The taxonomic status of *Macrophoma flaccida* and *Macrophoma reniformis* and their relationship to *Botryosphaeria dothidea*

Alan J. L. Phillips & Maria T. Lucas

Departamento de Fitopatologia, Estação Agronómica Nacional, 2780 Oeiras, Portugal

Phillips, A. J. L. & M. T. Lucas (1997). The taxonomic status of *Macrophoma flaccida* and *Macrophoma reniformis* and their relationship to *Botryosphaeria dothidea*. – Sydowia 49(2): 150–159.

Botryosphaeria dothidea and its anamorph *Fusicoccum aesculi* were associated with excoriose-type symptoms of grapevine canes, a disease attributed in the past to *Macrophoma flaccida*. Examination of herbarium material filed under *M. flaccida*, *M. reniformis* and *Phoma flaccida* revealed that these fungi would be better accommodated under *F. aesculi*.

Keywords: *Botryosphaeria dothidea, Fusicoccum aesculi,* grapevines, *Macrophoma flaccida, Macrophoma reniformis,* systematics.

Macrophoma flaccida (Viala & Ravaz) Cav. (syn. *Phoma flaccida* Viala & Ravaz) is a frequently reported associate of excoriose and false black rot of grapevines in France (Ravaz & Verge, 1925; Gaudineau, 1961; Bisson, 1965), Greece (Pantidou, 1973), Herzegovina (Radman, 1973), Portugal (d'Almeida & Prego, 1894; Dias & Lucas, 1980; Tomaz, 1985; Tomaz & Rego, 1990) and South Africa (Doidge & al., 1953). Although the pathogenic potential of *M. flaccida* has been questioned (e.g. Branas, 1967; Bugaret, 1987; Dias, 1980), Tomaz & Rego (1990) regarded it as the most important cause of excoriose in Portugal. Much of the controversy arises from the similarity of the symptoms caused by *M. flaccida* to those induced by *Phomopsis viticola* (Sacc.) Sacc. For many years *M. flaccida* was thought to be the main cause of excoriose in Europe while in the U.S.A. the cause was attributed to *P. viticola*. Indeed, many researchers considered them as separate diseases and they were known as European and American excoriose, respectively.

Phoma flaccida and *Phoma reniformis* Viala & Ravaz were first described on ripe grapes from the eastern Pyrenées, France (Viala & Ravaz, 1886a, 1886b). After Berlese & Voglino (1886) raised *Phoma* subgenus *Macrophoma* to generic rank as *Macrophoma* (Sacc.) Berl. & Vogl., Cavara (1888) transferred the names of the two species to *Macrophoma* as *M. flaccida* and *M. reniformis* (Viala & Ravaz) Cav.

In their comprehensive revision of *Macrophoma*, Petrak & Sydow (1927) transferred *M. reniformis* to *Dothiorella reniformis* (Viala & Ravaz) Petr. & Syd. According to Ainsworth (1961), however, the name *Dothiorella* is used in more than one sense. Therefore, we consider it best to avoid the use of this name until its status is clarified. The genus *Macrophoma* is also rather controversial and despite the work of Petrak & Sydow (1927), the name *Macrophoma* has often been used for *Phoma*-like species with relatively large conidia. As stated by Sutton (1980), many genera are available to accommodate the species that have been, and still are, described in *Macrophoma*. Furthermore, Sutton (1980) reduced *Macrophoma* to synonymy with *Sphaeropsis* Sacc. Since the fungus referred to as *M. flaccida* has thin-walled, hyaline, smooth conidia, in contrast to the thick-walled, internally ornamented ones characteristic of *Sphaeropsis*, it cannot be accommodated here. More recently, Zachos & Tzavella-Klonari (1980) considered that, because of the longitudinal and transverse septation in conidia of some of the isolates that they studied, *M. flaccida* should be re-classified as *Camarosporium flaccidum* (Viala & Ravaz) Zachos & Tzavella-Klonari. Judging from the photographs in their paper, however, only a small proportion of the conidia had this type of septation and we therefore consider this disposition to be questionable.

During the course of a study of grapevine diseases in Portugal, a coelomycete resembling *M. flaccida* was frequently found on mature grapevine canes with symptoms similar to those of excoriose. An ascomycete with characteristics of the genus *Botryosphaeria* Ces. & De Not. was also found on the same material. Given the controversy surrounding the genus *Macrophoma* (Sutton, 1980), the present study was initiated to determine the taxonomic status of *M. flaccida* and the correct identity of the fungus responsible for the excoriose-type symptoms on vines in Portugal.

Materials and methods

Grapevine canes with symptoms of excoriose were collected between January and May 1996. Ascomata and conidiomata were excised and crushed in a drop of sterile water on a flamed glass slide. A portion of the water was spread over a plate of 2% Difco malt extract agar (MA) and single ascospore and single conidium cultures were prepared from germinating spores. Preliminary identification of the fungi was by microscope examination of the crushed ascomata and conidiomata mounted in 100% lactic acid. For further diagnoses, ascomata and conidiomata were sectioned on a freezing microtome and the sections mounted in lactophenol containing 0.1% cotton blue. Dried herbarium material was rehydrated by soaking in

3% KOH, washed with water and then sectioned on a freezing microtome. All dimensions were determined at a magnification of × 1250 of material mounted in 100% lactic acid and 50 measurements were made for each preparation.

Isolates were cultured on Difco potato-dextrose agar (PDA) and oatmeal agar (OA) prepared as described by Anonymous (1968). Incubation was at 25° C with continuous light from daylight and near u.v. fluorescent tubes.

Specimens examined.

Macrophoma flaccida: On ripe berries of *Vitis vinifera*: Italy, P. A. Saccardo, PAD; Stradella, Italy Aug. 1887, F. Cavara, PC.

Macrophoma reniformis: On ripe berries of *Vitis vinifera*: Transcaucasia Telow 1897, A. von Jaczewski, K38226; Montubeccaria, Italy 1898, L. Montemartini, PAV; Stradella, Italy 1887, F. Cavara, PAV; Stradella, Italy 1887, F. Cavara, PC.

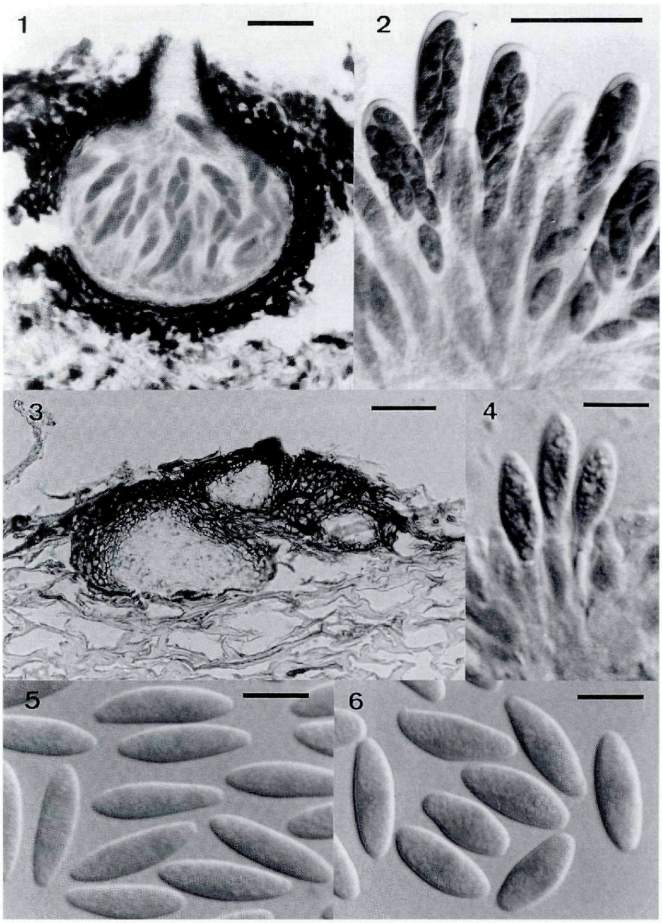
Phoma reniformis: On ripe berries of *Vitis vinifera*, Ponta Delgada, S. Miguel, Azores 1923, M. Bensaude, LISE 79207.

Botryosphaeria dothidea: On mature canes of *Vitis vinifera*: Parede Jan. 1963, B. d'Oliveira, LISE 62871; Quinta do Marquês, Oeiras Jan. 1996, A. J. L. Phillips, LISE 94069; Quinta do Marquês, Oeiras Mar. 1996, A. J. L. Phillips, LISE 94070; Sintra Apr. 1996, A. J. L. Phillips, LISE 94071; Alcobaça Apr. 1996, A. J. L. Phillips, LISE 94072; Montemor-o-Novo May 1996, A. J. L. Phillips, LISE 94074.

Results and discussion

The most conspicuous symptom on mature canes was a grey to white patch extending over three to four internodes and spotted with black fruit-bodies. Transverse and longitudinal cracks occurred mainly on the first and second internodes from the base of the canes and the epidermis flaked off readily. These symptoms are similar to those associated with excoriose on mature canes (Ravaz & Verge, 1925, 1928; Bugaret, 1987).

The general characters of the ascomycete on the diseased canes were those of the genus *Botryosphaeria*. Ascomata were immersed in the host (Fig. 1) and either separate or grouped in a stroma. Asci (Fig. 2), were bitunicate, 92–132 × 16–24 μm and the ascospores were thin-walled, hyaline, unicellular 15–28 × 6–12 μm. Characters of the ascomata (Fig. 1), asci and ascospores (Fig. 2) and their dimensions were similar on material from each locality and best fitted those ascribed to *Botryosphaeria dothidea* (Moug. : Fr.) Ces. & De Not. (Pennycook & Samuels, 1985). Although some researchers regard *B. dothidea* and *Botryosphaeria ribis* Gross. & Dugg. as two separate species (e.g. Punithalingam & Holliday, 1973; Rumbos, 1987; Ramos & al., 1991) others regard them as synonyms (e.g. Witcher & Clayton, 1963; Maas & Uecker, 1984; Michailides, 1991). However, the synonymy proposed by von Arx & Müller (1954) will be followed here.



Figs. 1–6. – *Botryosphaeria dothidea* on grapevine canes. – 1. Ascoma of LISE 94072. – 2. Asci. of LISE 94070. – 3. Eustromatic conidioma of LISE 94070. – 4. Conidiogenous cells and developing conidia of LISE 94070. – 5. Conidia of LISE 94072. – 6. Conidia of LISE 94070. – All material mounted in lactophenol with cotton blue. – Scale bars: Figs 1–3 = 50 µm, Figs 4–6 = 10 µm.

The forms of conidiomata on the host ranged from eustromatic, multilocular (Fig. 3), to unilocular structures that could be interpreted as being pycnidial. Conidiogenesis was usually holoblastic (Figs. 4 and 7), but 'phialides' (*sensu* Sutton, 1980) were also seen in some preparations. Conidia were hyaline, aseptate, fusiform or fusiform elliptical to elliptical, 15.0–22.5 × 4.5–7.5 µm (Figs 5–7). In most collections the base of each conidium was distinctly truncate (Fig. 5), but in others some conidia were rounded at both ends (Fig. 6). Pale brown conidia with one or two septa were sometimes seen in older cultures.

Cultures prepared from single ascospores were identical with those from single conidia, thus tending to confirm the connection between the anamorph and the teleomorph. On PDA, colonies were initially white but gradually became darker with dense aerial mycelium. The reverse of most colonies was initially white, but gradually turned dark olive green from the centre. This colouration spread radially to the edge of the colony and ultimately turned black. On OA, conidiomata were partially immersed in the medium, globose with a short papilla and covered with olive green to buff hyphae. Conidia were exuded either in a cirrhus or oozed from the ostiole within 10 days of their formation, and sometimes after as short a period as 5 days. Conidiogenesis in culture was holoblastic and the conidia were fusiform with a truncate base 13.5–24 × 4.5–7.5 µm (Fig. 7).

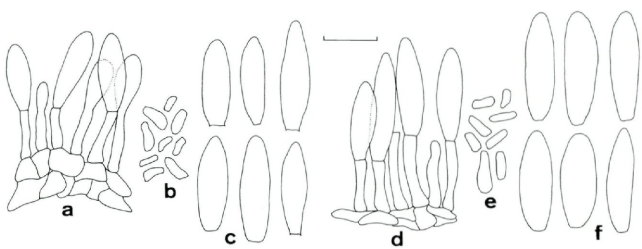


Fig. 7. – *Botryosphaeria dothidea*, LISE 94070: a. conidiogenous cells, b. microconidia, c macroconidia from conidiomata on grapevine canes; d. conidiogenous cells, e. microconidia, f. macroconidia from conidiomata on oatmeal agar cultures. – Scale bar = 10 µm.

Characters of the anamorph on the host and in culture fall within the range applied to the *Fusicoccum* Corda anamorph of *B. dothidea* which is often referred to *Fusicoccum aesculi* Corda (Pennycook & Samuels, 1985). These characters are listed in Tab. 1. Although the conidia were somewhat shorter than those described by

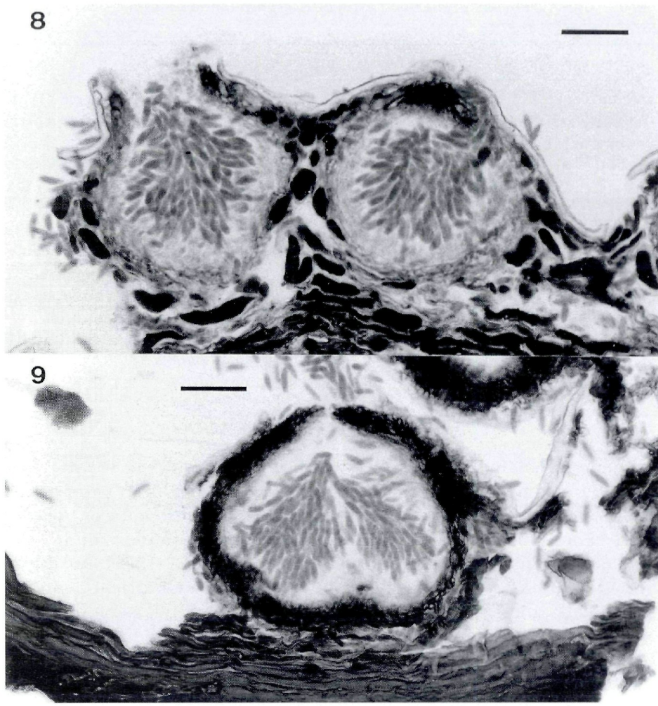
Tab. 1. – Characteristics of the anamorph of *Botryosphaeria dothidea* reported here and in previous studies.

Conidiomata	Conidiogenesis	Conidia (µm)	Reference
Pycnidial		16.0–25.0 × 4.5–7.5	Grossenbacher & Duggar (1911)
Stromatic		18.0–31.0 × 4.5–8.0	Grossenbacher & Duggar (1911)
Eustromatic	Holoblastic	18.0–25.0 × 4.0–4.5	Sutton (1980)
	Holoblastic and phialidic	20.0–26.0 × 5.0–6.0	Pennycook & Samuels (1985)
Eustromatic	Holoblastic	14.0–23.0 × 3.0–4.5	Morgan-Jones & White (1987)
Pycnidial	Holoblastic and phialidic	16.5–20.2 × 6.3–8.0	Maas & Uecker (1984)
Eustromatic	Holoblastic and phialidic	15.0–22.5 × 4.5–7.5	This study
Pycnidial	Holoblastic	15.0–25.5 × 4.0–6.0	*Macrophoma flaccida* PAD
Pycnidial	Holoblastic	15.0–21.0 × 4.5–6.0	*Macrophoma flaccida* PC
Pycnidial	Holoblastic	16.0–25.5 × 4.5–7.0	*Macrophoma reniformis* PAV
Pycnidial	Holoblastic	16.5–25.5 × 4.5–6.0	*Macrophoma reniformis* PC
Pycnidial	Holoblastic	12.0–21.0 × 4.5–6.0	*Macrophoma reniformis* K38226
Eustromatic	Holoblastic	16.0–25.5 × 4.5–6.0	*Phoma reniformis* LISE 79207

Pennycook & Samuels (1985), they fall within the range reported by Maas & Uecker (1984) and Morgan-Jones & White (1987). The current concept of *F. aesculi* encompasses fungi with conidiomata of various forms ranging from unilocular pycnidia to quite complex multilocular, eustromatic structures (Morgan-Jones & White, 1987); the first-formed conidia are produced holoblastically on cylindrical conidiogenous cells and the conidia are thin-walled, hyaline, aseptate, fusiform with a distinctly truncate base (Pennycook & Samuels, 1985). Dimensions of conidia are quite variable (Tab. 1). According to Morgan-Jones & White (1987), microconidia have not been recorded in *F. aesculi* but are present in the *Fusicoccum* anamorph of *B. ribis*. In the present work, microconidia were found in some isolates but were absent from others, although the teleomorphs could not be separated from one another. Pennycook & Samuels (1985) also reported microconidia in the isolates of *B. dothidea* that they studied.

The state of confusion surrounding the taxonomy of the *Fusicoccum* anamorph of *B. dothidea* has been discussed critically and in detail by Sutton (1980), Pennycook & Samuels (1985) and Morgan-Jones & White (1987) and will not be repeated here. However, following the concept of *F. aesculi* discussed by Pennycook & Samuels (1985), we are satisfied that the fungus with which we are dealing is *F. aesculi*, the anamorph of *B. dothidea*.

The similarity of this fungus to the one described as *P. flaccida* (Viala & Ravaz, 1886a) and *M. flaccida* (Cavara, 1888) prompted us to examine material filed under *P. flaccida*, *M. flaccida* and *M. reniformis*. Unfortunately, the types of *P. flaccida* and *P. reniformis* could



Figs. 8–9. – 8. Conidiomata of *Macrophoma flaccida* ex PC. – Scale bar = 50 µm. – 9. Conidioma of *Macrophoma reniformis* ex PC. – Scale bar = 50 µm.

not be located. However, we examined Saccardo's specimen of *M. flaccida* and Cavara's specimens of *M. flaccida* and *M. reniformis*. The specimens of *M. flaccida* and *M. reniformis* in PC and *M. reniformis* in PAV were identified by Cavara and so they represent his interpretation of these two species. Conidiomata in all these samples were unilocular pycnidial (Figs 8 and 9) as opposed to the eustromatic ones we found on vine canes. Conidia were produced holoblastically on cylindrical conidiogenous cells (Fig. 10) and were hyaline, smooth, aseptate, fusiform-elliptical with the base distinctly truncate. Dimensions of the conidia were within the range of those reported for the anamorph of *B. dothidea* (Tab. 1). Although the pycnidial conidiomata contrasted with the eustromatic conidiomata

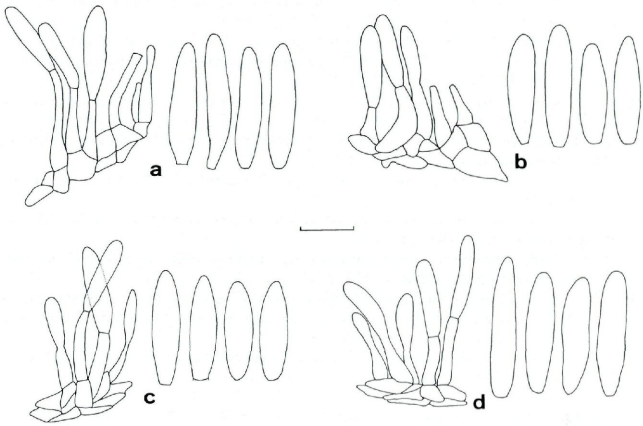


Fig. 10. – Conidia and conidiogenous cells of a. *Macrophoma flaccida* ex PAD; b. *Macrophoma reniformis* ex PAV; c. *Macrophoma flaccida* ex PC; d. *Macrophoma reniformis* ex PC. – Scale bar = 10 μm.

that we found on the grapevine canes, previous studies on the anamorph of *B. dothidea* have shown that the form of conidiomata is influenced by the substrate on which they are produced. This is clearly illustrated in the work of Maas & Uecker (1984). Characters of all the specimens examined (Tab. 1) correlated with the current concept of the *Fusicoccum* anamorph of *B. dothidea* (Pennycook & Samuels, 1985). On the basis of the material available, this study has shown that *M. flaccida* cannot be distinguished from the *Fusicoccum* anamorph of *B. dothidea* and should be called *F. aesculi*.

Acknowledgments

We thank the Director, Estação Agronómica Nacional for providing facilities for this work and the various herbaria mentioned for the loan of material. Dr. J. Mouchacca helped to locate some of the material studied and provided a copy of Viala & Ravaz's paper. Dr. G. J. Samuels gave valuable comments on an earlier draft of this paper and J. Gonçalves Passos prepared the photographs. A. J. L. Phillips was supported by a grant from Junta Nacional de Investigação Cientifíca e Tecnológica under the programme PRAXIS XXI, BCC/6412/95.

References

Ainsworth, G. C. (1961). Ainsworth & Bisby's Dictionary of the Fungi. Fifth Edition. – C.M.I., Kew, England. 547 pp.

Almeida, V. d' & J. M. Prego (1894). Les maladies de la vigne en Portugal pendant l'année 1894. – Bull. Soc. mycol. Fr. 10: 170–182.
Anonymous (1968). Plant Pathologist's Pocketbook. – C.M.I., Kew, England. 267 pp.
Arx, J. A. von & E. Müller (1954). Die Gattungen der amerosporen Pyrenomyceten. – Beitr. Kryptfl. Schweiz 11: 1–434.
Berlese, A. N. & P. Voglino (1886). Sopra un nuovo genere di funghi sferopsidei. – Atti Soc. Venet.-Trent. Sc. nat. 10: 176.
Bisson, J. (1965). Observations de l'excoriose de la vigne dans le Centre en 1963 et 1964. – Progrès agric. vitic. 82: 52–61.
Branas, J. (1967). Sur l'excoriose, sa cause et ses traitement. – Progrès agric. vitic. 84: 295–309.
Bugaret, Y. (1987). Comment lutter efficacement contre l'excoriose de la vigne. – Progrès agric. vitic. 104: 121–126.
Cavara, F. (1888). Intorno al disseccamento dei grappoli della vite. – Atti Ist. bot. Univ. Lab. crittogam. Pavia 1: 313–317.
Dias, M. R. de S. (1980). *Phomopsis viticola* (Sacc.) Sacc., fungo responsável pela "excoriose" da videira. – Proceedings I Congresso Português de Fitiatria e Fitofarmacologia, 15–19 Dez. 1980, pp 175–192.
—— & M. T. Lucas (1980). Fungi of Madeira and Selvagem Islands. – Bol. Soc. Brot. 53: 469–476.
Doidge, E. M., A. M. Bottomley, J. E. van der Plank & G. D. C. Pauer (1953). A revised list of plant diseases in South Africa. – Sci. Bull. Dep. Agric. S. Afr. 346: 1–122.
Gaudineau, M. (1961). Esca, excoriose, anthracnose de la vigne. – Bull. Off. int. Vin 34: 21–32.
Grossenbacher, J. G. & B. M. Duggar (1911). A contribution to the life history, parasitism, and biology of *Botryosphaeria ribis*. – Tech. Bull. N.Y. St. agric. Exp. Stn Geneva 18: 114–188.
Maas, J. L. & Uecker, F. A. (1984). *Botryosphaeria dothidea* cane canker of thornless blackberry. – Pl. Dis. 68: 720–726.
Michailides, T. J. (1991). Pathogenicity, distribution, sources of inoculum, and infection courts of *Botryosphaeria dothidea* on pistachio. – Phytopathology 81: 566–573
Morgan-Jones, G. & J. F. White Jr. (1987). Notes on coelomycetes. II. Concerning the *Fusicoccum* anamorph of *Botryosphaeria ribis*. – Mycotaxon 30: 117–125.
Pantidou. M. E. (1973). Fungus-host index for Greece. – Benaki Phytopathological Institute, Kiphissia, Athens. 382 pp.
Pennycook, S. R. & G. J. Samuels (1985). *Botryosphaeria* and *Fusicoccum* species associated with ripe fruit rot of *Actinidia deliciosa*, kiwifruit in New Zealand. – Mycotaxon 24: 445–458.
Petrak, F. & H. Sydow (1927). Die Gattungen der Pyrenomyzeten, Sphaeropsideen und Melanconieen. – Repert. Spec. nov. Regni. veg. 42: 1–551.
Punithalingam, E. & P. Holliday (1973). *Botryosphaeria ribis*. Descriptions of Plant Pathogenic Fungi and Bacteria, No. 395. – C.M.I., Kew, England.
Ramos, L. J., S. P Lara, R .T. McMillan Jr., & K. R. Narayanan (1991). Tip dieback of mango (*Mangifera indica*) caused by *Botryosphaeria ribis*. – Pl. Dis. 75: 315–318.
Radman, L. (1973). Contribution à l'étude de l'excoriose de la vigne en Hercegovine. – Zašt. Bilja 24: 299–309.
Ravaz, L. (1929). Chronique – l'excoriose. Progrès agric. vitic. 46: 77–83.
—— & Verge, G. (1925). Sur une maladie de la vigne, l'excoriose. – C. R. Séanc. Acad. Sci. 180: 313–315.
—— & G. Verge (1928). L'excoriose. – Progrès agric. vitic. 45: 450–458.
Rumbos, C. (1987). Twig and branch dieback of walnut trees induced by *Botryosphaeria ribis*. – Pl. Path. 36: 602–605.

Sutton, B. C. (1980). The Coelomycetes. – C.M.I., Kew, England.
Tomaz, I. L. (1985). Doenças do lenho, grandes responsáveis do declínio das videiras. – Gazeta das Aldeias 2915: 14–19.
—— & M. C. N. F. Rego (1990). Fungos do complexo responsável pelo declínio das videiras em Portugal. – Vida Rural 1493: 12–20.
Viala, P. & L. Ravaz (1886a). Mémoire sur une nouvelle maladie de la Vigne: le Black-Rot. – Bureaux du Progrès Agricole et Viticole, Montpellier.
—— & —— (1886b). Sur des nouvelles espéces du genre *Phoma* se développant sur les fruits de la vigne. – Bull. Soc. bot. Fr. 33: 61–67.
Witcher, W. & C. N. Clayton (1963). Blueberry stem blight caused by *Botryosphaeria dothidea* (*B. ribis*). – Phytopathology 53: 705–712.
Zachos, D. G. & K. Tzavella-Klonari (1980). Recherches sur les caractères et la position systématique du champignon qui cause la maladie de la vigne connue sous le noms «Faux Black-Rot» et «Excoriose». – Annls Inst. phytopath. Benaki. N. S. 12: 208–215.

(Manuscript accepted 3rd May 1997)