

First Report of *Aculeastrum (Pucciniastrum) americanum* Causing Late Leaf Rust on Raspberry in Slovenia

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In October 2024, a raspberry plant (*Rubus idaeus* cv. Polana) was collected in a raspberry farm near Ljubljana showing uredinia on sepals, fruits and the underside of leaves with brownish accompanying spots on the upper side. Disease incidence was high, as symptoms were observed on most plants, while disease severity varied, with some plants showing only incompletely ripening fruits. Microscopic observations revealed aculeate ostiolar cells and yellowish-orange, echinulate urediniospores. Spores were ellipsoidal to obovoidal with obtuse ends and measured 18.2 to 30.5 (average 24.1) × 11.7 to 19.4 (average 16.0) μm (n=60). The host species, occurrence of uredinia on fruits and morphological characteristics suggested *Aculeastrum americanum* (Farl.) M. Scholler & U. Braun (*Coleosporiaceae*, *Pucciniales*) as the causal agent. Genomic DNA extraction from an excised uredinium, PCR with primers (i) ITS1F and ITS4rust and (ii) Rust2inv and LR6, and Sanger sequencing with these and LR3 and LR0R as additional sequencing primers confirmed the identity of the involved species and thus, the disease as late leaf rust of raspberry. The ITS sequence from the Slovenian sample (GenBank accession PQ932524 for ITS and partial LSU) differs from *A. americanum* sequences MT022020–2 (from USA on *R. strigosus* or *R. pubescens*) published by Scholler et al. (2022) in a single nucleotide position; the LSU sequence is identical to MG907237 and MW147084 from *R. strigosus* or *Rubus* sp. (Aime and McTaggart 2021). Pathogenicity of the Slovenian isolate

was tested by spraying urediniospores as a watery Tween 20 (0.01%) suspension on leaves of three dormancy broken raspberry transplants cv. Heritage. Approximately 6×10^5 urediniospores, obtained from air-dried and refrigerated leaves from the originally obtained plant, were used per plant. Parallel tests suggested that 38% of spores produced germ tubes on 2% water agar. Sterile 0.01% Tween 20 solution was used for two negative control plants. Plants were covered with plastic bags and incubated in a growth chamber at 80% humidity, 20°C and darkness. After 42 h, plastic bags were removed and plants incubated under a 12-h light cycle by using Osram Fluora bulbs. After 14 days, individual leaves showed up to 5 uredinia on their abaxial surfaces. Spores from these uredinia were morphologically identical to the originally obtained urediniospores. No symptoms were present on control plants. *Aculeastrum americanum* is frequently reported from North America (Mycoportal 2025), where it can infect red raspberry species and host-alternates with *Picea glauca* (Scholler et al. 2022), while it has been rarely reported from South America so far [e.g., Argentina (Lucero et al. 2008)]. It was intercepted in quarantine in Japan (Motokura et al. 1999) and reported from New Zealand (Hofer et al. 2025) and a botanical garden in Switzerland (Gross et al. 2024). Varela et al. (2025) detected it from germplasm collections and plants in experimental field settings in Portugal. No other case of *A. americanum* was so far reported from Europe. If the pathogen could establish, it may impact raspberry production as it reduces leaf performance and damages berries.

References:

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Figure S1. *Aculeastrum americanum* on *Rubus idaeus*. Habit of uredinia on fruits (**a**), sepals (**b**), and leaf of field-infected cv. Polana (**c**, abaxial surface, left; adaxial surface, right; scale bar = 3 mm) and on leaf of cv. Heritage after artificial infection (**d**, scale bar = 3 mm). Urediniospores with germ tubes after 7 h incubation on 2% water agar (**e**, scale bar = 50 μ m); echinulate urediniospores (**f**, scale bar = 20 μ m) and aculeate ostiolar cells from cv. Polana (**g**, scale bar = 10 μ m)

177x144mm (300 x 300 DPI)