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

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1 **CONTAMINATION OF MOTH MULLEIN (*VERBASCUM BLATTARIA* L.)**

2 **SEEDS BY *PHOMA NOVAE-VERBASCICOLA***

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

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25 **SUMMARY**

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27 *Verbascum blattaria* (Scrophulariaceae family) is a hardy perennial species that is used for the
28 edges and flower beds of low-maintenance gardens. *Phoma novae-verbascicola* causes light
29 brown necrotic spots on the leaves of *V. blattaria* seedlings. In order to demonstrate the seed
30 transmission of this pathogen, several *V. blattaria* seeds belonging to three samples collected in
31 2013, were tested *in vitro* to detect the presence of *P. novae-verbascicola*. Two samples were
32 found to be contaminated and colonies of the pathogen were isolated from the tested seeds. *P.*
33 *novae-verbascicola* was identified from the morphological features observed *in vitro* and through
34 an ITS (Internal Transcribed Spacer) analysis. The virulence of one isolate was confirmed by
35 means of a pathogenicity test. This work demonstrates that *P. novae-verbascicola* can be
36 transmitted by affected *V. blattaria* seeds.

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38 *Key words:* ornamental plants, seed-borne pathogens, *Phoma poolensis* var. *verbascicola*,
39 *Phyllosticta novae-verbascicola*.

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

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

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

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

88 In order to confirm the morphological identification, genomic DNA of the DB15GIU13
89 isolate obtained from seeds was extracted from a pure culture grown on PDA, using the
90 Nucleospin Plant II Kit (Macherey Nagel), according to the manufacturer's instructions. The
91 internal transcribed spacer (ITS) region was then amplified and sequenced using the ITS1/ITS4
92 primer (White *et al.*, 1990). BLAST analysis (Altschul *et al.*, 1997) of the 504-bp amplicon

93 (GenBank Accession No. KU559629) showed 99% homology with the KJ192364 sequence of *P.*
94 *novae-verbascicola*, thus confirming the morphological identification of the pathogen.

95 In order to test the pathogenicity, the DB15GIU13 isolate of *P. novae-verbascicola* obtained
96 from seeds was grown in Petri dishes for 26 days on PDA, at temperatures ranging from 21 to
97 24°C. A conidial suspension was then prepared from pure cultures and adjusted to the final
98 concentration of 5×10^7 CFU/ml. The inoculum was sprayed onto healthy 60-day-old *V.*
99 *blattaria* plants grown in pots containing a steamed soil mixture (peat moss:perlite:clay, of
100 70:20:10, respectively). Ten plants (1 plant/pot) were inoculated (1ml of inoculum/plant), and 10
101 control plants were sprayed with only sterilised water. All the plants were covered with a plastic
102 bag to maintain an elevated relative humidity and were kept in a greenhouse, where the daily
103 average temperatures ranged from 18 to 20°C. The plants were checked daily and the humid
104 chamber was removed 4 days after the inoculation.

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

109 This study demonstrates that the contamination of *V. blattaria* seeds by *P. novae-verbascicola*
110 may be a potential source of inoculum and could favour the diffusion of this pathogen. This
111 result is in agreement with the results of other seed-borne *Phoma* spp., such as *P. pinodella*,
112 which has been reported on several hosts, including species belonging to Leguminosae (Kinsey,
113 2002) and on *Phoma digitalis* found on Scrophulariaceae species, especially on *Digitalis*
114 *purpurea* (Boerema *et al.*, 2004).

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

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