Session 4A

High Throughput Screening as an Approach to New Product Discovery (2)

Chairman	Professor A H Cobb
Session Organiser	Dr J C Ormrod
Papers	4A-1 to 4A-2

TECHNOLOGICAL DEVELOPMENTS IN HIGH THROUGHPUT SCREENING (1) EXPLOITING THE MOLECULAR DIVERSITY OF NATURAL PRODUCTS (2) MEASUREMENT OF BIOMOLECULAR INTERACTIONS AND THE USE OF ROBOTICS

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ABSTRACT

High throughput screening is used widely in the pharmaceutical industry to identify from diverse chemical libraries new drug molecules which are active against disease targets. Sources of molecular diversity for high throughput screening programmes range from synthetic and natural product libraries to those generated from newer techniques such as combinatorial chemistry, all of which also have potential to provide new agrochemical and pharmaceutical leads. Natural product libraries are generally considered to be a good source of molecular diversity but with the perceived disadvantage that the time to lead discovery is longer and more expensive than for chemical libraries. The competitiveness of natural product discovery can be improved by technological innovations such as analytical fingerprinting of samples and advances in chemical isolation methods which can exploit their inherent molecular diversity. The use of state-of-the-art screening technology is important to ensure the adequate sensitivity of assays and to minimize the effect of interferences. The natural product discovery process as a whole results in the generation of a wealth of data from the disciplines involved, so that a powerful informatics system is required to expedite its effective use. Robotic screening systems are now widely regarded as an integral part of the discovery process as they can facilitate lead discovery through high throughput screening, with significantly less manual resources. Using a combination of these technologies, natural product resources can rapidly deliver new chemical entities which are currently beyond the scope of chemical synthesis programmes.

INTRODUCTION

High throughput screening (HTS), a widely used term in the pharmaceutical industry, is the process by which very large numbers of compounds extracted from biological materials or derived by chemical syntheses are tested in assay systems to identify potential new drug molecules. The screening of diverse chemical libraries, if carried out efficiently and cost effectively, can be used to identify lead compounds rapidly.

The properties of these leads can be improved using novel drug design and synthesis technologies. Full advantage may be taken of molecular diversity existing in synthetic and natural product libraries and generated using new chemistries such as combinatorial synthesis. While combinatorial synthesis can provide a rapid and cost effective way of generating vast numbers of compounds for screening, the chemical diversity which can be achieved is not, at present, as great as that of natural products. The competitive position of the latter as a source of new drugs or agrochemical leads, however, must lie in technological innovation to significantly improve screening.

A growing number of measuring technologies may be incorporated into high throughput assays in order to detect inhibitors of key enzymes, receptors and protein-protein interactions implicated in disease states and organism pathogenicity. Where complex processes such as signalling pathways, cell growth and morphology or gene transcription are targeted, whole cell-based assays are used. The development of automated techniques for performing routine laboratory operations has been taken a stage further as a result of the growing need to perform such assays on a high throughput basis. Today, fully integrated robotic systems can be constructed or purchased from specialist manufacturers to reduce the manual element of the screening process. These systems, if carefully designed, can be programmed to run unattended overnight, reducing the cost of screening, and allowing the capital cost to be recovered.

HTS generates a vast amount of data which must be stored and made easily available for manipulation. The computational power of modern desk top computers now enables sophisticated analyses to be performed by the use of statistical and artificial intelligence methods such as neural networks. Considerable quantities of data may be collected relating to each extract, and these can be analyzed along with the data from screening tests to identify patterns for both grouping and predicting activities. Chemically fingerprinting extracts by, for example, mass spectrometry and correlating the data with biological activity offers a powerful means of selecting the most interesting leads and speeding up the evaluation process.

(1) EXPLOITING THE MOLECULAR DIVERSITY OF NATURAL PRODUCTS

NATURAL PRODUCTS AS SOURCES OF MOLECULAR DIVERSITY

Natural products have traditionally provided a rich source of chemical diversity in the search for biologically active molecules. From the numbers of species estimated by Groombridge (1992) (Table 1), if natural product diversity equates to the production of diverse secondary metabolites, nature would appear to be an inexhaustable source of new chemical entities.

Throughout history, plants have been used for therapeutic applications; approximately 25% of all prescription drugs used today are of plant origin. Microorganisms, on the other hand, have only been seriously exploited for their medicinal properties over the last 50 years since the discovery of penicillin. However, during this period, screening has been largely for antiinfectives, and it is only in recent years that the broader potential of their metabolites in non-infectious disease has been realised. Today, there are two groups of top selling medicines originally discovered from fungi which are not antibiotics; mevinolin, used for reducing serum cholesterol levels and cyclosporin, an immunosuppressant used to prevent rejection after organ transplantation. The latter also has a growing number of applications in the treatment of certain autoimmune diseases. Even the largest selling animal health pharmaceutical, the endectocide Ivermectin, is a natural product derivative produced by the bacterium, *Streptomyces avermitilis*. The use of the avermectins in the form of abamectin (avermectin B_{1a} plus avermectin B_{1b}) has been extended to control mites and other insect pests on cotton, citrus, ornamental and high value vegetable crops.

Table 1. Number of Species : Described and Estimated Figures *

	Described species ($x 10^3$)	Estimated species, highest figure ($x 10^6$)	Estimated species, working figure ($x 10^6$)	No. of species held in culture collections ($x 10^3$)
Plants	25	0.5	0.3	
Fungi	70	1.5	1.0	11.5
Viruses	5	0.05	0.5	2.2
Bacteria	4	3.0	0.4	2.3
Vertebrates	45	0.05	0.05	
Crustaceans	1	0.15	0.15	
Arachnids	75	1.0	0.75	
Insects	950	100	8.0	

* After Groombridge (1992)

Rationale for screening natural products

The majority of natural products belong to a class of biological molecules collectively called secondary metabolites-compounds of immense structural diversity that are not essential for growth, but are thought to play important roles in survival and ecological adaptation. The functions of secondary metabolites in defence and competition have been well documented, and similar roles have been proposed for fungal metabolites (Harborne, 1988, Porter & Fox, 1993). Indeed, in many cases secondary metabolites appear to play a central regulatory role in the complex relationships between organisms. These observations suggest that the molecules themselves possess inherent biological activity and, depending on their target, have potential applications in medicine or agriculture.

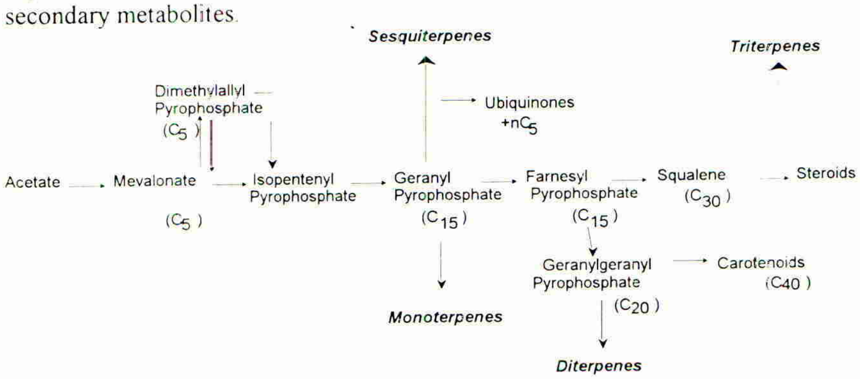
The sheer numbers and diversity of the structures of secondary metabolites has long intrigued scientists, particularly with regard to the evolution of the often complex pathways by which they are synthesised and to the purpose of these reactions, their intermediates and end-products. The biosynthetic origins of secondary metabolites lie in primary metabolism, where fundamental building blocks and intermediates feed into highly specific and tightly regulated secondary pathways (Table 2).

Table 2. Primary metabolites which act as precursors of secondary metabolism

Acetyl CoA and its condensation products
Saccharides
Shikimic acid or aromatic amino acids
Aliphatic amino acids
Tricarboxylic acid cycle intermediates
Purines and pyrimidines

In the primary steroid pathway and the secondary terpenoid pathway, for example, which predominate in plants and fungi, condensation reactions involve sequential addition of isoprenoid units which can be cyclised and modified in a very precise manner to give the final product (Figure 1). The diverse range of monoterpene and sesquiterpene secondary metabolites are produced as a result of terpenoid cyclases acting on the acyclic precursors geranyl and farnesyl diphosphate respectively (see Cane, 1992).

Figure 1. Intermediates in the steroid pathway which act as precursors for the terpene secondary metabolites.



Such reactions give rise to secondary metabolites with far more diverse structures than molecules currently being synthesised by combinatorial techniques. Some secondary metabolites can be quite large and exhibit complex stereochemistry, a feature which can significantly affect the affinity and specificity of binding to large macromolecules. The manufacture of greater quantities of such compounds may require fermentation processes, but this has not precluded their development into profitable pharmaceuticals and agrochemicals. More commonly, natural products may be perceived as a route to the provision of valuable novel chemical templates for "rational design" and synthesis by traditional chemical means. The latter is often the preferred route for exploiting natural product diversity.

Future Prospects

Considering the short lead compound identification time for the latest combinatorial chemistry technology what is the justification for the continued screening of natural products? The advantages and disadvantages of the two approaches are illustrated in Table 3. While natural product extracts offer the greater source of molecular diversity, the overall cost of new chemical template discovery is higher, and lead delivery times are longer. Any new technology, such as combinatorial chemistry (which can be competitive on cost and time and deliver a reasonable level of molecular diversity) will be very attractive to drug and agrochemical screening operations. However, it is possible to reduce the cost and time involved in screening natural products by ensuring that sufficient raw material is available to complete early chemical and biological evaluation rapidly. Effective project management can then be used to focus resource on key activities in the discovery process, thereby making it more competitive with compound mixtures derived by combinatorial synthesis.

Table 3. Lead Discovery: comparative assessment of natural product extracts versus combinatorial libraries.

	Natural products extracts	Combinatorial libraries
Molecular diversity	Very high	Questionable
Track record of success	Products in use	Leads identified
Generation cost	High	Low
Sample generation time	Slow/medium	Fast
Structure determination	Sometimes complex	Straightforward
Material required for structure determination	High	Low
Ease of compound resupply	Variable	Straightforward

Analytical fingerprinting

Fingerprinting can be carried out by a number of methods, but the ideal description of each individual sample is a chemical fingerprint representing-as far as possible-all the components in the mixture. Magee (1993) has recently reviewed chemically-based fingerprinting (chemometric) methods which can be performed on intact microorganisms and used for discrimination and classification (Table 4).

Such techniques are convenient and rapid but they generate large amounts of data. The composition of the cells themselves is reflected in the data as statistically complex, multiply-stacked responses. This means that none of the parameters measured are uniquely representative of a single molecular cell constituent. The key to exploiting chemometrics lies in not only understanding the basis of the analytical technique but also in having an awareness of the statistical approaches to data analysis. Mathematical techniques such as principal components analysis (PCA) are very useful methods of reducing the dimensionality of multivariate data while preserving most of the variance. As such, PCA is an excellent technique for observing the natural relationships between samples. However, it is a method of "unsupervised" learning which relies on linear transformations of the raw data and therefore is limited in providing the best analytical discriminations. "Supervised" learning methods such as artificial neural networks (ANNs) can map both simple linear and complex non-linear relationships in multivariate data. The use of ANNs therefore opens up new analytical capabilities for existing technologies.

Table 4. Chemical fingerprinting techniques used for microbial classification

Technique	References
Pyrolysis mass spectrometry (PyMS)	Meuzelaar *et al.* (1976)
Fourier transform infrared spectrometry (FT-IR)	Naumann *et al.* (1991)
Ultraviolet-resonance Raman spectroscopy (UVRRS)	Nelson & Sperry (1991)

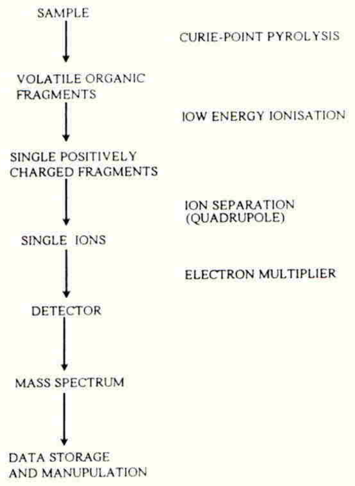
Pyrolysis mass spectrometry

Of the three analytical techniques indicated in Table 4, PyMS has been developed and evaluated to the greatest degree. This technique will be discussed further with respect to natural product fingerprinting. Currently however, there is considerable interest in FT-IR and UVRRS, which have the benefit over PyMS in that these methods are non-destructive and can facilitate the localised analysis of living material.

Details of PyMS have been described in detail by Magee (1993) and will not be repeated here but an outline of procedures is illustrated in Figure 2. To date, the predominant use of PyMS has been as a taxonomic tool in bacterial and fungal systematics. It has been successfully used to discriminate between species and strains within a range of microbial genera, including for example *Salmonella* (Freeman *et al.*, 1990), *Listeria* (Freeman *et al.*, 1991), *Streptococcus* (Magee *et al.*, 1991) and *Candida* (Magee *et al.*, 1988). Indeed Goodacre and Berkeley (1990) were able to distinguish between strains of *Escherichia coli* differing only in the presence and absence of a single plasmid, such is the discriminatory power of the technique.

The introduction of ANNs has made a significant impact on the application of PyMS. Goodacre *et al*, (1992,1993) were the first to use ANNs to discriminate biological samples based on the PyMS spectra. In a double blind study and with spectra from just 12 pure olive oils and 12 adulterated olive oils they were able to train a neural network to recognize unequivocally all adulterated oils amongst the samples presented for testing, a task which had previously been difficult and labour intensive. Significantly, statistical methods, including PCA, based on traditional "unsupervised" multivariate techniques, failed to distinguish between pure and adulterated oils. This combination of PyMS and ANNs has also been successfully applied to the identification of microbial strains, for example, *Streptomyces* (Chun *et al.*, 1993a,b).

Figure 2. Schematic of pyrolysis mass spectrometry



Neural networks can also be made to function in an " unsupervised" mode to create self organising maps termed Kohonen ANNs (Kohonen, 1989). This approach has been used to classify strains of the bacterium *Propionobacterium acnes.* Strains were clustered by applying Kohonen networks to the pyrolysis mass spectra and the clusters were found to be similar to those based on the multivariate techniques of canonical variates analysis and hierarchical cluster analysis (Goodacre *et al.*,1994b).

Another significant application of ANNs to PyMS has been the quantification of individual components of complex biological samples. Of these the most relevant to the current discussion is the successful measurement of individual secondary metabolites following PyMS analysis of fermentation broths (Goodacre *et al.*, 1994 a & b, 1995).

PyMS, ANNs and natural product screening

The application of ANNs to PyMS has been shown to be successful not only in characterizing and clustering biological materials, including micro-organisms, but also in quantifying individual components. Characterizing natural product samples in this way and carrying out comparisons with the distribution of biological activity would undoubtedly aid the selection of the most interesting samples for further evaluation. If screening is performed on a batch basis, the results of the selection process from the initial batch can also be used to train an ANN to select or eliminate samples in subsequent batches. FT-IR and UVRRS show similar promise when combined with

ANNs with the added advantage that these techniques are non- destructive, and can be non-invasive.

Chemical characterisation

As indicated in Table 3, one of the rate limiting steps in natural products discovery is the effort required to characterise active metabolites. Affinity chromatography is a technique used extensively to purify proteins and other macromolecules but there are few examples of its use when the ligand and ligate are of low molecular weight. However, the natural binding of glycopeptide antibiotics to diacetyl-L-lys-D-ala-D-ala has been exploited to develop a purification system based on affinity chromatography (Wasserman *et al.*, 1987). Using miniature columns of Affi-gel-10-D-ala-D-ala it was possible to concentrate and desorb pure glycopeptides which could be further resolved by HPLC. Fractions were submitted to fast atom bombardment mass spectrometry to facilitate the rapid identification of novel glycopeptides (Jeffs & Nisbet, 1988). Affinity purification still remains a very attractive technique for the purification of natural products, particularly today, when many of the high throughput screens are receptor based. However, constructing an affinity column using the same protein receptor used in a biological assay may not be economically or scientifically feasible.

In recent years there have been tremendous developments in mass spectrometry techniques and instrumentation. Machines have become smaller, cheaper, more powerful and easier to use. It may not be necessary to construct affinity columns to separate and concentrate metabolites prior to analysis by mass spectrometry, but simply to carry out mass spectrometric analysis of a bound metabolite desorbed directly from an assay well. Indeed, a mass spectrometric immunoassay has recently been described using matrix-assisted laser desorption/ionisation (MALDI) time-of-flight mass spectrometry (Nelson *et al.*, 1995). Using immunoaffinity capture and MALDI techniques for detection, these workers were able to detect and measure blood levels of myotoxin a and Mojave toxin (from rattlesnake venoms) with sub-nanomolar sensitivity. These toxins have molecular weights of approximately 5 kDa and 14 kDa; MALDI machines can also measure accurately relatively small organic molecules with molecular weights below 500 Da. The potential use of the technique in high throughput natural product screening is obvious. MALDI mass specrometry opens up the possibility of characterising receptor binding agents directly from an assay format.

(2) MEASUREMENT OF BIOMOLECULAR INTERACTIONS AND THE USE OF ROBOTICS

MEASUREMENT OF BIOMOLECULAR INTERACTIONS

As mentioned previously a diverse array of assays have been used in agrochemical and pharmaceutical screening programmes to detect compounds with novel activities for development into new products.

In the case of natural products screening, some key criteria must be met by the screening used. Natural products screens must be designed to function in the presence of samples with a range of physicochemical properties. Extracts may be prepared in a range of solvents, or simply filtered to remove high molecular weight material. In the latter case, samples may be highly coloured and may have a low pH, such that the screen used must be able to detect active metabolites above a background of potential interference. Assuming that active metabolites in the extracts have an average molecular weight of 500Da and are diluted in the assay 20 to 100-fold, the required detection limit of the screen is in the 20-200nM range. To enhance metabolite detection, the assay should be highly specific for the molecular or cellular target, and it is advantageous to compare data generated from the screening programme with that from similar and unrelated targets before progressing samples to metabolite isolation and structure elucidation.

The need to address such issues and to apply high throughput screening across a broad range of therapeutic and agrochemical targets has resulted in the development of a wealth of assay technology, both generic and specialised.

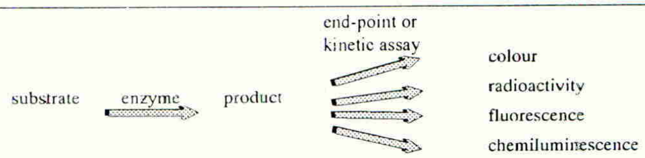
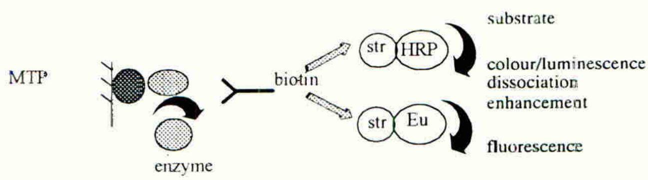
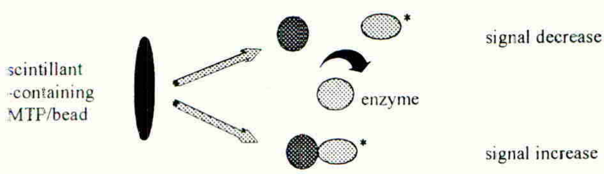
Enzyme assay screening technologies

A range of techniques including solution phase assays (Walker *et al.*, 1993, Gopalakrishna *et al.*, 1992) immobilizd substrate assays (Farley *et al.*, 1992, Sadick *et al.*, 1995) and scintillation proximity assays (SPA) have been used in the search for new enzyme inhibitors.

The scintillation proximity assay (SPA) is a homogeneous radioisotopic technique which relies on the limited path length a β particle will travel through aqueous media. If the electron collides with a scintillant particle, energy is transferred and light is emitted. In SPA, scintillant is coated on to the surface of microtitre plate (MTP) wells or incorporated into beads or the MTP itself, making it possible to assay hydrolytic enzymes and transferases using appropriately labelled substrates (Taylor *et al.*, 1994).

One possible alternative as a detection system for immobilized substrate assays is to use time resolved fluorescence (TRF). TRF utilizes lanthanide chemistry to overcome problems such as quenching and background fluorescence which are often observed with standard fluorescence systems. Under appropriate conditions, lanthanides produce a high fluorescence intensity with a sharp emission peak and long decay time, and exhibit a large Stoke's shift (i.e. difference between the excitation and emission wavelength). In practice this means that fluorescence can be measured after background has decayed and that assays are highly sensitive and have a wide dynamic range (Dickson *et al.*, 1995). In addition, a stable signal is generated making the technology ideal for automation (Hill, 1995). Streptavidin, antibody and protein-lanthanide complexes are available commercially and most proteins can be readily labelled to take advantage of this versatile technology which is easily adaptable to natural products drug screening (MacAllan, 1995).

Table 5. Overview of a range of enzyme assay screening technologies.

Assay type	Schematic
solution phase	
immobilised substrate	
SPA	

Receptor-ligand binding and protein-protein interaction screening technologies

Traditional receptor-ligand binding assays were carried out using filtration or precipitation to separate the bound from the free ligand. More recent developments in screening technology for receptor-ligand and other protein-protein interactions include capture assays, SPA (Holland *et al.*, 1994, Pernelle *et al.*, 1993) and fluorescence polarization.

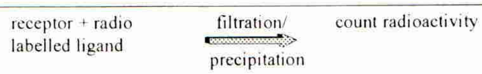
SPA and fluorescence polarization assays both allow monitoring of 'real time' kinetics of association or dissociation of the receptor-ligand/protein-protein complex as they do not require a separation step.

In the case of fluorescence polarization assays, the ligand is labelled with a fluorescent tag and used in a technique based on the rotation of molecules which measures the decrease in depolarization of fluorescence exhibited by small molecules when their rotation is slowed by binding to large molecules (Checovich *et al.*, 1995). The quantification of biomolecular interactions can then be carried out using competition methods, making this technique useful as a high throughput screening method (Sportsman *et al.*, 1995).

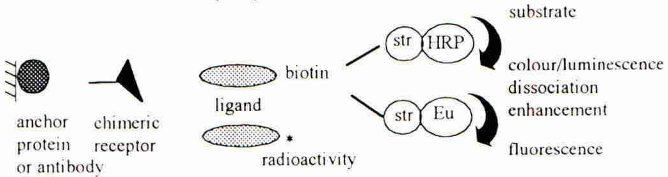
Table 6. Overview of a range of receptor-ligand binding and protein-protein interaction screening technologies.

Assay type — **Schematic**

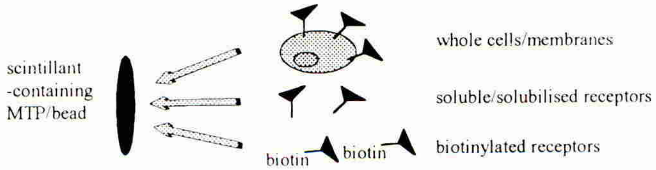
solution phase



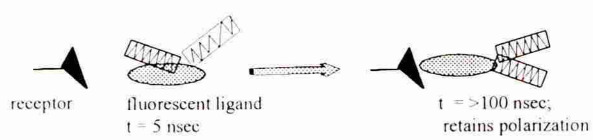
"capture" assays



SPA



fluorescence polarization

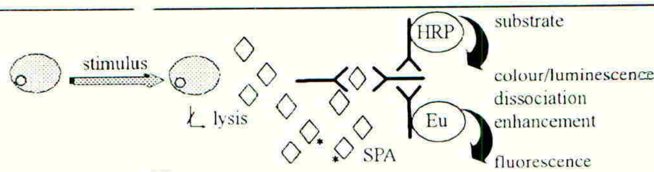
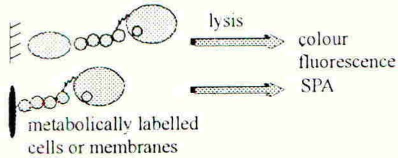
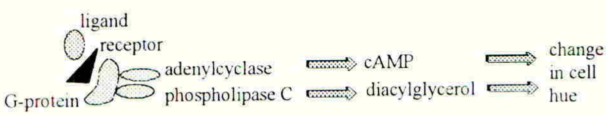


Cell-based screening technologies

Cell-based screening technology is becoming more widely used as a lead discovery tool, as the interest in targets which affect cell function and regulate cellular responses increases. Assay types used in screening programmes to date include those for inhibitors of extracellular protein secretion, adhesion assays, and reporter gene assays (Abe *et al.*, 1994).

Melanophore technology is a recent innovation which uses cells from the frog *Xenopus laevis*. The movement of pigment in this system is sensitive to intracellular concentrations of cAMP and diacylglycerol. If receptor stimulation activates adenyl cyclase and phospholipase C, darkening of cells is induced. Conversely, stimulation of a receptor which inhibits adenyl cyclase induces cell lightening. Recombinant receptors can be stably transfected into this system and pigment hue measured following receptor stimulation. Such changes are appreciable within minutes, making this technology a useful tool for high throughput screening (Lerner, 1993).

Table 7. Overview of a range of cell-based screening technologies.

Assay type	Schematic
extracellular protein detection	
adhesion	
reporter gene	
melanophore technology	

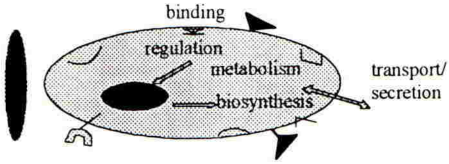
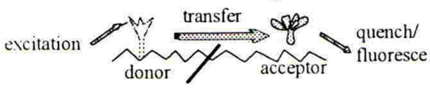
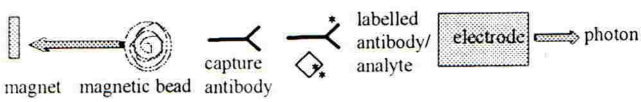
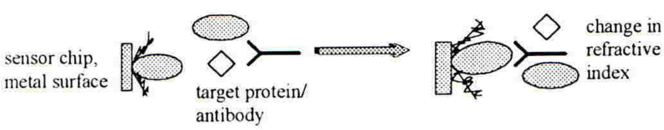
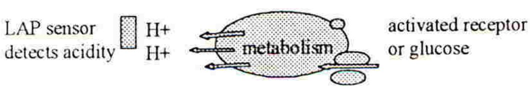
Recently available and future prospects in screening technologies

Some recent developments in assay technology include use of SPA for cell-based assays, delayed fluorescence resonance energy transfer (DEFRET), electrochemiluminescence (ECL), surface plasmon resonance (SPR) and microphysiometry (the principles of which are summarised in Table 8).

For cell-based SPA the scintillant is incorporated into the base of the MTP. The walls of the well are opaque to exclude optical cross talk and the base is transparent to facilitate visualisation of cells during culture. Cells are grown in monolayers in the wells and radioisotope in solution is too distant from the scintillating base plate to generate a signal. Using this type of assay it is possible to monitor *in situ* receptor binding as well as cellular metabolism and motility (Cook, 1995).

Time resolved fluorescence technology can be used in DEFRET techniques which enable homogeneous assays to be carried out, as fluorescence is generated by the close proximity of the lanthanide to an acceptor molecule. The acceptor molecule used in this technique may either transmit or quench fluorescence, dependent on the assay of choice (Mathis, 1995).

Table 8. Recently available/future prospects in screening technology.

Assay type	Schematic
cell-based SPA	
DEFRET	
ECL	
surface plasmon resonance	
micro-physiometry	

Electrochemiluminescence is another emerging assay technology which utilizes labelling of one of the assay reagents. In this case diffusion of a precursor molecule onto an electrode surface is followed by rapid electron transfer, which initiates the excitation of the reporter molecule and results in the emission of a photon of light at a specific wavelength. Use of an electrode allows reactions to take place in a variety of solvents, and gives the capability of increasing assay sensitivity to the fmol/L range by applying different electrode potentials (Yang *et al.*, 1994).

Surface plasmon resonance (SPR) is a label free, real time optical detection technique which facilitates quantification of molecular interactions. The basis of SPR is that a metal (usually gold) coated sensor chip is used to give rise to electronic oscillations, or surface plasmons, at a metal surface. These decay exponentially as a function of distance and their refractive index will change if a complex is formed on the sensor chip surface. This change can be detected and recorded using appropriate instrumentation (Brigham-Burke *et al.*, 1992).

Finally, the microphysiometer is a silicon-based device which can be used to detect and monitor the response of whole cells to a variety of chemical substances as well as facilitating the investigation of cell function and biochemistry. In this instance the machine uses a light addressable potentiometric sensor (LAPS) to measure the rate at which cells acidify their environment. The cells are retained in a flow chamber in aqueous diffusive contact with the pH-sensitive surface of a LAPS chip. Cellular acidification occurs mainly via glycolysis. However, when there is a ligand-receptor interaction at the cell surface there are substantial metabolic consequences in the cell which alter the acidification rate and can be measured using this instrument (McConnell *et al.*, 1992).

Future prospects for screening technology may involve modification of the above or other new techniques to move away from the 96-well MTP into alternative formats. These include other MTP designs (e.g. 384-well plates), and the development of technology which is completely different from the MTP. To this end, companies involved in screening programmes have been investigating use of high density spotting techniques, ink jet technology, biosensors and nanotechnology, to name but a few. The publication of assay formats and data resulting from the use of such technology is eagerly awaited by the screening community.

Analysis of data from natural products screening programmes

Much of the technology previously described is applicable to screening all sources of molecular diversity. However when screening natural products, data are generated from a broad range of sources, including organism characteristics, assay validation, high throughput screening and chemical characterization of active metabolites. To fully investigate this vast amount of data an advanced informatics system is required (Hill & Clifton, 1995). Such a system should be able to store and analyze information on the ecology, taxonomy and fermentation of the organism collection, facilitate an investigation of assay performance in the presence of natural products samples, enable statistical analysis and pattern recognition of test sample activity, and allow the matching of cross screen data, chromatographic profiles and chemical structures to each sample and across a broad range of screens. This powerful combination of appropriate assay technology and targeted informatics is the key to rapid discovery of lead compounds in natural products screening.

ROBOTICS

As previously discussed, the focal point of lead discovery in industry today is to find new lead compounds more quickly and efficiently than ever before. This means that the pressure is on to screen sources of molecular diversity more rapidly and with significantly less manual resource (Hook, 1995).

This requirement for cost-effective lead identification, together with the desire to increase reliability of the screening process and, where possible, to eliminate the more mundane manual tasks, has led to an increasing investment in robotics. To this end, several companies now offer complete or custom-built robotics systems for screening, and some companies involved in high throughput screening have developed their own systems. To date the majority of screening robots are based on the 96-well MTP format and require good liquid handling capability, storage and incubation facilities, plate readers, a reliable robotic arm and effective integration and communication software.

Liquid handling

Appropriate automated liquid handling is essential for reproducible assay performance. For high throughput screening, the final assay data should have a coefficient of variation (cv) of less than 10% with data acceptable up to a cv of 15% for more complex assays. Robotics systems usually incorporate an intelligent workstation for specific liquid handling tasks, together with 96-well dispensers for bulk reagent addition. Dispensers may have disposable tips for specific assay requirements, but one of the advantages of a robotic system is that these are often not required thereby reducing the cost per test.

Plate washers of the 96-well simultaneous operating format are the most commonly used in robotics systems. The advent of robotics has eliminated the requirement for stacking units on such equipment, but stacking plate instruments continue to be used widely as stand-alone units where complete automation of an assay is not under consideration.

In addition to these functions, automated plate harvesting is available on some robotic systems. The manual processing of filtration assays has been very time consuming in the past. However, automated plate harvesters can now perform this task in less time and with minimal operator intervention, again significantly introducing the cost of operation of such assays.

Storage and incubation facilities

All robotics systems require a storage area with sufficient capacity to complete the desired number of plates. These usually consist of stacking units and reagent storage areas which may be refrigerated to aid stability.

Several robotic systems are now available which have integrated incubation facilities. These may be provided via heating a shaking platform, for example, or if gas control is required, incubators with either drawers or fully opening doors can be used. For cell based assays the cells can either be dispensed into MTPs prior to assay and loaded into the incubator, or the robot can be situated in a clean room to allow hands free operation.

Plate readers

Most modern plate readers have an RS232 serial port which will allow communication with the robot controller. When selecting plate readers for use with a screening robot it is essential that access for the robotic arm is checked. A variety of instruments, from spectrophotometers, through to luminometers, scintillation counters and TRF readers have been integrated into robotics systems, and plate reader manufacturers are now more aware of the need to design their instruments with integration into a robotics system in mind.

Robotic arms and system design

Several companies provide robotic arms which can be used with assay systems. These arms must have controlling software which allows them to be positioned along their track or carousel with great accuracy and precision. They must be very reliable, and the ability to modify the gripper unit to allow the arm to handle MTP and other articles is desirable. Some robotic arms are able to change tools for different tasks and may even act as the pipetting head themselves. Robotic arms are available which operate either on a radial co-ordinate system or a linear track. In addition the arm may be cylindrical, moving instruments in the horizontal plane only, or Cartesian with a jointed elbow to allow inversion of instruments if required. Cylindrical robots facilitate access to a greater working surface area but there is a balance to be met between the size of the working area and speed of operation.

In any robotics system it is essential that there is a degree of versatility and control over assay and equipment integration as the very nature of high throughput screening means that molecular targets and assay formats may change rapidly. Custom built robotics systems are now rarely used as they could well be obsolete before delivery! The modular approach to system design is often the solution, allowing the independent replacement of separate sections as required by new assay formats. Rapid access to an in-house or closely linked external engineering facility is desirable, as well as the employment of an instrument specialist dedicated to maintenance and effective operation of the system.

Integration and communication software

The key to an effective robotics system is the ability to integrate any peripheral equipment and ensure reliable communication between its component parts. Robotics systems usually have specialized software for control of the arm, a core programme which manages equipment communication, and a front end assay planner and scheduler to allow those without programming expertise to set up their own assays. However, it remains difficult to obtain machine codes and access to proprietary software which would facilitate integration of some types of peripheral equipment, so that the employment of an in-house programmer dedicated to the development of robotics systems is highly desirable.

Capacity and operation of robotics systems

Robotics systems are now available which can run different assays in parallel and operate 24 hours a day. The rate limiting steps tend to be incubation times and pipetting rates. Depending on the assay type and duration, most 96-well MTP based robotics systems are now capable of screening between 1 and 5 million samples per annum (Hill, 1995, Loeber 1995).

The role and organization of robotics operations in the pharmaceutical industry-where much of the work has focused to date-has been a subject of much debate. Both centralised and decentralised approaches have been used (Scypinski *et.al.*, 1995, Babiak *et. al.*, 1995) but there is no clear leader in terms of success, as much depends on the degree of integration already required or present in an organization.

CONCLUSIONS

Technological advances in high throughput screening encompass a number of activities, from the generation of libraries as sources of molecular diversity, through to bioassay design, informatics, screen operation and chemical detection. Natural products provide a significant opportunity in the discovery of new drug leads, and in combination with such technology the competitiveness of the natural products approach can be increased. Integration of the disciplines involved in the natural products discovery process, to ensure focus of the operation on discovery of lead compounds, is the key to success. To this end, the maximisation of secondary metabolite production is essential and the provision of sufficient sample to carry out preliminary chemistry, including the integration of chemical fingerprinting, can shorten process times considerably. In addition, the use of appropriate assay technology to ensure sample batch throughput, and the focus of effort on assay performance and data analysis, permits the selection of quality hits early in the discovery process. Finally, the deployment of valuable natural products chemistry resource on fewer, better characterized samples, enables characterization of new, biologically active, chemical entities which are currently beyond the capability of chemical synthesis.

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STRATEGIES FOR MANAGING DATA FROM HIGH THROUGHPUT SCREENING

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ABSTRACT

A distributed information system strategy is presented for managing the process of screening and the large volumes of data expected from enterprise-wide High Throughput Screening (HTS) programs. Project-level HTS is managed on a dedicated, laboratory-based server (the WORKGROUP), in which all aspects of HTS data management, inventory management, and automation integration are performed. The individual WORKGROUPs are linked together though a central screening data server that integrates summary data from across the organization and coordinates workflow-based information shared across all WORKGROUPs. This architecture gives the greatest flexibility for location, performance, data access, and growth for HTS.

INTRODUCTION

Screening Information Management Systems (SIMS) for High Throughput Screening (HTS) are challenged with managing a complex, multicomponent process. As compared to conventional screening, HTS deals with a more diverse input of samples and has more information that must be tracked and correlated (Fig 1).

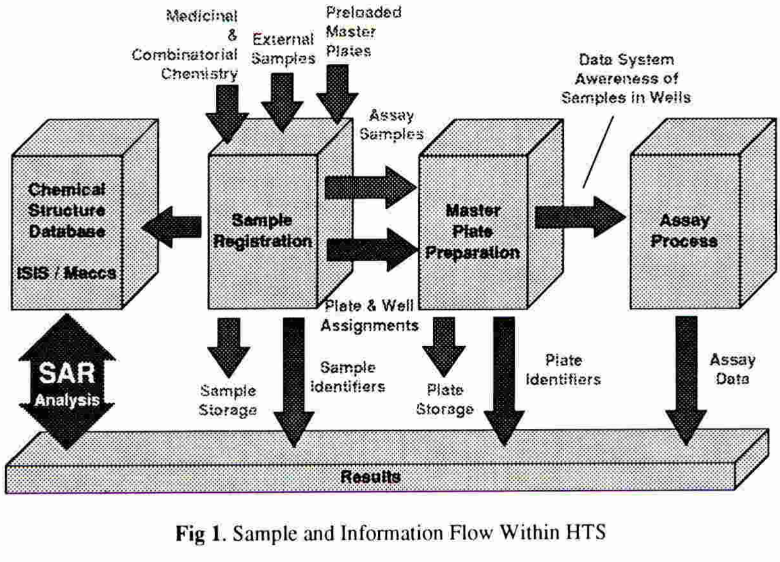

Fig 1. Sample and Information Flow Within HTS

The SIMS participates all facets of the HTS workflow (Fig 2): As an active partner in the laboratory with the screening scientist, the SIMS is expected to perform in near real time managing a wide variety of different information types and assay results.

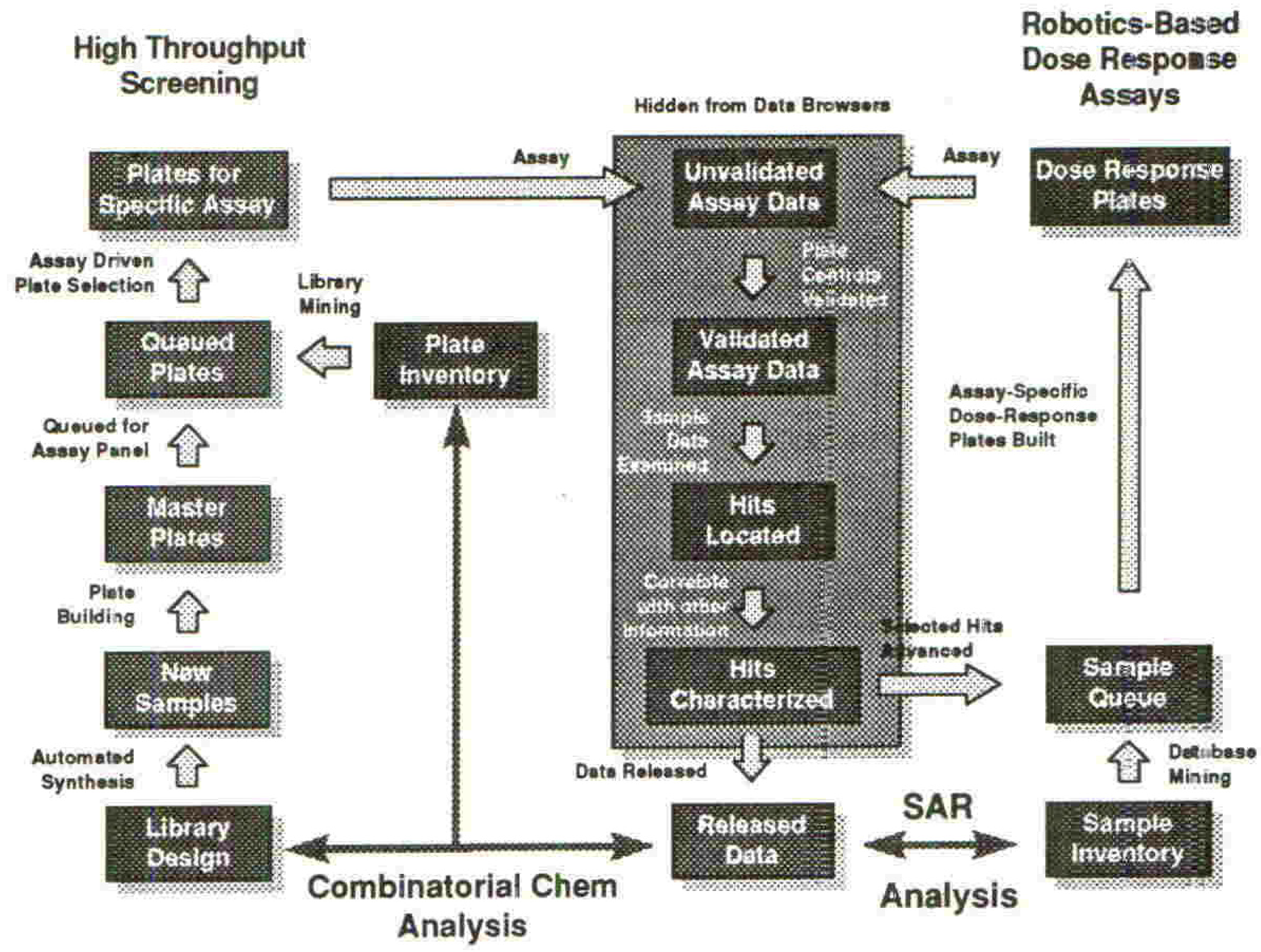

Fig 2. The HTS Workflow

HTS, and therefore the data and process the SIMS manages, work in conjunction with other disciplines within the Agricultural Chemical Research Environment (Fig 3). Data must be passed between all components in a timely manner to avoid bottlenecks that slow down the HTS process.

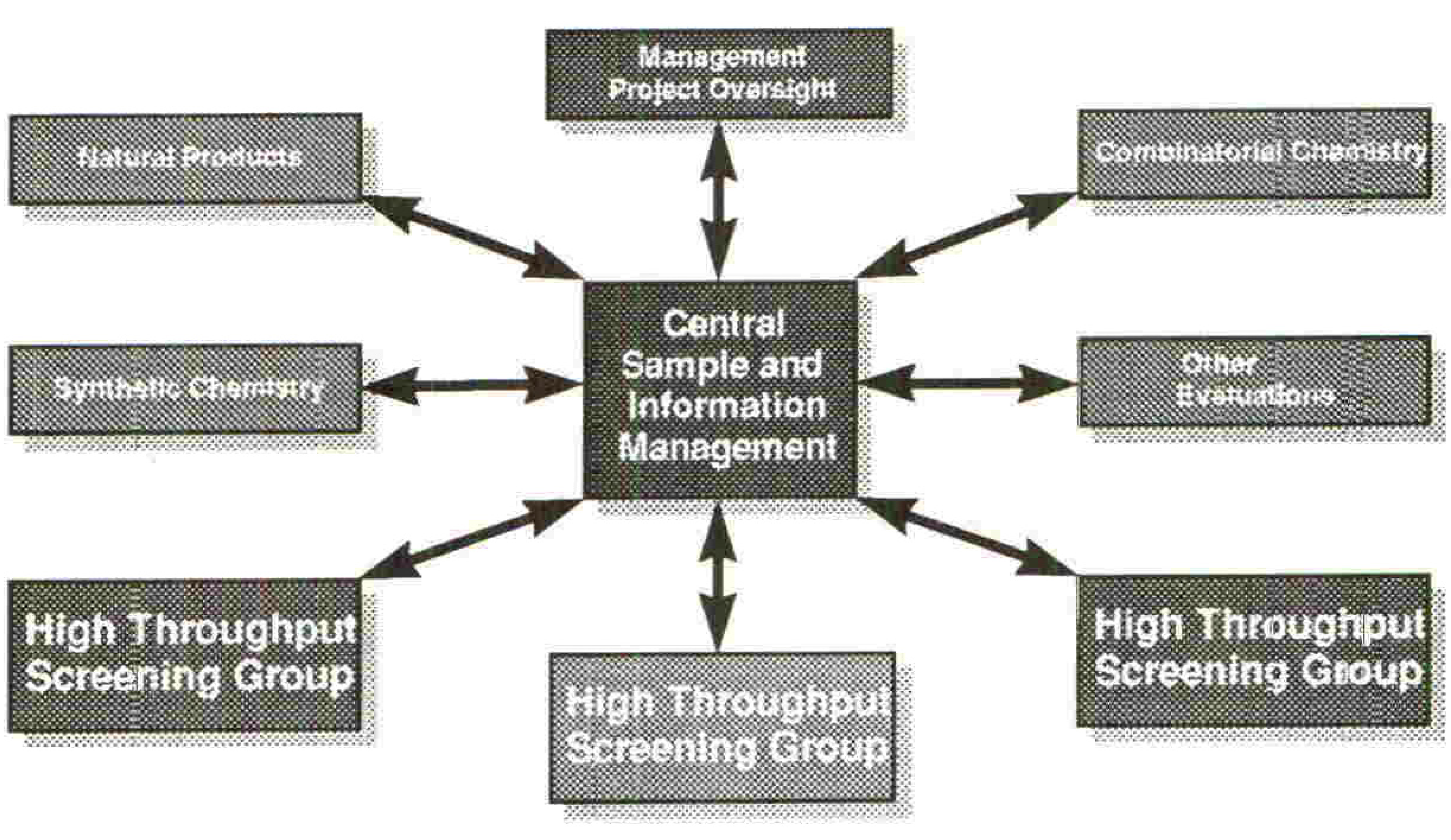

Fig 3. Interrelationship between Consumers and Producers of Information in the Agricultural Chemical Environment.

The problems of timely coordination of samples and information is compounded in a large, international organization (Fig 4). Samples and results must now be tracked between separate facilities, increasingly in multiple countries. The problem of tracking is compounded by different time zones, the transit times for samples, and collection and integration of the resulting screening data.

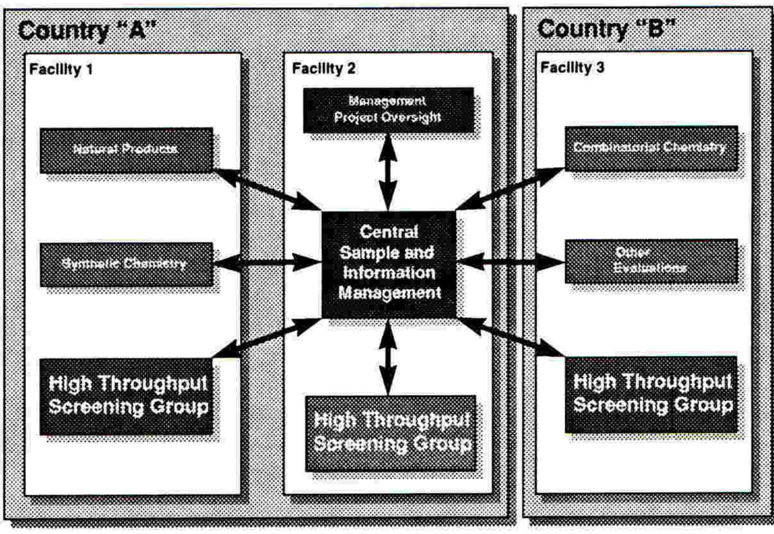

Fig 4. Organizational Components in a Large, International Organization.

From the analyst's perspective, all the screening results need to be available no matter where the work was performed. Summary data, which usually takes the form of a single value averaged for replicate experiments but can also be the "actives," is acceptable as a starting point for data analysis as long as the full body of available data is accessible.

DISTRIBUTED INFORMATION MANAGEMENT STRATEGY

One approach to distributed systems is to organize the SIMS as workgroups coordinated by a central server (Fig 5). Each workgroup is built around its own dedicated server that handles the data and inventory management tasks associated with the HTS workflow for a specific group of the screening scientists. Additional screening laboratories would have their own dedicated server. This allows each group to operate independently of each other and affords all the greatest possible compute performance while minimizing reliance on networks.

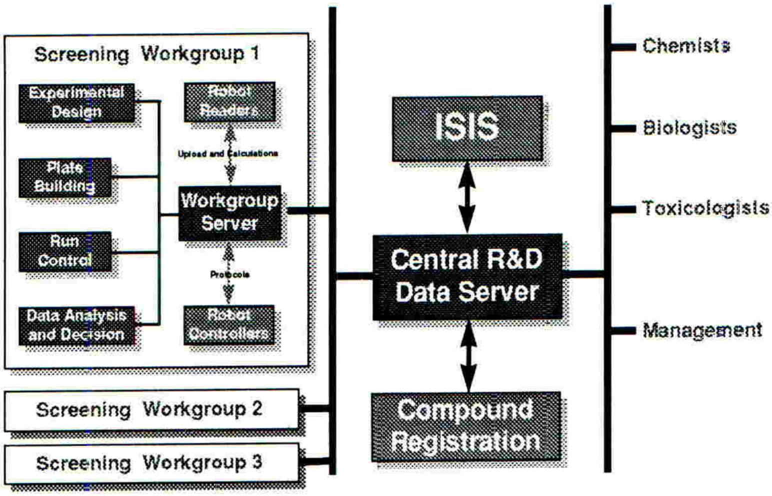

Fig 5. Screening Workgroups

The central SCREEN server acts as a resource coordinator for the individual workgroups that may be located in separate facilities in separate countries (Fig 6). Shared resources include experimental information such as assay protocols and plate layouts. It also includes making inventory data available to the workgroups when plates are prepared centrally. For multiple workgroup operations, the central server can track and coordinate plates through all the workgroups to improve productivity and not loose samples.

This model for a distributed SIMS is also easily scalable. Additional workgroups can be added without disrupting other elements of the system. The location of the new workgroups is not critical because the communication link between workgroup and central serve does not require maximum possible performance and can perform on existing T1 type (Telephone) communications links.

The central SCREEN server also acts as a data accumulator for summary results coming from all the screening workgroups (Fig 7). The nature of the summary data is specific for individual organizations. Because the workgroup and central servers can share information between them, there is no compelling reason to move all the data from the workgroup server to the central server. Negative results from single point data, such as percent inhibition commonly produced by HTS, are of little value for modeling and data analysis purposes, a situation that is compounded by the high volume of negative results expected from properly functioning HTS assays. This is a viable strategy as long as the data browser is aware that samples have been screened and results can be obtained on demand.

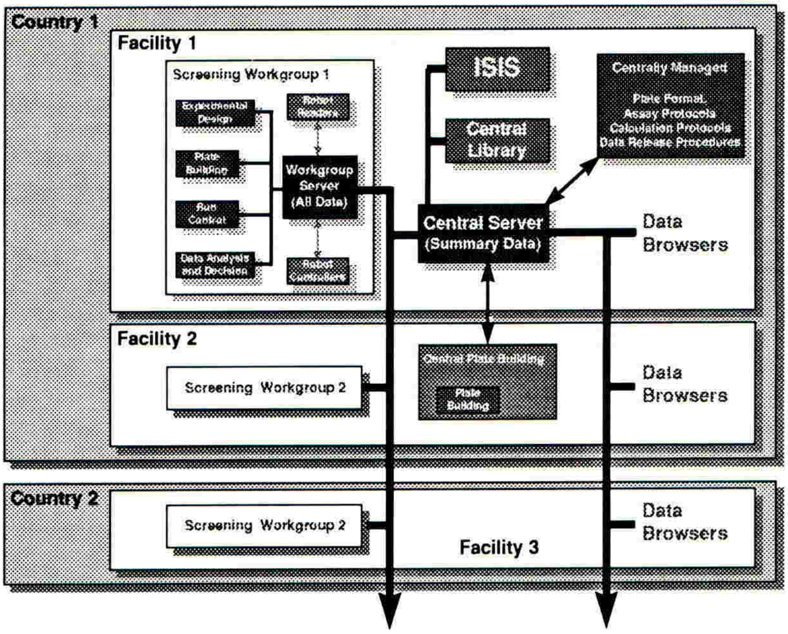

Fig 6. Interrelationship Between Workgroups, Central SCREEN Server and Data Browsers in the Full Distributed SIMS Environment..

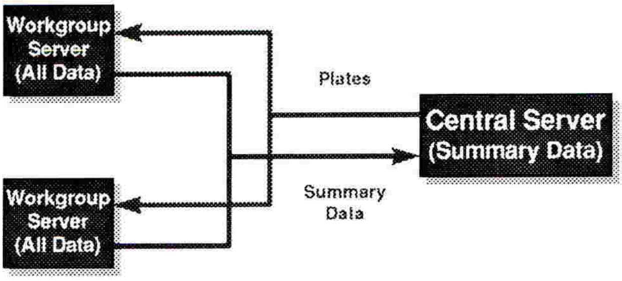

Fig 7. Sample and Data Flow Between Workgroup and Central Servers

The central server also functions as a buffer between data browsers and workgroups (Fig 8). The demands on computer and network resources are quite different for screening scientists involved in the process of screening and data browsers looking for information. By keeping the browsers off the workgroups, data system performance is greatly improved for the screeners.

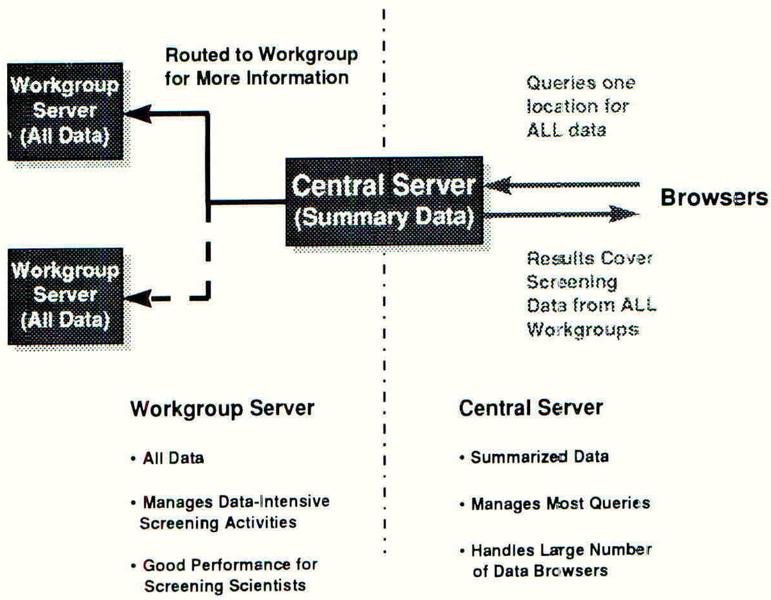

Fig 8. Buffering function of the Central Server

The data browsers also benefit from the use of the central server. Initial queries for data can be made without concern for which workgroup generated the data and the results from multiple workgroups are available. Queries such as screening results for a single sample across all screening programs, especially when the screening activity is internationally distributed, are simplified. Answers can be gained in a timely manner and with reduced risk of information loss.

CONCLUSION

A distributed SIMS is one approach to managing multiple-facility, multiple country HTS programs. It provides a balance between data access and performance and allows a large, distributed organization to maintain control over its screening program.

Session 4B

The Impact of the Management of Uncropped Land on Weed Control in Following Crops

Chairman	Mr G M Trevelyan
Session Organiser	Mr J H Orson
Papers	4B-1 to 4B-5

SET-ASIDE IN THE EU: PAST, PRESENT AND AN UNCERTAIN FUTURE

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ABSTRACT

Guaranteed prices and production control go together like a horse and carriage - to borrow from the song. To offer a fixed price for a commodity which is readily grown in the European Community's (EC) temperate climate, and to pitch the price well above world prices, is to encourage a rush into production of all able-bodied producers. This was well understood in the UK context before 1973, when the cereals guarantee was limited eventually to standard quantities; and the Community chose to limit milk production with some brutality from 1984, when it became clear that to set a milk price at a level which would be dissuasive to increasing production was beyond political reality. The longer-term future of set-aside in the European Union's (EU) arable sector depends now, as in 1992, on EU's ability to embrace price levels which allow a measure of international trade to take place without export subsidy.

INTRODUCTION

In the later 1980s the EC faced both budget and international pressures to restrict arable production. Financial 'stabilisers' were agreed in 1988 which imposed a Maximum Guaranteed Quality on cereals production for the first time, and the pressure which eventually led to the inclusion of agriculture in the General Agreement on Tariff and Trade (GATT) Uruguay Round became irresistible towards the end of the decade. We tend to forget how recently the EC became a major exporter of subsidised grains onto world markets. In the 1970s EC wheat exports rarely exceeded 5 million tonne, but by 1979 they reached 10 million and by 1989 20 million: the controversy over the international impact of the EC's oilseeds regime may be better remembered.

It is doubtful that it is necessary to dwell on the EC's hesitant steps towards a policy of full-blown obligatory set-aside from 1988 to 1992 but the important point to bear in mind is that, despite the seeming radicalism of the Macsharry proposals, the Common Agricultural Policy (CAP) Reform package as adopted represented a classic EC compromise, in particular between those member-states like the UK, who wanted lower prices, less subsidy and less production control, others who saw production-control as an acceptable price to pay to keep prices relatively high, and others again who put a high priority on maintaining subsidised exports and contested both the Blair House agreement and the emerging consensus around a significant set-aside percentage. All three points of view are still well represented in an EU of 15, and the Community is likely to have adjusted its arrangements again in recognition of one or other of them by the end of 1995.

The UK's implementation of set-aside in 1992 reflected on the one hand its desire to interpret Community rules as faithfully as possible, and thus to minimise audit query and disallowance; but also to take full account of the UK's concern for the environment, both in terms of pollution control and wildlife protection. Examples of this are our rules on green cover, the requirement not to damage or destroy hedgerows and other features, and the care taken to protect nesting sites, to the extent in some cases from derogating from normal management rules in the interests of species such as the stone curlew (*Burhinus oedicnemus*).

The same concerns have led us to campaign in Council over more than two years for the rules to allow producers putting land into farm woodland, nitrate sensitive areas and habitat schemes to count this against market set-aside. We achieved success this summer, in time for producers to benefit in their 1996 applications.

CHANGE IN STORE?

Set-aside has now been a condition of participation in the main arable support programme for three growing seasons, and UK farmers will submit their fourth applications in May 1996 in relation to the crops then in the ground. Before they do so, a number of issues are up for discussion either by Ministers or the Commission, where the outcome may well affect their actions. These do not result from any formal review of CAP reform, but rather the day-to-day pressures of running a major support programme, the experience acquired in the process, and a desire for simplification.

The rotational set-aside percentage

The Council will decide in the Autumn of 1995 what rate of rotational set-aside should be required for the 1995/96 production season. This will be the second year running when a figure other than the standard 15% will be in force as a result of anxieties about the short-term supply situation, so the practice is becoming well established of treating the percentage as one of the tools of market management available to the Community each year. Although producers have responded well to the late decision-taking which the EU's political process imposes, the search is on for an accelerated procedure for future years. This will be especially desirable in a year when a return to a higher set-aside rate becomes necessary: bad new arguably requires longer notice than good!

Flexible set-aside

In 1993 the Community agreed to allow 'flexible' set-aside as the alternative to rotational, and carried over the requirement for a five-point supplement from the earlier non-rotational regime. This figure represented the Council's view of the supplement required to eliminate the risk to production-control ('slippage') through yield increases on cropped land which may arise through allowing complete freedom to choose the sequence in which set-aside land is rotated. However, as the rotational rate reduces, the five-point supplement becomes a higher proportion of the total, amounting to 100% in the case of a 5% rotational rate. This bears no relation to any rational fear of slippage, and means that a Minister who seeks a reasonable rate for many producers who are now in flexible set-aside must press for an excessively low rate for those who are not.

A reduction in the higher rate for flexible set-aside is thus on the cards: but because slippage remains a real concern, any move towards a combined rate is likely to imply a higher rate for rotational set-aside than would otherwise be the case. The future of the UK's special 2-point derogation for flexible set-aside, which expired on 1 July 1995, is likely to be decided in the same context.

<u>The two-year rule and transfers</u>

We attempted a rationalisation of our implementation of the two-year rule this year (restricting producers from taking on new land with the intention of setting it aside immediately). However the Commission has let it be known that their response to the pressure for simplification of scheme rules is likely to be a proposal to suspend the rule entirely. Once the restriction is lifted, the limited arrangements allowing producers to export set-aside with a 3-5% supplement (transfers) would be suspended as well, since they would no longer have any function.

At the time of writing we are consultation on the implications of this proposal for the industry and other interested groups. At issue will be the extent to which the present two-year rule acts as a disincentive to 'exporting', the need for the restrictions under the transfer rules, and any environmental or economic impact of the change that can be foreseen.

<u>Penalty set-aside</u>

The Community's plan to restrict price-compensated production to a limited base area has to have an effective enforcement system if it is to be meaningful: but since there are no on-farm quotas the signal to the industry has to be generalised, through a requirement that all producers operating in any overshot base-area must make an additional uncompensated set-aside contribution. This has only operated unabated in France to date (apart from maize in England in 1995). While no government strongly contests the principle, there is some resentment that as set-aside itself counts against base area, it is possible for increases in voluntary and flexible set-aside to generate a penalty set-aside themselves. A proposal is now making progress in Council to provide that voluntary set-aside should not require matching penalty set-aside.

CONCLUSIONS

With diverse objectives in the Council of Ministers it is not surprising that it is hard to discern a clear direction for the development of set-aside policy. The present trend is towards reducing the burden on farm business, and on simplifying the rules. With the UK's success in obtaining permission for agri-environment schemes to count against set-aside, the pressure for 'greening' set-aside in the Community as a whole seems to be diminishing. However, one consequence of the current emphasis on market effects and simplification is that the protection which was built in against slippage in the original rules is likely to be eroded. This will mean that the Community will have to fix a basic rate of set-aside which assumes a higher rate of slippage than would otherwise be the case.

For the longer term set-aside could increase or diminish in significance, depending on the price policy the EU follows. The possibility of an increasing role was amply demonstrated by the Ministers CAP Reform Group, which on the assumption showed Community cereal production in 2005 exceeding demand (including permitted exports under GATT) by some 20m tonnes, implying a set-aside rate of 32%. The opportunity to avoid this scenario is demonstrated by the current market situation for cereals in Autumn 1995, when, at the time of writing, the EU's wheat export refund has been suspended for two months with a further month in prospect, and export trade is taking place without subsidy, and therefore does not count against the GATT ceiling. World prices are exceptionally high at present, but it should not be beyond the skill of policy-makers to establish a price-level for the EU which normally allows a proportion of production to be exported without subsidy; to decouple any compensation system for producers so that it does not stimulate production, and to remove the projected need for set-aside.

LONG TERM PROSPECTS FOR SET-ASIDE IN NORTHERN EUROPE

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ABSTRACT

The prospects for the long term future of set-aside will depend on how the factors which drive the European Union cereal balance and overall policy of the European Union, particularly the Common Agricultural Policy, develop. Historically the trend of cereal production in the European Union is one of steady increase, driven by average annual yield improvements of about 1.8%. Domestic consumption is virtually static. There are limited prospects for increasing cereal use in industrial processes but there are some indications that inclusion rates for use in animal feedstuffs will increase as the change in consumption from red meats to white meats continues. With overall cereal self-sufficiency currently over 120%, surplus production is exported, mainly with the aid of subsidies. The European Union's ability to subsidise exports is now constrained by the General Agreement on Tariffs and Trade (Uruguay Round) agreement. Thus, unless the European Union is able to export at world prices or there is relaxation of the rules in the next General Agreement on Tariffs and Trade agreement, then set-aside will be essential as the main means of managing over production. A slight shift in the world supply and demand for cereals could significantly alter the scenario.

INTRODUCTION

Set-aside is one of the primary mechanisms employed by European Union (EU) to manage arable production, and as such, the prospects for its retention into the next millennium will rely on the dynamic balance between three main factors, production, domestic consumption and exports of cereals. Numerous other factors, some within the political and economic control of the EU, but many outwith the control, will also influence its future.

Before trying to predict some future outcome, it is useful to first understand why set-aside was introduced. A simple examination of the recent levels of EU cereal production, consumption and exports (Table 1) will reveal the source of the problem.

Since the late 1970s, the EU has spent an increasing amount of money, initially storing, and more latterly subsidising cereal exports onto the world market. The simple solution would be to increase the volume of exports to keep pace with the widening gap between production and consumption. There is however a problem, or rather two problems. The first is the General Agreement on Tariffs and Trade (GATT) Uruguay Round (UR) agreement, and the second is the cost to the EU of exporting its surplus cereals. The two are inextricably linked. The GATT agreement strove to resolve the differences between its aim to liberalise world trade with the EU's policy of strongly supporting its agricultural industry via the Common Agricultural Policy (CAP).

Table 1. The EU cereals balance between 1986 and 1993 in million tonnes (Source: IGC)

Harvest Year	Production	Consumption	Exports
1986	153.7	139.6	27.2
1987	154.6	136.9	26.8
1988	163.5	135.8	35.3
1989	162.3	135.4	33.8
1990	170.1	141.3	30.1
1991	181.0	140.3	34.8
1992	168.8	134.1	37.4
1993	165.0	139.4	33.7

The most important elements of the GATT agreement for agriculture are the commitments to a :-

- 36% reduction in the cost of subsidising exports compared to the average level for 1986-1988
- 21% reduction in the volume of subsidised exports compared to the average for 1986-1989
- 20% reduction, compared to the average for 1986-1988, of internal fiscal support with exclusions for payments not directly linked to production.

The GATT (UR) agreement has therefore placed a major constraint on the EU continuing its past policy of accepting continued increases in cereal production and subsidising the disposal of surplus onto world markets. As a consequence, the 1992 CAP reforms included the introduction of set-aside as a means of curtailing production and reducing exportable surpluses.

The issue of whether CAP would reduce production sufficiently to ensure the reduction in subsidised exports agreed with GATT has been frequently debated. At present, such concerns have faded somewhat as a sequence of modest harvests and buoyant exports have significantly reduced cereal surpluses in store. However, the problem of compatibility between GATT and CAP was never immediate but it may still arise towards the end of the century (ADAS, 1995). For wheat in the year 2000 the limit for subsidised exports is 13.44 million tonnes compared to 20.255 million tonnes exported in 1991/92, for barley the limit is 4.5 million tonnes in the year 2000 compared to 5.7 million tonnes in 1991/92 (North, 1995).

The long term prospects for set-aside will depend therefore on how the pressures which gave birth to its introduction evolve, the opportunities for unsubsidised exports onto an expanding world market and the developing political and economic environment within the EU. Examination of each main area in turn will provide some pointers as to how set-aside may evolve.

EU CEREAL PRODUCTION AND CONSUMPTION

Production

Until the introduction of set-aside within the arable area payments scheme, in the harvest year 1993, the area of cereals grown in the EU had remained relatively stable, other than due to enlargement by the entry of new member states. The average yield (Table 2) has increased on average by about 2% per annum over the last decade. Improvements in husbandry standards, new technology and new varieties will continue this trend.

Table 2. Changes in EU cereal area and yield between 1986 and 1994 (Source: HGCA)

Harvest Year	Area (million ha)	Yield (t/ha)
1986	35.48	4.31
1987	34.99	4.46
1988	34.66	4.72
1989	34.95	4.64
1990	35.75	4.76
1991	35.78	5.06
1992	35.20	4.81
1993	32.12	5.14
1994	31.87	5.12

The potential for an increase in average yields is considerable. Introduction of improved varieties has in the past been responsible for driving much of the increased yield. Whilst there is some debate about the rate at which further increases will evolve, there is little disagreement that yields will continue to rise. While there are climatic limitations, particularly rainfall, that could constrain yield increases in some areas, within North Europe average yields are still well below those achieved by the better growers, indicating continuing scope to increase production via enhanced management. On the other hand falling prices in Europe and increasing environmental concerns may lead to more discerning use of inputs, particularly agrochemicals, but based on current trends, these are unlikely to offset the factors driving increased production

Consumption

However, it is difficult to identify the factors which will lead to significant growth in domestic consumption to utilise the potential additional production. Currently 60% of total consumption in the EU goes into animal feed, 26% is used for direct human consumption, 8% for industrial processes, 5% for seed and 1% for waste and other uses (HGCA, 1994).

Human consumption

The size of the population in Europe is relatively stable with no indication of any major changes in the pattern of consumption giving little prospect of increased cereal use for direct human consumption.

Animal feed

The continuing change in dietary habits in North Europe of increasing white meat consumption at the expense of red meat, has given rise to increasing production of compound animal feeds (Table 3). However this has been achieved by an increasing proportion of non-cereal ingredients leaving the volume of cereals fed to livestock little changed (Table 4). Whilst there is some indication recently of increasing use of cereals, prices will have to become much more competitive for this to continue.

Table 3. Growth in EU production of compound animal feed in million tonnes, 1985 to 1993 (Source: FEFAC)

Calendar Year	Cattle	Pigs & poultry	Total
1985	29.0	47.6	80.0
1986	30.1	48.9	82.6
1987	31.1	60.1	96.4
1988	32.2	62.0	99.7
1989	32.4	61.6	100.3
1990	32.0	63.7	102.1
1991	34.9	68.8	110.0
1992	33.8	70.1	110.5
1993	35.8	72.6	115.0

The EU Commission have projected an increased use of cereals of between 8 and 12 million tonnes by the end of the century. It is difficult to see exactly how this will arise. In the event that it does occur, this increase will do no more than balance the reduction in the volume of exports the EU is required to achieve to meet the commitments under GATT.

Table 4. EU cereal usage for animal feedingstuffs in million tonnes, 1986 to 1993.
(Sources: FEFAC, Eurostat)

Marketing Year	Total compound	Cereals usage
1986/87	82.6	84.7
1987/88	96.4	82.6
1988/89	99.7	81.5
1989/90	100.3	80.5
1990/91	102.1	83.6
1991/92	110.0	83.1
1992/93	110.5	81.1
1993/94	115.0	84.4

Industrial uses

Without some form of support, the prospects for the increased industrial use of cereals are low as they will have to compete, on economic grounds, with other sources of chemical feedstock. Similarly the use of cereals as a substrate for the production of biofuel is unrealistic with oil currently at US$ 18-20 per barrel. Oil prices would have to rise to US$ 40 per barrel or more for bioethanol production to become competitive.

Hence, from an EU perspective there are no likely factors which will significantly change the continuing need for some form of constraint on grain production, unless it becomes possible to export substantial volumes of cereals to the world market without the help of subsidies.

PROSPECTS FOR THE WORLD MARKET

As world population expands, the demands for grain will increase and as the economies of less developed countries evolve, changing dietary habits will also enhance demand for grain, especially wheat (Anderson, 1995).

With the world population forecast to rise substantially well into the next millennium, the demand for grain can also be expected to grow (Table 5).

The vast majority of the predicted increase in world population however will occur in the developing countries (OECD, 1994). They will undoubtedly seek to improve their own agricultural production in an attempt to feed themselves. Indeed several countries, notably India and China, have already had considerable success in doing so (Table 6). Other countries, such as the USA, are also expanding their grain production in the expectation of capturing some of this increasing world demand.

Table 5. Projected human population by 2025 in less and more developed countries (Source: United Nations, 1993)

	Human Population (billions)	
	1995	2025
Less Developed Countries	4.5	7.1
More Developed Countries	1.2	1.4
Total	5.7	8.5

Current trading agreements only allow the EU to increase its exports above agreed volumes without the aid of subsidies. The question therefore is not whether the demand will materialise, but whether the EU can produce grain for the world market without direct support at a price which these countries can afford.

World prices have recently risen to levels similar to those in the EU, to the extent that some wheat is being exported without subsidy. If this situation continues, it raises the prospect of an unsubsidised export market for EU grain, which may obviate the need for production control and hence set-aside.

Table 6. Production and consumption of wheat in India and China in million tonnes, 1986-1994 (Sources: USDA, IGC)

Marketing Year	India		China	
	Production	Consumption	Production	Consumption
1986/87	47.1	n/a	90.0	101.5
1987/88	44.3	n/a	85.8	102.8
1988/89	46.2	n/a	85.4	104.4
1989/90	54.1	52.7	90.8	104.5
1990/91	49.9	51.5	98.2	106.0
1991/92	55.1	56.4	96.0	111.7
1992/93	55.7	55.6	101.6	109.1
1993/94	56.8	56.4	106.4	110.7
1994/95	57.8	57.5	103.0	113.5

EU ENLARGEMENT

The EU itself is likely to change markedly in the future. It has a history of expansion with current prospects for a number of Central and Eastern European Countries (CEECs) joining the EU early in the next century. Those likely to be the first to join include, Bulgaria, Czech Republic, Slovak Republic, Hungary, Poland and Romania. These countries all have major agricultural industries and have the potential to increase EU grain production by over 50% (Table 7). While their current production and consumption are in balance, their reported levels of production were significantly higher while they were still members of the Eastern Block. If they can resolve their current problems, there is considerable capacity to increase productivity which would generate the potential for additional surplus grain for an enlarged EU to accommodate.

Table 7. The production and consumption balance for cereals in the CEECs in million tonnes, 1986-1994 (Sources: USDA, IGC)

Marketing year	Consumption	Production
1986/87	97.4	99.1
1987/88	97.5	92.5
1988/89	97.2	96.2
1989/90	97.2	98.6
1990/91	96.1	92.6
1991/92	95.5	103.2
1992/93	80.3	69.6
1993/94	78.0	74.8
1994/95	81.2	79.5

BUDGETARY AND POLITICAL PRESSURES

Inertia in the decision making process will inevitably lead to a reluctance by the EU to abandon the control that the current set-aside policy undoubtedly provides. The policy is however expensive for the tax payer who will become increasingly sensitive to its cost and the benefit it provides to the wider community. They are therefore likely to want an increasing say in the way any set-aside policy evolves. This will lead to increasing pressures to use land which is set-aside for the wider general benefit including a wide diversity of environmental and possibly recreational uses. Other pressures may also develop to use set-aside land for alternative crops for wider environmental and other benefits, such as a source of renewable energy crops.

CONCLUSIONS

In a solely European context, grain production potential seems inevitably set to increase alongside relatively static demand in the absence of some form of control. EU expansion will not resolve this problem and indeed seems likely to exacerbate it in due course.

In this environment the policy makers will be unlikely to forego the management control that current set-aside policy offers. The cost of this policy in a enlarged EU may necessitate some changes in the longer term, if the total CAP budget is considered to have become too high.

World markets do offer the potential opportunity to absorb EU surpluses and avoid the requirement for set-aside in the longer term. To exploit this opportunity, current CAP policy will have to change and growers will have to be able to compete at world market prices and accept the pressures which this will bring. It remains to be seen how the next GATT round, due for implementation early in the next century, will seek to change this position.

If set-aside does continue to be required, public pressure for greater community benefit in the way that set-aside is used and managed will be inevitable. This may well materialise in the form of a greater emphasis on longer term as opposed to rotational set-aside.

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COMPARISON OF THE EFFECT OF WEED CONTROL STRATEGIES FOR ROTATIONAL SET-ASIDE IN UNITED KINGDOM, DENMARK AND FRANCE

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ABSTRACT

The options for weed control in rotational set-aside are quite different in each country, reflecting different priorities. This paper outlines briefly the rules appropriate to weed control issues in each country and discusses the weed concerns that these address satisfactorily and those which are harder to satisfy. The options for weed control discussed are sowing a plant cover, cutting, cultivation and herbicide use. The relevant importance and benefits of each are described for the three countries. In the United Kingdom, herbicides have a major role in controlling weeds which has resulted from environmental pressure to reduce damage to wildlife. In Denmark, the most effective control of weeds results from sowing a plant cover. In France, both herbicides and cutting are important.

INTRODUCTION

The first European Community (EC) set-aside scheme came into operation in July 1988. The 5-year scheme was voluntary and designed to reduce surpluses of arable crops. In return for taking at least 20% of eligible arable land out of production on a holding, annual compensation payments were available. Set-aside land could be rotated annually or remain in the same place for up to 5 years. Set-aside land had to be managed within defined conditions.

In August 1991 another scheme, the EC one-year set-aside scheme, was introduced. Again this was voluntary. Under this scheme farmers were offered an area payment plus a refund of co-responsibility levy money paid on all cereals produced between 1 July 1991 and 30 June 1992. In return, farmers had to set-aside at least 15% of their arable area used to produce specified crops for the 1991 harvest. Within this requirement they also had to reduce their cereals area by 15%.

In May 1992 it was announced that set-aside would be part of the reform of the EC Common Agricultural Policy. Farmers were required to set-aside 15% of their total area of cereals, oilseeds, protein crops and set-aside as rotational set-aside, or from 1993 a higher 18% (in UK and Denmark) or 20% (in France) as non-rotational set-aside. These three countries represent nearly half of the set-aside in the EU. Of the 5,939,000 ha of CAP reform set-aside in the 12 Member States of the EU in 1994, there were 1,868,000 ha (31.5%) in France, 655,000 ha (11.0%) in UK and 267,000 ha (4.5%) in Denmark (Renshaw, 1994).

Where required, weed control on set-aside land can be achieved by cutting, cultivating, sowing a plant cover or by using herbicides (Anon, 1994). The relative importance of these will vary according to individual objectives and the rules of set-aside in each country. This paper aims to explain the rules in each country, explore the value of each of the options and to suggest how lessons in one country can influence change in the rules in another.

RULES

The rules concerning management of set-aside vary slightly in each Member State of the EC. These are determined by local circumstances and priorities. Of the countries covered by this paper, only Denmark has clear targets for reducing pesticide use and nitrate leaching in arable farming. This means that set-aside rules have been formulated to assist the objectives of reducing pesticide usage by 50% from the average amount used in 1981-1985 by 1 January 1997 and to reduce annual nitrate leaching by 50% to an estimated 260,000 t of nitrogen before 1 January 2000. This requires a good plant cover to be established and maintained for most of the set-aside period to prevent loss of nitrates and for herbicide use to be restricted to meet the target for a reduced use of pesticides (Anon, 1995). In both UK and France environmental criteria have created a framework for the rules which are designed to minimise nitrate leaching and maximise environmental benefits, although no specific targets have been set. In all cases the rules must ensure the prime objective of set-aside, which is to reduce production, is met.

CONCERNS

It is normally important to control weeds on rotational set-aside land. In particular grass weeds such as barren brome (*Bromus sterilis*), wild-oats (*Avena* spp.), loose silky-bent (*Apera spica-venti*) and black-grass (*Alopecurus myosuroides*) may need controlling. In many cases it may also be desirable to remove volunteer cereals and, especially in the case of longer term set-aside, to encourage a diversity of natural flora with little agronomic importance. For some species complete control is neither necessary nor desirable. It is not cost-effective to control many annual broad-leaved weeds or annual meadow-grass (*Poa annua*) in one-year set-aside, as there is a large seedbank in the soil and certain species can be of benefit to wildlife. Species which produce high seed numbers and can survive many years in soil, such as common poppy (*Papaver rhoeas*), scentless mayweed (*Tripleurospermum inodorum*), fat-hen (*Chenopodium album*) and shepherd's purse (*Capsella bursa-pastoris*), have the potential if left uncontrolled to become very numerous in following crops. By contrast species which produce few seeds which do not survive many years in soil, such as field pansy (*Viola arvensis*), are less important.

Table 1. Summary of the rules for rotational set-aside which affect weed control issues in UK, Denmark and France.

	UK	Denmark	France
Plant cover			
Natural regeneration	Allowed	Allowed (max. 5% of total set-aside area)	Allowed
Bare fallow	Not allowed	Not allowed	Not allowed
Species allowed	Grass, non-agricultural crops, mixtures	Grass, grass mixtures	Positive list of 39 species which includes grasses
Legumes	Max. 5% by weight	Max. 25% by seed number	No restriction from 21 species on list
Establishment date	As soon as possible	By 1 October (or within 14 days after harvest of a late maturing crop)	By 1 May
Earliest destruction date	1 May	20 October (15 July if followed by autumn sown crop)	31 August (16 June if followed by oilseed rape, lucerne, grass; 31 January if followed by spring crop)
Cutting			
Minimum requirement	Between 15 July and 15 August if cover still in place on 31 August	At least once in August	Must stop seed production from cover and weeds
Restrictions	None, but advised to avoid bird nesting season (April to mid-July)	Not allowed 1 May to 30 June (except to prevent cross pollination, or to control *Avena fatua*, *Bromus* spp. or noxious weeds, or if kept very short)	
Cultivation			
Earliest date allowed before autumn sown crop	1 May	15 July	15 July
Earliest date allowed before other crops	1 May	20 October	31 August
Herbicides			
Selective	Non-residuals allowed but must meet other plant cover requirements	Not allowed 1 October to 31 August, except to control *Avena fatua*	Only if on positive list of 17 a.i. (changes expected in 1995/6 to allow wider range - see text for details)
Non-selective	Allowed from 15 April	Not allowed 1 October to 31 August	Only if on positive list of 5 a.i. (changes as above)

OPTIONS

Cutting

This can be an effective method of weed control (Clarke, 1992). Cutting has been especially effective at controlling weed seed return when a good plant cover is present. In the UK on heavy soils, or in moist seasons on all soil types, up to five operations may be required (Clarke, 1995). In France up to seven cuts have been required to prevent seeding of black-grass. At around £10/ha/cut this could be a very expensive option. Some weeds, especially grass weeds, have produced seed at a height below the cutter bar and rolling by tractor wheels can also leave some uncut seedheads in both UK (Clarke & Cooper, 1992) and France. Cutting is less effective on prostrate species such as *Polygonum* spp., speedwells (*Veronica* spp.) which can seed below cutter height and those which regrow and produce seed very rapidly such as black-grass. In both UK (Clarke & Cooper, 1992) and France cutting has needed to start early, sometimes in March, to prevent seed production. Unless started early, birds will nest and may be killed by the cutting operation. Other wildlife, such as hares (*Lepus europaeus*) and deer (e.g. *Capreolus capreolus, Dama dama, Muntiacus reevesi*) are also at risk. Cutting is not a significant option in Denmark.

Cultivation

Like cutting, cultivation can be a very effective method of weed control. Cultivation for weed control is only allowed after 1 May in the UK and after 15 July in Denmark and France. In the UK this may be done by ploughing or deep cultivation. Early ploughing is likely to result in more nitrate mineralisation and may require subsequent cultivation to keep on top of regrowth of weeds. It may also lead to poorer soil conditions, especially in a wet season and can increase the risk from wheat bulb fly (*Delia coarctata*). However, well managed cultivation will offer the opportunity of timely establishment of following crops. Cultivation before early July can be very damaging to wildlife. In particular cultivating in May and June will destroy nest sites. However, for some bird species, and after a derogation has been obtained from MAFF, cultivation before 1 May to leave bare soil is essential to create nesting sites for birds such as stone curlew (*Burhinus oedicnemus*).

Plant cover

A vigorous plant cover, such as after undersowing or autumn sown, can give very good suppression of weed species. In all countries late heading perennial rye-grass (*Lolium perenne*) has been an appropriate plant cover choice. This option is especially relevant for set-aside left down for more than one year. Choice of plant cover will depend upon objectives and individual country's rules, as will cutting frequency and timing. Mixtures of plant species can be more difficult to manage than straight species covers since these may cause difficulties in choosing an appropriate selective herbicide or in the timing of cutting.

Data collected from three sources (Table 2) clearly demonstrate the ability of a plant cover of winter cereals to suppress weed seed production when compared to in natural regenerated set-aside. Left uncontrolled, these could produce significant increases in seed numbers in the soil with the potential to pose problems in crops following set-aside (Table 3).

Table 2. Comparison of the numbers of seeds produced per plant for several species in natural regeneration set-aside, winter cereals and sugar beet.

Weed species	Common name	Natural regeneration	Winter cereals	Row crops e.g. Sugar beet
Arabidopsis thaliana	Thale cress	22,200 (1)	2,600 (2) 3,800 (1)	
Avena sterilis ludoviciana	Wild-oat	1,700 (1)	700 (2)	
Cerastium glomeratum	Sticky mouse-ear	16,500 (1)	3,000 (1)	
Chenopodium album	Fat-hen	21,500 (1)		7,300 (3)
Conyza canadensis	Canadian fleabane	68,000 (1)		4,000 (3)
Digitaria sanguinalis	Hairy finger-grass	13,900 (1)		1,350 (3)
Echinochloa crus-galli	Cockspur	18,880 (1)		990 (3)
Lamium purpureum	Red dead-nettle	3,200 (1)	150 (3)	640 (3)
Poa trivialis	Rough meadow-grass	29,000 (1)	14,000 (2)	
Setaria pumila	Yellow bristle-grass	1,500 (1)		370 (3)
Sonchus asper	Prickly sowthistle	15,600 (1)	512 (2)	5,800 (3)
Stellaria media	Chickweed	1,200 (1)	120 (3) 298 (2)	
Veronica hederifolia	Ivy-leaved speedwell	1,200 (1)	450 (3)	
Veronica persica	Common speedwell	3,000 (1)	700 (1)	180 (3)
Viola arvensis	Field pansy	12,400 (1)	198 (2)	

Refs.: Rodriguez & Mamarot, 1994 (1); Pawlowski, 1966 (2); Slavnic, 1962 (3).

Table 3. Comparison of weed numbers in a wheat crop following set-aside or sunflowers (Rodriguez & Mamarot, 1994).

Preceding crop	Number of species	Number of weeds/m^2
Natural regeneration set-aside	19	166
Sunflowers	13	25

Herbicides

A correctly chosen and well timed herbicide application can offer very good weed control. Rules and attitudes in the three countries differ considerably. A well timed herbicide can also be a very cost effective option which both reduces the need for subsequent weed control and benefits wildlife.

UK

In the UK, following changes to the rules in 1993/94, herbicide use on set-aside areas is more straightforward. These changes resulted from public concern over the damage being caused to ground nesting birds from cultivating or mowing set-aside during the nesting season. Previously, herbicides were only allowed after seeking permission from the local MAFF Regional Service Centres, but they are now allowed without such prior permission, provided they fall within clearly defined criteria. The set-aside rules allow herbicide use under the following conditions:

- in general only non-residual products are allowed. Residual products can only be used if they have a specific label approval for use on set-aside land
- non-selective products (such as glyphosate and glyphosate-trimesium) only after 15 April, but if set-aside is to be left for a further year a replacement plant cover must be sown.
- non-selective products, at any time, when changing between plant cover options
- to create or maintain a bare strip where permitted
- using spot treatment or a wick applicator
- selective products at any time

Users must comply with the Food and Environment Protection Act 1985 and Control of Pesticides Regulations 1986. Under these regulations, products can only be used if they are approved for the appropriate crop or situation. Non-residual products are defined as those which are absorbed into the plant mainly through the leaves or stem.

The advantages of delaying destroying the cover with herbicides include reduced nitrate leaching risk, better soil conditions and greater environmental benefit to insects and birds. The risk is of viable seed being produced. Research has examined the spray timing required to prevent viable seed return. An initial MAFF-funded experiment at ADAS Boxworth in 1995 has looked at spraying four herbicides at a range of timings on the level of vegetation control and seed viability of black-grass and brome. These results (Figure 1) show very satisfactory levels of control from late applications of glyphosate but lower control and less flexibility with glufosinate and paraquat. Viability of the seed has not yet been tested.

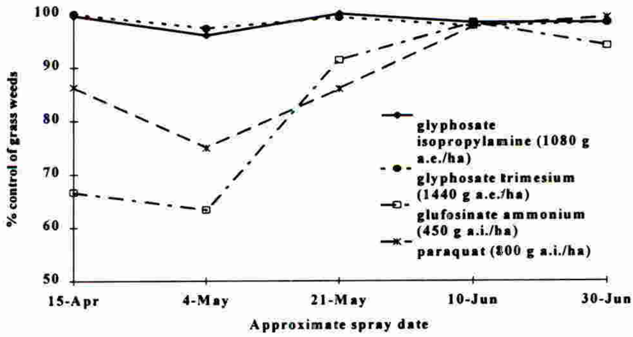

Figure 1. Control of grass weeds (% control of green material, assessed on 19 July) with four herbicide treatments at timings from 15 April to end of June in 1995.

Denmark

Herbicide use is not allowed from 1 October to the following 31 August except for selective control of wild-oats. Spraying must only be done with an herbicide approved for that use and the plant cover must not be damaged significantly by the treatment.

France

Twenty two active ingredients are authorised for use on set-aside. The list includes asulam, clopyralid, MCPA, fluroxypyr, metsulfuron-methyl, 2,4-D, alloxydim, dalapon, fluazifop-P-butyl, haloxyfop-ethoxyethyl, quizalofop-ethyl, fosamine-ammonium, triclopyr, CMPP, CMPP-P, dichlorprop, dichlorprop-P, diquat, paraquat, glufosinate, glyphosate, sulfosate (glyphosate trimesium). There is no restriction on the timing when these can be used. Changes are expected in 1995/96 which will allow any herbicide in the following circumstances:

- for selective weed control in the cover during establishment
- to restrict growth and seed production
- to destroy the plant cover

Research work on herbicides in France has demonstrated the benefits of broad spectrum products, such as mixtures of metsulfuron-methyl and glyphosate to control seed production of both the plant cover and weeds. Best weed control has resulted from application made at the start of flowering of broad-leaved species and to be effective two treatment have been required to prevent seed production until 15 July. In practice most farmers have thought it necessary to spray a selective herbicide during the establishment of the cover and a subsequent spray to stop growth in the spring.

Some work has investigated sequential applications of glyphosate. However, species such as phacelia (*Phacelia tanecetifolia*) a single full dose treatment has been shown to be optimum. On perennial rye-grass and Italian rye-grass (*Lolium multiflorum*), after a first application of 150-180 g a.e./ha glyphosate it has proved difficult to determine the second timing and cutting has been shown to be preferable. On some clover species (*Trifolium* spp.) and vetches (*Vicia* spp.) the rate of glyphosate has been shown to be very important with a single dose of 240 g a.e./ha giving similar results to two applications of 150 g a.e./ha. Sequential applications are however sometimes difficult to fit in with other work on the farm and it is essential to avoid drift to neighbouring crops.

SOLUTIONS

UK

In the UK in the first year of the present scheme (1993) the majority of the rotational set-aside land was natural regeneration (72%) either mown (60%) or cultivated (28%) (Abel, 1993). Widespread concern was expressed about the damage to nesting birds. This lead to pressure for greater flexibility in allowing the use of herbicides, especially non-selectives such as glyphosate, which were accepted as being the least damaging option. Although the plant cover is destroyed this is usually slow and allows birds and insects present to find alternative habitats. Herbicide application, as the first management operation to set-aside, rose from 10% in 1993 to 72% in 1994 according to Game Conservancy Trust survey (Thompson, 1995). Glyphosate has been the main herbicides used. The current debate now centres around the timing of the glyphosate spray.

Early destruction of the cover increases the risk of nitrate leaching, reduces the benefit to wildlife and can result in poorer soil conditions. Recent work by ADAS and others (Boatman, Edwards & Merritt, 1995) has demonstrated that satisfactory control of black-grass and barren brome can result from June applications of glyphosate. This is still early enough to prevent viable seed return and late enough to give the environmental benefits of reduced nitrate leaching risk and provide cover for birds. Following this with cultivation in mid-July has given good seedbed conditions for establishment of the following crop.

In the UK, provided seed return is controlled during set-aside, no significant weed problems have occurred succeeding crops (Clarke, 1995). Well managed set-aside has also provided the opportunity to control difficult grass weeds, including those resistant to herbicides (Table 4). New experiments and practical experience are demonstrating similar or better results with the herbicide and cultivation options now available. The only increased weed problems in following crops have been where inadequate control was achieved in set-aside, often associated with poor management. There are also indications, as predicted by Clarke (1993), that the plant covers themselves are becoming weeds, particularly Italian rye-grass, perennial rye-grass, phacelia and mustard (*Brassica alba*). For instance assessments made on 27 October 1994 in a crop of oilseed rape at ADAS Boxworth contained the following levels of volunteers: after natural regeneration/spring sown white mustard 7 white mustard plants/m², after natural regeneration/spring sown phacelia 36 phacelia plants/m² and after autumn sown perennial rye-grass 33 rye-grass plants/m². Numbers after other treatments were negligible.

Table 4. Comparison of weed numbers/m^2 in crops after different set-aside treatments (mean 4 UK sites set-aside in 1993, assessed in the following crop before a herbicide spray).

Treatment during set-aside year	Total number of broad leaved weeds/m^2	Total number of grass weeds/m^2
Normal arable rotation	77.9	27.5
Natural regeneration (mown to prevent seeding until cultivated in July)	70.7	22.3
Natural regeneration cultivated 1 May	72.6	12.6
Natural regeneration cultivated 1 July	70.4	21.6
Autumn sown perennial rye-grass (mown to prevent seeding)	77.9	27.5
Spring sown mustard after natural regeneration	59.1	10.7

Denmark

The authors estimate that of the set-aside in Denmark about one third is rotational set-aside, equivalent to about 3% of the total agricultural land area. The rules of set-aside offer very few options to the farmer concerned with controlling the undesirable side-effects of set-aside. Poorly managed rotational set-aside can result in large increases in weed numbers resulting in problems in future crops (Melander & Jacobsen, 1994). A well established plant cover is the most effective and economical measure that can be taken to control undesirable weed growth on rotational set-aside land. It is usually recommended to under-sow the grass cover in a cereal crop where broad-leaved weeds should be controlled with herbicides if possible. This results in a well established grass cover with few weeds after the harvest of the cereals. Late heading perennial rye-grass is most useful in this respect.

France

The area of rotational set-aside is thought to be about half of the nearly 2,000,000 ha in France (Viaux, pers. comm.). ITCF advice is to sow a plant cover which can regrow after cutting, such as a grass species. In terms of weed control within set-aside there is then little difference between cutting or herbicide use. If a plant cover which is susceptible to herbicides is chosen, primary treatment with herbicide is advised followed by cutting 4-8 weeks later to remove subsequent weed regrowth.

CONCLUSIONS

Advisers in all three countries have been very concerned about the potential weed problems caused by set-aside. In most cases, satisfactory solutions have been found to minimise the risks. In all countries reducing nitrate leaching has been an important consideration in framing the rules. The greatest potential for weed problems appears to be in Denmark where there are very few options for control other than competition from a sown cover. It remains to be seen whether the cover itself will become a weed of the future. The use of herbicides has been denied to farmers during set-aside at the risk of increased usage in subsequent crops. The UK allows the greatest flexibility in the choice of weed control measures. Avoiding damage to nesting birds is an issue in both UK and Denmark. The solutions have been different due to different pressures from outside farming. In UK, the use of herbicides was considered the least damaging option between cutting, cultivation and herbicide use. However, in Denmark the pressure to reduce total use of pesticides has resulted in this option not being available on set-aside. It remains to be seen what the effect of not allowing herbicides on set-aside will be on weed control and herbicide use through the rotation. There is evidence from UK to suggest that the well timed use of herbicides in set-aside can reduce the need for herbicides in following crops. Strategies employed in France result in satisfactory weed control. However, because the rules insist on prevention of seed production during the set-aside period this results in high inputs to control weeds. The authors suggest that the approach in each country is a result of the different level of pressure from groups outside farming. On current evidence the UK approach appears to offer the best opportunities for cost-effective weed control. Here well managed set-aside can provide good weed control whilst giving benefit to wildlife.

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MANAGEMENT OF DIFFERENT COVERS OF SET-ASIDE FOR WEED CONTROL

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ABSTRACT

In autumn 1992 and 1993, set-aside was sown in an experimental site in Mid-Belgium. Several species and mixtures were sown to compare their aptitude to cover the soil and to prevent the development of weeds. Clover has been more overgrown with weeds than grasses and grasses plus legumes in the spring. The cutting carried out in the spring reduced the differences. The comparison between the management by herbicides plus cutting and by cutting only showed plots treated with herbicide were less infested by weeds in the spring. In September, the differences between plots had disappeared. The management by herbicides plus cutting rapidly reduced the number of weeds and thus the risks of an increase in the seed bank. However, this treatment was more expensive than the management by cutting only.

INTRODUCTION

The introduction of set-aside in the crop rotation has strongly altered the farming practices. The obligation to keep a part of the farm in set-aside has engendered some questions. For the farmers, the challenge consists in sowing and managing the set-aside at a low cost while preserving the soil fertility and maintaining or reducing the weed seed bank in the soil. The choices of cover species and the management are therefore very important.

Weed growth and persistence, during the set-aside, are a very important problem to manage. Indeed, these weeds may increase the seed bank and could become a threat in the subsequent crops.

MATERIALS AND METHODS

A site was sown in autumn 1992 and another in 1993 on fertile and homogeneous sites (Table 1). These fields experiments were designed as complete randomised blocks with four replicates.

The assessment of the vegetation was measured by percentage ground cover of grasses, legumes and weeds on the whole surface of the plot.

Table 1. Soil analyses.

	pH H_2O	OM%	P (mg/100g)	K (mg/100g)	Ca (mg/100g)	Mg (mg/100g)
Site 1	7.2	1.7	6.5	32.5	191.8	13.0
Site 2	7.7	1.7	11.8	20.7	241.7	7.6

Extraction method : Acetate-EDTA (Lakanen & Erviö, 1971)

Site 1.

This first trial was sown in order to compare the cover of different species of legumes in a pure stand or in mixtures as well as the management by herbicide or cutting. The cut vegetation was left on the plots. During the growing period, ground cover was assessed in view to determining the most adapted mixtures.

Table 2. Treatments on site 1.

Factors	Treatment (sowing rate : kg/ha)
Mixtures	
	Red clover (20)
	White clover (10)
	Lucerne (20)
	Red clover + White clover (15 + 5)
Management	Herbicides[1] + 2 cuts [2]
	Cutting only [3]

1 Pyridate (450 g a.i./ha) + bentazone (720 g a.i./ha) + fluazifop-P-butyl (187.5 g a.i./ha) applied on 30/10/92
2 7/06 and 20/8/1993
3 30/4, 24/5, 17/6, 20/8/1993
Date of sowing : 10/9/92

Site 2.

On this second site, several cover mixtures were chosen. The comparison between different dates of sowing was also included (Table 3). The sowing was on 20th August 1993 for species sown before the winter and on 3rd May 1994 for species sown after the winter. The management of these covers is shown in Table 4.

Table 3. Treatments on site 2.

	Period of sowing	Cover (sowing rate : kg/ha)	Management
1	Before winter	Late perennial ryegrass (20)	Cutting only
2		Very late perennial ryegrass (20)	Cutting only
3		Perennial ryegrass + white clover (20 + 4)	Cutting only
4		Red fescue + common bent (*Agrostis capillaris*) + white clover (30 + 5 + 5)	Cutting only
5	After winter	Perennial ryegrass (20)	Cutting only
6		Perennial ryegrass (20)	Herbicide + cutting
7		Perennial ryegrass + white clover (20 + 4)	Cutting only
8		Perennial ryegrass + white clover (10 + 2)	Cutting only
9		Fodder kale (*Brassica rapa campestris*) (15)	Cutting only
10		Phacelia (*Phacelia tanacetifolia*) (10)	Cutting only

Table 4. Management on site 2.

Date of management	Sown before winter Cutting only	Sown after winter Cutting only	Sown after winter Perennial ryegrass Herbicide + cutting
10/5/94	Cutting		
30/5/94			Herbicide[3]
14/6/94	Cutting		
25/6/94		Cutting[2]	
15/7/94	Cutting[1]	Cutting[2]	Cutting
17/8/94	Cutting	Cutting	Cutting

1 Except red fescue + common bent + white clover
2 Except fodder kale and phacelia
3 Herbicide : bentazone (1200 g a.i./ha)

RESULTS AND DISCUSSION.

Site 1.

The comparison between chemical plus mechanical treatment and mechanical treatment only showed a difference between the two approaches. The herbicide application in the spring rapidly reduced the percentage of weeds. In April there was a difference of 14% in ground cover between the two treatments (Table 5 and Figure 1). The chemical treatment also permitted a reduction in the number of cuts from 4 to 2. This treatment also led to a decrease in the number of weeds and prevented them from setting seed. The weed growth and persistence were in this way strongly reduced. It is very important to reduce rapidly the weeds before they set seed. In August, there were no differences appeared between treatments.

Table 5. Ground cover of sown species (%) on site 1

Date	Herbicide and cutting	Cutting only	Difference
7 April 1993	97	83	**
1 July 1993	98	95	**
5 August 1993	99	98	NS
7 October 1993	99	100	NS
Mean	98	94	**

Significant level of difference (Newman-Keuls Test): ** = Very significant (p= *1%*); N.S. = not significant; Replicates: 16

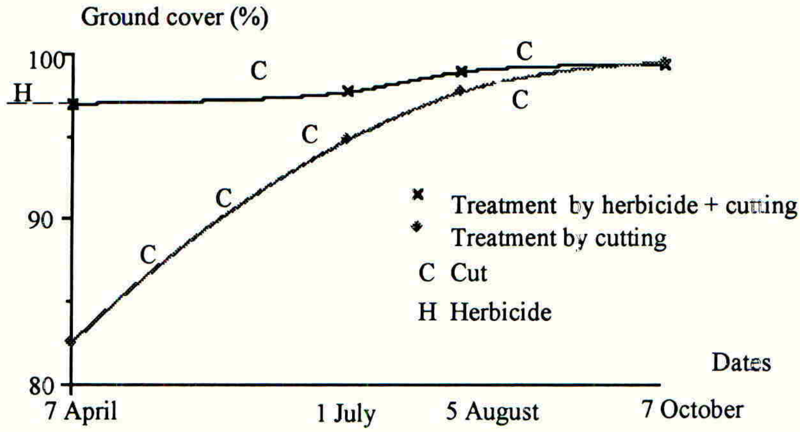


Figure 1. Percentage of cover ground of sown species on site 1

The white clover managed by cutting did not cover the ground so good as the other mixtures. This crop grew more slowly and thus its competition towards the weeds is weak. For the chemical treatment, the differences in weed ground cover between the species were low.

Concerning the management cost, if the cost of one cut is about 1500 FB/ha and herbicide application cost is about 950 FB/ha, the cost of treatment by cutting only was 6000 FB/ha and the chemical plus mechanical management was more expensive, at 9550 FB/ha. It is worth noting that the price of the chemical product was very expensive (5600 FB/ha).

Site 2.

Mixtures sown before the winter.
There was little difference between the covers as far as the percentage of weed ground cover is concerned. In March and April, differences were perceptible, but the weed had almost disappeared at the end of the period (on average 2% of ground covered by the weeds). In comparison with another trial, set-aside composed a natural re-generation was 80% covered by weeds, on October 7th. This set-aside type is difficult to manage because it is impossible to avoid the setting of seed of all weeds.

The ryegrass covered more ground than the red fescue plus common bent association. Regarding the white clover, it grew better when it was associated to these two grasses than when it was mixed with ryegrass. Between the two types of ryegrass, late and very late, there was no visible difference.

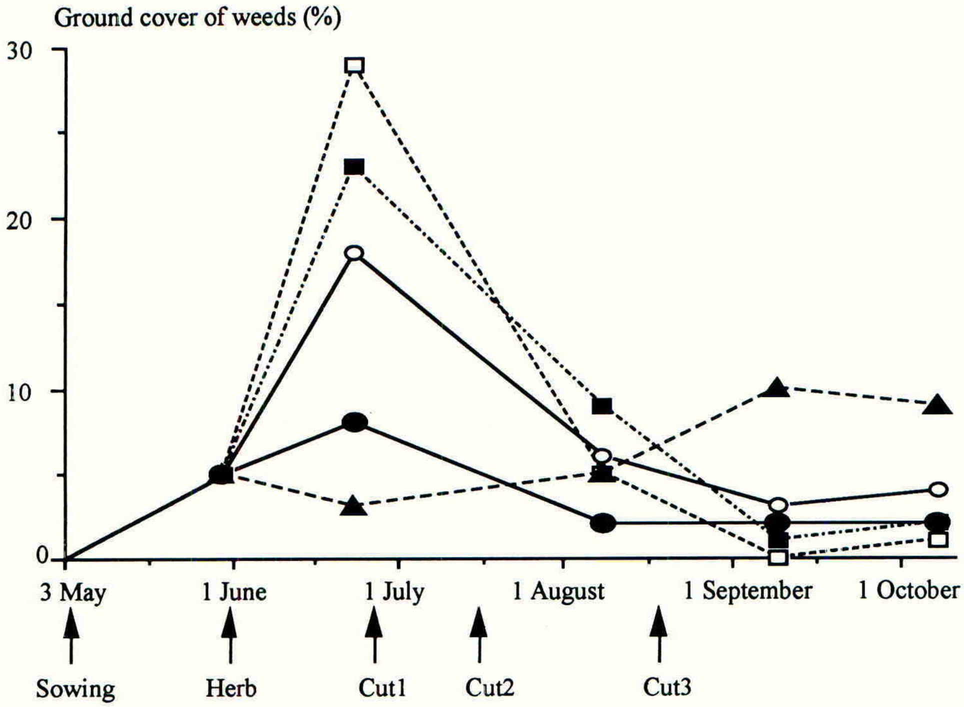
Mixtures sown after the winter.
There was no difference in weed ground cover between mixtures in May but big differences were visible in June. The growth of the fodder kale and the phacelia did not permit the development of many weeds. Although, at the end of the season, the cut which is obligatory in Belgium in the second fortnight of August resulted in the disappearance of the two covers. The soil therefore stayed bare and weeds developed (60 % ground cover of weeds in the plots of phacelia in September). The management cost of fodder kale and phacelia was lower than the other set-aside types sown in spring, but according to Clarke (1993), the phacelia, due to the production of seeds during the set-aside, risks creating problems in the subsequent crops.

Table 6. Approximate costs of different managements (FB/ha).

	Sown before winter Cutting only	Sown after winter Cutting only	Sown after winter Herbicide + cutting
Herbicide			2400
Spraying			950
Cutting	6000 [1]	4500 [2]	3000
Total cost	6000	4500	6350

1 Cost of red fescue + common bent + white clover : 4500 FB/ha (only three cuts required)
2 Cost of fodder kale and phacelia : 1500 FB/ha (only one cut required)
Cost of one cut estimated at 1500 FB/ha

The ryegrass chemically treated did not have so many weeds as the mechanical treatment, but at the end of the season, there was no difference. However, the beginning of the season is the critical period for the weeds growth; it is therefore very important to reduce the number of weeds in this period. The herbicide use on ryegrass is thus interesting from this point of view since it reduced the weed cover by half in July (Figure 2). In spite of the use of cheap herbicide (bentazone 1200 gr a.i./ha), the combined treatment (herbicide plus cutting) was still more expensive than the mechanical management by cutting only (Table 6).



Legend :

Herb : Herbicide on perennial ryegrass (bentazone 1200 g a.i./ha)

Cut1 : Cut of all plots except fodder kale and plot treated by herbicide

Cut2 : Cut of all plots except fodder kale

Cut3 : Cut of all plots

Perennial ryegrass treated by cutting

Perennial ryegrass treated by herbicide and cutting

Perennial ryegrass + White clover (20+4 kg/ha) treated by cutting

Perennial ryegrass + White clover (10+2 kg/ha) treated by cutting

Fodder kale

Figure 2. Ground cover of weeds on plots sowed after winter - Site 2

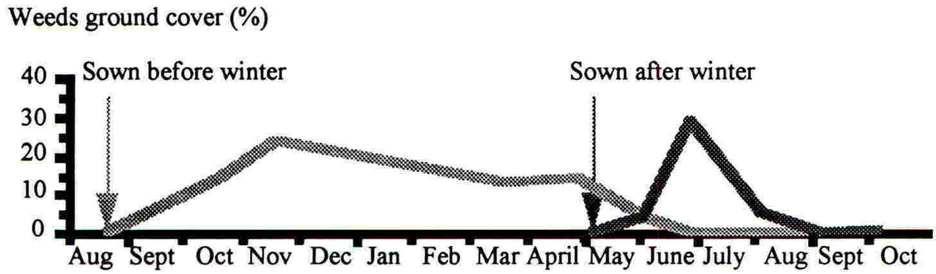
The total cost of mixtures in Site 2 (seed, management) was for mixtures 1 and 2 : 7600 FB/ha, 3 : 8080 FB/ha, 4 : 7500 FB/ha, 5 : 6100 FB/ha, 6 : 7950 FB/ha, 7 : 6580 FB/ha, 8 : 5540 FB/ha, 9 : 3300 FB/ha and 10 : 4000 FB/ha.

The reduction of seed rates of ryegrass with white clover did not lead to a greater abundance of weeds. A reduced seed rate is thus an interesting solution to decrease the cost of set-aside.

Other trials have also showed that the sowing of grasses + legumes is better than the sowing of legumes alone, as far as weeds are concerned (Lechner *et al.* 1992).

Comparison between sowing before and after winter.
The ryegrass sown before winter had less weeds after May than the same cover sown after winter. In the spring, this effect was understandable since the ground cover of ryegrass sown before the winter was thicker than ground cover of the ryegrass sown after the winter. This cover prevents the proliferation of spring germinated weeds. As a matter of fact, most of the weeds existing before winter were destroyed by the cold and by the competition with the sown cover. At the end of the season, no difference between the two sowing dates was visible. The same remarks could be made for the sowing of ryegrass plus white clover. Lechner *et al.* (1992) and Fisher & Davies (1991) came to the same conclusions.

Sowing before winter permits having the soil covered during the winter period and thus reducing the risks of soil erosion and of nitrate leaching. The sowing after winter should preferably be reserved for the late harvested land which do not permit a good establishment of cover before winter period.



Management : cutting only
Dates of cuts :
Sowing before winter : 10/5/94, 14/6/94, 15/7/94, 17/8/94
Sowing after winter : 14/6/94, 15/7/94, 17/8/94

Figure 3. Comparison of weed ground cover in the perennial ryegrass plus white clover mixture sown before or after the winter - Site 2

Figure 3 shows the evolution of weeds cover in a grass-clover mixture sown before or after the winter. The mixtures sown before winter were quite overgrown with weeds in November (about 25%). The cold and the competition with the cover sown reduced the weeds abundance during the winter. The first cut destroyed them almost completely. In the cover sown in the spring, the weed cover rapidly increased in June and was not destroyed by cutting by the end of July.

CONCLUSIONS

Sowing set-aside before winter results in having fewer weeds in the spring. The cold and the competition with the cover reduce the number of weeds during the winter and the first cut in the spring destroys them almost completely. Moreover, with the cover sown at this time, the risks of erosion and of nitrate leaching are strongly reduced. For set-aside beginning in August (after the cereal harvest) and ending in November of the next year, a cover of grasses, or even better grasses plus legumes, is perfectly suitable.

The sowing after winter permits a reduction in the number of cuts and thus the cost. The number of weeds is however higher in the sown cover and the risk of weeds seeding is higher. The phacelia and the fodder kale are two very competitive species which prevent the development of weeds but after the obligatory cutting in the second fortnight of August, they disappear. If the subsequent crop is not sown rapidly, the fields will be overgrown with weeds. Some maintain that the sowing of phacelia with clover reduced the weeds after the cutting, but it depends on the type of soil and climatic conditions.

The cover management by herbicide and cutting rapidly reduces weeds; this is an important point particularly if the cover is sown after winter. Nevertheless this treatment remains, in Belgium, more expensive than the treatment by cutting only. Whatever the treatment, either mechanical or chemical then mechanical, the weeds cover in September is the same.

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CONTROL OF BLACK-GRASS (*ALOPECURUS MYOSUROIDES*) AND BARREN BROME (*BROMUS STERILIS*) IN ROTATIONAL SET-ASIDE AND THE PREVENTION OF VIABLE SEED RETURN USING A NEW FORMULATION OF GLYPHOSATE

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ABSTRACT

Since their use was first permitted on rotational set-aside in the 1993/94 cropping year, glyphosate-based herbicides have proved to be the most widely used method of weed control. Trials have been carried out over three years to investigate the effect of dose and timing on annual grass weed control, using a new glyphosate formulation with improved safety characteristics. Later applications (late May/June) gave better control of black-grass *(Alopecurus myosuroides)* and barren brome *(Bromus sterilis)* than earlier (late April/early May) sprays, in terms of both foliage kill and viable seed production, although more biomass was produced. Early sprays reduced biomass but viable seed was produced from secondary regrowth. A sequential treatment programme controlled both biomass and viable seed production effectively. It was found that spraying black-grass after the time at which potentially viable seed is present on the plant still resulted in complete control, whereas cutting at this time would have returned viable seed to the soil.

INTRODUCTION

The current UK Arable Area Scheme was introduced in the 1992/93 cropping year. In the first season herbicides were not permitted except after 15 July, or as spot treatments, or otherwise by special derogation. This led to widespread concern among wildlife conservationists over destruction of nests and young animals by mowing or cultivation operations carried out for weed control purposes during the breeding season. Poor control of weeds, especially annual grasses and perennials, also often resulted. Weeds responded to cutting by producing further seeds closer to the ground, and up to five cuts were needed to give complete control (Clarke & Cooper, 1992), whilst early cultivation produced a further flush of weeds during the set-aside period.

New rules introduced in the 1993/94 cropping year allowed the use of non-residual herbicides, provided that the green cover was not destroyed before 15 April. This method of weed control immediately found favour with farmers; for example, in a survey carried out by the Game Conservancy Trust, herbicide use as the first management option applied to set-aside increased from 10% in 1993 (under derogation) to 72% in 1994 (Thompson, 1995). Glyphosate products have been the main herbicides used on set-aside, because of wide spectrum of weeds controlled, the favourable toxicological profile and safety to wildlife. In the Game Conservancy survey, 98% of farmers who sprayed used glyphosate-based products (P G L Thompson, unpublished data). A new formulation introduced by Monsanto offers the highest level of environmental and operator safety among the available products and also more consistent performance in terms of weed control than the standard formulation (Clemence & Merritt, 1993). This paper reports a trials programme over three seasons to determine the effect of dose and timing on the control of annual weeds. Because of the great importance of seed return from pernicious grass weeds, notably black-grass (*Alopecurus myosuroides*) and barren brome (*Bromus sterilis*), and their frequent abundance on set-aside, research was focused on the control of these species, including the viability of seed from treated and untreated plants.

MATERIALS AND METHODS

Field trials were laid out as randomised complete blocks with four replicates in farm fields under set-aside with a naturally regenerated green cover. Glyphosate as Roundup Biactive (Monsanto) was applied using small plot sprayers with flat fan nozzles. Further details are given in Table 1.

Table 1. Details of experimental sites

Year	Site	Major weed species	Plot size (m)	Volume rate (l/ha)	Spray pressure (bar)
1993	Sudbrooke, Lincs.	Volunteer wheat Barren brome Annual meadow-grass *(Poa annua)*	6x6	200	2
1994	Houghton-on-the-Hill, Leics.	Black-grass	3x6	250	2
	Loddington, Leics. (2 trials)	(a) Black-grass Annual meadow-grass Rough meadow-grass (*Poa trivialis)*	2x6	250	2.5
		(b) Barren brome Annual meadow-grass Rough meadow-grass	2x6	250	2.5
1995	Normanton, Leics.	Black-grass	3x6	200	2
	Loddington, Leics.	Black-grass	3x6	250	2.5

Foliage kill (combined chlorosis and necrosis symptoms) was visually assessed as a percentage in relation to control plots (untreated = 0, complete death = 100) at intervals after spraying. Percentage reduction of biomass (living or dead) in comparison with untreated plots was also assessed visually.

Seed samples of black-grass or barren brome were taken at random within plots during 1994 trials, on at least two occasions to minimise errors due to shedding or premature sampling. Seed samples were stored dry to allow loss of short term dormancy and germination tests done by growing seed in trays of compost from October (Moss, 1981). Seed heads were counted and head length measured for each plot, and these data used to calculate seed production per square metre, assuming an average of 1.32 seeds per mm (Moss, 1981).

Viability of seed collected on 29 July was much greater than that collected on 22 June, which was apparently too early for full maturity. Germination tests commencing in October showed similar trends between treatments, but much more rapid germination than tests commencing in August, indicating a loss of short term dormancy following a period of storage of the seed.

Data were analysed by analysis of variance, after angular (arcsine square-root) transformation of percentages.

RESULTS

Data for foliage kill in trials where there was a wide spread of spray dates are given in Tables 2 and 3. Better control of both species resulted from later application (Table 2). Complete foliage kill of barren brome was achieved from rates as low as 90g a.e./ha applied on 3 June.

Table 2. Mean percentage foliage kill, 29 days after spraying, of barren brome and volunteer wheat at Sudbrooke in 1993. (Means within columns followed by the same letter do not differ significantly at $P<0.05$; Duncan's Multiple Range Test after angular transformation)

	Barren brome		Volunteer wheat	
Spray date	5 May	3 June	5 May	3 June
dose (g a.e./ha)				
90	-	100	-	95 b
180	87 b	100	81 c	99 a
270	93 ab	100	96 b	100 a
540	99 a	100	99 ab	100 a
1080	100 a	100	100 a	100 a

There was a trend for control of black-grass to increase as application date became later, although statistical comparisons are difficult because assessments were carried out at different times for different spray dates (Table 3). At the Houghton site, where later assessments were made, there was evidence of recovery from earlier application. Later applications also had a more rapid effect at Loddington in 1995 (Figure 1). Application on 14 June had a significantly greater effect ($P<0.001$) seven days after treatment than earlier applications. At 10 and 21 days after treatment, there were significant differences between all timings averaged over the two doses ($P<0.001$), but at 28 days after treatment there was no significant difference between the June applications, though control by the 14 May application was still lower ($P<0.001$).

Table 3. Mean percentage foliage kill of black-grass

(a) Houghton-on-the-Hill, 1994

Dose (g a.e./ha)	540			1080		
Days after spraying	14-16	30	45-51	14-16	30	45-51
Spray date						
8 May	51.3	86.3	45.0	-	-	-
8 June	71.3	80.0	58.8	96.3	99.0	98.4
30 June	-	-	-	99.3	100	-

(b) Normanton, 1995

Dose (g a.e./ha)	540		1080	
Days after spraying	12-21	29-35	12-21	29-35
Spray date				
21 April	43	85	63	95
9 May	96	96	100	100
1 June	88	100	91	100
14 June	99	100	100	100
29 June	100	-	100	-

Biomass

Generally, biomass was much lower following early applications than later ones, which may be preferable to ease following cultivation, though the increased cover following later applications is beneficial to nesting birds, including game species. Where it is desirable to keep biomass low, a sequential treatment of two applications was found to reduce early growth and also gave a high level of final control at the Normanton site in 1995 (Figure 2).

A dose of 540 g a.e./ha on 15 April reduced biomass to 70% of untreated by 17 July ($P<0.001$), but plants recovered so that the score for foliage kill on 17 July (Figure 2) was zero. The split application achieved the same reduction in biomass but also 100% kill, equivalent to a single application in mid-June. Applications at 540 or 1080 g a.e./ha in mid-June did not significantly reduce biomass by 17 July.

Production of viable seed

The earliest date on which viable seed was produced was recorded at Loddington in 1993 and 1994, and also for some samples collected on set-aside in Hampshire in 1993 (Table 4).

In the 1994 trial at Loddington, seedheads emerging at three different times were marked as they reached Growth Stage 55 (ear 50% emerged; Tottman, 1987); the first batch of ears emerging on 6 May (black-grass) or 9 May (barren brome). These early-emerging ears took longer to mature and achieved much lower levels of germination on untreated plots than those harvested later (Figure 3).

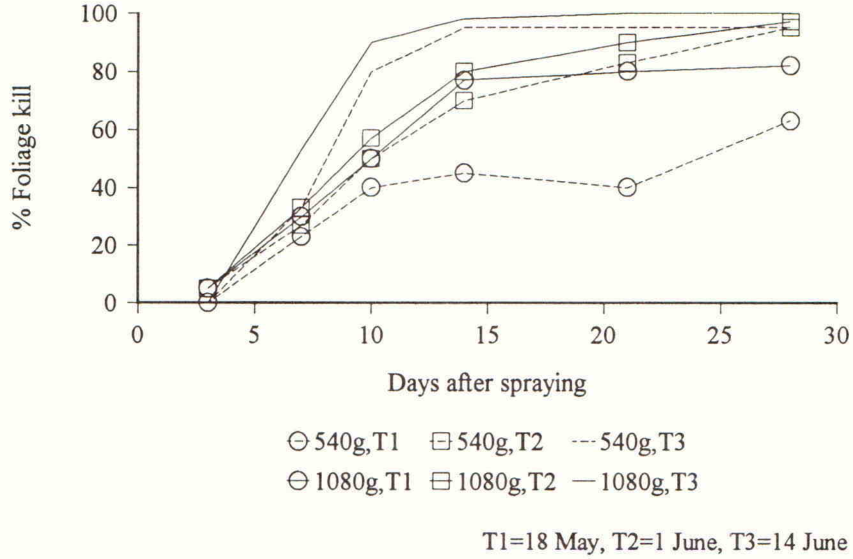

Figure 1: Percentage foliage kill of black-grass, Loddington 1995

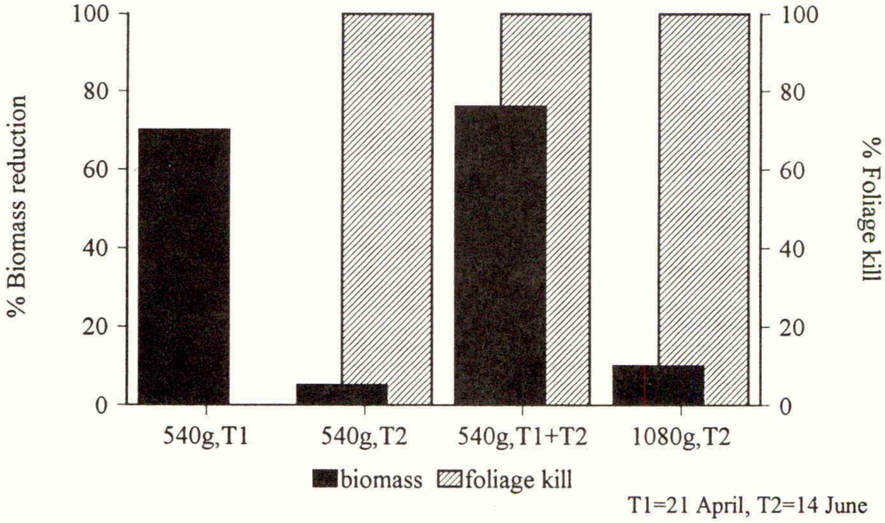

Figure 2: Foliage kill and biomass reduction (as percentage of untreated on 17 July) of black-grass, Normanton 1995

Table 4. Dates on which first viable seeds of black-grass and barren brome were recorded.

		Black-grass	Barren brome
1993:	Hampshire	18 May	24 May
	Leicestershire	24 May	7 June
1994:	Leicestershire	24 May	11 June

Effect of glyphosate on seed germination

Table 5. Effect of glyphosate dose and timing on seed germination percentage of black-grass, Houghton-on-the-Hill, 1994.

Date applied	Dose (g a.e./ha)	Heads/m^2 22 June	Seed numbers '000/m^2	Seed Viability %	Viable seeds '000/m^2
Untreated		1890	180.8	41	74.1
8 May	540	1005	48.0	76	36.5
8 June	540	1380	104.8	13	13.6
8 June	1080	1350	109.3	0	0
30 June	1080	2065	170.3	0	0

On plots treated in May, plants recovered and produced secondary tillers which formed seed with a percentage germination at least as high as seeds from untreated plots (Table 5). However, seeds from later-treated plots, where most seedheads had emerged by the time of spraying, had lower germination. A dose of 540 g a.e./ha of glyphosate applied in early June gave an 82% reduction in numbers of viable seeds, whilst application of 1080 g a.e./ha at any time in June completely prevented seed germination.

DISCUSSION

The choice of natural regeneration as the green cover on rotational set-aside offers an economic and environmentally favourable management option (Myers & Parish, 1994). However, good control of weeds is essential to avoid serious problems in returning the land to a cropping rotation, and excessive use of herbicides in following crops. Glyphosate offers excellent control of problem weeds, including annual grasses. The use of glyphosate to give complete control of black-grass, including populations resistant to selective herbicides (such as the substituted urea, aryloxyphenoproprionate and cyclohexanedione groups), offers a major opportunity as part of a programme to control this weed (Moss & Clarke, 1994). For example, subsequent analysis revealed that black-grass at the Houghton-on-the-Hill site was highly resistant to fenoxaprop, and only slightly resistant to isoproturon.

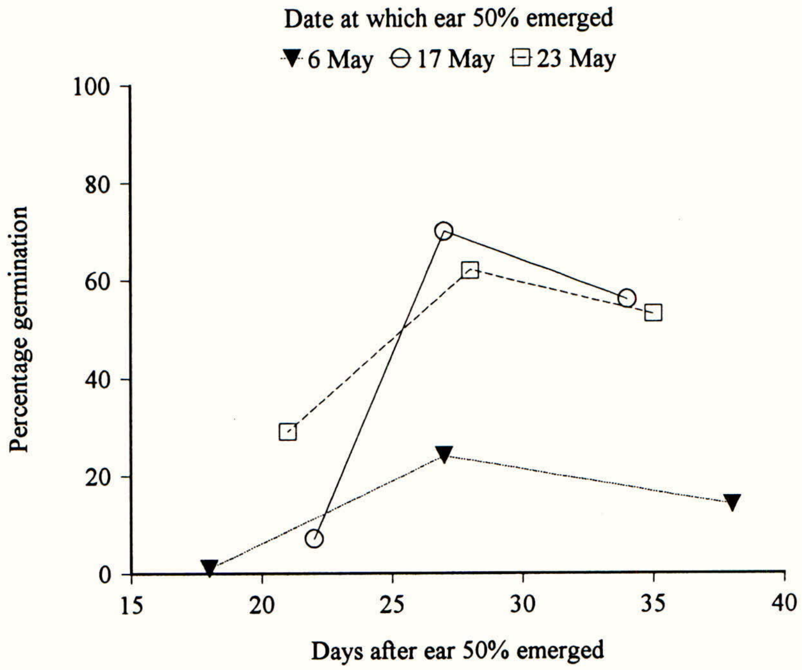

Figure 3a: Percentage germination of black-grass, Loddington 1994(a)

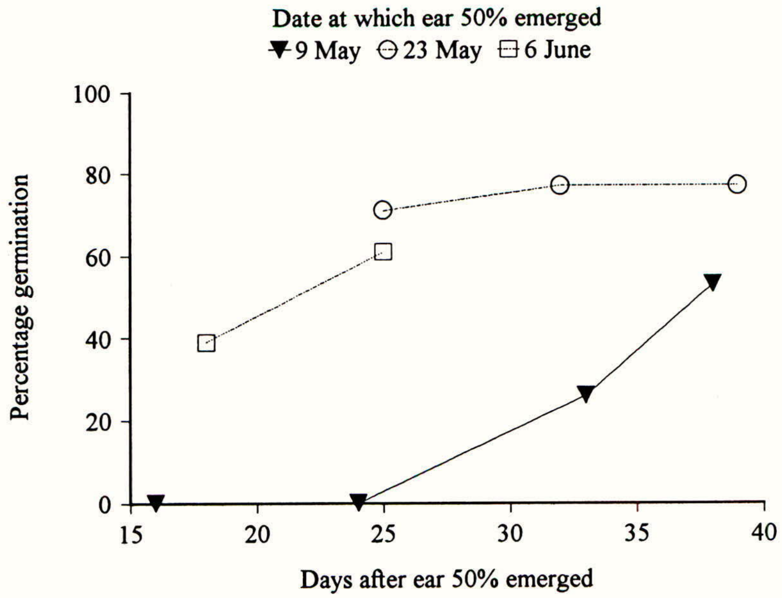

Figure 3b: Percentage germination of barren brome, Loddington 1994(b)

Timing and dose are key to the successful use of glyphosate on set-aside. Early timings (late April to Mid May) gave good reduction in biomass which may help subsequent cultivation and trash burial, but annual grasses in particular, such as black-grass and barren brome, are capable of regrowth from early sprays, depending on seasonal conditions. Use of a higher dose at these times did not ensure complete control. Later timing (mid May to late June in these trials) gave better control of annual grasses and volunteer cereals, and most importantly gave the best control of viable seed return. Indeed, the data indicate, at least for black-grass, that spraying after potentially viable seed is present on the plant still gave complete control. If cutting had been carried out instead, the seed would be capable of germination in the following crop.

Later timing of glyphosate also allows maximum use of set-aside by wildlife, including game species, as breeding cover and feeding areas (Sotherton *et al.*, 1994). In this respect the very favourable safety profile of the formulation used in this study was a major consideration.

ACKNOWLEDGEMENTS

Thanks are due to Leena Malik, Kirsten Whittaker and Sally Evans for technical help with the Loddington trials.

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Session 4C

Post Graduate Student Posters

Session Organiser Mr J Smith

Poster Papers 4C-1 to 4C-5

BIOLOGICAL WEED CONTROL IN ARABLE HEDGEROWS.

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ABSTRACT

Spatial relations between plants can give important information about positive and negative associations. Such associations may be due to allelopathy or competition. This study assesses plant distributions along arable hedgerows to identify species which may have these capabilities. The results are discussed in the context of biological weed control.

INTRODUCTION

Hedgerows have been considered to be problem structures as they may harbour agricultural pests (Marshall *et al.*,1987), diseases and, weeds. Nowadays, their value to wildlife and their benefits in providing shelter (Brooks *et al.*,1986) has been recognised. Consequently, hedgerow removal in the UK is reducing and grants are being provided to encourage new hedgerows to be established (Chapman,1994). Additionally, hedgerows are being structured to attract beneficial invertebrates that act as as an effective biological control against weeds, pathogens and pest invertebrates (Sotherton, 1984).

Hedgerows and field margins may provide a suitable habitat in which wild plants may use competitive strategies such as allelopathy and resource competition to remove agricultural weeds.This study has investigated the spatial distribution of weeds in new and established hedgerows. Hence, it will determine if plants have positive or negative associations with other species, and have the potential to act as a biological weed control against species.

MATERIALS AND METHODS

Two hedgerows of *Prunus spinosa* were established at Stockley Farm in Cheshire in late 1994. One was 180m long separating *Lolium perenne* from Setaside; the other 360m long separating *L. perenne* from *Zea mays.* Line transects were placed at 20m intervals across both lengths of hedgerow. Quadrats were then placed along these transects at 1m, 3m, 5m, 7m, 9m and 11m intervals away from the hedge. Data collected from the quadrats included species composition and percent cover. This data was examined using chi squared analysis.

RESULTS AND DISCUSSION

Preliminary results demonstrate significant negative associations between *Polygonum spp.* and other plants. *P. persicaria* and *P. aviculare* show a negative association with *Matricaria perforata*. This may result from preferential soil conditions for *Polygonum spp.* allowing them to dominate an area. Alternatively, it may be as a result of one species having an inhibitory effect on another.Both *P. persicaria* and *P. aviculare* are propagated by seed but have an aggressive ruderal habit which results in large expanses of ground cover (Grime,1979). However, *M. perforata* is the first to appear in the season and both of the *Polgonum spp.* studied were found mainly in positions other than those previously or presently occupied by *M. perforata* which may therefore be inhibiting either germination or growth of the other species.

Table1. Association between weed species in newly planted hedgerows using chi squared analysis

		Species					
		A	B	C	D	E	F
Species	A	*	0.937	(0.01)	0.918	(0.001)	0.524
	B	0.937	*	(0.001)	0.191	0.945	(0.018)
	C	(0.01)	(0.001)	*	0.613	0.604	0.210
	D	0.918	0.191	0.613	*	0.945	0.648

Each point shows the probability that any two species are not associated in hedgerow quadrats (n=670). Figure in brackets represents significant values where p=0.005.

KEY
A, *Polygonum aviculare*, B, *Polygonum persicaria*, C, *Matricaria perforata*
D, *Cirsium arvense*, E, *Viola tricolor*, F, *Galeopsis segetum*

A similar negative relationship could be seen between *P. avicular* and *Viola tricolor*. Viola also appears first in the season and could be exhibiting a similar effect to that of *M. perforata*.

ACKNOWLEDGEMENTS

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THE EFFECT OF HERBICIDE SAFENERS ON CHLOROTOLURON SUSCEPTIBLE AND RESISTANT BLACK-GRASS (*ALOPECURUS MYOSUROIDES*).

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ABSTRACT

Glutathione S-transferase activity in black-grass plants that are resistant to chlorotoluron was at least twice that in susceptible ones. Treatment with the herbicide safeners flurazole, fenclorim and benoxacor further elevated this enzyme activity. Furthermore, benoxacor may be able to reduce chlorotoluron toxicity in both biotypes.

INTRODUCTION

Chlorotoluron (CTU) resistant black-grass (*Alopecurus myosuroides* Huds.) originated in the UK in Peldon, Essex and has since spread to affect over 70 farms (Moss & Clarke 1994). There is evidence that resistance is due to enhanced CTU metabolism by the cytochrome P-450 dependant monooxygenases (Caseley *et al* 1990), however the possible role of other detoxification enzymes has yet to be established. The aim of this study was to investigate glutathione S-transferase (GST) activity in susceptible and resistant biotypes, for which the ED_{50} values (dose required to reduce fresh weight by 50%) have already been established, giving a resistance factor of 35 (Sharples *et al* 1995).

MATERIALS AND METHODS

Susceptible (Herbiseed Ltd, UK) and resistant (Peldon, UK) plants were grown under glasshouse conditions, and all chemicals were applied at the 2-3 leaf stage. CTU was sprayed at field rate (3.5 Kg ai ha^{-1}) using a Mardrive evaluation unit (fixed 80^0 flat fan tip, 200 l ha^{-1}, 3 bar pressure). To allow the delivery of safeners, plants were transplanted into shallow boxes containing a nutrient solution. The safeners fenclorim, flurazole and benoxacor were dissolved in DMSO and then added to 100 ml of nutrient solution such that the final concentration was 100 μM, and final DMSO concentration was 0.5% (v/v), which did not alter GST activity. All plants were harvested 24 hours after treatment. GST activity was measured using CDNB (1-chloro-2,4-dinitrobenzene) as the substrate (Sharples *et al* 1995) and expressed on a protein basis. Photosynthesis was measured using an infra-red gas analyser (LCA4, ADC Ltd, Herts, UK).

RESULTS AND CONCLUSIONS

The results in Table 1 show that the endogenous level of GST activity in resistant plants was more than twice that in susceptible biotypes when CDNB was used to assay GST. Chlorotoluron had no effect on GST activity.

Table 1. The specific activity of GST measured 24 hours after plants had either been sprayed with field rate CTU or received 100 µM flurazole, fenclorim or benoxacor, means ± SE (n = 2 or 3).

Biotype	GST specific activity (µmol h^{-1} mg^{-1} protein)				
	control	chlorotoluron	flurazole	fenclorim	benoxacor
Susceptible	4.24 ± 0.94	4.92 ± 0.72	7.37 ± 0.83	6.54 ± 0.75	10.50 ± 2.19
Resistant	11.69 ± 2.25	9.41 ± 1.16	14.00 ± 3.32	16.65 ± 1.18	18.27 ± 0.13

Benoxacor appeared to be the most effective at elevating GST activity, causing a 2.5 fold increase in susceptible plants and a further 1.5 fold increase in resistant plants. In additional experiments plants grown in hydroponics were pretreated with benoxacor for 24 hours (which had no effect on photosynthesis) and then dosed with CTU at 2.4 µM. Photosynthesis was measured to assess CTU toxicity 24 hours later. Pretreatment with benoxacor reduced the decline in photosynthesis caused by CTU (Table 2). The increase in GST activity due to benoxacor correlated with the reduction in chlorotoluron toxicity, thus GST may be involved in CTU detoxification.

Table 2. Carbon dioxide uptake by black-grass, means ± SE, n = 5 leaves.

Biotype	Carbon dioxide uptake (µmol m^{-2} s^{-1}) 24 hours after 2.4 µM CTU		
	control	chlorotoluron	benoxacor + CTU
Susceptible	12.00 ± 0.78	3.88 ± 0.28	5.86 ± 1.58
Resistant	14.22 ± 1.79	9.29 ± 0.77	12.51 ± 0.67

ACKNOWLEDGEMENTS

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THE EFFECT OF WHEAT DWARFING GENES ON COMPETITION AGAINST BLACKGRASS

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ABSTRACT

The effect of dwarfing genes Rht1 and Rht2 on the competitive ability of Maris Widgeon isolines against blackgrass was investigated in field plots sown at two dates, with and without grazing in spring. Sowing in November reduced blackgrass numbers compared to September. Dwarfing genes increased the number of blackgrass heads and mean head weight but this latter effect interacted with grazing.

INTRODUCTION

The competitive ability of wheat (*Triticum aestivum* L.) varieties against weeds is particularly important in systems where the use of synthetic herbicides is restricted. Competitive ability has sometimes been related to variety height, early dry matter production and ground cover although such relationships are often weak or not detectable (Gooding *et al.*, 1993). To aid clarification isolines of a tall variety, cv. Maris Widgeon, differing in the presence of Rht dwarfing genes were grown in a field experiment without the use of herbicides. The relative importance of dwarfing genes for controlling blackgrass (*Alopercurus myosuroides*) was assessed within other potential non-chemical weed control methods such as delayed sowing, and grazing the wheat in the spring.

MATERIALS AND METHODS

A field experiment was conducted at Harnhill Manor Farm, Gloucestershire, UK. in 1994/5 on Soil Association approved land for organic production. Two randomised blocks were divided into grazing main plots 25 x 12m (either grazed with 42 x 50kg sheep ha between 22 and 24 March or not grazed) which were further split into sowing date plots 12.5 x 12m (either sown on 29 September or 15 November). Within each sowing date plot four lines of Maris Widgeon differing in dwarfing genes (rht, Rht1, Rht2 and Rht1+2) were each sown in 1 x 2m plots (kindly supplied by Dr. J E Flintham, Cambridge Laboratory, Norwich, UK.). Crop height was measured at three random locations per plot using a polystyrene 350cm^2 rising disc apparatus on 10 July. All blackgrass plants from each plot were harvested on 9 July. The number of blackgrass heads per plot, the length of ten randomly selected heads and length of fifteen randomly selected stems was recorded. The samples were then dried at 85°C for 36 hours and the dry weights recorded. Statistical analysis was restricted to the September sown plots as levels of blackgrass were very low in the later sowing, contributing to excessive variance heterogeneity. A factorial, split plot analysis of variance was then applied to the remaining data and standard errors of the difference between means (SED) calculated.

RESULTS AND DISCUSSION

Average blackgrass head densities were reduced from 55 to 3.4 m^{-2} by sowing in November compared to September. For September sown plots there was a significant ($P<0.05$) increase in blackgrass heads per m^{-2} with Rht1 from 36.2 to 73.9 (SED=13.5). There was a similar increase with Rht2 from 39.4 to 70.8 but this was not quite significant ($P=0.058$).

Table 1. Interactions between dwarfing genes and grazing on head weight of blackgrass

	Mean head *wt* (g d.m.)		SED(a)
	No grazing	With grazing	
	Rht1 (averaged over Rht2 treatments)		
No Rht1	0.08	0.13	0.139
With Rht1	0.11	0.12	
	Rht2 (averaged over Rht1 treatments)		
No Rht2	0.07	0.13	0.139
With Rht2	0.13	0.12	
SED(b)	0.024		

SED(a) is for Rht means within grazing treatments, SED(b) is for grazing means within Rht treatments

There was a significant interaction between Rht2 and grazing on mean head weight whereby both treatments increased weights individually but effects were not additive (Table 1). A similar Rht1 x grazing interaction was apparent (Table 1) but not significant ($P=0.073$). There were no significant effects on blackgrass stem or head length. The presence of Rht1 and Rht2 each seperately reduced wheat plant height by 15cm (SED=1.41). There was a synergistic reduction in plant height (42cm) when both genes were included as evidenced by a significant ($P<0.001$) Rht1 x Rht2 interaction.

The results demonstrate that dwarfing genes alter the ability of wheat to compete with blackgrass with an increase in wheat height being associated with a decrease in the number of blackgrass heads and, in some circumstances, in mean head weight. This effect was small compared to apparent sowing date influences but large compared to grazing in spring which was ineffective at reducing blackgrass head production and increased mean head weight.

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INTRA- AND INTERSPECIFIC COMPETITION AMONG BROMUS SPECIES AND WINTER WHEAT

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ABSTRACT

A field experiment was designed to investigate intra- and interspecific competition among populations of *Bromus sterilis*, *B.diandrus* and *B.mollis*. In the absence of interspecific competition from winter wheat, height, fresh weight, panicle number and size of *B.sterilis* was not significantly different between the low and high density. Seed production per panicle was not significantly different at either low or high density of *B.sterilis* and *B.diandrus* in presence or absence of the crop. In the presence of interspecific competition, density of *Bromus spp.* was largely unaffected, but panicle number and size were significantly reduced whereas stem height was increased. Seeds per panicle of *B.mollis* were significantly reduced in presence of winter wheat. Seed production/m^2 in presence of interspecific competition was not significantly different for the three brome species.

INTRODUCTION

Bromus spp. occur as weeds in cereal crops where they lead to a reduction in crop grain yield and increased crop lodging.
The aim of the experiment was to investigate intra- and interspecific competition among populations of *B.sterilis*, *B.diandrus*, *B.mollis* and winter wheat.

MATERIALS AND METHODS

An additive design field competition experiment was conducted between autumn 1993 and summer 1994 at the University of Reading experimental grounds. Three *Bromus spp.* were used, *B.sterilis*, *B.diandrus* and *B.mollis*, and winter wheat, *cv.* Avalon, as the crop. Winter wheat was sown on 4th November 1993 in rows 10cm apart achieving a seed rate of 400 grains / m^2. *Brome* was broadcast at a low density (50 seeds / m^2) and a high density (200 seeds / m^2) in the presence and absence of the crop. Seed production was determined by bagging ten panicles / plot and counting number of seeds. Final harvest took place on 5-8th of September 1994.

RESULTS

Weed density was greater at the higher seed rate but not significantly different if the crop was present. Seedling establishment was significantly less for *B.mollis* compared with the other two *Bromus spp.* with only 50% of sown seeds resulting in established plants at final harvest. *B.sterilis* had greatest establishment.

Mean height in absence of winter wheat decreased in the order *B.sterilis* > *B.diandrus* > *B.mollis*. Plants were significantly shorter at low density than at high density except for *B.sterilis* and height was significantly increased at both densities in the presence of winter wheat.

Number of panicles was significantly greater at high compared to low density for *B.diandrus* but not significantly different for *B.sterilis* and *B.mollis*. The presence of winter wheat led to a significant reduction in panicle number for the three *Bromus spp*. At low density the order was *B.sterilis* > *B.diandrus* > *B.mollis* whereas at high density the order was *B.sterilis* = *B.diandrus* > *B.mollis*.

In the presence of interspecific competition, there was a substantial reduction in brome panicle weight, especially for *B.mollis* with the least value occurring at low density in winter wheat.

There was no significant difference in seed number/panicle (mean 75 seeds/panicle) if *B.sterilis* and *B.diandrus* were growing at either a low or high density in presence or absence of winter wheat. However, *B.mollis* seed number per panicle was severely reduced by the presence of the crop, from 300 to 100 seeds / panicle (Table 1).

Table1. Brome height(Ht), panicle number(pano) and seeds/panicle(se/pa).
Original data for pano and se/pa are in brackets.

		Nature of competition					
		Interspecific			Intraspecific		
		Ht (cm)	pano (log10)	se/pa	Ht (cm)	pano (log10)	se/pa
B.sterilis	Low	94.4	2.19(163)	1.86(75)	92.1	2.76(575)	1.93(89)
	High	92.7	2.53(358)	1.69(52)	89.2	3.05(1125)	1.73(55)
B.diandrus	Low	81.0	1.89(79)	1.95(91)	65.3	2.73(559)	2.01(104)
	High	87.3	2.60(403)	1.86(73)	77.9	3.03(1079)	2.03(108)
B.mollis	Low	50.5	1.59(53)	2.10(134)	36.6	2.72(546)	2.48(311)
	High	55.6	2.13(137)	1.94(91)	41.8	2.98(958)	2.47(300)
	LSD(5%)	3.75	0.30	0.13	3.75	0.30	0.13

DISCUSSION

Weed density was not reduced in presence of the crop but the reproductive output, i.e. the final seed production was reduced. Intraspecific competition between brome does occur but is less important compared to interspecific competition with the crop. Brome shows a plastic response with higher number of tillers/plant at low density whereas at high density tiller number on a per plant basis was reduced. *B. mollis* was the least competitive brome *sp.* in presence of the crop and is most negatively affected in terms of panicle number, panicle weight and seed number per panicle. Owing to the transient seed bank nature of *Bromus spp.*, the extent of seed production will determine the potential infestation of the next crop.

THE IMPACT OF INTEGRATED FARMING SYSTEMS ON THE ARABLE WEED FLORA.

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ABSTRACT

Integrated weed management has become more widely practised as reduced input systems of farming have gained acceptance. This study compares the effects of three factors *viz.* sowing date, cultivar and nitrogen on weed biomass and weed species composition in winter wheat.

INTRODUCTION

Interest in reduced input and lower cost systems of crop production has resulted in a number of national projects being undertaken to evaluate and define integrated crop production systems. This study is in addition to the LINK integrated farming systems (IFS) project enabling validation in small plot trials of methods used on the six site on-farm investigations.

MATERIALS AND METHODS

The experiment was designed to investigate the effects of sowing date on two cultivars of wheat at two levels of nitrogen application. Winter wheat cvs. Tonic and Spark were sown at a seed rate of 350 seeds/m^2 in plots 1.8x6m at the University field unit at Shinfield near Reading on 22 September and 13 October 1994. Fertiliser as 'Nitram' was applied as a split application in spring. Each plot received either 160 or 80 kg N/ha which was the full and half recommended rate for the site. The treatments were replicated four times in a randomised block design. Assessments were taken once in the autumn of 1994 and then monthly from March to June 1995 inclusive. Samples of above ground material were collected from one quadrat of 400 cm^2 per plot. Samples were separated by species and the number and fresh weight of each were recorded before dry weights were obtained for both weed and crop.

RESULTS AND DISCUSSION

Weed biomass

Significant differences in the amount of weed present were found between the two sowing dates. The earlier sowing date was more heavily infested with weeds than the later sowing date. At sample dates in November and March differences were significant at the 1% level, this dropped to the 5% level in April and were not significant from May onwards. This effect of sowing date is well known and is due to later drilling cultivations removing the weeds already present but these remain in earlier sown crops. Differences between the sowing dates diminished as the season progressed and the developmental stage of the two crops became similar. No significant differences in weed biomass present were found between the cultivars or levels of fertiliser used and no significant interactions of the treatments were observed.

Weed composition

The percentage fresh weight contribution of each species is presented in Table 1 for the March sampling. Since fertiliser had only just been applied it was considered not to have affected the weeds present.

Table 1. Percentage contribution of weed species groups to total fresh weight per plot.

Sampling date	Sowing date	Cultivar	*Stellaria media*	*Lamium* spp.[1]	*Veronica* spp.[2]	Other spp.[3]
20 March 1995	Early	Spark	30.31	51.38 a	14.40 c	3.91 e
	Early	Tonic	17.86	47.33 a	33.98 d	0.84 e
	Late	Spark	46.14	3.52 b	13.08 c	24.76 f
	Late	Tonic	17.82	2.77 b	59.10 d	20.31 f

[1] *Lamium* species recorded were *L. purpureum*, *L. amplexicaule*, and *L. album*

[2] *Veronica* species recorded were *V. persica* and *V. hederifolia*.

[3] Other species included *Urtica urens*, *Fumaria officinalis* and *Viola arvensis*.

Column means not followed by the same letter are significantly different at $p<0.01$ for *Lamium* and *Veronica* spp. and $p<0.05$ for the other spp. (data arc sine transformed).

A greater proportion of weed fresh weight was represented by *Lamium* spp. at the earlier sowing date compared to the later one indicative of a marked periodicity for germination in September. There was no apparent difference between cultivars. The proportion of biomass represented by *Veronica* spp. demonstrated a clear cultivar effect. The cultivars had very different growth habits with Spark being prostrate with good early ground cover whilst Tonic was more erect in habit early in the season. This indicates that *Veronica* spp. may be susceptible to light competition. No significant differences between cultivars or sowing date were seen for *Stellaria media*. Later assessments did not show a response to applied fertiliser.

Sowing date greatly affected weed biomass. Delayed sowing reduced weed biomass and this persisted until April. Choice of cultivar and sowing date affected weed flora composition but did not affect total weed biomass suggesting that compensation occurred.

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