

# **Grapevine pruning wound protection:**

## **EFFICACY OF THE BIOFUNGICIDE ESQUIVE®**

**Cecília Rego and Pedro Reis**

Instituto Superior de Agronomia, Universidade de Lisboa  
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# Esquive®

Biofungicide (W<sub>atable</sub> P<sub>owder</sub>)

*Trichoderma atroviride*

strain I-1237

Patent :



# Esquive<sup>®</sup>

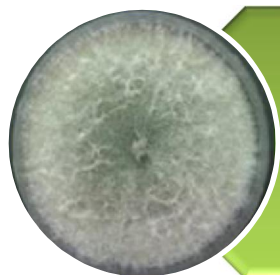
## Fast growing fungi

- 1) Ability to fast colonize pruning wounds without harming the plant

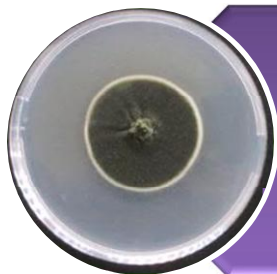
## Antagonist activity

- 1) Compete with pathogenic fungi for nutrients and space
- 2) Stimulate plant host defenses (metabolites/toxines)
- 3) Ability to attack and parasitize plant pathogens (mycoparasitisme)

The goal of the field trials conducted in Portugal was to test the efficacy of a formulation of *Trichoderma atroviride*, strain I-1237, **ESQUIVE®**, against :



*Neofusicoccum parvum* (Np)  
*Botryosphaeria dieback*



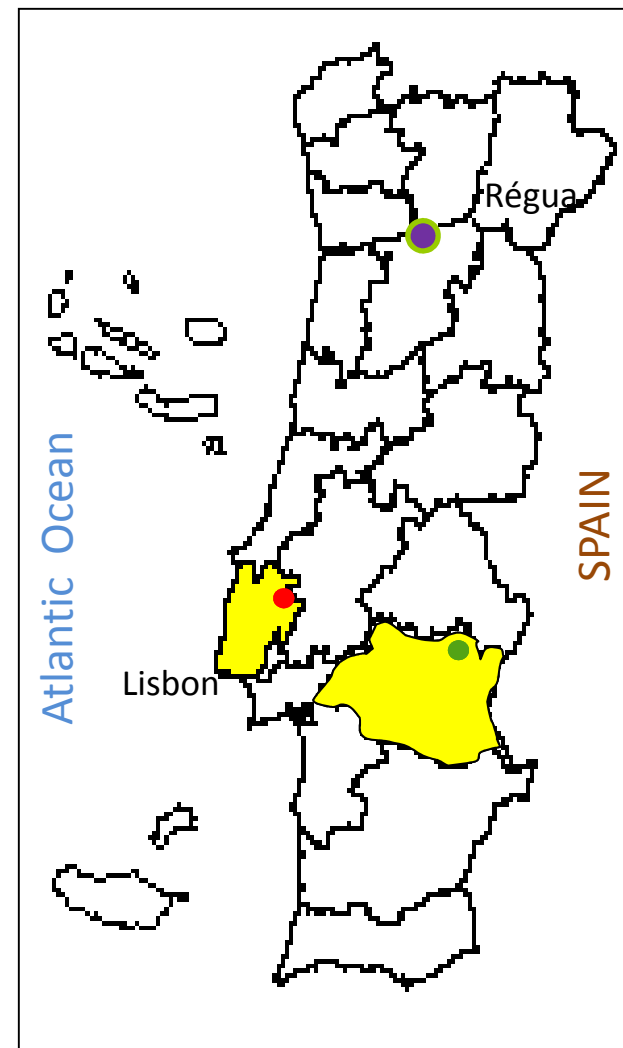
*Phaeomoniella chlamydospora* (Pch)  
*Petri disease*

# Materials and Methods

## Experimental conditions

(Selection of crop, cultivar and location)

- Two similar vineyards were selected for establishing the assays
- Age: 12 and 15 years old
- Cultivar: Aragonez (= Tempranillo)
- **1<sup>st</sup> year** for the field assays : **2013**  
Location: Estremadura (Alenquer) ●
- **2<sup>nd</sup> year** for the field assays : **2014**  
Location: Alentejo (Estremoz) ●



## Trial design

- Layout: completely randomized design (CRD)
- Plot size: Size sufficient to allow inoculation and sampling of 30 shoots/treatment
- Replications: 30 replicates /treatment





## Pruning method

- **one-year old shoots** with a similar appearance (length, internodes, buds) were selected for the trial
- shoots were **pruned at 2 cm above the 3rd bud** (shoot length > 30 cm to avoid infection of the older branches)
- a total of **210 shoots** were used in the trial





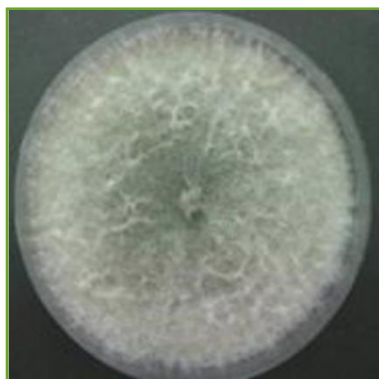
## Application method

- **ESQUIVE** was applied **on the same day** as pruning
- **Spraying: one spray per shoot** was done during favorable weather conditions (not windy)
- **Date:** February (2013, 2014)



## Inocula preparation

***Neofusicoccum parvum***      ***Phaeomoniella chlamydospora***



- one local pathogen strain of *Neofusicoccum parvum* and *Phaeomoniella chlamydospora* was selected
- spores were removed from the cultures and placed in sterile distilled water to obtain a conidial suspension which was adjusted to the concentration of  $10^5$  spores

## Inoculation method

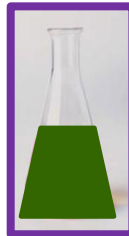
- **one droplet** of 40  $\mu$ l of each spore solution (2000 spores) was placed **on each wound** with a micropipette, **one day after the treatment**

**Np** spores  
suspension



&

**Pch** spores  
suspension



- the inoculated wounds were **protected with parafilm** for 2 weeks after inoculation, which prolongs the humidity on the wound

## Treatments, application, product, concentration, inoculation and spore solution volume

Treatments	Application	Product	Concentration	Inoculation	Spore solution volume*
1	-	Not treated		- (negative control)	-
2	-	Not treated		artificial infection Pch (positive control)	40µl**
3	-	Not treated		artificial infection Np (positive control)	40µl**
4	After pruning	ESQUIVE	27g/l*	artificial infection Pch	40µl**
5	After pruning	ESQUIVE	27g/l	artificial infection Np	40µl**
6	After pruning	ESQUIVE	27g/l	natural infection Pch	-
7	After pruning	ESQUIVE	27g/l	natural infection Np	-

\*- 4kg/ha with 150l of water/ha **Pch** - *Phaeomoniella chlamydospora* **Np** - *Neofusicoccum parvum* \* \* - 1 day after pruning

## Assessments

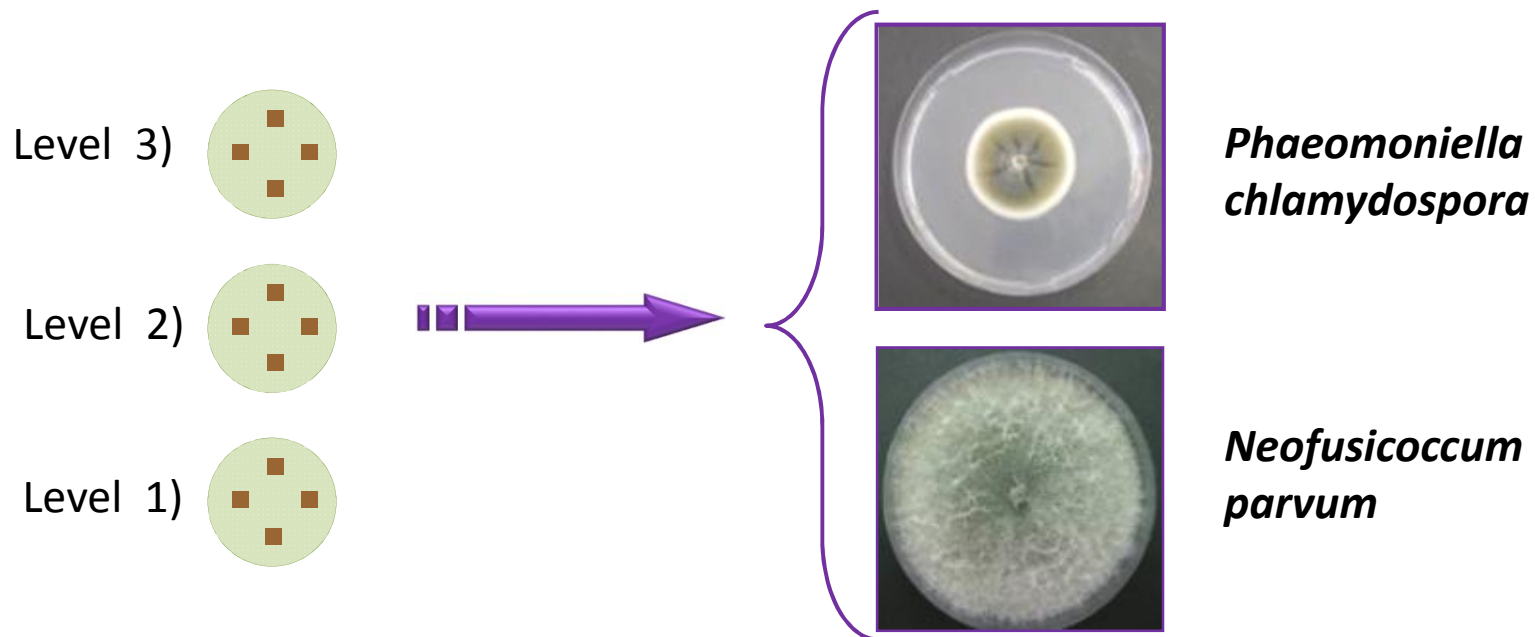
(October after harvest)

- **Length of internal necrosis** were recorded
- **Incidence** (infected/not infected shoots)
- **Severity** (% of infection in infected shoots)
- **Reisolation at different levels of the shoots:**
  - Level 3)** just bellow the cut
  - Level 2)** just bellow the third bud
  - Level 1)** just bellow the second bud



## Assessments

- From each level four pieces of internal wood tissues were plated onto PDA + chloramphenicol
- A total of 2520 fragments of wood were analyzed.
- Cultures were identified according to their morphocultural features

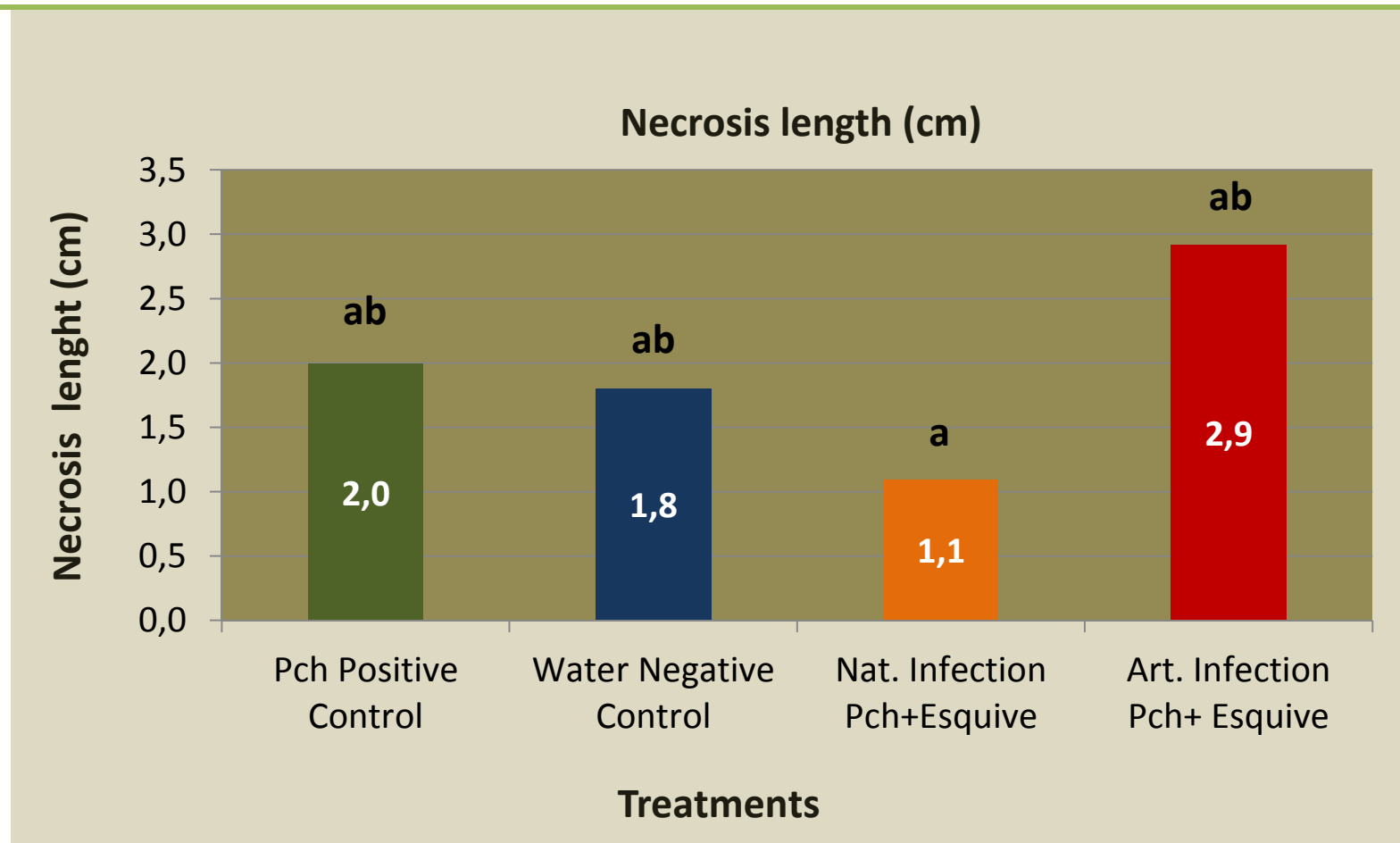


## Results

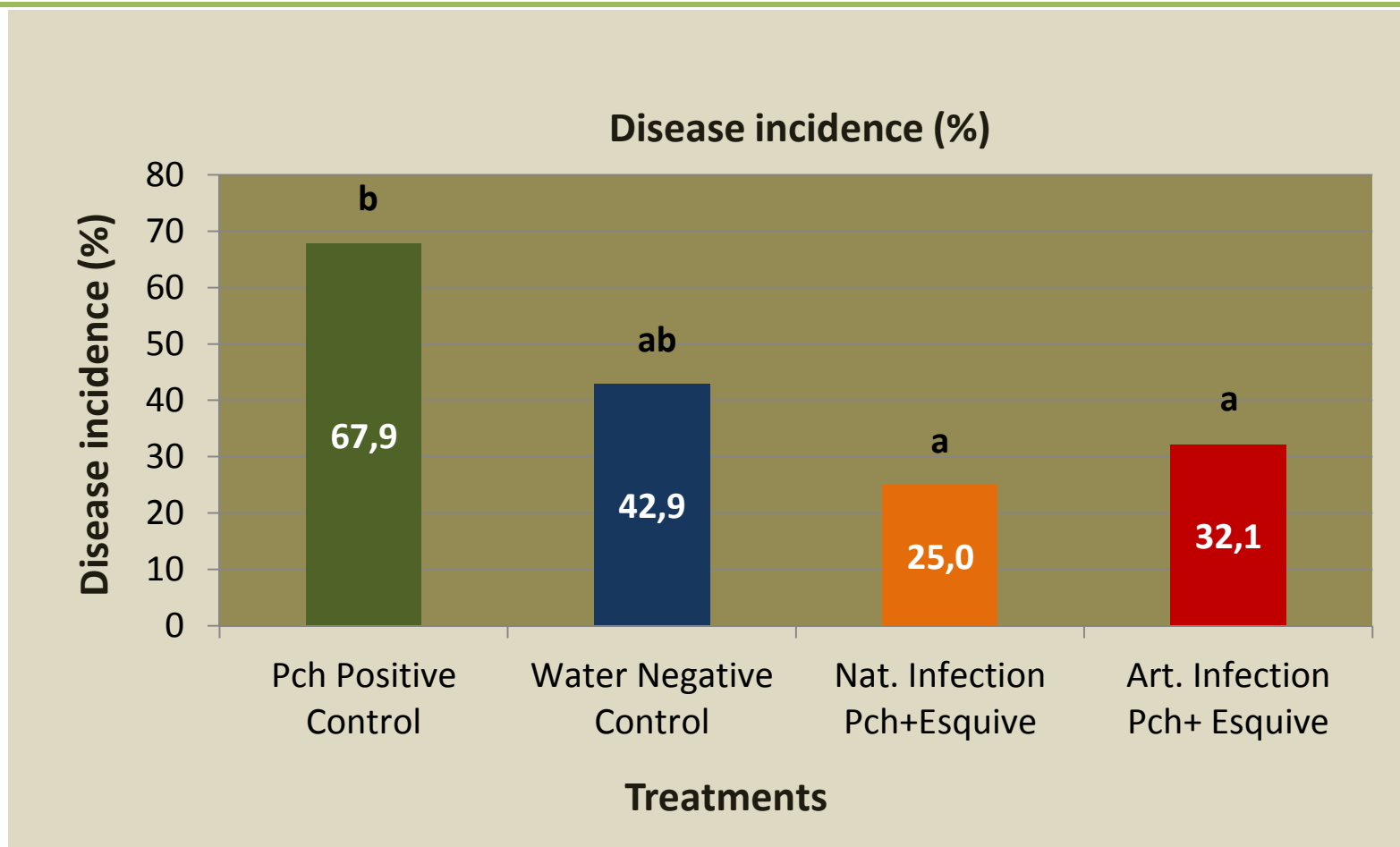
*Phaeomoniella chlamydospora*

*Neofusicoccum parvum*

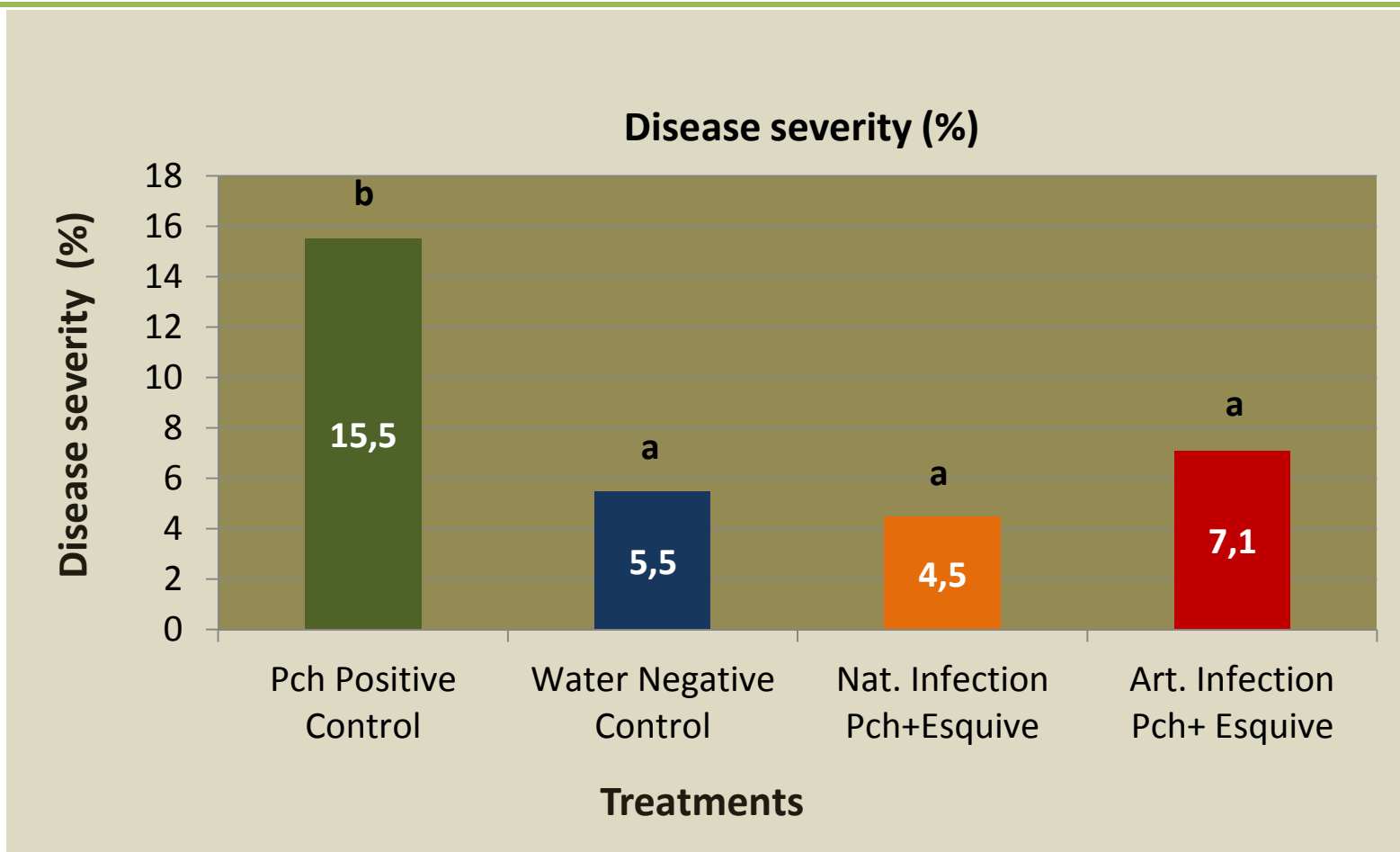




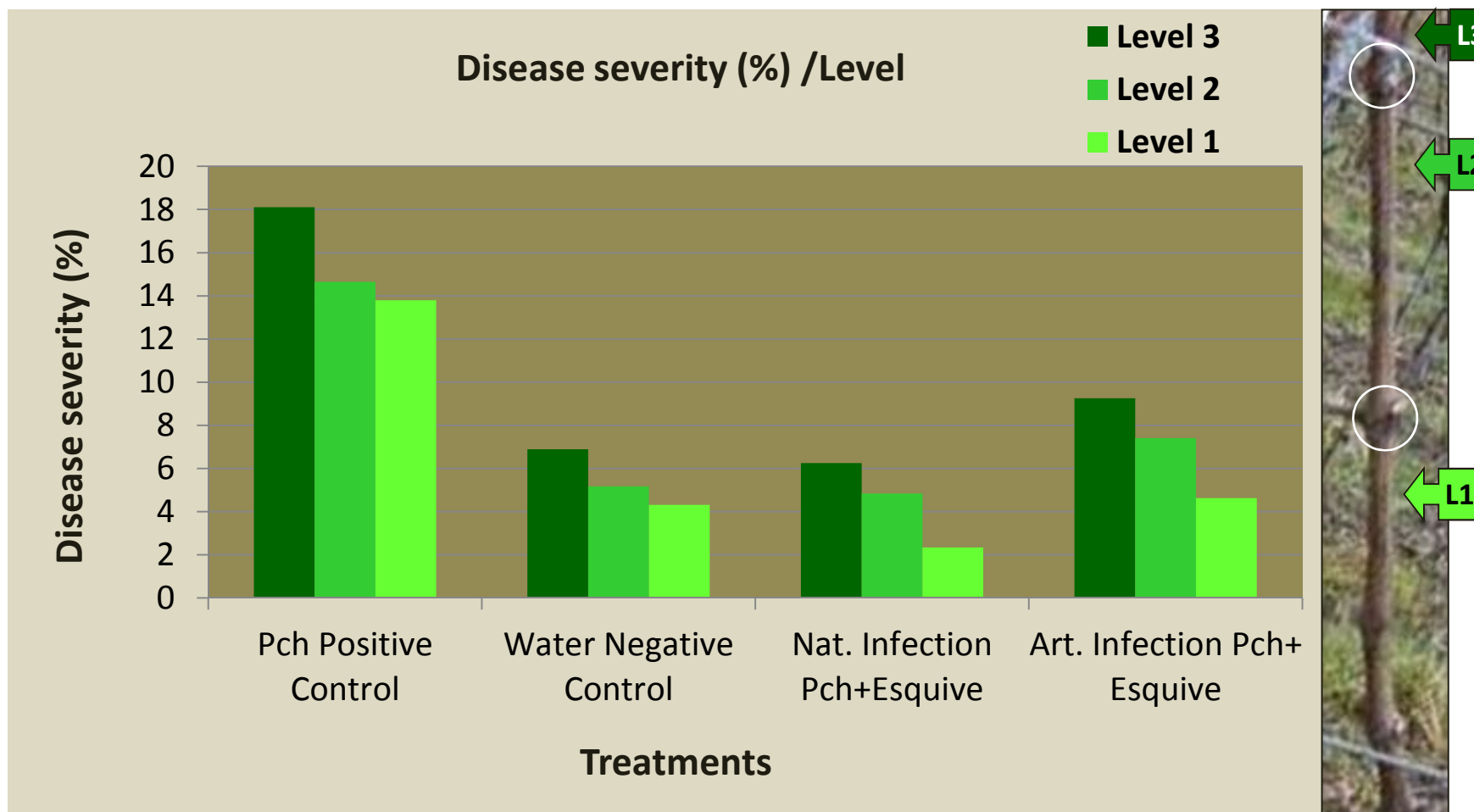
- The **lowest value** of necrosis length was obtained for **Esquive + natural infection** of *Pa. chlamydospora* (Pch)
- There were **no significant differences** among other treatments



- The lowest value of **incidence** was obtained for **Esquive + natural infection** of *Pa. chlamydospora* (Pch)
- Treatments with **Esquive** **differ significantly** from *Pa. chlamydospora* positive control.



- The lowest value of **severity** was obtained for **Esquire + natural infection** of *Pa. chlamydospora* (Pch)
- *Pa. chlamydospora* **positive control** differ significantly from all the treatments

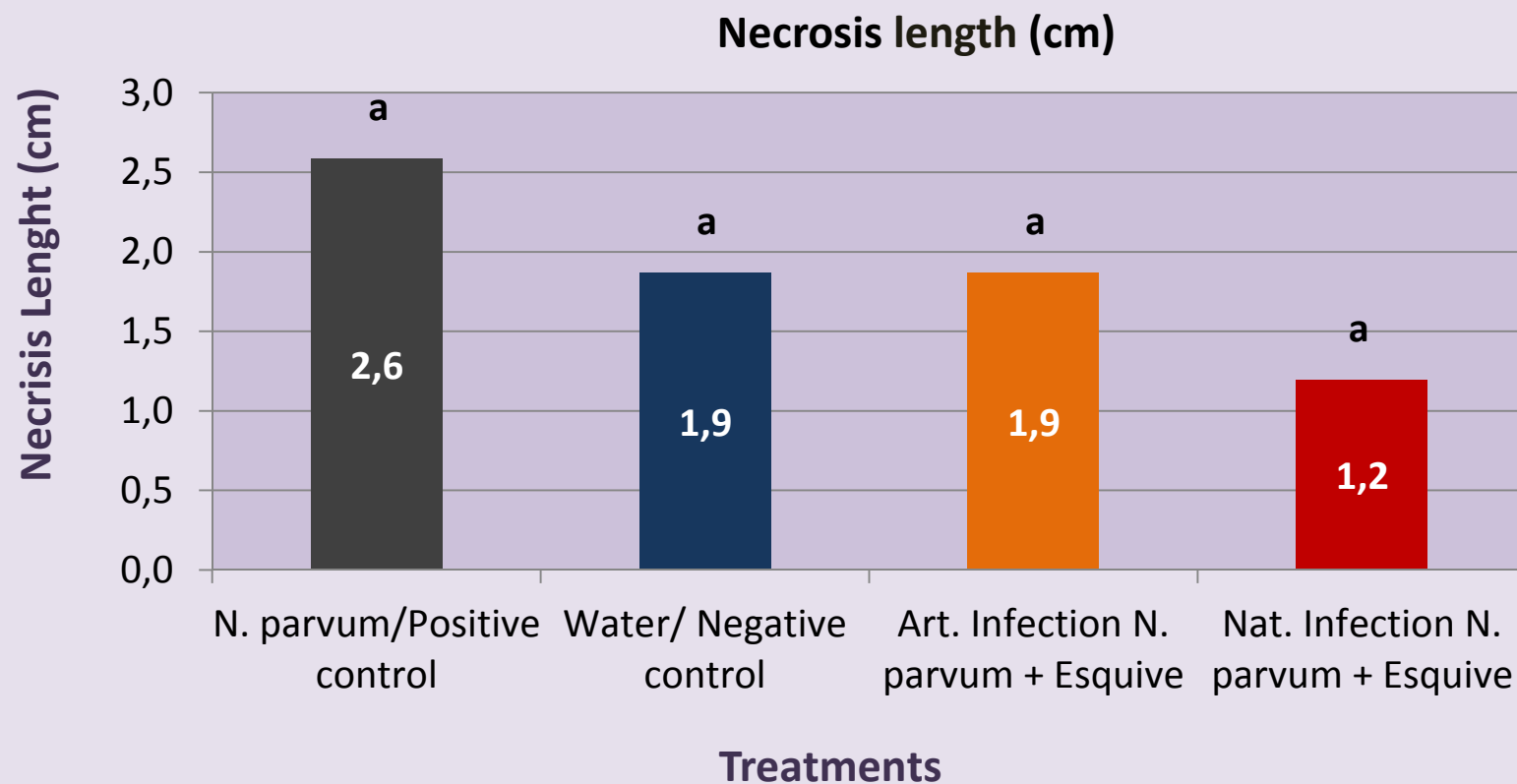


- Disease severity of *Pa. chlamydospora* (Pch) was **higher in level 3** than in levels 2 and 1 for all the treatments

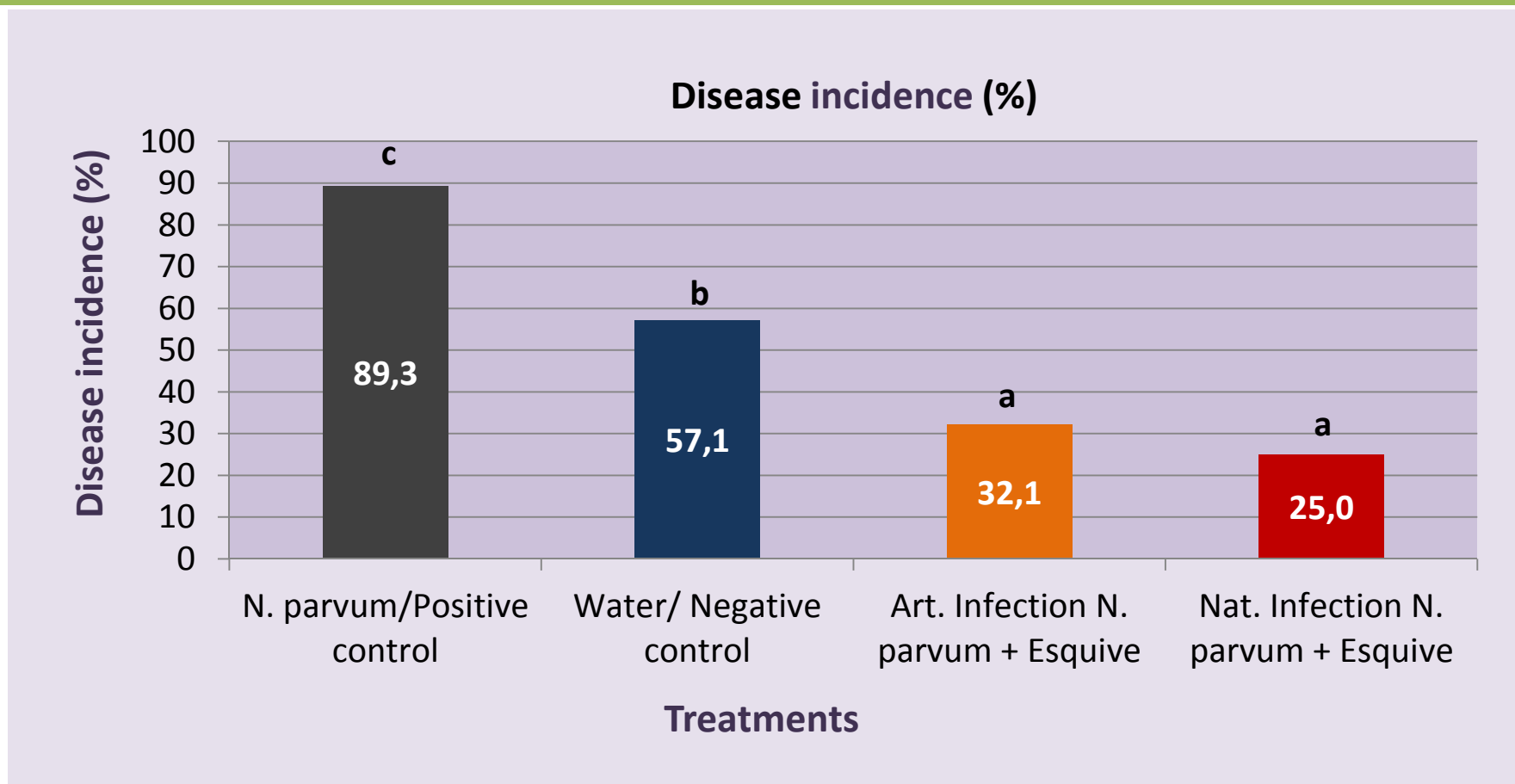
## Results

*Phaeomoniella chlamydospora*

*Neofusicoccum parvum*

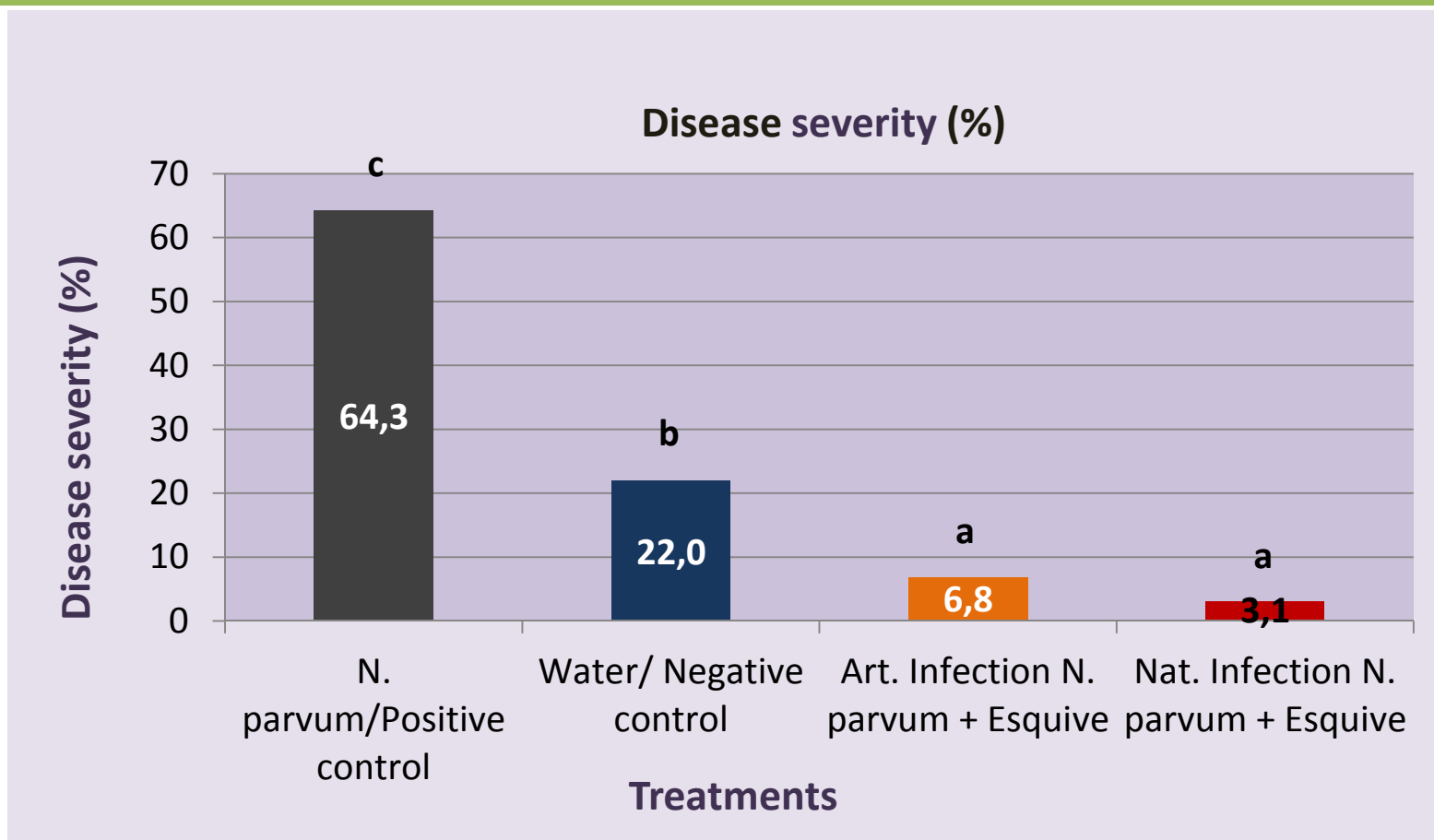


- **Necrosis length** caused by *Neofusicoccum parvum* (Np) **did not differ significantly** among treatments

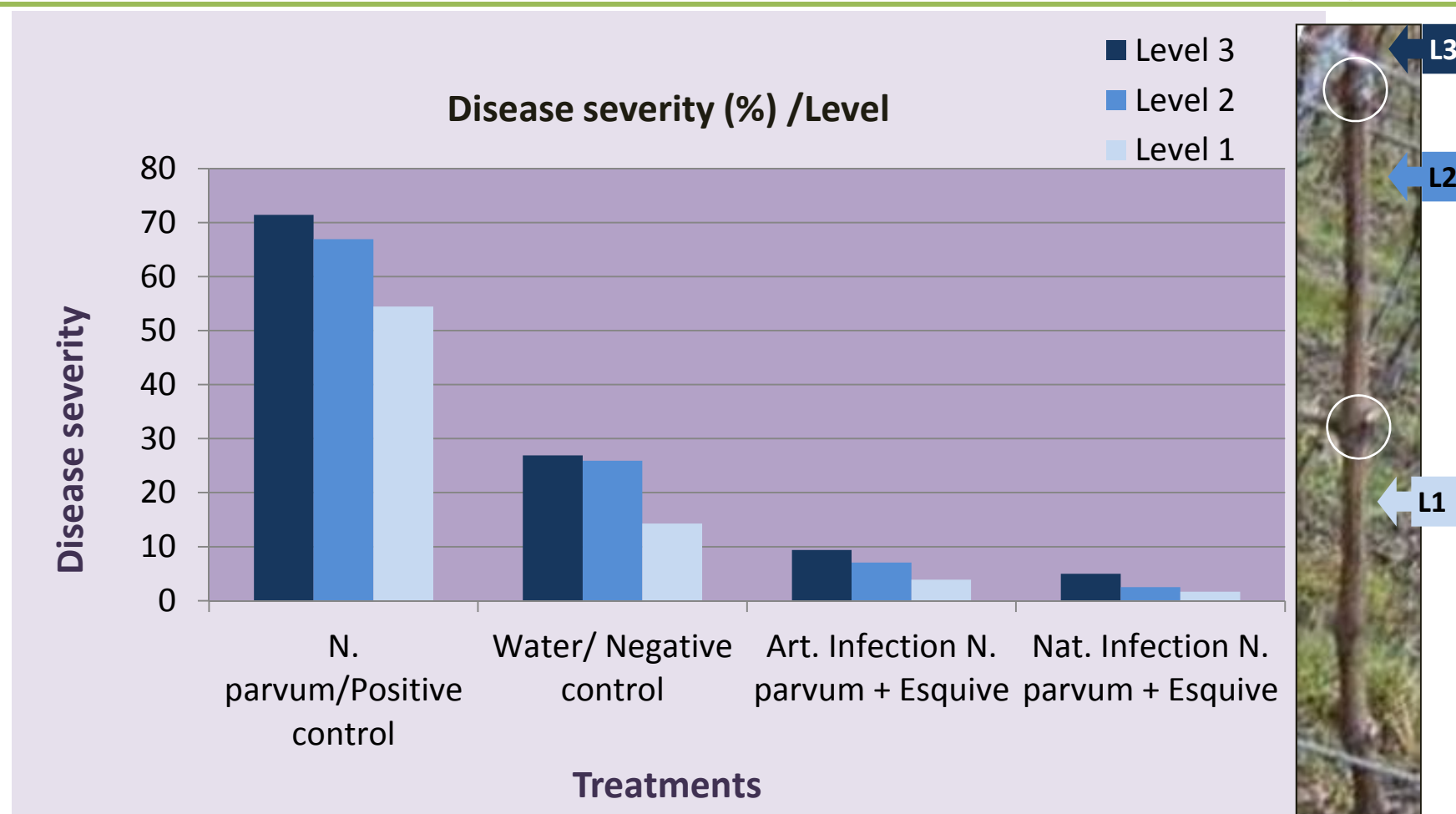


- The lowest value of **incidence** was obtained for **Esquive + natural infection** of *Neofusicoccum parvum* (Np)
- Treatments with **Esquive** **differ significantly** from water negative control and from *Neofusicoccum parvum* (Np) positive control.





- The **lowest value of severity** was obtained for **Esquive+natural infection** of *Neofusicoccum parvum* (Np)
- Treatments with **Esquive** **differ significantly** from water negative control and from *Neofusicoccum parvum* (Np) control.

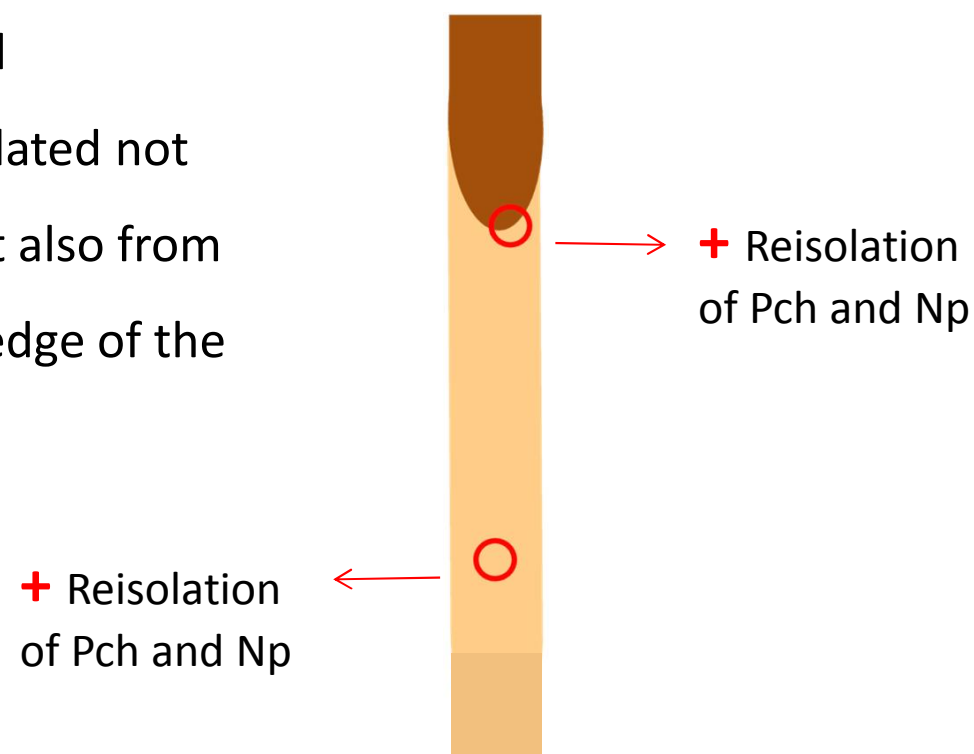


- Disease severity *Neofusicoccum parvum* (Np) **was higher in level 3** than in levels 2 and 1 for all the treatments

# Conclusions

- The lowest **extensions of necrosis were recorded** in the shoots treated with **Esquive** after pruning

- *Phaeomoniella chlamydospora* and *Neofusicoccum parvum* were reisolated not only from **symptomatic tissues** but also from **asymptomatic tissues** bellow the edge of the necrosis



- Results revealed that **Esquive** significantly reduced the **incidence** and **severity** of both pathogens (*Phaeomoniella chlamydospora* and *Neofusicoccum parvum*) when compared with the other treatments
- **Esquive** significantly reduced **the progress of both fungi** along the three **levels** of the shoots under analysis

Considering the importance of

- **preventing diseases** infection through pruning wounds
- **slowing down colonisation** of wood diseases

WE STRONGLY CONSIDER THAT **ESQUIVE** IS A POTENTIAL BIOSOLUTION FOR  
SUSTAINABLE CONTROL OF **GWD**.

# Thanks

