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***Balansia hypoxylon* (Ascomycota: Clavicipitaceae) in Canada: a mycosymbiont with *Danthonia spicata* (Poaceae)**

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Abstract: A description of *Balansia hypoxylon*, based primarily upon relatively recent Canadian collections from 1981 and 1983, is presented. Because *B. hypoxylon* is known in Canada from only 11 collections it is perceived as being rare. The range of the fungus in Canada is extended from Nova Scotia into southeastern Ontario. This note, hopefully, will encourage surveys to define the northern limit of the fungus and determine whether it is rare in Canada or simply overlooked.

Key words: *Balansia hypoxylon*, *Acremonium*, *Atkinsonella hypoxylon*, *Ephelis*, Clavicipitaceae, Canadian fungi, endophytic fungi, mycosymbiont, *Danthonia spicata*, Poaceae

Introduction: The fungus *Balansia* (*Atkinsonella*) *hypoxylon*, a North American endemic, is widespread in the eastern half of the United States and occurs in extreme southeastern

Canada (Diehl 1950, Farr and Rossman 2011, Wehmeyer 1950). There are extralimital reports and Diehl (1950) concluded they were misdeterminations. The fungus infects live

grasses and its host range is restricted to a few species. Early North American literature (Diehl 1950 and see compilation in Farr and Rossman 2011) reported it on species of the *Danthonia* and *Stipa*. Diehl recognized that some macro-morphological features in the specimens on *Stipa* differed from those on *Danthonia* and proposed the *Stipa* collections be recognized as *A. hypoxylon* var. *texensis* Diehl.

By 1989 enough evidence had accumulated on the differences between populations of the fungus on *Stipa* and those on *Danthonia* that Leuchtmann and Clay (1989) segregated the *Stipa* group at the species level as *Atkinsonella texensis* (Diehl) Leuchtm. & Clay. For example, strains of *B. hypoxylon* from one host genus were completely incompatible (failed to grow) on seedlings of the alternative host genus (Leuchtmann and Clay 1989a) and the result of isozyme activity in 291 strains grouped all strains from *Stipa* in a “cluster” (clade) distinct from the *Danthonia* strains (Leuchtmann and Clay 1989b). Consequently the hosts accepted for *B. hypoxylon* are a few species of *Danthonia*.

Balansia hypoxylon was first reported from Canada when Ellis and Everhart (1885) described one of its two anamorphs as *Ephelis borealis* from a 1883 collection in Nova Scotia. Having collected the distinctive *B. hypoxylon* in southwestern Quebec in 1981, I was surprised to find that in the previous 98 years only four additional Canadian collections (three from Nova Scotia and one from southern Quebec on the United States border) had been reported (Diehl 1950, Wehmeyer 1950).

The explanation for its apparent rarity in Canada is uncertain as the fungus is obvious to the naked eye and is widespread, as well as relatively common, in the United States, abutting the international border from Maine to Ohio (Diehl 1950, Farr and Rossman 2011).

Balansia hypoxylon is well known through the numerous studies published on its biology (see overviews by Clay 1988 and Schardl 2010). The fungus is a systemic, perennial epiphyte (Leuchtmann and Clay 1988) and is entirely dependent upon four species in the grass genus *Danthonia* (Leuchtmann and Clay 1996). Two of the grasses are rare hosts and have been found infected only in a few small localities in the southern Appalachian Mountains. The other two, *D. spicata* (poverty oatgrass) and *D. compressa* (flattened oatgrass), occur in Canada. The

northern edge of the native range of *D. compressa* is thought to be southernmost Quebec and its occurrence along the Saint Lawrence and Ottawa river systems due to inadvertent introductions by early river travellers such as lumberjacks and fur traders (Dore and McNeill 1980), i.e., it is of limited distribution in Canada. *Danthonia spicata* is widespread, occurs across the continent and in all the Canadian Provinces and Territories (Darbyshire and Cayouette 1989).

The purposes of this note is to update the status of *B. hypoxylon* in Canada, facilitate the identification of specimens by providing a detailed description and illustrations, report extensions to its range and raise awareness of the fungus that will lead to clarification of its northern limits and/or its limited distribution in Canada.

Materials and Methods: The description is taken principally from collections DAOM 180299 and 211269. Microscopic features of the anamorphs and ascomata were examined using Melzer's iodine, 2% (w/v) potassium hydroxide (KOH), and 0.05% (w/v) cotton blue in lactic acid (Kirk et al. 2001). Phloxine, ca 1% in KOH, was used to stain cytoplasm. Abbreviations for herbarium names, i.e. DAOM, are those listed in Thiers (<http://sweetgum.nybg.org/ih/>).

Results:

Balansia hypoxylon (Peck) G. F. Atk.

Journal of Mycology 11:254. 1905.

Figures 1–7

≡ *Epichloë hypoxylon* Peck, Annual Report of the New York State Museum 27:108. 1875.

≡ *Hypocrella hypoxylon* (Peck) Sacc., Sylloge Fungorum 2:581. 1883.

≡ *Hypocrea hypoxylon* (Peck) Ellis & Everh., Journal of Mycology 2:67. 1886.

≡ *Dothichloë hypoxylon* (Peck) G. F. Atk., Bulletin of the Torrey Botanical Club 21:223. 1894.

≡ *Ophiodothis hypoxylon* (Peck) Sacc. ex G. F. Atk, Cornell University (Science) Bulletin 3:10. 1897.

≡ *Atkinsonella hypoxylon* (Peck) Diehl, United States Department of Agriculture Monograph 4:49. 1950.

= *Ephelis borealis* Ellis & Everh., Journal of Mycology 1:86. 1885. Anamorph.

Figure 1. *Balansia hypoxylon* in June. Infected culm of *Danthonia spicata* with grey *Acremonium*-like state covering the sclerotium, and several erumpent patches of the *Ephelis* state. From DAOM 180299. Scale = 5 mm.

Sclerotia 9-15 x 2 mm, fusoid, slightly to distinctly lunate, grey becoming dingy blackish grey, forming around the grass stem at a flowering axil. The ***Acremonium*-like** state appearing in early summer, a palisade of conidiogenous cells and paraphysoid hyphae on the surface of the sclerotium. ***Acremonium*-like conidiogenous cells** phialidic, cylindrical, 10 x 4 μ m with a relatively large attenuated apex 7 μ m long and 1 μ m diam. ***Acremonium*-like conidia** ellipsoid, ovoid to oblong, 3.8-5.2 x 2.0-2.4 μ m, walls hyaline, thin, smooth. The ***Ephelis*** state about 2 x 1 mm, erumpent through the *Acremonium*-like state, discoid to elongate, slightly raised, margin distinct, hymenial surface plane, dark grey (darker than the sclerotial surface), forming in mid-summer on the surface of the sclerotia. ***Ephelis* conidiogenous cells** elongate, about 15 x 2.5 μ m, producing conidia sympodially. ***Ephelis* conidia** blastic, filiform, (15-) 25-30 (-50) x 1.5-2.0 μ m, borne singly, rarely in chains of two, walls hyaline, thin, smooth. **Paraphysoid hyphae** scattered

Figure 2. *Balansia hypoxylon* in October. Sclerotium with black stroma bearing perithecia. From DAOM 211269. Scale = 5 mm.

between the *Ephelis* conidiogenous cells, 2.4-3.2 μ m diam, simple, the walls hyaline, thin, smooth. **Teleomorph stroma** ca. 2 mm diam, ca. 1 mm thick, pulvinate, black, broadly attached to a sclerotium. **Perithecia** produced in late summer, imbedded in a black stroma, ovoid, ca. 220 x 160 μ m. **Asci** cylindrical, ca 130-170 x 5-6 μ m, the apex 3 μ m thick. **Ascospores** 8 per ascus, fasciculate, filiform, 1.0-1.5 μ m diam, in the ascus disarticulating into segments 13-31 x 1.5-2.0 μ m, apical segment often slightly clavate and swollen to 7 μ m diam, basal segment narrowing to a blunted point.

Habitat: In Canada fruiting on live and dead stems of current year's growth of *Danthonia spicata* (L.) Beauv.

Canadian distribution: Nova Scotia, southwestern Quebec, southeastern Ontario.

Collections studied: Nova Scotia: Colechester Co.: Truro, east of Experimental Farm, on *D. spicata*, 15 August 1909, J. Dearness (DAOM in Dearness Herb. as Species No. 3160); Kings Co.:

Figures 3–7. *Balansia hypoxylon*. 3–5. *Ephelis* state. 3. Hymenial hypha. 4. Three filiform conidia. 5. Three conidiogenous cells and two developing conidia. 6–7. *Acremonium*-like state. 6. Three ellipsoid conidia. 7. Two conidiogenous cells with attenuated apices. All from DAOM 180299. Scale = 5 μ m.

Cape Blomidon, on *D. spicata*, 29 June 1883, J. Macoun 1 (DAOM 211270), isotype of *Ephelis borealis*; Lunenburg Co.: Lunenburg: Petite Riviere bridge, on *D. spicata*, 9 July 1949, J. F. Hockey (DAOM 23236); Pictou Co.: Green Hill, on *D. spicata*, 23 August 1941, R. R. Hurst (DAOM 7528). Québec: Gatineau Co.: Cantley (12 km NE of Ottawa): Sainte-Elisabeth road: Maurice Millson's farm, on *D. spicata*, 21 June 1981, A. & J. Ginns 6309 (DAOM 180299); 260 Sainte-Elisabeth road, 11 July 1983, J. Ginns 7386 (DAOM 187602) and 9 October 1983, J. Ginns 7904 (DAOM 211269); Ile-de-Montreal Co.: Saint-Hippolyte, 7 July 1966, Fr. Rolland-Germain 37139 (DAOM 196984). Ontario: Carleton Co.: Bells Corners (ca. 10 km W of Ottawa), on *D. spicata*, 9 October 1983, J. Ginns 7905 (DAOM 189873).

Discussion: *Balansia hypoxylon* has been included in the genus *Atkinsonella* Diehl since the name was proposed (Diehl 1950) but molecular phylogenies using sequence data from the nuclear rDNA–*rrnITS1* and *rrnITS2* (Schardl et al. 1991), the 28S r DNA region (Kuldau et al. 1997) and the rDNA–ITS1 (Reddy et al. 1998) all, albeit tentatively, placed the type species of *Atkinsonella* (*A. hypoxylon*) in a clade with species of *Balansia* Speg.

This fungus produces two anamorphs. One has been named *Ephelis borealis* and the other is referred to as *Acremonium*-like (Morgan-Jones and White 1992). Ellis and Everhart (1892, p. 91) were the first to connect the *Ephelis* anamorph with Peck's teleomorph name.

When Ellis and Everhart (1885) described *Ephelis borealis* only one collection ("John Macoun...On leaves of living grasses, Nova Scotia, June 1883") was cited. The June 1883 collection in DAOM is likely the portion that Macoun retained and it is designated isotype.

In the synonymy *Ephelis borealis*, the name of one of *B. hypoxylon*'s anamorphs, is included although no teleomorph has been found on the type specimen. Also the name *Ophiodothis hypoxylon* although attributed to Saccardo by Atkinson has not been found in any of Saccardo's writings and Diehl (1950) thought the combination was in a letter to Atkinson. The context in which Saccardo used *O. hypoxylon* is not known and the authorities are herein cited as Sacc. ex G. F. Atk.

The description herein presents the spore bearing states in the chronological order as they appear during the eastern Canadian growing season. Collections between June 21–29 had only the two conidial stages, collections dated July 7–11 had smooth, black stromata, i.e., the perithecia had not matured, and collections from August 15 into October had mature ascospores. The succession of spore producing states was observed *in vitro* on sclerotia when in July 1983 collection G 7386 was incubated in a moist chamber and sporulating conidial states and subsequently black stroma with perithecia developed. The role of ascospores and conidia in transmission is unknown (Clay and Jones 1984). But in the related *Epichloe typhina* (Pers.:Fr.) Tul. conidia are transferred between stromata by the parasitic fly *Phorbia phrenione*, whose larvae feed only on fungus tissues and an insect vector is possible in *B. hypoxylon* (Leuchtman and Clay 1989b).

The structure herein labelled sclerotium has been called a “pseudosclerotium” (Atkinson 1905) and a “hypothallus” (Diehl 1950). Atkinson and Diehl examined more collections than I and gave the sclerotial size-range as 4–15 x 1.5–2 mm. Atkinson’s diameter of “1–5 mm,” presumably is a typographical error for 1.5 mm.

Even though collections in 1981 and 1983 in southwestern Quebec and southeastern Ontario suggested that the fungus was not rare in Canada only 11 Canadian collections are known; nine have been studied by me. Diehl (1950) cited two Canadian specimens. The 1909 Dearness collection from Truro, Nova Scotia has been studied but I have not seen the other (“Quebec.-1938, I. H. Crowell”). The latter’s specimen label states *Atkinsonella hypoxylon*, [on] *Danthonia spicata*, Canada: Quebec: [Stanstead Co.:] N[orth] Hatley [south of Montreal], I. H. Crowell, 1938 July 16, det: W. W. Diehl (BPI 635513, Farr and Rossman 2011). Wehmeyer (1950), in a compilation of the fungi of the Canadian provinces of New Brunswick, Nova Scotia and Prince Edward Island, listed 3 collections. Those by Macoun and Hurst were studied but not the R. M. Lewis collection from Hillcrest [Orchards], Kings Co., Nova Scotia.

The host grass is unusual in that it produces wind-pollinated chasmogamous flowers and self-fertilized cleistogamous flowers. Infected plants are partially sterilized, i.e., they fail to produce chasmogamous flowers, and most seeds from cleistogamous flowers contain mycelium and give

rise to infected plants (Clay and Jones 1984). In addition, infected plants of *D. spicata* have higher survival and growth rates, greater interspecific competitive ability and greater resistance to insect herbivores than uninfected ones (Leuchtman and Clay 1989b). The fungus produces a variety of ergot alkaloids with physiological activity against animals (Leuchtman and Clay 1988).

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