CHAPTER 4
LOCALIZED CARBOHYDRATE AND ESSENTIAL NUTRIENT DEPRIVATION AS CAUSAL FACTORS IN GRAPEVINE BUD NECROSIS

Abstract. Bud necrosis (BN) of grape is observed as an abortion and drying of one or more primordia of the developing compound winter bud. The causes are uncertain, but high vigor, and canopy shade increase BN incidence under some conditions. This work was conducted to determine if localized carbohydrate or mineral nutrient deprivation increased the incidence of BN observed with 'Riesling' grapes in Virginia. Changes in carbohydrate levels in 'Riesling' grapevines was attempted by shading and shoot tip removal. In 1994, 92% shade was applied at 20, 40, or 60 days after budbreak in one vineyard, and starting 40 days after budbreak in another vineyard for three-week periods. In 1996, a second shade experiment at 25 and 65 days after budbreak for a 40-day period was conducted. Shade reduced PPF in the fruit zone of canopies to <2% of ambient PPF in both experiments. The first experiment did not increase BN. However, the second experiment increased BN in the distal nodes of the shaded vines compared to the control vines. Shoot vigor, measured as shoot diameter and internode length at season's end, was positively correlated with BN in shaded as well as unshaded vines. The frequency of necrotic buds was greater at nodes 5 through 16 than at nodes 1 to 4 in both shaded and unshaded vines. Levels of total nonstructural carbohydrates (TNC) measured spectrophotometrically, were not significantly affected by shade treatment. Levels of sucrose, glucose, fructose, and starch in bud, leaf, and stem tissues analyzed by high performance liquid chromatography were lower in shaded vines as determined at the time of shade cloth removal. Shoot tip removal increased BN at nodes distal to 12. Bud tissues of shoot-tipped vines had lower levels of sucrose, glucose, fructose, and starch than did the control vines. Mineral nutrient surveys conducted on 'Riesling' and 'Chardonnay' vines indicated that BN is not caused by essential nutrient deficiency. Carbohydrate surveys revealed that 'Riesling' buds had lower levels of sucrose compared to 'Chardonnay' buds. Visual starch rating revealed that 'Chardonnay', the BN-insensitive cultivar, had greater levels of bud starch deposits than did the BN-susceptible cultivars, 'Riesling', 'Syrah', and 'Viognier'. Starch deposits in grape buds was negatively correlated with BN incidence. 'Riesling' and 'Chardonnay' vines showed a node position effect where in nodes 1to 6 had higher levels of starch deposits compared to nodes 7 to 13. From these studies it can be concluded that a negative correlation between carbohydrate levels in vines and BN incidence exisits.

INTRODUCTION

Bud necrosis (BN) of grape (*Vitis vinifera* L.) is observed as an apparent abortion and drying of the primary bud and, in severe cases, secondary buds of the developing winter bud (Dry and Coombe, 1994; Lavee et al., 1981; Naito et al., 1989; Perez, 1991; Wolf and Warren, 1995). Secondary buds usually continue to develop after primary bud death, and produce normal shoots the following season; however, crop yields are significantly reduced due to the destruction of the more fruitful primary buds. BN has been reported in Australia (Dry and Coombe, 1994), California, USA (Morrison and Iodi, 1990), Chile (Perez and Kliewer, 1990), India (Bains et al., 1981; Bindra and Chohan, 1975), Israel (Lavee et al., 1981; Lavee, 1987; Ziv et al., 1981), Japan (Naito et al., 1986), and Virginia, USA (Wolf and Warren, 1995). The causes of this apparent physiological disorder are uncertain, but high shoot vigor, quantified by shoot growth rate or judged from cane size (Dry and Coombe, 1994; Lavee et al., 1981; Wolf and Warren, 1995), excessive irrigation (Bindra and Chohan, 1975; Perez, 1991), low carbohydrate levels (Naito et al., 1987), shade (Perez and Kliewer, 1990; Wolf and Warren, 1995), and high gibberellin-like activity (Lavee, 1987; Ziv et al., 1981) have all been reported to increase BN. Shoot thinning and artificial shade studies have yielded conflicting responses. Severe shoot thinning ("75-85% shoot removal") increased BN of ‘Shiraz’ in Australia (Dry and Coombe, 1994), while a more modest level of shoot thinning decreased BN incidence of ‘Thompson Seedless’ in Chile (Perez and Kliewer, 1990). In Australia, severe thinning apparently stimulated shoot vigor (judged by cane diameter and lateral development), which probably increased BN. The timing of shade may also be critical to the occurrence of BN. Previous research in Virginia indicated that a three-week period of shade (either 64% or 92% reduction in PPF), applied shortly before veraison, had no effect on BN (Wolf and Warren, 1995). Preliminary anatomical studies, however, revealed that tissue destruction began as early as 66 days after bud break. Thus, shading experiments were conducted to determine if early-season shading caused BN in ‘Riesling’ grapevines grown in Virginia. It was hypothesized that as shade reduced the rate of photosynthesis in leaves, axillary buds would suffer from carbohydrate deprivation resulting in BN. Carbohydrates in buds of shaded and non-shaded grapevines were analyzed to test this hypothesis.

Shoot vigor has been reported to be one of the causes of BN (Dry and Coombe, 1994; Lavee et al., 1981; Wolf and Warren, 1995). Carbon resources are preferentially translocated to actively growing shoot tips at the expense of other plant parts such as developing fruit clusters and axillary buds (Candolfi-Vasconcelos and Koblet, 1990). Studies in Japan and California, USA attempted to correlate carbohydrate concentrations in bud tissues with BN incidence (Morrison and Iodi, 1990; Naito et al., 1987). Research in Japan on the cultivar ‘Kyoho’ revealed that starch was lower in strong shoots where “strong” was quantified as greater overall shoot length, greater internode length, and larger bud diameter (Naito et al., 1987). Strong shoots had higher BN incidence (8.3%) at basal nodes compared to the weak shoots (0% BN). The level of starch was 5% of dry matter in strong shoots whereas it was around 8% in weak shoots at 40 days after bloom (Naito et al., 1987). Morrison and Iodi (1990) also observed a negative correlation between starch level and necrosis. Therefore, it was hypothesized that shoot tipping would remove the sink that would compete with fruit clusters and buds for carbon resources early in the

shoot's growth period. Removal of shoot tips would cause preferential translocation of organic nutrients to fruit clusters and other organs on the current shoot including axillary buds. Therefore, the objective of this study was to increase carbohydrate translocation into the axillary buds by removing shoot tips.

A few studies in grapevines have indicated that low nutrient (nitrogen, phosphorus, potassium) levels in overvigorous vines are responsible for flower bud death in the grape cultivar 'Anab-e-Shahi' (Bains et al., 1981; Bindra and Chohan, 1975). However, other conflicting reports in literature have shown no correlation between nutrient levels and BN (Naito et al., 1987; Perez, 1991). Naito et al. (1987) examined the levels of nitrogen, phosphorus, potassium, calcium, magnesium, and boron in the lateral buds of 'Kyoho' grape. Strong shoots and weak shoots classified on the basis of shoot length, internodal cross section, and bud size were used. Strong shoots had a higher percentage of BN (44%) compared to the weak shoots (9%). However no correlation between nutrient levels and BN was observed (Naito et al., 1987). Similarly, Perez (1991) found no effect of nitrogen or potassium fertilizer application on BN. It cannot be judged from these conflicting reports whether nutrient deficiency is involved in BN occurrence in Virginia. Necrotic buds generally form because of cell compression and breakage beneath the primary axis (Chapter 3). As calcium is essential for cell wall formation, it was hypothesized that BN-prone vines may be deficient in calcium, leading to cell distortion and cell breakage. Calcium deficiency is reported to cause lettuce tip burn (Collier and Tibbetts, 1983), blossom-end rot in tomatoes (Ho et al., 1995), marginal bract necrosis in poinsettia (Woltz and Harbaugh, 1986), and bract necrosis in sunflower (de la Guardia et al., 1990). Foliar application of calcium and magnesium reduced rachis necrosis in 'Canada Muscat' grape (Cline, 1987). In plants, rapid death of cells in the apical meristem and stunted growth results when supplied with nutrients lacking in calcium (Epstein, 1972). Deficiency of certain other nutrient elements in plants can also cause nutritional disorders that resemble necrosis. For instance, deficiency of boron usually kills cells in meristematic regions. Boron deficiency in lettuce sometimes caused a form of tip-burn similar to that caused by calcium deficiency (Collier and Tibbetts, 1983).

In addition to mineral nutrient reductions, overly vigorous vines might be expected to have high BN (Dry and Coombe, 1994; Lavee et al., 1981; Wolf and Warren, 1995) because of actively growing shoot tips that are strong photosynthate sinks. Carbohydrate resources and mineral nutrients preferentially go to the growing tips rather than to the fruit clusters (Candolfi-Vasconcelos and Koblet, 1990). Fruit clusters form the next dominating sink followed by stems and axillary buds (Edson et al., 1995; Motomura, 1990). It was therefore hypothesized that either mineral nutrients, carbohydrates, or both may be locally deficient in the lateral buds and that such deficiencies might lead to BN. Therefore, essential elements such as calcium, magnesium, potassium, phosphorus, manganese and boron and carbohydrates were analyzed in the lateral buds, leaves, and stem tissues of a BN-prone and a BN-resistant cultivar.

It has been proposed that another grapevine disorder, inflorescence necrosis (IN) in grapes may also be caused by carbohydrate deprivation (Keller and Koblet, 1994, 1995). The vines developed severe symptoms of IN when exposed to light levels of 30 $\mu E \cdot m^{-2} \cdot s^{-1}$. Keller and

Koblet (1995) also observed low rates of photosynthesis at those low light levels, which led them to conclude that stress induced carbon depletion, combined with competitive interactions among sinks, were responsible for IN. Shading studies have revealed a reduction in the rate of photosynthesis in single leaves (Cartechini and Palliotti, 1995) leading to carbohydrate deprivation in leaves. At fruit set and fruit ripening, vegetative shoots are weak carbohydrate sinks (Edson et al., 1995; Motomura, 1990), and therefore a vine at this phenological stage, when subjected to stress situations like shade, experiences carbohydrate deprivation in leaves, stems, and other vegetative parts. A similar argument can be forwarded for BN as well. It was hypothesized that BN develops from localized carbohydrate deprivation in the lateral grape buds. Therefore, towards this end, four grape cultivars that showed differential BN-sensitivity, were selected for visual starch analysis. Visual starch rating studies have been performed in the past with apple fruits. A starch rating chart was developed to evaluate the quantity of starch in the fruits (Hesse and Hitz, 1938; Marini et al., 1990; Poapst et al., 1959). A similar key was created to evaluate the cultivars 'Riesling', 'Viognier', and 'Syrah' that have been reported to have high BN levels (Wolf, unpublished data) and compared with the BN-insensitive cultivar, 'Chardonnay'.

Therefore, the objectives of this study was to determine the potential role of carbohydrate or mineral nutrient deprivation in BN incidence of 'Riesling' grapevines. Changes in carbohydrate levels of grapevine organs was attempted by shading and shoot tipping. Carbohydrate and mineral nutrient surveys were conducted in BN-prone and BN-insensitive vineyards. Visual starch analyses were also performed on four cultivars showing differential BN sensitivity.

MATERIALS AND METHODS

Studies were conducted from 1993 to 1996 to evaluate the effect of artificial shade, vigor, mineral nutrients and carbohydrate deprivation on BN occurrence in the cultivars 'Riesling' and 'Chardonnay' at four vineyards in Virginia. A brief description of the procedures used in the experiments is followed by a description of each experiment. All general laboratory chemicals were obtained from Fisher Scientific (Springfield, NJ 07081-3193) and Sigma (St. Louis, MO 63178).

Mineral Analysis

Calcium, magnesium, potassium, boron, manganese, and phosphorus were analyzed at Virginia Tech's Soil Testing and Plant Analysis Laboratory, Blacksburg using a simultaneous inductively coupled plasma (ICP) spectrometer (Thermo-Jarrell-Ash ICAP-61). The procedure involves heating samples to volatilize the organic components, dissolving the resulting ash using perchloric acid, and filteration. The filterate was then analyzed for its elemental concentration using an ICP. The lyophilized plant tissue was ground and 0.5 g of the tissue was weighed into a Folin Wu digestion tube to which 4 ml of concentrated nitric acid was added. The tubes were then allowed to stand overnight, and then 2 ml perchloric acid was added to each tube. The tubes were heated until all the red fumes of nitric acid and the white fumes of perchloric acid had disappeared, and only 1 ml of the solution remained in the tubes. The tubes were cooled and 15 ml of distilled

deionized water was added. The samples were then filtered through Whatman #42 filter paper into plastic bottles.

All elements were analyzed in the same extract by an ICP equipped with a modified Technicon IV autosampler. A blank and a standard were analyzed with each set of plant samples. The quantity of each mineral nutrient present in the plant organ was reported as $mg \cdot g^{-1}$ of dried plant tissue.

Carbohydrate Analysis

Two representative shoots per treatment plot were collected every 10 days, starting 50 days after budbreak and continuing until 90 days after budbreak. Buds, leaves, and stem tissues were separated in the field and immediately submerged in liquid nitrogen. Stem and leaf tissues from nodes 5 through 13 were collected for analysis. Buds from all nodes (1-17) were collected. The plant tissues were then transported to the lab on dry ice and stored at -80C.

Carbohydrates were analyzed using high performance liquid chromatography (HPLC) (adapted from Mullen and Koller, 1988 and Wellso et al., 1989). Stem, leaf, and bud tissues were lyophilized (Virtis Sentry Lyophilizer) and ground to pass through a 1-mm screen. Subsamples from individual samples were used to determine glucose, fructose, sucrose, and starch levels. The sugars (glucose, fructose, and sucrose) were extracted by placing 100 g of each sample into prelabeled 25 ml centrifuge tubes with 5 ml of 80% ethanol and vortexing thoroughly to mix the samples. The samples were then placed in a heated sonicator (Branson Ultrasonic Cleaner B-32) (60C) for 1 hour. The samples were then centrifuged (Beckman centrifuge Model J2-31) for 5 minutes at 3080×g. The supernatant extract was removed and heated to evaporate the ethanol to approximately 1 ml and then diluted by adding 2 ml distilled, deionized water. The samples were again sonicated for 30 minutes and centrifuged at 3080×g for 5 minutes. The supernatant was drawn off and evaporated. The samples were then dried in a lyophilizer. Starch was hydrolyzed and measured as equivalents of glucose. Starch was hydrolyzed by adding 3 ml of 0.02 N sulfuric acid to the tissue remaining in the centrifuge tubes after the second centrifugation and placing the tubes in a boiling water bath for 1 hour to gelatinize the samples. The samples were cooled and 3 ml of 0.1M sodium acetate buffer, adjusted to pH 4.5, and 1 ml of amyloglucosidase (1 mg amyloglucosidase in 5 ml of 0.1M sodium acetate buffer) were added to the samples. The samples were then placed in a water bath at 55C for 48 hours. The samples were centrifuged at 3080×g for 5 minutes, and the supernatant was drawn off into centrifuge tubes, evaporated to 1 ml, and lyophilized. Lyophilized samples were diluted by adding 200 µl of deionized water. The samples were then filtered through a nylon filter (Nylon Acrodisc, 13 mm diameter, 0.45 µm pore size) before injecting 20 µL of the sample into the HPLC. The carbohydrates were separated using a Bio-Rad Aminex HPX-87P 3007.8 mm column (Bio-Rad Laboratories, Hercules, CA) heated to 85C with a mobile phase of degassed filtered double distilled water at a flow rate of 0.6 $ml \cdot min^{-1}$. A guard column (with Carbo-P micro guard cartridge from Bio-Rad Laboratories, Hercules, CA) at room temperature was used between the injector and analytical column. The carbohydrates in the extract were detected by a refractometer (Waters 410 Differential Refractometer). The area under each peak and the retention times were determined using Axiom

717 chromatography data system by referencing with sugar standards of sucrose, glucose and fructose.

BN Assessment

BN was evaluated on 10 shoots per vine in October by making sequential lateral razor cuts through the buds from node 1 to 20. Each shoot was divided into 5 sections (nodes 1-4, nodes 5-8, nodes 9-12, nodes 13-16, and nodes 17-20). Percentage of BN was determined by counting the number of dead and live buds for each section. Primary buds were judged dead if they appeared dry, crushed, and/or darkened. Buds were judged alive if green.

Effect of Shade on BN in 'Riesling' Grapevines, 1994

Vineyards: Shade experiments were conducted during 1994 in two mature (10 years old) 'Riesling' vineyards: Somerset, in central Virginia and Willowcroft, in northern Virginia. At Somerset Vineyard, 92% neutral black shadecloth, was applied 20 (Shade I), 40 (Shade II), or 60 (Shade III) days after budbreak over a 3-week period. Shade cloth was suspended over 2-vine plots, each replicated six times in a completely randomized design. An equal number of unshaded control plots (Control I, II, and III) were used for each shade period. Vines were trained to a casarsa, unilateral cordon system at Somerset Vineyard. The vines were spur pruned and shoots were positioned to grow vertically upright. The vines were spaced 3.7 m apart in rows 3.7 m wide.

At Willowcroft Vineyard, one shade treatment along with a control treatment (with no shade) was applied 40 days after budbreak to three-vine plots replicated five times in a completely randomized design. Vines were trained to bilateral cordons at Willowcroft Vineyard. Vines were spur-pruned, and shoots were positioned to grow vertically upright, similar to Somerset Vineyard. Spacing at Willowcroft Vineyard was 3.1 m (row) × 2.1 m (vine).

Point Quadrat Analysis: Shoot density of all plots at both vineyards was standardized to 15 shoots per meter of canopy 15 days after budbreak. Canopy point quadrat analyses (Smart et al., 1990) were also done about 100 days after budbreak. A thin metal rod was passed horizontally through the fruiting region of the canopy at equal intervals fifteen times per plot. Contacts of the probe with leaves, fruit clusters and canopy gaps were recorded. The number of leaf layers, percent gaps, percent exterior fruit clusters, and percent interior leaves was calculated for both vineyards.

Light Measurement: Photosynthetic photon flux (PPF) measurements were made in canopy fruit zones of each treatment plot with a 1.0-m line quantum sensor (LI-COR model LI-191SB) and a photometer (LI-COR model 185B) in both vineyards the day after each shade treatment was applied. Light measurements were made between 1100 and 1400 HR at both vineyards. Two PPF measures were recorded in each plot in the fruit zone. Each of those records was an average of three separate readings: one with the sensor face oriented vertically upright (0°), one facing 45 ° left, and one facing 45° right of the rows. Additionally, a single exterior PPF reading was made

at each plot by holding the sensor above the vine canopy with the sensor oriented skyward (0°). Ambient PPF values ranged from 1400 to 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ on all days of measurement.

Ten representative shoots per plot were selected 20 days after budbreak in both vineyards. The fruit clusters on all selected shoots were removed to avoid the confounding effect of cluster development on shoot growth rate. Average internode length (from nodes 1 to 20) and maximum diameter of the second internode were also measured on the tagged shoots of all treatments on 24 October 1994 at Somerset and on 8 November 1994 at Willowcroft. BN assessment, as described previously, was also done on those dates.

Total Nonstructural Carbohydrate Analysis: Total nonstructural carbohydrates (TNC) were measured for shade I and control I panels at Somerset Vineyard in bud and leaf tissues. Two representative shoots were sampled at 10-day intervals starting at 40 days after budbreak when the shading treatment finished, until 90 days after budbreak. TNC analysis was also done for shade and control panels of Willowcroft Vineyard. Here as well, several samplings were performed when the shade cloth came off 50 days after budbreak. Subsequent samplings were done at 10-day intervals until 90 days after budbreak. Leaves and buds were sampled from nodes one to 17 and were lyophilized. The samples were then stored at 4C until analysis. Analysis of TNC was conducted using procedures adapted from Davis (1976) and Smith (1981). The samples were ground and 200 mg of each sample was weighed into Erlenmeyer flasks. Samples were then treated with 50 ml of 0.01N hydrochloric acid and heated for 30 minutes at 100C. The samples were then cooled and 20 ml of the 0.5% enzyme solution (5 g of Mylase 100 enzyme dissolved in 1000 ml of distilled water) was added to each sample and incubated for 42 hours. Mylase 100 has rapid saccharogenic activity and no apparent hemicellulolytic or cellulolytic activity (Smith 1981). Mylase hydrolyzes starches and disaccharides to monosaccharides (Smith, 1981). The samples were then tested on a standard Technicon Autoanalyzer and the total nonstructural carbohydrates were determined by the p-hydroxy benzoic acid hydrazide (PAHBAH) method of Ferraro et al. (1976).

Statistical Analysis: In both Somerset and Willowcroft Vineyards, the designs were completely randomized. There were 6 treatments, which consisted of shaded and non-shaded panels, replicated six times in Somerset Vineyard. In Willowcroft Vineyard, there were 2 treatments of shaded and unshaded panels, replicated five times. PPF data were square root transformed, and BN rating data were arcsin-square root transformed before analysis by ANOVA (SAS Institute, 1990). Shoot density data (point quadrat analysis), shoot diameter, average internode length, and TNC data were also compared by ANOVA (SAS Institute, 1990). Mean separation was done using Duncan's procedure. Contrast analysis was done between Shade I vs Control I, Shade II vs Control II, Shade III vs Control III, Shade I vs Shade II, Shade I vs Shade III, and Shade II vs Shade III (treatments at Somerset Vineyard) for the variables, average BN, shoot diameter, and average internode length (SAS Institute, 1990).

Effect of Shade on BN in 'Riesling' Grapevines, 1996

Vineyard: A second shade experiment was conducted at Willowcroft Vineyard in 1996. Shade treatments commenced at 25 (Shade I) and 65 (Shade II) days after budbreak with each lasting 40 days. Shade treatment consisted of neutral black 92% shade cloth suspended over three-vine plots. Each shade treatment was replicated 4 times and compared to an equal number of control plots for each shade period (Control I and Control II). Vines were trained to bilateral cordons as previously described and spur pruned. Shoots were positioned to grow vertically upright.

Canopy point quadrat analyses (Smart et al., 1990) were also done about 100 days after budbreak as previously described. The number of leaf layers, percent gaps, percent fruit clusters and percent interior leaves were calculated. Light measurements (PPF) were also made as previously described, between 1100 and 1400 HR, 30 days after each shade treatment was erected using a LI-COR 1.0 m line quantum sensor and a LI-COR photometer. Average PPF was calculated for each treatment plot. Fruit cluster counts on the vines were made for Shade I and Control I panels after the shade cloth was removed. Net photosynthesis was measured for Shade II and Control II panels using a portable infra-red gas analyzer (ADC LCA2, The Analytical Development Co., Ltd.) with a leaf chamber (ADC PLC-7504). Ten measurements were made for each three-vine plot between 1000 and 1200 HR, 40 days after shade II was applied, at ambient light levels of 1600 to 1900 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Net photosynthetic rate was determined using the formula $P_n = (\Delta CO_2 \cdot F \cdot K) / A$ where

P_n = net photosynthesis in $\mu mol\ CO_2 \cdot m^{-2} \cdot sec^{-1}$

ΔCO_2 = change in CO_2 concentration between interior and exterior of the leaf as ppm by volume $\times 10^{-6}$

F = air flow in $L \cdot sec^{-1}$

K = ((44000mg of CO_2)/22.4) $\times$ (273/294) $\times$ (10^3/44) (an approximate conversion for liter of CO_2 to μmol of CO_2)

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

Two shoots were sampled per panel for carbohydrate analysis as soon as the shade cloth was removed: 66 days after budbreak for Shade I and Control I and 106 days after budbreak for Shade II and Control II panels. Subsequent samplings were made at 86 days after budbreak and 126 days after budbreak for Shade I and 126 days after budbreak for Shade II. The shoots were separated into bud, leaf, and stem tissues. The buds were sampled from nodes 1 through 20. Leaf and stem tissues were sampled from node 5 through 10. The tissues were freeze-dried as previously described and stored at 4C until analysis of carbohydrates. Levels of sucrose, glucose, fructose, and starch were analyzed by HPLC. BN assessment was also done as previously described on 10 shoots per vine on 28 October, 1996.

Statistical Analysis: The design was completely randomized with four treatments consisting of shaded and non-shaded three-vine plots, each replicated four times. The treatments were Shade I and Control I applied 25 days after budbreak and Shade II and Control II applied 65 days after budbreak with each lasting 40 days. PPF data were square root transformed, and BN rating data were arcsin-square root transformed before comparison by ANOVA (SAS Institute, 1990). The

shoot density data, photosynthesis data, and carbohydrate concentration data were also analyzed by ANOVA. Carbohydrate levels at different sampling dates within each organ were compared using the PDIFF option of SAS's GLM procedure.

Effect of Shoot Tipping on 'Riesling' BN, 1996

Vineyard: This experiment was conducted at the Alson H. Smith Agricultural Research and Extension Center, Winchester (AHS AREC) Vineyard in 1996. Six 'Riesling' vines were selected for the experiment. The vines were trained to a Geneva Double Curtain system and were spaced at a distance of 2.1m apart in rows 3.7 m wide. The treatments consisted of shoot-tipped and non-shoot-tipped single vines replicated three times. Budbreak was on 25 April, 1996. The shoot density was standardized to 15 shoots per meter of canopy 15 days after budbreak. All shoots of three vines were tipped 40 days after budbreak. After shoot tip removal the shoots had approximately 17 to 18 nodes. Ten representative shoots, each with two fruit clusters, were selected per vine for assessment of fruit set and BN. Vine measurements consisted of fruit set rating, carbohydrate analysis of stem, leaf and bud tissues, and BN rating.

Fruit Set Rating: As the hypothesis was shoot tip removal would remove a competing sink causing carbon reserves to be diverted to fruit clusters and other vegetative parts, shoot-tipped vines should have greater fruit set than control vines. Therefore, fruit set rating was done by determining the percent of flowers that set fruits (Matthews and Anderson, 1988). Both clusters on selected shoots were bagged with cloth bags (5" W × 8" L) approximately two weeks before anthesis (75% bloom was on 15 June, 1996). Approximately 20 days after full bloom (7 July, 1996) percent fruit set was determined after counting all set berries, shot berries, and aborted ovaries.

Representative shoots were sampled for carbohydrate analysis by HPLC four times at 10-day intervals starting 50 days after budbreak. Stem and leaf tissue from nodes 5 through 13, and buds from all nodes (1-17) were collected for analysis. BN assessment was also performed as previously described in October, 1996.

Statistical Analysis: The design was completely randomized with two treatments, which consisted of shoot-tipped and non-shoot-tipped vines. A single vine formed an experimental unit. The variables of interest, fruit set, percentage of BN, and carbohydrate levels, were analyzed by ANOVA (SAS Institute, 1990). The BN rating data were arcsin-square root transformed before analysis by ANOVA. Mean separation was done using least squares means with the PDIFF option of GLM procedure of SAS (SAS Institute, 1990).

Mineral Nutrient and Carbohydrate Survey of 'Riesling' Grapevines, 1995

Vineyards: 'Riesling' and 'Chardonnay' vines of Prince Michel Vineyard, Virginia and 'Riesling' vines of AHS AREC Vineyard were surveyed for mineral nutrient and carbohydrate levels in bud, leaf, and stem tissues from May 1995 to July 1995. Prince Michel 'Riesling' vines had a history of high BN occurrence (67% BN) while 'Chardonnay' vines annually expressed only 7% BN (Wolf and Warren, 1995) and Winchester 'Riesling' vines expressed 7.3% BN. The vines in both

the vineyards were trained to a bilateral cordon system with vertical shoot positioning. The vines at Prince Michel Vineyard were spaced at a distance of 1.1 m in rows 2.7 m wide. At AHS AREC, Winchester, the row × vine spacing was 3.7 m × 2.1 m. The shoot density in both vineyards was standardized to 15 shoots per meter of canopy 45 days after budbreak. The sampling strategy for tissue analysis was completely randomized and involved three replications of 5 vines each per cultivar ('Riesling' and 'Chardonnay') in Prince Michel Vineyard and four replications of 3 'Riesling' vines each in Winchester. Random sampling of two representative shoots per panel was done at 10-day intervals starting 50 days after budbreak until 90 days after budbreak. The bud, leaf, and stem tissues were frozen separately by submerging in liquid nitrogen in the field. The tissues were then transported to the lab on dry ice and stored in a freezer at -80C. The tissues were then lyophilized and stored at 4C. Nutrient analysis was done on leaf, bud, and stem tissues collected from 'Riesling' and 'Chardonnay' vines of Prince Michel Vineyard by the ICP technique described earlier. Carbohydrate analysis was done on tissues collected from 'Riesling' and 'Chardonnay' vines of Prince Michel Vineyard as well as from 'Riesling' vines of AHS AREC, Winchester by HPLC.

BN assessment was done on both 'Riesling' and 'Chardonnay' from Prince Michel Vineyard and on 'Riesling' from AHS AREC, Winchester at the end of the growing season in October, 1995. Twelve shoots were sampled for BN rating.

Statistical Analysis: Mineral nutrient and carbohydrate data were analyzed by ANOVA (SAS Institute, 1990). Mean separation for mineral nutrients data and carbohydrate concentration data at different sampling dates within each organ was done using least squares means with the PDIFF option of GLM procedure of SAS (SAS Institute, 1990).

Starch Rating of Grape Cultivars, 1996

Vineyard: This study was conducted in AHS AREC, Winchester in 1996. Three BN-prone cultivars, 'Riesling', 'Viognier', and 'Syrah', were selected and compared with the BN-resistant cultivar, 'Chardonnay' for the relative amount of starch in the axillary buds during summer. All vines were at least three years old and were spaced at a distance of 2.1 m in rows 3.7 m wide. The vines were trained to bilateral cordons with vertical shoot positioning. Two representative shoots were sampled randomly from three vines per cultivar at each sample date. The shoots were sampled at intervals of 10 days, starting 50 days after budbreak, until 80 days after budbreak. Buds at nodes 1 through 13 from the base were examined for starch levels. Each shoot was divided into two sections: a basal section of nodes 1 to 6 and an apical section of nodes 7 to 13. A total of twelve buds from each section was examined for starch. Budbreak for 'Riesling', 'Syrah' and 'Viognier' was on 24 April, 1996 and for 'Chardonnay' on 21 April, 1996. But all the cultivars were sampled using 24 April, 1996 as a reference point for budbreak.

Starch staining: Buds of sampled shoots were longitudinally sectioned through the center with a razor. One half of the bud was then stained in iodine-potassium iodide (IKI) solution (0.2 g of iodine dissolved in 100 ml of 2% potassium iodide solution in water) for 1 HR and examined under a dissection microscope at 2X magnification. On interaction with iodine, the amylose part

of starch stains blue and the amylopectin part reddish violet (Hesse and Hitz, 1938). A visual key (Table 4.1) was created based on different intensities of starch stain as well as parts of the buds stained, to enable quantification of starch in the buds with progression of summer, 1996.

A BN assessment was made with all cultivars in October, 1996. Ten shoots per replicate were sampled randomly for each cultivar for BN rating.

Statistical Analysis: The design was completely randomized with four treatments, which consisted of cultivars, replicated three times. A single vine formed the experimental unit. Numerical starch rating data were analyzed by a multivariate repeated measures procedure using the REPEATED statement of SAS's GLM procedure (SAS Institute, 1990). Interactions of sampling date, cultivar, and node position were explored by using the least squares means with the PDIFF option of SAS's GLM procedure. Regression analysis of starch data with BN data of 'Riesling', 'Viognier', 'Syrah', and 'Chardonnay' was performed using PROC GLM procedure of SAS (SAS Institute, 1990).

RESULTS

Shade Experiment, 1994

Three-week periods of shade, applied 20, 40, or 60 days after budbreak at Somerset Vineyard, or at 40 days after budbreak at Willowcroft Vineyard, did not increase BN (Table 4.2). Light measurements of the shade and control plots demonstrated that shading was effective in excluding 92% of PPF in the fruit zone of shaded vines (Table 4.2). Plant canopies were uniform and relatively thin (Smart et al., 1990) at Somerset Vineyard with 2.4 leaf layers, 31.1% interior leaves, 24.4% exterior fruits and 6.9% canopy gaps. Uniform canopy density was observed in Willowcroft Vineyard as well, where canopies averaged 2.0 leaf layers, 26.9% interior leaves, 48.6% exterior fruits, and 10.0% canopy gaps. Contrasts between shade treatments and their respective controls (Table 4.2), as well as between the different shade treatments (data not shown) revealed no significant difference in BN incidence despite the different light levels in shaded and control plots. The percentage of BN incidence, averaged over all treatments, was higher in Somerset Vineyard (29%) than at Willowcroft Vineyard (15%) (Table 4.2). An evaluation of different sections of the tagged shoots revealed a higher incidence of BN for nodes 5 to 16 (25 to 46% at Somerset and 10 to 26% at Willowcroft) compared to the basal four nodes, which had a very low rate of BN incidence (11% at Somerset and 3% at Willowcroft) (Table 4.3).

Regression analysis revealed a weak but positive correlation between BN and shoot vigor in both vineyards, where "vigor" was expressed as a function of shoot diameter (Fig. 4.1), or average internode length (data not shown).

Table 4.1. Visual key for iodine-potassium iodide starch staining rating

Analysis of total nonstructural carbohydrates (TNC) in bud tissues of 'Riesling' vines at Somerset Vineyard revealed no significant difference between shade (6.8% dry weight) and control (6.6% dry weight) panels (Table 4.4). TNC levels in leaf tissues did not reveal any significant difference either (Table 4.4). TNC level was lower at 40 days after budbreak in the bud (3.1%) and leaf (3.0%) then increased at 50 days after budbreak before leveling off (Table 4.4). At Willowcroft Vineyard, TNC levels did not reveal any significant difference between shade and control plots in bud or leaf tissues. Here, the TNC level was higher at 50 and 60 days after budbreak than at 70 and 80 days after budbreak (Table 4.5). Leaf tissues showed a different trend. The level of TNC was highest at 60 days after budbreak followed by a decrease at subsequent sampling intervals (Table 4.5).

Shade Experiment, 1996

Forty-day periods of shade, applied either at 25 or 65 days after budbreak, did not increase BN at Willowcroft Vineyards in 1996 (Table 4.6). Light measurements of shade and control panels again demonstrated that shading was effective in excluding 92% of PPF (Table 4.6). Canopy density was also uniform.2 leaf layers, 27.8% interior leaves, 28.2% exterior fruits, 11.5% canopy gaps (Table 4.6). Fruit cluster number was much lower in Shade I compared to Control I (Table 4.6). Yield of Shade II and Control II panels could not be measured because of very high bird and deer depredation. Net photosynthetic rate was significantly higher for Control II panels (11.8 $\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$), compared to Shade II panels (3.3 $\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$) (Table 4.6). Contrasts between shade and control treatments, as well as between the shade treatments (data not shown) did not reveal any significant increase in BN in shaded panels despite the different light levels (Table 4.6).

An evaluation of BN at different node sections revealed a higher incidence of BN at nodes 5 to 20 compared to the basal four nodes (Fig. 4.2). There was no significant difference between shaded and control panels in the first two node sections (1-4 and 5-8). Shade I increased BN in nodes distal to 9 while shade II increased BN in the last two node sections, 13-16 and 17-20 than did control 'Riesling' vines (Table 4.6).

Carbohydrate analyses of bud, leaf, and stem tissues revealed that sucrose, glucose, fructose, and starch levels were significantly lower immediately after the shade was removed in the shaded panels in bud, leaf, and stem tissues, compared to the control panels (Table 4.7). However, at subsequent samplings in shade I, the levels of carbohydrates were comparable between shade and control panels which was due to an increase in sugar levels in buds from shaded vines. In shade II, even though the levels of sucrose, glucose, fructose, and starch increased at the subsequent sampling, the levels of carbohydrates were significantly lower than in unshaded vine tissues. In the control vines, the levels of sucrose, glucose, and fructose in bud, leaf, and stem tissues decreased steadily. Starch levels increased in all the treatments (Table 4.7).

Table 4.2. Effect of different timings of artificial shade on bud necrosis (BN) incidence in 'Riesling' at Somerset and Willowcroft Vineyards, 1994.

	Point quadrat analysis							
Treatments	**Leaf layers**	**Interior leaves (%)**	**Exterior fruits (%)**	**Gaps (%)**	**PPF[z] (%)**	**Shoot diameter (mm)**	**Shoot internode length (cm)**	**BN[y] (%)**
Somerset Vineyard								
Shade I	1.8	18.8	16.7	14.5	2.4	7.1	7.0	20.0
Control I	2.2	28.5	44.5	6.8	37.0	7.6	5.5	34.5
Shade II	3.0	41.7	23.0	5.5	2.0	6.5	5.2	31.0
Control II	2.3	30.0	24.7	4.5	10.8	7.1	5.3	32.0
Shade III	2.5	33.2	28.3	4.5	0.4	6.6	5.3	14.8
Control III	2.6	34.2	9.0	5.8	3.8	6.8	5.1	20.9
Contrasts[x]								
Shade I vs Control I	ns	ns	ns	ns	**	ns	ns	ns
Shade II vs Control II	ns	ns	ns	ns	**	ns	ns	ns
Shade III vs Control III	ns	ns	ns	ns	**	ns	ns	ns
Willowcroft Vineyard								
Shade	2.0	26.0	44.8	10.6	1.3	6.7	6.0	12.9
Control	2.0	27.8	52.4	9.4	5.1	7.0	6.0	16.7
Significance[x]	ns	ns	ns	ns	*	ns	ns	ns

[z]Photosynthetic photon flux measured at ambient levels of 1400 to 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ is the percentage of the ambient within the canopy and was transformed by the square root method

[y]Bud necrosis percentage data were transformed by arcsin-square root method before analysis

[x]ns, *, ** are nonsignificant, significant at $P<0.01$, $P<0.001$, respectively

Shade I, Shade II, Shade III with the respective controls (Control I, Control II, Control III) were applied 20, 40, and 60 days after budbreak for a 3-week period in Somerset Vineyard.

Shade and Control treatments in Willowcroft Vineyard were applied 40 days after budbreak for a 3-week period.

Table 4.3. Effect of node position on bud necrosis (BN) incidence in 'Riesling' grapevines at Somerset and Willowcroft Vineyards, 1994.

	BN (%)					
	Node position[x]					
Treatments	**1 - 4**	**5 - 8**	**9 - 12**	**13 - 16**	**17 - 20**	**Significance[y,z]**
Somerset Vineyard						
Shade I	8.8	11.9	31.8	53.9	56.3	*
Control I	12.1	32.0	50.1	53.1	79.7	*
Shade II	15.9	37.1	41.8	49.5	54.3	*
Control II	14.2	34.6	44.7	55.5	61.2	*
Shade III	6.7	13.4	21.2	24.1	57.3	*
Control III	10.2	23.3	33.4	42.3	56.8	*
Significance[w,y]	ns	ns	ns	ns	ns	
Willowcroft Vineyard						
Shade	2.2	4.3	28.9	20.1	41.7	*
Control	3.1	12.0	25.0	32.4	16.0	*
Significance[w,y]	ns	ns	ns	ns	ns	

[z]Analysis was done using repeated measures analysis of variance.

[y]ns, * are nonsignificant and significant at P<0.001 respectively

[x]Bud necrosis percentage is expressed in each section of the shoot defined by the node position. Bud necrosis percentage data were transformed by arcsin-square root method before analysis.

[w]Data was analyzed by the analysis of variance procedure.

Shade I, Shade II, Shade III with the respective controls (Control I, Control II, Control III) were applied 20, 40, and 60 days after budbreak for a 3-week period in Somerset Vineyard.

Shade and Control treatments in Willowcroft Vineyard were applied 40 days after budbreak for a 3-week period.

Table 4.4. Total nonstructural carbohydrates level in bud and leaf tissues of 'Riesling' grapevines at Somerset Vineyard, 1994.

	Total Nonstructural Carbohydrates (%)						Total Nonstructural Carbohydrates (%)					
	Bud						Leaf					
	Sampling Intervals (days after budbreak)						Sampling Intervals (days after budbreak)					
Treatments[y]	**46**	**56**	**66**	**76**	**86**	**96**	**46**	**56**	**66**	**76**	**86**	**96**
Shade I	3.1d	7.4a	5.8b	5.0b	4.4c	5.0b	6.6d	13.2a	11.0c	12.6b	11.6c	11.1c
Control I	3.0d	6.7a	5.4b	5.4b	4.4c	5.3b	8.7d	10.1bc	9.3c	11.9a	10.4bc	9.4c
Significance[z]	ns	ns	ns	ns	ns	ns	***	***	ns	ns	ns	**

[z]ns, **, ***, are non significant, and significant at P≤0.01, P≤0.001 respectively

[y]Treatment values are means of 72 observations.

Means followed by the same letters are not significant at P=0.05 significance level across sampling intervals using Duncan's method of mean separation.

Shade I and Control I were applied 20 days after budbreak for a 3-week period.

Table 4.5. Total nonstructural carbohydrates level in bud and leaf tissues of 'Riesling' grapevines at Willowcroft Vineyard, 1994.

	Total Nonstructural Carbohydrates (%)				Total Nonstructural Carbohydrates (%)			
	Bud				Leaf			
	Sampling Intervals (days after budbreak)				Sampling Intervals (days after budbreak)			
Treatments[y]	56	66	76	86	56	66	76	86
Shade	4.5a	5.0a	4.1b	3.8b	6.8c	11.0a	9.4b	8.6ab
Control	5.1a	5.3a	4.0b	3.8b	9.0b	10.8a	8.3b	8.1b
Significance[z]	ns	ns	ns	ns	**	ns	ns	ns

[z]ns, ** are non-significant and significant at P≤0.01, respectively

[y]Treatment values are means of 40 observations.

Means followed by the same letters are not significant at P=0.05 significance level across sampling intervals using Duncan's method of mean separation.

Shade and control treatments were applied 40 days after budbreak for a 3-week period.

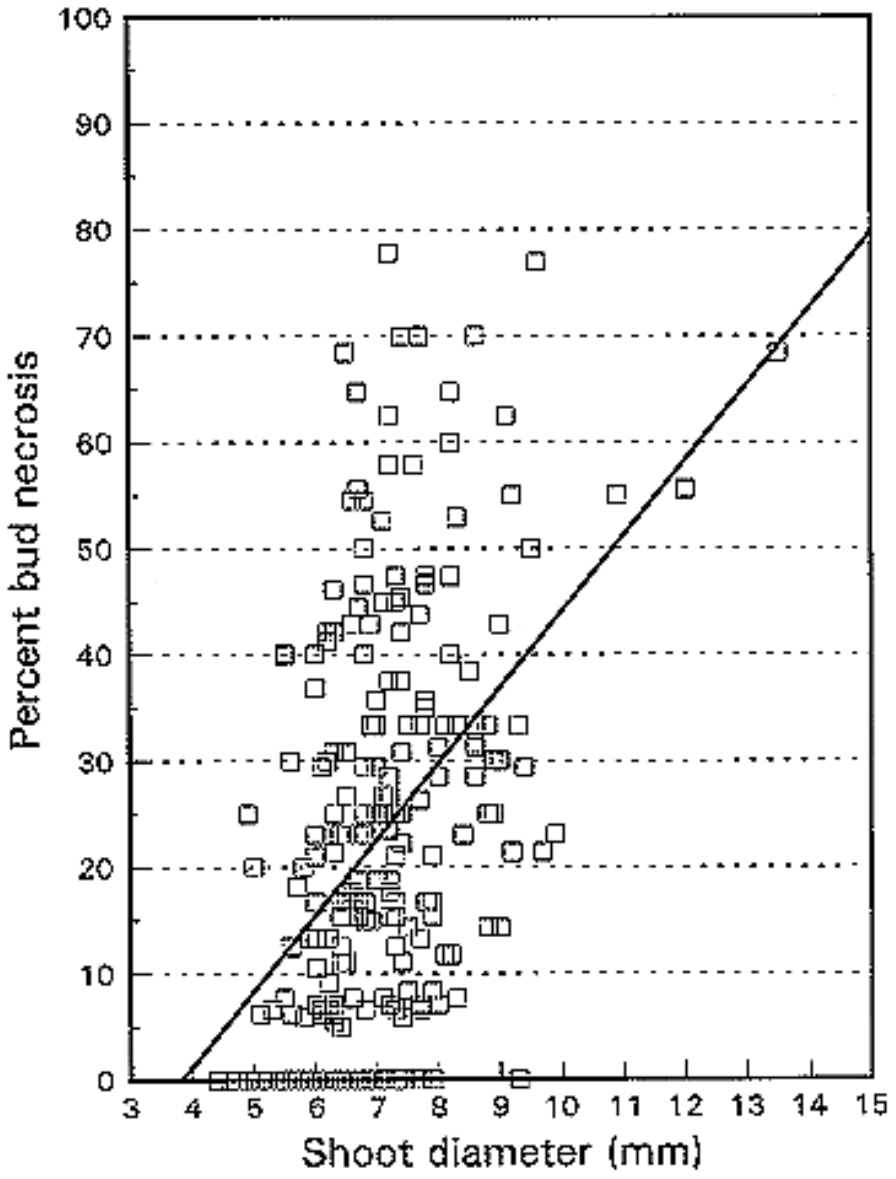


Figure 4.1. Relationship between shoot diameter (measured at the widest point of the second basal internode) and bud necrosis (BN) incidence in 'Riesling' at Somerset Vineyard. Bud necrosis is the mean incidence among nodes 1 - 20 of 120 shoots, pooled over all treatments. Linear regression slope (y= -27.6 + 7.6x; r^2 = 0.20) was significant at P < 0.001.

Table 4.6. Effect of shade at different timings on bud necrosis (BN) in 'Riesling' during 1996

	Point quadrat analysis							
Treatments	Leaf layer	Interior leaves (%)	Exterior fruits (%)	Gaps (%)	PPF[y] (%)	Number of clusters[x]	Pn[w] (μmol $CO_2 \cdot m^{-2} \cdot sec^{-1}$)	BN[v] (%)
Shade I	2.2	28.8	33.3	12.7	0.7	1.3	-	43.1
Control I	2.0	28.8	0.0	13.9	2.9	36.5	-	38.9
Significance[z]	ns	ns	ns	ns	**	***	NA	ns
Shade II	2.2	28.3	29.2	8.3	0.2	-	3.3	55.1
Control II	2.3	25.3	50.0	11.1	1.3	-	11.8	40.9
Significance[z]	ns	ns	ns	ns	**	NA	**	ns

, *, ns indicate significance at P = 0.05, 0.01, 0.001 and nonsignificance, respectively

[z]Contrasts were used to compare Shade I with Control I and Shade II with Control II

[y]Photosynthetic photon flux (PPF) percentage of ambient PPF was measured in canopy fruit zones. PPF percentage data was square root transformed for analysis but are shown untransformed. Ambient PPF values ranged from 1600 to 1900 $\mu mol \cdot m^{-2} \cdot s^{-1}$ measured during 1100 HR to 1400 HR.

[x]Cluster counts were taken on Shade I and Control I just before the shading period ended

[w]Photosynthesis measurements were taken on Shade II and Control II plots and net photosynthetic rate (Pn) was determined.

[v]BN percentage data were arcsin-square root transformed for analysis but are shown untransformed here.

NA not applicable.

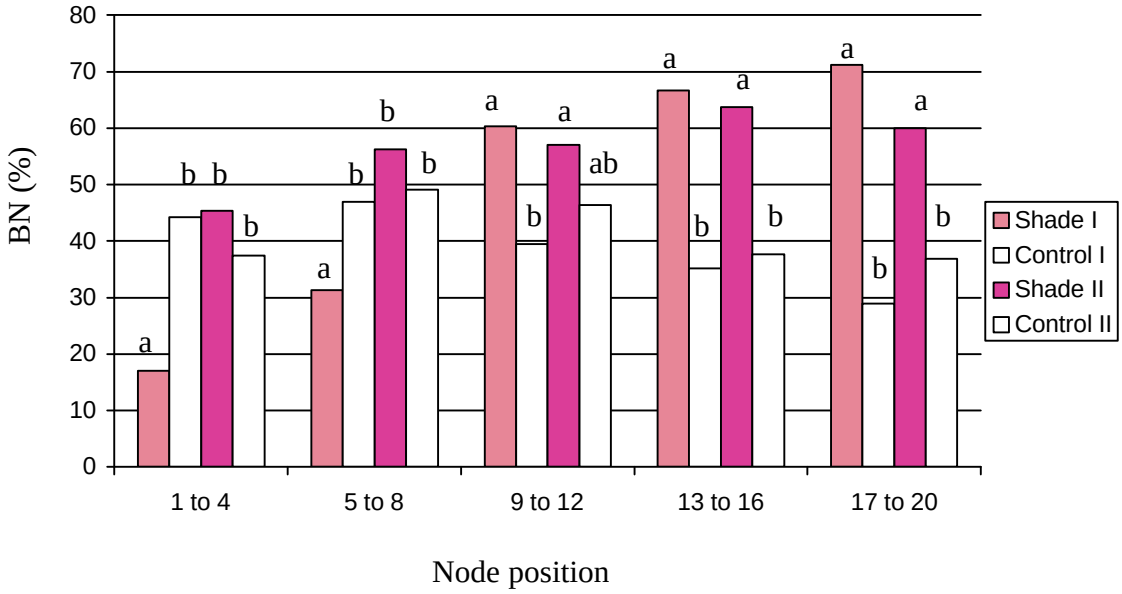


Figure 4.2. Effect of node position on bud necrosis (BN) in shaded and non-shaded 'Riesling' vines at Willowcroft Vineyard during 1996. Bars indicated by same letters are not significantly different at P = 0.05; mean separation done using least squares means with PDIFF option of GLM procedure of SAS.

Table 4.7. Carbohydrate analysis of shaded and unshaded 'Riesling' vines at Willowcroft Vineyard, Virginia during 1996.

				Carbohydrates ($mg \cdot g^{-1}$ of dried plant tissue)[x]			
Shade period	Organ	Treatment[z]	Sampling stage[y] (days)	Sucrose	Glucose	Fructose	Starch
I	Bud	Shade	66	0.20b	0.29c	0.45c	0.006c
(25 days			86	0.28a	0.36b	0.56b	0.012b
after			126	0.27a	0.15d	0.09d	0.016a
budbreak)		Control	66	0.28a	0.69a	0.84a	0.011b
			86	0.28a	0.44b	0.60b	0.012b
			126	0.25a	0.17d	0.09d	0.019a
	Leaf	Shade	66	0.35b	0.31c	0.53c	0.076b
			86	0.84a	0.71a	0.63b	0.079b
			126	0.87a	0.47c	0.59bc	0.082a
		Control	66	0.99a	0.77a	0.97a	0.107a
			86	1.12a	0.63a	0.79b	0.112a
			126	1.04a	0.52b	0.52c	0.113a
	Stem	Shade	66	0.74c	0.79c	0.29c	0.006c
			86	1.02a	1.06b	0.34b	0.009c
			126	0.79c	0.83c	0.19d	0.013b
		Control	66	1.03a	1.21a	0.44ab	0.020b
			86	0.99b	1.03b	0.53a	0.019b
			126	0.86bc	0.97b	0.22d	0.034a
II	Bud	Shade	106	0.26b	0.07a	0.15a	0.007c
(65 days			126	0.27b	0.06a	0.08b	0.011b
after		Control	106	0.33a	0.10a	0.10a	0.014b
budbreak)			126	0.36a	0.07a	0.10a	0.021a
	Leaf	Shade	106	0.62c	0.37b	0.46c	0.057d
			126	0.99b	0.43b	0.44c	0.072c
		Control	106	0.96b	0.53a	0.82a	0.101b
			126	1.23a	0.50a	0.55b	0.119a
	Stem	Shade	106	0.32c	0.30c	0.25b	0.007c
			126	0.39c	0.49b	0.26b	0.011c
		Control	106	0.78a	0.95a	0.37a	0.026b
			126	0.57b	0.83a	0.22b	0.036a

Table 4.7. contd.

[z]Treatments were analyzed by Analysis of Variance using the PROC GLM procedure of SAS and equality of treatments was tested within each plant organ.

[y]Sampling intervals were measured as number of days after budbreak.

[x]Means are averages of three observations and means followed by same letters are not significant at P=0.05. The mean separation was done using adjusted with the PDIFF option of SAS. The mean separation was done only within each treatment across the sampling intervals.

Shoot Tipping Experiment, 1996

The hypothesis of this study was that shoot tipping would divert more photosynthates to the developing buds and fruit clusters, as opposed to translocation to shoot tips, resulting in an increased fruit set and reduced BN in shoot-tipped vines. However, tipping of shoots did not result in any significant effect on percentage of aborted fruits, normal fruit set, or shot berries in the lower or upper clusters (Table 4.8) suggesting that the intended reduction did not occur.

The amount of aborted flowers per lower cluster ranged from 59% to 71%, shot berries from 9% to 10%, and normal berries from 19% to 29% in shoot-tipped vines and the control vines. The upper clusters had 57% to 54% aborted berries, 8% to 11% shot berries, and 31% to 36% normal berries in shoot-tipped vines and controls (Table 4.8). Levels of carbohydrates such as sucrose, glucose, fructose, and starch were also determined in bud, leaf, and stem tissues of shoot-tipped and control vines (Table 4.9). The levels of sucrose, glucose, and fructose decreased from 50 days after budbreak to 80 days after budbreak in bud, stem, and leaf tissues in both shoot-tipped and control vines. Starch, however, showed an increase in all the tissues (Table 4.9). The control vines had significantly higher levels of sucrose than shoot-tipped vines (Table 4.9). A similar trend was observed in leaves and stems of both shoot-tipped and control vines (Table 4.9). However, there was no significant difference in the levels of sucrose, glucose, fructose, and starch between shoot-tipped and control vines.

Statistical analysis of the BN rating data revealed that there was no significant difference between shoot-tipped and control vines (Table 4.8). No statistically significant difference was revealed in BN incidence between shoot-tipped vines (47%) and control vines (33%). Statistical analysis of average BN for each node section revealed a significant difference between shoot-tipped vines and controls at nodes 13-16 and nodes 17-20 (Fig. 4.3). Shoot-tipped vines had lower BN at both section 13-16 and section 17-20 (58%, 50%, respectively) than in controls (22%, 16%, respectively).

Table 4.8. Bud necrosis (BN) and fruit set measurements of shoot-tipped and non-shoot-tipped 'Riesling' vines during 1996.

Treatments	Aborted flowers (%)		Shot berries (%)		Normal fruit set (%)		
	Lower cluster	Upper cluster	Lower cluster	Upper cluster	Lower cluster	Upper cluster	BN (%)[y]
Shoot-tipped	71.3	57.3	9.1	11.1	19.6	31.6	46.6
Control	59.5	54.8	10.9	8.5	29.6	36.7	32.8
Significance[z]	ns	ns	ns	ns	ns	ns	ns

[z]Treatments were analyzed by Analysis of Variance using the PROC GLM procedure of SAS and equality of treatments was tested. ns indicates non-significance

[y]BN is the bud necrosis rating done at the end of the season in October, 1996. BN assessment was done from nodes 1-20 and the average BN was determined. The data was transformed by arsin square root transformation method before analysis. The data presented here are the untransformed values.

Table 4.9. Carbohydrate analysis of shoot-tipped and non-shoot-tipped 'Riesling' vines at AHS Agricultural Research and Extension Center, Winchester, VA during 1996.

			Carbohydrates ($mg \cdot g^{-1}$ of dried plant tissue)[x]			
Organ	Treatment	Sampling stage[y] (days)	Sucrose	Glucose	Fructose	Starch
Bud	Shoot-tipped	50	0.07a	0.36b	0.49a	0.015b
		60	0.06a	0.45a	0.35ab	0.020b
		70	0.03b	0.29c	0.23b	0.031a
		80	0.03b	0.29c	0.37ab	0.0361a
	Control	50	0.16a	0.83a	0.87a	0.021b
		60	0.13a	0.61b	0.81a	0.026b
		70	0.09a	0.64b	0.73ab	0.039a
		80	0.07a	0.73b	0.67b	0.040a
	Significance[z]		**	***	*	*
Leaf	Shoot-tipped	50	1.40a	0.86a	0.52b	0.031b
		60	1.15ab	0.78a	0.58b	0.040b
		70	0.86b	0.94a	0.74a	0.047ab
		80	0.75b	0.74a	0.46b	0.054a
	Control	50	1.50a	0.83a	0.47b	0.043b
		60	1.33a	0.86a	0.89a	0.042b
		70	0.99b	0.99a	0.54b	0.057a
		80	0.82b	0.59a	0.40b	0.056a
	Significance[z]		ns	ns	ns	ns
Stem	Shoot-tipped	50	1.39a	1.69a	0.29a	0.038b
		60	0.94b	1.31b	0.34a	0.040b
		70	1.07b	1.32b	0.60a	0.057a
		80	1.04b	1.09b	0.30a	0.061a
	Control	50	1.13a	2.02a	0.36a	0.038b
		60	1.01a	1.92a	0.64a	0.042b
		70	0.79a	0.79b	0.45a	0.590a
		80	0.80a	0.90b	0.63a	0.063a
	Significance[z]		ns	ns	ns	ns

Table 4.9. contd.

[z]Treatments were analyzed by Analysis of Variance using the PROC GLM procedure of SAS and equality of treatments was tested within each plant organ

[y]Sampling stage was measured as number of days after budbreak

[x]Means are averages of three observations and means followed by same letters are not significant at P=0.05. The mean separation was done by using adjusted means with the PDIFF option of SAS. The mean separation was done only within each treatment across the sampling intervals.

*, **, ***, ns indicate significance at P=0.05, 0.01, 0.001 and non significance respectively

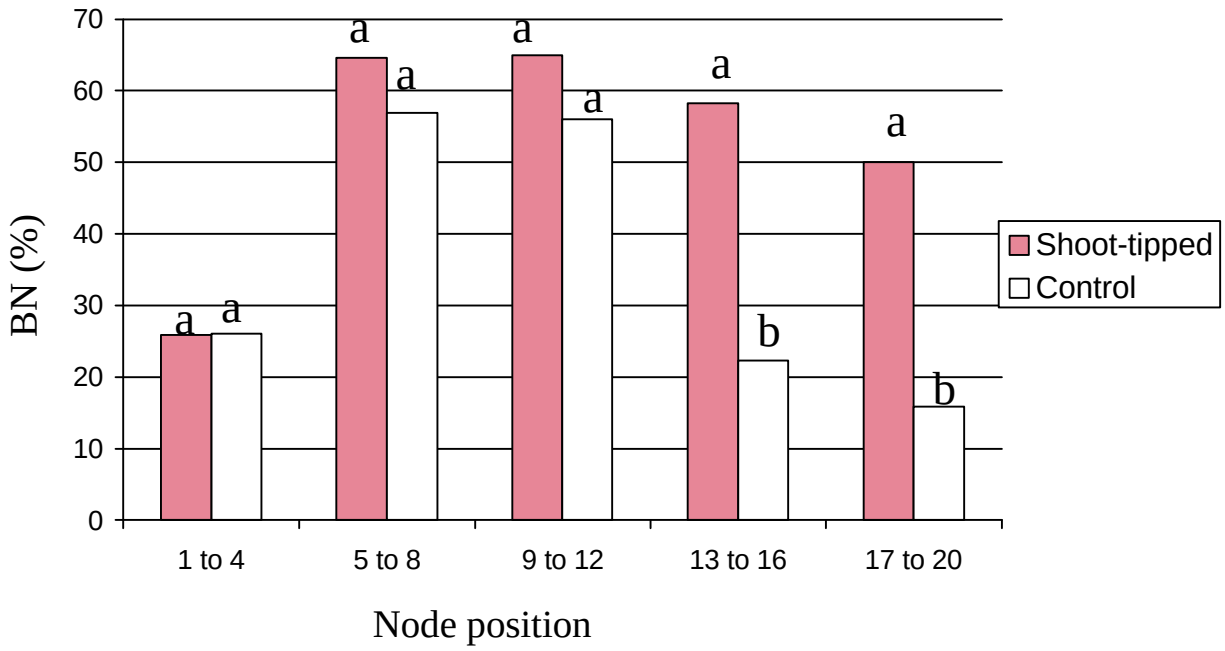


Figure 4.3. Effect of node position on bud necrosis (BN) in shoot-tipped and non-shoot-tipped 'Riesling' vines during 1996. Bars indicated by same letters are not significantly different at P = 0.05; mean separation done using least squares means with PDIFF option of GLM procedure of SAS.

Mineral Nutrient and Carbohydrate Survey, 1995

Mineral Nutrient Survey: Average BN was higher for 'Riesling' (44%) than 'Chardonnay' (15%) (Table 4.10). However, the level of nutrients did not show any difference between cultivars (Table 4.10). Calcium was higher in 'Riesling' compared to 'Chardonnay' bud tissue. The levels of magnesium, potassium, boron, and manganese did not show any difference between the two cultivars. Phosphorus was, however, higher in 'Riesling' than 'Chardonnay' in stem, leaf, and bud tissues. The level of nutrients was also compared at 10-day intervals. Calcium in all the tissues, and potassium and boron in leaf and stem tissues in both 'Riesling' and 'Chardonnay' showed very little difference among the sampling dates. The other mineral nutrients did show a difference over the sampling dates. However, there was no definite pattern followed in the two cultivars. Generally, the level of nutrients increased in leaves and remained constant in stem tissue from 70 to 90 days after budbreak. All the nutrients decreased in bud tissues over time with the exception of manganese. The level of manganese decreased from the first sampling at 50 days after budbreak but increased at 90 days after budbreak (Table 4.10).

Carbohydrate Survey: The level of monosaccharides such as glucose and fructose and the disaccharide sucrose decreased at each sampling, starting at 50 days after budbreak until 90 days after budbreak (Table 4.11). The level of starch, however, increased with each sampling interval. In the bud tissues of 'Riesling' vines of Prince Michel Vineyard, there was a decrease in the levels of sucrose and fructose, and an increase in starch, but no change in glucose was observed (Table 4.11). In 'Chardonnay' of Prince Michel Vineyard, there was a decrease in sucrose, glucose, and fructose, and an increase in starch (Table 4.11). In 'Riesling' of AHS AREC, Winchester, there was a decrease in sucrose and glucose, and an increase in starch, but no change in fructose levels was observed as the summer progressed (Table 4.11). A similar trend was observed in leaves (Table 4.12) and stems (Table 4.13) for both cultivars. The levels of carbohydrates were higher in leaves and stems than in buds at any given sampling stage. Upon examination of buds, leaves, and stems it was observed that Prince Michel 'Riesling' vines had the lowest levels of carbohydrates followed by AHS AREC, Winchester 'Riesling' vines and Prince Michel 'Chardonnay' vines.

Rating for BN at the end of the growing season in October revealed a higher incidence of BN in Prince Michel 'Riesling' (44%) compared to 'Chardonnay' (15%) vines. Prince Michel 'Chardonnay' had a lower percentage of BN at all nodes compared to 'Riesling' vines. Winchester (50%) and Prince Michel (44%) 'Riesling' vines had comparable BN incidence (Table 4.11, Table 4.12, Table 4.13).

Table 4.10. Concentrations of mineral elements in 'Riesling' and 'Chardonnay' cultivars sampled from Prince Michel Vineyard during 1995.

				Mineral nutrients ($mg \cdot g^{-1}$ of dried tissue)[z]						
Cultivar	BN (%)[w]	Organ	Sampling stage[yx] (days)	Ca	Mg	K	Ca+Mg / K	B	Mn	P
Riesling	44	leaf	50	9.45a	1.99ab	7.15a	1.62a	0.03a	0.23bc	2.86a
			60	9.17a	1.90b	7.86a	1.57a	0.03a	0.36b	2.08b
			70	10.25a	2.10ab	6.38a	1.92a	0.02a	0.25bc	1.50c
			80	9.14a	2.06ab	6.02a	1.84a	0.02a	0.21c	1.39c
			90	12.04a	2.56a	9.49a	1.60a	0.03a	0.50a	1.49c
		stem	70	4.01a	0.86a	7.03a	0.70a	0.02a	0.03b	1.45a
			80	3.37a	0.62b	6.48a	0.62a	0.02a	0.02b	1.33a
			90	5.20a	0.84a	7.19a	0.84a	0.02a	0.05a	1.51a
		bud	50	12.38a	3.27a	26.27a	0.61b	0.07a	0.15ab	4.10a
			60	10.38a	2.32b	18.75b	0.72ab	0.04b	0.16ab	2.96b
			70	10.61a	2.05b	13.13b	0.98ab	0.03b	0.10b	2.53b
			80	12.44a	2.09b	15.71b	0.97ab	0.04b	0.11b	2.49b
			90	11.44a	2.41b	12.81b	1.21a	0.04b	0.19a	2.22b

Table 4.10. contd.

Cultivar	BN (%)[w]	Organ	Sampling stage[yx] (days)	Mineral nutrients (mg·g^{-1} of dried tissue)[z] Ca	Mg	K	Ca+Mg / K	B	Mn	P
Chardonnay	15	leaf	50	9.57a	2.17bc	7.50a	1.63a	0.03a	0.33bc	1.92a
			60	12.09a	2.54ab	8.44a	1.78a	0.03a	0.53a	1.97a
			70	13.36a	2.93a	7.50a	2.26a	0.03a	0.47ab	1.60ab
			80	9.25a	1.85c	5.96a	1.98a	0.02a	0.27c	1.24b
			90	13.25a	3.00a	7.03a	2.42a	0.03a	0.61a	1.23b
		stem	70	6.09a	1.27a	6.13a	1.24a	0.02a	0.10a	1.10a
			80	5.41a	1.32a	6.41a	1.10a	0.02a	0.05a	1.06a
			90	6.38a	1.43a	8.84a	0.95a	0.02a	0.10a	0.81a
		bud	50	10.92a	2.88a	26.83a	0.53b	0.07a	0.18ab	2.66a
			60	10.93a	2.93a	19.65ab	0.74b	0.04b	0.19a	2.13ab
			70	9.60a	2.69a	19.12ab	0.64b	0.04b	0.15bc	1.85bc
			80	9.83a	2.61a	11.60b	1.20a	0.03b	0.13c	1.64bc
			90	9.18a	2.47a	15.40b	0.77b	0.03b	0.19a	1.35c

[z]Means of 3 panel replicates

[y]Sampling intervals were measured as number of days after budbreak

[x]The sampling intervals within each plant organ were analyzed by the PROC GLM procedure and REPEATED MEASURES of SAS. Means in columns followed by the same letters are not significantly different at 5% significance level using Duncan's mean separation method within each plant organ across sampling intervals.

[w]BN (Average bud necrosis) is the mean of 12 observations. BN assessment was done from nodes 1-20 at the end of the growing season in October, 1995 and the average BN was determined. The data was transformed by arsin square root transformation method before analysis. The data presented here are the untransformed values.

Table 4.11. Carbohydrate level in bud tissues of 'Riesling' and 'Chardonnay' of Prince Michel Vineyard and AHS Agricultural Research and Extension Station, Winchester during 1995.

Vineyard	Cultivar	BN[x] (%)	Sampling stage[z] (days)	Carbohydrates[y] ($mg \cdot g^{-1}$ dried tissue)			
				Sucrose	Glucose	Fructose	Starch
Prince Michel	Riesling	44	50	0.40a	0.17a	0.15a	0.014b
			60	0.43a	0.16a	0.12a	0.013b
			70	0.24b	0.15a	0.03b	0.017b
			80	0.24b	0.15a	0.05b	0.021a
			90	0.14c	0.14a	0.04b	0.022a
	Chardonnay	15	50	0.57a	0.40a	0.03a	0.034a
			60	0.55a	0.39a	0.06a	0.030a
			70	0.44b	0.37ab	0.06a	0.043a
			80	0.33c	0.35ab	0.04a	0.044a
			90	0.28c	0.33b	0.01a	0.044a
Winchester	Riesling	50	50	0.34a	0.31a	0.11a	0.026b
			60	0.30a	0.31a	0.13a	0.029b
			70	0.29a	0.28ab	0.11a	0.044a
			80	0.28b	0.24bc	0.12a	0.045a
			90	0.26b	0.23c	0.10a	0.041a

[z]Sampling stage measured in days after budbreak.

Means in columns followed by the same letter are not significantly different at 5% level; mean separation done using least squares means across sampling intervals by cultivar.

[y]Means of 4 single panel replicates.

[x]BN is the mean of 12 observations. BN assessment was done from nodes 1-20 at the end of the growing season in October, 1995 and the average BN was determined. Data were transformed by arcsin-square root transformation method before analysis. The data presented here are the untransformed values.

Table 4.12. Carbohydrate level in leaf tissues of 'Riesling' and 'Chardonnay' of Prince Michel Vineyard and AHS Agricultural Research and Extension Center, Winchester during 1995.

Vineyard	Cultivar	BN[x] (%)	Sampling stage[z] (days)	Carbohydrates[y] ($mg \cdot g^{-1}$ dried tissue)			
				Sucrose	Glucose	Fructose	Starch
Prince Michel	Riesling	44	50	0.45a	0.57a	0.39a	0.080b
			60	0.42a	0.55a	0.32a	0.070b
			70	0.39b	0.41b	0.27a	0.080b
			80	0.39b	0.39b	0.23a	0.099a
			90	0.36b	0.35b	0.16a	0.093a
	Chardonnay	15	50	0.54a	0.18a	0.35a	0.071a
			60	0.52a	0.16a	0.28a	0.054b
			70	0.48a	0.13a	0.27a	0.055b
			80	0.45a	0.17a	0.14b	0.069a
			90	0.45a	0.15a	0.17b	0.073a
Winchester	Riesling	50	50	0.54a	0.52a	0.27a	0.030d
			60	0.50a	0.49a	0.24a	0.053c
			70	0.57a	0.40b	0.19b	0.051c
			80	0.51a	0.37b	0.16b	0.066b
			90	0.55a	0.35b	0.08c	0.075a

[z]Sampling interval measured in days after budbreak.
Means in columns followed by the same letter are not significantly different at 5% level; mean separation done using least squares means across sampling intervals by cultivar.
[y]Means of 4 single panel replicates.
[x]BN is the mean of 12 observations. BN assessment was done from nodes 1-20 at the end of the growing season in October, 1995 and the average BN was determined. Data were transformed by arcsin-square root transformation method before analysis. The data presented here are the untransformed values.

Table 4.13. Carbohydrate level in stem tissues of 'Riesling' and 'Chardonnay' of Prince Michel Vineyard and AHS Agricultural Research and Extension Center, Winchester during 1995.

Vineyard	Cultivar	BN[x] (%)	Sampling stage[z] (days)	Carbohydrates[y] ($mg \cdot g^{-1}$ dried tissue)			
				Sucrose	Glucose	Fructose	Starch
Prince Michel	Riesling	44	70	0.49a	0.44a	0.16a	0.045a
			80	0.30b	0.37ab	0.13a	0.044a
			90	0.24b	0.11b	0.11a	0.041a
	Chardonnay	15	70	0.53a	0.44b	0.20a	0.044b
			80	0.50a	0.80a	0.19a	0.099a
			90	0.36a	0.45b	0.11b	0.098a
Winchester	Riesling	50	70	0.38a	0.43a	0.17a	0.034a
			80	0.39a	0.62a	0.07b	0.042a
			90	0.40a	0.36a	0.06b	0.055a

[z]Sampling interval measured in days after budbreak.
Means in columns followed by the same letter are not significantly different at 5% level; mean separation done using least squares means across sampling intervals by cultivar.
[y]Means of 4 single panel replicates.
[x]BN is the mean of 12 observations. BN assessment was done from nodes 1-20 at the end of the growing season in October, 1995 and the average BN was determined. Data were transformed by arcsin-square root transformation method before analysis. The data presented here are the untransformed values.

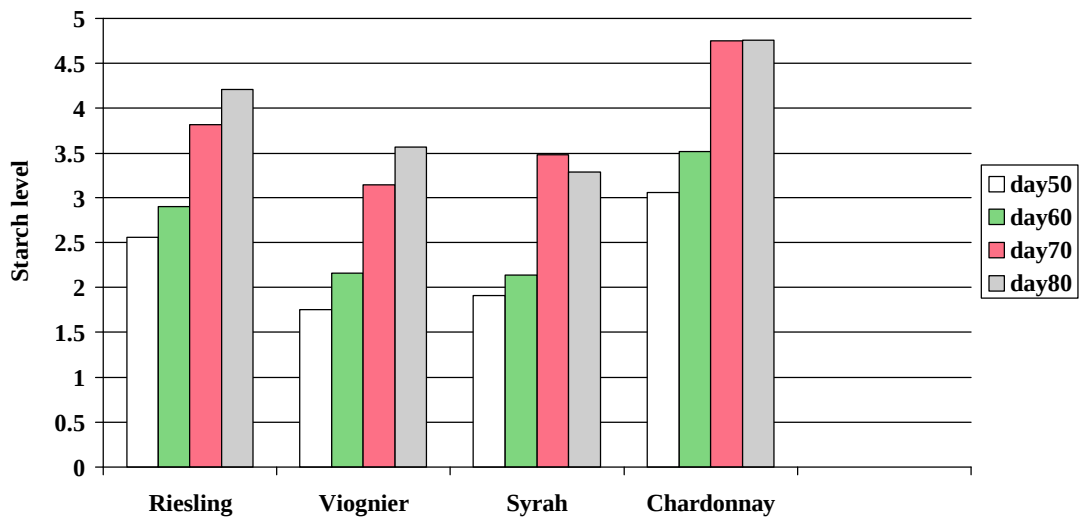
Starch Rating of Grape Cultivars, 1996

With the exception of the last sampling date with 'Chardonnay', bud starch ratings increased in all cultivars with seasonal progress (Fig. 4.4, Fig. 4.5). The greatest apparent starch increase occurred between 60 and 70 days after budbreak, which corresponded to 15 and 25 days after bloom. There was a significant difference in the amount of starch among the four cultivars (Fig. 4.4). At each date 'Chardonnay' had the greatest apparent amount of starch followed by 'Riesling', 'Viognier' and 'Syrah', with the latter two having approximately equal amounts of starch (Fig. 4.4, Fig. 4.5). 'Riesling' buds had significantly lower levels of starch than 'Chardonnay', but higher than 'Viognier' and 'Syrah' buds at comparable sampling dates ($P<0.001$) (Fig. 4.5).

'Viognier' and 'Syrah' showed almost equal ratings at 50 days (1.7 and 1.8, respectively) and 60 days (2.1 and 1.9, respectively) after budbreak. At 70 days after budbreak, 'Viognier' (2.6) had lower levels of starch than 'Syrah' (3.0) ($P<0.01$), while at 80 days after budbreak, 'Syrah' (2.9) had less starch than did 'Viognier' (3.1). Node position had a variable effect within each cultivar (Table 4.14). 'Riesling' revealed greater levels of starch in lower nodes compared to higher nodes at all sampling dates. 'Chardonnay' buds had significantly greater starch levels in the lower section than in the upper nodes only at 50 and 60 days after budbreak. Starch level in the two sections at 70 days and 80 days after budbreak did not show any significant difference in 'Chardonnay'. In 'Riesling' and 'Chardonnay', the basal 6 nodes generally had higher levels of starch than did the more apical nodes. 'Viognier' and 'Syrah', on the other hand, did not reveal any significant node position effect with respect to starch levels (Table 4.14). With the exception of 'Syrah', all cultivars had higher BN at nodes 7-13 compared to nodes 1-6. 'Syrah' did not reveal any significant node position effect on BN incidence. 'Viognier', 'Syrah', and 'Riesling' had higher BN than did 'Chardonnay' (Table 4.14). Regression analysis across all four cultivars revealed a negative correlation between BN and starch deposits in bud tissues ($r^2=0.55$) ($P<0.001$) (Fig. 4.6).

DISCUSSION

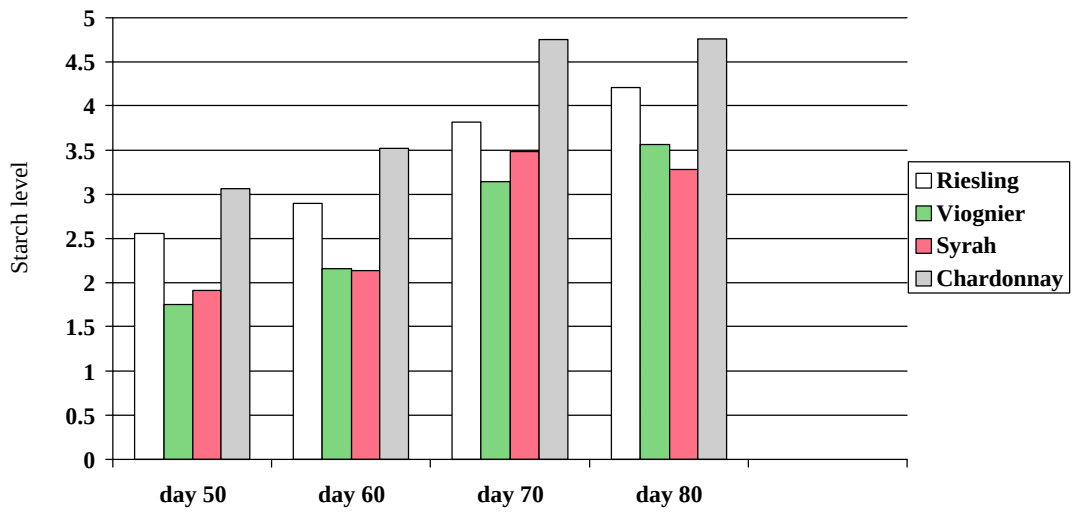
Shade, whether due to mutual leaf shading (Somda et al., 1991), periods of cloudy weather, or artificial, can cause tissue senescence and organ-specific dysfunctions, such as early fruit abscission in apple (Byers et al., 1991), soybean (Jiang and Egli, 1993), and flower bud or flower abscission in pepper (Wien et al., 1989). Research by Perez and Kliewer (1990) with 'Thompson Seedless' grapes in Chile indicated that 15-day periods of artificial shade (52%, 25%, or 14% of ambient PPF) increased BN if applied anytime from bloom until veraison. Shade was suggested as an integral aspect of the BN syndrome (Perez and Kliewer, 1990). Wolf and Warren (1995), however, found no increase in BN of 'Riesling' when either 64% or 92% shade was applied in the three-week period prior to veraison (starting at approximately 85 days after budbreak).



Significance level between sample days (P value):

	'Riesling'	'Viognier'	'Syrah'	'Chardonnay'
50 days vs 60 days	0.0012	0.0001	0.0340	0.0001
50 days vs 70 days	0.0001	0.0001	0.0001	0.0001
50 days vs 80 days	0.0001	0.0001	0.0001	0.0001
60 days vs 70 days	0.0001	0.0001	0.0001	0.0001
60 days vs 80 days	0.0001	0.0001	0.0001	0.0001
70 days vs 80 days	0.0002	0.0001	0.0497	0.8980

Figure 4.4. Comparison of bud starch levels as a function of date from 50 to 80 days after budbreak among four grape cultivars at AHS Agricultural Research and Extension Center, Winchester during 1996.



Significance level between cultivars (P value):

	Day 50	Day 60	Day 70	Day 80
'Riesling' vs 'Viognier'	0.0001	0.0001	0.0001	0.0001
'Riesling' vs 'Syrah'	0.0001	0.0001	0.0019	0.0001
'Riesling' vs 'Chardonnay'	0.0001	0.0001	0.0001	0.0001
'Viognier' vs 'Chardonnay'	0.0001	0.0001	0.0001	0.0001
'Syrah' vs 'Chardonnay'	0.0001	0.0001	0.0001	0.0001
'Viognier' vs 'Syrah'	0.1004	0.8054	0.0008	0.0105

Figure 4.5. Comparison of starch levels among four grape cultivars within each sampling date at AHS Agricultural Research and Extension Center, Winchester during 1996. Sampling was done starting 50 days after budbreak until 80 days after budbreak.

Table 4.14. Effect of node position on starch deposits and bud necrosis (BN) in bud tissues of grape cultivars, 'Riesling', 'Viognier', 'Syrah', and 'Chardonnay' at AHS Agricultural Research and Extension Center, Winchester during 1996.

	Sampling stage (days after budbreak)[y]													
	50			60			70			80			BN[x] (%)		
Cultivar	1-6	7-13	Sig[z]	1-6	7-13	Sig[z]	1-6	7-13	Sig[z]	1-6	7-13	Sig[z]	1-6	7-13	Sig[z]
Riesling	2.8	2.3	***	3.4	2.5	***	4.1	3.6	***	4.4	4.0	**	35.0	55.7	**
Viognier	1.9	1.6	*	2.2	2.1	ns	3.2	3.1	ns	3.7	3.5	ns	67.9	76.4	*
Syrah	1.9	1.9	ns	2.0	2.3	ns	3.7	3.3	*	3.5	3.1	*	58.9	61.7	ns
Chardonnay	3.2	2.9	*	3.4	3.7	*	4.9	4.6	ns	4.6	4.9	ns	1.7	7.2	*

ns, *, **, *** nonsignificant or significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively.

[z]Starch data analyzed by ANOVA and means within each node position indicated by 1-6 and 7-13, was tested by least squares means using PDIFF option of SAS.

[y]Starch rating data are means of 36 observations.

[x]BN assessment was done at the end of the growing season in October, 1996. BN is the average of 30 observations.

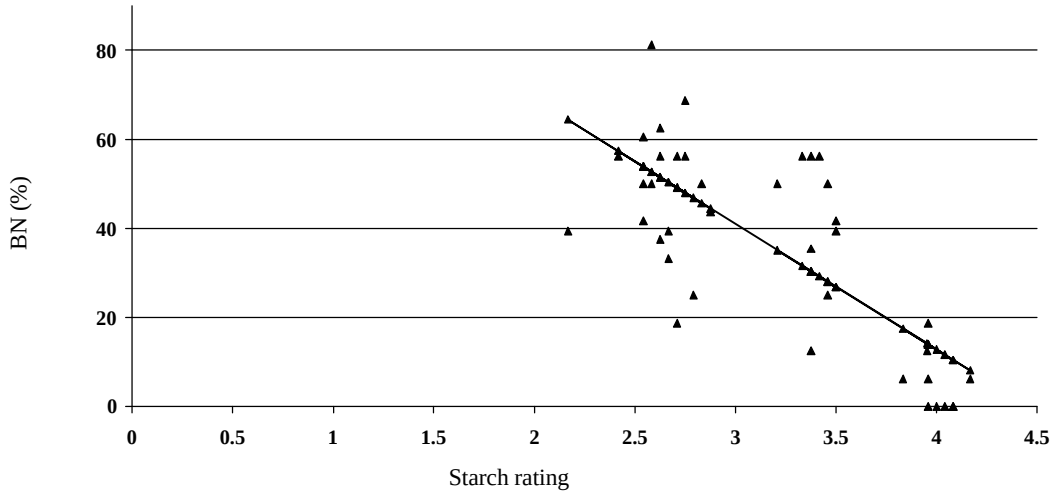


Figure 4.6. Correlation of BN with starch deposits in bud tissues of 'Riesling', 'Viognier', 'Syrah', and 'Chardonnay' at AHS Agricultural Research and Extension Center, Winchester, VA. Bud necrosis is the mean incidence among nodes 1-20 of 40 shoots pooled over all cultivars. Linear regression slope ($y = 125.46 - 28.16x$; $r^2 = 0.55$) was significant at $P<0.001$.

Anatomical studies (Chapter 3) have revealed that the earliest, visual evidence of BN in 'Riesling' buds is observed soon after bloom. Therefore, shade effects early in the bud development period could not be ruled out. The current study has shown that artificial shade applied at 20, 40, or 60 days after budbreak for a 3-week period did not increase BN of 'Riesling' grapevines in two vineyards. But shade applied at 25 or 65 days after budbreak for a 40-day period increased BN in 'Riesling' vines at the distal nodes than in the basal 8 nodes. Shade applied 25 days after budbreak increased BN in nodes distal to 8 and shade applied at 65 days after budbreak increased BN in nodes distal to 12 than in the basal nodes. Therefore, although a 21-day shading period was not long enough to produce a lasting effect on the buds, a season-long shade, which may occur with excessively dense canopies, may increase BN frequency. A higher frequency of BN was observed in nodes 5 to 16 than in the basal four nodes of current season shoots in all treatments (Table 4.3). Some studies have reported that the highest percentage of BN occurred among the basal buds (Dry and Coombe, 1994; Lavee et al., 1981; Morrison and Iodi, 1990; Perez and Kliewer, 1990). But in 'Kyoho' (Naito et al., 1989) and 'Riesling' (Wolf and Warren, 1995), a higher frequency of BN was observed in the more distal nodes, than in the basal nodes. The lower frequency of BN in the basal nodes of 'Riesling' does permit some yield compensation when affected vines are cordon-trained and spur-pruned.

Shoot diameter and average internode length showed positive but weak correlations with BN incidence. Positive correlations between similar growth parameters and BN incidence have also been reported by others (Dry and Coombe, 1994; Lavee et al., 1981; Naito et al., 1989; Wolf and Warren, 1995). In the cultivars, 'Shiraz' and 'Queen of Vineyard', shoots greater than 10 mm in diameter had a higher incidence of BN than did smaller shoots (<10 mm) (Dry and Coombe, 1994; Lavee et al., 1981). Wolf and Warren (1995) reported a positive relationship between BN and cane diameter, specific growth rate, and internode length. While a positive correlation between vigor and BN appears common to several BN studies, the establishment of a cause and effect relationship involving growth processes remains elusive.

One hypothesis tested here was that rapid growth led to carbohydrate or nutrient deprivation in buds and that, in turn, led to the specific visual symptoms associated with BN. Morrison and Iodi (1990) observed lack of starch grains in necrotic buds but they questioned whether it was the causal factor or effect of necrosis. Shade experiment conducted in 1996 revealed that shaded panels had a lower rate of photosynthesis compared to control vines (Table 4.6). Therefore, shaded panels should experience a reduction in carbohydrate levels in bud and leaf tissues. In 1994, TNC analysis of leaf and bud tissues of shaded and control panels did not reveal any significant difference between the treatments. Grapevines generally, mobilize carbohydrate reserves from trunk, arms and roots (Candolfi-Vasconcelos and Koblet, 1990; Candolfi-Vasconcelos et al., 1994) to the shoots during stress conditions. Therefore, in this situation enough carbon reserves might have been translocated, so that no significant difference was observed in TNC level. Another reason for observation of a lack of shade effect in the shade experiments of 1994 might have been contributed by the comparison of TNCs which are the sum total of reducing, non-reducing sugars, and starch. Levels of enough type of sugars might have been left unaltered in the shaded panels for the total amount of TNC to not show any significant

difference. For instance, Naito et al. (1987) observed higher levels of starch in the strong shoots which were more prone to BN than the weak shoots and an opposite trend was observed for total sugars and reducing sugars. Naito et al. (1987) observed no significant difference between total carbohydrate levels. Therefore, in 1996, the second shade experiment was conducted in order to measure the levels of the individual sugars at different sampling dates and to determine the trend of the sugar concentrations in bud, leaf, and stem tissues as the summer progressed. Sucrose, glucose, fructose, and starch were all at significantly lower levels in bud, leaf, and stem tissues of shaded panels than in controls as soon as the shade was removed (Table 4.7). However, the shaded vines made up the difference as was evident from the carbohydrate data from subsequent samplings. The vines might have mobilized carbohydrates or increased photosynthesis when exposed to higher light levels. Shaded vines had almost no crop yield compared to control vines, thereby eliminating a very dominant sink. Translocation becomes directed into fruit clusters during fruit set and veraison (Hunter and Visser, 1988; Motomura, 1990). Control panels, therefore, had a major part of their carbon resources diverted into fruit ripening whereas the shaded panels could divert these carbohydrates into the axillary buds enabling them to make up the reduction in photosynthates during shade as the summer progressed. But shade applied for a 40-day period did increase BN in the distal nodes. These distal nodes that developed necrosis were either very young when shade was applied or developed in shade. These young buds might have experienced carbohydrate deficiency during the shading period making them more sensitive to BN. Therefore, a negative correlation between carbohydrate deprivation and BN incidence is indicated. Radioactive studies with CO_2 and analyzing the levels of $^{14}CO_2$ in shaded basal and distal nodes would help to establish the sink strength of the nodes.

The objective of the shoot tipping study was to divert nutrients and photosynthates from the growing shoot tip to flower clusters and lateral buds. Expected results were an increase in bud carbohydrates and increased fruit set due to greater carbohydrate availability, concomitant to decrease in BN, if BN was due to decreased carbohydrate level. Nonetheless, analysis of fruit set revealed that there was no significant difference in normal fruit set between shoot-tipped vines and the controls. Shoot tipping was done rather early in the season at 40 days after budbreak and ≈17 to 18 nodes were left on the shoots. Shoot tipping resulted in production of lateral shoots. Laterals form sinks until veraison after which they act as source organs (Candolfi-Vasconcelos and Koblet, 1990). Therefore, in the shoot tipping study, the lateral shoots in shoot-tipped vines might have competed as alternate sinks with fruit clusters resulting in comparable levels of fruit set as the controls. A shoot tipping study on ‘Chardonnay’ vines caused a reduction in fruit soluble solids in shoot-tipped vines (Wolf et al., 1986). According to Wolf et al. (1986) prolific lateral growth observed in the shoot-tipped vines created a competitive sink resulting in poor quality fruits. In the current shoot tipping study, higher BN at nodes 13-16 and nodes 17-20 was also observed in shoot-tipped vines compared to the control vines. The buds in the basal portion of the shoots were less necrotic possibly because the buds were able to accumulate sufficient amount of carbohydrates to counteract the effect of shoot tipping. The distal buds were unable to store sufficient carbohydrates in time to withstand the production of competing sinks such as the lateral shoots produced after shoot tipping. The carbohydrates such as sucrose, glucose, fructose, and starch were much lower in the bud tissues of shoot-tipped vines than in controls ($P<0.05$).

This could have resulted from an increased transport of nutrients to the lateral shoots formed after shoot tipping instead of to the bottom part of the vines.

Studies conducted in Japan on the BN-prone Japanese cultivar 'Kyoho' revealed that nutrients such as nitrogen, phosphorus, potassium, calcium, magnesium, manganese, and boron were not correlated with BN occurrence (Naito et al., 1987). This was similar to the findings of the current mineral nutrient analysis of bud, leaf, and stem tissues of Virginia 'Riesling' and 'Chardonnay' vines. Naito et al. (1987) observed equal amounts of the mineral nutrients in strong and weak shoots of 'Kyoho' as well as shoots of BN-resistant cultivars such as 'Delaware' and Muscat Bailey A' 25, 40 and 57 days after budbreak. Bains et al. (1981) observed that overvigorous vines had higher levels of nitrogen compared to devitalized vines. Bains et al. (1981) proposed that this high nitrogen and low carbohydrate levels in overvigorous vines was responsible for unfruitfulness in 'Anab- e- Shahi' grapevines. Similarly, Bindra and Chohan (1974) observed that overmanuring increased flower bud killing in Anab-e-Shahi. However, studies in Chile did not reveal any correlation between nitrogen and potassium fertilization on BN occurrence (Perez, 1991). The current study revealed that there was a decrease in the level of mineral nutrients in the buds in both 'Chardonnay' and 'Riesling' vines with seasonal progress. However, the decrease in mineral nutrients between cultivars was comparable. Therefore, the results of this study provide no compelling evidence that BN is caused by or related to essential element deficiency. Also, the BN-prone cultivar 'Riesling' expressed higher BN incidence compared to the BN-resistant cultivar 'Chardonnay'. If a particular nutrient deficiency is a cause of BN, then that nutrient should exist at a higher level in 'Chardonnay' than in 'Riesling' vines. However, both cultivars contained the same levels of nutrients.

Analysis of carbohydrates by HPLC revealed that the level of sucrose, glucose, and starch was higher in the bud tissues of 'Chardonnay' vines compared to 'Riesling' vines. The higher levels of carbohydrates observed in 'Chardonnay' vines compared to the 'Riesling' vines might have resulted from mobilization of carbon resources from trunks and cordons of vines or high rates of photosynthesis. There are inherent differences in photosynthetic capacities among different cultivars within a species (Winkler et al., 1974). The demand or need for photosynthates can also influence the rate of photosynthesis. Therefore, the difference observed in the levels of carbohydrate between the two cultivars might be a cultivar effect.

Sucrose is the main sugar translocated in grapevines, while starch is the major storage form of polysaccharide (Winkler et al., 1974). A large amount of carbon is accumulated as starch in chloroplasts and sucrose in vacuoles and cytosol during photosynthesis (Kruger, 1990). About 30% of carbon fixed by leaves is converted into starch from sucrose during the day. During the ensuing dark period starch is mobilized into sucrose and transported via the phloem to the different plant parts including axillary buds (Kruger, 1990). Any carbohydrate deprivation experienced by the buds would be demonstrated as a decrease in the levels of starch or sucrose. In the present study sucrose concentration was higher in bud tissues of 'Chardonnay' vines compared to 'Riesling'. This indicates that either the rate of sucrose synthesis from photosynthesis is lower than in 'Riesling' or sucrose converted from starch at night is being

translocated to alternate sinks such as the vigorously growing shoot tips. And as BN was much lower in 'Chardonnay' vines compared to 'Riesling' vines, a negative relationship between sucrose levels and BN is indicated.

Results of the visual starch rating study revealed a continuous increase in starch in buds as the summer progressed with all cultivars except 'Chardonnay' in which no increase was observed from 70 to 80 days after budbreak, and 'Syrah' in which there was a small reduction in starch level from 70 days (3.5) to 80 days after budbreak (3.3). Starch level usually increases in grape shoots from May to November (northern hemisphere) as sucrose decreases (Pickett and Cowart, 1941; Chaumont et al., 1994). A major portion of sucrose synthesized during photosynthesis is used by the developing shoots for growth. The remaining part is converted into starch (Candolfi-Vasconcelos and Koblet, 1990; Koblet et al., 1993). But starch accumulation occurs when the rate of photosynthesis exceeds the leaf's capacity for sucrose export. Therefore, low levels of starch at the start of the season indicate that it is not being synthesized in sufficient amounts. Starch is the chief storage form of available carbohydrates (Winkler and Williams, 1945) and is found in parts of the vine such as the trunk, arms, roots, and green tissues (Koblet et al., 1993). As shoot growth starts in spring, carbon sources are preferentially translocated to the growing shoot tip. As more leaves are produced, the basal leaves direct the assimilates into the trunk and other permanent structures of the vine. Inflorescences are very poor sinks but at fruit set and ripening, fruit clusters become very strong sinks (Edson et al., 1995; Motomura, 1990). Under stress situations, carbon is believed to be mobilized from the permanent parts of the vines into the developing fruit clusters; and a compensation effect occurs with a change in partitioning in favor of developing fruit clusters at the expense of the vegetative parts (Koblet et al., 1993).

The intensity of starch staining was the highest for 'Chardonnay' followed by 'Riesling', 'Viognier' and 'Syrah'. Low levels of starch in these cultivars therefore, could be due to preferential partitioning of photosynthates into fruit clusters rather than to the axillary buds or due to low rates of starch production. 'Riesling', 'Viognier' and 'Chardonnay' vines have higher BN in nodes 7-17 than in the basal 6 nodes. 'Riesling' and 'Chardonnay' vines had greater levels of starch deposits in the basal section (nodes 1-6) compared to the distal section. 'Viognier' had greater levels of starch deposits in the basal node section compared to the distal section at the first sampling; and 'Syrah' had higher levels at the last two samplings. Correlatively, at least these data support the hypothesis that BN results from carbohydrate starvation. However, it cannot be ruled out that the low starch levels in bud tissues is an effect of the senescence process caused by BN. Morrison and Iodi (1990) suggested that the observed lack of starch grains in necrotic 'Thompson Seedless' buds could be caused by the senescence process that occurs during the initial stages of BN, rather than the *cause* of necrosis. BN might cause a dysfunction in phloem transport of sucrose causing a reduction in starch levels. This can be tested by radioactive $^{14}CO_2$ leaf feeding and measuring radioactive sucrose levels in bud tissues; examination of sieve elements of bud and adjoining nodal tissues for callose deposition using an electron microscope would also help to determine if phloem dysfunction has occurred.

CONCLUSION

Shade studies indicated a three-week period of artificial shade applied either 20, 40, or 60 days after budbreak did not increase BN in 'Riesling', while a 40-day period applied 25 or 65 days after budbreak did increase BN in distal nodes (9-20). Lack of sunlight over long periods does appear to be involved in BN incidence. Shoot vigor, expressed as shoot diameter or average internode length, was positively correlated with BN of 'Riesling'. A lower incidence of BN was observed in the basal nodes of shoots than in the more distal (5 - 16) nodes. Therefore, as suggested by Wolf and Warren (1995) management practices that regulate vigor as well as spur pruning are recommended compensatory practices. In 1994, TNC levels were analyzed and no significant difference was observed between shaded and non-shaded vines. Although shade did decrease sucrose, glucose, fructose and starch levels in bud, leaf, and stem tissues as determined at the point of shade removal, the carbohydrate concentrations in bud, leaf, and stem tissues of vines soon increased to levels equal to those in controls. But as distal nodes exhibited necrosis when shaded for a 40-day period, carbohydrate deprivation caused by shading does appear to be involved in BN occurrence.

Shoot tipping at ≈18 nodes increased BN at nodes distal to node 12 compared to control vines which were not shoot-tipped. Shoot-tipped vines had lower bud levels of sucrose, glucose, fructose, and starch than did the controls. Therefore, a negative relationship between sucrose, glucose, and starch and BN incidence is indicated.

Results of the mineral nutrient survey in 1995 suggested that BN is not caused by whole vine or localized bud deficiency of an essential element. The nutrients examined decreased in the bud tissue as the summer progressed in both 'Riesling' and 'Chardonnay' vines. Carbohydrate analyses indicated that sucrose levels might be related to BN incidence. However, there is not sufficient evidence to prove causality.

Starch increased in buds as the summer progressed. The BN-insensitive cultivar 'Chardonnay' had significantly higher levels of starch compared to the BN-susceptible cultivars, 'Riesling', 'Viognier', and 'Syrah'. 'Chardonnay' had the lowest BN incidence followed by 'Riesling', 'Syrah', and 'Viognier'. There was a node position effect as well in 'Chardonnay' and 'Riesling' cultivars in that the basal six nodes had a higher quantity of starch than nodes 7-13. BN was higher in the distal 7 to 13 nodes than in the basal six nodes for 'Riesling' and 'Chardonnay'. However, no node position effect was observed in 'Viognier' or 'Syrah'. Therefore, it can be concluded that there is a negative correlation between BN susceptibility and level of starch in the buds.

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