Etiology of Sweet Potato Russet Crack Disease

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ABSTRACT

Sweet potato russet crack is characterized by necrotic lesions on fine roots of the cultivar 'Jersey' grown in pasteurized soil in the glasshouse. These lesions appeared 4 wk after graft inoculation of virus-free stock plants, or 6.5 wk after vine cuttings were rooted. As fleshy roots enlarged, they also showed necrotic lesions. The causal agent was confirmed to be graft-transmissible from sweet potato to sweet potato or to *Ipomoea setosa,* thence to sweet potato. Additionally, *I. nil* and *I. tricolor* were shown to be hosts and the causal agent was sap-transmissible from sweet potato to *I. nil* and from *I. nil* to *I. nil.* The causal agent was transmitted in a stylet-borne manner by *Myzus persicae,* and was associated with a flexuous, rod-shaped virus particle. The sweet potato feathery mottle virus (FMV), as redefined from previous literature, also occurred in the russet crack-affected plants and it was sometimes isolated from russet crack by sap transmission and once by aphid transmission. It was not possible to isolate a distinct russet crack causal agent free from FMV. The only difference between FMV and the russet crack culture was the necrotic root reaction of 'Jersey' sweet potato inoculated with the russet crack culture. In cross-protection trials, sweet potatoes infected by FMV were protected from russet crack symptom expression. It is hypothesized that russet crack is caused by a strain of FMV; i.e., RC-FMV.

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Additional key words: sweet potato feathery mottle and internal cork viruses.

The viruses causing diseases of sweet potato [*Ipomoea batata* (L.) Poir.] are poorly known. Most have been characterized by host range and symptomatology and some by vector. Symptom differences are useful to distinguish between strains of a virus but they are unsuitable as the basis for describing new viruses (27). Thus, vector relationship is the sole characteristic by which affinities of various viruses described from sweet potato may be determined. On this basis there seem to be two major groups: one that is stylet-borne by aphids, and one that is transmitted by whiteflies. Sweet potato mosaic virus apparently constitutes a minor third group; a strain of tobacco mosaic virus has been isolated from such plants (4). There may be other groups but they have not been satisfactorily distinguished.

Martyn (26) lists four aphid-borne viruses: sweet potato internal cork virus, sweet potato leaf spot virus, sweet potato mosaic virus A, and sweet potato ringspot virus. Steinbauer and Kushman (37) list three: internal cork virus,chlorotic leaf spot virus,and russet crack virus. There is reason to question whether these are caused by 1, 2, or more distinct viruses. Seldom has the author of these names attempted to compare a "new" virus with previously described viruses. For example, sweet potato ringspot virus was described in an abstract containing only a name characteristic of the observed foliar symptoms (11). The same name was used independently for an aphid-transmitted virus in Israel (22).

Considering that sweet potatoes are vegetatively propagated, that aphids carry a stylet-borne virus of sweet potato, and that symptoms of this virus in sweet potato are commonly masked, we believe the general principle that vegetatively propagated plants are usually infected by one or more viruses deserves prominent consideration. In view of this and the stated shortcomings of earlier descriptions we propose to apply Occam's razor—entities shall not be multiplied beyond necessity—and to examine the literature with the view that there is an aphid-transmitted, stylet-borne virus of sweet potato that may consist of numerous strains displaying symptomatological differences but encompassing vein-clearing, vein-banding or -feathering, chlorotic spots or ringspots. With this in mind, most of the literature on sweet potato viruses can rather readily be explained.

The name to be applied to this aphid-borne virus is not as easily resolved; but, on the basis of priority, we propose to use "sweet potato feathery mottle virus" (FMV). This name was used by Doolittle and Harter (3) for the graft-transmissible agent(s) in an introduced sweet potato. They showed photographs of vein-clearing and vein-banding symptoms developing 2-4 wk after grafting into new plants. Further characterization of FMV was provided by Webb and Larson (41) and Webb (40) who showed that an aphid- and sap-transmitted virus occurred on sweet potato in Louisiana and that these isolates were similar to an aphid- and sap-transmitted virus they recovered from feathery mottle plants provided by Doolittle. Stubbs and McLean (38) and McLean (28, 29) provided confirmation of the limited host range of the virus and the stylet-borne aphid transmission. They also introduced *Ipomoea setosa* Ker. as a valuable indexing host (38); unfortunately, photographs of the symptoms (28) were not published. McLean (28) found flexuous rods, 850-nm long, in infected plants. In 1967, another isolate was partially purified and shown to have 800-nm particles and to cause the formation of pinwheel inclusions (D. H. Hall and J. F. Shepard, *unpublished*).

If FMV is unacceptable as the name for this virus, the next earliest name is internal cork virus (30). Nusbaum (30, 31) described internal cork disease in roots of Porto Rico plants and observed virus-like symptoms on the leaves of affected plants. This virus was transmissible by core grafts causing vein-clearing and mottle. Eventually,

in anthocyanin-containing cultivars, purple rings were observed (31). This virus has been called the internal cork virus (ICV) and shown to be sap transmissible to *I. nil* (9) and to be aphid-borne (16, 17, 32, 33).

The internal cork symptom is apparently caused by an agent that has been transmitted by core grafts (31). The agent has also been transmitted by aphids (17, 33) but only low percentages of plants show internal cork symptoms in their roots while almost all plants show foliar symptoms of the ICV. In the same manner, Martin (23) observed that one lot of sweet potatoes had internal cork, whereas several lots had the foliar symptoms ascribed to ICV. The evidence that the internal cork symptom is produced by the so-called ICV is not satisfactory. As Martin (23) speculated, there may be two agents involved, one causing corky spots in roots and one causing foliar symptoms. We propose that the viruses universally isolated from internal cork-affected sweet potato are one or more strains of FMV; the foliar symptoms of the so-called ICV are not an adequate basis to distinguish it from FMV and both have similar vector relationships. It seems particularly inappropriate to name a virus for a cultivar-specific symptom which it has not been proven to cause (internal cork) rather than for a symptom that it can cause on all cultivars (vein feathering).

Salama et al. (34) claimed to have isolated the internal cork virus by differential filtration and to have reproduced internal cork in roots and purple rings in leaves. The particles were stated to be isometric with a diam of 45-47.5 nm which would provide the basis for establishment of a separate virus. The particles, however, appear to be heterogeneous in size (34, Fig. 4). Furthermore, we measured 27 particles and found that the diam of 21 particles were 16-19 nm if calculations were based on the 100 nm scale printed on the photograph and 30 to 37.5 nm if calculations were based on the stated magnification of $\times$ 80,000. These serious discrepancies plus the differences between internal cork shown by Salama et al. (34) and authentic internal cork (31), plus the unknown source of their "virus-free" assay plants indicates that the results are of questionable validity.

The recognition of a more or less universally present virus such as FMV provides a ready explanation of other references in addition to "internal cork virus". We regard Sheffield's mosaic virus A (35), sweet potato ringspot virus (22), and sweet potato leafspot virus (12) as other isolates of FMV. The rod-shaped virus found in sweet potato little-leaf plants and identified on the basis of symptomatology in *I. setosa* and *I. nil* as russet crack virus and internal cork virus (20) is probably another strain of FMV. This shows that FMV is widespread, as would be expected by the principle cited above.

A whitefly-transmitted agent has been recognized in sweet potatoes since Sheffield described sweet potato mosaic virus B in East Africa (35, 36). A similar agent was recognized in the U.S. by Girardeau (6, 7) and in Israel by Loebenstein and Harpaz (22). This agent has been assumed to be a virus, but there is no characterization of the agent. We will not discuss nomenclature of this agent but will use the common name applied by California growers, yellow dwarf. The discovery of a similar agent in the original FMV source plant of Doolittle and Harter (10, 12) explains why the total symptom pattern was not reproduced by the FMV that was recovered by sap- or aphid-transmission by Webb and Larson (41) and by Webb (40). This whitefly-transmitted agent probably always occurs in field-grown plants as a second agent in addition to a strain of FMV. In California, yellow dwarf occurs in the south coastal sweet potato growing area and has been nearly eliminated by roguing infected plants from sprout beds, but earlier collections consistently yielded FMV by sap transmission.

Russet crack disease was described on Jersey-type sweet potatoes in 1964 as a probable virus disease (2). It was reported to be graft-transmissible from sweet potato to sweet potato or to *I. setosa* and then back to sweet potato. The necrotic symptoms on sweet potato roots were illustrated as well as mosaic symptoms on *I. setosa* and ringspots on sweet potato leaves. These authors also reported another unidentified virus (that we would identify as an FMV isolate) that was graft-transmitted to *I. setosa* and that was aphid-transmissible but did not cause internal cork (25). Later Hildebrand (13), illustrated russet crack symptoms on sweet potato roots and on *I. setosa* leaves and suggested *I. setosa* was a good indicator plant. Hildebrand was unable to show whitefly or aphid transmission in the greenhouse, but attempted to deduce that aphids transmitted the agent because of its spread in field plots (14). He (15) later suggested that soil pathogens, especially *Rhizoctonia,* were involved in the development of the root lesions and emphasized that foliar symptoms were the best assay for the russet crack "virus". None of these authors was able to sap-transmit russet crack. Martin (24) recovered russet crack from two other sweet potato varieties—'Porto Rico' with virus leaf spot and 'Goldrush' with internal cork. Apparently these varieties carry the russet crack agent, but do not express root symptoms.

The recommendation that *I. setosa* be used as an indexing host for russet crack virus (13) has been the basis for identification of the virus studied by Lawson et al. (21) as the russet crack virus and for identification of the disease in material received in Australia (39). In neither case were sweet potato root symptoms used in the diagnosis. The foliar symptoms of *I. setosa* shown by Hildebrand are exactly the same as those shown by McLean for FMV in 1959 (28).

The present study was begun to isolate and characterize the agent causing russet crack of sweet potato and, assuming that it was a virus, to compare it to the previously recognized viruses of that host. We shall use RC to indicate the russet crack agent in the text. An abstract has been presented (1). We considered three hypotheses as most likely: (i) RC was a distinct virus; (ii) RC was a strain of FMV; or (iii) RC was a virus dependent on its helper virus FMV.

MATERIALS AND METHODS.—The sweet potato cultivar 'Jersey' was used throughout this study. It is a yellow-fleshed selection from the Jersey type introduced to California many years ago. It is not 'Jersey Orange' as the editor assumed when he erroneously amended the earlier abstract (1). All plants used in this study were propagated by shoot cuttings from a single plant that was one of several freed of FMV by meristem culture. The virus-free progeny were maintained and

multiplied in an isolated, screened greenhouse and indexed at intervals by grafting to *I. setosa* to ensure that they remained free of FMV. The plants were grown in a sandy loam soil that was autoclaved prior to use.

Sap transmissions were done by triturating leaves showing virus symptoms in a mortar and pestle with 0.05 M phosphate buffer (pH 7.6) and finger-rubbing the sap over corundum-dusted leaves. Graft transmission was done by cleft-grafting a scion onto the stem of a stock plant. The scions were single-node sections, or occasionally two-node sections, with the leaves removed. After insertion, the graft was bound with elastic latex bandage material and the exposed, cut surfaces of the scion were coated with vaseline and a plastic bag was placed over the stock and scion for at least a week. The scions were always green at this time and usually continued to grow.

Stylet-borne aphid transmissions were done with *Myzus persicae* (Sulz.) raised on 'Tendergreen' mustard (*Brassica campestris* L. Perviridis group) from which they were removed and starved for at least 2 h before being placed on detached leaves of the virus-source plant. They were observed and allowed a 15-s probe before being transferred in groups of five to the test plant. Approximately 2 h later they were killed with a nicotine sulfate spray.

Virus particles were detected in infected plants by the epidermal dipping technique (19). The dips were made in potassium phosphotungstate.

Purification attempts were made with several buffers and clarifying agents. The most satisfactory method was to homogenize infected *I. nil* leaves with 1:1.5 w/v of 0.05 M or 0.5 M borate buffer (pH 8.2 or 9.0) containing 0.1% mercaptoethanol. After the homogenate was strained through cheesecloth, 1/10 volume of n-butanol was added and the mixture stirred for 0.5 h. Precipitated material was sedimented at 10,000 rpm for 20 min in a Sorvall SS-34 rotor. Virus was sedimented from the supernatant at 27,000 rpm for 2 h in a Beckman No. 30 rotor or at 42,000 rpm for 1.5 h in a 50.1 rotor. Another cycle of differential centrifugation was done before the virus solution was centrifuged into a 10-40% sucrose density-gradient column made with 0.05 M borate (pH 8.2) buffer. Centrifugation was for 3.0 h at 22,500 rpm in a Beckman SW-25.1 rotor. The virus band was removed, in most cases, by a hypodermic syringe needle punctured through the side of the tube. Virus was sedimented from the sucrose solution and resuspended in 0.05 M borate buffer.

RESULTS.—*Establishment of RC and FMV cultures.*—Roots were collected from a commercial field in Merced County, California, during harvest in November 1969. Six roots with mild to severe RC symptoms, and three symptomless roots were each placed in a pot of sand in the greenhouse to sprout. About 1 mo later three sprouts from each root were grafted to healthy plants. Foliar symptoms of vein-banding and ringspots began to appear in 3 wk and were observed on at least one of the three plants grafted from each source root. Roots of one grafted plant were washed free of soil after 4 wk and there were necrotic lesions on the fine roots (Fig. 1). Other roots washed free of soil at 7 wk had necrotic lesions on young fleshy roots (Fig. 2-4) as well as on fine roots. At 15 wk, roots of all the plants were examined and 23 of the 27 grafted plants had necrotic lesions on fine roots or on fleshy roots. These necrotic lesions are regarded as the characteristic symptom expression of RC and hereafter 'RC symptoms' will refer to such lesions. One replicate from each of four source roots (including three with mild-to-severe RC symptoms) was without necrotic lesions, as were three nongrafted controls interspersed among the experimental plants.

Additional plants were propagated as vine cuttings from sprouts on the field-collected roots and examined at intervals for RC symptoms. At 6.5 wk there were necrotic lesions on fine roots of plants from each source plant, and at later dates there were necrotic lesions on fleshy roots. Examination of freehand sections of roots 1-3 mm in diam with the light microscope showed that the necrotic reaction started with groups of cells in the outer cortical tissue just under the periderm, and in more advanced lesions the entire cortex was necrotic between the endodermis and the periderm. Similar lesions developed on fine roots of cuttings maintained in aerated or nonaerated nutrient solution for 2 mo (Fig. 5). Roots of noninfected plants were free of these lesions. Two of the infected plants were selected as the RC stock culture and used throughout this study.

An FMV culture was established from the same field-collected roots. When collected there were remnants of vine on some roots with RC symptoms; these vines were grafted to a few *I. setosa* of which one developed FMV symptoms. Since the vector of RC was unknown, stylet-borne aphid transmission was chosen to isolate FMV free of RC. Viruliferous aphids were transferred to five *I. setosa* of which three developed FMV symptoms and had FMV-type particles. Five control *I. setosa* exposed to nonviruliferous aphids remained symptomless. The infected *I. setosa* were grafted back to sweet potatoes and these did not develop RC symptoms. One plant was selected as the FMV stock culture. During 3 yr no RC symptoms have developed on the stock plants of this FMV culture or on plants inoculated from it. Later the *I. setosa* used as the source plant for acquisition of FMV by aphids was grafted into five sweet potatoes of which three developed RC symptoms. Thus, *I. setosa* was confirmed as a host for RC but a FMV culture was separated from it by aphid transmission. Additionally, this demonstrated that the RC source plants contained a mixture of RC and the universally present FMV. These results also confirmed that RC could be transmitted by grafting or propagated by cuttings. The latter method was used to maintain the RC culture because it was easier and more reliable than graft transmission. Since at least 6.5 wk were necessary before RC symptoms could be found on the roots, 9 wk has been the standard incubation period for experiments.

Host range, host reaction, and sap transmission.—Other species of *Ipomoea* were tested for susceptibility to RC. These hosts were inoculated by grafting and were indexed for RC by grafting back onto sweet potato. Three sprouts from each of eight field-collected roots were grafted to healthy *I. setosa* plants, all of which developed foliar symptoms. After 1 mo, five plants were selected and each was indexed to five sweet potatoes. From two *I. setosa*, five of five sweet potatoes

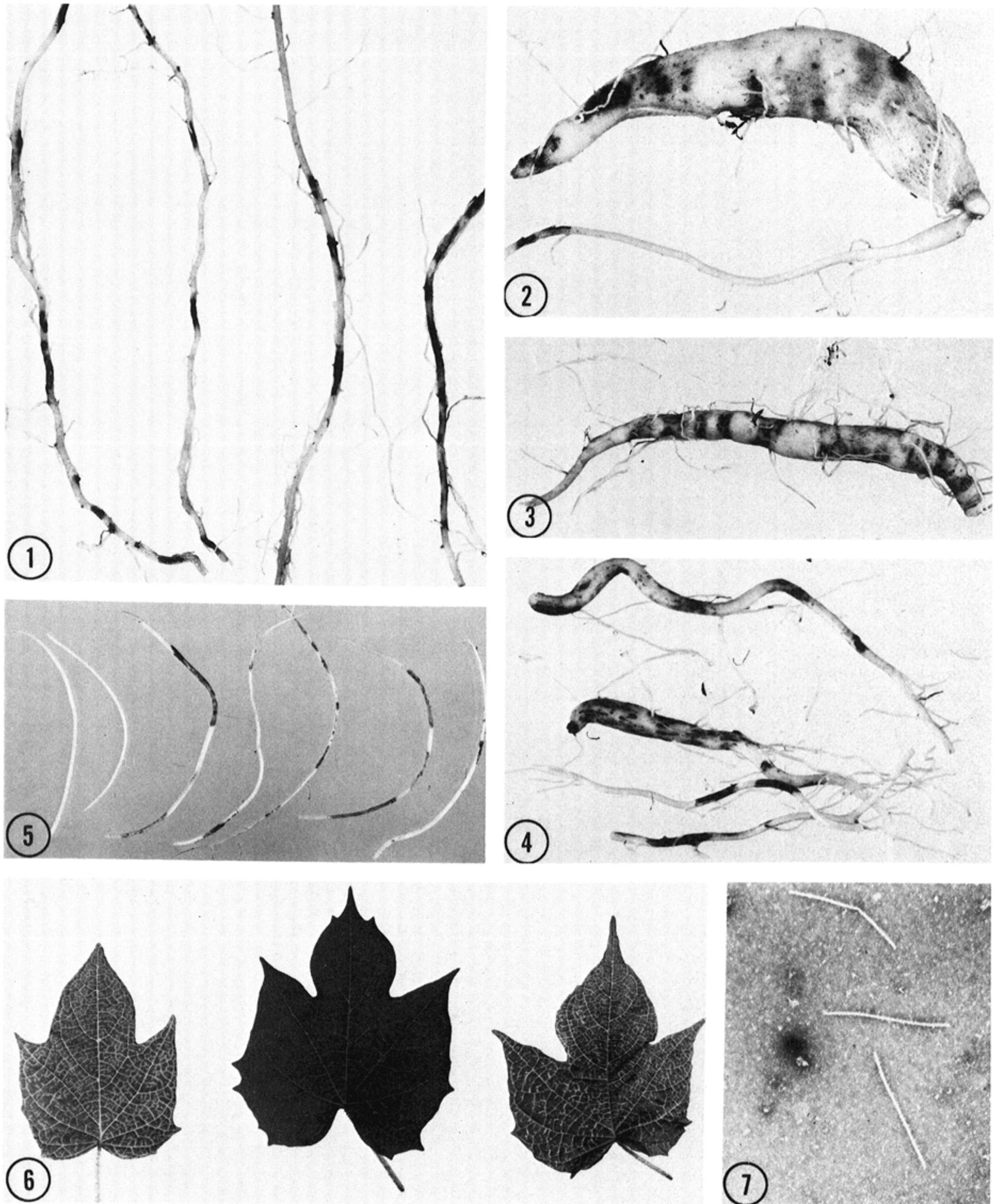

Fig. 1-7. 1) Necrotic lesions on fine roots of sweet potato 4 wk after grafting with RC culture. **2-4)** Necrotic lesions on fine roots and small fleshy roots of sweet potato 7 wk after grafting with RC culture. **5)** Necrotic lesions on fine roots of vine cuttings of RC-infected sweet potato after 9 wk in aerated nutrient solution. Two roots on left are from virus-free control plants. **6)** Early symptoms of FMV culture (left) and RC culture (right) on *Ipomoea setosa* 11 days after graft inoculation. Noninoculated control in center. **7)** Flexuous, rod-shaped virus particles in negatively stained epidermal dips from RC-infected *I. setosa* (× 26,400).

TABLE 1. Infection of *Ipomoea nil* and *I. tricolor* by graft transmission of russet crack (RC) and feathery mottle (FMV) cultures

Virus culture	Host inoculated	Results[a] Foliar symptoms	Index on sweet potato[a] Foliar symptoms	RC symptoms
RC	*I. nil*	9/10	8/9	6/9
RC	*I. tricolor*	9/10	4/10	6/10
FMV	*I. nil*	4/5	4/4	0/4
FMV	*I. tricolor*	5/5	2/5	0/5

[a](Number of plants with symptoms)/(Number of plants grafted).

developed severe RC symptoms; from another *I. setosa*, three of five sweet potatoes developed moderate RC symptoms; and from two other *I. setosa*, no RC symptoms developed although three of ten index plants had foliar vein-banding symptoms.

In another trial, the FMV culture in *I. setosa* and the RC culture in sweet potato were inoculated to five plants each of *I. setosa* and of sweet potato. All *I. setosa* developed a fine vein-clearing in 11 days (Fig. 6); by 5 wk this had changed to a broad vein-banding. Noninoculated controls remained symptomless. It was not possible at any time to distinguish between the symptoms induced by the RC and FMV cultures. Likewise, all sweet potato plants developed similar foliar vein-banding symptoms from either virus culture. The only difference was on the roots; the five plants grafted with RC had RC symptoms, whereas those grafted with FMV and the five controls were free of RC symptoms.

In another experiment, the FMV and RC cultures were inoculated to *I. nil* and *I. tricolor*. *I. nil* developed vein-clearing, vein-banding, and ringspots that were similar for both cultures. *I. tricolor* showed chlorotic spots followed by necrosis of the shoot with both cultures. In other trials, the plants sometimes died; in the present experiment, they all survived as stunted, slowly growing plants. After 6 wk, each plant was indexed and RC was recovered from *I. nil* and *I. tricolor* (Table 1). The leaves of the index plants showed vein-clearing, vein-feathering or vein-banding, and chlorotic ringspots regardless of the virus culture, but not all plants showed symptoms. Only the RC culture induced RC on the sweet potato roots. Again there was an incomplete correlation in sweet potato between foliar symptoms and RC symptoms; some plants had foliar symptoms with no RC symptoms and others had RC symptoms with no foliar symptoms.

FMV was known to be present in the RC culture because the FMV culture was established from the RC-affected plants. This was further confirmed by the presence of flexuous, rod-shaped particles about 800 nm long (Fig. 7) in epidermal dips made from hosts infected by either the FMV or the RC culture. Sap transmission was tested in some of the preceding experiments but there was little or no transmission of any virus as judged by lack of foliar symptoms on *Ipomoea* spp. or lack of RC symptoms on sweet potato roots. When some plants became infected, indexing showed that only FMV was present. *I. nil* seemed, however, to be the most susceptible host and was used for further sap transmission attempts with the RC culture. Indexing for RC was always done by means of grafts to sweet potato.

Sap transmission of RC was demonstrated in an experiment in which sweet potato leaves from RC stock culture plant were sap inoculated to *I. nil*, *I. tricolor* and sweet potato. The only plants that developed symptoms were four of five *I. nil* plants. These four plants were indexed on one sweet potato each. Three of the index sweet potatoes developed foliar symptoms and two of these, as well as the symptomless plant, had RC symptoms on their roots.

A similar trial was done using sap inoculation of the RC culture to nine *I. nil* and five sweet potatoes. Only two *I. nil* developed foliar symptoms and indexing showed only one was infected by RC. The remaining symptomless plants, seven *I. nil* and five sweet potatoes, were also indexed for RC in case RC did not cause foliar symptoms in the absence of FMV but none had RC. Additional treatments in this experiment consisted of similar inoculations with the FMV culture and of noninoculated controls. All these plants remained symptomless and those inoculated with the FMV culture indexed as free of RC.

Another trial was done with the RC culture. Sap transmissions were done separately from each of five RC sweet potatoes to three or four *I. nil*. Fifteen plants inoculated from four source plants remained symptomless, as did four controls. Only one of three plants inoculated from the fifth source plant developed symptoms. Two serial transmissions to *I. nil* were made at three-wk intervals from this plant. A month after the last transfer, six plants regardless of their foliar symptoms were indexed on *I. setosa* and sweet potato. Four plants induced foliar symptoms in *I. setosa* and in sweet potato and induced RC symptoms on sweet potato roots. The other two plants indexed to be free of FMV or RC.

After sap transmission of RC was recognized, a wider host range was inoculated by grafting the RC culture into *I. nil* or *I. setosa* and using the leaves with symptoms for sap transmissions to an herbaceous host range. At least two plants were inoculated in each of two trials. No symptoms were observed on the following hosts: *Vigna sinensis* (Torner) Savi 'Blackeye', *Datura stramonium* L., *Nicotiana glutinosa* L., *N. tabacum* L. 'Havana 425', 'Turkish', 'Bel W-3', *N. glutinosa* × *N. tabacum* ['Samsun' × 'Xanthi'] cultivar Turkish, nc. 'Glurk', *Gomphrena globosa* L., *Chenopodium quinoa* Willd., *C. amaranticolor* Coste & Reyn., *C. capitatum* (L.) Asch., and Tendergreen mustard.

Aphid transmission.—Aphid transmission of RC to sweet potatoes was tested using detached leaves from stock culture sweet potato plants or from graft-inoculated *I. nil* and *I. setosa* as virus sources. About 1 mo after the aphid transmission attempts each plant in the trial was grafted to a healthy *I. setosa*. Epidermal dips were examined from any of these latter plants developing foliar symptoms. The presence of FMV-like symptoms and 800-nm rods constituted presumptive evidence for the presence of FMV. Transmission of RC was determined by examination of the sweet potato roots about 1 mo later.

In six of seven trials there was transmission of both FMV and RC (Table 2) but in no case was RC found in

plants without FMV-type of particles. In trial 2 all plants were propagated and grown for 2 mo to determine if RC symptoms might develop later on symptomless plants, but the results were the same as shown in Table 2. The dual transmission of both FMV and RC in these trials compared to the earlier establishment of the FMV culture is of interest. The results in trial 7 offer some explanation. The one plant listed as having been infected was indexed as FMV-infected but the roots were free of RC symptoms. Since this was unique, confirmation was attempted by propagating four vine cuttings. These were grown for 2 mo along with two cuttings from a control plant. The control plants were free of RC symptoms and only one of the four progeny from the infected plant had RC symptoms. On this basis, the plant was rated as doubly infected with FMV and RC. Probably the aphids had transmitted FMV and a small amount of RC that had not systemically infected the host.

TABLE 2. Transmission of feathery mottle virus (FMV) and russet crack agent (RC) to sweet potatoes (*Ipomoea batatas*) by aphids after a 15-s acquisition probe on infected plants

		(No. plants with FMV & RC[a])/ (No. in each group)		
Trial no.	Virus source host	Viruliferous aphids	Aphid controls	Noninoculated controls
1	*I. batatas*	1/10	0/10	
2	*I. batatas*	2/15	0/5	0/5
3	*I. batatas*	1/10	0/5	0/5
4	*I. setosa*	5/15	0/5	0/5
5	*I. setosa*	0/10	0/5	0/5
6	*I. setosa*	6/10	0/5	0/5
7	*I. nil*	1/10	0/5	0/5

[a]FMV presumed to be present if ca. 800-nm-long rods were detected in *I. setosa* index plants; RC detected by symptoms on sweet potato roots. All infected plants were positive for both items. See text.

Two attempts were made to demonstrate circulative transmission of RC or FMV. In the first trial, ten groups of five aphids were transferred to sweet potatoes following a 24 h acquisition access period and they were allowed a 24 h transmission access period. In another trial, aphids reared on a RC culture plant were transferred in groups of five to five sweet potatoes. None of these plants developed RC symptoms.

Cross-protection.—Cross-protection trials were done to test the relatedness of RC and FMV. Challenge inoculations were done by grafting RC scions onto FMV-infected plants. As controls, similar scions were grafted onto healthy plants; in addition, nongrafted healthy or FMV-infected plants were interspersed among the grafted plants. FMV protected against RC in five tests done during a year (Table 3). As earlier, there was poor correlation between the development of foliar symptoms on graft-inoculated healthy plants and RC symptoms on their roots. The RC symptoms appeared on fine feeder roots (Fig. 8) and on enlarged storage roots (Fig. 9).

In the last experiment, five of 15 challenge-inoculated, FMV-infected plants had a few lesions on roots near the stem (Fig. 10, 11). These were rated as possible RC. To determine whether these or other challenge-inoculated plants would develop typical RC symptoms in the next propagative generation, two cuttings of each plant in the experiment were grown for 2 mo. Only one of 30 plants from the FMV-infected, challenge-inoculated plants had typical RC symptoms; this was on one of the progenies of a plant rated as possible RC. Both plants from another parent with possible RC had only a few lesions on roots near the stem (as in Fig. 10, 11). The six progeny from the other three possible RC plants had no lesions. Plants from the other ten previously symptomless, FMV-infected parents remained symptomless except for two that had a few lesions on fine roots near the stem. Of the 20 healthy plants grafted with RC, 18 had typical RC symptoms and only two were symptomless. Plants derived from both types of nongrafted controls remained free of RC symptoms throughout the experiment. The plants rated as possible RC are considered to have been protected against typical RC symptom expression because they did not consistently develop RC symptoms in two propagative generations.

Purification attempts.—Since RC was sap-transmissible, it seemed feasible to purify RC with the objective of demonstrating either that there were two distinct types of virus particles by electron microscopy and rate-zonal density-gradient centrifugation or that RC symptoms could be produced by particles of the FMV type. RC was transferred to *I. nil,* or occasionally *I. setosa,* by graft inoculation of a few plants. When these showed symptoms, sap transfers were made to *I. nil* plants that served as increase hosts. Sometimes two serial sap transfers were made. In each purification trial, 4-5 randomly selected increase plants were indexed for RC or else *I. nil* plants inoculated with crude homogenate were similarly indexed. Infectivity was assayed during purification by mechanical transmission to *I. nil* that showed symptoms in 2-4 wk and was indexed for RC by grafting to sweet potato.

In five of the ten purification attempts, RC was not present in the source plants and these trials provided no support for either of the objectives. In three of the five trials, however, there was enough FMV to give a distinct band in density-gradient centrifugation and the presence of FMV in the RC culture was directly confirmed. The

TABLE 3. Cross-protection experiments in which challenge inoculations were made by grafting russet crack scions onto healthy or feathery mottle virus (FMV)-infected sweet potatoes

	Challenge inoculations to:		
Trial no.	Healthy plants	FMV-infected plants	Nongrafted controls[a]
1	4/5[b]	0/5	0/10
2	2/5	0/5	0/10
3	7/9	0/9	0/10
4	5/10	0/15	0/16
5	10/10	0/15	0/12
Total	28/39	0/49	0/58

[a]Includes equal numbers of FMV-infected and of virus-free plants.

[b]Results expressed as (No. of plants with RC symptoms)/(No. in test).

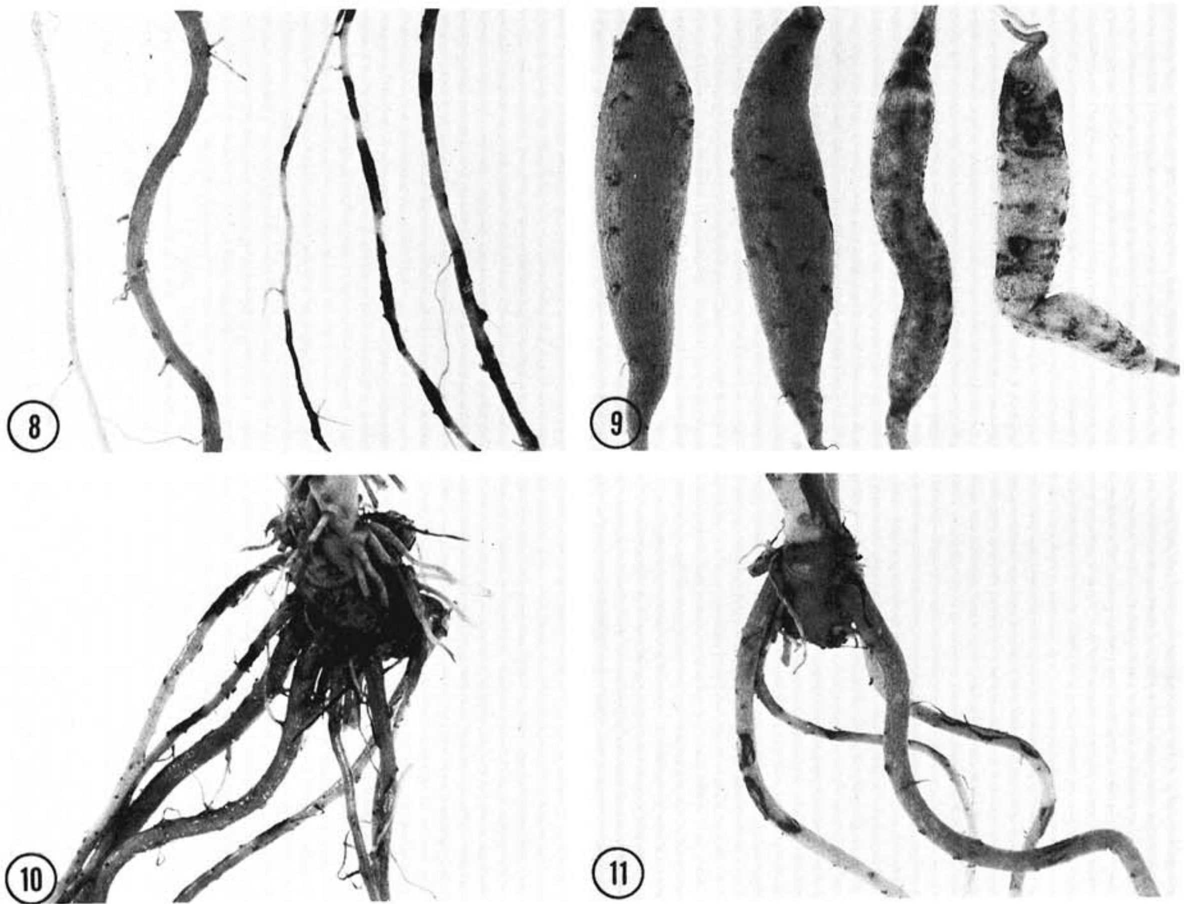

Fig. 8-11. Symptoms on roots of plants from cross-protection experiments comparing RC challenge inoculations to FMV-infected and to healthy sweet potatoes. **8)** Two fine roots from FMV-infected plants (left) compared to three fine roots with RC lesions from grafted healthy plant (right). **9)** Two fleshy roots from grafted FMV-infected plants (left) and from grafted healthy plants (right). **10, 11)** Possible RC lesions found only near stem of FMV-infected plants. One of two progenies of plant in Fig. 10 had typical RC symptoms. Neither of the two progenies of plant in Fig. 11 had RC symptoms.

FMV band was 20-26 mm below the meniscus in different trials and lay below several bands of host material. The virus recovered from these bands was rod-shaped, about 800 nm long, and in three trials infected four, six, and six of ten *I. nil* plants. These *I. nil* had typical FMV symptoms and in two trials they were grafted to sweet potato, some of which developed foliar symptoms, but none of which developed RC symptoms.

In three of the five trials in which RC was present at the start of purification, there was enough virus after two cycles of differential centrifugation to warrant density-gradient centrifugation. In these density-gradient columns there were the same normal host bands and the FMV band at about 25 mm below the meniscus. Virus from this band was infective to *I. nil* with three, two, and none of ten plants becoming infected in each of the three trials. In the first experiment, the columns had been analyzed with an ISCO density-gradient fractionator and only two of the three infected *I. nil* were grafted to sweet potato (two each). All four of these sweet potatoes developed RC symptoms. In the next trial all ten *I. nil*, regardless of foliar symptoms, were grafted to sweet potato but none developed RC symptoms. In both trials the material above the FMV band was not infectious to *I. nil*.

Purification attempts that did not yield virus after differential centrifugation involved the use of phosphate buffer with polyethylene glycol (PEG) precipitation with urea for resuspending the virus (18), the use of PEG precipitation with borate buffer, or the use of hydrated calcium phosphate as a clarifying agent (5).

DISCUSSION.—These results confirm the description of sweet potato russet crack (2) as an infectious disease that is graft-transmissible from sweet potato to sweet potato and *I. setosa*. This report also demonstrates that *I. nil* and *I. tricolor* are hosts, that the causal agent is transmitted in stylet-borne manner by *M. persicae*, that it can be sap-transmitted occasionally from sweet potato to *I. nil* and more regularly from *I. nil* to *I. nil*, that FMV occurs in the RC culture, and that foliar symptoms induced by RC cannot be distinguished from those induced by FMV. The vector relationships and

symptomatology support the view that russet crack is caused by a virus. There is limited evidence from the purification attempts to support this hypothesis.

The next problem is that of nomenclature. We do not favor the use of a new virus name unless the proposed new virus can be shown to be distinct from known viruses. FMV and RC have similar vector relationships, are similar in their host range (limited to Convolvulaceae), and are similar in symptomatology. We have been unable to demonstrate a virus other than the universally present FMV in the RC culture. Thus, we propose the hypothesis that the causal agent of russet crack is a strain of FMV; i.e., RC-FMV. The designation of the agent as "russet crack virus" in a previous abstract (1) was made by the editor, not the authors. Our hypothesis is supported by the cross-protection experiments in which FMV protected against RC symptom expression. The cross-protection test is admittedly imperfect (27), but it is one of the few that can be applied to the problem.

Our hypothesis about the nature of RC-FMV implies that two strains of the virus are present in the RC stock culture plant. The presence of FMV in these plants has been shown in sap-transmission experiments and in the purification trials. Since there is no local lesion host for FMV or RC-FMV, the converse proof that a subculture from the RC stock culture contains RC-FMV alone is difficult to achieve. It may be that some aphid or sap transfers have given RC-FMV alone but we have not done the extensive subtransfers that would be necessary to prove this. Our hypothesis explains why we have been unable to cause RC symptoms in the absence of FMV-type rod-shaped virus particles. If two strains of the virus occur in the same source plant, it would be expected that both would readily invade a virus-free plant whereas they would not readily invade an FMV-infected plant. Thus, transfers by grafting or aphids results in RC symptoms when virus-free plants are inoculated, but not when FMV-infected plants are inoculated as in the cross-protection trials. In these trials there was evidence for the slow establishment of RC-FMV in the FMV-infected plants; one plant of thirty developed RC in the second vegetative generation. These results provide an explanation for field observations that virus-free stocks of Jersey have a low incidence (8-10%) of RC symptoms at the first harvest in commercial fields. If such stocks are used for seed for the following year, the plants have a high incidence (70-80%) of RC symptoms. On the other hand, stocks that have been maintained by growers for a long time are universally infected with FMV and have a low percentage of RC disease each year (D. H. Hall, and R. W. Scheuerman, *unpublished*). The fact that sap transmission and purification trials with the RC-FMV culture sometimes yielded FMV alone suggests that the titer of FMV is greater than that of RC-FMV.

Ipomoea setosa was introduced as a useful indicator plant for FMV (38). Later Hildebrand (13) promoted the use of *I. setosa* as a specific-indexing host to separate his russet crack and internal cork viruses. The symptoms shown in his photographs, however, are the same as earlier illustrated by McLean (28) for FMV and are the same that we observe with either our FMV or RC-FMV cultures. It is impossible to distinguish between plants inoculated with either culture when several are grown side by side for several wk. We strongly support the use of *I. setosa* as an indexing host for FMV or RC-FMV; it rapidly shows distinct symptoms but it is not a differential host. Hildebrand (15) reported abandoning the root-lesion criterion for the diagnosis of RC disease. We find that the root lesion is the only distinguishing characteristic of RC-FMV. Hildebrand (15) also suggested that RC is a fungal disease that is accentuated by the so-called russet crack virus. We have raised our plants in pasteurized soil and propagated from shoot cuttings to avoid complications with soil-borne pathogens such as *Monilochaetes infuscans* Ell. & Halst. ex Harter and *Rhizoctonia* spp. In our trials, RC lesions are reproducible on roots of infected plants but not on virus-free controls. Furthermore, signs of *M. infuscans* and *Rhizoctonia* spp. cannot be observed on the surface of RC-affected roots. Thus, we regard the RC disease as virus-induced. Soil-borne fungi doubtless colonize necrotic tissue in field soils and cause more severe symptoms.

The FMV culture in this study agrees with all the earlier characteristics given for FMV: host range and host symptomatology (mosaic of Convolvulaceae), sap transmissibility (inconsistent), aphid relationship (stylet-borne), and particle morphology (flexuous, approximately 800-nm rods). We conclude that our FMV was correctly identified. The virus is longer than most viruses of the PVY group. Limited tests with $MgCl_2$ and EDTA (8) have not significantly changed the apparent length of the particles. A subsequent study of several hundred particles in negatively stained preparations from *I. setosa* has established that the normal length of FMV particles is 844 nm whereas the normal length of particles in the RC-FMV culture is 876 nm (F. Nome and T. A. Shalla, *personal communication*).

Several techniques for working with FMV and RC-FMV should be emphasized. Sap transmission from sweet potato has only been achieved when leaves showing symptoms are used as a source of virus; even then sap transmission is erratic, thus graft transmission is preferred to move virus to or from sweet potato. Foliar symptoms on sweet potato are unreliable for detection of infection by FMV or RC-FMV. *I. setosa* (38) is a preferred indexing host for FMV or RC-FMV because it shows distinct symptoms rapidly. *I. nil* is more susceptible to sap transmission than other species.

LITERATURE CITED

1. CAMPBELL, R. N., N. M. MIELINIS, and D. H. HALL. 1972. Transmission of sweet potato russet crack virus. Phytopathology 62:750 (Abstr.).
2. DAINES, R. H., and W. J. MARTIN. 1964. Russet crack, a new virus disease of sweet potatoes. Plant Dis. Rep. 48:149-151.
3. DOOLITTLE, S. P., and L. L. HARTER. 1945. A graft transmissible virus of sweet potato. Phytopathology 35:695-704.
4. ELMER, O. H. 1960. Etiology and characteristics of sweet potato mosaic. Phytopathology 50:744-749.
5. FULTON, R. W. 1959. Purification of sour cherry necrotic ringspot and prune dwarf viruses. Virology 9:522-535.
6. GIRARDEAU, J. H. 1958. The sweet potato whitefly, Bemisia inconspicua Q., as a vector of a sweet potato

mosaic in south Georgia. Plant Dis. Rep. 42:819.

7. GIRARDEAU, J. H., and T. J. RATCLIFFE. 1960. The vector-virus relationship of the sweet potato whitefly and a mosaic of sweet potatoes in south Georgia. Plant Dis. Rep. 44:48-50.
8. GOVIER, D. A., and R. D. WOODS. 1971. Changes induced by magnesium ions in the morphology of some plant viruses with filamentous particles. J. Gen. Virol. 13:127-132.
9. HILDEBRAND, E. M. 1956. Sweet potato internal cork virosis indexed on Scarlett O'Hara morning glory. Science 123:506-507.
10. HILDEBRAND, E. M. 1958. Two syndromes caused by sweet potato viruses. Science 128:203-204.
11. HILDEBRAND, E. M. 1959. Sweet potato ringspot virus associated with internal cork virus. Phytopathology 49:524 (Abstr.).
12. HILDEBRAND, E. M. 1960. The feathery mottle virus complex of sweet potato. Phytopathology 50:751-757.
13. HILDEBRAND, E. M. 1967. Russet crack—a menace to the sweet potato industry. I. Heat therapy and symptomatology. Phytopathology 57:183-187.
14. HILDEBRAND, E. M. 1968. Russet crack—a menace to the sweet potato industry. II. Natural transmission. Plant Dis. Rep. 52:967-971.
15. HILDEBRAND, E. M. 1969. Russet crack—a menace to the sweet potato industry. III. Role of fungi in the root-lesion phase of russet crack. Plant Dis. Rep. 53:103-107.
16. HILDEBRAND, E. M., and F. F. SMITH. 1956. Aphid transmission of sweet potato cork virus in the greenhouse. Phytopathology 46:468 (Abstr.).
17. HILDEBRAND, E. M., and F. F. SMITH. 1958. Aphid transmission of a virus associated with sweet potato internal cork and masked in feathery mottle-infected sweet potatoes. Plant Dis. Rep. 42:1148-1153.
18. HILL, J. H., and R. J. SHEPHERD. 1972. Biochemical properties of turnip mosaic virus. Virology 47:807-816.
19. HITCHBORN, J. H., and G. J. HILLS. 1965. The use of negative staining in the electron microscopic examination of plant viruses in crude extracts. Virology 27:528-540.
20. KAHN, R. P., R. H. LAWSON, R. L. MONROE, and S. HEARON. 1972. Sweet potato little-leaf (witches'-broom) associated with a mycoplasmalike organism. Phytopathology 62:903-909.
21. LAWSON, R. H., S. S. HEARON, and F. F. SMITH. 1971. Development of pinwheel inclusions associated with sweet potato russet crack virus. Virology 46:453-463.
22. LOEBENSTEIN, G., and I. HARPAZ. 1960. Virus diseases of sweet potatoes in Israel. Phytopathology 50:100-104.
23. MARTIN, W. J. 1950. Internal cork of sweet potatoes in Louisiana. Phytopathology 40:789 (Abstr.).
24. MARTIN, W. J. 1970. The reproduction of russet crack in Jersey Orange sweet potatoes by grafting on plants affected with either sweet potato virus leaf spot or internal cork. Phytopathology 60:1302 (Abstr.).
25. MARTIN, W. J., and R. H. DAINES. 1964. An aphid-transmitted virus from russet crack-affected sweet potatoes. Phytopathology 54:900 (Abstr.).
26. MARTYN, E. B. 1968. Plant virus names. Commonw. Mycol. Inst. Phytopathol. Pap. No. 9. 204 pp.
27. MATTHEWS, R. E. F. 1970. Plant Virology. Academic Press, New York and London. 778 pp.
28. MC LEAN, D. L. 1958. Vector-virus relationships of the severe isolate of the feathery mottle virus of sweet potato and the green peach aphid Myzus persicae (Sulzer). Ph.D. thesis, University of California, Berkeley, Calif. 107 pp.
29. MC LEAN, D. L. 1959. Some aphid vector-plant virus relationships of the feathery mottle virus of sweet potato. J. Econ. Entomol. 52:1057-1062.
30. NUSBAUM, C. J. 1947. Studies of internal cork, a probable virus disease of sweet potato. Phytopathology 37:435 (Abstr.).
31. NUSBAUM, C. J. 1950. Internal cork of sweet potatoes. S. C. Agric. Exp. Stn. Bull. 381. 23 pp.
32. RANKIN, H. W. 1952. Progress report on internal cork of sweet potatoes. Phytopathology 42:287 (Abstr.).
33. RANKIN, H. W., and J. H. GIRARDEAU. 1958. Transmission by Myzus persicae (Sulz.) of the internal cork virus of sweet potatoes. Plant Dis. Rep. 42:581-582.
34. SALAMA, F. M., C. M. LYMAN, and U. G. WHITEHOUSE. 1966. Isolation of the internal cork virus from sweet potato leaves having the purple ring structure by use of differential filtration. Phytopathol. Z. 57:89-95.
35. SHEFFIELD, F. M. L. 1957. Virus diseases of sweet potato in East Africa. I. Identification of the viruses and their insect vectors. Phytopathology 47:582-590.
36. SHEFFIELD, F. M. L. 1958. Virus diseases of sweet potato in East Africa. II. Transmission to alternative hosts. Phytopathology 48:1-6.
37. STEINBAUER, C. E., and L. J. KUSHMAN. 1971. Sweet potato culture and diseases. U.S. Dep. Agric. Agric. Handb. 388. 74 pp.
38. STUBBS, L. L., and D. L. MC LEAN. 1958. A note on aphid transmission of a feathery mottle virus of sweet potato. Plant Dis. Rep. 42:216.
39. VICTORIAN PLANT RESEARCH INSTITUTE. 1962-1967. Sweet potato. Victorian Plant Research Institute, Reports 3 and 4. Melbourne, Australia. p. 65.
40. WEBB, R. E. 1954. Some hosts of feathery mottle virus of sweet potato. Plant Dis. Rep. 38:448-452.
41. WEBB, R. E., and R. H. LARSON. 1954. Mechanical and aphid transmission of the feathery mottle virus of sweet potato. Phytopathology 44:290-291.