**INTRODUCTION**

*Cornus florida* (flowering dogwood) and *C. nuttallii* (Pacific dogwood), two native dogwood species of North America, are extremely susceptible to anthracnose caused by the fungus *Discula destructiva*. The first reports of this disease in Europe dated back to 1995 when it was intercepted in UK on plants imported from North America, then in 2002 the dogwood anthracnose was reported in Germany. The disease in 2003 was included in the “Eppo alert list” (the list identify the pathogens which can cause phytosanitary risks inside Eppo and Oepp region) as a pathogen of new introduction in Europe. (www.eppo.org/QUARANTINE/Alert_List/alert_list.html). During the spring of 2003, we observed anthracnose symptoms on *C. florida* and *C. nuttallii* plants cultivated in several nurseries in Lombardia and during 2004 the disease was found on flowering dogwoods in private gardens. After mycological and genetic investigations *D. destructiva* was identified as the causal agent. *D. destructiva* is known only to reproduce by asexual spores, with no sexual state having been observed. Populations of *D. destructiva* in the eastern and western North America are morphologically indistinguishable but genetically distinct. A fixed G-A transition at the 5’ end of the gene translation elongation factor-1 alpha (EF-1α), for example, distinguishes the eastern and western USA populations. Several other less variable loci, including the internal transcribed spacer (ITS) of the ribosomal RNA repeat region and partial chitin synthase-1 (CHS-1) gene, are identical between the eastern and western isolates. In this study, we sequenced these three genes in the isolate obtained from diseased dogwoods in Italy in order to (1) determine whether they were infected with *D. destructiva* and (2) to determine a potential genetic connection with eastern or western USA isolates.

**MATERIALS AND METHODS**

The infected dogwood leaves collected from nurseries of Lombardia Region were placed in moist chamber for 4 days at 20°C in order to promote the development of the fruiting bodies (acervuli) and the extrusion of the spores. The isolates were obtained in PDA from little pieces of the leaf blade (1-2 mm diameter) showing acervuli. The genomic DNA was
extracted from the mycelia of purified isolates obtained from conidia. Mycelia were ground in liquid nitrogen. Total nucleic acids were extracted by Dneasy Plant Mini Kit (Qiagen) which protocol was modified in the lysis step. The obtained genomic DNA was analyzed in the Fusarium Research Center of The Pennsylvania State University. ITS region, partial EF-1α gene, and partial CHS-1 gene were amplified and sequenced with primers ITS5/NL4, EF1-728F/EF1-986R, and CHS-79F/CHS-354R, respectively, followed the protocols of Zhang and Blackwell (2002).

RESULTS

The anthracnose symptoms were observed on the upper and lower side of Cornus leaves as black spots with red halo. The unabscised infected leaves stayed on trees for one or more seasons. Observations by stereoscope of the leaf sample from moist chamber showed conidiomata that were superficial or subcuticular, brown or black, pulvinate-globose, 40-100 µm in diameter with openings, conidia were exuded in white-gray cirrhi. Cultures were slow-growing (20 mm of radial-growth in 10 days on PDA). Colonies were white with hyphae submerged, then became gray, tan with age. The microscope observation at 400× showed hyaline, elliptical-fusiform conidia, 5,5-10 µm by 1,5-3 µm, which often contain one or two polar oil droplets. The symptoms and the morphological characteristics observed match with the descriptions in literature about Discula destructiva (Redlin, 1991).

On molecular level, the Italian isolates had identical ITS (555 bp), EF-1α (164 bp, partial), and CHS-1 (261 bp, partial) gene sequences. ITS and CHS-1 sequences of the Italian samples were identical to the USA isolates, and their EF-1α sequences were identical to all of those of analyzed previously from the western USA isolates. Because these three genes usually are powerful as a species-level diagnostic of fungi, our results provide strong support that the Italian isolates are, indeed, Discula destructiva and that they are close relatives of the U.S. western coast population if not identical. A more sensitive fingerprinting method may further distinguish the Italian D. destructiva strains, and a population study would shed light on the origin and spread of the disease in southern Europe.

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REFERENCES

