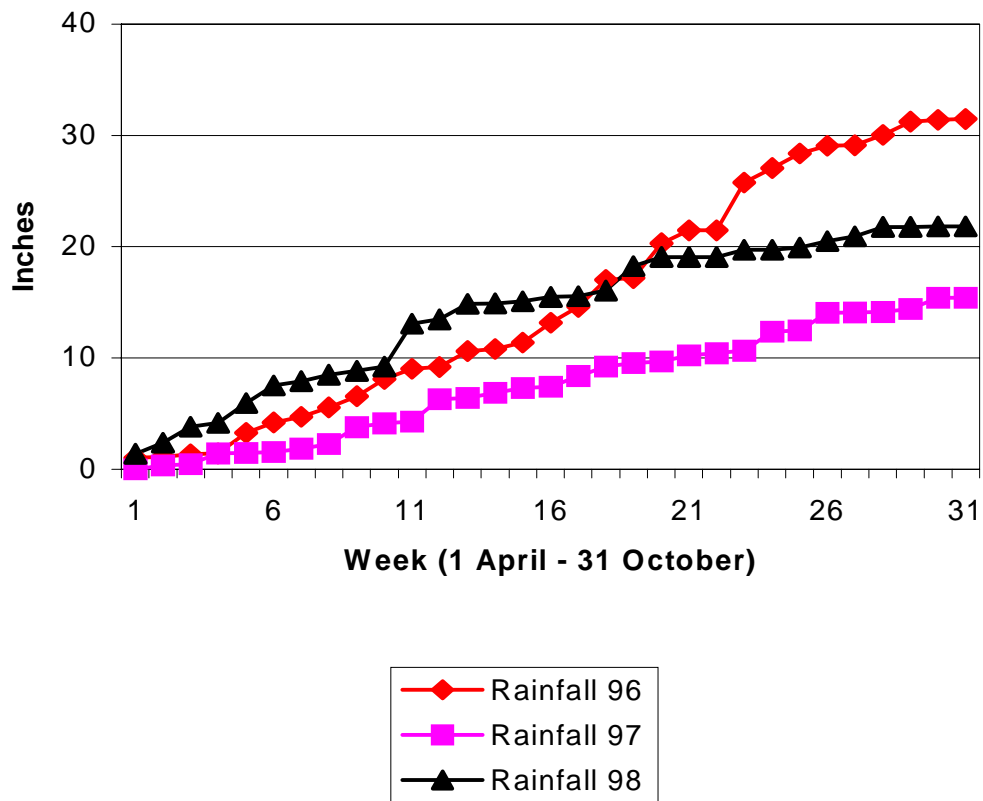
Figure 3.4. Cumulative rainfall (inches) at Winchester vineyard from 1 April – 31 October 1996-1998.

Discussion

Soil was moderately fertile at both vineyard sites. Although soil nitrogen levels were not assessed, analysis of soil elemental composition suggested similar soil nutrient concentrations existed among plots at completion of the Leesburg study. Soil pH was lower in the treatments that received N fertilizer at the completion of the Leesburg study. This pH decrease presumably occurred because to the soil samples were collected shortly after the final addition of the N fertilizer. The application of NH_4^+ fertilizer can temporally decrease pH. Soil Mg concentrations were increased in all treatments at the 20 – 40 cm depth, which was probably due to the application of $MgSO_4$ fertilizer (rate unknown) over the entire vineyard by the vineyard manager during September 1997. Soil Mg concentration was increased in Mg treatment plots at Winchester vineyard over the three-year period of the study. Regardless, little change was observed in the treatment vines' Mg status. This lack of response may have been due to the high level of K in the soil competing with Mg uptake. High levels of soil K can interfere with Mg uptake by plants (Tisdale *et al.*, 1993) presumably because the uptake rate of Mg is lower than the uptake rate of K (Mengel and Kirkby, 1987). Soil analyses may not reflect the amount of nutrients actually absorbed by the plant (Brady and Weil, 1996).

BSN incidence was low ($\leq 10\%$) in both years at Leesburg and was not significantly affected by treatment. Fertilizer treatments had little effect on petiole nutrient concentration over the two-year study at Leesburg. A possible reason for this lack of effect is that vine size and crop load was less than desired (Smart and Robinson, 1991) and vine nutrient status was satisfactory. Treatment vines that received N fertilizer consistently appeared to have larger leaves and a deeper green coloration. However, bloom-time leaf petiole analysis in both years revealed a N deficiency based on Virginia standards. The lack of increase in the N status of N treatment vines revealed by bloom-time and véraison leaf petioles was surprising, and was in contrast to most N fertilizer studies (Christensen *et al.*, 1994; Spayd *et al.*, 1993). Leaf petiole elemental changes were not observed over the two-year period of the study. This suggests that two years was not sufficient time to distinguish differences due to fertilizer treatment at this site.

Since no statistically significant difference between treatment plots was revealed for BSN of Cabernet Sauvignon at Leesburg, the discussion of fertilizer effects on BSN will focus on the Winchester vineyard, unless otherwise specified.

No consistent differences in berry chemistry were observed due to treatment over the study period. Some differences in cluster weight and crop load were observed, but no apparent relationship to BSN incidence was revealed. It was therefore unlikely that fertilizer treatments were simply delaying the onset of BSN through a delay in fruit maturity or differences in crop load.

Deficiencies of Mg and Ca have been associated with BSN (Boselli *et al*. 1983; Brendal *et al*. 1983). However, no evidence of such a relationship was observed at Winchester. Applications of Mg increased the rachis Mg concentration in 1996, but Mg reduced BSN symptoms only when combined with N. Applications of Mg and Ca slightly reduced BSN in the 1997 season; however, the overall expression of BSN during 1997 was low. Thus, the effect of Mg and Ca appear insignificant. Applications of Mg and Ca had no effect on BSN in the 1998 season. The results suggest that Mg and Ca were not involved with BSN at Winchester. Brechbuhler, (1975) reported a higher $K/(Mg + Ca)$ ratio was associated with BSN incidence. No relationship between BSN expression and the $K/(Mg + Ca)$ ratio was observed over the three-year period of the study. Christensen and Boggero (1985) reported a relationship of low $K/(Mg + Ca)$ ratio in the rachis being associated with BSN. This relationship was observed only once in the three-year period. It was therefore unlikely that tissue $K/(Mg + Ca)$ ratio had any bearing on BSN incidence at this site.

Split soil application of nitrogen increased bloom-time petiole N concentration and reduced the incidence of BSN in all three years of the study at Winchester vineyard. This was in contrast to Christensen and Boggero's (1985) report that application of N fertilizer increased BSN.

In 1997, bloom-time petiole N concentration in all treatment plots were within Virginia sufficiency range for N concentration. BSN expression was low in 1997 season compared to 1996 and 1998 season. The1997 season experienced less rainfall and fewer growing degree units prior to bloom than the 1996 and 1998 seasons. It is possible that vine demand for N was reduced due to these environmental factors. This may explain the increased bloom-time leaf petiole N concentration in all treatment plots with a corresponding reduction of BSN in the 1997 season. A common feature of BSN is the annual variability of expression (Haystead *et al.* 1988; Holzapfel and Coombe, 1995) in a given vineyard. The annual variability of BSN expression in the control plots observed in this study appears to be associated with the annual variability of the bloom-time leaf petiole N concentration of the control plots. Bloom-time leaf petiole concentrations below 1% dry weight appears to be associated with BSN expression at Winchester. Véraison rachis analysis also revealed an increase in N concentration due to application of N fertilizer. However, véraison leaf petioles and bloom-time rachis N concentrations did not consistently indicate an increase in N concentration due to applications of N fertilizer. This suggests that véraison rachis analysis may be a reliable indicator of vine N status. When considering the vine size (pruning weight per vine) and control vines were summer pruned twice during each season to maintain 15-17 nodes, the vines do not seem to be candidates for N deficiency as tissue analysis revealed. This suggests that vine size may not be a good indicator of N status. But it also suggests that a sufficiency range for N concentration for a specific grape tissue at a given time of sampling has not been established.

When the net assimilation rate (NAR) is high, N and other inorganic nutrients must be high for the conversion of photosynthates to metabolites such as carbohydrates needed for vegetative growth (Mengel and Kirkby, 1987). Temperature is known to influence growth rate more than light intensity. Growth rate is increased with temperature increases, which can result in a dilution of carbohydrates and chlorophyll due to rapid cell division. This particularly occurs under cloudy conditions (Mengel and Kirkby, 1987). The dilution of chlorophyll along with N deficiency can reduce NAR by

influencing the CO_2 fixation rate and reducing entry of CO_2 through the stomata (Marschner, 1995).

Warm temperatures and cloudy weather was observed during the 1998 season. Deficient N along with a lower NAR was revealed in the control and Mg + Ca plots compared to those plots that received N fertilizer, which may be a result of the previously described situation. Leaves of N treatment vines had a darker green coloration with a higher NAR, which may have increased the carbohydrate status of those vines. Carbohydrate status of a vine may be associated with BSN. Keller and Koblet (1995) suggested the hypothesis that carbon starvation is associated with BSN in a previous study of carbon starvation and Inflorescence Necrosis (Keller and Koblet, 1994). If the NAR was greater in the plots that received N fertilizer during the entire 1998 growing season, a greater carbohydrate status in those vines may have occurred reducing the incidence of BSN.

Conclusion

Annual variability of BSN was observed at Winchester. In 1998, no BSN incidence (control) of Cabernet Sauvignon at Leesburg vineyard was observed when BSN incidence of 23% (control) of Cabernet Sauvignon occurred at Winchester vineyard. These observations suggest a soil and environmental effect on BSN and that BSN prone vineyards should be examined individually.

Fruit chemistry did not suggest a delay of BSN incidence due to fertilizer treatments. Mineral nutrient surveys could not be associated with BSN at Leesburg due to the low incidence of BSN. Applications of Mg and Ca appeared to have no effect on BSN during the study at Winchester. No relationship was apparent with the petiole and/or rachis K/(Mg + Ca) ratio and BSN incidence. The results suggest that these divalent cations and the K/(Mg + Ca) ratio are not involved with the occurrence of BSN of Cabernet Sauvignon at Winchester.

Result of the mineral nutrient surveys in 1996-1997 at Winchester suggested that BSN was associated with bloom-time petiole N concentration below 1% dry weight. Split applications of N increased petiole N concentration and reduced the incidence of BSN. Nitrogen application also increased N concentration of non-symptomatic véraison rachis tissue during each year of the study at the Winchester vineyard. However, there is not sufficient evidence to prove causality. Therefore, it can be concluded that there was a negative correlation between BSN incidence and bloom-time petiole and véraison rachis N concentration.

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APPENDICES

Appendix A. Nutrient sufficiency ranges used for routine plant tissue analysis of grapevines.

Region	N %	P %	K %	Ca %	Mg %	Mn ppm	Fe ppm	Cu ppm	B ppm	Zn ppm	S ppm
					Mineral nutrient concentration of petioles (% or ppm dry weight)						
VA[z]	1.2-2.2	0.15-?	1.5-2.5	1.0-3.0	0.3-0.5	25-1000	40-300	7-15	30-100	35-50	unknown
OR[y]	0.7-1.5	0.1-0.4	1.1-3.0	1.3-3.0	0.5-1.3	61-650	31-100	6-20	25-50	41-100	0.13-0.35
BC[x]	1.7-3.0	0.2-0.5	1.5-2.0	1.0-3.0	0.3-1.5	30-150	40-300	7-15	30-100	25-100	unknown

[z]Virginia (VA), bloom time petioles collected from leaf opposite a flower cluster (Wolf and Poling, 1995).

[y]Oregon (OR), petioles collected from first fully expanded leaf of fruiting shoots during August (Brown,1992).

[x]British Columbia (BC), bloom time petiole analysis collected from leaf opposite a flower cluster (Ministry of Agriculture, Fisheries and Food, 1996).

Appendix B. Soil analysis prior to application of the first fertilizer treatment, Leesburg May 1997.

Soil sample depth	P1 Weak Bray	P2 Strong Bray	K	Mg	Ca	Mn	Fe	Cu	B	Zn	S	pH
					Mineral nutrient concentration in ppm							
0 – 20 cm	6.2	9.7	121	454	997	38	14	4.9	0.7	6.2	11	7.0
20 – 40 cm	4.7	6.6	73	245	740	14	13	1.4	0.5	1.1	13	5.8

Data presented are the mean mineral nutrient concentration and pH of three soil samples collected across an experimental vineyard.

Appendix C. Soil analysis by treatment near completion of the experiment, Leesburg July 1998.

Treatment	Soil sample depth	P1 Weak Bray	P2 Strong Bray	K	Mg	Ca	Mn	Fe	Cu	B	Zn	S	pH
						Mineral nutrient concentration in ppm							
Control	0 – 20 cm	2.0	4.0	94	444	1280	44	24	5.1	0.6	6.9	21	6.5
Control	20 – 40 cm	2.0	4.0	46	445	1280	18	28	2.6	0.4	2.0	68	6.0
N only	0 – 20 cm	3.0	5.0	113	261	760	54	22	5.7	0.5	8.5	28	4.7
N only	20 – 40 cm	2.0	4.0	91	361	1060	22	25	2.8	0.4	2.5	113	5.1
Mg + Ca	0 – 20 cm	3.0	5.0	199	411	810	43	19	6.8	0.7	7.9	80	6.3
Mg + Ca	20 – 40 cm	2.0	4.0	151	429	760	23	19	3.7	0.5	3.5	121	6.0
N + Mg + Ca	0 – 20 cm	2.0	4.0	113	410	810	38	22	5.4	0.5	6.1	128	5.1
N + Mg + Ca	20 – 40 cm	2.0	4.0	61	438	1210	29	33	2.9	0.4	2.8	152	5.7

Data presented are results from one composite soil sample per treatment.

Appendix D. Bloom-time leaf petiole elemental composition of Cabernet Sauvignon, Leesburg 1997 and 1998.

Treatment	Mean nutrient concentration (% or ppm dry weight) of petioles[z]									
	%N	%P	%K	%Mg	%Ca	B ppm	Cu ppm	Fe ppm	Mn ppm	Zn ppm
1997										
Control	1.04 b	0.13	2.44	0.59	2.00	40	13 b	53	173	44
N only	1.20 a	0.18	2.02	0.61	1.74	40	12 b	54	186	41
Mg + Ca	1.08 ab	0.11	2.88	0.48	1.70	39	12 b	43	173	40
N + Ca + Mg	1.06 b	0.11	2.28	0.59	2.28	38	17 a	60	133	44
1998										
Control	1.01	0.10	1.66 b	0.53 bc	1.94	29	8	53	221	28
N only	0.97	0.08	1.16 b	0.71 a	1.92	30	8	57	253	30
Mg + Ca	0.88	0.94	2.50 a	0.40 c	1.72	28	8	49	266	26
N + Ca + Mg	0.99	0.08	1.39 b	0.61 ab	1.92	28	8	57	194	26

[z]Elemental concentrations are means of five separate samples. Means within columns followed by the same letter, or by no letter, are not significantly different at $P \leq .05$, using Duncan's multiple range test.

Appendix E. Véraison leaf petiole elemental composition of Cabernet Sauvignon, Leesburg 1997 and 1998.

Treatment	%N	%P	%K	%Mg	%Ca	B ppm	Cu ppm	Fe ppm	Mn ppm	Zn ppm
					Mean nutrient concentration (% or ppm dry weight) of petioles[z]					
1997										
Control	1.08	0.06	2.28 b	0.96	1.52	27 b	4 a	58	325	53
N only	1.06	0.06	2.73 b	1.18	1.44	28 b	4 a	62	299	35
Mg + Ca	1.10	0.05	5.40 a	0.47	1.54	30 b	3 b	58	240	46
N + Ca + Mg	1.10	0.05	2.28 b	1.04	1.66	26 b	5 a	60	178	37
1998										
Control	0.73	0.05	1.62 b	1.36 a	1.78	26	3 b	55	459	43
N only	0.74	0.04	1.59 b	1.80 a	1.66	28	4 b	54	529	41
Mg + Ca	0.67	0.05	4.12 a	0.77 b	1.80	29	4 ab	56	476	40
N + Ca + Mg	0.77	0.04	2.14 b	1.41 a	1.90	26	5 a	52	353	40

[z]Elemental concentrations are means of five separate samples. Means within columns followed by the same letter, or by no letter, are not significantly different at $P \leq .05$, using Duncan's multiple range test.

Appendix F. Point qaudrat analysis, photosynthetically active radiation (PAR), and net assimilation rate (NAR) of Cabernet Sauvignon, Leesburg 1997 and 1998.

	Point quadrat analysis[z]						PPF[x] $\mu mol \bullet m^{-2} \bullet s^{-1}$	NAR[y] $\mu mol CO_2 \bullet m^{-2} \bullet s^{-1}$
	Leaf layers		% Exterior fruit		% Gaps			
Treatment	1997	1998	1997	1998	1997	1998	1997	1998
Control	2.7	3.0	24b	40	4	0	7.3	13.1
N only	2.9	2.8	32b	23	0	0	6.3	13.9
Mg + Ca	2.2	3.2	40b	56	2	0	5.9	12.8
N + Mg + Ca	2.5	2.9	72a	33	0	2	5.3	12.5

[z]Refer to Materials and Methods for explanation.

[y]Refer to Materials and Methods for explanation.   Net photosynthesis rate was not performed in 1997.

[x]Photosynthetic photon flux (PPF) is the ratio of interior to exterior PPF and was expressed as photosynthetically active radiation ($\mu mol \bullet m^{-2} \bullet s^{-1}$). PPF was not performed in 1998.

Means within columns are not significantly different at $P \leq .05$, using Duncan's multiple range test.

Appendix G. Pruning weight, cluster weight, crop per vine, and crop load of Cabernet Sauvignon, Leesburg 1997 and 1998.

Treatment	Mean pruning weight/vine (kg)		Mean cluster weight (g)		Mean crop/vine (kg)		Crop load[z]	
	1997	1998	1997	1998	1997	1998	1997	1998
Control	0.5	0.7	58.1	47.9	2.3	2.3	5.1	3.3
N only	0.6	1.1	68.4	50.8	3.0	2.4	4.9	2.2
Mg + Ca	0.5	0.8	61.5	60.2	2.6	2.6	5.1	3.8
N + Mg + Ca	0.5	1.0	63.8	54.4	2.8	2.5	6.1	2.6

[z]Crop load is mean crop per vine divided by mean pruning weight per vine.

Pruning weight, cluster weight, crop per vine are means of 10-treatment vines.

Means within columns are not significantly different at $P \leq .05$, using Duncan's multiple range test.

Appendix H. Soil analysis prior to application of the first fertilizer treatment, Winchester April 1996.

| Soil sample | Mineral nutrient concentration in ppm | | | | | | | | | | | |
| | P1 | P2 | | | | | | | | | | |
depth	Weak Bray	Strong Bray	K	Mg	Ca	Mn	Fe	Cu	B	Zn	S	pH
0 – 20 cm	13	21	120	153	803	53	10	2.2	0.7	5.2	11	6.4
20 – 40 cm	8	11	83	133	770	30	11	1.5	0.7	2.1	11	6.7

Data presented are the mean mineral nutrient concentration and pH of 3 soil samples collected across an experimental vineyard.

Appendix I. Soil analysis by treatment near completion of the experiment, Winchester, August 1998.

| | Soil sample | Mineral nutrient concentration in ppm | | | | | | | | | | | |
| | | P1 | P2 | | | | | | | | | | |
Treatment	depth	Weak Bray	Strong Bray	K	Mg	Ca	Mn	Fe	Cu	B	Zn	S	pH
Control	0 – 20 cm	31	35	98	43	150	128	40	2.4	0.6	6.4	13	4.3
Control	20 – 40 cm	9	11	103	55	330	164	21	2.1	0.5	3.4	9	4.1
N only	0 – 20 cm	45	69	130	91	710	49	16	8.1	0.6	27.0	5	5.6
N only	20 – 40 cm	7	12	53	153	680	22	8	2.3	0.5	3.5	5	6.5
Mg + Ca	0 – 20 cm	16	24	117	346	530	69	8	2.9	0.8	5.7	18	6.3
Mg + Ca	20 – 40 cm	35	51	163	422	380	62	7	4.4	0.8	12.0	10	6.2
N + Mg + Ca	0 – 20 cm	6	8	159	267	540	59	12	2.5	0.5	5.2	27	4.5
N + Mg + Ca	20 – 40 cm	3	5	153	328	1300	25	6	2.3	0.9	2.4	30	5.7

Data presented are results from one composite soil sample per treatment.

Appendix J. Bloom-time leaf petiole elemental composition of Cabernet Sauvignon, Winchester 1996 - 1998.

	Mean nutrient concentration (% or ppm dry weight) of petioles[z]									
Treatment	%N	%P	%K	%Mg	%Ca	B ppm	Cu ppm	Fe ppm	Mn ppm	Zn ppm
1996										
Control	0.80 b	0.45 a	5.09	0.37	1.97 a	43	22	70	189	48
N only	1.85 a	0.18 b	4.60	0.46	1.84 a	38	12	94	282	43
Mg[y]	0.93 b	0.47 a	4.82	0.37	1.85 a	38	14	82	153	44
N + Mg [y]	1.66 a	0.14 b	4.95	0.48	1.61 b	37	11	113	201	42
1997										
Control	1.32 b	0.12	2.38	0.23 b	1.38	36	6 b	37	85 bc	39
N only	1.85 a	0.10	1.86	0.26 ab	1.30	33	7 ab	39	163 a	41
Mg + Ca	1.34 a	0.19	2.88	0.27 a	1.40	37	8 a	38	71 c	40
N + Ca + Mg	1.56 ab	0.10	2.88	0.28 a	1.42	35	8 ab	39	114 b	41
1998										
Control	0.88 b	0.09 b	3.16	0.25 b	1.42 b	29	8 a	47	152 c	35
N only	1.18 a	0.10 ab	3.72	0.30 ab	1.80 a	29	7 ab	45	425 a	33
Mg + Ca	0.89 b	0.12 a	3.16	0.33 a	1.78 a	31	7 b	47	161 c	36
N + Ca + Mg	1.09 a	0.11 a	3.58	0.35 a	1.72 a	32	6 b	48	295 b	36

[z]Elemental concentrations are means of five separate samples. Means within columns followed by the same letter, or by no letter, are not significantly different at $P \leq .05$, using Duncan's multiple range test.

[y]Calcium was not included in the fertilizer treatments in 1996.

Appendix K. Bloom-time cluster stem (rachis) elemental composition of Cabernet Sauvignon, Winchester 1997 and 1998.

Treatment	%N	%P	%K	%Mg	%Ca	B ppm	Cu ppm	Fe ppm	Mn ppm	Zn ppm	K/Mg + Ca
Mean nutrient concentration (% or ppm dry weight) of petioles[z]											
1997											
Control	2.72	0.36	2.38	0.29	0.98	36	15	73	143	61	1.9
N only	2.76	0.35	2.34	0.27	0.96	36	15	74	165	61	1.9
Mg + Ca	2.76	0.34	2.26	0.27	0.94	33	15	71	122	55	1.9
N + Ca + Mg	2.88	0.33	2.28	0.28	0.92	31	16	69	141	56	1.9
1998											
Control	2.38 b	0.31	2.26 c	0.29	1.26	30	13	78 b	227 c	72	1.5 b
N only	2.78 a	0.30	2.70 a	0.27	1.10	28	14	87 a	424 a	69	2.0 a
Mg + Ca	2.38 b	0.33	2.32 bc	0.28	1.22	29	13	77 b	222 c	68	1.6 b
N + Ca + Mg	2.70 a	0.31	2.50 ab	0.28	1.22	29	15	85 a	322 b	69	1.7 ab

[z]Elemental concentrations are means of five separate samples. Means within columns followed by the same letter, or by no letter, are not significantly different at $P \leq .05$, using Duncan's multiple range test.

Bloom-time cluster stem (rachis) tissue was not collected in 1996.

Appendix L. Véraison leaf petiole elemental composition of Cabernet Sauvignon, Winchester 1997 and 1998.

Treatment	%N	%P	%K	%Mg	%Ca	B ppm	Cu ppm	Fe ppm	Mn ppm	Zn ppm
				Mean nutrient concentration (% or ppm dry weight) of petioles[z]						
1997										
Control	1.00 b	0.22 a	1.34 b	0.19 b	0.68 b	13 b	11	276 a	259	26
N only	1.08 a	0.06 b	6.54 a	0.44 a	1.66 a	37 a	8	43 b	374	34
Mg + Ca	1.08 a	0.10 b	6.22 a	0.42 a	1.60 a	41 a	11	37 b	178	40
N + Ca + Mg	1.05 ab	0.06 b	6.10 a	0.38 a	1.70 a	40 a	11	41 b	273	36
1998										
Control	0.78 b	0.09 b	5.68 b	0.39 b	1.60	25 b	2 c	37	225 b	46
N only	0.90 a	0.07 b	6.58 a	0.47 ab	1.80	30 a	3 b	42	319 b	55
Mg + Ca	0.80 b	0.20 a	5.74 b	0.50 a	1.98	32 a	3 b	41	603 a	56
N + Ca + Mg	0.88 a	0.07 b	6.34 ab	0.56 a	1.84	32 a	4 a	39	603 a	58

[z]Elemental concentrations are means of five separate samples. Means within columns followed by the same letter, or by no letter, are not significantly different at $P \leq .05$, using Duncan's multiple range test.

Véraison leaf petioles were not collected in 1996.

Appendix M. Véraison cluster stem (rachis) elemental composition of Cabernet Sauvignon, Winchester 1996 – 1998.

Treatment	%N	%P	%K	%Mg	%Ca	B ppm	Cu ppm	Fe ppm	Mn ppm	Zn ppm
				Mean nutrient concentration (% or ppm dry weight) of petioles[z]						
1996										
Control	1.16 b	0.35 a	4.71	0.17 b	0.86 b	43	11 ab	73	151	45a
N only	2.18 a	0.23 b	4.38	0.18 b	0.77 b	39	9 c	83	200	47 a
Mg[y]	1.02 b	0.40 a	4.74	0.23 a	0.98 a	43	12 a	67	137	48 a
N + Mg[y]	1.84 a	0.22 b	4.28	0.20 b	0.79 b	38	10 bc	78	166	32 b
1997										
Control	1.04 b	0.26 a	1.30 b	0.17 b	0.34 b	5 b	2 b	64	963 a	22 b
N only	1.40 a	0.13 b	5.00 a	0.23 a	0.91 a	79 a	53 a	62	216 b	29 a
Mg + Ca	1.04 b	0.24 a	4.56 a	0.27 a	1.01 a	81 a	55 a	57	138 b	29 a
N + Ca + Mg	1.26 a	0.14 a	4.64 a	0.26 a	1.02 a	85 a	64 a	67	191 b	31 a
1998										
Control	0.98 b	0.27 ab	4.32 c	0.28	1.14	26	10	55	188 b	44
N only	1.34 a	0.17 b	5.22 a	0.23	1.00	25	7	50	369 a	44
Mg + Ca	1.01 b	0.34 a	4.70 cb	0.29	1.16	27	10	46	213 b	42
N + Ca + Mg	1.26 a	0.18 b	5.16 ab	0.25	1.11	28	8	55	310 a	42

[z]Elemental concentrations are means of five separate samples. Means within columns followed by the same letter, or by no letter, are not significantly different at $P \le .05$, using Duncan's multiple range test.

[y]Calcium was not included in the fertilizer treatments in 1996.

Appendix N. Point quadrat analysis (PQA) of Cabernet Sauvignon, Winchester 1996-1998

| | Point quadrat analysis[z] | | | | | | | | |
| | Leaf layers | | | % Exterior fruit | | | % Gaps | | |
Treatment	1996	1997	1998	1996	1997	1998	1996	1997	1998
Control	0.9 b	1.2 b	1.2 b	65 b	64	79 a	12 b	10	10
N only	0.9 b	1.6 a	1.8 a	67 b	60	59 b	15 ab	6	7
Mg + Ca[y]	1.0 a	1.6 a	1.7 a	81 a	59	55 b	15 ab	7	6
N + Mg + Ca[y]	0.9 b	1.9 a	1.8 a	64 b	57	71 ab	16 a	4	3

[z]Refer to Materials and Methods for explanation. Means within columns followed by the same letter, or by no letter, are not significantly different at $P \leq .05$, using Duncan's multiple range test.

[y]Calcium was not included in the fertilizer treatments in 1996.

Appendix O. Photosynthetically active radiation (PAR), Winchester 1996 and 1997, and net assimilation rate (NAR) of Cabernet Sauvignon, Winchester 1997 and 1998.

Treatment	PPF^z μmol•m^{-2}•s^{-1}		NAR^y μmol•CO$_2$•m^{-2}•s^{-1}	
	1996	1997	1997	1998
Control	5.5 a	3.6 a	10.7 a	11.9 c
N only	4.0 b	1.9 b	9.2 b	12.7 b
Mg + Ca^x	5.4 a	2.1 b	9.3 b	11.3 d
N + Mg + Ca^x	3.7 b	1.8 b	9.9 ab	13.3 a

^{z}Photosynthetic photon flux (PPF) is the ratio of interior to exterior PPF and was expressed as photsynthetically active radiation (μmol•m^{-2}•s^{-1}). PPF measurements were not performed in 1998.

^{y}Refer to Materials and Methods for explanation. Net assimilaition rate was not performed in 1996.

Means within a column followed by the same letter, or by no letter, are not significantly different at $P \leq .05$, using Duncan's multiple range test.

^{x}Calcium was not included in the fertilizer treatments in 1996.

Appendix P. Pruning weight, cluster weight, crop per vine, and crop load of Cabernet Sauvignon, Winchester 1996-1998

Treatment	Mean pruning weight/vine (kg)			Mean cluster weight (g)			Mean crop/vine (kg)			Crop load[z]		
	1996	1997	1998	1996	1997	1998	1996	1997	1998	1996	1997	1998
Control	3.6 b	2.6 c	2.1 b	94 b	139 b	111	14.8	18.6	10.6	4.7	7.5 a	7.0 a
N only	4.2 ab	3.3 a	3.3 a	121 a	153 a	118	12.9	18.2	11.5	3.4	5.8 c	3.7 b
Mg + Ca[y]	3.7 b	3.0 b	2.4 b	123 a	149 ab	113	15.3	18.6	10.7	3.8	6.7 b	4.8 b
N + Mg + Ca[y]	4.4 a	3.3 a	3.7 a	118 a	139 b	122	14.7	17.3	11.7	3.5	5.4 c	3.3 b

[z] Crop load is mean crop/vine divided by mean pruning weight vine.

Pruning weight, cluster weight, crop per vine are means of 15 treatment vines.

Means within a column followed by the same letter, or by no letter, are not significantly different at $P \leq .05$, using Duncan's multiple range test.

[y] Calcium was not included in the fertilizer treatments in 1996.

VITA

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Eric was born in San Antonio, Texas in 1963 to Bonnie and Dean Capps.

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Eric graduated from York High School, located in Yorktown Virginia in 1981. Eric received a B. S. in Biology from Christopher Newport College, located in Newport News, Virginia in 1987. Eric started his M. S. studies at Virginia Polytechnic Institute and State University in 1997.

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