

ABSTRACT

Almond is one of the main crops of Majorca Island. Since 2008, symptoms of severe decline of almond trees have been observed in several orchards from different areas of the Island. Disease symptoms are similar to those described by different authors in other parts of the world caused by fungal trunk pathogens. In order to study the etiology of this problem, surveys were conducted on almond orchards distributed throughout the main growing regions in Majorca for six consecutive years (2009-2014). Disease symptoms included leaf chlorosis, general decay, and died branches. Internal wood symptoms ranged from brown to black vascular streaking, visible in cross sections as spots or circular discolouration of the xylem tissue, and wedge-shape necrosis or soft wood. Based on morphological and molecular identification, 14 fungal species were recovered from almond wood samples: *Collophora hispanica*, *Diplodia olivarum*, *D. seriata*, *Eutypa lata*, *E. leptoplaca*, *Fomitiporia mediterranea*, *Neofusicoccum luteum*, *N. mediterraneum*, *N. parvum*, *Omphalotus olearius*, *Phaeoacremonium amygdalinum*, *Pm. iranianum*, *Phellinus pomaceus* and *Pleurostomophora richardsiae* and two species were recovered from one apricots orchard near almonds orchards: *Pm. minimum* and *Pm. venezuelense*. Based on the DNA sequence analyses and morphological features, *C. hispanica* and *Pm. amygdalinum* proved distinct from all known species, and have been described. The most common species recovered from almond samples were *P. richardsiae* and *D. seriata*, followed by other species belonging to the family Botryosphaeriaceae and *C. hispanica*. The most frequently species isolated were also widely distributed and present in more regions. Subsequently, two pathogenicity tests were carried on almond trees by using representative isolates of some of the most frequent species. The first one was held for two consecutive years (2013 and 2014) with five species of Botryosphaeriaceae (*D. olivarum*, *D. seriata*, *N. luteum*, *N. mediterraneum* and *N. parvum*) and two species of Diatrypaceae (*E. lata* and *E. leptoplaca*). Fungi were inoculated on 1-2 years old almond trees of four different cultivars ('Jordi', 'Ferragnes', 'Pons' and 'Vivot') under field conditions. Nine months after inoculation, the total length of internal necrosis was evaluated. All species were pathogenic on almond. *Neofusicoccum luteum* caused the longest average lesion during the first year, and *N. mediterraneum* and *N. parvum* caused the longest lesion during the second year. *Eutypa leptoplaca* caused the shortest lesion length in both years of study. In addition, fungal lesion length varied depending on the variety of almond evaluated. In the first year of study, the more tolerant variety was 'Jordi', while in the second year, 'Ferragnes' and 'Vivot' varieties showed the highest degree of tolerance to fungal infection. In the second trial, almond seedlings variety 'Ferragnes' were inoculated with *C. hispanica*, *Pm. amygdalinum*, *Pm. iranianum* and *P. richardsiae*. Six months after inoculation the lesion length was evaluated. All species inoculated were pathogenic on almond, being *P. richardsiae* the most virulent species. Finally, the ability of some commercial fungicides to protect pruning wounds from infection by four species of Botryosphaeriaceae (*D. seriata*, *N. luteum*, *N. mediterraneum* and *N. parvum*) was evaluated. This study was conducted in two phases, an initial *in vitro* evaluation (mycelial growth assay) with 10 fungicides, followed by an evaluation

of five fungicides, which proved to be effective in the *in vitro* trial, applied on pruning wounds at 1 and 7 days after inoculation. Internal lesion length and the percentage of re-isolation of the pathogen were calculated. tebuconazole and pyraclostrobin were the most effective fungicides in the *in vitro* evaluation, followed by cyproconazole and thiophanate-methyl. Thiophanate-methyl was the most effective fungicide to protect pruning wounds from infections caused by species of Botryosphaeriaceae.