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Editorial Article: Protecting chocolate supplies: Scientists devise biosensor tests to tackle cocoa disease blighting West Africa

Dr. Jacqueline Barnett and Dr. Joel Allainguillaume discuss their work to develop handheld detectors to combat the spread of cacao swollen shoot virus and arrest the decline in the cocoa industry

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In this exclusive interview, we speak with Dr. Jackie Barnett, Senior Research Fellow, Institute of Bio-Sensing Technology, and Dr. Joel Allainguillaume, Associate Professor in Conservation Science Faculty of Applied Science at the University of the West of England (UWE), to find out more about their research developing on-site biosensing platforms to monitor and detect circulating strains of cacao swollen shoot virus (CSSV). Barnett, Allainguillaume, and their expert team of fellow scientists hope to use these innovative biosensors to help revitalize West Africa's endangered cocoa industry.

CSSV can have a devastating impact on cocoa yield and has killed millions of cocoa trees

The cocoa crisis

There has been remarkable progress in biosensing technology since the earliest discovery of the biosensor in 1962 by Clark and Lyons. Since then, extraordinary advancement has been made and the evolving biosensor field has resulted in many collaborations among a range of diverse scientific disciplines, including bioelectronics, nanotechnology, and biological sciences. Biosensors are demonstrating their valuable application within society, extending from clinical diagnostics to agriculture, food and water safety, as well as environmental monitoring. The aim of the Institute of Bio-Sensing Technology, Faculty of Applied Science, UWE is to develop highly sensitive and specific bio-sensing platforms to target a range of different diseases affecting humans, animals and even plants. "One of the most recent projects funded by Innovate UK, is to develop a biosensor to detect cacao swollen shoot virus, better known as CSSV, in cocoa plants in West Africa," explains Barnett. The research group at UWE is collaborating with Mars Wrigley on this project, as they work towards a shared goal of tackling the prevalence of CSSV and enhancing cocoa production in the region.

Producing chocolate is a surprisingly complex process that requires cocoa seeds to be fermented, dried, roasted and then ground. Most consumers of chocolate are not aware of the devastating infections that persist and damage cocoa production, the most important in West Africa being CSSV infection. CSSV is transmitted by different species of mealybug that feed on the sap of cocoa trees, leading to damaging effects on cocoa yield and even the death of cocoa trees. "CSSV is devastating for cocoa production. There have been many different strategies set out by governments in West Africa to try to limit its spread," states Allainguillaume. The disease can lead to several unwanted symptoms, such as swelling of the stems and roots of the plant, and it can take up to three years for these symptoms to appear. "Within that time, the mealybug vector can spread the infection to other surrounding trees at great distances, whilst remaining undetected," explains Barnett.

Made in West Africa

Côte d'Ivoire and Ghana supply around 70% of the world's cocoa production market. The cocoa decline has led to CSSV eradication programs to fight the infection head on by destroying countless infected cocoa trees. So far these efforts have failed to prevent the spread of CSSV, resulting in the death of many more cocoa plants and threatening the livelihoods of cocoa farmers. "Our aim is to be able to monitor the epidemiology of the disease, as well as test new planting material," says Barnett. "As a result of developing highly sensitive and specific tests to detect CSSV in pre-symptomatic trees, we can make sure that new plants are not sent out that would grow into infected trees." The timely detection of disease enables early intervention to prevent it from spreading further, as well as offering clear economic benefits, as Allainguillaume explains: "Early detection will not only improve yield, but support farmers in knowing that their crops are less vulnerable and that new planting material is free of infection, therefore we can help secure and better their livelihoods."

Testing on the move

Sequencing studies of CSSV isolates have identified a number of different strains of CSSV that can be grouped depending on their sequence homology. The team at UWE is developing a biosensor to detect different strains of the virus. "Many CSSV papers are outdated and many have developed immunoassays using polyclonal antibodies," says Barnett. "Using polyclonal antibodies generated against the purified virus via animal immunization typically results in a high level of unwanted background in the assay." Although researchers can absorb some of this unwanted background, it is often too high, which in turn reduces sensitivity. To combat this, Barnett and Allainguillaume chose to collaborate with Bio-Rad, using its custom antibody reagents to develop sensitive immunoassays for the detection of pre-symptomatic CSSV infections. "In addition to the antibody tests, we use quantitative PCR assays at UWE for comparison; these tools enable us to analyze the CSSV infection status of cocoa plants grown in the Envirotron at UWE," explains Barnett.

As well as working in the lab, the team traveled to Ghana in an early project to pursue their research efforts further at the Cocoa Research Institute of Ghana (CRIG). The group has tested a range of infected samples in Ghana, and gained some promising preliminary results.

Antibodies: The key to early disease detection

To develop the most sensitive and specific immunosensors for detection of distinct antibody-antigen interactions, high-quality antibodies are required for the bioreceptor components. Barnett chooses to apply customized antibodies produced by phage display to help reach her research aims, explaining: "There are many reasons to select antibodies generated via phage display, as they fulfill a certain set of criteria we are following, in particular, UWE and our collaborators at Mars Wrigley were keen for us not to involve animals in our research." Allainguillaume and Barnett's inventiveness was to identify a novel common antigen target on various strains of CSSV. Thus, in contrast to previous antibodies which only detect some strains of CSSV, the UWE team's antibodies enable different strains of the virus to be detected. This will allow broad and effective monitoring of the potentially lethal CSSV and enable farmers to mitigate the risks the virus poses to their livelihoods.

The EU directive 210/63/EU described in Gray *et al*, 2020 recommends that animals are no longer used to produce novel antibodies. "By collaborating with Bio-Rad, we were able to ensure that no animals were used in production of the monoclonal antibodies, also avoiding the drawbacks of polyclonal antibodies," says Barnett. "I found that the custom antibodies we commissioned from Bio-Rad, based on the antigen target information we gave them, were the most cost effective and most reliable way to go, whilst maintaining the highest level of sensitivity we required. As a result, the antibodies Bio-Rad produced for us were ideal for our research."

The biosensor will work by detecting a specific biological interaction and converting the chemical information obtained into a quantifiable output signal. "We now know our antibodies work well, and our goal is to make sure the sensor is reliable and robust," adds Barnett.

Future outlooks

Looking ahead, the team ultimately intends its sensor to function as an optical sensor. At present, they are developing a bespoke device to help them achieve exceptional sensitivity in the presence of plant material. “We are also working hard to define concentrations and learn how to better process plant tissue samples,” explains Barnett. “We will have cassettes made for us to our design specification and a reader; these will be used to perform field trials in Côte d’Ivoire and in Ghana.” Barnett and Allainguillaume’s expert team will be working with the World Agroforestry Institute (ICRAF) to explore a range of cocoa samples from sites across Côte d’Ivoire and Ghana. “Realistically, we cannot remove CSSV as there are far too many CSSV infected trees, however, we can create areas where you can develop plant material and control a particular area of growth,” concludes Allainguillaume.

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