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Leaf spot caused by *Alternaria crassa* on *Datura stramonium* in Turkey

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Abstract
In August 2021, jimson weed (*Datura stramonium*) plants growing as weeds in potato fields in Bolu province, Turkey, exhibited leaf spots with dark concentric rings. Sunken and lens-shaped lesions with a light center were also frequently observed on petioles, branches, and stems. Based on morphological characteristics and phylogenetic analysis of the transcription elongation factor 1-α, RNA polymerase second largest subunit, and glyceraldehyde-3- phosphate dehydrogenase loci, the causal agent was identified as *Alternaria crassa*. The pathogen was successfully re-isolated from inoculated jimson weed plants in the pathogenicity assay, proving Koch's postulates. *Alternaria crassa* caused necrotic lesions on potato plants, similar to those of early blight, confirming them as an alternative host of the pathogen. This is apparently the first report of leaf spot caused by *A. crassa* on jimson weed in Turkey.

Keywords Jimson weed · Potato · Alternaria leaf spot

Datura stramonium, also known as jimson weed or thorn apple, is a wild Solanaceae species that competes with cultivated solanaceous species and many other crops in temperate, tropical, and subtropical regions around the world (Holm et al. 1997). It is considered a pest in agricultural areas, being an aggressive colonizer ability. The seeds contain a variety of substances, some with significant pharmacological and toxicological effects on humans and animals, even at low doses (Friedman and Levin 1989). This species occurs naturally in agricultural areas of Turkey and is a potential reservoir host for mites in solanaceous crops (Kumral and Çobanoğlu 2015).

A number of *Alternaria* species have been identified as causing Alternaria leaf spot on *D. stramonium* plants: *Alternaria crassa* (Simmons 2007; Woudenberg et al. 2014; Camino-Vilaro et al. 2019; Bessadat et al. 2020; Nishikawa and Nakashima 2020), *A. protenta* (Blagojevic et al. 2020), *A. solani,* and *A. alternata* (formerly *A. tenuis*) (Mułenko et al. 2008). In August 2021, irregular lesions with dark pigmented concentric rings and chlorotic haloes were observed on leaves of jimson weed plants (Fig. 1a) in commercial potato fields in the Köprücüler village (40.699353°N, 31.584826°E), Bolu Province, Turkey. More than 90% of plants were affected in the fields. Sunken and lens-shaped lesions with a light center were also observed on petioles, branches, and stems (Fig. 1b, c). The lesions on the leaves spread to the main vein, causing leaf curl and defoliation (Fig. 1d).

More than 10 symptomatic jimson weed leaves and stems were randomly sampled from naturally infected plants in two fields. For each plant, leaf pieces about 0.5 cm^2 were cut from the margins of necrotic lesions, surface-sterilized in 1% NaOCl for 60 s, and then 75% ethanol for 45 s before being rinsed three times with sterile distilled water.

Samples of leaves were placed on Petri plates containing V8 agar and incubated at 25 °C with a 12-h photoperiod for 5 days. Grey to brownish-black *Alternaria*-like colonies with concentric zones of very intensive sporulation developed, and four isolates were subcultured onto fresh V8 agar plates (Fig. 2a). All isolates produced a large conidial body with long apical beaks that tapered gradually from a wide base to a narrow tip. Conidia on V8 were mainly solitary and rarely in chains, pale brown, with a long-ovoid or ellipsoid body measuring 52–128 × 16–27 µm in size ($n = 30$) and long apical beaks measuring 90–445 µm ($n = 30$) (Fig. 2b, c). Conidia had 5–12 transverse and 1–5

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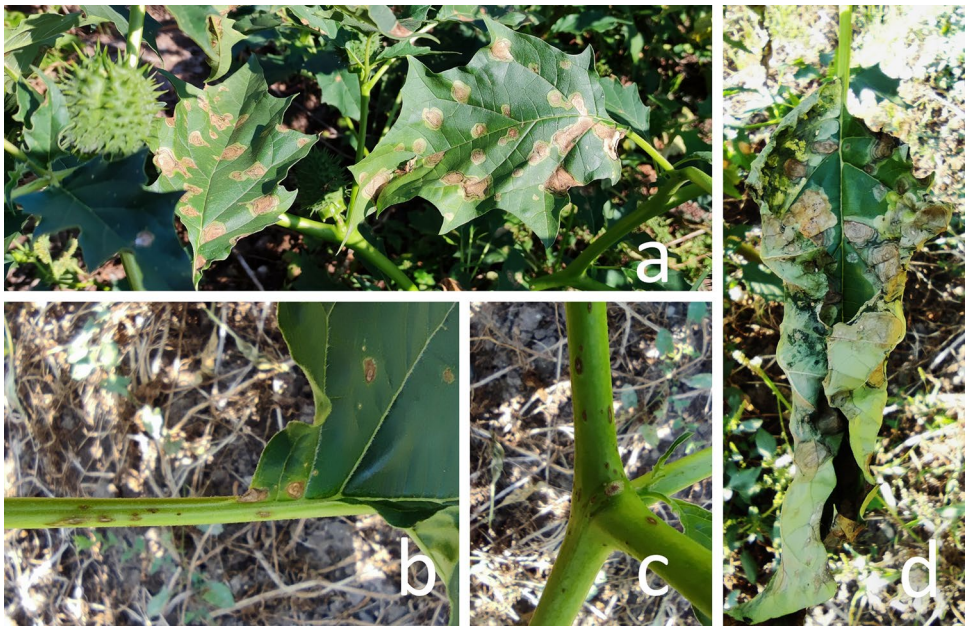

Fig. 1 Symptoms caused by *Alternaria crassa* on jimson weed plants: leaf spots **a**, lens-shaped lesions on petioles **b** and branches **c**, leaf curl **d**

longitudinal septa. Conidiophores, which emerged directly from the agar surface, were short and broad, rarely branched, and 29–72 × 6–9 µm in size ($n = 30$) (Fig. 2d). These morphological features were consistent with those of *Alternaria crassa*, as described by Simmons (2007), Nishikawa and Nakashima (2013), and Bessadat et al. (2020). One representative isolate, Tb_Ac01 (EUCC-22081 M), was randomly selected for molecular and pathogenic characterizations.

Using a DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, genomic DNA of the isolate EUCC-22081 M was extracted from the mycelial mass harvested by scraping the surface of a 7-day-old V8 culture. The transcription elongation factor 1-α (TEF1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and RNA polymerase second largest subunit (RPB2) loci were amplified as described in Woudenberg et al.

Fig. 2 Characteristics of *Alternaria crassa* isolates: colony morphology on V8 medium **a**, conidia with long apical beaks **b**, transverse and longitudinal septa **c**, conidiophores **d**

(2013) with primers EF1-728F and EF1-986R (Carbone and Kohn 1999), gpd1 and gpd2 (Berbee et al. 1999), and 5f2 (Reeb et al. 2004) and 7cr (Liu et al. 1999), respectively.

The PCR products were bi-directionally sequenced by Macrogen Inc. (Seoul, Republic of Korea) with the same primers. The acquired sequences were edited with MEGA X software (Kumar et al. 2018) and underwent a BLAST search within the GenBank sequence database of the National Center for Biotechnology Information (NCBI). The sequences were then deposited in GenBank (TEF1: OM362373; GAPDH: OM362374; RPB2: OM362375). The sequences of the isolate EUCC-22081 M with additional sequences retrieved from GenBank were aligned in the MAFFT v.7 online interface (Katoh et al. 2019, http://mafft.cbrc.jp/alignment/server/) using default settings. A maximum likelihood (ML) tree of concatenated sequence data from the TEF1, GAPDH, and RPB2 loci was inferred using the command-line version of IQ-TREE 1.6.7 (Nguyen et al. 2015) with ultrafast bootstrapping implemented with 1000 replicates at the CIPRES web portal (Miller et al. 2010, https://www.phylo.org/). Parsimony analysis was conducted in PAUP 4.0b10 (Swofford 2003) using the heuristic search option with 1000 random addition sequences and tree bisection reconnection branch swapping. Phylogenetic trees were visualized and edited using FigTree v1.4.2 software. The isolate EUCC-22081 M was clearly separated from the other *Alternaria* isolates and grouped with *A. crassa* isolates in a single clade (Fig. 3). The isolate was deposited in Erciyes University Culture Collection (EUCC-WDCM 1202) with accession number EUCC-22081 M.

Pathogenicity was tested on healthy 1-month-old jimson weed plants. Foliar parts of five plants were sprayed till runoff with a conidial suspension (1×10^4 conidia/ml) of *A. crassa* isolate EUCC-22081 M cultured on a V8 medium. Five control plants were treated with sterile water. After inoculation, the plants were covered with plastic bags to maintain moisture for 24 h. The plants were then transferred to a growth room at 25 °C with a 16-h light/dark photoperiod. After 5 days, the first symptoms on the leaves of all inoculated plants were observed (Fig. 4a). Typical Alternaria blight symptoms appeared on the leaves and petioles 10 days

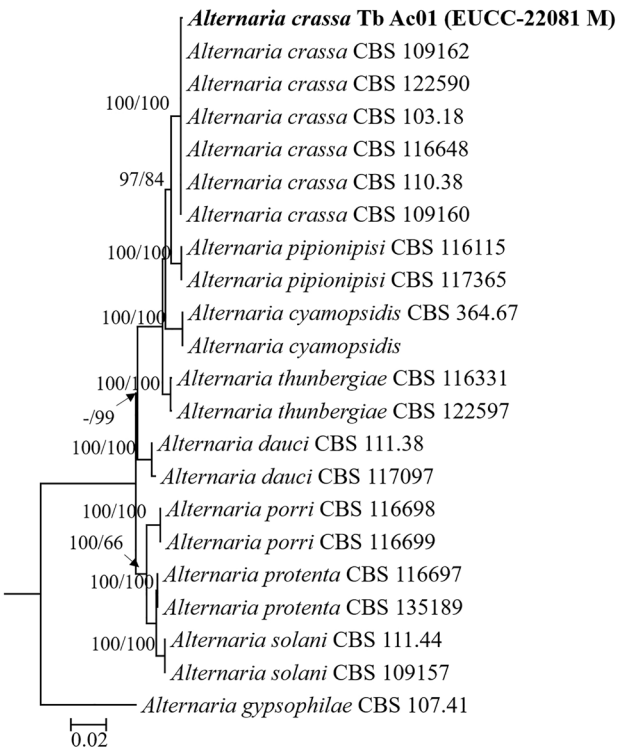

Fig. 3 Maximum likelihood and maximum parsimony tree of *Alternaria* species reconstructed utilizing the TEF1, GAPDH, and RPB2 sequences. Bootstrap percentages (based on 1000 replications) from maximum-likelihood/maximum parsimony (50% and greater) are shown at each node. The isolate EUCC-22081 M obtained in the present study is demonstrated in bold

after inoculation (Fig. 4b). Fifteen days after inoculation, 40% of the *A. crassa*-inoculated plants were dead (Fig. 4c). Similar pathogenicity assays were conducted on *Solanum tuberosum* cv. Marfona plants, and early blight-like symptoms were observed on the potato plants (Fig. 4d). The plants in the control group remained symptom-free (Fig. 4e). To prove Koch's postulates, the pathogen was re-isolated from inoculated plants and identified by conidial morphology.

This is the first report of *A. crassa* causing leaf spots on *D. stramonium* in Turkey (Farr and Rossman 2022). It has previously been evaluated as a potential biological

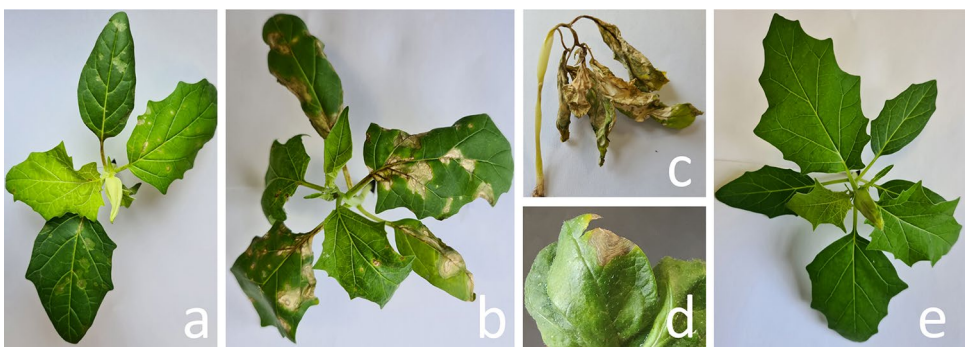

Fig. 4 Pathogenicity test results: 5 days after inoculation **a**, 10 days after inoculation **b**, a fully dead plant 15 days after inoculation **c**, leaf spots with concentric rings on leaves of potato plants **d**, control **e**

herbicide (Boyette 1986). However, *A. crassa* can also cause leaf spot disease in members of cultivated plants such as solanaceous crops, potato (Cash 1953), tomato, and eggplant (Bessadat et al. 2020). The observed aggressiveness of the pathogen to potato, as well as susceptibility in other crop and ornamental species (Stewart-Wade et al. 1998; Nishikawa and Nakashima 2013; Bessadat et al. 2020), makes developing *A. crassa* as a mycoherbicide difficult. This is due to the widespread presence of *D. stramonium* in solanaceous vegetable fields throughout Turkey and the susceptibility of solanaceous plants to *A. crassa*. Because infected jimsonweed near solanaceous plants can threaten practices to manage solanaceous crop production, the use *A. crassa* to control *D. stramonium* plants in these locations can not be recommended.

Declarations

Conflict of interest The authors of this work declare that there is no conflict of interest.

References

Berbee ML, Pirseyedi M, Hubbard S (1999) *Cochliobolus* phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. Mycologia 91(6):964–977. https://doi.org/10.1080/00275514.1999.12061106

Bessadat N, Hamon B, Bataillé–Simoneau N, Chateau C, Mabrouk K, Simoneau P (2020) Occurrence of leaf spot disease caused by *Alternaria crassa* (Sacc.) Rands on Jimson weed and potential additional host plants in Algeria. Plant Pathol J 36(2):179–184. https://doi.org/10.5423/PPJ.NT.01.2020.0003

Blagojevic J, Janjatovic S, Ignjatov M, Trkulja N, Gasic K, Ivanovic Z (2020) First report of a leaf spot disease caused by *Alternaria protenta* on the *Datura stramonium* in Serbia. Plant Dis 104(3):986. https://doi.org/10.1094/PDIS-06-19-1335-PDN

Boyette CD (1986) Evaluation of *Alternaria crassa* for biological control of jimsonweed: host range and virulence. Plant Sci 45(3):223–228. https://doi.org/10.1016/0168-9452(86)90143-3

Camino-Vilaro M, Castro-Hernandez L, Abreu–Herrera Y, Mena–Portales J, Cantillo-Perez T (2019) Fungi associated with invasive plant species in Cuba. Phytotaxa 419(3):239–267. https://doi.org/10.11646/phytotaxa.419.3.1

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91:553–556. https://doi.org/10.2307/3761358

Cash EK (1953) A record of the fungi named by J.B. Ellis Part II. U.S. Department of Agriculture Special Publication, Beltsville, Maryland

Farr DF, Rossman AY (2022) Fungal Databases, U.S. National Fungus Collections, ARS, USDA. https://nt.arsrin.gov/fungaldatabases/. Accessed 02 April 2022

Friedman M, Levin CE (1989) Composition of jimson weed (*Datura stramonium*) seeds. J Agric Food Chem 37(4):998–1005. https://doi.org/10.1021/jf00088a040

Holm L, Doll J, Holm E, Pancho JV, Herberger JP (1997) World weeds: natural histories and distribution. John Wiley and Sons Inc., New York, NY

Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform 20(4):1160–1166. https://doi.org/10.1093/bib/bbx108

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35(6):1547–1549. https://doi.org/10.1093/molbev/msy096

Kumral NA, Çobanoğlu S (2015) A reservoir weed for mites: *Datura stramonium* L. (Solanaceae) in the vicinity of cultivated solanaceous plants in Turkey. Int J Acarol 41(7):563–573. https://doi.org/10.1080/01647954.2015.1084043

Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. Mol Biol Evol 16(12):1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. 1–8. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA

Mułenko W, Majewski T, Ruszkiewicz–Michalska M (2008) A preliminary checklist of micromycetes in Poland. In: Biodiversity of Poland 9:752. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków

Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ (2015) IQ–TREE: a fast and effective stochastic algorithm for estimating maximum–likelihood phylogenies. Mol Biol Evol 32(1):268–274. https://doi.org/10.1093/molbev/msu300

Nishikawa J, Nakashima C (2013) Taxonomic characterization and experimental host ranges of four newly recorded species of *Alternaria* from Japan. J Phytopathol 161(9):604–616. https://doi.org/10.1111/jph.12108

Nishikawa J, Nakashima C (2020) Japanese species of *Alternaria* and their species boundaries based on host range. Fungal Syst Evol 5:197–281

Reeb V, Lutzoni F, Roux C (2004) Contribution of RPB2 to multilocus phylogenetic studies of the euascomycetes (Pezizomycotina, Fungi) with special emphasis on the lichen–forming Acarosporaceae and evolution of polyspory. Mol Phylogenet Evol 32(3):1036–1060. https://doi.org/10.1016/j.ympev.2004.04.012

Simmons EG (2007) Alternaria: An Identification Manual. CBS Fungal Biodiversity Centre: Utrecht, Netherlands

Swofford DL (2003) PAUP* ver 4.0. b10. Phylogenetic Analysis Using Parsimony and Other Methods. Sunderland, MA: Sinauer Associates, Sunderland

Stewart-Wade SM, Lawrie AC, Bmese E (1998) An Australian isolate of *Alternaria crassa* shows potential as a mycoherbicide to control the weed *Datura stramonium*. Australas Plant Pathol 27(3):186–197

Woudenberg JHC, Groenewald JZ, Binder M (2013) *Alternaria* redefined. Stud Mycol 75:171–212. https://doi.org/10.3114/sim0015

Woudenberg JHC, Truter M, Groenewald JZ, Crous PW (2014) Large-spored Alternaria pathogens in section Porri disentangled. Stud Mycol 79:1–47. https://doi.org/10.1016/j.simyco.2014.07.003