Interactions Between Maize Streak Virus and Downy Mildew Fungi in Susceptible Maize Cultivars

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ABSTRACT
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The effects of systemic infection by Peronosclerospora philippinensis and P. sorghi (both downy mildew fungi) and maize streak virus (MSV) on host reaction and pathogen reproduction were quantified in two maize cultivars. The maize cultivars tested (DeKalb XL 43A and Pioneer 3369A) were susceptible to all pathogens. Infection by MSV masked symptoms of downy mildew infection. Reduction in height and biomass were significantly greater with pathogen combinations than with single pathogens. Systemic infection by either downy mildew fungus had no effect on MSV titer. Infection by MSV reduced plant biomass, leaf area, and total sporulation of either fungus; however, sporulation per unit leaf area was not affected.

Additional keywords: Cicadulina mbila, synergism

There are numerous reports of interactions between two or more etiologic agents infecting a common host. Such interactions produce antagonistic or protective (2,7,11,13,15,24), mutually exclusive (10,21), additive (8,16), or synergistic effects (17) in the host. Plants systemically infected with any of several plant viruses are protected against infection by selected nonsystemic fungal pathogens (6,11,14,15,24). The nature of these resistance mechanisms is varied and may include inhibition of spore germination (24), altered host metabolism (15), and the triggering of protective mechanisms by viral infection (6,11,15,24).

Maize streak virus (MSV) and downy mildew fungi are important pathogens of maize (Zea mays L.). MSV causes one of the most important viral diseases of maize (4,5). It is not found in the Western Hemisphere. The virus has a single-stranded DNA genome and is classified as a monopartite geminivirus (22). It is transmitted by several Cicadulina species (leafhoppers), of which C. mbila Naude is considered the most common and important vector (18). The vector deposits the virus into the phloem. From there, virions are translocated to the apical meristem, where they infect dividing cells of the meristem. As new cells form and elongate, the virus multiplies and moves with the cells.

The downy mildew fungi, Peronosclerospora philippinensis (W. Weston) C.G. Shaw and P. sorghi (W. Weston & Uppal) C.G. Shaw cause important diseases in maize in tropical and temperate areas of the world, respectively. Both downy mildew species readily infect one- to three-leaf seedlings of susceptible maize by asexual conidiospores. Conidial infection occurs by germination and penetration of conidial germ tubes through stomates in the presence of free moisture. From the substomatal cavities, mycelia grow interveinally through leaves to the apical meristem. There, mycelia ramify within the tissue and grow systemically with newly developing tissues. Under favorable environmental conditions, conidial reproduction occurs on upright conidiophores protruding from stomates, primarily on the undersurface of the leaves (1).

As a part of the Foreign Disease-Weed Science Research mission to determine the potential threat of exotic diseases (12), studies were conducted to determine the recognizability of MSV and downy mildews when both infected the same maize plants, and to discover the interactions between P. philippinensis (exotic) and MSV, and P. sorghi (endemic) and MSV. No reports could be found describing co-infections of maize by MSV and downy mildews, although they occur in the same habitats. The studies reported here were conducted to evaluate the symptom expression of MSV and downy mildews alone and in combination infections, and to obtain quantifiable data on the effects of double infections on pathogens and host.

MATERIALS AND METHODS
MSV and its vector C. mbila were obtained from Potchefstroom, South Africa (4,5). The P. philippinensis isolate was obtained from Los Banos, Philippines (O. R. Exconde), and the P. sorghi isolate was obtained from Port Lavaca, Texas (R. A. Fredericksen). All pathogens were maintained in maize, cultivar DeKalb XL 43, or sorghum (Sorghum bicolor (L.) Moench), cultivar Tx 412.

Two maize cultivars (DeKalb XL 43A and Pioneer 3369A) were selected for the study based on their susceptibility to MSV and the downy mildews. Plants were grown and all experiments were conducted in the quarantine containment facility at Frederick, Maryland.

All initial inoculations were made when seedlings were in the one- to two-leaf stage. The second inoculation was done within 3 days of the initial inoculation, because maize is only susceptible to conidial infection by Peronosclerospora up to the three-leaf stage (1).

Plants were inoculated with MSV by caging five C. mbila adults, previously given a 24-hr acquisition-access period on infected maize, with test seedlings in 5- × 15-cm cellulose butyrate tube cages for a 24-hr inoculation-access period. Leafhoppers were aspirated from all inoculated plants, and the plants were treated with acephate insecticide.

Peronosclerospora species were inoculated by the technique described by Schmitt (20). Conidiospore concentrations were adjusted to 12,000 ± 1,000 spores per milliliter. Approximately 0.5 ml of inoculum was atomized onto each test seedling before incubation. Seedlings were incubated for at least 16 hr under dew at 21–22 C. Control plants for each treatment were caged with nonviruliferous C. mbila or incubated in dew without pathogens.

Treatments consisted of four plants of each cultivar in individual 10-cm clay plots inoculated with MSV, P. philippinensis, P. sorghi, MSV followed by P. philippinensis or P. sorghi, and P. philippinensis or P. sorghi followed by MSV; plus the uninoculated controls. All treatments were replicated three times, and the complete experiment was replicated on two dates.

The plants were placed on glasshouse benches under natural daylight (12- to 13-hr day) at 18–24 C, as randomized

blocks to minimize environmental effects. Symptoms were allowed to develop for 28 days, after which all plants were harvested. Data were obtained on the number of days from inoculation to symptom development, the percentage of plants systemically infected with each pathogen, the symptomatology, height, fresh weight, total leaf area, relative viral titer, and amount of fungal sporulation per unit of leaf area.

Conidia were collected from all leaves of each replication of inoculated plants by leaf washings in distilled water (20). Aliquots of conidiospore suspension were mixed with Isoton II electrolyte solution (Coulter Diagnostics, Hialeah, FL), and spore densities were determined by a microcomputerized ELZONE "/ADC-80XY" particle counter (Particle Data, Inc., Elmhurst, IL). Spores per unit of leaf area were calculated by multiplying spores per sample times the dilution factor of the sample times the total volume of the suspension, and dividing it by the total leaf area sampled. Leaf-area measurements were obtained with an electronic leaf-area meter. All green leaves of each test plant of selected treatments were removed at the stem and passed through the meter, and the total leaf area was calculated for each plant.

One-gram leaf samples were taken from each plant in all treatments containing MSV, and in the healthy controls, and placed into small, plastic sample bags, labeled, and frozen at −20 C. The small sample bags were placed inside larger plastic bags, then sealed in a double-walled container and shipped on ice to the third author for analysis by the direct double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (3). The ELISA procedure was modified from one developed for beet western yellows virus (9). Positive thresholds were set at A_{405nm} values more than two times those of the comparable uninoculated controls (23). Upon completion of the assay, all samples were autoclaved before disposal. ELISA values were analyzed by SAS

least squares means ($P = 0.05$) (19).

Proof of initial infection by *Peronosclerospora* pathogens was verified by local-lesion symptoms. Systemic infection was confirmed by visible symptoms, by conidial sporulation induced by placing test plants under lights for 14 hr prior to placement in dew for 6 hr, and/or by microscopic examination of leaf pieces from each test plant. Quantitative data were subjected to analysis of variance, and the means were ranked by SAS least squares means ($P = 0.05$).

RESULTS

In both maize cultivars, MSV symptoms appeared in 4–6 days as white-to-cream dots at the base of the youngest leaf. Initial streaking symptoms consisted of an intense chlorosis at the base of the first affected whorl leaf. This intense chlorosis never covered the whole leaf length and was related to the stage of leaf development at the initiation of infection of the apical meristem cells. Associated with this intense chlorosis were leaf-margin serrations and leaf distortion. Later-formed leaves exhibited a general chlorotic streaking typical of MSV infection.

The downy mildew fungi produced local lesions in 5–6 days (chlorotic, later necrotic leaf areas at the infection site) and systemic symptoms in 13–16 days. Systemic symptoms varied somewhat between species but usually consisted of characteristic chlorotic half-leaf symptoms on the first affected leaf. Later-formed leaves were characterized by broad, chlorotic stripe patterns running most of the leaf length.

Plants infected with MSV and either of the downy mildew fungi exhibited the leaf-streaking symptoms of MSV but did not show the broad downy mildew stripe patterns. When these doubly inoculated plants were exposed to 14 hr of light followed by at least a 6-hr dew period, conidial development on leaf undersurfaces indicated systemic infection by the downy mildew fungi.

Growth was measured as height from

the soil to the tip of the extended upper leaves. Each downy mildew alone reduced plant height, but not to the degree caused by MSV or by either combination with MSV (Table 1). There was no significant reduction in biomass with *P. philippinensis*, and its combination with MSV did not differ from MSV alone. However, *P. sorghi* caused significant reduction in biomass, and its combination with MSV resulted in greater reduction than did either alone (Table 1). Because there was no difference in the reactions of the two cultivars, the data were pooled for both cultivars and experimental dates.

When plants were previously inoculated with downy mildew, the average incubation period for MSV (inoculation to visible symptoms) was shortened from 5.1 to 3.6 days (*P. philippinensis*) and from 5.0 to 4.2 days (*P. sorghi*), compared to the period for plants inoculated with MSV only or with MSV as the first pathogen in the sequence. Plants infected with only downy mildew pathogens had more leaf area than doubly infected or MSV-infected plants. There was no treatment effect on sporulation per unit of leaf area (Table 1), although total sporulation was much lower in doubly infected plants because of the reduced leaf area.

Because there was little variation in ELISA values (A_{405nm}) between cultivars or experimental dates, the values for the cultivars were pooled for analysis. There were no significant differences among treatments except for *P. sorghi* + MSV (Table 1). ELISA values of all viral treatments exceeded the healthy controls by more than four times, except for the MSV + *P. sorghi* treatment (3.5 times).

DISCUSSION

Challenge inoculations in cross-protection studies are normally made after the initial pathogen has become established. However, maize leaves are susceptible to infection by *Peronosclerospora* species for only a few days (one- to three-leaf stage); and before test plants could be confirmed to be systemically

Table 1. Effects of single and concomitant systemic infections of *Peronosclerospora philippinensis* (*P.p.*) or *P. sorghi* (*P.s.*) and maize streak virus (MSV) in two susceptible maize cultivars

Treatments	Incubation period (days)	Infection (%)	Height[v] (cm)	Fresh wt. (g)	Leaf area/plant (cm²)	spores/cm²[w] (Log+y)	ELISA A_{405nm}
Healthy control		NA	87.3a[x]	23.0a	530.6a	NA	0.106a[y]
P. p.	14	100	73.5b	20.3ab	420.5b	8.0a	NA
P. s.	15	100	63.9c	16.4b	346.0c	7.4a	NA
MSV	5.2	100	37.8d	9.9c	136.2d	NA	0.485c
MSV + *P.p.*	5.1/—[z]	100/100[z]	34.2e	6.0cd	112.0d	7.9a	0.491c
MSV + *P.s.*	5.0/—	100/100	30.9e	4.6d	88.2d	6.9a	0.443c
P.p. + MSV	—/3.6	100/100	34.1e	6.3cd	112.7d	8.8a	0.451c
P.s. + MSV	—/4.2	100/100	31.7e	4.7d	93.5d	7.6a	0.378b

[v] Measurement from soil to tip of fully extended top leaves; data of two cultivars on two dates pooled for analyses because of little variability within treatments/cultivars/dates.

[w] Number of spores per sq cm of leaf area transformed to Log+y to normalize data.

[x] Values in column followed by same letter are not significantly ($P = 0.05$) different by SAS least-squares means.

[y] 0.106 is the mean absorbance value for uninoculated plants.

[z] Dually infected plants: each number refers to different pathogen, /— = systemic infection not confirmed until harvest.

infected by MSV, they were no longer susceptible to *Peronosclerospora*. Therefore, all *Peronosclerospora* inoculations were made within 2 days of MSV inoculations, and MSV inoculations were made within 2 days of the removal of fungi-inoculated plants from dew. The short incubation period of MSV (5 days) compared to the longer incubation period for systemic infection of the downy mildew fungi (13 days) resulted in MSV symptoms appearing earliest in all combinations of inoculations.

Doubly infected plants were lighter green than singly infected or healthy plants, and the streaking patterns were indicative of MSV only. Several of these chlorotic, doubly infected plants were less able to survive normal environmental stress, and they died prematurely.

Most fungal-viral interactions reported in the literature have demonstrated the viral infection's protective influence against nonsystemic fungal infection. Latch and Potter (13) indicated that the presence of barley yellow dwarf virus (phloem-limited) does not affect the severity of crown rust infection in ryegrass. MSV infections are strongly phloem-associated, and the downy mildews are systemic within the same leaf tissues. The results of our experiments indicate that the presence of MSV did not protect the maize against infection by either downy mildew fungus, nor was the reverse true. The cultivars tested were known to be susceptible to each pathogen when singly inoculated. When MSV and the *Peronosclerospora* species were co-inoculated in either cultivar, there was a greater (though not significant) reduction in height, fresh weight, and leaf area than with either pathogen alone. There was no evidence of increased or decreased susceptibility of the cultivars. In a related experiment, *P. philippinensis*

did not affect the resistance of the cultivar Revolution to MSV, and MSV did not affect the resistance of the cultivar Suwan I to *P. philippinensis* (*unpublished*).

The *Peronosclerospora* species and MSV both infect the apical meristems of susceptible maize plants. However, the virus multiplies intracellularly and the fungi grow extracellularly. The presence of MSV did not affect the sporulation of the downy mildew per unit leaf area, nor did the mildew affect virus multiplication. If either *P. sorghi* or *P. philippinensis* were present in the same field with MSV, the virus symptoms could mask early recognition of downy mildew symptoms. The lack of protection or strong interaction between virus and fungus indicate that neither would affect the establishment of the other.

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