Ustilago maydis Infection of the Nonnatural Host *Arabidopsis thaliana*

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ABSTRACT

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The experimental infection of *Arabidopsis thaliana* by the maize phytopathogenic hemibasidiomycete *Ustilago maydis* under axenic conditions is described. When plantlets were inoculated with mixtures of compatible haploids, the fungus was able to grow on the plant surface of inoculated seedlings in the form of white mycelium and invade the tissues, probably penetrating through stomata; however, it did not form teliospores. Symptoms of disease were increased anthocyanin formation, development of chlorosis, increased formation of secondary roots, induc-

tion of malformations in the leaves and petioles, induction of tissue necrosis, and stunting. In several cases, death of the invaded plants occurred. Interestingly, inoculation of single *U. maydis* haploid strains produced similar symptoms in *Arabidopsis* plantlets. In contrast, several mutants avirulent to maize also were avirulent or less virulent than wild-type strains on *Arabidopsis*. Collectively, the reported data suggest that the *U. maydis*-*Arabidopsis* pathosystem may constitute a useful experimental model for the analysis of some aspects of the virulence factors of the fungus. With the study of nonhost responses and their comparison to those occurring during maize infection, we will be able to elucidate some obscure aspects of *U. maydis* pathogenicity in the future.

Ustilago maydis (DC.) Cda. is a pathogenic hemibasidiomycete with a very narrow host range. This fungus normally infects only maize (*Zea mays* L.) and its putative ancestor, teozintle (*Z. mays* subsp. *parviglumis*). *U. maydis* research has a long tradition in fungal biology and genetics (21). It has been used to study the molecular basis of such diverse phenomena as mating-type determination, homologous recombination, and signaling pathways (reviewed in 3–5,10). Pathogenicity and the sexual cycle of the fungus are closely intertwined processes (12,21). They start by the fusion of two haploid cells (sporidia) of compatible mating types carrying different alleles at two loci: *a* and *b*, the first with two, and the second with multiple alleles (11,25,26,40). After cell fusion a pathogenic dikaryotic hypha is formed that invades the plant host and grows into its tissues in the form of mycelium. After a complex series of morphological changes the mycelium septates to form diploid teliospores (9). At the end of the infection cycle, millions of dark teliospores fill the galls characteristic of the disease. Meiosis occurs during germination of teliospores and leads to the eventual formation of basidiospores, which reinstate the cycle (reviewed in 4,5,10,37).

In nature, the mycelial form of growth is only observed in dikaryotic cells growing in the plant, whereas the free-living saprophytic form is haploid and grows as budding yeasts. Nevertheless, transient mycelial growth may be obtained on solid complex medium when two compatible haploid strains are mixed. This reaction, named “Fuz” (6–8,14), is a useful test for mating compatibility. The dimorphic transition of both haploid and diploid cells can

be triggered by a change in the pH of the culture medium (36). At neutral or near neutral pH, cells grow in the yeastlike form, whereas at a pH value below 5.0, mycelial growth is displayed.

According to these data, it is apparent that the pathogenic process of *U. maydis* is complex, involving morphogenetic transitions and the completion of its sexual cycle. Accordingly, the need for a more amenable system to analyze the pathogenic process was foreseen. The aim of this work was not a mere observational approach, but to explore the experimental infection of *Arabidopsis thaliana* plants as an alternative system to analyze various aspects of *U. maydis* pathogenicity. Previous work in our laboratory has revealed that *U. maydis* infects other plants besides maize under axenic experimental conditions (27). *A. thaliana* has several advantages over other hosts: its small size, short life cycle, the availability of the complete genome sequence (46), and the existence of tagged mutant collections (1,41). Further, *A. thaliana* has been developed as a model for plant-pathogen interactions (13,22,24). In the present work we provide evidence that *A. thaliana* is a useful model for nonhost responses, and to elucidate some aspects of the *U. maydis* pathogenicity.

MATERIALS AND METHODS

Plant and fungal strains, media, and culture conditions.

A. thaliana L. wild-type *Arabidopsis* Landsberg *erecta* (Ler) was used as host for *U. maydis*. Seeds were surface-sterilized by soaking in 95% ethanol for 5 min, transferred to a sodium hypochlorite solution (1.2% free chlorine) for 5 min, and washed at least three times with sterile distilled water. The seeds were placed over plates of sterile solid (0.6% agar) MS medium (31) normally without sucrose (20 seeds per plate) and incubated in a chamber maintained at 26 to 27°C and 99% humidity with a photoperiod of 12 h light.

Z. mays cv. Cacahuazintle seeds were germinated in soil as described previously (28).

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The strains of *U. maydis* used in this study are described in Table 1. Strains were maintained at -70°C in 50% (vol/vol) glycerol. When necessary, aliquots were transferred to 3 ml of liquid complete medium (CM) (21) held in test tubes and shaken at 28°C for 2 to 3 days. These cultures were used as inocula for all subsequent experiments. Aliquots of the cultures (0.02 ml) were inoculated into 3 ml of CM or minimal medium (MM) (21) maintained in test tubes and incubated with shaking at 28°C for 24 h. When necessary, hygromycin (150 $\mu\text{g/ml}$) or carboxin (40 μM) were added to the culture media. For plant inoculation, *U. maydis* cells were recovered by centrifugation, washed twice with sterile distilled water (to eliminate growth media in the inoculum), and resuspended in sterile distilled water to obtain a concentration of 10^8 cells per ml.

Recovery of fungal cells from infected plants. Plant tissue was ground with a sterile mortar and pestle and appropriately diluted with sterile distilled water, and aliquots were inoculated on plates of pH 7.0 MM (21) containing 1% (wt/vol) charcoal to obtain isolated colonies. Plates were incubated at 26°C for 18 h. Haploid cells were distinguished from diploids by means of the Fuz reaction (6). Haploid *U. maydis* cells grew as smooth colonies, whereas diploids grew as white colonies with aerial mycelium (Fuz phenotype). The phenotype was confirmed by re-inoculation on fresh plates. Confirmation of their diploid stage was obtained by inoculation of maize plants (described below).

Inoculation–*Arabidopsis* plants. Plantlets were inoculated with single haploid strains or mixtures of strains belonging to opposite

mating types. For this purpose, aliquots of the cell suspensions (described previously; 0.2 μl) containing 10^1 to 10^5 cells per inoculum (described below) were deposited at the vegetative apex of *Arabidopsis* seedlings (usually 6 days old) with the help of a 2- μl micropipette equipped with a thin tip. Seedlings were incubated as described previously and observed periodically for the appearance of signs and symptoms of infection.

Soil-grown maize plants. Aliquots (100 μl , 1×10^8 cells per ml) of the *U. maydis* cell suspensions of a mixture of haploid cells, or diploids recovered from Fuz colonies, were inoculated with a syringe and needle in the region around the second internode of 9 to 10 maize plantlets per isolate and incubated as described (29).

Microscopic observations. Infection and development of *U. maydis* in *Arabidopsis* were followed by both macroscopic and microscopic observation. Whole leaves or hand-made sections of infected plants obtained with a razor blade were stained with lactophenol-cotton blue and observed under bright-field optics or differential interference contrast (DIC). In some experiments, fresh tissue was heated at 95°C in 70% ethanol for 5 min to remove chlorophyll, washed with distilled water, and stained as described for microscopic observation.

Leaves or sections of plants inoculated with a strain of *U. maydis* expressing the green fluorescent protein (GFP) were observed by confocal microscopy.

Nuclei of fungal cells were stained with DAPI (4'6'-diamidino-2-phenylindole). Dye solution in distilled water (1 $\mu\text{g/ml}$) was ap-

TABLE 1. Strains of *Ustilago maydis* used in this study

Strain	Significant genotype	Significant phenotype	Origin or reference
1/9	<i>a1b1 uac1::Ble</i>	Adenylate cyclase minus, nonvirulent	(17)
1/68	<i>a1b1 ubc1::hyg</i>	Regulatory PKA subunit minus, not producing galls	(16)
GFPX	<i>a2b2 GFP Cbx</i>	Expressing the green fluorescent protein	From A. Martínez (University of Georgia), derived from a diploid from Spellig et al. (44)
BX5	<i>a2b2 X::Cbx</i>	Carboxin resistant, nonvirulent	(39)
BX28	<i>a2b2 X::Ben</i>	Benomyl resistant, nonvirulent	(29)
CL21B	<i>a1b1 myc⁻</i>	Yeast monomorphic, nonvirulent	(28)
CL211	<i>a2b2 myc⁻</i>	<i>Ibid</i>	(28)
CL235	<i>a2b2 myc⁻</i>	<i>Ibid</i>	(28)
FB1	<i>a1b1</i>	Wild-type	F. Banuett, California State University Long Beach
FB2	<i>a2b2</i>	Wild-type	<i>Ibid</i>
GP25	<i>a2b2 gcn5::hyg</i>	DNA acetylase minus, nonvirulent, mycelial	(18)
LG1	<i>a1b1 odc::hyg</i>	Ornithine decarboxylase minus, low virulence	(19)

Fig. 1. Disease progression in *Arabidopsis* seedlings inoculated with *Ustilago maydis*. Seven-day-old seedlings of *Arabidopsis* were inoculated with different densities of a mixture of sexually compatible haploid strains of *U. maydis* (FB1 $\times$ FB2). At the times indicated, disease index was assessed: **A**, 10^1 cells; **B**, 10^2 cells; **C**, 10^3 cells; **D**, 10^4 cells; and **E**, 10^5 cells. Standard deviation values are shown as error bars.

plied to the plant tissue preparations and observed by epifluorescence. Observations at low magnification were carried out with a stereomicroscope (Leica MZ-8; Leica, Herrbrugg, Switzerland). Microscopic observations under bright field, DIC, or epifluorescence were carried out in a Leica DMRE Microscope (Leica, Wetzlar, Germany). Photographs were obtained with a Spot digital camera (Diagnostic Instruments, Sterling Heights, MI). Confocal microscopy was performed with a confocal microscope (Leica).

Determination of disease index. To obtain a quantitative description of the infection severity in *Arabidopsis*, we searched for typical disease signs and symptoms: fungal growth on the plant, increased anthocyanin production, development of chlorosis, necrosis of tissues, etc. The following disease rating values were given: 0, no signs or symptoms; 1, significant amounts of mycelial growth in or on the plant; 2, mycelial growth in or on the plants and anthocyanin accumulation; 3, mycelial growth and development of chlorosis; 4, mycelial growth, accumulation of anthocyanins, and development of chlorosis; and 5, death of the plant. Disease index was calculated as Σ disease ratings divided

by the number of plants (16). Data were subjected to statistical analyses using two-sample comparison by the Mann-Whitney test. Differences between controls and problems were considered significant at P values of <0.05 , and very significant at P values of <0.01 .

Cells were counted with a hemacytometer. Wet weight was used to measure plant growth. Pathogenicity experiments on *Arabidopsis* were repeated at least three times, with at least 15 plants per experiment. Data shown correspond to representative experiments.

RESULTS

Establishment of experimental conditions for *Arabidopsis* infection by *U. maydis*. To determine conditions that allowed infection of *Arabidopsis* plants by *U. maydis*, a mixture of wild-type sexually compatible *U. maydis* sporidia (FB1 $\times$ FB2) was deposited on the epidermis of different plant tissues. Best results were obtained when the inoculum was placed at the vegetative apex. Wounding of the plant tissue by abrasion or injection was attempted, but was unnecessary because it did not result in an

Fig. 2. Signs and symptoms of the infection of *Arabidopsis* seedlings inoculated with a mixture of FB1 and FB2 strains of *Ustilago maydis*. **A and E**, Uninoculated control plants. **B, C, D, and F**, Infected plants. **B**, White mycelium growing on *Arabidopsis* tissues 48 h after inoculation. **C**, Anthocyanin accumulation. **D**, Plants showing necrotic areas and mycelial growth (yellow arrows). **E**, Close-up of petioles in 14-day-old control plants. **F**, Malformations observed in the petioles of infected plants (arrow) 7 days after inoculation.

increase in infection rate. Although infection could be obtained in soil-grown *Arabidopsis*, use of in vitro-grown plantlets gave more consistent results under controlled conditions. Additionally, these conditions guaranteed that the symptoms of the infection observed were due to *U. maydis*. The invading fungus was identified by microscopic observation and could be recovered from the infected tissues.

Effect of the density of inoculum on the infection of *Arabidopsis*. Seedlings of *Arabidopsis* seedlings were extremely sus-

ceptible to infection by *U. maydis*. Under axenic conditions, inoculation with as few as 10 fungal cells (a mixture of compatible FB1 and FB2 wild-type cells) resulted in a productive infection (Fig. 1). For practical reasons we normally used an inoculum of 10^4 cells. Application of larger inocula sometimes resulted in invasion of the medium by the fungus, which grew in the form of slimy smooth masses and produced damage to the plants, possibly by interference with oxygen diffusion. Whenever this happened, contaminated plates were discarded. Elimination

Fig. 3. Effect of *Ustilago maydis* infection on growth and development of *Arabidopsis* seedlings. Photographs of whole **A**, control and **B**, infected plants 14 days after infection with a mixture of FB1 and FB2 strains of *U. maydis*. Photographs showing root development of **C**, control and **D**, infected plants. Notice the increased number of secondary roots in infected plants.

of sucrose from the culture medium almost completely eliminated this invasion problem.

Signs and symptoms during the infection process. Symptoms of infection of *Arabidopsis* seedlings by *U. maydis* were similar to those observed with other dicot and monocot species (27). The first sign of infection in seedlings inoculated with the mixture of wild-type strains of *U. maydis* was the appearance of white mycelial growth at the site of inoculation. Mycelial growth was observed on the epidermis of infected tissues as early as 18 h after inoculation. After 48 h the fungal mycelium extended along the hypocotyl and invaded the surface of newly formed leaves (Fig. 2B). About 72 h after inoculation, accumulation of anthocyanins occurred (Fig. 2C), sometimes accompanied by the appearance of necrotic zones (Fig. 2D). Development of chlorosis and malformations in the leaves and petioles in the form of bulges were observed in numerous seedlings about 1 week after inoculation (Fig. 2F). At later stages of infection, we observed a net reduction in plant size when compared with noninoculated plants (Fig. 3A to B). In a typical experiment, 17 days after inoculation, wet weight of the inoculated seedlings was reduced by about 50% compared with control plants. At this age, healthy noninoculated plants weighed an average of 18.6 ± 0.70 mg ($n = 36$), while inoculated plants weighed 9.8 ± 0.24 mg ($n = 78$). Infection with

U. maydis also resulted in a noticeable alteration in root development: roots of infected plants were shorter and more branched than those of uninfected control plants (Fig. 3C and D).

U. maydis cells could be recovered from tissue sections not showing superficial growth of the fungus (described previously). When inoculated on charcoal plates, more than 85% of the fungal colonies grew in the form of mycelium (Fuz reaction). Previously, we have observed that Fuz⁺ strains recovered from the tissues of some nonhost plants infected with *U. maydis* are pathogenic to maize (27). The same results were obtained when several randomly selected Fuz⁺ colonies recovered from *Arabidopsis* plants were tested. Plants inoculated with cell suspensions of these colonies grown on liquid medium induced the appearance of chlorosis and the formation of tumors. These results are evidence that the fungus recovered from infected *Arabidopsis* plants is mainly in a diploid stage.

Microscopic observations of infected plant tissues. As indicated previously, during early stages of the infection the fungus grew in the form of white mycelium on the plant surface. Whole mounts of infected plants stained with cotton blue and observed under the compound microscope revealed that the fungus was growing not only on the epidermal surface (Fig. 4A) but also in the intercellular spaces between epidermal cells (Fig. 4B). In leaves stained with cotton blue, hyphae were observed forming appressorium-like structures and entering the plant tissues through stomata (Fig. 4C to D). Penetration of *U. maydis* through stomata was also observed in live plants infected with mixtures of sexually compatible strains, one of which constitutively expressed GFP (GFPX strain), as early as 18 h after inoculation (Fig. 4E). Using the same GFP expressing strain, mycelium was also seen growing inside the epidermal tissues, surrounding plant cells (Fig. 4F). To make a more thorough analysis of the mycelial growth in infected tissues, we examined a series of optical transverse sections through infected leaves. From these observations, it was clear that *U. maydis* mycelium was present not only within the plant tissues but also inside *Arabidopsis* cells (Fig. 5). Mycelial cells appeared long and branched, as has been previously described at the initial infection stages in maize (6), and their diameters did not differ significantly. DAPI staining of nuclei revealed the presence of a single nucleus per segment (data not shown). These data indicated that the fungus invading the plants was not in the dikaryotic stage.

Infection of *Arabidopsis* seedlings with *U. maydis* single haploid cells. It is known that in nature only heterokaryons or diploids that are formed by conjugation of sexually compatible strains of *U. maydis* are able to infect maize, the natural host of the fungus (reviewed in 2–4,38). To determine if this was the case with *Arabidopsis*, we analyzed the ability of haploid strains of *U. maydis* to infect the plant. We observed that haploid strains FB1 and FB2 were virulent, although to a lower degree than their mixture (Table 2). The mycelium observed in *Arabidopsis* plant tissues was not significantly different from that produced by the sexually compatible haploid mixtures (described previously). Development of malformations in seedlings inoculated with a single haploid strain also occurred, without a significant difference to plants inoculated with mixtures of compatible haploid strains (6 to 50% of plants inoculated with either haploid strain and 9 to 40% of plants inoculated with a mixture of sexually compatible wild-type strains showed malformations in the different experiments performed).

Infection of *Arabidopsis* seedlings with *U. maydis* mutants. Several *U. maydis* mutants that have an impaired ability to infect the natural host, maize, have previously been described (Table 1). To gain information about the similarities and differences between the infection process in *Arabidopsis* and maize, we examined the ability of these mutants to infect *Arabidopsis* seedlings. Three different monomorphic, avirulent mutants of *U. maydis* unable to grow as mycelium in vitro (28) proved to be

Fig. 4. Microscopic examination of the development of *Ustilago maydis* in *Arabidopsis* plant tissues. Seedlings of *Arabidopsis* were inoculated with a mixture of compatible strains of *U. maydis* and processed for microscopic observation. **A to D**, Plants inoculated with FB1 and FB2 strains (cotton blue stain). **E and F**, Plants inoculated with FB1 and GFPX strains, confocal microscopy. **A**, Mycelium growing on the plant epidermis. **B to D**, Detail of fungal growth after removal of aerial mass of mycelium. **B**, *U. maydis* growing in the intercellular spaces. **C and D**, Fungal growth and appressorium-like structures (arrows) at stomatal openings on the leaf surface. **E**, Composition of an array of confocal sections to show mycelial growth into the plant tissue. Notice a fungal hypha entering through a stomatal opening (arrow). **F**, Confocal section of an infected leaf showing mycelium growing inside the epidermal tissues and surrounding plant cells. Scale bar in **A**, 50 µm; scale bars in **B and D**, 10 µm; scale bars in **C, E, and F**, 25 µm.

less virulent on *Arabidopsis* plants when compared with the wild-type haploid strains (Table 3). The only sign of disease in plants inoculated with these monomorphic mutants was the superficial growth of fungal mycelium.

Several other nonvirulent strains of *U. maydis* were also found to be less virulent on *Arabidopsis* plants, especially strains BX5, BX28, and GP25. In the most extreme case, BX5 strain failed to grow at all on the plant surface (Table 3). On the other hand, *uac1* (1/9) and *ubc1* (1/68) mutants (16) were as virulent to *Arabidopsis* plants as the wild-type strains.

DISCUSSION

The results obtained in this work demonstrate that *U. maydis* is able to invade and cause significant damage to *Arabidopsis* plants under experimental conditions. The use of *Arabidopsis* as an alternative host for plant and even animal pathogens has proven to be a very powerful tool for dissecting aspects of their disease processes (13,22). *Verticillium longisporum* and *V. dahliae*, fungi that are not normal pathogens of *A. thaliana*, were found to be able to infect it under experimental conditions in the laboratory,

Fig. 5. Differential interference contrast optical sections of an infected leaf showing *Ustilago maydis* growth inside mesophyll cells (cotton blue stain). **A**, Optical section 10 μm above the epidermal surface showing the external mycelial growth. **B**, Optical section at the epidermal surface showing an open stomata. Mycelium can be seen growing below the stomatal opening. **C and D**, Optical sections 3 μm (**C**) or 6 μm (**D**) under the epidermal surface. Hyphae, now in correct focus are seen at the same focal plane as chloroplasts. **E**, Optical section 18 μm under the epidermal surface showing hyphae and chloroplasts at the same focal plane. **F**, Lower magnification of mycelium growing into infected cells. Scale bars **A to E**, 10 μm ; scale bar **F**, 25 μm .

setting the basis for the analysis of virulence factors of both fungi (45). More impressive are the studies by Rahme et al. (33) who utilized the infection of *Arabidopsis* by *Pseudomonas aeruginosa*, a bacterium that normally infects mammals, as a test model for the analysis of its virulence factors. These authors demonstrated that exotoxin A and phospholipase C, identified as virulence factors for *Arabidopsis*, were also important for the dissemination of the pathogen in its natural animal hosts. These results led Rahme et al. (33) to search for nonvirulent mutants in the plant, a simpler system than the use of small mammals (24,32).

The intimate relationship of the pathogenic phase and the sexual cycle of *U. maydis* makes it difficult to discern the specific factors involved in each one of these two important aspects of the biology of the fungus. Different authors including ourselves have previously demonstrated that some processes can be separated under artificial conditions. Mycelial growth of the transient dikaryotic state or of stable diploids occurs on solid media containing charcoal (the Fuz reaction) (6,14) or in liquid media under nitrogen deprivation (8). By means of pH control we have been able to induce mycelial growth of haploid strains of the fungus in vitro (36). Growth of *U. maydis* dikaryons or diploids and their eventual haploidization and formation of structures morphologically similar to teliospores were obtained when sexually compatible haploids were inoculated on permeable membranes sitting over embryonic maize calli (35).

We have described that under experimental conditions, *U. maydis* is able to infect a number of monocot and dicot species unrelated to maize (27). Using similar axenic conditions, we have now obtained evidence that *U. maydis* can also infect *A. thaliana*. The first sign of infection of the plants by *U. maydis* was the development of aerial mycelium, similar to that observed in the Fuz reaction. At later stages of infection, the fungus was seen penetrating the host tissues through stomata and growing abundantly in the mycelial form. Evidence that *U. maydis* invades *Arabidopsis* cells is provided by the observation that fungal hyphae were observed

in the same focal plane as the chloroplasts. Penetration by *U. maydis* in maize plants through stomata has been reported, although penetration can occur directly through the plant surface by appressorium-like structures, or through wounds (5,9 [reviews]). As occurs in maize (4,9,12), *U. maydis* induced development of chlorosis and increased synthesis of anthocyanins in *Arabidopsis* plants. Also, *U. maydis* infection interfered severely with plant growth and development, as commonly observed in experimentally infected maize plants. An interesting symptom of the infected *Arabidopsis* plants was a noticeable change in the development of the root system, a reaction possibly induced by an excess of indoleacetic acid (IAA) produced by the fungus, or by the plant through stimulation by *U. maydis*, and transported by the plant to the root system. IAA is known to be synthesized by *U. maydis* in vitro (43,47) and to induce root development in seedlings obtained by tissue culture (42). The development of malformations in the infected plants is of particular interest, since this effect is likely related to tumor formation in maize, the natural host plant. The process of tumor induction is not well understood, but may involve alterations in the physiological equilibrium in the plant, possibly by growth regulators produced by the fungus (30) or by stimulation of their production in the plant. Similar alterations in root development and malformation induction were observed in some other dicots experimentally infected with *U. maydis* (27).

One fundamental difference between the *U. maydis*-*Arabidopsis* and *U. maydis*-maize pathosystems is that only in the former was the haploid stage of *U. maydis* found to be virulent. A similar phenomenon was observed in papaya plants inoculated with single haploid strain of *U. maydis*, where some disease symptoms (but no malformation induction) were observed (27). It has been described that a heterodimer, formed by different bWp and bEp products encoded by compatible alleles, acts as a transcription factor required for the synthesis of proteins involved in the sexual cycle and pathogenesis during invasion of maize (15,20,23). However, it is also known that each single haploid

TABLE 2. Comparison of infection of *Arabidopsis thaliana* with single haploid strains and mixtures of sexually compatible *Ustilago maydis* cells^a

Experiment	Cells	Inoculum density	Number of plants	Disease rating						Disease index
				0	1	2	3	4	5	
A	FB1	10 ⁴	29	0	24	1	0	0	4	1.59
	FB2	10 ⁴	65	0	55	0	0	0	10	1.62
	FB1 + FB2	10 ⁴	22	0	13	0	2	0	7	2.45
B	FB1	10 ⁵	25	3	2	20	0	0	0	1.68
	FB2	10 ⁵	22	0	0	22	0	0	0	2.00
	FB1 + FB2	10 ⁵	36	1	0	8	21	4	2	2.92

^a *Arabidopsis* seedlings were inoculated and observed after 7 days. Comparison of disease indices of each haploid strain with the mixture of strains was made for each inoculum density. Disease rating values: 0, no signs or symptoms; 1, significant amounts of mycelial growth in or on the plant; 2, mycelial growth in or on the plants and anthocyanin accumulation; 3, mycelial growth and development of chlorosis; 4, mycelial growth, accumulation of anthocyanins and development of chlorosis; and 5, death of the plant.

TABLE 3. Infection of *Arabidopsis thaliana* with *Ustilago maydis* monomorphic and other low virulence mutants^a

Strains inoculated	Number of plants	Disease rating						Disease index
		0	1	2	3	4	5	
FB1	39	0	16	20	2	1	0	1.69
FB2	50	0	19	31	0	0	0	1.62
CL21B	49	6	43	0	0	0	0	0.88 (0.0000)
CL211	48	4	44	0	0	0	0	0.92 (0.0000)
CL235	30	1	29	0	0	0	0	0.97 (0.0000)
1/9	26	0	0	21	3	1	1	2.31 (0.0004)
1/68	38	1	7	26	2	1	1	1.90 (0.0970)
BX5	31	0	0	0	0	0	0	0.00 (0.0000)
BX28	32	10	16	3	2	1	0	1.00 (0.0002)
GP25	26	10	16	0	0	0	0	0.62 (0.0000)
LG1	23	0	20	3	0	0	0	1.13 (0.0004)

^a Plants were inoculated with 10⁴ cells, and data were recorded 7 days after inoculation. Comparison of disease indexes of each haploid strain with the corresponding wild-type strain was made for each inoculum density; *P* values from the Mann-Whitney test are shown in parentheses.

form of the fungus contains the target genes involved in pathogenesis. Accordingly, solo-pathogenic haploid strains have been constructed carrying compatible whole *b* genes, or *bE* and *bW* alleles (20,34). Additionally, diploidy at the *b* locus is non-essential for induced mycelial growth in vitro, both in liquid and solid media by control of external pH (36), or in *uac1* mutants (17). Therefore, alternative mechanisms may exist to activate genes involved in morphogenesis. It is feasible, then, that the conditions found in *Arabidopsis* may activate at least some of the *U. maydis* genes involved in virulence and morphogenesis independently of *b* genes; but not those genes necessary for sexual development of the fungus, as evidenced by the results obtained. Results obtained with several mutants avirulent on maize give further support to this hypothesis, since most of them were also avirulent on *Arabidopsis*. The only exceptions to the rule were the *uac1* and *ubc1* mutants which were as virulent as the wild-type strains. It is interesting to note, however, that the *ubc1* mutant dikaryon is virulent to maize but unable to form teliospores or induce galls in maize (16). It is also important to note that *ubc1* as well as the other monomorphic mutants developed mycelium over the plant surface. This result suggests that a different stimulus conveyed by an alternative route is responsible for mycelium induction under these conditions.

In summary, our results are evidence that the *U. maydis*–*Arabidopsis* pathosystem may constitute a useful experimental model for the study of nonhost responses and for the analysis of some aspects of fungal virulence. As a continuation of these studies, we are now in the process of identifying *U. maydis* and *A. thaliana* genes up-regulated during the infection process, through the construction of differential gene expression libraries, and in the case of the host, by the use of the available collections of gene and enhancer traps.

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