Morphological characterization of *Diaporthe foeniculacea* and its *Phomopsis* anamorph on *Foeniculum vulgare*

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Diaporthe foeniculacea is redescribed from two collections from *Foeniculum vulgare* in Portugal. The connection between the anamorph and teleomorph was established in cultures. The anamorph correlated well with *Phoma foeniculina*, and the transfer to *Phomopsis* is supported. The fungus is self-fertile and readily produces perithecia in culture. Three morphologically distinct *Phomopsis* species were found also on *F. vulgare* stems and one of these corresponded to the anamorph of *D. foeniculacea*.

Keywords: Diaporthales, *Diaporthe*, *Phomopsis*, systematics.

The Ascomycete genus *Diaporthe* includes several plant pathogenic and endophytic species that are most commonly seen as their *Phomopsis* anamorphs. Species in *Diaporthe* were characterized largely on host association until Wehmeyer (1933) reassessed the genus on a morphological basis. He rejected about 100 names, placed others in closely related genera (*Apioporthe* Höhnel, *Diaporthopsis* Fabre, *Diaporthella* Petrak, and *Cryptodiaporthe* Petrak) and reduced many of the remainder to synonymy. In so doing, he reduced the number of species from over 600 to about 70. Species in *Phomopsis* were also based largely on host association and more than 1000 species have been described (Uecker, 1988). It is now recognised that *Phomopsis* species are not necessarily host-specific (Rehner & Uecker, 1994) and more than one species can occur on a single host species (Zhang & al., 1998; Mostert & al., 2001). The genus *Phomopsis* has not been revised and many *Phomopsis* species have not been connected with their corresponding *Diaporthe* states. Furthermore, many of the reported connections were based on association on the host and not on cultural studies.

Foeniculum vulgare Millar, or wild fennel, is a common weed found on waste ground and road verges throughout Portugal. It was so common on the island of Madeira that the capital, Funchal, was

named after the local name for the plant (funcho). During late autumn and early winter, the senescent and moribund stems turn white or grey and are dotted with pycnidia of a *Phomopsis* species. The only species of *Phomopsis* reported on *F. vulgare* is *Phomopsis foeniculina* (Sacc.) Câmara, collected from Sacavém, near Lisbon, Portugal (Câmara, 1947). Although *Diaporthe foeniculacea* Niessl was described on *F. vulgare* (under *Foeniculum officinale* L.) from Coimbra, Portugal (von Thümen, 1880), no reports of it have been made since then. No other species of *Diaporthe* have been reported on *Foeniculum*, although *D. inquilina* (Wallr.) Nitschke, *D. faberae* Kze. and *D. umbellatarum* (Schw.) Ell. & Everh. have been reported from other members of the Umbelliferae, but all are morphologically indistinguishable from *D. arctii* (Lasch.) Nitschke (Wehmeyer, 1933). Furthermore, Wehmeyer (1933) considered *D. foeniculacea* to be more suitably placed in *Diaporthopsis*.

Recently, the author found a *Diaporthe* sp. at the base of a dead *F. vulgare* stem at Serra da Agua on the island of Madeira, and later at São Marcos near Lisbon. Therefore, the aim of the research reported in this paper was to clarify the taxonomic status of *D. foeniculacea* and to determine its relationship with *P. foeniculina*.

Materials and methods

Isolates and morphology

Conidia or ascospores were spread over the surface of plates of Difco potato dextrose agar (PDA) and incubated at 25 °C overnight. Individual, germinating spores were transferred to fresh plates of PDA and checked microscopically to ensure that only a single spore had been transferred. Cultures were maintained and colony morphology determined on oatmeal agar (OA) (Anonymous, 1968). To induce sporulation, cultures were grown on OA plates bearing a piece of autoclaved *F. vulgare* stem. Cultures for sporulation and colony morphology were incubated at room temperature (ca. 25 °C) exposed to indirect sunlight. Growth rates were determined on plates of PDA incubated in darkness at 25 °C. Plugs of agar cut from the margin of actively growing cultures on PDA were placed about 1 cm from the edge of a freshly prepared plate of PDA. Colony radii, measured from the edge of the agar plug, were recorded daily. Growth rates are expressed as the average increase in radius in mm per day of three replicate plates.

Asci and ascospores were mounted in lactophenol. Pycnidia were cut through horizontally; the conidiogenous layer was excised and mounted in lactophenol or lactofuchsin (0.1 g acid fuchsin in 100 ml of 100% lactic acid). Conidia oozing from pycnidia were

transferred to a spot of water on a microscope slide, spread in a thin layer and allowed to dry. A drop of lactophenol or lactofuchsin was applied and covered with a cover slip. Observations on micromorphological features were made with a Leica DMR HC microscope with bright field or Nomarski differential interference contrast illumination. Digital images were recorded with an Olympus C-3030 camera. Measurements were made with the UTHSCSA *ImageTool* version 3 program (http://ddsdx.uthscsa.edu/dig/download.html). At least 50 ascospores or conidia of each isolate were measured on images taken with a 100X objective lens. Mean, standard deviation and 95% confidence intervals were calculated. Data for spore measurements are presented as the lower and upper 95% confidence limits, with the minimum and maximum dimensions in parentheses. Dimensions of other structures are given as the range of at least 20 measurements. Herbarium abbreviations follow Holmgren & al. (1990).

Sexual compatibility

For each collection, ten single ascospore or single conidium isolates were crossed in all combinations in attempts to induce formation of the teleomorph. Plugs of agar cut from the periphery of actively growing colonies on PDA were placed at either end of 4 cm long segments of autoclaved *F. vulgare* stems placed on water agar. Two different isolates were paired in a Petri dish. Plates were then sealed with Parafilm and incubated at 25 °C for 7 days before incubating in darkness at 10 °C for up to 3 months. Successful matings were regarded as those combinations that produced fertile perithecia containing asci and ascospores.

Results

The general characteristics of the fungus found at Serra da Agua and São Marcos were perithecia immersed in a stroma surrounded by a narrow black line. Cylindrical asci with a refractile ring at the tip, and containing eight, two-celled ascospores confirmed it to be a *Diaporthe* sp. Cultures derived from single ascospores (CBS 111553, CBS 111554) of both collections were indistinguishable from one another and they could not be separated on dimensions of their conidia (Tab. 1).

Pycnidia of a *Phomopsis* were found adjacent to the stromata of the *Diaporthe* species, and on other parts of the stems of the two collections. Cultures derived from single conidia (CAP 076, CAP 079, CAP 105–107) could be placed in three groups according to colony morphology, growth rates, and morphology and dimensions of the

Tab. 1. – Dimensions and shapes of alpha-conidia of the species of *Phomopsis* studied

Specimen or culture number	Locality	Identity	Dimensions (µm)*	Shape
COI 285	Coimbra	*Phomopsis* type 2	(6.6–)9.5–10.5(–12) × (2.3–)2.8–3.0(–3.5)	Fusiform
PAD 281	Unknown	*Phoma foeniculina*	(5.8–)6.7–6.9(–8.0) × (2.2–)2.4–2.5(–2.9)	Fusiform
CBS 111553	Serra da Agua	*Phomopsis foeniculina*	(5.4–)7.0–7.3(–8.0) × (2.3–)2.5–2.6(–3.1)	Fusiform
CBS 111554	São Marcos	*Phomopsis foeniculina*	(6.3–)7.1–7.6(–9.0) × (2.0–)2.1–2.2(–2.6)	Fusiform
CAP 105	São Marcos	*Phomopsis* type 1	(4.9–)5.5–5.7(–6.4) × (2.3–)2.4–2.5(–2.8)	Oval
CAP 106	São Marcos	*Phomopsis* type 1	(4.1–)5.4–5.8(–6.4) × (2.2–)2.4–2.5(–2.7)	Oval
CAP 107	Quinta da Torre, Caparica	*Phomopsis* type 2	(7.7–)9.1–9.9(–12.2) × (2.6–)2.2–3.1(–3.7)	Fusiform
CAP 076	Quinta do Marquês, Oeiras	*Phomopsis* type 2	(5.5–)7.1–8.0(–9.1) × (2.0–)2.5–2.7(–3.2)	Fusiform
CAP079	Gualtar, Braga	*Phomopsis* type 2	(8.7–)10.7–11.6(–14.3) × (1.8–)2.4–2.6(–2.9)	Fusiform

* Dimensions are from conidia formed in culture, except in COI 285 and PAD 281.

conidia (Tab. 1). One isolate (CAP 076) corresponded with the cultures derived from single ascospores (CBS 111553, CBS 111554) as described above. The two other groups are referred to here as *Phomopsis* type 1 and *Phomopsis* type 2.

Specimens filed under *D. foeniculacea* and *Phoma foeniculina* were studied. In the protologue, von Thümen (1880) cites a specimen N° 133 collected by P. G. Mesnier from Coimbra in 1877. This specimen could not be found in COI (F. Sales, pers. comm.), but two other specimens in COI were examined; one from Chaupal (collected May 1880) and the other from Penedo da Meditação (collected May 1881), both have the number 285. The COI specimens bore a *Phomopsis* sp., and because the dimensions in both were similar, data from the two were combined (Tab. 1). In the specimen from Penedo da Meditação a *Diaporthe* sp., was present. Ascospores were two-celled and measured (11.5–)12.9–13.5(–14.6) × (3.4–)3.7–4.0(–4.5) µm. Two examples of Mycotheca Universalis N° 2260 were examined; one from PAD and the other from COI, but neither contained any fungus resembling a *Diaporthe* or *Diaporthopsis*.

A specimen of *D. foeniculacea* in PAD labelled "ex herb. von Thümen, *Diaporthe foeniculacea* Niessl" was also examined. The ascospores were aseptate, few asci were present, no free ascospores were seen and the perithecia contained numerous paraphyses. For these reasons, this specimen was considered to be immature and thus unrepresentative of this species.

The type specimen of *Phoma foeniculina* Sacc. (PAD 281) was examined. This is a typical *Phomopsis* with conspicuous conidiogenous cells up to 11 µm long and 2 µm wide. Alpha conidia (Table 2) were similar to those of the conidia of the single ascospore cultures (CBS 111553, CBS 111554) described above, but differ from the species considered by Câmara (1947), who gives them as 8.5–12.5 × 3–4 µm.

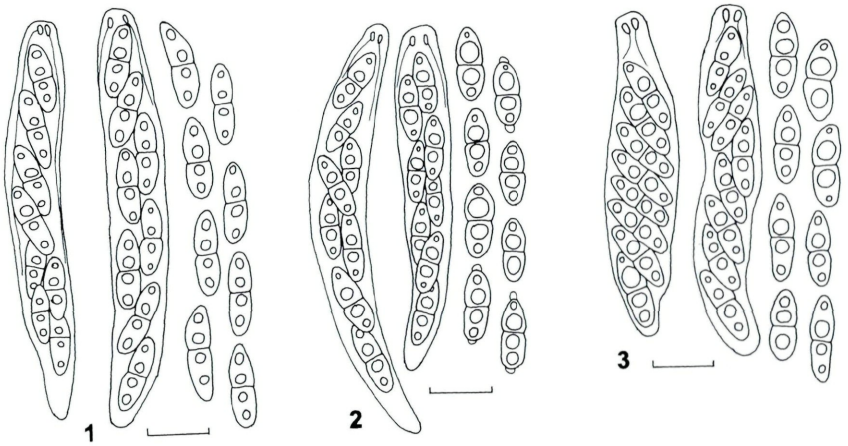
Taxonomy

Diaporthe foeniculacea Niessl, in litt. ad von Thüm., Contr. ad Fl. Myc. Lusit. 2: 30. 1880. – Figs. 1–21.

Anamorph: *Phomopsis foeniculina* (Sacc.) Câmara, Agron. Lusit. 9: 85–128. 1947.

≡ *Phoma foeniculina* Sacc., Sylloge Fungorum III, 125. 1884.

Appearing on the host as irregular black patches at the base of dead, partially decorticated stems. – Ostioles emerging separately, barely protruding through the host epidermis. – Perithecia globose to subglobose, 210–350 × 200 µm, not clustered, embedded in the host within a stroma surrounded by a narrow black line. –

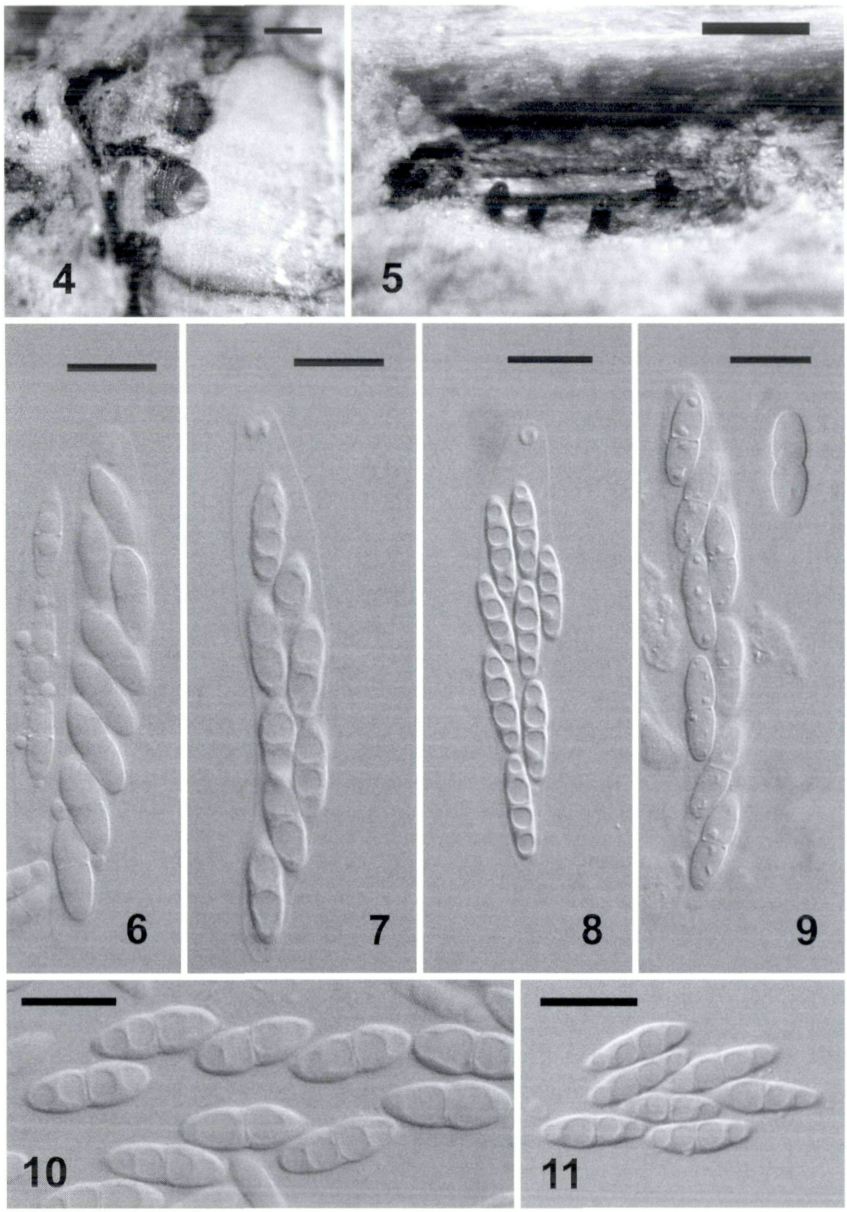


Figs 1–3. *Diaporthe foeniculacea*. Asci and ascospores. – 1. COI 285. – 2. LISE 94792. – 3. LISE 94791. Scale bars = 10 µm.

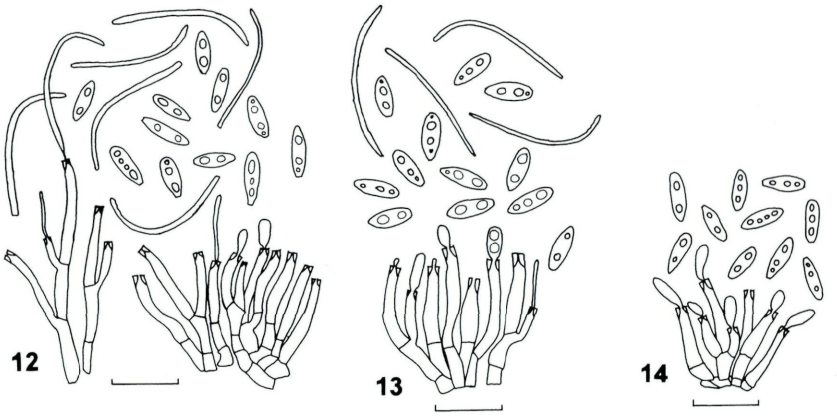
Entostroma differentiated and paler than the surrounding host tissues, sometimes with a yellow tint, not restricted in area, dorsal blackening strongly developed, partially obscured by the host epidermis, lateral blackened zone well developed, ventral zone strong, on the pith cavity. – Asci cylindrical to clavate, 50–60 × 8–10 µm (n=30) with a refractive apical ring. – Ascospores (9.5–)11.8–12.5 (–15) × (3–)3.7–4.9(–5.4) µm (mean ± S.D. = 12.5 ± 1.1 × 4.3 ± 0.5 µm, n = 50), biseriate or obliquely uniseriate, fusiform, hyaline, two-celled, slightly constricted at the septum, inequilateral, four-guttulate, upper cell slightly wider than the basal cell, rarely bearing minute apical appendages.

This species is self-fertile and perithecia formed in culture were indistinguishable from those formed in nature, except that when developing under conditions of high humidity in culture the ostioles became long and sinuous. Ascospores from culture were similar to those from nature.

Colonies on PDA increasing in diameter at a rate of 10 mm per day at 25 °C, initially white, becoming pink after 7 days and developing brown patches that later turn dark brown. – Pycnidia formed after 15 days at 25 °C on pieces of autoclaved *F. vulgare* stem placed on OA eustromatic, dark brown to black, separate, up to 560 µm wide and 350 µm tall, wall composed of two regions of *textura angularis*; outer region brown, 3–4 cells thick, 15–20 µm wide; inner region light brown to hyaline, 4–5 cells thick, 25–35 µm wide. – Conidial masses pale yellow to cream or white. – Alpha conidiophores 19–25 × 2–3 µm, cylindrical, branched, septate. – Alpha conidiogenous cells



Figs 4–11. *Diaporthe foeniculacea*. – 4. Stroma surrounded by a thin black line containing embedded perithecia with necks emerging through the host epidermis. – 5. Short perithecial necks emerging from the host. – 6–9. Asci. – 10, 11. Ascospores. – 6, 7: LISE 94972, 8: LISE 94971, 9: COI 285, 10: LISE 94972, 11: LISE 94971. Bars: 4, 5 = 0.5 mm; 6–11 = 10 µm.



Figs 12–14. *Phomopsis foeniculina*. – 12. Alpha and beta conidia and conidiophores of CBS 111554. – 13. Alpha conidia and conidiophores of CBS 111553. – 14. Alpha conidia and conidiophores of PAD 281. Bars = 10 µm.

10–13 × 1.5–3 µm, cylindrical, filiform, tapering towards the apex, straight or slightly curved, periclinal thickenings present. – Alpha conidia (5.4–)6.8–7.0(–9.0) × (2.0–)2.3–2.4(–3.1) µm; average of 184 conidia = 6.9 ± 0.6 × 2.4 ± 0.2 µm and L/W = 2.9 ± 0.4, fusiform, mostly biguttulate, sometimes four-guttulate. – Beta conidiophores not different from alpha conidiophores. – Beta conidia (16.8–)19.6–21.0(–24.2) × (1.1–)1.3–1.4(–1.7) µm, hyaline, aseptate, eguttulate, curved or hamate.

Non *P. foeniculina* isolates. – Figs. 22–24.

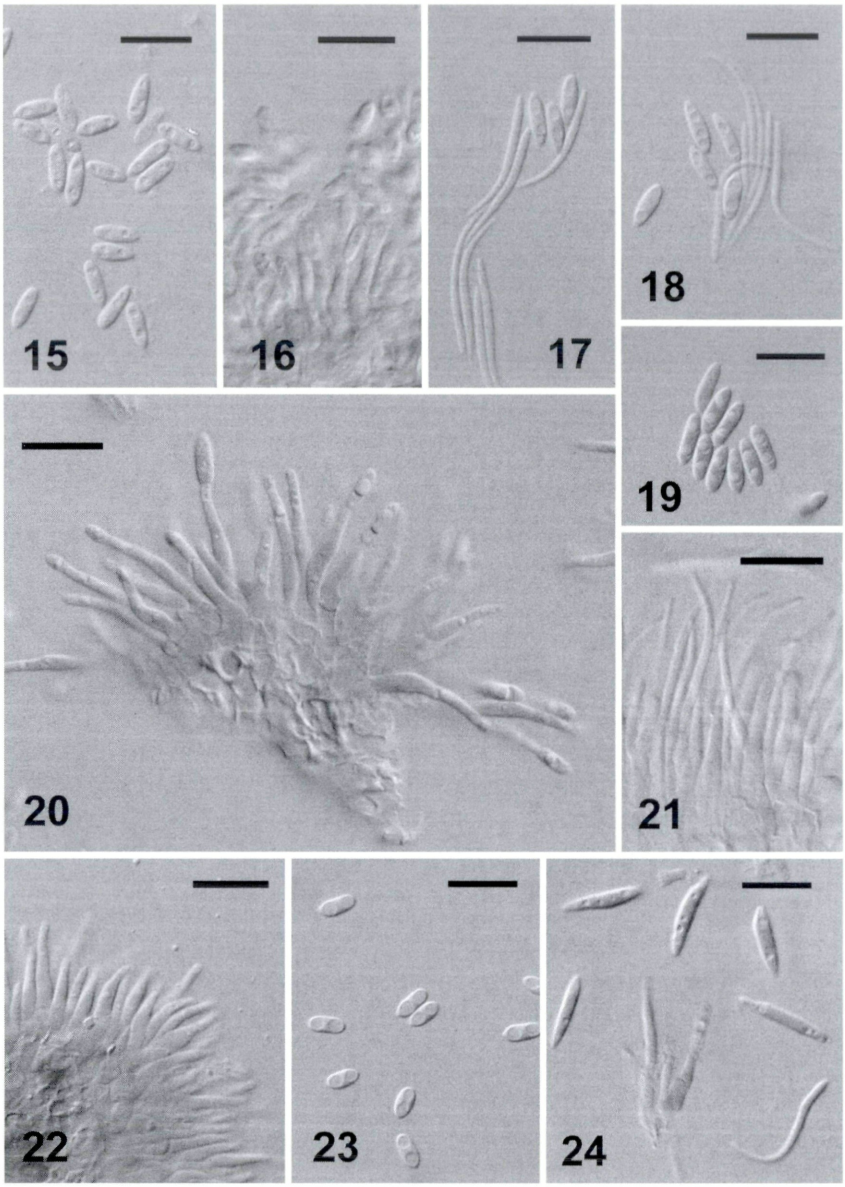
Phomopsis type 1

Colonies initially white, later becoming buff and ultimately dark grey brown with dark brown to black patches. Colonies increased in diameter at a rate of 16–18 mm per day. – Conidiophores cylindrical, sparingly branched, septate, 15–20 × 2–3 µm. – Conidiogenous cells cylindrical, tapering towards the apex, straight or slightly curved, periclinal thickenings present, 10–15 × 1.5–3 µm. – Alpha conidia were oval with both ends rounded, biguttulate, (4.1–)5.5–5.8(–6.5) × (2.2–)2.4–2.5(–3.0) µm; mean ± S.D. of 64 conidia = 5.6 ± 0.5 × 2.5 ± 0.1 µm, L/W = 2.3 ± 0.3. – Beta conidia hyaline, aseptate, eguttulate, curved or hamate, (18–) 19–24 × 1–1.5 µm.

These isolates are not *P. foeniculina*, nor are they the species that Câmara (1947) described.

Phomopsis type 2

Colonies on OA initially white becoming cream after 7 days and developing dark brown patches, increasing at a rate of 4–6 mm



Figs 15–24. *Phomopsis* species on *Foeniculum vulgare*. – 15–21. *Phomopsis foeniculina* (15, 16: PAD 281; 17, 20: CBS 111553; 18, 19, 21: CBS 111554). – 22–24. Non *P. foeniculina* species. – 22, 23. *Phomopsis* type 1 (CAP 106). – 24. *Phomopsis* type 2 (CAP107). Bars = 10 µm.

per day at 25 °C. – Conidiophores cylindrical, branched, septate 14–22 × 2–3 µm. – Conidiogenous cells cylindrical, filiform, tapering towards the apex, straight or slightly curved, periclinal thickenings present, 8–12 × 1.5–3 µm. – Alpha conidia fusiform and bi- or multiguttulate, measuring (5.5–)9.4–9.8(–14.3) × (1.8–)2.7–2.9(–4.1) µm; average of 150 conidia = 9.6 ± 1.5 × 2.8 ± 0.4 µm, L/W = 3.5 ± 0.9. – Beta conidia abundant, hyaline, aseptate, curved to hamate, eguttulate, 19.5–28 × 1–1.5 µm.

Conidia of these type 2 isolates correspond well with those of the species described by Câmara (1947).

Material examined. – *Diaporthe foeniculacea*: PORTUGAL: Madeira, Serra da Agua, at the base of a 2-yr-old stem of *Foeniculum vulgare*, August 2001, A. J. L. Phillips, LISE 94791, culture from single ascospore CBS 111553; São Marcos, at the base of a stem of *F. vulgare*, 25 April 2002, A. J. L. Phillips, LISE 94792, culture derived from single ascospore CBS 111554; Coimbra, Penedo da Meditação, on *F. vulgare*, May 1881, Moller (COI 285); Coimbra, Chaupal, on *F. vulgare*, May 1880, Moller (COI 285); Coimbra, on *F. vulgare*, Fr. Moller (PAD ex herb. von Thümen). – *Phoma foeniculina*: locality and date of collection unknown (PAD 281, type). – *Phomopsis foeniculina*: PORTUGAL: Oeiras, Quinta do Marquês, October 1998, A. J. L. Phillips CAP 076. – Non *Phomopsis foeniculina* isolates from *Foeniculum vulgare*: PORTUGAL: Braga, Gualtar, September 1999, A. J. L. Phillips CAP 079 (*Phomopsis* type 2); São Marcos, September 2001, A. J. L. Phillips CAP 105 (*Phomopsis* type 1); Caparica, Quinta da Torre, September 2001, A .J. L. Phillips CAP 106 (*Phomopsis* type 1); Caparica, Quinta da Torre, September 2001, A. J. L. Phillips CAP 107 (*Phomopsis* type 2).

Discussion

Diaporthopsis Fabre differs from *Diaporthe* in having aseptate ascospores (Hanlin, 1990). Wehmeyer (1933) examined *Diaporthe foeniculacea* Myc. Univ. 2260 in NYS, and a specimen ex herb. von Thüm., Coimbra, Portugal and reported aseptate ascospores. For this reason he considered these specimens to be representative of *Diaporthopsis* and transferred them to *Diaporthopsis angelicae* (Berk.) Wehm. The two exsiccati of Myc. Univ. 2260 studied in the present work bore no fungus corresponding to *Diaporthe* or *Diaporthopsis*. The specimen in PAD that was examined in the present work is labelled "ex herb. von Thümen, *Diaporthe foeniculacea* Niessl. – *F. officinalis*, Lusitania: Coimbra, leg. Moller". It is unclear what specimen ex Herb. von Thüm. Wehmeyer (1933) studied, but if it was part of the collection in PAD, his transfer to *Diaporthopsis* is understandable because the ascospores in this specimen are aseptate. The specimen in PAD, however, is immature and thus not representative of the species. Therefore, Wehmeyer's transfer to *Diaporthopsis* is considered erroneous. The only specimens of *D. foeniculacea* extant at COI were examined in the present study, and one of them (from Penedo da Meditação) bore a fungus that clearly belongs

to the genus *Diaporthe*. The ascospores of this were larger than von Thümen (1880) described for *D. foeniculacea*. It was, however, collected by Moller (who also collected the specimens of this species that von Thümen distributed in his Mycotheca Universalis N° 2260, and the specimen in PAD) and is here considered to be representative of *D. foeniculacea*. As the type specimen of *D. foeniculacea* could not be located, specimen COI 285 from Penedo da Meditação is herein proposed as lectotype.

Morphologically, the two collections made in this study correlated with COI 285 and the name *D. foeniculacea* was considered to be suitable. When grown in culture, colonies of single ascospore isolates of these two collections were indistinguishable from one another. Conidium morphology and dimensions correlated well with the type of *Phoma foeniculina* (PAD 281) and the name *Phomopsis foeniculina* was considered to be applicable. The connection between the anamorph and teleomorph was confirmed by the production of both sexual and asexual states in culture.

Of the three morphological types of *Phomopsis* found on *F. vulgare*, only one corresponded to the anamorph of *D. foeniculacea* as determined in this paper. Câmara (1947) transferred *Phoma foeniculina* to *Phomopsis* based on the samples he collected from Sacavém. Host association was used in the past as a key feature in the identification of *Phomopsis* species (Uecker, 1988; Brayford, 1990; Wechtl, 1990), and presumably Câmara (1947) used this to identify the species he found on *F. vulgare*. However, recent studies (Rehner & Uecker, 1994; Farr & al., 1999) have shown that *Phomopsis* species can infect more than one host. The observations in the present study, in which three morphologically different *Phomopsis* species were found on *F. vulgare*, confirm the findings of Mostert & al. (2001) and Farr & al. (2002) that a single host species can accommodate several different *Phomopsis* species.

The characteristics and dimensions of the conidia of the species that Câmara (1947) described correspond to one of the other, non-*P. foeniculina* species found in the present work. Therefore, the fungus that Câmara described is not the anamorph of *D. foeniculacea*. However, the correct name and authority for the anamorph of *D. foeniculacea* is *P. foeniculina* (Sacc.) Câmara, because Câmara effected the transfer of the name, based on *Phoma foeniculina* Sacc., even though he transferred a different species.

No attempts were made in this work to apply a name to the *Phomopsis* sp. described by Câmara, nor to the other non-*P. foeniculina* species isolated from *F. vulgare*. As it is possible that these are previously described species that may occur on other hosts, identification will have to wait until further studies using molecular techniques have been completed.

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