North American Fungi

Volume 3, Number 7, Pages 215-229
Published August 29, 2008
Formerly *Pacific Northwest Fungi*

Characterization of *Seuratia millardetii* on *Camellia* species and in artificial culture

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Gillis, I. M., and D. A. Glawe. 2008. Characterization of *Seuratia millardetii* on *Camellia* species and in artificial culture. *North American Fungi* 3(7): 215-229. doi: 10.2509/naf2008.003.00714

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Abstract: A sooty mold-like disease of *Camellia* species is observed frequently in the Pacific Northwest but the identity of the causal organism has not been reported. To clarify taxonomy of the fungus associated with the disorder, affected *Camellia* leaves were collected from locations in Seattle, WA, and from the *Camellia* collection of the Washington Park Arboretum, University of Washington. The fungus on affected leaves was determined to be *Seuratia millardetii* (anamorph: *Atichia glomerulosa*), a member of the Myriangiales *sensu* Barr known previously from conifer species in the region. Fungal colonies ranged from less than 0.1 mm to 5 mm in diameter, and were cushion-shaped to lobed. Colonies consisted of a peridium of melanized cells containing

enclosing globose, hyaline cells in a gelatinous matrix; hyphae were lacking. Dimorphic macroconidia and microconidia (determined in this study to be phialospores) typical of *A. glomerulosa* were observed. The teleomorph included bitunicate asci and ascospores typical of *S. millardetii*. Observation with SEM confirmed that colonies grew superficially on leaves without penetrating them. Colonies in culture resembled those on host leaves, produced dimorphic macroconidia, but grew indeterminately to become several cm across. The fungus was observed on several named cultivars of *C. japonica*, *C. sasanqua*, and *C. x williamsii*. Although known previously from a wide variety of angiosperm and conifer hosts, this is the first time *S. millardetii* has been reported on *Camellia* spp. This is also the first report of the teleomorph of this fungus in the Pacific Northwest, and the first description of the fungus in artificial culture.

Key Words: exobiology, microcolonial fungi, Myriangiales, phylloplane, plant pathogen.

Introduction: The term “sooty mold” refers to dark-colored fungal mycelia and sporulating structures growing superficially on leaves and produced by Ascomycetes in the orders Capnodiales, Chaetothyriales, or Meliolales (Agrios, 1988; Kirk et al., 2001). Thought to be most diverse and abundant in tropical regions, they derive their nutrition from honey dew secreted by insects. For many years a similar disorder of *Camellia* species has been observed in the Pacific Northwest (Pscheidt and Ocamb, 2007) but no reports indicated the identity of the associated fungus. Accordingly, the present study was undertaken to clarify which fungus or fungi are associated with sooty mold symptoms on *Camellia* species collected in Seattle.

Materials and Methods: Symptomatic plant material was collected on the campus of the University of Washington, Seattle, King Co., and the Washington Park Arboretum in Seattle from February 1 to July 1, 2007. Voucher specimens were deposited in the mycology herbarium in the Plant Pathology Department at Washington State University (WSP).

For isolation into pure culture, colonies were removed from leaves using a razor blade and hydrated in tap water, causing them to

enlarge. To remove other organisms, hydrated colonies were washed by vortexing in tap water, sedimenting them by centrifugation in a microfuge, resuspending them in 95% ethanol and vortexing again. This process was repeated 3 times. The colonies were then placed on BBL brand Potato-Dextrose agar (PDA) and maintained under ambient laboratory temperature (approximately 20°C) and light conditions. When growth of contaminating organisms was observed, colonies were removed from the agar and the purification procedure repeated. When colonies in pure culture were about 2-mm they were subdivided and replated on PDA to produce additional colonies for macroscopic and microscopic observations.

Characters of microscopic features were observed, dimensions recorded, and photographs taken with a Leica DMR compound microscope equipped with brightfield and differential interference contrast optics and a Leica MZ95 stereo microscope, both equipped with Leica DC300 digital cameras. For scanning electron microscopy, a razor blade was used to excise 2-mm-square sections of leaf tissue with fungal colonies which then were fixed using Karnovsky’s fixative (Karnovsky, 1965), critical point dried using a Denton DCP-1 critical point dryer, and gold-coated using a

Technics Hummer V sputter coater. Processed material was observed and photographed using a JEOL 840A scanning electron microscope at the Department of Biology Imaging Facility, University of Washington.

Results: Colonies (Figs. 1, 3-6) on leaves of *Camellia* species usually ranged from 0.1 mm to 0.3 mm diam. but sometimes attained diam. of 5 mm. Colonies were pulvinate and tended to become lobed as they enlarged. When dry they appeared shrunken and cartilaginous, and when immersed in water they quickly enlarged and became gelatinous. Colonies consisted of a outer peridium of melanized, brown, slightly echinulate cells (Fig. 7, 9) and an inner region with globose, hyaline cells (Figs. 8, 10-12) measuring (3.5-)4-9(-15) μm and embedded in a colorless gelatinous matrix. No hyphae were observed and cells appeared to reproduce via a budding-like process. Conidiogenous regions of colony surfaces appeared as small, whitish areas sometimes produced two kinds of macroconidia; globose conidia (Figs. 13, 15) were (15-)16-24.5(-28) μm , and cheirospores (Fig. 18) were 20-32 x 10-16 μm . Phialospores (sometimes termed microconidia) (Figs. 17, 18) were produced within locules near colony surfaces from globose to pyriform conidiogenous cells and were rod- to femur-shaped, hyaline, and 5.5-7(-7.5) x 1-2(-2.5) μm . The teleomorph consisted of locules (Fig. 19) with globose to subclavate, bitunicate asci (Figs. 20, 21) that measured (24.5-)28-39(-50) x (20-)21-28.5(-30.5) μm and contained eight ascospores that were hyaline, one-septate, and (12.5-)13.5-18.5 x (6-)6.5-9 μm . Colonies grew superficially on host leaves, exhibited

no indication of penetrating host tissues, and were easily dislodged (Figs. 31-34).

Three-month-old colonies on PDA (Figs. 22-24) were cerebriform, dark brown to black with minute whitish flecks, and grew indeterminately, attaining diameters of several cm. Colonies were anatomically similar to those on host tissue but internal cells, embedded in a transparent gelatinous matrix, ranged in color from brownish to hyaline (25-27, 29, 30). Whitish areas on colony surfaces contained globose macroconidia (Fig. 28).

The fungus was observed on several named cultivars of *Camellia japonica* L., *C. sasanqua* Thunb., and *C. x williamsii* (Table 1). No signs of insect infestation were observed. Colonies also were observed on needles of *Pseudotsuga menziesii* (Mirb.) Franco trees growing near or among colonized *Camellia* plants (Fig. 2).

Table 1. *Camellia* species and cultivars on which *Seuratia millardetii* was observed in the Washington Park Arboretum, Seattle, WA.

Genus	Species	Cultivar
<i>Camellia</i>	<i>japonica</i> L.	Akebono
<i>Camellia</i>	<i>japonica</i> L.	Masterpiece
<i>Camellia</i>	<i>japonica</i> L.	Monte Carlo
<i>Camellia</i>	<i>japonica</i> L.	Sol de Oro
<i>Camellia</i>	<i>japonica</i> L.	Sylvia
<i>Camellia</i>	<i>japonica</i> L.	Ville de Nantes
<i>Camellia</i>	<i>japonica</i> L.	Warrior
<i>Camellia</i>	<i>japonica</i> L.	Willmeta
<i>Camellia</i>	<i>sasanqua</i> Thunb.	Apple Blossom
<i>Camellia</i>	<i>sasanqua</i> Thunb.	Tago-no-tsuki
<i>Camellia</i>	<i>X williamsii</i>	Stryker

Discussion: The fungus described in this report from *Camellia* spp. matches previous descriptions of the teleomorph and anamorph of *Seuratia millardetii* made from host material (Meeker, 1975; Dennis, 1978; Funk, 1981; Sivanesan, 1984; Barr, 1987). No previous reports of the fungus from artificial culture were found. Noteworthy features of this organism in culture include the production of indeterminate colonies consisting of globose, budding cells embedded in a gelatinous matrix and bounded by melanized peridial cells, and production of globose macroconidia. As on host material, hyphae were lacking.

This is the first report of *Seuratia millardetii* from *Camellia* species. The fungus was found growing on leaves of 11 of the more than 200 named cultivars in the *Camellia* collection at the Washington Park Arboretum in Seattle. The observation of this fungus on only a small percentage of the cultivars in the collection suggests the need for further research to assess whether or not cultivars differ in the ability to support growth of the fungus.

Sooty mold of *Camellia* has been listed in editions of the *Plant Disease Control Handbook for the Pacific Northwest* (e.g., Pscheidt and Ocam, 2007) for some years and the fungus likely has occurred on *Camellia* species in the region for many years. The lack of a previous report of *S. millardetii* on *Camellia* probably reflects the incomplete knowledge of the fungi of the region. Previous reports of *S. millardetii* on a variety of other hosts were made from widespread locations in temperate and tropical regions (Table 2). Within the Pacific Northwest, *S. millardetii* has been known for many years to occur on conifer species (Shaw, 1973; Sherwood and Carroll, 1974; Funk, 1981). The widespread occurrence of

S. millardetii, its broad host range, and the popularity of *Camellia* species in many regions suggest that *S. millardetii* will be found on *Camellia* species in additional locations.

The fungus grew epiphytically, apparently obtaining nutrients by absorbing them from its environment on the leaf, rather than directly from the leaf itself. No insects were found associated with colonies, which was surprising because control recommendations include eliminating *Camellia*-infesting insects which presumably produce honey dew on which the fungus could subsist. These observations call into question the effectiveness of controlling insects in order to control this disorder on *Camellia* species. Although at present the source of nutrients is uncertain, observation of *Camellia* leaves revealed a wide variety of dust particles, pollen grains, and other materials that could potentially be sources of nutrients. It is also possible that insect honeydew originating on other nearby plant species, or from transient populations of insects on the examined *Camellia* species were supporting the *S. millardetii* colonies.

The unusual, cushion-shaped to lobed colonies may enhance the ability of this fungus to scavenge nutrients from its environment. Perhaps absorption of nutrients is enhanced by the way colonies shrink and collapse under dry conditions, yet quickly absorb water and enlarge when free water is available. We hypothesize that small molecules such as sugars or amino acids could be absorbed quickly into colonies as they imbibe free water from the environment. The development of lobes in some older colonies increases the ratio of surface area to internal volume. In addition, lobes on colonies immersed in water tended to move back and forth, a process that might aid the absorption of solutes from surrounding water. The

Table 2. Previous reports of *Seuratia millardetii*, or the associated anamorph *Atichia glomerulosa*, or *Atichia* sp. (likely anamorphic *S. millardetii*).

<i>Seuratia millardetii</i> and <i>Atichia glomerulosa</i> records	
Austria	on <i>Dryopogon jubulum</i> , <i>Salix</i> sp. (Meeker, 1975)
Brazil	on <i>Citrus limonia</i> , <i>C. limeta</i> , <i>Xylopia brasiliensis</i> , <i>X. frutescens</i> (Meeker, 1975); on <i>Citrus limonia</i> , <i>Guarea trichilioides</i> Mendes et al., 1998)
Canada (BC)	on bark of <i>Pseudotsuga menziesii</i> , <i>Abies grandis</i> (Funk, 1981)
Canada	on <i>Abies balsamea</i> , <i>Abies grandis</i> , <i>Picea mariana</i> (Ginns, 1986)
Caribbean	on <i>Cameraria orientensis</i> , <i>Clethra cubensis</i> , <i>Ilex macfadyenii</i> , <i>Jacaranda arborescens</i> (Minter et al., 2001)
Costa Rica	<i>Anthurium</i> sp., <i>Cestrum</i> sp., <i>Roupala</i> [as <i>Roupalae</i>] <i>veraguensis</i> , <i>Xylosma salzmannii</i> (Meeker, 1975)
Dominican Republic	on <i>Eugenia</i> sp. (Ciferri, 1961)
Ecuador	on <i>Podocarpus</i> sp., <i>Weinmannia</i> sp. (Dennis, 1970)
Gambiers Islands	on <i>Coffea</i> sp. (Meeker, 1975)
Germany	on <i>Abies pectinata</i> , <i>Tilia</i> sp. (Meeker, 1975)
Hawaiian Islands	on <i>Coffea</i> sp., <i>Gardenia</i> sp., <i>Metrosideros collina</i> subsp. <i>polymorpha</i> , <i>Pipturus albidus</i> , <i>Schinus terebinthifolius</i> , <i>Wikstroemia</i> sp. (Meeker, 1975); on <i>Gardenia</i> sp., <i>Passiflora</i> sp., <i>Pipturus albidus</i> , <i>Scaevola</i> sp., <i>Schinus terebinthifolius</i> , <i>Smilax</i> sp., <i>Straussia</i> sp., <i>Suttonia</i> sp., <i>Syzygium</i> sp. (Petrak, 1953)
Idaho	on <i>Abies grandis</i> (Shaw, 1973)
Java	on <i>Ficus elastica</i> , <i>Psychotria divergum</i> , <i>P. sylvatica</i> (Meeker, 1975)
Malaysia	on <i>Mangifera foetida</i> <i>Psidium guajava</i> (Turner, 1971); <i>Schizostachym</i> sp. (Liu, 1977; Petrak, 1954)
New Caledonia	on <i>Psidium guajava</i> (Huguenin, 1966)
Papua New Guinea	on <i>Nothofagus</i> sp. (Shaw, 1984)
Puerto Rico	on <i>Jambosa jambos</i> (Stevenson, 1975)
Sweden	on <i>Abies</i> sp. (Eriksson, 1992)
Tahiti	on <i>Coffea</i> sp. (Meeker, 1975)
UK	on <i>Rhododendron</i> sp., <i>Escallonia</i> sp. and <i>Lonicera</i> sp. (Cannon et al., 1985)
Virgin Islands	on <i>Jambosa jambos</i> (Stevenson, 1975)
Washington	on <i>Pinus ponderosa</i> , <i>Pseudotsuga menziesii</i> (Shaw, 1973)
<i>Atichia</i> sp. record	
Oregon	on <i>Pseudotsuga menziesii</i> (Sherwood and Carroll, 1974)

melanized peridial cells could aid in protecting the colonies from UV radiation and from grazing arthropods and other hazards.

Interestingly, similar colony forms have been reported in fungi associated with rock varnishes. Such fungi are termed

“microcolonial fungi” and generally form cushion-shaped colonies approximately 0.10 mm in diameter, filled with globose cells embedded in gelatinous material and surrounded by a peridium of melanized cells (Staley et al., 1982). The ability of such fungi to grow under harsh, nutrient-poor conditions has made them of interest to researchers

concerned with possible life forms in extra-terrestrial environments (Borgushina, 2003). The discovery of similar colony forms in fungi adapted both to growing on rock surfaces and plant leaves suggests common adaptations in diverse fungi growing on exposed surfaces with relatively low levels of available nutrients.

Observations reported herein suggest that *S. millardetii* grows epiphytically on its hosts and does not parasitize them directly. The fungus appears to absorb nutrients dissolved in the free water on leaf surfaces that periodically immerses the colonies. The lack of direct parasitism of hosts on which colonies grow could cause one to question whether *S. millardetii* can be considered a plant pathogen, and whether its effect on the host can be considered a plant disease. Heald (1926) noted that “In discussing disease, we may consider plants from two different points of view: first, as individuals whose place in nature is filled by growth to maturity with provision for the reproduction of their kind; and, second, as producers of crops, economic products, or possessors of qualities desired by man.” He (Heald, 1926) provided definitions of plant disease appropriate for both perspectives. For the first point of view he (Heald, 1926) noted: “First, disease in plants may be defined as any variation from the normal, as expressed either by the checking or by the interruption of physiological activities or by structural changes, which are sufficiently permanent to check development, cause abnormal formations or lead to premature death of a part of the plant or the entire individual.” In this sense, *S. millardetii* could be regarded either not as a pathogen, since it grows epiphytically on hosts without apparent parasitism or destruction of plant tissues, or as a pathogen if colony populations became great enough to interfere with photosynthesis or other physiological

functions of colonized leaves. Heald’s (1926) second definition of plant disease is “Second, disease may be defined as a ‘failure of thrifty development of the plant to produce a commercial product of satisfactory quality and quantity’”, a definition he attributed to an earlier (1911) report by R. E. Smith and E. H. Smith. In this sense *S. millardetii* can be viewed as pathogenic on *Camellia* and other ornamental plant species where the appearance of the fungus detracts from the ornamental value of affected plants and has prompted plant pathologists to provide information on how to eradicate it (Pscheidt and Ocamb, 2007).

Acknowledgments: PPNS No. 0487, Department of Plant Pathology, College of Agricultural, Human, and Natural Resource Sciences, Agricultural Research Center, Project No. WNP00313, Washington State University, Pullman, WA 99164-6430, USA. We thank Pang Chan, Biology Imaging Facility, University of Washington, for instruction and assistance in scanning electron microscopy. It is a pleasure to acknowledge the helpful suggestions of Professor J. D. Rogers.

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Figs. 1-6. *Seuratia millardetii* colonies on *Camellia* and *Pseudotsuga menziesii*. Fig. 1. *Seuratia millardetii* colonies on leaf of *Camellia* sp. Bar = 1 mm. Fig. 2. Colonies of *S. millardetii* on needle of *P. menziesii*. Bar = 1 mm. Fig. 3. Dry pulvinate colonies of *S. millardetii* on leaf of *Camellia* sp. Bar = 0.5 mm. Fig. 4. Same colonies as shown in Fig. 3 after immersion in water for 60 sec. Bar = 0.5 mm. Fig. 5. Dry lobed colony of *S. millardetii* on leaf of *Camellia* sp. Bar = 0.5 mm. Fig. 6. Same colony as shown in Fig. 5 after immersion in water for 60 sec. Bar = 0.5 mm.

Figs. 7-12. *Seuratia millardetii* from *Camellia* sp. Fig. 7. Free-hand section through pulvinate colony. Note melanized peridial cells enclosing transparent internal cells. Bar = 25 μ m. Fig. 8. Internal cells. Bar = 15 μ m. Fig. 9. Peridial cells with melanized, slightly echinulate walls. Bar = 15 μ m. Fig. 10. Internal cells adhering after budding to form chains. Note faint lines that indicate presence of gelatinous substance in which cells are embedded. Bar = 15 μ m. Fig. 11. Internal cells, one producing a bud-like extension. Bar = 15 μ m. Fig. 12. Dyad of internal cells resulting from budding-like process. Bar = 15 μ m.

Figs. 13-18. *Seuratia millardetii* from *Camellia* sp. Fig. 13. Globose conidia sloughing from surface of colony. Bar = 25 μ m. Fig. 14. Locule lined with conidiogenous cells and rod-shaped spores. Bar = 15 μ m. Fig. 15. Globose conidium. Bar = 15 μ m. Fig. 16. Cheirospores. Bar = 15 μ m. Fig. 17. Conidiogenous cells (one designated with arrow) and rod-shaped spores. Bar = 15 μ m. Fig. 18. Rod-shaped spores. Bar = 15 μ m.

Figs. 19-21. *Seuratia millardetii* from *Camellia* sp. Fig. 19. Group of asci forming near margin of colony. Bar = 50 μ m. Fig. 20. Asci with ascospores. Bar = 15 μ m. Fig. 21. Asci with ascospores. Bar = 15 μ m.

Figs. 22-24. *Seuratia millardetii* in artificial culture. Fig. 22. One-month old colonies. Fig. 23. Close up of colony shown in Fig. 22. Note cerebriform morphology and whitish areas. Bar = 1 cm. Fig. 24. Surface of colony. Note cerebriform folds and whitish area (arrow). Bar = 2.5 mm.

Figs. 25-30. *Seuratia millardetii* from artificial culture. Figs. 25, 26. Clusters of brownish cells suspended in gelatinous material. Fig. 25. Bar = 0.25 mm. Fig. 26. Bar = 50 μ m. Fig. 27. Chains of internal cells. Bar = 25 μ m. Fig. 28. Globose conidia produced in whitish area on colony surface. Bar = 25 μ m. Fig. 29. Internal brownish and hyaline cells embedded in gelatinous material. Bar = 25 μ m. Fig. 30. Chains of internal cells embedded in gelatinous material. Bar = 15 μ m.

Figs. 31-34. *Seuratia millardetii* on *Camellia* sp. viewed with SEM. Fig. 31. Colonies. Bar = 50 μm . 32. Colony. Bar = 25 μm . Fig. 33. Portion of leaf surface with circular areas indicating attachment points of colonies that detached during processing for SEM. Bar = 100 μm . Fig. 34. Leaf surface previously covered by colony of *S. millardetii*. Evidence of penetration of plant epidermis is lacking. Bar = 25 μm .