Foliar Application of Absciscic Acid Induces Dormancy Responses in Greenhouse-grown Grapevines

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Abstract. The purpose of this study was to investigate the influence of foliar application of abscisic acid (ABA) on grapevine dormancy, specifically to: 1) determine the optimum foliar application concentration of ABA and 2) evaluate the morphological and physiological changes of greenhouse-grown grapevines in response to exogenous ABA application. *Vitis vinifera* ‘Cabernet Franc’ and *Vitis* spp. ‘Chambourcin’ with different leaf ages (40, 50, 80, 100, 110, and 120 days) were subjected to foliar ABA application at different concentrations (0, 100, 200, 400, 600, 800, 1600, and 3200 mg·L⁻¹) and to a cold-acclimated regime. Concentrations of 800 mg·L⁻¹ or higher were phytotoxic and the optimum concentrations were between 400 and 600 mg·L⁻¹. Optimum concentrations of ABA inhibited shoot growth and advanced growth cessation, periderm formation, and leaf senescence, which led to advanced dormancy in both cultivars. In this study, it was concluded that exogenous ABA induced endodormancy because single cuttings (not paradormant) under favorable growing conditions (not ecodormant) were used. Furthermore, grapevine response to ABA was influenced by leaf age and cold treatment. ABA was effective in inhibiting shoot growth and increasing periderm formation in the young vines with 40- to 50-day old leaves and the old grapevines with 80- to 120-day old leaves. However, ABA was effective in inducing early shoot cessation, leaf senescence and abscission, and dormancy in old vines with 100- to 120-day old leaves only. The advanced morphological and physiological changes induced by exogenous ABA mimicked those triggered by environmental cues during the cold acclimation process. It was suggested that advancing the cold acclimation process using foliar ABA application may be beneficial for long-season grape cultivars grown in regions with short growing seasons and early fall frost events.

The grape and wine industries in Midwestern and Eastern states have been expanding rapidly and demand for premium wine grapes has also increased. Unfortunately, several premium cultivars do not ripen properly in those regions as a result of the short growing season.

This impacts not only the quality of the fruit and wine, but also the cold acclimation process and ultimately the winter hardiness of grapevines (Zabadal et al., 2007). Freeze protection methods have been developed for grapevines, including active and passive methods. Active protection methods include wind machines, heaters, and over-vine sprinkling (Poling, 2007). Passive methods include site and variety selection (Striegler, 2007; Zabadal et al., 2007) and application of chemical protectants (Dami and Beam, 2004; Dami et al., 2000). Among chemicals, growth regulators such as ABA have been applied to crops including *Secale cereale* L. (Churchill et al., 1998) and *Malus domestica* (L.) Borkh. (Guak and Fuchigami, 2001) to improve their cold hardiness.

ABA is a plant hormone that plays several physiological roles, including regulation of

shoot and root growth (Saab et al., 1990), stomatal closure (Liang and Zhang, 1999), and leaf senescence (Thomas and Stoddart, 1980). Additionally, ABA has been suggested as a dormancy-inducing hormone because it accumulates in dormant nodes and increases after the tissue is exposed to low temperatures (Dogramaci et al., 2010). The variation of ABA concentration in plant tissues, mostly in nodes and xylem tissues, correlates with the cold acclimation process of plants (Mader et al., 2003; Rinne et al., 1994). Therefore, ABA has been proven to play an important role in the development of cold acclimation and ultimately the increase of freezing tolerance in plant species such as *Arabidopsis thaliana* (L.) Heynh. (Mantyla et al., 1995), *Betula pendula* Roth. (Li et al., 2003), *Hordeum vulgare* L. (Bravo et al., 1998), *Secale cereale* L. (Churchill et al., 1998), *Triticum aestivum* L. (Dallaire et al., 1994), *Solanum tuberosum* L. (Mora-Herrera and Lopez-Delgado, 2007), and *Acer saccharum* Marsh. (Bertrand et al., 1997).

Exogenous application of ABA has also been investigated with grapes. ABA was used to delay budburst for spring frost protection but was not effective on field-grown grapevines (Hellman et al., 2006). ABA has also been used on table grapes to enhance color development and advance fruit maturity (Amiri et al., 2009; Peppi et al., 2007). To our knowledge, there are no published reports on the effect of exogenous ABA on inducing dormancy of grapevines. The purpose of this study was to investigate the influence of foliar application of ABA on grapevine dormancy. Our hypothesis stated that ABA would induce morphological and physiological changes in grapevines that mimic environmental cues including low temperature and/or a short photoperiod that lead to dormancy. The specific objectives were to: 1) determine the optimum foliar application concentration of ABA; and 2) evaluate the morphological and physiological changes of greenhouse-grown grapevines in response to exogenous ABA application.

Materials and Methods

Plant materials, growing conditions, and abscisic acid treatment

In the first year, 1-year-old dormant *Vitis vinifera* ‘Cabernet Franc’ (CF) grafted on *V. riparia* × *V. rupestris* ‘Couderc 3309’ were planted in 7.6-L pots and placed on benches in the greenhouse in Dec. 2008. In the second year, the same potted vines were used after being pruned back to two nodes and stored in a 4 °C cooler to satisfy their chilling requirement. In Year 2, 1-year-old *Vitis* spp. ‘Chambourcin’ (CHB) grapevines were also used. Before applying ABA, the average leaf number and shoot length per vine were 16 and 120 cm, respectively.

In the first year, the plants were grown in a climate-controlled greenhouse for the non-acclimated condition and a growth chamber (Convion, Pembina, ND) for the cold-acclimated regime. The greenhouse conditions were as follows: 22/19 °C and 50/50% relative humidity (day/night). The light intensity in the

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greenhouse was maintained at $300\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ using 1000-W metal halide and 1000-W high-pressure sodium lights (Sunlight Supply, Woodland, WA) with a 12-h photoperiod. The growth chamber conditions for the cold acclimation experiment were $23.0/15.0\ ^\circ\text{C}$ and 50/50% relative humidity (day/night) in the first week and then $15.0/7.0\ ^\circ\text{C}$ (day/night) for 3 weeks (Grant et al., 2009). The light intensity in the growth chamber was maintained at $300\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with an 8-h photoperiod. All grapevines were watered daily and fertilized every other day with $100\ \text{mg}\cdot\text{L}^{-1}$ 20–20–20 fertilizer (Peters Professional, Marysville, OH). In the second year, the conditions in the greenhouse were the same as in Year 1 with the exception that the growth chamber for the cold acclimation experiment was maintained at 12-h photoperiod. The ABA (VBS 350001) sample was provided by Valent Bioscience (Libertyville, IL). The a.i. was 20.0% (w/w) S-ABA. The ABA sample was dissolved in deionized water with 0.05% Tween-20 (Acros Organic, Hampton, NH). Whole vines were sprayed with ABA solutions to runoff with a 7.6-L handheld sprayer (Gilmour Gardening Innovation, Peoria, IL) averaging a spray volume of 0.2 L/vine.

Leaf age was determined based on Eichorn-Lorenz (EL) stages of shoot development (Eichhorn and Lorenz, 1977) with 1-d leaf age corresponding to the first unfolded leaf (EL stage 7) originating from the third basal node.

In Year 1, two experiments were conducted. The first experiment consisted of applying ABA to CF in the greenhouse at a concentration of 0, 100, 200, 400, 800, 1600, and $3200\ \text{mg}\cdot\text{L}^{-1}$ when leaf age averaged 40 d. The second experiment consisted of applying ABA to CF in the greenhouse at concentrations of 0, 200, 400, and $600\ \text{mg}\cdot\text{L}^{-1}$ in the greenhouse and growth chamber when leaf age averaged 110 d. In Year 2, experiments were repeated to confirm ABA concentrations and plant response relative to leaf age and growing conditions. The first experiment was conducted at 50- and 100-d leaf age stages in the greenhouse (non-acclimated) and growth chamber (cold-acclimated) with ABA concentrations of 0, 200, 400, and $600\ \text{mg}\cdot\text{L}^{-1}$. The second experiment was conducted in the greenhouse by applying ABA (0 and $600\ \text{mg}\cdot\text{L}^{-1}$) at 40, 80, and 120-d leaf age stages. In the Year 1 experiment, a randomized block design was used with five replications per treatment of two vines per replication; in the Year 2 experiment, four replications per treatment were used with one vine per replication. Four vines of uniform growth per cultivar

were selected for ABA treatments. In all experiments in both years, the shoot tips of the grapevines were cut back to 14 to 16 nodes a week before ABA application.

Absciscic acid phytotoxicity

The phytotoxicity of ABA was evaluated in leaves and nodes. Visual observation of leaf distortion was made 24 h after ABA application and leaf injury was assessed and recorded a week after ABA treatments and based on leaf damage incidence (LDI) and leaf damage severity (LDS). LDI is the ratio of number of damaged leaves to the total number of leaves. Leaves with scorching-like dots and flecks were recorded as damaged leaves. Damaged leaf area larger than $0.1\ \text{cm}^2$ was counted in severity assessment. Abscised leaves were counted as damaged leaves as well. LDS assessed the percentage of damaged leaf area using a score rating as follows: 0: 0% (no symptom); 1: 1% to 25%; 2: 26% to 50%; 3: 51% to 75%; and 4: 76% to 100% damaged leaf area. An LSD = 4 was also assigned to abscised leaves. Bud injury assessment was measured by assessing shoot growth resumption ≈ 2 months after ABA application. Nodes that did not break were excised and visually evaluated whether they were alive (green) or injured (brown).

Vegetative growth

Shoot growth. Each shoot was marked at 15 cm from the tip before ABA application. Then the distance between the tip and marker was measured three times a week and the shoot growth rates ($\text{cm}\cdot\text{d}^{-1}$) were calculated as the total new growth divided by the number of days.

Growth cessation. In the first year, all lateral shoots were removed during the course of the experiment. Shoot tip abscission was used to determine growth cessation but it did not occur for CF. Therefore, in the second year, growth cessation was estimated by proxy using the methods by Garriss et al. (2009) on both CF and CHB. This method consisted of monitoring the summer lateral shoot emergence. The number of nodes with a lateral shoot having at least one fully expanded leaf was recorded. Growth cessation was determined when no summer laterals were produced (Garriss et al., 2009).

Periderm development and leaf senescence

Periderm development. Periderm formation was determined by counting shoot internodes that changed color from green to tan or

brown 4 weeks after ABA application. Periderm formation was expressed as the ratio of number of brown to total number of internodes per shoot.

Leaf senescence and abscission. This physiological stage was assessed by monitoring chlorophyll concentration and eventually counting leaf abscission. The measurement of chlorophyll concentration was conducted twice a week using a SPAD-520 chlorophyll meter (Spectrum Technologies, Inc., East Plainfield, IL) for 4 weeks. Five random measurements were taken on the upper surface of the fifth basal leaf. These measurements were conducted on CF and CHB grapevines in the second year. Leaf abscission was determined as the percent of abscised leaves to the total number of leaves 30 d after ABA application (DAA).

Dormancy

Lignified shoots with periderm formation from the base were excised into one-node cuttings $\approx 5\ \text{cm}$ long, then inserted into $2.5\ \text{cm} \times 2.5\text{-cm}$ foam medium (Smithers-Oasis, Kent, OH) and placed in $55\ \text{cm} \times 25\ \text{cm} \times 7\ \text{cm}$ plastic trays (T.O. Plastics, Clearwater, MN) filled with water. For each replication, 10 to 12 brown cuttings were used with the exception of young grapevines (leaf age = 40 or 50 d), from which green cuttings were used. Trays were then placed under forcing conditions on benches in a growth chamber (Conviron, Pembina, ND) with the following settings: 24-h photoperiod with $300\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, $22\ ^\circ\text{C}$, and 80% relative humidity. Budburst was recorded as EL stage 5 and monitored three times a week for 30 d. Dormancy was estimated as the number of days to 50% budburst (D50BB). The higher D50BB, the more nodes are dormant. An increased D50BB indicated that nodes went into endodormancy.

Statistical analysis

All data were subjected to analysis of variance using Minitab statistical software (Minitab Inc., State College, PA). The model tested for main effects of application timing and concentration and for potential interaction between timing and concentration. When appropriate, means were separated using least significant difference ($\alpha = 0.05$).

Results

Absciscic acid phytotoxicity. Leaf damage symptoms in CF grapevines were observed 24 h after ABA spray application at the concentrations of $800\ \text{mg}\cdot\text{L}^{-1}$ and above. CF

Fig. 1. Leaf damage shown on 'Cabernet Franc' leaves 1 week after abscisic acid (ABA) application. (A–G) ABA concentration of 0, 100, 200, 400, 800, 1600, $3200\ \text{mg}\cdot\text{L}^{-1}$.

grapevines treated with ABA concentrations of 100, 200, 400, and 600 mg·L⁻¹ showed the same symptoms 6, 5, 3, and 2 d after ABA spray application, respectively (Fig. 1). A week after ABA application, leaf damage was detected on all ABA-treated grapevines in both cultivars. On mature leaves treated with ABA at all concentrations, the damage started with black spots on the lower surface of the blade, then the spots expanded and appeared on the upper surface as yellow (CF) or red (CHB) spots and flecks ≈0.25 cm² in size (Fig. 1). At the shoot apical zone, ABA concentrations of 1600 and 3200 mg·L⁻¹ burned shoot tips and young leaves. ABA concentrations of 800 mg·L⁻¹ and lower caused shoot stunting but growth resumed later. Two to 4 DAA, leaf deformation was apparent on the first unfolded leaf treated with ABA at or above 400 mg·L⁻¹. Deformed leaves had abnormal margins and failed to develop the typical five-lobed leaf shape in CF. ABA concentrations of 400, 800, 1600, and 3200 mg·L⁻¹ caused a proportional increase of deformed leaves of one, three, five, and five, respectively. The growth of normally shaped leaves resumed after the emergence of the apical five leaves. This demonstrated that ABA concentrations equal to 400 mg·L⁻¹ or higher caused a transient disturbance of the normal development of shoot tips, which led to leaf deformation. A week after ABA application, LDI increased proportionally with ABA concentrations. ABA concentrations of 0, 100, 200, 400, 800, 1600, and 3200 mg·L⁻¹ led to LDI values of 0%, 8%, 24%, 26%, 52%, 64%, and 72%, respectively. LDS followed a similar pattern as LDI and was concentration-dependent as well (Fig. 2A). ABA concentrations at 1600 mg·L⁻¹ or above had LDS values above 1, indicating damage above 25% leaf area (Fig. 2A). Bud injury occurred at ABA concentrations of 800 (28%), 1600 (47%), and 3200 mg·L⁻¹ (57%). In Year 2, the highest concentration of ABA application was 600 mg·L⁻¹ and resulted in no bud injury (data not shown). Therefore, it was concluded that the treatments with concentrations of 800 mg·L⁻¹ or higher caused not only extensive leaf damage, but also bud injury.

In the cold acclimation experiment, CF grapevines with 110-d-old leaves were used. LDI and LDS also increased with increasing ABA concentrations, which indicated foliar application of ABA was phytotoxic under both cold- and non-acclimated conditions (Fig. 2B). Therefore, the combination of ABA application and cold treatment increased leaf damage, and the severity of the damage was concentration-dependent (Fig. 2B).

LDS values in the grapevines with older leaves (leaf age = 110 d; Fig. 2B) were generally higher than those with younger leaves (leaf age = 40 d; Fig. 2A). This prompted a leaf age experiment to verify whether ABA phytotoxicity increased with leaf age. This experiment demonstrated an increase of LDI (data not shown) and LDS, which were proportional to leaf age (Fig. 2C). Therefore, phytotoxicity of ABA was also leaf age-dependent.

Fig. 2. (A) Effect of abscisic acid (ABA) and concentration on leaf damage severity (LDS) of potted 'Cabernet Franc' grapevines (leaf age = 40 d) a week after ABA foliar application. (B) Effect of ABA and concentration on LDS of potted 'Cabernet Franc' grapevines under non-acclimated and cold-acclimated conditions (leaf age = 110 d) 1 week after ABA application. (C) Effect of ABA (0 mg·L⁻¹ or control, and 600 mg·L⁻¹) and leaf age on LDS of potted 'Cabernet Franc' grapevines a week after ABA application.

To verify whether these responses were cultivar-dependent, CHB was also used. Generally, results from CHB were similar to those from CF with the exception that the color of CHB leaf damage was red, which was different from the yellow color damage on CF leaves. Additionally, the values of LDI and LDS of CHB were less than those of CF grapevines, which indicates that different cul-

tivars have different concentrations of tolerance to ABA (data not shown).

Vegetative growth. Shoot inhibition was observed at the first measurement, 48 h after ABA application. The shoot length was reduced by ABA and was concentration-dependent with 200 mg·L⁻¹ having the least inhibition and 600 mg·L⁻¹ having the most inhibition (Fig. 3). At 28 DAA, shoot length and growth

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