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Influence of bicarbonate salts, used against apple scab, on selected primary and secondary metabolites in apple fruit and leaves

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ABSTRACT

In a field experiment, the effectiveness and phytotoxicity of inorganic fungicides such as potassium bicarbonate (PBC) and sodium bicarbonate (SBC) were compared with water applications for the control and fungicide treatments as a classical method for controlling scab infections in cultivar 'Braeburn'. To examine all possible effects of this inorganic fungicide disease severity, external quality parameters (mass, firmness, colour), and the content of primary and secondary metabolites were measured and recorded on fruit; and disease severity, the content of secondary metabolites and accumulation of potassium were monitored in apple leaves. The results indicate that both inorganic fungicides are effective against apple scab and do not cause any phytotoxicity at given application doses. External fruit quality parameters were comparable with fruit produced with fungicide treatments. Similarly, the PBC treatments exhibited a positive effect on higher content of sugars and organic acids in comparison to fungicide treatments. No significant differences between fungicide and PBC or SBC treatments were observed in the content of analysed phenolic compounds. The content of phenolic compounds in leaves, were comparable to those in fungicide treated trees. However, this only indicates the activity of defence mechanisms in apple leaves. The use of PBC also positively affected the potassium accumulation in leaves through the growing season. Given that these products are not toxic to human health, PBC and SBC can potentially present a perspective protection in the apple orchards especially for the control of apple scab in organic fruit growing.

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1. Introduction

Apple scab, also known as black spot, caused by *Venturia inaequalis* ((Cook.) Wint.) is one of the most serious diseases of apple reported in almost all apple producing regions causing huge economic losses (up to 70% reduction in apple production). Scab infection leads to deformation in shape and size of the fruit, premature leaf and fruit drop, and enhanced susceptibility of tree to chilling and freezing injuries (McHardy, 1996).

Apples are prone to infection by pathogenic microorganisms during and after the growing season. Fungal pathogens are the major cause of economic losses in apple production and a challenge to crop protection strategies. The awareness and concern about possible health risks and the environmental impact of agrochemicals intensively used in the recent decades in conventional farming (Gessler and Pertot, 2012; Reganold et al., 2001; Tilman, 1999) increased the consumer's demand for healthier fruit produced in a more environmentally friendly manner. For these reasons, integrated and organic farming have become more widespread in apple production (Peck et al., 2005).

Chemical control of apple scab caused by *V. inaequalis* presents a considerable part of the pest control measures necessary for apple orchard protection when it is planted with one or several cultivars susceptible to the disease. However, these intensive treatments have a negative impact on the beneficial fauna and can lead to the outbreak of certain pests (Cuthbertson and Murchie, 2003), as well as to problems of *V. inaequalis* resistance to the active ingredient used (Parisi et al., 1994; Steinfeld et al., 2001).

In order to control apple scab in organic fruit growing, copper or sulphur products are widely used (Jamar et al., 2010; Mitre et al., 2009, 2010). These have several disadvantages, such as copper accumulation in soil, reduced efficiency at low temperatures, low fruit quality in terms of commercially important appearance etc. Therefore, a replacement for copper and sulphur products must be considered for more environmentally friendly and economical apple production (Kelderer et al., 2008).

In recent years, a broad range of products based on potassium bicarbonate (PBC) have been used as an alternative to products based on copper and sulphur (Jamar et al., 2007, 2008, 2010). Bicarbonate is considered harmless from an ecotoxicological and toxicological point of view (Environmental Protection Agency–EPA,

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1999) and this product has already been introduced in Annex II of European Regulation EEC 2091/92 list of active substances, which may be used as plant protection products in organic farming (Kelderer et al., 2005, 2006). Ilhan et al. (2006) reported that sodium bicarbonate (SBC) effectively inhibited spore germination and germ tube elongation of *V. inaequalis* in vitro. Favourable conditions for scab infection are still rather undefined in the orchard and also, the course of infection is not fully researched. However, combinations of sulphur and PBC offer increased effectiveness during distribution and development of the spore population in the orchard. Applied at the initial time of the infection or soon afterwards, sulphur is effective on the spores that are still in their germination phase, and the bicarbonates stop the spores that are already penetrating into the leaf.

The practical experience on apple scab control with bicarbonates is still limited, but laboratory and field trials indicate that the highest efficacy is shortly after the moment the infection has started. The objectives of this study were: (1) to evaluate the effectiveness of inorganic fungicides such as SBC and PBC for scab control (effects on fruit weight, firmness, disease severity on fruit and leaves, visible discolourations of the skin); (2) to evaluate the influence of this compounds on primary and secondary metabolites; and (3) to evaluate the effect of PBC on the content of potassium in apple leaves in apple orchard system. We hypothesised that both bicarbonate salts could influence plant secondary metabolism, which in return could induce higher plant resistance to apple scab.

2. Materials and methods

2.1. Chemicals

The following standards were used for the quantification of sugars and organic acids: sucrose, glucose and fructose; and citric and malic acids from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). The following standards were used for the quantification of phenolic compounds: chlorogenic acid (3-caffeoylquinic acid), phloretin and rutin (quercetin-3-*O*-rutinoside) from Sigma–Aldrich, ferulic acid, (−)-epicatechin, quercetin-3-*O*-rhamnoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, *p*-coumaric acid, procyanidin B2 and phloridzin dihydrate from Fluka Chemie (Buchs, Switzerland), quercetin-3-*O*-arabinofuranoside and quercetin-3-*O*-xyloside from Apin Chemicals (Abingdon, UK) and (+)-catechin from Roth (Karlsruhe, Germany). Methanol for the extraction of phenolics was acquired from Sigma–Aldrich. The chemicals for the mobile phases were HPLC–MS grade acetonitrile and formic acid from Fluka. Water for mobile phase was twice distilled and purified with the Milli-Q system (Millipore, Bedford, MA). For the total phenolic content, Folin–Ciocalteu phenol reagent (Fluka), sodium carbonate (Merck, Darmstadt, Germany), gallic acid and methanol (Sigma–Aldrich) were used.

2.2. Experimental site, design, and treatment

The study was performed on apple trees (*Malus domestica* Borkh.), cv. 'Braeburn', a scab-susceptible cultivar, in the growing season 2010. Fruits were harvested from 9-year-old trees grafted on the M9 rootstock growing in the University experimental orchard in Ljubljana (latitude: 46°2′, longitude: 14°28′).

The experimental treatments and the application protocol for the treatments were as followed: (1) water-treated control, (2) fungicide treated in accordance with the integrated pest management guidelines (Rules on integrated production of fruit), (3) 1% SBC (Merck) and (4) 1% PBC (Merck). The first application was on April 19th. The treatments were repeated every 10 days until harvest and 14 applications were completed in the growing period. Spray solutions were applied to the trees with a hand gun sprayer and each tree received 1 L of water/fungicide/SBC/PBC solution per application.

For the leaf phytotoxicity severity assessment, 10 shoots per tree were monitored during the whole growing season and 10 fully developed leaves per shoot were surveyed. The following leaf phytotoxicity levels were recorded: 0 = no damage; 1 = 0–2% damage; 2 = 2–5% damage; 3 = 5–20% damage; and 4 = >20% of the leaf surface damaged. The leaf damage of apple scab severity was evaluated using the grading scale described by Parisi et al. (1993): score 0 = no visible symptoms; score 1 = 0% < percentage of scabbed leaf surface (sls) ≤ 1%; score 2 = 1% < sls ≤ 5%; score 3 = 5% < sls ≤ 10%; score 4 = 10% < sls ≤ 25%; score 5 = 25% < sls ≤ 50%; score 6 = 50% < sls ≤ 75%; score 7 = sls > 75%.

Samples for the potassium content analysis were taken three times during the growing period (20 May, 20 June, and 20 September). A combined sample of 10 mature leaves per tree from different types of one-year shoots were analysed in five repetitions for each treatment. Plant samples were dried at 40 °C till constant weight was achieved. The sample was reduced to ashes in a muffle furnace at 550 ± 15 °C. The ash was then dissolved in hydrochloric acid and the silica compounds present are removed by precipitation and filtration. The filtrate was diluted to the desired volume (100 mL) with twice distilled water and the concentration of potassium was determined by atomic absorption spectrometry (Analyst 800, Perkin Elmer). The absorbance of potassium in the sample solution was determined by comparison with the absorbance of calibration solutions and was proportional to the concentration of this element in the solution. The ionisation and chemical interferences were controlled by the addition of caesium and lanthanum buffer solutions to standards and samples.

Fruit sampling was performed at technological maturity, which was determined by the starch iodine test. The harvesting date was October 10th. Five fruit (five repetitions for each treatment) were peeled as thin as possible and peel and pulp (separately) were immediately shock-frozen in liquid nitrogen and stored at −20 °C until analysis of the samples. Fruit disease assessment was monitored at harvest and disease severity was quantified using a scale developed by Tomerlin and Jones (1983). The scale consists of five categories; (0) no visible lesion; (1) <10% fruit surface infected; (2) 10–25% fruit surface infected; (3) 25–50% fruit surface infected; (4) >50% fruit surface infected.

2.3. External fruit characteristics

Fruit firmness, colour and mass were evaluated at technological maturity in order to determine the effect of different treatments on the external quality parameters. The measurements were done on 25 randomly selected fruits per each treatment. Fruit mass was measured for each fruit separately on electronic scales. Firmness was measured on four peeled sides of each fruit with a penetrometer (tr Italy, TR Turoni, Forli, Italy) equipped with a 11 mm tip.

Peel colour was measured by a portable colorimeter (CR-10 Chroma; Minolta, Osaka; Japan) with C illuminant. The colorimeter was calibrated with a white standard calibration plate before use. In CIE $L^*a^*b^*$ system of colour representation, the L^* value corresponds to a dark–bright scale and represents the relative lightness of colours with a range from 0 to 100 (0 = black, 100 = white). The a^* and b^* values extend from −60 to 60: a^* negative is for green and a^* positive is for red and b^* negative is for blue and positive for yellow. The hue angle ($h°$) is expressed in degrees from 0 to 360 (0° = red, 90° = yellow, 180° = green and 270° = blue). Colour was measured in the middle of each fruit (two repetition per fruit on different sides) to ensure equal measurement conditions.