Introduction

During August 2012, vertical oozing cankers have been observed on trunks and branches of walnut trees (Juglans regia L.) in the city of Zánka, near Lake Balaton and other parts of Hungary. Cankers were observed on trunks and branches, where brown-to-black exudates staining appeared on the bark mainly in the summer. The causative agent, a bacterium was first reported in California (Wilson et al., 1957; Hauben et al., 1998), and was also recorded in Iran, Spain, France and several Italian locations from walnut trees. The infected shoots were analysed in the laboratory of Department of Plant Pathology at Corvinus University of Budapest.

Materials and methods

The bacteria were isolated from walnut trees. The pathogens were identified with classical (morphological, biochemical and physiological properties, pathogenicity test), and molecular (16S rRNA) methods. After the identification artificial infections were performed on fruits and scions of ten different walnut cultivars (Alsószentiváni 117', 'Alsószentiváni késel', 'Bonifác', 'Milotai 10', 'Milotai intenzív', 'Milotai késel', 'Milotai bőtermő', 'Tiszacsécsi 83', 'BD6', and Juglans nigra). The formerly mentioned analytical methods were used. To determine the susceptibility/resistance of cultivars we relied on the symptoms on fruits and scions.

Results and discussion

The bacterial strain isolated from walnut trees was gram negative and did not induce a hypersensitive reaction in tobacco (Nicotiana tabacum L. 'White Burley') leaves. Colonies on KB medium were white and non-fluorescent. The results of substrate utilization profiling using the API 20E kit (Biomerieux, Marcy l’Etoile, France). The isolate showed positive reaction for citrate utilization, H₂S and acetoin production and also in urease, glucose, inositol, saccharose and arabinose reactions. Pathogenicity was tested by injecting five young healthy walnut branches with bacterial suspension of 10⁶ CFU/ml. Branches were enclosed in plastic bags and returned to the greenhouse under 80% shade at 26°C day and 17°C night temperatures. Three months after inoculation, necrotic lesions were observed in the inner bark and dark lines were observed in internal wood, but no external cankers were observed on inoculated branches.

For molecular identification of the pathogen the 16S rDNA amplification was performed from strain Bn-WalnutZa-Hun1 (Accession No. HF936707). On the basis of the symptoms, colony morphology, biochemical test and 16S rDNA sequence analysis, the pathogen was identified as Brenneria nigrifluens. On walnut fruits and scions natural infection through superficial scars was imitated in vitro. Green walnut fruits (15 fruits/cultivar) and scions (5 scions/cultivars) were used for this purpose. One punctures per fruit and scions were made with a needle previously dipped into the bacterial suspensions (10⁵ CFU/ml). Evaluation began on the five day (fruits) and three month (scions) after inoculation. Cultivars were divided into two categories: symptomless fruit and scion or very susceptible (water soaked spots, necrotic brown to black lesions with a exudates). The walnut fruits had not different resistance, but the Juglans nigra showed stronger symptoms. The scions were examined in the inner bark, and dark lines were observed in the internal wood, but no external cankers were appeared on inoculated branches. The walnut scions had not different resistance. According to our knowledge, this is the first report of a natural outbreak of bacterial bark canker on walnut in Hungary. The presence of the pathogen may seriously influence local orchards and garden production in the future. This appearance may have serious consequences for the future walnut production in Hungary.