Pseudocercospora macadamiae sp. nov., the cause of husk spot of macadamia

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Abstract. Pseudocercospora macadamiae sp. nov. is described from diseased husks of plantation-grown *Macadamia integrifolia* in Queensland and northern New South Wales, Australia.

Introduction

During a continuing taxonomic study by the first author of cercosporoid pathogens of Australian native plants, symptoms have usually been observed on vegetative parts (leaves, phyllodes or occasionally petioles) of affected plants from a wide range of genera. A striking exception to this generalisation is the damaging and economically important husk-spotting disease of *Macadamia integrifolia*, its cultivars and its hybrids with *M. tetraphylla* (Proteaceae). In nature, the disease is restricted to the husk (pericarp) of the fruit. Foliar, petiole or twig symptoms have not been observed under Australian conditions despite epidemics developing annually in mature orchards. Husk spot has been reported only from Australia, more specifically from Queensland and New South Wales.

In 1951, B.L. Oxenham deposited the first known Australian herbarium specimen of husk spot (as *Cercospora* sp.), from Nambour, Qld (BRIP 4604). J.H. Simmonds collected the second specimen, also identified as *Cercospora* sp., in 1971 from Maleny, Qld (BRIP 6137). With the exception of one specimen collected by R.D Fitzell at Whian Whian, NSW, in 1992 (DAR 68433), all specimens of macadamia husk spot deposited in eastern Australian plant pathology herbaria between 1981 and 2001 were provided by the second author who studied the disease during those years and who identified the causal pathogen as a species of *Cercoseptoria*, a genus into which similar cercosporoid taxa had been placed by Deighton (1976). Following Deighton's (1987) recombination of a number of species of *Cercoseptoria*, including the type species (*C. chamaescyses* (Stev. & Dalbey) Petrak), into *Pseudocercospora*, the

macadamia fungus is best treated as a *Pseudocercospora* and is described in this paper as a new species.

Macadamia is the only plant indigenous to Australia that has been developed into an edible food crop industry. The current value of the macadamia nut crop is A\$100m at the farm gate, the nuts being exported to the USA, Europe and parts of Asia, including Japan.

Macadamia nuts are enclosed in a leathery (fleshy) green fibrous husk which is commonly 3 mm thick and which, when stripped from the fruit ('de-husking'), exposes a conical to spherical or slightly flattened hard brown shell (testa) 17–35 mm in diameter. At opposite poles of the nut the smooth to slightly rough shell surface bears a broad hilum scar and a white circular to elliptical micropyle pore that is usually 1 mm wide but can be up to 3 mm wide. Enclosed is the creamy white kernel consisting of an embryo and the two edible cotyledons.

Depending on cultivar and region, the macadamia crop is usually mechanically harvested on two or more occasions throughout the progressive nut-fall period (February to October), as hand picking is too expensive and impractical on a large scale. Most growers harvest only from the ground, the harvesting machine at the same time de-husking those fruits which are freshly fallen and from which the husk has not already dehisced. Cultivars tolerant of the disease have been identified as those that sustain only cosmetic damage to the husk and which hold their crop until full maturity (which may be as late as September), whereas intolerant cultivars drop their infected crop prematurely or early. In the latter case, commercial crop yield is reduced by up to 40% because of the need to discard the first pre-harvest cleanup of

immature diseased nuts. In fact, healthy and diseased nuts of some cultivars fall simultaneously and continuously for 6–8 months of the year, and consequently are harvested as mixed populations of healthy and diseased nuts which cannot be distinguished after they are dehusked. Quality parameters, including nut roasting and shelf life, kernel discoloration and pinching, are adversely affected by the disease-induced drop of immature nuts being admixed with subsequent harvests. Higher sugar levels are present in immature kernels because the sugars have not been fully converted into oils.

In contrast to unselected trees in their natural habitat, which may flower throughout the year, commercial cultivars of *M. integrifolia* (and its hybrids with *M. tetraphylla*) flower mainly in September and October. Disease expression is first seen in December and January, and marked disease symptoms are evident on 4- to 5-month-old nuts. Field inoculation experiments have shown the optimum nut size for infection to be 12–14 mm in diameter. Early symptoms of the disease are pale chlorotic spots with diffuse margins, 2–10 mm in diameter, on the developing husks. As the disease progresses, the lesions may coalesce into irregular blotches, or become circular, zonate 'cat's eyes' which are orange, tan or brown and which develop necrotic centres. The developed lesions are often covered with a thin to felty, pale-brown mass of external hyphae. No fully resistant cultivars of *M. integrifolia* have been found, but breeding for tolerance, combined with cultural and fungicidal control measures, is proving effective in limiting the impact of the disease.

Taxonomy

Pseudocercospora macadamiae Beilharz, Mayers & Pascoe, **sp.nov.** (Fig. 1)

Etym: from *Macadamia*, the host genus.

Mycelium immersum intercellulare, pallide brunneum, ex hyphis septatis ramosis anastomosantibus 1.75–4 μm latis compositum. *Mycelium superficiale* ex stromata substomatis vel stromata erumpentia ad basim formatum, ex hyphis pallide olivaceo-brunneis laevibus ramosis septatis anastomosantibus 1.5–3.5 μm latis compositum; *conidiophora* terminalia vel lateralia ex mycelio vegetativo formata, 1–3 septata, 20–59 μm longa × c. 3 μm lata. *Stromata* subepidermalia vel substomatalia, pallide- vel medio-brunnea, compacta, ex textura angularis composita; *stromata subepidermalia* erumpentia, c. 34–90 μm diam. × 20–64 μm profunda; *stromata substomatalia* non alta, cum massa parva cellularum tumidarum supra stoma demum evoluta. *Conidiophora* pallide olivaceo-brunnea cylindracea curvata vel sinuata saepe 1–3 ramosa, laevia vel verruculosa, anastomosantia, usque 10-septata, 20–95 μm longa × (2–)2.5–3.5 μm lata. *Loci conidiogeni* 1–3, non-incrassati, non-fuscati, plerumque 2.5 μm diam. *Conidia* solitaria, pallide olivaceo-brunnea filiformia curvata sinuata vel recta, apicem obtusum versus leniter deminuta, basim versus

interdum leniter diminuta, (0–)5–9 septata, (17–)45–69 μm longa × 2–2.5 μm lata. *Hila* leniter convexa, non-incrassata, non-fuscata.

Lesions on the husk epicarp (outer surface) are circular or irregular and initially chlorotic, later necrotic in the centre with an orange, tan or brown margin. The central necrotic zone shows pale-brown, felty fungal colonies which sporulate profusely. The fungus extends through the mesocarp, which becomes tan or light-brown and fibrous due to the development of sclerified tissue, and into the endocarp. The inner surface of the husk can appear water-soaked and later pale-brown, in correspondence with the external lesions. On mature husks of most cultivars, the endocarp is brown to dark brown, largely obscuring the lesion. *Immersed mycelium* intercellular, light brown, septate, branching, anastomosing, 1.75–4 μm wide, the broader hyphae often being constricted at the septa. *Superficial mycelium* profuse on some lesions, emerging mainly from the stomata but also developing from the bases of erumpent stromata, pale olivaceous brown, smooth, branching, septate, anastomosing, 1.5–3.5 μm wide, bearing lateral and terminal conidiophores or conidiogenous cells which are delimited by a basal septum, have 0–2 additional septa, and are 20–59 μm long × c. 3 μm wide. *Stromata* subepidermal or substomatal, light to medium brown, irregular, compact, composed of *textura angularis*; *subepidermal stromata* become protuberant following rupture of the epidermis and cuticle, 34–90 μm wide × 20–64 μm deep; *substomatal stromata* shallow, giving rise to a few hyphae which pass through the stoma and proliferate to form a small external knot of swollen cells on which numerous caespitose conidiophores are borne. *Conidiophores* light olivaceous brown, cylindrical, curved or sinuate, often 1–3 branched or bifurcating, smooth or verruculose, having a slight swelling below some septa, anastomosing, up to at least 10-septate, 20–95 μm long × (2–)2.5–3.5 μm wide. *Conidiogenous loci* not darkened, unthickened, non-refractive, left on sloping shoulders following 1–3 sympodial proliferations of the conidiogenous cell, mostly 2.5 μm diam. *Conidia* formed singly, pale olivaceous brown, filiform, curved, sinuate or straight, usually tapering slightly to an obtuse apex and narrowing slightly if at all towards a flared, slightly convex base, (0–)5–9 septate, (17–)45–69 μm long × 2–2.5 μm wide. Hila not darkened, unthickened, non-refractive, 2–2.5 μm diam. Spermogonia forming within conidiomatal stromata or *de novo*, uniloculate and lenticular or 2–3 loculate, with pale olivaceous walls 3–5 cells thick and opening by a split; some lesions in VPRI 22745 showed a broad band of spermogonia, some of which were oozing yellowish-green cirrhi, surrounding a central field of conidiomata. Spermatiophores lining the spermogonial cavities are pale, subulate, straight to curved, 0–3 septate, often with 1–2 branches inserted below septa, mostly 25–40 μm long × 1.5–2 μm wide.
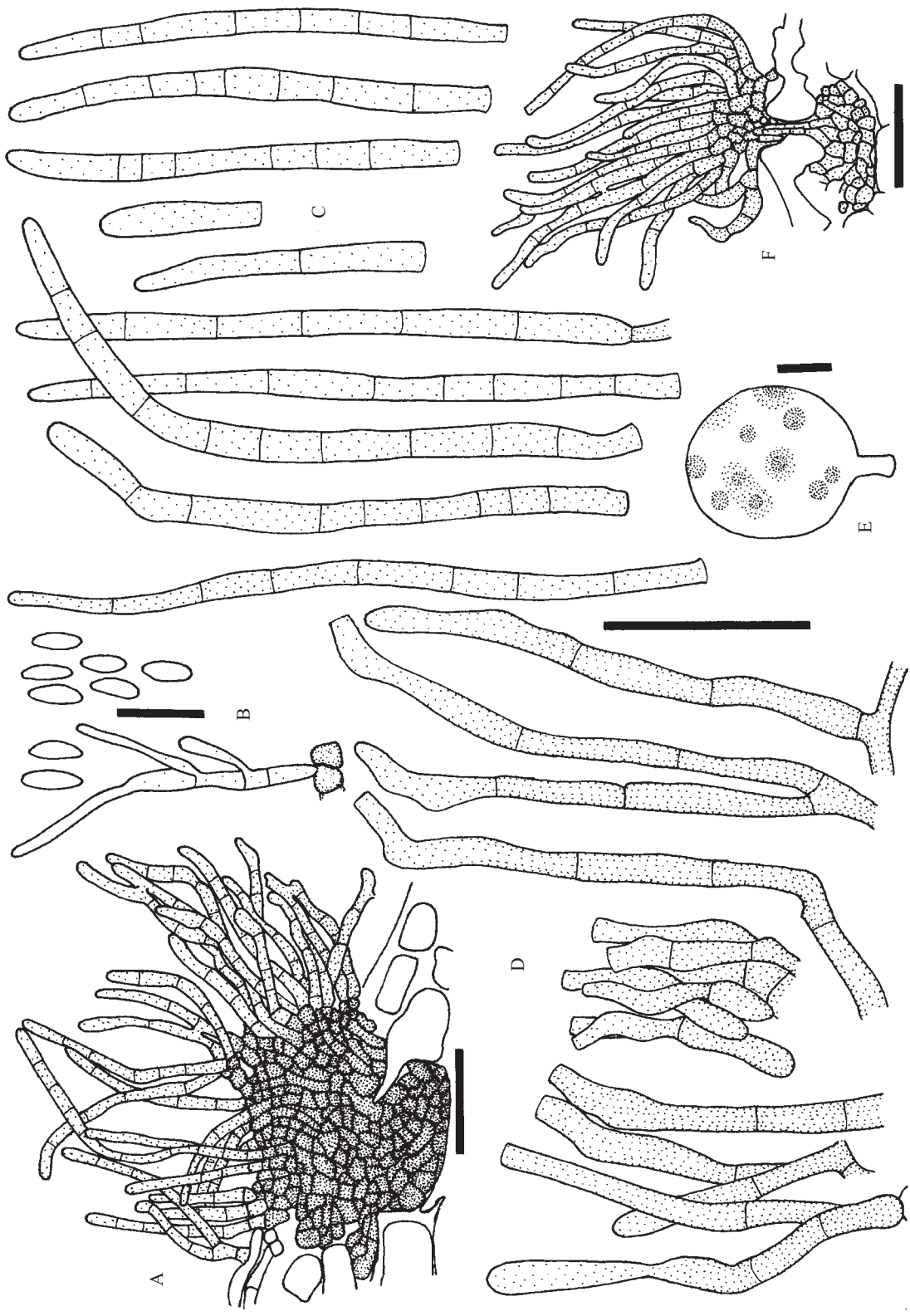
Fig. 1. *Pseudocercospora macadamiae,* VPRI 21900. (A) Erumpent conidioma, (B) branched spermatiophore and spermatia, (C) conidia, (D) caespitose conidiophores and one external conidiophore, (E) nut of *Macadamia integrifolia,* showing symptoms, (F) stomatal conidioma. Bars: 20 μm (A, C, D, F); 10 μm (B); 1 cm (E).

Spermatia hyaline, fusiform, with a minute flattened basal scar, 6–7 μm long × 2–2.5 μm wide.

Holotype: Australia, Qld, on living husks of *Macadamia integrifolia* Maiden & E.Betche cv. H2 (Proteaceae), Sahara Farms, Sahara Rd, Glasshouse Mountains, 5 Feb. 1999, *P. Mayers*, VPRI 21900.

Additional specimens examined: Qld: on *M. integrifolia* (as *M. ternifolia* F. Muell.), Nambour, *B.L. Oxenham*, 6 Apr 1951, BRIP 4604; on *M. integrifolia*, *ibid*, Maleny, *J.H. Simmonds*, 6 May 1971, BRIP 6137; on *M. integrifolia*. cv. Hinde, *ibid*, 17 Feb 1981, *P.E. Mayers*, BRIP 13798; on *M. integrifolia* cv. Own Choice, Didillibah, 20 Aug 1985, *P.E. Mayers,* BRIP 14869; on *M.* sp., *ibid*, 12 Aug 1985, *P.E. Mayers*, BRIP 15051; on *M.* sp., Maleny, 1985, *P.E. Mayers*, BRIP 15059; on *M. integrifolia* cv. 508, Peachester, 10 May 1991, *P.E. Mayers*, BRIP 18131; on *M. integrifolia*, Maleny, 19 Aug. 1991, *P.E. Mayers*, BRIP 19467. N.S.W.: on *M. integrifolia* cv. Hinde (H2), Clunes, 16 Mar 2001, *P.E. Mayers*, VPRI 22745; on *M. integrifolia* cv. Own Venture, *ibid*, 16 Mar 2001, *P.E. Mayers* VPRI 22746; on *M. integrifolia* cv. HAES 344, *ibid*, *P.E. Mayers*, VPRI 22747; on *M. integrifolia* cv. NG 18, *ibid*, *P.E. Mayers*, VPRI 22748; on *M. integrifolia* cv HAES 781, *ibid*, *P.E. Mayers*, VPRI 22749.

P. macadamiae is one of a number of *Pseudocercospora* species which fit the description of the former genus *Cercoseptoria* Deighton (Deighton 1976) which Deighton himself subsumed into *Pseudocercospora* in 1987. *Cercoseptoria* was typified in part by its pale-brown, narrow, acicular, multiseptate conidia which are not obclavate, but rather taper gradually from close to the base towards the apex. Whereas *Cercoseptoria* was specifically described as sporulating from substomatal stromata, the macadamia fungus sporulates also from large sub-epidermal stromata and from external hyphae.

These variations in mode of egress and sporulation are not considered taxonomically significant in the cercosporoid fungi, and the relative presence or absence of stromata is now regarded as being not a useful distinguishing characteristic at the generic level in *Mycosphaerella* anamorphs (Crous *et al.* 2001). Stomata are relatively sparse on the epidermis of the macadamia husk, and sporulation from substomatal stromata probably contributes much less to propagule dissemination than does the sporulation from the larger and more numerous erumpent stromata. Vertical sections through several erumpent stromata have shown conidiophores emerging through a rupture in the epidermis very close to a stoma,

even though stomatal egress would appear to be a more energy-efficient mode of egress. Beilharz (1994) has observed this behaviour in several other species of *Pseudocercospora* including *P. correae* B. Sutton & Pascoe (in Sutton *et al.* 1987) and a photograph published by Evans (1984, Fig. 113) shows a similar occurrence in *Pseudocercospora pinidensiflorae* (Hori & Nambu) Deighton on pine needles.

Two other species of *Pseudocercospora* are known on hosts in the Proteaceae. *P. protearum* (Cooke) U. Braun & Crous (in Braun and Hill 2002) and *P. stromatosa* J.E. Taylor & Crous (Taylor and Crous 2000) both differ from *P. macadamiae* in the characteristics and dimensions of their conidiophores and conidia.

Acknowledgements

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