Trans. Br. mycol. Soc. **87**, (1), 23–44 (1986) *Printed in Great Britain*

ARKOOLA NIGRA GEN. ET SP.NOV. (VENTURIACEAE) CAUSING BLACK LEAF BLIGHT OF SOYBEAN IN AUSTRALIA

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Arkoola nigra gen. et sp.nov. (Venturiaceae) is described, causing black leaf blight of soybean in New South Wales. It forms a superficial black mycelial web on infected plants and penetrates the host from large appressoria, causing severe spotting and blighting of leaves, stems and pods. Ascocarps develop on dead fallen leaves and on detached leaves inoculated in culture. They are black, setose and contain large cylindrical bitunicate asci with pale greenish two-celled ascospores and abundant pseudoparaphyses. The connexion between ascosporic and mycelial states has been proved by pure culture studies, and plant inoculations reproduce the disease symptoms. It is transmitted to a limited extent with seed. A range of native legumes were infected in artificial inoculation tests. The relationship of *A. nigra* to other genera of Venturiaceae, its possible origin, host range and associated matters are discussed.

In March 1982 a severe leaf, branch and pod spot disease occurred in a maturing crop of soybean (*Glycine max* (L.) Merr.) at Wauchope in the Manning District of coastal New South Wales (Fig. 1). A superficial black mycelial web was present on diseased parts of infected plants. A short account of this outbreak has been published (Stovold & Walker, 1983; Stovold *et al.*, 1984) and the similarity of the disease to various tropical aerial blights noted. Because of the dark septate hyphae and the absence of any spores, the associated mycelium was referred to as a *Rhizoctonia*-like fungus. Since then the disease has been seen each year in coastal soybeans, and at the time of writing (Sept. 1985) is known in crops from Wauchope in the south to the Casino district in the north (almost to the Queensland border) (Fig. 1). Early in 1985 it was recorded in the Tabulam district on the eastern fringe of the northern tablelands (Fig. 1), and it is now one of the most serious soybean diseases in eastern N.S.W.

Investigations have shown that the dark superficial fungus is the cause of the disease. Its teleomorph has been found and is a previously undescribed ascomycete in the family Venturiaceae. This paper gives details of the disease and a description of the causal fungus and discusses its possible relationships.

THE DISEASE

Infected plants show severe spotting and blighting of leaves, stems and pods (Figs 2, 3). Leaf spots are grey to dark grey to brown with a thin distinct very dark brown margin separating them from healthy tissue. They are roughly circular to oval and from 1 to 10, mainly 2–7 mm diam. Larger more irregular dead areas result from joining of two or more spots, which tear easily, giving heavily infected plants a tattered and blighted appearance. Leaf spots occur on both surfaces, penetrate the thickness of the leaf and, on severely spotted leaves, the surrounding leaf tissue is yellowed. Lesions developed on stems and pods are darker brown and oval to elongated rather than circular. Heavily infected pods are shrunken, and seed directly below spots may be discoloured.

The most striking feature of infected plants is the web of fine dark brown to black glistening aerial mycelium growing over them (Figs 4, 5). It is composed of strong dark brown branched septate hyphae 15–20 μm wide (Fig. 21). Some develop raised shiny dark brown to black appressoria at their tips in contact with the plant (Figs 6, 7, 10). Appressoria are up to 300 μm diam and consist of several short hyphal branches under which penetration occurs through small circular holes, 3–5 μm diam (Fig. 10). One or more appressoria are present on each spot and infection by hyphae without formation of appressoria has not been seen. Infection occurs on either leaf surface, but appressoria are more abundant on the lower (abaxial) surface than on the upper. Inside the plant, hyphae are pale brown, intercellular, branched and somewhat more variable in width than surface hyphae (Fig. 22).

On the ground, there is abundant mycelial development on trash resulting from dropping of infected leaves, pods and stems and from diseased residue remaining after harvest. Mycelium may

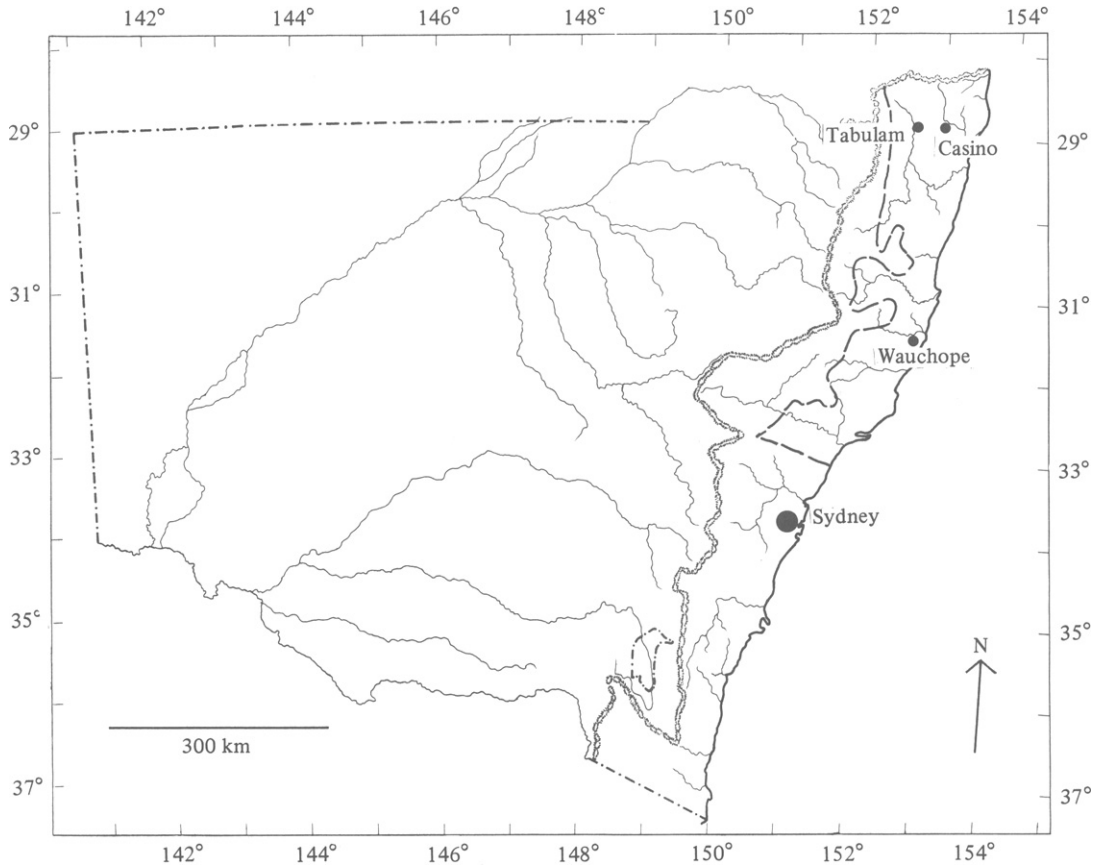


Fig. 1. Map of New South Wales showing the area on the north coast where *Arkoola nigra* has been found. ---, Boundary of the north coast botanical eco-geographic region (from Jacobs & Pickard, 1981).

grow quite strongly on soil under and surrounding infected trash. Dead leaves are heavily colonized by hyphae both externally and internally.

In 1982 the fungus was known only in its mycelial state on living and dead plants (Stovold & Walker, 1983). In July 1983 black setose bodies were found amongst the dark mycelium on diseased trash collected from soybean land at Wauchope (Fig. 8). It was suspected that they were immature ascocarps. They were matured by wetting and drying trash placed on soil in pots in the glasshouse at Rydalmere. Cultures (Figs 23, 24) obtained from single ascospores were identical with those obtained by isolating from leaf lesions (Stovold *et al.*, 1984).

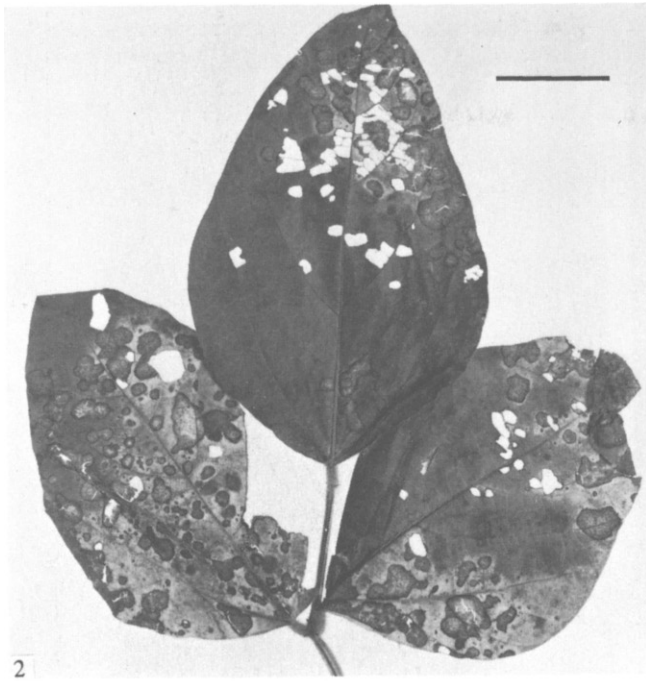
Although immature ascocarps have now been found several times on soybean trash in the field, mature ascocarps have not yet been seen in field collections. In addition to producing mature ascocarps by incubating infected field trash in the glasshouse, they have been developed by growing the fungus on detached soybean leaves in culture. Five-day-old Petri dish cultures on potato dextrose agar (PDA), covered with a thin layer of sterile sand

Fig. 2. Leaf spotting and tearing on soybean caused by *Arkoola nigra*, Wauchope, N.S.W., March 1982 (DAR 41445). Bar = 2 cm.

Fig. 3. Leaf spots on soybean from artificial inoculation with culture of *Arkoola nigra* in glasshouse, Rydalmere. Bar = 2 cm.

Fig. 4. Superficial mycelium of *Arkoola nigra* on dead soybean leaf collected in diseased crop. Bar = 500 μm.

Fig. 5. Superficial mycelium of *Arkoola nigra* on leaf of *Crotalaria medicaginea* artificially inoculated, Rydalmere (DAR 49794). Bar = 500 μm.



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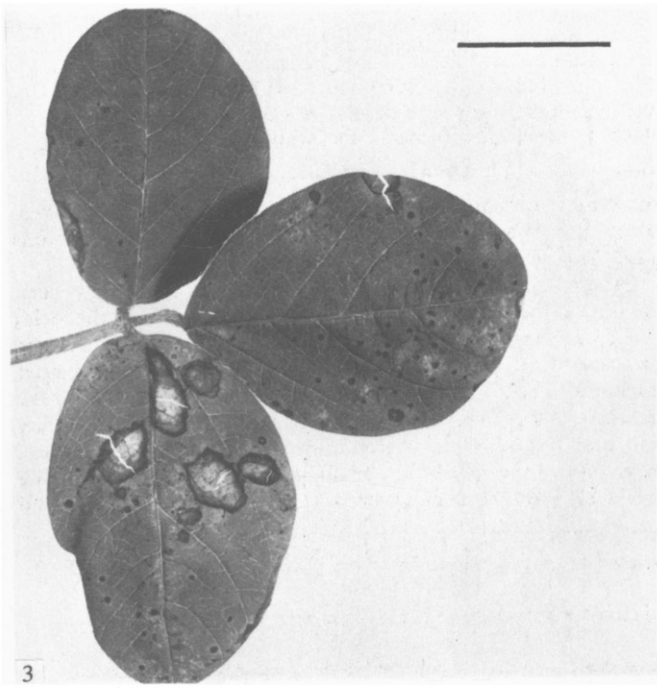

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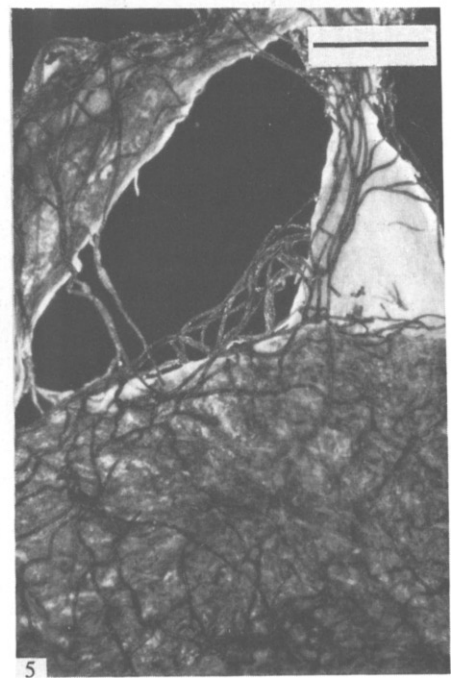

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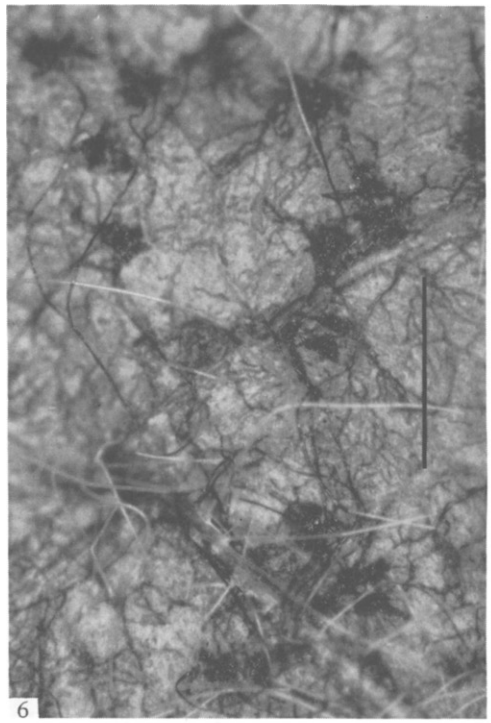
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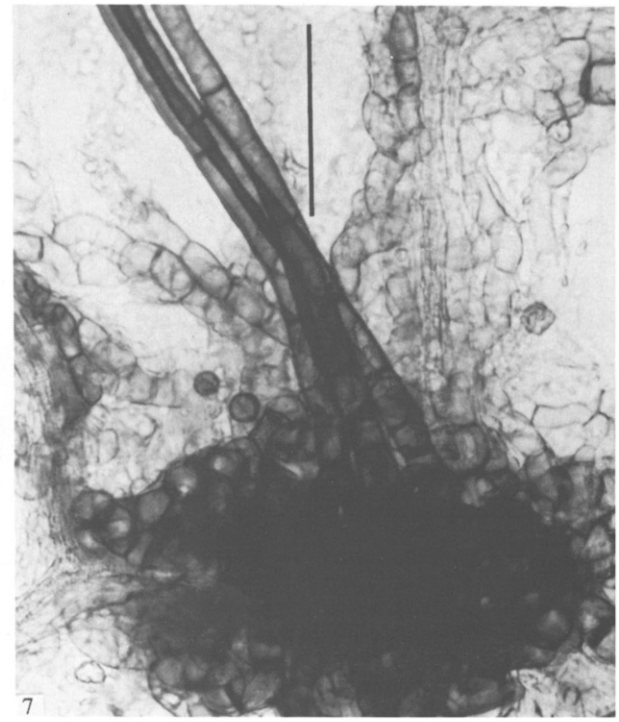
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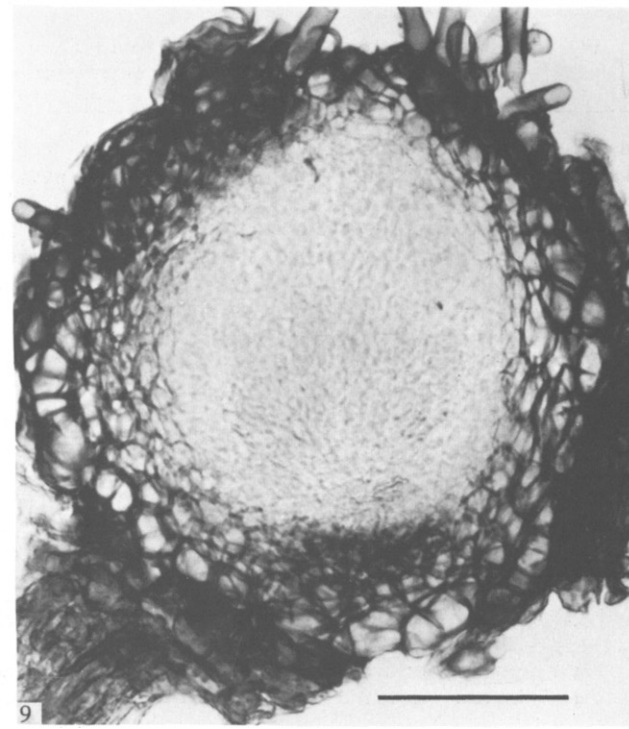
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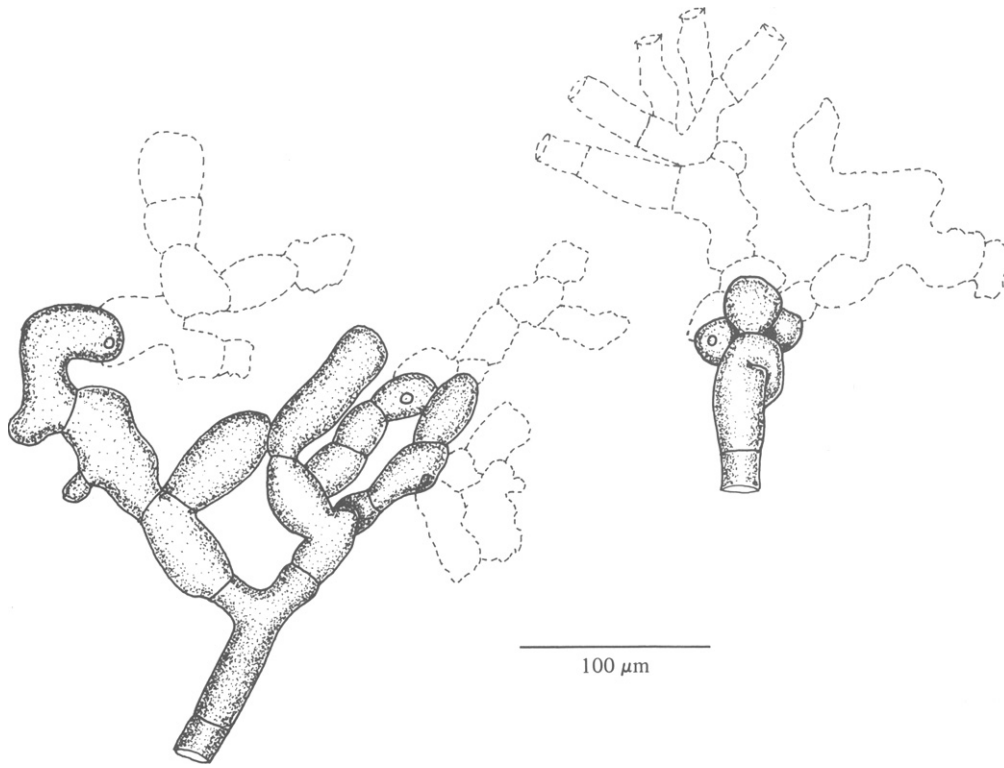


Fig. 10. Appressoria of *Arkoola nigra* showing branching, penetration holes and mycelium (dotted) in leaf tissue (DAR 41445). Bar = 100 μm.

on which several washed soybean leaves were placed, were held on a south-facing bench at room temperature (about 20–25 °C). The mycelium grew through the sand and colonized the leaves. Ascocarps developed on the superficial mycelium on leaves and some also on mycelium covering sand grains. Mature asci with ascospores were produced in 5–8 weeks. Single ascospore cultures were similar in all respects to those obtained by isolation from mycelium and plant lesions.

Inoculation of young soybean plants in the glasshouse with macerated agar cultures obtained from ascospores or from diseased plants reproduced the disease (Fig. 3). Infection also occurred on plants growing in pots containing trash with mature ascocarps and it is suspected that ascospores were responsible. The fungus is re-isolated readily from surface-sterilized lesions.

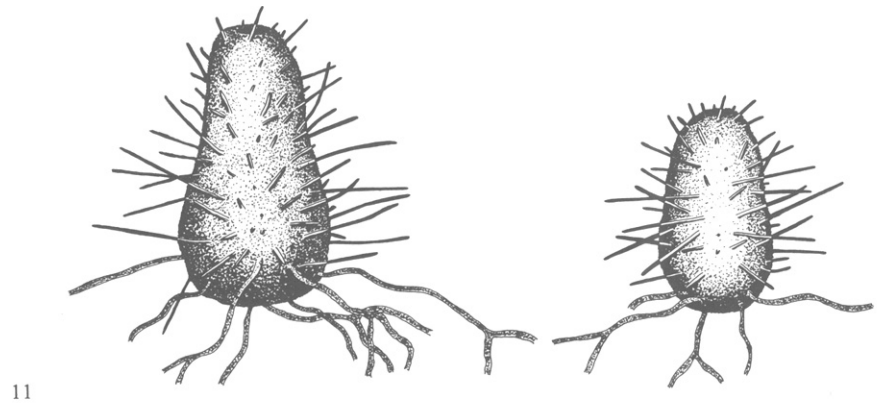
No similar disease of soybean appears to have been described (Anon., 1975). When first seen in 1982, the disease and the fungus were thought to be related to the aerial blights seen on various crops in warmer regions and caused by species of *Rhizoctonia* with *Thanatephorus* Donk, *Corticium* Pers. and related teleomorphs and by *Marasmius* spp. (Anon., 1975; Matz, 1917; Mordue, 1974; Petch, 1924; Stovold & Walker, 1983; Talbot, 1965; Wellman, 1972). With the finding of an ascomycetous teleomorph in August 1983, it was realized that the disease had not been described previously. Because of the leaf blighting in heavily infected crops and the distinctive dark aerial

Fig. 6. Superficial hyphae and raised black appressoria of *Arkoola nigra* on dead fallen soybean leaf in crop (DAR 45788). Bar = 1 mm.

Fig. 7. Detail of superficial hyphae and appressorium on soybean leaf (DAR 45788). Bar = 100 μm.

Fig. 8. Immature pseudothecia of *Arkoola nigra* developing on dead leaf of soybean (DAR 43447). Bar = 1 mm.

Fig. 9. Longitudinal section of immature pseudothecium of *Arkoola nigra* (DAR 43447). Bar = 100 μm.



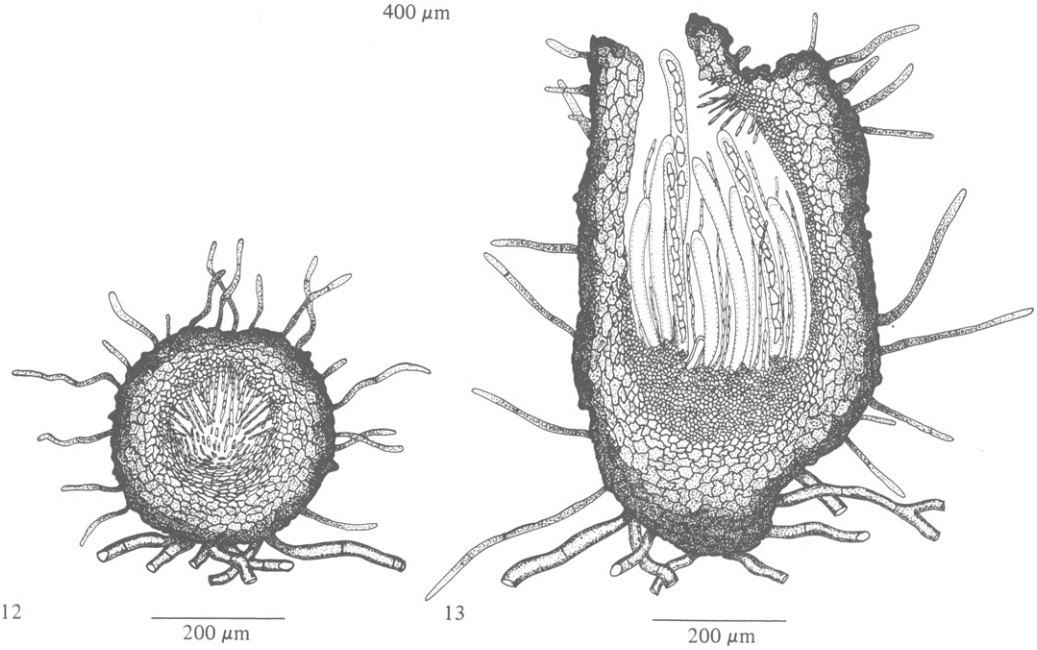


Fig. 11. Two pseudothecia of *Arkoola nigra* showing setae and hyphae attached at base (DAR 43446). Bar = 400 μm.

Fig. 12. Longitudinal section of immature pseudothecium of *Arkoola nigra* showing descending pseudoparaphyses (DAR 43446). Bar = 200 μm.

Fig. 13. Longitudinal section of mature pseudothecium of *Arkoola nigra* showing thick setose wall, asci, ascospores and pseudoparaphyses (DAR 43446). Bar = 200 μm.

mycelium on infected plants, the disease was named black leaf blight of soybean (Stovold *et al.*, 1984). The causal fungus is an undescribed species showing characteristics of the family Venturiaceae. It is distinct from all known genera in this family and is described below.

THE FUNGUS

Herbarium abbreviations used are taken from Holmgren, Keuken & Schofield (1981).

Description

Arkoola gen.nov.

(Etym. *arkoola*, pilus vel capillus, in lingua una aboriginum Australiae, mycelium nigrum superficiare abundans et pseudothecia setosa referens)

Pseudothecia praecipue superficiaria interdum erumpentia, in mycelio in organis emortuis delapsis plantarum infectarum evoluta, magna, nigra, setosa, subglobosa vel late ovoidea vel obpyriformia, uniloculata, ostiolata.

Paries pseudotheciorum ex stratis pluribus cellularum ovalium vel oblongarum constans, texturam angularem formans. Asci cylindrici, brevi stipitati, octospori, bitunicati cum dehiscentia fissitunicata, cubiculum oculatum adest. Ascosporae magnae, uniseptatae, ellipsoideae vel fusiformes, pallide virides, cum vagina gelatinosa tenui tectae. Pseudoparaphyses abundantes, hyalinae, filiformes, ramosae, cellulosae. Mycelium superficiare abundans, atrobrunneum vel nigrum, ex hyphis magnis septatis ramosis atrobrunneis constans. Parasitica in plantis vivis et saprophytica in foliis delapsis et organis ceteris emortuis plantarum infectarum. Genera *Protoventuria* Berlese & Saccardo et *Macroventuria* van der Aa simulantia, sed a *Protoventuria* absentia hypostromatis evoluti et magnitudine differt et a *Macroventuria* morphologia centri, mycelio superficiare abundanti, magnitudine et habitu parasitico differt.

Typus generis: *Arkoola nigra* J. Walker & G. E. Stovold

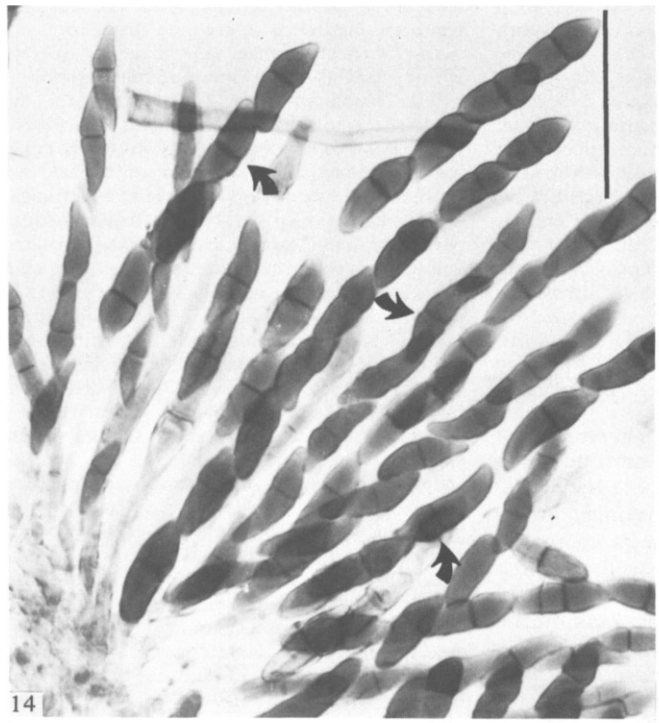
Pseudothecia mainly superficial, sometimes erumpent, developed from mycelium on dead fallen organs of infected plants, large, black, setose, subglobose to broadly ovoid to obpyriform, uniloculate, ostiolate. *Wall* of pseudothecia composed of several layers of oval to oblong cells forming a textura angularis. *Asci* cylindrical, shortly stipitate, 8-spored, bitunicate with fissitunicate dehiscence, ocular chamber present. *Ascospores* large, 1-septate, ellipsoidal to fusiform, pale greenish, surrounded by a thin gelatinous sheath. *Pseudoparaphyses* abundant, hyaline, filiform, branched, cellular. *Superficial mycelium* abundant, dark brown to black, composed of large septate branched dark brown hyphae. Parasitic on living plants and saprophytic on fallen leaves and other dead organs of infected plants. Similar to the genera *Protoventuria* Berlese & Saccardo and *Macroventuria* van der Aa, but differing from *Protoventuria* in the absence of a well-developed hypostroma and in size, and from *Macroventuria* in centrum morphology, abundant superficial mycelium, size and parasitic habit.

Arkoola nigra sp.nov. (Figs 4–25)

Pseudothecia praecipue superficiaria sed interdum erumpentia in mycelio in foliis emortuis delapsisque plantarum evoluta, nigra, singularia sed 6–10 laxe aggregata, initio subglobosa demum late ovoidea vel obpyriformia, uniloculata, (400) 500–800 (900) μm alta, 300–450 μm lata in dimidio inferiore, super 200–350 μm lata, ostiolum 40–70 μm lata adest, setosa. Setae rectae vel flexuosae, septatae, atrobrunneae, apice dilutiore et semicirculare, cylindricae, (7) 10–12 μm latae sed basi latiores 12–18 μm, 45–140 μm longae supra pseudotheciorum sed longiores ad 250–380 μm in dimidio inferiore pseudotheciorum, septa 30–60 μm distantia, pseudothecia immatura tegentes sed sparsae vel absentes prope ostiolum pseudotheciorum maturorum et in pseudotheciis erumpentibus. Paries pseudotheciorum 60–90 μm, basi ad 130 μm, latus, ex 4–6 stratis cellularum ovalium vel oblongarum 20–40 × 15–20 μm constans, strata externa atrobrunnea vel fere nigra, strata interna pallide brunnea vel hyalina, texturam angularem in sectione transversali et externe visus formans, hyphae vegetativae ad parietem basi affixae. Asci 35–50 in omnibus pseudotheciis, a strato hyalino basali 40–80 μm crasso exorientes, cylindrici, praecipue octospori sed ut videtur abortii ascosporae pauciores, 230–300 × 24–26 (30) μm, cum stipite brevi basi applanato 20–25 × 6–9 μm, bitunicati, ectotunica hyalina, valde tenuis, 0·5–1 μm lata, endotunica 5–6 μm lata in ascis inexpansis, 10 μm lata in ascis dehiscentibus, per totam longitudinem ascis extensa, 6–7 μm lata ad apicem, cubiculum oculatum 5–6 μm latum cum zonale refringente tenui in dimidio superiore adest; asci cum dehiscentia fissitunicata et ad longitudinem fere bis expansi. Ascosporae uniseriatae vel partim superpositae, uniseptatae plerumque parum supramedio vel in medio, (40) 50–70 (76) × (13) 16–22 (24) μm, ellipsoideae vel fusiformes, rectae vel parum curvatae, ad septum constrictae, cellula supera super septum dilatata et ad apicem late papillata attenuata vel fere hemisphaerica, cellula infera angustior et ad basem late rotundatam attenuata, pallide virides, paries laevis, cum vagina gelatinosa tenui 2 μm lata tectae, aliquae ascosporae abnormales adsunt. Pseudoparaphyses abundantes, hyalinae, filiformes, ramosae, cellulosae, cellulae in longitudine variabiles 10–26 μm, 4–7 μm latae. Mycelium superficiare super folia, caules et legumina viva et emortua delapsaque plantarum crescentia, atrobrunneum vel nigrum, abundans, nitidum, ex hyphis ramosis atrobrunneis septatis (12) 14–20 (22) μm latis compositum, septa (25) 60–100 (180) μm distantia, paries (1) 1·5 (2) μm latus, hyphae laeves vel subtiliter verruculosae, ad apices arcte ramosae et appressoria elevata atrobrunnea vel nigra nitida 250–300 μm lata formata plantarum organarum insidens. Anamorphosus conidialis ignotus.

Holotypus: in foliis emortuis *Glycinis magis* cum culturo agaro infectis, Rydalmere, Nova Wallia Australis, Australia, Nov. 1983. G. E. Stovold & J. Walker, DAR 43446.

Pseudothecia (Figs 8–13) mainly superficial but sometimes erumpent, developing from mycelium on dead and fallen leaves, black, single or up to 6–10 loosely clustered, at first subglobose then broadly ovoid to obpyriform, uniloculate, (400) 500–800 (900) μm high, 300–450 μm wide in the lower half, 200–350 μm wide above, ostiole present 40–70 μm wide, setose. *Setae* (Fig. 20) straight or flexuous, septate, dark brown with a paler semicircular apex, cylindrical (7) 10–12 μm wide but wider to 12–18 μm at the base, 45–140 μm long on the upper part of the pseudothecium, longer to 250–380 μm on the lower half, septa 30–60 μm apart, immature pseudothecia covered with setae but sparse to absent around the ostiole of mature pseudothecia and on erumpent pseudothecia. *Wall* of pseudothecia 60–90 μm thick, to 130 μm at the base, made up of 4–6 layers of oval to oblong cells 20–40 × 15–20 μm, the external layers dark brown to black, inner layers paler brown to hyaline, cells forming a textura



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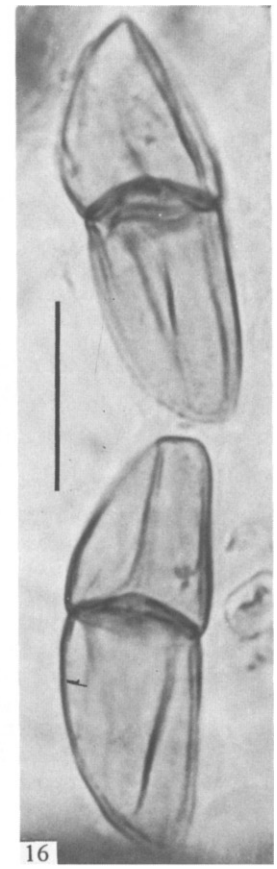

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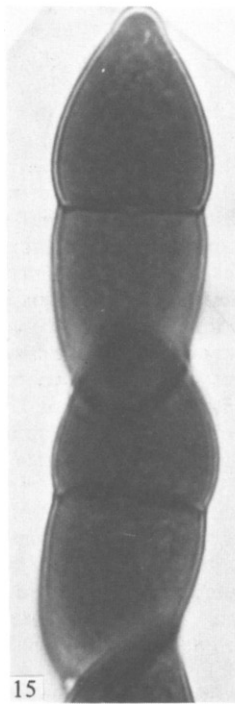

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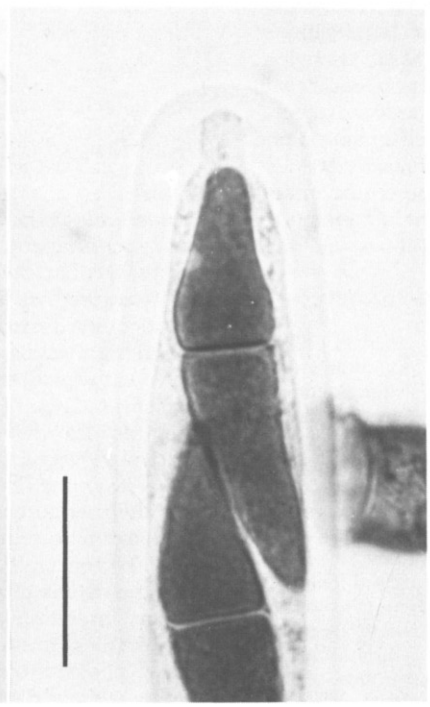

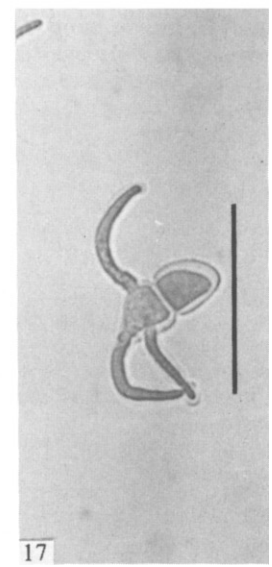

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angularis in section and in surface view, vegetative hyphae attached to wall at base. *Asci* (Figs 14, 15, 18) 35–50 per pseudothecium arising from a hyaline basal layer 40–80 μm thick, cylindrical, mainly 8-spored but sometimes fewer ascospores owing to abortion, 230–300 × 24–26 (30) μm, with a short basally flattened stalk 20–25 × 6–9 μm, bitunicate, ectotunica hyaline, very thin 0·5–1 μm wide; endotunica 5–6 μm thick in unexpanded asci, to 10 μm in expanded asci, extending the complete length of the ascus, 6–7 μm thick at the apex where an ocular chamber 5–6 μm wide surrounded in its upper half by a thin refringent zone is present; asci with fissitunicate dehiscence and expanding to almost twice their length. *Ascospores* (Figs 14–19) uniseriate or partly overlapping, uniseptate usually just above the middle or centrally, (40) 50–70 (76) × (13) 16–22 (24) μm, ellipsoidal or fusiform, straight or slightly curved, constricted at the septum, the upper cell widening above the septum and then narrowing to a broadly papillate apex or sometimes hemispherical, the lower cell thinner and narrowed to a broadly rounded base, pale greenish, wall smooth covered by a thin gelatinous sheath 2 μm thick, some abnormal ascospores present. *Pseudoparaphyses* (Figs 12, 13, 19) abundant, hyaline, filiform, branched, cellular, cell length variable from 10 to 26 μm, 4–7 μm wide. *Superficial mycelium* (Figs 4, 5) growing over living, dead and fallen leaves, stems and pods, dark brown to black, abundant, shining, made up of dark brown branched septate *hyphae* (12) 14–20 (22) μm wide (Figs 21, 22), septa (25) 60–100 (180) μm apart, wall (1) 1·5 (2) μm thick, hyphae smooth or finely verruculose, closely branched at their apices and forming raised dark brown to black glistening *appressoria* 250–300 μm wide seated on the organs of the plant (Figs 6, 7, 10). Conidial anamorph unknown.

Pseudothecia of *Arkoola nigra* produced on colonized soybean leaves in culture are identical with those developed on field-infected trash. Optimum conditions for development have not been defined, but high humidity and senescing or dead host tissue (rather than living leaves) seem to be essential. Ascocarps have not been seen on spots on living leaves either in the field or in artificial inoculation experiments, and their development has been observed only on dead plant tissue or on mycelium on sand grains in culture (see above). Immature ascocarps have been found on dead fallen infected leaves of four hosts other than soybean in pot inoculation tests (Table 4). These are *Glycine* sp. aff. *clandestina* Wendl., *Glycine* sp. aff. *tomentella* Hayata, *Indigofera hirsuta* L. and *Vigna luteola* (Jacq.) Benth., indicating that *A. nigra* can probably complete its life-cycle in the field on hosts in several genera of Fabaceae at least.

Ascocarps are formed mainly on superficial mycelium but in a few cases have been found on well-rotted leaves pushing up through the epidermis and developing from mycelium in the leaf tissue. Leaves are disintegrating rapidly at this stage and, although the ascocarps can be said to be erumpent, the tissue is offering very little resistance to them. Such ascocarps have much sparser development of setae than those formed superficially.

Mature pseudothecia (Fig. 13) have a well-developed ostiole but its method of formation has not been studied. No periphyses have been seen. Abundant pseudoparaphyses develop from the upper part of the centrum (Fig. 12) and persist in mature pseudothecia.

In normal asci there are eight ascospores arranged in a single row or slightly overlapping, but abnormalities occur frequently enough to deserve comment. In some asci, fewer than eight spores occur and asci with 5, 6 or 7 ascospores have been seen in ascocarps with normal asci. Sometimes their place was taken by either a small shrivelled aborted spore or a full-sized abnormal spores (Fig. 19). The latter may be (i) without a septum and oval to cylindrical or reniform, (ii) with a shrivelled, pale brown apical cell and normal pale greenish basal cell, (iii) of an unusual shape (thinner or thicker or more pointed at the ends), or (iv) empty, without contents and with a pale brown wall. Similar empty pale brown spores have been seen also on the dead leaf surface surrounding mature ascocarps. In a few asci spores were upside down (Fig. 14), with their apical cell towards the base of the ascus and their basal cell towards its tip. The reason for these abnormalities is not known. As mature asci and ascospores are known at present only from ascocarps developed on plant tissue in the laboratory, it will be interesting to see if similar abnormalities occur in mature field collections.

In water, ascospores are very pale greenish,

Fig. 14. Ascospores of *Arkoola nigra* in asci (lacto-phenol acid fuchsin); arrows show some 'upside-down' ascospores (others are present) (DAR 43446). Bar = 100 μm.

Fig. 15. Ascospores of *Arkoola nigra* showing variation in size and shape, and ascus tip (lacto-phenol acid fuchsin) (DAR 43446). Bar = 25 μm.

Fig. 16. Two empty ascospores of *Arkoola nigra* with pale brown walls (DAR 43445). Bar = 25 μm.

Fig. 17. Ascospore of *Arkoola nigra* germinating on PDA (DAR 48919). Bar = 100 μm.

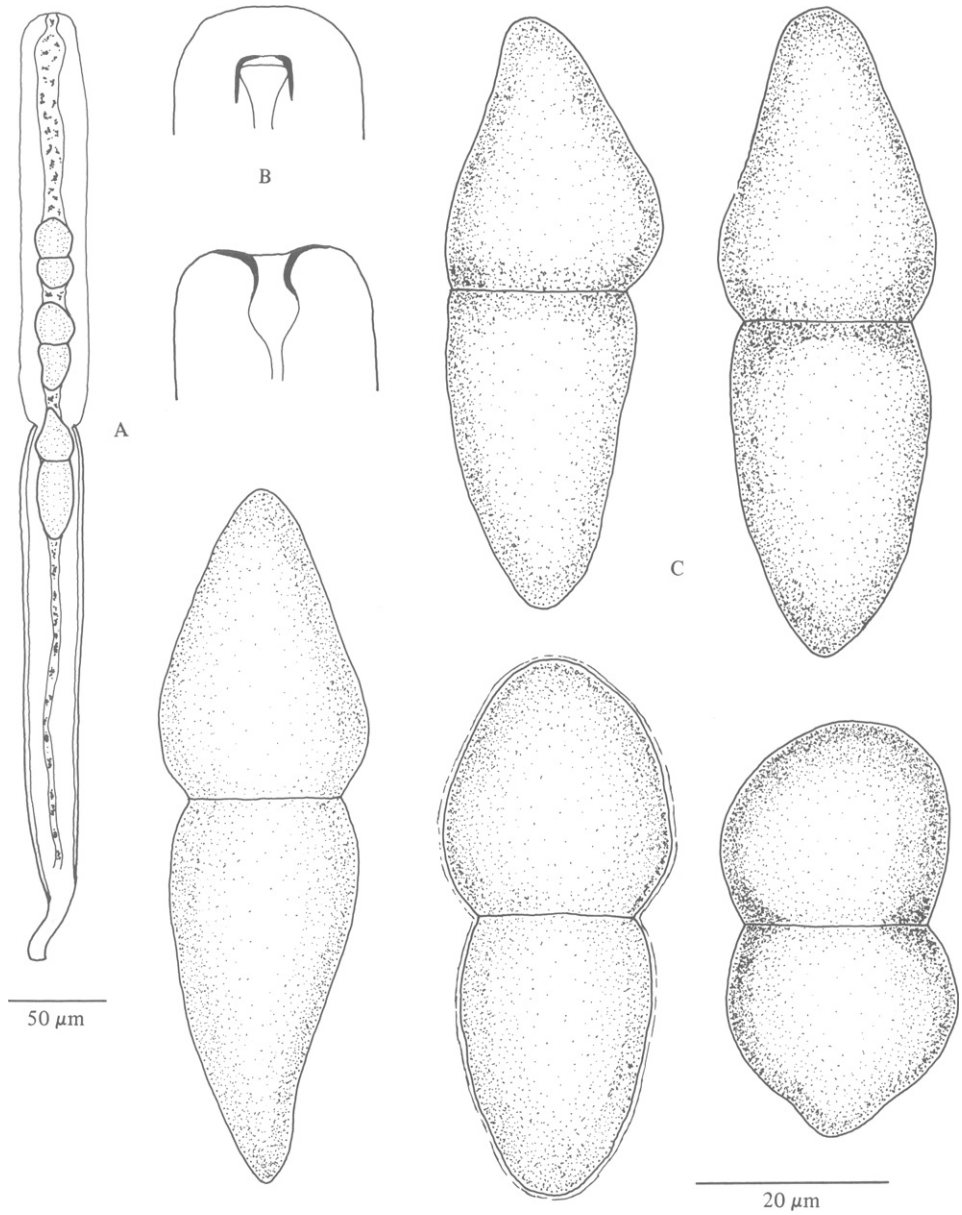


Fig. 18. *Arkoola nigra* (DAR 43446). (A) Bitunicate ascus showing fissitunicate dehiscence and thick endotunica still containing three ascospores; (B) ascus tip of unexpanded (above) and expanded (below) ascus, showing ocular chamber and refringent staining zone; (C) five ascospores, one showing thin sheath. Bar = 50 μm (A), 20 μm (B and C).

approaching Pale Glass Green (Ridgway, 1912) or 2·5 GY 9/2 (Munsell, 1967). They stain readily in both water and lactophenol with cotton blue or acid fuchsin. No trace of brown is seen in normal ungerminated ascospores but, as mentioned above, the wall of empty ascospores or of distorted apical cells is usually pale brown.

The bitunicate asci (Figs 15, 18) have a thin (< 1 μm) colourless ectotunica that does not stain in 0·1 % aqueous congo red and a thicker (to 4–6 μm)

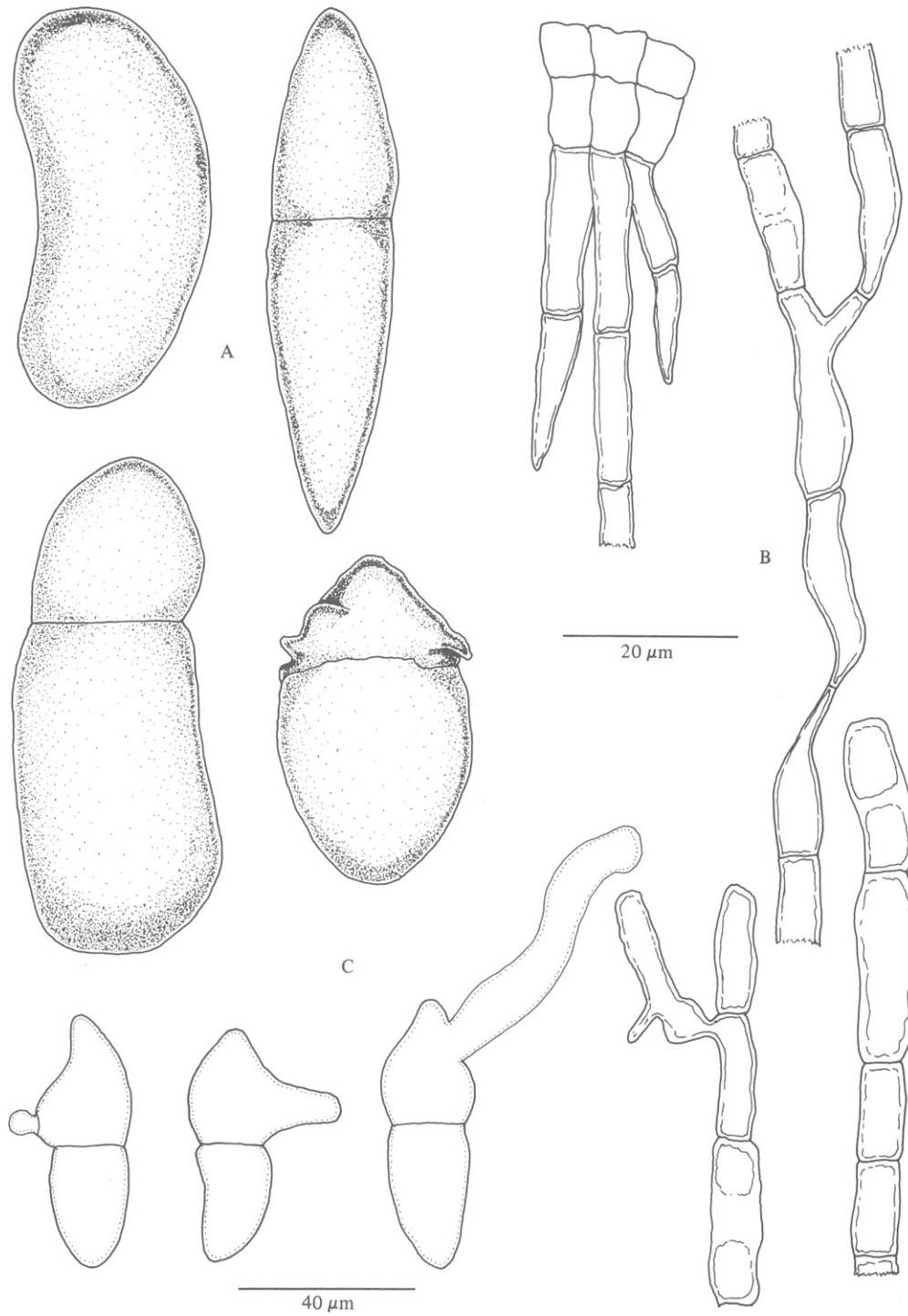


Fig. 19. *Arkoola nigra* (DAR 43446). (A) Abnormal ascospores, one with shrivelled apical cell; (B) pseudoparaphyses; (C) ascospores germinating in water mount. Bar = 20 μm (A and B), 40 μm (C).

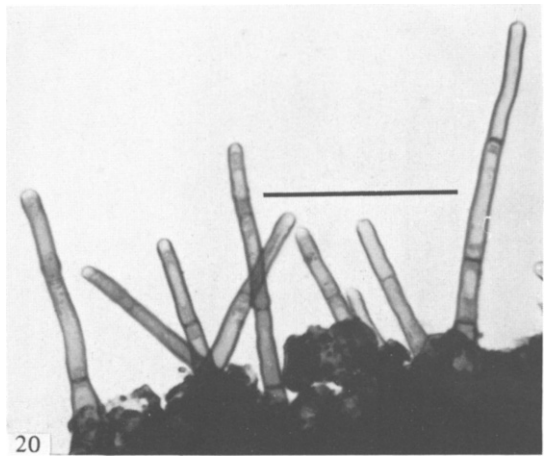


Fig. 20. Setae on wall of pseudothecium of *Arkoola nigra* (DAR 43446). Bar = 100 μm.

endotunica that does. The endotunica shows no sign of layering and extends to the base of the ascus. Fissitunicate dehiscence occurs readily when mature asci are placed in water, and sometimes a cap of ectotunica is left at the top of the expanded endotunica. Asci have a well-developed ocular chamber at the apex of the endotunica. In an undischarged ascus the ocular chamber is surrounded in its upper half by a thin refringent zone readily seen in water mounts (Fig. 18B). This zone stains faintly in 0·1% aqueous cotton blue but does not in 0·1% aqueous congo red or chlorazol black E. In a discharged ascus it is present on the outside of the endotunica surrounding the pore through which the ascospores have been ejected (Fig. 18B). Further study of the apical structure of asci and of ascospore discharge in *A. nigra* would be worthwhile and facilitated by the large asci.

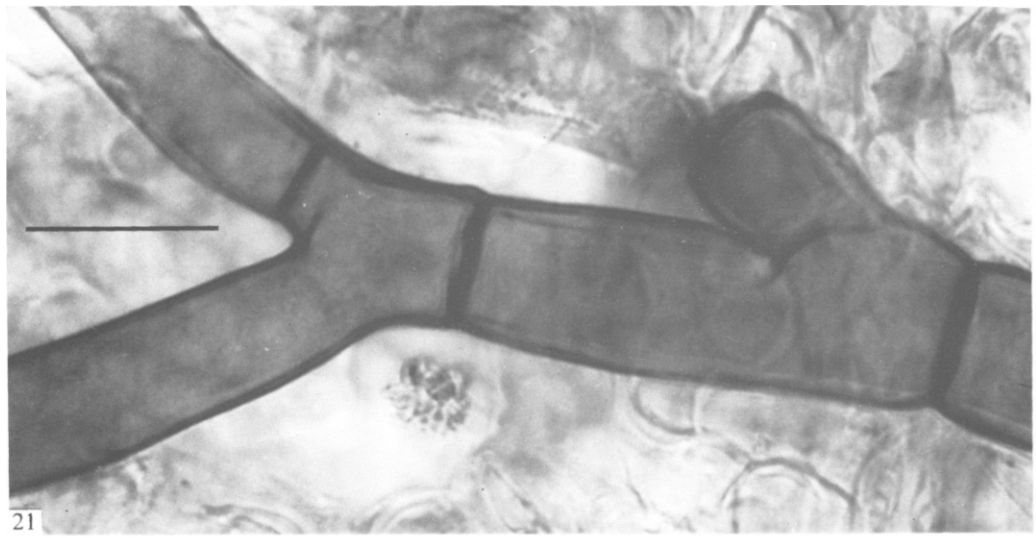
Taxonomic position

Arkoola nigra is a Loculoascomycete and, of the orders accepted by Luttrell (1973), falls into the Pleosporales. With its abundant superficial mycelium developing large setose ascocarps having a thick pseudoparenchymatous wall enclosing a centrum containing large cylindrical bitunicate asci with fissitunicate dehiscence, greenish two-celled asymmetric ascospores and abundant cellular pseudoparaphyses, it is placed best in the family Venturiaceae Müller & v.Arx ex Barr (1979).

The concept of the family accepted here is that conceived by Barr (1968, 1979) and Eriksson (1981, 1984) and, to a lesser extent and with somewhat varying circumscriptions, by Müller & v.Arx (1962) and Luttrell (1973). The family name Stigmateaceae Theissen (1916) used by v.Arx & Müller (1975) to accommodate many genera placed by the above workers in the Venturiaceae is not used here, in accord with reasons given by Barr (1979) and Eriksson (1981) and the usage in Hawksworth, Sutton & Ainsworth (1983).

Arkoola nigra is a much larger fungus than any placed previously in the Venturiaceae. The large ascospores, with their thin gelatinous sheath, are reminiscent of those of some Pseudosphaeriaceae, e.g. species of *Wettsteinina* Höhnel, but ascus and centrum morphology is quite different. In young ascocarps of *Arkoola* descending pseudoparaphyses are seen clearly (Fig. 12) and development of the centrum closely resembles that described and illustrated for *Venturia myrtilli* Cooke (= *Gibbera myrtilli* (Cooke) Petrak) by Parguey-Leduc (1966). Asci of *Arkoola* are long cylindrical and shortly stipitate rather than broadly saccate to obclavate and sessile as in most Pseudosphaeriaceae (Barr, 1972). It also differs from many genera of Venturiaceae in not forming a well-developed hypostroma in the infected tissue. Instead, diseased organs are invaded by a well-developed intercellular mycelium which develops further, extensively colonizing the tissue, when the plant part dies and falls.

Arkoola shows some similarity to the Dimeriaceae Müller & v.Arx ex v.Arx & Müller (1975), but members of this family all have small, relatively thin and soft-walled, globose to subglobose ascocarps and are either completely superficial or with comparatively little delicate mycelium in the host tissue (Farr, 1965, 1966; v.Arx & Müller, 1975; Barr, 1979; Eriksson, 1981). There is also a distinct resemblance to the genus *Herpotrichia* Fuckel (Pleosporaceae), whose species produce superficial or basally embedded pseudothecia on a brown superficial mycelial mat. Some have hairy ascocarps or two-celled ascospores with a gelatinous sheath, and numerous branched pseudoparaphyses are present (Sivanesan, 1971, 1984). However, ascospores of *Herpotrichia* spp. become pale to dark brown at maturity, and the asci have a well-developed nasse apicale (Sivanesan, 1984). The ostiole in several species, including the type, *H. herpotrichoides* (Fuckel) P. F. Cannon (1982), is lined with hyaline hyphae (Sivanesan, 1971) and, in the plant parasitic species such as *H. coulteri* (Peck) Bose and *H. juniperi* (Duby) Petrak, pseudothecia develop on diseased organs still attached to the plant. These features are not present in *Arkoola* which, on balance of characters, is more similar to the Venturiaceae than to the Pleosporaceae (in this context it must be noted that Chadefaud (1972) described and figured a nasse apicale in asci of *Venturia rumicis* (Desm.) Winter).



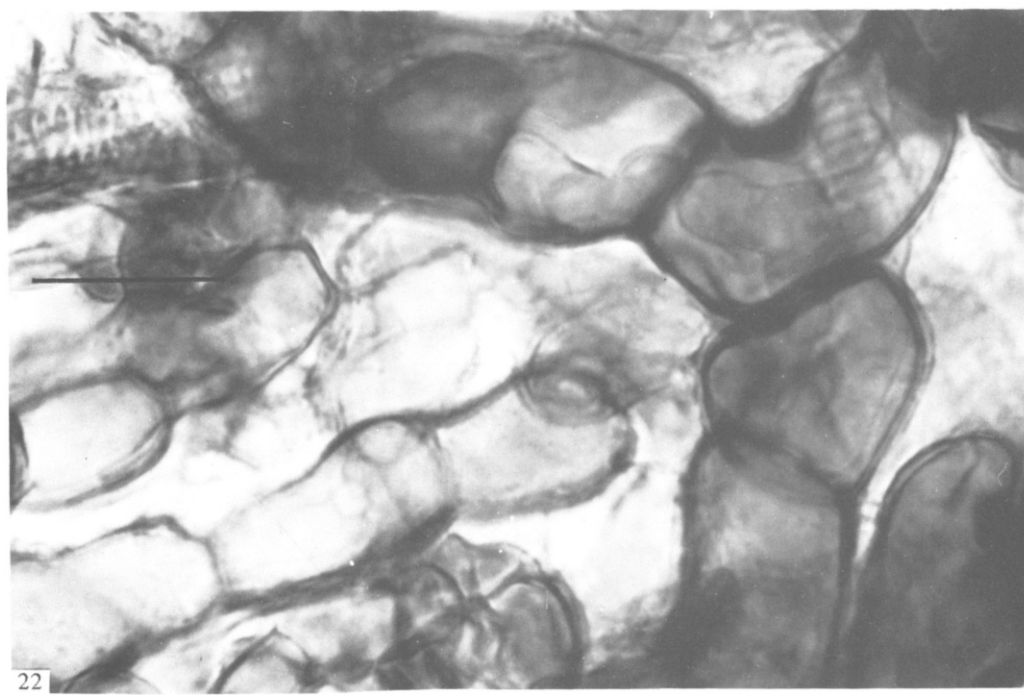
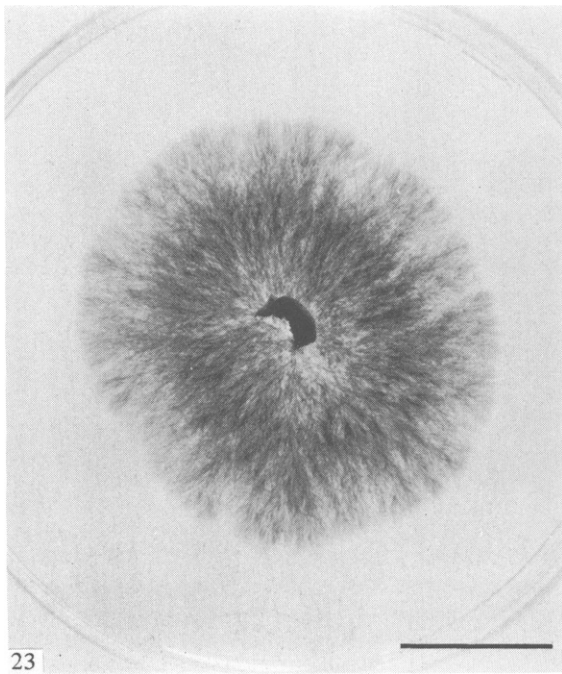


Fig. 21. Superficial hypha of *Arkoola nigra* on diseased soybean leaf (DAR 41445). Bar = 25 μm.
Fig. 22. Hyphae of *Arkoola nigra* within tissue of diseased soybean leaf (DAR 43446). Bar = 25 μm.

Of the described genera of Venturiaceae, *Arkoola* shows most resemblance to *Protoventuria* Berlese & Sacc. and a more superficial resemblance to *Macroventuria* van der Aa. Several species are now placed in *Protoventuria* (in some earlier works as *Antennularia* Reichenb.; for discussion and transfers see Hughes, 1970; Barr, 1971; Sivanesan, 1974). Most are plant parasites developing on living leaves and stems and forming a well-developed hypostroma in the infected tissue. This produces



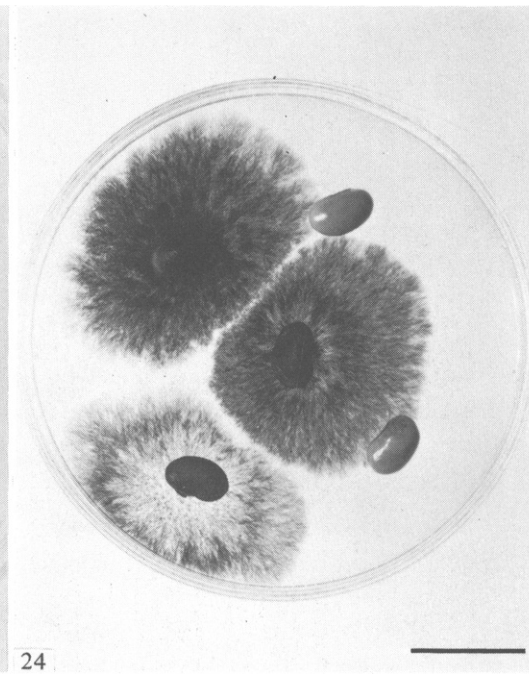


Fig. 23. *Arkoola nigra*, culture on PDA, 20 °C, 5 days (DAR 48919). Bar = 2 cm.

Fig. 24. *Arkoola nigra* isolated in culture from surface-sterilized immature soybean seed. Bar = 2 cm.

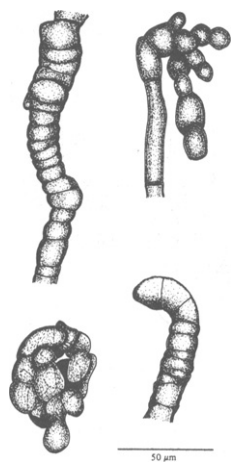


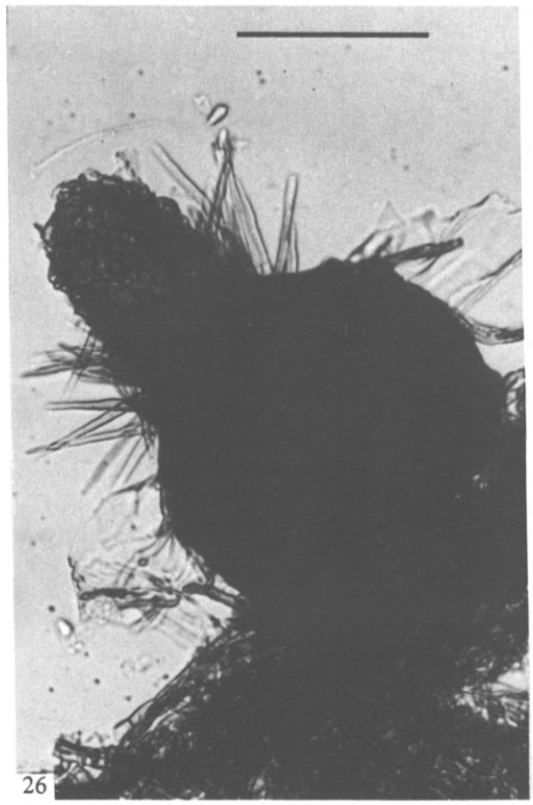
Fig. 25. *Arkoola nigra*. Thick-walled septate hyphae and clusters and moniliform chains of dark brown cells in old (6 months) PDA culture (DAR 44510). Bar = 50 µm.

surface hyphae or erumpent stromata on which pseudothecia develop. The superficial mycelium is usually of limited extent and does not extend much beyond the pseudothecia. In *Arkoola*, although dead leaves are heavily colonized internally and externally by mycelium, no distinct hypostroma is formed. Moreover, the superficial mycelium is abundant as a loose web on infected plants and spreads infection over the plant. Finally, in *Arkoola* pseudothecial development has not been seen on living plants but only on dead fallen infected tissue. Ascocarps, asci and ascospores of all species of *Protoventuria* are much smaller than those of *A. nigra*. One of the largest species, *P. hennessyi* A. Sivanesan (1974), has ascocarps 200–300 µm diam with wall 20–25 µm thick, asci 100–130 × 20–26 µm and ascospores 22–32 × 9–13·5 µm. This is much smaller than comparable structures in *A. nigra*. Examination of specimens of *P. arxii* (E. Müller) M. E. Barr, *P. engleriana*

Fig. 26. *Macroventuria anomochaeta*. Ascocarp with setae, from holotype in CBS. Bar = 100 µm.

Fig. 27. *Macroventuria anomochaeta*. Asci, ascospores and remnants of interascal tissue (lacto-phenol acid fuchsin), from holotype in CBS. Bar = 25 µm.

Fig. 28. *Macroventuria wentii*. Asci, ascospores and inter-ascal tissue (lacto-phenol acid fuchsin), from holotype in CBS. Bar = 25 µm.



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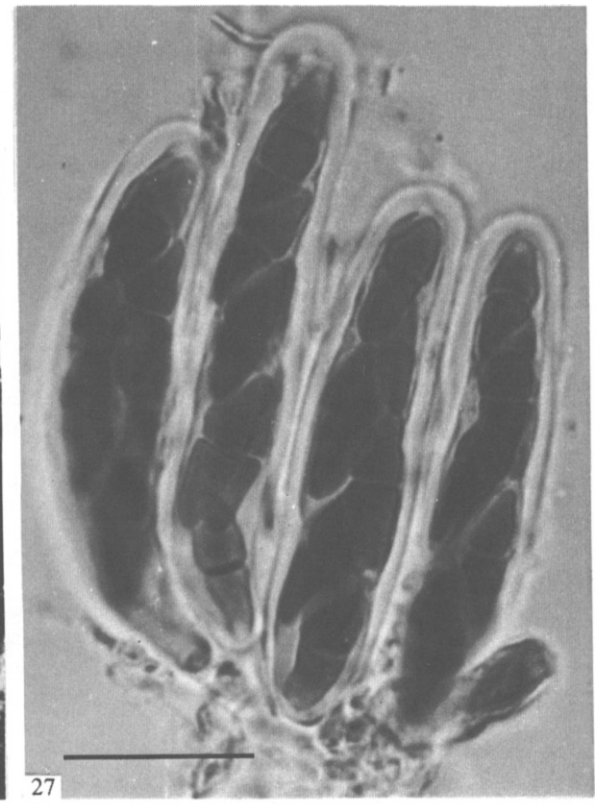

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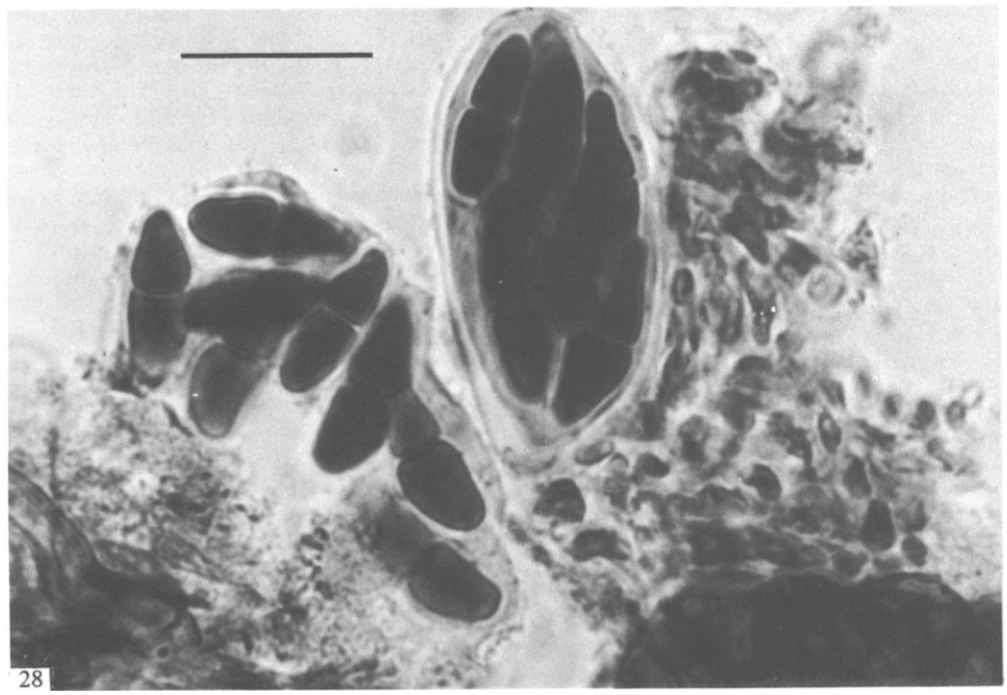

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(P. Henn.) A. Sivanesan and *P. straussii* (Sacc. & Roum.) A. Sivanesan has confirmed the characteristics of the genus and its distinctness from *Arkoola*.

Macroventuria contains two species, the type *M. wentii* van der Aa (1971) isolated from dead litter, other dead substrates and air in Death Valley, Nevada, U.S.A. and *M. anomochaeta* van der Aa (1971) isolated from decayed canvas in the Karoo Desert, South Africa. Both are known only in culture where they form setose ascocarps containing relatively few ellipsoidal to saccate bitunicate asci with eight two-celled ascospores within the range 21–32 × 8–14 μm. There is sparse development of tissue between the asci as slender cellular threads which tend to disappear at maturity. The genus was placed by van der Aa (1971) in the Venturiaceae and said to differ from *Venturia* by the small number of relatively large asci, the large almost hyaline ascospores and the saprophytic mode of life. Arx & Müller (1975) considered this combination of characters to be more typical of the Pseudosphaeriaceae, and transferred *Macroventuria* to this family. This was accepted by Barr (1979).

Examination of the type collections of both *Macroventuria* species has shown that *Arkoola nigra* is quite distinct (Figs 26–28). All have setose ascocarps, but those of *A. nigra* are much larger and differ in structure from those of *Macroventuria*. No well-developed pseudoparaphyses were seen in *Macroventuria*. Some sparse tissue is present between asci, but this appears to be remnants of the ground tissue of the pseudothecium. It is made up of cells 7–8 × 5–7 μm or, where squashed between asci, (1) 1·5 (2) μm wide and up to 6–8 μm long. In sections of young pseudothecia of *M. wentii*, young asci are in separate cavities of the stroma, separated from each other by stromatal tissue and not by descending pseudoparaphyses. The structures present in *Macroventuria* are paraphysoids sensu Eriksson (1981). The bitunicate asci of *Macroventuria* are mainly ellipsoidal to narrowly saccate, widest near or below the middle, approaching short cylindrical in *M. anomochaeta*, with relatively thin ecto- and endotunicae. They are not long cylindrical with a thickened endotunica as in *Arkoola*. Ascospores of *Macroventuria* are centrally septate, the upper cell generally slightly wider than the lower, and pale greenish in colour. They are much smaller (range 21–32 × 8–14 μm) than those of *A. nigra* (range (40) 50–70 (76) × (13) 16–22 (24) μm). The two species of *Macroventuria* are known only in culture and both appear to be saprophytes. Two isolates of *M. wentii* were obtained from young burr of *Franseria* sp. (probably an *Ambrosia* sp., Asteraceae – J.W.) and from a male inflorescence of *Hymenoclea* sp. (Asteraceae) (van der Aa, 1971) but no mention is made of any association with disease. As these fungi have not been studied in nature, the presence or absence of a superficial mycelium is not known, although one isolate of *M. wentii* was said to have been obtained from 'mycelium of Veromessor nest' (van der Aa, 1971). In culture, *M. wentii* colonies grow to 4·5–5 cm diam in 10 days and *M. anomochaeta* colonies to 1 cm diam in the same period (van der Aa, 1971). This is much slower than *A. nigra*, which can cover a 9 cm Petri dish in 7 days at 20°. Ascocarps are produced readily in culture by *Macroventuria* spp. but not by *A. nigra*.

Macroventuria shows a mixture of characters. Ascospore shape and greenish colour are similar to that seen in many Venturiaceae, but the structure of the centrum and the presence of paraphysoids rather than pseudoparaphyses indicates a closer relationship to the Pseudosphaeriaceae, as suggested by v.Arx & Müller (1975). The resemblance to *Arkoola* is only superficial, with both genera having in common only setose ascocarps, and relatively large two-celled pale greenish ascospores.

Of the other genera of Venturiaceae listed by v.Arx & Müller (1975), Barr (1968), Eriksson (1984) and Luttrell (1973), the only one with any resemblance to *Arkoola* is the monotypic genus *Metacoleroa* Petrak (type species *M. dickiei* (Berk. & Br.) Petrak), a leaf parasite of *Linnaea* (Caprifoliaceae) in North America. The resemblance is due to the setose (around the ostiole) ascocarps of *Metacoleroa* which develop on a thin superficial mycelium. However, as in *Protoventuria*, this arises from a well-developed hypostroma, which is absent in *Arkoola*. In all other respects the two genera are quite distinct.

The main characteristics used to delimit genera of the Venturiaceae are the origin and position of the ascomata, the degree of development of stromatic tissue, the location and amount of mycelium formed and the position of the septum in the two-celled ascospore (v.Arx & Müller, 1975; Barr, 1968; Luttrell, 1973). Using these criteria, the soybean black leaf blight fungus is quite distinct from all described genera. As far as can be seen, no similar genus is known with such an abundant web-like superficial mycelium or with such large pseudothecia, asci and ascospores.

Ascospore germination

Ascospores germinated readily when mounted in water for microscopic examination and also germinated and produced germ-tubes up to 100 μm long in 0·1 % aqueous congo red and 0·1 % aqueous chlorazol black E. Germination started within an hour of mounting and germ-tubes grew up to 60 μm in 3 h on the slides (at about 20°). Ascospores inside

Table 1. *Ascospore germination and germ-tube growth on PDA at 5–37°*

Temperature (°C)	Time (h)	Germination (%)	Mean germ-tube length (μm)
5	4	22	18
10	2	80	33
15	2	98	78
20	2	98	123
25	2	96	151
30	2	98	146
35	2	4	5
37	2	0	0

Table 2. *Growth on PDA at 5–37°*

Temperature (°C)	Diam (mm) 4 days
5	13
10	38
15	58
20	74
25	66
30	59
35	0
37	0

asci also germinated under these conditions. In all cases, germination was from the apical cell mainly by one, but often by up to three, germ-tubes. Ascospores discharged on to PDA germinated similarly (Figs 17, 19).

In order to study the effect of temperature on ascospore germination, ascospores discharged from ascocarps matured on infected trash in the glasshouse (DAR 48919) were collected on the surface of potato dextrose agar (PDA) plates. Within thirty minutes plates were placed at constant temperatures and ascospore germination observed. Mean length of germ-tubes after 2 h (4 h at 5°) is shown in Table 1. For each temperature, thirty germ-tubes were measured.

Ascospores germinated over the range 5–35° but germination was much reduced at 5° and 35°. Most vigorous germination occurred from 15 to 30°, with best germ-tube growth from 20 to 30°. A sudden decline in both occurred above 30°.

Growth in culture

Arkoola nigra grows readily in agar culture from infected tissue, mycelium or ascospores. Cultures are black, with a relatively open mycelium in the agar composed of branched radially oriented hyphae and a short sparse black aerial mycelium (Fig. 23). Ascocarps or other spore states have not been seen in agar cultures. In old (6 months) PDA plate cultures, clusters and chains of thickened dark brown cells are present (Fig. 25).

To determine the optimum temperature for linear growth of mycelium in culture, circular plugs (5 mm diam) were taken from the margin of an actively growing colony on PDA (DAR 41445) and transferred to freshly poured PDA plates (10 ml). Diameter growth was measured over the range 5–37°. There were four replicate plates for each temperature, and each colony was measured across two diameters at right angles. Results are given in Table 2.

The optimum temperature for growth was 20°, but good growth occurred also at 15°, 25° and 30°. Growth was markedly reduced at 5° and none was recorded at 35° or 37°.

The effect of various media on linear growth of *A. nigra* was also investigated. Inoculum plugs prepared as above were placed on the test media in 9 cm Petri dishes (10 ml). Two single-ascospore cultures of the type isolate (DAR 43446) were used,

Table 3. *Growth on various agar media*

	Mean colony diam (mm) 7 days	
	DAR 43446-1	DAR 43446-2
Potato Dextrose Agar (PDA)	79	82
PDA + novobiocin (100 ppm)	77	77
Potato Vegemite® Dextrose agar (PVDA)	62	58
V8 Juice agar + β-sitosterol	52	66
Oat agar	47	54
Prune agar	48	50
Cornmeal agar	35	34
Malt agar	26	26
Malt Vegemite® agar	16	16
Acidified PDA (pH 4·0)	7	9
Czapek Dox agar	6	6

with two plates for each medium. Plates were incubated at 25° and colony diameters measured after 7 days. Results are shown in Table 3.

PDA was superior to all other media in terms of radial growth and colony density. On all media, only sterile mycelium was produced. *Arkoola nigra* is not tolerant of acid media but will tolerate 100 p.p.m. novobiocin, which can be used to free cultures from bacteria.

A. nigra does not survive well in agar culture. Serial transferring every 2–3 months on PDA or PVDA slopes results in a gradual decline in vigour, slower growth and eventual death. Cultures on agar slopes kept under mineral oil or water, or kept as pieces of agar culture in sterile water, do not survive longer than one year at the most. The most successful method found so far has been a modification of the L-drying method used for *Gaeumannomyces graminis* (Sacc.) v.Arx & Olivier var. *tritici* J. Walker by Fang & Parker (1981). Small strips cut from the margin of an actively growing PDA culture are allowed to dry slowly in a sterile Petri dish for 7–10 days before being placed in the ampoules and vacuum dried. Cultures prepared in this way have survived so far for two years with full retention of original culture characteristics and pathogenicity. *A. nigra* has also survived for at least two years on samples of field-infected trash air-dried and kept at ambient temperature in the laboratory. Mycelium can be seen clearly on such leaves, and fresh isolates obtained by plating on PDA.

Survival in the field

The methods by which *A. nigra* survives in the field are not known at present. Although it can survive on air-dried field-infected trash in the laboratory for over two years, in the field infected trash breaks down rapidly and is often difficult to find after 3–4 months. However, trash is often left in heaps following harvest, and the fungus can be detected in these 6 months later. This is long enough to allow its survival from one soybean crop to the next if successive crops are grown in the same paddock. Reinfection of self-sown soybeans germinating in accumulated trash has been observed in several instances. These are infected mainly on the lower stem, although leaf spotting has been seen on seedlings in some self-sown crops. Generally, infection with black leaf blight is not observed in commercial crops until after flowering, when there is a dense canopy of foliage. Just how the fungus survives from early to late season and what forms of inoculum are involved in initiating post-flowering infection has not been determined. Although immature ascocarps have been found on trash in the field, the cycle of ascocarp development and ascospore release and dispersal has not yet been worked out.

In artificial infection studies, infected leaf trash was incorporated into the surface of pasteurized potting soil in pots and soybeans sown. Some pre-emergence death of seedlings occurred, but usually plants grew satisfactorily without obvious symptoms. If such plants were removed from the soil and examined under the microscope, mycelium could be seen on the lower stem and taproot. Mycelium was also seen commonly on the underside of cotyledons until such time as they fell off. Such seedling infections may have significance in the establishment of infection in the field and in the survival of the fungus on the host during the early stages of crop development.

Transmission with seed

Arkoola nigra commonly infects maturing pods and seeds, and can be isolated readily from infected immature green seed (Fig. 24). The possibility of its being carried with mature harvested seed was investigated.

During the seasons 1982–4, seed of various cultivars from many coastal crops was assayed for the presence of several seed-borne fungi, especially *Phomopsis phaseoli* (Desm.) Sacc. Two hundred seeds from each bulk sample were surface-sterilized for 2 min in 1% sodium hypochlorite, rinsed twice in sterile water and plated on PDA. All seed samples assayed using this method were examined for *A. nigra*. In addition, lots of 200 seeds from crops infected with black leaf blight were incubated without surface sterilization in moist germination trays at room temperature (20–25°) and examined after 10 days for the presence of mycelium of *A. nigra*. During the three years, a total of 19,000 seeds were tested by the two methods and only two seeds from separate samples, one surface sterilized and one unsterilized, were found infected by *A. nigra*. Infected seeds did not germinate, and the characteristic dark mycelium grew over the seed surface and on to the moist pad in the germination tray or on to the agar.

The results show that *A. nigra* is not carried commonly on or in seed even when heavy infection is present on maturing pods. Its detection at a very low level (0·5%) in two samples suggests that caution be exercised in sending seed from infected to disease-free areas. There is the possibility that the disease could be spread with seed as fragments of infected plant trash or shrivelled infected immature seed, but commercial soybean seed is cleaned carefully, and cleaned seed generally contains very little plant trash. Seed infected in the

Table 4. *Reaction of several legume species to inoculation with* Arkoola nigra (DAR 48919)

	Disease rating†			
	11 days	22 days	Re-isolation (11 days): successful/total plants	Ascocarps on dead leaves (22 days)
Aeschynomene indica L.	0	0	—	−
**Crotalaria lanceolata* E. Meyer (DAR 49793)	1·5	1·5	29/30	−
C. linifolia L.f. (DAR 49795)	1	3	5/25	−
C. medicaginea Lamk. (DAR 49794)	1	2	6/15	−
**C. pallida* Ait. (DAR 49796)	3	3·5	18/25	−
Desmodium heterocarpon (L.) DC. (DAR 49797)	1	1	2/15	−
D. varians (Labill.) Endl. (DAR 49798)	1	1	9/15	−
Galactia tenuiflora (Willd.) Wight & Arn. (DAR 49799)	1·5	1·5	13/15	−
Glycine tabacina (Labill.) Benth. (DAR 49802)	2	3	12/15	−
G. sp. (aff. *clandestina* Wendl.) (DAR 49801)	2	3	20/23	+
G. sp. (aff. *tomentella* Hayata) (DAR 49803)	2·5	3	15/15	+
Indigofera hirsuta L. (DAR 49804)	3	4	20/25	+
Kennedia rubicunda (Schneev.) Vent. (DAR 49805)	2·5	2·5	19/25	−
K. rubicunda var. *robusta* Maiden & Betche (DAR 49806)	3	3	13/25	−
Lespedeza juncea (L.f.) Pers. (DAR 49807)	0·5	0·5	1/15	−
L. striata Hook. & Arn. cv. Kaloe	0	0	—	−
Rhynchosia minima (L.) DC. (DAR 49809)	1	1	13/15	−
Vigna luteola (Jacq.) Benth. (DAR 49810)	3	3	23/25	+
Zornia dyctiocarpa DC.	0	0	—	−

* Introduced species.

† 0, no symptoms; 1, minor leaf spotting (0·5–5 mm diam) on older leaves; 2, medium leaf spotting (> 4 mm diam) on older leaves; 3, extensive blighting of older leaves, spreading spots (0·5–5 mm diam) on young leaves; 4, extensive blighting of all leaves, stem lesions present; 5, plants completely blighted and dead.

immature green stages fails to develop further and, because of its small size and light weight, is not harvested by the machinery commonly used.

Hosts

With one exception, *A. nigra* has been found in the field only on soybean. Next to a heavily infected crop at Upper Kangaroo Creek (near Grafton), one plant of the introduced weed Paddy's lucerne (*Sida rhombifolia* L., Malvaceae), showed leaf spotting and blighting similar to that on soybean (DAR 51599*a*). The characteristic dark aerial mycelium and black shiny appressoria were present on infected leaves and the fungus was isolated readily from surface-sterilized lesions. Although searches have been made in the vicinity of infected soybean paddocks, no other host has been found in the field.

Of the 77 genera of Fabaceae known in N.S.W., 56 have been recorded in the north coast ecogeographic region (Jacobs & Pickard, 1981), which includes the total area where black leaf blight has so far been found. In the area there are 44 genera with a total of 147 native species, and 17 genera with a total of 46 introduced species (Jacobs & Pickard, 1981; Beadle, 1982). Possibly one or more of the many native species could be host to *A. nigra* and the source of the new soybean disease. In order to test the susceptibility of some of these plants, 17 of the native and 2 of the introduced species were inoculated with *A. nigra*. These are listed in Table 4. Seed was sown into 10 cm diam pots of steam-pasteurized potting mix. After emergence, plants were thinned to 6–10 per pot and grown in the glasshouse at 20–25° for 8 weeks before inoculation. Inoculum was prepared by macerating, in a blender with sterile water, 7-day-old PDA plate cultures of *A. nigra* (12 plates of DAR 48919 in 900 ml sterile distilled water). This was sprayed on to the foliage of test plants with a power sprayer. Plants were kept in a shaded inoculation chamber, with humidity maintained by misters operated for 2 s every 10 min. After 72 h, plants were returned to the glasshouse bench. Susceptibility was noted at 11 and 22 days after inoculation and specimens from susceptible plants were taken at 11 days to see if the fungus could be re-isolated. The development of ascocarps on dead fallen leaves in the pots was checked after 22 days. Results are shown in Table 4.

No sign of infection was seen on *Aeschynomene indica*, *Lespedeza striata* or *Zornia dyctiocarpa*, but all other species were infected in varying degrees

and the fungus re-isolated from surface-sterilized lesions. The introduced *Crotalaria pallida* was severely blighted and, of the native species, *Glycine* spp., *Kennedia rubicunda* (including var. *robusta*), *Indigofera hirsuta* and *Vigna luteola* were the most susceptible. Some hosts, e.g. *Crotalaria linifolia*, *Glycine* spp. and *Indigofera hirsuta*, showed an increase in disease severity from 11 to 22 days. Re-isolation from surface-sterilized lesions was obtained from all infected hosts but, of those showing mild (0–2) symptoms re-isolation was possible only from a small proportion of the lesions, e.g. *Desmodium heterocarpon* and *Lespedeza juncea*. Immature ascocarps were found on dead fallen leaves of four species and the fungus could conceivably complete its life cycle in the field on these hosts. The results show that several potential native leguminous hosts grow in the general area where soybean black leaf blight occurs.

DISCUSSION

The finding in Australia of an apparently undescribed fungus causing a new disease of an introduced crop such as soybean raises a number of questions about its origin. It is most unlikely to have been introduced, as no similar soybean disease has been seen elsewhere and the fungus does not agree with any previously described either on soybean or other hosts. Most probably, *Arkoola nigra* is a native species present at a low level on a native host (or hosts) with which it is in ecological balance. Several native Fabaceae are susceptible in artificial inoculation tests, and these or related species are possible native hosts. The ability of the fungus to attack in the field a non-leguminous host such as *Sida rhombifolia* also raises the possibility that hosts in other botanical families may be present. More intensive surveys to try and find the hypothetical native hosts are needed. The Wauchope district, the first outbreak area from which subsequent spread has occurred northwards, is especially worthy of attention. The recent expansion of soybean culture in coastal New South Wales has obviously provided large areas of a susceptible host and allowed the fungus to develop in abundance and, indeed, be found.

Soybeans are grown currently on over 10000 ha in coastal N.S.W., and black leaf blight seriously threatens the future of the industry. Losses in diseased crops can be as high as one-third of the potential yield, and the development of effective measures to ensure that such severe losses do not occur regularly is of major importance. High rainfall and humidity on the coast in March, April and May are usually favourable for fungal infections at a time when crops are in the post-flowering, pod-filling stage. Mean temperatures at this time range from a minimum of 14–15° to a maximum of 24–26°, and this is within the range of temperatures suitable for ascospore germination and linear hyphal growth in culture (Tables 1, 2). This period is the time that most severe crop infections have been seen. So far, black leaf blight has not been found in inland irrigation districts in the northwest and Riverina areas of N.S.W. Conditions in these areas during the pod-fill stage are drier and hotter than on the coast and generally not as favourable for fungal foliar pathogens. It is unlikely that black leaf blight would be a problem in these areas, but every care should be taken to prevent its spread into them. As the fungus can be carried at a low level in seed from infected plants, the possibility exists of spread with seed to other soybean growing areas, both in Australia and overseas.

Studies on factors which favour development and spread of black leaf blight have been started. Knowledge of conditions favouring infection, persistence of the disease from season to season, other possible hosts, the relative importance of ascospores and mycelium as sources of inoculum and other aspects of the life-cycle is essential basic information for any work on control measures.

Because of its pale greenish ascospores, borne in cylindrical bitunicate asci in superficial setose pseudothecia, *Arkoola* is placed in the Venturiaceae. Most members of this family occur in cool temperate areas, and *Arkoola* does not seem to be related closely to any of them. It does not fit readily into any of the four groups of genera distinguished by Barr (1968). Its production of a loose freely growing mycelial web on living plants, producing appressoria under which infection occurs, appears to be unique in the family. This points perhaps to an origin in a warm, humid environment similar to that which favours the web and thread blights of tropical and subtropical regions. Although other genera of Venturiaceae produce superficial mycelium on living leaves and twigs, it does not extend much beyond the pseudothecia and serves as a base for them. It is more a product of the internal mycelium than an agent for spreading new infections over the host. These other genera with superficial mycelium, such as *Protoventuria* and *Apiosporina* Höhnel, also develop their pseudothecia primarily on the living plant rather than on dead, fallen and rotting organs of the host. The absence of a well-developed hypostroma and presence in the ascus tip of a faintly staining refringent zone also distinguish *Arkoola* from most other genera of Venturiaceae. However, so few details of ascus tip structure are known for most species that valid comparisons of the latter are not

possible. In centrum structure, *Arkoola* is relatively advanced in the scheme put forward by Parguey-Leduc (1966). With its well-developed parasitic superficial mycelium, it may represent a slightly different line of development from that taken by other members of the family.

Specimens examined

Arkoola nigra J. Walker & G. E. Stovold, all on *Glycine max*, N.S.W., Australia: (*a*) with mature ascocarps: on leaves in culture, Rydalmere, Nov. 1983, G. E. Stovold & J. Walker, DAR 43446 (Holotype); on trash on soil surface in pot, Rydalmere, 6 Aug. 1983, M. J. Priest, DAR 43445; on inoculated leaves in pot, Rydalmere, 18 Nov. 1983, A. Francis, DAR 48919; (*b*) with immature ascocarps, all on field-collected trash: Wauchope, D. McCoy, 25 July 1983, DAR 43450; 26 July 1983, DAR 43451, 45786; 6 Sept. 1983, DAR 43447; 7 Oct. 1983, DAR 49554; 3 Aug. 1984, DAR 50260; Byabarra, 29 July–3 Aug. 1983, D. McCoy, DAR 45787; (*c*) mycelial state only (a selection only listed): Wauchope, 24 Mar. 1982, D. McCoy, DAR 41445 (dup. as IMI 277447), first record; Wauchope, 3 Mar. 1983, D. McCoy, DAR 44510 (dup. as IMI 277448): Willi Willi, near Kempsey, 24 July 1983, G. Fenton, DAR 48848*a*; Ellenborough, 29 July 1983, D. McCoy, DAR 48853; Kundabung, 6 Feb. 1984, D. McCoy, DAR 49580; South Grafton, 15 May 1984, J. Betts, DAR 50267; Wauchope, 14 Feb. 1985, H. Smith & A. Francis, DAR 51512; Heron's Creek, 14 Feb. 1985, H. Smith & A. Francis, DAR 51511; Tabulam, 13 Feb. 1985, B. Clarke, DAR 51596; Tabulam, 21 Mar. 1985, B. Clarke, DAR 51597; Casino district, 19 Mar. 1985, B. Clarke, DAR 51598.

Macroventuria anomochaeta van der Aa: culture from decayed canvas, Karoo Desert, Republic of South Africa, date not given, M. C. Papendorf 278, Herb. Mycol. van der Aa 2427 ex CBS, Holotype (slide as DAR 51215).

Macroventuria wentii van der Aa: culture from leaf litter, Death Valley, Nevada, U.S.A., 1970, F. W. Went, Herb. Mycol. van der Aa 2592 ex CBS 526.71, Holotype (slide as DAR 51214).

Protoventuria arxii (E. Müller) M. E. Barr: on twig of *Rhododendron* sp., Radnor, Wales, U.K., 1960, collector not given, IMI 80930*a* (slide as DAR 51207).

Protoventuria engleriana (P. Henn.) A. Sivanesan: on branch of *Erica algida*, Republic of South Africa, collector not given, 1979, IMI 241049 (slides as DAR 51209).

Protoventuria straussii (Sacc. & Roum.) A. Sivanesan: on branch of *Erica carnea* L., Germany, collector not given, 1941, IMI 30614 (slide as DAR 51208).

The assistance of D. McCoy, G. Fenton, J. Betts and B. Clarke, District Officers of the Department of Agriculture N.S.W., with field work and collection of specimens is gratefully acknowledged. Mr G. P. M. Wilson, Senior Research Agronomist, Grafton Agricultural Research and Advisory Station, supplied seed of native and naturalized coastal legumes and advised on their distribution. Miss A. Francis, Technical Officer (Scientific) carried out much of the culture and plant inoculation work and Miss C. Nuzum, Technical Officer (Scientific) devised the L-drying modification for keeping the fungus in culture. Mr M. J. Priest, Plant Pathologist (Mycology) helped with field inspections and examination of specimens. We also thank Dr H. A. van der Aa (CBS) and Dr B. C. Sutton (IMI) for lending specimens of *Macroventuria* spp. and *Protoventuria* spp. respectively. Dr M. Barr Bigelow (MASS) kindly read the taxonomic section in draft.

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(Received for publication 21 November 1985)