



# First report of *Alternaria alternata* causing black spots in carob (*Ceratonia siliqua*) leaves in Europe

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In March 2024, in several carob (*Ceratonia siliqua*) orchards in the Ragusa province (Sicily, Italy), a high incidence of black spots (mean diameter  $\pm 2$  mm) on leaves and defoliation of trees was observed. Isolations from symptomatic leaves on potato-dextrose-agar amended with streptomycin (200.00  $\mu\text{g/ml}$ , consistently yielded a fungal species tentatively identified as *Alternaria alternata*. This identification was based on the morphology of colonies and conidia carried out as in Aloï et al. (2021). DNA of 12 selected isolates was extracted using the PowerPlant<sup>®</sup> Pro Kit. Their identity was confirmed by multigene phylogenetic analysis of ITS1–58S–ITS2, translation elongation factor 1- $\alpha$  (EF-1 $\alpha$ ), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and SCAR marker (OPA 10-2) regions amplified using ITS1/ITS4, EF1-728F/EF1-986R, GPD1/GPD2, and OPA10-2R/OPA10-2L primers, respectively (Aloï et al. 2021). All 12 isolates showed identical sequences. Pairwise alignments of ITS, TUB2, and GAPDH sequences showed 487/487, 193/194, 580/580 and 629/634 bp similarity, respectively, with those of *A. alternata* ex-type isolates in GenBank (Accession Nos. AF347031, KC584634, AY278808 and KP124632 respectively). The sequences of a representative isolate were deposited in GenBank (PP824821, PP821113, PP821114 and PP821115). The pathogenicity of the isolates was tested on detached carob leaves (10 leaves per isolate). Leaves were disinfected with 1% NaClO, rinsed with sterile distilled water, and inoculated by placing sporulating mycelium plugs (5 mm diameter) on the abaxial surface (Zhou and Xu 2014). PDA plugs were used for control leaves. Inoculated leaves showed necrotic spots after 7–10 days at

24 °C in a humid chamber, controls remained asymptomatic. *Alternaria alternata* was re-isolated from symptomatic leaves. Similar leaf diseases caused by *A. alternata* have been reported in Turkey (Antalya province) and Libya (Castello et al. 2023). This is the first report of *A. alternata* as a carob pathogen in Europe.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s42161-024-01741-w>.

**Data availability** All data of this study are included in this published article.

## Declarations

**Ethical approval** This article does not contain any studies with animals or human participants performed by any of the authors.

**Conflict of interest** The authors declare no conflicts of interest.

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