Digital farming technologies for disease surveillance, pathogen detecting & forecasting

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A seminar presented by Professor Jean Ristaino from the Ristaino Lab, North Carolina State University on 26 February 2020 at the Department of Primary Industries, Research and Development

The Ristaino Lab works on emerging plant diseases that threaten global food security. Pathogens constantly change and evolve. Emerging new races of wheat rust are a global concern and capable of causing 100% losses in susceptible crops. New technology being explored in wheat may have direct transferability to other crops like berries.

For example, a new strain of wheat stem rust (the Ug99 pathotype of *Puccinia graminis*) is acknowledged as a major threat to Australia's grain industries.

UG99 has overcome many wheat resistance genes and can attack all above ground plant parts. Since it was first recognised in Uganda in 1999, it has evolved into six different races. Other emerging strains of wheat rust include TTTTF, first found in Sicily in 2016 and AF2012 which started in Ethiopia and Uzbekistan, also in 2016. A major focus of Ristaino Lab is to understand the factors that contribute to disease emergence. This includes the epidemiology and population genetics of Oomycete plant pathogens in the genus *Phytophthora*. Late blight of potatoes (*Phytophthora infestans*) has been around for well over 150 years, having been famously responsible for the Irish potato famine. But it is re-emerging in new locations such as Guam with surprising intensity and in 2009 a new strain of late blight (now called US-22), that is highly pathogenic to tomatoes, was found on tomato seedlings in the US.

Evolution of technologies

Jean Ristaino and her team first developed the platform USAblight.org to help growers manage late blight of potatoes. Growers send in disease samples which are diagnosed and mapped. That information is available on the website for other growers. Genotype specific strains are identified as well as susceptibility to fungicides. Growers are thus made aware of the extent of the current threat. The platform also contains best management practices for the disease including a decision support system.

One deficiency of the current platform is that growers must physically go out and collect samples for diagnosis and then submit them. A time-consuming process in terms of sampling and mailing off specimens and the time lag for testing, diagnosis and results. The other problem is that growers are deciding to spray according to the information generated by the decision support tool which is based on weather data - not knowing whether the pathogen is actually present.

Rapid DNA extraction from plant tissues using microneedles (LAMP method)

This new technique uses microneedle patches to collect DNA from plant tissues in one minute, rather than the hours needed for conventional techniques. Previously, microneedle patch technology was developed and used in the delivery of drugs in human medicine. This is a new application of the technology.

Typically, DNA is extracted from a plant sample using a method called CTAB (cetyl trimethylammonium bromide) extraction, which must be done in a lab, requires a lot of equipment, and takes at least 3 to 4 hours. CTAB extraction is a multi-step process involving everything from tissue grinding to organic solvents and centrifuges.

This new DNA extraction technique involves only a microneedle patch and an aqueous buffer solution. The patch is about the size of a postage stamp and is made of an inexpensive polymer (2-3 cents each). The surface on one side of the patch is made up of hundreds of needles that are only 0.8 millimeters long.

The farmer or researcher applies the microneedle patch to a leaf of the suspect plant, holds the patch in place for a few seconds, then peels it off. The patch is then rinsed with a buffer solution, washing genetic material off the microneedles and into a sterile container. The entire process takes about a minute.

This plant DNA extraction approach doesn't rely on the usual concept of DNA isolation through tissue and cell lysis. Instead, it uses puncturing with a microneedle (MN) patch into leaf tissue to extract intracellular DNA and other molecules without the need for cell lysis. Since the DNA is extracted without using any chemicals, it is directly amplifiable without purification and therefore able to be used 'as is', for subsequent molecular analysis, such as polymerase chain reaction (PCR).

The MN patches are made from a poly vinyl alcohol (PVA) material. It is a cost-effective water-absorbing polymer that can rapidly absorb 10–30 % of its own weight in water within minutes. It is that swelling-driven capillary flow which is one of the main forces to concentrate intracellular DNA molecules around the microneedle tips. While this technique is new, and much quicker than previous methods, it still requires physically collecting samples in the field and it still requires the use of a laboratory.

Non-invasive diagnosis of plant disease by volatile organic compound (VOC) analysis

This pathogen sniffer work based on volatile sensor strips was published in the journal Nature last summer. The project at Ristaino Lab developed a smartphone-integrated plant VOC profiling platform using a paper-based colourimetric sensor array with functionalised gold nanomaterials and chemo-responsive organic dyes for accurate and early detection of late blight in tomato leaves. The system is calibrated to detect specific VOCs emitted by plants infected with specific pathogens. It will also differentiate between healthy and diseased plants. The process involves placing a leaf in a 20mL vial for an hour. Fine tubing attached to the vial connects with a VOC sensor attached to an Android smartphone. The sensor is scanned in real time by the smartphone reader. The readings generated by the sample can be compared with those from previously calibrated samples of VOCs emitted by healthy and unhealthy plants infected with a specific pathogen.

Trials to date have shown a detection accuracy of ≥95% in diagnosis of P. *infestans* in both laboratory-inoculated and field-collected tomato leaves in blind pilot tests. The sensor platform has also been tested for detection of P. infestans in symptomless tomato plants in the greenhouse setting.

In the work published in Nature, ten different compounds were each able to be identified within one minute. Compounds were also able to be detected only two days after inoculation – well before visible symptoms.

The environment-induced signal drift of the VOC strips is much better than that of commercial e-nose sensors (<5% versus <30%). In addition, the cost of the chemical sensor array is estimated to be about 15 cents per test and the smartphone attachment is about US$20 (excluding the smartphone), which is orders of magnitude less expensive than commercial e-nose sensors.

Although this work used leaves placed in glass vials for an hour to collect VOCs it is possible to reduce this time to about 15 minutes. However, the future is to eliminate this step entirely and use sensor patches attached directly to the plant leaves which emit signals to be continuously received by remote monitoring devices for long-term monitoring of symptomless plants and deployment of larger numbers of sensors over a large scale to more efficiently detect early infections in fields.

It has been observed that undetached leaves produce 10–15% less volatile emissions than those from detached leaves, but better sensor and gas sampling design in future may help compensate for that deficiency. The current smartphone-based VOC pathogen sensors can potentially be integrated into a disease forecasting system for late blight and used by field extension officers for farmers to trigger a spray event, whereas current late blight forecast systems are mostly weather-based. To that end, earlier this year, the Ristaino Lab was one of four recipients of the next phase of the Game-Changing Research Incentive Program (GRIP).

The project proposal, entitled: Plant-Aid: A Data-Driven and Sensor-Integrated Platform for Monitoring Emerging Plant Diseases aims to develop a platform to monitor crops for signs of early plant disease, enabling farmers to make real-time, data-driven decisions. Flexible, cost-effective (2-3 cents each) sensors will be attached to tomato plants, checking for physical, chemical, environmental and biomolecular markers of disease or plant stress. The data, collected wirelessly on smartphones, will be integrated with a bioinformatics and geospatial database, which will be able to alert growers of new outbreaks nearby via SMS and model their spread for a more effective, rapid response.

While this current project is focussed on late blight in tomato it is hoped that if this model proves successful, it can be extended to other crops and pathogens.

Find out more about the Ristaino Lab at ristainolab.cals.ncsu.edu