Reaction of Rice (*Oryza sativa*) Cultivars to Penetration and Infection by *Curvularia tuberculata* and *C. oryzae*

Lilian Z. de Luna, Graduate Student, Department of Plant Science, McGill University, 21,111 Lakeshore Road, Ste-Anne-de-Bellevue, Québec, Canada H9X 3V9 and Scholar, International Rice Research Institute, DAPO 7777, Metro Manila, Philippines; **Alan K. Watson**, Professor, Department of Plant Science, McGill University, and Weed Scientist (Seconded from McGill University), International Rice Research Institute; and **Timothy C. Paulitz**, Associate Professor, Department of Plant Science, McGill University

ABSTRACT

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Isolates of *Curvularia* species were collected from weedy Cyperaceae species and are being evaluated as possible biocontrol agents of sedge weeds in rice (*Oryza sativa*). *Curvularia* species have been reported from rice; thus cultivars of rice were tested to determine rice seedling responses to these potential biocontrol agents. All 13 rice cultivars were resistant to *Curvularia tuberculata* isolate 93-022, 12 were resistant to *C. tuberculata* isolate 93-020, and 7 were resistant to *C. oryzae* isolate 93-061. In the resistant cultivars, lesions on the leaf laminae were small, light to dark brown, with a dry appearance. Spots on the leaf margins and leaf tips were light brown to cream and dry. In the susceptible cultivars, the brown lesions coalesced with necrotic centers. Sporulation was observed in the lesions on susceptible cultivars but not on the resistant cultivars. The histopathology of *C. tuberculata* and *C. oryzae* was studied in two resistant rice cultivars, IR 64 (IRRI Acc. no. 66970) and Norin 21 (IRRI Acc. no. 7686), by light microscopy. *C. tuberculata* exhibited polar germination beginning at 4 h postinoculation (HPI); whereas *C. oryzae* was characterized by bipolar germination starting at 2 HPI. Simple terminal or intercalary appressoria were initiated at 24 HPI over stomatal apertures, or rarely, on the epidermal cell walls and bulliform cells. No infection cushions were formed. Penetration occurred by the formation of a fine penetration peg beneath the appressorium. A chlorotic reaction was observed in areas beneath and adjacent to the appressoria and germ tubes and in the infected cells. Resistance of IR 64 and Norin 21 to *C. tuberculata* and *C. oryzae* infection was mainly expressed after penetration as a slow and restricted mycelial growth and no sporulation. *C. tuberculata* isolate 93-022 is the preferred isolate for further study as a biological control agent against *Cyperus difformis*, *C. iria*, and *Fimbristylis miliacea*.

Additional keywords: globe fingerush, mycoherbicides, rice flat sedge, small flower umbrella sedge

Rice (*Oryza sativa* L.) is one of the most important crops in the world, being the staple food for millions of people in Asia. Weeds are considered a major constraint to world rice production and commonly cause yield losses in rice from 10 to 40% and, occasionally, complete crop failure (31). Poaceae and Cyperaceae species are the major weeds in irrigated rice, and yield

reductions of 12 to 50% have been attributed to sedge weeds (1). Current weed management practices of mechanical, cultural, and chemical methods need to be reassessed in the wake of increasing concerns about economical and environmental sustainability of intensive rice production (31,44).

Biological control of weeds may have a place as a component of integrated pest management (IPM) programs for rice in Southeast Asia (44). Two isolates of *Curvularia tuberculata* Jain, one from diseased *Cyperus difformis* L. (small flower umbrella sedge) (93-020) and the second from *Cyperus iria* L. (rice flat sedge) (93-022), and one isolate of *Curvularia oryzae* Bugnicourt (93-061), from *Fimbristylis miliacea* (L.) Vahl (globe fingerush) in the Philippines, were evaluated as possible biological control agents for these sedge weeds. Foliar application of conidial suspensions of these three *Curvularia* isolates killed seedlings of *Cyperus difformis*, *C. iria*, and *Fimbristylis miliacea* (See ADDENDUM).

Species of *Curvularia* occur mostly as tropical and subtropical facultative plant pathogens with teleomorphic states in *Cochliobolus* and *Pseudocochliobolus* (37). *C. tuberculata* is reported as a pathogen of guava fruit (*Psidium guajava*) (20), citrus (23), sorghum (*Sorghum bicolor*) (22), sugarcane (*Saccharum officinale*) (39), coconut (*Cocos nucifera*) (29), mango (*Mangifera indica*) (24), *Cosmos* (9), and purple nut-sedge (*Cyperus rotundus*) (30). *C. tuberculata* has also been recovered from soil, wood, and fabric (37,40).

Curvularia oryzae was originally reported from rice grains and also causes a fruit rot in okra (*Abelmoschus esculentus*) (21). *C. verruculosa* (2), *C. cymbopogonis* (38), and *C. lunata* (4) may cause leaf spots on rice under certain conditions, but these diseases are not economically important (33).

Safety of a potential weed biocontrol agent, especially to the crop where it will be used, is one of the primary considerations for bioherbicide development (43). Therefore, it is necessary to demonstrate that these fungal isolates will not cause damage to rice. Information about the resistance mechanisms operating during the pre- and postpenetration stages of the infection process will be important in assessing the compatibility between the weed biological control agent and the crop plant. There is no record of *C. tuberculata* or *C. oryzae* causing a foliar disease on rice. However, *C. tuberculata* and *C. oryzae* have been isolated from rice grains (3,16) and implicated in the black kernel disease of rice along with 12 other *Curvularia* species (33,45). Black kernel is considered a minor disease of rice and is little studied (45). The mode of pathogen entry and the infection process in black kernel disease have not been elucidated. There is no information on resistance to this disease in different rice cultivars. This work reports the host reactions of seedlings of several rice cultivars to inoculations with *C. tuberculata* and *C. oryzae*. The histological responses of highly to moderately resistant cultivars of rice to the three *Curvularia* isolates were also studied.

MATERIALS AND METHODS

Fungal isolates. *C. tuberculata* was isolated from diseased *Cyperus difformis*

Corresponding author: Alan K. Watson
E-mail: alan.watson@mcgill.ca

Current address of L. Z. de Luna: USDA-ARS, Land Management and Water Conservation, 221 Johnson Hall, Washington State University, Pullman, WA 99164-6421.

Current address of T. C. Paulitz: USDA-ARS, Root Disease and Biological Control Lab, 363 Johnson Hall, Washington State University, Pullman, WA, 99164-6430.

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(isolate 93-020) and also from diseased *C. iria* (isolate 93-022) from Lian, Batangas, Philippines. *C. oryzae* (isolate 93-061) was obtained from diseased *F. miliacea* from Manicahan, Zamboanga, Philippines. All fungal isolates were cultured on half-strength potato dextrose agar (½PDA) plates for initial observation and later stored on agar-oil slants and/or in soil cultures.

Host plants. Seeds of 13 rice cultivars were obtained from the International Rice Research Institute (IRRI) Genetic Resources Center and included indica, japonica, and javanica types as well as IRRI hybrid cultivars. Rice cultivars were chosen on the basis of their contrasting agronomic traits. Seeds of *Cyperus difformis*, *C. iria*, and *F. miliacea* were obtained from mature plants in the rice fields in the vicinity of Los Baños. Seeds of rice and weeds were germinated in petri dishes lined with moist filter paper. One-week-old seedlings were transplanted into 10-cm-diameter standard plastic pots filled with field soil (Maahas clay, Suborder Haplustic, Order Alfisol). The pots were then placed in a large pushcart, and a 2- to 3-cm layer of water was maintained in the pushcart to keep the soil moist. The seedlings were maintained in the greenhouse at 35/25 ± 5°C day/night temperature.

Preparation and application of inoculum. Agar plugs from 1-week-old cultures of *C. tuberculata* and *C. oryzae* were used to seed ½PDA plates for the mass production of the inoculum. Seeded plates of isolates 93-020 and 93-022 were incubated in the dark at 28°C, while cultures of 93-061 were maintained under continuous light (30 µE·m⁻²·s⁻¹) at 28°C. Spore suspensions were prepared by flooding each dish with distilled water and lightly scraping the surface of the colony with a clean glass slide or a camel's hair brush. The suspension was filtered through cheesecloth to separate mycelial fragments from the conidia. Spore concentration was determined using a hemacytometer, and 2-week-old seedlings were sprayed at the rate of 1 × 10⁸ conidia m⁻². Tween 20 (oxysorbic [20 POE] polyoxyethylene sorbitan monolaurate) was added at the rate of two drops per 100 ml of spore suspension. The suspensions were applied with an atomizer connected to a pressure source. At this stage, rice, *Cyperus difformis*, and *C. iria* had three to four expanded leaves, and *F. miliacea* had five to six leaves. Control plants were sprayed with distilled water with Tween 20.

The control and inoculated seedlings were incubated in dew chambers in the dark at 28°C and 100% relative humidity (RH) for 24 h and then transferred to the mist room of the greenhouse at 24 to 28°C and 80 to 95% RH (47). The experiment was a completely randomized design with six replications. The experiment was repeated once.

Evaluation of varietal response to *C. tuberculata* and *C. oryzae*. The inoculated and control plants were scored at 7 and 14 days after inoculation (DAI) using a key (Table 1) modified from Mardinus (26). Symptom expression on rice cultivars did not change from the 7 DAI to the 14 DAI observation, thus only the 7 DAI data are presented. Scores for each of the three isolates were analyzed using the Kruskal-Wallis ANOVA on ranks (SigmaStat for IBM PC, version 1.0, Jandel Scientific Corporation, 1992-1994). The reactions of rice seedlings were reported as median scores. Results of the two trials were pooled as homogeneity of variance was confirmed by Bartlett's test (10). Plant height differences, if present, were evaluated visually. Relative sporulation of the *Curvularia* isolates was assessed by excising leaf pieces with lesions, placing them in moist chambers, and observing microscopically after 24, 48, and 72 h. Sporulation was visually rated as sparse, moderate, or heavy.

Histopathology of infection. The third and fourth leaves of IR 64 and Norin 21 rice from infected and uninfected (control) plants were randomly harvested at 2, 4, 8, 16, 20, 24, 36, 48, 72, 96, 120, 144, and 168 h after inoculation (HPI). Two sets of leaf samples were obtained: the first for whole leaf clearing and the second for paraffin imbedding. Leaf segments for whole leaf clearing were placed into vials containing 70 parts ethanol:30 parts glacial acetic acid, while the leaf segments for cross-sectioning were fixed in formaldehyde-acetic acid-alcohol solution (FAA) (17). Leaf samples obtained after 2 and 4 HPI were observed under the microscope for spore deposition before they were subjected to leaf clearing. Twenty or more 1-cm leaf segments from the leaf tips, the middle portions of the lamina, and the leaf bases from each isolate-cultivar combination were examined.

Leaf segments were immersed several times in the alcohol-acetic acid clearing solution until they appeared translucent, and then stained in lactophenol-acid fuchsin for 15 min or until a good contrast was achieved between the epidermal cells (colorless) and

the fungal pathogen (deep pink) (6). Stained tissues were dipped in lactophenol to remove excess stain and mounted on glass slides using 50% glycerin. Microscopic observations were conducted and photographs were taken using a compound microscope mounted to a Nikon 35-mm camera.

Leaves for cross-sectioning were prepared following the method of Johansen (17). Twenty or more leaf segments from each of the isolate-cultivar-harvest time combinations were dehydrated in a graded ethanol-tertiary butyl alcohol series, cleared, and then infiltrated with Paraplast (Fisher Scientific, Montreal, QC) in a 56°C oven. After three changes, they were embedded in the same medium and sectioned using a rotary microtome. The sections (12 µm thick) were affixed to clean glass slides using egg albumin and stained with safranin-fast green. Microscopic examinations were performed and photographs taken as described above.

RESULTS

Evaluation of varietal responses to *C. tuberculata* and *C. oryzae*. The response of rice cultivars to *Curvularia* isolates 93-020, 93-022, and 93-061 ranged from immune to susceptible, but most cultivars were resistant to the isolates (Fig. 1A to C). However, most inoculated rice seedlings developed only small leaf spots and browning of the leaf tips and margins, indicating an incompatible disease reaction. Lesions were formed only on fully expanded leaves and were first observed between 3 and 5 DAI, demonstrating susceptibility of older leaves. Younger leaves that unfolded after inoculation did not show any symptoms and remained healthy throughout the experimental period. The leaf tips and margins of susceptible rice cultivars were the most vulnerable parts to invasion as necroses were concentrated in these areas. Clumps of conidia were trapped by trichomes present on these sites, thus increasing the probability of infection. In resistant rice cultivars, leaf tips and margins were also invaded, but appeared dry and cream to light brown.

C. oryzae (93-061) was more pathogenic to the rice cultivars tested than the two

Table 1. Disease severity key used to score the reaction of rice seedlings to three isolates of *Curvularia*^a

Score	Host reaction	Description
0	I – immune	No visible symptoms
1	HR – highly resistant	Few to many brown specks, 0.5 to 1.0 mm in diameter, grayish-green or light brown leaf tips
2	MR – moderately resistant	Discrete brown lesions with or without chlorotic halo, 1.0 to 2.0 mm long, brown leaf tips and margins
3	MS – moderately susceptible	Many brown spots, 2.0 to 3.0 mm long, a few lesions coalescing, brown leaf tips and margins
4	S – susceptible	Many lesions with necrotic centers, 3 to 5 mm long, 10 to 50% of leaf area killed
5	HS – highly susceptible	Numerous large and expanding lesions, more than 50% of leaf area killed, plant collapsed

^a Modified from Mardinus (26).

Fig. 1. A, Disease severity score of rice (*Oryza sativa*) cultivars inoculated with *Curvularia oryzae* isolate 93-061. **B,** Disease severity score of rice cultivars inoculated with *Curvularia tuberculata* isolate 93-020. **C,** Disease severity score of rice cultivars inoculated with *C. tuberculata* isolate 93-022. Seven days after inoculation. Data represent median scores from two trials. Boxed area represents the 25th and 75th percentile, a line within the box indicates the median, and the whiskers represent the 10th and 90th percentile. The median is indicated by a dot when it occurs at the border of the boxed area. Sporulation is indicated by – (no sporulation), + (sparse sporulation), or ++ (moderate sporulation).

isolates of *C. tuberculata*. Of the 13 cultivars, two were susceptible, four were moderately susceptible, six were moderately resistant, and one was highly resistant to *C. oryzae* (Fig. 1A). By comparison, only one variety was susceptible to *C. tuberculata* isolate 93-020 and the other 12 were moderately to highly resistant (Fig. 1B); meanwhile, all of the 13 cultivars were moderately to highly resistant to *C. tuberculata* isolate 93-022 (Fig. 1C).

There was no mortality associated with disease development in any of these rice cultivars. Seven days after inoculation, slight stunting was evident in those cultivars expressing susceptibility to the *Curvularia* isolates. Sparse to moderate sporulation from the necrotic spots was detected in the susceptible, but not in the resistant, cultivars (Fig. 1A to C). Untreated rice and weed seedlings were healthy and did not develop any symptoms. However, when *Cyperus difformis*, *C. iria*, and *F. miliacea* were inoculated with their respective *Curvularia* pathogen, leaf spots developed after 3 days and rapidly coalesced to form large blighted areas. The infected weeds eventually collapsed and died 7 to 14 DAI.

Histopathology of infection. Reactions of IR 64 and Norin 21 to the *Curvularia* isolates ranged from moderately to highly resistant (Fig. 1A to C). The infection process of the three isolates was similar, and the histopathological reactions of the highly resistant rice cultivars differed from the moderately resistant ones only with respect to the number and size of lesions produced. Necrotic areas in the leaf tips and margins also were observed more frequently on moderately resistant cultivars.

Responses to *C. tuberculata* (isolates 93-020 and 93-022). Spores germinated after 4 h on IR 64 and Norin 21, and germ tubes emerged only from the hilar or the apical ends of the spore. By 24 HPI, most of the spores had germinated. The direction of germ tube growth was predominately ($\pm 75\%$) random, although some were found to follow the long axis of the epidermal cells. Appressoria were mainly ($\pm 85\%$) formed over the stomata (Fig. 2A) beginning 24 HPI. These prepenetration events of the two isolates of *C. tuberculata* in IR 64 and Norin 21 were similar to those that occurred in the sedge weed hosts (L. Z. de Luna, A. K. Watson, and T. C. Paulitz, unpublished). After penetration, infection hyphae were observed within the epidermal cells, but their growth was restricted and slow. Yellowing and browning of the infected cell(s) was pronounced. Small lesions were present on the leaf lamina (Fig. 2B) at 168 HPI.

In Norin 21, appressoria developed preferentially over stomata, but some were formed over the epidermal or bulliform cells (Fig. 2C). They were round, oval, or clavate and 3 to 7 μm in diameter. Some epidermal cells in the vicinity of the germ

tubes or appressoria became granular in appearance (Fig. 2D).

C. tuberculata isolate 93-022 caused small lesions in the epidermal and mesophyll cells of Norin 21 (Fig. 2E). Although this rice variety had a median disease severity score of "0" (immune) when inoculated with 93-022, some plants developed tiny flecks that became more apparent when histological examinations were performed. Cross-sections of the lesions revealed that the growth of the infection hyphae was sparse and that a yellowing reaction was present at the border of the lesion (Fig. 2E). This indicates that Norin 21 exhibits a high degree of resistance to isolate 93-022. No sporulation was observed in IR 64 or in Norin 21 when inoculated with these *C. tuberculata* isolates.

Responses to *C. oryzae* (isolate 93-061). The reactions of IR 64 and Norin 21 to *C. oryzae* were similar. Spores germinated within 2 h, and germ tube growth was both perpendicular and parallel to the epidermal cells. Appressorium formation began 24 h after inoculation and preferentially developed over the stomatal region, although a few were formed over the epidermal cells. Appressoria were round or clavate (Fig. 2F). Attempted penetration was immediately accompanied by the yellowing of the guard cells or epidermal cells underneath the infection structure. Infection hyphae were detected within the leaf tissues (Fig. 2G).

A narrow penetration peg (Fig. 2H) accomplished the penetration process. When the appressorium was removed, a small hole was observed where the penetration peg entered the cell (Fig. 2H). The upper cuticle where the appressorium was formed appeared yellow, while the lower cuticle and the rest of the upper cuticle remained red from the safranin stain (Fig. 2H). A stoma directly underneath a branched germ tube appeared yellow and granular (Fig. 2I). Initially, yellowing was confined in the intercellular regions of the mesophyll layer (Fig. 2I), but it later spread intercellularly even without evidence of penetration (Fig. 2J).

Immediately after penetration, primary infection hyphae were formed and grew within the epidermal cells. The spread of the fungus to the neighboring cells was, however, slow. By 72 HPI, only a few cells in the mesophyll region closest to the epidermis had been infected and further colonization of this region was slow. Small, brown spots were produced at 168 HPI consisting of collapsed epidermal and mesophyll cells, but no sporulation occurred in these lesions.

DISCUSSION

A compatible disease reaction occurs when symptoms are clearly expressed as opposed to an incompatible reaction where the symptoms do not develop and the effect on the plant is minimal (25). When

inoculated with their respective *Curvularia* pathogens, *Cyperus difformis*, *C. iria*, and *F. miliacea* rapidly developed coalescing foliar lesions and a blighting reaction that resulted in plant death within 1 to 2 weeks. However, the effect of the pathogens on rice was, for the most part, an incompatible reaction.

A high degree of resistance was exhibited by rice to *C. tuberculata* and *C. oryzae*. *C. oryzae* was the most virulent to the rice cultivars, although half of the cultivars tested were resistant. *C. tuberculata* was less virulent, with most of the rice cultivars resistant to isolate 93-020 and all resistant to isolate 93-022. Host specialization has apparently occurred in *C. tuberculata*. A *C. tuberculata* isolate from Rangpur lime (*Citrus limonia*) was not pathogenic to rice (Taichung Native 1), but it caused severe blighting and death of a local maize variety (23). In our study, isolate 93-022 from *Cyperus iria* was less pathogenic to rice compared with isolate 93-020 from *C. difformis*.

Sporulation of the *Curvularia* isolates did not occur on the resistant rice cultivars. Although sporulation is never an isolated component of resistance (5), it is often the most sensitive indicator of race-specific (vertical) and race-non-specific (horizontal) resistance (18). In most cases, inhibited infection and colonization are accompanied by reduced sporulation (5). Rice cultivars that were resistant to the *Curvularia* isolates, such as Norin 21 and Red Khosha Cerma, were characterized by limited lesion development and inhibition or absence of sporulation. Moderately susceptible to susceptible rice cultivars such as IR 58 and PSBRC 20 exhibited more leaf necrosis and sparse to moderate sporulation.

Older leaves were more susceptible than younger leaves to inoculations of *C. tuberculata* and *C. oryzae*. Resistance to pathogens is often affected by the developmental stage of the plant or of the plant organ (8), with older plants appearing to be more resistant (15), although this is not always the case (35). Lesions on the leaf margins grew inward from the edges of the leaf. In rice, hydathodes are present at the margins and leaf tips (14), and these could have facilitated the entry of the fungus. Hydathodes serve to secrete excess water from the interior of the leaves but may also provide an opening on the epidermis through which pathogens can enter (32).

Surface development of *C. tuberculata* and *C. oryzae* on the leaves of IR 64 and Norin 21 did not differ and was similar to the development events recorded for the sedge hosts (L. Z. de Luna, A. K. Watson, and T. C. Paulitz, unpublished). Sivanesan (40), however, reported that *C. tuberculata* exhibited bipolar germination and sometimes lateral germination. There were no obvious signs of orientation for the direction of the germ tube growth. Germ tubes developed perpendicularly and parallel to

the long axis of the rice epidermal cells, but there was generally less branching than observed on the sedge host's leaves.

Only simple appressoria were formed in the rice hosts treated with any one of the three isolates. Compound appressoria or infection cushions as described by Emmett and Parberry (7) were not observed. It is possible that the different surface architecture of rice plants did not provide the appropriate stimulus for the infection cushion formation.

Most appressoria in this study were formed over the stomatal aperture in rice, and penetration of epidermal cell walls occurred infrequently. This is in contrast to what occurs in sedge hosts, where epidermal cell wall junctions were the preferred penetration sites. Since there was no indication of the orientation of the germ tube growth in both *C. tuberculata* and *C. oryzae* in rice, it is possible that a substance present in the area of the stomatal aperture could be the stimulus for appressorium formation. Evidence of the involvement of chemical stimuli in appressorium formation has been identified for the rust fungi and include K⁺ and Ca²⁺ ions (19), sucrose (13), and a volatile compound from the host plants (11).

The occasional penetration of the epidermal cells could be due to the leaching of stimulatory substance(s) from cuticular breaks that resulted from the opening and closing of the stomata (46). Few appressoria were formed over bulliform cells. Bulliform cells have been reported as the preferred penetration sites for *Pyricularia oryzae* (12).

Stomata occur on the adaxial and abaxial sides of the rice leaf in files between the veins (14), while in the sedges, they are found only on the abaxial side (28). Huge hydathodes or hydropores occur along the leaf margins at the edges of the rice leaf blade. These structures have pore diameters more than twice as large as those of ordinary stomata and are involved in the guttation of water from the leaf when active water uptake is occurring in the plants (14). Since the lesions observed along the leaf margins developed from the edge to the inner part of the leaf lamina (Fig. 2G), this suggests that hydathodes are also the entry sites of *C. tuberculata* and *C. oryzae* into rice.

In sedge hosts, infection structures were observed between 8 and 12 HPI, but in rice, appressoria did not form until 20 to 24 HPI. The repression of the ability of pathogens to form infection structures can be viewed as a defense mechanism to disease (27).

The mode of penetration of rice by the three isolates followed the same pattern as that observed in the sedge hosts. A fine penetration peg that emerged from the appressorium (Fig. 2C) accomplished penetration. Both mechanical force and enzymatic action were apparently required

Fig. 2. Histopathology of infection of *Oryza sativa* cvs. IR64 and Norin 21 by *Curvularia tuberculata* (isolates 93-020 and 93-022) and *C. oryzae* (isolate 93-061). **A**, Appressorium (A) formation on stomata of cv. IR64 by isolate 93-022, 24 hours postinoculation (HPI). **B**, Transverse section of a small lesion (L) on the leaf blade of cv. IR64 by isolate 93-020, 72 HPI. **C**, Transverse section of a conidium (C) and appressorium (A) of isolate 93-020 over bulliform cells of cv. Norin 21, 24 HPI. **D**, Granular appearance of mesophyll cells (MC) of cv. Norin 21 beneath a stomata with a germ tube (GT) and an appressorium (A) from isolate 93-022, 72 HPI. **E**, Restricted lesion (L) formation in cv. Norin 21 by isolate 93-022 at 168 HPI. **F**, Penetration of a stomata by a penetration peg (PP) arising from an appressorium (A) of isolate 93-061 in cv. IR64, 36 HPI. **G**, Infection hyphae (IH) of isolate 93-061 in transverse leaf section of cv. IR64, 144 HPI. **H**, A hole is revealed in the penetration site (PS) where an appressorium (A) of isolate 93-061 penetrated the epidermal cell wall of cv. Norin 21, 72 HPI. Note yellowing of cuticle (CT). **I**, Appressorium (A) of isolate 93-061 penetrating a stomata that has turned yellow. Note yellowing reaction (YR) in some mesophyll cells (MC) underneath the invaded stomata of cv. Norin 21, 72 HPI. **J**, Yellowing reaction in some mesophyll cells (MC) of cv. Norin 21 underneath conidia (C) and germ tubes (GT) of isolate 93-061, 72 HPI. EC, epidermal cell; GT, germ tube; CT, cuticle.

for penetration since a hole was present (Fig. 2D) and yellowing of the cuticle beneath and adjacent to the appressorium was observed (Fig. 2F). Differential staining of the area beneath, adjacent to, or just ahead of the hyphal tips and infection structures has been previously reported (41).

After penetration, primary infection hyphae were observed but their growth was restricted and slow. Restricted colony and lesion formation are considered components of resistance, since these reflect the growth rate of the pathogen in the host and therefore its spore production (34). In the resistant type of host-pathogen combinations of Peta rice and Philippine races of *P. oryzae*, browning of the tissues occurred at a faster rate, inhibiting lesion formation, which resulted in a resistant lesion type (42). The expression of race-specific resistance to *P. oryzae* involves the inhibition of fungal hyphae growth, accompanied by light browning of epidermal cells in rice leaves (36).

In this study, partial resistance of IR 64 and Norin 21 to *C. tuberculata* and *C. oryzae* was operative during the prepenetration phase as expressed in the delay of the initiation of infection structure formation. Postpenetration resistance mechanisms were expressed as inhibition of fungal growth after penetration and lack of sporulation.

Species of *Curvularia* are commonly saprophytic or weakly pathogenic, and rarely virulent (45). Seshadri et al. (39) noted that dense spotting and extensive blighting of sugarcane leaves from a *C. tuberculata* infection was only observed on a few severely affected plants, and concluded that the disease was of negligible importance. It is possible that *Curvularia* leaf spots do occur on rice in fields, but the disease is so minor that it has gone completely unnoticed. The implication of minor foliar lesions on rice in the appearance of black kernel is not known. Similarly, the possible role of *Curvularia* blights of sedge weeds in the rice field on black kernel disease of rice is unknown.

This study has been part of a biological weed control project, and although extreme host specificity is not needed for indigenous plant pathogens that are to be developed as bioherbicides, the pathogens must be "sufficiently safe" to desirable plant species (43). Resistance to *C. tuberculata* isolate 93-022 was expressed in all rice cultivars from the three ecogeographic races or types (indica, japonica, and javanica). Further studies of isolate 93-022 are needed to clearly determine its host range, to evaluate its safety and potential as a biocontrol agent for sedge weed species in rice production.

ADDENDUM

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