Incidence and Pathogenicity of *Colletotrichum orbiculare* and a *Phomopsis* sp. on *Xanthium* spp.

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ABSTRACT

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Two diseases of *Xanthium spinosum*, caused by *Colletotrichum orbiculare* and an unidentified *Phomopsis* sp., were widely distributed in New South Wales. *Phomopsis* sp. occurred less frequently on *X. occidentale, X. italicum*, and *X. orientale* than on *X. spinosum*. Isolates of *C. orbiculare* were highly virulent on *X. spinosum* but were only weakly virulent to other *Xanthium* spp. in NSW and in the United States. Isolates of *Phomopsis* sp. were pathogenic to all *Xanthium* spp. However, isolates of *Phomopsis* sp. from *X. spinosum, X. occidentale*, and *X. italicum* were more virulent to *X. spinosum* than to the other *Xanthium* spp.

Additional keywords: biological control, *Macrophomina phaseolina*, mycoherbicide, *Sclerotinia sclerotiorum, Verticillium dahliae, Xanthium strumarium*

Xanthium spp. are important weeds worldwide (9). In Australia, *X. spinosum* L. (Bathurst burr, spiny clotbur) is one of the most widespread and important weeds, causing losses in grazing land and some cropping areas (8). *X. occidentale* Bertol. (Noogoora burr) and *X. italicum* Mor. (hunter burr) are considered major weeds of sheep-grazing land because of their competition with pasture grasses and contamination of wool with burrs, which causes a reduction in wool prices (10). In eastern Australia, these weeds also cause problems in cotton and soybeans where 2,4-D cannot be used (11). *Xanthium* spp. in the *X. strumarium* L. complex (common cockleburr) are among the most serious weeds of cotton and soybean crops in the southern United States (2,18). In the United States, the weeds are usually recognized as one taxon, *X. strumarium* sensu lato. *X. spinosum* is not a widespread weed problem in annual crops in the United States (9) and is not present in Arkansas.

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Butler (3) reported the widespread distribution of an anthracnose of *X. spinosum* caused by a species of *Colletotrichum* in New South Wales (NSW). He showed that artificial release of the pathogen largely eliminated the necessity for chemical control during seasons conducive to disease development. However, this approach was not pursued.

Much interest has been generated in the development of mycoherbicides as alternatives to chemicals for weed control following the successful use of *C. gloeosporioides* (Penz.) Penz. & Sacc. in Penz. f. sp. *aeschynomene* to control *Aeschynomene virginica* L. in rice and soybeans in the southern United States (20) and *Phytophthora palmivora* (Butler) Butler for control of *Morrenia odorata* Lindl. in Florida citrus groves (14). This study was initiated to determine the incidence of fungi causing diseases of *Xanthium* spp. in NSW and to test the pathogenicity of isolates to determine their potential for development as mycoherbicides in Australia and the United States.

MATERIALS AND METHODS

Collection and isolation. During the late summer of 1984, *Xanthium* spp. showing symptoms of dieback and stem lesions were collected from burr-infested areas in NSW. Between one and 18 plants were collected from each of 52 sites in the eastern and southwestern half of the state. Stem tissue pieces of *X. spinosum* with lesions were placed in a humid chamber for 12 hr. Conidia of *Colletotrichum* produced in acervuli on lesions were transferred with a sterile needle to acidified potato-dextrose agar (PDA). Stem pieces of the remaining specimens of *Xanthium* that did not produce fungal fructifications were surface sterilized in one percent sodium hypochlorite for 2 min. Small pieces of tissue were excised, transferred to petri dishes containing acidified PDA, and incubated at 25 C.

Fungi growing from the tissue pieces were subcultured onto PDA after 2 days. All fungal cultures and specimens were deposited in the herbarium, NSW Agriculture & Fisheries, Rydalmere, NSW (Herb DAR).

Pathogenicity and virulence tests. The pathogenicity of isolates of a *Colletotrichum* sp., subsequently identified as *C. orbiculare* (Berk. & Mont.) Arx (Walker, Nikandrow, and Millar, *unpublished*) was tested on *Xanthium* spp. from eastern Australia and from the southern United States. In NSW, 17 isolates of *C. orbiculare*, representative of the major collection sites, were tested on *X. spinosum* in two experiments. Test cultures were incubated on plates containing PDA in the dark at 25 C for 7–10 days. Conidia were washed from the plates with sterile distilled water, centrifuged at low speed, resuspended in sterile distilled water, and standardized to 1×10^6 conidia per milliliter with a haemocytometer. Six-week-old plants of *X. spinosum*, growing in potting soil in 10-cm pots, were sprayed to runoff with the conidial suspensions from each isolate. Plants were placed in a darkened dew chamber at 25 C for 24 hr after inoculation and then placed in a growth chamber at 25 C with a 12-hr photoperiod at a light intensity of 500 $\mu E^{-2} s^{-1}$. Each single-plant treatment was replicated 10 times in a completely randomized design.

Disease development was recorded daily after inoculation (t_1) using a rating system from 1 (no symptoms) to 6 (death) until half of the plants infected by the most virulent isolate were dead (t_2) (12). In the comparison, the arithmetic mean daily rating for each isolate for the period t_2-t_1 was compared. Isolate 1 was common to both experiments.

Additionally, stems of 6-wk-old seedlings of *X. occidentale* and *X. orientale* Bl., growing in potting soil in 10-cm pots, were injected with 0.5 ml of a water suspension of 1×10^6 conidia per milliliter of each of the 17 isolates and maintained under the same conditions as used previously. Each treatment was replicated 10 times in a completely randomized design. Disease was recorded as positive when a necrotic lesion developed on the stem beyond the point of inoculation, and the fungus was reisolated.

In Arkansas, burrs of *X. italicum* (= *X. strumarium*) and *X. occidentale* (= *X. strumarium*) were collected from common Arkansas biotypes grown at the University of Arkansas Agricultural Experiment Station Farm, Fayetteville, and stored at 10 C until used. Test isolates of *C. orbiculare* were incubated on plates containing PDA under cool-white fluorescent lights with a 12-hr photoperiod at ambient air temperature. After 4 days, conidia were washed from the plates with distilled water, vacuum filtered through Whatman No. 4 filter paper, centrifuged at low speed, and resuspended in distilled water. The conidial suspensions were standardized at 2×10^6 conidia per milliliter with a haemocytometer. Conidial suspensions were sprayed to runoff on 4-wk-old plants of *X. italicum* and *X. occidentale* growing in potting soil in 10-cm pots. The inoculated plants were immediately placed in a dew chamber at 28 C for 24 hr in the dark and then transferred to a growth chamber at 28 C with a 16-hr photoperiod, at a light intensity of 330 $\mu E\ m^{-2}\ s^{-1}$. Disease ratings were made at weekly intervals for 2 wk on a scale of 0 (no disease) to 9 (dead). Each single plant treatment was replicated six times in a completely randomized design.

The pathogenicity of isolates of the *Phomopsis* sp., obtained from each of the *Xanthium* spp., was tested on ecotypes of *X. spinosum, X. occidentale, X. italicum*, and *X. orientale* from NSW. The stems of 6-wk-old plants of each species were wound-inoculated with mycelial plugs taken from the outside edge of actively growing cultures on PDA. The wounds were sealed with petroleum jelly, and the plants were placed in the greenhouse at temperatures between 20 and 25 C. The treatments were replicated nine times, although four of the treatments had only seven replicates. Plants were rated weekly for 6 wk for disease using the scale: 0, no disease; 1, lesion up to 2 cm beyond the point of inoculation; 2, lesion up to 4 cm beyond the point of inoculation; 4, lesion up to 8 cm beyond the point of inoculation; and 5, lesion up to 10 cm beyond the point of inoculation. A sixth category recorded the number of dead plants. For isolate groups from each source/test *Xanthium* host combination, disease severity ratings (0–5 scale) were analyzed as a normally distributed variate using an unbalanced analysis of variance. A generalized linear model, assuming a binomial distribution, was used to analyze the proportion of dead plants for the isolate group.

RESULTS

Symptomatology. In the field, anthracnose of *X. spinosum* caused by *C. orbiculare* was found most frequently on mature plants. Initially, small, black, lenticular lesions appeared on the stems, frequently occurring at nodes, where spines are attached to the stem. Lesions became sunken and black, with an irregular outline but with a definite margin. Under humid conditions, numerous salmon-colored spore masses developed in acervuli in the centre of the lesions. Lesions eventually girdled the stem, killing the tissue above the lesion. Lesions that occurred near ground level usually killed the plant. Less frequently, blighting of seedlings occurred as a result of infection of the hypocotyl.

Disease of *X. spinosum* caused by *Phomopsis* sp. was only observed in the field on mature plants that had set seed. Lesions initially developed on the surface of the stem as tan and dark brown mottled areas, lenticular in shape, with a diffuse margin. Lesions often developed at axils on the main branch, eventually girdling the stem. Branches usually died above the lesion. Margins of older lesions became more sharply defined, remaining light brown at the advancing edge and becoming dark brown in the center. Lesions became sunken and deeply furrowed as the dead tissue dried. Pycnidia were detected only rarely on the surface of such tissue.

The *Phomopsis* sp. was also consistently associated with lesions on mature plants of *X. occidentale, X. italicum*, and *X. orientale*. Light brown lesions with a diffuse margin usually occurred at nodes on the main stem and appeared to originate from the base of petioles of infected leaves. Lesions later became dark brown to black, sunken with a definite margin, and, with age, developed longitudinal wrinkles. Eventually, transverse cracks developed on the surface in lesions that became grayish white. Sometimes black pycnidia were embedded in the epidermis within such lesions. Lesions that girdled the stem resulted in the death of tissue above the lesion. However, lesions that developed down only one side of the plant killed leaves and branches only on the diseased side. In most instances, disease of these *Xanthium* spp. was relatively minor and was restricted to lesions on only some branches. At one particularly wet site, however, many young plants of *X. italicum* were killed by this pathogen.

Collection and isolation. Two hundred and thirty-six plants with disease symptoms were collected from 52 sites

Table 1. Isolations of plant pathogenic fungi from species of *Xanthium* in New South Wales, Australia

	Isolation frequency[z]			
Host	*Phomopsis*	*Colletotrichum orbiculare*	*Sclerotinia sclerotiorum*	Other
X. spinosum	44	84	9	12
X. occidentale	12	0	0	3
X. italicum	8	1	0	1
X. orientale	1	0	0	3

[z] Number of plants from which the fungus was isolated.

in NSW, and pathogenic fungi were isolated from 151 plants at 38 of the sites. Isolates obtained from *X. spinosum* (Table 1) were primarily *C. orbiculare* (84 plants) and a *Phomopsis* sp. (44 plants). *Sclerotinia sclerotiorum* (Lib.) de Bary was isolated from 9 plants but occurred only at three sites. *C. orbiculare* was isolated only on one occasion from a *Xanthium* sp. (*X. italicum*) other than *X. spinosum*. A different species of *Colletotrichum* was isolated on one occasion from stem and burr lesions of a plant of *X. occidentale*. A *Phomopsis* sp. was isolated from 12 plants of *X. occidentale* and eight plants of *X. italicum*. Only one isolate of the *Phomopsis* sp. was obtained from *X. orientale*. Other fungi recorded infrequently included *Macrophomina phaseolina* (Tassi) Goidanich from *X. spinosum, X. occidentale*, and *X. orientale* and *Verticillium dahliae* Klebahn from *X. spinosum* and *X. italicum*.

On *X. spinosum, Phomopsis* was widely distributed, occurring at 33% of the collection sites. *C. orbiculare* was collected at 31% of the sites sampled. Both pathogens were collected from the same location at only 8% of the sites.

Anthracnose caused by *C. orbiculare* was restricted to the wetter parts of the northeastern NSW, while the disease caused by *Phomopsis* extended into the drier, western portions of the state.

Pathogenicity and virulence tests. A comparison of 17 isolates of *C. orbiculare* on *X. spinosum* (Table 2) in two separate experiments showed the isolates to vary in virulence on *X. spinosum*. Most of the isolates caused a significant amount of plant damage to *X. spinosum* following foliar inoculation.

C. orbiculare caused only limited disease when wound-inoculated to *X. occidentale* and *X. orientale*. Of the 17 isolates tested on *Xanthium*, only six were pathogenic to *X. occidentale* or *X. orientale*. Lesions spread only a few millimeters beyond the point of inoculation. Initial tests with conidial suspensions of a range of isolates sprayed onto foliage of 6-wk-old plants of these species failed to produce symptoms; however, subsequent foliar inoculations with isolates 13/5 and 22/8 produced minor local leaf lesions on *X. italicum* and *X. occidentale*, whereas *X. orientale* was immune (Nikandrow and Miller, *unpublished*). No leaf lesions were noted on these species in the field.

C. orbiculare was only weakly virulent when spray-inoculated on an ecotype of *X. italicum* (= *X. strumarium*) from the southern United States. Of the 51 isolates tested, disease severity ranged from 0 to 1.2 out of a possible 9 (plant death). In most instances, disease was limited to minor leaf spotting, which resulted in the death of inoculated leaves in only nine out of 51 isolates. An ecotype of *X. occidentale* (= *X. strumarium*) was immune to six isolates of the fungus and not tested further. Subsequent tests (Weidemann, *unpublished*) with *X. strumarium* ecotypes obtained from 21 locations in 13 states demonstrated high levels of resistance or immunity to *C. orbiculare*. Collections of *X. spinosum* were not tested.

All isolates of the *Phomopsis* sp. from *X. spinosum, X. occidentale*, and *X. italicum* were pathogenic when wound-inoculated to the four species of *Xanthium* (Table 3), and the fungus was reisolated in all cases from necrotic tissue away from the original point of inoculation. Analysis of variance showed a significant interaction ($P < 0.001$) between the source host of the isolates and disease severity on the test host. Isolates of the *Phomopsis* sp., irrespective of origin, were more virulent ($P < 0.05$) on *X. spinosum* than on the other species of *Xanthium*. Isolates from *X. occidentale* and *X. italicum* were least virulent ($P < 0.05$) on *X. italicum*. The most virulent isolates ($P < 0.05$) on *X. spinosum, X. occidentale*, and *X. orientale* were those from *X. italicum*. The generalized linear model showed that only the test host *Xanthium* sp. had a significant ($P < 0.001$) main effect on the proportion of dead plants following inoculation with the *Phomopsis* sp. The *Phomopsis* sp., irrespective of source, killed a higher proportion of *X. spinosum* ($P < 0.05$) than other *Xanthium* spp. tested. An isolate from *X. orientale* was weakly virulent on all *Xanthium* sp. tested. Foliar inoculation with conidial suspensions of the *Phomopsis* sp. was attempted on *X. spinosum* and *X. occidentale*, but local necrotic lesions on stems of these species were produced only rarely up to 15 wk after inoculation. Preliminary examination of cultures and spore measurements suggests that disease on all four species of *Xanthium* is caused by the same species of *Phomopsis. Diaporthe arctii* (Lasch) Nitschke was found on debris of *X. spinosum* from which a *Phomopsis* sp. was previously isolated, but a cultural connection between the two fungi has not been made.

DISCUSSION

C. orbiculare and a *Phomopsis* sp. were frequently associated with diseased plants of *X. spinosum* in NSW. Pathogenicity tests showed that most isolates of *C. orbiculare* were highly virulent to *X. spinosum* but showed only limited virulence to other *Xanthium* spp., both in NSW and in the United States. *C. orbiculare* may have potential as a mycoherbicide for *X. spinosum* but is not sufficiently virulent to other species of *Xanthium* tested.

Anthracnose has been previously reported on *X. spinosum*. Veitch (21) reported a disease of *X. spinosum* similar to that reported in this study caused by a species of *Colletotrichum* in Queens-

Table 2. Disease severity caused by *Colletotrichum orbiculare* spray inoculated onto 6-wk-old plants of *Xanthium spinosum*

Experiment 1		Experiment 2	
Isolate	Disease severity[y,z]	Isolate	Disease severity
1	4.5 a	12/2	4.6 a
5/11	4.3 ab	13/5	4.6 a
1/3	3.9 b	22/8	4.3 a
4/15	3.9 b	14/6	3.8 b
10/5	2.6 c	24/2	3.6 b
10/12	2.1 cd	1	3.4 b
11/1	1.7 d	22/9	3.4 b
		3/3	3.3 b
		2/2	2.2 c
		15/3	1.0 c
		19/1	1.0 c

[y] Mean disease severity rating of 10 replicates recorded daily from inoculation (t_1) until death of half of the replicates in the most effective treatment (t_2). Rating: 1 = no disease, 2 = basal lesion (below cotyledonary node) only, 3 = leaf lesions only, 4 = leaf and stem lesions, 5 = basal lesion and leaf and stem lesions, and 6 = plant death.

[z] Means followed by the same letter are not significantly different ($P < 0.05$) within experiments using Duncan's multiple range test.

Table 3. Disease severity caused by *Phomopsis* sp. wound inoculated on species of *Xanthium*

Isolate source	Number (n)	Disease severity on *Xanthium*[v]			
		X. spinosum	*X. occidentale*	*X. italicum*	*X. orientale*
X. spinosum	35	2.4a[w] B[x]	1.8b B	1.7b A	1.7b B
X. occidentale	7	2.5a B	1.7b B	1.2c B	1.6b B
X. italicum	9	3.6a A	2.4b A	1.2c B	2.5b A
All isolates	51	0.20[y]a[z]	0.12b	0.06c	0.11b

[v] Disease severity rating: 1 = lesion up to 2 cm beyond point of inoculation, 2 = lesion up to 4 cm beyond point of inoculation, 3 = lesion up to 6 cm beyond point of inoculation, 4 = lesion up to 8 cm beyond point of inoculation, 5 = lesion up to 10 cm beyond point of inoculation. Mean of n isolates (7–9 replicates per isolate).

[w] Means in the same source host group followed by the same lower case letter do not differ significantly ($P < 0.05$) using Duncan's multiple range test.

[x] Means in the same test host group followed by the same upper case letter do not differ significantly ($P < 0.05$) using Duncan's multiple range test.

[y] Proportion of dead plants. Mean of n isolates.

[z] Means followed by the same letter do not differ significantly ($P < 0.05$) using Duncan's multiple range test.

land. Butler (3) also noted a severe outbreak of anthracnose of *X. spinosum* in NSW and attributed the disease to *C. xanthii* Halst. Although no material from Butler's collections remains, circumstantial evidence suggests that his report of *C. xanthii* was most likely *C. orbiculare* (Walker, Nikandrow, and Millar, *unpublished*). *C. orbiculare* (syn. *C. lagenarium* (Pass.) Ellis & Halst.) is generally considered a widespread pathogen of the Cucurbitaceae (6). Simmonds (16) has also recorded *C. orbiculare* on *X. spinosum* in Queensland. *C. orbiculare* has not been previously recorded on *X. italicum*.

There are no other records of a *Phomopsis* sp. causing disease of *Xanthium* spp. in NSW. Saccardo (15) reported *Phomopsis* pau Gonz. Fragoso on dead twigs of *X. spinosum* in Spain; however, spore measurements indicate that this is a different species from that on *Xanthium* spp. in NSW (Nikandrow, *unpublished*). Both Herr et al (7) and Muntanola-Cvetkovic (13) have reported a similar disease on sunflower (*Helianthus annuus* L.), attributed to *Diaporthe helianthi* Munt. Circumstantial evidence suggests that the *Phomopsis* sp. reported here may be the anamorph of *D. arctii*. *D. arctii* has been reported on *X. saccharatum* Wallr. (= *X. italicum* = *X. strumarium*) and on *X. strumarium* L. var. *canadense* (Mill.) Torr. A. Grey (= *X. italicum*) in Georgia (5) and on *X. spinosum* and *X. strumarium* in South America (22). Our studies showed that some isolates are sufficiently virulent to *X. spinosum* and possibly other *Xanthium* spp. to merit further study as a mycoherbicide.

Some of the other fungi recorded on *Xanthium* spp. in this study have been reported elsewhere. *S. sclerotiorum* has been recorded on *X. strumarium* in Taiwan (19), on *X. pensylvanicum* Wallr. (= *X. orientale* = *X. strumarium*) in Maryland (1), and on *X. pungens* Wallr. (= *X. occidentale*) in Australia (17). Evans (4) reported infection of *X. pungens* with *V. dahliae*. There are no records of *S. sclerotiorum* from *X. spinosum*, of *V. dahliae* from *X. spinosum* or *X. italicum*, or of *M. phaseolina* from *X. spinosum, X. occidentale*, or *X. orientale*.

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