Greenhouse inoculation of banana plantlets for Fusarium wilt resistance

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Plant material:

Pathogen-free micro-propagated banana plantlets must be obtained from a reputable tissue culture laboratory. The plants are then replanted in small bags or pots containing a potting soil mixture, and hardened off for 2-3 months to a height of 20-30 cm. For sufficient root development to occur, the plantlets also need to be fertilized appropriately. If tissue culture bananas are not available, experimental plants can be generated by macro-propagation from clean planting materials. Resistant and susceptible control plants also need to be multiplied, along with the experimental plants. For Foc Lineage VI isolates, resistant varieties are East African Highland banana (EAHB) cultivars, Cavendish bananas and Calcutta-4. Susceptible varieties include Gros Michel, Sukari Ndizi and Pisang Awak.

Millet seed preparation:

Millet kernels must be sterilised in 1-L Erlenmeyer flasks or Schott bottles. The flasks/bottles are first filled with 250 g millet kernels and covered with 200 ml distilled water, and left to soak overnight. All access water is drained off the millet seed the following morning, and the flasks/bottles covered with cotton wool or a lid, and autoclaved on two consecutive days. This is done to ensure that all microbial contaminants are killed, including endospore-forming bacteria. The millet kernels in each flask and bottle are thereafter shaken to loosen the grain, and a small sample is collected for plating onto PDA to ensure that it is sterile. The bottles can also then be stored in a cold room until use.

Inoculum preparation:

A virulent Foc isolate must be used for pathogenicity testing. The isolate should be isolated from diseased banana plants, single-spored, and identified to VCG level. It then needs to be stored at 4^{o}C on carnation leaf agar (CLA) or in 15% glycerol at -80^{o}C to prevent loss of virulence. For inoculum, the fungus needs to be grown on ½-strength potato dextrose agar (PDA) for 7 days. Five mycelial plugs, 0.5 cm in diameter, then needs to be cut from the margins of the culture and used to inoculate the sterilized millet kernels in the Erlenmeyer flasks or Schott bottles. After white fungal growth begins to show on the surface of the millet kernels, the flasks and bottles are shaken every 2nd day to distribute the spores equally and to prevent the kernels from clumping together. After 14 days, individual millet kernels will appeared discoloured with a reddish taint. A small sample must be removed from each flask/bottle and placed onto PDA to ensure that they are colonised by Foc only. The millet can then be stored until use, but not for longer than 3 months.

Greenhouse trial:

For greenhouse pathogenicity testing, the Foc-infested millet kernels are mixed with a sand-soil blend at a concentration of 2-10 g millet kernels/1 kg soil. The sand-soil combination has to be steam-sterilized before Foc inoculation. This is to ensure that unwanted micro-organisms do not affect the outcome of the trial. The Foc-potting mixture is then deposited into planting bags or pots, into which the experimental plants are replanted. The banana roots are not intentionally bruised during replanting. Three to 15 plants need to be included for each treatment, depending on the nature of the trial, and the experiment needs to be repeated.

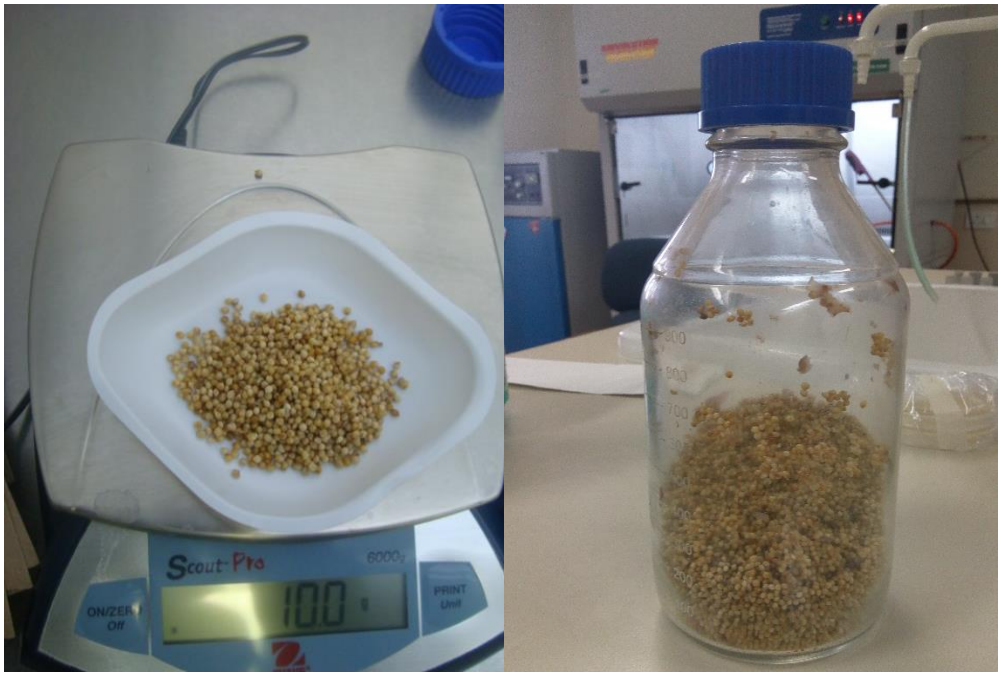
The experimental plants need to be kept at a greenhouse for the duration of the trial. The daylight photoperiod should be at least 12 hrs, with the minimal day/night temperatures set at 25/20^{o}C. The experimental pots should be clearly marked, and then randomly arranged to prevent any bias. When greenhouses are not available, screen houses can be used. In such a situation, care should be taken not to not to expose trial plants to unnecessary contamination by other pests or pathogens. Plants should be appropriately fertilised and irrigated to prevent any unnecessary environmental stresses.

External Fusarium wilt symptoms usually develop within 3-8 weeks after inoculation, but can take longer. Leaf symptoms may be inconsistent, and it is therefore recommended that the rhizome be cut open to score the discolouration of the inner rhizome. Cut needs to be made in the middle of the rhizome, where discolouration is more pronounced than in the lower or upper rhizome. The rating scale for rhizome discolouration ranges from 1 to 6, with 1 meaning no disease, and 6 meaning that the entire rhizome is discoloured (Viljoen et al., 2017). As rhizome scoring is a destructive method, additional resistant and susceptible control plants need to be included in the experiment. When a disease rating of 4 and more is found in the susceptible control plants, the experiment is ready to be rated. Alternatively, rhizomes can be dissected once leaf symptoms are visible on 50% of the susceptible control plants.

It is important to realise that growing conditions and disease development in different greenhouses and at different locations may affect results. It is, therefore, important to adjust the inoculation procedure to best suit the local conditions. Also, the method and amount of inoculum applied can be changed according to the objective of the experiment. For instance, more inoculum can be used for pathogen confirmation, while less is used when comparing resistance of different varieties.

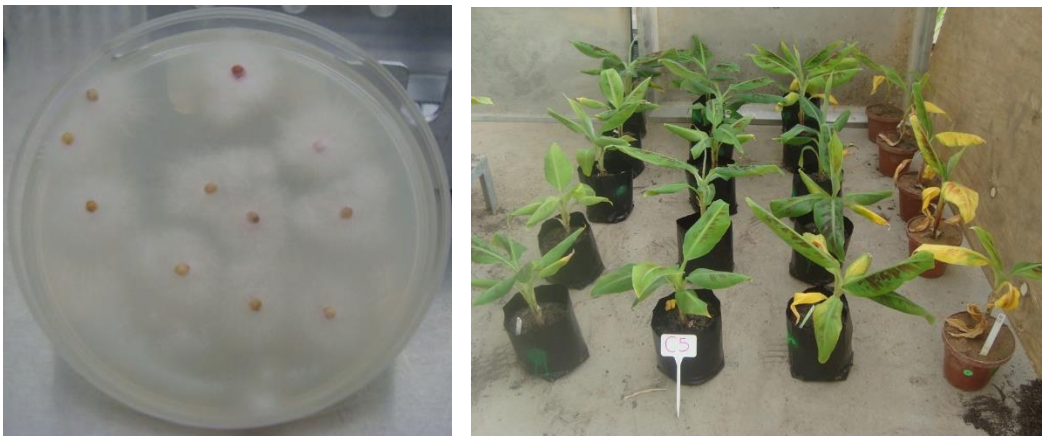
Recommended reading:

Viljoen, A., Mahuku, G., Massawe, C., Ssali, R.T., Kimunye, J., Mostert, D., Ndayihanzamaso, P. and Coyne, D. 2017. Banana pests and diseases: field guide for disease diagnostics and data collection. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. ISBN: 978-978-8444-80-0. http://www.musalit.org/seeMore.php?id=16927

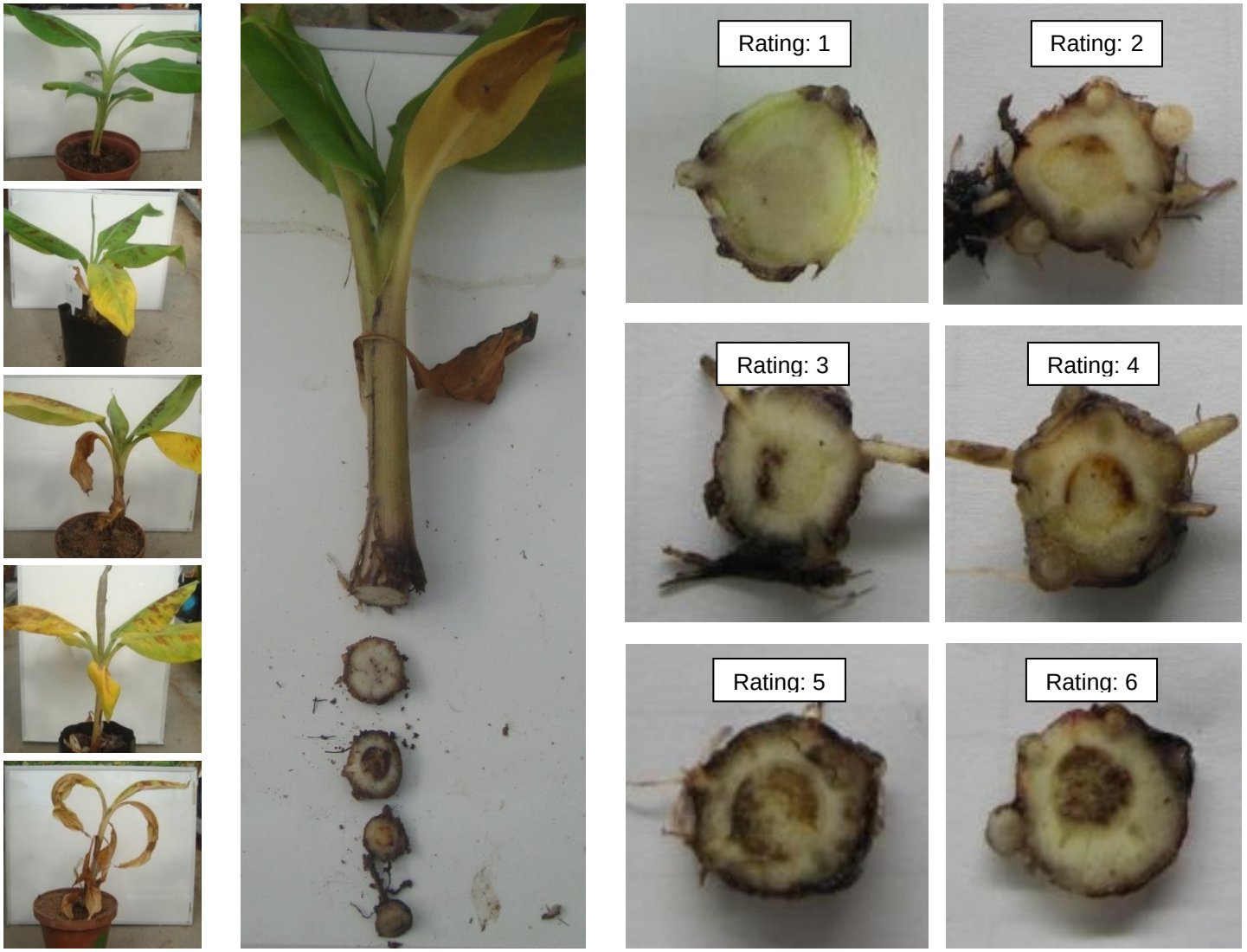


Millet seeds used for the experiment can be placed in an Erlenmeyer flask or Schott bottle.

Mixing of millet seed with potting soil.



Growth of Foc-colonised millet seeds on PDA plates (left), the development of external leaf symptoms (right).



Scoring of disease development according to the rhizome discolouration index.