SHORT COMMUNICATION

CONTAMINATION OF MOTH MULLEIN (VERBASCUM BLATTARIA L.) SEEDS BY PHOMA NOVAE-VERBASCICOLA

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SUMMARY

Verbascum blattaria (Scrophulariaceae family) is a hardy perennial species that is used for the edges and flower beds of low-maintenance gardens. Phoma novae-verbascicola causes light brown necrotic spots on the leaves of V. blattaria seedlings. In order to demonstrate the seed transmission of this pathogen, several V. blattaria seeds belonging to three samples collected in 2013, were tested in vitro to detect the presence of P. novae-verbascicola. Two samples were found to be contaminated and colonies of the pathogen were isolated from the tested seeds. Phoma novae-verbascicola was identified from the morphological features observed in vitro and through an ITS (Internal Transcribed Spacer) analysis. The virulence of one isolate was confirmed by means of a pathogenicity test. This work demonstrates that P. novae-verbascicola can be transmitted by affected V. blattaria seeds.

Keywords: ornamental plants, seed-borne pathogens, Phoma poolensis var. verbascicola, Phylllosticta novae-verbascicola.

The genus Verbascum (Scrophulariaceae family) includes several spontaneous hardy perennial Italian flora species (Pignatti, 1982). These plants and their cultivars are suitable for the edges and flower beds of low-maintenance gardens in which they produce yellow, white or purple flowers, densely grouped together in long-lasting eye-catching inflorescences.

Several fungal pathogens belonging to the genus Phoma have been reported on Verbascum spp. (USDA, Fungal Databases). A phylogenetic analysis on this genus has led to the identification of some new species, such as P. novae-verbascicola (Syn.: Phylllosticta novae-verbascicola; P. poolensis var. verbascicola) (Aveskamp et al., 2010). This pathogen has recently been detected on black mullein (Verbascum nigrum L.) plants (Garibaldi et al., 2013) and on moth mullein (Verbascum blattaria L.) seedlings (Garibaldi et al., 2014), both grown in Italy.

The transmission of plant diseases through the diffusion of affected seeds is already well known for several fungal pathogens and can favour the long-distance transport of parasites, as in the case of Fusarium species (Elmer, 2012), and can cause the outbreak of diseases, starting from a small source of infection (Elmer, 2002). Several seed-pathogens have also been found on ornamental plants, for example, Cryptocline cyclaminis and Ramularia cyclaminicola on cyclamen, Colletotrichum sp. on anemone (Daughtrey et al., 1995), Fusarium oxysporum f. sp. cyclaminis on cyclamen (Tomkpsin and Snyder, 1972), F. oxysporum f. sp. callistephi on China aster (Orlicz-Luthard, 1998) and F. oxysporum f. sp. papaveris on Papaver nudicaule (Bertetti et al., 2015). The spread of P. novae-verbascicola to several V. blattaria seedlings has suggested the need to evaluate the contamination of seeds by this pathogen. Therefore, the aim of this work was to test the transmission of P. novae-verbascicola by affected V. blattaria seeds.

Three seed samples of V. blattaria, collected in 2013, were checked in this work. In order to test the presence of the pathogen, 400 unwashed seeds/sample were distributed on a PDA (Potato Dextrose Agar) medium contained in Petri plates (20 seeds/plate). The plates were covered with parafilm and incubated at room temperatures. The development of fungal colonies around the seeds was checked daily. Two out of three seed samples of V. blattaria were contaminated and developed two or three colonies of P. novae-verbascicola, respectively. These colonies were subcultured on PDA to obtain pure isolates, which were coded and stored at 7°C. These isolates were then cultured on PDA and MEA (Malt Extract Agar) for about 15 days, at temperatures ranging from 21 to 24°C, to observe the morphological characteristics produced in vitro. The isolates on the PDA produced a rather soft mycelium, with alternating green-olivaceous and whitish circles at maturity, and dark olivaceous pigments in the agar medium. The isolates on the MEA produced a feltly...
mycelium. Pycnidia were produced both on the agar and in the agar. They were globose to subglobose, solitary or confluent, glabrous, with one ostiolum (sometime two), and measured 44–244 × 44–235 (mean: 101 × 94) μm. The conidia were non-septate, hyaline, ellipsoid, and measured 2.5–5.0 × 0.9–2.2 (mean: 3.2 × 1.3) μm. These features are similar to those described for the colony morphology of *P. novae-verbascicola* in Q-bank.eu. (http://www.q-bank.eu/).

In order to confirm the morphological identification, genomic DNA of the DB15GIU13 isolate obtained from seeds was extracted from a pure culture grown on PDA, using the Nucleospin Plant II Kit (Macherey Nagel), according to the manufacturer's instructions. The internal transcribed spacer (ITS) region was then amplified and sequenced using the ITS1/ITS4 primer (White et al., 1990). BLAST analysis (Altschul et al., 1997) of the 504-bp amplicon (GenBank Accession No. KU559629) showed 99% homology with the KJ192364 sequence of *P. novae-verbascicola*, thus confirming the morphological identification of the pathogen.

In order to test the pathogenicity, the DB15GIU13 isolate of *P. novae-verbascicola* was grown in Petri dishes for 26 days on PDA, at temperatures ranging from 21 to 24°C. A conidial suspension was then prepared from pure cultures and adjusted to the final concentration of 5 × 10⁷ CFU/ml. The inoculum was sprayed onto healthy 60-day-old *V. blattaria* plants grown in pots containing a steamed soil mixture (peat moss:perlite:clay, 70:20:10, respectively). Ten plants (1 plant/pot) were inoculated (1 ml of inoculum/pot), and 10 control plants were sprayed with sterile water only. All the plants were covered with a plastic bag to maintain an elevated relative humidity and were kept in a greenhouse, where the daily average temperatures ranged from 18 to 20°C. The plants were checked daily and the humid chamber was removed 4 days after the inoculation.

The first light brown necrotic spots appeared 5 days after the artificial inoculation, but only on the inoculated leaves, from which *P. novae-verbascicola* was constantly reisolated. During the following days, necrosis extended to the leaves of all the seedlings, all of which died within 20 days. The control plants remained symptomless.

This study demonstrates that the contamination of *V. blattaria* seeds by *P. novae-verbascicola* may be a potential source of inoculum and could favour the diffusion of this pathogen. This result is in agreement with the results of other seed-borne *Phoma* spp., such as *P. pinodella*, which has been reported on several hosts, including species belonging to Leguminosae (Kinsey, 2002) and on *Phoma digitalis* found on Scrophulariaceae species, especially on *Digitalis purpurea* (Boerema et al., 2004).

Seed dressing with registered and effective fungicides should be adopted as a solution to avoid the presence of *P. novae-verbascicola* on *V. blattaria* seedlings, in particular on the more aesthetically appreciated cultivars. This procedure could control the spread of the disease in low-maintenance gardens, in which *V. blattaria* is suitable for planting.

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**REFERENCES**


Fungal Nomenclature Databases. Systematic Mycology and Microbiology Laboratory. Online publication. ARS, USDA. Available at: http://nt.ars-grin.gov.


Q-bank.eu. Colony morphology of *Phoma novae-verbascicola*. Available at: http://www.q-bank.eu/Fungi/BioloMICS.aspx?TableKey=611592400000011&Rec=58&Fields=All.
