Characterization of Maize Streak Virus Isolates Using Monoclonal and Polyclonal Antibodies and by Transmission to a Few Hosts

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ABSTRACT

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The pathogenic and serological properties of maize streak virus (MSV) were characterized by studying isolates from maize, sugarcane, and other Poaceae species in Réunion. Transmission tests of six isolates indicated they could be divided into two groups on the basis of their virulence. The isolates in the first group showed differences in the percentage of plants infected and the severity of symptoms and reacted with polyclonal antibodies prepared against an MSV isolate from maize. The second group contained one isolate from sugarcane that only reacted with the polyclonal antibodies after purification. With use of five monoclonal antibodies prepared against a maize isolate from Réunion, three distinct serotypes were identified from leaf samples collected in Africa and islands in the Indian Ocean. The maize isolates from 11 countries all belonged to the same serotype (SP). In contrast, sugarcane was the host for three serotypes (SP, 94, and 180). One monoclonal antibody reacted with the three serotypes and appears to be a useful tool for the diagnosis of all isolates of MSV.

Maize streak virus (MSV) is the cause of the most serious viral disease of maize (*Zea mays* L.) in Africa and in the Mascarenes Islands (7,10,25). Typical streak symptoms have been described not only on maize but also on sugarcane (*Saccharum* sp.) and several Poaceae species (4,15,16). The virus is transmitted by *Cicadulina mbila* (Naude) (Homoptera: Cicadellidae) and other species from the same genus (20). Typical geminivirus particles have been isolated from infected plants (4). Several MSV isolates have been differentiated according to host range (15,16) and by serology (4). On the basis of host range studies, Ricaud and Felix (19) suggested that isolates from sugarcane from Mauritius and Réunion differ from those from Africa (22). Studies of polyclonal and monoclonal antibodies (6) have shown that MSV is highly variable but that all the isolates are serologically related. The most distantly related isolate was from sugarcane from Mauritius. All serological studies on MSV have used antibodies prepared against African isolates from maize and sugarcane (4,6) and a Digitaria streak virus (DSV) isolate (8) from Vanuatu but never against isolates from islands in the Indian Ocean. Consequently, in the present study we analyzed isolates from various origins using monoclonal and polyclonal antibodies produced against maize isolates from Réunion and compared these results with transmission to a range of hosts for Réunion isolates.

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MATERIALS AND METHODS

Viral isolates and leaf samples with streak symptoms. An MSV reference isolate (hereafter designated SP) was collected in Saint Pierre, Réunion, from a maize plant with particularly severe symptoms and maintained on a maize hybrid (cv. INRA 508) through leafhopper transmission without any symptom variation. After two successive transmissions with individual insects and an acquisition access period of a few minutes, two isolates were selected from the SP isolate—one causing severe symptoms (strong SP) and another causing mild symptoms (attenuated SP). Eight samples of maize leaves showing streak symptoms were collected in different parts of the island. *C. mbila* were allowed to feed on them overnight and were then released onto young INRA 508 plants. Several grasses showing streak symptoms were collected in Saint Pierre and maintained in pots. The species collected were from the following genera: *Brachiaria, Cenchrus, Chloris, Coix, Digitaria, Eleusine, Rottboellia,* and *Setaria.* The leafhopper vectors were allowed to feed on them, then were released onto the maize and the *Digitaria* sp. Three cultivars of sugarcane showing streak symptoms were maintained in a greenhouse: R 574 from Réunion, M 112/34 from Mauritius, and Co 1223 from Burkina Faso. For extracts prepared from plants showing streak symptoms and that gave positive reactions with antibodies to MSV, we used the term "isolate," provided the infected plant material could be maintained in a greenhouse either on perennial hosts or by transmission with *C. mbila.* Leaf samples showing streak symptoms were received from Africa, Madagascar, and Mauritius. For these samples, the term "field-collected leaf samples" was preferred because the infected material was not maintained in a greenhouse. These samples and an isolate of DSV from Vanuatu (8) maintained on *D. sanguinalis* (L.) Scop. were used only for serological tests.

Transmission tests. Transmission experiments were conducted in Réunion using leafhopper vectors (*C. mbila*) reared on pearl millet (*Pennisetum glaucum* (L.) R. Brown) and selected for its improved transmission ability. The percentage of active vectors in this experiment was 80%. Plant species tested for susceptibility to the different isolates included an MSV-susceptible maize hybrid (INRA 508), an MSV-resistant composite cultivar (IRAT 297), a sugarcane cultivar (R 574), and wild grass species from plant seedlings, including *Brachiaria plantagina* (Link) Hitch., *B. ramosa* (L.) Stapf., *Cenchrus echinatus* L., *Coix lacryma-jobi* L., and *Digitaria ciliaris* (Retz.) Koeler. For host range studies, nonviruliferous adults of *C. mbila* were placed on the different species of source plants showing streak symptoms for an acquisition access period of 48 hr. Each test plant (maize, sugarcane, and grasses) was caged with 10 of these insects for 10 days. Plants were kept 1 mo for symptom observations. Symptom severity and time of appearance of symptoms were determined on INRA 508 sown in pots. Plants were inoculated by individual insects for

only 24 hr. The severity of the symptoms and the effect on growth of the MSV isolates from Réunion were determined on the susceptible maize hybrid INRA 508 and on the resistant composite cultivar IRAT 297. Three maize isolates (SP, strong SP, and attenuated SP) were compared with three grass isolates (*C. echinatus, Chloris gayana* Kunth, and *B. plantagina*). For each isolate, 12 plants were sown in insect-proof greenhouses on an artificial substrate with a nutrient supply by drip irrigation. They were inoculated at the three-leaf stage with three viruliferous insects kept for 3 hr on the second leaf from the top in a small plastic box attached to the leaf with a clip (23). The aggressiveness of the isolates was evaluated using a visual scale from 1 (no streaking) to 5 (severe streaking and stunting), adapted from Soto et al (21).

Production of polyclonal antibodies and their use in double-immunodiffusion and in enzyme-linked immunosorbent assays (ELISA). Maize streak virus was purified from maize collected in fields near Saint Pierre, Réunion, following the technique of Von Wechmar and Milne (24). Two rabbits were immunized by four intramuscular injections of purified virus that was emulsified volume-to-volume in Freund's complete (FCA) and incomplete adjuvant (FIA) for the first and subsequent injections, respectively. Blood was taken on the 10th day after the fourth injection over a period of 110 days, maintaining the titer by booster injections. The immunoglobulins (Ig) were purified by the procedure of Hardie and Van Regenmortel (12) and coupled to alkaline phosphatase (AP) by the glutaraldehyde method (1).

Leaf samples with streaking were tested by the double-immunodiffusion method of Ouchterlony using crude antiserum. The gel was prepared with 0.75% noble agar, 0.85% NaCl, and 0.02% NaN_3 in a 0.02 M phosphate buffer, pH 7.2. This buffer was also used to homogenize the test plant extracts.

Extracts of purified isolates were compared by direct double-antihody sandwich (DDAS) ELISA (5). In the first step, the wells of polystyrene microtiter plates were coated with the Ig at 500 ng/ml in a 0.05 M carbonate buffer, pH 9.6. The antigens were prepared in phosphate-buffered saline with 0.05% Tween and 2% polyvinylpyrrolidone (PBS-T-PVP) and antivirus Ig-AP conjugate in PBS-T-PVP buffer with 0.2% bovine serum albumin (BSA). After addition of 200 µl of *p*-nitrophenyl phosphate substrate at 1 mg/ml in 0.1 M diethanolamine buffer, pH 9.8, to each well, the absorbance was read at 405 nm in a Titertek Multiskan photometer.

Sixteen isolates from Réunion were analyzed by two other ELISA techniques using the Ig directed against the MSV from Réunion and Ig purified by salt precipitation from a rabbit antiserum prepared by Bock (2) against a maize isolate of MSV from Kenya. The two enzyme immunoassays used were indirect ELISA and indirect double-antibody sandwich (IDAS) ELISA. For each type of assay, the 16 isolates were tested simultaneously on the same microtiter plate. The buffers used to coat the plates and to dissolve the substrate were the same as those used from the DDAS-ELISA. For the indirect ELISA, the extracts of the test plants were absorbed directly onto the plastic in the carbonate buffer. For the IDAS-ELISA, the antigens prepared in PBS-T-PVP buffer were complexed with mouse anti-MSV monoclonal antibodies that had been coated on the plates in the first step at 200 ng/ml. For both techniques, the coating step was followed by incubation in 1% BSA in PBS-T. The next steps were common to both techniques and were carried out in PBS with the exception of substrate addition. The Ig directed against the MSV from Réunion were incubated at 200 ng/ml and those from Kenya at 1,000 ng/ml in PBS-T. Alkaline phosphatase-labeled goat antirabbit Fc-specific Ig were diluted 1/1,000 in PBS-T. Substrate was added and the OD read as described above.

Production of anti-MSV monoclonal antibodies and serotype identification by ELISA. Four BALB/c mice were immunized over a period of 80 days with the strong SP isolate purified from maize according to the technique of Larsen and Duffus (13). Each mouse received 50 µg of virus per injection. The immunization started with an intraperitoneal injection with FCA on day −81. It was followed by intramuscular and intraperitoneal injections with FIA on day −52 and by intraperitoneal injection with FIA on day −38. The two mice that gave the highest titer in indirect ELISA and IDAS-ELISA were given an intravenous injection of 10 µg of virus on day −4 and an intraperitoneal booster of 100 µg on day −3.

The protocol used for the fusion was adapted from that described by Fazekas de St Groth and Scheidegger (9). One week after fusion, the wells were screened by indirect ELISA and IDAS-ELISA using the strong SP isolate. The hybridoma cultures whose supernatants remained positive for antibody during the period of cellular multiplication were cloned by the limiting dilution technique. The hybridoma clones secreting antibodies directed against the SP isolate were injected intraperitoneally into BALB/c mice. The mice were primed with pristane 15 days before the injection of at least 10^7 hybrid cells. Collection of ascites fluid began 10–15 days after injection.

Serotype identification. Crude extracts of the various plants showing streak symptoms were tested by the indirect ELISA and IDAS-ELISA procedures used to screen the supernatants. Ascites fluid was used instead of supernatant and crude extracts of virus-infected plants instead of extracts of purified virus. Serotype variability was noted by comparing the absorbance readings obtained for each isolate with those obtained with the strong SP isolate taken as control.

RESULTS

Transmission tests. During a 3-yr survey in Réunion, streak symptoms were found on 12 Poaceae species, five of which belong to the Paniceae tribe (*B. plantagina, B. ramosa, Digitaria* sp., *D. ciliaris, Setaria barbata* (Lam.) Kunth), two to the Eragrostideae tribe (*Eleusine* sp., *E. indica* (L.) Gaertner), one to the Chlorideae tribe (*Chloris gayana*), two to the Androponeae tribe (*Rottboellia cochinchinensis* (Lour.) W. Clayton, *Saccharum* sp.), and two to the Maydeae

Table 1. Host range of six isolates of maize streak virus from Réunion

	Plants tested							
						Coix lacryma-jobi		
Origin of isolate	**Maize cv. INRA 508**	**Sugarcane cv. R 574**	***Brachiaria plantagina***	***B. ramosa***	***Cenchrus echinatus***	**Réunion ecotype**	**Mauritius ecotype**	***Digitaria ciliaris***
Maize cv. INRA 508	9/10[x]	0/6	8/8	0/8	8/9	0/7	2/2	1/2
Sugarcane cv. R 574	0/26	1/20	0/5	0/5	7/8	0/8	0/5	0/8
B. plantagina	14/14	0/4	8/8	1/5	13/13	...[y]	5/5	4/8
B. ramosa	6/6	0/2	...	0/20	...	0/2	...	2/4
C. echinatus	3/9[z]	0/8	4/4[z]	0/8	5/8	0/2	3/4[z]	1/1
D. ciliaris	4/7	0/3	8/8	0/8	5/8	...	4/4	5/9

[x]Number of plants infected/number of plants inoculated.
[y]Transmission not tested.
[z]Attenuated symptoms.

tribe (*Coix lacryma-jobi, Z. mays*). *B. plantagina, C. echinatus*, and *D. ciliaris* were encountered often, whereas sugarcane cv. R 574, *B. ramosa, Chloris gayana, Coix lacryma-jobi, E. indica, R. cochinchinensis*, and *S. barbata* were rarely found. Transmission tests from these symptomatic grasses (except sugarcane) to maize seedlings using virus-free *C. mbila* resulted in symptomatic maize plants after 3–15 days.

Six MSV isolates were characterized by their host range (Table 1). The isolate from sugarcane infected sugarcane cv. R 574 and *C. echinatus* but not maize. The individual insect inoculation to maize (Table 2) confirmed this result. All the other isolates infected INRA 508 and *D. ciliaris* but not R 574. When maize was inoculated with individual insects, differences in infection rate and symptom severity (Table 2) were observed among the isolates. The isolates from maize, *B. plantagina*, and *B. ramosa* were transmitted to INRA 508 at frequencies very close to the theoretical transmission ability of *C. mbila* (80% active vectors) and caused severe symptoms, rating 5 on the 1–5 scale. Isolates from *C. echinatus* and *D. ciliaris*, on the other hand, were transmitted at low frequencies and caused attenuated symptoms, rating 2 or 3.

The results of the host range study also revealed the differences in susceptibility among the plant species. Sugarcane cv. R 574 was only infected by the R 574 isolate and *B. ramosa* only by the *B. plantagina* isolate. *C. echinatus*, however, was infected at a high frequency by all the isolates. Contrary to *Coix lacryma-jobi* from Réunion, a Mauritius ecotype, when inoculated under the same conditions, was found to be susceptible to MSV isolates from Réunion (Table 1).

Symptom severity and the effect on growth of INRA 508 and IRAT 297 plants were determined for several MSV isolates (Table 3). Classification of isolates according to severity of symptoms caused was the same for the resistant cultivar and the susceptible hybrid. Inoculation with the isolate from *C. echinatus* was without effect. Infection with the isolate from *Chloris gayana* was so weak that the symptoms obtained on IRAT 297 could hardly be distinguished from those obtained on the healthy control. The three SP isolates caused the most severe symptoms and a greater than 50% reduction in the height of the INRA 508 plants. On IRAT 297, the attenuated SP isolate could be differentiated from SP and strong SP by symptoms on day 15 and mean reduction of plant height. On the basis of the severity of the symptoms, the isolate from *B. plantagina* showed an aggressiveness intermediate between the maize isolate and the *C. echinatus* and *Chloris gayana* isolates.

Serological studies using polyclonal antibodies. In the double-immunodiffusion tests, the antiserum to MSV from maize (Réunion) reacted not only with the infected maize but also with crude extracts from several Poaceae species from Réunion showing the streak symptoms: *Brachiaria* sp., *C. echinatus, Digitaria* sp., and *Eleusine* sp. In contrast, no reaction was observed with crude extracts from *D. sanguinalis* infected with DSV or with extracts from sugarcane cv. R 574 from Réunion. The precipitin line obtained with the SP isolate was continuous with that obtained with crude extracts from *C. echinatus* and *Digitaria* sp. showing streak symptoms.

By indirect ELISA and IDAS-ELISA, the anti-MSV antibodies from Réunion and Kenya reacted with 15 of the 16 isolates from Réunion: *B. ramosa, C. echinatus, Chloris gayana, Digitaria* sp., *R. cochinchinensis*, and 10 isolates from maize. Neither of the two antibodies, however, reacted with the sugarcane cv. R 574 isolate. No difference in specificity was noted between the immunoglobulins prepared against MSV from Réunion and Africa.

When the sugarcane cv. R 574 isolate was purified, it was detected by the DDAS-ELISA procedure. Comparison with a purified extract from maize showed that to obtain the same absorbance value in the ELISA tests, a far greater concentration of antigen was necessary from sugarcane than from maize (Table 4).

Serological studies using monoclonal antibodies. The antiserum titers of the two mice used for the fusions were 1/100,000 by indirect ELISA. We obtained five stable hybridomas secreting monoclonal antibodies (MAbs) directed against the strong SP isolate: 94 × 2, 114 × 1, 114 × 2, 180 × 3, 180 × 4. These MAbs reacted in the IDAS-ELISA as well as the indirect ELISA. Titers of 10^{-5} to 10^{-6} were obtained for these five MAbs produced in ascites fluids. The working dilutions of the ascites fluids used in ELISA were 10^{-5} for 114 × 1, 114 × 2, and 94 × 2 and 10^{-4} for 180 × 3 and 180 × 4.

Isolates from maize. The reactivity of the five MAbs with the different isolates and field-collected leaf samples was compared with that of the strong SP isolate, the immunogen (Fig. 1). All the maize isolates from Réunion, SP,

Table 2. Transmission by only one *Cicadulina mbila* per plant of seven isolates of maize streak virus from various Poaceae to maize cv. INRA 508

Origin of isolate	Number of transmission attempts	Percentage of transmission	Average time of appearance of symptoms (days)	Severity of symptoms[z]
Maize cv. INRA 508	22	73	5	5
Brachiaria ramosa	12	67	9	5
B. plantagina	25	52	6	5
Eleusine sp.	34	35	10	5
Cenchrus echinatus	22	32	6	2
Digitaria ciliaris	49	20	18	3
Sugarcane cv. R 574	25	0	···	1

[z] On a scale of 1–5, where 1 = no streaking, 5 = severe streaking and stunting.

Table 3. Effect of different isolates of maize streak virus on susceptible (INRA 508) and resistant (IRAT 297) cultivars of maize

	INRA 508			IRAT 297		
	Mean infection severity rating[x]		Average height of plants	Mean infection severity rating		Average height of plants
Origin of isolate	15 days[y]	60 days	(cm)	15 days	60 days	(cm)
Control	1.00 a[z]	1.00 a	214 cd	1.00 a	1.00 a	203 cd
Cenchrus echinatus	1.00 a	1.00 a	223 d	1.00 a	1.00 a	200 cd
Chloris gayana	2.25 bc	2.25 bc	189 cd	1.08 a	1.17 a	180 cd
Brachiaria plantagina	3.17 d	3.67 d	136 ab	1.42 a	1.83 ab	191 cd
Maize attenuated SP	4.17 e	4.25 d	120 a	1.75 ab	2.25 c	192 cd
Maize strong SP	4.75 e	4.17 d	120 a	2.42 bc	2.75 c	169 bc
Maize SP	4.42 e	4.33 d	111 a	2.92 cd	2.75 c	171 bc

[x] On a scale of 1–5, where 1 = no streaking, 5 = severe streaking and stunting.
[y] Days after inoculation.
[z] Means within a column followed by the same letter are not significantly different at $P < 0.05$ according to the Newman-Keuls multiple range test.

Table 4. Detection of maize and sugarcane isolates of maize streak virus from Réunion by direct double-antibody sandwich ELISA using polyclonal antibodies

Source of isolate	Virus concentration[z] (ng/ml)	Absorbance at 405 nm[z]
Sugarcane	3,900	0.034
	19,500	0.691
Maize	400	0.536

[z] Isolates were purified extracts.

attenuated SP, and eight others from different parts of the island were serologically indistinguishable from the strong SP isolate, irrespective of the ELISA procedure used, indirect or IDAS. The reactivity of the five MAbs with crude extracts of 21 maize samples received from 10 countries (Benin, Cameroon, Chad, Congo, Côte d'Ivoire, Madagascar, Mali, Mauritius, Mozambique, and Zimbabwe) was similar to that with the strong SP.

Isolates from sugarcane. The isolate from sugarcane cv. Co 1223 from Burkina Faso could not be distinguished from strong SP. The isolate from sugarcane cv. R 574, however, was not recognized by MAbs 114 × 1 and 114 × 2 (Fig. 1). This isolate was well recognized by MAb 94 × 2 but poorly recognized by MAbs 180 × 3 and 180 × 4. On the basis of the difference in reactivity between the strong SP and the sugarcane cv. R 574 isolate with the MAbs, two serotypes could be distinguished and were designated SP and 94, respectively. In contrast to serotypes SP and 94, the sugarcane M 112/34 isolate was better recognized by MAbs 180 × 3 and 180 × 4 than by the other MAbs (Fig. 1). This isolate was poorly recognized by MAbs 114 × 1 and 114 × 2 and was designated serotype 180.

Isolates from other Poaceae species. The MAbs could not distinguish between the strong SP isolate and the isolates from grasses originating from Réunion: *B. ramosa, Brachiaria* sp., *C. echinatus, Chloris gayana, Coix lacryma-jobi, Digitaria* sp., *E. indica, R. cochinchinensis*, and *S. barbata*. The reactivity of the different MAbs with *C. echinatus* and *Chloris gayana* isolates was identical regardless of whether *Digitaria* sp. or INRA 508 was used for propagation. Similarly, the reactivity of the MAbs with isolates originally from *C. echinatus, Digitaria* sp., and *R. cochinchinensis* were the same whether obtained from the original host or from INRA 508.

The reactivity of the MAbs with most of the crude extracts of grass samples from Mauritius and Africa could not be distinguished from that with the strong SP isolate: *Digitaria* sp. and *Pennisetum* sp. from Mali, *Coix lacryma-jobi* and *Panicum* sp. from Mauritius, and *E. indica* from Zimbabwe. However, the reactivity of the MAbs with crude extracts from *Setaria verticillata* (L.) P. Beauv. from Zimbabwe and from *D. sanguinalis* infected with DSV was similar to that with serotype 94 (Fig. 1). MAb 94 × 2 reacted stronger with these two crude extracts than did the other MAbs.

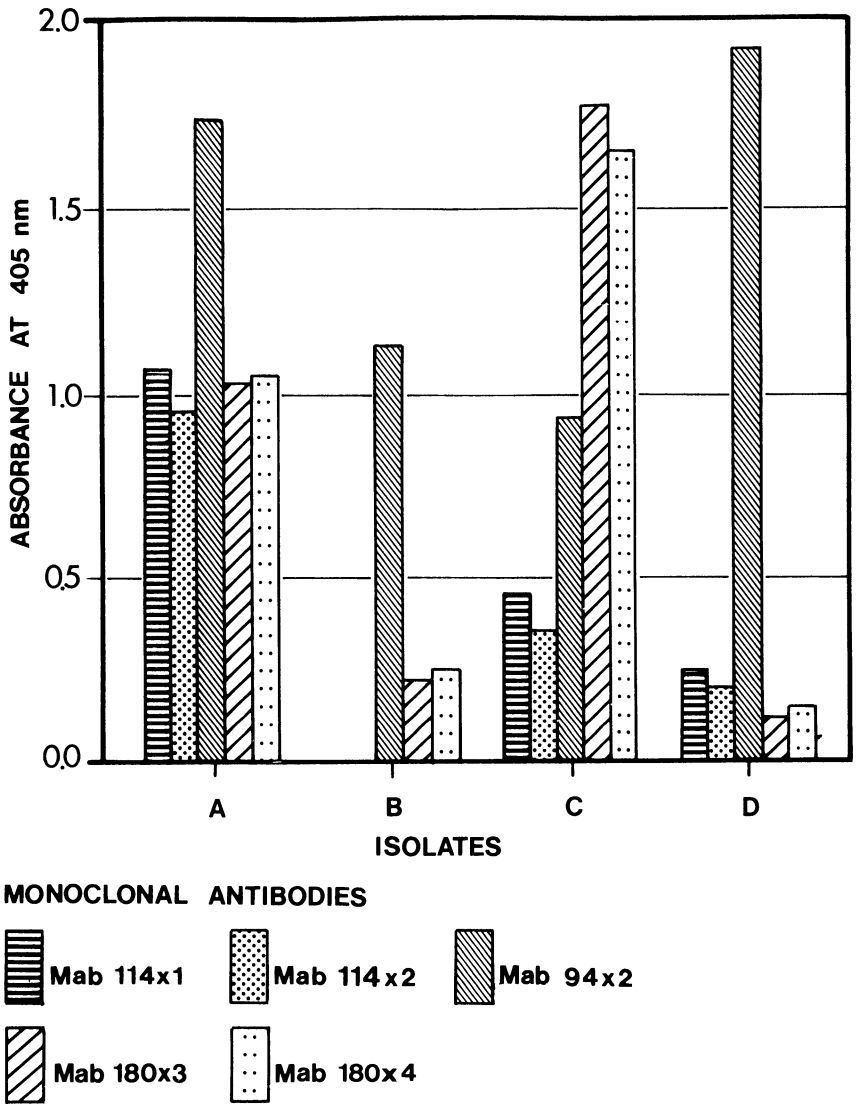

Fig. 1. Reactivity in IDAS-ELISA of five monoclonal antibodies raised against the maize streak virus (MSV) strong SP isolate with three MSV isolates and one Digitaria streak virus (DSV) isolate. A = strong SP isolate from maize (serotype SP), B = isolate from sugarcane cv. R 574 (serotype 94), C = isolate from sugarcane cv. M 112/34 (serotype 180), and D = DSV isolate from *Digitaria sanguinalis*.

DISCUSSION

Two types of virulence were distinguished by the host range study; one isolate from sugarcane cv. R 574 represented one type, and all the other isolates tested belonged to the other type. The failure to transmit the sugarcane isolate to maize is consistent with the results of studies performed in Mauritius (19). A sugarcane isolate from Mauritius was transmitted, however, by Pinner et al (17) to the Golden Cross Bantam maize hybrid. We were able to infect only sugarcane with the isolate from sugarcane—and even then at a very low rate (1/20). This difficulty in infecting sugarcane is probably best explained by resistance of this species (nonpreference resistance to transmission or virus resistance sensu stricto) rather than by inability of this MSV serotype to circulate in *C. mbila*, which was found to transmit the same isolate to *C. echinatus* at an excellent rate. The low rate of infection of sugarcane is consistent with the fact that Pinner et al (17) were unable to transmit an MSV isolate to this plant regardless of its host origin.

ELISA tests showed that the types of virulence corresponded to two different serotypes. The isolate from sugarcane is serotype 94, whereas the other isolates tested are serotype SP. Differences in aggressiveness were observed within the SP group, however. The isolates from *C. echinatus* and *D. ciliaris* were remarkable by their weak aggressiveness; they caused attenuated symptoms and were transmitted to maize at a low rate. On the basis of the percentages of transmission and the mean infection severity ratings, the isolates from maize were found to be the most aggressive, followed by the *B. plantagina* isolate. The isolate from *Chloris gayana* had an aggressiveness intermediate between the isolate from *B. plantagina* and the least aggressive isolates cited above. Classification of SP isolates by their aggressiveness correlates with that of the Mauritius isolates deduced from the study of their host range (16); the virulence of the isolates from *Cenchrus* sp. and *Digitaria* sp.

differs from that of the isolate from maize, whose host range is the same as that of the isolate from *Brachiaria* sp. Immunoenzymatic analyses of the Mauritius isolates from maize and from *Digitaria* sp. did not confirm this difference in virulence because both of them were found to be serotype SP. For reproducible results, however, test plants of the same origin must be used. Indeed, there can be great variation between ecotypes of the same wild plant species, as in the case of *Coix lacryma-jobi*, in which the ecotype from Réunion was resistant to all the isolates inoculated, whereas an ecotype from Mauritius was susceptible under the same inoculation conditions. When isolates from different regions are compared, serological analyses are easier and more reliable than transmission studies, unless the same batches of seeds or, better still, cuttings are used.

The reactivity of the MAbs with field-collected maize leaf samples from 10 different countries could not be distinguished from that with serotype SP. Furthermore, antiserum received from Kenya was probably prepared against an SP isolate because its reactivity against a series of isolates from Réunion was identical to that obtained with an anti-SP antiserum from Réunion. The similarities of the serological reactions observed with maize isolates, as well as crude extracts of maize leaves showing streak symptoms, correlate with the homogeneousness of the symptoms caused by isolates from maize originating from various geographic sites (17) and confirm the serologic results of Dekker et al (6). Therefore, it may be that the serotype most commonly detected in maize by Dekker et al (6) is the same as serotype SP. However, the fact that the attenuated SP isolate was obtained from the SP isolate shows that this homogeneity of the maize isolates is only apparent and confirms the results of Mac Lean (15) and Dekker et al (6). Maize could be simultaneously infected by several isolates, but the severe symptoms caused by the SP isolate would mask the symptoms of any other isolate. Also, its serological reaction pattern would mask the pattern of lower concentrated isolates.

In contrast to maize, sugarcane is the host for three different serotypes: SP, 94, and 180. Detection of serotype 94 in Réunion and of serotype 180 in Mauritius suggests that the serotype of the isolates from sugarcane varies according to their geographic origin. This hypothesis has been proposed by Ricaud and Felix (19) on the basis of transmission tests and correlates with the results of Dekker et al (6), who were able to distinguish an isolate from South Africa sugarcane from an isolate from Mauritius sugarcane.

In addition to sugarcane cv. R 574 from Réunion, only one other Poaceae species—*S. verticillata* from Zimbabwe—is host for serotype 94. The apparent rarity of this serotype is in agreement with the results found with the sugarcane isolate from Réunion whose host range is more restricted than that of serotype SP. In contrast to serotype 94 from sugarcane, the DSV isolate cannot be transmitted by *C. mbila* (17), but by *Nesoclutha declivata* (J. F. Julia and M. Dollet, *personal communication*). MSV and DSV appear to share a common epitope, as revealed by their reactivity with MAb 94 × 2.

Serotype 180 was detected in Mauritius only on the M 112/34 cultivar of sugarcane. This isolate reacted with all the MAbs, but the profile was different from that of serotype SP, suggesting that the antigenic sites recognized were slightly different. Among the isolates studied by Dekker et al (6), the isolate from Mauritius sugarcane was only distantly related to the reference isolate from maize and was not recognized by any of their 15 MAbs. If we assume that their sugarcane isolate from Mauritius is similar to the one we studied, our five MAbs have a different specificity than theirs. On the basis of the different reactivities of the MAbs with the various isolates tested, it seems that the five MAbs recognize three different antigenic sites. No difference in specificity was found between MAb 114 × 1 and MAb 114 × 2 and between MAb 180 × 3 and 180 × 4. MAb 94 × 2 seems to be the best MAb for the diagnosis of a larger range of isolates because it is specific for an antigenic determinant present on all the isolates analyzed.

Most of the isolates from Africa and the Indian Ocean are serotype SP, dominant in Réunion. This serological homogeneity validates the regionalization of the work concerning the selection of resistant maize cultivars in Réunion—all the more so since maize cv. Revolution from Réunion was found to be resistant to MSV in Togo (14), Kenya (3), and Nigeria (21). The study of the biological properties of isolates from Réunion illustrates, however, the great differences in aggressiveness depending on the host origin. This diversity must be taken into consideration in an artificial infestation program for plant screening. This is the case in the maize breeding program in Réunion, not only to increase the resistance of the IRAT 297 (11) donor but also to transfer it to susceptible material (18). By choosing isolates from maize, a suitable selection should be possible because they are the most aggressive, not only in Réunion but also in other geographic regions (17). In addition, in accordance with the classification scheme of isolates as a function of their aggressiveness, no "specialized variant" could be identified on the basis of isolate/cultivar interaction.

Certain perennial grasses, such as *Brachiaria* sp., can be harmful sources of streak disease because they are frequent hosts for aggressive isolates. Nevertheless, the importance of the alternative host plants may be less than previously thought; they may be infected by avirulent or weakly aggressive isolates. When a plant is infected by several different isolates, it is difficult to predict to what extent the isolates will be transmitted to the cultivated plants. Because different isolates can be rapidly identified by monoclonal antibodies, it is now possible to study the transmission by leafhoppers of several serotypes infecting the same plant. On the basis of the studies described herein, it should be possible to improve the present knowledge of the epidemiology of MSV.

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