Integrated management of viruses infecting *Musa* spp.

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Virus diseases

Viruses are infectious agents consisting of nucleic acid (ribonucleic acid or deoxynucleic acid) and a protein coat. Some virus groups additionally have a phospholipid membrane. Viruses are inert outside a living organism but once they infect a living cell, the infected cell replicates the viral nucleic acid and coat protein that is then assembled into infectious viral particles.

Unlike animals, plants do not possess an immune system and therefore, once a plant is infected it remains infected. The viruses are then transmitted through vegetative propagules (including micropropagation). Viruses can also be transmitted through true seed and by vectors (usually insects, but also nematodes and fungi).

Virus diseases usually induce conspicuous foliar symptoms and usually reduce the vigour of the plant. This results in stunting and often an increased susceptibility to other pests and diseases. Virus diseases lead to reduced yields, in terms of both quantity and quality.

Virus diseases can usually be controlled by using healthy planting material, eliminating vectors (insects, nematodes, fungi), removing sources of infection or alternative hosts and through the use of resistant cultivars or varieties. Development of integrated control strategies for virus diseases requires a thorough understanding of the viruses, their field transmission and their epidemiology.

Virus diseases of *Musa* spp.

Virus diseases are a major constraint to banana and plantain production. Four virus diseases of *Musa* spp. are known to occur in Africa: banana bunchy top, banana mosaic, banana streak and banana die-back. Banana bunchy top causes severe disease outbreaks where the vector is present (Diekmann and Putter 1996). Banana mosaic can cause losses if severe strains of the causal agent, cucumber mosaic virus (CMV), are present (Jones 1994) and banana streak may cause severe losses where severe strains occur (Lockhart 1994) and susceptible cultivars are grown. The significance of banana

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die-back (Hughes *et al.* 1998) is not known, but as with almost all virus diseases, any infection can cause yield losses due to diversion of plant resources to virus replication. In addition to effects on growth and yield, viruses are a constraint to international germplasm distribution because, for quarantine reasons, only pathogen-free vegetative material of banana and plantain can be distributed. All the viruses infecting *Musa* spp. are transmitted vegetatively and through tissue culture or micropropagation.

The virus diseases of *Musa* spp. occurring outside Africa, abaca mosaic and banana bract mosaic, will be described as they remain potential quarantine issues. The causal agents of these two diseases are 'conventional' potyviruses. The other viruses known to infect *Musa* spp. occur in Africa. The distribution of the viruses is given in Table 1. The viruses that are found in Africa fall into the potyvirus, cucumovirus, badnavirus and nanavirus groups. With the exception of the badnavirus, the viruses are 'conventional' and control methods for each of them are similar. The badnavirus, banana streak badnavirus (BSV), is a pararetrovirus and it has been shown that BSV sequences are integrated into the host chromosomes. The potential control measures for the disease caused by this virus will be discussed separately.

Table 1. Geographical distribution of viruses infecting *Musa* spp.

Disease	Causal virus	Distribution[1]	Distribution in sub-Saharan Africa[1]
Abaca mosaic	Abaca mosaic potyvirus (possibly a strain of sugarcane mosaic potyvirus)	Asia (Philippines)	Not reported
Banana bract mosaic	Banana bract mosaic potyvirus	Asia (Philippines, India, Sri Lanka)	Not reported
Banana bunchy top	Banana bunchy top nanavirus	Africa, Asia, Australia and Pacific Islands	Burundi, Central African Republic, Congo, Egypt, Gabon, Rwanda and Zaire
Banana mosaic	Cucumber mosaic cucumovirus	All continents	The virus is found continent-wide
Banana streak	Banana streak badnavirus	Europe, Africa, Asia and Oceania	Benin, Cameroon, Cape, Verde, Côte d'Ivoire, Ghana, Guinea, Kenya, Madagascar, Malawi, Mauritius, Morocco, Nigeria, Rwanda, South Africa, Tanzania, Uganda and Zanzibar[2]
Banana die-back	Banana die-back virus	Africa	Nigeria[3]

[1] Distribution data from Diekmann and Putter (1996)
[2] Vuylsteke *et al.* (1998)
[3] Hughes *et al.* (1998)

Abaca mosaic

The natural hosts of abaca mosaic, caused by abaca mosaic potyvirus (possibly a strain of sugarcane mosaic potyvirus, SCMV), are *Musa textilis*, *Marantha arundinacea* and

Canna indica. Banana is an experimental host. Leaves of infected plants may have yellow or light green streaks and the petioles may be mottled dark green with yellowish streaks. In addition to vegetative transmission, aphids (*Rhopalosiphum maidis* and *Aphis gossypii*) transmit abaca mosaic potyvirus in a non-persistent manner. Diagnosis of abaca mosaic potyvirus is by enzyme-linked immunosorbent assay (ELISA) (Diekmann and Putter 1996).

Banana bract mosaic

Banana bract mosaic potyvirus (BBrMV) infects *Musa* spp. and cultivars. The virus causes dark streaks on the bracts of the inflorescence (Ploetz 1994). Streaks may also occur on the petioles and spindle-shaped chlorotic streaks may occur on the laminae. Vector transmission is by aphids (*R. maidis*, *A. gossypii* and *Pentalonia nigronervosa*). The virus is transmitted vegetatively. Detection of BBrMV is by ELISA (Diekmann and Putter 1996) using monoclonal or polyclonal antibodies.

Banana bunchy top

Musa spp. and cultivars are naturally infected by banana bunchy top nanavirus (BBTV). *C. indica* and *Hedychium coronarium* may be alternative hosts and the virus has been transmitted experimentally to *Ensete ventricosum* (Diekmann and Putter 1996). Infected plants may exhibit dark green streaks on the petioles and typically exhibit 'bunching' of the leaves due to progressive shortening of leaves and internodes. The leaves tend to develop chlorotic margins. Symptomless plants and attenuated symptoms have also been observed. Transmission is vegetative and by the aphid *P. nigronervosa*. Detection is by ELISA using monoclonal and polyclonal antibodies as well as by using DNA probes (Wu and Su 1990, Burns *et al.* 1995).

Banana mosaic

The causal agent of banana mosaic, CMV, is distributed worldwide and is found in many dicotyledon and monocotyledon families (Brunt *et al.* 1990). Infected *Musa* spp. exhibit chlorotic streaking or flecking, mosaics and leaf distortion. Severe strains can cause severe symptoms including cigar leaf and pseudostem necrosis. Transmission is by aphids (*A. gossypii*, *R. maidis*, *R. prunifoliae* and *Myzus persicae*) in a non-persistent manner and also by true seed. Detection of this virus is by ELISA using polyclonal or monoclonal antibodies, by mechanical inoculation to diagnostic herbaceous indicator plant species (Francki *et al.* 1979) and polymerase chain reaction (Singh *et al.* 1995).

Banana streak

The natural hosts of banana streak badnavirus (BSV) are *Musa* spp. and cultivars. A bacilliform virus likely to be BSV has been found in *Ensete ventricosum* (Mesfin *et al.* 1995). *Ensete* spp. are also experimental hosts for BSV. The symptoms vary between cultivars, but generally consist of chlorotic streaks or spindle-shaped lesions that may turn necrotic. Cigar leaf necrosis may occur and lead to death of the plant (Dahal *et al.* 1998a). Symptoms are sporadic and appear to be environment dependent. There is a

correlation between ambient temperatures and symptom expression (Dahal *et al.* 1998b). In addition to vegetative transmission, the virus is reported to be transmitted through true seed (Daniells *et al.* 1995) as well as by the citrus mealybug (*Planococcus citri*) (Lockhart and Autrey 1991). The virus particles can be detected by several means: by ELISA using polyclonal antibodies (Thottappilly *et al.* 1997, 1998), by immunosorbent electron microscopy (ISEM) (Diekmann and Putter 1996) and by immunocapture (IC-) polymerase chain reaction (PCR) (Hull and Harper 1998). The viral nucleic acid sequences that are integrated into the *Musa* spp. genome can be identified using direct PCR (Hull and Harper 1998).

Banana die-back

Banana die-back infects banana plants causing symptoms of leaf necrosis and die-back of the plant. Subsequent suckers that develop are progressively more stunted until the entire mat is dead (Hughes *et al.* 1998). The mechanism of transmission of banana die-back virus (BDBV) is not known although some limited field spread has been observed. Diagnosis of this virus is at present through the use of ELISA using polyclonal antibodies and mechanical inoculation of herbaceous indicator plants.

Control of virus diseases

Healthy planting material

Healthy planting material is a very important starting point for the control of plant virus diseases. Virus-infected plants are unable to eliminate the virus and therefore remain infected throughout their life and, in the case of vegetatively propagated crops such as *Musa* spp. and *E. ventricosum*, throughout the lives of subsequent generations that were taken as suckers from the infected mother plants. Healthy planting material therefore at least allows the chance of preventing infection from outside occurring and therefore maintaining healthy crops in the field.

In crops propagated through true seed, 'virus-free' seeds are often used. These are seeds collected from virus-tested mother plants. In reality the term 'virus-free' is rarely used, as it is not possible to test a whole plant or seed in its entirety in a non-destructive way. Theoretically, even one virus particle can cause virus infection of the whole plant. The term 'virus-tested' is usually used, indicating that the plant has been tested for viruses and the tests were negative. Seeds can be obtained from *Musa* spp., but these are not used for multiplication of planting material. However, where it is intended that seeds will be used, for example in breeding programmes, the female and male parents should be indexed for viruses. If one or other of the parents is virus-infected, the seedling(s) may also be virus-infected (Daniells *et al.* 1995, Gold 1972).

The use of virus-tested vegetative propagules (micropropagated plantlets) are the most effective means of ensuring that new planting material is free from virus diseases at planting. Specific guidelines are followed for the testing procedures (Diekmann and

Putter 1996) and the plantlets are certified that they tested negative for viruses. A Germplasm Health Statement is usually issued but this does not substitute for a phytosanitary certificate from the exporting country. Micropropagated plantlets are the only accepted means of distributing *Musa* spp. germplasm internationally. Where micropropagated plantlets are not available, multiplication of suckers from virus-tested mother plants may be done, preferably in an insect-proofed screenhouse. These suckers will not have virus-tested status unless re-testing is done, but they do provide some likelihood that the propagules will be healthy. In cases where even virus indexing is not available, at the very least, vegetative propagules should be taken from mother plants that have never expressed virus-like symptoms.

It is possible to use virus-tested planting material to saturate an area with healthy plants. Provided all the infected plants are first removed from the site, this will remove sources of infection within the area (with the possible exception of alternative host plants for the virus) and may delay re-infection. This technique is however ineffective with vector transmitted viruses if the replanting is done on a small scale, as the vectors will come in to the replanted area from the surrounding infected areas (Ollennu and Hughes 1991). The efficacy of this method of treatment of virus-infected areas is dependent on the policy-makers being effective in the rigorous implementation of the introduction of the healthy material and a thorough knowledge of the means of spread of the disease so that natural barriers can be used to prevent vector spread.

Vector control

The vectors of virus diseases of *Musa* spp. are aphids (*R. maidis*, *R. prunifoliae*, *A. gossypii*, *P. nigronervosa* and *M. persicae*) and mealybugs (*P. citri*). Two main types of vector control would normally be considered: biological control and vector control.

Biological control is not normally considered effective for aphids out of a controlled environment, although it can be effective in glasshouse conditions (Hall 1985). Biological control of mealybugs has been achieved (Neuenschwander 1996), but is not applicable at the present time for the control of the putative vector of BSV, *P. citri*.

The use of insecticides to control these vectors of virus diseases is not economically feasible for subsistence agriculture although they can be used in commercial plantations to control aphids. Control of mealybugs through insecticides is not practical on *Musa* spp. Mealybug colonies have been found on different sites on the plant: under the leaf sheaths of the pseudostem, just under the soil surface on the roots and on the inflorescence (Hughes 1998). Due to the cryptic nature of the mealybugs, contact insecticides do not find their targets and systemic insecticides may have toxicity problems and taint fruit (Thorold 1975).

Removal of sources of infection

In the case of vegetatively propagated crops such as *Musa* spp., ratoon crops can be a source of virus for the next planting season. Infected crop plants that perennate from one growing season to another can provide a significant source of inoculum. For

example, if a single *Musa* spp. sucker infected with BBTV is left in a field, it can serve as a source of inoculum for the vector aphids when the new healthy material is planted. Ideally all material infected with viruses should be removed from the farm or plantation and burnt to prevent it being a source of infection and also to prevent any vectors which may be on the removed plant material from migrating to adjacent plants.

Weeds around the farm can also harbour viruses. Some of these may be transmissible to *Musa* spp. In particular, CMV has many alternative hosts in weed species. Removal of weeds is good farming practice in any case and will also serve to remove possible sources of infection. CMV is also known to infect many crops species, for example cowpea, soybean, fodder legumes, yams and many vegetable and salad crops (Brunt *et al.* 1990) that may be grown together with *Musa* spp. in subsistence agriculture.

Phytosanitation

Removal of symptomatic, virus-infected plants from within a crop reduces the chances of vector transmission within the crop. Viruses generally reduce crop yields. Even when the symptoms are not particularly severe, there may be a yield advantage to be gained by substituting a healthy plant for an infected one.

Without virus-indexing, farmers will be unable to rogue infected but symptomless plants from within the crop, and these can remain a source of inoculum for vector transmission to adjacent healthy plants.

Geographical or temporal isolation

Geographical isolation is where the crop is grown at a distance from sources of inoculum. The barrier to infection may simply be that a sufficiently large distance has been left between the plantings to preclude vector transmission. In other cases, geographic barriers such as mountain ranges or lakes can prevent or reduce spread of diseases, as the vectors are less likely to cross those natural barriers. Interestingly, geographic barriers, more than political ones, are likely to reduce the movement of people between areas, thus reducing spread of disease through infected planting material. Diseases, spread through infected planting material in this way, may initially occur along roads, and other transportation routes such as rivers, before being spread locally between farmers.

Temporal isolation requires the crop to be grown at a different time from other host plants. This is not appropriate for *Musa* spp. which are grown over more than one season.

The use of geographical or temporal isolation is not appropriate for subsistence agriculture. However, under intensively managed commercial conditions, these options should be investigated as a means of controlling the spread of vector-borne virus diseases.

Resistant cultivars or species

Breeding for resistance to virus diseases of *Musa* spp. or *E. ventricosum* has not been a significant component of breeding programmes, even though host plant resistance is probably the most effective form of virus disease control. Producing viable seeds has, however, been a

major difficulty in *Musa* spp. and there has only been limited attention given in the past to the significance of virus diseases of *Musa* spp. by the research community.

In the past there has probably been some natural selection in response to disease pressure. However many selections, even at present, are based on fortuitous observation of absence of symptoms. Some breeding programmes are now paying increased attention to breeding for virus resistance and some virus-resistant or tolerant clones are available. These include BITA-3, a starchy banana with partial resistance to black sigatoka (Ortiz and Vuylsteke 1998a), and tolerance to streak virus, and PITA-14, a black Sigatoka-resistant tetraploid hybrid plantain with virus tolerance (Ortiz and Vuylsteke 1998b).

Integrated disease management

The integrated management of plant virus diseases must be part of an integrated disease management strategy that, in turn, is part of the management of the farm and farming environment. For all of the virus diseases of *Musa* spp., except for banana streak, conventional disease management strategies apply. Therefore, for abaca mosaic, banana bract mosaic, banana bunchy top, banana mosaic and probably banana die-back, appropriate disease management strategies using healthy planting material, controlling the vectors, removal of sources of infection (whether of the crop or other species), geographic or temporal isolation and the use of resistant cultivars or species will control the diseases (Figure 1). In most subsistence farming conditions, the most appropriate means of control will be the use of healthy, resistant planting material in combination with roguing/phytosanitation.

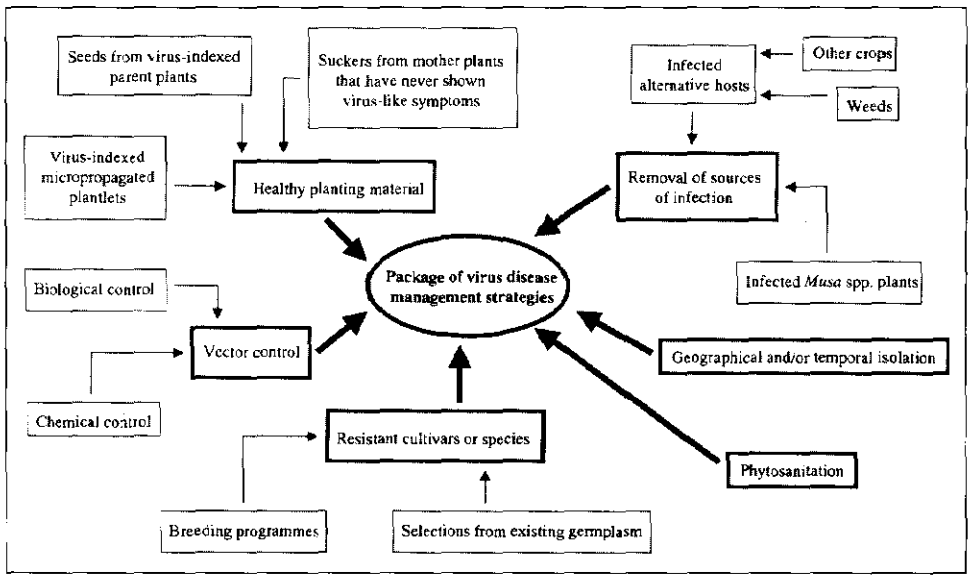

Figure 1. Components of an integrated package to manage virus diseases of *Musa* Spp.

Banana streak is, however, a non-conventional virus disease. It is known that the virus exists as infective virus particles and that virus DNA is integrated into the *Musa* spp. genome (LaFleur *et al.* 1996). It is also postulated that the virus may exist as a super-coiled DNA replicative intermediate within the host cells. In addition it appears that all *Musa* spp. and cultivars have integrated BSV sequences. Transcription of the integrated sequences, giving rise to infective virus particles (Lockhart *et al.* 1998), appears to be activated by stress. It has been suggested that the following may be considered stressful events: environmental stresses (for example drought (water stress), poor nutritional status, abnormal climatic conditions), pest and disease pressure (including weed competition) and tissue culture/micropropagation (Frison and Sharrock 1998).

While methods to control this non-conventional, potentially stress-induced disease are not obviously apparent, there are techniques that may be used to manage the disease. There is little evidence of natural transmission or field spread (Hughes 1998, Su 1998, Thomas *et al.* 1998). The major cause of spread appears to be the dissemination of highly susceptible planting material. The apparent spread of virus symptoms may be simply a 'switching-on' of symptoms by some form of stress (Dahal *et al.* 1998b), or due to an increased awareness of the disease and its symptoms by researchers and extension staff. The best form of management for banana streak seems to be to grow resistant or tolerant species or cultivars and to manage stress-induced transcription of the integrated sequences through 'good' farming practices.

In conclusion, it is clear that healthy virus-resistant or tolerant species or cultivars and 'good' farming practices are prerequisites to avoid major *Musa* spp. or *E. ventricosum* yield losses from virus infection. Both these requirements can easily be put, with recommendations to control other pathogens and pests, into a comprehensive integrated pest and disease management package for use by subsistence farmers. This package can be adapted for the commercial banana growers as the basic principles remain the same regardless of the size of the farm. The main difference between commercial and subsistence farmers, apart from the size of the farms, monoculture vs. mixed cropping and quality of the end product required, is the ability of the different growers to provide high cost inputs. With a range of options available within the integrated pest management package, the management of pests and diseases of *Musa* spp. and *E. ventricosum* should lead to improved, sustainable yields of plantain, banana and ensete in sub-Saharan Africa.

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