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Effectiveness of pre- and post-veraison calcium applications to control decay and maintain table grape fruit quality during storage

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ABSTRACT

A two year research was carried out on a table grape vineyard, cv. Italia, to evaluate the effectiveness of pre- and post-veraison calcium applications for controlling postharvest table grape rots and maintaining high fruit quality during cold storage. Two calcium application timings (from fruit set to veraison and from veraison to harvest) were compared to an untreated control. Clusters were sprayed with calcium chloride as Ca EDTA 44%. After each calcium application, bunch samples were collected and Ca^{2+} concentration was measured in berry compartments (skin, flesh and seeds). The main mechanical and chemical characteristics were measured on bunch samples at harvesting and during storage. In addition, the incidence of *Botrytis cinerea* rots, computed as McKinney index, was evaluated in field on natural inoculum and after harvesting on bunches artificially inoculated and maintained at room temperature. The highest Ca^{2+} concentrations were detected in skin tissues and after pre-veraison applications. Calcium accumulation in skin and flesh tissues stopped after veraison, whereas it continued up to ripening in seeds since the axial flow, differently from the peripheral, remains functional. In both years, calcium applications to bunches were effective both in maintaining postharvest fruit quality, as shown by flesh firmness and berry breaking force, and in reducing *B. cinerea* rots during storage. The applications were particularly efficacious if carried out between fruit set and veraison when stomata are functional and the re-translocation of calcium not directly absorbed by the bunches may occur via xylem transport.

1. Introduction

Table grape is a non-climateric fruit with a low rate of physiological activity but is subject to serious physiological and parasitic disorders after harvest and during long term storage (Combrink et al., 1978). Gray mold, due to *Botrytis cinerea* Pers., is the most economically important postharvest disease for this crop; it may cause mild losses during cultivation which could become severe after harvest, particularly in years when heavy rainfall during fruit ripening occurs (Cappellini et al., 1986).

B. cinerea is especially troublesome because of its vigorous growth rate and ability to spread among berries even at cold temperatures (−0.5 °C). Infections causing serious postharvest losses can originate from spores on the surface of the berries, from microscopic latent infections occurred before harvest, or from visibly infected berries that escape removal during packaging (de Kock and Holz, 1994). Gray mold infections are particularly serious in Apulia (Southern Italy), where "tendone" training system is mainly adopted and table grape bunches are left on vines under plastic coverings to delay the harvest. The control of gray mold is very difficult since postharvest treatments with synthetic fungicides or food additives are not allowed by European legislation (European Parliament and Council Directive 95/2/EC, 1995). In addition, preharvest fungicide applications are not always effective to prevent and control gray mold infections during long term storage and their efficacy is strictly related to the timing of application (Smilanick and Mansour, 2009).

Postharvest gray mold is usually controlled by an initial sulphur dioxide fumigation (SO_2), followed by weekly fumigations during cold storage (Smilanick et al., 1990). However, the amount of sulfite residues, remaining on fruits at final market, may represent a serious risk for human health. Therefore, there is a need for alternative disease management practices, safe, efficacious and cost effective, thereby able to reduce risks for consumers and workers but also to meet fruit quality requirements. New control strategies are under investigation (De Boer et al., 2003) and have already shown promising results under field conditions. These include bio-control agents like yeast and bacteria (Janisiewicz and Korsten, 2002), pesticides of natural origin such as plant extracts and essential oils, natural substances (Ippolito and Nigro, 2003) and physical agents (Nigro et al., 1998). Among the natural substances a key role may be played by mineral nutrients, which can have both a direct and an indirect

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effect consisting in the control of pathogen infections and in the improvement of the endogenous tissues resistance.

Calcium (Ca), known for its ability to reduce or delay parasitic and/or physiological disorders in fruit and vegetables, gave promising results in controlling storage rots when applied both as organic and inorganic salts (Biggs, 1999; Conway et al., 1999; Punja and Grogan, 1982). It is thought that Ca reduces postharvest disorders by strengthening cell walls and maintaining membrane selective permeability and integrity (Demarty et al., 1984). Postharvest calcium applications maintain cell turgor, tissue firmness and delay membrane lipid catabolism, extending storage life of fresh fruits (Garcia et al., 1996; Picchioni et al., 1998).

In grape berry, a high Ca concentration has been found able to delay senescence and to increase resistance to *B. cinerea*, as a consequence of calcium release from the host cell walls which increase concentration in the free space between cells (Chardonnet and Donèche, 1995). For instance, applications with calcium chloride to table grape bunches in post-veraison showed to be effective in reducing *B. cinerea* rots, providing either good results after harvesting, and ensuring an adequate protection of grape berries up to six weeks of cold storage (Nigro et al., 2006). Although several studies demonstrated the crucial role of calcium in improving shelf life during storage, most of them was focused on the use of calcium salts, often in combination with biological control agents or physical treatments, in the final stages of crop cycle. In addition, these applications are inappropriate for fruits such as strawberries and table grapes since their cosmetic appeal can be seriously reduced by excessive mishandling (Ippolito and Nigro, 2000). Finally, there are only few reports about pre-veraison salts applications to control storage rots.

Most of the studies concerning calcium salts applications has not deepened and explained the physiological processes determining their efficacy, with particular regard to the transport and the accumulation of calcium in fruits and to its absorption by epidermal tissues.

In plants, calcium is well known to move in xylem but to be substantially immobile in phloem. Many studies have shown that calcium accumulates in grape berries throughout their development (Ollat and Gaudillere, 1996; Rogiers et al., 2000; Schaller et al., 1992), whereas others indicate that calcium accumulation stops after veraison (Cabanne and Donèche, 2001; Creasy et al., 1993; Possner and Kliewer, 1985), because xylem vessels become non-functional (Bondada et al., 2005). In addition, it has been demonstrated that calcium absorption through berry skin declines drastically around lag phase as a consequence of loss in stomata functionality (Blanke and Leyhe, 1987; Rogiers et al., 2001). Therefore, it is fundamental to enhance Ca accumulation during the first stages of berry growth, through the definition of the most appropriate application timings. In particular, timing and number of applications need to be investigated, as well as the effectiveness of these treatments as a function of calcium absorption and accumulation in the fruits.

The present work was performed to study the influence of calcium application timing, as related to fruit development stage, in the accumulation and partitioning of the element in fruit tissues and consequently in the control of postharvest gray mold and in the improvement of postharvest fruit quality.

2. Materials and methods

A field research was carried out over a 2-year period in Apulia (Southern Italy, latitude 40°55′N, longitude 17°01′W, elevation 230 m), on *Vitis vinifera* cv. Italia, grafted in 2005 onto *Vitis berlandieri* × *Vitis riparia* SO 4 rootstock, at a spacing of 2.50 m × 2.50 m apart (1.600 vines ha^{-1}). Vines were pruned with mixed-pruning system with four canes (12 buds each) and 2 two bud spurs per vine (52 buds per vine) and trained to overhead tendone system (Apulia type). Vines were drip-irrigated and covered with hail protection net and plastic film, from veraison, to delay the harvesting.

The climate of the region is generally typical of a Mediterranean environment, characterized by a drier season between May and September, with high summer evaporation and low relative humidity. The average annual rainfall in the region is moderate (500 mm $year^{-1}$) and mostly concentrated during the winter months.

The soil was characterized on average by a low chemical fertility (0.5 g 100 g^{-1} of organic carbon and 0.8 g kg^{-1} of total nitrogen); soil texture was silty-clay, $CaCO_3$ content was of 30 g 100 g^{-1}. Water content at field capacity and wilting point were 30 and 16 g 100 g^{-1}, respectively, and total available water was 14 g 100 g^{-1}.

Two calcium application timings ("Ca before veraison", applications from fruit set to veraison; "Ca after veraiosn", applications from veraison to harvest) were compared to a untreated control ("Non-treated", no applications). Treatments were arranged in a completely randomised block design with three replicates. All clusters per vine were sprayed with calcium chloride (as CaEDTA, 44%, CALCIUM LG 44, Gobbi, Italy) at 0.1 (w/v) in about 1000 L ha^{-1} of water. Specifically, calcium applications were performed: 22–37 and 69 days after flowering (DAF) in 2009 and 28, 42, 67 DAF in 2010, under pre-veraison treatment ("Ca before veraison"); 102, 116 and 130 DAF in 2009 and 97, 106 and 125 DAF in 2010, under post-veraison treatment ("Ca after veraison"). One week after each calcium application, three bunch samples in each experimental unit were collected and immediately frozen at −18 °C. Calcium concentration was measured in each berry compartment (flesh, skin, seeds). To this aim, berry tissues were first oven-dried at 65 °C and then ashed in a muffle furnace at 550 °C (Baker et al., 1964). The ashes were dissolved with 10 ml of 0.1 N chloridric acid and total calcium concentration was determined by an atomic absorption spectrometer. Calcium concentration was expressed as g 100 g^{-1} of dry matter (d.m.).

At the commercial harvest (end of September) the main quantitative and qualitative yield parameters were determined on 5 bunches and 100 berries collected in each experimental unit.

In addition, to evaluate the efficacy of pre and post-veraison calcium applications on postharvest fruit quality, 200 fruit samples per plot were packaged and stored in cool room at −0.5 °C and 80% of relative humidity (RH). Then, at weekly intervals (7, 14 and 21 days after storage, DAS) the main physical (flesh firmness, berry removal force, berry breaking force) and chemical (juice pH, total soluble solids and titratable acidity) fruit characteristics were determined. Flesh firmness was quantified by puncture test using a digital penetrometer (Digital Fruit firmness tester, TR Turoni S.r.l., Forlì, Italy) fitted with a 3 mm diameter plunger. After skin removal the pit was inserted, at berry equator, on opposite sides, to a depth of 7 mm. Berry breaking force was tested by measuring the force, expressed in Newton (N), required to compress a berry through a flat cylinder probe (8 mm diameter) to reach a depth of 5 mm. Total soluble solids (TSS) were measured using a digital refractometer in juice obtained by squeezing, homogenizing and filtrating peeled berries; juice pH was quantified by a pH combined probe. Titratable acidity, expressed as grams of tartaric acid per 100 ml, was determined by titrating the berry juice with a sodium hydroxide solution to a bromothymol blue end point.

Finally, to evaluate Botrytis bunch rots occurrence, the incidence of occurring decay was evaluated in the field (one week before harvest) on natural inoculums on three vines per plot, and during storage on artificially inoculated berries. To create artificial inoculum conditions, immediately after harvesting, three bunch samples within each experimental unit were sprayed with