Vitis 37 (3), 143–144 (1998)

Research Note

Salicylic acid treatment of grape berries retards ripening

ELENA KRAEVA, C. ANDARY, A. CARBONNEAU and A. DELOIRE

K e y w o r d s : berry, ripening delay, salicylic acid.

Introduction: Veraison is a key stage of grape berry development, and few studies have been made to identify the nature of the signal that initiates ripening. Hormones such as abscisic acid (ABA) and auxin have been studied in relation to berry ripening. ABA increases in concentration during berry ripening, and treatments that delay this increase seem to delay ripening (COOMBE and HALE 1973; DÜRING *et al.* 1978). Recently, DAVIES *et al.* (1997) have shown that treatment of grapes with the synthetic auxin BTOA (benzothiazole-2-oxyacetic acid), delays the onset of berry ripening by ca. two weeks. The nature of the signal that initiates ripening in nonclimacteric fruits remains unidentified. We have used salicylic acid (SA) as an elicitor of phenolics and hydrolytic enzymes involved in grapevine defense (RENAULT *et al.* 1996; KRAEVA *et al.* 1997). In this paper we demonstrate that SA is able to delay, or even inhibit berry ripening.

Material and methods: The grapevine variety used was *Vitis vinifera* L. cv. Shiraz, a black variety. Experimentation was carried out in the vineyard (Domaine du Chapitre, INRA Montpellier), in the 1996/1997 season. The SA treatment was performed at three phenological stages of berry growth, according to LORENZ *et al.* (1995): "berry touch complete" when the berries are green and firm (July); "beginning of berry softening" (veraison, August); and in the "ripening period" (September). Only healthy looking berries were chosen.

S a l i c y l i c a c i d (S A) t r e a t m e n t s : SA (Sigma) prepared according to RENAULT *et al.* (1996) was injected into berries (7.2 mM, 5 µl), using a syringe. Half of the berries of different bunches, from different grapevines, were treated. For each replicate, 100 berries were treated. Treatments were performed 6-7 weeks after flowering, at « berry touch complete » stage. The controls were non-treated berries and berries wounded with the syringe.

H i s t o c h e m i s t r y : Transverse sections (100 µm) of entire berries were stained with Neu's reagent and observed under epifluorescence, according to DAI *et al.* (1995). Neu's reagent is a solution of 1 % 2-amino-ethyldiphenyl-borinate (Fluka) in absolute methanol, which gives yellow fluorescence with flavonoids under blue light.

Correspondence to: Dr. A. DELOIRE, UFR de Viticulture, Agro.M-INRA, 2 place Viala, F-34060 Montpellier cedex 1, France. Fax: +33-4-99612064. E-mail: deloire@ensam.inra.fr

Results and Discussion: If the SA treatment was applied 6 or 7 weeks after anthesis, berry ripening was delayed for 2-4 weeks (no berry softening and no skin colour development) (Table). SA treatment provoked cell necrosis at the berry skin (Figure, a). The necrotic area of each berry was limited and stabilised 12 h after SA treatment. In a previous work, we have shown that flavonoids act as good markers of grapevine stresses (DAI *et al.* 1995). Histological observations (Figure, d), made at this time, showed that the necrosis concerned only a limited number of cells of the exocarp and the outer mesocarp (between the epidermis and the peripherical vascular bundles). The necrotic tissues were recognisable by their lack of flavonoids (Figure, d). Two to 3 weeks before veraison when the fruits are green and firm (Figure, a), the injection of SA into half of the total berries of a bunch induced a delay of ripening in the majority of berries as compared to the non-treated berries of the same bunch (Figure, b), and to the control (Figure, c). When the SA treatment was made at veraison and during ripening, no particular effects were observed. This experiment has also showed that the treated berries did not affect the ripening of the non-treated berries at the same bunch (Table).

T a b l e

Percentage of coloured fruit of treated (Tr) and non-treated berries of the same bunch, compared with the control (C). Anthesis was in June, veraison at the beginning of August (8 weeks after anthesis); observations were made 2-5 weeks after veraison

Weeks post anthesis	10	11	12	13
Tr	3b	9b	44b	70b
non-Tr	64a	80a	100a	100a
C	67a	81a	100a	100a

Calculation was done on 3 replicates of 100 berries each. For each period, the values followed by the same letter are not significantly different.

It is clearly demonstrated that SA treatment of grapevine berries is able to delay or inhibit ripening when applied before veraison (2-3 weeks before veraison corresponding to 6-7 weeks after anthesis). SA is produced from the phenylpropanoid metabolism via decarboxylation of *E*-cinamic acid to benzoic acid and its subsequent hydroxylation. It is known to be involved in plant defense mechanisms, particularly in the systemic acquired resistance (SAR) (RYALS *et al.* 1996), but also in other developmental events, e.g. flowering (CLELAND and AZAMI 1974). SA is known to be an antagonist of ABA (RAY 1986). It has been suggested that an increase of ABA levels, which begins at veraison, may be the trigger for grape berry ripening (COOMBE and HALE 1973; DÜRING *et al.* 1978; SCIENZA *et al.* 1978), and in parallel, it has been demonstrated that SA could inhibit the effect of ABA (RAY 1986; RASKIN 1992). This would support, in part, the role of exogenous SA treatment, which retards berry ripening of grapevines. We have demonstrated that exogenously applied SA has the same effect as the exogenously applied auxin-like BTOA (DAVIES *et al.* 1997). These

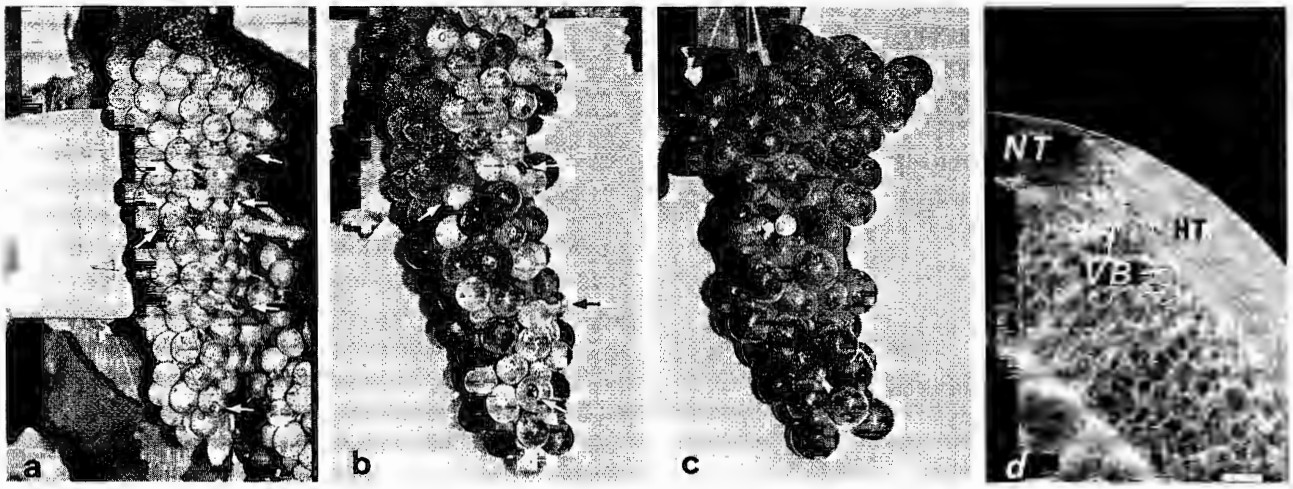

Figure: **a**: SA was injected into half of the berries (right part of the bunch). The phenological stage is "berry touch complete" (bunch closure), ca. 2-3 weeks before ripening. The berries are green and firm. Limited necrotic areas (arrows), due to SA, are visible on the treated berries. Photo has been taken 10 d after the treatment; **b** and **c**: The majority of SA-treated berries (arrows, Figure b) were still green and firm 15 d after the onset of ripening (12 weeks after anthesis) as compared to the non-treated berries of this bunch (Figure b, left part of the bunch) and to a control bunch (Figure c); **d**: Transverse section of a treated green berry, stained with Neu's reagent. Note that the site of cell death (necrotic tissue: NT) included only a limited zone of the berry tissue: between the exocarp to the outer part of the mesocarp (delimited by the system of vascular bundles: VB). Dead cells were devoid of flavonoids, which were present in the surrounding healthy tissues (HT), (bar = 240 μm).

substances are able to delay berry ripening and seem to be involved in the control of gene expression that contributes to nonclimacteric fruit ripening.

We would like to thank Dr. C. ROMIEU (IPV-INRA, Montpellier) for helpful discussion on grape berry ripening, Dr. A. F. ADAM-BLONDON (INRA-GAP Montpellier) for critical reading and Dr. N. WYNN for editorial assistance.

CLELAND, C.; AJAMI, F.; 1974: Identification of the flower-inducing factor isolated from aphid honeydew as being salicylic acid. Plant Physiol. **54**, 904-906.

COOMBE, B. G.; HALE, C. R.; 1973: The hormone content of ripening grape berries and the effects of growth substance treatments. Plant Physiol. **51**, 629-634.

DAI, G. H.; ANDARY, C.; MONDOLOT-COSSON, L.; BOUBALS, D.; 1995: Histochemical responses of leaves of *in vitro* plantlets of *Vitis* spp. to infection with *Plasmopara viticola*. Phytopathology **85**, 149-154.

DAVIES, C.; BOSS, P. K.; ROBINSON, S. P.; 1997: Treatment of grape berries, a nonclimacteric fruit with a synthetic auxin, retards ripening and alters the expression of developmentally regulated genes. Plant Physiol. **115**, 1155-1161.

DÜRING, H.; ALLEWELDT, G.; KOCH, R.; 1978: Studies on hormonal control of ripening in berries of grapevines. Acta Hort. **80**, 397-405.

KREAVA, E.; RENAULT, A. S.; TERRIER, N.; TESNIERE, C.; ROMIEU, C.; SAUVAGE, F. X.; BIERNE, J.; CARBONNEAU, A.; DELOIRE, A.; 1997: Beta-1,3-glucanase mRNA synthesis in grapevine leaves after *Botrytis* inoculation, and in berries after wounding or during ripening. 5th International Symposium on Grapevine Physiology, Jerusalem, Israel, May 25-30 (Acta Horticulturae, in press).

LORENZ, D. H.; EICHHORN, K. W.; BLEIHOLDER, H.; KLOSE, R.; MEIER, U.; WEBER, I.; 1995: Phenological growth stages of the grapevine (*Vitis vinifera* L. ssp. *vinifera*) - Codes and descriptions according to the extended BBCH scale. Austr. J. Grape Wine Res. **1**, 100-110.

RASKIN, I.; 1992: Role of salicylic acid in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **43**, 439-463.

RAY, S. D.; 1986: GA, ABA, phenol interaction and control of growth: Phenolic compounds as effective modulators of GA-ABA interaction in radish seedling. Biol. Plant. **28**, 361-369.

RENAULT, A. S.; DELOIRE, A.; BIERNE, J.; 1996: Pathogenesis-related proteins in grapevines induced by salicylic acid and *Botrytis cinerea*. Vitis **35**, 49-52.

RYALS, J. A.; 1996: NEUENSCHWANDER, U H., WILLITS M G., MOLINA, A., STEINER, H Y., and HUNT, M. D. - Systemic acquired resistance. Plant Cell **8**, 1809-1819.

SCIENZA, A.; MIRAVALLE, R.; VISAI, C.; FREGONI, M.; 1978: Relationship between seed number, gibberellin and abscissic acid levels and ripening in Cabernet Sauvignon grape berries. Vitis **17**, 361-368.