WINETWORK

European Knowledge Transfer

TECHNICAL DATA SHEET

Trichoderma application

for protecting grapevine pruning wounds

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

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Trichoderma application

Introduction

Trunk diseases significantly limit the productivity and longevity of vineyards in most of the winegrowing regions all over the world. Trunk diseases **attack the permanent woody structure** of the vine, including the trunk, cordons and spurs (Baumgartner, 2013). The pathogens - **a set of taxonomically-unrelated Ascomycete fungi** - associated with grapevine trunk diseases are able to **infect healthy vines** mainly by **pruning wounds** and these wounds can remain susceptible for several months. It is important to highlight that there are no curative methods to control GTDs; the only way is to **prevent or limit the infection of wood diseases using different cultural practices**. The prevention of wound infection applying **biocontrol agents** is one of an **alternative technique** to control trunk diseases. Species of the **genus *Trichoderma*** (an ascomycete fungus, originally present in the soil) have been investigated several times as a potential biocontrol agent by spatial and nutritive competition.

Application area

Use of *Trichoderma* to protect pruning wounds is very popular in Europe and implement on the field by many winegrowers.

Figure 1: European wine-growing areas where *Trichoderma* application is applied, red dot (result from Winetwork interviews). White dots are showing project's partners.

Practical application

The different strains of *Trichoderma* spp. are able to **colonise about 1-2 cm of the pruning wounds** and **prevent the penetration** (into the wood) of pathogens associated with GTDs. The colonisation of grapevine pruning wounds by the *Trichoderma* spp. depends on the **physiological state** of the vines as well as the **weather conditions** at pruning. The pruning season coincides with the period of pathogen spore release which usually originates from infected wood. **Wounds may remain susceptible for a long time** (up to 4 months or more, according to the GTD), but the most critical time for **infection ranges from 2 to 8 weeks after pruning** (Eskalen et al. 2007, Van Niekerk et al. 2011b).

1- Time of application

Normally, *Trichoderma* spp. are not limited by climatic conditions, being able to start wound colonization at 10° C, but the time of the treatment could improve its efficiency in colonizing wounds and thus, its protection capability. The correct timing is above 0°C temperature, though some *Trichoderma* species require higher temperature (exceeds 10 °C). It is important to highlight that *Trichoderma* spp. as a biocontrol agent is susceptible to the frost. The best timing could be **as soon as possible after pruning**, to limit the wound susceptibility period to new GTDs infections. Different studies attested that better colonization results could be achieved with treatments done within 5 or 6 hours after pruning (Harvey et al., 2006, Mutawila et al., 2016).

Some producers recommend distributing *Trichoderma* products during bleeding, since the sap presence helps the antagonist in colonizing the wounds faster. At the same time it is important to check the weather forecast before the application because **heavy rain** can interfere with the beginning of colonization, **washing away the spores**.

Scientists recommend to plant vines that have been inoculated with *Trichoderma* spp in nursery during the propagation process and repeat field treatment 2 or 3 years after planting. Then it is highly recommended to **repeat the application each year thereafter** (Sosnowski, 2016). Both small and

large wounds should be treated with the biocontrol agent either by spraying or painting, according to the economic possibilities or the value of the vineyard.

2- Mode of application

Preventive wound protection practices should start in **1-year-old grapevines** following the first pruning and **continue each year thereafter** (Sosnowski, 2016). Both the small and the large wounds should be treated with the biocontrol agent, using a canopy sprayer with nozzles targeting the cordon (Sosnowski, 2016). When canopy sprayers are used, **maximum coverage of wounds** can be achieved by turning off fans (no air), applying high water rates at low pressure, selecting spray nozzles that produce **large droplet size** and focussing nozzles towards the pruning wound zone.

Different canopy sprayers (modified weed sprayer, recycle sprayer, tangential sprayer and air-shear sprayer) have been tested in cordon- and in cane pruned vines varying the amount of water volume. According to the obtained results, it is important to select adequate sprayer and the required amount of water to achieve the maximum coverage of the vines.
When preparing for the treatment it is highly recommended to **clean carefully the tank** from previous fungicide residues in order to not 'disactivate' *Trichoderma*.

One of the most important obstacles for the use and diffusion of *Trichoderma* is often related to the variable results observed by winegrowers. Indeed, numerous factors could influence the biocontrol capability of a *Trichoderma*-based product, namely the *Trichoderma* species utilized, the method used for its distribution, the phenological stage of the vines, the time between pruning and the *Trichoderma* treatment, the interaction of the antagonist with host plant and, least but not last, with environmental factors (Di Marco et al., 2004.). Furthermore, the biocontrol activity could vary according to the different cultivar (Mutawila et al. 2011a). All these factors, if not properly managed or not take in consideration, could lead to unsatisfactory results.
Thus it is important to not consider a *Trichoderma* treatment similar to a chemical one.
Treatment can also be applied with backpack sprayer and the spray need to be directed on the wounds surface and well cover the wounds (Fig.3) on the entire grapevine.

Outcomes

Among *Trichoderma* species and strains, several are used in European countries in pruning wound protection: *Trichoderma atroviride* SC1 and I1237, T*richoderma asperellum* ICC012, *Trichoderma gamsii* ICC 080 and *Trichoderma harzianum* ICC012 :

- *Trichoderma atroviride* SC1 has been isolated from dead hazelnut wood and selected for its high colonization capability and its high productivity of Lytic enzimes

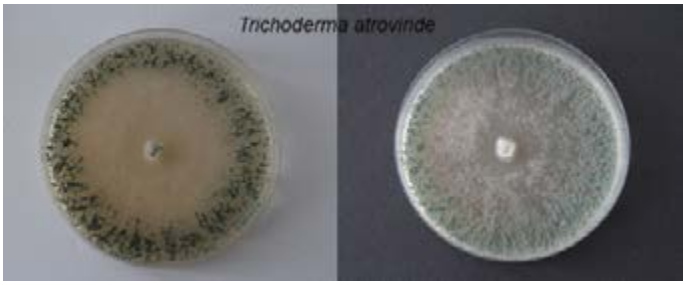
(chitinases, proteases and cellulases). Trichoderma atroviride SC1 is highly competitive and efficiency antagonizes *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora* so is able to reduce the yearly infections on the pathogens associated to esca disease (D'Enjoy et al., 2016.)

- *Trichoderma atroviride* I1237 has the ability to fast colonize pruning wounds, to compete with pathogenic fungi for nutrients and space and properties of antibiosis and mycoparasitism.

- *Trichoderma asperellum* and *Trichoderma gamsii* ICC 080 can have an effect on GTDs pathogens (especially on *Phaeomoniella chlamydospora*) at 10°C and 15°C respectively. Both species remain viable at 5°C.

For future practical applications, experimental trials should be carried out to **confirm its efficacy with a wide combination of application conditions**.

The effectiveness of protection based on *Trichoderma* spp. treatments depends on the ability of these fungi to colonize grapevine pruning wounds (John et al., 2008). For a complete colonization of the wound, *Trichoderma* species usually need some time, and during which grapevine is susceptible to infection from GTDs pathogens and to washing off by rainfall. However, more field tests are needed and necessary to conclude on their effect on the short and long term and to determine how it could be optimied by a combination of other management strategies (such as combination with other biological or chemical products, remedial surgery, reducing the number and size of pruning wounds and application of sanitation methods) (Bertsch et al., 2013).



Trichoderma atroviride SC1 (DLR Rheinpfalz)

Trichoderma application

Figure 3: Treatment of pruning wounds in a vineyard damaged by Esca (Eszterházy Károly University, N. Burghardt)

Some scientific elements

One way to control grapevine trunk diseases is to protect pruning wounds with fungicide applications, which can be problematic because of the limited number of registered products (not authorized in all european countries), the difficulty for these products to control numerous taxonomically unrelated organisms, the challenge of these products to protect during the entire period of wound susceptibility and the difficulties and costs associated with hand application of protection treatments (Rolshausen et al., 2010).

The integration of fungicide and biological wound protection could provide better control, but is limited by the susceptibility of the biocontrol agents to the fungicides.

The major way of managing trunk diseases in field grapevines is to prevent pathogen entry through pruning wounds. Product for wound protection should be effective against the whole range of trunk pathogens while also protecting the wound for the whole period of wound susceptibility. Generally, the goal for pruning wound treatments is to inhibit mycelial growth on the wound itself and/or physically seal the wood to prevent infection (Newsome, 2012.). *Trichoderma* are well known as fungi that exhibit antagonistic activity and hyper-parasitism in regard to other microorganisms and it is used for biological control against several diseases. Although their mode of action is not fully understood, they seem to be associated with mycoparasitism, the production of inhibitory compounds, competition for nutrients and space with pathogenic fungi, stimulation of plant growth and enhanced host resistance (Di Marco et al., 2004). Since 2000s, several trials were conducted in order to evaluate the efficacy of *Trichoderma* spp. to control GTDs pathogens (table 1). Results of these studies globally showed that *Trichoderma* spp. have a partial efficiency according to assessment methods used in controlling the main GTDs pathogens on both pruning wounds in the field and cuttings at nursery, avoiding new infections. Furthermore, thanks to its broad spectrum activity, *Trichoderma* is able to delay infections of a wide range of GTDs pathogens, staying viable in the woody tissues below wound up to 1 year. Being a “living” product, its efficiency could be influenced by the environment. In particular wound colonization capability and persistence of the *Trichoderma* species may depend on intrinsic wound factors and hence may vary between cultivars and on the vine physiological stage in which *Trichoderma* is applied (Bruez et al, 2014; Di Marco, 2007).

Botryosphaeria dieback	Eutypa dieback	Esca complex
T. harzianum, T. atroviride, and Benzimidazole-resistant mutant strain **TESTED: pruning wound protection**	*Trichoderma* spp *T. harzianum, T. atroviride*, Benzimidazole-resistant mutant strain **TESTED: for Eutypa toxic metabolites degradation activity for pruning wound protection**	*Trichoderma* spp *T. harzianum, T. atroviride, T. longibrachiatum* and Benzimidazole-resistant mutant strain **TESTED: pruning wound protection**
Bacillus subtilis EE isolate TESTED: pruning wound protection	*Bacillus subtilis* EE isolate **TESTED: pruning wound protection**	*Bacillus subtilis* EE isolate **TESTED: pruning wound protection**
		Pythium oligandrum **TESTED: induced resistance by root colonization**

Table 1: BCAs used to control GTDs (Esca, Botryosphaeria and Eutypa dieback)

Furthermore, the *Trichoderma* wound protection effect also depends on its interaction with the grapevine, since it is not only due to the direct suppressive effect of *Trichoderma* on pathogens, as reported by some researchers (Mutawila et al, 2011).
A recent study (Aloi et al., 2014) showed the ability of *Trichoderma* gamsii+*Trichoderma asperellum* in reducing the incidence of Esca symptoms when applied as wound protectant.

Key points for success

Trichoderma spp. have a preventive effect on the infection of grapevine trunk diseases pathogens, to maximise its preventive effect, several conditions need to be respected:

- Application of *Trichoderma*-based product as **soon as possible after pruning**
- Application can be done either by sprayer (canopy sprayer or backpack sprayer) and paintbrush.
- Respect ***Trichoderma* strain characteristics** (temperature during application) and if possible apply the product on dry conditions and before rain.
- to maximise preventive action, start the application of *Trichoderma* on the first year and r**enew each winter** at the pruning period

Trichoderma species (DLR Rheinpfalz)

More information on

www.winetwork-data.eu

Technical datasheets: Good pruning practices
Global vineyard strategy to manage GTDs

Video seminars:

- Scientific overview of grapevine trunk diseases (Dr. Vincenzo Mondello, URCA)
- Symptomatology and epidemiology of grapevine trunk diseases (Dr. Vincenzo Mondello, URCA)

Source of information

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Forscher testen Mittel gegen «Rebenkiller»-Pilz Esca

G. E. Harman, Cornell University, Geneva, NY 14456 https:/biocontrol.entomology.cornell.edu/pathogens/trichoderma.php

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 litterature.