

DIAGNOSTICS IN CROP PRODUCTION

Derek Hollomon and Sheila Kendall from IACR Long Ashton Research Station, UK, describe the increasing importance of diagnostics in crop production as an aid to decision-making as described at a recent conference¹

Worldwide trade in foodstuffs, including perishable fruit and vegetables, has increased considerably in recent years. Consumer pressures in many countries have generated a need for rapid and cost-effective diagnostic tools for a number of food safety indicators. At the same time, the production side of agriculture creates a demand for diagnostics to improve the precision inputs, and to lessen the environmental impact of fertilizers, pesticides and veterinary products. Pesticide residues must not only be below certain levels, but in organic produce, they should be absent. As a result, there is a growing market for diagnostics in this area, particularly as world trade in organic produce expands.

Immunodiagnosis

Immunodiagnosis, particularly enzyme-linked (ELISA) offers many advantages, especially in plant health and situations where “yes” or “no” answers are required.

A number of recent advances promise to breathe new life into serological methods. The successful incorporation of antibodies into bio-sensors will permit “real-time” monitoring of target analytes whilst recombinant antibody technology offers an alternative source of reagents and even “designer” antibodies. Lateral flow devices will allow inspectors, growers and consultants to perform antibody-based testing in true “field” locations and should allow more rapid and targeted decisions to be made on the safe movement of planting material. Growers should also be able to use the technology to target and better manage their inputs to the benefit of the environment and profitability.

Aphid resistance to insecticides

The peach-potato aphid, *Myzus persicae*, is an important agricultural pest in the UK and other parts of the world, causing direct feeding damage and transmission of viral diseases on a variety of crops. However, because of heavy selection pressure over a number of years, resistance is now well documented for three of the major insecticide classes; organophosphates, carbamates and pyrethroids.

Three separate mechanisms have been identified that contribute to resistance in this pest. The first (and best char-

acterised) involves the increased production of carboxylesterases (termed E4 or FE4) that degrade or sequester certain insecticidal esters. This mechanism was originally thought to confer broad-spectrum cross-resistance to all ester-containing insecticides (*i.e.* organophosphates, carbamates and pyrethroids). However, more recent studies have identified two target-site-based mechanisms, involving changes in the nerve membrane sodium channel and acetylcholinesterase that contribute more selective resistance to pyrethroids and certain carbamates respectively.

Immunological (esterase), biochemical (AChE) and DNA-based (sodium channel) diagnostic assays have been developed for identifying each of these mechanisms in individual aphids. By applying these tests to field and glasshouse aphids, useful information is being accumulated on the relative frequencies and spatial distributions of the three mechanisms within the aphid populations. As a result researchers are able to provide informed advice for farmers and growers on the most effective choice of insecticide within a particular region.

Precision spraying

Various imaging techniques provide opportunities to diagnose *in situ* weeds, and crop nutrient status, and offer the exciting prospect of instantly coupling this information to a response of a sprayer or fertiliser spreader. Research has shown that there is the potential to make significant savings in herbicide use by targeting applications to match the spatial distributions of weeds and, that this can have important financial and environmental consequences. Considerable progress has been made in developing application systems that are capable of delivering targeted doses of sprayed treatments over a wide range of dose rates and spatial scales without compromising spray quality or the accuracy of delivery. However, the detection of weed patches and the generation of weed treatment maps in a timely and cost-effective manner, remains an important component of the total system that requires further development. The use of a treatment map approach to the patch spraying of weeds enables the dose rates and mixtures of herbicides to be matched to the weed species and density. This then gives an important interface between computer programmes that are transforming weed patch maps into treatment maps and decision support software.

Methods of automatically detecting weed patches have been based on spectral reflectance differences between crop and weed, an analysis of shape differences by image analysis or a combination of these approaches. While differences in

¹ This article is based on the proceedings of a recent meeting on “Diagnostics in Crop Production” organised by the Crop Protection Group of SCI and held in Brighton on 13th November 2000. Full abstracts of the talks given at the meeting can be found on <http://sci.mond.org/groups/cropprot/cropevents/abstiagnostics.pdf>

spectral reflectance characteristics have been shown to be useful in discriminating between, for example, given weeds and crops, the differences are a function of a wide range of production variables including seasonal effects, soil type, variety lighting and orientation at the time of detection. Difficulties associated with automated weed detection have led to the development of improved detection systems based on human operators. Logging weed patch positions, tagged with location from global positioning data, on hand-held and vehicle-mounted computing systems has used push buttons and voice recognition at the operator interface.

Prophylactic fertilising of the crop is no longer an option as the cost conscious and environmentally aware farmer moves towards precision farming and sustainable farming practices. Existing plant tests are largely rudimentary and empirical with little physiological basis and, reliable diagnosis of plant nutrient status is far from simple especially in the fluctuating environment of a field. However, there have been considerable advances in the analytical technology for the measurement of crop nutrient status. Instruments such as specific ion electrodes, reflectometers, chlorophyll meters and radiometers, suitable for *in situ* testing, are now being used to target plant organs of a fixed physiological age, *e.g.* the newest fully expanded leaf, by expressing concentrations on a tissue water basis, and by targeting specific nutrient pools, *e.g.* the storage pool in the case of N, P and S, and the chlorophyll pool as a tracker for N.

For the future it seems that the targeted spraying will have an increasingly important role, and that technological developments particularly associated with the rapid computer analysis of images collected under variable light conditions will make automated detection systems feasible.

Disease identification and quantification

Disease complexes pose particular problems with respect to identification and implementation of relevant disease control measures. Two such complexes of economic importance in wheat are stem base disease and fusarium ear blight (also known as scab or Fusarium head blight). Each of these diseases may be caused by a number of fungal pathogens, often occurring together in the same crop or even the same plant. Stem base disease itself consists of three recognised diseases – eyespot, sharp eyespot and brown foot rot. PCR assays have been developed for the major causal agents, and these assays have been developed to enable quantification of the relative amounts of each of the pathogens in plant tissues and identify differences in efficacy of fungicides and cultivars against the particular pathogens.

Quantitative PCR is being used in studies of fusarium ear blight. In this disease toxin producing *Fusarium* species may occur alongside non-toxin producing species such as *Microdochium nivale*. To date studies have revealed that particular fungicides, such as tebuconazole, control true *Fusarium* species while appearing to be ineffective against *M. nivale*. In contrast, azoxystrobin is particularly effective at controlling *M. nivale* whilst not appearing to control *Fusarium* species. Such differential control is of particular significance as it appears that the two groups may compete with one another, and that suppression of one may result in

an increase in the other. Under some circumstances this may result in an increase in the levels of trichothecene mycotoxins such as deoxynivalenol (DON).

Recent developments in PCR-based technologies provide greater opportunity for quantification in areas such as disease levels, and the frequency of pesticide resistance. The PCR reaction has succeeded as a result of its elegance and simplicity. In a single reaction, of only a few microlitres volume, it is possible to amplify a specific nucleic acid sequence more than a million-fold. A highly stable enzyme from *Thermus aquaticus*, Taq Polymerase, enables this process. The technology is easily and rapidly applied to any DNA or RNA target for which the nucleotide sequence is known. PCR is now in routine use in tens of thousands of laboratories around the globe, perhaps more visibly in Forensic and Medical sciences. In less than two hours, it is possible to move from a single, undetectable, molecule of nucleic acid to millions of identical, amplified copies that can be used for subsequent analysis.

Real-time PCR

The modification of fluorescence-based PCR (TaqMan®) gave rise to “Real-time PCR” producing a complete “video” of the reaction rather than a single “photograph” of an assay at a defined point in time. The benefit for users is truly quantitative PCR data across a wide dynamic range in, a low cost, high throughput format. The combination of the sensitivity of the PCR process with the reliable quantification data obtained by performing “Real-time assays” enabled rapid discovery of many processes. Perhaps the most widely cited application is classical gene expression studies. “Real-time PCR” is used to monitor changes in gene expression such as response or resistance to infection. The assay can be used to accurately determine viral or fungal load in samples whilst the specificity of the amplification is capable of amplifying and detecting specific variants. The spread of strobilurin (QoIs) resistance in several important plant pathogens has prompted development of rapid PCR assays to identify accurately the presence and frequency of the target-site mutation linked to resistance. In a dual probe PCR assay it is possible to screen populations for specific point mutations, deletions and insertions. Such changes in the chromosomal make-up of the organism may influence disease susceptibility or resistance. The only analytical method for the comprehensive analysis of GMO's in processed foods is PCR. Using “Real-time PCR”, the precise amount of modified DNA can be determined even in complex matrices that have been subject to varying degrees of processing.

GMO detection

The incorporation of genetically modified soya and maize into the food we eat has sparked a massive interest in detection systems for GMOs. Again there are many diagnostic opportunities in this area, and it is surprising how rapidly industry has responded to provide diagnostic tools to meet the demand.

Over recent years, rapid test systems have been developed for GMOs based on the detection of novel proteins rather

than novel DNA. This system was originally developed to meet the needs of an industry favouring GM crops and prepared to pay the premium for such crops. However, because of public concern over GM crops, this technology is now offered, as a suite of immunoassay-based test kits, for the detection and quantification of GM material in crops and primary processed ingredients.

New BioChip microarray technology offers the exciting possibility of comprehensive detection of GMO traits in one analysis. Using micro droplets deposited robotically, literally thousands of analyses can be conducted in an area no larger than a postage stamp. Rapid turnaround and a comprehensive report confirming plant species, specific GMO traits, screening results and controls represent a significant advance in GMO analysis.

Future prospects

Commercial application of many diagnostic technologies may still be some way off, but as research tools they are already providing valuable novel information on, epidemiology, for instance. This knowledge should improve how crop protection inputs are managed.

With all these openings for diagnostics in crop production there is a feeling perhaps that too much rests on assumptions surrounding sampling, and that these protocols are not yet robust enough to meet the challenges.

There are still serious gaps in our understanding at the production level as to how to translate effectively accurate diagnostic information into decisions about when to spray, and with what dose. Certainly diagnostics can provide a lot more detailed information on how crops are growing. Before many of these systems become useful commercial

products we must learn how to interface all this information with computer based decision-making systems.

Further reading

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Derek Hollomon's research career has concentrated on several aspects of fungicide use. Over the past ten years he has explored how developing diagnostic technologies can improve the effectiveness of fungicide use against cereal diseases. Although recently retired, Derek continues an active research involvement in diagnostics as a visiting worker at the Long Ashton Research Station (LARS). A past-chairman of the Crop Protection Group of SCI he is now the groups' Honorary Recorder.

Sheila Kendall's research at LARS has been concerned with the development and evaluation of strategies to combat fungicide resistance in diseases of arable crops. In recent years she has been involved with the development of strategies for the use of novel diagnostic technologies for the control of diseases of field grown crops.

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