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Exogenously Applied Abscisic Acid Did Not Consistently Delay Budburst of Deacclimating Grapevines

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Abstract

An experimental formulation of abscisic acid (ABA; Valent Biosciences VBC-30025) was evaluated for potential to delay budburst of *Vitis vinifera* L. winegrapes. Five experiments were conducted during 2004 and 2005 on dormant grapevine cuttings, container-grown vines, and field-grown vines to evaluate rates and/or timing of ABA applications. Two application methods were tested – spray application to buds or soil application. Spray application of ABA solutions to unopened buds increased the number of days to budburst by 3.5 days in one of four laboratory trials on single-bud cuttings, and delayed budburst by one day in established field-grown vines in one of two years. Further study to enhance the efficacy of ABA spray applications should examine materials or methods to improve penetration of ABA through bud scales. Soil applications of ABA to container-grown vines provided the greatest delay in budburst (up to 7 days) and gave the most consistent response. Soil application of ABA to established field-grown vines, however, produced no response and this may not be a practical or economical application method for commercial vineyards.

Introduction

Spring frost damage is a significant production risk in many grape growing regions of the U.S. and crop losses can negatively impact vineyard profitability. Frost protection methods are sometimes employed including wind machines, heaters, and various forms of sprinkler irrigation, but these methods are expensive, present logistical difficulties, and have limitations on the extent of protection provided against cold temperatures.

Often, damage from a spring frost could be avoided if grapevine budburst were delayed until after the last frost event. Closed buds are more frost tolerant than green shoots or opening buds in various stages of development (9). Grape cultivars differ in their time of budburst and vineyard managers commonly use this characteristic to plant grape cultivars with later budburst in their most frost-prone locations and earlier cultivars in the least frost-prone sites. Few management practices are available to postpone budburst. Delayed pruning or double-pruning methods can be used to postpone budburst a few days, but these present additional costs and logistical problems for larger vineyards. Budburst has been delayed by lowering soil and air temperature within the vineyard by evaporative cooling with sprinkler irrigation (12), but this method is unavailable to growers without irrigation and may present logistical difficulties.

Manipulation of the time of budburst in grapevines has been demonstrated experimentally by application of certain chemical compounds and plant growth regulators. Budburst has been advanced and its uniformity improved with the application of hydrogen cyanamide in viticultural regions with inadequate chilling temperatures to break dormancy (11, 25). Budburst delay has been achieved experimentally with the application of mineral oils (11), vegetable oils (3, 8), alginate (3), cycocel (7, 22), gibberellin (20, 21), and ethephon (13, 19, 23), but none

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of these materials has been utilized in commercial production to delay budburst. The response to applied ethephon was related to enhanced synthesis of abscisic acid (ABA) within grapevines (13). ABA has long been considered to play a role in the induction and maintenance of bud dormancy in grapevines and numerous reports have demonstrated ABA levels to be related to dormancy of grapevine buds (1,2,4,5,6,10,16)

Exogenous ABA was reported to delay budburst by Weaver et al. (23). Grape cuttings collected after the chilling requirement had been met were immersed in one of three concentrations of ABA, then placed in containers with 7.6-10.2 cm water and observed for bud growth twice per week. Of the 30 cuttings receiving treatment with 10, 100, or 1000 mg/L ABA, 15, 8, and 0 (respectively) had exhibited budburst after 34 days compared to 17 of 30 cuttings of the untreated control. After 69 days, the number of cuttings with budburst was 24, 26, and 21 respectively. Thus the two higher ABA concentrations delayed budburst without significantly inhibiting their eventual growth.

No reports of exogenous ABA applications to field-grown vines for budburst delay were found in the literature. A likely factor restricting field applications of ABA has been the absence of a practical commercial formulation of the growth regulator. Recently, the experimental ABA formulation VBC-30025 has been made available for testing by Valent BioSciences Corporation. The demonstrated potential for exogenous ABA to delay budburst in grapevines strongly suggests that it should be tested for its efficacy and potential utility in commercial vineyards.

Materials and Methods

VBC-30025 (Valent BioSciences Corporation, Libertyville, IL) was formulated as a crystalline powder containing 90% (+)-cis, trans-abscisic acid. Test solutions were prepared by dissolving appropriate quantities of VBC-30025 in 100 ml ethanol and diluting with distilled water. Budburst for all experiments was defined as the point at which the first leaf had fully released from the bud base (24), corresponding to growth stage 11 (14). The number of days from the start of the experiment to budburst was calculated for each treatment and the total number of open buds was counted at the completion of each experiment. Analysis of variance for treatment effects was conducted for days to budburst and total budburst for all experiments. Linear regression analysis was conducted when 3 or more rates of ABA were tested.

Preliminary ABA rate and timing trial. This study was designed and conducted in 2004 as a factorial experiment with 4 rates of ABA and 5 application dates (timing). Treatments were replicated 3 times in a completely randomized design with each plot consisting of 10 single-bud cuttings of 'Sangiovese' (clone 3) collected from a commercial vineyard near Ropesville, Texas. Cuttings were collected on 8 February, stored overnight at 2°C and the experiment was begun on 9 February. All cuttings were placed in propagation trays containing moist vermiculite and maintained at room temperature (20-21°C) for spray applications, bud development and budburst assessment. Rates of applied ABA were: 0, 10, 100, and 1000 mg/L prepared as solutions with 0.5 ml/L of Tween 20 (Fisher Scientific, Hampton, NH). Timing treatments consisted of single spray applications of ABA made at one-week intervals for four weeks (0, 7, 14, 21) and one double treatment (0+21) receiving ABA applications three weeks apart. Treatments were applied using a hand sprayer until each bud was thoroughly wetted to the point of dripping. Budburst of cuttings was assessed every two days. Data were analyzed by two-way ANOVA with rates and timing as the main effects.

ABA Rate and timing trial on field-grown grapevines. This field study was designed as a factorial experiment with 3 rates of ABA and 4 application dates (timing) in a completely randomized design. Treatments were replicated 4 times with each plot consisting of 3 mature (6th leaf) vines of 'Sangiovese' (clone 3) grafted to 5C rootstock growing in a commercial vineyard in Ropesville, Texas. All vines were pruned to 12 two-bud spurs, six spurs per cordon. All plots received the same management practices throughout the season.

Solutions were prepared to contain ABA rates of 0, 100, and 1000 mg/L with 0.5 ml/L of Tween 20. Application treatments were initiated approximately 19 and 25 days prior to budburst in 2004 and 2005, respectively. Timing treatments included three application dates at 7-day intervals (0, 7, 14) and a double application treatment (0+14) that was initially sprayed at the earliest application date and a second time at the last application date. ABA solutions were applied using a hand spray bottle until each two-bud spur was thoroughly wet. Budburst was assessed every three days. This experiment was repeated in 2005 using the same treatment plots and experimental procedures as in 2004.

ABA spray application to cuttings at wool stage. In 2005 we evaluated ABA applications at the "wool" stage of bud development, growth stage 5 of Meier (14). The experiment was conducted in a series of 3 identical trials on dormant single-bud cuttings of 'Sangiovese' (clone 3) obtained from a commercial vineyard in Ropesville, Texas. Dormant canes consisting of node positions 3 to 4 were collected from the field for trials 1, 2, and 3 on the following dates: 19 January, 14 February, 10 March. Canes were prepared into single-bud cuttings and a large quantity were placed with their basal ends submerged in a water bath held at room temperature (approximately 22°C) in the laboratory. Bud development was closely monitored and when sufficient numbers of cuttings had buds at stage 5, 100 of these cuttings were selected and 20 cuttings were randomly assigned to each of five treatment groups. Treatments consisted of spray applications of ABA rates: 0, 100, 250, 500, and 750 mg/L, plus 0.5ml/L Tween 20. Treatments were applied external to the water bath and the cuttings were replaced into the water as soon as spray treatments were observed to be absorbed into the bud. Cuttings were maintained in the water bath throughout the trial and observations of budburst were made every two days.

ABA soil application to container-grown grapevines. ABA was applied to soil of container-grown vines with 4 rates of ABA on two grape cultivars: 'Sangiovese' (clone 3) and 'Cabernet Sauvignon' (clone 4). Plants were one-year-old, dormant, bare-rooted vines grafted on 1103P rootstock and planted in Metro-Mix 700 growing medium (Sun Gro Horticulture, Bellevue, WA) in 3-gallon containers. Treatments were replicated 6 times per cultivar in single-vine plots arranged in a completely random design. Treatments were four rates of ABA (0, 10, 100, 1000 mg/L) applied in a one-liter solution to the soil at the time vines were planted into containers. A second ABA application was made one week later at the same volume and rates for each treatment. This experiment was repeated in 2005 using the same plants and procedures used in 2004. ABA treatment rates (0, 10, 100, and 1000 mg/L) were applied in a one-liter solution to the soil three days after the container-grown vines were removed from cold storage in 2005. A second, identical ABA application was made one week later at the same volume and rate for each treatment. Vines were monitored every two days for budburst.

ABA soil application to field-grown grapevines. In 2005 we evaluated ABA soil application treatments to field-grown vines of 6th leaf 'Sangiovese' vines in a commercial vineyard in Ropesville, Texas. The soil type is a Patricia loamy fine sand, a thermic Aridic Paleustalf (15). Treatment plots consisted of single-vines replicated 6 times in a completely randomized design. ABA treatments were applied to the soil at a rate of 0, 250, 500, or 1000 mg/L. Soil application treatments were prepared in 8 liters of water and applied twice, 7 days apart beginning approximately 2 weeks prior to expected budburst for untreated vines. Vines were observed twice per week to assess budburst.

Results and Discussion

Preliminary ABA rate and timing trial. Single-node cuttings of 'Sangiovese' treated with spray applications of ABA at different rates and times required an average of 27.7 days to reach budburst from the beginning of the experiment (Table 1). Two-way ANOVA demonstrated that the main effect of ABA rate was non-significant and the main effect of application timing was significant ($p < 0.05$). The interaction of rate x timing was also significant at $p < 0.05$.

Cuttings treated with ABA rates of 0, 10, 100, or 1000 mg/L did not differ in time required to attain budburst (Table 1) defined as growth stage 11(14). The percentage of buds that were open at the completion of the experiment (total budburst) was not affected by ABA rate (Table 1). However, total budburst was low (44%) for all treatments in this experiment, despite a high rate of budburst (95%) in field-grown vines that were the source of the cuttings. We attribute the low budburst rate in the laboratory to inadequate water availability of cuttings placed in moist vermiculite. This problem was resolved in subsequent experiments by placing cuttings in a water bath.

Timing of ABA applications significantly influenced the number of days to budburst (Table 2). Later applications (Day 21 or Day 0 + 21) delayed budburst by about 8 days compared to the earliest ABA treatment (Day 7). There was no difference between the single (Day 21) and double (Day 0 plus Day 21) late application treatments. The significant interaction of rate x timing indicates that days to budburst was most responsive to later applications of higher rates of ABA. Total budburst was also influenced by timing of ABA treatments, with later applications showing the greatest reduction of the total number of buds that opened (Table 2). The apparent inhibition of budburst by ABA was not observed in any subsequent experiments and perhaps was influenced by the reduced water availability to cuttings in this trial.

ABA rate and timing trial on field-grown grapevines. Field-grown 'Sangiovese' grapevines subjected to an ABA rate and timing experiment over two seasons took an average of 19.7 days after the first treatment was applied to reach budburst in 2004 and 24.7 days in 2005 (Table 3). Total budburst assessed at the end of the experiment was high in both years, with a mean of 23.7 (99%) open buds in 2004 and 20.7 (86%) in 2005. No inhibition of budburst by ABA was observed; experimental treatments were not significantly different in total budburst in either year.

Table 1. Mean number of days to budburst and total budburst of single-bud cuttings of 'Sangiovese' grapevines treated with four rates of ABA in 2004.

ABA (mg/L)	Days to budburst[z]	Total budburst[y] (%)
0	28.5	48.7
10	25.7	43.3
100	27.4	47.0
1000	29.1	37.0

[z]Mean number of days from Day 0 to growth stage 11.

[y]Percentage of open buds out of 30 at completion of experiment.

Table 2. Mean number of days to budburst and total budburst of single-bud cuttings of 'Sangiovese' grapevines treated with a spray application of ABA by timing of treatment application in 2004.

Time of ABA application	Days to budburst[z,y]	Total budburst[x,y] (%)
Day 0	24.0b	52.0a
Day 7	23.6b	61.0a
Day 14	27.5ab	44.9ab
Day 21	31.3b	30.8b
Day 0 plus Day 21	32.2b	30.8b

[z]Mean number of days from Day 0 to growth stage 11.
[y]Means, within a column, followed by different letters are significantly different at $p < 0.05$ by Fisher's Protected Least Significant Difference Test.
[x]Percentage of open buds out of 30 at completion of experiment.

ABA rate had a significant, but slight effect on number of days to budburst in 2004. Both rates of ABA (100 and 1000 mg/L) delayed budburst compared to the untreated control, but only by a little more than one day (Table 3). Timing of ABA had no significant effect on days to budburst in either year and the interaction of rate x timing was non-significant.

Table 3. Mean number of days to budburst of field-grown 'Sangiovese' grapevines treated with three rates of ABA applied as a spray application to dormant buds in 2004 and 2005.

ABA (mg/L)	Days to budburst[z,y]	
	2004	2005
0	18.9b	24.6
100	19.9a	24.7
1000	20.3a	24.8
F	5.63**	ns
R^2	0.0426*	ns

[z]Mean number of days from Day 0 to growth stage 11.
[y]Means, within a column, followed by different letters are significantly different at $p < 0.05$ by Fisher's Protected Least Significant Difference Test.
**ABA rate significant by ANOVA at $p < 0.01$.
*Linear regression significant at $p < 0.05$.

ABA spray application to cuttings at wool stage. Mean total budburst of single-bud cuttings (20 per treatment) was generally good in this laboratory experiment, averaging 16.6 (83%), 14.6 (73%), and 19 (95%) for trials 1, 2, and 3, respectively. There was no effect of experimental treatments on total budburst in any of the trials, indicating that ABA rates were not inhibitory to budburst. Across all treatments, the number of days to reach budburst following treatment applications was consistent for trials 1, 2, and 3, averaging 11.8, 10.7, and 11.8, respectively. Days to budburst were considerably less in this experiment compared to others because applications were made at the wool stage (stage 5) of bud development.

ABA spray applications to stage 5 buds significantly delayed budburst in only one of three trials. In trial 3 (Table 4), ANOVA demonstrated a significant effect of ABA rate on days to budburst, with all ABA rates reaching budburst later than the untreated control. Linear regression of ABA rate on days to budburst was also significant, although R^2 was quite low (0.0520). Spray applications of ABA to stage 5 buds resulted at best in only a few days of delay in budburst and the response was inconsistent among trials.

Table 4. Mean number of days to budburst of 20 single-bud cuttings of 'Sangiovese' grapevine treated with a spray application of five rates of ABA in 2005.

ABA (mg/L)	Days to budburst[z]		
	Trial 1	Trial 2	Trial 3
0	9.3	9.6	9.5
100	12.1	13.4	11.9
250	11.5	9.0	13.3
500	13.8	10.4	11.4
750	12.3	11.2	13.0
F	ns	ns	4.24**
R^2	ns	ns	0.0520*

[z]Mean number of days from time of treatment at growth stage 5 to growth stage 11.
**ABA rate significant by ANOVA at $p < 0.01$.
*Linear regression significant at $p < 0.05$.

Overall, spray applications of ABA (VBC-30025) to deacclimating buds gave inconsistent results for delaying budburst of grapevine. The same formulation of ABA was also reported to give variable response across seasons and table grape cultivars when applied to ripening fruit to enhance berry color development (17, 18). ABA is known to be readily inactivated by sunlight, which may contribute to inconsistent results from exogenous applications. In our experiments, another limiting factor could be the inability of spray solutions to rapidly penetrate the bud scale layer. The use of spray adjuvants to improve penetration of ABA through bud scales should be investigated.

ABA soil application to container-grown grapevines. Mean total budburst of container-grown grapevines was high in both years of this study, ranging from 92-100% for three of the trials and 83% for 'Cabernet Sauvignon' in 2004. There was no effect of ABA treatments on total budburst in any of the trials, thus our ABA rates were not inhibitory to budburst (Table 5). The number of days to reach budburst following treatment applications was comparatively short in the first year of this study and similar for both cultivars ('Cabernet Sauvignon': 12.7 days, 'Sangiovese': 12.6 days), suggesting that buds were already in later stages of development at the time of treatment. In year two, days to budburst were longer than year one and more consistent with field observations of the two cultivars; 'Sangiovese' broke bud in 16.8 days and 'Cabernet Sauvignon' in 22.3 days, averaged across all treatments.

Soil applications of ABA to container-grown vines consistently increased the number of days to budburst of 'Cabernet Sauvignon' compared to untreated control vines. For both years of this trial (Table 5), the highest rate (1000 mg/L) of ABA significantly delayed budburst of 'Cabernet Sauvignon' by 3 to 7 days compared to the untreated control and the low rate (10 mg/L) of ABA.

'Sangiovese' vines treated with the higher rates of ABA also demonstrated delayed budburst compared to untreated control vines in one of two years. In 2005, ABA at 100 and 1000 mg/L delayed budburst by almost 3 and 6 days, respectively, compared to the control and the low rate (Table 5).

ABA soil application to field-grown grapevines. Field-grown 'Sangiovese' vines exhibited a high level of total budburst in this trial, averaging 89.5% across all treatments in 2005. The average number of days to reach budburst following initiation of treatment applications was 17.2 days. In contrast to the

Table 5. Mean number of days to budburst for container-grown grapevines treated with a soil application of 4 rates of ABA in 2004 and 2005.

ABA (mg/L)	Days to budburst[z]			
	'Cabernet Sauvignon'		'Sangiovese'	
	2004	2005	2004	2005
0	11.3	20.3	12.0	13.3
10	8.8	20.7	10.0	15.0
100	11.8	22.8	12.0	17.7
1000	19.0	25.5	16.5	21.2
F	3.24*	7.03**	ns	9.92***
R^2	0.3671**	0.4390***	0.2505*	0.4797***

[z]Mean number of days from time of treatment to growth stage 11.
ABA rate significant by ANOVA at $p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$.
Linear regression significant at $p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$.

results achieved with container-grown vines, soil applications of ABA to field-grown vines were not effective in delaying budburst compared to untreated control vines.

Several factors of importance were different in the two soil application experiments that could have contributed to varying experimental results. There were large differences between the commercial potting mixture used in containers and the field soil at the vineyard site, and potential interactions of exogenous ABA with physical, chemical, and microbial components of the soil are unknown. Furthermore, field-grown and container-grown 'Sangiovese' were grafted onto different rootstocks (5C versus 1103P, respectively), making comparison of results between experiments problematic. There was also a large difference in the effective area of treatment. Soil application treatments to container-grown vines wetted the entire rootzone of the small vines, whereas only a small portion of the rootzone of large, field-grown vines was treated with soil application of ABA despite a higher total volume per treatment. Treating a significantly larger portion of the rootzone in the field with ABA may be impractical and uneconomical.

Conclusions

Exogenous application of an experimental formulation of ABA (VBC-30025) to grapevines prior to budburst demonstrated inconsistent effects on delaying the time of budburst. Spray application of ABA solutions to unopened buds induced only a short delay (1-3 days) in budburst and the response was inconsistent. ABA did not inhibit budburst at the rates used in this study. Rapid penetration of spray solution through bud scales may be a limiting factor for efficacy; evaluation of spray adjuvants to improve penetration and potentially enhance efficacy of ABA warrants further research. Soil applications of ABA were effective in delaying budburst of container-grown vines, but the lack of a similar response in established field-grown vines suggests that this method may be impractical for commercial vineyards.

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