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Agroscope

Grapevine trunk diseases: what's new?

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Grapevine trunk diseases: what's new?

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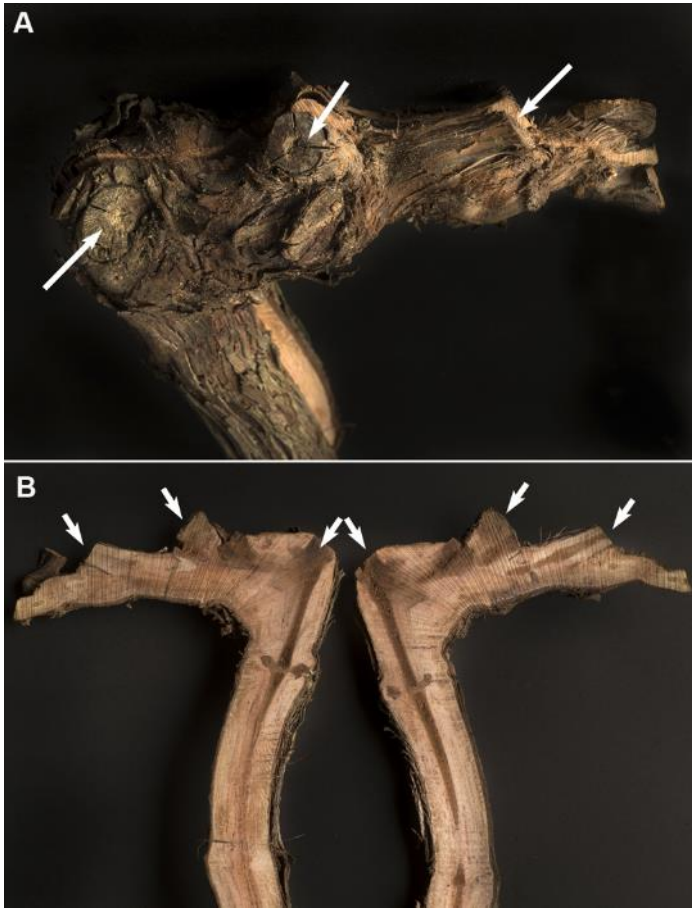
Grapevine trunk diseases (GTD) : what we know.

The **fungi** associated to GTD have never been isolated from grapevine leaves and are thought to live **exclusively** in **the wood** of grapevine plants.



GTD fungi mainly **infect** the plants through **pruning wounds**.

Eskalen A, Feliciano AJ, Gubler WD (2007) Susceptibility of grapevine pruning wounds and symptom development in response to infection by *Phaeoacremonium aleophilum* and *Phaeomoniella chlamydospora*. Plant Disease 91:1100–1104

Munkvold GP, Marois JJ (1995) Factors associated with variation in susceptibility of grapevine pruning wounds to infection by Eutypa lata. Phytopathology 85:249–256

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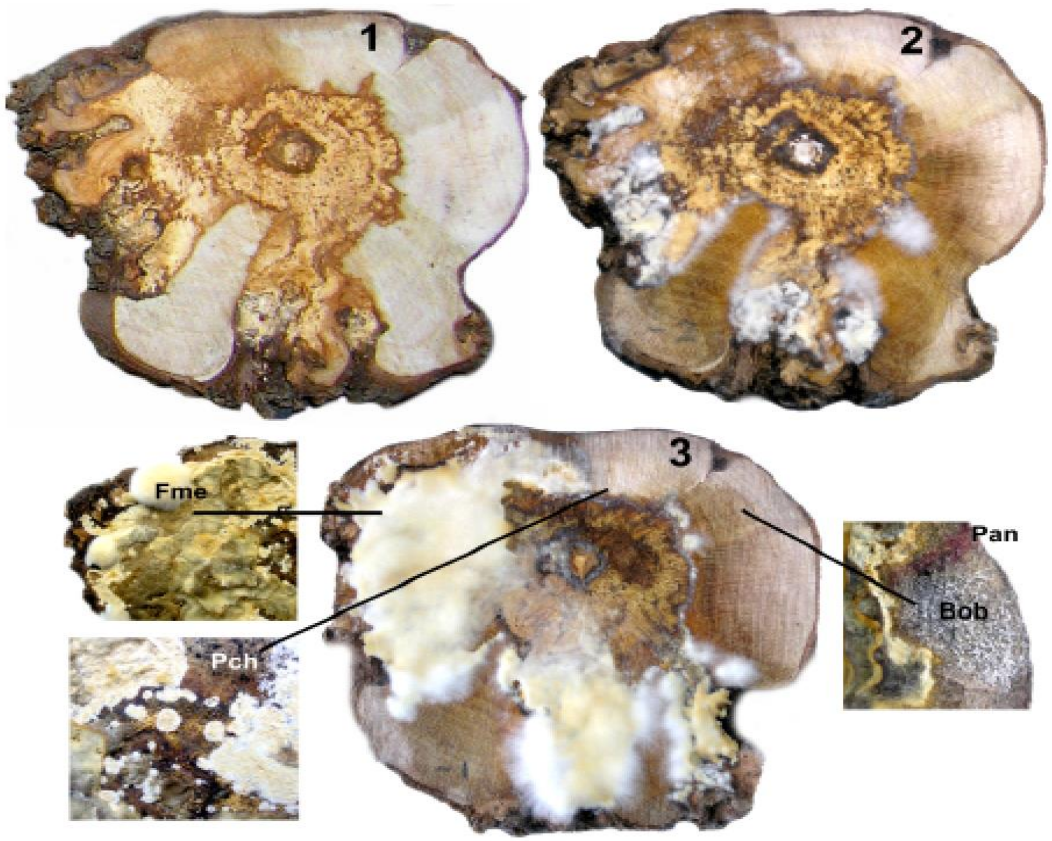
The **same fungal species** have been **repeatedly isolated** from **wood necroses**, reason why they are considered responsible for GTD.



Cross-section of a grapevine trunk suffering from esca disease. 1. day 1; 2. day 3; 3. day 7.

Main GTD are
recognizable by the **foliar symptoms** and the type of **wood necroses** they produce.

- **Eutypa dieback** (pathogen: *Eutypa lata*)

- **Black dead arm (BDA)** (pathogens: Botryosphaeriaceae spp.)

- **Esca** and **young vine decline** (pathogens: *Phaeomoniella chlamydospora*, *Phaeoacremonium spp., Cadophora spp.,* and, only for esca, *Basidiomycetes spp.*

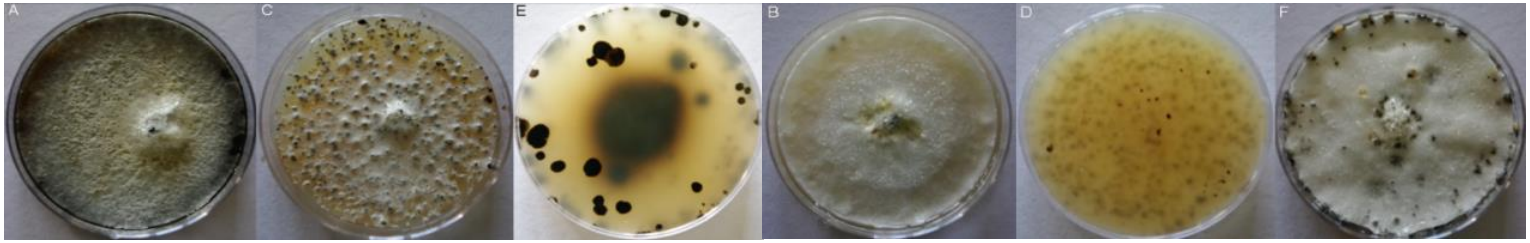
- **Excoriosis** (pathogen: *Phomopsis viticola*)

- **Black foot disease** (pathogen: *Cylindrocarpon spp.*)

These diseases are **present everywhere grapevine is cultivated** and in almost all vineyards worldwide. The high **majority of diseased plants die**.

Eutypa dieback

Fungus : ***Libertella blepharis*** (teleomorph), ***Eutypa lata*** (anamorph) but other **Diaporthales** species may be involved .

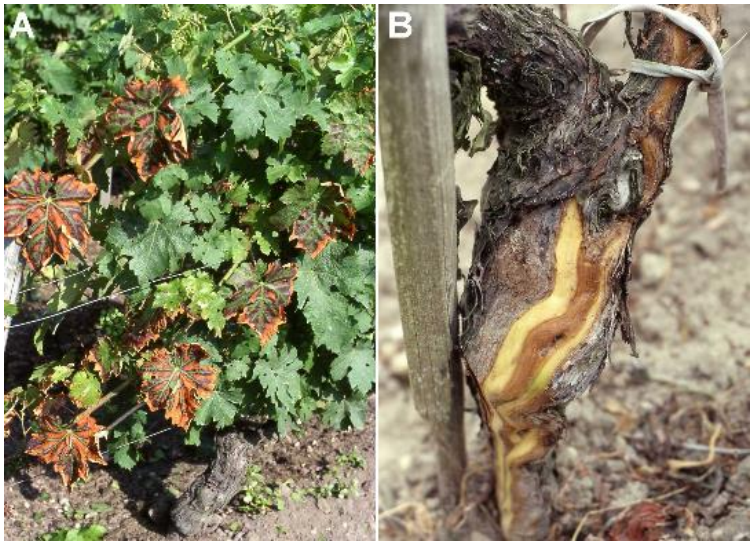


Symptoms

Black dead arm (BDA)

Fungi : ***Botryosphaeria obtusa***, ***Botryosphaeria parva*** (teleomorphs); ***Diplodia seriata***, ***Neofusicoccum parvum*** (anamorphs) and other **Botryosphariaceae** species.

Symptoms



Foliar symptoms (A) and wood necroses (B) generated by BDA disease.

Esca (as redefined by Surico, 2009) and **young esca**

Fungi : ***Phaeomoniella chlamydospora***, ***Phaeoacremonium spp.*** and, only for esca, ***Fomitiporia mediterranea*** and other **Basidiomycetes** species.

Symptoms

slow evolving form

apoplexy

Young esca appears on **young plants** and **esca** on **adult plants** (10 years old or more)

Esca (as redefined by Surico, 2009)

Fungi : ***Phaeomoniella chlamydospora***, ***Phaeoacremonium spp.***, ***Fomitiporia mediterranea*** and other species of **Basidiomycetes**.

Esca **development** is generally **patchy** and does not spread from a particular point of infection.

Grapevine trunk diseases (GTD)

Foliar **symptoms** of **esca** (on the left) and of **BDA** (on the right) are nearly similar.
(Luque *et al.*, 2009, Viret & Gindro, 2014)

These two diseases are **difficult to distinguish one from the other in the vineyard**.
Diagnosis implies to isolate and identify the fungal species present in wood necroses.

Some authors think that ***Eutypa lata*** (responsible for eutypa dieback) may also **play a role** in **esca** disease.
(Larignon & Dubos, 1997; Lehoczky & Szabolcs, 1983; Bertch et al., 2013).

Grapevine trunk diseases (GTD)

GTD are considered **emerging diseases** because their **incidence** has considerably **increased** during these last decades.

The **reasons** for their **emergence** remain **elusive**.

GTD-associated **fungi** are considered **latent pathogens**, even if their **action mode** is **not** yet clearly **understood**. They **infect** the plants through **pruning wounds**.

Pedoclimatic factors (rainfall, soil water holding capacity), **cultural practices** (canopy management, pruning system, leaf/fruit ratio, rootstock choice) and **disease susceptibility** of **individual cultivars** have been reported to influence the incidence and severity of GTD.

There is **no efficient treatment** to control GTD.

Grapevine trunk diseases (GTD): what we don't know.

GTD are **difficult to study** because they involve more than a single actor (plant-fungus(i)) and are influenced by many abiotic factors. Also the progression of the fungi in the wood is not directly observable:

1. GTD **emergence causes**, especially of **esca**?
2. Main factor(s) leading to **disease expression**?
3. Efficient **control strategy**?

Gaps in the **knowledge** we presently have about GTD.

1. Epidemiology: sparse data, local surveys (one GTD and a single grapevine cultivar).

2. Fungal pathogens: action mode(s) of the GTD-associated fungi?

3. Plant physiology:

1. Impact of pedoclimatic conditions on grapevine physiological state?
2. Impact of GTD-associated fungi on grapevine physiological state?

4. Culture practices:

1. Impact of the pruning mode on the incidence and abundance of GTD-associated fungi?
2. Impact of soil management?
3. Impact of the various rootstocks on vine vigor?

5. Differences in disease suceptibility between grapevine cultivar?

6. The interactions plant-fungal pathogen(s) are very poorly documented

7. GTD control: efficiency of marketed products? Biocontrol agents?

Agroscope research on GTD

We try to **fill part of the gaps** in the **knowledge** we presently have about GTD. We think that these gaps **impede** the discovery of an efficient way to control GTD.

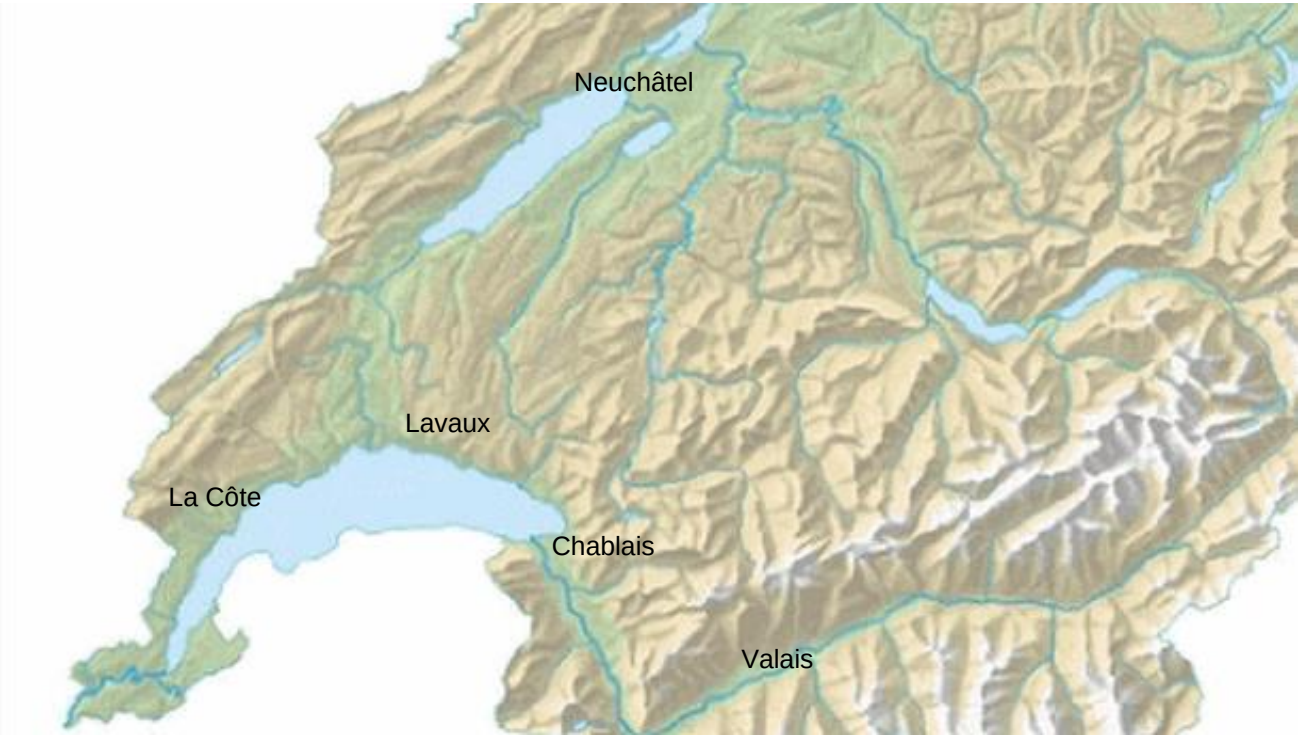
Main factor(s) leading to **disease expression**

1. **Epidemiology** of GTD.
2. **Fungal community** of grapevine, including GTD-associated fungi.
3. **Fungal secondary compounds (metabolomics)**
4. **Plant physiology**.
5. **Pedoclimatic conditions** («**terroir»**) and **culture practices** (pruning mode) in relation with GTD-associated fungi and plant physiology.

1. Epidemiology of GTD

Agroscope research essentially focus on **esca** disease, the most destructive wood disease in Switzerland, and on **eutypa dieback**.

Surveys of the **incidence** and **severity** of these two diseases have been performed since 2001 in La Côte and Chablais, and have been progressively extended to the whole Romandie (Chablais, Valais, Neuchâtel).



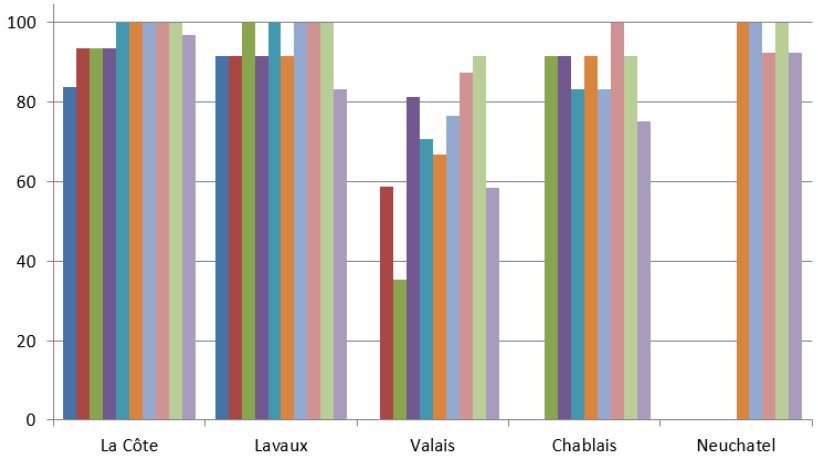
1. Epidemiology of GTD

Esca disease, the most destructive grapevine trunk disease in Switzerland

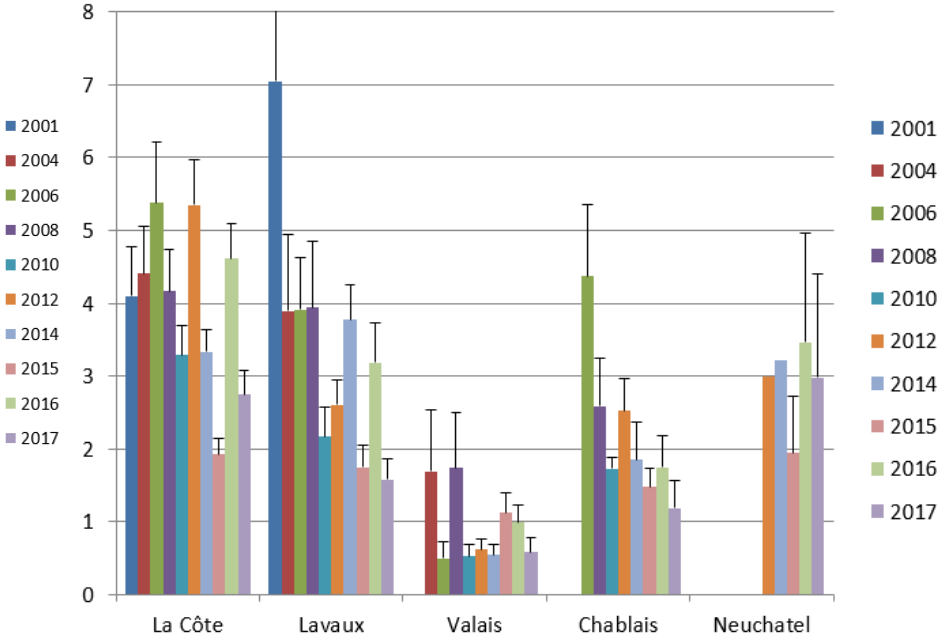
Nowadays, these surveys are effected in:

87 plots (each including **4 replicates** of a **100 plants**)
The presence or absence of symptoms is checked for **34'800 plantes**.

Esca incidence (% of plots)



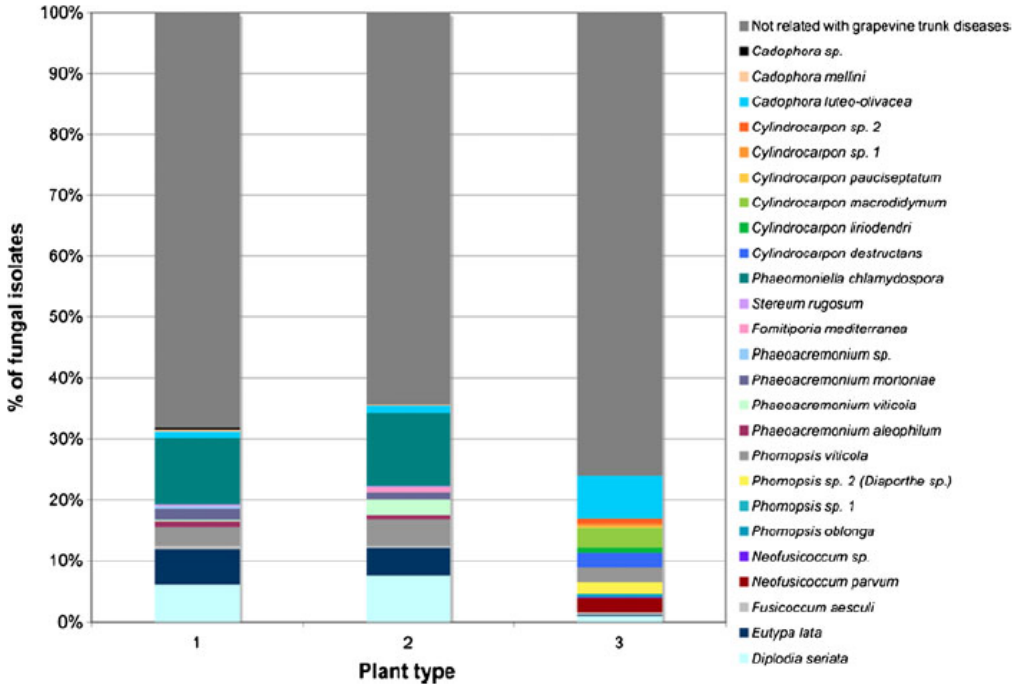
Esca severity (% of plants/plot)



2. Fungal community of grapevine

Main factor(s) leading to **disease expression**
Hypothesis 1: **Invasion** of the wood by GTD-associated fungi?

Fig. 4 Abundance of wood disease associated fungi in each plant type. Abundance is defined as the number of fungal isolates of a given OTU as a percentage of the total number of fungal isolates obtained from each plant category. Plant types: 1. asymptomatic, 2. esca-symptomatic, 3. nursery



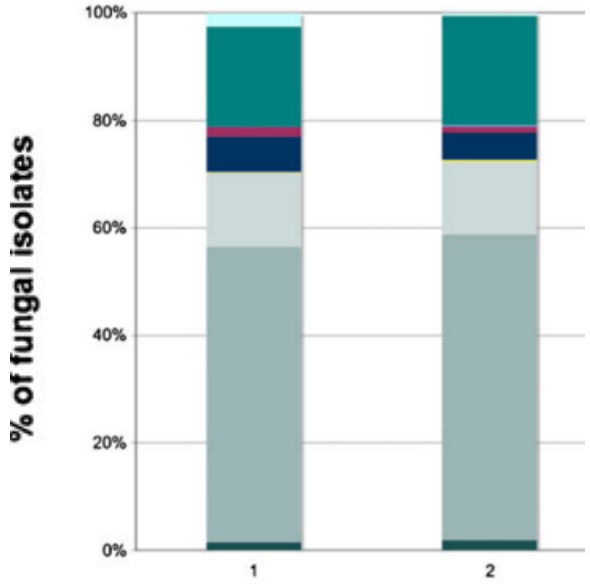
Colonization rate by **GTD-associated fungi** is **not significantly different** between **esca-symptomatic** and **asymptomatic** plants. **Fungal invasion** of the wood is **not** the **limitant factor** in the **apparition** of **esca foliar symptoms**.

(Hofstetter V, Buyck B, Croll D, Viret O, Couloux A, Gindro K, 2012. What if esca disease of grapevine were not a fungal disease? *Fungal Diversity* 54 : 51–67.)

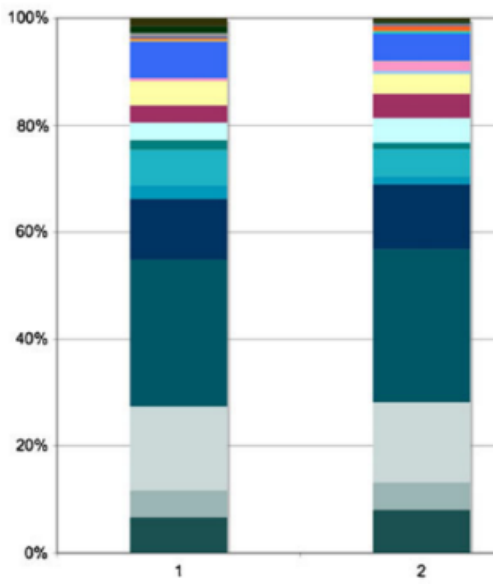
2. Fungal community of grapevine

Main factor(s) leading to **disease expression**?
Hypothesis 1: **Invasion** of the wood by **other fungi** than GTD-associated fungi?

Fungal Classes



Fungal Orders



1. Asymptomatic plants
2. Esca symptomatic plants

Overall **esca symptomatic** and **asymptomatic** plants host **nearly similar fungal communities**.

(Hofstetter V, Buyck B, Croll D, Viret O, Couloux A, Gindro K, 2012. What if esca disease of grapevine were not a fungal disease? *Fungal Diversity* 54 : 51–67.

2. Fungal community of grapevine

Main factor(s) leading to **disease expression** ?
Hypothesis 1: **Invasion** of the wood by GTD-associated fungi or other fungi?

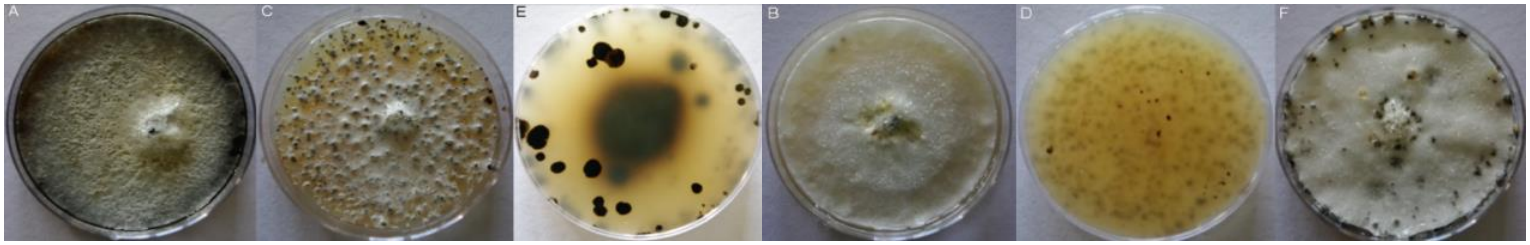
Similar results were obtained for **eutypa dieback** symptomatic plants.
(Hofstetter *et al.* 2018, in prep.)

What about the **fungal community** of plants in **an asymptomatic vineyard**? Are GTD-associated fungi present? Does the fungal community of an asymptomatic vineyard differ from the one associated with an esca and eutypa dieback symptomatic vineyard?
(Hofstetter *et al.* 2018, in prep.)

2. Fungal community of grapevine

Main factor(s) leading to **disease expression** ?
Hypothesis 2: Do **diseased plants** host **more virulent pathotypes** of GTD-associated fungi than asymptomatic plants?

Diatrypaceae isolates from **asymptomatic**, **esca symptomatic** and **eutypa dieback symptomatic** plants: molecular characterization.



(Hofstetter et al., 2018. in prep.)

Hypothesis 2: Do **diseased plants** host **more virulent pathotypes** of GTD-associated fungi than asymptomatic plants?

4 different species of Diatrypaceae

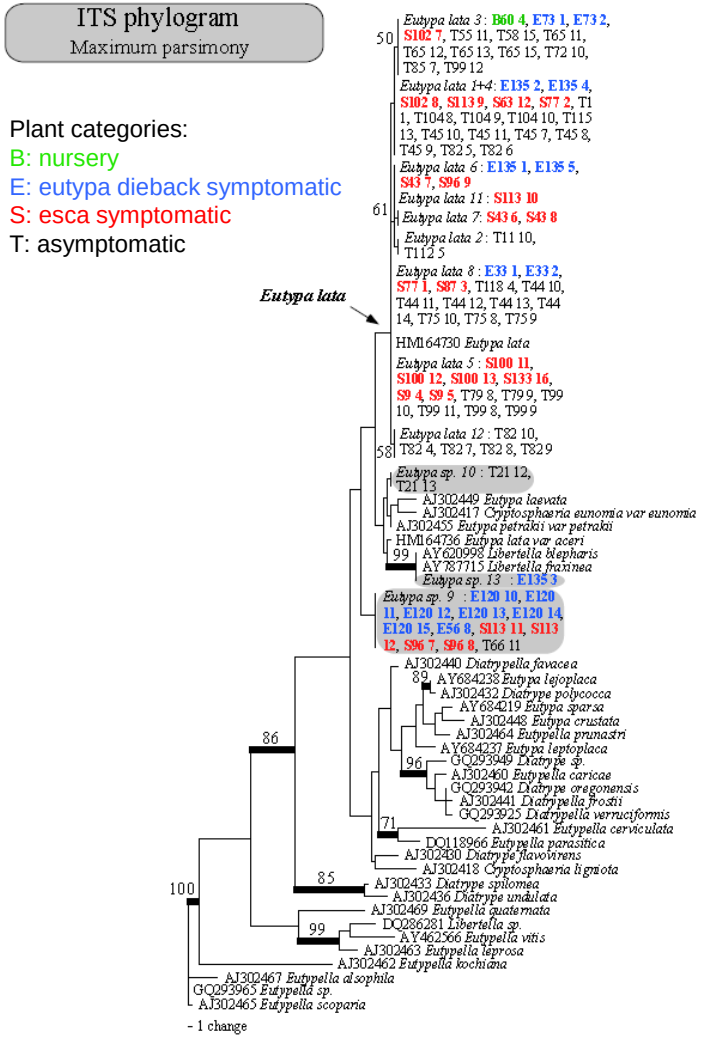
Eutypa lata
3 other *Eutypa* species (two out of them never sequenced before)

(Hofstetter et al., 2018. in prep.)

13 different ITS genotypes

1 genotype isolated from all plant categories
4 genotypes isolated from 3 plant categories
5 genotypes isolated only from diseased plants
3 genotypes isolated only from asymptomatic plants

5 out of these 13 genotypes have been isolated from a **unique plant**.



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Hypothesis 2: Do **diseased plants** host **more virulent pathotypes** of GTD-associated fungi than asymptomatic plants?

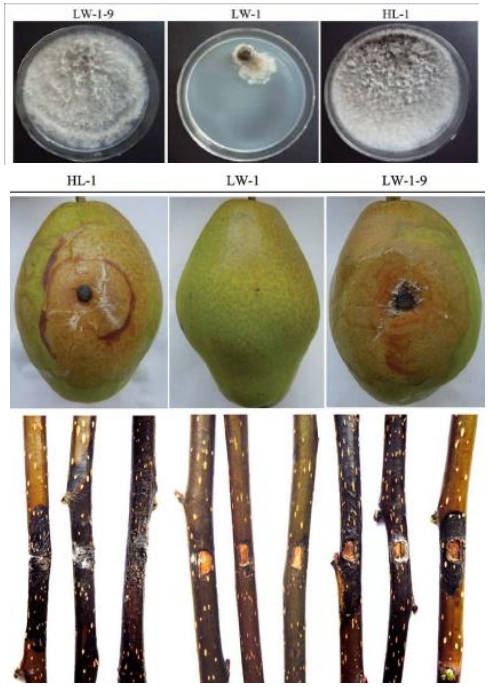
Grapevine virome is **dominated** by **mycoviruses** (Rwahnih *et al.* 2011).

It has been shown recently (Yu *et al.* 2015, Wang *et al.* 2014) that the **presence of particular viruses in fungal pathogens** (*Botrytis cinerea, Botryosphaeria dothidea*) make them **hypovirulent**.

Yu et al. 2015. Novel Hypovirulence-Associated RNA Mycovirus in the Plant-Pathogenic Fungus *Botrytis cinerea*: Molecular and Biological Characterization.

Wang et al. 2014. Hypovirulence of the phytopathogenic fungus *Botryosphaeria dothidea*: 2 associations with a co-infecting chrysovirus and a partitivirus.

Rwahnih et al. 2011. Deep sequencing evidence from single grapevine plants reveals a virome dominated by mycoviruses.



3. Fungal metabolomics

Main factor(s) leading to **disease expression**?
Hypothesis 3: **Production** of **phytotoxic compounds** by GTD-associated fungi.

GTD-associated fungi in pure culture produce **secondary metabolites** that are **phytotoxic** (Andolfi *et al.* 2011, Bertsch *et al.* 2013).

?

The **action mode(s)** of most of these secondary metabolites are **unknown**.

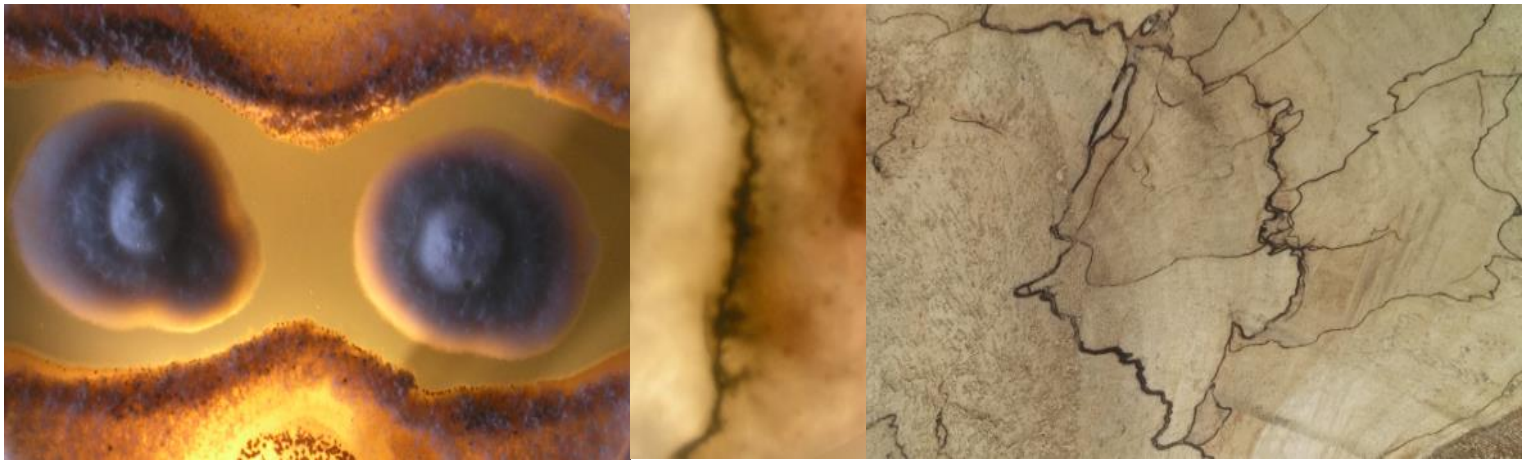
GTD-associated fungi live in the wood (they have never been isolated from the leaves): foliar symptoms may only result from the **transportation** of these fungal phytotoxic compounds **from the wood to the leaves** by the plant vascular system, this in **sufficient amount** to generate foliar symptoms or apoplexy.

Main factor(s) leading to **disease expression**?

Hypothesis 3: **Production** of **phytotoxic compounds** by GTD-associated fungi and/or other fungi?

Fungi are also known to be able to ***de novo* induce secondary compounds** when they are **co-cultured**.

(Bertrand S., Bohni N., Schnee S., Schumpp O., Gindro K. & Wolfender J.-L., 2014. Metabolite induction via microorganism co-culture: A potential way to enhance chemical diversity for drug discovery. *Biotechnology Advances* **32**, 1180–1204.)



Esca foliar symptoms as well as apoplexy might result from the the **chemical war between fungal species when they meet in the wood**, fungi starting to produce de novo phytotoxic secondary compounds.

4. Physiology of grapevine

Main factor(s) leading to **disease expression**?
Pedoclimatic factors are known to influence the expression of **esca disease** (Dubos *et al.* 2002)

1. **Soils** with **high water holding capacity** (deep and clayey soils)

2. **Climate variations** during the summer (wet and fresh weather alternating with hot and wet conditions leading to an important leaf and canopy production).

(Fischer 2003; Surico, Mugnai and Marchi 2006)

Increase the risks of **sap flow breaking** and **favor** grapevine **apoplexy**.

4. Physiology of grapevine

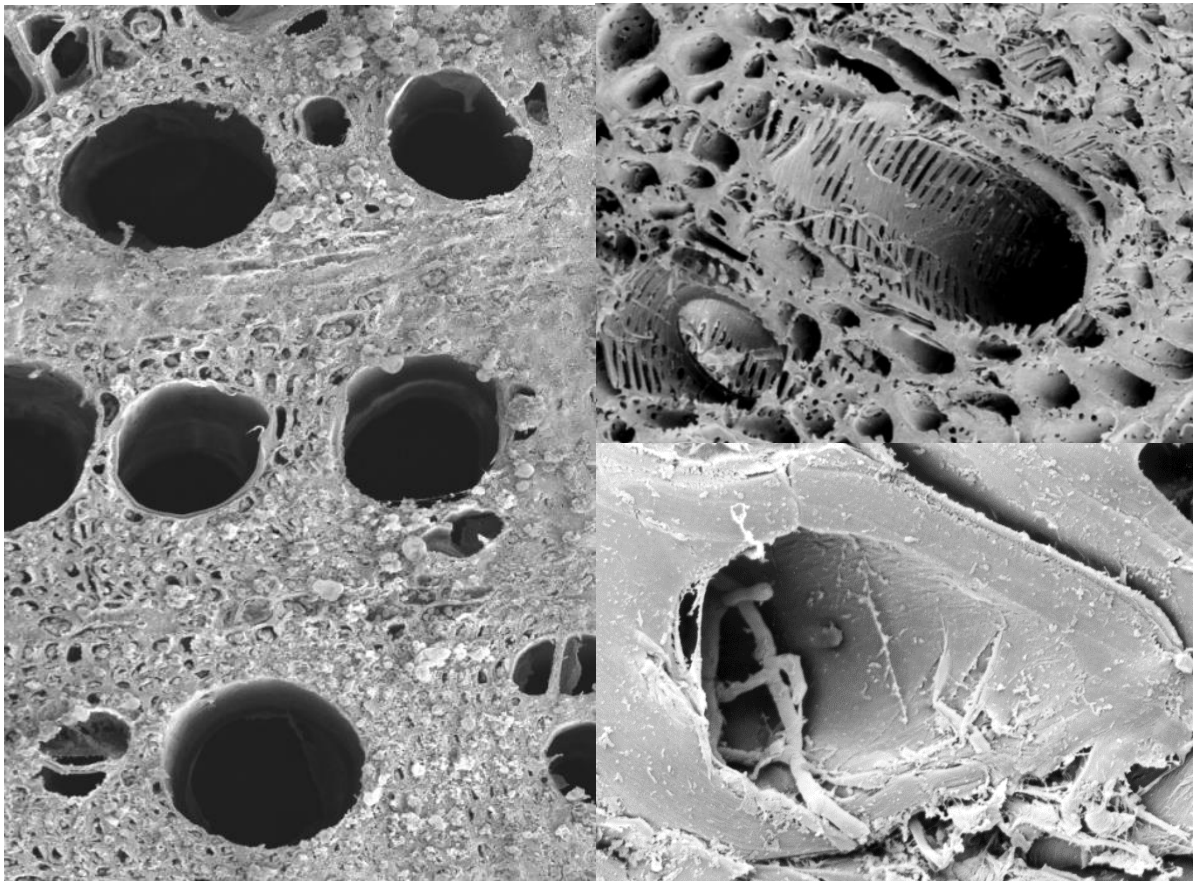
Main factor(s) leading to **disease expression**?

Preliminary studies show that **vines exhibiting esca foliar symptoms**, even **weakly**, are **already suffering** from **important hydraulic failures** (decrease of hydraulic conductance in the petioles and the shoots) compared to healthy plants.

Causes of these failures?

Tylose and **gels** (in link with pruning) **and/or fungi obstructing** the **vascular system** of the plant?

Main factor(s) leading to **disease expression**
Hypothesis 4: **Other fungi** (or GTD-associated fungi or both?) **precluding sap flow** into plant **leaves**?



Xylem vessels in petioles of grapevine leaves of **assymptomatic** (A) and **esca symptomatic** (B, C).

Main factor(s) leading to **disease expression**
Hypothesis 4: **Other fungi** (or GTD-associated fungi or both?) **precluding sap flow** into plant **leaves**?

When wood physiology was compared between cultivars, the vessels occupied from 21.8% ('Chasselas') to 47.2% ('Humagne') of the xylem lumen in cross section (Table 4 and Fig. 3), with mean values from 6.32E+03 um^2 ('Chasselas') to 8.4E+03 um^2 ('Arvine'). SEM observations showed many starch granules in most of the cells surrounding the vessels (Fig. 4) and in a few cases there were fungal conidia in the xylem vessels (Fig. 5).

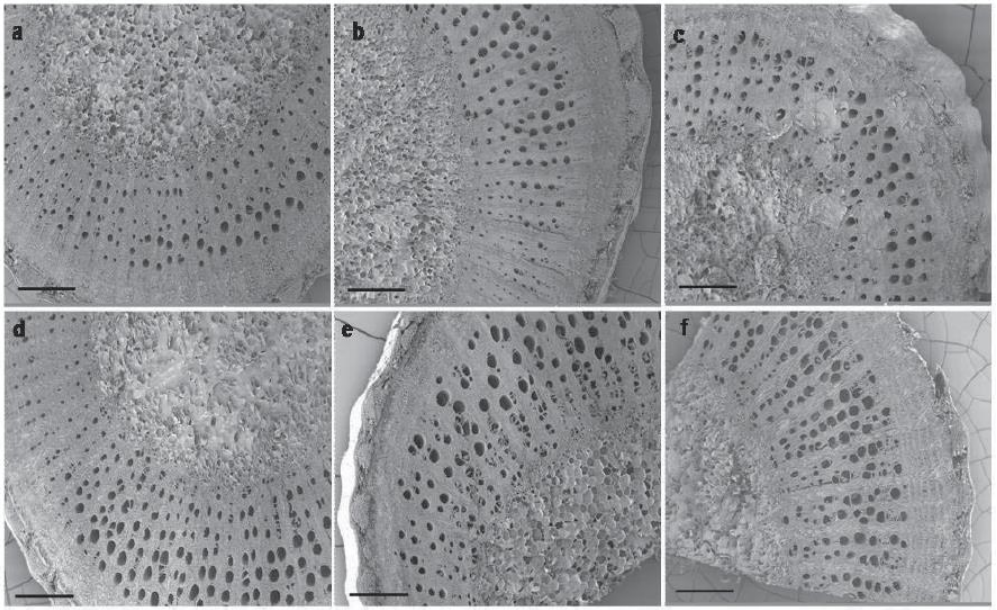


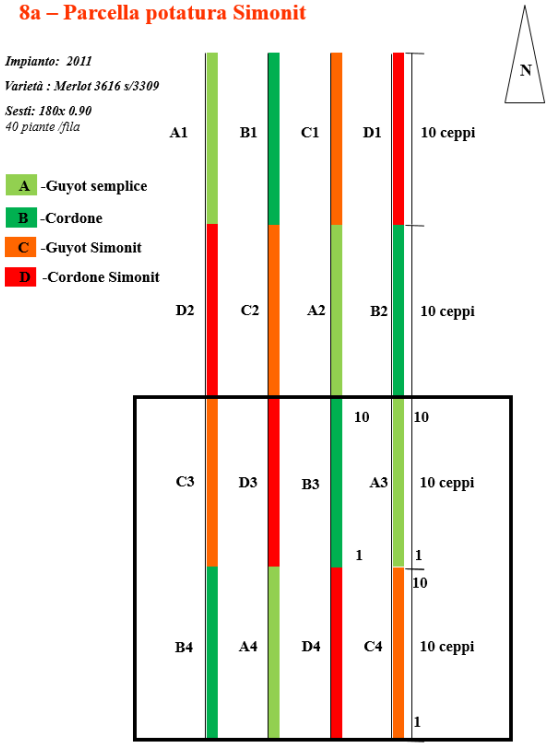
Fig. 3. Example SEM images of vessels surface in semi-thin section dormant canes. a, Chasselas; b, Gamay; c, 3309 (rootstock); d, Gamaret; e, Arvine; f, Humagne. Scale bar=1 mm.

Casieri L, Hofstetter V, Viret O, Gindro K, (2009). Fungal communities living in the wood of different cultivars of young *Vitis vinifera* plants. *Phytopathol. Mediterr.* 48, 73–83

Is **disease suceptibility** linked to the **variable vessel size** of the **different** grapevine **cultivars**?

4. Cultural practices

Impact of the **pruning parctices** on GTD and on plant physiology.



Agroscope vineyard at Cugnasco (Tessin) planted to test for the impact of pruning practices on GTD

The **fungal community** of **nursery plants** was isolated and characterized before plantation

⇩

Plants were pruned under **4 different modes**
Fungal community analysed for each pruning mode
5 years after plantation

⇩

(**10 years after? 15 years after?...**)

Comparison between the fungal communities of plants pruned differently: **which is the best way to prune vines to reduce GTD**, if any?

4. Main factor(s) leading to disease expression

Systemic approach linking **epidemiology**, **fungal community** and **plant physiology** approaches taking in account the influence of **local** pedoclimatic conditions («**terroir**»)

Systemic approach linking **epidemiology, fungal community** and **plant physiology** approaches taking in account the influence of **local** pedoclimatic conditions («**terroir**»)

Further research (2018-2021)

Project:

Search for the **causes** of **Gamaret vine decline** in the Vaud state in relation with pedoclimatic conditions.

Gamaret is the **most planted cultivar** in Switzerland (400 ha)

Occupies the **3rd rank** of the red cultivars in **Vaud state** (148 ha) after **Pinot noir** and **Gamay**.

Is also the **most sensitive** cultivar to **esca disease** (apoplexy)

4. Main factor(s) leading to disease expression

Search for the **causes** of **Gamaret vine decline** in the Vaud state in relation with pedoclimatic conditions.

1) Do the **inventory** of Gamaret plots used in a previuos study of the « terroirs vaudois », which was implemented in 2003 (20 plots, rootstock PG 3309)

2) Assess the **sanitary state** of the plants in these plots in relation with the « terroir » (soils profiles de sols, climat ...)

3) Assess the **physiological behavior** of vines in relation with « terroir » (vigor, hydric status of the plants, of the leaves, and of the grapes…)

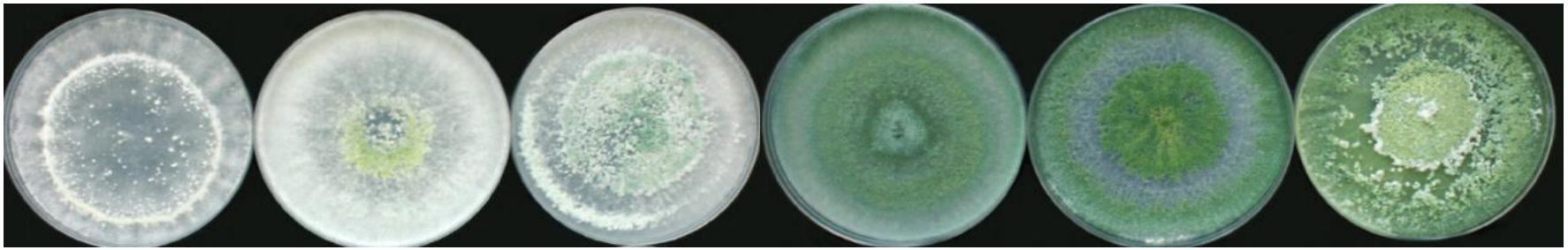
4) Conducting **in-depth mycological analyses** of symptomatic and asymptomatic plants for **each « terroir » type**.

Results should allow us to **define the most adapted « terroirs »** to cultivate **Gamaret** and to provide **culture recommendations** for the Vaud state vinyards.

5. GTD biocontrol?

Trichoderma as antagonist and/or inducer of grapevine defense mechanisms.

Genus *Trichoderma* includes **mycoparasitic species** already used by some winegrowers and grapevine nurserymen. Le product **Esquive®WP** (Bayer S.A.S, Lyon, France), made of a *Trichoderma atroviride* natural fungal strain), was recently **marketed**. This product is sold as a **biocontrol solution** against **major GTD** (esca, black dead arm and eutypa dieback).



However, a study from the Institut Français de la Vigne et du Vin (IFV) has shown that *Trichoderma* (13 tested species) were **not efficient to protect pruning wounds** from GTD-associated fungi, with the exception of *Eutypa lata* for which the growth was slicely reduced by this treatment (Larignon 2009).

But some *Trichoderma* can **activate** grapevine **defense** mechanisms (Palmieri et al., 2012) and have shown some efficiency to control young esca associated fungi (Pertot et al. 2016).

***Trichoderma* efficency is disputed and needs further study!**

5. GTD biocontrol?

Endomycorrhiza (Glomeromycota / arbuscular mycorrhizal fungi [AMF]) as inducers of grapevine resistance.

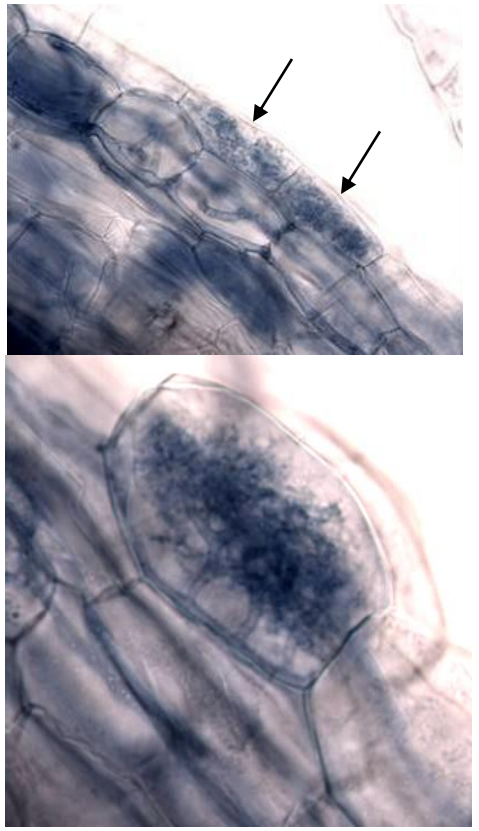
-AMF **increase plant resistance** to at least one GTD (black foot disease; Petit & Gubler 2006).

- AMF provide **nutrients** to the plant (Schreiner 2007; Redecker *et al.* 2008).

- A **large proportion** of grapevine **roots** is **colonized** by AMF (Karagiannidis & Nikolaou 1999, Nappi *et al.* 1985, Schubert & Cravero 1985). However, this might be true **only** for **dry locations** (Schreiner, Tarara & Smithyman, 2007) and for **soils poor in phosphorus** (Redecker *et al.* 2008).

- AMF **diversité** is **highly variable** depending of vineyard **location** (Schreiner & Mihara 2009; Bouffaut *et al.,* 2016).

The effects of AMF inoculation on grapevine performances and GTD need **further study**.



Rhizophagus irregularis
(*Glomus intraradices*)

5. GTD biocontrol?

Endomycorrhiza potentiates **grapevine defenses** in response to **downy mildew** (*B. cinerea*) and **grey mould** (*P. viticola*)

Bruisson S, Maillot P, Schellenbaum P, Walter B, Gindro K, Deglene-Benbrahim L (2016) Arbuscular mycorrhizal symbiosis stimulates key genes of the phenylpropanoid biosynthesis and stilbenoid production in grapevine leaves in response to downy mildew and grey mould infection

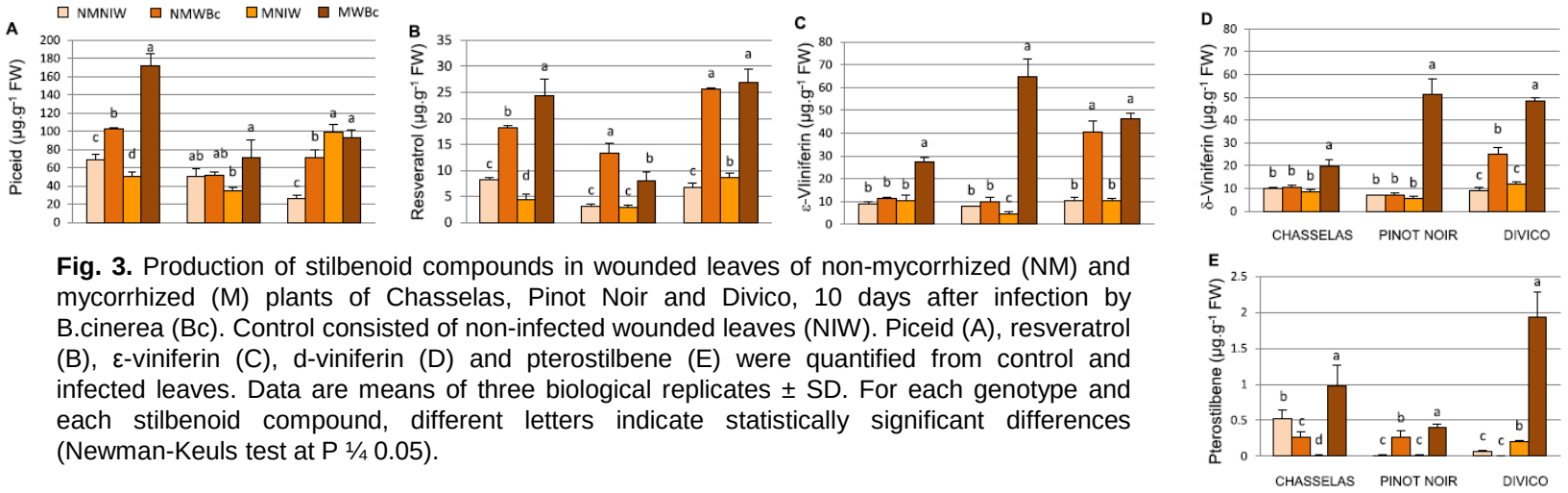


Fig. 3. Production of stilbenoid compounds in wounded leaves of non-mycorrhized (NM) and mycorrhized (M) plants of Chasselas, Pinot Noir and Divico, 10 days after infection by B.cinerea (Bc). Control consisted of non-infected wounded leaves (NIW). Piceid (A), resveratrol (B), ε-viniferin (C), d-viniferin (D) and pterostilbene (E) were quantified from control and infected leaves. Data are means of three biological replicates ± SD. For each genotype and each stilbenoid compound, different letters indicate statistically significant differences (Newman-Keuls test at P ¼ 0.05).

AMF colonization **stimulates** the **stilbenoid** pathway for the production of **resveratrol** and **fungi toxic derivatives** in leaves **after infection** by a **fungal pathogen** (*B. cinerea*). However the production of stilbenoid compounds induced by pathogen infection is **highly variable** depending on the **cultivars**.

Agroscope

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Systemic approach linking **epidemiology**, **fungal community** and **plant physiology** approaches taking in account the influence of **local** pedoclimatic conditions («**terroir**»)

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Thank you for your attention!