Peanut Web Blotch: I. Cultural Characteristics and Identity of Causal Fungus[1]

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ABSTRACT

A foliar disease of peanuts, previously unreported in the USA, was found in Texas in 1972. The pathogen was identified as a species of *Ascochyta*. Further cultural studies have revealed this fungus to be *Phoma arachidicola* Marasas, Pauer, and Boerema. Pycnidia form profusely at 20 C and 25 C. Pycnidiospores are borne on short pycnidiosphores and are predominantly one-celled in culture. Spores produced in pycnidia on infected leaflets become 1 septate. Large 1-septate spores, as well as an occasional 2-septate spore, may form in culture. Optimum temperature for mycelial growth in 20 C; little or no growth occurs at 5 C or above 30 C. The teleomorphic state develops in the field on fallen leaflets and can be induced to form in the laboratory on sterilized peanut leaflets between 15 and 20 C. Cultures derived from single ascospores form pseudothecia. Pycnidiospores, ascospores, and chlamydospores are all infective units. Because this fungus produces hyaline ascospores and pseudoparaphyses, it has been transferred to the genus *Didymella* as *Didymella arachidicola* (Choch.) comb. nov. Comparisons with 15 isolates causing web blotch of peanut in the USA, Argentina, and South Africa indicate that web blotch symptoms are produced by the same fungal species.

Key Words: *Ascochyta, Phoma arachidicola, Arachis hypogaea,* groundnuts.

A foliar disease of peanuts (*Arachis hypogaea* L.), was first observed in the USA by peanut growers in Frio County, TX in early June 1972. Symptoms appeared on leaflets as tan net-like bronzing and large light brown blotches and the disease progressed rapidly to epidemic proportions in several fields (20). A. L. Harrison (then Director of the Texas A&M Research Station at Yoakum) and Dugan Wells (Smith Co. of Uvalde, TX) first recognized the disease as new to this country. The incitant was originally identified as a species of *Ascochyta* by the senior author (20,30). Infected plants were initially confined to South Central Texas; however, the disease was subsequently confirmed in the major peanut-growing area of North Central Texas in and around Comanche County (22). Disease development progressed in the prevailing humid, warm environment regardless of fungicide treatments. Virginia peanut cultivars are less susceptible than spanish cultivars (28). Since 1972, the disease has appeared less frequently in Texas, which may be due to the introduction of the more resistant Florunner cultivar; however, web blotch was still a problem in some areas of Texas in 1973 and 1974. The disease has since been observed in Oklahoma, Virginia, Georgia. North Carolina, and Florida (18,21,23,29,31).

Texas Agricultural Experiment Station Research Paper No. 19630. Supported in part by Texas Peanut Producers Board.

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Peanut Science (1984) 11:109-114

Ascochyta spp. have been reported to parasitize peanut plants in several other countries. In 1924, *Ascochyta arachidis* Woron. was observed by Woronichin on dead peanut leaflets in the USSR (32). He reported that the fungus produced pycnidiospores 6-7 x 3.5 μm in diameter. In 1934, the teleomorphic state, *Mycosphaerella arachidicola* Choch. (12), of *A. adzamethica* Schos. (25) was reported in USSR. More recently, Marasas *et al.* (18) quoted the description of *A. adzamethica* from Shoshiashvili (25) and noted pycnidial sizes ranged from 60-100 μm, and that hyaline 1-septate pycnidiospores were produced that measured 8-12 (14.5) x 3-3.5 μm. In 1962, Cruz *et al.* (16) described "muddy spot" of peanuts in Brazil and indicated that an *Ascochyta* sp was the causal agent. Eight years later Frezzi (17) reported *A. arachidis* on peanut foliage in Argentina. In 1972, *A. adzamethica* was reported from Rhodesia (24). In 1977, Blamey *et al.* (4) published information on the epiphytology of web blotch in South Africa.

There are several reports of the occurrence of the teleomorphic state. Frezzi (17) described it as *Mycosphaerella argentinensis* Frezzi on the basis of immersed aparaphysate pseudothecia (75-170 X 75-126 μm) and cylindrical, hyaline asci (40-59 X 12.5-17 μm) containing hyaline, 2-celled ascospores (14-21 X 6-8.5 μm). Chochryakov (12) characterized *M. argentinensis* as having pseudothecia 80-100 μm in diameter, cylindrical asci (36-42 X 9-10 μm), and hyaline, 1-septate ascospores that measured 12-15 X 5-7 μm. Alcorn *et al.* (1) redescribed the sexual state as *Didymosphaeria arachidicola* (Choch.) Alcorn, Punith., and McCarthy based on the presence of pseudoparaphyses and brown ascospores. In 1980, Luttrell and Smith (18) indicated that this fungus may belong in the genus *Didymella.*

More recently, Marasas *et al.* (19) reported the disease in South Africa and indicated it has been present there for the previous 10 years. They were unable to obtain isolates of *Ascochyta* from the USSR for comparison. No teleomorphic state was found in South Africa. In 1980, Young *et al.* (33) reported additional information on the occurrence and epidemiology of web blotch in South Africa. Using criteria for genetic delimitation of *Phoma* and *Ascochyta* as outlined by Brewer and Boerema (11), Marasas *et al.* (19) transferred the web blotch fungus to the genus *Phoma* as *P. arachidicola* Marasas, Pauer, and Boerema. This reassignment was based on the fact that the pycnidiospores of *Phoma* spp., as determined by Boerema *et al.* (5,6,7,8,9,10) and Brewer and Boerema (11), are produced on phialides, are 1-celled when formed, but *in vitro* often become secondarily 2-celled by an annular septal ingrowth from the lateral wall. Zherbele (34) and Boerema and Dorenbosch (7) stated that many of the species described as *Ascochyta* would be better housed in the genus *Phoma.*

This paper documents our conclusion that the web

blotch disease in the USA is caused by the same fungal species that infects peanuts in South Africa and Argentina.

Materials and Methods

To determine the causal agent of this disease new to the USA, leaflets from infected plants from South Texas were surface-sterilized 1 min. in 70% ethyl alcohol and 10% Clorox. Diseased leaf tissues 1-2 cm^2 were placed on potato dextrose agar, rose-bengal streptomycin agar, malt agar, and water agar. Fungi originating from these pieces were isolated in pure culture. Water suspensions of spores of each isolate were sprayed on 60-day-old Starr cultivar peanuts growing in outdoor box plots at College Station, TX. Box plots were 3' X 3' enclosures bounded by 1' aluminum sheeting. They were unprotected from direct sunlight. Clear plastic bags were tied over the inoculated leaflets (to increase humidity) and removed after 5 days.

Inoculations of peanut leaflets were conducted using 10 Texas isolates from infected leaflets (including the first USA isolate, designated 1-1) and five isolates from other areas. These five isolates were from infected leaflets and/or cultures from Argentina (isolate designated *Ar* from J. J. Frezzi), South Africa (isolate *A* from G. H. Boerema), Georgia (isolate *G*from R. H. Littrell), Virginia (isolate *V.* from P. M. Phipps), and Oklahoma (isolate *O* from D. H. Smith). Each isolate was tested for pathogenicity and symptoms by swabbing a spore suspension from 10-day-old malt agar (MEA) cultures onto Starr cultivar peanuts in greenhouse pots. Plastic bags provided moist chambers over inoculated leaflets. Inoculated plants were placed in subdued light for 48 hrs. under the greenhouse bench and then returned to the bench. Fungi were isolated from leaflets that developed typical symptoms.

Optimum temperatures for radial growth of the isolates from Argentina, South Africa, and South Texas were determined on Difco malt extract agar. Small agar plugs were cut from actively growing colonies on MEA with a #1 (3.6 mm) cork borer. Plugs were placed on MEA plates and placed in incubators set at 5-35 C in 5-degree increments.

Cultural characteristics and sporulation patterns were determined by transferring 7-day-old MEA cultures grown in 20 C incubators, to incubators at 10-30 C with a Sylvania fluorescent blacklite (F15T8-BL) 28 cm above the plate in each incubator. All observations were made at 14 days. Treatments were conducted in triplicate, including comparable cultures grown in darkness.

Production of pycnidia and pseudothecia was tested by placing conidia on autoclaved peanut leaflets and incubating them 14 days at 10-30 C. In addition, isolates were grown on V-8 juice agar, Czapeks agar, peanut decoction agars, and MEA supplemented with 1 ppm ergosterol, cholecalciferol, and/or cholesterol to induce pseudothecia formation in culture.

Spore size and septation were measured from spores harvested from MEA cultures and peanut leaflets. Spores were mounted in lacto-phenol cotton blue.

Results and Discussion

Seven different fungi were isolated from peanut leaflets that exhibited web blotch symptoms in South Texas, including *Alternaria* spp., *Penicillium* spp., a *Nigrospora* sp., and a pycnidial fungus designated Isolate 1-1. Isolate 1-1 produced typical webbing and blotch symptoms on the inoculated peanut leaflets in the box plots (Fig. 1A) and was reisolated from such typical leafspots. Examination of this isolate initially led to its identification as a species of *Ascochyta,* based on its production of pycnidia and 2-celled pycnidiospores both in agar cultures and on peanut leaflets (Figs. 1B and C; Figs. 2A and B).

All web blotch isolates from Texas, Argentina, South Africa, Georgia, Virginia, and Oklahoma grew well on MEA. Plants inoculated with these isolates developed identical symptoms after 5 days. Webbing symptoms predominated.

Optimum temperature for radial mycelial growth of all isolates was 20 C (Table 1). No growth occurred at 0 C or 35 C and very little occurred at 5 C. Cultures subjected to near ultraviolet light frequently formed

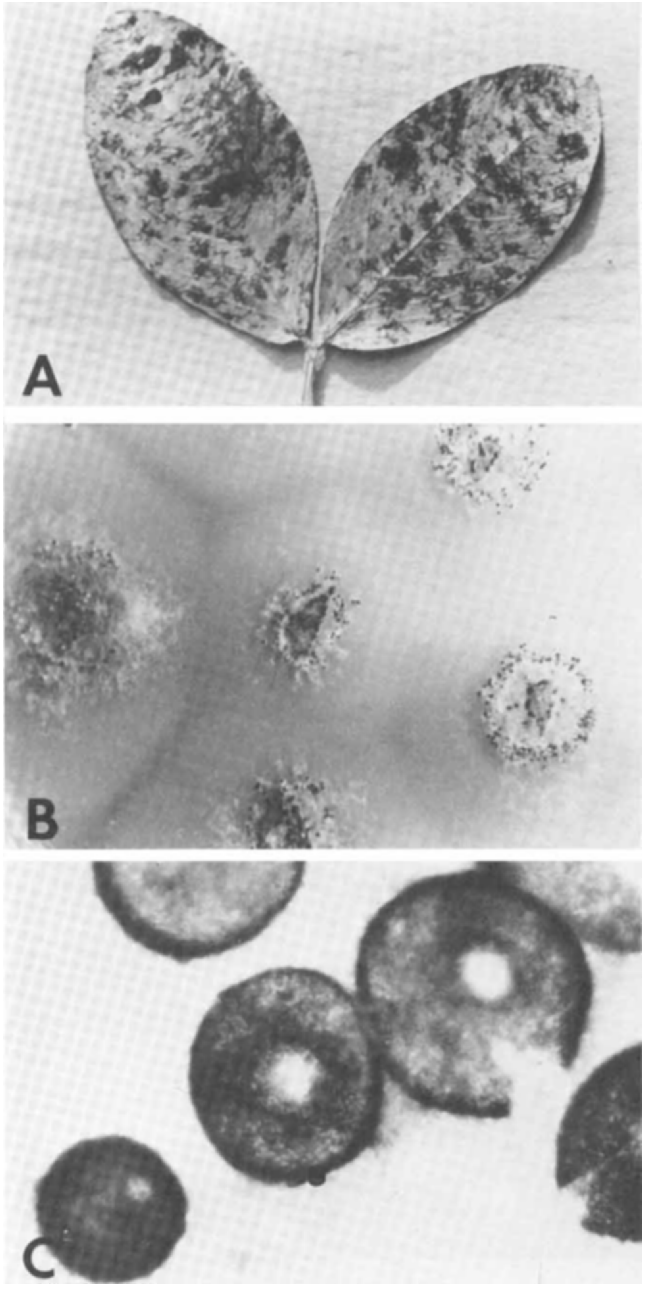

Fig. 1. A. Web blotch symptoms on Spanish peanuts in Texas. B. Pycnidia of the web blotch fungus produced in agar culture, C. Higher magnification of pycnidia showing ostioles.

pycnidia with long cirrhi of spores (Fig. 2C). Isolates taken from leaflets exhibiting web blotch symptoms typically produced uniform mycelial growth on agars at 20 C (Fig. 3A); however, at 25 C differences in colony pigmentation occurred with some isolates (Fig. 3B).

Pycnidiospores from pycnidia on infected leaflets (Figs. 4B and C) were large and became 1-septate with maturity. Spores were significantly smaller and mostly non-septate when produced in agar cultures. Less size

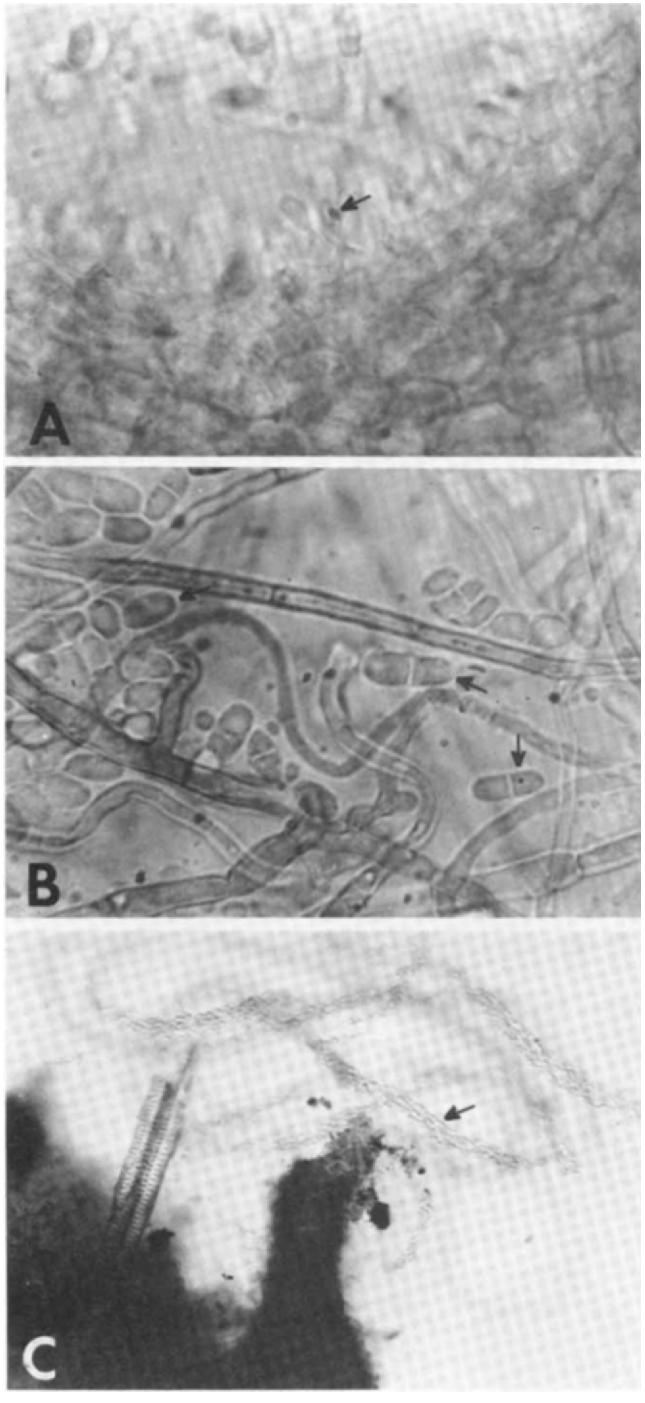

Fig. 2. Web blotch fungus. A. Short pycnidiosphores with attached spore (arrow), B. Septate and non-septate pycnidiospores (arrows) produced in agar culture, C. Cirrhus (arrow).

variation occurred on MEA than on autoclaved leaflets. The Argentina isolate tended to form more septations at 25 C than the African or South Texas isolates; however, overall spore dimensions were similar (ca 6x3 μm). The largest pycnidiospores (8-12 x 3-4.5 μm) were produced after 14 days growth at 25 C on MEA. Considering all spores examined from cultures grown at 25 C, 20-90% of the spores were large and septate, whereas 50-85% of spores were classified as large and septate from cultures grown at 15 C. Between 10-40% of the spores from cultures grown at 25 C were small (4-6 x 2.5 μm) whereas 10-20% were small from cultures grown at 15 C. An increase in spore size, as we found in these studies, is also common in other fungi grown at lower temperatures.

Numbers of pycnidia and pseudothecia formed/cm² by isolates Ar, P, A, and Tx 1-1 on sterilized peanut leaflets are summarized in Table 2. No pseudothecium formed on agar cultures under any condition tested. The most favorable temperature for pycnidium production by all isolates was 25 C, whereas pseudothecia were produced at lower temperatures (Fig. 4A). This finding is consistent with the function of the teleomorph as an over-wintering state. Production in the field would also

Table 1. Colony diameter (mm) of 5 web blotch isolates grown at various temperatures on malt extract agar for 12 days in darkness.

	Temperature, °C							
Isolate	0	5	10	15	20	25	30	35
Argentina (Ar)	0	2	7	11	70	42	10	0
Tx Pearsall (P)	0	3	8	13	57	52	13	0
Tx 1-1	0	3	6	12	53	47	12	0
Tx 1-3	0	2	7	11	49	44	12	0
Africa (A)	0	1	4	8	52	36	14	0

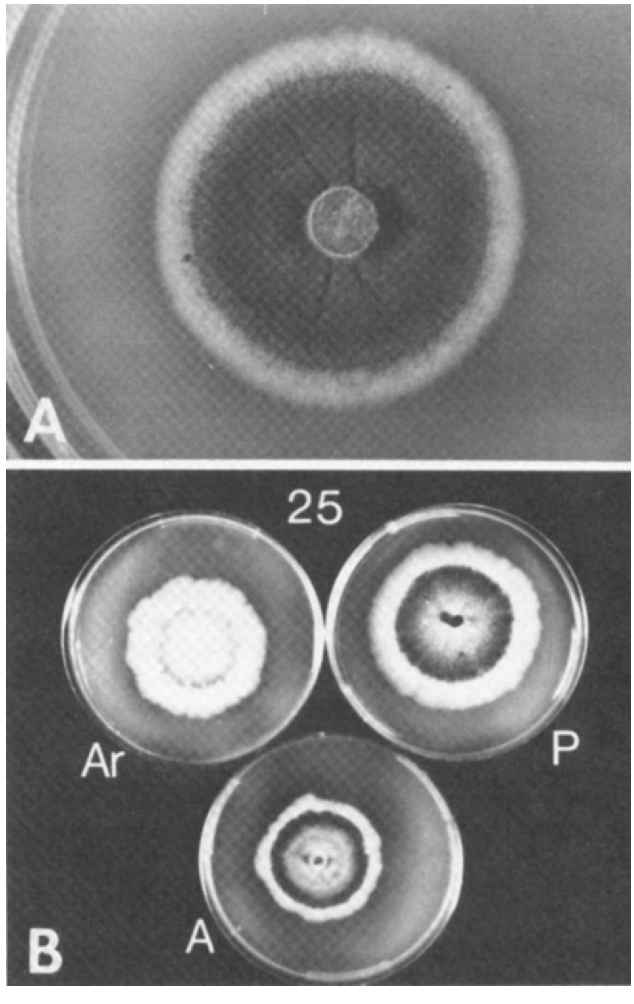

Fig. 3. Cultures of the web blotch fungus. A. Typical culture on agar, B. Pigmentation formed by some isolates at 25 C. Ar = Argentina isolate; P = Pearsall isolate; A = African isolate.

be influenced by other factors such as water, temperature, and light. Three isolates formed pseudothecia at 15 and 20 C whereas the Argentina isolate produced no pseudothecia at any temperature (Table 2). Pseudothecia did form, however, in desiccated infected leaflets on moist filter papers in Petri dishes that were exposed to near UV (300-400 nm) for 48 hours. It is interesting to note that the African isolate produced pseudothecia under Texas conditions (Table 2) although Marasas *et al.* (19) did not observe them in their studies. Single ascospores, as well as chlamydospores from cultures, initiated infection that resulted in disease symptoms on peanut leaflets.

Pseudothecia of all Texas isolates were dark brown, sub-globose to globose, separate, and 65-154 m in diamter (Fig. 5A). Asci were hyaline, bitunicate, and 8-spored (Fig. 5, B & C). Ascospores at discharge were

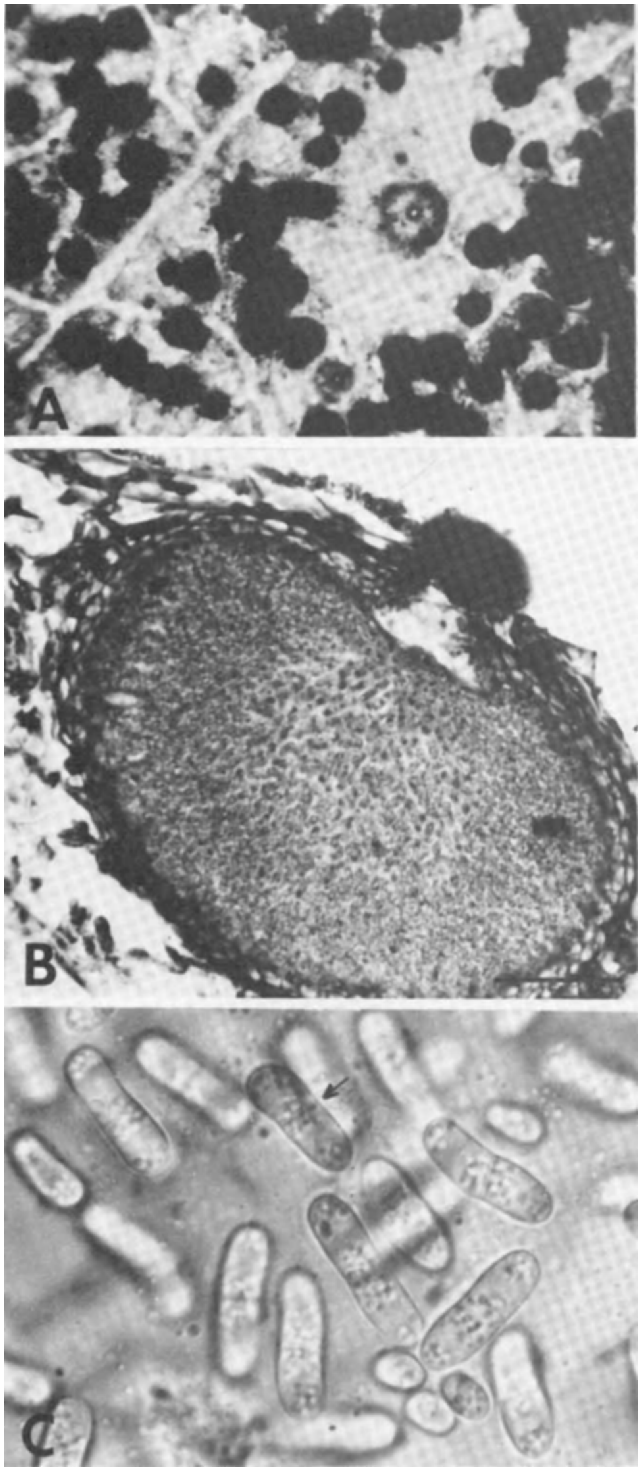

Fig. 4. Morphology of the web blotch fungus. A. Pycnidium (large light structure) and numerous pseudothecia on autoclaved spanish peanut leaflet, B. Cross - section of a pycnidium, C. Wet mount showing pycnidiospores. Septate spore at arrow.

Table 2. Pycnidia and pseudothecia formed by web blotch isolates on autoclaved peanut leaflets after 14 day incubation. (no/cm2/14 days).

Temperature	Pycnidia[1]				Pseudothecia			
°C	Ar	P	Tx 1-1	A	Ar	P	Tx 1-1	A
10	1157	297	285	263	0	0	0	0
15	1473	200	210	446	0	1524	1333	1138
20	1467	935	630	596	0	1094	960	1416
25	1517	1555	1500	1030	0	0	0	0
30	522	0	7	97	0	0	0	0

[1]Ar = Argentina isolate; P = Pearsall Texas isolate; Tx 1-1 = first web blotch isolate from South Texas; A = Africa isolate.

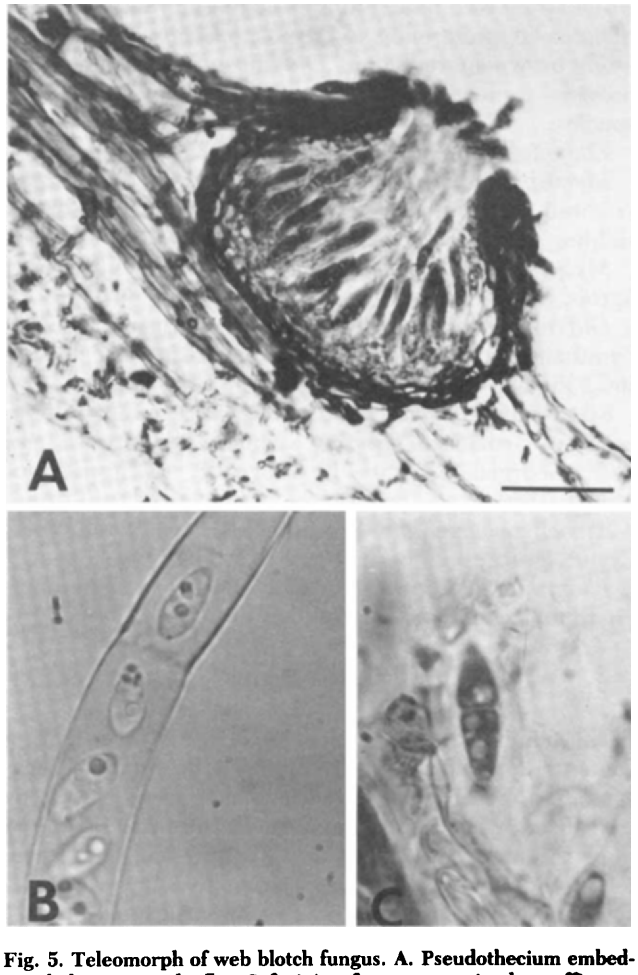

Fig. 5. Teleomorph of web blotch fungus. A. Pseudothecium embedded in peanut leaflet. Safrainin - fast green stained paraffin section, B. Young bitunicate ascus containing dislodged, normally distichous immature ascospores as in Fig. 5A, C. Mature ascospore stained with lactophenol cotton blue.

hyaline, smooth, 2-celled, and 13-16.5 X 4.5-6.5 μm. Pseudoparaphyses were hyaline and 1-1.8 μm in diameter.

There is confusion in the literature over generic distinctions between *Mycosphaerella, Didymella,* and *Didymosphaeria.* Ascospores of *Didymosphaeria* are typically brown-spored at discharge whereas those of *Didymella* and *Mycosphaerella* are hyaline. Corlett (14) emphasized the problem of species identification and the separation of *Didymella* and *Mycosphaerella.* He recently described, illustrated, and provided keys for 15 species of *Didymella* and *Didymella*-like species (14) and listed distinguishing characteristics between *Mycosphaerella* and *Didymella* as described by Corbaz (13): hyaline, slightly constricted, relatively narrow ascospores and fasciculate asci arising from a basal cushion in *Mycosphaerella,* absence of pseudoparaphyses in *Mycosphaerella,* and smaller pseudothecia in *Mycosphaerella.* He listed anamorphs of *Mycosphaerella* to be genera such as *Ramularia, Septoria, Cladosporium,* and *Cercospora,* whereas anamorphs of *Didymella* are *Ascochyta* and *Phoma.* Von arx (2) also recently discussed generic differences between *Mycosphaerella* and *Didymella.* In view of the presence of pseudoparaphyses and hyaline mature ascospores formed by our test isolates of the web blotch fungus, we agree with Luttrell (18) that the web blotch fungus belongs in the genus *Didymella* in preference to *Mycosphaerella* or the definitely brown-spored genus *Didymosphaeria,* and herein provide a new combination in this genus for this species:

Didymella arachidicola (Chochrjakov) comb. nov.

Mycosphaerella arachidicola Chochrjakov, Bolezni i vrediteli maslichnykh kul'ter (Diseases and pests of oil-yielding plants) I (2), 29 (1934).

Mycosphaerella argentinensis Frezzi, Rev. Invest. Agrop. Serv. 5, 6:149 (1969).

Didymosphaeria arachidicola (Chochrjakov) Alcorn, Punithalingam, & McCarthy. Trans. Brit. Mycol. Soc. 66(2):351-355 (1976).

Stat. conid. *Ascochyta adzamethica* Schoschiaschvili, Izvestiya Gruzinskoi Opytnoi Stantsii Zashchity Rastenii (Bull. Georgian Exp. Sta. Pl. Prot. Ser. A, Fitopat, No. 2, 272 (1940).

Ascochyta arachidis Woronichin, Not. Syst. Inst. Crypt. Horti. Bot. Reip. Ross. 3:31 (1924).

Phoma arachidicola Marasas, Pauer & Boerema, Phytophylactica 6:200 (1974).

Conclusions

The comparison of cultures of 17 fungi representing incitants of web blotch symptoms in South Africa, Argentina, and the USA indicates that these symptoms are caused by the same fungus in all countries. Pycnidia were commonly produced on the plant and in culture; pseudothecia occurred less commonly, but were induced in the laboratory on sterilized peanut leaflets. Based on the generic concepts of *Phoma* and *Ascochyta* as outlined by Brewer and Boerema (10), this fungus is *Phoma arachidicola* Marasas, Pauer, and Boerema. Because of the presence of pseudoparaphyses and hyaline ascospores (which only darken with over-maturity) it seems desirable to provide a new combination in *Didymella* for this species.

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Accepted December 10, 1984