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Morphology of the rust fungus *Puccinia boroniae* revisited

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Abstract: *Puccinia boroniae* Henns. is a rust fungus endemic to Australia, infecting various *Boronia* spp. This study describes and illustrates, using light and scanning electron microscopy, the telial stage, teliospore germination and basidiospore production of specimens collected from commercial *Boronia* plantations in Western Australia. Unusual formation of a single basidiospore per germinating teliospore, and the pycnial stage, observed on *Boronia megastigma* leaves, are reported for the first time for *P. boroniae*.

Key words: basidiospores, *Boronia*, leptosporic, microcyclic, monokaryotic haustoria, pycnia, Rutaceae, teliospore germination, Uredinales

INTRODUCTION

Puccinia boroniae Henns. (Uredinales) is an endemic rust fungus of Australia, with its known host range including several species of *Boronia*, a native Australian wildflower (Sampson and Walker 1982, Cook and Dubae 1989, Shivas 1989). In southwestern Western Australia *Boronia* is cultivated commercially, with harvested stems primarily destined for export cutflower markets (Lidbetter and Plummer 2004). Several of the more commonly cultivated species and varieties, such as *Boronia heterophylla* and *B. megastigma*, are susceptible to *P. boroniae*. Infected plants exhibit telial pustules on stems and leaves, with defoliation of infected leaves eventually occurring. Although the disease does not directly result in plant death, the presence of rust in a plantation drastically reduces the number of harvestable stems, resulting in economic loss to the growers.

P. boroniae was described by several authors in the beginning of the 20th century (Hennings 1903, Sydow and Sydow 1904, McAlpine 1906). All descriptions are similar, recording only the telial stage (teliospores and mesospores, and the gross morphology of the telium) of the rust fungus from one specimen (on branches of *Boronia spinescens* Benth. from Western Australia, collected by L. Diels). The records also described *P. boroniae* as a Leptopuccinia, a microcyclic rust fungus in which mature teliospores germinate without a period of dormancy. However descriptions of the germination structures and basidiospores were not recorded. No modern descriptions of *P. boroniae* have been published subsequent to these, a likely reflection of the low economic importance of *Boronia* before its extensive commercial cultivation. The objectives of this study were to provide a current and more detailed morphological description of the various spore stages of *P. boroniae* observed in the field and to describe the mode of teliospore germination and basidiospore formation. Classification of *P. boroniae* as a microcyclic rust fungus was assessed through controlled inoculation trials.

MATERIALS AND METHODS

Fortnightly to monthly examination of *B. heterophylla*, *B. megastigma* and other susceptible species and varieties of *Boronia* grown on commercial plantations in the great southern region of Western Australia were made Feb 2003–Jul 2004. Fresh specimens were obtained for spore and sorus examinations, as well as teliospore germination. Herbarium specimens of *P. boroniae* collected from within the same region also were examined.

Sorus morphology.—Color designation of the various sorus stages was made from Kornerup and Wanscher (1967). Telia, pycnia and the intra/intercellular fungal structures were observed in cleared and stained, stained only and unstained hand sections of fresh leaf and stem material. Cleared sections were prepared using a modified version of the methods of Quilliam and Shattock (2003). Stained only and unstained sections were mounted directly in 0.05% lactoglycerol cotton blue and lactoglycerol, respectively. Sections were viewed under oil at 1000× magnification with an Olympus BH-2 microscope with bright field and differential interference contrast (DIC) and photographed with an Olympus DP10 digital camera. Images were edited for clarity where necessary with Adobe Photoshop® 7.0.

The surface morphology of the telia on leaves and stems was examined by scanning electron microscopy (SEM). Several telial samples were hand-sectioned through the middle of the telium before fixation. Specimens were fixed overnight at 4 C in 3% glutaraldehyde in 0.025M phosphate buffer (pH 7.0), washed several times in buffer, dehydrated in a graded series of ethanol, with a final wash of amyl acetate. Each specimen was critical point dried, adhered to an aluminium stub with carbon paste, sputter-coated with

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gold in a Balzers Union SCD 020 (Balzers Union Ltd, Liechtenstein) and examined under a Philips XL20 scanning electron microscope at 5–10 kV.

Spore morphology.—Teliospores and pycniospores were mounted in lactoglycerol, gently heated to expand collapsed spores and examined by bright field and DIC microscopy as described previously. Spore dimensions were determined with Olysia BioReport Imaging Software version 3.2 (Olympus, Australia) and digital images recorded with an Olympus BX51 microscope attached to a MicroPublisher 3.3 RTV photographic unit (Olympus, Australia).

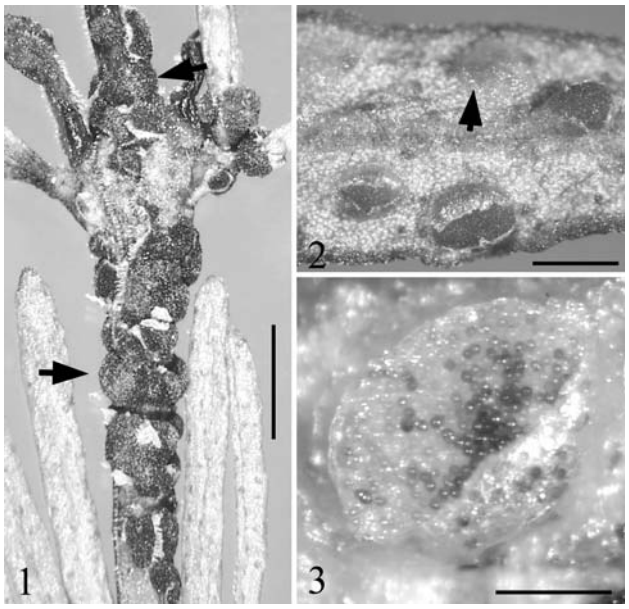
Teliospore germination.—Freshly collected leaves bearing telia were soaked 1–4 h in sterile distilled water at 15 C in the dark and blotted dry with sterile filter paper to remove excess water. Intact telia retained on the host leaves were incubated in sealed Petri dishes lined with moist filter paper and incubated at 15 and 20 C in the dark. Preliminary studies had shown that teliospore germination and basidiospore formation was optimal at 15–20 C in the dark (Driessen unpublished). Germination also was assessed on whole telia detached from the underling plant material under a dissecting microscope by placing detached telia upright onto 2% distilled water agar plates and incubating at 10, 15, 20 and 25 C in the dark . Telia were examined for the presence of germination structures and basidiospores at hourly intervals for 8 h at 400× with bright field and DIC microscopy. After 24 h germinating teliospores were gently teased from each sorus with a fine needle, mounted in lactoglycerol and examined as previously described.

Host inoculation.—Several branches from a heavily infected *B. heterophylla* plant were soaked 2 h in sterile distilled water at 20 C in the dark. The inoculum was blotted dry with sterile filter paper and placed among the top branches of mature healthy *B. heterophylla* plants (18–24 mo old) to allow for natural dispersal of the basidiospores produced from the intact telia (Morin et al 1993). Inoculated plants were gently misted with water, covered with thick opaque plastic bags to ensure high humidity was maintained and placed in a controlled growth cabinet maintained at 20 ± 1 C with a 12 h photoperiod. Plants were misted daily for 2 d after which the inoculum and plastic bags were removed, and inoculated plants then were checked daily for signs of infection. The removed inoculum was examined immediately under a dissecting microscope to ensure that teliospore germination and basidiospore formation had occurred.

TAXONOMY

Puccinia boroniae Henns. *Hedwigia* 42:73. 1903. FIGS. 1–16

Uredia and aecia not observed. *Telia* erumpent and pulvinate, amphigenous on leaves, stems, peduncles and sepals, reddish brown (9E7-8) to dark brown



FIGS. 1–3. Telia of *Puccinia boroniae*. 1. Confluent telia (arrow) along stem of *Boronia megastigma*. Bar = 5 mm. 2. Mature telia on adaxial surface of *Boronia heterophylla* leaf. Arrow indicates a telium not yet erupted through the leaf epidermis. Bar = 1 mm. 3. Semimature telium surrounded by ruptured epidermis of the adaxial surface of *B. megastigma* leaf with mature (pigmented) teliospores visible. Bar = 0.2 mm.

(9F6-8), mostly scattered or moderately concentric, individually up to 2.5 mm wide, often confluent on stems and peduncles, subepidermal, in the leaf arising between the mesophyll and epidermis, and in the stem between the cortex and epidermis from a hyaline, dense pseudoparenchymatous layer up to 20 μm thick, composed of globose to angular hyaline cells, 4–6 μm diam, giving rise to aseptate, hyaline, cuboidal to rectangular teliospore initials. *Teliospore initials* developing a single horizontal septum, forming the pedicel and primary teliospore, initially rectangular, unicellular and hyaline, broadening with maturity to become more ellipsoid, two-celled and pigmented, forming a compact, erumpent mass of teliospores. *Teliospores* predominantly 2-celled, with

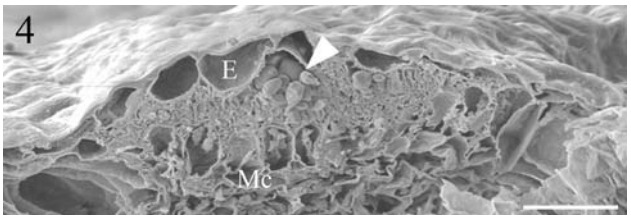
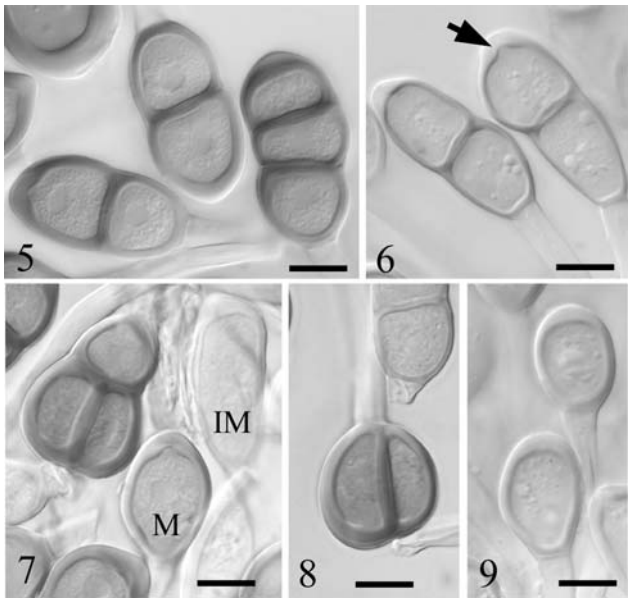
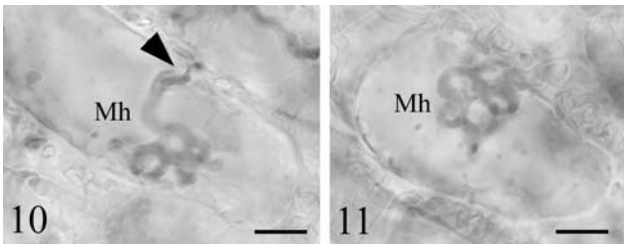


FIG. 4. SEM of cross section of a telium, showing mature teliospores just beneath the leaf epidermis (arrow). E = epidermal cell. Mc = mesophyll cell(s). Bar = 50 μm.



FIGS. 5–9. Teliospores and mesospores of *Puccinia boroniae*. 5 and 6. Two and three-celled teliospores of *P. boroniae* with apical germ pore (arrow) visible. 7 and 8. Variation in septum formation in teliospores. M = mesospore; IM = immature (primary) teliospore. 9. Mesospores. Bars = 10 μm.

a single, horizontal, occasionally oblique or vertical septum, rarely 3-celled or 4-celled, slightly constricted at septum, cinnamon to dark brown, smooth, pedicellate, broadly ellipsoidal, (22–)24–35(–37) × (13–)14–19(–20) μm (average 28.8 ± 2.8 × 16.6 ± 1.4 μm; n = 235), a single germ pore observed in each cell, apical in upper cell and septal in lower cell, germinating without dormancy. *Mesospores* ellipsoid to obovoid with single apical germ pore, coloration similar to teliospores, 18–29(–32) × 13–18(–20) μm (average 23.5 ± 3.1 × 15.7 ± 1.9 μm; n = 99). *Pedicel* persistent, hyaline to pale yellow, up to 130 μm long, attached at bottom of basal cell of mesospores and teliospores, occasionally obliquely or laterally inserted. *Haustoria* coiled and branched, with a well defined neckband at the entry point into host mesophyll cells. *Basidiospores* formed on single sterigma at subterminal end of the metabasidium, ovate to elliptical with a prominent apiculus, 13.5–18.6 × 8.8–11.7 μm (average 16.4 ± 1.0 × 10.5 ± 0.6 μm; n = 80). *Pycnia* amphigenous, arranged in small clusters, individually up to 350 μm wide, yellowish-orange (4A7-8) to orange (6A/B8), subepidermal, arising from an extensive network of intercellular hyphae within host tissue, aggregating beneath stomata to form pycnial primordials, mature pycnium ampulliform with numerous straight, unbranched, spine-like periphyses present above the hymenium and just below the ostiole, hymenium



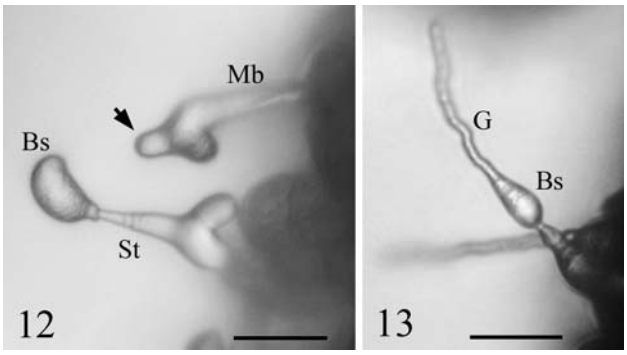
FIGS. 10, 11. M-haustoria of *Puccinia boroniae* in mesophyll cells beneath a telium. Note constricted neckband region at point of entry into cell (arrow). Bar = 10 μm.

consisting of a layer of pseudoparenchymatous cells giving rise to long, slender pycniosporophores up to 30 μm long. *Pycniospores* subpyriform to ellipsoidal, hyaline, smooth, 2.8–5.0 × 1.6–2.6 μm (average 3.8 ± 0.5 × 2.0 ± 0.2 μm; n = 80), released in a honey-like fluid through the ostiole of the pycnium.

Specimens examined. AUSTRALIA. WESTERN AUSTRALIA: Albany, commercial nursery, (35°01′S, 117°50′E), *Boronia heterophylla*, Jul 2004, *S.A. Driessen* (WAC 12425); Mount Barker, private flower plantation, (34°34′S, 117°46′E), *Boronia megastigma*, May 2000, *S.A. Driessen* (WAC 12424); same local, *Boronia megastigma*, Feb 2004, *S.A. Driessen* (WAC 12426); Redmond, private flower plantation, (34°54′S, 117°33′E), *Boronia clavata*, Jun 2000, *S.A. Driessen* (WAC 12427); same local, *Boronia heterophylla*, Jun 2000, *S.A. Driessen* (WAC 12428).

Commentary. Examination of the specimens in this study showed that telia predominantly formed on leaves in a scattered arrangement. Although telia also were observed on stems in a number of specimens in this study, we observed that extensive confluent formation of telia on the stems, as described by Hennings (1903), Sydow (1904) and McAlpine (1906), was restricted to specimens from *B. megastigma* (WAC 12426). Telium structure of *P. boroniae* was typical of many *Puccinia* spp., being subepidermal, erumpent, with a well developed basal layer of sporogenous cells from which teliospores were born singly on pedicles (Cummins and Hiratsuka 1983, Mendgen 1984). Morphology of the teliospores agreed with previous descriptions, although a greater range of mesospore dimensions were recorded in this study in comparison to those reported by McAlpine (1906) (ca. 30–34 μm × 17–18 μm).

The confinement of inter- and intracellular hyphae to the host mesophyll layers indicated a nonsystemic mode of infection. The observation of M-haustoria, the formation of which are associated with infections by basidiospores in rust species (Littlefield 1981, Quilliam and Shattock 2003), suggested a microcyclic lifecycle, in which telia develop from successful basidiospore infection (with or without pycnia pro-

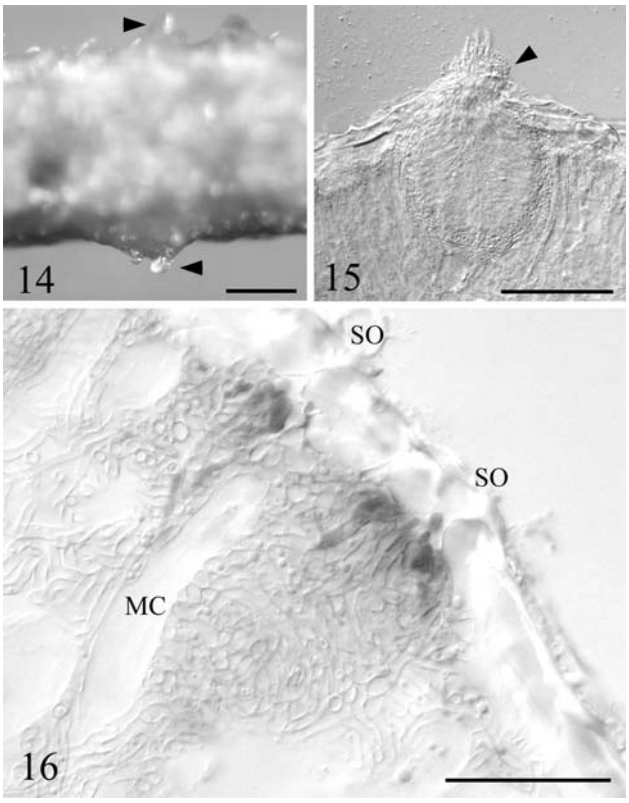


FIGS. 12, 13. Teliospore germination structures and basidiospores of *Puccinia boroniae*. Bs = basidiospore; G = germ tube; Mb = metabasidium; St = sterigma. Bar = 20 μm.

duction) without the intermediates of aeciospores or urediospores, which have never been observed on infected hosts in the field.

Teliospores of *P. boroniae* germinated immediately without a period of dormancy, confirming the description provided by earlier authors (Hennings 1903, Sydow and Sydow 1904, McAlpine 1906). Consistent and reproducible formation of a single basidiospore from each metabasidium was observed under all experimental conditions, indicating this to be the normal behavior of the rust pathogen. A whip-like germ tube occasionally formed on the sterigma instead of a basidiospore. This abnormal germination structure was associated with germinating teliospores that remained submerged in excessive water, an occurrence reported by other authors (Gardner 1996, Ono 2002a). Several authors (Peterson 1974, Hiratsuka and Sato 1982, Ono 2002b) have reviewed the diversity of basidial development of rust fungi, and though the formation of a single basidiospore is unusual, it previously has been reported for *Puccinia rutainsularа* (Gardner 1994) and *Uromyces alyxiae* (Gardner 1987).

The pycnial stage of *P. boroniae* was detected on a single host plant species (*B. megastigma*) at a single commercial plantation in Mount Barker, Western Australia. The structure of the pycnium conformed to type 4 as described by Hiratsuka and Cummins (1963), typical of *Puccinia* spp. and characterized by determinate growth, subepidermal and strongly convex hymenia, with well developed bounding structures (periphyses). Accompanying the periphyses with acute apical tips were hyphae that were blunt at the distal end, presumed to be flexuous hyphae. However these were morphologically difficult to distinguish with the techniques employed. Unlike other *Puccinia* spp., such as *P. recondita* (Gold et al 1979), ostiole formation was seen to occur by the extension of



FIGS. 14–16. Pycnial stage of *Puccinia boroniae* on *Boronia megastigma*. 14. Honey-like fluid being released from pycnia (arrow). Bar = 1 mm. 15. Type 4 pycnial structure of *P. boroniae* with released pycniospores present near ostiole (arrow). Bar = 100 μm. 16. Subepidermal pycnial primordia showing focused growth towards stomatal opening. SO = stomatal opening; MC = mesophyll cell. Bar = 40 μm.

periphyses through stomatal openings as reported for the rust fungus *Melampsora lini* (Gold and Littlefield 1979), rather than rupturing through the leaf epidermis. Because the material examined was naturally infected rather than inoculated with a single basidiospore, it was impossible to determine whether the cluster of multiple pycnia observed on each leaf surface developed from a single basidiospore or from multiple spores. Although pycnia were present on leaves of many *B. megastigma* plants within this plantation, many infected plant parts also exhibited only telia at varying stages of maturity. Furthermore no other specimens of *P. boroniae* collected from different locations exhibited the pycnial stage despite extensive sampling. This would suggest that the pycnial stage may not be a prerequisite for the development of the telial stage and, although still present, may be nonfunctional in the rust lifecycle.

Inoculation of *B. heterophylla* with basidiospores of *P. boroniae* resulted in development of telia on the leaves within 21 d, confirming the lifecycle as micro-

cyclic as suggested by Hennings (1903) and McAlpine (1906). Small yellow discolorations initially were detected on the leaf surface 15–17 d after inoculation, developing into mature telia that ruptured through the leaf epidermis within 3–5 d. Once teliospores had ruptured the leaf surface, they were mature enough to germinate and produce basidiospores, assessed by incubating leaves bearing telia in a moist chamber after 1 h exposure to water. Basidiospore formation was observed within 3–6 h. No pycnia were observed on the inoculated *B. heterophylla* plants. However the specimen exhibiting pycnia in the field was not employed in the trial due to decreased viability of the teliospores, and whether the pycnial stage is functional in the lifecycle of *P. boroniae* remains inconclusive.

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