



# *Trichoderma* spp. and *Bacillus subtilis* for control of *Dactylonectria macrodidyma* in grapevine

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(short note: *Phytopathologia Mediterranea* (2016) 55, 2, 293–300)

***Logroño, 7 October 2016***



COST is supported by the EU  
RTD Framework programme

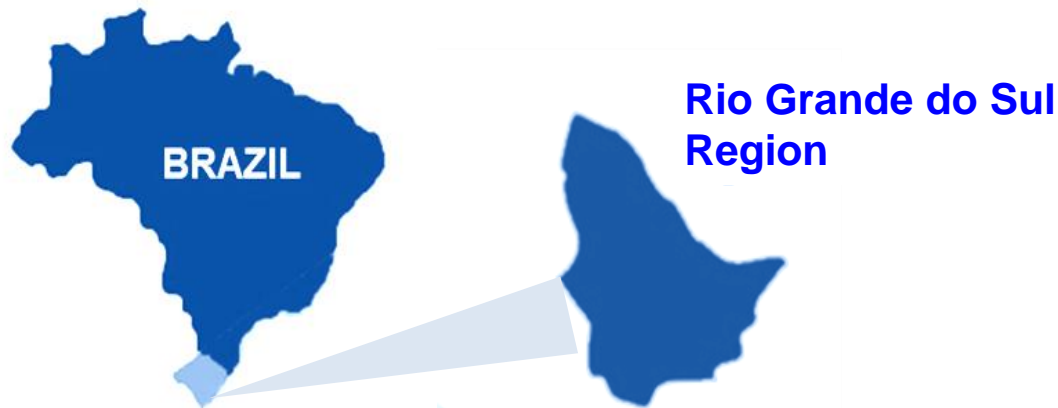


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## Biocontrol of *Dactylonectria macrodidyma* in grapevine

- ❖ Since 1999, **black foot disease (BFD)** has been observed in South Brazil and causes several losses mainly in Serra Region vineyards.



- ❖ In recent studies, *Dactylonectria macrodidyma* and *Campylocarpon pseudofasciculare* were the species more frequently associated with BFD in South Brazil.



## Biocontrol of *Dactylonectria macrodidyma* in grapevine

- ***Trichoderma atroviride*** and ***T. harzianum*** and ***Bacillus subtilis*** have been reported as a potential biological control agents (**BCA**) against grapevine trunk disease pathogens
- The aim of this study was to evaluate the potential of *Trichoderma spp.* and *Bacillus subtilis*, *in vitro* and *in vivo*, as **BCA** against *Dactylonectria macrodidyma*



# Materials and Methods

(*All methods: 10.14601/Phytopathol\_Mediterr-18048*)



## Biocontrol of *Dactylonectria macrodidyma* in grapevine

**Six isolates** of *Dactylonectria macrodidyma* used in this study

<b>Isolate</b>	<b>Origin (Region of Brazil)</b>	<b>Cultivar/rootstock</b>	<b>GenBank access numbers (Histone H3)</b>
<b>Cy4UFSM</b>	<b>Erechim</b>	<b>Isabel/VR 043-43</b>	<b>KF633167</b>
<b>Cy5UFSM</b>	<b>Garibaldi</b>	<b>Niágara Branca*</b>	<b>KF633168</b>
<b>Cy7UFSM</b>	<b>Garibaldi</b>	<b>Isabel/16149</b>	<b>KF633170</b>
<b>Cy11UFSM</b>	<b>Bento Gonçalves</b>	<b>Merlot/Gravesac</b>	<b>KF633155</b>
<b>Cy15UFSM</b>	<b>Flores da Cunha</b>	<b>Niágara Branca*</b>	<b>KF633159</b>
<b>Cy16UFSM</b>	<b>Flores da Cunha</b>	<b>Bordô*</b>	<b>KF633160</b>

\* Own-rooted cultivar



## Biocontrol of *Dactylonectria macrodidyma* in grapevine

Characteristics of six biological control agents (BCA) studied.

Treatment	Species	Formulation	Trade name	Company
T1	<i>T*. harzianum</i>	Concentrated suspension	Trichodermil SC1306®	Itaforte Bio Produtos, <b>Brazil</b>
T2	<i>T. asperellum</i>	Emulsifiable concentrate	TrichoderMax EC®	Novozymes BioAg, <b>Brazil</b>
T3	<i>T.asperellum</i>	Water-dispersible granules	Quality WG®	Grupo Farroupilha, <b>Brazil</b>
T4	<i>T. harzianum</i> , <i>T. viride</i> , <i>T. atroviride</i> .	Concentrated suspension	Trichodel®	Empresa Caxiense de Controle Biológico Ltda, <b>Brazil</b>
T5	<i>B**. subtilis</i>	Concentrated suspension	Rizos®	Grupo Farroupilha, <b>Brazil</b>
T6	<i>B. subtilis</i>	Concentrated suspension	Rizolyptus®	Grupo Biosoja, <b>Brazil</b>

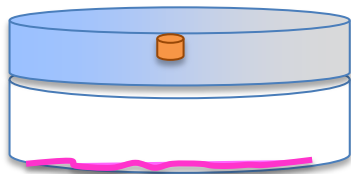
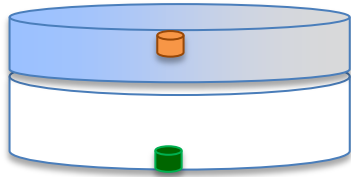
\* - *Trichoderma* \*\* - *Bacillus*



## Biocontrol of *Dactylonectria macrodidyma* in grapevine

### *In vitro* experiments

#### ❖ Volatile metabolites assay



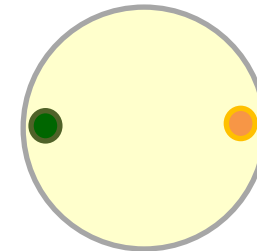
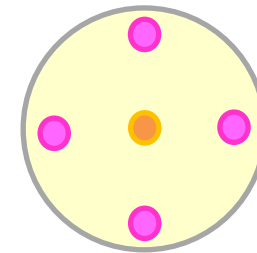
● *Dactylonectria macrodidyma*

● *Trichoderma* spp.

● *B. subtilis*

(10 replicates/treatment)

#### ❖ Paired culture assay



### Assessments:

Inhibitions of pathogen mycelial growth Percentage were evaluated  
[(treatment - control) / control × 100]



## *In vivo* experiment

- ❖ 10 replicates of **one year old plants** of cv. Merlot/1103 P / per treatment
- ❖ **Substrate** were treated (drenched) with the BCA, **14 days prior** to inoculation with the pathogen
- ❖ After 14 days, plants were transplanted and, **simultaneously**, the substrate was inoculated with ***D. macrodidyma*** (highly aggressive isolate Cy5UFSM)
- ❖ 30 days after the planting, **treatment with BCA** (*Trichoderma* spp. and *B. subtilis*) **was repeated**.
- ❖ Control treatments:
  - T7** positive control (only with the pathogen);
  - T8** negative control (without the pathogen/antagonist)

(All methods: 10.14601/Phytopathol\_Mediterr-18048)





## *In vivo* experiment

**Assessments:** after 4 months

1. Length: longest root (LR, cm)  
primary shoot (LPS, cm)
2. Number of nodes in the primary shoot (NNPS)
3. Total number of nodes (TNN) and shoots (TNS)
4. Dry weight: shoot (SDW, g)
5. root (RDW, g)

Pathogen reisolation (RI,%)



# Results



## Biocontrol of *Dactylonectria macrodidyma* in grapevine

Mean inhibition of mycelial growth (%) of isolates of *Dactylonectria macrodidyma* exposed to **volatile metabolites** of (4) *Trichoderma* spp. and (2) *Bacillus subtilis*

Inhibition of mycelial growth of <i>D. macrodidyma</i> (%)							
Treat. <sup>1</sup>	Cy4UFSM	Cy5UFSM	Cy7UFSM	Cy11UFSM	Cy15UFSM	Cy16UFSM	Mean
<b>T1</b>	66.2 aA <sup>2</sup>	71.5 aA	66.2 aA	62.7 aA	68.8 aA	65.6 aA	<b>66.8 a</b>
<b>T2</b>	61.1 aA	63.1 bA	50.5 cB	65.6 aA	59.0 bA	61.2 aA	60.1 b
<b>T3</b>	45.8 bB	44.4 dB	58.6 bA	47.7 bB	50.4 cB	46.5 cB	48.8 c
<b>T4</b>	67.8 aA	53.1 cB	51.1 cB	61.6 aA	63.5 bA	52.7 bB	58.3 b
<b>T (Mean)</b>							<b>59</b>
<b>T5</b>	40.9 aA	50.5 aA	32.9 aA	50.9 aA	47.2 aA	68.8 aA	48.6 a
<b>T6</b>	45.5 aA	48.5 aA	41.0 aA	44.2 aA	29.5 aA	69.1 aA	46.3 a
<b>T (Mean)</b>							<b>47</b>

<sup>1</sup> Treatment: **T1**: *Trichoderma harzianum* (**Trichodermil SC1306**<sup>®</sup>); **T2**: *Trichoderma asperellum* (TrichoderMax EC<sup>®</sup>); **T3**: *Trichoderma asperellum* (Quality WG<sup>®</sup>); **T4**: *Trichoderma harzianum*, *Trichoderma viride/atroviride* and *Trichoderma viride* (Trichode<sup>®</sup>); **T5**: *Bacillus subtilis* (Rizos<sup>®</sup>); **T6**: *Bacillus subtilis* (Rizolyptus<sup>®</sup>). <sup>2</sup> Means within each **column** followed by the same **lowercase letter** and within each **row** followed by **uppercase letter** within are not different by the Scott-Knott test ( $P \leq 0.05$ ).



## Biocontrol of *Dactylonectria macrodidyma* in grapevine

Mean inhibition of mycelial growth (%) of isolates of *Dactylonectria macrodidyma* in *in vitro* paired culture with isolates of (4) *Trichoderma* spp. and (2) *Bacillus subtilis*

Inhibition of mycelial growth of *D. macrodidyma* (%)

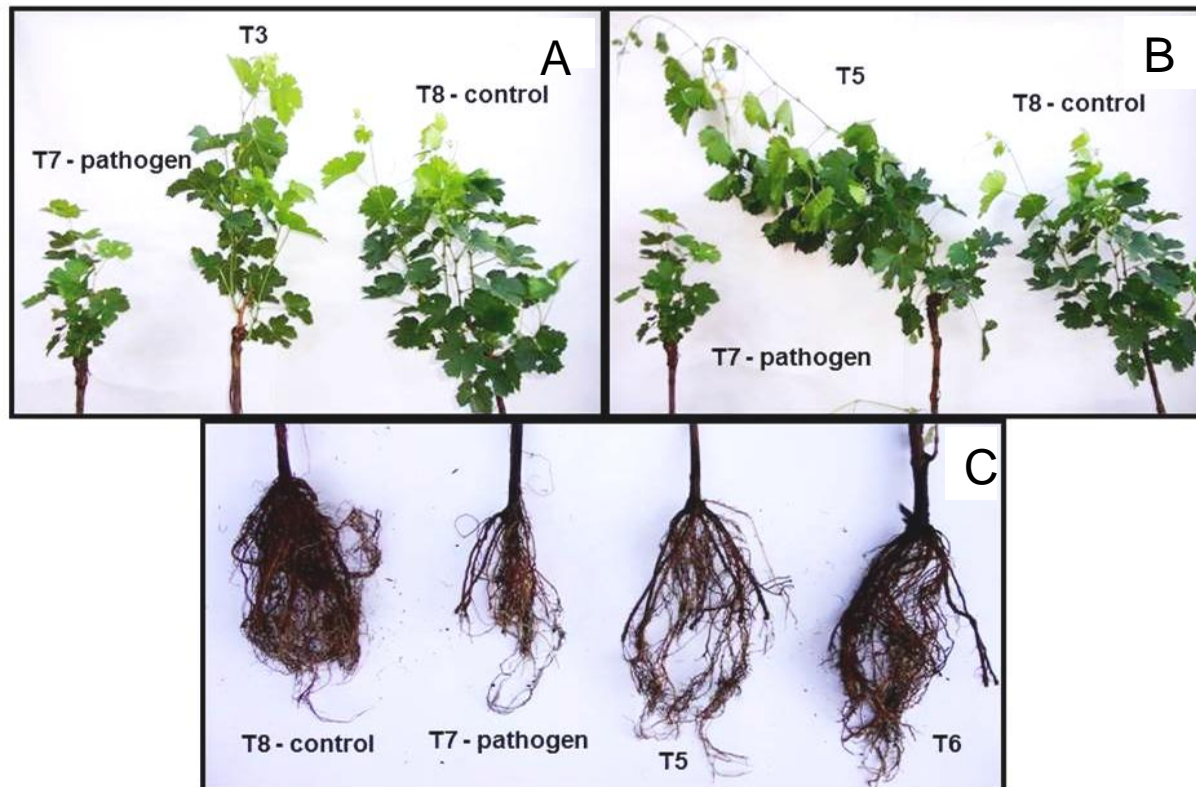
Treat. <sup>1</sup>	Cy4UFSM	Cy5UFSM	Cy7UFSM	Cy11UFSM	Cy15UFSM	Cy16UFSM	Mean
<b>T1</b>	33.9 bB <sup>2</sup>	37.1 aA	34.5 bB	38.6 aA	41.7 bA	35.0 bB	36.8 c
<b>T2</b>	34.2 bB	37.5 aB	44.0 aA	35.2 aB	40.9 bA	35.5 bB	37.9 c
<b>T3</b>	44.2 aA	38.2 aB	40.9 aA	37.2 aB	40.6 bA	41.3 aA	40.4 b
<b>T4</b>	44.2 aA	40.9 aA	42.8 aA	40.9 aA	46.0 aA	43.7 aA	<b>43.1 a</b>
<b>T (Mean)</b>							<b>40</b>
<b>T5</b>	26.8 bC	26.5 bC	20.9 bD	36.1 bA	34.0 bA	29.5 bB	29.0 b
<b>T6</b>	50.6 aA	37.4 aC	29.8 aD	45.8 aA	40.5 aB	42.9 aB	<b>41.2 a</b>
<b>T (Mean)</b>							<b>35</b>

<sup>1</sup> Treatment: **T1**: *Trichoderma harzianum* (Trichodermil SC1306®); **T2**: *Trichoderma asperellum* (TrichoderMax EC®); **T3**: *Trichoderma asperellum* (Quality WG®); **T4**: *Trichoderma harzianum*, *Trichoderma viride/atroviride* and *Trichoderma viride* (**Trichodel**®); **T5**: *Bacillus subtilis* (Rizos®); **T6**: *Bacillus subtilis* (**Rizolyptus**®). <sup>2</sup> Means within each column followed by the same lowercase letter and within each row followed by uppercase letter within are not different by the Scott-Knott test ( $P \leq 0.05$ ).



## Biocontrol of *Dactylonectria macrodidyma* in grapevine

Black foot symptoms were visible in inoculated plants with *D. macrodidyma* in contrast with control plants: reduce of vigour, sudden wilting of foliage, root rot, reduced in root biomass and root necrosis



**Figure 1.** *Trichoderma* spp. and *B. subtilis* in the control of *Dactylonectria macrodidyma* in cv. Merlot. **A, B)** effect of treatment in the development of the aerial part of the plants: **T3** (*Trichoderma asperellum*), **T5** (*B. subtilis*), **T7** (pathogen only) and **T8** (control treatment); **C)** effect on the development of the root system: **T5** (*B. subtilis*), **T6** (*B. subtilis*), **T7** (pathogen only) and **T8** (control treatment).



## Biocontrol of *Dactylonectria macrodidyma* in grapevine

Mean growth parameters of cv. Merlot plants treated with BCA and inoculated with *D. macrodidyma*: length of the longest root formed (**RL**, cm), length of the primary shoot (**LPS**, cm), nodes number in the primary shoot (**NNPS**), total number of nodes (**TNN**), total number of shoots (**TNS**), shoot dry weight (**SDW**, g), root dry weight (**RDW**, g) and the frequency of reisolation (**RI**, %)

Treat. <sup>1</sup>	RL (cm)	LPS (cm)	NNPS	TNN	TNS (g)	SDW (g)	RDW (g)	RI (%)
<b>T1</b>	31.8 a	53.5 b	16.6 b	29.0 c	2.8 b	8.5 c	4.9 c	58.9 b
<b>T2</b>	24.8 a	80.2 a	27.0 a	34.2 c	3.1 b	10.8 b	4.0 c	52.2 b
<b>T3</b>	29.6 a	71.6 a	24.1 a	41.4 b	2.8 b	11.4 b	4.4 c	61.1 b
<b>T4</b>	28.6 a	55.3 b	16.9 b	27.9 c	2.3 b	5.6 c	3.5 c	64.4 b
<b>T5</b>	38.7 a	80.6 a	22.4 a	36.6 b	3.1 b	13.7 a	6.4 b	61.1 b
<b>T6</b>	33.2 a	67.2 a	18.7 b	40.3 b	3.8 b	10.1 b	6.8 b	44.4 b
<b>T7 positive c</b>	27.4 a	47.7 b	13.9 b	24.0 c	2.4 b	6.1 c	4.1 c	74.4 b
<b>T8 negative c.</b>	31.4 a	66.1 a	19.1 b	56.9 a	5.6 a	17.7 a	9.4 a	0 a

<sup>1</sup> Treatment: **T1**: *Trichoderma harzianum* (Trichodermil SC1306®); **T2**: *Trichoderma asperellum* (TrichoderMax EC®); **T3**: *Trichoderma asperellum* (Quality WG®); **T4**: *Trichoderma harzianum*, *Trichoderma viride/atroviride* and *Trichoderma viride* (Trichodel®); **T5**: *Bacillus subtilis* (Rizos®); **T6**: *Bacillus subtilis* (Rizolyptus®); **T7**: pathogen (positive control); **T8**: negative control.



# Conclusions



## Biocontrol of *Dactylonectria macrodidyma* in grapevine

- ❖ This study is a first step of a research involving *Trichoderma* spp. and *B. subtilis* biocontrol agents, to reduce *D. macrodidyma* in grapevine
- ❖ This study demonstrates that *Trichoderma* spp. and *B. subtilis* **have potential** for the biocontrol of *Dactylonectria macrodidyma*
- ❖ Further studies must be carried out to evaluate efficacy of these BCA to reduce natural infections in vineyards and nurseries
- ❖ Similar results were obtained using these BCA for control of *Campylocarpon pseudofasciculare* in grapevine

(Master's Degree Dissertation of Leise Heckler, 2015)





## Biocontrol of *Dactylonectria macrodidyma* in grapevine



“Miolo” Company – Bento Gonçalves

*Thank you for your attention*