TCP/SLC/3402
Technical Manual

A HOLISTIC INTEGRATED MANAGEMENT APPROACH TO CONTROL BLACK SIGATOKA DISEASE OF BANANA CAUSED BY *Mycosphaerella fijiensis*

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

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INTRODUCTION

Banana and plantains (*Musa* spp.) are grown throughout the tropical and subtropical regions of the world. They are a key staple food in many developing countries and a source of income for subsistence farmers.

Bananas are one of the main agricultural crops in the world, and the main fruit crop, with an annual production of about 117 Mt (Lescot, 2011). Bananas are also a major, multibillion dollar export commodity for consumption primarily in developed countries. International trade of bananas represents 14 Mt/year with a value of more than 4 billion US$ (Loeillet, 2005). The production of bananas for this international trade is grown in a small number of tropical countries mainly located in Latin America (80%), in African and Asian countries. In all these countries, this industry is economically important and a source of direct and indirect employment. But one of the main problems is that it relies on a very narrow genetic base, the Cavendish subgroup, which exposes the industry to threats from a number of key pests and diseases.

Globally, plantains are grown on 5.4 million ha, with a production of 36 million Mt. Both plantain and banana play an important role in food security and the livelihood of the growers.

BLACK SIGATOKA DISEASE

The fungal pathogen *Mycosphaerella fijiensis* (anamorph *Pseudocercospora fijiensis*) causes black Sigatoka disease (BSD) on the majority of edible banana cultivars grown worldwide. The disease appeared in America (Honduras) in 1972 and disseminated in the Caribbean in two independent introductions: In 1990 in Cuba (then Jamaica, Dominican Republic, Haiti and Puerto Rico) and in 2003 Trinidad, spreading along the Lesser Antilles. Currently, only Dominica remains free of the disease.

Musa spp. are the primary hosts of *M. fijiensis* and exhibit a range of symptoms depending on the level of resistance of individual hosts. The ornamental plant *Heliconia psittacorum* is the only known alternative host of *M. fijiensis* (Gasparotto et al., 2005).

BSD is one of the main constraints to banana and plantain production, impacting directly on yields and on the environment due to the control measures to cope with the disease. Wherever *M. fijiensis* has been introduced, it has replaced *M. musicola* due to its higher spore production, shorter disease cycle and higher aggressiveness to varieties normally resistant to *M. musicola* (Pérez *et al.*, 2002).

There are two major impacts of the disease on production. The first is a reduction in yield due to negative effects on the photosynthetic assimilation (50-100%) and thus on dry matter production, strongly affecting the bunch (Ramsey & Daniells, 1990). The second is a reduction in the "green-life" of fruits, i.e. the time lag between harvest and ripening, a direct effect of foliar diseases on fruit physiology. The impact

on export quality is thus very critical as there is a high risk of early maturation (Stover, 1972). Therefore banana growers must control BSD to a level that does not alter fruit export and marketability. BSD has a serious, negative impact on the economics of banana production. The cost of control can reach more than 25% of the production cost (Stover & Simmonds, 1987) and this cost has been steadily increasing over the years (Table 1).

TABLE 1. Impact of BSD in banana production according to different sources.

Country	Year	Observations	References
Honduras	1973	10-20 % of bunches with early ripening in 1200 ha.	Stover and Dickson (1976); Stover (1986)
Costa Rica	1978-1980	Losses of > 3.000 ha of plantains in San Carlos zone.	Guzmán and Jaramillo (1979)
Costa Rica	1980 - 1986	Losses of 40% of plantain production.	Romero (1986)
Panamá	1980-1984	Plantain production decrease in 40%	Bureau (1990)
Colombia	1991	Reduction of plantain production and high increase of prices	Belalcázar (1991)
México	1980 -	Destruction of 2000 ha of banana in Tabasco: 50-100% fruit losses	Orozco, (1998) Orozco et al, (2001)
México	1989-1991	Reduction of 50% banana cultivated surface (5000 ha) Colima.	Orozco, (1998
Cuba	1990-1995	Three fold increase of fungicide costs in Cavendish banana. Reduction of Cavendish banana (AAA) planted surface from 14,000 ha to less than 600 ha and from 45,000 ha of plantains (AAB) to less than 12,000.	Pérez-Vicente et al., (2000)
Venezuela	1987-1990	40-45% increase of costs of production	Martínez et al., (2002)
Brazil	2000-2002	Losses of 50-100% of production. Changes in preferences and changes in the gustative preferences	Gasparoto, 2003 according Guzman (2006)

SYMPTOMS

Six stages of lesion development following infection by the fungus have been described (Fouré, et al., 1984; Figure 1):

First stage: is a faint reddish-brown speck less than 0.25 mm diameter visible on the underside of the leaves.

Second stage: is characterized by an elongation of the specks turning into reddish brown streaks along the long axis of the streak, parallel to the leaf veins.

Third stage: the streaks coalesce, reaching about 20 x 2 mm, and change to dark brown. Conidia are produced at the second and third stages. If the streaks are numerous at this stage, the entire leaf can turn black.

Fourth stage or the first spot stage: when lesions are scarcer, they develop into spots becoming fusiform or elliptical with water soaked borders.

Fifth stage: Is reached when the dark brown or black center of the spots becomes depressed and the spots are surrounded by a yellow halo. At this stage conidia as well as ascospores are produced.

Sixth stage: the center of the spot is light gray and dry. Where the spots coalesce, entire sections of leaves become necrotic and in these sections ascospore production is high. After flowering and fruit production, plants can lose all leaves (Figure 2).

Figure 1. Black Sigatoka symptoms stages.

Figure 2. Severely affected plants in the rainy season.

Early ripening and fruits with creamy pulp are manifested in severely-defoliated plants resulting in rejection at the packinghouse as well as the marketplace (Figure 3).

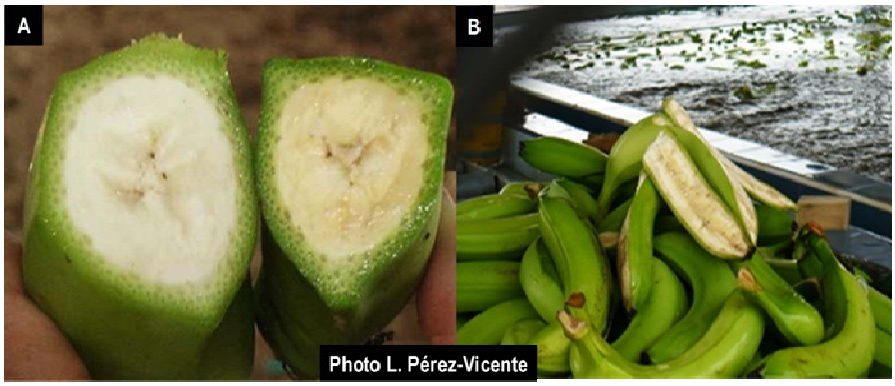

Figure 3. A). Fruits with white and cream pulp from healthy (left) and BS severely diseased plants respectively; B) Rejected fruits in packinghouse due to cream pulp (Photographs: L. Pérez-Vicente).

The pathogen

Mycospherella fijiensis belongs to the class Dothideomycetes, order Capnodiales and family Mycosphaerellaceae and is the most destructive member of the 'Sigatoka disease complex', which includes *M. musicola* (anamorph *Pseudocercospora musae*), the causal agent of Sigatoka leaf spot disease (yellow Sigatoka leaf spot) and *M. eumusae* (anamorph *P. eumusae*), which causes Eumusae leaf spot disease. The three species are hemibiotrophic and heterothallic (Stover, 1963; Mourichon and Zapater, 1990).

The fungus produces multicellular conidia from conidiophores in culture and *in planta*, in the latter case arising from stomata primarily on the abaxial (lower) surface of the infected leaves. Conidiophores arise from hyphae present in the sub-stomatic chamber and can produce multiple conidia from a single conidiophore (Figure 4). Conidiophores are produced on the lower surface of initial specks (Stage 1, Fig. 1A) or at the first streak stage (Stage 2, Fig. 1B) up to the second spot stage (Stage 5; Fig. 1 E). Conidia are produced almost continuously between the second streak (Stage 3; Fig. 2B) and second spot (Stage 5; Fig. 1D)

In black Sigatoka spots samples from Cuba (Pérez-Vicente, 1993; Pérez-Vicente, 1996), **conidiophores** were observed to develop from four to six engrossed cells of the substomatic chamber and emerge through stomata, in fascicles of two to four. The conidiophores are pale brown, straight to variably curved, with a wider basal cell and 0-5 septa, 27-71 x 3-5 µm and with up to six scars at the apex or on a light lateral shoulder. At the 8- to 12-day stage, single-spore isolations cultures developed hyaline conidiophores producing conidia at the extreme (Figure 4). **Conidia** are obclavated, hyaline to brown olivaceous, 5-8 septa, 27-110 x 2-5 µm and with a well

marked hilum that allow a ready dispersal by wind. **Pseudothecia,** which appear in spots at stages 4 to 6, are amphigenous and erumpent, with a papillated dark ostiole, and walls composed by two or three layers of polygonal dark brown cells. They are more abundant on abadaxial side of leaves, with a diameter between 43 and 86.5 µm, with bitunicated hyaline ascae, without paraphyses, with two rows of hyaline, fusiform to cleaved bi-celled **ascospores,** having a larger anterior cell and a marked constriction at the level of septa, 12-15.4 x 2.5-5.0 µm. Spermogonia are more abundant in the abadaxial side of leaves, globose, obpyriform, with pale brown walls of 23-55 µm of diameter with an ostiole slightly prominent that emerges by stomata and have rows of unicellular, bacilliform, hyaline trunked by its extremes spermatia of 2.0-4.5 x1.5-3.0 µm.

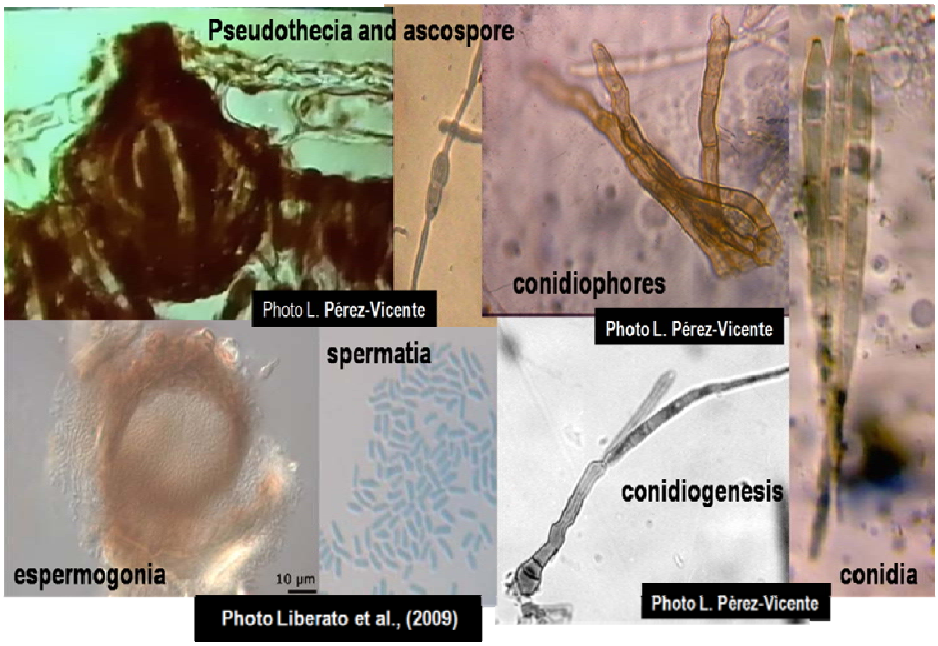

Figure 4. *M. fijiensis* morphologic traits.
Both anamorphs and teleomorphs of *M. fijiensis* can be present on infected leaves concurrently. Abundant development of asexual and sexual spores takes place in warm, rainy weather. Wet leaf surfaces favour spermatia release, followed by fertilization of protopseudothecia and development of ascospores, which are released by rainfall and in the presence of a water film on the leaves (Fig. 5).

The disease cycle of *M. fijiensis* consists of four distinct stages that include spore germination, penetration of the host, symptom development and spore production. After a period of epiphyllic growth of generally 2-3 days, germ tubes penetrate stomata. Under favourable conditions, the first symptoms appear generally 10-14 days after incubation (Fig. 6); the symptoms then gradually evolve from stage 1 (streaks) to first stage of spots (necrosis, transition period) (Fig. 6) and stages 5 and 6 (Fouré, 1982). Conidia are produced in early phases (stages 2-4); short-distance dispersal is primarily by water, although wind-borne dispersal may also occur.

Ascospores, produced at later stages, are wind-dispersed (after the pseudothecia burst) and are dispersed at longer distances than conidia.

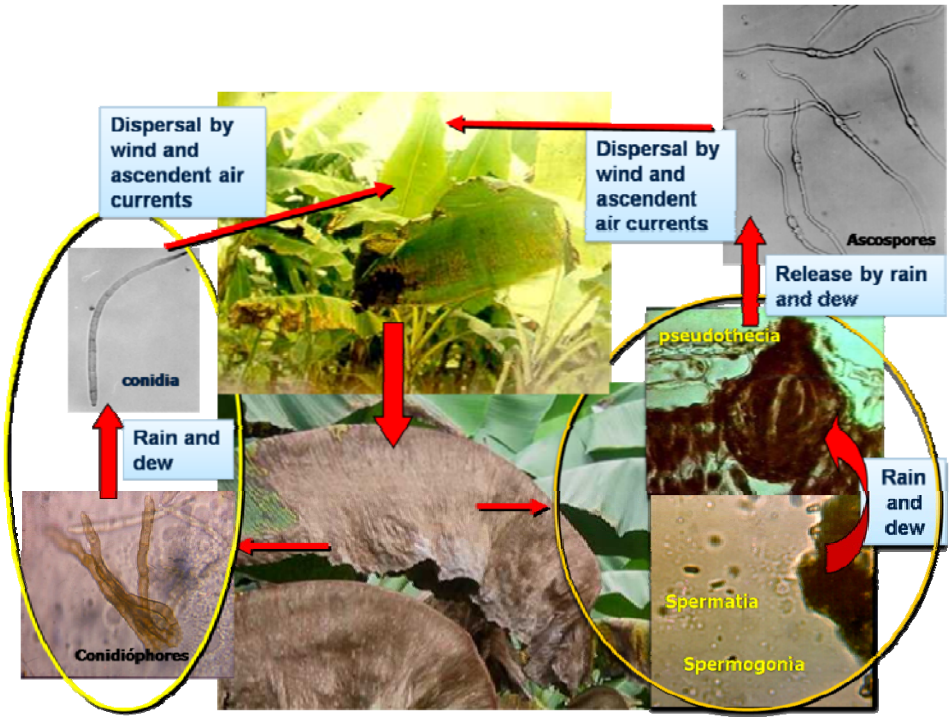

Figure 5. Biological cycle of *M. fijiensis* (Pérez-Vicente, 1998)

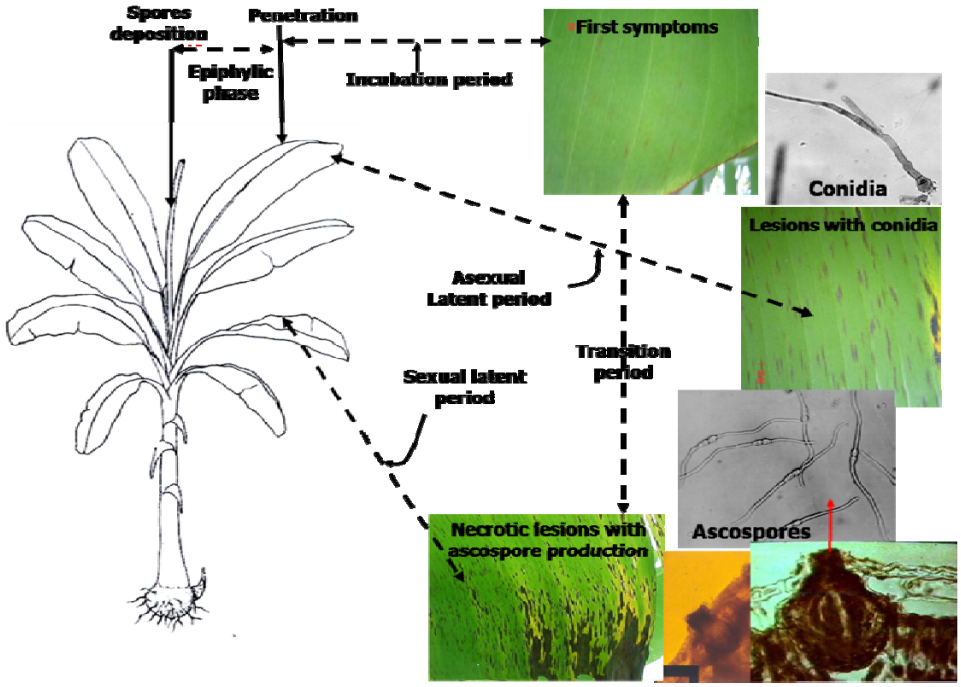

Figure 6. Infection cycle. Incubation and transition periods.

Development of BSD leaf spots is closely related to the rainfall pattern at each site (Calpouzos, 1955;, Pérez et al., 1983; Gauhl, 1990, Pérez et al., 2000). Ascospores can be wind-dispersed over large distances while conidia are dispersed in dew and rain water deposited on the leaves and by splashing. Conidia and ascospores become wind-borne within infected plantations and travel up to several tens of kilometers away from disease sources, but ascospores play the most important epidemiological role in the spread of the disease through wind-borne dispersal (Gauhl *et al.*, 2000)

Duration of incubation, transition periods and intensity of spore production depend on the resistance or susceptibility (reaction) of the host to the pathogen, weather conditions and the severity of infection. With susceptible cultivars (e.g. Cavendish and plantains), warm, humid weather and high inoculum pressure, incubation and transition periods are shorter and the production of conidia and ascospores higher.

The seasonal trends of the BLS epidemic development are closely related to the local rainfall patterns and the successive infection cycles in a growing season, with the accumulated amount and duration of rainfalls over the previous 10 and 14 days (Pérez *et al.* 2000 a and b).

INTEGRATED BLACK SIGATOKA MANAGEMENT PROGRAM

The goal of the present programme is to achieve the control of black Sigatoka disease (BSD) of banana and plantain with a minimum of fungicide applications resulting in lower impact in banana production, lower cost of control, lower risk of fungicide resistance and lower environmental negative impact. The program comprises:

1) Standardization of control measures at the national/regional levels, via organizational and legal measures to eliminate or reduce the sources of inoculum,
2) Cultural practices during the vegetative, reproductive and harvest phases of plant development aimed at boosting plant development and its resistance, making the environment unfavorable to disease development, improving fruit develop quality and reducing disease severity and losses by early ripening,
3) Biological and weather monitoring procedures to determine weekly changes on speed of disease evolution in order to optimize the time of treatments and efficacy assessment of the fungicidal treatments,
4) In areas where fungicide treatments and control measures are not feasible, plant resistant cultivars in the field or backyard,
5) Appropriate use of fungicides from different chemical families and organization of applications at each locality,
6) Fungicide resistance monitoring to prevent losses due to lack of efficacy.

7) Improving the quality of fungicide applications and assessment of the quality of applications

1. Standardization of the control measures at national/regional scale

The management against BSD should be focused on eliminating or reducing inoculum pressure. Management should be organized on an area-wide scale (can include several growers or communities) through the following measures/ mechanisms:

1. Centralization of decisions and operations is essential and the best situation is when banana growers are grouped in an association that undertakes the control strategy. Since ascospores are transported by wind over long distances, the control strategy should be the same in all banana plantations to prevent any disruption. The organization of the treatments is more efficient when a centralization of the decision is performed by a single technical service operating according to rational guidelines rather than if each grower implements his own strategy.
2. Weekly monitoring at geo-climatologically different areas in order to time applications and assess fungicide treatment efficacy and sanitary conditions. The monitoring should be carried out by a well-trained core-team capable of taking treatment decisions based on weather and biological parameters, and the dynamic of the sensitivity of *M. fijiensis* to the fungicides.
3. Well-timed application of effective fungicides with a good coverage of droplets on the leaves including the complete area.
4. Elimination of abandoned fields of susceptible varieties and volunteer plants, which must receive fungicide applications until complete destruction).
5. Systematic sanitation of plants in areas which pose difficulties for good systematic spraying or in unsprayed buffer areas close to houses, buildings or water sources. Replanting of these areas with resistant varieties is the best option, but requires taking into account such aspects as the acceptability of these varieties by the consumers.
6. Monitoring the sensitivity of populations to the main chemical groups of fungicides used in disease management.

To achieve this goal, it is necessary to enforce the Ministry of Agriculture's legal plant protection regulations (Plant Protection Act) and use coercive measures such as imposition of penalties/fines for non-compliance of sanitary measures of eradication-confinement or suppression-containment and management.

2. Cultural practices

Nutrition

A balanced nutrition results in high rates of leaf emergence, leading to better tolerance and possible escape from the effects of *Mycosphaerella* leaf spots.

Banana plants are more prone to higher attack of BS in soils deficient in potassium or with unbalanced N/K relationship (Holderness *et al.*, 1998; Pérez, *et al.*, 2002).

1. Nitrogen. Studies in Costa Rica soils showed a relationship between the annual N supply and the incidence of BSD (Méndez, 1998). Fields with less than 100 kg/ha/ year exhibited twice the severity of BSD compared to fields that received between 200 and 500 kg/ha/year.
2. Potassium. There is an inverse relationship between potassium application and BSD severity. Studies carried out in Costa Rica, showed that fields receiving less than 150 kg/ha/year had more severe BSD attack than those fertilized with rates between 150 and 750 kg/ha/year (Méndez, 1998). In Cuba, it was found that severity of BSD-infected fields with the resistant FHIA-18 hybrid was negatively correlated with the potassium content in soil and leaves (Pérez-Vicente, 2000). Potassium is related to the accumulation of products of high molecular weight as proteins, carbohydrates, etc.
3. Calcium. Studies carried out show that plants with calcium deficiency exhibit more severe BSD symptoms.

In organic production, the use of different systems of composting, vermi-compost and lixiviates of compost, as well as the so-called 'biols' (products of anaerobic and aerobic fermentations with efficient microorganisms, weeds, manure and plant parts) to supply the nutritional needs of plants. These products have not only proved to provide plant nutrition but also when applied to the soil to improve the population of actinomycetes, bacteria and soil microflora and reduce the population of nematodes.

Irrigation

The duration of leaf wetness has a direct influence on the epidemiology of BS and other banana diseases. Water plays a vital role in the disease cycle and a film of moisture is essential for spore germination, germ tube growth and penetration, sporulation and dispersal.

Application of irrigation under the plant canopy is the best method of watering the plants in order to avoid wetting of the leaves and creating high humidity. In fields irrigated with under-canopy systems such as mini-sprinkler, micro-spray and drip irrigation, disease severity is lower than areas with overhead irrigation (Wielemaker, 1990).

Drainage improvement

The quick removal of the excess of water after storms or during periods of heavy rainfall is important in reducing the humidity in the field, in order to prevent BS build-up and avoid root hypoxia and the outbreak of bacterial and fungal root and rhizome rots. Generally, two weeks after rain or storms, there is a severe outbreak of BSD in the fields. Improving drainage to eliminate the excess of water of the fields is essential to reduce high humidity on the air and duration of leaf wetness.

Sanitation. Control of inoculum pressure in the field

Leaf sanitation is achieved through prophylactic leaf removal and has to be carried out coupled with other management practices that boost plant development and disease management such as plant density, nutrition and proper irrigation practices. Systematic, timely pruning of leaves has a marked effect on available inoculum. Mycosphaerella leaf spots reduce the life span of leaves, increasing leaf drying and senescence that are hastened by about 30 days in unprotected plants. On the other hand the incubation period is shorter and symptom development is faster when leaves are inoculated with high concentration of spores (Pérez, 1980).

BSD is more easily controlled in isolated fields that are far from sources of external contamination. Thus, keeping the inoculum sources at low level is important for the success of the chemical control strategy. Furthermore, the aim of sanitation is to reduce the risk of early fruit ripening and aid the chemical control strategy. In the presence of extensive spotting, new infections develop quickly because the fungicide sprays do not remove the pathogen from infected leaves and the only solution is eliminate them mechanically from banana plant through sanitation. The removal of infected leaves reduces the inoculum pressure and enhances the efficiency of fungicide sprays. In turn, this reduces the exposure of the pathogen to fungicides as well as the risk of build up of populations that are fungicide-resistant.

Sanitation practices are particularly necessary in areas regular spraying may not be possible due to natural obstacles, or regulatory measures (e.g. buffer areas where spraying is prohibited close to houses, gardens, rivers, etc.). Leaf removal is mandatory on BSD susceptible plants in home gardens, near houses, along roads and buildings even when is such cases is better to replace with resistant varieties.

Sanitation during vegetative banana growth.

Leaf removal/pruning. The systematic pruning of BSD affected leaves and the removal of fragments of leaf lamina with necrotic spots is important to reduce *M. fijiensis* and other leaf and fruit pathogens that grow saprophytically (Stover, 1972, Pérez-Vicente, 1996). During vegetative growth, partial removal is done when a portion of the leaf is affected, while leaves removed completely when more than 50% of leaf area is diseased. After pruning, the leaves should be placed on the soil underside down, piled one on top the other to reduce ascospore release and windblown spread and aid decomposition (Gauhl, 1994; Pérez-Vicente, 1998) and to reduce the leaf surface capable of release

ascospores. Observations carried out in Cuba (Pérez, 1998) and Costa Rica (Guzmán and Villalta, 2006), showed that ascospore availability is reduced by several weeks in the leaves placed in soil compared to those kept hanging on plants.

Early removal of leaf tips. Under humid weather, leaves are infected in the early stages of emergence. It has been determined that first symptoms appear earlier in the extreme left half of leaf. Conidia production takes place on lesions that develop from early streaks. To cope with this, early leaf removal practice is recommended: this consists of a systematic elimination of 15% of the length of the leaf tip (before the appearance of the lesion) from the 3rd open leaf downwards in the plant, to avoid conidia production and secondary infections (Martínez-Acosta *et al.*, 2006).

Leaf defoliation can however depress plant growth and successive seasons of severe leaf pruning may have detrimental effects on plant growth. The intensity of sanitation should be locally adapted, taking into account the economical and biological efficacy.

Sanitation during the reproductive phase of banana plants.

Controlled leaf pruning during reproductive stage of the plants (at shooting). Soon after flowering, during the change of position of the fingers, the bunch should be clear from transition leaves, bracts, the bunch terminal bud and the rest of flowers of fingers and bagged with sleeves. There is an axiom that disease severity increases as the leaf ages. Vargas, et al. (2009) have shown that early removal of the ninth and older leaves at the time when the bunch changes position, reduces infection (compared to those that not defoliated), increases fruit development and bunch weight and does not affect the growth of flowers.

Harvesting practices

Harvesting schedule and organization. Banana plants do not produce new leaves after flowering. Disease severity increases as the plant ages. To obtain a marketable bunch, it is necessary to have eight or more functional leaves at flowering and no less than six at harvest. To avoid losses caused by creamy pulp (as a result of BSD), a careful control of the following is of great importance:

- Age and grade of the fruit to be harvested in a given week: The age of the fruit has to be strictly controlled. Age of bunches should be precisely controlled with color ribbons at shooting of the flower. Two aspects have to be considered:
  - ✓ Over-grade: The fruits that could not be harvested or packaged in a given week should not be included in the following harvest.
  - ✓ Selective harvesting: when the plant is infected, it is advisable to eliminate firstly the bunch of the badly-affected plants to avoid the risk of early ripening. Fruits of affected plants are physiologically two weeks older than those not affected and can induce a problem of early ripening during

packaging and transport. So, the bulk of fruits should be removed from severely-affected plants prior to harvesting.

- Plant elimination. Plants with poor functional canopy should be eliminated, since they act as inoculum sources and hinder good coverage of treatments on the adjacent plants.
- De-handing intensity at flowering: At the reproductive stage, it is a normal practice to remove some lower hands (de-handing) of the bunch to get faster and uniform grading of the fruit. Under high BS pressure periods, a more rigorous de-handing may be considered, to achieve the harvest grade in a shorter time and help to reduce the effect of the disease on bunch ripening. The severity of de-handing to reach faster the grade for harvest should be adapted locally based on BSD severity and banana growth conditions.

Postharvest treatment of fruits.

To reduce the effect of BSD in ripening after de-handing and fruit washing, gibberellic acid AG3 can be applied at rates between 300-1000 ppm on crowns. These can mitigate the early ripening of the fruits.

3. Biological and weather monitoring procedures to determine weekly changes on speed of BSD development. Bioclimatic warnings.

The objective is to detect as early as possible changes in the speed of development of BSD based on current information on the level of infection and the speed of development, complemented by weather data to forecast the most probable future trend of BSD, and then decide whether or not fungicide applications are necessary.

The bioclimatic warnings for Sigatoka were developed as early as 1955 by French workers in Guadeloupe (Guyot and Cuillé, 1955; Ganry and Meyer, 1972 a and b) and modified for BSD (Ternisien, 1985; Fouré, 1988; Bureau, 1990; Pérez-Vicente, 1996, 1997, 1998; Pérez-Vicente et al., 2000 a; Pérez-Vicente et al. 2000 b). This success story relies on some basic requirements:

- Application of standard procedure over large, well-characterized geo-climatic areas including several growers
- The centralization of decisions in a well-trained and very specialized team
- An integrated approach to control BSD of bananas addressed to reduce inoculum, while reducing fungicide use and environmental impact.
- Rotation of systemic fungicides with different mode of action and a regular monitoring of fungicide resistance in order to adapt the fungicide use strategy.

- Excellent spraying logistics to reduce, as much as possible, the period between the decision of the fungicide application in accordance with the warning system and the completion or execution of applications.
- A strong curative effect of fungicide application resulting from:
  I. good coverage of droplets of the aerial and ground applications (application under suitable weather conditions, with properly-calibrated spraying equipment),
  II. combination of systemic fungicides with mineral oil (oil compatible formulations)
  III. good quality oil and appropriated dosage.
- A strictly localized control of hotspots (through ground sprays or alternated and adapted crop practices).

The basic components of the bioclimatic warnings are:

Weather records:

Weather records are important information to take into account when

- Duration of leaf surface wetness. Most of the events of life cycle of *M. fijiensis* occur when leaves have a film of water on the surface. Pérez et al. (2000 a; 2000 b) reported a high correlation between the duration of leaf wetness and the speed of development of the disease four weeks later in Cavendish banana.
- Rainfall (daily and accumulated in 10 and 14 days). The curve of the amount and duration of rainfall accumulated for periods of 10 and 14 days has a predictive value of the speed of development of the disease four and five weeks later in Cavendish banana and plantains respectively (Jiménez et al., 1994; Thierry et al., 1998; Pérez-Vicente et al., 1998, 2000 a and 2000 b).
- Piche evaporation weighed weekly. Piche evaporation is collected in a simplified shelter and its value is that it integrates the combination of factors affecting the micro-climate at leaf surface (such as air temperature, relative humidity and wind speed) and is negatively correlated to speed of development of the disease (Ganry and Meyer, 1972 b). Because disease development is the result of the prevalent conditions of more than one week, Piche evaporation is weighed weekly to adjust the interval from the date when the last application was done. For Sigatoka, it is very useful to determine the suitability of the conditions for disease development and to forecast and adjust the interval between two consecutive treatments.
- Temperature. Temperature is collected in standard weather station. The speed of development of plants and fungus is closely relationed to temperature (Ganry, 1973, 1975). Guyot and Cuillé (1958) were the first to use Livingstone's thermophysiologic method, $SEV= \Sigma n_i.V_j$, where: n_i is the number of hours at each

temperature in the day and Vj is the constant of growth of the pathogen at the given temperature, according to the law of action of the temperature on the fungus growth. A model to calculate the constant of development of the germinative tubes of *M. fijiensis* as a function of temperature ($V_f=79.35^{e^{-0.015(t-27.13)^2}}$) was developed (Porras and Pérez, 1998), to calculate sums of speed evolutions using Livingstone's termophysiologic method. Temperatures under 20ºC are not suitable for fast disease development. In Cuba, when accumulated sum of speed of development at a particular week is under 12000 units, BSD does not progress and treatments can be stopped. However, *M. fijiensis* has a shorter cycle and a higher production of spores than *M. musicola* and as soon as temperature rises, BSD development speeds up.

Biological records:

Biological assessments are carried out weekly on 10-20 fixed plants at vegetative stage with more than nine expanded leaves, in plots representative of the banana fields at a locality or region. When plants shoot are changed, a new group of representative plants are selected and used in the following evaluations.

Banana development (number of leaves and stage of development of the new emerging leaf.

The success or failure of control measures is the result of a balance between the rate of necrosis formation and the rate of leaf emergence, so is important to know the rate of leaf emergence. If not taken into account, it could result in under- or over-estimation of the real rate of BSD development in the field. So, estimation of the rate of emergence of leaves is important. The development of a plant can be represented by a number composed by an entire part (total number of open leaves) and a decimal (the stage of development of the emerging leaf) that is determined according the phases described in figure 7.

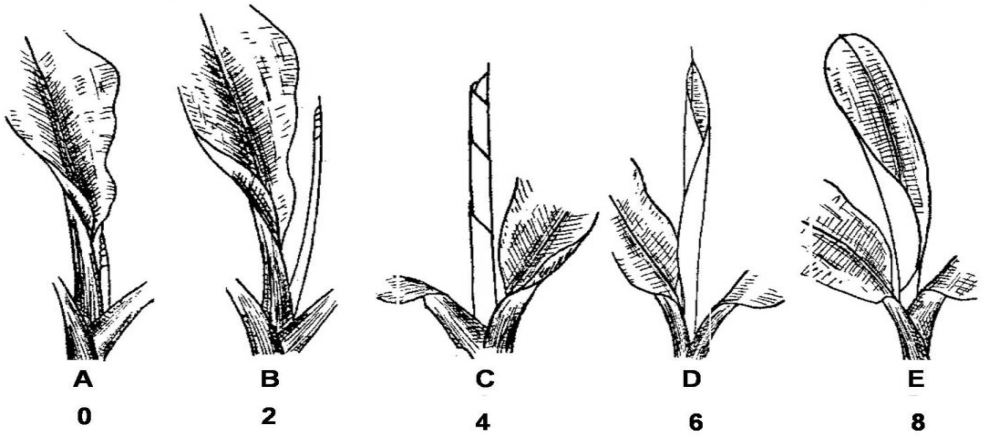

Figure 7. Stages of development of a new leaf according description of Brun (1958).

Leaf emergence rates are determined by the difference between the data of two consecutive assessments. If ten plants are assessed and recorded in an observation

plot, the rate of leaf emergence for a period of ten days is determined by the sum of the difference between the records of two consecutive assessments divided by the number of days between the assessments. If twenty plants are observed, then the sum of the difference between the records of two consecutive assessments is divided by the number of days between the assessments multiplied by two.

BSD development assessments.

A number of assessments should be obtained weekly to provide complementary information

- *Stage of evolution of disease (SED)*. SED is the main parameter of assessment and can detect weekly changes in the development of the disease. It was designed (Ganry and Meyer, 1972 b) for early detection of new attacks of Sigatoka disease in a warning system and was adapted to BSD taking into consideration the density of symptoms and coalescence of spots leading to necrotic development (Fouré, 1988, Ternisien, 1985). SED is also an indicator of the efficacy of chemical control in the youngest banana leaves, and reflects weather conditions and the intensity of the infection. Because ascospore production starts in necrotic tissues, the attacks have to be stopped in the early stages of symptom development before necrosis. Treatments should be decided on the basis of the intensity of the SED curve.
- *Youngest leaf bearing streaks* (YLSt): this is the position of the youngest leaf that exhibits BSD symptoms and reflects the incubation period, which depends on weather conditions and fungicide control. YLSt is scored together with SED. If symptoms do not appear on the fourth-youngest leaf, then the older leaves should be examined. The frequency of plants with (% YLS <5) is negatively correlated with SED and a good appraisal of the shortening of the incubation period and the existence of favourable conditions.
- Youngest leaf spotted (YLS). Should be determined according Stover's method (Stover, 1971). This is the youngest leaf bearing 10 necrotic lesions (stage four or more advanced). If none of the leaves bear lesions, then the arbitrary YLS is N+1; N= total number of leaves of banana plant. All plants should be homogeneous. The parameter is useful to understand the efficacy of the control. If YLS decreases, then the control is failing because necrotic formation is faster than leaf emissions and vice-versa. The frequency of plants with spots in leaves younger than 8 (% YLS <8), should never be higher than 20%.
- *The number of functional leaves at harvest (NLH).* This is a final estimator of the effectiveness of control strategy and an indicator of fruit marketability. A functional leaf should have less than 30% of necrotic surface. Banana companies use an empiric threshold of NLH (3-5) in order to decide which bunches are exportable or not. NLH should be regarded as an indicator of the efficiency of chemical strategy at harvest stage.

Table 1 provides a template for recording data of the field assessments.

TABLE 1. MODEL FOR ASSESSMENT OF BLACK SIGATOKA **PLOT _____** **DATE_____**

PLANT	LCL	LCC	LER	EC	YLSt	yLN	L II				L III					L IV					
							1	2	3	4	1	2	3	4	5	1	2	3	4	5	6
1	16.2	17.0	0.8	0	2	6	+					+					-				
2	16.6	17.0	0.4	0	4	9												+			
3	14.6	15.0	0.4	0	3	7							-								
4	15.2	15.4	0.2	4	3	8							-								
5	15.0	15.4	0.4	0	5	10															
6	15.0	15.8	0.8	8	4	8											-				
7	13.2	14.0	0.8	0	3	6						-						-			
8	12.4	13.0	0.6	0	2	6	-														
9	13.0	13.8	0.8	8	3	7						+									
10	15.8	16.0	0.2	0	4	9						-					+				
11	12.0	12.4	0.4	0	6	11															
12	13.2	14.0	0.6	0	2	6	+						+				+				
13	11.0	12.0	1.0	0	6	9															
14	12.4	13.0	0.6	0	3	7						-					+				
15	11.0	12.0	1.0	0	6	10															
16	13.0	13.4	0.4	12	2	6	-						+					-			
17	15.2	16.0	0.8	0	5	9															
18	12.0	12.8	0.8	8	3	7						+									
19	15.0	16.0	1.0	0	6	10															
20	12.4	12.6	0.2	12	2	7															
TOTAL=			12.4	52	74	158	**+ 80**	**120**	**160**	**200**	**60**	**100**	**140**	**180**	**220**	**40**	**80**	**120**	**120**	**200**	**240**
N x 2 =	14						**- 60**	**100**	**140**	**180**	**40**	**80**	**120**	**160**	**200**	**20**	**60**	**100**	**100**	**180**	**220**
LERi =	0.88						+160					300	280				240	120			
LERpi=(LERp+LERi)/2=					0.74		-120					300	240				120	200			

+ = MORE THAN 50 LESIONS	**BS=** 2020	**ES = (BS-EC)/2 =** 984	**YLSt =** 3.7	**% YLSt (1-5) =** 90
- = LESS THAN 50 LESIONES	**SE4H = Ss x LERpi =** 728	**%HJR<5 =** 70	**YLSp =** 7.9	**% YLS <8 = 5**0

Forecasting strategies should be implemented in specific areas: a) areas free of fungicide resistance, b) new banana areas, and c) low disease pressure areas. Warning systems are more efficient in an environment of low inoculum pressure and in regions with annual rainfall <2000 mm. Such strategies should be implemented under specific conditions: a) banana areas with none or low resistance to available fungicide families or those where adverse climatic conditions exist during a part of the year (dry season); b) availability of curative fungicides with different modes of action that can be used in alternation; c) logistic capacity exist for spraying without delay and in the best conditions; d) strong involvement of managers of plantations, and cooperation of the administration and general population to contribute to the sanitation of banana plants.

The efficacy of the warning system depends on the quality of treatments and the curative effect of the systemic fungicides and does not work in situations where there is a loss of sensitivity and build-up of fungal populations resistant to fungicides. Where fungicide-resistance becomes established, the bioclimatic warnings do not work well and multisite fungicides should be introduced in the schedule. The reintroduction of on-site forecasting strategies relies on possible reversion of fungicide resistance and introduction of fungicides that have a new mode of action, with a high curative effect.

4. Timing and quality of treatments.

The time of fungicide application is determined by field assessments together with an analysis of multiple parameters. Hence, it should be carried out by a highly skilled team. The decision on actions to be taken should be based on the date of last treatment, the accumulation of 10 days of rainfall, and BSD assessments SE4L. A stable increase of ESD in more than 200 units in two consecutive assessments, together with the presence of environmental conditions favorable to BSD indicates the need for fungicide applications within two-three days (Figure 8). In Cuba, the number of sprays and the costs of protection in enterprises that applied bio-climatic system were 31% lower than in farms in the same localities applied on a scheduled program.

Fungicide application can be carried out by airplanes, helicopter, or from the ground with mist blowers attached on tractors or trucks or by motorized knapsack mist blowers for small areas.

Special care should be taken to carry out spraying during appropriate conditions in order to obtain good coverage and active ingredient deposit in vertical leaves (at least 50 drops/cm^2 with a VMD of 150 µm is required). Such conditions usually occur during early mornings or late evenings. Aerial application is not possible on rainy and windy days. To obtain a good coverage, applications should be carried out avoiding hours with temperatures >28ºC, wind speed over 2m/seg and conditions of air inversion

over plants that do not permit a good deposition of the spray on the leaves. Since it is a) important that leaves to be protected are the ones that are vertical and b) fine droplets spray cloud need to penetrate the air cushion over the leaves surface, it is better spray when there is a light wind of 1m/seg. Logistics availablility is therefore essential to optimize spraying during this small window.

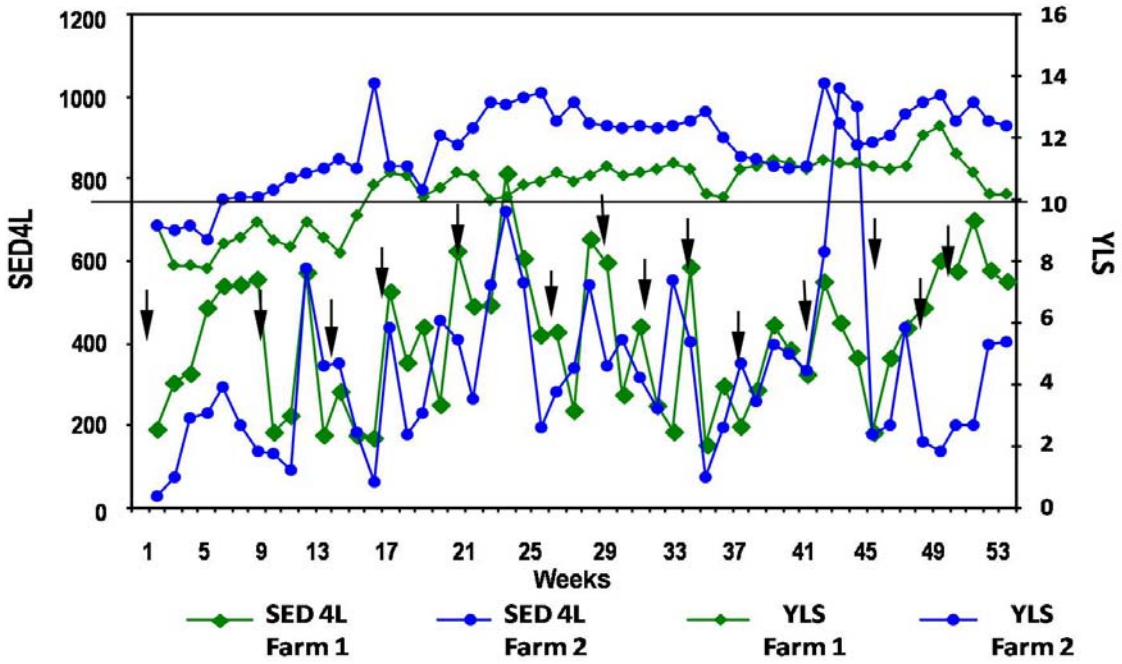

Figure 8. BSD control in Grand Nain (Cavendish) in 'La Cuba Enterprise, Ciego de Avila, 1996 by bio-climatic warnings (Pérez, 1996). Arrows indicate moment of applications of oil in water emulsions of systemic fungicides.

Sprayers and nozzles must be cleaned every day and calibrated frequently (calibration depends on the use of water or oil and on the rate of mixture used per ha). Significant technical improvement has been achieved when aircraft are guided precisely by GPS.

A good synergy is desirable between the spraying company and the growers to obtain the best results from the application. The shape of the fields, the density of plants and the elimination of obstacles are essential to achieve a good quality of treatments.

The use of straight mineral oils or oil in water emulsions as a carrier in the sprays, considerably improves the quality of coverage with low volumes (12–25 L/ha^{-1}). In order to increase productivity, it is essential to protect the maximal area possible in the span of time with good environmental conditions for spraying.

5. Fungicides used in BSD control programmes.

The seven main classes of fungicide in current use are demethylation inhibitors (DMIs), amines, quinone outside inhibitors (QoIs; strobilurins), anilinopyrimidines (APs), benzimidazoles (BCMs), succinate dehydrogenase inhibitors (SDHIs) and guanidines (Anonymous, 2010 b).

1. Protectants (multisite inhibitors):

1.1. Dithiocarbamates: mancozeb, propineb

1.2. Cloronitrilos (phtalonitrilos): clorotalonil

1.3. Guanidines : dodine

2. Systemics (unisite inhibitors)

1.4. *Mitosis inhibitors* (ß-tubulina assembly inhibitors) very high risk of resistance widely present in most countries. Rates of use vary between 150 y 250 g ai/ha.

1.4.1. Methyl carbamate benzimidazoles (benomyl, carbendazim)

1.4.2. Thiophanates: methyl thiophanate, thiophanate.

1.5. *Sterol biosynthesis inhibitors (SBI's)*:

1.5.1. SBI's group I (medium risk of resistance, widely distributed): C_{14}-α demethylase inhibitors (triazole fungicides): bitertanol, difenoconazole, cyproconazole, epoxiconazole, fluzilazole, propiconazole, tebuconazole, triadimenol. Rates of use are between 80 and 100 g ai/ha

1.5.2. SBI's group II (low risk of resistance): Δ14reductase and Δ7-Δ8 isomerase inhibitors (amines).

- Morpholines (tridemorph, fenpropimorph). Rates of use: 450 g ai/ha
- Spiroketalaminas (spiroxamine)

1.6. *Complex III: cytochrome bc1 (ubiquinol oxidase; quinona outside inhibitors) Qol's (very high risk of resistance)*:

1.6.1. Methoxy-acrylates: azoxystrobin

1.6.2. Methoxy-carbamates: pyraclostrobin.

1.6.3. Oximino-acetates. Trifloxystrobin

1.7. *Metionine biosynthesis inhibitors Anilinopyrimidine*s: pyrimethanil (Medium risk of resistance; sensitivity stable in M. fijiensis so far). Rate of use 300-400 g ai/ha.

1.8. *Complex II Succinate dehidrogenase inhibitor*s (SDHI's):

1.8.1. Carboximides: boscalid,

1.8.2. Fluopyram; isopyrazam.

Fungicides are mixed in oil alone or in oil-water emulsions. They can be used singly, in mixtures of systemic with protectants and as mixtures of two systemics with different mechanisms of action and translocation properties in the leaves (so leaves are better and uniformly protected due to different distribution properties of active ingredients).

The use of oil in banana sprays facilitates (1) the production of small droplets (120-300 µm) that do not evaporate before reaching the target and (2) reduction of the final solution/ha. This increases appreciably the productivity of spraying equipment. Among the benefits of using oils are:

- ✓ As adjuvants: improve systemic fungicide uptake by leaves.
- ✓ Improved recovery of the leaf due to the composition of the paraffin, which results in the reduction of surface tension.
- ✓ Improved adherence and the rate of absorption of fungicides in leaf
- ✓ Protection against runoff of fungicides due to low solubility in water.
- ✓ Fungistatic action

Oils have a fungicidal/fungistatic action. Fungicide uptake and distribution in banana are strongly affected by the wax layer on the cuticle of leaves. This wax layer is a barrier to the uptake of fungicides into the leaf tissues. Beside the previously mentioned benefits, the use of oil-fungicide mixtures or fungicide-oil-water emulsions improve the uptake and distribution of active ingredients in the leaves because oil develops a bridge between the cuticle and fungicide molecules.

6. Monitoring *M. fijiensis* population sensitivity to fungicides (fungicide resistance)

Mycosphaerella-Musa spp. Pathosystems form a continuum in space and time. The fungus exhibits a great capacity of recombination and adaptation, so selection pressure for the build-up of population resistant to fungicides is high especially during humid weather when conditions are favorable to spore production.

Special attention should be paid to the management of fungicide resistance that might develop following the repetitive use of curative fungicides with a monosite action. Alternating fungicides with different mechanism of action, or mixtures with contact fungicide, is essential to reduce selection pressure and delay the emergence of such resistance.

Monitoring the degree of sensitivity of the populations has to be carried out periodically to reformulate the strategy of fungicide use. At the very least, monitoring must be done at the beginning and the end of the rainy season to get information: a) of the sensitivity of the population after the dry season, and b) of changes in sensitivity during the rainy season which provides a better environment for spore production and when spraying is more frequent.

Monitoring can be carried out by biological and molecular (PCR) methods. Together with sensitivity tests, it is necessary to collect information of disease severity in the field and the frequency and rates of fungicides used.

A base-line of sensitivity of populations should first be established using biological tests. These consist of sampling infected, diseased leaf fragments (at least 50 pieces /field), which are thoroughly mixed and induced to discharge ascospores on water agar plates amended with different active ingredients. The plates are then incubated for 48 hours. The results are compared with the results from the base-line as well as from previous tests.

Benzimidazoles: the tests are carried out using 0, 0.1, 0.5, 1, 5, and 10 µg/ml of active ingredient. After incubation for 48 hours at 27 ºC, insensitive spores produce normal tubes meanwhile sensitive ones do not germinate or produce short, distorted germ tubes. Sensitive populations are totally inhibited at 5 µg/ml ai. The minimal inhibitory concentration (lowest concentration that inhibit 100 % of spore germination; MIC)

SBI's. With SBI's of Group I (triazole and imidazole), tests are carried out using the concentrations 0, 0.001, 0.003 0.01, 0.03, 0.1, 3 and 10 µg/ml ai. In the case of group 2 SBIs (amines), concentrations to be used are 0, 0.001, 0.01, 0.1, 1 and 10 µg/ml. After discharge of spores, the plates are incubated 48 hours at 27 ºC and the germination stopped by incubating the plates at 5 ºC. The length of the germination tubes of 50 spores are measured and the percentage of inhibition compared to the control plate is calculated. The distribution of frequencies of spores inhibited at each concentration is compared with the distribution obtained from a reference, untreated field.

QoI's. (methoxyacrilates, metoxycarbamates and oximino acetates). Tests are carried out at concentrations of 0, 0.01, 0.03, 0.1 and 1.0 µg ai/ml. After incubation the germination and growth of germination tubes are measured. The % of growth inhibition (GI) is calculated for each strain at a specific concentration. A strain is considered as resistant if germ tube length or GI is over a threshold value. The ED50 of the populations of treated and untreated fields are calculated and compared.

*Metionine biosynthesis inhibitors Anilinopyrimidine*s: pyrimethanil: Concentrations to be tested are 0, 1, 3, 10, 30 and100 µg ai/ml. After incubation, the germination and length of germ tubes are measured. The ED50 of the populations of treated and untreated fields are calculated and compared.

7. Use of resistant cultivars.

Management of BSD by fungicide applications alone is unsustainable over time due to environmental problems linked to their use and the considerable adaptation capacity shown by the fungus. The most sustainable and environmentally-friendly strategy for BSD management is the use of resistant varieties. However, due to difficulties with hybridization and breeding, current banana and plantain production is based on landraces domesticated by man since early times. Partially-resistant banana and plantain cultivars have been developed at FHIA, IITA and CIRAD incorporating genes from highly-resistant wild varieties. A common feature of resistance of these varieties is the longer duration for disease development (longer incubation and transition period between streaks and spots) and a reduction in spore production, resulting in more healthy leaves at harvest.

Unfortunately, the varieties can also exhibit undesirable traits like finger-dropping, soft pulp and low acceptance of flavour by consumers. Some varieties have been accepted and are extensively cultivated. Resistant cultivars are the best solution for BSD hot spots and in areas where spraying is difficult.

The use of resistant varieties also requires phytosanitary surveillance of the production of planting material that can carry not only *M. fijiensis* but also other important diseases.

8. Capacity building and public information.

The execution of the BSD Management Program requires:

- capacitating the growers on best cropping and cultural practices
- training personnel in monitoring skills as well as in leading and organising the treatment programs
- developing a cadre of trained persons in fungicide resistant testing procedures
- public information on the program

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