



EVALUATION OF SIX RED GRAPEVINE CULTIVARS INOCULATED WITH *NEOFUSICOCCUM PARVUM* IN A “TERROIR” OF LA MANCHA WINE REGION (SPAIN)

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Abstract

Aim: Among Botryosphaeriaceae species associated with Botryosphaeria dieback of grapevines, *Neofusicoccum parvum* is one of the most virulent and fastest wood-colonizing fungi. This study aimed to evaluate the susceptibility of six red grapevine cultivars (“Bobal”, “Monastrell”, “Garnacha Tinta”, “Moravia Agría”, “Tinto Velasco” and “Moribel” to *N. parvum*, under field conditions.

Methods and Results: Pathogenicity studies were conducted, over two consecutive years, with one-year old grapevine rooted cuttings inoculated with a *N. parvum* isolate. Rooted cuttings were wounded between the two upper internodes with a cork borer. A mycelial agar plug, from a 3-weeks-old culture on potato dextrose agar (PDA), was placed in the wound. Wounds were sealed with parafilm and wrapped with foil paper to prevent drying. Ten rooted cuttings per cultivar were inoculated with the fungus and two others with uncolonized PDA plugs, as negative controls. After inoculating, rooted cuttings were planted in a plot and irrigated by a drip system with two drippers per plant. Plants were collected after eight months and inspected for lesion development. Extent of wood necrosis was measured upward and downward from the inoculation point. Three rooted cuttings for each cultivar were selected and small pieces, of necrotic tissue from the edge of each lesion, were cut and placed on malt extract agar supplemented with 0.5 g/L of streptomycin sulphate (MEAS), in an attempt to recover the inoculated fungus and complete Koch’s postulates. *N. parvum* was identified by morphological and molecular approaches. Mean percentage of infected rooted cuttings ranged from 42.1% (“Monastrell” cultivar) to 93.3% (“Tinto Velasco” cultivar). Mean lengths of the extent of wood necrosis caused by *N. parvum* on inoculated one-year-old grapevine wood ranged from 21.2 (“Bobal” cultivar) to 87.2 mm (“Tinto Velasco” cultivar). *N. parvum* was reisolated from the edge of each lesion in 90.3% of the cultivars. The results of statistical analysis showed that “Bobal” and “Monastrell” cultivars were significantly more tolerant than “Tinto Velasco”.

Conclusions: All tested grapevine cultivars were susceptible to infection by *N. parvum*, evidencing that there was no qualitative resistance to this fungus. “Bobal” and “Monastrell” cultivars highlighted for their lower wood response susceptibility to *N. parvum*.

Significance and Impact of the Study: Interactions between Botryosphaeriaceae species and grapevine cultivars are poorly understood and there is currently little data available. This study allowed classifying different *Vitis vinifera* cultivars based on their degree of quantitative resistance to *N. parvum*. “Bobal” and “Monastrell” cultivars could be potential candidates to create tolerant varieties to *N. parvum* fungus. Using tolerant varieties would be the safest, easiest, the least expensive and the most effective means of controlling this disease.

Keywords: Botryosphaeria dieback, grapevine, grapevine trunk diseases, *Neofusicoccum parvum*

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ABSTRACT

Botryosphaeria dieback (BD) is a serious grapevine trunk disease (GTD) that causes large losses in yield and shortens the productive life of vineyards. *Neofusicoccum parvum* is one of the main causal agents of BD. Currently there are no curative fungicides to eradicate this disease; therefore, the use of tolerant cultivars to BD could be considered an interesting and sustainable alternative for its control. For this purpose, rooted cuttings of six red *Vitis vinifera* cultivars were selected and inoculated with *N. parvum* over two consecutive years. Eight months after inoculation, plants were collected and inspected for lesion development. The fungal incidence varied depending on the cultivar and ranged from 42.1% in "Monastrell" cultivar to 93.3% in "Tinto Velasco" cultivar, evidencing a lack of qualitative resistance to *N. parvum*. The severity of internal wood symptoms caused by *N. parvum* differed considerably amongst the cultivars, being "Bobal" and "Monastrell" more tolerant than "Tinto Velasco" cultivar.

INTRODUCTION

Botryosphaeria dieback is one of the most damaging GTDs affecting vineyards in all major grape-producing areas worldwide. BD is one of the major threats for the economic sustainability of viticulture reducing yields, productivity and longevity of vines and vineyards. Among Botryosphaeriaceae species associated with BD of grapevines, *N. parvum* is one of the most virulent and fastest wood-colonizing fungi. Plants affected by this pathogen can show symptoms such as, wedge-shaped perennial cankers in spurs, cordons and trunks that end up causing the death of the vines. Previous research have shown that grapevine cultivars and rootstocks have different levels of susceptibility to Botryosphaeriaceae species. The use of tolerant cultivars to BD would be the safest, easiest, the least expensive and the most effective way of controlling the disease.



METHODS AND RESULTS

Pathogenicity studies were conducted, over two consecutive years, with one-year old grapevine rooted cuttings inoculated with a *N. parvum* isolate. Rooted cuttings were wounded between the two upper internodes with a cork borer. A mycelial agar plug, from a 3-weeks-old culture on potato dextrose agar (PDA), was placed in the wound. Wounds were sealed with parafilm and wrapped with foil paper to prevent drying. Ten rooted cuttings per cultivar were inoculated with the fungus and two others with uncolonized PDA plugs, as negative controls. After inoculating, rooted cuttings were planted in a plot and irrigated by a drip system with two drippers per plant. Plants were collected after eight months and the extent of wood necrosis was measured upward and downward from the inoculation point.



Figure 1. *N. parvum* isolate.



Figure 2. Inoculation process.



Figure 3. Plot of rooted cuttings.



Figure 4. Wood necrosis symptoms.

The incidence of the fungus on different cultivars, expressed as the mean percentage of infected rooted cuttings from the total of inoculated rooted cuttings, ranged from 42.1% ("Monastrell" cultivar) to 93.3% ("Tinto Velasco" cultivar). Mean lengths of the extent of wood necrosis caused by *N. parvum* on inoculated one-year-old grapevine wood ranged from 21.2 ("Bobal" cultivar) to 87.2 mm ("Tinto Velasco" cultivar).

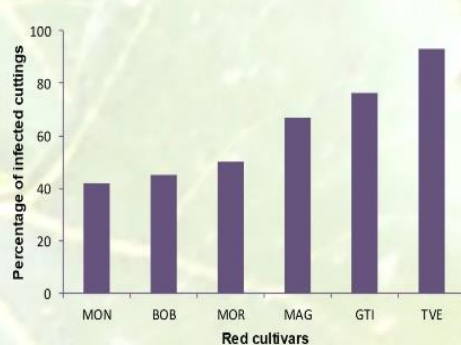


Figure 5. Mean percentage of infected rooted cuttings by *N. parvum* on grapevine cultivars, considering the two years. Cultivar abbreviations: BOB: "Bobal"; MON: "Monastrell"; GTI: "Garnacha Tinta"; MOR: "Moribel"; MAG: "Moravia Agria" and TVE: "Tinto Velasco".

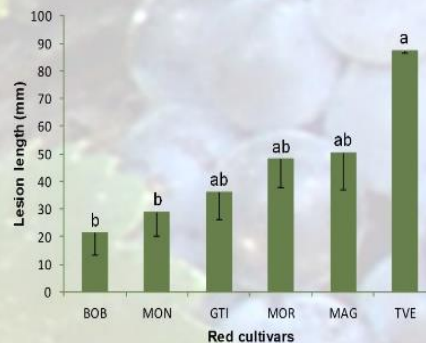


Fig. 6. Mean lesion length caused by *N. parvum* on grapevine cultivars at eight months after inoculation. Letters indicate statistically significant differences between varieties, according Tukey's honestly significant difference test at $P \leq 0.05$. Bars represent standard error of the mean. Cultivar abbreviations: BOB: "Bobal"; MON: "Monastrell"; GTI: "Garnacha Tinta"; MOR: "Moribel"; MAG: "Moravia Agria" and TVE: "Tinto Velasco".

CONCLUSIONS

This study allowed classifying different *Vitis vinifera* cultivars based on their degree of quantitative resistance to *N. parvum*. Among them, "Bobal" and "Monastrell" cultivars showed lower wood response susceptibility to fungal infection and in consequence, this feature would make them potential candidates to create tolerant varieties to *N. parvum* fungus.

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