Instituto de Ciencias de la Vid y del Vino

Gobierno de La Rioja

CSIC

Universidad de La Rioja

ARC • LNR

Excellence in Research and Development

Grapevine trunk diseases: from nurseries to vineyards

David Gramaje and Francois Halleen

Economic impact

Australia: losses of 1,500 kg/ha in 47% Syrah due to Eutypa dieback (**AU$ 2,800/ha**) (Wicks and Davies, 1999)

California: Botryosphaeria and Eutypa diebacks (**$USD 260 M/year**) (Siebert, 2001)

France: 12% of affected vineyards (**1 billion €/year**) (Lorch, 2014)

Replant: Tempranillo in La Rioja

Plant cost: 1.5 €
Annual maintenance: 0.3 €
Cost of plantation: 2.8 €

Total cost per plant: 4.6 €

PLANT DENSITY: 3,000 plants/ha

1% (GTD)

Total cost of replant: 138 € / ha

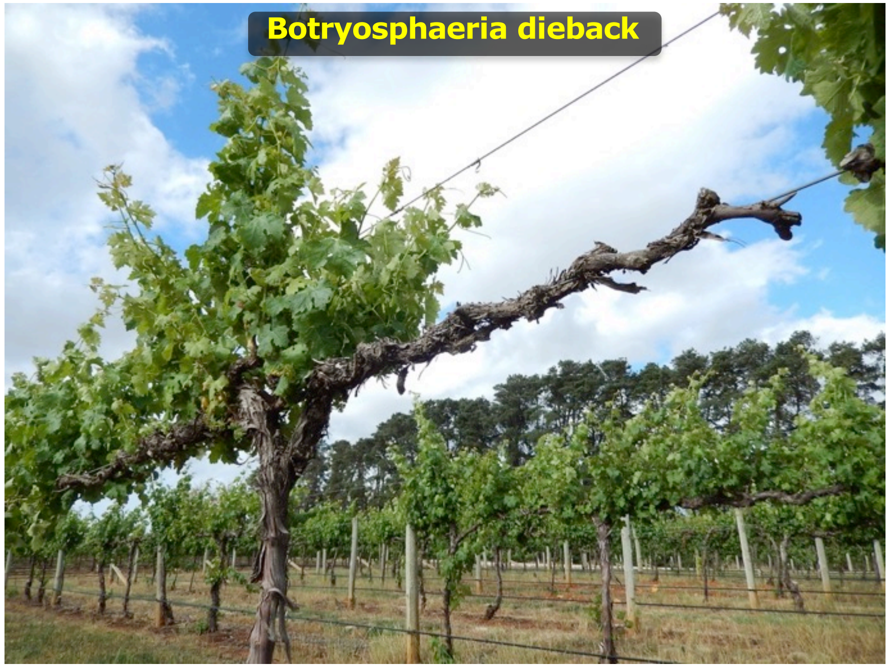
51,896 ha

7.16 M € /year

+ losses in wine production until full yield

Martínez-Diz et al. (2019) Sci. Hortic. 246:104-109

Fungal grapevine trunk diseases: external symptoms



Fungal grapevine trunk diseases: internal symptoms

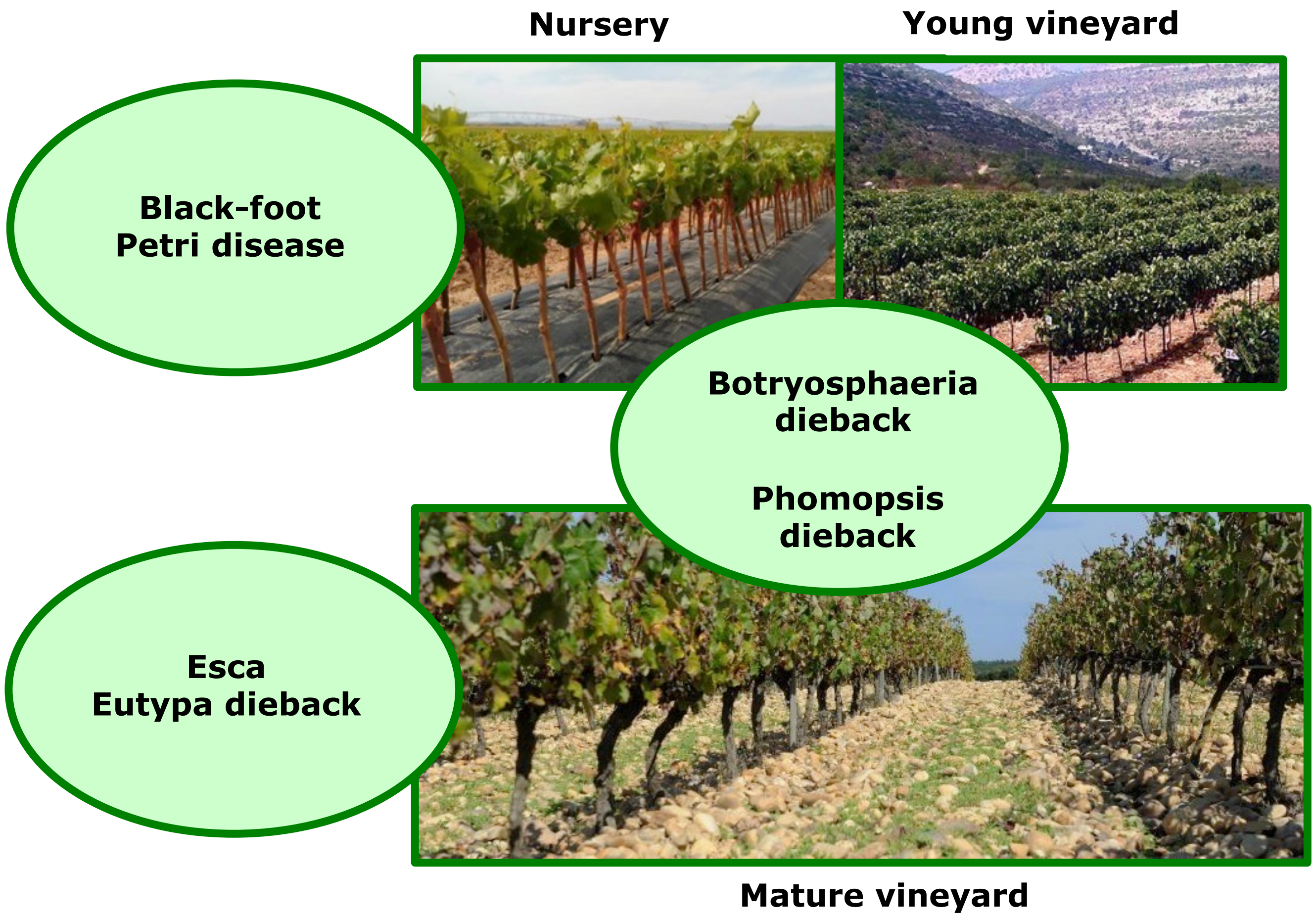





GRAPEVINE TRUNK DISEASES (GTD)

Complexity of this pathosystem

1. **Many fungal species associated with GTDs symptoms**
   135 species – 35 genera



GRAPEVINE TRUNK DISEASES (GTD)

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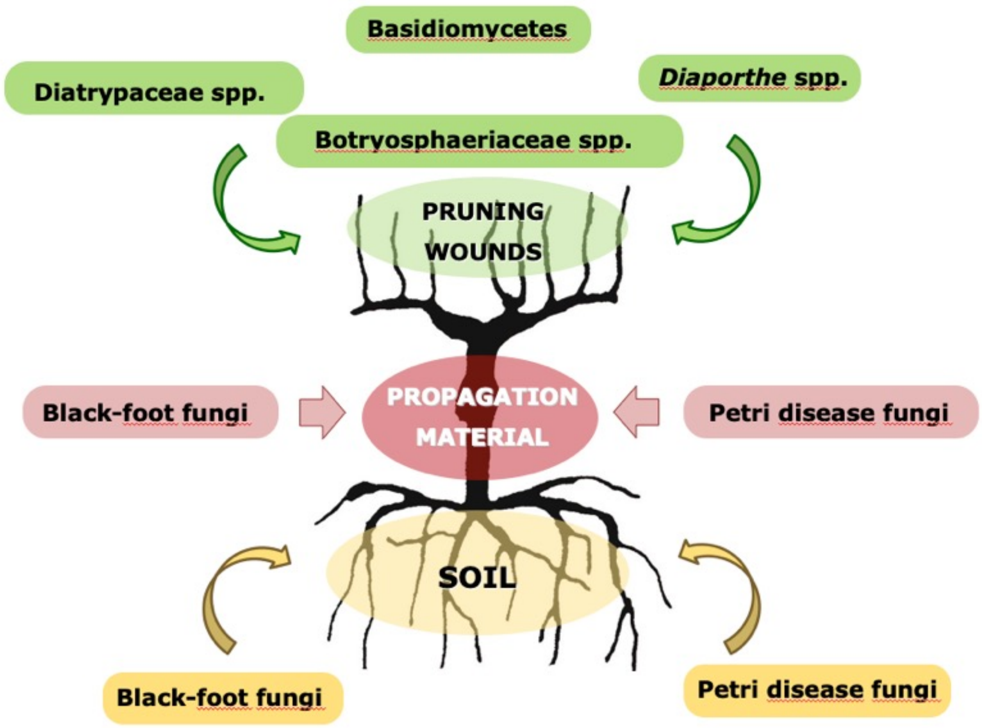
2. **Fungi with different biology and epidemiology**



GRAPEVINE TRUNK DISEASES (GTD)

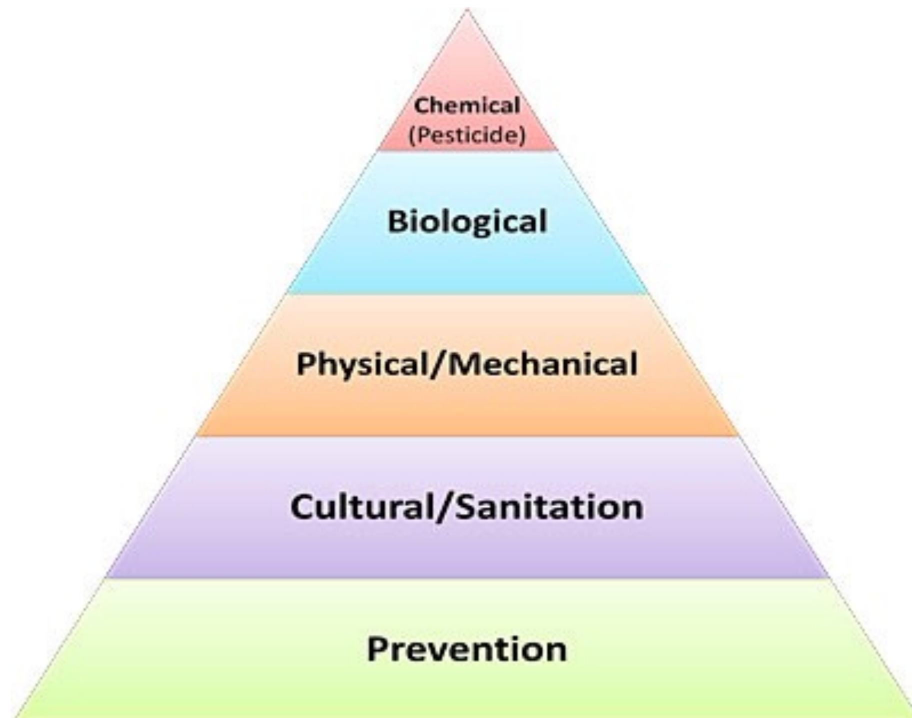
Complexity of this pathosystem

1. **Many fungal species associated with GTDs symptoms**
   135 species – 35 genera

2. **Fungi with different biology and epidemiology**

3. **No curative measures are known for control of GTD**

INTEGRATED DISEASE MANAGEMENT STRATEGY



Integrated management strategy

Nursery mother blocks

Propagation processes

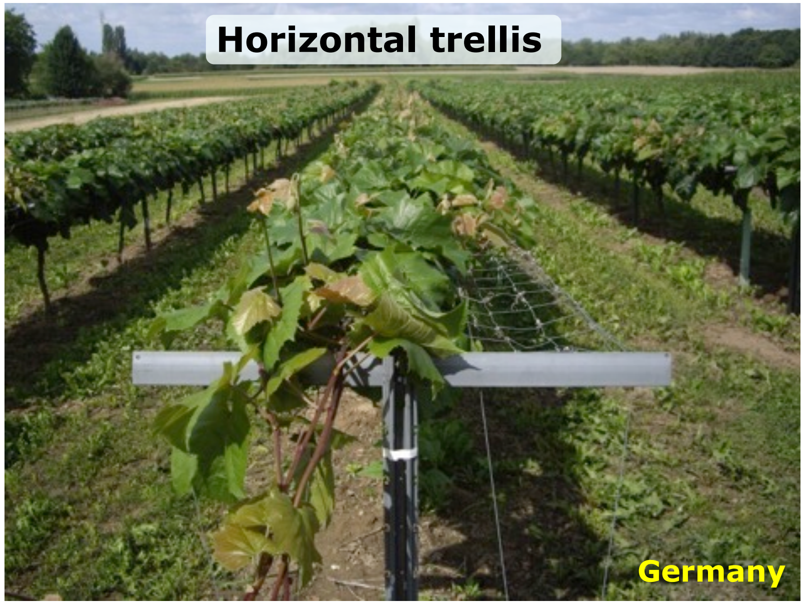
Nursery field

Newly established vineyards

Mature vineyards

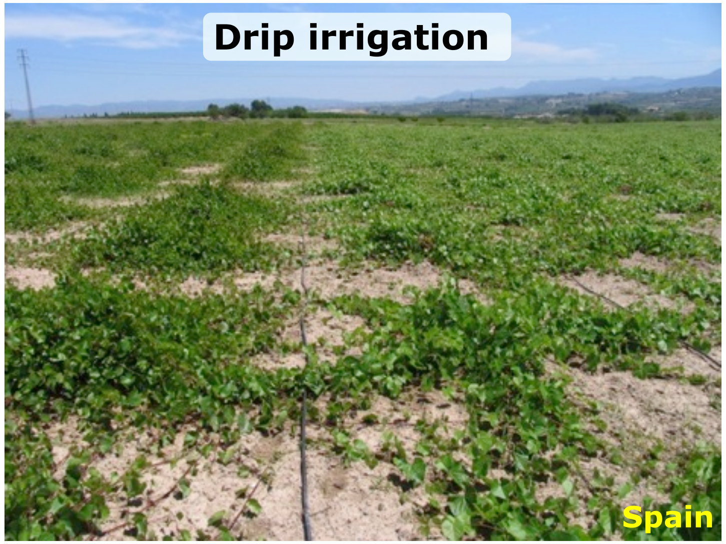
Mother plants

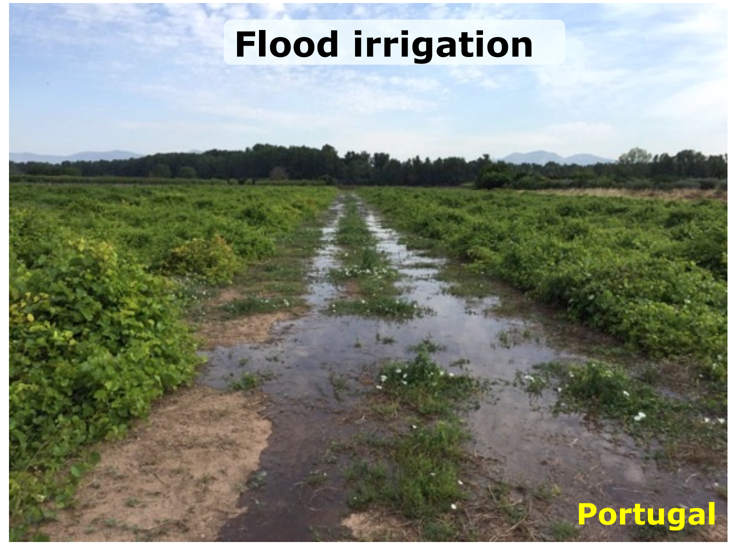
Cultural practices and sanitation: rootstock mother vine cultivation

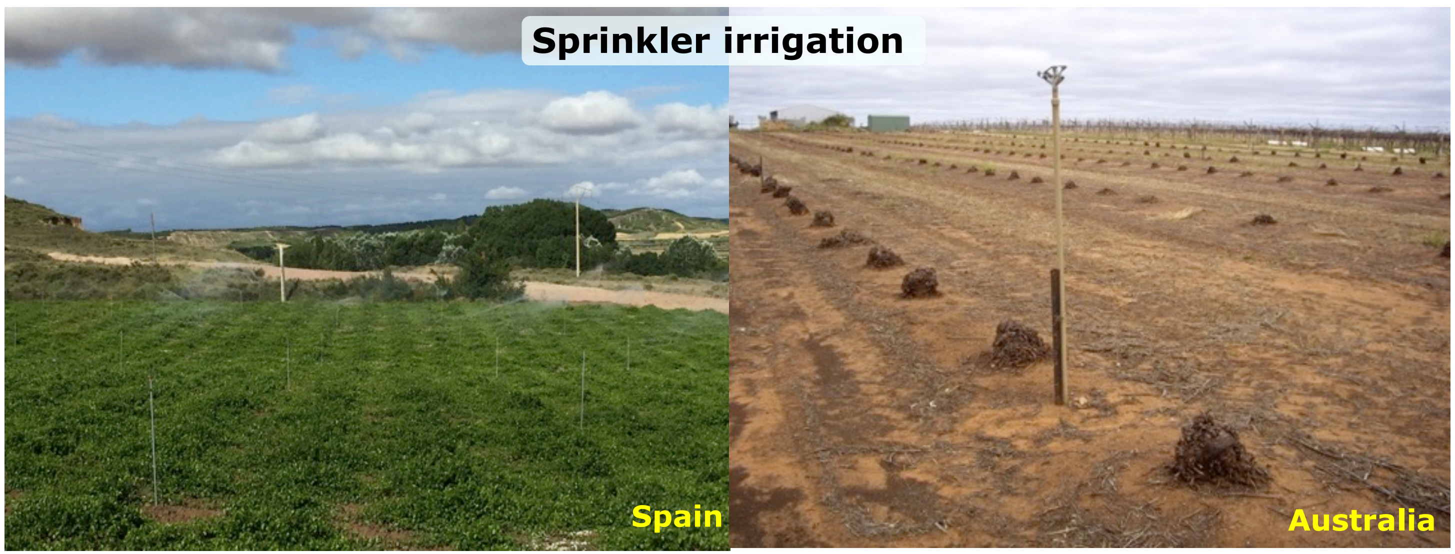


Mother plants

Cultural practices and sanitation: irrigation





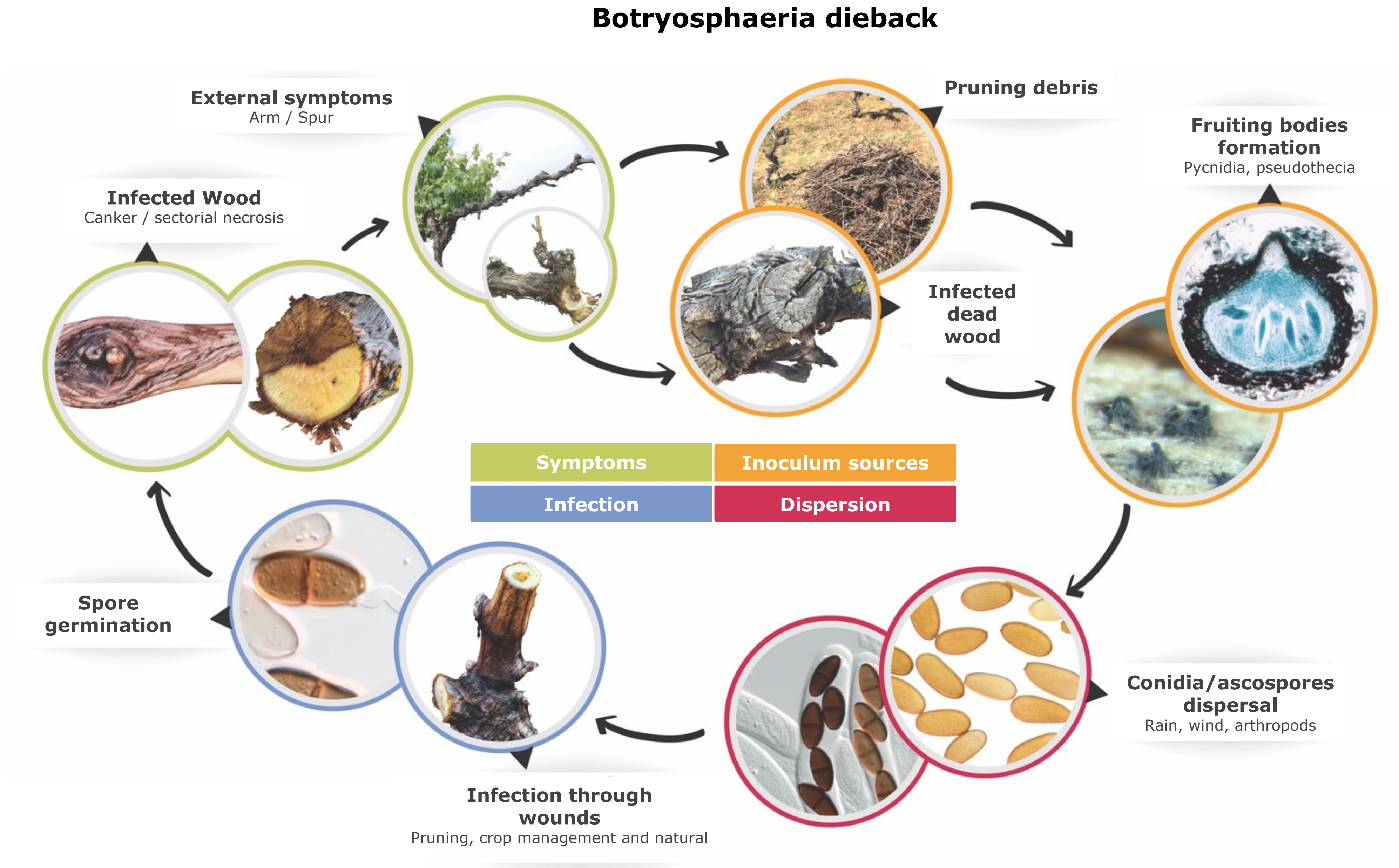


Botryosphaeriaceae spp.
Úrbez-Torres et al. 2010

***Phaeoacremonium* spp.**
Gubler et al. 2013

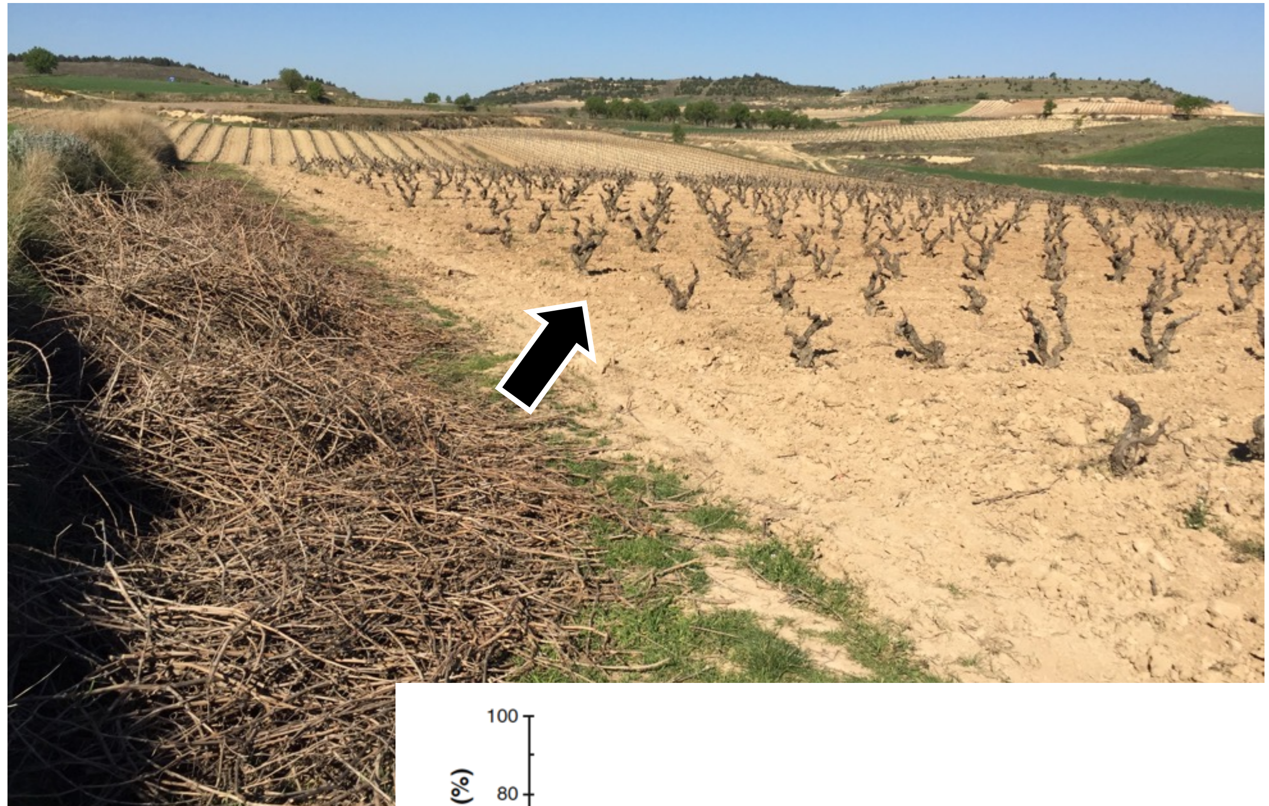
Diatrypaceae spp.
Úrbez-Torres et al. 2019

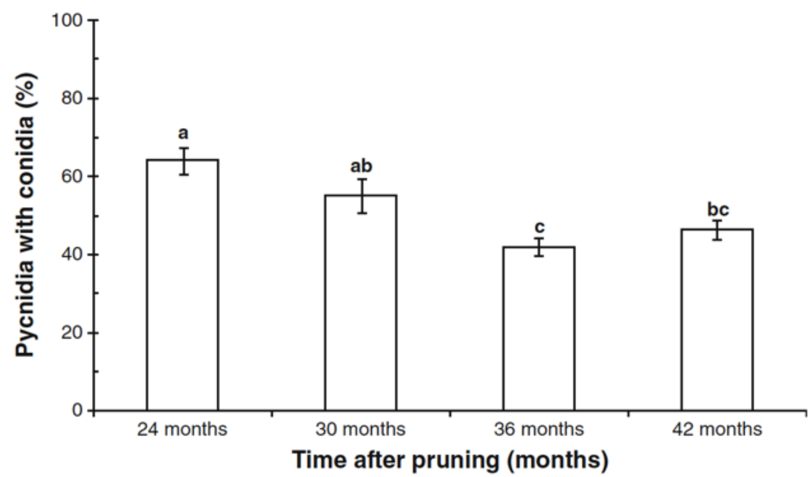
Epidemiology. Disease cycle



Mother plants & mature vineyards

Cultural practices and sanitation: removal of dead wood or pruning debris





Elena and Luque 2016 Eur. J. Plant Pathol

Mother plants & mature vineyards

Mother plants & mature vineyards

Cultural practices and sanitation: removal of dead wood or pruning debris

BURNING

GRIND AND COVER

500 µm

10 µm

MULCHING

COMPOSTING

140 m³ of plant material (pruning debris)
+
125 m³ of sheep manure
60 m³ of garden residues

Year 2

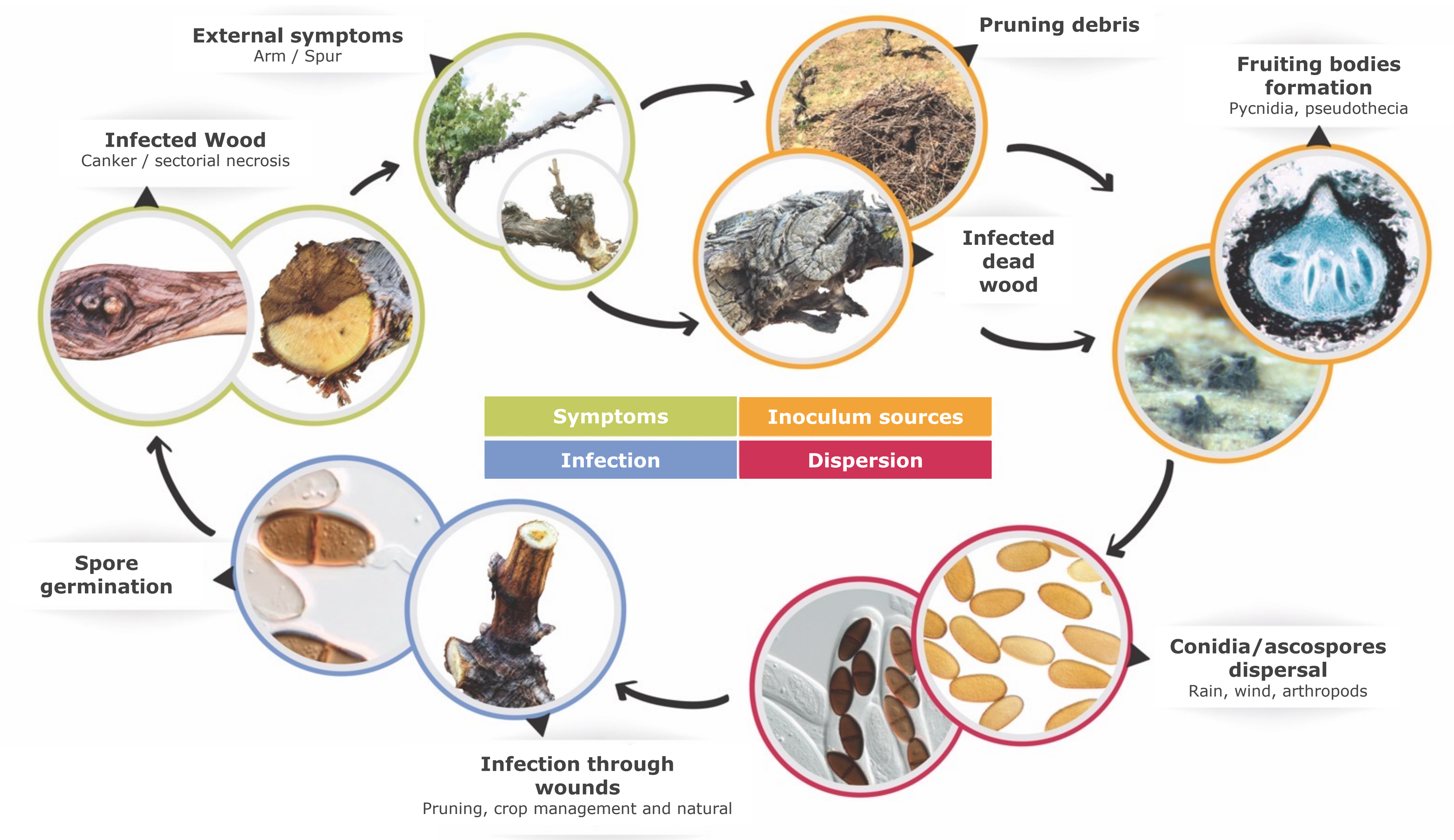
40-50 ºC (75ºC)

Botryosphaeriaceae spp. (60 % - 0%)
***P. chlamydospora* (93% - 0%)**
***P. minimum* (50% - 0%)**

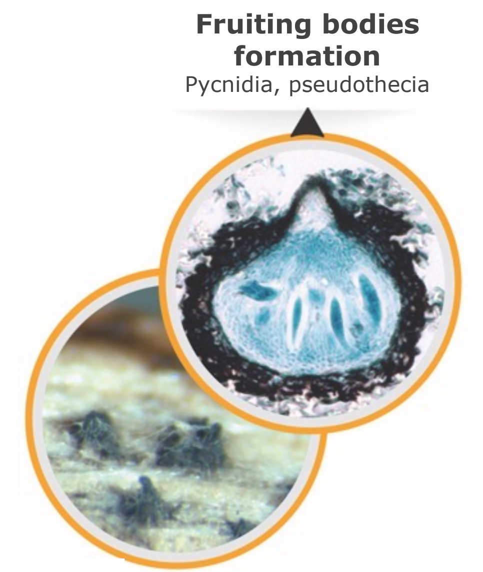
Lecomte et al. 2006 Phytopathol. Mediterr 45

Epidemiology. Disease cycle

Botryosphaeria dieback



Epidemiology. Alternative woody hosts



Fungal Ecology

Diatrypaceae species overlap between vineyards and natural ecosystems in South Africa

P. Moyo [a], L. Mostert [a], F. Halleen [a, b, *]

2019

Plant Pathology

Fungal trunk diseases: a problem beyond grapevines?

D. Gramaje[a*], K. Baumgartner[b], F. Halleen[cd], L. Mostert[d], M. R. Sosnowski[e], J. R. Úrbez-Torres[f] and J. Armengol[g]

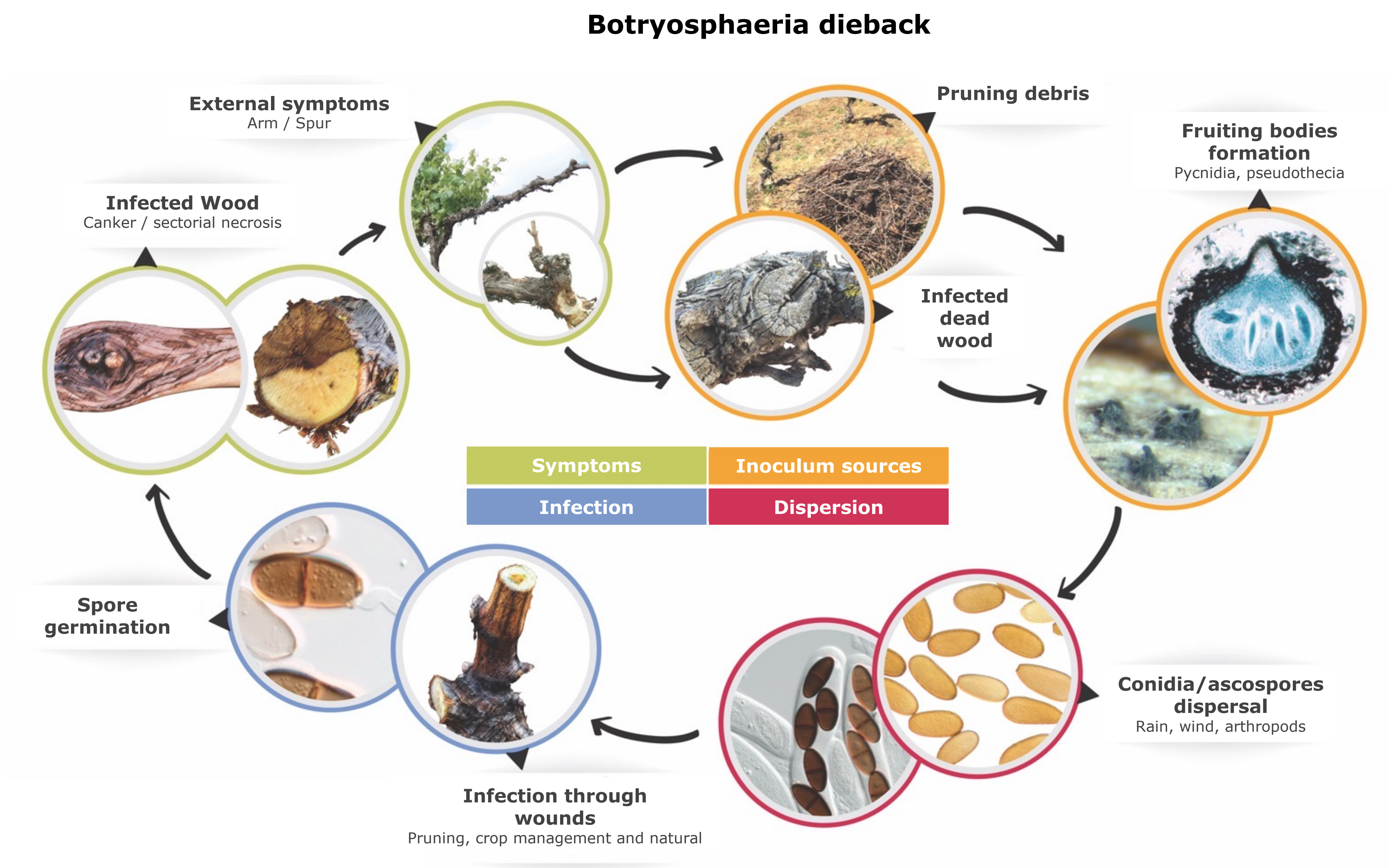
2016

Almond

Pear

Walnut

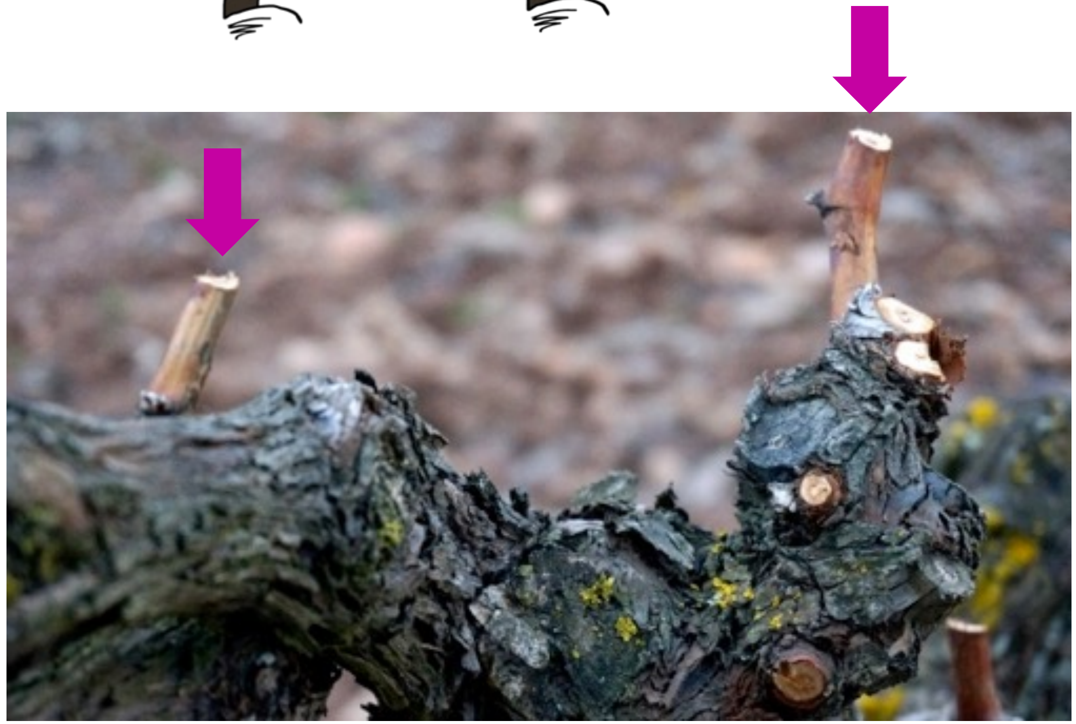
Epidemiology. Disease cycle



Winter pruning

Diatrypaceae spp.
Botryosphaeriaceae spp.
Basidiomycetes
Phaeomoniella chlamydospora
***Phaeoacremonium* spp.**

Mother vine



Mature vine

Pruning wound protection: chemical treatments

Mastic/paste + fungicides

Benzimidazole carbamate mode of action group (benomyl, carbendazim & thiophanate methyl)

Eutypa dieback
Australia (Sosnowski et al. 2008 Aus. J. Grape Wine Res. 14)
USA (Rolshausen et al. 2010 Am. J. Enol. Vitic. 61)

Botryosphaeria dieback
South Africa (Halleen et al. 2010)
Chile (Díaz and Latorre 2013 Crop Prot. 46)
USA (Rolshausen et al. 2010 Am. J. Enol. Vitic. 61)

Esca
Chile (Díaz and Latorre 2013 Crop Prot. 46)
South Africa (Mutawila et al. 2015 BioControl 60)

The demethylation inhibitors (tebuconazole & flusilazole)

Eutypa dieback
Australia (Ayres et al. 2017 Aus. J. Grape Wine Res. 23)
South Africa (Halleen et al. 2010 S. Afr. J. Enol. Vitic. 31)

Botryosphaeria dieback
Chile (Díaz and Latorre 2013 Crop Prot. 46)
New Zealand (Amponsah et al. 2012 Pest. Manag. Sci. 68)
Australia (Pitt et al. 2012 Plant Dis. 96)
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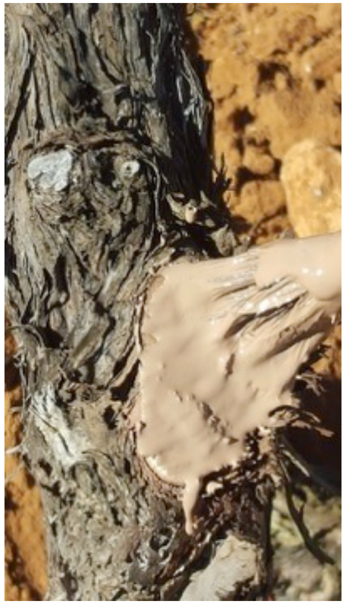

Mastic or paste

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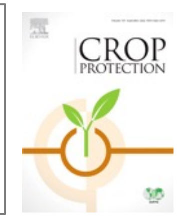
pyraclostrobin + boscalid
with or without a liquid polymer

Protection of grapevine pruning wounds against *Phaeomoniella chlamydospora* and *Diplodia seriata* by commercial biological and chemical methods

María del Pilar Martínez-Diz [a,b], Emilia Díaz-Losada [a], Ángela Díaz-Fernández [a], Yolanda Bouzas-Cid [a], David Gramaje [c,*]



Pruning-wound protectants for trunk-disease management in California table grapes

Albre A. Brown [a], Renaud Travadon [a], Daniel P. Lawrence [a], Gabriel Torres [b], George Zhuang [c], Kendra Baumgartner [d,*]

The demethylation inhibitors (tebuconazole & flusilazole)

Eutypa dieback
Australia (Ayres et al. 2017 Aus. J. Grape Wine Res. 23)
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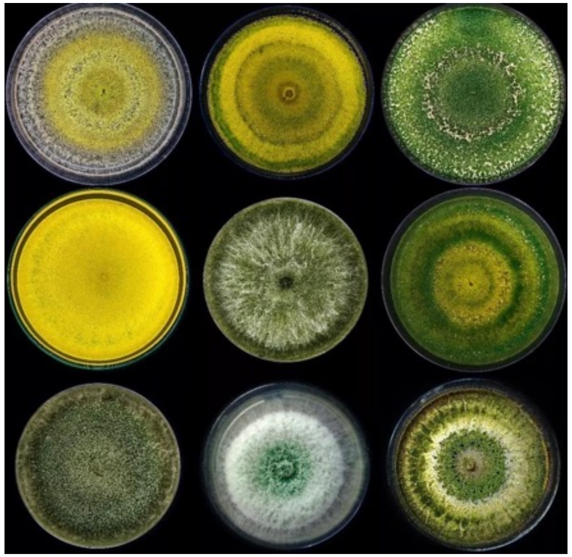
South Africa (Halleen et al. 2010 S. Afr. J. Enol. Vitic. 31)

Restrictions and difficulties that chemicals are facing in most countries around the world

Pruning wound protection: *Trichoderma* spp.

ALTERNATIVE: Biological Control Agents (BCA)

***Trichoderma* spp.**



MECHANISMS OF ACTION
Mycoparasitism
Secretion of secondary metabolites
Competition for nutrients and space

In general, BCA have shown variable results for preventing infection by GTD pathogens

Grape growing region (climatic conditions, genotype)
Experimental design: artificial vs natural infection

***Trichoderma asperellum* ICC 012 + *T. gamsii* ICC 080**

ARTIFICIAL PATHOGEN INFECTION

Italy (Di Marco *et al*. 2022)

***Trichoderma* strains reduced *Phaeomoniella chlamydospora* colonization and esca incidence from 66 to almost 90%**

California (Blundell and Eskalen, 2022)

***Trichoderma*-based biological fungicides performed the best against two fungal pathogens evaluated**

NATURAL PATHOGEN INFECTION

Spain and France (Bujanda *et al*. 2022)

***Trichoderma* spp. yielded inconsistent outcomes, which were contingent on the year, location, and the specific disease in question**

Pruning wound protection: *Trichoderma* spp.

ALTERNATIVE: Biological Control Agents (BCA)

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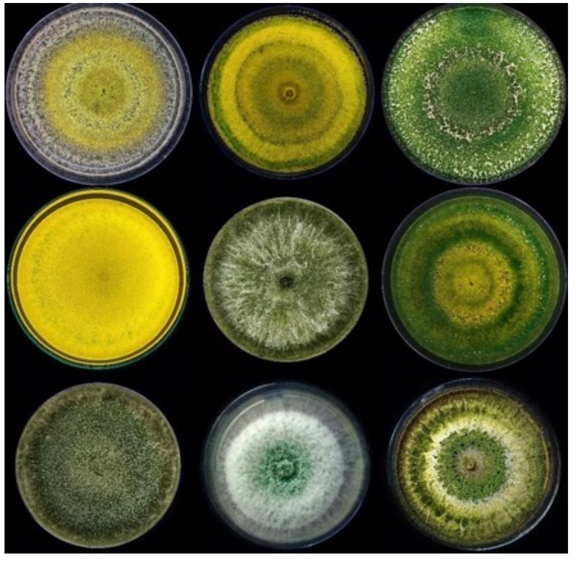

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Experimental design: artificial vs natural infection

***Trichoderma atroviride* I-1237**

ARTIFICIAL PATHOGEN INFECTION

Galicia, Spain (Martínez-Diz *et al*. 2021)

***Ta. atroviride* I-1237 did not reduce infection by *D. seriata* compared to the untreated inoculated control**

NATURAL PATHOGEN INFECTION

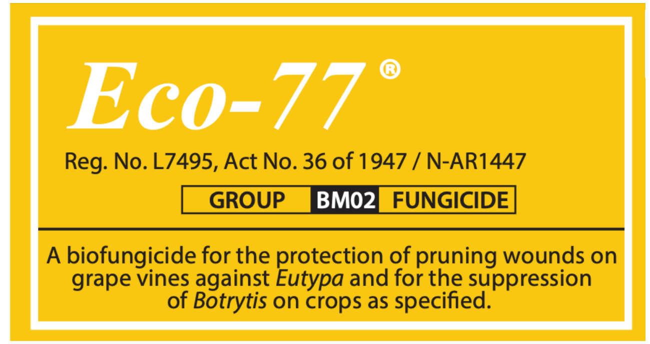
La Rioja, Spain (Bujanda *et al*. 2022)

***Ta. atroviride* I-1237 was the most effective treatment against Botryosphaeriaceae spp. among several physical, chemical, biological strategies**

Pruning wound protection: *Trichoderma* spp.

SOUTH AFRICA

Steriseal: 8-Hydroxyquinoline sulphate
Trichoderma atroviride* T-77 (Eco-77®): *Eutypa lata



Andermatt Madumbi

SOUTH AFRICA: Halleen et al. 2010 S. Afr. J. Enol. Vitic. 31

ARTIFICIAL PATHOGEN INFECTION

The fungicides benomyl and flusilazole were the most effective treatments, although the *Trichoderma* treatment T77 caused a significant reduction in *E. lata* infection.

NATURAL PATHOGEN INFECTION

Eco77 not only reduced *E. lata*, but they also reduced the incidence of other grapevine trunk disease pathogens.

CANADA: Pollard-Flamand et al. 2023 S. Am. J. Enol. Vitic. 74

ARTIFICIAL PATHOGEN INFECTION

Eco77 provided effective pruning wound protection against Botryosphaeriaceae spp. for up to 60 days

Pruning wound protection: cost of treatments

Spraying

Fungicides

Biological control agents

US$ 87/ha

Manual panting

Mastic/paste with or without fungicides

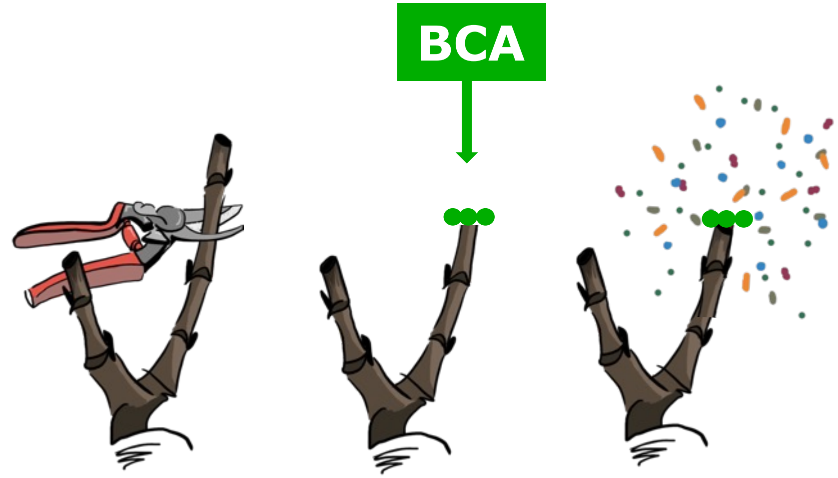
US$ 166/ha: guyot

US$ 333/ha: double cordon

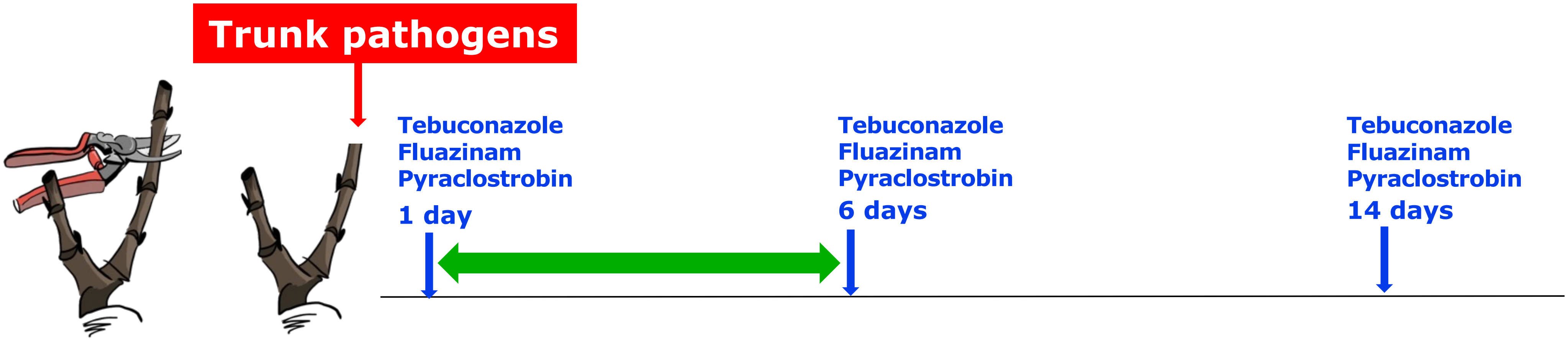
Sosnowski and McCarthy (2017) Wine Vitic J 32:42-48

When should we apply the preventive treatment?

Biological control agent (*Trichoderma* spp.)
Immediately after pruning



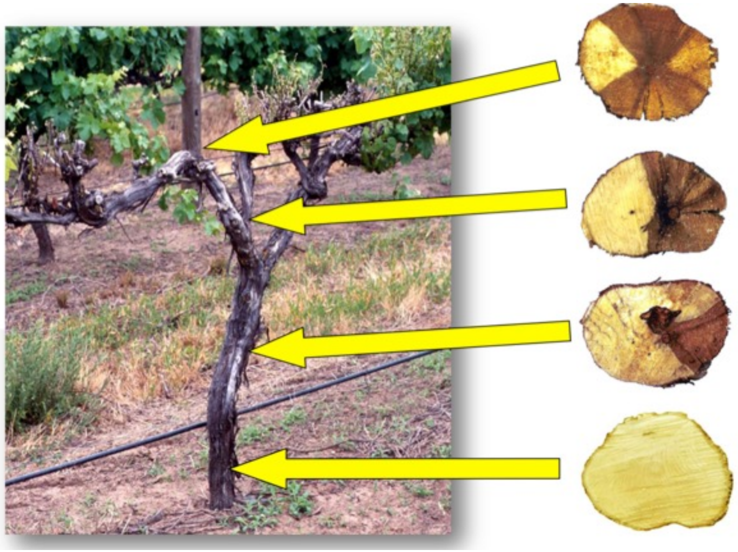
Chemical control: fungicides



- ✓ **Chemical product: similar efficacy <u>up to 6 days after pruning</u>**
- ✓ **Flexibility for the grower**

Ayres et al. 2021 Aus. J. Grape Wine Res. 21

Trunk renewal. Remedial surgery



Mark Sosnowski, SARDI, Australia

Trunk renewal. Remedial surgery

Calzarano et al. (2004) Phytopathol. Mediterr. 43

ESCA DISEASED VINES

- trunk renewal does not excise all the necrotic wood
- necrosis was still visible on the cut trunk surface of all trunk- renewed vines

Integrated management strategy

Nursery mother blocks

Propagation processes

Nursery field

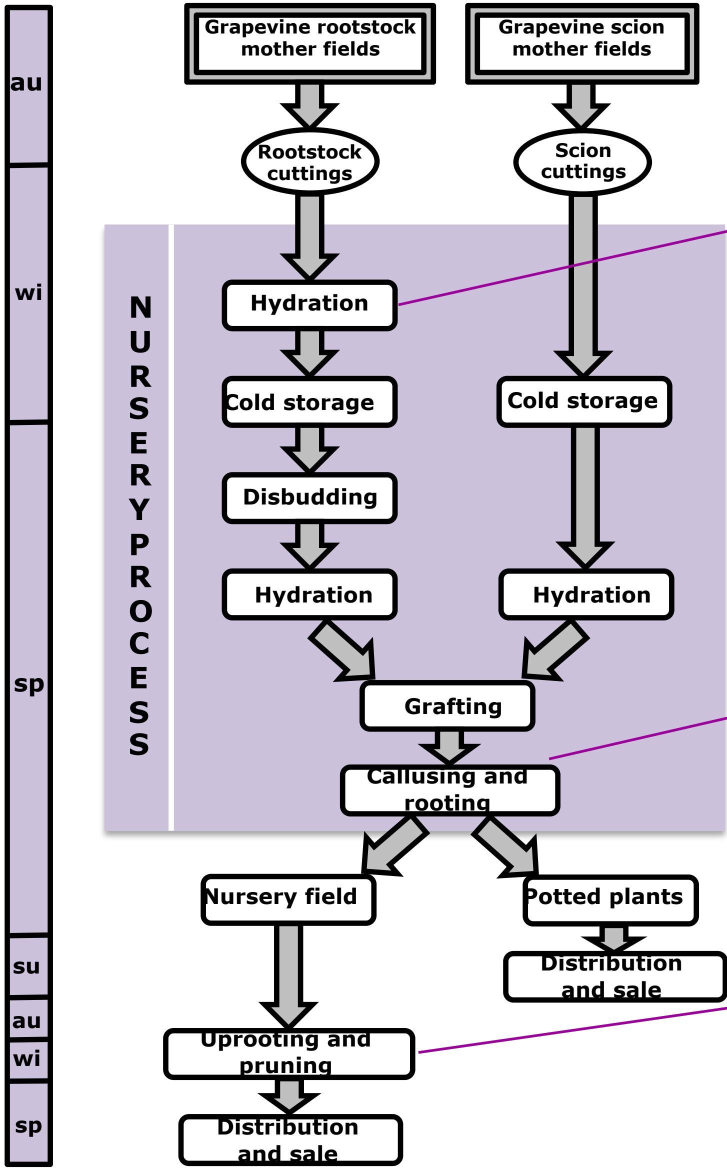
Newly established vineyards

Mature vineyards

NURSERIES ARE SOURCES OF DISEASED MATERIAL

1 Nurseries are favorable for fungal trunk pathogens

- ✓ Wet and humid conditions
- ✓ High root density
- ✓ Close spacing of plants



Hydration tanks

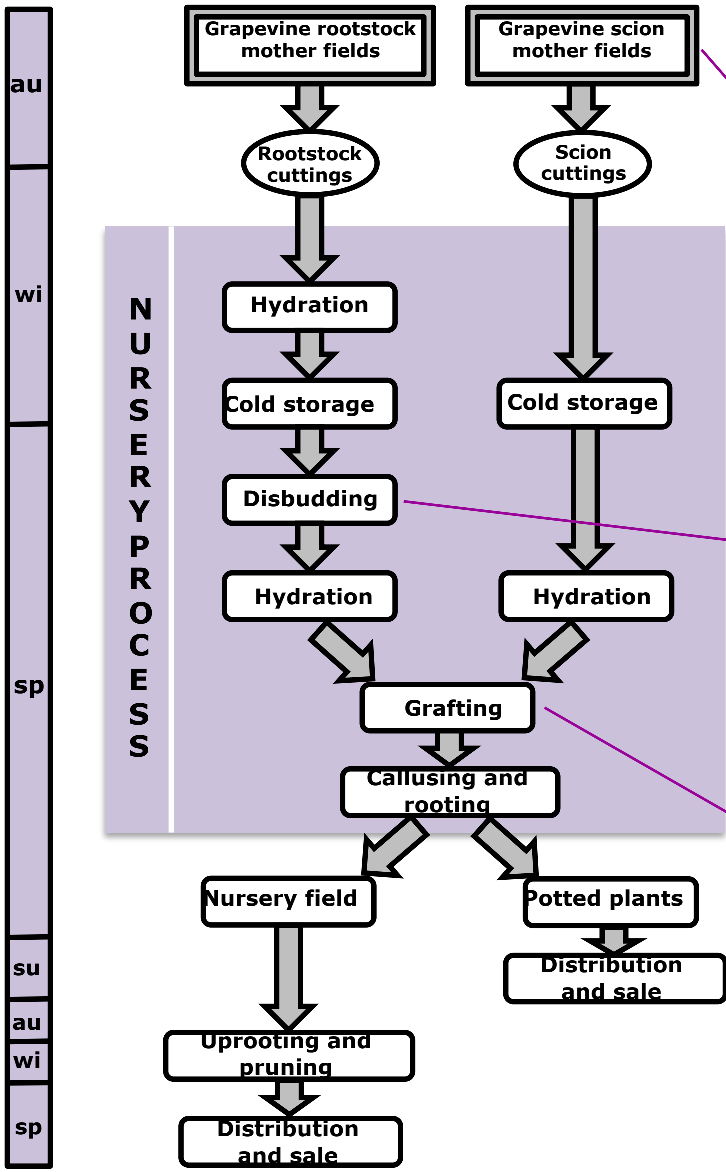
Callusing

Rooting

Gramaje and Armengol 2011. Plant Disease 95

NURSERIES ARE SOURCES OF DISEASED MATERIAL

2 Practices increase infection risk



Pruning wounds

Disbudding

Grafting

Gramaje and Armengol 2011. Plant Disease 95

NURSERIES ARE SOURCES OF DISEASED MATERIAL

3 Diseased plants are difficult to detect

External symptomless plants

Latent pathogens: asymptomatic tissues

Pathogenic: biotic and/or abiotic stress factors

Destructive sampling

Hrycan et al. 2020 Phytopath. Mediterr. 59

THE SOLUTION: CLEAN PLANT PRODUCTION

START CLEAN, KEEP IT CLEAN

- ✓ **Use pathogen-free plant material**
- ✓ **Clean grafting machines frequently**
- ✓ **Maintain a high standard of general cleanliness in the nursery, particularly the grafting room, callusing room and cool room**
- ✓ **Never reuse callusing media**
- ✓ **Avoid soaking cuttings**

Propagation processes in the nursery

CHEMICAL CONTROL

Sporekill®

Agricultural disinfectant / plant sanitizer
Active ingredient: Didecyldimethylammonium Chloride

SOUTH AFRICA: Fourie and Halleen 2006 Eur. J. Plant Pathol. 116
SPAIN: Gramaje et al. 2009 Crop Protec. 28

Propagation processes in the nursery

CHEMICAL CONTROL

BIOLOGICAL CONTROL

Trichoderma atroviride **SC1 (Vintec®)**

Pertot et al. 2016. BioControl 61
Berbegal et al. 2019. Pest Manag. Sci.

HOT-WATER TREATMENT (HWT)

Standard treatment: 50ºC – 30 min.
Several pests and diseases: Phytoplasma organisms.

1. **Some *Vitis vinifera* varieties are more sensitive to HWT than others** (Waite et al. 2001)
2. **Tolerance of plants to HWT is affected by the climate in which the cuttings are grown**
3. **HWT is not completely effective in eliminating fungal trunk disease pathogens growth**

Propagation processes in the nursery

USE OF RESISTANT ROOTSTOCKS

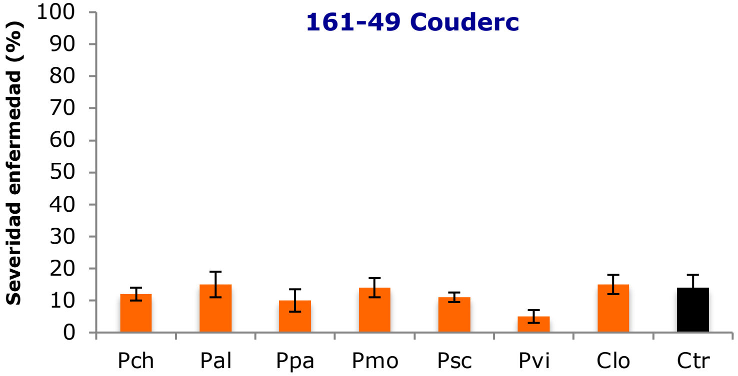
New Zealand
Jaspers et al. 2007; Billones-Baaijens et al. 2014
Spain
Alaniz et al. 2010; Gramaje et al. 2010
U.S.A
Eskalen et al. 2001; Gubler et al. 2004

None of the rootstocks tested have shown complete resistance to black-foot and Petri disease pathogens

101-14 Millardet et de Grasset: ***Cylindrocladiella parva*** (Brown et al. 2013 N.Z. Plant Prot. 66)
161-49 Couderc: Petri disease pathogens (Gramaje et al. 2010 Am. J. Vitic. Enol. 61)

161-49 Couderc (*V. riparia* x *V. berlandieri*)
Phaeomoniella chlamydospora **and** ***Phaeoacremonium*** **spp.**

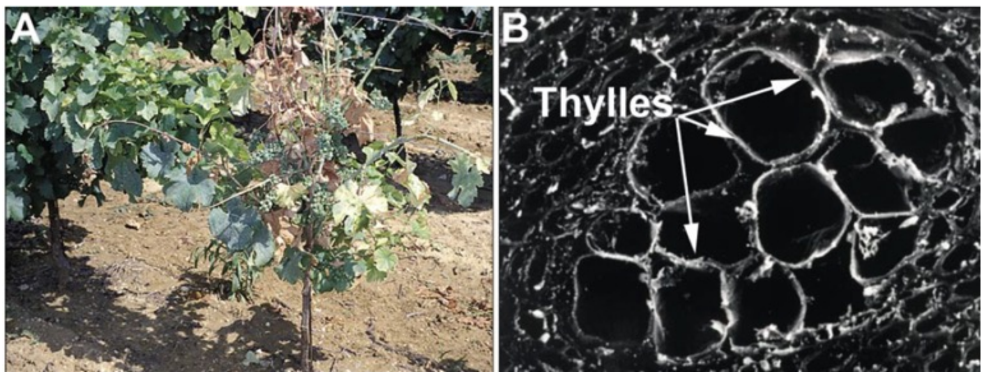
Gramaje et al. 2010 Am. J. Enol. Vitic. 61



Propagation processes in the nursery

161-49 Couderc

Tyloses
Physiological problems
Poor behaviour in compacted and wet soils



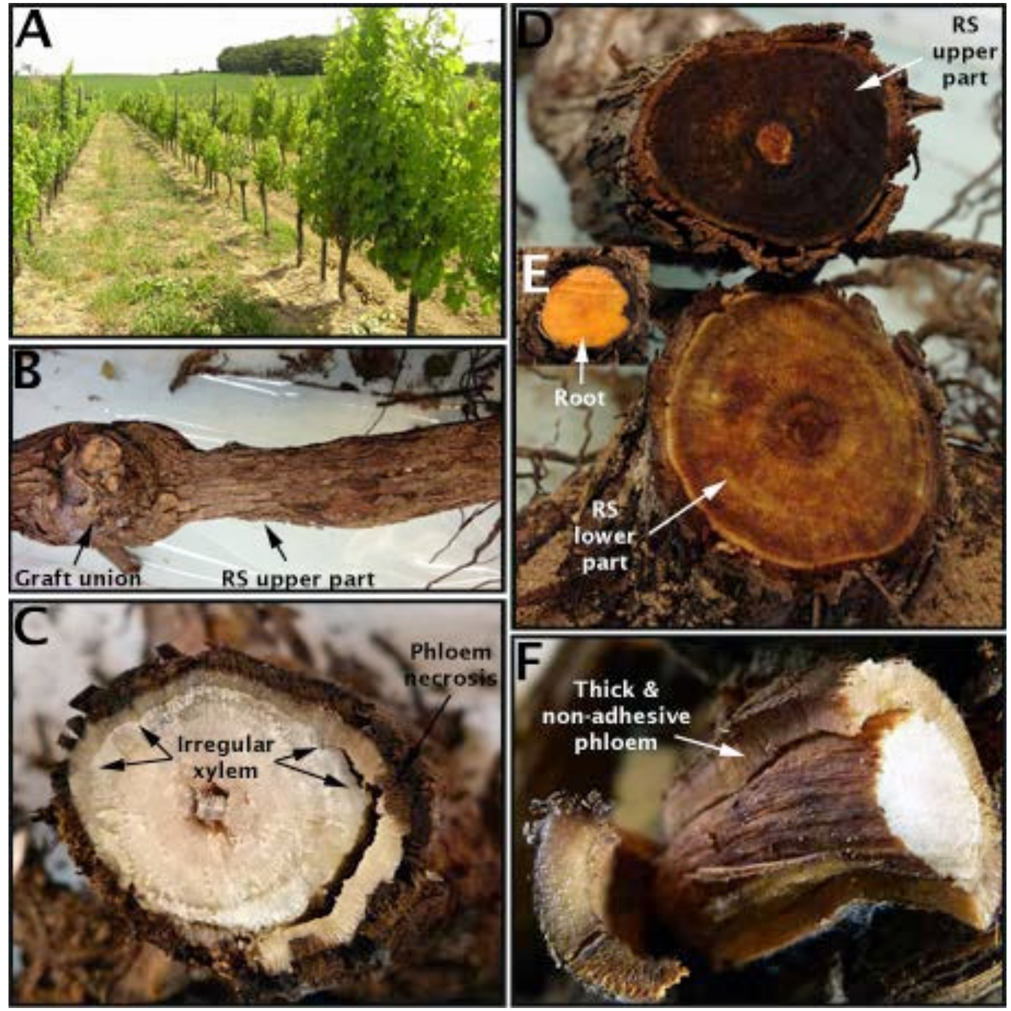
161-49 C decline

Decrease of vigour (3-4 y old plants)
Trunk diameter variation
Thick and non-adhesive phloem
Very small amount of starch in roots

The decline of the young vines grafted onto 161-49 C

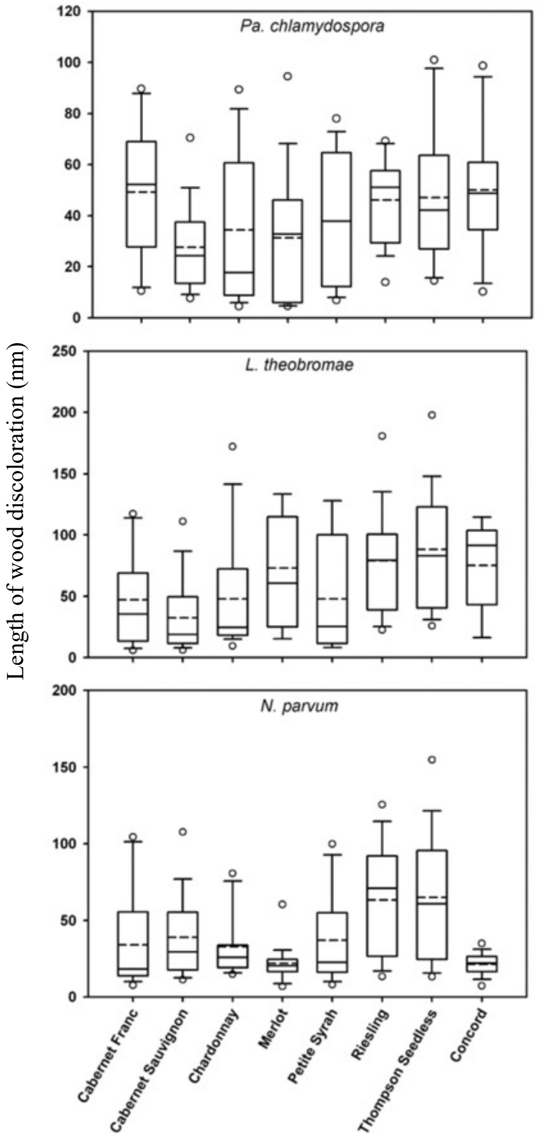
A.S. Spilmont[1*], C. Sereno[1], N. El khoti[1] and L. Torregrosa[2]

Spilmont et al. (2014) Acta Hortic. 1136



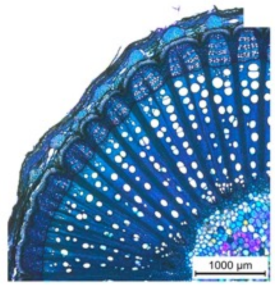
Propagation processes in the nursery

CULTIVARS



Travadon et al. 2013 Plant Dis. 97

There is a relationship between the mean diameter of the xylem and susceptibility to infection



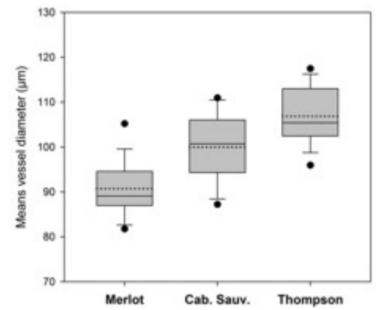


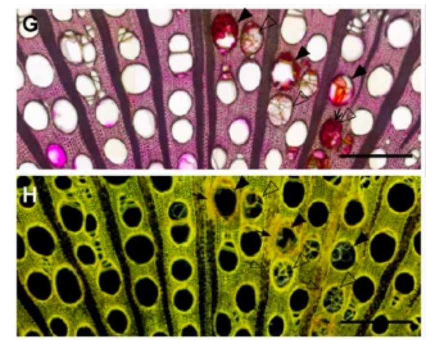
Table 1 | Mean of equivalent vessel diameter measured in 1 years old stems of *V. vinifera* cvs. Merlot, Cabernet Sauvignon, and Thompson Seedless.

Vitis vinifera cvs.	Merlot	Cabernet Sauvignon	Thompson Seedless
Level of susceptibility to fungal vascular diseases	Low	Medium	High
Mean vessel diameter	90.7 ± 5.8 a	99.9 ± 7.1 b	106.9 ± 6.3 c

Pouzoulet et al. 2014 Frontiers Plant Sci. 5

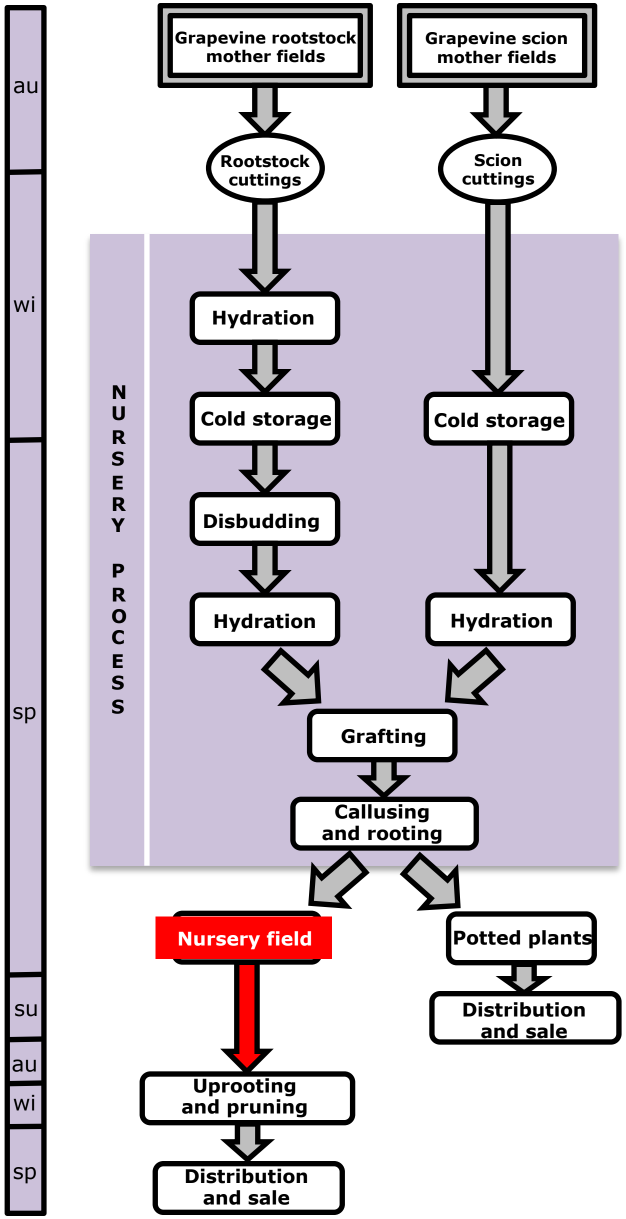
frontiers in Plant Science

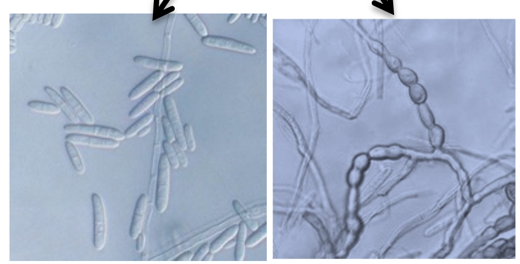
Xylem Vessel Diameter Affects the Compartmentalization of the Vascular Pathogen *Phaeomoniella chlamydospora* in Grapevine



Pouzoulet et al. 2017 Frontiers Plant Sci. 8

Nursery propagation beds





BLACK-FOOT DISEASE PATHOGENS

Gramaje and Armengol 2011. Plant Disease 95

Nursery propagation beds

CROP ROTATION IN NURSERY FIELDS

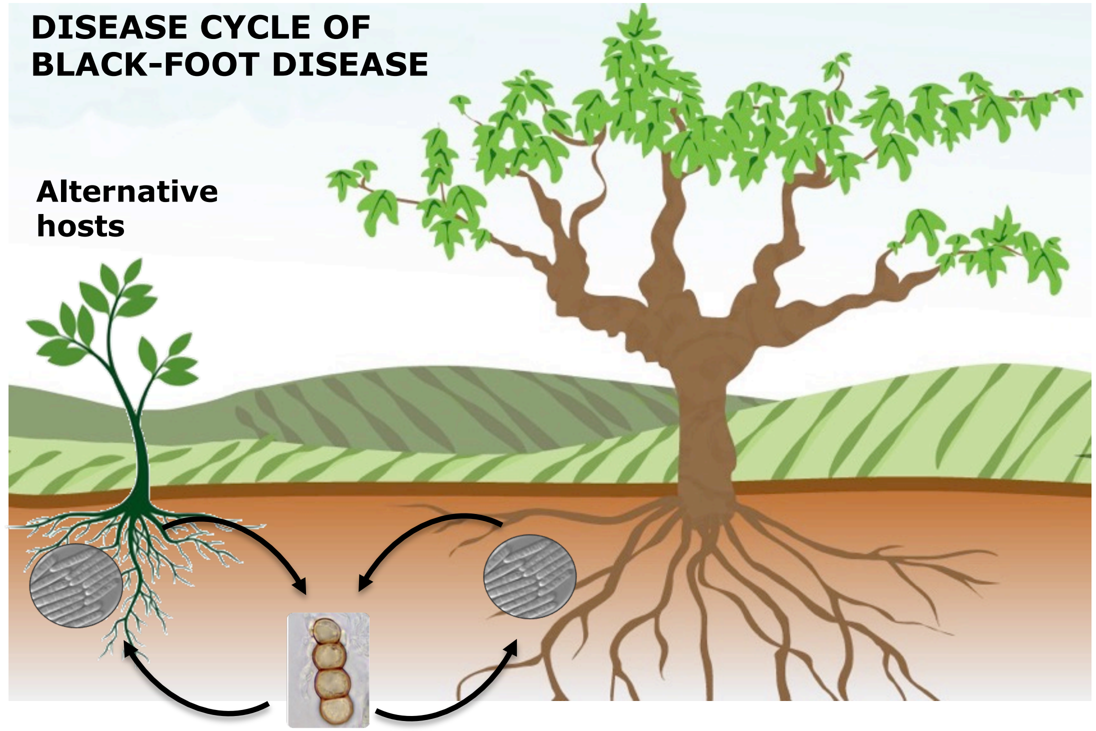
Increase of black-foot disease incidence

Portugal
Grapevines + 3-year rotation (e.g. potato, garlic, carrot, cereals)
(Rego et al. 2009 Phytopathol. Mediterr. 48)

South Africa
Grapevines every second year + cover crop
(Halleen et al. 2003 Australa. Plant Pathol. 32)

Fungal detection in soil during rotation

Wheat and barley
Portugal (Cardoso et al. 2013 Phytop. Mediterr. 52)
Spain (Berlanas et al. 2017 Plant Soil 417)



Resting spores (chlamydospores)
Mycelium in rotten root fragments

CHEMICAL SOIL FUMIGATION

✓ **Biofumigation**

Indian mustard seed meal

New Zealand
Barbour et al. 2014 Phytopathol. Mediterr. 53
Bleach et al. 2010 Phytopathol. Mediterr. 49

White mustard

Spain
Berlanas et al. 2018 Pest Manag. Sci. 74

Canada
Richards et al. 2020 Diversity 12

Reduction of black-foot disease incidence and fungal inoculum in soil

Commercial production settings

PREPLANTING TREATMENTS: Biological control agents

Pest Management Science

Investigation of *Trichoderma* species colonization of nursery grapevines for improved management of black foot disease

Wynand J van Jaarsveld,[a,b] Francois Halleen,[a,b] Michael C Bester,[a] Romain JG Pierron,[c] Elodie Stempien[a] and Lizel Mostert[a*]

→ ***Trichoderma* spp. were not sufficient to prevent infections by BFD pathogens, but a certain degree of protection was obtained in the basal ends.**

Field evaluation of biocontrol agents against black-foot and Petri diseases of grapevine

María del Pilar Martínez-Diz,[a,b] Emilia Díaz-Losada,[a] Marcos Andrés-Sodupe,[c] Rebeca Bujanda,[c] María M Maldonado-González,[c] Sonia Ojeda,[c] Amira Yacoub,[d] Patrice Rey[d] and David Gramaje[c*]

→ **The combination of the disease-suppressive activity of two or more beneficial microbes is required to prevent fungal infection**

- ***Streptomyces* sp. E1 + R4**
- ***Pythium oligandrum* Po37**
- ***Trichoderma atroviride* SC1**
- ***Trichoderma koningii* TK7**
- ***Pseudomonas fluorescens* + *Bacillus atrophaeus***

Biocontrol. Preplanting

Arbuscular mycorrhizal fungi

- ✓ **Improvement of nutrient adquisition (N, Cu, Fe, Zn)**
- ✓ **Reduction of water loss**
- ✓ **Increase of plant resistance to abiotic and biotic stresses**

horticulturae

Does Inoculation with Arbuscular Mycorrhizal Fungi Reduce Trunk Disease in Grapevine Rootstocks?

Taylor Holland [1], Patricia Bowen [2], Vasilis Kokkoris [1], Jose Ramon Urbez-Torres [2] and Miranda Hart [1,*]

PeerJ

Commercial arbuscular mycorrhizal fungal inoculant failed to establish in a vineyard despite priority advantage

Corrina Thomsen [1], Laura Loverock [1], Vasilis Kokkoris [2,4], Taylor Holland [1], Patricia A. Bowen [3] and Miranda Hart [1]

agriculture

Performance and Establishment of a Commercial Mycorrhizal Inoculant in Viticulture

Daniel Rosa [1,*], Antreas Pogiatzis [1], Pat Bowen [2], Vasilis Kokkoris [3], Andrew Richards [1,*], Taylor Holland [1] and Miranda Hart [1]

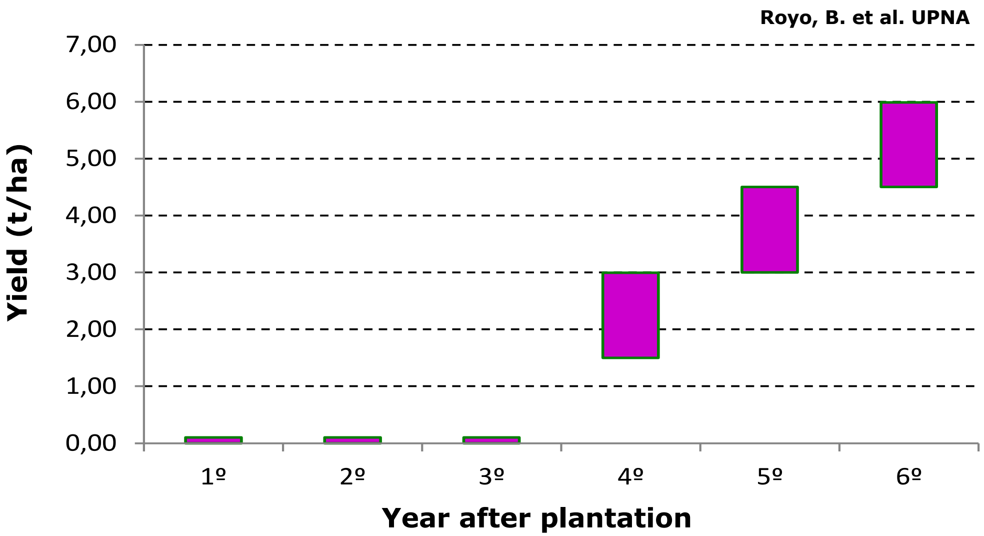
Low level of mycorrhiza establishment in the vineyard

Inefficacy to prevent black-foot disease infection

Lack of significant differences in agronomical features between mycorrhiza inoculated and non-inoculated plants

Management of young vineyards

Avoid placing a heavy fruit load on vines in the early production years



AVOID OVERCROPPING
Vines that are stressed are more susceptible to disease than unstressed vines.

2016
Plantation in 2014
Cultivar Macabeo

3rd YEAR

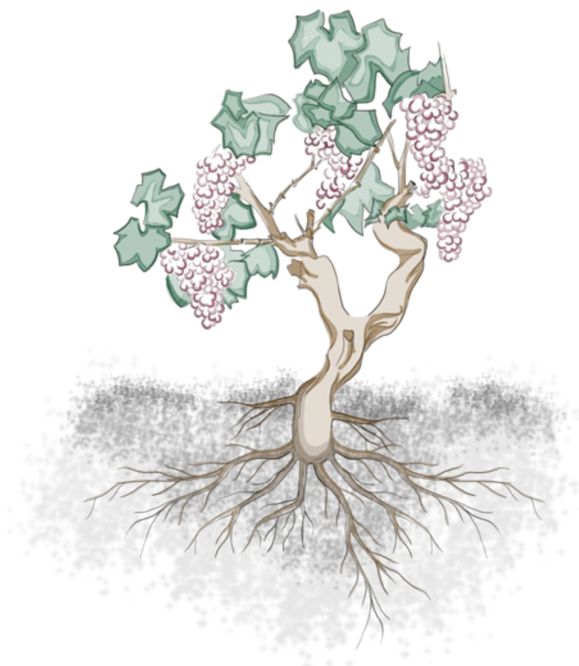
PHYTOPATHOLOGIA MEDITERRANEA

Review

Grapevine trunk disease fungi: their roles as latent pathogens and stress factors that favour disease development and symptom expression

Jared HRYCAN[1,2], Miranda HART[2], Patricia BOWEN[1], Thomas FORGE[1], José Ramón ÚRBEZ-TORRES[1,*]

Influencing factors



Influencing factors

Disease/pest management

Nursery

Pruning

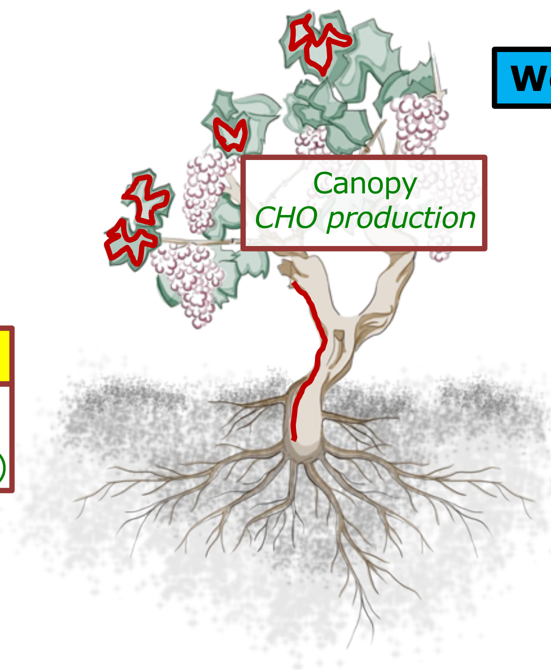
Outer inoculum

Foliar symptoms

Wounds

Rootstock

Cultivar, (clone)
Defenses, vessel num &
Size, select microbiota?
Indirect factors: vigour,
Productivity, acrotony



Colonization by fungi, decayed and discoloured wood, inner inoculum (*biomass*, *composition*)

Defenses

Functional sapwood
Quality
Quantity (vessels nb & size)

Leaves

Grapes

Reserves

Green harvest, de-budding

Fertilization

Irrigation

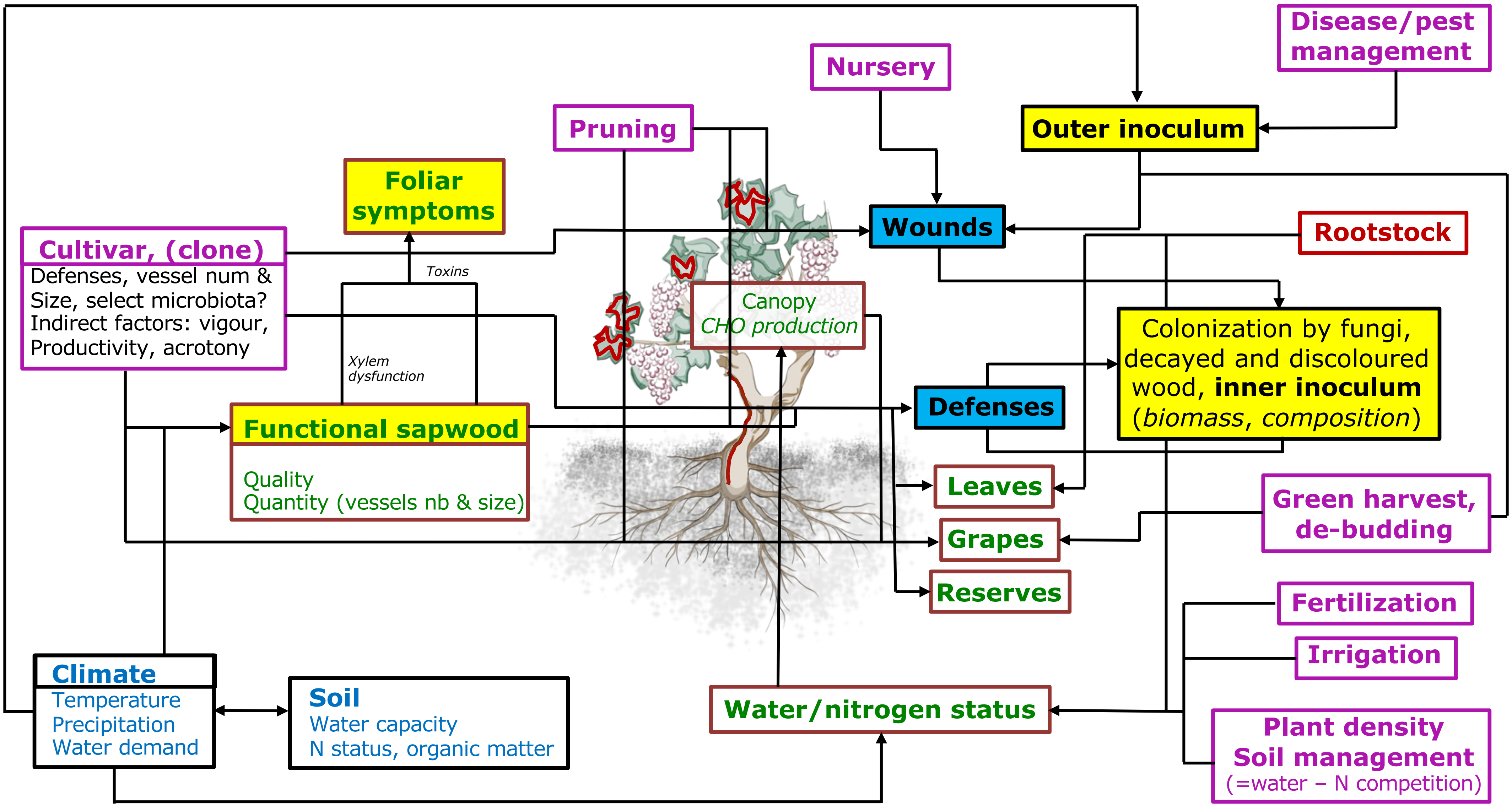
Climate
Temperature
Precipitation
Water demand

Soil
Water capacity
N status, organic matter

Water/nitrogen status

Plant density
Soil management
(=water – N competition)

Influencing factors



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Thanks for your attention!

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