# Evidence for Soil Transmission of Sugarcane Mosaic Virus

W. P. Bond and T. P. Pirone

Department of Plant Pathology, Louisiana State University, and Department of Plant Pathology, University of Kentucky, respectively. Senior author is now Assistant Professor of Biology, Southeastern Louisiana College, Hammond, 70401; junior author is Associate Professor of Plant Pathology, University of Kentucky, Lexington, 40506.

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ABSTRACT

Noninoculated sorghum plants became infected with sugarcane mosaic virus when grown in containers with infected plants. Control plants grown in separate containers, but at an equal distance from the inoculated plants, did not become infected. The fact that transmission is associated with the presence of roots of healthy and infected plants in the same container, rather than the proximity of healthy and infected leaves, suggests that soil transmission, rather than transmission by aphids or other aerial vectors, is occurring. Examination of the soil ruled out nematodes or root aphids as potential vectors. Root contact was not necessary for transmission. The possibility of transmission by fungi or other microorganisms could not be ruled out. Phytopathology 60:437-440.

In the course of routine bioassays of strain H of sugarcane mosaic virus (SCMV), we noticed that noninoculated sorghum plants often became infected when grown in greenhouse flats containing infected plants. Transmission occurred only to plants growing in the same flat with inoculated plants; infected plants were never found in flats containing noninoculated sorghum seedlings. The frequent occurrence of this phenomenon under screened greenhouse conditions suggested that the virus was being spread by some means other than aphids.

The experiments described in this paper were designed to attempt to determine how SCMV spread to noninoculated plants. Some of the data have been published in abstract (1).

MATERIALS AND METHODS.—Strain H of SCMV, obtained from the collection maintained at the USDA Sugarcane Field Station, Houma, Louisiana, was used in the initial experiments. An isolate of the virus which infected Johnson grass collected from "maize dwarf mosaic"-infected corn at Frankfort, Kentucky, was also used in later experiments.

The sorghum-sudan hybrid Beefbuilder T (*Sorghum vulgare* × *Sorghum vulgare sudanense*) was used in most experiments. The hybrid Lindsay 77 was used in several tests with the Johnson grass strain. In the initial experiments with strain H of SCMV, the plants were grown in a mixture of Mississippi sandy loam, peat moss, and sand in a 3:2:1 ratio. In later experiments with strain H, and in experiments with the Johnson grass strain, plants were grown in nonamended silt loam. The soil was steam-sterilized for 3 or 4 hr at 15 psi before use. When metal containers were used they were sterilized with the soil. Plastic containers were disinfested with a 1% sodium hypochlorite solution before use.

Plants were mechanically inoculated when about 5 cm tall. They were maintained under screened greenhouse conditions and watered with tap water. The greenhouses were fumigated weekly with TEPP or Vapona.

Data on transmission to the test plants were recorded 4 to 5 weeks after mechanical inoculation of the virus source plants.

RESULTS.—*Preliminary trials.*—Rows of sorghum plants inoculated with strain H were alternated, in metal flats, with rows containing an equal number of noninoculated plants. To check for possible leaf-to-leaf or aphid transmission, control plants were grown in the same flat, but were contained in metal pans to keep their roots separate from those of the inoculated plants. Table 1 shows the results of such experiments. In the first five experiments, transmission to test plants growing in soil containing infected plants ranged from 0.7 to 5.4%. Two to 4 weeks were required for symptom expression in the test plants. In two of the experiments, however, a plant in the controls also became infected.

No attempt had been made, in the first five experiments, to prevent water-splashed soil from entering the pans containing the control plants. This could possibly account for the occurrence of infection in the control plants. In the next five experiments, therefore, care was taken to avoid splashing water and soil into the controls. In these tests, transmission to test plants was from 0.8 to 5.0%, with no transmission to controls (Table 1, tests 6 to 9).

*Transmission of strain H in the absence of root contact.*—Some infection occurred in the control plants when care was not taken to keep water from the soil around infected plants out of control containers, suggesting that root contact might not be necessary to obtain transmission. Two types of experiments were set up to test this possibility. In the first type, porcelain pans that contained noninoculated plants were set in flats containing infected plants. The pans were set below the soil level to allow water to run into the pan from the flat containing infected plants. The roots of plants inside the pans did not contact those of infected plants. The controls were plants grown under similar conditions, except that the pan was set above the soil level and care was taken to avoid water splash-

TABLE 1. Evidence of transmission of strain H of sugarcane mosaic virus from infected plants to noninoculated test plants grown in the same flat[a]

| Experiment | Test plants | | Transmission % | Check plants | |
|---|---|---|---|---|---|
| | Exposed | Infected | | Exposed | Infected |
| 1 | 201 | 11 | 5.4 | 138 | 0 |
| 2 | 288 | 8 | 2.7 | 141 | 1 |
| 3 | 318 | 13 | 4.1 | 60 | 0 |
| 4 | 273 | 10 | 3.7 | 138 | 1 |
| 5 | 286 | 2 | 0.7 | 185 | 0 |
| 6 | 254 | 2 | 0.8 | 193 | 0 |
| 7 | 288 | 9 | 3.1 | 141 | 0 |
| 8 | 200 | 10 | 5.0 | 154 | 0 |
| 9 | 399 | 18 | 4.5 | 702 | 0 |
| 10 | 610 | 4 | 0.7 | 633 | 0 |

[a] Rows of sorghum plants inoculated with the virus were alternated, in metal flats, with rows of noninoculated plants. Control plants were grown in the same flat, but were contained in metal pans, to keep their roots separate from those of infected plants.

ing into it when the infected plants were watered. The total transmission, in two experiments, to plants inside pans into which soil water was allowed to wash from infected plants was 6.2% (10/160 plants infected). No transmission occurred to plants in the control pans. The flats which contained infected plants also contained noninoculated plants to compare the level of soil transmission which developed when root contact could occur. Transmission to these plants was 8.8% (22/248 plants infected) and 4.5% (20/442 plants infected).

In the second type of test, sorghum seedlings were planted in 4-inch peat pots at each end of a rectangular polyethylene container. The plants at one end were mechanically inoculated. The plants at the other end were not inoculated. Since there was no soil between the pots, roots could be kept separated for the duration of the experiment. The only contact between the plants at either end was through water at the bottom of the polyethylene container. Control plants were grown in adjacent containers at an equal distance from the inoculated plants, but there were no infected plants in the containers. The results are shown in Table 2. Transmission was about 5% in each of 3 replicates. No infection occurred in the controls.

These two types of experiments were also made inside screened cages in a screened greenhouse. The area inside the cages was sprayed with phosdrin. Seeds were then germinated in the cages, and plants were inoculated as in other tests. Transmission occurred using either method. No transmission occurred to controls (Table 3).

TABLE 2. Transmission of strain H of sugarcane mosaic virus in the absence of root contact[a]

| | Test plants | | Transmission % |
|---|---|---|---|
| | Exposed | Infected | |
| Plants grown in pans with infected plants | 37 | 2 | 5.4 |
| | 44 | 2 | 4.5 |
| | 39 | 2 | 5.1 |
| Controls grown in adjacent pans | 85 | 0 | 0 |
| | 93 | 0 | 0 |

[a] Infected sorghum plants were grown in 4-inch peat pots at one end of a polyethylene container; test plants were grown at the other end. Water in the container contacted both sets of plants. Control plants were grown in adjacent pans that contained no infected plants.

*Comparative transmission tests with the H and Johnson grass strains.*—Soil transmission of these strains was compared in greenhouse tests. Beefbuilder T seedlings were grown in nonamended silt loam in polyethylene trays. The plants in one-half of each tray were mechanically inoculated with either strain of the virus. The plants in the other half were not inoculated. Controls were plants grown in trays which contained no inoculated plants. Each container was mounted on a separate base which kept it 3 inches above the bench, so that soil water from trays which contained inoculated plants did not contact the controls. Trays containing inoculated plants were alternated with control

TABLE 3. Transmission of strain H of sugarcane mosaic virus under screened cages in the absence of root contact[a]

| Experiment | Replication | Test plants | Transmission | |
|---|---|---|---|---|
| | | | % | To controls |
| 1 | 1 | 6/59[b] | 10.1 | 0/73 |
| | 2 | 6/72 | 8.3 | 0/70 |
| | 3 | 3/68 | 4.4 | 0/65 |
| | 4 | 1/66 | 1.6 | 0/72 |
| 2 | 1 | 2/226 | 0.9 | 0/235 |
| | 2 | 6/232 | 2.5 | 0/227 |
| 3 | 1 | 8/193 | 4.1 | 0/137 |
| 4 | 1 | 3/115 | 2.6 | 0/94 |
| | 2 | 2/96 | 2.0 | 0/86 |
| | 3 | 0/104 | 0.0 | 0/108 |

[a] In experiments 1 and 4, root contact was avoided by growing test sorghum plants in pans set slightly below the surface of flats containing infected plants. Water was allowed to flow into the pan from the flat containing infected plants. Controls were plants grown in pans set above the surface of flats containing infected plants; water from flats containing infected plants was kept out. In experiments 2 and 3, root contact was avoided by growing infected plants in 4-inch peat pans at one end of a polyethylene container; test plants were grown at the other end. Water in the container contacted both sets of plants. Control plants were grown in adjacent pans which contained no infected plants.

[b] Numerator, plants infected; denominator, plants exposed.

TABLE 4. Comparative transmission of the H and Johnson grass strains of sugarcane mosaic virus[a]

| Experiment | Transmission of Strain H | Transmission of Johnson grass strain | Replications | Transmission to controls | Replications |
|---|---|---|---|---|---|
| 1 | 2/1083[b] | 3/1056 | 10 | 0/3518 | 15 |
| 2 | 1/1145 | 3/1035 | 10 | 0/3238 | 14 |
| 3 | 2/1350 | 5/1347 | 12 | 1/3655 | 19 |

[a] Sorghum plants in one half of a polyethylene tray were inoculated with one of the SCMV strains; noninoculated test plants were in the other half. Controls were plants grown in adjacent trays which contained no inoculated plants.
[b] Numerator, plants infected; denominator, plants exposed.

trays on the bench. The frequency of transmission was measured by determining the number of infected plants in the noninoculated half of the tray. The results of three experiments are shown in Table 4. Similar levels of transmission were obtained with the H and Johnson grass strains, but the levels were considerably below those obtained in earlier tests with strain H (Table 1).

*Transmission of the Johnson grass strain in the absence of root contact.*—These experiments were made using the second type of test as described above. Lindsay 77 was grown in nonamended silt loam in peat pots. Plants were grown in a Sherer-Gillette CEL-25 HL chamber illuminated with about 2,000 ft-c for 14 hr/day. A temperature of 32 C was maintained during light hours, and 26 C at night. The results (Table 5) indicate that this strain can also be transmitted in the absence of root contact.

*Retention of virus in the soil.*—To determine whether transmission of virus retained in the soil could occur in the absence of infected plants, the upper portions of plants were removed from soil in which transmission of strain H had occurred previously. The roots were allowed to remain in the soil. Beefbuilder T seeds were then planted in this soil, and the resulting plants were examined after 1 month to determine whether transmission had occurred. No evidence of transmission was obtained. In two tests, none of 1,260 and 1,497 plants, respectively, was infected.

TABLE 5. Transmission of the Johnson grass strain of sugarcane mosaic virus in the absence of root contact[a]

| Experiment | Replication | Transmission to Test plants | Transmission to Controls |
|---|---|---|---|
| 1 | 1 | 0/85[b] | 0/112 |
| | 2 | 1/92 | 0/95 |
| | 3 | 1/88 | 0/106 |
| 2 | 1 | 1/109 | 0/108 |
| | 2 | 0/112 | 0/121 |
| | 3 | 0/111 | 0/115 |
| 3 | 1 | 1/90 | 0/83 |
| | 2 | 2/117 | 0/117 |
| | 3 | 0/145 | 0/136 |

[a] Infected sorghum plants were grown in 4-inch peat pots at one end of a polyethylene container; test plants were grown at the other end. Water in the container contacted both sets of plants. Control plants were grown in adjacent pans which contained no infected plants.
[b] Numerator, plants infected; denominator, plants exposed.

*Attempts to implicate an organism as a vector.*—Experiments were conducted in an attempt to gain evidence for the role of a vector in soil transmission. Soil in which transmission had occurred in a previous experiment (used soil) was compared with freshly sterilized soil. One half of a flat of Lindsay 77 seedlings was mechanically inoculated with strain H, and transmission to plants in the noninoculated half was determined. There was no increase in the level of transmission to plants grown in the used soil.

Total transmission, in two experiments, to test plants grown in freshly sterilized soil was 0.8% (8/975 plants infected). Transmission to test plants grown in used soil was 0.5% (5/1,098 plants infected). None of 2,584 control plants were infected.

Two fungi, a *Pythium* sp. and a *Colletotrichum* sp., could frequently be isolated from roots of sorghum plants in these tests. Liquid cultures of these fungi were made, and mycelial suspensions were added to sterilized soil. Transmission of the virus was compared with that in sterilized soil. No increase in transmission occurred when these fungi were added to the soil.

Soils from tests in which high levels of transmission occurred were examined for the presence of nematodes. No plant-parasitic nematodes were found, although free living forms were sometimes present in the soil. Roots and soil were examined for the presence of root aphids; none were found.

*Identity of the virus involved in transmission.*—Virus was extracted from plants which had been infected through soil transmission. Serological and infectivity tests and electron microscope pictures of leaf dip preparations all verified that the virus being transmitted was SCMV.

DISCUSSION.—The levels of transmission which occurred in most of the tests reported here are extremely low and, if observed under normal greenhouse conditions, would probably be attributed to spread by aphids. The lack of transmission to controls in greenhouse tests, if proper precautions in watering were observed, and the fact that transmission occurred under screened cages and in growth chambers, in which rigorous measures were taken to exclude aphids, suggest that aphid transmission is not involved here. Transmission by other aerial vectors such as mites, while not excluded by the caging experiments, seems unlikely in view of the lack of transmission to control plants whose leaves were actually in contact with those of infected plants. The possibility that transmission occurred due to handling of the plants seems unlikely, for similar reasons.

Any handling of plants necessary to the experiment was done with extreme care, and applied to test and control plants alike.

Harrison (4) has defined a soil-borne virus as a virus with an underground natural method of spread which does not depend simply on contact between tissues of infected and healthy plants. That SCMV is transmitted from infected plants to test plants grown in the same container in the absence of root contact (Tables 2, 3, 5) indicates that the type of transmission reported here is in this category.

Much lower levels of transmission were obtained in later experiments with strain H (Table 5) than in earlier ones (Tables 1-3). Although techniques used were essentially similar, there were unavoidable differences in the materials used, including differences in seed lot, soil type, and water source. Differences in environmental conditions in Baton Rouge and Lexington also exist.

Both Cadman (2) and Grogan & Campbell (3) suggest that soil transmission of viruses is a function of specific biological agents. Although the soil used in these tests was sterilized before use, the seeds were not surface-sterilized, and the plants were commonly affected with root and stem rots. Differences in any of the materials mentioned above might affect the population of soil organisms which might be involved in transmission. Even if no organisms are involved in transmission, some of these factors now being studied could conceivably affect the virus once it is outside the root.

LITERATURE CITED

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