Contamination of moth mullein (Verbascum blattaria L.) seeds by Phoma novae-verbascicola.

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

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Running title: *Phoma novae-verbascicola* on *Verbascum blattaria* seeds

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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. *P. 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.

Key words: ornamental plants, seed-borne pathogens, *Phoma poolensis* var. *verbascicola*, *Phyllosticta 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.: *Phyllosticta 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 (Tompkins 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 felty mycelium. Pycnidia were produced both on the agar and in the agar. They were globose to subglobose, solitaries 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* obtained from seeds 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 5×10^7 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, of 70:20:10, respectively). Ten plants (1 plant/pot) were inoculated (1ml of inoculum/plant), and 10 control plants were sprayed with only sterilised water. 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.

AKNOWLEDGEMENTS

The research leading to these results has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 634179 "Effective Management of Pests and Harmful Alien Species - Integrated Solutions" (EMPHASIS).

REFERENCES

Altschul S.F., Madden T.L., Schaffer A.A., Zhang Z., Miller W., Lipman D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programme. *Nucleic Acids Research* **25**: 3389-3402.

Aveskamp M.M., de Gruyter J., Woudenberg J.H.C., Verkley G.J.M., Crous P.W., 2010. Highlights of the *Didymellaceae*: a polyphasic approach to characterise *Phoma* and related pleosporalean genera. *Studies in Mycology* **65**: 1-60.

Bertetti D., Ortu G., Gullino M.L., Garibaldi A., 2015. Contamination of seeds of Iceland poppy (*Papaver nudicaule* L.) by *Fusarium oxysporum*. *Phytoparasitica* **43**: 189-196.

Boerema G.H., de Gruyter J., Noordeloos M.E., Hamers M.E.C., 2004. *Phoma* Identification Manual. Differentiation of specific and infra-specific taxa in culture. CABI Publishing, Wallingford, UK.

137 Daughtrey M.L., Wick R.L., Peterson J.L., 1995. Compendium of Flowering Potted Plant
138 Diseases. APS Press, St. Paul, Minnesota, USA.
139 Elmer W.H., 2002. Seeds as vehicles for pathogen importations. *Biological Invasions* **3**: 263-
140 271.
141 Elmer W.H., 2012. Biology and epidemiology. In: Gullino M.L., Katan J., Garibaldi A. (eds).
142 Fusarium wilt of greenhouse vegetable and ornamental crops, pp. 11-19. APS Press, St. Paul,
143 Minnesota, USA.
144 Garibaldi A., Bertetti D., Poli A., Gullino M.L., 2013. A Leaf Spot caused by *Phoma novae-*
145 *verbascicola* on Black Mullein (*Verbascum nigrum* L.) in Italy. *Plant Disease* **97**: 1660.
146 Garibaldi A., Bertetti D., Ortu G., Gullino M.L., 2014. First report of *Phoma novae-verbascicola*
147 on moth mullein (*Verbascum blattaria* L.) in Italy. *Journal of Plant Pathology* **96**: 436.
148 Kinsey G.C., 2002. *Phoma pinodella*. *IMI Descriptions of Fungi and Bacteria* **151**: sheet 1505.
149 Fungal Nomenclature Databases. Systematic Mycology and Microbiology Laboratory. Online
150 publication. ARS, USDA.
151 Orlicz-Luthard T., 1998. On the transfer of Fusarium wilt by seeds in China aster. *Seed Sci.*
152 *Technol.*, **26**: 67-76.
153 Pignatti S., 1982. Flora d'Italia. Edagricole, Bologna, Italia, 2324 pp.
154 Tompkins C. M., Snyder W. C., 1972. Cyclamen wilt in California and its control. *Plant Dis.*
155 *Rep.*, **56**: 493-497.
156 Q-bank.eu. Colony morphology of *Phoma novae-verbascicola*. Available at: http://www.q-
157 bank.eu/Fungi/BioloMICS.aspx?TableKey=6115924000000011&Rec=58&Fields=All
158 USDA, Fungal Databases. Available at: http://nt.ars-grin.gov/

159 White T.J., Bruns T., Lee S., Taylor J.W., 1990. Amplification and direct sequencing of fungal
160 ribosomal RNA genes for phylogenetics. In : Innis M.A., Gelfand D.H., Sninsky J.J., White T.J.
161 (eds). PCR Protocols: a guide to methods and applications, pp. 315-322. Academic Press, Inc.,
162 New York, USA.