Euphytica 21 (1972): 344–351

PANGOLA STUNT VIRUS STUDIED IN PANGOLAGRASS AND *DIGITARIA* HYBRIDS[1]

S. C. SCHANK, J. R. EDWARDSON, R. G. CHRISTIE and M. A. OVERMAN

Institute of Food and Agricultural Sciences, Department of Agronomy, University of Florida, Gainesville, Florida 32601, USA

Received 16 August 1971

SUMMARY

Two *Digitaria* hybrids were compared with pangolagrass, *Digitaria decumbens* STENT., using both the light and electron microscope. The purpose of the study was to develop a diagnostic method of screening breeding lines for resistance or tolerance to pangola stunt virus (PSV). Pangolagrass from Surinam and Guyana, naturally infected with PSV, was found to have occassional bundle sheath cells with very thick cell walls. One of the two hybrids, which appeared to be highly susceptible to PSV, also showed similar thick cell walls. It had spherical particles 70 nm in diameter, resembling PSV, maize rough dwarf virus (MRDV) and rice dwarf virus (RDV). Sieve element cells of the phloem were the primary site of particles and the probable site of virus replication. The second hybrid, an apparently resistant line, was found to be completely free of thick-walled bundle sheath cells, and no viruslike particles were found in the bundle sheath or phloem cells. The presence of thick-walled bundle sheath cells appears to offer an excellent diagnostic test for PSV.

INTRODUCTION

Pangolagrass is a clone derived from *Digitaria decumbens* STENT., PI 111110, which was collected in E. Transvaal, S. Africa in 1935. At present it is a very popular tropical forage grass which occupies millions of hectares throughout the world (HODGES et al., 1967; GARZA et al., 1970; DAVIES and HUTTON, 1970). Since pangolagrass is a sterile triploid which is propagated only by vegetative methods, all plantings of this cultivar represent the same genotype. Therefore, all pangolagrass would theoretically be susceptible to certain plant diseases, including virus infections, or to insect attack to the same degree if environmental conditions were stabilized.

A devastating disease of pangolagrass was carefully studied in Surinam (DIRVEN and VAN HOOF, 1961), and was named pangola stunt virus (PSV). This virus was found to be transmitted by the planthopper vector, *Sogata furcifera* HORVATH. PSV has also been suspected and descriptive symptoms reported from Guyana, Brazil, Peru, and Fiji. The descriptions given could be due to causes other than a viral infection, and therefore

[1] Journal Paper No 4036 from the Florida Agricultural Experiment Station, Gainesville, Florida. In cooperation with the Plant Virus Laboratory, Department of Plant Pathology, IFAS, University of Florida.

rapid presumptive test for the virus was needed. Electron microscopy (EM) methods were used on pangolagrass leaves infected with PSV collected in the field in Surinam (SCHANK and EDWARDSON, 1968). Spherical particles found within the leaves measured 70 nm in diameter and were observed in both free and crystalline states. Additional studies of PSV using the EM have been recently reported (KITAJIMA and COSTA, 1970). The morphology and size of the particles was similar to rice dwarf virus (SHIKATA, 1969), maize rough dwarf virus (GEROLA and BASSI, 1966), and an unknown virus in *Peregrinus maidis* ASHM. (HEROLD and MUNZ, 1967). Just recently, maize rough dwarf virus was found to be present in *Digitaria sanguinalis* (L.) SCOP. (LUISONI and CONTI, 1970). Symptoms expressed in *D. sanguinalis* are very similar to those found in pangolagrass infected with PSV.

Inasmuch as pangolagrass was known to be susceptible to PSV, introductions of digitgrass[2] from South Africa have been used in an interspecific hybridization program (SCHANK and TAN 1967; SCHANK, 1968). Thousands of new genotypes have been synthesized, and it was the purpose of this investigation to evaluate some of these newly bred digitgrasses for tolerance to the stunt virus. A rapid cytological technique was needed to establish whether a given genotype was susceptible to the virus. Further, correlation of such light microscope studies with EM work would be extremely helpful in evaluating breeding lines.

MATERIALS AND METHODS

Pangolagrass and other digitgrass samples were collected in Surinam, Guyana and Venezuela in December 1970. Infected plants showed characteristic stunting, purple discoloration at leaf margins, and a twisted blade of the first fully-expanded leaf. Normal plants did not show any of these characteristics. Epidermal strips, sections of young fully-expanded leaves, and apical shoot meristems were immediately killed and fixed in Karnovsky's fluid (KARNOVSKY, 1965) buffered to pH 7.2, or in 6.5% glutaraldehyde buffered with phosphate to pH 7.2. Later, the samples were prepared for light microscopy by embedding in cryoform and sectioning at 10 μm, using an International Cryostat. Sections for light microscopy were stained with luxol brilliant green and calcomine orange according to CHRISTIE (1967). Processing for EM included post-fixation in osmium tetroxide for 4 h, dehydration in ethanol, and embedding in Epon-Araldite. Thin sections were obtained with a Porter-Blum ultramicrotome, and stained with uranyl acetate for 2 h, followed by 15 min with lead citrate according to REYNOLDS (1963). Since collections were made in the field in several tropical countries over a 10-day period, it was not possible to control the temperature at time of fixation nor subsequently. Also, osmium post-fixation followed the glutaraldehyde treatment by several weeks.

In order to more precisely correlate light and EM images, thick sections (1 μm) of plastic embedded material were made on the ultramicrotome and stained with 1% Gentian Violet. When the desired tissue was observed with light microscopy, thin sections were then cut for EM observations.

[2] Digitgrass has been suggested by McCaleb and Hodges (1969) to be the common name for the genus *Digitaria*. We therefore use digitgrass to include all the perennial *Digitaria* introductions, hybrids, cultivars or varieties of this highly cross-pollinated genus.

Pangolagrass was used as the control in this experiment since both normal and infected materials of this cultivar were available. Infected samples were from the Ebini Research Station, Guyana and the Government Livestock Farm in Surinam. Virus-free samples were collected near Maracaibo, Venezuela, and from the University of Florida Agronomy Greenhouse. Two hybrid digitgrasses were examined, X 254–15, a line believed to be susceptible to PSV and X 125–1, believed to be resistant to the virus. Both had been established since December, 1969 in an area at Paramaribo, Surinam, where PSV is known to occur. The Surinam location had been chosen because pangolagrass was susceptible to PSV, and yet other digitgrass clones had shown a degree of resistance or tolerance to stunting symptoms (HUNKAR, pers. comm.). Hybrid × 254–15 was an intraspecific hybrid between *D. valida* STENT., USDA PI 299861, and *D. valida* STENT. USDA PI 299864. Hybrid × 125–1 utilized an unnamed *Digitaria* species, USDA PI 299892 as female parent and *D. pentzii* STENT. USDA PI 299748 as pollen parent. The first cross had a very susceptible male parent and the second cross had parents with possible PSV resistance.

RESULTS AND DISCUSSION

Pangolagrass and digitgrass hybrid × 254–15 had strong PSV symptomology, and were sampled in Surinam as the most likely plants to be infected with PSV. Table 1 summarizes the data obtained from light microscopic studies of leaf morphology of young fully-expanded leaves. The primary morphological feature observed in all infected samples was the presence of some bundle-sheath cells with extremely thick cell walls; whereas non-infected samples did not have this feature. Examples of the thickened cell wall are shown in both transverse and longitudinal sections (Fig. 1–3). When stained according to the classical cellulose-lignin test (JENSEN, 1962), the thickened cell wall was composed primarily of lignin, with concentric rings of cellulose. Normal bundle sheath cell walls were composed primarily of cellulose.

Sections for the light microscope were most easily handled with the cryostat or freezing microtome, but free-hand or paraffin techniques also were suitable on digitgrass tissue. Although the frequency of thick-walled cells is not as high as one might like to

Table 1. Frequency of thick-walled bundle sheath cells in pangolagrass, hybrid × 125–1, and hybrid × 254–15. Young fully expanded leaves were collected from three locations within each plot.

	Total number of bundle sheath cells	Number of thick-walled cells
Samples showing PSV symptoms		
Pangolagrass from Ebini, Guyana	24,656	135
Pangolagrass from Paramaribo, Surinam	13,520	79
Hybrid × 254–15 from Paramaribo, Surinam	6,320	36
Samples having normal growth characteristics		
Pangolagrass from Maracaibo, Venezuela	15,910	0
Pangolagrass from Gainesville, Florida	18,631	0
Hybrid × 125–1 from Paramaribo, Surinam	9,306	0

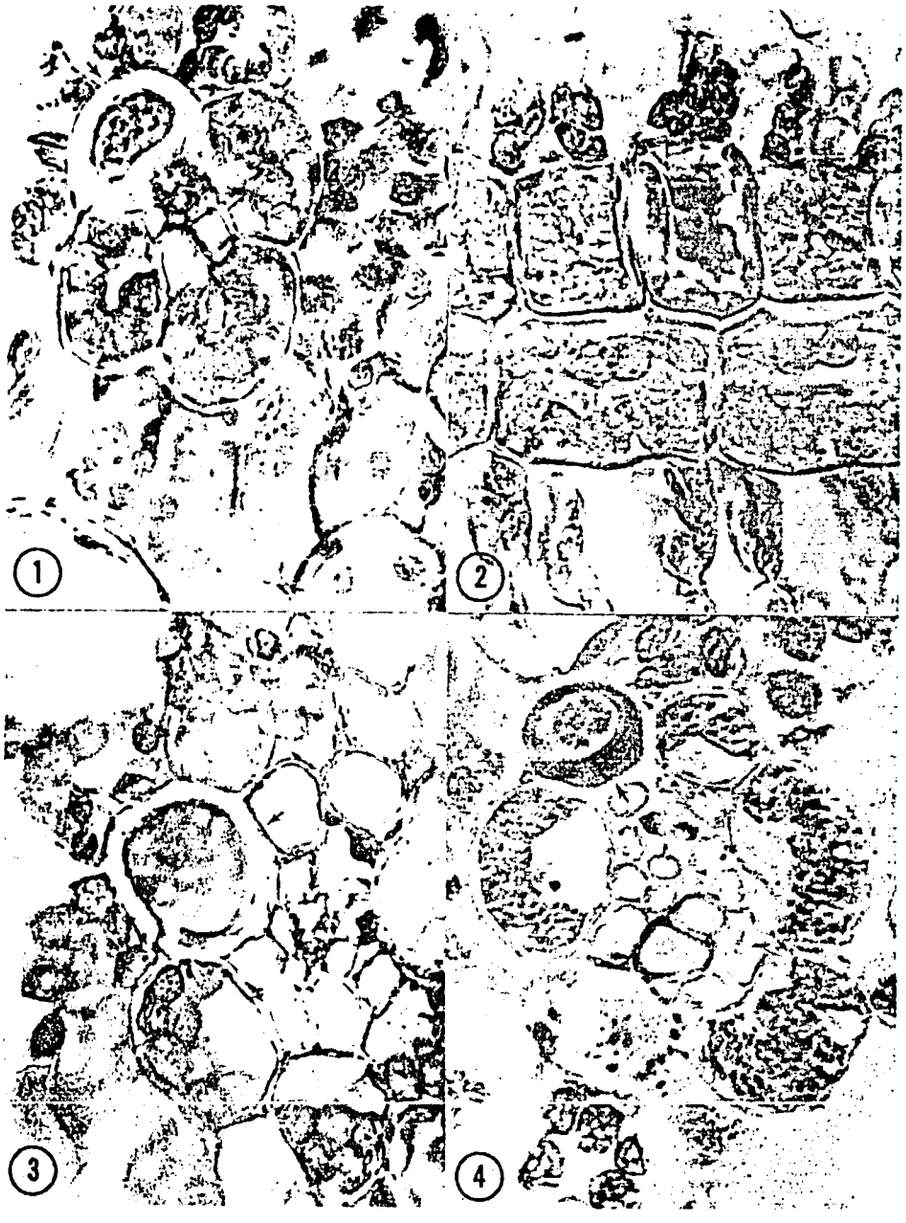

Fig. 1–4. Light micrography of cross and longitudinal sections of typical thick-walled cells in the leaf bundle sheaths of digitgrasses; Fig. 1 and 2. Pangolagrass from Ebini, Guyana, 10 μm; × 1000; Fig. 3. *Digitaria* hybrid 254–15, collected at Paramaribo, Surinam, 10 μm; × 1200. Fig. 4. Epon-Araldite embedded pangola leaf from Ebini, Guyana, 1 μm; × 1215.

obtain in a presumptive test, their striking morphology in the small vascular bundles, their presence in serial sections, and their occurrence in all infected plants is significant. Non-infected plants which had been stunted by drought and lack of fertilizer did not have thick walled bundle sheath cells.

Shown in Fig. 4 is a typical thick-walled cell in the bundle sheath of pangolagrass which had been embedded in plastic, cut at 1 μm, and stained with crystal violet. The same cell as it appears in the EM is shown in Fig. 5.

The electron microscope further demonstrated the laminar structure of the cell wall

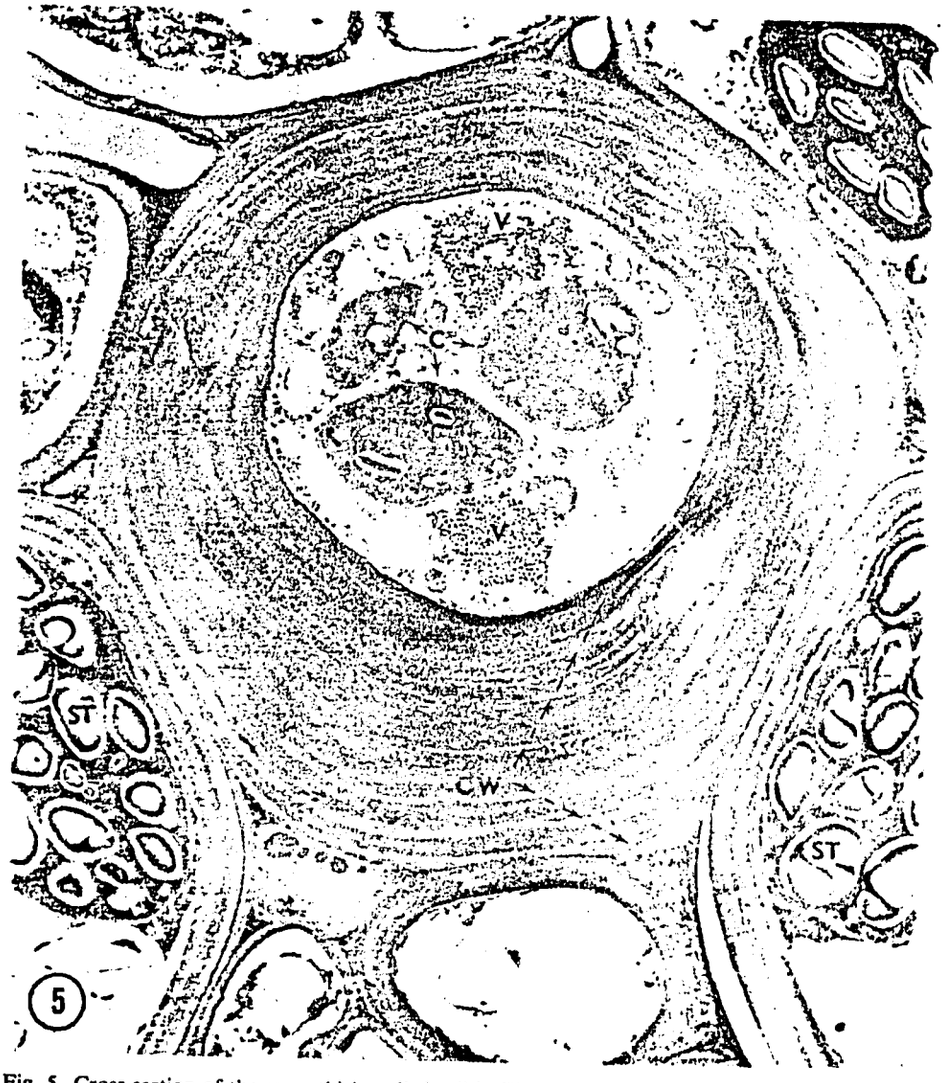

Fig. 5. Cross section of the same thick-walled cell in Fig. 4 as seen with the EM. Note presence of virus particles (V), degenerating chloroplasts (C), and cell wall (CW) layering. Starch grains (ST) are within chloroplasts of adjoining bundle sheath cells. × 8500.

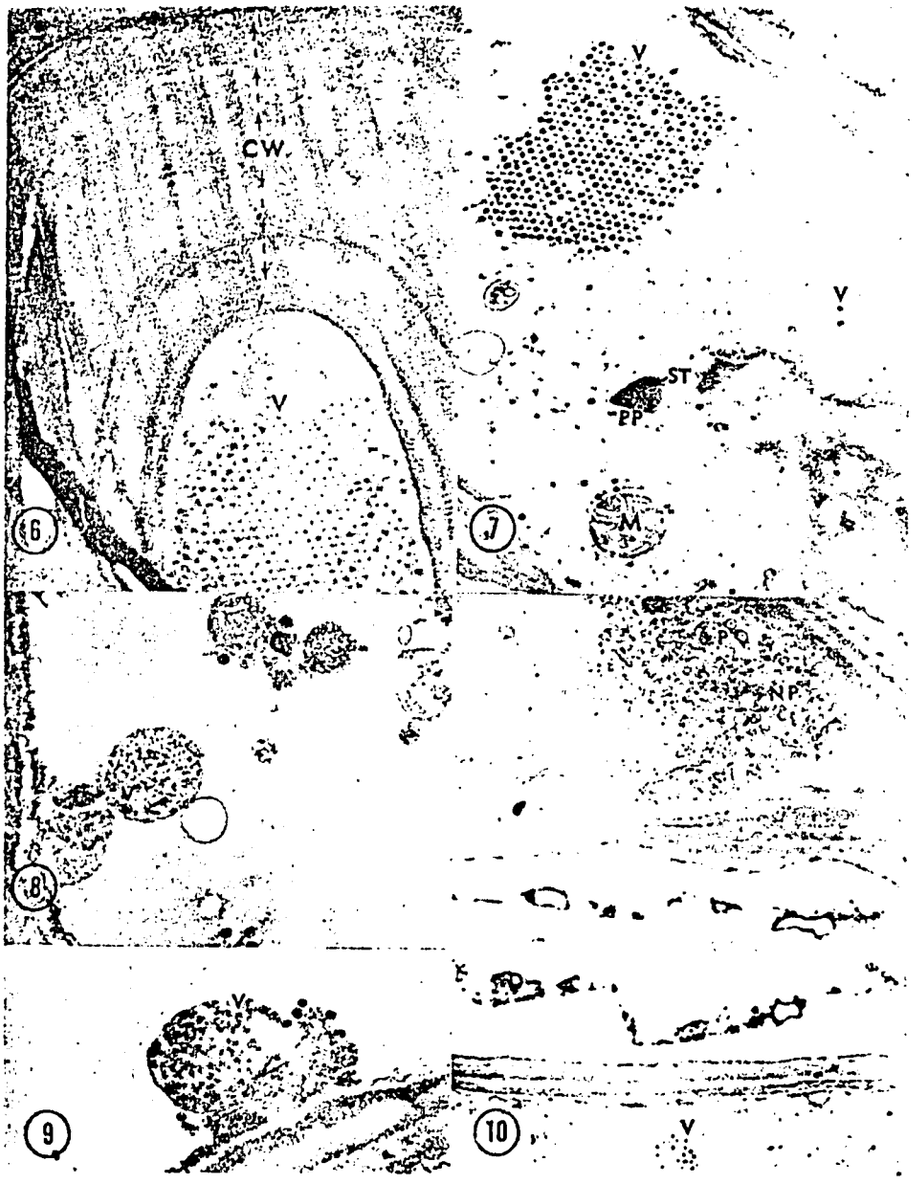

Fig. 6–10. Spherical virus particles within bundle sheath and phloem cells of pangolagrass and hybrid 254-25. Fig. 6. Pangolagrass leaf from Ebini, Guyana containing virus particles in orderly array; × 20,000; Fig. 7. Longitudinal section of leaf sieve tube cell showing aggregated and free virus particles (V), mitochondrion (M), phloem plastid (PP), and starch (ST); × 13,650; Fig. 8 and 9. Membranous structures within sieve tube cells containing viral particles; × 20,000; Fig. 10. Comparison of spherical virus (V), nuclear pores (NP), and plasmadesmata (P) within phloem tissue of hybrid 254-15; × 8200.

and confirmed that spherical viruslike particles of 70 nm diameter were present in the thick-walled cells (Fig. 5). In these bundle sheath cells, the chloroplasts were degenerate and starch grains were reduced in size. Although the layered deposition of cell-wall material is an unusual response to virus infection, the phenomenon is not unknown in certain healthy specialized plant tissues. For example, SRIVASTAVA and O'BRIEN (1966) have shown a layered deposition of cell wall in *Pinus strobus* L., and the concentric growth rings of cotton fibres (*Gossypium*) are deposited on a daily growth basis (KERR, 1937). Several of the festucoid grasses also have thickened cell walls near leaf vascular tissue (GOULD, 1968). The layering of the cell wall and presence of a virus crystal are shown in the pangolagrass bundle sheath cell from Ebini, Guyana. This is the first verification by electron microscopy of the presence of PSV in Guyana (Fig. 6).

Mature sieve element cells were found to be the most prevalent site of the viruslike particles. This would be expected in a phloem transmitted virus, and we agree with JENSEN (1969) that longitudinal sections of phloem allow most readily the detection of virus particles. Since cytoplasm is reduced in quantity in the enucleate sieve element cells, the usual cell organelles are fewer in number and particles are easily observed. The virus particles are often aggregated as shown in Fig. 7, or occur as isolated particles, often near mitochondria.

Replication of the virus is believed to take place in the sieve element cells within membranous structures shown in Fig. 8 and 9. The membranous structures appeared to rupture and fully mature virus particles were then seen within the sieve element cells. Virus crystals were regularly observed in these specialized cells, but never near the sieve plate. The virus probably does not stunt the plant by plugging the phloem, but instead simply uses the high energy metabolites for its own replication. SHIKATA et al. (1964) reported that clusters of rice stunt virus occur inside sacs or surrounded by membrane-like envelopes. They believed the virus had entered and multiplied or accumulated in mitochondria or some other defined structure of the cell similar in size to mitochondira.

The relative sizes of the spherical virus, nuclear pores, and plasmodesmata cut in cross section are clearly shown in Fig. 10. The protoplast in the center phloem cell appears to have degenerated.

Spherical particles believed to be PSV were regularly found in phloem tissue of infected pangolagrass and of hybrid × 254–15. Bundle sheath cells possessing thick cell walls also contained such particles. Hybrid × 125-1 appeared to be free of viruslike particles in the phloem and had no bundle sheath cells with thickened walls. No stunting symptoms of × 125–1 were observed in the field.

Additional breeding lines of digitgrass will be analyzed for thick cell walls to further document the occurrence of this unusual phenomenom in PSV-infected plants.

ACKNOWLEDGMENT

Appreciation is extended to Dr A. R. Tjong A Hung, Paramaribo, Surinam and Mr Noel Holder, Ebini, Guyana, for providing plant materials for analysis. We also thank Drs H. E. Warmke and Dan E. Purcifull of the Plant Virus Laboratory for their help and cooperation. This research was partially supported by a National Institutes of Health Biomedical Sciences grant.

REFERENCES

CHRISTIE, R. G., 1967. Rapid staining procedures for differentiating plant virus inclusions in epidermal strips. Virology 31:268–271.

DAVIES, J. G. & HUTTON. E. M., 1970. Tropical and sub-tropical pasture species. In: R. M. Moore, (Ed.), Australian grassland, p.273–302. Australian National University Press, Canberra.

DIRVEN, J. G. P. & VAN HOOF, H. A., 1961. A destructive disease of pangolagrass. Tijdschr. PlZiekt. 66:344–349.

GARZA, R. T., ARROYO, D. & PEREZ, A. S., 1970. Produccion de carne con los zacates pangola y jaragua fertilizados en el tropico Aw. Téc. Pecuar. 14:20–24.

GEROLA, F. M. & BASSI, M., 1966. An electron microscopy study of leaf vein tumours from maize plants experimentally infected with maize rough dwarf virus. Caryologia 19:13–40.

GOULD, F. W., 1968. Grass systematics. McGraw Hill, New York, pp.382.

HEROLD, F. & MUNZ, K., 1967. Virus particles in apparently healthy *Peregrinus maidis*. J. Virol. 1: 1028–1036.

JENSEN, S. G., 1969. Occurrence of virus particles in the phloem tissue of BYDV-infected barley. Virology 38:83–91.

JENSEN, W. A., 1962. Botanical histochemistry: principles and practices. Freeman, San Francisco, pp.408.

KARNOVSKY, M. J., 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol. 27:137A.

KERR, T., 1937. The structure of the growth rings in the secondary wall of the cotton hair. Protoplasma 27:229–241.

KITAJIMA, E. W. & COSTA, A. S., 1970. In: P. Favard (Ed.), Microscopie electronique. Vol. III, pp.323–324.

LUISONI, E. & CONTI, M., 1970. *Digitaria sanguinalis* (L.) SCOP., a new natural host of maize rough dwarf virus. Phytopathol. Mediterranea 9:102–105.

MCCALEB, J. E. & HODGES, E. M., 1969. Slenderstem digitgrass. Circ. S-201. Fla agric. Exp. Stn 11pp.

REYNOLDS, E. S., 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17:208–212.

SCHANK, S. C. & TAN, N. V., 1964. Improvement of forage grasses by interspecific hybridization within the genus *Digitaria*. Proc. 61st a. Conv. Ass. s. Agric. Workers. 62:63.

SCHANK, S. C. & EDWARDSON, J. R., 1968. Cytological examination of pangolagrass (*Digitaria decumbens* STENT.) infected with stunt virus. Crop Sci. 8:118–119.

SCHANK, S. C., 1968. Breeding grasses for resistance to pangolagrass stunt virus. Sunshine State agric. Res. Rep. 13:5–7.

SHIKATA, E., 1969. Electron microscopic studies of rice viruses. p.223–240. In: The virus diseases of the rice plant. John Hopkins Press, Baltimore.

SHIKATA, E., ORENSKI, S. W., HIRUMI, H., MITSUHASHI, J., & MARAMOROSCH, K., 1964. Electron micrographs of wound-tumor virus in an animal host and in a plant tumor. Virology 23:441–444.

SRIVASTAVA, L. M. & O'BRIEN, T. P., 1966. On the ultrastructure of cambium and its vascular derivatives. II. Secondary phloem of *Pinus strobus* L. Protoplasma 61:277–293.