

Pathogenic Capacity of *Botrytis cinerea* on Leaves of *Pyrus mamorensis*, an Endemic Tree of Mamora Forest in Morocco

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Abstract

A survey in the Mamora forest was done in the spring of 2010 and revealed that 67% of buds and 27% of leaves of *Pyrus mamorensis* (Trabut) samples collected had lesions with a gray felting. The pathogenic fungus was identified as *Botrytis cinerea* by the filter – paper technic. Koch’s postulate was verified by inoculating healthy leaves. The estimated disease severity on *P. mamorensis* leaves was respectively 75.56% and 68.81% for inoculation by conidial suspension and the mycelial disks. Conidia production of *Botrytis cinerea* on inoculated leaves by conidial suspension was $1.03.10^5$ conidia.cm⁻² and by mycelial disks was $0.60.10^5$ conidia.cm⁻². This was the first report of gray mold disease of Mamora pear caused by *Botrytis cinerea* in Morocco.

Keywords: Morocco, *Pyrus mamorensis*, *Botrytis cinerea*, gray mold, inoculation.

Introduction

Mamora pear (*Pyrus mamorensis*, Traub) is a perennial plant belonging to Rosaceae. Its abundant in Mamora forest (Morocco) is particular but does not form large stands. This species does not receive any conservation effort despite its endemism and rarity (Fennane et al., 1998). In addition, the reasons of the declining health of this tree over the long term and especially in periods of drought where stress by human activity, livestock grazing and attack by *Pestalotia subcuticularis* (Yamni et al., 2006). Other fungi were also isolated from leaves, flowers, fruits or trunk of this tree as *Aspergillus niger*, *A. fumigatus*, *A. Versicolor*, *Alternaria alternata*, *Cladosporium herbarum*, *Drechslera australiensis*, *Mucor* sp., *Rhizopus stolonifer*, *Epiccocum nigrum*, *Trichoderma harzianum*, *Curvularia lunata*, *Hysterium pulicare*, *H. asymmetricum*, and *Trematosphaeria pertusa* (Sellal et al., 2012).

In the spring of 2010, the survey in the Mamora forest revealed that 67% of 100 buds and 27% of 100 leaves collected from eight trees of *Pyrus mamorensis* had lesions with a gray felting. Leaf blights were gray with brown contour (Fig. 1a). The other lesions were brown spots with a gray center (Fig. 1b). The buds were brown with a gray felting (Fig. 1c). This was the first time that these symptoms were observed on *Pyrus mamorensis*, endemic species in Morocco.

The objective of this work was to achieve isolation of the pathogenic fungus from the diseased leaves and buds of *Pyrus mamorensis* Traub and to verify the Koch’s postulate.

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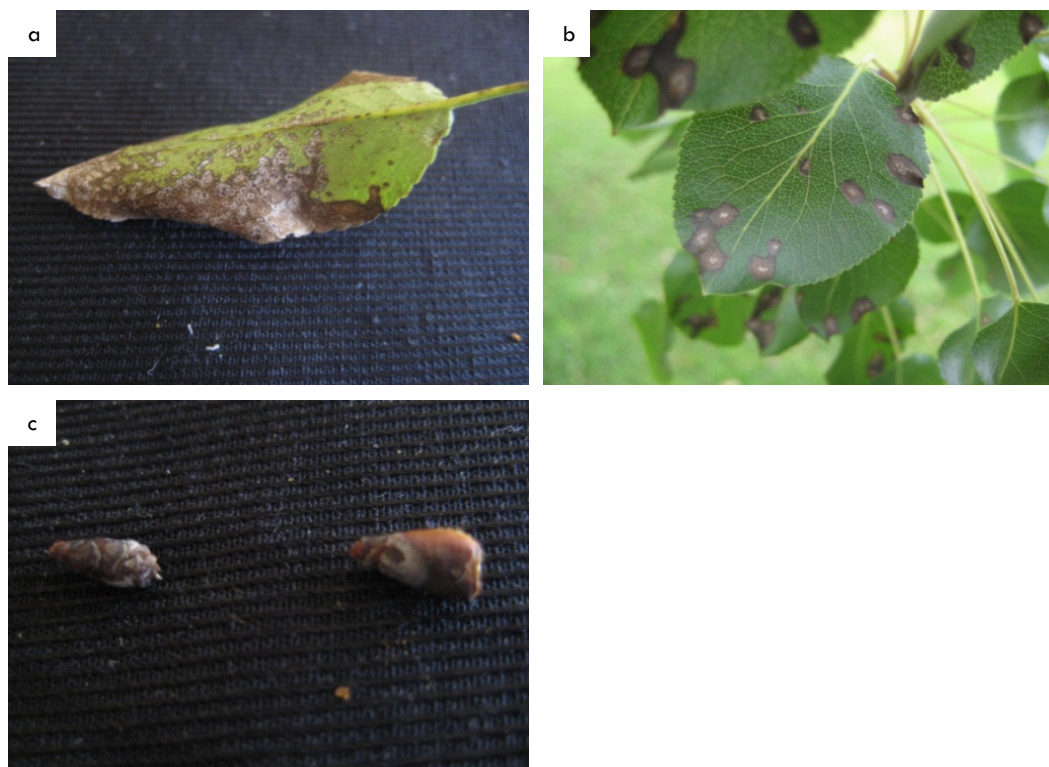


Fig. 1. *Botrytis cinerea* symptoms developed on leaves (a, b) and buds (c) of *Pyrus mamorensis*.

Material and Methods

Twenty leaves and ten buds showing lesions with a gray felt-like growth were collected from eight trees of *Pyrus mamorensis* and cut into fragments, washed in tap water, disinfected with alcohol and placed in Petri dishes on filter paper moistened with sterile distilled water. After incubation at 22°C under continuous lighting for 2–3 days, leaves and buds fragments were examined using an optical microscope and the conidia emerging from the lesions were taken with a capillary tube and displayed on agar medium (15 g Agar-agar, distilled water 1000 ml). A single spore was placed on PSA medium (Potato Sucrose Agar: 200 g potato, 20 g sucrose, 15 g Agar-agar, distilled water 1000 ml) and incubated in the dark for five days at 28°C (Benkirane, 1995). The developed colonies represented as pure cultures, were retained and used for species determination.

Pathogenicity tests were realized by inoculating healthy leaves of *Pyrus mamorensis* using two techniques. The surface of sixty leaves was disinfected with 5% sodium hypochlorite, washed with sterile distilled water and dried on a filter paper. Fifteen leaves were inoculated with mycelial disks of the fungus, the other fifteen by a conidial suspension adjusted to a final concentration of 10⁵ conidia.ml⁻¹ with sterile distilled water containing 0.05% Tween 20 and 5% gelatin. Thirty leaves were used as a control, the half of them were inoculated with distilled water containing Tween 20 and gelatin and the other half with medium plugs. Every bunch of five leaves was placed in 120-mm

Petri dish containing small glass beads and sterile distilled water. Inoculated leaves were incubated at 28 ± 1 °C under black plastic sheeting that was removed after 24 hrs.

The disease severity was scored after 7 days of inoculation using the scale of Stover modified by Gauhl et al., (1995).

Class	1	2	3	4	5	6	7
%Diseased leaf area	-0,5% of the limbus with symptoms	0,6 à 5%	6 à 15%	16 à 30%	31 à 50%	51 à 80%	81 à 100%

The severity index (IS) of disease was calculated using the formula:

$$IS = (\sum nb / (N - 1) \times T) \times 100$$

n = Number of leaves for each degree of the scale

b = Degree of the scale

N = Number of the degrees used in the scale

T = Total number of the scored leaves

The conidia production (conidia cm⁻²) of *Botrytis cinerea* on the inoculated Mamora pear leaves was estimated according to the technique of Hill and Nelson (1983). Ten days after inoculation, the leaves that had shown lesions were cut into pieces of 1 cm² and placed in 90 mm Petri dishes on three filter paper discs moistened with sterile distilled water. The dishes were in-

cubated for 48 hours under continuous fluorescent lighting. Then each fragment was placed in a test tube containing 1 ml of sterile distilled water and agitated by a vortex mixer for 2 min. The conidia of the pathogen were counted using a Malassez slide under an optical microscope at magnification $\times 100$ with 10 counting of each sample.

Results and Discussion

On PSA, the growing colonies of the isolated fungus were white at first, gray as it ages and whitish gray in reverse (Fig. 2a). The mycelium was very large and dense, 12 μm of wide, cylindrical, often banded, especially when cultures are older. It appeared light brown to olive. The conidiophores were clear colored and shiny. Their back always branched (Fig. 2b) and supported small sterigmata. The conidia were ellipsoidal or ob-ovoid, with slightly protuberant hilum colorless to pale brown 7-15 \times 5-9 μm (Fig. 2c) and abundant sclerotia developed as the fungus culture ages. Based on the morphological description Ellis (1971), the fungus was identified as *Botrytis cinerea* Pers. 1794.

Botrytis species were important pathogens of nursery plants, vegetables, ornamental, field and orchard crops, stored and transported agricultural products (Elad et al., 2007).

The *Botrytis cinerea* Pers. ex Fr. caused gray mold disease in a wide variety of hosts (Coley-Smith et al., 1980; Elad et al., 2004), and was a serious economic problem in crops such as table grapes, vines, strawberries, raspberries, lettuce, cucum-

bers, broad beans, tomatoes, beans, flowers, and forest plants produced in containers (Elad et al., 2004). Gray mold was an important sanitary problem in *Pinus radiata* D. Don and *Eucalyptus globulus* Labill. in Chilean forest nurseries (Butin and Paredo, 1986). *Botrytis* sp. and *B. cinerea* were also isolated from the young diseased plants of *Cedrus atlantica* (Endl.) Manetti ex Carriere respectively in Morocco (Bakry et Abourouh, 1992) and in France (Abourouh et Morelet, 1999). *B. cinerea* seemed to prefer senescent leaves but were also found in other stages in *Quercus rotundifolia* Lam. in a holm oak forest (High Atlas, Morocco) (Sadaka and Ponge, 2003).

Two days after inoculation of leaves of *Pyrus mamorensis* with conidial suspension, the first symptoms of *Botrytis cinerea* appeared at the limbus and then covered the entire leaf surface. The lesions were brown to black (Fig. 3b). The second type of lesions were black that appeared first at the tips then grew and covered the leaf surface (Fig. 3c). The lesions appeared also on the petioles.

The inoculated leaves with mycelial disks showed brown lesions, grayish to blackish in the disk and then cover leaf surface with blackening of the petiole (Fig. 4b and c). Seven days after inoculation, the estimated disease severity on leaves of *P. mamorensis* was 75.56% and 68.81% for inoculation by conidial suspension and the mycelial disks. No disease was observed on the control leaves (Fig. 3a and Fig. 4a).

Ten days after inoculation, *Botrytis cinerea* produced conidia abundantly on leaves of *P. mamorensis* inoculated both by conidial suspension ($1.03.10^5$ conidia. cm^{-2}) and the mycelial disks

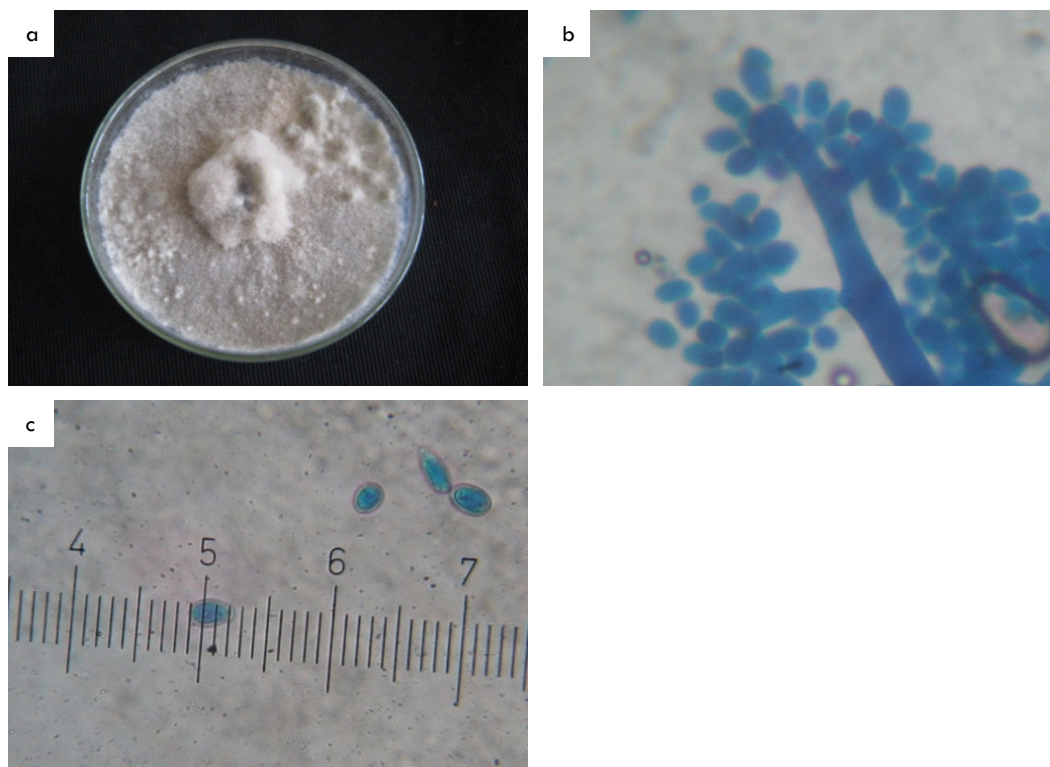


Fig. 2. *Botrytis cinerea* on PDA (a), conidiophores (b) and conidia (c) on cotton blue ($\times 400$).

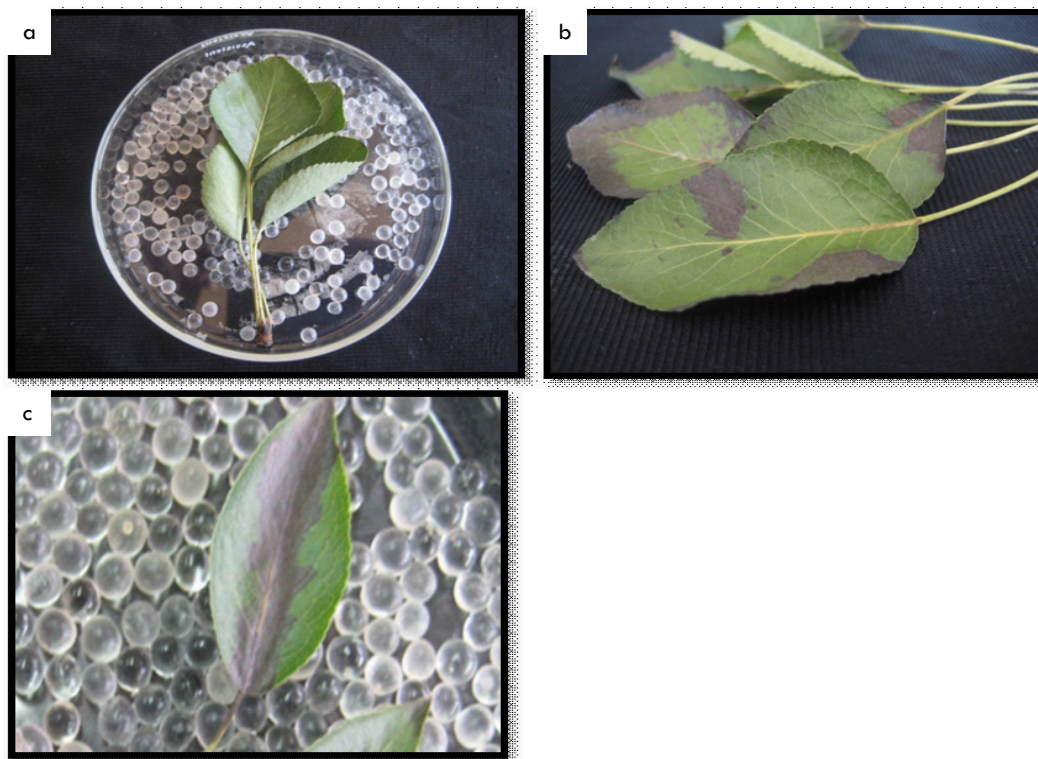


Fig. 3. Symptoms caused by *B. cinerea* on leaves of *Pyrus mamorensis* inoculated with conidial suspension (b, c), control (a).

($0.60 \cdot 10^5$ conidia.cm⁻²).

The pathogen was a common fungus that damaged flowers, leaves, stems, fruit and other parts of many plants (Ellis, 1971; Elad and Shteinberg cited by O'Neill et al., 1997). The fungus exists in different habitats as mycelia, micro- and macroconidia, chlamydospores, sclerotia, apothecia and ascospores and these are dispersed by diverse means (Jarvis, 1980). If it is to infect, the fungus must conquer space (Zadocs and Scein, 1979) that is to move from the primary source and land on susceptible tissue. Each part of the fungus thallus can serve as a dispersal unit. These propagules are dispersed by wind, rain and insect.

Botrytis cinerea was observed causing gray mold in *Pyrus communis* (Sommer et al., 1985; Shiva, 1989; Crous et al., 2000), *P. serotina* (Cho and Shin, 2004), *P. serotina* var. *culta* and *P. betulifolia* (Kobayashi, 2007).

However, to our knowledge, this was the first report of gray mold disease of *Pyrus mamorensis* caused by *Botrytis cinerea* in Morocco. The pathogen was a real threat to this tree already weak.

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