

1 **First Report of Leaf Spot Caused by *Colletotrichum kahawae* on Cultivated Rocket (*Eruca***
2 ***sativa*) in Italy.**

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8 *Eruca sativa* [syn. *E. vesicaria* subsp. *sativa* (Miller) Thell.], common name cultivated rocket or
9 arugula, is an annual species belonging to the Brassicaceae that is grown widely for fresh
10 consumption, mostly in mixed, ready-to-eat salads. During September and October 2014, in a
11 commercial plastic-house (0.3 ha) in Piedmont, northern Italy, where rocket had been grown for
12 7 years in the same soil under intensive cultivation, a leaf spot was observed on rocket plants (cv.
13 Coltivata) from 7 to 35 days after sowing, corresponding to the cotyledon stage to harvest. The
14 incidence of plants affected ranged from 5 to 15% in the more humid area of the tunnel (75 to
15 90% relative humidity) at 20 to 28°C, with 5 to 75% of the affected leaf area showing white
16 spots. Symptoms first appeared as pale white or cream-colored, circular spots, each 5 to 50 mm
17 in diameter, on the tip or margins of the leaf. Each spot had a thin, dark brown or black border.
18 Leaf spots enlarged, with a round or irregular shape, to form extensive dead areas. Symptoms
19 differed from those produced by *Alternaria japonica* and *Fusarium equiseti* on this crop (Gilardi
20 et al. 2013). Isolations were carried out from symptomatic leaf sections (each 1 mm²) dipped in
21 1% sodium hypochlorite for 1 min, then rinsed in sterilized water, and placed onto PDA
22 amended with 25 mg/liter of streptomycin sulfate. A *Colletotrichum* sp. (Bailey and Jeger 1992)

1 was recovered consistently at a frequency of 70% from twenty leaf sections incubated at 20 to
2 23°C and a 12 h photoperiod/day. Hyaline, cylindrical, aseptate, and thin-walled conidia (9.3 to
3 16.5 x 3.4 to 6.3 μm , average 14.4 x 4.7 μm ; n = 41 conidia) were produced abundantly in
4 acervuli (latter 79.0 to 91.5 μm ; n=15) in gray mycelium. Genomic DNA was extracted from 10
5 mg of mycelium collected from PDA plates of one representative, single-conidium isolate. The
6 ITS1-5.8S-ITS2 region of ribosomal DNA (rDNA), and a fragment of the beta-tubulin 2 gene
7 (TUB2) between exons 2 and 6, were amplified using 200 ng genomic DNA as template (Faedda
8 et al. 2011). Purified amplicons (ExoSAP-IT) were sequenced in both directions. Sequence
9 analysis using BLASTN (Morgulis et al. 2008). The 489 and 600 bp fragments of ITS rDNA
10 (GenBank Accession No. KT259854) and TUB2 (KT259853), respectively, showed 100%
11 similarity with ITS and TUB2 sequences of *Colletotrichum kahawae* (JN715847.1 and
12 KC425710.1, respectively). Pathogenicity tests were performed on healthy, 25-day-old *E. sativa*
13 (cv. Coltivata) plants by spraying the leaves with a conidial and mycelial suspension (1×10^5
14 conidia/ml) prepared from PDA plates of the same sequenced isolate of the pathogen. Control
15 plants were sprayed with sterilized water. Fifteen plants/treatment were used. The plants were
16 then covered with plastic bags for 5 days to maintain relative humidity close to saturation, and
17 kept in a growth chamber at 25°C under white fluorescent lamps (12 h photoperiod/day). Small,
18 necrotic spots enlarged, and each formed a distinct black margin similar to those on the original
19 plants, 7 to 10 days after inoculation, while control plants remained asymptomatic. A
20 *Colletotrichum* sp. with morphological characteristics similar to *C. kahawae* was re-isolated
21 consistently from inoculated plants only, following the re-isolation protocol described above. No
22 fungi colonies were re-isolated from asymptomatic leaves of control plants. The pathogenicity
23 test was repeated with the same results. *C. kahawae* has been reported in Italy on olive (*Olea*

1 *europaea* L.) fruit (Scheda et al. 2014). This is, to our knowledge, the first report of *C. kahawae*
2 on *E. sativa* in Italy as well as worldwide. Due to the wide host range of *C. kahawae* and the
3 economic value of cultivated rocket, this disease could be a threat in other rocket production
4 areas of Italy and rocket, in turn, could be an inoculum reservoir for other susceptible crops.

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