THE USE OF DNA PROBES TO STUDY THE ECOLOGY OF HERBICIDE-DEGRADING MICROORGANISMS IN SOILS

T. Duxbury, Department of Microbiology, Faculty of Agriculture, University of Sydney, NSW 2006.

Background to the project

Herbicides are now established as essential components of modern agricultural practice and will probably find increasing application as conservation farming becomes more widespread. However, many herbicides presently used in Australia and elsewhere are limited in their application because of potential damage to susceptible crops. For example, atrazine, a low cost residual herbicide, has been used to control weeds in corn and sorghum crops, but it also has considerable potential as a fallow herbicide in no-tillage crop production. However, the list of susceptible crops is long and includes linseed, cotton, soybeans, barley, wheat, oats and sunflower. Other herbicides pose similar problems. Trifluralin, triallate and chlorsulfuron are recommended herbicides for pre-sowing and salvage seedbed weed control. The former two may cause carryover injury in sorghum and maize, while the latter causes problems with soybeans, sunflowers and some varieties of wheat as well.

Microorganisms play an important role in the degradation of most herbicides in current use. However, the rapidity with which microbiological breakdown can occur will clearly depend on the number of organisms present in the soil which have the capacity to participate in the degradation process. Until now such organisms could only be enumerated by growing them on selective media supplemented with the herbicide as a growth substrate. However, this only provides quantitative data for a fraction of the actual community involved, i.e. those organisms that use the herbicide as a nutrient. But there are other organisms that play a part in herbicide degradation that may not be able to use the parent compound as a growth substrate.

Rationale behind the project

Most herbicides are complex molecules and their decomposition often requires several different chemical conversions, each mediated by a variety of organisms. Until now the detailed investigation of the ecology of such organisms has been hampered because of the need to have sufficient quantities of herbicide degradation products for inclusion in the selective media required for their enumeration. The rationale behind the approach to the present work is that each of the chemical conversions performed by microbes will be catalysed by enzymes. The quantitative detection of

the genes coding for these enzymes in soil microbial communities should indicate whether or not a soil contains organisms able to degrade a herbicide and, if it does, whether the rate of degradation will be relatively fast or slow. DNA probes are labelled pieces of DNA which are copies of the individual genes, in this case herbicide degradation genes. Under appropriate conditions these probes will selectively bind to the same genes contained in microorganisms, even when they are growing on non-selective media. Consequently, by judicious selection of probes it is now possible to identify and, more significantly, enumerate the many different organisms involved in the degradation pathway of particular herbicides. Studies of the ecology of these organisms, using DNA probes, could reveal ways in which the soil environment might be manipulated to temporarily stimulate the activity of such organisms and so reduce herbicide persistence.

A model system

Presently, the work is at an early stage and the herbicide 2,4-D is being used as a model compound to develop techniques for the construction of DNA probes and to overcome some of the difficulties of using them with soil microbial communities. The 2,4-D system in Alcaligenes eutrophus is fairly well characterised and the degradation pathway is shown in Fig. 1. Each of the degradation steps is catalysed by a different

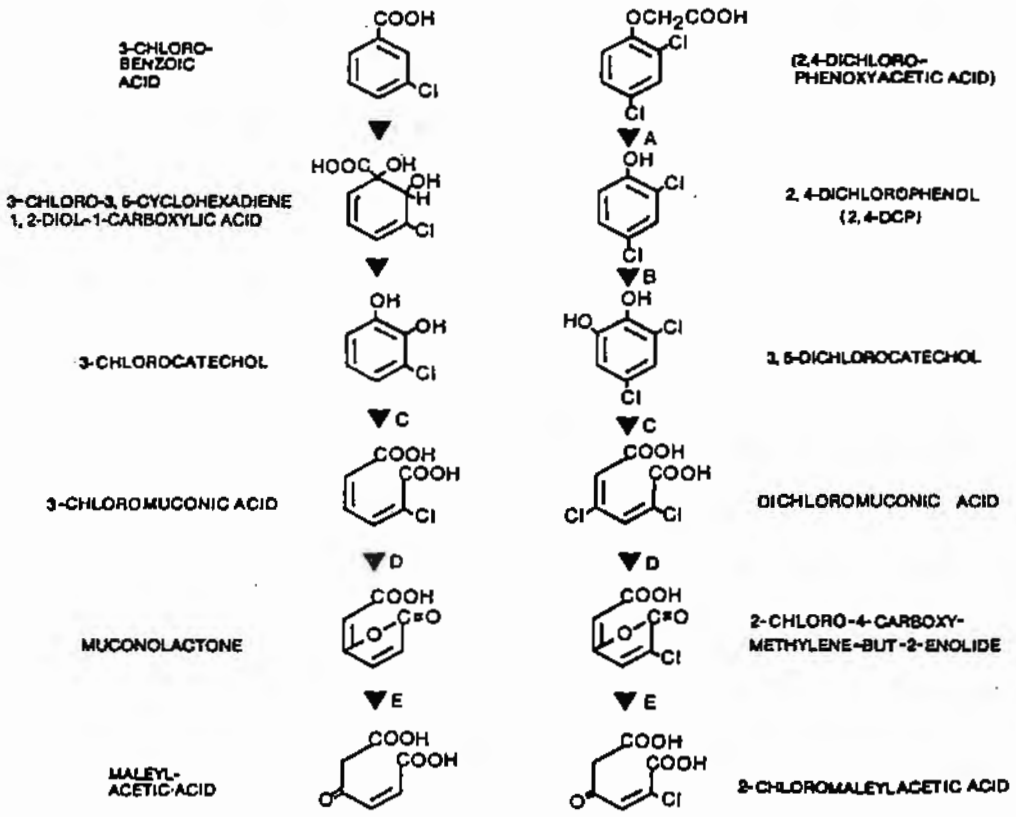

Fig. 1. Degradation pathways for 3-CB and 2,4-D.

enzyme, each coded for by genes A to F. (Gene F is thought to be an isomerase.) But, as can also been seen in Fig. 1, the degradation of another aromatic compound, 3-chlorobenzoate (3-CB), contains chemical conversions common to the 2,4-D pathway. Thus, enumeration of 2,4-D-degrading organisms by traditional methods of growing them on 2,4-D-supplemented medium would not enumerate the 3-CB degraders, even though the latter would be involved in the breakdown of some of the degradation products of 2,4-D. Consequently, DNA probes have been constructed for the genes A, B, and the cluster C-D-E-F to

determine which combinations provide the best correlative data with 2,4-D degradation under field conditions.

The ultimate aim of the project is to use gene probes to study the disappearance of atrazine, chlorsulfuron, triallate and trifluralin from agricultural soils. So far most efforts have been directed towards the isolation and characterisation of microbes involved in atrazine degradation. These organisms will be used to construct gene probes so that a more complete picture of atrazine disappearance from Australian agricultural soils can be obtained.

Postscript

If any of the conference participants are currently using any of the abovementioned herbicides and are willing to provide soil samples, if required, would they please contact me at the above address. In addition, if anyone has experienced problems with other herbicides under particular conditions I would be pleased to hear of them.

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