

Research Article

Early Mass Diagnosis of Fusarium Wilt in Banana Cultivations using an E-Nose Integrated Autonomous Rover System

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Abstract

Nucleic acid based diagnostics are the standard means for diagnosis of infected plant material. However, these methods are expensive and time-consuming, but they are accurate. On the contrary, disease prediction methods based on Volatile organic compound (VOC) emission from plants are less accurate but, allow for screening of large volumes of samples. This work reports the methodology for development of an inexpensive electronic nose for implementation as early warning systems intended to prevent plant disease outbreaks using VOC pattern analysis. It is proven that plants emit VOCs in response to pathogenic attacks. In this project, efforts were made to register the pattern of VOCs released by the diseased plants. The disease taken for this purpose was Fusarium wilt disease of banana. The E-Nose was successfully fabricated using five MOS sensors connected to a microcontroller, which along with a microSD card module was able to store the acquired VOC data. The VOC data analysis was done in MS-Excel, using NeuroXL Predictor, a neural networking add-in. A small scale banana field containing 35 plants, divided into disease, test and control groups, was established. The disease and test sets were subjected to similar disease induction protocols and VOC data was collected over a period of 40 days. NeuroXL Predictor was trained to recognize odours corresponding to diseases by feeding the neural network with the disease set VOC data. Finally, the training model was validated by providing the test set VOC data to the neural network and the results were found to be accurate. Efforts were made to automate the VOC data acquisition from the plants, as it will be impractical to carry around, a device, through several hectares of plantation. Therefore, a simple autonomous rover was fabricated using DC motors connected to a microcontroller. A DC motor placed on top was used to move the E-nose towards the plants in left and right of the rover. The microcontroller was programmed to stop, move forward and turn the E-nose towards left or right as per the measurements of the field.

Keywords: Electronic-nose; Artificial Neural Network; Plant disease diagnosis; MOS sensors and Volatile organic compounds.

Introduction

Most of the rural population all around the world is dependent on agriculture. Banana, despite not being a staple crop, it is highly preferred by tropical farmers for its profitability. However, banana requires a lot of time before significant yield can be obtained, which makes it prone to several diseases. Serious disease outbreaks in these cultivations can destroy entire harvests. Of all the banana diseases, Fusarium wilt is highly destructive, it is caused by the soil-borne fungus *Fusarium oxysporum f.spp. cubense*. It exists as spores in the soil for an indefinite amount of time and is highly resistant to fungicides (Vezina, 2016). Mass monitoring of fields and early detection of diseases in plants is vital when maladies of this magnitude

are considered. Current plant disease diagnosis technologies include polymerase chain reaction (PCR), immunofluorescence (IF), fluorescence in-situ hybridization (FISH), enzyme-linked immunosorbent assay (ELISA) and flow cytometry (FCM) (Webster *et al.*, 2004). These are expensive, time consuming and cannot be implemented for monitoring in large farms, where several thousand plants are cultivated. In order, to achieve mass monitoring, non - invasive technologies should be used. It is a well-established fact that plants emit VOCs in response to infections (Freeman, 2008). Hence by regularly monitoring the change in VOCs, the outbreak of plant diseases can be identified at early stages and is far more advantageous than any existing invasive technologies. Current VOC detection methods include Gas chromatography-mass spectrometry,

Selected ion flow tube–mass spectrometry, Proton transfer reaction mass spectrometry etc., (Materić *et al.*, 2015). These techniques require a laboratory setup, expensive equipment and sample processing. However, by creating an electronic analog of the mammalian olfactory system, such a portable, less expensive, mass monitoring device can be materialized.

E-noses are already existent in the market but they are highly expensive which makes it out of reach for normal research purposes and real life applications. Therefore, an inexpensive electronic nose system was fabricated using MOS Sensors, which quantify analytes by providing a hike in output voltage that is directly proportional to the analyte concentration. By exposing an array of MOS sensors (sensitive to different functional groups) to several disease positive plants, an odour fingerprint can be obtained in the form of sensor output voltages. This output was stored by the device in a microSD card, which can be plugged into a PC and opened in MS-Excel, to observe the data. This data was fed to NeuroXL Predictor, a neural networking add-in for MS-Excel, as training set, so that it can predict the presence of diseases in new set of plants.

Materials and Methods

Banana Field Setup

A small-scale banana plantation containing 35 plants (45 days old) was set up in college nursery facility. Tissue cultured Grand nain banana plants were used in this study and were procured from Sunglow Biotech, Coimbatore. The 35 plants were divided into 3 groups: control, healthy and disease. The control group contained 5 plants, healthy group contained 10 plants and the disease group contained 20 plants and all these plants were enclosed with polyethene bags a small incision was made and sealed with a tape. The incision was used to water the plants on a regular basis.

E-Nose Development

The Fig. 1, illustrates the assembly scheme of the E-nose device. The VOCs from the diseased plants was be sucked in through the suction unit and the MOS Sensors were exposed to the VOCs. The corresponding sensor readings were stored in a microSD card.

The E-nose was programmed to take a reading every 6 seconds and an LED was used to indicate data acquisition. All the sensors and the sensors and microSD card module were procured from Sparkfun. The MOS Sensors taken for this experiment were MQ-2, MQ-3, MQ-4, MQ-5, MQ-135.

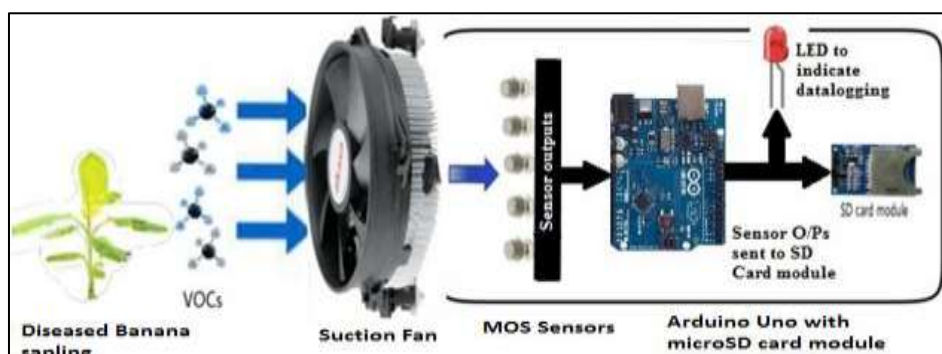


Fig.1: Assembly scheme of the E-nose.

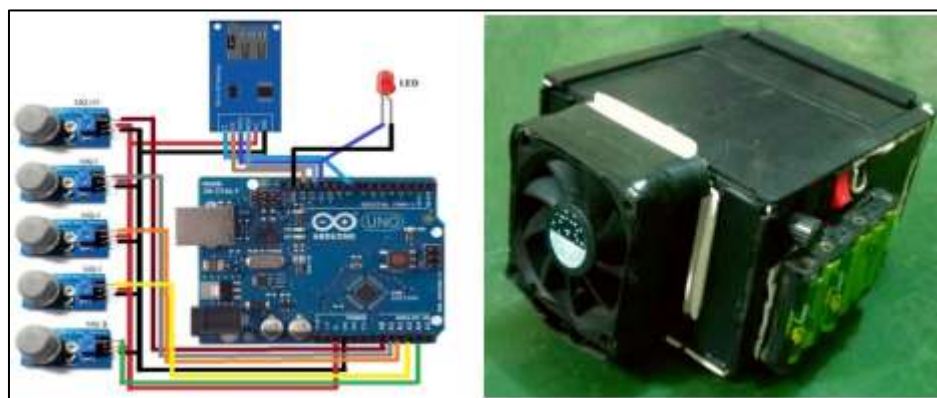


Fig. 2: The circuitry of the E-Nose (Left) and picture of the actual E-nose (Right).

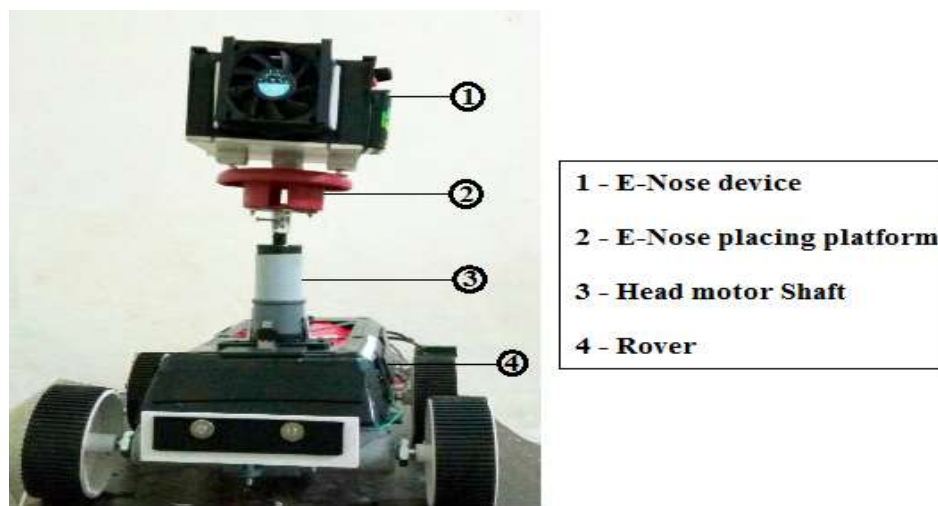


Fig.3: Picture of the actual rover with the E-nose mounted on top

Rover Development

A rover was constructed by connecting attaching four 100 RPM DC motors to an acrylic chassis. A digital pin of an Arduino Uno was connected to an IRF540N mosfet which was inturn the motors. Additionally another 100 RPM DC motor (head motor), were a platform for attachment of E-nose was placed. The head motor moves clockwise and anticlockwise so that the E-nose can take readings from plants on both the sides of the rover. The head motor was programmed to turn towards the left, stay as such for 18 seconds to take 3 readings from the left-side plant then, turn right side and stay for 18 seconds to take 3 readings from the right -side plant. The 18 second delay was set because the E-nose acquires readings for every 6 seconds. The rover was programmed to move forward for 1500 microseconds and wait for 36 seconds (time taken to acquire 3 readings from right side plant and 3 readings for left side plant). The ground motors connected to the IRF540N MOSFET was made to switch the ground motors on and off for 1500 milliseconds and 36 seconds respectively. All the motors were connected to a 6V lead acid battery.

Inoculum Preparation and Disease Induction

Fusarium oxysporum f spp. cubense Tropical Race- 4 (Foc-TR4) culture was procured from Department of Plant Pathology, Tamilnadu Agricultural University, Coimbatore. The culture was isolated from *Fusarium* wilt affected Grand Nain banana plants. The culture was mass multiplied in Malt Yeast Extract broth. All the constituents of the broth were procured from HiMedia Labs. The inoculated broth was kept agitated at 20 C for 2 weeks. At this time, the inoculum was adjusted to 1.5×10^6 microspores per ml with a hemacytometer. The inoculum was injected at the end of the pseudostem near the rhizome of each plant in the disease group (Boyhan *et al.*, 2001), additionally 10 ml of the broth was added to the soil around all the disease group plants. After induction, the control,

disease and test plants were enclosed in polythene bags, to facilitate collection of VOCs within the bag.

VOC Sampling and Model Development

The VOC patterns for air, disease and control groups were recorded for every 5 days. Three readings were taken for every plant and its mean value was taken as the final reading of the plant. Initially the data collection was done manually. The suction unit of the E-nose was switched on and placed next to the polythene bag, after opening the incision on the polythene bag. After collecting data from each plant, the E-nose was switched off and the suction unit was switched on for 3 seconds, to flush out the previous plant VOCs. The overall mean of the constituents of each group was used to plot a radar graph. The radar graph contained 5 axes, one each for the 5 sensors containing the sensor outputs in millivolts (mV). The number of symptomatic plants was also recorded during the days of data collection, to determine the relation between sensor outputs and the number of symptomatic plants over the experimental period.

Disease Prediction

NeuroXL Predictor, an MS- Excel add-in tool, was used for prediction of infected and normal plants. The VOC data from the disease set plants was fed as the training set. In the neural network, the disease set plants were represented with '1'. The air VOC data and control set data were represented with '0'. Thus, when the test data is provided, the neural network will return a value '0' or '1', corresponding to infected or normal VOC pattern (Fillela *et al.*, 2014). The VOC data from the test group plants was taken as the test data. The prediction process using NeuroXL Predictor was performed in three steps: i) Selection of training and test data ii) Prediction the test set results and iii) Reduction the number of decimal places in the results to get whole numbers since whole numbers make it easier to differentiate the infected from the normal plants.

Results and Discussion

VOC Pattern for Fusarium Wilt

In this experiment, 5- axes radar graphs were used to represent the disease VOC patterns, with one axis allotted for each of the five sensors. This enables visual differentiation of the disease and normal plant patterns. Three readings were taken for each plant and the mean of the three readings were considered as the final reading. The overall mean reading of a group was calculated by taking the average of the final readings of all the members of the set. This overall reading was used to plot the radar graph for each group. The radar graphs provide vital information on where the clustering occurred for a particular odour. In this case, the odour of normal plants, the infected plants and the odour of air VOCs. Data acquisition was carried out for 40 days at 5 days interval. The difference between the control and the diseased plants were very distinct on the 25th day.

The Fig. 5 shows the sensor outputs obtained by exposing the sensor array to the environmental air. As expected, the air VOC data remained almost the same throughout the experimental period. The small fluctuations in air VOC data can be due to environmental factors (Kim et al., 2014). Plants emit VOCs even under the absence of stress conditions and the flux of VOCs increase by several folds under stress and infection (Seco et al., 2006). This statement takes effect in this experiment, which is evident from the

control set VOC data. There is a sharp contrast between the air VOC data and control set VOC data, as observed from Fig. 6, which indicates that even normal plants emit VOCs, further proving the statement. However, a comparison between the control set and the disease set shows that the flux of VOCs is higher in the disease. From Fig. 7, it was observed that the VOC flux in disease set increased until the 25th day, after which there is a sharp decline. The increase can be attributed to the defense mechanisms of the plant (Freeman, 2008) whereas the decline can be assumed to be due to wilting which reduces the number of plant parts that can emit VOCs. The results obtained are consistent with the findings of Rizzolo et al., 2012, where the VOC emissions peaked on the 23rd day after which the VOC data recording was not performed. The Figure.8, shows the physical comparison of the control and the disease induced plants. The diseased plants exhibited the classic symptoms of Fusarium wilt such as drooping leaves, wilting and yellowing whereas the control plant was healthy as seen from Fig. 8 and Fig. 7, portrays the pseudostem cross sections of the banana plants whereas the control plants were normal. The vascular tissue turned from pale green to pale red along the circumference and black in the interiors of diseased plants. The girth of the pseudostem of control plants was also bigger than the disease and test set plant. This showed that the pathogen also hindered the growth of the plants.

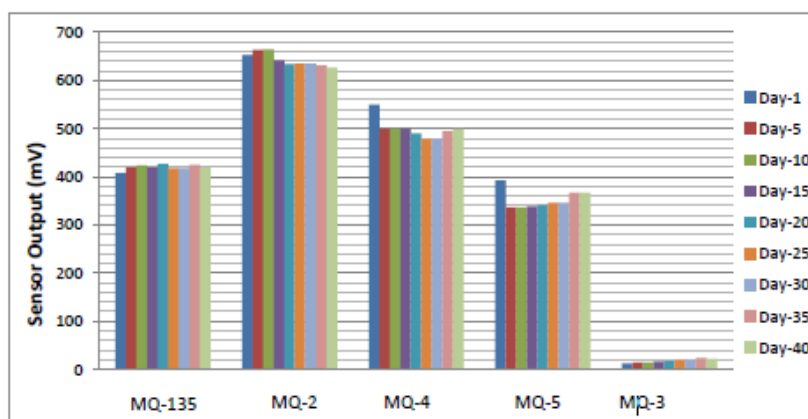


Fig. 4: Graph indicating the variation of outputs of all the sensors for air over the experimental period.

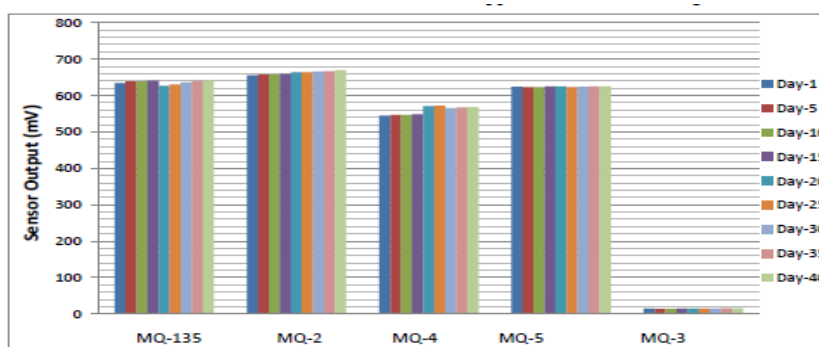


Fig. 5: Graph indicating the variation of outputs of all the sensors for control group plants over the experimental period.

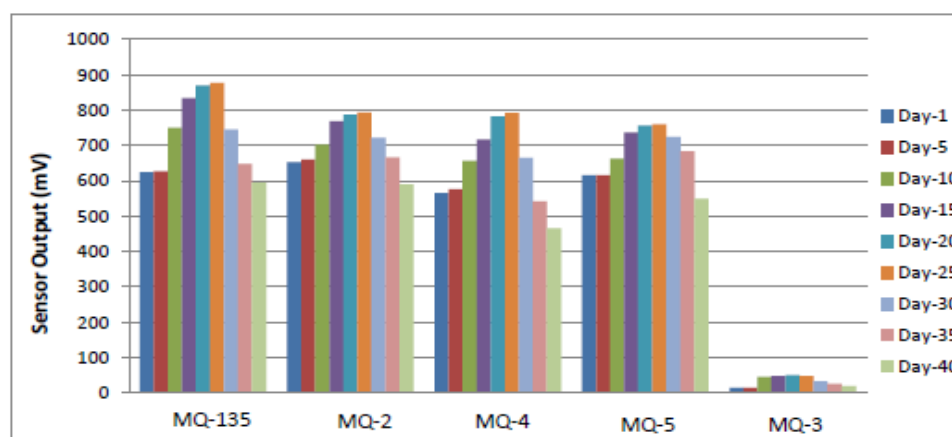


Fig. 6: Graph indicating the variation of outputs of all the sensors for disease group plants over the experimental period.



Fig. 7: Cross section of the pseudostems of Disease (A) Test (B) and Control set (C) plants from left to right.



Fig. 8: Comparison of Control (A), Disease (B) and Test set (C) plants respectively from left to right.

Comparison of Sensor Outputs and Symptomatic Plants

A comparison of the sensor outputs and the number of symptomatic plants is required to establish the Optimal Detection Period (ODP). OPD is the time at which the disease can be detected using the device, before the disease becomes widely prevalent i.e., before the plants start exhibiting symptoms. This period is very essential for quarantining or uprooting the suspected plants.

As seen from Figure 9 and Figure 10, a close relation between the trend of symptomatic plants and the trend of

sensor output was observed. During the 10th day, when just two plants exhibited wilting symptoms, the VOC data was strikingly different from the 5th day VOC data. This indicates that the device was able to detect subtle differences in VOC patterns after infection, when only 1% of the total disease set plants showed symptoms. This proves the effectiveness of the device in detecting Fusarium wilt at early stages. Hence, the OPD is approximately after ten days of contracting an infectious dose of the pathogen.

Conclusion

This research emphasizes the importance of the electronic nose in disease diagnosis. The key objective of this research was to develop an inexpensive electronic nose system for mass monitoring of diseases in agricultural cultivations. E-noses are already existent in the market but they are highly expensive which makes it out of reach for normal research purposes and real life applications. MOS Sensors, quantify analyses by providing a hike in output voltage, which is directly proportional to the analytic concentration. By exposing an array of MOS sensors (sensitive to different functional groups) to several disease positive plants and analyzing the output of sensors in the form of a radar graph, a graph pattern (odour fingerprint) corresponding to disease positives was made. This was taken as the training set and fed to an Artificial Neural Network (ANN). The device can then detect new disease positive plants. Since, this technology is non-invasive it is suitable for mass monitoring and this can be automated by fitting the device to an autonomous rover platform. Though, *Musa acuminata*, is considered for this study, by standardizing the device to different plants, disease diagnosis in several species of plants is possible

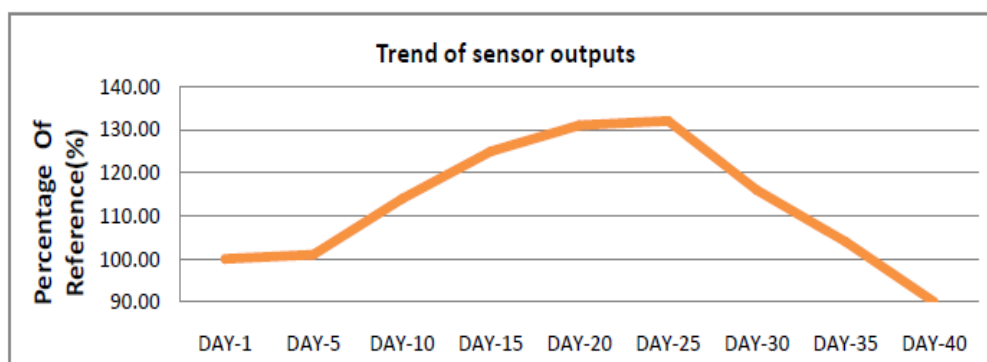


Fig. 1: The trend of output of all the sensors during the experimental period.

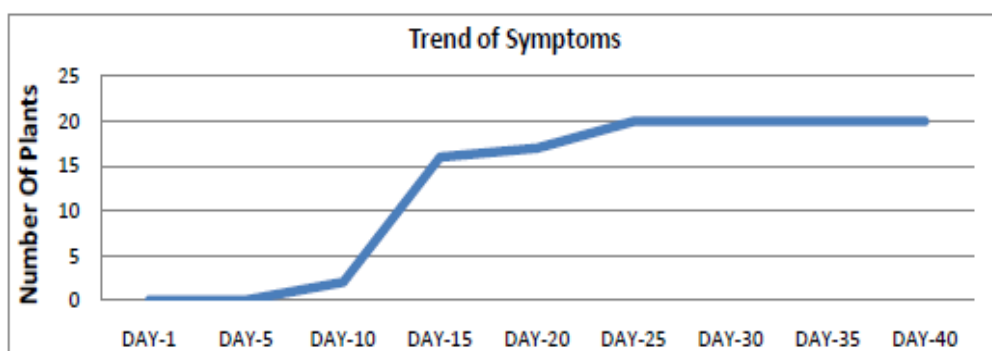


Fig. 2: Plot showing the progression of number of symptomatic plants observed over the experimental period

References

- Boyhan GE, Langston DB, Lewis PM and Linton MO (2001) Use of an Insulin Syringe for Fusarium Wilt Inoculation of Watermelon Germplasm. *Cucurbit Genetics Cooperative Report* **24**(5): 50–52.
- Filella X, Foj L, Alcover J, Augé JM, Molina R and Jiménez W (2014) The influence of prostate volume in prostate health index performance in patients with total PSA lower than 10µg/L. *Clinica Chimica Acta* **436**: 303-307. DOI: [10.1016/j.cca.2014.06.019](https://doi.org/10.1016/j.cca.2014.06.019)
- Freeman BC (2008) An Overview of Plant Defenses against Pathogens and Herbivores. *The Plant Health Instructor, Iowa State University Digital Repository*, pp.3-8. DOI: [10.1094/phi-i-2008-0226-01](https://doi.org/10.1094/phi-i-2008-0226-01)
- Kim JY, Chu CH and Shin SM (2014) ISSAQ: An integrated sensing systems for real-time indoor air quality monitoring. *IEEE Sensors Journal* **14**(12): 4230-4244. DOI: [10.1109/JSEN.2014.2359832](https://doi.org/10.1109/JSEN.2014.2359832)
- Materić D, Bruhn D, Turner C, Morgan G, Mason N and Gauci V (2015) Methods in plant foliar volatile organic compounds research. *Applications in Plant Sciences* **3**(12): 137-143. DOI: [10.3732/apps.1500044](https://doi.org/10.3732/apps.1500044)
- Rizzolo A, Bianchi G, Lucido P, Cangelosi B, Pozzi L, Villa G and Curir P (2012) Electronic nose for the early detection of Red Palm Weevil (*Rhynchophorus ferrugineus* Olivier) infestation in Palms: preliminary results. *II International Symposium on Horticulture in Europe*, pp. 347-355.
- Seco R, Penuelas J and Filella I (2007) Short-chain oxygenated VOCs: Emission and uptake by plants and atmospheric sources, sinks, and concentrations. *Atmospheric Environment* **41**(12): 2477-2499. DOI: [10.1016/j.atmosenv.2006.11.029](https://doi.org/10.1016/j.atmosenv.2006.11.029)
- Vezina A (2017) Fusarium Wilt of Banana. Retrieved from: www.promusa.org/Fusarium+wilt.
- Webster CG, Wylie SJ and Jones GK (2004) Diagnosis of plant viral pathogens. *Current Science* **86**(12): 1604-1607.