THE IMPORTANCE OF USING FULLY CHARACTERISED POPULATIONS FOR WEED EXPERIMENTS

THE NEED FOR CLEARLY DEFINED POPULATIONS

The composition of any weed population used in an experiment has a crucial influence on the outcome of the experiment, whether it is a laboratory screen or a field trial. Variation introduced by an incorrectly identified species or an inadequately defined population will compromise the experiment and can limit the validity of its results.

The experimentalist wishes to reduce the uncontrolled variation in an experiment so that it does not confound the effects of the experimental treatments. It is therefore crucial to correctly identify the species of weed present, and to ensure that the population of each species is clearly defined and as homogenous as possible.

THE PROBLEM

Several species of weeds are difficult to distinguish at the vegetative stage from closely related species. This problem is often encountered with field populations of grasses, *Matricaria spp., Veronica spp., Amaranthus spp.* etc. Even within a species, individual biotypes with significantly different tolerances of a herbicide may be widespread (Appendix 1 gives a few examples). Failure to define the weed population can have a crucial impact on the validity of experimental results as illustrated by the following examples;

Example 1). In a field trial which included chlortoluron and tralkoxydim to control *Alopecurus myosuroides* in cereals, one treatment killed 90% of the grasses in the plot. The facile conclusion was that the treatment gave 90% control of *A. myosuroides*. However, a closer examination of the grass weed population showed that it was composed mainly of *A. myosuroides* but with up to 20% of *Lolium perenne* and *Poa annua*. The true interpretation was that the tralkoxydim treatment gave 100% control of *A. myosuroides* but no control of the *Poa*. In the chlortoluron treatments the level of control achieved was strongly influenced by the tolerance level of the *Alopecurus* biotype(s) present and the interpretation would probably have been invalid if the presence of the more tolerant *Lolium perenne* had not been recognised.

Example 2). High throughput glasshouse screens tend to have very low numbers of plants per treatment. This makes it crucial to use a homogenous and clearly defined population of each species, since a small number of atypical plants randomly distributed can represent a high proportion of the experimental population in a particular treatment. What conclusion does the screener draw from a treatment which kills seven out of ten plants of a species in a screen but leaves the remaining three undamaged? Is the chemical inconsistent or is the experimental plant population composed of different biotypes? Or even different species? The wrong interpretation could result in the rejection of a potentially valuable treatment.

SOLUTIONS

In both of the above examples, experimental accuracy, ease of interpretation and confidence in the value of the results would be improved by ensuring, before starting the experiment, that the weed population was clearly defined, contained a uniform population of a single species and was appropriate to the objectives of the experiment. How can this best be achieved?

In field trials: Identification of weed seedlings to the species level is difficult with some species, particularly some grass seedlings, *Veronica spp., Chenopodium spp., Matricaria spp.* and *Amaranthus spp.* Gaining some practice on live plants, aided by a good key or picture book can overcome problems with these species. A greater problem arises with species in which herbicide tolerant biotypes are widespread. This phenomenon is becoming of increasing importance, particularly in Europe, Australia and the USA. A knowledge of the field's weed flora and agronomy in the previous seasons is a great help in reducing the risks of encountering unwanted population characteristics. A more reliable technique is to select a field in which these species do not occur and to establish the experimental population from seed derived from a correctly identified and well characterised population.

In glasshouse tests: Critical attention to the origin of the seed used to produce plants for glasshouse tests is the key to achieving confidence in the results. The seed must be species-pure and of a uniform biotype appropriate to the objectives of the experiment.

The source of seed: Establishing an experimental population from seed does not achieve a uniform population if the seed itself is not homogenous. Seed collected from farm crop cleanings, or worse, the cleanings from communal silos, will inevitably include several biotypes and possibly several species. A single batch of what was labelled '*Amaranthus retroflexus'* seed collected from 'elevators' in the USA was once determined to consist of 72% *A. hybridus*, 17% *A. retroflexus*, 5% *A. rudis* 2% other unidentified *Amaranthus* species and 4% other genera! It is imperative that the seed used to produce experimental plants is of a single well characterised population.

THE HERBISEED SOLUTION

Herbiseed employs a well thought out system to ensure that the seeds of the major weed species which we supply are correctly identified, well characterised and appropriate to agrochemical experiments. An initial seed stock is hand collected from an accurately identified and clearly defined population growing in a habitat (usually an agricultural field) typical for the species. This is cultivated on one of our farms under controlled conditions to produce the seed which we supply. During growth it is rogued to remove atypical plants and weeds of other species. Isolation measures are employed to avoid cross contamination between different populations of the same species. After about five generations, this population is replaced with another population newly selected from the field to counter the potential effects of genetic drift.

Herbiseed's database stores records of the significant characteristics and production details of each seed batch . These can be printed out as a 'Population Certificate' to provide the experimenter with essential information to assist in selecting a seed batch and in interpreting the experimental results It also provides documentary authentication of the seed origin and identity for formal GLP/GEP.

APPENDIX 1.

1. DIFFERENT POPULATIONS WITHIN A SPECIES

Alopecurus myosuroides. Most field populations in lowland Britain show a level of elevated tolerance to chlortoluron, which varies continuously up to the high tolerance of the East Anglian 'Peldon' population. Partial tolerance of chlortoluron and some graminicides is also present in some populations in France, Germany and Spain.

Avena fatua. Populations of this species show a large variation in levels of dormancy and seedling frost tolerance. Nutrition of the mother plant and degree of seed maturity at harvest are also involved in subsequent behaviour of the seed. There is increasing evidence that some populations of this species show quantitative and qualitative differences in herbicide metabolism and tolerance.

***Galium aparine*.** A notoriously variable species. 'Northern' and 'Southern' populations differ considerably in competetiveness, habit and reaction to drought. 'Hedgerow' populations have been found to be less tolerant of some herbicides than 'Field' populations. Seed size varies by up to 200% between batches, and appears to have a genetic component. Germination behaviour varies considerably between batches of seed, and that of the same batch varies with time and storage conditions.

Lolium rigidum. Different populations of this species throughout the world exhibit varying levels of tolerance to a range of herbicides. In some cases this is specific to a single herbicide, other populations show tolerance of several unrelated herbicides.

***Viola arvensis*.** There are significantly different populations of this species across Europe, with some indications of genetic introgression with the habit, flower size and colour of *V. tricolour*. There is significant variation between populations in their response to hormone and urea herbicides.

Xanthium strumarium. Another species renowned for its variability in morphology, reaction to daylength and germination behaviour. In practice, timing and depth of germination has a significant effect on this species' ability to survive pre-emergence herbicide application in maize crops.. In glasshouse tests, the use of a population which remains vegetative at winter light levels is highly desirable.

2. POPULATIONS OF CLOSELY RELATED SPECIES

A field population of weeds seldom consists of a single species. This can pose difficulties in field trials on natural populations where it will be necessary to identify all of the species present before any treatments are applied. Failure to do so risks missing important information if a treatment is effective against one species but not against a closely similar one.

Seed collected directly from field populations poses similar risks if it is used in glasshouse experiments. Where two or more morphologically similar species grow together, seed collected from that population will not be species-pure, and will give rise to difficulties in interpreting the results of the experiments in which it is used.

Some weed genera in which morphologically similar species growing together give rise to problems of apparantly variable response to herbicide treatments are listed below.

***Amaranthus retroflexus*.** The morphology of this species varies widely across Europe, with spreading branches and compact inflorescences being common in the North and upright plants with lax inflorescences common in the Balkans. *Amaranthus chlorostachys* is frequently present, unnoticed, in fields infested with *A. retroflexus.*

In America, mixed infestations of *A. retroflexus* and *A. hybridus* occur, with neither species occuring in a pure population except where an atrazine resistance biotype (usually of *Amaranthus hybridus*) is the only weed present.

Chenopodium album. This species has several sub-species, at least one of which, (*Chenopodium album amaranticolor*) is different to *C. album album* in its tolerance of some herbicides. Other superficially similar species such as *C. ficifolium* frequently grow among populations of *C. album.*

Matricaria and ***Anthemis* species.** These closely related genera tend to be lumped together as 'mayweeds'. However the reactions of individual species to herbicides differ, with A*nthemis arvensis* and *Matricaria recutita* being relatively susceptible to hormones, *A. arvensis* can be more tolerant of chlortoluron and *M. perforat*a is the most tolerant of paraquat.

Veronica spp. Many field populations of 'Speedwell' comprise mixtures of *V. arvensis, V. agrestis, V. polita* and *V. persica.* These species appear superficially similar but differ in their sensitivity to herbicides, with for instance, *V. arvensis* being susceptible to chlortoluron and *V. persica* being tolerant.

THE MORAL

In any experimental work with weeds, it is safer, easier and more cost effective to use a clearly defined population of weeds produced by sowing known batches of seed into the experiment than to risk the possible presence of atypical biotypes in a population which occurs naturally in a field with an unknown history.

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