ΦYTON
REVISTA INTERNACIONAL DE BOTÁNICA EXPERIMENTAL
INTERNATIONAL JOURNAL OF EXPERIMENTAL BOTANY
FUNDACION ROMULO RAGGIO
Gaspar Campos 861, 1638 Vicente López (BA), Argentina
www.revistaphyton.fund-romuloraggio.org.ar

First report of *Botrytis cinerea* on *Lilium polyphyllum*, a critically endangered herb in Uttarakhand, India

Primera cita de *Botrytis cinerea* sobre *Lilium polyphyllum*, especie en peligro de extinción en Uttarakhand, India

Dhyani A[1], BP Nautiyal[2], MC Nautiyal[1], MC Rivera[3], D Prasad[4], KP Singh[4]

Abstract. *Lilium polyphyllum* is a critically endangered herb in the wilds of Uttarakhand, India. Gray mold appeared as a new disease on this species from July to August (2008-2009) causing death of inflorescences and shoots. *Botrytis cinerea* was consistently isolated on PDA, from infected inflorescences. Pathogenicity was established by inoculation of potted plants of *L. polyphyllum* in a glasshouse. *Botrytis cinerea* was recorded as a gray mold pathogen on *L. polyphyllum* for the first time in India, causing flower and shoot blight. This disease may seriously affect reproductive stages of this endangered host. To our knowledge, *B. cinerea* had not been reported previously on *L. polyphyllum*.

Keywords: *Lilium polyphyllum*; Gray mold; *Botrytis cinerea*; First report; India.

Resumen. *Lilium polyphyllum* es una planta herbácea que se encuentra en estado de peligro en ambientes naturales de Uttarakhand, India. La enfermedad conocida como moho gris fue detectada sobre esta especie desde Julio a Agosto (2008-2009) causando muerte de inflorescencias y varas. *Botrytis cinerea* fue aislado consistentemente de inflorescencias. Su patogenicidad fue confirmada mediante inoculación de plantas de *L. polyphyllum* cultivadas en macetas en un invernáculo. Como resultado, se cita a *Botrytis cinerea* por primera vez como causante de moho gris sobre *Lilium polyphyllum* en India, cuyos síntomas son tizón de flores y varas. Esta enfermedad puede afectar seriamente a este hospedante en riesgo durante su estado reproductivo. A nuestro entender, no existe otro antecedente de *B. cinerea* sobre este hospedante.

Palabras clave: *Lilium polyphyllum*; Moho gris; *Botrytis cinerea*; Primera cita; India.

[1] High Altitude Plant Physiology Research Centre, Srinagar, Pauri Garhwal, Uttarakhand, India.
[2] Department of Horticulture, Aromatic and Medicinal Plant, Mizoram University, Aizawl, India.
[3] Phytopathology. School of Agriculture. University of Buenos Aires, Argentina.
[4] Plant Pathology Section, College of Forestry & Hill Agriculture, G. B. Pant University of Agriculture & Technology, Ranichauri, India.
Address correspondence to: Anurag Dhyani. High Altitude Plant Physiology Research Centre, Srinagar, Pauri Garhwal, Pin- 246174, Uttarakhand, India. *e-mail*: anuragdhyani@gmail.com
Recibido / Received 23.IX.2011. Aceptado / Accepted 9.XI.2011.

INTRODUCTION

The genus *Lilium* L. (Liliaceae) comprises approximately 100 species distributed throughout cold and temperate regions (Siljak-Yakovlev et al., 2003), 11 of which have been recorded in India (Hooker, 1990). *Lilium polyphyllum* D. Don ex Royle is a perennial, bulbous herb with a wide range of medicinal and ornamental uses (Dhyani et al., 2010). These plants are 30-90 cm high, with alternate leaves, and racemes of pendulous flowers of a creamy white color speckled with pink (Fig. 1a). It is distributed from North-West Himalaya to Westward of Afghanistan (Hooker, 1990; Gaur, 1999) in China, Tibet, Nepal, Pakistan, the Hunza Valley (which borders on Russia, Afghanistan, China, India) and Wanga Valley (in the North-Western Himalaya) (Dhyani, 2009). In India, it is found in the states of Jammu and Kashmir, Himachal Pradesh and Uttarakhand, at 1800-3600 m.a.s.l. The bulb is used in traditional and modern medicine (Warrier et al., 1997; Dhyani et al., 2010). This species was categorized as critically endangered due to specific habitat requirements and fast decline caused by human intervention for medicinal, ornamental and fodder uses (Ved et al., 2003). In addition, forest fires and pests are also threatening its survival (Dhyani, 2007).

A new disease was observed on *L. polyphyllum* plants in the temperate region of Garhwal Himalaya, Uttarakhand (Dhanaulti; 30° 25' N, 78° 19' E, 2200 m.a.s.l.) in 2008 and 2009. Petals showed circular necrotic spots that enlarged until the whole flowers became blighted and covered with a grey mold (Fig. 1b). Flower infections occasionally spread towards the base of the plants, leading to premature drying and death of entire shoots. The disease appeared during May after the onset of the Monsoon. Incidence was 35-45% during the flowering period (June to middle of July) and became 50-60% (end of July to first week of August) for both years, respectively.

MATERIALS AND METHODS

For pathogen isolation, small pieces from diseased flowers were sterilized by immersion in 0.01% mercuric chloride for 1 min, rinsed with sterile distilled water three times and cultivated on potato dextrose agar (PDA). The pathogenicity of the obtained fungal isolate was evaluated by inoculating healthy *L. polyphyllum* plants. Inoculum was prepared from 10-day old PDA cultures by superficially scraping conidia from the surface of the colonies in a 0.1 M $MgSO_4$ solution. Inflorescences of ten healthy potted plants (average two flowers/plant) were sprayed with a conidial suspension (1×10^7 conidia/mL). Flowering plants treated with 0.1 M $MgSO_4$ served as controls. Both inoculated and control plants were kept in a humidity chamber for 48-72 h and then transferred to a glasshouse bench and monitored for symptom development. The inoculated fungus was re-isolated from symptomatic flowers by surface disinfection and growth on PDA as previously described. The cultural and morphological characteristics of the isolate were registered to identify the pathogen to the species level.

RESULTS AND DISCUSSION

Pinpoint water-soaked lesions developed on flowers and flower buds 72 h after inoculation. Lesions turned necrotic, increased in size, and often coalesced to form patches within 7 to 10 days. Shoots became blighted and died in 12-14 days. All the infected organs were covered with gray mold within 16-18 days. Control plants did not show any symptom of the disease. Re-isolation from the inoculated plants on PDA consistently yielded the inoculated fungus.

Colonies were gray. Septate hyphae (8 to 16 µm wide) beared smooth, botryous, ovoid, colourless conidia (11 to 15 × 8 to 11 µm) (Fig. 1c-d). The isolate produced black sclerotia. These characteristics agree with the descriptions of *Botrytis cinerea* (Ellis & Waller, 1974, Chaube & Pundhir, 2005). The fulfillment of Koch's postulates lets us state that *Botrytis cinerea* is the causal agent of flower and shoot blight of *Lilium polyphyllum*. There is no report of the occurrence of *B. cinerea* or other fungal disease on *L. polyphyllum* from India and other regions (Farr et al., 2011). To our knowledge, this is the first record of gray mold on *L. polyphyllum*.

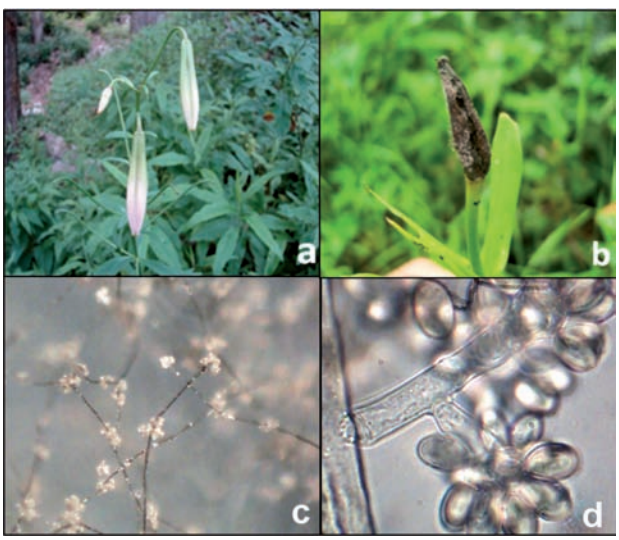

Fig. 1. Gray mold of *Lilium polyphyllum* caused by *Botrytis cinerea*, a. Healthy flower buds; b. Infected flower bud; c. *Botrytis cinera* grey mold; d. Detail of conidiophores and botryose conidia.
Fig. 1. Moho gris causado por *Botrytis cinerea* sobre *Lilium polyphyllum*, a. Inflorescencia sana; b. Pimpollo infectado; c. Conidioforos y conidios de *B. cinerea*; d. Detalle de conidióforo y conidios.

In the fields, disease symptoms were mostly visible on the inflorescences. The pathogen was able to develop downward towards the base of the plants in all cases, under laboratory conditions. Although *B. cinerea* has been reported to damage shoots even of lignified plants (Rivera & Wright, 2002; Vasquez et al., 2007), flower tissues were always the most susceptible to infection. According to Staats (2007) gray mold disease symptoms produced by *Botrytis* species on bulb crops are known as blight, fire or bulb rot. In this work, no lesions were detected on bulbs either during surveys or after inoculations.

Botrytis species are important pathogens of numerous ornamentals, vegetables, orchard crops and stored agricultural products (Jarvis, 1977; Elad et al., 2004). Many *Lilium* species are susceptible to *Botrytis* infection (Cotton, 1933; Maclean, 1951; Alippi, 1969; Hou & Chen, 2003). Among 22 *Botrytis* species, ten have been reported to host on bulbous plants and damage their leaves, stems, bulbs, flowers, and rhizomes (Hennebert, 1973; Yohalem et al., 2003; Staats, 2007). *Botrytis cinerea* is a broad host range necrotrophic pathogen, responsible for great economic loss and its control is difficult (Faretra & Pallastro, 1991; Lamondia & Douglas, 1997).

ACKNOWLEDGEMENTS

G.B. Pant Institute of Himalayan Environment and Development, IERP, Almora, acknowledges the financial support.

REFERENCES

Alippi, H.E. (1969). Tizón de la azucena *(Lilium longiflorum)* producido por *Botrytis elliptica* (Berk.) Cooke en la Argentina. *Revista Facultad de Agronomía (La Plata)* 45: 44-51.

Chaube, H.S. & V.S. Pundhir (2005). Crop diseases and their management. Prentice Hall of New Delhi, New Delhi. 724 pp.

Cotton, A.D. (1933). The detection and control of lily diseases. *Lily Yearbook* 2: 194-210.

Dhyani, A. (2007). Exploring *Lilium polyphyllum* in Uttarakhand, India. In: Sims, D. (ed.). The Lily Yearbook of North American Lily Society. pp. 79-82.

Dhyani, A. (2009). *Lilium polyphyllum*- rarest of rare lilies. In: Mitchell, A. (ed.). Lilies and related plants. Royal Horticulture Society, London. pp. 85-91.

Dhyani, A., B.P. Nautiyal & M.C. Nautiyal (2010). Importance of Astavarga plants in traditional systems of medicine in Garhwal, Indian Himalaya. *International Journal of Biodiversity Science, Ecosystem Services and Management* 6: 13-19.

Elad, Y., B. Williamson, P. Tudzynski & N. Delen (2004). *Botrytis*: biology, pathology and control. Kluwer Academic Publishers. Dordrecht, The Netherlands. 416 p.

Ellis, M.B. & J.M. Waller (1974). *Sclerotinia fuckeliana* (conidial state: *Botrytis cinerea*). *CMI Descriptions of pathogenic fungi and bacteria* 431: 1-2.

Faretra, F. & S. Pallastro (1991). Genetic basis of resistance to benzimidazole and dicarboximide fungicides in *Botryotinia fuckeliana (Botrytis cinerea). Mycological Research* 95: 943-51.

Farr D.F., A.Y. Rossman, M.E. Palm & E.B. McCray (2011). Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Available at http://nt.ars-grin.gov/fungaldatabases/

Gaur, R.D. (1999). Flora of the district Garhwal North West Himalaya with ethnobotanical notes. Transmedia Publications, Srinagar Garhwal, India. 812 p.

Hennebert, G.L. (1973). *Botrytis* and *Botrytis*- like genera. *Persoonia* 7: 183-204.

Hooker, J.D. (1990). The Flora of British India. Bishen Singh Mahendra Pal Singh. Dehradun, India. 6:792 pp.

Hou P.F. & C.Y. Chen (2003). Early stages of infection of lily leaves by *Botrytis elliptica* and *B. cinerea*. *Plant Pathology Bulletin* 12: 103-108.

Jarvis, W.R. (1977). *Botryotinia* and *Botrytis* species: taxonomy, physiology and pathogenicity. Monograph No. 15, Canadian Department of Agriculture, Ottawa. 195 p.

Lamondia, J.A. & S.M. Douglas (1997). Sensitivity of *Botrytis cinerea* from Connecticut greenhouses to benzimidazole and dicarboximide fungicides. *Plant Disease* 81: 729-732.

Maclean, N.A. (1951). *Botrytis* on Lilies. *Phytopathology* 41: 941.

Rivera, M.C. & E.R. Wright (2002). First report of blight caused by *Botrytis cinerea* on China rose in Argentina. *Plant Health Progress* http://www.plantmanagementnetwork.org/php/briefs/chinarose/top.cfm.

Siljak-Yakovlev, S., S. Paccenini, E. Muratovic, V. Zoldos, O. Robin & J. Valles (2003). Chromosomal differentiation and genome size in three European mountain *Lilium* species. *Plant Systematics and Evolution* 236: 165-173.

Staats, M. (2007). *Botrytis* species on flower bulb crops: Phylogeny, genetic variation and host specificity. PhD Thesis Wageningen University. The Netherlands. 168 p.

Vasquez, P., J.A. Baldomá, E.R. Wright, A. Pérez, M. Divo de Sesar & B.A. Pérez (2007). First report of blueberry *Botrytis* blight in Buenos Aires, Entre Ríos and Córdoba (Argentina). *Plant Disease* 91: 639.

Ved, D.K., G.A. Kinhal, K. Ravikumar, V. Prabhakaran, U. Ghate, V.R. Shankar & J.H. Indresha (2003). CAMP Report: Conservation assessment and management prioritization for medicinal plants of Himachal Pradesh, Jammu, and Kashmir, and Uttarakhand. Workshop FRLHT. Shimla, Himachal Pradesh, Bangalore, India.

Warrier, P.K., V.P.K. Nambiar & C. Ramankutty (1997). Indian medicinal plants. A compendium of 500 medicinal plants. Arya Vaidya sala. Orient Longman, 3: 423 p.

Yohalem, D.S., K. Neilson & M. Nicolaisen (2003). Taxonomic and nomenclatural clarification of the onion neck rotting *Botrytis* species. *Mycotaxon* 85: 175-182.