OCCURRENCE AND HOST RANGE OF *ASCOCHYTA PHASEOLORUM* IN QUEENSLAND

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[*Manuscript received April* 26, 1968]

Summary

The presence of *Ascochyta phaseolorum* as a pathogen of *Phaseolus vulgaris* in Queensland is reported. The fungus has been found to have a wide host range including vegetable crops, pastures, weeds, and indigenous species. Natural infections were found in 48 hosts in 14 families, and an additional 12 species proved susceptible when inoculated experimentally. Pathogenicity of isolates from French bean towards many of the natural hosts has been demonstrated, and in reciprocal inoculations cultures from field-infected hosts produced symptoms on bean typical of those produced by bean isolates. The fungus is shown to be a weak parasite, requiring some form of wounding to initiate infection under Queensland conditions.

I. Introduction

A disease of French bean (*Phaseolus vulgaris* L.) in Queensland caused by a species of *Ascochyta* was first reported in 1956, from the Atherton Tableland (Simmonds 1966). Following a period of continuous light rain with below-average temperatures in October 1965, the disease was found at several localities in the Nambour district in south-eastern Queensland (Pegg and Alcorn 1967). It has occurred in subsequent bean-growing seasons in this area. Studies on the identity and host range of the causal fungus are reported here.

The two commonly reported species of *Ascochyta* on bean are *A. phaseolorum* Sacc. and *A. boltshauseri* Sacc. The latter is readily distinguished from *A. phaseolorum* by its much larger, often multiseptate conidia (Sutton and Waterston 1966). Other species described on *Phaseolus* are *A. oró* Viégas on *P. panduratus* Mart. and *A. bornmülleri* Syd. on *P. acutifolius* Gray.

II. Materials and Methods

Cultures were obtained by isolation from freshly collected specimens on potato dextrose agar. Abundant sporulation was induced by maintaining isolates on sterilized potato cylinders as recommended by Crossan (1958). Inoculum was prepared by filling the culture tube with water and gently rubbing the surface of the cylinder with a sterile needle.

When only one isolate was used, the spore suspension was atomized onto test plants. To minimize the possibility of cross contamination when using isolates from different sources, inoculum was applied directly from the test tubes containing the potato cylinder cultures. After the conidial suspensions were prepared, each tube was stoppered by a cork bearing protruding pins, with a narrow V-cut to allow the suspension to escape when the tube was inverted. Simul-

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taneous wounding and inoculation was achieved by pressing the cork against the leaf to be inoculated. The inoculum was also shaken over unwounded parts of the same leaf.

Inoculated plants were maintained in a moist chamber at high relative humidity for 3–5 days, or occasionally as long as 8 days, then placed on glasshouse benches. Lesions were usually visible 2–3 days after inoculation. Samples were taken from infected plants and the presence of the pathogen determined by microscopic examination or culture on potato dextrose agar.

III. Observations and Results

(*a*) *The Disease on French Bean*

Leaf spots are roughly circular, drab-grey to brown with a well-defined dark margin, and up to 2 cm in diameter. Concentric zonation occurs in some lesions, and in the larger ones the centres tend to tear and disintegrate (Fig. 4). On the lower surface a darkening of the larger veins extending out from the lesion is sometimes produced. When plants have been grown in locations exposed to wind, marginal infection of leaves is more common, resulting in semicircular lesions as the infection extends inwards. In more sheltered areas the sites of infection are usually insect injuries, rust pustules, or other wounds. Leaf spotting may be severe enough to cause premature defoliation, and is often accompanied by elongate lesions on stems and petioles, uniformly dark in colour or with paler brown centres.

Infection of the calyx and pedicel also occurs, and may progress down into the pod to produce a dark, dry stem-end lesion of limited extent. A darkening of the dorsal and ventral sutures of the pod is sometimes associated with this condition. Larger dark lesions are also formed at other sites on the pod, often originating at small superficial scars (Fig. 2). Pycnidia are produced abundantly in the necrotic tissue of leaves, petioles, stems, and pods.

Isolates from bean were shown to be pathogenic to wounded bean leaves in the glasshouse. Senescent bean petals soaked in a spore suspension and placed on unwounded leaves also served as sites of infection. In artificial infections such as these, lesions are often paler at maturity and zonation is less pronounced than in those developed under natural conditions. Shattering of the necrotic tissue is also less in evidence.

(*b*) *Identity of the Causal Fungus*

The pycnidia are mostly epiphyllous, pale yellow to dark brown, immersed, globose to flattened, 70–240 μ in diameter, and darkened around the circular ostiole, which measures 11–19 μ in diameter. Conidia are hyaline, mostly without a septum or uniseptate, oblong with the ends obtuse, or with the basal cell sometimes tapered and truncate, straight or variously bent; constriction at the septum is slight or absent. Most two-celled conidia mounted in water are within the range 7–12 (15) by 2·5–5 μ. Unicellular conidia are smaller, measuring 5–9 by 2·5–4 μ (Fig. 1). Conidiophores are hyaline, unicellular, globose, phialidic, and measure 5–9 by 4–7·5 μ. Of the species of *Ascochyta* recorded on *Phaseolus*, this fungus agrees most closely with published descriptions of *A. phaseolorum*, and therefore these collections are referred to this species. Spore length is less than that reported by Sutton and Waterston (1966), but is in agreement with dimensions given by Saccardo (1884),

Grove (1935), Sneep (1945), and Crossan (1954). Comparison of the local collections with a specimen from the Commonwealth Mycological Institute (on *P. vulgaris* IMI 47179) offered no grounds for considering the Queensland material distinct from this species. The Commonwealth Mycological Institute kindly confirmed this identification for one of the collections on French bean (17076, IMI 127009).

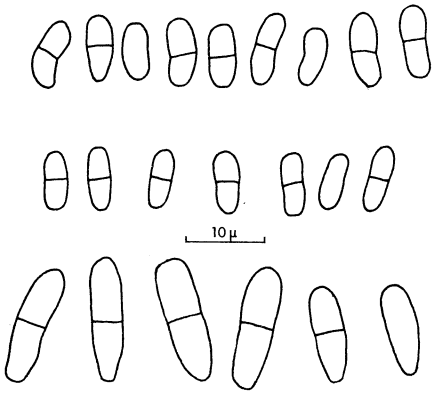


Fig. 1.—Camera lucida drawings of *Ascochyta* conidia. Top row, *A. phaseolorum* IMI 127009; middle row, *A. phaseolorum* IMI 47179; bottom row, *A. arida*.

Because of the close agreement between these specimens and the description of *A. oró* (Viégas 1945), the type collection for this species was examined (IACM 436). The fungus present on the specimen is indistinguishable from *A. phaseolorum*, and in addition an isolate obtained locally from the type host was pathogenic to bean (Table 1). *A. oró* is therefore considered to be a synonym of *A. phaseolorum*.

(*c*) *Host Range*

A fungus very similar to *A. phaseolorum* on French bean has been found occurring naturally on a wide range of unrelated plants in south-eastern Queensland (Table 1). While in most instances these plants were growing near affected bean crops, sometimes the collections were made in areas remote from bean-producing districts. The variability in fungal morphology between these collections is no greater than that shown by different collections from French bean, and, as shown below, cultures from many of these hosts are pathogenic to bean. As with French bean, infection in these hosts is often obviously associated with wounds. Lesions are usually more or less circular, uniformly dark brown or more often paler in the centres and zonate, with the margins dark and sometimes surrounded by a faint chlorotic halo (Fig. 3). On *Hibiscus rosa-sinensis* a pronounced reddish discoloration is present around some spots. Pycnidia are formed abundantly in natural infections on many of these hosts, but sparingly in some, e.g. *Glochidion supra-axillare*, *Ipomoea plebeia*, and *Trema aspera*.

(*d*) *Infection Experiments*

Isolates of *Ascochyta* from a selection of the hosts listed in Table 1 were tested for pathogenicity to bean. All isolates tested were pathogenic, producing symptoms identical with those produced by *A. phaseolorum* from bean (Figs. 6–9).

TABLE 1

NATURAL HOSTS OF A FUNGUS RESEMBLING *A. PHASEOLORUM*

Ageratum houstonianum Mill.*
Anoda cristata (L.) Schlecht.
Asclepias sp.*
Baccharis halimifolia L.*
Cajanus cajan (L.) Millsp.*
Capsicum frutescens L.
Citrullus vulgaris Schrad.
Crotalaria mucronata Desv.*
Cucurbita pepo L.
Datura sp.
Desmodium distortum (Aubl.) Macbride
Desmodium intortum (Mill.) Urb.
Dolichos falcatus Klein ex Willd.
Dolichos formosus A. Rich.
Dolichos lablab L.*
Dolichos lignosus L.
Dolichos uniflorus Lam.
Erigeron floribundus (H.B.K.) Schultz-Bip.*
Eustrephus latifolius R. Br.*
Galinsoga parviflora Cav.
Glochidion supra-axillare (Benth.) Domin*
Glycine javanica L.*
Hibiscus esculentus L.
Hibiscus rosa-sinensis L.*
Ipomoea plebeia R. Br.*
Lantana camara L.*
Nicandra physalodes (L.) Gaertn.
Passiflora subpeltata Ortega*
Phaseolus atropurpureus DC.*
Phaseolus bracteatus Nees & Mart.
Phaseolus caracalla L.*
Phaseolus lathyroides L.
Phaseolus panduratus Mart. ex Benth.*
Phaseolus vulgaris L.*
Physalis minima L.
Phytolacca octandra L.*
Richardia brasiliensis (Moq.) Gomez.*
Sechium edule Sw.*
Sida rhombifolia L.*
Solanum mauritianum Scop.*
Tagetes minuta L.*
Teramnus uncinatus Sw.*
Teramnus volubilus Sw.*
Trema aspera (Brongn.) Bl.*
Verbena bonariensis L.
Vigna luteola Benth.*
Vigna marina (Burm.) Merr.*
Vigna sinensis (L.) Endl. ex Hassk.

* Sources of isolates pathogenic to bean.

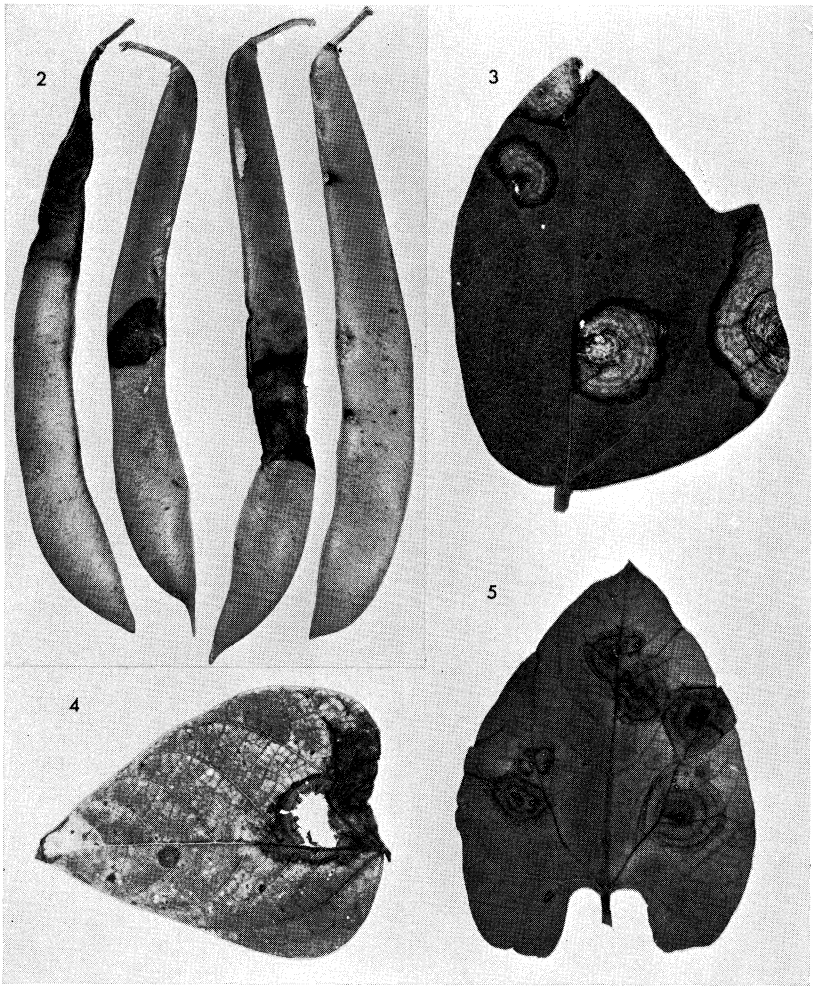
TABLE 2

HOSTS SUSCEPTIBLE TO *A. PHASEOLORUM* ISOLATED FROM FRENCH BEAN

Asclepias fruticosa L.*
Cajanus cajan (L.) Millsp.
Capsicum frutescens L.†
Citrullus vulgaris Schrad. cv. Candy Red
Crotalaria mucronata Desv.*
Cucumis sativus L. cv. Marketer
Cucurbita pepo L. cv. Long White Bush
Cyamopsis tetragonoloba (L.) Taub.*
Datura stramonium L.
Desmodium intortum (Mill.) Urb.†
Dolichos axillaris E. Mey.
Dolichos lablab L.*
Dolichos lignosus L.
Dolichos uniflorus Lam.*
Glycine max (L.) Merr. cv. Leslie†
Glycine javanica L.
Gossypium hirsutum L. cv. Miller
Hibiscus esculentus L. cv. Clemson's Spineless*
Hibiscus rosa-sinensis L.†
Lantana camara L.
Lycopersicon esculentum Mill. cv. Grosse Lisse
Nicotiana tabacum L.†
Phaseolus atropurpureus DC.
Phaseolus aureus Roxb.
Phaseolus caracalla L.
Phaseolus lathyroides L.*
Phaseolus panduratus Mart. ex Benth.
Phaseolus vulgaris L.*
Sechium edule Sw.*
Sida rhombifolia L.
Solanum melongena L.†
Teramnus uncinatus Sw.
Verbena bonariensis L.
Vigna hosei (Craib) Back.
Vigna marina (Burm.) Merr.
Vigna sesquipedalis (L.) Fruwirth
Vigna sinensis (L.) Endl. ex Hassk. cv. Caloona*
Vigna vexillata (L.) A. Rich.*

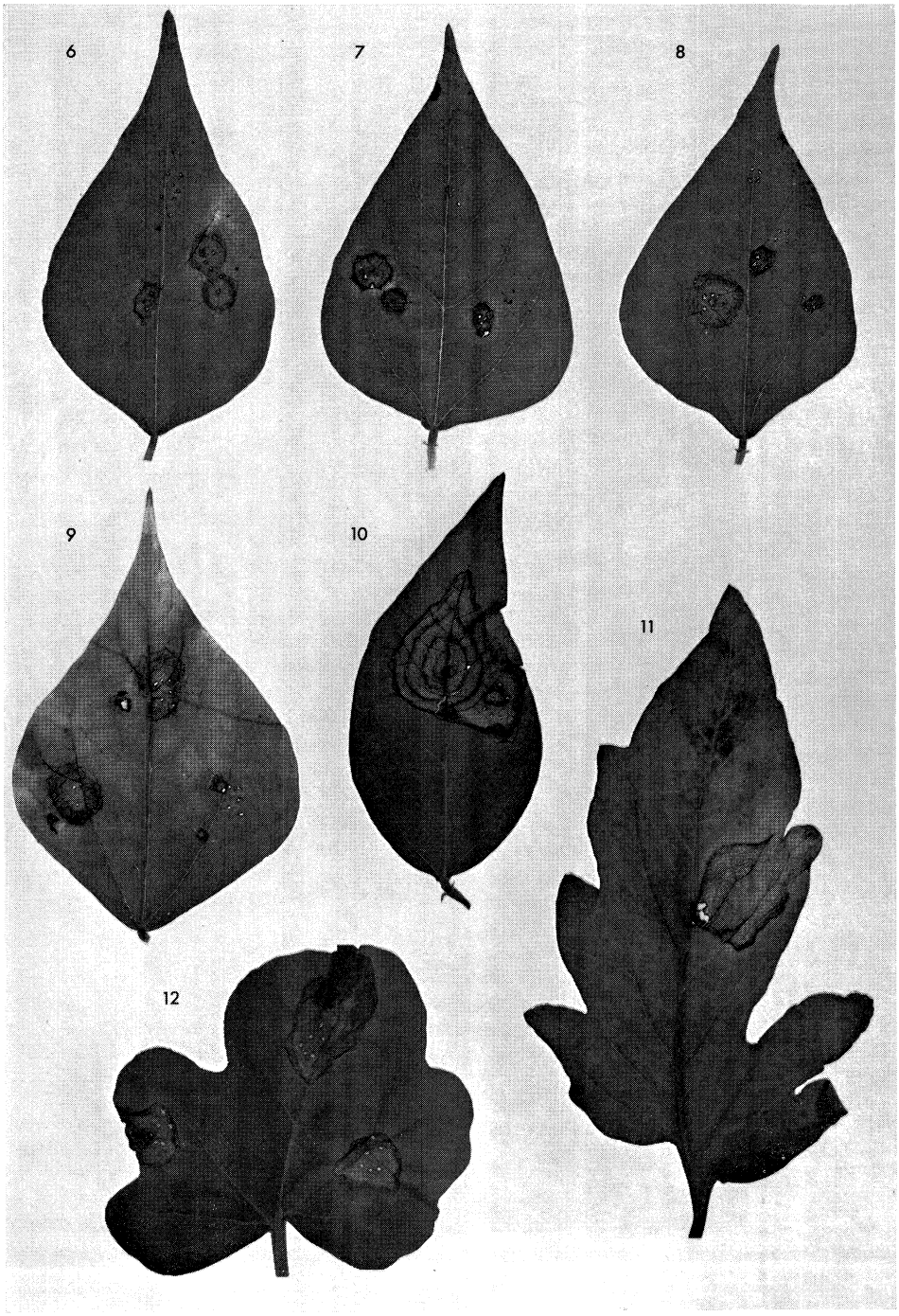
* Very susceptible. † Resistant.

Of 40 species tested, 38 were infected in the glasshouse by *A. phaseolorum* isolated from bean (Table 2; Figs. 5, 10–12). A very susceptible reaction was shown by some, for example *Vigna sinensis*, while others such as *Glycine max* were resistant. Immune to infection were *Ageratum houstonianum* and *Pisum sativum* L. Where sufficient data is available, the more susceptible and resistant species have been indicated in Table 2.



Figs. 2–4.—Natural infection by *A. phaseolorum* on pods of *Phaseolus vulgaris* (Fig. 2), and on leaves of *P. atropurpureus* (Fig. 3) and *P. vulgaris* (Fig. 4).
Fig. 5.—Infection of *Dolichos lablab* by an isolate of *A. phaseolorum* from *P. vulgaris*.

In these experiments infection at sites not deliberately wounded was extremely rare, despite periods of up to 8 days in a moist chamber in some instances. However, it is possible that minute injuries were present but not detected.



Figs. 6–9.—Infection of *Phaseolus vulgaris* by isolates of *Ascochyta* from *Glochidion supra-axillare* (Fig. 6), *Baccharis halimifolia* (Fig. 7), *Eustrephus latifolius* (Fig. 8), and *P. vulgaris* (Fig. 9). Figs. 10–12.—Infection of *Dolichos uniflorus* (Fig. 10), tomato (Fig. 11), and watermelon (Fig. 12) by *A. phaseolorum* from *P. vulgaris*.

A wide host range was also demonstrated for one other isolate. A culture from *Ipomoea plebeia* infected *Asclepias fruticosa, Capsicum frutescens, Citrullus vulgaris, Crotalaria mucronata, Cucumis sativus, Dolichos axillaris, D. uniflorus, Glycine javanica, G. max, Phaseolus atropurpureus, P. lathyroides, Sechium edule, Solanum melongena, Vigna sinensis,* and *V. vexillata,* but not *D. lablab, P. aureus,* or *V. marina.*

An isolate from bean was compared with a culture of the cucurbit gummy stem blight fungus, *Mycosphaerella melonis* (Pass.) Chiu & Walker (*Ascochyta cucumis* Fautr. & Roum.), which also occurs on watermelon leaves. The latter was non-pathogenic on bean leaves, but caused typical symptoms of gummy stem blight when introduced into the cotyledonary node of young watermelon plants. In the same tests, the bean isolate did not invade watermelon stem, but was virulent on bean leaves.

Lesion development following removal of plants from the moist chamber to glasshouse benches may be arrested by hot, dry weather. On some occasions such an incident was followed by a period of cool, overcast conditions, and it was observed that lesion expansion would recommence. Similarly, returning plants to the moist chamber would sometimes produce similar results.

Rapid drying of lesions was found to suppress pycnidial production. If leaves bearing such lesions were placed on moistened filter paper in a Petri dish, numerous pycnidia usually developed within 3–4 days. A similar increase in numbers of pycnidia was also observed to take place on abscissed leaves which had fallen onto the surface of the peat–sand potting mixture used in these experiments. Naturally senescent leaves, unwounded but previously inoculated by atomizing with a spore suspension, were also observed to support an abundant development of pycnidia after several days on the potting medium surface.

Tobacco was difficult to infect with *A. phaseolorum* in these experiments. In Queensland, *A. arida* McAlp. occurs naturally on this host and on *Nicotiana glauca* Graham. Although conidial shape is similar for both species, in *A. arida* most two-celled spores are in the range 12–20 by 3·5–5 μ (Fig. 1). It was therefore of interest to find a specimen of tobacco leaf spot from North Carolina in the Plant Pathology Herbarium, Queensland Department of Primary Industries. The packet is labelled *Phyllosticta nicotianae* and was sent to this Department in 1937. The fungus present is a species of *Ascochyta* with spores similar in size and shape to those of *A. phaseolorum,* and this specimen conceivably provides an earlier record than Crossan's (1954) of this disease in North Carolina.

IV. Discussion

A fungus morphologically identical with *A. phaseolorum* has been found on eight genera of legumes in south-eastern Queensland. On the basis of morphology and pathogenicity to French bean, the same fungus has been found in this study to occur on a wide range of non-leguminous hosts, including crop plants, weeds, and indigenous species. Pathogenicity of isolates from bean to many unrelated plants has been demonstrated (Table 2).

Undetermined species of *Ascochyta* have previously been recorded in Queensland on *Cyamopsis tetragonoloba* (1965), *Dolichos biflorus* L. (1964), *Phaseolus atropurpureus* (1960), *Vigna hosei* (1964), and *V. marina* (1959) (Simmonds 1966). Specimens were not available for examination, but the infection experiments indicate that *A. phaseolorum* may have been responsible for the disease on these hosts (Table 2). Diseases caused by *Ascochyta* had not been recorded in Queensland on the other hosts in Table 1 prior to this study.

Crossan (1954, 1958) compared the morphology, physiology, and pathogenicity of seven species of *Ascochyta* occurring on 10 hosts in North Carolina, and found that his isolates could not be differentiated using these criteria. In cross-inoculation tests using unwounded plants, identical symptoms were produced on each host irrespective of the original source of inoculum. He proposed that *A. abelmoschi* Harter, *A. althaeina* Sacc. & Bizz., *A. capsici* Bond.-Mont., *A. gossypii* Syd., *A. lycopersici* Brun., and *A. nicotianae* Pass. be considered synonyms of *A. phaseolorum*, on the basis of these results. A European isolate of *A. phaseolorum* differed considerably in virulence from the American isolates, being non-pathogenic on bean and tomato, and showing differences in response to temperature and pH.

In contrast to Crossan's results with American isolates, unwounded leaves were infected only rarely even when inoculated plants were maintained for long periods in a moist atmosphere. Field observations in Queensland support the conclusion that the association of this fungus with wounds is an obligate one here. Infection of weakened or damaged crops was also noted by Sutton and Waterston (1966), and Sneep (1945) found that small wounds on bean pods were conducive to infection by *A. boltshauseri* and *A. phaseolorum*. The occurrence of *A. phaseolorum* on crops, weeds, pasture plants, and other species in addition to French bean is in agreement with the wide host range reported for this species (Sutton and Waterston 1966). These plants could probably serve as reservoirs of infection between seasons. In south-eastern Queensland *Baccharis halimifolia*, *Dolichos lablab*, *D. uniflorus*, *Hibiscus rosa-sinensis*, *Ipomoea plebeia*, *Passiflora subpeltata*, *Phaseolus atropurpureus*, *P. lathyroides*, *Sida rhombifolia*, and *Vigna marina* have been found infected by this fungus during summer, when beans are not widely grown.

The results reported here emphasize the risk of using host identity as a basis for the application of different specific epithets to fungi of similar morphology, in the absence of infection studies. They also suggest the possible non-validity of several specific names in the form-genus *Ascochyta*. Synonomy was confirmed for one of these species, *A. oró*.

V. Acknowledgments

I am indebted to the Commonwealth Mycological Institute for confirming the determination of *A. phaseolorum* on French bean; to Mr. K. G. Pegg for generous assistance in collecting in the field; to Miss S. M. Way for technical assistance; and to the Photography Section, Department of Primary Industries, for the photographs.

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