Insect Transmission of Sweet Potato Disease Agents in Nigeria

G. A. Schaefers and E. R. Terry

Visiting Entomologist (present address: N. Y. State Agric. Exp. Station, Geneva, N. Y. 14456) and Plant Pathologist, International Institute of Tropical Agriculture, PMB 5320, Ibadan, Nigeria, respectively.

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ABSTRACT

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Two infective agents have been separated from a sweet potato disease complex in Nigeria. One is a nonpersistent virus [a filamentous rod (850 nm)] which is transmitted most efficiently by the aphids *Myzus persicae* and *Aphis gossypii* and less efficiently by *A. citricola*. It is latent in sweet potato, but produces marked veinclearing and leaf twisting in *Ipomoea setosa*. Following infection, symptoms disappear after about eight new leaves are produced. The disease is referred to as sweet potato veinclearing. The second agent is transmitted by the whitefly, *Bemisia tabaci*. It also is latent in the sweet potato cultivars tested. In *I. setosa* it produces mild chlorosis and severe stunting of the plant. The disease is referred to as sweet potato chlorotic stunt. In combination, the two disease agents produce severe symptoms in sweet potato. Depending on the cultivar, veinclearing, puckering, leaf strapping, chlorosis, and stunting may occur. Dual infection in *I. setosa* results in stunting, severe chlorosis followed by necrosis of older infected leaves, and a severe reduction in leaf lamina.

Additional key words: transmission dependency, symptomless.

A wide range of "virus" disease symptoms have been reported from sweet potatoes, *Ipomoea batatas* L. wherever this crop is grown. In Nigeria, Hahn (4) reported that symptomless plants of a high-yielding cultivar produced about 43 metric tons of fresh yield and 10 tons of dry yield per hectare compared with 9 tons and 2 tons, respectively, from plants showing disease symptoms. Infection in another cultivar reduced fresh weight yield about 64 percent.

Robertson (7) described a veinclearing disease of sweet potato in Nigeria. He concluded that the symptoms resembled those reported from Ghana (3), and those produced by certain strains described from Israel (6) and East Africa (8), but found no evidence that they were related. Although whitefly- as well as aphid-transmissible components were reported from these areas, Robertson (7) was unable to demonstrate insect transmission in experiments with a "veinclearing" disease in Nigeria. Here, we report the results of insect transmission studies of a sweet potato disease with symptoms closely resembling those described by Roberton (7).

MATERIALS AND METHODS

Symptoms vary widely among the sweet potato lines maintained in the breeding program at the International Institute of Tropical Agriculture in Ibadan, Nigeria. Virus source material was selected from plants in the field that showed severe symptoms of various combinations of leaf strapping, veinclearing, puckering, and stunting. Hereafter, this disease syndrome will be referred to as the sweet potato virus disease (SPVD).

Test plants were obtained by growing seedlings from parental lines known to be sensitive to SPVD. The seedlings were then clonally propagated and their sensitivity was evaluated by grafting to infected tubers Although a number were selected, those most frequently used were TIS-2247 and TIS-2361 seedlings. All seedling material was grown under greenhouse and insecticide protection and was presumed to be virus free.

Observations from preliminary grafting experiments indicated that one of the most SPVD-sensitive sweet potato selections was TIB-10. About 100 cuttings of TIB-10 from field material were planted in the greenhouse. Although symptomless at the time of collection, about 15% of the cuttings exhibited SPVD symptoms during the first month in the greenhouse. Plants that exhibited symptoms were destroyed and during subsequent months only an occasional SPVD-infected plant was observed among the stock supply.

The Brazilian morning glory (*Ipomoea setosa* Ker.) was found useful by Stubbs and McLean (10) in their studies on sweet potato feathery mottle in the USA. Therefore, *I. setosa* seedlings at the first true leaf stage, also were used as virus indicators in the present study.

The vectors were those reported by Sheffield (8): the tobacco whitefly (*Bemisia tabaci* Genn.) and the green peach aphid (*Myzus persicae* Sulz.). The whitefly was maintained on mosaic-free cassava seedlings, and the aphid was maintained on cabbage (*Brassica oleracea* L.) or on radish (*Rhaphanus sativus* L.). Plastic pot cages and transfer aspirators used in the whitefly tests were similar to those described by Bird (1).

Standard procedures consisted of transferring adult whiteflies from the stock colony to a short plastic cage covering a single virus source plant. The short cage, approximately 15 cm high, topped with a piece of black plastic, forced the whiteflies into close proximity with the foliage and facilitated feeding. After a 24- to 48-hour access feeding period, the insects were released into a large, glass-topped sleeve cage where they were collected

and distributed in groups of 50-100 to individually caged test plants. After a 24-hour inoculation feed, they were released and the test plants were sprayed with methidathion.

In tests which involved aphids, adults were brushed from the stock plants into stender dishes and starved for periods ranging from 30 to 120 minutes. Single aphids were then transferred with a camel's hair brush to a young leaf on a source plant. Aphids were observed under magnification and removed approximately 30 seconds after they had assumed a probing attitude. The aphids were then transferred to test plants and allowed 24 hours of inoculation feeding, after which they were removed and the plants sprayed with malathion or methidathion.

All test plants were further isolated in fine-mesh cages in the greenhouse for at least 2 months to await symptom development. Temperatures were uncontrolled and ranged from 24-40 C in the greenhouse during the day.

RESULTS

Whitefly studies.—Whiteflies were transferred from various diseased source plants to 36 symptomless cuttings of field-collected TIB-10 sweet potato. Of these, 28 plants or 78% showed severe SPVD symptoms which included veinclearing, followed by some puckering, dwarfing, and rolling under of leaf edges. Ten plants, to which virus-free whiteflies from the stock colony were transferred for feeding (nontreated checks), did not develop disease symptoms. In most cases, symptoms developed in about 4 weeks, but actual times ranged from 19 to 32 days.

Attempts were made to transmit the whitefly-vectored agent from the above source plants to clonally propagated sweet potato seedlings. These included 12 different seedlings from five parental lines selected for their sensitivity to SPVD in the field. Of 29 test plants that received infective whiteflies, none showed disease symptoms.

Whiteflies successfully transmitted the agent from symptomatic TIB-10 plants to *I. setosa* seedlings in three out of four trials. Further, a single transmission to *I. setosa* resulted when an *I. setosa,* which had been grafted to a tuber from a field-infected plant, was used as a disease-agent. The agent was recovered and transmitted from the above test plants to TIB-10 sweet potato in five out of five trials. When the *I. setosa* test plants were held under conditions of reduced but continuous light, a temperature range of 27.8 - 34.5 C, and a relative humidity of about 80%, symptoms were observed in about 2 weeks. This consisted of stunting of growth, shortening of internodes, and a mild chlorosis (Fig. 1-C). After 3 weeks, the infected plants were approximately 20 cm in height, compared to about 75 cm for the nontreated checks. After 5 weeks, the average height of the infected plants was still about one-fourth that of the nontreated checks. No veinclearing or malformation of the leaf blades was noted. Based on the symptoms produced by this disease-agent in *I. setosa,* the whitefly-transmitted disease will hereafter be referred to as sweet potato chlorotic stunt (SPCS). No virus particles, however, were detected by electron microscopy of leaf-dip preparations from *I. setosa* with the SPCS agent.

Attempts were made to transmit the SPCS agent using only one or 10 insects after 1 hour of access feeding. Ten trials were conducted with each number of insect vectors and no transmissions resulted.

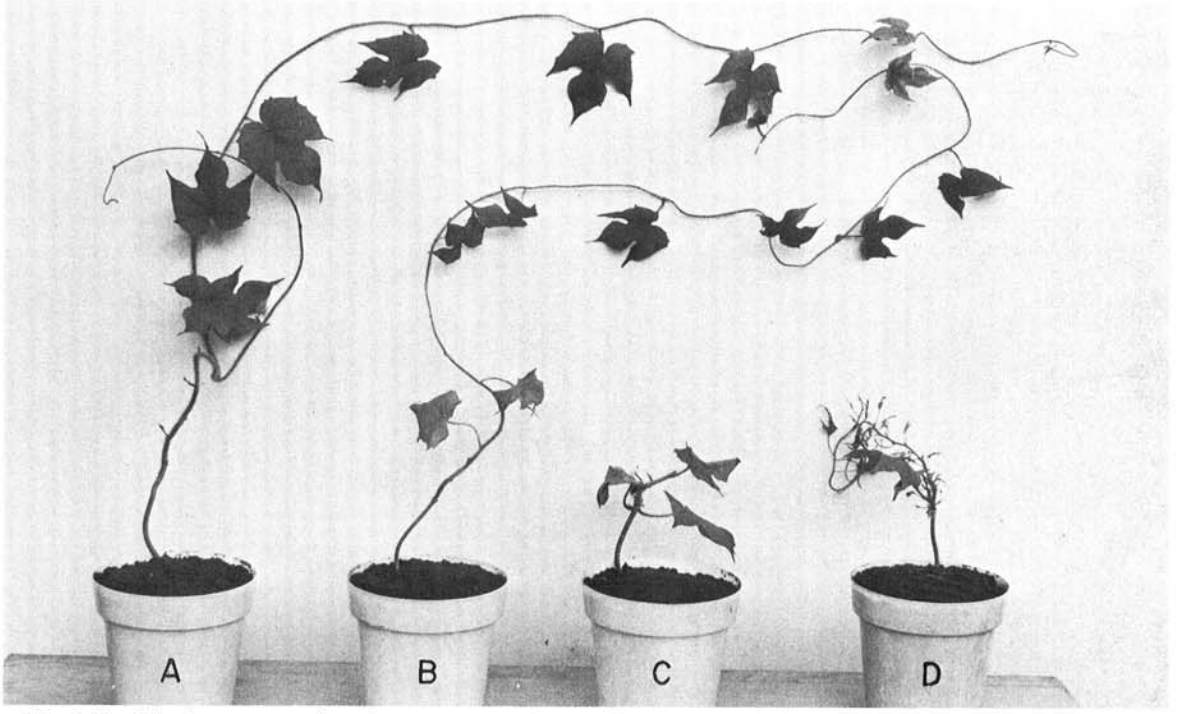

Fig. 1-(A to D). Sweet potato virus disease components in Brazilian morning glory, *Ipomoea setosa* Ker. **A)** nontreated check, **B)** sweet potato veinclearing (SPVCV), **C)** sweet potato chlorotic stunt agent (SPCS agent), and **D)** sweet potato virus disease (SPVD = SPVC + SPCS).

Aphid studies.—In tests that involved 10-50 *M. persicae* transferred from various source plants expressing SPVD symptoms to *I. setosa* seedlings, 22 of 24 test plants began to show symptoms in about 6 days. When infected *I. setosa* were grown under conditions as reported under whitefly studies, the first symptoms consisted of a net-like appearance of the leaves which resulted from clearing of most veins. In successive leaves there was a puckering and distortion of the leaf blade, reduction in leaf size, and restriction of veinclearing to mid- and secondary veins and finally to the mid-vein only (Fig. 1-B). No chlorosis was apparent and the plants were only slightly stunted. Symptoms were transient in this host; postinfection growth returned to a symptomless state after about 4 weeks or at about the eighth true leaf after infection. On the basis of the symptom expressed in *I. setosa,* the aphid-transmitted agent of SPVC will hereafter be referred to as sweet potato veinclearing virus (SPVCV). Filamentous rods that measured 850 nm were detected by electron microscopy of leaf-dip preparations of *I. setosa* infected with SPVCV.

In a further test, adult cotton aphids, *Aphis gossypii* Glover, were collected from *I. tuberosa* L. and given the standard starvation treatment. As a nontreated check, 15 aphids were selected at random from the petri dish and placed directly on a *I. setosa* seedling. Of the remaining aphids, 15 were given access feeds on an SPVCV-infected *I. setosa* plant and then transferred to a healthy *I. setosa* seedling. All five test plants developed SPVC symptoms; the nontreated check plants remained healthy.

The above test was duplicated using the *A. citricola* van der Goot, collected from *Citrus reticulata* Blanco. Of five *I. setosa* seedlings infested with aphids from the SPVCV source plant, one developed symptoms characteristic of SPVC. The nontreated check plant remained healthy. Because of the low transmission rate, the test was repeated. In the second test, two of the five test plants developed SPVC symptoms.

Investigations on virus persistence were conducted using single adults of *M. persicae.* In each test, the aphid was given a 30-second access feeding on an SPVD-infected TIB-10 sweet potato and then serially transferred to five *I. setosa* seedlings. The aphid was permitted a 5-minute inoculation feed on the first plant, a 30-minute feed on each of the next three plants, and 60 minutes on the fifth plant. The five-plant series was replicated 14 times and SPVC symptoms appeared in seven of the replicates. In six of seven cases, symptoms appeared only in the initial plant which received the 5-minute inoculation feed. In one case, both the 5-minute plants and the first of the 30-minute inoculated plants exhibited symptoms.

Dual infection.—Tests were conducted to study the effects of dual infection by the SPCS agent and SPVCV (*M. persicae*-vectored) on symptom development in sweet potato seedlings. The disease-agent source consisted of sweet potato showing SPVD in all these tests except one in which the SPVD source consisted of infected *I. setosa.* In three of these tests, SPVD symptoms appeared only in those test plants that received both SPVC and SPCS components. Those seedlings that received only SPVCV or the SPCS agent components, as well as 15 nontreated check plants, remained symptomless.

A similar experiment was conducted using *I. setosa* as test plants. The source plant consisted of a single TIB-10 sweet potato exhibiting SPVD symptoms. The symptom expression of the *I. setosa* test plants in this test were consistent with those produced on sweet potato. In addition, there occurred a pronounced strapping and reduction in size of the leaf blades and the older leaves became necrotic. The symptoms of the plants with either SPVC or SPCS were persistent in successive growth.

Virus recovery from latent infections.—Initial efforts to recover a virus from symptomless sweet potato seedlings, which were previously exposed to viruliferous aphids only, were unsuccessful. Aphid recovery of the SPVC component from sweet potatoes showing SPVD symptoms was accomplished with ease, however.

Results from whitefly studies indicated that a high percentage of the greenhouse population of TIB-10 cuttings had a latent infection of SPVCV. Five of the above 10 TIB-10 series were used in further tests. Indirect evidence for the presence of SPVCV in the five selected series was obtained by stem graft. One plant from each of the five series was used as the stock. The scions were obtained from sweet potato seedlings, which were symptomless following their exposure to the infective whitefly-borne agent. Of four successful graft unions, three developed SPVD symptoms which indicated dual infection. When virus-free scions were grafted to representatives of the five-plant series, 0/4 successful graft unions developed disease symptoms.

TIB-10 plants showing SPVD symptoms from previous tests were used as SPVCV source plants. Thirty *M. persicae* were given access feeds on each of the 10 source plants and then transferred to *I. setosa* seedlings. Five of five test plants that received aphids from the SPVD source developed SPVC symptoms.

Cuttings were made from another five test plants of the TIB-10 series and exposed to the whitefly-borne agent of SPCS and their respective basal sections were retained as nontreated checks. Three of the cuttings exposed to whitefly-borne agent developed SPVD symptoms, whereas their respective three basal sections remained symptomless. Thirty *M. persicae* were then given access feeds on each of the latter six plants and then transferred to *I. setosa* seedlings. Typical SPVC symptoms developed on the three test plants, which received aphids from the TIB-10 source plants that showed SPVD symptoms, but no symptoms developed on those that received aphids from their respective symptomless basal sections. All six source plants were reindexed to *I. setosa* with identical results.

DISCUSSION

The above results suggest either that TIB-10 sweet potato was particularly sensitive to the SPCS agent or that it contained a latent virus, which is part of a disease complex manifested by severe symptoms observed in field plantings. The existence of an aphid-transmitted component was discovered when adult *M. persicae* were fed on sweet potatoes showing severe symptoms and then transferred to *I. setosa.*

It was shown that the aphid-vectored SPVCV, as well as whitefly-vectored SPCS agent, was latent in sweet potato seedlings, at least under our greenhouse conditions. When both of these agents were added in

combination, however, severe symptoms of the sweet potato virus disease complex resulted. This finding suggests that infection of the single sweet potato seedling, which developed severe symptoms following the addition of SPVCV as reported above, probably resulted from accidental infection with the whitefly-borne SPCS agent. The appearance of severe symptoms in field-collected TIB-10 cuttings following the addition of the SPCS agent suggested that these plants are carriers of latent SPVCV in the field.

Further tests showed that when sweet potato cuttings were made from a single plant systemically infected with SPVCV, the virus could not be acquired and transmitted by *M. persicae* unless the source plant also contained the SPCS agent. The occurrence of such transmission dependency is not unusual and several such cases have been discussed by Carter (2) under the heading of "complex transmissions". The practical implications of such a dependency; i.e. the value of rogueing only obviously diseased plants and the futility of using aphids to index cultivar material, await further investigation. Further, to resolve the question of TIB-10 sensitivity to the SPCS agent alone, it will be necessary to use alternative methods for determining the presence of SPVCV. Preliminary investigations involving whitefly recovery of the SPCS agent from latent infections in sweet potato suggest that coincident infection of the source plant with SPVC is not a prerequisite; but in the absence of SPVCV, the SPCS agent is acquired by the vector with greater difficulty.

Relationships between SPVC and SPCS and those sweet potato diseases reported elsewhere (3, 5, 6, 8) remain obscure. Variations in technique and in symptom development in different sweet potato cultivars has led to confusion in the literature.

In general, the symptoms described by Robertson (7) in Nigeria resemble those of SPVD reported here. He failed to get infection with either *B. tabaci* or with *A. gossypii,* but he did not report attempts with dual infections.

Disease symptoms on sweet potato in Ghana (3) again resemble those reported for SPVD here except that "small bright yellow spots" have not been consistently observed on the lamina of our test cultivars. Clerk (3) used "small healthy rooted cuttings" as test plants for his successful disease transmission with whitefly, but the existence of a latent aphid-transmitted component in his test plants cannot be discounted. The incubation period of 5-6 weeks in Ghana approximates that found in Nigeria.

Loebenstein and Harpaz (6) reported a whitefly-transmitted veinclearing disease and an aphid-transmitted ringspot disease on sweet potato in Israel. Their sweet potato test plant material was derived from meristematic tips and was repeatedly checked for virus content by various methods. Symptoms of their veinclearing disease differs from the latent whitefly-borne component in Nigeria and also from SPVD by the presence of chlorotic specks and a later brilliant mosaic, but this may be due to cultivar differences. The aphid-transmitted component in Israel produced ringspot symptoms on the cultivar Gokoku, but it was latent in other cultivars. We have not consistently observed ringspot or any symptoms among our test cultivars which were infected with SPVCV.

Sheffield (8) reported the presence of *B. tabaci*- and *M. persicae*-transmitted components of a sweet potato disease in East Africa. These were named virus "B" and virus "A", respectively. Test plants included sweet potato seedlings (8) and a wide range of alternate host plants (9). Virus "A" was rare in occurrence and produced a mild mottle or chlorotic spots on sweet potato seedlings in 3-4 weeks. Much more severe symptoms resulted when virus "A" was combined with virus "B", and it was of the nonpersistent type relationship. It differs from SPVCV in Nigeria in that it does not cause veinclearing in alternate hosts, including other *Ipomoea* species, it expresses mild symptoms in sweet potato, and it can be recovered from symptomless sweet potatoes by aphids.

The mild symptoms described and pictured for virus "B" in East Africa by Sheffield (8) resemble the early symptoms observed with dual infections in Nigeria. Her "severe" strain resembles an advanced stage of infection in some of our cultivars infected with both SPVCV and the SPCS agent. The long incubation period of virus "B" (i.e., 4-6 months) differs markedly from that reported here for SPVDV as well as from those reported from Ghana (3) and Israel (6).

The relationships between the SPVD components in Nigeria, those discussed above, and the feathery mottle components (5) in the United States require further investigation. However, the evidence that the aphid-transmitted component is a filamentous rod should confirm that at least the SPVCV component of the sweet potato disease is induced by a virus. No evidence is available that the SPCS agent is a virus other than symptomatology. Because of the symptom differences among sweet potato cultivars, it is suggested that the indicator plant *I. setosa* should be utilized in future investigations.

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