Potassium bicarbonate: a conceivable alternative control measure towards scab on pome fruits

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Abstract

Scab on apple and pear, caused by the ascomycete Venturia inaequalis *and* V. pirina*, respectively, is one of the most important diseases in fruit growing, due to high economic losses if no control measurements are undertaken. Seventy five % of the pesticide use in apple production is related with control of fungal diseases, in which apple scab has a share of 70 %. With the new regulation replacing Directive 91/414 and the Framework Directive on the sustainable use of Pesticides (2009/128/EC), products will disappear from the European market which will give a new challenge in IPM strategies to control pests and diseases. Besides there is the pressure of environmental and consumer groups on supermarkets to reduce residues on fruits. As such, the use of pesticides is at the moment under debate. Reduction in pesticide use is possible, for example, by taking alternative control measures against scab. Pear scab, and to a lesser extent apple scab, does not only infect fruits and leaves, but gives rise to twig lesions as well. The presence of twig scab represents a major problem, especially in organic pear growing, as fruit growers lack satisfying measurements to put an end to the disease. Furthermore, as the use of copper is under discussion, new alternative control strategies are a must in the organic farming. To this end, the efficacy of potassium bicarbonate (pbc) to control scab was investigated. The first experiments on the efficacy of kbc towards scab were performed on apple. The mode of action of pbc on conidial infection and the timing of applications in function of the infection period was determined. Out of the first trials it was concluded that pbc has a fungistatic activity and has the best activity towards apple scab when it is applied curatively, shortly after the infection around 300 degree hours.*

Keywords: Scab, potassium bicarbonate, conidia, fungistatic

Introduction

The most important disease on pome fruits is scab. This disease can be present on apple (*V. inaequalis*) and pear (*V. pirina*) and if no control measurements are undertaken, high economic losses will appear. At this moment about 50 % of the pesticide use in apple production is related with control of scab disease. With the new regulation replacing Directive 91/414 and the Framework Directive on the sustainable use of Pesticides (2009/128/EC), chemical products will disappear from the European market which will give a new challenge in IPM strategies to control diseases. Besides that, a competition between retailers is present, which is based on residue levels that are much stronger compared with the legally MRL (Maximum Residue Level) and eventually are combined with a maximum of active ingredients present on the fruit. Furthermore there is the public concern about pesticide residues present on the fruits. This will all lead to a reduced use of fungicides starting from the post bloom period. As such, the challenge for the present and the future research will be how to manage pest and diseases starting from the post blossom period until harvest. The basis to achieve this goal in scab control will be directed in sanitation measures and in obtaining a total control during the primary scab season. In the secondary season alternative control measures such as biological control organisms, salts or elicitors could be contribute to manage the disease. The past decade much

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research concerning alternative control of scab by means of biological antagonists or elicitors has been performed (Köhl *et al.*, 2009; Spinelli *et al.,* 2010). Besides those alternative techniques also salts can be used to control fungal diseases in vegetables and fruits (Deliopoulos *et al.*, 2010). The past years different carbonates have been tested for the control of apple scab with varying efficiencies (Jamar *et al.*, 2007; Tamm *et al.*, 2006; Trapman, 2008; Kunz *et al.,* 2008). In this article the potential of Vitisan as an alternative control measure to manage scab on apple will be discussed. Much attention has been paid to determine the best application time of the product and the influence of additives on the efficacy of the product and this both in *in vitro* and *in vivo* trials.

Material and Methods

In vitro germination tests

A *V. inaequalis* spore solution of 300.000sp/ml was made out of infected seedling leaves. The solution was prepared by cutting the leaves in small pieces and shaking them in a solution containing 0.5ml/L Tween20. Out of this stock solution a concentration range was prepared for the different tests. The *in vitro* germination tests were performed in microtiter plates. In each well 100µl of a specific spore concentration was added in combination with 100µl of a specific concentration of Vitisan, or captan, which was included as a control treatment. The plates were incubated at room temperature for specific time periods whereafter the percentage of spore germination was counted. To test whether Vitisan has a fungitoxic or fungistatic activity the spore solution, after a specific contact time with Vitisan, was plated out on PDA medium to test if the spores would continue germinating once again. The percentage of germination was counted 24h after incubation of the plates at room temperature.

Seedling trials

Preventive or curative efficacy of Vitisan

For this trial, apple seedlings were inoculated with a spore solution of 150.000sp/ml, prepared as described above, and treated with Vitisan (0.5% or 1%) at specific time points prior to or after infection. As a control treatment preventive application of captan (0.15%) was included. Seedlings were then incubated for 48h at 100% RH and room temperature. Thereafter, they were placed in climate chambers (20°C, 90% RH, 12h light/dark cycle) for up to 2 weeks. Two and three weeks after inoculation seedlings were analysed for the presence of scab symptoms. The degree of infestation and the efficacy were determined with the formula of Townsend-Heuberger and Abbott (see below).

Townsend-Heuberger formule: $$TH_{v\,max} = \frac{\Sigma\,(n \times v)}{v_{max} \times N} \times 100$$

($TH_{v\,max}$ = degree of infestation (%), v = infection classes (0,1,2,3), v_{max}= highest infection class, n = amount leaves/fruits in each class, N = total amount of evaluated leaves/fruits)

Abbott formule: $$ABB = \frac{C - T}{C} \times 100$$

(C = degree infestation untreated object, T = degree infestation treated object)

Rain fastness and efficacy of additives

For this trial, apple seedlings were inoculated with a spore solution of 150.000sp/ml, prepared as described above, and treated with Vitisan (0.5% or 1%) 24h after inoculation. As a control treatment curative application of lime sulphur (1.5%) was included in the test. To test the rain fastness of the product a rain event was created at specific moments after application of the fungicide by means of a spraying device (<1.0l/m^2, flow rate: 0.1l/m^2). In order to test the efficacy of additives different products were applied in combination with Vitisan (0.5%). The incubation of the seedlings and the evaluation of the symptoms was done as described above.

Field trial

The objective of this trial, performed in 2010, was to check the optimal timing and the effect of Vitisan in comparison with sulphur, copper and lime sulphur treatments (Table 4) towards scab on leaves and fruits of apple trees cultivar Jonagold. This trial was performed during the primary scab season (08/04/2010-19/05/2010) and covered 4 ascospore releases (8-9/04; 03-05/05; 12-14/05 and 17-19/05). Object 2 was treated on the following dates: April 8, 26 and 30, May 5, 8, 11 and 17. For object 3 the treatment dates were April 8 and 26, May 3, 11, 12 and 17. Object 4 till 10 was treated on April 9 and May 4, 14 and 19 and object 11 and 12 were treated on April 8, 26 and 30 and May 4, 8, 14, 17 and 19. Four times 9 trees were included per object. Assessment of scab on leaves and fruits was done by analyzing 100 different leaves at the sun and shadow side and 100 fruits per plot in the field. The degree of infestation and the efficacy were determined with the formula of Townsend-Heuberger and Abbott.

Statistical analysis

Statistical analyses were performed on TH-value using the Unistat Statistical Package, version 5.5 (Unistat Ltd. 1998). Means were compared using Duncan's multiple range test.

Results

In vitro germination tests

The first trials pointed out that Vitisan can inhibit the germination of apple scab conidia and that the efficacy of the product is dependent on the concentration of the spore solution and of the product. For example, addition of Vitisan (13.7g/L) resulted in 50% inhibition of spore germination when a concentration of 100.000sp/ml was used. On the other hand, addition of Vitisan at a concentration of 6.85g/L was enough to get the same results when the spore solution was diluted till 25.000sp/ml. In a second trial the fungistatic or fungitoxic activity of Vitisan was determined (Table 1).

After addition of Vitisan for a specific time, all spore solutions were able to restart germination when the spores were incubated on PDA. On the contrary, when the spores were in contact with Captan for a specific time prior to incubation on PDA medium, no spores could germinate any more. Based on these results it can be stated that Vitisan has a fungistatic activity towards scab conidia.

Table 1: Fungistatic activity of Vitisan

object	incubation time (h)	% germination (after 24h on PDA)
Vitisan (13,7 g/L)	1,5	88
	3	87
	6	84
	12	68
	24	73
	32	74
	48	86
Captan (1,5 g/L)	1,5	0
	3	0
	6	0
	16	0
	24	0
	32	0
	48	0

Preventive or curative efficacy of Vitisan

The results of the seedling trial to reveal the preventive or curative efficacy are shown in Table 2. Overall, captan as a preventive treatment had the highest efficacy. In comparison, the use of Vitisan prior to infection only had an efficacy of 35 to 45%. However, when Vitisan was applied in a curative way better efficacies were obtained. Out of this trial it can be concluded that curative application of Vitisan at 24h after inoculation resulted in the best control of scab on the seedlings. A dose range effect of the product could only be observed when the product was applied at suboptimal time points.

Table 2: Preventive and curative efficacy of Vitisan on apple seedlings

objects		% infestation	TH13		ABB
1	untreated	68,8	22,8	d	
2	0,5% Vitisan (preventive)	50,0	14,7	d	35,8
3	1,0% Vitisan (preventive)	37,5	12,5	d	45,3
4	0,15% captan (preventive)	9,4	0,7	a	96,8
5	0,5% Vitisan (8hpi)	59,4	10,3	d	54,7
6	1,0% Vitisan (8hpi)	40,6	8,2	c,d	64,2
7	0,5% Vitisan (16hpi)	21,9	5,5	b,c	75,8
8	1,0% Vitisan (16hpi)	15,6	3,4	a,b	85,3
9	0,5% Vitisan (24hpi)	21,9	3,8	b,c	83,2
10	1,0% Vitisan (24hpi)	15,6	3,4	a,b,c	85,3
11	0,5% Vitisan (32hpi)	62,5	17,3	d	24,2
12	1,0% Vitisan (32hpi)	46,9	11,3	d	50,5

* TH13: degree of infestation; ABB: efficacy of the product, hpi: hours post inoculation; different letters indicate significant differences in degree of infestation.

Rain fastness of Vitisan and effect of additives towards efficiency of Vitisan

The results of the seedling trial to reveal the rain fastness of Vitisan and the effect of additives are shown in Table 3. As can be seen in this table, the efficacy of Vitisan declines even after a small (<1.00l/m^2) rain event (ABB:73% vs ABB 37-55%). On the other hand, addition of additives, like T/S Forte, Cocana and Trend had a synergistic effect towards the efficacy of Vitisan. Although the infestation on the seedlings was rather low, differences between objects could be observed. The combination of Vitisan with additives performed in general much better than Vitisan alone, however nog significant differences were observed. Between the different additives applied also no significant differences has been observed.

Table 3: Rain fastness of Vitisan and effect of additives towards efficiency of Vitisan towards scab on apple seedlings

		% A	TH13*		ABB
1	**Vitisan 0,5% curative**	4,7	1,4	abc	73,0
2	**Vitisan 1% curative**	3,6	0,3	ab	94,9
3	**Lime sulphur 1,5% curative**	1,6	0,2	a	95,5
4	**untreated + rain (after 15 min)**	15,1	5,3	c	
5	**Vitisan 0,5% curative + rain (after 15 min)**	7,8	2,4	abc	55,0
6	**untreated + rain (after 2u)**	9,4	2,5	abc	
7	**Vitisan 0,5% curative + rain (after 2u)**	11,0	3,4	c	36,8
8	**untreated + rain (after 24u)**	11,8	4,6	bc	
9	**Vitisan 0,5% curative + rain (after 24u)**	12,7	3,7	c	31,2
10	**Vitisan 0,5% curative + T/S Forte**	1,6	0,5	ab	91,0
11	**Vitisan 0,5% curative + cocana**	0,0	0,0	a	100,0
12	**Vitisan 0,5% curative + trend**	1,6	0,6	ab	88,8

* different letters indicate significant differences in degree of infestation.

Field trial

The results of the field trial are shown in Table 4. In the untreated object, the scab infestation on the leaves was very high (92%). The best efficacy towards scab on leaves were obtained with sulphur applied during the germination window (object 3) and with schedule 12 in which sulphur was used preventively according to the weather forecast, in complement with a curative treatment with Vitisan about 300 degree hours (DH) after start RIM-curve.

Table 4: Assessment of scab symptoms on leaves and fruits in field trial

Objects		leaves 11/08				fruits 15/09			
		%A	TH3	ABB		%A	TH3	ABB	
1	Check	91,88	78,46			42,90	24,30		
2	preventive Cu (0,025%)/after bloom S (0,3%)	21,00	11,29	85,61	ab	3,60	1,40	94,20	ab
3	S (0,3%) in germination window	14,75	7,00	91,08	a	3,50	1,40	94,20	ab
4	lime sulphur (1%) 300 DH	26,88	16,13	79,45	bc	5,60	2,40	90,20	b
5	Vitisan (0,33%) 300 DH	23,75	13,88	82,32	bc	12,00	5,60	77,00	c
6	Vitisan (0,33%) + S (0,3%) 300 DH	25,50	14,38	81,68	bc	6,10	2,50	89,70	b
7	Vitisan (0,33%) + T/S Forte (0,133%) 300 DH	34,88	22,96	70,74	d	13,30	6,00	75,40	c
8	Vitisan (0,5%) 300 DH	35,00	23,00	70,69	d	14,80	6,50	73,20	c
9	Vitisan (0,5%) + S (0,3%) 300 DH	19,75	10,96	86,03	ab	2,80	1,00	95,90	ab
10	Vitisan (0,5%) + T/S Forte (0,133%) 300 DH	28,88	19,00	75,78	cd	11,30	4,80	80,20	c
11	optimal schedule S or lime sulphur 300DH*	19,50	10,25	86,94	ab	1,60	0,50	97,80	a
12	optimal schedule S and Vitisan 300 DH**	14,75	6,96	91,13	a	1,90	0,70	97,30	a

* S (sulphur) preventive or in germination window (RIM<150) or Vitisan 300DH (RIM between 150 and 300) or lime sulphur 300 DH (RIM >300), DH: degree hours, different letters indicate significant differences in degree of infestation.
** idem object 11 but without lime sulphur; so also Vitisan at 300DH with RIM>300

In general a good efficacy (70-82%) was obtained with the curative application of Vitisan at about 300 DH. Although not significantly different, the application of Vitisan at 300 DH seems to have a better efficacy compared with the application with Lime sulphur at the same time point. When sulphur was added to Vitisan applied at the highest dose rate, a slightly better efficacy was obtained compared with application of Vitisan alone, although results were not significantly different. However, when T/S forte was added, a decline in efficacy was observed, although results out of the *in vitro* trial on seedlings indicated a synergistic effect when this product was added.

At harvest time about 43% of the fruits in the untreated object were infested with scab. The best efficacy towards scab on fruits was also obtained with schedule 11, where sulphur was applied preventively combined with a curative application of lime sulphur or Vitisan according to the RIM value and with schedule 12 in which sulphur was used preventively according to the weather forecast, in complement with a curative treatment of Vitisan at about 300 degree hours (DH) after start of the RIM-curve. No significant differences have been observed between the two dose rates of Vitisan that were tested. Again, when sulphur was applied in a tank-mixture with Vitisan, at the highest dose rate applied, an increase in efficacy towards scab on fruits was obtained. As observed for the leaves, the synergistic effect when an additive was applied was lost. Overall, it can be concluded that Vitisan has a potential as an alternative control measure against scab on apples.

Discussion

With the new regulation replacing Directive 91/414 and the Framework Directive on the sustainable use of Pesticides (2009/128/EC), chemical products are under discussion and some of them will disappear from the European market. These facts will give a new challenge in IPM strategies to control diseases. One solution for this problem is to look for alternative techniques or strategies as a substitution for the use of fungicides. In this work, the potential of unformulated potassium bicarbonate (Vitisan) to control scab on apples was investigated. A lot of research on the potential of bicarbonates to control fungal diseases is already performed. The activity of (sodium) bicarbonates towards scab on apples was reported earlier (Ilhan *et al.*, 2006). *In vitro* trials pointed out that sodium bicarbonate could strongly reduce the germination of *V. inaequalis* conidia. Here, we reported that Vitisan has a fungistatic activity towards conidia of *V. inaequalis*. Inhibitory activity of carbonates towards micro-organisms is mostly fungistatic (Smilanick *et al.,* 1999). Therefore, it is probable that a residue of carbonate or bicarbonate must remain on the fruit, or at least within the wound infection courts occupied by this pathogen to be able to control it.

Furthermore, field trials based on a 10 days schedule revealed that efficacy of sodium bicarbonate (1%) was comparable with that of tebuconazole (Ilhan et al., 2006). Sodium bicarbonate applied at a concentration of 2% resulted in phytotoxicity. In our trials, no phytotoxicity on leaves was observed after application of Vitisan (0.5% and 1%). On the other hand, it was also reported previously that a during-infection application of Armicarb resulted in a good efficacy towards scab on leaves and fruits of apple trees (Jamar *et al.,* 2008). Besides that, Trapman (2008) reported a varying activity depending on the orchard when Vitisan was applied shortly before a rain event or during the germination window of the spores.

However, in the trials described here, it was pointed out that for the control of scab, potassium bicarbonate can best be applied as a curative treatment at 240 - 320 degree hours. The same results were also obtained by Kunz et al. (2008). Furthermore, it was stated that the efficacy of Vitisan could be enhanced when it was applied in combination with sulphur (Kelderer *et al.*, 2008) and this was also confirmed in our trials. However, it was observed that not only addition of sulphur but also tank-mixtures with additives enhanced the efficacy of Vitisan. A possible explanation is that due to the tank mixture a better formulation of the product is obtained. Previous studies with Armicarb (Kelderer *et al.* 2006; 2008) have shown that the formulation can definitely affect the efficacy of the product. Overall, it can be concluded that Vitisan has a potential as an alternative control strategy towards scab on apple, when applied as a curative treatment. Further research concerning a correct timing of the product (in relation to its rain fastness) and the efficacy of this product when it is applied in combination with other alternative techniques is still needed.

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