




Communication

# First Report of *Phaeoacremonium iranimum* Causing Olive Twig and Branch Dieback

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**Abstract:** In an olive orchard on the western part of Istria, Croatia, twig and branch dieback was observed on several olive trees. In total, seven samples from symptomatic trees were collected. Samples were analyzed, and four fungal isolates showed morphological similarities to the species *Phaeoacremonium*. One isolate, chosen as a representative, was taken for molecular identification and pathogenicity tests. Based on the DNA sequence data of the ITS, TUB, and EF1 $\alpha$  gene regions, the isolate was identified as *P. iranimum*. Pathogenicity tests were conducted on detached olive branches and olive trees in the greenhouse. To the best of our knowledge, this is the first report of twig and branch dieback on olive caused by *Phaeoacremonium iranimum*.

**Keywords:** *Phaeoacremonium iranimum*; olive; dieback



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## 1. Introduction

Olive (*Olea europaea* L.) is one of the most important crops in the Mediterranean part of Croatia. According to the latest statistical data, the Croatian national production of olives is approximately 23,000 tones [1]. Olive trees are known to be drought-resistant and hardy but susceptible to several major diseases [2]. Recently, however, olive trees are becoming more susceptible to diseases caused by phytopathogenic fungi. We believe the main reasons for this increased susceptibility are changes in cultivation methods, the planting of infected plant material, increasing resistance of pathogens to fungicides, and climate extremes. In recent years, there have been various occurrences of new diseases in olive trees in Istria that were unknown even to experienced olive growers. Unfortunately, studies of pathogens associated with olive decline in Croatia are few. In order to create a plant protection strategy (within the framework of sustainable olive production) and for proper tillage of the soil before planting (especially if a crop that hosts the same diseases as olive was grown on the plot), the detection of the causal agents of these unusual olive diseases is crucial.

## 2. Materials and Methods

### 2.1. Sampling and Fungal Isolation

In 2021, olive trees which showed signs of twig and branch dieback, discoloration of the bark, and necrotic lesions were spotted in an olive orchard on the western side of Istria, Croatia. The area of the orchard was 0.43 ha and contained approximately 70 olive trees. Disease incidence was reaching 40%. Olive trees of the orchard (100% local cultivar ‘Buza’) were over 30 years old and grown on the soil where grapevine had been grown beforehand. In total, seven samples from seven trees (one sample per tree) of branches from symptomatic trees of ‘Buza’ were collected and brought to the laboratory for analysis. Small pieces of branches (4 × 4 mm) were rinsed under tap water, surface sterilized in 70% ethanol for one minute, rinsed two times in sterile distilled water, and placed on a sterile paper sheet in a laminar flow cabinet until dry. Pieces of branches were plated on

Article

# Identification and Characterisation of *Pseudomonas savastanoi* pv. *savastanoi* as the Causal Agent of Olive Knot Disease in Croatian, Slovenian and Portuguese Olive (*Olea europaea* L.) Orchards

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**Abstract:** Strains of *Pseudomonas savastanoi* pv. *savastanoi* (*Pss*), isolated from infected olive trees (*Olea europaea* L.) in three European countries (Croatia, Slovenia and Portugal) were identified and characterised according to their colony morphology, physiological and biochemical features. According to the LOPAT scheme, 38.6% of *Pss* isolates were grouped in the Ib cluster. The Portuguese *Pss* strains were fully consistent with the typical LOPAT profile for this bacterium. Conversely, most Slovenian *Pss* strains showed delayed oxidase activity, whilst Croatian *Pss* strains did not produce any fluorescent pigment when grown in vitro. For *Pss* molecular identification, both end-point and real-time PCR were used, as well as MALDI-TOF, which was additionally used for proteomic analysis and the subsequent species identification of a number of strains that showed deviations from expected LOPAT results. *Pss* was confirmed as a causal agent of olive knot disease in 46.6% of olive orchards screened. Overall, these data suggests a possible correlation of certain *Pss* features with the geographical origin and the ecological niche of *Pss* isolates.

**Keywords:** LOPAT; colony morphology; strain; molecular identification; MALDI-TOF; olive variety

## 1. Introduction

*Pseudomonas syringae* (*P. syringae*) is a species complex of bacteria currently known to include at least 15 species, 13 phylogroups, and more than 60 pathovars [1–3]. Most of the strains belonging to the *P. syringae* species complex are plant pathogens or were originally detected in the agro-environment; however, several strains have also been isolated from habitats unrelated to agriculture [4]. *Pseudomonas savastanoi* (*P. savastanoi*) is one of the plant pathogenic species belonging to the *P. syringae* complex and has established infections in a wide array of wild and cultivated plants, including both herbaceous and woody species. However, each pathovar has a specific host range, as expected by definition [5]. The main host of Gammaproteobacterium *P. savastanoi* pv. *savastanoi* (*Pss*) is the olive tree (*Olea europaea* L.). Olives trees are grown in a large area worldwide, and are a typical feature of Mediterranean countries such as Portugal, Croatia and Slovenia, whose productions account for around 722, 150 and 33 thousand tons of olives per year, respectively [6]. Olive oil is important for its benefits to human health, due to its high content of secondary plant metabolites and antioxidants [7]. Olive trees are susceptible to many abiotic and biotic stresses that negatively impact yield and the organoleptic and/or biochemical traits of