Preliminary Studies on a Virus Disease of a Sapogenin-Producing *Dioscorea* Species in Puerto Rico

Earl G. Ruppel, Herminio Delpín, and Franklin W. Martin[1]

INTRODUCTION

Tubers of several *Dioscorea* species constitute one of the major sources of the sapogenin, diosgenin, which is used for the commercial synthesis of valuable therapeutic compounds such as cortisone and progesterone (*4*).[2] Studies are being conducted at the Federal Experiment Station in Mayagüez, P.R., to increase the diosgenin content of these species through hybridization and selection.

Few serious diseases have been observed during the course of these studies. However, a survey of 227 *Dioscorea* plants in a field at Las Mesas, Mayagüez, P.R., in December 1963 revealed five distinct viruslike symptoms in about 10 percent of the plants. The two predominant symptoms included a nondescript mosaic, and a "green-banding" one. The symptoms of the latter disease, by far the most common, consisted of a dark-green banding of the main veins of the leaves, while the interlaminate areas were light yellowish-green (fig. 1). Since the planting consisted of several *Dioscorea* species and hybrids, it was not evident whether the various symptoms were the results of more than one virus, or of differential responses of the plants.

A second disease survey of the same *Dioscorea* plants in autumn 1964 revealed a disease incidence of more than 95 percent. All the symptoms observed in 1963 were found again; however, the green-banding symptom greatly predominated. The marked increase in disease incidence precipitated the following studies on the nature and spread of the viruslike diseases. Attention was concentrated on the green-banding disease. *In vivo* therapy measures were also investigated.

METHODS AND RESULTS

MECHANICAL TRANSMISSION BY RUBBING

We attempted to determine whether the different viruslike symptoms observed in the field could be transmitted by the usual rubbing technique.

[1] Plant Pathologist (present address: Univ. Ariz. Agr. Exp. Sta., Mesa, Ariz.), Agronomist, and Plant Geneticist, respectively, USDA, Agricultural Research Service, Crops Research Division, Federal Experiment Station, Mayagüez, P.R.

[2] Numbers in parenthesis refer to Literature Cited, p. 157.

We also made cross-inoculations of *Dioscorea composita* Hemsl., *D. floribunda* Mart. & Gal., and *D. spiculiflora* Hemsl. to ascertain whether one or more viruses were responsible for the various symptoms in the field. We prepared inoculum of each symptom-type by macerating leaves of *Dioscorea* species or hybrids with a mortar and pestle in 0.05-mol phosphate buffer (pH 7.0) containing 0.01-mol cysteine hydrochloride (5 gm. tissue

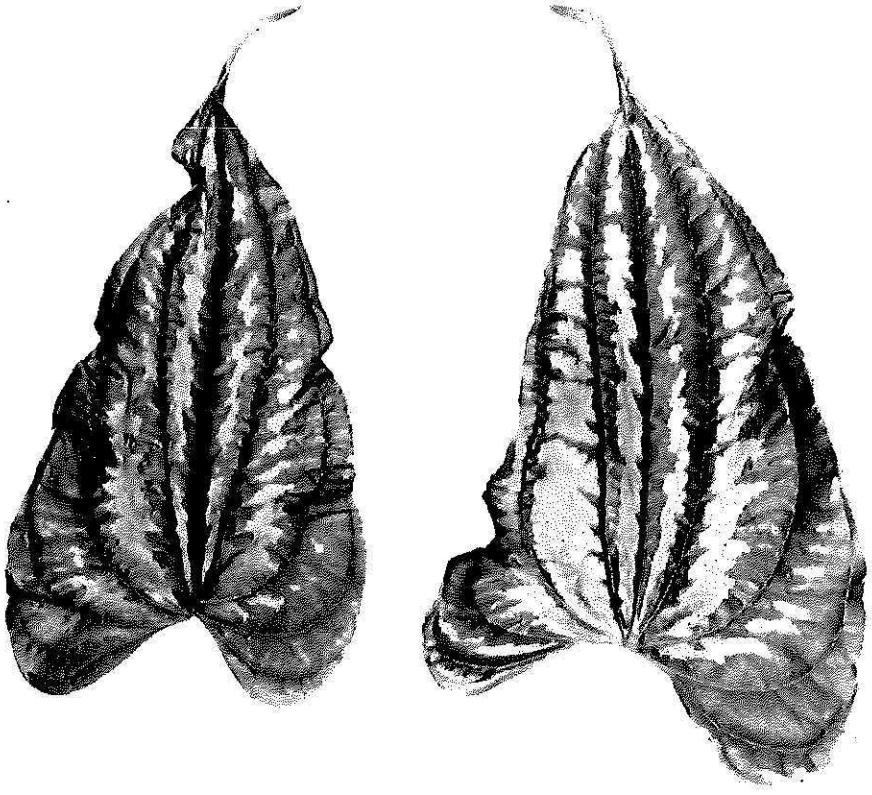

FIG. 1.—Leaves of *Dioscorea composita* naturally infected with the green-banding virus. Leaves of healthy plants are uniformly green.

per 1 ml. buffer). Each inoculum was rubbed onto the carborundum-dusted leaves of three plants of each species with small cheesecloth pads. An additional carborundum-dusted plant of each species was rubbed with buffer-cysteine hydrochloride solution for controls.

One plant of *D. composita* and one of *D. floribunda* rubbed with inoculum from mosaic-diseased plants developed green-banding symptoms. One plant of *D. composita* and two of *D. floribunda*, when rubbed with inoculum from plants exhibiting the green-banding symptom, developed the green-banding disease. Transmission of the other virus-like diseases mechanically

by sap inoculation was unsuccessful. The results indicate that the mosaic and green-banding symptoms were induced by the same virus, which was named the *Dioscorea* green-banding virus (DGBV).

Transmission of DGBV in three additional tests with inoculum from infected *D. composita* plants was low and erratic. In one test, only one of five inoculated plants of *D. composita* became infected, and only one of five inoculated *D. floribunda* plants was infected in a second test. None of five *D. composita* plants became infected in a third test.

TRANSMISSION BY CUTTING KNIFE

Mechanical transmission of DGBV was difficult by the usual rubbing technique. Nevertheless, some success was obtained. Consequently, we attempted transmission of the virus by a cutting-knife, one of the usual methods of tuber propagation in the field. To test such transmission, 10 DGBV-infected tubers of *D. composita* were cut in half with a knife. Then 10 healthy greenhouse-grown tubers were cut with the same knife. All tuber halves were planted in individual pots and the developing vines were observed for virus symptoms. After 5 months we observed no evidence of virus transmission by means of the cutting-knife. Only vines from previously infected tubers exhibited virus symptoms.

TRANSMISSION BY TUBER-GRAFT

We removed tissue cylinders (8 × 5 mm.) from DGBV-infected tubers of *D. floribunda* with a sterile cork borer, and similar cylinders from healthy tubers. We inserted the cylinders from the diseased tubers into the healthy tubers, and those from the healthy tubers into the diseased tubers. We covered the wounds with anhydrous lanolin to prevent desiccation, and planted the tubers (five replications) in individual pots.

Only two of the five previously healthy tubers germinated, and these developed typical DGBV symptoms, which indicated successful transmission through the tuber-grafts.

HOST RANGE

Five plants each of *Capsicum annuum* L. 'California Wonder', *Crotalaria juncea* L., *C. striata* DC., *Cucumis sativus* L. 'PR 39', *Dioscorea floribunda*, *D. spiculiflora*, *Gomphrena globosa* L., *Lycopersicon esculentum* Mill. 'Atom', *Nicotiana glutinosa* L., *N. tabacum* L. 'Havana 38', *Phaseolus vulgaris* L., and *Vigna sinensis* (Torner) Savi. were dusted with carborundum and inoculated with sap extracted from DGBV-infected leaves of *D. composita*. We also inoculated healthy *D. composita* plants to check the viability of the inoculum. Two plants of each species, dusted with carborundum and rubbed with buffer, served as controls.

Only *C. striata*, *D. composita*, *D. floribunda*, and *N. glutinosa* developed symptoms. Inoculated plants of *C. striata* grew apparently unaffected for almost 30 days. Then terminal growth ceased and many axillary buds began to grow, especially in the upper half of the plant. Older leaves exhibited vein-yellowing or a mild mottling. Leaflets of the axillary shoots were reduced in size, malformed, and markedly curled downward at the margins. Inoculated *D. floribunda* plants, and the *D. composita* control plants, exhibited typical green-banding symptoms. Inoculated *N. glutinosa* plants grew slowly compared to the controls. After 30 days the leaves yellowed, wilted, and then became necrotic, beginning at the base of the plant and progressing upwards. Eventually, the plants died. The virus was recovered from infected *D. floribunda* and *N. glutinosa* plants by back-inoculation to *D. composita*.

Inoculated *N. tabacum* plants remained symptomless; however, the virus was recovered from this host upon back-inoculation to *D. composita*.

INSECT TRANSMISSION

The cotton aphid (*Aphis gossypii* Glov.)[3] often was observed infesting *Dioscorea* vines in the Las Mesas area. The rapid spread of the virus in the plantings also suggested insect dissemination. To determine whether the cotton aphid was a vector of DGBV, portions of aphid-infested healthy and diseased vines were collected in the field and placed on healthy *D. composita* plants in insectproof cages in the greenhouse. Approximately 20 aphids were placed on each plant in each of 4 replications. The aphids left the dying vines and infested the healthy plants. After 1 week all the aphids had died. No aphid multiplication occurred on the plants.

Two plants infested with aphids from diseased field plants developed DGBV symptoms within 30 days. All other plants remained healthy. These results suggested that some of the aphids transferred from diseased field plants were viruliferous, and were able to transmit DGBV to the test plants.

THERMAL THERAPY OF INFECTED TUBERS

Some viruses have been inactivated *in vivo* through high-temperature treatment of whole plants or plant parts (*2,6,7,8,9,10*). We attempted to inactivate DGBV within viable, infected tubers of *D. floribunda* by heating them in a water bath at 50°C. for 5, 10, 30, and 60 minutes. We heated four healthy and four infected tubers at each time interval and then immersed them in ice water for 5 minutes before planting in individual pots

[3] Identified by George W. Miskimen, Entomologist, USDA, Agricultural Research Service, Entomology Research Division, Federal Experiment Station, Mayagüez, P.R.

of sterilized soil. None of the thermal treatments inactivated the virus in the infected tubers.

In another experiment we placed six healthy and six DGBV-infected tubers of *D. floribunda* for 2 weeks in an incubator at 37°C. with a high relative humidity. We planted the tubers and observed the germinating vines for virus symptoms. Three healthy and three infected tubers germinated. All of the former produced healthy vines, while the latter produced vines with DGBV symptoms.

DISCUSSION

The green-banding virus of *Dioscorea* was extremely difficult to transmit mechanically by rubbing healthy plants with sap extracted from diseased plants. Cysteine hydrochloride, a strong reducing agent, was added to the inoculum to counteract the rapid oxidation that occurred when *Dioscorea* sap was extracted. Nevertheless, we rarely obtained more than 20-percent transmission from diseased to healthy *Dioscorea* plants. Consequently, only positive results in the host-range studies are considered significant.

The positive infection of *C. striata* may have special significance. This plant is a common weed growing in the Las Mesas area and in other parts of Puerto Rico. Further studies may reveal that this plant serves as a reservoir for the *Dioscorea* virus. This species may also prove useful as a source-plant for inoculum in future investigations.

The rearing of cotton aphids on caged plants in the greenhouse was not feasible. Thus, we could make only preliminary tests. The meager evidence obtained, however, suggests that aphids are responsible for the rapid spread of the virus in the field. More precise aphid-transmission studies are necessary to confirm the mode of virus dissemination.

Thermal-therapy treatments under the conditions of our study failed to inactivate the virus *in vivo*. However, the longer thermal periods tended to stimulate tuber germination. Only two of eight tubers (healthy and infected) sprouted after a 5-minute treatment; three of eight after 10 minutes; eight of eight after 30 minutes; and six of eight after 60 minutes. Thus, higher temperatures or longer durations could be used that may prove more effective in freeing tubers from the virus.

The difficulty in transmitting the virus by sap-inoculation prevented studies of physical properties of the virus. Consequently, it was impossible to compare our *Dioscorea* virus with that reported by Adsuar (*1*). However, he reported successful infection of *Cucumis sativus*, whereas our test with this species was negative. Since we were unable to germinate tubers of *D. rotundata* Poir, symptom comparisons between our isolate could not be made with Adsuar's virus, which produced a mosaic of this host. However, our isolate induced the green-banding symptom in all *Dioscorea* species

in the field, including *D. composita*, *D. floribunda*, *D. friedrichsthalii*, and *D. spiculiflora*. Nevertheless, the occasional induction of mosaic symptoms by our isolate may indicate that it is identical with the one reported by Adsuar.

Cook (*3*) reported a mosaic disease of *Dioscorea* sp. from Puerto Rico, and Deighton (*5*) described a similar disease from Africa. Since these investigators did not study the causal virus, comparisons with our isolate could not be made.

SUMMARY

Virus incidence increased from 10 to more than 95 percent in a field-planting of *Dioscorea* spp. between 1963 and 1964. The predominant symptom consisted of dark-green bands of tissue bordering the main veins, while the interlaminate areas of the leaves were yellowish-green. The virus was mechanically transmitted to *Dioscorea composita*, *D. floribunda*, *Crotalaria striata*, *Nicotiana glutinosa*, and *N. tabacum*. *N. tabacum* was a symptomless host. Symptoms observed on *D. friedrichsthalii* and *D. spiculiflora* in the field were similar to those observed on other *Dioscorea* spp. in the greenhouse. *C. striata*, a common weed in Puerto Rico, may serve as a reservoir for the virus. The virus was not transmitted with a tuber-cutting knife from diseased to healthy tubers, but transmission was effected through tuber-grafts. The virus could not be inactivated *in vivo* with high temperature treatments of the tubers. Preliminary evidence suggested that the virus is transmitted by the cotton aphid (*Aphis gossypii*).

RESUMEN

La incidencia de un virus que ataca el ñame aumentó de menos de 10 a más de 95 por ciento en una siembra de ñame (*Dioscorea* spp.) que se sembró en 1963 y se cosechó en 1964. El síntoma predominante consistió de franjas verde obscuras en el tejido próximo a las venas principales, mientras que las áreas interlaminadas de las hojas eran de un color verde-amarillento. El virus se transmitió mecánicamente a tubérculos de *Dioscorea composita*, *D. floribunda*, *C. striata*, *Nicotiana glutinosa* y *N. tabacum*. *N. tabacum* no reveló ningún síntoma del virus. Se observaron síntomas similares en *D. friedrichsthalii* y *D. spiculiflora*, en el campo, así como en otras especies de *Dioscorea* en el invernadero. *C. striata*, un yerbajo común en Puerto Rico, puede servir como fuente de contaminación del virus. El virus no se transmitió de los tubérculos enfermos a los sanos con la cuchilla que se usó para cortarlos, sino mediante la implantación de injertos a los tubérculos. El virus no se puede inactivar *in vivo* sometiendo los tubérculos a altas temperaturas. La evidencia preliminar pareció indicar que el virus lo transmite el pulgón del algodón (*Aphis gossypii*).

LITERATURE CITED

1. Adsuar, J., A mosaic disease of the yam, *Dioscorea rotundata*, in Puerto Rico. *J. Agr. Univ. P.R. 39*(3): 111–3, 1955.
2. ——, Susceptibility of some sugarcane varieties to the heat treatment used in the control of chlorotic streak, *J. Agr. Univ. P.R., 40*(1): 67–9, 1956.
3. Cook, M. T., Ann. Rept. Agr. Exp. Sta., Univ. P. R., Rio Piedras, 1935–36, pp. 39–46, 1937.
4. Correll, D. S., Schubert, B. G., Gentry, H. S., and Hawley, W. O., The search for plant precursors of cortisone, *Econ. Bot. 9*(4): 307–75, 1955.
5. Deighton, F. C., Rept. Dept. Agr., S. Leone, 1935, pp. 22–6, 1936.
6. Holmes, F. O., Rose mosaic cured by heat treatments, *Plant Dis. Reptr. 44*(1): 46–7, 1960.
7. Kassanis, B., Potato tubers freed from leaf-roll virus by heat, *Nature 164*: 881, 1949.
8. —— ——, Heat-therapy of virus-infected plants, *Ann. Appl. Biol. 41*(3): 470–4, 1954.
9. ——, Effects of changing temperature on plant virus diseases, *Advances in Virus Res. 4*: 221–42, 1957.
10. Kunkel, L. O., Heat treatments for the cure of yellows and other virus diseases of peach, *Phytopathology, 26*(9): 809–30, 1936.