Objectives:
To unravel the confused picture of mineral and hormone composition of leaves and bunches associated with bunchstem necrosis (BSN), seeking its cause and control.

Staff and facilities:
The project was supervised by Dr. B.G. Coombe, Reader in Horticultural Science of the University of Adelaide. The research was done primarily by Mr. Bruno Holzapfel, a visitor from Bonn University with B.G.C acting as an external supervisor of his Ph.D. candidacy. The vines used for the research were Flame Seedless grown under glass at Murray Bridge (Mr C Schuetz) and Cabernet Sauvignon grown at three sites - in the Waite Experimental Orchard, at Langhorne Creek (Mr M Cleggett) and at Lenswood (Mr G Weaver). A gas chromatograph with electron capture detector for measuring abscisic acid was purchased on ARC funds; minerals were assayed by the Waite IPC service.

Background:
BSN afflicts grapes in all viticultural countries. It is an important limitation to the production of some varieties of grapes in many regions in Australia. There is an enormous literature on BSN, most of it from Europe (Stellwaag-Kittler, 1983). Much of it is conflicting and the general conclusion is that the control of BSN is difficult and its cause has yet to be established. No pathogenic organisms have been found associated with it and the present consensus is that it is a physiological disorder of the localised calcium-deficiency type (Pearson and Goheen, 1988).

The only commercial treatment that has been partially successful against it is a series of vine sprays with Mg and Ca salts e.g. Brechbuhler and Meyer (1977). However, these treatments, widely used in Europe, have erratic effects and are generally ineffective in non-European vineyards. There is therefore a valid questioning of the proposal that BSN is caused by Mg/Ca deficiency.

Assessments of the damage caused by BSN in Australianvineyards are unavailable. This is both a reflection of the inadequate attention BSN has been given by industry and researchers, and of the lack of any useful control measures. It is contended that these omissions are directly related to the absence of an explanation of its cause; once the cause is discovered, research should eventually lead to the provision of control measures.

Methodology:
Recent findings have provided new ideas about BSN:

a. Nitrogen nutrition. Christensen and Boggero (1985) suggested that BSN may be due to ammonium toxicity effects in bunchstem tissue. They showed that BSN symptoms developed when NH4 concentrations exceeded 3 mg NH4-N per g tissue dry weight. The concept has been supported by similar research in New Zealand and Oregon (Jordan, 1989).

b. Hormone imbalance. The possibility of a connection between BSN and endogenous phytohormone levels was suggested by Theiler (1977) who found that gibberellin treatments at flowering reduced BSN symptoms at veraison. This finding did not lead to a commercially useful treatment but provided a background for other work on the role of hormones. Recently, BSN symptoms were induced by perfusing bunches with abscisic acid (ABA), a hormone with effects opposite to those of gibberellins (Baldocchino et al., 1987). Since ABA is regarded as a stress hormone, many new ideas present themselves.

The present research was designed to explore the interconnection, if any, between NH4, ABA and BSN under Australian conditions. This was approached, firstly, by testing the relationship of viticultural conditions to the endogenous levels of both compounds, and secondly, by treating bunches with both compounds as inducers of BSN and testing whether exogenous compounds would counter their inductive effects. As the work developed, attention returned to the concentration of mineral nutrients in bunchstem tissue.

Results and Discussion:

1) Flame Seedless growing in a glasshouse in Murray Bridge and showing severe BSN symptoms had a reduced incidence of BSN after being sprayed with MgS04. This effect was significant and is the first response of this type reported in Australia. However the response was not enough to prevent the grower from uprooting the vines because of unprofitability due to BSN occurrence. The vines showed leaf symptoms resembling Mg deficiency, probably because they were young and therefore with a limited root distribution.

2) Cabernet Sauvignon vines grown in three regions by diverse viticultural methods showed a widely different incidence of BSN between regions and years (Table 1) confirming the picture reported in the literature. Unlike the response to Mg mentioned above, three years of Mg spraying failed to reduce BSN; it was noted that these vines showed no symptoms of Mg deficiency. A new finding was the significantly lessened incidence shown in minimally-pruned vines compared with spur-pruned; such a difference may be associated with the divergent physiology of their two types of shoot growth (Downton and Grant, 1992). These plots were invaluable for the subsequent research comparing mineral and hormone differences.

3) Analyses of free NH4 in bunchstems of Cabernet in the samples reported in Table 1 showed that the concentrations were 10 to 100 times lower than the values reported by Christensen and Boggero (1985) and showed no correlation with BSN incidence. The Waite vines, with no BSN, had the highest level of bunchstem NH4 and also especially high concentrations of releasable NH4. (The major contributor to the latter was found to be the amine, glutamine; this is an interesting result since the adjacent berry tissue accumulates arginine more than glutamine). These results do not support the notion that BSN is due to NH4 toxicity.

4) Analyses of cis,trans abscisic acid (ABA), in the same samples as used in 3), resembled those of NH4 in showing highest levels where BSN was nil. These results do not support the suggestion that BSN is caused by an increase in ABA. The concentrations of ABA in bunchstems, and of the trans,trans isomer t-ABA, were similar to those found by Baldocchino et al. (1987).

5) Cabernet bunches at veraison were perfused by a wick method with four concentrations of either ABA, NH4 or agmatine (since NH4 can be toxic, agmatine was included as it releases NH4 when metabolised to the polyamine putrescine). The effects on BSN and on endogenous levels in bunchstems of ABA and NH4 are shown in Table 2. The results confirm that administration if ABA does induce BSN, as was shown by Baldocchino et al (1987), but that this effect was not accompanied by an increase in endogenous NH4, either free or releasable. This supports the suggestion in 3) that NH4 is not acting as a causal agent.

6) In a parallel experiment, perfusion of NH4 or agmatine also induced BSN but here there was an associated significant increase in the endogenous level of ABA (Table 2). Further, another inducing compound, the auxin BTO, resulted in an increase in the levels of endogenous ABA (see Figure 1). These results would permit the conclusion that ABA may be the causal agent (but note the conclusion in 4)).

7) In other perfusion experiments, ABA, NH4 and BTO treatments, as inducers, were followed by Mg and Ca treatments as possible countering agents. The results in Table 3 show that the addition of Mg and Ca did not reduce the level of BSN following treatment with ABA, NH4 or BTO; in fact, they did the reverse - both cations increased the small amount of BSN following NH4 and BTO to the level caused by ABA. This result raises further questions about the role of Mg and Ca in this disorder.

   In one of these experiments the level of endogenous ABA was assessed and found to be high where BSN was high (Figure 1), agreeing with the conclusion in 6).

   Analyses were also made of endogenous NH4 in these experiments; these showed that all bunchstems had the same level of free NH4 but, wherever BTO was perfused, the level of releasable NH4 was lowered by two thirds. This effect was not related to any difference in BSN, and no explanation can be offered for it.

8) The role of minerals other than Ca, Mg and NH4 was explored further by comparing their levels in bunchstem tissues. There is only one published paper in which this has been done extensively by Redl (1983) in Austria; his analyses show that bunchstems have generally lower levels than do leaves. Analyses of Australian Cabernet bunchstems showed generally similar levels to those found by Redl except that the nutrients Ca, Mg, Zn and Mn were lower. Of more interest were the differences in mineral level between the experimental plots. Simple linear regression analyses of concentration and BSN percentage showed poor correlations; the best was Na which accounted for 32% of the variation; of the others, Mn accounted for 25%, NH4 17%, Ca 13% and others had less than 10%. However multiple regressions gave useful relationships. 'Modified backward elimination' yielded the following 'best' formula, accounting for an encouraging 66% of the variance :

$$\% \text{ BSN} = k - (0.037 * \text{Fe}) - (0.033 * \text{Mn}) + (0.00244 * \text{Na})$$

   The factor eliminated last was Zn and before that ABA, Mo, Mg, NH4, Ca in that order - but the complex formula did not account for any greater proportion of the variance. This formula shows that, under the test conditions, high Na and low Mn and Fe appear to be associated with high levels of the disorder.

9) Trials are in progress currently (1993 vintage) to test the effects of treatments with multiple mineral sprays.

Conclusions:

The aims of finding the cause and control of bunchstem necrosis (BSN) have not been met, but encouraging progress has been made towards both aims. The disorder in Australia differs from that in Europe in being without symptoms of magnesium (Mg) deficiency and unresponsive to Mg and Ca sprays. Some support was found from treatment and analytical work that the stress hormone, abscisic acid (ABA) could be causal except that endogenous ABA levels were high in bunchstems where BSN was absent. No support was found for the idea that NH4 toxicity causes BSN. NH4 perfusion of bunches did increase its incidence, as did the auxin BTO, but high NH4 levels, natural or induced, did not correlate with BSN incidence. Nevertheless, the possibility remains that nitrogenosus compounds may be involved because of the potent stimulation of BSN caused by the polyamine precursor, agmatine.

Finally, the possible involvement of multiple mineral deficiencies has been shown by correlations between BSN incidence and the levels of several minerals in bunchstems. These leads hold potential for unravelling the problem.

Table 1

Natural incidence of BSN (% bunches affected) in Cabernet Sauvignon vines grown in different regions, years and with different training

Region	Pruning	1990	1992
Southern	spur	26	11
	minimal	12	1
Hills	spur	1	3
Waite	spur	0	0
	minimal	0	0

Table 2

Generalised effects of perfusing bunches at veraison with ABA and NH4 or agmatine

Perfused substance	BSN incidence	Effect on concentration of	
		ABA	NH4
ABA	++	+++	0
NH4, agmatine	+++	+	+++

+ = increased, 0 = no change

Table 3

Incidence of BSN (%) after perfusion of bunches at veraison with a combination of inducing agents (ABA, NH4 and BTO) and possible countering cations (Mg and Ca). Mean of three experiments.

Chemical	H20	Mg	Ca
H20	0	2	0
ABA	12	14	12
NH4	2	11	11
BTO	3	17	19

Figure 1.

Endogenous levels of ABA in the bunchstems of Cabernet after perfusing mixtures of H20, NH4S04 and BTO followed by H20, MgS04 and CaCl2, and the amount of BSN that developed.

a = NH4 + Mg
b = BTO + Mg
c = BTO + Ca