

# Doenças do Lenho (DL)

## Meios de luta

**PEDRO REIS E CECÍLIA REGO**





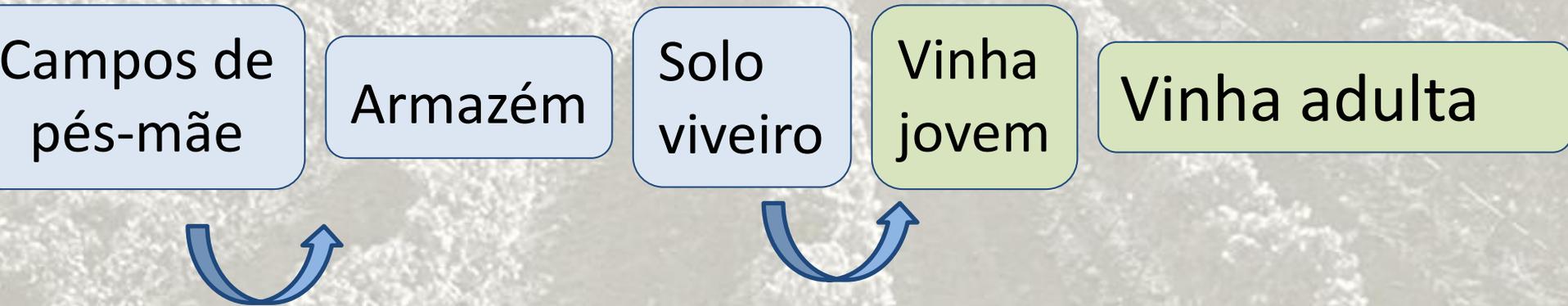
**VIVEIRO**

**VINHA**

Prevenção de infecções de fungos do lenho

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Prevenção de infecções de fungos do lenho

# Meios de luta para DL em viveiros vitícolas

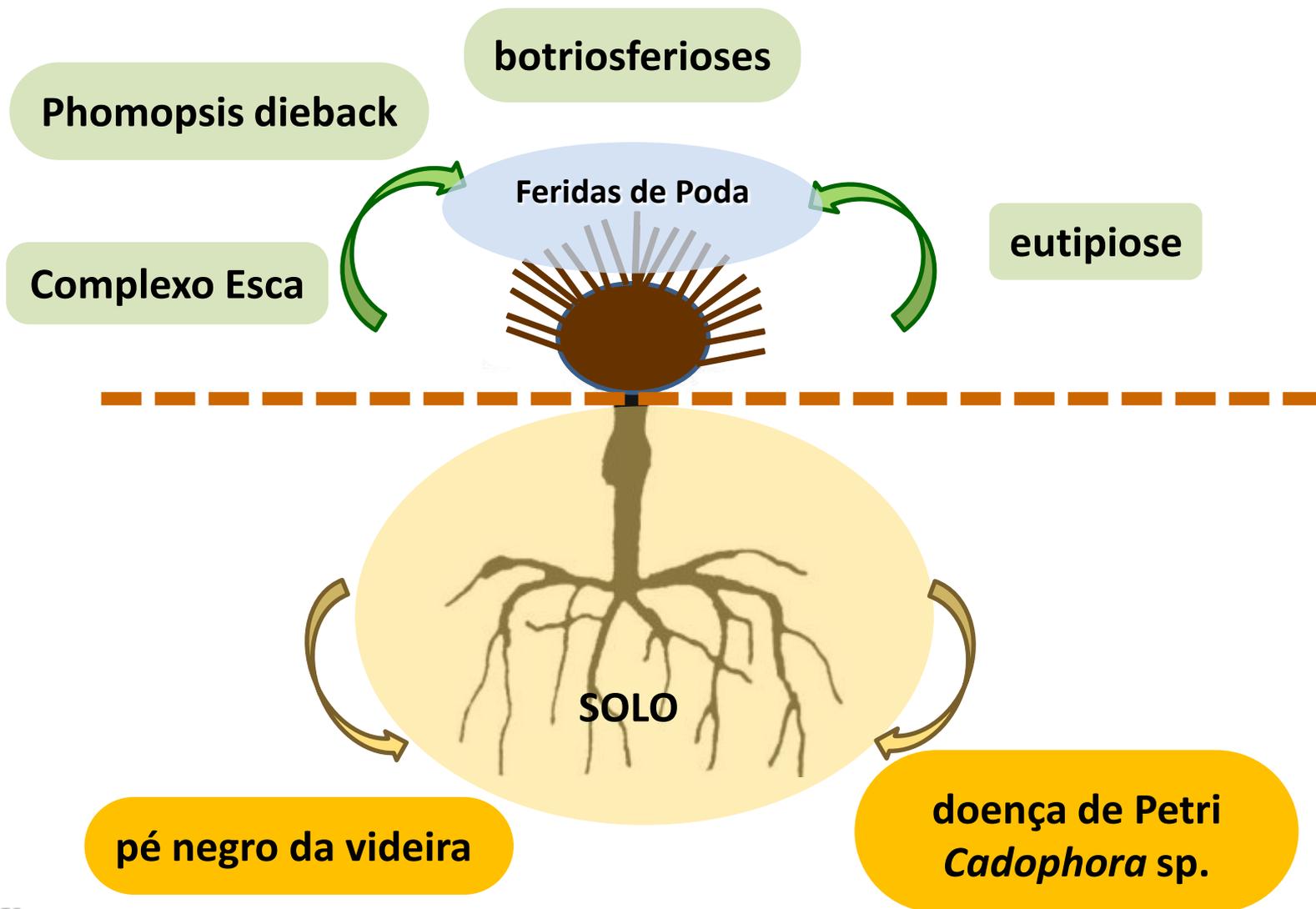


Campo de pés-mãe de porta-enxertos mostrando o **grande número de cortes de poda**.

A proximidade com a **superfície do solo** predispõe à infecção de DL

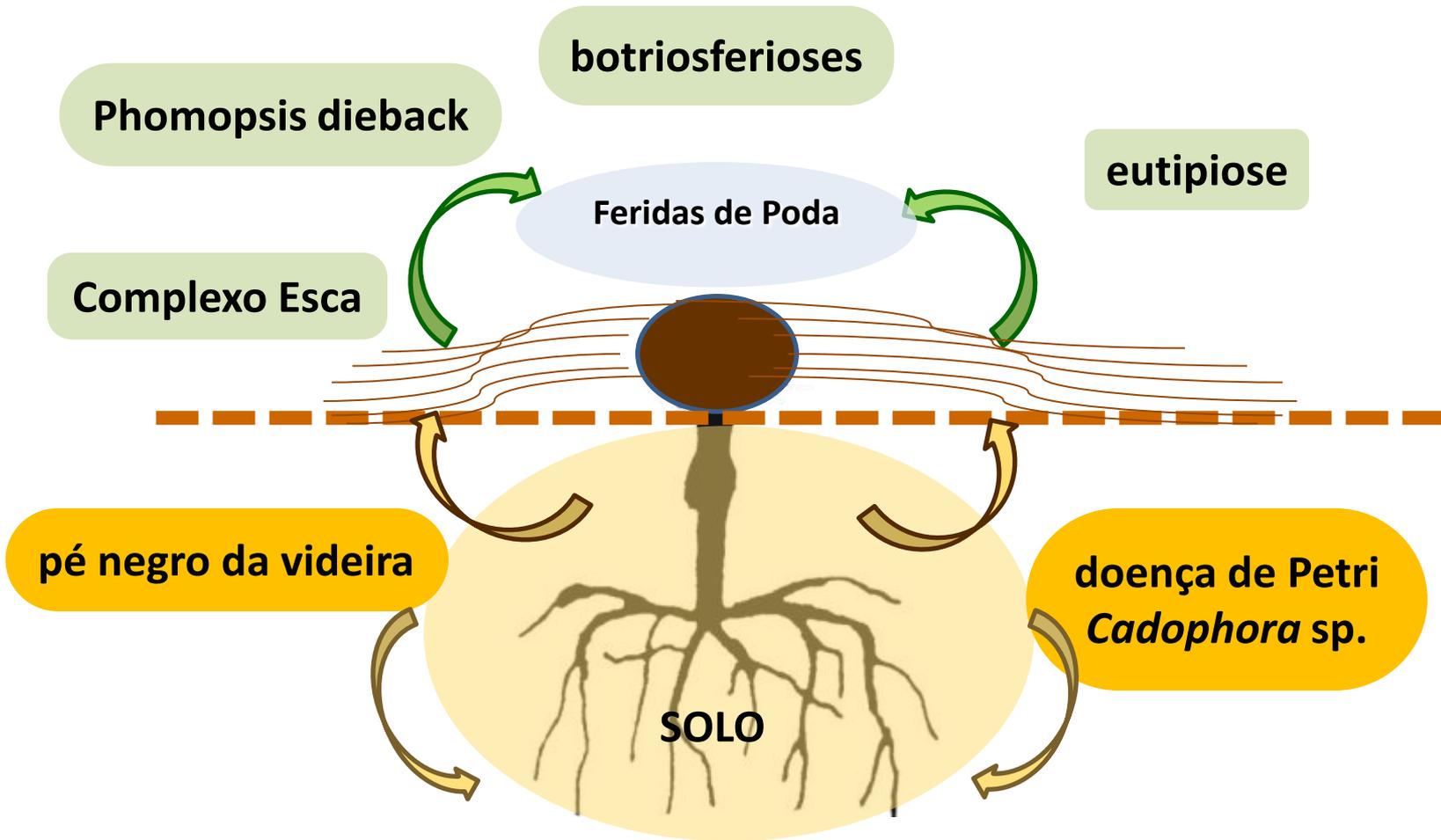


# Pés-mãe de porta-enxertos após o corte das varas





# Pés-mãe de porta-enxertos durante o desenvolvimento – infecções DL



# Vinha de garfos



# Vinhas de garfos - DL - Infecções

Phomopsis dieback

botriosferioses

Comp. esca

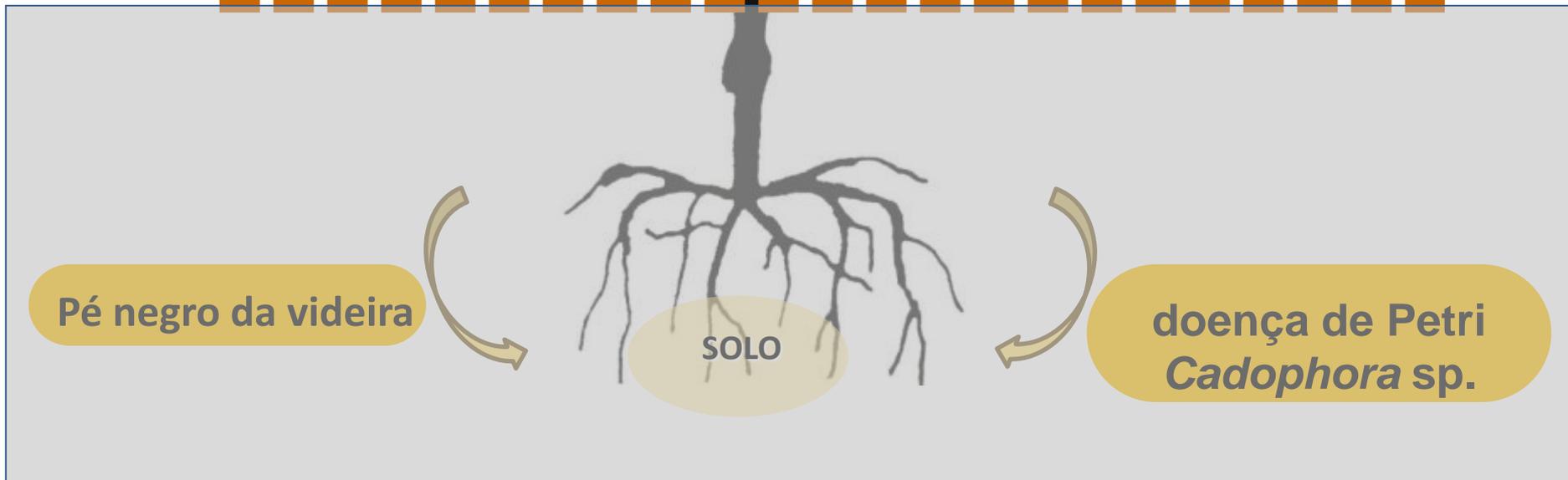
Feridas de Poda

eutipiose

Pé negro da videira

SOLO

doença de Petri  
*Cadophora sp.*



# Prevenção de infecções de fungos do lenho

Em campos de pés-mãe:

1. meios de luta culturais
2. biofungicidas/fungicidas



**Campos de pés-mãe de porta-enxertos:  
varas espalhadas sobre **tela****



**Condução de campos de pés-mãe de porta-enxertos:  
diagonal (a, b e c), horizontal (d)**





## Condução vertical

# Plantas com armação

## Vantagens

- Aumento da madeira produzida, com varas de maior qualidade e de diâmetro mais uniforme
- Eliminar a contaminação potencial pelos patógenos de solo-superfície

## Desvantagens

- Mais mão de obra
- Mais caro

# Plantas espalhadas no solo

## Vantagens

- Menos mão de obra
- Mais barato

## Desvantagens

- As varas diferem no grau de exposição à luz solar (efeito sobre as reservas e atempamento; (as varas não atempam uniformemente e isso pode eventualmente conduzir variadas % de sucesso)
- Maior susceptibilidade a pragas e doenças (especialmente patógenos de solo)

# Rega

gota-a-gota



inundação



aspersão



**Evitar rega por aspersão  
ou por inundação para  
reduzir transmissão de  
inóculo DL**

# Vinhas de garfos: proteção das feridas da poda:

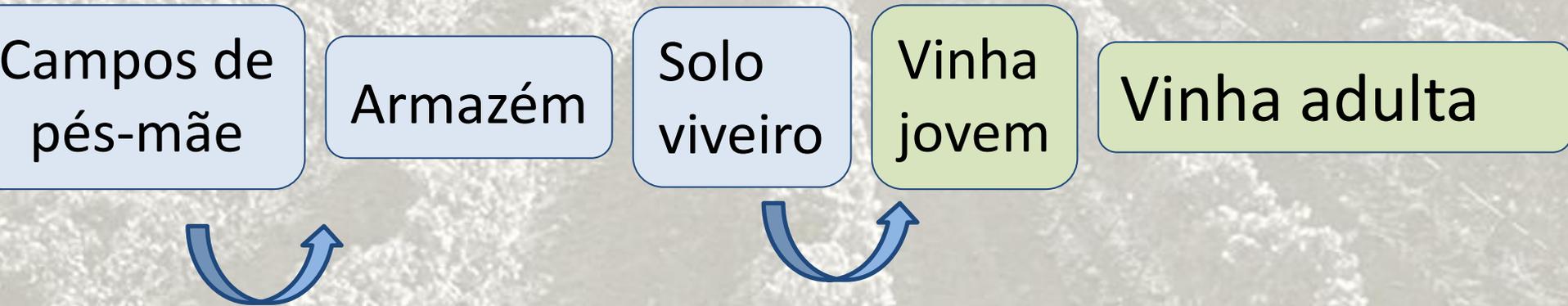


Feridas da poda



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Prevenção de infecções de fungos do lenho

Camaras de frio	Tesouras	Água e tanques de hidratação	Máquinas de enxertia	Parafina	Calogénese
					
					
Pé negro		Doença de Petri		botriosferiose	

# Prevenção de infecções de fungos do lenho

No armazém:

1. meios de luta culturais
2. meio de luta físico - TAQ.
3. biofungicidas/fungicidas

# Prevenção da transmissão de DL

- Limpeza regular de todos os espaços no armazém (câmaras, bancadas de enxertia,
- Desinfecção das tesouras de poda regularmente.
- Evitar a imersão das estacas por períodos mais longos (**não mais de 30 min a 1 hora**). A imersão favorece a contaminação cruzada e amolece os tecidos (pode ajudar a penetração de fungos causadores de DL).



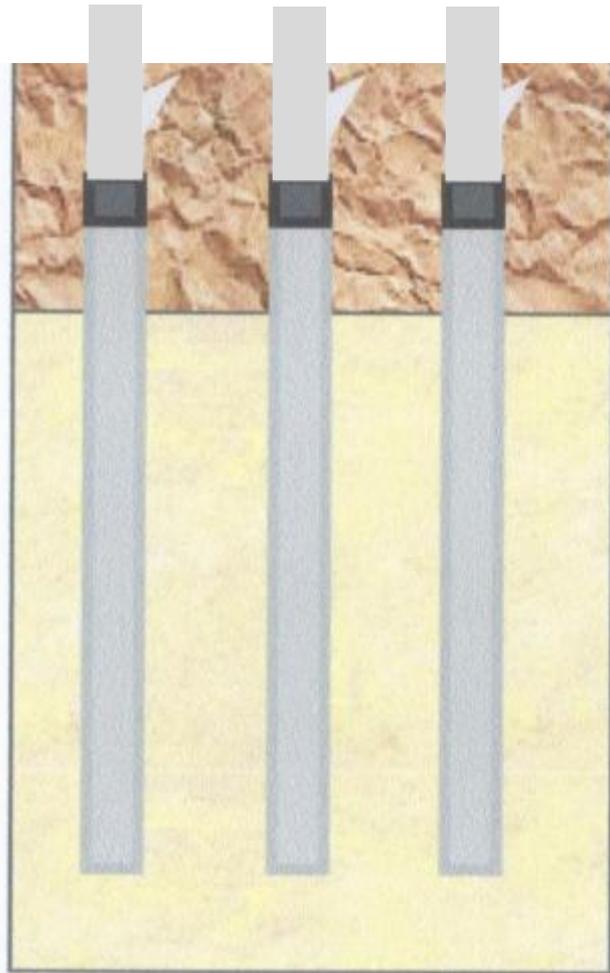
# Caixas de estratificação: Substituição das caixas de madeira por caixas em plástico



Caixas estratificação em plástico

# Caixas de estratificação:

## Substituição da turfa por um substrato inerte hidratado



Vermiculite

perlite

Approx 2/3 of total volume

6 (100L ) sacos de vermiculite + 5 litros de água  
4 sacos de perlite humedecida até escorrer  
(saturada)

-----  
5cm  
-----

28°C ± 2°C e humidade relativa entre 70% and 90%



Caixas estratificação com vermiculite e perlite

TAQ: 50 - 53°C / 30min, Hidratação: 1 hora

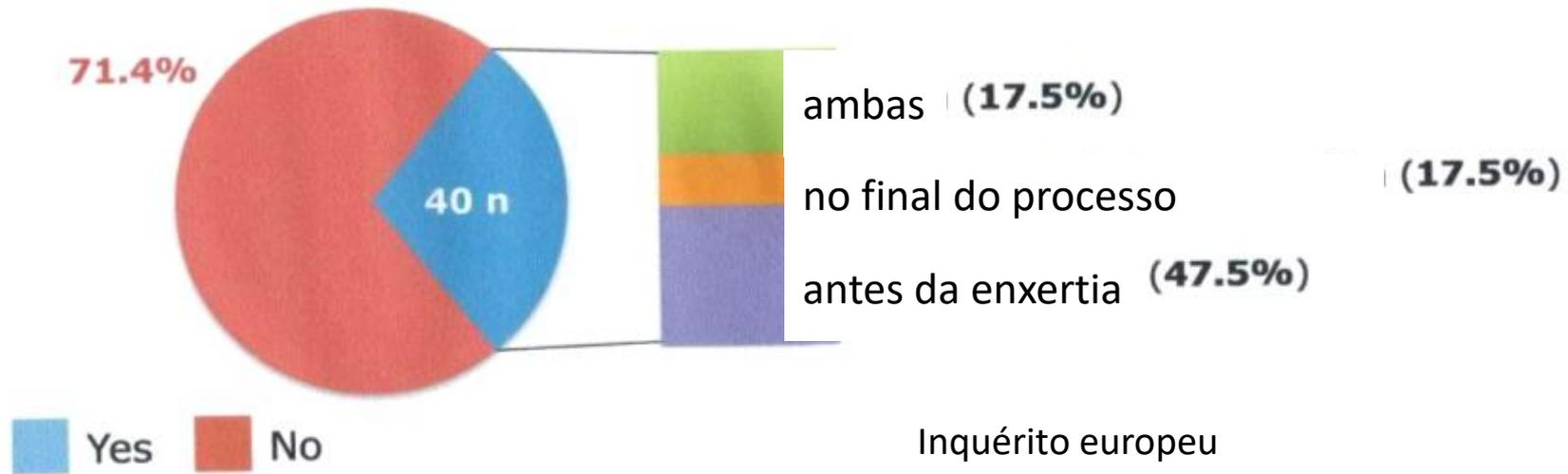
## TAQ

Tratamento com  
água quente





# Tratamento com água quente - TAQ



Inquérito europeu  
Gramaje e Di Marco (2015). *PM*, 54

## Eficácia:

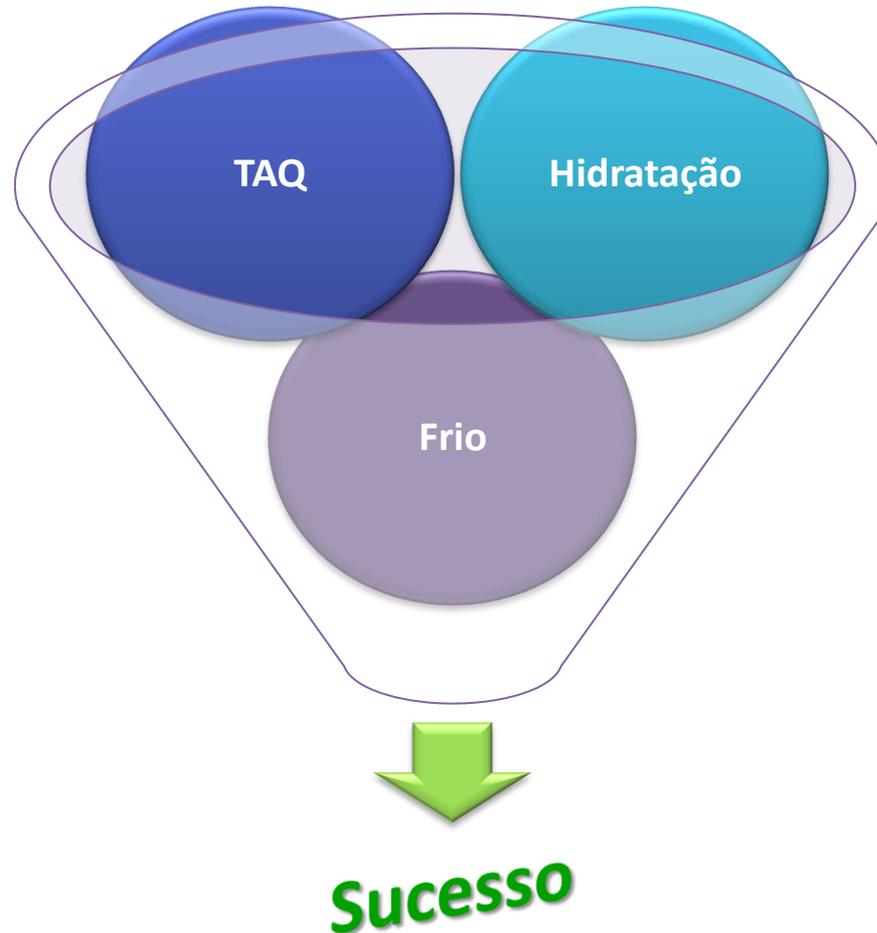
Patogénios <sup>✓</sup> causadores de doenças do lenho da videira

Outros patogénios: *Agrobacterium vitis*, *Xylella fastidiosa* e Flavescência dourada

## Eficiência: €

maior custo das plantas

# Tratamento com água quente - TAQ



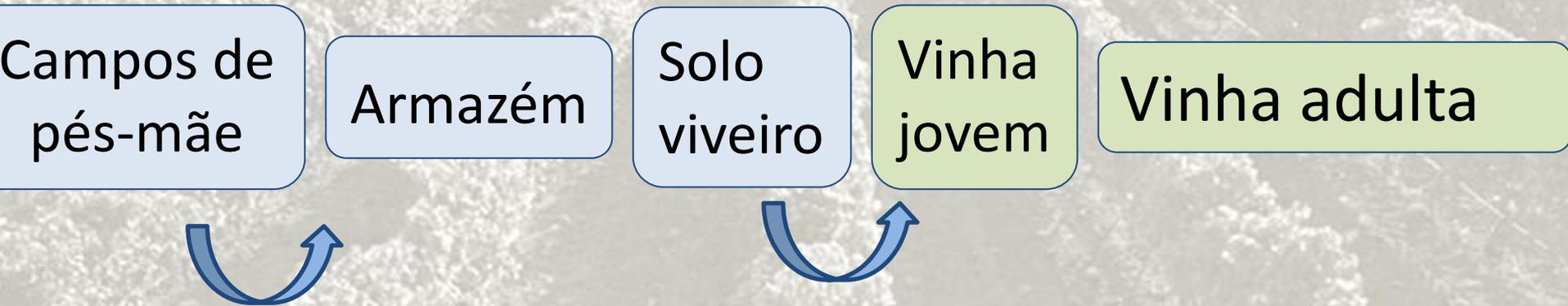
O material tratado com TAQ pode à posteriori ser sujeito a stresses causados por práticas incorrectas tais como o armazenamento prolongado em câmaras de frio depois do tratamento.

Tratamento	Doença	País	Resultados
50 °C / 30 min	BD BF PD	South Africa	Completely eliminated fungi stems of treated cuttings (3)
50 °C / 30min	PD	Australia	Not very effective as a curative treatment (1)
51 °C / 30min	PD	USA	<i>In vitro</i> , slight reduction in growth rate of <i>P. c.</i> but no effect on <i>P. in.</i> (2)
51 °C / 30min	PD	USA	
50 °C / 30min	PD	Australia	Reduced the infection level of <i>P.c.</i> , but it was not effective against <i>P. m.</i> (2)
50 °C / 30 min	PD	South Africa	Effective in reducing the infection caused by <i>P.c.</i> and <i>P. spp.</i> (3)
50 °C / 30 min	PD	New Zealand	Reduced the incidence of the pathogen (3)
50 °C / 30 min	BF PD	South Africa	Effective in eradicating fungal infection from uprooted dormant plants (3)
49, 50, 51, 52, 52, 54, 55 °C / 30, 45 or 60 min	PD	Spain	<i>In vitro</i> , up to 53°C for 30 min required to reduce growth and germination (3)
50, 51, 52, 53, 54 °C / 30, 45 or 60 min	PD	Spain	53°C for 30 min significantly reduced the incidence pathogens (3)
50 °C / 45 min	PD	Italy	Reduced the frequency of isolation of the pathogen (3)
41, 42, 43, 44, 45, 46, 47, 48, 49 °C / 30, 45 or 60 min	BF	Spain	<i>In vitro</i> , 48°C for 30 min inhibited growth and germination (3)
50 °C / 45 min	BD PD	France	Reduced pathogen infections (3)
53 °C / 30 min	BD PD	Peru	Highly effective against <i>L.t.</i> , and reduced <i>P.p.</i> in dormant cuttings (3)
50°C / 30 min	BD	New Zealand	Reduced the incidence of <i>N.l.</i> but not <i>N.p.</i> (2)
50, 51, 53 °C / 30 min	BD	Spain	Reduced survival in artificially inoculated canes after 30 min at 51°C (3)
50°C / 30 min	BD BF PD	South Africa	Eradicated black-foot pathogens and reduced the incidence of <i>P.c.</i> , <i>P. spp</i> and <i>Bot. spp</i> in dormant grafted vines (3)

BF – Pé negro, BD – botriosferiose; PD – Doença de Petri

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Prevenção de infecções de fungos do lenho

# Botriosferiose, Complexo da esca e Phomopsis dieback



**Pé negro e Doença de Petri**

# Control of grapevine wood fungi in commercial nurseries.

*Phytopathologia Mediterranea* 48: S128-S136.

Rego, C., Nascimento, T., Cabral, A., Silva, M.J., Oliveira, H. 2009.

Os avultados prejuízos causados pela ocorrência de declínio em videiras jovens, resultante da utilização de plantas infectadas, tornaram prioritário e fundamental o desenvolvimento de meios de luta relativamente a esta síndrome complexa, que envolve a presença de doenças recentemente assinaladas, nomeadamente o pé negro da videira e a doença de Petri, para além das tradicionais doenças do lenho.

## Prospecção em viveiros

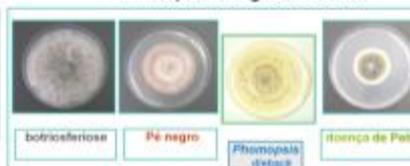


## Sintomas



Internamente, observam-se necroses com forma e dimensão variáveis (tipo pontuações ou anel contínuo), no lenho, mais intensas na base do porta-enxerto.

## Principais fungos isolados



Porta-enxertos: 1103P

Método: imersão de estacas em calda fungicida, 50 minutos, antes da enxertia



No final do processo de enraizamento (11 meses), os bacelos enraizados são analisados relativamente à presença de fungos do lenho

Análise da incidência e severidade das infestações naturais causadas por fungos do lenho



Garfos: cultivar Aragonés



**Delineamento experimental**  
 • Unidade experimental: 20 enxertos prontos  
 • Repetições: 4  
 • Distribuição aleatória

	Incidência (%)		
	Pé negro	Botriosferiose	Phomopsis dieback
ciprodinil + fludioxonil (Switch)	40,00 a	20,00 b	0,00 a
piraclostrobina + metirame (Cabrio Top)	30,00 a	35,00 bc	5,00 a
fludioxonil (Geoxe)	25,00 a	40,00 bc	10,00 a
ciprodinil (Chorus)	65,00 b	15,00 a	5,00 a
água (Controlo)	75,00 b	50,00 c	10,00 a

Tratamentos	Severidade (%)		
	Pé negro	Botriosferiose	Phomopsis dieback
ciprodinil + fludioxonil (Switch)	4,17 a	1,67 a	0,00 a
piraclostrobina + metirame (Cabrio Top)	4,17 a	4,17 a	1,76 a
fludioxonil (Geoxe)	2,92 a	4,58 ab	2,50 a
ciprodinil (Chorus)	10,83 b	0,83 a	0,52 a
água (Controlo)	10,42 b	13,33 b	2,08 a



## Abstract

**Grapevine trunk disease** pathogens of economic importance in nurseries include *Ilyonectria* spp., *Dactyloctenocyon* spp., *Cylindrocarpon* spp. and *Caryospora* spp. (Schuck et al., 1998; 1999; Hansen et al., 2004, 2005, 2006; Lombard et al., 2014). *Botryosphaeria* long (Van Nieuwen et al., 2004, 2006, 2010) and *Phaeoannellaria chrysosporium* and *Phaeoannellaria* spp. (Másson et al., 2000; Møller et al., 2009) and *Diaporthe* spp. They, either individually or collectively, can be responsible for graft failure and/or also be responsible for early mortality when cuttings remain latent until the vines are exposed to predisposing stress and/or conditions favorable for disease development once planted out in a vineyard (Fernes et al., 1990; Van Nieuwen et al., 2010). It is, therefore, important to test new solutions to reduce the incidence of these fungi in order to try to obtain **healthy** planting materials which may reduce the impact that these disease have on **arepays**. Field trials were conducted, during two years in a commercial nursery in Bombarda, Portugal, where the majority of Portugal's grapevine nurseries are located. The objective of the experiments was to assess the effectiveness of *Trichoderma atroviride* strain I-1237 (EJP12) under commercial nursery conditions (natural infection) for the protection of cuttings against *Botryosphaeria*-*Diaporthe*, *Phaeoannellaria*, *Ilyonectria*, *Dactyloctenocyon*, *Caryospora* and *Phaeoannellaria* trunk diseases. The effectiveness of the treatments was evaluated through the ability of this particular strain of *T. atroviride* in reducing natural infections by the fungi in propagating materials.

## Material and Methods

### Plant materials, Treatments, products and immersion

Treatments	Products	Immersion (weeks to)
• cuttings - 100 Paulsen certified rootstock mother plants	1 <i>Trichoderma atroviride</i> strain I-1237(EJP12)	24h
	2 <i>Trichoderma atroviride</i> strain I-1237(EJP12)	3h
• cuttings - culture Aragonez were used as stock	3 sprayed + reduced (SulfoP)	24h
	4 sprayed + reduced (SulfoP)	3h
Both rootstocks and stocks are thought to be susceptible to GTD fungi	5 water control	24h
	6 water control	3h

Prior to grafting, dormant rootstock and scion cuttings were dipped for **1 hour or 24 hours** in a *Trichoderma atroviride* suspension, sprayed + reduced suspension (recommended fertilizer) or in water

cuttings: 100P  
 scion: culture Aragonez

1 hour & 24 hours

### Nursery procedures

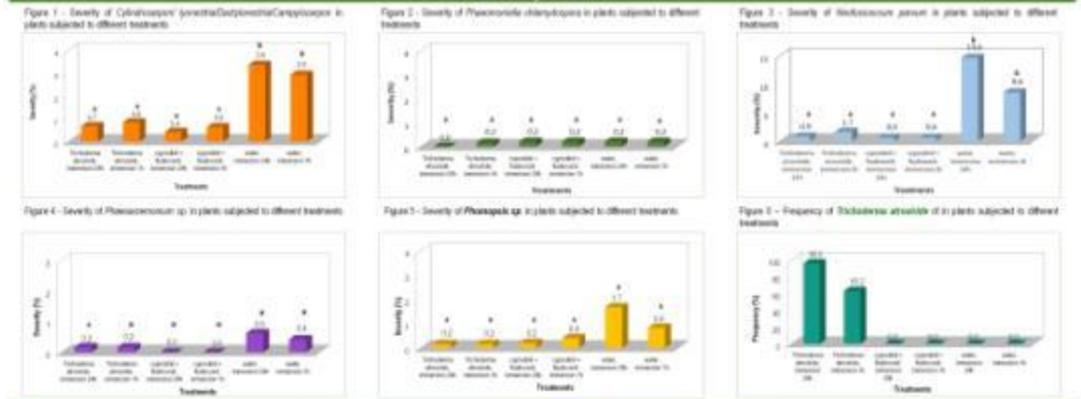
- After **treatments**, cuttings were grafted following standard practices.
  - A total of 6 treatments were set up on grafted cuttings in a completely randomized design with 100 repetitions.
  - The grafted cuttings were stocked in separate culturing boxes for 3 weeks at 20°C and 100% relative humidity.
  - After that period, the grafted cuttings were randomly planted in the nursery field following standard nursery practices.
  - Grafting practices were followed according to nursery guidelines. **After 3 months**, the plants were sprayed and isolations were made from necrotic tissues located 2 cm above the basal end of the rootstock.
- 

### Isolation

Fungi isolation was complete by placing 12 wood fragments from each plant onto two PCA medium Petri dishes amended with chloramphenicol (200 mg l<sup>-1</sup>) and incubated at 20°C, in darkness for 4 weeks. Isolated fungi was identified according to their morphological features. Severity of the fungi present in **colony affected** plants was evaluated. The colonization of cuttings by *Trichoderma atroviride* strain I-1237(EJP12) was also recorded.

Colony affected: *Ilyonectria complex*, *Dactyloctenocyon complex*, *Caryospora*, *Phaeoannellaria chrysosporium*, *Phaeoannellaria*, *Botryosphaeria parvum*, *Phaeoannellaria*.

## Severity Results



## Conclusions

The objective of the trial was to assess the effectiveness of *Trichoderma atroviride* strain I-1237 (EJP12) under commercial nursery conditions for the protection of cuttings against wood fungal pathogens. The trial was conducted successfully under local environmental conditions. **EJP12** showed the ability to provide a good control of severity of all pathogens, despite the duration of the treatment strategy (3h versus 24h). Results obtained were similar to the ones obtained for SulfoP, used as standard control. Although chemical fungicides have a huge but short live effect in pathogenic fungi, biologicals, on the contrary, are able to **colonize** **sterilizing** the wood along the canes and offer a longer protection to infection, as it can be confirmed by the high frequency of *Trichoderma atroviride* isolation in the treated cuttings. In conclusion, ***Trichoderma atroviride* strain I-1237 (EJP12)** can be regarded as a good and efficient biological control agent for protection of grapevine propagation materials against grapevine trunk diseases.

DDAV

[http://www.dgav.pt/fitofarmaceuticos/guia/finalidades\\_guia/Insec&Fung/Culturas/videira.htm](http://www.dgav.pt/fitofarmaceuticos/guia/finalidades_guia/Insec&Fung/Culturas/videira.htm)

DOENÇA	SUBSTÂNCIA ACTIVA	FORM	CONCENTRAÇÃO (g sa /hl)	IS dias
Doenças do lenho:	<u>boscalide+piraclostrobina</u>	SD **	20L/ha	-
Escoriose Europeia*/BDA*, doença de Petri, Esca , Eutipiose	<u>difenoconazol (111)</u>	<u>EC</u>	<u>25 g s.a./ha</u>	<u>21</u>
	<u>trichoderma atroviride SC1 (159)</u>	WG ***	<u>30 g s.a./ha</u>	-
	<u>trichoderma atroviride I-1237</u>	WP	(98)	1
	<u>trichoderma asperellum+trichoderma gamsil</u>	WP	20+20g s.a./ha	-

\*. Botriosferiose

\*\* . Aplicação direta nas feridas de poda.

\*\*\*. Durante o processo de viveiro, aplicação por imersão de porta enxertos e plantas já enxertadas.



## Controlo eficaz de DL

Minimizar as infecções no material de propagação vegetativa

Melhorar as práticas culturais no **VIVEIRO**

Controlo das DL nos **campos de pés-mãe**

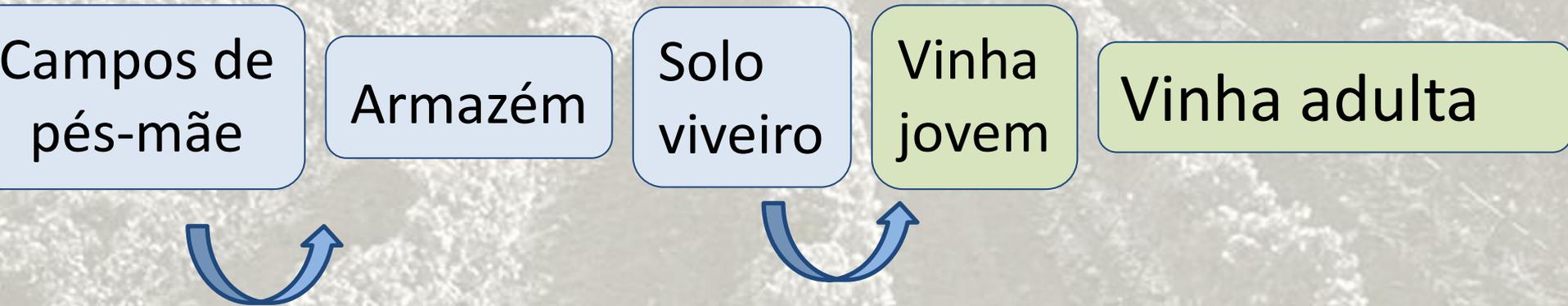
**Desinfecção dos espaços** (armazém), rotações no solo do viveiro, tratamento com água quente

**Melhor conhecimento dos limites máximos de infecção**



VIVEIRO

VINHA



Prevenção de infecções de fungos do lenho

Na vinha:

1. meios de luta culturais
2. biofungicidas
3. fungicidas

Prevenção de infecções de fungos do lenho

# 1. Solo e rizosfera/sistema radicular

- Histórico da parcela
- Remoção de materiais Infectados
- Rotações
- Microbiota do solo; quantificação do inóculo de patogéneos DL existentes

- Evite a replantação imediata de vinhas!
- Preparação adequada do solo (surriba, drenagem, etc).

## 2. Material de propagação vegetativa

- Use materiais de propagação vegetativa certificado, preferencialmente

### Directiva Europeia/Directiva Nacional

Ausência de norma específica relativamente a infecções latentes causadas por patogénios do lenho da videira.

A qualidade do material vegetal é determinante na produção e longevidade da vinha;

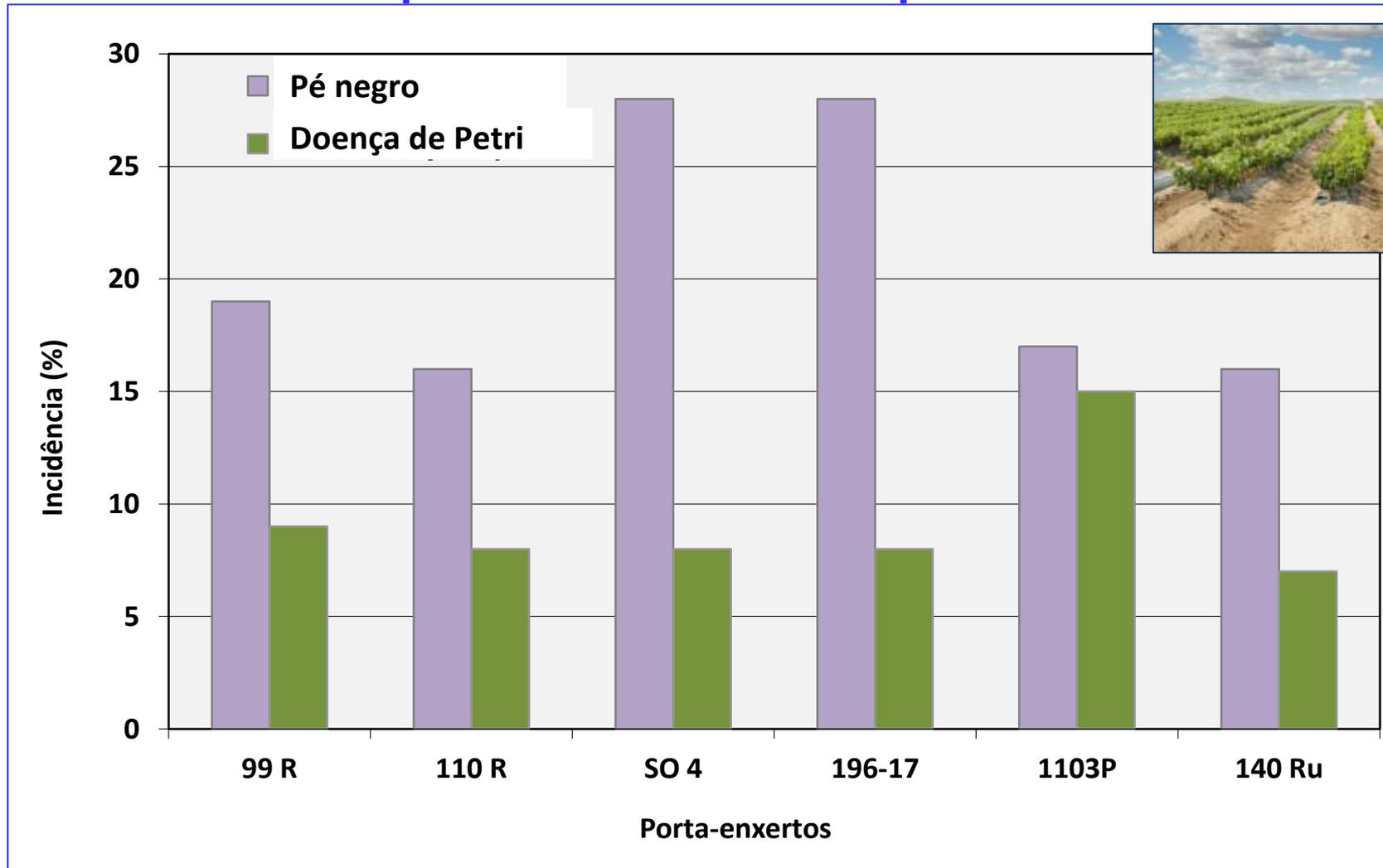
Parâmetros a avaliar para assegurar:

- qualidade da enxertia,
- ausência de feridas desnecessárias ou de tecidos necróticos,
- bom diâmetro do caule
- raízes adequadas

### 3. Escolha o porta-enxerto/tipo de solo

Cultivares	Vigor	Resistência ao calcário ativo	Resistência à secura	Comportamento em relação à		Ação sobre o ciclo vegetativo
				Humidade	Salinidade	
<b>Rup. Du Lot</b>	Muito vigoroso	14%	Sensível	Muito sensível	Tolerante	Retarda
<b>99 R</b>	Muito vigoroso	17%	Média	Sensível	Resistência nula	Retarda
<b>110 R</b>	Muito vigoroso	17%	Elevada	Sensível	Resistência nula	Retarda
<b>140 Ru</b>	Muito vigoroso	17-20%	Elevada	Sensível	Resistência nula	Retarda um pouco
<b>1103 P</b>	Muito vigoroso	17-19%	Elevada	Tolerante	Tolerante	Retarda um pouco
<b>420 A</b>	Fraco a médio	20%	Média	Tolerante	Resistência nula	Avança
<b>S O 4</b>	Vigoroso	17-18%	Sensível	Bastante tolerante	Resistência nula	Avança
<b>5 B B</b>	Médio	20%	Sensível	Bastante tolerante >161-49	Resistência nula	-
<b>161-49 C</b>	Fraco a médio	25%	Média	Tolerante	Resistência nula	Avança
<b>5 C</b>	Médio	20%	Sensível	Tolerante	Tolerante	Avança>5BB
<b>125-AA</b>	Médio	13%	Sensível	Tolerante	-	-
<b>3309 C</b>	Médio	11%	Sensível	Sensível	Resistência nula	Avança
<b>41 B</b>	Médio	40%	Média	Sensível	Sensível	Avança
<b>196-17 CI</b>	Médio	6%	Média	-	Tolerante	-

## 4. Susceptibilidade dos porta-enxertos



Porta-enxertos menos susceptíveis: **Esca**  
161-49, *Vitis berlandieri* x *V. riparia*/

Porta-enxertos mais susceptíveis: **Botriosferioses**  
5C e S04, *Vitis berlandieri* x *V. riparia*

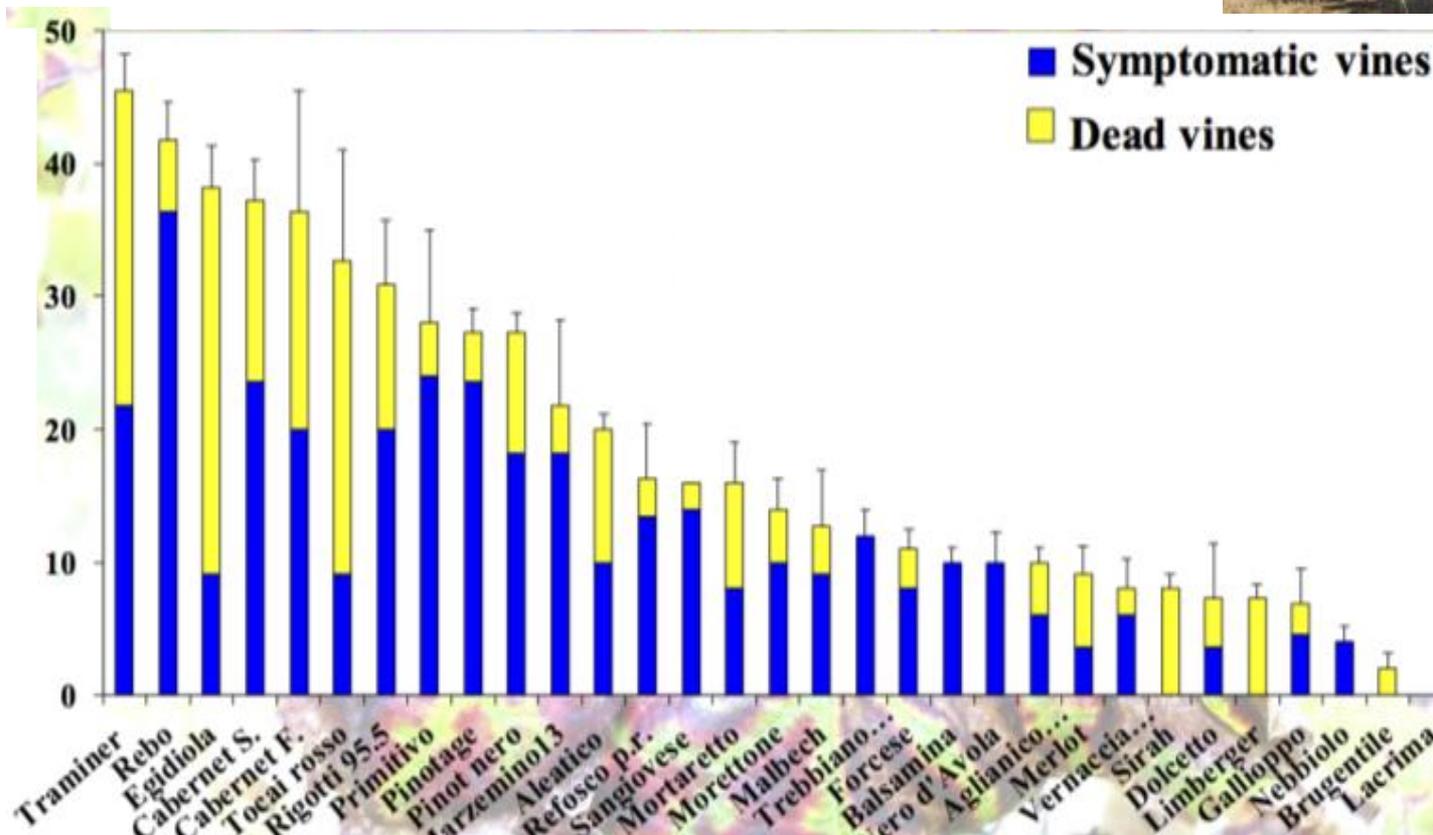
**Conclusão: susceptibilidade variável  
em função da doença!**

# 5. Susceptibilidade das castas

Influencia dos factores ambientais!!!  
chuva, temperatura, ...



Esca



Romanazzi et al., 2014

# Susceptibilidade variável!

## Exemplo: Incidência de Esca

Table 2. Cumulative esca incidence in a vineyard planted with the cv. Sangiovese (CBSI-1) and Trebbiano (CBSI-2), on five different rootstocks and own-rooted. The incidence is the percentage of all plants that exhibited symptoms at least once in the five-year period 1995-1999.

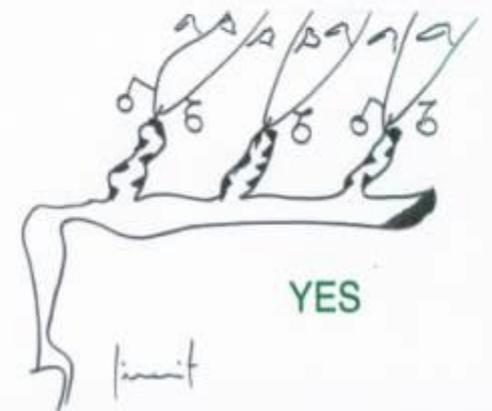
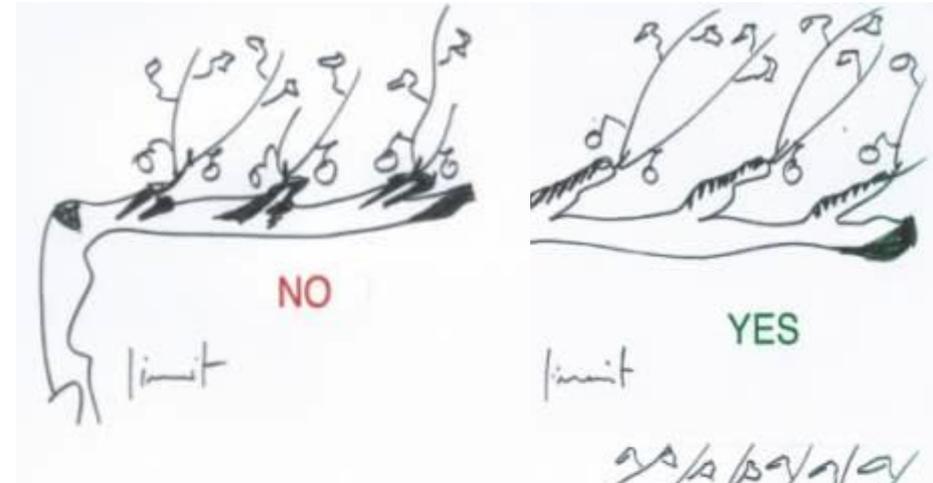
Vineyard	Cultivar	Rootstock	No. standing plants in 1995	Cumulative incidence (%)	Average incidence (%)
CBSI-1	Sangiovese	140RU	80	16.2 <sup>a</sup>	15.3 <sup>c</sup>
		1103P	90	11.1	
		K5BB	87	24.1 ←	
		SO 4	90	8.8 ←	
		420A	90	17.7	
		own-rooted	75	13.3 ←	
CBSI-2	Trebbiano	140RU	85	9.4 <sup>b</sup>	17.4
		1103P	90	24.4 ←	
		K5BB	91	18.6 ←	
		SO 4	91	8.7 ←	
		420A	92	21.7	
		own-rooted	84	21.4	

## 6. Sistema de condução e poda

Sistema de condução e poda:  
“diminuir o número e o tamanho das feridas”

**Sistema Guyot-Poussard:** Preserva a passagem do fluxo da videira de um ano para outro, devido à implementação de uma poda que posiciona as feridas principalmente na parte superior do sistema de condução.

**Poda Dupla:** Pré-poda mecânica + poda desejada (remove a madeira infectada)



7. Poda dupla: pré-poda precoce seguida de poda no fim do Inverno
8. Podar com tempo seco
9. Remoção da madeira de poda (compostagem durante 6 meses)
10. Arranque e queima de todas as plantas mortas ou doentes
11. Eliminar as vinhas abandonadas

12. Fertilização equilibrada

13. Rega adequada, quando possível

14. Evitar a disseminação de inóculo através de práticas culturais

15. Enrelvamento semeado de forma a evitar espécies hospedeiras das doenças

# 15. Enrelvamento semeado de forma a evitar espécies hospedeiras das doenças

*Ilyonectria macrodidyma* foi isolado a partir de 15 famílias e 26 espécies de infestantes (Reis e Oliveira, 2013). *Pa. chlamydospora* foi isolado a partir de *Convolvulus arvensis* (Agustí-Brisach *et al.*, 2011)



# Meios de luta

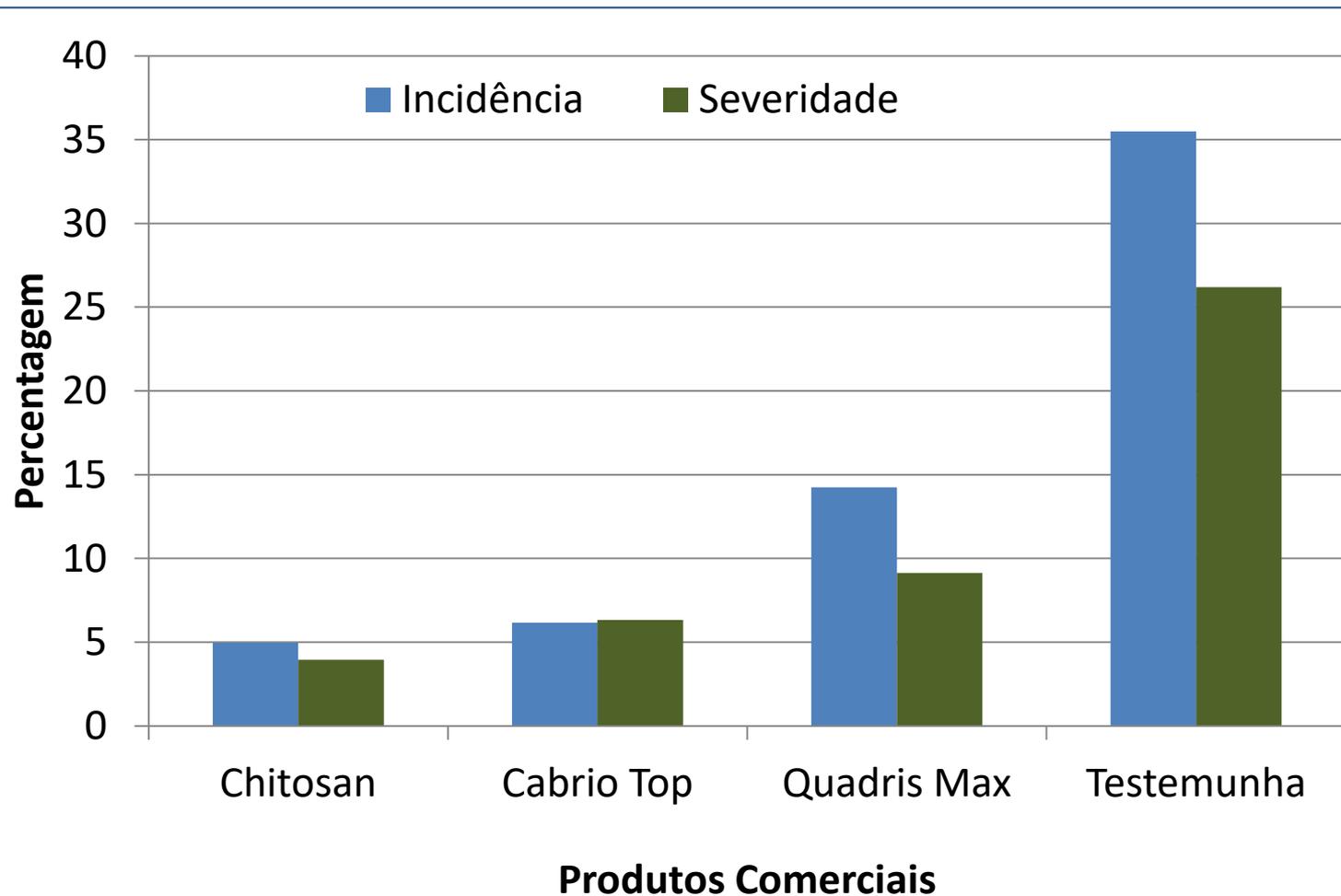
## Compostos naturais e biológicos

## Compostos naturais

substancia activa	Botriosferiose			Eutipiose			Comp. Esca		
	lab	campo	Prot. feridas	lab	campo	Prot. feridas	lab	campo	Prot. feridas
Extracto de alho			++			+++			+++
quitosana	+++			+++			+++		
vanilina			+++			+++			+++
quitosana + extracto de alho + vanilina	+++		+++	++		++	+++		+++



# Ensaio de campo na casta Castelão em Reguengos ao longo de 3 anos



# Agentes de biocontrolo (BCAs)

Botriosferiose	Eutipiose	Comp. Esca
<p><b><i>Trichoderma</i> spp</b></p> <p><i>T. harzianum, T. atroviride, and Benzimidazole-resistant mutant strain</i></p> <p><b>TESTED: Pruning Wound Protection</b></p>	<p><b><i>Trichoderma</i> spp</b></p> <p><i>T. harzianum, T. atroviride, Benzimidazole-resistant mutant strain</i></p> <p><b>TESTED: Pruning Wound Protection</b></p>	<p><b><i>Trichoderma</i> spp</b></p> <p><i>T. harzianum, T. atroviride, T. longibrachiatum, T. asperellum, T. gamsii and benzimidazole-resistant mutant strain</i></p> <p><b>TESTED: Pruning Wound Protection</b></p>
<p><b><i>Bacillus subtilis</i> EE isolate</b></p> <p><b>TESTED: Pruning Wound Protection</b></p>	<p><b><i>Bacillus subtilis</i> EE isolate</b></p> <p><b>TESTED: Pruning Wound Protection</b></p>	<p><b><i>Bacillus subtilis</i> EE isolate</b></p> <p><b>TESTED: Pruning Wound Protection</b></p>
		<p><b><i>Pythium oligandrum</i></b></p> <p><b>TESTED: induced resistance by root colonization</b></p>

*Outras espécies de fungos: Fusarium lateritium, Chaetomium spp.*

# Fungicidas de síntese

substancia activa	Botriosferiose			Eutipiose			Comp. Esca		
	lab	campo	Prot. feridas	lab	campo	Prot. feridas	lab	campo	Prot. feridas
<b>Benomyl</b> (FORBIDDEN)			+++			+++			+++
Boric acid			+++			+++			+++
<b>Carbendazim</b> (FORBIDDEN)	+++	+++		+++	+++		+++	+++	
Fluazinam		+++			+++	+++			
Paste + cyproconazole + iodocarb			+++			+++			+++
Tebuconazole		+++	+++		+++	+++		++	++
Thiophanate-methyl	+++		+++			+++	+++		+++
Copper oxichloride+ gluconates								+++ Vs Symptoms	
Tetraconazole								+++ Vs Symptoms	
Fludioxinil+Cyprodinil		+++							
Iprodione		+++							
Procloraz			+++						
Acrylic paint						+++			
Penconazole						+++			
Pyrimethanil						+++			

# Ensaio no campo Esquive (biofungicida)

# Esquive<sup>®</sup>

Biofungicida (W<sub>etable</sub> P<sub>owder</sub>)

*Trichoderma atroviride*

strain I-1237

Patent :



# Condições experimentais

Duas vinhas foram seleccionadas para montagem dos ensaios:

**Cultivar:** Aragonez (= Tempranillo)

**Idade:** 12 anos

**Localização:** Estremadura (Alenquer)

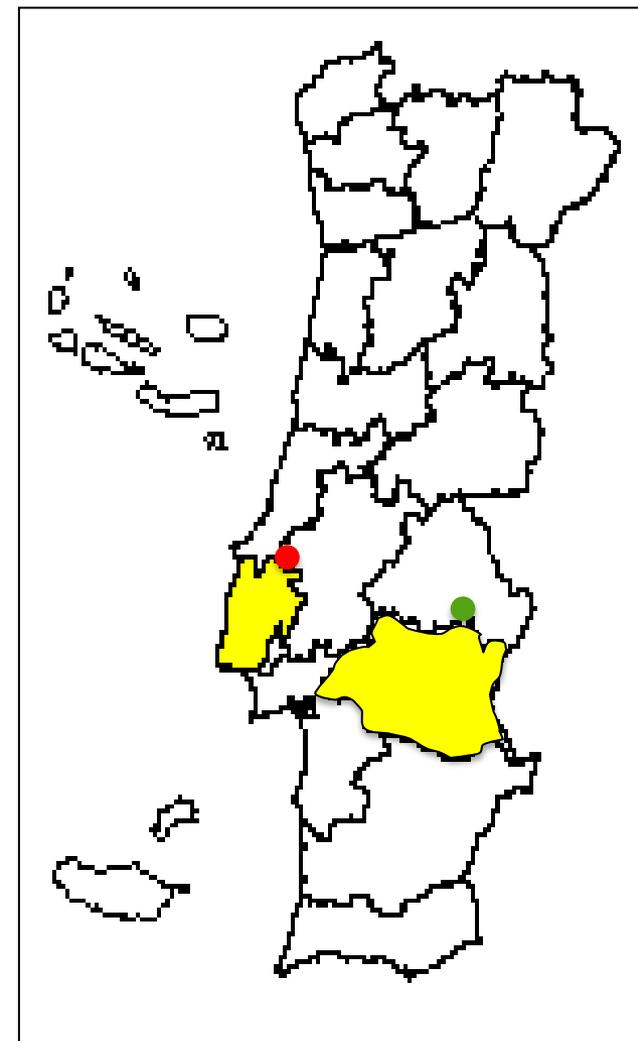
**Classificação climática:** Csb

**Cultivar:** Aragonez (= Tempranillo)

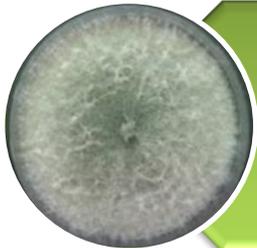
**Idade:** 15 anos

**Localização:** Alentejo (Estremoz)

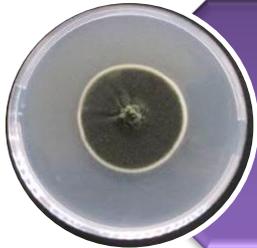
**Classificação climática:** Csa



- O principal objectivo dos ensaios:
  - Testar a eficácia de uma formulação de *Trichoderma atroviride*, strain I-1237, **ESQUIRE**<sup>®</sup>, contra:



*Neofusicoccum parvum* (Np)  
Botriosferiose



*Phaeomoniella chlamydospora* (Pch)  
GLSD

## Aplicação do biofungicida

**ESQUIVE** foi aplicado **no mesmo dia** em que se efectuou a poda

**Aplicação: uma pulverização** directamente sobre a ferida de poda, efectuada com condições climatéricas adequadas

**Data:** Fevereiro de 2013 e 2014



# Inoculação

Suspensão de esporos colocada sobre a **ferida de poda** com o auxílio de uma micropipeta, **um dia após o tratamento**



As feridas tratadas e inoculadas foram protegidas com parafilm durante 2 semanas, por forma a prolongar a humidade

## Avaliações

(Outubro – após a vindima)

**Incidência** (infectado/não infectado) e **Severidade** (% de lançamentos infectados)



# Trichoderma atroviride strain I-1237: colonization of pruning wounds against grapevine wood diseases

Pedro REIS<sup>1</sup>, Patricia LETOUSEY<sup>2</sup>, Cecília REGO<sup>3</sup>

<sup>1</sup> Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, University of Lisbon, Lisbon, Portugal

<sup>2</sup> Agronomie Lesaffre Plant Care, 264 Rue Henri Becquerel - 69003 BEAUCOUZE - France

Email: pedroreis@isag.ucp.pt



## Introduction

On grapevine, *Neofusicoccum parvum* (Np) and *Phaeoannellaria chlamydospora* (Pch) are among the pathogens associated to Botrytis-like rot (BlR) (Miller & Kaimowitz, 1978; Laignon & Dubou, 1987; Guedes et al., 2000; Foster, 2006; Laignon et al., 2009; Utrero Torres, 2011; Spagnolo et al., 2014a; Laignon et al., 2015) and Grapevine leaf stripe, one of the diseases within the necra complex (Girou et al., 2008), respectively. These trunk diseases are of major importance worldwide. They are associated with wood necrosis and affect both young and mature vines. Infection by these fungal pathogens occurs primarily through pruning wounds (Patel et al., 1981; Chapiro et al., 1996; van Nieuwen et al., 2011; Mutzels et al., 2015), being the higher risk of infection during the pruning period, from late fall to early spring, because of the high number of wounds made on a single vine and the frequency of raining events that occur during that period. Therefore, in order to test a formulation of *Trichoderma atroviride* strain I-1237 (Ecovine® RP, Agronomie Lesaffre Plant Care) as wound protectant, trials in two different Portuguese vineyards (i.e., Pinel Noir and Aragonez) were carried out. The effectiveness of the treatments was evaluated through the ability of the product in reducing the infections on pruned canes, artificially inoculated with the two pathogens.

## Material and Methods

### Experimental conditions

Two similar vineyards were selected to establish the assays

**1<sup>st</sup> year for the field assays - 2013**  
Cultivar: Aragonez (> Pinel Noir)  
Age: 17 years old  
Location: Estremoz (Alentejo)  
Vigour: average (moderate); Cab

**2<sup>nd</sup> year for the field assays - 2014**  
Cultivar: Aragonez (> Tempranillo)  
Age: 15 years old  
Location: Alentejo (Estremoz)  
Vigour: average (moderate); Cab

Training system: double curtain  
Pruning system: long pruning

Layout: completely randomized design (CRD)

Plot size was large enough to allow vine inoculation in each branch of the plot



### Pruning Method

One-year old shoots with a similar appearance (length, orientation, buds) were selected for the trial  
Shoots were being pruned at 2 cm above the 3rd leaf (shoot length = 30 cm) to avoid infection of the older branches  
A total of 200 shoots were used in the trial



### Treatment, application date, product, concentrations and spore solution volume

Treatment	Application date	Product	Concentration	Inoculation	Spore solution volume
1	10/10/2013	Ecovine® RP	1000 mg/L	10/10/2013	100 ml
2	10/10/2013	Ecovine® RP	1000 mg/L	10/10/2013	100 ml
3	10/10/2013	Ecovine® RP	1000 mg/L	10/10/2013	100 ml
4	10/10/2013	Ecovine® RP	1000 mg/L	10/10/2013	100 ml
5	10/10/2013	Ecovine® RP	1000 mg/L	10/10/2013	100 ml
6	10/10/2013	Ecovine® RP	1000 mg/L	10/10/2013	100 ml
7	10/10/2013	Ecovine® RP	1000 mg/L	10/10/2013	100 ml
8	10/10/2013	Ecovine® RP	1000 mg/L	10/10/2013	100 ml
9	10/10/2013	Ecovine® RP	1000 mg/L	10/10/2013	100 ml
10	10/10/2013	Ecovine® RP	1000 mg/L	10/10/2013	100 ml

1 - High vine (Pinel Noir); 2 - Phaeoannellaria chlamydospora; 3 - Neofusicoccum parvum

### Application conditions

Ecovine® RP was applied on the same day as pruning

Pruning and spray on shoots was done during favorable weather conditions (high humidity and low wind)



Date: February (2013, 2014)

### Inocula preparation

Leafy twigs of 10 years old P. chlamydospora and Neofusicoccum parvum were collected from the vineyard and subjected to sterile conditions to obtain a reliable suspension which was adjusted to the concentration of 10<sup>7</sup> spores



### Inoculation method

One shoot (10 cm) of each vine (200 shoots per vine) was cut and placed in a plastic bag (200 bags per vine) and subjected to sterilization

Neofusicoccum parvum spore suspension  
Phaeoannellaria chlamydospora spore suspension



The inoculated shoots were embedded with parafilm for 2 weeks after inoculation, which prevents the fungus to be washed

### Assessments

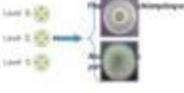
Incidence (total infected shoots)  
Severity (5 infection classes)



Five cm long leaf for pieces of internal wood were excised with PDA microtome/section

A total of 250 fragments of wood were analyzed

Cultures were identified according to the morphological features



## Results

### Phaeoannellaria chlamydospora

#### Incidence and severity for all treatments in Alentejo (2013) and in Estremoz (2014)

Treatments	Alentejo 2013		Estremoz 2014	
	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)
Water	30.0 a	37.0 a	43.0 ab	37.0 a
negative control				
Artificial inoculation Pch	60.0 b	50.0 a	37.0 ab	55.0 b
positive control				
Ecovine® RP + artificial inoculation Pch	33.0 a	34.0 a	33.0 a	37.0 ab

#### Severity in level 1, 2, 3 for all treatments



### Neofusicoccum parvum

#### Incidence and severity for all treatments in Alentejo (2013) and in Estremoz (2014)

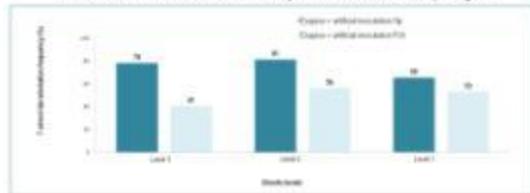
Treatments	Alentejo 2013		Estremoz 2014	
	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)
Water	47.0 a	35.0 a	37.0 a	23.0 a
negative control				
Artificial inoculation Np	55.0 a	47.0 a	33.0 a	49.0 b
positive control				
Ecovine® RP + artificial inoculation Np	33.0 a	34.0 a	33.0 a	34.0 a

#### Severity in level 1, 2, 3 for all treatments



### Trichoderma atroviride canes colonization

T. atroviride was reisolated from artificially infected canes with both pathogens



## Conclusions

The objective of the experiment was to assess the potential of Ecovine® RP BCA, under different environmental conditions, for the protection of pruning wounds against fungal pathogens, P. chlamydospora and N. parvum, being the effectiveness of the treatments evaluated through the ability to reduce artificial infections of pruned grapevine canes by these two fungi.

Results revealed that Ecovine® RP significantly reduced the incidence and severity of both pathogens (Phaeoannellaria chlamydospora and Neofusicoccum parvum).

Chemical fungicides have an immediate but short term effect in pathogens fungi. On the contrary, biofungicides, like Ecovine® RP, are able to colonize the wood along the canes and offer a longer protection to infection.

The best conditions for the wood colonization by pathogenic fungi (pathogens) are similar to those for the colonization of biofungicides, and therefore can prevent GTDs infection through pruning wounds and slow down colonization of canes.

Considering the importance of preventing wood pathogens infection through pruning wounds and slowing down colonization of wood fungi, we strongly believe that Ecovine® RP can be regarded as a potential BCA product for pruning wound protection against P. chlamydospora and N. parvum associated with the accurate cultural practices for a sustainable control of GTDs.

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# Ensaaios no campo Tessior



# Tessor

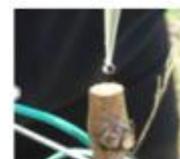
## Field part



1- Pruning



2- Treatment



3- Inoculation



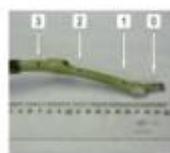
4- Incubation



## Laboratory part



1-Bark removal



2- Three-slice method



3- Petri-dish  
cultivation

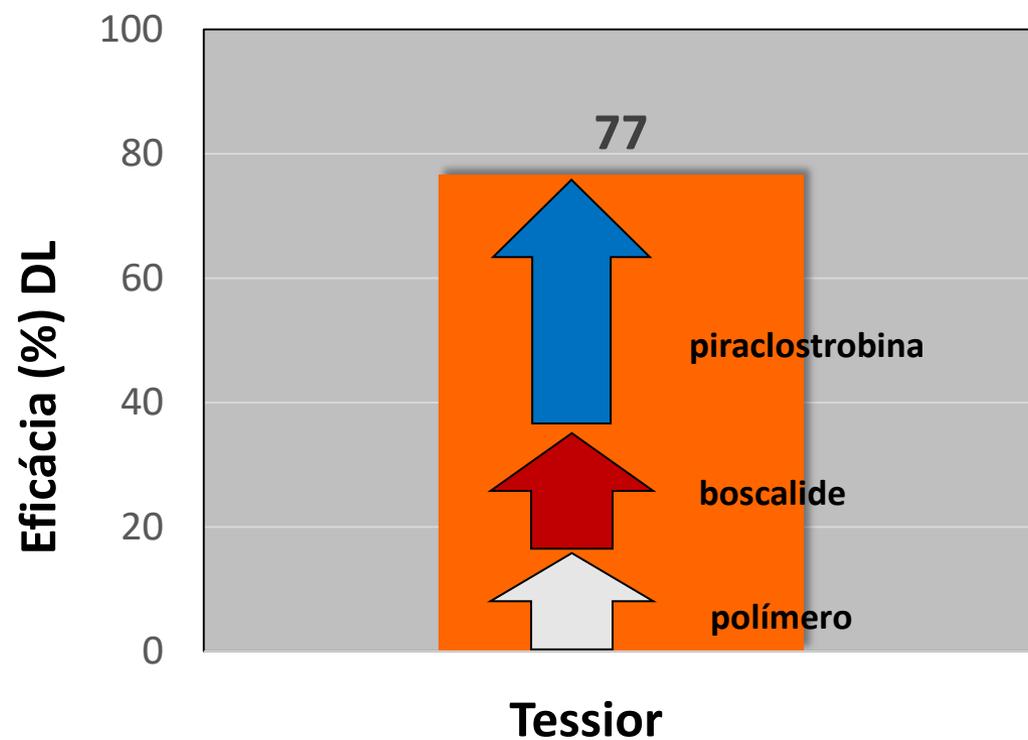


4- Assessment



**Ensaio de Campo durante 8 anos**

# Proteção das feridas de poda





Tessior



Proteção das feridas de poda



ESQUIVE ou VINTEC

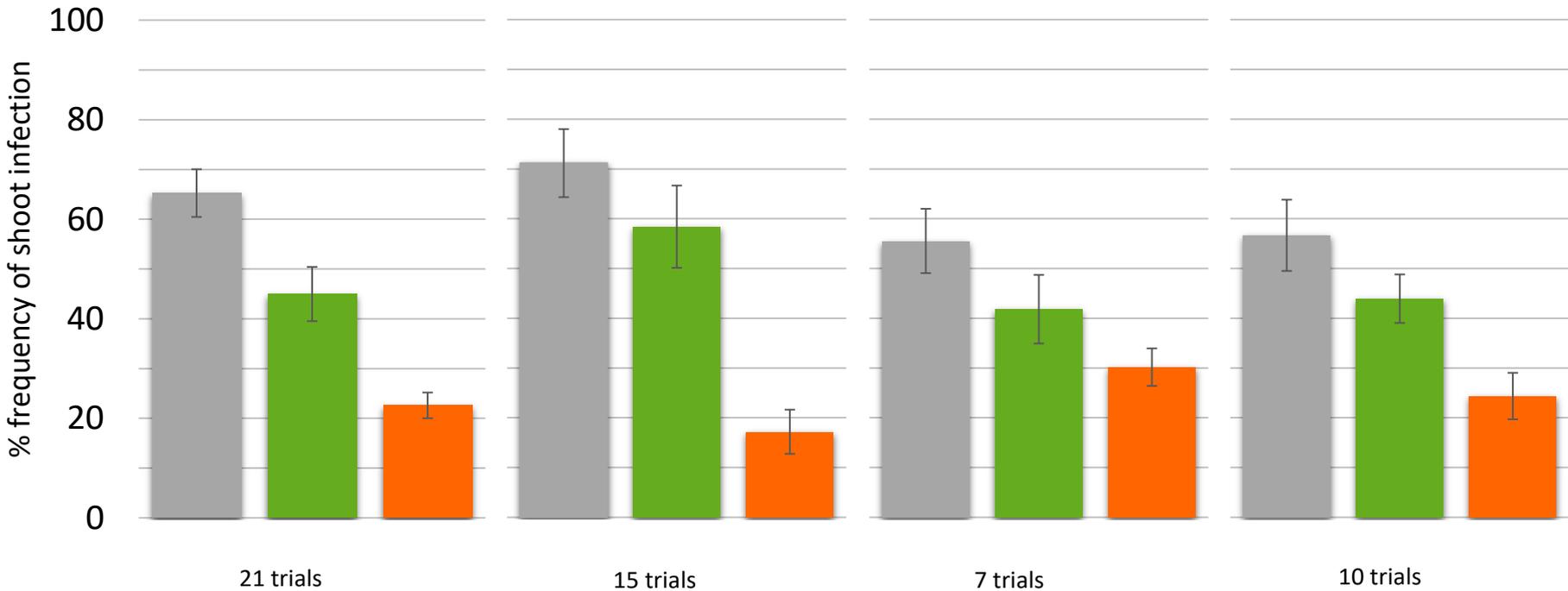


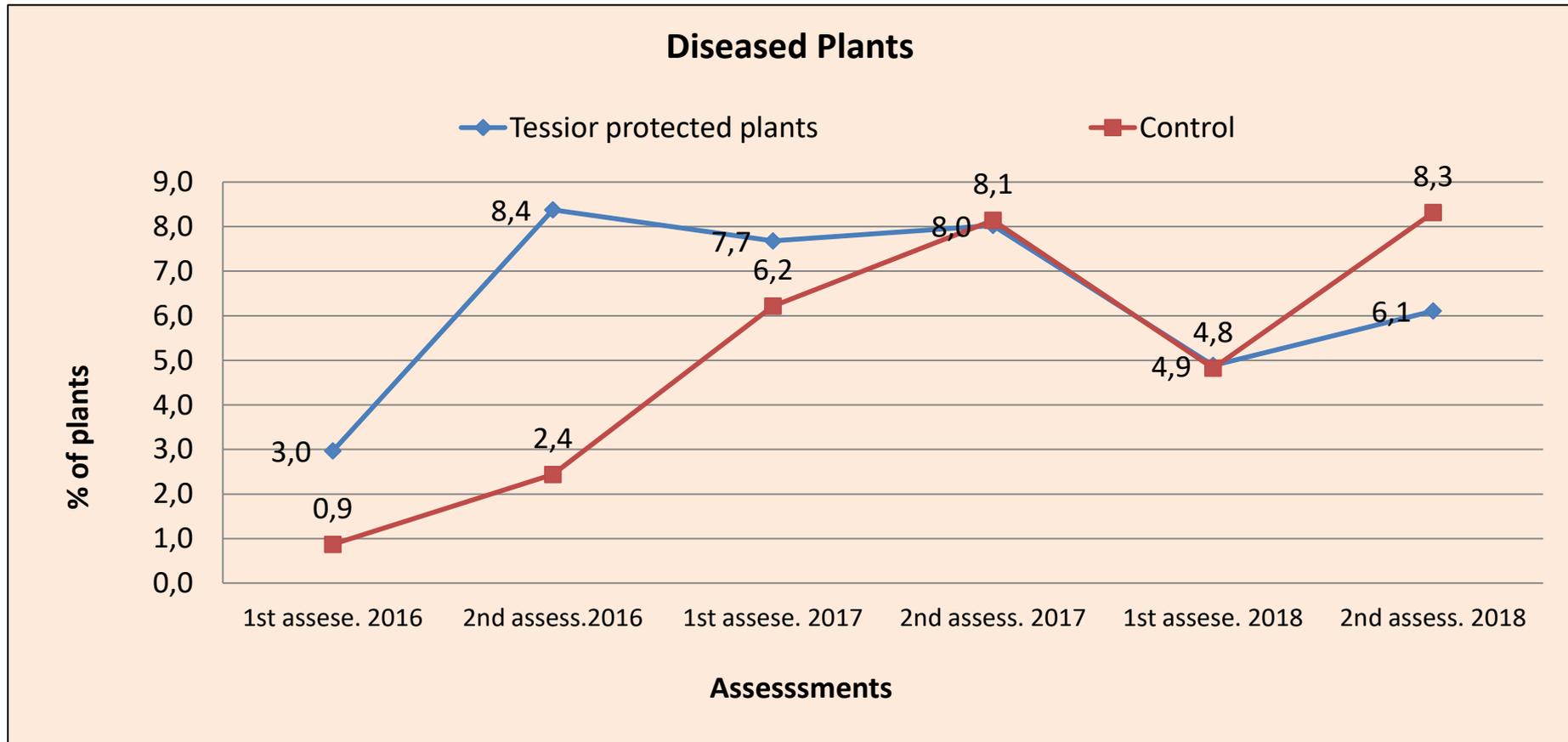
*Phaeomoniella  
chlamydospora*

*Botryosphaeria  
obtus*

*Phaeoacremonium  
aleophilum*

*Eutypa lata*





# CA3356 – Hidroxiapatite nano estruturada com compostos de cobre Cu(II) como pesticida

- Tentativa de redução da quantidade de substância activa aplicada.
- Aplicação de nano tecnologia permite:
  - sistemas mais lentos de libertação de pesticidas.
  - Baixa concentração do pesticida aplicado, que atinge as zonas “alvo” da planta.
  - Menor impacto eco-toxicológico

Início dos ensaios de campo!



# Ensaio preliminares

## Research Article

Received: 17 November 2017 | Revised: 1 February 2018 | Accepted article published: 19 February 2018 | Published online in Wiley Online Library: (wileyonlinelibrary.com) DOI 10.1002/ps.4892

## Functionalization of a nanostructured hydroxyapatite with Cu(II) compounds as a pesticide: *in situ* transmission electron microscopy and environmental scanning electron microscopy observations of treated *Vitis vinifera* L. leaves

Enrico Battiston,<sup>a\*</sup> Maria C Salvatici,<sup>b</sup> Alessandro Lavacchi,<sup>b</sup> Antonietta Gatti,<sup>c</sup> Stefano Di Marco<sup>d</sup> and Laura Mugnai<sup>a</sup>

### Abstract

**BACKGROUND:** The present study evaluated a biocompatible material for plant protection with the aim of reducing the amount of active substance applied. We used a synthetic hydroxyapatite (HA) that has been studied extensively as a consequence of its bioactivity and biocompatibility. An aggregation between HA nanoparticles and four Cu(II) compounds applied to *Vitis vinifera* L. leaves as a pesticide was studied. Formulations were characterized by X-ray diffraction (XRD), dynamic light scattering (DLS) and electron microscopy and applied *in planta* to verify particle aggregation and efficiency in controlling the pathogen *Plasmopara viticola*.

**RESULTS:** The XRD patterns showed different crystalline phases depend on the Cu(II) compound formulated with HA particles, DLS showed that nanostructured particles are stable as aggregates out of the nanometer range and, in all formulations, transmission electron microscopy (TEM) and environmental scanning electron microscopy (ESEM) microscopy showed large aggregates which were partially nanostructured and were recognized as stable in their micrometric dimensions. Such particles did not show phytotoxic effects after their application *in planta*.

**CONCLUSION:** A formulation based on HA and a soluble Cu(II) compound showed promising results in the control of the fungal pathogen, confirming the potential role of HA as an innovative delivery system of Cu(II) ions. The present work indicates the possibility of improving the biological activity of a bioactive substance by modifying its structure through an achievable formulation with a biocompatible material.  
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**Keywords:** pesticide; toxicology; hydroxyapatite; Cu; drug delivery

### 1 INTRODUCTION

Nanotechnological research has led to innovative solutions in many fields, including electronics (nano-sensors), medicine (drug release) and agriculture (nano-silver pesticides). Through the development of revolutionary smart materials, nanotechnology has provided solutions in the fields of agriculture and food sciences to improve plant productivity and crop quality, to produce food with nano-sized nutrients, and to develop new tools for molecular and cellular biology in these fields.

In agriculture, the nanotechnological approach has significant potential in the development of slow-release systems for pesticides.<sup>1,2</sup> This opportunity arises from several critical aspects, mainly linked to the very low concentration of the applied pesticide that generally reaches the plant target site and the consequent eco-toxicological impact of the remaining pesticide.

\* Correspondence to: E. Battiston, Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente - Sezione Patologia Vegetale ed Entomologia, Università degli Studi di Firenze, P. le delle Cascine 28, Firenze I-50144, Italy. E-mail: enrico.battiston@uni.fi

a Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente - Sezione Patologia Vegetale ed Entomologia, Università degli Studi di Firenze, Firenze, Italy

b Centro di Microscopia Elettroniche "Laura Bonzi" - Istituto di Chimica dei Composti Organici Metallici, Consiglio Nazionale delle Ricerche, Sesto Fiorentino, Italy

c Nanodiagnosics srl, Modena, Italy

d Istituto di Biometeorologia, Consiglio Nazionale delle Ricerche, Bologna, Italy

# Ensaio a decorrer no ISA

CA3356 Treatments	Concentration	Status	Date
Immediately after harvest	400l/ha	Done	13/11/2018
After pruning (Winter)	250l/ha	Done	19/02/2018
4 leaves	250l/ha	Done	02/04/2019
Summer pruning	400l/ha		
Veraison	400l/ha		

## Ensaio Fungicidas - Lasiodiplodia

**Responsável:** Cecília Rego e Pedro Reis

**Castas:** Cabernet e Touriga Nacional

**Sistema de condução:** Cordão duplo

**Linhas:** Touriga Nacional - 13 a 19 (inclusive)

Cabernet Sauvignon - 6 e 13 a 19

Treatment	Product	Inoculation	Spore solution volume	Color
1	Trichoderma + CA3356	Water (control)	-	White+Green
2	Trichoderma + CA3356	L. theobromae (PT)	40µl	White+Red
3	Trichoderma + CA3356	L. theobromae (CL)	40µl	White+Blue
4	Trichoderma + CA3356	L. mediterranea (CBS)	40µl	White+Gray
5	BASF 516 17F + CA3356	Water (control)	-	Black+White
6	BASF 516 17F + CA3356	L. theobromae (PT)	40µl	Black+Red
7	BASF 516 17F + CA3356	L. theobromae (CL)	40µl	Black+Blue
8	BASF 516 17F + CA3356	L. mediterranea (CBS)	40µl	Black+Gray
9	BASF 516 17 F	Water (control)	-	Black
10	BASF 516 17 F	L. theobromae (PT)	40µl	Red
11	BASF 516 17 F	L. theobromae (CL)	40µl	Blue
12	BASF 516 17 F	L. mediterranea (CBS)	40µl	Gray
13	Trichoderma	Water (control)	-	Green&Yellow
14	Trichoderma	L. theobromae (PT)	40µl	Green&Yellow+Red
15	Trichoderma	L. theobromae (CL)	40µl	Green&Yellow+Yellow
16	Trichoderma	L. mediterranea (CBS)	40µl	Green&Yellow+Gray
17	Inoculated non treated Control	L. theobromae (PT)	40µl	Orange
18	Inoculated non treated Control	L. theobromae (CL)	40µl	Green
19	Inoculated non treated Control	L. mediterranea (CBS)	40µl	Yellow

**Próximo passo:** Análise molecular do impacto dos produtos sobre a reacção da planta à infecção com os fungos em estudo, por real-time PCR.

## DDAV

[http://www.dgav.pt/fitofarmaceuticos/guia/finalidades\\_guia/Insec&Fung/Culturas/videira.htm](http://www.dgav.pt/fitofarmaceuticos/guia/finalidades_guia/Insec&Fung/Culturas/videira.htm)

DOENÇA	SUBSTÂNCIA ACTIVA	FORM	CONCENTRAÇÃO (g sa /hl)	IS dias
Doenças do lenho:	<a href="#"><u>boscalide+piraclostrobina</u></a>	SD **	20L/ha	-
Escoriose Europeia*/BDA*, doença de Petri, Esca , Eutipiose	<a href="#"><u>difenoconazol (111)</u></a>	<a href="#"><u>EC</u></a>	<a href="#"><u>25 g s.a./ha</u></a>	<a href="#"><u>21</u></a>
	<a href="#"><u>trichoderma atroviride SC1 (159)</u></a>	WG ***	<a href="#"><u>30 g s.a./ha</u></a>	-
	<a href="#"><u>trichoderma atroviride I-1237</u></a>	WP	(98)	1
	<a href="#"><u>trichoderma asperellum+trichoderma gamsil</u></a>	WP	20+20g s.a./ha	-

\*. Botriosferiose

\*\* . Aplicação direta nas feridas de poda.

\*\*\*. Durante o processo de viveiro, aplicação por imersão de porta enxertos e plantas já enxertadas.





## Mario de la Fuente

*Head of unit "viticulture"* International Organisation of Vine and Wine



“Nursey protocol for  
managing GTD”

**PROTEC meeting  
in April 2018**

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Mondello et al. 2017. **Grapevine trunk diseases: a review of fifteen years of trials for their control with chemicals and biocontrol agents.**

<https://doi.org/10.1094/PDIS-08-17-1181-FE>



# Obrigada

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**Pedro Reis & Cecília Rego**