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# Lectotypification and characterization of the natural phenotype of *Fusarium bactridioides*[1]

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Abstract — Specimens of *Fusarium bactridioides* deposited in the US National Fungus Collections and New York Botanical Garden were examined and a lectotype is selected based on naturally infected galls of *Cronartium conigenum* collected in Arizona. This species was previously only described from culture, and its natural phenotype is presented and illustrated here. Historical experiments involving the attempted use of *F. bactridioides* as a biocontrol of pine blister rusts in Oregon and New Hampshire are reviewed, but the unpublished records of the ultimate fate of these experiments could not be located.

Key words — *Hypocreales, Nectriaceae*, anamorph taxonomy, biological control

## Introduction

*Fusarium bactridioides* was the last species of the genus to be formally described in the august pages of the journal Science. The single page outlined the circumstances of the discovery of the fungus by the American botanist Arthur Hinckley and forest pathologist L.N. Goodding. The *Fusarium* "thoroughly parasitized" a cone of *Pinus leiophylla* var. *chihuahuana* (Chihuahua pine) attacked by the cone blister rust, *Cronartium conigenum* Hedgc. & N.R. Hunt. It was originally collected in the remote Chiricahua Mountains in Arizona (ca. 31°50′N 109°17′W), a so-called 'sky island' range of eroded volcanic rhyolite rock arising from the surrounding grassland desert.

[1] This article, the first of a projected series on the typification and nomenclature of species of *Fusarium* and allied genera, is dedicated to our friend and mentor Emory G. Simmons, whose erudite and articulate '*Alternaria:* Themes and Variations,' published in this journal, inspired this pale imitation.

Following the formal description is a remarkable story of what must be among the earliest deliberate releases of one fungus as a possible biological control of another. The *Cronartium* host is one of several rusts infecting *Pinus* species in the southwestern United States and adjacent Mexico, with uredinia and telia developing on various *Quercus* spp. (Cannon 2007). In July 1932, only three months after the discovery of the species, conidia of *F. bactridioides* were sprayed onto living *C. conigenum* cankers on *Pinus monticola* in Clackamas County Oregon. The following July, all the inoculated cankers were dead. Inoculations were then attempted on galls of *C. harknessii* E. Meinecke and *C. filamentosum* Hedgc. on *P. contorta* in two locations in Oregon, where sporulating *Fusarium* colonies were observed on the galls during a survey four months later. Field inoculations in four localities in Idaho were reported, but only sketchy details were provided of observations of the *Fusarium* sporulating on the cankers afterwards and there are no subsequent literature reports. Unfortunately, we were unable to locate any unpublished notes of L.N. Goodding that might give more information on the ultimate fate of these inoculations.

*Fusarium bactridioides* was classified in *Fusarium* sect. *Discolor* by Wollenweber (1934), Wollenweber & Reinking (1935), and Gerlach & Nirenberg (1982). It was considered a synonym of *F. sambucinum* Fuckel (Booth 1971, Nelson et al. 1983) and *F. bactridioides* vaguely fits the broad concept of *F. sambucinum* adopted by these authors. However, the macroconidia lack the asymmetrical, 'dolphin-nose' apical cell typical of the latter species. Of the species segregated from *F. sambucinum* by Nirenberg (1985), the macroconidia of *F. bactridioides* have some morphological similarity to those of the potato pathogen *F. venenatum* Nirenberg, but the latter species lacks microconidia. The phylogenetic and taxonomic studies of O'Donnell et al. (1998) and Nirenberg & O'Donnell (1998) accept *F. bactridioides* as a distinct species in the American clade of the *Fusarium fujikuroi* complex.

Wollenweber (1934) did not illustrate the species with the protologue, but later published a drawing as *Fusarium* autographice delineata no. 1153 (Wollenweber 1935), which was excerpted in his subsequent monographs. All published descriptions and illustrations of this species are probably based on one culture, exemplified by the modern species description and microscopic photographs of Gerlach & Nirenberg (1982: 267). This in vitro characterization is not repeated here, but the natural 'wild type' of this species on pine rust galls is illustrated and described. No holotype was designated in the protologue, and this shortcoming is remedied below by the selection of a lectotype.

**Typification**

The protologue of *F. bactridioides* explicitly mentions three specimens, the first probably the original collection from Arizona, and two from the inoculation


Figs. 1–2. *Fusarium bactridioides,* habit photographs. 1. Naturally infected *Cronartium conigenum* gall (BPI 451322). 2. Detail of sporodochia (lectotype, composite photograph assembled with CombineZ, Hadley 2006).

experiments in Oregon. There is no designation of a holotype. The description was based on a culture, and it is unlikely that Wollenweber ever saw any of the specimens that he listed.

Eight specimens of *F. bactridioides* are deposited in BPI and one in NY. Apart from one specimen collected and identified by J.R. Hansbrough in Waterville, New Hampshire (BPI US0451805) after the publication of the protologue, these collections are authentic and represent; a) material collected by A. Hinckley in the Chiricahua Mountains (NY 00936830 from 1932 on a host identified as *Cronartium quercuum* (Berk.) Miyabe; BPI US0451322 from 1933 on a host identified as *C. conigenum*); b) dried cultures from "cones collected by Arthur, Mar. 15, 1934" (BPI US0451803) and four specimens collected in 1933 from the inoculated locations in Oregon. It seems reasonable to designate the galls of *C. quercuum* collected by Hinckley in 1932 as the lectotype, despite the absence of the variety name for the host. The dates and locality match precisely and the identification of the host probably was changed between the times of specimen deposit and publication. This is presumably the specimen from which the cultures and subsequent specimens from inoculated localities were derived. The 1933 specimen in BPI appears also to be a natural infection from the type locality and although it predates publication, it is not mentioned in the protologue.

An argument could be made to designate the dried cultures in US0451803 as the lectotype, because they probably represent the strain(s), but not the actual transfers, that Wollenweber described. Unfortunately, the six dried PDA slants have no evident sporodochia or macroconidia, although there are abundant

microconidia in the cottony, white aerial mycelium. The cultures are heavily contaminated with coccoid bacteria. The date on the package containing the dried cultures is "Mar. 15, 1934", a scant three months before Wollenweber's description was published. The notes inside this packet suggest that the 1934 date is actually when the preserved cultures were dried. Only one strain of this fungus now exists, i.e. Wollenweber 4748 → CBS 177.35 (→ BBA 63602, CBS → NRRL 22201, NRRL → DAOM 225115, NRRL → CBS 100057). The data for CBS 100057 record *P. leiophylla* as the plant host and the location as Arizona, suggesting that this strain, which was regarded as an 'ex-type culture' by Gerlach & Nirenberg (1982) and Nirenberg & O'Donnell (1998), was derived from material from the Chiricahua Mountains. The protologue is ambiguous about how many strains were originally isolated and how many were sent to Wollenweber by his American colleagues. One of these strains was illustrated as *Fusarium* autographice delineata no. 1153, and we must assume that the culture now represented by CBS 100057 is the same strain. We consider this to be the ex-lectotype strain, and see no need to formalize this by epitypification.

For formal typification, we prefer to emphasize the concept of the species as a parasite of *Cronartium*, and designate the lectotype accordingly below.

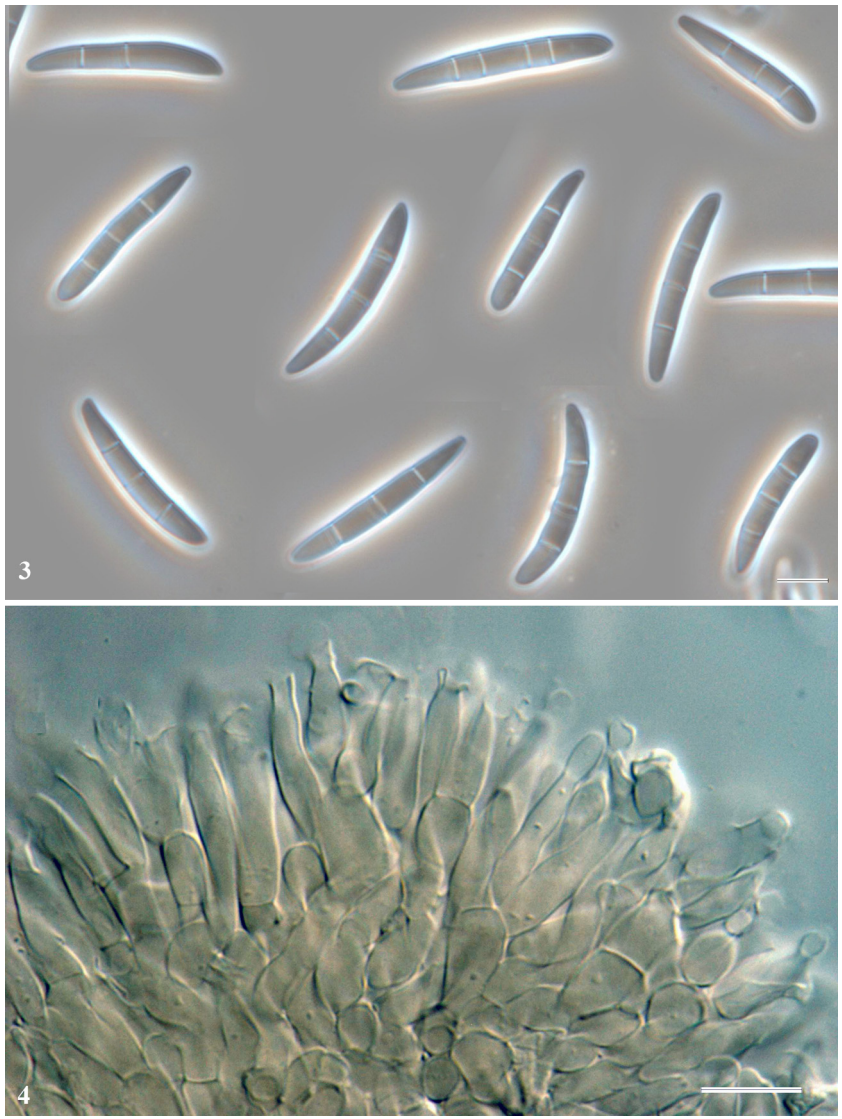
## Taxonomy

***Fusarium bactridioides*** Wollenw., Science 79: 572. 1934. Figs 1–5

MycoBank MB 258078

Type: On *Cronartium quercuum* on *Pinus chichuahuana* (sic), USA, Arizona, Chiricahua Mt., Cave Creek, III.1932, Arthur Hinckley (**lectotype designated here**, NY 00936830).

Sporodochia are erumpent from host tissue and visible as Orange-White (5A2, Kornerup & Wanscher 1978) to Light Orange (5A5) masses of conidial slime. On the naturally infected specimens, they are irregular in outline and form lesions up to 1 cm × 5 mm on the galls. On inoculated specimens, the lesions are scattered and smaller, about 200–1000 µm diam. The stroma is poorly developed, about 100 µm thick, and in optical section appears as a textura angularis of thin- to slightly thick-walled cells about 3.5–7 µm wide, but its hyphal character is evident with changes of focus. A hymenium-like layer of conidiophores and phialides arises from the stroma, with the conidiophores more or less biverticillate, but with phialides also often arising at the first level of branching. Metulae are cylindrical, doliiform to slightly clavate, 8.5–11.5 × 3.5–7 µm at the broadest part. Phialides are 9.5–15 × 3–5 µm at the broadest part, narrowly doliiform, sometimes with a central constriction, in terminal pairs or whorls of 3, or arising singly, in pairs or in whorls with metulae, with a flared collarette about 1–1.5 µm long and periclinal thickening sometimes visible; conidiogenous aperture about 2 µm wide.




Figs 3–4. Microphotographs of *Fusarium bactridioides*, BPI 451804. 3. Macroconidia. (composite image). 4. Hand section of the sporodochial stroma, showing conidiophores and phialides. Scale bars = 10 µm.

There is no obvious distinct difference between 'microconidial' and 'macroconidial' sporodochia and there is more or less a continuum between the two conidial types. Few macroconidia occur on the naturally infected

specimens, but they are more abundant on the lectotype and inoculated specimens. Macroconidia are 3–6 septate; 3-septate predominate and are 30.5–45.5 × 4.5–6 µm (mean ± SE = 38.3 ± 0.8 × 5.3 ± 0.1, n = 20), l/w = 5.5–9; 4-septate 27–36.5 × 5–6 µm (mean ± SE = 31.9 ± 0.8 × 5.4 ± 0.1, n = 10), l/w = 5–7; 5-septate 29–39.5 × 5.5–6.5 µm (mean ± SE = 34.6 ± 0.9 × 5.8 ± 0.1, n = 13), l/w = 5–7; 6-septate 35.5–41 × 5–6 µm (n = 3), l/w = 6–7. In side view, the ventral surface is more or less straight or gently curved, and the dorsal surface is moderately curved, with the walls more or less parallel in the central two cells of the conidia, with the widest point near the middle or above the middle. The apical cell is bluntly rounded, and roughly the same length as the penultimate cell. The basal cell is tapered more acutely than apical cell; the base is rounded, flat, or has a slight indentation on the dorsal side or central papilla, indicating a foot cell. In front view, the macroconidia appear somewhat clavate. 4–6 septate macroconidia tend to have unequal lengths of cells, with the additional septa dividing one but not all of the original four cells. Microconidia are abundant in the sporodochia, are 0–3 septate, and vary in shape and size from small, ellipsoidal, oblong-ellipsoidal or allantoid cells that are obviously microconidia to fusiform to clavate, septate spores that intergrade with macroconidia. Aseptate conidia 6–18 × 3.5–6.5 µm (mean ± SE = 11.8 ± 0.6 × 4.9 ± 0.1, n = 25), l/w = 1.5-4. 1-septate conidia 9.5–19.5 × 4–6.5 µm (mean ± SE = 14.9 ± 0.5 × 5.1 ± 0.1, n = 25), l/w = 2–4. 2-septate conidia are infrequent and 15–24 × 5–6.5 µm. 3-septate microconidia also occur and can be distinguished from macroconidia by their shorter length and their more clavate shape; they are 16–31 × 5–7 µm (mean ± SE = 24.0 ± 0.7 × 6.1 ± 0.1, n = 25), l/w = 3–5. In general, the bases of microconidia are conical, symmetrical or asymmetrical, with a flat secession scar or relatively conspicuous papilla; the apical cell or part of the conidium is rounded.

Additional specimens examined: **USA, Arizona,** Chiricahua Mt. (as Chricicalma), Cave Creek, on *Cronartium conigenum* on *Pinus leiophylla,* 25.VIII.1933, Arthur Hinckley (BPI US0451322); **Oregon**, Mt. Hood National Forest, On *Cronartium harknessii* on *Pinus contorta,* 8.VII.1933, L.N. Goodding (BPI US0451323); on *Cronartium ribicola,* 24.X.1933, L.N. Goodding (BPI US0451325); Eagle Creek, on *Cronartium ribicola* on *Pinus monticola,* 24.X.1933, L.N. Goodding (BPI US0451326); six dried slant cultures on PDA from "cones collected by Arthur" (BPI US0451803); Hood River Co., Eagle Creek, on *Cronartium ribicola* on *Pinus* sp., 24.X.1933, L.N. Goodding (BPI US0451804); **New Hampshire**, Waterville, on *Pinus strobus,* 1.IX.1935, J.R. Hansbrough (BPI US0451805).

## Discussion

The micromorphology of *F. bactridioides* reported here from natural material is comparable to that reported from cultures of this species by Wollenweber (1934) and Gerlach & Nirenberg (1982). The main distinction is that on the natural substrate the microconidia are produced from the

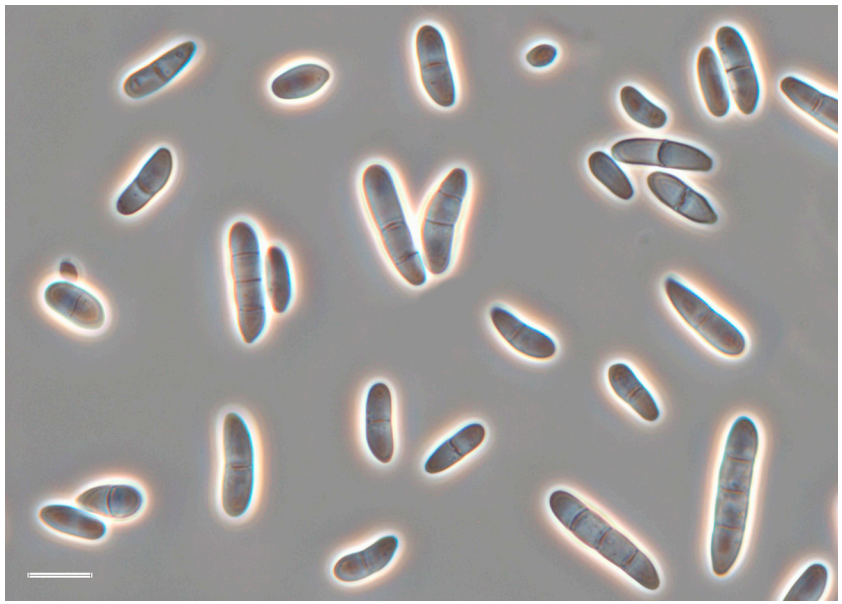


Fig. 5. Microphotograph of microconidia of *Fusarium bactridioides,* lectotype, NY 00936830 (composite image). Scale bar = 10 µm.

same sporodochia as the macroconidia, whereas in culture microconidia are produced independently. In nature, there is a continuum of shape and size between macro- and microconidia, but in culture the two kinds seem to be morphologically distinct. Gerlach & Nirenberg (1982) reported macroconidia with up to 11 septa in culture, with 3–5 septa being the typical condition. On the specimens, the macroconidia with three septa predominated, with a small number of 4–6 septate macroconidia seen on some specimens. Morphologically, *Fusarium bactridioides* has more robust macroconidia than is typical for the *F. fujikuroi* complex, and the 0–1 septate oval macroconidia are produced on the agar surface in vitro, rather than in the aerial mycelium as they are in related species. The mycoparasitic habit is also unusual in this group.

To our knowledge, *Fusarium bactridioides* has not been collected after 1935 and no natural infections have been reported outside of Arizona. The Hansbrough specimens (USO451805) collected in 1935 are the result of inoculation experiments in New Hampshire in 1934, about which apparently nothing was ever published. We were unsuccessful in locating the typewritten report by Hansbrough cited in a note included with the specimens. Whether *F. bactridioides* is indigenous and perhaps restricted to the remote Chiricahua Mountains, and whether the inoculations of blister rust cankers in Oregon,

Idaho and New Hampshire still persist, seem questions worthy of investigation. Such inoculation experiments would not easily be conducted today, with the need for risk assessments and legal permits from regulatory agencies. *Fusarium bactridioides* may provide a unique opportunity to search for the lingering fingerprints of innovative scientific activities of a more innocent time.

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