A reassessment of the anamorphic fungus *Fusicoccum luteum* and description of its teleomorph *Botryosphaeria lutea* sp. nov.

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When *Fusicoccum luteum* was first described in 1985, it was not found sporulating in nature and its teleomorph was not known. Recently, a *Botryosphaeria* sp. was found on grapevines in Portugal, and the anamorph in culture resembled *F. luteum*. Nucleotide sequence of the nuclear rDNA internal transcribed spacer ITS2 distinguished isolates of this fungus from others in the *B. dothidea* complex, and the teleomorph, *Botryosphaeria lutea*, is described here as new. Although the teleomorph of *B. lutea* is morphologically indistinguishable from others in the *B. dothidea* complex, the holomorph is clearly distinguished by a yellow pigment produced in culture.

Keywords: *Botryosphaeria*, *Fusicoccum*, grapevine, *Vitis vinifera*, systematics.

Botryosphaeria dothidea (Moug. : Fr.) Ces. & de Not., the type species of *Botryosphaeria* Ces. & de Not., is associated with cankers and diebacks on a wide range of woody hosts. Although von Arx & Müller (1954) cited extensive synonymy for this species, their work was based solely on an examination of herbarium specimens of the teleomorph, no cultural work was done and no anamorphic characters were taken into account. However, since the teleomorphs are rarely seen in nature and their characters vary little between species (Shoemaker, 1964; Laundon, 1973), taxonomy of the group is based largely on characters of the anamorphs. The taxonomic problems surrounding the anamorphs of *Botryosphaeria* spp. have been explained and, to a large extent, resolved by Crous & Palm (1999). Among various genera, *Fusicoccum* Corda is acknowledged to be a suitable name for at least some of the species, including *B. dothidea*.

In a study of *Botryosphaeria* and *Fusicoccum* species on kiwifruit in New Zealand, Pennycook & Samuels (1985) concluded that *B. dothidea* is a complex of species that can be distinguished on the

basis of cultural and morphological details of the anamorphs. In this way they distinguished three cultural types of the *Fusicoccum* anamorph. Although all three fell within the broad concept of the anamorph of *B. dothidea*, they were separated on minor differences in conidium size and shape and on cultural characters. Since the differences were consistent within a large number of isolates, they regarded them as three separate species, namely, *Fusicoccum aesculi* Corda (anamorph of *B. dothidea*), *F. parvum* Pennycook & Samuels (anamorph of *B. parva* Pennycook & Samuels) and *F. luteum* Pennycook & Samuels. *Fusicoccum luteum* differed from the other two species in producing a transient yellow pigment that diffused into the agar medium. It was isolated from lesions on over-ripe kiwifruits, but conidiomata were not found in nature and the teleomorph was not seen. *Fusicoccum luteum* has rarely been mentioned in the literature. The single isolate that Jacobs & Rehner (1998) included in their comparative study of morphological and cultural characters and ITS sequence phylogeny of *Botryosphaeria* species was distinct from the other species they studied.

In a study of fungi on grapevine canes in Portugal (Phillips & Lucas, 1997; Phillips, 1998), a number of *Botryosphaeria* species were collected and deposited in the National Herbarium, LISE, at Estação Agronómica Nacional, Oeiras, Portugal. At the time of collection, Phillips & Lucas (1997) considered them to be morphological variants of *B. dothidea* and they were filed under that name. The work of Jacobs & Rehner (1998), however, prompted a re-examination of these specimens and cultures derived from their ascospores. Cultures from LISE 94070 and LISE 94072 produced a yellow pigment in agar media and could be representative of *F. luteum*, in which case the ascomycete from which the cultures were derived would be the undescribed teleomorph. Therefore, the purpose of this work was to reassess the taxonomic status of *F. luteum*, to establish a connection between the teleomorph and the anamorph, and to describe the teleomorph.

Materials and methods

Collection and isolation

Single ascospore and single conidium cultures were prepared as follows. Ascomata or conidiomata were crushed in a drop of sterile water on a flamed glass slide. A portion of the water was spread over a plate of Difco potato dextrose agar (PDA). After 8 h at 25°C, germinating ascospores or conidia were transferred to fresh plates of PDA and checked microscopically to ensure that only a single spore had been transferred.

Tab. 1. – Sources of *Botryosphaeria* and *Fusicoccum* species studied.

Isolate	GenBank number	Identity	Source	Symptom	Host	Locality	Morphological group
CAP002	AF 286255	*Botryosphaeria lutea*	Ascospore	Cane blight	*Vitis vinifera*	Carcavelos vineyard	1
CAP007	AF 286256	*Botryosphaeria dothidea*	Ascospore	Cane blight	*Vitis vinifera*	Montemor-o-Novo	2
CAP008	AF 286257	*Botryosphaeria stevensii*	Conidium	Wood necrosis	*Vitis vinifera*	Montemor-o-Novo	–
CAP009	AF 286258	*Botryosphaeria obtusa*	Conidium	Wood necrosis	*Vitis vinifera*	Montemor-o-Novo	–
CAP022	AF 286259	*Botryosphaeria parva*	Ascospore	Cane blight	*Vitis vinifera*	Montemor-o-Novo	2
CAP025	AF 286260	*Botryosphaeria parva*	Ascospore	Cane blight	*Vitis vinifera*	Clone vineyard	2
CAP032	AF 286261	*Botryosphaeria parva*	Ascospore	Cane blight	*Vitis vinifera*	Carcavelos vineyard	2
CAP035	AF 286262	*Botryosphaeria lutea*	Conidium	Necrotic leaf	*Vitis vinifera*	Carcavelos vineyard	1
CAP037	AF 286263	*Botryosphaeria lutea*	Ascospore	Cane blight	*Vitis vinifera*	Sintra	1
CAP038	AF 286264	*Botryosphaeria parva*	Ascospore	Cane blight	*Vitis vinifera*	Alcobaça	2
CAP039	–	*Botryosphaeria obtusa*	Conidium	Wood necrosis	*Vitis vinifera*	Alcobaça	–
CAP040	–	*Botryosphaeria parva*	Ascospore	Trunk dieback	*Vitis vinifera*	Montemor-o-Novo	2
CAP041	–	*Botryosphaeria parva*	Ascospore	Trunk dieback	*Vitis vinifera*	Montemor-o-Novo	2
CAP042	AF 286265	*Botryosphaeria parva*	Ascospore	Trunk dieback	*Vitis vinifera*	Cantanhede	2
CAP043	–	*Botryosphaeria parva*	Ascospore	Trunk dieback	*Vitis vinifera*	Cantanhede	2
CAP056	AF 286266	*Botryosphaeria lutea*	Conidium	Leaf blight	*Fraxinus angustifolia*	Oeiras	1
CAP058	AF 286267	*Botryosphaeria lutea*	Ascospore	Twig dieback	*Sophora japonica*	Oeiras	1
CAP060	–	*Botryosphaeria parva*	Mycelium	Wood necrosis	*Vitis vinifera*	Montemor-o-Novo	2
CAP061	–	*Botryosphaeria parva*	Mycelium	Wood necrosis	*Vitis vinifera*	Montemor-o-Novo	2
CAP062	–	*Botryosphaeria stevensii*	Conidium	Wood necrosis	*Vitis vinifera*	Montemor-o-Novo	–
CAP067	AF 286268	*Botryosphaeria parva*	Ascospore	Cane blight	*Vitis vinifera*	Santo Tirso	2
CAP068	–	*Botryosphaeria parva*	Ascospore	Cane blight	*Vitis vinifera*	Santo Tirso	2
CAP071	AF 286269	*Botryosphaeria parva*	Mycelium	Wood necrosis	*Vitis vinifera*	Matosinhos	2

Morphological characterization

Cultures were grown on PDA, potato carrot agar (PCA), or oatmeal agar (OA) at 23°C with 12 h of light per day from mixed UV and daylight fluorescent tubes, and 12 h of darkness. PCA and OA were prepared according to the recipes given in the Plant Pathologist's Pocket Book (Anonymous, 1968).

Conidia, asci and ascospores were mounted in lactophenol and at least 50 conidia, 50 ascospores, and 20 asci of each collection were measured at a magnification of 1250× with a calibrated eyepiece micrometer. Since the teleomorph did not form in culture, dimensions of asci and ascospores were taken from material on the host. Hand-cut sections of conidiomata and ascomata were mounted in lactophenol and examined by bright field and Nomarski differential interference contrast microscopy.

DNA extraction

DNA was extracted by means of the DNeasy Plant Mini Kit (Quiagen Inc.). From 100 mg fresh mycelium, the DNA concentration in the eluate was 50 ng/µl.

RAPD amplification

In each PCR reaction 1 µl of the eluate diluted 1:10 in water was used as the template. RAPD profiles were generated using decamer primers (Operon Technologies Inc., Alameda, CA). Amplification reactions were performed in a volume of 15 µl, containing 1× Taq buffer (Fermentas Ab, Vilnius, Lithuania), 3 mM $MgCl_2$, 0.2 mM of each dNTP, 1µM of primer, 1 Unit of Taq DNA Polymerase (Fermentas Ab) and 1 µl of template DNA. For each primer, genomic DNA was omitted from control reactions to confirm that the observed bands were amplified DNA and not primer artefacts. Amplification was performed with a DNA thermocycler (Uno II, Biometra, Göttingen, Germany) programmed to give one cycle of 3 min at 94°C, 45 cycles of 1 min at 94°C, 1 min. at 36°C, and 1 min. at 72°C with a final extension period of 5 min. at 72°C followed by 10 min at 21°C. Amplification reactions for each isolate were done at least twice in separate experiments. The products were separated in 2% agarose gel and detected with ethidium bromide. In the analyses of RAPD patterns, clearly visible and reproducible bands were taken into account. Hence only bands generated by amplification with primers OPA10, OPG2, OPG5, OPK7, OPL5, OPM10 and OPN4 were used in the analysis. Primers sequences are available at http://www.operon.com/store/merkits.php.

Amplification of ITS1 and ITS2, and SSCP analysis

Based on published sequences (Jacobs & Rehner, 1998, GenBank numbers AF027741–AF027763) two pairs of primers were designed

to amplify the ITS1 and ITS2. The primers, designed with the aid of the program OSP (Hillier & Green, 1991) and synthesized by Life Technologies Ltd., were as follows: BotDot3 (5′-GGAAGGATCAT-TACCGAGT-3′) and BotDot4 (5′-CCGTTGTTGAAAGTTTTAG-3′) located respectively on the 18S and 5.8S units of the rDNA and amplifying the ITS1; and BotDot5 (5′-TGCCTGTTCGAGCGTCATT-3′) and BotDot6 (5′-ATCCGAGGTCAACCTTGAG-3′) located respectively on the 5.8S and 28S units of the rDNA and amplifying the ITS2. Amplification was performed with the thermocycler programmed as follows: one cycle for 3 min. at 94°C, 45 cycles of 1 min. at 94°C, 1 min. at 54°C, and 1 min. at 72°C. The experiment was repeated once.

Amplified fragments were analysed for single strand conformation polymorphism (SSCP) (Orita & al., 1989). One µl of the amplification product was diluted 1:10 in water and 1 µl of the dilution was heat denatured for 5 min at 90 °C. The SSCP patterns were analysed by electrophoresis through 8% polyacrylamide gel (1 × TBE), at 4°C with 200 V/h in a 10 cm high vertical apparatus. SSCP patterns were detected by staining with silver nitrate.

DNA sequencing

Based on the SSCP banding patterns, isolates were chosen to represent the various ITS2 rDNA groups. Fresh PCR products were inserted into the pGEM-Teasy vector, according to the pGEM-Teasy System I Kit protocol (Promega Corporation, Madison, USA) and insertion was confirmed by PCR with BotDot5 and BotDot6. Both strands of the PCR product were sequenced by 4-Base Lab (Reutlingen, Germany), using the M13 universal and M13 reverse primers. Sequences were checked between complementary strands for reading errors, and ambiguities were resolved.

Data analysis

In the analysis of RAPD's, the profile of bands was determined for each isolate to assess genetic identity and to examine the patterns of genetic distance between isolates. The genetic analysis was performed on all isolates. To position these isolates amongst other *Botryosphaeria* species, two isolates of *B. obtusa* and two of *B. stevensii* were used. The presence/absence binary matrix was built with RAPDistance (Armstrong & al., 1996) input data routine. Pair-wise genetic divergence between isolates was estimated for RAPD's by using Jaccard's similarity coefficient. Levels of genetic diversity, and its partition into within and between group components, was assessed by Shannon's Diversity Index (SDI) (King & Shaal, 1989). Analysis of molecular variance (AMOVA, Excoffier & al., 1992) was

determined from the pair-wise genetic Euclidean distance matrix. Dendrograms were generated with the Neighbour-Joining (NJ) algorithm of the Treecon program (Van de Peer & De Wachter, 1994). Robustness of the branches was estimated by 500 bootstrap replicates (Felsenstein, 1985). A Permutation Test Probability (PTP) analysis was performed to test whether the resulting tree reflects an actual phylogenetic signal in the data or merely an artefact of the algorithm (Faith & Cranston, 1991), using RAPDistance (Armstrong & al., 1996) to calculate the Z statistic and Instat (GraphPad, USA) to assert its significance.

DNA sequences were edited with BioEdit (Hall, 1999), aligned with CLUSTAL W (Thompson & al., 1994), and visually corrected where necessary. Default settings for gap weight, and gap length penalties were used to maximize alignment of homologous character sites. Gaps resulting from the alignment were treated as missing data. Nucleotide composition was calculated by BioEdit (Hall, 1999).

Nucleotide sequence divergence between isolates was calculated by Kimura's two-parameter method (Kimura, 1980). A maximum-likelihood tree based on sequence data was produced using quartet-puzzling (Strimmer & von Haeseler, 1996; Strimmer & von Haeseler, 1997) Empirical transition:transversion ratios and base frequencies were estimated from the data set following the HKY substitution model (Hasegawa & al., 1985).

Results and discussion

Isolates

Between 1996 and 1998, 19 isolates of *Fusicoccum* spp. were obtained from diseased plants (Tab. 1). The strains were isolated from single ascospores, single conidia, or directly from diseased tissues. Pseudothecia were either unilocular or multilocular and often united with conidiomata on a common basal stroma. Within the locules, interspersed with pseudoparaphyses, were clavate, bitunicate asci, each with eight more or less biseriate, hyaline, aseptate ascospores. These characters clearly placed the ascomycete within the genus *Botryosphaeria* (Denman & al., 2000).

Conidiomata on canes and other woody parts were mostly multilocular and eustromatic but on leaves only unilocular pycnidial conidiomata were found. Conidiophores were hyaline, cylindrical, branched at the base, smooth, and 0–1 septate. First-formed conidia were produced holoblastically on cylindrical conidiogenous cells with subsequent conidia produced enteroblastically, proliferating percurrently with 1–2 indistinct percurrent proliferations, or determinate with typical phialides and periclinal thickening (*sensu*

Sutton, 1980). In all isolates the conidia were hyaline, thin-walled, smooth, aseptate with shapes varying from elliptical to fusiform. These characters fall within the current concept of *Fusicoccum* (Pennycook & Samuels, 1985; Phillips & Lucas, 1997; Crous & Palm, 1999).

Morphological characters

Isolations from single ascospores and single conidia gave rise to two distinct colony types on agar media. In one type (Tab. 1), hereafter referred to as morphological group 1 (MG1), colonies on PDA were initially pale to colourless, gradually darkening with age and ultimately becoming grey to dark grey. A distinctive feature of this group was the production of a yellow pigment which diffused into the agar ahead of the leading edge of the colony. The colour was most intense after 3 days at 25°C, thereafter it became violaceous and by 6–7 days the yellow colour could no longer be seen. Finally, the violaceous colour darkened and was obscured by the dense growth of dark mycelium. The yellow pigment was also formed by cultures growing on MA and PCA, but on these media it was less intense than on PDA. Although Witcher & Clayton (1963) mentioned a yellow pigment in isolates of what they regarded as *B. dothidea* from blueberry, they did not consider it to be of taxonomic value. However, this yellow pigment was the main character that Pennycook & Samuels (1985) used to separate *F. luteum* from *F. aesculi* and *F. parva*. The MG1 isolates consistently produced unilocular, pycnidial conidiomata on oatmeal agar and spores started to ooze from the ostioles within 5–7 days at 23°C. Up to 800 conidiomata were produced by a colony in a 9 cm diameter Petri dish. They were partially immersed in the medium, globose, usually papillate and covered with olive green appendage-like hyphae. Conidia were hyaline, thin-walled, aseptate, fusiform to fusiform elliptical, with a subobtuse apex and a truncate or rounded base often bearing a minute basal frill. Conidia were (15–)18–22.5(–24) × 4.5–6(–7.5) µm; mean ± standard deviation of 242 conidia = 19.7 ± 1.8 × 5.6 ± 0.6 µm. Length/width ratios were (2.4–)3.4–3.9(–5.3), and the mean ± standard deviation of 242 conidia = 3.6 ± 0.5. Microconidia were produced by some isolates. They were rod-shaped to reniform with either truncate or rounded ends 3–5 × 1–2 µm. These isolates were regarded as *Fusicoccum luteum*.

Colonies of the other group (Tab. 1), referred to in this paper as morphological group 2 (MG 2), on PDA were initially white with abundant aerial mycelium, which gradually became grey to dark grey. The reverse side of colonies on PDA was initially white, but after 2–3 days the centre became dark green to olive green. This colouration gradually spread to the edge of the colony which became

darker from the centre until the entire colony became black. Conidiomata on OA were multilocular, non-papillate, and conidia started to ooze from their ostioles after 13–15 days of incubation at 23°C. Between 36–52 conidiomata were formed on each plate of OA.

Two distinct types of conidium morphology were identified amongst the MG2 isolates. In most isolates, the conidia were hyaline, guttulate, thin-walled, non-septate, smooth, fusiform to narrowly ellipsoidal with an obtuse apex and truncate or rounded base, often with a minute basal frill. They were widest in the middle or upper third with dimensions of (12–)15–20(–24) × (4–)4.5–6(–7.5) µm; mean ± standard deviation of 320 conidia = 17.1 ± 2.1 × 5.5 ± 0.8 µm. Length/width ratios were (1.8–)2.5–4(–5), and the mean ± standard deviation of 320 conidia = 3.2 ± 0.6. Older conidia became olivaceous and some developed a septum before germination. Microconidia, which were seen in some isolates, were hyaline, smooth, rod-shaped and truncate at either end, 3–5 × 1–1.5 µm. These isolates were considered to be the *Fusicoccum parvum* anamorph of *Botryosphaeria parva*.

One isolate (Tab. 1) in MG2 (CAP007) produced fusiform conidia that were 21–28.5 × 4–6 µm. Mean ± standard deviation of 50 conidia = 24.7 ± 1.9 × 4.7 ± 0.5 µm. Length width ratios were 3.8–6.3, with the mean ± standard deviation of 50 conidia = 5.3 ± 0.6. This isolate correlated most closely with the *Fusicoccum aesculi* Corda anamorph of *Botryosphaeria dothidea* (Pennycook & Samuels, 1985; Crous & Palm, 1999).

Although there was considerable overlapping of conidium dimensions, the three species of *Fusicoccum* could be distinguished when the average dimensions of many conidia were taken into account. Thus, *F. luteum* and *F. parva* had shorter conidia than *F. aesculi* confirming the conclusions reached by Pennycook & Samuels (1985). *Fusicoccum luteum* was distinguished from the other two species by production of a short-lived yellow pigment that diffused into the agar medium. Furthermore, *F. luteum* formed unilocular, pycnidial conidiomata on OA, while conidiomata of the other two species were multilocular. There was also a difference in the maturation time of the conidiomata of the three species as indicated by the time taken before the first spores started to ooze from the ostioles. Although several studies have shown that size, shape and type of conidiomata in *Fusicoccum* spp. vary according to the growth medium or host substrate on which they are produced (e.g. Witcher & Clayton, 1963; Pennycook & Samuels, 1985; Morgan-Jones & White, 1987; Rayachhetry & al., 1996), in the present study they were consistent when isolates were cultured on OA under controlled conditions of temperature and light. Ascomata were not formed in culture by any isolate, even when single ascospore and single conidium cultures were paired in all possible combinations.

RAPD analyses

Pair-wise distances between isolates, based on RAPD data, were between 0.11111 and 0.94444. The most similar strains were CAP060 and CAP061, and the most different were CAP008 and CAP007. Genetic diversity was estimated as a partition of the diversity of all the isolates into diversity within morphological types for each primer. Diversity estimates varied considerably for the different primers. The lowest single primer level of within-type diversity was found in MG1 (H_O = 0.1022), and the mean diversity values over all primers was also lower in MG1 (H_O = 0.2260) compared to 0.3878 for MG2. Partitioning of genetic variation, examined by AMOVA, revealed that most of the variation (82%) was found within the morphological groups, but a significant proportion ($P < 0.001$) was attributable to differences between groups.

Neighbour-Joining analysis with all isolates showed a low degree of clustering (Fig. 1). Both isolates of *B. stevensii* clustered together with high branch support. Of the other three main clusters, one corresponded to MG1 isolates and *B. obtusa*, while the other two clusters corresponded to MG2 isolates. Terminal branches tended to be longer than the basal branches, suggesting that most of the RAPD distances in the data set exist between isolates rather than between

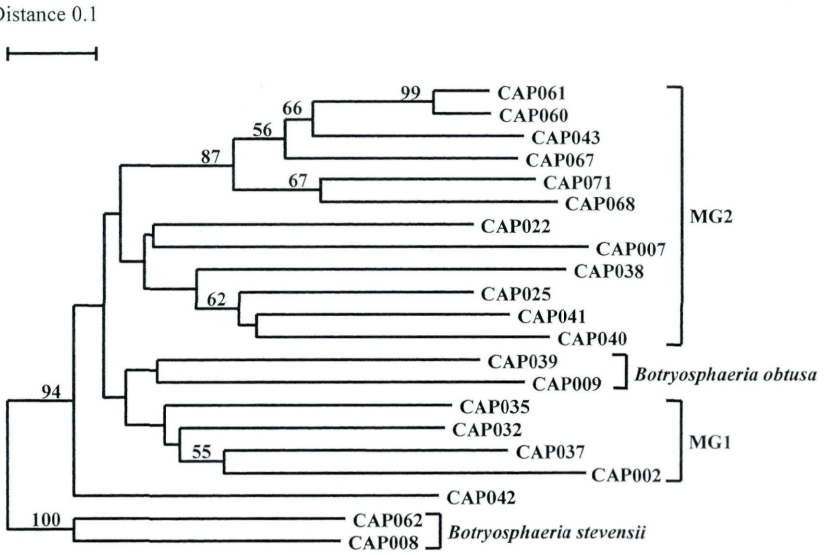


Fig. 1. – Neighbour-joining tree based on RAPD markers. Bootstrap values (%) from 500 bootstrap replications are indicated above the branches when the values are over 50%. The scale bar indicates genetic distance of 1-Jaccard's similarity index. MG1 and MG2 refer to morphological groups 1 and 2 described in the text. The tree was rooted to CAP062.

groups. The PTP gave a Z-value of 12.56 implying that the probability of this tree occurring simply by chance is null.

SSCP analysis of the ITS1 and ITS2 for the 19 isolates studied revealed five different patterns. Single patterns corresponded to *B. stevensii*, *B. obtusa*, and MG1 isolates, while two patterns were associated with MG2 isolates. Based on these patterns, representative isolates of each pattern were chosen for sequencing the rDNA ITS2. ITS1 was not sequenced because the SSCP patterns were too scattered among the isolates and incongruent with morphological group and geographical origin. DNA sequences were deposited in GenBank (Tab. 1).

An initial analysis of the sequence alignment in 15 isolates resulted in 196 sites, with 28 (14.3%) variable sites, corresponding to 32 nucleotide differences and 19 sites (9.7%) were parsimony informative. Base frequencies were estimated as $\pi_A = 0.172$, $\pi_C = 0.310$, $\pi_G = 0.268$, $\pi_T = 0.250$, showing a statistically significant deviation from equal base composition. Over all positions, the average transitions:transversions (ti:tv) ratio between these isolates was 2.48 (s.d. = 1.00) and expected pyrimidine transitions exceeded purine transitions by a factor of 1.68. Haplotype diversity was 0.886 (s.d. = 0.062) and nucleotide diversity was 0.04357 (s.d. = 0.00994). When the two morphological groups were considered separately, MG1 was found to be more homogeneous since the nucleotide diversity was much smaller than that of MG2 (Tab. 2).

A complete sequence multiple alignment was done including the 23 ITS2 sequences published by Jacobs & Rehner (1998). An analysis of the 203 nucleotide sites resulted in 59 (29.1%) variable sites and 26 (12.8%) that were parsimony informative. Base frequency parameters were estimated as $\pi_A = 0.175$, $\pi_T = 0.248$, $\pi_C = 0.309$, $\pi_G = 0.268$, indicating a statistically significant deviation from equal base composition. This was also true when the outgroup was excluded.

The mean number of differences between sequences was 12.569 (6.6%), the gene diversity was 0.930 (s.d. = 0.018) and the nucleotide diversity was 0.0705 (s.d. = 0.00934). Over all positions, the average transitions:transversions (ti:tv) ratio between all possible isolate combinations was found to be 1.26 (s.d. = 0.30) and expected pyrimidine transitions exceeded purine transitions by a factor of 1.64.

There was congruence between both tree-building methods used in the nucleotide sequence analysis. Maximum likelihood (Fig. 2) and Neighbor-Joining (not shown) trees recovered the same topology with all 38 isolates showing a high degree of clustering. Of the seven isolates in MG2, the six considered to represent *F. parvum* clustered together in clade I of Jacobs & Rehner (1998). The single MG2 isolate regarded as *F. aesculi* (CAP007) clustered with other *F. aesculi* isolates in Jacob & Rehner's clade III. All six MG1 isolates, whether

Tab. 2. – Measures of sequence diversity between morphological group 1 (MG1) and morphological group 2 (MG2) isolates of *Botryosphaeria* spp.

Parameter	MG1	MG2	All isolates
Number of sequences	6	7	15
Haplotype diversity (± s.d.)	0.600 ± 0.215	0.714 ± 0.181	0.886 ± 0.062
Nucleotide diversity (± s.d.)	0.0054 ± 0.0024	0.0227 ± 0.0121	0.0436 ± 0.0099

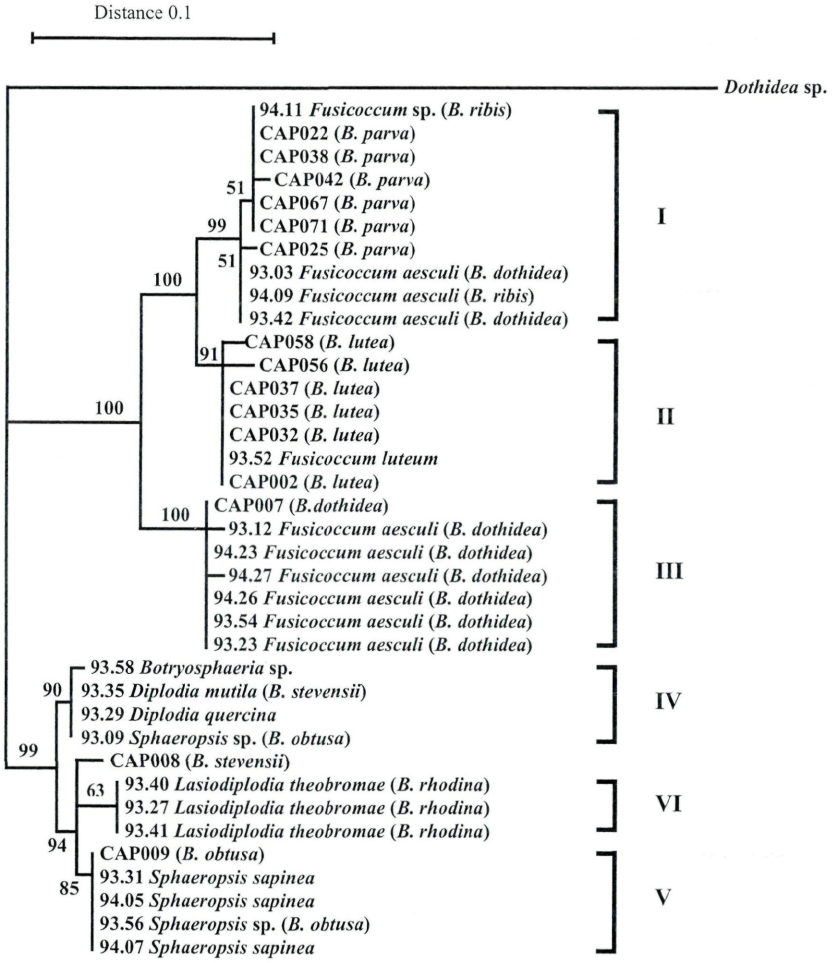


Fig. 2. – Maximum-likelihood phylogenetic tree calculated from the HKY substitution model based on ITS2 sequences. Base frequencies were estimated from the data set and transition:transversion ratios of 1.26. Bootstrap values greater than 50% are indicated above the branches. Strains preceded by CAP are listed in Table 1, all other strains are described by Jacobs & Rehner (1998). The Roman numerals I–VI refer to the strain groupings used by Jacobs & Rehner (1998). The tree was rooted to *Dothidea* sp.

they originated from single ascospores or single conidia, clustered in clade II with the single isolate of *F. luteum* (strain 93.52) studied by Jacobs & Rehner (1998). This strain of *F. luteum* was isolated from kiwifruit and identified by G. J. Samuels (Jacobs & Rehner, 1998) and can, therefore, be regarded as authentic.

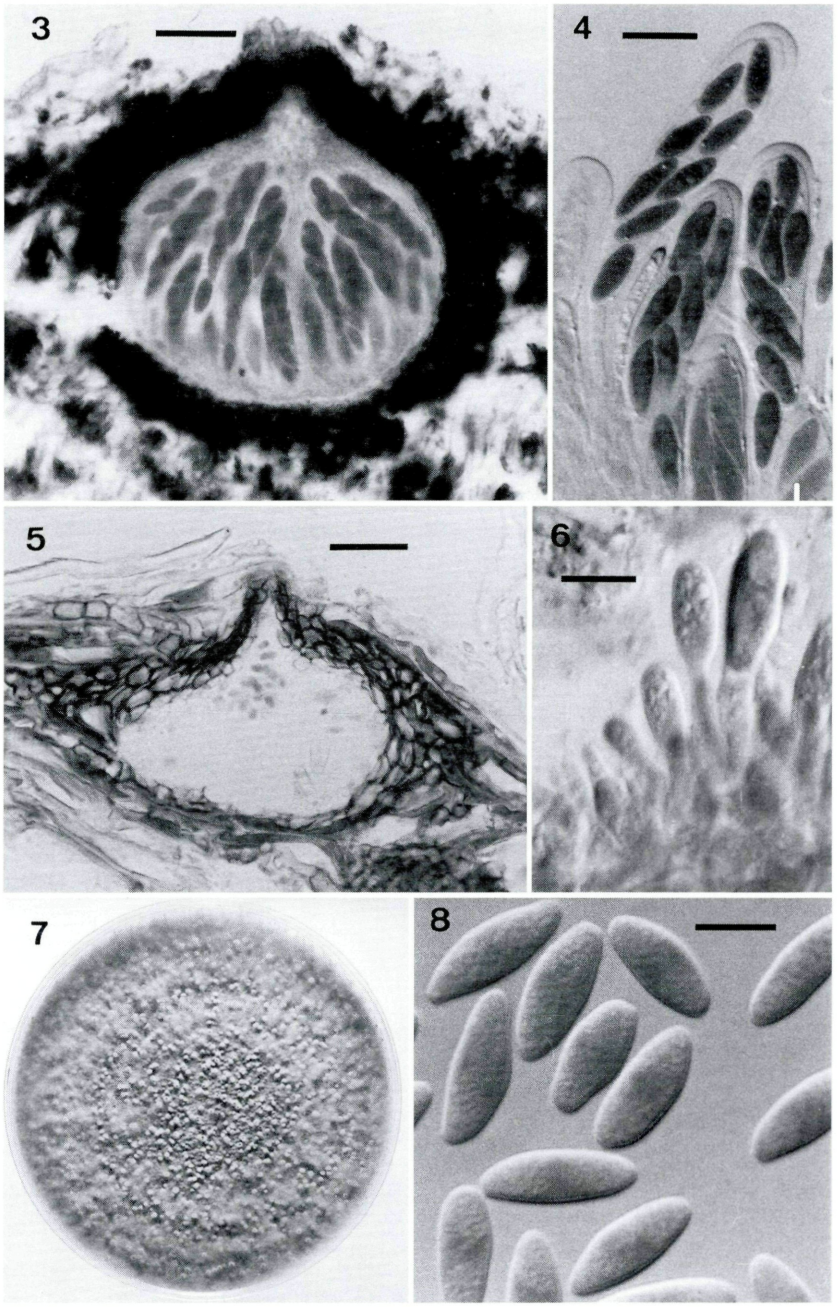
In addition to the morphological and cultural characters that define *F. luteum* we have shown that the sequence of the ITS2 region of the rDNA differed from other isolates in the *B. dothidea* complex. Thus, the isolates of *F. luteum* formed a clear group together with *F. luteum* strain 93.52 studied by Jacobs & Rehner (1998). These characters taken together support the contention that *F. luteum* is a species distinct from others in the *B. dothidea* complex. Some of the isolates studied here originated from single ascospores of a *Botryosphaeria* sp. that is considered to be the teleomorph of *F. luteum*. Since no teleomorph has been described for *F. luteum* it is described here as *Botryosphaeria lutea* sp. nov. In the absence of anamorphic characters collected from pure culture it would not be possible to distinguish *B. lutea* from other species in the *B. dothidea* complex.

Botryosphaeria lutea A. J. L. Phillips **sp. nov.** Figs 3–16.

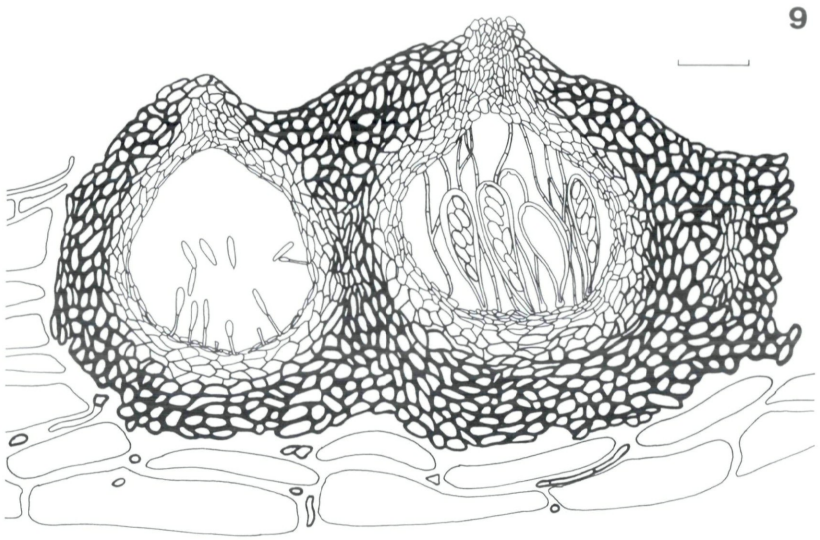
Stromata immersa dein per texturam hospitis erumpentia, nigra, <0.5 mm diam., uni- vel multiloculata. Loculi sphaerici vel ovati, ostiolati, 150–200 µm diam. Pseudothecia et conidiomata saepe consociata in eodem stromate. Pseudothecia collo brevi, per ostiolum periphysibus carens aperentia; paries e 8–12 stratis cellularum constans, textura angulari, ad 60 µm crassa. Asci bitunicati, cylindrici vel clavati, stipitati, 84–176 × 16–24 µm, octospori, pseudoparaphysibus filamentosis intersparsi. Ascosporae irregulariter biseriatae, ovales ad late fusiformes, guttulatae, laeves, aseptatae, hyalinae, basim et apicem obtusos versus angustatae, 18–22.5 × 7.5–12 µm. Pseudoparaphysae hyalinae, septatae, ramosae, 2–3.5 µm latae. *Botryosphaeriae dothideae* similis sed anamorphosi differens, coloniis in agaro pigmentum lutescens diffundentibus.

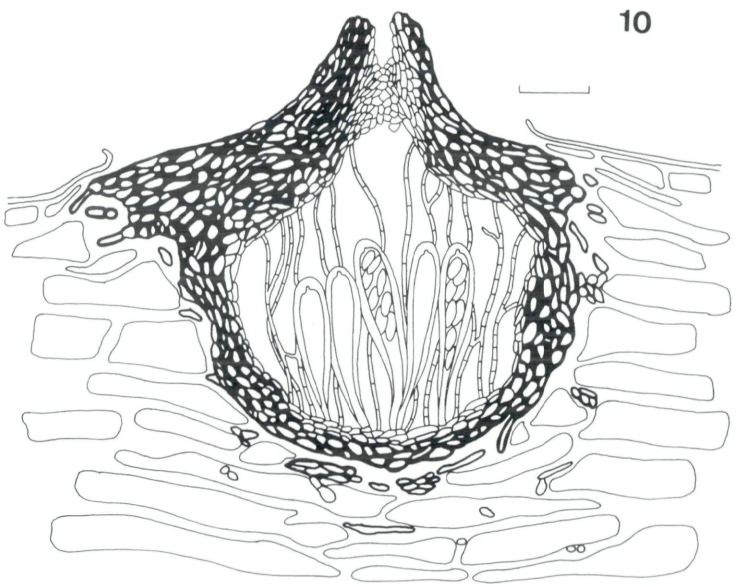
Anamorph. – *Fusicoccum luteum* Pennycook & Samuels (Pennycook & Samuels, 1985).

Stromata initially immersed, later becoming erumpent through the host tissue, black, <0.5 mm diam., uni- or multilocular, locules spherical to ovoid, 150–200 µm diam. Pseudothecia and conidiomata often formed in the same stroma. – Pseudothecia with a short neck, opening through a nonperiphysate ostiole, wall consisting of 8–12 layers of dark brown to black, thick-walled cells, forming pseudoparenchymatic *textura angularis*, up to 60 µm thick, with 3–4 layers of thin-walled, hyaline cells lining the cavity. – Asci bitunicate, cylindrical, to clavate, stipitate, 84–176 × 16–24 µm, 8-spored, associated with filamentous pseudoparaphyses. – Ascospores



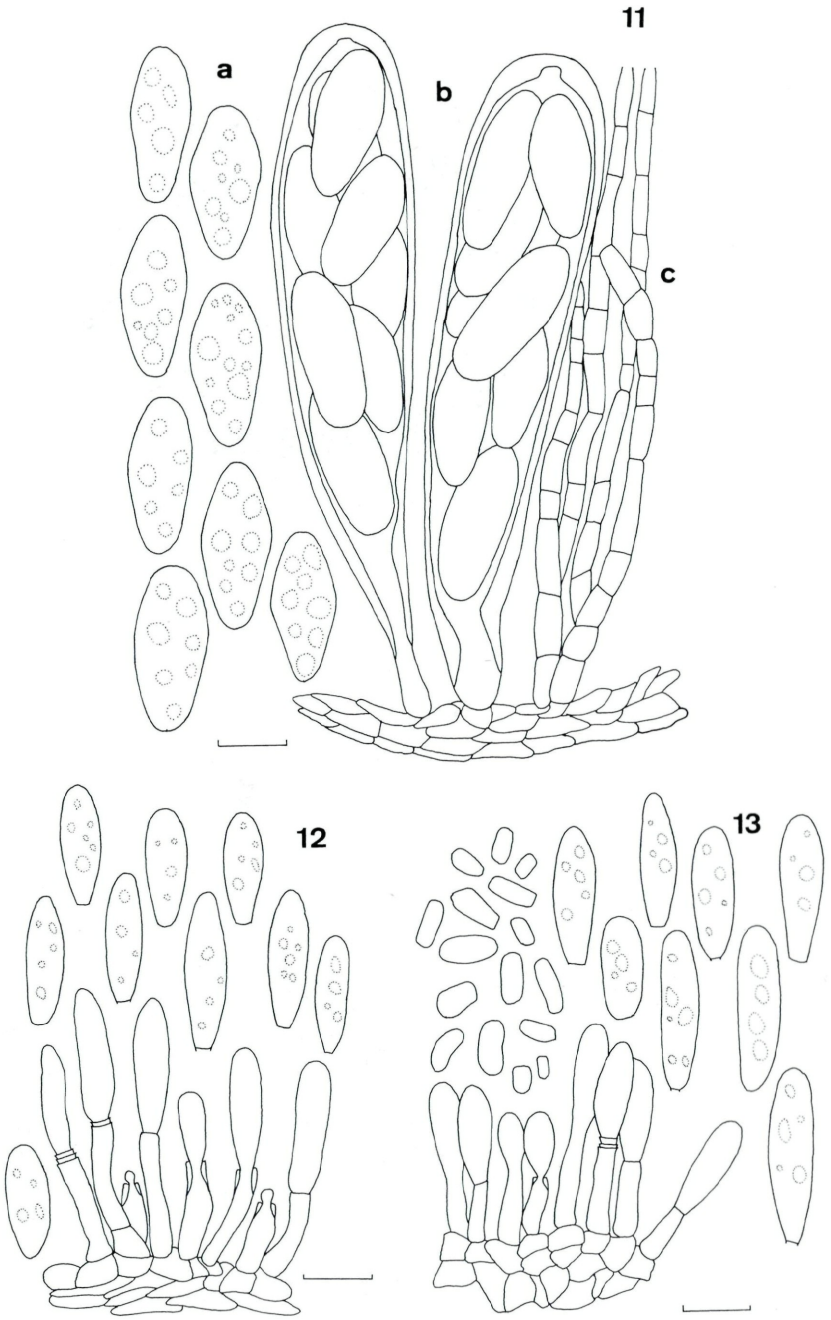
Figs 3–8. – *Botryosphaeria lutea* LISE 94070 and its anamorph *Fusicoccum luteum*. – 3. Longitudinal section through an ascoma on a grapevine cane. – 4. Asci and ascospores. – 5. Longitudinal section through a conidioma on a grapevine cane. – 6. Conidiophores with developing conidia. – 7. Pycnidia developed in culture on oatmeal agar. – 8. Conidia from oatmeal agar culture. – Bars = 50, 25, 50, 10; and 10 µm, respectively, in Figs 3, 4, 5, 6, and 8.



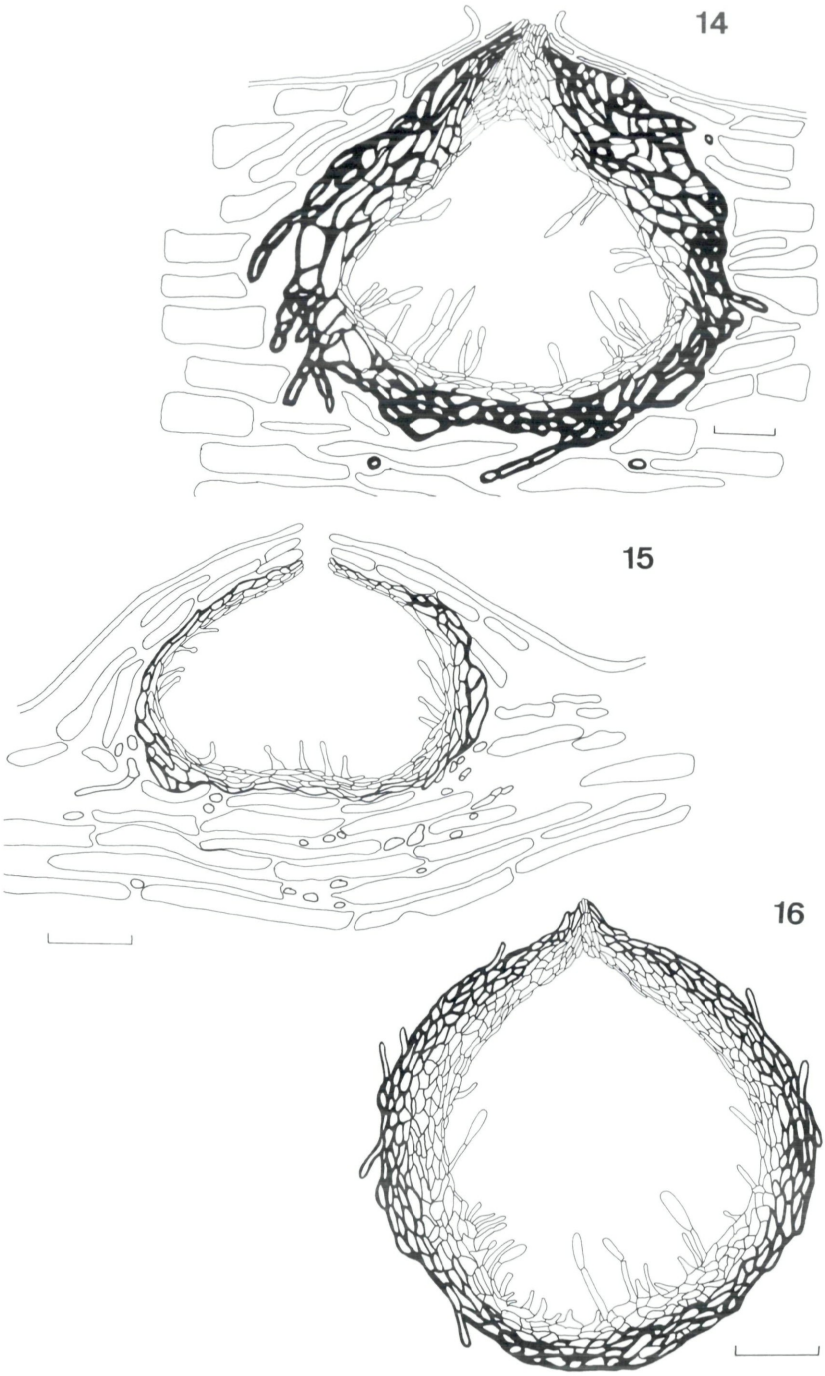


Figs 9–10. – *Botryosphaeria lutea* LISE 94070 on grapevine canes. – 9. Ascoma and conidioma formed in the same stroma. – 10. Unilocular ascoma. – Bars = 40 µm.

irregularly biseriate, hyaline, guttulate, smooth, aseptate, oval to broadly fusiform, widest in the middle or upper third of the ascospore, tapering to the obtuse base and apex 18–22.5(–24) × 7.5–12 µm. – Pseudoparaphyses hyaline, septate, branched, 2–3.5 µm wide. Similar to *B. dothidea* but differing in anamorphic characters



Figs 11–13. – *Fusicoccum luteum*. – 11. a. Ascospores, b. Asci, c. Pseudoparaphyses – 12. Conidiophores and conidia from a pycnidium formed on a grapevine cane (LISE 94070). – 13. Conidiophores, conidia and microconidia from oatmeal agar culture (CAP002). – Bars = 10 μm.



Figs 14–16. – Longitudinal sections through conidiomata of *Fusicoccum luteum*. – 14. From a grapevine cane (LISE 94070). – 15. From a grapevine leaf (LISE 94073). – 16. From oatmeal agar culture (CAP002). – Bars = 40, 20 µm, respectively, in Figs 14, 15–16.

formed in culture, of which the formation of a yellow pigment is the most discriminative.

The type of *F. luteum* is a dried culture deposited in the herbarium of the Plant Disease Division, DSIR, Aukland, New Zealand (PDD 45400) with ex-type cultures PDDCC 8004 and ATCC 58193. Since the anamorph has not been reported in nature, LISE 94070 is herein designated as an epitype. The following description is based on LISE 94070.

Conidiomata eustromatic, separate or confluent, dark brown to black, uni- or multilocular immersed in the host, sub-peridermal, locules up to 150 µm diam., walls consisting of a dark brown *textura angularis*, becoming smaller, thinner-walled and hyaline towards the conidiogenous region. – Ostioles papillate, circular. – Conidiomata frequently formed on the same stromata as the ascomata. – Conidiophores hyaline, smooth, thin-walled, rarely branched at the base, cylindrical, formed from the cells of the inner locule wall, 8–19 × 3–4 µm. – Conidiogenous cells discrete, integrated, hyaline, smooth, cylindrical, producing the first conidium holoblastically and subsequent conidia enteroblastically, proliferating percurrently with 2–3 indistinct percurrent proliferations, or determinate with typical phialides and periclinal thickening (*sensu* Sutton, 1980), (6–)8–16(–18) × (2.5–)3–4(–4.5) µm. – Conidia hyaline, thin-walled, aseptate, smooth, fusiform, widest in the middle or upper third of the conidium, apex sub-obtuse, base truncate (12–)16.5–22.5(–24) × 4.5–6(–7.5) µm (mean and standard deviation of 115 conidia = 17.2 ± 2.2), and length width ratio of 2.8 ± 0.5, often with a minute basal frill.

The form of the conidiomata varied according to the host substratum. Thus, on grapevine canes they were thick-walled, eustromatic, ostiolate (Fig. 14), while on leaves they were sub-epidermal, thin-walled, globose, ostiolate (Fig. 15).

Holotype. – PORTUGAL. ESTREMADURA: Oeiras, Quinta do Marquês, on cane of *Vitis vinifera* L. cv. Galego Dourado, March 1996, A. J. L. Phillips (LISE 94070, culture ex-type CAP002).

Additional specimens examined. – *Botryosphaeria lutea*: PORTUGAL. ESTREMADURA: Sintra, Magoito, cane of *V. vinifera*, April 1996, Luísa Castro Freitas (LISE 94071); Oeiras, Quinta do Marquês, on leaf of *V. vinifera* cv. Galego Dourado, April 1996, A. J. L. Phillips (LISE 94073, anamorph only); Oeiras, Rua Cândido dos Reis, on dead fruits of *Sophora japonica* L., May 1998, A. J. L. Phillips (HAP073).

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