# BANANA STREAK BADNAVIRUS INFECTION IN *MUSA*: EPIDEMIOLOGY, DIAGNOSIS AND CONTROL

B.E. Lockhart
Department of Plant Pathology,
University of Minnesota, St. Paul,
MN 55108, U.S.A.

*ABSTRACT*

*Viral leaf streak of banana, caused by banana streak virus (BSV) infection, is a recently-described disease which occurs in most banana-producing countries. The disease, which is known to affect only Musa and Ensete, is spread by vegetative propagation, mealybugs and through seed. BSV infection in banana can adversely affect yield and fruit quality. Isolates of the virus differ in symptoms produced, and are serologically and genomically heterogeneous. This has led to problems in developing reliable indexing methods for virus identification and disease control. Integration of segments of viral genome into Musa nuclear DNA may limit the usefulness of PCR amplification for BSV detection in Musa.*

## INTRODUCTION

Banana streak badnavirus (BSV) is the causal agent of viral leaf streak of banana and plantain (*Musa* sp.). The symptoms of viral leaf streak consist of broken or continuous streaks which vary in color from yellow to brown to black. Isolates of BSV differ widely in the severity of symptoms produced. Symptoms may vary from faint broken chlorotic lines to necrosis of emerging leaves, internal necrosis of the pseudostem and plant death (Figs. 1-4). Symptoms induced by BSV infection may sometimes resemble those caused by cucumber mosaic virus (CMV) (Figs. 5-6), and there is evidence that some earlier reports of virus infection in *Musa* incorrectly attributed the cause to CMV rather than to BSV.

### Distribution of BSV in *Musa*-Producing Countries

Viral leaf streak of banana was first described in Ivory Coast, Africa in 1968 (Lassoudiere 1974), and the causal agent, BSV, was identified in Morocco in 1985 (Lockhart 1985). The disease is probably distributed worldwide in cultivated banana and plantain. The occurrence of BSV has been confirmed in *Musa*-producing areas of Asia, Africa, Australasia, the Americas and Europe. Countries affected include Malaysia, China, Indonesia, India, Philippines, Vietnam, Thailand, Sri Lanka, Australia, Papua New Guinea, New Caledonia, Tonga, Western Samoa, Madagascar, Mauritius, South Africa, Tanzania, Malawi, Kenya, Uganda, Ghana, Nigeria, Benin, Rwanda, Togo, Sierra Leone, Cameroon, Cape Verde, Guinea Bissau, Jordan, Brazil, Ecuador, Colombia, Costa Rica, Honduras, Nicaragua, Puerto Rice, Jamaica, Trinidad, Cuba, Guadeloupe, Venezuela, the USA (Florida), Portugal (Madeira) and Spain (Canary Islands) (Jones and Lockhart 1993, Vuylsteke *et al.* 1996).

## IMPORTANCE OF BSV INFECTION IN *MUSA*

BSV infection in banana and plantain may be important for one of three reasons:

- Its effect on plant growth, and fruit yield and quality
- It is a hindrance to germplasm exchange
- The need for certification of *in vitro* plantlets for international trade

The effects of BSV infection on plant growth,

Keywords: banana badnavirus, banana viral leaf streak.

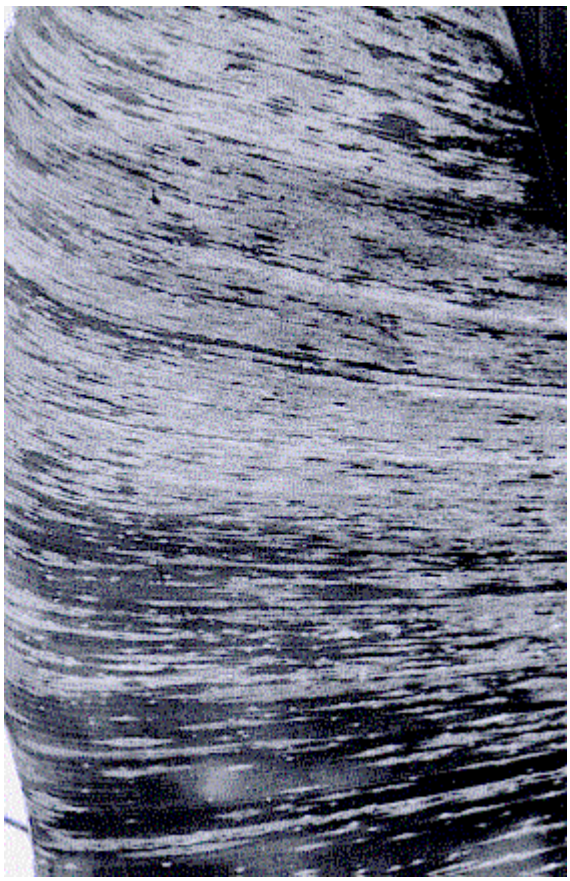

Fig. 1. Chlorotic streak symptoms caused by banana streak virus (BSV, Morocco isolate) infection in Cavendish banana cv. Williams (AAA).

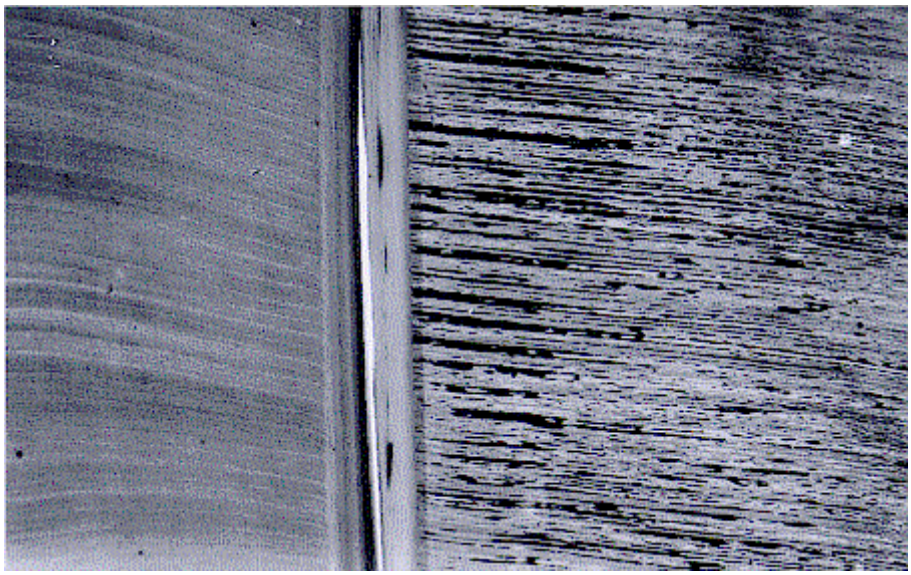

Fig. 2. Black necrotic streak symptoms caused by banana streak virus (BSV, Rwanda isolate) infection in Musa cv. Kamaramasenge (AAB). Healthy leaf on left

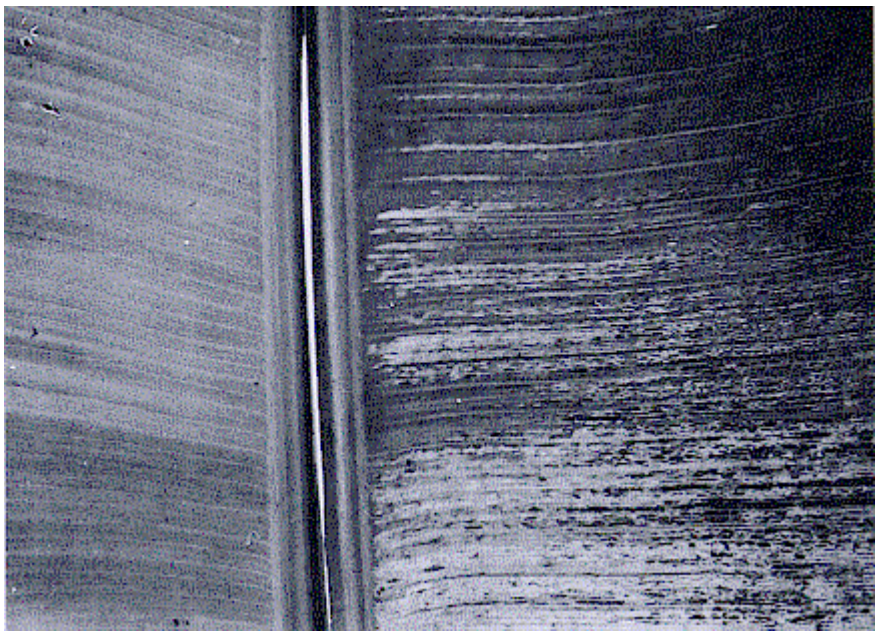

Fig. 3. Chlorotic and brown necrotic streak symptoms caused by banana streak virus (BSV) infection in *Musa* cv. Mysore (AAB). Healthy leaf on left.

bunch yield and fruit quality may be very variable, for two reasons. Firstly, as mentioned previously, isolates of BSV differ greatly in the severity of symptoms produced, ranging from occasional chlorotic streaking to lethal systemic necrosis. Secondly, the symptoms caused by BSV infection characteristically occur sporadically over the course of the year, and are correlated with variations in virus concentration. Yield losses due to BSV invection in banana have been documented for the AAA cultivar 'Poyo' in Ivory Coast (Lassoudiere 1979). Reduction in bunch size and malformation of fingers have also been reported for BSV infection in 'Dwarf Cavendish' (Lockhart 1986). It has been suggested that BSV infection may have a significant effect on bunch size and fruit quality when floral initiation and early bunch development coincide with a period of increased virus synthesis (Lockhart and Olszewski 1993). Plant death due to BSV infection (Fig. 4) has been recorded from Rwanda (Lockhart, unpublished) and Nigeria (Gauhl and Pasburg-Gauhl, personal com-munication).

The occurrence of BSV in banana and plantain is likely to have an adverse effect on the international exchange of *Musa* germplasm. This is particularly true of the tetraploid hybrids recently produced by several *Musa* breeding programs. These new hybrids have been bred for resistance to attack from fungal pathogens (black leaf streak) and nematodes, but the occurrence of BSV in a number of them will undoubtedly lead to restrictions on their distribution to, and acceptance by, some countries.

Finally, the awareness of potential infection by BSV, BBTV and other viruses has led to a need for methods to verify that *in vitro* plantlets, are virus free. The production and international movement of these plantlets has greatly increased in recent years.

## Properties of BSV

BSV is a member of the plant virus genus Badnavirus, representing viruses which have bacilliform particles averaging 30 x 150 nm in size and which contain a circular double-stranded DNA genome 7.2 kb in size (Fig. 7) (Lockhart and Olszewski 1994). Badnaviruses are only the second group of ds DNA plant viruses to be described (Lockhart 1990). Like the caulimoviruses, the other genus of ds NDA plant viruses (Hull 1984), badnaviruses belong to the family Pararetroviridae. This family includes plant and animal viruses in which the virions contain a ds DNA genome, and which replicate their genomic DNA via an RNA intermediate using reverse transcription (Medberry *et al.* 1990).

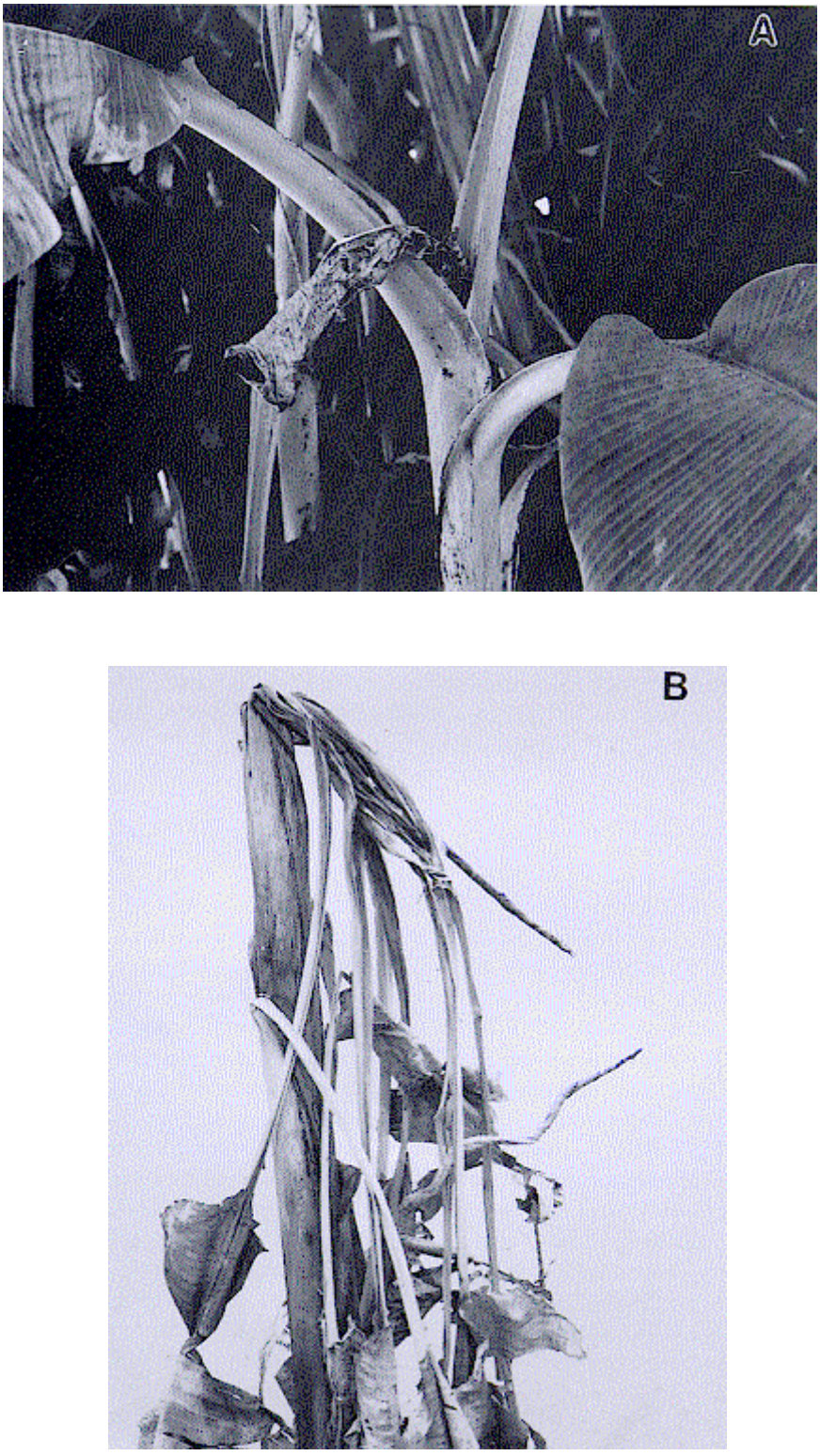


Fig. 4. Cigar-leaf necrosis (A) and systemic necrosis and pseudostem collapse (B) caused by banana streak virus (BSV, Rwanda isolate) infection in *Musa* cv. Kamaramasenge (AAB).

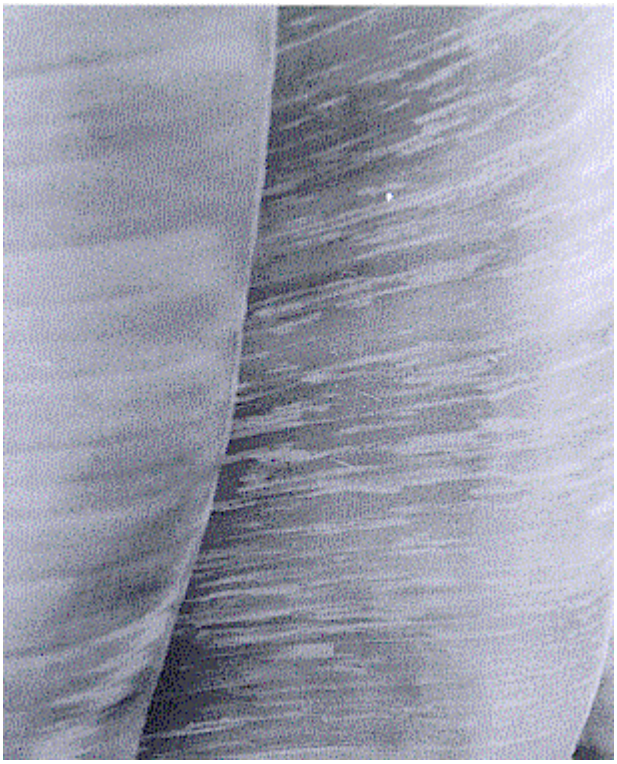

Fig. 5. Chlorotic mosaic symptoms caused by cucumber mosaic virus (CMV) infection in *Musa* cv. Grand Nain (AAA). Healthy leaf on left.

Fig. 6. Initial cigar-leaf necrosis caused by the heart-rot strain of cucumber mosaic virus (CMV) in *Musa* cv. Grand Nain (AAA)

The genus Badnavirus at present includes twelve definitive members. With one exception (rice tungro bacilliform virus, RTBV) badnaviruses occur in vegetatively-propagated perennial crops in the tropics and subtropics. In addition to *Musa*, crops infected with badnaviruses include sugarcane, cacao, pineapple, black pepper, citrus and yam (*Dioscorea* spp.). It is interesting to note that with a few exceptions (cacao and Kalanchöe*), the natural hosts of badnaviruses are plants with centers of origin and/or diversity in southeast Asia and Australasia (Lockhart and Olszewski 1994).

BSV shares with other badnaviruses the property of infecting only a very limited number of plant host species. BSV has been found naturally only in *Musa* (banana, plantain, bluggoe*), and has been transmitted experimentally only to *Musa* and *Ensete**. Experiment transmission of BSV to *Musa textilis* and the related genera *Heliconia* and *Strelitzia* has been unsuccessful (Lockhart, unpublished). The significance of the restricted host range of BSV is that spread of BSV must of necessity occur from banana to banana. Weed hosts do not play a role in the epidemiology of viral leaf streak as they do in infection by CMV, which infects a very wide range of plant species (Francki *et al.* 1979).

## SPREAD OF BSV UNDER NATURAL CONDITIONS

Like the majority of badnaviruses, BSV occurs in a clonally-propagated crop. Therefore, vegetative propagation is the principal method of virus spread, since all progeny plants originating from a BSV-infected mother plant eventually develop viral leaf streak symptoms.

BSV and nine of the other 11 definitive members of the genus Badnavirus are transmitted by mealybugs (Pseudococcidae) (Lockhart and Olszewski 1994). Again, RTBV is the notable exception, being transmitted by rice leafhoppers (Cicadellidae) (Hibino *et al.* 1979). Mealybug vectors of badnaviruses include species of *Pseudoccus, Planococcus, Planococcoides, Ferrisia, Saccharicocus* and *Dysmicoccus* (Brunt 1970, Lockhart *et al.* 1992). BSV has been shown to be transmitted by *Planococcus citri* (Fig. 8) and *Saccharicoccus sacchari* (Fig. 9), both of which colonize banana (Lockhart *et al.* 1992). Two unidentified species of mealybug have also been found colonizing pseudostems and roots of *Musa* in Nigeria (Gauhl and Pasburg-Gauhl, personal communication) and Ghana (Lockhart, unpublished), but there is as yet no experimental evidence that these mealybugs are capable of transmitting BSV.

Although adult mealybugs are sedentary, the early instars or "crawlers" are highly mobile. Mealybugs, which transmit badnaviruses in a semipersistent manner (Lockhart and Olszewski 1994), are very efficient vectors, and transmission rates higher than 90% have been obtained using single insects (Ayala-Navarrete 1992). It is therefore feasible to assume that field transmission of BSV could be readily accomplished by early mealybug instars when they are crawling between adjacent plants or are carried by wind to neighboring plants.

The banana scale, *Aspidiotus nerii* (Coccidae) is very frequently found colonizing banana. However, this species failed to transmit BSV from infected to healthy banana (Lockhart, unpublished). Several aphid species, such as *Aphis gossypii* and *Rhapalosiphum padi*, which briefly colonize or visit banana, also failed to transmit BSV (Lockhart 1985).

Although BSV can be readily transmitted experimentally to banana by mealybugs, all attempts to transmit the virus by mechanical inoculation have failed, even when young *in vitro* plants were inoculated (Lockhart 1985). This may be due to the very high levels of phenolic compounds, latex and other inhibitory substances present in bananas. This also suggests that the spread of BSV on cutting tools or during any cultural operations is highly unlikely.

## SEED TRANSMISSION OF BSV

Several definitive or tentative members of the badnavirus group have been shown to be transmitted through seed, in some instances at rates of 60-90% (Hearon and Locke 1984, Martin and Kim 1987). One badnavirus, *Kalanchöe* top-spotting virus (KTSV), is also pollen-transmitted (Hearon and Locke 1984).

In 1924, it was observed that seed progeny from crosses involving the AAB clone 'Mysore' showed foliar chlorotic streaks similar to those present in the parent (Wardlaw 1972). It was concluded that this was due to a physiological effect. It was later shown that the symptoms in 'Mysore' were due to BSV infection (Lockhart and Olszewski 1993), and it was suggested that BSV, like other badnaviruses, might be seed-transmitted in *Musa*. This has recently been confirmed in Australia (Daniells *et al.* 1995). This finding means that care needs to be taken to avoid the use of BSV-infected parents in banana breeding programs.

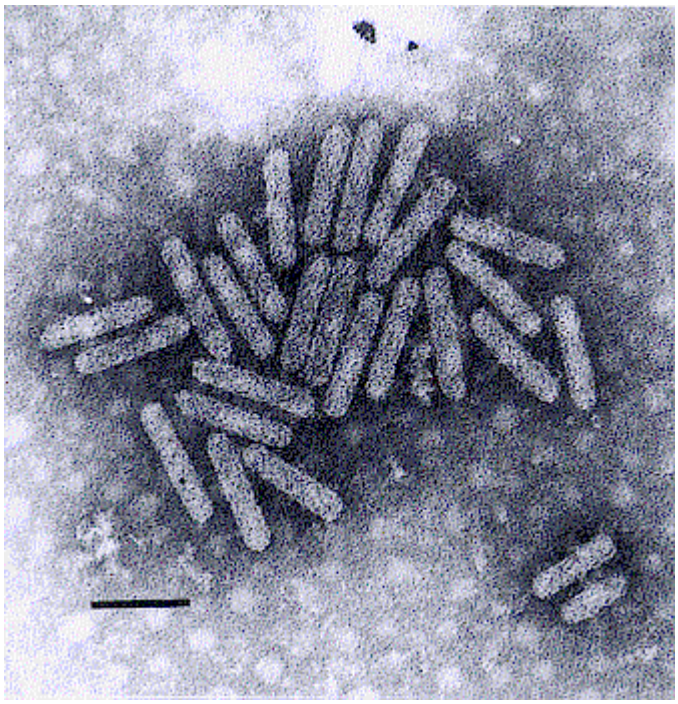

Fig. 7. Particles of banana streak virus (BSV) detected by immunosorbent electron microscopic (ISEM) indexing using BSV antiserum. Scale bar represents 100 nm

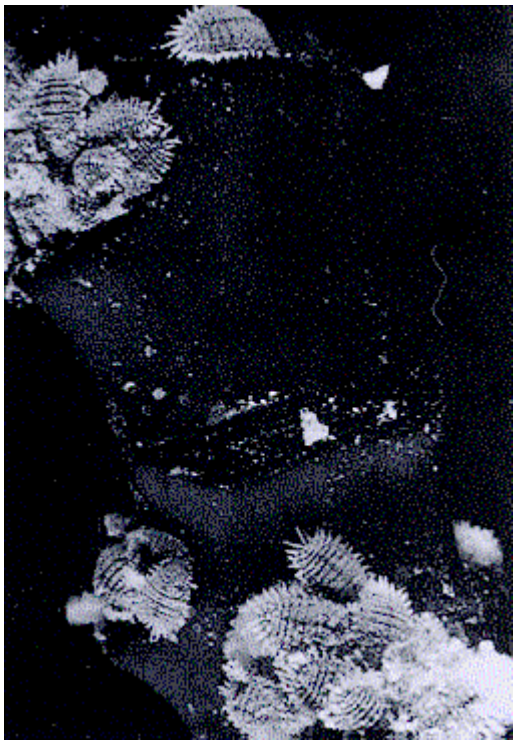

Fig. 8. The citrus mealybug, *Planococcus citri* (Hemiptera, Pseudo-coccidae), a vector of banana streak virus (BSV), colonizing banana.

Fig. 9. The pink sugarcane mealybug, *Saccharicoccus sacchari*, a vector of surgarcane bacilliform virus (ScBV), colonizing sugarcane.

### Relationship of BSV to Sugarcane Bacilliform Virus

Sugarcane bacilliform virus (ScBV) (Lockhart and Autrey 1988) is a mealybug-transmitted badnavirus which occurs worldwide in noble sugarcanes (*Saccharum officinarum* L.), related species (*S. robustum, S. sinense, S. barberi, S. spontaneum*), and in a number of commercial hybrids (Comstock and Lockhart 1990). ScBV differs from the majority of badnaviruses in being able to infect a wider range of plant species, including *Sorghum, Rottboellia, Panicum*, rice (*Oryza sativa*) and banana (Bouhida *et al.* 1993). ScBV was transmitted by the mealybugs *Planococcus citri* (Fig. 8) and *Saccharicoccus sacchari* (Fig. 9) from infected sugarcane to healthy 'Dwarf Cavendish' banana, which developed symptoms identical to those caused by BSV (Fig. 10). Although *P. citri* is rarely found on sugarcane, *S. sacchari* is common on this crop, and also readily colonizes banana. Many isolates of BSV are serologically closely related to isolates of ScBV (Lockhart and Autrey 1988, Lockhart and Olszewski 1993). It is possible that viral leaf streak in banana could on occasion be the result of ScBV infection carried by mealybugs from adjacent sugarcane. It is also interesting to note that recently described badnaviruses occurring in pineapple in Hawaii (J. Hu, personal communication) and Australia (Wakman *et al.* 1995) and in citrus in India (Ahlawat *et al.* 1996) are also closely related serologically to ScBV. This means that ScBV, which is able to infect a fairly wide range of plant species, may occasionally be transmitted by mealybugs to new hosts, including banana.

## DETECTION AND CONTROL OF BSV IN BANANA

While CMV (Bouhida and Lockhart 1990) and banana bunchy top virus (BBTV) (Wardlaw 1972) are both transmitted by aphids, BSV in contrast is spread by a slow-moving insect vector and infects only banana. Since the principal method of disease spread is by vegetative propagation, disease control must be based on the use of virus-free stock plants for propagation by suckers or *in vitro* plantlets. It is also important to avoid the introduction of BSV into banana breeding lines. Hybridization in bananas, the majority of which are sterile triploids, is a very slow process. Infection of new hybrids by BSV can effectively nullify a long and arduous effort. Detection of BSV is therefore the key to the control of viral leaf streak.

Reliable diagnosis of BSV in banana is

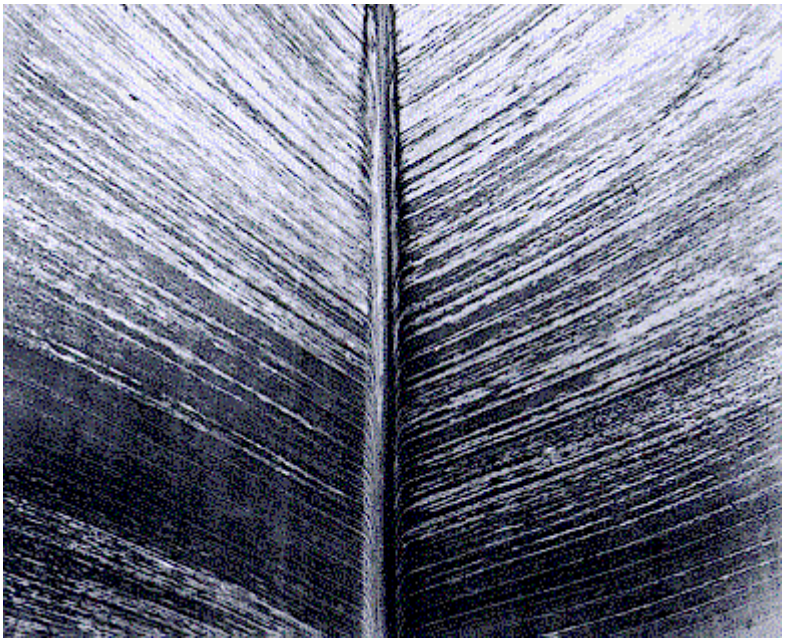

Fig. 10. Chloritic leaf streak symptoms in Dwarf Cavendish banana (AAA) caused by infection with sugarcane bacilliform virus (ScBV) transmitted by *Saccharicoccus sacchari* from sugarcane.

complicated by several factors arising from the nature of the disease, and of the causal agent itself. Firstly, diagnosis based on foliar symptoms may be very unreliable because of the sporadic nature of symptom expression throughout the year (Jones and Lockhart 1993). Symptoms may be totally absent, or may be indistinct, under certain conditions. Although the effects of environmental conditions (temperature, day length) on symptom expression have been noted, the precise conditions that trigger symptom development have not been identified. Symptom expression is also usually totally lacking in plants derived from *in vitro* multiplication. For many plant viruses, biological indexing using indicator plants represents a simple and inexpensive method of testing propagating material for virus infection. Because BSV infects only *Musa* and *Ensete*, and is not transmissible to these species by mechanical inoculation, this method of indexing cannot be used to test for BSV.

Attempts to develop sensitive, reliable methods for BSV indexing using antigen-based (serology) and genome-based (amplification by polymerase chain reaction, PCR) have been only partly successful. Both these approaches have been compromised by the very high degree of both serological and genomic heterogeneity that exists among isolates of the virus (Lockhart and Olszewski 1993). The high degree of genomic heterogeneity that exists among BSV isolates can be explained by the mechanism of replication of badnaviruses and other pararetroviruses. The virions of these viruses contain a ds DNA which is replicated by reverse transcription via an RNA intermediate. This process is inherently prone to the generation of genomic variants. Because these variants are maintained and disseminated by vegetative propagation, there is little selection pressure applied against these variants. Some isolates of BSV are serologically unrelated to others, and the degree of cross-hybridization between different isolates is low (Lockhart and Olszewski 1993).

Enzyme-linked immunosorbent assay (ELISA) indexing protocols, using polyclonal antisera raised against a mixture of BSV antigens, have been partially successful in screening *Musa* banana and plantain for BSV, but the procedure is not totally reliable because it fails to detect a number of isolates of the virus (Lockhart and Olszewski 1993). The most reliable method for serological detection of BSV in banana is immunosorbent electron microscopy (ISEM) (Fig. 10), using partially purified extracts prepared from small (5-7 gm) samples of leaf tissue (Ahlawat *et al.* 1996).

Using two pairs of oligonucleotide primers based on conserved badnavirus sequences, PCR-mediated amplification was shown to be a potentially useful method for detection of all BSV isolates (Lockhart and Olszewski 1993). However, while this technique has worked when partially-purified virus extracts are used, far less success is obtained when the template consists of total DNA extracted from plants using a variety of isolation methods. This failure may be due in part to the presence of inhibiting substances from banana which contaminate the DNA sample, or to the lack of sufficient homology between primers and template. In addition, there is evidence that segments of the BSV genome have become integrated into the nuclear DNA of a wide range of bananas and plantains (Lockhart and Olszewski, unpublished), further compromising the reliability of PCR detection of BSV. Efforts are now under way to overcome the problems posed by serological and genomic heterogeneity in BSV, and to develop more sensitive and reliable indexing procedures than are currently available.

## REFERENCES


Ahlawat, Y.S., R.B. Pant, B.E.L. Lockhart, M. Srivastava, N.K. Chakraborty and A. Varma. 1996. Association of a badnavirus with citrus mosaic disease in India. *Plant Disease* 86: (In press).

Ayala-Navarrete, L.I. 1993. Genetic Analysis of Commelina Yellow Mottle Virus (CoYMV). Unpub. M.S. Thesis, University of Minnesota, St. Paul, MN, USA. 82 pp.

Bouhida, M. and B.E. Lockhart. 1990. Increase in importance of cucumber mosaic virus infection in greenhouse-grown bananas in Morocco. *Phytopathology* 80: 981.

Bouhida, M., B.E.L. Lockhart and N.E. Olszewski. 1993. An analysis of the complete nucleotide sequence of a sugarcane bacilliform virus genome infectious to banana and rice. *Journal of General Virology* 74: 15-22.

Brunt, A.A. 1970. Cacao swollen shoot virus. *Descriptions of Plant Viruses No. 10.* Commonwealth Mycological Institute/ Association of Applied Biologists, Kew, Surrey, England. 4 pp.

Comstock, J.C. and B.E. Lockhart. 1990.

Widespread occurrence of sugarcane bacilliform virus in U.S. sugarcane germplasm collections. *Plant Disease* 74: 530.

Daniells, J., J.E. Thomas and M. Smith. 1995. Seed transmission of banana streak virus confirmed. *Infomusa* 4,1:7.

Francki, R.I.B., D.W. Mossop and T. Hatta. 1979. Cucumber mosaic virus. *Descriptions of Plant Viruses No. 213.* Commonwealth Mycological Institute/ Association of Applied Biologists, Kew, Surrey, England. 6 pp.

Hearon, S.S. and J.C. Locke. 1984. Graft, pollen, and seed transmission of an agent associated with top spotting in *Kalancöe blossfeldiana*. *Phytopathology* 74: 347-350.

Hibino, H., N. Saleh and M. Roechan. 1979. Transmission of two kinds of rice tungro-associated viruses by insect vectors. *Phytopathology* 69: 1266-1268.

Hull, R. 1984. Caulimovirus group. *Descriptions of Plant Viruses No. 295.* Commonwealth Mycological Institute/ Association of Applied Biologists, Kew, Surrey, England. 4 pp.

Jones, D.R. and B.E.L. Lockhart. 1993. Banana Streak Disease. *Musa Disease Fact Sheet No. 1.* International Network for the Improvement of Banana and Plantain, Montpellier, France.

Lassoudie`re, A. 1974. La mosaïque dite "a` tirets" du bananier Poyo en Côte d'Ivoire. *Fruits* 29: 349-357. (In French).

Lassoudiere, A. 1979. Mise en évidence des répercussion économiques de la mosaïque en tirets du bananier en Côte d'Ivoire. Possibilitiés de Lutte par éradication. *Fruits* 34: 3-34. (In French).

Lockhart, B.E.L. 1986. Purification and serology of a bacilliform virus associated with banana streak disease. *Phytopathology* 76: 995-999.

Lockhart, B.E.L. 1990. Evidence for a circular double-stranded DNA genome in a second group of plant viruses. *Phytopathology* 80: 127-131.

Lockhart, B.E.L. and L.J.C. Autrey. 1988. Occurrence in sugarcane of a bacilliform virus related serologically to banana streak virus. *Plant Disease* 72: 230-233.

Lockhart, B.E.L. and N.E. Olszewski. 1993. Serological and genomic heterogeneity of banana streak badnavirus: Implications for virus detection in *Musa* germplasm. In: *Breeding Banana and Plantain for Resistance to Diseases and Pests*. J. Ganry, (ed.). CIRAD/INIBAP, Montpellier, France, pp. 105-113.

Lockhart, B.E.L. and N.E. Olszewski. 1994. Badnavirus Group. In: *Encyclopedia of Virology*, Vol. I, R.G. Webster, and A. Granoff (eds.), Academic Press, New York. pp. 139-143.

Lockhart, B.E.L., L.J.C. Autrey and J.C. Comstock. 1992. Partial purification and serology of sugarcane mild mosaic virus, a mealybug-transmitted clorterolike virus. *Phytopathology* 82: 691-695.

Martin, E.M. and K.S. Kim. 1987. A new type of virus causing striped chlorosis of mimosa. *Phytopathology* 77: 935-940.

Medberry, S.L., B.E.L. Lockhart and N.E. Olszewski. 1990. Properties of *Commelina* yellow mottle virus' complete DNA sequence: Genomic discontinuities and transcript suggests that it is a pararetrovirus. *Nucleic Acids Research* 18: 5055-5513.

Vuylsteke, D.R., C.T. Chizala and B.E. Lockhart. 1996. First report of banana streak virus in Malawi. *Plant Disease* 80: 224.

Wakman, W., D.S. Teakle, J.E. Thomas, and R.G. Dietzgen. 1995. Presence of a clostero-like and a bacilliform virus in pineapple plants in Australia. *Australian Journal of Agricultural Research* 46: 947-958.

Wardlaw, C.W. 1972. *Banana Diseases. Including Plantains and Abaca.* Second Edition. Longman, London. 878 pp.

## DISCUSSION

Dr. Lockhart was asked whether he had attempted to transmit ScBV to sugarcane by means of mealybugs. He confirmed that he had, and added that when the virus in transmitted in this manner, if symptom expression is strong in sugarcane, it is also strong in Dwarf Cavendish banana. Although it is not possible to transmit the closely related BSV virus to sugarcane, ScBV moves easily from sugarcane into banana.

However, BSV and ScBV are not a single virus. Any one plant does not have a single genomic population, but a vast number of them. If the virions present in a single banana plant are islaoted and a PCR amplitude carried out, around 20 products can be obtained. If these are then sequenced, they will all be different. BSV includes thousands, perhaps even millions, of different genotypes. If a banana plant is to be certified as free of BSV virus, then we must be sure that all isolates are detectable, and that none of them are present. To be able to detect the presence of any one isolate is not very meaningful.