

**Paredes-Machado, C., Bogaj, V., Papp, V., Balázs, G. & Papp, D. (2025). *Alternaria* and *Curvularia* leaf spot pathogens show high aggressivity on watermelon, and are emerging pathogens in cucurbit production. *Phytopathologia Mediterranea* 64(1): 41–55 doi: 10.36253/phyto-15955**

**Table S1.** Thermocycler settings used in this study, for three barcoding markers.

| Marker         | Initial denaturation | Denaturation | Annealing    | Extension    | Final extension | N° cycles | Reference                                           |
|----------------|----------------------|--------------|--------------|--------------|-----------------|-----------|-----------------------------------------------------|
| HIS3           | 94°C, 120 sec        | 94°C, 20 sec | 56°C, 30 sec | 72°C, 60 sec | 72°C, 300 sec   | 35        | (Steenkamp <i>et al.</i> , 2000) Modified protocol. |
| TEF1- $\alpha$ | 94°C, 300 sec        | 94°C, 30 sec | 59°C, 30 sec | 72°C, 45 sec | 72°C, 420 sec   | 40        | (Woudenberg <i>et al.</i> , 2013)                   |
| ITS            | 94°C, 120 sec        | 94°C, 30 sec | 48°C, 30 sec | 72°C, 90 sec | 72°C, 300 sec   | 35        | (Woudenberg <i>et al.</i> , 2013)                   |

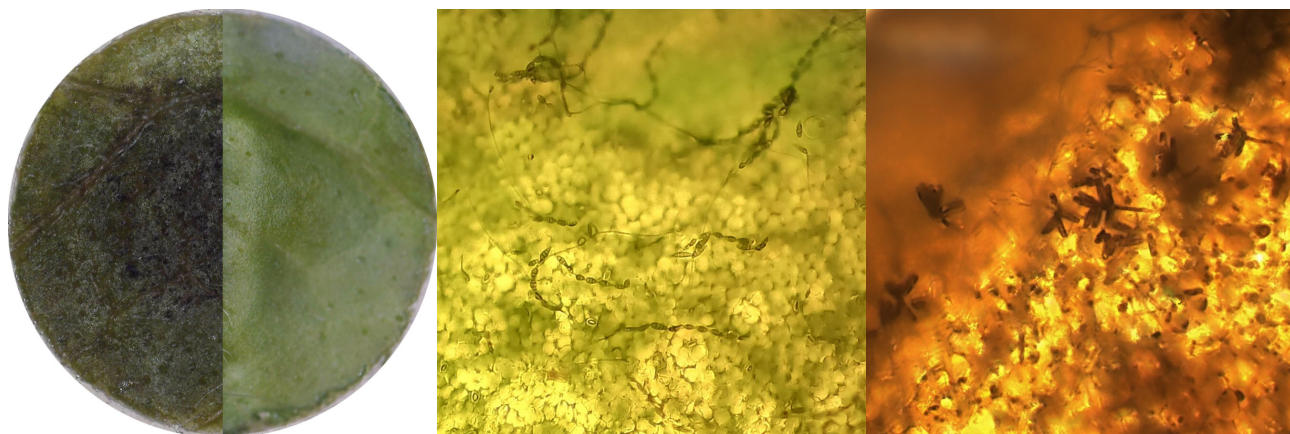
**Table S2.** *Alternaria* and *Curvularia* GenBank accession numbers of downloaded and newly obtained sequences used for phylogenetic analyses in this study.

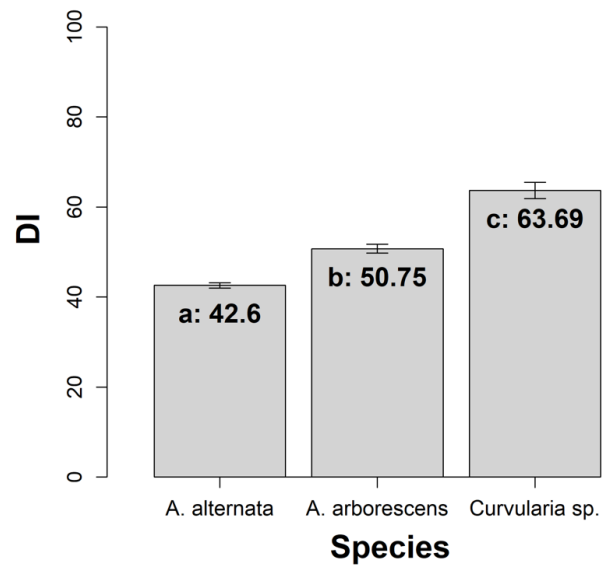
| Species                       | Isolate      | GenBank accession number |                |            |
|-------------------------------|--------------|--------------------------|----------------|------------|
|                               |              | ITS                      | TEF1- $\alpha$ | HIS3       |
| <i>Alternaria abundans</i>    | CBS 534.83 T | JN383485                 | KC584707       | na         |
| <i>Alternaria abundans</i>    | UWR_011      | KX426947.1               | na             | na         |
| <i>Alternaria alternata</i>   | CBS 130254   | KP124383                 | KP125161       | na         |
| <i>Alternaria alternata</i>   | CBS 130255   | KP124384                 | KP125162       | na         |
| <i>Alternaria alternata</i>   | YL-1         | MK860132                 | MN072924       | na         |
| <i>Alternaria alternata</i>   | HB-ZXJ-1     | MK684044.1               | na             | MK683949   |
| <i>Alternaria alternata</i>   | BJ-DW-3      | MK684035.1               | na             | MK683940   |
| <i>Alternaria alternata</i>   | BJ-FLD-1     | MK684037.1               | na             | MK683942   |
| <i>Alternaria alternata</i>   | CBS 916.96 T | AF347031                 | KC584634       | na         |
| <i>Alternaria alternata</i>   | MRV1         | MG661807.1               | MK210168.1     | na         |
| <i>Alternaria alternata</i>   | BC1          | KY548068.1               | na             | KY548070.1 |
| <i>Alternaria alternata</i>   | BC2          | KY548069.1               | na             | KY548071.1 |
| <i>Alternaria alternata</i>   | cc_3         | PV368657                 | PV613798       | PV619037   |
| <i>Alternaria alternata</i>   | cc_6         | PV368662                 | PV613804       | PV619039   |
| <i>Alternaria alternata</i>   | cc_7         |                          | PV613805       | PV619040   |
| <i>Alternaria alternata</i>   | cc_11        |                          | PV613785       | PV619044   |
| <i>Alternaria alternata</i>   | cc_12        | PV368650                 | PV613786       | PV619045   |
| <i>Alternaria alternata</i>   | cc_13        | PV368651                 | PV613787       | PV619046   |
| <i>Alternaria alternata</i>   | cc_14        | PV368652                 | PV613788       | PV619047   |
| <i>Alternaria alternata</i>   | cc_15        |                          | PV613789       | PV619048   |
| <i>Alternaria alternata</i>   | cc_16        |                          | PV613790       | PV619049   |
| <i>Alternaria alternata</i>   | cc_17        |                          | PV613791       | PV619050   |
| <i>Alternaria alternata</i>   | cc_20        | PV368653                 | PV613793       | PV619051   |
| <i>Alternaria alternata</i>   | cc_21        | PV368654                 | PV613794       | PV619052   |
| <i>Alternaria alternata</i>   | cc_25        |                          | PV613797       | PV619055   |
| <i>Alternaria alternata</i>   | cc_36        |                          | PV613799       | PV619056   |
| <i>Alternaria alternata</i>   | cc_41        | PV368659                 | PV613801       | PV619057   |
| <i>Alternaria alternata</i>   | cc_42        | PV368660                 | PV613802       | PV619058   |
| <i>Alternaria alternata</i>   | cc_45        | PV368661                 | PV613803       | PV619059   |
| <i>Alternaria arborescens</i> | CBS 102605 T | NR_135927                | KC584636       | na         |
| <i>Alternaria arborescens</i> | CBS 124281   | KP124414                 | KP125192       | na         |
| <i>Alternaria arborescens</i> | cc_1         | PV368649                 | PV613783       | PV619035   |
| <i>Alternaria arborescens</i> | cc_2         |                          | PV613792       | PV619036   |

(Continued)

**Table S2.** (Continued).

| Species                       | Isolate      | GenBank accession number |                |            |
|-------------------------------|--------------|--------------------------|----------------|------------|
|                               |              | ITS                      | TEF1- $\alpha$ | HIS3       |
| <i>Alternaria arborescens</i> | cc_4         | PV368658                 | PV613800       | PV619038   |
| <i>Alternaria arborescens</i> | cc_8         | PV368663                 | PV613806       | PV619041   |
| <i>Alternaria arborescens</i> | cc_9         |                          | PV613807       | PV619042   |
| <i>Alternaria arborescens</i> | cc_10        |                          | PV613784       | PV619043   |
| <i>Alternaria brassicae</i>   | CBS 116528   | KC584185                 | KC584641       | na         |
| <i>Alternaria brassicae</i>   | HEYD10       | MW867250.1               | MW882930.1     | na         |
| <i>Alternaria brassicae</i>   | HEYD8        | MW867249.1               | MW882929.1     | na         |
| <i>Alternaria breviramosa</i> | CBS 121331   | FJ839608                 | KC584700       | na         |
| <i>Alternaria cetera</i>      | CBS 121340   | JN383482                 | KC584699.1     | na         |
| <i>Alternaria cucumerina</i>  | LN-STG-9     | MK684058                 | Na             | MK683963   |
| <i>Alternaria cucurbitae</i>  | CBS 483.81   | FJ266483                 | KC584743       | na         |
| <i>Alternaria gaisen</i>      | CBS 118488 T | KP124427                 | KP125206       | na         |
| <i>Alternaria gaisen</i>      | HB3-1        | ON954512.1               | ON961776.1     | ON961777.1 |
| <i>Alternaria infectoria</i>  | CBS 210.86 T | DQ323697                 | KC584662       | na         |
| <i>Alternaria infectoria</i>  | IM-HT-2      | MK684063.1               | Na             | MK683968.1 |
| <i>Alternaria obclavata</i>   | CBS 124120   | KC584225                 | KC584701       | na         |
| <i>Alternaria tenuissima</i>  | AH-HCG-4     | MK683973                 | Na             | MK683878   |
| <i>Alternaria tenuissima</i>  | AH-AHH-1     | MK683972                 | Na             | MK683877   |
| <i>Alternaria tenuissima</i>  | 34-2         | MZ182356.1               | MZ246599.1     | MZ202356.1 |
| <i>Alternaria tenuissima</i>  | BJ-FS-X1     | KP701115.1               | Na             | KP701179.1 |
| <i>Curvularia buchloes</i>    | CBS 246.49 T | KJ909765                 | Na             | na         |
| <i>Curvularia rouhaniai</i>   | CBS 144674 T | KX139030                 | MG428687       | na         |
| <i>Curvularia</i> sp.         | cc_22        | PV368655                 | PV613795       | PV619053   |
| <i>Curvularia</i> sp.         | cc_24        | PV368656                 | PV613796       | PV619054   |
| <i>Curvularia spicifera</i>   | HN43-6-2     | MT033079                 | MT036975.1     | na         |
| <i>Curvularia hawaiiensis</i> | BRIP 11987 T | KJ415547                 | KJ415445       | na         |
| <i>Curvularia spicifera</i>   | CBS 274.52   | JN192387                 | JN601023       | na         |
| <i>Curvularia spicifera</i>   | EML-KWD01 T  | KT351794.1               | na             | na         |

**Figure S1.** Watermelon leaf discs (diam = 6 mm) infected with *Alternaria alternata* (left) and mock inoculated (right). The two micrographs are of leaf discs with sporulation of *Alternaria alternata* (left, 10 $\times$  magnification) and *Curvularia* sp. (right, 20 $\times$  magnification).



**Figure S2.** Mean disease indices (DIs) caused by isolates of *Alternaria alternata*, *A. arborescens*, or *Curvularia* sp. after inoculations of 73 watermelon accessions.