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Disease Notes

# First Report of the Natural Infection of *Robinia pseudoacacia* with *Alfalfa mosaic virus*

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## Abstract

*Robinia pseudoacacia* L. (family Fabaceae), commonly known as black locust, is native to the southeastern United States, but has been widely planted and naturalized in temperate regions worldwide. In Europe it is often planted alongside streets and in parks, not only because of the dense canopy and impressive flower clusters in spring, but also because it tolerates air pollution well. In June 2012, several black locust trees exhibiting yellow leaf spots accompanied by mottling and leaf deformation were observed in a park in Backa Topola, North Backa District, Serbia. Numerous aphid colonies were found colonizing symptomatic trees. Leaves collected from nine symptomatic and 10 asymptomatic trees were tested for the presence of three

common aphid-transmitted viruses, *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus*, and *Potato virus Y*, using double-antibody sandwich (DAS)-ELISA with commercial polyclonal antibody (Bioreba AG, Reinach, Switzerland). Commercial positive and negative controls and extracts from healthy black locust leaves were included in each assay. AMV was serologically detected in all symptomatic and also in four of the asymptomatic trees, while no other tested viruses were found. Sap from affected leaves of a ELISA-positive sample (373-12) was mechanically inoculated onto five plants each of *Chenopodium quinoa* and *Nicotiana benthamiana* using 0.01 M phosphate buffer (pH 7). Symptoms including local chlorotic leaf lesions followed by mosaic on *C. quinoa* and a bright yellow mosaic on *N. benthamiana* were observed on all inoculated plants 5 and 10 days post-inoculation, respectively. The identity of the virus was confirmed using reverse transcription (RT)-PCR analysis. Total RNAs from all naturally and mechanically infected plants were isolated using RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). RT-PCR was carried out using the One-Step RT-PCR Kit (Qiagen) with primer pair CP AMV1 and CP AMV2 specific to the partial CP gene and 3'-UTR of AMV RNA 3 (1). Total RNAs from Serbian AMV isolate from alfalfa (GenBank Accession No. FJ527748) and RNA extract from healthy leaves of *R. pseudoacacia* were used as positive and negative controls, respectively. All tested plants, as well as the positive control, yielded an amplicon of the correct predicted size (751 bp), while no amplicon was recorded in the healthy control. The amplified product of isolate 373-12 was purified with QIAquick PCR Purification Kit (Qiagen) and sequenced on ABI PRISM 3700 DNA analyzer (Macrogen, South Korea) in both directions (KC288155). Pairwise comparison of the 373-12 isolate CP sequence with those available in GenBank, conducted with MEGA5 software (4), revealed the maximum nucleotide identity of 99% (99% amino acid identity) with the soybean isolate (HQ185569) from Tennessee. AMV has a worldwide distribution and its natural host range includes over 150 plant species, including many herbaceous and several woody plants (2). To our knowledge, this is the first report of *R. pseudoacacia* as a natural host of AMV worldwide. This finding has potentially significant implications for the successful production of susceptible crops, considering that black locust could act as an important link in the epidemiology of AMV as it may serve as a virus reservoir (3).

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