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DISEASE NOTES

First Report of Anthracnose Disease Caused by Colletotrichum acutatum on Lupinus albus in Italy

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ABSTRACT

Approximately 5 million ha of lupin species are cultivated worldwide (Jansen 2006). Anthracnose is a very destructive disease of lupins and may cause complete crop losses in susceptible varieties. Typical anthracnose symptoms were observed in spring 2013 on leaves, pods, and stems of white lupin (Lupinus albus L.) plants on ~200 ha out of the total cultivated area (600 ha) in Lecce Province (southern Italy). Disease incidence ranged from 60 to 80% in investigated fields. The infected organs, leaves, and apical stems initially showed small, circular, brown spots 2 to 3 mm in diameter that evolved into larger sunken necrotic lesions with orange conidial masses in their center. In order to isolate the likely pathogen, small pieces of symptomatic plant tissues taken from the lesions or from their nearby zones were previously surface-disinfested with 1% sodium hypochlorite for 1 min, rinsed three times with sterile distilled water, and then plated in petri dishes containing potato dextrose agar (PDA). Plates were incubated under alternating light and dark conditions at 25°C and, after 10 days, the grown colonies were retransferred to the same media to obtain pure cultures. Conidia produced by the pure isolates were hyaline, smooth-walled, aseptate, and cylindrical to fusiform. Their dimensions ranged from 19.5 to 21.5 (mean = 20.5) \times 2.7 to 4.0 (mean = 3.4) μm . On the basis of colony and conidia morphology (Damm et al. 2012), isolates were identified as Colletotrichum acutatum (J.H. Simmonds). The ITS1-5.8S-ITS2 region of 10 representative isolates were amplified with primers ITS5/ITS4 (White et al. 1990) and sequenced. The obtained sequences when compared with those present in GenBank were highly similar (99%) to C. acutatum from lupin (accessions AJ749674 and JN543068). The ITS sequences of two C. acutatum isolates were deposited in GenBank under accessions LN877887 and LT160697. In order to determine if the C. acutatum isolates under study were the cause of anthracnose symptoms on lupins in fields, healthy potted 1.5-month-old lupin seedlings were each wound-inoculated with a suspension of conidia obtained from 7- to 10-day-old PDA cultures, obtained from the two deposited isolates, grown at 25°C and adjusted to 1×10^6 conidia/ml in sterile deionized water. Fifty plants were used for inoculation tests. A

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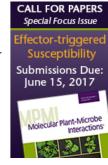
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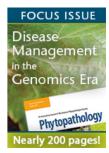
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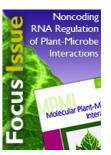
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lypodermic syringe needle (0.2 mm in diameter and 0.3 mm in depth) was used to wound

lupin leaves and apical main stem. A 20-µl droplet of conidia suspension (10⁶ conidia/ml) was used for inoculation and placed on the wound. Ten control plants were inoculated with $20~\mu l$ of sterile distilled water. Inoculated and control plants were maintained at $25^{\circ}C$ and 90% RH with a 12-h photoperiod. Anthracnose symptoms very similar to those observed in fields developed on all inoculated lupin plants after 14 days. No symptoms appeared on control plants. Pathogenicity tests were carried out twice. C. acutatum colonies identical to those of the isolate used for inoculation were obtained from all the inoculated lupin leaves and apical main stems. Colletotrichum species have a broad host range and are known to be pathogenic on several crops (Damm et al. 2012) and can affect lupin in many countries (Talhinhas et al. 2002). To our knowledge, this is the first report of *C. acutatum* on lupin in Italy.

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