Effect of Maize Dwarf Mosaic Virus Infection on Sweet Corn Pollen and Silk

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ABSTRACT

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The effects of maize dwarf mosaic virus (MDMV) on pollen vigor and on silk receptivity were examined as possible causes of sterility observed on ears from MDMV-infected sweet corn plants. Pollen was collected from six *Zea mays* L. cultivars (Aztec, BiQueen, Cherokee, Seneca Scout, Sugar Loaf, and Wintergreen) of MDMV-infected and uninfected sweet corn and germinated on an artificial medium. Pollen germination and germ tube length in vitro were measured after 2 hr of incubation at 25 C. Germination in vitro for pollen from healthy and MDMV-infected plants was not significantly different in five of six cultivars. In four cultivars (Aztec, Cherokee, Seneca Scout, and Sugar Loaf), pollen from MDMV-infected plants had significantly shorter germ tubes in vitro than did pollen from uninfected plants. In vivo germ tube lengths were measured in silks of crosses made with MDMV-infected (V) and uninfected (H) plants of the cultivars Sugar Loaf and Wintergreen. Within each cultivar, germ tube lengths in the cross H♀ × H♂ were significantly longer than those in the crosses H♀ × V♂, V♀ × H♂, or V♀ × V♂. The presence of MDMV in the silks of infected Sugar Loaf and Wintergreen was established by infectivity tests. MDMV was not detected in pollen grains of Sugar Loaf or Wintergreen by either enzyme-linked immunosorbent or infectivity assays.

Yield reduction in dent corn (*Zea mays* L.) caused by maize dwarf mosaic virus (MDMV) is the result of reduced kernel number per ear and reduced kernel weight (5,9,10). In sweet corn (*Zea mays* L.), early MDMV infection has been observed to cause reduced plant height, delayed maturity, smaller ear diameter, smaller ear length, reduced ear weight, and sterility expressed as missing kernels (6,7). Missing kernels reduce the quality of fresh market ears.

On MDMV-infected plants, missing kernels could result from reduced pollen vigor, an alteration of silk receptivity (functionally, stigma and style receptivity) for pollen germ tube growth, or an alteration in the ovule. If pollen is less vigorous or silk receptivity is reduced in diseased plants, germ tubes may be unable to grow the entire length of silks, resulting in missing kernels. We studied pollen vigor and pollen receptivity in MDMV-infected and uninfected sweet corn plants.

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MATERIALS AND METHODS

Sweet corn cultivars Aztec, BiQueen, Cherokee, Seneca Scout, Sugar Loaf, and Wintergreen were planted on 30 May 1979. Seeds were spaced 97 cm between and 33 cm within north-south oriented rows, with approximately 50 seeds per row and three rows per cultivar. On 21 June, when plants were at the three to four leaf stage, 14 plants at the north end of each row were inoculated with MDMV strain B. Inoculum consisted of 1 g of freshly harvested

MDMV-infected corn tissue (infected 10–15 days) per 4 ml of chilled 0.05 M sodium phosphate, pH 7.0, extracted in a Waring Blendor for 30 sec. The homogenate was expressed through a triple layer of cheesecloth and through a layer of Miracloth. Before inoculation, 15 g/L of 22-μm Carborundum was added to the inoculum. Plants were inoculated using a Wren artist's airbrush (Binks Manufacturing Company, Franklin Park, IL), with air supplied from an air compressor operating at 4.9 kg/cm^2. The plots were examined weekly, and control plants showing symptoms and plants in MDMV treatments not showing symptoms were tagged. Those plants were not sampled.

Pollen was collected at approximately 9 a.m. and germinated on a medium containing 15% sucrose, 0.03% calcium nitrate, 0.01% boric acid, and 0.6% Bacto agar (8). After 2 hr of incubation at 25 C, growth was stopped by storage at 4 C. The percent germination was determined with a stereoscope at ×70 magnification. Those pollen grains with one or more emerging germ tubes were considered germinated; those that had ruptured during germination were not counted (8). A minimum of 2,000 grains from 10–20 plants were counted for each cultivar.

After germination counts were made, pollen was covered with an aqueous solution (38% glycerin/glacial acetic acid/formaldehyde [20:3:5, v/v]) to stop growth and again stored at 4 C. Germinated pollen grains were photographed through a stereoscope (8), and parallel photographs of a stage micrometer were taken for accurate magnification calculations (Fig. 1). Slides projected on a screen at a magnification of ×186 were measured for germ tube lengths (in micromillimeters) with a planimeter (4). Multiple germ tubes from the same pollen grain were not measured (8). Comparisons between pollen from MDMV-infected and uninfected plants for percent germination and germ tube length were analyzed, using Student's *t*-test at the 5% level of confidence (12,13).

Samples from the cultivars Sugar Loaf and Wintergreen were taken from the same field for the in vivo germ tube growth study. Young ears were bagged before silk emergence. After silk emergence they were hand-pollinated with freshly collected pollen and rebagged until harvest. Four crossing combinations were made between healthy (H) and MDMV-infected (V) plants as follows: H♀ × H♂, H♀ × V♂, V♀ × H♂, and V♀ × V♂. Five hours after pollination, ears were removed and immediately stored at 4 C until fixation. Before fixation, husks were removed from the ears and silks tied with string to prevent entanglement. Silks attached to ovules at the basal end of the ears were prepared for observation as described by Adams and MacKay (1). Karpechenko's modification of Navashin's fixative was vacuum infiltrated into the silks. Detached silks were then dehydrated through an ethanol series (15–80%) and rehydrated through 50 and 15% ethanol. Fixed samples were boiled in water approximately 1 min, then immersed for 1 hr sequentially in 1% potassium permanganate and 1% oxalic acid solutions containing equal parts of concentrated HCl and 95% ethanol (v/v). After rehydration in 70% ethanol, silks were immersed in lactophenol for 2–5 min, then placed on microscopic slides coated with lactophenol. The pollen germ tubes were measured under dark-field microscopy at ×160 magnification with the aid of a calibrated ocular lens (Fig. 1).

For each treatment (crossing) of both cultivars, the first three measurable pollen germ tubes per silk for 20 silks on each of two ears were measured. The experimental design was hierarchal classification analyzed according to Fisher's least significant difference test at the 5% level of significance.

For the cultivars Sugar Loaf and Wintergreen, the presence of MDMV in the silks was indexed by infectivity. For each cultivar, six silk samples (each sample from a separate ear, five silks per sample) from MDMV-infected and three samples from uninfected plants were ground in 0.05 M sodium phosphate, pH 7.0 (1 g/4 ml)

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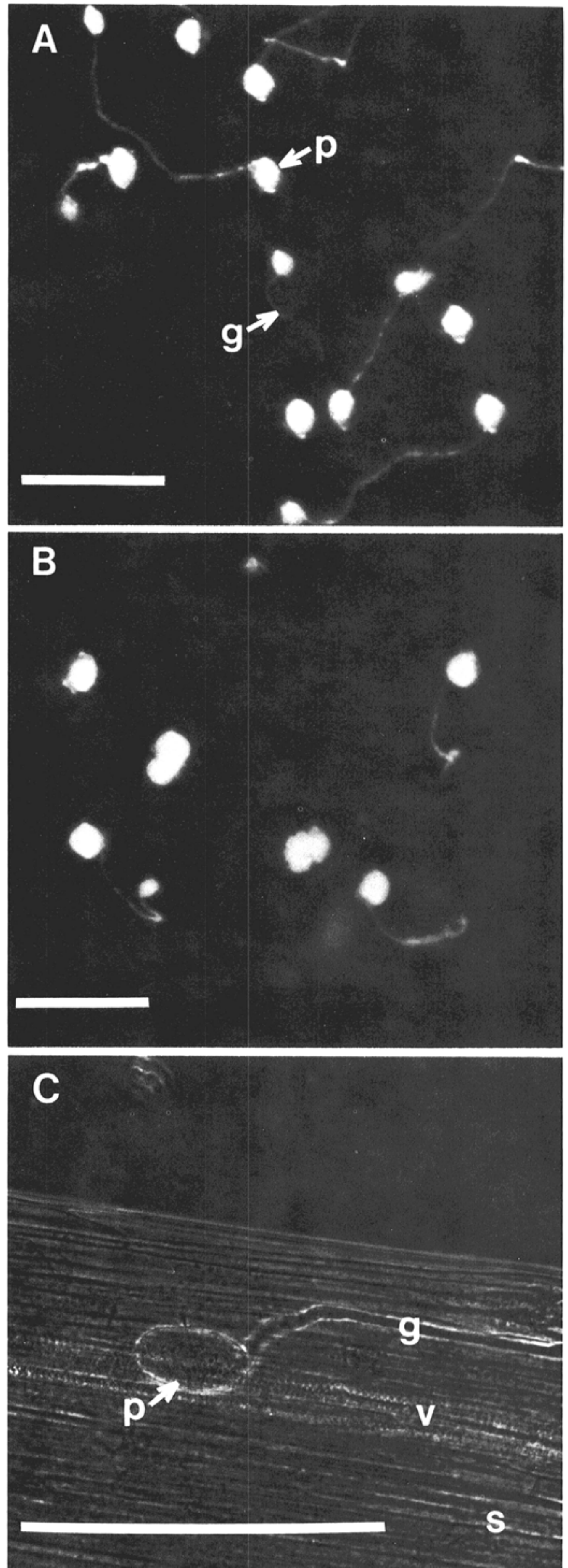

Fig. 1. Germination of sweet corn pollen in vitro and in vivo. Pollen in vitro after 2 hr of incubation at room temperature: **A,** pollen from an uninfected plant; **B,** pollen from a plant infected with maize dwarf mosaic virus; **C,** pollen germ tube growing in silk. p = Pollen grain, g = pollen germ tube, s = silk, v = fibrovascular bundle. Bar = 300 μm.

and inoculated to susceptible sweet corn seedlings. Test plants were rated for infection by visual symptoms 14 days later.

Pollen from greenhouse-grown Sugar Loaf and Wintergreen inoculated at the four to five leaf stage was assayed for MDMV by an infectivity assay and by enzyme-linked immunosorbent assay (ELISA). Fresh pollen was collected daily by shaking tassels into paper bags. Ten and six samples, respectively, were assayed from MDMV-infected Sugar Loaf and Wintergreen cultivars; six samples from uninfected plants were also assayed. Pollen was washed in phosphate-buffered saline (1 g/10 ml) consisting of 0.14 M NaCl, 5 mM KH_2PO_4, 0.02 M Na_2HPO_4, 2.6 mM KCl, 3 mM NaN_3 with 0.5% polyoxyethylene sorbitan monolaurate (PBS-Tween) and collected in a pellet by centrifugation (10,000 rpm in a Sorvall SS-34 rotor for 10 min). The supernatant fluid was placed directly into Cooke Microtitre® plates (200 μl per well) for ELISA. The pellet was resuspended in PBS-Tween (1 g/4 ml), ground until ruptured in a tissue homogenizer, decanted into a mortar, reground, and placed into microtiter wells (200 μl per well) for ELISA. The procedures of Clark and Adams (3) were followed for ELISA. Samples having an absorbance at 405 nm equal to or more than twice that of the control (identically treated healthy pollen extract) were considered positive for MDMV. Extracts were also assayed for infectivity by inoculation to healthy corn seedlings.

RESULTS

Of the six cultivars tested, only pollen from MDMV-infected Sugar Loaf was significantly reduced in in vitro germination when compared with pollen from healthy plants (Table 1). The pollen germ tubes from healthy plants in four of the six cultivars grew significantly longer in vitro than those from MDMV-infected plants (Table 1). Although differences in pollen germ tube lengths from healthy and MDMV-infected BiQueen and Wintergreen were not statistically significant, the data suggest a tendency for the pollen germ tubes from healthy plants to be longer.

In Sugar Loaf and Wintergreen, average in vivo germ tube length was significantly longer for the H♀ × H♂ cross than for the other three crosses, which were statistically similar (Table 2). In comparison to those of the H♀ × H♂ cross, the germ tube lengths of the H♀ × V♂, V♀ × H♂, and V♀ × V♂ crosses were 56, 57, and 43%, respectively, for Sugar Loaf and 62, 48, and 40%, respectively, for Wintergreen. Thus, when either or both parents were MDMV-infected, the pollen germ tube length in vivo was significantly reduced from that of the cross of healthy plants.

Infectivity assay of silk extracts from the cultivars Sugar Loaf and Wintergreen showed that all MDMV-infected plants contained virus in the silks and that no virus was detected in silk extracts from uninfected plants. MDMV was not detected by ELISA or infectivity assay on the surface of or within pollen of either Sugar Loaf or Wintergreen. Infected and healthy leaf sap controls verified the testing procedure. Purified MDMV could be detected by ELISA at a concentration of 50 but not at 25 ng/ml.

TABLE 1. In vitro germination and germ tube length of pollen from six healthy sweet corn cultivars and six infected with maize dwarf mosaic virus, strain B (MDMV-B)

Cultivar	Germination percentage[a]		Average germ tube length (μm)[b]	
	Healthy[c]	MDMV-B-infected	Healthy	MDMV-B-infected
Aztec	42	32	495[d]	386[d]
BiQueen	57	55	469	400
Cherokee	58	61	542[d]	465[d]
Seneca Scout	41	32	473[d]	306[d]
Sugar Loaf	45[d]	19[d]	625[d]	491[d]
Wintergreen	26	39	486	444

[a] For each cultivar, more than 2,000 pollen grains (from 10–20 plants) were counted.
[b] More than 110 pollen grains were measured per cultivar.
[c] No MDMV was detected in healthy plants.
[d] Difference between paired values was significant at the 5% level with Student's *t*-test.

TABLE 2. Length of pollen germ tubes in silks of two healthy (H) sweet corn cultivars and two infected (V) with maize dwarf mosaic virus, strain B

Cultivar	Treatment mean[a] (μm)			
	H♀ × H♂	H♀ × V♂	V♀ × H♂	V♀ × V♂
Sugar Loaf	1,149 a	641 b	654 b	496 b
Wintergreen	905 a	560 b	431 bc	364 c

[a] Mean of 120 germ tube measurements. Experiment was designed as a hierarchal classification analyzed according to Fisher's least significant difference test ($P = 0.05$). Means within cultivars separated by more than 172 μm are significantly different and are followed by different letters.

DISCUSSION

Little work had been done to date on the effect of virus infection of the parent plant on pollen production and vigor. Barley stripe mosaic virus in barley (*Hordeum vulgare*) causes pollen sterility (2). Yang and Hamilton (14) found that pollen from soybeans (*Glycine max*) infected with tobacco ringspot virus was reduced in germination and that germ tube elongation was slower. By electron microscopy they found viruslike particles in the pollen grains and concluded that virus infection caused the observed reduction in pollen vigor. The situation for MDMV is different. The in vitro germination of pollen from MDMV-infected sweet corn did not seem to be different from that of healthy plants, but the growth of pollen germ tubes in vitro and in vivo, as measured by germ tube length, was significantly reduced. Serological and infectivity tests were negative for the presence of MDMV within pollen of sweet corn. The reduced vigor of pollen from MDMV-infected plants thus appears to be due to the stress of virus infection on the parent plant rather than to direct infection of the pollen.

Any factor that reduces pollen germ tube elongation contributes to sterility if the pollen germ tubes thereby cannot grow the full length of the silk necessary to fertilize the egg and to set seed. We have demonstrated that MDMV-infection is one of the factors causing reduced pollen germ tube elongation, which, in turn, may contribute to reduced kernel set.

The results of in vitro tests of pollen vigor cannot be relied on exclusively because the interaction of the pollen germ tube with the silk is not taken into consideration. During the early stages of growth, the pollen germ tube is dependent upon its own food reserves. During the later stages of growth, it is dependent upon the food material furnished by the style (11). At the growth stage of the pollen germ tubes in our experiments, the germ tubes were beginning to acquire energy from the surrounding style (11). When pollen from uninfected plants was used to pollinate diseased silks, the pollen germ tube was significantly reduced in length, suggesting that MDMV influenced the receptivity of silks to germ tube growth and development. The mechanism responsible for the healthy pollen-diseased silk relationship is unknown, but possible explanations are that MDMV may alter the morphology of the style or that the supply of nutrients in the silk is reduced. Reduced style receptivity and lower pollen vigor are two mechanisms that might contribute to sterility in MDMV-infected sweet corn.

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