Reduction of Bunch Stem Necrosis of Cabernet Sauvignon by Increased Tissue Nitrogen Concentration

ERIC R. CAPPS[1] and TONY K. WOLF[2]*

Field experiments were conducted over three years at two vineyards in northern Virginia to examine relationships between specific nutrients and the incidence of bunch stem necrosis (BSN) of Cabernet Sauvignon. Nitrogen, magnesium, and calcium were applied alone or in combination. Only one of the vineyards (Winchester) showed appreciable BSN incidence during the study period. During the 1996 season at Winchester vineyard, bloom-time leaf petiole and veraison rachis nitrogen concentration of unfertilized (control) vines were 0.80% and 1.16%, respectively. The corresponding control BSN incidence was 41% at harvest time. Seasonally split application of nitrogen fertilizer at 112 kg/ha actual nitrogen increased bloom-time leaf petiole and veraison cluster stem nitrogen concentration to 1.85% and 1.82%, respectively, in the nitrogen plots. 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 tissue nitrogen levels in 1997. Application of nitrogen fertilizer in 1998 was associated with bloom-time leaf petiole and veraison rachis nitrogen concentrations of 1.18% and 1.34%, respectively. Corresponding BSN was reduced to 3% at harvest time, versus 17% to 23% in treatments that did not receive nitrogen. Magnesium and calcium had minimal (1997) or no impact on BSN incidence. Measures of canopy density, cane pruning weights, crop yield, and fruit chemistry suggest that the ameliorating effects of nitrogen on BSN incidence were directly related to increased tissue nitrogen concentration, and not an indirect effect of vigor stimulation or crop ripening rate. A low incidence of BSN at the second vineyard precluded a definitive explanation of prior BSN expression at that location. We conclude that low tissue [N] may be one cause of BSN, and that vineyards should be examined individually for contributing factors.

KEY WORDS: bunch stem necrosis, dessèchement de la rafle, fertilizer, grapevine, nitrogen, nutrient analysis, palo negro, shanking, stiellähme, waterberry

Late-season bunch stem necrosis (BSN) is a physiological disorder of the bunch stem (rachis) of grapevines [7]. The terminology used with BSN is diverse and descriptive of the observed effects on the entire cluster. Hence, *waterberry* (California) [3,10], *shanking* (New Zealand) [24], *stiellähme* (Germany) [34], *dessèchement de la rafle* (France) [36], *palo negro* (Chile) [28], *disseccamento del rachide* (Italy) [14], and *rachis necrosis* (Canada) [11] are synonymously used in the literature.

Bunch stem necrosis may develop at or around anthesis, or later, at or after veraison. The former condition is referred to as *early* bunch stem necrosis [EBSN] [22], or inflorescence necrosis [16] [but see 23]. While EBSN and post-veraison BSN may have similar etiologies, they are temporally distinct. In our experience, occurrence of the latter is not usually preceded by the occurrence of EBSN. The research described in this paper involved the late-season, or post-veraison expression of bunch stem necrosis which, for brevity, will be called BSN.

Initial BSN symptoms appear as 1- to 3-mm darkened lesions on the rachis and/or pedicels of grape clusters [10,34]. Lesions can expand to girdle the rachis, which leads to desiccation of the rachis distal to the lesion. Affected portions of clusters either abscise or remain on the cluster in a dry condition. Cluster shoulders and/or tips are frequently symptomatic, while the remainder of the cluster normally develops. BSN-affected berries are initially soft and dull in appearance before withering. BSN-affected fruit has elevated tartaric and titratable acidity, while soluble solids concentration, pH, total phenols and potassium are depressed, relative to healthy fruit [27,36].

Climate, edaphic factors, vine nutrient status, cultivar, rootstock, method of dormant pruning and crop load have all been associated with BSN symptoms, making it difficult to ascribe a central cause [8]. Etiology has been further confounded by the annual variability of BSN expression within a particular vineyard [20]. No pathogen has been linked to the disorder and research has focused on environmental, hormonal, and nutritional imbalances as potential causes. Environmental parameters that have been positively correlated to BSN expression include:

- low mean maximum temperatures at bloom [35]
- low temperatures during the 20 days preceding bloom, but not during bloom itself [20]
- high humidity (80%) during the flowering to harvest period [24]
- excessive or irregular rainfall, especially after veraison [5,29]

[1]Graduate Research Assistant and [2]Professor, AHS Agricultural Research and Extension Center, Virginia Tech, 595 Laurel Grove Rd., Winchester, VA 22602

* Corresponding author [vitis@vt.edu].

Manuscript submitted for publication 20 December 1999; revised 1 March 2000.

Given the divergence of some of these reports, it is likely that climatic conditions are simply correlative or serve to mediate more fundamental causes.

An abundance of data, much derived from vineyard fertilizer trials, indicates that some occurrences of BSN result from nutrient imbalances, in particular a high ratio of potassium (K) to magnesium (Mg) and/or calcium (Ca) [5,6,12,34]. Application of Mg and occasionally Ca fertilizers often reduced the incidence of BSN in Europe [7,18,29]. In contrast, BSN was unaffected by applications of Ca and Mg in California, but was increased by applications of nitrogen (N) [10,17]. Total N and ammonium (NH_4^+) levels were greater in BSN-symptomatic rachis tissue compared to non-symptomatic rachis tissue [10]. Others have also observed the occurrence of elevated NH_4^+ levels in BSN-symptomatic tissues [28], but not consistently. Rather than being causal, elevated NH_4^+ levels associated with BSN were hypothesized to reflect tissue senescence or secondary ramifications of the disorder [26].

In addition to climatic and nutritional causes of BSN, the role of hormonal imbalances [21] and accumulation of stress metabolites [28] have also been explored.

Bunch stem necrosis occurs with some regularity in mid-Atlantic US vineyards [8,37]. In Virginia, BSN commonly affects Cabernet Sauvignon, but many cultivars show the disorder in some years (our unpublished data). Visual and plant mineral analysis surveys were conducted for several seasons in six Cabernet Sauvignon vineyards that had histories of BSN expression. Results were ambiguous; however, data from two vineyards suggested a correlation between BSN and a deficit of tissue N. One case was particularly intriguing in that tissue N concentrations were contrarily associated with heavy crop yields and cane pruning weights, both conventional indicators of sufficient N [37]. In light of those findings, and previous research on nutritional aspects of BSN, we initiated experiments in 1996 to determine if application of N, Ca, and Mg fertilizers affected BSN incidence.

Materials and Methods

Vineyards: Cabernet Sauvignon (FPMS clone #7) vines (7 years old in 1996), grafted to rootstock C-3309 and grown at the AHS Agricultural Research and Extension Center in Winchester, Virginia (39°17′ N, 78°17′ W), were used during the 1996 - 1999 seasons. Vines at Winchester were spaced 2.1 m apart in 3.7-m wide rows. Vines were cordon-trained to an open-lyre, divided canopy system, and were spur-pruned with shoots vertically positioned upright. The incidence of BSN at Winchester averaged 14% in 1995. Cabernet Sauvignon (unknown clone) vines (10 years old), grafted to rootstock SO4 and grown at Leesburg, Virginia (39°10′ N, 77°56′ W), were used in a second experiment during 1997 and 1998. Grapevines at Leesburg were spaced 1.5 m apart in 3.7-m wide rows. Vines at Leesburg were cordon-trained and spur-pruned on a non-divided canopy system, with all shoots vertically positioned upright. The incidence of BSN was not recorded in 1995. The vineyards were selected for study due to fairly consistent histories of BSN expression, and because plant tissue analysis results suggested that both vineyards were somewhat low in tissue (N). The soil at Winchester was a Frederick-Poplimento loam, primarily limestone-derived with some contribution from sandstone deposits. The effective rooting zone was greater than 100 cm deep, with moderate to abundant (*ca.* 118 to 238 mm/m) available water capacity. Soil pH was slightly acidic to neutral (*i.e.,* 6.0 to 7.0). Soil at Leesburg was a Braddock cobbly loam, primarily granite-derived, but also well-drained with depth to bedrock greater than 2 m.

A one to 1.5-meter weed-free strip was maintained under the trellis by herbicide application at both vineyards. Row middles were perennial grass (*Festuca spp.*), mowed as necessary. Pest management followed regional recommendations and was effective.

Treatment and experimental design: Fertilizer treatments were applied to two-vine plots at Leesburg and three-vine plots at Winchester, each replicated five times in completely randomized designs. Unfertilized guard plots in the row and guard rows separated individual treatment plots. The initial plot arrangements remained unchanged over the course of both experiments. Treatment vines at both vineyards were shoot-thinned to 15 shoots per meter of cordon prior to bloom each season. Shoot length was maintained at about 17 nodes/shoot by trimming, typically once or twice per season. Four fertilizer treatments (2 × 2 factorial) were applied at both vineyards in the following fashion [treatment designation in brackets]:

(1) N_0 and $Mg_0 + Ca_0$: No fertilizer application [**Control**].

(2) N_{112} and $Mg_0 + Ca_0$: Ammonium nitrate (NH_4NO_3); treatment consisted of 112 kg/ha of actual N applied to soil in a split application of 28 kg/ha at budburst, 56 kg/ha at bloom, and 28 kg/ha 30 days after bloom. No magnesium or calcium was applied [**N**].

(3) N_0 and $Mg_{280} + Ca_{94}$: Magnesium sulfate ($MgSO_4$) + calcium chloride ($CaCl_2$); treatment consisted of 280 kg/ha of $MgSO_4$ applied to soil in a split application of 140 kg/ha at budburst and 140 kg/ha bloom. In addition, seven foliar applications of $CaCl_2$ (13.4 kg/ha $CaCl_2$ per application) were made every two weeks, commencing when shoots were approximately five nodes long. No nitrogen was applied. The $CaCl_2$ was not included at Winchester in 1996 [**Mg + Ca**].

(4) N_{112} and Mg280 + Ca94: NH_4NO_3 + $MgSO_4$ + $CaCl_2$ all applied, as above [**N + Mg + Ca**].

Ammonium nitrate and $MgSO_4$ were shallowly incorporated into the soil under the trellis. A backpack sprayer was used to apply the $CaCl_2$ to the entire vine canopy to the point of runoff. Except as noted here and in the tables, treatments were applied each year. Only

treatments 1 and 2 were applied at Winchester in 1999, although BSN was evaluated on all previously used plots.

Soil analysis: Soil was sampled at depths of 0 to 20 cm and from 20 to 40 cm, prior to application of the first fertilizer treatment. Three soil samples, 15 probes per sample, at each depth were collected across intended treatment plots at both vineyards. Subsequent soil samples were collected by treatment (n = 1; 10 soil probes per treatment) from both vineyards and at both soil depths in July 1998. Finally, the top 20 cm of soil was sampled at both vineyards by treatment plot (n = 5; for pH assessment in October of 1999. No lime was applied to either vineyard during the course of study.

Tissue analysis: Plant tissue samples were collected each year at bloom and veraison, except as noted in tables. Samples consisted of 80 leaf petioles per treatment plot. Leaves opposite flower clusters were sampled at bloom, while mid-shoot leaves were sampled at veraison. In addition, 20 rachises were collected from each treatment plot at bloom and veraison in the Winchester plots. Berries were removed and discarded with the veraison sample. The veraison rachis samples did not have visible BSN symptoms, even if BSN was present on other clusters. Bloom-time leaf petioles and veraison rachises were the only tissue samples collected at Winchester in 1996. All tissues were promptly dried at 90°C and processed by a commercial tissue analysis laboratory.

Canopy descriptors: Point quadrat analyses [31] were conducted on all treatment plots approximately 60 days after budburst to quantify canopy characteristics. At this point the canopy had developed and shoots had been hedged once. A thin metal rod was inserted horizontally through the fruiting zone of the vine canopy at equal intervals, 42 and 10 probes per treatment plot at Winchester and Leesburg, respectively. Probe contacts were recorded to describe leaf layers and fruit exposure. Photosynthetic photon flux (PPF) was measured within canopy fruit zones to further describe canopy density. PPF was measured prior to veraison with a 1.0-meter line quantum sensor (model LI-191SB, LI-COR, Inc. Lincoln, NE 68504) with photometer (LI-COR model 185B). Light was measured between 1100 and 1600 hours EDT on a clear day at both vineyards during July, as described in Wolf and Warren [38].

Bunch stem necrosis: Each treatment plot was rated for BSN incidence every two weeks at both vineyards. The ratings started at veraison and continued through harvest. Fifty clusters at Leesburg vineyard and 100 clusters at Winchester vineyard, chosen at random from each treatment plot, were visually rated and percent BSN was calculated for each plot. Each rating was done on a uniquely random selection of clusters. Clusters were considered affected if they bore at least five shriveled berries; however, BSN symptoms typically involved 20 or more berries on a given cluster.

Components of yield and berry chemistry: Berry samples were collected at both vineyards from every treatment plot at each BSN rating, including harvest. Berry samples did not include visibly 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 after frozen storage. At harvest, fruit from each vine was harvested and weighed. Other yield components included clusters per vine and cluster weight. Cane pruning-weights were collected after each growing season.

Statistical analysis: All dependent variables were subjected to two-way analyses of variance using type III ANOVA of the General Linear Model procedure of the Statistical Analysis System (SAS Institute, Cary, NC) to determine the significance of the main effects (N and Mg + Ca) and their interaction. Data that consisted of multiple measures within a treatment replicate, such as crop yield and PPF data, were averaged by treatment replicate prior to ANOVA. Percentage data, such as BSN incidence, were arcsin-transformed for ANOVA; however, non-transformed data are presented for ease of interpretation.

Results

Winchester

Soil analysis: Soil samples collected in 1996, prior to establishment of fertilizer treatments, indicated the Winchester site to be moderately fertile with a pH of 6.4 at 0 to 20 cm and 6.7 at 20 to 40 cm [8]. Soil samples collected in 1998 revealed soil [Mg] was increased from 43 ppm in control plots (0 to 20 cm depth) to an average of 306 ppm in the Mg + Ca and N + Mg + Ca plots. Calcium and other nutrients were not appreciably affected by treatment, while soil [N] was not quantified. Soil pH (0- to 20-cm depth) at the termination of the experiment was (mean ± S.D.; n = 5): 6.2 ± 0.1 (control), 6.2 ± 0.1 (N only), 6.3 ± 0.1 (Mg + Ca), and 6.1 ± 0.1 (N + Mg + Ca).

Tissue elemental analysis: Nitrogen application, whether alone or in combination with Mg and Ca, increased tissue N concentration relative to concentrations of vines that received no N. The increased tissue N concentration was consistent and significant with petioles collected at bloom (Table 1). Using petioles collected at veraison, only the 1998 data showed a significant N response. Interaction between N and Mg + Ca was noted with veraison-sampled petioles in 1997, with petiole [N] apparently increased by both treatments. Rachis tissue was also sensitive to applied N if clusters were sampled at veraison, less so if collected at bloom (Table 1). Although they received no applied N, control, and Mg + Ca plots had elevated N levels in 1997, which was reflected in both petiole and rachis tissue samples. Petiole [N] appeared to increase between bloom and veraison of 1999, at least in the control and Mg + Ca plots.

Fertilizer treatments were inconsistently associ-

Table 1. Cabernet Sauvignon leaf petiole and rachis nitrogen composition as influenced by fertilizer treatments at Winchester from 1996 to 1999.

	Nitrogen concentration (%) of leaf petioles				Nitrogen concentration (%) of rachis		
Treatment	**1996**	**1997**	**1998**	**1999**	**1996**	**1997**	**1998**
				Samples collected at bloom time			
Control	0.80	1.32	0.88	0.80	—[y]	2.72	2.38
N	1.85	1.85	1.18	0.99	—[y]	2.76	2.78
Mg + Ca[z]	0.93	1.34	0.89	0.78	—[y]	2.76	2.38
N + Mg + Ca[z]	1.66	1.56	1.09	—[y]	—[y]	2.88	2.70
Significance (Pr > F)[x]							
N	0.0001	0.006	0.0001	0.04	—[y]	ns	0.0006
Mg + Ca	ns	ns	ns	ns	—[y]	ns	ns
N + Mg + Ca	ns	ns	ns	—[y]	—[y]	ns	ns
				Samples collected at veraison			
Control	—[y]	1.00	0.78	0.94	1.16	1.04	0.98
N	—[y]	1.08	0.90	0.95	1.82	1.40	1.34
Mg + Ca[z]	—[y]	1.08	0.80	1.28	1.02	1.04	1.01
N + Mg + Ca[z]	—[y]	1.05	0.88	—[y]	1.84	1.26	1.26
Significance (Pr > F)[x]							
N	—[y]	ns	0.0009	ns	0.0001	0.0002	0.0001
Mg + Ca	—[y]	ns	ns	0.006	ns	ns	ns
N*Mg+Ca	—[y]	0.01	ns	—[y]	ns	ns	ns

[z]Calcium (Ca) was not applied in the 1996 or 1999 seasons.

[y]Data not collected.

[x]Significance of treatment main effects and their interaction. ns = non-significant.

ated with altered K, Mg, and Ca concentrations. As sampled at bloom, N application increased petiole [K], but only in 1998 and 1999 (Table 2). Nitrogen appeared to have a positive effect on petiole Mg or Ca concentrations, at least as indicated by veraison-sampled tissues. The significant N effect on bloom petiole [Ca] in 1996, however, appeared to be negative. Magnesium application increased petiole Mg levels in 1997 and 1998, with a persistent effect obtained in 1999 (Table 2). Calcium levels were significantly increased by Mg + Ca application, as indicated by veraison-sampled tissues. An increased [Ca] by Mg + Ca at bloom of 1999 was, curiously, associated with a significant reduction at veraison. Sampled at veraison, we again saw a slight increase in petiole [K] with N application, and all fertilizer treatments tended to increase Mg and Ca levels relative to the control vines (Table 2). Significant treatment effects on rachis concentrations of other elements were observed, but the relationships among those differences were in no instance consistent with the observed pattern of BSN incidence (data not shown). Other

Table 2. Cabernet Sauvignon leaf petiole elemental composition as influenced by fertilizer treatments at Winchester from 1996 to 1999.

	Potassium				Magnesium				Calcium			
Treatment	**1996**	**1997**	**1998**	**1999**	**1996**	**1997**	**1998**	**1999**	**1996**	**1997**	**1998**	**1999**
					Petioles collected at bloom time							
Control	5.09	2.38	3.16	2.00	0.37	0.23	0.25	0.22	1.97	1.38	1.42	1.42
N	4.60	1.86	3.72	2.52	0.46	0.26	0.30	0.28	1.84	1.30	1.80	1.41
Mg + Ca[z]	4.82	2.88	3.16	1.66	0.37	0.27	0.33	0.34	1.85	1.40	1.78	1.73
N + Mg + Ca[z]	4.95	2.88	3.58	—[y]	0.48	0.28	0.35	—[y]	1.61	1.42	1.72	—[y]
Significance (Pr > F)[x]												
N	ns	ns	0.01	0.06	0.02	ns	ns	0.07	0.03	ns	ns	ns
Mg+Ca	ns	ns	ns	ns	ns	0.01	0.007	0.002	0.03	ns	ns	0.01
N*Mg+Ca	ns	ns	ns	—	ns	ns	ns	—	ns	ns	0.03	—
					Petioles collected at veraison							
Control	—[y]	1.34	5.68	5.58	—[y]	0.19	0.39	0.69	—[y]	0.68	1.60	1.96
N	—[y]	6.54	6.58	6.20	—[y]	0.44	0.47	0.59	—[y]	1.66	1.80	1.72
Mg + Ca[y]	—[y]	6.22	5.74	5.52	—[y]	0.42	0.50	0.46	—[y]	1.60	1.98	1.64
N + Mg + Ca[y]	—[y]	6.10	6.34	—[y]	—[y]	0.38	0.56	—[y]	—[y]	1.70	1.84	—[y]
Significance (Pr > F)[x]												
N	—	0.0001	0.003	0.07	—	0.02	0.04	ns	—	0.008	ns	0.02
Mg+Ca	—	0.0001	ns	ns	—	0.03	0.008	0.001	—	0.01	0.04	0.003
N*Mg+Ca	—	0.0001	ns	—	—	0.001	ns	—	—	0.02	ns	—

[x] Significance of treatment main effects and their interaction. ns = nonsignificant.

[z] Calcium (Ca) was not applied in the 1996 or 1999 seasons.

[y] Data not collected.

Table 3. Cabernet Sauvignon leaf petiole nutrient composition at bloom time, averaged across fertilizer treatments at Winchester.

	Percent of dry weight					μmg•mg^{-1} dry weight				
	N	P	K	Mg	Ca	B	Cu	Fe	Mn	Zn
1996	1.31	0.31	4.87	0.42	1.82	39	15	90	206	44
1997	1.52	0.13	2.50	0.26	1.38	35	7	38	108	40
1998	1.01	0.11	3.41	0.31	1.28	30	7	47	258	35
1999	0.86	0.20	2.06	0.28	1.52	23	9	32	220	30
Standards[z]	1.20 – 2.20	0.15 - ?	1.50 – 2.50	0.30 – 0.50	1.00 - ?	30 - 100	7 - 15	40 - ?	25 – 1K	35 - 50

[z]Currently accepted sufficiency range as used in Virginia [37].

essential nutrients were generally at or above bloom sufficiency levels used in the mid-Atlantic (Table 3).

Correlation coefficients between BSN incidence at harvest and individual nutrients and various nutrient ratios revealed strong inverse relationships between tissue [N] and BSN incidence (Table 4). Significant, positive correlations were also observed between phosphorus concentration and BSN incidence for bloom-sampled petioles and veraison-sampled rachises. Ratios of K/Mg and K/(Mg + Ca) were significant but *negative* with bloom-sampled rachis tissues. Bloom-sampled tissues produced more significant correlations between nutrients and BSN incidence than did veraison-sampled tissues.

Table 4. Correlation coefficients between BSN and selected nutrients and nutrient ratios at four combinations of tissue and sample period. Data are only for Winchester Vineyard and include four years' data.

BSN correlated with:	Bloom-sampled petioles[z]	Bloom-sampled rachises[z]	Veraison sampled petioles[z]	Veraison sampled rachises[z]
N	- 0.43 (0.0005)	-0.55 (0.0001)	ns	- 0.25 (0.04)
P	0.53 (0.0001)	ns	ns	0.69 (0.0001)
K	ns	-0.37 (0.007)	ns	ns
Mg	0.22 (0.08)	ns	ns	ns
Ca	0.33 (0.009)	0.36 (0.01)	ns	ns
K/Mg	ns	-0.26 (0.07)	ns	ns
K/(Mg + Ca)	ns	-0.46 (0.0008)	ns	ns

[z]Pearson product-moment correlation coefficients and, in parentheses, $p > |r|$. Each correlation coefficient is based on approximately 60 observations. ns = non-significant correlation.

Canopy descriptors: Vines of all treatments, including control, produced an abundance of healthy foliage, and two or three (1996) shoot hedgings were required to keep shoots at about 17 nodes in length. Point quadrat analyses conducted each season (except 1999) illustrated that canopy dimensions were generally uniform among all treatments with respect to leaf layers, percent exposed fruit, and percent gaps (Table 5). Sunlight penetration of fruit zones differed little among treatments; however, N decreased PPF in 1996, and both N and Mg + Ca decreased PPF in 1997, with some interaction apparent (Table 5). Vines that received either N treatment had perceptibly greener foliage than observed with other treatments.

Table 5. Canopy characteristics and sunlight penetration of fruit zones [photosynthetic photon flux (PPF)] of Cabernet Sauvignon vines as affected by fertilizer treatment at Winchester from 1996 to 1998.

	Canopy (fruit zone) point quadrat analysis									PPF	
	Leaf layers			% Exterior fruit			% Gaps			(μmol•m^{-2}•s^{-1})	
Treatment	1996	1997	1998	1996	1997	1998	1996	1997	1998	1996	1997
Control	0.9	1.2	1.2	65	64	79	12	10	10	5.5	3.6
N	0.9	1.6	1.8	67	60	59	15	6	7	4.0	1.9
Mg + Ca[z]	1.0	1.6	1.7	81	59	55	15	7	6	5.4	2.1
N + Mg + Ca[z]	0.9	1.9	1.8	64	57	71	16	4	3	3.7	1.8
Significance (Pr > F)[y]											
N	ns	0.003	0.02	0.007	ns	ns	0.03	ns	ns	0.0001	0.003
Mg + Ca	ns	0.002	0.04	0.02	ns	ns	ns	ns	ns	ns	0.02
N + Mg + Ca	ns	ns	ns	0.0005	ns	ns	ns	ns	ns	ns	0.05

[z]Calcium (Ca) was not applied in the 1996 or 1999 seasons.

[y]Significance of treatment main effects and their interaction. ns = nonsignificant.

Table 6. Average cane pruning weights, crop per vine, and cluster weight of Cabernet Sauvignon vines as influenced by fertilizer treatment at Winchester.

	Pruning wt./vine (kg)			Crop/vine (kg)			Cluster wt. (g)		
Treatment	**1996**	**1997**	**1998**	**1996**	**1997**	**1998**	**1996**	**1997**	**1998**
Control	3.6	2.6	2.1	14.8	18.6	10.6	94	139	111
N	4.2	3.3	3.3	12.9	18.2	11.5	121	153	118
Mg + Ca[z]	3.7	3.0	2.4	15.3	18.6	10.7	123	149	113
N + Mg + Ca[z]	4.4	3.3	3.7	14.7	17.3	11.7	118	139	122
Significance (Pr > F)[y]									
N	0.0001	0.0001	0.0001	ns	ns	ns	ns	ns	ns
Mg+Ca	ns	0.05	ns	ns	ns	ns	ns	ns	ns
N*Mg+Ca	ns	ns	ns	ns	ns	ns	0.04	0.02	ns

[z]Calcium (Ca) was not applied in the 1996 season.

[y]Significance of treatment main effects and their interaction. ns = nonsignificant.

Table 7. Soluble solids concentration (SSC) and pH of Cabernet Sauvignon at harvest as influenced by fertilizer treatment at Winchester.

	SSC%			pH[z]		
Treatment	**1996**	**1997**	**1998**	**1996**	**1997**	**1998**
			Winchester experiment			
Control	19.9	22.0	22.7	3.71	3.61	3.61
N	19.5	21.7	21.2	3.84	3.82	3.82
Mg + Ca[y]	19.9	21.8	22.1	3.76	3.70	3.70
N + Mg + Ca[y]	19.7	21.9	22.1	3.85	3.73	3.73
Significance (Pr > F)[x]						
N	0.04	ns	0.0008	0.002	0.01	0.02
Mg + Ca	ns	ns	ns	ns	ns	ns
N*Mg+Ca	ns	ns	0.002	ns	ns	ns

[z]Analyses conducted on previously frozen berry samples, with pH increased 0.1 to 0.2 pH units (33).

[y]Calcium was not included in 1996.

[x] Significance of treatment main effects and their interaction. ns = nonsignificant.

Cane pruning weights, components of yield, and fruit quality: Nitrogen application had a significant, positive, and seasonally consistent effect on cane pruning weights (Table 6). Crop per vine and berry weights (data not shown) varied little between treatments, although cluster weights were lower on control vines than on other treatments in 1996, with a significant treatment interaction in both 1996 and 1997 (Table 6). Crop load did not differ among treatments (data not shown). Fruit soluble solids concentration was unaffected by treatment in 1997, and was slightly depressed by N in 1996 and 1998 (Table 7). Relative to control, the greatest decrease (1.5° Brix) occurred with the N-only treatment of 1998. Fruit pH was increased from 0.1 to 0.2 units by N application in all years, significant in 1996 and 1997. Fruit titratable acidity was unaffected by treatment (data not shown).

Percent BSN: BSN symptoms observed in this study were comparable to those described elsewhere; symptoms initially appeared as a discoloration of the cluster stem at or around the time that berry color began to transition from green to blue. Berries distal to that lesion were retarded in sugar accumulation and were flaccid by the time that unaffected berries of the cluster were pigmented. Typically, only the distal 15% to 20% of a cluster was affected. The temporal relationship between sugar accumulation and BSN symptoms is illustrated for the 1996 data by Figure 1. Symptoms rapidly appeared when fruit soluble solids concentration reached about 15° Brix and continued to increase for approximately one month, or until about 19° Brix. Affected portions of clusters occasionally abscised with time, leading to an apparent decrease in BSN inci-

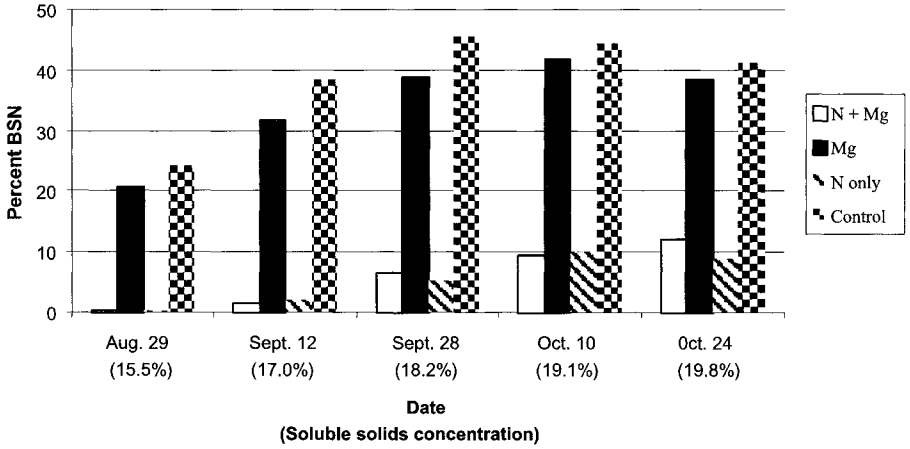

Fig. 1. Sampling date and corresponding percent bunch stem necrosis (BSN) and soluble solids concentration of Cabernet Sauvignon grapevines at Winchester during 1996. BSN levels of the two treatments that included N were significantly ($p \leq 0.05$) less than BSN levels of the two treatments that did not include N at each sample date. Soluble solids concentration is the mean of all fertilizer treatments for that date.

Table 8. Incidence of bunch stem necrosis (BSN) as influenced by fertilizer treatments in Winchester and Leesburg experiments.

Treatment	Percent BSN prior to harvest 1996	1997	1998	1999
		Winchester experiment		
Control	41	7	23	4
N	14	3	3	3
Mg + Ca[z]	39	2	17	33
N + Mg + Ca[z]	9	2	3	7
Significance (Pr > F)[y]				
N	0.0001	ns	0.0001	0.02
Mg + Ca	ns	0.02	ns	0.005
N*Mg+Ca	ns	ns	ns	0.03
		Leesburg experiment		
Control	—[x]	1	0	—[x]
N	—[x]	2	10	—[x]
Mg + Ca	—[x]	1	0	—[x]
N + Mg + Ca	—[x]	5	4	—[x]
Significance (Pr > F)[y]				
N	—[x]	ns	0.06	—[x]
Mg + Ca	—[x]	ns	ns	—[x]
N*Mg+Ca	—[x]	ns	ns	—[x]

[z]Calcium (Ca) was not applied in the 1996 or 1999 seasons.
[y] Significance of treatment main effects and their interaction.
ns = nonsignificant.
[x]Treatments not applied.

dence, as illustrated by the October data of Figure 1.

BSN incidence was decreased by N, whether the N was applied alone or in combination with Mg + Ca (Table 8). By contrast, control vines, and those that received only Mg + Ca, exhibited as much as 41% BSN incidence (Table 8). Mg + Ca had a slight negative effect on BSN in 1997, but a stronger positive effect in 1999. The overall low level of BSN in 1997 casts doubt on the actual significance of the 1997 Mg + Ca effect. Seasonally, the 1996 and 1998 seasons produced the highest incidence of BSN symptoms.

Meteorology: Accumulated heat units at Winchester (10°C base, April-October period) were 1808 (1996), 1721 (1997), 2225 (1998), and 2060 (1999). The 1996 season had the greatest frequency and total amount of precipitation, the 1997 season the least (data not shown).

Leesburg

Percent BSN: Very low levels of BSN were observed at Leesburg in the course of this study (Table 8). A follow-up examination in September 1999 was consistent with that trend (data not shown). Nitrogen application was associated with slightly increased BSN at Leesburg in 1998; however, that response was quite variable among plots. For example, 92% of the BSN-affected clusters in the N treatment were found in two of the five treatment plots. Given the short-term nature and generally insignificant results of the Leesburg experiment, the results of that experiment are presented in an abbreviated fashion here, but are available elsewhere [8].

Soil analysis: Soil samples collected in May 1997, prior to treatment application, showed the soil to be moderately fertile with a pH of 7.0 at 0 to 20 cm and 5.8 at 20 to 40 cm [8]. Soil Mg levels (July 1998) averaged 382 ppm in the top 20 cm and did not appear to be influenced by treatment at Leesburg [8]. Soil [K], which averaged 121 ppm at the start of the experiment (0 to 20 cm), was greatest (199 ppm) in the Mg + Ca plots at the termination of the experiment and essentially unchanged among other treatments. The soil samples collected in 1999 revealed pH values of 6.5 (control), 6.8 (N only), 6.9 (Mg + Ca), and 6.4 (N + Mg + Ca).

Tissue elemental analysis: Vines that received only N in 1997 showed elevated N concentrations in petioles collected at bloom (1.20% *vs.* 1.04% in control vines); however, that response did not persist to veraison, was not evident with the N + Mg + Ca treatment, and was not observed in 1998 (data not shown). Other than a possible increase in tissue [Mg] and decrease in [K] in 1998, applied N had no other impact on tissue macronutrient concentration, or on micronutrients (data not shown). Magnesium application was associated with *increased* K and *depressed* Mg in 1998. Calcium application produced no meaningful effects on macro- or micronutrients (data not shown).

Canopy descriptors: Canopy density was unaffected by treatment at Leesburg (data not shown). Canopies averaged 2.2 to 3.2 leaf layers and 0 to 4% gaps among treatments, and all were associated with a majority of exposed fruit clusters. Average sunlight penetration of fruit zones ranged from 5 to 7%. Vines that received N had perceptibly greener canopies by midsummer, relative to control and Mg + Ca vines.

Cane pruning weights, components of yield, and fruit quality: Nitrogen application at Leesburg led to increased vine pruning weight in 1998, but crop yield components were unaffected by treatment in the two years examined (data not shown). Vine size increased from 1997 to 1998, but crop per vine remained essentially unchanged. Fruit soluble solids concentration was unaffected by treatment in either year, while pH was increased by either N or Mg + Ca (data not shown).

Discussion

Vines at Winchester responded predictably to added N, showing increased tissue N concentrations, increased cane pruning weights, and visually greener foliage from the first year of treatment, relative to vines that received no N. Slightly increased canopy density was measured after the first year, possibly as a function of increased lateral shoot development, larger leaves, or both [2,30], as shoot density was held constant. Increased vine size, or cane pruning weights, is a common response to N fertilization, at least where unfertilized vines are deficient in N [2,30]. Cane pruning weights at Winchester ranged, on treatment average, from 0.5 to 1.0 kg/m of canopy. That range was

within or in excess of the range (0.3 to 0.6 kg/m) that Smart *et al.* [31] suggested was desirable for non-shaded canopies, especially in light of the fact that our canopies were shoot-trimmed at least once per year. Canopy leaf layers, gaps, and PPF measures at Winchester all suggested that indirect effects of N on BSN incidence (*e.g.,* via canopy shade) were minimal. Response to Mg was less pronounced at Winchester, and increases in tissue Mg occurred with N alone, as well as the two Mg treatments.

Vines at Leesburg, by contrast, failed to predictably respond to treatments: Tissue N concentration and leaf color suggested that vines were on the low end of the N sufficiency range. Cane pruning weights (0.3 to 0.7 kg/m of canopy) indicated N sufficiency, or borderline insufficiency. Nevertheless, aside from greener foliage, the Leesburg vines showed little benefit from the added N in the two-year period of study. Magnesium application either had no effect or was accompanied by depressed Mg tissue levels. The Leesburg vines did have very high tissue Mg levels, compared to standards used in the Mid-Atlantic region [37], and that high Mg status might have mitigated further Mg accumulation. We suspect that the apparent increase in tissue [K] with Mg + Ca application was partially due to the high soil availability of K in those plots.

The Winchester data implicate depressed tissue N concentration in the expression of BSN at that vineyard. The lower threshold of that response was at approximately 0.9% N, as assessed in bloom-sampled leaf petioles. An upper threshold was not identified, and regardless of tissue N level, vines at Winchester annually expressed up to 10% BSN. It therefore appears that factors other than N were *also* involved in BSN expression at Winchester. In that regard, we found no reports in the literature of complete BSN control through vineyard fertilization practices. Treatment efficacy was typically reported on the order of 60% to 70% reduction in incidence or BSN severity (*e.g.,* 4), and in cases only 20% to 30% [19].

To our knowledge, the decrease in BSN symptoms by N application has not been previously reported. Furthermore, the reductions in BSN incidence by increased tissue N appear, qualitatively, to contradict several other reports [10,13,17]. Quantitatively, however, our results are not necessarily at odds with previous studies. For example, the average N concentration of rachis tissue ($\leq$ 1.84%) in our work was less than that (2.50%) which Christensen and Boggero [10] found in rachises collected from symptomatic clusters in "high incidence" vineyard areas. In the Chilean work [13], N application (129 kg/ha) was associated with 15% affected grapes, compared to 4.7% with control vines, not a dramatic difference. And, in the Swiss work [17], increased N availability (up to 240 kg/ha) increased BSN incidence slightly in only two of eight years of trial.

A common feature of BSN is the annual variability of expression [17,20] in a given vineyard. The annual variability of BSN expression at Winchester was strongly correlated with the annual variability of bloom-time leaf petiole N concentration. Bloom-time leaf petiole concentrations below 0.9% increased the likelihood of BSN development in that season; however, 1999 provided an exception to that pattern. The incidence of BSN at Winchester in 1999 was puzzling in that BSN failed to appreciably develop in control plots (0.8% N in bloom-time petioles). Those vines did, however, show somewhat increased N concentration at veraison, opposite the normal seasonal decline of tissue N concentration. Bloom-time rachis tissue N concentration was also predictive of BSN symptoms, but rachis tissue at bloom did not show the response to N fertilizer as discriminatively as petioles did. Veraison petioles and rachis tissue were also predictive of BSN incidence, but not as precisely as bloom-time petioles. Given their cane pruning weights and the need to summer prune to maintain 15-17 nodes per shoot, the Winchester vines were not obviously deficient in N. This observation raises some questions about N allocation within the vine and discourages the use of visual indicators alone for judging N fertilization needs.

Deficiencies of Mg and Ca have been associated with BSN [11,18,34]. We do not believe that either nutrient was directly involved with BSN expression at Winchester. First, Mg application alone (1996) or in combination with Ca, had no or very little (1997) impact on BSN expression. Secondly, the year with the least BSN (1997) produced the lowest tissue levels of Mg and Ca. The K/[Mg + Ca) ratio did correlate significantly with BSN incidence, but the relationship was negative, meaning that BSN incidence *decreased* with increasing K/(Mg + Ca) ratio. That is opposite to the response reported in certain European studies [5,6], but comparable to results reported by Christensen and Boggero [10].

It is interesting to note that the one instance of elevated BSN occurrence at Leesburg (N treatment in 1998) was associated with a low to deficient level of K (1.16% in bloom time petioles). Christensen and Boggero [10] observed depressed [K], and increased NH_4^+ levels in BSN-symptomatic rachis tissues across several years and numerous field studies. Despite previous episodes of BSN, the incidence of BSN was essentially nil in both years at Leesburg. Our treatments had little effect on petiole nutrient concentration, and little or no effect on BSN. The short-term nature of the Leesburg experiment could partially explain the lack of vine response; however, we might have at least expected greater increases in vine N status. Vines that received N consistently appeared to have larger leaves and deeper green foliage, compared to vines that did not receive N. However, bloom-time leaf petiole analysis in both years revealed N deficiency by Virginia standards. This does raise the possibility that other nutrient deficiencies were constraining growth or limiting the effectiveness of the applied N.

We considered the possibility that N application merely delayed the expression of BSN through a delay

in fruit maturity. That possibility was discounted, however, in light of the minimal effects that N had on fruit ripening. The increased fruit pH [above that due to sample freezing [33] observed here with increased N availability, often reported in canopy management literature, does have undesirable enological implications and would have to be considered in an optimized N nutrition program.

Nitrogen has been linked to BSN expression chiefly through a surplus or toxic mode [10]. Here we illustrate that *insufficient* tissue N may also increase the frequency of BSN. The apparent disparate causes of BSN might gain some degree of unification under an hypothesis in which BSN can result from a range of stresses, mediated perhaps through "carbon starvation" [25], or the accumulation of stress metabolites such as putrescine [28]. BSN incidence was also associated with elevated NH_4^+ and putrescine, both of which were linked to insufficient K levels [28]. Putrescine levels have been linked to K deficiency in many plants [32], including grape [1]. Putrescine accumulation in grape leaves has also been observed under N deprivation [15].

The potential, manifold causes of "shank" [BSN] were quaintly proposed more than a century ago. Discussing production of hot-house grapes, William Chorlton [9] stated, "... we are led to believe, that anything which arrests, or interferes with the healthful action of the vine at [veraison] will be likely to produce shank." Chorlton's description of shank is no different from contemporary descriptions of BSN. Regardless of timing, we reiterate Chorlton's premise that BSN may be caused by multiple factors. In the case of the Winchester vineyard, the prime contributor appeared to be insufficient tissue N. Nitrogen, however, was probably not the sole factor, in that a low level of BSN persisted even when N was increased to sufficient or even supra-optimal levels. The historical cause(s) of BSN at Leesburg were uncertain given the low level of BSN occurrence in our two years of study.

Conclusions

(1) BSN at Winchester was closely associated with low (<0.90%) tissue N concentration within a given year, especially as measured in leaf petioles collected at bloom-time.

(2) Annual variability of BSN incidence at Winchester was correlated with seasonal fluctuations in bloom-time [N], regardless of fertilizer treatment.

(3) Potassium, Mg, and the K/(Mg + Ca) ratio had no apparent bearing on BSN at Winchester. Our results therefore differ from many of the European reports.

(4) With the possible exception of the 1998 Leesburg data, N application did not promote BSN.

(5) Our data present an interesting paradox for vine N assessment. Traditional, visual measures of vine N status (canopy fill, crop level, cane pruning weights) did not necessarily agree with tissue analysis results. The latter more accurately defined the need for additional N to minimize (but not eliminate) BSN occurrence.

(6) Results from the Leesburg experiment were equivocal due to the low incidence of BSN and the lack of vine response to treatment.

Literature Cited

1. Adams, D. O., K. E. Franke, *et al.* Elevated putrescine levels in grapevine leaves that display symptoms of potassium deficiency. Am. J. Enol. Vitic. 41:121-125 (1990).

2. Bell, S.-J. and A. Robson. Effect of nitrogen fertilization on growth, canopy density, and yield of *Vitis vinifera* L. cv. Cabernet Sauvignon. Am. J. Enol. Vitic. 50:351-358 (1999).

3. Bioletti, F. Blackmeasles, waterberries and related troubles. California Agricultural Experiment Station Bulletin. 358:1-15 (1923).

4. Boselli, M., and M. Fregoni. Possibilities of control of stem dieback (stiellähme) of grape by foliar applications. Dev.Plant Soil Sci. 22:214-230 (1986).

5. Boselli, M., A. Scienza, *et al.* Possibilità di previsione del disseccamento del rachide mediante il controllo della nutrizione minerale. Vignevini 10:35-38 (1983).

6. Brechbuhler, C. Ergebnisse von untesuchungen zur Berkämpfung der Stiellähme Mitt. Klosterneuburg 25:19-24 (1975).

7. Brendel, G., F. Stellwaag-Kittler, *et al.* Die patho-physiologischen Kriterien der Stiellähme. Mitt. Klosterneuburg 33:100-104 (1983).

8. Capps, E. R. The relationship between mineral nutrition and late-season bunch stem necrosis of Cabernet Sauvignon (*Vitis vinifera* L.) grapevines. MS Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia (1999).

9. Chorlton, W. The American Grape Grower's Guide. 204 pp. Orange Judd Co, New York (1882).

10. Christensen, P., and J. Boggero. A study of mineral nutrition relationships of waterberry in Thompson Seedless. Am. J. Enol. Vitic. 36:57-64 (1985).

11. Cline, R. Calcium and magnesium effects on rachis necrosis of interspecific hybrids. J. Plant Nutr. 10:1897-1905 (1987).

12. Cocucci, S., M. Morgutti, *et al.* A possible relationship between stalk necrosis and membrane transport in grapevine cultivars. Sci. Hortic. 34:67-74 (1988).

13. Cooper, T., D. Castro, *et al.* Facteurs influencant le dessèchment de la rafle chez la Sultanine raisin de table, au Chili. Controle chimique de cet accident. Progrès Agric. Vitic. 104:467-471 (1987).

14. Fregoni, M., and A. Scienza. Aspetti ormonici del disseccamento del rachide del grappolo di viti. Riv. Ortoflorofruttic. Italiana, n. 5-6:760-773 (1972).

15. Geny, L., M. Broquedis, *et al.* Effect of excessive nitrogen nutrition on free and conjugated polyamines content of leaves and berries of *Vitis vinifera* L. during their development. J. Intl. Sci. Vigne Vin 30:121-131 (1996).

16. Gu, S., P. B. Lombard, *et al.* Inflorescence necrosis induced from ammonium incubation and deterred by α-keto-glutarate and ammonium assimilation in Pinot noir grape vines. Am. J. Enol. Vitic. 45:155-160 (1994).

17. Gysi, C. Einfluss der Stickstoffmenge und-form Auf das auftreten der Stiellähme an Riesling X Sylvaner. Mitt. Klosterneuburg 33:122-126 (1983).

18. Haup, G. Control of stiellähme (grape stalk necrosis) with foliar fertilizers. *In:* Developments in Plant and Soil Science 22: pp. 231-241. Martinus Hijhoff Publisher, Netherlands (1986).

19. Holzapfel, B., and B. Coombe. The effect of magnesium sprays on the incidence of grapevine bunchstem necrosis (BSN). Australian Grapegrower and Winemaker 336:25-28 (1994).

20. Holzapfel, B., and B. Coombe. Incidence of grapevine bunchstem necrosis in South Australia: effects of region, year and pruning. Austral.

J. Grape Wine Res. 1:51-54 (1995).

21. Holzapfel, B., and B. Coombe. Relationship of ammonium ion and abscisic acid in bunchstem tissue to the incidence of the disorder bunchstem necrosis in grapevines. Austral. J. Grape Wine Res. 3:127-132 (1997).

22. Jackson, D. I., and B. G. Coombe. Early bunchstem necrosis in grapes — a cause of poor fruit set. Vitis 27:57-61 (1988).

23. Jackson, D. I., and B. G. Coombe. Early bunchstem necrosis – A matter of nomenclature. Am. J. Enol. Vitic. 46:579-580 (1995).

24. Jordan, D. Narrowing the research focus. Southern Hortic. Grape-grower Winemaker 3:53-55 (1985).

25. Keller, M., and W. Koblet. Is carbon starvation rather than excessive nitrogen supply the cause of inflorescence necrosis in *Vitis vinifera* L.? Vitis 33:81-86 (1994).

26. Keller, M., and W. Koblet. Stress-induced development of inflorescence necrosis and bunch-stem necrosis in *Vitis vinifera* L. in response to environmental and nutritional effects. Vitis 34: 145-150 (1995).

27. Morrison, J., and M. Iodi. The influence of waterberry on the development and composition of Thompson Seedless grapes. Am. J. Enol. Vitic. 41:301-305 (1990).

28. Ruiz, S., and A. Moyano. Bunchstem necrosis in grapes and its relationship to elevated putrescine levels and low potassium content. Austral. NZ Wine Ind. J. 13:319-324 (1998).

29. Rumbos, I. Occurrence of Stiellähme (grape stalk necrosis) in viticultural areas of Greece and preliminary results on its control. *In:* Influence of Environmental Factors on the Control of Grape Pests, Diseases and Weeds. Proceedings of a meeting of the EC Expert's Group. R. Cavallaro (Ed.). pp 275-285. Rotterdam (1989).

30. Smart, R. E. Canopy microclimate implications for nitrogen effects on yield and quality. *In:* Proceedings of the International Symposium on Nitrogen in Grapes and Wine. J.M. Rantz (Ed.). pp 90-101. Am. Soc. Enol. Vitic., Davis, CA (1991).

31. Smart, R. E., J. K. Dick, *et al.* Canopy management to improve grape yield and wine quality – principles and practices. S. Afr. J. Enol. Vitic. 11:3-17 (1990).

32. Smith, T. Polyamines. Ann. Rev. Plant Physiol. 36:117-143 (1985).

33. Spayd, S. E., C. W. Nagel, *et al.* Effect of freezing fruit on the composition of musts and wines. Am. J. Enol. Vitic. 38:243-245 (1987).

34. Stellwaag-Kittler, F. Äussere Symptomatik der Stiellähme an Trauben. Mitt. Klosterneuburg 33:133-137 (1983).

35. Theiler, R., and H. Müller. Beziehungen zwischen Klimafaktoren und dem Stiellähmebefall bei Riesling X Sylvaner. Vitis 25:8-20 (1986).

36. Ureta, F., J. Boidron, *et al.* Influence of dessèchement de la rafle on grape quality. Am. J. Enol. Vitic. 32:90-92 (1981).

37. Wolf, T. K., and E. B. Poling. The Mid-Atlantic Winegrape Grower's Guide. 126 pp. Department of Agricultural Communications, North Carolina State University, Raleigh, North Carolina (1995).

38. Wolf, T. K., and M. K. Warren. Shoot growth rate and density affect bud necrosis of 'Riesling' grapevines. J. Am. Soc. Hortic. Sci. 120:989-996 (1995).