Reaction of selected accessions of forage *Cassia* spp. to some fungal pathogens

S. CHAKRABORTY,[1] R. CHARUDATTAN[2] AND J.T. DE VALERIO[2]
[1]*Division of Tropical Crops and Pastures, CSIRO, Brisbane, Queensland, Australia*
[2]*Department of Plant Pathology, University of Florida, Gainesville, Florida, USA*

Abstract

Eleven accessions of *Cassia rotundifolia*, including the cv. Wynn, and one of *C. pilosa* were screened against 6 fungal pathogens, of which two are exotic to Australia. *Colletotrichum dematium* f.sp. *crotalariae* and *Sclerotium rolfsii* were pathogenic on several accessions, whereas isolates of *Phomopsis* sp., *Macrophomina phaseolina* and *Pseudocercospora nigricans* were non-pathogenic on *Cassia*. *Alternaria cassiae*, a possible mycoherbicide for the control of sicklepod (*C. obtusifolia*), produced small necrotic lesions on young leaves and stems in 8 of the accessions. Accessions Q9862, 57503, 86178 and cv. Wynn were considered susceptible and the others were grouped as being moderately resistant to resistant. Symptoms were mainly restricted to the youngest leaves, making the 2.5-week old seedlings more prone to severe damage than the 5 or 9-week old seedlings with many mature leaves. This could have serious implications for Wynn-based pastures as regular seedling recruitment is essential for long-term persistence of Wynn. The work demonstrates the potential damage that may be caused to *Cassia*-based pastures if *A. cassiae* was introduced for the biological control of sicklepod weed in Australia or if the fungus is already present in Australia.

Correspondence: Dr S. Chakraborty, CSIRO Division of Tropical Crops and Pastures, 306 Carmody Road, St. Lucia, Qld 4067, Australia

Introduction

In Australia, the improvement of native pastures for increased beef production has relied heavily on the successful introduction, establishment and persistence of legume species. Among other genera, species of *Cassia*, native to tropical and subtropical regions, have been tested for their potential as pasture legumes. Accession CPI 34721[1] of *C. rotundifolia*[2], originally collected from Brazil in 1964, was released in 1984 in Australia as the cultivar Wynn (Oram 1990). Wynn has proved superior to several other accessions and species of *Cassia* tested in the 600–1000 mm annual rainfall areas of northern Australia (Strickland *et al.* 1985). It is a productive, summer-growing legume of moderate palatability with dry matter yields in excess of 7000 kg/ha and seed yields of 700 kg/ha. It is tolerant to heavy grazing and combines well with most pasture grasses.

A mild leaf spotting on old leaves caused by a *Pleospora* sp. was the only disease reported on Wynn at the time of its release (Oram 1990). Since then, tip blight caused by *Periconia byssoides* Pers., leaf spot caused by a *Pestalotiopsis* sp., stem spot caused by *Phomopsis phaseoli* (Desmaz.) Sacc., and root and crown rots caused by *Rhizoctonia solani* Kuehn. and *Sclerotium rolfsii* Sacc. have been recorded on *C. rotundifolia* in Australia (J.L. Alcorn, personal communication). No information is available on the level of damage these fungi may be causing to *Cassia*-based pastures. *C. rotundifolia* is only one of 42 native and 10 naturalised species of *Cassia* in Australia (Anning *et al.* 1989). While there are several pathogens,

[1]All accession numbers without an alphabetic prefix used in this paper are Australian Commonwealth Plant Introduction (CPI) numbers.
[2]In a taxonomic review, Irwin & Barneby (1982), placed *C. rotundifolia* Pers. into *Chamaecrista rotundifolia* (Pers.) Greene. We have retained the more popular nomenclature for clarity.

some causing serious diseases on many of these species in Australia (J.L. Alcorn, personal communication) and elsewhere (Lenne 1990), it is not known if fungi reported on other *Cassia* species will infect Wynn.

Some *Cassia* species, like *C. obtusifolia* L. (sicklepod), which infests some 10 000 ha from Cooktown to Sarina in Queensland (Anning *et al.* 1989), are serious weeds. Several fungal pathogens have been shown to be effective as potential biocontrol agents of sicklepod. Of these, *Alternaria cassiae* Jurair & Khan and *Pseudocercospora nigricans* (Cooke) Deighton have shown promise (Charudattan 1991). In the USA, a formulation of *A. cassiae* has been developed and tested as a mycoherbicide to control sicklepod in crops such as soybean and peanut (Bannon 1988). It is imperative that these mycoherbicides do not destroy beneficial pasture species of *Cassia*.

In the past, introduced pasture plants have sometimes been released in Australia without adequate testing of their susceptibility to potential pathogens. As a result, pathogens gaining a bridgehead in Australia have subsequently devastated large stands of susceptible plants such as *Stylosanthes* cultivars Endeavour, Schofield and Fitzroy. Lenne (1990) recommended that Wynn and other selected accessions of *Cassia* be screened against exotic pathogens to avoid a similar situation arising.

In this work, 11 accessions of *C. rotundifolia* and one of *C. pilosa* were screened against *A. cassiae* and 5 other fungal pathogens. Three of these pathogens are potential biocontrol agents of weeds, and the other 2, *Sclerotium rolfsii* Sacc. and *Macrophomina phaseolina* (Tassi) Goid., are pathogens with broad host ranges. In addition, the influence of plant age on susceptibility to *A. cassiae* was examined.

Materials and methods

Pathogenicity of Alternaria cassiae

Seeds of 12 accessions of *Cassia* which have shown promise as pasture legumes were obtained from the CSIRO Tropical Forage Genetic Resource Centre (Table 1). Seeds of sicklepod, used as a control, were collected from local populations around Gainesville, Florida. Scarified seeds were sown in seedling flats in a commercial potting mix (Terra-lite Metro-Mix 500, Grace Sierra Horticulture Products Co., 1001 Yosemite Drive, Milpitas, CA 95035) in a glasshouse. Three seedlings, transplanted in a 7 cm diameter clay pot, constituted a replicate and 3 replicates were used for control and inoculated treatments. The experiment was repeated and the inoculum concentration was doubled in the repeat run. An experimental mycoherbicide formulation of *A. cassiae*, MYX-104 100% WP (Mycogen Corporation, 5451 Oberlin Drive, San Diego, CA 92121) was used as inoculum. Conidia were suspended in distilled water containing 1% surfactant (MYDM, Mycogen Corporation) to obtain 3×10^7 spores/ml. Four-week old seedlings, with 3-4 leaves each, were sprayed with the conidial suspension to incipient run-off using a garden sprayer and incubated in a dew chamber at 27 °C in darkness for 18 h. Control plants were similarly sprayed with distilled water containing the surfactant and incubated. All plants were inspected daily for symptoms. Leaves were visually inspected to assess the size of individual lesions and the percentage of leaf area covered by lesions. Pieces of tissue with symptoms were surface sterilised in 0.5% Na-hypochlorite solution and plated on appropriate media after 2 washings with sterile distilled water. The fungus was reisolated to apply Koch's postulates.

Influence of plant age on susceptibility of Cassia *to* A. cassiae

Three different age-groups of accessions Q9862, 86178, 93094 and 37234 and cv. Wynn, were used in this experiment. The ages of the 3 groups at inoculation were 2.5, 5 and 9 weeks with approximately 2, 10 and 25 leaves/seedling, respectively. Plants in all age groups were in a vegetative state. Three seedlings in a pot constituted a replicate and 3 replicates were used for each accession per age-group for both inoculated and control treatments. Two-week old seedlings of sicklepod were used as a control. Plants were inoculated with the Mycogen formulation of *A. cassiae* and assessed as in the previous experiment. Control plants were sprayed with an autoclaved suspension of conidia.

Pathogenicity of other fungi

Methods used to grow, inoculate and incubate seedlings, their age at inoculation and the number of replicates used were the same as in the previous experiment for *A. cassiae* inoculation, except where indicated. The host species from which the pathogen was originally isolated was used as a control. The 12 *Cassia* accessions were inoculated with each of the following pathogens:

(i) *Phomopsis* sp. (isolate 2365 from R. Charudattan's collection) from *Baccharis halimifolia*, a shrubby weed, native to Florida and the eastern United States that has invaded large areas of subtropical Queensland. Plants were inoculated with a conidial suspension (3.5×10^6 conidia/ml) from 2-week old potato dextrose agar (PDA) cultures with 0.5 ml/l of an adjuvant, Triton X-100 (Octyl phenoxy polyethoxy-ethanol, Sigma Chemical Co.).

(ii) *Colletotrichum dematium* f.sp. *crotalariae* (SS5 from R. Charudattan's collection), a potential biological control agent of showy crotalaria, *Crotalaria spectabilis* (Charudattan 1986), was grown on V-8 juice agar (3 g $CaCO_3$, 15 g bacto agar, 200 ml V-8 juice in one l water). A conidial suspension of 50 000 spores/ml with 0.5 ml/l Triton X-100 was used to spray seedlings. The test was later repeated with an inoculum concentration of 8×10^6 spores/ml.

(iii) *Pseudocercospora nigricans* cultures (isolated from sicklepod plants by Hofmeister and Charudattan (1987)) were grown using methods described by these authors. An inoculum containing 4.4 g of fresh blended mycelia and conidia in 100 ml water with 0.5 ml/l Triton X-100 was sprayed on to the seedlings.

(iv) *Sclerotium rolfsii* (isolate SR5 from peanut was obtained from Dr David Mitchell, Plant Pathology Department, University of Florida) was grown in potato dextrose broth in Roux bottles for about 2 weeks. Mycelial mats were washed in sterile distilled water and 200 g of fresh mycelia and sclerotia were blended with 360 ml distilled water for 10 sec. Ten ml of hyphal suspension (with about 8–10 sclerotia) was poured around the base of the 3 seedlings in each pot. All inoculated seedlings were incubated in a dew chamber for 18 h. The peanut (*Arachis hypogea*) cultivar Florunner was used as a control.

(v) *Macrophomina phaseolina* (isolated from peanut by Dr F. Shokes, University of Florida, Quincy) was grown on PDA for 2–3 weeks and plates were scraped for an inoculum containing mycelial and sclerotial fragments. Roots of seedlings were wounded with a sterile needle and 10 ml of the inoculum was poured around the base of seedlings. Seedlings were also sprayed to incipient run-off using this suspension and a post-inoculation dew period of 18 h was applied. Florunner peanut was used as a control.

Results

Pathogenicity of Alternaria cassiae

On sicklepod seedlings, disease symptoms could be first seen 2 days after inoculation. Leaves and hypocotyl were covered with small necrotic lesions, less than 1 mm in diameter. Often infected seedlings wilted with severe curling of leaves. Within a week after inoculation, most sicklepod plants wilted and died. In other *Cassia* spp. small necrotic lesions were evident on the youngest 1–2 leaves in 8 of the 12 accessions including cv. Wynn. Accessions could be placed into 3 groups according to the type of lesions: group 1 (resistant — accessions 93094, 78916 and 78355) had only a few small chlorotic lesions without any necrosis; group 2 (moderately resistant — accessions Q10057, 37234, 86172, 85836 and 92931) produced only a few necrotic spots, less than 0.5 mm, on each leaf; and group 3 (susceptible — accessions Q9862, 57503, 86178 and cv. Wynn) had necrotic lesions surrounded by a chlorotic area, up to 1.5 mm in diameter. In the repeat run, the youngest unexpanded leaf in many accessions was severely distorted and often blighted. Overall, the accessions maintained their groupings. In the susceptible group, the percentage of diseased seedlings of 3 of the 4 accessions (57503, 86178 and cv. Wynn) was higher than 80%, and in the resistant group, it was less than 35% for 2 of the 3 accessions (Table 1).

Influence of plant age on susceptibility of Cassia *to* A. cassiae

Symptoms appeared on inoculated sicklepod seedlings 2 days after inoculation, while the sicklepod (control) seedlings remained healthy.

Table 1. Source of seeds of the different species and accessions of *Cassia* used in the present study with the percentage of seedlings infected and the type of lesion caused by *Alternaria cassiae*.

Accession	Year & Source	Seedlings infected	Lesion type[2]
		(%)	
C. rotundifolia			
Q10057	1984, CSIRO[1]	55	mn
Q9862	1982, CSIRO	66	nc
93094	1982, CSIRO	67	sc
92931	1982, CSIRO	44	mn
86178	1982, CSIRO	89	nc
86172	1982, CSIRO	100	mn
85836	1982, CSIRO	44	mn
78916	1981, CSIRO	33	sc
78355	1982, CSIRO	22	sc
37234	1984, CSIRO	100	mn
34721 (cv. Wynn)	1986, Commercial	89	nc
C. pilosa			
57503	1979, CSIRO	89	nc
C. obtusifolia	1991, Gainesville	100	
LSD (P<0.05)		47.5	

[1]High quality early generation seed from the CSIRO germplasm collection.
[2] Lesion type: sc = small chlorotic lesions without necrosis
mn = medium <0.5 mm necrotic lesions
nc = necrotic lesions, surrounded by a chlorotic area.

Symptoms on all other *Cassia* accessions were first noted 3–4 days after inoculation, irrespective of their age. Symptoms of typical necrotic lesions with surrounding chlorotic regions were mainly restricted to the youngest 1–2 leaves. Small necrotic lesions were also noted on stems of older plants in accession Q9862. All seedlings were assessed for disease severity (percentage of infected leaves) 7 days after inoculation. In some accessions, seedlings which were sprayed with an autoclaved suspension of *A. cassiae*, also showed blighting of some young leaves. The blighted areas were largely brown in colour with some areas of affected leaves appearing chlorotic and bleached. These were distinguishable from the typical necrotic lesions caused by the live inoculum of the pathogen by the lack of curling of leaf tissue and the absence of fungal conidia. Several *Alternaria* spp. are known to produce toxins (Nishimura and Kohmoto 1983) and the chlorosis may have been caused by a toxin in the autoclaved suspension.

The 2.5-week old seedlings showed significantly ($P < 0.05$) higher severity (percentage of infected leaves) than the other 2 age-groups (Table 2). Among accessions, 37234 and Q9862 appeared more susceptible than the other 3, although this effect was not statistically significant. There was a significant ($P < 0.05$) accession x age interaction. The increased severity in the 2.5-week old seedlings is a reflection of the influence of leaf age on susceptibility. The 2.5-week old seedlings with fewer mature leaves showed a higher severity than seedlings in the other 2 age-groups.

Table 2. Influence of plant age of *Cassia rotundifolia* at inoculation on the percentage of leaves infected by *Alternaria cassiae*.

Accession	Age (weeks) 2.5	5	9
		(%)	
Q9862	42	4	9
93094	28	6	4
86178	6	8	9
37234	34	12	10
Wynn	6	15	5
Mean	23	9	7

LSD (P<0.05) between age groups = 8.3.

Pathogenicity of other fungi

C. dematium *f.sp.*crotalariae. Only mild symptoms were present on *Cassia* in the first run. However in the repeat run, symptoms appeared as water-soaked brown lesions with a chlorotic margin, about 1 mm in diameter, 6 days from inoculation. Lesions were more clearly visible on the lower surface of leaves. The lesions on stems and petioles were dark brown to tan in colour and somewhat larger in size. Surface-sterilised pieces of infected leaves and stems yielded *C. dematium* f.sp. *crotalariae* when plated on PDA. Details of disease incidence on the different accessions are given in Table 3. Incidence was high in accessions 37234 and 86178 with over 80% of seedlings infected, and moderate in accession 85836 with 44% of seedlings infected.

Table 3. Percentage of seedlings of *Cassia* spp. infected by *Colletotrichum dematium* f.sp. *crotalariae (Cdc)* and *Sclerotium rolfsii (Sr)*.

Species	Pathogen *Cdc*	*Sr*
Cassia rotundifolia		
Q10057	22	—[1]
Q9862	—	33
93094	—	—
92931	—	11
86178	89	11
86172	—	8
85836	44	—
78916	11	—
78355	—	—
37234	89	—
34721 (cv. Wynn)	—	22
C. pilosa		
57503	—	22
Control[2]		
Crotalaria spectabilis	100	
Arachis hypogea (cv. Florunner)		11

[1]No symptoms were detected.
[2]Represents the host from which the pathogen was originally isolated.

On crotalaria (control), the severity of symptoms was dependent on the inoculum concentration. The lower inoculum concentration used in the first inoculation, produced brown to tan lesions with chlorotic margin on leaves and petioles within 5-6 days of inoculation. Lesions were 1-8 mm wide with a circular or irregular outline. Petiole lesions caused some defoliation but no seedling mortality. In the repeat run, the majority of plants were dead within about 10 days of inoculation.

S. rolfsii. A typical collar-rot symptom with a water-soaked region around the base of infected stems appeared within a week from inoculation with *S. rolfsii*. Symptoms were first noted on accession Q9862. In 2 weeks these plants were completely dead and whitish mycelial growth with small white to light brown sclerotial initials was visible at the collar region. In *C. pilosa* accession 57503, one peanut plant and 4 accessions of *C. rotundifolia*, symptoms of collar rot were observed on only some of the branches while the other accessions were apparently free of any symptoms. In peanut, this partial wilting was the only symptom noted in one of the 3 pots.

The other 3 pathogens, *P. nigricans*, *Phomopsis* sp., and *M. phaseolina*, failed to produce any symptoms on the accessions of *Cassia* used in this study.

Discussion

Among the pathogens tested, *A. cassiae* was the most virulent, infecting all 12 accessions of *Cassia* used in this study. Small chlorotic lesions without any necrosis in accessions 93094, 78916 and 78355 suggest a high level of resistance; disease reaction of Q10057, 37234, 86172, 85836 and 92931 was that of moderate resistance, whereas Q9862, 57503, 86178 and cv. Wynn gave susceptible reactions. It is of interest to note that accessions 86172 and 85836 are currently on a pre-release status with the Queensland Herbage Plant Liaison Committee. Although both of these accessions were scored as moderately resistant based on the type of lesion, 44% and 100% of 85836 and 86172 seedlings, respectively, developed the disease. Accession 85836 was also moderately susceptible to *C. dematium* f.sp. *crotalariae*. In an earlier study, when the same 12 accessions were inoculated with the same commercial formulation of *A. cassiae*, accessions 78355, 37234, 57503 and Q9862 were considered resistant based on the lack of seedling mortality (Charudattan and DeValerio, unpublished data). Of these 4 accessions, both 57503 and Q9862 were considered susceptible based on lesion type in the present study. No *Cassia* seedling mortality was detected in any accession in the present study. It appears that the variable seedling density of 1 — 20 per pot and contamination by other pathogens such as a *Fusarium* sp. may have confounded results of the earlier study.

Of the other 5 fungi tested, *S. rolfsii* and *C. dematium* were pathogenic on some accessions. Both of these pathogens originated from a host other than a *Cassia* sp. Only *S. rolfsii* has been reported on *C. rotundifolia* in Australia. *Macrophomina phaseolina*, recorded on *C. absus* in

Australia (J.L. Alcorn, personal communication), was not pathogenic on the *Cassia* accessions used. The *Phomopsis* sp. used in this study is a weak pathogen of *Baccharis halimifolia* and this isolate failed to cause any visible symptoms on any accessions of *Cassia* tested. Virulent isolates of *Phomopsis*, including *P. cassiae*, need to be tested in the future. Of the fungi which Lenne (1990) considered as potentially important, *Corynespora casiicola, Pseudoperonospora cassiae* and *Ravenelia berkeleyii*, could not be tested due to our failure to locate live cultures, partly as a result of strict domestic quarantine regulations within the USA.

The fact that a formulation of *A. cassiae* designed to control sicklepod can seriously infect Wynn and other *Cassia* accessions of pastoral interest has serious implications for the use of this mycoherbicide in Australia. The other potential biocontrol agent of sicklepod, *P. nigricans*, which produces leaf mottling and mildew-like symptoms on sicklepod (Hofmeister and Charudattan 1987), did not infect any *Cassia* accessions tested.

This work highlights the potential damage that may be caused by *A. cassiae* if it enters Australia and emphasises the need for strict quarantine regulations. One of the main strengths of Wynn is its high level of seedling recruitment (Strickland *et al.* 1985). The fact that young seedlings are more severely affected by *A. cassiae* than adult plants, re-enforces this concern. A small collection of fungal isolates, including a *Pestalotiopsis* sp. and a *Phomopsis* sp., obtained from Wynn pastures in Australia is now available. These and other fungi pathogenic on other pasture plants in Australia, such as species of *Colletotrichum*, should be screened on a range of *Cassia* accessions alongside likely exotic pathogens.

References

Anning, P., Bishop, H.G., Lambert, G. & Sutherland, B. (1989) Sicklepod replaces overgrazed tropical pastures. *Queensland Agricultural Journal*, **115**, 188–192.

Bannon, J.S. (1988) CASST^{TM} herbicide (*Alternaria cassiae*): a case history of a mycoherbicide. *American Journal of Alternative Agriculture*, **3**, 73–76.

Charudattan, R. (1986) Biological control of showy crotalaria (*Crotalaria spectabilis*) with two fungal pathogens. *Abstract of the Weed Science Society of America*, **26**, 51.

Charudattan, R. (1991) The mycoherbicide approach with plant pathogens. In: TeBeest, D.O. (ed.) *Microbial Control of Weeds*. pp. 24–57. (Chapman & Hall: New York).

Hofmeister, F.M. and Charudattan, R. (1987) *Pseudocercospora nigricans*, a pathogen of sicklepod (*Cassia obtusifolia)* with biocontrol potential. *Plant Disease*, **71**, 44–46.

Irwin, H.S. and Barneby, R.C. (1982) The American Casiinae — A synoptical review of the Leguminosae Tribe Cassieae Subtribe Cassiinae in the New World. *Memoirs of the New York Botanical Garden*, **35**, 1–918.

Lenne, J.M. (1990) Diseases of *Cassia* species — a review. *Tropical Grasslands*, **24**, 311–324.

Nishimura, S. and Kohmoto, K. (1983) Host-specific toxins and chemical structures from *Alternaria* species. *Annual Review of Phytopathology*, **21**, 87–116.

Oram, R.N. (1990) *Register of Australian Herbage Plant Cultivars*. 3rd Edn. pp. 284. (CSIRO: Melbourne).

Strickland, R.W., Greenfield, R.G., Wilson, G.P.M. and Harvey, G.L. (1985) Morphological and agronomic attributes of *Cassia rotundifolia* Pers., *C. pilosa* L. and *C. trichopoda* Benth., potential forage legumes for northern Australia. *Australian Journal of Experimental Agriculture*, **25**, 100–108.

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