The antifungal properties of the Frucol preparates

Received for publication, November 8, 2007
Accepted, December 1, 2007

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Abstract

The product Frucol was designed to hasten fruit ripening and to improve all the crop quality parameters. Along with its primary nutritive functions, some of the factual active entities born straight on the leaf and fruit surface – nanoparticulate elemental sulphur and potassium carbonate/sulphide/sulphite salts – bear significant antifungal abilities. This paper iterates the Frucol actual antifungal properties according to a treatment protocol chosen to disclose which fungi species can be controlled and the best way to apply the product before and after harvesting.

Keywords: foliar fluids, fertilizers, biostimulants, fungicide

Introduction

Frucol is a new multiple function nutritive fluid originating from a new class of micro emulsified agricultural products[1-3], designed to induce natural precocious crop ripening and to promote genuine color hue over an extended fruit surface area. It was formulated on the basis of ecological friendly components for foliar application along the entire vegetative period, as well as for post harvest fruit treatment. By its biological entities freshly substantiated on foliage and fruit surfaces, some fungal properties were affixed to the nutritive primary functions. Detailed mechanism, including matrix incorporating the factual biological active entities (nanoparticulate elemental sulphur, potassium hydrogen carbonate/hydrogen sulphide and sulphite, and carbonated products of the hydrolyzed naphthenic and oleic potassium overbasic salts) and their gradual release after application, was presented elsewhere[2,4] . This paper deals with Frucol fungicide activity over vegetative, harvesting and post harvesting periods according to a preliminary tested protocol of treatment applied to apples trees.

Materials and Methods

A. Sample collecting. Samples of the organic materials (fruitlets, apparently contaminated and apparently healthy fruits before and after harvesting) were randomly collected from the experimental plots. Then, selected samples were brought into laboratory and assay samples were selected by visual scanning, holding back both healthy and contaminated parts of fruits. All the assay samples were isolated after their immersion in deionized and sterilized water.

B. Inoculation, identification and specimen confirmation of fungi and lees were described before[1].

C. Experimental development. All the experiments were carried out on the allotted surfaces in the orchards where Jonathan and Idared apple varieties are grown. The experimental plots were organized on 6 lines as follows: ***V1*** – reference; no treatment before and after harvesting and storage; ***V2*** – four treatments with Frucol 4 (commercial product) during the entire vegetation period; no treatment before storage; ***V3*** – four treatments with Frucol 3 (intermediary product) during the entire vegetation period; no treatment before storage; ***V4*** – five treatments with Frucol 4 during the entire vegetation period (the first one after fruit let and the last one two weeks before harvesting); no treatment before storage; ***V5*** – two treatments with Frucol 4, the first one after apple physiological maturation, and the second one in the harvesting day; ***V6*** – two treatments with Frucol 4, first one in the harvesting day and the second one before storage (next day to the harvesting day); the fruits have been collected from the V1 reference plot. Experimental design was completely randomized over the above

experimental set with three replicates and 10 fruits per plot. Representative picture were taken to illustrate the effect of Frucol product on the fungal status of 2003/2004 year apple crop. The product was diluted to 0.5% concentration and subsequently applied by spraying at a rate of 5 l/ha.
Representative samples were collected from each plot before first treatment, after first and second treatment and during the harvesting day. Some representative samples collected during the harvesting day from each plot were packed separately in polyethylene bags and stored at 3ºC under no controlled atmosphere.

Results and Discussions

All the experimental data concerning fungi identification and their density on growth medium are given with their subsequent interpretation in the tables 1 – 4.
A. Vegetative period. Before the first treatment the fungal load observed on the contaminated fruits of both varieties was accepted as a natural status of orchard. Identified species are the initial orchard dower (tables 1 and 3) and their capacity to grow (figure 1) may be taken for a maximum in development and extent evolution. The figure 1 concerns only the species growing on contaminated fruits, bearing merely a qualitative relevance and ascertaining the source for an eventual incoming fungal escalation. Unfortunately, this less convenient state of contamination hardly impair a quantitative evaluation of experimental data, but some confident results may assign the efficiency of Frucol product and protocol application under heavy fungal stress. After the first treatment (tables 1 and 3, figure 2), *Botrytis spp* fungi were removed completely from Jonathan plot and partially from Idared contaminated apples. Also, the entire fungal growth was consistently decreased in number and surface density.

Table 1. Jonathan variety – distinctly contaminated fruits

Sample collection date	Experimental plot	Identified fungi	Density	Comment
05.07.2004	V1	*Aspergillius spp.* *Botrytis spp.* *Geotricum spp.* *Penicillium spp.*	Medium	Samples collected before Frucol application
	V4	*Alternaria spp.* *Botrytis spp.* *Geotricum spp.* *Penicillium spp.*	Maximum	
14.07.2004	V1	*Penicillium spp.*	Poor	Samples collected after the first Frucol application
	V4	*Acremonium spp.* *Penicillium spp.*	Medium	
04.08.2005	V1	*Acremonium spp.* *Geotricum spp.* *Penicillium spp.*	Maximum	Samples collected after the second Frucol application
	V4	*Acremonium spp.* *Fusarium spp.* *Penicillium spp.*	Poor	
	V5	*Acremonium spp.*	Poor	
21.09.2004	V1	*Alternaria spp.* *Botrytis spp.* *Geotricum spp.*	Medium	Harvested untreated sample
	V2	*Alternaria spp.* *Botrytis spp.* *Geotricum spp.*	Medium	Full treatment at harvesting, according to the protocol

	V3	*Alternaria spp* *Candida spp.* *Monillia spp.*	Poor	
	V4	*Alternaria spp.* *Botrytis spp.* *Geotricum spp.*	Medium	
	V5	*Alternaria spp.*	Very poor	
25.01.2005	V1	*Botrytis spp.*	Medium	Untreated sample. *Botrytis spp*.infected
	V2	*Candida spp.* *Geotricum spp.*	Very poor	*Botrytis spp*., *Fusarium spp*. and *Monillia spp*. complete removal
	V3	*Aspergillius spp.*	Very poor	
	V4	-	Absent	
	V5	*Aspergillius spp.*	Very poor	
	V6	*Candida spp.*	Very poor	
23.03.2005	V1	*Aspergillius spp.* *Candida spp.* *Geotricum spp.* *Scopulariopsis spp.*	Medium	*Botrytis spp*., *Fusarium spp*. and *Monillia spp*. complete removal
	V2	*Aspergillius spp.* *Geotricum spp.* *Penicillium spp.* *Scopulariopsis spp.*	Very heavy	
	V3	*Acremonium spp.* *Geotricum spp.*	Very poor	
	V4	*Aspergillius spp.* *Penicillium spp.*	Maximum	
	V5	*Candida spp.* *Penicillium spp.* *Scopulariopsis spp.*	Medium	
	V6	*Acremonium spp.* *Aspergillius spp.* *Mucor-Rizhopus* *Penicillium spp.*	Heavy	

Table 2. Jonathan variety – apparently healthy fruits

Sample collection date	Experimental plot	Identified fungi	Density	Comment
23.03.2005	V1	*Botrytis spp.*	Poor	*Botrytis spp*. survived on apparently healthy apples
	V4	*Candida spp.*	Very poor	Best results concerning fungal state after 5 months of storage

	V6	*Aspergillius spp.* *Geotricum spp.* *Penicillium citrin*	Medium	

a. – Jonathan V1 *b. – Jonathan V4* *c. – Idared V1* *d. Idared V4*

Figure 1. Fungal status before first tree treatment, 05.07.2004

a. – Jonathan V1 *b. – Jonathan V4* *c. – Idared V1* *d. Idared V4*

Figure 2. Fungal status after first tree treatment, 14.07.2004

a. – Jonathan V1 *b. – Jonathan V4* *c. – Idared V1* *d. Idared V4*

Figure 3. Fungal status after second tree treatment, 04.08.2004

Second treatment (tables 1 and 3, figure 3) removed completely *Botrytis spp* fungi on both varieties of apples. But, significant drop in colonies number and surface density was recorded only for Jonathan apples. At the end of treatment program, all the contaminated samples should summarize the effect of both product efficiency and treatment protocol. As it is shown in tables 1 and 3, as well as in figure 4 and 5, *Acremonium spp, Alternaria spp*, *Aspergillius spp, Candida spp* and *Geotricum spp* fungi survived on the surface all treated samples. Some colonies of *Botrytis spp* fungi on both apple varieties and *Monillia spp* only on Idared apples proliferate at low rate with less significant share in total contamination. An overall lay out of the results, under the unavoidable natural convection of contamination inside the orchard and fungi displacement from one plot to another, relieves the Frucol capacity to neutralize *Botrytis spp* fungi and to prevent contamination with *Monillia spp* and *Fusarium spp* fungi.

a. – Jonathan V1 *b. – Jonathan V4* *c. – Jonathan V5*

Figure 4. Fungal status at the end of tree treatments. September 2004. Jonathan apple.

a. – Idared V1 *b. – Idared V4* *c. – Idared V5*

Figure 5. Fungal status at the end of tree treatments. September 2004. Idared apple.

It seems that no one of Frucol active entities can eradicate *Acremonium spp, Alternaria spp*, *Aspergillius spp, Candida spp* and *Geotricum spp* fungi, although a significant slow down in their proliferation has been certainly noticed. May be an increase în concentration of the diluted applied micro emulsion from 0.5 to 1.0% will bring about major improvement in product antifungal capacity. According to the above mentioned data, there was found that V_3 and V_4 ways of treatment are the most convenient for Frucol application. Former for its contribution to healthy nutrition accompanied by fungal control over entire fruit growth, and the last for a punctuate over dosage during the fruit maturation.

B. Postharvest treatment. Apples storage was extended till the end of January (first party), respectively till end of March (second party). From each party, according to validated protocol, samples of contaminated and non-contaminated apple were withdrawn, and assay samples were processed as described above. Fungal load of all the samples described in tables 1-4 and illustrated with selected pictures from figures 6-11, display once again all the advantages of product application under alternatives V_3 and V_4 of the protocol with particular emphasis for Jonathan apple (figures 6 and 8). These two alternatives let the product to change fruit nutrition state, lessening chemical stress and clearing fruit epidermis for better fungi control.

a. – Jonathan V1 *b. – Jonathan V4* *c. – Jonathan V5*

Figure 6. Fungal status at the end of January 2005. Jonathan apple.

a. – Idared V1 *b. – Idared V4* *c. – Idared V5*

Figure 7. Fungal status at the end of January 2005. Idared apple.

a. – Jonathan V1 *b. – Jonathan V4* *c. – Jonathan V5*

Figure 8. Fungal status at the end of March 2005. Jonathan apple.

a. – Idared V1 *b. – Idared V4* *c. – Idared V5*

Figure 9. Fungal status at the end of March 2005. Idared apple.

Failure of the alternative V_6 is a proof of the missing nutritive support to alleviate chemical stress or to face a fungi charge. Uncontaminated fruits (tables 2 and 4, and figures 10 and 11) exhibit the same species of fungi as contaminated ones, but in the latent state. On treated samples their growth was partially or totally inhibited. *Penicillium spp* presence on these samples may be explained only by improper aerial micro flora in the storage room.

It should be mentioned that the entire set of data, recorded both before and after harvesting was impaired by unfriendly meteorological conditions during crop growth and maturation, as well by uneven crop maturation at harvesting time.

Table 3. Idared variety – distinctly contaminated fruits

Sample collection date	Experimental plot	Identified fungi	Density	Comment
05.07.2004	V1	*Acremonium spp.* *Botrytis spp.* *Geotricum spp.* *Penicillium citrin*	Maximum	Samples collected before Frucol application
	V4	*Acremonium spp.* *Alternaria spp.* *Geotricum spp.* *Penicillium spp.*	Maximum	
14.07.2004	V1	*Geotricum spp.* *Penicillium spp.*	Poor	Samples collected after the first Frucol application
	V4	*Acremonium spp.* *Alternaria spp.* *Botrytis spp.* *Geotricum spp.* *Penicillium spp.*	Medium	
04.08.2005	V1	*Geotricum spp.* *Penicillium spp.*	Medium	Samples collected after the second Frucol application
	V4	*Acremonium spp.* *Alternaria spp.* *Geotricum spp.*	Maximum	
	V5	*Acremonium spp.* *Geotricum spp.*	poor	
21.09.2004	V1	*Alternaria spp.* *Yeasts* *Penicillium spp.*	Very heavy	Harvested untreated sample
	V2	*Aspergillius spp.* *Candida spp.* *Monillia spp.*	Heavy	Full treatment at harvesting, according to the protocol
	V3	*Alternaria spp.* *Monillia spp.*	Poor	

	V4	*Acremonium spp.* *Alternaria spp.* *Botrytis spp.*	Medium	
	V5	*Alternaria spp.* *Botrytis spp.* *Geotricum spp.*	Poor	
25.01.2005	V1	*Penicillium spp.*	Maximum	*Botrytis spp*., *Fusarium spp*. and *Monillia spp*. complete removal
	V2	*Aspergillius spp.*	Maximum	
	V3	*Aspergillius spp.*	Very poor	
	V4	*Aspergillius spp.*	Very poor	
	V5	*Geotricum spp.*	Very poor	
	V6	-	Absent	
23.03.2005	V1	*Candida spp.* *Penicillium spp.*	Maximum	Samples completely destroyed by maximum density of contaminating
	V2	*Acremonium spp.* *Aspergillius Niger* *Aspergillius spp.* *Monillia spp.*	Maximum	
	V3	*Candida spp.* *Penicillium spp.* *Scopulariopsis spp.*	Maximum	
	V4	*Botrytis cinerea*	Maximum	
	V5	*Penicillium spp.*	Maximum	
	V6	*Aspergillius spp.* *Penicillium spp.*	Maximum	

a – Jonathan V1 *b - Jonathan V4* *c - Idared V1* *d - Idared V4*

Figure 10. Fungal status of uncontaminated apples. March 2005

Table 4. Idared variety – apparently healthy fruits

Sample collection date	Experimental plot	Identified fungi	Density	Comment
23.03.2005	V1	*Botrytis spp.*	Medium	*Botrytis spp*. survived on apparently healthy apples
	V4	*Acremonium spp.* *Botrytis spp.*	Poor	Good results, but *Botrytis spp*. survived
	V6	*Aspergillius spp.* *Botrytis spp.* *Penicillium spp.*	Heavy	Inefficient treatment with complete lost of fruits

Conclusions

1. Fungal activity of Frucol product was investigated over two significant time intervals: fruit growth and maturation period, and postharvest and storage period. Experimental plot were organized on 6 lines, including: reference, three variants of tree treatment, one variant of combined tree and postharvest treatment and one variant of postharvest treatment.

2. Recorded data have disclosed the reference fungal state of orchard, product capacity to remove partially/totally during vegetative period and to prevent postharvest fruit contamination with *Botrytis spp*., *Fusarium spp*. and *Monillia spp*. Some other fungi species development was slowed down with substantial diminishing in contamination power. Eventual increase in dosage may extent product area of action.

3. The entire set of experimental data has validated the best protocol for product application.

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