

Responses of *Echinochloa* Species and Rice (*Oryza sativa*) to Indigenous Pathogenic Fungi

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ABSTRACT

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Six pathogenic fungal species were isolated from naturally infected *Echinochloa* species and evaluated as biological control agents of *Echinochloa* species in rice. *Curvularia lunata* var. *aeria* and *Exserohilum oryzae* were pathogenic to both rice and *Echinochloa* species and were not evaluated further. *Bipolaris sacchari*, *Curvularia geniculata*, *Dactylaria dimorphospora*, and *Exserohilum monoceras* were pathogenic only to *Echinochloa* species and were further compared for virulence under controlled environment conditions in the greenhouse. When provided a 24-h dew period, *Exserohilum monoceras* killed seedlings of all three *Echinochloa* species tested: *E. crus-galli*, *E. colona*, and *E. glabrescens*; *B. sacchari* resulted in 100% mortality of seedlings of *E. colona* and *E. glabrescens*; *C. geniculata* killed seedlings of only *E. colona*; and *D. dimorphospora* did not cause any plant death. When given a 12-h dew period, *Exserohilum monoceras* still killed the three *Echinochloa* species, whereas the other fungi did not cause plant death. *Echinochloa* seedlings at the one- and two-leaf stages were more susceptible to these fungi than were seedlings at the three- and four-leaf stages. *B. sacchari*, *Exserohilum monoceras*, and *E. oryzae* produced phytotoxins that caused 100% leaf area chlorosis and wilting of intact seedlings of the *Echinochloa* species placed in cell-free culture filtrates.

Additional keyword: bioherbicide

Barnyard grass, *Echinochloa crus-galli* (L.) Beauv., and jungle rice, *Echinochloa colona* (L.) Link, are ranked as the world's third and fourth worst weed species, respectively, and are two of the most serious weeds in rice (*Oryza sativa* L.) (11). *Echinochloa glabrescens* Munro ex Hook. f. is another important weed species in rice, especially in the tropical regions (6,16). These species severely reduce both yield and quality of rice (11,19).

Cultural measures, hand weeding, mechanical methods, and herbicides are available to control these weeds (10,15). Labor and water costs limit the utility of most procedures, resulting in increased dependence on herbicides (13). Large rice production areas are shifting from transplanting to direct seeding, resulting in increased weed populations and chemical herbicide use (13). The increased use of herbicides will accelerate development of herbicide resistance in weed populations and will increase environmental and societal concerns related to pesticide use. The use of

biological control agents is one alternative or complementary tactic to reduce herbicide inputs, but it has received limited attention in the major rice-producing areas of Asia (25). In Korea, a fungal pathogen identified as *Exserohilum monoceras* (Drechs.) K. J. Leonard & E. G. Suggs was found to cause leaf blight of *E. crus-galli* but this isolate was also pathogenic to several important crops including rice (5). In Japan, a fungal pathogen, identified as *Drechslera monoceras* (Drechs.) Subram. & Jain, is being evaluated as a bioherbicide for control of *Echinochloa* species in rice (7,9).

Recently, six different indigenous fungal species have been isolated from naturally infected *E. crus-galli*, *E. colona*, and *E. glabrescens* in the Philippines (2,12,25). To select the best candidate for further development as a biocontrol agent for *Echinochloa* species in rice, this study was designed to (i) determine the pathogenicity of these fungi on *Echinochloa* species and rice, (ii) compare the virulence of these fungi to *Echinochloa* species, and (iii) assess potential phytotoxin production by these fungi.

MATERIALS AND METHODS

Isolation and identification of fungi. From 1990 to 1994, diseased leaves of *Echinochloa* spp. were collected in the Philippines, air dried in a paper press, cut

to appropriate size, and stored at 4°C in envelopes. Leaf pieces with lesions were surface sterilized with 0.5% sodium hypochlorite solution and incubated on fresh potato dextrose agar (PDA; Difco, Detroit, MI). Fungi that grew from the lesions were isolated and Koch's postulates were completed for most samples shortly after each collection trip. Monoconidial isolates of the recovered fungi were maintained on half-strength PDA slants in test tubes as stock cultures. Cultures of these fungi were submitted to the International Mycological Institute (IMI), Kew, UK, for confirmation of identification.

Inoculum production. Small pieces of mycelium from the stock culture of each fungus were aseptically transferred to fresh PDA. Plates were sealed with Parafilm and incubated in the dark at 28°C for 7 days. Agar plugs (6 mm diameter) from the margins of young colonies were used as seed inoculum (23).

Rice polish agar (RPA; 20 g of rice polish, 17 g of agar, and 1 liter of water) was used as a medium for conidia production (23). Agar plugs of seed inoculum were placed in the center of each petri dish; plates were then sealed with Parafilm and incubated at 28°C in the dark. Conidia were harvested 15 days after incubation by flooding the plates with 10 ml of distilled water and scraping the surface of the colonies with a glass slide. Resulting suspensions were filtered through a layer of cheesecloth and conidial concentrations were determined with a hemacytometer.

Pathogenicity of the tested fungi. Cultivars Dee-Geo-Woo-Gen and IR72, Chianan and Chianung 242, and Brondol putih and Rodjolele were selected as representatives of indica, japonica, and tropical japonica rice types, respectively. A single lot of seeds of each of the *Echinochloa* species, i.e., *E. crus-galli*, *E. colona*, and *E. glabrescens*, collected from the International Rice Research Institute (IRRI) farm, was used in each test. Seeds of each *Echinochloa* species and rice cultivar were incubated in petri dishes on moistened filter paper at room temperature for 48 h. Five germinated seeds (coleoptile and radicle just emerged) were planted per 10-cm-diameter plastic pot filled with saturated soil (Maahas clay, suborder Haplustic). Seeded pots were placed on a pushcart in the greenhouse and a 2 to 3 cm depth of

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water was maintained in the pushcart throughout the experiment. Greenhouse conditions were 35/25 ± 5°C day/night temperature, a 12-h photoperiod, and average light intensity of 20 MJ/m² per day. Seedlings at the one- to two-leaf stage were inoculated with 10⁵ to 10⁶ conidia per ml to run-off with 0.05% Tween 20 as a wetting agent, using a motorized sprayer (Arthur H. Thomas Co. Scientific Apparatus, Philadelphia) at 100 kPa. After spraying, pots were placed in a dark dew chamber with 100% relative humidity at 25°C for 24 h. Subsequently, pots were transferred to a corner of the greenhouse having a temperature of 24 to 28°C and 85 to 95% relative humidity. The disease reactions of *Echinochloa* species and rice to each of the tested fungal species were evaluated 14 days after inoculation (DAI). On the basis of lesion type and size, plant response was rated at four levels: 0 = lesions absent; 1 = small, unexpanded lesions; 2 = slightly to moderately expanded lesions; and 3 = large lesions. The experimental design was a randomized complete block with four replications (five plants per replication). The experiment was performed twice. The control treatment was sprayed with distilled water containing only the wetting agent.

Comparison of disease severity. The fungal species pathogenic to rice were excluded from further evaluation. Those fungi pathogenic only to *Echinochloa* species were further evaluated to assess the effect of dew period duration on disease severity at the one-, two-, three-, and four-leaf stages. Seeding was done at 7, 9, 11, and 13 days before inoculation for *E. crus-galli* and *E. glabrescens* and at 6, 8, 10, and 12 days before inoculation for *E. colona* in order for the four leaf stages to be inoculated on the same day. Five seedlings were established within each pot before inoculation. Seedlings were sprayed until run-off with 10⁵ to 10⁶ conidia per ml containing 0.05% Tween 20 as a wetting

agent. Control treatments were sprayed with distilled water containing only the wetting agent. After spraying, pots were immediately placed in a dark dew chamber at 25°C for a 12-h or a 24-h dew period. Immediately after dew treatment, pots were transferred to a corner of the greenhouse having a temperature of 24 to 28°C and 85 to 95% relative humidity. Disease severity was assessed as percent leaf area damage (LAD) and was estimated visually at 2, 4, 6, 8, and 10 DAI. The mean LAD for each pot was recorded. For comparative purposes, the standardized area under the disease progress curve (SAUDPC) was calculated for each replication by dividing the AUDPC value by the total duration of disease development (22). Ten days after inoculation, mortality was evaluated for each plant, and results pooled and averaged for each pot. Completely collapsed seedlings were considered dead. The experimental design consisted of a split-split-plot in randomized complete block with four replications. The dew period was the main plot, the fungal species was the subplot, and plant growth stage was the sub-subplot. Data were collected individually for each plant. Mean values of five plants were used for statistical analyses. The experiment was performed twice.

Bioassay for phytotoxin production.

Agar plugs (6 mm diameter) of each fungus taken from the edge of 7-day-old PDA cultures were placed into 500-ml Erlenmeyer flasks containing 100 ml of modified Fries liquid medium (30.0 g of sucrose, 5.0 g of ammonium tartrate, 1.0 g of NH₄NO₃, 1.0 g of KH₂PO₄, 0.5 g of MgSO₄, 0.1 g of NaCl, 0.1 g of CaCl₂, 0.5 g of casein hydrolysate, and 1.0 g of yeast extract in 1,000 ml of distilled water) (23). Flasks containing only modified Fries medium served as controls. The flasks were placed on rotary shakers (Lab-Line Instruments, Melrose Park, IL) at 150 rpm at room temperature. After a 2-week incubation, cultures in each flask were separately

centrifuged (Beckmann, Palo Alto, CA) at 3,000 rpm for 20 min. The supernatant in the centrifuge tubes was filtered through a membrane filter (pore size 0.45 μm) to obtain cell-free culture filtrate, 30 ml of which was dispersed into sterilized 50-ml vials (20 mm diameter). The roots of healthy *Echinochloa* seedlings at the two-leaf stage were immersed in the cell-free filtrates. The experimental design was a randomized complete block with four replications. After 48 h, data were recorded. Chlorosis and wilting of leaves were assumed to indicate the presence of phytotoxins in the culture filtrate. Living leaf area was measured in cm² with a leaf area meter (LI-3100 Area Meter, LI-COR, Inc., Lincoln, NE). This test was performed twice.

Data analyses. All percentage data were arc sine-transformed before analysis (8). Factorial analysis of variance of experiments considered the effect of each factor individually and their interaction. Results from the two trials of each experiment were pooled if homogeneity of variances was confirmed by Bartlett's test (8). However, for experiments in which the variance of trials was not homogenous, results from one trial are presented if a similar trend was observed in them. Mean values of five plants were used for statistical analyses and treatment means were separated with the least significant difference or Duncan's multiple range test at the 5% level of significance.

RESULTS

Isolation and identification of fungi.

Six different species of pathogenic fungi—two *Exserohilum* spp. (92-044 and 93-136), two *Curvularia* spp. (92-074 and 93-128), one *Bipolaris* sp. (91-097), and one *Dactylaria* sp. (91-106)—were isolated from naturally infected *Echinochloa* plants. Isolates 91-106, 93-128, and 93-136 were isolated from *E. crus-galli*, isolates 91-097 and 92-044 from *E. colona*, and isolate 92-074 from *E. glabrescens*. These fungi were identified by IMI as *Exserohilum monoceras* (92-044), *Exserohilum oryzae* Sivan. (93-136), *Curvularia lunata* (Wakk.) Boedijn var. *aeria* (Batista, Lima & Vasconcelos) M. B. Ellis (92-074), *Curvularia geniculata* (Tracy & Earle) Boedijn (93-128), *Bipolaris sacchari* (E. J. Butler) Shoemaker (91-097), and *Dactylaria dimorphospora* Veenbaas-Rijks (91-106).

Pathogenicity of the tested fungi. Both *Exserohilum* species caused large, necrotic lesions on all *Echinochloa* species tested (Table 1). Necrotic flecks appeared within 24 h and a blightlike reaction was observed 2 DAI, characterized by chlorosis and a diffuse, general, water-soaking reaction that was followed by rapid collapse and necrosis of affected tissues. Distinct lesions were usually not observed. *E. oryzae* was also pathogenic to rice and appeared to be more virulent on the japonica and tropi-

Table 1. Pathogenicity of six fungi on various *Echinochloa* species and rice (*Oryza sativa*)^w

Fungus	Host responses ^x								
	Rice cultivar ^y						<i>Echinochloa</i> species ^z		
	IN-1	IN-2	JP-1	JP-2	JV-1	JV-2	ECHCG	ECHCO	ECHGL
<i>Bipolaris sacchari</i>	0	0	0	0	0	0	2	3	3
<i>Dactylaria dimorphospora</i>	0	0	0	0	0	0	1	1	1
<i>Curvularia lunata</i> var. <i>aeria</i>	1	1	1	1	1	1	2	2	2
<i>Curvularia geniculata</i>	0	0	0	0	0	0	3	3	2
<i>Exserohilum monoceras</i>	0	0	0	0	0	0	3	3	3
<i>Exserohilum oryzae</i>	1	1	2	2	2	2	3	3	3

^w Plants at the one- to two-leaf stage were inoculated with 10⁵ to 10⁶ conidia per ml, placed in a dew chamber at 25°C for 24 h and subsequently transferred to a corner of the greenhouse having a temperature of 24 to 28°C and 85 to 95% relative humidity.

^x Host responses rated 14 days after inoculation with a 0 to 3 grading system: 0 = lesions absent; 1 = small, unexpanded lesions; 2 = slightly to moderately expanded lesions; and 3 = large lesions.

^y IN-1 = Indica rice cv. Dee-Geo-Woo-Gen; IN-2 = Indica rice cv. IR72; JP-1 = Japonica rice cv. Chianan; JP-2 = Japonica rice cv. Chianung 242; JV-1 = Tropical japonica rice cv. Brondol Putih 20; JV-2 = Tropical japonica rice cv. Rodjolele.

^z ECHCG = *Echinochloa crus-galli*; ECHCO = *Echinochloa colona*; ECHGL = *Echinochloa glabrescens*.

cal japonica rice cultivars than on the indica rice cultivars. *E. monoceras*, however, was nonpathogenic to rice.

Lesions induced by both *Curvularia* species appeared on *Echinochloa* leaves within 24 h after inoculation and wilting of the top portion of leaves occurred 2 to 3 DAI. *C. geniculata* caused large, necrotic lesions on *E. crus-galli* and *E. colona* and moderately expanded lesions on *E. glabrescens*; *C. lunata* var. *aeria* resulted in slightly expanded lesions on all *Echinochloa* species. However, *C. geniculata* was only pathogenic to *Echinochloa* species, whereas *C. lunata* var. *aeria* was pathogenic to both rice and the *Echinochloa* species (Table 1).

B. sacchari resulted in large, necrotic lesions on *E. colona* and *E. glabrescens* and moderately expanded lesions on *E. crus-galli*. Lesions induced by *B. sacchari* appeared on *Echinochloa* species within 24 h after inoculation. This fungus also caused a blightlike symptom similar to that caused by *E. monoceras* and was also nonpathogenic to rice (Table 1).

D. dimorphospora caused small, unexpanded lesions on *Echinochloa* species.

Lesions induced by *D. dimorphospora* appeared on *Echinochloa* species 2 to 3 DAI and infected leaves wilted 5 to 7 DAI. Fewer lesions, however, were observed on plants inoculated with *D. dimorphospora* than on those inoculated with the other fungi. This fungus was nonpathogenic to rice (Table 1).

Comparison of disease severity. Since *E. oryzae* and *C. lunata* var. *aeria* were pathogenic to rice, they were excluded from further evaluation. The other four fungal species—*E. monoceras*, *B. sacchari*, *C. geniculata*, and *D. dimorphospora*—were pathogenic only to *Echinochloa* species and their virulences to the *Echinochloa* species were further compared.

Disease progress over time was significantly different among the four fungal species tested (Fig. 1). Inoculation of *Echinochloa* species at the two-leaf stage with *E. monoceras* resulted in 100% disease severity of all *Echinochloa* species within 4 DAI when provided a 24-h dew period and within 8 DAI when provided a 12-h dew period. When inoculated with *B. sacchari* and given a 24-h dew period, *E. colona* and *E. glabrescens* showed 100%

disease severity 2 DAI and 8 DAI, respectively, whereas *E. crus-galli* did not show 100% disease severity and limited regrowth occurred. When provided a dew period of 12 h, disease severity of *B. sacchari* on *Echinochloa* species decreased dramatically. Following inoculation with *C. geniculata* with 24 h of dew, only *E. colona* expressed 100% disease severity, whereas *E. crus-galli* and *E. glabrescens* had less disease and showed some regrowth. When provided a dew period of 12 h, inoculation with *C. geniculata* resulted in less than 20% LAD for the three *Echinochloa* species. Finally, inoculation with *D. dimorphospora* resulted in <20% LAD for all three *Echinochloa* species even after a 24-h dew period was provided and almost no disease occurred when the dew period was shortened to 12 h.

When provided a 24-h dew period, the maximum SAUDPC value was 90% for *E. glabrescens* inoculated with *E. monoceras* and *E. colona* inoculated with *B. sacchari*. Similar high SAUDPC values were also obtained for plants of *E. crus-galli* and *E. colona* inoculated with *E. monoceras* and for *E. crus-galli* and *E. glabrescens* plants

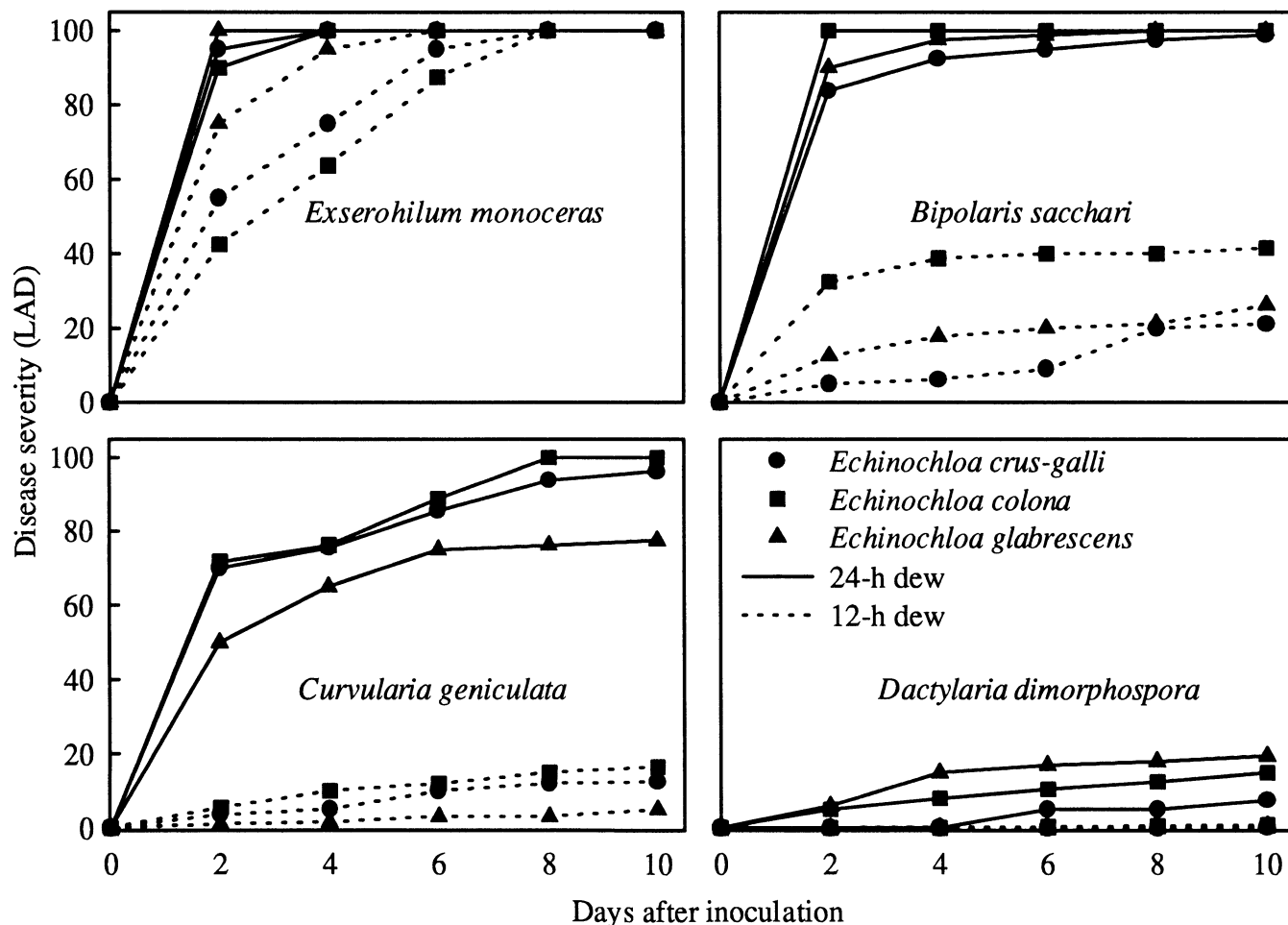


Fig. 1. Disease development of four pathogenic fungi on *Echinochloa* species subjected to 12-h and 24-h dew periods following inoculation, expressed as the mean percentage of leaf area damage (LAD). Seedlings of *E. crus-galli*, *E. colona*, and *E. glabrescens* at the two-leaf stage were inoculated with 10^5 to 10^6 conidia per ml of *Exserohilum monoceras*, *Bipolaris sacchari*, *Curvularia geniculata*, and *Dactylaria dimorphospora*, respectively. Data from two trials were not pooled because the variances were heterogeneous, but trends for two trials were similar. Data points represent means of four replications of one trial. The least significant difference (5%) values between dew periods, fungi, and weed species are 8.5, 8.9, and 8.7%, respectively.

inoculated with *B. sacchari*. The *C. geniculata* SAUDPC value for *E. crus-galli* was not significantly different from that obtained for *B. sacchari*, but it was significantly lower than that produced by *E. monoceras*. The *C. geniculata* SAUDPC value for *E. colona* was significantly lower than the *E. monoceras* and *B. sacchari* SAUDPC values. The same trend was observed for *E. glabrescens*. The SAUDPC of *D. dimorphospora* for *Echinochloa* species was below 1%. When provided a 12-h dew period, the highest SAUDPC values were recorded for *E. monoceras*. The SAUDPC of *B. sacchari* subjected to a 12-h dew period was significantly lower than the SAUDPC value produced by *E. monoceras*. With a 12-h dew period, inoculation with *C. geniculata* and *D. dimorphospora* produced low SAUDPC values (Fig. 2).

Echinochloa species mortality varied with fungal species, weed species, plant leaf stage, and dew period duration (Fig. 3). When provided a 24-h dew period, *E. monoceras* inoculation killed the seedlings of all three *Echinochloa* species at all leaf stages. The application of *B. sacchari* caused 100% mortality of *E. colona* seedlings at the one- and two-leaf stages and *E. glabrescens* seedlings at the two-leaf stage,

with lower mortality being observed at the three- or four-leaf stages, but it did not kill all *E. crus-galli*. *C. geniculata* killed only *E. colona* seedlings at the one- and two-leaf stages, with some mortality observed at the three- or four-leaf stages, and did not kill all *E. crus-galli* and *E. glabrescens* seedlings. *D. dimorphospora* inoculation did not cause any plant death. When given a 12-h dew period, only *E. monoceras* caused 100% mortality of the three *Echinochloa* species at one-, two-, or three-leaf stages. The other fungi did not cause any plant death when provided a 12-h dew period.

E. monoceras, therefore, required the shortest dew period duration for 100% kill of *Echinochloa* species, followed by *B. sacchari*, *C. geniculata*, and *D. dimorphospora*. Seedlings at the one- to two-leaf stage were generally more susceptible to disease from these fungi than seedlings at the three- to four-leaf stage (Fig. 3).

Bioassay for phytotoxin production. Chlorosis and wilting of *Echinochloa* species were observed 24 h after placing the roots of intact seedlings at the two-leaf stage in the cell-free filtrates of *E. monoceras*, *E. oryzae*, and *B. sacchari*. Symptoms were similar to those observed in plants inoculated with conidial suspensions. Forty-eight hours after immersion, seedlings of all *Echinochloa* species were dead (Table 2). Thus, these three fungi produced secondary metabolites that were highly active to all *Echinochloa* species. The culture filtrate of *C. lunata* var. *aeria* significantly reduced the living leaf area of *E. crus-galli* and *E. colona*, by 53 and 64%, respectively, but did not affect the living leaf area of *E. glabrescens*. The cell-free culture filtrate of *C. geniculata* did not influence the living leaf area of *E. crus-galli* and *E. glabrescens*, but reduced the living leaf area of *E. colona* by nearly 80%. The culture filtrate of *D. dimorphospora* significantly reduced the living leaf area of *E. crus-galli*, but did not influence that of the other two *Echinochloa* species (Table 2).

DISCUSSION

The responses of *Echinochloa* species and rice to the six indigenous pathogenic fungi varied. *E. oryzae* and *C. lunata* var. *aeria* were pathogenic to the three *Echinochloa* species but also to rice. The former is a pathogen of rice that causes rice brown spot disease and the latter was reported to cause chlorotic and brown spots of bean (*Phaseolus vulgaris* L.) (3). *D. dimorphospora*, *C. geniculata*, *B. sacchari*, and *E. monoceras* were not pathogenic to rice, but were pathogenic to the *Echinochloa* species.

It is often assumed that a virulent, highly aggressive pathogen (i.e., one that causes a high level of mortality) is a preferred bioherbicide candidate (26). *D. dimorphospora* caused limited disease on the *Echinochloa* species; *C. geniculata* caused 100% mortality of *E. colona*; *B. sacchari* resulted in 100% mortality of *E. colona* and *E. glabrescens*; and *E. monoceras* caused 100% mortality of *E. crus-galli*, *E. colona*, and *E. glabrescens*. Therefore, *C. geniculata*, *B. sacchari*, and *E. monoceras* have potential to control *Echinochloa* species.

C. geniculata is reported as a pathogen causing seed rot of soybean (*Glycine max* (L.) Merr.) and a weak pathogen causing leaf spot disease of a turfgrass species, Kentucky bluegrass (*Poa pratensis* L.) (21). *B. sacchari* is the causal fungus of eyespot disease of sugarcane (*Saccharum* spp.) (14). The development of *C. geniculata* and *B. sacchari* as part of bioherbicide strategies to control *Echinochloa* must consider the potential risks to these crop species.

E. monoceras was first reported in 1970 as a beneficial organism to protect wheat (*Triticum aestivum* L.) against powdery mildew (*Erysiphe graminis* DC.) (1). Since then, there were no reports on *E. monoceras* until the 1990s. In 1990, *E. monoceras* was reported to cause leaf blight of *E. crus-galli* in Korea but this isolate was also pathogenic to several im-

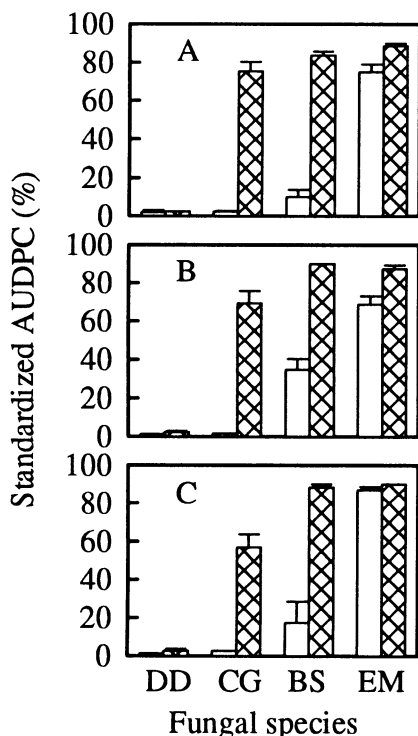


Fig. 2. Disease development of four pathogenic fungi on *Echinochloa* species subjected to 12-h and 24-h dew periods following inoculation, expressed as the standardized area under the disease progress curve (SAUDPC). Bars represent means of four replicates. A = *E. crus-galli*; B = *E. colona*; C = *E. glabrescens*; DD = *Dactylaria dimorphospora*; CG = *Curvularia geniculata*; BS = *Bipolaris sacchari*; EM = *Exserohilum monoceras*. Open bars = 12-h dew, and shaded bars = 24-h dew.

Table 2. Area of living leaf tissue of three *Echinochloa* species 48 h after roots were placed in cell-free filtrates of six fungi^x

Fungus	Living leaf area (cm ²) ^y		
	<i>E. crus-galli</i>	<i>E. colona</i>	<i>E. glabrescens</i>
<i>Dactylaria dimorphospora</i>	0.63 bc ^z	0.70 a	0.83 a
<i>Curvularia lunata</i> var. <i>aeria</i>	0.53 c	0.33 b	0.47 a
<i>Curvularia geniculata</i>	0.98 ab	0.22 b	0.61 a
<i>Exserohilum monoceras</i>	0 d	0 c	0 b
<i>Exserohilum oryzae</i>	0 d	0 c	0 b
<i>Bipolaris sacchari</i>	0 d	0 c	0 c
Control	1.12 a	0.92 a	0.60 a

^x Healthy two-leaf-stage seedlings of *Echinochloa* spp. were separately immersed in vials containing 30 ml of cell-free cultural filtrates from the fungi. Modified Fries medium served as control. Data from two trials were pooled because variances were homogeneous. Data represent means of eight replicates.

^y Living leaf area was measured in cm² with a leaf area meter (LI-3100 Area Meter, LI-COR, Inc. Lincoln, NE).

^z In a column, means having a common letter are not significantly different according to Duncan's multiple range test at the 5% level of significance.

portant crops including rice (5). In 1992, *Drechslera monoceras* was reported to effectively control *Echinochloa* species in rice in Japan (7). The Philippines isolate was also identified as *Exserohilum monoceras* by IMI. According to Sivanesan (18), *Drechslera monoceras* and *Exserohilum monoceras* are the same species. However, differences among the three isolates in terms of conidial characteristics, host specificity, and phytotoxin production are apparent. Our isolate appears to be more similar to that found in Japan because neither infect rice. However, whether or not these three isolates can be differentiated at either forma specialis or race level remains to be seen. DNA fingerprinting might provide an approach for further comparison.

Phytotoxins have been reported to be produced by *E. oryzae*, *B. sacchari*, and *C. lunata* var. *aeria* (3,20,24). Our results confirm this and have demonstrated that these phytotoxins are biologically active on *Echinochloa* spp. Further studies are needed to determine the role of these phytotoxins in the control of *Echinochloa*. Monocerin was the first component isolated from *E. monoceras* (1). It is an anti-

biotic that protects wheat against powdery mildew and not a phytotoxin. Subsequently, monocerin has also been isolated from *Exserohilum turcicum* (Pass.) K. J. Leonard & E. G. Suggs and has been found to have phytotoxic activity on johnsongrass (*Sorghum halepense* (L.) Pers.) and Canada thistle (*Cirsium arvense* (L.) Scop.) (17). However, to our knowledge, there have been no reports on phytotoxin production by *E. monoceras*. Further studies can be directed to isolate, purify, and characterize the phytotoxins produced by *E. monoceras*.

Dew period duration is a key factor in the evaluation of weed pathogens as potential bioherbicides. Chiang et al. (4) proposed a relative dew requirement index (RDRI) for evaluation. RDRI was the ratio of disease severity with a 12-h dew period to disease severity with 24-h dew period. In most cases, the RDRI reflects the dew requirement for specific candidates. However, in some cases (especially when there are equal RDRI values), it does not reflect a difference in dew period requirements. For example, the RDRI of *B. sacchari* for *E. crus-galli* is equal to the RDRI of *C. geniculata* for *E. crus-galli*, but the former actually requires a shorter dew period du-

ration than the latter. Multiplying RDRI with average disease severity at two dew period durations showed the difference in actual dew requirements between *B. sacchari* and *C. geniculata*. Use of SAUDPC values might provide a more accurate estimation because SAUDPC values are an average of the disease severity over time while disease severity is only a single observation in the process of disease development.

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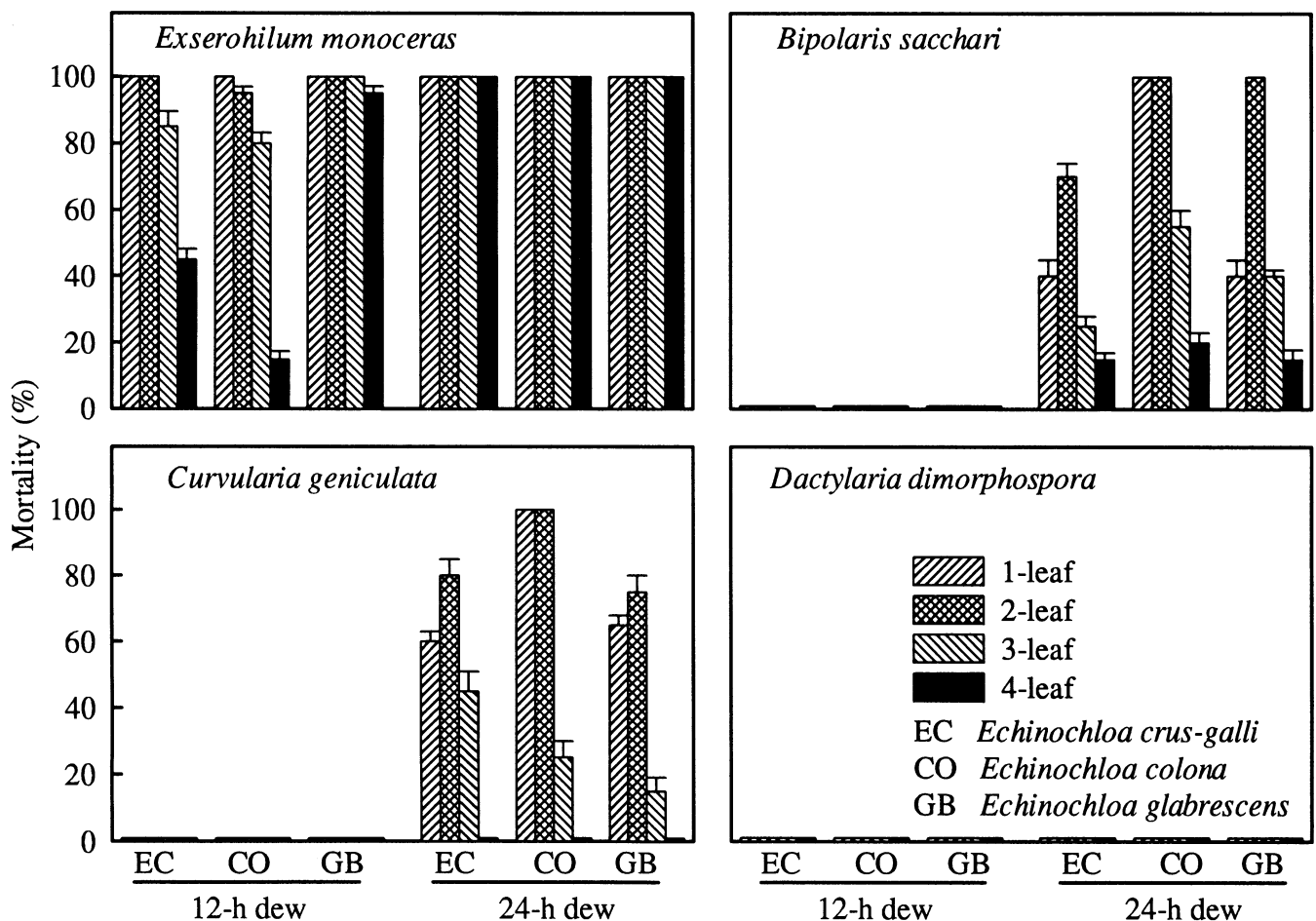


Fig. 3. Mortality of *Echinochloa* species at different leaf stages caused by pathogenic fungi for either 12-h or 24-h dew period. Seedlings of *E. crus-galli*, *E. colona*, and *E. glabrescens* at the two-leaf stage were inoculated with 10^5 to 10^6 conidia per ml of *Exserohilum monoceras*, *Bipolaris sacchari*, *Curvularia geniculata*, and *Dactylaria dimorphospora*, respectively. Data from two trials were not pooled because variances were heterogeneous, but trends for two trials were similar. Data points represent means of four replicates of one trial. The least significant difference (5%) values between dew periods, fungi, weed species, and leaf stages are 16.9, 16.9, 17.0, and 17.6%, respectively.

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