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An Australian isolate of *Alternaria crassa* shows potential as a mycoherbicide to control the weed *Datura stramonium*

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Abstract

An isolate of *Alternaria crassa* obtained from *Datura stramonium* was tested for its potential as a mycoherbicide in south-eastern Australia. Sporulation of *A. crassa* in culture was greatest under the following conditions: on V-8 juice agar, with a 12 h photoperiod of 'Warm White' light, with cultures arranged in a monolayer, at 25–30°C, at about 13 days after inoculation. Spores rather than mycelial fragments were required to kill seedlings of *D. stramonium*. Preliminary experiments indicated that a minimum dew period of 9 h and a spore concentration of 10^5/mL were required to kill cotyledonary-stage seedlings of *D. stramonium*. In host range studies in the glasshouse, *A. crassa* killed or severely reduced the growth of all *Datura* species tested and other solanaceous weeds. Also, *A. crassa* caused disease symptoms on some crops and ornamental species, including four tomato and two eggplant cultivars, *Petunia × hybrida* 'Mardi Gras' and a soybean cultivar. Microscopic examination of the infection process after inoculation showed that spores germinated, formed appressoria and penetrated equally well on different species and so resistance was intracellular. This local isolate of *A. crassa* is potentially an effective mycoherbicide for *D. stramonium* and other solanaceous weeds in south-eastern Australia.

Additional keywords: bioherbicide, biological control, thornapple

Introduction

Datura stramonium L. (thornapple) is a broadleaf weed in south-eastern Australia. It is a major weed of cotton, maize, wheat, sweet corn, sorghum, soybeans and vegetable crops and invades native bushland (Felton 1974; Lamp and Collet 1976; Auld and Medd 1987). It is a declared noxious weed in several states and, due to its toxicity to humans and animals, has a tolerance of two seeds/L as a contaminant in wheat (Anonymous 1992). Non-selective herbicides control growth effectively, but cannot be used in dicotyledonous crops or native vegetation. Control in solanaceous crops is difficult because few selective herbicides are available (Parsons and Cuthbertson 1992). It is thus a suitable target weed for biological control.

D. stramonium is also a problem in the mid-west of the United States of America, where it is called jimsonweed. Previous studies on the potential of the fungus *Alternaria crassa* (Sacc.) Rands to control *D. stramonium* showed that a United States isolate of *A. crassa* (NRRL #18136) killed seedlings and even larger plants when applied as a suspension of more than $1x10^6$ spores/mL (Boyette and Turfitt 1988). At least 9–10 h of dew were required by *A. crassa* to kill seedlings of *D. stramonium* and the host range was restricted to *D. stramonium* and two cultivars of tomato (Boyette 1986; Boyette and Turfitt 1988). However, importation of foreign organisms requires rigorous testing over many years. The aim of this study was therefore to determine if an Australian isolate of *A. crassa* showed potential for use as a mycoherbicide to control thornapple in Australia. The steps taken were to (i) isolate *A. crassa* from infected plants in Australia (ii) determine the most effective propagules of *A. crassa* (iii) optimise propagule production in artificial culture (iv) optimise infection of *D. stramonium* and (v) determine the host range of the selected isolate of *A. crassa*.

Methods

Isolation and preparation of inoculum of *A. crassa* A single-spore culture of *A. crassa* (VPRI 20404) was obtained from a diseased plant of *D. stramonium* at Orbost, Victoria, using standard procedures (Tuite 1969). This isolate was grown on V-8 juice agar (Miller 1955) (modified to 35% V-8 juice plus 1.5% agar), contained in plastic Petri dishes and incubated at 25°C in a monolayer under Osram Wattsaver 36 W 'Warm White' fluorescent tubes (12 h photoperiod), 25 cm from the light source (Walker 1982), unless stated otherwise. Spore suspensions of *A. crassa* for inoculation were prepared by flooding 6–day–old plates with 10 mL/plate of a solution of 0.1% Tween 20 in water and rubbing the surface with a glass rod to dislodge the spores. The concentration of each spore suspension was assessed with a haemocytometer. Mycelial inoculum was produced by growing the fungus on Czapek-Dox agar for 6 days, then removing the mycelial mat and grinding it with a mortar and pestle to produce viable mycelial fragments. The viability of the mycelial propagules was tested by inoculating 1 mL aliquots of serial dilutions (to 1 in 10^5) onto V-8 juice agar plates and observing the number of colonies after 12 days (conditions as above).

Seed germination and growth of *D. stramonium* Mature field-collected seeds of *D. stramonium* were germinated on sterile moist filter paper in glass Petri dishes in an alternating 12 h thermoperiod and photoperiod of 35°C dark/25°C light (Reisman-Berman *et al.* 1991). Seeds were sown in 2:1 Hortico potting mix:Barry & Orenshaw composite garden mixture in 8 cm pots. Unless stated otherwise, five plants per pot were grown and maintained in a glasshouse with natural daylight at 15–25°C and 30–60% relative humidity.

General inoculation procedures Unless stated otherwise, spore suspensions (10^6 spores/mL) were applied to plants to run-off (about 2 mL/plant) with a hand-held sprayer. Control plants were sprayed with 0.1% Tween 20. Dew was applied for 48 h in custom-made dew chambers made from glass aquaria (0.6 m × 0.3 m × 0.3 m), with polyethylene covers over the open sections and an ultrasonic room humidifier (Model KT-100A, Humidaire Pty. Ltd) providing humid air via rubber hoses.

Optimisation of spore production *A. crassa* was grown on V-8 juice agar in plastic Petri dishes and incubated at 25°C in a monolayer under Osram Wattsaver 36 W 'Warm White' fluorescent tubes (12 h photoperiod), 25 cm from the light source, unless stated otherwise. All agar plates were inoculated with a 2 mm^2 plug from an actively growing culture of *A. crassa* on V-8 juice agar, unless stated otherwise. Spores were collected as described previously except that sterile deionised water was used instead of 0.1% Tween 20. In each experiment, ten replicate plates or flasks of each treatment were used.

Medium *A. crassa* was inoculated centrally onto plates containing 16 different media (amounts of all ingredients are per litre). Cornmeal agar (Difco), cornmeal-plus agar (cornmeal agar powder 15.5 g/L, yeast extract powder 1 g/L, malt extract 1 g/L), cornmeal agar plus biotin (200 µg/L), cornmeal agar plus thiamine (200 µg/L), cornmeal agar plus biotin and thiamine (200 µg/L each), Czapek-Dox agar (Oxoid), malt-extract agar (Oxoid), half-strength malt-extract agar, oatmeal agar (oatmeal 25 g, agar 12 g; oatmeal boiled in 500 mL of water, strained, agar added and made up to 1 L), potato-dextrose agar (Oxoid), half-strength potato-dextrose agar, tomato agar (tomato juice 350 mL, agar 20 g), V-8 juice agar (V-8 juice 350 mL, agar 15 g), half-strength V-8 juice agar, V-8 juice/potato-dextrose agar (V-8 juice 350 mL), yeast/phosphate/soluble-starch agar (yeast extract 4 g, potassium phosphate 1 g, soluble starch 15 g, magnesium sulphate 0.5 g, agar 20 g). Ten µL of a spore suspension of *A. crassa* (1 x 10^6 spores/mL) was inoculated onto 20 mL V-8 juice agar plates, into 20 mL V-8 juice liquid in Petri dishes, or into 20 mL V-8 juice liquid (35%) in flasks (volume 100 mL) which were incubated either still or in a Gallenkamp Compenstat Cooled Orbital Incubator at 60 rpm. Solid cultures were assessed for sporulation after 10 days as described above. Liquid cultures were assessed for spore production by adding 10 mL of deionised water to the fungal mat on top of the liquid medium and rubbing the surface with a glass rod to dislodge the spores.

Photoperiod Cultures were exposed to photoperiods of 0 h (constant dark), 12 h or 24 h (constant light).

Culture position and light type Cultures were arranged in a monolayer or in vertical stacks of ten

plates and were incubated under Osram Wattsaver 36 W 'Warm White' or Osram L 36 W 77 'Fluora' light, each at approximately 27 μmoles/sec/m^2. The monolayer and base plates of the vertical stacks were 25 cm from the light source.

Temperature Cultures were incubated at 15, 20, 25, 30 or 35°C.

Culture age Cultures were assessed for sporulation at 24 h intervals from 3–14 days after inoculation.

Effect of inoculum Ten plants of *D. stramonium*, in two pots of five and at the three- to five-leaf stage of growth, were inoculated with 0.1% Tween 20 containing one of: (i) 10^5/mL spores of *A. crassa* (ii) 10^5/mL viable mycelial propagules of *A. crassa* or (iii) no fungal propagules. About 2 mL of each suspension was applied to run-off on each plant with a fine, hair paintbrush (Grade 1, Rubens 12). Each pot was covered with a plastic bag to create a humid micro-environment and simulate dew for 48 h. Plants were then placed in a glasshouse (conditions as above) and assessed for disease symptoms after 2, 4 and 7 days.

Optimisation of infection The following preliminary experiments were conducted to confirm trends observed in previous studies (Boyette and Turfitt 1988).

Inoculum concentration Ten seedlings of *D. stramonium*, in two pots of five and at the three- to five-leaf stage of growth, were each inoculated to run-off with spore suspensions adjusted to the following concentrations (spores/mL): 0 (control), 10^3, 10^4, 10^5, 10^6 or 10^7. Plants were covered with a plastic bag to create a humid micro-environment and simulate dew for 48 h. The numbers of diseased and dead plants were recorded after 5 and 14 days in the glasshouse.

Dew period and plant growth stage Plants at two growth stages (cotyledonary stage and three- to four-leaf stage) were assessed for their susceptibility to *A. crassa*. Ten seedlings of *D. stramonium* of each growth stage, in two pots of five, were each inoculated as described previously. The plants were incubated in dew chambers for periods of 0, 6, 7, 8, 9, 10, 11, 12, 18, 24 or 48 h. The numbers of diseased and dead plants were recorded after 3, 7 and 14 days in the glasshouse. Plants were inoculated on two dates due to constraints in glasshouse space (cotyledonary-stage plants in September 1993 and three- to four-leaf-stage plants in October 1993).

Host range Thirty-six plant species or cultivars from six plant families were tested (four *Datura* species, fourteen of the most common cultivars of solanaceous crop and ornamental plants, six native and weedy solanaceous species and seven other crop plants on which *D. stramonium* has been recorded). Two forms of *D. stramonium* were tested: two accessions of the green-stemmed, white-flowered form and one accession of the purple-stemmed, lavender-flowered form, all obtained from the Adelaide Herbarium. Selection of species was based on the centrifugal phylogenetic method (Wapshere 1974) and the previous work of Boyette (1986). The most commonly grown or readily available commercial cultivars were used. Ten plants of each species or cultivar at the two- to nine-leaf stage were inoculated to run-off with either spore suspension or 0.1% Tween 20 (control) and incubated in dew chambers for 48 h. Disease was assessed after 3, 7 and 21 days in the glasshouse. Macroscopic disease symptoms on plants were rated on a scale of 1 (immune) to 7 (highly susceptible), similar to that used by Bruzzese and Hasan (1986).

Leaf disks (two per plant at 1 cm diameter) were cut from inoculated leaves at 3 and 7 days after inoculation, cleared and stained with a chloral hydrate/aniline blue solution followed by concentrated chloral hydrate (Bruzzese and Hasan 1986) and mounted in polyvinyl alcohol (Omar *et al.* 1978). Spore germination, appressorium formation and leaf penetration were examined microscopically. Twenty spores on each leaf disk were counted and the numbers of each event were calculated as proportions and are presented as percentages.

Lesions on non-solanaceous crop plants were excised, surface sterilised and cultured on V-8 juice agar as described previously, to check that *A. crassa* was the causal organism.

General All experiments were repeated at least once with the exception of the 'optimisation of infection' studies which were not repeated due to agreement of the results with earlier findings (Boyette and Turfitt 1988). Results were analysed by analysis of variance and linear regression with the MINITAB statistical package. Proportions were probit transformed prior to analysis. In all figures, the vertical bar above and below the mean is the standard error.

Results

Optimisation of spore production Spore production was significantly different among media (Figure 1) and was greatest on V-8 juice agar. Sporulation was greater on solid than in liquid V-8 medium (Table 1). When cultures were exposed to a 12 h photoperiod, sporulation was almost doubled compared to when cultures were under constant light or dark (Table 1). Spore production was greatest when plates were arranged in a monolayer under 'Warm White' fluorescent light (Table 1). Sporulation was significantly reduced at temperatures less than 25°C or greater than 30°C (Table 1). Spore production increased with culture age, reaching a maximum at 13 days after inoculation (Figure 2). Repetition of the experiments gave results that followed the same trends.

Effect of inoculum Spores of *A. crassa* were the only inoculum to cause necrotic lesions within 2 days and to kill all seedlings of *D. stramonium* within 7 days. All seedlings inoculated with mycelia of *A. crassa* developed necrotic lesions within 4 days, but did not die. Seedlings inoculated with 0.1% Tween 20 developed no disease symptoms.

Optimisation of infection

Inoculum concentration Seedling death increased with increasing inoculum concentration (Table 2). A minimum concentration of 10^6 spores/mL was necessary for all plants to die after only 5 days, while a lower concentration of 10^5 spores/mL killed all plants after 14 days (Table 2). All inoculated plants were infected at all spore concentrations.

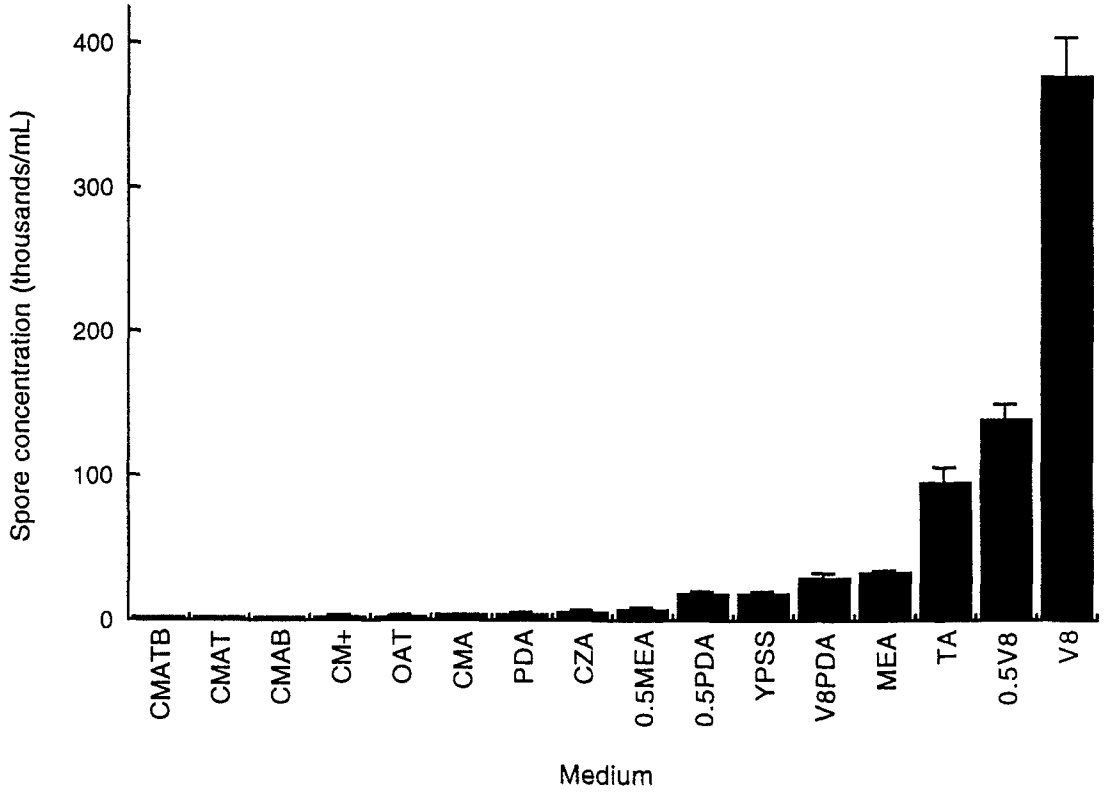

Figure 1 Effect of medium on spore production by *Alternaria crassa* in plastic Petri dishes at 25°C with a 12 h photoperiod in a monolayer under 'Warm White' light, after 6 days. Media were: CMA = cornmeal agar, CM+ = cornmeal-plus agar, CMAB = cornmeal agar plus biotin, CMAT = cornmeal agar plus thiamine, CMATB = cornmeal agar plus biotin and thiamine, CZA = Czapek-Dox agar, MEA = malt extract agar, 0.5MEA = half-strength malt-extract agar, OAT = oatmeal agar, PDA = potato-dextrose agar, 0.5PDA = half-strength potato-dextrose agar, TA = tomato agar, V8 = V-8 juice agar, 0.5V8 = half-strength V-8 juice agar, V8PDA = V-8 juice/potato-dextrose agar, YPSS = yeast/phosphate/soluble-starch agar. The vertical line above each bar represents the standard error.

Table 1 Effect of type of V-8 medium, photoperiod, culture position and light type, and temperature on spore production (in thousands/mL) by *Alternaria crassa* after 6 days[A]

Medium				
Shake flask	Still flask	Liquid plate	Solid plate	
148±12[B]	698±57	1164±82	1859±72	
Photoperiod (h)				
0	12	24		
638±32	1239±64	709±28		
Culture position and light type[C]				
Stack/F	Stack/WW	Mono/F	Mono/WW	
43±4	50±50	466±38	623±53	
Temperature (°C)				
15	20	25	30	35
391±46	125±11	1203±53	1361±74	1±0

[A]Variables (medium, photoperiod, culture position and light type, and temperature) were tested in separate experiments as shown in the table and described in the text.
[B]Spore concentration data are the mean ± standard error.
[C]Position: Stack = cultures in stacks of ten plates, Mono = cultures in monolayer; Light type: F = 'Fluora' light, WW = 'Warm White' light.

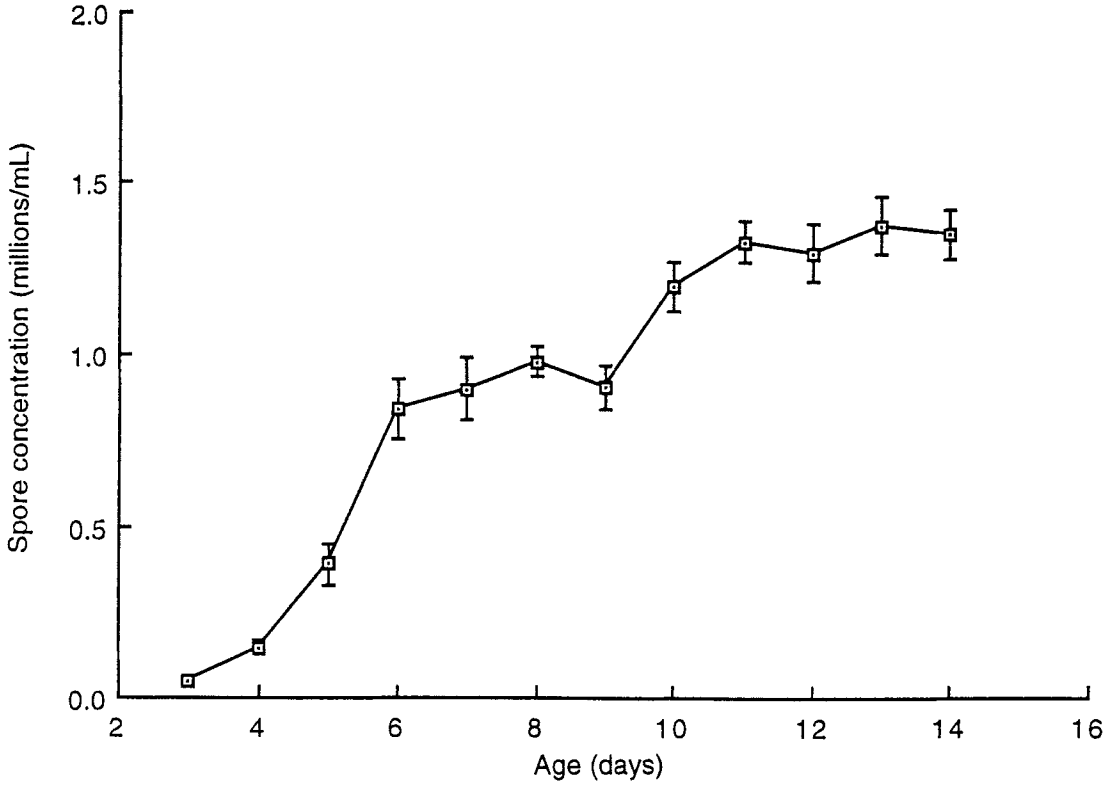

Figure 2 Effect of culture age on spore production by *Alternaria crassa* on V-8 juice agar in plastic Petri dishes at 25°C with a 12 h photoperiod in a monolayer under 'Warm White' light. The regression equation is: spore concentration = – 0.1296 + 0.1212 age (days). The vertical bar above and below each mean represents twice the standard error.

Dew period and plant growth stage *A. crassa* killed cotyledonary-stage *D. stramonium* over a broad range of dew periods (Table 3). Both infection and mortality increased with dew period and time after inoculation. Minimum dew periods of at least 6 h and 9 h were required for all cotyledonary-stage plants to be infected and killed, respectively, 14 days after inoculation.

Longer dew periods were required for *A. crassa* to kill older plants (three- to four-leaf stage) of *D. stramonium* (Table 3). *A. crassa* required a dew period of at least 7 h to infect plants. A dew period of at least 18 h was necessary for all inoculated plants to become infected and die, 14 days after inoculation.

Host range The three accessions of *D. stramonium* tested were highly susceptible to *A. crassa*, as most plants were dead within 7 days of inoculation (Table 4). All other *Datura* species were highly susceptible, with all plants dead after 7 days (Table 4).

The solanaceous crops varied in susceptibility to *A. crassa* (Table 4). Two capsicum cultivars (Clovis and Target) were immune, while two were resistant. Similarly, the tobacco cultivar was resistant. The tomato and eggplant cultivars were susceptible, with up to 20% mortality in cvv. Carmelo and Pirate (tomato) and 100% mortality in cv. Blackbell (eggplant). The potato cultivar developed limited lesions but did not die.

Of the solanaceous ornamentals, both cv. Mardi Gras petunia and *N. alata* developed limited lesions and one plant of *N. alata* died (Table 4). The native solanaceous plants *S. linearifolium*, *S. aviculare*, *S. aviculare* f. *albiflora* and *S. laciniatum* were highly susceptible. *Physalis* sp. (i) and *P. philadelphica* were susceptible to highly susceptible. Two other solanaceous species, *L. pennellii* and *W. avistata*, which are both introduced and of no economic importance, were highly susceptible.

Most non-solanaceous crop plants tested exhibited very slight reaction (resistant) or no reaction

Table 2 Effect of spore concentration of *Alternaria crassa* on infection and mortality of *Datura stramonium* 5 and 14 days after inoculation

Spore concentration (spores/mL)	% infection		% mortality	
	5 days	14 days	5 days	14 days
0	0	0	0	0
10^3	100	100	0	0
10^4	100	100	0	40
10^5	100	100	20	100
10^6	100	100	100	100
10^7	100	100	100	100

Table 3 Effect of dew period on infection and mortality of *Datura stramonium* at two growth stages, caused by *Alternaria crassa* at 3, 7 and 14 days after inoculation

Dew (h)	Cotyledonary stage						Three- to four-leaf stage					
	% infection			% mortality			% infection			% mortality		
	3	7	14	3	7	14	3	7	14	3	7	14
0	60	60	90	0	30	70	0	0	0	0	0	0
6	50	80	100	0	10	50	0	0	0	0	0	0
7	90	90	100	0	20	50	0	10	10	0	0	0
8	90	100	100	0	0	70	0	20	20	0	0	0
9	100	100	100	0	20	100	10	30	30	0	0	0
10	100	100	100	0	70	100	20	90	90	0	0	0
11	100	100	100	40	100	100	20	80	90	0	0	0
12	100	100	100	10	100	100	50	80	80	0	0	0
18	100	100	100	50	100	100	100	100	100	0	50	100
24	100	100	100	100	100	100	100	100	100	10	50	100
48	100	100	100	100	100	100	100	100	100	20	100	100

Table 4 Effect of inoculating plant species with *Alternaria crassa* on spore germination, appressorium formation, plant penetration and disease rating[A]

Family and species	Date[B]	Time (days)[C]	Disease rating	Mean % germ.[D]	Mean % app.[D]	Mean % pen.[D]
Solanaceae						
Datura stramonium	8/93	3	4(1)–6(9)[E]	100	38	22
(White flowered		7	6(1)–7(9)	–[F]	–	–
thornapple)[G]		21	7			
Datura stramonium	8/93	3	6	–	–	–
(M88) (White flowered		7	7	–	–	–
thornapple)[G]		21	7			
Datura stramonium	8/93	3	3(5)–6(5)	99	67	42
(Lavender flowered		7	5(3)–7(7)	–	–	–
thornapple)[G]		21	5(2)–7(8)			
Datura inoxia	8/93	3	6	–	–	–
(Downy thornapple)[G]		7	7	–	–	–
		21	7			
Datura metel f. *chlorantha*	8/93	3	6	–	–	–
(Double yellow flowered		7	7	–	–	–
thornapple)[G]		21	7			
Datura metel f. *fastuosa*	8/93	3	6	–	–	–
(Double flowered		7	7	–	–	–
thornapple)[G]		21	7			
Datura velutina[G]	8/93	3	6	100	76	42
		7	7	–	–	–
		21	7			
Capsicum annuum	8/93	3	1	95	54	25
cv. Clovis (Capsicum		7	1	85	5	5
or Bell pepper)		21	1			
Capsicum annuum	8/93	3	2	94	43	17
cv. Magnum (Capsicum		7	2	90	3	1
or Bell pepper)		21	2			
Capsicum annuum	8/93	3	2	95	47	14
cv. Sultan (Capsicum		7	2	94	7	3
or Bell pepper)		21	2			
Capsicum annuum	8/93	3	1	98	45	9
cv. Target (Capsicum		7	1	87	6	6
or Bell pepper)		21	1			
Lycopersicon esculentum	8/93	3	4	84	10	8
cv. Armada (Tomato)		7	5	69	16	15
		21	5			
Lycopersicon esculentum	8/93	3	4	87	10	5
cv. Buccaneer (Tomato)		7	5	77	14	12
		21	5			
Lycopersicon esculentum	8/93	3	5	82	16	11
cv. Carmelo (Tomato)		7	5	64	8	6
		21	5(9)–7(1)			
Lycopersicon esculentum	8/93	3	4.5	90	20	11
cv. Pirate (Tomato)		7	5	70	9	5
		21	5(8)–7(2)			

Table 4 continued

Lycopersicon pennellii	11/93	3	6	71	22	5
		7	6(2)–7(8)	–	–	–
		21	7			
Physalis philadelphica[G]	8/93	3	3	99	14	5
		7	4	85	3	3
		21	4(9)–5(1)			
Physalis sp. (i)[G]	11/93	3	4	53	17	13
		7	4(6)–7(4)	–	–	–
		21	4(2)–7(8)			
Solanum aviculare	8/93	3	5	96	66	32
(Kangaroo apple)[GH]		7	6	–	–	–
		21	6(9)–7(1)			
Solanum aviculare	8/93	3	6	97	26	10
f. *albiflora* (Kangaroo		7	7	–	–	–
apple)[GH]		21	5(4)–7(6)[I]			
Solanum laciniatum[GH]	8/93	3	6(7)–7(3)	100	6	1
		7	7	–	–	–
		21	7			
Solanum linearifolium[GH]	8/93	3	6(5)–7(5)	80	4	1
		7	7	–	–	–
		21	7			
Solanum melongena	8/93	3	5	79	16	6
cv. Blackbell		7	6(6)–7(4)	–	–	–
(Eggplant)		21	6(3)–7(7)			
Solanum melongena	11/93	3	4	52	6	3
cv. Blackmail		7	5	54	9	8
(Eggplant)		21	5			
Solanum tuberosum	8/93	3	2	97	52	16
cv. Sebago C		7	3	–	–	–
(Potato)		21	4			
Withania avistata	8/93	3	5	99	70	18
		7	6(5)–7(5)	–	–	–
		21	6(1)–7(9)			
Nicotiana alata	8/93	3	2	78	22	8
		7	3	66	9	3
		21	3(9)–7(1)			
Nicotiana tabacum	8/93	3	1	89	27	8
cv. Samsun		7	2	80	12	9
(Tobacco)		21	2			
Petunia x hybrida	11/93	3	2	73	31	17
cv. Mardi Gras		7	4	76	19	13
		21	4			
Vitaceae						
Vitis vinifera cv. H5	12/93	3	1	34	32	
(Grapevine)		7	1	27	1	1
		21	1			
Fabaceae						
Glycine max cv. Davis	8/93	3	4	97	8	1
(Soybean)		7	4	89	2	0
		21	4			

Table 4 continued

Malvaceae						
Gossypium hirsutum	11/93	3	1	78	23	13
cv. L22 (Cotton)		7	1	63	6	5
		21	1			
Cannabaceae						
Humulus lupulus	5/94	3	1	64	13	7
cv. T6 (Hops)		7	1	63	12	4
		21	1			
Poaceae						
Sorghum bicolor	8/93	3	2	96	60	49
(cultivar unknown)		7	2	79	1	0
(Forage sorghum)		21	2			
Triticum aestivum	8/93	3	1	76	7	0
(cultivar unknown)		7	1	72	1	1
(Wheat)		21	1			
Zea mays	8/93	3	1	86	61	27
(cultivar unknown)		7	1	98	6	0
(Sweet corn)		21	1			
LSD ($P = 0.05$)				16	16	12

[A]Disease rating scale: 1 = Immune, no lesions on leaves or stems; 2 = Resistant, production of anthocyanin pigments and/or very small (up to 1 mm diameter), limited lesions and/or curling of leaves; 3 = Susceptible, small lesions coalescing to produce large lesions (up to 5 mm diameter) and/or some wrinkling/puckering of leaves; 4 = Susceptible, large lesions and/or wilting of plants to a slight degree, limited (<25% of all leaves) abscission of infected leaves, limited (<25% of all leaves) leaf death; 5 = Susceptible, large lesions, moderate wilting of plant and moderate (<50% of all leaves) abscission of infected leaves, new growth moderately to very healthy; 6 = Highly susceptible, all leaves with significant lesions (>50% of the whole leaf), greater wilting of plant and greater abscission of leaves, no new growth evident and plant with little chance of recovery; 7 = Highly susceptible, plant dead (i.e. no green living material present).
[B]Date of inoculation.
[C]Time after inoculation.
[D]% germ. = percentage of spores germinating, % app. = percentage of spores forming appressoria, % pen. = percentage of appressoria penetrating plant tissue.
[E]Numbers in brackets in the disease rating column indicate the number of plants out of ten that scored that rating, if there were differences among plants.
[F]– Plants could not be sampled due to severe disease symptoms (dehydration of leaves).
[G]Weed species.
[H]Native species.
[I]At 7 days, all plants appeared dead (no green living material), but by 21 days, four plants produced new apical growth.

(immune) to *A. crassa* (Table 4). Soybean was the only non-solanaceous plant to develop lesions due to *A. crassa*; these were limited and the plants did not die. Repetition of the host range studies gave results that followed the same trends.

Spores of *A. crassa* germinated, formed appressoria and penetrated all plant surfaces (Table 4). Spore germination, appressorium formation and plant penetration varied significantly among plant species, although these data could not be obtained from some highly susceptible species because of the extent of leaf damage (Table 4). Some other *Datura* species (e.g. lavender-flowered *D. stramonium* and *D. velutina*) had greater percentages of appressorium formation and plant penetration than the common white-flowered *D. stramonium*, whereas some solanaceous and non-solanaceous species (e.g. *V. vinifera*) had less (Table 4).

At 3 days, spore germination was significantly correlated with appressorium formation ($r = 0.563$, $P<0.001$) and plant penetration ($r = 0.419$, $P<0.01$), but none of these was significantly correlated with disease rating. At 7 days, plant penetration was significantly correlated with both appressorium formation ($r = 0.832$, $P<0.001$) and disease rating ($r = 0.390$, $P<0.05$). In the species in which spore germination, appressorium formation and plant penetration could be assessed at both 3 and 7 days, there was either no change or a significant decrease in the percentages of all events with time (Table 4).

Discussion

The results of the spore production experiments suggest that maximum sporulation occurs on V-8 juice agar exposed to a 12 h photoperiod of 'Warm White' light, with cultures arranged in a monolayer at 25–30°C at about 13 days after inoculation. Spores of *A. crassa* were readily produced *in vitro* under standard cultural and environmental conditions, similar to those used previously by Walker (1982) and Boyette (1986). However, for commercial use, other more efficient methods of mass production are required. Reduced spore production in liquid culture indicates that further work is needed on submerged culture conditions such as aeration and agitation. Alternatively, the combined use of liquid and solid culture may be a feasible option, for example by producing mycelia in submerged culture followed by sporulation in large trays (Boyette *et al.* 1991*a*).

Spores of *A. crassa* were more efficient than mycelium in infecting and killing *D. stramonium*, as found also by Boyette *et al.* (1991*b*) with the NRRL#18136 strain of this fungus. The preliminary experiments indicated an increase in infection and mortality with increasing inoculum concentration and dew period. Also, longer dew periods were required for infection and mortality of three- to four-leaf-stage plants of *D. stramonium* compared to cotyledonary-stage plants. These results were comparable with those obtained by Boyette and Turfitt (1988) with the NRRL #18136 isolate of *A. crassa*. The dew-period requirement is a limiting factor in the development of the fungus as a commercial product but this may be overcome with formulation technology and product label directions specifying the most suitable time of application.

The rapid death of the three accessions of *D. stramonium*, all species of *Datura* and six other solanaceous weed species (four native *Solanum* and two introduced *Physalis* spp.) suggested that *A. crassa* may be an effective mycoherbicide for several solanaceous weeds. The potential of *A. crassa* to kill a number of weed species increases the commercial viability of the mycoherbicide by creating a wider market for the product (Bowers 1982).

The susceptibility of the crop and ornamental species tested, except soybean, should not pose a problem to the further development of *A. crassa* as a mycoherbicide. This is because *D. stramonium* has not been recorded on tomato, eggplant or potato crops and is not a problem in nurseries amongst petunia or ornamental *Nicotiana*. Also, the site-directed application of *A. crassa* means that non-target plants should not be directly exposed to inoculum (Leonard 1982). For example, the mycoherbicide DeVine was successfully marketed with label directions outlining the potential problem of host susceptibility (Kenney 1986). Differences in the reported reaction of some of the solanaceous plant cultivars to *A. crassa* in this study and that of Boyette (1986) indicate that cultivars of one species can respond differently to *A. crassa*. Therefore it is desirable to test all the most commonly grown cultivars of solanaceous crop plants in commercial use. However, such differences could be due to variation in the use of terms describing host reaction between studies or variation within isolates (Watson 1985). All of the non-solanaceous crop plants, except soybean, were immune or resistant to *A. crassa*. These results were expected because the plants are not closely related to *D. stramonium* (Wapshere 1974) and the results are similar to those obtained by Boyette (1986), except for soybean. As biocontrol agents of weeds commonly attack a greater range of hosts under controlled conditions than they do in the field (Watson 1985), the testing of soybean cultivars in common use in the field is highly desirable.

As some of the susceptible weed species are native, it would be desirable to test other native solanaceous species, from a conservation standpoint. Future host range testing should also include *Brugmansia* species, as they are common garden ornamentals (Parsons and Cuthbertson 1992). Due to the site-directed application of *A. crassa* as a mycoherbicide, any susceptibility of these species should not pose a major problem.

Microscopic observation of spore germination, appressorium formation and penetration gave no indication that *A. crassa* would cause disease in a given plant, as the frequency of events did not

correlate significantly with disease rating (except penetration at 7 days). This was because whether the plants were immune, resistant or susceptible, the appressoria still penetrated the plant. It was only after penetration that the plant was able to overcome the effects of the pathogen (immune or resistant) or not (susceptible) (Watson 1985).

The percentage of spores that germinated was greater than that which formed appressoria, which was greater again than the percentage that penetrated the plant. This was expected, as these are sequential events in the infection process (Ellingboe 1968). The lack of change or significant decrease in the percentages of all microscopic events (spore germination, appressorium formation and plant penetration) observed from 3 days to 7 days was unexpected. It was assumed that, being sequential events (Ellingboe 1968), the percentage of each would increase with time. The spores may have germinated, formed appressoria and penetrated the plant, but the spore body and associated infection structures may have broken down and evidence for these events was lost, leading to a decrease in some but not all resistant or immune species.

This local isolate of *A. crassa* has shown potential as a mycoherbicide for the control of *D. stramonium* and other solanaceous weeds in glasshouse studies. Advances in fermentation and formulation technologies should help to overcome the production and environmental constraints of this mycoherbicide.

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