THE CHEMOTAXONOMY OF THE FUNGAL GENUS ENDOTHIA FR.

by

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(ABSTRACT)

The taxonomic status of the fungal genus *Endothia* was recently changed in a controversial monograph by Barr (Barr, M. E. 1978. The Diaporthales of North America. Mycol. Mem. 7. J. C. Cramer. 232 p.), who divided the genus into two separate genera, *Endothia* and *Cryphonectria*, based on differences in ascospore shape and septation, stromatic configuration and distribution of stromatic tissues. This group of fungi traditionally contains some important plant pathogens; its taxonomic position needs to be resolved. The morphological criteria used by Barr were re-investigated. Polyacrylamide gel electrophoresis and fungicide sensitivity assays were also used to examine biochemical relationships among the organisms and to establish additional means of distinguishing among the closely related taxa.

The morphological features of 12 species of *Endothia* were examined. Those species with 2-celled, ovoid ascospores produced valsoid stromata, while organisms associated with nonseptate, allantoid ascospores formed diatrypoid stromata. Pseudo-parenchymatous tissue was observed along the edge of the stroma, while prosenchyma was confined to the stromatic center. The

major criteria used by Barr were confirmed.

Polyacrylamide gel electrophoresis was used to separate the buffer-soluble proteins of 78 isolates, representing 13 species of Endothia and Cryphonectria cubensis. Intraspecific variation of banding patterns was less than interspecific differences; the species were separated by this technique. The banding patterns of E. eugeniae isolates closely resembled those of C. cubensis; these organisms may be conspecific. Hypovirulent isolates of E. parasitica could not be distinguished from wild isolates. The banding patterns of specific isozymes were species specific; the use of isozyme analysis has great potential for future taxonomic and genetic studies.

The sensitivities of E. parasitica and E. gyrosa were determined for 23 different fungitoxicants. The two species were differentially sensitive to cycloheximide, with ED_{50} values of 0.01 - 0.03 µg/ml and 1.0 - 2.0 µg/ml for E. gyrosa and E. parasitica respectively. Differential sensitivities were not exhibited toward the remaining fungitoxicants; these fungi probably share many biochemical processes and response mechanisms.

Barr's classification system is technically correct and it seems to organize relationships within the entire order in a uniform manner. Its adoption is recommended with some hesitation since the influence of host on stromal development is not fully understood.

The classification of the Ascomycetes constitutes the most difficult taxonomic problem in mycology. The large number of species and the morphological complexities and variations in the Ascomycetes have led to an understandable hesitancy on the part of the majority of mycologists to accept revisions of the class which attempt to incorporate the meager data that have been obtained into the system of classification.

E. S. Luttrell, Taxonomy of the Pyrenomycetes. 1951.

"Grau, teurer Freund, ist alle Theorie

Und grün des Lebens goldner Baum."

Goethe, Faust.

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provided technical assistance throughout the study and remains a good and loyal friend.

first opened my eyes to the natural world and taught me to appreciate the intricacies of nature. My parents,

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CHAPTER 1

INTRODUCTION

The fungal genus Endothia Fr. is one of the most notorious groups of plant pathogens in the history of phytopathology. This is due to Endothia parasitica (Murr.) P. J. & H. W. And., the causal agent of chestnut blight. Since its introduction to the United States in the early 1900's, E. parasitica has virtually destroyed the American chestnut, Castanea dentata Borkh., and has vastly changed the composition of the eastern hardwood forest. Endothia parasitica is perhaps the only plant pathogen which has brought a host species close to extinction (81, 193).

Endothia parasitica, however, is not the only species of Endothia; thirteen species are currently recognized throughout the world (15). The genus is presently placed in the Diaporthales of the Ascomycotina (124). Its principal morphological characteristics include the production of yellow, orange or red stromata (green in one species) which are subcortical to erumpent and which contain pycnidia and perithecia. Pycnidia consist of simple cavities to complex, labyrinthiform chambers; conidia are produced by conidiophores which cover the surface of the chambers. The minute conidia accumulate within the pycnidial chambers and are bacilliform to oblong in shape. The perithecia are usually deeply immersed within the stroma, while the perithecial necks grow up to the stromal surface. Asci are clavate to oblong

fusoid and usually contain eight ascospores. Paraphyses are absent. Ascospores are hyaline, one- or two-celled and oblong fusoid, subellipsoid to cylindrical or allantoid-cylindric in shape (150, 168). Species are traditionally differentiated by the size and color of the stroma and the size, shape and degree of septation of the ascospores (93, 150, 168).

The genus Endothia was first erected by Fries in 1849 based on collections made by Schweinitz in North Carolina and New England. Schweinitz described these specimens under the name Sphaeria gyrosa in 1822 (160), and Endothia gyrosa (Schw.) Fr. became the type species of the genus (168). In 1832, Schweinitz (161) also recognized a second species, Sphaeria radicalis; the differences between these two species were not well understood. Schweinitz did not include microscopic characteristics in his descriptions and most of the specimens in his herbarium collections did not contain perfect stages of the fungi (60). The true identities of these native species of Endothia became quite important after the introduction of E. parasitica, for it was not known whether the disease was caused by an introduced pathogen or by environmental stress which predisposed the chestnuts to colonization by native species of Endothia (59). The ambiguity of the early nomenclature was traced by Farlow (59, 60) and was finally resolved in a monograph by Shear, Stevens and Tiller (168).

The virulence of E. parasitica to the American chestnut is

not typical of the genus; most of the other species of Endothia are opportunistic pathogens or saprophytes. Of the four additional species of Endothia indigenous to temperate North America, only E. gyrosa is associated with a specific plant disease, pin oak blight. This disease was first described in southeastern Virginia in 1971 (196) and has been shown to be water stress-related (13,14). The fungus is most frequently associated with pruned branch stubs and exposed, wounded roots on pin oak, Quercus palustris Muenchh; stromata have also been observed in the crown and on the bole of severely affected trees. Appel (13) and Appel and Stipes (14) have demonstrated that the rate of canker development is dependent upon the degree of water stress to which the tree is subjected; colonization is greatly restricted in well watered trees. Endothia gyrosa has also been reported to cause a serious canker disease on Formosan sweetgum, Liquidambar formosana Hance in the southern United States; as with pin oak, infection is most frequently associated with pruned branch stubs (187). Other hosts of E. gyrosa include various species of Quercus L., Acer saccharinum Mill., Liquidambar styraciflua L., Fagus grandifolia Ehrh., Fagus sylvatica L., Castanea dentata, Ilex opaca Ait, Ulmus americana L. and Vitis L. sp. (168, 195). The remaining species of Endothia distributed within the temperate United States are collected infrequently and are presumably saprophytic. Endothia radicalis (Schw.) Ces. and DeNot. [= E. fluens (Sow.) Shear and Stevens] was frequently reported on the exposed roots of large specimens of American

chestnut before their death due to E. parasitica. Other hosts include several species of oak in the United States; in Europe it has been reported on Quercus pedunculata Ehrh., Castanea sativa Mill., Alnus glutinosa (L.) Gaertn., Ulmus campestris L., Carpinus betula L. and Corylus sp., while in Japan it has been found on oriental species of Castanea Mill. and Pasania (Miq.) Oerst. (168). Fresh specimens have not been collected in the United States in recent years, and only a few isolates have been located in Europe. It is unknown whether the fungus was unable to compete with E. parasitica, whether its habitat was destroyed with the death of the large American chestnuts or whether its presence is simply masked by the overwhelming numbers of E. parasitica stromata (S. L. Anagnostakis, personal communication).

Endothia singularis (H. and B. Syd.) Shear and Stevens is another native species of Endothia; it has been collected on various oaks in Colorado. Morphologically this fungus resembles E. gyrosa except for its much larger stromata (168). Endothia viridistroma Wehmeyer, which was collected on Cercis canadensis L. in Athens, Georgia in 1934, is the only species of Endothia with green stromata. Wehmeyer (207) placed this species in Endothia because of its other morphological characteristics. Additional specimens have not been collected.

Other species of Endothia are distributed throughout the tropical and subtropical regions of the world; several of these are suspected of causing economically important diseases.

Endothia havanensis Bruner was first described in Cuba in 1916 (31). It was discovered on several species of Eucalyptus L'Herit., Persea gratissima Gaertnif., Mangifera indica L. and Spondias mombin L. where it appeared to be growing as a saprophyte or weak parasite. In 1968, E. havanensis was suspected of causing a serious canker disease among Eucalyptus grandis Hill ex Maiden and Eucalyptus saligna Sm. in plantations in Surinam (12, 23). After the appearance of this disease in Brazil, Hodges (84) questioned the identity of this fungus based on its morphology. He believed that the eucalyptus canker fungus was actually Diaporthe cubensis Bruner, which he renamed Cryphonectria cubensis (Bruner) Hodges (84). This organism was originally described in an obscure Cuban journal in 1917 and was mistaken for E. havanensis by Boerboom and Maas (23). Cryphonectria cubensis is highly pathogenic to eucalyptus and causes significant yield reductions throughout the tropics (15, 83). This fungus is not an opportunistic pathogen; the disease is more akin to chestnut blight than to the other diseases caused by species of Endothia (4). Endothia havanensis is still a valid species, however. Davidson and Tay (44) recently isolated it from Eucalyptus marginata Donn ex Sm. and Eucalyptus calophylla R. Br. in western Australia where it is frequently associated with frost damage.

Another tropical species of Endothia associated with a specific disease is Endothia eugeniae (Nutman and Roberts) Reid and Booth. This fungus was originally classified as

Cryptosporella eugeniae Nutman and Roberts, and was later transferred to Endothia (142) based on its morphological characteristics. Endothia eugeniae is responsible for "acute dieback" of cloves, Syzygium aromaticum (L.) Merr. and Perry, in Zanzibar, Tanzania. Once again, the fungus enters the plant through wounds which are often formed during harvest of the flower buds. Branches die back to the trunk, while the leaves turn a distinctive russet color and are retained. Root injuries and trunk wounds can also lead to infection (129, 130). Hodges (personal communication) and Alfenas (personal communication) believe that E. eugeniae is conspecific with C. cubensis on the basis of cross-inoculation studies and isozyme analysis.

Other tropical species of Endothia do not appear to be pathogenic. Endothia longirostris Earle was originally collected on the "bark of fallen log in wet woods" in Puerto Rico (53). It has also been found in French Guiana but has never been associated with a specific host (168). Kobayashi (93) considered it to be synonymous with E. radicalis, but this was rejected by Roane and Stipes (148, 150) on the basis of pigment content and quantitative characters which allow the separation of taxa by numerical analysis. Endothia coccolobii Vizioli was originally described on fallen green fruits of the sea grape, Coccolobis uvifera (L.) Jacq. (204), while E. tropicalis (Berk. and Br.) Shear and Stevens was found on rotten logs and stumps of Elaeocarpus glandulifer Mast. in Hook. in Ceylon (present-day Sri

Lanka) (168). Kobayashi and Ito (94) considered E. tropicalis to be synonymous with E. havanensis with the latter species maintaining priority. Roane and Stipes (150) deferred judgement on this matter due to a lack of fresh material.

Three additional species of Endothia are known only in Japan; all are presumably saprophytic. Endothia japonica Kobayashi and Ito was found on cut branches and bark of dead specimens of Quercus mongolica var. grosseserrata (Bl.) Rehd., Quercus glandulifera Bl. and Quercus acutissima Carr. It is quite similar to E. tropicalis but contains larger asci and ascospores (95). Endothia macrospora Kobayashi and Ito was discovered on the bark of dead specimens of Castanopsis cuspidata var. sieboldii (Makino) Nakai and produces the largest asci and ascospores in the genus (94, 95). Endothia tetraspora Kobayashi produces only four ascospores per ascus; it was collected on dead twigs of Carpinus yedoensis Maxim. (92). A complete description of all the species of Endothia found in Japan is presented by Kobayashi (93).

Several other representatives of the genus have been described but are no longer considered valid; these species were discussed by Roane and Stipes (150). Endothia sordida Fuckel, which was associated with Agave L. sp. in the southwestern United States, was removed from Endothia because of its brown stromata and large, brown, guttulate ascospores. Endothia nitshkei (Otth.) has also been removed from the genus due to its lack of stromal development, converging perithecial necks and absence of

alcohol-soluble pigments. A final species, Endothia parryi Farl., was reassigned to the genus Dothidella Speg. by Theissen and Sydow in 1915.

In 1978, a major revision of the genus was made by Barr (16) in her monograph on the Diaporthales; the genus was partitioned into two separate genera belonging to two different families. The characteristics used by Barr include the stromatic tissue texture, stromal configuration and the shape and degree of septation of the ascospores. Under this system, the genus Endothia was placed in the Gnomoniaceae and was restricted to those fungi with brightly colored pseudoparenchymatous stromata, a diatrypoid configuration and one-celled, allantoid ascospores. Species which possess these characteristics are: E. gyrosa (the type species), E. singularis and E. viridistroma. The majority of the fungi that were traditionally included in Endothia were transferred to the genus Cryphonectria Sacc. of the family Valsaceae. These fungi are distinquished by prosenchymatous stromata, a valsoid configuration and two-celled, ellipsoid or ovoid ascospores. The new species designations are: Cryphonectria gyrosa (Berk. and Br.) Sacc. (=E. tropicalis Shear and Stevens) which is the type species, Cryphonectria parasitica (Murr.) Barr, comb. nov., Cryphonectria radicalis (Schw. ex Fries) Barr, comb. nov., Cryphonectria havanensis (Bruner) Barr, comb. nov., Cryphonectria nitschkei (Otth.) Barr, comb. nov. (= E. japonica Kobayashi and Ito) and Cryphonectria macrospora (Kobayashi and Ito) Barr, comb.

nov. Endothia tetraspora was transferred to the genus Cryptosporella Sacc. of the Melanconidaceae because of its one-celled, ellipsoid fusoid, thick-walled ascospores. Several other current species of Endothia were not mentioned by Barr but are presumably reassigned to Cryphonectria because of their morphological characteristics. These include E. eugeniae, E. longirostris and E. coccolobii. The eucalyptus canker fungus, which was formerly Diaporthe cubensis, has been renamed Cryphonectria cubensis (84).

Barr's revision greatly changed the taxonomic position of these organisms. This new classification system has not been universally accepted, although it is being used by some researchers. The objective of this study was to reexamine and clarify the relationships among the fungi which have traditionally been placed in the genus Endothia. This problem was approached by three different techniques:

A) The morphology of the different species were reexamined to test the accuracy of Barr's observations;

B) Polyacrylamide gel electrophoresis (PAGE) was used to determine whether the profiles of buffer-soluble proteins were species specific and whether they could be used to study interspecific relationships; and

C) The reactions of the fungi to a variety of antibiotic and xenobiotic agents were studied to determine whether differential sensitivities to these compounds could be used to investigate the relationships among the species.

Endothia nomenclature will follow that of Roane and Stipes (150). The taxon *Cryphonectria cubensis* is firmly established in the literature (3, 4, 15, 83) and will be utilized. Preliminary findings have been reported (110-119).

CHAPTER 2

Morphology of the genus Endothia.

LITERATURE REVIEW

The first major contribution to the taxonomy of the genus Endothia was published in 1917 by Shear et al (168). Prior to this work, the taxonomy of the genus was in a state of disarray due to the inadequacy of the original descriptions and the loss of much of the type material upon which the various species were based. Extreme confusion existed over the identities of the native American species of Endothia (10, 166, 167) and the relationship of the causal agent of chestnut blight to these organisms (11, 38, 59, 60, 165). Shear et al (168) published the first comprehensive guide to the genus and resolved many of the previous controversies. Six species and one variety were recognized after a thorough examination of all major herbarium specimens in the United States and Europe and over 600 field collections from the United States, Europe and Asia.

The genus was generally described in these terms:

> Stromata subcorticular in origin, variable in size and shape, pustular to subspherical, subcoriaceous to friable, sometimes confluent, surface light auburn or chestnut to mahogany red, capucine yellow or cadmium orange to scarlet within, pycnidial and perithecial stromata the same or similar; pycnidia few to numerous, consisting of simple cavities or complex and irregular chambers; pycniospores minute, simple, bacilliform to oblong, yellowish to reddish in mass; perithecia deeply immersed, in one or more irregular layers, usually black when mature, with long necks, black within, colored like the stroma without; asci clavate to oblong

> fusoid, 8-spored, usually without paraphyses; ascospores oblong fusoid or subellipsoid to cylindric or allantoid cylindric, uniseptate or nonseptate, hyaline to pale yellowish (168).

It was divided into two sections based on the shape and degree of septation of the ascospore. Section 1 was composed of those species which produce a short cylindric to allantoid, continuous or pseudoseptate ascospore, and included E. gyrosa and E. singularis. Section 2 consisted of those fungi which produce oblong fusiform to oblong ellipsoid, uniseptate ascospores, and included E. parasitica, E. tropicalis, E. longirostris, E. fluens and E. fluens var. mississipiensis. Individual species were distinguished by the dimensions of stromata, ascospores and conidia. Endothia fluens var. mississipiensis was differentiated from E. fluens on the basis of cultural characteristics (168).

Additional species were added to the genus in individual papers. These included E. viridistroma (207), E. havanensis (31), E. eugeniae (142), E. coccolobii (204), E. japonica, E. macrospora (95) and E. tetraspora (92). The creation of these species was based on differences in stromal characteristics, the size of perithecia and ascospores and host.

The next major revision of the species was done by Orsenigo (133). He divided the genus into two subgenera using similar criteria as those used by Shear et al (168) in their erection of sections. The subgenus Proendothia was used for the nonseptate ascospored species E. gyrosa and E. singularis. The uniseptate

ascospored species, E. radicalis, E. longirostris and E. tropicalis, were placed in the subgenus Euendothia. Endothia radicalis was further divided into two subspecies; ssp. aflabellata (=E. fluens) and ssp. parasitica (=E. parasitica). Endothia parasitica was not accorded separate species status; this was later disputed by Roane and Stipes (150) on the basis of pigment content and enzyme analysis. Orsenigo's revision is not widely used.

The genus was reexamined by Müller and von Arx (124) in 1962. Among their revisions was the substitution of the name Endothia radicalis (Schw. ex Fr.)Ces. et de Not. for E. fluens, based on nomenclatural priority. This was supported by Kobayashi (93) and Roane and Stipes (150).

The Japanese species of Endothia were summarized by Kobayashi (93) in an expansion of a previous work (95); descriptions of E. singularis, E. radicalis, E. havanensis, E. parasitica, E. macrospora and E. tetraspora were included. The binomial E. nitschkei was substituted for E. japonica based on nomenclatural priority. Additionally, E. tropicalis was placed in synonomy with E. havanensis, while E. longirostris and E. fluens were made synonymous with E. radicalis. The descriptions of E. singularis, E. radicalis and E. parasitica were quite similar to those of Shear et al (168) with minor variations in spore and stromatic measurements.

Another review of the genus was completed by Roane and Stipes in 1978 (150). This is the first work in which all pre-

viously published species were discussed. The genus was again divided into two sections based on the degree of ascospore septation. Endothia gyrosa, E. singularis, E. viridistroma and E. tetraspora were grouped in the section with nonseptate ascospores; Endothia radicalis, E. longirostris, E. parasitica, E. havanensis, E. tropicalis, E. coccolobii, E. japonica, E. macrospora and E. eugeniae were placed in the section with uniseptate ascospores. Several older species were removed from the genus; these included E. sordida, E. nitschkei, E. parryi and the imperfect form species Endothiella robiniae Chona and Munjal. Endothia eugeniae was not removed from the genus, but its status was questioned due to its lack of bisanthraquinone pigments and the presence of oval conidia.

The status of Kobayashi's synonyms was also discussed in this review (150). Endothia longirostris was not thought to be synonymous with E. radicalis due to differences in pigment content and the lack of grouping of the two species by numerical analysis. The specific epithet japonica was reinstated because of the removal of the type specimen of E. nitschkei due to the latter's relative lack of stromal tissue, absence of bisanthraquinone pigments and valsoid arrangement of perithecia. The proposed synonomy of E. tropicalis and E. havanensis was not resolved due to the lack of suitable material of E. havanensis.

A major revision of the Diaporthales, including significant changes in the status of Endothia, was made by Barr (16). The

genus was divided into two separate genera placed in two different families. This was done on the basis of ascospore septation and shape, stromatic configuration and the type of fungal tissue present within the stroma. Those species with allantoid, one-celled ascospores, pseudoparenchymatous tissue and upright perithecia in diatrypoid stromata were retained in the genus Endothia of the Gnomoniaceae. These include E. gyrosa, the type species, E. singularis and E. viridistroma. The remaining species were removed to the genus Cryphonectria of the Valsaceae on the basis of their two-celled, ellipsoid or ovoid ascospores, prosenchymatous tissues and oblique perithecia within valsoid stromata. Barr included the following species in this genus: C. gyrosa (= E. tropicalis), the type species, C. parasitica (=E. parasitica), C. radicalis (=E. radicalis), C. nitschkei (=E. japonica) and C. macrospora (= E. macrospora). Barr rejected Kobayashi's (93) synonymy of E. havanensis with E. tropicalis, and subsequently created C. havanensis. This has been disputed by Hodges (84) who compared the type specimen of E. havanensis to samples of E. tropicalis authenticated by Shear and Stevens. He found only minor differences in ascosporic and stromatic dimensions and concluded that Kobayashi's decision was correct. Barr transferred E. tetraspora to the genus Cryptosporella because of its large, ellipsoid fusoid, one-celled ascospores. Several species previously placed in Endothia were not specifically mentioned by Barr; they are presumably transferred to Cryphonectria because of their morphology. These species are: E. eugeniae, E.

coccolobii and E. longirostris. Diaporthe cubensis, the causal agent of eucalyptus canker, was transferred to Cryphonectria by Hodges (84). It is suspected of being conspecific with E. eugeniae (C. S. Hodges, personal communication and A. C. Alfenas, personal communication).

In order to analyze Barr's work, it is necessary to have a full understanding of the terminology employed. The terms "valsoid" and "diatrypoid" are used to describe the general configuration of stromata: they have been defined differently by various authors. Hawksworth, Sutton and Ainsworth (80) described "valsoid" as "having groups of perithecia with their beaks pointing inward, (convergent), or even parallel to the surface, as in Valsa." This was further elucidated by Snell and Dick (183) who described this term in the manner of Wehmeyer (206). They defined valsoid as:

> Like the genus Valsa, having an imbedded or erumpent stroma in which the perithecia are clustered in a group of limited extent and with the perithecial necks convergent and erumpent as a fascicle or in a stromatic disc of limited area. The disc in a valsoid stroma is usually less strongly developed and less widely erumpent than in the 'diatrypoid' type, and the disc is usually 'ectostromatic'.

Diatrypoid was then defined as:

> Like the genus Diatrype, having an imbedded, somewhat widely erumpent stroma, in which the perithecia are clustered in limited or widely effuse areas and with the perithecial necks erumpent separately over the entire stromatic surface. The stroma is usually more strongly developed and more widely erumpent in the diatrypoid than in the 'valsoid' type, and the disc is usually

'entostromatic' (183).

This term was not included in Hawksworth et al (80).

It now becomes necessary to understand the terms "ecto-" and "entostroma". Ectostroma has been defined by Wehmeyer (206) as:

> that portion of the stroma which is formed on the surface of the bark, beneath or within the periderm and which consists typically of fungous tissues only, except that when it is developed within the periderm it may contain the remnants of periderm cells, but never of the bark cortex cells.

The ectostroma correspondingly consists of "that portion of the stroma which develops within the cortical or woody tissue of the host or substratum and is made up of both fungous and host tissue or substratum tissue" (206). The configuration of the valsoid and diatrypoid stroma, with the regions of ecto- and entostroma delineated, is presented in Figure 1.

Ruhland (155) involved the position of pycnidia and perithecia in the definitions of these terms, with the pycnidia arising in the ectostroma and the perithecia forming in the entostroma. Although this is generally true, Wehmeyer (206) disputed this association because of the many exceptions which frequently occur. He preferred to limit these terms to the description of structure and position and not of function.

The stroma of the genus Endothia has been interpreted by different authors in different ways. Shear et al (168) did not believe that the terms ecto- and entostroma could be applied to stromata of Endothia due to the large amount of variability

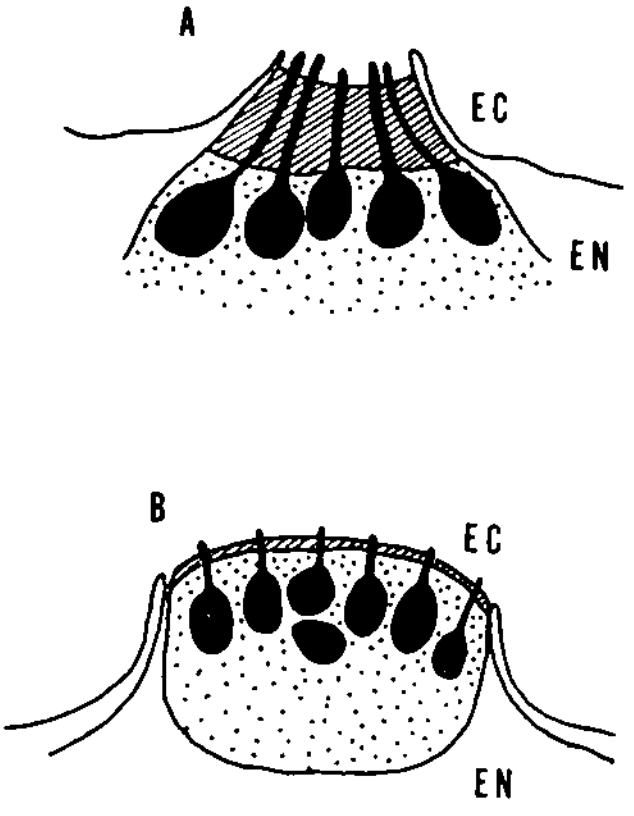

Figure 1. Representation of a valsoid (A) and diatrypoid (B) stroma. Regions of ectostroma (ec) and entostroma (en) are delineated (183, 198).

associated with pycnidial and perithecial position. They were employing the terms as defined by Ruhland (155). Wehmeyer (206) maintained that the stroma of Endothia could be described in this manner, although he admitted that the structure was highly variable. He reported that the stroma of Endothia originated as a hyaline ectostromatic cushion which subsequently expanded and produced pycnidia when it was sufficiently well developed. This ectostromatic tissue then ruptured the periderm and became yellow to orange-red in appearance. Entostroma and perithecial initials developed beneath the ectostroma. The entostroma frequently proliferated and a large stromatic mass with the remnants of the bark cells was pushed up through the periderm. The ectostroma was usually lost although it occasionally remained as a conic orange disc. This description fits the definition of a diatrypoid stroma; if Wehmeyer was describing the type species, his appraisal would correspond with that of Barr's. Wehmeyer (206) added, however, that the use of specific terms to descibe stromal morphology was quite limited in that only a small number of fixed types were designated. Stromal development is a very complex process and involves many independent events which cannot be described by a single term.

The terms "pseudoparenchymatous" and "prosenchymatous" refer to the arrangement and morphology of fungal hyphae within the stroma. Prosenchymatous tissue, as defined by Alexopoulos and Mims (2), consists of a "rather loosely woven tissue in which the component hyphae lie more or less parallel to one other, and

their typically elongated cells are easily distinguishable as such". Pseudoparenchymatous tissue is composed of "closely packed, more or less isodiametric or oval cells resembling the parenchyma cells of vascular plants....the hyphae have lost their individuality and are not distinguishable as such." Similar definitions are given by Bessey (22), Butler (34), and Hawksworth, Sutton and Ainsworth (80). These tissue types are illustrated in Figure 2. Barr is not the first to apply these terms to the stromata of Endothia. Kobayashi (93) noted in his genus description that the stromata are "compact plectenchymatous [pseudoparenchymatous] at the upper part and prosenchymatous containing decomposed tissue of substrata at lower part." Similar phrases are included in each of his species descriptions except for that of E. tetraspora.

Although Barr described pseudoparenchymatous tissue in stromata of Endothia and prosenchymatous tissue in those of Cryphonectria, the terms are not meant to be mutually exclusive (16, 17 - text presented in Appendix I). Barr described the diatrypoid stroma of Endothia as "typically pseudoparenchymatous, quite well developed ... may be more loosely arranged in spaces between perithecia and margins and may then appear prosenchymatous". The valsoid stromata of Cryphonectria were viewed as "prosenchymatous, may be slightly or well developed ... is more condensed at the margins, especially in the ectostromatic disc, and there may appear pseudoparenchymatous in these re-

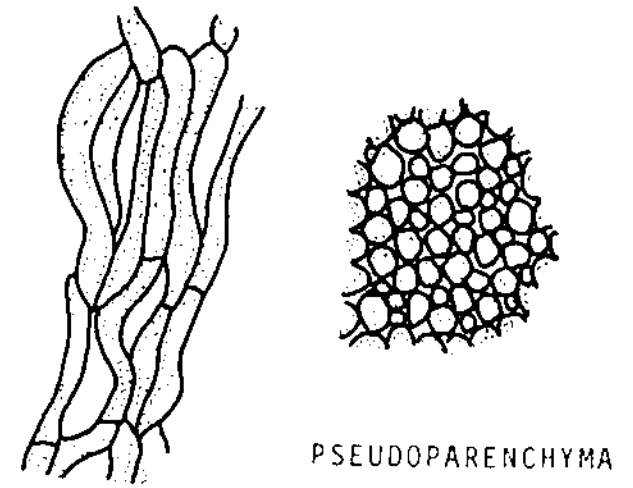

Figure 2. Representation of prosenchyma and pseudoparenchyma (2).

gions." (17). These descriptions are more flexible than those presented in the monograph and correspond with the observations of Kobayashi (93). Barr's descriptions are meant to give an overall impression of the fungi rather than to rigidly describe them in mutually exclusive terms (17).

Pigments

The production of spectacular yellow and orange pigments in both stromata and culture is one of the key characteristics of members of the genus Endothia. References to stromal color were made by both Schweinitz (160, 161) and Fries (64) in the original descriptions of the genus. Hawkins and Stevens (79) and Shear et al (168) characterized the various species of Endothia by their orange and yellow stromata. The first hypothesis about the chemical nature of these pigments was made by Pantanelli (134) who ascribed the yellow color to the presence of lipochrome in the hyphae. P. J. Anderson (9) suggested that this yellow pigmentation was due to an aurin. These views were both disputed by Hawkins and Stevens (79) who distinguished two water-insoluble yellow pigments and one water-soluble red pigment from cultures of E. parasitica, E. radicalis and E. tropicalis. This was the first use of pigment content to distinguish among species: E. radicalis produced all three compounds, E. tropicalis formed the two yellow pigments, and E. parasitica produced only one yellow compound. Although the authors did not identify these pigments, they did not believe that there was enough evidence to classify

them as either aurins or lipochromes.

The majority of modern chemical information known about these pigments was described in a series of papers by Shibata and his coworkers (170-175). Four yellow to orange compounds have been identified. Three of these, skyrin, oxyskyrin and skyrinol, are dimeric bisanthraquinones, while the fourth, rugulosin, is a modified bisanthraquinone. Chemical structures of these pigments are presented by Roane and Stipes (151). These compounds are synthesized by condensation reactions of malonate and acetate units onto protein-bound intermediates (201).

The first systematic attempt to associate specific pigments with various species of Endothia was made by Roane and Stipes (148). Alcohol and ethyl acetate extracts of stromal tissue were analyzed by spectrophotometric scans (270-700 nm), high pressure liquid chromatography and thin-layer chromatography. Although specific pigments were located by these techniques, the results were somewhat indefinite. The use of thin-layer chromatography was expanded (151); distinct pigment contents were associated with isolates of 12 species. These results are summarized in Table 1. The pigment contents of stromal tissue were similar to those of culture extracts. Many of the pigment distributions were shared by several species. The presence of skyrin in E. longirostris and E. tropicalis confirmed a previous report (30).

A red, water-soluble pigment has been reported from several Endothia species when they are grown on white cornmeal. It was

Table 1. Pigment content of 12 species of Endothia as determined by silica gel thin-layer chromatography of stromal and cultural extracts (151).

Species	Skyrin	Oxyskyrin	Rugulosin	Skyrinol
E. gyrosa	+[a]	+	+	-
E. macrospora culture	+	+	+	-
E. parasitica	+	+	+	-
E. havanensis culture (CBS505.63)	+	+	+	-
stromal	+	-	-	-
E.radicalis American cultures	+	+	+	-
European & New Zealand cultures	+	-	-	-
E. japonica	+	-	+	-
E. coccolobii stroma	-	+	+	-
E. longirostris stroma	+	-	-	-
E. singularis	+	-	-	-
E. tropicalis	+	-	-	-
E. viridistroma	-	-	+	-
E. eugeniae	-	-	-	-

[a]Indicates results of stromal and cultural extracts unless otherwise specified; "+" indicates presence, "-" indicates absence of pigment.

initially described by Shear et al (168) as "perilla purple" based on the nomenclature of the Ridgway color chart (147). It was originally reported from cultures of E. gyrosa, E. radicalis (as E. fluens) and E. singularis (168), and has also been associated with E. japonica and E. eugeniae (149). Hawkins and Stevens (79) determined that it was water-soluble and that is was deposited as red crystals within the hyphae. Sando (156) named it "endothine red" and reported its empirical formula as $C_7H_5O_4$. Its identity and synthesis are not known, but it is believed to be a phenolic compound with two hydroxyl groups and acidic properites indicative of anthraquinone, flavone, xanthone or ketone moieties (156).

MATERIALS AND METHODS

1. Microscopic examination of Endothia specimens

Fresh and dried material of eleven species of Endothia were examined microscopically. The sources of these specimens are listed in Table 2. Small specimens, bearing one to several stromata, were removed from the larger pieces with a razor blade and fixed in FAA [ethanol: glacial acetic acid: formaldehyde: water (5.0: 0.5: 1.0: 3.5)] for 48 to 72 h. Trapped air was removed from the specimens during fixation by the application of a vacuum (10 - 20 lbs psi) for several minutes. After fixation, the specimens were dehydrated at room temperature in a standard TBA series (20); solutions were changed every 3 h. Before infil-

Table 2. Source of fresh and dried material used to prepare sections[a].

E. coccolobii
Cornell #11899 (type)
Fresh Material:
Barnard, 10/82, Fort Lauderdale, FL, Coccolobis uvifera (L.) Jacq.
Dow, 2/26/84, Grape Bay, Bermuda, Coccolobis uvifera

E. eugeniae
IMI 45440
IMI401954 (type)

E. gyrosa
Fresh Material:
Nash, 10/19/82, Durham, NC, Quercus palustris Muenchh.
Micales, 3.29/82, Blacksburg, VA, Quercus palustris
Harris, 11/15/82, Arlington, VA Quercus palustris
Mahoney, 8/12/82, Harrison Experimental Station, Gulfport, MS, Quercus palustris
Stipes, 5/14/81, Norfolk, VA, Quercus velutina Lam.
Stipes, 5/81, Richmond, VA Quercus palustris
Micales, 11/14/80, Fairfax, VA, Quercus palustris
Micales, 11/14/80, Fairfax, VA, Quercus phellos L.

E. havanensis
Nat. Fungus Coll. #740 (type)

E. japonica (= E. nitschkei)
Nat. Fungus Coll. #2486 (type)
E59, Dept. PPWS,(VPI & SU)
Kobayashi #1048
Kobayashi #1049

E. longirostris
Dingley #28477
Nat. Fungus Coll. #4340 (type)

E. macrospora
Kobayashi isotype
Kobayashi #3662

E. parasitica
Fresh Material:
Micales, 10/17/82, Doylestown, PA, Quercus velutina
Micales, 9/3/82, Blacksburg, VA Castanea dentata Borkh.
Micales, 10/14/82, Buckingham, PA, Castanea dentata
Micales, 5/18/83, Blacksburg, VA, Castanea dentata
Micales, 7/18/83, Blacksburg, VA, Castanea dentata

Table 2 (continued)

E. parasitica
Fresh Material (continued)
Micales, 5/18/83, Arlington, VA, *Castanea crenata* Sieb. and Zucc.
Micales, 10/12/80, Matthew's Courthouse, VA, *Quercus virginiana* Mill.

E. radicalis (= *E. fluens*)
Dingley, #23586
National Fungus Collection (2 isolates, one as *E. fluens mississippiensis*, numbers not recorded)

E. singularis
E58 Dept. PPWS, Blacksburg, VA (type)

E. tropicalis
Dingley, #18377

E. viridistroma
Cornell #3634 (type)

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[a]Preserved specimens listed by herbarium location and number. Freshly collected specimens listed by collector, date, origin and host.

tration, the specimens were transferred to TBA: paraffin oil: chloroform (6: 3: 1). The specimens were infiltrated with Paraplast® and embedded in plastic sectioning blocks. Before sectioning, the specimen was exposed by the removal of the top layer of wax with a rotary microtome; the block was then soaked in detergent water overnight at 40 °C. Blocks were dried and 8 - 12 µm sections produced with a rotary microtome; sections were attached to slides with Haupt's adhesive as described by Sass (157). Slides were allowed to dry overnight on a slide warmer (45 °C), and sections were stained in a modified safranin - fast green series:

1. Xylene - 6 min
2. Xylene - 6 min
3. Xylene : Absolute ethanol - 3 min
4. Absolute ethanol - 3 min
5. 95% ethanol - 3 min
6. 70% ethanol - 3 min
7. 50% ethanol - 3 min
8. 0.75% aqueous safranin - 5 to 12 min
9. 50% ethanol - rinse
10. 50% ethanol - rinse
11. 70% ethanol - 3 min
12. 95% ethanol - 3 min
13. 0.75% fast green in 95% ethanol - 30 sec
14. 95% ethanol - rinse
15. Absolute ethanol - 3 min
16. Absolute ethanol - 3 min
17. Clove oil: xylene: absolute ethanol (1:1:1) - 6 min
18. Carbol xylene [phenol: xylene (1:1)] - 3 min
19. Xylene - 5 min
20. Xylene - 5 min
21. Xylene - 3 min

Coverslips were attached to the slides with Permount® at the end of this procedure.

Sections were examined for the amount of stromal tissue

present, the type and distribution of tissues within the stroma, and position and characteristics of perithecia, pycnidia, ascospores and conidia. Measurements were not made because of the distortion associated with dehydrated material (10).

2. Thin-layer chromatography of pigment extracts.

Pigment extractions and thin-layer chromatography were conducted as described by Roane and Stipes (151) with some modifications. The following species and isolates were used in this study: E. radicalis - E16, E42, E56, E64, E67, E76, E92, E150, E152; E. eugeniae - H173, H174, H176, H184, H185, H186, H189, E138, E139; E. havanensis - E40, H183, E158, E159; C. cubensis - H91, H137, H151, H154, H175, H187, H188. Sources, hosts and dates of collection of these isolates appear in Table 3. Colonies were grown at 25 °C for a minimum of 14 d on autoclaved white cornmeal (2.5 g corn meal: 15 ml dHOH) in 90 mm diameter glass Petri plates. Pigmented areas in the medium were excised, placed in 250 ml Erlenmeyer flasks and extracted in 20 ml absolute ethanol on a rotary shaker (Gyrotory® shaker-model G2, New Brunswick Scientific Co.). The pigment extracts were removed with a pipette and placed in glass culture tubes. Any remaining cornmeal was allowed to settle, and the clarified liquid was decanted and stored at 4 °C. Between 30 and 70 µl of pigment extract were spotted on Eastman Chromagram 6061 silica gel thin layer chromatography sheets; purified solutions of skyrin and oxyskyrin were used as standards. Chromatography was

Table 3. Isolate numbers, sources, dates of collection and hosts of *Endothia* species and *Cryphonectria cubensis* used in this study.

Species	Isolate Number[A]	Source of Collection	Collection Date	Host
Endothia parasitica	E24 V	Tifton, GA	4/72	*Castanea mollissima* Blume
	E85 V	Cherokee National	4/76	*Castanea dentata* Borkh.
	E86 V	Alum. Ridge, VA	4/76	*Castanea dentata*
	E87 V	Floyd County, VA	4/76	*Castanea dentata*
	E88 V	Alaben, NY	6/76	*Castanea dentata*
	E89	Williamsburg, VA	7/63	*Quercus virginiana* Mill.
	E95 V	Hampton, ME	8/76	*Castanea dentata*
	E96 V	Camp Hill, PA	8/76	*Castanea dentata*
	E106	Camp Hill, PA	8/76	*Castanea dentata*
	E107	Augusta County, VA	9/76	*Castanea dentata*
	E108	Blacksburg, VA	5/77	*Castanea dentata*
	E112 V	Vicker, VA	7/77	*Quercus rubra* L.
	E113 V	Vicker, VA	7/77	*Quercus coccinea* Muenchh.
	E114 V	Vicker, VA	7/77	*Quercus rubra*
	E115 V	Vicker, VA	7/77	*Quercus coccinea*
	E137 V	Boyce, VA	2/77	*Castanea mollissima*
	E153 V	Blacksburg, VA	10/82	*Castanea dentata*
	E155 V	Craig County, VA	12/82	*Quercus velutina* Lam.
	J1	Blacksburg, VA	7/80	*Castanea dentata*
	J2	Buckingham, PA	7/80	*Castanea dentata*
	J4 V	Barrington, NH	7/80	*Castanea dentata*
	J5 V	Barrington, NH	7/80	*Castanea dentata*
	J6 V	Barrington, NH	7/80	*Castanea dentata*
	J7 V	Barrington, NH	7/80	*Castanea dentata*
	J8 V	Panorama, VA	8/80	*Castanea dentata*
	J9 V	Panorama, VA	8/80	*Castanea dentata*
	J10 V	Panorama, VA	8/80	*Castanea dentata*

Table 3 (continued)

Species	Isolate Number[A]	Source of Collection	Collection Date	Host
Endothia parasitica (continued)	J11 V	Panorama, VA	8/80	*Castanea dentata*
	J12 V	Mountain Lake, VA	10/80	*Castanea dentata*
	J13 V	Mountain Lake, VA	10/80	*Castanea dentata*
	J14 V	Mountain Lake, VA	10/80	*Castanea dentata*
	J15 V	Mountain Lake, VA	10/80	*Castanea dentata*
	J16 V	Matthew's Court-house, VA	10/80	*Quercus virginiana*
	RW341P-MG1[B]	Parsons, WV		*Castanea dentata*
	RW992P-MG2	Parsons, WV		*Castanea dentata*
	RW51P-MG3	Parsons, WV		*Castanea dentata*
	RW911P-MG4	Parsons, WV		*Castanea dentata*
	RW494B-MG5	Bartow, WV		*Castanea dentata*
	RW178B-MG6	Bartow, WV		*Castanea dentata*
	RWEp113	France		*Castanea dentata*
Endothia gyrosa	E18 V	Hampton, VA	9/70	*Quercus palustris* Muenchh.
	E20 V	Hampton, VA	9/70	*Quercus palustris*
	E30 V	Eastern Shore, VA	11/72	*Quercus palustris*
	E37 V	Alexandria, VA	8/73	*Quercus palustris*
	E38 V	Alexandria, VA	9/73	*Acer saccharinum* L.
	E48	Auburn, AL	5/74	*Quercus palustris*
	E50 V	Franklin County, VA	7/74	*Quercus palustris*
	E51 V	South Boston, VA	7/74	*Quercus marilandica* Muenchh.
	E72 V CBS509.76	Surry County, VA	10/75	*Quercus palustris*
	E73	Hampton, VA	10/75	*Quercus palustris*
	E74 V CBS510.76	Norfolk, VA	12/75	*Quercus suber* L.
	E98 V	Lynchburg, VA	10/76	*Quercus palustris*

Table 3 (continued)

Species	Isolate Number[A]	Source of Collection	Collection Date	Host
Endothia gyrosa (continued)	E110 V	Wadswoth, OH	1/78	*Fagus grandifolia* Ehrh.
	E111 V	Fortress Monroe, VA	4/77	*Quercus phellos* L.
	E123 V	Langley Air Force Base, VA	2/78	*Quercus phellos*
	E140 V	Washington, D.C.	8/79	*Prunus caroliniana* (Mill.) Ait.
	E145 V	Charleston, WV	2/80	*Fagus grandifolia*
	E146 V	Charleston, WV	2/80	*Quercus palustris*
	E147 V	Charleston, WV	2/80	*Quercus palustris*
	E154 V	Arlington, VA	11/82	*Quercus palustris*
	J17 V	Fairfax, VA	11/80	*Quercus phellos*
	J18 V	Fairfax, VA	11/80	*Quercus palustris*
Endothia radicalis	E16 CBS202.54			*Castanea sativa* Mill.
	E42 CBS202.54			*Castanea sativa*
	E56	Auckland, New Zealand		*Fuchsia excortica* L.
	E64 V	Auckland, New Zealand		
	E67 V	Auckland, New Zealand		
	E75 CBS239.54			*Castanea sativa*
	E76 CBS240.54			*Castanea sativa*
	E92 CBS117.13	Connecticut		*Quercus* sp.
	E149	Skiti, Greece		*Castanea sativa*
	E150	Skiti, Greece		*Castanea sativa*
	E151	Skiti, Greece		*Castanea sativa*
	E152	Skiti, Greece		*Castanea sativa*

Table 3 (continued)

Species	Isolate Number[A]		Source of Collection	Collection Date	Host
Endothia eugeniae	H173[C]	IMI279035	Zanzibar, Tanzania	4/83	Syzgium aromaticum (L) Merr. & Perry
	H174	IMI279036	Zanzibar, Tanzania	4/83	Syzygium aromaticum
	H176	IMI279618	Zanzibar, Tanzania	4/83	Syzygium aromaticum
	H184		Itubara, Bahia, Brazil	12/83	Syzygium aromaticum
	H185		Funao, E.S., Brazil	12/83	Syzygium aromaticum
	H186		Valenca, B.A., Brazil	12/83	Syzygium aromaticum
	H189		Valenca, B.A., Brazil	12/83	Syzygium aromaticum
	E138	IMI44845	Zanzibar, Tanzania	1951	Syzygium aromaticum
	E139	IMI56425a	Barns, Malaya	1954	Syzygium aromaticum
Endothia havanensis	E40	CBS505.63	Loandijli, Congo		Eucalyptus saligna Sm.
	E158		Tampa Bay, FL	3/82	Eucalyptus camaldulensis Dehn.
	E159		Tampa Bay, FL	3/82	Eucalyptus camaldulensis
	E183		Darling Range, Australia	4/81	Eucalyptus marginata Donn ex Sm.
Endothia macrospora	E54 V		Komakuro, Japan	12/71	Castanopsis cuspidata (Thunb.) Schottkg.
Endothia japonica	E53 V		Nagano, Japan	10/65	Quercus mongolica var. grosseserrata (Bl.) Rehd
	E59 V			12/74	Quercus mongolica var. grosseserrata
Endothia singularis	E52		Chiyoda, Japan	10/60	Castanea crenata Sieb. Zucc.
	E58 V		Silver Mountain, CO		Quercus gambelii Nutt.

Table 3 (continued)

Species	Isolate Number[A]		Source of Collection	Collection Date	Host
Endothia tropicalis	E57		Auckland, New Zealand		Elaeocarpus dentatus Vahl.
	E70		Auckland, New Zealand		Elaeocarpus dentatus
Endothia viridistroma	E41	CBS202.36	Athens, GA		Cercis canadensis L.
	E82	CBS249.54	Athens, GA		Cercis canadensis
Endothia coccolobii	E157	V	Fort Lauderdale, FL	10/82	Coccoloba uvifera (L.) Jacq.
Cryphonectria cubensis	H91		Lihue-Koloa Forest Reserve, HI	3/80	Eucalyptus saligna
	H182	IMI268904	Jarrahdale, Australia	4/82	Eucalyptus marginata
	H137		Botanical Garden, Hong Kong	8/81	Eucalyptus sp.
	H151		Wynaud, Calicut, India	11/82	Eucalyptus grandis Hill ex Maid.
	H154		Uverito, Venezuela	1/82	Eucalpytus grandis
	H187		Aracruz, E.S., Brazil	12/83	Eucalyptus alba Reinw. ex Blume

Table 3 (continued)

Species	Isolate Number[A]	Source of Collection	Collection Date	Host
Cryphonectria cubensis (continued)	H188	Ceplac Itabuna, Brazil	12/83	*Eucalyptus grandis*
	H175	IMI279614 Mangombe, Cameroon	4/83	*Eucalyptus urophylla* S. T. Blake

[A]Isolates designated "V" represent those with vouchered specimens in herbarium of the Department of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA.

[B]Hypovirulent isolates of *Endothia parasitica* representative of six different morphology groups as defined by Willey (210).

[C]Isolate numbers that begin with "H" obtained from the culture collection of C. S. Hodges, USDA Forest Service, Honolulu, HI.

conducted for 20 min at ambient temperature (27 °C). The position and color of spots were recorded under natural and ultraviolet (254 nm) light; Rf values were calculated.

RESULTS AND DISCUSSION

Stromata of 11 species of Endothia were sectioned and examined for the distribution of perithecia, pycnidia and stromatic tissue types (prosenchyma and pseudoparenchyma). The general arrangement of the stromata (valsoid or diatrypoid) and the shape of the ascospores were also observed. Both pycnidial and perithecial stromata were located among sections of E. parasitica, E. radicalis, E. tropicalis, E. longirostris, E. coccolobii, E. eugeniae, E. japonica and E. gyrosa. Perithecial stromata alone were found in isolates of E. macrospora, E. havanensis and E. viridistroma, while only pycnidial stromata were observed for E. singularis. Freshly collected specimens were used whenever possible because of the superior sections which were produced. Older material, especially many of the type specimens, was extremely brittle; sectioning artifacts, particularly collapsed perithecia, were common in these samples. Typical stromata of each species are presented in Figures 3 - 17.

Stromatic Type

The production of valsoid and diatrypoid stromata was one criterion used by Barr to differentiate Endothia from Cryphonectria. This designation is correct if the terms are used

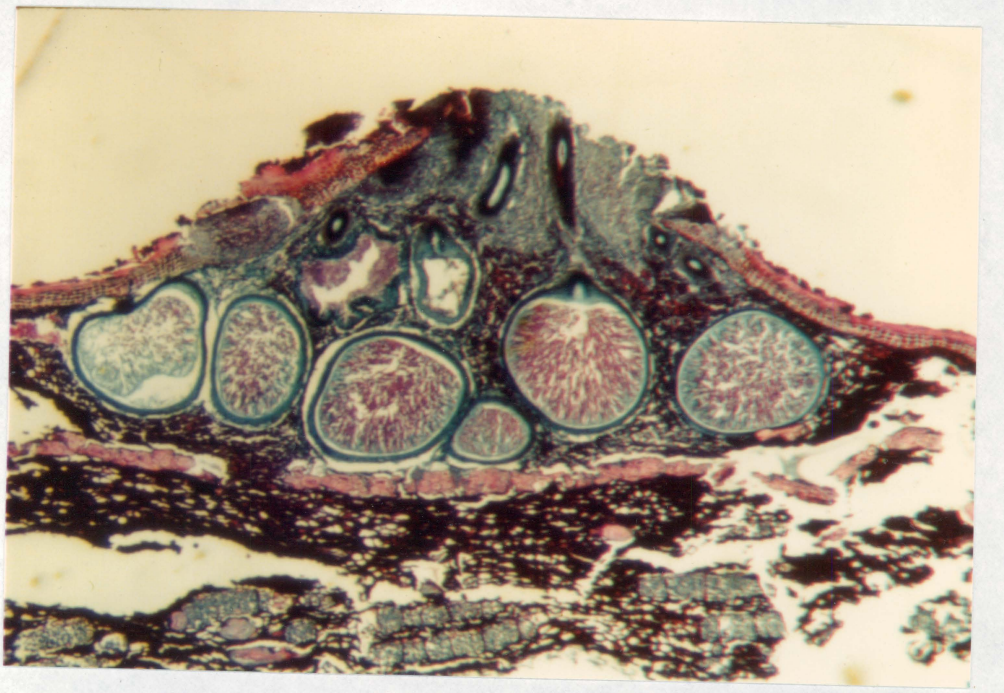


Figure 3. Perithecial and pycnidial stroma of Endothia parasitica (50X). Stroma is valsoid with an erumpent ecto-stromatic disc and immersed entostroma. Perithecia along stromal periphery are tilted while those in the center are upright. Perithecial necks converge within the ectostromatic disc.

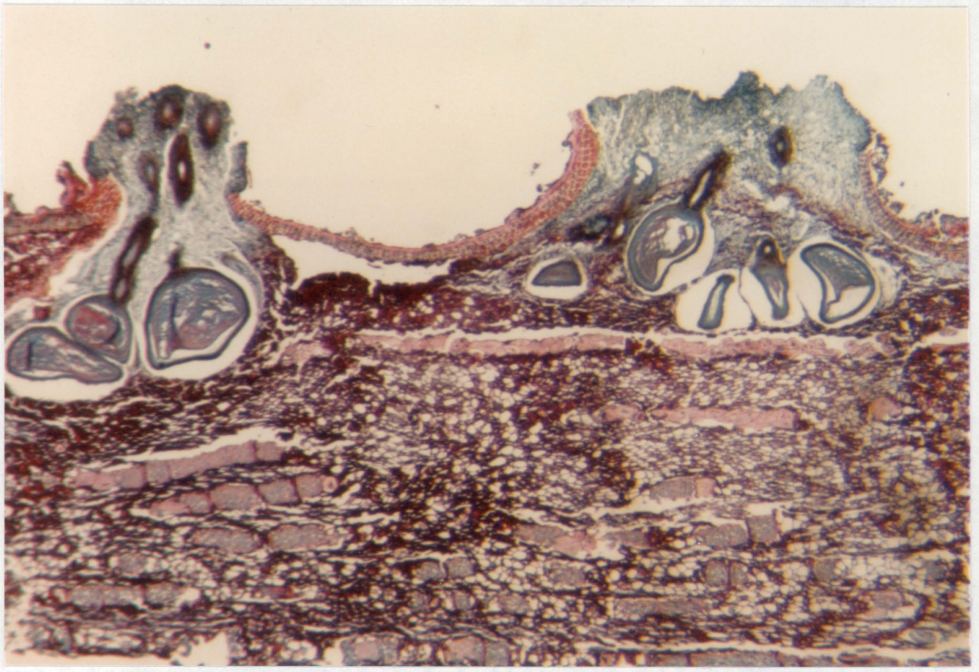


Figure 4. Perithecial stromata of Endothia radicalis (50X). Stromata are valsoid with easily distinguished ecto- and entostroma. Perithecia along the periphery of the stroma are tilted while those in the center are upright. Perithecial necks converge within the ectostromatic disc.

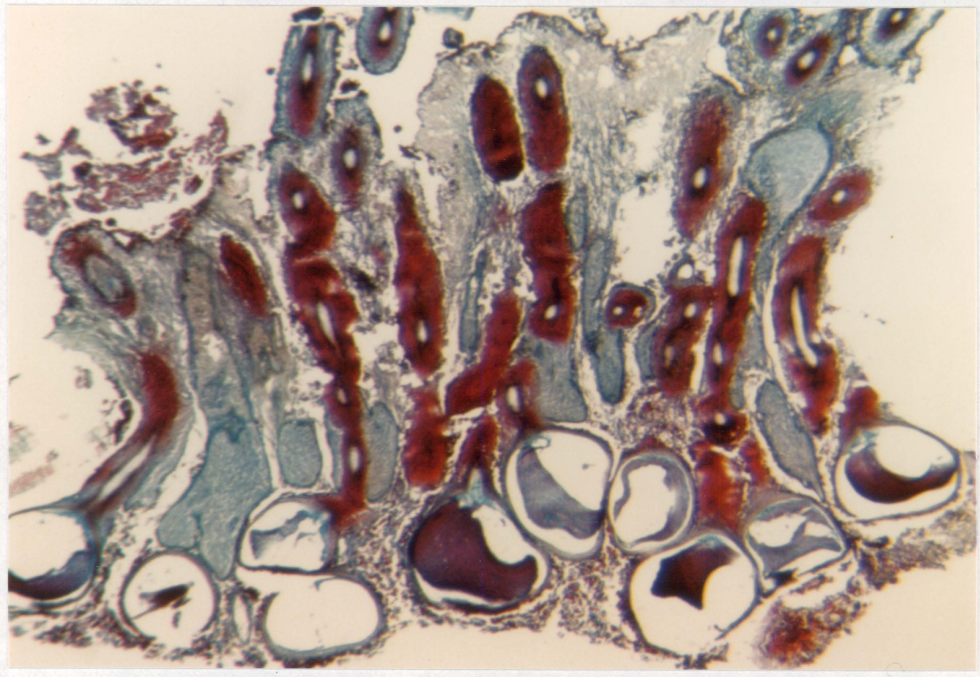


Figure 5. Perithecial and pycnidial stroma of Endothia longirostris (50X). Perithecial necks converge within the ecto-stromatic disc; they are surrounded by a sheath of pseudoparenchymatous cells. Note the tilted perithecia along the periphery of the stroma and the upright perithecia in the stromal center.

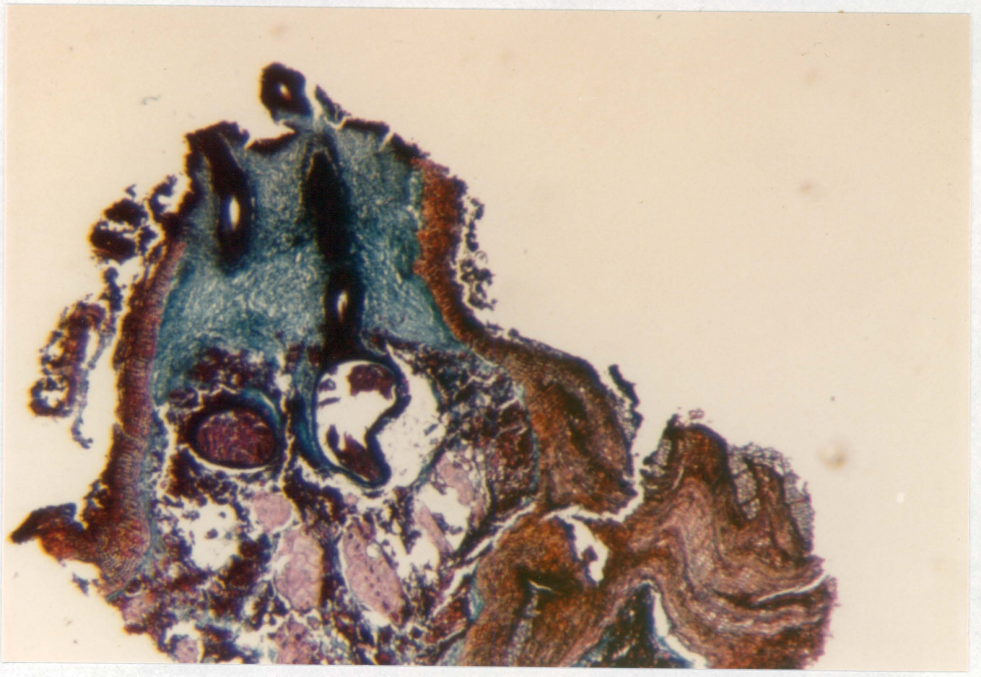


Figure 6. Perithecial stroma of Endothia macrospora (50X) with two collapsed perithecia in entostroma; perithecial necks in ectostroma. Perithecium along the periphery of the stroma is tilted, while the one within the stromal center is upright.

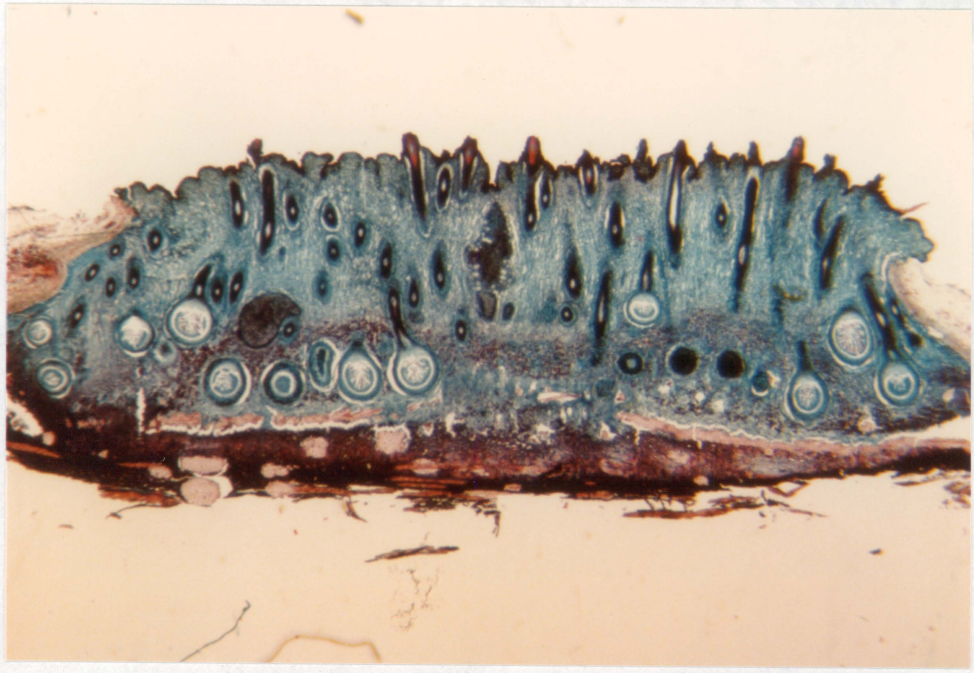


Figure 7. Perithecial and pycnidial isolate of Endothia japonica (25X). Stroma is clearly divided into regions of ecto- and entostroma. Perithecia along the periphery of the stroma are tilted while those in the center are upright. Perithecial bases are embedded in the entostroma and the perithecial necks grow up through the ectostroma to the surface. Several small perithecia are located entirely within the ectostroma.

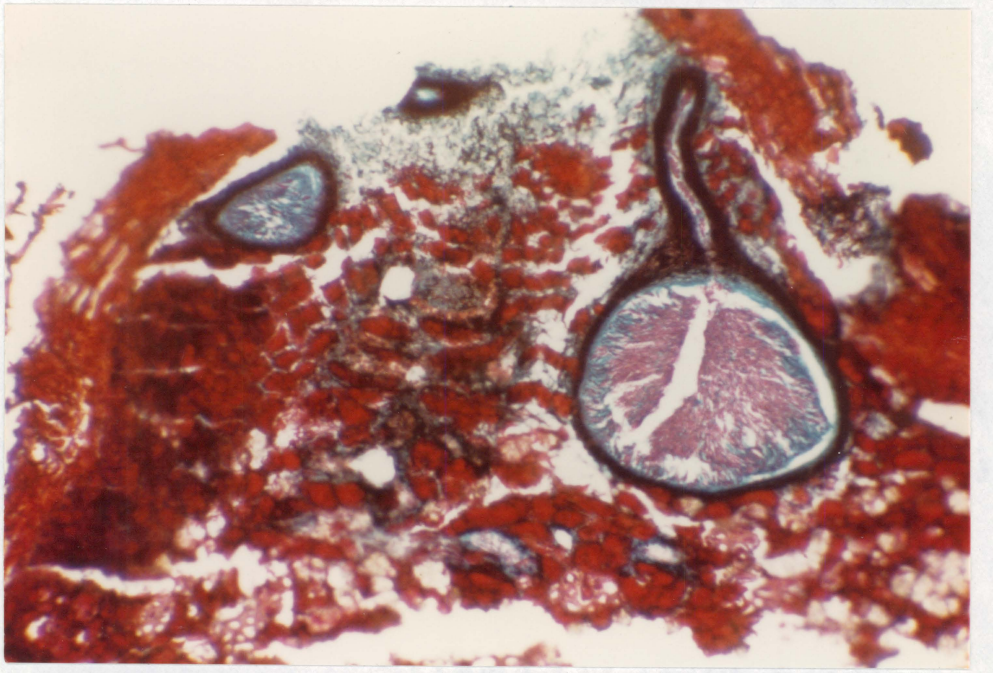


Figure 8. Perithecial and pycnidial stroma of Endotha coccolobii on exposed roots of Coccolobis uvifera (100X). Top of ectostromatic disc partially lost; valsoid configuration is still evident. Perithecial bases embedded in entostroma and tilted perithecial neck growing through ectostroma to surface. Individual pycnidia visible deep within the entostroma.

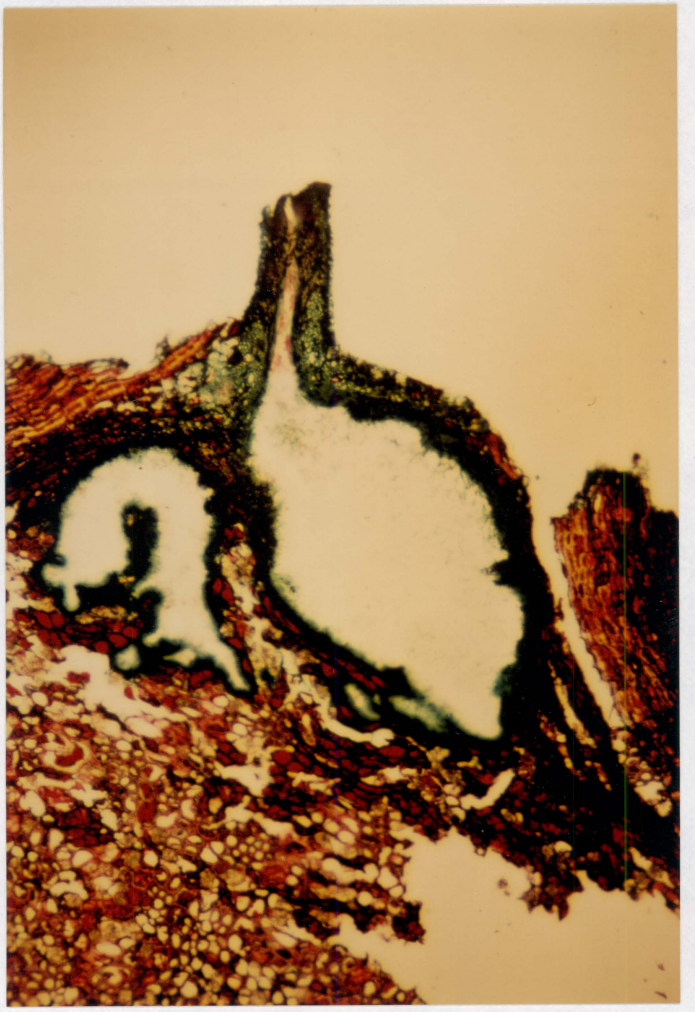


Figure 9. Beaked pycnidial stroma of Endothia coccolobii on exposed root of Coccolobis uvifera (100X). Valsoid appearance of stroma obscured by the large pycnidia and relative lack of vegetative tissue.

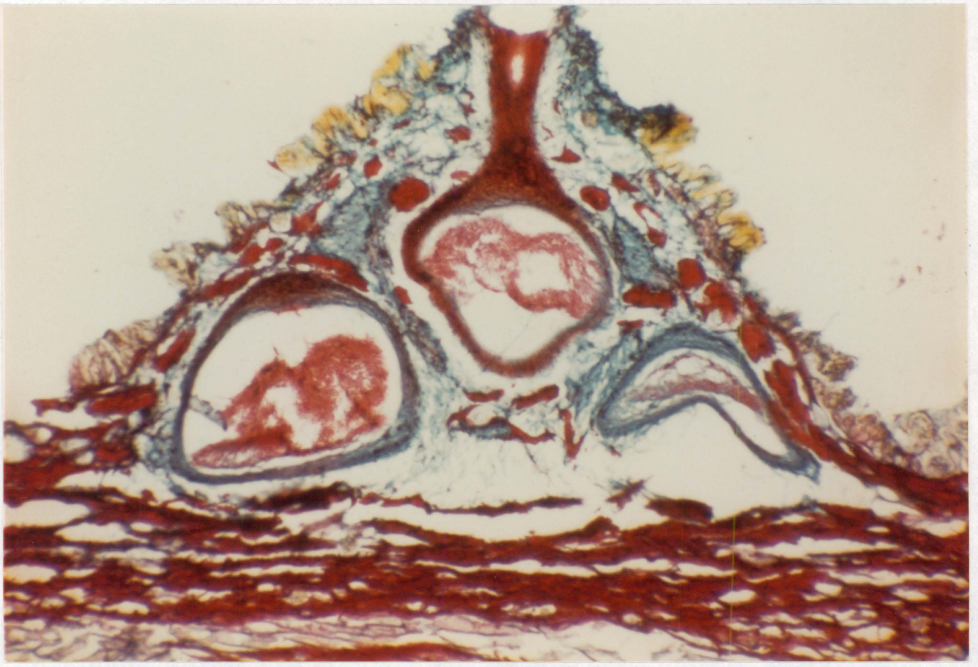


Figure 10. Perithecial stroma of Endothia coccolobii on seed coat of Coccolobis uvifera (type specimen) (100X). Stroma unable to penetrate within the host tissue; typical valsoid appearance is obscured.

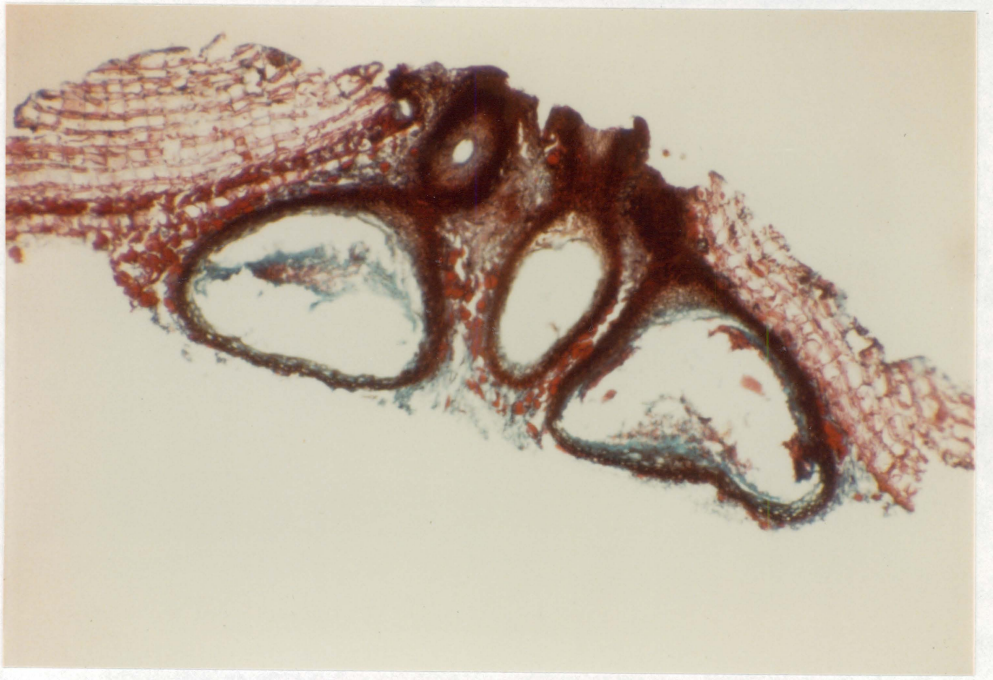


Figure 10B. Perithecial stroma of Endothia eugeniae. Valsoid appearance obscured by the relative lack of vegetative tissue.

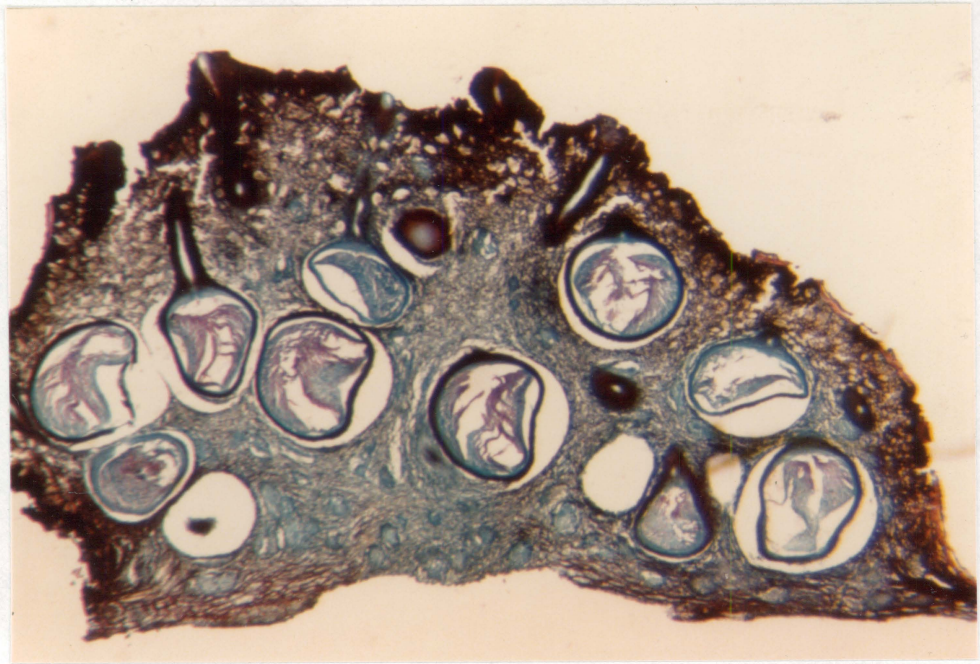


Figure 11. Perithecial stroma of the Dingley isolate of Endothia radicalis (50X). This specimen has a surface-limited stroma in which there is no delineation of ecto- and entostroma; its configuration is not valsoid. Perithecia are distributed within the erumpent portion of the stroma. This isolate may have been misidentified; it bears no resemblance to the other specimens of Endothia radicalis.

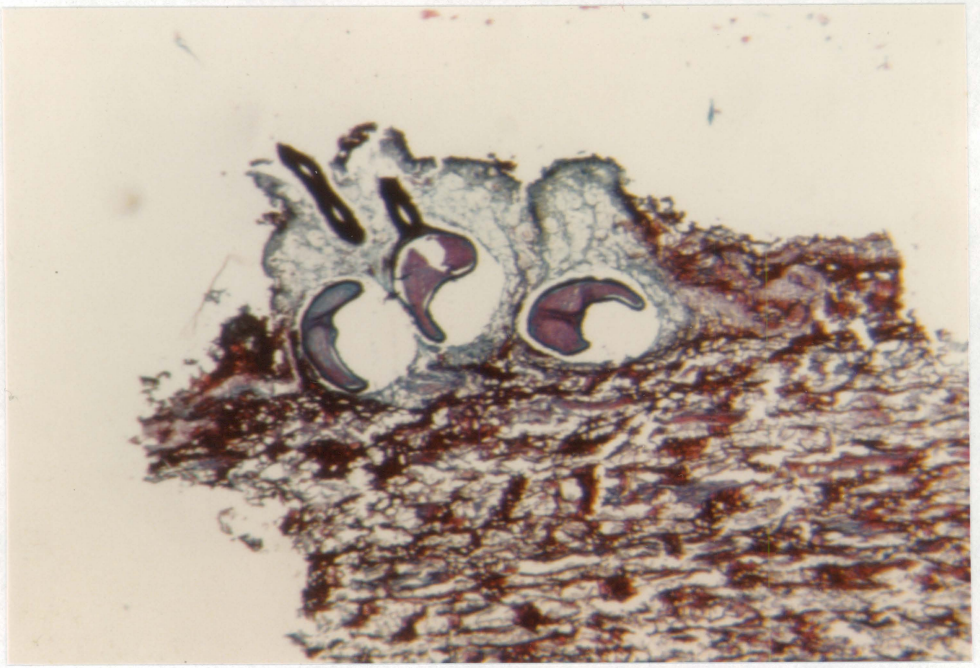


Figure 12. Perithecial stroma of Endothia havanensis (type specimen) (50X). Stroma is surface limited; perithecia have direct access to stromal surface and are not tilted. This stroma does not appear valsoid and may demonstrate the influence of host on stromal development.

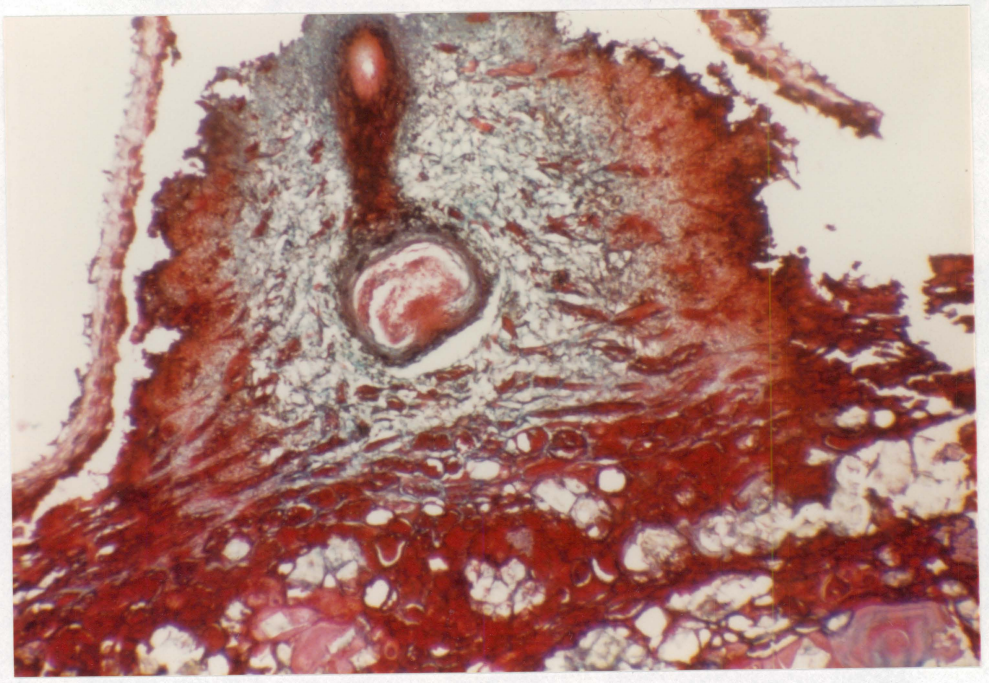


Figure 13. Perithecial stroma of Endothia gyrosa (100X) with upright perithecium in center of stroma. Pseudoparenchymatous tissue (red) along edge of stroma and prosenchymatous tissue in stromal center (green). Stroma is diatrypoid with entostromatic disc; note host cells evenly distributed throughout stromal center.

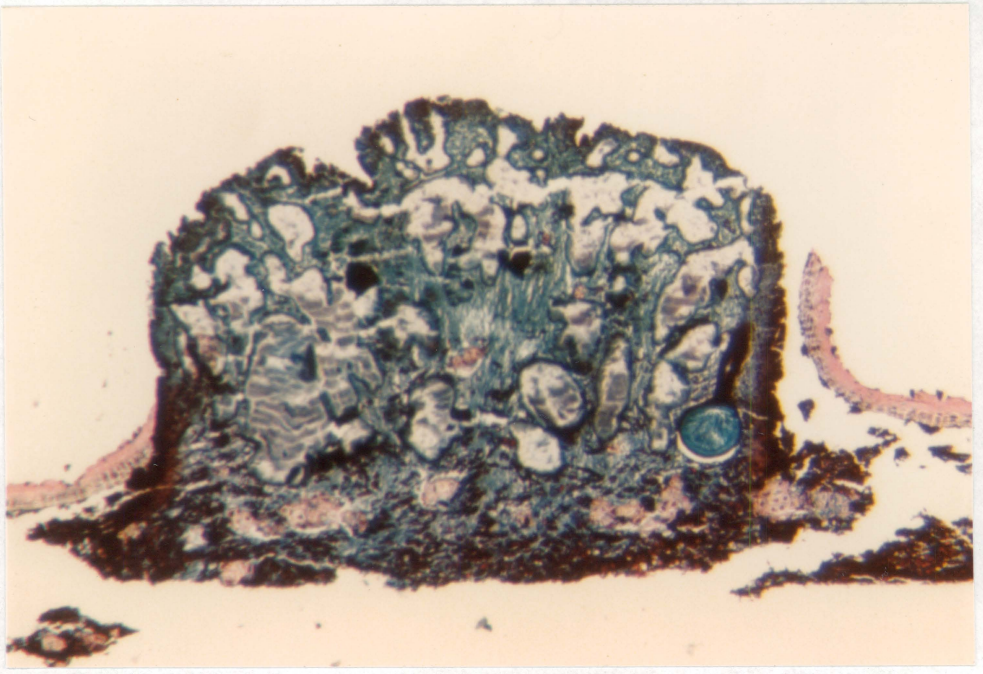


Figure 14. Well-developed perithecial and pycnidial stroma of Endothia gyrosa (50X). Stroma is diatrypoid in appearance with widely erumpent entostromatic disc. Perithecium is tilted towards stromal edge.

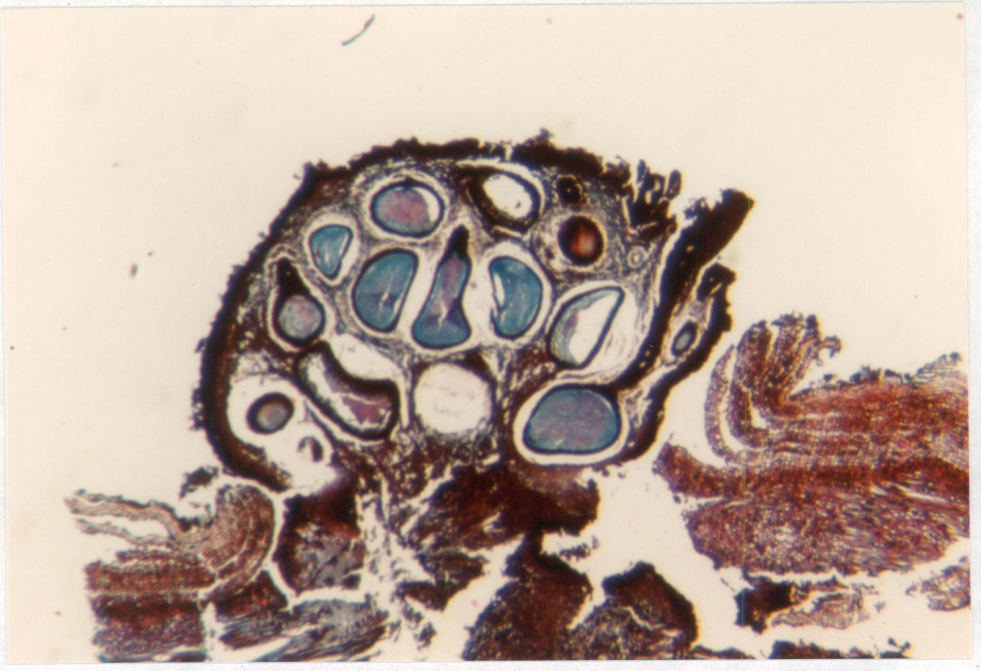


Figure 15. Perithecial stroma of Endothia viridistroma (type specimen) (50X). Stroma is diatrypoid with well-developed entostromatic disc. Perithecial necks diverge to stromal edge.

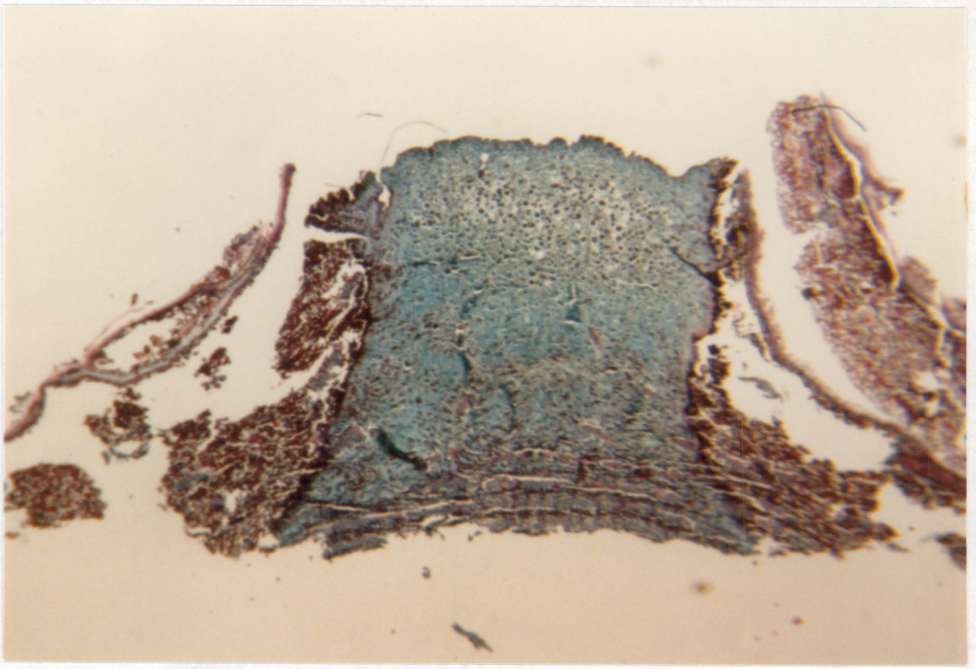


Figure 16. Sterile stroma of Endothia singularis (25X) composed of a widely erumpent, entostromatic disc. Host cells (red) uniformly distributed throughout stroma.

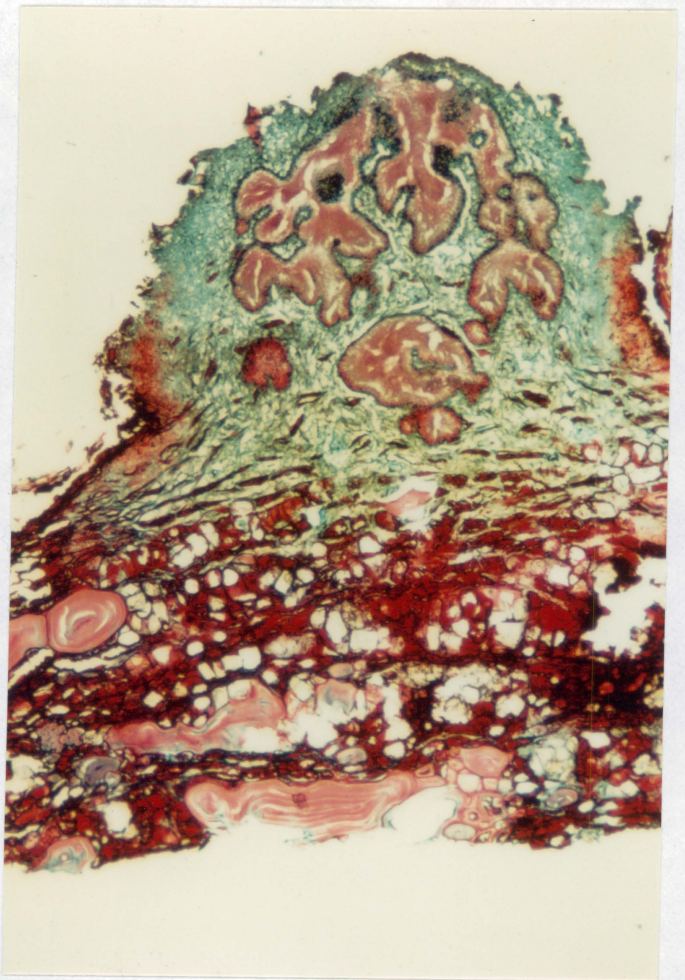


Figure 17. Pycnidial stroma of Endothia gyrosa (100X). Note compressed pseudoparenchymatous tissue (red) along edge of stroma.

in the manner of Snell and Dick (183) and Wehmeyer (206). The terminology of Hawksworth et al (80) is not adequate to completely describe these stromata. Species which would belong in Barr's genus Cryphonectria typically produced two layers of stromal tissue: an erumpent ectostroma composed primarily of fungal tissue, and a deeply immersed entostroma formed of both fungal hyphae and host bark cells. In most cases, pycnidia were confined to the ectostroma while the perithecial bases were located in the entostroma. The perithecial necks grew through the ectostroma to, and often beyond, the stromal surface. Exceptions to this pattern were frequently observed; an entire perithecium was located in the ectostroma of E. japonica (Figure 7), while an isolated pycnidium was observed deep within the entostroma of E. coccoloba (Figure 8). The placement of perithecia and pycnidia were not considered in the definitions of ecto- and entostroma by Wehmeyer (206); these observations support his concepts.

The typical valsoid appearance of these stromata was sometimes obscured and difficult to discern. This was especially common among smaller stromata which contained large numbers of pycnidia or perithecia (Figure 9). Vegetative stromal tissue was frequently limited in these stromata and it was difficult to differentiate the layers of ecto- and entostroma. The nature of the host tissue also altered the appearance and the size of the stroma. This was especially striking with different samples of

E. coccolobii on Coccoloba uvifera. Stromata produced on the roots (Figure 8) displayed the typical valsoid configuration; those formed on the seed coat (Figure 10) were not able to penetrate into the host tissue and were confined to the seed surface. A much smaller stroma was subsequently formed on the seed; the boundary between ecto- and entostroma was obscured. The Dingley isolate of E. radicalis (Figure 11) also produced surface-limited stromata which were not valsoid; this specimen may have been misidentified. It did not resemble other isolates of E. radicalis which did form valsoid stromata (Figure 4). Some of the specimens included only the top portion of the stroma; the entostromatic region was lost due to the extreme fragility of the samples. It is important to examine several stromata from different sources before the final stromal type is defined. In most cases, a valsoid appearance was associated with species that produced two-celled ascospores.

The unicellular ascosporic species, classified by Barr as members of the true Endothia, exhibited a diatrypoid stroma (Figures 13 - 16). These stromata were not as deeply emersed as in those of the previous group; a larger portion of the stroma was erumpent. There was also no division of ecto- and entostroma; host cells were distributed uniformly throughout the entostromatic disc. Presumably the ectostroma had been lost; developmental studies would be necessary to confirm this.

Distribution and Position of Perithecia

One aspect of the terms "valsoid" and "diatrypoid", as defined by Snell and Dick (183), is the position of the perithecia and perithecial necks within the stroma. In the valsoid stroma, perithecial necks converged towards the center of the stroma and then became erumpent. In a diatrypoid stroma, the perithecial necks were separately erumpent to the stromal surface. This difference in the growth of the perithecial necks was due to the general morphology of the stroma. In a valsoid stroma, the entostroma frequently extended laterally beneath the ectostromatic disc. Perithecia produced along the perimeter of the stroma had no perpendicular pathway to the top of the stroma and must align their necks toward the center of the stroma before orienting themselves to the stromal surface. These peripheral perithecia were therefore oblique in orientation. Perithecial necks originating from perithecia in the center of the stroma could grow straight to the surface; their appearance was generally upright. This can be seen in stromata of E. parasitica (Figure 3), E. radicalis (Figure 4) and E. japonica (Figure 7). The size of the stroma and the number of perithecia influenced the general pattern of growth since crowding produced irregularities.

The situtation was quite different within the diatrypoid stroma. Perithecia were generally produced within the erumpent portion of the stroma and therefore had no obstructions to the stromal surface. Although the perithecia were generally upright,

the necks did not necessarily lead directly to the stromal surface. Perithecia within stromata of E. gyrosa were frequently produced along the edge of the stroma; the necks grew on an angle away from the center and toward the side of the stroma (Figure 14). Perithecia within stromata of E. viridistroma also produced divergent necks which were not necessarily upright (Figure 15). Unfortunately, most of the unicellular ascospored species produced few perithecia; the perithecial position could not be noted under conditions of crowding.

Barr (16) used the orientation of the perithecia as another criterion to separate Endothia from Cryphonectria. Members of Cryphonectria were said to produce "perithecia grouped, ± oblique, beaks elongate, converging through stromatic disc." This description is essentially correct, especially since the term "oblique" was qualified with a "±". This allows the upright position of the perithecia within the center of the stroma to be recognized. Species within the true genus Endothia were described with "perithecia upright, numerous in one layer or several layers, beaks ± central, elongate, erumpent separately to or beyond stromal surface." This account is also accurate since it allows some latitude in the orientation of the perithecial necks.

Distribution of Prosenchyma and Pseudoparenchyma

The distribution of prosenchymatous and pseudoparenchymatous tissue was determined for the 11 species of Endothia examined in

this study. The interpretation of Kobayashi (93) and the more flexible interpretation by Barr (17) were confirmed. Pseudoparenchyma, textura angularis, was consistently observed on the surface and along the upper edges of the stromata of the various species (Figures 18 and 19). The center of the stroma was composed of prosenchyma, textura intricata (Figure 18 and 19). Pseudoparenchyma aligned the edges of the pycnidia and also made up the perithecial walls. Each tissue type could be located at a magnification of 1000X and was also distinguished at lower magnifications by differences in staining patterns; pseudoparenchyma was usually darker in appearance than prosenchyma due to its closely compacted cells (Figure 17). Barr (16, 17) stated that the overall appearance of the stroma was prosenchymatous for members of Cryphonectria and pseudoparenchymatous for species retained in Endothia. This was somewhat more difficult to discern. Sections of E. gyrosa (Figure 14), E. singlularis and E. viridistroma (Figure 15) differed in their staining and sectioning properties from those of the other species; they appeared much darker under the same stain regimen and were subject to more sectioning damage. This may indicate a more tightly compressed texture. The ectostromatic disc of species transferred to Cryphonectria was much more filamentous and open in appearance than the entostromatic tissue found in members of the true Endothia (Figures 3 and 14); prosenchyma was noted in the stromal centers of both groups.

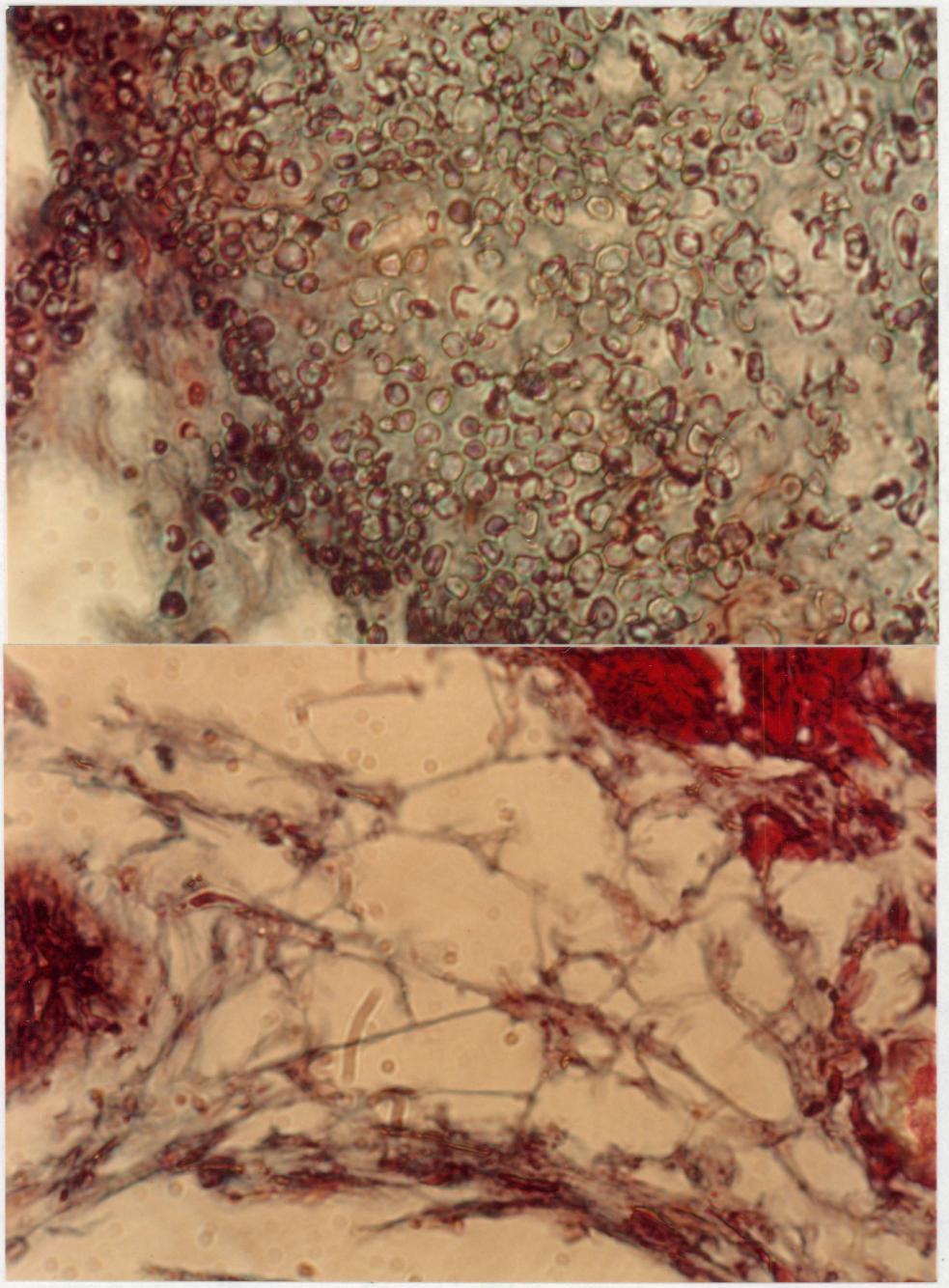


Figure 18. Pseudoparenchymatous (ps) and prosenchymatous (ps) tissue within a single section of Endothia gyrosa (1000x). The pseudoparenchyma is oriented along the edge of the stroma and the prosenchyma is found within the stromal center.

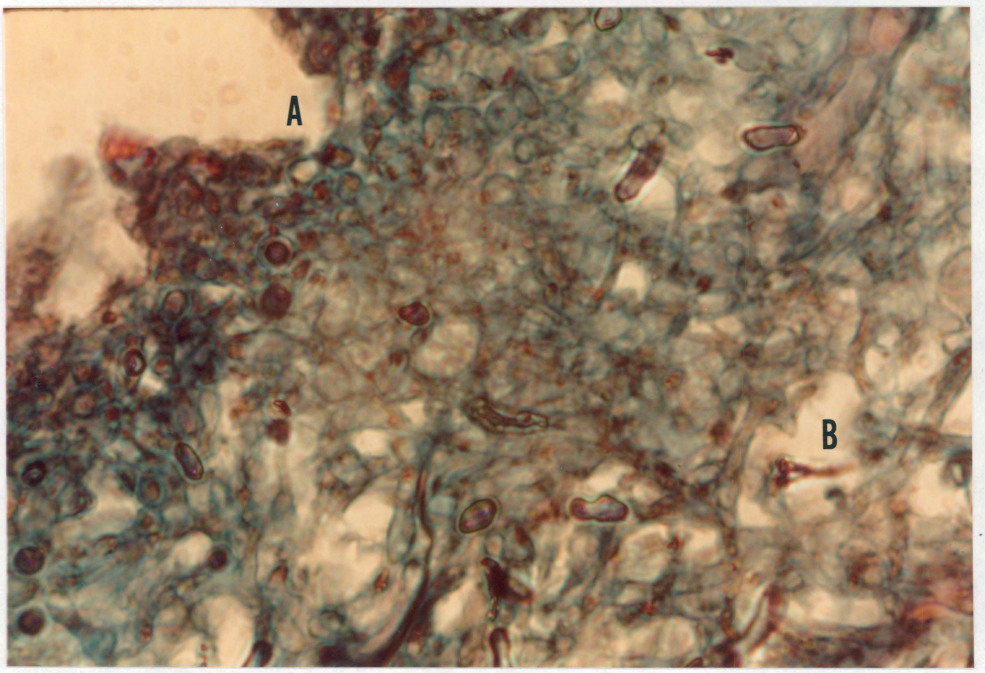


Figure 19. Pseudoparenchymatous tissue (A) and prosenchymatous tissue (B) within a single stroma of Endothia radicalis (1000x). Pseudoparenchyma located along the edge of the stroma while prosenchyma limited to the stromal center.

Ascospore Shape and Septation

The most important criterion used by Barr, and that subject to the least interpretation, was the shape and degree of septation of the ascospore. Species contained within Cryphonectria produced two-celled, ovoid or ellipsoid ascospores, while members of the true genus Endothia formed allantoid, nonseptate ascospores. This is quite straightforward; representative differences in cell septation and shape are presented in Figures 20-23. Ascospores produced by E. gyrosa (Figure 20) were somewhat difficult to discern; ascospores of E. viridistroma (Figure 21) were more easily observed and fit Barr's description of the genus.

Barr was technically correct in her description of these fungi. Those species retained in Endothia produced nonseptate, allantoid ascospores in upright perithecia. The perithecia produced central beaks and were found within diatrypoid stromata. The species transferred to Cryphonectria produced 2-celled, ellipsoid or allantoid ascospores. The perithecia were frequently tilted and the oblique necks converged within the ectostromatic disc before growing to the stromatic surface. The configuration of the stroma was valsoid with an immersed entostroma and an erumpent ectostroma.

The real issue is whether differences in general stromatic configuration and ascosporic shape and septation are sufficient to redistribute these species into different genera of different

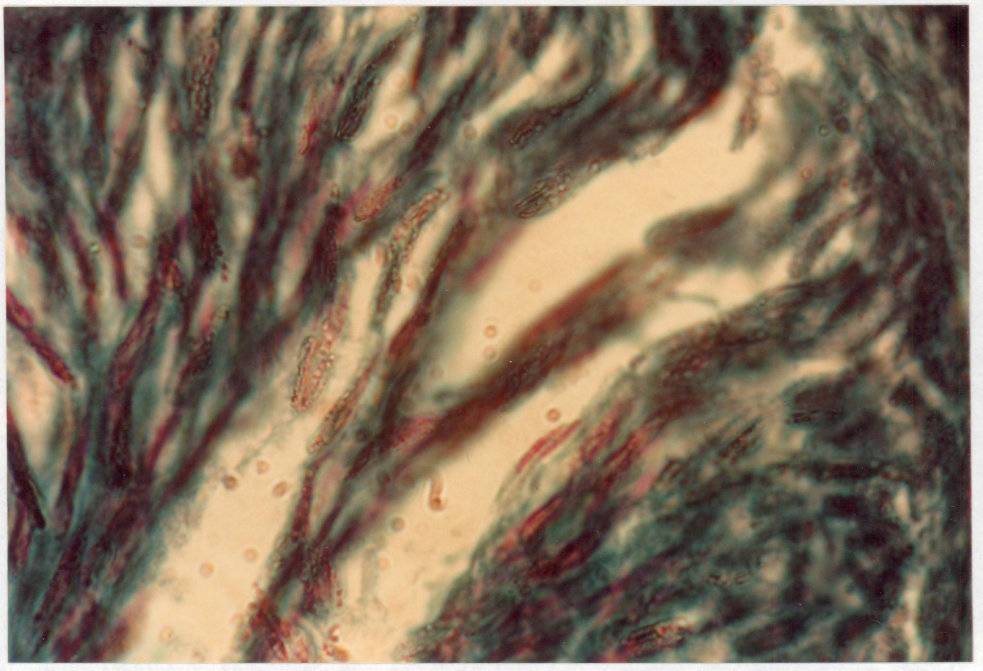


Figure 20. Allantoid, nonseptate ascospores of Endothia gyrosa (1000x).

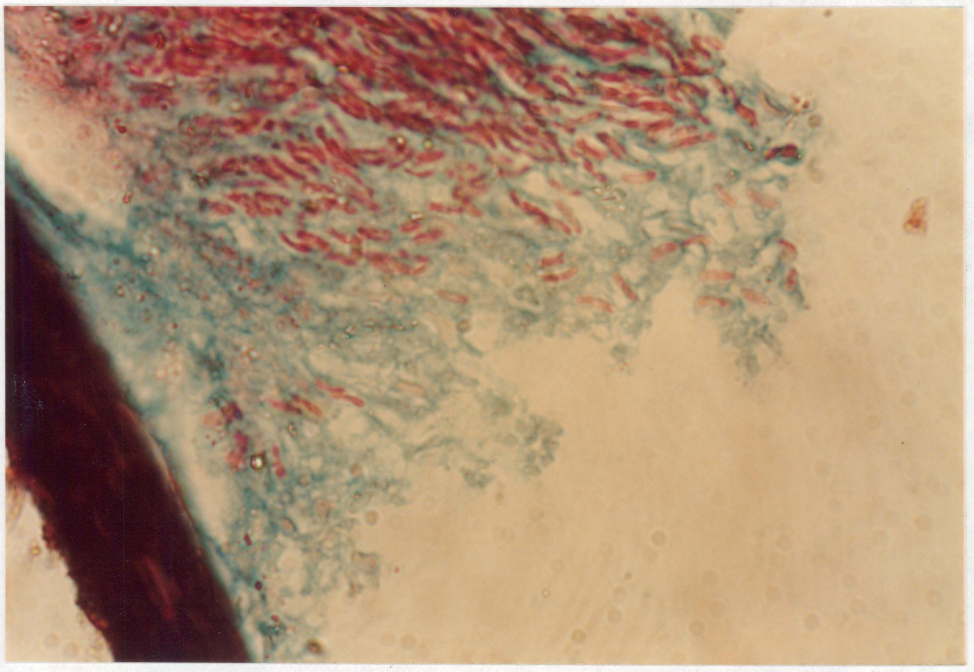


Figure 21. Allantoid, nonseptate ascospores of Endothia viridistroma (1000X).

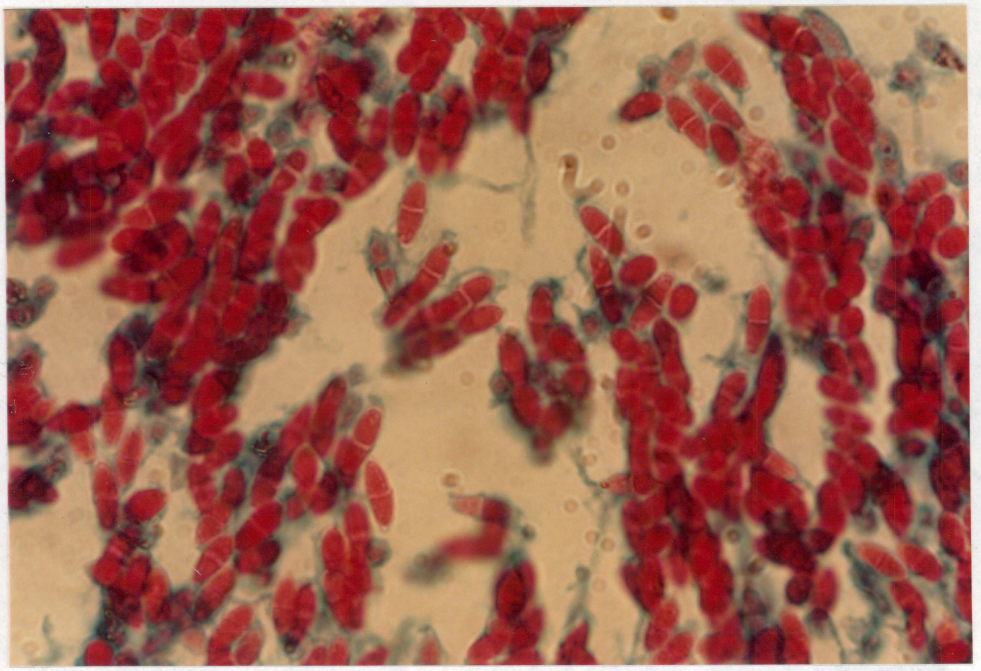


Figure 22. Oblong to ellipsoid, two-celled ascospores of *Endothia parasitica* (1000X).

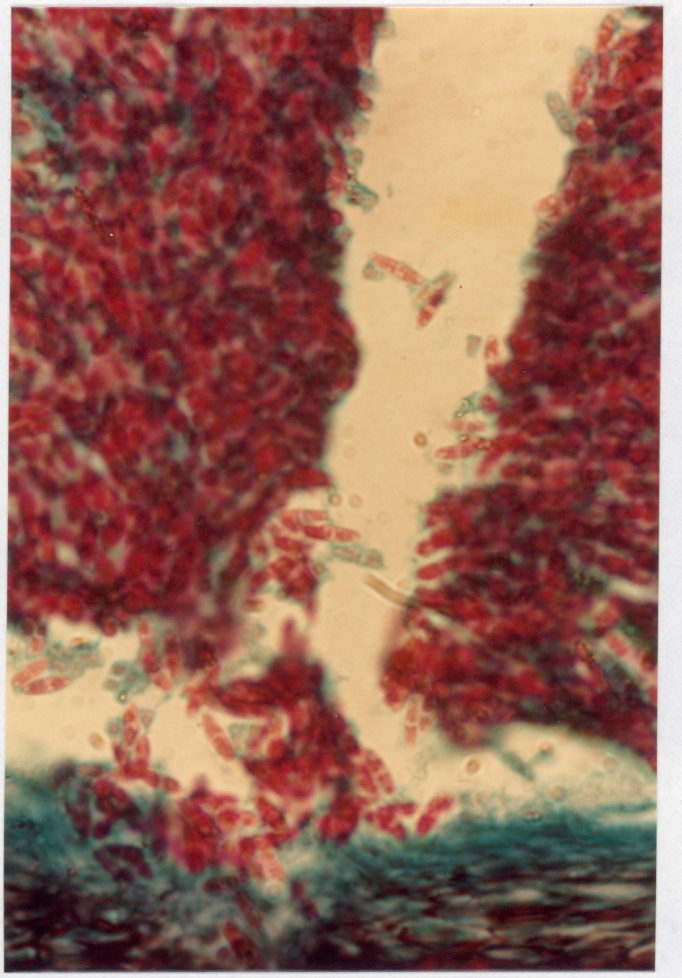


Figure 23. Oblong to ellipsoid, two-celled ascospores of *Endothia coccolobii* (1000X).

families. Barr included over 50 genera in her monograph on the Diaporthales, a project far beyond the scope of this investigation. She therefore has a far better idea of the relationships within the order and among closely related species. On the other hand, her classification greatly changes the identification of these organisms. These fungi are quite similar macroscopically due to their brilliant, erumpent stromata. More extensive investigation will now be required to differentiate Endothia from Cryphonectria; this will be particularly difficult because of the frequent absence of perithecia and ascospores in field collections. The traditonal concept of Endothia united these organisms by their pigment content and pathogenicity as well as by their general morphology.

One potential problem with Barr's classification system is the possible influence of host on stromal morphology. This effect was observed in this study with E. coccolobii on the roots and seed coat of Coccolobis uvifera. Hodges (personal communication) determined that stromal development is extremely variable in C. cubensis. The fungus produced rudimentary to well-developed stromata on clove, Syzygium aromaticum. Stromal development was severely limited on eucalyptus; perithecia and pycnidia were usually superficial and little or no stromal tissue was observed. Traditional taxonomy did not implicate this variability; the clove fungus was designated E. eugeniae, while the eucalyptus pathogen was named Diaporthe (and later

Cryphonectria) cubensis. Cross-inoculation studies with both hosts were needed to verify the unity of these organisms. The influence of host on stromal morphology could have a drastic impact on the taxonomy of the stromatic Ascomycetes; the traditional system and Barr's classification would both be inadequate to allow for this amount of variability. Further studies are needed to determine whether this diversity occurs frequently in nature.

At issue is the fundamental difference in goals between the pathologist and the systematist. Most pathologists view taxonomy as a tool to be used to identify, describe and communicate to others about the organisms with which they work. They require rapid means of identification even in the absence of the sexual stage of the fungus. Systematists are more interested in probing the relationships among organisms and use classification to express these relationships. The two views are not mutually exclusive; a firm understanding of Barr's classification system would allow pathologists to distinguish among all the different genera of the Diaporthales in an organized manner. This investigation has confirmed the accuracy of Barr's observations; there is no technical reason why it should not be adopted. The genus *Cryphonectria* is being used with increasing frequency in the literature (4, 8). The pathologists and mycologists who work with these organisms will make the final determination on the classification through the adoption or rejection of the available systems.

Pigments

Thin-layer chromatography with silica gel plates was used to detect the bisanthraquinone pigments skyrin and oxyskyrin in ethanolic extracts from the mycelia of isolates of E. radicalis, E. coccolobii, E. havanensis, E. eugeniae and C. cubensis. Certain of these isolates represent new acquisitions which were not previously tested by Roane and Stipes (151), while others were reexamined due to uncertainties in identification. Pigment contents by isolate and Rf values are presented in Tables 4 - 9 and summarized in Table 10.

The pigment content of E. radicalis isolates was somewhat variable. All isolates, except E56, produced skyrin, while isolate E92 formed both skyrin and oxyskyrin. These data corroborate those of Roane and Stipes (151) who detected both pigments in American isolates of this species but found only skyrin in isolates from Europe, Australia and New Zealand. Isolate E56, from New Zealand, did not produce any identifiable pigments. This culture did not resemble any of the other isolates in its colony morphology; its identification must be suspect.

Rugulosin, a third bisanthraquinone pigment frequently associated with Endothia species (148, 150) was not formally assayed in this study due to the decomposition of the purified standard. It is characterized by a brilliant yellow color (148,

Table 4. Pigment content of ethanol extracts of mycelium of selected isolates of *E. eugeniae* and *C. cubensis* separated on silica gel thin-layer chromatography plates with a 20:1 ethyl acetate: glacial acetic acid solvent at ambient temperature. Plate 1.

Standards[a]	Species	Isolate	Color[b]	Rf value	Pigment
Oxyskyrin			orange	0.58	
Skyrin			orange	0.65	
	E. eugeniae	E138	none		
		E139	yellow (uv)	0.63	
			orange	0.66	skyrin
		H173	orange (uv)	0.23	
			orange	0.59	oxyskyrin
			orange	0.66	skyrin
			yellow (uv)	0.85	
		H174	orange (uv)	0.22	
			orange	0.58	oxyskyrin
			orange	0.66	skyrin
			yellow (uv)	0.84	
		H176	orange (uv)	0.22	
			orange	0.58	oxyskyrin
			orange	0.65	skyrin
			yellow (uv)	0.79	
	C. cubensis	H91	orange	0.58	oxyskyrin
			orange	0.66	skyrin
		H137	orange (uv)	0.22	
			orange	0.59	oxyskyrin
			orange	0.66	skyrin

Table 4 (Continued)

Standards[a]	Species	Isolate	Color[b]	Rf value	Pigment
		H151	orange (uv)	0.23	
			orange	0.60	oxyskyrin
			orange	0.66	skyrin
			orange (uv)	0.84	
		H154	orange	0.59	oxyskyrin
			orange	0.66	skyrin
			yellow (uv)	0.84	
		H175	orange (uv)	0.22	
			orange	0.59	oxyskyrin
			orange	0.65	skyrin
			orange (uv)	0.80	

[a]Purified standards of skyrin and oxyskyrin applied in solution (1×10^{-2}M) with absolute ethanol as the solvent.

[b]Color recorded under natural light unless otherwise indicated. Colors designated "uv" recorded under ultraviolet light (254 nm).

Table 5. Pigment content of ethanol extracts of mycelium of selected isolates of E. eugeniae and C. cubensis separated on silica gel thin-layer chromatography plates with a 20:1 ethyl acetate: glacial acetic acid solvent at ambient temperature. Plate 2.

Standards[a]	Species	Isolate	Color[b]	Rf value	Pigment
Oxyskyrin			orange	0.60	
Skyrin			orange	0.68	
	E. eugeniae	E138	blue (uv)	0.61	
			blue (uv)	0.76	
		E139	blue (uv)	0.61	skyrin
			orange	0.68	
			blue (uv)	0.78	
		H173	orange	0.60	oxyskyrin
			orange	0.70	skyrin
			blue (uv)	0.84	
		H174	orange	0.61	oxyskyrin
			orange	0.70	skyrin
		H176	orange	0.59	oxyskyrin
			orange	0.68	skyrin
	C. cubensis	H91	orange	0.67	skyrin
		H137	orange	0.57	
			orange	0.67	skyrin

Table 5 (continued)

Standards[a]	Species	Isolate	Color[b]	Rf value	Pigment
		H151	orange	0.67	skyrin
		H154	orange	0.70	
		H175	orange	0.59	oxyskyrin
			orange	0.68	skyrin

[a]Purified standards of skyrin and oxyskyrin applied in solution (1×10^{-2}M) with absolute ethanol as the solvent.

[b]Color recorded under natural light unless otherwise indicated. Colors designated "uv" recorded under ultraviolet light (254 nm).

Table 6. Pigment content of ethanol extracts of mycelium of selected isolates of *E. eugeniae*, *E. havanensis* and *C. cubensis* separated on silica gel thin-layer chromatography plates with a 20:1 ethyl acetate: glacial acetic acid solvent at ambient temperature. Plate 3.

Standards[a]	Species	Isolate	Color[b]	Rf value	Pigment
Oxyskyrin			orange	0.65	
Skyrin			orange	0.73	
	E. havanensis	E40	blue (uv)	0.79	
			orange	0.72	skyrin
		H183	orange	0.73	skyrin
	E. eugeniae	H184	orange	0.66	oxyskyrin
			orange	0.74	skyrin
		H185	orange	0.65	oxyskyrin
			orange	0.75	skyrin
		H186	orange	0.65	oxyskyrin
			orange	0.75	skyrin
		H189	orange	0.73	skyrin
	C. cubensis	H182	blue (uv)	0.66	
			blue (uv)	0.83	

Table 6 (continued)

Standards[a]	Species	Isolate	Color[b]	Rf value	Pigment
		H187	none		
		H188	orange	0.64	oxyskyrin
			orange	0.72	skyrin

[a]Purified standards of skyrin and oxyskyrin applied in solution (1×10^{-2} M) with absolute ethanol as the solvent.

[b]Color recorded under natural light unless otherwise indicated. Colors designated "uv" recorded under ultraviolet light (254 nm).

Table 7. Pigment content of ethanol extracts of mycelium of selected isolates of *E. eugeniae* separated on silica gel thin-layer chromatography plates with a 20:1 ethyl acetate: glacial acetic acid solvent at ambient temperature. Plate 4.

Standards[a]	Species	Isolate	Color[b]	Rf value	Pigment
Oxyskyrin			orange	0.79	
Skyrin			orange	0.85	
	E. eugeniae	E139	orange	0.82	
			orange	0.85	skyrin
			blue (uv)	0.93	
			blue (uv)	0.79	
		E139 (broth)	blue (uv)	0.77	
			blue (uv)	0.93	
		E138 (broth)	blue (uv)	0.77	
			orange	0.85	
			blue (uv)	0.92	

[a]Purified standards of skyrin and oxyskyrin applied in solution (1×10^{-2} M) with absolute ethanol as the solvent.

[b]Color recorded under natural light unless otherwise indicated. Colors designated "uv" recorded under ultraviolet light (254 nm).

Table 8. Pigment content of ethanol extracts of mycelium of selected isolates of *E. radicalis* and *E. coccolobii* separated on silica gel thin-layer chromatography plates with a 20:1 ethyl acetate: glacial acetic acid solvent at ambient temperature. Plate 5.

Standards[a]	Species	Isolate	Color[b]	Rf value	Pigment
Oxyskyrin			orange	0.56	
Skyrin			orange	0.63	
	E. radicalis	E76	orange	0.62	skyrin
		E149	orange	0.62	skyrin
			yellow	0.65	
		E150	orange	0.62	skyrin
			yellow	0.64	
		E151	orange	0.62	skyrin
			yellow	0.65	
		E152	orange	0.62	skyrin
			yellow	0.65	

Table 8 (continued)

Standards[a]	Species	Isolate	Color[b]	Rf value	Pigment
	E. coccolobii	E157	yellow	0.34	
			orange	0.61	skyrin

[a]Purified standards of skyrin and oxyskyrin applied in solution (1×10^{-2} M) with absolute ethanol as the solvent.

[b]Color recorded under natural light unless otherwise indicated. Colors designated "uv" recorded under ultraviolet light (254 nm).

Table 9. Pigment content of ethanol extracts of mycelium of selected isolates of E. radicalis separated on silica gel thin-layer chromatography plates with a 20:1 ethyl acetate: glacial acetic acid solvent at ambient temperature. Plate 6.

Standards[a]	Species	Isolate	Color[b]	Rf value	Pigment
Oxyskyrin			orange	0.71	
Skyrin			orange	0.78	
	E. radicalis	E16	orange	0.80	skyrin
		E42	orange	0.80	skyrin
			yellow	0.82	
		E56	yellow	0.70	
		E64	orange	0.79	skyrin
		E67	orange	0.79	skyrin
			yellow	0.81	
		E76	orange	0.79	skyrin
		E92	yellow (uv)	0.53	
			orange	0.71	oxyskyrin
			orange	0.75	
			orange	0.79	skyrin
			yellow	0.83	
		E150	orange	0.79	skyrin
			yellow	0.81	

Table 9 (continued)

Standards[a]	Species	Isolate	Color[b]	Rf value	Pigment
		E152	yellow (uv)	0.50	
			orange	0.77	skyrin
			yellow	0.81	

[a]Purified standards of skyrin and oxyskyrin applied in solution (1×10^{-2} M) with absolute ethanol as the solvent.

[b]Color recorded under natural light unless otherwise indicated. Colors designated "uv" recorded under ultraviolet light (254 nm).

Table 10. Presence or absence of skyrin, oxyskyrin and perilla purple in isolates of Endothia species and Cryphonectria cubensis.

Species	Isolate	Skyrin[a]	Oxyskyrin	Perilla purple[b]
E. radicalis	E16	+[c]	-	+
	E42	+	-	+
	E56	-	-	-
	E64	+	-	-
	E67	+	-	-
	E76	+	-	-
	E92	+	+	-
	E149	+	-	+
	E150	+	-	+
	E151	+	-	+
	E152	+	-	+
E. tropicalis	E57	ND[d]	ND	-
	E70	ND	ND	-
E. singularis	E52	ND	ND	+
	E58	ND	ND	+
E. viridistroma	E41	ND	ND	-
	E82	ND	ND	-
E. coccolobii	E157	+	-	-
E. havanensis	E158	-	-	-
	E159	-	-	-
	E40	+	-	-
	H183	+	+	-
E. eugeniae	E138	-	-	-
	E139	+	-	-
	H173	+	+	-
	H174	+	+	-
	H176	+	+	-
	H184	+	+	-
	H185	+	+	-
	H186	+	+	-
	H189	+	-	-

Table 10 (continued)

Species	Isolate	Skyrin[a]	Oxyskyrin	Perilla purple[b]
C. cubensis	H91	+	-	-
	H137	+	+	-
	H151	+	+	-
	H154	+	-	-
	H175	+	+	-
	H182	-	-	-
	H187	-	-	-
	H188	+	+	-

[a]Skyrin and oxyskyrin assayed on silica gel thin-layer chromatography plates with ethanol extracts of mycelial pigments. See text for details of extract preparation and chromatography procedure.

[b]The presence of perilla purple was assayed after a minimum of 14 days in isolates of Endothia species and C. cubensis cultured on white cornmeal agar at 25°C.

[c]"+" denotes presence of pigment; "-" denotes absence of pigment.

[d]"ND" signifies data not determined.

151). Its presence is suspected in E. radicalis isolates E42, E92, E149, E150, E151 and E152; if this could be confirmed it would be the first report of this pigment in European isolates of the fungus.

Roane and Stipes (151) detected oxyskyrin and rugulosin in stromal extracts of the type specimen of E. coccolobii. Skyrin alone was obtained from the mycelial extract of a freshly collected isolate of this fungus. This discrepancy should be further investigated with other isolates of E. coccolobii since the pigment content of stromal tissue and mycelia corresponded among the other species (151).

Skyrin and oxyskyrin were obtained from most isolates of E. eugeniae and C. cubensis. These two species are suspected of being conspecific (5); similarities in pigment content would help support this contention. Certain isolates produced only skyrin (E139, H189, H91 and H154), while a few failed to synthesize either skyrin or oxyskyrin (E138, H182, H187). This is the first report of bisanthraquinone pigments in these fungi; no pigments were detected by Roane and Stipes (151) in isolates E138 and E139; these particular isolates were subsequently single-spored and began to produce large amounts of pigment in culture. The orange pigment produced by E138 may be glycosidic since it was detected in the culture broth but not in ethanolic extracts of mycelium. Hodges (personal communication) noted a great deal of variability in pigment production in cultures of C. cubensis; this variation was positively correlated with pycnidial produc-

tion. Lack of pycnidial formation may explain why certain isolates failed to produce one or both pigments. Isolate H182, which did not produce any identifiable pigments, was subsequently shown to have an electrophoretic protein pattern and cycloheximide sensitivity different from those of the other fungi in this group. The lack of pigment production supports, but does not prove, its misidentification. The inability to produce bisanthraquinones led Roane and Stipes (150) to question the inclusion of E. eugeniae in the genus Endothia; this objection has now been removed.

Two isolates of E. havanensis (E40, H183) produced at least one bisanthraquinone. These isolates are suspected of belonging to the E. eugeniae - C. cubensis group due to their electrophoretic patterns; their pigment contents do not negate this supposition. Two additional isolates tentatively identified as E. havanensis (E158 and E159) did not produce any identifiable pigments. The exact identification of these isolates is not possible due to the lack of reliable reference cultures of E. havanensis.

Perilla purple was produced on white cornmeal by certain isolates of E. radicalis (E16, E42, E149, E150, E151 and E152) and all available isolates of E. singularis (E52, E58). It was not produced by isolates of E. tropicalis, E. viridistroma, E. coccolobii, E. havanensis, E. eugeniae and C. cubensis. Roane and Stipes (149) reported the production of perilla purple by

colonies of E. eugeniae; this was not confirmed in this study. The production of perilla purple is a key diagnostic characteristic of E. radicalis (168); the lack of its formation by supposed isolates of this species emphasizes the heterogeneity of this group. Several of these isolates may have been misidentified; this suspicion is supported by electrophoretic evidence. A pigment similar to perilla purple was observed in isolates of E. parasitica on glucose-yeast extract agar (media preparation detailed in Appendix II) when contaminated with an unidentified fungus; neither the contaminant nor the E. parasitica isolates produced the pigment when cultured separately. The contaminating mold may have formed some intermediate compound in the synthesis of perilla purple which was further metabolized to the pigment by E. parasitica. Pigment production in Endothia is extremely dependent on the amino acid content of the medium (D. F. Hindal, personal communication) and should not be relied on for species identification.

Chapter 3

Polyacrylamide gel electrophoresis of *Endothia* species.

LITERATURE REVIEW

Fungal taxonomy is traditionally based on morphological criteria; reproductive and vegetative structures are described and comparisons made among taxa. Difficulties frequently arise, however, and fungal taxonomy is fraught with inconsistencies and uncertainties. The morphology of some fungi may be too simple for good differentiation. Some organisms, such as *Rhizoctonia* DC. and *Sclerotium* Tode, produce only sterile mycelia, while others, such as *Cephalosporium* Corda, have extremely simple, nondescript, reproductive features. When morphology is complex and can be used to distinguish taxa, it may not correspond to genetic relationships among organisms. Many form genera are based on sophisticated morphological structures but are associated with diverse perfect genera. The form genus *Monilia* Bonorden, for example, includes dermatophytes with no known perfect forms, as well as the imperfect stages of the saprophytic, perithecial Ascomycete *Neurospora* Shear and B. Dodge and the parasitic, apothecial ascomycete *Sclerotinia* Fuckel. The classification of these forms as one genus does not reflect their true genetic relationship. At the same time, differences in morphology may not correspond with genetic diversity. Fungi can

be extremely pleomorphic; a single genotype can often produce a large number of morphological characteristics (74). Morphological features can also vary with the environment and nutrient availability. Finding the particular fungal structure required for identification is often difficult. Sexual or asexual fruiting bodies may form only rarely or they may require particular conditions for their induction. These requirements are usually not known by the investigator (69, 74).

Molecular approaches to taxonomy are frequently used when traditional taxonomic techniques cannot be applied. They are also quite useful when the phylogenetic relationships of a group of organisms are being studied (202). Since polypeptides are the result of gene activity, protein analysis can be used to study the genetic relationships among organisms (74). Theoretically, this technique can be used to study the differences among closely related strains of an organism or among saprophytic and pathogenic forms of one species (162).

Electrophoresis is based on the movement of charged particles in an electric field. Proteins vary in their net charge due to differences in their amino acid composition; they will migrate at different rates in an electrical field. The molecular weight and the shape of the molecule will also affect their movement; the supporting matrix can act as a molecular sieve and decrease the movement of larger proteins, so that lower molecular weight compounds migrate faster. The final position of the proteins can be visualized by general protein stains or

specific activity stains that detect particular enzymes (65, 131, 164). Although this technique has been utilized in biochemistry since the 1930's, it was not applied to fungal material until 1962, when Chang, Srb and Steward (35) studied the soluble proteins of Neurospora. In this work, three different supporting matrices were used which resulted in different resolutions of the protein bands. Paper electrophoresis resulted in the separation of six to eight fractions, while gel electrophoresis with starch and polyacrylamide resolved 18 and 25 fractions, respectively. Although the primary objective of this experiment was not to study taxonomy, it suggested several important points which could be useful to taxonomists. The technique was shown to yield reproducible results over duplicated runs of the same protein extract and by extracts prepared at different times from the same isolate. Proteins from different isolates varied from each other.

In the following year, Clare (36) was the first to apply electrophoresis to a taxonomic problem. He compared eight species of Pythium Pringsh. by starch gel electrophoresis, and found that different species produced dissimilar protein patterns. Intraspecific variation was minimal, and the age of the culture did not seem to influence the electrophoretic pattern.

These two papers led to a virtual explosion of research, and many different fungi were studied by electrophoresis. The

technique was applied to different taxonomic levels, including genus, species, race, strain and formae specialis. This profusion of research lead to conflicting opinions about the validity of electrophoresis and its application to fungal taxonomy. The results of most of these studies suggested that the technique could be used to distinguish among species of a genus; intraspecific variation in protein patterns was usually less than interspecific variation. This was confirmed with species of the genera Ceratocystis Ell. and Halst. (191, 192), Endothia (55, 194, 197), Sclerotinia (40, 213, 215), Candida Berkhout (169), Puccinia Pers. (33, 178) Polyporus Micheli ex Adans. (98, 163), Pleurotus (Fr.) Kummer (24), Fusarium Link (1, 70, 85, 141, 158), Phymatotrichum Bonorden (71), Septoria Sacc. (52), Aspergillus Micheli ex Link (96, 127) Nasuno, 126), Penicillium Link (18), Entomophthora Fres. (105), Phytophthora deBary (37, 61, 68, 69), Peronosclerospora C. Shaw (27) and Verticillium Nees (73, 120, 137, 162). In some cases, electrophoresis has been used to address controversial taxonomic issues. In the past, mycologists could not agree on the taxonomic status of the dark mycelial and microsclerotial forms of Verticillium albo-atrum Reinke and Berth. European workers recognized these two forms as different species; V. albo-atrum was used for those fungi which produce thick, dark-walled resting hyphae, while V. dahliae Kleb. referred to microsclerotia-producing isolates. Whitney, Vaughn & Heale (209) demonstrated that these forms are readily differentiated by electrophoresis, and they concluded that each

deserved species status. This work was later confirmed by other workers (73, 120, 162), although one study showed that the two species were closely related (137, 138).

Electrophoresis has also been used to identify unknown fungal isolates and to find inconsistencies in established taxonomic groupings. An unidentified species of Phytophthora on milkweed, Morrenia odorata Lindl., was shown to have a protein distribution pattern similar to that of P. palmivora (Butl.) Butl., a pathogen of cacao, Theobroma cacao L. This identification was confirmed with studies of colony morphology, sporangial structure, oospore production and pathogenicity (61). An extensive survey of 47 isolates of Sclerotinia (213, 215) resulted in the formation of three distinct groups, corresponding to the species S. minor Jagger, S. trifoliorum Erikss. and S. sclerotiorum (Lib.) deBary. This information contradicted the work of Purdy (140) which established only one species, S. sclerotiorum. Three morphological forms of Phytophthora palmivora were readily distinguished by electrophoresis; Kaosiri & Zentmeyer (91) have suggested that they are sufficiently dissimilar to be considered separate species. Two species of Phymatotrichum, P. fimicola Dring and P. fungicola Zeller, were shown to be closely related physiologically, despite morphological differences (71), while electrophoresis of Armillariella mellea (Vahl ex Fr.) Karst. has confirmed that this species is probably a complex of taxonomically distinct fungi (123).

In other cases, however, electrophoresis has not been successful in distinguishing among morphologically distinct species. Sorenson, Larsh and Hemp (190) showed that isolates of different Aspergillus species were quite variable in the number and position of protein bands. Other fungi which have not been resolved at the species level include Taphrina Fr. (185, 186), Ustilago (Pers.) Roussel (28), Pholiota (Fr.) Kummer (87) and Drechslera Ito (179). The use of electrophoresis at taxonomic levels higher and lower than the species has been shown to be even more unreliable. Glynn and Reid (70) demonstrated that electrophoretic patterns of Verticillium albo-atrum isolates were very similar to those of Fusarium oxysporum f. sp. cubense (E. F. Sm.) S. and H. This was confirmed by Glynn and Reid (70). The production of similar protein profiles by taxonomically unrelated fungi is probably due to the co-migration of nonhomologous proteins (184). Distinguishing among subspecies, races, strains and formae specialis of fungi is also quite difficult. Differentiation was successful between races 21 and 111 of Puccinia graminis Pers. f. sp. tritici Erikss. and Henn. (103), while no differences were seen among banding patterns of races of P. graminis f. sp. avenae Erikss. and Henn. (177, 178), P. graminis f. sp. secalis Erikss. and Henn., P. recondita Rob. ex Desm. f. sp. secalis (Erikss.) Carl., P. recondita f. sp. tritici (Erikss.) Carl., P. coronata Corda f. sp. avenae Eriks., P. hordei Otth., or races 1581, 32 and 56 of P. graminis f. sp. tritici (178). Gill and Powell (67) were not able to distinguish

individual banding patterns among eight races of Phytophthora fragariae Hickman. In most cases, different formae speciales of Fusarium could not be recognized (70, 109, 158), and the few researchers who claimed identification (90, 108, 141) did not use sufficient material to establish normal levels of variation among isolates. Protein patterns of formae specialis of Puccinia were found to be more consistent than among races but less variable than those observed for different species (33, 178). Gill and Zentmeyer (69) were unable to distinguish between two mating types of Phytophthora cinnamomi Rands, while electrophoresis could be used to predict successful crosses among strains of Rhodotorula Harrison and Rhodosporidium Banno (216). Differentiation among pathologically different strains is also unpredictable. Aggressive and nonaggressive isolates of Ceratocystis ulmi (Buism.) C. Moreau were easily discerned by both isozyme analysis (19) and general protein patterns (89), while "progressive" virulent and "fluctuating" less-virulent (88) strains of Verticillium albo-atrum could not be detected (120). Differences in geographic origin and relative degree of pathogenicity were detected in five isolates of Cryphonectria cubensis by the enzymes esterase, peroxidase, phosphoglucomutase and hexokinase (3). Schmidt, Curtis and Bean (159) could not differentiate aflatoxin-producing strains of Aspergillus flavis Link ex Fries or A. parasiticus Speare, while the five genetically distinct anastomosis groups of Rhizoctonia solani Kuehn could

be easily identified (143). It is apparent that small differences in genotype, such as those that affect pathogenicity or mating type, may not be detected by electrophoresis (177, 178). Races and formae speciales may require interactions with the host before any enzymatic differences are expressed; in vitro experiments would not reveal any electrophoretic variation (67).

Because of this diversity of results, researchers have re-investigated the factors which are responsible for intraspecific variation which tend to obscure interspecific differences. It appears that a large amount of this variation can be due to the developmental stage of the fungus at the time of sampling as well as the cultural conditions used in the study.

Preliminary tests should be conducted before an electrophoresis project is started. It is essential to determine whether the age of the culture will influence the protein pattern; this varies with the organism that is being studied. Milton, Rogers and Isaac (120) demonstrated that the number, position and intensity of protein bands varied with the age of cultures of Verticillium albo-atrum. Similar results have been obtained with other species of Verticillium (137, 138) as well as with species of Penicillium (18) and Fusarium (209). This does not seem to occur in all fungi, however, for the age of culture reportedly had no influence on protein patterns obtained from isolates of Pythium (36), Septoria (52) or Phytophthora (69).

Intraspecific variation due to the age of the culture is frequently due to the morphological development of the fungus.

Many enzymatic changes are associated with different stages of sclerotial development in Sclerotinia sclerotiorum (214). Comparisons among isolates at different stages of sclerotial formation yield highly inconsistent results. Pelletier and Hall (137, 138) determined that the protein composition of mycelium was quite different from that of conidia for species of Verticillium. A similar situation was found among the catalase isozymes of Fusarium solani (Mart.) Appel and Wr. (72). Of the four catalase bands which were resolved, two arose from the mycelium and two originiated from the conidia. The number and position of bands was therefore dependent on the relative amounts of spores and mycelium in the culture, which varied with the morphological stage of the fungus. A similar phenomenon was demonstrated with two species of Drechslera (179).

Reproducible protein patterns can frequently be obtained with cells at the same stage of development. It is quite desirable to produce protein extracts solely from conidia, but this is usually not possible due to the large quantity which would be required. Another alternative is to use cultures which are at some known developmental stage, such as in the early or late logarithmic phase of growth. This may be quite difficult to determine for cultures which have radically different growth habits (184). Another important consideration is the portion of the thallus which should be sampled; this is especially important in the higher basidiomycetes, where differences in protein con-

tent have been demonstrated in various portions of the basidiocarp. This variation may be associated with the developmental stage of the fungus. In Lentinus edodes (Berk.) Sing., the distribution of esterase isozymes varied between the pileus and the stipe of the mature basidiocarp, between the primordial tissue of the fruiting body and the pileus, and between the vegetative, aerial mycelium and the basidiocarp primordium (132). Similar variations have been reported for enzymes of Coprinus cinerens (Schaeff.) Cke. (121) and Agaricus bisporis (Lange) Imbach (136). Dramatic variations in enzyme content have been attributed to sexual morphogenesis and the formation of the dikaryon in Coprinus congregatus (Bull.) Fr. and Schizophyllum commune Fr. (47, 205). Evers and Ross (57) have criticized these experiments, however, and maintain that these large variations are due to dye artifacts and protein aggregation in the heterokaryon. In any case, taxonomic studies should be conducted with tissues in the same developmental stage.

Another agent which may be responsible for intraspecific variation is the length of time which the fungus has been maintained in culture. It is usually best to use freshly isolated organisms or those which have been lyophilized immediately after isolation. Cultures which are maintained by periodic transferring are often subject to mutation; such mutations will be expressed immediately when a haploid genome is involved. Fungi can also selectively adapt to the medium upon which they have been maintained. For these reasons, the electrophoretic profiles

may not truly reflect the actual genetic relationships which would be expressed by patterns from freshly isolated organisms (184).

The type of medium which is used is another important consideration. Although Durbin (52) found no differences in electrophoretic patterns produced by Septoria avenae Frank on three different media, Glynn and Reid (70) reported that the medium composition had a definite influence on the profile of Fusarium oxysporum f. sp. cubense. Variations in protein pattern may also result when the nitrogen source becomes depleted or whether the organism is growing in shake or static culture (18). It is important to use the same type of medium throughout the study in order to ensure that variation is not due to different cultural conditions (184). Electrophoretic patterns may be difficult to interpret even when interspecific variation is greater than intraspecific differences due to the difficulty in representing and analyzing variations in protein content. In many of the earlier papers, interpretative drawings were published, often with a photograph or densitometer tracing. Another popular way of interpreting the banding pattern has been through the use of Rf values, where the distance that a band has traveled is related to the movement of a tracker dye. This technique has been criticized, however, due to the close proximity and variable thicknesses of many bands. Minor variations of the band positions may also occur on different gels due to

irregularities of current, voltage or resistance. Shipton and Fleischmann (177, 178) determined that Ef values could express a large degree of variation depending on how they were measured.

The recent application of numerical taxonomy has helped to correct some of the problems associated with representation and interpretation. Different authors have used different numerical techniques. In a study of Candida (169), 31 isolates representing seven species were compared on the basis of presence or absence of protein bands of both acidic and basic protein fractions. Computer analysis created similarity coefficients by comparing the number of bands common between each of the 465 pairs of isolates. Each isolate was considered to be an operational taxonomic unit (OTU). A taxonomic hierarchy was then constructed by the complete linkage method of cluster analysis. In almost all cases, isolates within a species were grouped before species were delineated. Both acidic and basic protein fractions were used to provide a significant number of characters for a meaningful analysis. Only those bands which were present or absent in all isolates of a species were used; this is probably one reason why the species were so accurately separated. The authors concluded that it is probably best to use purely qualitative differences, such as the presence or absence of bands, rather than quantitative information, such as Ef values or optical density measurements.

Numerical analysis has also been applied in a study involving the genus Polyporus (98). In this work, the banding pat-

terns of nine enzymes were compared among nine species. The data were collected by dividing the gel into five millimeter sections, and the presence or absence of a band in each division was recorded. The analysis was then based on the probability of obtaining, due to chance alone, the observed number of matched bands between each pair of isolates. When a large number of the bands matched, there was only a low probablility that the similarity was due to chance; the fungi were assumed to be closely related. This type of analysis assumes that the production of each isozyme is under independent genetic control. This may be a fallacious assumption, however, and the genetics of the organisms should be studied to confirm or refute this. In this study, isolates within a species were more closely related than those from different species, as long as standardized cultural conditions were maintained. For this reason, the application of electrophoresis was seen as a valuable taxonomic tool within the Polyporaceae.

Computer analysis in other groups of fungi has not revealed significant interspecific differences, however. Snider and Kramer (185) studied 50 isolates of 31 species of Taphrina. In this study, bands were recorded as Rf values. A Fortran program was then used to calculate the mean number of homologous bands per gel for the 31 species, as well as for species grouped by host family or ascus morphology. A biomedical computer program, termed BMDP1M, was then utilized to cluster the species on the

basis of similarity coefficients between isolates. Distance computations were also calculated by this program. Unfortunately, there was no clustering of species belonging to different host family groups or ascus types, and isolates of the same species did not always cluster together. One possible explanation for this inconsistency is that the fungi had mutated in culture and no longer reflected the typical characteristics of the species. The authors also postulated that the large number of species involved may have complicated the analysis so that interspecific differences were obscured. When only two species were examined (186), a more realistic clustering was obtained. Since the electrophoretic pattern of each isolate varies within the species, increasing the number of species analyzed raises the probability that isolates from different species will be considered similar. For this reason, Snider and Kramer (185, 186) concluded that electrophoresis should be used in conjunction with other biochemical and morphological criteria, such as information obtained from G/C content, serology, morphology and nutrient studies.

Little work has been done to relate electrophoresis of fungal proteins with genetic systems. It is possible that this may be more difficult for fungi than for plants and animals due to the presence of haploid or dikaryotic genomes as well as their high level of mutation in culture. The technique of electrophoresis is heavily biased in detecting genetic changes. Small differences in amino acid sequences may result in large altera-

tions of electrophoretic mobility, while a large number of mutations may not be detected if they do not affect the net charge of the protein. Proteins may have similar migration rates even if they differ in size, net charge or amino acid composition (73). Recent techniques, which involve two dimensional electrophoresis, isoelectric focusing and immunoelectrophoresis are much more sensitive and are being used increasingly to study genetic and taxonomic problems (1, 85, 86, 90, 197). Isozyme analysis has also been used to interpret genetic relationships (27, 154).

Despite its limitations, electrophoresis has made, and will continue to make, significant contributions to our understanding of fungal taxonomy. Protein patterns are quite reproducible if cultural conditions and morphological stages of the fungus are standardized. Most studies have shown that electrophoresis can be used at the species level since interspecific variation is usually larger than intraspecific differences. The technique appears to be more limited at the subspecies and generic level, but more rigorous studies are needed to confirm this.

Preliminary studies have been conducted with electrophoresis and Endothia taxonomy. Isoelectric focusing was used to differentiate three isolates of E. radicalis from five isolates of E parasitica, including representatives of the hypovirulent strain (197). Seven isolates of E. gyrosa were separated from five isolates of E. parasitica by the distribution of soluble proteins with polyacrylamide tube gels (55). This differentia-

tion was confirmed with a subsequent study in which general esterase and β-D-glucosidase activity was detected in addition to general soluble proteins (194). These experiments indicate that differences among species of Endothia can be detected; in the current investigation, the number of species and isolates was increased to further study inter- and intraspecific relationships.

MATERIALS AND METHODS

Experiment 1: Effect of harvest date on electrophoretic pattern.

A. Production of inoculum and measurement of growth curve.

Four monoconidial isolates each of E. parasitica (J4, J5, J10, J16) and E. gyrosa (E38, E110, J17, J18) and two monoconidial isolates of E. radicalis (E16, E42) were maintained at 4 °C on "double" glucose-yeast extract agar slants (see Appendix II for formulations of all culture media). Hosts, collection dates and geographic origins of these isolates are listed in Table 3. Five mm mycelial plugs, taken from the margins of 6 to 17-day-old colonies grown on glucose-yeast extract agar (gyea) plates were used to inoculate 50 ml of glucose-yeast extract broth (gyb) in 250 ml Erlenmeyer flasks. The initial pH of the broth was measured at the time of inoculation. The liquid cultures were incubated in darkness at 25 °C and harvested at 4 d intervals; twenty flasks per isolate were collected at 4 and 8 d and 15 flasks per isolate were harvested at 12 and 16 d. An

additional three flasks per isolate were collected at each harvest date for dry weight determinations. Mycelial mats were gathered by vacuum filtration in a Buchner funnel and were thoroughly washed with distilled water to remove all traces of broth. The mycelia of a given isolate were combined and frozen at 0 °C; they were then lyophilized for 24 h in a VirTis® freeze-drier. Dry weight determinations were made by harvesting the mycelium of each flask on preweighed filter paper; the mycelium-laden paper was then oven-dried for 24 h and reweighed with a Mettler balance. Dry weight was calculated as the difference of these two measurements. The growth curve was obtained by plotting the average dry weight of an isolate against the number of days of growth. The pH of the broth and the degree of pigmentation of the culture were recorded for each flask used in the dry weight analysis. The pH of a noninoculated control flask, which was incubated for the same amount of time as those harvested, was also measured.

B. Production of protein extract and protein assay.

The lyophilized mycelium was ground into a fine powder in a chilled mortar and pestle and stored in glass culture tubes over desiccant at 0 °C. The mycelial powder was examined microscopically for the presence of ruptured hyphae. Proteins were extracted overnight at 4 °C in 0.125 Tris-Cl buffer (pH 6.8) + 10% glycerol at the rate of 50 mg powder / ml buffer. The supernatant was collected by centrifugation for 20 min in an IEC

clinical centrifuge and was further clarified by passage through a Swinney filter fitted with a penicillin sensitivity disk (Schleicher and Schuell, Inc., No. 740-E).

The protein concentrations of the extracts were determined by the Bradford Coomassie blue assay (29) using a Bausch and Lomb Spectronic 20. A standard curve was obtained with known concentrations of BioRad Protein Standard II, a preparation of bovine serum albumin. Individual standard curves were formed for separate electrophoretic runs. The standard curve was plotted on graph paper and regression analysis was performed with a TI-55 pocket calculator. Protein concentrations of the unknown extracts were determined by interpolation from the regression line. The sample volume which contained 80 µg of protein was then calculated for each isolate.

C. Electrophoresis.

A modification of the Laemmli technique (97), employing a BioRad Model 220 dual vertical slab gel electrophoresis cell, was used for all electrophoresis. The system was discontinuous and nondissociating and was composed of a 3.9% acrylamide stacking gel (pH 6.8), a 12% acrylamide separating gel (pH 8.8) and a 0.05 M Tris- 0.38 M glycine electrode buffer (pH 8.3). Bromophenol blue (0.05%) was incorporated into the stacking gel as a tracking dye. Teflon well-forming combs (28 X 8 X 1 mm) were inserted into the stacking gel before polymerization. The length of the

stacking gel was 20 mm, while the final dimensions of the separating gel were (155 X 93 x 1 mm). Additional details of gel preparation are presented in Appendix III.

Eighty µl of each protein extract were loaded into the sample wells; ten samples were analyzed per gel. Electrophoresis was conducted with constant current using 30 mA as the tracking dye moved through the stacking gel and 60 mA as it moved through the separating gel. The interior of the cell was cooled with ice water (0 °C) pumped through the unit with a Beckman Thermocirculator. Electrophoresis proceeded until the tracking dye reached the bottom of the separating gel (2 - 2.5 h). The gels were then removed and stained overnight with 0.1% Coomassie blue R250 in water: methanol: glacial acetic acid (5: 5: 2). Destaining was achieved with 12.5% isopropanol, 10% acetic acid and usually required 6 to 8 hours. Gels were stored at 4 °C in 7% acetic acid in Ziploc® sandwich bags. The banding patterns of the gels were delineated with a spectrodensitometer (Schoeffel Instrument Corporation - Model SD 3000) fitted with a density computer (Schoeffel Instrument Corporation - Model SDC 300) and a recorder (Houston Instruments - Omniscribe). The densitometer tracings, as well as visual observations and manual measurements, were used to produce diagrams of each gel. Bands separated by at least 1 mm were resolved as individual proteins. The similarity coefficient of banding patterns between each pair of protein samples was computed by the formula:

$$\text{Similarity Coefficient} = \frac{\text{No. similar bands}}{\text{No. similar bands + No. different bands}}$$

The percent similarity of each isolate pair was obtained by multiplying the similarity coefficient by 100. Comparisons were made between the similarity coefficients and the position of the fungus on the growth curve.

Experiment 2: Inter- and intraspecific variation in electrophoretic protein distribution.

The protein banding patterns of 78 monoconidial isolates, representing 11 species of Endothia were compared by polyacrylamide gel electrophoresis. Isolates used in this study were E. parasitica - E24, E85, E86, E87, E88, E89, E95, E96, E106, E107, E108, E112, E113, E114, E115, E137, E153, E155, J1, J2, J4, J5, J6, J7, J8, J9, J10, J11, J12, J13, J14, J15, J16; E. gyrosa - E18, E20, E30, E37, E38, E48, E50, E51, E72, E73, E74, E98, E110, E111, E123, E146, E147, E154, J17, J18; E. radicalis - E16, E42, E56, E64, E67, E75, E76, E92, E150, E152; E. eugeniae - E138, E139; E. macrospora - E54; E. japonica - E53, E59; E. singularis - E52, E58; E. tropicalis - E57, E70; E. viridistroma - E41, E82; E. coccolobii - E157; E. havanensis - E40, E158, E159. The hosts, collection dates and geographic origins of these isolates are listed in Table 3. Maintenance of fungal cultures, sample preparation and electrophoresis were conducted as in the previous experiment with some modifications. The pH of the broth was standardized to 5.70 by the addition of 1% lactic acid or 1% NaOH

after autoclaving. Ten flasks were inoculated for each isolate, and cultures were harvested after 8 d. Pigment production and pH were recorded from three randomly chosen flasks per isolate; dry weights were not obtained. The quantity of mycelial powder used for extraction was increased to 60 mg/ml buffer. The volume of sample which contained 30 µg of protein was calculated from the protein assay; this volume, to a maximum of 35 µl, was loaded into the 18 X 4 X 1 mm sample wells. Twenty samples were analyzed per gel. The length of the stacking gel was increased to 30 mm, while the dimensions of the separating gel remained unchanged. Electrophoresis was conducted with two separate extractions of two replicate sets of powders.

Intraspecific variation was initially studied with 33 isolates of E. parasitica and 20 isolates of E. gyrosa. Ten isolates of each species, which represented the range of intraspecific variability, were then selected for comparisons of protein distribution among the remaining species. These isolates were: E. parasitica - E24, E85, E86, E87, E88, E89, E95, E96, E153, E155; E. gyrosa - E18, E20, E30, E37, E38, E48, E50, E98, E145, E154. Similarity coefficients were calculated for each pair of isolates; all combinations of isolates were produced on a series of gels since comparisons could not be made between different gels. The percent similarities were arranged in a similarity matrix, and a dendrogram was constructed by unweighted pair group average cluster analysis.

Experiment 3: Influence of abnormal morphology types associated with hypovirulent strains of E. parasitica on electrophoretic protein pattern.

The electrophoretic patterns of eight suspected hypovirulent isolates of E. parasitica (RW911P, RW9929, RW17B, RW51P, RW494B, RWEp113) were compared to those of wild, virulent strains of the fungus (E24, E85, E86, E89, E155, J10, J16). The suspected hypovirulent isolates were obtained from R. L. Willey at West Virginia University. With the exception of RWEp113, these cultures were isolated from cankers of American chestnut previously formed by inoculation with known hypovirulent strains of E. parasitica. Isolate RwEp113 is a single spore culture of a known hypovirulent French strain isolated by Grente. Each isolate contained at least one species of double-stranded RNA (dsRNA). The cultures displayed abnormal colony morphologies when grown on Difco potato dextrose agar (PDA) supplemented with 5 µg/l biotin and 100 mg/l methionine and were categorized into six morphological groups (Willey, 1982). The morphological groups of the suspected hypovirulent isolates, hosts, geographic origins and dates of collection are presented in Table 3. Electrophoresis was performed with mass cultures and single spore isolates of the suspected hypovirulent strains; all wild-type isolates were monoconidial. Maintenance of fungal cultures, sample preparation and electrophoresis were conducted as in the previous experiment.

Experiment 4: Comparison of electrophoretic patterns of C. cubensis and E. eugeniae.

The electrophoretic protein distribution of seven monoconidial isolates of C. cubensis (H91, H137, H151, H154, H175, H182, H187, H188) and nine monoconidial isolates of E. eugeniae (H173, H174, H176, H184, H185, H186, H189, E138, E139) were compared. Additional isolates of E. havanensis (E40, H183, E158) and E. coccolobii (E157) were also included in this study. The hosts, dates of collection and geographic origins of these isolates are listed in Table 3. Maintenance of fungal cultures, sample preparation and electrophoresis were conducted as in Experiment 2.

Experiment 5: Use of specific activity stains with different Endothia species.

Specific activity stains, which detect the presence of acid and alkaline phosphatase, α- and β-esterase and β-D-glucosidase were used to examine representative isolates of 11 species of Endothia and Cryphonectria cubensis. The isolates utilized were: E. parasitica - E87, E88, E89; E. gyrosa - E48, E50, E154; E. radicalis - E16, E42, E56, E64, E67, E76, E92, E150, E152; E. singularis - E52; E. macrospora - E54; E. coccolobii - E157; E. viridistroma - E41, E82; E. japonica - E53, E59; E. tropicalis - E57, E70; E. havanensis - E158, E159, E40, H183; E. eugeniae - E138, E139, H173, H185, H186, H189; C. cubensis - H137, H151, H182, H188. The hosts, dates of collection and geographic origins of the isolates are listed in Table 3.

Acetone powders were prepared as described by Stipes (192) with some modifications. Mycelial mats were obtained as in the previous section and stored in distilled water overnight at 4 °C. The mycelial mats were then homogenized in cold acetone (-75 °C) in a Waring blender with an Eberbach stainless steel jar and blending assembly for 1 min. The homogenate was collected by vacuum filtration and rehomogenized in cold acetone for an additional 30 sec. The resultant powder was collected by vacuum filtration and stored in glass culture tubes over desiccant at 0 °C. Electrophoresis was performed as in the previous experiments except that constant volumes (20 or 30 µl) of protein extract were applied to each well. Staining procedures were conducted as in Shaw and Prasad (164) for α - and β-esterase, Nealson and Garber (127) for acid and alkaline phosphatase and Cohen, Rutenburg, Tsou, Woodbury and Seligman (39) for β-D-glucosidase. After development, gels were photographed and stored at 4 °C in 5% acetic acid. Duplicate electrophoretic runs were made with protein extracts obtained from two sets of acetone powders.

RESULTS AND DISCUSSION

1. Effect of harvest date on electrophoretic protein pattern.

This preliminary test was conducted to determine whether the stage of the growth cycle at time of mycelial collection influenced the number and position of protein bands obtained by

PAGE. The optimum harvest time was also determined for subsequent studies. Changes in broth pH, mycelial dry weight and protein concentration of the samples were monitored at 4, 8, 12 and 16 d for selected isolates of E. parasitica, E. gyrosa and E. radicalis. These data are presented in Tables 11, 12 and 13; growth curves of each species are shown in Figures 24, 25 and 26.

The initial pH of the broth was highly variable between tests. In subsequent experiments, the pH was standardized after the broth was autoclaved and before it was inoculated. The pH values of noninoculated control flasks generally decreased by approximately 0.1-0.2 pH units by the end of the 16 d experiment. Changes in the broth pH of inoculated flasks were much more dramatic; the extent of the change was species dependent. The pH of broth inoculated with isolates of E. parasitica generally dropped more than one pH unit by the end of the study; those inoculated with isolates of E. gyrosa decreased by 0.2-0.8 pH units. The two isolates of E. radicalis were quite diverse; the pH of broth inoculated with E16 increased by almost 2 pH units while that of E67 decreased slightly. Only one noninoculated control flask was used at each harvest date due to spatial limitations; these data are somewhat limited. Similar trends were observed in subsequent experiments.

The dry weight of each isolate increased in a sigmoid curve. The four different isolates of E. parasitica and E. gyrosa were quite uniform in their response. Isolates of E. parasitica produced shallow growth curves, while those of E. gyrosa were

Table 11. Effect of date of harvest on broth pH, dry weight of mycelium and protein concentration of isolates of E. parasitica cultured in glucose-yeast extract broth at 25°C.

Date of Harvest (Days)	Isolate	pH[a] (Initial)	pH[b] (Control)	pH[c] (Sample)	Dry Weight[d] (mg)	Protein Concentration[e] (µg/ 100 µl)
4	J4	5.74	5.76	5.04 (0.04)	16.3 (2.1)	130
	J5	5.82	5.74	5.09 (0.03)	9.7 (1.5)	130
	J10	5.89	5.73	5.01 (0.04)	9.3 (2.5)	130
	J16	5.25	5.08	4.60 (0.03)	3.7 (2.1)	130
	Average				9.8 (5.1)	130
8	J4		5.72	4.58 (0.04)	19.7 (1.5)	--
	J5		5.75	4.63 (0.05)	20.3 (3.2)	44
	J10		5.68	4.58 (0.02)	22.7 (0.6)	99
	J16		5.19	4.27 (0.01)	13.8 (1.0)	130
	Average				19.1 (3.9)	91
12	J4		5.67	4.48 (0.01)	29.7 (2.1)	82
	J5		5.69	4.52 (0.01)	43.3 (2.1)	94
	J10		5.68	4.42 (0.04)	36.7 (1.5)	67
	J16		5.23	4.28 (0.11)	26.8 (3.3)	97
	Average				34.1 (7.4)	85
16	J4		5.67	4.55 (0.06)	37.3 (1.2)	--
	J5		5.65	4.55 (0.02)	49.0 (0.0)	107
	J10		5.68	4.55 (0.02)	40.0 (1.0)	93
	J16		5.15	4.46 (0.07)	29.0 (2.0)	103
	Average				38.8 (8.2)	101

Table 11 (continued)

[a]Initial pH determined after autoclaving and before inoculation.

[b]Control pH determined from noninoculated broth at time of harvest; one flask per isolate.

[c]Sample pH determined from inoculated flasks at time of harvest; average of three flasks per isolate. Standard error follows in parentheses.

[d]Dry weight determined gravimetrically after mycelium collected on tared filter paper and dried overnight at 100°C. Average of three colonies. Standard error follows in parentheses.

[e]Protein concentration calculated by Bradford assay (29) from a supernatant derived from a suspension of lyophilized mycelium in 0.125 M Tris-Cl buffer + 10% glycerol (50 mg lyophilized mycelium/ml buffer). Significant differences were found among protein concentrations at different harvest dates by single factor analysis of variance (ANOVA) at $p = 0.001$. Least significant difference of the means at $p = 0.01$ (LSD) = 25.3.

[f]Data not recorded.

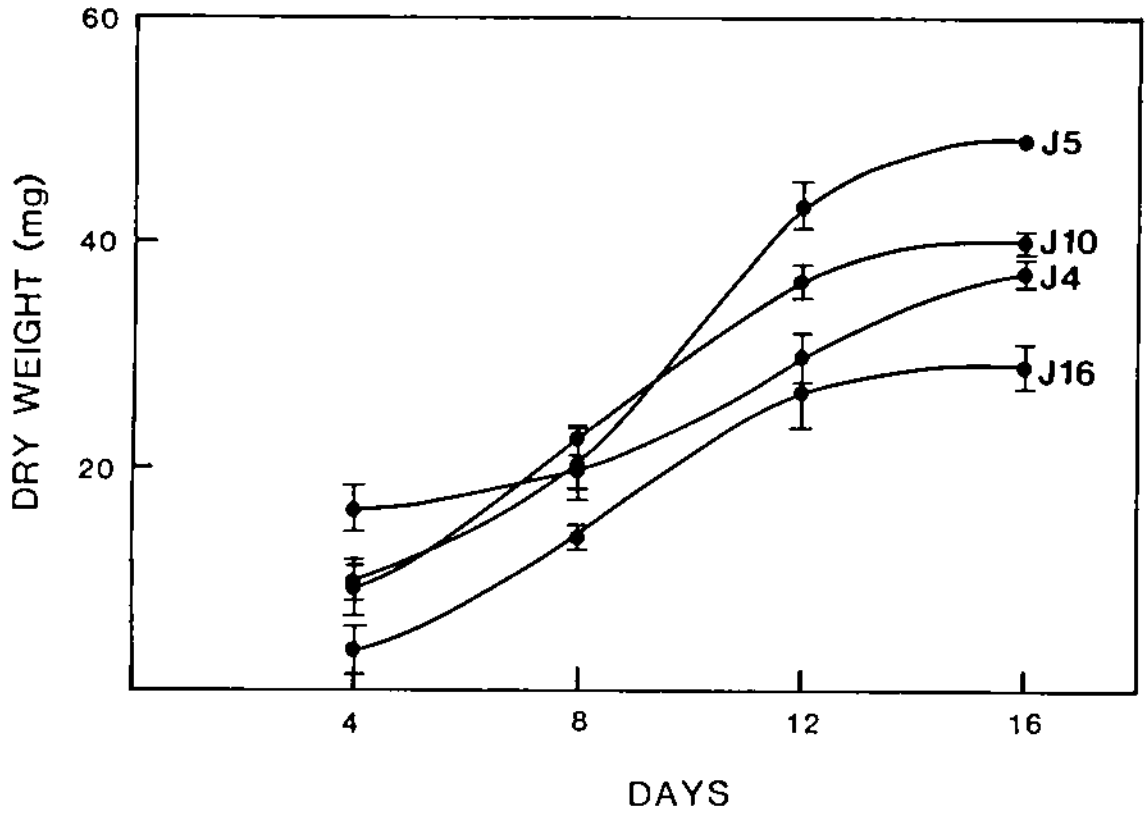


Figure 24. Dry weight (mg) of four isolates of Endothia parasitica grown in glucose-yeast extract broth at 25 °C for 4, 8, 12 and 16 d. Dry weight determined gravimetrically on tared filter paper after mycelium dried overnight at 100 °C. Average of three colonies per isolate; vertical bars represent standard error of the mean.

Table 12. Effect of date of harvest on broth pH, dry weight of mycelium and protein concentration of isolates of E. gyrosa cultured in glucose-yeast extract broth at 25°C.

Date of Harvest (Days)	Isolate	pH[a] (Initial)	pH[b] (Control)	pH[c] (Sample)	Dry Weight[d] (mg)	Protein Concentration[e] (µg/ 100 µl)
4	J17	5.84	--[f]	5.40 (0.02)	1.0 (0.0)	213
	J18	5.35	--	5.15 (0.02)	1.0 (0.0)	119
	E38	5.49	5.22	4.85 (0.08)	1.0 (0.0)	202
	E110	5.38	5.30	5.04 (0.02)	1.0 (0.0	193
	Average				1.0 (0.0)	182
8	J17		5.73	4.74 (0.02)	27.3 (4.7)	167
	J18		5.32	4.58 (0.02)	29.7 (6.8)	195
	E38		5.14	4.66 (0.11)	55.3 (5.5)	185
	E110		5.30	4.71 (0.05)	22.8 (4.2)	160
	Average				35.3 (13.4)	177
12	J17		5.69	4.74 (0.04)	80.7 (0.6)	110
	J18		5.19	4.37 (0.11)	71.3 (3.7)	203
	E38		5.24	5.06 (0.15)	96.0 (3.6)	178
	E110		5.12	4.49 (0.08)	82.8 (2.1)	175
	Average				82.7 (10.2)	166
16	J17		5.83	5.33 (0.11)	104.3 (4.9)	93
	E38		5.23	4.99 (0.17)	99.0 (13.8)	120
	E110		--	--	91.0 (8.2)	106
	Average				98.1 (6.7)	106

Table 12 (continued)

[a]Initial pH determined after autoclaving and before inoculation.

[b]Control pH determined from noninoculated broth at time of harvest; one flask per isolate.

[c]Sample pH determined from inoculated flasks at time of harvest; average of three flasks per isolate Standard error follows in parentheses.

[d]Dry weight determined gravimetrically after mycelium collected on tared filter paper and dried overnight at 100°C. Average of three colonies. Standard error follows in parentheses.

[e]Protein concentration calculated by the Bradford assay (29) from a supernatant derived from a suspension of lyophilized mycelium in 0.125 M Tris-Cl buffer + 10% glycerol (50 mg lyophilized mycelium/ml buffer). No significant differences were found among protein concentrations at different harvest dates by single factor analysis of variance (ANOVA).

[f]Data not recorded.

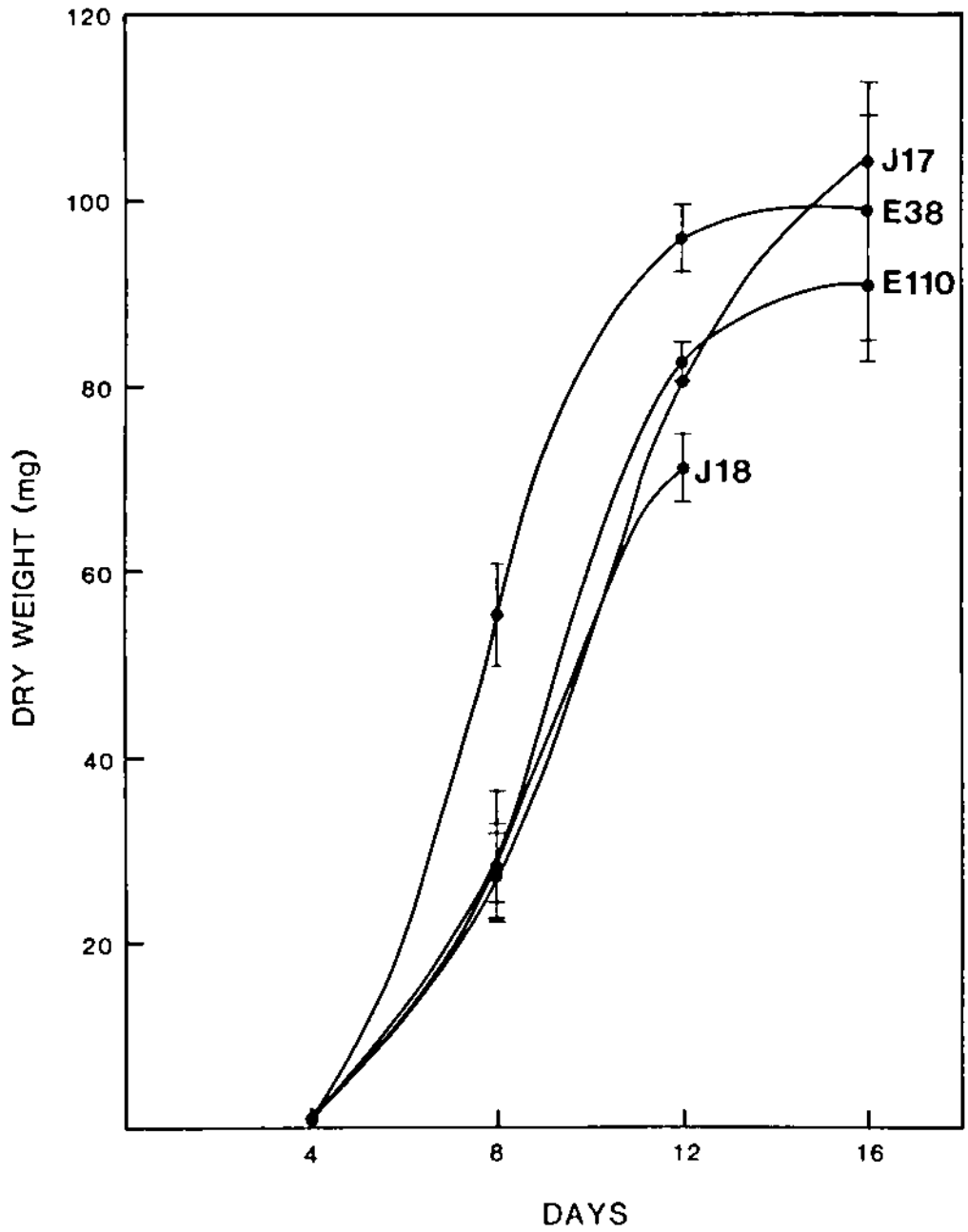


Figure 25. Dry weight (mg) of four isolates of Endothia gyrosa grown in glucose-yeast extract broth at 25 °C for 4, 8, 12 and 16 d. Dry weight determined gravimetrically on tared filter paper after mycelium dried overnight at 100 °C. Average of three colonies per isolate; vertical bars represent standard error of the mean.

Table 13. Effect of date of harvest on broth pH, dry weight of mycelium and protein concentration of isolates of E. radicalis cultured in glucose-yeast extract broth at 25°C.

Date of Harvest (Days)	Isolate	pH[a] (Initial)	pH[b] (Control)	pH[c] (Sample)	Dry Weight[d] (mg)	Protein Concentration[e] (µg/ 100 µl)
4	E16	5.96	--[f]	5.22 (0.06)	--	260
	E67	5.40	5.38	5.24 (0.05)	5.0 (1.0)	244
	Average				5.0 (1.0)	252
8	E16		6.02	6.40 (0.17)	74.7 (7.5)	216
	E67		5.36	5.76 (0.02)	33.0 (1.0)	196
	Average				53.8 (29.5)	206
12	E16		5.99	7.28 (0.19)	113.7 (2.5)	170
	E67		5.42	5.39 (0.06)	50.7 (3.0)	194
	Average				82.2 (44.5)	182
16	E16		6.00	7.87 (0.02)	112.7 (2.5)	84
	E67		5.33	5.03 (0.25)	57.3 (8.5)	260
	Average				85.0 (39.2)	172

Table 13 (continued)

[a]Initial pH determined after autoclaving and before inoculation.

[b]Control pH determined from noninoculated broth at time of harvest; one flask per isolate.

[c]Sample pH determined from inoculated flasks at time of harvest; average of three flasks per isolate. Standard error follows in parentheses.

[d]Dry weight determined gravimetrically after mycelium collected on tared filter paper and dried overnight at 100°C. Average of three colonies. Standard error follows in parentheses.

[e]Protein concentration calculated by the Bradford assay (29) from a supernatant derived from a suspension of lyophilized mycelium in 0.125 M Tris-Cl buffer + 10% glycerol (50 mg lyophilized mycelium/ml buffer). Significant differences found among protein concentrations at different harvest dates by single-factor analysis of variance (ANOVA) at $p = 0.005$. Least significant difference of means at $p = 0.01$ (LSD) = 24.3.

[f]Data not recorded.

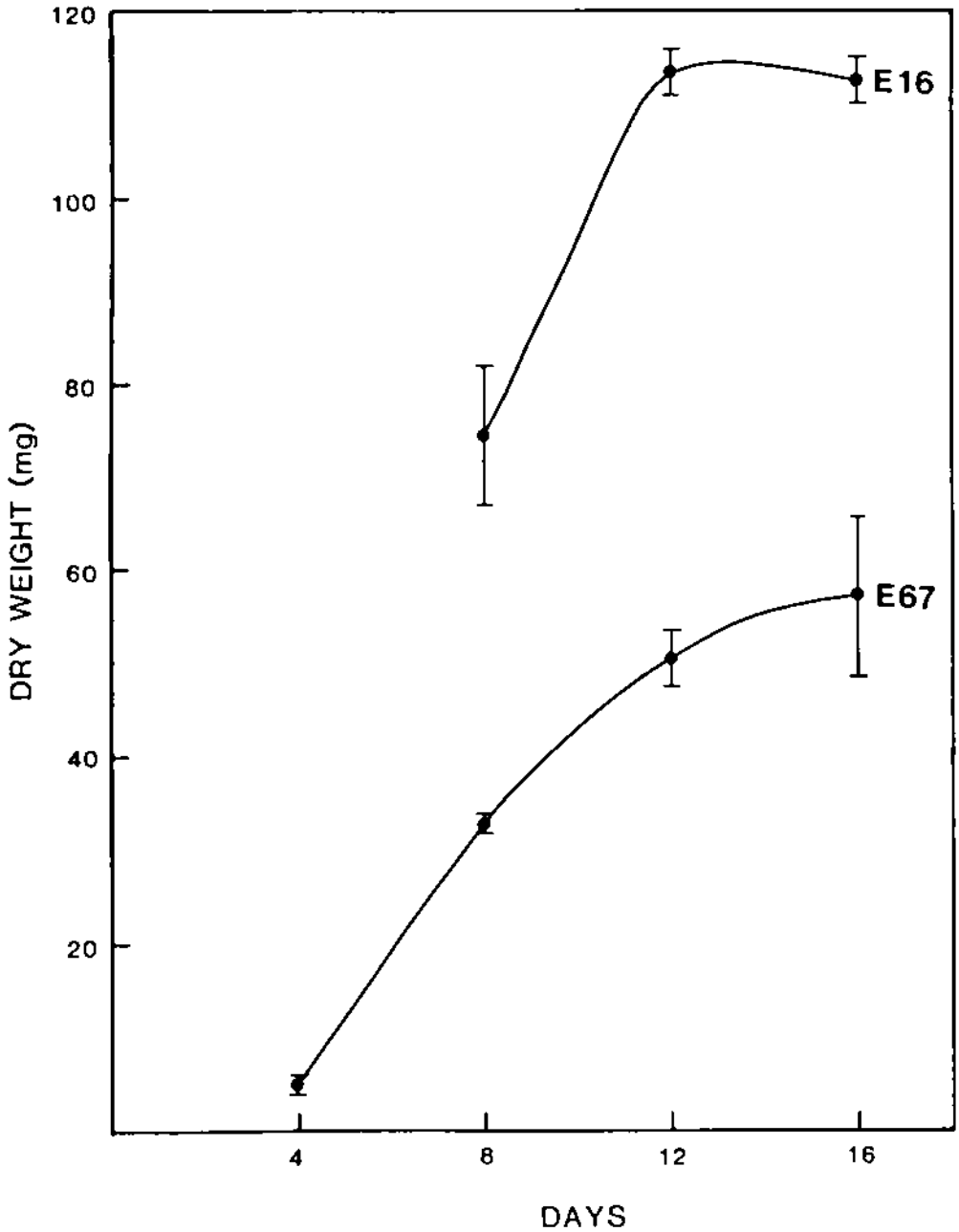


Figure 26. Dry weight (mg) of two isolates of Endothia radicalis grown in glucose-yeast extract broth at 25 °C for 4, 8, 12 and 16 d. Dry weight determined gravimetrically on tared filter paper after mycelium dried overnight at 100 °C. Average of three colonies per isolate; vertical bars represent standard error of the mean.

steeper and showed greater mycelial production. The two isolates of E. radicalis were once again diverse; E16 accumulated almost twice as much mycelium as did E67. Log phase occurred from 4 to 12 d for each isolate.

The concentration of protein was also determined for each isolate at the different dates of harvest. A characteristic standard curve obtained for the protein assay is presented in Figure 27. Single-factor analysis of variance was used to determine whether significant differences existed among protein concentrations from mycelia harvested at different times. Significant differences were found ($p = 0.001$) for E. parasitica and E. radicalis; no significant differences were detected for E. gyrosa. The least significant difference of the means (LSD) was used as an a priori test to determine whether higher concentrations of protein were produced in the early stages of the growth curve. Significantly higher ($p = 0.01$) concentrations of proteins were detected at 4 d for E. parasitica and 4 and 8 d for E. radicalis than for those collected at the remaining times. The LSD values are listed in tables 11 and 13.

Differences in banding patterns were detected at different harvest dates for all isolates; interpretative drawings and similarity matrices for each set of gels are presented in Figures 28 - 32 and Tables 14 - 23. In most cases, only minor variations were apparent (similarity coefficients 0.85 - 1.0 between different harvest dates), although larger differences were also

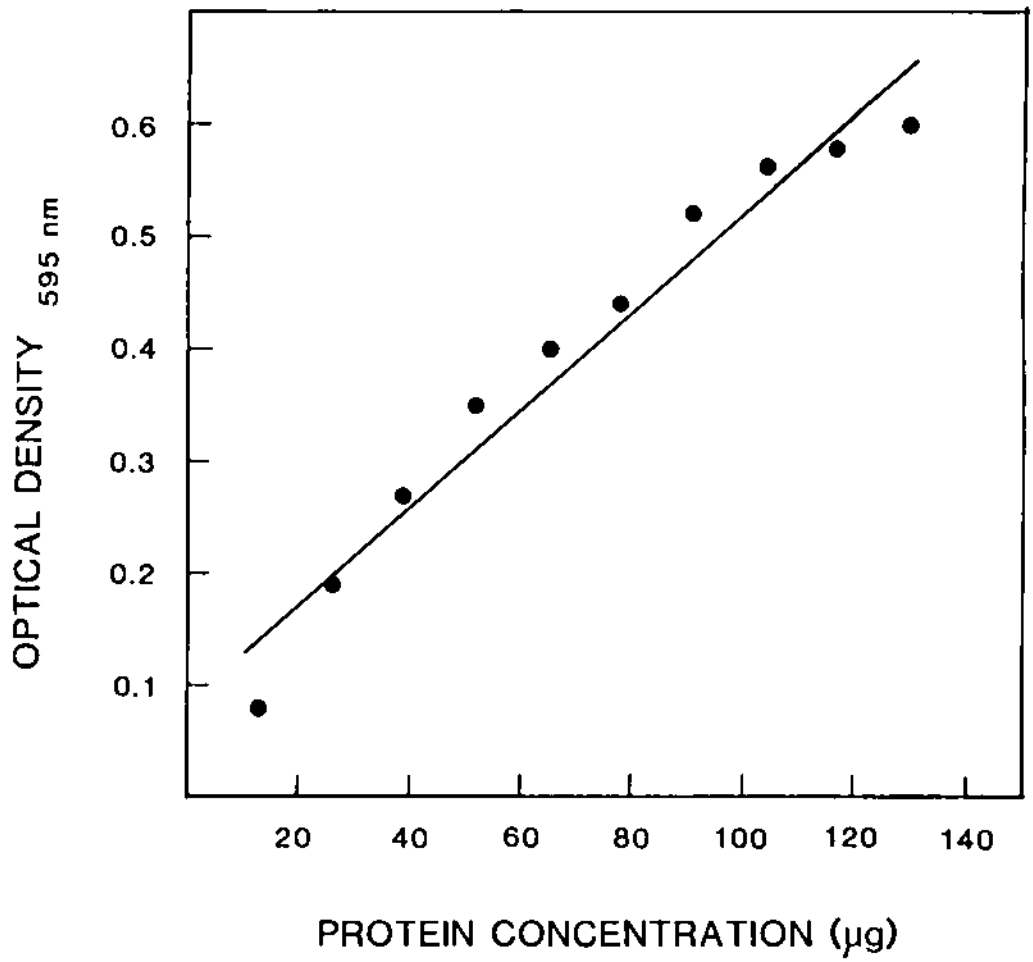


Figure 27. Representative standard curve obtained with serial dilutions of BioRad Protein Standard II and the Bradford Coomassie blue protein assay (28). Linear regression plotted with a TI-55 pocket calculator: R^2 value = 0.986. Protein concentrations of samples calculated from region of curve between 0.20 to 0.55 optical density units.

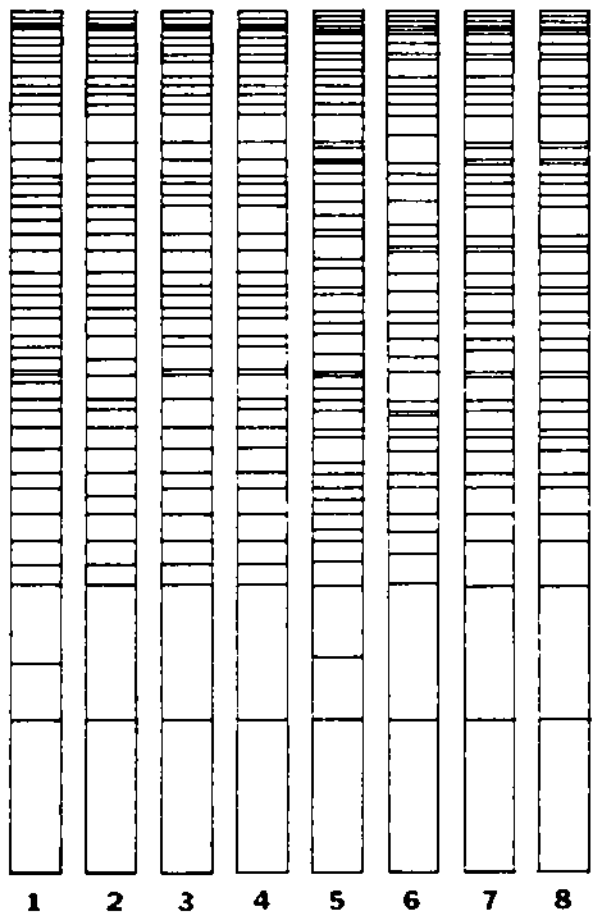


Figure 28. Interpretative drawing of polyacrylamide electrophoretic gel of two isolates of Endothia parasitica grown for 4, 8, 12 and 16 d. Isolate and date of harvest by lane number: 1 - J4 (4 d); 2 - J4 (8 d); 3 - J4 (12 d); 4 - J4 (16 d); 5 - J5 (4 d); 6 - J5 (8 d); 7 - J5 (12 d); 8 - J5 (16 d).

Table 14. Percent similarity of protein banding patterns from lyophilized mycelia of isolate J4 of *E. parasitica* incubated for 4, 8, 12 and 16 days.

Days	Percent Similarity			
4	100			
8	87	100		
12	89	84	100	
16	89	86	98	100
Days	4	8	12	16

Table 15. Percent similarity of protein banding patterns from lyophilized mycelia of isolate J5 of *E. parasitica* incubated for 4, 8, 12 and 16 days.

Days	Percent Similarity			
4	100			
8	53	100		
12	62	69	100	
16	62	69	100	100
Days	4	8	12	16

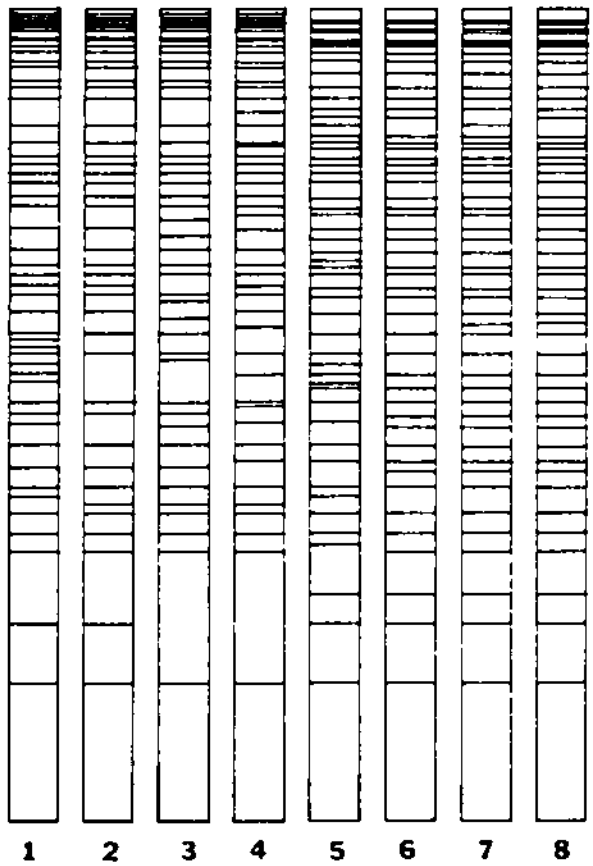


Figure 29. Interpretative drawing of polyacylamide electrophoretic gel of two isolates of Endothia parasitica grown for 4, 8, 12 and 16 d. Isolate and date of harvest by lane number: 1 - J10 (4 d); 2 - J10 (8 d); 3 - J10 (12 d); 4 - J10 (16 d); 5 - J16 (4 d); 6 - J16 (8 d); 7 - J16 (12 d); 8 - J16 (16 d).

Table 16. Percent similarity of protein banding patterns from lyophilized mycelia of isolate J10 of E. parasitica incubated for 4, 8, 12 and 16 days.

Days	Percent Similarity			
4	100			
8	84	100		
12	71	80	100	
16	77	76	64	100
Days	4	8	12	16

Table 17. Percent similarity of protein banding patterns from lyophilized mycelia of isolate J16 of E. parasitica incubated for 4, 8, 12 and 16 days.

Days	Percent Similarity			
4	100			
8	80	100		
12	79	94	100	
16	79	98	96	100
Days	4	8	12	16

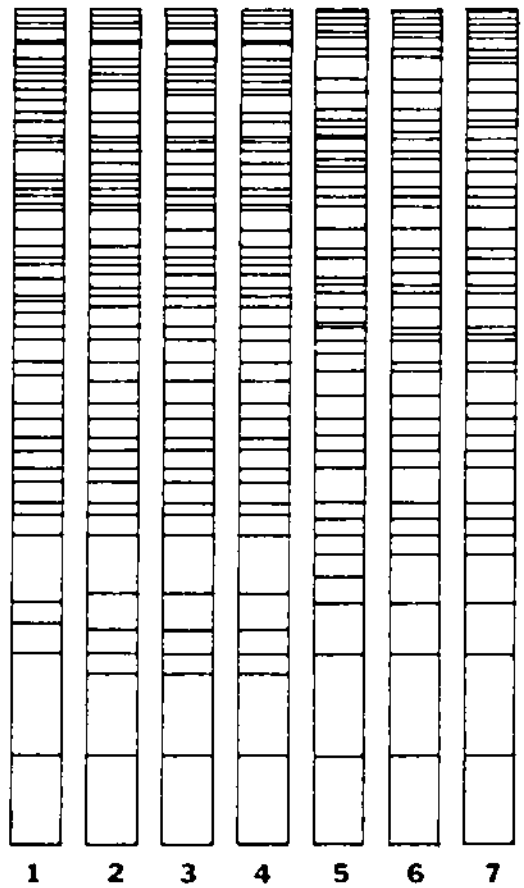


Figure 30. Interpretative drawing of polyacylamide electrophoretic gel of two isolates of Endothia gyrosa grown for 4, 8, 12 and 16 d. Isolate and date of harvest by lane number: 1 - J17 (4 d); 2 - J17 (8 d); 3 - J17 (12 d); 4 - J17 (16 d); 5 - J18 (4 d); 6 - J18 (8 d); 7 - J18 (12 d).

Table 18. Percent similarity of protein banding patterns from lyophilized mycelia of isolate J17 of E. gyrosa incubated for 4, 8, 12 and 16 days.

Days	Percent Similarity			
4	100			
8	73	100		
12	70	96	100	
16	70	96	100	100
Days	4	8	12	16

Table 19. Percent similarity of protein banding patterns from lyophilized mycelia of isolate J18 of E. gyrosa incubated for 4, 8 and 12 days.

Days	Percent Similarity		
4	100		
8	78	100	
12	76	89	100
Days	4	8	12

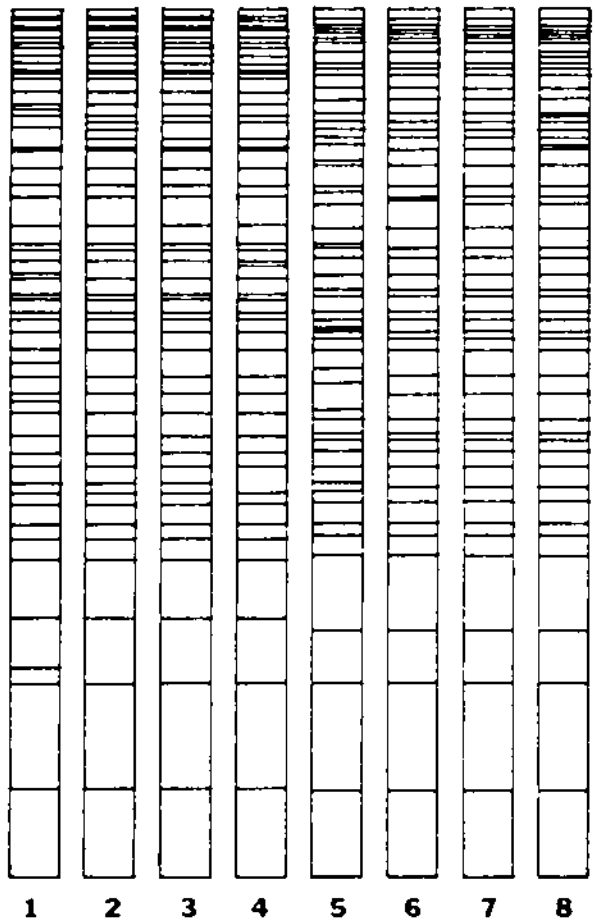


Figure 31. Interpretative drawing of polyacylamide electrophoretic gel of two isolates of Endothia gyrosa grown for 4, 8, 12 and 16 d. Isolate and date of harvest by lane number: 1 - E110 (4 d); 2 - E110 (8 d); 3 - E110 (12 d); 4 - E110 (16 d); 5 - E38 (4 d); 6 - E38 (8 d); 7 - E38 (12 d); 8 - E38 (16 d).

Table 20. Percent similarity of protein banding patterns from lyophilized mycelia of isolate E110 of *E. gyrosa* incubated for 4, 8, 12 and 16 days.

Days	Percent Similarity			
4	100			
8	87	100		
12	85	98	100	
16	82	94	96	100
Days	4	8	12	16

Table 21. Percent similarity of protein banding patterns from lyophilized mycelia of isolate E38 of E. gyrosa incubated for 4, 8, 12 and 16 days.

Days	Percent Similarity			
4	100			
8	72	100		
12	74	98	100	
16	73	92	94	100
Days	4	8	12	16

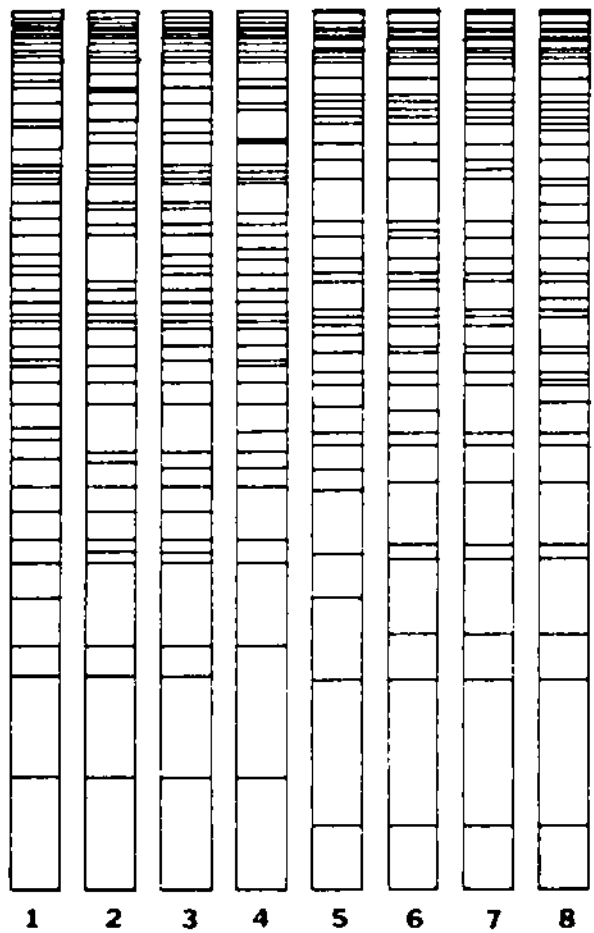


Figure 32. Interpretative drawing of polyacrylamide electrophoretic gel of two isolates of Endothia radicalis grown for 4, 8, 12 and 16 d. Isolate and date of harvest by lane number: 1 - E16 (4 d); 2 - E16 (8 d); 3 - E16 (12 d); 4 - E16 (16 d);5 - E67 (4 d); 6 - E67 (8 d); 7 - E67 (12 d); 8 - E67 (16 d).

Table 22. Percent similarity of protein banding patterns from lyophilized mycelia of isolate E16 of *E. radicalis* incubated for 4, 8, 12 and 16 days.

Days	Percent Similarity			
4	100			
8	63	100		
12	71	82	100	
16	56	62	67	100
Days	4	8	12	16

Table 23. Percent similarity of protein banding patterns from lyophilized mycelia of isolate E67 of E. radicalis incubated for 4, 8, 12 and 16 days.

Days	Percent Similarity			
4	100			
8	74	100		
12	74	91	100	
16	77	79	83	100
Days	4	8	12	16

found. The protein distribution of E. parasitica isolates were the most uniform between 12 and 16 d with the exception of isolate J10. Variations in protein concentration were frequently observed among the different lanes on the gel despite the application of supposedly uniform amounts of protein. Some variation in banding patterns can be ascribed to this difference in concentration; fewer bands are resolved if less protein is present and more are detected if the protein concentration is too high. This increases the number of unmatched bands between any pair of harvest dates and artificially lowers the similarity coefficient. This phenomenon was responsible for the extremely low similarity coefficients of J5 (0.53-0.69) (Table 15) and J10 (0.64-0.84) (Table 15). Isolates of E. gyrosa (Tables 18 - 21) were much more uniform at different harvest dates than were isolates of E. parasitica (0.70-1.00) (Tables 14 - 17); the greatest differences were observed between the banding patterns produced at 4 d when compared with those obtained at other times. The banding pattern of E. radicalis isolate E67 (Table 23) was more uniform with time than was that of isolate E16 (Table 22); no harvest date seemed particularly variable among these two isolates.

An important issue was decided from this preliminary study. It was necessary to determine some uniform time at which all samples would be collected since the banding patterns were somewhat dependent upon the position of the growth curve at time of harvest. An incubation time of 8 d was decided upon for several

reasons. The growth curve of all isolates was in the log phase at this time. Protein concentrations, although somewhat lower for isolates of E. parasitica than those cultured for 4 d, were still at high levels and enough mycelium had grown so that adequate samples could be produced. Sporulation was minimal at 8 d; conidia were not observed by microscopic examination of broth and mycelium and pigment production, which has been correlated with sporulation (82), was still not extensive. Ideally, growth curves should be produced for each isolate and the mycelium collected at a specific point on the curve. This is not possible on a practical level, however, and a uniform harvest date of 8 d was seen as the best alternative.

2. Electrophoresis of different species of Endothia.

Initial electrophoretic runs with 33 and 21 isolates of E. parasitica and E. gyrosa, respectively, revealed little intraspecific variation. For this reason, ten representative isolates of each of these two species were chosen that would represent the range of protein banding patterns observed. Representatives from different geographic sources and isolates which had been in culture for varying amounts of time were also selected. Geographic origin and length of time in culture did not seem to influence protein distribution. This is evident in the final dendrogram where isolates of E. parasitica from Georgia (E24), Tennessee (E85), Virginia (E87, E153, E155) and Pennsylvania (E96) were all grouped with 100% similarity; these isolates had

been in culture for one to eleven years.

The measurements of broth pH at time of harvest and protein concentration of the final samples also revealed some specific taxonomic trends; these data are presented in Table 24. The pH of E. parasitica isolates was dependent on the amount of pigment released into the broth. Samples which were classified as "deep straw" were always 0.3-0.4 pH units lower than those in which pigments were absent or at low levels. The standard errors of the pH readings of E. parasitica isolates were subsequently higher than those of E. gyrosa and many of the other species. In most cases, broth samples were chosen that represented the range of pigmentation. This resulted in quite divergent pH values for each E. parasitica isolate.

Nested analysis of variance was used to determine that significant differences existed among pH values in both sets of collections. Unfortunately, the large variation in sample size precluded the use of a priori or a posteriori tests to locate the differences among the mean pH values of each species. An examination of the data does reveal consistent trends. The pH values of E. eugeniae isolates (3.82 - 4.18) were generally lower than those of the other species, while those of E. tropicalis were markedly higher (5.21-5.42). The majority of the isolates produced pH values in the range of 4.50 - 5.00. Isolates identified as E. radicalis displayed quite diverse pH values; several of these were quite high (E16, E42, E64, E67 and E76) while the

Table 24. Date of inoculation, pH of broth and concentration of soluble proteins from lyophilized mycelia of Endothia species used for electrophoresis.

Species	Isolate	Set 1				Set 2			
		Date of Inoculation	pH of Broth[a]	Protein[b] Concentration 1	2	Date of Inoculation	pH of Broth	Protein Concentration 1	2
E. parasitica	E24	7/31/83	4.27 (0.20)	75	74	8/27/83	4.31 (0.25)	77	92
" "	E85	7/31/83	4.32 (0.22)	78	84	8/27/83	4.48 (0.34)	77	92
" "	E86	7/31/83	4.32 (0.02)	70	73	8/27/83	4.32 (0.26)	65	79
" "	E87	7/31/83	4.28 (0.02)	96	107	8/27/83	4.38 (0.20)	87	101
" "	E88	7/31/83	4.44 (0.04)	78	76	8/27/83	4.75 (0.04)	116	280
" "	E89	7/31/83	4.27 (0.16)	66	65	8/27/83	4.59 (0.14)	105	115
" "	E95	7/31/83	4.53 (0.06)	82	76	8/27/83	4.35 (0.25)	94	100
" "	E96	7/31/83	4.26 (0.30)	89	90	8/27/83	4.28 (0.26)	85	104
" "	E153	7/31/83	4.36 (0.32)	68	71	8/27/83	4.43 (0.32)	80	96
" "	E155	7/31/83	4.25 (0.40)	87	85	8/27/83	4.47 (0.28)	73	85
E. gyrosa	E18	7/29/83	4.66 (0.08)	308	283	9/7/83	4.64 (0.09)	320	290
" "	E20	7/29/83	4.82 (0.03)	332	330	9/7/83	4.77 (0.04)	293	327
" "	E30	7/29/83	4.79 (0.05)	332	313	9/7/83	4.8 (0.07)	293	300
" "	E37	7/29/83	4.70 (0.02)	314	313	9/7/83	4.56 (0.09)	327	320
" "	E38	7/29/83	4.72 (0.05)	320	320	9/7/83	4.66 (0.03)	317	300
" "	E48	7/29/83	4.71 (0.04)	338	303	9/7/83	4.76 (0.06)	317	267
" "	E50	7/29/83	4.70 (0.02)	332	307	9/7/83	4.60 (0.05)	313	327
" "	E98	7/29/83	4.71 (0.04)	367	300	9/7/83	4.71 (0.10)	327	327
" "	E145	7/29/83	4.78 (0.03)	314	303	9/7/83	4.67 (0.03)	380	393
" "	E154	7/29/83	4.71 (0.01)	320	263	9/7/83	4.67 (0.03)	300	290

Table 24 (continued)

Species	Isolate	Set 1 Date of Inoculation	Set 1 pH of Broth[a]	Set 1 Protein[b] Concentration 1	Set 1 Protein Concentration 2	Set 2 Date of Inoculation	Set 2 pH of Broth	Set 2 Protein Concentration 1	Set 2 Protein Concentration 2
E. radicalis	E16	7/27/83	5.00 (0.14)	213	313	10/3/83	5.07 (0.33)	373	363
"	E42	7/27/83	4.98 (0.11)	204	273	8/26/83	5.19 (0.12)	343	357
"	E56	7/27/83	4.45 (0.07)	153	187	7/27/83	4.45 (0.07)	243	247
"	E64	8/25/83	5.43 (0.10)	212	333	10/3/83	5.19 (0.13)	373	360
"	E67	8/7/83	6.07 (0.36)	171	237	10/3/83	6.29 (0.14)	343	373
"	E75	7/16/83	4.94 (0.10)	54	72	10/3/83	4.82 (0.17)	80	79
"	E76	7/27/83	5.30 (0.08)	650	353	10/3/83	5.42 (0.09)	403	573
"	E92	8/17/83	4.32 (0.08)	213	327	10/3/83	4.28 (0.06)	367	383
"	E150	7/27/83	4.82 (0.12)	206	337	10/3/83	3.99 (0.22)	533	403
"	E152	8/7/83	4.44 (0.23)	196	327	10/3/83	4.74 (0.12)	390	373
E. eugeniae	E138	8/25/83	4.10 (0.09)	562	360	8/26/83	4.18 (0.02)	403	587
	E139	7/27/83	3.92 (0.07)	568	360	6/3/83	3.82 (0.03)	380	580
E. havanensis	E40	7/27/83	4.40 (0.23)	164	237	8/26/83	4.92 (0.15)	247	273
	E158	8/25/83	4.12 (0.10)	196	303	8/26/83	4.14 (0.03)	343	343
	E159	7/27/83	4.55 (0.17)	210	313	8/26/83	4.42 (0.04)	357	360
E. macrospora	E54	7/27/83	4.30 (0.04)	220	330	8/26/83	4.45 (0.03)	357	383
E. japonica	E53	8/26/83	4.42 (0.14)	148	197	7/16/83	4.45 (0.04)	250	243
	E59	7/27/83	4.38 (0.11)	180	260	----[c]	----	320	290

Table 24 (continued)

Species	Isolate	Set 1 Date of Inoculation	Set 1 pH of Broth[a]	Set 1 Protein Concentration 1	Set 1 Protein Concentration 2	Set 2 Date of Inoculation	Set 2 pH of Broth	Set 2 Protein Concentration 1	Set 2 Protein Concentration 2
E. singularis	E52	9/7/83	4.18 (0.24)	548	347	9/7/83	4.16 (0.32)	373	387
	E58	8/26/83	4.34 (0.15)	192	277	7/16/83	5.01 (0.20)	290	280
E. tropicalis	E57	7/27/83	5.21 (0.26)	213	347	6/3/83	5.22 (0.39)	343	337
	E70	7/27/83	5.42 (0.10)	206	303	8/26/83	5.30 (0.06)	320	320
E. viridistroma	E41	8/26/83	4.75 (0.11)	686	453	8/26/83	4.63 (0.07)	403	620
	E82	8/26/83	4.39 (0.04)	538	460	7/16/83	4.21 (0.10)	547	600
E. coccolobii	E157	7/27/83	4.53 (0.02)	772	377	8/26/83	4.65 (0.07)	547	647

[a]Average pH of contents of three flasks. Standard error of mean in parentheses. Initial pH adjusted to 5.70 after autoclaving by the addition of 0.1% NaOH or 0.1% lactic acid.

Table 24 (continued)

[b]Protein concentration of samples expressed in μg/buffer. Protein concentration determined by the Bradford Coomassie blue assay (29) from a supernatant derived from a suspension of lyophilized mycelium (see text for details) in 0.125 M Tris-Cl buffer + 10% glycerol (60 mg lyophilized mycelium/ml buffer).

[c]Same lyophilized mycelium used as in Set 1 due to contamination of second sample.

others were more typical of the genus.

Protein concentrations were also quite varied. Isolates of E. parasitica consistently produced extracts with very low concentrations of soluble proteins. It is possible that the majority of proteins in this species are membrane-bound or that they were destroyed or inactivated during sample preparation. Endothia radicalis isolate E75 also produced a very low quantity of protein. Extremely high protein concentrations were obtained for several isolates, including E76, E41, E82 and E157. The actual concentration of the extracts was not as important as the relative concentrations in this study. The objective of the protein assay was to determine the proper volume of sample to apply to the gel in order to deliver a uniform amount of protein. The assay was adequate for this purpose.

Since only 40 isolates could be studied at one time (two 20-sample gels) and a total of 45 isolates were to be analyzed after the final 10 isolates of E. parasitica and E. gyrosa were chosen, it was necessary to eliminate five isolates whose banding patterns were already represented. In an initial test, it was determined that the following pairs of isolates showed virtually identical banding patterns: E. radicalis - E150 and E152 (similarity coefficient = 0.98), E. viridistroma - E41 and E82 (similarity coeffiecient = 1.00); E. tropicalis - E57 and E70 (similarity coeffiecient = 1.00); E. singularis - E52 and E58 (similarity coefficient = 0.98); and E. havanensis - E158 and E159 (similarity coefficient = 1.00). In each series of electro-

phoretic runs, an initial gel was produced to reconfirm these similarities. A second gel, which included only one representative of each pair, was then analyzed for similarities to isolates of other species. The isolates which were not directly compared were added to the dendrogram after the positions of their partners were determined. In two cases, the banding patterns of one of the samples changed with repetitive runs so that the isolate pairs were no longer identical. This occurred with the two isolates of E. singularis; the banding pattern of isolate E58 altered substantially between the first and second set of powders. This culture apparently became contaminated during the study; it was not included in the final dendrogram. The banding pattern of E57, the isolate of E. tropicalis which was analyzed with the other species, showed extremely poor resolution in extracts from the second powder. Since individual lines could not be easily distinguished, an artificially low similarity coefficient was obtained when compared to the other isolates. Because this would be misleading, both isolates of E tropicalis were dropped from the final dendrogram.

The remaining isolates showed very consistent banding patterns among electrophoretic runs of extracts from the same and from replicate powders. Two series of gels produced from extracts of different lyophilized mycelia and their interpretative drawings are presented in Figures 33 - 58. The interpretative drawings for the remaining gels are presented in Appendix IV.

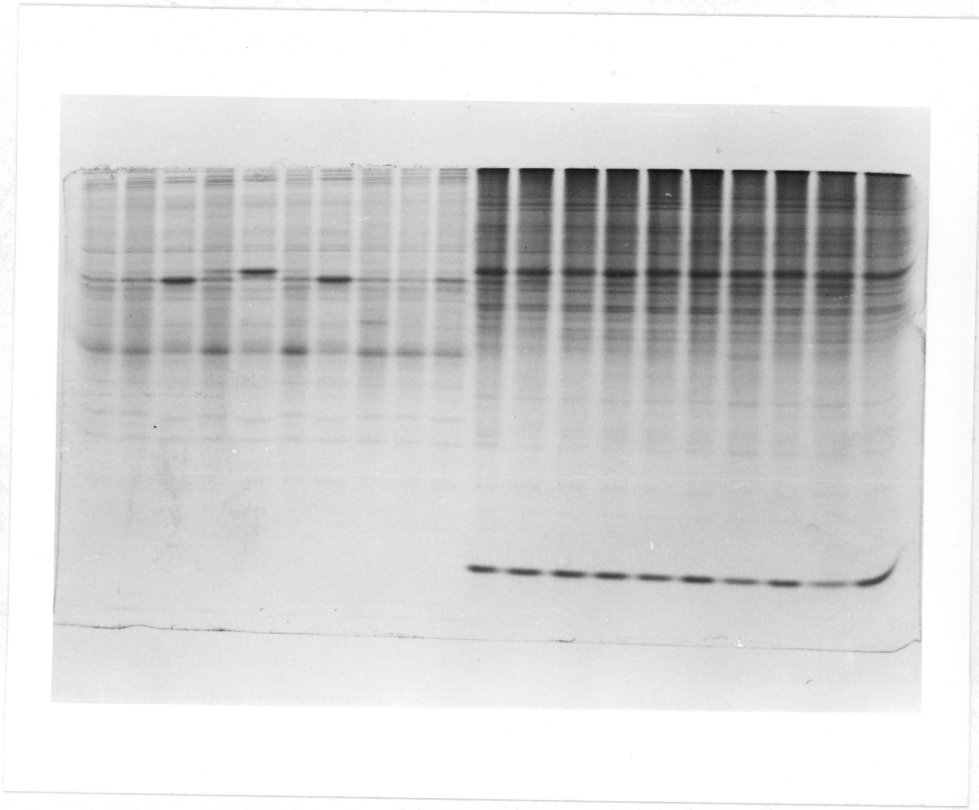


Figure 33. Polyacrylamide electrophoretic gel of buffer-soluble proteins of isolates of Endothia parasitica and E. gyrosa. Species and isolates by lane number (from left to right): E. parasitica 1 - E24, 2 - E85, 3 - E86, 4 - E87, 5 - E88, 6 - E89, 7 - E95, 8 - E96, 9 - E153, 10 - E155; E. gyrosa 11 - E18, 12 - E20, 13 - E30, 14 - E37, 15 - E38, 16 - E48, 17 - E50, 18 - E98, 19 - E145, 20 - E154. Second extraction of first set of lyophilized mycelial powders.

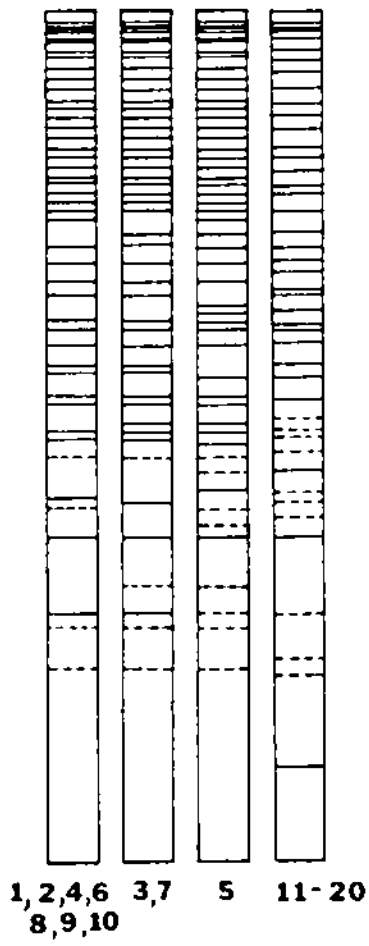


Figure 34. Interpretative drawing of polyacrylamide electrophoretic gel of buffer-soluble proteins of isolates of Endothia parasitica and E. gyrosa. Species and isolates by lane number (from left to right): E. parasitica 1 - E24, 2 - E85, 3 - E86, 4 - E87, 5 - E88, 6 - E89, 7 - E95, 8 - E96, 9 - E153, 10 - E155; E. gyrosa 11 - E18, 12 - E20, 13 - E30, 14 - E37, 15 - E38, 16 - E48, 17 - E50, 18 - E98, 19 - E145, 20 - E154. Second extraction of first set of lyophilized mycelial powders.

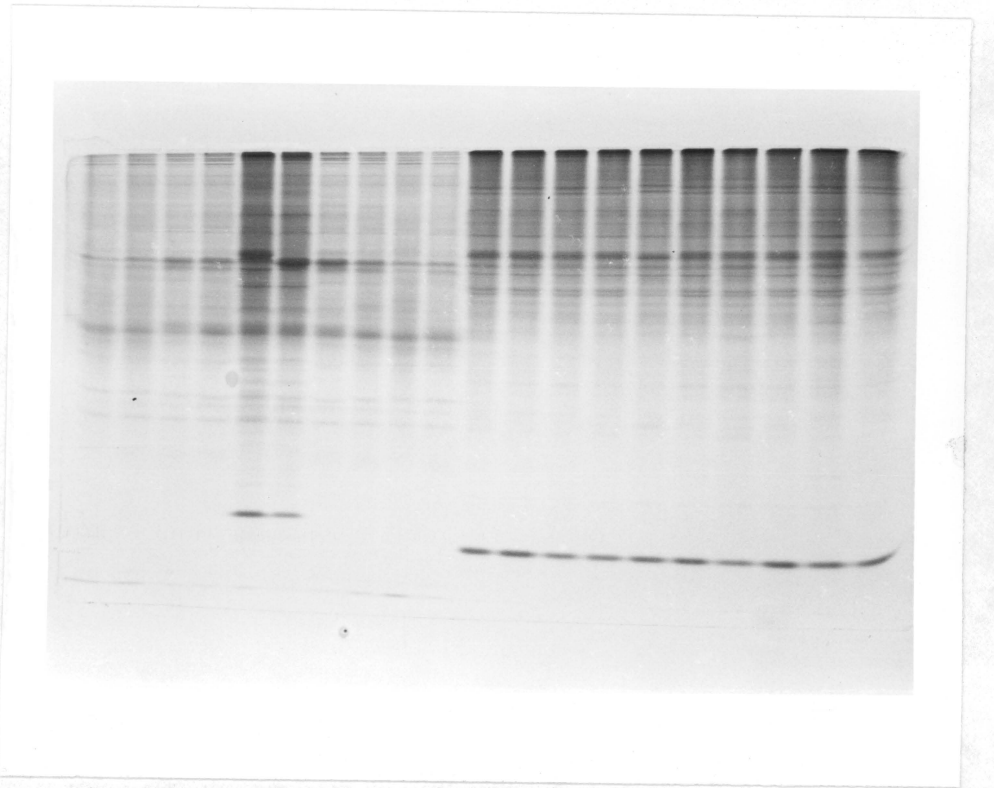


Figure 35. Polyacrylamide electrophoretic gel of buffer-soluble proteins of isolates of Endothia parasitica and E. gyrosa. Species and isolates by lane number (from left to right): E. parasitica 1 - E24, 2 - E85, 3 - E86, 4 - E87, 5 - E88, 6 - E89, 7 - E95, 8 - E96, 9 - E153, 10 - E155; E. gyrosa 11 - E18, 12 - E20, 13 - E30, 14 - E37, 15 - E38, 16 - E48, 17 - E50, 18 - E98, 19 - E145, 20 - E154. First extraction of second set of lyophilized mycelial powders.

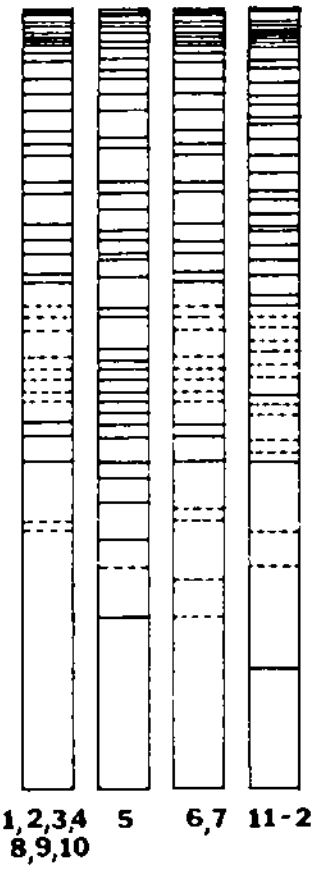


Figure 36. Interpretative drawing of polyacrylamide electrophoretic gel of buffer-soluble proteins of isolates of Endothia parasitica and E. gyrosa. Species and isolates by lane number (from left to right): E. parasitica 1 - E24, 2 - E85, 3 - E86, 4 - E87, 5 - E88, 6 - E89, 7 - E95, 8 - E96, 9 - E153, 10 - E155; E. gyrosa 11 - E16, 12 - E20, 13 - E30, 14 - E37, 15 - E38, 16 - E48, 17 - E50, 18 - E98, 19 - E145, 20 - E154. First extraction of second set of lyophilized mycelial powders.

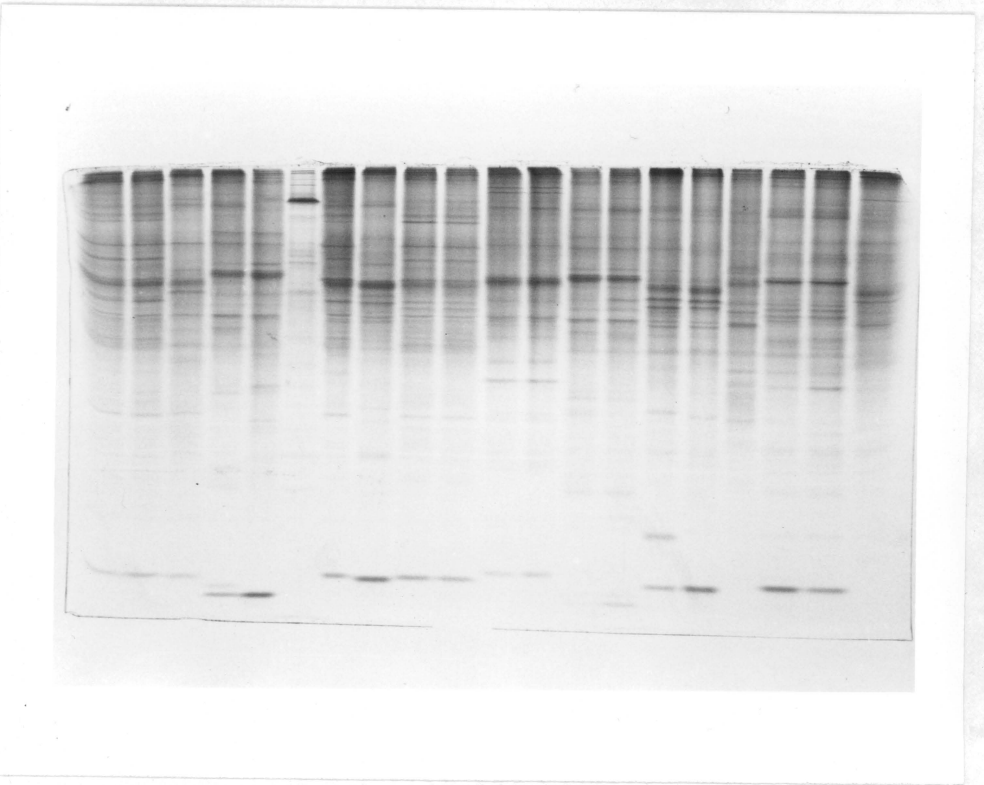


Figure 37. Polyacrylamide electrophoretic gel of buffer-soluble proteins of Endothia species. Species and isolates by lane number (from left to right): E. radicalis 1 - E16, 2 - E42, 3 - E56, 4 - E64, 5 - E67, 6 - E75, 7 - E76, 8 - E92, 9 - E150, 10 - E152; E. viridistroma 11 - E41, 12 - E82; E. tropicalis 13 - E57, 14 - E72; E. singularis 15 - E52, 16 - E57; E. havanensis 17 - E40, 18 - E158, 19 - E159; E. coccolobii 20 - E157. This gel was not used in the construction of the similarity matrices but did establish the identical intraspecific banding patterns of certain isolate pairs (E150 and E152; E41 and E82; E57 and E70; E52 and E58; E158 and E159). Second extraction of first set of lyophilized mycelial powders.

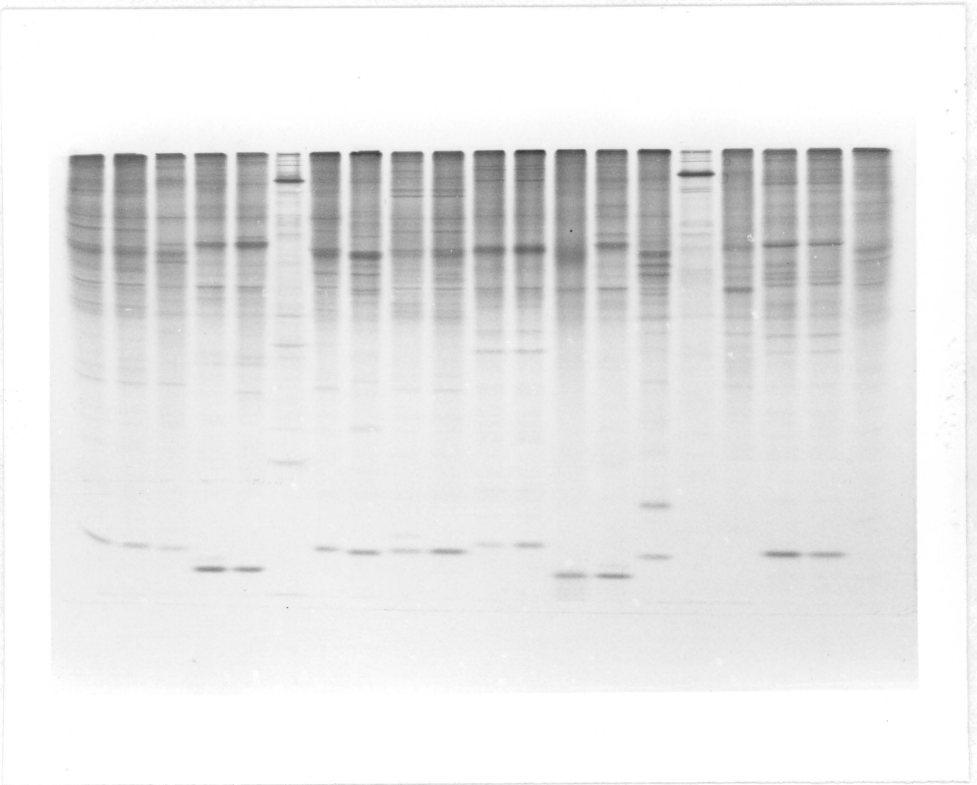


Figure 38. Polyacrylamide electrophoretic gel of buffer-soluble proteins of Endothia species. Species and isolates by lane number (from left to right): E. radicalis 1 - E16, 2 - E42, 3 - E56, 4 - E64, 5 - E67, 6 - E75, 7 - E76, 8 - E92, 9 - E150, 10 - E152; E. viridistroma 11 - E41, 12 - E82; E. tropicalis 13 - E57, 14 - E72; E. singularis 15 - E52, 16 - E57; E. havanensis 17 - E40, 18 - E158, 19 - E159; E. coccolobii 20 - E157. This gel was not used in the construction of the similarity matrices but did establish the identical intraspecific banding patterns of certain isolate pairs (E150 and E152; E41 and E82; E57 and E70; E52 and E58; E158 and E159). First extraction of second set of lyophilized mycelial powders.

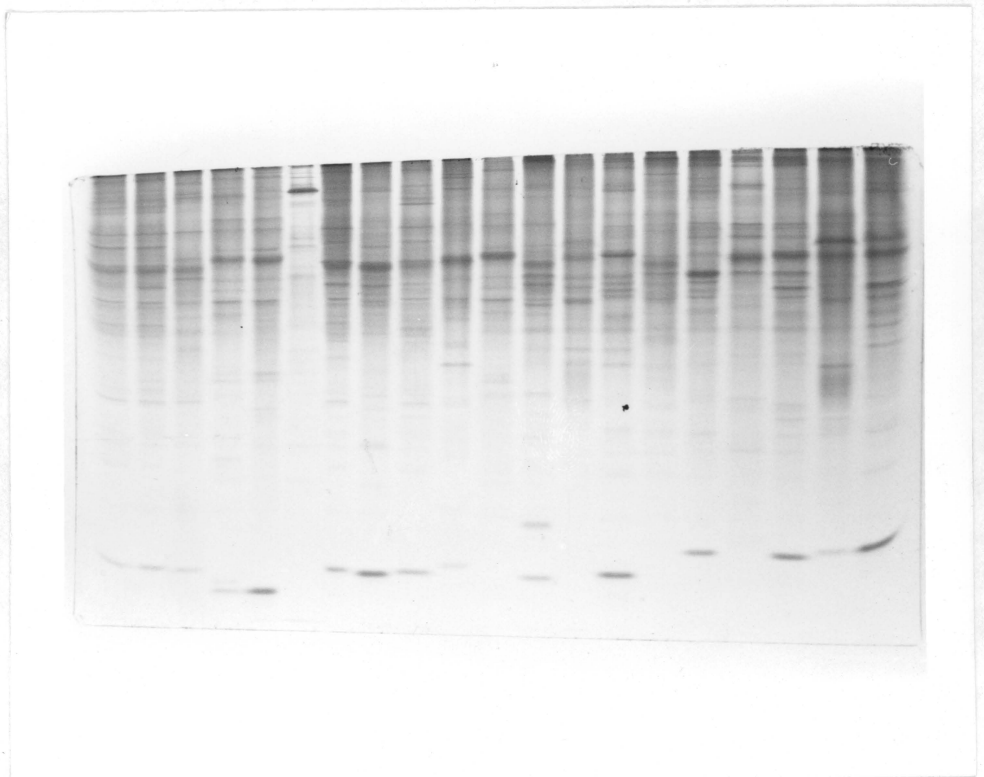


Figure 39. Polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia species. Species and isolates by lane number (from left to right): E. radicalis 1 - E16, 2 - E42, 3 - E56, 4 - E64, 5 - E67, 6 - E75, 7 - E76, 8 - E92, 9 - E150; E. viridistroma 10 - E41; E. tropicalis 11 - E57; E. singularis 12 - E52; E. havanensis 13 - E40, 14 - E158; E. coccolobii 15 - E157; E. macrospora 16 - E54; E. japonica 17 - E53, 18 - E59; E. eugeniae 19 - E138, 20 - E139. Second extraction of first set of lyophilized mycelial powders.

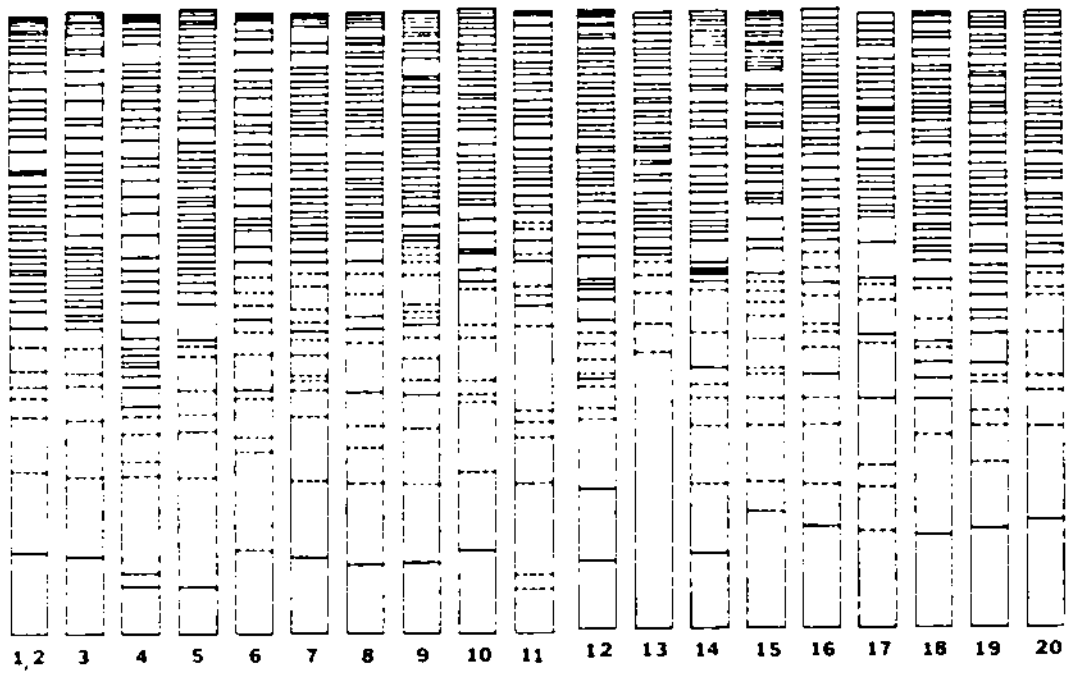


Figure 40. Interpretative drawing of polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia species. Species and isolates by lane number (from left to right): E. radicalis 1 - E16, 2 - E42, 3 - E56, 4 - E64, 5 - E67, 6 - E75, 7 - E76, 8 - E92, 9 - E150; E. virgistroma 10 - E41; E. tropicalis 11 - E57; E. singularis 12 - E52; E. havanensis 13 - E42, 14 - E158; E. coccolobii 15 - E157; E. macrospora 16 - E54; E. japonica 17 - E53, 18 - E59; E. eucalyptorum 19 - E138, 20 - E139. Second extraction of first set of lyophilized mycelia, powders.

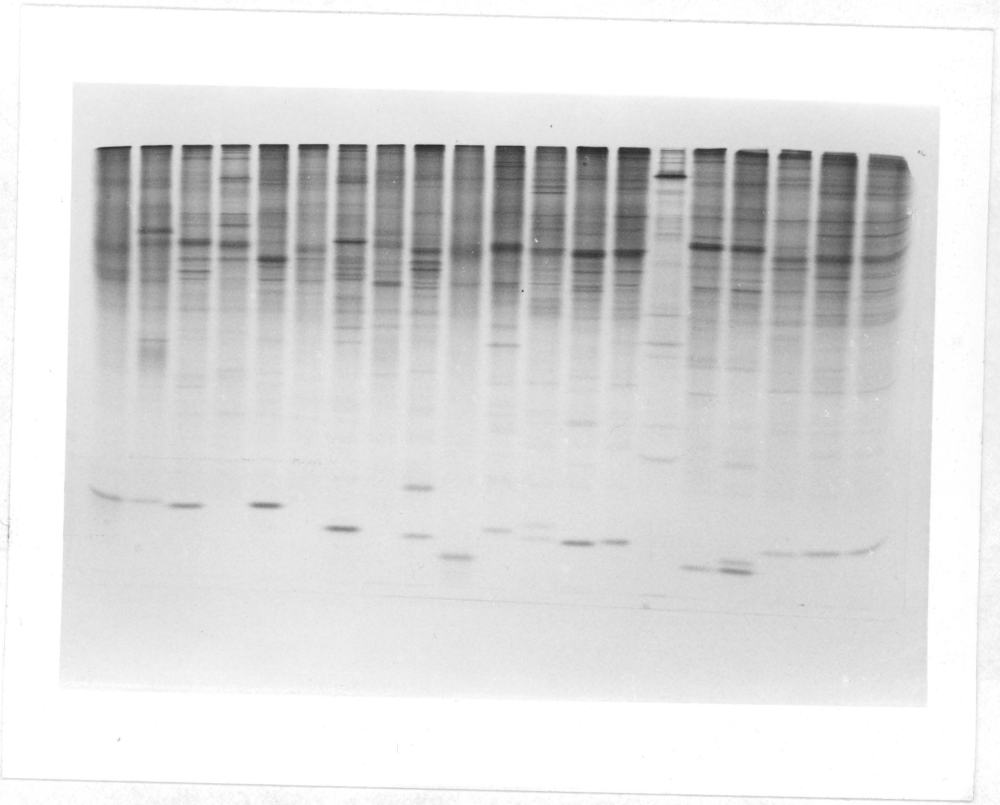


Figure 41. Polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia species. Species and isolates by lane number (from left to right): E. radicalis 1 - E16, 2 - E42, 3 - E56, 4 - E64, 5 - E67, 6 - E75, 7 - E76, 8 - E92, 9 - E150; E. viridistroma 10 - E41; E. tropicalis 11 - E57; E. singularis 12 - E52; E. havanensis 13 - E40, 14 - E158; E. coccolobii 15 - E157; E. macrospora 16 - E54; E. japonica 17 - E53, 18 - E59; E. eugeniae 19 - E138, 20 - E139. First extraction of second set of lyophilized mycelial powders.

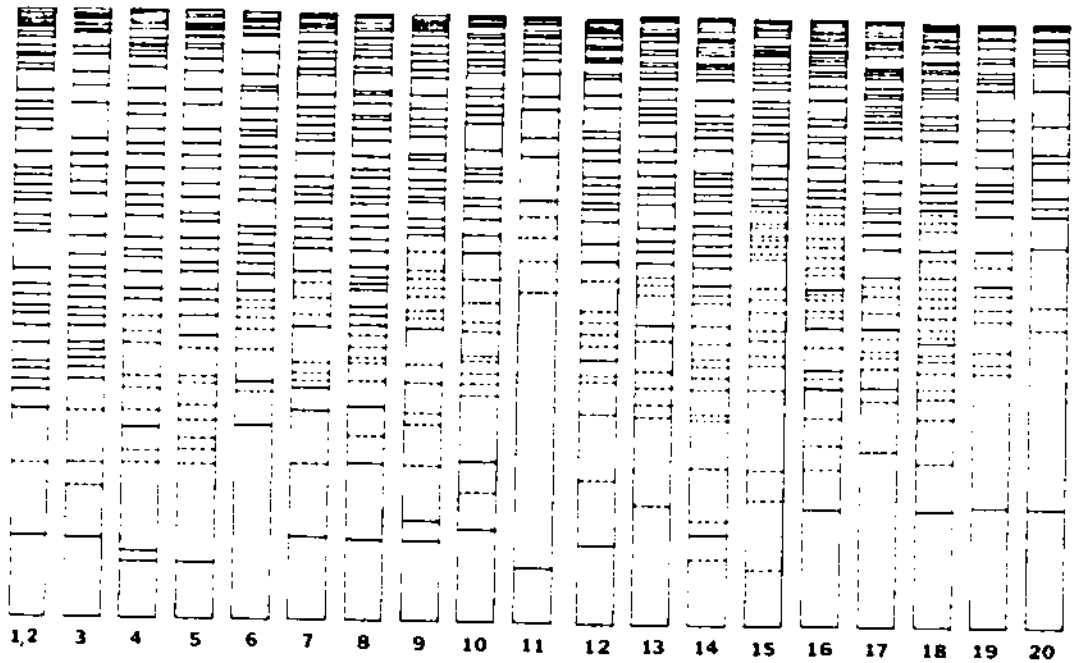


Figure 42. Interpretative drawing of polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Encoelia species. Species and isolates by lane number (from left to right): E. radicalis 1 - E16, 2 - E42, 3 - E56, 4 - E64, 5 - E67, 6 - E75, 7 - E76, 8 - E92, 9 - E150; E. viridistroma 10 - E41; E. tropicalis 11 - E57; E. singularis 12 - E52; E. navanensis 13 - E40, 14 - E158; E. coccolobii 15 - E157; E. macrospora 16 - E54; E. japonica 17 - E53, 18 - E59; E. eugeniae 19 - E138, 20 - E139. First extraction of second set of lyophilized mycelial powders.

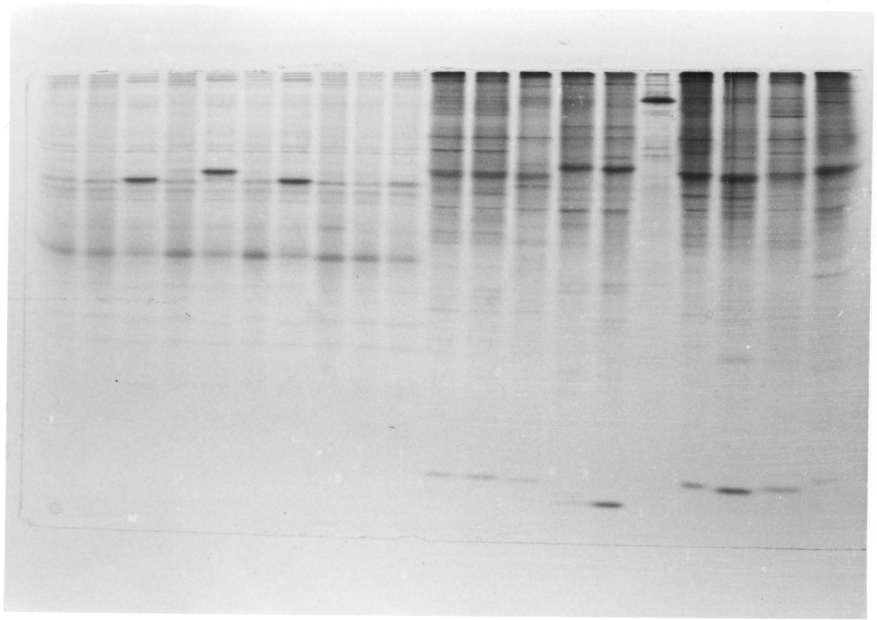


Figure 43. Polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia parasitica, E. radicalis and E. viridistroma. Species and isolates by lane number (from left to right): E. parasitica 1 - E24, 2 - E85, 3 - E86, 4 - E87, 5 - E88, 6 - E89, 7 - E95, 8 - E96, 9 - E153, 10 - E155; E. radicalis 11 - E16, 12 - E42, 13 - E56, 14 - E64, 15 - E67, 16 - E75, 17 - E76, 18 - E92, 19 - E150; E. viridistroma 20 - E41. Second extraction of first set of lyophilized mycelial powders.

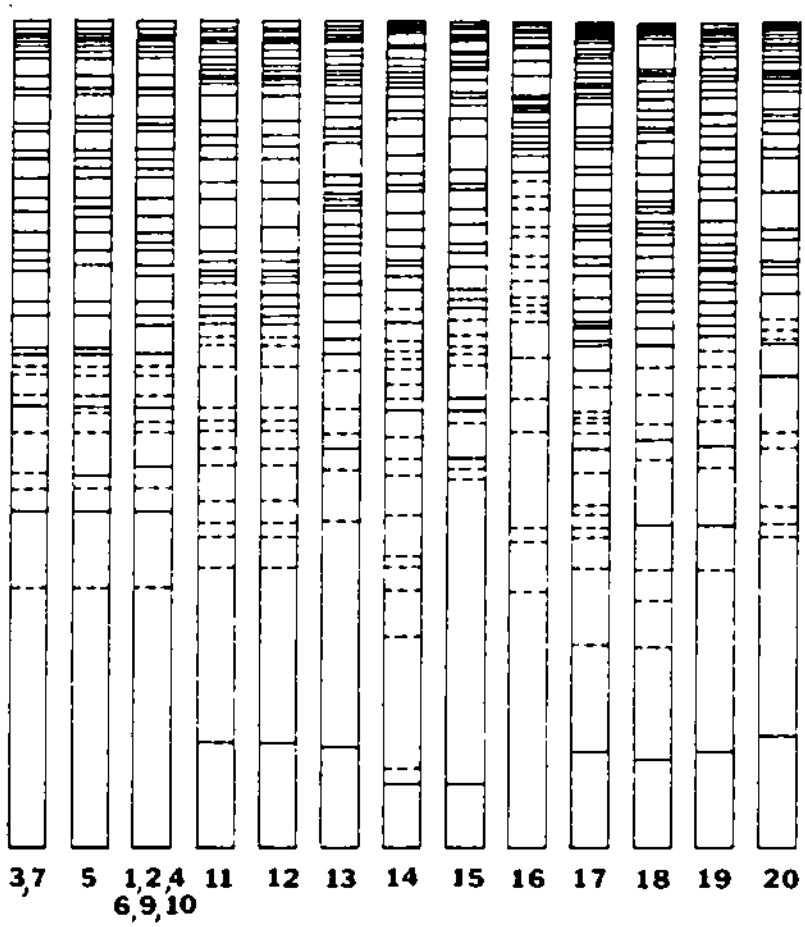


Figure 44. Interpretative drawing of polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia parasitica, E. radicalis and E. viridistroma. Species and isolates by lane number (from left to right): E. parasitica 1 - E24, 2 - E85, 3 - E86, 4 - E87, 5 - E88, 6 - E89, 7 - E95, 8 - E96, 9 - E153, 10 - E155; E. radicalis 11 - E16, 12 - E42, 13 - E56, 14 - E64, 15 - E67, 16 - E75, 17 - E76, 18 - E92, 19 - E150; E. viridistroma 20 - E41. Second extraction of first set of lyophilized mycelial powders.

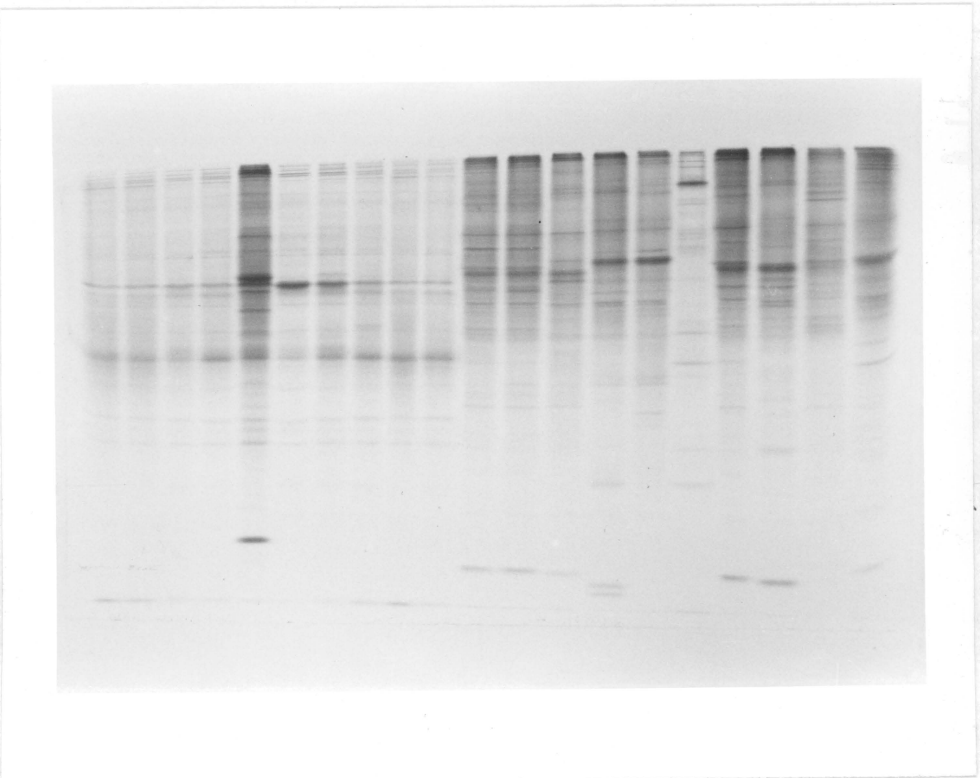


Figure 45. Polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia parasitica, E. radicalis and E. viridistroma. Species and isolates by lane number (from left to right): E. parasitica 1 - E24, 2 - E85, 3 - E86, 4 - E87, 5 - E88, 6 - E89, 7 - E95, 8 - E96, 9 - E153, 10 - E155; E. radicalis 11 - E16, 12 - E42, 13 - E56, 14 - E64, 15 - E67, 16 - E75, 17 - E76, 18 - E92, 19 - E150; E. viridistroma 20 - E41. First extraction of second set of lyophilized mycelial powders.

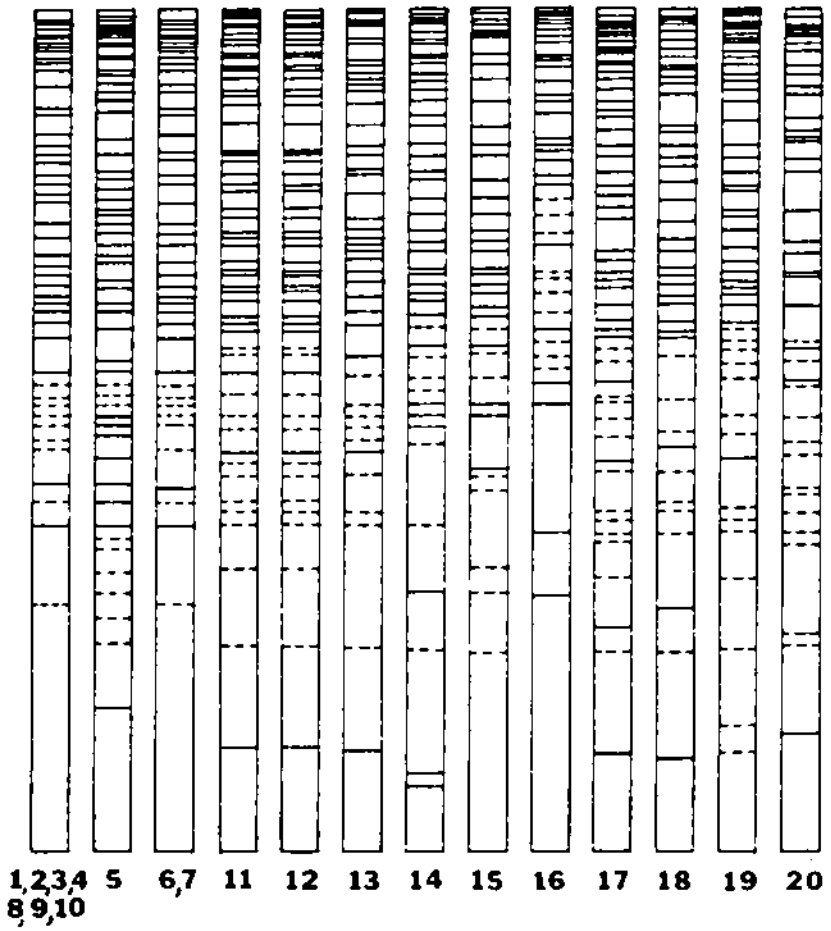


Figure 46. Interpretative drawing of polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia parasitica, E. radicalis and E. viridistroma. Species and isolates by lane number (from left to right): E. parasitica 1 - E24, 2 - E85, 3 - E86, 4 - E87, 5 - E88, 6 - E89, 7 - E95, 8 - E96, 9 - E153, 10 - E155; E. radicalis 11 - E16, 12 - E42, 13 - E56, 14 - E64, 15 - E67, 16 - E75, 17 - E76, 18 - E92, 19 - E150; E. viridistroma 20 - E41. First extraction of second set of lyophilized mycelial powders.

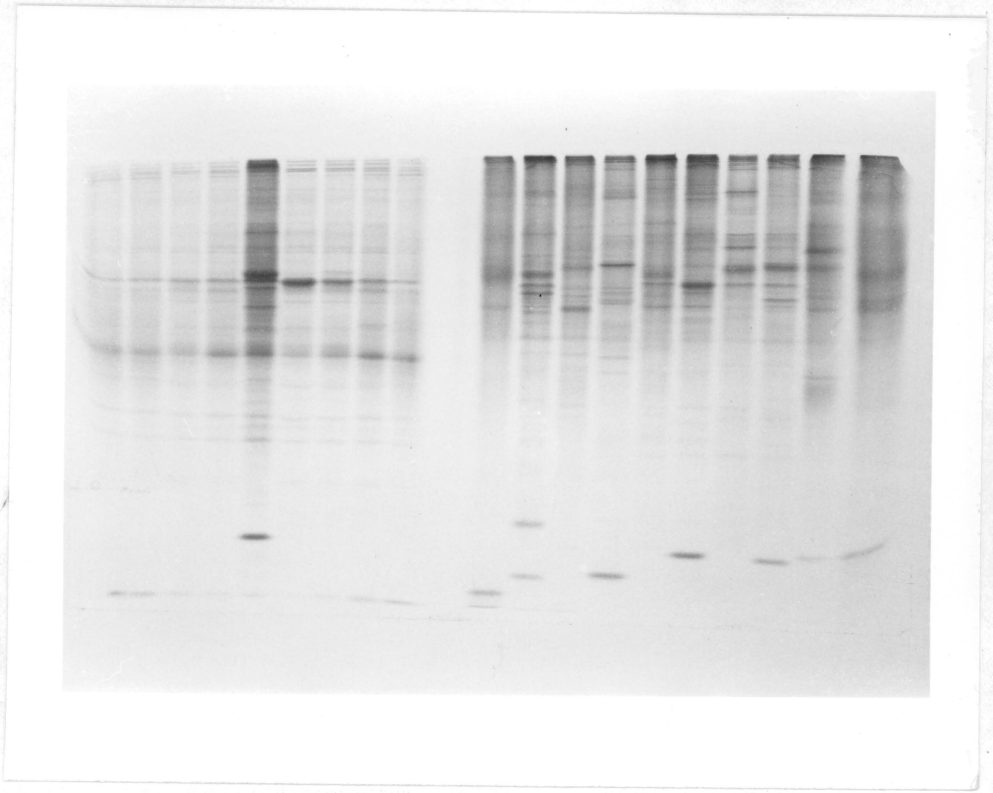


Figure 47. Polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia parasitica and uncommon Endothia species. Species and isolates by lane number (from left to right): E. parasitica 1 - E24, 2 - E85, 3 - E86, 4 - E87, 5 - E88, 6 - E89, 7 - E95, 8 - E96, 9 - E153, 10 - E155; E. tropicalis 11 - E57; E. singularis 12 - E52; E. havanensis 13 - E40, 14 - E158; E. coccolobii 15 - E157; E. macrospora 16 - E54; E. japonica 17 - E53, 18 - E59; E. eugeniae 19 - E138, 20 - E139. First extraction of second set of lyophilized mycelial powders.

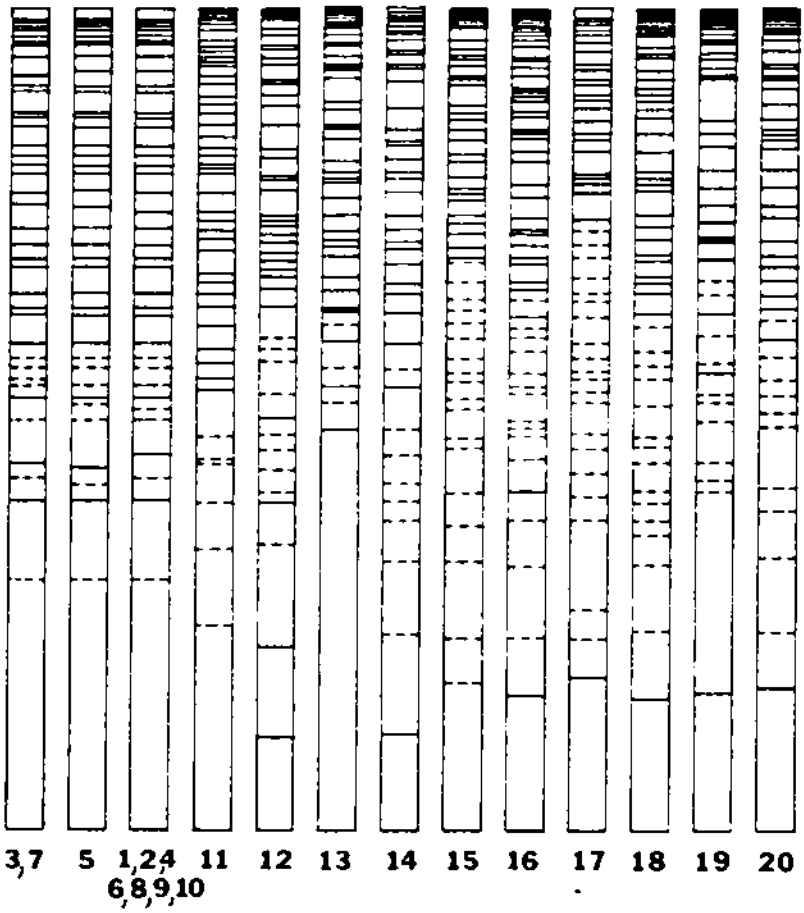


Figure 48. Interpretative drawing of polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia parasitica and uncommon Endothia species. Species and isolates by lane number (from left to right): E. parasitica 1 - E24, 2 - E85, 3 - E86, 4 - E87, 5 - E88, 6 - E89, 7 - E95, 8 - E96, 9 - E153, 10 - E155; E. tropicalis 11 - E57; E. singularis 12 - E52; E. havanensis 13 - E40, 14 - E158; E. coccolobii 15 - E157; E. macrospora 16 - E54; E. japonica 17 - E53, 18 - E59; E. eugeniae 19 - E138, 20 - E139. Second extraction of first set of lyophilized mycelial powders.

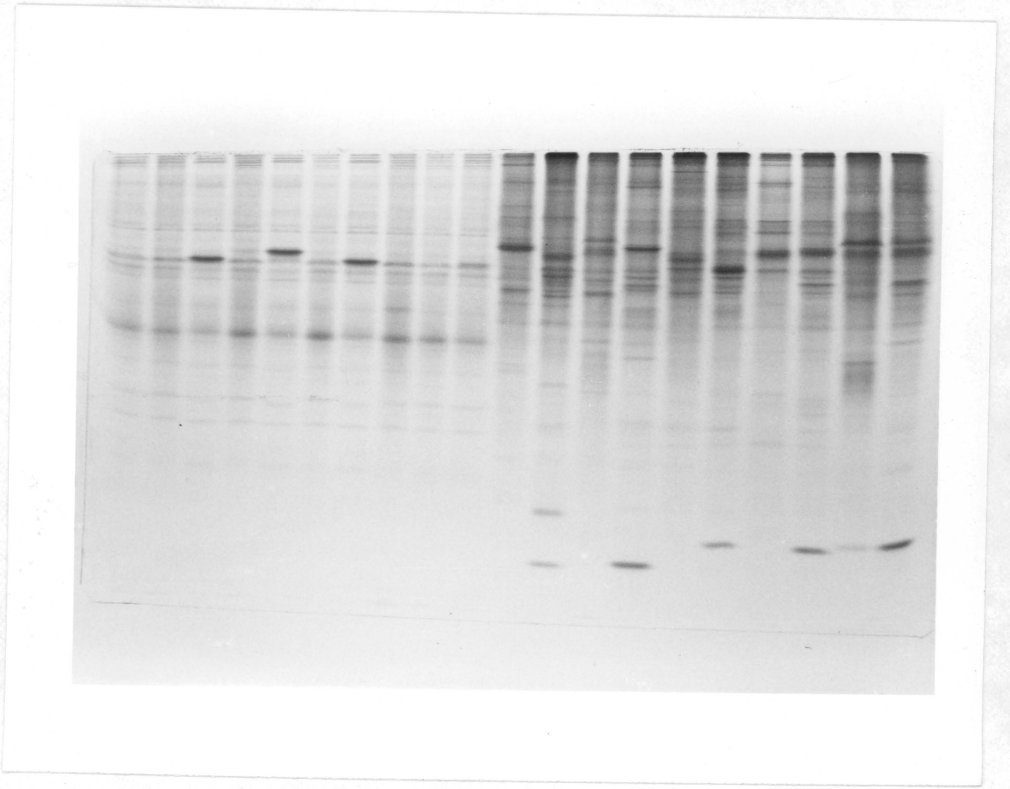


Figure 49. Polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia parasitica and uncommon Endothia species. Species and isolates by lane number (from left to right): E. parasitica 1 - E24, 2 - E85, 3 - E86, 4 - E87, 5 - E88, 6 - E89, 7 - E95, 8 - E96, 9 - E153, 10 - E155; E. tropicalis 11 - E57; E. singularis 12 - E52; E. havanensis 13 - E40, 14 - E158; E. coccolobii 15 - E157; E. macrospora 16 - E54; E. japonica 17 - E53, 18 - E59; E. eugeniae 19 - E138, 20 - E139. Second extraction of first set of lyophilized mycelial powders.

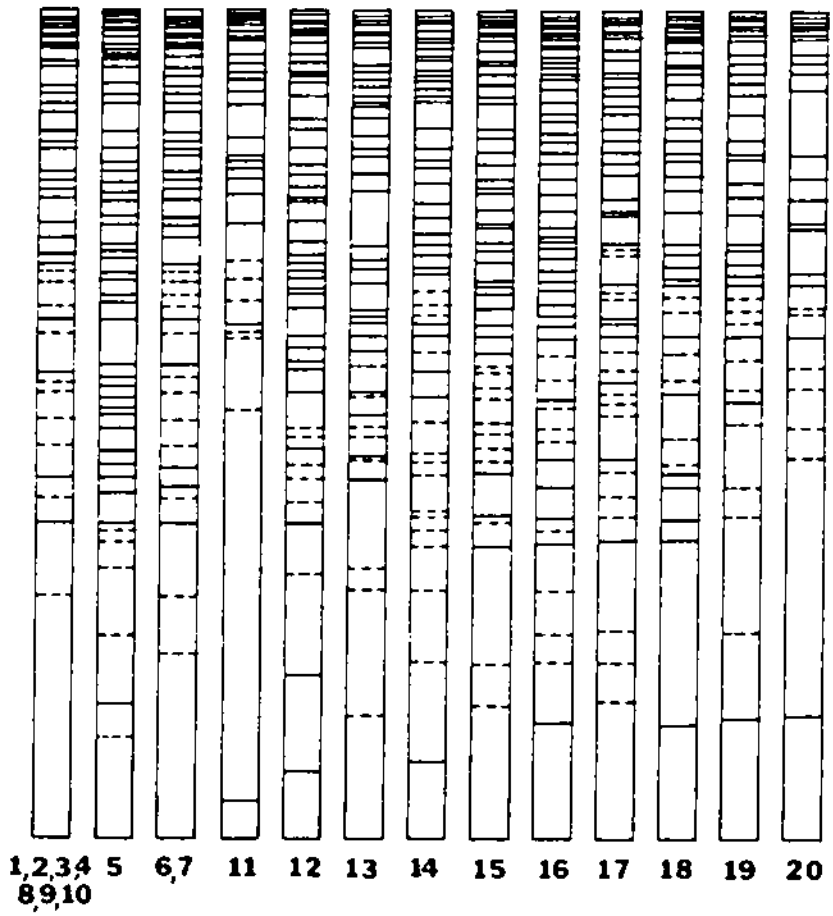


Figure 50. Interpretative drawing of polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia parasitica and uncommon Endothia species. Species and isolates by lane number (from left to right): E. parasitica 1 - E24, 2 - E85, 3 - E86, 4 - E87, 5 - E88, 6 - E89, 7 - E95, 8 - E96, 9 - E153, 10 - E155; E. tropicalis 11 - E57; E. singularis 12 - E52; E. havanensis 13 - E40, 14 - E158; E. coccolobii 15 - E157; E. macrospora 16 - E54; E. japonica 17 - E53, 18 - E59; E. eugeniae 19 - E138, 20 - E139. First extraction of second set of lyophilized mycelial powders.

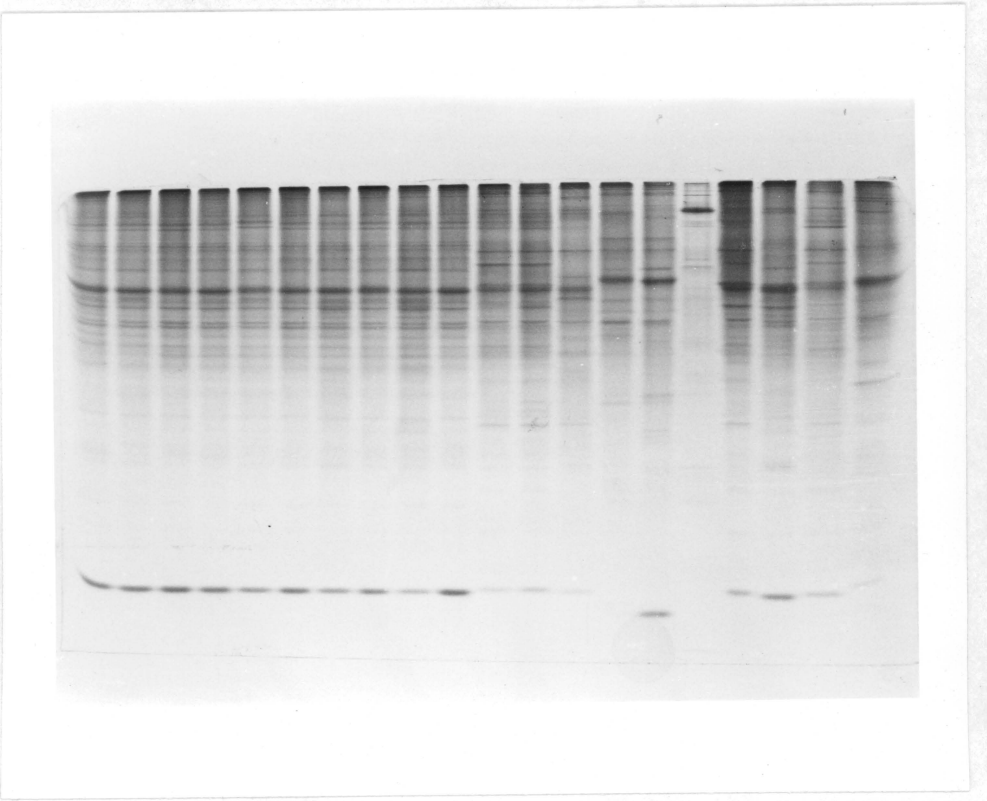


Figure 51. Polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia gyrosa, E. radicalis and E. viridistroma. Species and isolates by lane number (from left to right): E. gyrosa 1 - E18, 2 - E20, 3 - E30, 4 - E37, 5 - E38, 6 - E48, 7 - E50, 8 - E98, 9 - E145, 10 - E154; E. radicalis 11 - E16, 12 - E42, 13 - E56, 14 - E64, 15 - E67, 16 - E75, 17 - E76, 18 - E92, 19 - E150; E. viridistroma 20 - E41. Second extraction of first set of lyophilized mycelial powders.

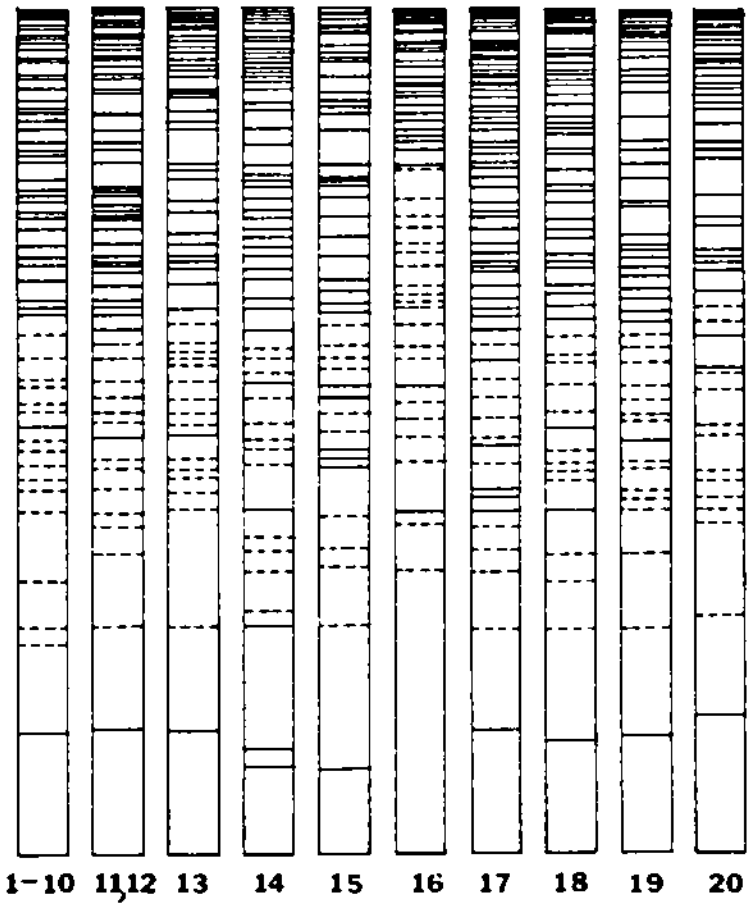


Figure 52. Interpretative drawings of polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia gyrosa, E. radicalis and E. viridistroma. Species and isolates by lane number (from left to right): E. gyrosa 1 - E18, 2 - E20, 3 - E30, 4 - E37, 5 - E38, 6 - E48, 7 - E50, 8 - E98, 9 - E145, 10 - E154; E. radicalis 11 - E16, 12 - E42, 13 - E56, 14 - E64, 15 - E67, 16 - E75, 17 - E76, 18 - E92, 19 - E150; E. viridistroma 20 - E41. Second extraction of first set of lyophilized mycelial powders.

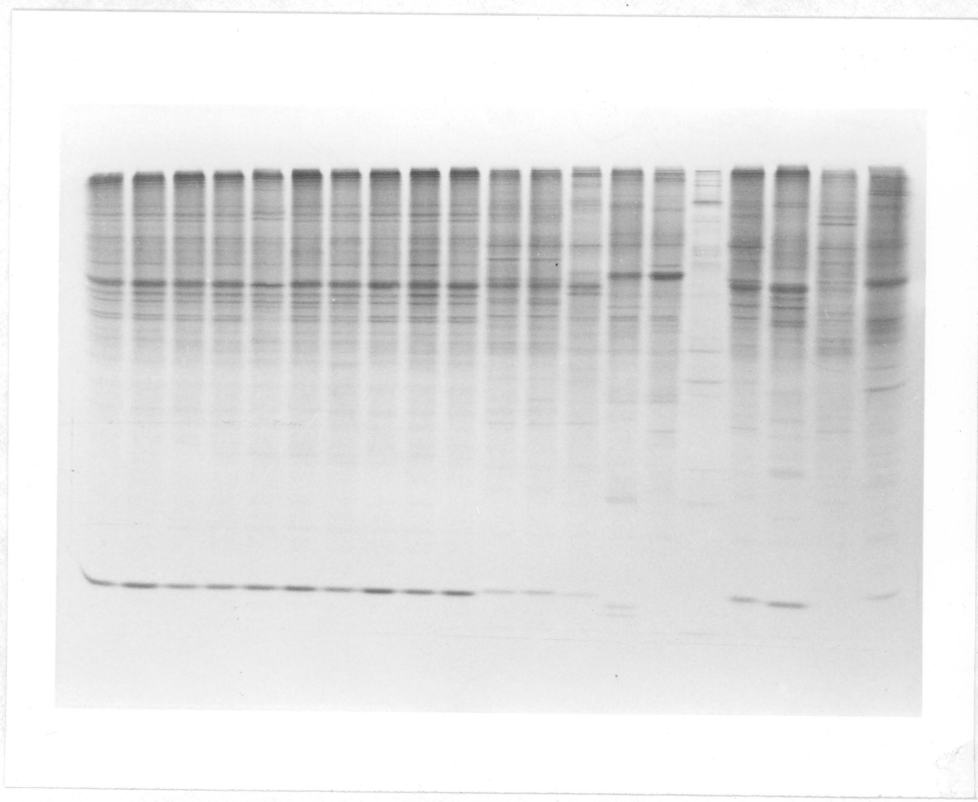


Figure 53. Polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia gyrosa, E. radicalis and E. viridistroma. Species and isolates by lane number (from left to right): E. gyrosa 1 - E18, 2 - E20, 3 - E30, 4 - E37, 5 - E38, 6 - E48, 7 - E50, 8 - E98, 9 - E145, 10 - E154; E. radicalis 11 - E16, 12 - E42, 13 - E56, 14 - E64, 15 - E67, 16 - E75, 17 - E76, 18 - E92, 19 - E150; E. viridistroma 20 - E41. First extraction of second set of lyophilized mycelial powders.

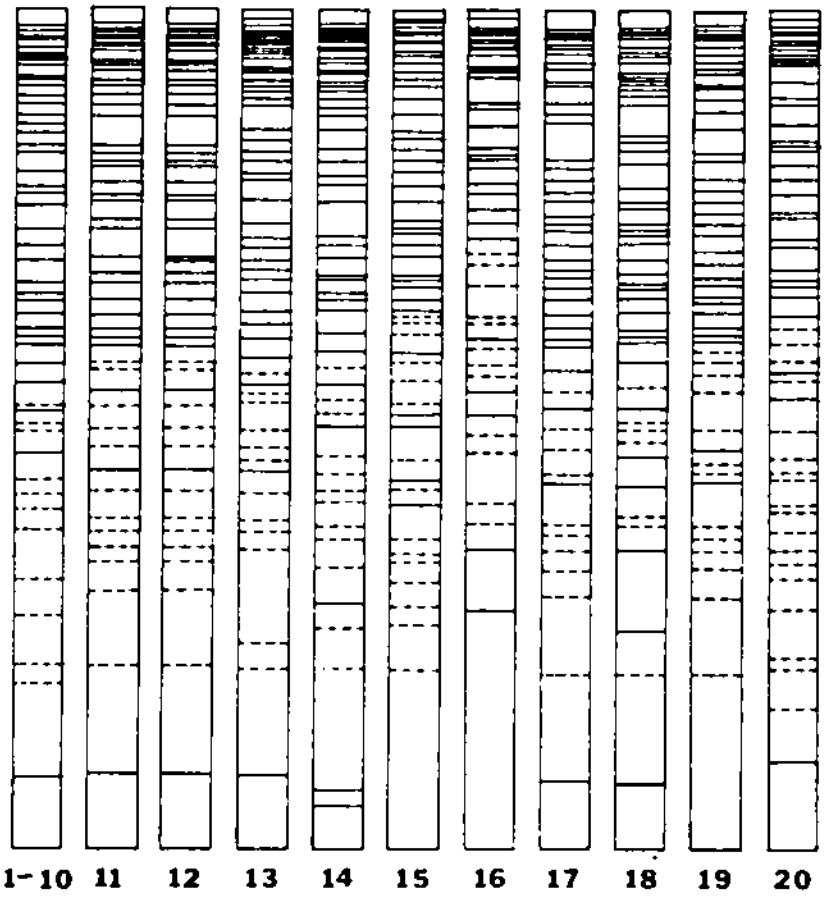


Figure 54. Interpretative drawings of polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia gyrosa, E. radicalis and E. viridistroma. Species and isolates by lane number (from left to right): E. gyrosa 1 - E18, 2 - E20, 3 - E30, 4 - E37, 5 - E38, 6 - E48, 7 - E50, 8 - E98, 9 - E145, 10 - E154; E. radicalis 11 - E16, 12 - E42, 13 - E56, 14 - E64, 15 - E67, 16 - E75, 17 - E76, 18 - E92, 19 - E150; E. viridistroma 20 - E41. First extraction of second set of lyophilized mycelial powders.

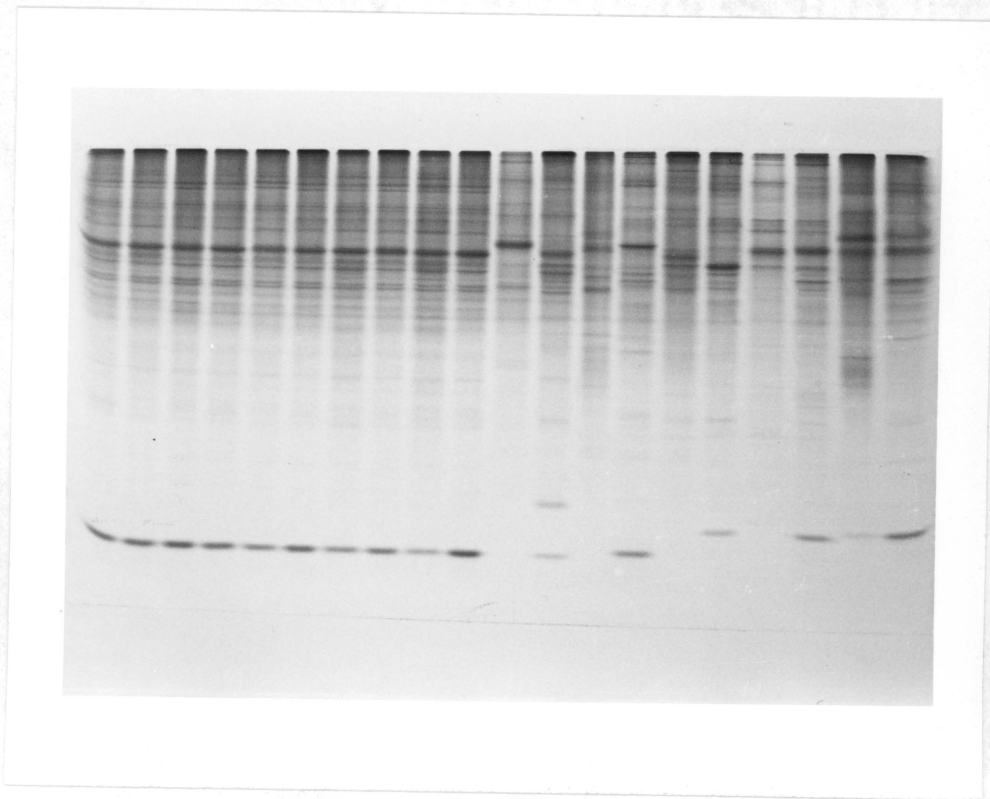


Figure 55. Polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia gyrosa and uncommon species of Endothia. Species and isolates by lane number (from left to right): E. gyrosa 1 - E18, 2 - E20, 3 - E30, 4 - E37, 5 - E38, 6 - E48, 7 - E50, 8 - E98, 9 - E145, 10 - E154; E. tropicalis 11 - E57; E. singularis 12 - E52; E. havanensis 13 - E40, 14 - E158; E. coccolobii 15 - E157; E. macrospora 16 - E54; E. japonica 17 - E53, 18 - E59; E. eugeniae 19 - E138, 20 - E139. Second extraction of first set of lyophilized mycelial powders.

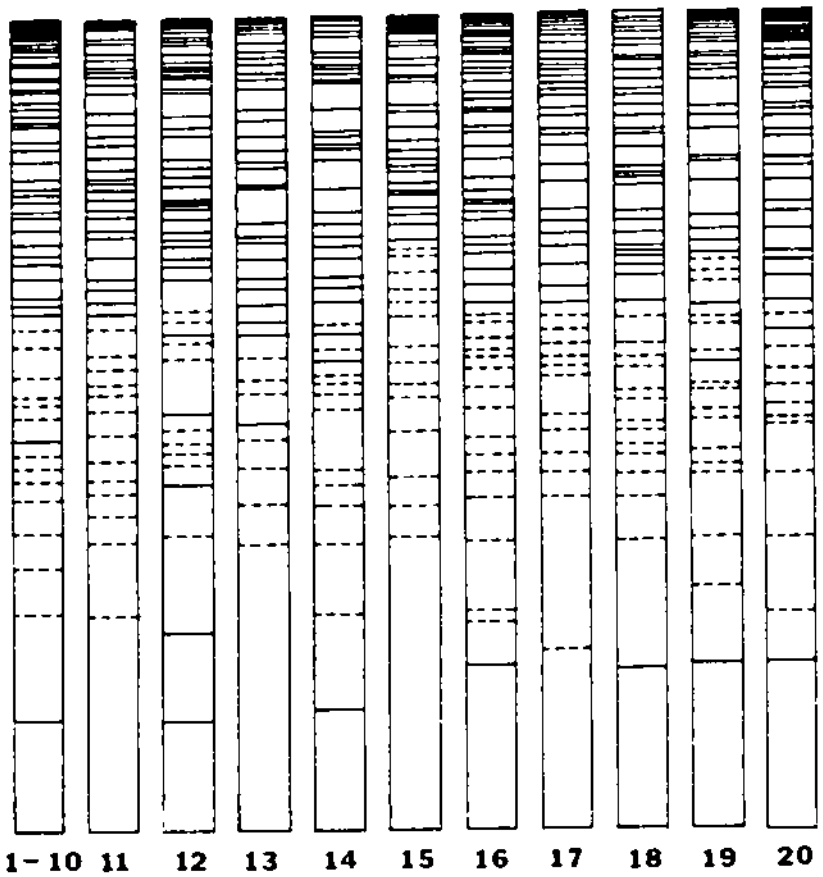


Figure 56. Interpretative drawing of polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia gyrosa and uncommon species of Endothia. Species and isolates by lane number (from left to right): E. gyrosa 1 - E18, 2 - E20, 3 - E30, 4 - E37, 5 - E38, 6 - E48, 7 - E50, 8 - E98, 9 - E145, 10 - E154; E. tropicalis 11 - E57; E. singularis 12 - E52; E. havanensis 13 - E40, 14 - E158; E. coccolobii 15 - E157; E. macrospora 16 - E54; E. japonica 17 - E53, 18 - E59; E. eugeniae 19 - E138, 20 - E139. Second extraction of first set of lyophilized mycelial powders.

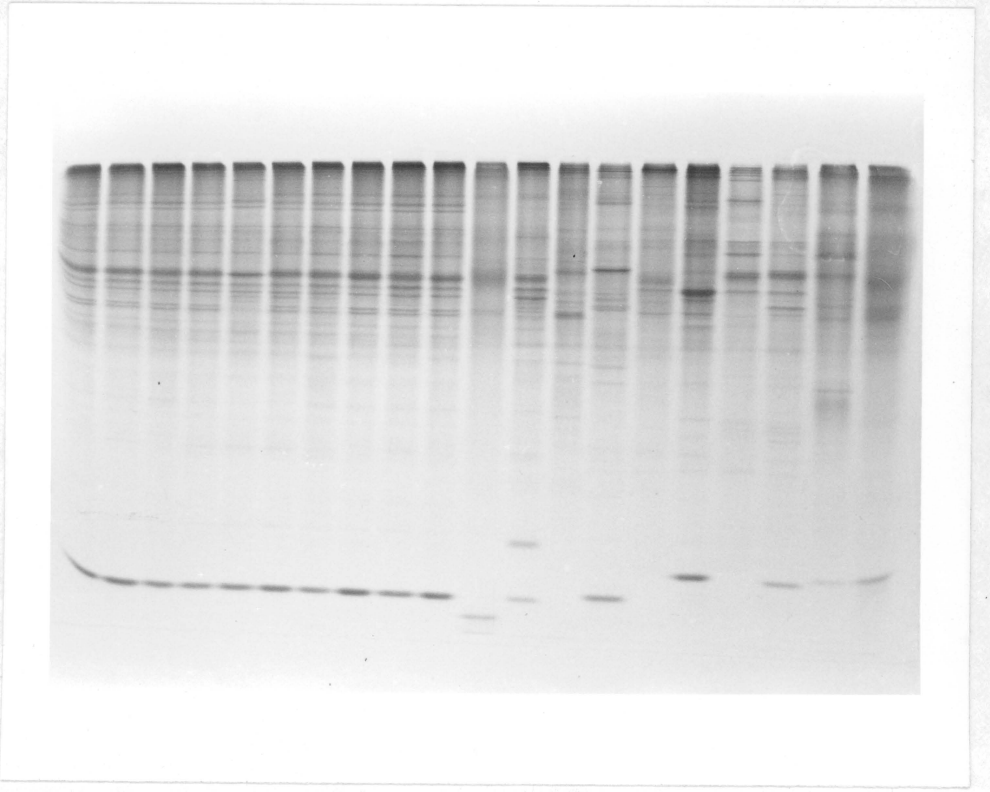


Figure 57. Polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia gyrosa and uncommon species of Endothia. Species and isolates by lane number (from left to right): E. gyrosa 1 - E18, 2 - E20, 3 - E30, 4 - E37, 5 - E38, 6 - E48, 7 - E50, 8 - E98, 9 - E145, 10 - E154; E. tropicalis 11 - E57; E. singularis 12 - E52; E. havanensis 13 - E40, 14 - E158; E. coccolobii 15 - E157; E. macrospora 16 - E54; E. japonica 17 - E53, 18 - E59; E. eugeniae 19 - E138, 20 - E139. First extraction of second set of lyophilized mycelial powders.

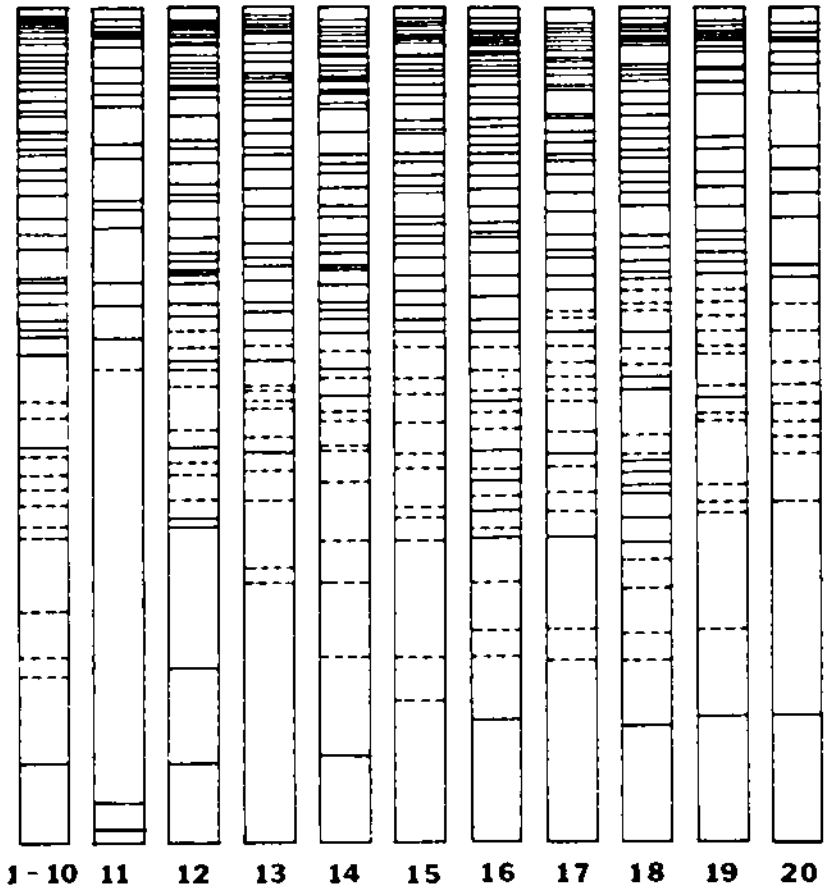
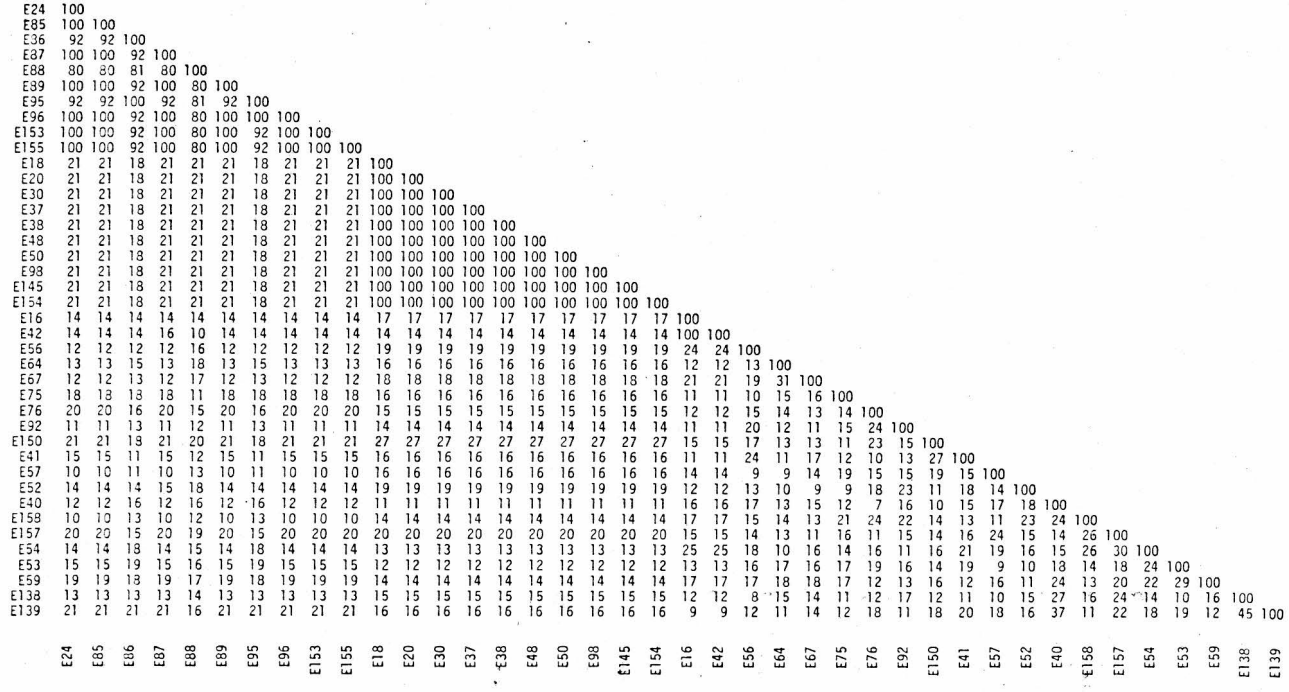


Figure 58. Interpretative drawing of polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia gyrosa and uncommon species of Endothia. Species and isolates by lane number (from left to right): E. gyrosa 1 - E18, 2 - E20, 3 - E30, 4 - E37, 5 - E38, 6 - E48, 7 - E50, 8 - E98, 9 - E145, 10 - E154; E. tropicalis 11 - E57; E. singularis 12 - E52; E. havanensis 13 - E40, 14 - E158; E. coccolobii 15 - E157; E. macrospora 16 - E54; E. japonica 17 - E53, 18 - E59; E. eugeniae 19 - E138, 20 - E139. First extraction of second set of lyophilized mycelial powders.

The similarity matrices are presented in Tables 25 - 29; the dendrogram is depicted in Figure 59. Minor differences are found in the similarity matrices; the dendrogram is based on the average percent similaritiy obtained from four sets of gels (two different extractions from each of two sets of powders). One reason for minor differences among replicate experiments was the manner in which the bands were counted. The densitometer was able to detect many minor bands which were barely visible. Slight variations in protein concentrations placed some of these bands below the level of detection; the similarity coefficient was then artificially reduced. Higher similarity coefficients would have been obtained if only major bands had been counted. The values in the similarity matrices often seem artifically low compared to visual observations of the gels. For this reason, many closely related isolates produce similarity coefficients of only 0.40 to 0.50. Banding patterns of isolates at each end of the gel were usually somewhat distorted and often showed a loss of resolution; this may explain the low similarity coefficient (0.41) between isolates E138 and E139 of E. eugeniae. When these two isolates were compared in the center of the gel (in Experiment #4) the similarity coefficient was increased to 0.70.

In most cases, isolates within a species were uniform in their banding patterns. Isolates of E. gyrosa were extremely consistent; this is quite evident in the photographs. Isolates of E. parasitica were somewhat more variable; this is particularly evident with a major band in the top third of the



	E24	E85	E86	E87	E88	E89	E95	E96	E153	E155	E18	E20	E30	E37	E38	E48	E50	E98	E145	E154	E16	E42	E56	E64	E67	E75	E76	E92	E150	E41	E57	E52	E40	E158	E157	E54	E53	E59	E138	E139
E24	100																																							
E85	100	100																																						
E86	92	92	100																																					
E87	100	100	92	100																																				
E88	80	80	81	80	100																																			
E89	100	100	92	100	80	100																																		
E95	92	92	100	92	81	92	100																																	
E96	100	100	92	100	80	100	100	100																																
E153	100	100	92	100	80	100	92	100	100																															
E155	100	100	92	100	80	100	92	100	100	100																														
E18	21	21	18	21	21	21	18	21	21	21	100																													
E20	21	21	18	21	21	21	18	21	21	21	100	100																												
E30	21	21	18	21	21	21	18	21	21	21	100	100	100																											
E37	21	21	18	21	21	21	18	21	21	21	100	100	100	100																										
E38	21	21	18	21	21	21	18	21	21	21	100	100	100	100	100																									
E48	21	21	18	21	21	21	18	21	21	21	100	100	100	100	100	100																								
E50	21	21	18	21	21	21	18	21	21	21	100	100	100	100	100	100	100																							
E98	21	21	18	21	21	21	18	21	21	21	100	100	100	100	100	100	100	100																						
E145	21	21	18	21	21	21	18	21	21	21	100	100	100	100	100	100	100	100	100																					
E154	21	21	18	21	21	21	18	21	21	21	100	100	100	100	100	100	100	100	100	100																				
E16	14	14	14	14	14	14	14	14	14	14	17	17	17	17	17	17	17	17	17	17	100																			
E42	14	14	14	16	10	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	100	100																		
E56	12	12	12	12	16	12	12	12	12	12	19	19	19	19	19	19	19	19	19	19	24	24	100																	
E64	13	13	15	13	18	13	15	13	13	13	16	16	16	16	16	16	16	16	16	16	12	12	13	100																
E67	12	12	13	12	17	12	13	12	12	12	18	18	18	18	18	18	18	18	18	18	21	21	19	31	100															
E75	18	18	18	18	11	18	18	18	18	18	16	16	16	16	16	16	16	16	16	16	11	11	10	15	16	100														
E76	20	20	16	20	15	20	16	20	20	20	15	15	15	15	15	15	15	15	15	15	12	12	15	14	13	14	100													
E92	11	11	13	11	12	11	13	11	11	11	14	14	14	14	14	14	14	14	14	14	11	11	20	12	11	15	24	100												
E150	21	21	18	21	20	21	18	21	21	21	27	27	27	27	27	27	27	27	27	27	15	15	17	13	13	11	23	15	100											
E41	15	15	11	15	12	15	11	15	15	15	16	16	16	16	16	16	16	16	16	16	11	11	24	11	17	12	10	13	27	100										
E57	10	10	11	10	13	10	11	10	10	10	16	16	16	16	16	16	16	16	16	16	14	14	9	9	14	19	15	15	19	15	100									
E52	14	14	14	15	18	14	14	14	14	14	19	19	19	19	19	19	19	19	19	19	12	12	13	10	9	9	18	23	11	18	14	100								
E40	12	12	16	12	16	12	16	12	12	12	11	11	11	11	11	11	11	11	11	11	16	16	17	13	15	12	7	16	10	15	17	18	100							
E158	10	10	13	10	12	10	13	10	10	10	14	14	14	14	14	14	14	14	14	14	17	17	15	14	13	21	24	22	14	13	11	23	24	100						
E157	20	20	15	20	19	20	15	20	20	20	20	20	20	20	20	20	20	20	20	20	15	15	14	13	11	16	11	15	14	16	24	15	14	26	100					
E54	14	14	18	14	15	14	18	14	14	14	13	13	13	13	13	13	13	13	13	13	25	25	18	10	16	14	16	11	16	21	19	16	15	26	30	100				
E53	15	15	19	15	16	15	19	15	15	15	12	12	12	12	12	12	12	12	12	12	13	13	16	17	16	17	19	16	14	19	9	10	18	14	18	24	100			
E59	19	19	18	19	17	19	18	19	19	19	14	14	14	14	14	14	14	14	14	14	17	17	17	18	18	17	12	13	16	12	16	11	24	13	20	22	29	100		
E138	13	13	13	13	14	13	13	13	13	13	15	15	15	15	15	15	15	15	15	15	12	12	8	15	14	11	12	17	12	11	10	15	27	16	24	14	10	16	100	
E139	21	21	21	21	16	21	21	21	21	21	16	16	16	16	16	16	16	16	16	16	9	9	12	11	14	12	18	11	18	20	18	16	37	11	22	18	19	12	45	100

Table 25. Similarity matrix of ten Endothia species composed of percent similarity values of each isolate pair obtained by polyacylamide gel electrophoresis of the first extraction of the first set of lyophilized mycelial powders.

	E24	E85	E86	E87	E88	E89	E95	E96	E153	E155	E18	E20	E30	E37	E38	E48	E50	E98	E145	E154	E16	E42	E56	E64	E67	E75	E76	E92	E150	E41	E57	E52	E40	E158	E157	E54	E53	E59	E138	E139
E24	100																																							
E85	100	100																																						
E86	73	73	100																																					
E87	100	100	73	100																																				
E88	71	71	86	73	100																																			
E89	100	100	73	100	71	100																																		
E95	73	73	100	73	86	73	100																																	
E96	100	100	73	100	71	100	73	100																																
E153	100	100	73	100	71	100	73	100	100																															
E155	100	100	73	100	71	100	73	100	100	100																														
E18	19	19	16	19	14	19	16	19	19	19	100																													
E20	19	19	16	19	14	19	16	19	19	19	100	100																												
E30	19	19	16	19	14	19	16	19	19	19	100	100	100																											
E37	19	19	16	19	14	19	16	19	19	19	100	100	100	100																										
E38	19	19	16	19	14	19	16	19	19	19	100	100	100	100	100																									
E48	19	19	16	19	14	19	16	19	19	19	100	100	100	100	100	100																								
E50	19	19	16	19	14	19	16	19	19	19	100	100	100	100	100	100	100																							
E98	19	19	16	19	14	19	16	19	19	19	100	100	100	100	100	100	100	100																						
E145	19	19	16	19	14	19	16	19	19	19	100	100	100	100	100	100	100	100	100																					
E154	19	19	16	19	14	19	16	19	19	19	100	100	100	100	100	100	100	100	100	100																				
E16	16	16	15	16	13	16	15	16	16	16	22	22	22	22	22	22	22	22	22	22	100																			
E42	14	14	15	14	13	14	15	14	14	14	22	22	22	22	22	22	22	22	22	22	100	100																		
E56	16	16	13	16	14	16	13	16	16	16	23	23	23	23	23	23	23	23	23	21	21	100																		
E64	8	8	11	8	8	8	11	8	8	8	22	22	22	22	22	22	22	22	22	18	18	19	100																	
E67	15	15	11	15	15	15	11	15	15	15	19	19	19	19	19	19	19	19	19	15	15	26	33	100																
E75	21	21	20	21	15	21	20	21	21	21	26	26	26	26	26	26	26	26	26	14	14	13	8	11	100														
E76	16	16	12	16	11	16	12	16	16	16	26	26	26	26	26	26	26	26	26	17	17	22	14	13	16	100														
E92	16	16	13	16	13	16	13	16	16	16	22	22	22	22	22	22	22	22	22	14	14	14	10	15	14	52	100													
E150	19	19	15	19	14	19	15	19	19	19	23	23	23	23	23	23	23	23	23	15	15	12	12	13	11	28	23	100												
E41	10	10	11	10	12	10	11	10	10	10	15	15	15	15	15	15	15	15	15	19	19	13	13	20	10	13	21	27	100											
E57	15	15	12	15	17	15	12	15	15	15	21	21	21	21	21	21	21	21	21	22	22	21	14	20	16	15	20	16	8	100										
E52	16	16	11	16	18	16	11	16	16	16	22	22	22	22	22	22	22	22	22	16	16	18	11	14	11	23	28	22	27	10	100									
E40	15	15	19	15	15	15	19	15	15	15	18	18	18	18	18	18	18	18	18	19	19	14	12	14	11	11	24	12	19	12	18	100								
E158	16	16	13	16	18	16	13	16	16	16	13	13	13	13	13	13	13	13	13	14	14	14	11	16	18	17	16	12	12	11	16	19	100							
E157	20	20	18	20	12	20	18	20	20	20	20	20	20	20	20	20	20	20	20	13	13	11	8	15	13	12	13	9	15	16	11	13	27	100						
E54	19	19	14	19	16	19	14	19	19	19	21	21	21	21	21	21	21	21	21	18	18	18	13	14	13	16	12	19	24	25	17	18	20	19	100					
E53	14	14	12	14	14	14	12	14	14	14	20	20	20	20	20	20	20	20	20	11	11	10	11	8	21	21	21	14	15	12	12	14	13	12	13	100				
E59	17	17	15	17	19	17	15	17	17	17	15	15	15	15	15	15	15	15	15	18	18	16	21	18	10	9	8	19	15	14	14	18	16	14	26	40	100			
E138	14	14	20	14	16	14	20	14	14	14	14	14	14	14	14	14	14	14	14	19	19	18	10	16	5	13	13	20	23	9	15	37	11	16	15	7	19	100		
E139	15	15	18	15	15	15	18	15	15	15	19	19	19	19	19	19	19	19	19	13	13	19	17	22	7	15	17	21	19	16	17	34	12	17	19	7	18	58	100	

Table 26. Similarity matrix of ten Endothia species composed of percent similarity values of each isolate pair obtained by polyacylamide gel electrophoresis of the second extraction of the first set of lyophilized mycelial powders.

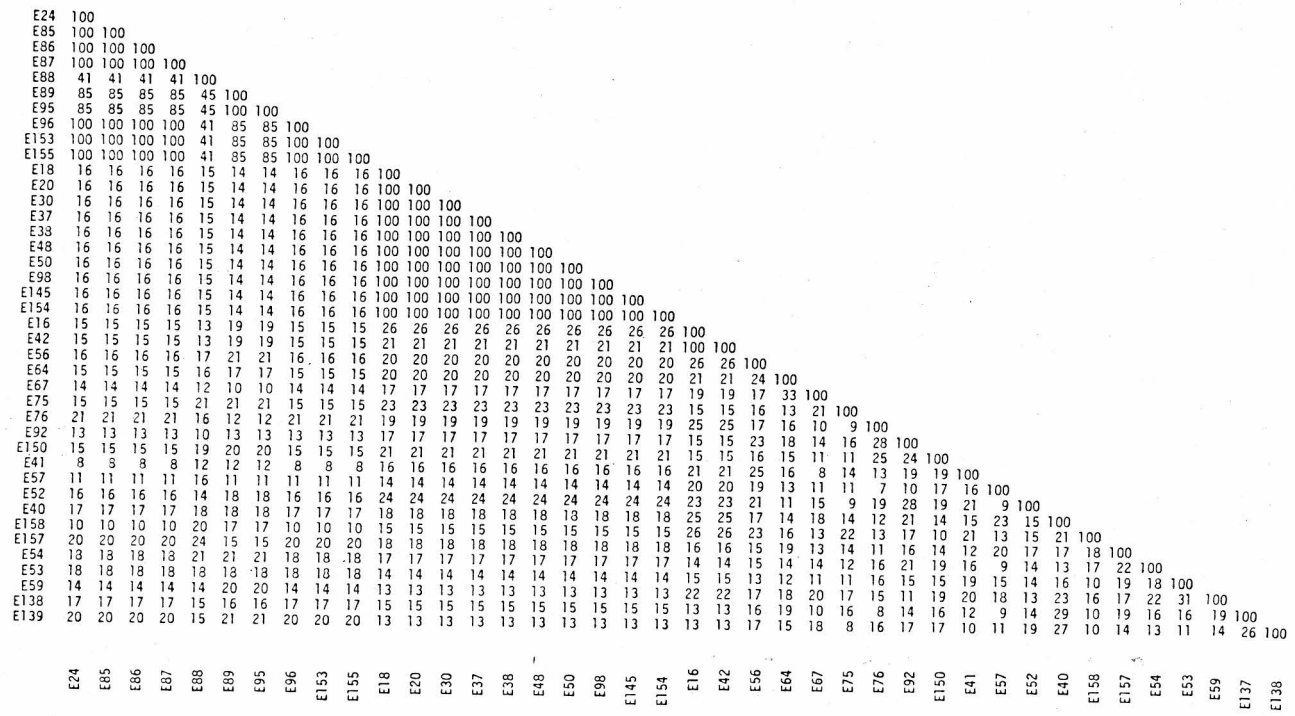
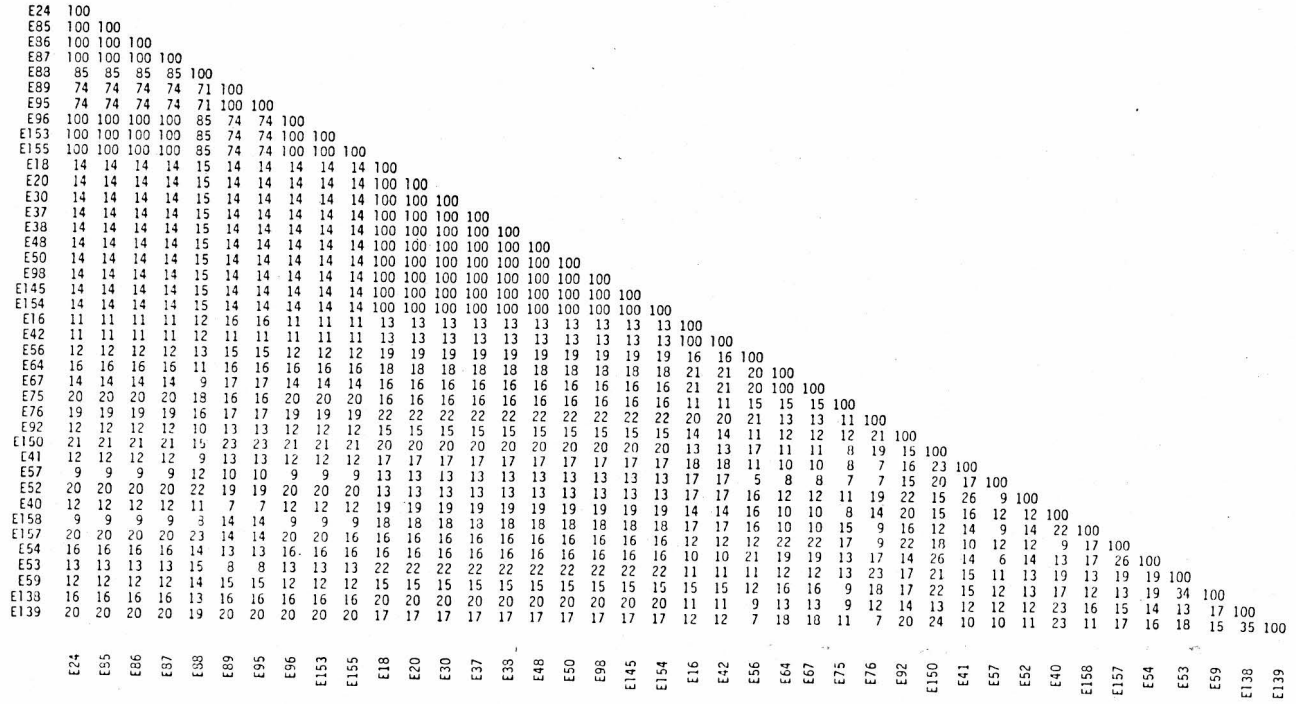


Table 27. Similarity matrix of ten Endothia species composed of percent similarity values of each isolate pair obtained by polyacylamide gel electrophoresis of the first extraction of the second set of lyophilized mycelial powders.



	E24	E85	E86	E87	E88	E89	E95	E96	E153	E155	E18	E20	E30	E37	E38	E48	E50	E98	E145	E154	E16	E42	E56	E64	E67	E75	E76	E92	E150	E41	E57	E52	E40	E158	E157	E54	E53	E59	E138	E139
E24	100																																							
E85	100	100																																						
E86	100	100	100																																					
E87	100	100	100	100																																				
E88	85	85	85	85	100																																			
E89	74	74	74	74	71	100																																		
E95	74	74	74	74	71	100	100																																	
E96	100	100	100	100	85	74	74	100																																
E153	100	100	100	100	85	74	74	100	100																															
E155	100	100	100	100	85	74	74	100	100	100																														
E18	14	14	14	14	15	14	14	14	14	14	100																													
E20	14	14	14	14	15	14	14	14	14	14	100	100																												
E30	14	14	14	14	15	14	14	14	14	14	100	100	100																											
E37	14	14	14	14	15	14	14	14	14	14	100	100	100	100																										
E38	14	14	14	14	15	14	14	14	14	14	100	100	100	100	100																									
E48	14	14	14	14	15	14	14	14	14	14	100	100	100	100	100	100																								
E50	14	14	14	14	15	14	14	14	14	14	100	100	100	100	100	100	100																							
E98	14	14	14	14	15	14	14	14	14	14	100	100	100	100	100	100	100	100																						
E145	14	14	14	14	15	14	14	14	14	14	100	100	100	100	100	100	100	100	100																					
E154	14	14	14	14	15	14	14	14	14	14	100	100	100	100	100	100	100	100	100	100																				
E16	11	11	11	11	12	16	16	11	11	11	13	13	13	13	13	13	13	13	13	13	100																			
E42	11	11	11	11	12	11	11	11	11	11	13	13	13	13	13	13	13	13	13	13	100	100																		
E56	12	12	12	12	13	15	15	12	12	12	19	19	19	19	19	19	19	19	19	19	16	16	100																	
E64	16	16	16	16	11	16	16	16	16	16	18	18	18	18	18	18	18	18	18	18	21	21	20	100																
E67	14	14	14	14	9	17	17	14	14	14	16	16	16	16	16	16	16	16	16	16	21	21	20	100	100															
E75	20	20	20	20	18	16	16	20	20	20	16	16	16	16	16	16	16	16	16	16	11	11	15	15	15	100														
E76	19	19	19	19	16	17	17	19	19	19	22	22	22	22	22	22	22	22	22	22	20	20	21	13	13	11	100													
E92	12	12	12	12	10	13	13	12	12	12	15	15	15	15	15	15	15	15	15	15	14	14	11	12	12	12	21	100												
E150	21	21	21	21	15	23	23	21	21	21	20	20	20	20	20	20	20	20	20	20	13	13	17	11	11	8	19	15	100											
E41	12	12	12	12	9	13	13	12	12	12	17	17	17	17	17	17	17	17	17	17	18	18	11	10	10	8	7	16	23	100										
E57	9	9	9	9	12	10	10	9	9	9	13	13	13	13	13	13	13	13	13	13	17	17	5	8	8	7	7	15	20	17	100									
E52	20	20	20	20	22	19	19	20	20	20	13	13	13	13	13	13	13	13	13	13	17	17	16	12	12	11	19	22	15	26	9	100								
E40	12	12	12	12	11	7	7	12	12	12	19	19	19	19	19	19	19	19	19	19	14	14	16	10	10	8	14	20	15	16	12	12	100							
E158	9	9	9	9	8	14	14	9	9	9	18	18	18	18	18	18	18	18	18	18	17	17	16	10	10	15	9	16	12	14	9	14	22	100						
E157	20	20	20	20	23	14	14	20	20	16	16	16	16	16	16	16	16	16	16	16	12	12	12	22	22	17	9	22	18	10	12	12	9	17	100					
E54	16	16	16	16	14	13	13	16	16	16	16	16	16	16	16	16	16	16	16	16	10	10	21	19	19	13	17	14	26	14	6	14	13	17	26	100				
E53	13	13	13	13	15	8	8	13	13	13	22	22	22	22	22	22	22	22	22	22	11	11	11	12	12	13	23	17	21	15	11	13	19	13	19	19	100			
E59	12	12	12	12	14	15	15	12	12	12	15	15	15	15	15	15	15	15	15	15	15	15	12	16	16	9	18	17	22	15	12	13	17	12	13	19	34	100		
E138	16	16	16	16	13	16	16	16	16	16	20	20	20	20	20	20	20	20	20	20	11	11	9	13	13	9	12	14	13	12	12	12	23	16	15	14	13	17	100	
E139	20	20	20	20	19	20	20	20	20	20	17	17	17	17	17	17	17	17	17	17	12	12	7	18	18	11	7	20	24	10	10	11	23	11	17	16	18	15	35	100

Table 28. Similarity matrix of ten Endothia species composed of percent similarity values of each isolate pair obtained by polyacylamide gel electrophoresis of the second extraction of the second set of lyophilized mycelial powders.

	E24	E85	E86	E87	E88	E89	E95	E96	E153	E155	E18	E20	E30	E37	E38	E48	E50	E98	E145	E154	E16	E42	E56	E64	E67	E75	E76	E92	E150	E41	E57	E52	E40	E158	E157	E54	E53	E59	E138	E139
E24	100																																							
E85	100	100																																						
E86	91	91	100																																					
E87	100	100	100	100																																				
E88	69	69	73	69	100																																			
E89	90	90	90	90	67	100																																		
E95	81	81	90	81	71	91	100																																	
E96	100	100	91	100	69	90	81	100																																
E153	100	100	91	100	69	90	81	100	100																															
E155	100	100	91	100	69	90	81	100	100	100																														
E18	18	18	16	18	16	17	16	18	18	18	100																													
E20	18	18	16	18	16	17	16	18	18	18	100	100																												
E30	18	18	16	18	16	17	16	18	18	18	100	100	100																											
E37	18	18	16	18	16	17	16	18	18	18	100	100	100	100																										
E38	18	18	16	18	16	17	16	18	18	18	100	100	100	100	100																									
E48	18	18	16	18	16	17	16	18	18	18	100	100	100	100	100	100																								
E50	18	18	16	18	16	17	16	18	18	18	100	100	100	100	100	100	100																							
E98	18	18	16	18	16	17	16	18	18	18	100	100	100	100	100	100	100	100																						
E145	18	18	16	18	16	17	16	18	18	18	100	100	100	100	100	100	100	100	100																					
E154	18	18	16	18	16	17	16	18	18	18	100	100	100	100	100	100	100	100	100	100																				
E16	14	14	14	14	12	16	16	14	14	14	20	20	20	20	20	20	20	20	20	20	100																			
E42	14	14	14	14	12	14	15	14	14	14	18	18	18	18	18	18	18	18	18	18	100	100																		
E56	14	14	13	14	15	16	15	14	14	14	20	20	20	20	20	20	20	20	20	20	22	22	100																	
E64	13	31	14	13	13	14	15	13	13	13	19	19	19	19	19	19	19	19	19	19	18	18	19	100																
E67	14	14	13	14	13	14	13	14	14	14	18	18	18	18	18	18	18	18	18	18	19	19	20	49	100															
E75	18	18	13	18	16	19	19	18	18	18	20	20	20	20	20	20	20	20	20	20	13	13	14	13	16	100														
E76	19	19	17	19	14	16	14	19	19	19	20	20	20	20	20	20	20	20	20	20	18	18	19	14	12	12	100													
E92	13	13	13	13	11	13	13	13	13	13	17	17	17	17	17	17	17	17	17	17	14	14	17	13	13	14	31	100												
E150	19	19	17	19	17	21	19	19	19	19	23	23	23	23	23	23	23	23	23	23	14	14	16	13	12	11	24	19	100											
E41	11	11	10	11	10	12	12	11	11	11	16	16	16	16	16	16	16	16	16	16	17	17	18	13	14	11	11	17	24	100										
E57	11	11	11	11	14	12	11	11	11	11	18	18	18	18	18	18	18	18	18	18	18	18	18	14	11	13	13	11	15	18	14	100								
E52	16	16	15	17	18	17	16	16	16	16	20	20	20	20	20	20	20	20	20	20	17	17	17	11	12	10	20	25	17	23	11	100								
E40	14	14	15	14	15	13	15	14	14	14	14	14	14	14	14	14	14	14	14	14	18	18	16	12	14	11	11	20	13	16	16	16	100							
E158	11	11	11	11	14	14	14	11	11	11	14	14	14	14	14	14	14	14	14	14	18	18	17	13	13	19	16	18	12	15	11	17	22	100						
E157	20	20	18	20	20	17	16	20	20	20	20	20	20	20	20	20	20	20	20	20	14	14	13	16	15	15	11	16	14	13	18	14	13	22	100					
E54	17	17	16	18	16	17	16	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	18	14	16	13	16	14	20	21	15	15	15	20	24	100				
E53	15	15	16	15	16	14	14	15	15	15	16	16	16	16	16	16	16	16	16	16	12	12	12	13	12	16	20	17	16	17	12	12	17	12	17	18	100			
E59	16	16	15	16	16	18	17	16	16	16	14	14	14	14	14	14	14	14	14	14	18	18	16	18	18	13	14	12	19	16	15	13	20	14	16	22	34	100		
E138	15	15	16	15	14	15	16	15	15	15	15	15	15	15	15	15	15	15	15	15	14	14	13	14	13	10	11	14	15	14	10	14	29	13	18	15	12	18	100	
E139	19	19	20	19	16	19	19	19	19	19	16	16	16	16	16	16	16	16	16	16	16	12	14	15	18	10	14	16	20	15	14	16	30	11	18	16	11	15	41	100

Table 29. Mean similarity matrix of percent similarity values of ten Endothia species. This matrix was obtained by averaging the percent similarity values from both extractions of each set of lyophilized mycelial powders.

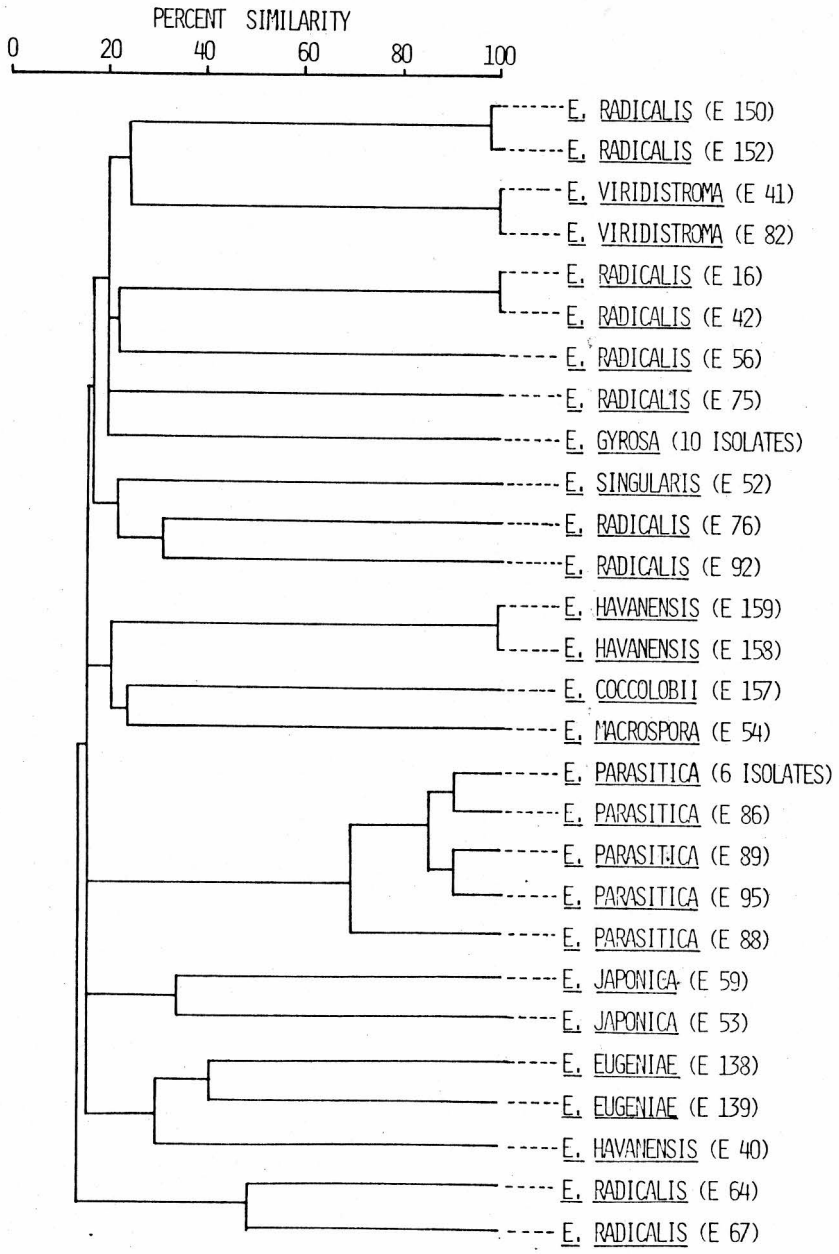


Figure 59. Dendrogram of isolates of ten Endothia species constructed by unweighted pair group average cluster analysis of the mean similarity matrix.

[a] Endothia gyrosa isolates: E18, E20, E30, E37, E38, E48, E50, E98, E145, E154.

[b] Endothia parasitica isolates: E24, E85, E87, E96, E153, E155.

gel (Rf = 0.77) that did not migrate as far in isolate E88 (Rf = 0.78) as it did in the others. A closer examination of the gel reveals that this may be a difference in the quantity of protein produced, rather than an actual difference in the position of the band. A minor band is visible among the other isolates at this location; at the same time, a minor band is visible in the banding pattern of E88 at Rf = 0.77. Since different proteins can share electrophoretic mobilities, several proteins may be involved. Two-dimensional electrophoresis or isozyme analysis would be needed to resolve this matter. Isolate E88 appears to be the least like the other isolates of E. parasitica (its average similarity coefficient was only 0.70 when compared to other isolates after unweighted pair group average cluster analysis). One additional reason for this low value was the artifically depressed percent similarities with the other isolates in the first extraction of the second set of lyophilized powders. The banding pattern of this isolate was much darker than were the others on the gel despite the attempt to compensate for higher protein concentrations by decreasing sample size. Similarity coefficients with the other isolates were artificially lowered to 0.41-0.45 (compared to values of 0.71-0.85 in the other tests). No doubt there are some legitimate differences in this isolate from the others; it often displayed a white colony morphology similar to that associated with hypovirulent strains of the fungus. The amount of difference reported in the

similarity matrix and accompanying dendrogram is somewhat inflated.

The banding patterns of E. parasitica were more variable than were those of E. gyrosa. It would seem that E. gyrosa would have a broader genetic background since it is endemic to North America while E. parasitica was introduced from the Orient. One possible explanation for this is the mode of reproduction of the two species. Endothia parasitica frequently produces perithecia; these were consistently observed in samples collected from different geographic sources and at different times of year for the morphologic studies of Chapter 2. Perithecia were found in less than one percent of the sections of E. gyrosa; pycnidia and conidia were common. Limited sexual reproduction in E. gyrosa would decrease the amount of genetic exchange and would keep genetic variability quite low.

Only a limited number of isolates were available for many of the uncommon and exotic species. The two isolates of E. viridistroma are presumably different subcultures of the same original isolate since the fungus was only collected upon a single occasion. No estimation of intraspecific variation can be made for isolates of E. coccolobii and E. macrospora as only single isolates were available. Endothia havanensis was represented by three isolates; E158 and E159 were collected in Florida, while E40 is a CBS culture which originated in Africa. While the banding patterns of E158 and E159 were identical, they were quite different from that of E40, which was grouped (at a

similarity coefficient of 0.30) with isolates of E. eugeniae. The grouping of E40 with isolates of E. eugeniae and C. cubensis was confirmed in Experiment #4. Since isolates E158 and E159 were tentatively identified as E. havanensis due to their isolation from eucalyptus (ascospores and perithecia were absent from the sample), their identity is not certain and the position of E. havanensis on the dendrogram is suspect.

Isolates of E. radicalis showed the greatest amount of intraspecific variation of all species examined; isolates were scattered throughout the dendrogram and were not clustered together. Isolates E16 and E42 showed identical banding patterns; this is not surprising since they are both subcultures of CBS202.54 obtained at different times. Similarly, isolates E150 and E152 share virtually identical banding patterns (similarity coefficient = 0.98); these isolates were derived from different conidia of the same mass culture. Isolate E75 was not closely related to any other isolates of E. radicalis (similarity coefficient = 0.20); its banding pattern was quite diverse from any isolate of Endothia. It most closely resembled the banding pattern of the contaminated sample of E58 in the second group of powders and should be dismissed from the dendrogram; any similarity to other members of Endothia is probably coincidental. Isolates E64 and E67 were also quite different from the other isolates of E. radicalis. Morphologically these cultures resembled isolates of E. tropicalis, whose banding patterns were

not represented here. Isolates of E. radicalis have not been found in North America in recent years, and the identification of this organism is difficult due to its morphological similarity to E. parasitica. The isolates in our culture collection are a diverse group of organisms, some of which have been incorrectly identified.

Can electrophoresis be used on a practical level to identify and differentiate the various species of Endothia? It is most effective when a limited number of species are being compared; the difference in banding patterns between isolates of E. parasitica and E. gyrosa were quite obvious. When a large number of species or isolates are involved, the analysis becomes much more difficult and time-consuming. It probably could not be used in a clinical situation. While the banding patterns of general proteins are just too complex to be used on a practical level, it is quite possible that individual isozymes may be used to differentiate isolates of various species.

Although most of the species were differentiated from each other, there was no clustering of species into two separate groups corresponding to the genera Endothia and Cryphonectria. Snider (184) observed that genera often cannot be distinguished by electrophoresis due to similar electrophoretic mobilities of nonhomologous proteins coded for by different alleles at different loci. Since genera consist of "closely related" species, usually based on a limited number of morphological criteria, there is no reason to expect that these similarities

would necessarily extend to the biochemical level. For this reason, electrophoresis cannot be used to support or refute Barr's classification system.

Experiment 3: Influence of abnormal morphological types associated with hypovirulent strains of E. parasitica on electrophoretic protein patterns.

Willey (210) delineated six morphological classes into which suspected hypovirulent isolates of E. parasitica with abnormal colony morphologies could be classified. These particular morphologies were obtained when the isolates were grown on potato dextrose agar supplemented with biotin and methionine. The morphological groups were distinguished by the following criteria:

Morphological Group	Description
1	radially symmetrical growth; white mycelium; yellow-pigmented colony center
2	radially symmetrical growth; white
3	slow, erratic growth; white mycelium mycelium
4	slow, erratic growth; orange-pigmented mycelium
5	radially symmetrical growth; orange-pigmented mycelium; aggregates of curved hyphae within colony
6	radially symmetric growth; orange-pigmented mycelium

One representative isolate from each group was compared by PAGE; dsRNA had been detected in each of these isolates (50). An additional isolate, EP113, is a hypovirulent strain from France which is frequently used as a reference among researchers who study hypovirulence.

Some of the representative colony morphologies were not retained on glucose - yeast extract agar (gyea). Isolate RW51P, representative of Group 3, produced radially symmetric growth and resembled the representative isolate of Group 1. Isolates from Group 4 and Group 6 both produced symmetrical growth and could not be differentiated. Isolate Ep113, which was not classified by Willey, most resembled isolate RW341P, the representative of Group 1. Because single-spored isolates of hyoovirulent strains often revert to the wild-type morphology (7), both mass and single-spored cultures were examined. Single-conidial cultures could not be obtained from isolates Ep113 and RW494B due to a complete lack of sporulation (which is quite common among hypovirulent strains). The morphological groups, date of inoculation, pH of broth and protein concentration of each isolate are presented in Table 30.

There were no obvious differences in broth pH or protein concentration among wild and hypovirulent isolates. The banding patterns of the hypovirulent strains did not differ from those of wild-type reference isolates in both Test 1 and Test 2 despite some intraspecific variation among both wild and hypovirulent groups (Figure 62). Hypovirulent isolate RW311P, the representa-

Table 30. Date of inoculation, pH of broth and protein concentration of protein extracts produced by wild and hypovirulent isolates of E. parasitica.

Isolate	Morphological Group[b]	Test 1[a]			Test 2		
		Date of Inoculation	pH of Broth[c]	Protein Concentration	Date of Inoculation	pH of Broth	Protein Concentration
J10	Wild	11/21/82	4.37 (0.03)	112			
J16	Wild	12/5/82	4.28 (0.16)	115			
E153	Wild	12/5/82	4.28 (0.03)	270			
E24	Wild				8/27/83	4.31 (0.25)	103
E85	Wild				8/27/83	4.48 (0.34)	112
E86	Wild				8/27/83	4.32 (0.26)	95
E89	Wild				8/27/83	4.59 (0.14)	142
E155	Wild				8/25/83	4.47 (0.28)	95
911P	4	1/25/83	4.15 (0.24)	170	10/24/83	4.46 (0.03)	184
992P	2	1/25/83	4.37 (0.08)	377	10/24/83	4.36 (0.01)	212
Ep113[e]	-	1/25/83	4.16 (0.05)	213			
178B	6	1/7/83	4.32 (0.18)	108	10/24/83	4.47 (0.01)	180
51P	3	1/25/83	4.31 (0.01)	343	10/24/83	4.24 (0.04)	196
341P	1	1/25/83	4.35 (0.05)	377	10/24/83	4.20 (0.04)	57
494B	5	1/25/83	4.28 (0.33)	197			

Table 30 (continued)

[a]Hypovirulent isolates in Test 1 are all mass cultures; those in Test 2 are all derived from single conidia. All wild isolates in Test 1 and Test 2 are derived from single conidia.

[b]Morphological groups as defined by Willey (210).

[c]Average pH of contents of three flasks. Standard error of mean in parentheses. Initial pH adjusted to 5.70 after autoclaving by the addition of 0.1% NaOH or 0.1% lactic acid.

[d]Protein concentration of samples expressed in μg protein/100 μl buffer. Protein concentration determined by the Bradford Coomassie blue assay (29) from a supernatant derived from a suspension of lyophilized mycelium (see text for details) in 0.125 M Tris-Cl buffer + 10% glycerol (50 mg lyophilized mycelium/ml buffer).

[e]Standard hypovirulent reference isolate obtained from Grente. Willey (210) did not classify this culture into a specific morphological group.

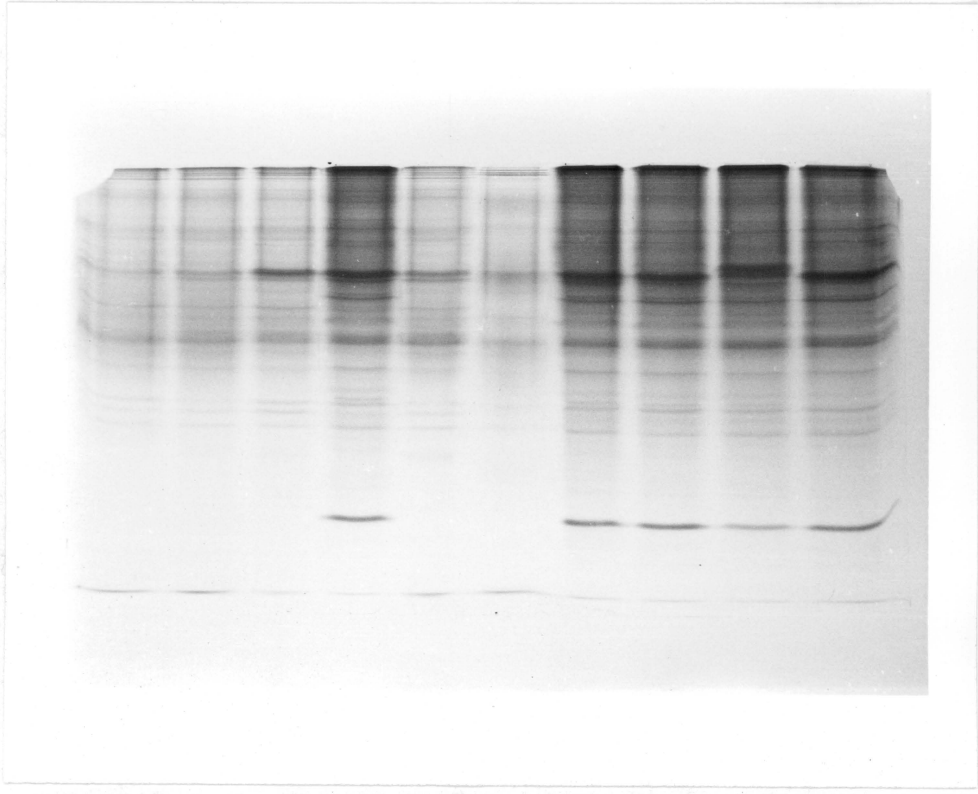


Figure 60. Polyacrylamide electrophoretic gel of buffer-soluble proteins of wild and hypovirulent strains of Endothia parasitica. Virulence level and isolate number by lane number (from left to right): Wild 1 - E24, 2 - E85, 3 - E86, 4 - E89, 5 - E155; Hypovirulent 6 - RW341P, 7 - RW911P, 8 - RW992P, 9 - RW178B, 10 - RW51P.

tive of group #4, appeared the most dissimilar to the other isolates in Test #1; this was not confirmed in Test #2. The low protein concentration of this isolate in Test #1 made the banding pattern somewhat difficult to resolve.

From these studies, it appears that PAGE cannot be used with a general protein stain to differentiate hypovirulent strains of E. parasitica from those of the wild type. This is not surprising since no gene product has been consistently associated with hypovirulence. These tests should be considered preliminary, however, since a limited number of hypovirulent strains were examined. It is possible that specific activity stains, which detect particular enzymes and which are much more sensitive than general protein stains, may be more effective in detecting hypovirulence.

Experiment 4: Electrophoretic comparisons of banding patterns of E. eugeniae and C. cubensis.

The banding pattern of general soluble proteins of isolates of E. eugeniae and C. cubensis were examined; the date of inoculation, pH of broth and protein concentration of each isolate are presented in Table 31 while the gel is shown in Figure 61. The similarity matrix is presented in Table 32, and the banding diagram and dendrogram are shown in Figures 62 and 63. There was no consistent differentiation of isolates of C. cubensis and E. eugeniae, although the geographic origin of the isolate did seem to influence the banding pattern slightly. Four

Table 31. Date of inoculation, pH of broth and protein concentration of lyophilized mycelium of *E. eugeniae*, *C. cubensis*, *E. havanensis* and *E. coccolobii* used in electrophoresis.

Species	Isolate	Date of Inoculation	pH of Broth	Protein Concentration
E. eugeniae	E138	8/25/83	4.10 (0.09)	410
" "	E139	7/27/83	3.92 (0.07)	400
" "	H173	10/24/83	4.12 (0.09)	320
" "	H174	10/24/83	4.07 (0.06)	330
" "	H176	10/24/83	4.03 (0.09)	320
" "	H184	3/5/84	3.90 (0.03)	580
" "	H185	3/5/84	4.02 (0.05)	390
" "	H186	3/5/84	3.85 (0.01)	400
" "	H189	3/5/84	3.92 (0.05)	713
C. cubensis	H91	8/26/83	4.05 (0.13)	337
" "	H137	8/25/83	4.17 (0.06)	230
" "	H151	10/3/83	3.74 (0.26)	320
" "	H154	10/3/83	3.81 (0.05)	287
" "	H175	10/24/83	3.83 (0.01)	330
" "	H182	3/5/84	5.03 (0.07)	330
" "	H188	3/5/84	3.81 (0.04)	347
E. havanensis	E40	7/27/83	4.40 (0.23)	250
" "	E158	8/25/83	4.12 (0.10)	383
" "	H183	3/5/84	4.07 (0.02)	520
E. coccolobii	E157	8/26/83	4.73 (0.12)	553

[a]Average pH of contents of three flasks. Standard error of mean in parentheses. Initial pH adjusted to 5.70 after autoclaving by the addition of 0.1% NaOH or 0.1% lactic acid.

[b]Protein concentration of samples expressed in µg protein/100 µl buffer. Protein concentration determined by the Bradford Coomassie blue assay (29) from a supernatant derived from a suspension of acetone powdered mycelium (see text for details) in 0.125 M Tris-Cl buffer + 10% glycerol (50 mg acetone powder/ml buffer).

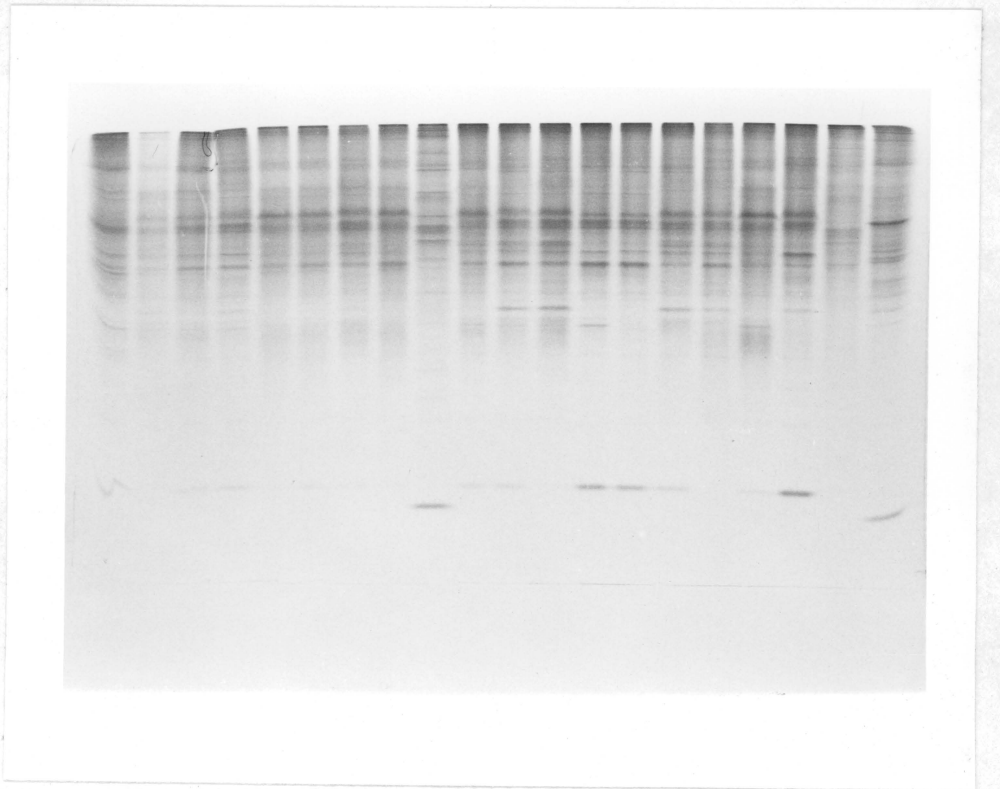


Figure 61. Polyacrylamide electrophoretic gel of buffer-soluble proteins of Endothia eugeniae, E. havanensis, E. coccolobii and Cryphonectria cubensis. Species and isolates by lane number (from left to right): C. cubensis 1 - H91, 2 - H137, 3 - H151, 4 - H154, 7 - H175, 9 - H182, 14 - H188; E. eugeniae 5 - H173, 6 - H174, 8 - H176, 11 - H184, 12 - H185, 13 - H186, 15 - H189, 17 - E138, 18 - E139; E. havanensis 10 - H183, 16 - E40, 20 - E158; E. coccolobii 19 - E157.

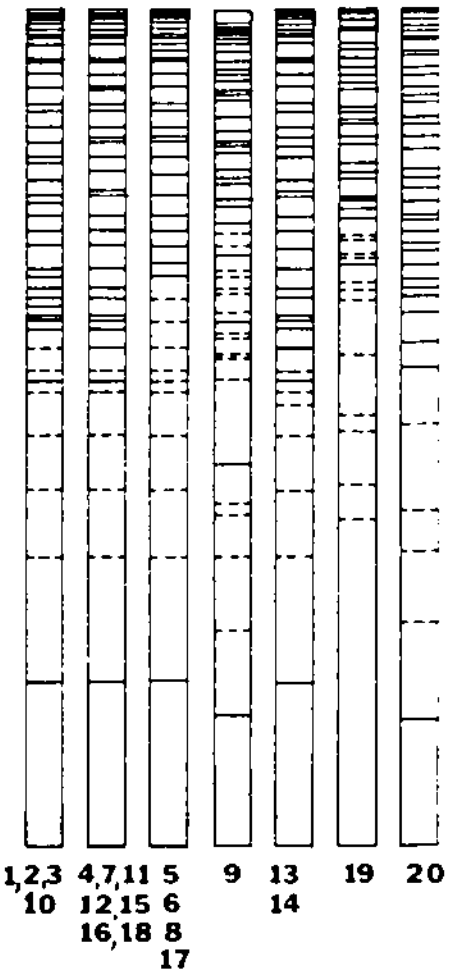


Figure 62. Interoretative drawing of polyacrylamide electrophoretic gel of buffer-soluble proteins of Endothia eugeniae, E. havanensis, E. coccolobii and Cryphonectria cubensis. Species and isolates by lane number (from left to right): C. cubensis 1 - H91, 2 - H137, 3 - H151, 4 - H154, 7 - H175, 9 - H182, 14 - H188; E. eugeniae 5 - H173, 6 - H174, 8 - H176, 11 - H184, 12 - H185, 13 - H186, 15 - H189, 17 - E138, 18 - E139; E. havanensis 10 - H183, 16 - E40, 20 - E158; E. coccolobii 19 - E157.

Table 32. Similarity matrix of percent similarity values obtained by polyacrylamide gel electrophoresis of lyophilized mycelium of isolates of Endothia eugeniae, E. coccolobii, E. havanensis and Cryphonectria cubensis.

	H91	H137	H151	H154	H173	H174	H175	H176	H182	H183	H184	H185	H186	H188	H189	E40	E138	E139	E157	E158
H91	100																			
H137	100	100																		
H151	100	100	100																	
H154	79	79	79	100																
H173	65	65	65	70	100															
H174	65	65	65	70	100	100														
H175	79	79	79	100	70	70	100													
H176	65	65	65	70	100	100	70	100												
H182	15	15	15	18	14	14	18	14	100											
H183	100	100	100	79	65	65	79	65	15	100										
H184	79	79	79	100	70	70	100	70	18	79	100									
H185	79	79	79	100	70	70	100	70	18	79	100	100								
H186	61	61	61	70	53	53	70	53	13	61	70	70	100							
H188	61	61	61	70	53	53	70	53	13	61	70	70	100	100						
H189	79	79	79	100	70	70	100	70	18	79	100	100	70	70	100					
E40	79	79	79	100	70	70	100	70	18	79	100	100	70	70	100	100				
E138	65	65	65	70	100	100	70	100	14	65	70	70	53	53	70	70	100			
E139	79	79	79	100	70	70	100	70	18	79	100	100	70	70	100	100	70	100		
E157	10	10	10	15	16	16	15	16	14	10	15	15	15	15	15	15	16	15	100	
E158	13	13	13	10	16	16	10	16	12	13	10	10	15	15	10	10	16	10	15	100

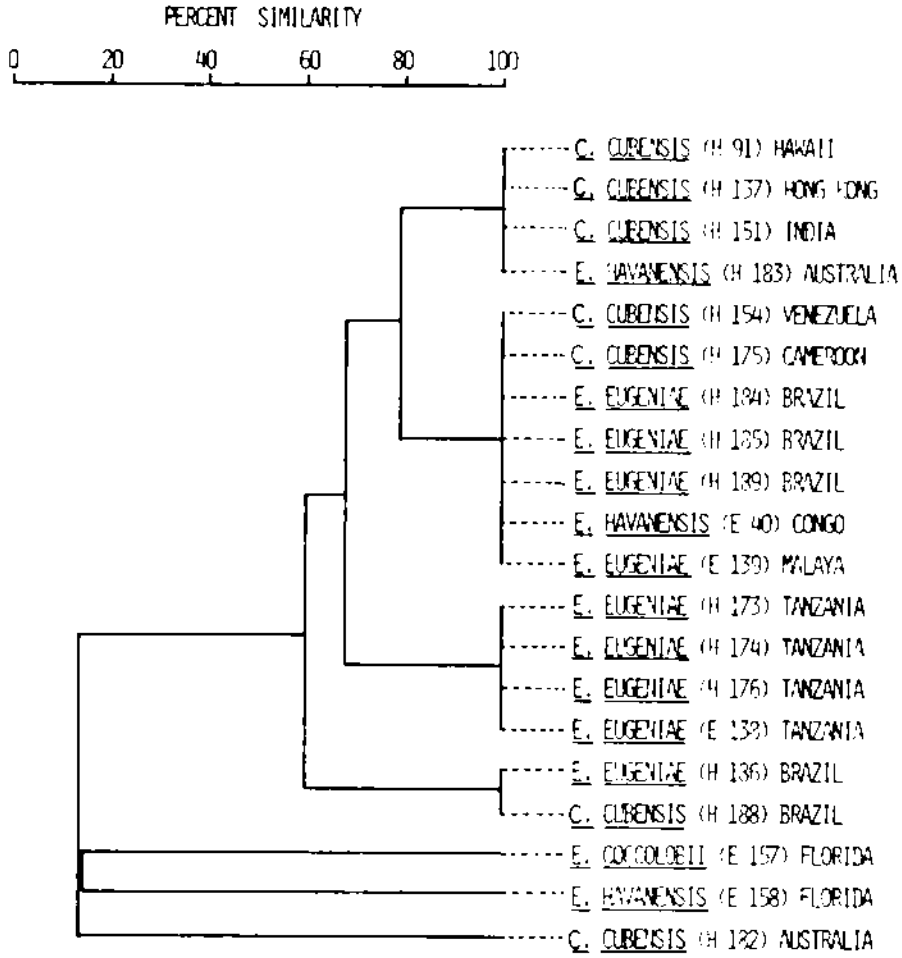


Figure 63. Dendrogram of isolates of Endothia eugeniae, E. coccolobii, E. havanensis and Cryphonectria cubensis constructed by unweighted pair group average cluster analysis of the similarity matrix (Table 32).

isolates of E. eugeniae from Tanzania showed identical banding patterns, as did three isolates from Brazil. Geographic origin did not necessarily guarantee identical banding patterns; two isolates from Brazil (H186 and H188) were grouped with other Brazilian isolates at a similarity coefficient of only 0.60. One isolate from Australia, originally identified as E. havanensis, produced banding patterns identical to isolates of C. cubensis from Hawaii, Hong Kong and India; in all liklihood it also belongs to the E. eugeniae - C. cubensis group. Another isolate originally identified as E. havanensis from the Congo, E40, produced banding patterns similar to members of the E. eugeniae - C. cubensis group. As this isolate is a CBS culture and is often used as a reference of E. havanensis, its identification as a member of this group is quite important. The banding pattern of one isolate of C. cubensis from Australia (H182) did not resemble those of the other isolates; it was probably misidentified or contaminated in culture. Two other reference isolates which were included, representing E. coccolobii (E157) and a suspected isolate of E. havanensis from Florida (E158), were also quite dissimilar in banding pattern. The identity of this E. havanensis isolate cannot be certain since there are no reference strains for comparison.

The data from this experiment support the idea that E. eugeniae and C. cubensis are conspecific. There are some minor differences in banding patterns, as there are among isolates of

E. parasitica. This is not surprising since these isolates were collected from all over the world. The consolidation of these two species is supported by the work of Alfenas (3,5), who demonstrated the similarities of organisms by isozyme analysis, and by Hodges (personal communication), who conducted cross-inoculation studies and obtained infection and colonization on both clove and eucalyptus with isolates of both E. eugeniae and C. cubensis.

Experiment 5: Use of specific activity stains with different species of Endothia and C. cubensis.

Because specific activity stains are dependent upon the presence of functioning enzymes, it was necessary to insure the preservation of extracted isozymes in an active state. For this reason, extracts from acetone powders were used for these tests rather than lyophilized mycelium. Acetone powders are prepared at a colder temperature than lyophilization, and the acetone rapidly dehydrates the cell, thus preserving the enzymes in a functional state by preventing hydrolysis (J. R. Elkins, personal communication). The dates of inoculation, pH of broth and protein concentration of each isolate are presented in Table 33. Protein concentrations of extracts prepared from acetone powders were somewhat lower than those obtained from lyophilized mycelium, but they were adequate for the tests which were used. Since specific activity stains are generally more sensitive than general protein stains, lower quantities of proteins were needed. Standard volumes of sample were applied to the gel since the optimum staining concentrations were not known; these volumes

Table 33. Date of inoculation, pH of broth and concentration of acetone powders produced from Endothia species and Cryphonectria cubensis used for electrophoresis.

Species	Isolate	Set 1				Set 2		
		Date of Inoculation	pH of Broth[a]	Protein[b] Concentration 1	2	Date of Inoculation	pH of Broth	Protein Concentration
E. parasitica	E87	2/3/84	4.51 (0.03)	29	67	11/7/83	4.38 (0.14)	93
" "	E88	2/3/84	4.58 (0.06)	57	88	11/7/83	4.35 (0.22)	81
" "	E89	2/3/84	4.39 (0.02)	65	90	11/7/83	4.28 (0.14)	86
E. gyrosa	E48	2/3/84	4.71 (0.25)	166	175	11/7/83	4.73 (0.02)	184
" "	E50	2/3/84	4.52 (0.03)	148	175	11/7/83	4.73 (0.01)	160
" "	E154	2/3/84	4.64 (0.07)	148	173	11/7/83	4.64 (0.02)	160
E. radicalis	E16	12/5/83	5.02 (0.10)	126	157	11/7/83	5.54 (0.11)	102
" "	E42	12/5/84	4.97 (0.03)	107	133	11/7/83	4.93 (0.17)	163
" "	E56	2/3/84	4.77 (0.13)	47	62	2/3/83	4.77 (0.13)	69
" "	E64	2/3/83	5.52 (0.26)	175	203	11/7/83	5.56 (0.14)	247
" "	E67	2/3/84	4.98 (0.03)	155	180	11/7/83	4.43 (0.03)	138
" "	E76	12/5/83	4.97 (0.06)	144	153	11/18/83	5.26 (0.14)	187
" "	E92	2/14/84	4.43 (0.04)	215	255	11/18/83	4.54 (0.29)	187
" "	E150	12/5/83	4.58 (0.14)	175	186	11/18/83	4.32 (0.16)	163
" "	E152	2/3/84	4.41 (90.9)	170	136	11/18/83	4.49 (0.14)	116
E. eugeniae	E138	2/14/84	4.03 (0.06)	186	192	11/18/83	4.14 (0.09)	70
" "	E139	2/14/84	3.95 (0.07)	179	205	11/18/83	4.02 (0.02)	172
" "	H173	12/5/83	4.08 (0.06)	153	186	11/26/83	4.25 (0.04)	143

Table 33 (continued)

Species	Isolate	Set 1				Set 2		
		Date of Inoculation	pH of Broth[a]	Protein[b] Concentration 1	2	Date of Inoculation	pH of Broth	Protein Concentration
E. eugeniae (continued)	H186	3/5/84	3.87 (0.12)	219	273	3/5/84	3.87 (0.12)	247
" "	H189	3/5/84	3.92 (0.05)	219	346	3/5/84	3.95 (0.01)	201
C. cubensis	H137	12/5/84	4.03 (0.01)	59	92	11/26/83	3.95 (0.07)	92
" "	H151	12/5/83	3.74 (0.15)	102	153	11/26/83	3.99 (0.02)	121
" "	H154	12/5/83	3.89 (0.02)	117	138	11/26/83	3.96 (0.08)	124
" "	H182	2/14/84	4.96 (0.04)	177	201	3/5/84	5.10 (0.19)	221
" "	H188	3/5/84	3.81 (0.04)	175	298	3/5/84	3.82 (0.03)	201
E. japonica	E53	12/5/83	4.39 (0.05)	97	155	11/26/83	4.42 (0.01)	129
" "	E59	2/14/84	4.48 (0.06)	158	192	11/18/83	4.50 (0.04)	124
E. singularis	E52	2/14/84	4.58 (0.19)	97	201	11/26/84	4.42 (0.29)	182
E. tropicalis	E57	2/14/84	5.45 (0.02)	219	408	11/18/84	5.17 (0.02)	172
" "	E70	2/14/84	5.66 (0.19)	175	210	11/18/84	5.35 (0.11)	163
E. viridistroma	E41	2/14/84	4.88 (0.06)	135	153	11/18/84	4.48 (0.09)	153
" "	E82	2/14/84	4.87 (0.05)	186	188	11/18/84	4.26 (0.05)	153

Table 33 (continued)

Species	Isolate	Set 1				Set 2		
		Date of Inoculation	pH of Broth[a]	Protein Concentration[b] 1	2	Date of Inoculation	pH of Broth	Protein Concentration
E. coccolobii	E157	12/5/83	4.57 (0.06)	153	162	11/18/83	4.40 (0.02)	163
E. havanensis	E158	12/5/83	4.00 (0.13)	121	140	11/18/83	4.00 (0.09)	172
" "	E159	2/14/84	5.08 (0.27)	184	205	11/18/83	4.00 (0.09)	172
" "	E40	2/14/84	4.73 (0.07)	103	114	11/26/83	4.00 (0.08)	148

[a]Average pH of contents of three flasks. Standard error of mean in parentheses. Initial pH adjusted to 5.70 after autoclaving by the addition of 0.1% NaOH or 0.1% lactic acid.

[b]Protein concentration of samples expressed in µg protein/100 µl buffer. Protein concentration determined by the Bradford Coomassie blue assay (29) from a supernatant derived from a suspension of acetone powdered mycelium (see text for details) in 0.125 M Tris Cl buffer + 10% glycerol (50 mg acetone powder/ml buffer).

were then adjusted by trial and error to produce gels of good resolution. As in the previous tests, the pH measurements of isolates of E. eugeniae and C. cubensis, with the exception of isolate H182, were markedly lower than those of the other species; those of E. tropicalis were much higher.

Photographs of the gels and diagrams of the banding patterns are presented (Figures 64 - 73). The activity of β-D-glucosidase was very faint in all isolates (Figures 64 and 65). The bands did not migrate very far into the gel; this may indicate that the acrylamide concentration was too high, the pH of the gel (8.3) was not optimal for enzyme movement or the protein had aggregated within the sample before electrophoresis (77). Isolates E57, E70 (E. tropicalis) and E64 and F67 (E. radicalis) produced a pair of bands on at least one of the gels. All other isolates were characterized by a single band. The positions of the individual bands were difficult to ascertain due to their proximity to the edge of the gel. It does seem that at least two different alleles were represented, but further experiments with greater movement and concentration would be necessary to confirm this.

The resolution of acid and alkaline phosphatase was rather poor; large blots of stain were present instead of precise bands (Figures 66 - 69). Interpretation of the gels was therefore difficult. Several characteristic bands did emerge, however. Isolates of E. gyrosa had a distinctive banding pattern with

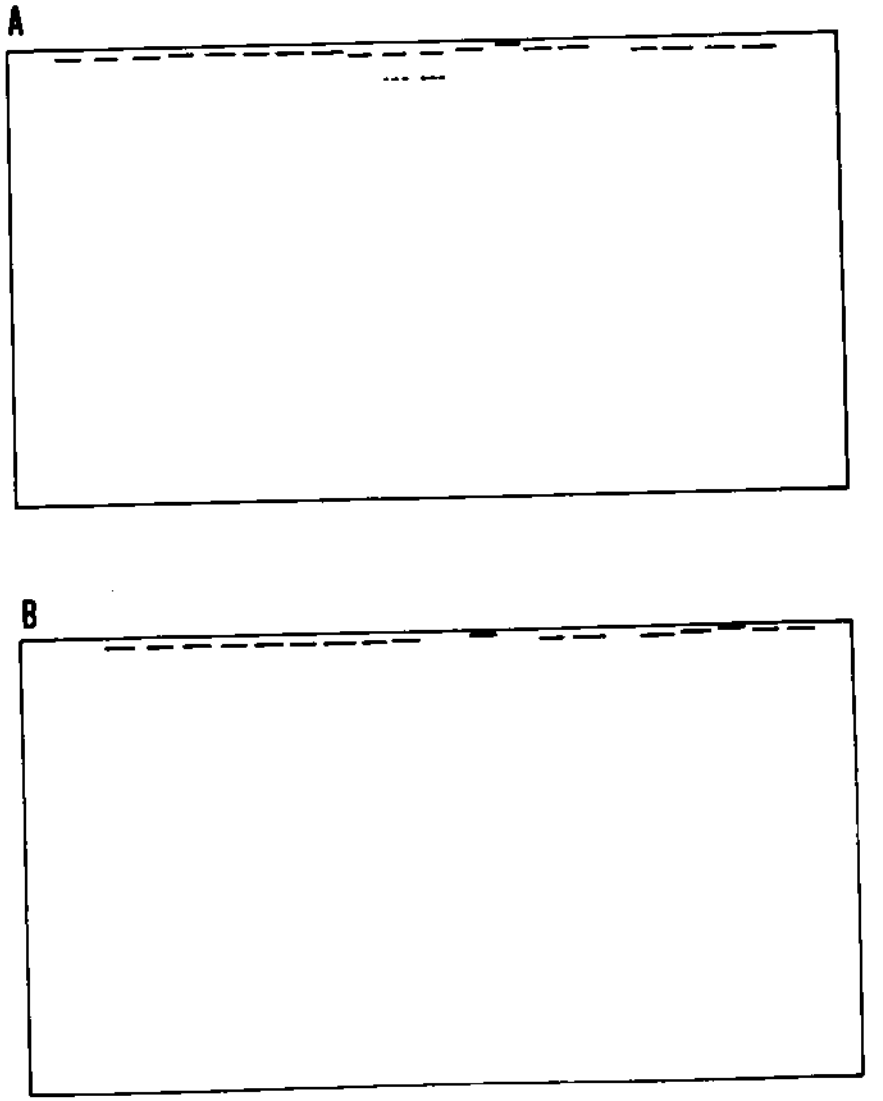


Figure 64. Interpretative drawings of β -D-glucosidase activity of acetone powder-extracts of 11 species of Endothia and Cryphonectria cubensis from the first set of acetone powders. Species and isolates by lane number (from left to right):

Front plate (A) E. parasitica 1 - E87, 2 - E88, 3 - E89; E. gyrosa 4 - E48, 5 - E50, 6 - E154; E. radicalis 7 - E16, 8 - E42, 9 - E56, 10 - E64, 11 - E67, 12 - E76, 13 - E92, 14 - E150, 15 - E152; E. singularis 16 - E52; E. macrospora 17 - E54; E. coccolobii 18 - E157; E. viridistroma 19 - E41, 20 - E82.

Back plate (B) E. japonica 1 - E53, 2 - E59; E. parasitica 3 - E87; E. gyrosa 4 - E154; E. tropicalis 5 - E57, 6 - E70; E. havanensis 7 - E158, 8 - E159, 9 - E40; E. eugeniae 10 - E138, 11 - E139, 15 - H173, 17 - H185, 18 - H186, 20 - H189; C. cubensis 12 - H137, 13 - H151, 14 - H154, 16 - H182, 19 - H188.

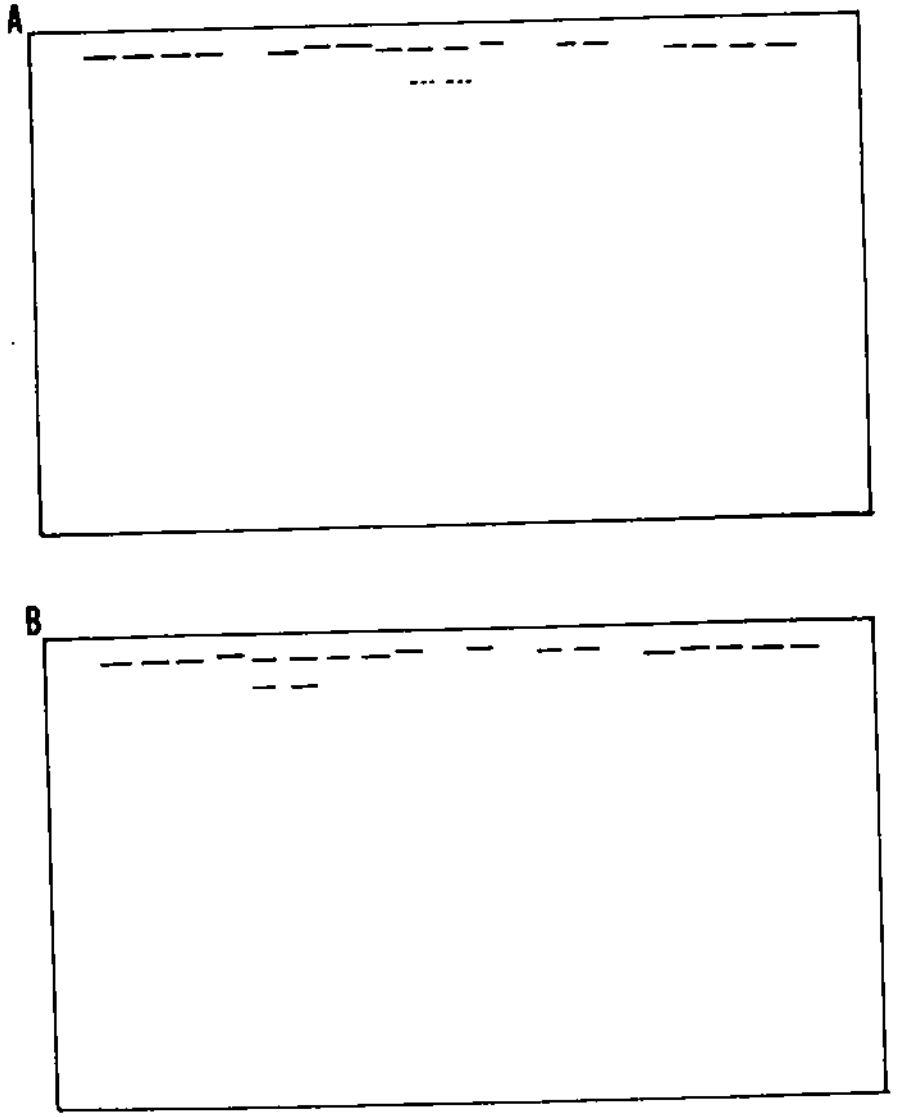


Figure 65. Interpretative drawings of β-D-glucosidase activity of acetone powder-extracts of 11 species of Endothia and Cryphonectria cubensis from the second set of acetone powders. Species and isolates by lane number (from left to right):

Front plate (A) E. parasitica 1 - E87, 2 - E88, 3 - E89; E. gyrosa 4 - E48, 5 - E50, 6 - E154; E. radicalis 7 - E16, 8 - E42, 9 - E56, 10 - E64, 11 - E67, 12 - E76, 13 - E92, 14 - E150, 15 - E152; E. singularis 16 - E52; E. macrospora 17 - E54; E. coccolobii 18 - E157; E. viridistroma 19 - E41, 20 - E82.

Back plate (B) E. japonica 1 - E53, 2 - E59; E. parasitica 3 - E87; E. gyrosa 4 - E154; E. tropicalis 5 - E57, 6 - E70; E. havanensis 7 - E158, 8 - E159, 9 - E40; E. eugeniae 10 - E138, 11 - E139, 15 - H173, 17 - H185, 18 - H186, 20 - H189; C. cubensis 12 - H137, 13 - H151, 14 - H154, 16 - H182, 19 - H188.

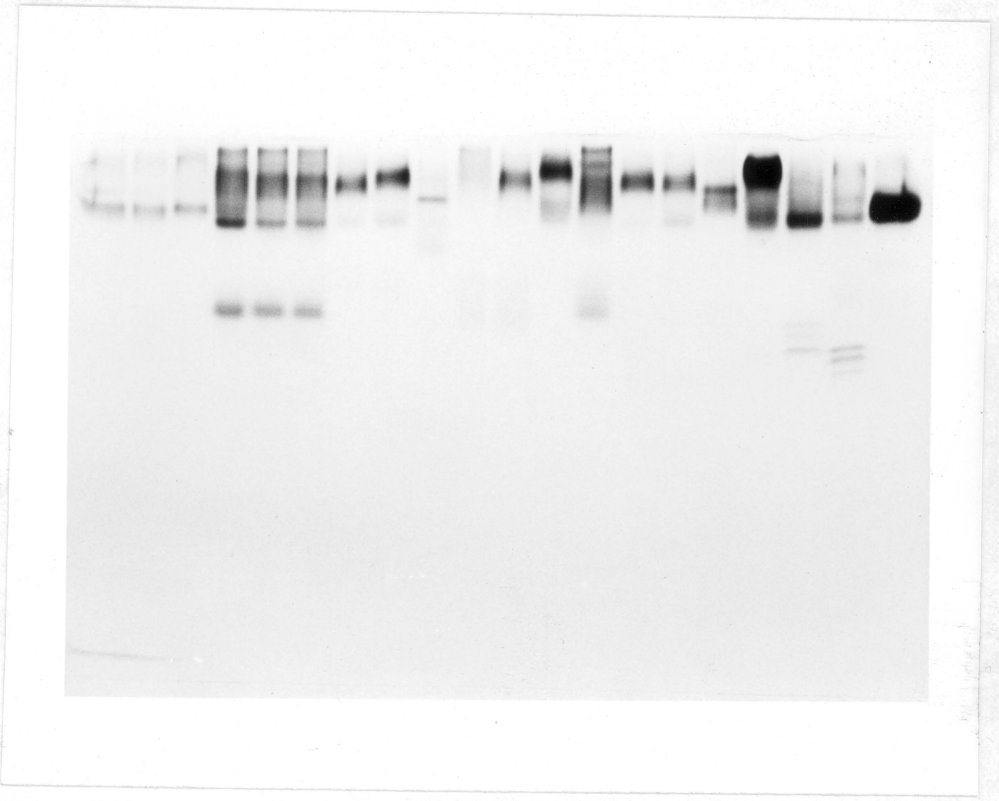


Figure 66. Polyacrylamide electrophoretic gel of acid and alkaline phosphatase activity of acetone powder-extracts of 11 species of Endothia and Cryphonectria cubensis. Species and isolates by lane number (from left to right): E. parasitica 1 - E87, 2 - E88, 3 - E89; E. gyrosa 4 - E48, 5 - E50, 6 - E154; E. radicalis 7 - E16, 8 - E42, 9 - E56, 10 - E64, 11 - E67, 12 - E76, 13 - E92, 14 - E150, 15 - E152; E. singularis 16 - E52; E. macrospora 17 - E54; E. coccolobii 18 - E157; E. viridistroma 19 - E41, 20 - E82.

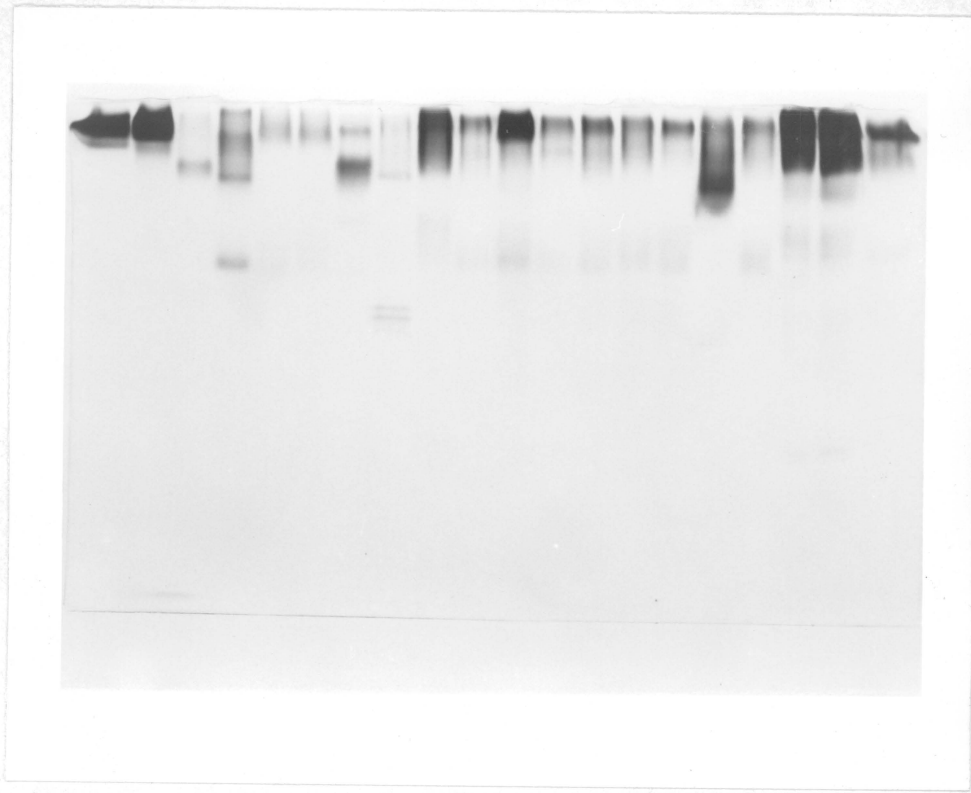


Figure 67. Polyacrylamide electrophoretic gel of acid and alkaline phosphatase activity of acetone-powder extracts of 11 species of Endothia and Cryphonectria cubensis. Species and isolates by lane number (from left to right): E. japonica 1 - E53, 2 - E59; E. parasitica 3 - E87; E. gyrosa 4 - E154; E. tropicalis 5 - E57, 6 - E70; E. havanensis 7 - E158, 8 - E159, 9 - E40; E. eugeniae 10 - E138, 11 - E139, 15 - H173, 17 - H185, 18 - H186, 20 - H189; C. cubensis 12 - H137, 13 - H151, 14 - H154, 16 - H182, 19 - H188.

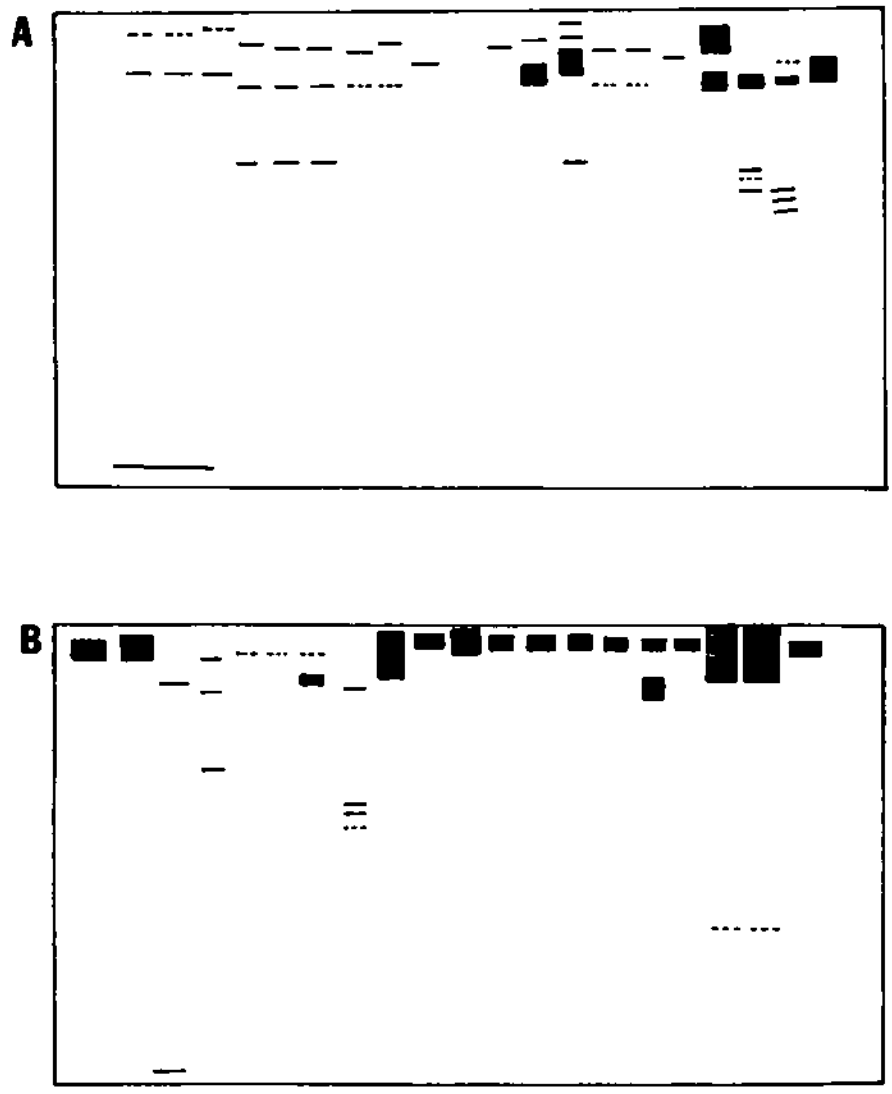


Figure 68. Interpretative drawings of acid and alkaline phosphatase activity of acetone powder-extracts of 11 species of Endothia and Cryphonectria cubensis from the first set of acetone powders. Species and isolates by lane number (from left to right):

Front plate (A) E. parasitica 1 - E87, 2 - E88, 3 - E89; E. gyrosa 4 - E48, 5 - E50, 6 - E154; E. radicalis 7 - E16, 8 - E42, 9 - E56, 10 - E64, 11 - E67, 12 - E76, 13 - E92, 14 - E150, 15 - E152; E. singularis 16 - E52; E. macrospora 17 - E54; E. coccolobii 18 - E157; E. viridistroma 19 - E41, 20 - E82.

Back plate (B) E. japonica 1 - E53, 2 - E59; E. parasitica 3 - E87; E. gyrosa 4 - E154; E. tropicalis 5 - E57, 6 - E70; E. havanensis 7 - E158, 8 - E159, 9 - E40; E. eugeniae 10 - E138, 11 - E139, 15 - H173, 17 - H185, 18 - H186, 20 - H189; C. cubensis 12 - H137, 13 - H151, 14 - H154, 16 - H182, 19 - H188.

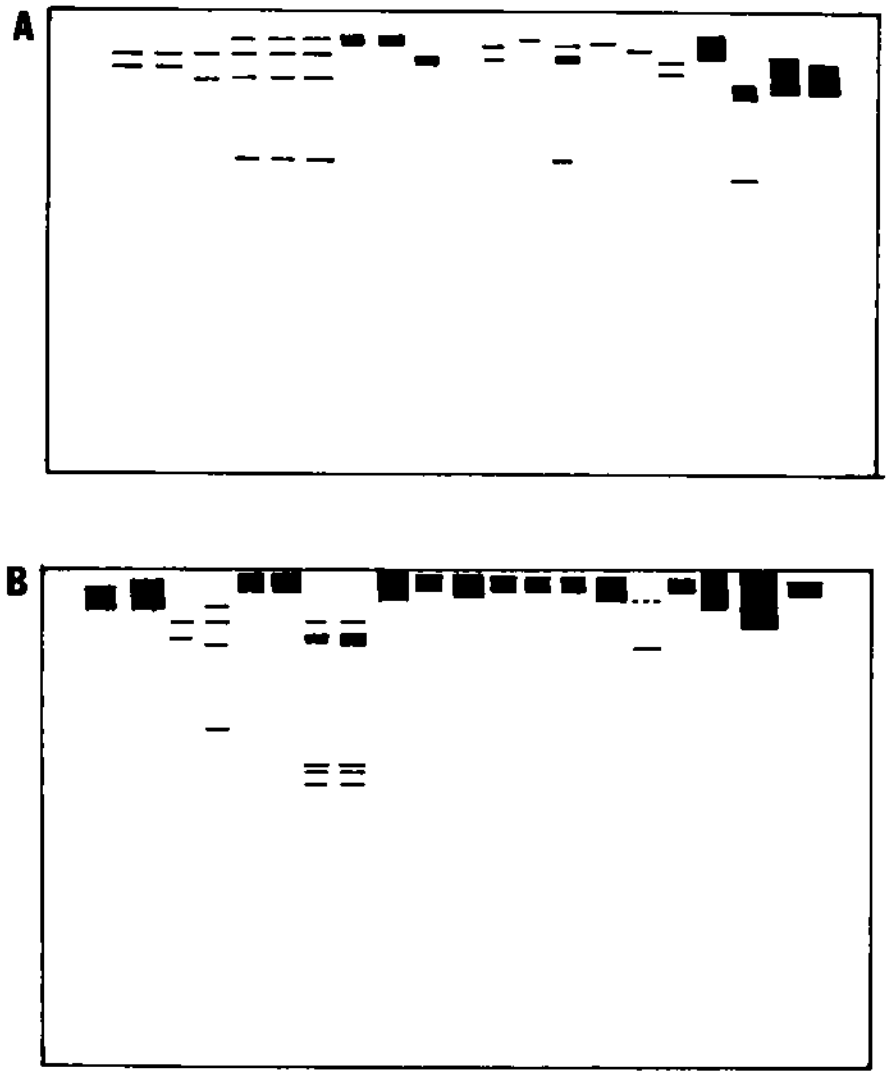


Figure 69. Interpretative drawings of acid and alkaline phosphatase activity of acetone-powder extracts of 11 species of Endothia and Cryphonectria cubensis from the second set of acetone powders. Species and isolates by lane number (from left to right):

Front plate (A) E. parasitica 1 - E87, 2 - E88, 3 - E89; E. gyrosa 4 - E48, 5 - E50, 6 - E154; E. radicalis 7 - E16, 8 - E42, 9 - E56, 10 - E64, 11 - E67, 12 - E76, 13 - E92, 14 - E150, 15 - E152; E. singularis 16 - E52; E. macrospora 17 - E54; E. coccolobii 18 - E157; E. viridistroma 19 - E41, 20 - E82.

Back plate (B) E. japonica 1 - E53, 2 - E59; E. parasitica 3 - E87; E. gyrosa 4 - E154; E. tropicalis 5 - E57, 6 - E70; E. havanensis 7 - E158, 8 - E159, 9 - E40; E. eugeniae 10 - E138, 11 - E139, 15 - H173, 17 - H185, 18 - H186, 20 - H189; C. cubensis 12 - H137, 13 - H151, 14 - H154, 16 - H182, 19 - H188.

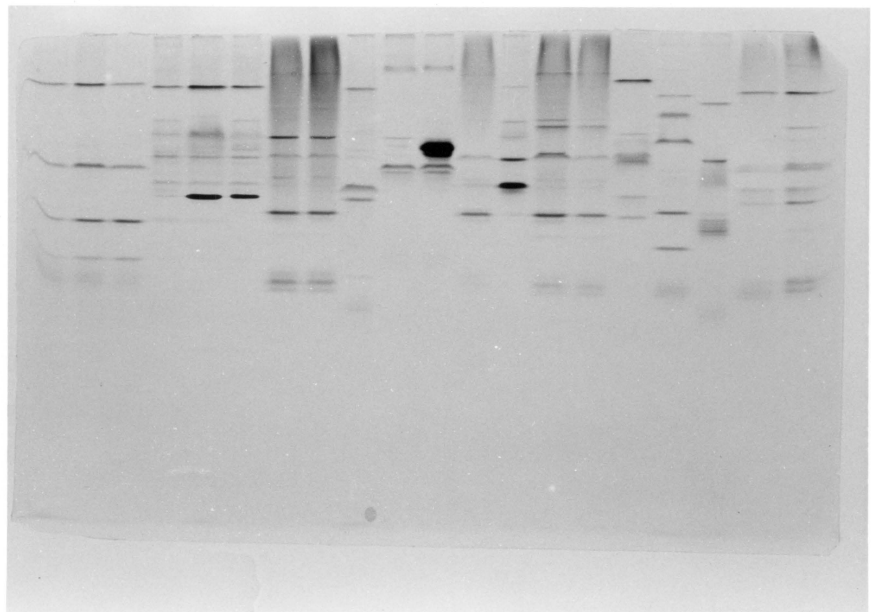


Figure 70. Polyacrylamide electrophoretic gel of α- and β - esterase activity of acetone powder-extracts of 11 species of Endothia and Cryphonectria cubensis. Species and isolates by lane number (from left to right): E. parasitica 1 - E87, 2 - E88, 3 - E89; E. gyrosa 4 - E48, 5 - E50, 6 - E154; E. radicalis 7 - E16, 8 - E42, 9 - E56, 10 - E64, 11 - E67, 12 - E76, 13 - E92, 14 - E150, 15 - E152; E. singularis 16 - E52; E. macrospora 17 - E54; E. coccolobii 18 - E157; E. viridistroma 19 - E41, 20 - E82.

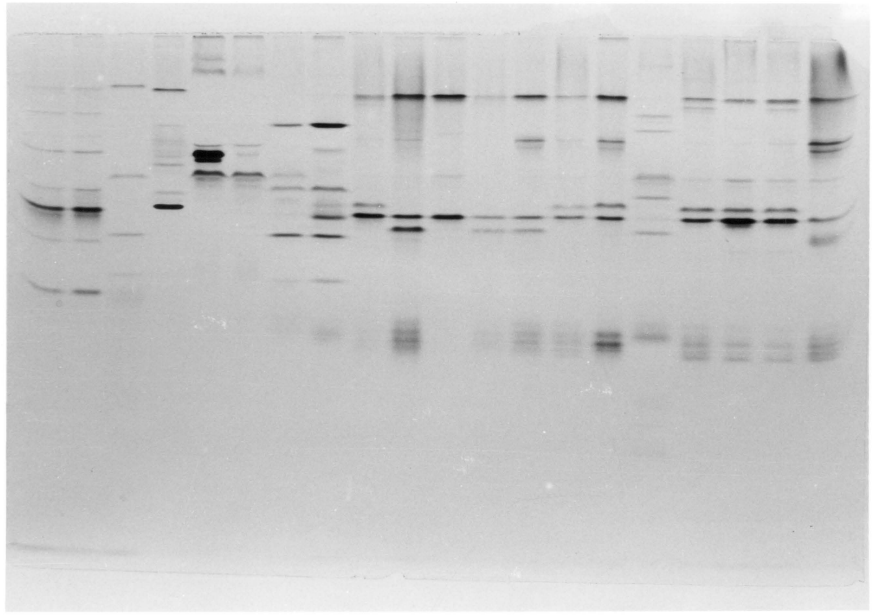


Figure 71. Polyacrylamide electrophoretic gel of α- and β-esterase activity of acetone powder-extracts of 11 species of Endothia and Cryphonectria cubensis. Species and isolates by lane number (from left to right): E. japonica 1 - E53, 2 - E59; E. parasitica 3 - E87; E. gyrosa 4 - E154; E. tropicalis 5 - E57, 6 - E70; E. havanensis 7 - E158, 8 - E159, 9 - E40; E. eugeniae 10 - E138, 11 - E139, 15 - H173, 17 - H185, 18 - H186, 20 - H189; C. cubensis 12 - H137, 13 - H151, 14 - H154, 16 - H182, 19 - H188.

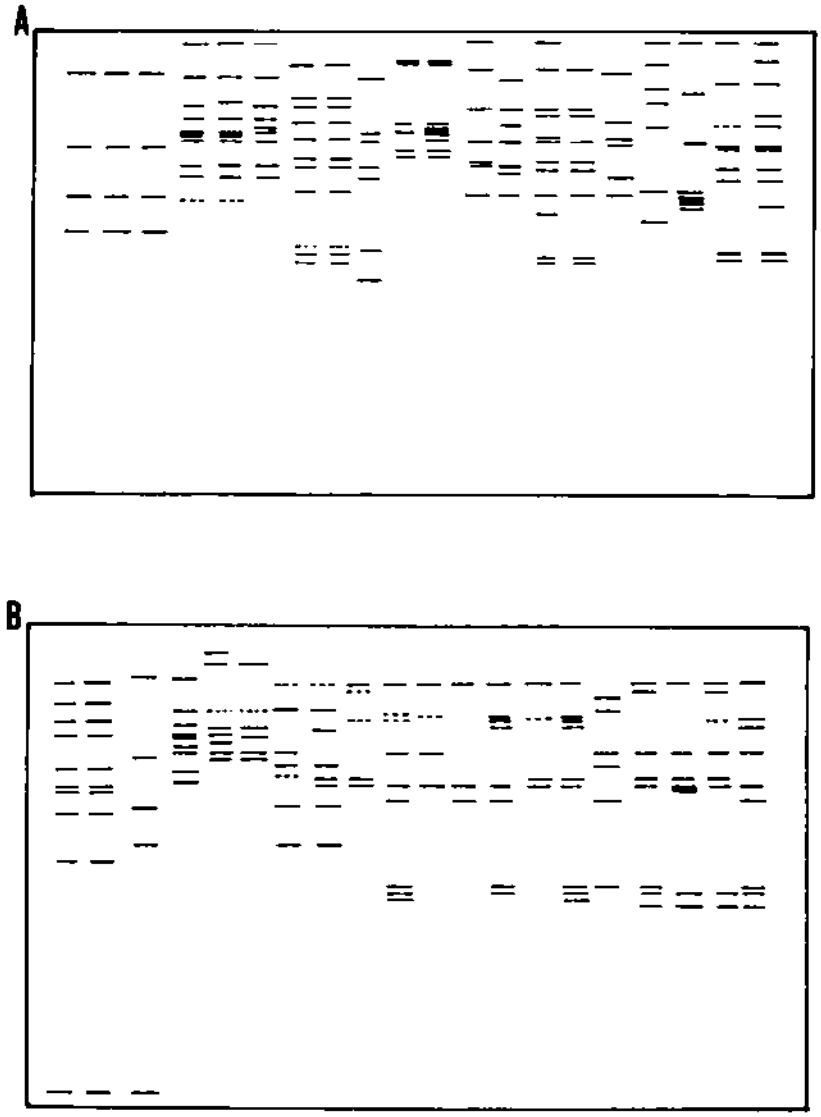


Figure 72. Interpretative drawings of α- and β-esterase activity of acetone powder-extracts of 11 species of Endothia and Cryphonectria cubensis from the first set of acetone powders. Species and isolates by lane number (from left to right):

Front plate (A) E. parasitica 1 - E87, 2 - E88, 3 - E89; E. gyrosa 4 - E48, 5 - E50, 6 - E154; E. radicalis 7 - E16, 8 - E42, 9 - E56, 10 - E64, 11 - E67, 12 - E76, 13 - E92, 14 - E150, 15 - E152; E. singularis 16 - E52; E. macrospora 17 - E54; E. coccolobii 18 - E157; E. viridistroma 19 - E41, 20 - E82.

Back plate (B) E. japonica 1 - E53, 2 - E59; E. parasitica 3 - E87; E. gyrosa 4 - E154; E. tropicalis 5 - E57, 6 - E70; E. havanensis 7 - E158, 8 - E159, 9 - E40; E. eugeniae 10 - E138, 11 - E139, 15 - H173, 17 - H185, 18 - H186, 20 - H189; C. cubensis 12 - H137, 13 - H151, 14 - H154, 16 - H182, 19 - H188.

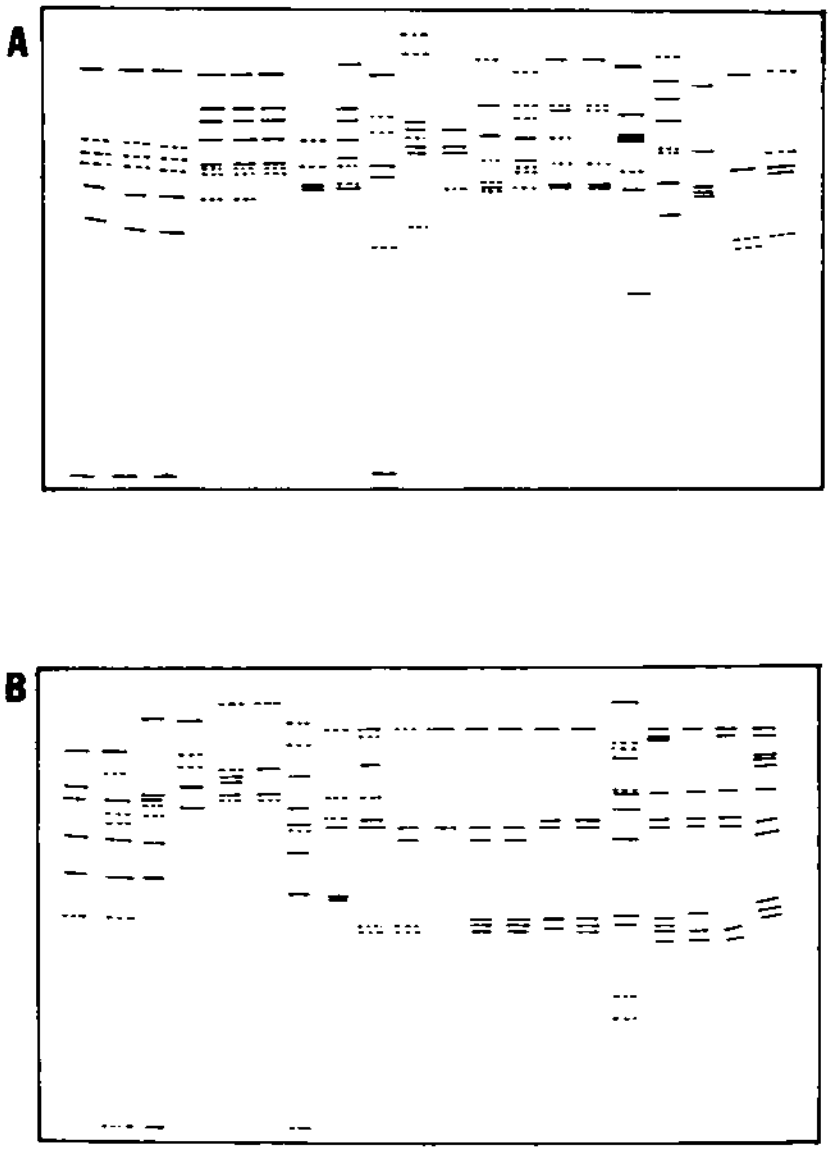


Figure 73. Interpretative drawings of α- and β-esterase activity of acetone powder-extracts of 11 species of Endothia and Cryphonectria cubensis from the second set of acetone powders. Species and isolates by lane number (from left to right):

Front plate (A) E. parasitica 1 - E87, 2 - E88, 3 - E89; E. gyrosa 4 - E48, 5 - E50, 6 - E154; E. radicalis 7 - E16, 8 - E42, 9 - E56, 10 - E64, 11 - E67, 12 - E76, 13 - E92, 14 - E150, 15 - E152; E. singularis 16 - E52; E. macrospora 17 - E54; E. coccolobii 18 - E157; E. viridistroma 19 - E41, 20 - E82.

Back plate (B) E. japonica 1 - E53, 2 - E59; E. parasitica 3 - E87; E. gyrosa 4 - E154; E. tropicalis 5 - E57, 6 - E70; E. havanensis 7 - E158, 8 - E159, 9 - E40; E. eugeniae 10 - E138, 11 - E139, 15 - H173, 17 - H185, 18 - H186, 20 - H189; C. cubensis 12 - H137, 13 - H151, 14 - H154, 16 - H182, 19 - H188.

three major bands in the top quarter of the gel and a fourth band with an Rf value of approximately 0.67. No other isolate shared this particular banding pattern. This is significant because the extreme sensitivities of isolates E158 and E159 to cycloheximide suggested that they may be members of E. gyrosa. These particular isolates were unique in the production of three major bands in the center of the gel; this banding pattern was not observed among isolates of the other species. Isolates of E. radicalis were quite diverse, as with the results obtained from general protein stains. Definite similarities were apparent in the banding patterns of isolates E16, E42, E150 and E152; these may represent the true isolates of E. radicalis. All isolates of E. eugeniae and E. cubensis, with the exception of H182, produced dark-staining areas at the very top of the gel; differences in protein concentration account for the variation in thickness of these bands. Further interpretation could not be made without better resolution. It is quite possible that a different stain, which does not combine acid and alkaline phosphatase, would offer higher resolution and provide more information about these isolates.

The best resolution was obtained with gels stained for α- and β-esterase activity; four to twelve discrete bands were obtained for each isolate. A similarity matrix was not produced since all isolate combinations were not made on the same gel. Some obvious similarities can be distinguished from the photographs and banding drawings (Figures 70 - 73). Isolates of E.

parasitica, E. gyrosa, E. viridistroma, E. tropicalis and E. japonica all produced identical intraspecific banding patterns yet were quite variable among species. Unique protein patterns were obtained with the single isolates of E. coccolobii, E. macrospora and E. singularis. Banding patterns produced by certain isolates of E. radicalis were quite diverse, although those formed by isolates E16, E42, E76, E150 and E152 were fairly uniform. The banding patterns of E64 and E67 resembled those produced by E. tropicalis. This corresponds with the general appearance of the isolates in culture; all produced white mycelia with orange stromata; other isolates of E radicalis had different colony morphologies. These two isolates may be legitimate members of E. tropicalis.

The α- and β-esterase patterns of E. eugeniae and C. cubensis were quite uniform with the exception of isolate H182. One set of bands in the center of the gel was particularly variable and may represent enzymes coded by two different alleles. One band had an Rf value of 0.69; this band was detected in isolates E40, H154, H173, H185, H186 and H188. The position of the corresponding band was at an Rf value of 0.65; this enzyme was located in isolates E138, H137, H151 and H189. Neither band was visible in isolate E139. The variation in this set of bands had no relationship to the original identification of the organism or to its geographic source; it is therefore of little taxonomic significance. The banding pattern of α- and

β-esterase enzymes confirmed the close relationship and probable conspecificty of these two organisms.

The banding patterns produced by the two suspected isolates of E. havanensis from Florida (E158 and S159) did not resemble those formed by any other isolate. This confirmed the phosphatase test which demonstrated that these isolates were not members of E. gyrosa.

The use of specific activity stains greatly facilitates the application of PAGE in taxonomic studies. The interpretation of protein patterns is simplified due to the lesser number of bands; it is much easier to "fingerprint" an organism. It is even possible to give genetic interpretations to the data when a single enzyme is involved. This could not be done here since two different sets of isozymes (α- and β-esterase; acid and alkaline phosphatase) were resolved and the banding pattern was too complex. These studies are not conclusive since only a limited number of the available isolates were examined. The use of isozyme analysis with Endothia taxonomy has a great deal of potential and should be pursued.

Chapter 4

The differentiation of *Endothia* species by their sensitivities to fungitoxicants.

LITERATURE REVIEW

The limitations of morphology as a means of identification and classification have been discussed in the previous chapter. An attractive alternative to the use of morphological characteristics is the development of biochemical techniques which can be used to differentiate among organisms. Such procedures, which generally test the assimilation or degradation of specific metabolites, are frequently used in the identification of bacteria, actinomycetes and certain yeasts (125). Another type of biochemical trait is the degree of sensitivity of an organism to antibiotics or other xenobiotic agents (63). It is this latter approach which will be discussed in relation to fungal taxonomy.

The term "xenobiotic" is derived from the Greek "xenos", meaning a stranger, alien or guest, and "bio", which translates as "life" (223). Xenobiotic compounds can therefore be defined as any synthetic chemical agent and include man-made fungitoxicants and other pesticides (176). Certain fungitoxicants have been shown to be differentially toxic to specific fungi; they effectively inhibit the growth of some organisms but are not active against others. This differential sensitivity is usually observed at the class or family level; oxathiins are particularly

active against Basidiomycetes, while benzimidazoles are most toxic to Ascomycetes and Deuteromycetes. Some compounds are differentially toxic among genera and species. The spectrum of activity of a compound, which is a listing of sensitive and tolerant organisms, is often used to predict which diseases will be controlled by a certain compound. This information can also be useful to the mycologist, for the degree of sensitivity of an organism to various fungitoxicants provides additional phenotypic characteristics. This information can be used to develop differential and selective media for the isolation and purification of specific organisms (200). Differential sensitivities can also be utilized in the identification of particular fungi and to predict the biochemical properties and taxonomic relationships of certain organisms (76).

The degree of sensitivity of an organism to a specific compound is determined by the production of a dosage-response curve, which presents the response of the fungus, typically as a percent inhibition per unit concentration of fungitoxicant. Assays are usually conducted by germinating spores or growing mycelium on a fungitoxicant-amended medium. These techniques result in two general categories of dosage-response curve; those in which there is a progressive change in fungal response, as in the degree of inhibition of radial growth, and those in which the response is scored into two categories, as in the number of germinated and nongerminated spores (106).

Two important values which can be calculated from the dosage-response curve are the "effective dose - 50", or ED_{50}, and the slope. The ED_{50} is the concentration of fungitoxicant at which 50% of the spores fail to germinate or at which the growth or some aspect of the physiology of the organism is inhibited by 50%. This value is used to compare the toxicity of various compounds. The slope of the dosage-response curve gives an indication of the toxicity under conditions of changing concentration. A steep slope indicates that the compound elicits a fairly uniform response (48). Dosage-response curves are typically sigmoidal when the logarithm of the concentration is plotted against the response. They can frequently be converted to a linear function by the probit transformation (188). This is done by charging percentage into units of standard deviation, so that the 50% point becomes 0 standard deviation units, the 84.13% point becomes +1 standard deviations and 97.73% represents +2 standard deviations. These standard deviation units are called "normal equivalent deviates" and are transformed to probits by the addition of 5.0, which eliminates the need for negative values. Calculations of slope and ED_{50} can then be obtained by linear regression (188).

Dosage-response curves plotted on log-probit paper occasionally are not linear. Bimodal curves, in which inhibition decreases after an initial rise and then returns to the previous level, have been reported for some dithiocarbamates. This can be explained by the formation of metal - toxicant complexes. The

initial zone of inhibition is due to the presence of a 1:1 metal - toxicant complex. The subsequent decrease in activity is due to the formation of a 2:1 metal - toxicant complex which is not as toxic as the former and which probably acts by a different mode of action. Toxicity then increases due to the dissociation of the 2:1 metal - toxicant complex. Another deviation from a linear relationship is the "plateau" type of dosage-response curve. This occurs when the response levels off after an initial rise so that complete inhibition is never reached. Plateau-type curves have been produced by Colletotrichum acutatum Simmonds (25), Colletotrichum coccodes (Wallr.) Hughs, Fusarium solani (Mart.) Sacc. and Glomerella cingulata (Ston.) Spauld. and Schrenk (145) in response to benomyl and some of its derivatives. Other deviations from linearity have also been encountered; Dimond et al (48) hypothesized that most of these can be explained by association and dissociation of the fungitoxicants into compounds of lower and higher toxicity.

Differences in sensitivities among fungal species and genera have been reported for several groups of fungitoxicants. One of the most selective classes of fungitoxicants is the benzimidazoles. Sensitivities among the Deuteromycetes is correlated with the mechanism of conidiogenesis. Members of the Phialosporae, Arthrosporae, Sympodulosporae and Aleurosporae are very sensitive to these compounds (ED_{50} = 0.4 - 4.0 µg/ml), while representatives of the Porosporae are insensitive (ED_{50}) 50 µg/ml)

(54). Members of the Blastosporae are generally sensitive to the benzimidazoles (ED_{50} = 0.1-1 µg/ml) (26), although Candida humicola (Daszewska) Diddens and Lodder, which has its perfect stage in the Hemiascomycetes, is tolerant (ED_{50} > 100 µg/ml) (54). Inconsistencies have also been found among members of the Annellosporae; species of Leptographium Lagerb. and Melin and Pestalotia de Not. are highly sensitive to benomyl (ED_{50} = 0.2 and 0.08 µg/ml, respectively), while species of Doratomyces Corda and Scopulariopsis Bainier are highly resistant (ED_{50} = 100-500 µg/ml) (25, 26, 54). Such biochemical inconsistencies confirm the heterogeneity of Deuteromycete form genera.

Isolates of Fusarium solani can be easily differentiated from other species of Fusarium by their unique plateau type dosage-response curve formed upon exposure to benomyl and thiabendazole. Other Fusarium species typically produce a steep, linear dosage-response curve (145). The reaction of Fusarium solani var. coeruleum (Sacc.) C. Booth [= Fusarium coeruleum (Libert) Sacc.] does not resemble that of F. solani; it produces a linear dosage- response curve comparable to F. oxysporum and other Fusarium species. The classification of this organism as a variety of F. solani should be reexamined (128).

Significant differences were found among ED_{50} values of Verticillium dahliae (0.36 µg/ml), V. albo-atrum (0.26 µg/ml) and V. nigrescens (Pethybridge) (0.19 µg/ml). The slope of V. nigrescens was also flatter than those of the other species. The ED_{50} values and slopes could not be used to identify unknown

isolates, however, due to the considerable range formed by different isolates of each species (75). Differential sensitivity towards benomyl is also exhibited among species of Mortierella Coemans; M. alpina Peyr., M. candelabrum van Tiegh. and LeMonn. and M. elongata Linnemann are all relatively sensitive (ED_{50} = 10 - 25 µg/ml), while M. isabellina Oudemans and Koning and M. vinacea Dixon-Stewart are quite resistant (ED_{50} > 500 µg/ml). Phoma betae Frank, which has Pleospora bjoerlingii Byford as its perfect stage, is very tolerant of benomyl (ED_{50} = 500 µg/ml). Other Phoma Sacc. species, which have perfect stages unrelated to that of P. betae, are very sensitive (ED_{50} = 1 - 10 µg/ml) (26). In both cases, a benomyl-amended medium could be used to distinguish among the different taxa.

Other compounds have also been used to differentiate among fungi. Sensitivity to cycloheximide was used to partition Ceratocystis Ellis and Halst. into two diverse groups. Those species with Chalara-like anamorphs, which produce enteroblastic conidia and lack detectable levels of cellulose and rhamnose, were inhibited by cycloheximide at concentrations of 100 µg/ml. Other species of Ceratocystis, which include the Fimbriata, Ophiostoma and Ceratocystopsis groups and which produce holoblastic conidia and contain detectable levels of cellulose and rhamnose, were not affected by cycloheximide. This difference in tolerance supports the division of Ceratocystis into two genera (78).

Martin, Lucas and Campbell (104) utilized sensitivities to a variety of fungitoxicants, including benomyl, carboxin, PCNB, iprodione, chlorothalonil and triadimefon, to study relationships among isolates of Rhizoctonia DC. This form genus is quite diverse and is composed of fungi from the Ascomycetes, Basidiomycetes and Deuteromycetes. Because of this taxonomic diversity, one would expect to find differences among sensitivities to fungitoxicants. Rhizoctonia solani Kuehn could be distinguished from R. zeae Voorhees by its greater sensitivity towards benomyl (ED_{50} (10 and) 50 µg/ml, respectively). Anastomosis groups of R. solani varied in their responses to carboxin, PCNB, chlorothalonil and triadimefon; a differential series of amended media could potentially be used to assign unknown isolates into their proper groups.

Fungitoxicants can elicit responses other than growth inhibition to differentiate organisms. Two morphologically identical varieties of Phoma, P. exigua Desm. var. exigua Desm. and P. exigua var. foveata (Foister) Boerema, can be distinguished by their growth on malt agar amended by thiophanate methyl. This compound stimulates the production of yellow anthraquinone pigments by P. exigua var. foveata. The pigment creates a striking difference between the two colony types and identification is simplified (199).

Several different mechanisms exist which can explain why closely related fungi are differentially sensitive to a compound. Sensitive fungi may selectively accumulate the fungitoxicant due

to differences in membrane permeability. Susceptible species of Phytophthora and Pythium can collect up to nine times the amount of streptomycin as do resistant members of the Mucorales and Aphanomyces. Decreased uptake may also result from the absence or decreased affinity of internal binding sites. Another common mechanism of selectivity is detoxification, where resistant organisms accumulate a fungitoxicant, but are able to inactivate it. One common detoxification process is to react strong alkylating agents with soluble thiols, thus protecting the thiol groups of enzymes. Neurospora crassa Shear and Dodge and other fungi can break down captan by reacting it with a cellular pool of soluble thiols composed primarily of glutathione. This pool can then be regenerated by reducing the glutathione with NADPH (189). Detoxification may also be achieved by binding the compound to sites on the cell wall (146).

Selectivity may result from differences in biochemical pathways which would convert an inactive compound to one which is fungitoxic; this process is termed "lethal synthesis". Pyrazophos, which is active against powdery mildews, is changed to a fungitoxic pyrimidine derivative by sensitive organisms. Resistant fungi, such as Pythium debaryanum Hesse and Saccharomyces cerevisiae Meyen, are not able to make this conversion (45). Certain organisms may be able to circumvent pathways which are affected by a fungitoxicant. Ustilago maydis (DC.) Corda is normally resistant to antimycin A, a compound which

inhibits electron transport between cytochromes b and c. Resistance can be traced to the transfer of electrons to an alternate terminal oxidase at a site previous to cytochrome b. Sensitive mutants lack this alternative pathway. Metabolic compensation, in which less of an inhibited product is required or more of an affected compound is produced, is one final mechanism of selectivity. This has been shown to occur in 6-azauracil-resistant strains of Cladosporium cucumerinum Ell. and Arth. in which up to three times as much of the target enzyme is produced as in wild, sensitive strains (45).

Fungitoxicants are frequently divided into two major categories. Protective fungicides are usually toxic to a large number of organisms because their modes of action are nonspecific and involve many biochemical reactions in the cell. These compounds typically react with sulfhydryl, amino, carboxylic and hydroxylic groups on proteins, amino acids or amino acid precursors. Some fungi may be tolerant to these fungitoxicants due to large pools of soluble thiols, as previously mentioned in the case of captan resistance. Differences in permeability and accumulation may also result in differential sensitivities of organisms to these compounds. Fungi usually do not develop resistant strains against protectants due to the large number of cellular processes which are affected by them (102). Systemic fungitoxicants have quite specific modes of action and are usually effective against a small number of taxonomically related organisms. It is common for these compounds to act at a single

metabolic site, and fungi frequently develop resistance to them because of the limited number of biochemical processes involved (184).

Differential sensitivities of organisms to a particular compound can lead to a better understanding of the biochemical differences among fungi. A thorough knowledge of the modes of action of each fungitoxicant is necessary to allow a physiological interpretation of sensitivity data. The final portion of this discussion will describe the modes of action of the protectant and systemic compounds used in this study.

Protectant Fungitoxicants

Polyene antibiotics

Polyene antibiotics are a group of compounds characterized by a macrolid structure with a β-hydroxylated hydrophillic area and a rigid, conjugated double bond system which forms a lactone ring. The size of the molecule determines its toxicity. Smaller tetraenes are good lysing agents, while the larger haptaenes, which include nystatin (Figure 74), amphotericin (Figure 74) and candicidin (Figure 75), are more effective in the inhibition of fungal growth (100). Polyene antibiotics bind to membrane-bound sterols, such as ergosterol and cholesterol, and destroy the selective permeability of the cell. Sensitivity to these compounds is correlated to the sterol content of the membranes; Oomycetes, which lack membrane-bound sterols, are generally

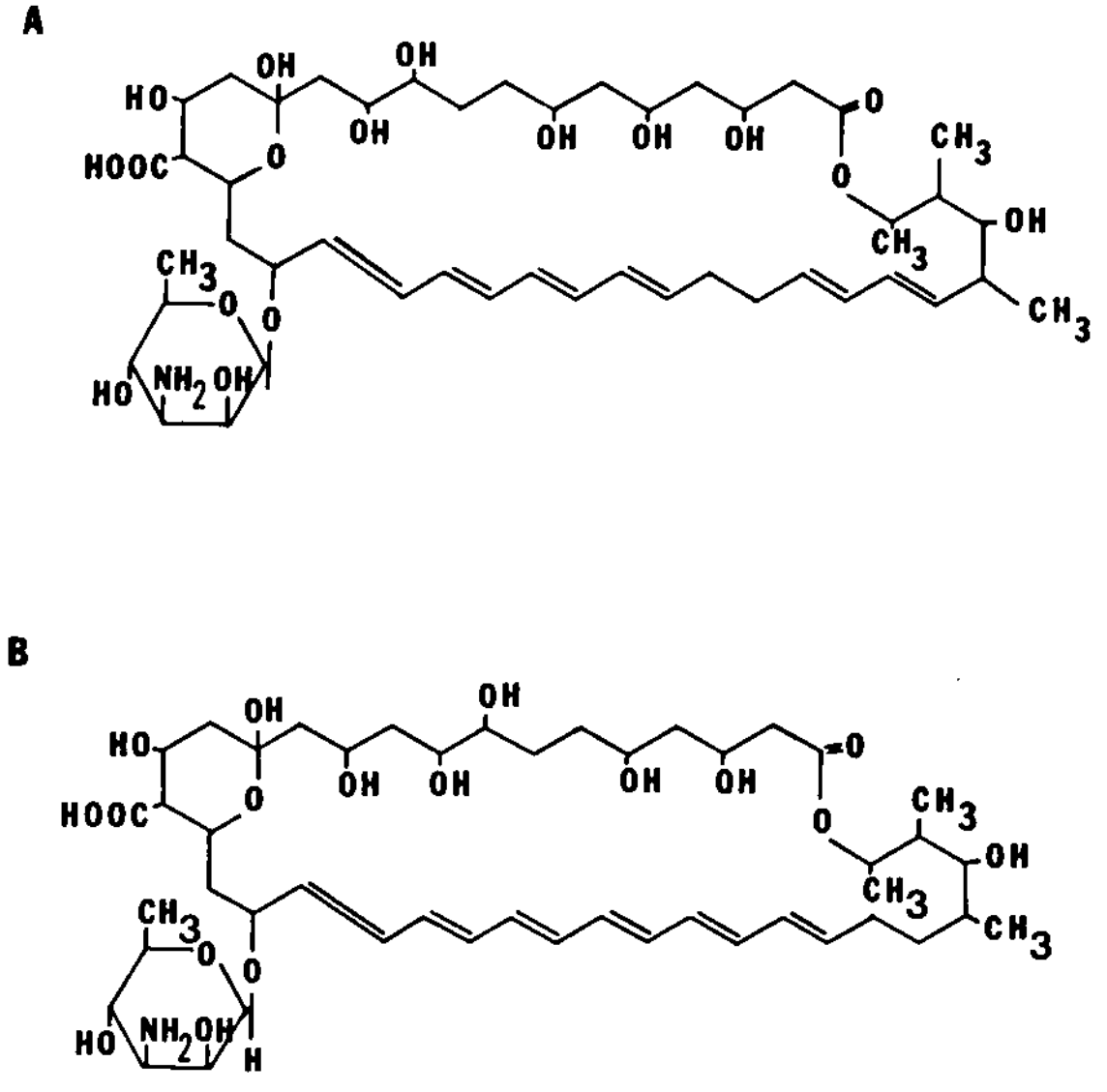


Figure 74. Molecular structure of nystatin (A) and amphotericin (B).

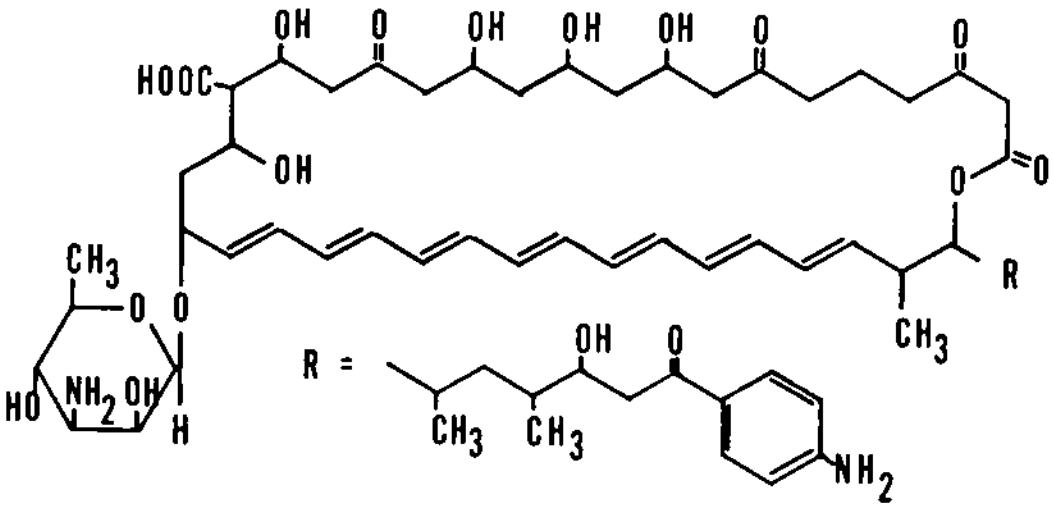


Figure 75. Molecular structure of candicidin D.

resistant. Nystatin-resistant mutants of certain yeasts contain ergosterol precursors in the membrane but not the sterol itself. These precursors do not react with polyenes, and the cells are resistant to the compounds (100, 102).

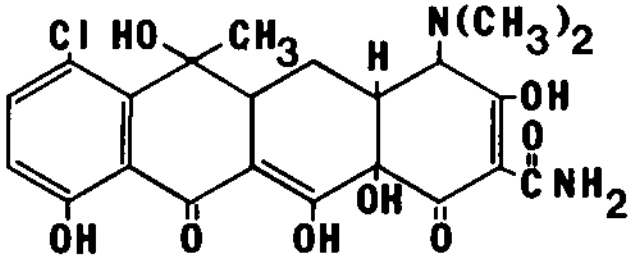
Tetracyclines

The tetracycline antibiotics are potent inhibitors of protein synthesis in prokaryotes and eukaryotes, including Phytophthora and Pythium. Tetracyclines interfere with the elongation process of protein synthesis by blocking the aminoacyl receptor site of both eukaryotic and prokaryotic ribosomes. Release factors are also excluded from the site so peptide chain termination is inhibitied (99, 180). Lack of protein synthesis in the mitochondria leads to a deficit of cytochromes a, a_3, b and c, and a subsequent drop in respiratory activity (183). The molecular structure of chlortetracycline is presented in Figure 76.

Chloramphenicol

The antimicrobial activity of chloramphenicol (Figure 76) is similar to that of the tetracyclines, although Phytophthora and Pythium are not as sensitive to it. Chloramphenicol inhibits protein synthesis of 70S ribosomes by blocking the peptidyl transfer reaction by peptidyl transferase (99, 180). Mycelium grown in the presence of chloramphenicol is frequently devoid of cytochromes a, a_3, and b, but shows elevated levels of cytochrome c (183). Resistance in eukaryotes is often achieved by an

A



B

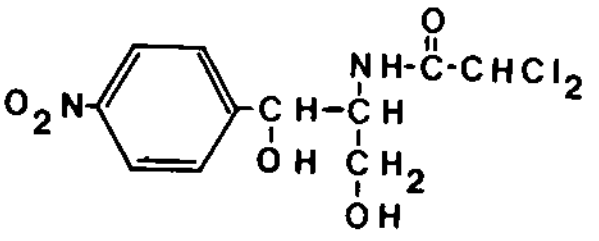


Figure 76: Molecular structure of chlortetracycline (A) and chloramphenicol (B).

acetylation detoxification reaction (180), although decreased cell permeability to the compound has also been reported (183).

Quinones

Quinones are α, β - unsaturated ketones which have a broad range of activity as strong oxidation-reduction reagents. The molecular structures of o-phenyl phenol and hydroquinone, the compounds used in this study, are presented in Figure 77. Their mechanism of action is relatively nonspecific, and includes binding with thiol and amino moieties and disruption of electron transport pathways (184). Fungi can frequently detoxify quinones into nontoxic secondary metabolites by the action of polyphenol oxidase and other enzymes (144).

Ethylenebisdithiocarbamates

The ethylenebisdithiocarbamates are a large group of protectant compounds which are derivatives of ethylenediamine and which contain cations such as manganese, zinc and sodium. Mancozeb (Figure 78) is a coordination product of zinc ion and manganous ethylenebisdithiocarbamate. The mode of action of these compounds is through the formation of isothiocyanate by the reaction:

$$R - \underset{H}{N} - \overset{S}{\overset{\|}{C}} - S^{-} \longrightarrow S - H^{-} + C - N = S$$

Isocyanate inactivates thiol groups and is quite toxic to a large number of cellular processes (184).

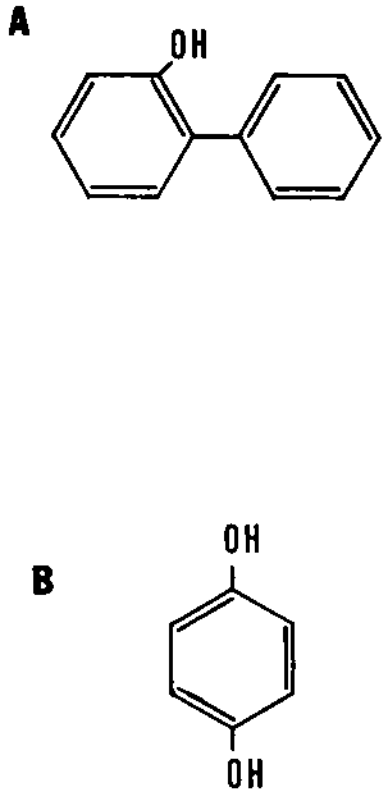


Figure 77. Molecular structure of o-phenyl phenol and hydroquinone.

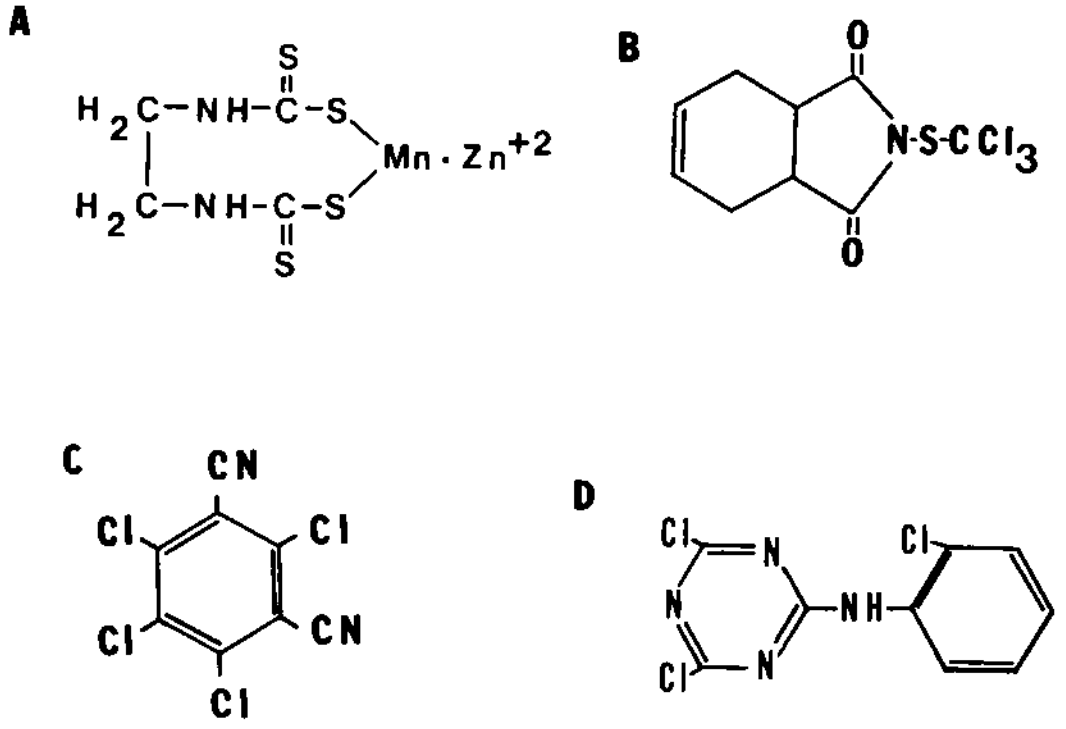


Figure 78. Molecular structure of mancozeb (A), captan (B), chlorothalonil (C) and anilazine (D).

Captan

Captan is a broad spectrum protectant phthalimide (Figure 78). It is a strong biological alkylating agent, and reacts with cellular thiol, imidazole and amino groups. Various reactions occur, including the oxidation of thiol moieties, the inactivation of thiol and other groups by the production of $-SCCl_3$ and $-\overset{S}{\overset{\|}{C}}Cl$ derivatives, and the linkage of properly spaced thiol and amino groups by fungicide-derived thiocarbonyl moieties. Thiophosphene, $SCCl_2$, is frequently formed as a by-product of these reactions. This compound is also highly reactive and may combine with other thiol groups or be hydrolyzed to carbonyl sulfide, hydrochloric acid and hydrogen sulfide. These various reactions lead to the disruption of many cellular processes. The enzymes glyceraldehyde-3-phosphate dehydrogenase, carboxylase, hexokinase and aldolase are particularly sensitive to this compound. Variations in sensitivity to captan are dependent on the cell's ability to decompose it. Cellular pools of low molecular weight thiols, such as glutathione, must be exhausted before cellular processes are affected (181, 184).

Chlorothalonil

Chlorothalonil (Daconil®) is a substituted aromatic (Figure 78) which is a broad spectrum protectant. Like captan, it acts as a biological alkylating agent which reacts with cellular thiol moieties. The enzymes alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase and malate dehydrogenase

are particularly sensitive to this compound. Low molecular weight thiol pools help protect the cell from chlorothalonil (181, 184).

Anilazine

Anilazine (Dyrene®) is a triazine (Figure 78) with broad spectrum protectant properties. Its mode of action is similar to that of captan and chlorothalonil; free thiol and amino groups react with the halogen atoms in the s-triazine ring (184). It may also disrupt membrane function by reacting with functional groups of membrane proteins (100).

Pentachloronitrophenol

Pentachloronitrophenol (PCNB) is highly selective and is effective against species of Rhizoctonia DC., Sclerotium Tode, Sclerotinia Fuckel and Botrytis Micheli ex Pers. It is a substituted aromatic hydrocarbon (Figure 79). Although its exact mode of action is not fully understood, it apparently binds to certain hydrophobic sites in the cell and disrupts various processes such as membrane function, mitosis and cell division (184). Variation in sensitivity is due to differences in uptake (102).

Dicloran

Dicloran (Botran®) is also a member of the substituted aromatic hydrocarbon group (Figure 79). It is effective against species of Botrytis, Rhizopus Ehrenb. and Monilinia Honey. Dicloran is a nonspecific fungitoxicant which disrupts cell

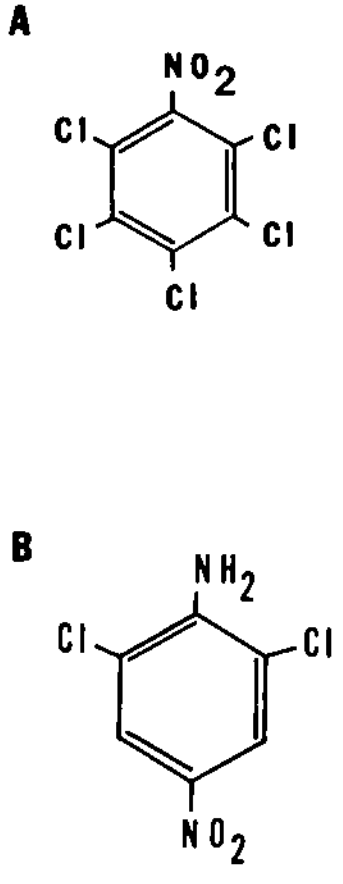


Figure 79. Molecular structure of PCNB (A) and dicloran (B).

growth and division; the exact fungitoxic mechanism is not known. Inhibition of protein synthesis seems to be a secondary effect (184).

Systemic Fungitoxicants

Cycloheximide

Cycloheximide is an antifungal antibiotic produced by Streptomyces griseus Waksman and Henrici (212). It is a derivative of glutarimide (Figure 80) (180) and has a broad spectrum of activity (102, 208). Sensitivities of closely related fungi are often quite diverse; some organisms are inhibited at concentrations of 0.1 µg/ml, while others are not affected at 1000 µg/ml (184). The primary mode of action is the inhibition of the elongation stage of protein synthesis of the 80S ribosome; the binding or reactive site is on the 60S subunit. The compound inhibits the transfer of amino acids from aminoacyl-tRNA into the protein chain by blocking the EF_2-GTP dependent translocation of peptidyl-tRNA from the A site to the P site of the ribosome. The release of the deacylated tRNA from the ribosome is also inhibited (180). Secondary effects may include inhibition of RNA and DNA biosynthesis (46, 153, 184). Sensitivity is apparently associated with the presence of susceptible ribosomal protein synthesizing systems. In Neurospora crassa, ribosomal proteins of three cycloheximide-resistant mutants showed differences in electrophoretic and antigenic properities. Other mechanisms of

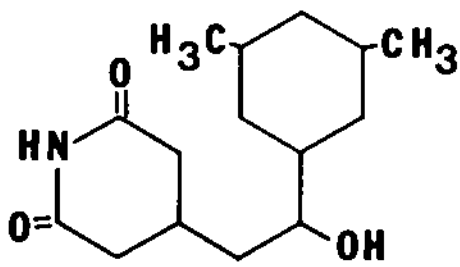


Figure 80. Molecular structure of cycloheximide.

cyclcheximide resistance also exist. Cunninghamella blakesleeana Lendner can convert cycloheximide to a nontoxic acetate derivative. Some mutants are resistant due to a lack of permeability of the cell to the compound (184).

Benzimidazoles

The benzimidazoles are the most important group of systemic fungicides currently utilized. They are active against most classes of fungi, except Oomyetes and Zygomycetes, although individual species or genera may be resistant. Important members of this group include benomyl (Benlate®) (Figure 81), which is transformed to its active ingredient, methyl-2-benzimidazole carbamate (MBC, carbendazim) (Figure 81) by cleavage of a butylcarbamoyl moiety, and thiabendazole (Mertect®) (Figure 81). Cationic salts of MBC and thiabendazole are also fungitoxic and are more effective in treating vascular diseases than are the parent compounds due to their increased solubility (184). These salts include Lignasan BLP® (MBC phosphate) and Arbotect 20S® (thiabendazole hypophosphite). Fungisol® is a mixture of MBC (0.3%) and 2-(2-ethoxyethoxy)ethyl-2-benzimidazole carbamate (1.7%). The benzimidazole moiety itself (Figure 81) is generally not fungitoxic (183).

Benzimidazoles inhibit nuclear division in fungi by binding to tubulin, the protein subunits of microtubules. This prevents proper spindle development, and mitosis can not be completed (42, 43). These compounds also seem to interfere with the assembly or

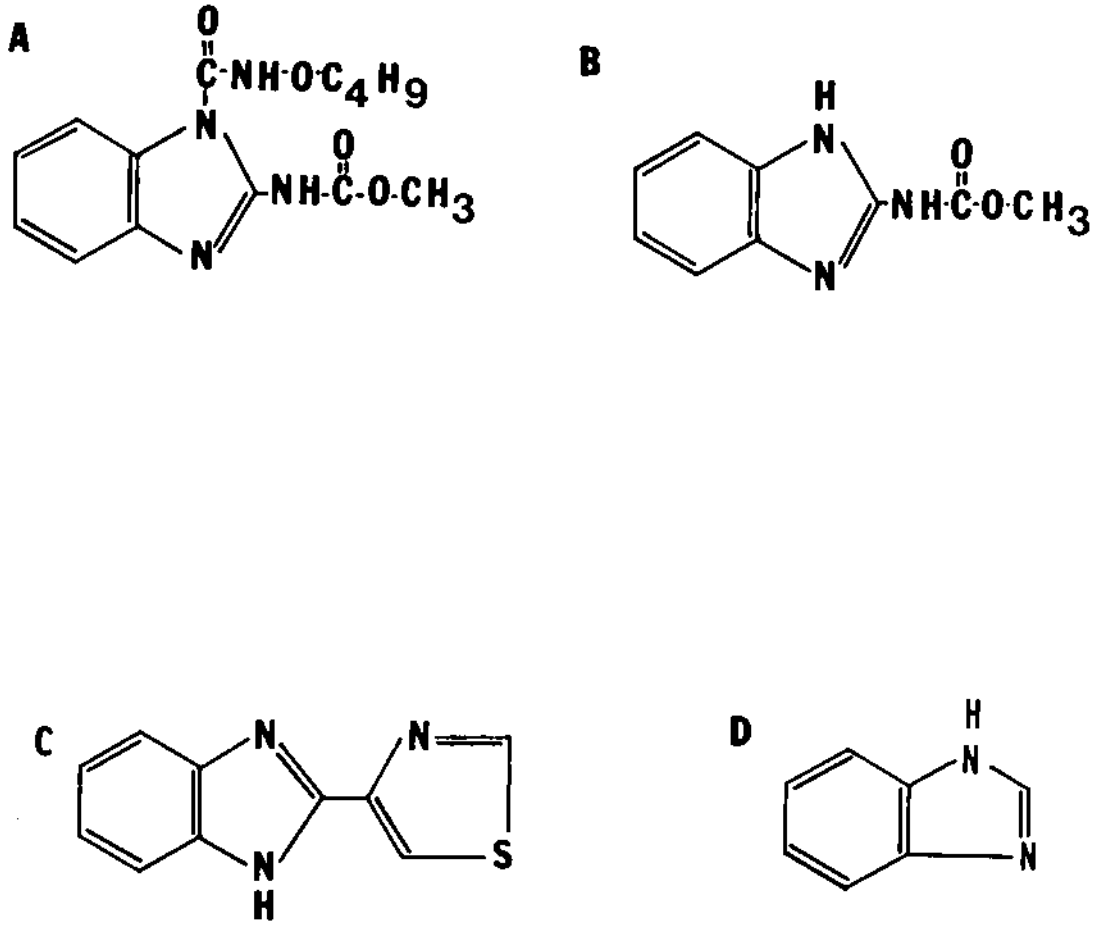


Figure 81. Molecular structures of benomyl (A), MBC (B), thiabendazole (C) and benzimidazole (D).

function of cytoplasmic microtubules which would disrupt hyphal orientation (184). Variation in sensitivity is correlated with the affinity of tubulin for these compounds. Resistant organisms frequently contain tubulin subunits which will not bind with these agents due to differences in amino acid content. Resistance may also develop by nonspecific mechanisms which increase the affinity of microtubule ends for tubulin dimers, thereby masking the benzimidazole binding sites (122).

Chloroneb

Chloroneb (Demosan®) is a narrow spectrum systemic fungitoxicant which is highly toxic to certain species of Rhizoctonia, Pythium, Sclerotinia, Typhula (Pers.) Fr. and Ustilago (Pers.) Roussel. It is a substituted aromatic hydrocarbon (Figure 82). The mode of action of this compound is not fully understood, although it appears to disrupt growth by binding to certain hydrophobic sites within the cell or upon the cell surface. This may result in the inhibition of DNA synthesis, as in Rhizoctonia solani, disruption of cell division, as in Ustilago maydis, or interference with nuclear function, as in Aspergillus nidulans (Eidam) Wint. Sensitivity is not due to increased cell permeability, for sensitive fungi take up as much as resistant organisms (183, 184).

Dicarboximides

The dicarboximides, vinclozolin (Ronilan®) and iprodione

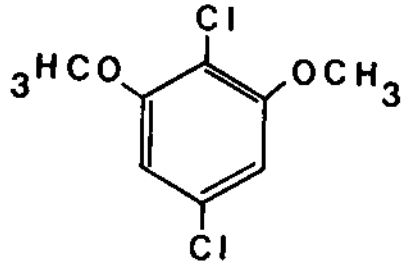


Figure B2. Molecular structure of chloroneb.

(Rovral®) (Figure 83), are relatively new systemic compounds for which the modes of action have not been determined. Aberrations in lipid metabolism (135) and mitosis (56, 66) have been observed. Japanese researchers believe that cytoplasmic microfilaments, such as those that control cytoplasmic streaming, may be disrupted or inactivated (H. D. Sisler, personal communication).

Triadimefon

Triadimefon (Bayleton®), a triazole (Figure 84), is a broad spectrum fungitoxicant with a relatively large antifungal spectrum. Its primary effect on the cell is to inhibit the biosynthesis of ergosterol, the major sterol component in most fungal membranes. Inhibition seems to involve C_{14} demethylation and leads to a build-up of several sterol intermediates and the accumulation of free fatty acids (32, 184).

Fosetyl-Al

Fosetyl-Al (efosite Al, phosethyl Al, Aliette®) is a recently released systemic fungicide which is particularly effective against members of the Peronosporales. It is an aluminum derivative of phosphonate (Figure 84) and is thought to release orthophosphorous acid in plant tissue. The exact mode of action is not understood; it exhibits limited activity against *in vitro* mycelial growth except under conditions of low phosphate. Mechanisms of resistance have not been reported (58, 62).

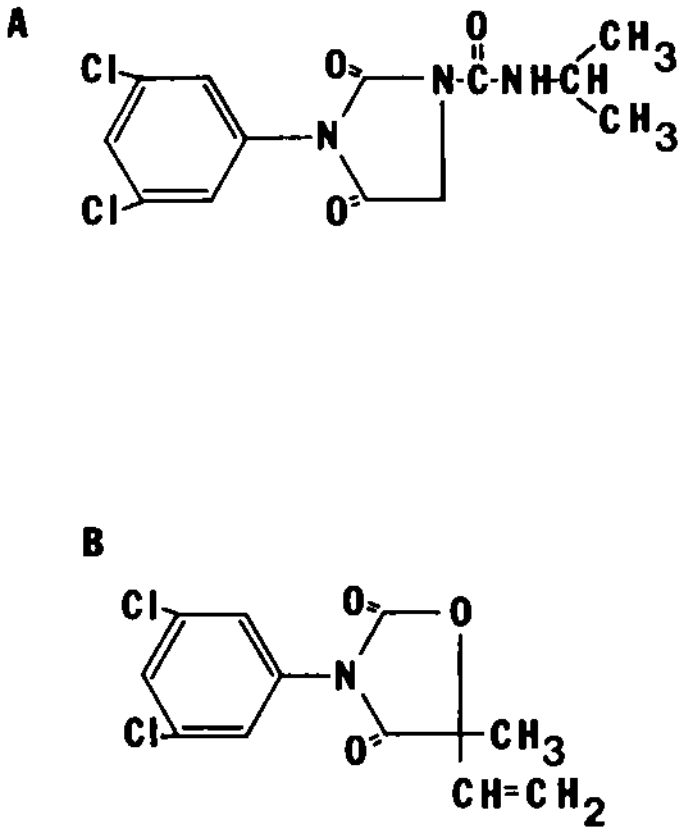
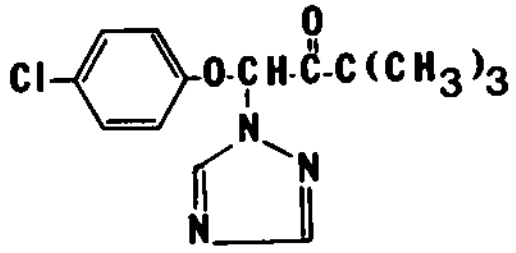


Figure 83. Molecular structure of iprodione (A) and vinclozolin (B).

A



B

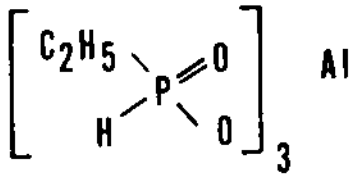


Figure 84. Molecular structure of triadimefon (A) and fosetyl-Al (B).

MATERIALS AND METHODS

1. Influence of medium volume on radial growth of fungal cultures.

Fifteen, 20 and 25 ml of molten Difco potato dextrose agar (PDA) were dispensed into 85 mm Petri plates. These plates were inoculated along one edge with 5 mm mycelial plugs taken from 3 monoconidial isolates each of E. parasitica (E87, E107, E158) and E. gyrosa (E72, E50, E98) grown for 10 d on Difco PDA. Three replicate plates were used for each volume. The cultures were incubated at 25 °C for 8 d after which the colony radii were measured. Single factor analysis of variance (ANOVA) was used to determine whether radial growth was influenced by variations in medium volume.

2. Sensitivity of Endothia species to selected fungitoxicants.

Assays with selected fungitoxicants were performed with the following isolates: E. parasitica - E24, E85, E86, E87, E88, E89, E95, E96, E107, E108, E137, E153, E155; E. gyrosa - E18, E20, E30, E37, E38, E48, E50, E51, E72, E73, E74, E98, E145, E154; E. radicalis - E16, E42, E56, E64, E67, E76, E92, E150, E152; E. eugeniae - E138, E139; E. havanensis - E40, E158, E159; E. macrospora - E54; E. japonica - E53, E59; E. singularis - E58; E. tropicalis - E70; E. viridistroma - E41, E82; E. coccolobii - E157; C. cubensis - H151, H154. The sources, dates of location and hosts of these isolates are listed in Table 3. Cultures were

stored at 4 °C on double glucose-yeast extract agar (dgyea) slants (see Appendix II). The common, trade and chemical names, as well as the chemical class, formulation and manufacturer of all fungitoxicants are presented in Table 34.

Stock solutions of fungitoxicants were added to molten PDA in 1 ml aliquots per liter of medium. The following solvent - fungitoxicant combinations were used: sterile distilled water - chlortetracycline; absolute ethanol - chloramphenicol, thiabendazole hypophosphite, Fungisol®, MBC phosphate, benzimidazole, triadimefon, fosetyl-Al, o-phenylphenol, hydroquinone, cycloheximide; acetone - chloroneb, iprodione, mancozeb, anilazine, chlorothalonil; methanol - nystatin; DMSO - candicidin, amphotericin B; chloroform - PCNB; acetone:ethanol (1:1) - captan; acetone:methylene chloride (1:1) - dicloran, vinclozolin. Stock solutions were prepared on a weight per volume basis except for liquid formulations which were made as volume per volume. The final concentrations of the fungitoxicants in the amended media varied with the individual compound, although initial screening tests usually included the concentrations: 0.01, 0.1, 1, 10, 100 and 500 µg/ml. The nonamended control medium in each test contained 1 ml solvent / l PDA.

Approximately 20 ml of medium were dispensed into 85 mm diameter Petri plates with 3 replicate plates per concentration for each isolate tested. Individual plates were then inoculated along one edge with a 5 mm plug taken from the margin of 7 - 30 d

Table 34: Technical, trade and chemical names, chemical class, formulations and manufacturers of the fungitoxicants used in this study.

Technical name	Trade name	Chemical name or formula	Chemical class	Formulation	Manufacturer
cycloheximide	Actidione®	3-(2-(3,5-Dimethyl-2-oxocyclo-hexyl)2-hydroxyethyl-glutarimide	antifungal antibiotic	technical 100%	Upjohn, Co.
chlortetracycline HCl		7-Chloro-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide	antifungal antibiotic	technical 100%	U. S. Biochemical Corp.
nystatin		$C_{47}H_{75}NO_{17}$	polyene antibiotic	technical 100%	American Cyanamid
candicidin		$C_{63}H_{85}N_{21}O_{19}$ (tentative)	polyene antibiotic	technical 100%	Julius Schmid, Inc.
amphotericin B type 1		$C_{47}H_{73}NO_{17}$	polyene antibiotic	technical 100%	Squibb and Sons
chloramphenicol	Chloromycetin®	D(-)-Threo-2,2-dichloro-N-(B-hydroxy--a-(hydroxymethyl)-p-nitrophenyl-2-amino-1,3-propanediol	antifungal antibiotic	technical 100%	Parke-Davis
captan	Orthocide®	cis-N-((Trichloromethyl)thio)-4 cyclohexene-1,2-dicarboximide	dicarboximide	technical 93%	Chevron
iprodione	Rovral®	3-(3,5-Dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1-imida-zolidinecarboxamide	dicarboximide	50WP	Rhone-Poulenc Inc.
vinclozolin	Ronilan®	3-(3,5-Dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione	dicarboximide	50WP	BASF Wyandotte
PCNB	Terrachlor®	pentachloronitrobenzene	substituted aromatic	technical 100%	Olin Corp.
dicloran	Botran®	2,6-Dichloro-4-nitroanaline	substituted aromatic	75WP	Upjohn Co.
chlorothalonil	Daconil® 2787	tetrachloroisophthalonitrile	substituted aromatic	75WP	Diamond Shamrock
chloroneb	Demosan®	1,4-Dichloro-2,5-dimethoxybenzene	substituted aromatic	technical 90%	DuPont Co.
o-phenyl phenol		ortho-phenyl phenol	substituted aromatic	technical 100%	Aldrich

Table 34: (continued)

Technical name	Trade name	Chemical name or formula	Chemical class	Formulation	Manufacturer
hydroquinone		1,4-benzenediol	substituted aromatic	technical 100%	Aldrich
benzimidazole		N,N'-Methenyl-o-phenylenediamine	benzimidazole	technical 100%	Eastman Kodak
thiabendazole hypophosphite	Arbotect® 20S	2-(4-Thiazolyl)benzimidazole, hypophosphite salt	benzimidazole	solution 26.6%	Merck & Co.
MBC phosphate	Lignasan® BLP	Methyl-2-benzimidazolecarbamate phosphate	benzimidazole	solution 0.7%	DuPont Co.
	Fungisol®	2-(2-ethoxyethoxy)ethyl-2-benzimidazole carbamate (1.7%) + methyl-2-benzimidazole carbamate (0.3%)	benzimidazole	solution 2.0%	J. J. Mauget Co.
mancozeb	Fore®	manganese ethylene bisdithiocarbamate + zinc ion	dithiocarbamate	80WP	Rohm & Haas Co.
anilazine	Dyrene®	4,6-Dichloro-N-(2-chlorophenyl)-1,3,5-triazin-2-amine	triazinamine	50WP	Mobay Chemical Co.
triadimefon	Bayleton®	1-(4-Chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-trizol-1-yl)-2-butanone	triazole	50WP	Mobay Chemical Co.
fosetyl-Al	Aliette®	aluminum tris(-O-ethyl phosphonate)	phosphonate	80WP	Rhone-Poulenc, Inc.

old isolates grown on Difco PDA. Colonies were incubated for 8 d at 25 °C. The average colony radius was then determined for each concentration of fungitoxicant and the control. The percent growth inhibition for each concentration was calculated by the formula:

$$\text{Percent Inhibition} = (1 - ra/rc) \times 100$$

where ra = the average colony radius on amended medium and rc = the average colony radius on nonamended medium. Linear regression analysis, as performed by Statistical Analysis Systems (SAS), was used to establish the dosage-response curve and to estimate the ED_{50} value of each species when differential sensitivities were found. Each fungitoxicant was initially screened with one to five isolates each of E. parasitica and E. gyrosa. Compounds to which differential sensitivities were detected were further tested with 10 - 12 isolates of these two species; the experiment was then repeated. The sensitivities of additional species were determined when extreme differences were found among isolates of E. parasitica and E. gyrosa.

RESULTS AND DISCUSSION

I. Influence of medium volume on radial growth of fungal isolates.

The effect of medium volume on radial growth was determined for three monoconidial isolates each of E. parasitica and E. gyrosa. These data are presented in Table 35. With the exception of isolate E85, there were no significant differences in

Table 35. Effect of volume of Difco potato dextrose agar on radial growth of three monoconidial isolates each of Endothia parasitica and E. gyrosa after eight days at 25°c.

	Radial Growth (mm)					
	E. parasitica			E. gyrosa		
Volume (ml)	E87	E107	E153	E50	E72	E98
15	41[a]	46	46	43	8	28
	41	46	45	40	15	31
	41	45	47	36	8	30
20	42	47	47	39	8	32
	44	45	46	42	17	28
	44	45	48	39	19	29
25	47	46	45	34	22	32
	45	47	48	28	17	31
	45	47	48	34	19	31
F value	20.6*	1.5	0.6	6.7	3.2	1.3

[a]Radial growth in mm; three plates per isolate per volume.

[b]* represents a significant difference at p = 0.01 by single factor ANOVA. $F_{[2,6]}\ 0.01 = 10.9$

radial growth at the p = 0.01 level; the F value of each isolate is given in Table 35. The volume of medium in this test was increased in 5 ml increments per plate; this represents a variation in medium volume much greater than would be found in plates prepared by experienced personnel. In subsequent tests, media were poured into the Petri plates by hand rather than employing automatic dispensing equipment. This lowered the chance of contamination and accelerated the procedure.

II. Sensitivity of Endothia species and Cryphonectria cubensis to selected fungitoxicants.

The effects of selected fungitoxicants on the radial growth of E. parasitica and E. gyrosa are presented in Tables 36 - 72; the accompanying dosage-response curves are shown in Figures 86 - 126.

Cycloheximide was the only compound to which E. parasitica and E. gyrosa were differentially sensitive at the visual level (Figure 85). These species were easily separable by the gross response of the isolates on cycloheximide-amended medium; E. parasitica was able to grow at concentrations which totally inhibited the growth of E. gyrosa. This differential sensitivity was confirmed by the percent inhibition of radial growth (Tables 36 - 42) and dosage-response curves (Figures 86 - 90). The compound was tested against 11-13 isolates of each species; the results were consistant among isolates, and the tests were successfully repeated. The ED_{50} values were calculated from

Figure 85A. Endothia parasitica on cycloheximide-amended potato dextrose agar. Concentrations:

Top row: 0, 0.1, 0.25 µg/ml
Bottom row: 0.5, 0.75, 1.0 µg/ml

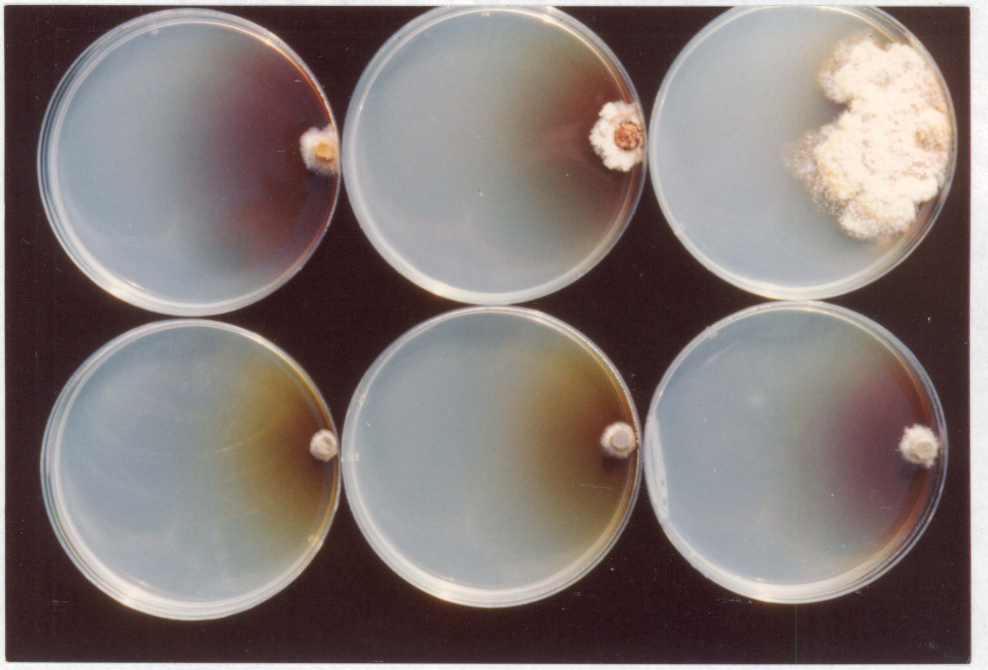


Figure 85B. Endothia gyrosa on cycloheximide-amended potato dextrose agar. Concentrations:

Top row: 0.25, 0.1, 0 µg/ml
Bottom row: 1.0, 0.75, 0.5 µg/ml

Table 36. Average radial growth and percent inhibition of average radial growth of isolates of E. parasitica, E. gyrosa and E. radicalis on cycloheximide-amended potato dextrose agar at 25°C for seven days. Test 1.

Species	Isolate	Radial Growth (mm) Concentration (µg/ml) 0	1	5	25	100	500	Percent Inhibition[a] Concentration (µg/ml) 1	5	25	50	100
E. parasitica	E24	53.3[b]	30.0	14.0	0.0	0.0	0.0	43.8	73.4	100.0	100.0	100.0
" "	E85	50.7	33.3	16.0	0.0	0.0	0.0	34.2	68.4	100.0	100.0	100.0
" "	E86	47.3	32.3	15.7	0.0	0.0	0.0	36.6	66.9	100.0	100.0	100.0
" "	E87	47.7	34.7	14.7	0.0	0.0	0.0	27.3	69.2	100.0	100.0	100.0
" "	E88	61.0	37.3	12.7	0.0	0.0	0.0	38.8	79.2	100.0	100.0	100.0
" "	E89	53.0	33.3	15.3	0.0	0.0	0.0	37.2	71.1	100.0	100.0	100.0
" "	E95	53.7	35.2	10.7	0.0	0.0	0.0	34.5	80.1	100.0	100.0	100.0
" "	E96	49.0	32.7	15.0	0.0	0.0	0.0	33.3	69.4	100.0	100.0	100.0
" "	E107	50.0	34.7	14.7	0.0	0.0	0.0	30.7	77.3	100.0	100.0	100.0
" "	E108	50.7	32.3	13.0	0.0	0.0	0.0	36.2	74.3	100.0	100.0	100.0
" "	E137	45.7	33.3	17.3	0.0	0.0	0.0	27.0	62.0	100.0	100.0	100.0
Average		51.1	33.5	14.5	0.0	0.0	0.0	34.5	72.0	100.0	100.0	100.0
Standard error		4.2	1.9	1.8	0.0	0.0	0.0	4.9	5.5	0.0	0.0	0.0
E. gyrosa	E18	26.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	100.0
" "	E20	37.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	100.0
" "	E30	32.7	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	100.0
" "	E37	23.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	100.0
" "	E38	30.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	100.0
" "	E48	27.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	100.0

Table 36 (continued)

Species	Isolate	Radial Growth (mm)						Percent Inhibition[a]				
		Concentration (μg/ml)						Concentration (μg/ml)				
		0	1	5	25	100	500	1	5	25	50	100
E. gyrosa (continued)	E51	27.7	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	100.0
" "	E72	21.3	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	100.0
" "	E74	33.5	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	100.0
" "	E98	34.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	100.0
Average		29.2	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	100.0
Standard error		5.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E. radicalis	E64	22.0	9.5	2.0	0.0	0.0	0.0	56.8	90.9	100.0	100.0	100.0
" "	E76	41.0	5.0	0.0	0.0	0.0	0.0	87.8	100.0	100.0	100.0	100.0
" "	E149	75.5	11.5	0.0	0.0	0.0	0.0	84.8	100.0	100.0	100.0	100.0
" "	E150	71.0	17.0	3.5	0.0	0.0	0.0	76.1	95.1	100.0	100.0	100.0
" "	E151	79.0	14.5	2.5	0.0	0.0	0.0	81.6	96.8	100.0	100.0	100.0
" "	E152	73.5	8.0	0.0	0.0	0.0	0.0	89.1	100.0	100.0	100.0	100.0
Average		60.3	10.9	1.3	0.0	0.0	0.0	79.4	97.1	100.0	100.0	100.0
Standard error		23.3	4.4	1.5	0.0	0.0	0.0	12.0	3.7	0.0	0.0	0.0

Table 36 (continued)

[a]Percent inhibition was calculated from radial growth based on the formula:
Percent inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average radial growth of 3 colonies per isolate.

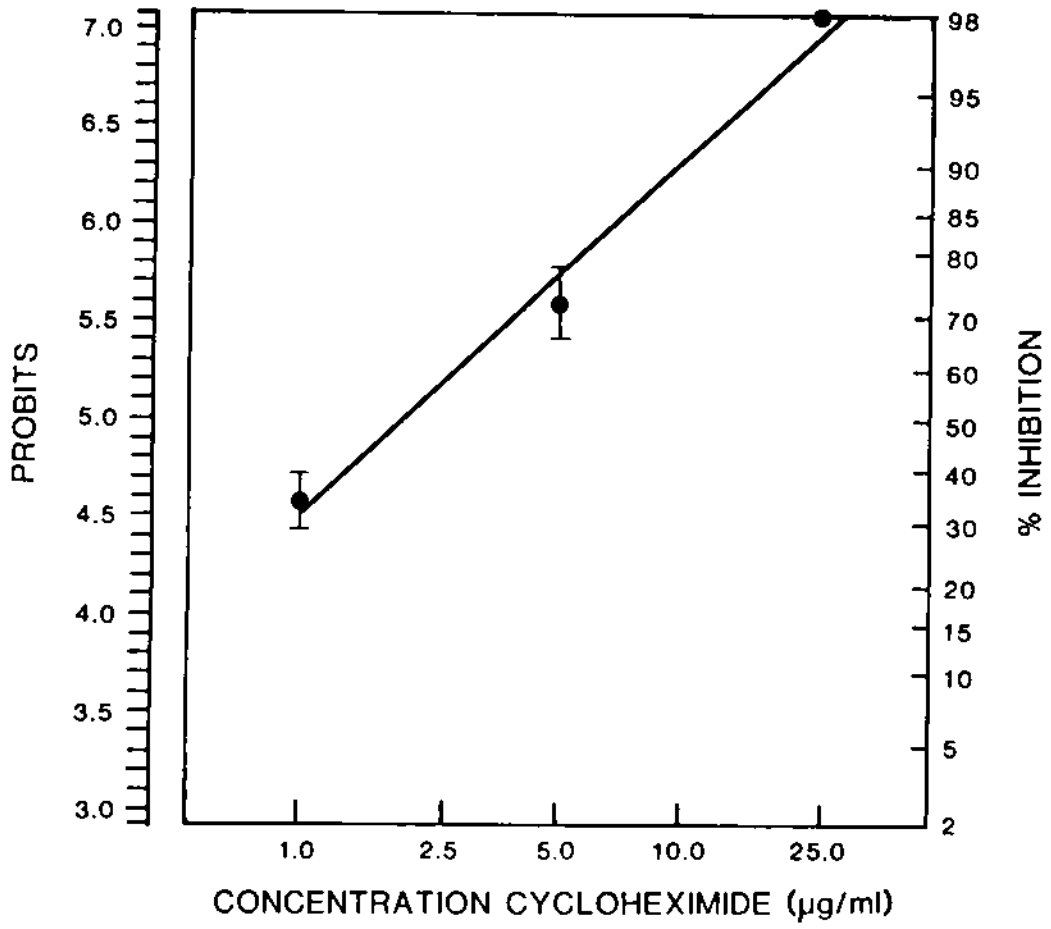


Figure 86. Dosage-response curve of Endothia parasitica grown on cycloheximide-amended potato dextrose agar at 25 °C for 8 d. Average of 11 isolates; three replicate plates per isolate. Vertical bars represent the standard error of the mean. Linear regression with log - probit transformation plotted by Statistical Analysis Systems (SAS). Test #1. Concentration plotted on a logarithmic scale.

Table 37. Average radial growth and percent inhibition of average radial growth of E. parasitica, E. gyrosa and E. radicalis cultured on cycloheximide-amended potato dextrose agar at 25°C for eight days. Test #1.

Species	Isolate	Radial Growth (mm)						Percent Inhibition[a]				
		Concentration (µg/ml)						Concentration (µg/ml)				
		0	0.1	0.25	0.5	0.75	1	0.1	0.25	0.5	0.75	1
E. parasitica	E24	54.0[b]	50.3	50.0	49.0	43.3	41.0	6.8	7.4	9.3	19.8	24.1
" "	E85	49.7	45.3	44.0	40.3	38.0	35.7	8.8	11.5	18.9	23.5	28.2
" "	E86	47.7	44.0	43.0	40.0	39.0	38.3	7.7	9.8	16.1	18.2	19.7
" "	E87	46.3	42.7	40.3	38.7	36.3	34.0	7.8	12.9	16.4	21.6	26.6
" "	E88	60.3	55.7	54.7	48.7	47.3	42.0	7.6	9.3	19.2	21.6	30.3
" "	E89	51.3	50.3	46.0	44.7	43.3	42.3	1.9	10.3	12.9	15.6	17.5
" "	E95	54.3	50.0	47.3	46.7	44.0	42.3	7.9	12.9	14.0	19.0	22.1
	E107	56.0	52.0	49.3	45.7	43.7	43.7	7.1	12.0	18.4	22.0	22.0
" "	E108	45.0	44.3	44.3	41.0	40.0	38.0	3.7	3.7	10.9	13.0	17.4
" "	E137	44.7	41.0	40.3	36.7	36.0	34.3	8.2	9.8	17.9	19.4	23.2
" "	E153	46.0	43.7	40.7	38.7	34.7	35.3	5.0	11.5	15.9	24.6	23.3
" "	E155	60.3	52.7	49.7	48.0	46.3	45.5	12.6	17.6	20.4	23.2	24.5
Average		51.3	47.7	45.8	43.2	41.0	39.4	7.1	10.7	15.8	20.1	23.2
Standard error		5.7	4.7	4.5	4.4	4.2	3.9	2.7	3.4	3.5	3.5	3.9
E. gyrosa	E18	35.0	5.7	3.7	3.0	1.7	0.0	83.7	89.4	91.4	95.1	100.0
" "	E20	32.0	3.3	1.7	1.0	0.7	1.0	89.7	94.7	96.9	97.8	98.4
" "	E30	26.0	4.7	3.5	1.3	1.3	0.7	81.9	86.5	95.0	95.0	100.0
" "	E37	28.0	6.7	5.0	2.0	1.7	0.3	76.1	82.1	92.9	93.9	98.9
" "	E38	33.7	6.3	5.3	3.7	1.3	1.0	81.3	84.3	89.0	96.1	97.0

Table 37 (continued)

Species	Isolate	Radial Growth (mm)						Percent Inhibition[a]				
		Concentration (µg/ml)						Concentration (µg/ml)				
		0	0.1	0.25	0.5	0.75	1	0.1	0.25	0.5	0.75	1
" "	E48	32.0	6.7	4.0	2.3	1.0	0.0	79.1	87.5	92.8	96.9	100.0
" "	E51	33.7	6.0	4.7	2.7	1.0	0.7	74.7	86.1	88.6	95.8	97.0
" "	E72	27.0	6.3	3.0	2.3	1.3	1.0	76.7	88.9	92.6	95.2	96.3
" "	E74	44.3	7.7	4.7	2.3	1.0	1.0	86.1	90.6	90.6	94.1	98.2
" "	E98	30.3	8.0	4.3	2.3	1.0	1.0	73.6	85.8	92.4	96.7	96.7
" "	E145	44.0	10.7	5.7	2.0	1.0	0.0	75.7	84.8	95.4	100.0	100.0
" "	E154	44.3	7.7	4.7	2.3	1.0	1.0	82.6	91.6	94.8	97.7	97.7
Average		34.2	6.6	4.2	2.3	1.2	0.6	80.1	87.7	92.7	96.2	98.4
Standard error		6.6	1.8	1.1	0.7	0.3	0.4	5.0	3.5	2.5	1.7	1.4
E. radicalis	E64	23.5	20.5	18.5	17.5	15.5	16.0	12.8	21.3	25.5	34.0	31.9
" "	E76	28.7	4.0	2.7	2.7	1.7	0.5	21.4	35.7	48.8	59.5	64.3
" "	E149	68.0	57.0	49.5	39.0	24.5	28.5	16.2	27.2	42.6	64.0	58.1

Table 37 (continued)

Species	Isolate	Radial Growth (mm)										
		Concentration (μg/ml)										
		0	0.1	0.25	0.5	0.75	1	0.1	0.25	0.5	0.75	1
E. radicalis (continued)	E152	65.0	51.0	33.5	27.5	24.0	22.5	19.0	46.8	56.3	61.9	64.3
Average		46.3	33.1	26.0	21.7	16.4	16.9	17.4	32.8	43.3	54.8	54.6
Standard error		23.4	25.1	20.1	15.4	10.6	12.0	3.7	11.1	13.1	14.0	15.4

[a]Percent inhibition was calculated from radial growth based on the formula:
Percent inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony on nonamended medium.

[b]Average radial growth of 3 colonies per isolate.

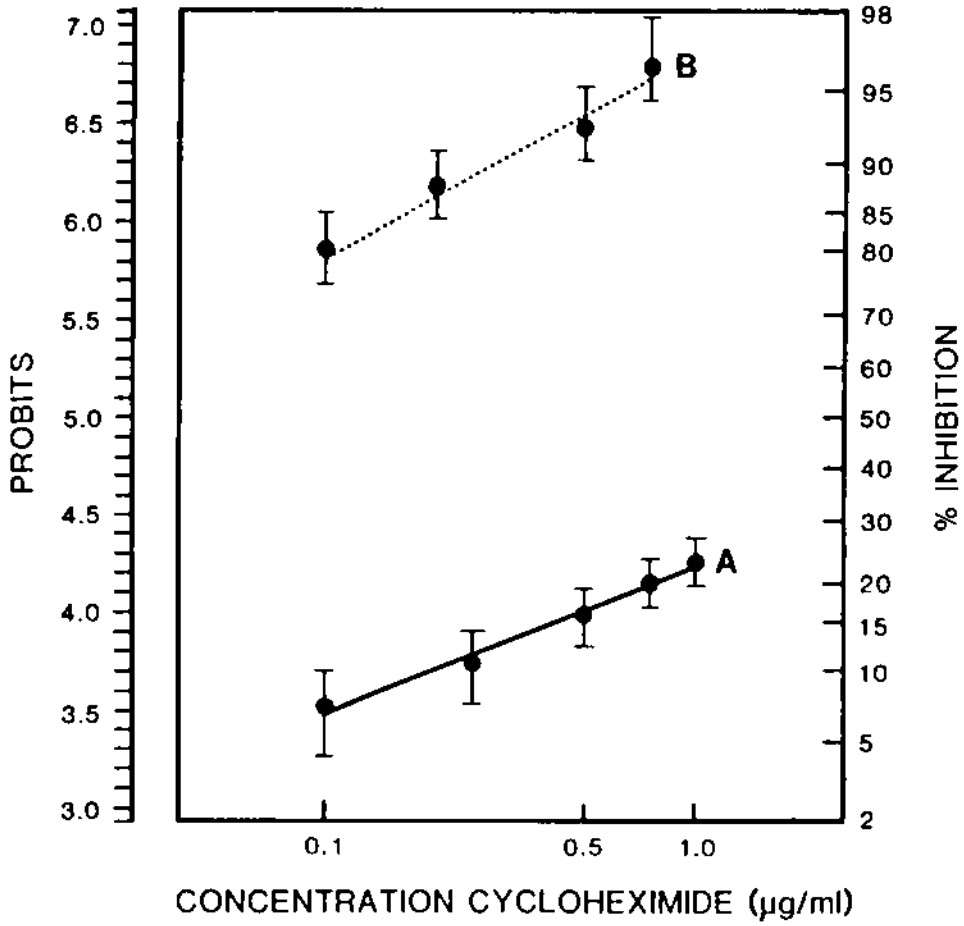


Figure 87. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on cycloheximide-amended potato dextrose agar at 25 °C for 8 d. Average of 11 isolates; three replicate plates per isolate. Vertical bars represent the standard error of the mean. Linear regression with log - probit transformation plotted by Statistical Analysis Systems (SAS). Test #1. Concentration plotted on a logarithmic scale.

Table 38. Average radial growth and percent inhibition of average radial growth of E. gyrosa on cycloheximide-amended potato dextrose agar at 25°C for eight days. Test #1.

Species	Isolate	Radial Growth (mm) Concentration (µg/ml) 0	0.01	0.025	0.05	0.075	0.1	Percent Inhibition[a] Concentration (µg/ml) 0.01	0.025	0.05	0.075	0.1
E. gyrosa	E18	30.7	13.5	7.7	6.0	4.0	4.3	56.8	74.9	80.5	87.0	86.0
" "	E20	34.0	10.7	6.0	5.7	3.3	3.3	68.5	82.4	83.2	90.3	90.3
" "	E30	25.7	9.3	6.3	5.3	5.0	4.0	63.8	75.5	79.4	80.5	84.4
" "	E37	23.0	11.0	6.3	5.3	4.3	4.7	52.2	72.6	77.0	81.3	79.6
" "	E38	29.7	14.7	7.7	7.3	6.7	5.3	50.5	74.1	75.4	77.4	82.2
" "	E48	20.3	12.0	7.7	7.7	6.3	4.3	40.9	62.1	62.1	69.0	78.8
" "	E50	32.0	23.0	12.7	10.7	7.7	5.7	28.1	60.3	66.6	75.9	82.2
" "	E51	28.0	12.7	8.7	7.3	7.0	5.7	54.6	68.9	73.9	75.0	79.6
" "	E72	21.3	12.0	9.0	6.0	5.3	4.0	43.7	57.7	71.8	75.1	81.2
" "	E74	30.7	12.7	8.3	6.0	4.0	5.7	58.6	73.0	80.4	87.0	81.4
" "	E98	24.0	14.0	8.3	7.7	7.3	6.0	41.7	65.4	67.9	69.6	75.0
" "	E145	35.7	20.0	18.8	11.3	6.7	6.3	44.0	50.4	68.3	81.2	82.4
" "	E154	28.0	13.0	10.3	8.0	7.3	6.0	53.6	63.2	71.4	73.9	78.6
Average		27.9	13.7	9.1	7.2	5.8	5.0	50.5	67.7	73.7	78.7	81.7
Standard error		4.8	3.8	3.4	1.9	1.5	1.0	10.7	8.8	6.4	6.6	3.8

Table 38 (continued)

[a]Percent inhibition was calculated from radial growth based on the formula:
Percent inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average radial growth of 3 colonies per isolate.

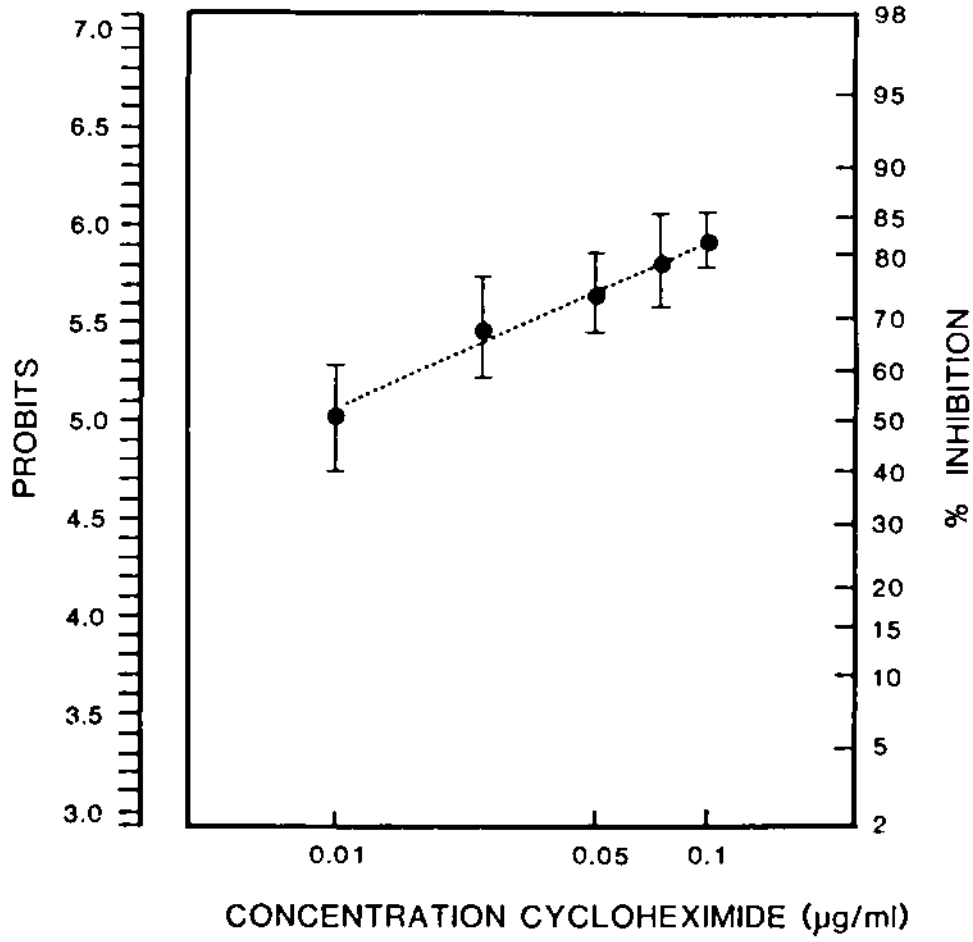


Figure 88. Dosage-response curve of E. cyrosa grown on cycloheximide-amended potato dextrose agar at 25 °C for 8 d. Average of 13 isolates; three replicate plates per isolate. Vertical bars represent the standard error of the mean. Linear regression with log - probit transformation plotted by Statistical Analysis Systems (SAS). Test #1. Concentration plotted on a logarithmic scale.

Table 39. Average radial growth of E. parasitica, E. gyrosa and E. singularis cultured on cycloheximide-amended potato dextrose agar at 25°C for seven days. Test #2.

		Radial Growth (mm)										
		Concentration (µg/ml)										
Species	Isolate	0	0.01	0.025	0.05	0.075	0.1	0.25	0.5	0.75	1	5
E. parasitica	E24	37.0[a]					39.0	35.3	31.7	31.0	29.0	9.0
" "	E85	38.0					33.0	33.0	31.3	31.0	28.7	8.0
" "	E86	38.0					35.0	34.3	31.0	31.3	30.3	7.3
" "	E87	36.0					29.5	26.5	27.3	24.5	23.0	9.7
" "	E88	45.0					42.3	38.7	33.0	37.0	36.0	9.7
" "	E89	39.3					36.3	36.3	33.0	32.3	31.7	12.3
" "	E95	46.7					40.0	37.7	35.0	32.3	33.7	11.3
" "	E107	39.0					34.7	34.3	33.3	32.0	31.3	7.3
" "	E108	39.3					36.0	34.3	31.0	28.0	29.0	7.3
" "	E153	31.0					---[b]	31.0	28.5	27.7	25.7	10.7
" "	E155	41.0					36.3	35.0	31.3	29.3	27.0	12.3
Average		39.1					36.2	34.2	31.5	30.6	29.6	9.5
Standard error		4.2					3.6	3.3	2.2	3.2	3.6	1.9
E. gyrosa	E18	16.7	12.3	8.3	8.0	5.3	4.3	3.7	2.2	1.3	0.7	0.0
" "	E20	13.0	6.0	6.0	4.0	3.7	1.0	1.0	0.8	0.8	0.2	0.0
" "	E30	15.7	6.0	6.3	4.0	4.5	4.2	3.7	1.7	1.5	1.0	0.0
" "	E37	14.3	8.2	7.7	7.3	4.7	7.7	3.0	1.7	0.8	0.0	0.0
" "	E38	14.7	9.7	7.3	7.7	7.3	4.3	2.0	1.8	1.0	0.3	0.0
" "	E48	11.0	7.0	5.0	4.0	5.3	5.3	3.3	2.3	1.5	1.2	0.0
" "	E50	32.0	17.0	10.0	8.0	5.0	8.7	3.0	1.3	0.7	0.0	0.0
" "	E51	17.3	8.0	7.0	7.7	6.3	4.0	3.3	0.8	1.0	1.0	0.0
" "	E98	13.7	8.0	8.3	5.7	6.0	5.7	3.0	1.0	1.0	0.3	0.0

Table 39 (continued)

Species	Isolate	Radial Growth (mm) Concentration (µg/ml) 0	0.01	0.025	0.05	0.075	0.1	0.25	0.5	0.75	1	5
E. gyrosa (continued)	E145	45.0	26.7	19.0	14.0	8.0	6.0	2.0	0.5	0.0	0.0	0.0
" "	E154	15.0	7.7	6.0	5.3	4.7	5.0	3.3	1.3	0.8	0.0	0.0
Average		18.9	10.6	8.3	6.9	5.5	5.1	2.8	1.4	0.9	0.4	0.0
Standard error		10.2	6.2	3.8	2.9	1.3	2.0	0.8	0.6	0.4	0.5	0.0
E. singularis	E58	10.0	4.2	2.7	1.3	0.8	0.3	0.0	0.0	0.0	0.0	0.0

[a]Average radial growth of 3 colonies per isolate.

[b]Data missing due to contamination.

Table 40. Percent inhibition of average radiaal growth of isolates of E. parasitica, E. gyrosa and E. singularis on cycloheximide-amended potato dextrose agar at 25°C for seven days. Test #2.

Species	Isolate	Percent Inhibition[a] Concentration (µg/ml) 0.01	0.025	0.05	0.075	0.1	0.25	0.5	0.75	1	5
E. parasitica	E24					-5.4	4.6	14.3	16.2	21.6	73.8
" "	E85					13.2	13.2	17.6	18.4	24.5	78.9
" "	E86					2.8	4.7	13.9	13.0	15.8	79.7
" "	E87					18.0	26.4	24.2	31.9	36.1	73.0
" "	E88					6.0	14.0	26.7	17.8	20.0	78.4
" "	E89					7.6	8.4	16.0	17.8	19.3	68.7
" "	E95					14.3	19.3	25.0	30.8	27.8	75.8
" "	E107					11.0	12.0	14.6	17.9	19.7	81.3
" "	E108					8.4	12.7	21.1	28.7	26.2	81.4
" "	E153					--[b]	0.0	8.1	10.6	17.1	65.5
" "	E155					11.5	14.6	23.7	28.5	34.1	70.0
Average						8.7	11.8	18.6	21.0	23.8	75.1
Standard error						6.6	7.3	5.9	7.5	6.7	5.4
E. gyrosa	E18	26.3	50.3	52.1	68.3	74.2	77.8	86.8	92.2	95.8	100.0
" "	E20	53.8	53.8	69.2	71.5	92.3	92.3	93.8	93.8	98.5	100.0
" "	E30	61.8	59.9	74.5	71.3	73.2	76.4	89.2	90.4	93.6	100.0
" "	E37	42.7	46.1	48.9	67.1	46.1	79.0	88.1	94.4	100.0	100.0

Table 40 (continued)

Species	Isolate	Percent Inhibition[a] Concentration (μg/ml) 0.01	0.025	0.05	0.075	0.1	0.25	0.5	0.75	1	5
E. gyrosa (continued)	E38	34.0	50.3	47.6	50.3	70.7	86.4	87.7	93.2	98.0	100.0
" "	E48	36.4	54.5	63.6	51.8	51.8	70.0	79.1	86.4	89.1	100.0
" "	E50	46.9	68.8	75.0	84.4	72.8	90.6	95.9	97.8	100.0	100.0
" "	E51	53.8	59.5	55.5	63.6	76.9	80.9	95.4	94.2	94.2	100.0
" "	E98	41.6	39.4	58.4	56.2	58.4	78.1	92.7	92.7	97.8	100.0
" "	E145	40.7	57.8	68.9	82.2	86.7	95.5	98.9	100.0	100.0	100.0
" "	E154	48.7	60.0	64.7	68.7	66.7	78.0	91.3	94.7	100.0	100.0
Average		44.2	54.6	61.7	66.8	70.0	82.3	90.8	93.6	97.0	100.0
Standard error		10.1	8.0	9.8	11.0	13.8	7.8	5.5	3.5	3.5	0.0
E. singularis	E58	58.0	73.0	87.0	92.0	97.0	100.0	100.0	100.0	100.0	100.0

[a]Percent inhibition was calculated from average radial growth (based on the measurement of three plates per isolate) by the formula:
Percent inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

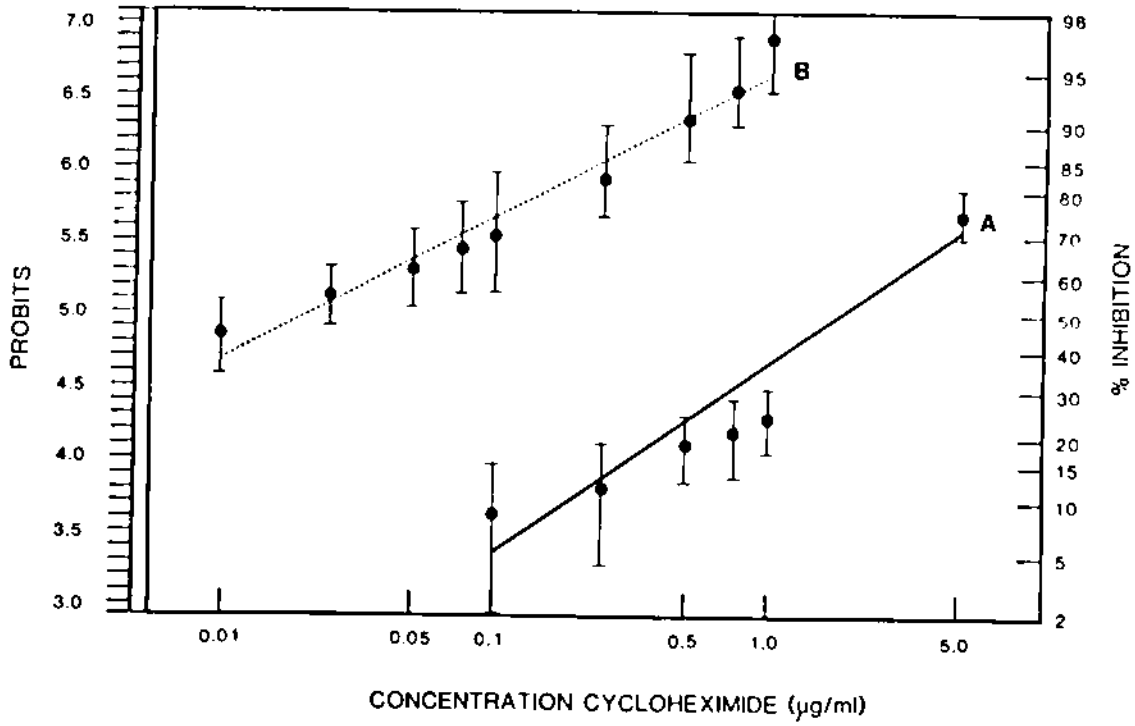


Figure 89. Dosage-response curve of E. parasitica (A) and E. pyrosa (B) grown on cycloheximide-amended potato dextrose agar at 25 °C for 7 d. Average of 11 isolates; three replicate plates per isolate. Vertical bars represent the standard error of the mean. Linear regression with log - probit transformation plotted by Statistical Analysis Systems (SAS). Test #2. Concentration plotted on a logarithmic scale.

Table 41. Average radial growth of isolates of E. parasitica, E. gyrosa and E. singularis cultured on cycloheximide-amended potato dextrose agar at 25°C for 14 days. Test #2.

Species	Isolate	Radial Growth (mm) Concentration (µg/ml) 0	0.01	0.025	0.05	0.075	0.1	0.25	0.5	0.75	1	5
E. parasitica	E24	75.3[a]					72.0	55.7	45.7	38.0	24.7	16.3
" "	E85	69.0					68.0	65.0	57.7	45.7	34.0	15.0
" "	E86	68.3					61.3	58.0	40.0	48.0	41.7	11.0
" "	E87	49.0					36.0	30.5	29.7	22.3	22.0	16.3
" "	E88	77.7					51.7	46.0	29.7	33.7	43.0	18.0
" "	E89	68.7					64.7	64.0	42.7	46.0	40.0	19.0
" "	E95	78.0					72.7	73.7	69.0	56.7	58.0	17.0
" "	E107	63.0					61.7	39.3	39.7	37.3	36.7	15.7
" "	E108	66.3					70.5	68.0	49.3	35.7	46.3	13.7
" "	E153	71.5					---[b]	54.0	32.5	36.0	29.3	18.3
" "	E155	71.0					71.3	66.0	48.3	38.7	32.3	17.7
Average		68.9					63.0	56.4	44.0	39.8	37.1	16.2
Standard error		8.1					11.5	13.2	12.0	9.0	10.3	2.3
E. gyrosa	E18	28.3	23.7	15.3	14.3	12.0	10.3	9.3	6.3	4.0	3.3	0.0
" "	E20	21.3	12.0	11.3	9.7	7.7	6.3	6.3	3.3	2.7	1.0	0.0
" "	E30	27.0	15.7	15.0	8.7	9.0	8.3	8.3	5.7	5.3	4.7	0.0
" "	E37	23.7	15.3	14.3	14.7	13.3	14.7	12.0	4.7	2.7	0.7	0.0
" "	E38	27.3	18.0	14.3	13.7	12.3	9.3	7.7	5.3	3.7	1.3	0.0
" "	E48	21.0	15.3	11.7	11.3	15.0	12.0	7.3	4.3	3.3	2.3	0.0
" "	E50	70.3	35.3	21.7	16.0	13.7	15.7	6.3	3.3	3.3	0.8	0.0

Table 41 (continued)

Species	Isolate	Radial Growth (mm) Concentration (μg/ml) 0	0.01	0.025	0.05	0.075	0.1	0.25	0.5	0.75	1	5
E. gyrosa (continued)	E51	38.3	16.0	13.7	15.7	13.0	10.3	8.3	6.0	5.3	4.3	0.0
" "	E98	25.0	18.7	16.3	14.0	13.7	14.3	10.3	5.0	4.7	4.0	0.0
" "	E145	77.0	57.7	44.0	30.7	19.3	11.0	4.7	3.0	1.0	0.3	0.0
" "	E154	38.0	14.7	11.0	10.7	11.3	11.0	8.0	5.3	3.3	2.0	0.0
Average		35.2	22.0	17.1	14.5	12.7	11.2	8.0	4.7	3.6	2.2	0.0
Standard error		19.6	13.4	9.4	5.9	3.0	2.8	2.0	1.1	1.3	1.6	0.0
E. singularis	E58	20.7	12.0	9.3	7.0	4.7	2.7	1.3	0.0	0.0	0.0	0.0

[a]Average radial growth of 3 colonies per isolate.

[b]Data missing due to contamination.

Table 42. Percent inhibition of radial growth of isolates of E. parasitica, E. gyrosa and E. singularis on cycloheximide-amended potato dextrose agar at 25°C for 14 days. Test #2.

Species	Isolate	Percent Inhibition[a] Concentration (µg/ml) 0.01	0.025	0.05	0.075	0.1	0.25	0.5	0.75	1	5
E. parasitica	E24					4.4	26.0	39.3	49.5	67.2	78.3
" "	E85					1.4	5.8	16.4	33.8	50.7	78.3
" "	E86					10.2	15.1	41.4	29.7	38.9	83.9
" "	E87					26.5	37.7	39.4	54.5	55.1	66.7
" "	E88					33.5	40.8	61.8	56.6	44.7	76.8
" "	E89					5.8	6.8	37.8	33.0	41.8	72.3
" "	E95					6.8	5.5	11.5	27.3	25.6	78.2
" "	E107					2.1	37.6	37.0	40.8	41.7	75.1
" "	E108					-6.3[b]	-2.6	25.6	46.1	30.2	79.3
" "	E153					---[c]	24.5	54.5	49.6	59.0	74.4
" "	E155					-0.4	7.0	32.0	45.5	54.5	75.1
Average						8.4	18.6	36.1	42.4	46.3	76.2
Standard error						12.3	15.4	14.7	10.1	12.4	4.4
E. gyrosa	E18	16.2	45.9	49.5	57.6	63.6	67.1	77.7	85.9	88.3	100.0
" "	E20	43.7	46.9	54.5	63.8	70.4	70.4	84.5	87.3	95.3	100.0
" "	E30	41.8	44.4	67.8	66.7	69.3	69.3	78.9	80.4	82.6	100.0
" "	E37	35.4	39.7	38.0	43.9	38.0	49.4	80.2	88.6	97.0	100.0
" "	E38	34.1	47.6	49.8	54.9	65.9	71.8	80.6	86.4	95.2	100.0
" "	E48	27.1	44.3	46.2	28.6	42.9	65.2	79.5	84.3	89.0	100.0
" "	E50	49.8	69.1	77.2	80.5	77.7	91.0	95.3	95.3	98.9	100.0

Table 42 (continued)

Species	Isolate	Percent Inhibition[a] Concentration (μg/ml) 0.01	0.025	0.05	0.075	0.1	0.25	0.5	0.75	1	5
E. gyrosa (continued)	E98	25.2	34.8	44.0	45.2	42.8	58.8	80.0	81.2	84.0	100.0
" "	E145	25.1	42.9	60.1	74.9	85.7	93.9	96.1	98.7	99.6	100.0
" "	E154	47.5	60.7	61.8	59.6	60.7	71.4	81.1	88.2	92.9	100.0
Average		36.7	49.1	55.3	58.3	62.7	67.1	83.5	87.5	92.0	100.0
Standard error		12.7	10.7	11.3	14.8	15.4	14.5	6.4	5.4	5.8	0.0
E. singularis	E58	42.0	55.1	66.2	77.3	87.0	93.7	100.0	100.0	100.0	100.0

[a]Percent inhibition was calculated from average radial growth (based on the measurement of three plates per isolate) by the formula:
Percent inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Negative values of percent inhibition indicate growth stimulation.

[c]Data missing due to contamination.

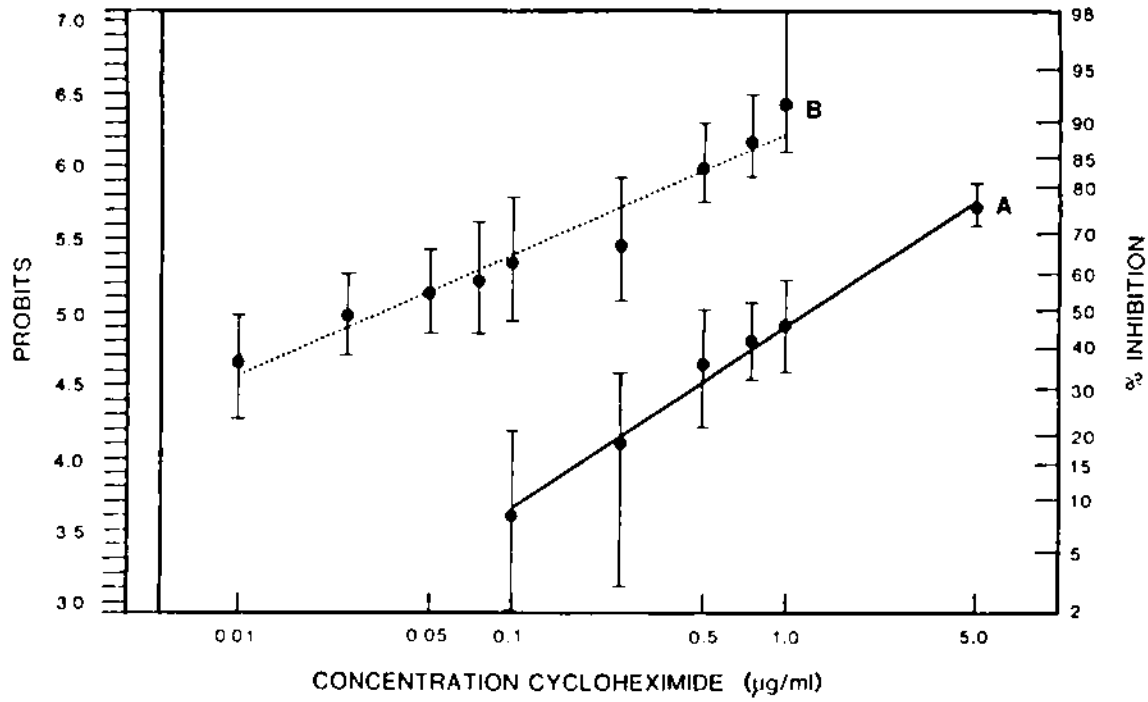


Figure 90. Dosage-response curve of E. parasitica (A) and E. pyrosa (B) grown on cycloheximide-amended potato dextrose agar at 25 °C for 14 d. Average of 11 isolates; three replicate plates per isolate. Vertical bars represent the standard error of the mean. Linear regression with log - probit transformation plotted by Statistical Analysis Systems (SAS), Test #2. Concentration plotted on a logarithmic scale.

the dosage-response curve and are presented in Table 43. These values were 1-2 µg/ml for E. parasitica and 0.01-0.03 µg/ml for E. gyrosa. This represents a hundred-fold difference in sensitivity to cycloheximide and may reflect disparate ribosomal protein fractions. Other resistance mechanisms may also be involved. Endothia parasitica may be able to detoxify the compound, as do resistant strains of Cunninghamella blakesleeana, or the cell membrane may be less permeable to it (184) Endothia parasitica is not totally resistant to the compound; concentrations of 25 µg/ml and above do result in the total inhibition of radial growth. Isolates of E. gyrosa will not grow above 1 µg/ml so the relative resistance of E. parasitica is quite high. This level of selectivity indicates that some important biochemical differences exist between the two species.

Sensitivity to cycloheximide was tested at both 7 and 14 d in Test #2 (Figures 89 and 90). Although there are slight differences in the position and slopes of the dosage-response curves, the standard errors of the means overlap and the variation is not significant. This indicates that the response of the fungus is stable with time and that the compound does not break down in the medium.

Cycloheximide also seemed to alter the colony morphology of certain isolates; pigment production was particularly affected. Isolates of E. gyrosa frequently produced large amounts of perilla purple at concentrations lower than 1 µg/ml, while a brilliant yellow pigment was released into the medium at 1 and 5

Table 43. ED_{50} values of isolates of Endothia species for cycloheximide obtained by linear regression of percent inhibition of fungal growth on cycloheximide-amended potato dextrose agar for 7 and 14 days at 25°C.

Species	Isolate	Test 1	Test 2	
		7 days	7 days	14 days
E. radicalis	E16	0.48	0.26	0.36
" "	E42	0.26	0.76	0.60
" "	E56	0.28	0.27	0.42
" "	E64	1.19	0.50	0.44
" "	E67	1.52	ND[a]	ND
" "	E76	0.44	0.60	0.56
" "	E92	ND	ND	0.18
" "	E150	0.53	0.68	1.41
" "	E152	1.04	ND	ND
E. tropicalis	E57	2.72	ND	1.43
" "	E70	1.54	0.49	0.68
E. singularis	E58	0.01	0.01	0.02
E. macrospora	E54	2.40	1.72	1.73
E. havanensis	E40	1.38	0.04	2.96
" "	E158	0.05	0.06	0.18
" "	E159	0.05	0.05	0.12
" "	H183	ND	3.53	3.24
E. viridistroma	E41	1.64	0.80	ND
" "	E82	0.91	0.72	2.60
E. coccolobii	E157	0.23	ND	ND
E. eugeniae	E138	1.00	1.20	ND
" "	E139	2.24	2.06	2.76
" "	H184	ND	2.06	ND
C. cubensis	H151	3.61	3.02	ND
" "	H154	2.04	0.88	ND
" "	H182	ND	0.03	0.03

Table 43 (continued)

Species	Isolate	Test 1	Test 2	
		7 days	7 days	14 days
E. parasitica	Average of 11 isolates	1.93	2.40	1.19
E. gyrosa	Test 1 - average of 13 isolates Test 2 - average of 11 isolates	0.01	0.02	0.03

[a]"ND" = not determined.

µg/ml despite the complete absence of mycelial growth. Apparently the fungi, unable to produce proteins and possibly nucleic acids because of the cycloheximide, were shunting their metabolites into secondary, pigment-producing pathways. Isolates of E. parasitica did not display any alterations in pigment metabolism, even at concentrations of 100 µg/ml.

A cycloheximide-amended medium could be used in a clinical situation to differentiate E. parasitica from E. gyrosa. The difference in sensitivity is stable over at least two weeks, and detailed measurements are not necessary to distinguish between the two organisms. Potato dextrose agar was used as the basal medium in these tests, but the same phenomenon was also observed with cycloheximide-amended glucose-yeast extract agar (gyea); the formula for this medium is presented in Appendix II. A cycloheximide concentration of 1 µg/ml in the basal medium would be optimal; at this level the growth of E. gyrosa is totally inhibited while that of E. parasitica is reduced by only 30-50%. Both of these organisms are found on a variety of forest and landscape trees; diagnosis is often difficult due to the frequent lack of perithecial formation. Proper identification is important for management strategies; Endothia gyrosa is an opportunistic pathogen which can frequently be controlled by allievating water stress. This would not be effective for trees colonized by E. parasitica. Rapid diagnosis would allow the proper recommendation to be given. One note of precaution must be

added: cycloheximide is a highly toxic compound [ED_{50} = 150 mg/kg in mice (212)] and must be used with extreme care.

Cycloheximide sensitivity was also determined for nine other species of Endothia and C. cubensis. These data are presented in Tables 44 - 46; dosage-response curves are shown in Figures 91 - 100 and ED_{50} values are included in Table 43. The ED_{50} values were somewhat variable for some isolates between Test #1 and Test #2, but some general trends are evident. Most isolates responded to cycloheximide as did E. parasitica with ED_{50} values between 0.5-5.0 µg/ml. Several isolates, however, were much more sensitive. Endothia singularis isolate E58 had an ED_{50} value of 0.01 µg/ml at 7 d; its response was similar to that of E. gyrosa. Both of these species produce nonseptate ascospores and remain in the genus Endothia under Barr's classification system. Cycloheximide sensitivity cannot be used to separate Endothia from Cryphonectria, however, since isolates of E. viridistroma, the third species in Barr's Endothia, consistently produced higher ED_{50} values (0.7 - 1.6 µg/ml at seven days). Its response more closely resembled that of E. parasitica. Endothia viridistroma is the only species of the traditional Endothia which produces green stromata; perhaps its taxonomic position should be reexamined in light of these biochemical irregularities.

The two suspected isolates of E. havanensis from Florida (E158,E159) were quite sensitive to cycloheximide and produced ED_{50} values of 0.05-0.06 µg/ml. The identification of these

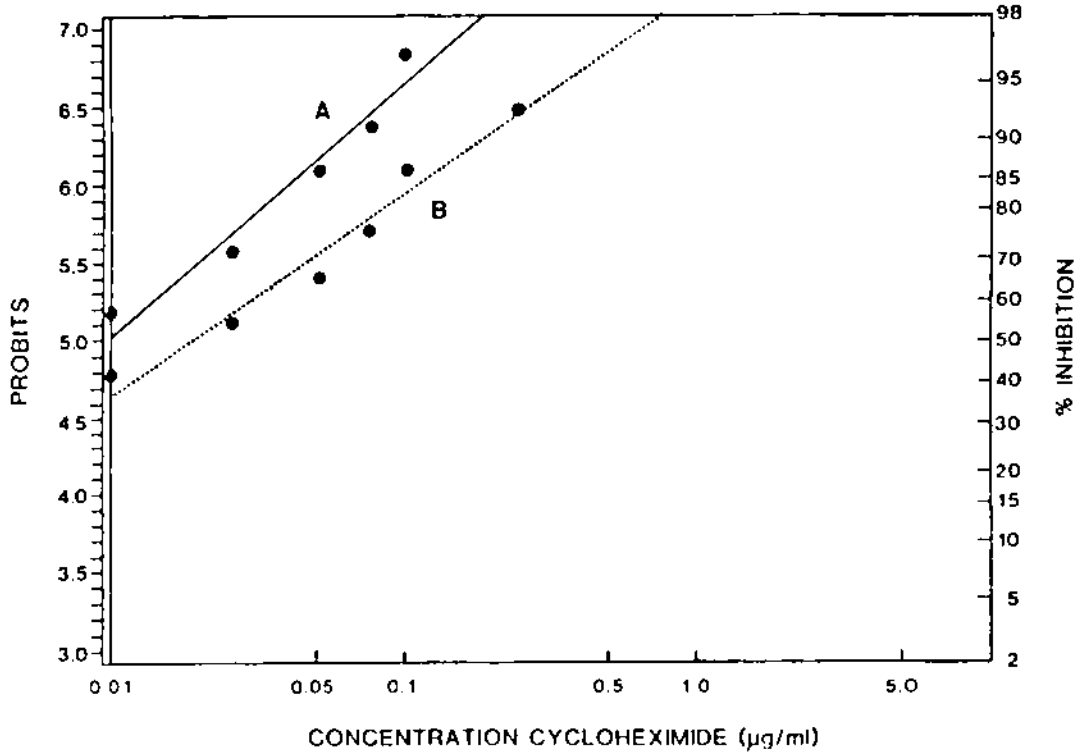


Figure 91. Dosage-response curve of E. singularis isolate E58 grown on cycloheximide-amended potato dextrose agar at 25 °C for 7 (A) and 14 (B) d; average of three replicate plates. Linear regression with log - probit transformation plotted by Statistical Analysis Systems (SAS). Test #2. Concentration plotted on a logarithmic scale.

Table 44. Average radial growth and percent inhibition of average radial growth of isolates of Endothia species and Cryphonectria cubensis cultured on cycloheximide-amended potato dextrose agar at 25°C for seven days (Test 3).

Species	Isolate	Radial Growth (mm)						Percent Inhibition[a]				
		Concentration (µg/ml)						Concentration (µg/ml)				
		0	0.01	0.1	1	10	100	0.01	0.1	1	10	100
E. radicalis	E16	50.7[b]	51.0	42.0	16.7	0.0	0.0	-0.6[c]	17.2	67.1	100.0	100.0
" "	E42	46.3	44.7	26.7	13.7	0.0	0.0	3.4	42.3	70.4	100.0	100.0
" "	E56	40.7	37.3	29.3	10.3	0.0	0.0	8.4	28.0	73.7	100.0	100.0
" "	E64	22.3	21.0	20.3	18.3	1.3	0.0	5.8	9.0	17.9	94.2	100.0
" "	E67	17.0	17.7	15.7	10.0	0.0	0.0	-4.1	7.6	41.2	100.0	100.0
" "	E76	42.0	38.0	33.0	18.0	3.3	0.0	9.5	21.4	57.1	92.1	100.0
" "	E92	12.0	12.0	9.3	0.0	0.0	0.0	0.0	22.5	100.0	100.0	100.0
" "	E150	53.7	51.3	48.0	29.3	1.3	0.0	4.5	10.6	45.4	97.6	100.0
" "	E152	48.0	46.0	39.7	23.7	0.0	0.0	4.2	17.3	50.6	100.0	100.0
E. macrospora	E54	38.3	41.0	40.1	28.0	6.0	0.0	-7.0	-4.4	26.9	84.3	100.0
E. japonica	E53	36.3	35.0	32.0	23.0	0.0	0.0	3.6	11.8	36.6	100.0	100.0
	E59	43.3	42.3	40.3	20.7	0.3	0.0	2.3	6.9	52.2	99.3	100.0
E. tropicalis	E57	24.0	23.7	24.3	19.0	5.0	0.0	1.2	-1.2	20.8	79.2	100.0
	E70	25.7	28.7	25.3	16.3	0.0	0.0	-11.7	1.6	36.6	100.0	100.0
E. viridistroma	E41	79.3	79.0	53.3	5.0	0.0	0.0	0.4	0.4	32.8	93.7	100.0
	E82	79.0	78.7	67.7	40.7	0.0	0.0	0.4	14.3	48.5	100.0	100.0

Table 44 (continued)

Species	Isolate	Radial Growth (mm)						Percent Inhibition[a]				
		Concentration (μg/ml)						Concentration (μg/ml)				
		0	0.01	0.1	1	10	100	0.01	0.1	1	10	100
E. eugeniae	E138	69.0	65.3	74.7	43.0	10.0	0.0	5.4	-8.3	37.7	85.5	100.0
	E139	33.5	34.7	36.7	20.3	10.3	0.0	-3.6	-9.5	39.4	69.2	100.0
C. cubensis	H151	74.1	76.3	76.5	67.0	11.7	0.0	-2.1	-2.4	10.3	84.3	100.0
	H154	79.0	80.0	78.7	45.0	10.3	0.0	-1.3	0.3	43.0	87.0	100.0
E. havanensis	E40	58.0	61.7	59.3	34.7	5.7	0.0	-6.4	-2.2	41.9	90.1	100.0
	E158	63.7	46.7	27.3	5.0	0.0	0.0	26.7	57.1	92.2	100.0	100.0
	E159	51.0	37.3	21.7	3.7	0.0	0.0	26.9	57.4	92.7	100.0	100.0
E. singularis	E58	17.0	10.0	2.0	0.0	0.0	0.0	41.1	88.2	100.0	100.0	100.0
E. coccolobii	E157	61.0	47.0	47.0	6.0	0.0	0.0	-2.8	22.9	90.2	100.0	100.0

[a]Percent inhibition was calculated from radial growth based on the following formula: Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average of three colonies.

[c]Negative values of percent inhibition indicate growth stimulation.

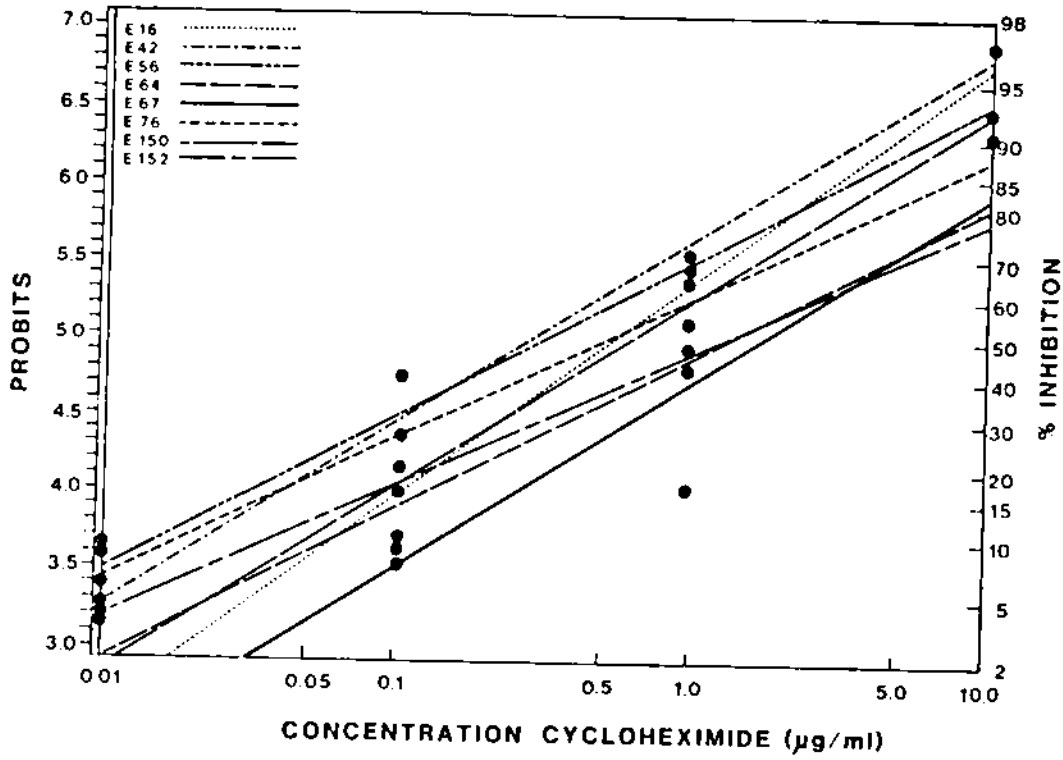


Figure 92. Dosage-response curve of E. radicalis grown on cycloheximide-amended potato dextrose agar at 25 °C for 7 d; average of three replicate plates per isolate. Linear regression with log - probit transformation plotted by Statistical Analysis Systems (SAS), Test #3. Concentration plotted on a logarithmic scale.

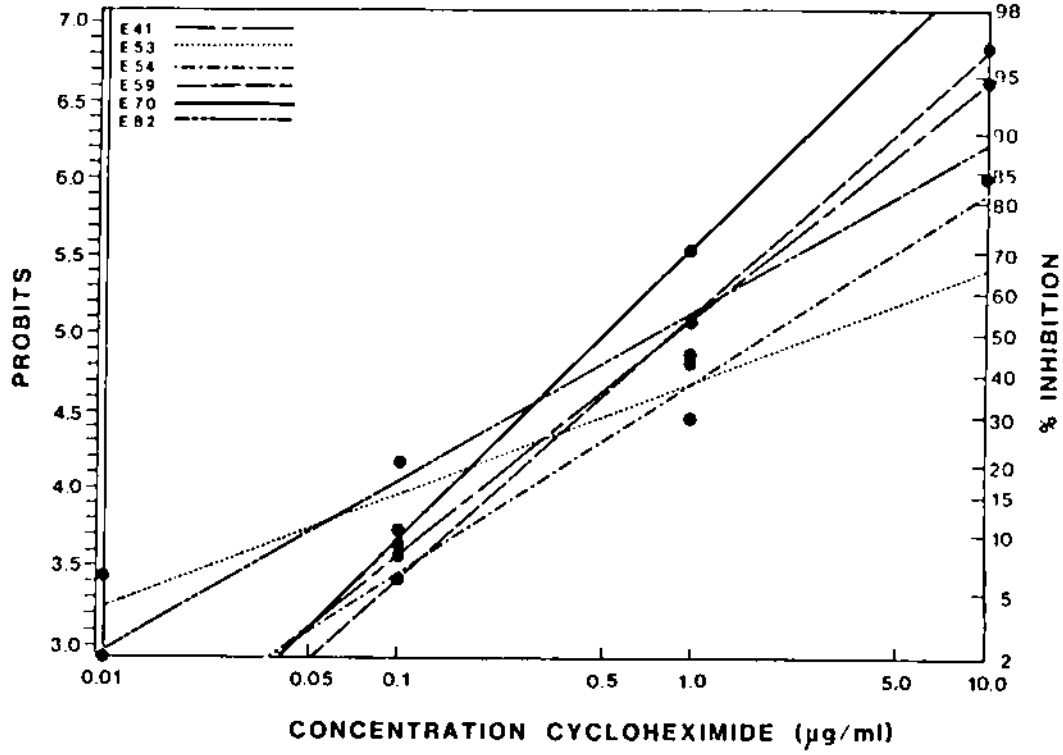


Figure 93. Dosage-response curve of Endothia viridistroma (E41, E82), E. japonica (E53, E59), E. macrospora (E54) and E. tropicalis (E57) grown on cycloheximide-amended potato dextrose agar at 25 °C for 7 d; average of three replicate plates per isolate. Linear regression with log - probit transformation plotted by Statistical Analysis Systems (SAS), Test #3. Concentration plotted on a logarithmic scale.

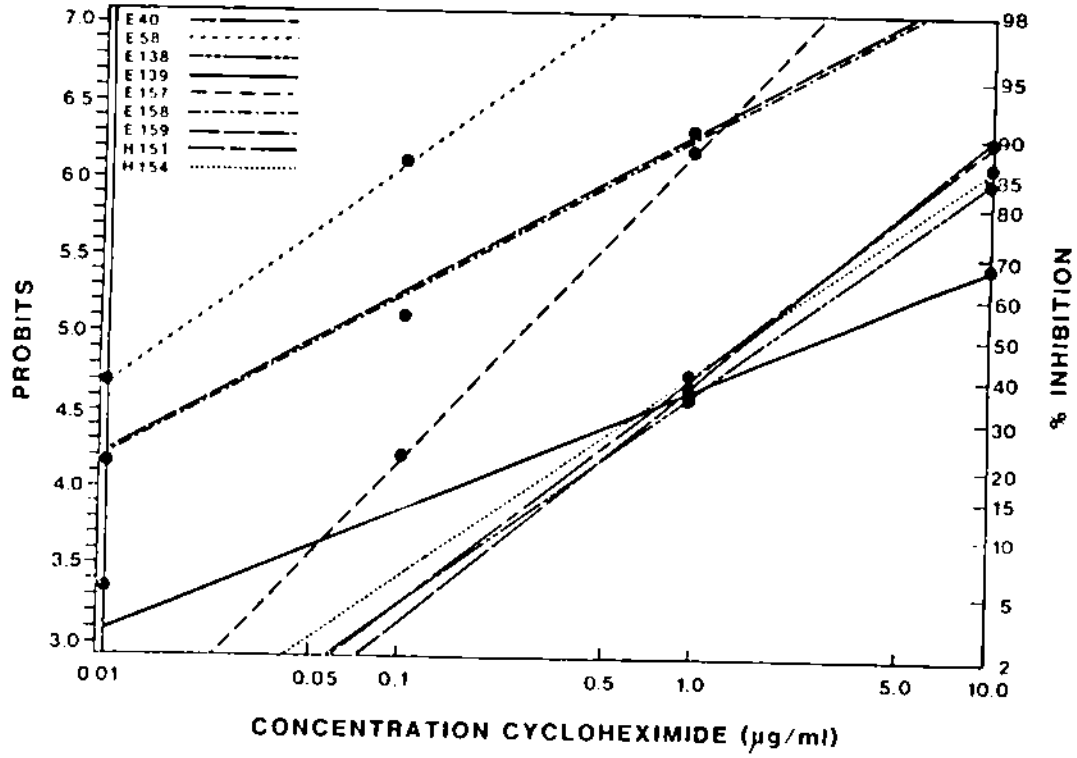


Figure 94. Dosage-response curve of Endothia havanensis (E40, E158, E159), E. singularis (E58), E. eugeniae (E138, E139), E. coccolobii (E157) and Cryphonectria cubensis (H151, H154) grown on cycloheximide-amended potato dextrose agar at 25 °C for 7 d; average of three replicate plates per isolate. Linear regression with log - probit transformation plotted by Statistical Analysis Systems (SAS). Test #3. Concentration plotted on a logarithmic scale.

Table 45. Average radial growth and percent inhibition of average radial growth of isolates of Endothia species and Cryphonectria cubensis cultured on cycloheximide-amended potato dextrose agar at 25°C for seven days (Test 4).

Species	Isolate	Radial Growth (mm)						Percent Inhibition[a]				
		Concentration (µg/ml)						Concentration (µg/ml)				
		0	0.01	0.1	1	10	100	0.01	0.1	1	10	100
E. radicalis	E16	39.0[b]	39.0	27.7	8.0	2.0	0.0	0.0	29.0	79.5	94.9	100.0
" "	E42	42.0	41.7	34.0	20.3	0.0	0.0	0.7	19.0	51.7	100.0	100.0
" "	E56	34.0	33.0	25.7	7.0	0.0	0.0	2.9	24.4	79.4	100.0	100.0
" "	E64	16.0	16.3	11.0	2.7	0.0	0.0	-1.9[c]	1.2	83.1	100.0	100.0
" "	E76	33.0	33.0	28.3	2.7	0.0	0.0	0.0	14.2	68.8	91.8	100.0
" "	E92	8.7	10.3	6.0	0.0	0.0	0.0	-18.4	31.0	100.0	100.0	100.0
" "	E150	44.7	46.7	39.3	18.3	0.0	0.0	-4.5	12.1	59.1	100.0	100.0
E. macrospora	E54	25.0	30.3	23.0	17.3	3.7	0.0	-21.1	8.0	30.8	85.2	100.0
E. japonica	E53	32.0	30.0	29.0	17.3	0.0	0.0	6.2	9.4	45.9	100.0	100.0
	E59	37.3	37.3	35.0	16.7	0.0	0.0	0.0	6.2	55.2	100.0	100.0
E. tropicalis	E57	16.0	17.7	18.3	7.3	0.0	0.0	-10.6	-14.4	54.4	100.0	100.0
	E70	24.0	24.7	21.7	6.7	0.0	0.0	-2.9	9.6	72.1	100.0	100.0
E. viridistroma	E41	78.7	78.7	70.3	43.7	2.7	0.0	0.0	10.7	44.5	96.6	100.0
	E82	76.3	74.7	60.3	35.0	0.0	0.0	2.1	21.0	54.1	100.0	100.0
E. eugeniae	E138	58.7	57.7	55.0	31.7	73.0	0.0	1.7	6.3	46.0	87.6	100.0
" "	E139	36.0	40.3	41.3	22.3	9.3	0.0	-11.9	-14.7	38.1	74.2	100.0
	H184	66.0	74.0	65.3	38.0	11.3	0.0	-12.1	1.1	42.4	82.9	100.0

Table 45 (continued)

Species	Isolate	Radial Growth (mm) Concentration (µg/ml) 0	0.01	0.1	1	10	100	Percent Inhibition[a] Concentration (µg/ml) 0.01	0.1	1	10	100
E. eugeniae (continued)	H186	12.0	15.0	17.7	13.3	3.3	0.0	-25.0	-47.5	-10.8	72.5	100.0
C. cubensis	H151	64.3	64.7	64.3	51.7	11.3	0.0	-0.6	0.0	19.6	82.4	100.0
" "	H154	62.2	69.7	65.0	28.7	2.3	0.0	-12.4	-4.8	53.7	96.3	100.0
	H182	30.7	23.0	8.0	0.0	0.0	0.0	25.1	73.9	100.0	100.0	100.0
E. havanensis	E40	58.7	58.0	53.3	26.7	5.0	0.0	1.2	9.2	54.5	91.5	100.0
" "	E158	51.0	40.0	22.7	3.7	0.0	0.0	21.6	55.5	92.7	100.0	100.0
" "	E159	41.3	30.7	17.3	2.7	0.0	0.0	25.7	58.1	93.5	100.0	100.0
	H183	30.7	32.3	31.7	24.7	7.3	0.0	-5.2	-3.2	19.5	76.2	100.0

[a]Percent inhibition was calculated from radial growth based on the following formula: Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average of three colonies.

[c]Negative values of percent inhibition indicate growth stimulation.

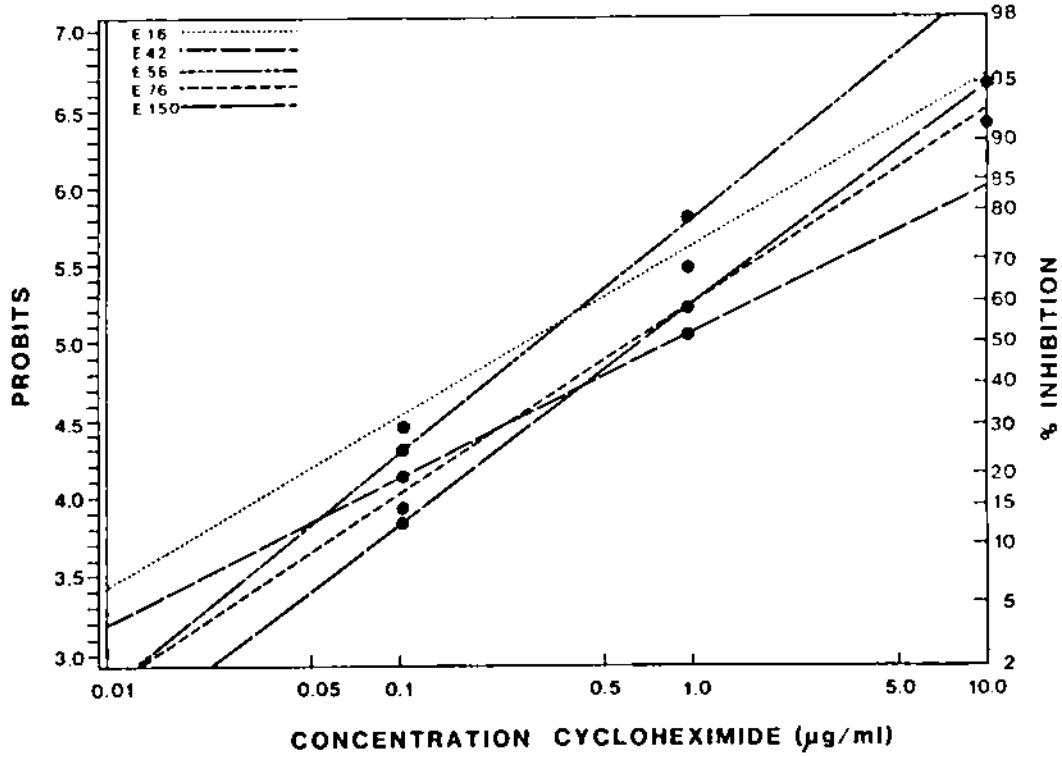


Figure 95. Dosage-response curve of E. radicalis grown on cycloheximide-amended potato dextrose agar at 25 °C for 7 d; average of three replicate plates per isolate. Linear regression with log - probit transformation plotted by Statistical Analysis Systems (SAS). Test #4. Concentration plotted on a logarithmic scale.

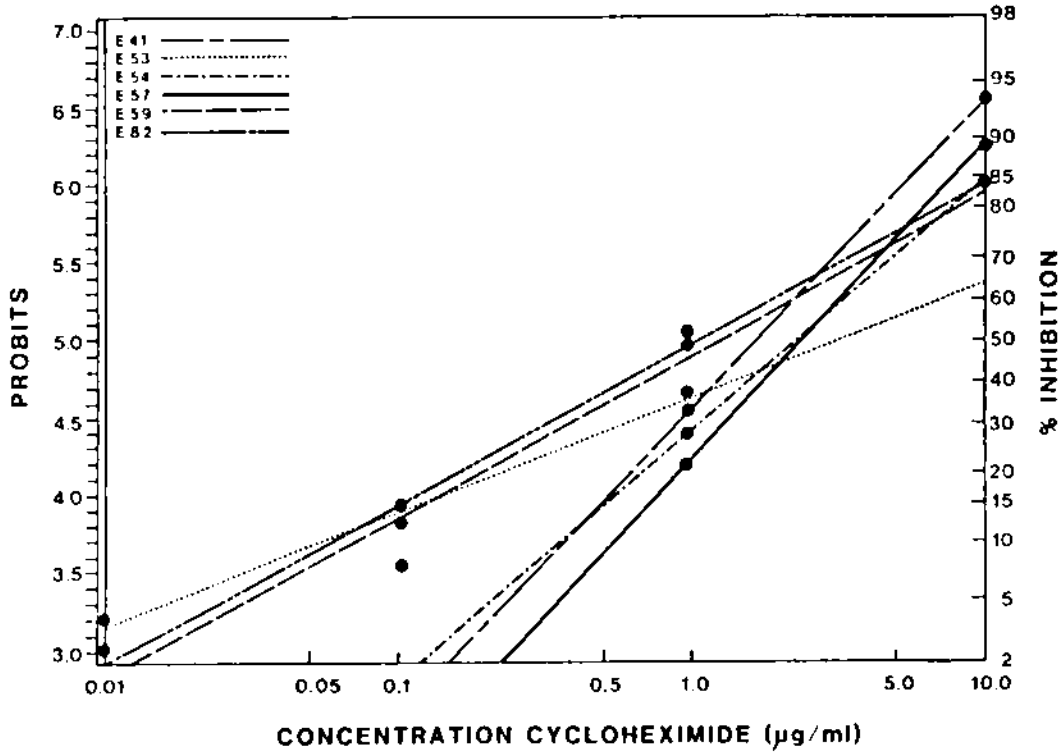


Figure 96. Dosage-response curve of Endothia viridistroma (E41, E82), E. japonica (E53, E59), E. macrospora (E54) and E. tropicalis (E57) grown on cycloheximide-amended potato dextrose agar at 25 °C for 7 d; average of three replicate plates per isolate. Linear regression with log - probit transformation plotted by Statistical Analysis Systems (SAS). Test #4. Concentration plotted on a logarithmic scale.

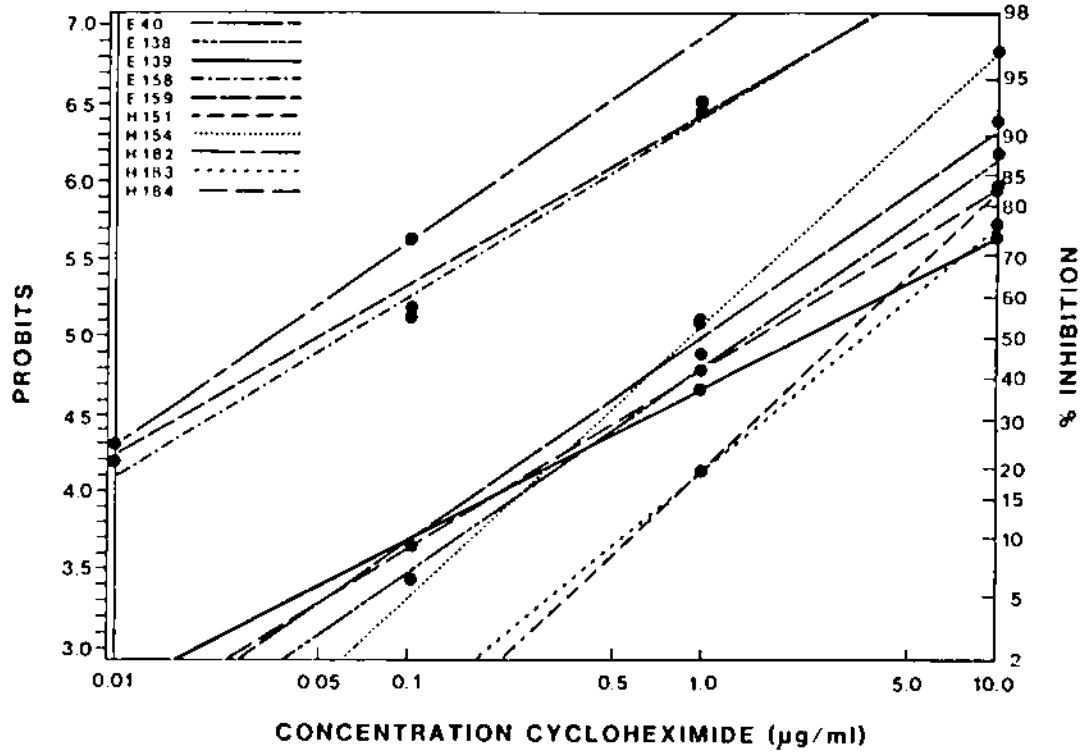


Figure 97. Dosage-response curve of Endothia havanensis (E40, E158, E159, H183), E. eugeniae (E138, E139, H184) and Cryphonectria cubensis (H151, H154, H182) grown on cycloheximide-amended potato dextrose agar at 25 °C for 7 d; average of three replicate plates per isolate. Linear regression with log - probit transformation plotted by Statistical Analysis Systems (SAS). Test #4. Concentration plotted on a logarithmic scale.

Table 46. Average radial growth and percent inhibition of average radial growth of isolates of Endothia species and Cryphonectria cubensis cultured on cycloheximide-amended potato dextrose agar at 25°C for 14 days. (Test #4).

Species	Isolate	Radial Growth (mm)						Percent Inhibition[a]				
		Concentration (µg/ml)						Concentration (µg/ml)				
		0	0.01	0.1	1	10	100	0.01	0.1	1	10	100
E. radicalis	E16	78.0[b]	78.0	59.0	21.7	3.3	0.0	0.0	24.3	72.2	95.8	100.0
" "	E42	78.0	78.0	65.3	36.0	2.7	0.0	0.0	16.3	53.8	96.5	100.0
" "	E56	77.3	76.0	61.7	23.7	0.0	0.0	1.7	20.2	69.3	100.0	100.0
" "	E64	35.7	34.7	29.0	11.3	0.5	0.0	2.8	18.8	68.3	98.6	100.0
" "	E76	65.0	64.0	55.0	23.3	3.7	0.0	1.5	15.4	64.1	94.3	100.0
" "	E92	19.0	22.0	13.7	1.0	0.0	0.0	-15.8[c]	27.9	94.7	100.0	100.0
" "	E150	78.0	78.0	78.0	48.7	2.7	0.0	0.0	0.0	37.6	96.5	100.0
E. macrospora	E54	59.0	73.7	61.7	36.0	11.0	0.0	-24.9	-4.6	39.0	81.4	100.0
E. japonica	E53	66.0	63.3	60.7	42.7	1.2	0.0	4.1	8.0	35.3	98.2	100.0
	E59	76.0	74.3	69.0	35.0	1.0	0.0	2.2	9.2	53.9	98.7	100.0
E. tropicalis	E57	37.0	40.3	42.3	24.7	0.3	0.0	-8.9	-14.3	33.2	99.2	100.0
	E70	45.7	47.7	45.3	18.0	0.0	0.0	-4.4	8.7	60.6	100.0	100.0
E. viridistroma	E41	78.0	78.0	78.0	78.0	10.3	0.0	0.0	0.0	0.0	86.8	100.0
	E82	78.0	78.0	78.0	74.3	0.7	0.0	0.0	0.0	4.7	99.1	100.0

Table 46 (continued)

Species	Isolate	Radial Growth (mm)						Percent Inhibition[a]				
		Concentration (µg/ml)						Concentration (µg/ml)				
		0	0.01	0.1	1	10	100	0.01	0.1	1	10	100
E. eugeniae	E138	78.0	78.0	78.0	78.0	31.7	0.0	0.0	0.0	0.0	59.4	100.0
" "	E139	78.0	78.0	78.0	54.7	21.0	1.0	0.0	0.0	29.9	73.1	98.7
" "	H184	78.0	78.0	78.0	78.0	32.0	0.0	0.0	0.0	0.0	59.0	100.0
" "	H186	35.0	44.7	63.0	42.0	8.0	0.0	-27.7	-80.0	-20.0	77.1	100.0
C. cubensis	H151	78.0	78.0	78.0	78.0	38.7	0.0	0.0	0.0	0.0	50.4	100.0
" "	H154	78.0	78.0	78.0	78.0	17.0	0.0	0.0	0.0	0.0	78.2	100.0
" "	H182	63.3	50.0	19.7	0.7	0.0	0.0	26.8	71.2	99.0	100.0	100.0
E. havanensis	E40	78.0	78.0	78.0	62.7	9.7	0.0	0.0	0.0	19.6	87.6	97.0
" "	E158	78.0	78.0	50.3	12.0	0.0	0.0	0.0	35.5	84.6	100.0	100.0
" "	E159	78.0	73.0	41.3	8.7	0.0	0.0	6.4	47.0	88.8	100.0	100.0
" "	H183	77.3	76.3	75.7	60.0	18.7	0.0	1.3	2.1	22.4	75.8	100.0

[a]Percent inhibition was calculated from radial growth based on the following formula: Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average of three colonies.

[c]Negative values of percent inhibition indicate growth stimulation.

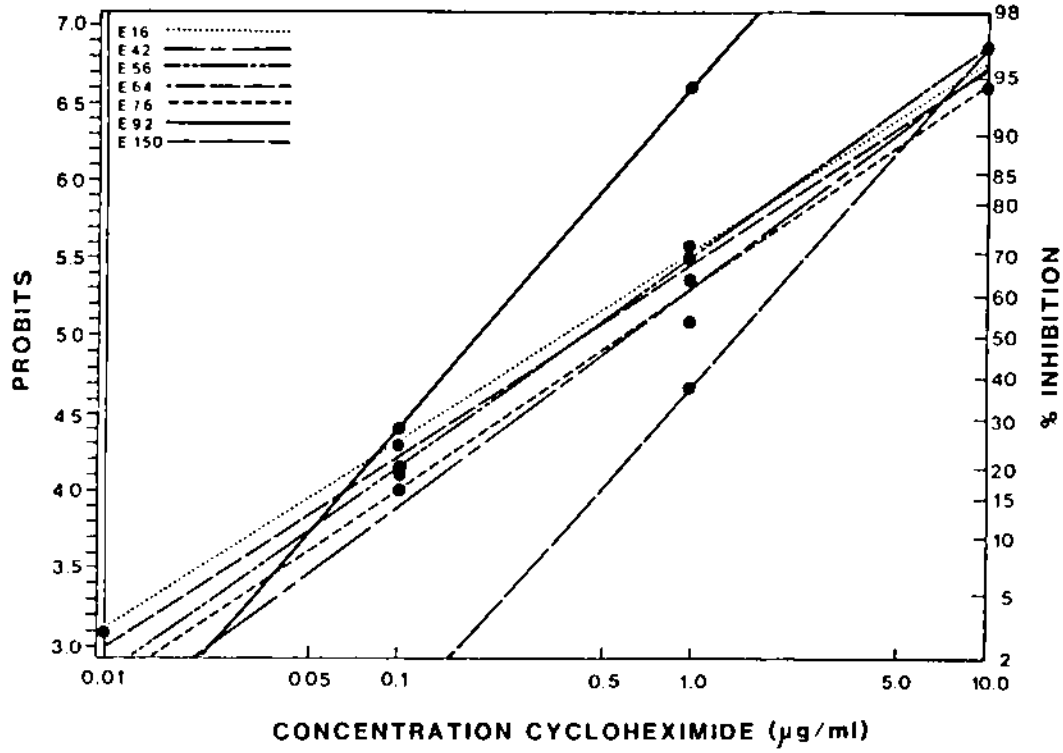


Figure 98. Dosage-response curve of E. radicalis grown on cycloheximide-amended potato dextrose agar at 25 °C for 14 d; average of three replicate plates per isolate. Linear regression with log - probit transformation plotted by Statistical Analysis Systems (SAS). Test #4. Concentration plotted on a logarithmic scale.

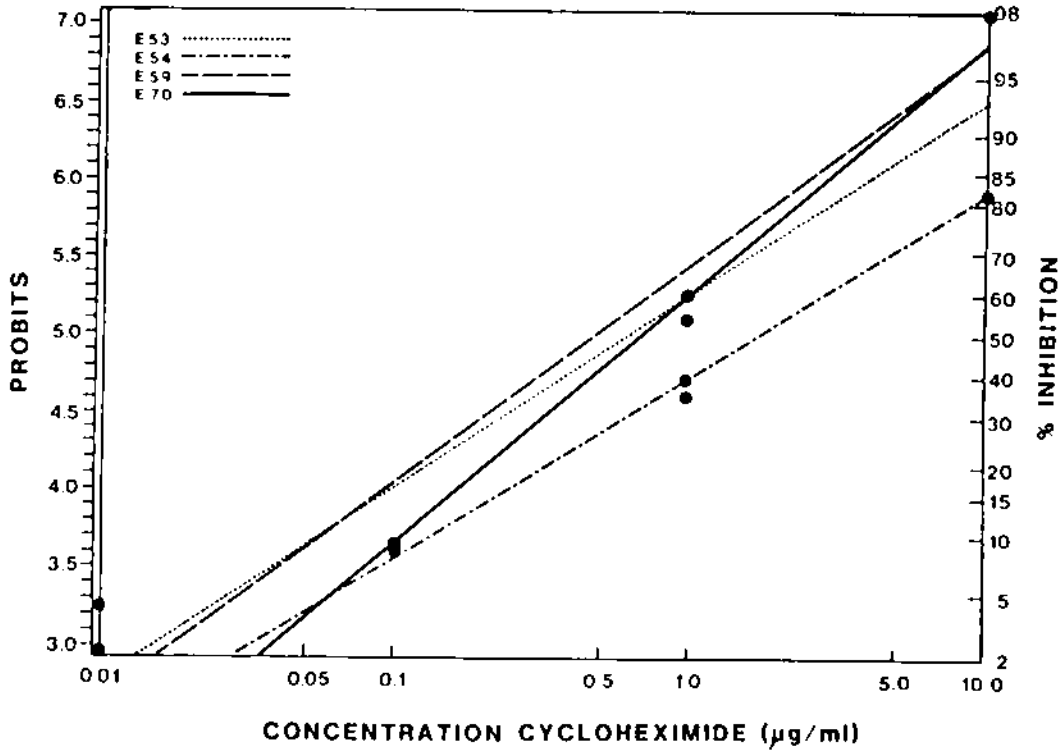


Figure 99. Dosage-response curve of Endothia japonica (E53, E59), E. macrospora (E54) and E. tropicalis (E70) grown on cycloheximide-amended potato dextrose agar at 25 °C for 14 d; average of three replicate plates per isolate. Linear regression with log - probit transformation plotted by Statistical Analysis Systems (SAS). Test #4. Concentration plotted on a logarithmic scale.

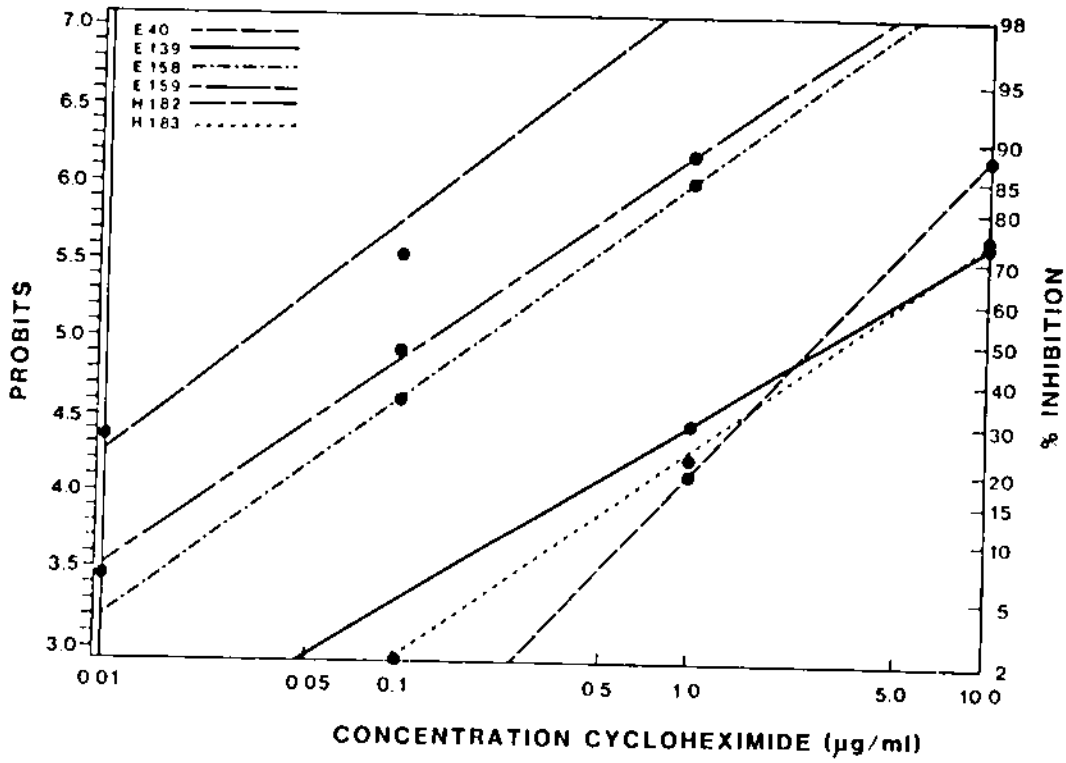


Figure 100. Dosage-response curve of Endothia havanensis (E40, E158, E159, H183), E. eugeniae (E139) and Cryphonectria cubensis (H182) grown on cycloheximide-amended potato dextrose agar at 25 °C for 14 d; average of three replicate plates per isolate. Linear regression with log - probit transformation plotted by Statistical Analysis Systems (SAS), Test #4. Concentration plotted on a logarithmic scale.

organisms is not certain due the lack of reliable reference strains of this species and the absence of perithecia and ascospores in the field collection. They may actually be members of E. gyrosa; their extreme sensitivity to cycloheximide would support this hypothesis. Endothia gyrosa was recently reported on eucalyptus in Australia (K. M. Old, personal communication) so species identification by host is not possible. Electrophoretic studies with general protein and specific activity stains do not support the hypothesis that these isolates are actually E. gyrosa; the banding patterns between these two groups were not similar (average similarity coefficient with the general protein stain = 0.14). Fresh isolates of E. havanensis would be needed to determine whether this species is especially sensitive to cycloheximide. If these isolates are legitimate members of this species, it would not support the conspecificity of E. havanensis and E. tropicalis since the latter was much more resistant to the compound (ED_{50} = 0.5 - 3.0 µg/ml). The other two isolates of E. havanensis used in this study, E40 and H183, were less sensitive to cycloheximide (ED_{50} = 0.8 - 1.4 µg/ml and 3.5 µg/ml for E40 and H183 respectively). These particular isolates produced electrophoretic patterns similar to those of the E. eugeniae - C. cubensis group, so their identity is also suspect. Isolate H182, originally identified as C. cubensis, was extremely sensitive to cycloheximide (ED_{50} = 0.03 µg/ml). This isolate was shown to have a protein profile unlike other members of the E. eugeniae -

C. cubensis group and was probably misidentified or contaminated in culture.

Several isolates appeared to be more resistant to cycloheximide at 14 d than at 7 d. This is reflected in the diverse ED_{50} values of E150, E40, and E82 produced at the two different dates. In several cases, dosage-response curves could not be analyzed on a log - probit scale due to growth stimulation or complete inhibition of growth; such values cannot be included in this transformation. This was especially common at 14 d when the radial growth of certain isolates ranged from no inhibition at low concentrations to complete inhibition at higher levels; there were no readings in the center of the scale. ED_{50} values were not calculated from such data due to insufficient data points. It would be necessary to expand the concentrations used in certain portions of the scale to obtain an accurate dosage-response curve. The data in this study should be considered preliminary; more work needs to be done with more concentrations and greater numbers of isolates per species to obtain curves as detailed as those of E. parasitica and E. gyrosa. Standard deviations of the mean were not included in these dosage-response curves since individual isolates, rather than groups of isolates representing one species, were being depicted.

Although the ED_{50} values were somewhat variable between tests, they can be used to divide the genus into two groups based on cycloheximide-sensitivity. The natural dividing line seems to be at 0.1 µg/ml. The species can be assigned as such:

Sensitive	Insensitive
E. gyrosa	E. parasitica
E. singularis	E. radicalis
Isolates E158 and E159 (= E. havanensis?)	E. tropicalis
	E. macrospora
	E. viridistroma
	E. coccolobii
	E. eugeniae
	C. cubensis

Abnormal pigment production by several of these cultures was also noted on the cycloheximide-amended medium. Perilla purple was produced in isolates of E. radicalis (E16, E42, E56, E92, E150 and E152) and E. singularis (E58), while a dark brown to black coloration was associated with isolates of E. japonica (E59), E. eugeniae (H186) and a culture labelled E. havanensis, but which probably belongs to the E. eugeniae-C. cubensis group (H183). Additional yellow pigments were observed in isolates of E. eugeniae (E138, E139, H184, H186), a reported isolate of C. cubensis which is probably misidentified (H182), E. radicalis (E42, E56) and two of the suspected isolates of E. havanensis which seem to belong to the E. eugeniae-C. cubensis group (E40, H183). One of the unidentified Florida isolates (E158), which may be E. havanensis, also produced a yellow pigment. In other isolates, pigment production was inhibited at 1 µg/ml; this occurred among isolates of E. coccolobii (E157), E. viridistroma (E41), E. macrospora (E54), C. cubensis (H154) and E. radicalis (E76).

Another alteration in colony morphology associated with the

presence of cycloheximide was a decrease in the number of pycnidia produced in culture. This was observed among isolates of E. radicalis (E42, E64), E. tropicalis (E57, E70), E. coccolobii (E157) and the E. eugeniae - C. cubensis group (H151, H154). The concentration which elicted this response varied among the different isolates. Some cultures were quite sensitive and displayed decreased pycnidial production at 0.01 µg/ml (E42), while others required up to 10 µg/ml (H151) for the same affect. In a few instances, high concentrations of cycloheximide (1 µg/ml) resulted in irregularities in growth patterns, as in the production of sectors (E139) or the formation of a thin, web-like mycelium (H151, H154, H184). In most cases, the growth of the colony was quite regular, although greatly inhibited. The presence of cycloheximide radically altered the metabolism of all these fungi.

None of the other fungitoxicants elicited such drastic differences in growth among isolates of E. parasitica and E. gyrosa. Several compounds induced differential sensitivies over at least a portion of the dosage-response curve; this was observed for candicidin (Table 47, Figure 101), MBC phosphate (Table 48, Figure 102), thiabendazole hypophosphite (Table 49, Figure 103) and chlorothalonil (Table 50, Figure 104). Differences were extremely limited, however, and could only be detected by taking measurements and plotting the response curves; they would not be useful in a clinical situation. These tests

Table 47. Average radial growth and percent inhibition of radial growth of isolates of *E. parasitica* and *E. gyrosa* cultured on candicidin-amended potato dextrose agar at 25°C for eight days.

Species	Isolate	Radial Growth (mm)						Percent Inhibition[b]				
		Concentration (µg/ml)						Concentration (µg/ml)				
		0	1	5	10	50	100	1	5	10	50	100
E. parasitica	E87	37.0[b]	12.5	7.0	7.0	2.5	1.5	66.2	81.1	81.1	93.2	95.9
" "	E88	51.0	20.5	9.0	5.5	3.0	0.0	59.8	82.3	89.2	94.1	100.0
" "	E107	43.0	15.5	5.5	4.0	2.0	1.5	64.0	87.2	90.7	95.3	96.5
" "	E137	38.0	13.0	7.0	5.0	2.0	0.0	65.8	81.6	86.8	94.7	100.0
" "	E153	42.5	13.5	8.5	5.5	1.0	2.0	68.2	80.0	87.0	97.6	95.3
Species average		42.3	15.0	7.4	5.4	2.1	1.0	64.8	82.4	87.0	95.0	97.5
Standard error		5.5	3.3	1.4	1.1	0.7	0.9	3.2	2.8	3.6	1.7	2.3
E. gyrosa	E18	21.7	3.5	1.5	2.0	2.0	2.0	83.9	93.1	90.8	90.8	90.8
" "	E38	21.0	1.0	2.0	1.5	2.0	2.5	95.2	90.5	92.9	90.5	88.1
" "	E48	10.5	3.0	1.5	1.0	1.0	1.5	71.4	85.7	90.5	90.5	85.7
" "	E98	25.0	2.0	2.0	1.5	1.5	2.5	92.0	92.0	94.0	94.0	90.0
" "	E154	28.0	1.5	1.0	1.5	2.0	3.0	94.6	96.4	94.6	92.9	89.3
Species average		21.2	2.2	1.6	1.6	1.7	2.3	87.4	91.5	92.6	91.7	88.8
Standard error		6.6	1.0	0.4	0.4	0.4	0.6	10.0	3.9	1.8	1.6	2.0

Table 47 (continued)

[a]Percent inhibition was calculated from radial growth based on the following formula: Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average of two colonies.

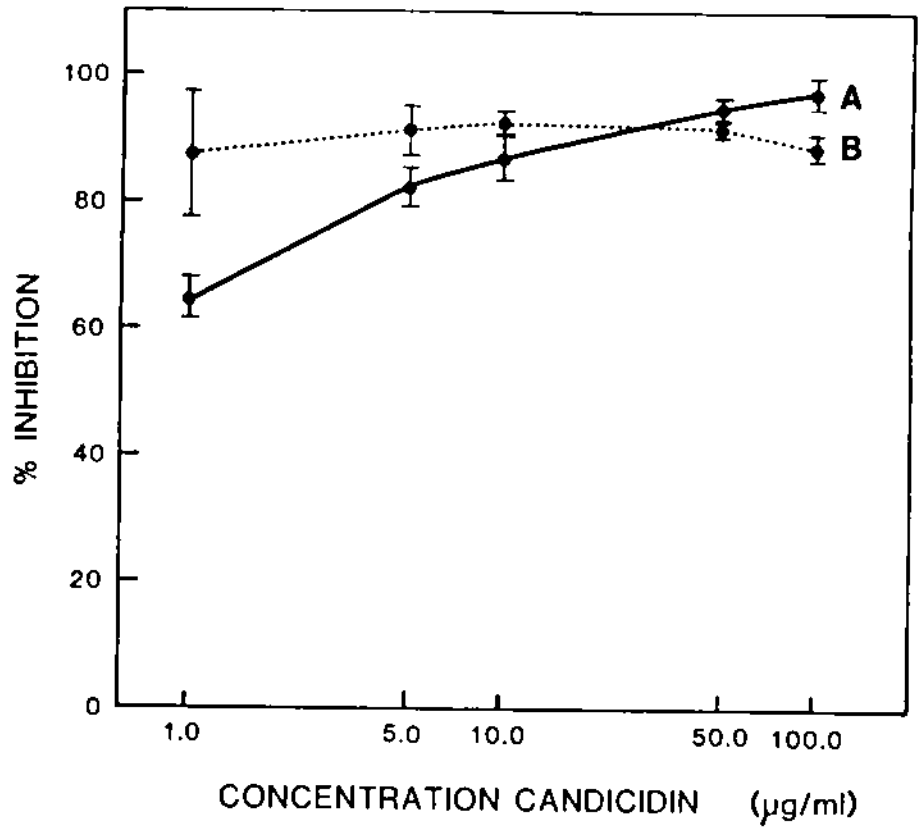


Figure 101. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on candicidin-amended potato dextrose agar at 25 °C for 8 d. Average of five isolates with two replicate plates per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 48. Average radial growth and percent inhibition of average radial growth of E. parasitica and E. gyrosa cultured on MBC phosphate-amended potato dextrose agar at 25°C for eight days.

Species	Isolate	Radial Growth (mm)						Percent Inhibition[a]				
		Concentration (µg/ml)						Concentration (µg/ml)				
		0	0.01	0.1	1.0	5.0	10.0	0.01	0.1	1.0	5.0	10.0
E. parasitica	E85	51.5[b]	51.0	30.5	0.0	0.0	0.0	1.0	40.8	100.0	100.0	100.0
" "	E87	52.0	47.5	30.5	0.0	0.0	0.0	8.6	41.3	100.0	100.0	100.0
" "	E88	56.5	56.5	39.0	0.0	0.0	0.0	0.0	31.0	100.0	100.0	100.0
" "	E95	57.2	54.5	39.8	0.0	0.0	0.0	4.7	30.4	100.0	100.0	100.0
" "	E96	52.2	52.8	33.8	0.0	0.0	0.0	-1.1	35.2	100.0	100.0	100.0
Species average		53.9	52.5	34.7	0.0	0.0	0.0	2.6	35.7	100.0	100.0	100.0
Standard error		2.7	3.4	4.5	0.0	0.0	0.0	4.0	5.2	0.0	0.0	0.0
E. gyrosa	E22	30.5	21.5	29.0	--[c]	0.0	0.0	29.5	4.9	--	100.0	100.0
" "	E51	26.0	25.0	21.5	0.0	0.0	0.0	3.8	17.3	100.0	100.0	100.0
" "	E72	20.5	21.0	19.5	0.0	0.0	0.0	-2.4	4.9	100.0	100.0	100.0
" "	E74	21.5	18.0	20.5	0.0	0.0	0.0	16.3	4.6	100.0	100.0	100.0
Species average		24.6	21.4	22.6	0.0	0.0	0.0	11.8	7.9	100.0	100.0	100.0
Standard error		4.6	2.9	4.3	0.0	0.0	0.0	14.1	6.2	0.0	0.0	0.0

Table 48 (continued)

[a]Percent inhibition was calculated from radial growth based on the following formula:
Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average measurment of two colonies

[c]Data missing due to contamination.

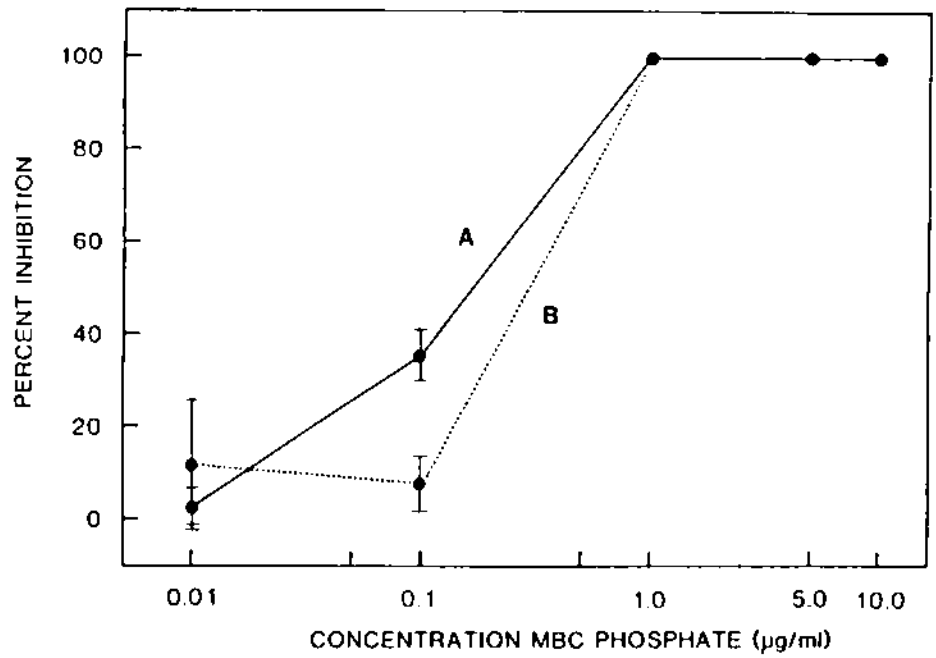


Figure 102. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on MBC phosphate-amended potato dextrose agar at 25 °C for 8 d. Average of five isolates of E. parasitica and four isolates of E. gyrosa ; two replicate plates per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 49. Average radial growth and percent inhibition of average radial growth of isolates of E. parasitica and E. gyrosa on thiabendazole hypophosphite-amended potato dextrose agar at 25°C for eight days.

Species	Isolate	Radial Growth (mm)							Percent Inhibition[a]					
		Concentration (µg/ml)							Concentration (µg/ml)					
		0	0.1	0.25	0.5	0.75	1	5	0.1	0.25	0.5	0.75	1	5
E. parasitica	E24	54.3[b]	55.0	55.0	39.0	18.3	14.3	0.0	-1.3[c]	-1.3	28.2	66.3	73.7	100.0
" "	E85	48.7	47.3	50.7	33.7	16.3	12.7	0.0	2.9	-4.1	30.8	66.5	73.9	100.0
" "	E86	45.7	48.0	47.0	31.7	17.3	12.7	0.0	-5.0	-2.8	30.6	62.1	72.2	100.0
" "	E87	40.0	45.0	43.0	29.0	20.1	13.3	0.0	-12.5	-7.5	27.5	49.8	66.8	100.0
" "	E88	62.7	61.0	63.0	48.3	17.7	3.0	0.0	2.7	-0.5	23.0	71.8	95.2	100.0
" "	E89	57.7	55.0	57.0	45.0	15.0	0.0	0.0	4.7	1.2	22.0	74.0	100	100.0
" "	E95	60.3	60.0	60.3	52.0	21.7	14.3	0.0	0.5	0	13.8	64.0	76.3	100.0
" "	E107	41.7	43.0	46.3	38.3	20.3	18.3	0.0	-3.1	-11.0	8.2	51.3	56.1	100.0
" "	E108	44.7	52.7	50.7	35.0	17.3	10.7	0.0	-17.9	-13.4	21.7	61.3	76.1	100.0
" "	E137	38.0	36.3	35.3	16.7	15.0	9.3	0.0	4.5	7.1	56.0	60.5	75.5	100.0
Average		49.4	50.3	50.8	36.9	17.9	10.9	0.0	-2.4	-3.2	26.2	62.8	76.6	100.0
Standard error		8.8	7.8	8.4	10.2	2.2	5.5	0.0	7.5	6.1	12.7	7.8	12.7	0.0
E. gyrosa	E18	33.3	35.0	33.3	31.0	22.3	17.3	2.3	-5.1	0.0	6.9	33.0	48.0	93.1
" "	E20	34.0	36.5	24.7	20.7	16.7	12.7	1.7	-7.3	27.4	39.1	50.9	62.6	95.0
" "	E30	25.0	24.7	23.3	23.3	19.0	17.0	0.7	1.2	6.8	6.8	24.0	32.0	97.2
" "	E37	25.0	24.7	26.7	23.0	19.7	16.0	3.7	1.2	-6.8	8.0	21.2	36.0	85.2

Table 49 (continued)

Species	Isolate	Radial Growth (mm)							Percent Inhibition[a]					
		Concentration (µg/ml)							Concentration (µg/ml)					
		0	0.1	0.25	0.5	0.75	1	5	0.1	0.25	0.5	0.75	1	5
E. gyrosa (continued)	E38	31.7	28.3	27.0	30.0	18.3	15.0	1.0	10.7	14.8	5.4	42.3	52.7	96.8
" "	E48	22.7	22.3	24.7	22.0	16.7	13.7	0.0	1.8	-8.8	3.1	26.4	39.6	100.0
" "	E51	35.0	31.7	31.7	26.3	23.0	19.5	2.7	9.4	9.4	24.8	34.3	44.3	92.3
" "	E72	25.0	25.7	26.3	21.0	17.7	13.3	2.3	-2.8	-5.2	16.0	29.2	46.8	90.8
" "	E74	24.0	23.3	24.7	23.7	21.3	14.7	1.0	2.9	-2.9	1.2	11.2	38.8	95.8
" "	E98	34.3	33.7	34.3	31.7	25.7	16.3	3.0	1.7	0.0	7.6	25.1	52.5	91.3
Average		29.0	28.6	27.7	25.3	20.0	15.6	1.8	1.4	3.5	11.9	29.8	45.5	93.8
Standard error		5.0	5.2	3.9	4.2	3.0	2.1	1.2	5.7	11.1	11.7	11.1	9.2	4.1

[a]Percent inhibition was calculated from average radial growth by the formula: Percent inhibition = (1 - ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average of three colonies.

[c]Negative values of percent inhibition indicate growth stimulation.

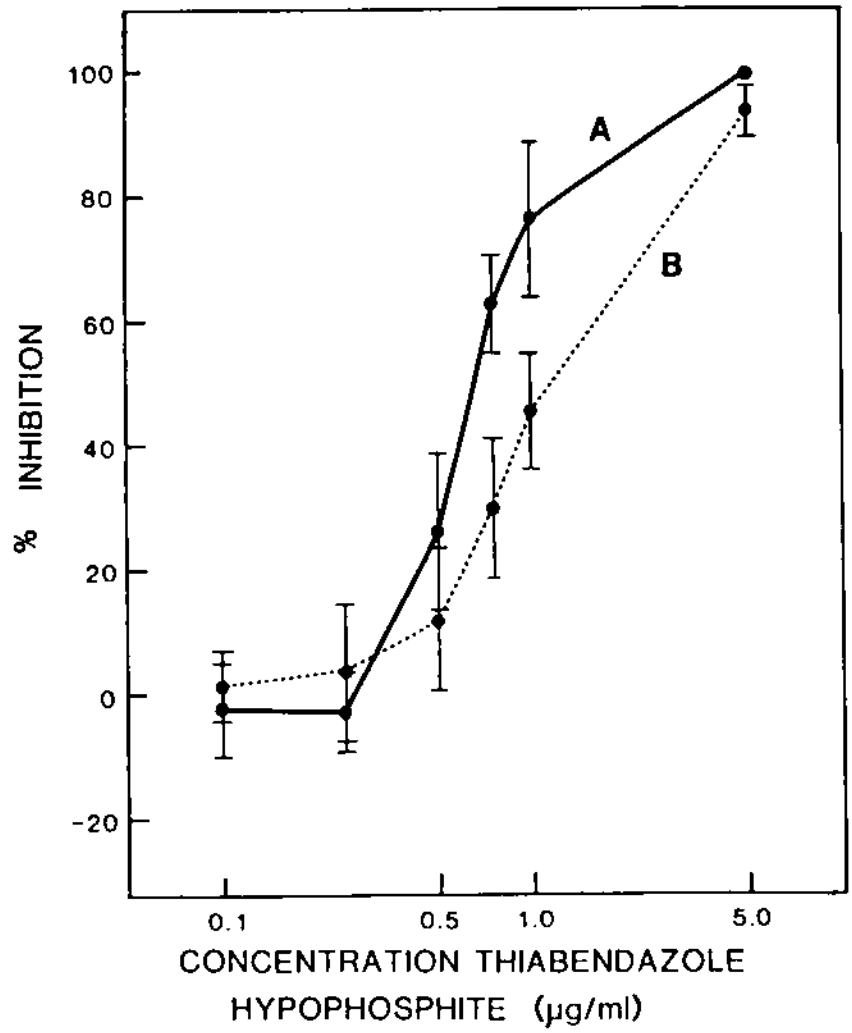


Figure 103. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on thiabendazole hypophosphite-amended potato dextrose agar at 25 °C for 8 d. Average of ten isolates with three replicate plates per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 50. Average radial growth and percent inhibition of average radial growth of E. parasitica and E. gyrosa cultured on chlorothalonil-amended potato dextrose agar at 25°C for eight days.

Species	Isolate	Radial Growth (mm)						Percent Inhibition[a]				
		Concentration (μg/ml)						Concentration (μg/ml)				
		0	0.5	1.0	5.0	10.0	50.0	0.5	1.0	5.0	10.0	50.0
E. parasitica	E86	30[b]	22	21	22	20	16	27	30	27	33	47
" "	E87	27	21	22	20	19	14	22	18	26	30	48
" "	E88	30	24	24	30	26	13	20	20	0	13	57
" "	E95	29	28	26	29	18	17	3	10	0	38	41
Species average		29	24	23	25	21	15	18	20	13	28	48
Standard error		1	3	2	5	4	2	10	8	15	11	6
E. gyrosa	E98	20	13	9	11	11	0	35	55	45	45	100
" "	E154	34	14	13	13	8	6	59	62	62	76	82
Species average		27	14	11	12	10	3	47	58	53	61	91
Standard error		10	1	3	1	2	4	17	5	12	22	12

Table 50 (continued)

[a]Percent inhibition was calculated from radial growth based on the following formula: Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Measurement of one colony.

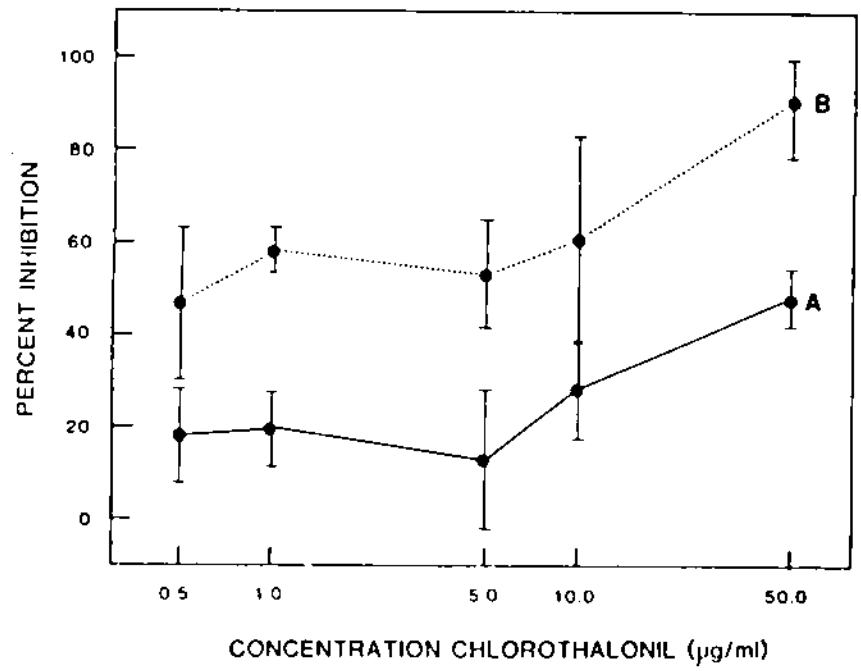


Figure 104. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on chlorothalonil-amended potato dextrose agar at 25 °C for 8 d. Average of four isolates of E. parasitica and two isolates of E. gyosa; one plate per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

were not replicated due to the marginal amount of selectivity. Amphotericin B (Table 51, Figure 105) and dicloran (Tables 53 - 54, Figures 107 - 108) produced promising initial results; this differential selectivity was not maintained when the tests were repeated (Tables 52 and 55, Figures 106 and 107). Endothia parasitica and E. gyrosa also responded differently to hydroquinone (Table 56, Figure 110); E. parasitica was weakly inhibited at 100 and 500 µg/ml, while the growth of E. gyrosa was stimulated at these concentrations. There was a large amount of variability among the individual isolates, however, and the accompanying standard deviations of the mean were quite large. Another phenolic compound, o-phenyl phenol, was also tested (Table 57, Figure 111); both species responded similarly to it. The test with hydroquinone was not replicated due to the extremely variable response.

The dosage-response curves for most of the fungitoxicants followed the sigmoid curve; this was expected since the data were plotted on a log - percent scale. Hydroquinone produced the most unusual response with high concentrations actually stimulating E. gyrosa. Abnormal curves were also obtained for chlortetracycline (Table 58, Figure 112), benzimidazole (Table 59, Figure 113) and fosetyl-Al (Table 60, Figure 114); these can be explained by the lack of toxicity of these compounds to the fungi. Fosetyl-Al rarely inhibits in vitro growth of most fungi (58, 62) and benzimidazole is not particularly fungitoxic (183). Chlortetracycline should affect the mitochondrial ribosomes; it may be

Table 51. Average radial growth and percent inhibition of average radial growth of isolates of E. parasitica and E. gyrosa cultured on amphotericin B-amended potato dextrose agar at 25°C for eight days. Test 1.

Species	Isolate	Radial Growth (mm)						Percent Inhibition[a]				
		Concentration (µg/ml)						Concentration (µg/ml)				
		0	0.1	1	10	50	100	0.1	1	10	50	100
E. parasitica	E86	45.0[b]	42.5	23.5	9.0	6.0	6.0	5.6	47.8	80.0	86.7	86.7
" "	E87	41.5	43.5	14.5	7.0	5.5	4.5	-4.8[c]	65.1	83.1	86.7	89.2
" "	E88	55.5	53.0	25.0	8.5	7.0	4.0	4.5	55.0	84.7	87.4	92.8
" "	E107	45.0	48.0	24.0	6.0	3.0	3.0	-6.7	46.7	86.7	93.3	93.3
" "	E155	48.0	46.5	23.5	7.5	6.0	4.5	3.1	51.0	84.4	87.5	90.6
Species average		47.0	46.7	22.1	7.6	5.5	4.4	0.3	53.1	83.8	88.3	90.5
Standard error		5.3	4.2	4.3	1.2	1.5	1.1	5.7	7.4	2.5	2.8	2.7
E. gyrosa	E30	24.0	21.0	2.5	1.0	1.0	1.0	12.5	89.6	95.8	95.8	95.8
" "	E37	25.0	19.0	2.5	0.0	1.0	0.5	24.0	90.0	100.0	96.0	98.0
" "	E48	22.0	22.5	6.0	1.0	1.0	1.5	-2.3	72.7	95.4	95.4	93.2
" "	E98	24.0	23.5	1.5	1.0	0.5	0.5	2.1	93.8	95.8	97.9	97.9
Species average		23.8	16.5	3.1	0.8	0.9	0.9	9.1	86.5	96.8	96.3	96.2
Standard error		1.2	9.5	2.0	0.5	0.2	0.5	11.7	9.4	2.2	1.1	2.3

Table 51 (continued)

[a]Percent inhibition was calculated from radial growth based on the following formula: Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average of two colonies.

[c]Negative values of percent inhibition indicate growth stimulation.

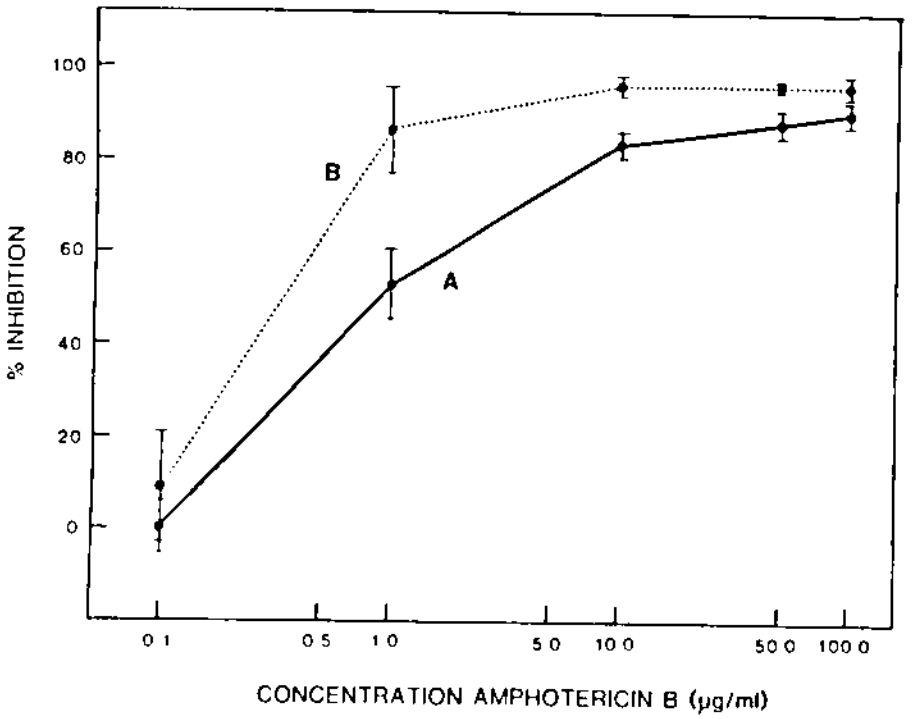


Figure 105. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on amphotericin B-amended potato dextrose agar at 25 °C for 8 d. Average of five isolates of E. parasitica and four isolates of E. gyosa; two replicate plates per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 52. Average radial growth and percent inhibition of average radial growth of isolates of E. parasitica and E. gyrosa cultured on amphotericin B-amended potato dextrose agar at 25°C for eight days. Test 2.

Species	Isolate	Radial Growth (mm)						Percent Inhibition[a]				
		Concentration (µg/ml)						Concentration (µg/ml)				
		0	0.1	0.5	1	5	10	0.1	0.5	1	5	10
E. parasitica	E24	50.7[b]	50.7	35.4	23.7	9.0	5.7	0.0	30.4	53.2	82.2	88.7
" "	E85	42.0	42.3	34.7	23.7	8.7	5.7	-7.1[c]	17.4	43.6	79.3	86.4
" "	E86	42.3	35.0	12.0	10.0	7.0	5.0	17.3	71.6	76.4	83.4	88.2
" "	E87	44.7	40.3	13.0	10.0	6.0	4.7	9.8	70.9	77.6	86.6	89.5
" "	E89	49.0	45.3	12.3	9.7	6.0	4.7	7.6	74.9	80.2	87.7	90.4
" "	E96	44.3	38.3	24.3	13.3	6.3	6.3	13.5	45.1	70.0	85.8	85.8
" "	E107	46.7	44.3	10.7	9.7	5.7	5.7	5.1	77.1	79.2	87.8	87.8
" "	E108	45.0	9.0	30.3	22.7	8.7	6.3	80.0	32.7	49.5	80.7	86.0
" "	E137	36.7	39.7	10.0	9.3	6.3	5.3	-8.2	72.7	74.7	82.8	85.6
" "	E153	46.0	42.7	13.0	9.3	8.0	6.0	7.2	71.7	79.8	82.6	87.0
Species average		44.7	38.8	19.6	14.1	7.2	5.5	12.5	56.4	68.4	83.9	87.5
Standard error		3.9	11.3	10.4	6.5	1.3	0.6	25.1	22.6	14.1	2.9	1.6
E. gyrosa	E18	20.3	19.0	9.0	4.0	4.3	4.5	6.4	55.7	80.3	78.8	77.8
" "	E20	15.7	15.3	6.7	2.3	1.0	1.0	2.5	57.3	85.4	93.6	93.6
" "	E30	21.3	22.7	8.3	5.3	1.7	3.0	-6.6	61.0	75.1	92.0	85.9
" "	E37	16.0	14.7	11.7	7.7	5.7	6.0	8.1	26.9	51.9	64.4	62.5
" "	E38	16.3	14.7	11.0	6.7	3.7	4.3	9.8	32.5	58.9	77.3	73.6
" "	E48	15.0	17.3	11.3	4.3	5.3	3.0	-15.3	24.7	71.3	64.7	80.0

Table 52 (continued)

Species	Isolate	Radial Growth (mm) Concentration (µg/ml) 0	0.1	0.5	1	5	10	Percent Inhibition[a] Concentration (µg/ml) 0.1	0.5	1	5	10
E. gyrosa	E50	37.0	34.3	11.0	5.7	6.0	4.0	7.3	70.3	84.6	83.8	89.2
" "	E51	21.0	17.0	10.0	6.3	5.0	4.0	19.0	52.4	70.0	76.2	80.9
" "	E98	17.3	17.3	11.7	6.7	2.3	3.7	0.0	32.4	61.3	86.7	78.6
" "	E154	18.0	19.3	8.7	5.3	3.3	1.3	-7.2	51.7	70.6	81.7	92.8
Species average		19.8	19.2	9.9	5.4	3.8	3.5	2.4	46.5	70.9	79.9	81.5
Standard error		6.5	5.8	1.7	1.6	1.7	1.5	10.0	16.0	11.1	9.9	9.4

[a]Percent inhibition was calculated from radial growth based on the following formula: Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average of three colonies.

[c]Negative values of percent inhibition indicate growth stimulation.

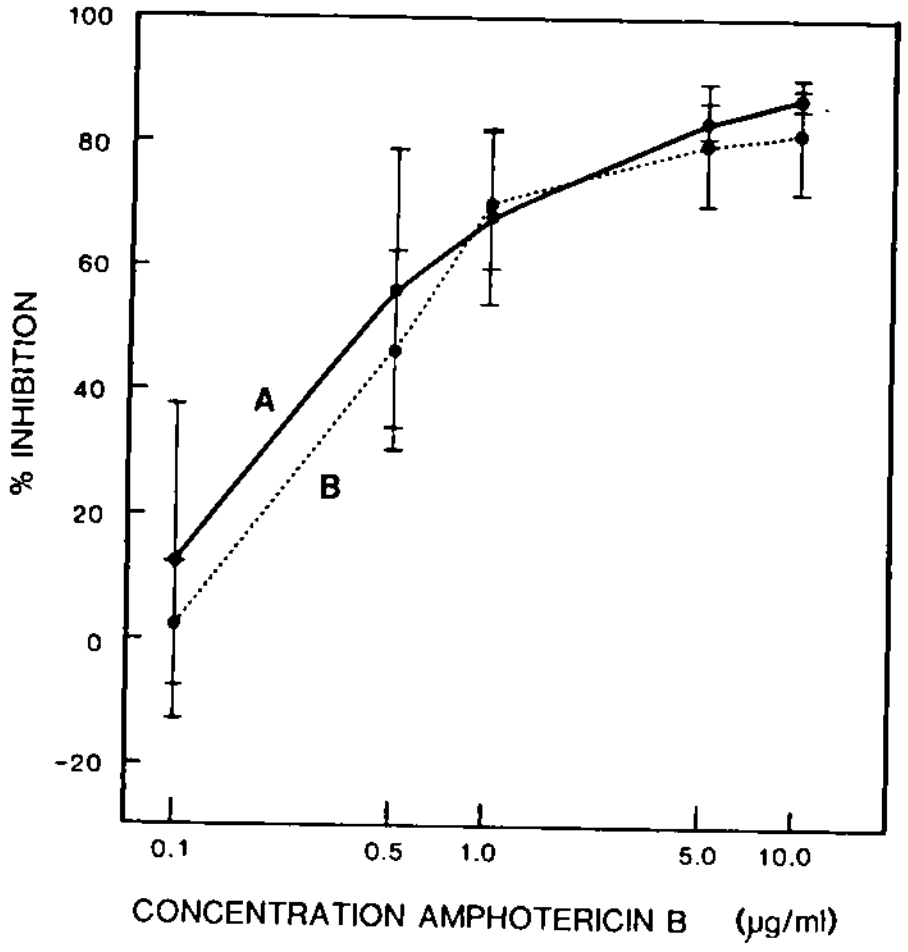


Figure 106. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on amphotericin B-amended potato dextrose agar at 25 °C for 8 d. Average of ten isolates with three replicate plates per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 53. Radial growth and percent inhibition of radial growth of isolates of E. parasitica and E. gyrosa cultured on dicloran-amended potato dextrose agar at 25°C for eight days. Test 1.

Species	Isolate	Radial Growth (mm) Concentration (µg/ml) 0	0.5	1	10	50	Percent Inhibition[a] Concentration (µg/ml) 0.5	1	10	50
E. parasitica	E24	41.0[b]	32.0	11.0	14.0	8.0	22.0	73.2	65.8	80.5
" "	E85	38.0	28.0	18.0	9.0	5.0	26.3	52.6	76.3	86.8
" "	E88	45.0	33.0	16.0	16.0	10.0	26.7	64.4	64.4	77.8
" "	E95	48.0	33.0	14.0	18.0	9.0	31.2	70.8	62.5	81.2
" "	E96	40.0	28.0	17.0	15.0	7.0	30.0	57.5	62.5	82.5
Species average		42.4	30.8	15.2	14.4	7.8	27.2	63.7	66.3	81.8
Standard error		4.0	2.6	2.8	3.4	1.9	3.6	8.7	5.8	3.3
E. gyrosa	E18	15.0	17.0	15.0	12.0	9.0	13.3[c]	0.0	20.0	40.0
" "	E37	18.0	14.0	13.0	12.0	7.0	22.2	27.8	33.3	61.1
	E48	19.0	12.0	11.0	10.0	5.0	36.8	42.1	47.4	73.7
	E98	22.0	21.0	17.0	11.0	9.0	4.5	22.7	50.0	59.1
Species average		18.5	16.0	14.0	11.2	7.5	12.6	23.2	37.7	58.5
Standard error		2.9	3.9	2.6	1.0	1.9	21.8	17.5	13.9	13.9

Table 53 (continued)

[a]Percent inhibition was calculated from radial growth based on the following formula: Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Measurement of one colony.

[c]Negative values of percent inhibition indicate growth stimulation.

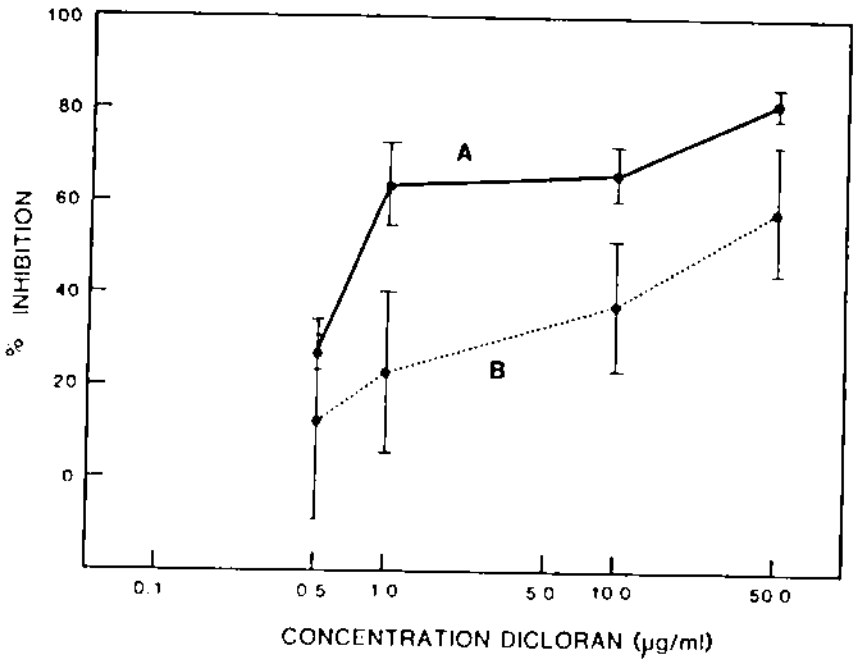


Figure 107. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on dicloran-amended potato dextrose agar at 25 °C for 8 d. Average of five isolates of E. parasitica and four isolates of E. gyosa; one plate per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 54. Average radial growth and percent inhibition of average radial growth of isolates of E. parasitica and E. gyrosa cultured on dicloran-amended potato dextrose agar at 25°C for eight days. Test 2.

Species	Isolate	Radial Growth (mm)						Percent Inhibition[a]				
		Concentration (µg/ml)						Concentration (µg/ml)				
		0	1	2.5	5	7.5	10	1	2.5	5	7.5	10
E. parasitica	E24	54.0[b]	46.0	35.3	27.7	19.5	15.0	14.8	34.6	48.7	63.9	72.2
" "	E85	49.3	45.0	33.3	27.7	21.0	16.0	8.7[c]	32.4	43.8	57.4	67.5
" "	E86	51.0	44.7	34.0	26.0	19.3	20.0	12.4	33.3	49.0	62.2	60.8
" "	E87	46.7	42.3	32.0	23.0	17.0	13.3	9.4	31.5	50.7	63.6	71.5
" "	E89	58.0	48.7	32.7	27.0	20.7	19.0	16.0	43.6	53.4	64.3	67.2
" "	E95	55.0	50.3	37.3	27.7	22.3	18.7	8.5	32.2	49.6	59.4	66.0
" "	E107	54.0	47.7	35.3	26.7	20.7	17.3	11.7	34.6	50.6	61.7	68.0
" "	E108	52.3	45.7	32.0	27.0	19.7	14.0	12.6	38.8	48.4	62.3	73.2
" "	E153	50.7	47.3	33.7	29.7	20.3	18.0	6.7	33.5	41.4	60.4	64.5
" "	E155	55.3	48.7	33.7	26.3	21.0	15.3	11.9	39.1	52.4	62.0	72.3
Species average		52.6	46.6	33.9	26.9	20.2	16.7	11.3	35.4	61.7	61.7	68.3
Standard error		3.3	2.4	1.6	1.7	1.4	2.3	2.9	3.9	2.2	2.2	4.0
E. gyrosa	E18	27.7	26.7	24.0	20.7	18.3	15.0	3.6	13.4	25.3	33.9	45.8
" "	E20	34.0	31.7	25.0	20.3	16.7	14.0	6.8	26.5	40.3	50.9	58.8
" "	E30	23.0	21.7	22.3	21.0	19.0	18.0	5.6	3.0	8.7	17.4	21.7
" "	E37	24.0	25.3	23.7	19.0	16.7	15.0	-5.4	1.2	20.8	30.4	37.5
" "	E48	23.3	23.7	23.5	18.3	16.3	16.0	-1.7	-0.9	21.5	30.0	31.3
" "	E50	40.3	38.0	30.3	25.3	22.3	21.3	5.7	24.8	37.2	44.7	47.1

Table 54 (continued)

Species	Isolate	Radial Growth (mm) Concentration (μg/ml) 0	1	2.5	5	7.5	10	Percent Inhibition[a] Concentration (μg/ml) 1	2.5	5	7.5	10
E. gyrosa (continued)	E51	33.3	30.7	25.3	18.3	16.0	15.3	7.8	24.0	45.0	51.9	54.0
" "	E98	26.7	27.3	26.3	21.3	19.6	15.3	-2.2	1.5	20.2	28.8	42.7
" "	E154	29.0	26.0	25.3	20.7	18.7	18.0	10.3	12.8	28.6	35.5	37.9
Species average		29.0	27.9	25.1	20.5	18.2	16.4	3.4	11.8	27.5	35.9	41.9
Standard error		5.8	4.9	2.3	2.1	2.0	2.3	5.3	11.1	11.5	11.3	11.4

[a]Percent inhibition was calculated from radial growth based on the following formula: Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average of three colonies.

[c]Negative values of percent inhibition indicate growth stimulation.

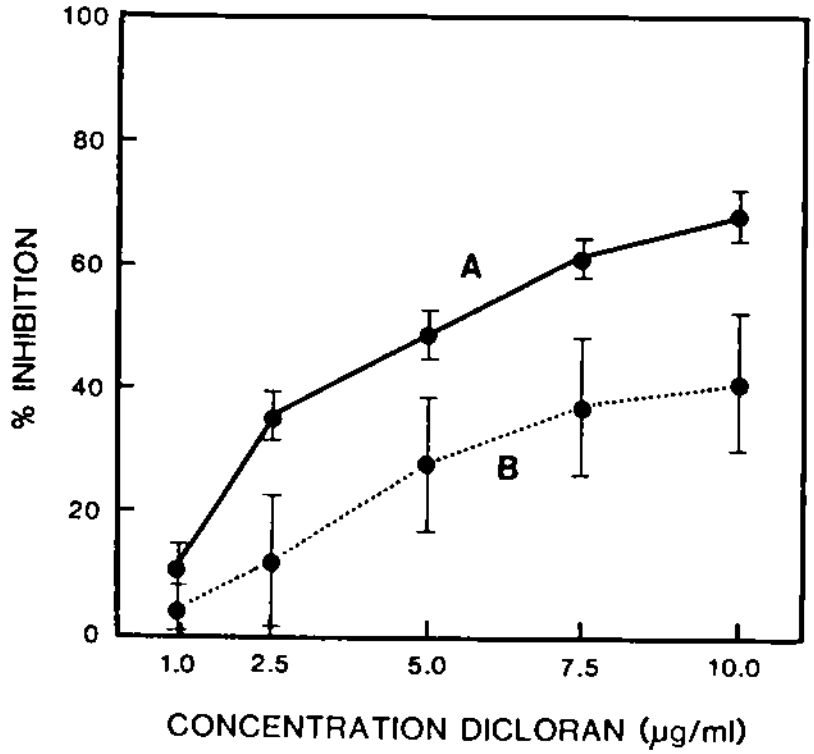


Figure 108. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on dicloran-amended potato dextrose agar at 25 °C for 8 d. Average of ten isolates of E. parasitica and nine isolates of E. gyosa; three replicate plates per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 55. Average radial growth and percent inhibition of average radial growth of isolates of E. parasitica and E. gyrosa cultured on dicloran-amended potato dextrose agar at 25°C for eight days. Test 3.

Species	Isolate	Radial Growth (mm)						Percent Inhibition[a]				
		Concentration (µg/ml)						Concentration (µg/ml)				
		0	0.5	1	10	25	50	0.5	1	10	25	50
E. parasitica	E24	43.3[b]	40.0	38.3	16.3	11.0	8.7	7.6	11.5	62.4	74.6	79.9
" "	E85	40.7	39.0	35.7	11.7	6.7	7.7	4.2	12.3	71.2	83.5	81.1
" "	E86	39.7	38.7	37.0	13.0	9.0	7.7	2.5	6.8	67.3	77.3	80.6
" "	E87	39.0	37.7	37.7	17.7	9.7	8.7	3.3	3.3	54.6	75.1	77.7
" "	E89	45.0	42.7	40.0	14.7	8.3	8.0	5.1	11.1	67.3	81.6	82.2
" "	E95	46.0	43.7	42.0	11.7	8.3	6.7	5.0	8.7	74.6	82.0	85.4
" "	E107	45.0	36.7	39.7	15.0	9.0	7.7	18.4	11.8	66.7	80.0	82.9
" "	E108	41.0	41.0	38.7	17.7	9.0	8.7	0.0	5.6	56.8	78.0	78.8
" "	E153	38.0	39.0	36.7	14.7	8.3	6.3	-2.6[c]	3.4	61.3	78.2	83.4
" "	E155	40.7	39.7	37.0	12.3	7.7	7.7	2.5	9.1	69.8	81.1	81.1
Species average		41.8	39.8	38.3	14.5	8.7	7.8	4.6	8.4	65.2	79.1	81.3
Standard error		2.8	2.2	1.9	2.3	1.2	0.8	5.6	3.4	6.3	3.0	2.3
E. gyrosa	E18	25.7	26.7	24.7	11.7	6.0	5.0	-3.9	3.9	54.5	76.7	80.5
" "	E20	28.7	24.7	21.0	8.7	5.7	2.0	13.9	26.8	69.7	80.1	93.0
" "	E30	23.3	28.0	26.7	14.7	8.3	5.3	-20.2	-14.6	36.9	64.4	77.3
" "	E37	23.3	22.0	22.7	12.7	7.3	3.0	5.6	2.6	45.5	68.7	87.1
" "	E48	22.3	20.0	21.7	9.3	7.3	4.7	10.3	2.7	58.3	67.3	78.9

Table 55 (continued)

Species	Isolate	Radial Growth (mm) Concentration (µg/ml) 0	0.5	1	10	25	50	Percent Inhibition[a] Concentration (µg/ml) 0.5	1	10	25	50
E. gyrosa (continued)	E51	31.7	29.3	28.3	11.3	5.7	4.3	7.6	10.7	64.4	82.0	86.4
" "	E98	26.7	31.0	25.0	14.0	8.0	6.0	-16.1	6.4	47.6	70.0	77.5
" "	E145	42.7	39.0	36.7	19.0	16.0	11.3	8.7	14.0	55.5	62.5	73.5
" "	E154	31.7	29.7	27.3	13.7	7.0	4.3	6.3	13.9	56.8	77.9	86.4
Species average		28.4	27.8	26.0	12.8	7.9	5.1	1.4	7.4	54.4	72.2	82.3
Standard error		6.4	5.5	4.7	3.1	3.2	2.6	12.1	11.3	9.9	7.1	6.2

[a]Percent inhibition was calculated from radial growth based on the following formula:
Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average of three colonies.

[c]Negative values of percent inhibition indicate growth stimulation.

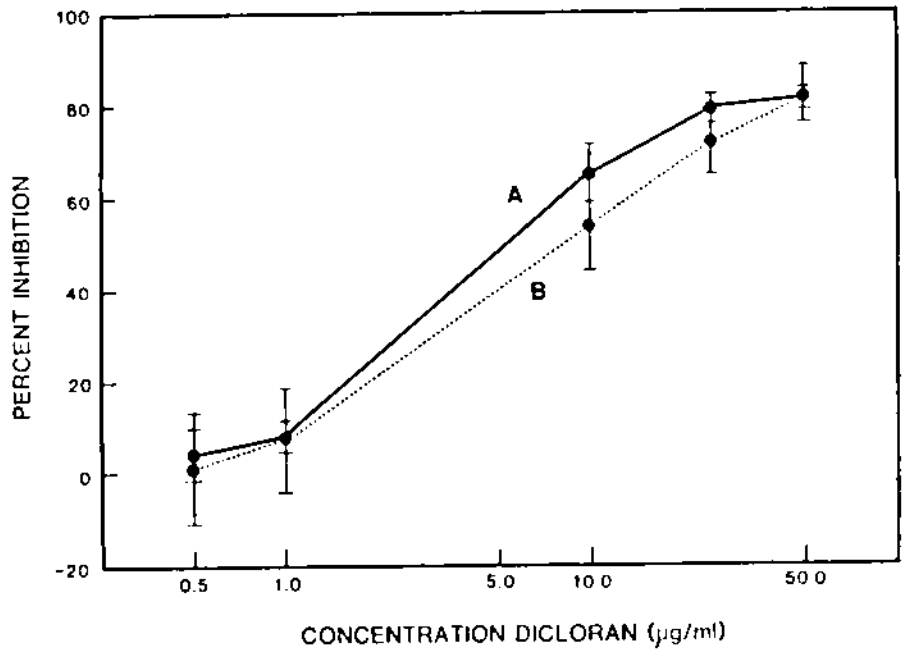


Figure 109. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on dicloran-amended potato dextrose agar at 25 °C for 8 d. Average of ten isolates of E. parasitica and nine isolates of E. gyosa; three replicate plates per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Test #2. Concentration plotted on a logarithmic scale.

Table 56. Average radial growth and percent inhibition of average radial growth of isolates of E. parasitica and E. gyrosa cultured on hydroquinone-amended potato dextrose agar at 25°C for eight days.

Species	Isolate	Radial Growth (mm)						Percent Inhibition[a]				
		Concentration (µg/ml)						Concentration (µg/ml)				
		0	1	10	50	100	500	1	10	50	100	500
E. parasitica	E24	32.0[b]	36.0	35.0	32.3	29.0	23.0	-12.5[c]	-9.4	-0.9	9.4	28.1
" "	E85	34.0	32.0	23.0	28.7	27.0	21.5	5.9	32.3	15.6	20.6	36.8
" "	E86	27.0	14.0	32.5	31.3	25.3	21.3	48.1	-20.4	-15.9	6.3	21.1
" "	E89	34.3	36.7	36.3	32.0	31.0	21.0	-7.0	-5.8	6.7	9.6	9.6
" "	E96	31.7	29.7	32.0	31.0	29.7	24.7	6.3	-0.9	2.2	6.3	22.1
" "	E108	28.0	30.0	33.0	31.5	24.3	18.3	-7.1	-17.9	-12.5	13.2	34.6
" "	E137	30.0	33.5	29.0	23.0	10.3	15.0	-11.7	3.3	23.3	65.7	50.0
" "	E153	32.7	33.5	32.0	32.3	30.0	24.0	-2.4	2.1	1.2	8.2	26.6
" "	E155	27.0	33.3	33.0	30.0	30.3	24.0	-23.3	-22.2	-11.1	-12.2	11.1
Species average		30.7	31.0	31.7	30.2	26.3	21.4	-0.4	-4.3	0.9	17.4	26.7
Standard error		2.9	6.8	3.9	2.9	6.4	3.1	20.4	16.7	13.0	20.1	12.7
E. gyrosa	E18	16.3	15.3	17.0	19.5	23.7	22.0	6.1	-4.3	-19.6	-45.4	-35.0
" "	E19	21.0	15.7	15.7	17.0	25.3	21.7	28.0	28.0	19.0	-20.5	-3.3
" "	E20	15.3	12.3	14.0	16.3	33.0	19.0	19.6	8.5	-6.5	-115.7	-24.2
" "	E37	13.7	15.0	14.0	13.7	25.3	19.0	-9.5	-2.2	0.0	-84.7	-38.7
" "	E38	20.3	15.3	16.0	24.0	25.3	24.7	24.6	21.2	-18.2	-24.6	-21.7
" "	E48	12.0	14.0	12.3	19.3	22.0	19.0	-16.7	-2.5	-60.8	-83.3	-58.3

Table 56 (continued)

Species	Isolate	Radial Growth (mm) Concentration (µg/ml) 0	1	10	50	100	500	Percent Inhibition[a] Concentration (µg/ml) 1	10	50	100	500
E. gyrosa (continued)	E50	30.3	39.0	32.7	31.7	31.7	23.7	-28.7	-7.9	-4.6	-4.6	21.8
" "	E98	23.7	27.7	22.0	26.3	25.3	22.0	-16.9	7.2	-11.0	-6.7	7.2
" "	E154	14.3	20.0	14.3	21.0	33.0	24.0	-39.9	0.0	-46.8	-130.8	-67.8
Species average		18.5	19.4	17.6	16.7	27.2	21.7	-3.7	5.3	-16.5	-57.4	-24.4
Standard error		5.8	8.7	6.3	7.5	4.2	2.2	24.2	12.2	24.2	47.6	29.4

[a]Percent inhibition was calculated from radial growth based on the following formula: Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average of three colonies.

[c]Negative values of percent inhibition indicate growth stimulation.

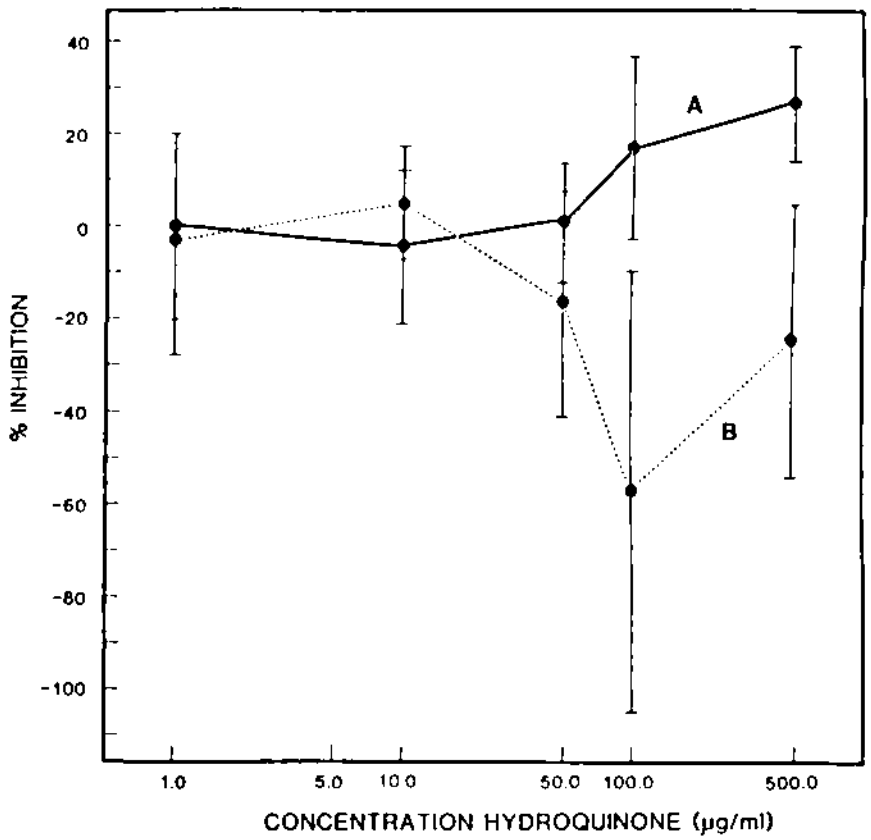


Figure 110. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on hydroquinone-amended potato dextrose agar at 25 °C for 8 d. Average of nine isolates with three replicate plates per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 57. Average radial growth and percent inhibition of average radial growth of E. parasitica and E. gyrosa cultured on o-phenyl phenol-amended potato dextrose agar at 25°C for eight days.

Species	Isolate	Radial Growth (mm)							Percent Inhibition[a]					
		Concentration (µg/ml)							Concentration (µg/ml)					
		0	1	5	10	25	50	100	1	5	10	25	50	100
E. parasitica	E85	32.3[b]	27.3	7.7	9.3	14.0	7.7	0.0	15.5	76.2	71.2	56.6	76.2	100.0
" "	E86	28.3	32.7	25.7	12.0	17.3	4.3	0.0	-15.5[c]	9.2	57.6	38.9	84.8	100.0
" "	E87	15.7	11.7	11.0	11.3	7.0	8.0	0.0	25.5	29.9	28.0	55.4	49.0	100.0
" "	E88	54.3	52.0	49.7	41.7	28.7	18.0	0.0	4.2	8.5	23.2	47.1	66.8	100.0
" "	E89	46.0	45.7	35.7	14.7	16.0	7.0	0.0	0.6	22.4	68.0	65.2	84.8	100.0
" "	E96	39.3	20.3	11.0	10.0	14.5	8.0	0.0	48.3	72.0	74.5	63.1	79.6	100.0
" "	E108	9.5	10.5	10.0	10.0	7.0	4.0	0.0	-10.5	-5.3	-5.3	26.3	57.9	100.0
" "	E137	8.0	10.3	11.0	10.0	8.3	3.7	0.0	-28.8	-37.5	-25.0	-3.8	53.8	100.0
" "	E153	38.7	37.0	22.0	22.3	24.0	11.0	0.0	4.4	43.1	42.4	38.0	71.6	100.0
" "	E155	42.3	41.0	32.3	14.3	20.7	5.7	0.0	3.1	23.6	66.2	51.1	86.5	100.0
Species average		31.4	28.8	21.6	15.6	15.8	7.7	0.0	4.7	24.2	40.1	43.8	71.1	100.0
Standard error		15.8	15.3	14.1	10.0	7.3	4.2	0.0	21.7	34.2	34.4	20.6	13.7	0.0
E. gyrosa	E18	22.0	23.0	19.3	15.0	9.0	3.7	0.0	-4.5	12.3	31.8	59.1	83.2	100.0
" "	E19	21.3	27.7	19.0	15.3	10.3	4.0	0.0	-30.0	10.8	28.2	51.6	81.2	100.0
" "	E20	17.0	15.7	14.7	12.3	7.7	0.3	0.0	7.6	13.5	27.6	54.7	98.2	100.0
" "	E37	19.7	17.0	19.0	16.0	9.7	5.3	0.0	13.7	3.5	18.8	50.8	73.1	100.0
" "	E38	21.0	23.3	19.7	16.3	11.0	3.0	0.0	-10.9	6.2	22.4	47.6	85.7	100.0
" "	E48	14.3	17.3	16.0	15.0	12.7	4.0	0.0	-21.0	-11.9	-4.9	11.2	72.0	100.0
" "	E50	41.7	37.3	31.3	21.0	9.7	0.7	0.0	10.5	24.9	49.6	76.7	98.3	100.0

Table 57 (continued)

Species	Isolate	Radial Growth (mm) Concentration (μg/ml) 0	1	5	10	25	50	100	Percent Inhibition[a] Concentration (μg/ml) 1	5	10	25	50	100
E. gyrosa (continued)	E51	24.0	26.0	24.7	18.3	11.0	5.3	0.0	-8.3	-2.9	23.8	54.2	77.9	100.0
" "	E98	23.7	25.7	22.3	16.3	11.7	2.7	0.0	-8.4	5.9	31.2	50.6	88.6	100.0
" "	E154	18.0	21.3	20.7	18.7	7.3	1.2	0.0	-18.3	-15.0	-3.9	59.9	83.3	100.0
Species average		22.3	23.4	20.4	16.4	10.0	3.0	0.0	-7.0	4.7	22.5	51.6	84.2	100.0
Standard error		7.5	6.4	4.7	2.4	1.7	1.8	0.0	14.2	12.0	16.4	16.4	9.1	0.0

[a]Percent inhibition was calculated from radial growth based on the following formula: Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average of three colonies.

[c]Negative values or percent inhibition indicate growth stimulation.

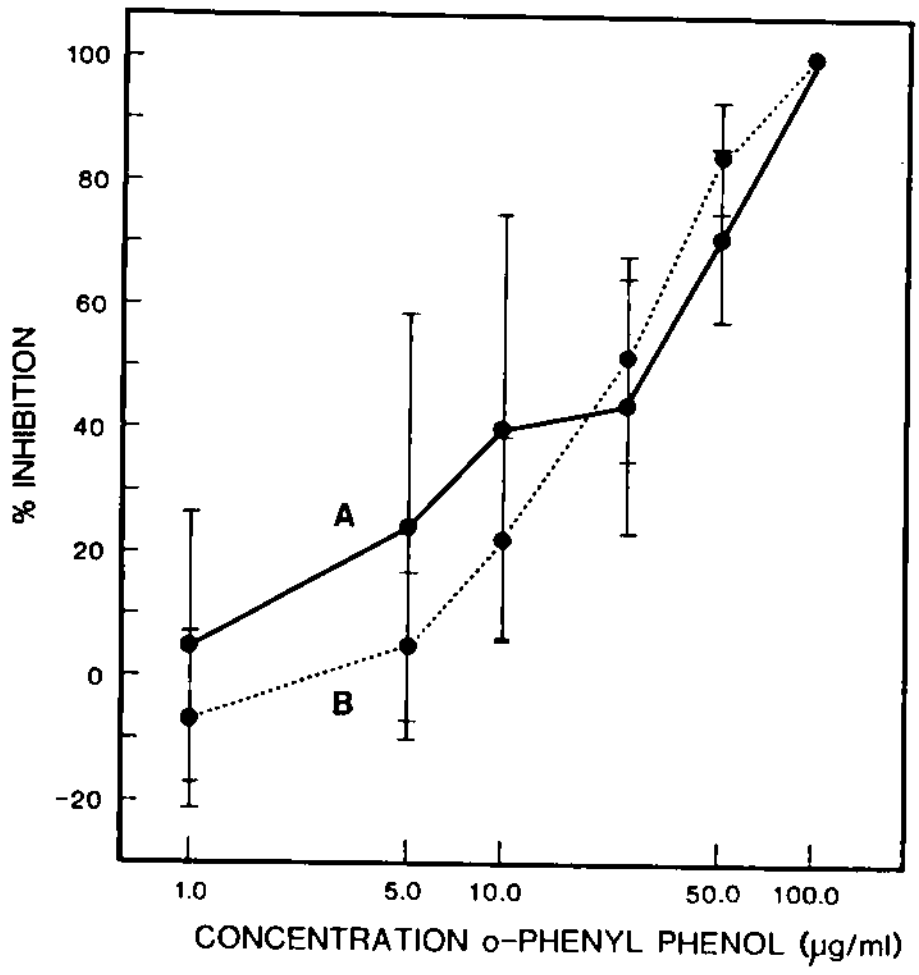


Figure 111. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on o-phenyl phenol-amended potato dextrose agar at 25 °C for 8 d. Average of ten isolates with three replicate plates per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 58. Radial growth and percent inhibition of radial growth of isolates of E. parasitica and E. gyrosa cultured on chlortetracycline-amended potato dextrose agar at 25°C for eight days.

Species	Isolate	Radial Growth (mm) Concentration (μg/ml) 0	0.1	1	10	100	Percent Inhibition[a] (Concentration μg/ml) 0.1	1	10	100
E. parasitica	E85	37.0[b]	36.0	37.0	35.0	32.0	2.7	0.0	5.4	13.5
" "	E88	47.0	--[c]	--	43.0	34.0	--	--	8.5	27.7
" "	E95	44.0	46.0	43.0	44.0	34.0	-4.5[d]	2.3	0.0	22.7
" "	E96	39.0	40.0	39.0	38.0	34.0	-2.6	0.0	2.6	12.8
Species average		41.8	40.7	39.7	40.0	33.5	-1.5	0.8	4.1	19.2
Standard error		4.6	5.0	3.0	4.2	1.0	3.7	1.3	3.7	7.3
E. gyrosa	E18	19.0	18.0	22.0	19.0	13.0	5.3	-15.8	0.0	31.6
" "	E37	18.0	18.0	16.0	23.0	16.0	0.0	11.1	-27.8	11.1
" "	E48	16.0	18.0	22.0	24.0	15.0	-12.5	-37.5	-50.0	6.2
" "	E98	25.0	28.0	25.0	25.0	21.0	-12.0	0.0	0.0	16.0
Species average		26.5	20.5	21.2	22.8	16.2	14.3	-10.6	-19.4	16.2
Standard error		7.9	5.0	3.8	2.6	3.4	6.6	21.1	24.2	11.0

Table 58 (continued)

[a]Percent inhibition was calculated from radial growth baseed on the following formula: Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Measurement of one colony.

[c]Data missing due to contamination.

[d]Negative values of percent inhibition indicate growth stimulation.

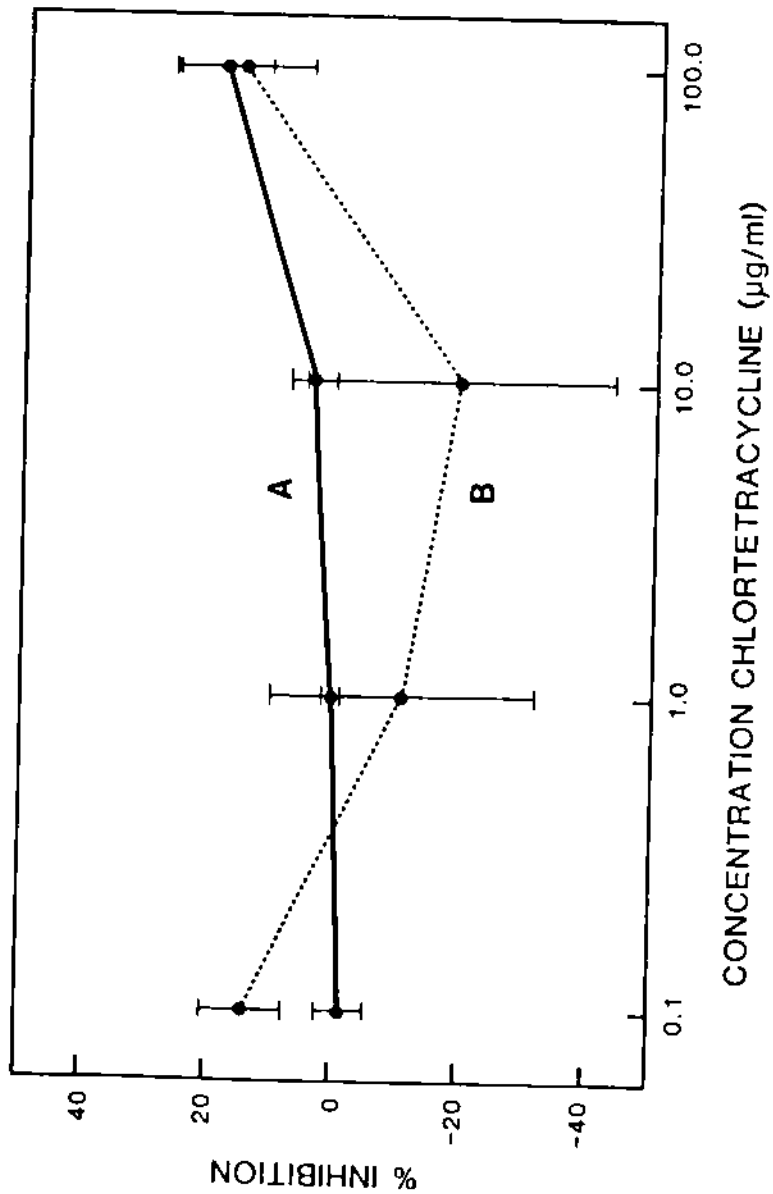


Figure 112. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on chlortetracycline-amended potato dextrose agar at 25 °C for 8 d. Average of four isolates; one plate per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 59. Average radial growth and percent inhibition of average radial growth of isolates of *E. parasitica*, *E. gyrosa* and *E. radicalis* cultured on benzimidazole-amended potato dextrose agar at 25°C for eight days.

Species	Isolate	Radial Growth (mm) Concentration (µg/ml) 0	0.01	0.1	1	10	100	Percent Inhibition[a] (Concentration (µg/ml) 0.01	0.1	1	10	100
E. parasitica	E86	40.0[b]	38.5	39.5	38.0	37.5	34.0	3.8	1.2	5.0	6.2	15.0
" "	E89	42.0	42.0	42.0	44.0	42.5	38.0	0.0	0.0	-4.8[c]	-1.1	9.5
" "	E96	45.5	44.0	45.0	44.0	47.0	41.5	3.3	1.1	3.3	3.3	8.8
" "	E108	40.0	37.5	38.0	39.5	37.5	37.0	6.2	5.0	1.2	6.2	7.5
Species average		41.9	40.5	41.1	41.4	41.1	37.6	1.8	1.8	1.2	3.6	10.2
Standard error		2.6	3.0	3.1	4.6	3.1	2.0	2.0	2.2	4.3	3.4	3.3
E. gyrosa	E37	32.5	20.5	25.5	30.5	14.0	22.5	36.9	21.5	6.2	56.9	30.8
" "	E38	30.5	28.0	28.0	33.5	27.0	25.5	8.2	8.2	-9.8	11.5	16.4
" "	E48	28.0	24.0	24.5	26.5	22.0	26.5	14.3	12.5	5.4	21.4	5.4
Species average		30.3	24.2	26.0	30.2	21.0	24.8	19.8	14.1	0.6	29.9	17.5
Standard error		2.2	3.7	1.8	3.5	6.6	2.1	15.1	6.8	9.0	23.9	12.7

Table 59 (continued)

Species	Isolate	Radial Growth (mm) Concentration (µg/ml) 0	0.01	0.1	1	10	100	Percent Inhibition[a] (Concentration (µg/ml) 0.01	0.1	1	10	100
E. radicalis	E64	21.0	20.5	21.5	23.0	21.5	18.0	2.4	-2.4	-9.5	-2.4	14.3
	E150	54.5	58.0	59.5	59.0	54.5	46.5	-6.4	-9.2	-8.3	0.0	14.7
Species average		37.8	39.2	40.5	41.0	38.0	32.2	-2.0	-5.8	-8.9	-1.2	14.5
Standard error		23.7	26.5	26.9	25.5	23.3	20.1	6.2	4.8	0.8	1.7	0.3

[a]Percent inhibition was calculated from radial growth based on the following formula: Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average of two colonies

[c]Negative values of percent inhibition indicate growth stimulation.

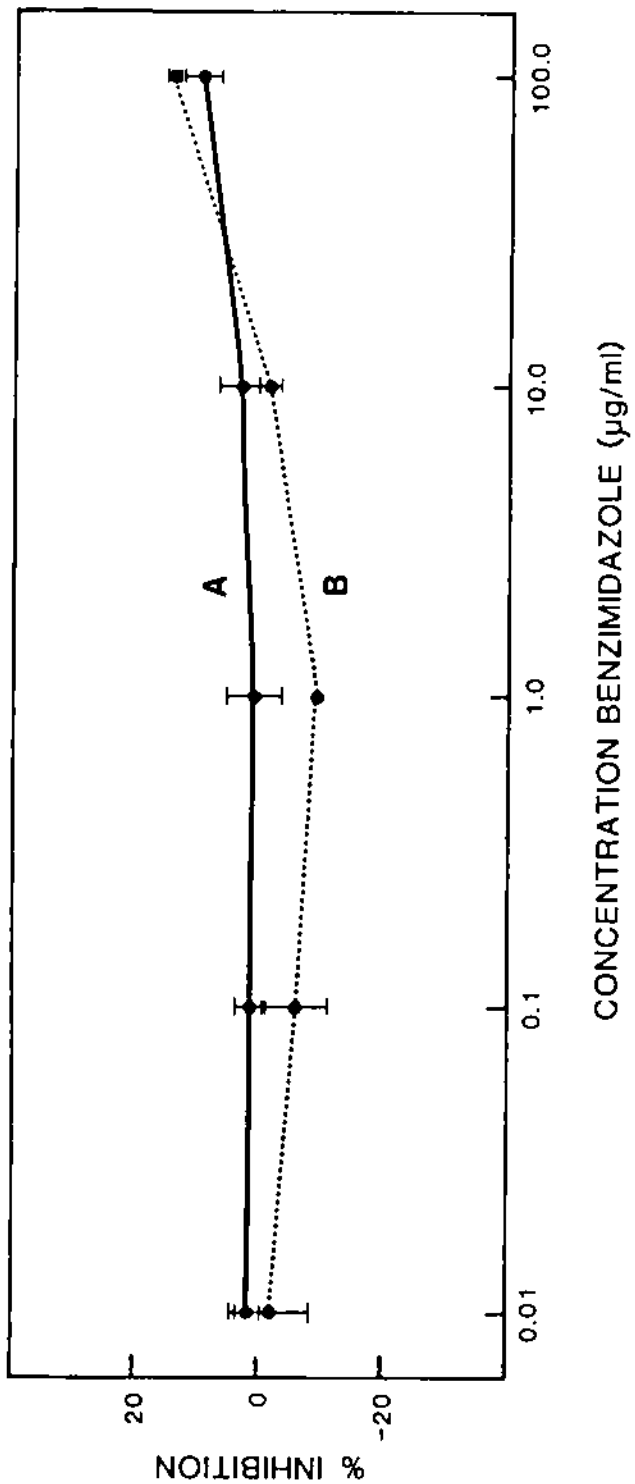


Figure 113. Dosage-response curve of Sclerotia sclerotiorum (A) and E. gyrosa (B) grown on benzimidazole-amended potato dextrose agar at 25 OC for 6 d. Average of four isolates of E. parasitica and three isolates of E. gyrosa; two replicate plates per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 60. Average radial growth and percent inhibition of average radial growth of *E. parasitica* and *E. gyrosa* cultured on fosetyl-Al-amended potato dextrose agar at 25°C for seven days.

Species	Isolate	Radial Growth (mm) Concentration (µg/ml) 0	0.01	0.1	1	10	100	Percent Inhibition[a] (Concentration (µg/ml) 0.01	0.1	1	10	100
E. parasitica	E86	32.5[b]	32.0	33.5	31.5	32.0	13.8	1.5	-3.1[c]	3.1	1.5	57.7
" "	E88	39.0	40.5	40.5	39.5	38.0	37.0	-3.8	-3.8	-1.3	2.6	5.1
" "	E89	36.5	35.5	37.5	37.0	37.0	30.8	2.7	-2.7	-1.4	-1.4	15.8
" "	E95	31.0	35.5	34.5	37.0	37.5	34.0	-14.5	-11.3	-19.4	-21.0	-9.7
" "	E155	37.0	36.0	36.0	34.5	34.5	33.5	2.7	2.7	6.8	6.8	9.5
Species average		35.2	39.5	36.4	35.9	35.8	29.8	-2.3	-3.6	-2.0	-2.3	15.7
Standard error		3.3	3.0	2.7	3.0	2.5	9.2	7.3	5.0	10.2	10.9	25.3
E. gyrosa	E30	12.0	11.0	11.0	10.0	10.5	12.0	8.3	8.3	16.7	12.5	0.0
" "	E38	13.5	12.0	11.2	11.5	12.5	12.5	11.1	16.5	14.8	7.4	7.4
" "	E48	11.0	10.5	11.0	8.5	8.5	10.5	4.5	0.0	22.7	22.7	4.5
" "	E51	13.2	14.5	14.5	14.0	13.2	13.5	-9.4	-9.4	-5.7	0.0	-1.9
" "	E154	11.0	13.8	14.0	11.5	11.0	12.0	-25.0	-27.3	-4.5	0.0	-9.1
Species average		12.1	12.4	12.3	11.1	11.1	12.1	-2.1	-2.4	8.8	8.5	0.2
Standard error		1.2	1.7	1.7	2.0	1.8	1.1	15.0	16.9	13.0	9.5	6.3

Table 60 (continued)

[a]Percent inhibition was calculated from radial growth based on the following formula: Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average of two colonies.

[c]Negative values of percent inhibition indicate growth stimulation.

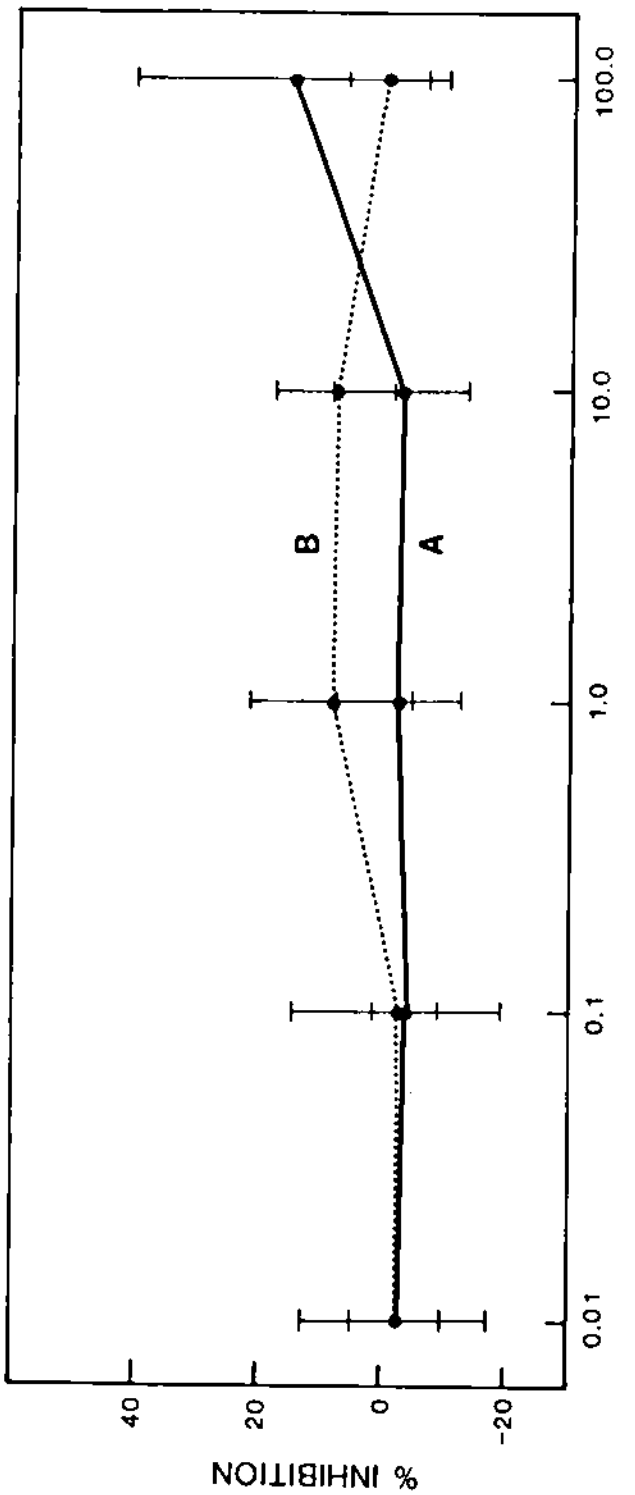


Figure 114. Dosage-response curve of Endoconidia parasitica (A) and E. gyrosa (B) grown on fosetyl Al-amended potato dextrose agar at 25 oC for 5 d. Average of five isolates with two replicate plates per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

excluded from the cell or detoxified in some manner.

Endothia parasitica and E. gyrosa responded similarly to the remaining compounds. These were: nystatin (Table 61, Figure 115), chloramphenicol (Table 62, Figure 116), captan (Table 63, Figure 117), iprodione (Table 64, Figure 118), vinclozolin (Table 65, Figure 119), PCNB (Table 66, Figure 120), chloroneb (Table 67, Figure 121), Fungisol® (Tables 68 and 69, Figures 122 and 123), mancozeb (Table 70, Figure 124), anilazine (Table 71, Figure 125) and triadimefon (Table 72, Figure 126). This uniformity in response suggests that the organisms may share many biochemical pathways and resistance mechanisms to fungitoxicants. It would be necessary to determine whether other members of the Diaporthales produce similar dosage-response curves before these biochemical similarities could be used to challenge Barr's classification system. The remaining species of Endothia were not tested with these compounds due to the lack of differentiation between E. parasitica and E. gyrosa.

Table 61. Average radial growth and percent inhibition of average radial growth of isolates of *E. parasitica* and *E. gyrosa* cultured on nystatin-amended potato dextrose agar at 25°C for eight days.

Species	Isolate	Radial Growth (mm)					Percent Inhibition[a]			
		Concentration (μg/ml)					Concentration (μg/ml)			
		0	0.1	1	10	100	0.1	1	10	100
E. parasitica	E87	38.5[b]	38.5	13.5	3.5	0.0	0.0	64.9	90.9	100.0
" "	E88	49.5	49.0	22.5	5.5	0.0	1.0	54.5	88.9	100.0
" "	E107	42.0	43.5	17.0	0.0	0.0	-3.6[c]	59.5	100.0	100.0
" "	E137	36.0	37.0	14.0	0.0	0.0	-2.8	61.1	100.0	100.0
" "	E153	40.5	42.0	13.5	1.5	0.0	-3.7	66.7	96.3	100.0
Species average		41.3	42.0	16.1	2.1	0.0	-1.8	61.3	95.2	100.0
Standard error		5.1	4.7	3.9	2.4	0.0	2.2	4.8	5.1	0.0
E. gyrosa	E18	21.0	24.5	8.5	0.0	0.0	-16.7	59.5	100.0	100.0
" "	E38	25.5	21.0	2.0	0.0	0.0	17.6	92.2	100.0	100.0
" "	E48	11.5	9.5	4.0	0.0	0.0	17.4	65.2	100.0	100.0
" "	E98	27.0	26.5	6.0	0.8	0.0	1.8	77.7	97.2	100.0
" "	E154	28.5	25.5	5.0	0.0	0.0	10.5	82.5	100.0	100.0
Species average		22.7	21.4	5.1	0.2	0.0	6.1	75.4	99.4	100.0
Standard error		6.9	7.0	2.4	0.4	0.0	14.3	13.2	1.2	0.0

Table 61 (continued)

[a]Percent inhibition was calculated from radial growth based on the following formula: Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average of two colonies.

[c]Negative values of percent inhibition indicate growth stimulation.

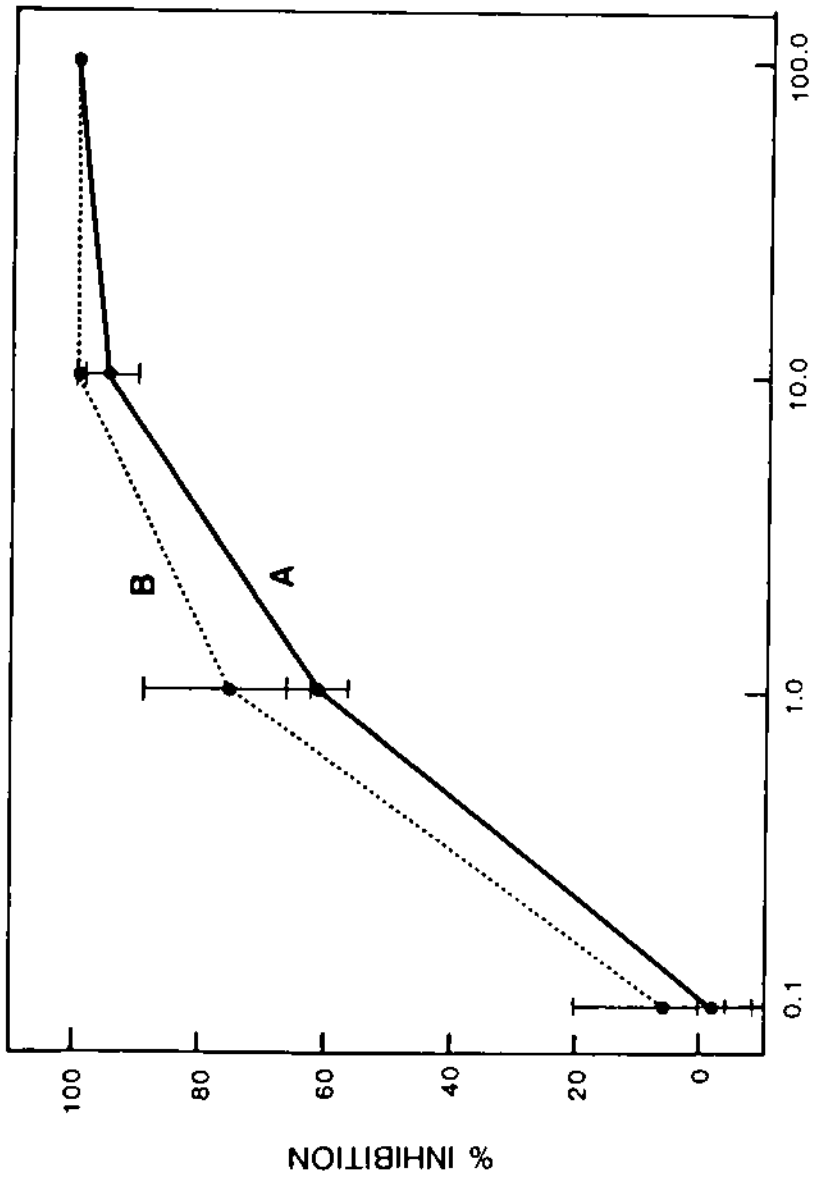


Figure 115. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on nystatin-amended potato dextrose agar at 25 oC for 8 d. Average of five isolates with two replicate plates per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 62. Average radial growth and percent inhibition of average radial growth of E. parasitica and E. gyrosa cultured on chloramphenicol-amended potato dextrose agar at 25°C for eight days.

Species	Isolate	Radial Growth (mm) Concentration (µg/ml) 0	100	500	1000	Percent Inhibition[a] Concentration (µg/ml) 100	500	1000
E. parasitica	E24	25[b]	24	21	0	4	16	100
" "	E89	30	24	16	0	20	47	100
" "	E137	18	20	16	0	-11	11	100
Species average		24	23	18	0	4	25	100
Standard error		6	2	3	0	15	19	0
E. gyrosa	E20	16	--[c]	11	0	--	31	100
" "	E51	25	26	13	0	-4	48	100
" "	E145	31	25	26	0	19	16	100
Species average		24	26	17	0	8	32	100
Standard error		8	1	8	0	16	16	0

[a]Percent inhibition was calculated from radial growth based on the following formula:
Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Measurement of one colony.

[c]Data missing due to contamination.

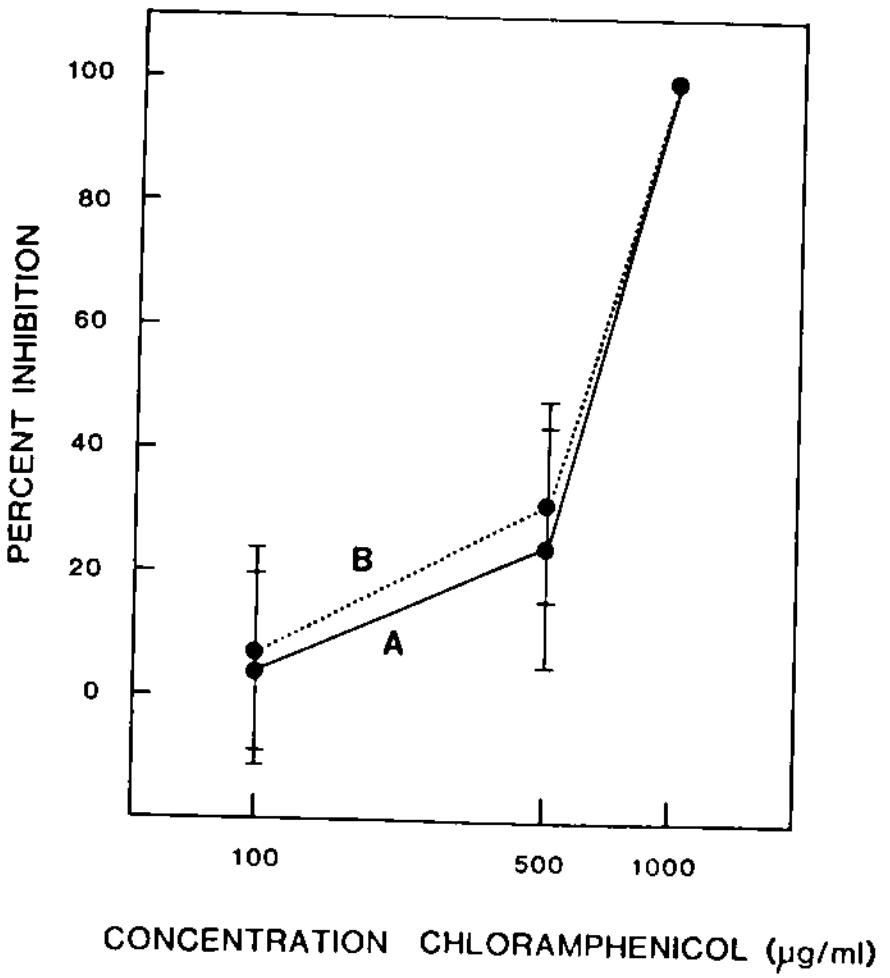


Figure 116. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on chloramphenicol-amended potato dextrose agar at 25 °C for 8 d. Average of three isolates; one plate per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 63. Radial growth and percent inhibition of radial growth of isolates of E. parasitica and E. gyrosa cultured on captan-amended potato dextrose agar for 25°C for eight days.

Species	Isolate	Radial Growth (mm)					Percent Inhibition[a]			
		Concentration (µg/ml)					Concentration (µg/ml)			
		0	0.1	1	10	100	0.1	1	10	100
E. parasitica	E24	40.0[b]	39.0	40.0	13.0	5.0	2.5	0.0	67.5	87.5
" "	E85	40.0	39.0	38.0	12.0	0.5	2.5	5.0	70.0	98.8
" "	E88	47.0	47.0	46.0	39.0	0.0	0.0	2.1	17.0	98.9
" "	E95	46.0	46.0	45.0	36.0	3.0	0.0	2.1	17.0	98.9
" "	E96	36.0	39.0	38.0	30.0	0.5	-8.3[c]	-5.6	16.7	98.6
Species average		41.8	42.0	41.4	25.0	1.8	-0.7	0.7	37.6	96.5
Standard error		4.6	4.1	3.8	14.5	2.1	4.4	4.0	28.4	5.0
E. gyrosa	E18	20.0	25.0	18.0	23.0	0.5	-25.0	10.0	-15.0	97.5
" "	E37	21.0	21.0	23.0	23.0	1.0	0.0	-9.5	-9.5	95.2
" "	E48	20.0	17.0	16.0	19.0	4.0	15.0	20.0	50.0	80.0
" "	E98	26.0	26.0	25.0	23.0	2.0	0.0	3.8	11.5	92.3
Species average		21.8	22.2	20.5	19.8	1.9	-2.5	6.1	9.2	91.2
Standard error		2.9	4.1	4.2	6.5	1.5	16.6	2.5	29.5	7.8

Table 63 (continued)

[a]Percent inhibition was calculated from radial growth based on the following formula:
Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Measurement of one colony.

[c]Negative values of percent inhibition indicate growth stimulation.

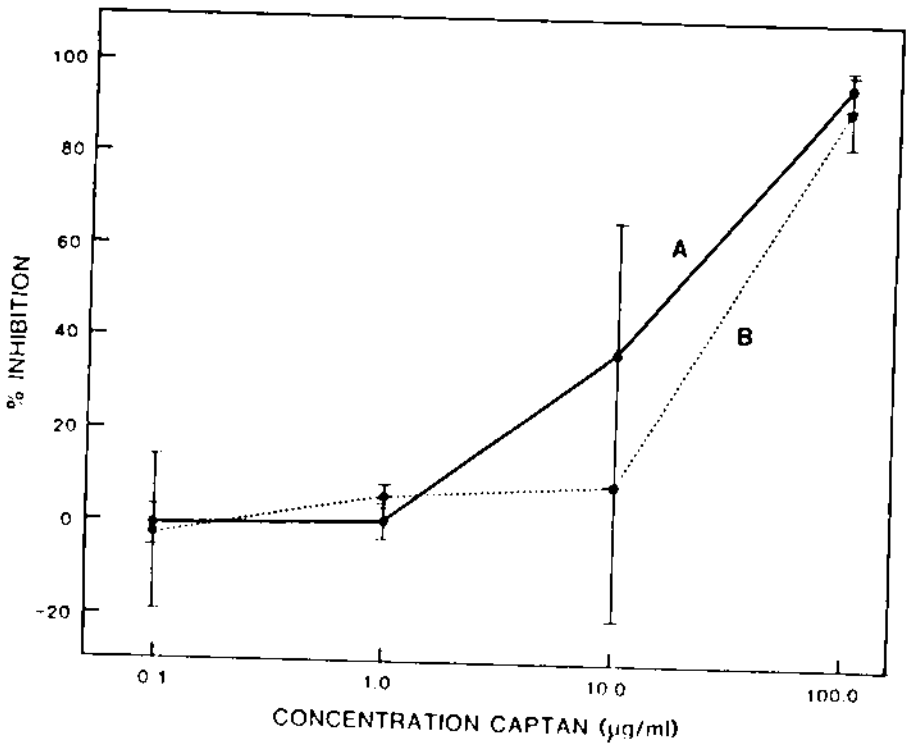


Figure 117. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on captan-amended potato dextrose agar at 25 °C for 8 d. Average of five isolates of E. parasitica and four isolates of E. gyrosa; one plate per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 64. Average radial growth and percent inhibition of average radial growth of isolates of *E. parasitica* and *E. gyrosa* on iprodione-amended potato dextrose agar at 25°C for eight days.

Species	Isolate	Radial Growth (mm)					Percent Inhibition[a]			
		Concentration (µg/ml)					Concentration (µg/ml)			
		0	0.1	1	10	100	0.1	1	10	100
E. parasitica	E86	49.0[b]	48.0	37.5	1.0	1.0	2.0	23.5	98.0	98.0
" "	E87	46.0	43.0	35.5	12.5	6.0	6.5	22.8	72.8	87.0
" "	E88	58.5	60.0	37.5	21.0	5.5	-2.6[c]	35.9	64.1	90.6
" "	E107	51.0	49.5	35.0	11.0	11.0	2.9	31.4	78.4	78.4
" "	E155	50.0	48.5	35.5	14.5	6.0	3.0	29.0	71.0	88.0
Species average		50.9	49.8	36.2	12.0	5.9	2.4	28.5	76.9	88.4
Standard error		4.6	6.2	1.2	7.2	3.5	3.3	5.5	12.9	7.0
E. gyrosa	E30	24.0	20.5	14.0	1.5	1.8	14.6	41.7	93.8	92.7
" "	E37	27.5	29.5	15.5	0.0	2.5	-7.3	43.6	100.0	90.9
" "	E48	23.0	24.0	16.0	0.0	1.0	-4.3	30.4	100.0	95.6
" "	E98	31.5	29.0	17.0	0.5	2.0	7.9	46.0	98.4	93.6
Species average		26.5	25.8	15.6	0.5	1.8	2.7	40.4	98.0	93.2
Standard error		3.8	4.3	1.2	0.7	0.6	10.3	6.9	2.9	1.9

Table 64 (continued)

[a]Percent inhibition was calculated from radial growth based on the following formula:
Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average of two colonies.

[c]Negative values of percent inhibition indicate growth stimulation.

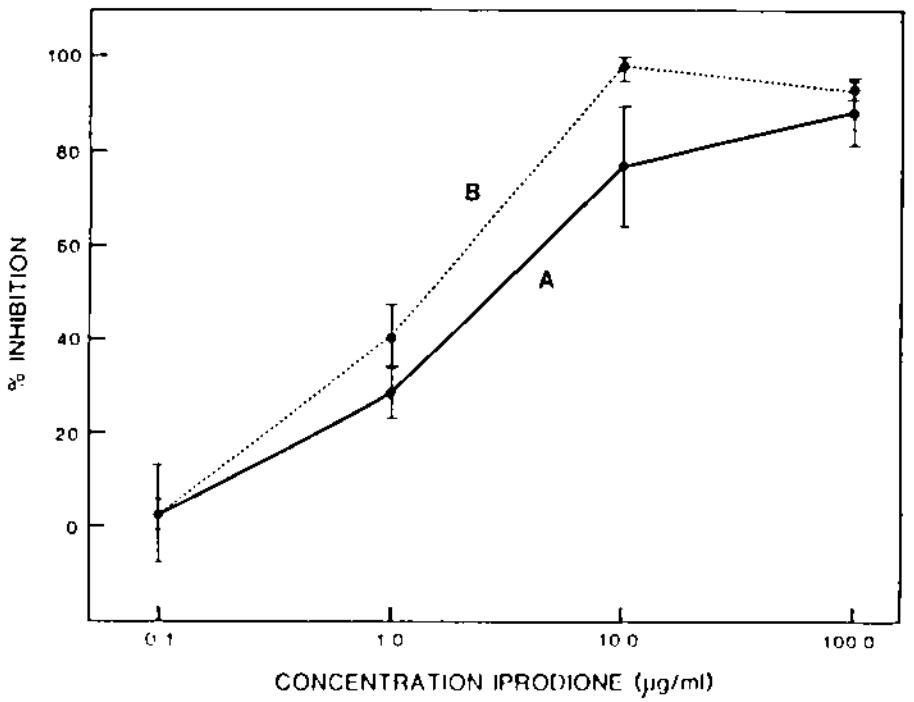


Figure 118. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on iprodione-amended potato dextrose agar at 25 °C for 8 d. Average of five isolates of E. parasitica and four isolates of E. gyrosa; two replicate plates per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 65. Radial growth and percent inhibition of radial growth of isolates of E. parasitica and E. gyrosa cultured on vinclozolin-amended medium at 25°C for eight days.

Species	Isolate	Radial Growth (mm) Concentration (µg/ml) 0	0.5	1	10	50	Percent Inhibition[a] Concentration (µg/ml) 0.5	1	10	50
E. parasitica	E24	41.0[b]	42.0	36.0	--[c]	3.0	-2.4[d]	12.2	--	-2.7
" "	E85	37.0	38.0	--	8.0	4.0	-2.7	--	78.4	89.2
" "	E88	45.0	46.0	40.0	21.0	6.0	-2.2	11.1	53.3	86.7
" "	E95	45.0	45.0	36.0	10.0	--	0.0	20.0	77.8	--
" "	E96	39.0	37.0	32.0	12.5	5.0	5.1	17.9	69.2	87.2
Species average		41.4	41.6	36.0	12.9	4.5	-0.4	15.3	69.7	89.0
Standard error		3.6	4.0	3.3	5.7	1.3	3.3	4.3	11.7	2.7
E. gyrosa	E18	23.0	21.0	19.0	11.0	3.0	8.7	17.4	52.2	87.0
" "	E37	18.0	18.0	15.0	8.0	1.0	0.0	16.7	55.6	94.4
" "	E48	17.0	18.0	17.0	8.0	1.0	-5.9	0.0	52.9	94.1
" "	E98	32.0	23.0	16.0	7.0	1.0	28.1	50.0	77.1	96.9
Species average		22.5	20.0	16.8	8.5	1.8	7.7	21.0	59.7	93.1
Standard error		6.8	2.4	1.7	1.7	1.0	14.8	20.9	12.3	4.2

Table 65 (continued)

[a]Percent inhibition was calculated from radial growth based on the following formula:
Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Measurement of one colony.

[c]Data missing due to contamination.

[d]Negative values of percent inhibition indicate growth stimulation.

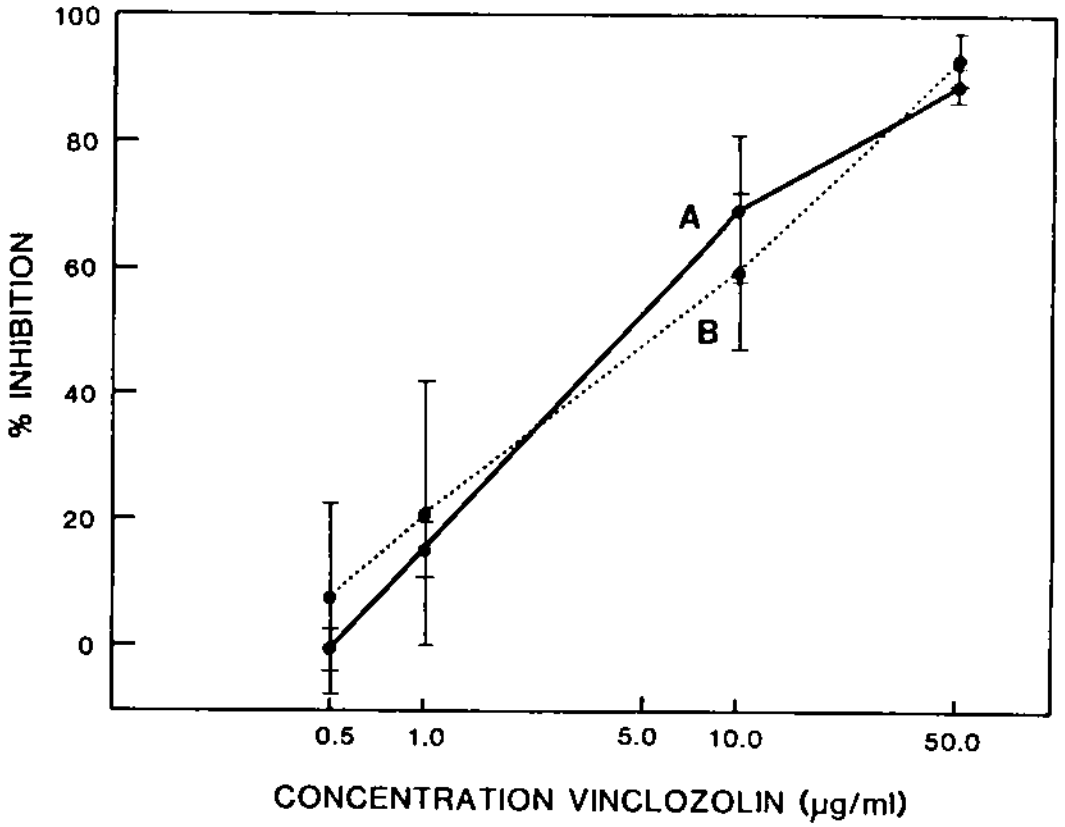


Figure 119. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on vinclozolin-amended potato dextrose agar at 25 °C for 8 d. Average of five isolates of E. parasitica and four isolates of E. gyrosa; one plate per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 66. Average radial growth and percent inhibition of average radial growth of E. parasitica and E. gyrosa on PCNB-amended potato dextrose agar at 25°C for eight days.

Species	Isolate	Radial Growth (mm)					Percent Inhibition[a]			
		Concentration (µg/ml)					Concentration (µg/ml)			
		0	1	10	100	500	1	10	100	500
E. parasitica	E86	42.5[b]	44.5	38.5	15.5	17.5	-4.7[c]	9.7	63.5	58.8
" "	E87	42.0	42.0	34.5	18.5	16.0	0.0	17.8	56.0	61.9
" "	E88	52.5	54.0	45.0	19.5	17.5	-2.8	14.3	62.8	66.7
" "	E107	47.5	46.5	39.0	14.5	13.5	2.1	17.9	69.5	71.6
	E155	47.5	46.5	36.0	18.5	13.5	2.1	24.2	61.0	71.6
Species average		46.4	46.7	38.6	17.3	15.6	-0.7	16.7	62.6	66.1
Standard error		4.3	4.5	4.0	2.2	2.0	3.0	5.4	4.9	5.7
E. gyrosa	E30	20.0	22.0	16.5	9.5	5.5	-10.0	17.5	52.5	72.1
" "	E37	21.5	27.5	16.5	8.5	6.0	-27.9	23.2	60.5	72.1
" "	E48	30.0	19.5	16.5	8.5	8.0	35.0	45.0	71.7	73.3
	E98	24.0	24.5	15.0	8.5	8.0	35.0	45.0	71.7	73.3
Species average		23.9	23.4	16.1	8.8	6.2	-13.3	30.8	62.3	73.6
Standard error		4.4	3.4	0.8	0.5	1.2	13.2	12.7	8.0	2.4

Table 66 (continued)

[a]Percent inhibition was calculated from radial growth based on the following formula:
Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average of two colonies.

[c]Negative values of percent inhibition indicate growth stimulation.

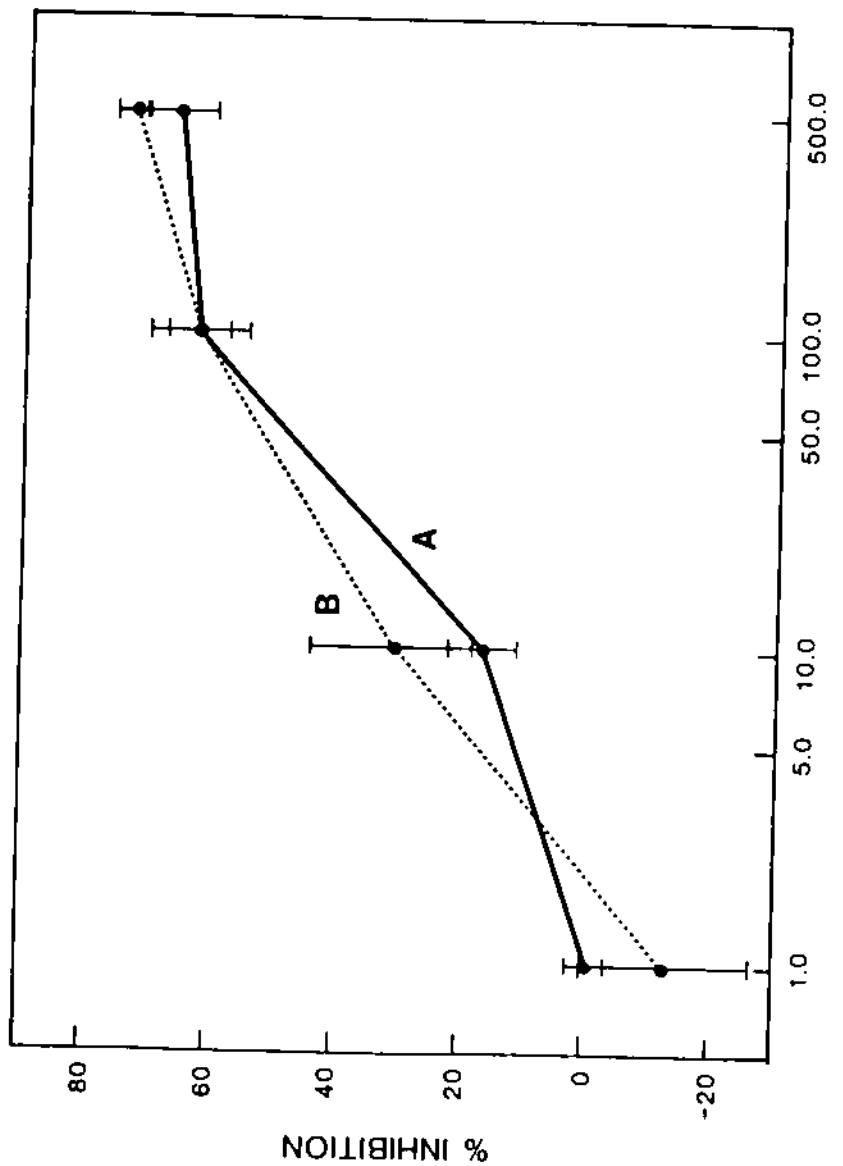


Figure 120. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on PCNB-amended potato dextrose agar at 25 °C for 8 d. Average of five isolates of E. parasitica and four isolates of E. gyrosa; two replicate plates per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 67. Average radial growth and percent inhibition of average radial growth of E. parasitica and E. gyrosa cultured on chloroneb-amended potato dextrose agar at 25°C for eight days.

Species	Isolate	Radial Growth (mm) Concentration (μg/ml) 0	10	100	Percent Inhibition[a] Concentration (μg/ml) 10	100
E. parasitica	E24	25[b]	19	6	24	76
" "	E89	30	13	3	57	90
	E137	18	7	3	61	83
Species average		24	13	4	47	83
Standard error		6	6	2	20	7
E. gyrosa	E20	16	7	1	56	94
" "	E51	25	11	2	56	92
	E145	31	19	7	39	77
Species average		24	12	3	50	88
Standard error		8	6	3	10	9

[a]Percent inhibition was calculated from radial growth based on the following formula.

Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Measurement of one colony.

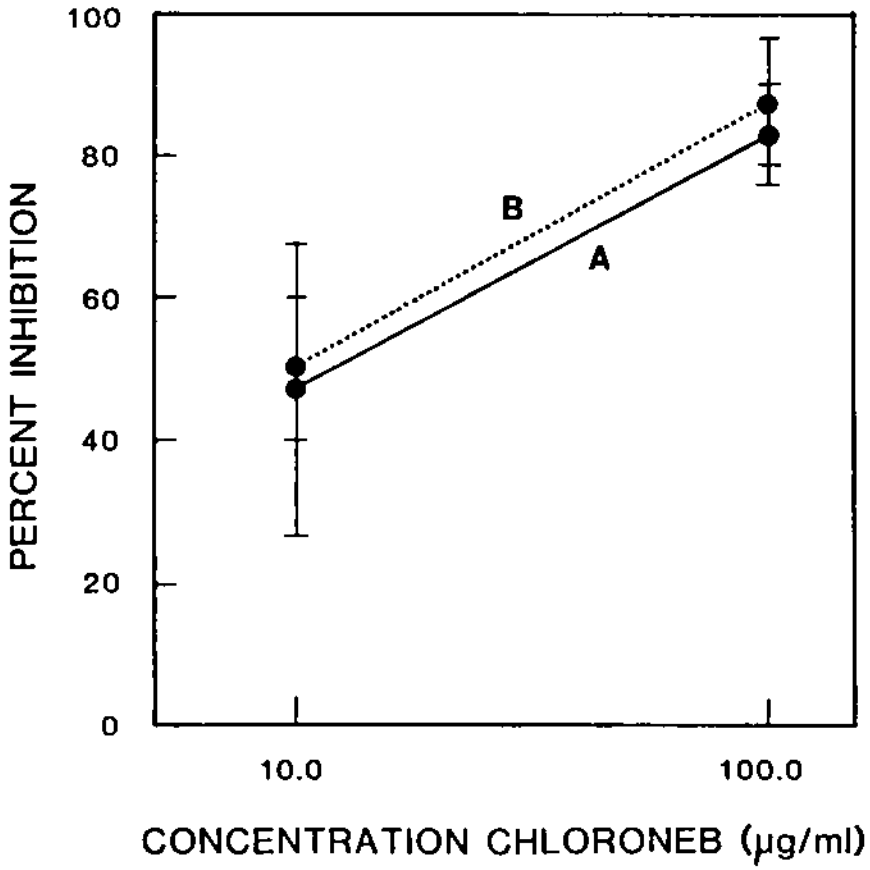


Figure 121. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on chloroneb-amended potato dextrose agar at 25 °C for 8 d. Average of three isolates; one plate per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 68. Radial growth and percent inhibition of radial growth of isolates of E. parasitica and E. gyrosa cultured on Fungisol^{R}-amended potato dextrose agar at 25°C for eight days. Test 1.

Species	Isolate	Radial Growth (mm)					Percent Inhibition[a]			
		Concentration (µg/ml)					Concentration (µg/ml)			
		0	0.1	0.5	1	5	0.1	0.5	1	5
E. parasitica	E24	42.0[b]	42.0	43.0	9.0	0.0	0.0	-2.4[c]	8.6	100.0
" "	E85	39.0	40.0	39.0	10.0	0.0	-2.6	0.0	74.4	100.0
" "	E88	48.0	47.0	45.0	15.0	0.0	2.1	6.2	68.8	100.0
" "	E95	47.0	47.0	46.0	14.0	0.0	0.0	2.1	70.2	100.0
" "	E96	39.0	41.0	39.0	12.0	0.0	-5.1	0.0	69.2	100.0
Species average		43.0	43.4	42.4	12.0	0.0	-1.1	1.2	72.2	100.0
Standard error		4.3	3.4	3.3	2.5	0.0	2.8	3.2	4.2	0.0
E. gyrosa	E18	25.0	21.0	21.0	21.0	0.5	16.0	16.0	56.0	98.0
" "	E37	24.0	16.0	17.0	11.0	0.0	33.3	29.2	54.2	100.0
" "	E48	23.0	23.0	20.0	10.0	0.0	0.0	13.0	56.5	100.0
" "	E98	29.0	26.0	26.0	13.0	0.0	10.3	10.3	55.2	100.0
Species average		25.2	21.5	21.0	11.2	0.1	14.9	17.1	55.5	99.5
Standard error		2.6	4.2	3.7	1.3	0.2	13.9	8.4	1.0	1.0

Table 68 (continued)

[a]Percent inhibition was calculated from radial growth based on the following formula:
Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Measurement of one colony.

[c]Negative values of percent inhibition indicate growth stimulation.

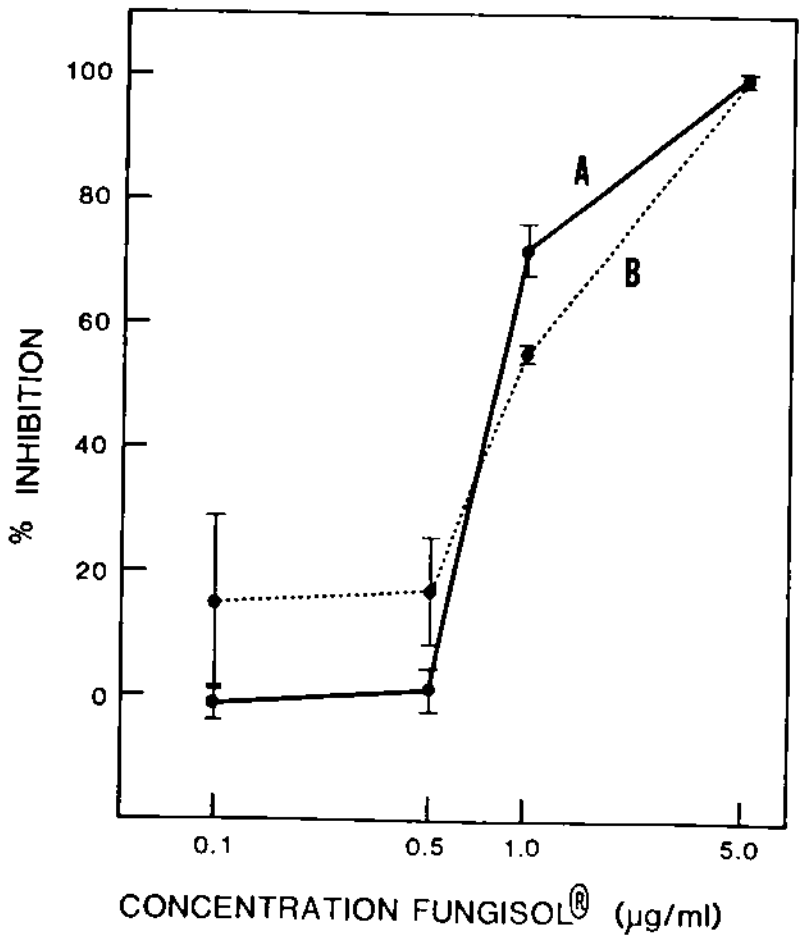


Figure 122. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on Fungisol®-amended potato dextrose agar at 25 °C for 8 d. Average of five isolates of E. parasitica and four isolates of E. gyrosa; one plate per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 69. Average radial growth and percent inhibition of average radial growth of E. parasitica, E. gyrosa and E. radicalis cultured on Fungisol^{R}-amended potato dextrose agar at 25°C for eight days. Test 2.

Species	Isolate	Radial Growth (mm) Concentration (µg/ml) 0	0.1	0.5	1	2.5	5	Percent Inhibition[a] (Concentration (µg/ml) 0.1	0.5	1	2.5	5
E. parasitica	E24	45.7[b]	47.0	33.3	2.0	0.0	0.0	-2.8	27.1	95.6	100.0	100.0
" "	E85	42.7	43.0	19.0	0.0	0.0	0.0	-0.7	55.5	100.0	100.0	100.0
" "	E86	44.0	43.3	18.0	4.7	0.0	0.0	1.6	59.1	89.3	100.0	100.0
" "	E87	39.7	40.3	17.3	1.3	0.0	0.0	-1.5	56.4	96.7	100.0	100.0
" "	E88	51.0	54.3	28.3	0.7	0.0	0.0	-6.5	44.5	98.6	100.0	100.0
" "	E89	46.3	46.3	14.3	0.0	0.0	0.0	0.0	69.1	100.0	100.0	100.0
" "	E95	49.7	48.0	21.7	2.7	0.0	0.0	3.4	56.3	94.6	100.0	100.0
" "	E107	48.0	47.3	43.7	0.2	0.0	0.0	1.5	8.6	99.6	100.0	100.0
" "	E108	43.7	43.7	18.7	3.3	0.0	0.0	0.0	57.2	92.4	100.0	100.0
" "	E137	38.7	39.0	12.7	1.3	0.0	0.0	-0.8	67.2	96.6	100.0	100.0
" "	E153	40.7	40.3	29.0	0.0	0.0	0.0	1.0	28.7	100.0	100.0	100.0
" "	E155	43.7	42.3	14.0	0.0	0.0	0.0	3.2	68.0	100.0	100.0	100.0
Species average		44.5	44.6	22.5	1.4	0.0	0.0	-0.1	49.8	97.0	100.0	100.0
Standard error		3.8	4.3	9.3	1.5	0.0	0.0	2.7	18.9	3.5	0.0	0.0
E. gyrosa	E18	27.3	29.0	20.7	4.0	0.0	0.0	-6.2	24.2	85.3	100.0	100.0
" "	E20	25.3	27.7	25.0	8.3	0.3	0.0	-9.4	1.2	67.2	98.8	100.0
" "	E30	19.7	27.5	12.7	0.0	0.0	0.0	-39.6	35.5	100.0	100.0	100.0
" "	E37	25.7	23.0	22.0	2.3	0.0	0.0	10.5	14.4	91.0	100.0	100.0
" "	E38	28.0	28.3	27.3	4.3	1.7	0.0	-1.1	2.5	84.6	93.9	100.0

Table 69 (continued)

Species	Isolate	Radial Growth (mm) Concentration (µg/ml) 0	0.1	0.5	1	2.5	5	Percent Inhibition[a] (Concentration (µg/ml) 0.1	0.5	1	2.5	5
E. gyrosa (continued)	E50	36.0	36.3	27.7	0.0	0.0	0.0	-0.8	23.0	100.0	100.0	100.0
" "	E51	32.3	27.3	21.0	6.7	0.7	0.0	15.4	35.0	79.3	97.8	100.0
" "	E98	28.7	30.3	18.3	0.7	0.7	0.0	-5.6	36.2	97.6	97.6	100.0
" "	E145	41.0	29.7	29.0	1.0	0.0	0.0	27.6	29.3	97.6	100.0	100.0
" "	E154	32.0	32.0	19.7	4.0	0.0	0.0	0.0	38.4	87.5	100.0	100.0
Species average		29.6	29.1	22.3	3.1	0.3	0.0	-0.9	24.0	89.0	98.8	100.0
Standard error		6.0	3.5	5.0	2.8	0.6	0.0	17.7	13.8	10.5	2.0	0.0
E. radicalis	E64	20.3	19.7	0.0	0.0	0.0	0.0	3.0	100.0	100.0	100.0	100.0
" "	E67	14.0	17.7	0.0	0.0	0.0	0.0	-26.4	100.0	100.0	100.0	100.0
" "	E76	38.0	38.0	0.0	0.0	0.0	0.0	0	100.0	100.0	100.0	100.0
" "	E149	53.0	54.7	9.0	0.0	0.0	0.0	-3.2	83.0	100.0	100.0	100.0
Species average		31.3	32.5	2.2	0.0	0.0	0.0	-6.6	95.8	100.0	100.0	100.0
Standard error		17.7	17.4	4.5	0.0	0.0	0.0	13.4	8.5	0.0	0.0	0.0

Table 69 (continued

[a]Percent inhibition was calculated from radial growth based on the following formula: Percent Inhibition = (1-ra/rc) x 100.0, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average of three colonies.

[c]Negative values of percent inhibition indicate growth stimulation.

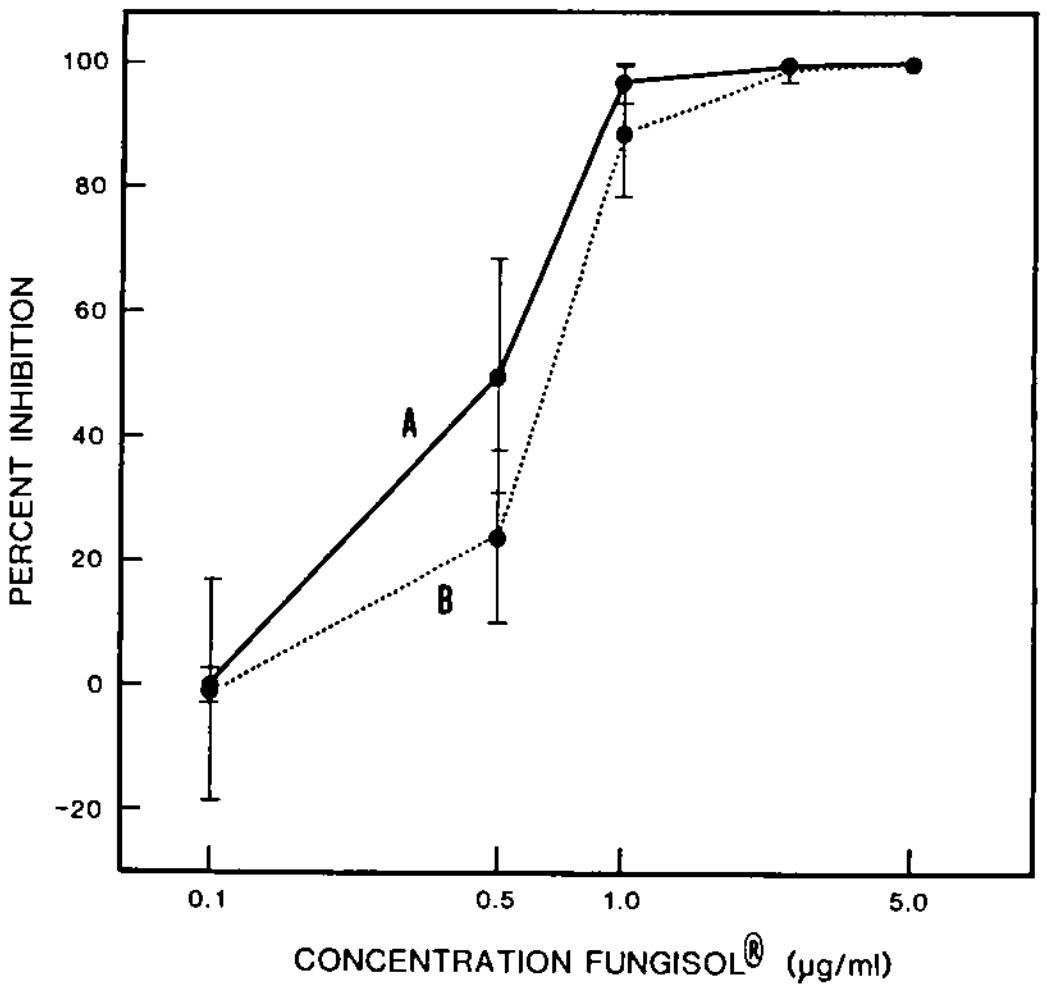


Figure 123. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on Fungisol®-amended potato dextrose agar at 25 °C for 8 d. Average of 12 isolates of E. parasitica and 10 isolates of E. gyrosa; three replicate plates per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 70. Average radial growth and percent inhibition of average radial growth of isolates of E. parasitica and E. gyrosa cultured on mancozeb-amended potato dextrose agar at 25°C for eight days.

Species	Isolate	Radial Growth (mm)					Percent Inhibition[a]			
		Concentration (µg/ml)					Concentration (µg/ml)			
		0	0.1	1	10	100	0.1	1	10	100
E. parasitica	E86	49.5[b]	49.0	48.5	39.5	3.0	1.0	2.0	20.2	93.9
" "	E87	45.0	45.5	46.0	37.5	2.5	-1.1[c]	-2.2	16.7	94.4
" "	E88	59.0	57.5	57.5	49.5	32.5	2.5	2.5	16.1	44.9
" "	E107	49.0	51.0	50.0	41.0	0.0	-4.1	-2.0	16.3	100.0
" "	E155	49.0	49.0	49.0	40.0	0.0	0.0	0.0	18.4	100.0
Species average		50.3	50.4	50.2	37.5	7.6	-0.3	0.1	17.5	86.6
Standard error		5.2	4.4	4.3	10.3	14.0	2.5	2.2	1.7	23.5
E. gyrosa	E30	22.5	25.0	19.0	15.5	12.0	-11.1	15.6	31.1	46.7
" "	E37	25.0	28.5	24.0	20.5	11.0	-14.0	4.0	18.0	56.0
" "	E48	25.5	24.5	24.0	20.0	10.0	3.9	5.9	21.6	60.8
" "	E98	28.5	35.5	32.0	25.0	11.0	-24.6	-12.3	12.3	61.4
Species average		25.4	28.4	24.8	20.2	11.0	-11.4	3.3	20.8	56.2
Standard error		2.5	5.1	5.4	3.9	0.8	11.8	11.6	7.9	6.8

Table 70 (continued)

[a]Percent inhibition was calculated from radial growth based on the following formula:
Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average of two colonies.

[c]Negative values of percent inhibition indicate growth stimulation.

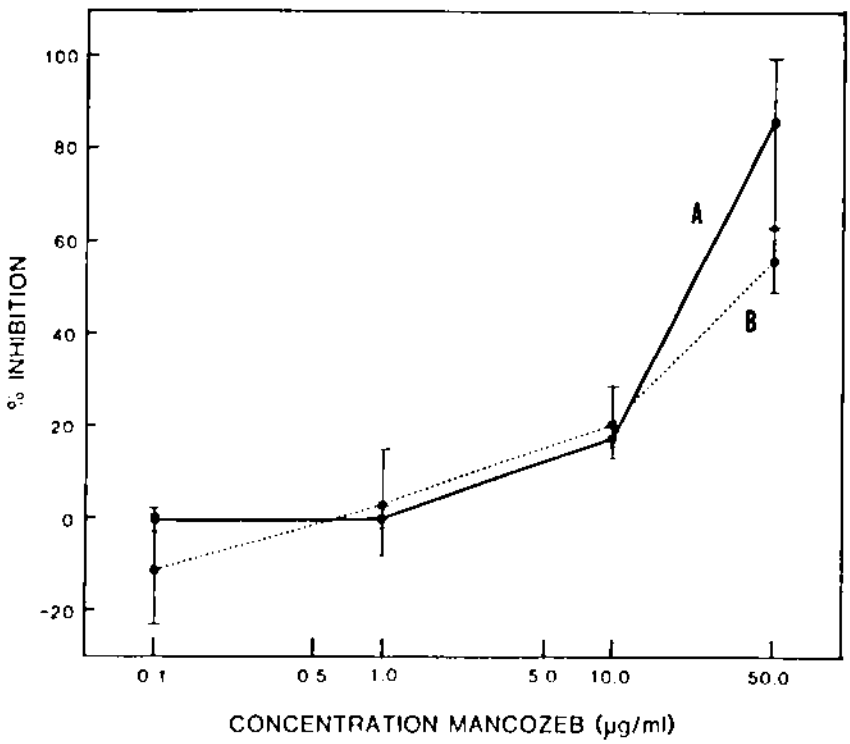


Figure 124. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on mancozeb-amended potato dextrose agar at 25 °C for 8 d. Average of five isolates of E. parasitica and four isolates of E. gyrosa; two replicate plates per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 71. Radial growth and percent inhibition of radial growth of isolates of E. parasitica and E. gyrosa cultured on anilazine-amended potato dextrose agar at 25°C for eight days.

Species	Isolate	Radial Growth (mm)					Percent Inhibition[a]			
		Concentration (µg/ml)					Concentration (µg/ml)			
		0	0.1	1	10	100	0.1	1	10	100
E. parasitica	E86	47.5[b]	50.0	49.0	38.0	6.0	-5.3[c]	-3.2	20.0	87.4
" "	E87	43.5	43.0	42.5	37.0	6.5	1.1	2.3	14.9	85.0
" "	E88	66.0	61.0	57.5	40.0	12.5	-1.7	4.2	33.3	79.2
" "	E107	51.0	50.0	48.5	36.0	12.5	2.0	4.9	29.4	75.5
" "	E155	48.5	50.0	48.5	37.5	4.0	-3.1	0.0	22.7	91.7
Species average		50.1	50.8	49.2	37.7	8.3	-1.4	1.6	24.1	83.8
Standard error		6.2	6.5	5.4	1.5	3.9	3.0	3.3	7.3	6.5
E. gyrosa	E30	22.5	27.5	22.0	16.5	8.0	-22.2	-2.2	26.7	64.4
" "	E37	27.0	28.5	27.0	19.5	7.5	-5.6	0.0	27.8	72.2
" "	E48	21.0	24.5	22.5	16.5	2.0	-16.7	-7.1	21.4	90.5
" "	E98	26.5	30.5	30.0	19.0	8.5	-15.1	-13.2	28.3	67.9
Species average		24.2	27.8	25.4	17.9	6.5	-14.9	-5.6	26.0	73.8
Standard error		3.0	2.5	3.8	1.6	3.0	6.9	5.9	3.2	11.6

Table 71 (continued)

[a]Percent inhibition was calculated from radial growth based on the following formula:
Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Measurement of one colony.

[c]Negative values of percent inhibition indicate growth stimulation.

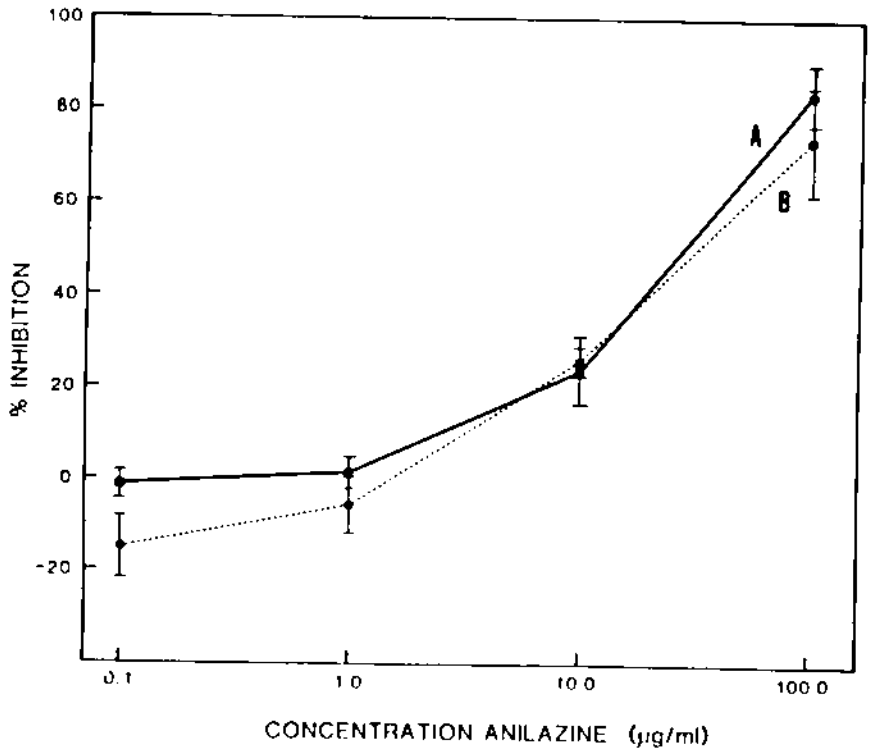


Figure 125. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on anilazine-amended potato dextrose agar at 25 °C for 8 d. Average of five isolates of E. parasitica and four isolates of E. gyrosa; one plate per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Table 72. Average radial growth and percent inhibition of average radial growth of E. parasitica and E. gyrosa cultured on triadimefon-amended potato dextrose agar at 25°C for eight days.

Species	Isolate	Radial Growth (mm)						Percent Inhibition[a]				
		Concentration (μg/ml)						Concentration (μg/ml)				
		0	0.01	0.1	1	10	100	0.01	0.1	1	10	100
E. parasitica	E86	32.0[b]	32.0	31.0	10.0	0.0	0.0	0.0	3.1	68.8	100.0	100.0
" "	E88	41.0	41.0	40.0	19.0	0.0	0.0	0.0	2.4	53.7	100.0	100.0
" "	E89	35.0	36.0	28.0	12.0	0.0	0.0	-2.9[c]	20.0	65.7	100.0	100.0
" "	E95	37.5	37.0	31.5	6.5	0.0	0.0	1.3	16.0	82.7	100.0	100.0
" "	E155	35.0	36.0	33.5	15.5	0.0	0.0	-2.9	4.3	55.7	100.0	100.0
Species average		36.1	36.4	32.8	12.6	0.0	0.0	-0.9	9.2	65.3	100.0	100.0
Standard error		3.4	3.2	4.5	4.8	0.0	0.0	1.9	8.2	11.6	0.0	0.0
E. gyrosa	E30	12.5	11.5	11.0	6.5	0.0	0.0	8.0	12.0	48.0	100.0	100.0
" "	E38	11.5	11.5	10.5	6.0	0.0	0.0	0.0	8.7	47.8	100.0	100.0
" "	E48	9.5	9.0	9.5	4.5	0.0	0.0	5.3	0.0	52.6	100.0	100.0
" "	E51	20.0	22.5	8.5	5.0	0.0	0.0	-12.5	57.5	75.0	100.0	100.0
" "	E154	12.5	13.5	11.5	4.5	0.0	0.0	-8.0	8.0	64.0	100.0	100.0
Species average		13.2	13.6	10.2	5.3	0.0	0.0	-1.4	17.2	57.5	100.0	100.0
Standard error		4.0	5.2	1.2	0.9	0.0	0.0	8.7	22.9	11.8	0.0	0.0

Table 72 (continued)

[a]Percent inhibition was calculated from radial growth based on the following formula:
Percent Inhibition = (1-ra/rc) x 100, where ra = colony radius on amended medium and rc = colony radius on nonamended medium.

[b]Average of two colonies.

[c]Negative values of percent inhibition indicate growth stimulation.

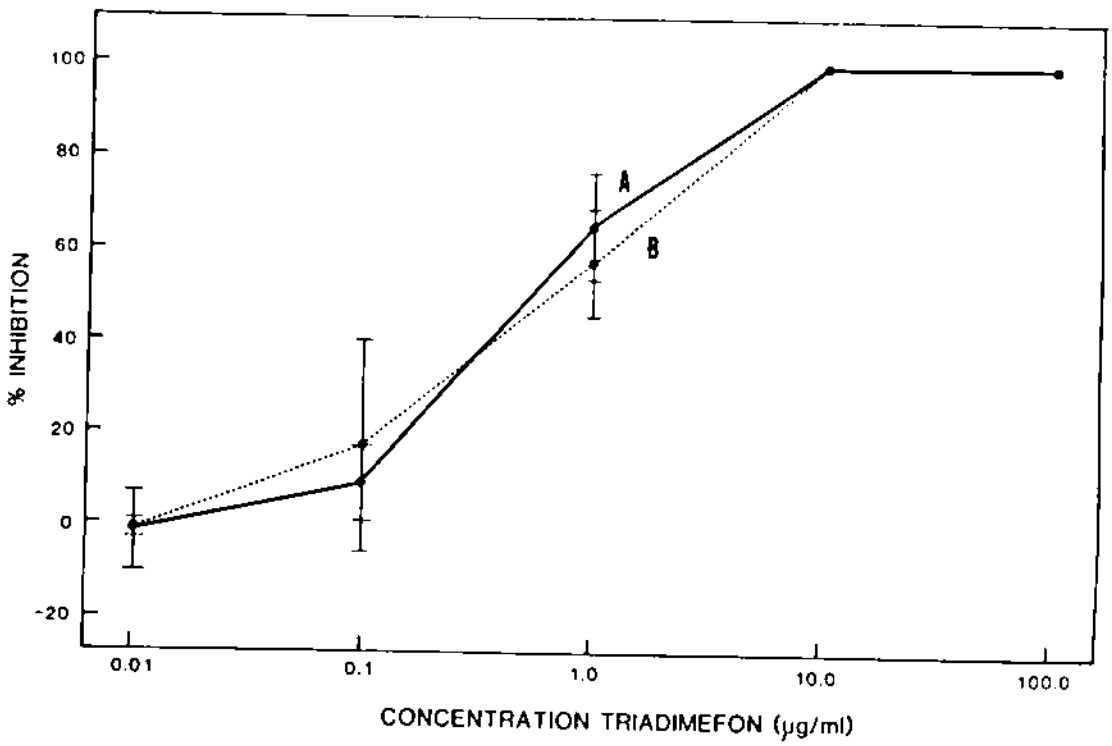


Figure 126. Dosage-response curve of Endothia parasitica (A) and E. gyrosa (B) grown on triadimefon-amended potato dextrose agar at 25 °C for 8 d. Average of five isolates; two replicate plates per isolate. Vertical bars represent standard error of the mean. Linear regression plotted by Statistical Analysis Systems (SAS). Concentration plotted on a logarithmic scale.

Chapter 5

Conclusions

Barr's recent revision of the order Diaporthales (16) included a drastic alteration in the classification and composition of the genus Endothia. Although the familial position of this genus has varied over the years, its placement in the Diaporthaceae by Müller and Von Arx (124) was not questioned until Barr's modification. Members of Endothia were traditionally distinguished by the production of 1-celled, allantoid or 2-celled, ovoid or ellipsoid ascospores within long-necked perithecia. Pycnidia and perithecia are both produced in brilliantly pigmented, erumpent stromata. Barr reassigned the genus to the family Gnomoniaceae and greatly restricted its parameters. Only those species which produce unicellular, allantoid ascospores in upright perithecia with central beaks are included in this new delineation of the genus. Additional criteria include the production of pseudoparenchymatous tissue within diatrypoid stromata. The only species retained in the genus are: E. gyrosa, the type species, E. singularis and E. viridistroma. The remaining fungi previously placed in Endothia were transferred to the genus Cryphonectria of the Valsaceae. This genus is char acterized by the production of 2-celled, ovoid or ellipsoid ascospores in tilted, circinate perithecia. The perithecial necks are usually oblique and converge in the ecto-stromatic disc. Stromata are prosenchymatous and valsoid in

arrangement. The species contained within this genus are: C. gyrosa (= E. tropicalis), the type species, C. parasitica (= E. parasitica), C. radicalis (= E. radicalis), C. havanensis (= E. havanensis), C. nitschkei (= E. japonica) and C. macrospora (= E. macrospora). Former members of Endothia which were not mentioned by Barr but which are presumably included in Cryphonectria due to their morphology are: E. coccolobii, E. eugeniae and E. longirostris. An additional species, C. cubensis, is suspected of being conspecific with E. eugeniae (C. S. Hodges, personal communication).

The genus Endothia traditionally includes some extremely important plant pathogens. Endothia parasitica is the causal agent of one of the most devastating diseases in the history of phytopathology. Chestnut blight is possibly the only plant disease in which the host has been brought close to extinction; the fungus also produces cankers on oaks, primarily Quercus virginiana. Eucalyptus canker, induced by C. cubensis, is significantly reducing yields of eucalyptus throughout the tropics (83), while E. gyrosa is responsible for the blighting of pin and willow oak in the southeastern United States. The discovery of hypovirulent strains of E. parasitica has caused a resurgence of interest in these fungi. For these reasons, the classification of these organisms needs to be resolved so that researchers can identify and communicate their findings about this group of fungi. The goals of this investigation were to clarify the relationships among the species of Endothia and to

determine whether Barr was justified in splitting the genus. It included: a reexamination of the morphological criteria used by Barr in separating the genera, a study of electrophoretic banding patterns produced by different species and a search for differential sensitivities of the fungi to a variety of antibiotics and xenobiotic agents.

The criteria used by Barr were correct. Species retained in Endothia produced allantoid, 1-celled ascospores in upright perithecia. Perithecial necks were usually perpendicular to the stromal surface although they occassionally diverged to the stromal edge. The diatrypoid stromata were widely erumpent and consisted of an entostromatic disc with host cells distributed evenly throughout its interior. Those fungi transferred to Cryphonectria produced 2-celled, ovoid or ellipsoid ascospores. Perithecia along the periphery of the stroma were oblique; their necks converged to the stromal center before growing through to the stromal surface. Perithecia in the center of the stroma were generally upright and produced necks perpendicular to the stromal surface. The stroma itself was valsoid, with an ectostromatic, erumpent disc and deeply immersed entostroma. The valsoid appearance was sometimes obscured in smaller stromata which produced large numbers of perithecia or pycnidia due to the reduction of vegetative tissue.

In her monograph, Barr (16) reported the presence of pseudoparenchymatous tissue in Endothia and prosenchymatous tissue in

Cryphonectria. These terms are meant to give an overall impression of the stroma and are not mutually exclusive. Barr (17) readily admitted that the stromata of Cryphonectria are more condensed at the margins, especially in the ectostromatic disc, and may appear pseudoparenchymatous in these regions. The stromata of Endothia are more loosely arranged in spaces between the perithecia and the margins and may then appear prosenchymatous. This more flexible interpretation corresponds to the descriptions by Kobayashi (93) and to the observations in this study. Pseudoparenchymatous cells were observed at magnifications of 1000X along the borders of the stromata, while prosenchyma was noted in the center of the same sections. The staining properties of the two groups were somewhat different; stromata of E. gyrosa, E. singularis and E. viridistroma often stained darker and were more difficult to section than those of species transferred to Cryphonectria. This indicates a difference in the texture or chemistry of the stromata and therefore reflects taxonomic differences in the two groups.

If Barr's classification system is adopted, it will be necessary for plant pathologists and mycologists to be able to differentiate between the two genera and among the different species of each genus. Species identification is difficult even when the species are all contained within a single genus. Specimens collected in the field often do not contain perithecia and ascospores; differences among anamorphs are not sufficient to distinguish among species. For these reasons, a series of chemo-

taxonomic studies were conducted to determine whether alternative means of identification could be used.

The pigment content of certain isolates was determined. The production of skyrin and oxyskyrin is reported for the first time in E. eugeniae and C. cubensis. Rugulosin, a third bisanthraquinone frequently found in Endothia cultures, was not formally assayed since the purified standard had broken down with time. Rugulosin is a brilliant yellow pigment; no such pigment was detected in extracts of E. eugeniae and C. cubensis. Its absence is therefore suspected but cannot be substantiated due to the lack of purified standard. Isolates of E. radicalis did not have consistent pigment distributions; this substantiates the work of Roane and Stipes (151) and may indicate that some of these organisms have been misidentified. Pigment content cannot be used to differentiate among the various species of Endothia and Cryphonectria as there are considerable overlaps and inconsistencies in presence and synthesis.

Polyacrylamide gel electrophoresis of soluble proteins was also used to examine relationships among isolates of the different species. A total of 101 monoconidial isolates, representing eleven species of Endothia plus Cryphonectria cubensis, was studied by PAGE in a nondissociating, discontinuous system. Preliminary studies demonstrated that slight variations in banding patterns occurred at different dates of harvest. The collection date was therefore standardized at 8 d ; all isolates

studied in preliminary tests were in the logarithmic portion of the growth curve and minimal sporulation was observed at this time. Variations in banding patterns could then be attributed to differences in taxonomy since cultural conditions were standardized.

Interspecific differences in banding patterns were greater than intraspecific variation among isolates of all species except E. radicalis. Isolates of E. parasitica were easily differentiated from those of E gyrosa. The banding patterns of E. gyrosa isolates were strikingly uniform, even when collected from different hosts and different geographical origins. This uniformity may relate to the infrequency of perithecia and ascospores located in field specimens. The ten isolates of E. radicalis produced diverse banding patterns and did not cluster together. It is possible that some of these cultures may have been misidentified. No one has isolated this fungus in recent years, and its morphological resemblance to E. parasitica complicates its identification. Acquisitions in established culture collections may represent a variety of nonrelated fungi.

A limited number of isolates were available for studies with the remaining species. These isolates clustered by species, but frequently with a rather low coefficient of similarity. The amount of intraspecific variation cannot be accurately estimated for these species due to the lack of sufficient isolates. Hypovirulent strains of E. parasitica could not be differentiated from wild-type isolates. This is not surprising since hypo-

virulence has not been associated with specific gene products.

There was no separation of species into two major groups representing the genera Endothia and Cryphonectria. Electrophoresis frequently fails to delineate intergeneric differences and probably should not be used at taxonomic levels higher than the species. For this reason, PAGE could not be used to support or refute Barr's classification system.

Some interesting relationships were found among the different isolates. Protein profiles from isolates of E. eugeniae, a pathogen of cloves, could not be distinguished from those of C. cubensis, a pathogen of eucalyptus, despite some intraspecific variation in each group. This indicates that the two species are closely related or are actually conspecific. Hodges (personal communication) and Alfenas (personal communication) have argued for the conspecificity of these organisms on the basis of morphology, cross-inoculation studies and isozyme analysis. This study supports their contention. CBS isolate 505.16 (E40) produced a banding pattern similar to those formed by other isolates of this group. This culture is widely used as a reference of E. havanensis; its identity is questioned. Isolate H183, also originally identified as E. havanensis, displayed patterns similar to those of the E. eugeniae - C. cubensis group, while two unidentified fungi (E158 and E159) obtained from eucalyptus in Florida did not produce this protein profile. These two isolates may represent legitimate specimens of E.

havanensis, but their identification must remain tentative due to a lack of reference cultures of this species. Isolate H182, originally identified as C. cubensis, did not appear to belong to this group.

Results obtained by general protein studies were confirmed with specific activity stains for the enzymes: α- and β-esterase, acid and alkaline phosphatase, and β-D-glucosidase. Endothia parasitica and E. gyrosa were easily separated on gels stained for α- and β-esterase and acid and alkaline phosphatase. Isolates of E. radicalis produced fairly diverse banding patterns, although some similarities were noted in isolates E16, E42, E76, E150 and E152 for α- and β-esterase and acid and alkaline phosphatase. These may represent true isolates of E. radicalis. All of these cultures produced perilla purple when grown on white cornmeal - a classical test for E. radicalis isolates. Cultures E64 and E67, previously identified as E. radicalis from Australia, produced banding patterns similar to those of E. tropicalis for β-D-glucosidase and α- and β-esterase. The colony morphologies of these isolates were also quite similar.

Extracts from isolates of E. eugeniae and C. cubensis were studied with specific activity stains. Similar banding patterns were obtained for these taxa, as with the general protein stain. The similarities were especially striking for α- and β-esterase; gels stained for acid and alkaline phosphatase showed poor resolution and the fungi were monomorphic for β-D-glucosi-

dase. CBS isolate 505.63 and H183 also belonged to this group, while isolate H182 was obviously misidentified.

The remaining species produced unique banding patterns for these enzymes and could be easily differentiated from each other. These procedures are no more difficult than is the general protein stain, and the results are much easier to interpret. Isozyme analysis can also be explained at the genetic level; this would be an interesting area for future research. A determination of genetic similarity may be able to resolve the suitability of Barr's classification system.

Sensitivity to a series of antibiotics and xenobiotic agents was also used as a chemotaxonomic trait. The objective of these tests was to determine whether compounds could be found to which the species would be differentially sensitive. This information could then be used to construct a differential medium which would distinguish among species in the anamorphic state. Differential sensitivities to a particular compound would also reveal information about biochemical differences between the organisms, especially if the mode of action of the fungitoxicant is known. The sensitivities of isolates of E. parasitica and E. gyrosa were determined for 23 fungitoxicants representing different classes of antibiotics and xenobiotic agents. These two species were differentially sensitive to cycloheximide, an antifungal antibiotic. This was determined by the incorporation of cycloheximide into molten PDA at increasing concentrations after autoclaving;

inhibition of radial growth was then determined by comparison to growth of colonies on a nonamended medium. The approximate ED_{50} values of E. parasitica and E. gyrosa were 1-2 µg/ml and 0.01-0.03 µg/ml respectively. This represents a hundred-fold difference in sensitivity to the compound and may represent a disparity in the protein content of the ribosomal subunits of the two species. Subsequent tests with eight other species of Endothia plus C. cubensis revealed that the genus could be divided into two groups based upon cycloheximide sensitivity. Species which were extremely sensitive to cycloheximide (ED_{50} < 0.1 µg/ml) included: E. gyrosa, E. singularis, and the two unidentified cultures isolated from eucalyptus in Florida. The remaining species were more tolerant (ED_{50} > 0.1 µg/ml) and produced dosage- response curves similar to those of E. parasitica. These species included: E. radicalis, E. tropicalis, E. macrospora, E. coccolobii, E. viridistroma, E. eugeniae and C. cubensis. Isolates E40 (CBS505.63) and H183, originally identified as E. havanensis but subsequently shown to resemble E. eugeniae and C. cubensis by PAGE, also belonged to this group. This separation of species corresponds with the division of Endothia and Cryphonectria by Barr with the exception of E. viridistroma; this species is much more resistant to cycloheximide than are the other unicellular ascosporic species. Endothia viridistroma is the only member of the genus which does not produce brilliantly pigmented stromata, and it was collected only upon a single occassion. The identification of this

organism should be reexamined in light of this biochemical difference.

Isolates of E. parasitica and E. gyrosa were not differentially sensitive to the remaining compounds; each responded in a similar manner or intraspecific variation among isolates was too great to detect interspecific differences. Various modes of action were represented by these compounds; the two major species responded similarly to all of these agents and likely possess many identical biochemical processes. One interesting area for future research would be to explore the differences in cycloheximide sensitivity among the various species at the biochemical level. Are different proteins present in the ribosomal subunits of each species, or is the selective toxicity due to differences in permeability or detoxification mechanisms?

An important limitation in any taxonomic study is the lack of any single, unifying definition of the term "species". Even Darwin (41) refused to elucidate his concept of the term and stated "no one definition has satisfied all naturalists; yet every naturalist knows vaguely what he means when he speaks of a species." Two opposing concepts have developed over the years. In the view of the "traditional" taxonomist, species are real entities; taxonomic systems are equivalent to scientific theories and can be proven or disproven (51). The philosophy here is that "individuals do not belong in the same taxon because they are

similar, but they are similar because they belong to the same taxon (182). The alternative concept is that "nature produces individuals and nothing more...species have no actual existence in nature. They are mental concepts... species have been invented in order that we may refer to great numbers of individuals collectively" (21). In this view, classification systems are merely ways of summarizing information and cannot be judged correct or incorrect; they are evaluated on their usefulness (51). These two views have been reconciled by the adoption of different species concepts. Mycologists have traditionally utilized the morphological species, in which taxonomic decisions are based on similarities and differences in morphology and physiology. This concept is dominated by the type system, which rigidly maintains that all members of a taxon conform to a single type specimen. This system minimizes any variation that can exist within the taxon and has lead to the creation of many extraneous species (107). Barr's classification system and the traditional view of Endothia taxonomy are both representatives of the morphological species concept.

Most modern taxonomists recognize that there are dynamic populations of organisms whose members may be quite variable in terms of morphology and physiology. One example of this is the proposed conspecificity of E. eugeniae and C. cubensis by Hodges (personal communication) and Alfenas (personal communication) which recognizes the influence of the host on the morphology of the organism. Type specimens lose their descriptive significance

and are reduced to nomenclatural references when such variation is recognized (107).

Alternative species concepts are the biological and ecological species. The biological species concept utilizes reproductive isolation as the sole means of distinguishing among species. Members of the same species share a common gene pool; the species is therefore dynamic and varies with the evolutionary process. This definition would be difficult to apply to the taxonomy of Endothia on a practical level. Isolates of E. parasitica have been successfully crossed in the laboratory (6); isolates of the other species have not. This is a very time-consuming process and would not take into account differences in geographic location and environmental factors which influence reproductive success in nature.

The evolutionary species is philosophically appealing but also has limitations in practical use. Dobzhansky (49) defines an evolutionary species as an array of related gene combinations that occupy an adaptive peak; the summit of that peak is held by those individuals most fit for a given environment. Variation within the species is represented by individuals at different levels on the peak. This concept allows the recognition of individual species without intermediate genotypes; adaptive peaks are not available for all gene combinations. Studies of population and evolutionary genetics are necessary to discern evolutionary species; Endothia taxonomy has not progressed to

this level.

Evolutionary relationships may be more readily detected in developmental studies than by morphology alone. The developmental process of Endothia has been studied (101). Members of the genus produce a Diaporthe-type centrum, in which branches of the ascogonium or neighboring vegetative cells envelop the ascogonium to produce the perithecial initial. The outer layer then differentiates into the perithecial cell wall, while the central portion forms a pseudoparenchymatous centrum. These pseudoparenchymatous cells disintegrate upon the expansion of the asci to form the perithecial cavity. The asci are clavate, short-stalked and thin-walled except at the apex where the wall is thickened and penetrated by a pore; this pore is surrounded by a light-refractive ring. The stalks of the asci deliquesce at maturity, and the intact asci move up through the perithecial neck. At the ostiole, the asci rupture and the ascospores are simultaneously discharged. This is termed an "Endothia-type" ascus. Additional developmental differences may be found between species of Endothia and those transferred to Cryphonectria which would further support Barr's (16) division of the genus. Developmental studies were not attempted in this study due to the lack of perithecial formation by E. gyrosa in inoculated pin oaks and the risk of releasing exotic plant pathogens into the environment.

The taxonomy of Endothia is still dependent on morphology and physiology. Barr's classification system is technically

correct and should probably be adopted since it organizes relationships within the entire order. This recommendation is made with some hesitation since further studies are needed to determine the influence of host on stromal development. It would also be beneficial for other researchers to reexamine Barr's placement of additional genera to confirm the usefulness of this system. Future studies in developmental morphology and population genetics may allow a different interpretation of this group of fungi. Certain anamorphic forms of these organisms can be distinguished by polyacrylamide gel electrophoresis and their differential sensitivities to cycloheximide. Additional chemotaxonomic procedures should be pursued to facilitate species identification and to support or refute Barr's classification system.

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Appendix I. Correspondence with Dr. M. E. Barr Bigelow concerning the definitions of "valsoid" and "diatrypoid" stromata and the distribution of prosenchymatous and prosenchymatous tissue.

The Commonwealth of Massachusetts
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BOTANY DEPARTMENT

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5 November 1984

Jessie A. Micales
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Dear Ms. Micales,

I will try to answer your questions concerning terminology. My interpretation of the terms "valsoid" and "diatrypoid" includes not only stromatic configuration but the position and arrangement of perithecia also. The classical use is as "distinctive stroma configuration" well illustrated by Talbot, P.H.B. 1971 (Principles of fungal taxonomy, fig. 63, p. 180), but he too included perithecia in his illustrations. Typically, the valsoid stroma in species of Cryphonectria is prosenchymatous, may be slightly or well developed, may and often does include substrate cells, is more condensed at margins, especially the ectostromatic disc, and there may appear pseudoparenchymatous in these regions. The diatrypoid stroma in species of Endothia is typically pseudoparenchymatous, quite well developed, may sometimes incorporate substrate cells, may be more loosely arranged in spaces between perithecia and margins and may then appear prosenchymatous. (So some convergence of the two types here.)

Perithecia in species of Cryphonectria are typically circinate and tilted, with oblique beaks often converging to or through the usually small ectostromatic disc; beaks may reach only to surface or may extend far beyond. And in some cases the stromatic tissue is reduced to a weft around the upper parts of perithecia. Perithecia in species of Endothia are typically upright, mono- or polystichous, with ventral beaks erumpent to the surface separately, usually not far beyond the surface of the stroma.

With both of these features defined, you can see a certain amount of overlap -- perhaps the best way of observing is at low magnification, or the details obscure the overall aspects. I use the dissecting microscope considerably for the "feel" of the fungus, before facing sections under low and high magnifications. And as I attempt to set down in words, I think it is indeed the impression that people are attempting to convey, rather than a precise regimentation of features that are mutually self-exclusive. Of course, the final point in morphological/anatomical details is that of the ascospores. To me, there is no way of reconciling the differences between those of the two genera as merely of specific value. There may be some valuable differences in anamorphs, and I hope that does come to be of use, especially since the fungi are more frequently collected in the anamorphic state. What about chemotaxonomic findings?

I shall be most interested to learn if they corroborate or not the other characters. That seems to be a prevalent hope, for more data to prove or disprove the classical ones that we use in separation.

I hope my ramblings will be of some assistance to you, and wish you well in defending your dissertation.

Sincerely,

M. E. Barr Bigelow

Appendix II: Formulations of culture media used in this study.

Glucose - yeast extract agar (gyea)

Glucose	5 g
Yeast extract	1 g
Micronutrient solution	2 ml
Agar	15 g
Distilled HOH	1000 ml

Micronutrient solution:

$Fe(NO_3)_3 \cdot 9H_2O$	723.5 mg
$ZnSO_4 \cdot 7H_2O$	439.8 mg
$MnSO_4 \cdot 4H_2O$	203.0 mg

q.s. to 1000 ml with distilled HOH; clarify by adding concentrated H_2SO_4 and filter.

"Double" glucose - yeast extract agar (dgyea)

Glucose	10 g
Yeast extract	2 g
Micronutrient solution	2 ml
Agar	15 g
Distilled HOH	1000 ml

Glucose - yeast extract broth (gyb)

Glucose	5 g
Yeast extract	1 g
Micronutrient solution	2 ml
Distilled HOH	1000 ml

Appendix III: Formulations and procedures for polyacrylamide electrophoresis.

A. Gel preparation

Stock solutions

A Acrylamide: Bis (30: 0.8)

15.0 g acrylamide
0.4 g N'N'-Bismethyleneacrylamide
q.s. to 50 ml in volumetric flask with dHOH, filter and store at 4 °C in the dark. Be extremely careful with this solution; it is highly toxic.

B 1.5 M Tris-Cl, pH 8.8

18.15 g Tris
50 ml dHOH; adjust to pH 8.8 with 2N HCl
q.s. to 100 ml in volumetric flask with dHOH.

C 0.5 M Tris-Cl, pH 6.8

6.0 g Tris
50 ml dHOH; adjust to pH 6.8 with 2N HCl
q.s. to 100 ml in volumetric flask with dHOH.

Separating gel (12% acrylamide, pH 8.8)

10.4 ml dHOH
7.5 ml 1.5 M Tris-Cl buffer, pH 8.8 (stock solution B)
12.0 ml A:Bis (stock solution A)
0.1 ml 10% ammonium persulfate (freshly prepared)
Degas under vacuum (20 psi) until all air bubbles are released from solution.
7.5 µl TEMED
Pour gel between horizontal glass plates. Overlayer with dHOH and allow approximately 40 min for polymerization. Pour off dHOH and rinse gel surface with 1.5 Tris-Cl buffer.

Stacking gel (3.9% acrylamide, pH 6.8)

5.7 ml dHOH
0.4 ml 0.05% bromophenol blue
2.5 ml 0.5 Tris-Cl, pH 6.8 (stock solution C)
1.3 ml A:Bis (stock solution A)
0.1 ml 10% ammonium persulfate (freshly prepared)
Degas as above.
5.0 µl TEMED
Pour gel and insert well forming combs. Allow approximately 30 min for polymerization, remove combs and rinse wells three times with 0.5 M Tris-Cl buffer. Remove excess buffer from wells with microsyringe.

Electrode buffer (0.05 M Tris + 0.38 M glycine):

6.0 g Tris
28.8 g Glycine
q.s. to 1 l in volumetric flask with dHOH.

Sample buffer:
1: 4 dilution of 0.5 M Tris-Cl buffer, pH 6.8 (stock solution C) + 10% glycerol.

B. Staining solutions

General protein stain (0.1% Coomassie brilliant blue):

1.0 g Coomassie brilliant blue - R250
416.5 ml dHOH
416.5 ml methanol
166.6 ml glacial acetic acid
Filter. Stain gels overnight.

Destaining reagent: 12.5% isopropanol + 10% glacial acetic acid in dHOH. Replace as dye diffuses from gel; usually takes several hours.

Acid and alkaline phosphatase (127):

25 mg Na-α-naphthylphosphate
100 mg Fast Red TRN
100 ml 0.6 M acetate buffer
Incubate at room temperature.

0.6 M acetate buffer, pH 5.0:
74 ml glacial acetic acid
176 ml 1.2 M sodium acetate

α- and β-esterase (164):

Substrate solution (1% α- and β-naphthylacetate):
1 g α-naphthyl acetate
1 g β-naphthyl acetate
50 ml acetone
50 ml dHOH

Stain solution:
100 mg Fast Blue RR
10 ml 0.5 M Tris-Cl buffer, pH 7.1
3 ml Substrate solution
87 ml dHOH
Incubate at room temperature.

β-D glucosidase (39):

Substrate solution:
100 ml absolute ethanol
100 ml dHOH
Bring to boil and add:
50 mg 6-bromo-2-naphthyl-B-D-glucopyranoside
100 ml phosphate citrate buffer, pH 4.95
200 ml dHOH
Place gel in substrate solution at 37 °C for 5 hr.
Increase pH to 7.5 with 19 ml 2.2 M Na_3PO_4
and couple with 50 ml tetrazotized diorthoanisidine
(100 mg/ 1000 ml) in cold dHOH. Fix and destain
gels in 7% acetic acid.

Appendix IV. Interpretative drawings of polyacrylamide electrophoretic gels from the first extraction of the first set and the second extraction of the second set of lyophilized mycelial powders.

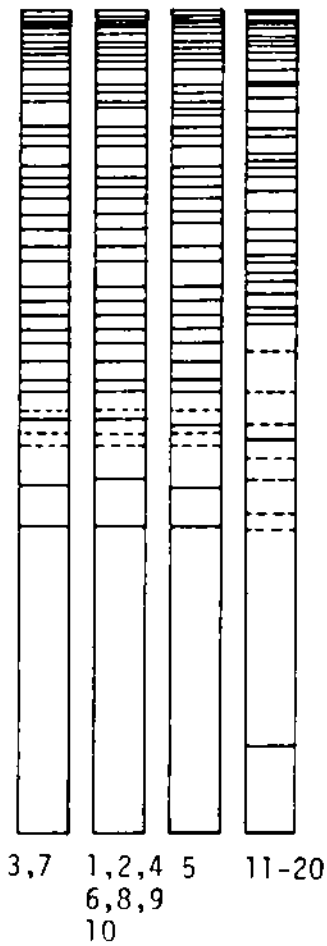

Figure 127. Interpretative drawing of polyacrylamide electrophoretic gel of buffer-soluble proteins of isolates of Endothia parasitica and E. gyrosa. Species and isolates by lane number (from left to right): E. parasitica 1 - E24, 2 - E85, 3 - E86, 4 - E87, 5 - E88, 6 - E89, 7 - E95, 8 - E96, 9 - E153, 10 - E155; E. gyrosa 11 - E18, 12 - E20, 13 - E30, 14 - E37, 15 - E38, 16 - E48, 17 - E50, 18 - E98, 19 - E145, 20 - E154. First extraction of first set of lyophilized mycelial powders.

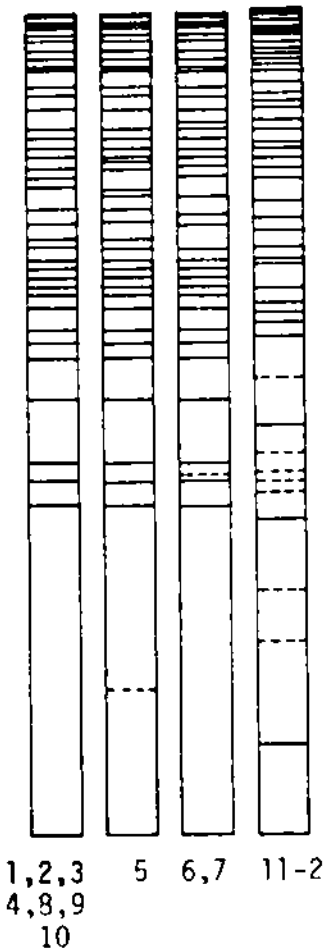

Figure 128. Interpretative drawing of polyacrylamide electrophoretic gel of buffer-soluble proteins of isolates of Endothia parasitica and E. gyrosa. Species and isolates by lane number (from left to right): E. parasitica 1 - E84, 2 - E85, 3 - E86, 4 - E87, 5 - E88, 6 - E89, 7 - E95, 8 - E96, 9 - E153, 10 - E155; E. gyrosa 11 - E18, 12 - E20, 13 - E30, 14 - E37, 15 - E38, 16 - E48, 17 - E50, 18 - E98, 19 - E145, 20 - E154. Second extraction of second set of lyophilized mycelial powders.

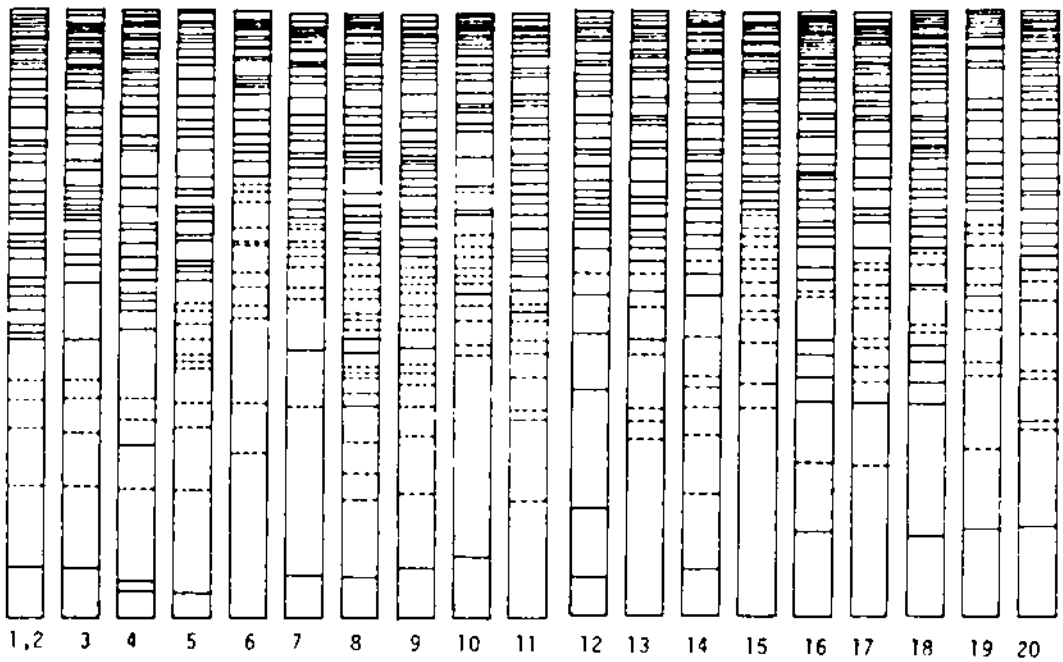

Figure 129. Interpretative drawing of polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia species. Species and isolates by lane number (from left to right): E. radicalis 1 - E16, 2 - E42, 3 - E56, 4 - E64, 5 - E67, 6 - E75, 7 - E76, 8 - E92, 9 - E150; E. viridistroma 10 - E41; E. tropicalis 11 - E57; E. singularis 12 - E52; E. havanensis 13 - E40, 14 - E158; E. coccolobii 15 - E157; E. macrospora 16 - E54; E. japonica 17 - E53, 18 - E59; E. eugeniae 19 - E138, 20 - E139. First extraction of first set of lyophilized mycelial powders.

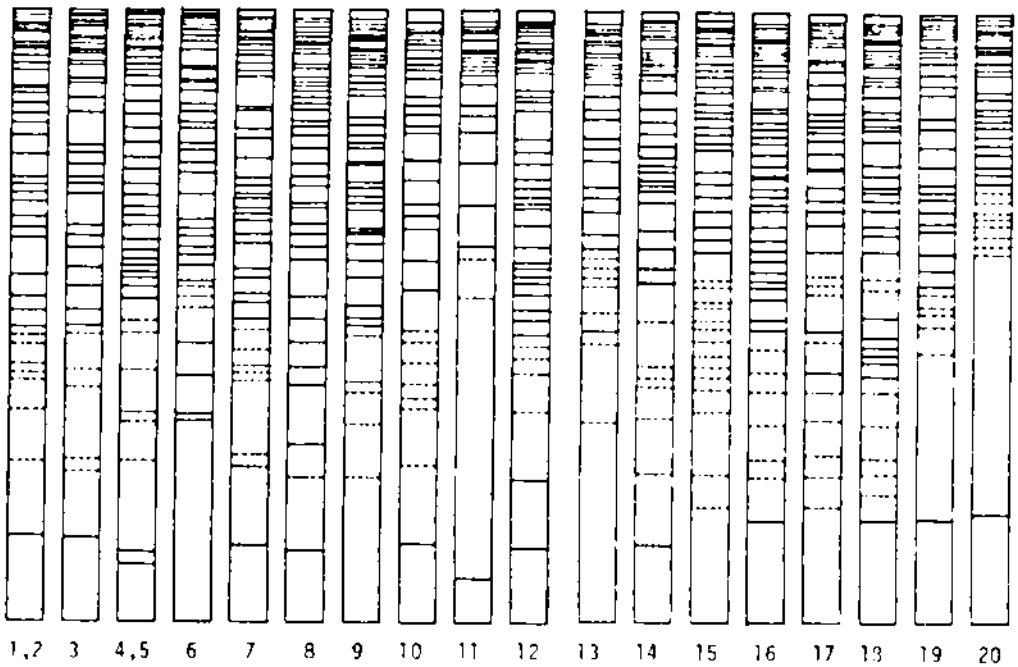

Figure 130. Interpretative drawing of polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia species. Species and isolates by lane number (from left to right): E. radicalis 1 - E16, 2 - E42, 3 - E56, 4 - E64, 5 - E67, 6 - E75, 7 - E76, 8 - E92, 9 - E150; E. viridistroma 10 - E41; E. tropicalis 11 - E57; E. singularis 12 - E52; E. havanensis 13 - E40, 14 - E158; E. coccolobii 15 - E157; E. macrospora 16 - E54; E. japonica 17 - E53, 18 - E59; E. eugeniae 19 - E138, 20 - E139. Second extraction of second set of lyophilized mycelial powders.

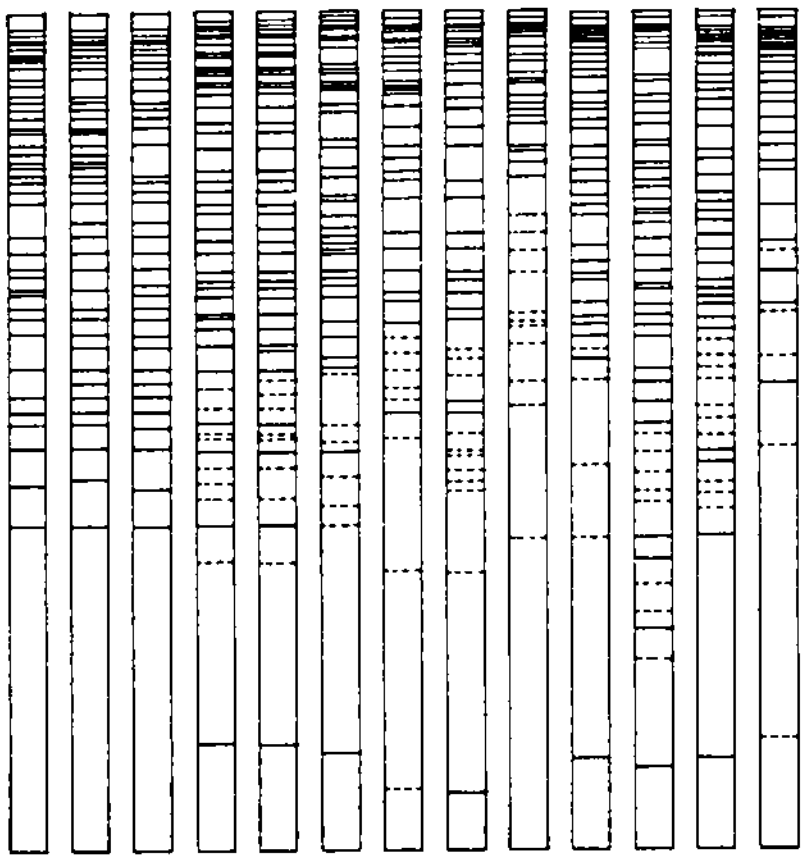

Figure 131. Interpretative drawing of polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia parasitica, E. radicalis and E. viridistroma. Species and isolates by lane number (from left to right): E. parasitica 1 - E24, 2 - E85, 3 - E86, 4 - E87, 5 - E88, 6 - E89, 7 - E95, 8 - E96, 9 - E153, 10 - E155; E. radicalis 11 - E16, 12 - E42, 13 - E56, 14 - E64, 15 - E67, 16 - E75, 17 - E76, 18 - E92, 19 - E150; E. viridistroma 20 - E41. First extraction of first set of lyophilized mycelial powders.

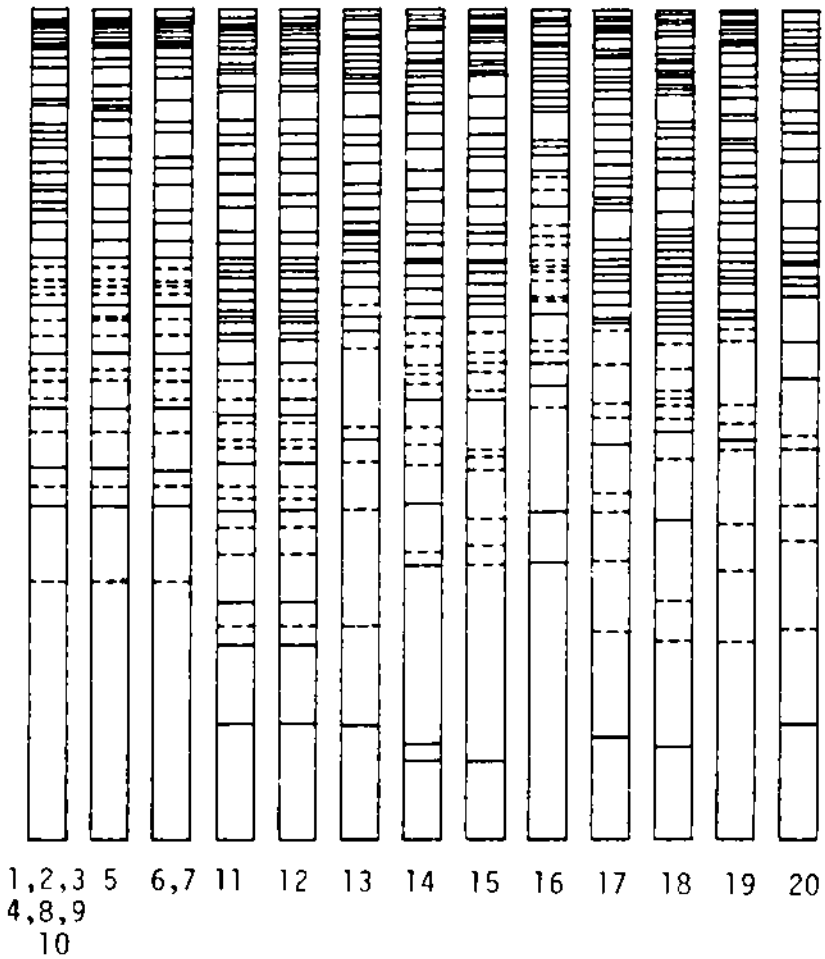

Figure 132. Interpretative drawing of polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia parasitica, E. radicalis and E. viridistroma. Species and isolates by lane number (from left to right): E. parasitica 1 - E24, 2 - E85, 3 - E86, 4 - E87, 5 - E88, 6 - E89, 7 - E95, 8 - E96, 9 - E153, 10 - E155; E. radicalis 11 - E16, 12 - E42, 13 - E56, 14 - E64, 15 - E67, 16 - E75, 17 - E76, 18 - E92, 19 - E150; E. viridistroma 20 - E41. Second extraction of second set of lyophilized mycelial powders.

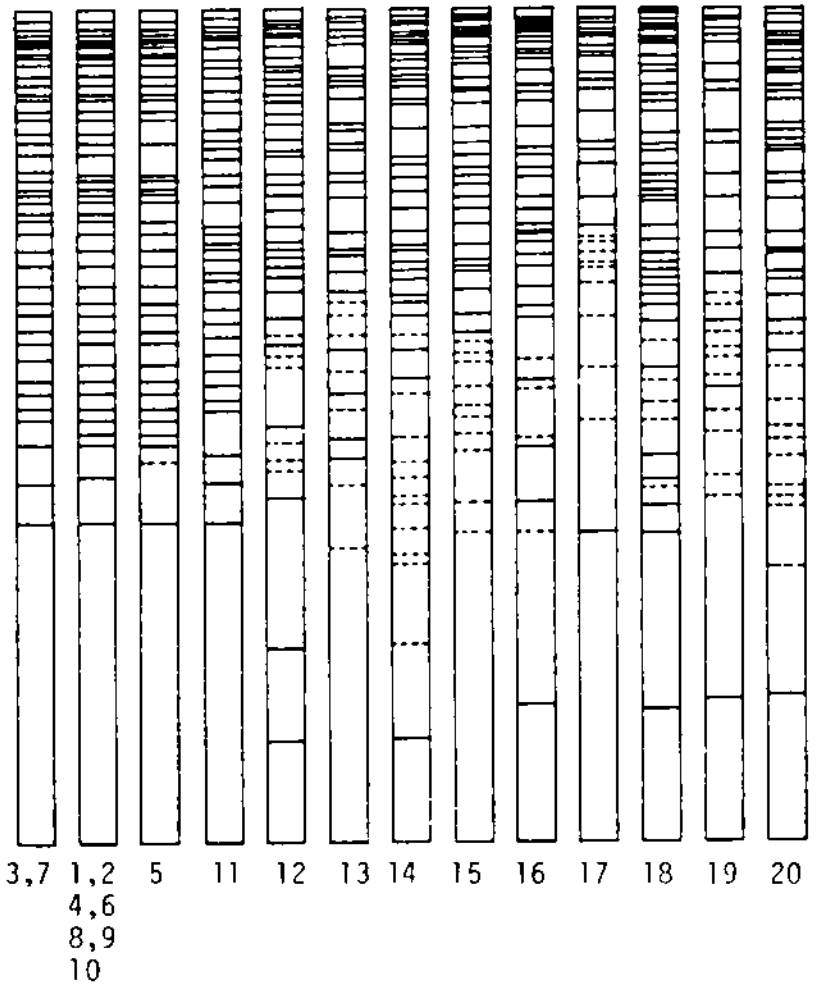

Figure 133. Interpretative drawing of polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia parasitica and uncommon Endothia species. Species and isolates by lane number (from left to right): E. parasitica 1 - E24, 2 - E85, 3 - E86, 4 - E87, 5 - E88, 6 - E89, 7 - E95, 8 - E96, 9 - E153, 10 - E155; E. tropicalis 11 - E57; E. singularis 12 - E52; E. havanensis 13 - E40, 14 - E158; E. coccolobii 15 - E157; E. macrospora 16 - E54; E. japonica 17 - E53, 18 - E59; E. eugeniae 19 - E138, 20 - E139. First extraction of first set of lyophilized mycelial powders.

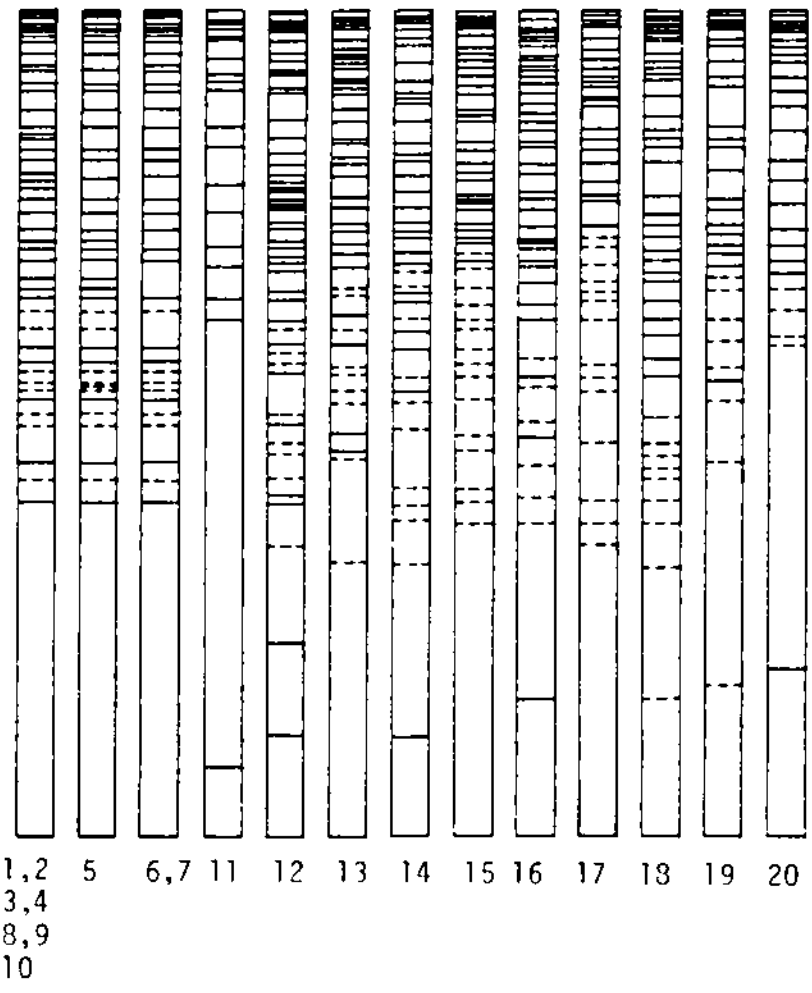

Figure 134. Interpretative drawing of polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia parasitica and uncommon Endothia species. Species and isolates by lane number (from left to right): E. parasitica 1 - E24, 2 - E85, 3 - E86, 4 - E87, 5 - E88, 6 - E89, 7 - E95, 8 - E96, 9 - E153, 10 - E155; E. tropicalis 11 - E57; E. singularis 12 - E52; E. havanensis 13 - E40, 14 - E158; E. coccolobii 15 - E157; E. macrospora 16 - E54; E. japonica 17 - E53, 18 - E59; E. eugeniae 19 - E138, 20 - E139. Second extraction of second set of lyophilized mycelial powders.

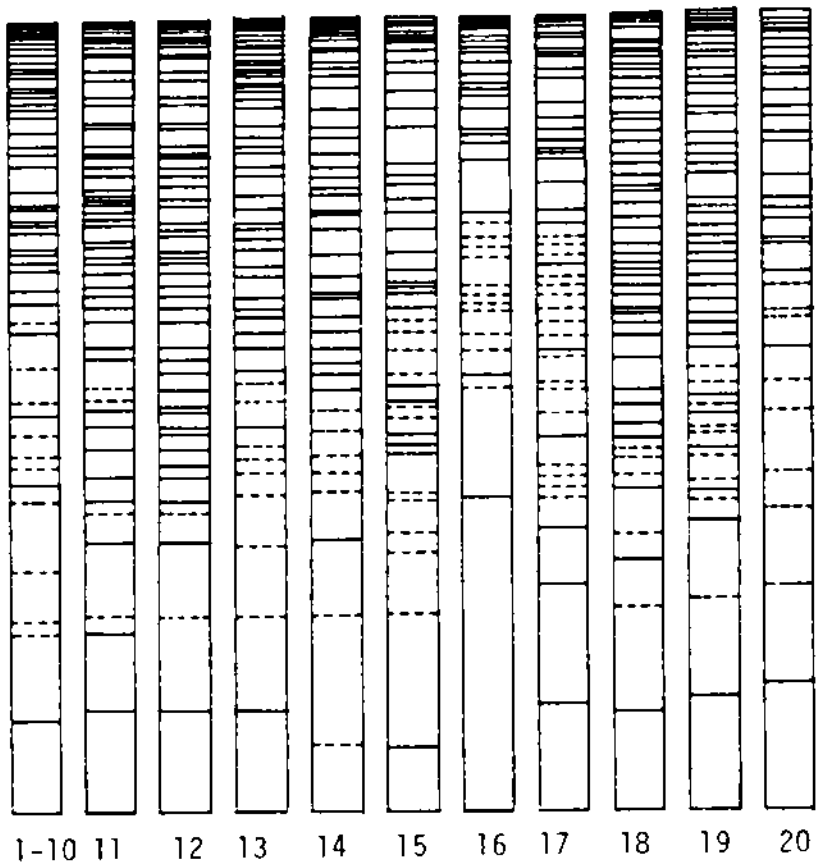

Figure 135. Interpretative drawings of polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia gyrosa, E. radicalis and E. viridistroma. Species and isolates by lane number (from left to right): E. gyrosa 1 - E18, 2 - E20, 3 - E30, 4 - E37, 5 - E38, 6 - E48, 7 - E50, 8 - E98, 9 - E145, 10 - E154; E. radicalis 11 - E16, 12 - E42, 13 - E56, 14 - E64, 15 - E67, 16 - E75, 17 - E76, 18 - E92, 19 - E150; E. viridistroma 20 - E41. First extraction of first set of lyophilized mycelial powders.

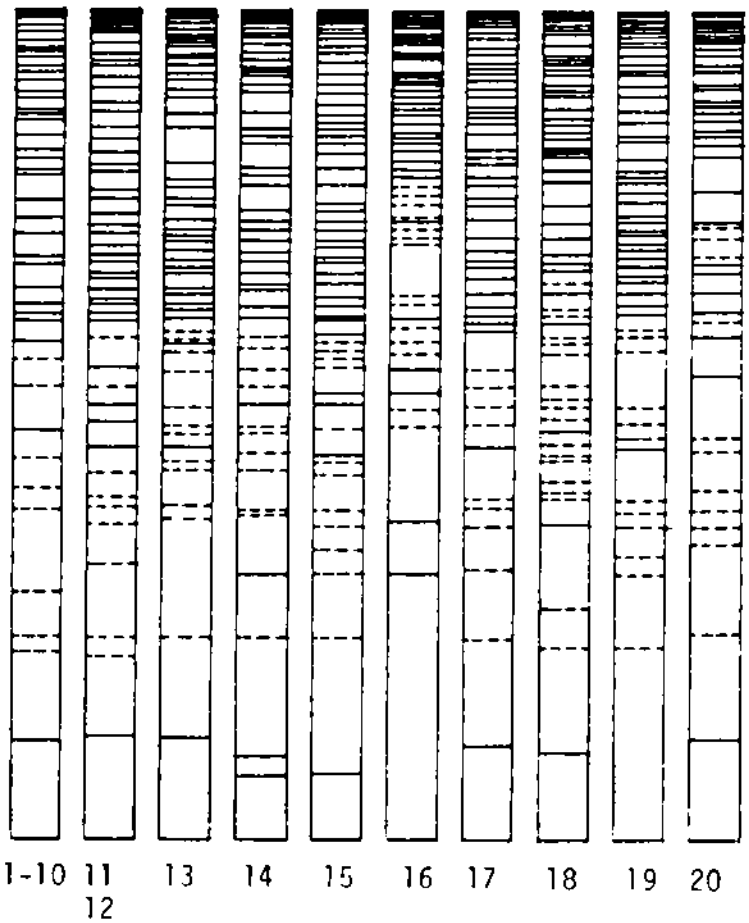

Figure 136. Interpretative drawings of polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia gyrosa, E. radicalis and E. viridistroma. Species and isolates by lane number (from left to right): E. gyrosa 1 - E18, 2 - E20, 3 - E30, 4 - E37, 5 - E38, 6 - E48, 7 - E50, 8 - E98, 9 - E145, 10 - E154; E. radicalis 11 - E16, 12 - E42, 13 - E56, 14 - E64, 15 - E67, 16 - E75, 17 - E76, 18 - E92, 19 - E150; E. viridistroma 20 - E41. Second extraction of second set of lyophilized mycelial powders.

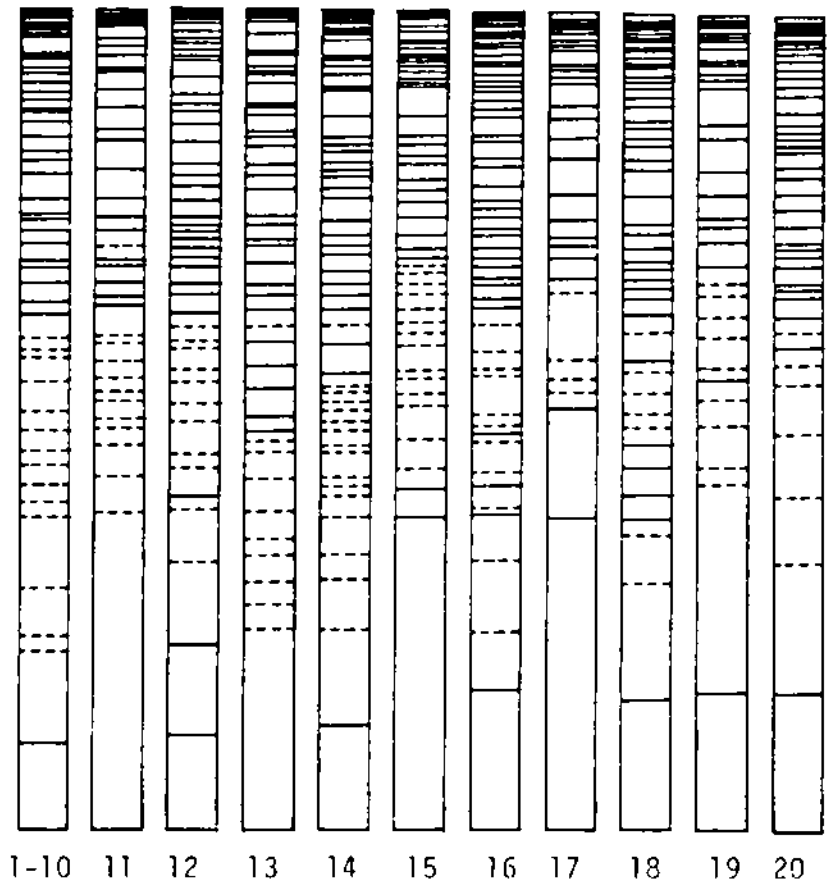

Figure 137. Interpretative drawing of polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia gyrosa and uncommon species of Endothia. Species and isolates by lane number (from left to right): E. gyrosa 1 - E18, 2 - E20, 3 - E30, 4 - E37, 5 - E38, 6 - E48, 7 - E50, 8 - E98, 9 - E145, 10 - E154; E. tropicalis 11 - E57; E. singularis 12 - E52; E. havanensis 13 - E40, 14 - E158; E. coccolobii 15 - E157; E. macrospora 16 - E54; E. japonica 17 - E53, 18 - E59; E. eugeniae 19 - E138, 20 - E139. First extraction of first set of lyophilized mycelial powders.

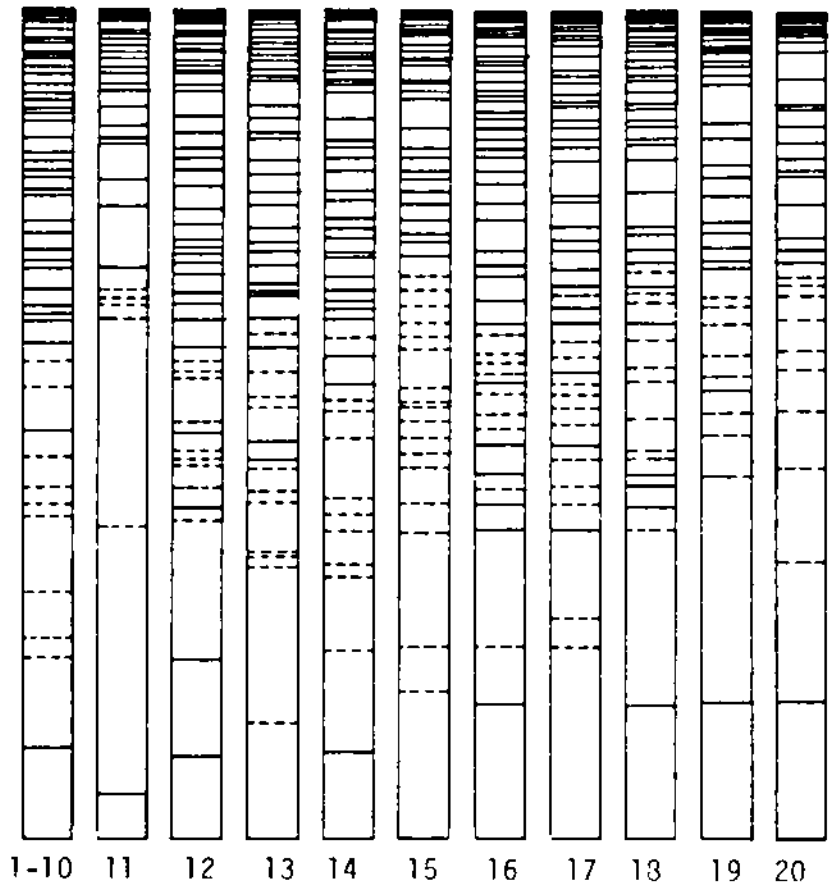

Figure 138. Interpretative drawing of polyacrylamide electrophoretic gel of buffer-soluble protein extracts of Endothia gyrosa and uncommon species of Endothia. Species and isolates by lane number (from left to right): E. gyrosa 1 - E18, 2 - E20, 3 - E30, 4 - E37, 5 - E38, 6 - E48, 7 - E50, 8 - E98, 9 - E145, 10 - E154; E. tropicalis 11 - E57; E. singularis 12 - E52; E. havanensis 13 - E40, 14 - E158; E. coccolobii 15 - E157; E. macrospora 16 - E54; E. japonica 17 - E53, 18 - E59; E. eugeniae 19 - E138, 20 - E139. Second extraction of second set of lyophilized mycelial powders.

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