Transmission of Maize Dwarf Mosaic Virus with Solid-Stream Inoculum

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ABSTRACT

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In the absence of 600-mesh silicon carbide, solid-stream inoculum formed by solid-stream nozzle tips was as effective as aerosol-abrasive inoculum formed by airbrush for maize dwarf mosaic virus (MDMV) inoculation to corn (*Zea mays*). In one test, inoculum (1:20, w/v) applied from a distance of 10 cm using a nozzle with a 0.7-mm-diameter orifice and line pressures of 2.1, 4.2, 6.3, 8.5, 10.6, and 12.7 kg/cm^2 resulted in 65, 81, 82, 93, 96, and 96% transmission, respectively. When 600-mesh silicon carbide was added to the same inoculum, transmission values for solid-stream inoculation ranged from 87 to 100% but were not significantly different from the 88% transmission for airbrush inoculation. In solid-stream inoculation with a 0.7-mm-diameter nozzle, percent transmission increased with a second and third inoculation pass. A nozzle orifice of 0.7-mm-diameter and line pressure of 6.3 kg/cm^2 were optimal for greenhouse use, but in the field, a nozzle orifice of 0.8 or 1 mm diameter and pressure of 12.3 kg/cm^2 were optimal. Compared with airbrush inoculation, advantages of solid-stream include fast rates of inoculation, consistent transmission, use of more dilute inoculum, and inoculation without silicon carbide. A major disadvantage is use of a greater volume of inoculum. In greenhouse inoculations, this problem is not insurmountable but in the field, the system is impracticable without a recirculating system, as described in this paper.

Additional key words: mechanical inoculation

Richards and Munger (13) developed an aerosol method of inoculum dispersal that efficiently transmitted some plant viruses. The airbrush (6) was a greatly improved modification. Subsequent modifications, especially for inoculations of monocotyledonous plants, usually resulted in larger and more powerful machines (2,9,14). Except in one case (3), inoculum in these modified apparatuses still included silicon carbide and dispersed as an aerosol. Aerosol dispersal of inoculum with smaller equipment (eg, airbrush) required an operator to maintain a nearly constant distance between plant and nozzle orifice for extended periods to properly place the virus-silicon carbide mixture on the inoculation site. With monocot seedlings, digital dexterity was also required to gently separate and hold individual plants during inoculation. Larger equipment often produced a cloud of virus-silicon carbide aerosol and required additional protective equipment to prevent aerosol inhalation.

Regardless of available equipment, an inoculation method that is quick and efficient over a wide range of operating conditions was still needed to evaluate corn (*Zea mays* L.) for resistance to virus diseases. Equally important was adaptation of the technique for field use with commonly used spraying methods. This last requirement precluded use of an abrasive that might damage pumps or clog filter systems. Mumford's (10) report of a solid-stream inoculum under pressure suggested an alternative method that might be as effective as the more exacting airbrush method for virus transmission.

We report a modification of the solid-stream inoculum method that increases efficiency of inoculation and rate of transmission and is efficient over a wide range of operating conditions for maize dwarf mosaic virus strain A (MDMV-A). A preliminary report has been made (7).

MATERIALS AND METHODS

Greenhouse tests. Seeds of inbred Oh28 corn (25–30 seeds per row) were sown in three rows in flats (30.4 × 45.7 cm) filled with a greenhouse soil mix. When seedlings produced three to five leaves and reached a height of 13–15 cm (at 17–21 days), poorly growing plants were removed, leaving 21–23 plants per row. One row of plants was used for a replicate and three replicates were used for each treatment.

Oh28 seedlings (14–21 days old) were also used as source plants for MDMV-A inoculum. Leaves from infected plants were harvested about 21 days after inoculation, stripped of midribs, homogenized with a blender in buffer solution (0.01 M potassium phosphate buffer, pH 7.0), and the juice filtered through four layers of cheesecloth. The final dilution of infected leaves to buffer solution was usually 1:20 (w/v). In preliminary tests, a 1:20 instead of a 1:10 dilution was sufficient to detect differences among techniques. Silicon carbide (0.25%, 600-mesh) was added when needed and kept in suspension by frequently shaking the inoculum container during inoculation.

Airbrush inoculation was done with a Paasche model H3 airbrush (Paasche Airbrush Co., Chicago, IL 60614) powered by 4.2 and 8.5 kg/cm^2 air pressure for greenhouse and field use, respectively. During inoculation, the airbrush was held about 1 cm from corn seedlings. Inoculum was directed toward the base of whorl leaves held down by the thumb and slightly bent around the forefinger. An inoculation period of about 1 sec/plant (flow rate about 18 ml/min) was required to produce a slight water-soaked appearance that indicated sufficient treatment.

Solid-stream inoculation was done with a model 22H Gunjet and brass or hardened stainless steel UniJet spray nozzle tips, models 1501, 000050, 0001, 00015, and 0002 with 0.7, 0.5, 0.7, 0.8, and 1-mm-diameter orifices, respectively (Spraying Systems Co., Wheaton, IL 60187) (Fig. 1). The first two numbers and the next two to four numbers in each nozzle refer to the spray angles and flow rates, respectively, standardized at 2.4 kg/cm^2, eg, 000050 is 0 degree or a solid-stream nozzle with a flow rate of 0.02 L/min (0.005 gal/min). Hereafter, spray tip nozzle models 1501, 000050, 0001, 00015, and 0002 will be referred to as N1, N2, N3, N4, and N5, respectively. Inoculum was pressurized in a 2.3- or 11.4-L stainless steel cylinder (model SHD2250, Hoke, Inc., Saddle Brook, NJ 07662, and Sparton Model, Fox Equipment Co., Kansas City, MO 64100) by regulated compressed air. Neoprene hydraulic hoses were used to carry both

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air pressure and virus inoculum (Fig. 1).

During inoculation to compare nozzles and pressures, the Gunjet was held about 10 cm from test plants. Plants were supported by a backrest during inoculation, and depending on the treatment schedule, inoculated once each about 0.6, 2, and 6 cm above the bases of the whorl leaves. Inoculation time was about 1 sec/replicate. Excess inoculum collected from the backrest was reused when needed.

Inoculated plants were maintained in a greenhouse and rated for virus symptoms three times at weekly intervals. Greenhouse temperatures for day/night averaged 24/18 C. Plants were fertilized weekly with a water-soluble solution of 20-20-20 fertilizer (0.4%) and sprayed with 0.5% malathion for insect control when necessary.

Field tests. Field plants were Comanche and Harmony sweet corn hybrids planted in rows 76 cm apart with 20-cm spacings within rows. Each plot, planted to one hybrid, was 46 m long. Treatments were replicated twice in the two sweet corn hybrids. Test plants were inoculated 2–6 cm above the bases of the whorl leaves when they were in the five- to seven-leaf stage and 30–38 cm tall. Plants were rated for virus symptoms 2 and 3 wk after inoculation.

Inoculum preparation for field use was the same as for greenhouse tests, except an ice bath was used to keep the inoculum cool and 10 ml of Antifoam B (Dow Corning Corp., Midland, MI 48640 and Sigma Chemical Co., St. Louis, MO 63178) was added to 19 L of inoculum.

Figure 2 is a schematic drawing of a tractor-mounted version for solid-stream inoculation. Inoculum was placed in a 19-L container surrounded by an ice bath. A piston pump (model 6412, F. E. Myers & Bros. Co., Ashland, OH 44805) was driven by the tractor power-takeoff. Inoculum flowed through in-line filters before reaching the nozzles. Flow to the nozzles was turned on or off with a quarter-turn ball valve. Nozzle pressure (5.3, 8.8, or 12.3 kg/cm^2) was controlled with a pressure relief valve. A pair of nozzles (either N3 or N4) placed 2.5 cm apart on a vertical line directed the inoculum 20 cm across the row into a collector when corn plants were not in the path of the pressurized inoculum. The tractor was driven at 2.4 or 4.8 km/hr.

The collector was made of galvanized sheet metal and mounted on a skid. Nozzles, collector, and skids were fastened to the tractor with parallel linkages that permitted the unit to follow soil contours and maintain a relatively constant height. The collector had three sets of filters. A brass screen (eight-mesh) on the side opposite the nozzles excluded leaves; inside were two layers of foam filters supported by a 50-mesh stainless steel screen. Excess inoculum was collected and funneled to the bottom of the collector. Inoculum was withdrawn from the collector with a size one-half model V venturi (Pardee Engineering, Berkeley, CA 94710) and returned to the tank. Flow rate through the venturi was controlled by a throttling valve. Equipment was cleaned with a detergent wash and water rinse. The nozzles, filters, and virus container were cleaned separately.

Data analysis. Each experiment (nozzle diameter) was analyzed as a factorial with pressure as one main effect. Silicon carbide, number of inoculation passes, inoculation site, dilution, tractor speed, or variety were main effects where appropriate. Before analysis, data were transformed to arc sine ($\sqrt{x/100}$), where x is percent transmission. Contrasts of the treatment means were then conducted to determine the significance of the following effects: treatment (ie, silicon carbide vs. no silicon carbide, number of passes, site of inoculation, or inoculum dilution); linear or quadratic (curving) change in transmission with increase in pressure; and the interaction of linear and quadratic effects with treatment. A linear $\times$ treatment interaction, for example, would indicate different slopes for the lines corresponding to the two treatment levels (eg, silicon carbide vs. no silicon carbide). Contrasts were also used to determine if airbrush differed from solid-stream inoculation at the same pressure and if the various solid-stream inoculation treatments within an experiment differed from each other.

RESULTS

Greenhouse tests. Transmission rates increased to a maximum or reached 100% with increased nozzle pressures (Fig. 3). Use of silicon carbide generally improved transmission rates (especially at the lower pressures), and with some nozzles, partially nullified the effect of increasing inoculation pressures (Fig. 3C). Without silicon carbide, solid-stream inoculation at 12.7 kg/cm^2 for all nozzle sizes tested (N1–N5, inoculum diluted at 1:20, and inoculated with two passes) was greater than or equal ($P = 0.05$) to airbrush aided by silicon carbide. Without silicon carbide, percent transmission with nozzle N1 (Fig. 3A) did not appear as high as with N3 (Fig. 3C), but amounts generally appeared similar when silicon carbide was used. Volume of liquid delivered was similar except N1 had a spray angle of 15 degrees. The change in transmission means as related to increased pressure was linear ($P = 0.01$) for nozzles N1–N4; quadratic changes were significant for nozzle N4. Because percent transmission for N4 and N5 were high and very similar, they were not included in Figure 3. Percent transmission with nozzle N4 ranged from 85.5 to 100% and 97.2 to 100% for silicon carbide and no silicon carbide treatments, respectively, and

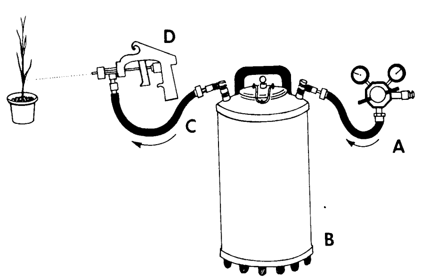

Fig. 1. A portable version of the equipment used for solid-stream inoculation: **(A)** air-pressure regulator, **(B)** virus inoculum container with rated working pressure of 8.8 kg/cm^2, **(C)** connecting hydraulic hoses, and **(D)** spray gun and nozzle.

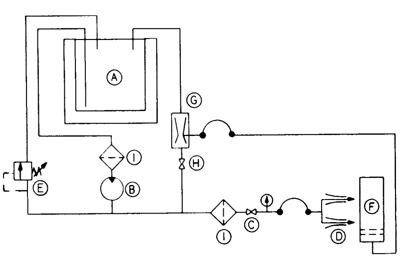

Fig. 2. Schematic drawing of the tractor-mounted version of equipment used for solid-stream inoculation: **(A)** virus container, **(B)** power-takeoff-driven piston pump, **(C)** quarter-turn ball valve, **(D)** nozzles, **(E)** pressure-regulating valve, **(F)** collector, **(G)** venturi, **(H)** throttling valve, and **(I)** in-line filters.

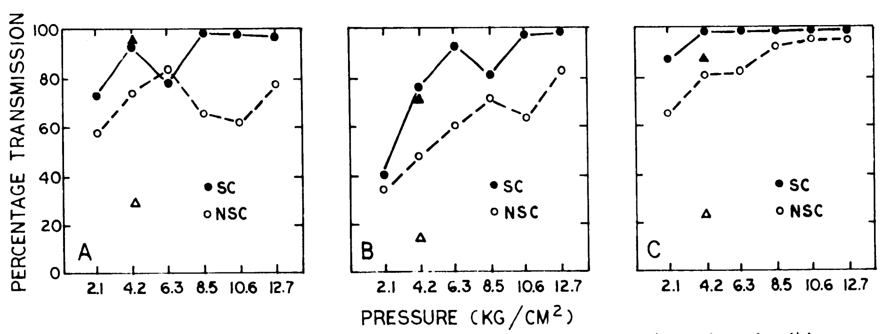

Fig. 3. Transmission of maize dwarf mosaic virus (MDMV-A) with airbrush and solid-stream inoculation, with or without silicon carbide treatments, using nozzle models **(A)** 150100, **(B)** 0050, and **(C)** 000100. Values are means of three replicates. Plants were inoculated once each about 0.6 or 2 cm above the bases of the whorl leaves with solid-stream inoculum (● or ○). SC = inoculation aided by adding 0.25% silicon carbide (● or ▲) and NSC = inoculation without silicon carbide (○ or △). Inoculum prepared at 1:20 (w/v) with 0.01 M phosphate buffer, pH 6.8–7.0. Airbrush inoculation (▲ or △) powered by air pressure of 4.2 kg/cm^2.

generally were not statistically different at 6.3 to 12.7 kg/cm^2. Percent transmission with nozzle N5 ranged from 87.6 to 95.6% and 93.8 to 100.0% for silicon carbide and no silicon carbide treatments, respectively. Although differences were small between treatments, transmissions by the addition of silicon carbide were statistically better at pressures of 4.2, 6.3, 8.5, and 12.7 kg/cm^2. In these two tests, percent transmission for both nozzles with or without silicon carbide treatments were statistically similar to the standard airbrush method with silicon carbide treatment (93.3 and 96.3% for the first and second airbrush tests, respectively) and better than airbrush without silicon carbide treatment (37.9 and 36.7% for the first and second tests, respectively). There was a significant ($P = 0.01$) linear change in response to increased pressures in all tests. The treatment × linear interactions with nozzles N2, N3, and N5 were not significant. Nozzle N3 had a significant quadratic ($P = 0.05$) change in response to increased pressure.

When plants were inoculated with the N2 and N3 nozzles, percent transmission increased (Fig. 4A–D) with second and third inoculation passes. The difference in percent transmission between the second and third passes with the N3 nozzle, however, was not significant. Inoculations of leaves with one pass (nozzle N3, Fig. 4E) either 0.6 or 2 cm above the base of the whorl were significantly different ($P = 0.01$) from each other. Most differences resulted from inoculations at the lower pressures. With the same nozzle, reducing the inoculum dilution from 1:20 to 1:80 (w/v) significantly reduced ($P = 0.01$) the transmission rate. Linear change in response to increased pressure was significant ($P = 0.01$) in all tests except in the comparison between the 1:20 and 1:80 dilution. The treatment × linear interaction also was significant ($P = 0.01$) in all tests except in the comparison between two and three passes with N3. The quadratic change in response to increased pressure was not significant in any of the tests.

Field tests. *F* values of data from the field test on effects of corn hybrid variety, pressure, pressure × variety interaction, tractor speed, and nozzle diameter were significant ($P = 0.05$) based on analysis of variance. Percent transmission for nozzles N3 and N4 increased linearly ($P = 0.01$) with increased pressure. The linear × variety and the quadratic × variety interactions were significant ($P = 0.05$ and 0.01, respectively) in that transmission in the two varieties behaved differently at different pressures. Transmission rates for N4 were significantly greater than for N3 (41.8 vs. 19.0%, respectively); significantly higher transmission resulted at 2.4 km/hr than at 4.8 km/hr (34.2 vs. 26.6%, respectively, $P = 0.05$). Solid-stream inoculations with one combination of treatments (N4 nozzle, ground speed of 2.4 km/hr, and pressure of 12.3 kg/cm^2) appeared as effective as airbrush inoculation aided by silicon carbide (51.9 vs. 55.6%, respectively). Ground speed of airbrush inoculation with a manually propelled air compressor averaged 0.2 km/hr; a tractor-mounted airbrush system with two proficient operators inoculating simultaneously could attain ground speeds (combined) as high as 0.8–1 km/hr.

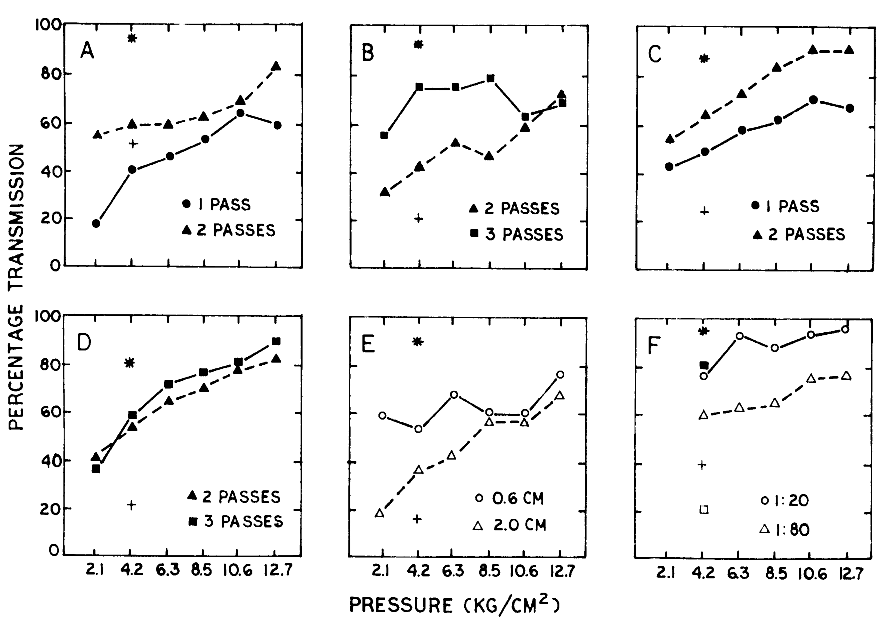

Fig. 4. Percentage transmission of maize dwarf mosaic virus (MDMV-A) with airbrush and solid-stream inoculations at different inoculation frequencies, sites, and dilutions using nozzle models **(A and B)** 00050 and **(C–F)** 000100. Solid-stream inoculation was without silicon carbide; airbrush inoculation was at 4.2 kg/cm^2 with (*) and without (+) 600-mesh silicon carbide (0.25%). **(A–C)** • = Plants inoculated once at 0.6 cm, ▲ = plants inoculated once each at 0.6 and 2 cm, and ■ = plants inoculated once each at 0.6, 2, and 6 cm above the bases of the whorl leaves. **(E)** Inoculation sites were 0.6 (o) and 2 (△) cm above the whorl leaves and plants were inoculated with one pass. **(F)** Dilution of inocula: o = 1:20 and △ = 1:80 (w/v). Plants were inoculated once each at 0.6 and 2 cm above the bases of the whorl leaves.

DISCUSSION

Mechanical inoculations of corn lines to evaluate MDMV resistance can be laborious, tedious, and expensive. Some techniques such as rubbing plants with thumb and forefinger dipped in a silicon carbide-inoculum mix require little input of equipment and can adapt easily to plant variations. Efficiency of the finger-rub method varies among individuals and the method requires many hours for diligent and competent personnel to inoculate large numbers of plants. In the field, the method is difficult to standardize and requires individuals to work in uncomfortable positions.

Use of a solid stream for inoculation substitutes equipment for hand labor. Major benefits of the solid-stream technique include an efficient and consistent transmission rate and elimination of silicon carbide to minimize wear on equipment. Inoculum diluted at 1:20 was not as effective in field tests as in greenhouse tests.

The main advantage of this method (it is efficient and easy to use) is partially nullified by greater inoculum usage. Generally, 0.1 ml/plant of inoculum is required for airbrush inoculation; the amounts used (0.15–0.20 ml/plant) for solid-stream inoculation depend on nozzle diameter, plant size, pressure, duration of inoculation, and whether a recovery system is included. For solid-stream inoculation in the greenhouse, the problem of increased inoculum use is relatively easy to overcome. One or 2 L of inoculum can easily be produced and used to inoculate 5,000–10,000 plants. Another major advantage of this method over the airbrush is the decreased time required for inoculation. In one comparison, solid-stream inoculation of 20 flats of about 60 plants each took about 10 min. Airbrush inoculation of 1,200 plants can be accomplished in 30 min by a proficient individual. Use of the airbrush generally requires dexterity and coordination of hands and test plants for high transmission rates. Consistently high transmission rates in airbrush inoculations have been achieved, often by rigidly controlling inoculation variables (3,8). Use of solid-stream equipment is not as exacting. Inoculating 1,200 plants by the finger-rub method can require 50–60 min. Savings in actual inoculation time under field conditions between the solid-stream and airbrush methods are greater; inoculation of 2,385 plots, each 6 m long, took about half a day and 2½ days, respectively. A shorter time requirement reduces the chances of cancellations of inoculation caused by inclement weather and minimizes environmental variations among replicates caused by a long inoculation period.

In the field, solid-stream inoculations require some subjective judgment and adjustments. Selection of an appropriate pressure, nozzle size, and ground speed depend on plant growth conditions. Inoculations at tractor speeds of 2.4–4.8 km/hr sometimes did not allow sufficient time to center the plants between nozzle and collector, but operating at a pressure of 12.3 kg/cm^2 seemed adequate for transmission and compensated for variations in distances (0–20 cm). Inoculum delivery is high at 12.3 kg/cm^2. Thus, field use without a recirculating system for inoculum recovery is impractical. Passage of inoculum through the recirculating system did not appear to adversely affect infectivity. In all tests, inoculum from the recirculating system assayed by rub-inoculation to test plants was as viable as that before the test.

Previous tests of nozzles with a 25- or 40-degree spray angle at pressures as high as 35.2 kg/cm^2 resulted in low infection levels. Adding 600-mesh silicon carbide to the inoculum did not always significantly aid transmission efficiency of those nozzles. Probably, these spray angles allowed the force of the pressure to quickly dissipate after leaving the nozzle, whereas the force of the pressure in a solid-stream remained concentrated. Use of solid-stream inoculum at high pressures (higher than 12.7 kg/cm^2 for greenhouse-grown plants) or with smaller orifices (<0.3 mm diam.), however, was also inefficient and shredded the leaves. Leaf shredding was most obvious when the injection gun proposed by Mumford (10) was used for inoculation of MDMV.

The distance from plant to nozzle orifice and the rate of nozzle travel in our studies of solid-stream inoculation were based on an arbitrary standard of feasibility. Preliminary tests indicated that the solid stream of inoculum began to disperse at distances greater than about 15 cm and plants were severely damaged by the stream when the plants were too close to the nozzle orifice (about 2–3 cm). Within these limits, the combination of nozzle size, pressure, inoculation site, dilution of inoculum, and number of inoculation passes were studied.

In the airbrush method, operator judgment is required to determine by chance manipulations which nozzle adjustments are necessary to produce the cone-shaped spray pattern (as viewed from the side) required for efficient inoculation. In our experience, calibration of flow rate, pressure, distance of nozzle from plant, etc., without due attention to spray patterns are insufficient to ensure efficient transmission. For example, a nozzle with a worn orifice similarly calibrated as an unworn nozzle may not achieve the same virus transmission rate because the spray pattern has been changed from one that is concentrated into a cone to one that is more diffused. Efficient use of the airbrush appears to be more of an art than an exact procedure.

Virus transmission since the report by Rawlins and Tompkins (12) has generally incorporated some kind of abrasive to the inoculum (5). Hence, efficient virus transmission in the absence of an abrasive was of particular interest. The mode of action of solid-stream inoculation is unclear. Injection of inoculum into intercellular spaces has not resulted in successful virus transmission (1,4) and probably is not the mode of action. Unlike the airbrush, which requires holding the nozzle in place over the inoculation site for about 1 sec or passing over the leaves numerous times (11), solid-stream inoculation with a suitable nozzle diameter can be effected quickly in a single pass. This indicates that the solid-stream inoculum formed by solid-stream nozzles with an orifice diameter of 0.7–0.8 mm and propelled by pressures of 10–13 kg/cm^2 is very efficient in producing the proper type of wounds necessary for virus entry and subsequent multiplication.

Future modifications will aim at inoculum conservation, eg, use of roller instead of piston pumps. Of equal interest will be the applicability of solid-stream inoculum in the transmission of other plant viruses.

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