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Control of apple scab (*Venturia inaequalis*) with bicarbonate salts under controlled environment

Bekämpfung des Apfelschorfs (*Venturia inaequalis*) mit Bicarbonatsalzen unter kontrollierten Bedingungen

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Summary

The effectiveness of potassium bicarbonate against *Venturia inaequalis*, the cause of apple scab, was studied. *In vitro* experiments with sodium, ammonium and potassium bicarbonate, as well as potassium phosphate used at 1% (w/v), reduced colony growth of *V. inaequalis* by 99, 98, 90 and 64%, respectively. Under controlled conditions in greenhouse experiments, a single spray of 0.5 or 1% (w/v) aqueous solution of sodium or potassium bicarbonate applied on young apple seedlings, 24 h before or 24 h after scab artificial inoculation, significantly controlled the disease. Greater effectiveness of potassium bicarbonate was recorded when the period of time before or after the inoculation was reduced. A significant increase of the fungicide activity of potassium bicarbonate was observed when salt was mixed with mineral oils. However, combining potassium bicarbonate with vegetable linseed oil and grapefruit seed extract did not increase its efficacy whereas these two vegetable products used alone reduced significantly scab infections. Formulated potassium bicarbonate, under the trade name Armicarb® 100 and containing surfactant compounds, was more effective than bicarbonate alone. A phytotoxicity effect of potassium bicarbonate was observed with a 0.75% dose. The potential and limitations of potassium bicarbonate used to control apple scab in the field are discussed.

Key words: alternative fungicides, Armicarb, copper, linseed oil, natural substances, organic farming

Zusammenfassung

Die Wirkung von Kaliumbicarbonat gegenüber *Venturia inaequalis*, dem Erreger des Apfelschorfs, wurde untersucht. Natrium-, Ammonium- und Kaliumbicarbonate sowie Kaliumphosphat in einer Konzentration von jeweils 1% (w/v) verminderten das Koloniewachstum von *V. inaequalis in vitro* um 99, 98, 90 bzw. 64%. Die Krankheit konnte unter kontrollierten Bedingungen im Gewächshaus durch Einzelspritzungen einer 0,5 oder 1%igen (w/v) wässrigen Lösung von Natrium- oder Kaliumbicarbonat 24 h vor oder nach der Inokulation junger Apfelsämlinge bekämpft werden. Eine Wirkungssteigerung von Kaliumbicarbonat wurde durch eine Verkürzung des Anwendungszeitraums vor oder nach der Inokulation erreicht. Eine Wirkungssteigerung wurde auch durch Mischung der Substanz mit mineralischen Ölen erzielt. Eine Zugabe von Leinöl oder Grapefruit-Samenextrakten hingegen steigerte die Wirkung von Kaliumbicarbonat nicht, während beide Produkte eine deutliche Einzelwirkung gegenüber dem Apfelschorf aufwiesen. Mit Netzmitteln unter dem Handelsnamen Armicarb® 100 formuliertes Kaliumbicarbonat wirkte stärker als das reine Salz, das in einer Konzentration von 0,75% eine phytotoxische Wirkung zeigte. Das Potential und die Grenzen von Kaliumbicarbonat als Pflanzenschutzmittel gegen den Apfelschorf werden diskutiert.

Stichwörter: Alternativfungizide, Armicarb, Kupfer, Leinöl, Naturstoffe, ökologischer Landbau

1 Introduction

Apple scab, caused by *Venturia inaequalis* (Cooke) G. Wint. is the most important apple disease, causing significant economic losses in many of the world's apple production areas, particularly in rainfed agricultural areas where intensive fungicide control is necessary for commercial apple production. With the cultivation of susceptible commercial apple cultivars, apple scab control is becoming more difficult, such that losses caused by apple scab would be about 70% if no control measures were taken. Even in Integrated Pest Management systems, scab is currently controlled by up to 15–20 applications of protective and curative fungicides during the growing season, regardless of the presence of ascospores in the orchards (Demeyere and de Turck 2002). Prediction systems have been developed for apple scab and used successfully to assist in timing fungicide applications (MacHardy 1996; Trapman and Polfliet 1997; Jamar and Lateur 2005). There is, however, a growing concern globally over the continuous use of synthetic chemicals on food crops because of their potential effects on human health and on the environment. Pathogen resistance is another factor militating against the continuous use of synthetic fungicides (Benaouf and Parisi 1998; Anonymous 2004; Koller et al. 2004).

Bicarbonate salts are one of several alternative control options that have recently received attention. These 'biocompatible' chemicals are particularly interesting because they have fungicidal properties combined with a very low mammalian and environmental toxicity profile. Bicarbonates are generally regarded as safe by United States Environmental Protection Agency (EPA) and therefore will be much easier to register. They are common food additives allowed in many applications by European and North American regulations. The actual use of these products in the control of plant disease is, however, still limited.

Bicarbonates have been shown to control a wide range of fungi, including food spoilage organisms and plant pathogens. The fungicidal effects of the carbonate and bicarbonate salts of ammonium, potassium and sodium have been reported on soil-borne pathogens, including *Sclerotium rolfsii* Sacc. (Punja and Grogan 1982). Homma et al. (1981b) found sodium bicarbonate to be inhibitive to powdery mildew on cucumber and green mould on citrus, and the addition of surfactants to improve the effectiveness of sodium bicarbonate against green mould on citrus. Horst et al. (1992) showed that rose powdery mildew (*Sphaeroteca pannosa*) and blackspot (*Diplocarpon rosae*) were significantly controlled by weekly sprays of 0.5% (w/v) aqueous solution of either potassium or sodium bicarbonate used alone or with 0.5% or 1.0% (v/v) Sunspray oils. Ziv and Zitter (1992) found pronounced detrimental effects of bicarbonates on the *in vitro* growth and disease incidence of several cucurbit foliar pathogens: *Alternaria cucumerina*, *Colletotrichum arbiculare*, *Didymella bryoniae*, and *Yulocladium cucurbitae*. PALMER et al. (1997) reported the inhibitory effects of bicarbonates on the *in vitro* colony growth of *Botrytis cinerea* and examined the contribution of pH and buffering capacity of these compounds. The pre-harvest application of 2% potassium bicarbonate on bell peppers significantly reduced post-harvested gray mould, caused by *B. cinerea* (FALLIK et al. 1997). Sodium bicarbonate controls post-harvest green mould, caused by *Penicillium digitatum* on citrus fruit, and is in common commercial use (SMILANICK et al. 1999). MLIKOTA GABLER and SMILANICK (2001) demonstrated the potential of carbonate and bicarbonate salts for the control of post-harvest gray mould on grapes. A novel fungicide (Armicarb SP) containing potassium bicarbonate reduced defoliation in citrus caused by *Mycosphaerella citri* (MCGOVERN et al. 2003). Potassium bicarbonate salt reduced powdery mildew (*Microsphaera pulchra*) in flowering dogwood in the field (MMBAGA and SAUVE 2004).

There are very few studies on the bicarbonate effects on apple scab: SCHULZE and SCHÖNHERR (2003) reported that calcium hydroxide prevents spore germination and kills the germ tubes of apple scab (*V. inaequalis*) at a concentration of 4.3 g l^{-1}. A successful field trial with potassium carbonate to reduce fruit and leaf scab has been conducted by GRIMM-WETZEL and SCHÖNHERR (2005). More recently, ILHAN et al. (2006) reported the effectiveness of sodium bicarbonate alone or in combination with a reduced dose of tebuconazol for controlling apple scab under field conditions.

The objective of this study was to evaluate the effectiveness of bicarbonates used alone or combined with horticultural oils for the control of apple scab in order to develop a successful strategy using environmentally friendly substances compatible with the organic production system. This is the first study we know of that demonstrates the effectiveness of potassium bicarbonate for controlling apple scab (*V. inaequalis*) on *in vivo* plantlets raised from seeds.

2 Materials and methods

2.1 Plant material

Experiments were carried out with highly susceptible apple seedlings from open-pollinated trees of cv. 'Golden Delicious' that had probably been pollinated by cv 'Gala', two highly scab susceptible cultivars. After dry storage, the seeds were stratified in moist peat at 2°C for 80–90 days. The apple seeds were raised in commercial potting soil mixture under greenhouse conditions at 18°C ± 2°C and 80% relative humidity in a 12-h light regime as described by OLIVIER and LESPINASSE (1980). Four-week-old plants at the four-leaf stage were used for the experiment.

2.2 Controlled inoculation

A mixture of strains of *Venturia inaequalis* isolated from diseased leaves from unsprayed orchards of various cultivars in central Belgium was used for the experiments. Dry leaves were conserved in the deep freeze at −18°C. The inoculum was prepared as described by SZKOLNIK (1978). For infection experiments, conidia were collected in distilled water and the suspension was adjusted to 1.5 x 10^5 living conidia ml^{-1}, using a heamocytometer. Quantitative seedling inoculations were carried out with an automatic bench sprayer machine in laboratory. The conidial suspension was sprayed at the 'just-before-run-off' stage. Immediately after inoculation, the plants were incubated in a dew chamber at 100% relative humidity for 48 h at 18°C to provide optimal infection conditions. The treatments were randomised within the mist chamber in a complete block design.

2.3 Fungicide preparations

The chemicals tested included potassium bicarbonate (99.5% $KHCO_3$, Sigma-Aldrich, Bornem, Belgium), sodium bicarbonate (99.5% $NaHCO_3$, Sigma-Aldrich), Armicarb®100 (85% $KHCO_3$, Helena Chemical Company, Collierville, TN, USA) and both 'Candit' (a synthetic fungicide containing 50% kresoxim-methyl, BASF, Antwerp, Belgium) and Thiovit jet (80% micronised sulphur, Syngenta Agro, Saint Cyr l'Ecole Cedex, France) as the positive control. The treatments included bicarbonate salts alone or combined with emulsified linseed oil (Vandeputte Oleochemicals, Mouscron, Belgium), Citripur grapefruit seed extract (containing 33% grapefruit seed extract without benzethonium from Pro-vera, Chaumont-Gistoux, Belgium) and Oviphyt mineral oil (a refined petroleum distillate marketed by Belchim Benelux, Londerzeel, Belgium). Armicarb is registered in US by the EPA and labelled as a biocompatible fungicide.

2.4 Performance of bicarbonate salts at varying concentrations for foliar disease control

In the first experiment, freshly prepared aqueous solutions containing 0.25, 0.5 and 1% of $KHCO_3$, 0.25, 0.5 and 1% of $NaHCO_3$, and 0.25, 0.5 and 1% of Armicarb were sprayed from a bench sprayer machine in the laboratory onto the upper surface of seedling leaves until just before run-off. A solution containing 0.02% Candit (50% kresoxim-methyl) was used as a reference treatment. Water-treated samples were used as the control. There were 160 seedlings per treatment (four replicates of 40 seedlings for each treatment). Pre-inoculation protective treatments were applied once 24 h before inoculation and post-inoculation curative treatments were applied 24 h after inoculation when the germination period had ended and the infection period had begun (MACHARDY 1996). The plants were then placed on the greenhouse bench at 18°C and 80% relative humidity for 3 weeks to promote plant and disease development. Disease incidence was assessed 21 days after inoculation by estimating the scab severity on the most infected leaf of the plant (LATEUR and POPULER 1996; LATEUR and BLAZEK 2002).

2.5 Use of potassium bicarbonate and oils for foliar disease control

The set-up of this experiment was as described above except for the following modifications. $KHCO_3$ was used as an active ingredient at a concentration of 0.5% (w/v). It was applied alone or mixed with soluble vegetable linseed oil, grapefruit seed extract or mineral oil at 0.5% (v/v). The choice of linseed oil and grapefruit seed extract was based on unpublished results that had been obtained in our laboratory. Armicarb was used as a comparative commercial formulated potassium bicarbonate. A sulphur solution (0.2% w/v) was used as a reference treatment. In this experiment, all treatments were applied with a hand sprayer until runoff 24 h before or 12 h after inoculation with *V. inaequalis*.

2.6 Effect of treatment timing on potassium bicarbonate effectiveness

In a third set of experiments, the plants were sprayed once up to run-off with a freshly prepared salt solution of $KHCO_3$ 0.85% (w/v) plus Tween-20 0.05% (v/v) as a surfactant compound, Armicarb 1% (w/v) and Thiovit 0.25% (w/v). According to previous assessment, Tween-20 at 0.05% did not express any inhibitory effects on apple scab. The treatments were applied 48, 24 and 3 h before the inoculation or 3, 24 and 48 h after inoculation. The seedling treatments, inoculation, incubation and experimental design were carried out as described above.

2.7 Phytotoxicity studies

In this experiment, solutions of sodium and potassium bicarbonates alone or combined with 0.5% mineral oil were prepared in distilled water at 0.5, 0.75, 1.0 and 2.0% (w/v). For comparative purposes, solutions of Armicarb were prepared with the same active ingredient dose. Each solution was applied with the bench sprayer machine in the laboratory onto the upper surface of 40 healthy seedlings until just before run-off. The plants were 4 weeks old at the time of treatment. Visible leaf phytotoxicity (necrotic area) was recorded on the 10^{th} day after treatment. A qualitative assessment was conducted on the third and fourth leaf of each seedling. Leaf phytotoxicity was scored thus: – = no damage; + = 0 to 2%; ++ = 2 to 5%; +++ = 5 to 20%; and ++++ = >20% of the leaf surface damaged.

2.8 In vitro *effect of bicarbonate salts on mycelial growth of* V. inaequalis

Two monoconidial strains of *V. inaequalis* isolated from Belgium apple cultivars were used to evaluate the bicarbonate inhibition of *in vitro* colony growth. The first strain was isolated from *Malus floribunda* and the second provided by 'Golden Delicious' (strain EU-B-04 INRA Anger). Malt extract at 25.0 g l^{-1} and agar at 20.0 g l^{-1} (Roberts and Crute 1994) were mixed with Na, K, NH_4^+ bicarbonate or K_2HPO_4 at three concentrations (100, 1000 and 10.000 ppm) and were autoclaved for 20 min, then incubated at 60°C and then poured onto sterile plastic Petri plates. Non-autoclaved captan at 10 and 100 ppm was used as a control. The *V. inaequalis* conidia were transferred to solidified plates with a heat-sterilized glass rod; the plates were then sealed with Parafilm. The media pH was not adjusted after amending with bicarbonate. Six plates differentiated by treatments and by strain were inoculated and then incubated at 18°C under dark conditions. The colony diameter was determined by measuring the average radial growth at 7, 14, 21 and 28 days. The control consisted of pathogen grown on standard malt agar.

2.9 Experimental design and data analysis

All the greenhouse experiments were arranged in a completely randomized split-plot design with four replicates of 40 seedlings for each treatment and repeated at least twice. The percentage data were transformed into arcsine angles before performing an analysis of variance. The data were analysed using statistical SAS software and the Student-Newman-Keuls test was applied as a mean variance analysis. All statistical analysis was conducted at a significance level of $P < 0.05$. For *in vitro* experiments, six replicates per strain and treatment were conducted and the experiment was repeated twice. The Student-Newman-Keuls multiple range test at $P < 0.05$ was also used to establish the differences among treatments.

3 Results

3.1 Effect of bicarbonate salts at varying concentrations under greenhouse conditions

All sprays of aqueous solutions of $NaHCO_3$ and $KHCO_3$ applied on apple plantlets 24 h before or 24 h after artificial inoculation with a conidial suspension of *V. inaequalis* significantly reduced the scab severity on the leaves (Table 1). Similar effects were recorded with both Na and $KHCO_3$, although $KHCO_3$ performed slightly better than $NaHCO_3$. When applied 1 day before inoculation, sodium and potassium bicarbonates at 1% (w/v) reduced scab severity to rates of 6.9 and 4.9%, respectively, compared with the infection rate of the controls that ranged from 41 to 43.8%, respectively. Similar values were recorded when treatments were applied 1 day after inoculation. The results indicated that Armicarb was more effective than $KHCO_3$ alone at the same active ingredient (a.i.) rate. Slight phytotoxicity was observed only when bicarbonates and Armicarb were used at a 1% a.i. dose.

3.2 Effect of potassium bicarbonate when mixed with oils

$KHCO_3$ at 0.5% applied 24 h before or 12 h after inoculation significantly reduced scab severity with an effectiveness of 69% compared with the water control. When $KHCO_3$ treatments were combined with vegetable oils, no significant

Table 1: Effect of $KHCO_3$, $NaHCO_3$ and Armicarb applied at increasing concentrations on apple scab development on artificially inoculated apple seedlings. The chemicals were applied 24 h before or 24 h after the conidial suspension was added

Treatment	a.i. dose %	Leaf area covered with scab (%)		Phytotoxicity[a]
		Pre-inoculation treatments	Post-inoculation treatments	
Water control	...	41.0 a[b]	43.8 a	–
$NaHCO_3$	0.25	30.6 b	33.9 b	–
$KHCO_3$	0.25	31.6 b	33.0 c	–
Armicarb	0.25	19.5 cd	22.7 c	–
$NaHCO_3$	0.5	19.3 cd	16.6 d	–
$KHCO_3$	0.5	17.9 d	15.6 d	–
Armicarb	0.5	06.5 e	08.4 e	–
$NaHCO_3$	1.0	06.9 e	06.5 e	+
$KHCO_3$	1.0	04.9 ef	04.1 ef	+
Armicarb	1.0	02.2 f	01.6 f	+
Kresoxym-methyl	0.01	01.0 f	01.0 f	–

[a] – = no phytotoxicity., + = slight phytotoxicity.
[b] Means of four replicates, each replicate including 40 seedlings. Two leaves per seedling were assessed. Means followed by the same letter are not significantly different according to the Student-Newman-Keuls multiple range test at $P < 0.05$. The experiment was repeated three times and similar results were recorded.

Table 2: Effect of pre-inoculation and post-inoculation application of potassium bicarbonate alone or combined with a vegetable oil, a mineral oil and a vegetable extract on the severity of apple scab in the greenhouse

Treatments[a]	pH	Leaf area covered with scab (%)[c]	
		Pre-inoculation[b]	Post-inoculation
Water control	7.6	52 a	52 a
0.5% MPO	7.8	48 a	46 a
0.5% VGE	7.6	26 b	23 bc
0.5% VLO	7.7	17 cd	20 bcd
0.5% $KHCO_3$	8.5	15 d	16 d
0.5% $KHCO_3$ + 0.5% MPO	8.6	08 e	07 e
0.5% $KHCO_3$ + 0.5% VLO	8.5	17 cd	19 cd
0.5% $KHCO_3$ + 0.5% VGE	8.5	17 cd	18 cd
0.5% Armicarb	8.5	05 e	06 e
0.25% Sulphur control	7.7	02 e	02 e

[a] MPO = mineral paraffinic, VLO = soluble vegetable linseed oil, VGE = soluble vegetable grapefruit seed extract.
[b] The pre-inoculation and post-inoculation treatments were made, 24 h before and 12 h after inoculation, respectively.
[c] Means followed by the same letter are not significantly different according to the Student-Newman-Keuls multiple range test at *P* < 0.05

increase of the fungicide activity of $KHCO_3$ was observed. However, significant improvement in the effectiveness of $KHCO_3$ was observed when it was mixed with 0.5% (v/v) mineral oil (Table 2). Apple scab was reduced by 88 and 86% compared with the controls on plants sprayed with Armicarb 24 h before or 12 h after inoculation, respectively. The effectiveness of $KHCO_3$ mixed with mineral oil at 0.5% (v/v) and Armicarb, were fairly similar. Linseed oil and grapefruit seed extract at 0.5% used alone significantly reduced infection, whereas mineral oil used alone did not reduce infection significantly. In another set of experiments, our observations indicated that the effectiveness of linseed oil increased as the concentration increased (data not shown).

3.3 *Effect of treatment timing on potassium bicarbonate effectiveness*

The effects of single spray of aqueous solutions of $KHCO_3$, Armicarb or sulphur applied from 48 h before inoculation to 48 h after inoculation are given in Figure 1. In this set of experiments, $KHCO_3$ at 0.85% reduced apple scab by 95 and 94.5% when applied 3 h before or 3 h after inoculation, respectively. Control of apple scab was significantly better with Armicarb than with $KHCO_3$ when the treatments were applied 48 h before inoculation. From 24 h before inoculation to 24 h after inoculation the effectiveness of $KHCO_3$ and Armicarb were as affective as sulphur. When the treatments were applied 48 h after inoculation, the control of the disease was significantly reduced. Hence, $KHCO_3$ was more effective when treatments were applied just before or just after artificial inoculation. Any phytotoxicity on leaves from either $KHCO_3$ or Armicarb was observed at this concentration (0.85% w/v).

3.4 *Phytotoxicity studies*

Phytotoxicity in the form of beige to light brownish necrotic areas was noted on healthy apple leaves treated with 0.5, 0.75, 1 and 2% of $NaHCO_3$ and $KHCO_3$ used alone or combined with oil and Armicarb (Table 3). Tests with 4-week-old seedlings showed that phytotoxicity symptoms were related to the concentration of the bicarbonate salts used. The importance of injury level is directly correlated to the level of salt concentration. With all the treatments, at 0.5% no injury at all was observed on young leaves. A few beige necrotic spots appeared on leaves treated with $NaHCO_3$ and $KHCO_3$ when used at concentrations of 0.75%. When bicarbonates salts were mixed with mineral oil leaves were far less susceptible to phytotoxic reactions. At a 0.75% concentration, neither bicarbonate plus oil (0.5% v/v) nor Armicarb showed any injury symptoms. Armicarb was less phytotoxic than potassium bicarbonate at the same active ingredient dose. In all cases, it was noted that the application of bicarbonate solutions that resulted of distinct droplets on the leave surface produced more phytotoxicity compared with a better standard leaf coverage like a continuous film.

3.5 In vitro *screening*

The *in vitro* results show that potassium and sodium bicarbonates reduced the growth development of *V. inaequalis* colony as the bicarbonate concentration increased (Table 4). At 1000 ppm, both $NaHCO_3$ and $KHCO_3$ significantly reduced colony growth in comparison with malt agar controls, but NH_4HCO_3 did not express any fungicide effect. No colony expansion was measurable with $NaHCO_3$, $KHCO_3$ and NH_4HCO_3 at 10.000 ppm. At low concentration, potassium phosphate dibasic stimulated colony growth and at 1000 ppm some inhibition was recorded. At 10.000 ppm, potassium phosphate

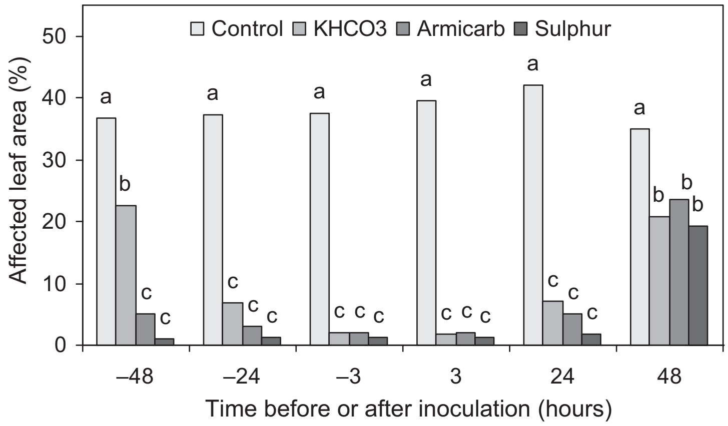

Fig. 1: Effect of a single foliar spray of 0.85% potassium bicarbonate, 1% Armicarb and 0.3% wettable sulphur solutions on apple scab severity (leaf area infected). Freshly prepared salt solutions of $KHCO_3$, Armicarb and wettable sulphur were sprayed until run-off on the upper surface of each leaf of the plants 48, 24 or 3 h before inoculation and 3, 24 or 48 h after inoculation. The numbers are means of 160 plants per treatment, including four replicates. Different letters denote a significant difference (*P* < 0.05) among treatment means according to the Student-Newman-Keuls multiple range test.

Table 3: Phytotoxicity of bicarbonates on apple seedlings under greenhouse conditions

Treatment	Active ingredient concentration (% w/v)			
	0.5	0.75	1	2
$KHCO_3$	–	+	++	+++
$NaHCO_3$	–	+	++	+++
$KHCO_3$ + MO 0.5%	–	–	+	++
$NaHCO_3$ + MO 0.5%	–	–	+	++
Armicarb	–	–	+	++

Phytotoxicity was recorded on the 10th day after treatment on healthy leaves of 4-week-old seedlings. A qualitative assessment was conducted on the third and fourth leaves of each seedling.
– = no damage, + = 0 to 2%, ++ = 2 to 5%, +++ = 5 to 20% of the leaf damaged.

Table 4: Mean diameters (mm) of *Venturia inaequalis* colonies 28 days after placement onto malt extract agar amended with $KHCO_3$, $NaHCO_3$ or NH_4HCO_3 applied at increasing concentrations

Treatment	100 ppm	1000 ppm	10.000 ppm
Control	16.6 b	16.7 b	16.6 b
K_2HPO_4	22.8 a	16.4 b	06.0 d
$KHCO_3$	15.0 b	10.0 c	01.8 e
$NaHCO_3$	17.8 b	10.2 c	00.2 e
NH_4HCO_3	15.6 b	16.4 b	00.4 e
Fungicide	00.1 e	–	–

Mean of 12 replicates. Values followed by the same letters are not significantly different according to the Student-Newman-Keuls multiple range test at $P < 0.05$.

reduced colony growth but was less effective than bicarbonate salts. Similar pH for all bicarbonate agar solutions were measured: 5.9, 6.4, 7.2 and 7.9 at 0, 100, 1000 and 10.000 ppm, respectively. A higher pH was observed for the phosphate agar solutions: 6.4, 7.4 and 8.2 at 100, 1000 and 10.000 ppm, respectively.

4 Discussion

The results presented in this paper describe the ability of $NaHCO_3$ and $KHCO_3$ to reduce the growth (*in vitro* and *in vivo*) of *V. inaequalis*. The ability of bicarbonate salts to reduce *in vitro* fungal development and disease incidence in several disease systems had already been reported (Punja et al. 1982; Horst et al. 1992; Ziv and Zitter 1992; Reh and Schlösser 1995; Osnaya-Gonzalez et al. 1998; Mlikota Gabler and Smilanick 2001; Mmbaga and Sauve 2004). Controlling apple scab with bicarbonate salts has previously been demonstrated in an *in vitro* study using isolated cuticles (Schulze and Schönherr 2003) and in three field studies conducted by Beresford et al. (1996), Grimm-Wetzel and Schönherr (2005) and Ilhan et al. (2006).

The mechanisms of fungistatic or fungicidal activity of bicarbonate salts have not been conclusively established. Because K_2HPO_4 was much less effective than $NaHCO_3$ and $KHCO_3$ in our *in vitro* experiments, it seems clear that bicarbonate anions are involved directly in the reduction of *V. inaequalis* colony growth. As reported by Palmer et al. (1997), bicarbonate anion appears to be the active portion of the bicarbonate salts even if cations might have some minor effects, as demonstrated by differing sensitivities to various bicarbonate salts. The same author reported that other bicarbonates salts, such as ammonium bicarbonate, were more effective than sodium or potassium bicarbonate salts in controlling the colony growth of *Botrytis cinerea*.

Bicarbonates might have several modes of action against fungi, including buffering and action to raise the pH level and the osmotic pressure of cells at the leaf surface, both factors leading to detrimental conditions for fungal spore (Palmer et al. 1997). Mlikota Gabler and Smilanick (2001) demonstrated that bicarbonate solutions were less toxic when tested at pH 7.2 than at a higher pH in the case of controlling post-harvest gray mold (*Botrytis cinerea*) in grapes. Bicarbonate concentration in solution is directly related to the pH of that solution. Bicarbonates are ineffective under acidic conditions because carbonic acid predominates in solutions below ph 6.5. H_2CO_3 is unstable and decomposes into carbon dioxide and water. As the pH increases to pH 8.5, the concentration of bicarbonate increases. Above pH 8.5, bicarbonate concentration decreases and the level of carbonate rises. However, high pH on leaf and fruit surfaces was shown to be effective as a control strategy for scab (Washington et al. 1998).

Our results and those described by Horst et al. (1992) show that the effectiveness of disease control by bicarbonates can be improved when bicarbonates are used in combination with horticultural oils. This improved effectiveness was attributable to the following factors: bicarbonate ions, the fungicidal characteristics of oils, the improved leaf coverage ability and the spreader-sticker characteristics of oils that keep the bicarbonate ions on foliar surfaces. Therefore, in order to enhance the stability on leaves and hence scab control effectiveness, bicarbonates could be mixed with mineral oils and with surfactant supplies in a well-buffered alkaline solution. This raises the question of whether the greater activity observed with Armicarb was due to the active ingredient itself ($KHCO_3$) or to other components of the unspecified formulation.

The remarkable reduction of apple scab with a single foliar spray of linseed oil and grapefruit seed extract was shown in our work. Other authors have reported detrimental effects of various vegetable oils on the disease incidence of several plant foliar pathogens (Cohen et al. 1991; Northover and Schneider 1996; Steinhauer and Besser 1997; Osnaya-Gonzalez and Schlösser 1998). Grapefruit seed antimicrobial effects as well as grapefruit seed effectiveness against apple scab had been reported (Trapman 2004) althought its relation to the preservative substances contained has been established earlier (Woedtke et al. 1999). Clayton et al. (1943) showed that oils from cottonseed, corn, linseed, peanut and soybean were fungicidal against *Phytophthora tabacina* and that the oils from castor bean, coconut, olive and palm were non-fungicidal. They concluded that linoleic acid "occurs in large amounts in most of the fungicidal oils, but not to any extent in the no fungicidal oils". They also concluded that there were "strong indications that linolenic acid (in linseed oil) is associated with positive fungicidal activity". These conclusions contrast with Northover and Schneider (1993) who showed that against three foliar pathogens there was no difference in fungicidal activity between two groups of oils which had either a high or a low linoleic acid concentration. Corroborating evidence was obtained by Cohen et al. (1990) using water sonicates of free unsaturated fatty acid instead of oils. Against *Phytophthora infestans*, they found that linoleic and linolenic acids were fungicidal, whereas oleic acid was not fungicidal. Linseed oil was more effective than paraffinic oil when used alone in our experiment, whereas linseed oil mixed to $KHCO_3$ did not have positive additive effect, unlike parraffinic oil. This might be related to the negative effect on the pH of the linseed mixed solution.

The effectiveness of bicarbonates salts in controlling scab on apple, as reported here, together with the improvement of several disease controls using bicarbonate salts (Homma et al.

1981a; Horst et al. 1992; Ziv and Zitter 1992; Schulze and Schönherr 2003; Smilanick et al. 2005), suggest that these simple compounds are good 'biocompatible' fungicides. The compounds are ubiquitous in nature, naturally present in human food, available to the general public for non-pesticide uses, and available for normal functions in human, animal, plant and environmental systems. The US EPA and the European Commission DG Health & Consumer Protection ruled that $NaHCO_3$ and $KHCO_3$ are exempt from residue tolerances. Bicarbonate salts have minimal environmental or worker safety issues associated with their use; they pose a minimal ingestion hazard because of their very low toxicity to animals.

Some issues need further examination, however, before the technology can be recommended for commercial adoption against apple scab. The fact that effectiveness of potassium bicarbonates fell when the time before or after inoculation increased indicates the short longevity activity of these salts when applied alone on the upper surface of the leaves.

Potassium bicarbonate acts as a contact fungicide and is not likely to be systemic or curative. Therefore, it is very important to apply such compounds with a very high foliar coverage quality. Armicarb is formulated with a surfactant system that increases its coverage ability. Consequently, the performance of Armicarb is much better than that of the pure potassium bicarbonate salt. The timing of their application is crucial since *V. inaequalis* causes deep-seated fungal infections after germination in contrast with *Podosphaera leucotricha*, the causal agent of powdery mildew. As shown in this study, a long-lasting action of potassium bicarbonate cannot be expected. Bicarbonate salts are quickly converted into an ineffective compound and are highly water soluble, and they will be washed off the leaves by a small amount of precipitation. They will therefore require frequent spray applications considering the presence of ascospores in the orchards and infection risk periods determined by modern local warning systems.

The practical relevance of this work on the use of bicarbonate salts for controlling apple scab includes the following points: (i) $NaHCO_3$ and $KHCO_3$ used at up to 0.5% were effective in controlling apple scab in greenhouse seedlings inoculated with a *V. inaequalis* suspension; (ii) the stability and performance of Armicarb is much better than that with straight $KHCO_3$; (iii) the addition of mineral oil to $KHCO_3$ improved its effectiveness in controlling apple scab; (iv) $NaHCO_3$, $KHCO_3$ and Armicarb could not be used at up to 0.75% without a phototoxic risk on seedling leaves under greenhouse conditions. Additional research is necessary to determine the effectiveness of $KHCO_3$ under field conditions, with an appropriate treatment formulation, timing and frequency. The results of field trials will be the subject of a future paper.

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