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Technical article

Use of *Trichoderma* spp. in the management of grapevine trunk diseases in Europe

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Eco-friendly alternatives to pesticides are at the centre of current concerns in the world of agriculture. In this context, the use of biocontrol products is more and more under investigation and requested by European producers and consumers alike. Viticulture does not escape this trend and, aside from bio-products capable of controlling important diseases (i.e. Ampelomyces quisqualis on powdery mildew), several biocontrol agents have been tested for the control of other grapevine diseases, such as Esca, Botryosphaeria and Eutypa diebacks. In this contest the fungal genus *Trichoderma*, widely used in organic and integrated agriculture to reduce and control diseases affecting several crops, could play an important role in offering an effective and sustainable control of such GTDs. For this reason, since the 2000s, the effects of *Trichoderma* use on grapevine trunk diseases have been studied in most of the world's main wine-growing areas.

The antagonism of *Trichoderma*

Trichoderma spp. are fungi presenting a well-known antagonism (since 1887) towards a significant number of plant pathogens and especially soil-borne pathogens. The first studies conducted showed its ability in protecting plant roots from infections caused by pathogens, and also described its modes of action. In detail, *Trichoderma* species could attack pathogens by: 1) Antibiosis, through the production of substances that, spreading in the substrate (soil, dead organic matter, etc.) inhibit the growth of other competitors, including plant pathogens, 2) competition for nutrients, since *Trichoderma* uses the same nutrition resources as the pathogens, 3) competition for space and infection sites, due to the high rate of growth of *Trichoderma* compared with those of other microorganisms and 4) hyper-parasitism, which is the destruction of pathogens through *Trichoderma*-produced enzymes such as lytic enzymes, lethal for the pathogen's cells. All these modes of action are species- or even strain-specific, being regulated at gene level. Thus *Trichoderma* species could pursue several strategies at the same time to control pathogens, depending on environmental factors (temperature, humidity, etc.) and/or on the physical, chemical and biological conditions of the matrix (that is, the grapevine woody tissues).

Trichoderma for the control of GTDs

Since the 2000s, several trials have been conducted in order to evaluate the efficacy of *Trichoderma* spp to control GTD pathogens. The main pathogens used in tests were *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum* for the Esca complex, *Diplodia seriata* and *Neofusicoccum parvum* for Botryosphaeria dieback and *Eutypa lata* for Eutypa dieback. In particular, and after the in vitro tests, they were used for artificial inoculation of pruning wounds or plants treated with the antagonist, to observe the ability of the *Trichoderma* strain to limit GTD infections. Results of these studies globally showed that *Trichoderma* spp. have a partial effectiveness according to the assessment methods used in controlling the main GTD pathogens on both pruning wounds in the field and cuttings in nurseries, avoiding new infections. Furthermore, thanks to its broad spectrum of activity, *Trichoderma* are able to delay infection by a wide range of GTD pathogens, remaining viable in the woody tissues below the wound up to 1 year. Being a "living" organism, its efficacy should be influenced by the environment. In particular, wound colonisation capability and persistence of the *Trichoderma* species may depend on intrinsic wound factors and hence may vary between cultivars and on the vine's physiological stage when *Trichoderma* is applied (Bruez et al, 2014; Di Marco, 2007). 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).

Several *Trichoderma* species have been tested. Currently the most commonly-used strains as biocontrol-agents in GTD management belong to the *T. atroviride, T. asperellum T.gamsii* and *T. harzianum* species. As mentionned before, the strains of these species differ in their antagonistic potential against different harmful fungi and are unlikely to have the same effect against all GTD pathogens. Here is some brief information about some of the more commonly-used strains:

Trichoderma atroviride SC1: the strain was isolated from the wood of the hazelnut tree. *T. atroviride* SC1 has rapid growth, germination and colonisation of the wood at low temperature. Its colonisation is fast and persistent, providing significant competition with pathogens. This strain produces lytic enzymes (cellulases and proteases) and antibiotics providing good biocontrol activity, enzyme-degrading spores and mycelium of pathogens on the wood surface.
Trichoderma atroviride I1237: has the ability to colonise pruning wounds quickly, to compete with pathogenic fungi for nutrients and space, and to attack pathogens by antibiosis and mycoparasitism.
Trichoderma asperellum and *Trichoderma gamsii* ICC 080: these two strains have an activity range from 10 to 28°C, with an optimum activity around 15°C (the strain is viable from 8 to 35°C). *Trichoderma gamsii* and *T.asperellum* can have an effect on P. chlamydospora at 10°C and 15°C respectively. Both species remain viable at 5°C, and, are able to grow when the temperature increases.

Trichoderma atroviride SC1

How *Trichoderma* acts in grapevine to control GTDs?

At first, *Trichoderma* spp. should be used primarily as a preventative treatment, since these species don't have a curative ability with regard to GTDs but can, for instance, effectively prevent infection of pruning wounds.
Trichoderma spp. are fast growing fungi, and in optimal conditions they could colonise pruning wounds. Furthermore, they induce a significant competitive capability at the colonisation site due to a successful and stable colonisation. Mycelium from *Trichoderma* spp. can colonise pruning wounds and is able to colonise the tissues below (up to 1 to 2 centimetres in a short time period). *Trichoderma* spp., could inhibit the germination of spores of different pathogens such as Botryosphaeriaceae (Kortekamp,. 2013)

How to use *Trichoderma*-based products to control GTDs?

Time and application method

After pruning, wounds may remain susceptible to GTD pathogens for a long time (up to 4 months, depending on the GTD concerned), but the most critical time for infection ranges from 2 to 8 weeks after pruning (Eskalen et al. 2007, Van Niekerk et al. 2011b).
Normally, *Trichoderma* spp are able to start wound colonisation at 10°C, and their efficacy and protection capability could be improved by the timing of the treatment. Application of *Trichoderma* -based product to pruning wounds could be done between grapevine dormancy and bleeding (BBCH 00 – BBCH 05). However, different authors have observed a faster wood colonisation if *Trichoderma* is applied during the break dormancy stage or at the bleeding stage (late winter) than during the dormancy period. Furthermore, a recent study has shown that when applied 6h after pruning, either early or late pruning, the colonisation of *Trichoderma* spp was higher than at other application times (0, 24, 48 and 96h - Mutawila et al, 2016). In order to obtain maximum efficacy, application needs to be done shortly after pruning. It is also important to consider the weather forecast before the treatment, since heavy rain could interfere with wound colonisation if the *Trichoderma* based product is washed away.
Wound protection practices should start immediately after planting. Scientists recommend planting vines that have been inoculated with *Trichoderma* spp in the nursery during the propagation process and repeating 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, depending on cost considerations or the value of the vineyard.
All *Trichoderma*-based products can be applied by spraying, (spores suspended in water, either with backpack sprayer or standard sprayer). Nozzles need to be directed onto the pruning zone in order to cover pruning wounds and a large volume of water needs to be used (from 400-600 l/ha and need to be adapted to the type of sprayer (Sosnowski et al., 2016).

Application of *Trichoderma* spp. in the management of grapevine trunk diseases in Europe

In order to improve the effect, spray quality needs to be well managed, reaching both wounds on the shoots and on the trunk. 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 sizes and focusing nozzles towards the pruning wound zone. If used in an Integraded Pest Management strategy, no fungicide should be applied prior/immediately after *Trichoderma* application, and any residual fungicides in the tanks used must have been totally removed.
Trichoderma-based products can also be applied by painting pruning wounds. This method is not commonly used in practice as it is time consuming but could be considered for valuable vineyards.
As the effectiveness of *Trichoderma* species varies with local conditions and has not been completely established in the field, it is essential to complement the use of *Trichoderma* with good management practices in the vineyard (good pruning methods, inoculum restriction, good vine balance, etc.).

The use of Trichoderma in some Winetwork areas

In Italy, *Trichoderma*-based products are always used as a preventive treatment (spray), after pruning (as soon as possible) in young vineyards, where no symptoms have yet appeared. In practice, *Trichoderma*-based products are used essentially in large vineyards with good productivity and high value, for instance the Soave or Prosecco areas, but their use is nevertheless recommended for all types of vineyards. Although its use in France is currently somewhat sporadic, *Trichoderma*-based products are used by some nurseries and winegrowers. When spraying in vineyards, both sprayer and backpack are used between 3 to 10 days after pruning as a preventive action against GTDs. In Germany, *Trichoderma* products are used in nurseries where the grapevines are immersed in a water bath contaianing *Trichoderma* for some hours before planting. Only a few winegrowers currently use *Trichoderma* in the vineyard because the product has only been available since 2016. They use it as a spray as soon as possible after pruning and only if they are fairly sure that frost is unlikely. Throughout Europe, nurseries have developed special processes to treat material and equipment with *Trichoderma* spp. Efficacy in these application conditions, both in nurseries and vineyards, has not been proven.

An unusual method of applying *Trichoderma* has been recorded in a Spanish vineyard. This consists of inserting small *Trichoderma*-inoculated wood dowels into previously drilled trunks and/or branches. Inoculation is performed at pruning time, at BBCH stage 11 to 13 or even later. The effectiveness of this practice still needs to be proven, since there are no scientifically sound results.

Products available in Winetwork regions

Biocontrol products are available in Europe for a large range of diseases. Depending on the country, several *Trichoderma*-based products are authorised for use against grapevine trunk diseases and, in some cases, represent the only products meeting the criteria for organic viticulture. As shown in the following table, different *Trichoderma* species and strains are available in different countries, whereas no products with *Trichoderma* are registered in others (Croatia,) or are still awaiting approval (Hungary, Portugal and Spain).

Country	Product	Quantity	Composition	Price (commercial product)
France	Esquive WP ®	4 kg/ha	*Trichoderma atroviride* I-1237	252€/ha
	Vintec®	200 g/ha	*Trichoderma atroviride* SC1	200€/ha
Italy	Patriot Dry®	1 kg/ha	*Trichoderma asperellum* ICC012+ *Trichoderma gamsii* ICC 080	From 45 to 50€/ha
	Remedier®	1 kg/ha	*Trichoderma asperellum* ICC 012 2% + *Trichoderma gamsii* ICC 080 2%	From 45 to 50€/ha
	Tellus WP®	1 kg/ha	*Trichoderma asperellum* ICC 0122% +*Trichoderma gamsii* ICC 080 2%	From 45 to 50€/ha
Germany	Vintec®	200 g/ha	*Trichoderma Atroviride* SC1	180€/ha
Croatia	No registered product			
Hungary	Ongoing process for homologation of Vintec®		*Trichoderma Atroviride* SC1	
Portugal	Ongoing process for homologation of Esquive WP ®		*Trichoderma atroviride* I-1237	
Spain	Ongoing process for homologation of Esquive WP ®		*Trichoderma atroviride* I-1237	

Esquive WP ®: can be used during the winter bud stage (phenological stage BBCH 00) up to bleeding with one application per hectare and per year. Product can be applied with paintbrush or, better, by spraying after pruning, (max 15 days after). The dose is 4kg/ha when spraying with 150L water/ha (100 g/l if painted into wounds). Minimum temperature for application is 4°C (below 0°C the strain doesn't germinate) and the weather needs to be dry, without rain in the 4 hours following treatment. The product can be used until 6 months after first use (if kept in its orignial package) and needs to be stored at room temperature, preferably below 20°C.

Vintec®: is presented as dispersible granules and can be applied from BBCH 00 to BBCH 05, after pruning and until bud break using a dose of 200 g/ha with 100L of water per ha. The suspension needs to be prepared just prior to use and may not be reused (but good survival of conidia until 72h afterwards). The minimum temperature for application is close to 10°C. For best results, no rain or frost should occur after the treatment. Product needs to be stored between 0 and 4°C.

Tellus WP®: can be used at BBCH 00 (winter bud), after pruning, at 250g/100L with a minimum volume of treatment of 400 L/ha in order to distribute at least 1kg of product/ha. Application must be done at a temperature close to 10°C and can't be carried out beyond vine bleeding. On dry soils, the vineyard can be irrigated before the treatment.
Remedier®: can be used at BBCH 00 (winter bud) after pruning and until bleeding at 250g/100L with a minimum volume of treatment of 400 L/ha. Avoid rain or additional watering after treatment. To promote spore germination, put the product in water 24 hours before application to allow spore pre-germination.

Patriot Dry®: can be used at BBCH 00 (winter bud) after pruning and until bleeding at 250g/100L with a minimum volume of treatment of 400 L/ha. The product needs to be stored at room temperature below 25°C.

Concerning the efficacy of *Trichoderma*

One of the most important obstacles for an extension of *Trichoderma* use is mainly related to the variable effectiveness observed by winegrowers in the field. The strain (where a choice is possible), the phenological stage of the vines and mode of application, the time gap between pruning and the treatment, weather conditions during and after the application and the level of GTD incidence in the vineyard treated, are all factors that could help or hinder the effectiveness of the treatment (Di Marco et al., 2004).

As the effectiveness of *Trichoderma* species varies with local conditions and has not been completely established in the field, it is essential to complement the use of *Trichoderma* with good management practices in the vineyard (good pruning methods, inoculum restriction, good vine balance, etc.).

Trichoderma-based product application in Eger vineyard, Hungary (EKU). Wounds must be well sprayed.

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