Purification, Serology, and Particle Morphology of Two Russet Crack Strains of Sweet Potato Feathery Mottle Virus

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ABSTRACT

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Three strains of sweet potato feathery mottle virus (FMV) were isolated from roots and leaves of *Ipomoea batatas* 'Porto Rico.' Serial aphid and local lesion transfers, as well as dilution series, were used to separate isolates from mixed infections. Two strains of FMV were differentiated by lesion development in *Chenopodium amaranticolor* and the severity of russet crack (RC) lesions on *I. batatas* 'Jersey' roots. The third strain was FMV common strain, which did not infect *C. amaranticolor* and caused no external symptoms on Jersey roots. The normal particle lengths for the two RC strains were 850 nm and 833 nm, and are similar to those reported for the common strain (830–850 nm). Heterologous serological titrations indicated that the RC strains were related, but not identical, to the common strain. Attempts to purify the two RC strains by procedures used for the common strain resulted in severe aggregation; however, a method was developed to purify these two and the common strain. Evidence is provided that shows the two strains inducing russet crack to be strains of FMV.

Additional key words: mild and severe russet crack.

The russet crack (RC) disease of sweet potato, *Ipomoea batatas* (L.) Lam., was first described by Daines and Martin in 1964 (6). They suggested that the disease was caused by a virus because it is graft transmissible and fungi isolated from diseased roots failed to induce the disease. The symptoms, necrosis of sweet potato roots and chlorosis on leaves of graft-inoculated *Ipomoea setosa* Ker. were described. Results of graft transmission experiments have suggested that leafspot, internal cork, and russet crack disease were caused by the same virus (9,11). In these experiments, two cultivars carried the russet crack virus but did not exhibit the necrotic root symptoms characteristic of the russet crack disease. Centennial, Porto Rico, and Jersey (*I. batatas*) scions grafted in different combinations into healthy cuttings of all three cultivars always produced the same symptoms in each cultivar (ie, Centennial foliage developed leaf spots, Jersey roots developed russet crack, and Port Rico roots developed internal cork [9]). In 1974, cross-protection tests suggested that russet crack disease was caused by a strain of sweet potato feathery mottle virus (FMV) (5). Confirmation of this hypothesis was prevented due to lack of differential hosts and an adequate means of separating the russet crack and common strains of FMV from mixed infections.

The research reported here was undertaken to critically examine the relationship between the russet crack virus and the common strain of FMV. Portions of this research were previously presented in abstracts (3,4).

MATERIALS AND METHODS

Roots and foliage of infected *Ipomoea batatas* 'Porto Rico' were used as the virus source. Virus isolates were obtained by graft, aphid, or mechanical transfer to *I. setosa* and *I. nil* (L.) Roth. These isolates produced symptom types in *I. nil* similar to those previously reported (12). Initial isolates were chosen for differential symptom expression in *I. nil*. Sap dilution series and serial single probe aphid transmissions were used to separate mixed infections and to establish the stability of symptom expression. Isolates were further identified by using a host range to differentiate FMV strains (12). These hosts included *I. nil*, *I. purpurea* (L.) Roth, *Chenopodium amaranticolor* Coste & Reyn, and grafting to Jersey sweet potato. Leaves from symptomless *I. purpurea* plants were indexed in *I. nil* to confirm infection. Symptoms on Jersey roots were observed 4 mo after inoculation. Plants were grown in a screened greenhouse that was fumigated weekly to prevent insect spread and subsequent cross-contamination. Causality of disease reactions in sweet potato was confirmed by inoculation of homogenates of root slices (in 0.05 M phosphate buffer, pH 7.2, containing 0.01 M sodium diethyldithiocarbamate), to cotyledons of eight *I. nil* seedlings.

Isolates that induced local lesons were cloned by three single lesion transfers in *C. quinoa* Willd followed by back inoculations to *I. nil*. All isolates were maintained on *I. nil* in a screencage. These plants served as source tissue for increase and subsequent purification of FMV. Periodic inoculations to test plants from stock cultures were used to monitor for cross-contamination; none was detected during the 2 yr these experiments were conducted.

Particle morphology. The particle morphology of the RC strains was determined by serologically specific electron microscopy (SSEM) (7) of leaf dips from infected *I. nil* plants. More than 790 particles of each strain were measured as previously described (13). A main maximum (2,14,15) of 700–1,000 nm, which contained over 75% of the particles for each strain, was used to calculate the normal length.

Purification. The purification procedure used for FMV common strain was unsatisfactory for both RC strains. Preliminary experiments designed to define optimal conditions for extraction and clarification of these strains used various buffers, ionic strengths (0.05–0.5 M) (pH 6–9) and organic solvents. Systemically infected *I. nil* leaf tissue harvested 14–17 days after inoculation was used as the virus source. Treatments were evaluated by using local lesion assays on *C. amaranticolor* or *C. quinoa* and heterologous precipitin reactions with common strain antiserum (13).

Results of these trials led to development of the following purification procedure. Extracts were maintained at 4 C and buffers and rotors were precooled. Symptomatic *I. nil* tissue was harvested, immediately homogenized for 2 min in 0.1 M borate buffer (1:3, w/v; pH 8), passed through a double layer of cheesecloth, and the filtrate was homogenized in chloroform (3:1, v/v) for 10 sec. The emulsion was broken by low speed centrifugation (Sorvall GSA rotor, 10,000 *g*, 10 min) and the

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aqueous phase was decanted and subjected to two cycles of differential centrifugation (Beckman Type 30 rotor, 90,000 *g* max). The first high speed centrifugation was for 2 hr with an 8-ml 20% sucrose cushion. The second cycle was for 2.5 hr with a discontinuous (8 + 8 ml) 20, 50% sucrose cushion. Sucrose solutions were made in 0.05 M borate buffer (pH 8) containing 0.5 M urea. Each pellet from the first high speed cycle was resuspended in 6 ml of 0.05 M borate buffer (pH 8) containing 0.5 M urea and 1% Triton X-100 (resuspension buffer) for 5 hr with vigorous stirring (Thermolyne stir plate, ~1/2 speed). Insoluble materials were removed by centrifugation (Sorvall SS-34 rotor, 8,000 *g*, 10 min). Each pellet from the second high speed centrifugation was resuspended overnight with vigorous stirring in 5 ml of resuspension buffer and centrifuged (Sorvall SS-34 rotor, 8,000 *g*, 10 min) to remove insoluble materials. A cesium chloride solution (1.14 g/ml) was added to the supernatant to a final density of $\rho = 1.39$ and centrifuged (Beckman Type 65 rotor, 218,000 *g* max) for 16 hr. The gradients were fractionated by using an ISCO Model 640 density gradient fractionator and the virus fraction was dialyzed exhaustively against resuspension buffer.

Serology. Virus to be used as an immunogen was collected from CsCl gradients and dialyzed against 0.05 M borate buffer (pH 8) containing 0.5 M urea. The virus was administered subcutaneously and intramuscularly to New Zealand white rabbits at 5-day intervals for 6 wk. Each injection consisted of 2–3 mg of virus in 3 ml of buffer homogenized with an equal volume of Freund's complete adjuvant. Virus concentration was estimated spectrophotometrically by using $E_{260\ nm}^{0.1\%} = 2.5$, uncorrected for light scattering (8). Antibodies to host proteins were removed by resuspending pellets of partially purified healthy *I. nil* tissue in antisera, incubating the mixture overnight at 4 C, and recovering the supernatant after low speed centrifugation. No healthy reactions were detected in microprecipitin tests with cross-absorbed antisera. Antiserum to the common strain of FMV was prepared as previously described (13). Homologous and heterologous antisera reactions in microprecipitin tests (1) were determined by using clarified virus extracts (12). Storage of purified virus previous to its use in serological titrations resulted in nonspecific precipitation; therefore, it was necessary to use freshly prepared clarified virus extracts. Homologous and heterologous reactions were measured against the same extract and repeated three times.

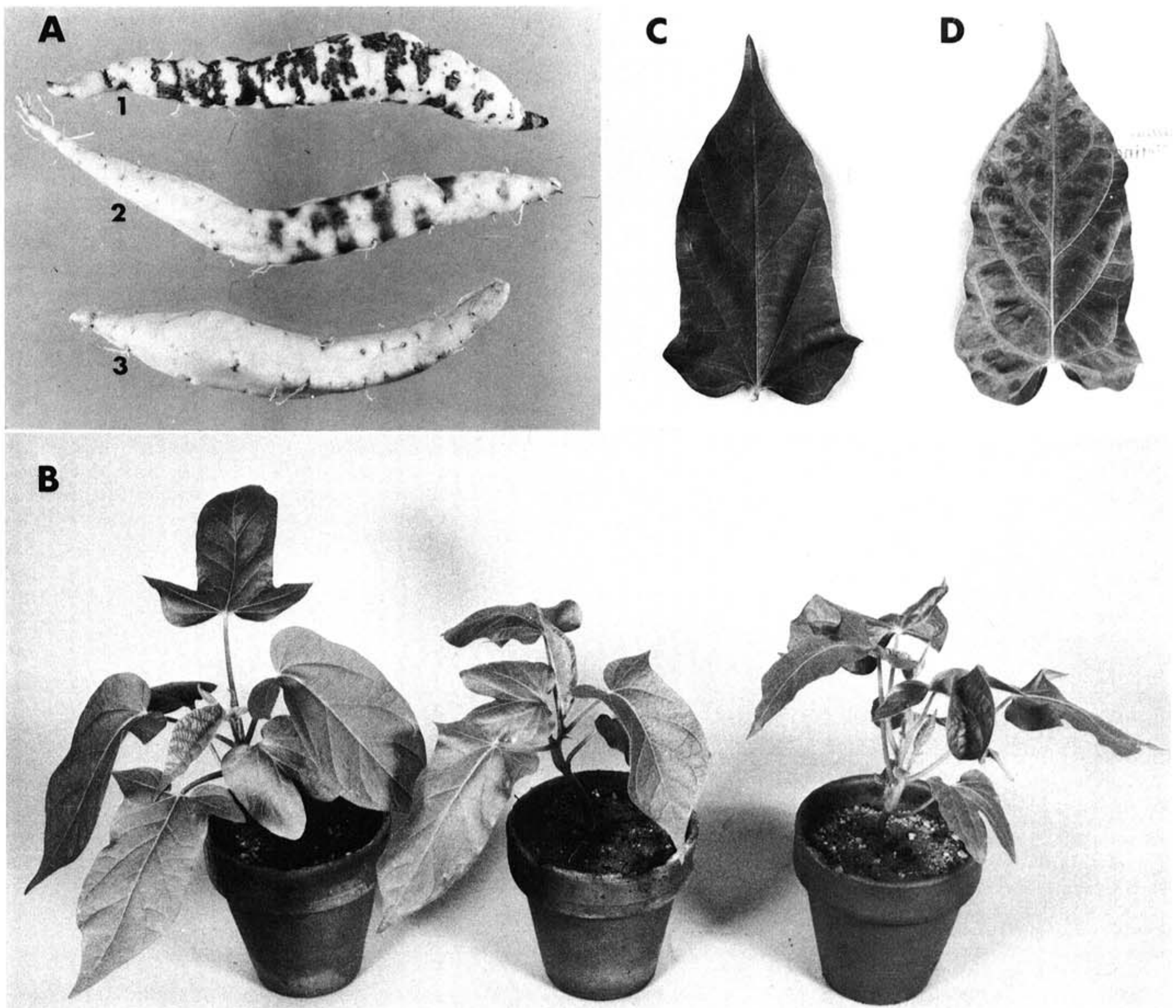

Fig. 1. A, Root symptoms characteristic of SRC (severe russet crack), MRC (mild russet crack), and common strains 1, 2, and 3, respectively, of sweet potato feathery mottle virus in *Ipomoea batatas* 'Jersey' roots. **B,** Symptoms characteristic of russet crack strains in *I. nil* 14 days after inoculation. From left to right: healthy, MRC, and SRC with distortion. **C,** Leaf from a healthy *I. nil* plant. **D,** Leaf from an infected *I. nil* plant exhibiting water-soaked veinbanding which is characteristic of both russet crack strains.

RESULTS

Testing of virus isolates from Porto Rico plants (following several transfers through *I. nil* with the precautions described above to prevent mixed infections) resulted in the identification of three distinct symptom types. One type was identified as the common strain of FMV (12,13) and the other two as russet crack strains of FMV (Table 1) designated as either MRC (mild russet crack) or SRC (severe russet crack) on the basis of the type of necrotic lesions produced on Jersey roots (Fig. 1A). The common strain of FMV produced no symptoms on Jersey roots. However, roots indexed on *I. nil* gave characteristic symptoms for common or RC strains in that host (Tables 1 and 2). The symptoms induced in the host differentials by the common strain were consistent with those previously reported (12,13). The symptoms induced by RC strains also were consistent with those previously described (12) for russet crack; however, SRC was distinguished from MRC based on symptom severity in *I. nil* and the time required for lesions to appear in *C. amaranticolor*. In *I. nil* the primary symptom induced by both RC strains was veinal chlorosis followed by water-soaked vein banding (Fig. 1D) in the first three to four true leaves. Vein banding was limited to the primary veins in subsequent leaves. Leaves (Fig. 1B) on SRC-infected plants were distorted while little or no distortion was exhibited by MRC-infected plants. In addition, chlorotic lesions developed within 4 days on inoculated leaves of *C. amaranticolor* while at least 9 days were required for chlorotic lesions to develop on MRC-inoculated leaves. Local lesion development in *C. quinoa* was similar to that in *C. amaranticolor*; however, the differential incubation period was less distinct. Consistent results and optimum lesion development for these viruses require that *C. amaranticolor* plants be less than 31 cm in height. Also, symptom expression was less distinct under greenhouse conditions from 1 November to 1 March than during the remainder of the year.

TABLE 1. Symptoms of three strains of sweet potato feathery mottle virus (FMV) on a differential host range

Hosts	FMV strains[a]		
	Common	MRC	SRC
Ipomoea nil	Cr, VCh[b]	WSVB, VCh	WSVB, D, VCh
I. purpurea	Symptomless	VC, VCh	VC, VCh
Chenopodium amaranticolor	Nonhost	Lesions[c]	Lesions[d]
I. batatas 'Jersey'[e]	Symptomless	Mild - N	Severe - N

[a]Common, mild russet crack (MRC), and severe russet crack (SRC) strains.
[b]Symptom abbreviations: D, distortion; S, stunting; WSVB, water-soaked veinbanding; CR, crinkling; VC, vein clearing: VCh, veinal chlorosis; and N, necrosis.
[c]Lesions required 9 days to appear.
[d]Lesions required 4 days to appear.
[e]Symptoms on roots.

TABLE 2. The consistent relationship of *Ipomoea nil* foliar symptom types to *I. batatas* 'Jersey' root symptom types confirmed by indexing root extracts on *I. nil*.

FMV strains[a]	Jersey root symptoms	*I. nil* symptom types	
		Common strain	RC strain
Common	0/10[b]	6/10[c]	0/10[d]
MRC	6/9	0/9	6/9
SRC	4/9	0/9	4/9

[a]Common, mild russet crack (MRC), and severe russet crack (SRC) strains of sweet potato feathery mottle virus (FMV).
[b]Denominator is the number of Jersey plants inoculated; the numerator is the number of Jersey plants with roots exhibiting russet crack (RC) symptoms.
[c]Denominator is the number of Jersey plants from which roots were indexed; the numerator is the number of Jersey plants from which root extracts induced the FMV symptom type on *I. nil*.
[d]Denominator is the number of Jersey plants from which roots were indexed; the numerator is the number of Jersey plants from which root extracts induced the russet crack symptom type in *I. nil*.

Purification. Severe aggregation of the RC strain virions prevented purification when methods for the common strain (13) were used. Clarification with butanol and precipitation with PEG reduced the yield and infectivity of the preparations. The addition of 0.5 M urea to all buffers and 1% Triton X-100 to resuspension buffers increased yields. However, after resuspension, infectivity was reduced when virus was stored in buffer with Triton X-100. Over half of the virus was found in the pellet at the bottom of the sucrose gradient column. Yields of purified virus obtained by using sucrose density gradients ranged from 1 to 5 μg/g of infected tissue vs 5 to 20 μg/g of infected tissue when CsCl gradients were used. Yields were determined by using an extinction coefficient of $E_{260\ nm}^{0.1\ \%} = 2.5$, corrected for light scattering (8). The predominant virus band in the CsCl gradients was located approximately 30 mm from the bottom of the gradient (Fig. 2A). This was confirmed by the presence of long flexuous rods and by infectivity assays; however, lesion counts were too low for quantiative measurement. Microprecipitin tests revealed a small amount of virus on either side of the major band (Fig. 2A). Neither infectivity nor serological reactions were detected in extracts from healthy tissue (Fig. 2B). This procedure was equally effective for all three FMV strains; yields were similar and virus collected from the gradients was infectious. These studies were conducted during winter months, and relative infectivity was low; however, virus samples from CsCl gradients during spring and summer months typically infected eight of 10 *I. nil* test plants and produced 20–100 lesions per leaf on *C. amaranticolor*. Purified virus inoculated to differential hosts, including cultivar Jersey, produced the same symptoms as the isolates maintained in *I. nil*. The A_{260}/A_{280} of purified preparations of both strains was 1.06 and 1.13, corrected and uncorrected, respectively, for light scattering.

Particle morphology. Normal particle lengths determined in leaf dips from infected *I. nil* tissue were 833 nm and 850 nm for SRC and MRC, respectively (Fig. 3). Particle measurements were grouped in 10-nm and 20-nm size classes; normal lengths of both strains were within their respective modal 10-nm and 20-nm size classes. Confidence limits set for both means indicated significantly different normal lengths ($P = 0.001$) for the isolate of each strain measured.

Serology. Relative homologous antisera titers prepared from common, SRC, and MRC strains by using freshly prepared clarified extracts were from 1/2,048 to 1/4,096. Heterologous precipitin reactions indicated a close, yet distinct, serological relationship between the common strain and the SRC and MRC strains. No serological difference could be detected between SRC and MRC strains (Table 3).

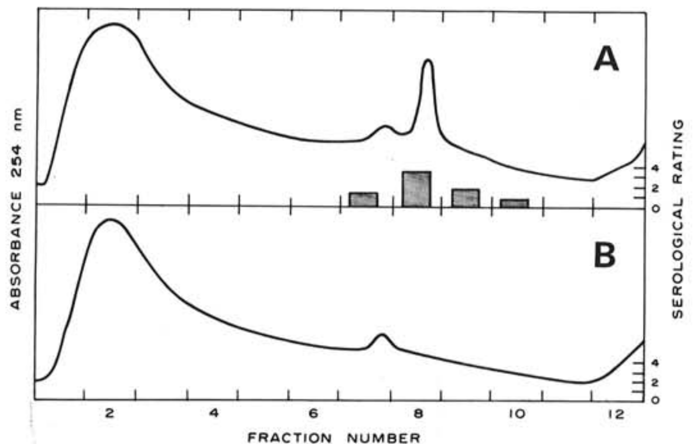

Fig. 2. Ultraviolet absorption profile of CsCl gradients (ρ = 1.39) centrifuged 16 hr (Beckman Type 65 rotor, 218,000 *g* max) containing partially purified extracts of the severe russet crack (SRC) strain of sweet potato feathery mottle virus from **A,** infected and **B,** healthy *Ipomoea nil* plants. Histogram bars indicate relative serological reactions (0–4) in microprecipitin tests.

TABLE 3. Homologous and heterologous serological reactions between strains of sweet potato feathery mottle virus (FMV) as measured in microprecipitin tests

Antigens[a]	Antisera prepared to:		
	Common	SRC	MRC
Common	2,048[b]	512	512
SRC	1,024	4,096	2,048
MRC	512	4,096	2,048

[a] Feathery mottle virus strains: the common strain, the severe russet crack (SRC) strain, and the mild russet crack (MRC) strain. Antigens were prepared as freshly clarified extracts of infected *Ipomoea nil* tissue. Homologous and heterologous reactions were measured against the same extract and repeated three times.

[b] Expressed as reciprocal of antiserum dilution end points.

DISCUSSION

In this report we have demonstrated the usefulness of differential hosts as an aid for isolating and separating the RC strains of FMV. Maintenance of contamination-free stocks and subsequent indexing of diseased roots on *I. nil* (Table 2) confirms that the RC disease is caused by strains of sweet potato feathery mottle virus. Development of a purification procedure suitable for all three strains has enabled us to support this hypothesis by investigating their serological relationships. The etiological significance of these findings is supported by the widespread occurrence of both common and RC strains in commercial sweet potato fields in North Carolina (12). Moderate and severe RC symptoms are mentioned in the literature (5); however, the recognition of two strains of this virus has not been previously reported. A recent survey of FMV isolates established the consistent reaction of differential hosts to common and RC strains (12). Symptom severity on these same hosts can be used to differentiate the mild and severe RC strains. Indexing on cultivar Jersey sweet potato requires maintenance of healthy stocks and a virus indexing period of 2–6 mo, compared to 7–14 days required for indexing on *I. nil* and *C. amaranticolor*. Foliar symptoms caused by common and RC strains in other *Ipomoea* spp. are very similar (12). This is true for *I. setosa*, a host proposed as a sweet potato virus indicator (5,10,16). In our studies, *I. setosa* was of no diagnostic value; however, it can be used as an assay host for graft isolations from sweet potato. It also can be inoculated by sap transmission, but less efficiently than *I. nil*.

The combined use of urea and Triton X-100 is essential to disrupt aggregates of virions formed during purification. The purified virus is a good immunogen, resulting in reciprocal homologous antisera dilution end points ranging from 2,048 to 4,096. Results of heterologous serological reactions showed that the common and the two RC strains were closely related, but not identical. No serological differences were detected between the RC strains.

In addition to these properties, the common and RC strains have previously been shown to have similar dilution end points (10^{-3}–10^{-4}) and thermal inactivation points (60–65 C) (3,12,13). Normal lengths, 833 nm and 850 nm, of the SRC and MRC strains, respectively, agree closely with those previously reported lengths 850 nm (13) and 844 nm (15) for FMV (determined without the use of SSEM) from purified and leaf dip preparations, respectively. They are slightly less than the length reported for a russet crack strain isolated in California (15). These properties, together with our findings, confirm a previous report based on cross-protection studies that the russet crack disease is caused by a strain of FMV (5). Other reports suggesting that FMV and RC were the same virus strains were shown to be inaccurate (9,11). Viral etiology is suspected for several diseases of sweet potato. Unfortunately, in only a few cases has the etiology been satisfactorily resolved. Lack of adequate means for separating mixed infections and inadequate characterization of the virus has resulted in much synonomy in the nomenclature of sweet potato viruses. A comprehensive review is available (5).

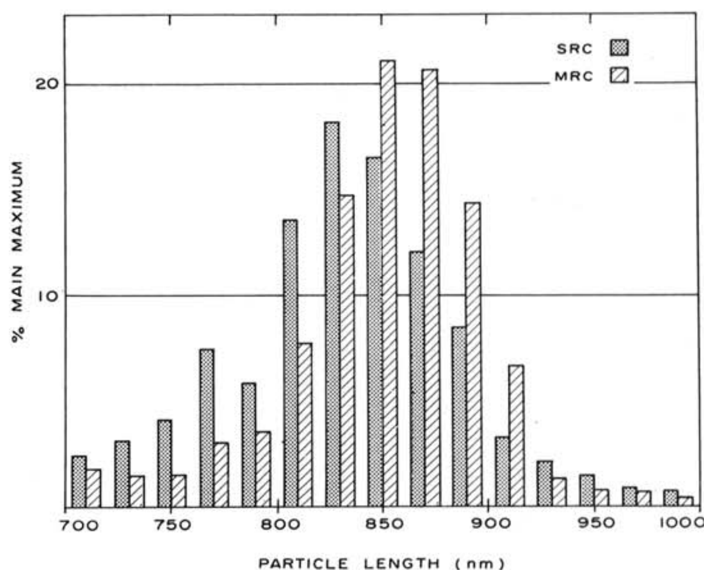

Fig. 3. A histogram by 20-nm size classes of main maximum (2) of particle lengths used to calculate normal length of severe russet crack (SRC) and mild russet crack (MRC) strains of sweet potato feathery mottle virus observed in leaf dip preparations. Values are percent of main maximum consisting of a minimum of 790 particles for each strain.

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