

TECHNICAL DATA SHEET

Hydrogen peroxide injection

Atypical practice applied in field to limit GTDs symptoms expression



This document is the result of interviews made on field with the principal objective to highlight the diversity of techniques used in field. To date, no assessment, no validation or checking efficacy of this practice was made. In the absence of any assessment, its success in different conditions of the ones exposed is not guarantee and the responsibility of Winetwork partners can't be involved.

Network for the exchange and transfer of innovative knowledge between European wine growing regions



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Hydrogen peroxide injection

General principle

This technique consist in one or more **injection(s) inside the trunk of grapevine of peroxide hydrogen** in order to limit symptoms expression. This technique is applied by winegrowers in South of France (Madiran), Spain

Application cases

1- In South-West

Injections were experimented in two plots characterized by the following:

Cultivar: **Cabernet Sauvignon**

Planting date: 2004

Rootstock: 3309 C

Pruning system: Guyot double

Yield: 60 hl/ha

Type of soil: clay and limestone

Pruning debris: pruning debris are crushed on the floor

Soil Management: natural grass cover between the rows.

Chemical management of grass under rows.

No irrigation system

Cultivar: **Gros Manseng**

Planting date: 2006

Rootstock: 3309 C

Pruning system: guyot

Yield: 30hl/ha

Type of soil: clay and limestone

Pruning debris: pruning debris are crushed on the floor

Soil Management: Chemical management of grass under rows, soil labour between the raw.

No irrigation system

Application area:

In South West region in the area denominated "Madiran" close to Pyrenees mountains.

Climatic conditions are characterised by high annual rainfall around 1100 mm and 670 mm of rain during vegetative period. Annual average temperature are comprised between 2°C minimum in winter and 25°C in summer. The region also show a good sunshine of around 1400 hours during vegetative season.

Concrete application:

As soon as first foliar symptoms appears, diseased grapevine are **marked**. Rapidly the injection is done.

(Galicia) and Portugal (Douro) on vines showing Esca or Botryosphaeria dieback. Application is easy and quick and results seems to be promising.

On the marked grapevine, **make one hole** with a driller, the best is to use a big wood wick. Hole need to be done **below vine arms** (in the previous years some experiments were done on the location on the hole ; at the top of the trunk, basis of the trunk, in one arm, below the arms ; the more effective appears to be below the arms). In order to drill properly and to retain the liquid, give a **inclination of 35 to 45°** in the trunk axis (pict. 1). Once the hole is made, **inject with a serynge 3 to 4 ml of hydrogen peroxide (10%)** in the hole. (pict. 2) In average, winegrower spend 1 minute per grapevine to make the injection.

Winegrower is using this technique for 5 years on Cabernet-Sauvignon and for 2 years on Gros Manseng.

Over time, technique was refined and the most efficient entry point was found.



Picture 1: Drill in the trunk and injection point (IFV South-West)



Picture 2: Injection of H2O2 in the hole (IFV South-West)

2- In Galicia

The injection with H₂O₂ was applied in 3 vines of a plot of cultivar **Albariño** (white) with unless 15 % of affection. The plot is characterized by the following:

Cultivar: **Albariño**

Vine age: 30-40 years old

Non rootstock or also american rootstock of 1^a generation (V rupestris de Lot)

Training system: "Parral"

Planting density: 5x4 m

Pruning system: Guyot.(Rod and spur pruning)

Yield: 10 t/Ha

Soil: clayey quite heavy

Pruning debris: Infected pruning debris are taken out. Rest of them are crushed and left on the floor.

Soil Management: natural grass cover and mechanical management 3 times along vine cycle (winter, spring and pre- harvest)

Fertilization: Organic fertilizer, usually horse manure is distributed every year in winter with milling machine or toothed cultivators (shanks)

No irrigation system

Application area:

Area denominated "O Salnés" in SW Galicia close to sea coast and belongs to Rías Baixas Designation of Origin.

Atlantic climate: High annual rain fall: 1566 mm and 593 mm during vegetative period and 1400 hours of sunshine.

Annual average temperature: 17.9 °C. Between 4.7 °C in winter and 27° C in summer and 1400 hours of sun during vegetative period.

Concrete application:

Previously vines were **marked in summer**, when foliar symptoms are clear.

The **application was done after pruning** on third week of February in phenology stage winter dormancy. Each vine is 2 m high so it was made **four holes with a drill** (drill bit: 8) in two parts of vine: **two at the base of trunk** and **two at the top** (1,8.m high) (pict. 3).

Hydrogen peroxide was injected with a syringe, and then **the hole was covered with 2 cm³ of pure eucalyptus oil**. It was injected H₂O₂ 30% p/v (110 vol) in three vines affected by Esca (pict 4, 5).

It was spent almost 3 minutes per vine including (drilling +injection both H₂O₂ and eucalyptus oil)

This practice was **applied only one year and results are unknown so far**.

The same winegrower will applied again this technique in other vines of the same plot affected by GTDs.



Picture 3: Drilling 8 mmm at the top and bottom of vine (INGACAL)



Picture 4: Injection Hydrogen peroxide 30%p/v (110 vol) (INGACAL)



Picture 5: Hydrogen peroxide 30%p/v and eucalyptus essential oil used for this trial(INGACAL)

Hydrogen peroxide injection

Some scientific elements

First, it is known that H₂O₂ is an oxidative species of oxygen (ROS) that plant produces in responses to biotic or abiotic actions. In the case of an pathogen attack, these oxidative species are fundamental in the defense against fungal infection inducing enzymatic or non-enzymatic defense reactions of the plant.

Hydrogen peroxide can **diffuse into the cell and activate the defense genes**, leading to programmed cell death (Davison et al., 2002, Grant and Loake, 2000, Hammond-Kosack and Jones, 2000), which contributes to limit the invasion of potential pathogens (Bokoch, 1994).

Correlation between ROS accumulation and the establishment of defenses suggests that **ROS could have a direct toxic function on the pathogens**, or on the cells where they occur.

ROS may have opposite functions in different plant / pathogen interactions, being in some cases positive regulators of defense and cell death reactions, while in others they act as negative regulators (Montillet et al., 2005).

Furthermore, different trials to check several products against Esca, eutypa dieback and botryosphaeria dieback in which grapevine rootstock and scion cuttings soaked in a product, like a Bio-Steriliser (hydrogen peroxide) results were inconsistent. (Fourie & Halleen, 2006).

However, information about US patent "Method of protecting growing plants from the effects of plant pathogens -US 6024986 A" which summary said "The direct introduction or injection of peroxy compounds into a transpiration layer in a plant structure found inside the protective layer, bark or husk of a growing plant is described as an effective method in treating plant disease and can achieve levels of protection unavailable in the simple application of the materials to the leaf, stem, root or plant environment including air or soil".

In the beginning of 2000's some studies were conducted in order to evaluate the efficacy of fungicides by injection inside the trunk in the control of grapevine trunk diseases. Several fungicides were injected into the trunk with a high pressure pump after drilling a hole. Injection were tested on several cultivars: Cabernet Sauvignon, Cabernet franc, Riesling, Pinot, Gamay, Fer Servadou and Sauvignon. Any of the studies gave positive results, efficiency of fungicides varying according to the region and cultivar, action seems to be limited in time, since as the second or third year of obser-

vation, percentage of diseased plants was similar to control (Sentenac et al., 2004, Lecomte et al., 2006). Furthermore, **the effect of an injected product and its mobility inside the trunk is unknown.**

Actually, other **scientific elements are missing** in order to understand and **assess peroxide hydrogen efficiency into the plant.** We don't know if sap is transporting peroxide hydrogen into plant vessels or if product stay at injection point and either what is the response of the plant to this product and **how product is affecting GTDs pathogens.**

Some winegrowers are using this technique on the field but are asking for further information and for more research on this field in order to **understand the effect of H₂O₂ on the plant and if H₂O₂ has a real efficiency on GTDs pathogens.** Furthermore, questions on application are raised: what is the correct dose? What happen if injected volume is higher? Does the plant die?

Other practical experience

In Spain one winegrower of Galicia has applied this practice in 2016, in Castilla- León exists another winegrower, who applies Hydrogen peroxide although with a different formulation -HUWA SAN 50A (formulated on the basis of a synergistic mixture of hydrogen peroxide and colloidal ionic silver). It has begun in 2015 with affected vines by Esca and results are being successful, although **only one year is few time to deduce favorable data.**

The application is done immediately after pruning making an opening with a wedge, ax or with a drill, to reach the heart of vine, and pour a 3% diluted solution (3L/100L water) into the opening of HUWA-SAN50A to gradually penetrate into the open hole. Once the solution has been absorbed into the open holes, it is sprayed on the whole plant and more specifically on the pruning wounds zone. In the following spring and when grapes are on pea size (phenological state setting to berries-peas size), it should be made a new directed spraying to canopy with this compound at doses between 0.3 and 0.5% (300 to 500 cm³/ 100 l water). Through this new leaf spray, it is intended to maintain the action of this product against Esca as well as preventing from other fungi and bacteria.

Outcomes

1- In South-West

Injections made in South-West gave some results. It is important to remember here that **it isn't a scientific trial but empirical approach**. In 2015, the winegrower made the injection on 20 grapevines of Cabernet-Sauvignon, the year after, none of the grapevine expressed GTDs foliar symptoms. In 2016 injections were made on **50 grapevines** of Cabernet-Sauvignon. It appears that after injections **symptoms are blocked** and does not evolve anymore and new healthy leaves appears.

The grapevine seems to recover and don't express the symptoms the following years but **more observations in the next years are needed** in order to follow the treated plants and see how they'll evolve. On Gros Manseng, injections were done on 20 grapevines in 2016, and showed the same results.

2- In Galicia

Although the 3 vines showed symptoms on leaves and branches, only one of them seems to show recovery because it had production although with smaller grains.

It should be noted that these vines had already showed symptoms of Esca during the last years and the summer of 2016 has registered strong heat strokes with 38°C of temperatures and vines have been bearing water stress. **The treatment will be repeated again** in the same vines and in more symptomatic vines of the same plot in order to have a more representative number of treated vines. In order to assess this trial, injections need to be done on a significant number of plants. No conclusion can be provided on that small amount of grapevine.

More experimentation is needed in order to determine the **real efficiency of hydrogen peroxide** on symptoms expression. In addition, residues analysis on leaves and clusters are necessary to determine if product is mobile into the plant.

Conditions of success	Risks
Treatment must be done as soon as first foliar symptoms appears, the sooner, the better	Phytotoxicity
Injection point below vine arms	Death of treated grapevine
Inclination of the hole	Residues in clusters?
On mature vines	

Innovative aspects

Only a few people are using this technique, probably mainly because there is no feedbacks on this technique neither enough validated scientific elements. Nevertheless, it is noticed on field that more and more producers are speaking of this technique, questioning, and some positive results are cited in press. On the field, people applying injections of peroxide hydrogen are confident even if some concerns are persisting.

Other requirements

This practice can be appropriated easily, it can be done on all farm size. Of course, practice is time consuming, so need to mobilize one person to realize the injections at a busy period. This technique don't need any particular training or background.

The bigger cost of this practice is labour cost (personal costs); in addition a driller is necessary, a bit and the hydrogen peroxide that can be found in commercial shops.

Warning: using H2O2 is not accepted by the law, hydrogen peroxide is not a registered product for grapevine and more particularly for grapevine trunk diseases.



Source of information

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More information on

www.winetwork-data.eu

Technical datasheet: Good pruning practices

Video clips:

- [Epidemiology and symptomatology of GTDs](#) (Dr. Vincenzo Mondello, URCA)
- [Scientific overview on Grapevine Trunk Diseases](#) (Dr. Vincenzo Mondello, URCA)



Work realized in common by the facilitators agents of Winetwork project. Data came from practice through the help of 219 interviews and from a review of scientific literature.

The practice described in this datasheet has not been assessed scientifically and the data provided is coming directly from practice.