Bio Fruit Advies

Marc Trapman Dorpsstraat 32 4111 KT Zoelmond

Evaluation of grapefruit seed extract as natural fungicide to control apple scab in organic apple growing

Marc Trapman, Bio fruit Advies, Netherlands.

Poster for: Eco Fruit Conference, 3rd – 5th February 2004, Weinsberg, Germany

Tel: +31 (0)345 502627 Fax +31 (0)345 501721 Mobiel: +31 (0)653 176118
m.trapman@wxs.nl www.biofruitadvies.nl

Evaluation of grapefruit seed extract as natural fungicide to control apple scab in organic apple growing

Marc Trapman, Bio fruit Advies, Netherlands.

Abstract

C-pro, an experimental fungicide based on grapefruit seed extract was compared to copper oxychloride for the control of apple scab in a field trial. Efficacy and possible phytotoxic effects where accessed. The C-pro formulation was analysed for possible chemical additives by HPTLC.
C-pro proved tot be more effective in controlling apple scab then the standard rate of 300 gram copper oxychloride per ha, and gave a better leaf quality and less fruit skin russeting then the standard program of copper oxychloride.
However C-pro contained 6.6 % of the synthetic preservation agent benzethonium chloride what excludes C-pro from use in organic agriculture.
There is a growing interest in grapefruit seed extract products as well as in other plant extracts as potential crop protection materials. To prevent disappointments and waist of resources it is necessary to analyse these materials on possible chemical additives before taking them into efficacy trials.

Introduction

During the informal meetings of the international apple scab working group Arne Stensvand (Planteforsk, Oslo, Norway) reported effective control of apple scab by both preventive and curative applications of a fungicide based grapefruit seed extract in 2001 and 2002 in Norway. The product used in these trials was C-pro, a Norwegian formulation of the company "CITRO Europe AS" containing 30-60% grapefruit seed extract. During the meeting of the working group in January 2003 on Sicily it was decided to do parallel trials with this product on apple scab in Norway, Denmark and the Netherlands..

Grapefruit seed extracts (GSE) and products based on GSE are being sold in Europe as food supplements, cosmetics, natural disinfectants and as health-enhancing drugs. Effects of GSE on a wide range of bacteria and fungi have been reported. More recently GSE formulations have been evaluated for use as crop protection material against fungal diseases in different crops.
In the commercial available GSE products the synthetic preservation agents Triclosan, Methylparabene and Benzethonium chloride can be percent. Benzethoium chloride is not allowed as food additive. Only for cosmetic products that are rinsed off immediately after use a maximum level of 0.1% is tolerated. In GSE products Benzethonium chloride contents ranging from 3 to 14 % have been reported. [1,2,3,4,5] benzethonium chloride also has been found in GSE formulations offered for use as fungicide. [6]
Woedtke and co-workers [7] proved that in the GSE products they tested only the GSE's that contained benzethonium chloride had antimicrobial efficacy.
CITRO Europe AS stated that their GSE product C-pro was free of benzethonium chloride.

In organic fruit growing C-pro could possibly be used as fungicide form natural origin to control apple scab provided that the product is effective and does not contain chemical additives. In this trial the efficacy of C-pro on apple scab was tested under field conditions and the product was analysed on the presence of benzethonium chloride.

Methods and materials

In the 2002 Norwegian trial C-pro caused fruit skin russeting on the apple variety Gravenstein For this reason CITRO Europe provided two formulations of C-pro for the 2003 trial. One was the original product and the other a "neutralized" formulation (pH ~ 6). As the trial was made in an organic apple orchard the proposed standard fungicide Delan (dithianon) could not be used. I stead a low rate of copper oxychloride was used as standard for scab control in organic orchards.

Table 1:
Treatements to be compared in the scab trial

	Objects	Dose
1	Untreated	
2	Copper oxychloride (50 % Cu)	300 gr. /ha.
3	C-Pro original (1358)	14 litre / ha.
4	C-Pro neutralized (1358 B)	14 litre /ha.

The treatments where scheduled preventively, following the weather predictions and actual rainfall. Apple scab infections where calculated with the program RIMpro [8] using weather data gathered with a Metos Compact weather station (Pessl Instruments, Weiz, Austria) standing in an orchard several kilometres from the trial orchard.
The trial was made in a full grown plantation of the variety Elstar. Planting distance 1.5 * 3.5 meter (= 1900 trees /ha.) A randomize block design in 4 replications was used with 10 trees per object and replication. The replications where ordered one behind the other in the east border row of the orchard.
Sprays where applied with a Stihl SR 400 mist blower using 665 litre of spraying liquid per hectare.

Scab infections and treatments
The grower made two applications with copper oxychloride (50% Cu) in 300 gram /ha. prior to the start of the trial, and one treatment with copper after the end of the trial at the end of June.

Table 2:
Recorded apple scab infections and spray dates

Date	Scab infections		Treatments				Three phenology
	Mils infection	RIM value	Time	T	RH	Wind	
12 March	M *)	26					
2 April	L	13					
18 April			8:30	9.9 °C	65 %	4 ONO	Pink
22 April	L	13					
25 April			9:00	13.7 °C	77 %	0	Beginning of bloom
25-28 April	S	1245					
28 April			12:30	16.4 °C	75 %	2 ZO	Full bloom
1-3 May	S	294					
6 May	M	52	9:00	10.4 °C	99 %	0	End of bloom
12 May	M	10					
13 May			13:00	12.6 °C	99 %	2 W	
15 May	M	199					
19- 21 May	S	139					
21 May			20:00	14. 0 °C	99 %	0	
3 June	M	3	20:00	22.6 ° C	75 %	0	
8 June	L						
18 June	S						
20 June	M						
30 June-4 July	S						

**) Mills infections:*
L= light infection according to Mills table
M= moderate infection according to Mills Table
S= severe infection according to Mills Table

***) RIM values:*
< 100 low infection risk according to RIMpro 2003
100-300 medium infection risk according to RIMpro 2003
300 High infection risk according to RIMpro 2003

Assessments on apple scab
On the 10th of June assessments where made on 40 randomly chosen shoots from the middle 8 trees of every object. Of each shoot the number of scabbed leaves was noted.

Caused by spring frost and attack by apple blossom weevil the number of fruits was limited. After harvest (4 September) all fruits of each object where checked for scab symptoms. Three categories where used: no scab, < 2.5 cm^3 and > 2.5 cm^3 scabbed surface/fruit. The percentage fruits in each category was calculated. Differences where tested for significance using ANOVA Tukey HSD test.

Assessments on phytotoxicity
On the 14 of June a group of 12 organic fruit growers made assessments on "leaf quality". Each individual grower gave scores for the leaf quality of all objects without knowing the treatments. A rating from 1 to 10 was used. 6 meaning "sufficient", 7= "optimal" > 7 to wealthy.
After harvest all fruits of each object where checked for russeting of the fruit skin. A scale from 1 to 5 was used.

1= no russeting of the fruit skin
2= <= 10% of the fruit surface
3= 10-30 % of the fruit surface
4= > 30 of the fruit surface

The percentage of fruits in each category was calculated. Differences where tested for significance using ANOVA Tukey HSD test.

Analysis on Benzethonium chloride
On October 2nd a sample of the C-pro batch 1358 that was used in the trials was analysed on Benzethonium chloride by the "Kantonales Laboraturum", Basel, Switzerland. Identification and quantification was made by HPTLC (RP2) and confirmed by HPTLC (KG60). (SOP 032)

Results

Efficacy on apple scab
C-pro provided better scab control then the standard treatments with copperoxychloride. The effectiveness of the 'original' product on fruit scab was significantly better than the 'neutralized' formulation.

Table 3
Results of the assessments on apple scab

	Objects	Scabbed leaves on 40 shoots 10th June	% scabbed fruits at harvest		
			< 2.5 cm^3	> 2.5 cm^3	total
1	Untreated	197 a	52.1	26.4	78.5 a
2	Copperoxychloride 50 %	22 b	13.7	3.5	17.0 b
3	C-Pro original (1358)	13 b	4.1	0.3	4.5 c
4	C-Pro neutralized (1358 B)	14 b	9.7	0.7	10.3 b

Figures in the same column followed by the same character do not differ significantly form each other. (P< 0.05)

Phytotoxicity and fruit skin quality
The C-pro treated plots had a visible better leaf quality than the copper treated and untreated plots. In the way the assessments where made and evaluated only for the neutralized C-pro objects this effect was statistically significant.

Table 4
Results of the assessments on phytotoxicity

	Objects	Leaf quality on 14th June
1	Untreated	4.8 a
2	Copperoxychloride 50 %	4.9 a
3	C-Pro original (1358)	5.9 ab
4	C-Pro neutralized (1358 B)	6.1 b

Figures in the same column followed by the same character do not differ significantly form each other. (P< 0.05)

All treatments increased the fruit skin russeting. The russeting caused by the copper treatments reduced the marketable crop by 27 %. For the original C-pro formulation the increase in fruit russeting was not significant.

Table 5
Results of the assessments on fruit skin russeting

	Objects	1	2	3	4	1+2
1	Untreated	16.2	54.3	28.1	1.3	70.5 a
2	Copperoxychloride 50 %	4.3	39.0	50.7	5.9	43.3 c
3	C-Pro original (1358)	7.2	54.7	37.1	0.9	61.9 abc
4	C-Pro neutralized (1358 B)	5.6	49.4	41.8	3.2	55.0 b

1= no russeting of the fruit skin
2= <= 10% of the fruit surface
3= 10-30 % of the fruit surface
4= > 30 of the fruit surface
Figures in the same column followed by the same character do not differ significantly form each other. (P< 0.05)

The presence of Benzethonium chloride.
In the C-Pro sample that was analyzed 6.6 % ± 0.6 % benzethonium was found.

Discussion and conclusion

With 94% effectiveness on fruit scab C-Pro provided excellent scab control. The effectiveness of the 'original' product on fruit scab was significantly better than the 'neutralized' formulation and there was a tendency that the neutralized C-Pro formulation caused more fruit skin russeting than the original product. So we conclude that the neutralized formulation is no improvement to the original product.

The C-pro batch used in this trial contented 6.6 % benzethonium chloride. Form the results of this trial no conclusion can be drawn on the whether benzethonium or an other substance in C-pro was the active compound. According to the industry the benzethonium content is a result of the synthesis that is performed on the dried and grounded grapefruit pulp and seed. During this synthesis ammonium chloride, glycerine and ascorbic acid are added. This process to prepare a antimicrobial grapefruit extract is patented by Jacob Harich. [9,10,11] Several chemists doubt whether this synthesis can take place as it is described and yield benzethonium chloride and if so how it can yield almost pure benzethonium chloride as reaction product rather then a mixture of quaternary ammonium chlorides. Also the toxicological information provided by the producers on their internet sites is found to be misleading. (5,7,12,13,14)

Whatever the reason for the presence of benzethonium in C-pro is: as product of the patented synthesis, as pollution during the extraction process or deliberately added at some point of the manufacturing process, C-pro can not be regarded as a fungicide from "natural origin". Therewith C-pro is not suitable for use in organic agriculture.

There is a growing interest in GSE products as well as other plant extracts as potential crop protection materials. To prevent disappointments and waist of resources it is necessary to analyse these materials on possible chemical additives before taking them into trials.

References

1-Kantonales Laboratorium Basel Stat. : Jahresbericht 1997 p. 50
2-Kantonales Laboratorium Basel Stat. : Jahresbericht 1998 pp. 59-60
3-Kantonales Laboratorium Basel Stat. : Jahresbericht 2000 pp. 83-84
4-Redaction Arznei-telegramm, 1998: Grapefruitkernextrakt – ein biologisches Breitbandantibiothikum ? Arznei-telegramm 2/89 p.25-26
5-Takeoka, G.R. 2001: Identification of benzethonium chloride in commercial grapefruit seed extracts. www.nal.usda.gov/ttic/tektran
6- Marchall,K. 2003: Personal communication. Marchall, Research Centre for Agriculture and Foresty Laimburg, Bolzano, Italy.
7-Woedtke, von, Th., Schlüter,P., Pfegel,P., Lindequist, U. & Jülich, W.D. 1999: Aspects of the antimicrobial efficacy of grapefruit seed extract and its relation tot the preservative substances contained. Pharmazie 54 vol. 6 pp. 452-456

8-Trapman, M.C. 1994: Development and evaluation of a simulation model for ascosporeinfections of *Venturia inaequalis*. Norwegian Journal of Agricultural Sciences, supplement No. 17: 55-67.
9-Harich, J. 1995: USA Patent: Antimicrobial Grapefruit Extract. Patent number 5,425,944 June 20, 1995
10- Grapefruit seed extract products. Brought Spectrum Antiseptics. www.itmonline.org/jintu/grapefruit.htm
11- Gesuntheitsservice.net . http://members.aon.at/gesundheitsinfo/nontoxic.html
12- Lucas Reijnders, Stichting Natuur en Milieu, personal communication
13- Hohl Christopher, Kantonales Laboratorium Basel Stat, personal communication.
14- McCluggage,D.V.M : Grapefruit Seed Extract. http://www.wellvet.com/grapefruitseedextracts.html