205

208

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A taxonomic reassessment of *Phyllachora proteae*, a leaf pathogen of Proteaceae

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Abstract: *Phyllachora proteae* is a well known leaf pathogen of *Protea* spp. In the present study this fungus was recollected from several genera and species of Proteaceae in the Western Cape province of South Africa, and its taxonomy was reassessed. Single ascospore cultures produced a *Fusicoccum* anamorph in culture, described here as *F. proteae*. A microconidial synanamorph with narrowly ellipsoidal, brown, thick-walled conidia was commonly associated with *F. proteae* in culture. Based on its bitunicate asci, as well as pseudothecial and ascospore morphology, a new combination for *P. proteae* is proposed in *Botryosphaeria*, as *B. proteae*.

Key Words: *Botryosphaeria, Fusicoccum, Protea,* systematics

INTRODUCTION

The Proteaceae, one of the oldest plant families, is estimated to be more than 140 Myr old. The family comprises at least 1400 species, of which 330 occur in the South African Fynbos biome, and is among the most predominant groups of flowering plants in the southern hemisphere (Rebelo 1995). The unique beauty and hardiness of *Protea* flowers make them highly desirable to local and international cut-flower markets. In 1996, 4.8 million kg of fresh proteas were produced in South Africa, of which 3.3 million kg were exported, earning an estimated R64.5 million (Wessels et al 1997). However, strict phytosanitary regulations of importing countries frequently prevent blemished blooms from reaching potential export markets. Additionally, the marketing of low quality flowers results in consumer dissatisfaction, a loss in credibility of South African products and ultimately the forfeiting of markets to other exporting countries (Wessels et al 1997).

Lesions induced by plant pathogenic organisms are a major cause of foliage and bloom spoilage. A large number of fungal pathogens is known to occur on Proteaceae in South Africa (Knox-Davies et al 1987). The taxonomy of some of these has, however, changed considerably since they were first reported. The correct identification of pathogenic fungi is necessary to ensure appropriate quarantine decisions and suitable control strategies. *Phyllachora proteae* Wakef., commonly associated with leaf spots and stem cankers of *Protea* L., and *Leucospermum* R. Br. species, is an example of a pathogen that requires taxonomic reassessment. This fungus was described by Wakefield (1922) as having unilocular ascomata that develop under a very small epidermal clypeus, cylindrical asci, pseudoparaphyses and hyaline, aseptate, ellipsoidal ascospores, 19–22 × 8–9 μm. In a reexamination of the type material, Doidge (1942) found the ascomatal wall to be continuous with, and similar in structure to the clypeus. She noted, however, that the ascomatal stromata differed from those of other South African *Phyllachora* spp. In his study of leaf pathogens of *Protea, Leucadendron* and *Leucospermum* spp., Van Wyk (1973) commented that the ascocarps of *P. proteae* appeared to be unilocular with pseudoparaphyses, and that the fungus should probably be transferred to *Guignardia* Viala & Ravaz or *Botryosphaeria* Ces. & De Not. The aims of this study were therefore to recollect *P. proteae,* study the type specimen, identify the anamorph, and to record new hosts and collection sites.

MATERIALS AND METHODS

Collection and isolation.—Several farms reporting proteas with severe leaf spots and stem cankers were visited. Affected plants were identified, symptoms recorded and diseased leaves and branches cut from bushes and brought back to the laboratory for study. Leaf and stem samples were incubated in Petri dishes containing moist filter paper. Single ascospore cultures were obtained from pseudothecia by squashing the contents in a drop of sterile water and spreading this onto the agar surface of dishes containing potato dextrose agar (PDA, Biolab). Alternatively, pseudoth-

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ecia were soaked in water for 2 h, attached to lids of Petri dishes, and ascospores ejected onto the agar surface of PDA plates. Single germinating ascospores were transferred to fresh PDA plates, and incubated at room temperature in the dark for 5 d. Subcultures were made from five single ascospore or conidial colonies per diseased plant.

Morphological characterization and culture.—To induce sporulation, two different techniques were used. In the first, cultures were transferred to divided plates containing carnation leaf agar (Fisher et al 1982) in one half of the dish and PDA in the other. In the second technique, isolates were grown on a sterilized piece of *Leucospermum* stem in full strength V8 broth (Englander and Turbitt 1979), and placed on tap water agar (Biolab). All plates were incubated in the laboratory at room temperature (20-25 C) under cool white and near-ultraviolet light with a 12 h photoperiod. Cultures were stored on PDA slants, with or without mineral oil, at room temperature. All fungal material was mounted in lactophenol, and at least 30 structures were measured. The range of dimensions is given with the extremes in parentheses. Reference specimens have been deposited at the National Collection of Fungi in Pretoria (PREM), and cultures are maintained in the culture collection of the Department of Plant Pathology at the University of Stellenbosch (STE-U).

Ten isolates derived from different hosts and localities were selected for cultural growth studies on PDA. Mycelial discs 5 mm diam were cut from the periphery of actively growing cultures and placed at the center of PDA plates, with three plates per isolate at each temperature (5–40 C at 5 C intervals). Linear growth and colony color (Rayner 1970) were determined after 7 d. Two perpendicular readings were taken for each colony, using a digital caliper. The mean growth rates for three replicates of ten isolates were plotted for each temperature tested.

TAXONOMY

In a reexamination of the type specimen of *Phyllachora proteae* (PREM 32915), it was found that this taxon had bitunicate asci that were borne in thick-walled, brown pseudothecia. Contrary to the protologue for the species, no clypeus was observed. These observations suggest that this species would be better accommodated in *Botryosphaeria* than *Phyllachora*, and a new combination is therefore proposed. Cultures derived from single ascospores of *B. proteae* produced a *Fusicoccum* Corda anamorph with a microconidial state when cultured on PDA. As no anamorph has thus far been reported for *B. proteae*, the *Fusicoccum* state is described as new.

Botryosphaeria proteae (Wakefield) Denman et Crous, comb. nov. FIGS. 2–13

≡ *Phyllachora proteae* Wakefield, Kew Bull. 1922: 164. 1922.

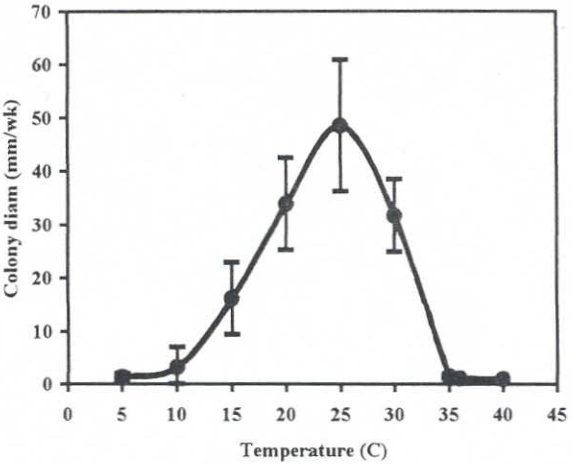

FIG. 1. Growth rate of *Botryosphaeria proteae* isolates on PDA after one wk at different temperatures. Each data point is the mean of three replicates of ten isolates at each temperature.

Anamorph. **Fusicoccum proteae** Denman et Crous, sp. nov. FIGS. 7, 8, 11

Conidiomata pycnidialia, eustromatica ad 450 μm diam, atrobrunnea, uni- ad multilocularia. Conidiophorae hyalinae, laeves, ramosae subcylindricae, 1–3-septatae, 20–40 × 3–4.5 μm, paraphysibus hyalinis, septatis inmixtae. Conidiogenae cellulae holoblasticae, hyalinae, laeves, cylindricae, enteroblastice et percurrenter proliferantes vel phialidibus typicus periclinaliter spissescentibus. Conidia hyalina, parietibus tenuibus, aseptata, laevia, clavata ad fusiformia, apice subobtuso, base truncata, (20–)22–25(–30) × (4.5–)5–6 μm.

Mycelium immersed, consisting of branched, septate, smooth, medium brown hyphae, 2.5–5 μm diam. Mycelial growth rates on PDA were maximal at 25 C, and growth virtually ceased at temperatures below 10 C and above 35 C (FIG. 1). Pseudothecia epiphyllous, separate, unilocular, initially solitary and discrete, becoming aggregated, immersed, substomatal, with a central, flattened ostiole, obovoid, slightly depressed, 200–300 μm wide, 200–240 μm high; wall consisting of 8–11 layers of brown pseudoparenchymatic *textura angularis,* up to 65 μm thick in upper, widest part (FIGS. 2, 9). Asci fissitunicate, clavate to cylindrical, stipitate, bitunicate, 90–150 × 12–15 μm; nasse apicale visible as a notch-like indentation at the apex (FIGS. 5, 10). Ascospores uni- to biseriate, hyaline, guttulate, smooth, ellipsoidal, clavate to fusiform, frequently widest in the upper third of the ascospore, tapering to obtuse ends, (15–)17–20(–21) × (5–)6–8(–9) μm (FIGS. 4, 10). Pseudoparaphyses hyaline, septate, branched, frequently attached to the top and base of the pseudothecial cavity, 2–3.5 μm diam (FIGS. 3, 10). Conidiomata pycnidial, eustromatic, to 450 μm diam, immersed, subepidermal, separate, dark brown, uni-