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Original paper

Pathogenicity of *Biscogniauxia mediterranea* (De. Not. Kunze.) to Quercus seedlings

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Abstract

Introduction: The fungus *Biscogniauxia mediterranea* (syn.: *Hypoxylon mediterraneum*) is one of the most dreaded pathogens on trees of the genus Quercus throughout the Mediterranean basin.The present work aims to study in vivo the pathogenicity of two isolates of *B. mediterranea* from symptomatic trees of the cork oak of the two forests of western Algeria.
Methods: The pathogenicity of the isolates was tested on young seedlings of *Q. suber* and *Q. ilex* aged one year, by inoculation method, under controlled conditions (T° ~ 19 to 24 °C).
Results: After 30 days of inoculation, the two isolates confirmed their pathogenicity towards cork oak and holm oak seedlings causing necrotic lesions on the stem reaching up to 45 mm in certain seedlings. Drying and chlorosis of the leaves are also observed.
Conclusion: Given the potential threat posed by the inherent aggressiveness of *Biscogniauxia* species, further research is deemed necessary to curb the development of charcoal disease on oak trees.

Keywords: *Biscognauxia mediterranea*, *Quercus suber*, *Quercus ilex*, pathogenicity, seedlings.

Introduction

The cork oak (*Quercus suber* L.) is an emblematic component of the western region of the Mediterranean basin where it plays a major role in shaping the landscape. Indeed, cork oak forests have always been highly valued by the Mediterranean population due to their high ecological and socio-economic value(Maghnia 2018). In addition, the holm oak (*Quercus ilex* L.) is also very important in the Mediterranean basin. Although the wood of this species is used locally and marginally, at an artisanal level, for the manufacture of small objects, agricultural utensils and tool handles, it is almost exclusively exploited for the production of firewood. The volumes harvested are much lower than organic production and populations (Roda et al. 2015).

Unfortunately, a significant decline in cork oak and holm oak forests has been reported throughout the Mediterranean region since the early 1980s.The causes of this decline in oaks are multiple and interactive. The interaction between the different biotic and abiotic factors leads to a progressive decrease in the vigor of the tree. This decrease in vitality leads to a reduction in their ability to defend themselves, thus creating favourable conditions for the installation of pathogens.The majority of these pathogens are opportunistic like the ascomycete *B. mediterranea*.

B. mediterranea is a causal agent of the disease “charcoal disease” (Belhoucine 2013; Smahi et al. 2014; Bendjebbar et al. 2020).Several studies confirm that the fungus is closely associated with the decline of trees of the genus Quercus (Santos 2001), particularly in the Mediterranean basin (Bakry and Abourouh 1996; Franceschini et al. 2005).In order to determine the aggressiveness of *B. mediterranea*strains isolated from asymptomatic trees in the forests of western Algeria, a pathogenicity test was carried out.

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The present study arose from a question about the susceptibility of young local seedlings of the genus Quercus to be infected by the fungus, under conditions of water stress.

Material and methods

In order to study the pathogenicity of *Biscogniauxia mediterranea*strains on young cork oak and holm oak seedlings, an in vivo pathogenicity test was performed.

The pathogen

Two isolates of *B. mediterranea* were used in this pathogenicity test. These two strains were all isolated from symptomatic cork oak trees in two representative forests of western Algeria. These are the Hafir forest (W. Tlemcen) and the Djebel Saadia forest (W. Relizane). These isolates were identified (by sequencing of their elongation factor 1-alpha) in the plant pathology laboratory, Kwazulu Natale University, South Africa.

Plant material

The pathogenicity test of the two isolates of *B. mediterranea* (B.M H1) and (B.M D1) was carried out on young seedlings (one year old) of *Q. suber* and *Q. ilex*.

The acorns are from the two forests: Hafir and Zarriffet (W. Tlemcen), and they are grown in the National Park of Tlemcen in plastic pots containing peat. The young seedlings were kept in a greenhouse at room temperature under natural light.

A total of sixty (60) oak seedlings were used in this pathogenicity test: thirty (30) cork oak and thirty (30) others of holm oak.

The process of inoculating the plants

The inoculation process was carried out according to the method of Linaldeddu et al. (2014). The protocol is as follows:

Young seedlings of each variety were inoculated at about 15 to 20 cm above ground level and in the internodes. The inoculation point was previously disinfected with 70% ethanol, in order to eliminate any saprophytic culture.Then, using a sterile scalpel, a portion of the bark about 1 to 2 cm was carefully removed. A 5 mm mycelial plug of each isolate studied was taken from the margin of an actively growing culture and placed on the part of the barked stem.

The inoculation point was covered with cotton soaked in sterile distilled water, then fixed with para-film and wrapped with a piece of aluminium foil.

A total of sixty (60) seedlings were inoculated, twenty-four (24) seedlings for each variety, and twelve (12) seedlings were reserved as a control.The latter were inoculated with sterile PDA plugs.

The labeled seedlings were placed in an airy, sunny place at a temperature ranging from 18 to 26 °C (Fig. 1). The infected seedlings were subjected to a different irrigation regime: Twelve (12) seedlings of each species underwent water stress (watering once a week), and twelve (12) others underwent normal irrigation according to the needs of the plant. Six (6) seedlings for each isolate (B.M H1 and B.M D1) (Fig.1).

The seedlings were watered gently with the sampling water at the same time (morning).

Monitoring and reading the results

The tested seedlings were observed daily throughout the pathogenicity test period, in order to note the evolution of symptoms.

Fig 1. The process of inoculation of seedlings by *B. mediterranea*: (a) the fungus on PDA, (b-f) inoculation of the fungus at the level of the seedling stem.

At the end of the experiment, the results were expressed according to the scoring system described by Hibar et al. (2005): The seedlings of each variety were divided into five classes of 0-4, depending on the severity of the symptoms.

- ➢Class 0: no symptoms, healthy plant.
- ➢Class 1: slight yellowing, slight rot (necrosis up to 10%).
- ➢ Class 2: significant yellowing of the leaves with or without wilting up to50% of the surface and necrosis up to 25% of the surface.
- ➢ Class 3: significant rot of the collar and browning of the stem vessels.
- ➢ Class 4: dead plant.

A disease index (DI) was calculated using the following formula:

$$\text{I.M} = \frac{\Sigma\,(g \times n)}{N}$$

g = damage class,n = number of samples belonging to each class,N = 30, the total number of seedlings in each variety.

Then, all seedlings were examined. Necrotic areas were assessed by cutting the stem longitudinally through the inoculation site.The extent of vascular discoloration, caused by each isolate tested, in all inoculated varieties was measured upward and downward from the inoculation point.Re-isolation of inoculated isolates was performed from necrotic lesions in symptomatic seedlings.

Statistical analyses

Statistical analysis of data relating to the length of infected areas (stroma/stem blackening) of each seedling with each isolate of *B. mediterranea* was performed. The statistical program used is Minitab 2019. Indeed, analyses of variance (ANOVA) and the Fisher multiple comparison test were used to test the effects of inoculations of the two isolates of *B. mediterranea* on the health status of young seedlings of the genus Quercus (*Q. suber* and *Q. ilex*).

Results and discussion

Effect of B. mediterranea isolates on Q. suber seedlings

Symptomatology

After the inoculation period (thirty days), all inoculated seedlings showed symptoms of *B. mediterranea* infection.In young seedlings that have undergone water stress, the most observed symptoms, on almost all inoculated plants, are leaf necrosis. Yellowing and chlorosis of the leaves were observed after twelve days of inoculation. Some plants show a strong leaf drop. On the other hand, others dry out completely above the inoculation point.

At the trunk level, necrosis was observed around the inoculation point. After removal of the bark, a dark brown stain tending towards black was observed in all inoculated seedlings. These spots spread on the vascular tissues up and down the inoculation points.No necrotic lesions were developed on the seedlings inoculated by PDA (Fig. 2.A-F).

Young seedlings inoculated with both isolates of *B. mediterranea* without water stress developed symptoms 15 days after inoculation. Almost all seedlings behaved in an equivalent manner. Yellowing and leaf necrosis were the first symptoms observed.In addition, both isolates of the fungus produced visible cankers on the stems of the inoculated seedlings. Under the bark, brownish lesions spread to the vascular tissues.In seedlings inoculated with sterile PDA, no necrotic lesions were observed(Fig. 2.G-K).

No seedling mortality was detected at the end of the experiment.

Fig 2. Effect of *B. mediterranea* isolates on young cork oak seedlings with (A-F) or without (G-K) water stress after 30 days of inoculation: (A) Plants at the beginning of the test, (B) Necrotic lesions in vascular tissues, (C) Leaf drop, (D) Leaf drying and drop, (E) Leaf yellowing, (F) Control plants, (G) Plants at the beginning of the test, (H) Leaf necrosis, (I) Discoloration of the outer bark, (J) Necrotic lesions in the xylem tissues, (K) Spots and drying at the leaf tips.

Statistical analysis

Both isolates of the fungus proved their virulence by developing necrotic zones at the xylem level.

Figure 3 shows a comparison between the length of necrotic lesions in *Q. suber* L. seedlings tested by the two *B. mediterranea* isolates with and without water stress.

Analysis of variance (two-way ANOVA), using Tukey's test with a confidence level of 95%, showed no significant difference in the mean length of necrotic lesions of seedlings inoculated with the two *B. mediterranea* isolates. These two strains reacted similarly in the vascular tissues of the plants.

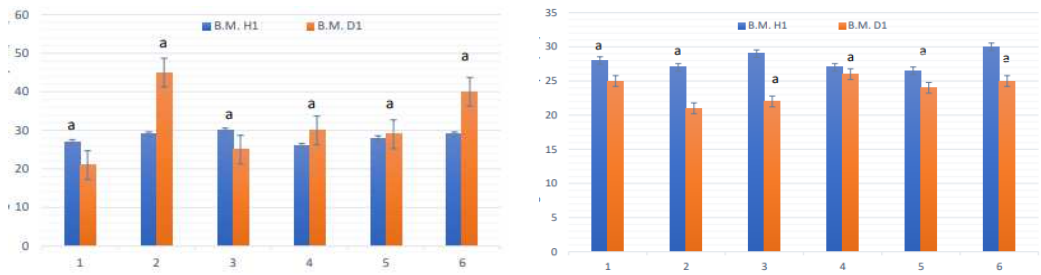

Fig 3. The length of necrotic lesions (in mm) caused by the two *B. mediterranea* isolates on cork oak plants with (Left) and without (Right) water stress after 30 days of inoculation. Letter (a) indicates significantly different means (Tukey test, $P<0.05$).

Effect of B. mediterranea isolates on Q.ilex seedlings

Symptomatology

Young holm oak plants inoculated with both*B. mediterranea* isolates began to show symptoms on the 20th day of inoculation. The aggressiveness of the B.M. D1 isolate towards holm oak was noted by the severe symptoms displayed on the aerial part of the plants compared to the second B.M. H1 isolate.At the aerial part of the plants, the isolates infected the leaves, causing drying and yellowing. The aggressiveness of the fungus is also reflected on the vascular tissues of the plant. The different strains developed necrotic areas on the stem. The length of each lesion differs depending on the isolate inoculated. On the other hand, no necrotic lesions were developed on the holm oak seedlings inoculated with sterile PDA (Fig 4.).

Fig 4. Effect of *B. mediterranea* isolates on young holm oak seedlings under water stress after 30 days of inoculation: (A) Control plants, (B, C) Length of necrotic lesions in vascular tissues, (D) Leaf drying and dropping, (E)Control plants, (F) Yellowing and chlorosis of the leaves, (G)Blackish spots on the inoculated stems, (H) The absence of necrosis in the control plants

Statistical analysis

Q. ilex seedlings present necrotic lesions which differ from one seedling to another depending on the type of isolate inoculated.

The results of the two-way ANOVA analysis of variance showed no statistically significant difference (95% confidence level) in the lengths of necroses developed in holm oak seedlings inoculated with the two B. mediterranea isolates (Tukey test with $p \leq 0.01$). This can be explained by the fact that, whatever the isolates inoculated, the difference between them, in terms of the length of necrotic lesions remained very low with or without water stress (Fig. 5).

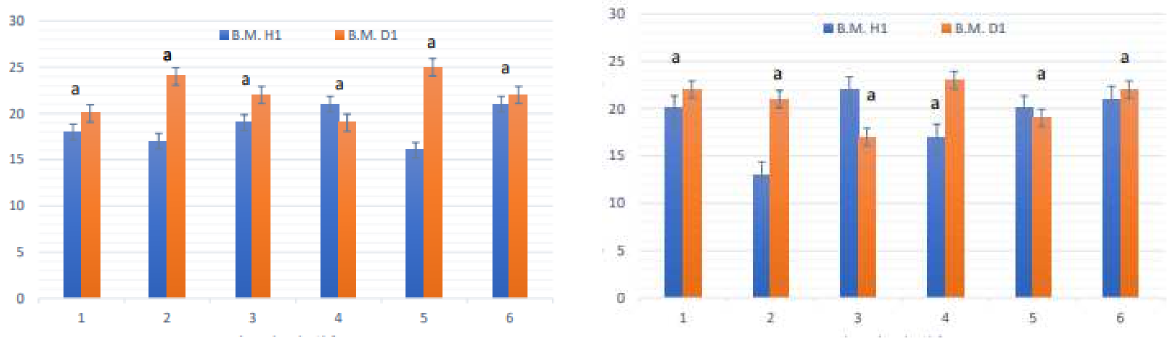

Fig 5. The length of necrotic lesions (in mm) caused by both *B. mediterranea* isolates on holm oak plants with (Left) and without (Right) water stress after 30 days of inoculation. Letter (a) indicates significantly different means (Tukey test, P<0.05).

The "I.M" disease index

The pathogenicity of *B. mediterranea* is reflected in the disease index "I.M". This index was calculated and compared between the two isolates B.M. H1 and B.M. D1 (Fig. 6).

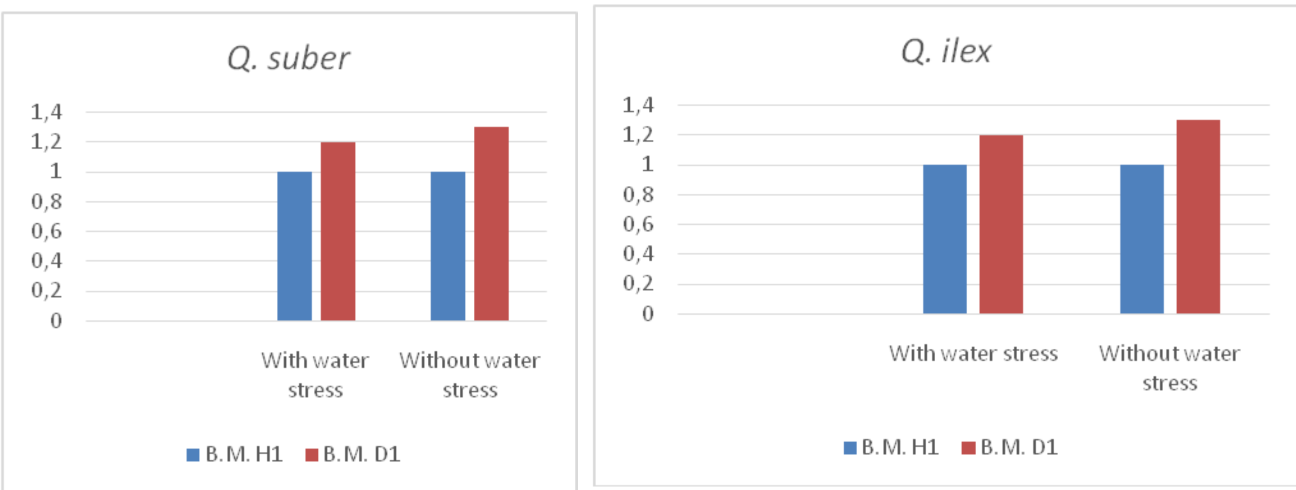

Fig 6. Disease index (D.I.) in *Q. suber* and *Q. ilex* seedlings with or without water stress.

B. mediterranea isolates were successfully re-isolated from necrotic lesions in inoculated symptomatic seedlings.

Conclusion

This work focuses on the study of the pathogenic power of *B. mediterranea* on young seedlings of Quercus (*Q. suber* and *Q. ilex*). The isolates were collected from the charcoal stromata of dying trees in two forests in western Algeria. These are the Hafir forest (W. Tlemcen) and the Djebel Saadia forest (W. Ghilizane).
The in vivo test carried out under controlled conditions confirmed the pathogenicity of the two *B. mediterranea* isolates after 30 days of inoculation. In fact, the B.M. D1 isolate proved to be the most aggressive species, causing significant necrotic lesions in the bark of the inoculated seedlings, followed by the B.M. H1 isolate. The aggressiveness of the *B. mediterranea* strains has already been confirmed on cork oak and holm oak by previous studies (Francheschini et al.2005).
In conclusion, the arsenal for combating plant diseases in forest ecosystems is based on a thorough knowledge of the pathogens involved, their biology and their interactions with host plants and the environment. In light of this conclusion, we consider that our study, like any other research, can only be participatory and absolutely requires the complementarity of other studies.
The arsenal for combating plant diseases in forest ecosystems is based on a study of the pathogens involved, their biology and their interactions with host plants and the environment.Finally, we consider that our study, like any other research, can only be participatory and absolutely requires the complementarity of other studies.

Acknowledgment

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Conflict of interest

There is no conflict of interest to be declared.

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