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DISEASE NOTES

**Iberian Wild Pear (*Pyrus bourgaeana*) is a New Host of *Erwinia amylovora*, the Causal Agent of Fire Blight**

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The Iberian wild pear (*Pyrus bourgaeana* Decne) is a deciduous tree in the Mediterranean forest and evergreen open woodlands (dehesas), which plays an important trophic role in ecological balance. Its populations are dispersed through central and western Spain, southern Portugal, and northwestern Africa, and has been considered a threatened species. In April 2010, unusual symptoms were observed in Iberian wild pear trees near the National Park of Cabañeros, Ciudad Real Province, Spain. The symptoms were similar to those of fire blight reported from other hosts, and included blossom necrosis, leaf and shoot blight, fruit mummification, and internal necrotic tissue in branches and twigs. In some cases, blight reached the entire tree. Samples were analyzed according to the EPPO diagnostic protocol for *Erwinia amylovora* ([EPPO 2013](javascript:popRefFull('b1'))) and this pathogen was recovered in pure culture in 70.5% of samples. Nine isolates, Gram-negative and negative for oxidase test, showed the typical colonial morphology of *E. amylovora* on King’s B, NSA, and CCT media. Their biochemical and physiological profiles in the API 20E and API 50CH strips (bioMérieux) showed that they were able to produce acid from glucose, mannitol, sorbitol, sucrose, and arabinose, and unable to use galacturonate and malonate as sole carbon sources, as the reference *E. amylovora* strain CFBP1430. Two conventional and two real-time PCR protocols specific for *E. amylovora* with primers G1-F/G2-R ([Taylor et al. 2001](javascript:popRefFull('b5'))), AJ75/AJ76, and PEANT1/PEANT2 in a nested PCR ([Llop et al. 2000](javascript:popRefFull('b3'))); primers Ams 116F/Ams 189R and probe Ams 141T ([Pirc et al. 2009](javascript:popRefFull('b4'))), and primers hpEaF/hpEaR and probe hpEaP ([Gottsberger 2010](javascript:popRefFull('b2'))), respectively, were performed. The results with all primer sets were positive, obtaining in the two conventional PCRs the expected products of 187 and 391 bp, respectively, and in the two real-time PCRs Ct values ranging from 13.8 to 15.1 and 13.4 to 18.3, respectively, just as with the French reference strain. Moreover, all strains were positive by the double antibody sandwich indirect (DASI)-ELISA test specific for *E. amylovora* (Plant Print Diagnòstics S.L.). The pathogenicity of all isolates was assayed on young shoots of *P. bourgaeana* and immature pear fruits of *P. communis*. Shoots were inoculated by cutting two young leaves to the main vein with scissors dipped into 109 CFU/ml bacterial suspensions in phosphate buffered saline solution 10 mM, pH 7.2 (PBS). The inoculated shoots were incubated in pots with sterile 1% agar at 25°C for 7 days. After day 2, leaves showed necrosis from the main vein to the entire leaf blade, typical symptoms of fire blight. For fruit assays, four small incisions were made on the peel of previously disinfected fruits, and then inoculated with 10 µl per cut of 109 CFU/ml bacterial suspensions in PBS. The inoculated fruits were incubated on sterilized plastic boxes at 25°C for 7 days. From day 3, the fruits exhibited severe necrosis and bacterial ooze at inoculated sites. In all cases, similar symptoms were observed after inoculation with a suspension of the reference strain. No symptoms were observed on PBS-only negative controls. Pure cultures of presumptive *E. amylovora* colonies recovered from symptomatic shoots and fruits were confirmed by DASI-ELISA and real-time PCR ([Gottsberger 2010](javascript:popRefFull('b2'))). Pathogenicity assays were repeated at least twice. In conclusion, these results confirm the identification of *E. amylovora* as the causal agent of the symptoms observed in Iberian wild pear trees. This is the first report of *P. bourgaeana* as a new host of *E. amylovora*. The adoption of measures to protect this tree species from fire blight is compelled by the high relevance and significance of Iberian wild pear for the fauna and forest ecosystem, the increasing interest of the dehesas, and the necessity of preserving the biodiversity harbored in the Mediterranean region.

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