Sweet Potato Little-Leaf (Witches'-Broom) Associated with a Mycoplasmalike Organism

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ABSTRACT

A plant introduction, *Ipomoea batatas* 'Nukua Loka' (P.I. 308200) from New Zealand, originally collected in Tonga, showed little-leaf, proliferation, yellowing, stunting, and witches'-broom symptoms when grown in quarantine at the U.S. Plant Introduction Station, Glenn Dale, Md. Sweet potato russet crack virus (RCV) and a mycoplasmalike organism (MLO) were both detected in P.I. 308200. However, the little-leaf, proliferation, and witches'-broom symptoms were associated with a MLO and not RCV on the basis of electron microscopy, short-term heat therapy, and oxytetracycline sensitivity data. Plants of P.I. 308200 without little-leaf or proliferation symptoms were developed either by heat therapy or oxytetracycline treatment.

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A plant introduction (P.I.) of sweet potato, *Ipomoea batatas* (L.) Lam., P.I. 308200, received from New Zealand, but originally collected in Tonga, was infected with a complex of two, if not three, viruses or agents (6). One virus was identified as sweet potato russet crack virus (RCV) on the basis of symptoms incited on *I. setosa* (4), and we suspected the presence of a second virus, sweet potato internal cork virus (ICV) on the basis of symptoms in the morning glory hybrid Scarlett O'Hara (3). However, RCV and ICV incited similar symptoms in Scarlett O'Hara in subsequent tests. Mycoplasmalike bodies were associated with little-leaf and proliferation symptoms, and the incitant was graft- but not aphid- or mechanically transmitted (7). We now present additional evidence that the little-leaf and proliferation symptoms are associated with a mycoplasmalike organism, and not with any virus which was also present in P.I. 308200.

MATERIALS AND METHODS.—The original source of all components in the complex was a single plant of *I. batatas* 'Nukua Loka', P.I. 308200 collected in Tonga. A known culture of RCV in the sweet potato cultivar Jersey Orange as supplied by R. H. Daines was used for comparison.

The indicator plants used were (i) seedlings of the hybrid morning glory, Scarlett O'Hara; (ii) seedlings of the Brazilian morning glory, *I. setosa*; (iii) rooted cuttings of USDA sweet potato seedling No. 21 furnished by E. M. Hildebrand; and (iv) healthy P.I. 308200 plants obtained after heat treatment of P.I. 308200. The original healthy plant, from which all other plants described as "healthy P.I. 308200" in this report were propagated, still remains free of any symptoms more than 3 years after heat treatment. This mother plant continued to test negatively for viruses when indexed periodically during the 2-year period following heat treatment. USDA Seedling No. 21 shows symptoms when grafted with sweet potato infected with viruses that incite feathery mottle or fanleaf symptoms. Plants were inoculated by grafting (tip grafts with latex bandage wrapping), sap transfer (5), tissue wipe (2), or aphid transmission. Green peach aphids, *Myzus persicae* (Sulz), were reared on healthy collard seedlings. Aphids brushed into a glass stendar dish were starved for 2 hr at about 21 C.

Small sections of leaves from the infected source plant were placed in another dish, and the aphids shaken onto them. A piece of leaf bearing 15 to 20 aphids was placed on a small paper square on each test plant after 10 min. Pieces of source tissue were removed as soon as the aphids moved to the test plants. Contact between the source pieces and the test plants was not permitted. Aphids were destroyed after about 25 hr by the immersing of plants in a mixture containing 10 ml of 40% nicotine sulfate and 15 ml of household detergent/gal of water.

Samples for electron microscopy were processed as before (8). Leaf samples, cut to include vascular tissue, were fixed in phosphate-buffered 1.5% acrolein and 2% glutaraldehyde. The samples were postfixed in 1% osmium tetroxide, dehydrated in ethanol, and embedded in Epon. Sections were stained with uranyl acetate and lead citrate.

RESULTS.—*Transmission.*—Aphids did not transmit an agent that incites little-leaf or proliferation symptoms in healthy P.I. 308200, USDA Seedling No. 21, *I. setosa*, or Scarlett O'Hara morning glory. Inoculum sources were plants of P.I. 308200, No. 21, and *I. setosa* showing little-leaf and proliferation symptoms. The aphids were tested for circulative and stylet-borne virus transmission. Although the little-leaf agent was not transmitted in several trials, a virus was transmitted in the stylet-borne manner from P.I. 308200 or a known culture of RCV to *I. setosa* and Scarlett O'Hara, but not to No. 21. In addition, aphids transmitted from P.I. 308200, a component that incited chlorotic spots in healthy P.I. 308200, but did not incite proliferation and little-leaf symptoms. The component was identified as RCV on the basis of symptoms incited on *I. setosa*.

An agent that incites little-leaf symptoms was not transmitted mechanically from P.I. 308200 with little-leaf and proliferation symptoms to healthy P.I. 308200, *I. setosa*, or No. 21, even with the use of chemical additives. In addition, it was not possible to mechanically transmit any agent that would incite viruslike symptoms in *Nicotiana tabacum* L. 'Samsun' and 'Ky 35', *Chenopodium amaranticolor* Coste & Reyn., *C. quinoa* Willd., *Datura stramonium* L., *D. metel* L., *Phaseolus vulgaris* L. 'Top Crop' and 'Pinto No. 111', *Cucumis sativus* L. 'Improved Long Green', *Gomphrena globosa* L., *Capsicum annuum* L. 'California Wonder', and *Vigna sinensis* Endl. 'California No. 5'. Chemical additives used were (i) 0.02 M mercaptoethanol; (ii) 0.01 M sodium thioglycollate plus 0.02 M sodium diethyldithiocarbamate; (iii) 0.5% caffeine plus 0.5% nicotine; (iv) cysteine hydrochloride; (v) 1% sodium sulfite; and (vi) 1% bentonite; all additives were in 0.1 M potassium phosphate buffer at pH 7.

Leaves or roots of infected P.I. 308200, or leaves or flowers of No. 21 (with little-leaf and proliferation symptoms) were ground in the presence of the additive. The expressed juice was rubbed on Carborundum (300 mesh) -dusted leaves of the test plants. In some trials, the time interval between grinding and inoculation was less than 1 min. The little-leaf agent was not transmitted by the tissue-wipe method from P.I. 308200 or No. 21 with little-leaf and proliferation symptoms to healthy P.I. 308200, No. 21, or *I. setosa*. Russet crack virus was transmitted mechanically, including the use of the tissue-wipe method, from the P.I. 308200 complex to *I. setosa* and Scarlett O'Hara morning glory, but not to No. 21. Russet crack virus incited chlorotic spots in P.I. 308200, but not proliferation or little-leaf symptoms.

An agent that incites little-leaf and proliferation symptoms was transmitted by grafting from P.I. 308200 to several cultivars of *I. batatas* and to *I. setosa*. The cultivars used were supplied by W. J. Martin from field-grown stocks and were Centennial, Porto Rico, Julian, Jersey Orange, and Goldrush as well as our own source of healthy P.I. 308200 and USDA Seedling No. 21. The little-leaf agent was transmitted from *I. setosa* (infected by grafting) back to healthy P.I. 308200. The incubation period was usually 20 to 40 days with healthy, vigorously growing young plants of P.I. 308200 and *I. setosa*. However, for well-established plants of *I. batatas* that were not showing flushes of growth at the time of grafting, the incubation period was often as long as 3 to 5 months. In general, healthy plants of P.I. 308200 and No. 21 had leaves that were 3 to 4 times larger, stems that were twice as thick, leaf margins that had 2 to 4 times as many lobes, and stems that had one-half to one-eighth as many branches as did infected plants. In addition, the internodes on healthy P.I. 308200 were twice as long as those on infected plants.

Heat therapy.—Plants of P.I. 308200 free of little-leaf and proliferation symptoms were obtained by a combination of heat treatment and propagation of one-node cuttings. In one test, 10 well-established infected plants were subject to heat treatment for 60 days at 38 to 39 C in a heat chamber with fluorescent and incandescent light at about 500 ft-c on a 16-hr day. Two hundred and forty cuttings were harvested between the 30th and 60th day from new growth produced in the heat chamber. Of the 120 cuttings that were established as plants, 62 were free of little-leaf and proliferation symptoms. All 62 remained symptomless for more than 1 year; and 10 plants still remain symptomless more than 3 years after treatment. Several plants, developed from cuttings removed from heat-treated plants, were grafted with scions from the original nonheat-treated plants, and the new growth showed typical little-leaf symptoms within 4 to 6 weeks after grafting.

Sixty-one of the 62 plants that were free of little-leaf and proliferation symptoms also indexed negatively for virus when tested 4 to 6 months after heat treatment. One plant without little-leaf symptoms indexed positive for virus when indexed on Scarlett O'Hara.

In a second heat-treatment test, at the end of 30 days, cuttings were taken from P.I. 308200 at the first through 12th nodes on each of 15 stems produced in a heat chamber. The percentage of cuttings that were free of little-leaf and proliferation

TABLE 1. Effect of 3- to 21-day heat treatment on *Ipomoea batatas* (P.I. 308200 and USDA Seedling No. 21) infected with the little-leaf agent

Experiment no. and cultivar	No. of days in heat chamber[a]	No. of cuttings harvested	No. plants	
			With delayed appearance of symptoms[b]	Remaining free of symptom[c]
P.I. 308200				
1	5	4	0	0
2	7	7	0	5
3	3	8	1	0
4	7	1	0	1
5	7	15	0	0
6	3	1	0	0
7	21	5	0	3
8	7	5	0	0
9	7	10	0	0
10	8	3	0	0
11	21	9	4	0
USDA seedling no. 21				
12	8	10	0	2
13	7	5	0	0
14	3	5	1	0
15	7	4	0	0
16	3	4	3	0
17	21	6	0	1
18	7	5	0	0
19	5	2	0	0
20	11	2	0	0
21	13	2	0	1
22	7	6	3	0
23	5	6	0	2
24	7	3	0	0

[a] 38 C, fluorescent and incandescent light at about 500 ft-c, 16-hr day.
[b] Symptom repression, plants not cured.
[c] Symptoms never appeared, plants cured.

symptoms 6 months after heat treatment were 70% from the 1st node, 55% from the 2nd node, 64% from the 3rd node, 75% from the 4th node, 66% from the 5th node, and 71% from the 6th through 12th nodes. Comparable numbers of cuttings were taken from the first through 12th nodes of nonheat-treated control plants. All cuttings from this infected control plant that rooted developed into plants with typical little-leaf and proliferation symptoms.

In a third test, plants of P.I. 308200 and No. 21, infected with the complex by grafting from P.I. 308200, were placed in a heat chamber and cuttings removed from new growth after 3 to 21 days' exposure. Results from 24 trials (Table 1) were variable, but symptomless plants were obtained in some trials with only 7 days' exposure. In five trials, the appearance of little-leaf symptoms was delayed since some plants produced obviously larger leaves for the first 6 weeks after heat treatment, but then the plants developed little-leaf and proliferation symptoms. Plants free of little-leaf symptoms have not been tested for virus.

Some heat-treated plants produced one or two long shoots without little-leaf symptoms and with normal internodes. Propagations from the long shoots developed into symptomless plants.

In two trials out of seven, plants of P.I. 308200 were obtained that were free of little-leaf and proliferation symptoms by heat treatment for at least 7 days. Symptom expression was delayed sometimes by a 3-day treatment. Symptom expression was not delayed with treatments of shorter duration at higher temperatures. The following treatments were ineffective in either eliminating the agent or delaying symptom expression: (i) immersing rooted or nonrooted cuttings in water at 41 C for 0.5, 1, 2, 4, or 6 hr; (ii) immersing nonrooted cuttings for 15 min at 42 to 43 C or 47 to 48 C; (iii) heat chamber treatment for 1.5, 2, or 3 days at 40 to 42 C; or (iv) immersing nonrooted cuttings of P.I. 308200 and No. 21 at 43 C for 2, 4, 6, 8, 16, 24, or 48 hr.

Antibiotic therapy.—Treatment of nonrooted cuttings and of intact plants of P.I. 308200 and No. 21 showing little-leaf and proliferation symptoms with the calcium salt of oxytetracycline (OTC) resulted in the production of plants without such symptoms (Table 2). Plants free of symptoms were produced by (i) soaking cuttings in OTC at 500 μg/ml for 48 hr; (ii) soaking cuttings in OTC at 100 μg/ml

for 48 hr followed by mist for 72 hr and a second OTC treatment for 72 hr; (iii) soaking cuttings in OTC at 250 μg/ml for 16 and 24 hr in the presence of 5% dimethylsulfoxide; and (iv) watering plants with OTC at 1,000 μg/ml for 6 weeks. The cured plants remained free of symptoms during a 2-year post-treatment observation period.

Electron microscopy.—Sieve elements in vascular tissue from leaves of P.I. 308200 contained mycoplasmalike bodies (Fig. 1). Bodies ranged in size from 100 to 1,000 nm. In many sieve cells, dense granules, assumed to be ribosomes, were incorporated in the bodies. In some cells, the bodies were bounded by a clearly defined unit membrane and were elongated and irregular in form (Fig. 2). Dense nuclear strands were not observed.

TABLE 2. Effect of the calcium salt of oxytetracycline (OTC) on the remission of little-leaf and proliferation symptoms in sweet potato

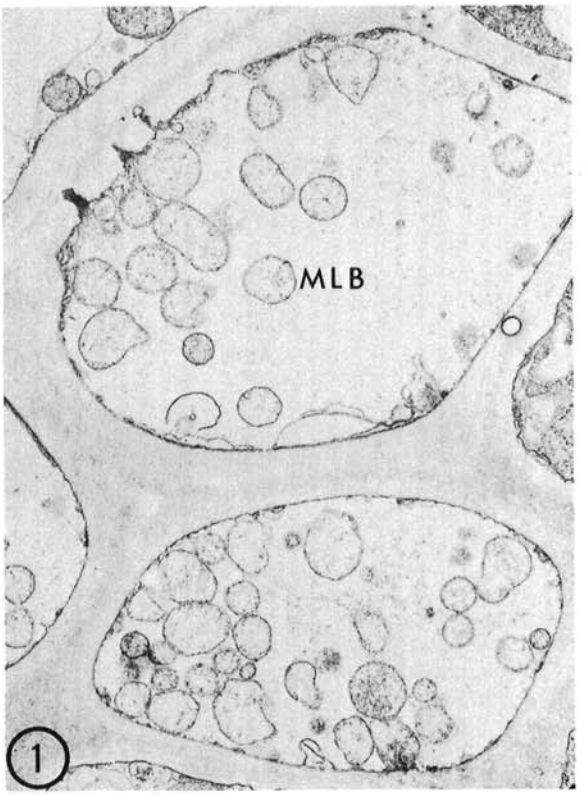
Infected source plants	OTC treatment	Propagation after treatment	No. cured/No. treated
Nonrooted cuttings			
P.I. 308200[a]	100 μg/ml, 48-hr soak	Grafted onto healthy P.I. 308200	0/5
	500 μg/ml, 48-hr soak	Grafted onto healthy P.I. 308200	4/5
	Tap water, 48-hr soak	Grafted onto healthy P.I. 308200	0/5
P.I. 308200	100 μg/ml, 48-hr soak, then mist for 72 hr, and 100 μg/ml for 72 hr	Rooted under mist	3/4
No. 21[b]	100 μg/ml, 48-hr soak, then mist for 72 hr, and 100 μg/ml for 72 hr	Rooted under mist	1/4
P.I. 308200	Tap water, 48-hr soak, then mist for 72 hr, and tap water for 72 hr	Rooted under mist	0/10
No. 21	Tap water, 48-hr soak, then mist for 72 hr, and tap water for 72 hr	Rooted under mist	0/10
P.I. 308200	250 μg/ml, no DMSO[c], 16-hr soak	Rooted under mist	0/4
	250 μg/ml, 5% DMSO, 16-hr soak	Rooted under mist	3/4
	Tap water, no DMSO, 16-hr soak	Rooted under mist	0/4
	Tap water, 5% DMSO, 16-hr soak	Rooted under mist	0/4
	250 μg/ml, no DMSO, 24-hr soak	Rooted under mist	0/4
	250 μg/ml, 5% DMSO, 24-hr soak	Rooted under mist	4/4
	Tap water, no DMSO, 24-hr soak	Rooted under mist	0/4
	Tap water, 5% DMSO, 24-hr soak	Rooted under mist	0/4
No. 21	250 μg/ml, no DMSO, 16-hr soak	Rooted under mist	0/4
	250 μg/ml, 5% DMSO, 16-hr soak	Rooted under mist	2/4
	Tap water, no DMSO, 16-hr soak	Rooted under mist	0/10
	Tap water, 5% DMSO, 16-hr soak	Rooted under mist	0/10
	250 μg/ml, no DMSO, 24-hr soak	Rooted under mist	0/4
	250 μg/ml, 5% DMSO, 24-hr soak	Rooted under mist	2/4
	Tap water, no DMSO, 24-hr soak	Rooted under mist	0/4
	Tap water, 5% DMSO, 24-hr soak	Rooted under mist	0/4
Intact plants			
P.I. 308200	1,000 μg/ml, foliar spray applied 3 to 5 times/week for 4 weeks		0/10
No. 21	1,000 μg/ml, foliar spray applied 3 to 5 times/week for 4 weeks		0/10
P.I. 308200	Plants watered with 100 μg/ml during 6-week period		2/3
	Plants watered with 500 μg/ml during 6-week period		0/3
	Plants watered with tap water		0/6
No. 21	Plants watered with 100 μg/ml during 6-week period		0/3
	Plants watered with 500 μg/ml during 6-week period		1/3
	Plants watered with tap water		0/6

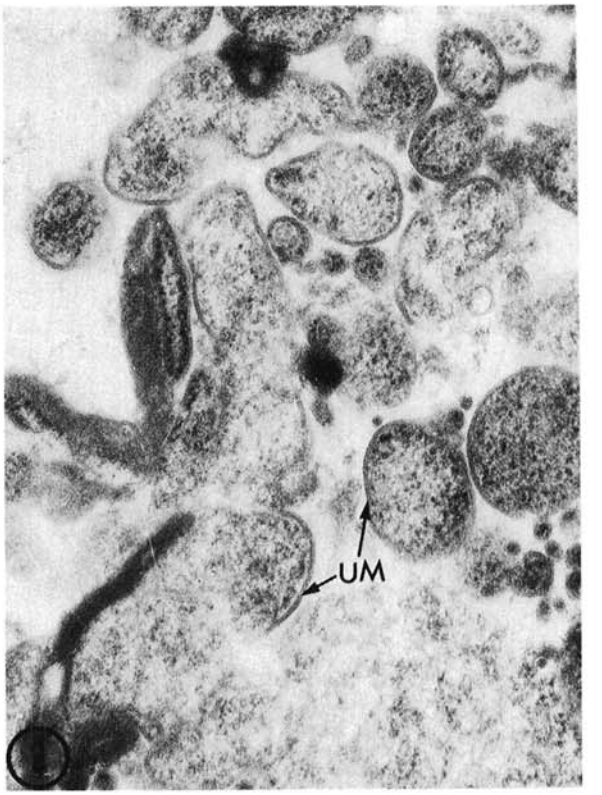
[a] *Ipomoea batatas* 'Nukua Loka', P.I. 308200, with little-leaf and proliferation symptoms (natural infection).

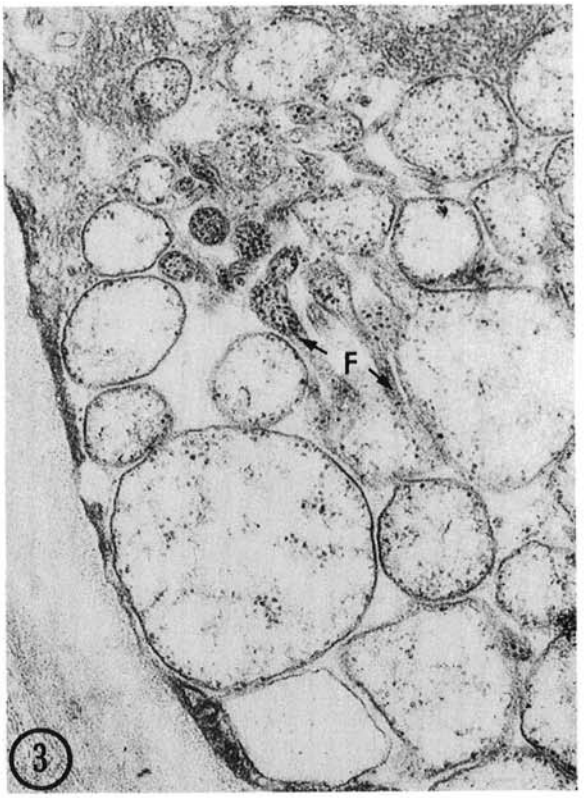
[b] USDA sweet potato seedling No. 21 with little-leaf and proliferation symptoms (infected as a result of graft transmission from P.I. 308200).

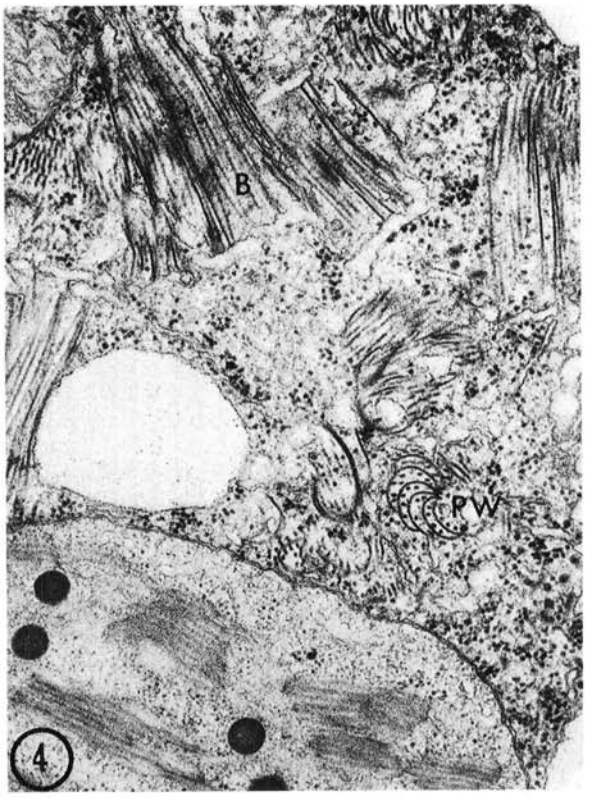
[c] DMSO = dimethylsulfoxide.

Fig. 1-4. Electron micrographs of ultrathin sections from *Ipomoea batatas* 'Nukua Loka', P.I. 308200, showing little-leaf and proliferation symptoms. **1)** Mycoplasmalike bodies (MLB) in the phloem (× 13,700). **2)** MLB with clearly defined unit membrane (UM) surrounding the bodies (× 63,100). **3)** Elongated or filamentous forms (F) of MLB (× 51,000). Note the increased electron transparency of the interior structure and the absence of a clearly defined UM. **4)** Bundle inclusions (B) and pinwheels (PW) in the cytoplasm of a parenchyma cell (× 38,500).


MLB
1


UM
2


F
3


B
PW
4

Other sieve cells contained pleomorphic bodies that were not so dense, and the bounding membrane did not show a well-defined trilaminar structure. There was a suggestion of elongated or filamentous forms (Fig. 3). In some cells, the bodies occurred in such high concentration that they completely filled the cell.

Bundle- and pinwheel-inclusions, similar to those observed for RCV from Jersey Orange, were observed in P.I. 308200 (Fig. 4). No evidence was found in thin sections for the existence of the 50 nm particle of internal cork virus (10).

Mycoplasmalike bodies were not observed in plants grown from heat-treated cuttings of P.I. 308200 that resumed normal growth. Pinwheel- and bundle-inclusions were absent in the heat-treated propagations that indexed negatively on *I. setosa* and Scarlett O'Hara, and no other inclusions or viruslike particles were present in these plants.

Mycoplasmalike bodies were present in phloem cells of USDA Seedling No. 21 showing little-leaf that was graft-inoculated with P.I. 308200. In serial section, the bodies changed shape only slightly in adjacent sections, but random examination of additional sections showed that the outline of the bodies changes, indicating a pleomorphic shape.

The plant developing from a cutting of No. 21 infected with the little-leaf agent and cured with two antibiotic soaks of 48 and 72 hr at 100 μg/ml was sampled 3 months after the cutting was treated. No mycoplasmalike bodies were observed in the phloem cells of the cured plant. A treated cutting of No. 21 that reverted to the little-leaf condition contained mycoplasmalike bodies in the phloem.

DISCUSSION.—Proliferation and little-leaf symptoms in P.I. 308200 and other sweet potato cultivars grafted with P.I. 308200 resemble the symptoms of sweet potato witches'-broom disease described in the Ryukyu Islands (11). We suggested that the pathogen in P.I. 308200 and the one in the Ryukyu Islands were probably the same, although we did not make direct comparisons (6). The disease agent in the Ryukyu Islands was reported to be a virus on the basis of leafhopper and graft transmission (9). Our data for P.I. 308200 shows that the little-leaf and proliferation symptoms in *I. batatas* P.I. 308200 were associated with a mycoplasmalike organism and not with any virus also present in P.I. 308200.

Mycoplasmalike organisms that are associated with other plants have most of the following characteristics in common: (i) transmission by leafhoppers and grafting, but not mechanically or by aphids; (ii) sensitivity to antibiotics of the tetracycline type; (iii) sensitivity to heat; (iv) presence of mycoplasmalike bodies in phloem cells; and (v) the production of yellowing, stunting, or proliferation symptoms. The agent that incites the little-leaf symptoms in sweet potato can also be characterized by all these criteria, with the exception of leafhopper transmission which was not tested. However, the vectors of the witches'-broom disease in the Ryukyu Islands are leafhopper species which are not known to occur in the United States (9). Proliferation and little-leaf symptoms in P.I. 308200 were eliminated by tetracycline treatment and by heat treatment at 38 to 39 C for at least 7 days. Mycoplasmalike bodies were observed in the sieve elements of P.I. 308200 and USDA Seedling No. 21 grafted with P.I. 308200, but not in No. 21 that had been cured, nor in nongraft inoculated No. 21.

Virus was also present in P.I. 308200 in addition to the mycoplasmalike organism, but the virus could not have incited the little-leaf and proliferation symptoms. Evidence for the presence of virus was transmission of an agent by aphids, or mechanically, that incited viruslike symptoms not of the proliferation type in *I. setosa* and Scarlett O'Hara, whereas the agent that incites little-leaf and proliferation symptoms was not aphid- or mechanically transmissible. On the basis of symptomatology, the aphid- or mechanically transmitted virus was RCV (1).

In addition to mode of transmission and tetracycline sensitivity, evidence that the virus in P.I. 308200 could not have incited the little-leaf and proliferation symptoms is as follows: (i) The incitant of little-leaf and proliferation symptoms is eliminated by heat therapy in as short as 6 days from a high percentage of cuttings and from an entire 1-node leaf cutting, whereas viruses usually require a much longer exposure, and the treatment usually produces a meristem (meristem plus one or more leaves) free of virus in a small percentage of cases. (ii) Sweet potato viruses isolated from 35 different imported sweet potato introductions showing russet crack, internal cork, and feathery mottle symptoms failed to incite little-leaf and proliferation symptoms when graft-inoculated to *I. setosa* and No. 21 (R. P. Kahn & R. L. Monroe, *unpublished data*). The agent that incites little-leaf and proliferation symptoms in P.I. 308200 does so in *I. setosa* and No. 21. (iii) In heat therapy tests (30 days at 38 to 39 C) of P.I. 308200 infected with the complex, one plant was found which was free of the proliferation agent but was still infected with a virus that could be mechanically transmitted to Scarlett O'Hara.

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