Carolina Foxtail (*Alopecurus carolinianus*): Susceptibility and Suitability as an Alternative Host to Rice Blast Disease (*Magnaporthe oryzae* [formerly *M. grisea*])

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ABSTRACT

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Carolina foxtail (*Alopecurus carolinianus*) has not been reported to host *Magnaporthe oryzae*. A collection of Carolina foxtail obtained from several Arkansas locations over a 4-year period was inoculated with four races of the fungus under greenhouse conditions and, in all cases, inoculation resulted in the formation of irregular, yellow and brown lesions without obvious gray centers that are characteristic for blast on rice. Differences in these lesions were not observed among our collection. These lesions appeared to differ from typical blast lesions on inoculated rice leaves but were evident following artificial inoculation of Carolina foxtail in the greenhouse. *M. oryzae* races that differed in pathogenicity toward rice cultivars also displayed differences in lesion development on Carolina foxtail. The most virulent race on rice cultivars also produced lesions most rapidly on Carolina foxtail. These lesions developed more quickly on Carolina foxtail than on the most susceptible rice cultivars tested, including a susceptible California cultivar, M202. *M. oryzae* isolates cultured from these lesions in the infected Carolina foxtail caused typical disease symptoms of blast on inoculated rice cultivars. We suggest that Carolina foxtail is a new and previously unrecognized host for the blast pathogen.

Additional keywords: *Pyricularia grisea*, *Oryza sativa*

Rice (*Oryza sativa* L.) is one of the most important food crops in the world. One of the most important diseases of rice worldwide is blast, caused by the fungal pathogen *Magnaporthe oryzae* (formerly *Magnaporthe grisea*) Cavara (anamorph: *Pyricularia grisea* (Cooke) Sacc.) (5,17,26,27). The pathogen can infect rice plants at any growth stage from seedling through grain formation, and causes leaf blast, collar rot, nodal blast, neck blast, or panicle blast (1).

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The rice blast community agrees to change the name of the rice blast fungus from the former name *Magnaporthe grisea* to *Magnaporthe oryzae* at the rice blast workshop in San Diego (January 14, 2007) of the Plant and Animal Genome conference.

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habitats makes it well suited to the flat, intermittently flooded agricultural fields of the southern United States that commonly are rotated between rice, soybean, and sometimes wheat. The susceptibility of Carolina foxtail to *M. oryzae* was discovered serendipitously during evaluation of rice blast disease in the greenhouse. Thus far, there have been no published studies documenting that the rice blast pathogen can infect Carolina foxtail. It is also unknown whether or not blast can infect weeds commonly present in fields rotated between rice and soybean, such as flatsedge, yellow nutsedge, hemp sesbania, northern jointvetch, and palmleaf morning glory.

The objectives of this study were to determine whether Carolina foxtail and several common weeds found in rice fields may be hosts for *M. oryzae* under favorable conditions for the pathogen.

MATERIALS AND METHODS

Plant materials and growth. Rice cvs. M202, Zhe733 (10), Katy (15), and an experimental line RU9101001 (10) were evaluated in this study. Rice seed were germinated on moist filter papers in petri dishes for 3 days in a 30°C dark incubator. Seedlings were transplanted to 13-cm-diameter pots containing a sterilized DeWitt silt loam soil from Stuttgart, AR without application of fertilizer. These pots were subirrigated in a water-filled tray in a greenhouse at 24 to 30°C with a cycle of 16 and 8 h of light and darkness, respectively, for 2 weeks, until plants were at the four-leaf stage.

Carolina foxtail seed (50 to 100) were sown in small pots containing moist potting soil (Scotts-Sierra Horticultural Products Co., Marysville, OH). The pot was covered with transparent plastic film to reduce moisture loss and maintained at 25°C under diffuse natural sunlight near a window in the laboratory until the seed germinated (usually within 7 days). Pots containing the young seedlings then were moved to the greenhouse. Subsequently, individual seedlings were subsampled, transplanted, and grown for 3 weeks. Seedlings were grown in 13-cm-diameter pots containing sterilized Dewitt silt loam soil

In the southern United States, epidemics of blast disease are erratic; thus, it is important to understand the primary source of overwintering inoculum. Seed as a primary source of *M. oryzae* inoculum has been well documented (8,11,14). Research on weed hosts is important in determining the *M. oryzae* reservoir for a portion of genetic diversity found in rice blast populations, in studying the biology of *M. oryzae*, and in determining host specificity. *M. oryzae* causes diseases in a number of plant species, including wheat, rye grass, and pearl millet (2,20,21,26). However, there has been no consensus in the literature regarding the cross-pathogenicity of *Magnaporthe* spp. occurring on rice and grass weeds even though Mackill and Bonman (13) reported successful cross-inoculation studies of *M. oryzae* isolates from several grass weeds and rice (1,13,16).

Carolina foxtail is a winter annual grass species commonly found in moist areas of fallow fields or in wetland areas throughout most of the United States (22,23). Under favorable environmental conditions or crop rotations, Carolina foxtail could occur in the same field as rice for at least part of its growth cycle. This preference for wet

from Stuttgart, AR, without the application of fertilizer. The pots were placed in a tray that contained water. Seedlings were grown until they reached a height of 10 to

20 cm. Small seedlings (less than 5 cm tall) of Carolina foxtail were obtained from the edges of a rice paddy and were grown in a greenhouse.

Seed of several weed species commonly present in Arkansas rice or soybean fields also were obtained from local rice fields. These were rice flatsedge (*Cyperus iria*

Fig. 1. Brown-spot lesions on Carolina foxtail in comparison with disease reactions of rice to *Magnaporthe oryzae*. **A**, Brown spot disease on Carolina foxtail (left pot) and noninoculated control (right pot); **B** and **D**, on Carolina foxtail, **C** and **E**, blast lesions on 'M202' rice, **F**, sporulated mycelia on Carolina foxtail, **G**, three *M. oryzae* spores and mycelia from F were magnified. A–C were taken with a Nikon digital camera; B–E were taken using a digital camera connected to a Leica Stereomicroscope; and F and G were taken with a Nikon camera DXM1200 connected to a light microscope under $\times 100$ magnification.

L.); yellow nutsedge (*C. esculentus* L.); hemp sesbania (*Sesbania exaltata* (Raf.) Cory), northern jointvetch (*Aeschynomene virginica* Britton, Sterns & Poggenb.), and palmleaf morning glory (*Ipomoea wrightii* A. Gray). They were grown to heights of 10 to 15 cm for inoculation.

Fungal isolates and culture. TM2 and ZN19, virulent field isolates that belong to race IE1k, initially were used for this study. Cvs. Katy and M202 and line RU9100101 are susceptible (9) and Zhe 733 is resistant to TM2 (10). Subsequently, a collection of differential races of *M. oryzae* (IB 49 [ZN61], IC17 [ZN60], and IE1 [ZN4]; 4) was used for pathogenicity assays and for verification of disease reaction on Carolina foxtail. The fungal spores were grown on filter papers and stored at -20°C after desiccation. The filter papers containing fungal mycelia were placed on oatmeal agar plates (25). The inoculated plates were placed under a cycle of 16 and 8 h of light and darkness, respectively, at 24°C for 7 days. Hyphal tips were transferred to oatmeal agar (24) and cultures were incubated for an additional 12 days for production of spores.

Spore suspensions. Spores were harvested in a sterile 0.25% (wt/vol) gelatin (ICN Biomedical Inc., Costa Mesa, CA) solution by scraping fungal mycelia on the culture plates. The spore suspensions then were filtered through eight layers of cheesecloth to remove mycelia and pieces of agar (25). The concentration of the initial spore suspension was determined using a hemacytometer as described by Sambrook and Russell (18).

Pathogenicity assays. The standard pathogenicity assay was a procedure described by Valent et al. (25). Five to seven rice plants were used for each experiment. Plants were grown to the four-leaf stage in 13-cm-diameter plastic pots in a greenhouse at 24 to 30°C with a cycle of 16 and 8 h of light and darkness, respectively, and then inoculated with 2-ml spore suspensions (5×10^5 spores/ml) using an airbrush (Paasche IVL double-action airbrush). Plants were inoculated inside a plastic bag that then was sealed to maintain the high humidity required for fungal penetration of

the host plant cuticle (25). Plants were exposed to 24 h of continuous fluorescent light (10 to 12 $\mu\text{Em}^{-2} \text{sec}^{-1}$) at room temperature (21 to 24°C) and then the plants were removed from bags and returned to the greenhouse. Greenhouse-grown Carolina foxtail seedlings (15 cm tall) and mature plants of similar heights removed from the edges of a rice paddy (from April to May in Arkansas) were used for inoculation using the same procedure described above. Pathogenicity assays were repeated three times.

Disease development was monitored daily, and plants were maintained in a greenhouse at 24 to 30°C with a cycle of 16 and 8 h of light and darkness, respectively, until disease symptoms were observed for rice (7 to 11 days; 24) and for Carolina foxtail (4 to 7 days).

RESULTS

Disease symptoms. Disease progression on Carolina foxtail was compared with disease progression on blast-susceptible cvs. Katy and M-202 and a susceptible experimental line, RU9100101. At 1 day post inoculation (dpi) with isolate TM2, no visible change was observed on inoculated rice leaves. At 2 dpi, yellow spots began to develop on Carolina foxtail, whereas brown spots appeared on the rice leaves regardless of the resistance and susceptibility to the isolates used, with the exception of the resistant cv. Zhe 733. At 3 dpi, the yellow lesions had expanded noticeably on Carolina foxtail and sporulating mycelia were not present based on observations with the unaided eye. On Katy, M202, and RU9100101, brown spots were enlarged with gray centers where faint mycelia were visible with the unaided eye. In subsequent days, yellow and water-soaked brown lesions without borders expanded on Carolina foxtail, while susceptible rice cultivars developed typical blast lesions with large gray centers (Fig 1). No symptoms were observed in the resistant cv. Zhe 733 (*data not shown*).

At 7 dpi, brown lesions on Carolina foxtail were readily distinguishable from blast lesions on rice, where an eye-shaped gray center normally was visible (Fig. 1A-C).

In contrast to rice, conidiospores on Carolina foxtail were visible only under bright light examined by the use of microscopes after diseased leaves were incubated for 1 to 3 days on moistened filter papers in a petri dish (Fig. 1F and G).

The susceptibility of Carolina foxtail to *M. oryzae* was discovered serendipitously during evaluation of rice blast disease in the greenhouse. Rice flatsedge, yellow nutsedge, hemp sesbania, northern jointvetch, and palmleaf morning glory also were inoculated with TM2. None of them supported *M. oryzae* growth like Carolina foxtail. No visible responses were observed even at 10 dpi (*data not shown*).

Race reactions. The different isolates of *M. oryzae* were verified first by fingerprinting using Rep-polymerase chain reaction (PCR) (*data not shown*); reactions of Carolina foxtail to these isolates are summarized in Table 1. Uniformly, Carolina foxtail was susceptible to five races (seven isolates) tested and the disease reaction was comparable with the most susceptible experimental line RU9101001 and cv. M202. By comparison, Katy was susceptible to some isolates and Zhe733 was resistant to all isolates. IE1k was the most virulent race for all rice cultivars except Zhe733. The disease symptoms on Carolina foxtail appeared 1 day faster after inoculation with IE1k than with the other races.

To verify that there was no differential reaction within Carolina foxtail, seed produced from plants grown in the greenhouse and field plots from Stuttgart, AR from 2001 to 2005 were germinated and analyzed using the seven isolates. Consistently, all Carolina foxtail were uniformly susceptible (*data not shown*). We concluded that there was no differential reaction of Carolina foxtail to differential races of rice blast fungus tested.

Cross-infection. Isolate TM2, belonging to race IE1k which infects Katy, was used for cross-infection. The spores were isolated from infected Carolina foxtail leaves (Fig. 1A-G), and these isolates were verified using Rep-PCR to be the same isolate IE1k initially used to inoculate Katy (*data not shown*). Due to limited sporulation, it was extremely difficult to recover spores from anywhere on infected leaves of Carolina foxtail. The extent of virulence with similar disease lesions on rice cultivars is identical to that of the original isolate TM2 after the same length of time post inoculation (*data not shown*). Passage of isolates through Carolina foxtail does not affect their pathogenicity on rice based on the fact that the same disease reaction was obtained on rice using conidia from foxtail as with conidia of the same race obtained from culture.

DISCUSSION

M. oryzae is one of the most deadly airborne pathogens of cereal crops. Resis-

Table 1. Disease reactions of rice and Carolina foxtail to common races (isolates) of *Magnaporthe oryzae*^a

<i>M. oryzae</i> race (isolate) ^b	Host				
	Carolina foxtail	Zhe733	Katy	M202	RU9101001
IB49 (ZN61)	S	R	R	S	S
IC17 (ZN60)	S	R	R	S	S
IC17 (ZN57)	S	R	R	S	S
IE1 (ZN 4)	S	R	R	S	S
IE1 (ZN 49)	S	R	R	S	S
IE1k (ZN19)	S	R	S	S	S
IE1k (TM2)	S	R	S	S	S

^a Disease reactions were determined using the method described in Valent (27). The data represent overall visual ratings from three independent experiments; S = susceptible and R = resistant.

^b Fungal isolates were differential races commonly found in the southern rice-growing areas in the United States (4).

tance to the pathogen has been characterized to be a gene-for-gene system (6–10,19). The pathogen utilizes diverse hosts for overwintering, and multiple means of transmission (seed and wind-dispersed spores) are strategies for the pathogen's survival (6,11–13). We have demonstrated that *M. oryzae* races that infect rice also could infect a widespread winter annual weed in the United States, Carolina foxtail, causing brown spots that are different from typical blast symptoms. In contrast, other common weeds found in rice fields, rice flatsedge, yellow nutsedge, hemp sesbania, northern jointvetch, and palmleaf morning glory did not develop symptoms when inoculated with *M. oryzae*. These findings suggest that *M. oryzae* potentially could use Carolina foxtail as a host between rice crops.

Adaptability is a characteristic of *M. oryzae* (28). Successful cross-infection previously performed with grass weeds by Mackill and Bonman (13) is an important demonstration of this adaptability. It is known that *M. oryzae* is the causal agent for many gramineous plants with specificity within several subgroups. However, it also is known that the blast fungus can damage plant species that are not known as primary hosts. For example, recent destructive epidemics of blast diseases were found on perennial ryegrass (*Lolium perenne*; 2) and on wheat (*Triticum aestivum*; 21). Carolina foxtail is closely related to blackgrass (*Alopecurus myosuroides* Huds.), a highly competitive and problematic weed of winter wheat in Europe (3). It is worthwhile to examine whether blackgrass can be a host of *M. oryzae* under favorable conditions for the pathogen.

Planting time for rice has shifted recently from April to March in Arkansas to save water and to increase yield. The earlier that rice is planted, the greater would be the overlap of rice and Carolina foxtail growth cycles and the more likely that the blast pathogen could be exchanged between these two plant species. Our results reveal that the brown spot symptoms on Carolina foxtail are quite distinct from those of typical blast symptoms. Lack of previous published reports of blast on Carolina foxtail could be attributable to no studies having been conducted on this weed species and, more straightforwardly, blast on Carolina foxtail may have been overlooked previously because the disease symptoms look significantly different from those on rice and other hosts. Successful isolation of *M. oryzae* from lesions on Carolina foxtail and infection of rice by

these isolates is indisputable evidence that the fungus is a pathogen of Carolina foxtail and causes brown spot disease symptoms under favorable conditions. Research should be initiated worldwide to determine whether Carolina foxtail could be a host for *M. oryzae* in nature.

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