Occurrence of Panicle Necrosis and Small Seed as Manifestations of Maize Dwarf Mosaic Virus Infection in Otherwise Symptomless Grain Sorghum Plants

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ABSTRACT

In 1970 at Manhattan, Kan., underdeveloped seed were manifest in plots of RS 671 hybrid sorghum where greenbug, *Schizaphis graminum* biotype C, populations occurred after exsertion and where cool weather with temperature minimums of <16 C prevailed 4 or more consecutive days while seed was in milk to soft dough. Symptoms included development of pigmented necrotic lesions on panicle branches followed by excessive shrinkage of seed. No fungus was consistently isolated from those lesions. A virus, identified as maize dwarf mosaic, strain A, was recovered from affected panicles. We reproduced similar manifestations in the greenhouse by inoculating upper leaves of RS 610 hybrid sorghum plants at anthesis with this virus and then lowering the temperature from 28 C to <16 while seed was in milk to soft dough. The manifestations could not be reproduced without lowering the temperature. Results agree with observations made among irrigated fields in southwestern Kansas where in recent years, large populations of both greenbug and corn leaf aphid, *Rhopalosiphum maidis*, have occurred after exsertion.

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In 1969 in southwestern Kansas, undersized seed in varying patterns on individual panicles of sorghum plants, whose vegetative development and vigor appeared to be normal, represented reductions in yield as high as 40%. Those small seed occurred in proximity to normal-sized seed within or between spiklets on the various branches of panicles. Other times the small seed was restricted to lateral, top, or bottom halves of the heads. Besides seed shrinkage, pigmented, necrotic lesions on branches of panicles were characteristic. Notably absent have been disease symptoms of vegetative plant parts before or after exsertion of the heads. Excessive shrinkage of seed as it ripens often is the first indication that damage to the crop has occurred. Affected seeds are lightweight, have chalky endosperms, shatter, and are attacked by such head molds as *Alternaria* and *Fusarium* more readily than are normal seeds in the same head. The problem has been associated with cool, wet weather coincident with maturation of grain. It has not been described heretofore, mainly because evidence of a pathogen could not be ascertained. In 1970, opportunity was available to follow daily development of those manifestations in experimental field plots at Manhattan, Kan.

MATERIALS AND METHODS.—Plots representing four row spacings and four planting rates were established on ca. 2 acres of silt loam that had not been planted to sorghum for at least 10 years and which had been fallowed in 1968 and 1969. RS 671, a hybrid grain sorghum well adapted to the area, was chosen as the test crop. For each of three planting dates, 3, 10, and 17 June, combinations of four planting rates, 25, 50, 75, and 100 thousand seeds/acre, and four row spacings, 10, 20, 30, and 40 inches, were used. Four, six, nine, and 18 rows, 30 ft

long, were planted in plots with 40-, 30-, 20-, and 10-inch spacings, respectively. Planting rates were randomized within each of three replications. A record was kept of pertinent observations concerning plant development, aphid populations, and symptoms. Weather data were secured from official meteorological records of a station less than one mile from the plots. Material was collected for extraction of virus and isolation of fungi within 48 hr after panicle lesions appeared.

To isolate fungi, collected material was stored in heavy kraft paper bags at 4 C. Platings of tissue from surface-sterilized (0.26% NaOCl from 5% Clorox), peduncles, rays, and seeds were made within 3 weeks on Nash-Snyder medium (3). Results were recorded after plates were incubated at 30 C for 7-10 days. For extraction of virus, separate homogenates of seed and supporting panicle parts were made from affected panicles within 1 hr after collection in the field. Those plant materials were homogenized in 4 times their weight in 0.05 M phosphate buffer, pH 7.0. Homogenates were strained through cheesecloth and centrifuged 10 min at 12,000 *g*. Supernatant fluids were made up to 8% polyethylene glycol 6000 and 0.2 M NaCl, and stored overnight at 4 C. Next they were centrifuged 10 min at 12,000 *g*. Final pellets were resuspended in 3 ml buffer, checked for opalescence in a thin beam of light, and applied to Carborundum-dusted Ohio 28 corn seedlings at the three-leaf stage. For identification, the virus was increased in corn and inoculated to other hosts.

Production of small seed with the virus was attempted in the greenhouse. RS 610 hybrid sorghum was planted in 6- X 8-ft soil beds at 2-inch intervals in rows 12 inches apart. Infected corn plants were homogenized in 5 times their weight of buffer. The homogenates were strained through cheesecloth, and Celite was added as an abrasive. The inoculum was applied at 35 psi onto upper leaves of test plants through a DeVilbiss No. 501 atomizer. Sorghum plants in two rows were inoculated at boot and those in two other rows at anthesis. Three rows were left noninoculated. Greenhouse temperature was maintained at 28 C except for a week while seed was in milk-soft dough when temperature was held at <16 C. The experiment then was repeated without lowering the temperature.

RESULTS AND DISCUSSION.–Colonies of greenbug, *Schizaphis graminum* (Rondani) biotype C (1), were first observed in field plots 5 August and last observed 17 August (Fig. 1). Maximum numbers were observed 14 August when the most heavily colonized leaves were visually estimated to have an average of 95 cm^2 of leaf area covered with aphids.

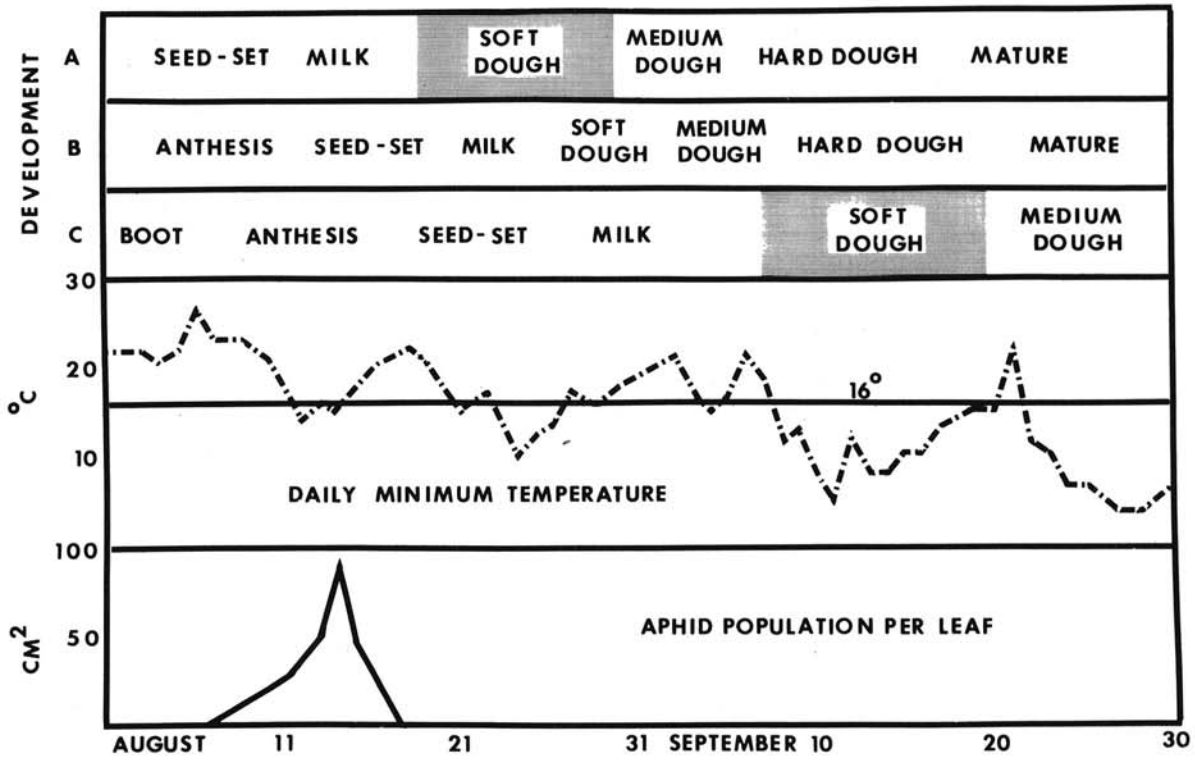

Fig. 1. Graphic representation of conditions prevailing when maize dwarf mosaic virus-associated necrosis (shaded areas) of panicle parts occurred in sorghum planted 3 June (A) and 17 (C), but not in that planted 10 June (B); post-exertion virus transmission (*Schizaphis graminum*), and low temperature (<16 C) for 4 or more consecutive days while seed was in soft dough, were associated with lesion development and subsequent seed shrinkage.

Presence of greenbugs at that time coincided with seed at milk in plants of the first planting and anthesis in plants of the third planting. Climatological cold fronts caused minimum temperatures of 10-17 C on 20-26 August, and 5-15 C on 8-20 September. Those below-normal temperatures coincided with seed at soft dough in the first and third plantings. Plants in the second planting were at seed set during maximum greenbug colonization, milk during the first cool period, and hard dough during the second cool period.

Lesions on panicle parts were noted 24 August in the first planting (Fig. 2). Terminal branches of the panicles often were first to exhibit those lesions. Sometimes damage was limited to points of seed attachment. Pith of peduncles often exhibited reddened areas (Fig. 3). This symptom, however, was not noted among plants in later plantings or among those in the greenhouse experiments. Undersized seed always could be traced to necrosis of supporting panicle parts at one or more points critical to development of the supported seed. Absence of other symptoms and presence of more or less random patterns of lesion development within individual panicles first led us to suspect that an airborne fungus was responsible for the damage. However, of more than 1,200 panicle lesion samples plated to isolate fungi, 767 yielded *Alternaria*; 260, *Fusarium*; the rest, miscellaneous fungi, bacteria, or nothing. Isolating more than one colony type from the same lesion was uncommon. No fungus was isolated from reddened pith tissues of peduncles.

No visible damage occurred in the second planting. In the third planting, all plants exhibited panicle lesions, including some whose seed had not begun to color (milk stage). Coincidence with

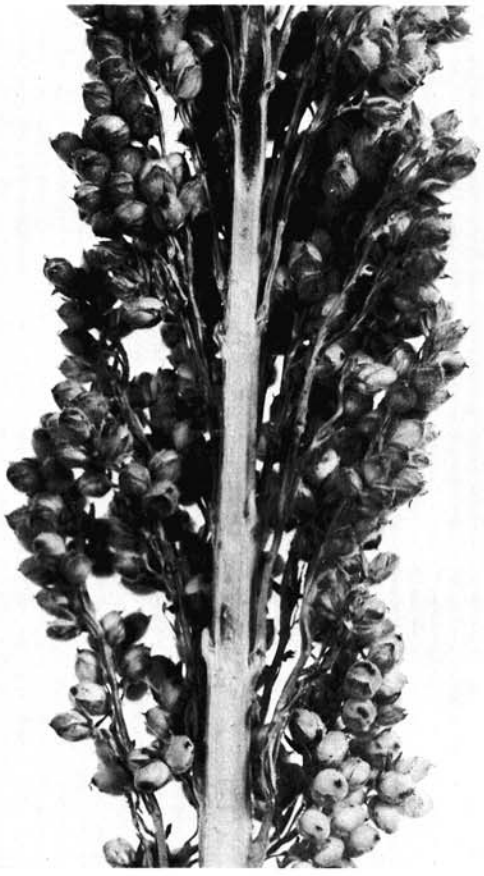

Fig. 2. Portion of a panicle affected by pigmented, necrotic lesions associated with premature seed shrinkage in grain sorghum.

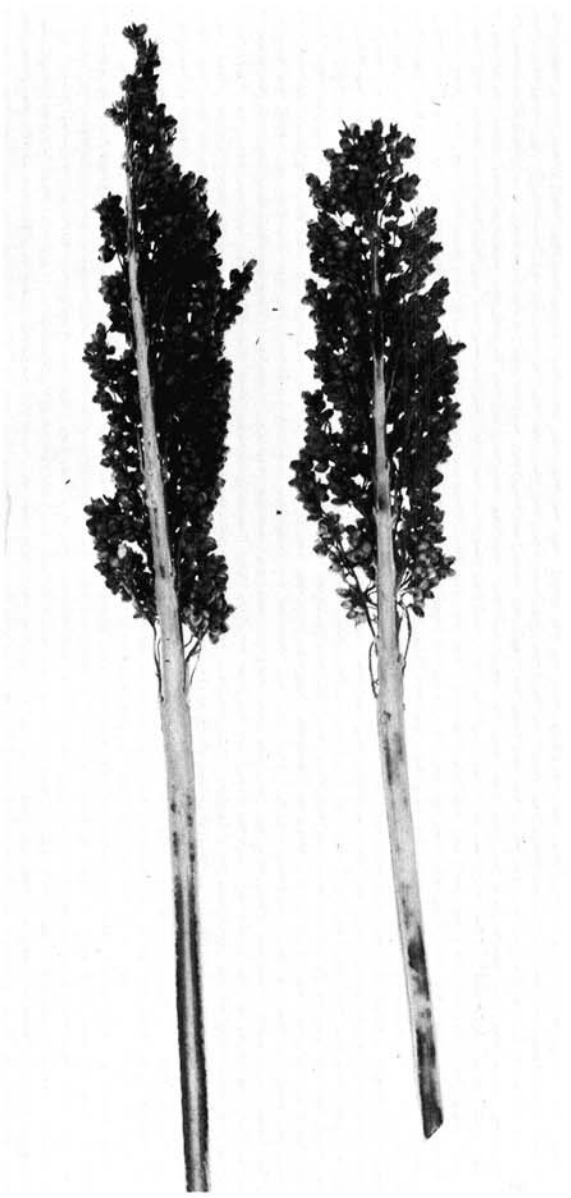

Fig. 3. Showing pigmented pith tissues of peduncles supporting heads with small seed.

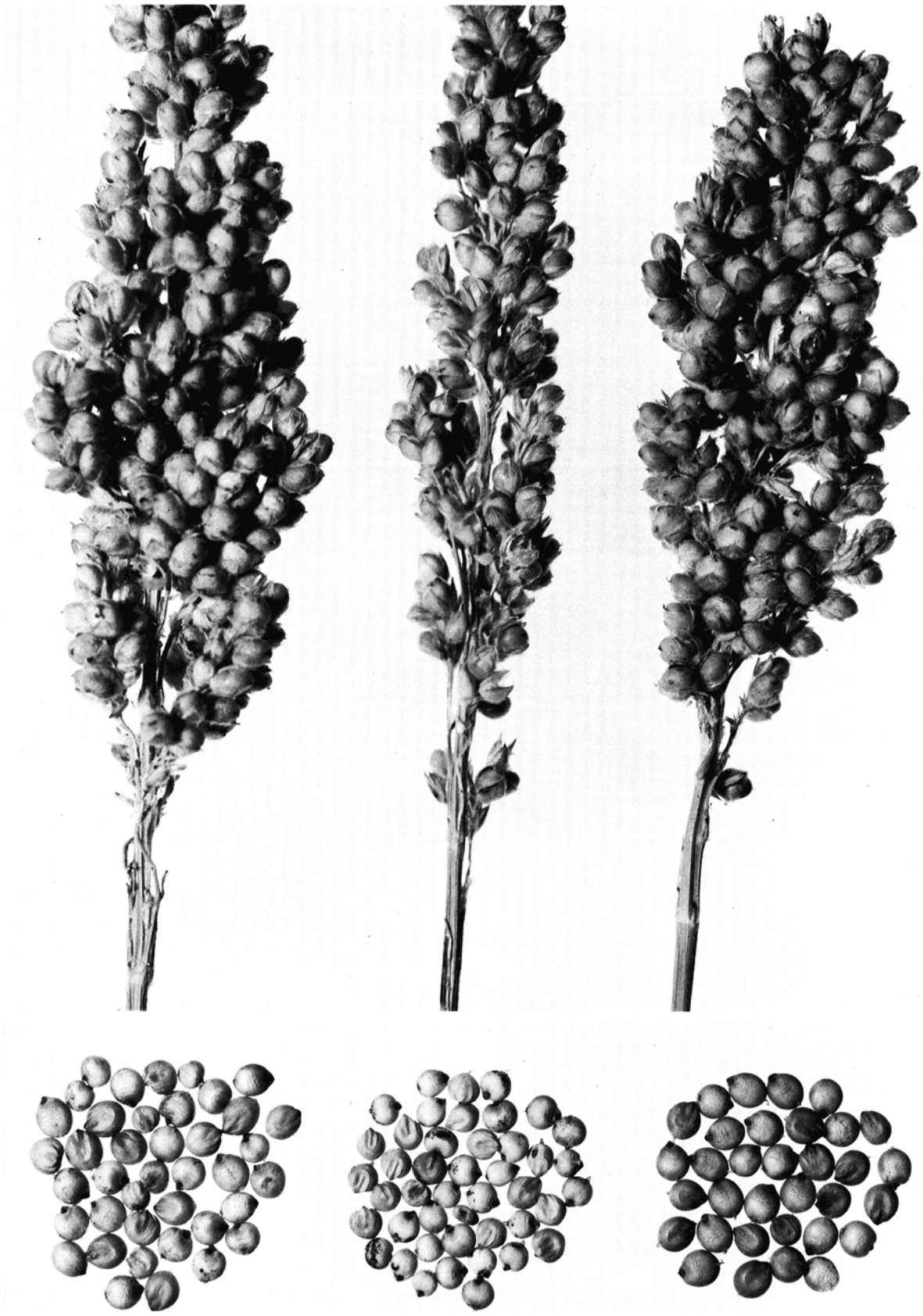

Fig. 4. Heads and seed samples from sorghum plants inoculated with maize dwarf mosaic virus at boot (left), anthesis (center), and not inoculated (right).

markedly cool nights and recollections that red leaf symptoms in maize dwarf-mosaic (MDM) are triggered by similar conditions (5) led us to make extractions for virus in affected panicles.

None of the partially purified, viral preparations exhibited opalescence, suggesting absence or low concentrations of virus. However, all inoculations of corn seedlings with preparations from seed-free panicle parts of 32 randomly selected panicles resulted in mosaic symptoms within 1-2 weeks. The virus was identified as maize dwarf mosaic virus (MDMV) on the basis of host range, and as MDMV-strain A by the reaction on Combine Kafir 60 sorghum (4). No virus was transmitted from preparations of seed.

In the greenhouse, RS 610 sorghum plants used for reproduction of disease tended to be short, small-headed, and often partially sterile. Nevertheless, 60 of 75 plants that had been inoculated with MDMV at anthesis developed typical panicle lesions while the temperature was kept at <16 C. After plants matured, affected seeds were smaller and of lighter weight than seeds of noninoculated plants or those inoculated at boot (Fig. 4). Average weight of 1,000 seeds was 38.9, 24.4, and 40.0 g for plants inoculated at boot, inoculated at anthesis, and noninoculated, respectively. Panicle lesions did not develop on any plants when the experiment was repeated without lowering the temperature. Average weight of 1,000 seeds was 45.6, 42.9, and 46.5 g for plants inoculated at boot, inoculated at anthesis, and noninoculated, respectively. Inoculation of corn seedlings with extracts from plants inoculated at both boot and anthesis of both exeriments resulted in development of mosaic symptoms.

The etiology of small seed associated with MDMV at Manhattan in 1970 closely followed that observed in southwestern Kansas in 1969. Since MDMV was extracted from affected panicles at Manhattan, and since the disease was reproduced under greenhouse conditions with this virus, we suspect that MDMV may be the primary incitant in many cases of undersized seed among grain sorghums in southwestern Kansas. We have not attempted to extract virus from affected panicles from fields in that area, nor have we directly determined the viruliferous nature of postexsertion aphid populations in fields that subsequently exhibited small seed. However, high incidence of MDM was noted in adjacent, late-planted sorghums which were in the five- to seven-leaf stage.

As described herein, small seed and MDMV have similar histories of occurrence and distribution on the High Plains. Such small seed was noted in southwestern Kansas 2 years after MDM appeared. Conditions favoring the described manifestations occur more commonly on the High Plains than farther east. On the High Plains, however, those manifestations have not been found farther north than MDMV-infected sorghum. Both postexsertion aphid populations and below-normal temperatures, however, have occurred as commonly in northwestern Kansas (where MDMV has not been found) as in southwestern Kansas. Although host pigments and necrosis of infected tissues in both leaves and panicles are triggered by low temperatures, those in panicles are relatively inconspicuous and remain most evident when subsequent weather favors rapid maturation of heads. When senescence of panicle parts proceeds more slowly because of sustained humid, cool weather after development of panicle lesions, those lesions tend to fade and disappear before excessive seed shrinkage occurs.

Failure of plants to develop symptoms when inoculated at boot may indicate that time between initial infection and panicle necrosis may be restricted, possibly because peak virus titer in affected tissues is transitory. Further transmission studies are needed to clarify this point.

The possibility that an airborne fungus might cause the panicle lesions has not been eliminated. However, inconsistency of isolations from very young lesions, and production of panicle lesions under greenhouse conditions by inoculation with MDMV, lead us to minimize this possibility.

The possibility that panicle lesions could result from toxigenic effect of greenbug activity also is recognized (2). In our tests, that possibility seemed remote because greenbugs did not colonize panicles and because panicle lesions developed as long as a month (third planting) after colonization of leaves ceased.

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