Evaluation of *Trichoderma atroviride* as a biocontrol agent against five Botryospahericeae grapevine trunk pathogens

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INTRODUCTION

Species of the genus *Trichoderma* have been widely investigated as biocontrol agents and could become a potential alternative to routine chemical control. *Trichoderma* species have been also tested as biological agents to protect pruning wounds against grapevine pathogens. In this study, *Trihoderma atroviride* was tested *in vitro* and in a greenhouse trial against five Botryosphaeriaceae fungi: *Diplodia corticola, Neofusicoccum australe, Neofusicoccum luteum, Neofusicoccum mediterraneum* and *Neofusicoccum parvum*.

DUAL CULTURE TESTS

One 6 mm agar plug of an actively growing colony of each Botryospaheriaceae isolate was placed on the agar surface of a 90 mm Petri dish, containing 20 ml of PDA, at 1 cm from the edge of the dish. A 6 mm diameter mycelial disc from an actively growing *Trichoderma atroviride* culture was then placed on the agar surface opposite the pathogen. Twelve replicate plates were maintained for each treatment.

T. atroviride opposite A: *D. corticola*, B: *N. australe*, C: *N. luteum*, D: *N. mediterraneum* and E: *N. parvum* after 7 days at 25°C

Plates were incubated for one month at 25°C in the dark. After the first seven days, the percent of inhibition of radial growth was calculated by Royse and Ries, 1978 and after one month cultures were scored using the Badalyan rating scale (2002).

	Inhibition of radial growth	Badalyan rating scale
Neofusicoccum parvum	60,74%	CA2
Neofusicoccum mediterraneum	57,46%	CA2
Neofusicoccum australe	57.00%	CA2
Neofusicoccum luteum	54,30%	CA2
Diplodia corticola	43,59%	CA2

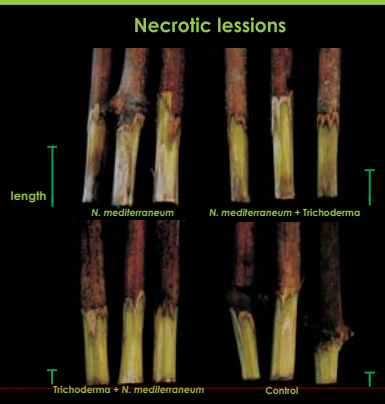
Mycelial growth inhibition of *Neofusicoccum* species was significantly greater than *Diplodia corticola*. Although all Botryosphaeriaceae isolates showed the same type of interaction, CA2, produced a complete replacement after initial deadlock with micelial contact in all plates. On the basis of these results *T. atroviride* was considered an active antagonistic fungi against our Botryosphaeriaceae isolates.

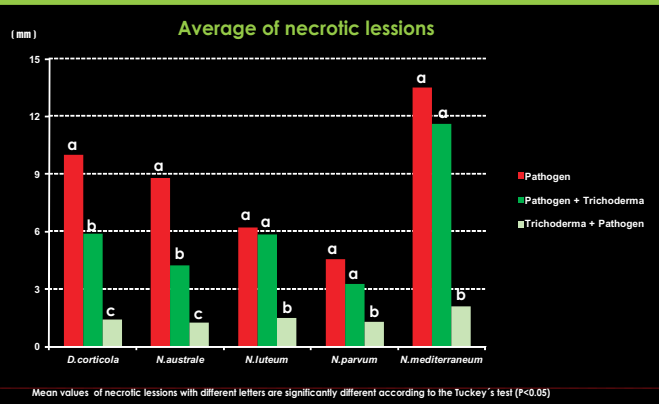
BIOCONTROL TESTS

Two additional biocontrol tests using detached one year-old grapevine canes in laboratory and potted vines in greenhouse were performed. Mycelial plugs of Botryosphaeriaceae species and *T. atroviride* were placed in the same inoculation sites following two procedures: *T. atroviride* was inoculated either three days before or three days later than the pathogens, respectively.

1. Grapevine canes

10 days

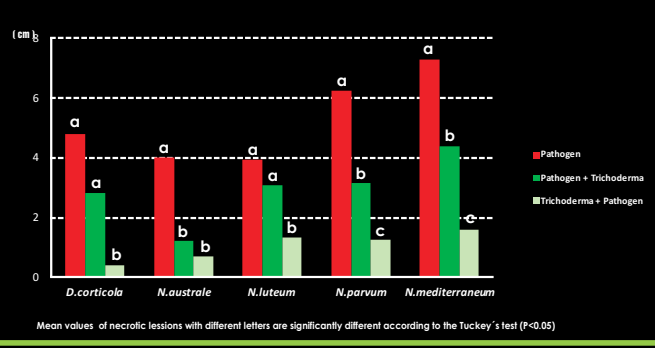




Mean values of necrotic lessions with different letters are significantly different according to the Tuckey´s test (P<0.05)

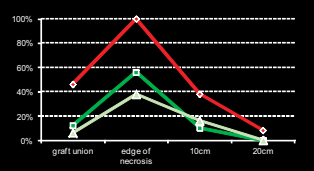
2. Grapevine plants

6 months

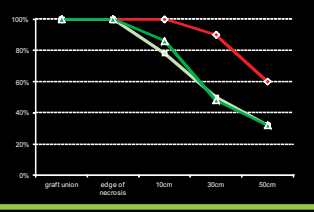


Mean values of necrotic lessions with different letters are significantly different according to the Tuckey´s test (P<0.05)

Reisolation of pathogens



Reisolation of *Trichoderma*



Post-inoculations with *T. atroviride* reduced the length of the pathogen necrosis in 6.7- 58.3% in detached canes and in 22.1- 69.2% in potted vines. Pre-inoculation with this antagonist had a stronger inhibition effect since it reduced the length of the necrosis in 90.4 -96.8 % in detached canes and 65.7-91.9 in potted vines. In adittion, reisolation of the pathogens decreased. Botryospaheriaceae species were on average reisolated in 56% of the cases when *T. atroviride* was inoculated after the pathogen, while in 38% when the antagonist was inoculated prior to the pathogen in the edge of the necrosis. *T. atroviride* was isolated in 100% of cases until 10 cm above edge of the necrosis and in 60% until 50 cm when it was inoculated alone. When it was inoculated together with the pathogen its recovery was lower but it could also be isolated until 50 cm in 32% six months after the inoculations.

CONCLUSIONS

According to these results *Trichoderma atroviride* application as a preventive method could be a good choice to avoid Botryosphaeriaceae grapevine diseases and to reduce the severity of the disease in plants recently affected by bot canker. *T. atroviride* can persist in plants for six months, proving it is a good protectant against pruning wounds because the susceptibility to infection can last up to 16 weeks after pruning. Further field studies need to be performed to confirm these results.

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