CHAPTER 3
ANATOMICAL DEVELOPMENTS IN THE ONSET OF GRAPEVINE BUD NECROSIS

Abstract. Bud necrosis (BN) of grapes occurs as an apparent abortion and drying of the primary bud and/or secondary buds of the developing compound grape bud. Buds from 'Riesling' vines were collected at 10-day intervals starting 46 days after budbreak until 96 days after budbreak in 1994, from two vineyards in Virginia, USA. In 1995, five bud samplings were made from 'Riesling' and 'Chardonnay' vines at 10-day intervals starting 50 days after budbreak from another vineyard in Virginia. 'Riesling' and 'Chardonnay' bud samplings were also made from a New York vineyard which were selected as controls for Virginia 'Riesling' vines. Buds were excised, fixed and embedded in plastic. Ultrathin sections (1μm) of 'Riesling' buds collected from the more severely affected vineyard revealed that many buds exhibited a zone of compressed cells immediately beneath the primary bud axis. Cells in that zone started to show irregular cell walls within 66 days after budbreak with cell compression and cell lysis occurring within 90 days after budbreak. Multiple zones of cell compression were observed in some buds. Zones of cell compression were lacking or much less obvious (localized groups of irregular cells in some buds) in 'Riesling' buds collected from the less severely affected vineyard. New York 'Rieslings' also revealed results similar to that from the less severely affected vineyard. Zones of compressed cells were absent in 'Chardonnay' buds collected from Virginia and New York, as well. Scanning electron microscopy confirmed the findings of light microscopy study. From this study it can be concluded that BN starts within 60 days after budbreak. A cultivar and an environmental effect on BN occurrence are indicated.

INTRODUCTION

Grapevine buds are formed in leaf axils and are compound buds composed of a primary bud and one or two secondary buds (Pratt, 1974). The primary bud is axillary to the prophyll on the summer lateral. The secondary buds are axillary to the prophylls on the primary bud. The compound bud is composed of a primary and secondary bud enclosed in the prophyll of the summer lateral (Pratt, 1974). The summer lateral grows in the same season it is formed. The primary bud axis forms about six to nine nodes before entering endodormancy in August or September. Inflorescence primordia are formed in the buds at the fourth or fifth node and tendrils are formed at subsequent nodes. The primary shoot develops the following season in the spring (Pratt, 1974). If the primary bud is killed, secondary buds develop into secondary shoots. The yield from secondary shoots is less than that from primary shoots because cluster differentiation occurs in the primary buds before the onset of dormancy while secondary bud differentiation is retarded or subordinate to primary bud differentiation (Pratt, 1974).

BN affects the primary buds, and occasionally, the secondary buds within the compound bud (Dry and Coombe, 1994; Lavee, 1987; Lavee et al., 1981; Morrison and Iodi, 1990; Naito et al., 1986; Wolf and Warren, 1995). BN is, one of the causes of low vine fruitfulness in vineyards throughout the world. The appearance of BN varies (Lavee et al., 1981; Morrison and Iodi, 1990). Some buds were reported to have developed a necrotic layer at the base of the primary axis (Morrison and Iodi, 1990), some from one to four nodes above the base of the primary axis (Lavee et al., 1981; Morrison and Iodi, 1990) while still others developed necrosis only at the apex of the primary axis (Morrison and Iodi, 1990). A few studies on BN indicated that in late summer, the color of the primary buds changed from a light green to brownish green, eventually turning brown (Perez and Kliewer, 1990; Wolf and Warren, 1995). Perez and Kliewer (1990) also observed the development of a necrotic zone followed by the death of the primary bud, though the time of occurrence of the necrotic zone was not mentioned.

The objective of this study was to explore anatomical differences if any, between BN-prone ‘Riesling’ and the less BN-sensitive ‘Chardonnay’ buds. The second objective was to investigate the temporal development of BN by light microscopy and scanning electron microscopy (SEM). ‘Riesling’ vines from vineyards with a history of high BN occurrence were also compared with ‘Riesling’ vines from vineyards with low BN incidence.

MATERIALS AND METHODS

Time frame of BN development in ‘Riesling’ grown in Virginia (1994)

Vineyards: ‘Riesling’ vines at Somerset and Willowcroft Vineyards were examined for BN occurrence from May 1994 to August 1994. In 1993, Somerset Vineyard had 41% BN while Willowcroft Vineyard had an incidence of 2.5% BN.

The vines at Somerset Vineyard were trained to a unilateral cordon system. The spacing in the vineyard was 3.7 m (row) X 3.7 m (vine) with two vines per panel. Vines at Willowcroft

Vineyard were trained to a bilateral cordon system with three vines per panel spaced at 2.1 m apart in rows 3.1 m wide. Vines were spur-pruned and shoots were positioned to grow vertically upright in both vineyards. The shoot density on the vines was standardized to 15 shoots per meter of cordon, 15 days after budbreak. Randomized sampling of two representative shoots per panel was done from plots where shoot density was controlled.

Light Microscopy: Representative shoots from both vineyards were collected at six 10-day intervals, starting 46 days after budbreak. Shoots were transported to the lab where buds from nodes five through seventeen were excised under a dissection microscope (Bausch and Lomb 31-26-84). The scales and hairs around the primary and secondary buds were removed after which the buds were embedded in plastic. All general laboratory chemicals were obtained from Fisher Scientific (Springfield, NJ 07081-3193) and Sigma (St. Louis, MO 63178). Buds were fixed in 5 percent gluteraldehyde in sodium phosphate buffer (0.1M, pH 7.2) for 48 hours. Buds were then rinsed three times, 30 minutes each, in sodium phosphate buffer to remove the unreacted gluteraldehyde and passed through an ethanol dehydrating series (25%, 50%, 75%, and 95%) for 48 hours each, followed by two rinses in 100% propylene oxide for 12 hours each. The buds were then subjected to the embedding process where the buds were passed through 25%, 50%, 75%, 90%, 95%, and 100% Spurr's resin (Spurr, 1969) in propylene oxide for 24 hours each. The buds were subjected to vacuum at a pressure of 40.6 KPa for 15 minutes while in 25% and 50% Spurr's resin to increase infiltration of the resin into the buds. The embedded buds were then cured in 100% Spurr's resin at 60C overnight. The buds were trimmed by a specimen trimmer (Reichert-Jung TM 60, Leica Inc., Deerfield, IL 60015) and ultrathin sections (1-2µm thickness) of buds were made with a ultra-microtome (LKB 2128, Leica Inc., Deerfield, IL 60015) using a glass knife. The sections were stained with 0.5% toluidine blue in 50 mM sodium acetate, pH 4.5, mounted in permount and examined under a light microscope (Nikon Optiphot 111652, Garden City, NY 11530). For both 'Riesling' and 'Chardonnay', at least 10 buds were sampled at each sampling stage from all the vineyards examined.

Scanning Electron Microscopy: Representative shoots were sampled from 'Riesling' and 'Chardonnay' vines of Somerset and 'Riesling' vines of Willowcroft Vineyards and buds were excised from nodes five through seventeen. Samples were collected at 10-day intervals from 40 days until 90 days after budbreak. No less than ten buds were sampled at each sampling stage for both 'Riesling' and 'Chardonnay' from the two vineyards. Buds were excised from shoots; and scales and hairs were removed from around the primordia. The buds were then fixed in formalin-acetic acid-alcohol (FAA) for 48 hours. The buds were then passed through a dehydration series of ethanol (25%, 50%, 75%, and 95%). The dehydrated buds were bisected longitudinally through the center of the compound bud with a razor, and critical-point-dried using CO_2 in a Samdri 780A critical point drier. The dried bud tissues were mounted on stubs with silver paint, sputter-coated with a 15/20 nm layer of gold and palladium to give them a good electrical ground. The sectioned bud faces were then examined using a scanning electron microscope (SEM) (Cambridge Model S 120, Leica Inc., Deerfield, IL 60015) at various magnifications.

Comparison of 'Riesling' and 'Chardonnay' at Prince Michel Vineyard, Virginia and Foxrun Vineyard, New York (1995)

Vineyards: A second BN survey was conducted at Prince Michel Vineyard, Virginia from May to August 1995 with two cultivars, 'Riesling', a BN-prone cultivar (66.5% BN), and 'Chardonnay', a BN-insensitive cultivar (6.8% BN) (data from Wolf and Warren, 1995). For each cultivar, five panels of three vines were selected for examining the occurrence of BN in the axillary buds. The vines were trained to a bilateral cordon system with vertical shoot positioning. The vines were spaced at a distance of 1.1 m in rows 2.7 m wide. The shoot density was standardized to 15 shoots per meter of row at 30 days after budbreak. Buds were sampled randomly as described in the previous experiment at intervals of 10 days, starting at 50 days after budbreak, continuing to 90 days after budbreak. Buds were also sampled randomly from 'Riesling' vines of Foxrun Vineyard, in the Finger Lakes region of New York from five panels trained to a modified Keuka high renewal system at a spacing of 2.44 m between vines in rows 2.74 m wide. New York 'Riesling' vines had no known history of BN, and so they were selected for comparison with the BN-prone 'Riesling' vines of Prince Michel Vineyard, Virginia.

Light microscopy: As described above, the buds were fixed in 5% gluteraldehyde for 48 hours and then passed through a dehydrating series of ethanol (25%, 50%, 75%, and 95%). The buds were then passed through a graded series of Spurr's resin and finally cured overnight at 60°C in 100% Spurr's resin. Ultrathin sections were made as described previously, stained with 0.5% toluidine blue and examined under a light microscope.

RESULTS

Time frame of BN development in 'Riesling' of Virginia (1994)

The Somerset buds from nodes one through five on current season shoots generally appeared healthy throughout the growing season when viewed under a dissection microscope. Necrotic buds were generally observed in nodes distal to node five on the current season shoots. At 46 and 56 days after budbreak, all the buds appeared healthy. Beyond 60 days after budbreak some 'Riesling' buds had dark brown hairs, and the nodal cushion also appeared dark brown to black (40 to 90%) (Table 3.1). At 70 days after budbreak a gradual browning in 55% of primary buds was observed (Table 3.1). Occasionally secondary buds also developed a brown color. With subsequent samplings buds were found to have darker brown hairs. Beyond 80 days after budbreak, the scales, hairs, and 73% of the primary buds and 20% of the secondary buds developed a crumbly texture. The buds at this stage detached very easily from the stem. The nodes at the point of attachment with the primary and secondary axes were dark brown to black in color. The primary buds in some instances had aborted and the secondary buds had expanded to fill the space left by the primary buds. Development of necrosis in the Willowcroft buds was similar to the Somerset 'Riesling' vines. Only 20% of buds were necrotic among the Willowcroft 'Riesling' grapes (Table 3.1). Visual symptoms of BN in Willowcroft buds appeared much later (86 days) than the Somerset buds (66 days). The necrosis was usually confined to the primary axis within the compound grape bud. The secondary buds were usually healthy and green.

Table 3.1. Comparison of BN in Virginia 'Riesling' with Virginia 'Chardonnay' and New York 'Riesling'.

Location of Vineyard	Cultivar	Days after budbreak	Total buds examined	Buds with BN	BN[z] (%)
Virginia					
Somerset	Riesling	46 days	10	0	0.00
		56 days	10	0	0.00
		66 days	10	4	40.00
		76 days	11	6	54.55
		86 days	15	11	73.33
		96 days	10	9	90.00
Willowcroft	Riesling	66 days	10	0	0.00
		76 days	10	0	0.00
		86 days	10	2	20.00
		96 days	10	2	20.00
Prince Michel	Riesling	50 days	10	0	0.00
		60 days	10	3	30.00
		70 days	10	6	60.00
		80 days	10	8	80.00
		90 days	10	9	90.00
	Chardonnay	50 days	10	0	0.00
		60 days	10	2	20.00
		70 days	10	1	10.00
		80 days	10	0	0.00
		90 days	10	3	30.00
New York					
Fox Run	Riesling	50 days	10	0	0.00
		60 days	10	2	20.00
		70 days	10	0	0.00
		80 days	10	1	10.00
	Chardonnay	70 days	10	0	0.00
		80 days	20	0	0.00

[z]BN is the percentage of buds showing distorted and compressed cells under the light microscope from the total examined.

Light microscopy: Ultrathin sections (1-5μm) of buds examined under a light microscope appeared healthy without any cellular abnormalities at 46 days and 56 days after budbreak (Fig. 3.1A, B). At 66 days after budbreak (third sampling), 40% of Somerset 'Riesling' buds had a zone of distorted cells (Fig. 3.2). This zone was confined to one or two cell layers at the base of the primary axis within the compound grape bud. The cell walls appeared irregular and distorted. Further samplings at 76, 86 and 96 days after budbreak showed advanced necrosis. The zone of distortion had advanced to greater number of cell layers. The cells were flattened or compressed. The zone of compression was generally observed at the junction of younger cells at the base of the primary axis and cells of the nodal cushion that had undergone some secondary thickening (Fig. 3.3). At 76 days after budbreak, three to four layers of compressed cells were observed (Fig. 3.4A, B). In some buds, the compressed zone extended from the base of the primary axis to the base of the secondary axis. At 86 and 96 days after budbreak a more advanced stage of compressed cells was seen (Fig. 3.5A). The compression had advanced to the nodes of the primary axis within the lateral bud. Three to four zones of compressed cells, each seven to nine cell layers thick, were observed. This was followed by cell wall collapse (Fig. 3.5B). The primary axis in approximately 50% of the buds had completely disintegrated. In some buds, the primary axis as well as the secondary axis had separated from the nodal tissue. The vascular bundles were also highly distorted in some instances. Approximately 20% of the Willowcroft 'Riesling' buds developed necrosis at 86 days after budbreak as was evident from the occurrence of the compressed cells. However, in the Willowcroft 'Riesling' buds the compressed cells were observed as localized groups of cells and not as a zone (Fig. 3.6). The cells had distorted cell walls similar to the Somerset buds in the initial stages of necrosis development at 66 days after budbreak. But the cells did not become as compressed as the Somerset 'Riesling' buds.

Scanning Electron Microscopy: 'Riesling' and 'Chardonnay' buds were examined by SEM to ascertain whether tissue disintegration observed with light microscopy was an artifact caused by improper embedding and microtome sectioning. The bisected buds from Somerset 'Riesling' showed a zone of compressed cells at the junction of the young cells at the base of the primary axis and older cells with secondary thickening and pitting of cell walls in the nodular cushion (Fig. 3.7A). The zone of compression was observed at the base of the primary axis in 70% of the buds and, in 40% of the buds observed, under the secondary bud axis as well (Fig. 3.7B). Thirty percent of the buds had their primary axis separated from the nodal cushion (Fig. 3.8). Willowcroft 'Riesling' and Somerset 'Chardonnay' buds examined under the SEM lacked the compressed zones of cells observed in the Somerset 'Riesling' buds. The SEM observations of bud deterioration were consistent with the results of light microscopy.

Fig. 3.1. Bud sections of the primary axis of 'Riesling' vines without any cellular abnormalities (40X) at 46 and 56 days after budbreak. A) 'Riesling' buds from Somerset Vineyard were sampled at 46 days after budbreak on 30 May, 1994. B) 'Riesling' buds from Somerset Vineyard were sampled at 56 days after budbreak on 9 June, 1994. C) A section of grapebud with primary bud axis and two secondary bud axes illustrating the region of visual symptoms of BN.

Fig. 3.2. A) Distorted and compressed zone of cells (40X) first appeared at 66 days after budbreak on 20 June, 1994 at the base of the primary bud axis of 'Riesling' buds from Somerset Vineyard, Virginia. B) A section of grapebud with primary bud axis and two secondary bud axes illustrating the region of visual symptoms of BN.

Fig. 3.3. A) A 'Riesling' bud section from Somerset Vineyard sampled at 76 days after budbreak on 30 June, 1994. The zone of compression (10X) is visible at the junction of the nodal cushion where the cells have undergone secondary thickening, and the younger cells at the base of the primary axis. B) A section of grapebud with primary bud axis and two secondary bud axes illustrating the region of visual symptoms of BN.

Fig. 3.4. A) and B) Three to four distorted cell layers (40X) were observed at 76 days after budbreak at the base of the primary bud axis of 'Riesling' buds from Somerset Vineyard, Virginia; sampling done on 30 June, 1994. C) A section of grapebud with primary bud axis and two secondary bud axes illustrating the region of visual symptoms of BN.

Fig. 3.5. A) Seven to eight distorted layers of cells (40X) are visible at 86 days after budbreak in at the base of the primary bud axis of 'Riesling' buds of Somerset Vineyard; sampling was done on 11 July, 1994. The nodes of the primary axis are also affected at this stage. B) Sections of 'Riesling' buds of Somerset Vineyard sampled at 96 days after budbreak on 25 July, 1994 showing cell wall collapse (40X) at the base of the primary bud axis. C) A section of grapebud with primary bud axis and two secondary bud axes illustrating the region of visual symptoms of BN.

Fig. 3.6. A) Localized groups of distorted cells (40X) at the base of the primary bud axis of 'Riesling' buds from Willowcroft Vineyard at 66 days after budbreak; sampling done on 18 July, 1994. B) A section of grapebud with primary bud axis and two secondary bud axes illustrating the region of visual symptoms of BN.

Fig. 3.7. A) Scanning electron microscopy micrograph of a 'Riesling' bud from Somerset Vineyard showing compressed layers of cells (288X) at the base of the primary axis observed at 76 days after budbreak (30 June, 1994). B) Another scanning electron microscopy micrograph of 'Riesling' bud from Somerset Vineyard sampled at 96 days after budbreak on 25 July, 1994; shows a zone of compression (27.6X) at the base of the primary axis and extending to the base of the secondary buds as well.

Fig. 3.8. Scanning electron microscopy micrograph (23.1X) of a 'Riesling' bud from Somerset Vineyard sampled at 96 days after budbreak on 25 July, 1994; the primary axis and the secondary axis have separated from the nodal cushion.

BN development in 'Riesling' vs 'Chardonnay' (1995)

Prince Michel 'Riesling' buds also showed compressed zones of cells at 60 days after budbreak. The occurrence of BN was similar to the Somerset 'Riesling' buds. Initially, the cells were slightly distorted by irregular cell walls followed by transverse compression of the entire cell. As the summer progressed, the zones of compressed cells increased until the primary axis disintegrated. The occurrence of this disorder was similar to the Somerset 'Riesling' vines previously described. 'Chardonnay' had a very low incidence of BN (14.8%) compared to 'Riesling' (44.7%). The pattern of necrosis in 'Chardonnay' buds was similar to the Willowcroft 'Riesling' buds. BN developed as localized zones of compressed cells at the base of the primary axis of the compound lateral bud. New York 'Riesling' buds also showed some necrosis (10 to 20%) (Table 3.1). Localized regions of compressed cells, similar to Willowcroft 'Riesling' and Prince Michel 'Chardonnay' vines, were observed in New York 'Riesling' buds. Anatomical observations of the buds showed no visual evidence of BN 46 and 56 days after budbreak in 'Riesling' vines at Somerset Vineyard. Prince Michel 'Riesling' vines also did not show any necrotic symptoms 50 days after budbreak. The buds first started to become necrotic at 60 days after budbreak (Table 3.1). BN incidence increased to 90% at 90 to 96 days after budbreak. This percentage is much higher than the BN rating done at the end of the season (Chapter 4). 'Chardonnay', the BN-insensitive vines showed a much lower percentage of BN with 20% of buds showing necrosis at 60 days after budbreak and 30% of buds showing necrosis at 90 days after budbreak. However, 'Chardonnay' vines did show necrosis to some extent, as indicated by the distorted cells, in the BN-prone vineyard, Prince Michel. 'Riesling' vines of Foxrun Vineyard selected as a BN-insensitive population showed some necrosis as well, at 60 days after budbreak (20%). A few cells at the base of the primary axis appeared compressed and distorted. 'Chardonnay' vines from Foxrun Vineyard, New York sampled at 70 and 80 days after budbreak did not reveal any BN.

DISCUSSION

Anatomical observations of Virginia 'Riesling' vines revealed that deterioration of the primary bud began approximately 60 days after budbreak (≈15 days after bloom). The first visual symptoms of BN were observed by Lavee et al (1981) 20 days following full bloom for a two-week period. Morrison and Iodi (1990) also reported that primary BN started 3 to 6 weeks after bloom but continued until the onset of dormancy. BN in 'Riesling' in both Somerset and Prince Michel Vineyards first appeared 60 days after budbreak and increased with time until the onset of dormancy which was similar to the finding of Morrison and Iodi (1990). In the current study, the first visible symptom of BN was marked by the presence of bands of distorted cells with irregular cell walls followed by a transverse band of apparently compressed cells at the base of the primary bud axis of the compound lateral bud. Sometimes, the transverse band of compressed cells extended all along the base of the secondary buds as well. But in contrast to the findings of this study, Lavee et al. (1981) observed the occurrence of necrosis at the 3rd or 4th node of the primary bud axis of the central bud and the basal tissues were found to be healthy. Moreover, Morrison and Iodi (1990) found that some buds died from the base, others from nodes 1 to 4 nodes, and still others from the apex. In the current study necrosis first affected the basal tissues

of the primary axis and later advanced to the nodes of the primary axis resulting in drying of the primary axis. Occasionally, in cases where the secondary buds were not affected, the secondary buds expanded and filled the space left by the primary bud. This is important for instances where BN occurs early in bud development because there is some compensatory effect in yield by the secondary buds. However, more commonly, as reported by Lavee et al. (1981) and Morrison and Iodi (1990) revealed that the bud above the necrotic zone dries, while the basal tissues remained healthy. During excision, the primary axis could be easily detached at 70 to 80 days after budbreak from the nodal cushion. The cell compression was followed by cell breakage which is consistent with the observations of Morrison and Iodi (1990). Therefore, there are some cultivar differences observed between this study and previous literature regarding the point of occurrence of the first visual symptoms of BN in the primary axis.

The percentage of BN from the light microscopic studies was higher than the BN rating data obtained at the end of the growing season in October (Chapter 4). This could be due to limited sample size because just ten to twelve buds were dissected from nodes five through seventeen from two representative shoots per panel. Conversely, the BN assessment of vines done in October was conducted on all the nodes of 10 representative shoots per panel. BN was lower at the basal four nodes compared to the more distal nodes (Chapter 4), and so this might explain the higher BN numbers in this study. Another reason could be due to the occurrence of non-lethal lesions and therefore, recovery of a few buds by the end of the growing season.

SEM revealed the same compression zone confirming that the cell compression observed under light microscope was not an artifact created by embedding in plastic and/or microtoming. At higher magnifications, the zones of compression revealed several layers of cells with distorted cell walls. These zones of compression were observed only in the Somerset 'Riesling' buds and not in the Willowcroft 'Riesling' or Somerset 'Chardonnay' buds.
A higher percentage of BN was observed with Somerset and Prince Michel 'Riesling' vines compared to Prince Michel 'Chardonnay' and New York 'Riesling' and 'Chardonnay', indicating cultivar as well as possible environmental effects on the occurrence of BN. Some of the factors believed to be responsible for BN are shade (Perez and Kliewer, 1990, Perez, 1991, Wolf and Cook, 1995), vigor (Bindra and Chohan, 1975, Lavee et. al., 1981, Naito et al., 1986, Wolf and Cook, 1995), and high soil nitrogen (Bindra and Chohan, 1975, Perez, 1991). Therefore, one of these or other as yet unknown factors, are possibly responsible for the high BN observed in Somerset and Prince Michel 'Riesling' vines.

CONCLUSION

From these studies it can be concluded that BN usually occurred at nodes distal to node five. When viewed under a light microscope the buds appeared necrotic with distorted cell walls at approximately 60 days after budbreak in 'Riesling' vines. This was confirmed by the SEM studies. A compressed zone of cells was observed at the base of the primary axis at the junction of the younger cells of the primary axis and the older cells of the nodal cushion within the compound lateral bud. BN started as localized groups of cells with distorted cell walls, followed

by apparent compression of cell walls with a reduction in the volume of the cells, ultimately leading to cell breakage. Initially, the zone of compressed cells appeared at the base of the primary axis, and as the summer progressed, the compression zones advanced to the nodes of the primary axis. In some cases, the vascular bundles were also affected. Very few 'Chardonnay' buds showed distorted cells. 'Chardonnay' vines had a lower incidence of BN than did 'Riesling'. A higher incidence of BN in Somerset 'Riesling' and Prince Michel 'Riesling' vines was also observed, compared to Willowcroft and New York 'Riesling', during this survey. These findings suggest a cultivar and an environmental effect on BN occurrence and intensity.

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