Short review of the applications of hydrogen peroxide and EcoClearProx® in viticulture

1. Effectivity of H2O2 and EcoClearProx® in suppression of diseases in grape vines

1.1. Short literature review

1.1.1. *Botrytis bunch rot or grey mould in grape vines*

In Australia there is a blend of H2O2 and peroxyacetic acid on the market, called Peratec, which is registered for suppression of Botrytis bunch rot or grey mould in grape vines. Peratec is not allowed to be used in the last 7 days before harvest (AWRI, 2009a; State of Western Australia, 2009).

Target pest	Activity and chemical group	Active ingredient	Some trade names	Rate**	Withholding period (days)	Restriction on use for wine grapes*	Comments
Botrytis bunch rot or grey mould (*Botrytis cinerea*) cont.	Group M4 Fungicides—multi-site activity (phthalimide)	captan	Captan Captan WG Orthocide WG Merpan	125 g/100 L	7	Use no later than 30 days before harvest.	*Table grapes:* **Do not** apply products that contain captan after berries reach 10 mm in diameter.
			Captan 900 WG	100 g/100 L			
	Group M Fungicides – multi-site activity (inorganic)	hydrogen peroxide + peroxyacetic acid***	Peratec fungicide (suppression only)	1–2 L/100 L	1	Use no later than 7 days before harvest.	Suppression only.

Reference: State of Western Australia, 2009

1.1.2. Powdery mildew, downy mildew and black rot

Oxidate, a commercial H2O2 product, is registered in several States of the US and Canada as broad spectrum bactericide/fungicide. In Oregon the product is registered in viticulture for use against black rot caused by Guignardia bidwellii, downy mildew caused by Plasmopara viticola and powdery mildew caused by Uncinula necator (Kaiser et al., 2008).

“Other alternatives are Nutrol (active ingredient: monopotassium phosphate), Armicarb 100 (active ingredient: potassium bicarbonate), and OxiDate (active ingredient: 27% hydrogen peroxide). These are protectants. Used on a weekly (7-day) basis, they appear to effectively control powdery.” (Wolf, 2005 and 2007).

Chart 3. Winegrape Fungicides and Bactericides Registered in Oregon, 2008

Grape disease	2,4-xylenol (Gallixa)	azoxystrobin (Abound Flowable Fungicide)	*Bacillus pumilus* strain qst 2808 (Sonata Biofungicide)	*Bacillus subtilis* qst713 strain (Serenade Max)	boscalid (Endura, Pristine)	captan (Captan 50 WP, Drexel Captan 4 L Fungicide)	chitosan (Elexa 4 Plant Defense Booster)	chloropicrin (67-33 Preplant Soil Fumigant)	cinnamaldehyde (Cinnacure)	clarified hydrophobic extract of neem oil (Trilogy Fungicide/ Miticide/Insecticide)	copper hydroxide (Kocide DF)	copper metallic (Copper-Count-N)	copper octanoate (Neudorff Cueva Fungicide Conc–OMRI, Scop-Shield Flowable Liquid Copper Fungicide)	copper oxide (cuprous oxide) (Nordox 75 WP)	copper oxychloride ($Cu_4Cl(OH)_6$) (C-O-C-S WDG)	copper salts of fatty and rosin acids (PRES Treatment Corrected Fungicide)	copper sulfate (-pentahydrate) (Triangle Brand Copper Sulfate Instant Powder)	copper sulfate basic (Basic Copper 53, Cuprofix Ultra 40 Disperss)	cyprodinil (Vanguard WG Fungicide)	DCNA-dichloran (Botran 6% Dust)	fenarimol (Rubigan EC)	fenhexamid (Elevate 50 WDG)	ferbam (Ferbam Granulo Fungicide)	fluopicolide (Presidio Fungicide)	hydrogen peroxide (Moxide) (Oxidate)
REI hours (h) or days (d)	S	4 h	4 h	4 h	24 h	48 h	4 h	48 h	4 h	4 h	24 h	12 h	4 h	24 h	24 h	12 h	24 h	24 h	12 h	12 h	12 h	12 h	24 h	12 h	1 h
PHI hours (h) or days (d)	–	14 d	0 d	0 d	14 d	0 d	–	P	0 d	B	–	–	0 d	–	–	0 d	–	66 d	7 d	1 d	21 d	0 d	7 d	21 d	0 d
Armillaria root rot								♦																	
Black rot (*Guignardia bidwellii*)		♦			♦	♦				♦	♦	♦	♦	♦	♦	♦		♦					♦		♦
Botrytis bunch rot		♦																	♦	♦		♦			
Crown gall	♦					♦																			
Downy mildew		♦	♦	♦	♦	♦	♦		♦	♦	♦	♦	♦	♦	♦	♦	♦	♦						♦	♦
Esca (grape measles)																									
Eutypa dieback											♦	♦		♦	♦	♦	♦								
Phomopsis (cane and leaf spot)		♦			♦	♦					♦	♦	♦	♦	♦	♦	♦	♦							
Powdery mildew		♦	♦	♦	♦	♦	♦		♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦		♦	♦			♦

3) Restricted-entry Intervals (REI) and Preharvest Intervals (PHI) are provided as a guide only. These restrictions change frequently, and similar products produced by different manufacturers may not have the same restrictions. Consequently, growers are advised to keep abreast of the latest information concerning individual label requirements and restrictions before selecting and applying a product. Remember, **THE LABEL IS THE LAW.**

Chart 4. Winegrape Fungicides and Bactericides Registered in Oregon, 2008

Active ingredient	Trade name (bold = OMRI registered)	Chemical group	Mode of action	Chemical activity	FRAC code
hydrogen peroxide (dioxide)	HDH Peroxy, **Oxidate Broad Spectrum Bactericide/Fungicide**, Peroxi Cide	inorganic	unknown (U)	contact	NC

Oxidate is allowed for use in organic farming (registered by the Organic Materials Review Institute of the USA)

1.2. Effectivity of EcoClearProx® in suppression of grape vine diseases – the organic way (based on Italian field work)

EcoClearProx® has been tested in viticulture successfully against Botrytis bunch rot and powdery mildew of grapevines by spraying 1% dilutions of EcoClearProx® (0.421% H_2O_2)[1] on a weekly (to 2-weekly) basis using 1000 litre of spray water per ha[2]. Also downy mildew can be controlled using EcoClearProx®, however the work with this pathogen revealed that (1) an alternation of 1% dilutions of EcoClearProx® (0.421% H_2O_2) once a month or 2 months with another substance with fungicidal

[1] EcoClearProx® was tested in preliminary trials using 1 to 2.5% dilutions. From the results it was concluded that 1% dilutions of EcoClearProx® already control these diseases, so in general this dilution is recommended.

[2] Field tests using 500 to 1000 litres of spraying water per hectare revealed that EcoClearProx® can longer protect the grape vines against these diseases when using 1000 litres of spraying water per ha.

activity (e.g. monopotassium phosphate, or eventually a classical synthetic fungicide or cupper sulphate) or (2) blends of diluted EcoClearProx® (0.421% H2O2) with monopotassiumphosphate are most effective. Finally, EcoClearProx® showed to control to some extend some pests (e.g. spider mites).

1.2.1. Powdery mildew

The suppression of powdery mildew of grapevines (caused by U. necator) is successful in Italian field experiments (2 years of experience – growth seasons 2008 and 2009) when spraying 1% dilutions of EcoClearProx® (0.421% H2O2) on a weekly to 2-weekly basis. The amount of water sprayed should be 1000 liter per hectare, as this has a positive impact on the prolongation of the protective/curative effect of EcoClearProx®.

1.2.2. Botrytis cinerea

The suppression of Botrytis cinerea in grapevines is successful in Italian field experiments (2 years of experience – growth seasons 2008 and 2009) when spraying 1% dilutions of EcoClearProx® (0.421% H2O2) on a weekly basis. The amount of water sprayed should be 1000 liter per hectare, as this has a positive impact on the prolongation of the protective/curative effect of EcoClearProx®.

1.2.3. Downy mildew

Only a few active substances with fungicide activity can be used in organic farming, above all copper and sulphur. The copper is the only substance that can be used against downy mildew (La Torre et al., 2008); however, since it causes problems of environmental impact, incompatible with organic farming's objective of environmentally friendly farming, the Commission of the European Communities has fixed a ceiling on use expressed in terms of kilograms of copper per hectare per year (EC, 2002).

The suppression of downy mildew caused by P. viticola in grapevines has been tested with EcoClearProx® on 4 different locations situated in 3 different regions of Italy representing 3 different ecological niches:

Only one field trial there was infestation of the vineyard by downy mildew, while in the 3 other field trials, both, the controls and the treated plants were not infested at all with the pathogen, due to too good weather conditions (no rainfall).

(1) Venice – Claudio Debortoli, the total area of the vineyard of this winehouse is approx. 800 hectares, result: no attack by downy mildew of any treatment in the whole experimental plot on this location.
(2) Piemonte – Michele Vigasio, the total area of the vineyard of this winehouse is approx. 500 hectares, result: control treatments (no treatment with EcoClearProx®) severely attacked by downy mildew, while in the treatments using EcoClearProx® the disease was under control (more details in next §)

(3) Lombardy – Mauro Bettini, the total area of the vineyard of this winehouse is approx. 70 hectares, result: no attack by downy mildew of any treatment in the whole experimental plot on this location.
(4) Lombardy – Foundation Fojanini – an experimental station that coordinate and follow up all the experiments, result: no attack by downy mildew of any treatment in the whole experimental plot on this location.

To conclude, the work at Piemonte revealed that the best control of downy mildew is obtained when:

(1) treatments with 1% dilutions of EcoClearProx® (0.421% H2O2) are varied once a month or 2 month with another substance with fungicidal activity (e.g. monopotassium phosphate, or eventually a classical synthetic fungicide or cupper sulphate), or
(2) treatments with EcoClearProx® and monopotassium phosphate[3] are combined in one treatment (instead of only EcoClearProx®), although both products should be added separately to the spraying tank as their concentrates are not 100% compatible. The effective doses to control downy mildew are 0.5 liter EcoClearProx® (0.421% H2O2) and 0.5 litre monopotassiumphosphate (30% P + 20% K) per 100 liter of spraying water.

2.4. Insects

Treatment with EcoClearProx® shows a general control of spider mite and some other insects, but not always consistent. Further work is planned because there are different responses to the treatments with EcoClearProx®.

2.5. Some reflections concerning the benefits of EcoClearProx® in viticulture

1. Traditional fungicides when applied in vineyards leave a residue behind on the grapes which stay on them even when they are pressed. This means that traces of the fungicide can be found in the wines produced from a vineyard. Because EcoClearProx® is an oxidizing agent that depletes in water and oxygen, absolutely no residue is left behind, thus there isn't any in the wine that is produced. This means a better quality wine is produced.

2. When the classical fungicides are washed into the soil they also kills the 'good' soil fungi. At the EcoClearProx® dose which is used for leaf treatment no effect is expected at this level.

Only when EcoClearProx® is washed into the soil at higher concentrations, also some effect on soil microbial life can expected, but the microbial life is restored very fast after treatment (in contrast with fungicide treatment), as nutrients are released after partial degradation of the soil organic matter, and the roots improve as they are being exposed to more oxygen due to an increased soil aeration. Over a period of time it would be reasonable to expect better yields as the quality of the

[3] A phosphate-foliar fertilizer which seems to be an important factor for inducing resistance and controlling foliar diseases and to reduce pesticide usage and which is normally used against powdery mildew

vine roots and soil is vastly improved, and no build up of rest concentrations of fungicides in the soil.

3. In seasons with frequent rainfalls more fungicide treatments are required, often resulting in soil and plant toxic levels of fungicides, while, under the same conditions, frequent treatments with EcoClearProx® will not result in soil and plant toxic levels.

4. Employees daily working in a vineyard, which is treated with EcoClearProx®, are not exposed to harmful fungicide levels, so they can perform their work in a less polluted area.

3. Safety of hydrogen peroxide and EcoClearProx® when applying in viticulture and agriculture

Hydrogen peroxide breaks down rapidly in the environment to oxygen gas and water, leaving no residue, and is not expected to cause adverse effects to humans or the environment when users follow label directions (EPA, 2002). This safety level does also apply for EcoClearProx®.

MRL values when treating vineyards with H2O2 or EcoClearProx®

The Australian Wine Research Institute (AWRI, 2009b) made an overview of the MRL values for several countries worldwide for the commercial product Peratec, in a document which is called "Agrochemicals registered for use in Australian viticulture - The Australian Wine Research Institute MRL Database".

Group Y Fungicides -multi-site activity: Peratec (hydrogen peroxide + peroxyacetic acid), dose 1-2 L/100 L, use no later than 7 days before harvest.

Of course, in the case of EcoClearProx® only the MRL of hydrogen peroxide itself is relevant, and because the reaction byproducts of EcoClearProx® are typically water and oxygen, no residues are expected when treating the vineyards with EcoClearProx®. Furthermore, based on the American legislation (Kaiser, 2008) it can be concluded that, in contrast with the situation of Peratec, there is with EcoClearProx® no need to respect restricted-entry intervals (REI) and preharvest intervals (PHI) longer than 1 hour and 0 days, respectively (See also "Chart 3. Winegrape Fungicides and Bactericides Registered in Oregon, 2008").

4. Further research and other potential applications of EcoClearProx® in viticulture

4.1. Adjuvants

Further research with EcoClearProx® in viticulture will focus on the combination of EcoClearProx® with adjuvants. The aims of this work is to investigate of adjuvants (1) can further reduce the number of sprayings with EcoClearProx®, (2) allow to spray with even lower concentrations of EcoClearProx® or (3) can reduce the volume of spraying water needed per ha.

4.2. Insects

Further work with EcoClearProx® is planned to better understand responses of insects to the treatments with EcoClearProx®.

4.3. Vapor phase hydrogen peroxide treatment during grape storage

Different hydrogen peroxide vapor applications were tested to decrease the spoilage after harvest and especially during storage, and to present a high quality product to the market for a longer time. Liquid hydrogen peroxide at 30–35 % concentration was applied for 5, 10, 15 and 20 minutes after vaporizing at 30, 40 and 60 °C. The grapes were stored for 3 months in cold rooms at 0 ± 1 °C, immediately after harvest. At the end of 3 months' storage, 10 and 20 minutes applications of 40 °C vaporized hydrogen peroxide and 5 and 10 minutes applications of 60 °C vaporized hydrogen peroxide gave better results with respect to decrease in microorganisms compared with other applications. In addition, when taste and appearance were considered, a 20-minute application of 40 °C vaporized hydrogen peroxide gave better results than the others in grapes stored for 3 months (Eris et al., 1994).

The potential use of vapor phase hydrogen peroxide (VPHP) to prevent decay caused by Botrytis *cinerea Pers. ex Fr. in table grapes (Vitis vinifera L.) was investigated. 'Thompson Seedless' and 'Red* Globe' grapes, inoculated with Botrytis cinerea spores, were placed in polyethylene bags and flushed for 10 minutes with VPHP generated from a 30% to 35% solution of liquid hydrogen peroxide at 40 °C. Immediately after treatment, bags were sealed and held at 10 °C. Vapor phase hydrogen peroxide significantly reduced the number of germinable Botrytis spores on grapes. The number of germinable spores on 'Thompson Seedless' and 'Red Globe' grapes had been reduced 81% and 62%, respectively, 24 hours following treatment. The incidence of decay on inoculated 'Thompson Seedless' and 'Red Globe' grapes was reduced 33% and 16%, respectively, after 8 days of storage at 10 °C compared with control fruit. Vapor phase hydrogen peroxide reduced the decay of non-inoculated 'Thompson Seedless' and 'Red Globe' grapes 73% and 28%, respectively, after 12 days of storage at 10° C. Treatment with VPHP did not affect grape color or soluble solids content (Forney et al., 1991).

According to Rij and Forney (1995) H2O2 vapours were very effective in killing B. cinerea spores on dry glass slides when spores were air-dispersed to the slides. A treatment of 0.27 mg/l H2O2 at 20 DEG C killed 99 % of the spores in <11 min. Thompson Seedless grapes appear to be fairly tolerant to H2O2 vapour treatments. The first visible sign of injury was a brown discoloration of the stem and fruit tissue which occurred after a 6 h exposure to H2O2 vapour at 40 DEG C. Grapes held in 0.27 mg/l H2O2 vapour at 20 or 30 DEG C for up to 24 h did not show visible injury. At 30 DEG C, 0.55 mg/l H2O2 vapour was effective in killing Botrytis spores in minutes. Results suggest that the tolerance of grapes to H2O2 vapour treatments could allow H2O2 vapour to be an effective treatment to control postharvest decay.

Also EcoClearProx® could be the organic alternative for this application.

4.4. Irrigation water in viticulture

OxiDate is labeled in the US as an irrigation disinfectant for vineyards (Vinebalance, 2007). Also EcoClearProx® is definitely the organic alternative for this application.

4.5. Water re-use in viticulture

The results of Barhorst and Kubiak (2009) indicate that hypochlorite has a high potential to form AOX in effluents of viticulture. The predominant by-products are chloroform, dichloroacetic acid and trichloroacetaldehyde. If possible, disinfection should be accomplished by the use of no chlorine-containing agents. By this means, negative influences of hypochlorite on the quality of wine can also be avoided. Also EcoClearProx® is such an alternative organic disinfection agent.

4.6. Root formation in grape grafts

Barabalchuk and Klimenko (1991) have shown that it is possible to promote root formation in grape grafts by means of hydrogen peroxide solutions. 2-4 solutions prove to be most efficient when immersing basal ends of grafts 1-2 cm deep into the solution for 24-28 h. Such action of hydrogen peroxide on morphologically upper ends of grafts exerts no negative effect on callus formation, bud termination and shoot growth.

4.7. Hydrogen peroxide as alternative for pasteurization of fruit juices, including juices of grape vines

Cryptosporidium parvum has historically been associated with waterborne outbreaks of diarrheal illness. Foodborne cryptosporidiosis has been associated with unpasteurized apple cider. Infectious oocysts are shed in the feces of common ruminants like cattle and deer in and near orchards. In this study, the ability of organic acids and hydrogen peroxide (H2O2) added to fruit juice to inhibit the survival of C. parvum was analyzed. Oocyst viability was analyzed by a cell culture infectivity assay with the use of a human ileocecal cell line (HCT-8) whose infectivity pattern is similar to that for human oral infectivity. Cell monolayers were infected with 10_6 treated oocysts or a series of 10-fold dilutions. Parasitic life stages were visualized through immunohistochemistry with 100 microscope elds per monolayer being counted. In vitro excystation assays were also used to evaluate these treatments. Organic acids and H2O2 were added to apple cider, orange juice, and grape juices on a weight/volume basis. Malic, citric, and tartaric acids at concentrations of 1 to 5% inhibited C. *parvum's infectivity of HCT-8 cells by up to 88%. Concentrations ranging from 0.025 to 3% H2O2* were evaluated. The addition of 0.025% H2O2 to each juice resulted in a >5-log reduction of C. *parvum infectivity as determined with a most-probable-number–based cell culture infectivity assay.* As observed with differential interference contrast and scanning electron microscopy, reduced infectivity may be mediated through effects on the oocyst wall that are caused by the action of H2O2 or related oxygen radicals. The addition of low concentrations of H2O2 can represent a valuable alternative to pasteurization (Kniel et al., 2003).

Also EcoClearProx® could be the organic alternative for this application.

5. References:

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