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Research Note

Reductions in bud carbohydrates are associated with grapevine bud necrosis

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Introduction: Bud necrosis (BN) develops in the season of bud initiation (LAVEE 1987; MORRISON and IODI 1990; WOLF and WARREN 1995). Rapid shoot growth (WOLF and WARREN 1995), low tissue carbohydrate levels (VASUDEVAN *et al.* 1998), shade (MORRISON and IODI 1990; PEREZ and KLIEWER 1990; WOLF and WARREN 1995; VASUDEVAN *et al.* 1998), and elevated gibberellin-like activity (LAVEE 1987), have all been associated with increased BN incidence. Nevertheless, fundamental causes of BN remain uncertain.

Transient, artificial (PEREZ and KLIEWER 1990) as well as canopy (WOLF and WARREN 1995) shade increased BN, and suggested that localized deficiencies of essential growth and development substrates may lead to BN. Localized reductions in carbohydrates may, for example, occur in response to strong shoot tip sink strength (CANDOLFI-VASCONCELOS and KOBLET 1990), as with rapid shoot growth, which has been associated with BN incidence (WOLF and WARREN 1995). Here, as part of a larger study (VASUDEVAN 1997), we examined sugar and starch levels in buds of grape cultivars that differed in susceptibility to BN.

Materials and methods: Buds of Riesling and Chardonnay vines were sampled at 10-day intervals from 50 to 90 d after bud break (DABB) in 1995 to quantify BN development as a function of time and cultivar. Samples were collected from Prince Michel Vineyard in central Virginia, and Fox Run Vineyard in the Finger Lakes Region of New York State. Prince Michel Riesling historically expressed 60 to 90 % primary BN, whereas Prince Michel Chardonnay expressed less than 10 % BN (WOLF and WARREN 1995). Fox Run Vineyard vines had no known history of BN with either cultivar. Details of sampling and vine training are found in VASUDEVAN (1997). Buds were excised, fixed and embedded in plastic as described in VASUDEVAN *et al.* (1998). Embedded buds were sectioned (1-5 µm) and examined under a light microscope for visual evidence of tissue destruction (VASUDEVAN *et al.* 1998).

Riesling and Chardonnay vines of Prince Michel Vineyard and Riesling vines at the Agricultural Research and Extension Center (AREC), Winchester Virginia, were sampled at 10-day intervals (50 to 90 DABB) in 1995 for carbohydrate levels in bud, leaf, and stem tissues. Sampled vines were arranged in 5 randomly selected, 3-vine plots of each cultivar at Prince Michel, and 4 random-ly selected 3-vine plots at Winchester. Buds and leaves were collected from the first 17 nodes of two shoots from each plot at each sampling; stem tissue was obtained from the corresponding internodes. Vine training and analytical procedures are detailed in VASUDEVAN (1997). Tissue carbohydrates were analyzed by HPLC (VASUDEVAN *et al.* 1998).

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Starch deposition in buds of Riesling, Chardonnay, Syrah, and Viognier vines grown at the AREC, Winchester, VA was evaluated during the 1996 season. The mean ± SD (n = 30 buds) BN incidence for those vines in November 1995 was 3 ± 6 % (Chardonnay), 77 ± 20 % (Syrah), and 60 ± 23 % (Viognier); Riesling BN was not quantified. Vine spacing, age, and canopy management details are provided in Vasudevan (1997). Two representative shoots were randomly sampled from each cultivar at 10-day intervals, from 50 to 80 d after budbreak. Each shoot was divided into two sections: a basal section of nodes 1 to 6 and an apical section of nodes 7 to 13. Twelve buds from each section were longitudinally sectioned through the center of the bud axis with a razor. One half of the bud was then stained in iodine-potassium iodide (IKI) solution and examined under a dissection microscope for intensity of starch staining. The degree of starch staining was rated as: 1 = no starch staining observed; 2 = thin layer of stain in nodal tissue below primary bud axis/some stain in one or two prophylls of the primary bud; 3 = 50 % of nodal tissue and > 2 prophylls stained; 4 = most nodes of primary axis and most prophylls stained; 5 = all nodal tissue, all prophylls, and primary axis (nodes and internodes) deeply stained. BN was evaluated by node position in October by assessing 10 randomly collected shoots per plot from each of the cultivars and vineyards used in the above studies. Buds were cross-sectioned with a razor, and the primary buds were judged dead if they appeared dry, crushed, or darkened, and alive if green.

Data were analyzed for variance using SAS Institute (SAS INSTITUTE 1990) software. Percentage data were either square root-transformed or arcsin-square root-transformed prior to analysis of variance. Numerical starch rating data were analyzed using a multivariate repeated measures procedure (SAS INSTITUTE 1990).

Results and Discussion: Microscopic evidence of BN was observed as early as 60 d after bud break in Prince Michel Riesling, and appeared as discrete groups or zones of cells with pleated cell walls. The onset of symptoms and the seasonal frequency of affected buds was comparable, although slightly earlier, to that reported in our companion study (VASUDEVAN *et al.* 1998). The microscopic appearance of affected buds was comparable to other reports (MORRISON and IODI 1990; PEREZ and KLIEWER 1990; VASUDEVAN *et al.* 1998). The percentage of examined buds that exhibited tissue destruction 90 d after bud break at Prince Michel was 90 % for Riesling and 30 % for Chardonnay. The corresponding BN percentages assessed in the field that fall were 44 % and 15 %, respectively. By contrast, only 10 % of Riesling buds and 0 % of Chardonnay buds sampled from vines at Fox Run vineyard exhibited tissue destruction when assessed 80 d after bud break. The corresponding fall field assessment at Fox Run revealed 5 % and 0 % BN, respectively. Possible reasons for the greater number of buds judged to be BN-affected when viewed microscopically, as opposed to in the field, are offered in VASUDEVAN *et al.* (1998).

Sucrose, glucose, and fructose concentrations decreased, while starch concentrations increased with sample date for both Riesling and Chardonnay buds at both vineyards (Table). Sucrose, glucose, and starch concentrations were somewhat greater in Chardonnay than in Riesling buds

Table

Carbohydrate level in bud tissues (mg·g^{-1} dried tissue)[z] of Riesling and Chardonnay of Prince Michel Vineyard and AHS Agricultural Research and Extension Station, Winchester during 1995

Vineyard	Cultivar	Sampling stage[x]	Sucrose	Glucose	Fructose	Starch
Prince Michel	Riesling	50	8.0 a	3.4 a	3.0 a	0.3 b
	44 %[y]	60	8.6 a	3.2 a	2.4 a	0.3 b
		70	4.8 b	3.0 a	0.6 b	0.3 b
		80	4.8 b	3.0 a	1.0 b	0.4 a
		90	2.8 c	2.8 a	0.8 b	0.4 a
Prince Michel	Chardonnay	50	11.4 a	8.0 a	0.6 a	0.7 a
	15 %[y]	60	11.0 a	7.8 a	1.2 a	0.6 a
		70	8.8 b	7.4 ab	1.2 a	0.9 a
		80	6.6 c	7.0 ab	0.8 a	0.9 a
		90	5.6 c	6.6 b	0.2 a	0.9 a
Winchester	Riesling	50	6.8 a	6.2a	2.2 a	0.5 b
	50 %[y]	60	6.0 a	6.2 a	2.6 a	0.5 b
		70	5.8 a	5.6 ab	2.2 a	0.9 a
		80	5.6 b	4.8 bc	2.4 a	0.9 a
		90	5.2 b	4.6 c	2.0 a	0.8 a

[z] Means in columns followed by the same letter are not significantly different at $P < 0.05$ level; mean separation done using least squares means across sampling intervals by cultivar.

[y] Percentage BN evaluated on nodes 1 to 20 in October, 1995. Data were transformed by arcsin-square root transformation before analysis; however, the data presented are untransformed values.

[x] Sampling stage measured in days after budbreak.

at a given collection date with vines grown at Prince Michel. Riesling buds collected at Winchester had somewhat greater concentrations of glucose and starch than did Riesling buds collected from Prince Michel vineyard; however, statistical support of these observations was not possible. Concentrations of sugars and starch were higher in leaves and in stems than in corresponding buds at any given sampling stage (data not shown). Significant reductions of sucrose or starch could potentially impair growth and development of meristematic tissues, and lead to disorders such as BN.

With the exception of the last sampling date with Chardonnay, bud starch ratings increased in all cultivars with seasonal progress (Figure). The greatest apparent starch increase occurred between 60 and 70 d after budbreak (15 and 25 d after bloom). In addition to significant changes in starch accumulation over time, there were significant differences in the starch rating among the 4 cultivars at a given date: Chardonnay had the greatest apparent amount of bud starch deposition, followed by Riesling, Viognier, and Syrah (data not shown). The inverse relationship between starch rating and BN frequency is consistent with the report of MORRISON and IODI (1990), who observed reduced starch deposition in BN-prone Flame Seedless buds compared to BN-resistant buds. While it is premature to draw cause and effect relationships between tissue carbohydrate levels and the incidence of BN, the associative relationships observed here were similarly observed in artificial shading studies (VASUDEVAN *et al.* 1998). Localized carbohydrate deficiencies may therefore be a contributing factor to BN in grape.

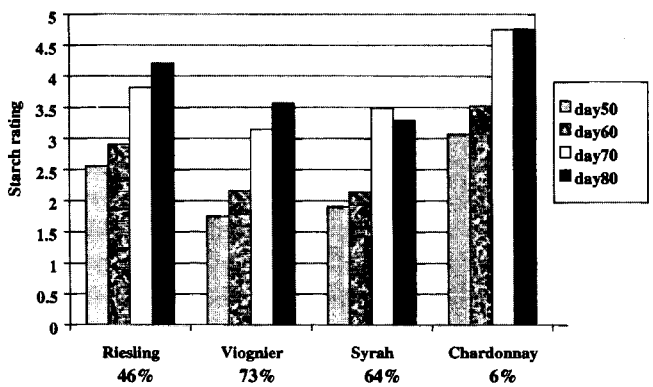

Figure: Comparison of bud starch levels as a function of date from 50 to 80 days after budbreak (dabb) among four grape cultivars at Winchester, VA during 1996. For each cultivar, the ratings for bud starch levels differed significantly ($P \leq 0.001$) between sample dates, except from 50 to 60 dabb and from 70 to 80 dabb for Syrah ($P \leq 0.05$) and from 70 to 80 dabb for Chardonnay ($P > 0.05$). Numbers under cultivar names are the percentages ($n > 150$ buds) of necrotic buds assessed in October 1996.

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