

Molecular Approaches- Fingerprinting made Easy

David Shaw¹

The following are frequently asked questions. How variable is the fungus in my field/region/country/hemisphere? Is migration, mutation, sexual or asexual recombination involved? Will answers to these questions affect the strategy for control?

At present, many populations are characterised using isozyme and DNA fingerprinting with probe RG57; the latter provides information (presence/absence) at many loci and increases the resolution of variation detected by isozymes. This marker combination appears to be ideal in characterising populations in regions with low diversity (e.g. the US and Canada) where several clonal lineages seem to predominate. In Europe, where migration some 20 years ago introduced A2 and many new genotypes, isozyme/RG57 data suggest sexual populations in non-commercial sites and widely dispersed clonal lineages in commercial crops.

Recent evidence using AFLP and RAPD markers indicates that many European isolates can have identical RG57 fingerprints yet very different AFLP/RAPD fingerprints. Lack of relatedness within an RG57 genotype suggests that convergent evolution frequently generates one of the common RG57 fingerprints. Perhaps the possession of a particular RG57 fingerprint confers a selective advantage. In these situations, RG57 suggests a clonal lineage that is not confirmed by other markers. Also, isolates with several RG57 genotypes from U.K. were identical at all 24 polymorphic AFLP loci scored. In such situations, RG57 alone does not seem to detect true relatedness. An unexpected finding was the close AFLP relatedness of almost all A2 isolates from U.K. These did

not appear to be hybridizing with co-occurring A1 genotypes (i.e. an A2 clonal lineage is probable).

What molecular markers should we use in the future? Ideal markers would be cheap, easy to use, robust and indicate true relatedness. Co-dominant markers detecting multiple alleles have great advantages in population genetics where understanding population structure depends on detecting gene (allele) frequencies and also in situations where more than two copies of a gene are present (trisomic or polyploid isolates). Isozyme genotypes at loci *Gpi-1* and *Pep-1* are quickly and easily determined, but are monomorphic in some populations of new genotypes. Single-locus RFLP markers —some with multiple alleles— are useful, but the method is laborious. Microsatellite markers (Simple Sequence Repeats) are close to ideal; once identified they can be detected by PCR. In our recent work, many such loci were, unfortunately, monomorphic. Sequence data from the genome project should provide many candidates for loci with varying numbers of simple-sequence repeats. Dominant markers (no multiple alleles) can still provide valuable information on relatedness of isolates. The AFLP method is ideal for relatedness studies and is efficient (many loci on a single gel) but technically fairly demanding. The RAPD method is easy and, with great care and suitable controls, is robust, but it is more time-consuming than AFLP. Some mitochondrial DNA polymorphisms can be detected very easily using a PCR method. Haplotype Ib seems to be diagnostic of the old A1 clone, US-1, easily controlled with metalaxyl.

I wish to acknowledge the help of my Bangor colleagues: Richard Shattock, Nick Pipe, Jenny Day, Nick Hardman and Andrew Purvis.

¹ University of Wales, Gwynedd, U.K.