Effects of Maize Dwarf Mosaic Virus Infection of Corn on Inoculum Potential of *Helminthosporium maydis* Race O

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ABSTRACT

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Conidia of *Helminthosporium maydis* race O produced in lesions on maize dwarf mosaic virus (MDMV)-infected corn leaves were compared with those produced on MDMV-free leaves and artificial media. Sporulation began sooner and was more abundant in lesions on MDMV-infected leaves. Conidia from these lesions were 24–87% longer, contained two to four more septa, showed higher rates of germination and appressorium formation, and incited more and larger lesions than did conidia produced on MDMV-free leaves, xylose or glucose media, or potato-dextrose agar. The increased inoculum potential associated with the increased size of conidia suggests there was increased energy available in the conidia for germination and infection. Enhancement of conidial size appeared to be related to increased leakage in MDMV-infected leaves, which led to increased availability of nutrients for growth and sporulation of *H. maydis*.

Numerous studies have shown that the nature of the substrate on which fungi are grown can markedly influence inoculum potential (4,10,11,13,17,22,23). Recently, Trainor and Martinson (20) reported that conidia of *Helminthosporium maydis* race T produced either on corn (maize) leaves or in a medium amended with corn leaf tissue had a higher inoculum potential than conidia produced on other media, and that this effect appeared to be nutritional. Earlier, enhanced availability of nutrients in virus-infected plants and consequent increased inoculum potential of fungal pathogens was proposed as a mechanism for increased susceptibility of virus-infected corn (21), peas (3), and gladiolus (2) to some fungal diseases. In a recent study (18), we found that the increased susceptibility of maize dwarf mosaic virus (MDMV)-infected corn to *H. maydis* race O (1) (as measured by numbers and size of lesions incited by the fungus) was associated with increased leakage in MDMV-infected leaves and increased numbers of germ tubes and appressoria. This suggests that the increased numbers and size of *H. maydis* lesions on MDMV-infected leaves were due in part to an increase in the inoculum potential of *H. maydis* conidia deposited on the leaves. The objective of the study reported here was to determine if the inoculum potential of conidia of *H. maydis* produced in lesions on MDMV-infected leaves differs from that of conidia produced on MDMV-free leaves.

MATERIALS AND METHODS

The sources for the MDMV (strain A) and *H. maydis* used in this study and the procedures for pathogen maintenance and test plant (*Zea mays* L. 'H60 × C103') inoculation were as described previously (18).

Spore production. Corn seedlings that had been infected with MDMV for 7 days (mosaic symptom visible) and MDMV-free seedlings were inoculated 15 days after planting by spraying them with a suspension of *H. maydis* conidia (10^4 conidia per milliliter; 20 ml per five plants). Plants were maintained under high humidity in a moist chamber and conidia were collected by washing sporulating lesions with distilled water.

Artificial media used for production of *H. maydis* conidia were potato-dextrose agar (PDA) and Garraway and Evans' medium (6) containing 2% (w/v) α-D-glucose or α-D-xylose, but without agar. Media were dispensed into plastic petri plates (15 × 60 mm) at the rate of 10 ml per plate and each plate was inoculated with a plug (10 mm in diameter) cut from a 7-day-old culture of *H. maydis* on PDA. Plates were incubated under continuous fluorescent light (650 lux) at 28 C for 7 days to promote sporulation, and conidia were washed from mycelial surfaces or mats either with 5% sucrose solution or distilled water.

Spore characterization. Numbers of septa and lengths of conidia produced on substrates described above were determined with a compound microscope fitted with an ocular micrometer. Germination rates and appressorium formation for the conidia were determined by using the procedures of Trainor and Martinson (20).

Inoculum potential of spores. Conidia produced on the various substrates were washed and centrifuged in distilled water, resuspended in 5% sucrose solution, and filtered through two layers of cheesecloth; Tween-20 was added at a rate of one drop per 100 ml of suspension. The concentration of conidia was adjusted to 600 conidia per milliliter as measured with a hemacytometer and the suspension was sprayed onto MDMV-free corn seedlings. Criteria for inoculum potential of the conidia were numbers and lengths of lesions at 2 and 3 days after inoculation, respectively, and subsequent sporulation. For determining sporulation, leaves were detached from corn seedlings 2 days after inoculation with conidia and cut into sections that were placed over moist filter paper in petri plates and incubated at 28 C under continuous light (650 lux). Leaf sections were examined at 24-hr intervals with a binocular microscope at ×30. Conidia were washed from leaf sections with distilled water and counted with a hemacytometer.

RESULTS

Sporulation on MDMV-infected and MDMV-free seedlings. Sporulation by *H. maydis* generally began sooner, and was two to three times more abundant on leaves of MDMV-infected corn seedlings than on those of MDMV-free seedlings (Table 1).

Spore characteristics. Conidia from lesions on MDMV-infected leaves were about 24% longer than those from lesions on MDMV-free leaves or those produced in xylose medium, and 63–87% longer than those produced on glucose medium or PDA (Table 2). The majority of conidia produced on MDMV-infected leaves were 111 μm in length or longer, whereas only 22 and 10% of the conidia from MDMV-free leaves and xylose medium, respectively, were

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that long and none of those produced on glucose medium or PDA exceeded 110 μm in length. Generally, the number of septa in the conidia increased with an increase in conidial length. Conidia from MDMV-infected leaves and from xylose medium had about two to four more septa on the average than those produced on MDMV-free leaves, glucose medium, or PDA.

Conidia from lesions on MDMV-infected and MDMV-free leaves and xylose and glucose cultures were typical of those described previously (16). Conidia from PDA were incompletely filled, darkly pigmented, and stained poorly with 1% acid fuchsin as compared to those produced on the other substrates.

Spore germination and appressorium formation. When conidia from leaf lesions and PDA were placed on water agar, 56% of those from MDMV-infected leaves germinated after 15 min of incubation compared to 15 and 0% of those from MDMV-free leaves and PDA, respectively (averages from 100–200 conidia). After 45 min, germination rates by conidia source were 83%, MDMV-infected leaves; 60%, MDMV-free leaves; and 24%, PDA. After 60 min, virtually all the conidia from MDMV-infected leaves had germinated as compared to 82 and 38% for those from MDMV-free leaves and PDA, respectively. When placed on sections from MDMV-free corn leaves, 72% of the conidia from MDMV-infected leaves germinated after 1 hr as compared to 59% of those from MDMV-free leaves and 2–15% of those from artificial media (Table 3). By 6 hr, most of the conidia from all sources had germinated, except those from PDA, which showed only a 61% germination rate. After 6 hr of incubation, the number of conidia from MDMV-infected leaves that had produced appressoria was about twice that for conidia from the other sources. By 12 hr, appressoria had formed from 50% of the conidia from MDMV-infected leaves compared to 32–34% of those from MDMV-free leaves and xylose, and less than 20% of those from the other sources. When conidia from MDMV-infected leaves, MDMV-free leaves, and PDA were incubated on MDMV-infected leaves, there was no difference in germination rates between conidia produced on MDMV-infected and MDMV-free leaves; however, germination rates of conidia from both of these sources were fivefold greater than that of conidia from PDA after 1 hr of incubation and 23–56% greater after 3–12 hr (averages from 89–350 conidia). After 6 hr of incubation on MDMV-infected leaves, the proportions of conidia that had produced appressoria were, by source: 89%, MDMV-infected leaves; 45%, MDMV-free leaves; and 20%, PDA.

Inoculum potential of spores. Conidia from MDMV-infected leaves incited larger lesions and greater numbers of lesions on corn leaves than did conidia from MDMV-free leaves or any of the other sources (Table 4). Conidia from MDMV-free leaves, in turn, incited more and larger lesions than those from the other sources except those from the xylose medium.

After 48 hr of incubation, 77% of the lesions incited by conidia from MDMV-infected leaves were sporulating as compared to 38 and 34% of those incited by conidia from MDMV-free leaves and PDA, respectively (averages from 200–300 lesions). After 96 hr, there were no apparent differences in numbers of sporulating lesions originating from conidia from MDMV-infected and

TABLE 1. Sporulation of *Helminthosporium maydis* race O in lesions on sections from maize dwarf mosaic virus (MDMV)-infected and MDMV-free corn leaves

	Lesions sporulating (%)[a]			Conidia/lesion[b,c]		
Plant	24 hr	48 hr	72 hr	48 hr	72 hr	96 hr
MDMV-infected	9	67	97	199.4*	496.0*	730.0*
MDMV-free	1	28	48	83.4	165.0	260.0

[a] Averages from 146 lesions in one experiment.
[b] From 905–908 lesions in three experiments.
[c] * = Significantly different ($P = 0.05$) from the corresponding value for a virus-free plant according to the F test.

TABLE 2. Lengths and septation of conidia of *Helminthosporium maydis* race O produced in lesions on maize dwarf mosaic virus (MDMV)-infected and MDMV-free corn seedlings and in culture on artificial media

	Length[a]			Septa[a]		
Source of conidia	Range (μm)	Proportion (%)	Mean (μm)[b]	Range (no.)	Proportion (%)	Mean (no.)[b]
MDMV-infected leaves	50–80	12		3–5	6	
	81–110	35		6–8	12	
	111–140	36		9–11	77	
	141–171	17	116 a	12–14	5	9.4 a
MDMV-free leaves	19–49	4		3–5	17	
	50–80	20		6–8	34	
	81–110	54		9–11	49	
	111–140	22	94 b			7.7 b
Xylose medium	50–80	21		3–5	7	
	81–110	69		6–8	38	
	111–140	10		9–11	49	
			93 b	12–15	6	9.2 a
Glucose medium	19–49	24		3–5	17	
	50–80	43		6–8	61	
	81–110	33		9–11	19	
			71 c	12–15	3	7.5 b
Potato-dextrose agar	19–49	17		3–5	42	
	50–80	68		6–8	54	
	81–110	15	62 d	9–11	4	5.5 c

[a] Averages from 50–100 conidia in two experiments.
[b] Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 3. Germination and appressorium formation on maize dwarf mosaic virus (MDMV)-free corn leaf sections by *Helminthosporium maydis* race O conidia produced in lesions on MDMV-infected and MDMV-free corn seedlings and in culture on artificial media

	Percentage of conidia[a]						
	Germinating				Producing appressoria		
Source of conidia	1 hr	3 hr	6 hr	12 hr	3 hr	6 hr	12 hr
MDMV-infected leaves	72	87	98	···	0	28	50
MDMV-free leaves	59	87	98	···	0	16	32
Xylose medium	15	50	94	96	0	18	34
Potato-dextrose agar	3	54	61	85	0	10	17
Glucose medium	2	31	89	95	0	9	13

[a] From 130–150 conidia on six to eight leaf sections for conidia from MDMV-infected and -free leaves, and from 300–700 conidia on six to eight leaf sections for conidia from the other sources.

TABLE 4. Numbers and lengths of lesions on corn leaves inoculated with *Helminthosporium maydis* race O conidia collected from lesions on maize dwarf mosaic virus (MDMV)-infected and MDMV-free corn seedlings and from cultures on artificial media

	Lesions[a]		
Inoculum source	No./leaf[b]	No./cm^2	Length (mm)[c]
MDMV-infected leaves	83.2 a	6.9 a	3.3 a
MDMV-free leaves	45.3 b	3.4 b	2.1 b
Xylose medium	14.4 c	2.4 b	2.0 b
Potato-dextrose agar	19.7 c	1.9 c	1.5 c
Glucose medium	8.3 c	1.2 c	1.4 c

[a] Values followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[b] From 17–44 plants in two experiments.
[c] From 26–50 lesions on 32–44 plants in two experiments.

MDMV-free leaves, but the numbers of sporulating lesions from inocula from these two sources were still 15–20% greater than the number of sporulating lesions that arose from PDA-produced conidia.

DISCUSSION

These results show that *H. maydis* not only sporulated more abundantly on MDMV-infected leaves, but also that the inoculum potential of these conidia was greater than that of conidia produced on MDMV-free leaves. This increased inoculum potential probably was related to the increased size of conidia and/or increased energy available in the conidia for germination and appressorium formation, penetration, and other aspects of the infection process. Garrett (7) indicated that larger propagules contain greater reserves of nutrients and hence are more likely to have a higher degree of infectivity than smaller propagules. An apparent increase in nutritional status of conidia was suggested as the basis for increased inoculum potential of *Fusarium roseum* f. sp. *cerealis* grown on a high nutrient medium (13).

The results of numerous studies show that conidial dimensions are affected by nutrients in the culture medium (5,8,9,12–15,19,20). Recently, we reported (18) that MDMV-infected leaves showed increased leakage and suggested that an enhanced nutrient status of these tissues was responsible, at least in part, for their increased susceptibility to *H. maydis*. Conceivably, the increase in size and presumed energy levels of conidia produced in lesions on these leaves was also related to enhanced availability of nutrients in the leaves. Increased leakage of nutrients onto the surface of MDMV-infected leaves apparently augmented the endogenous nutrition of conidia because conidia originating from MDMV-infected leaves, MDMV-free leaves, and PDA produced appressoria more rapidly on MDMV-infected leaf sections than on MDMV-free ones.

Trainor and Martinson (20) reported that the inoculum potential of *H. maydis* race T conidia produced on agar was inversely related to nitrogen concentration. Our results indicate that the conidial inoculum potential of *H. maydis* can also be affected by the type of carbohydrate in the medium. Conidia produced on the basal medium containing xylose were longer and had higher inoculum potential than those produced on the glucose-containing medium. Xylose also enhances sporulation by *H. maydis* race T (6). Our earlier study (18) showed that leachates from MDMV-infected leaves contained higher levels of all detected pentoses than did leachates from MDMV-free leaves. This and the facts that sporulation by *H. maydis* was enhanced on MDMV-infected leaves and that the characteristics and inoculum potential of conidia produced on the xylose medium approached those of conidia from MDMV-infected and MDMV-free leaves suggest that xylose or some pentose isomer is a major nutrient for the fungus in corn leaves (6) and that it is more readily available when the leaves are infected with MDMV.

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