First report in Chile of *Mycocentrospora acerina,* causal agent of Peony (*Paeonia lactiflora)* Red Spot

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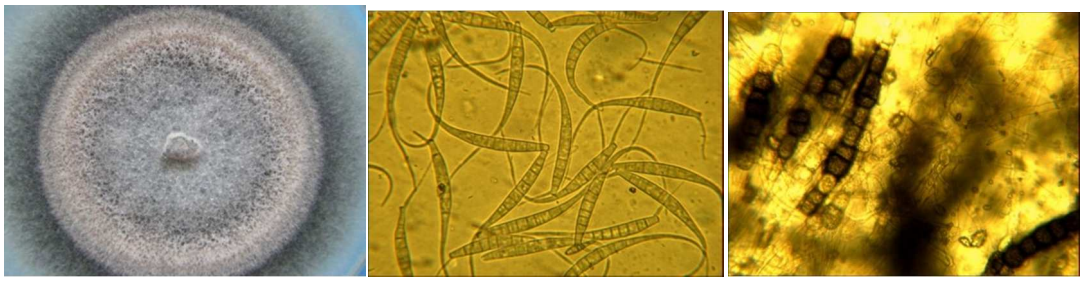
A survey to identify pathogens causing peony diseases in southern Chile ($37^0$95´ to $40^0$58´S) was conducted from 2008 to 2011. A noticeable symptom consisted of small red spots on leaves, stems, sepals, and first petals of the bud. The central part of the lesions (1 cm) enlarged, darkened and became necrotic, coincident with rainy and cold weather, including frosts. Lesions grew but never coalesced completely. At the edges of leaves the infected tissues cracked and produced a twisted and corrugated appearance. Rhizome symptoms were also observed and consisted of black spots, which enlarge to elongated lesions. Small pieces (~ 1 x 1 mm) of symptomatic leaves, previously washed with 2% NaOCl for 2 min were cultured on PDA and V8 juice agar (20%) media. The cultures were incubated in growth chambers at 20°C (dark) and 5°C (dark)/ 20°C (light) for 12/12 h (dark/light). Sporulation was obtained only with V8 under the dark and light regime. Mycelia growth was superficial and immersed in the media; hyphae were septate with thick black walls. Conidiophores were flexuous, not branched and produced a single spore. Spores were elongate, multiseptate, with a long, strongly curved beak. After one week, sporulation decreased and thick walled, round black clamydospores formed in the media. Spores ranged 125 to 235 µm long and most frequently 170 to 220 µm. The number of septa ranged from 5 to 12. Morphological and cultural characteristics fit the description of *Mycocentrospora acerina* (Harting) Deighton (1,2). DNA was obtained from fungal culture. The ITS region was amplified using ITS1/ITS4 primers (3), and part of the amplicon (502 of 550 pb) was sequenced. The sequence was deposited in GeneBank (Accession No KF015599) and showed 100% identity values with sequences of similar regions from *M. acerina* (Strain ATCC 16259, Accession No KF278454). Healthy leaves and rhizome pieces were washed with sterile distilled water and placed on sterile moist paper towel. A small agar disc with mycelium was placed on the leaves and rhizomes with and without wounding before inoculation. The inoculated materials were kept in closed boxes with high humidity and 5º to 20ºC. Tests were positive in leaves and rhizomes with and without wounds. Seven days after leaf inoculations necrotic symptoms developed. A second inoculation test in peony leaves of plants bagged with plastic under field conditions corroborated the *in vitro* test. Inoculated rhizomes developed dark orange lesions initially, then turning black with a watery consistency, similarly to affected rhizomes in the field. In both test controls showed no symptoms. To our knowledge, this is the first report of *M. acerina* on *P. lactiflora* globally (4). In an evaluation at Carillanca ($38^0$41´S, $72^0$25`W), using a scale from 0 (no symptoms) to 7 (necrotic lesions covering >60% of foliage, with death of stems), 11 out of 31 peony varieties scored 0-1. As a reference, a score of 3 is the maximum damage allowed on peonies for export. These varieties might help to keep Chilean peonies in the market without an economic impact.

References (1)M. B. Ellis, B. Dematiaceous Hyphomycetes. CMI, Surrey, England.1971. (2) B. C. Sutton and I.A.S Gibson. *M. acerina.* CMI. Descriptions of Pathogenic Fungi and Bacteria. No 537.1977 (3) T. J. White et al. PCR Protocols. Academic Press, San Diego, CA, 1990. (4) D. F. Farr and A. Y. Rossman. Fungal Databases. Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved April 16, 2014

a) b) C)

Peony Red Spot symptoms: Initial and coalescing reddish lesions on leaf (a); leaf margin damage (b) and stem lesions (c).



a) b) C)

Growth of *Mycocentrospora acerina* on V8 juice agar (20%) media (a), conidia (b) and clamydospores (c).