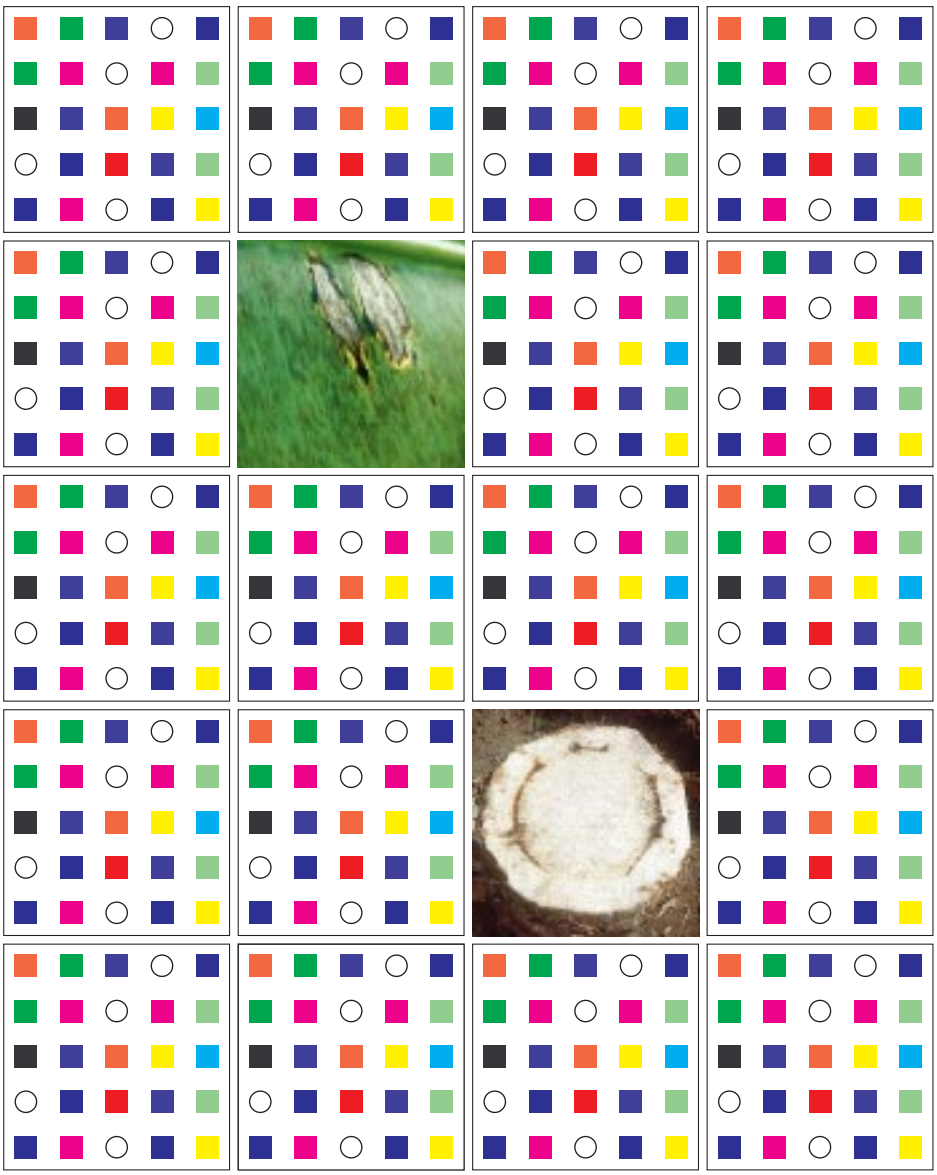
Inibap Technical Guidelines 3

Evaluation of *Musa* germplasm for resistance to Sigatoka diseases and Fusarium wilt

Gisella Orjeda in collaboration with the PRO*MUSA* working groups on Sigatoka and Fusarium



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- to organize and coordinate a global research effort on banana and plantain, aimed at the development, evaluation and dissemination of improved cultivars and at the conservation and use of *Musa* diversity;
- to promote and strengthen regional efforts to address region-specific problems and to assist national programmes within the regions to contribute towards, and benefit from, the global research effort;
- to strengthen the ability of NARS to conduct research on bananas and plantains;
- to coordinate, facilitate and support the production, collection and exchange of information and documentation related to banana and plantain.

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These technical guidelines are a revision and an expansion of IMTP II technical guidelines which were initiated by Sigatoka experts at a meeting organized by INIBAP-LACNET (Colombia, September 1992) and by Fusarium experts at a meeting organized by INIBAP-ASPNET (Taiwan, December 1992). The IMTP II guidelines were finalized by the Sigatoka and Fusarium wilt experts and breeders that attended the 1rst IMTP global conference held in Honduras in 1994 and edited by David Jones.

Revisions have been made on the basis of recommendations and practical comments made by the IMTP II site managers at the 2nd IMTP global meeting held in Guadeloupe in March 1997. The Sigatoka section of the present technical guidelines has been developed with the active contributions of the Sigatoka working group of PRO*MUSA*. The Fusarium wilt section was prepared during a meeting of the Fusarium working group of PRO*MUSA* held in Tenerife in December 1997.

INIBAP wishes to thank all the scientists that contributed to these guidelines.

Special thanks go to Inès van de Houwe who prepared the section on handling *in vitro* plant material, Suzanne Sharrock who assisted with the English editing and Claudine Picq who undertook the style editing of these guidelines.

Cover illustration:
Top: symptoms of black Sigatoka (Dr Eric Fouré, CIRAD-FLHOR)
Bottom: internal symptoms of Fusarium wilt (Dr Zilton Cordeiro, EMBRAPA-CNPMF).

Contents

1. Introduction 5
How to obtain plant material? 5

2. Handling the plant material 6
Proliferating tissue cultures 6
Multiplication 6
Regeneration 7
Handling of *in vitro* rooted plantlets 8

3. Technical guidelines for the evaluation of resistance to Sigatoka diseases 10
Clones to be evaluated for their reaction to black leaf streak / black Sigatoka and Sigatoka / yellow Sigatoka 10
Reference cultivars 10
3.1 Performance evaluation sites 10
3.2 In-depth evaluation sites 14

4. Technical guidelines for Fusarium wilt resistance evaluation 19
Clones to be evaluated for their reaction to Fusarium wilt 19
Reference cultivars 19
4.1 Performance evaluation sites 19
4.2 In-depth evaluation sites 24

Appendices 31
Appendix I. Stages of an unfolding banana leaf 33
Appendix II. Symptoms of black leaf streak / black Sigatoka 34
Appendix III. Symptoms of Sigatoka / yellow Sigatoka 35
Appendix IV. Sigatoka severity scoring and calculations 36
Appendix V. Field forms and database structures for collecting and recording agronomic and environmental data 38
Appendix VI. Field forms and database structures for collecting and recording data of Sigatoka disease resistance trials 43
Appendix VII. Field Forms and database structures for collecting and recording data of Fusarium wilt resistance trials 49
Appendix VIII. How to isolate Fusarium samples for analysis of vegetative compatibility group (VCG)? 58

References 60

List of Contributors 61

1. Introduction

This document gives details of protocols for Sigatoka and Fusarium disease evaluation. For both diseases, two protocols have been established, thus allowing two different levels of evaluation to be carried out. These protocols have been developed in response to a demand from a growing number of national programmes to evaluate germplasm under their local conditions, while at the same time recognising the need to carry out, at a limited number of sites, more detailed research on the pathogens and their hosts. The two types of evaluation are:

(i) **performance evaluations** - a simplified evaluation protocol is used to obtain information on cultivar / hybrid performance under local conditions as well as basic information on disease resistance / tolerance.

(ii) **in-depth evaluations** - more complete disease resistance evaluations are carried out at a smaller number of in-depth sites. These sites will be used to screen new improved hybrids and, if requested by breeding programmes, parental breeding lines. These sites will also provide a location where basic research on the pathogen and its relationship with its host can be carried out.

These guidelines have been written with the collaboration of the Sigatoka and Fusarium working groups of PRO*MUSA* and INIBAP staff with the objective of helping researchers to:

- design their trial,
- choose the appropriate location for the trial,
- artificially infest the trial site in case it is not sufficiently infested with the pathogen (only for Fusarium wilt sites),
- handle propagating tissue,
- handle *in vitro* rooted plantlets,
- harden and transfer the plants into the field,
- evaluate the genotypes for disease resistance / tolerance and agronomic traits.

How to obtain plant material?

A list of virus-indexed material available for evaluation is compiled by INIBAP from which genotypes can be selected. Please contact INIBAP.

2. Handling the plant material

Clones will be supplied by the INIBAP Transit Centre (ITC) preferably as proliferating tissue cultures to laboratories with tissue-culture facilities. Collaborators at these sites will be responsible for producing plantlets for use in the experiments. In those cases where the collaborators do not have tissue-culture facilities, clones can be supplied as tissue-culture-derived rooted plantlets. In both cases plantlets should be grown in plastic bags/pots filled with pasteurized soil or potting mix.

Proliferating tissue cultures

Proliferating shoot-tip cultures are distributed by ITC in sterile single-use transparent polystyrene vials, sealed with a screw cap. The cultures are grown in semi-solid multiplication medium. As soon as possible upon arrival, they should be sub-cultured onto fresh medium.

Proliferating shoot-tip cultures can be used for further multiplication and regeneration of rooted plantlets (suitable for transfer to soil) through sub-culturing on an appropriate growth medium. Relatively high levels of cytokinin in the medium tend to stimulate the formation of multiple shoots and buds whereas the development of individual plants is induced when propagules are transferred to a medium without or with low cytokinin concentration.

The composition of media used for *Musa* micropropagation at the INIBAP Transit Centre is described in Table 1.

Note: Aseptic conditions must be maintained throughout the culture manipulations.

Multiplication

The desired numbers of proliferating propagules can be obtained through repeated sub-culturing the cluster as follows:

- Unscrew the culture vial and flame the brim for a few seconds,
- Gently remove the cluster of shoots and buds out of the vial,
- Separate a cluster into smaller groups of 2 to 3 micro-shoots and/or buds,
- Trim away superfluous corm tissue and blackened tissue. Shorten shoots to a size of 5-7mm in height,
- Transfer each excised group of shoots/buds to fresh pre-sterilized multiplication medium,
- Incubate the cultures at a temperature of 28±2°C with a light intensity of 1,000-3,000 lux, and a 16h photoperiod,
- After 4-6 weeks, when new lateral shoots and/or buds are produced, the procedure can be repeated.

Note: The multiplication rate depends on the genotype of the cultivar and is influenced by the composition of the medium (particularly cytokinins), the explant size and age of culture.

Table 1. Composition of the modified Murashige and Skoog nutrient medium, used for *in vitro* propagation of bananas and plantains at INIBAP Transit Centre.

		mg/l
Macronutrients	NH_4NO_3	1,650
	KNO_3	1,900
	$CACl_2.2H_2O$	440
	$MgSO_4.7H_2O$	370
	KH_2HPO_4	400
Micronutrients	H_3BO_3	6.18
	$MnSO_4.H_2O$	16.90
	$ZnSO_4.7H_2O$	8.60
	KI	0.83
	$Na_2MoO_4.2H_2O$	0.24
	$CoCl_2.6H_2O$	0.024
	$CuSO_4.5H_2O$	0.025
Iron	$FeSO_4.7H_2O$	27.80
	$Na_2.EDTA.2H_2O$	37.22
Vitamins	Glycine	2.00
	Thiamine hydrochloride	0.10
	Nicotin acid	0.50
	Pyridoxine hydrochloride	0.50
Antioxidant	Ascorbic acid	10.00
Carbon source	Sucrose	30,000
Gelling agent	Gelrite®	2,000
	or Agar	8,000
Growth regulators	N^6-benzylaminopurine	2.25*
		0.225**
	Indole-3-acetic acid	0.175

Adjust the pH of the medium to 5.8 before autoclaving.
* for multiplication
** for regeneration

Regeneration

The aim of this step is to produce individual rooted plantlets for establishment in soil.

- Unscrew the culture vial and flame the brim for a few seconds,
- Gently remove the cluster of shoots and buds from the vial,
- Subdivide proliferating clusters into individual shoots,
- Remove superfluous corm tissue and blackened tissue. Trim away the upper part of the shoots to a height of 10mm,
- Place each trimmed shoot onto fresh pre-sterilized regeneration medium, inducing shoot elongation and promoting root growth,
- Grow cultures at an ambient temperature of 28±2°C and in a 12-16h light cycle. High light intensity between 5,000 and 10,000 lux is recommended,

- If new lateral shoots or buds arise, repeat subculturing until individual rooted shoots are obtained,
- Keep rooting shoots in culture for 4-6 weeks,
- *In vitro* plants that are 5-10 cm tall, with at least four leaves and with a well-developed root system are ready for soil planting.

Handling of *in vitro* rooted plantlets

In vitro rooted plantlets are distributed as sterile cultures, grown on a hardening medium in watertight Cultu saks®.

Plantlets that are 5-10 cm tall and have well-developed roots are ready for planting in pots. If plantlets are smaller or if transplanting is not immediately possible, it is advisable to place the plantlets in the Cultu saks® in an upright position under sufficient light (not direct sunlight), at a temperature of between 20 and 30°C. Under these conditions the plantlets can be kept for a few weeks.

To transplant rooted plantlets to the soil requires some care. Please proceed as follows or use your own proven method:

- Cut open each Cultu sak® chamber at one vertical edge,
- Carefully remove the plantlet from the Cultu sak® by holding the base gently with blunt-end forceps. Place the plantlet on the palm of your hand,
- Remove culture medium adhering to the roots and leaves by placing the plantlet in a container of water (bucket) and shaking gently. Do not damage the stem nor the root system,
- Transplant the plantlet to plastic pots or bags (15-cm diameter) filled with a pasteurized mixture 30:70 peat:sand of which the upper 2 to 3cm are fine (sifted). The upper roots should be covered by 2-3cm soil,
- After transplanting, water plantlets immediately.
- Keep the plants in a high humidity atmosphere:
  - A simple humidity chamber can be constructed by enclosing a wooden frame in strong transparent plastic. The humidity chamber (about 40-60 cm high) is placed over the pots in a shaded area where the temperature is kept at 25 to 32°C.
  - The humidity inside the chamber is maintained by spraying water regularly to saturate the air. The heat building-up inside the chamber is minimized by leaving a 2-3cm opening at the base to allow air to circulate.
  - During the first week after transplanting, mist the humidity chamber twice a day to saturate the atmosphere. This is very important as low relative humidity at this stage could easily destroy the plantlets. Water the plants once a day with a little tap water.
  - One week after transplanting, spray the humidity chamber and the plants once a day.
- One month after transplanting, remove the plants from the humidity chamber,

- Keep the plantlets on a raised surface (not directly on the ground) in a nursery in a shaded area until they are about 30cm tall (2-3 months before establishment in the field),
- Re-potting into a larger pot may be required,
- Transplant the plants into the field during the wet season, but not later than six weeks before the onset of the dry season.

3. Technical guidelines for the evaluation of resistance to Sigatoka diseases

CLONES TO BE EVALUATED FOR THEIR REACTION TO BLACK LEAF STREAK/BLACK SIGATOKA AND SIGATOKA/YELLOW SIGATOKA

A list of virus-tested material available for evaluation is compiled by INIBAP from which genotypes can be selected.

Reference cultivars

Yangambi Km 5	Highly resistant	ITC1123
Pisang Ceylan	Partially resistant	ITC0650
Grande Naine	Susceptible	ITC0180
Local cultivar	To be selected by collaborator at each site as an appropriate local standard to compare reactions	

3.1 Performance evaluation sites

Objective

To obtain information on the performance of introduced germplasm in Sigatoka-infested sites under local conditions and using local management practices. A simple evaluation protocol allows information on resistance/tolerance to Sigatoka diseases to be collected for feedback to plant breeders and pathologists. At the same time basic agronomic information of interest to local extension workers and farmers is collected.

Establishment of plots and appropriate location

Fields must be established in areas where there is sufficient presence of the pathogen. The evaluation site should be located in a farmer's field and it should be managed according to local banana growing practices. Moreover, the field layout must include plots of susceptible clones interspersed among those being evaluated. Susceptible local clones can be used. One possible example of a field layout is shown in Figure 1.

Experimental design

Once plants reach 0.3-0.5m in height, they can be planted in the experimental field. Experimental design will depend on the particular site characteristics and alternatives are described below. Where practical and possible, a randomized complete block design should be used. However, whichever the design chosen, plant density should not exceed 2,000 plants per hectare.

Completely randomized design (CRD)

A completely randomized design should be used when there is no identifiable source of variation at the evaluation site, i.e. the site is uniform for soil type, slope, planting date etc. This is a very powerful design, which allows statistical analysis even when there are different numbers of experimental units per treatment.

Plant spacing of 2m x 2.5m is recommended, but plants may be spaced differently if this is more appropriate for the normal management practices of the institute / farmer.

Layout

8-10 replicates per treatment. The experimental site should be surrounded by a row of border plants. Layout should be in quincunx (interspersing susceptible plants) as shown in Figure 1.

Randomized complete block design (RCBD)

A RCBD may be used when there is an identifiable source of variation and the experimental units can be meaningfully grouped. The object of grouping is to have the units in a block as uniform as possible so that observed differences will be largely due to the treatments (in this case to the genotypes). The source of variation, such as slope, pH gradient, planting date etc. should be identifiable and blocks should be arranged to account for this variation. Blocks, also called replications, should consist of a nearly square group of plots.

Layout

Three to four blocks, or replications should be planted. All genotypes should be randomly assigned to the plots within blocks. Each plot should have three to six plants. The number of blocks and plants per plot will depend on land and human resources available. Layout as shown in Figure 2.

For more information and advice on experimental design, please contact the IMTP coordinator at INIBAP.

Agronomic practices and duration of trial

Fertilization and irrigation should be optimized to the greatest extent possible. Do not apply any kind of fungicides by any means. In areas where it is normally practised, mats should be desuckered every three months using local methods leaving only the follower sucker. Experiments should be conducted for the plant crop and first ratoon.

Disease evaluation data

Only two variables should be recorded: Youngest leaf spotted at flowering (Vakili 1968) and number of standing leaves at flowering. These two variables should be recorded for every plant. Every plant in the trial should be assigned a unique identifier that will facilitate identification and follow-up of the plant. This identifier should be an integer between 1 and the total number of plants in the experiment assigned in a correlative way. Field form 4 in Appendix VI is provided as a model for the recordings. Please also record the block or replication number. This system enables data to be analysed using sub-sampling, which allows greater precision.

Figure 1. Completely randomized design in quincunx for Sigatoka diseases evaluation

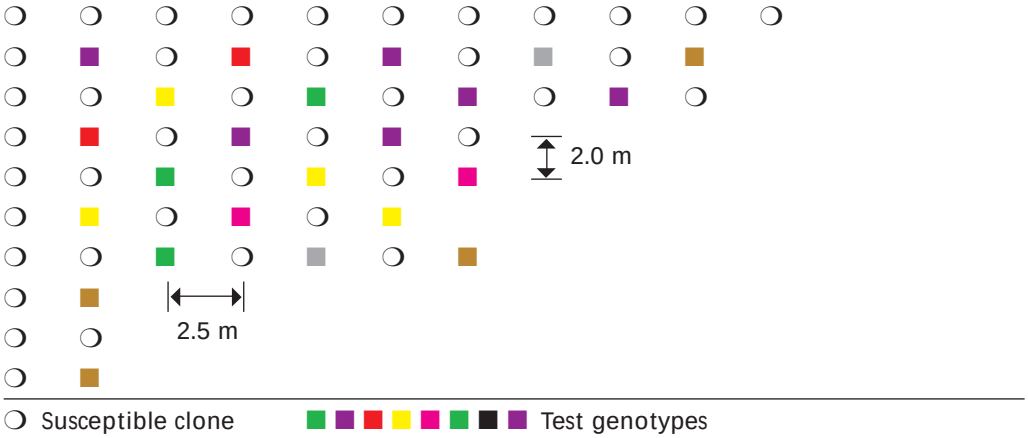
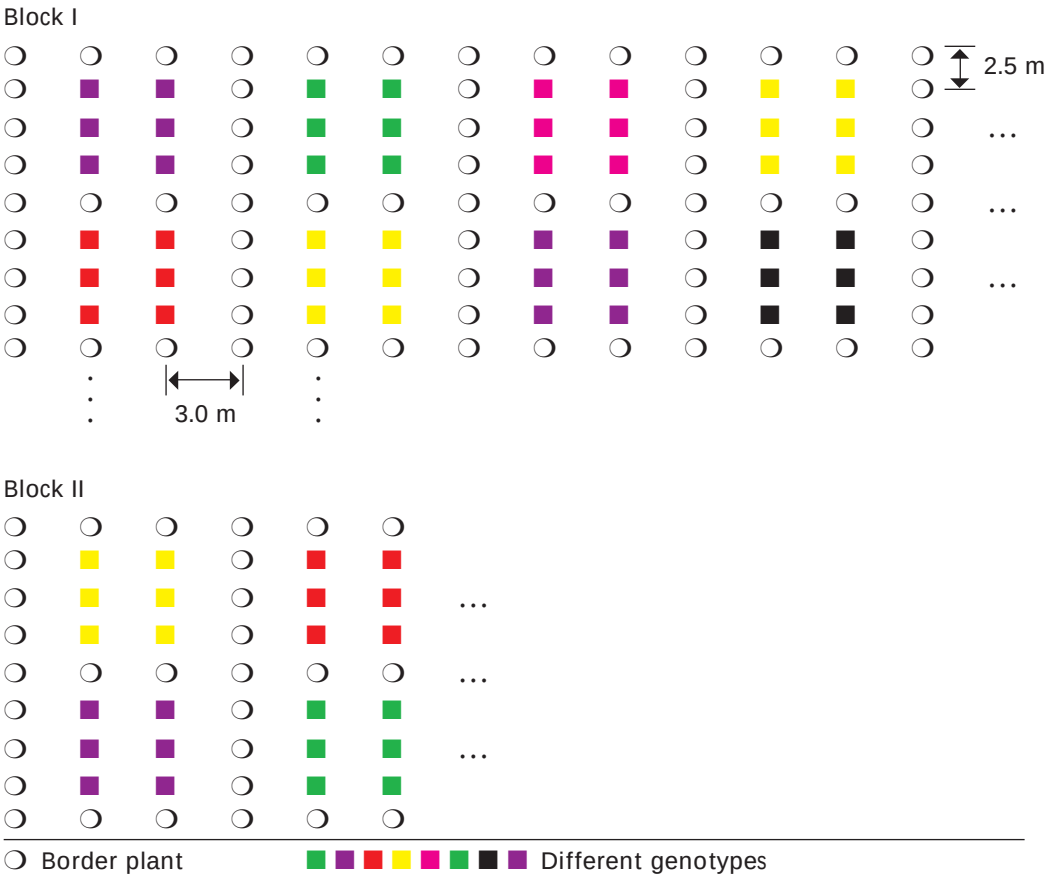


Figure 2. Randomized complete block design, layout of trial for Sigatoka evaluation



The two variables recorded will permit the calculation of the index of non-spotted leaves (INSL) (Ortiz *et al.* 1997) using the following equation:

$$\text{INSL} = \frac{100 * (YLS - 1)}{NSL}$$

where: YLS = Youngest leaf spotted
NSL = Number of standing leaves and,
INSL = Index of non-spotted leaves

Definitions

Youngest leaf spotted (YLS): The first leaf showing at least 10 spots with a necrotic dry centre.

Number of standing leaves (NSL): The number of leaves counting from the youngest (i.e. the highest and unrolled) leaf downwards. Do not count those leaves with the petiole bent back, other leaves should be recorded regardless of their colour or the colour of their petiole.

Index of non-spotted leaves (INSL): The proportion of standing leaves without the typical late-stage symptoms of black Sigatoka; that is a black spot with a necrotic centre. This index provides an estimation of available photosynthetic leaf area prior to fruit filling and is a measure of Sigatoka resistance in *Musa*. It also corrects the difference in the number of leaves produced by different types of bananas and plantains.

Agronomic data

The following data (INIBAP-IPGRI / CIRAD 1996) should be taken for all plants; you may use Field form 1 in Appendix V and the corresponding database structure to input your data in a database:

- **Date of planting**
- **Time from planting to shooting *(d)***
  This can be calculated in days derived from the dates of planting and bunch emergence.
- **Plant crop cycle *(d)***
  This can be calculated in days derived from the date of planting and harvest.
- **Pseudostem height at shooting (bunch emergence) *(cm)***
  This is the distance in cm from the ground to the angle made between the bunch stalk and bunch cover leaf.
- **Pseudostem girth at harvest *(cm)***
  Measured at 1m from base of pseudostem
- **Height of 'following' ratoon at shooting *(cm)***
  This is the distance in cm from the ground to the junction between the youngest and next youngest leaf of the 'follower' sucker at the time the bunch emerges from the mother plant. All other suckers except the 'follower' sucker should be rogued as they appear.

- **Bunch weight *(kg)***
  Cut the bunch stalk (peduncle) above the first hand at the level of the last scar and immediately below the last hand.
- **Number of hands in bunch at harvest**
  Cut the hands from each bunch following weighing, and record the number of hands.
- **Number of fruits at harvest**
- **Fruit weight *(g)***
  Average: Weigh all the hands cut from the peduncle and divide by the number of fruits.
- **Number of functional leaves at flowering**
  Functional leaves are leaves that have photosynthetic activity. Consider that a leaf is functional if it has more than 50% of green area.
- **Number of functional leaves at harvest**

Environmental data

The following data should be collected weekly from the nearest weather station. Field form 3 for recording this information is provided in Appendix V:

- Rainfall,
- Highest temperature,
- Lowest temperature,
- Average temperature,
- Highest relative humidity,
- Lowest relative humidity,
- Average relative humidity,
- Number of days of rain,
- Number of hours during which average humidity reached 90% or more.

3.2 In-depth evaluation sites

Objective

To carry out detailed evaluations of new improved hybrids and, if requested by breeding programmes, parental breeding lines, in order to obtain information on their resistance / tolerance to black and yellow Sigatoka.

In addition, these sites may be used for studies designed to answer key questions about the pathogen, disease and host. Such research may include epidemiological studies on pathogen populations, studies on host-pathogen relationships for different races of the pathogen and adaptability and productivity studies.

The following guidelines apply specifically to resistance / tolerance, adaptability and productivity evaluations.

Establishment of plots and appropriate location

Fields must be established in areas where there is sufficient presence of the pathogen. Moreover, the field layout must include plots of susceptible clones

interspersed among those being evaluated. Susceptible local clones can be used.

Experimental design

The experiment is a randomized complete block design with 4-6 plants per plot and five replications. Note that clones are independently randomized within each of the five replications. One possible example of a field layout is shown in Figure 2. The layout of blocks in the field should aim to minimize expected variations (e.g. soil changes, such as pH). There should be a 2.5m-space between plants in each row and 3m between rows.

Agronomic practices

- The trial should be managed according to the local agronomic practices of the collaborating organization,
- All management practices should be applied uniformly over the whole trial site,
- Leaf spot diseases should not be controlled,
- Experiments should be conducted for the plant crop and next first ratoon.

Data to be recorded

The data to be recorded are summarized by plant stage. Detailed guidelines and forms follow.

Planting

- Date of planting,
- Name of surveyor.

Growing Phase (three months after planting to shooting)

- Disease development time,
- Youngest leaf spotted,
- Leaf emission rate,
- Disease severity (six months after planting).

Shooting

- Disease severity,
- Time from planting to shooting *(d)*,
- Pseudostem height at shooting (bunch emergence) *(cm)*,
- Height of ‘following’ ratoon at shooting *(cm)*,

Shooting to harvest phase

- Youngest leaf spotted.

Harvest

- Disease severity,
- Plant crop cycle *(d)*,
- Pseudostem girth at harvest *(cm)*,
- Bunch weight *(kg)*,

- Number of hands in bunch at harvest,
- Number of fruits at harvest,
- Fruit weight *(g)*,

Environmental factors
- Environmental data.

Management factors
- Management practices.

Details of data to be collected

Generalities

The evaluation of Sigatoka resistance requires knowledge of the stages of both the process of leaf unrolling and symptom development. These are explained in Appendices I, II and III. Readings for the evaluation of resistance should begin three months after planting. Every test plant, except the extra plants at the ends of rows, should be used for data collection. Agronomic traits should also be recorded at shooting and at harvest. These are straightforward standard practices. It is also necessary to monitor the climatic conditions in a weather station as close as possible to the fields. Daily readings should be taken as early as possible every day. Climatic recordings should be made at the same hour. For your convenience, forms and database structures are provided in Appendices V and VI.

Disease development time (DDT)

Definition

The DDT is the time measured in days between stage B of cigar leaves and the appearance of 10 or more discrete mature necrotic lesions in that leave.
- Brun's stage B is a stage of the unrolling banana leaf (see Appendix I)
- A mature lesion of black leaf streak / black Sigatoka is defined as stage 6 by Fouré (Appendix II).
- A mature lesion of Sigatoka / yellow Sigatoka is defined as stage 6 or the 'third spot stage' by Meredith (Appendix III).

Field form 5 in Appendix VI is provided to make your recordings of DDT. You should use as many forms as you have plants in your experiment and use the same form for each plant throughout the cycle.

Procedure
- Plants should be inspected once a week. Plants with cigar leaves near Brun's stage B should be selected and marked (indelible black felt-tip pen, coloured ribbon, tags) with the date at which it was estimated that the leaf was at Brun's stage B. Annotate the date in the form.
- These leaves should be inspected once a week until the ultimate necrotic stage in the disease (stage 6) or one large necrotic area with 10 or more light-coloured dry centres is visible. This date should be recorded. The time at which mature lesions appear should be estimated if this occurs between

inspections. The DDT in days can then be worked out for this leaf and recorded on the form.

- This process should be repeated every week, i.e. every week plants with cigar leaves at Brun's stage B are selected for observations. Observations to check for 10 mature lesions on these leaves should also be made once a week.

Youngest leaf spotted (YLS)

Definition

This is the first fully unfurled leaf with 10 or more discrete, mature, necrotic lesions or one large necrotic area with 10 light-coloured dry centres, counting leaves down from the top of the plant.

Procedure

- This information should be recorded for each leaf that has been used to assess DDT (see above). After shooting, when leaves cease to be produced, the YLS value should be recorded weekly until harvest in Field form 5, Appendix VI.

Leaf emission rate (LER)

Definition

LER is a rate that expresses the 'number of leaves produced per week' and should be less than one.

Procedure

- This should be calculated regularly (at least monthly) for each test and reference plant beginning three months after planting and finishing at bunch emergence (shooting).
- LER can be worked out from DDT readings. Count the number of leaves produced between marked leaves at Brun's stage B on each plant and divide by the number of weeks between observations. Record your calculation in Field form 5, Appendix VI.

Disease severity

Definition

Disease severity is the amount of leaf area affected by the Sigatoka pathogen. It can be expressed in percentage or in disease grades.

Procedure

- Leaves should be scored for disease levels using the Gauhl's modification of Stover's system (see Appendix IV).
- Percentage of leaf area affected by the Sigatoka pathogen expressed as disease grades should be recorded for each leaf on each test plant (see Appendix IV).
- This information should be recorded:
  - six months after planting,

– at bunch emergence (shooting),
– at harvest.

- Only upright leaves should be recorded.

After Sigatoka severity has been recorded, the infection index for each test plant should be calculated as shown in Appendix IV. Field form 6, Appendix VI is provided to help you to record these data.

Agronomic traits

Please refer to page 13 for a complete list of variables to evaluate and to Field form 1 in Appendix V to record these data.

Sigatoka disease ratio

- Leaf samples should be collected from each accession six months after planting and examined to determine the ratio of black leaf streak / black Sigatoka and Sigatoka / yellow Sigatoka lesions.
- In most locations, it is expected that only one pathogen will be present, but both are possible at some sites where black leaf streak / black Sigatoka and Sigatoka / yellow Sigatoka may coexist.
- The ratio of black leaf streak / black Sigatoka to Sigatoka / yellow Sigatoka should be expressed as a percentage.

Remark: it is not always easy in the field and on a same leaf to differentiate between yellow Sigatoka and black Sigatoka symptoms. Although today's situation is not a very stable one and may undergo developments in the months to come (cf. Caribbean area), it would be preferable at the project initiation phase to choose well-defined sites. Furthermore, in the event of mixing two pathogens, it would be difficult to compare results obtained on this site with those of other evaluation sites.

Environmental data

- Environmental data should be collected from the weather station nearest the trial. Where trials are conducted in the grounds of collaborating institutes, this should not be a problem, as many centres have recording equipment.
- Daily fluctuations in temperature and in humidity should be monitored. Rainfall could be calculated for the week if daily readings cannot be taken.
- Readings should begin at planting and continue until harvest.
- The soil of the test site should be analysed.
- A climatic map on the long-term climatic trend should be provided, if possible, to give an overview of the annual fluctuations of temperature and rainfall.
- A format for recording environmental data is provided on Field form 3, Appendix V. For the complete list of variables refer to page 14.

Management practices

- Details of fertilizer application, nematode / weevil control measures and irrigation / drainage management should be recorded.

4. Technical guidelines for Fusarium wilt resistance evaluation

Clones to be evaluated for their reaction to Fusarium wilt

A list of virus-tested material available for evaluation is compiled by INIBAP from which genotypes can be selected.

Reference cultivars

Gros Michel (AAA)	Race 1 susceptible	ITC1122
Bluggoe (ABB)	Race 2 susceptible	ITC0643
Cavendish (AAA) cv. Williams or cv. Grande Naine	Race 4 susceptible	ITC0365
cv. Rose	Resistant	ITC0712
Local cultivar (if this is not Cavendish)	To be selected by collaborator at each test site as an appropriate local standard.	

4.1 Performance evaluation sites

Objective

To obtain information on the performance of introduced germplasm in Fusarium wilt infested sites under local environmental conditions, using local management practices. A simple evaluation protocol allows information on resistance/tolerance to Fusarium wilt to be collected for feedback to breeders and plant pathologists. At the same time basic agronomic information of interest to local extension workers and farmers is collected.

Establishment of plots and appropriate location

For a meaningful evaluation of germplasm resistance to Fusarium wilt, it is important that the evaluation site be located in an area of known occurrence of Fusarium wilt. If possible, the evaluation site should be located in a farmer's field and it should be managed according to local banana growing practices. Prior to establishing the evaluation trial, the identity of the particular strain of *Fusarium oxysporum* f.sp. *cubense* (*Foc*) at the site must be determined. If this is not known, samples should be collected from banana plants at the site and sent to a recognised laboratory for analysis. Full details of how to send samples for analysis are given in Appendix VIII. The site should be uniformly infested with only one strain of *Foc*. If possible, a clone of broad susceptibility (e.g. Gros Michel, Silk or the local susceptible variety) should be grown on the site for one season prior to establishing the trial. At harvest, these plants should be chopped and ploughed back into the soil. Before planting the experiment, the site should be deeply ploughed to evenly distribute the inoculum throughout the site. Care must be taken not to infest the site with more than one strain of *Foc*. To avoid contamination with other strains, it is recommended that infected plants and soil from other sites **not** be introduced into the evaluation site.

At sites which have not previously been used for evaluation for Fusarium wilt resistance, it is strongly recommended that the global standard reference clones (Gros Michel, Bluggoe and Cavendish cv. Williams or Grande Naine) be included for comparison.

Experimental design

Once plants reach 0.3-0.5m in height they can be planted in the experimental field. Experimental design will depend on the particular site characteristics and alternatives are described below. Where practical and possible, a randomized complete block design should be used. However, whichever the design chosen, plant density should not exceed 2,000 plants per hectare.

Completely randomized design (CRD)

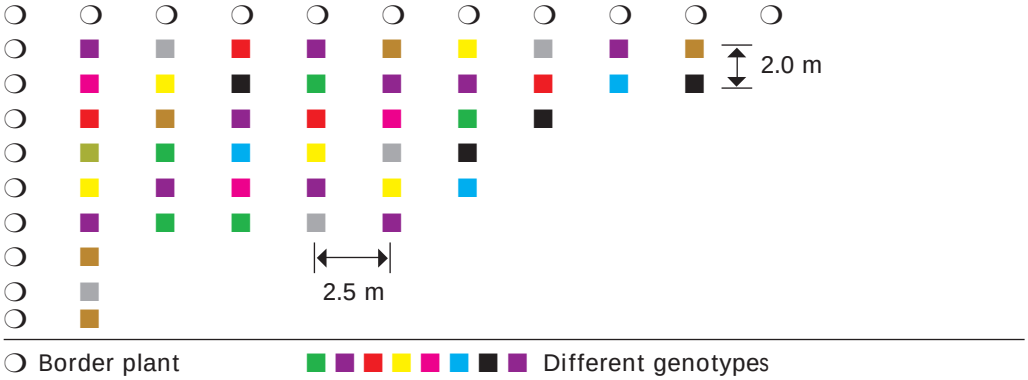
A CRD should be used when there is no identifiable source of variation at the evaluation site, i.e. the site is uniform for soil type, slope, planting date etc. This is a very powerful design, which allows statistical analysis even when there are different numbers of experimental units per treatment.

Plant spacing of 2m x 2.5m is recommended, but plants may be spaced differently if this is more appropriate for the normal management practices of the institute / farmer.

Layout

Twenty replicates per treatment. The experimental site should be surrounded by a row of border plants. Layout as shown in Figure 3.

Figure 3. Completely randomized design for evaluation of Fusarium wilt



Randomized complete block design (RCBD)

A RCBD may be used when there is an identifiable source of variation and the experimental units can be meaningfully grouped. The object of grouping is to have the units in a block as uniform as possible so that observed differences will be largely due to the treatments (in this case to the genotypes). The source of variation, such as slope, pH gradient, planting date etc. should be identifiable and blocks should be arranged to account for this variation. Blocks, also

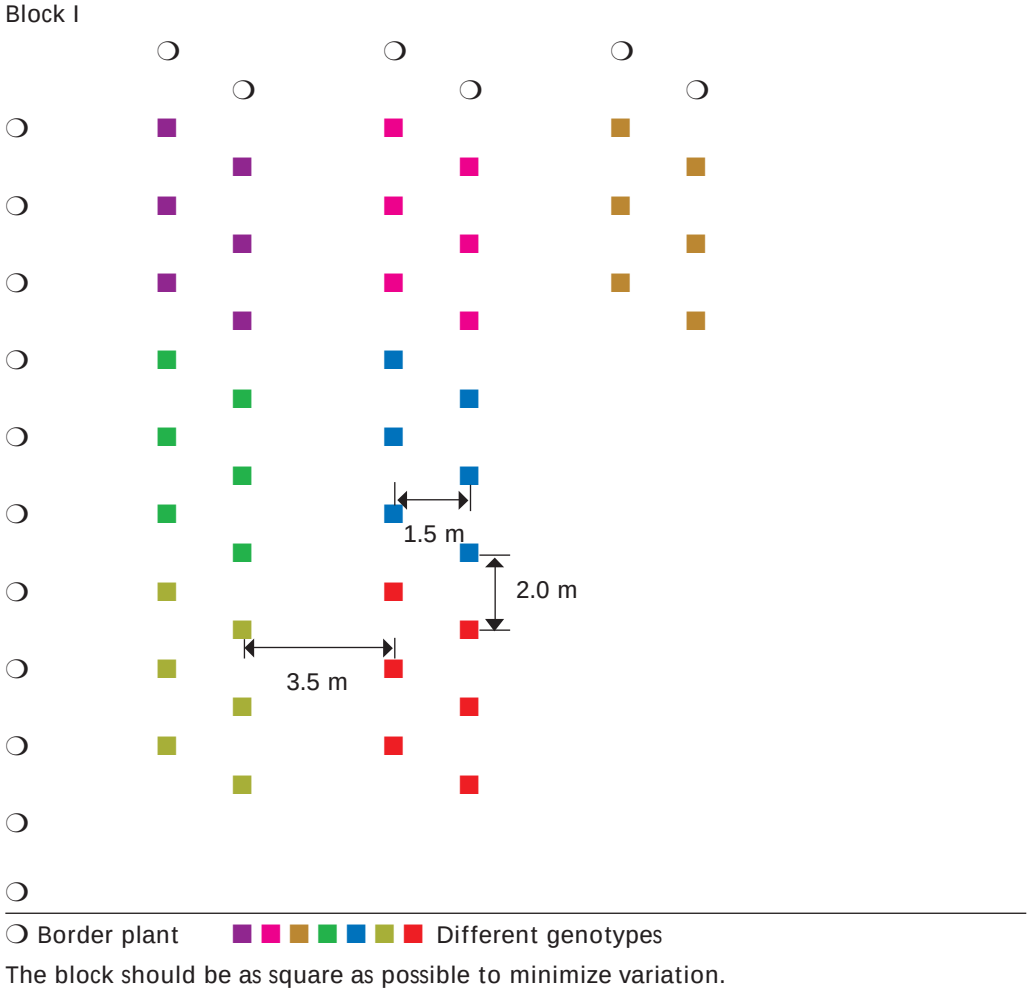
called replications should consist of a nearly square group of plots. Plots within blocks should be arranged in double staggered rows, as shown in Figure 4 in order to minimize competition effects between the different treatments (genotypes). There should be at least six plants per plot but data will be taken only from the central plants; the two outer plants are considered to be border plants.

Layout

Five blocks or replications should be planted. All genotypes should be randomly assigned to the plots within blocks. Layout as shown in Figure 4.

For more information and advice on experimental design, please contact the IMTP coordinator at INIBAP.

Figure 4. Randomized complete block design, plot layout for performance evaluation sites, Fusarium wilt.



The block should be as square as possible to minimize variation.

Agronomic practices and duration of trial

Fertilizer application and irrigation should be carried out according to local practices. Fungicides must not be applied. In areas where it is normally practised, mats should be desuckered every three months using local methods leaving only the follower sucker. Likewise, deleafing should be practised if and when it is normally conducted in the test location. Experiments should be conducted for the plant crop and first ratoon.

Disease evaluation data

On a monthly basis, beginning three months after planting, plants should be observed for symptom development. Plants, which are clearly affected by Fusarium wilt, should be recorded as diseased on Field form 7, Appendix VII and marked for future reference. Thereafter, these plants need only be observed either when they die or produce fruits. Fields should be inspected more frequently (weekly) near to flowering time in order to accurately determine the flowering date. If plants are going to die before yielding fruit, they should be examined internally to verify the presence of the disease. If plants ultimately yield fruit, internal ratings of disease severity should be taken at harvest.

For the plant crop cycle, internal symptoms for the pseudostem only should be collected as follows:

At harvest, the plant should be cut at the base of the pseudostem and the extent to which vascular discoloration extends up the pseudostem should be determined by making transverse cuts from the base of the pseudostem upwards and examining the internal tissues following each cut. The point at which discoloration is no longer visible should be noted and the distance from this point to the pseudostem base recorded. Use Field form 7.

For the first ratoon crop, symptoms within the pseudostem, and if practical, within the corm, should be assessed as described for the in-depth evaluation sites (See page 27). Use Field form 11 if you decide to record data from the corm.

Agronomic data

The following minimum data (INIBAP-IPGRI / CIRAD 1996) should be recorded for all plants on Field forms 1 and 2. If you have used a completely randomized design, discard the column plant #. If you have used a randomized complete block design, use the column plant # to take individual measurements of each plant in the plot.

- **Date of planting**
- **Time from planting to shooting *(d)***

  This can be calculated in days derived from the dates of planting and bunch emergence.
- **Plant crop cycle *(d)***

  This can be calculated in days derived from the date of planting and harvest.

- **Pseudostem height at shooting (bunch emergence)** ***(cm)***
  This is the distance in cm from the ground to the angle made between the bunch stalk and bunch cover leaf.
- **Pseudostem girth at harvest** ***(cm)***
  Measured at 1m from base of pseudostem.
- **Height of 'following' ratoon at shooting** ***(cm)***
  This is the distance in cm from the ground to the junction between the youngest and next youngest leaf of the 'follower' sucker at the time the bunch emerges from the mother plant. All other suckers except the 'follower' sucker should be rogued as they appear.
- **Bunch weight** ***(kg)***
  The bunch stalk (peduncle) above the first hand at the level of the last scar and immediately below the last hand.
- **Number of hands in bunch at harvest**
  Cut the hands from each bunch following weighing, and record the number of hands.
- **Number of fruits at harvest**
- **Finger characteristics** (Field form 2 optional for performance sites, highly desirable for in-depth sites)
  Length, diameter, and weight of individual fingers should be taken for the third and seventh hands. For varieties with a small number of hands (e.g. plantains) these measurements should be made on the second oldest and second youngest hands.
- **Fruit weight** ***(g)***
  Average: Weigh all the hands cut from the peduncle and divide by the number of fruits.
- **Number of functional leaves at flowering**
  Functional leaves are leaves that have photosynthetic activity. Consider that a leaf is functional if it has more than 50% of green area.
- **Number of functional leaves at harvest**

Environmental data

The following data should be collected weekly from the nearest weather station; you may use Field form 3 in Appendix V.

- Rainfall,
- Highest temperature,
- Lowest temperature,
- Average temperature,
- Highest relative humidity,
- Lowest relative humidity,
- Average relative humidity,
- Number of days of rain,

- Number of hours during which average humidity reached 90% or more (only if this data is available).

4.2 In-depth evaluation sites

Objectives

To carry out detailed evaluations of new improved hybrids and, if requested by breeding programmes, parental breeding lines, in order to obtain information on their resistance/tolerance to Fusarium wilt.

In addition, these sites may be used for studies designed to answer key questions about the pathogen, disease and host. Such research may include epidemiological studies on pathogen populations, studies on host-pathogen relationships for different races of the pathogen and adaptability and productivity studies.

The following guidelines apply specifically to resistance/tolerance, adaptability and productivity evaluations.

Establishment of plots

A site should be selected that has a severe natural infestation of Fusarium wilt (*Fusarium oxysporum* f.sp. *cubense* - *Foc*). Edaphic factors such as soil type and soil pH should be determined. The soil should also be analysed for mineral content. The cropping history of the site should be documented.

If possible, inoculum levels of *Foc* should be further increased by growing a crop of appropriate susceptible cultivars on the site prior to establishing the trial. Diseased pseudostems/corms should be cut up after harvest and incorporated into the soil. Before planting the trial, the site should be deeply ploughed and ripped to evenly distribute the inoculum. The soil should be tested for nematodes and if severe infestations are found, treated to reduce populations.

Planting should occur in shallow furrows or basins. These should be filled with soil once the plants are established. For successful establishment, plants will need to be monitored to ensure soil in the root zone remains moist. Ideally, an irrigation system should be installed. Fertilizer should be added to the soil according to local requirements or based on the results of soil analysis to boost growth in the early stages. Measures should be taken during establishment to prevent losses from weevil borers.

Experimental design

Once plants reach 0.3-0.5m in height they can be planted in the experimental field. Experimental design will depend on the particular site characteristics and alternatives are described below. Where practical and possible, a randomized complete block design should be used. However, whichever the design chosen, plant density should not exceed 2,000 plants per hectare.

Completely randomized design (CRD)

A CRD should be used when there is no identifiable source of variation at the evaluation site, i.e. the site is uniform for soil type, slope, planting date etc. This is a very powerful design, which allows statistical analysis even when there are different numbers of experimental units per treatment.

Plant spacing of 2m x 2.5m is recommended, but plants may be spaced differently if this is more appropriate for the normal management practices of the institute / farmer.

Layout

Twenty replicates per treatment. The experimental site should be surrounded by a row of border plants. Layout as shown in Figure 3 p. 20.

Randomized complete block design (RCBD)

A RCBD may be used when there is an identifiable source of variation and the experimental units can be meaningfully grouped. The object of grouping is to have the units in a block as uniform as possible so that observed differences will be largely due to the treatments (in this case to the genotypes). The source of variation, such as slope, pH gradient, planting date etc. should be identifiable and blocks should be arranged to account for this variation. Blocks, also called replications should consist of a nearly square group of plots. Plots within blocks should be arranged in double staggered rows, as shown in Figure 4 (p. 21) in order to minimize competition effects between the different treatments (genotypes). There should be at least six plants per plot but data will be taken only from the central plants; the two outer plants are considered to be border plants.

Layout

Five blocks or replications should be planted. All genotypes should be randomly assigned to the plots within blocks. Layout as shown in Figure 4.

For more information and advice on experimental design, contact the IMTP coordinator at INIBAP.

Agronomic practices and duration of trial

- The trial should be managed according to the agronomic practices of the NARS or the institute.
- Experiments should be conducted for the plant crop and first ratoon.
- All management practices should be applied uniformly over the whole trial site.
- Only the ‘follower’ sucker should be allowed to develop. Other suckers should be removed as they appear.
- Do not apply fungicides.

Data to be collected

The data to be collected are summarized according to plant stage. Detailed guidelines follow. The field forms to collect this data are provided in Appendix VII. You may use:

Field form 9 for external symptoms,

Field form 10 for internal symptoms of the pseudostem normally recorded for both the plant crop cycle and 1st ratoon,

Field form 11 for internal symptoms of the corm recorded only for the 1st ratoon,

Field forms 1 and 2 for agronomic and fruit traits and Field form 3 for environmental data.

Planting

- Date of planting.

Growing phase (three months after planting to shooting and harvest)

- Disease severity (external symptoms) every month until harvest.

Shooting

- Time from planting to shooting *(d)*,
- Pseudostem height at shooting (bunch emergence) *(cm)*,
- Height of 'following' ratoon at shooting *(cm)*,
- Number of living (functional) leaves at flowering.

Harvest

- Disease severity (internal symptoms),
- Plant crop cycle *(d)*,
- Pseudostem girth at harvest *(cm)*,
- Bunch weight *(kg)*,
- Number of hands in bunch at harvest,
- Number of fruits at harvest,
- Finger characteristics (Field form 2 Appendix V),
- Fruit weight *(g)*,
- Number of living (functional leaves) at harvest.

Environmental data

The following data should be collected weekly from the nearest weather station:

- Rainfall,
- Highest temperature,
- Lowest temperature,
- Average temperature,
- Highest relative humidity,
- Lowest relative humidity,
- Average relative humidity,
- Number of days of rain,
- Number of hours during which average humidity reached 90% or more (only if this data is available).

Management practices

- Details of fertilizer application, nematode / weevil control measures and irrigation / drainage management should be recorded.

Details of data to be collected

Monthly readings from three months after planting until harvest will give the rate of disease development. Fields should be inspected more frequently (weekly) near to flowering time in order to accurately determine the date of flowering. External symptoms may develop between flowering and harvest. Agronomic traits should also be recorded at shooting and at harvest. These are straightforward standard practices. It is also necessary to monitor the climatic conditions in a weather station as close as possible to the experimental site. Daily readings should be taken as early as possible at the same time every day. For your convenience, forms and database structures for agronomic and environmental data are provided in Appendix V and for disease data in Appendix VII.

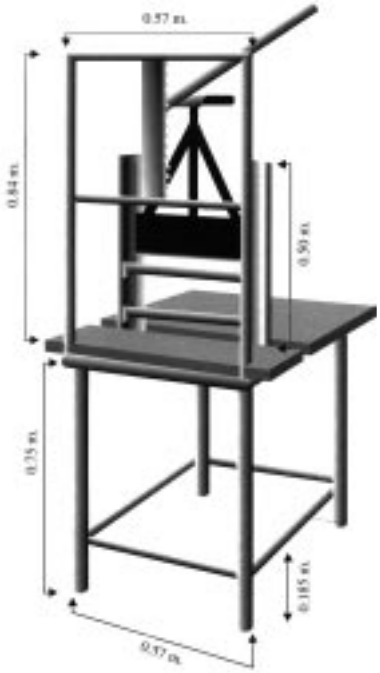
External symptoms

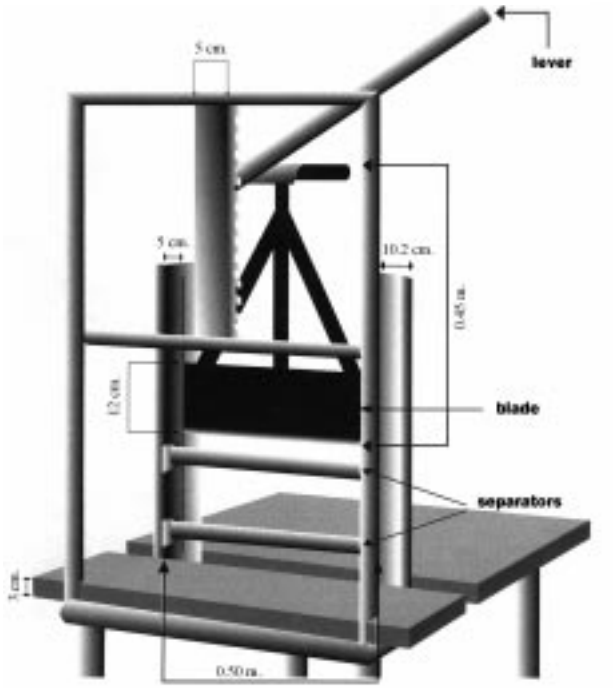
Yellowing of foliage (YF)	(1)	symptoms absent
	(2)	symptoms present
Splitting of pseudostem base (SP)	(1)	symptoms absent
	(2)	symptoms present
Changes in new leaves (CHN): irregular pale margins, narrowing, burning plus ripping of lamina and becoming more erect	(1)	symptoms absent
	(2)	symptoms present
Petiole collapse (PC)	(1)	symptoms absent
	(2)	symptoms present

Internal symptoms

- For the **plant crop cycle**, internal symptoms for the **pseudostem** only should be collected:
  Pseudostem: At harvest the plant should be cut at the base of the pseudostem and the extent to which vascular discoloration extends up the pseudostem should be determined by making transverse cuts from the base of the pseudostem upwards and examining the internal tissues following each cut. The point at which discoloration is no longer visible should be noted and the distance from this point to the pseudostem base recorded.
- For the **first ratoon crop**, symptoms within the **pseudostem** and the **corm** should be collected:
  Corm: The complete corm should be removed from the soil, the roots cut off and excess soil removed. Using a 'guillotine' (see Figure 5) or other suitable device, transverse sections of the corm should be cut to obtain five slices of equal thickness per corm. The upper surface of each cut section should be examined and the extent of vascular discoloration noted on a scale of 1-6 (see plates 1-6, p. 29).

Figure 5. Guillotine for cutting corms





5a. Global view of the guillotine.

5b. Details and measurements of the guillotine.

5c. Cutting area showing the blade and the notches which support the iron bars used to define the width of the slice.

5d. Detailed view of the lever and the rack.

5e. The guillotine in action.

(Photographs and design of the guillotine courtesy of Dr Mauricio Rivera, FHIA).

An average grade for the five sections (slices) should be calculated and recorded.

Note: Corm and pseudostem material must not be removed from the harvest site for, or following, assessment.

Agronomic traits

Field forms 1 and 2 in Appendix V are provided to help you record these data. Please refer to pages 22-23 for the complete list of variables to be recorded.

1. Corm completely clean, no vascular discoloration.

2. Isolated points of discoloration in vascular tissue.

3. Discoloration of up to 1/3 of vascular tissue.

4. Discoloration of between 1/3 and 2/3 of vascular tissue.

5. Discoloration greater than 2/3 of vascular tissue.

6. Total discoloration of vascular tissue.

Plates 1-6. Scale for rating the internal symptoms caused by Fusarium wilt (Photographs courtesy of Mr Zilton Cordeiro, EMBRAPA-CNPMF).

APPENDICES

Appendix I. Stages of an unfolding banana leaf (Brun J. 1963)

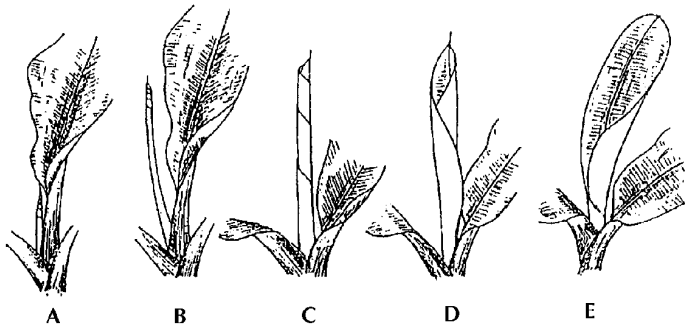


Figure 6. Stages of an unfolding banana leaf.

The young unfolded leaf is coiled into a double spiral. The right half of the leaf is situated in the hollow of the central petiole, while the left half of the leaf covers both the petiole and the right side.

The lapse of time in which a leaf unfolds varies. Under favourable climatic conditions, it takes about seven days, but it can take up to 15-20 days under poor conditions (drought, malnutrition, etc.).

In order to understand the unfolding process, it is important to recall that the formation of the leaf takes place within the pseudostem before shooting. The new leaf is tightly coiled, whitish, and particularly fragile.

The shooting of the leaf results in an extraordinarily rapid growth of the leaf sheath (4m in 10 days for Gros Michel). The young leaf slips into the petiolar canal of the preceding leaf and the development of a leaf corresponds to two successive phenomena, that of 'growth' and that of 'unfolding'.

To facilitate the description of the unfolding process, the latter has been divided into five successive stages. These stages are defined arbitrarily, since the process is in reality a continual one. The first two stages can be considered to correspond to the 'growth' phase, the third stage represents the end of the growth and the beginning of the unfolding process, and the fourth and fifth concern the unfolding itself. These different stages have been defined as follows:

- **Stage A -** The 'cigar', about 10cm in length, is still joined to the preceding leaf.
- **Stage B -** The 'cigar' is bigger, but has not yet reached its full length.
- **Stage C -** The 'cigar' is completely free. It reaches its full length and the diameter of its apex has considerably increased following the loosening of the spiral.
- **Stage D -** The left-hand side has already unfolded, and spreading takes place at the extreme apex.
- **Stage E -** The upper part of the leaf has unfolded and the base is in an open cornet shape.

Appendix II. Symptoms of black leaf streak/black Sigatoka (Fouré E. 1982)

7. Stage 1.

8. Stage 2.

9. Stage 3.

10. Stage 6.

Plates 7-10. Symptoms of black leaf streak/black Sigatoka (Courtesy of Dr Eric Fouré, CIRAD-FLHOR)

- **Stage 1** (Plate 7) is the first external symptom of the disease. It appears as a small whitish or yellow coloured spot that resembles the first stage of Sigatoka / yellow Sigatoka disease. These symptoms are not visible in transmitted light and can be observed only on the underside of the leaf.
- **Stage 2** (Plate 8) appears as a stripe, generally brown in colour and visible on the underside of the leaf; later the symptom also appears on the upper part of the limb as a stripe, the yellow colour of which resembles the stripe at Stage 1 of Sigatoka / yellow Sigatoka. The colour of this stripe will change progressively to brown and later to black on the upper side of the leaf, but will retain the brown colour on the underside.
- **Stage 3** (Plate 9) differs from the previous one in its dimensions. The stripe becomes longer, wider and, in certain conditions (weak inoculum and unfavourable climatic conditions), can reach a length of 2 or 3cm.
- **Stage 4** appears on the underside as a brown spot and on the upper side as a black spot.
- **Stage 5** is when the elliptical spot is totally black and has spread to the underside of the leaf. It is surrounded by a yellow halo with the centre beginning to flatten out.
- **Stage 6** (Plate 10) is when the centre of the spot dries out, turns light grey, and is surrounded by a well-defined black ring, which is, in turn, surrounded by a bright yellow halo. These spots remain visible after the leaf has dried out because the ring persists.

Appendix III. Symptoms of Sigatoka/yellow Sigatoka (Meredith D.S. 1970)

Plates 11-12. Symptoms of Sigatoka/yellow Sigatoka (Courtesy of Dr Eric Fouré, CIRAD-FLHOR).

11. Initial streak stage.

12. Second streak stage.

- **1. Initial streak stage** (Plate 11) **-** The spot is only just visible to the naked eye as a minute yellowish-green speck (about 1.0 x 5.0mm).
- **2. Second streak stage** (Plate 12) **-** The initial streak increases in size, notably in length rather than in breadth, still remaining yellowish-green.
- **3. Third streak stage -** The streak begins to broaden slightly as well as to increase in length and it begins to turn rusty red, usually near its centre.
- **4. First spot stage -** The streak turns dark brown and at the same time, or within 24 hours, a light brown water-soaked halo forms around the spot when the leaf is turgid. This halo is especially well seen if the spot is held up against the light in the early morning. The spot increases considerably in size during this stage. The streak has now reached the stage when it is clearly recognisable as a spot.
- **5. Second spot stage -** The dark brown part of the spot shrinks, appears sunken, and the water-soaked halo turns a darker brown.
- **6. Third spot stage -** The spot is fully developed, the sunken central area having turned grey and the halo usually dark brown or black, forming a well-defined ring round the spot. Such a spot remains well-defined even when the leaf is dead, the dark ring round the spot remaining quite distinct.

Appendix IV. Sigatoka severity scoring and calculations

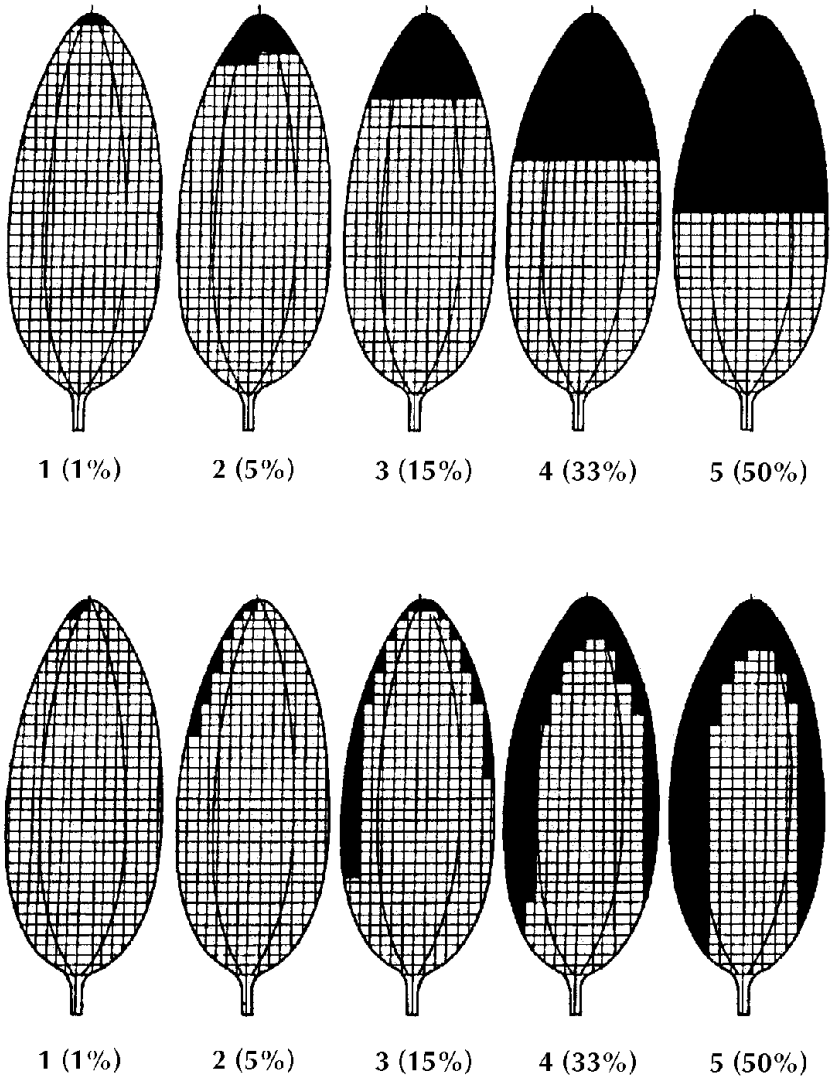


Figure 7. Sigatoka severity scoring.

The Gauhl's modification of Stover's Sigatoka severity scoring system is shown in Figure 7.

Table 2. Sigatoka severity scoring to be completed for upright leaves on each test plant at the growth stages indicated (scores have been included as an example).

Leaf Number	1*	2	3	4	5	6	7	8	9	10	11	12	13	14
Growth stage:														
Six months	0	0	0	1	2	2	2	3	5	6	6	6	–	–
Bunch emergence	0	0	0	1	2	2	–	4	5	6	6	6	–	–
Harvest	0	1	2	5	5	6	6	–	–	–	–	–	–	–

Key:

*	=	youngest completely unfurled leaf
0	=	no symptoms
1	=	less than 1% of lamina with symptoms (only streaks and/or up to 10 spots)
2	=	1 to 5% of lamina with symptoms
3	=	6 to 15% of lamina with symptoms
4	=	16 to 33% of lamina with symptoms
5	=	34 to 50% of lamina with symptoms
6	=	51 to 100% of lamina with symptoms
–	=	missing leaf or dead leaf hanging down the pseudostem (when a leaf is missing or dead and hanging down the pseudostem, it should not be included in the infection index calculations).

Calculation of infection index

Calculate the infection index for each plant in each replication at each growth stage:

$$\text{Infection index} = \frac{\sum nb}{(N-1)T} x100$$

Where
n = number of leaves in each grade
b = grade
N = number of grades used in the scale (7)
T = total number of leaves scored
Example: at bunch emergence as in Table 2:

$$\text{Infection index} = \frac{3(0) + 1(1) + 2(2) + 1(4) + 1(5) + 3(6)}{(7 - 1)11} x100$$

$$\frac{1 + 4 + 4 + 5 + 18}{6 \text{ x } 11} x\ 100 = \frac{32 \text{ x } 100}{66} = 48{,}5$$

(Infection index calculation courtesy of Dr Ronald Romero).

Appendix V. Field forms and database structures for collecting and recording agronomic and environmental data

Field form 1: Agronomic data

Site: .....

Surveyor: .....

ITC #	Replication	Plant #*	Planting date *(dd/mm/yy)*	Days from planting to shooting *(d)*	Plant crop cycle *(d)*	Plant height at shooting *(cm)*	Pseudostem girth *(cm)*	Follower sucker height at shooting *(cm)*	Weight of bunch *(kg)*	Number of hands	Number of fruits	Fruit weight *(g)*	# leaves at flowering	Number of leaves at harvest

* Use 'Plant #' column to take individual measurements of each plant in the plot only if experimental design is RCBD.

Database structure for agronomic traits

Name of database: "AGRxx.ext"

Where "xx" is the site number and "ext" is any extension your database assigns to files.

Fields	Type	Number of characters	
UI#	numeric	3 integers	
Rep.	numeric	2 integers	
ITC#	numeric	4 integers	
plant#	numeric	1 integer	
PL_date	date	dd/mm/yy	
Sh_date	date	dd/mm/yy	
Pl_heigh	numeric	2 integers	2 decimals
days_psh	numeric	3 integers	
H_date	date	dd/mm/yy	
days_pha	numeric	3 integers	
F_height	numeric	1 integer	2 decimals
B_weigth	numeric	2 integers	3 decimals
N_hands	numeric	3 integers	
N_finger	numeric	2 integers	
AW_finge	numeric	1 integer	2 decimals
LV_flr	numeric	2 integers	
LV_hv	numeric	2 integers	

Where:
UI# is a unique identifier for each plant.
Rep is the replication number: From 1 to 20 if experimental design is CRD and from 1 to the total number of blocks, if experimental design is RCBD.
ITC# is the INIBAP Transit Center code and refers to the clones being tested.
plant# is the number of the plant as it is in the plot, it has values of 1 to the total number of plants in the plot. To be used only when experimental design is RCBD.
PL_date is the actual planting date.
Sh_date is the shooting date.
Pl_heigh is the plant height at bunch emergence.
days_psh is the number of days from planting to shooting.
H_date is the harvest date.
days_pha is the number of days from planting to harvest.
F_height is the height of the follower sucker at harvest.
B_weight is the weight of the bunch at harvest.
N_hands is the number of hands in the bunch.
N_finger is the number of fingers in the bunch.
AW_finge is the average weight of each finger.
LV_flr is the number of functional leaves at flowering.
LV_hv is the number of functional leaves at harvest.

Field form 2: Agronomic data: Fruit characteristics

Site: .....

Surveyor: .....

ITC #	Replication	Plant #*	Fruit characteristics of 3rd hand				Fruit characteristics of 7th hand			
			Hand #*	Length (cm)	Diameter (cm)	Weight (g)	Hand #	Length (cm)	Diameter (cm)	Weight (g)

Database structure for agronomic data: Fruit characteristics

Name of database: FR_CHRxx.ext

Where "xx" is the site number and ".ext" is any extension your database assigns to files.

Fields	Type	Number of characters
ITC #	numeric	4 integers
Rep	numeric	2 integers
Plant #	numeric	1 integer
Hand #	numeric	1 integer
length3	numeric	3 integers
diam3	numeric	3 integers
weigh3	numeric	3 integers
length7	numeric	3 integers
diam7	numeric	3 integers
weigh7	numeric	3 integers

Where:
ITC # is the INIBAP Transit Centre code.
Rep is the replication number: From 1 to 20 if experimental design is CRD and from 1 to the total number of blocks, if experimental design is RCBD.
Plant #: is the number assigned to each plant in the plot. To be used only when the experimental design is RCBD.
hand # to be used if hand different from 3rd of 7th.
length3 is the length in cm of the central finger of the 3rd hand*.
diam3 is the diameter in cm of the central finger of the 3rd hand*.
weigh3 is the weight in g of the central finger of the 3rd hand*.
length7 is the length in cm of the central finger of the 7th hand*.
diam7 is the diameter in cm of the central finger of the 7th hand*.
weigh7 is the weight in g of the central finger of the 7th hand*.

* The hand number can be different from 3rd and 7th if the bunch has few hands (see guidelines).

Field form 3. Environmental data to be collected at each site from planting to harvest

Data to be collected	**Week**				
	1	**2**	**3**	**4**	**5**
Rainfall (mm)					
Highest temperature (°C)					
Lowest temperature (°C)					
Average temperature (°C)					
Highest relative humidity (%)					
Lowest relative humidity (%)					
Average relative humidity (%)					
No. days with rain					
No. of hours during which relative humidity ≥ 90%					

Appendix VI. Field forms and database structures for collecting and recording data of Sigatoka disease resistance trials

Field form 4. Data from IMTP performance evaluation for Sigatoka disease resistance trials

Site: .....
Surveyor: .....
Experimental design: .....
Latitude: ..... **Longitude:** ..... **Altitude:** .....

ITC number	Unique Identifier	Rep #	YLS	NSL	INSL

YLS: Youngest leaf spotted
NSL: Number of standing leaves
INSL: Index of non-spotted leaves

Field form 5. Evaluation of disease development time (DDT), youngest leaf spotted (YLS) and leaf emission rate (LER) to be collected for each test plant (in-depth trials)

Site: .....
Surveyor: .....
Block or Rep: ..... **ITC #:** .....
Plant #: ..... **Unique Identifier:** .....

Week #	Date stage B *dd/mm/yy*	Date 10 or more lesions *dd/mm/yy*	DDT	YLS	New leaves	LER

Database structure for DDT, YLS and LER

Name of the database: DDTxx.ext

Where xx is your site number and ext is the extension suffix that you database assigns automatically.

Fields	Type	Number of characters	
UI#	numeric	3 integers	
Rep	numeric	1 integer	
ITC#	numeric	4 integers	
Plant#	numeric	1 integer	
Week#	numeric	2 integers	
Date_B	date	dd/mm/yy	
Date_10	date	dd/mm/yy	
DDT	numeric	3 integers	
YLS	numeric	2 integers	
NLV	numeric	2 integers	
LER	numeric	1 integer	2 decimals

Where:
UI# is the unique identifier for each plant.
Rep refers to the block or replication number and holds values between 1 and 5.
ITC# refers to the clones being tested inclusive of the local cultivar.
Plant# is the number of the plant as it is in the plot, it has values of 1 to 5. This should be assigned once the plant is in the field and should be the same throughout the trial.
Week# is the number marked on the leaf when it was at Brun's stage B: First, second, every week.
Date_B is the date the leaf is marked at Brun's stage B.
Date_10 is the date 10 or more mature lesions are visible on marked leaf.
DDT is the disease development time in days. Subtract Date_10 - Date_B.
YLS is the youngest leaf spotted at Date_10.
NLV is the number of new leaves produced between consecutive Date_B readings.
LER is the average leaf emission rate calculated weekly.

Field Form 6. Disease severity (in-depth trials)

Site: .....
Surveyor: .....
Rep: ..... **ITC #:** .....
Plant #: ..... **Unique Identifier:** .....

		Leaf Number													
Evaluation at	**date**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Six months															
Bunch emergence															
Harvest															

		Leaf Number											
Evaluation at	**date**	**15**	**16**	**17**	**18**								**Sum**
Six months													
Bunch emergence													
Harvest													

Database structure for disease severity

Name of database: "SEVERxx.ext"

Where "xx" is the site number as it was assigned to you and "ext" is any automatic extension your database assigns to files.

Fields	Type	Number of characters
UI #	numeric	3 integers
Rep	numeric	1 integer
ITC #	numeric	4 integers
Plant #	numeric	1 integer
Eval_T	numeric	1 integer
LN1key	numeric	1 integer
LN2key	numeric	1 integer
LN3key	numeric	1 integer
LN4key	numeric	1 integer
LN5key	numeric	1 integer
LN6key	numeric	1 integer
LN7key	numeric	1 integer
LN14key	numeric	1 integer
Sum	numeric	3 integers

Where:
UI# is a unique identifier for each plant.
Rep refers to the block number and has values of 1 to 5.
ITC # refers to the clones being tested.
Plant # is the number of the plant as it is in the plot, it has values of 1 to 5. This should be assigned once at the beginning of the trial.
Eval_T refers to the time the evaluation was performed. Evaluations performed at six months after planting have the value 1, at bunch emergence have the value 2 and at harvest have the value 3.
LNxkey is the scoring grade for Sigatoka according to Appendix IV in these guidelines. It has values of 0 to 6. The 'x' in 'LNxkey' indicates the leaf number where the grade is scored.
Sum is the sum of LN1key + LNxkey values

Appendix VII. Field Forms and database structures for collecting and recording data of Fusarium wilt resistance trials

Field form 7: Evaluation of disease severity in performance trials: External and internal symptoms for the plant crop cycle

Planting date: .....
Site: .....
Surveyor: .....
Experimental design: .....

ITC number	Replication	Plant # *	Date recorded diseased (dd/mm/yy)	Date recorded dead (dd/mm/yy)	Extent of discoloration at harvest (cm)

* Use 'Plant #' column to take individual measurements of each plant in the plot **only** if experimental design is RCBD.

Database structure for disease severity: External and internal symptoms for the plant crop cycle

Name of the database: SYMPxx.ext

Where "xx" is the site number as it was assigned to you and "ext" is any automatic extension your database assigns to files.

Fields	Type	Number of characters
ITC #	numeric	4 integers
Rep	numeric	2 integers
Plant #	numeric	1 integer
diseased	date	dd/mm/yy
dead	date	dd/mm/yy
extcm	numeric	3 integers

Where:
ITC # is the INIBAP Transit Centre code.
Rep is the replication number: From 1 to 20 if experimental design is CRD and from 1 to 5 if experimental design is RCBD.
Plant # is the number assigned to each plant in the plot. To be used only where the experimental design is RCBD. Will have values of 1 to 4.
diseased is the date when symptoms where evident.
dead is the date the plant was recorded dead.
extcm is the extent of discoloration at harvest or when dead.

Field form 8. Summary of disease data of Fusarium wilt resistance in performance trials

Site: .....
Surveyor: .....
VCG: .....
Susceptible cultivar used: .....
Experimental design: .....
Latitude: ..... **Longitude:** ..... **Altitude:** .....

ITC number	Number of plants evaluated	% Plants with external symptoms	% Plants with internal symptoms

Field form 9. Monthly evaluation of Fusarium wilt disease severity at in-depth trials: external symptoms

Site: .....
Surveyor: .....
Planting date: .....
Experimental Design:.....

ITC #	Replication	Plant #*	MAP	YF	SP	CHN	PC

* Use 'Plant #' column to take individual measurements of each plant in the plot only if experimental design is RCBD.

MAP: months after planting
YF: yellowing of foliage
SP: splitting of pseudostem
CHN: changes in new leaves
PC: petiole collapse

Database structure for monthly evaluation of Fusarium wilt disease severity: external symptoms

Name of the database: EXTDSxx.ext

Where "xx" is your site number and "ext" is any extension that your database assigns to files.

Fields	Type	Number of characters
ITC #	numeric	4 integers
Rep	numeric	2 integers
Plant #	numeric	1 integer
MAP	numeric	2 integers
YF	numeric	1 integer
SP	numeric	1 integer
CHN	numeric	1 integer
PC	numeric	1 integer

Where:
ITC # is the INIBAP Transit Centre code.
Rep is the replication number: From 1 to 20 if experimental design is CRD and from 1 to 5 if experimental design is RCBD.
Plant # is the number assigned to each plant in the plot. To be used only in case the experimental design is RCBD. Will have values from 1 to 4.
MAP is months after planting.
YF is yellowing of foliage (YF4 at month 4, YF5 at month 5 after planting).
SP is splitting of pseudostem base at the same month.
CHN is changes in new leaves.
PC is petiole collapse.
Note: According to the guidelines, all disease variables have values of 1 to 2.

Field form 10. Evaluation of Fusarium wilt disease severity of the pseudostem at in-depth trials: internal symptoms

Site: .....
Surveyor: .....
Planting date: .....
Experimental Design: .....

ITC #	Replication	Plant #*	Extent of discoloration at harvest *(cm)*

Database structure for evaluation of Fusarium wilt disease severity of the pseudostem: internal symptoms

Name of the database: INTPDSxx.ext

Where "xx" is your site number and "ext" is any extension that your database assigns to files.

Fields	Type	Number of characters
ITC #	numeric	4 integers
Rep	numeric	2 integers
Plant #	numeric	1 integer
extcm	numeric	2 integers

Where:
ITC# is the INIBAP Transit Centre code.
Rep is the replication number: From 1 to 20 if experimental design is CRD and from 1 to 5 if experimental design is RCBD.
Plant # is the number assigned to each plant in the plot. To be used only in case the experimental design is RCBD. Will have values of 1 to 4.
extcm is the extent of discoloration at harvest or when dead.

Field form 11. Evaluation of Fusarium wilt disease severity of the corm at in-depth trials: internal symptoms

Site: .....
Surveyor: .....
Planting date: .....
Experimental Design:.....

ITC #	Replication	Plant #*	Slice #				
			1**	2	3	4	5

Database structure for evaluation of Fusarium wilt disease severity of the corm: internal symptoms

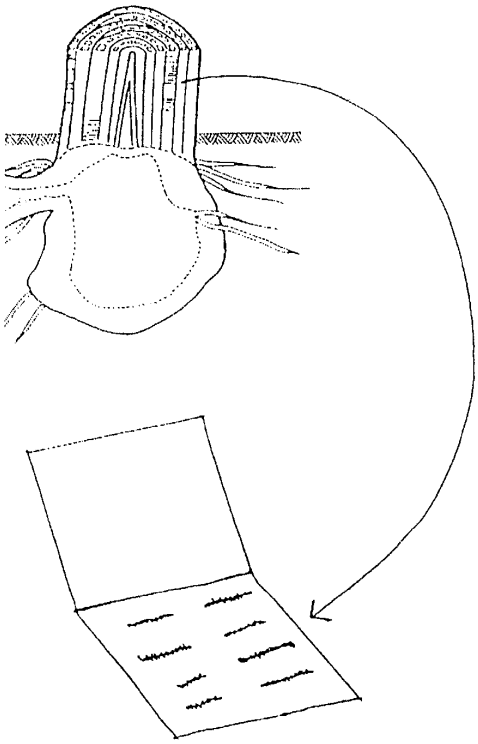
Name of the database: INTCDSxx.ext

Where "xx" is your site number and "ext" is any extension that your database assigns to files.

Fields	Type	Number of characters	
ITC #	numeric	4 integers	
Rep	numeric	2 integers	
Plant #	numeric	1 integer	
SL1	numeric	1 integer	
SL2	numeric	1 integer	
SL3	numeric	1 integer	
SL4	numeric	1 integer	
SL5	numeric	1 integer	
AvgDS	numeric	1 integer	2 decimals

Where:
ITC # is the INIBAP Transit Centre code.
Rep is the replication number: From 1 to 20 if experimental design is RCD and from 1 to 5 if experimental design is RCBD.
Plant # is the number assigned to each plant in the plot. To be used only where the experimental design is RCBD. Will have values of 1 to 4.
SL1 is the basal slice while **SL5** is the uppermost slice of the corm.
AvgDS is the average disease severity of the corm taking into account the 5 slices.

Appendix VIII. How to isolate Fusarium samples for analysis of vegetative compatibility group (VCG)?



Cut out chuncks of discolored tissue from the pseudostem and dissect out individual vascular strands from some.

Place strands and untouched chuncks between sterile blotting paper to dry.

Figure 8.
Steps in the preparation of *Fusarium oxysporum* f.sp. *cubense* (Foc) samples for posting (courtesy of K. Pegg, QDPI).

When dry, place the blotting paper, chuncks and strands in a paper envelope; seal and label with:
– isolate number;
– cultivar name;
– locality;
– collector's name.

Samples of infected pseudostem or corm tissue from diseased plants should be prepared and sent to a recognised laboratory for analysis.

If all plants of an accession become infected, samples should be taken from 4-5 randomly selected plants for analysis. If only a few plants of an accession become infected, samples from all diseased plants should be sent for analysis. Eight strands per plants should be prepared (See Plate 13).

Plate 13. Excised discoloured vascular tissue in blotting paper for recovery of *Foc* from banana plants. (Courtesy of Dr N. Moore, QDPI)

Loss of viability of the samples can be minimized if you are prompt in their preparation after collection and then, if you are prompt in posting them when the excised strands are dry (approximately three days in sterile blotting paper).

You should post them in paper envelopes as plastic causes samples to sweat and promotes the growth of bacterial contaminants. The things to keep in mind regarding the viability of the fungus inside the vascular strands are simply not to let the strands get too hot, too cold or too wet. Samples should be kept in paper at all times and dried on the bench under normal laboratory conditions **not in ovens.**

(Protocol courtesy of Dr Natalie Moore, QDPI)

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