Environmental and Hormonal Effects on Development of Early Bunch Stem Necrosis

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Factors influencing the development of early bunch stem necrosis (EBSN) were studied using fruiting cuttings of Riesling and Cabernet Sauvignon grapes. Riesling shaded to 10%. 20%, 50%, and 100% full greenhouse light developed more EBSN than unshaded control, and reducing leaf area of Cabernet Sauvignon held at 50% full greenhouse light further enhanced EBSN development. Reducing irrigation frequency without inducing wilt or leaf fall also increased the disorder in Cabernet Sauvignon. Similarly, reducing nutrient levels to 20% of control promoted EBSN in the same cultivar, and, in a parallel experiment, sprays of foliar nutrients pre-bloom reduced the disorder. Field experiments with Gewürztraminer showed that ethephon and chlormequat applied pre-bloom increased berry set. Only the ethephon treatment increased EBSN. Neither influenced final yield. The level of ethephon-induced EBSN was reduced if bunches were avoided during spraying. These results suggest the disorder is exacerbated by conditions inducing stress and may be mediated by endogenous or exogenous ethylene. They also indicate practical ways to reduce EBSN

KEY WORDS: grapevine, early bunch stem necrosis, fruit set, leaf area, light, water, nutrition, ethephon, chlormequat, CCC

A physiological disorder which causes death of sections of the bunch of grapevines has been described and termed early bunch-stem necrosis (EBSN) (9). It mostly develops between the time the bunch is 2 cm long and capfall; sections and sometimes entire bunches are observed to shrivel and die. No pathogens have been found on affected tissues, and the disorder appears to have a physiological cause.

The condition has similarities to bunch stem necrosis (BSN - also called Stiellähme, shanking, waterberry, dessèchement de la rafle) but occurs much earlier in the ontogeny of fruiting. Causes of the disorder have not been established, but trials have shown that similar symptoms can be induced by the cations Ca^{++} and NH_4^+ applied as sprays pre-capfall (10).

EBSN doubtless contributes to the phenomena generally termed poor set or coulure which may also be a consequence of poor pollination and fertilization. Set can be improved by girdling and by spraying with chlormequat which appear to reduce the tendency for seedless berries to fall (4,8). However, poor set is increased by sprays of ethephon (12,18,19,20,21). When used to control growth it is recommended that the sprays be directed at the foliage and away from the bunches (13).

Using pot-grown vines, this work studied the effects of several environmental factors on the development of EBSN. In addition, the response of field-grown plants to sprays of chlormequat and ethephon in terms of berry numbers and EBSN are reported.

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Materials and Methods

Glasshouse studies: Cuttings of the grape cultivars Riesling and Cabernet Sauvignon were rooted at 25°C root-zone temperature. When rooted they were transferred to 1-L pots and treated by the technique of Mullins and Rajasekaren (17) to enable them to retain their inflorescences. The greenhouse used for the subsequent experiments transmitted 95% of ambient light and maintained air temperatures between 16°C and 25°C. Potting compost contained pulverized bark:sand in the ratio 80:20, and the following fertilizers were added to 100 L of mix: 600 g dolomite, 100 g superphosphate, and 200 g 3- to 4-month Osmocote (15-4.8-10.8). Osmocote is manufactured by Sierra Chemical Europe BV, Nijerheidsweg 5, Heerlen, The Netherlands.

For low-nutrient mixes, Osmocote and superphosphate levels were reduced to 40 and 20 g, respectively. In foliar nutrition trials, 4 mL of `Foliar Nitrophoska' (BASF, P.O. Box 407, Auckland, NZ) in 1 L water was sprayed to run-off. This is a complete fertilizer containing trace elements and 10%, 2%, and 6% of N, P, and K, respectively. Most pots were watered to capacity each day, but those where water deficiency was to be investigated were watered only on Monday, Wednesday, and Friday. Plants did not wilt, but vegetative growth was significantly reduced.

Field study: Ten-year-old vines (cv. Gewürztraminer) were used to evaluate the effects of chlormequat and ethephon sprays. Vines were cane-pruned on a 1.8-m vertical trellis. Sprays of both chemicals were applied at 300 ppm a.i. plus Tween 80 at 500 ppm. There were three times of application (see tables) and two methods: *(a)* to the whole canopy and *(b)* to the canopy above the fruit zone. The trial was a 7 (treatments) × 2 (spray

Table 1. Effect of plant nutrition on the severity of EBSN on fruiting cuttings of Cabernet Sauvignon.

Nutrient treatment	No. of plants	EBSN score	No. of berries
Low root-zone nutrition	70	4.90	31
High root-zone nutrition	70	3.00	73
Significance ($p < 0.01$)		**	**
No root-zone or foliar nutrition	21	5.04	Not recorded
No root-zone nutrition (foliar nutrition only)	21	2.76	Not recorded
Significance ($p < 0.01$)		**	

zones) factorial with six single-plant replicates in a randomized complete-block design.

General: Unless indicated otherwise, EBSN was visually estimated at three to four days after 95% capfall. Damage was recorded on a scale from 0 (no observable damage) to 10 (all parts of the bunch affected). Set was determined as indicated in the **Results** section. Unless otherwise stated, all greenhouse experiments were treated and recorded over the mid-summer period between 20 November and 15 January 1988.

Results

Glasshouse studies. ***Effect of plant nutrition:*** Seventy Cabernet Sauvignon vines were grown in pots with normal fertilizer; another seventy were in the low-nutrient mix. At harvest, the number of berries per plant was recorded. The low-nutrient plants displayed significantly ($p < 0.01$) more EBSN and fewer berries at maturity (Table 1).

To test the effectiveness of foliar-applied nutrients on similar fruiting cuttings, 21 plants without fertilizer in the potting medium were sprayed with Foliar Nitrophoska commencing when the flower cluster was between 2 cm and 4 cm long and repeated three times at four day intervals. Another 21 plants served as untreated controls. The effectiveness of this treatment regime was indicated by the clear reduction in EBSN severity (Table 1) and by the fact that the leaf yellowing noted at capfall in unsprayed plants failed to appear on those receiving Foliar Nitrophoska (data not shown).

Effects of water stress: The reduction in irrigation frequency from seven to three times per week commenced approximately three weeks before flowering of 48 Cabernet Sauvignon plants. An equal number of plants were watered daily.

Table 2. Effect of water stress on vine weight, early bunch stem necrosis (EBSN), and final berry numbers of Cabernet Sauvignon.

	Weight of above-ground portion of plant (g)	EBSN	Berry no. per plant
Normal watering	43.1	1.77	60.8
Water limitation	35.3	2.60	47.8
Significance	*	**	*

*$p < 0.05$; **$p = < 0.01$

Table 3. Effects of light intensity on early bunch-stem necrosis

Treatment: % of glasshouse light	EBSN 12 May 1987	EBSN at capfall
100	3.9 b	4.1 b
50	8.1a	8.4a
20	6.0 ab	9.3 a
10	3.3 b	10.0a

Treatments with no common letter were significantly different at the 5% level.

Reducing the frequency of irrigation did not result in obvious moisture stress (*e.g.,* wilting, leaf yellowing), but vegetative growth was reduced (Table 2). The severity of EBSN was increased by this treatment, and consequently, berry number at harvest was significantly reduced. Other data not presented has shown that tendrils are also sensitive to moisture stress, displaying a withering not dissimilar to EBSN.

Effect of light: Riesling vines were grown from budburst to flowering under different light regimes beginning 14 April 1987. Shading was provided by growing under Sarlon screens. Sarlon is manufactured to transmit a given proportion of ambient light; by using different sheets, transmission of 10%, 20%, or 50% of glasshouse light was achieved. Greenhouse light intensity over the period of the experiment varied from below 100 μEm^2s^{-1} on days with heavy cloud to 2000 on bright sunny days. There were 15 plants placed into each of these environments, and another 15 were unshaded within the glasshouse. EBSN was scored as described above and recorded on two occasions; the first was 12 May 1987, the second was done on individual plants when each was judged to be at capfall - in fact over a period of 10 days.

Midday temperatures were, on average, slightly reduced by shading. Mean maximum temperature was highest in unshaded plants at 24.9°C compared with 22.5°C in the 90% shade treatment (the coolest). Night temperature differences were less and in reverse order, *viz.* 16.4°C unshaded, 17.5°C for 90% shade.

There was some visual evidence of etiolation in the 90% shade treatment but not in the other treatments.

The final assessment at capfall (Table 3) indicated that high levels of shading induced more EBSN; in fact with 90%, shade no bunches survived to produce fruit. The different results shown on the earlier assessment can probably be explained by different rates of develop-

Table 4. Effect of leaf number on development of early bunch-stem necrosis (EBSN).

Treatment	EBSN score
One leaf	4.86 a A
Two leaves	2.71 ab AB
Eight leaves	0.71 c B

Treatments with no common letter were significantly different at the 5% level (lower case) or 1% level (caps).

Table 5. Effects of chlormequat (CCC) and ethephon (Ethrel)* on early bunch stem necrosis (EBSN), fruit set, and yield of Gewürztraminer vines.

Effects of chemicals	EBSN recorded ** 13 Jan. 90	Set recorded ** 13 Jan. 90	Berry no./ bunch 10 Apr. 90	Yield g/6 canes
Control (Tween 80)	1.75 a ***	6.17 a	70.4 a	600 a
CCC 21 Nov. 90	1.58 a	8.00 cd	102.1 bc	626 a
CCC 1 Dec. 90	1.33 a	8.25 d	117.5 bc	669 a
CCC 8 Dec. 90	1.50 a	8.25 d	99.0 b	561 a
Ethrel 21 Nov. 90	3.58 b	6.75 b	120.1 bc	629 a
Ethrel 1 Dec. 90	3.58 b	7.67 c	108.2 bc	597 a
Ethrel 8 Dec. 90	3.17 b	8.25 d	127.1 c	727 a
Effects of spraying whole plant or leaves only				
Whole plant sprayed	2.54 a	7.62 a	109.6 a	594 a
Leaves only sprayed	2.17 a	7.62 a	103.0 a	678 a

*CCC and Ethrel were each applied at 300 ppm a.i. with Tween 80 at 500 ppm.

**EBSN recorded on scale 0 = no EBSN to 10 = all bunch destroyed. Set recorded on scale 0 = no berries set, 10 = all berries set.

***Treatments with no common letter are significantly different at the 5% level.

ment. Increased shade slowed down growth rate and inflorescence development. Thus, the low degree of EBSN recorded on 12 May 1987 in 90% shade was due to the fact that development of the inflorescence was slow, and most EBSN did not appear until after that date.

Effect of leaf number: Riesling vines were grown in 50% shade to encourage development of EBSN, and three treatments were applied to seven plants, each in 1-L a pot. In Treatment 1 only one leaf was allowed to develop, in Treatment 2 two leaves were permitted, and in Treatment 3 no restriction was placed on leaf development; in fact, there was an average of eight leaves at capfall. Average individual leaf area was 18 cm^2. Results (Table 4) show a marked increase in EBSN as leaf area was reduced.

Effects of chlormequat and ethephon: The sprays shown in Table 5 were applied until run-off. The following phenological data indicate the bunch development stage at each spray date.

21 November 1989	Inflorescence 7 cm long
1 December 1989	Inflorescence 8 cm long
8 December 1989	Inflorescence 9 cm long, 20 - 30% capfall, shoots contained 14 - 15 nodes

The appearance of EBSN caused by ethephon was indistinguishable from that occurring without treatment. It was increased by ethephon at three dates prior to capfall. There was a slight, but non-significant, reduction of EBSN if ethephon was applied to leaves only. However, the interaction:treatment × place of application (not presented in table) was significant and suggests that the two later ethephon sprays, if applied to foliage only, caused less EBSN (2.8 cf 4.3 and 2.0 cf 4.3, respectively). Chlormequat had no significant effects on EBSN.

Set was estimated by visual means on those parts of the bunch not affected with EBSN on 13 January 1990. At harvest, it was found by counting berry numbers on two bunches from six canes of each replicate. Both sets of data indicate that both chlormequat and ethephon increased set. Despite these results the final crop weights from six canes per replicate per treatment were not significantly different from control.

Discussion

The evidence from pot-grown vines (Table 1) suggests that in situations of low fertility incidence of EBSN increases. The data does not distinguish if the presence of any one element is of special significance in preventing EBSN.

Shortage of water similarly predisposes plants to the disorder (Table 2). Visible stress symptoms were not apparent, but vegetative growth was reduced, and as a consequence of EBSN, berry numbers were lower.

Field trials have not been established to confirm these results, but a high incidence of the disorder has been noted in those local vineyards showing water or nutrient stress. A number of reports indicate increased yield with irrigation and nutrient application (1,3,11,14,16). These responses often are not attributed to any specific cause (bunch number, berry number, or size). Champagnol (5), in a review, states that for most cultivars improved mineral nutrition, especially nitrogen, will improve set and berry number; however, in those susceptible to coulure, nitrogen application can reduce berry numbers. It is possible that vigorous growth increased shade and thus EBSN, which was then reported as coulure. Other studies show that water stress close to flowering reduces berry numbers much more than stress after their time (7,15). Such papers do not record EBSN, so its role in yield modification can only be implied.

Low light (Table 3) and low leaf area per plant (Table 4) increased the incidence of EBSN. There were some methodological problems with the light experiment. It was not possible to shade plants individually, so all 15 pots in each treatment were under one structure. The experiment was analyzed as a complete randomized-design block which is not strictly appropriate and does not discount the influence of position within the greenhouse. Minor differences in temperature between treatments were also noted. Despite these qualifications the results are consistent with the author's field observations in Canterbury which indicate more EBSN occurs in shaded canopies. Thus it seems not unreasonable to implicate low light intensity in the development of EBSN. Branas (2) describes a condition termed 'coulure accidentale' which could be the same as EBSN and reports work which suggests it may be due to a shortage of carbohydrates - an idea not inconsistent with the effects of low light and reduced leaf area inducing the disorder.

EBSN is a difficult disorder on which to work, since its appearance is unpredictable. The author's experience suggests it is more likely to occur in cutting- than field-grown plants, but even here its severity can be variable. Some of the variability could be due to weather conditions, such as ambient light intensity, and we have noted that greenhouse-grown plants were more likely to succumb if grown in winter than in summer. For example in Table 3, 50% shade induced an EBSN score in Riesling of over 8; in Table 4, with maximum leaf number and 50% shade, it was below 1. In the former experiment EBSN developed in autumn (April), in the latter in mid-summer (January).

Chlormequat did not significantly alter the level of EBSN in field-grown vines, but ethephon doubled the level (Table 5). Ethephon-induced EBSN appeared to be identical to that found in non-treated plants. Reduced berry number following ethephon application close to capfall is well known, but the symptoms which are responsible for this loss are either not described or desribed only briefly. Ethephon has been said to reduce set (21), cause flower abscission (19), cause abscission of parts of the cluster (20), and induce withering or a caducous response of rachides or whole bunches (18). That EBSN was not recorded earlier as a separate disorder was probably due to failure to distinguish between such withering and abscission typical of EBSN and poor pollination and fertilization.

Despite increasing EBSN, ethephon also increased set so that final yield was not significantly altered. Both chlormequat and ethephon reduce vegetative growth, and for the former it has been proposed that diversion of metabolites may favor fruit set (6). It is possible that ethephon exerts its effect by the same mechanism. If increase of EBSN due to ethephon could be eliminated, the possibility of using the compound to increase set may have practical significance. Some evidence given in this paper suggests spray direction to the upper parts of the shoot will lower ethephon-induced EBSN.

Results indicate that EBSN is a response to stress, and this may be induced by low nutrient levels, low water, heavy shading, or severely reduced leaf area. Lombard (personal communication, 1989) has collected data which suggests that it is associated with high ammonium and nitrate levels in bunch tissue; other work indicates that it may be exacerbated by solutions of calcium and ammonium salts (10). Work in this laboratory (not reported) has indicated no response to sprays of indoleacetic acid, naphthalene acetic acid, benzyladenine, or gibberellin A_3. However, the response to ethephon indicates that environmental effects may possibly be mediated via the ethylene system.

Clearly, the work on EBSN is just in its infancy, and the data reported herein must be regarded as the preliminary stages in the understanding of a complex disorder. Nevertheless, results do indicate methods which, if applied in the field, could probably reduce the incidence of EBSN. Low light intensity accentuates the disorder and can lead to drastically reduced berry numbers and crop yield. In many canopies, especially where shoots are tucked between parallel wires, congestion may cause leaf shading over much of the shoot's length. Canopy management to reduce shading prior to flowering is an obvious antidote. In those districts where water stress can occur prior to flowering, irrigation can be recommended. Stress due to low nutrition may be overcome by general fertilizers application; alternatively, it may be assisted by targeting nutrients at this specific time using foliar sprays or fertilizer addition via the irrigation water. Reduced leaf area by hail or damage by insects and disease may possibly accentuate EBSN. The latter two are normally capable of being remedied.

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