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Studies on *Diplodia* and *Diplodia*-like Fungi

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VI. Effects of Natural Substrates on Variability in Taxonomic Characters

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IV. Effects of pH, Temperature, Light, and Vitamins on Certain Taxonomic Characters

Fifteen isolates representing various genera and species of *Diplodia* and *Diplodia*-like fungi were grown on various synthetic media, for study of the effect of pH, temperature, light, and vitamins on growth, sporulation, and stability of morphological characters currently used to delimit members of the Phaeodidymous Sphaeropsidales taxon. Fungi tested grew over a wide pH range. A bimodal response in growth at pH levels near 4.5 and 7.0 was common for most but not all isolates tested. The pH of the culture medium within ranges allowing good growth had little influence on mycelial color or general colony appearance. Sporulation was influenced by pH, however, apparently more so by the buffering system. Although the pH of the culture medium influenced production of fruiting structures and spores, it had little effect on stabilizing characteristics used in classification of these fungi.

Temperatures ranging from 6° to 39°C had the usual expected effects. Growth of isolates increased as temperature increased, peaked at a range from 27° to 33°C, and then dropped rapidly to form a skewed curve. Temperature apparently had little influ-

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VI. Effects of Natural Substrates on Variability in Taxonomic Characters[1]

ABSTRACT

Isolates representing nine genera and 28 species were cultured on eight natural substrates and two media. The object was to compare the effect of natural substrates on the morphology of characters currently employed to delimit genera and species of this group of fungi. Observations clearly show that valid distinctions cannot be made among most of these fungi on the basis of characters such as relationship of pycnidia to substrate, rostrate or nonrostrate pycnidia, pycnidial hairs or setae, presence or absence of stromata and distribution of pycnidia, single vs. multiloculate stromata, and paraphysis. It is proposed that pycnidiospore characters, such as gross morphology, ornamentation, and size, would be more useful for distinguishing genera and species than are those characters now employed.

INTRODUCTION

THE LITERATURE dealing with the *Diplodia* and *Diplodia*-like fungi included in the Phaeodidymous:Sphaeropsidales taxon shows that considerable difficulty exists in regard to precise identification of genus as well as species. We have contended, and our previous studies have shown (Satour, Webster, and Hewitt, 1969*a, b;* Webster, Hewitt, and Polach, 1969) that, for the most part, this difficulty stems from an inadequate knowledge of the range of variation and usefulness of the majority of the morphological characters previously used to delimit taxa of this group. We have therefore attempted to determine experimentally the inherent variation and the effect of various culture conditions on those characters. In addition, many taxa included in this group were apparently given specific names totally on the basis of association with or occurrence on a particular host or substrate. A notable example of this is found in a report by Wehmeyer (1964) in which he describes six new species of *Diplodia.* There he stated, "In this genus again so many species have been described with similar or overlapping characters but differing only in the host occurrence that occurrences on new hosts cannot be definitely placed but must be erected as new species to complicate the confusion."

Previously, we reported (Satour, Webster, and Hewitt, 1969*a, b;* and the two papers preceding this one) that pycnidial characters varied considerably within single isolates on different carbon and nitrogen sources, and under varying culture conditions. Since most fungi of this group occur on natural substrates, we need to know if the effect of natural substrates on those char-

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acters was similar to the effect of the cultural media tested in the laboratory. The present report analyzes the effect of various natural substrates on variation of characters used earlier in the literature to delimit taxa in this group.

MATERIALS AND METHODS

Representative isolates of nine genera and 28 species, as identified by various contributors, were included in the present study. The isolate number and previously designated name are listed in table 1. The natural substrates included as growth media were: corn leaves, alfalfa stems, barley grains, citrus leaves, pea straw, rice hulls, and wheat grains. The isolates were also grown on potato-dextrose-agar (PDA) and sorbose agar, as described earlier (Satour, Webster, and Hewitt, 1969*a*), to allow for a comparison with the results obtained with these isolates in previous studies. The various plant parts mentioned were autoclaved for 50 minutes at 15 psi, and small portions were placed on the surface of fresh Sach's agar (Shoemaker, 1955). Plates were then inoculated with small agar blocks containing mycelium from fresh PDA cultures of the various isolates. Incubation was at room temperature (24°C ± 2) under constant fluorescent light of approximately 250 ft-c. Observations on morphological structures and measurements were made after 30 days' incubation by the methods described previously (Webster, Hewitt, and Polach, 1969; Satour, Webster, and Hewitt, 1969*a, b*). The statistical analysis was carried out with the aid of an IBM 7040 computer.

RESULTS AND OBSERVATIONS

Qualitative comparisons

Variations in pycnidial and pycnidiospore characters as observed in cultures of the isolates on various natural media are summarized in table 1. It is apparent that many of the characters listed were influenced in their expression by the substrate on which they were produced. Some isolates did not fruit on all of the substrates tested. This was particularly true in the case of isolates considered to represent the genera *Botryodiplodia, Rynchodiplodia,* and *Schizodiplodia.*

Relationship of pycnidia to substrates

Pycnidia produced were either superficial, erumpent, or submerged in all isolates when all test media are considered. When test media are considered separately, it can be seen, for example, in isolate 59, that the relationship of the pycnidium to the substrate varied with the different substrates.

Pycnidia—rostrate or nonrostrate

The presence or absence of "beaks" on pycnidia has been used as a diagnostic character for delimiting genera in this group. In the present study several of the isolates showed no variation in this character when grown on different substrates. On the other hand, isolates 123, 59, 157, 1, and 95 produced both beaked and nonbeaked pycnidia, sometimes in the same culture.

All pycnidia observed on the substrates tested produced discernible ostioles.

Pycnidial hairs or setae

Hairy, bristly (setae), or smooth appearance of pycnidia has also been used as a character in delineating genera in this group. This character, too, was affected by the various substrates for given isolates. Of the nine genera represented, none was consistent with respect to the presence or absence of hairs on pycnidia. Often both smooth and hairy pycnidia were observed on a single substrate.

Presence or absence of stromata and distribution of pycnidia

The data clearly show these characters to vary with the substrate, both between and within genera and species and single cultures.

Paraphysis

Zambettakis (1953, 1954*a*, *b*) has used the presence or absence and nature of paraphysis extensively in delimiting genera and species of this group. We have examined cultures at various stages of development, by squash techniques, and have found that at some point in development, structures resembling paraphyses can be found in most of the isolates. When cultures are examined at the time spores are mature, however, the paraphyses are at best difficult to discern, and we have observed very little differentiation in our material with respect to this character.

Single vs. multiloculate stromata

The variation observed in this character, both within and between isolates, was similar to that observed in previous studies by us and by others (Barbe and Hewitt, 1965; Taubenhaus, 1915; Wardlaw, 1932). Of all the characters previously used to distinguish genera of this group, the nature and extent of stromata development appear to be most affected by substrate and environment. As stromata development increases, so does the difficulty in ascertaining whether a stromata is multiloculate or is supporting aggregations of individual pycnidia. Even so, the occurrence of solitary single-loculate, solitary multiloculate, and clusters of both single and multiloculate pycnidia—both with and without distinct stromata in a single culture—or variations by a single isolate on different substrates suggest that continued use of these characters for identification purposes will simply compound the existing confusion.

Pycnidiospore characters

Number of septa. By definition, fungi included in the Phaeodidymous: Sphaeropsidales must produce dark, two-celled spores. We have therefore considered any variation in this character observed by us to mean that the deviating spores were not mature. This, in fact, has been the case, and is the main reason for our making observations after 30 days' incubation. In cases where no septa were observed (noted in table 1), most of the pycnidiospores were typically single-septate. In all cases but one in which a number of nonseptate spores were observed, a few single-septate spores were always found. The only exception was in isolate 49.

Constriction at septum

Maturity of the spore is also pertinent in considering constrictions at the septa. The occurrence of both constricted and nonconstricted spores in a single isolate apparently was not an effect of the various substrates. This conclusion gains support from the fact that a number of isolates showed no constriction on any media tested.

Ornamentation of pycnidiospores

Of the characters observed, ornamentation on the surface of mature spores appeared to be the most consistent and least affected by culture conditions and by the various substrates tested. A few exceptions were noted, mainly in distinguishing between smooth and granular as defined by Zambettakis (1953, 1954*a*, *b*). In those cases, most of the spores were considered to be smooth. We believe this character has taxo-

TABLE 1

SUMMARY OF VARIATION IN MORPHOLOGY OF SELECTED ISOLATES OF *DIPLODIA* AND RELATED FUNGI GROWN ON DIFFERENT NATURAL SUBSTRATES AND CULTURE MEDIA

Isolate and substrate	Pycnidium characters*								Pycnidiospore characters*			
	Rel. to substr.†	Beaks‡	Ostiole	Hairs§	Stromata			Para-physes	Septa		Mucus	Ornamen-tation**
					Pres. or abs.	Distri-bution¶	Develop-ment‖		No.	Constr.		
Botryodiplodia phaseoli 27:												
Corn leaf	S, E	NR	+	NH	+, −	I–C	SL	−	0–1	−	+, −	SM
B. hypodermia 29:												
Corn leaf	S, E	NR	+	H–NH	−	I–C	SL	−	0–1	−	+	SM
B. malorum 32:												
Corn leaf	S, E, SB	NR	+	H–NH	+, −	I–C	SL	−	0–1	−	+	SM
B. theobromae 43:												
Corn leaf	S, E	NR	+	H	+	I–C	SL–M	+	1	−	+, −	ST
B. theobromae 123:												
Corn leaf	S, E	R–NR	+	H–NH	+, −	I–C	SL–M	−	1	−	−	ST
PDA	S, E	NR	+	H–NH	+, −	I–C	SL–M	+	1	−	+, −	ST–SM
Lasiodiplodia theobromae 102:												
Corn leaf	S, E	NR	+	H–NH	+, −	I–C	SL	−	1	−	+, −	ST
Sorbose agar	S, E	NR	+	H	−	I	SL	−	1	−	−	ST
L. theobromae 105:												
Corn leaf	S, E, SB	NR	+	H	−	I–C	SL	−	1	0	+, −	SM
Alfalfa stems	S, E, SB	NR	+	NH	+, −	I–C	SL	−	1	+	+, −	SM
Barley grains	S, E	NR	+	H–NH	+, −	I–C	SL	−	1	+, −	+	SM
Citrus leaves (orange)	S, E	NR	+	NH	−	I	SL	−	0–1	+, −	+	SM
Pea straw	S, E	NR	+	NH	−	I	SL	−	1	+, −	+	SM
Rice hulls	S, E	NR	+	H–NH	−	I	SL	−	1	+, −	+	SM
Wheat grains	S, E, SB	NR	+	H–NH	−	I–C	SL	−	1	0, −	+	SM
PDA	S, SB	NR	+	H–NH	+, −	I–C	SL–M	−	1	+, −	+, −	SM
Rhyncodiplodia ursusiformae 59:												
Corn leaf	S	R–NR	+	H–NH	−	I	SL	−	1	+, −	+	SM
Alfalfa stems	S, E, SB	NR	+	NH	+, −	I–C	SL–M	−	1–0	−	+	SM
Barley grains	S, SB	NR	+	NH	−	I	SL	−	1	+	+	SM
Citrus leaves (orange)	S, E	NR	+	NH	−	I	SL	−	1–0	−	+	SM
Pea straw	S	NR	+	NH	−	I	SL	−	1	+	+	SM
Rice hulls	S, E, SB	NR	+	NH	−	I	SL	−	1–0	−	+	SM
Wheat grains	S	NR	+	NH	−	I	SL	−	1	+	+	SM
PDA	S–SB	NR	+	NH	−	I–C	SL–M	−	1–0	+, −	+	SM

***R. ursusiformae* 93:**											
Corn leaf	S–E	NR	+	NH	–	I	SL	–	1	–	+	SM
PDA	S–SB	NR	+	NH	+, –	I–C	SL–M	–	1	+, –	+	SM
Pellionella tetonensis 75:												
Corn leaf	S–E	NR	+	NH	–	I	SL	–	1	+, –	+	SM
Alfalfa stems	S	NR	+	NH	–	I	SL	–	1	–	+	SM
Barley grains	S–SB	NR	+	H–NH	+, –	I–C	SL	–	1	–	+	SM
Citrus leaves (orange)	S	NR	+	H–NH	–	I	SL	–	1	–	+	SM
Pea straw	S	NR	+	NH	–	I	SL–M	–	1	–	+	SM
Rice hulls	S	NR	+	NH	+, –	I–C	SL–M	–	1	–	+	SM
Wheat grains	S	NR	+	H–NH	–	I	SL	–	1	–	+	SM
PDA	S–SB	NR	+	NH	+, –	I–C	SL–M	–	1	+, –	+	SM
Schizodiplodia sp. 99:												
Sorbose agar	S–SB	R	+	H	+, –	I–C	SL	+	1	–	+	SM
Macrophoma sp. 95:												
Corn leaf	S–E	R–NR	+	H–NH	+, –	I–C	SL–M	–	1-0	–	+	SM
PDA	S–SB	NR	+	NH	+, –	I–C	SL–M	–	1	–	+	SM
Microdiplodia osmanthi 92:												
Corn leaf	S	NR	+	NH	–	I–C	SL	–	1	–	+	SM
Alfalfa stems	S–E	NR	+	NH	–	I	SL	–	1	–	+	SM
Barley grains	S–E	NR	+	NH	+, –	I–C	SL–M	–	1	–	+	SM
Citrus leaves (orange)	S–E	NR	+	NH	+, –	I–C	SL–M	–	1	–	+	SM
Pea straw	S	NR	+	NH	–	I–C	SL	–	1	–	+	SM
Rice hulls	S	NR	+	NH	–	I–C	SL	–	1	–	+	SM
PDA	S–E	NR	+	NH	+, –	I–C	SL	–	1	–	+	SM
Microdiplodia sp. 103:												
Corn leaf	S–E–SB	NR	+	H–NH	+, –	I–C	SL	–	1	+, –	+	SM
Alfalfa stems	SB	NR	+	NH	–	I	SL	–	1	+, –	+	SM
Barley grains	S–E–SB	NR	+	H	–	I	SL	–	1	+, –	+	SM
Citrus leaves (orange)	S–E	NR	+	H–NH	–	I	SL	–	1	+, –	+	SM
Pea straw	S–E	NR	+	H	–	I	SL	–	1	+, –	+	SM
Rice hulls	SB	NR	+	H	–	I	SL	–	1	+, –	+	SM
Wheat grains	S–E–SB	NR	+	H	–	I	SL	–	1	+, –	+	SM
PDA	S–E	NR	+	H	+, –	I–C	SL–M	–	1	+, –	+	SM
Sorbose agar	S–E	NR	+	NH	–	I	SL	–	1	+	+	SM

* + and – indicate present and absent, respectively.
† S = superficial; E = erumpent; SB = submerged.
‡ R = rostrate; NR = not rostrate.
§ H = hairy; NH = not hairy.
¶ I = solitary; C = clustered.
‖ SL = single-loculate; M = multiloculate.
** SM = smooth; ST = striate; GR = granular

Continued

TABLE 1—(*Continued*)

Isolate and substrate	Pycnidium characters*								Pycnidiospore characters*			
	Rel. to substr.†	Beaks‡	Ostiole	Hairs§	Stromata			Paraphyses	Septa		Mucus	Ornamentation**
					Pres. or abs.	Distribution¶	Development‖		No.	Constr.		
M. perpusilla 109:												
Corn leaf	S-E-SB	NR	+	NH	−	I	SL	−	1	+	+	SM
Alfalfa stems	S-E	NR	+	NH	−	I	SL	−	1	−	+	SM
Barley grains	S-E	NR	+	NH	−	I	SL	−	1	−	+	SM
Citrus leaves (orange)	S-E	NR	+	H-NH	−	I	SL	−	1	−	+	SM
Pea straw	S-E	NR	+	NH	−	I	SL	−	1	−	+	SM
Rice hulls	S-E	NR	+	NH	−	I	SL	−	1	−	+	SM
Wheat grains	S-E	NR	+	NH	−	I	SL	−	1	−	+	SM
PDA	S-E	NR	+	NH	+, −	I-C	SL	−	1	−	+	SM
Sorbose agar	S-E	NR	+	NH	−	I	SL	−	1	−	+	SM
M. myriospora 110:												
Corn leaf	S	NR	+	NH	−	I	SL	−	0	−	+	SM
PDA	S	NR	+	NH	−	I	SL	−	0	−	+	SM
M. myriospora 238:												
Corn leaf	S	NR	+	H	−	I	SL	−	0-1	−	+	SM
Alfalfa stems	S	NR	+	H	+, −	I-C	SL-M	−	0-1	−	+	SM
Barley grains	S	NR	+	H	+, −	I-C	SL-M	−	0-1	−	+	SM
Citrus leaves (orange)	S	NR	+	H	+, −	I-C	SL-M	−	0-1	−	+	SM
Pea straw	S	NR	+	H	+, −	I-C	SL-M	−	0-1	−	+	SM
Rice hulls	S	NR	+	NH	+, −	I	SL-M	−	0	−	+	SM
Wheat grains	S	NR	+	H	+, −	I-C	SL-M	−	0	−	+	SM
PDA	S	NR	+	H	+, −	I-C	SL-M	−	0	−	+	SM
M. agaveae 113:												
Corn leaf	S-E	NR	+	H	+, −	I-C	SL	−	1	−	+	SM
Alfalfa stems	S-E	NR	+	H	−	I	SL	−	1	−	+	SM
M. agaveae 112:												
Corn leaf	SB	NR	+	NH	−	I	SL	−	0	−	−	SM
Alfalfa stems	S	NR	+	NH	−	I	SL	−	0	−	−	SM
Barley grains	SB	NR	+	NH	−	I	SL	−	0	−	−	SM
Rice hulls	S-E-SB	NR	+	NH	−	I	SL	−	0	−	−	SM
PDA	S-E-SB	NR	+	NH	−	I	SL	−	0	−	−	SM

Diplodina lycopersici 46:												
Corn leaf	S–E	NR	+	H–NH	+	I–C	SL–M	–	1	+, –	+	SM–GR
Alfalfa stems	E–SB	NR	+	NH	+	I–C	SL–M	–	1–0	–	+	SM
Barley grains	S–E–SB	NR	+	NH	+	I	SL	–	1–0	+, –	+	SM
Citrus leaves (orange)	S	NR	+	NH	–	I	SL	–	0	–	+	SM
Pea straw	S–E	NR	+	H–NH	–	I–C	SL–M	–	1	+, –	+	SM
Rice hulls	S	NR	+	NH	–	I	SL	–	1–0	+, –	+	SM
Wheat grains	SB	NR	+	NH	+	I	SL	–	1–0	+, –	+	SM
PDA	S–SB	NR	+	H–NH	+	I–C	SL–M	–	1–0	+, –	+	SM–GR
D. coloradoensis 47:												
Alfalfa stems	E–SB	NR	+	NH	–	I–C	SL	–	0	–	+	SM
Barley grains	E–SB	NR	+	NH	–	I–C	SL	–	0	–	+	SM
Citrus leaves (orange)	E–SB	NR	+	NH	–	I–C	SL	–	0	–	+	SM
Pea straw	S–E–SB	NR	+	NH	–	I–C	SL	–	0	–	+	SM
Rice hulls	E–SB	NR	+	NH	–	I–C	SL	–	1–0	–	+	SM
Wheat grains	E–SB	NR	+	NH	–	I–C	SL	–	0	–	+	SM
PDA	SB	NR	+	NH	–	I–C	SL	–	1–0	–	+	SM
D. pyrethri 49:												
Corn leaf	S	NR	+	NH	–	I	SL	–	0	–	+, –	SM
Alfalfa stems	S	NR	+	NH	–	I	SL	–	0	–	+	SM
Barley grains	S	NR	+	NH	–	I	SL	–	0	–	+	SM
Citrus leaves (orange)	S	NR	+	NH	–	I	SL	–	0	–	+	SM
Pea straw	S	NR	+	NH	–	I	SL	–	0	–	+	SM
Rice hulls	S	NR	+	NH	–	I	SL	–	0	–	+	SM
Wheat grains	S	NR	+	NH	–	I	SL	–	0	–	+	SM
PDA	S–SB	NR	+	NH	–	I–C	SL–M	–	0–1	+, –	+	SM
Diplodia warburgiana 63:												
Corn leaf	S–E–SB	NR	+	H–NH	+	I–C	SL	–	1–0	+, –	+	SM
Alfalfa stems	E–SB	NR	+	NH	+	I–C	SL	–	1–0	+, –	+	SM
Barley grains	E–SB	NR	+	NH	+	I	SL	–	1–0	–	+	SM
Citrus leaves (orange)	E–SB	NR	+	NH	+	I	SL	–	1–0	–	+	SM
Pea straw	S–E–SB	NR	+	NH	+	I–C	SL–M	–	1–0	–	+	SM
Rice hulls	E–SB	NR	+	NH	+	I–C	SL–M	–	1–0	–	+	SM
Wheat grains	E–SB	NR	+	NH	+	I–C	SL	–	1–0	–	+	SM
PDA	SB	NR	+, 0	NH	+, 0	I–C	SL–M	–	1–0	+, –	+	SM

* + and – indicate present and absent, respectively.
† S = superficial; E = erumpent; SB = submerged.
‡ R = rostrate; NR = not rostrate.
§ H = hairy; NH = not hairy.
¶ I = solitary; C = clustered.
‖ SL = single-loculate; M = multiloculate.
** SM = smooth; ST = striate; GR = granular.

Continued

TABLE 1 (*Continued*)

Isolate and substrate	Pycnidium characters*								Pycnidiospore characters*			
	Rel. to substr.†	Beaks‡	Ostiole	Hairs§	Stromata			Para-physes	Septa		Mucus	Ornamen-tation**
					Pres. or abs.	Distri-bution¶	Develop-ment‖		No.	Constr.		
D. zea 65:												
Corn leaf	S-SB	NR	+	H-NH	+	I-C	SL	+, −	1	−	+	SM
Alfalfa stems	S-E	NR	−	H-NH	−	I-C	SL	−	1	−	+	SM
Barley grains	S	NR	−	H	−	I-C	SL	−	1	−	+	SM
Citrus leaves (orange)	S-E	NR	+	H-NH	−	I-C	SL	−	1	−	+	SM
Pea straw	S	NR	+	H	−	I-C	SL	−	1	−	+	SM
Rice hulls	S	NR	+	H	−	I	SL	−	1	−	+	SM
Wheat grains	S	NR	+	H-NH	−	I	SL	−	1	−	+	SM
PDA	S	NR	+	H	−	I-C	SL	+, −	1	−	+	SM
Sorbose agar	S-SB	NR	+	NH	−	I	SL	+, −	1	−	+	SM
D. philodendri 97:												
Corn leaf	S-E	R	+	H	−	I-C	SL	+	1-2	−	+, −	SM
Alfalfa stems	S-E	R	+	H	−	I-C	SL	+	1	−	+, −	SM
Barley grains	S-E	R	+	H	+	I-C	SL	+	1	−	+	SM
Citrus leaves (orange)	S-E	R	+	H	+	I-C	SL-M	+	1	−	+	SM
Pea straw	S-E	R	+	H	−	I-C	SL	+	1	−	+, −	SM
Rice hulls	S-E	R	+	H	+	I-C	SL-M	+	1	−	+	SM
Wheat grains	S-E	R	+	H	+	I-C	SL-M	−	1	−	+	SM
PDA	S-E	R	+	H	−	I-C	SL	+	1	−	+, −	SM
D. gossypina 122:												
Corn leaf	S	NR	+	NH	−	I-C	SL	+, −	1	−	+, −	ST
Citrus leaves (orange)	S	NR	+	NH	+, −	I	SL-M	+, −	1	−	−	ST
Rice hulls	S-SB	NR	+	NH	+, −	I-C	SL	−	1	−	+, −	ST
PDA	S-SB	NR	+	H	+, −	I-C	SL	−	1	−	+, −	ST
D. tubericola 148:												
Corn leaf	S-E	NR	+	H	+, −	I-C	SL-M	+	1	−	+, −	ST
Alfalfa stems	S	NR	+	H	+, −	I-C	SL-M	+	1	−	+, −	ST
Barley grains	S	NR	+	H	+, −	I-C	SL-M	+	1	−	+, −	ST
Citrus leaves (orange)	S	NR	+	H	+, −	I-C	SL	+	1	−	+, −	ST
Pea straw	S	NR	+	H	+, −	I-C	SL	+	1	−	+, −	ST
Rice hulls	S	NR	+	H	+, −	I-C	SL	+	1	−	+, −	ST
Wheat grains	S	NR	+	H	+, −	I-C	SL	+	1	−	+, −	ST
PDA	S-SB	NR	+	H	+, −	I-C	SL	+	1	−	+, −	ST
Sorbose agar	S-SB	NR	+	H	−	I	SL	+	1	−	+, −	ST

D. natalensis 157:												
Corn leaf	S-E	R	+	H-NH	+, −	I-C	SL-M	+	1	−	+, −	ST
PDA	S-SB	NR	+	H-NH	+, −	I-C	SL-M	+	1	−	−	ST
Sorbose agar	S-SB	NR	+	H-NH	+, −	I-C	SL	+	1	−	+, −	ST
D. subtecta 241:												
Corn leaf	E	NR	+	H	+	I-C	SL	−	1	−	+	SM-GR
Alfalfa stems	S	NR	+	H	−	I	SL	−	1	−	+	SM
Barley grains	S	NR	+	H	+	I	SL-M	−	1	−	+	SM
Citrus leaves (orange)	S-E	NR	+	H	+	I-C	SL-M	−	1	−	+	SM
Pea straw	S	NR	+	H	+, −	I	SL	−	1	−	+	SM
Rice hulls	S	NR	+	H	−	I	SL	−	1	−	+	SM
Wheat grains	S	NR	+	H	−	I	SL	−	1	−	+	SM
PDA	S-SB	NR	+	H	+, −	I-C	SL-M	−	1	−	+	SM
D. pinea 1:												
Corn leaf	S-E	NR	+	NH	+, −	I-C	SL-M	−	1	−	+, −	SM-GR
PDA	S-SB	R-NR	+	H-NH	+, −	I-C	SL	+, −	1	−	+, −	SM-GR
D. madis 145:												
Corn leaf	S-E	NR	+	H-NH	+, −	I-C	SL	+	1	−	−	SM
PDA	S-SB	NR	+	H-NH	+, −	I-C	SL	+	1	−	−	SM
D. ursusiformae 60:												
Corn leaf	S-E	NR	+	H	+, −	I	SL	+	1	−	−	ST
PDA	S-SB	NR	+	H	+, −	I-C	SL-M	−	1	−	+, −	ST
D. oleae 34:												
Corn leaf	S	NR	+	H	−	I-C	SL-M	+	1	−	+, −	ST
Alfalfa stems	S	NR	+	H	+	I-C	SL-M	+	1	−	+	ST
Barley grains	S	NR	+	H	+	I-C	SL-M	+	1	−	+, −	ST
Citrus leaves (orange)	S-E	NR	+	H	+	I-C	SL-M	+	1	−	+, −	ST
Pea straw	S-E	NR	+	H	+	I-C	SL-M	+	1	−	+, −	ST
Poplar twigs	S-E	NR	+	H	+	I-C	SL-M	+	1	−	+, −	ST
Rice hulls	S	NR	+	H	+	I-C	SL-M	+	1	−	+, −	ST
Wheat grains	S	NR	+	H	+	I-C	SL-M	+	1	−	+, −	ST
PDA	S	NR	+	H	+	I-C	SL-M	+	1	−	+, −	ST

* + and − indicate present and absent, respectively.
† S = superficial; E = erumpent; SB = submerged.
‡ R = rostrate; NR = not rostrate.
§ H = hairy; NH = not hairy.
¶ I = solitary; C = clustered.
‖ SL = single-loculate; M = multiloculate.
** SM = smooth; ST = striate; GR = granular.

nomic value, but most likely at the species level instead of the genus level as employed by Zambettakis (1953).

Pycnidiospores produced wet or dry

Whether pycnidiospores are produced dry or slimy (in a mucus) has been cited by some authors as indicative of important natural relationships among *Fungi imperfecti*. In our studies, both substrate and culture conditions exercised profound effects on this character. It is also common for spores to be produced on some pycnidia and in a mucus on others in the same culture of a particular isolate. Numerous examples of this are given in table 1.

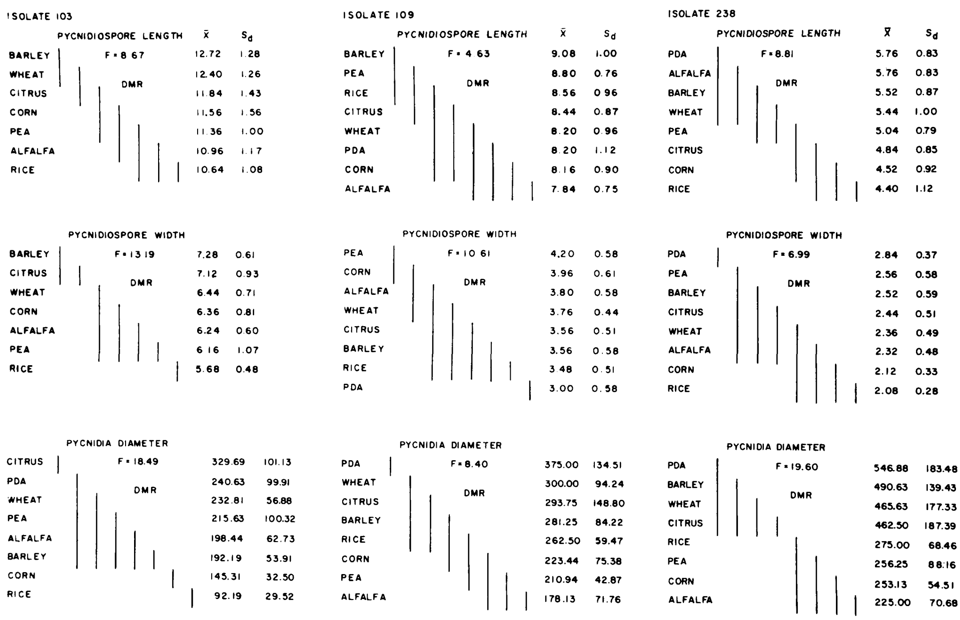

Fig. 1. Statistical comparison of pycnidial diameter and pycnidiospore length and width of isolates *Microdiplodia* sp. 103, *M. perpusilla* 109, and *M. myriospora* 238, grown on various substrates. Values represent measurements of 25 pycnidia and 25 pycnidiospores of each isolate from each of the substrates tested. DMR = Duncan's Multiple Range test. F values all significant at the 5 per cent level.

Quantitative comparisons

Measurements of 25 pycnidia (diameter) and 25 pycnidiospores (width and length) were made for each isolate and from each substrate upon which the isolates fruited. The data obtained were analyzed statistically to determine mean value and standard deviations for each. We also wanted to determine whether significant differences occurred within the individual isolates grown on various substrates. Results of this analysis are presented in figures 1 to 6.

Pycnidia diameter. As the figures show, diameters of pycnidia produced by individual isolates on various substrates varied considerably both on a given substrate and among substrates. Also to be considered is the difficult distinction between single vs. multiloculate pycnidia and the presence or absence and abundance of stromatal tissue. In nine of 18 isolates so compared, pycnidial diameter was the greatest when isolates were grown on PDA. In seven of the other nine iso-

ISOLATE 59

PYCNIDIOSPORE LENGTH — F = 11.42 — DMR

	$\bar{x}$	S_d
CITRUS	9.36	0.95
PEA	9.16	1.03
WHEAT	8.76	0.97
CORN	8.60	0.82
BARLEY	8.12	0.78
ALFALFA	7.88	0.88
PDA	7.84	1.14
RICE	7.72	0.84

PYCNIDIOSPORE WIDTH — F = 26.99 — DMR

PEA	5.00	0.65
WHEAT	4.64	0.49
CORN	4.64	0.64
CITRUS	4.44	0.51
BARLEY	4.04	0.61
ALFALFA	3.88	0.53
PDA	3.56	0.58
RICE	3.32	0.48

PYCNIDIA DIAMETER — F = 8.00 — DMR

PEA	275.00	95.44
PDA	264.06	62.07
CITRUS	243.75	66.18
BARLEY	229.69	49.96
WHEAT	226.56	42.87
CORN	223.48	87.38
ALFALFA	171.88	49.15
RICE	159.38	39.98

ISOLATE 75

PYCNIDIOSPORE LENGTH — F = 24.86 — DMR

	$\bar{x}$	S_d
RICE	14.56	2.64
ALFALFA	12.72	2.24
PDA	12.72	1.76
PEA	11.72	1.72
CORN	11.56	1.38
WHEAT	11.16	1.21
CITRUS	10.64	1.07
BARLEY	8.68	1.10

PYCNIDIOSPORE WIDTH — F = 22.17 — DMR

RICE	5.80	0.76
ALFALFA	5.64	0.56
PEA	5.56	0.58
CORN	5.52	0.77
PDA	5.40	0.57
WHEAT	5.00	0.57
CITRUS	4.84	0.47
BARLEY	4.08	0.40

PYCNIDIA DIAMETER — F = 9.99 — DMR

PEA	325.00	78.14
PDA	300.00	76.81
CORN	284.38	58.16
CITRUS	282.81	124.37
BARLEY	246.88	64.84
WHEAT	226.56	57.24
ALFALFA	198.44	61.90
RICE	168.75	61.17

ISOLATE 105

PYCNIDIOSPORE LENGTH — F = 18.50 — DMR

	$\bar{x}$	S_d
WHEAT	13.48	1.33
CORN	13.48	1.81
BARLEY	13.00	1.29
CITRUS	12.88	1.42
ALFALFA	12.88	1.30
PEA	11.36	1.25
PDA	10.76	1.23
RICE	10.60	1.44

PYCNIDIOSPORE WIDTH — F = 5.05 — DMR

WHEAT	7.56	0.82
CITRUS	7.48	0.59
PEA	7.20	0.76
CORN	7.04	1.17
ALFALFA	6.96	0.68
BARLEY	6.80	0.71
RICE	6.60	0.87
PDA	6.56	0.92

PYCNIDIA DIAMETER — F = 20.33 — DMR

CITRUS	362.50	151.20
BARLEY	289.06	74.42
PDA	235.94	61.23
WHEAT	231.25	65.24
CORN	207.81	65.93
PEA	195.31	69.42
RICE	170.31	44.74
ALFALFA	98.44	34.04

Fig. 2. Statistical comparison of pycnidial diameter and pycnidiospore length and width of isolates *Rhyncodiplodia ursusiformae* 59, *Pellionella tetonensis* 75, and *Lasiodiplodia theobromae* 105, grown on various substrates. Values represent measurements of 25 pycnidia and 25 pycnidiospores of each isolate from each of the substrates tested. DMR = Duncan's Multiple Range test. F values all significant at the 5 per cent level.

ISOLATE 46

PYCNIDIOSPORE LENGTH — F = 29.18 — DMR

	$\bar{x}$	S_d
PDA	10.44	1.00
CITRUS	8.16	1.46
CORN	7.68	1.38
WHEAT	7.08	1.15
PEA	6.96	0.93
RICE	6.72	1.21
BARLEY	6.48	1.26
ALFALFA	6.32	1.44

PYCNIDIOSPORE WIDTH — F = 53.17 — DMR

PDA	6.04	0.79
ALFALFA	3.96	0.68
RICE	3.84	0.37
CORN	3.80	0.50
WHEAT	3.76	0.60
CITRUS	3.68	0.63
PEA	3.64	0.57
BARLEY	3.00	0.65

PYCNIDIA DIAMETER — F = 1.54 — DMR

CORN	220.31	83.90
PDA	207.81	53.91
BARLEY	201.56	62.07
PEA	195.31	49.54
CITRUS	190.63	56.36
ALFALFA	184.38	99.81
RICE	176.56	48.91
WHEAT	160.94	64.34

ISOLATE 47

PYCNIDIOSPORE LENGTH — F = 3.93 — DMR

	$\bar{x}$	S_d
WHEAT	5.84	0.85
BARLEY	5.60	0.82
ALFALFA	5.48	0.71
PEA	5.32	0.85
CITRUS	5.20	0.65
RICE	4.96	0.79

PYCNIDIOSPORE WIDTH — F = 26.66 — DMR

ALFALFA	4.12	0.60
CITRUS	3.16	0.62
RICE	2.80	0.71
PEA	2.80	0.41
BARLEY	2.68	0.48
WHEAT	2.52	0.51

PYCNIDIA DIAMETER — F = 2.82 — DMR

ALFALFA	185.93	56.88
RICE	154.69	48.07
CITRUS	146.88	47.66
BARLEY	142.19	77.53
WHEAT	137.50	56.81
PEA	120.31	57.60

ISOLATE 49

PYCNIDIOSPORE LENGTH — F = 14.28 — DMR

	$\bar{x}$	S_d
BARLEY	6.56	1.04
CITRUS	6.44	1.19
ALFALFA	6.40	1.19
WHEAT	6.32	0.80
PDA	6.28	0.61
PEA	5.24	0.66
CORN	5.20	0.50
RICE	5.00	0.65

PYCNIDIOSPORE WIDTH — F = 37.39 — DMR

CITRUS	3.64	0.57
BARLEY	3.64	0.64
ALFALFA	3.44	0.65
WHEAT	3.32	0.48
PDA	3.32	0.48
PEA	2.28	0.54
RICE	2.24	0.44
CORN	2.20	0.41

PYCNIDIA DIAMETER — F = 5.24 — DMR

PDA	273.44	81.03
BARLEY	273.44	68.67
CORN	260.94	76.95
WHEAT	242.19	52.56
RICE	237.50	77.48
PEA	212.50	76.95
CITRUS	181.25	59.64
ALFALFA	175.00	81.99

Fig. 3. Statistical comparison of pycnidial diameter and pycnidiospore length and width of isolates *Diplodina lycopersici* 46, *D. coloradoensis* 47, and *D. pyrethri* 49, grown on various substrates. Values represent measurements of 25 pycnidia and 25 pycnidiospores of each isolate from each of the substrates tested. DMR = Duncan's Multiple Range test. F values all significant at the 5 per cent level.

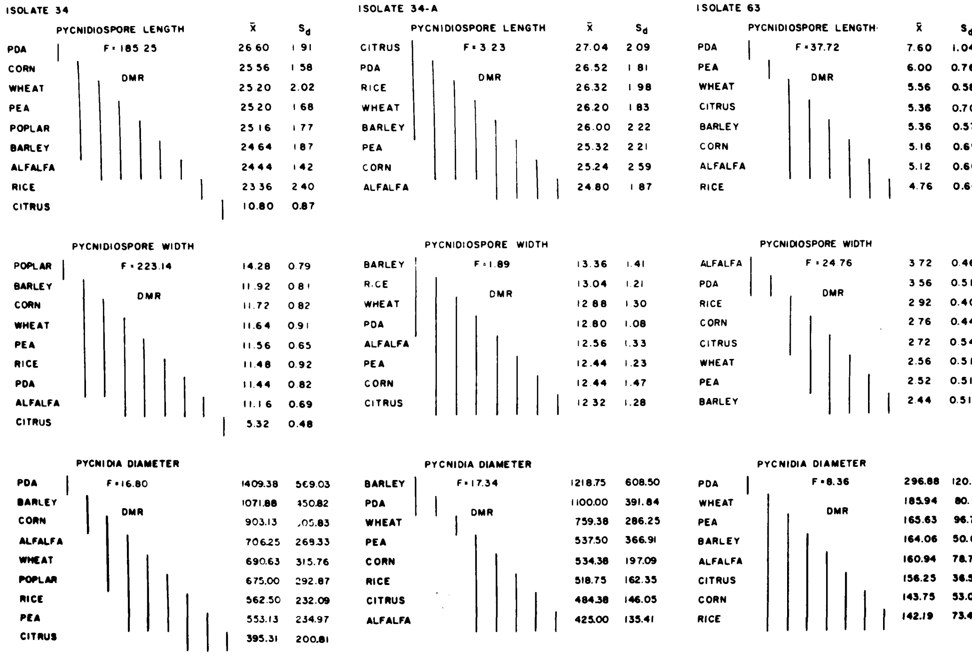

Fig. 4. Statistical comparison of pycnidial diameter and pycnidiospore length and width of isolates *Diplodia oleae* 34, *D. oleae* 34-A, and *D. warburgiana* 63, grown on various substrates. Values represent measurements of 25 pycnidia and 25 pycnidiospores of each isolate from each of the substrates tested. DMR = Duncan's Multiple Range test. F values all significant at the 5 per cent level.

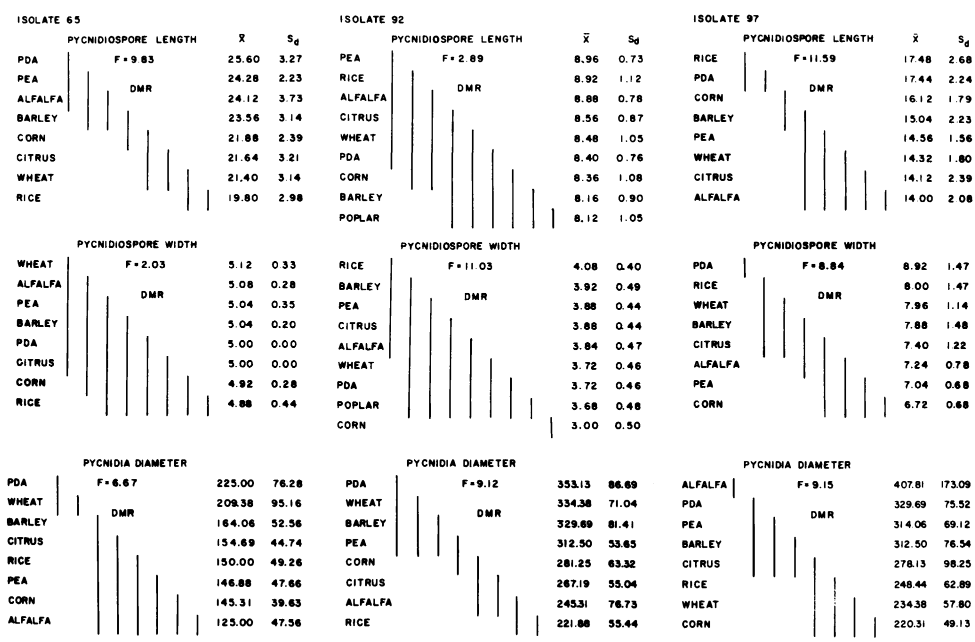

Fig. 5. Statistical comparison of pycnidial diameter and pycnidiospore length and width of isolates *Diplodia zea* 65, *Microdiplodia osmanthi* 92, and *D. philodendri* 97, grown on various substrates. Values represent measurements of 25 pycnidia and 25 pycnidiospores of each isolate from each of the substrates tested. DMR = Duncan's Multiple Range test. F values all significant at the 5 per cent level.

ISOLATE 122

PYCNIDIOSPORE LENGTH F = 3.65 DMR

	$\bar{x}$	S_d
CORN	25.52	3.08
RICE	23.96	2.57
PDA	23.56	1.94
CITRUS	23.08	3.27

PYCNIDIOSPORE WIDTH F = 5.84 DMR

	$\bar{x}$	S_d
RICE	11.96	1.06
CORN	11.80	0.96
PDA	11.36	0.76
CITRUS	10.96	0.93

PYCNIDIA DIAMETER F = 15.55 DMR

	$\bar{x}$	S_d
PDA	353.13	160.02
RICE	275.00	102.19
CITRUS	248.44	126.01
CORN	206.25	53.07
ALFALFA	131.25	44.89
PEA	120.31	65.14

ISOLATE 148

PYCNIDIOSPORE LENGTH F = 2.62 DMR

	$\bar{x}$	S_d
PEA	22.64	2.25
PDA	21.52	2.55
CORN	21.48	2.18
CITRUS	21.36	1.82
WHEAT	21.12	2.64
ALFALFA	20.72	1.81
BARLEY	20.44	1.80

PYCNIDIOSPORE WIDTH F = 3.26 DMR

	$\bar{x}$	S_d
ALFALFA	11.96	0.93
CORN	11.76	0.97
WHEAT	11.52	1.01
BARLEY	11.48	0.96
PEA	11.40	1.04
CITRUS	11.36	0.70
PDA	10.84	1.14

PYCNIDIA DIAMETER F = 1.63 DMR

	$\bar{x}$	S_d
PDA	537.50	259.21
CITRUS	521.88	160.02
PEA	484.38	220.16
BARLEY	468.75	181.94
CORN	459.38	127.08
ALFALFA	443.75	158.24
WHEAT	403.13	104.34
RICE	396.88	154.79

ISOLATE 241

PYCNIDIOSPORE LENGTH F = 4.52 DMR

	$\bar{x}$	S_d
BARLEY	9.40	0.91
WHEAT	9.36	0.81
CITRUS	9.24	0.78
ALFALFA	9.16	1.03
PEA	8.68	0.90
RICE	8.60	1.12
PDA	8.60	0.82
CORN	8.48	0.82

PYCNIDIOSPORE WIDTH F = 7.15 DMR

	$\bar{x}$	S_d
PDA	4.12	0.33
CORN	3.96	0.20
WHEAT	3.88	0.44
PEA	3.80	0.41
BARLEY	3.76	0.44
ALFALFA	3.60	0.50
RICE	3.48	0.51
CITRUS	3.48	0.51

PYCNIDIA DIAMETER F = 8.32 DMR

	$\bar{x}$	S_d
BARLEY	468.75	69.51
ALFALFA	418.75	103.59
CITRUS	390.63	155.59
PDA	385.94	97.92
WHEAT	356.25	98.51
PEA	328.13	66.87
CORN	271.88	66.56

Fig. 6. Statistical comparison of pycnidial diameter and pycnidiospore length and width of isolates *Diplodia gossypina* 122, *D. tubericola* 148, and *D. subtecta* 241, grown on various substrates. Values represent measurements of 25 pycnidia and 25 pycnidiospores of each isolate from each of the substrates tested. DMR = Duncan's Multiple Range test. F values all significant at the 5 per cent level.

lates, pycnidial size on PDA was not significantly different from that on the substrate upon which the largest pycnidia were observed for these isolates. This appeared to be the most consistent trend with regard to pycnidial diameter. It is obvious from the data that pycnidia diameter is so variable both within an individual isolate and among isolates on the same or different substrates that its use as a taxonomic character is not justified.

Pycnidiospore size. Pycnidiospore size has been used in some cases to distinguish genera in this group. The results show (figs. 1 to 6) that substrates do affect both width and length, and that in many cases the differences are statistically significant. The observations on isolate 34 show that on citrus, spores are only half the size of those produced on the other substrates tested. The main advantage of such a comparison is that it allows for a determination of the extremes in variability, i.e., the range in spore size of which a particular isolate is capable. In the case of isolate 34, according to early authors (Bender, 1934; Grove, 1937) this isolate would belong to the genus *Microdiplodia* when grown on citrus and to *Diplodia* when grown on the other substrates tested. With a knowledge of the range of spore size, species could be satisfactorily delimited, but use of this character to delimit genera does not appear warranted.

DISCUSSION

Bender (1934) distinguished 14 genera of Phaeodidymous: Sphaeropsidales, for the most part by pycnidial characters—beaked vs. not beaked, hairy vs. smooth, separate or grouped, superficial or submerged, stromata and subicle present or absent, and the nature of the ostiole. Further delimitation was made through differences in conidiophores, pycnidiospore size, and production of wet (mucus) or dry spores. Zambettakis (1953, 1954*a, b*) emphasized many of these characters and added those of paraphysis and pycnidiospore ornamentation. He retained 23 genera as a result of his studies. Opinions of other authors as to the number of genera in this group include those of Barnett (1960) two, Buchwald (1939) three, Grove (1937) five, Stevens (1913) 10, and Clements (1909) 11. Saccardo's *Syllogue Fungorum* lists 17 genera.

Most of the published works dealing with this group of fungi have been seriously deficient in critical evaluation of the stability and taxonomic usefulness of the characters discussed above. In the present report and in our previous studies (Satour, Webster, and Hewitt, 1969*a, b;* Webster, Hewitt, and Polach, 1969; and in the second paper presented here) the results clearly show that the emphasis placed on the characters used previously, such as presence of a stroma and its mode of formation, mucus around conidia, morphology of the pycnidium, e.g., beaks, setae, etc., and occurrence on a particular host substrate, has not resulted in a workable system of classification for this variable, heterogeneous group of fungi. We base this conclusion on the fact that our studies have repeatedly shown that, in many cases, a single isolate and single-spore derivatives from it produce structures that encompass the differences indicated above.

We do not claim that the cultural conditions in the studies reported here are identical with those encountered in nature. There is no doubt that many members of this group of fungi cause considerable disease in certain plant hosts. It cannot be disputed, however, that without exception, these fungi are very capable saprophytes and, in fact, may be more frequently encountered in nature on older plant parts and refuse. This alone seriously challenges the validity of naming species on the basis of host association, even if that were considered a valid criterion for species delimitation. Thus, the occurrence of a particular organism on bark, twigs, fallen or senescent leaves, prunings, etc., when disease is not evident could scarcely justify the naming of a species.

As early as 1915 Taubenhaus recognized the fallacy in using a number of the mentioned characters to delimit the genera *Botryodiplodia, Diplodiella, Chaetodiplodia,* and *Lasiodiplodia.* By culturing isolates representing these genera on sweet potato slices and other natural substrates, he was able to show that the characters used to delimit these taxa are not well based and that a continued separation of these genera is not justified. Nevertheless, a proliferation of genera and species in this group has continued, based largely on pycnidial, stromatal, and pycnidiospore characters and occurrence on new substrates.

In the present and previous studies, the morphology and ornamentation of pycnidiospores, when augmented by a knowledge of the variability and range in size, appear to be the most consistent characters regardless of the cultural conditions and substrates upon which the fungi were produced (Webster, Hewitt, and Polach, 1969).

The isolates included in the present study were obtained from a number of workers and collections throughout the world. Whether or not they truly represent the published descriptions to which they are attributed would not detract from the significance of the results, but merely substantiate our conclusion that most of the characters used previously in developing a taxonomy for this group are not well founded.

A suggested revision based mainly on characters of the pycnidiospores is being prepared and will be published elsewhere.

LITERATURE CITED

BARBE, G. DOUGLAS, and W. B. HEWITT
1965. The principal fungus in the summer bunch rot of grapes. Phytopathology **55**:815–16.
BARNETT, H. L.
1960. Illustrated genera of imperfect fungi. Minneapolis, Minn.: Burgess Publ. Co., 225 pp.
BENDER, H. B.
1934. Key to the genera Phaeodidymous Sphaeropsidales. Privately published. New Haven, Conn.: The Tuttle, Morehouse and Taylor Co., 52 pp.
BUCHWALD, N. F.
1939. *Fungi imperfecti.* Copenhagen: Kandrup und Wunsch Bogtrykkeri, 144 pp.
CLEMENTS, F. E.
1909. The genera of fungi. A key to Saccardo's *Sylloge Fungorum.* New York: H. W. Wilson Co., 227 pp.
GROVE, W. B.
1937. British stem and leaf fungi, vol. 2. London: Cambridge University Press, 406 pp.
SATOUR, M. M., R. K. WEBSTER, and W. B. HEWITT
1969*a*. Studies on *Diplodia* and *Diplodia*-like fungi: I. Effects of carbon sources on growth in agar culture. Hilgardia 39(22): 601–29.
1969*b*. Studies on *Diplodia* and *Diplodia*-like fungi: II. Effects of nitrogen sources on growth, sporulation, and certain taxonomic characters. Hilgardia 39(22):631–53.
SHOEMAKER, R. A.
1955. Biology, cytology, and taxonomy of *Cochliobolus sativus.* Can. Jour. Bot. **33**:562–76.
STEVENS, F. L.
1913. The fungi which cause plant disease. New York: The Macmillan Co., 754 pp.
TAUBENHAUS, J. J.
1915. Notes on the probable nonvalidity of some genera of Phaeodidymous Sphaeropsidales. Amer. Jour. Bot. **2**:324–31.
WARDLAW, C. W.
1932. Observations on the pycnidium of *Botryodiplodia theobromae* Pat. Ann. Bot. **46**:229–38.
WEBSTER, R. K., W. B. HEWITT, and F. J. POLACH
1969. Studies on *Diplodia* and *Diplodia*-like fungi: III. Variation in *Diplodia natalensis* from grape in California. Hilgardia **39**(22): 655–71.
WEHMEYER, L. E.
1964. Some fungi imperfecti of Punjab and Kashmir. Mycologia **56**:29–52.
ZAMBETTAKIS, C. E.
1953. Clés dichotomiques des généres et des espèces des Phaeodidymae de la famille des Sphaeropsidaceae. Ann. Inst. Phytopath. Benaki **7**:112–60.
1954*a*. Recherches anatomiques et biologiques sur les Sphaeropsidales-Phaeodidymae des fungi imperfecti. Arch. Mus. Nat. Hist. Nat. 7th Series **3**:43–146.
1954*b*. Recherches sur la systématique des "Sphaeropsidales-Phaeodidymae." Bul. Soc. Myc. Française **70**:219–350.

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4*m*-11,'71 (P6529) L.L. 141

ence on sporulation or characteristics used in classification. In general, isolates appeared to grow better and mature more favorably in light—either continuous or cyclic—than in continuous dark, although some did grow and fruit normally in continuous dark. Two isolates required light for initiation of pycnidia. Two required biotin for sporulation, while all others tested grew and sporulated on minimal medium without biotin or other added vitamins. Potato-dextrose-agar, available in all laboratories, is considered a satisfactory medium for growing these fungi for identification purposes.

V. Effects of Carbon:Nitrogen Ratio on Growth, Pycnidia, and Pycnidiospore Formation

Isolates of *Diplodia natalensis* and *Botryodiplodia theobromae* were grown on synthetic media containing various concentrations of carbohydrate and nitrogen sources in different carbon:nitrogen ratios to determine those ratios' effect on the growth and stability of taxonomic characters used to delimit these fungi. Growth of hyphae was favored as carbohydrate concentration increased, whereas increases of nitrogen above 1 gm sodium nitrate per liter had little effect.

Varying the carbon:nitrogen ratio of the growth media affected pigmentation, pycnidia shape and size, distribution of pycnidia, presence or absence and amount of stromata, presence or absence of hairs or setae on pycnidia, and number of pycnidiospores produced. Size and ornamentation of pycnidiospores were least affected, suggesting that these characters are least influenced by culture conditions. *D. natalensis* and *B. theobromae* are considered synonymous, since the results show that characters used previously to distinguish the fungi are significantly influenced by the media on which they are grown.

VI. Effects of Natural Substrates on Variability in Taxonomic Characters

Isolates representing nine genera and 28 species were cultured on eight natural substrates and two media. The object was to compare the effect of natural substrates on the morphology of characters currently employed to delimit genera and species of this group of fungi. Observations clearly show that valid distinctions cannot be made among most of these fungi on the basis of characters such as relationship of pycnidia to substrate, rostrate or nonrostrate pycnidia, pycnidial hairs or setae, presence or absence of stromata and distribution of pycnidia, single vs. multiloculate stromata, and paraphysis. It is proposed that pycnidiospore characters, such as gross morphology, ornamentation, and size, would be more useful for distinguishing genera and species than are those characters now employed.

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