Brief Report

Protocol

Mobile Image Ratiometry for the Detection of *Botrytis cinerea* (Gray Mold)

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Mobile Platform Informatics (MPI) and Smartphone Informatics (SPI) methods like Mobile Image Ratiometry (MIR) are potentially transformative point-of-use instantaneous analysis tools that are useful across a variety of industries. In agriculture, MIR-compatible immuno test strips allow early detection of a number of biotic stressors before devastating crop losses occur. Here we describe a low-cost and easy-to-use Smartphone and/or tablet-based protocol (Mobile Assay Inc., www.mobileassay.com) for the detection and on-sight instantaneous analysis of *B. cinerea*, a fungus that causes significant damage to a variety of plants and flowers. Early detection and tracking of the *B. cinerea* fungus before the visible gray mold appears has the potential to increase agricultural productivity especially in the developing world.

Introduction

Botrytis cinerea (gray mold) is a necrotrophic fungus that commonly appears as blossom blights and fruit rots. It is a very common organism that readily grows on dead, declining plant tissue and organic matter. Diagnosing gray mold requires careful examination and testing. Pathogens like *B. cinerea* have caused costly storage yield losses of 20 to 30% in Colorado onion crops, 50% in Idaho and 60% in Europe. To make testing for pathogens like *B. cinerea* more convenient, researchers at the University of Colorado, Boulder have developed Mobile Image Ratiometry (MIR; Mobile Assay Inc. www.mobileassay.com Boulder, CO), which allows users with Smartphone or tablet-based cameras and calibrated test strips to make rapid and accurate disease diagnoses on the spot (**Fig 1**). Having a quick test and result makes plant management decisions more efficient and effective. Proper identification of the disease needs to be made in a timely fashion, before the infection gets out of control leading to catastrophic losses. MIR technology allows rapid quantification and cloud-based analysis of immuno-based tests strips like those available for the diagnosis of *B. cinerea*. This newly developed rapid testing technology provides a result in less than 10 minutes and is designed to eliminate disease misdiagnoses and establish whether or not the symptoms are physiological or caused by a chemical or pathogen. This on the spot quantification also permits location geo-tagging to facilitate precise field location damage identification. Thus an entire crop can be tested and the *B. cinerea* outbreaks plotted on the crop map.

Symptoms of B. cinerea

Plants can be attacked at any stage of growth. *B. cinerea* usually appears first as lesions on leaves and stems that rapidly lead to the development of gray, furry spores. In onions, *B. cinerea* produces symptoms that often appear after the bulbs are stored. The fungus produces tan to brown stain of outer bulb scales. The pathogen either directly penetrates the scale to initiate the lesion or grows down into the scale from the leaf sheath or the leaf itself. Other species of Botrytis such as *B. allii* produce gray mold may form in the neck area causing it to become sunken as well as drying out the entire bulb. The fungus may partially rot the bulb before it is observed externally. Infected scales become soft and brown in color leaving the plant susceptible to secondary infection by other pathogens. The MIR immunostrip only detects *B. cinerea*, but the technology could be expanded in the future to include other pathogens such as *B. allii*. In addition, infection in flower petals produces a rapidly spreading infection that causes fruit tissue to disintegrate into a liquid mass.

Fig 1. MIR acquisition and analysis of rapid test strips on the Android and iOS platforms.

In grapes, small circular water-soaked spots are the first to appear. These spots may be faintly clear and relatively indistinct, however, when the grapes are rubbed, the skin over these spots cracks and reveals the firm inner pulp. Gradually affected fruit softens and turn brown. In dense bunches, *B. cinerea* may spread rapidly until entire bunches are rotted.

MIR-based quantification and analysis can be applied to a variety of plants susceptible to Botrytis including grapes,

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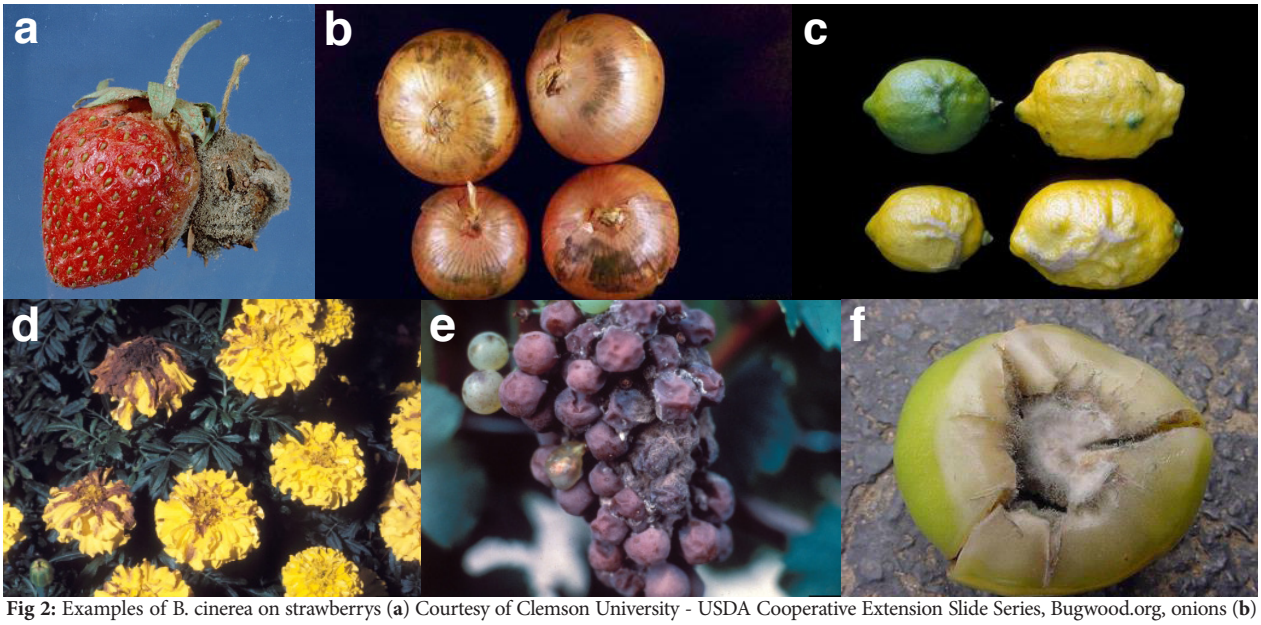

Fig 2: Examples of B. cinerea on strawberrys (**a**) Courtesy of Clemson University - USDA Cooperative Extension Slide Series, Bugwood.org, onions (**b**) Courtesy of Howard F. Schwartz, Colorado State University, Bugwood.org, lemons (**c**) Courtesy of Gerald Holmes, Valent USA Corporation, Bugwood.org, marrigolds (**d**) Courtesy of R.K. Jones, North Carolina State University, Bugwood.org, grapes (**e**) Courtesy of University of Georgia Plant Pathology Archive, University of Georgia, Bugwood.org, and a tomato (**f**) Courtesy of Paul Bachi, University of Kentucky Research and Education Center, Bugwood.org.

potatoes, onions, berries, lettuce, flowers **(Fig 2)** wheat, yams, and chick peas. The main factors that promote *B. cinerea* infection and the MIR-based protocol for its detection are listed below.

Five Conditions that Favor B. cinerea Infection

1. High relative humidity (>90%) and cool temperatures 50-75ºF near harvest
2. Poor plant management (e.g. dead leaves) and sanitation (e.g. debris)
3. High levels of nitrogen from fertilization
4. Inadequate air circulation among the plants
5. Light rain and condensation that lasts for several days

Testing for B. cinerea

Serious *B. cinerea* fungal infection can usually be visually observed in the field or vineyard. However, low-level infections and infections that arise during plant or fruit storage can be difficult to identify visually and can only be detected using immuno-based tests like an enzyme-linked immunosorbant assay (ELISA) or a lateral flow rapid test strip. Isolating infected plants is the standard method for eliminating *B. cinerea* infection, but it is often difficult to identify which plants carry the spores. Undetected *B. cinerea* can create a serious infectious outbreak that can be catastrophic. An ideal diagnostic test would be inexpensive, mobile, and simple to use while allowing growers to randomly sample plants in storage, in transit, or in the field in order to detect and begin treating the disease prior to visual symptoms. Such a test could be used by growers, packagers, and buyers at the plant receiving stage to provide an objective measure of *B. cinerea* infection and to gauge the effectiveness of any eradication program.

Procedure for MIR-Based B. cinerea Testing in the Field

To use MIR, only minimal training with a Smartphone or tablet with a camera and a test strip are required. The Smartphone app does not require the purchase of specialized and expensive laboratory instrument. Costly and time-consuming laboratory testing is not required. Tests may be performed at any chosen location in the field. To make a determination whether *B. cinerea* is present, a sample is taken from plant tissue showing signs of infection. If possible, a control sample should be taken from an uninfected area of the same plant. Samples are placed in a pouch or vial and macerated with liquid buffer. The liquid samples from the control and test regions are then tested with MIR and a test strip. A photo is taken of the test strip for automated analysis using the MIR application. A properly executed test strip procedure indicates one band (Control) and a second band (Test) if the sample tissue is positive for *B. cinerea.* The intensity of the second band is proportional to the degree of infection in the sample and can be quantified by MIR analysis. In addition to the results being available on the spot at test time, the results for each test are wirelessly transmitted securely to a cloud server for storage, detailed reporting, and analysis.

Mobile platform informatics (MPI) and Smartphone informatics (SPI) tools like MIR are designed to allow early detection of B. cinerea before infestation and devastating crop losses occur. As new MIR-compatible rapid tests are developed it will be possible to perform instantaneous analysis for other biotic stressors. Biotic stressors such as viruses, fungi, bacteria and other pathogens are a significant problem for agricultural productivity. Farmers with small plots of land in developing countries have sparse resources to prevent these stressors and can, therefore, experience devastating crop losses before and after harvests. MIR-compatible tests have the capacity for early detection and mitigation of these losses, which has the potential to be transformative for farmers in the developing world.

PROGRESS AND COLLABORATIONS

To see up to date progress on this project or if you are interested in contributing to this project visit: http://www.Neuro-Cloud.net/nature-precedings/botrytis

Donald C. Cooper is Co-Founder and Chairman of Mobile Assay Inc.

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For detailed references and expanded protocol please go to http://www.Neuro-Cloud.net/nature-precedings/botrytis