Differences in Conidial Morphology Among Isolates of *Sphaeropsis sapinea*

WANG CHENG-GUO, Visiting Scientist; ROBERT A. BLANCHETTE, Associate Professor, Department of Plant Pathology, University of Minnesota, St. Paul 55108; and WILLIAM A JACKSON, Technician, and MARGUERITA A. PALMER, Research Plant Pathologist, U.S. Department of Agriculture, North Central Forest Experiment Station, St. Paul, MN 55108

ABSTRACT

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Thirty isolates of *Sphaeropsis sapinea* (=*Diplodia pinea*) from 10 countries were separated into two groups on the basis of conidial morphology. Scanning electron microscopy of mature spores demonstrated that type A isolates had smooth surfaces and type B isolates had pits distributed over the conidium surface. The separation of isolates into two groups may help to explain the variation in cultural characteristics and results of pathogenicity tests observed by researchers working with *S. sapinea*.

Sphaeropsis sapinea (Fr.) Dyko & Sutton (=*Diplodia pinea* (Desm.) Kickx) is a destructive pathogen of conifer species in many areas of the world (2,4,9). Damage has been reported from natural stands, windbreaks, Christmas tree and ornamental plantings, and nurseries (2,4,5,7,9,10,12). Isolates of *S. sapinea* from various locations in the north central United States were previously shown to differ in several cultural characteristics and could be assigned to two categories (8). Type A cultures have fluffy white to gray-green mycelium, and those of type B have white to black mycelium closely appressed to the agar surface when grown on a variety of media. A representative type A isolate could infect nonwounded young shoots, whereas a type B isolate required wounds for infection. Type B isolates were apparently opportunistic fungi that colonized wounded or declining host tissues. Palmer (8) suggested that it is important to identify the isolate type involved before recommending management strategies for controlling *S. sapinea*. However, type identification based on cultural characteristics may not be possible because of the variation that can be observed when the fungus is grown on different media and under different environmental conditions. In a study that examined conidial size (8), spores from type A isolates produced in culture were significantly longer and wider ($P = 0.01$) than spores from type B isolates. Conidia from naturally infected tissues were larger than those produced in culture. Both isolates had spore sizes within the reported range for *S. sapinea*. This suggests that there is substantial variability in spore size, and conidial size alone may not be a reliable character for type identification. Preliminary investigations of a small number of isolates of *S. sapinea* demonstrated that types A and B isolates can be differentiated by conidial morphology (11).

This research was done to determine if *S. sapinea* isolates from a worldwide collection can be categorized into two groups on the basis of conidial morphology and to compare spores from pycnidia of types A and B isolates from field collections and cultures.

MATERIALS AND METHODS

Thirty single-spore isolates of *S. sapinea* from 10 countries (Table 1) were grown on Difco potato-dextrose agar (PDA). After 3–4 days, sterilized red pine (*Pinus resinosa* Ait.) needles were placed directly on the mycelium and plates were incubated at 23 C under a fluorescent lamp. Four to 20 days later, isolates were identified as types A, B, or intermediate on the basis of cultural characteristics (8), and small segments of pine needles with pycnidia were cut with a razor blade. Pycnidia from field-collected material obtained from several locations in Minnesota and Wisconsin that yielded either type A or B isolates when grown on PDA were also used. Samples were submerged in water and placed under a low vacuum for 5–10 min. Samples were mounted in Tissue Tek II embedding compound and frozen at −20 C, then the pycnidia were cut with a cryostat microtome. Pycnidia, cut in half to reveal the spores, were mounted and allowed to dry on aluminum stubs. Specimens were coated with a gold-palladium mixture and examined with a Philips 500× scanning electron microscope at 12 kV. Additional samples were viewed with light microscopy.

Two other methods of specimen preparation were used to compare results with different techniques. Pycnidia were fixed in 2.5% glutaraldehyde in pH 7.0 phosphate buffer for 8 hr, 2% osmium textroxide for 1 hr, saturated thiocarbohydrazide for 1 hr, and 2% osmium textroxide for an additional hour.

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Present address of first author: Department of Forest Resources and Protection, Northwestern College of Forestry, Yangling, Shaanxi, People's Republic of China.

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Samples were then dehydrated through a graded acetone series, critical-point dried, mounted, and coated with gold-palladium. Other specimens were frozen in liquid nitrogen, placed on a cold stage in a vacuum evaporator, and coated with gold-palladium. Samples were observed on a cryostage with the scanning electron microscope at −150 C (1).

RESULTS

Isolates of *S. sapinea*, grown on PDA, were separated into groups on the basis of cultural characteristics. Cultural growth of most isolates was either type A or B (Fig. 1). However the growth pattern of a few isolates was intermediate, with some characteristics of both types (Table 1). Conidia from types A, B, or intermediate isolates could not be easily differentiated by light microscopy.

Mature spores from all isolates with type A cultural characteristics (Table 1) had smooth surfaces when examined with a scanning electron microscope (Fig. 2A,B). Isolates with an intermediate growth habit also produced spores with smooth surfaces. All mature spores of type B isolates had distinct pits on the spore surface (Fig. 2C,D). These pits were present only in mature spores. When nonpigmented spores, determined with light microscopy, from young type B pycnidia were examined with a scanning electron microscope, the spore surface appeared smooth. The three methods of specimen preparation all showed type A and intermediate isolates with smooth spore surfaces and type B isolates with

Table 1. Collection data, cultural characteristics, and spore morphology of isolates of *Sphaeropsis sapinea*

Geographic origin	Host	Isolate number	Collector	Type designation	Spore surface
Australia N.S.W.	*Pinus radiata*	192	M. Palmer	A	Smooth
Chile	*P. radiata*	163	CMI 238736	A	Smooth
India	*P. patula*	157	CMI 248286	Intermediate	Smooth
Japan	*P. thunbergii*	185	T. Kobayashi	Intermediate	Smooth
Kenya	*P. radiata*	153	CMI 1181955	A	Smooth
New Zealand	*P. radiata*	165	ATCC 34924	A	Smooth
South Africa	*P. pinaster*	164	CMI 243925	A	Smooth
Tanzania	Wood	154	CMI 235273	A	Smooth
United States					
Hawaii	*P. radiata*	189	M. Palmer	A	Smooth
Illinois	*P. strobus*	201	K. Robbins	Intermediate	Smooth
Iowa	*P. mugo*	167	L. Sweets	A	Smooth
Indiana	*Abies concolor*	151	T. Mog	A	Smooth
Michigan	*P. banksiana*	131	M. Palmer	B	Pitted
	P. banksiana	113	M. Palmer	B	Pitted
	P. resinosa	120	M. Palmer	A	Smooth
	Pseudotsuga menziesii	141	K. Robbins	A	Smooth
Missouri	*Pinus nigra*	170	P. Gowan	A	Smooth
Montana	*P. ponderosa*	183	T. Nicholls	A	Smooth
Wisconsin	*Larix decidua*	147	K. Robbins	A	Smooth
	P. banksiana	124	M. Palmer	B	Pitted
	P. banksiana	171	M. Wingfield	B	Pitted
	P. banksiana	172	M. Wingfield	B	Pitted
	P. banksiana	175	M. Wingfield	B	Pitted
	P. resinosa	123	M. Palmer	A	Smooth
	P. resinosa	128	M. Palmer	A	Smooth
	P. resinosa	215	K. Robbins	B	Pitted
	P. strobus	173	M. Wingfield	A	Smooth
Texas	*Pinus* sp.	156	CMI 265070	Intermediate	Smooth
	Vine	155	CMI 264965	A	Smooth
Zimbabwe	Unknown	188	Y. Katerere	A	Smooth

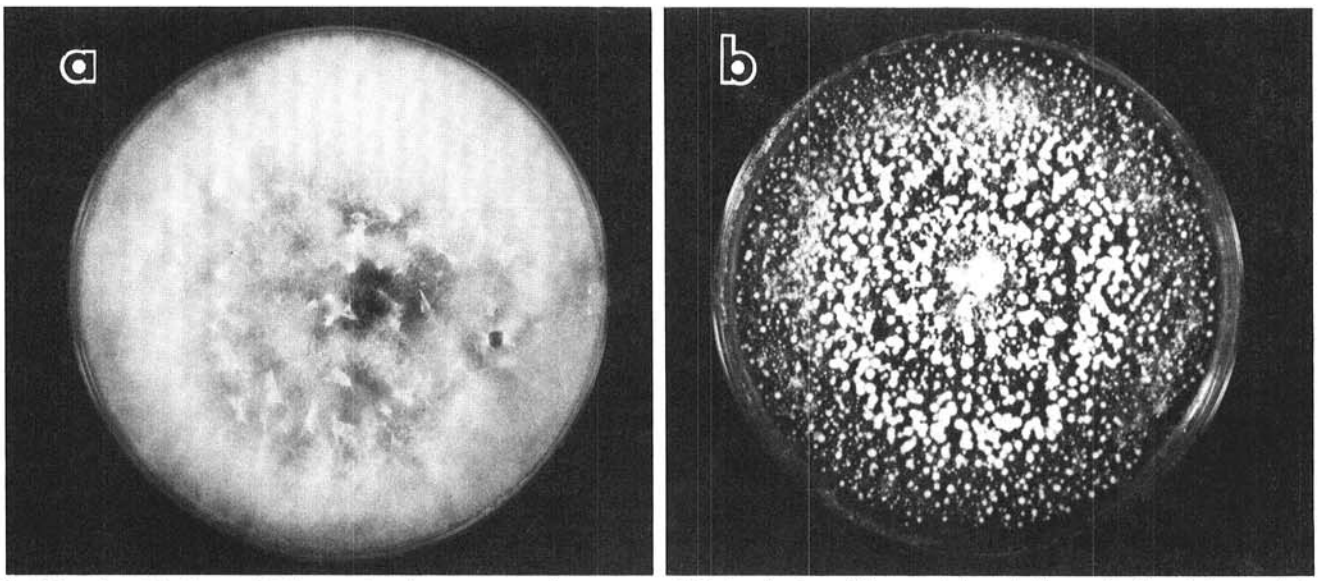

Fig. 1. Ten-day-old cultures of *Sphaeropsis sapinea* on potato-dextrose agar. **(A)** Type A cultural characteristics and **(B)** type B cultural characteristics.

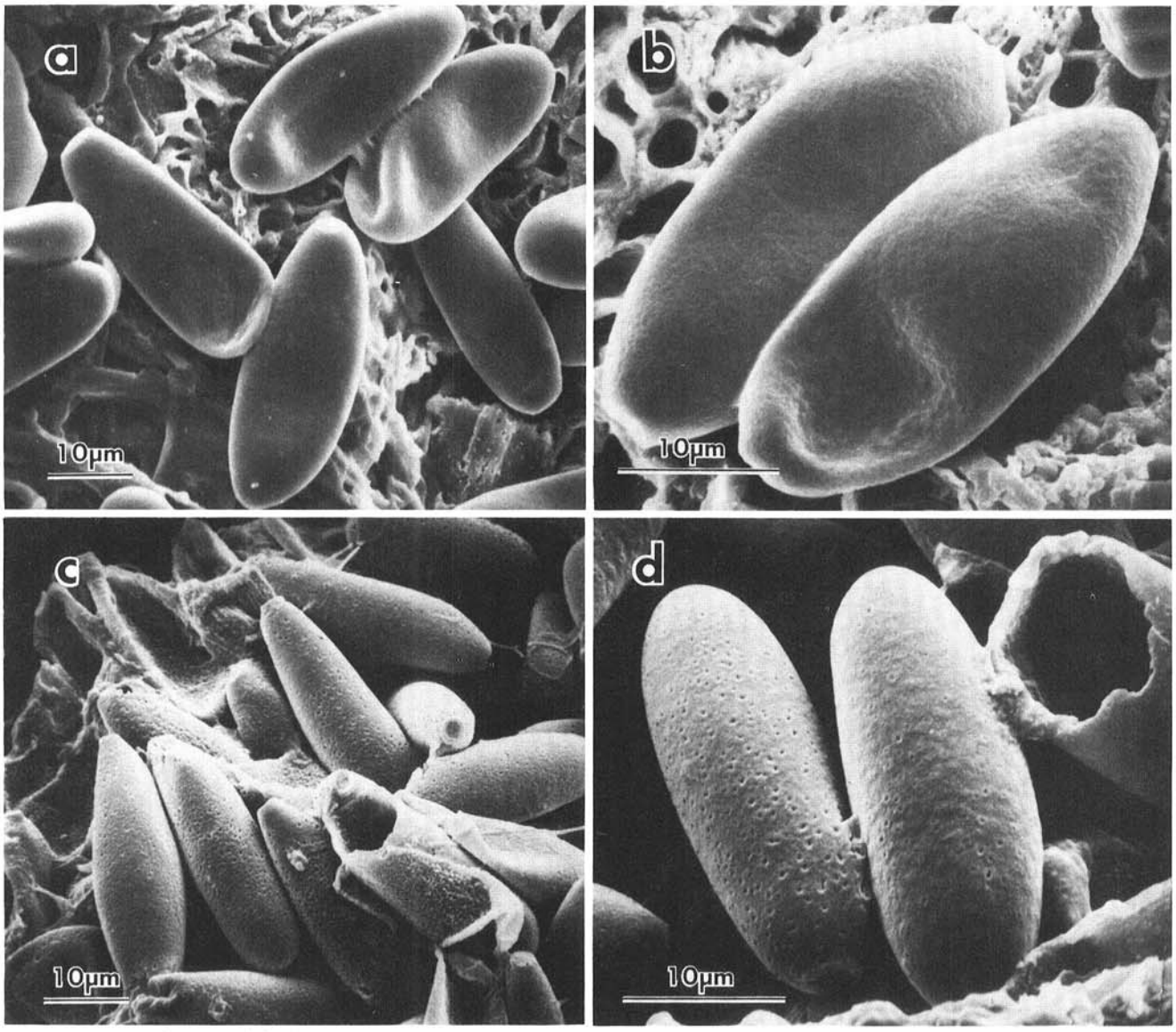

Fig. 2. Mature spores from pycnidia of *Sphaeropsis sapinea* grown on pine needles on potato-dextrose agar. **(A and B)** Type A isolate 120 and **(C and D)** type B isolate 215.

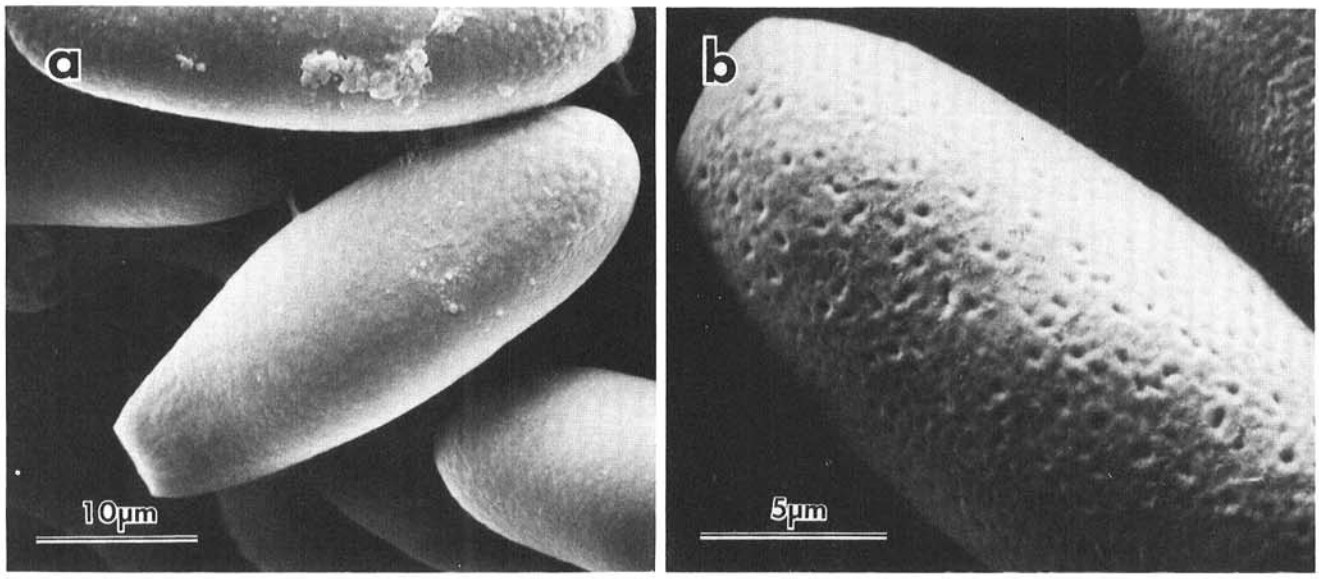

Fig. 3. Mature spores from field-collected pycnidia of *Sphaeropsis sapinea*. **(A)** Spores from the pycnidium that yielded type A isolate 123; pycnidium was on a cone. **(B)** Spores from the pycnidium that yielded type B isolate 215; pycnidium was on a diseased stem.

pitted spore surfaces.

Conidia from field-collected pycnidia were also smooth if the specimen yielded a type A isolate and pitted if the specimen yielded a type B isolate (Fig. 3). Pycnidia from cones of *P. resinosa* produced type A isolates on PDA and had mature spores with smooth surfaces (Fig. 3A). Pycnidia from diseased *P. resinosa* stems produced type B isolates with pitted spores (Fig. 3B).

DISCUSSION

Differences in conidial morphology can be used to separate isolates of *S. sapinea* into two groups. The pitted appearance of mature spores of type B isolates is distinct and easily observed with scanning electron microscopy. The small size of the pits and dark pigmentation within the conidium cell wall make identification impossible with bright-field microscopy. Careful observations, however, with differential interference-contrast microscopy using an oil immersion objective can differentiate the pitted appearance of type B spores.

The pits appear to be a stable characteristic of all mature spores of type B isolates produced in vitro or on field samples. Other differences that exist between isolates, i.e., cultural characteristics and pathogenicity test results, can also be used to separate *S. sapinea* into two groups (8). However, these characteristics are not always easily identifiable. Intermediate forms of *S. sapinea* occur, and cultural characteristics can change by using different growth media or by growing cultures under different environmental conditions. The existence of two strains of *S. sapinea* should prompt investigators working with this fungus to reexamine their isolates.

All of the type B isolates to date have been found in the north central United States. This is probably due to the concentrated effort in that region to look for type B isolates. The conflicting results reported among several researchers (3–7, 10,12) concerning the pathogenicity of *S. sapinea* indicates that type B isolates may occur in many other geographical locations. Identification and differentiation of *S. sapinea* isolate types should provide a better understanding of this serious forest pathogen. Additional study is warranted to determine if the two isolate types are different species of *Sphaeropsis*.

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