Host range of three isolates of *Alternaria zinniae*, a potential biocontrol agent for *Xanthium* spp.

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Summary

Three *Alternaria zinniae* isolates collected from *Xanthium occidentale* in eastern Australia were host range tested to assess their potential for development as bioherbicides for *Xanthium* spp. As well as five *Xanthium* spp. eleven other Asteraceae were screened together with representatives from Cucurbitaceae, Fabaceae, Malvaceae, Myrtaceae and Poaceae. With one exception all isolates were pathogenic to all *Xanthium* spp. *A. zinniae* had a wide host range within the dicotyledons including the Cucurbitaceae; grasses were generally immune. The relative resistance of cotton to *A. zinniae* may indicate some scope for development of a bioherbicide based on this fungus in cotton.

Introduction

Xanthium spp. are major summer annual weeds in Australia and the USA in crops and pasture. A potential mycoherbicide based on *Colletotrichum orbiculare* is being developed for *Xanthium spinosum* (Auld *et al.* 1990) but the other *Xanthium* spp., the Noogoora burr or cockleburr group are resistant to infection by this fungus.

Alternaria zinniae M.B. Ellis was isolated from *X. occidentale* Bertol. at Armidale NSW by M.A. Cholil in 1989 (IMI 336377 DAR 68373) and subsequently assessed as a potential mycoherbicide by Laidlaw (1990) and Nehl (1990) who performed limited host range studies with this isolate of *A. zinniae*. A degree of susceptibility was found in some cultivars of summer crops in which the fungus would be likely to be used if adopted as a biocontrol agent.

We collected two other isolates of the same species of fungus, one from fruits harvested from *X. occidentale* at Warren NSW in 1989 (IMI 352085 DAR 65024) and another from stem lesions on *X. occidentale* growing at Gympie Qld in 1990 (IMI 352084 DAR 68414) (Morin, Auld, Brown and Cholil unpublished data).

This paper reports a more extensive host range study of *A. zinniae* with the isolate from Armidale together with the two isolates we collected.

Materials and methods

The three isolates of *A. zinniae* from Armidale (A), Gympie (G) and Warren (W) were grown on potato dextrose agar (PDA) medium contained in petri dishes at 25°C in the dark for ten days before cutting into the mycelium and agar in checkboard fashion with a sterile scalpel and transferring to 20°C in near ultraviolet light to promote sporulation. After 7 days spores were collected by washing the plates with sterile water.

Plants to be tested (Table 1) were grown in steam sterilized soil-sand-sawdust mixture (1:3:1) in 10 cm diameter plastic pots in a temperature controlled glasshouse at 25°C. Seven plants of each species or cultivar were used: duplicate plants for each *A. zinniae* isolate and one as an untreated control.

Plants were inoculated with spore suspensions of approximately 4×10^4 spores ml^{-1} in sterile water applied through an atomizer and placed in a dew chamber (Percival) in the dark at 25°C for 18 to 24 hours as preliminary experiments had produced severe host infection with these conditions. Subsequently plants were moved back to the glasshouse.

Table 1. Host range test of *Alternaria zinniae* isolates

Family	Species	Leaf stage	*A. zinniae* isolate	Disease rating*	Isolation *A. zinniae* (+=yes,–=no)
Asteraceae	*Xanthium occidentale*	5	W	6	+
			G	6	+
			A	6	+
	X. italicum	4	W	5	+
			G	3	–
			A	5	+
	X. cavanillesii	4–5	W	6	+
			G	6	+
			A	5	+
	X. orientale	4–5	W	5	+
			G	6	+
			A	6	+
	X. spinosum	8–10	W	5	+
			G	5	+
			A	5	+
	Zinnia elegans	4	W	2	+
	cv. Sunnyface		G	0	
	zinnia		A	3	–
	Helianthus annuus	6–7	W	3	+
	cv. Hysun		G	3	+
	sunflower		A	3	+
	H. annuus	4	W	3	+
	cv. Suncross		G	3	+
	sunflower		A	2	+
	Cynara scolymus	4–5	W	3	+
	cv. Grand beurre		G	3	+
	globe artichoke		A	3	+
	Lactuca sativa	6	W	0	
	cv. Great lakes		G	1	–
	lettuce		A	1	–
	Carthamus tinctorius	5	W	3	+
	cv. Gila		G	1	+
	safflower		A	3	+
	Calendula officinalis	6–8	W	3	+
	cv. Flame beauty		G	3	+
	calendula		A	4	+
	Bellis perennis	4	W	3	+
	English daisy		G	3	+
			A	3	+

(continued)

Table 1. (continued). Host range test of *Alternaria zinniae* isolates

Family	Species	Leaf stage	*A. zinniae* isolate	Disease rating*	Isolation *A. zinniae* (+=yes,–=no)
	Carduus nutans	3	W	1	+
	nodding thistle		G	0	
			A	I	+
	C. tenuiflorus	4	W	0	
	winged slender thistle		G	0	
			A	3	–
	C. pycnocephalus	4	W	0	
	slender thistle		G	4	+
			A	3	+
Cucurbitaceae	*Citrullus lanatus* var. *caffer*	3–4	W	4	+
	cv. Country sweet		G	4	+
	watermelon		A	4	+
Fabaceae	*Pisum sativum*	5	W	0	
	cv. Yates telephone		G	5	+
	pea		A	3	+
	Glycine max	4	W	3	+
	cv. Forrest		G	4	+
	soybean		A	3	+
Malvaceae	*Gossypium hirsutum*	3	W	1	–
	cv. Siokra		G	1	+
	cotton		A	1	+
	G. hirsutum	3	W	1	–
	cv. Deltapine		G	1	+
	cotton		A	1	+
	G. hirsutum	3	W	1	–
	cv. CS189		G	1	+
	cotton		A	1	+
Myrtaceae	*Eucalyptus melliodora*	8–10	W	1	+
	yellow box		G	1	+
			A	1	+
Poaceae	*Triticum aestivum*	5	W	0	
	cv. Banks		G	0	
	wheat		A	0	
	Oryza sativa	4	W	0	
	cv. Amaroo		G	0	
	rice		A	0	
	Sorghum bicolor	5	W	0	
	cv. E57		G	1	+
	sorghum		A	0	

* **Disease rating scale:** 0 = no symptoms (immune); 1 = hypersensitive flecks on leaves only; 2 = hypersensitive flecks on leaves and stems; 3 = chlorosis on 1 or 2 leaves ± hypersensitive flecks; 4 = general chlorosis (at least 3 leaves) ± hypersensitive flecks; 5 = necrotic lesions on leaves and/or stems; 6 = large (> 3 mm diameter) necrotic lesions on leaves and stems.

Detailed observations were recorded from 3 weeks after inoculation when most symptoms appeared, and for the next 10 days.

Where disease symptoms were observed, isolations of the fungus were attempted from lesions or hypersensitive flecks. Small pieces of tissue were removed with a sterile scalpel, surface sterilized for 1 to 3 min in 2% sodium hypochlorite and placed on ¼ strength acidified PDA agar in petri dishes as above for a total of 14 days.

Results

With one exception, all the isolates were pathogenic to the five *Xanthium* species examined, producing necrotic lesions on leaves and stems. The isolate from Gympie, however, produced only limited chlorosis and hypersensitive flecks on *X. italicum* from which the fungus could not be reisolated (Table 1).

A. zinniae exhibited a wide host range both within and beyond the Asteraceae but the effects on other plants were less severe than on the *Xanthium* spp. Symptoms were graded from hypersensitive flecks on leaves (rating: 1); on leaves and stems (2); chlorosis on one or two leaves (± hypersensitive flecks) (3); more general chlorosis (at least three leaves affected) (± hypersensitive flecks) (4); necrotic lesions on leaves and/or stems (5); large necrotic lesions on leaves and stems (> 3 mm diameter) (6). No sporulation was observed.

Within the Asteraceae the crops sunflower and safflower displayed some susceptibility to the fungus. Outside the Asteraceae only the Gympie isolate produced significant necrosis and then only on one test species, pea, *Pisum sativum* cv. Yates telephone. However symptoms of hypersensitive leaf flecks and leaf chlorosis were widespread across plant families.

The monocotyledons, represented by three genera in the Poaceae were resistant with only hypersensitive leaf spots occurring on *Sorghum bicolor* cv. E57 inoculated with the Gympie isolate.

Discussion

The host ranges previously recorded for various isolates of *A. zinniae* include several genera from the Asteraceae (e.g., MacFarlane 1968, Neergaard 1979) and species within the following families: Malvaceae (Varshney 1986); Fabaceae (van der Heuval 1970); Cariaceae (Varshney 1986); Onagraceae, Balsaminiaceae, Gentianaceae, Solanaceae (MacFarlane 1968), Malvaceae (Laidlaw 1990) and Violaceae (Neergaard 1979). The results presented in this paper indicate that this list could now include the Cucurbitaceae.

Although these *A. zinniae* isolates produced limited symptoms on a wide range of plant species their virulence for *Xanthium* species is of interest. The one cultivar tested of the putative "original" host of these isolates *Zinnia elegans* was immune to the Gympie isolate and relatively resistant to the others. Other cultivars of *Z. elegans* may, however, be susceptible. The relative resistance of cotton to *A. zinniae*, especially the Warren isolate, is encouraging as *Xanthium* spp. are particularly troublesome in this crop. Nehl (1990) also recorded the comparative virulence of the Armidale isolate on *X. italicum* and *X. occidentale* when compared with its effects on cotton (CS #189), sunflower (Hysun 44) and *Zinnia elegans* cv. Pulcino.

The variability in the effects produced by these isolates indicates that further field collections of this fungus from *Xanthium* spp. may provide even more useful isolates. Andersen and Lindow (1985) found an isolate of an *Alternaria* sp. resembling *A. zinniae* with a host range restricted to *Z. elegans* and two weedy *Carduus* species.

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References

Andersen, G.L. and Lindow, S.E. (1984). Biological control of *Carduus pycnocephalus* with *Alternaria* sp. Proceedings of the Sixth International Symposium on the Biological Control of Weeds. ed Delfosse, E.S., Agriculture Canada: Vancouver. pp. 593-600.

Auld, B.A., Say, M.M., Ridings, H.I. and Andrews, J. (1990). Field applications of *Colletotrichum orbiculare* to control *Xanthium spinosum*. *Agriculture, Ecosystems and Environment* 32, 315-23.

Heuval, J. van der (1970). The influence of light and dark on attack of bean leaves by *Alternaria zinniae*. *Netherlands Journal of Plant Pathology*, 76, 192-5.

Laidlaw, J.C. (1990). The biological control of the Noogoora burr complex (*Xanthium occidentale* Bertol.) with *Alternaria zinniae* Pape. Diploma of Science in Agriculture Thesis, Department of Botany, University of New England, Armidale, N.S.W., Australia.

MacFarlane, H.H. (1968). Review of Applied Mycology. Plant Host-Pathogen Index to Volumes 1-40 (1922-1961). Commonwealth Mycological Institute: Kew.

Neergaard, P. (1979). Seed Pathology. Volume I. Macmillan Press: Copenhagen.

Nehl, D.B. (1990). The potential of *Alternaria zinniae* M.B. Ellis as a mycoherbicide to control *Xanthium* weeds in cotton. Bachelor of Science with Honours Thesis, Department of Botany, University of New England, Armidale, NSW Australia.

Varshney, J.L. (1986). Outbreaks and new records. India. *Alternaria zinniae*, a new record on seeds of papaya and okra. *FAO Plant Protection Bulletin*, 34, 216.