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Production Research:

Tackling an Old Foe with New Technology

Production research is a cornerstone of CAC's goal of achieving measurable gains in yield per acre for California's avocado growers to improve the industry's profitability. In this issue, we focus on two projects whose aim is to apply new technology to the fight against one of avocado's old enemies, Phytophthora.

By some accounts, Phytophthora costs the California avocado industry $30 million annually. Although there is no solid data to support this figure, no one would argue that Phytophthora is not a major issue for California avocado growers and probably deserves more attention. A good first step to tackling this issue is to determine exactly how much of the industry's acreage is affected by Phytophthora and how severe the disease is in those affected areas. Two CAC-funded research projects by the USDA and the private company GeoSpatial Partners LLC aim to do just that.

Remote Sensing for Pest and Disease Monitoring

Remote sensing, in its most basic form, is simply the use of modern technology, usually aerial or satellite sensing, to gather information about objects on Earth. In agriculture, remote sensing has been gaining a foothold mostly in agronomic-type row crops – corn, soy beans, wheat. In those crops, aerial and satellite sensing technology has been proven to be able to detect things such as drought stress, nutrient deficiency, or pest outbreaks. Based on this information, farmers are able to take corrective action (e.g., fertilizer application) only on those parts of a farm that need it. On a 10,000 acre soy bean farm in the Midwest this can be a tremendous savings that directly results in yield improvements.

Several factors have allowed this technology to be successful in agronomic crops: they are generally very uniform (spacing and plant height), they have a limited canopy (several inches to a few feet), they are grown on flat ground, and they are high dollar value crops grown on large acreage so they can afford to invest in cutting-edge technology. As these technologies have become more common and their costs have declined, there has been a growing interest to use them in tree crops. However, several factors make this challenging, not the least of which is the large, multilayered canopies that trees have. Some problems, such as drought stress, that affect a canopy uniformly are relatively easy to detect. But other problems, such as a pest outbreak, that may affect only a small part of a canopy in their early stages are more difficult to detect remotely.

CAC has been working with Dr. Alassane Toure, a remote sensing expert and president of GeoSpatial Partners, for several years. He has been producing CAC's avocado acreage survey and has developed methodology to accurately identify and distinguish avocados from other crops on aerial and satellite imagery. Dr. Toure was funded this year for a three year, $70,000, proof-of-concept project to determine if the algorithms he has developed can be refined to identify avocados of different health status.

The first step in developing remote sensing for a crop is to find a reliable source of imagery with high enough resolution to identify the condition of the crop that's of interest. Currently, the USDA Farm Service Agency has a program called NAIP (National Agriculture Imagery Program) that collects 1 meter (about 3 feet) resolution aerial images of U.S. agricultural land during the "leaf-on" season. There is also a commercially available source of satellite imagery called RapidEye, which uses five satellites to collect 6 meter (about 19 feet) resolution images of the Earth. These two sources collect images in true color as well as different spectral wavelengths (e.g., infrared). As plants develop stress, their reflectance (the wavelengths of visible and invisible

It is very difficult to identify different species of Phytophtora on avocado trees.

back to Dr. Toure's team to further improve the models.

Regardless of which resolution images are used for this project, it is unlikely that it will be able to identify individual trees with Phytophthora. In fact, it may not be able to distinguish Phytophthora from other issues, such as compact soil or poor drainage, that cause avocado trees to have thin canopies. However, it is likely that it will be able to identify areas of groves that have trees with poor canopy health so that they can be carefully investigated from the ground. This could allow growers to focus on correcting those areas of their groves with problems and spend less time trying to find them. Ultimately, a remote sensing program, such as the one described here, would be developed into an online pest/disease outbreak detection and monitoring system for avocado producing counties in California.

light reflected from a leaf's surface) changes. For Phytophthora, which tends to affect areas of a grove rather than scattered individual trees, the 6 meter resolution images will probably be sufficient. However, since these are from a commercial company they are more costly than the NAIP images, so they will only be used during times of the year that NAIP images are unavailable.

Dr. Toure and his team will be cooperating with UC Riverside researcher Dr. Akif Eskalen to use Dr. Eskalen's lab's ground-based data for Phytophthora, PSHB/Fusarium dieback, and other pest/disease incidence in commercial avocado groves. Dr. Toure's team will use the Eskalen lab's data to calibrate their models with areas of known Phytophthora infection. From these models they will then try to identify other areas that may be infected. Dr. Eskalen's lab will assist in "ground-truthing" these identified areas of potential Phytophthora infection. Their findings on the ground will be relayed

Diagnosing Phytophthora

Phytopthora is generally believed to be just about everywhere in California's avocado producing region, and decline of avocado trees from Phytophthora in California has been an issue since the 1920s. Despite this pathogen's status as the greatest limitation to avocado production, relatively little headway has been made in the fight against it. To make matters worse, there are many different species of Phytophthora that can cause disease and it is nearly impossible to tell them apart based on symptoms alone. The two most important species in California are *Phytophthora cinnamomi* and *P. mengei*, but other species such as *P. citricola* (from which *P. mengei* was recently separated), *P. nicotianae* and *P. palmivora* can also cause disease problems. Because of the importance of this disease to avocado, and the fact that not all of these species affect all avocado growing areas, it is important to have a rapid, sensitive and accurate method for detecting the different species.

Historically, to positively identify different Phytophthora species they had to be cultured and grown in a laboratory and identified morphologically. Recently, Dr. Frank Martin, a Research Plant Pathologist with the USDA-ARS lab in Salinas, was part of a team that endeavored to develop molecular markers to distinguish Phytophthora species using modern technology. This federally funded project was undertaken with the primary goal of ultimately developing a field test for *Phytophthora ramorum*, the species that causes sudden oak death. Knowing the severity of Phytophthora in avocado, Dr. Martin approached CAC about developing a similar test for the Phytophthora species important to avocado, capitalizing on the huge federal investment already made. CAC has funded Dr. Martin for a two year, $30,000, project to develop these diagnostic tools for the avocado industry.

Dr. Martin and his team will use a technique called polymerase chain reaction, or simply PCR, which is a biochemical technique used in molecular biology for creating copies of pieces of DNA. This technique is based on DNA's structure of two complementary strands of nucleic acids. This can be visualized as a ladder cut lengthwise into two long "backbones" with pieces of rungs – the nucleic acids – coming off. Each piece of ladder rung will only match up with one other one to form a complete ladder. If the ladder rungs were coded and you knew the code, you could produce a complete ladder from just one half. That is exactly what PCR does, but figuring out the code is the key step.

Based on the extensive work already completed in the sudden oak death project, Dr. Martin's group now has a library of genetic data for all known Phytophthora species. From this, they know where the species are different and where they are the same genetically. This allows them to create "probes," which are essentially halves of the ladders. These probes are unique to each species of Phytophthora, and by extracting the Phytophthora DNA from a soil sample the probes can be used to determine the Phytophthora species in the soil. This is done by pairing the probes to their matching half under precisely controlled conditions. The probes have a fluorescent molecule (marker) bound to them, and when the probe binds with a matching DNA strand the fluorescent marker is released and it can be detected. If no fluorescence is detected, then the marker was not released, indicating that the sample species did not bind with the probe and is not the species the probe was developed from. Different fluorescent markers can be attached to the probes for different species, so several different species can be tested against a soil sample at the same time.

Technology has advanced significantly since the PCR technique was developed in the early 1980s. Today "PCR machines" are common in research laboratories, and are about the size of a large laser printer. Once the probes are developed, samples can be run quickly and easily for only a few dollars each, and the method is extremely sensitive (only a small sample is needed) and accurate. However, there are newer technologies that can be used to accomplish the same results. One of these technologies, recombinase polymerase amplification or RPA, holds promise for being used in the field. A commercial company, Agdia, is currently working to commercialize RPA for the detection of *Phytophthora ramorum*. If Agdia succeeds, the probes developed in Dr. Martin's CAC-funded project will be made available for the development of a field-based diagnostic assay for Phytophthora in avocado.

Phytophthora is an old problem for avocado production in California. The use of new technology promises to provide us with details about the true extent of the problem, and to more easily determine which Phytophthora species are most important in different areas. While this work will not result in a cure for Phytophthora, it will provide us with valuable tools to better understand the problem and allow growers to better manage their groves.