Melanospora washingtonensis, a new species from potato

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Nadav Nitzan, Jack D. Rogers & Dennis A. Johnson (2004): *Melanospora washingtonensis*, a new species from potato. – Sydowia 56 (2): 61–67.

An undescribed species of *Melanospora*, *M. washingtonensis*, was discovered on incubated potato slices. The fungus features compressed ascospores and long setae at the apex of the perithecial neck. An anamorph with apparent annellides was seen sporadically. Possible affinities of the fungus with other *Melanospora* species are discussed.

Keywords: *Melanospora*, Pyrenomycetes, systematics

Tubers of potato (*Solanum tuberosum* L.) cultivar Shepody were collected in a field near Pasco, Franklin Co., Washington in August, 2003 with the intention of isolating the black dot fungus, *Colletotrichum coccodes* (Wallr.) S. Hughes, from them. Tubers were disinfected in NaClO for 10 min, rinsed in distilled water, and air-dried. Tuber stolon ends were excised and a piece of vascular tissue placed on potato dextrose agar that had been emended with streptomycin sulphate. Plates were incubated at 25°C in darkness for 14 days. Perithecia of a *Melanospora* formed on one slice of tuber and some of them were transferred to corn meal agar. Our examination and perusal of the literature led us to believe that the *Melanospora* represents an undescribed species. Slides were kindly examined by Josep Guarro and Alberto Stchichel who reinforced our belief that the fungus has not been previously described. It is thus described here.

Materials and Methods

Perithecia were picked from the tuber slice and transferred to 2% Potato Dextrose Agar (Difco), 2% Corn Meal Agar (Difco), SMEA medium (Kenerley & Rogers (1975), and Leonian's Agar (Booth, 1971). Growth and sporulation were optimal on the latter medium and all observation herein are recorded from it. A *Verticillium* species always contaminated the *Melanospora* colonies. Pure cultures were established by removing ascospore masses from peri-

thecial necks that protruded above the contaminating fungus and placing them on clean media. Length and width measurements of ascospores (20) and their narrow side (15) and of perithecia (10) were made in water mounts. Observations were made with bright field light microscopy (BF), differential interference contrast microscopy (DIC) and scanning electron microscopy (SEM).

Taxonomic Part

Melanospora washingtonensis N. Nitzan, J. D. Rogers & D. A. Johnson; **sp. nov.** – Figs. 1–19

Perithecia subhyalina vel flavida basi (115–)200–250(–300) µm diam. Collo 150–200(–266) µm longo setis 53–90(–115) µm longis praeditum. Asci clavati vel subglobosi, 8-spori, initio longe stipitati, tandem 22–40 × 17–22 µm, jam deliquescentibus. Paraphyses jam deliquescentibus. Ascosporae brunneae late ellipsoideae a latere compressae, leves, 10.5–12(–13.5) × 9–10.5(–12) x 7–9 µm, utrinque poro germinativo praeditae. Status asexualis inclusus.

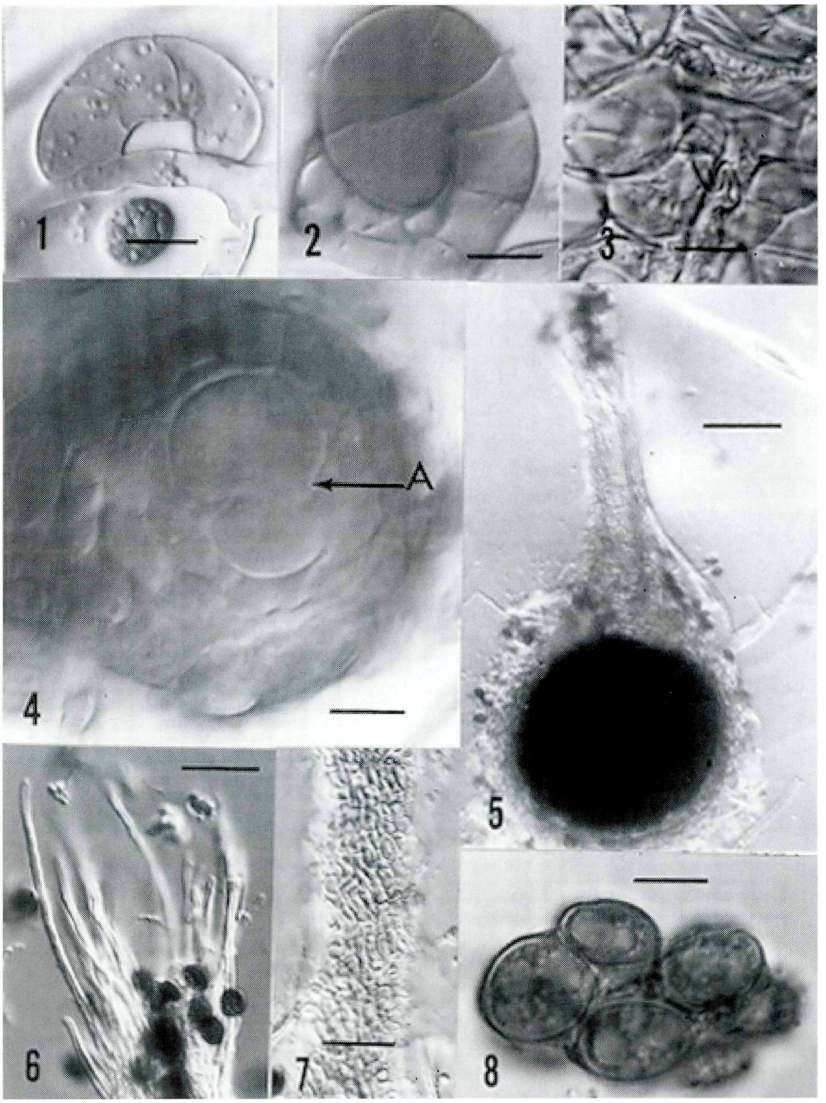
Perithecia subhyaline to yellowish,at the base (115–)200–250 (–300) µm diam. (Fig. 5, 17). With neck 150–200(–266) µm long, provided with setae 53–90(–115) µm long (Figs. 5–7, 18). Asci clavate to subglobose, 8-spored (Figs. 8, 9), initially long-stipitate, finally 22–40 × 17–22 µm, soon deliquescent. Paraphyses early deliquescent. Ascospores brown, broad ellipsoid, laterally compressed, smooth, 10.5–12(–13.5) × 9–10.5(–12) × 7-9 µm (ave. 11.7 ± SD 0.7 × 10.1 ± SD 0.9 × 7.7 ± SD 0.5 µm), with a germ pore at each end (Figs. 10, 11, 19). Anamorph included.

Colony on Leonian's agar incubated in darkness at ca. 20°C covering plate in about 3 wk, white, lanose, producing perithecia from the center outward. Frequent hyphal swellings, probably chlamydospores, often with thick walls, globoid to irregular, 7–14 × 5–10 µm produced (Figs 14, 15. Occasional flask-shaped cells, 4.5–5.5 × 2.2–2.7 µm, possibly annellides, producing subglobose conidia often with fattened bases, hyaline, 2.5–3 × 1.5–2.5 µm (Figs. 12, 13, 16). Conidia often collecting in globular masses on the medium.

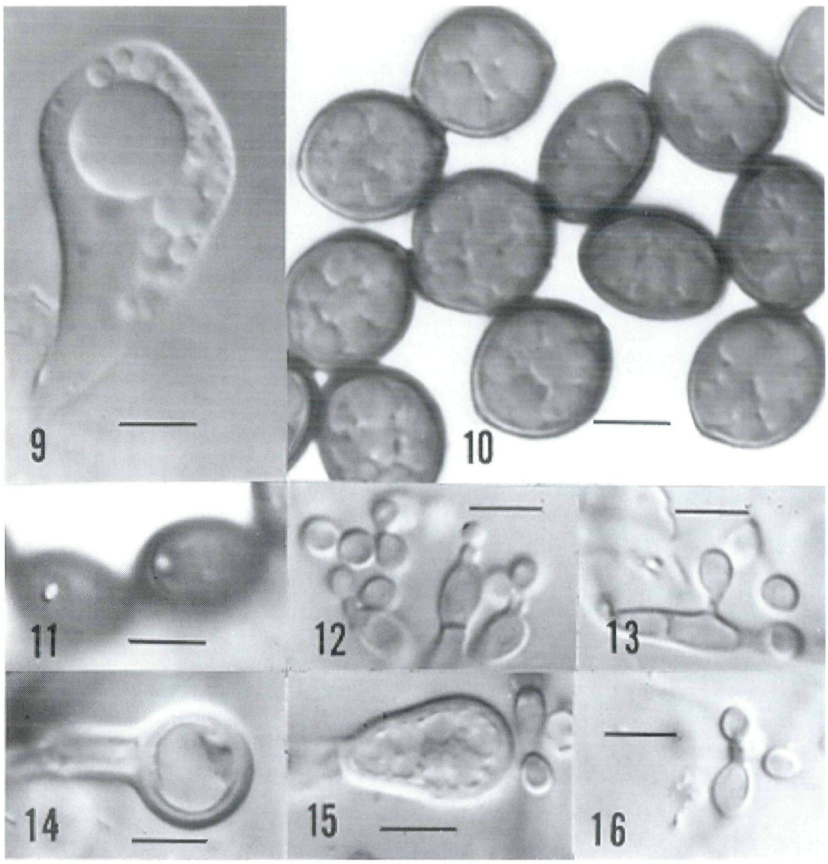
Etymology. – for Washington state, USA

Specimen examined. – USA, Washington state, Franklin Co., near Pasco, on slice of potato tuber, Aug 2003, Nadav Nitzan, WSP 70967 holotype. Dried cultures from holotype WSP 70968 and 70969. Culture deposited in ATCC.

Perithecia are initiated when an ascogonium is produced from a vegetative hypha (Fig. 1). The ascogonium enlarges, produces additional septa (Fig. 2), and becomes enveloped by branches from

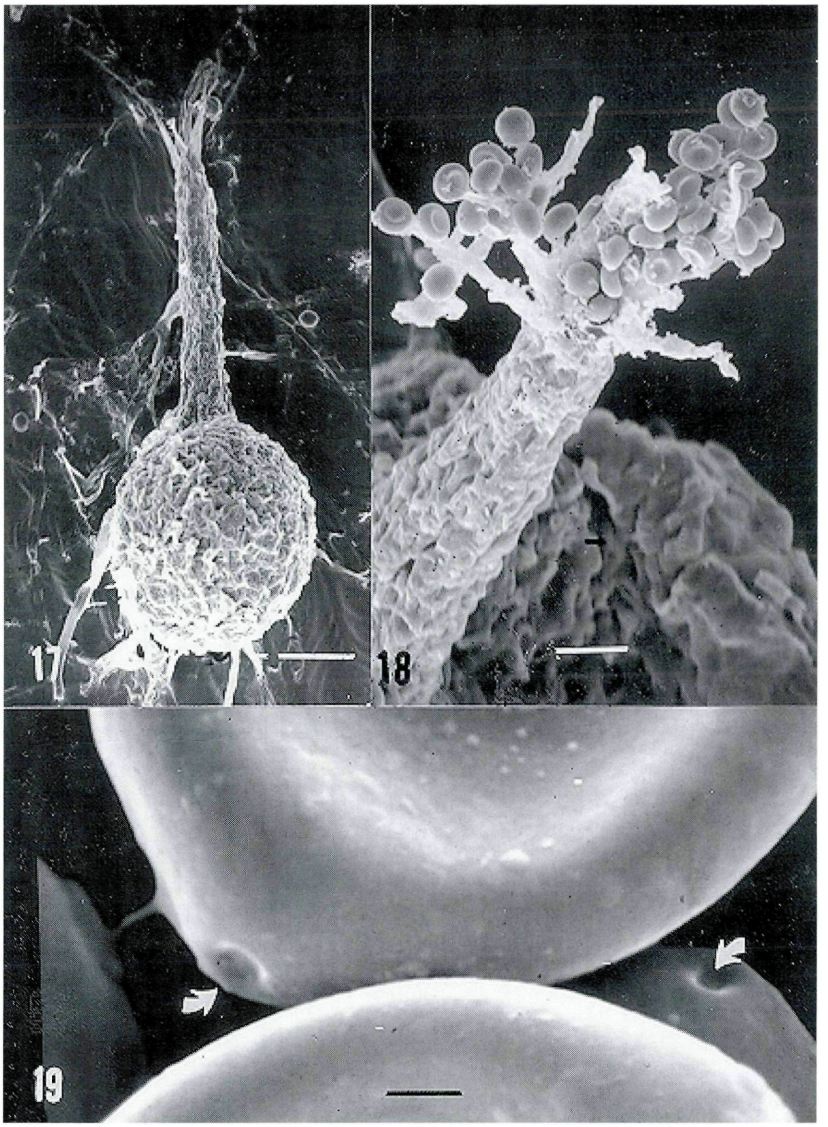


Figs. 1–8. *Melanospora washingtonensis*. – 1, 2. Ascogonia, the one depicted in Fig. 2 has become enlarged with numerous septa. – 3. Surface of perithecial wall. – 4. Protoperithecium showing prominent ascogonium (A) enclosed in hyphae destined to become the perithecium wall. – 5. Mature perithecium with centrum filled with ascospores. – 6. Hyphal ornamentation on apex of perithecial neck, with associated ascospores. – 7. Surface of perithecial neck wall. – 8. Six (of eight) ascospores in ascus. Ascal wall not visible. – All figs. by DIC. Figs 2, 4, 8 from mounts in aqueous phloxine; other figs. from water mounts. Scale bars: 1–4 = 14 µm; 5 = 53 µm; 6 = 18 µm; 7 = 46 µm; 8 = 7 µm.



Figs. 9–16. *Melanospora washingtonensis*. – 9. Young ascus. – 10. Ascospores. Note the two spores near center display the narrow dimension. – 11. Ascospores, each of which show one (of two) germination pores. – 12, 13. Conidiogenous cells and conidia. – 14, 15. Inflated, thick-walled hyphal cells, probably chlamydospores. – 16. Conidiogenous cell bearing a conidium. Elongated neck of conidiogenous cell might be annellated. All figs. by DIC. All figs. from water mounts. Scale bars = 7 μm.

the hypha that produced it and possibly other hyphae in the vicinity. The ascogonium comes to occupy the center of the enlarging globoid protoperithecium, apparently disarticulating (Fig. 4). Additional stages were not examined in detail, but the protoperithecium becomes pyriform, develops a periphysate neck, and produces asci from ascogenous hyphae that line the base and lower sides. Paraphyses are few and largely nonpersistent. Asci are clavate with a more or less long stipe (Fig. 9). Ascospores are produced in a ball within (Fig. 8) and the ascus quickly deliquesces; intact mature asci



Figs. 17–19. *Melanospora washingtonensis*. – 17. Perithecium. – 18. Ascospores among setae of perithecial neck. – 19. Ascospore germination pores (arrows). All figures by SEM. Scale bars: 17 = 75 µm; 18 = 21 µm; 19 = 1 µm.

are difficult to find. Ascospores, prior to becoming too opaque for observations, are quadrinucleate.

Melanospora washingtonensis grows well in a two-membered culture with an unidentified *Verticillium* species. On Leonian's agar

a cottony mycelium of *Verticillium* bears abundant perithecia of *Melanospora* on its surface. The relationship of these two fungi is uncertain. It seems most likely that the *Melanospora* is parasitic on the *Verticillium*. The *Verticillium* is white, produces no sclerotia or other obvious resting structures on the media employed, and produces a yellow diffusing pigment in Leonian's agar. It has some features of *V. tricorpus* I. Isaac (colony color and yellow diffusate), but lacks resting structures (Isaac, 1953). The *Verticillium* most commonly isolated from Washington potato fields is *V. dahliae* Kleb.

Discussion

Melanospora washingtonensis resembles *M. longisetosa* P. Cannon & D. Hawksw. and *M. chionea* (Fr.) Corda in the laterally compressed ascospores (Cannon & Hawksworth, 1982). It differs from the former species primarily in having smaller ascospores and eight-spored asci and from the latter species in ascospore dimensions. The biological activities of *M. washingtonensis* are unknown. It was associated with a *Verticillium* species when isolated and, indeed is difficult to separate from that fungus in order to obtain pure cultures. Cultures on Leonian's medium with the *Melanospora* and the *Verticillium* grow vigorously, with the *Melanospora* producing abundant perithecia. The *Verticillium* produces a yellow reverse on Leonian's agar and, thus, contaminated cultures are easily detected. Interestingly, *M. zamiae* Corda, a fusion biotroph, has a wide host range that includes *V. albo-atrum* Reinke & Berthold and *V. dahliae* (Jeffries & Young, 1994). *Melanospora damnosa* (Sacc. & Berlese) Lindau is a parasite of *Fusarium* species and *M. brevirostris* (Fuckel) Höhnel, *M. caprina* (Fr.) Sacc., *M. lagenaria* (Pers.) Fuckel, and *M. fusispora* (Petch) Douget are associated with a variety of other fungi (Jeffries & Young, 1994). Of great interest, a recent molecular study by Zhang & Blackwell (2002) suggests that *Melanospora* is allied to order Hypocreales. Many undoubted hypocreaceous fungi are mycoparasitic in manners similar to *Melanospora*.

A conidial fungus featuring small conidiogenous cells, possibly annellides, is seen occasionally. It is almost certainly the anamorph of *Melanospora washingtonensis*, but we were unable to isolate it and initiate cultures from it. It highly resembles the anamorph reported for *Melanospora singaporensis* Morinaga, Minoura & Udagawa (Moringa et al., 1978) and in some respects the anamorphs of *M. ornata* Zukal (Furuya & Udagawa, 1973) and *M. fusispora* (Petch) Doguet (Udagawa, 1970). The conidiogenous cells of these species were reported to be phialides.

Table 1. Comparison of *Melanospora* species with compressed ascospores

Species	Ascospore number and dimensions	Perithecial neck length	Anamorph	Host or substrate
M. washingtonensis	8 spores/ascus 10.5–12(–13.5) × 9–10.5(–12) × 7–9 µm	150–200(–266) µm	Annellidic hyphomycete	*Solanum tuberosum*. Associated with *Verticillium* sp.
M. longisetosa	4 spores/ascus 14–19 × 12–14 × 8–9 µm	50–70 µm	Unproven	Possibly parasitic on *Tubercularia*
M chionea	8 spores/ascus 7.5–16 × 6–12 × 4–7 µm	250–400 µm	Unknown	On *Pinus sylvestris*, possibly associated with fungi

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