THE IMPACT OF HERBICIDE TOLERANT OILSEED RAPE IN SELECTED AGRO-ECOSYSTEMS

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ALS	Acetolactate synthase
a.i.	Active ingredient
BAP	Biodiversity action plan
BRIGHT	Botanical and Rotational Implications of Growing Herbicide Tolerant Crops
°C	Degrees celsius
cv.	Cultivar
d.f.	Degrees of freedom
DNA	Deoxyribonucleic acid
e.g.	For example
Fpr	F-probability
g	Grammes
Glufosinate	Glufosinate ammonium-phosphinothricin
GM	Genetically modified
GMHT	Genetically modified herbicide tolerant
GS	Growth stage
ha	Hectare
IPL	Inverse power law
L/ha	Litres per hectare
LSD	Least significant difference
m	Metres
m^2	Square metre
MAFF	Ministry of agriculture fisheries and food
m.s.	Mean squares
n	Number of observations
NE	Negative exponential
NL	National List
REML	Residual Estimates by Maximum Likelihood
s.e.	Standard Error
s.s.	Sum of squares
µg/l	Micrograms per litre
%vaf	Percentage variance accounted for
v.r.	Variance ratio

ABSTRACT

A range of field studies of cross pollination between herbicide tolerant and conventional oilseed rape crops and plots were conducted to demonstrate the effects of variety, distance, pollen source and sink size and intervening crop on levels of outcrossing. Experiments investigating the influence of variety on outcrossing showed that hybrid oilseed rape varieties containing high proportions of male sterile plants (varietal associations) were pollinated at higher frequencies than standard fully fertile varieties. Studies conducted using various sizes of genetically modified herbicide tolerant (GMHT) pollen sources showed that small GMHT feral populations cross pollinated with crops in close proximity and the levels of contamination obtained depended on the genotype of the conventional crop. Long range cross pollination of male sterile oilseed rape receptor plots showed that pollination events were measurable at up to 600m from the large GMHT pollen source.

Results from a study of cross pollination between mixed populations of GMHT oilseed rape plants and conventional varieties also demonstrated that a hybrid rape type (varietal association) was cross pollinated at considerably higher levels than an open pollinated and a fully restored hybrid variety. Evidence gathered in the experiment suggested that, over a wide range of initial GMHT contamination rates, the final proportion of GMHT seed in the total population was a constant fraction of the initial contamination rate.

Outcrossing data was used to compare negative exponential and inverse power law models for their fit to describe the observed relationship between cross pollination and distance from source. Results showed that the inverse power law provided a better fit of the data. This demonstrated that dispersal described by the inverse power law was more likely to lead to cross pollination at both near and large distances from the pollen source compared to the negative exponential model. The consequences of the likely ecological behaviour of GMHT traits resulting from the dispersal curves for regulation and risk assessment are discussed.

The effect of the herbicides used in herbicide tolerant and conventional oilseed rape on weed populations were compared in a single season. Results suggested that the herbicides have different activity spectra thus resulting in a variety of surviving weed species in HT treatments. The change in active ingredient and the timing of herbicide application in HT winter oilseed rape crops will likely cause a change in the weed species that are being controlled or those that escape treatment. Levels of weed biomass recorded prior to harvest of the oilseed rape crop showed that there may be differences between treatments in terms of the quantity of seed returned to the seedbank from the range of weed species present. Limited data on the behaviour of herbicide tolerant volunteers showed that single and putative double-tolerant plants were as susceptible as conventional oilseed rape volunteers to normal selective herbicides used in cereal crops.

Data from a number of elements of the studies on GM contamination rates, weed control, and seed bank estimates were used to develop a simple population projection model. The model used a Markov process to examine the fate of volunteer and feral populations of oilseed rape comprising a mixture of conventional and herbicide tolerant types. Results from the model indicated that the prevalence of the GMHT trait in the weed or feral population was more sensitive to the efficacy of control practices used in the rotation than the levels of cross-pollination and competition between the herbicide tolerant and conventional varieties in the mixed population. Thus, although the cross-pollination studies suggested that varietal associations are more likely to be cross-pollinated by GM pollen than fully fertile varieties, the projection model suggested that resulting differences in the prevalence of the GM trait in volunteer and feral populations may not be very large. Population projections from the model are compared with results from other modelling studies which have used more complex simulation approaches.

1. INTRODUCTION

1.1 Weed control and the development of herbicide tolerant crops

Weed control became a major part of agronomy, botany, horticulture and plant physiology in the 1950s, when synthetic organic herbicides became widely available for the first time (Timmons, 1970). The 'herbicide era', a period between the end of the second world war and the late 1970s brought about a high expectation that herbicides represented the 'final solution' for controlling weeds (Mortensen, Bastiaans, Sattin, 2000). Although weed management is still dominated by the use of herbicides, there are indications that this may change in the future. In agriculture today, weeds are the main factor causing yield reductions, if measured by the effort used for their control and by global agrochemical sales (Powell and Jutsum, 1993). In the UK, fungicides accounted for 35% of the total area treated with pesticides in arable farm crops in 1998 and herbicides and desiccants accounted for 33%. In contrast, herbicides and desiccants accounted for 70% of the total weight of pesticide active ingredients applied to arable crops and fungicides accounted for 14% (Garthwaite and Thomas 1999).

Although weed control is still dominated by the use of herbicides in most of the important agricultural areas of the world, recently, sustainable systems of crop production have been developed where there is less complete reliance on herbicide use. Sustainable systems of crop production use a mixture of chemical, biological and mechanical methods to control weeds, pests and diseases to provide stable long term protection to the crop (Liebman and Davis, 2000; Muller-Sharer, Scheepens, Greaves, 2000). The development of sustainable integrated systems of pest management is mainly being driven by the increasing occurrence of herbicide resistant weed species (Mathews, 1994; Powles, 1997) and concern about environmental and food safety impacts of herbicides (Matteson, 1995). Where herbicides are still relied upon as the main tool for weed control, technological improvements are being made to maximise efficacy and minimise environmental impact, by improving application technology (Jensen, 1999; Lutman and Perry, 1999), application timing (Bond and Burston, 1996; Blair, Cussans, Lutman, 1999), using factor adjusted

dosages (Steckel, Deflice, Sims, 1990; Ketel, 1996; Salonen and Jaakkola, 1997) and developing herbicides with low environmental impact (Rasche, Cremer, Donn, Zink, 1995; Wells, 1995; Moll, 1997).

Herbicides have traditionally been designed for their efficacy in weed control as well as their effects on crop plants. Selective herbicides normally control only part of the weed species spectrum associated with a particular crop, which may lead to using additional chemicals or cultural practices to achieve acceptable levels of weed control. The utilisation of biotechnological techniques to incorporate herbicide tolerance into crops has enabled herbicides to be selected for their efficient weed control properties, environmental safety and economic acceptability.

The deployment of herbicide tolerant crops could further contribute to reducing chemical inputs into farming systems by utilising less environmentally damaging herbicides (Madsen and Jensen, 1995; Read and Ball, 1999a) and enabling improvements to be made in application timing (Madsen and Jensen, 1995; Moil, 1997; Read and Ball, 1999b). However, the repeated use of broad spectrum herbicides in HT crops raises further concerns, such as depletion of certain weed species from farming systems, selection for weed resistance to new herbicides, spread of resistant volunteer crops and transfer of tolerance genes to closely related species (Darmency, 1996). Plants with herbicide tolerance transgenes constitute the first major introduction of GM plants in Europe on which decisions about risk to the environment and agronomic management must be made.

1.2 Herbicide Tolerant Crops

Herbicide tolerant crops could provide a range of benefits and also additional disadvantages when compared with selective herbicides. Some of the potential advantages to crop production using this technology include; the development or use of existing herbicides with less persistence in the environment (Burnside, 1992), low mammalian toxicity (Dekker and Duke, 1995), using herbicide chemistry where there is less chance of the development of weed resistance, and the opportunity to

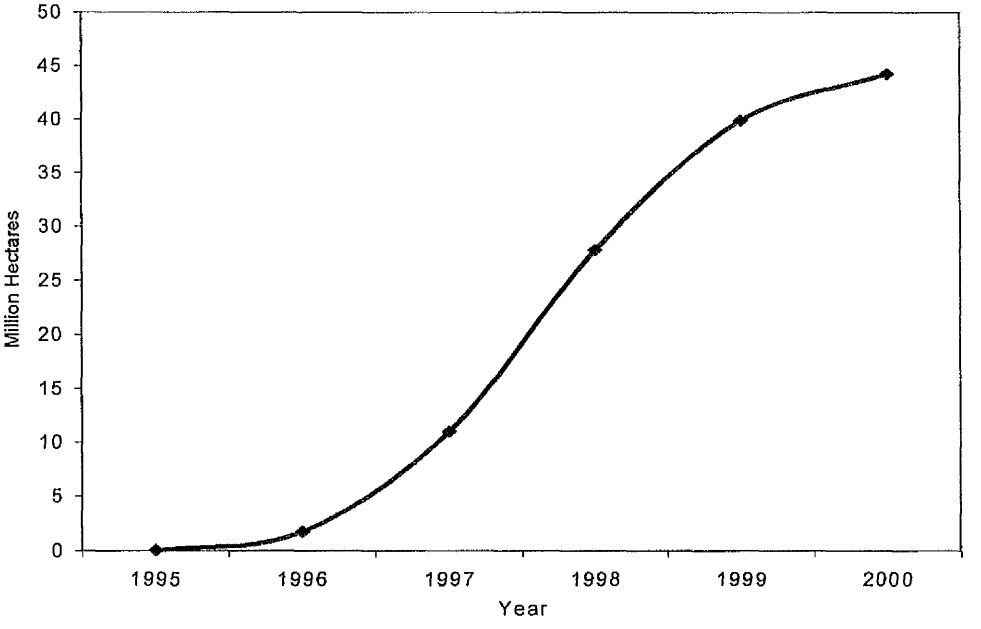
use HT crops to control currently resistant or difficult to control weeds (Gressel, 1992; Shaner, Bascomb, Smith, 1996), reduction in soil erosion through minimal cultivation methods (Marshall, 1995), use in minor crops and forestry (Marshall, 1995), crop rotation benefits due to reduced herbicide residues in soil (Dekker and Duke, 1995), improved weed control options (Lawson, 1993); applications based on the level of weed infestation present (Rasche *et al.*,1995), reductions in production costs (Singh, Bascomb, Shaner, 1994; Dekker and Duke, 1995).

Some of the suggested disadvantages of utilising herbicide tolerant crops include; potential increase in their use and reliance on a few herbicide chemistries (Burnside, 1992), adverse environmental impacts such as gene introgression in wild weed species related to the crop plants (Raybould and Gray, 1993 ; Chevre, Eber, Renard, Darmency, 1999), gene introgression to crop plants of the same species (Timmons, O'Brien, Charters, Dubbels, Wilkinson, 1995) resulting in potential difficulties with volunteer control (Derksen, Harker, Blackshaw , 1999), complication of crop rotation management, adverse impact of herbicides on botanical diversity (Sweet, Shepperson, Thomas, Simpson, 1997), the development of monopolistic seed/chemical companies and the general public concern of the use of genetic engineering (Marshall, 1995).

In the last fifteen years there has been considerable research by chemical and seed companies into the incorporation of herbicide tolerance into normally susceptible crop plant species. Plant biotechnology incorporating recombinant DNA (rDNA) technology has allowed the development of new herbicide tolerant varieties more precisely and in a shorter period of time than conventional breeding techniques. Herbicide tolerant crops, including oilseed rape were some of the first products of rDNA technology to be developed and utilised in worldwide agricultural systems.

The adoption rates for transgenic crops are the highest for any new technologies by agricultural industry standards due to their associated economic and agronomic benefits (James, 2000). In 1999 and 2000 the global area of transgenic crops started to plateaux reflecting the high adoption rates. Figure 1 shows the rapid increase in global area of transgenic crops from zero in

1995 to 44.2 million hectares in 2000. The areas of transgenic crops grown in 1998 and 1999 by crop are shown in Table 1 and by trait in Table 2.



Source: James 2001

Figure 1. The increase in the global area of transgenic crops from 1995-2000 (millions of hectares)

Table 1. Global area of transgenic crops in 1998 and 1999 by trait (millions of hectares)

Crop	1998	% of total area	1999	% of total area
Herbicide tolerance	19.8	71	28.1	71
Insect resistance (Bt)	7.7	28	8.9	22
Bt/Herbicide tolerance	0.3	1	2.9	7
Virus resistance/other	<0.1	<1	<0.1	<1
Total	27.8	100	39.9	100

(Source: James, 2000)

Table 2. Global area of transgenic crops in 1998 and 1999 by crop (millions of hectares)

Crop	1998	% of total area	1999	% of total area
Soybean	14.5	52	21.6	54
Corn	8.3	30	11.1	28
Cotton	2.5	9	3.7	9
Canola	2.4	9	3.4	9
Potato	<0.1	<1	<0.1	<1
Squash	0	0	<0.1	<1
Papaya	0	0	<0.1	<1
Total	27.8	100	39.9	100

(Source: James, 2000)

1.3 Herbicide tolerant oilseed rape

There are currently three main types of herbicide tolerant oilseed rape (*Brassica napus* L. ssp. *oleifera* (and turnip rape: *Brassica rapa* L. ssp. *oleifera*) that are being widely used in agriculture particularly in North America and Canada. Two transgenic types, glyphosate tolerant (marketed as Roundup Ready®) developed by Monsanto, glufosinate ammonium tolerant (marketed as Liberty Link®) developed by Aventis (Formerly Plant Genetic Systems/AgrEvo) and BASF (Formerly American Cyanamid/Pioneer Hi-Bred) have developed imazethapyr tolerance (marketed as Pursuit Smart®) and tolerance to both imazethapyr and imazamox (marketed as Odessey Smart®) through a combination of tissue culture and conventional breeding techniques.

A significant proportion of the spring oilseed rape (canola) grown in both the US and Canada is now herbicide tolerant. In Canada for example, where herbicide tolerant oilseed rape has been grown for several years, approximately 80% of the 5.6 million hectares of rape grown in 1999 were herbicide tolerant (Derksen *et al.*, 1999). This widespread adoption of the technology is partly due to the fact that production systems are based on spring crops which contain high densities of annual weeds (Derksen *et al.*, 1999). In this short season, spring cropping system, there is a high

reliance on effective and economic weed control compared to long season winter sown crops of oilseed rape in Europe where weed control is less critical (Marshall, 1995). The adoption of transgenic crops and particularly herbicide tolerant crops has been widespread in the USA, Argentina and Canada (Table 3), herbicide tolerance also accounts for 71% of the global area of all transgenic crops (Table 1). Notably some of the stacked gene systems (insect resistance and combined herbicide tolerance) are also being widely adopted in both maize and cotton in the USA, the areas of these crops increased from 1% in 1998 to 7% in 1999 (James, 2000).

Table 3. Global area of transgenic crops in 1998 and 1999, by country (millions of hectares)

Country	1998	% of total area	1999	% of total area
USA	20.5	74	28.7	72
Argentina	4.3	15	6.7	17
Canada	2.8	10	4.0	10
China	<0.1	<1	0.3	1
Australia	0.1	1	0.1	<1
South Africa	<0.1	<1	0.1	<1
Mexico	0.1	<0.1	<0.1	<1
Spain	<0.1	<1	<0.1	<1
France	<0.1	<1	<0.1	<1
Portugal	0.0	0	<0.1	<1
Romania	0.0	0	<0.1	<1
Ukraine	0.0	0	<0.1	<1
Total	27.8	100	39.9	100

(Source: James, 2000)

1.4 Glufosinate Ammonium – herbicide characteristics and mode of action

Glufosinate ammonium (phosphinothricin) is a widely used broad spectrum pre-emergence herbicide, it is also used for pre harvest desiccation in potatoes, legumes and oilseed rape by application to the leaves. Glufosinate ammonium interferes with amino acid synthesis by inhibition of GS. GS is the key enzyme in nitrogen metabolism that assimilates ammonia produced by nitrate

reduction, and recycles ammonia produced by processes such as photorespiration and deamination (Kishore and Shah 1988). As a structural analogue of the GS substrate, glutamate, glufosinate ammonium inhibits GS irreversibly. This inhibition triggers ammonia accumulation to levels up to 100 times higher than in control plants, resulting in cessation of photosynthesis and disruption of the chloroplast structure (Tachibana, Watanabe, Sekizuwa, Takematsu, 1986; Devine, Duke, Fedtke, 1993).

1.4.1 The development of glufosinate ammonium tolerance

Glufosinate (or phosphinothricin) tolerance in crops is based on the mechanism used by the microbial producers of phosphinothricin and bialaphos. These organisms protect themselves against the autotoxic effect of glufosinate by producing the enzyme phosphinothricin-N-transferase (PAT). This enzyme also plays a role in bialaphos biosynthesis (Kumada, Anzai, Takano, Murakami, Hara , Itoh,Imai, Satoh Nagaoka, 1988; Nap and Metz, 1996). Acetylation of the free NH_2 group of phosphinothricin by PAT causes the inactivation of phosphinothricin.

The PAT encoding *bar* gene was isolated from *Streptomyces. hygroscopicus* (Murakami, Anzai Imai, Satoh Nagaoka, Thompson, 1986) and the *pat* gene was cloned from *Streptomyces viridochromogenes* Tu494 (Strauch, Wohlleben, Puhler, 1988). Both of these genes code for proteins of the 183 amino acids, which show very high homology, variations of genes being confined to the 5" – noncoding region (Wohlleben, Arnold, Broer, Hillemann, Strauch, Puhler, 1988).

Successful introduction and expression of the *bar* gene in plants was achieved for a number of crops including tobacco, potato, oilseed rape, alfalfa, sugar beet, sunflower and wheat (De Block, Botterman, Vandewiele, Dock, Thoen, Grossele, Rao Movva, Thompson, Van Montagu, Leemans, 1987; De Greef, Delon, De Block, Leemans, Botterman, 1989; D'Halluin, Botterman, De Greef, 1990; Escandon and Hahne 1991; Vasil, Castillo, Fromm, Vasil, 1992). The *pat* gene was introduced and

expressed in crops such as tobacco (Wohlleben *et al.*,1988) and maize (Morocz, Donn, Nemeth, Dudits, 1990, Donn, Nilges, Morocz, 1990).

Genetically modified plants were shown to tolerate glufosinate doses 4-10 times higher than the dose required to kill untransformed control plants. The ammonia levels of genetically modified plants were unaltered following glufosinate application, indicating efficient glutamine synthetase (GS) protection and thus a high degree of tolerance to glufosinate ammonium (De Block *et al.*, 1987).

1.4.2 Glufosinate ammonium tolerant crops and weed control

A number of crops were developed by Plant Genetic Systems/AgrEvo (Aventis) with tolerance to glufosinate ammonium (proposed product name in the U.K - Liberty) including oilseed rape, maize, soybean and sugar beet. In 1995 two glufosinate tolerant spring oilseed rape varieties were registered in Canada for use in conjunction with the herbicide glufosinate ammonium.

In the UK, transgenic herbicide tolerant crop technology has not yet been fully approved by the government. Data on the effectiveness of glufosinate ammonium in tolerant crops in the UK is scarce, although there is significant experience with these crops in the US and Canada. There are now some published reports of weed control in Liberty tolerant crops in the UK and elsewhere in Europe, such as maize (Rasche *et al.*, 1995; Read and Ball 1999a), sugarbeet (Read and Bush, 1998) and oilseed rape (Rasche *et al.*, 1995; Read and Ball, 1999b; Booth, Green, de Both, 1999). Since 1993 trials have been carried out in the U.K. by Aventis. Recently, Read and Ball (1999b) reported on the initial findings of some of the trials that were conducted across the UK using GM herbicide tolerant varieties of winter and spring oilseed rape; weed control was compared with currently available selective herbicides. The results showed that a single treatment of glufosinate ammonium compared well with a normal two-spray programme of, for example metazachlor + quizalofop-ethyl for control of grass and broadleaved weeds. In Autumn applied treatments, some species such as *Viola arvensis* and *Papaver rhoeas* were found to be more difficult to control and

required higher dose rates of up to 0.8kg/ha (Read and Ball, 1999b). It was also noted that some species with prolonged germination periods such as *Galium aparine* could escape herbicide treatment, as glufosinate is principally a contact herbicide with some acropetal translocation.

In common with weed control in winter rape, a higher dose in spring rape crops controlled some of the recalcitrant weeds such as *Lamium pupureum* and *Fumaria officinalis* (Read and Ball, 1999b). It was concluded that there might be some economic benefit of the use of this technology as well as distinct agronomic benefits of controlling some of the more difficult and resistant weeds such as *Alopecurus myosuroides*. Further work conducted throughout Europe reported by Booth *et al.*, (1999) showed a great variation in yield response in winter oilseed rape when comparing glufosinate with currently used herbicides. This variation was on a site to site basis implying that differences in weed spectrum, weed density and application timing affected herbicide performance. Results from spring rape trials generally showed less variation and a better mean yield response from applying glufosinate compared to currently used herbicides. Booth *et al.*, (1999) also suggested that glufosinate tolerant oilseed rape may offer the opportunity to control Cruciferous weeds, not well controlled by conventional herbicides and provide rotational control of herbicide resistant grass weeds. The relative selectivity of glufosinate and other herbicides used in herbicide tolerant crops in Canada is shown in Table 4.

1.5 Glyphosate – herbicide characteristics and mode of action

Glyphosate is a non-selective, post emergence, foliar applied herbicide with systemic activity. It is the most widely used herbicide in the world and has been used for more than 20 years in all types of crop production (Duke, 1988; Wells, 1995). This widespread use of glyphosate is due to its effectiveness in broad spectrum weed control and its excellent environmental safety (Wells, 1995).

Glyphosate activity is based on the inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) which is part of the shikimate pathway (Amrhein, Deus, Gehrke,

Steinrucken, 1980; Steinrucken and Amrhein, 1980). The development of EPSPS enzymes which are tolerant to glyphosate has been the main focus in developing glyphosate tolerant crops. Inhibition of EPSPS by glyphosate prevents the synthesis of aromatic amino acids and secondary metabolites, which causes the accumulation of shikimate and benzoic acid derivatives which results in cell death (Comai, Facciotti, Hiatt, Thompson, Rose, Stalker, 1985; Lydon and Duke, 1989). The main metabolic degradation route for glyphosate in soil is through the cleavage of the glycyl moiety and formation of aminomethylphosphate (AMPA) plus glyoxylate (Jacob *et al.*, 1988,; Pipke and Amrhein 1988). This metabolic inactivation of the active ingredient has also been a primary target to developing glyphosate tolerant crops (Wells, 1995).

1.5.1 The development of glyphosate tolerance

Two main approaches have been developed to achieve glyphosate tolerance through genetic modification. Both of the approaches have been used in combination to develop commercial crops of oilseed rape with robust herbicide tolerance. Presently, only canola plants have been successfully engineered to contain a functional GOX enzyme. However, all the commercial glyphosate tolerant crops contain a tolerant EPSPS gene (APHIS, 2000).

i) Glyphosate tolerant EPSPS (5-enolpyruvylshikimate-3-phosphate synthase)

The first approach is the introduction of an EPSPS with a reduced affinity to glyphosate. There are several genes that have been isolated from microrganisms that encode for a modified EPSPS enzyme. An EPSPS was identified from a screen of glyphosate degrading bacteria (*Agrobacterium* sp.). CP4 EPSPS exerted high glyphosate tolerance while maintaining high catalytic efficiency (Padgette, Re, Barry, Eichholtz, Delannay Fuchs, Kishore, Fraley, 1996). The gene for CP4 EPSPS is fused to the chloroplast transit peptide coding sequences to target the protein to the plastids. This CP4 EPSPS gene has been evaluated in a number of species including oilseed rape and soybean where high levels of tolerance have been demonstrated at both vegetative and reproductive stages

(Barry, Kishore, Padgette, Taylor, Kolacz, Weldon, Eichholtz, Fincher, Hallas, 1992; Padgette, Kolacz, Dellanny, Re., La Vallee, Tinius, Rhodes, Otero, Barry, Eichholtz, Peschke, Nida, Taylor, Kishore, 1995).

ii) Glyphosate degradation by GOX

Glyphosate is known to be degraded by soil and water bacteria (Rueppell, Brightwell, Schaefer, Marvel, 1977). Several glyphosate metabolites have been observed in soils, the most important are aminomethylphosphonic acid (AMPA) and sarcosine (N-methylglycine) (Torstensson, 1985). The primary pathway for glyphosate breakdown to non-phytotoxic compounds in soil is the aminomethylphosphonate pathway (Torstensson, 1985; Jacob, Garbow, Hallas, Kimack, Kishore, Shaeffer, 1988). Glyphosate degrading bacteria (*Achromobacter* sp.) were first isolated from a glyphosate waste stream facility and Achromobacter sp. strain LBAA was selected from this screen (Hallas, Hahn, Korndorfer, 1988). The enzyme glyphosate oxidoreductase (GOX) from *Achromobacter* sp. strain LBAA catalyses the cleavage of the C-N bond of glyphosate yielding AMPA and glyoxylate. The gene expressing GOX was cloned and inserted in several plant species. High glyphosate tolerance levels have been observed in oilseed rape carrying the GOX enzyme, both in vegetative and reproductive organs (Barry *et al.*, 1992).

1.5.2 Glyphosate tolerant crops and weed control

Glyphosate (Roundup Ready®) crops were introduced commercially by Monsanto in 1996 (Moll, 1997). Soybeans were commercialised in Argentina and the United States and spring oilseed rape was marketed in Canada. Roundup Ready® cotton was introduced in the United States in 1997 (Moll, 1997). No Roundup Ready® crops have been commercialised in Europe, although the major crops targeted for introduction are sugar beet, oilseed rape, maize, soybean and cotton. There is very little published data on the efficacy of glyphosate in tolerant crops, although the herbicide has

been used non-selectively in arable farming for many years to clear land prior to drilling or on set aside areas and is especially effective against perennial grass weeds.

In Canada where Roundup Ready® crops have been successfully adopted into rotations some data has been published on the effects of herbicide tolerant spring oilseed rape (canola) on weed dynamics (Derksen *et al.*, 1999). Herbicide tolerant canola has become a popular clean up crop in rotations in Canada and has provided growers with the ability to control difficult or resistant weeds (Derksen *et al.*, 1999). Glyphosate tolerant canola has also encouraged the use of minimum tillage systems due to the reliance of these systems on the use of glyphosate for clearing land prior to drilling.

The use of glyphosate in HT oilseed rape has predictably given a wider spectrum of weed control than with previously available herbicides. Glyphosate and other herbicide tolerant systems have allowed farmers the opportunity to control *Cruciferae* species more effectively (Table 4). The systemic mode of action of glyphosate would be of benefit where there are specific weed problems such as infestations of perennial weeds or where there are populations of herbicide resistant weeds such as blackgrass in the UK (*Alopecurus myosuroides*) (Marshall, 1998) and *Setaria viridis* in Canada (Derksen *et al.*, 1999). Research in Europe conducted by Madsen and Jensen (1995) investigated weed control in glyphosate tolerant sugar beet. The effects of glyphosate on weeds were evaluated both in greenhouse bioassays and in a sugar beet crop in the field. Weed control with glyphosate was shown to be equivalent or superior to the mixtures of commonly applied herbicides in sugar beet crops. It was noted that control of *Galium aparine* was poor with all the herbicides tested after the weed development had exceeded cotyledon growth stage (Madsen and Jensen, 1995). The relative selectivity of glyphosate and other herbicides used in herbicide tolerant crops in Canada is shown in Table 4.

1.6 Imidazolinones – herbicide characteristics and mode of action

The imidazolinones belong to the acetolactate synthetase inhibiting herbicides, along with three other structurally diverse compounds i.e. sulfonylureas, triazolopyrimidines and pyrimidyl-oxy-benzoates (Subramanian, Hung, Dias, Miner, Butler, Jachetta, 1990). ALS is the first enzyme in the branched chain amino acid pathway that produces valine, leucine and isoleucine (Devine *et al.*, 1993). A large number of compounds have been found to be effective inhibitors of ALS binding a vestigal ubiqinone binding site (Schloss, Ciskanik, Van Dyk, 1988).

The imidazolinones have a broad spectrum of weed control, having activity against both monocotyledonous and dicotyledonous weeds. They can be absorbed by both foliage and roots and are translocated systemically within weeds (Shaner and Reider, 1986). The imidazolinones have demonstrated selectivity in a number of crops including legumes, cereals and plantation crops and are widely used because of their efficacy and low mammalian toxicity. Imidazolinones were first identified and developed by American Cyanamid, Co. Princeton, NJ (Los, Ciarlante, Ettinghouse, Wepplo, 1982; Los, Orwick ,Russell, Wepplo, 1983; Los, 1984; Cross, Johnson, Los, Orwick, 1983). Several active ingredients e.g. imazethapyr, imazapyr, imazaquin and the more recently developed imazamox are now being widely used in a range of crops.

Imidazolinones are absorbed by both foliage and roots of weeds and are translocated via the xylem and the phloem to the meristems where phytotoxicity is expressed (Shaner and Anderson, Stidham, 1984). Their herbicidal activity is influenced by a range of different interacting factors both biological and physical. The amount of herbicide that reaches the meristems is dependant upon the compound involved, the site of uptake and the plants ability to metabolise the herbicide (Shaner, 1989). Indeed, Shaner (1989) reviewed the factors in detail affecting soil and foliar availability of the imidazolinones, several general conclusions were reached:

1. Soil bio-availability: activity in soil is determined by the chemical structure of the herbicide, the concentration of herbicide in the soil solution, soil pH and soil texture.

2. Foliar bio-availability: absorption is limited by the amount of herbicide that crosses the cuticle, environmental conditions relating to plant growth and when the herbicide is applied. Uptake is enhanced with surfactants. Translocation of the herbicide is dependent upon the compound, the species and the limit of the amount of imidazolinone that can be translocated out of the leaf.

1.6.1 The development of imidazolinone tolerance

The use of acetolactate sythatase inhibitor (ALS) herbicides in agriculture, including the imidazolinones is based on the existence of various crops such as soybean and cereals with natural tolerance to these compounds. Selection for imidazolinone tolerant maize began in 1982 as collaboration between American Cyanamid and Molecular Genetics Inc. (Shaner *et al.*, 1996). This was driven by the need to develop herbicides that would control many of the problematic weeds in maize crops in the US such as *Sorghum bicolor* and *Sorghum halepense* (Shaner *et al.*, 1996). Maize is also often planted in rotation with soybean so that problems of crop phytotoxicity can occur with residual imidazolinones that have been used in the previous crop. Initial work carried out on maize utilised a combination of tissue culture and pollen mutagenesis (Shaner and Anderson, 1985; Anderson and Georgeson, 1989; Bright, Chang, Evans, MacDonald, 1990; Newhouse, Singh, Shaner, Stidham, 1991). Imidazolinone tolerant maize is now widely grown in the US.

1.6.2 The development of imidazolinone tolerant oilseed rape

Cyanamid and Pioneer Hi-Bred collaborated to develop imidazolinone (IMI) tolerant oilseed rape (canola), it was the first herbicide tolerant crop to be grown widely (Anon, 1998). IMI tolerant oilseed rape was developed using a non-transgenic process comprising of *in vitro* microspore mutagenesis and selection. This first reported use of microspore mutagenesis and selection resulted in chlorsulfuron tolerant *Brassica napus* plants (Swanson, Herrgesell, Arnoldo, Sippell, Wong, 1989). *B. napus* micropores were isolated, mutagenised and cultured, essentially using techniques

developed by Swanson, Coumans, Brown, Patel, Beversdorf, (1988). Microspores were mutagenized using 20mM ethyl nitro sourea and were cultured on a microspore medium containing 40 ug/l of imazethapyr (Anon, 1998; Swanson *et al.*, 1989). Small haploid plantlets with active root systems were recovered, they were treated with colchicine to double the chromosome numbers. Several mutants were developed in this way, two independent mutants PM1 and PM2 were identified as having the highest levels of tolerance, these were subsequently combined using conventional breeding techniques (Anon, 1998) eventually leading to the commercial release of varieties of IMI tolerant oilseed rape.

1.6.3 Imidazolinone tolerant crops and weed control

Three herbicides have been developed by American Cyanamid and have been successfully used with IMI tolerant oilseed rape. Imazethapyr is a broad-spectrum imidazolinone that is registered for use in the USA on soybeans other legumes and maize. It is registered in Canada for use on oilseed rape (Smart Canola®). Imazethapyr and imazamox combined (Odessey®) was designed specifically for use in canola in western Canada where it controls some of the major dicot and monocot weeds such as *Avena fatua, Setaria viridis, Echinochloa crus-galli* and *Galium sp*. The use of Odessey Smart Canola® means that growers can achieve control of emerged weeds and flushes of shallow germinating weeds. Imazamox is a new selective imidazolinone registered by Cyanamid for use on soybeans and other legumes.

Imazamox gives good control of both monocot and dicot weeds and has some residual soil activity for later germinating species. The product is not yet registered in the USA (in 1999) for use on herbicide tolerant canola. In Europe and Australia the registration of imazamox is in progress for its use on tolerant spring and winter oilseed rape varieties. Results have demonstrated that single applications of imazamox provide season long control of a broad spectrum of weed species (Anon

1998). Table 4 shows the relative selectivity of imidaziolinones and other herbicides used in herbicide tolerant crops in Canada.

Table 4. Relative selectivity of herbicide tolerant* and conventional canola varieties (Adapted from Derksen *et al.*, 1999)

Weed species	Glufosinate*	Glyphosate*	Imazethapyr*	Ethylfluralin
Setaria viridis	G	G	G	E
Avena fatua	G	G	F	G
Polygonum convolulus	G	F	G	G
Seline noctiflora	-	G	-	-
Stellaria media	G	G	G	G
Galium aparine	G	G	G	P
Taraxacum officinale	-	S	-	-
Galeopsis tetrahit	G	G	-	P
Kochia scoparia	G	G	-	G
Chenopodium album	G	G	P	E
Brassica kaber	G	G	E	-
Amaranthus retroflexus	G	G	G	E
Agropyron repens	F	S	-	-
Salsola pestifera	G	G	-	P
Capsella-bursa pastoris	G	G	-	-
Polygonum persicaria	G	G	G	-
Thalaspi arvensis	G	G	E	-
Sonchus arvensis	G	S	-	-
Cirsium arvense	F	S	-	-
Triticum aestivum	F	G	P	P
Hordeum vulgare	F	G	P	F

E = excellent control under wide ranging conditions (depending on specific graminicide chosen); G = good control under most conditions; F = fair control depends on conditions; P= poor control; S = suppression of perennial weeds; - = not registered. Based on rates of 593g a.i./ha for glufosinate, 440g a.i./ha for glyphosate, 50g a.i./ha for imazethapyr.

1.7 Weeds in the arable ecosystem

Most arable crop production systems aim to produce monocultures of crops or simple mixtures of species in order to maximise crop yield and profitability, thus the natural vegetation of an area must be changed by introducing the crop species or selecting out most of the other species. Weed control in arable systems is concerned with controlling the unwanted species that compete with crop plants for water, nutrients, space and light. A species may become a weed because it has the appropriate characters that enable it to exploit a niche created by a particular land use practice, weed abundance is therefore proportional to habitat size (Mortimer, 1990). Other species have a more general all purpose genotype which ensures persistence under unpredictable habitat conditions and may be common to a large number of different habitats and management practices (Mortimer, 1990).

Kropff, Bastiaans, Cousens, (1999) identified the main processes determining the life cycle of weeds as: germination and emergence of seedlings from seeds; establishment and growth of weed plants; seed production; seed shedding and seed mortality in the soil. Whole lifecycle models of weeds have been developed, and represented by a series of growth stages and the transitions from one stage to the next; e.g. germination rate and reproduction rate. Such models have been developed by Van der Weide and Van Groenendael (1990) for example. A number of characteristics have been identified as creating the 'ideal' weed shown in Table 5 (Adapted from Baker and Stebbins, 1965). Williamson (1994) raises questions over the ability of these characters to predict invasive species.

Table 5. Life history characteristics of a plant species that if combined would result in an ideal weed (adapted from Baker and Stebbins,1965)

No.	Weed characteristic
1	Seed germination requirements fulfilled in many environments
2	Discontinuous germination (through internal dormancy) and considerable longevity of seed
3	Rapid growth through the vegetative phase to flowering
4	Seed production in a wide range of environmental conditions; tolerant and plastic
5	Continuous seed production for as long as conditions for growth permit
6	Very high seed output in favourable environmental circumstances
7	Self compatible but not completely self pollinating or apomictic
8	Possession of traits for short and long distance dispersal
9	When cross pollinated unspecialised pollinator visitors or wind pollinated
10	If clonal species, has vigorous vegetative growth and regenerates from fragments
11	If clonal species, has brittleness of leafy parts ensuring survival of main plant
12	Shows a strong inter-specific competition by special mechanisms

1.7.1 Seed bank dynamics

The soil seed bank is the major source of infestations of annual weeds, periodical germination of weed seeds from the seed bank results in flushes of germinating seedlings (Roberts and Ricketts 1979; Mortimer, 1990; Murdoch, 1998). Many agronomic practices such as soil disturbance, crop rotation and herbicide applications influence the size of the weed seed bank by affecting survival and dormancy in the soil or seed production (Roberts, 1981). In order for a weed species to achieve temporal dispersal only a proportion of seeds should lose dormancy at one time. The main germination periods for common arable weed species are well known, three main patterns of germination can be identified, regardless of when land has been cultivated (Mortimer, 1990). Weeds are either: predominantly autumn germinators, predominantly spring germinators, or year round germinators. Seedling emergence patterns reflect the seasonal variation in edaphic and climatic factors and the extent to which weed species respond to aspects of these changes as stimuli or cues (Mortimer, 1990). Many dicotlyedonous weed species exhibit periodicity of germination in autumn and spring, spring emergence being exhibited by *Chenopodium album* and *Polygonum*

aviculare for example (Froud-Williams, 1999). Graminaceous weeds often exhibit peaks of autumn germination such as *Alopecurus myosuroides* as do the dicot species, *Veronica hederifolia* and *Urtica urens* (Mortimer, 1990). Good knowledge of periodicity is invaluable when making decisions on herbicide applications and also for mechanical weed control techniques.

Many weeds produce seeds that may remain in the seed bank for over a year, forming persistent seed banks. In order for seeds to survive, viability must be maintained and germination avoided by dormancy (Bradbeer, 1988). Dormancy determines how much of the total seed bank of a species is available for germination at a given time. There are two dormancy 'strategies' in weeds; predictive or consequential (Bradbeer, 1988). Innate (predictive) dormancy is when seeds enter into dormancy prior to adverse seasonal changes in the environment and is genetically determined. Innate dormancy develops on the mother plant and persists after shedding to ensure the temporal dispersal of seeds by preventing their immediate and synchronous germination. The consequential strategy for dormancy, defined as enforced or induced dormancy is a direct response to adverse conditions, such as lack of sufficient water for germination or an unfavourable temperature (Bradbeer, 1988; Mortimer, 1990; Murdoch, 1998).

1.7.2 Crop-weed interactions

Competition plays a major role in different stages of the weed life cycle and therefore strongly affects the population dynamics of weeds (Kropff *et al.*, 1999). Competition can be defined as the growth reduction of a plant due to the capturing of growth limiting resources by its neighbours, these resources can be light, water and nutrients (Kropff and Spitters, 1992). In integrated systems of weed control, where the systematic use of herbicides is avoided, tillage, cultivation and ecological practices have become more important for weed suppression (Liebman and Davis, 2000). In order to achieve this, accurate models for predicting crop yield loss due to given levels of weed interference need to be applied (Van Acker, Lutman, Froud Williams, 1997). Models can allow criterion to be

developed for determining whether a treatment against weeds is necessary or not (Onofri and Tei, 1994). The economic threshold is defined as the weed density at which the cost of control measures equals the benefit obtained as a result (Cussans, Cousens, Wilson, 1986). Application of weed control thresholds to decision making in weed management may contribute to the reduction in the use of herbicides (Auld, Menz, Tidsell, 1987). In order to establish a threshold level however, the degree to which a weed is likely to reduce the yield of a given crop must be known (Onofri and Tei, 1994). This information can be obtained from competition experiments (e.g. Firbank, Cousens, Mortimer, Smith, 1990; Weaver, 1991), where mathematical modelling is important for interpreting results. Hughes (1996) discusses difficulties in applying these concepts arising from the patchy nature of weed spatial patterns. McRoberts and Hughes (2001) discuss the limitations of decision tools imposed by sampling and the belief of the user.

There have been many studies investigating the competition between crops and weeds (e.g. Blackshaw, Anderson, Dekker, 1987; Gerowitt, 1993). The predictive accuracy of models of weed density and yield loss is limited because weed density does not account for the variation in time of weed emergence relative to the crop, consecutive weed flushes or variations in weed:crop vigour (Kropff and Spitters, 1991). Frequently studies on the prediction of yield loss concentrate on the effects of single species, although some multispecies approaches have been utilised (e.g. Hume, 1989; Wilson and Wright, 1990; Wright, Seavers, Wilson, 1997). More complex ecophysiological models (e.g. Graf, Gutierrez, Rakotobe, Zahner, Delucci, 1990; Kropff and Spitters, 1992; Ball and Schaffer, 1993; Lindquist and Kropff, 1996) simulating competition for light, water and nutrients between a crop and one or more weeds allow the exploration of the effects of crop management including sowing dates, crop density, fertilisation and weeding on weed biomass and weed seed production.

1.7.3 Weed dispersal

The dispersal of weed seeds regulates the inflow of weeds in arable ecosystems over a range of spatial scales (Kropff *et al.*, 1999). Pollen movement can also spread weedy traits such as herbicide tolerance between related weed and crop species (Thill and Mallory-Smith, 1997).

Most weed seeds are dispersed relatively close to the parent plant (Mortimer, 1990). The weed seed morphology of the majority of species shows some features which can be regarded as dispersal mechanisms (Bradbeer, 1988). Seeds may be adapted to wind dispersal by modification of the testa or pericarp to form a wing, or by the lightness of the seed that allows them to be blown for considerable distances. Animals and birds may either internally or externally carry seeds, such as the hooked or awned seeds of *Gallium aparine* or *Avena fatua*. Farm machinery and the activities of man is perhaps the mechanism by which seeds are most actively dispersed locally among farms (Mortimer, 1990). Studies on the movement of seeds by soil cultivations show that the type of cultivation influences the horizontal and vertical movement of seeds in the soil profile, ploughing moving seeds deeper than tine cultivations (e.g. Dessaint, Chadeouf, Barralis, 1996; Moss, 1988).

Studies looking at horizontal movement of seeds with various farm cultivation implements show that the majority of seeds are moved less than 1m from the source (Howard, Mortimer, Gould, Putwain, Cousens, Cussans, 1991; Rew and Cussans, 1997). The depth at which seeds are incorporated will affect the possibility of successful germination and emergence (Grundy, Mead, Bond, 1996; Froud-Williams, 1999). Harvesting machinery has the potential to move seeds considerable distances but the majority remain close to their source (Howard *et al.*, 1991; McCanny and Cavers, 1988). Most broad-leaved weeds will complete their lifecycle in the crops understorey and are therefore unaffected by harvesting machinery, cultivations are the main mechanism for seed movement (Rew and Cussans, 1997).

1.8 Environmental impacts of growing herbicide tolerant oilseed rape

The intensification of agriculture through the use of pesticides, fertilisers, drainage and cultivation techniques, increases in winter sown crops such as wheat and oilseed rape and reductions in mixed farming have all been associated with the general decline in arable biodiversity. There has been increasing concern in recent years about the decline in biodiversity associated with changes in the diversity of arable plants. A specific example is the increased scientific interest in the ecology of field margins/boundaries. Field margins are associated with benefits to crop growth by serving as a windbreak (Forman and Baudry, 1984), reducing soil erosion (Tim and Jolly, 1994) and enhancing natural populations of crop pollinators and other beneficial insects (Sotherton, 1984; Coombes and Sotherton, 1986). A large number of animal and plant species rely on field margins for shelter or as a place to grow (Tew, Todd, Macdonald, 1994).

There are number of policy initiatives that have been recently developed in response to this concern. One of the priority habitats under the UK Biodiversity Action Plan (BAP) are cereal field margins, targets have been set to restore 15000ha of cereal field margins by 2010 (Anon 1995). The Priority List in the UK BAP contains 62 vascular plant species, 14 are found exclusively in farm habitats or have a large proportion of their UK populations on farmland. There is also a list of 159 species which are of conservation concern, 24 of these are found predominantly in farmland ecosystems. Some plant species are associated almost entirely with arable land such as *Agrostemma githago* (Corncockle), *Ranunculus arvensis* (Corn buttercup), *Centaurea cyanus* (Cornflower) and *Galeopsis segetum* (Yellow hemp nettle) are either regarded as extinct or nationally scarce (Anon, 1995). The Arable Stewardship scheme has also been recently initiated specifically to encourage farming practices which conserve and enhance the flora and fauna of arable ecosystems and to contribute toward the BAP initiative (MAFF 1998).

The introduction of genetically modified herbicide tolerant (GMHT) crops has been regarded by many groups as a further intensification of agriculture who state that GM crops will increase or

encourage the use of pesticides thus further the continuing decline in diversity in arable ecosystems (World Wildlife Fund, 1995; Friends of the Earth, 1997; Gene Watch, 1998a/b; Royal Society for the Protection of Birds, 1997; English Nature, 1998; Fromwald and Strauss, 1998; Hill, 1999). Crops such as oilseed rape which carries transgenes conferring herbicide tolerance are thought to be of particular concern given its potential to spread and hybridise with related species (Raybould and Gray, 1993). The introgresssion of transgenes into related weed species and contamination of other rape crops with transgenes via cross pollination may complicate farm management and reduce the efficacy of the herbicide(s) in question (Marshall, 1998). The introduction of herbicide tolerant varieties of oilseed rape may increase problems with volunteer management particularly if plants have become tolerant to more than one herbicide. An increase in the complexity of rape volunteer management has been reported in North America, where no-tillage cropping systems are being used in conjunction with glyphosate and glufosinate tolerant oilseed rape varieties, with a resulting lack of control of volunteer plants with these chemicals (Derksen *et al.*, 1999). To ensure harvested crops of rape reach certain levels of genetic purity there is interest in the rates of cross pollination between crops of GM and non-GM oilseed rape. There has been considerable research effort directed at finding the rates of decline in cross pollination with distance and whether this can be related to quantifying isolation distances (e.g. Ingram, 2000). There is however, still a lack of published data reflecting real agricultural situations and information regarding any possible conventional variety effects on the levels of cross pollination obtained.

The ecological impact of the types of herbicides that will be used in HT oilseed rape and other HT crops is a further important consideration when assessing the impact of HT crops on agriculture. The herbicides used on HT crops have a broader spectrum of activity than the current pre- and post-emergence selective herbicides and may be applied later in the growing season thus; their use will have different effects on weed diversity. The use of broad-spectrum herbicides may lead to more rapid changes in botanical diversity and have additional effects on the seed bank in

arable ecosystems, particularly if several herbicide tolerant crops have been integrated into crop rotations. Shifts in botanical diversity could result in reductions of weed and invertebrate populations on which farmland birds and other wildlife depend (Kleijn and Verbeek, 2000).

It is the long term agronomic and ecological benefits and risks of GM and non-GM herbicide tolerant crops that require further examination and are now beginning to be addressed in the U.K. Up until recently there have been very few comparative studies of ecological effects of GMHT and conventional crops in Europe. Several U.K. government and industry sponsored projects such as SCIMAC (Supply Chain Initiative on Modified Agricultural Crops, 1999-2003) and the BRIGHT project (Botanical and Rotational Implications of Growing Genetically Modified Herbicide Tolerant Crops 1998-2002) have now been initiated which are investigating some of the direct and indirect effects on crop production and the environment that herbicide tolerant crops compared with conventional crops may have when grown in agricultural systems in the UK.

1.8.1 Pollen dispersal from oilseed rape

Oilseed rape is a predominantly self-pollinated crop with average outcrossing rates of between 15% and 45% (Rakow and Woods, 1987; Becker, Damgaard, Karlsson, 1992). Environmental factors such as high radiation and wind can influence these rates (McCartney and Lacey 1991; Becker *et al.*, 1992), it has also been demonstrated that outcrossing rates vary among flowers at different positions on the plant, between 11% at the top to 39% at the bottom of the plant (Becker *et al.*, 1992). Winter oilseed rape flowers in April into May in northern continental Europe, and in June-July in Scotland and Scandinavia. Most spring oilseed rape flowers about one month later. Consequently it is believed by many workers that insects are more important to cross pollination in spring sown or later flowering crops. The crop is self-fertile; however, both insects and wind are widely recognised to influence the transport of oilseed rape pollen. Several studies have demonstrated the role that

wind has in pollination and the transport of pollen downwind from oilseed rape crops (Mesquida and Renard, 1982; Williams, 1987; McCartney and Lacey, 1991).

McCartney and Lacey (1991) measured airborne pollen concentrations over five seasons and showed significant amounts of pollen were airborne above and within rape crops. Over all the seasons, pollen production lasted a similar length of time although its onset differed with favourable environmental conditions. The results from their pollen trapping experiments and dispersal modelling suggest that pollen concentration quickly declines with distance from the crop. They calculated that 100m downwind of the crop airborne pollen concentrations may only be between 2-10% of the values within the crop. They suggested that these levels would result in a cross pollination level of between 0.6 and 3%, thus airborne pollen would not play a significant role in the pollination of the crop of scales larger than a few tens of metres. The main insect pollinator of oilseed rape in the UK is the honeybee (*Apis mellifera*) which is numerically more common than *Bombus* sp. in most field crops (Ramsay, Thompson, Neilson, Mackay, 1999). Pollen dispersal by the honey bee has been previously reported (Landridge and Goodman 1982; Bilsborrow, Evans, Bowman, Bland, 1998) and some aspects of behaviour of the honeybee with respect to pollination have been studied in both conventional oilseed rape (e.g. Free, 1968; Williams, 1987, Cresswell, Bassom, Bell, Collins, Kelly, 1995) and transgenic oilseed rape (e.g. Ramsay *et al.*, 1999; Thompson, Squire, Mackay, Bradshaw, Crawford, Ramsay, 1999, Scheffler, Parkinson, Dale, 1993).

Ramsay *et al.* (1999) studied honeybees as vectors of GM oilseed rape pollen. Their observations suggested that bees can forage at significant distances from the hive (2-4km) and predicted that honeybees are capable of foraging and dispersing pollen over much larger distances. The pollen adhering to honey bees in their study was also found to be viable when used to pollinate flowers on male sterile oilseed rape plants. Scheffler *et al.* (1993) attempted to optimise cross pollination of an inner area of GM oilseed rape and non-GM surrounding area of rape by placing hives of honeybees on the perimeter of the field. They concluded that no obvious directional effects

could be detected from their measurements that could be ascribed to either wind or insect activity. It is difficult to correlate wind direction averaged over the flowering period and the degree of cross pollination in different directions in a rape crop, since rape flowers for a period of approximately one month (Scheffler and Dale, 1994), there is also a more limited peak period of flowering which could mean that there are a few critical days where wind direction is important (McCartney and Lacey, 1991). The significance of both wind and insects as vectors of oilseed rape pollen have been widely researched with many contradictory results that are probably influenced by varying environmental and topographical conditions, and the differences in research methodology used.

1.8.2 Gene flow between crops and plots of oilseed rape

The large number of studies of gene flow via pollen dispersal in oilseed rape crops has highlighted the variability of levels of outcrossing. Differences in experimental designs, genotypes and environmental conditions have likely contributed to the wide variation in reported gene flow frequencies. It is probably the differences in the relative size of the pollen source and receptors in experiments that are the main factors that cause much of this variation in results (Timmons *et al.*, 1995). Common to all studies however, frequencies of transgene dispersal generally decline rapidly with increasing distance from the pollen source. For example, Scheffler *et al.*, (1993) studied pollen dispersal from transgenic oilseed rape and found a rapid decline in frequency of pollination with increasing distance from the pollen source. Levels of 5% at 1m and 0.02% at 12m from the pollen source declining to 0.00033% at 47m. Champolivier,, Gasquez, Messean, and Richard-Molard, (1999) showed a decline in cross-pollination rates with distance in a field scale study. At zero distance, levels of cross pollination varied with site from 1.6 - 4%, falling to 0.8 - 2.5% at 5m, 0.6 - 1.8% at 10m and 0.2% - 0.6% at 30m.

Studies of pollen dispersal are often supported by measurements of airborne pollen density using volumetric spore traps. Pollen densities fall rapidly with distance from the edge of the pollen

source, decreasing by about 50% within 6-10m from the edge of the crop (Mesquida and Renard 1982). These and other results (e.g. McCartney and Lacey, 1991) suggest that the opportunity for pollen to be dispersed by wind over long distances and cross pollinate other rape crops is limited. However, data from experiments investigating pollen dispersal and cross pollination from large fields of oilseed rape (e.g. Timmons *et al.*, 1995; Thompson *et al.*, 1999) suggest that not only can pollen be dispersed for relatively large distances but it remains viable and can fertilise other oilseed rape plants. Timmons *et al.*, (1995) measured pollen density around large agricultural fields and found much higher densities at (27%-69% of those recorded at the field margin) 100m from the field margin.

There have been a number of reports of long range pollen dispersal from oilseed rape at distances from 360m – 4000m (Timmons *et al.*, 1995; Downey, 1999; Ramsay *et al.*, 1999; Simpson, Norris, Law, Thomas, Sweet, 1999; Thompson *et al.*, 1999). These studies investigating longer range pollen dispersal generally use emasculated or male sterile oilseed rape plants which are exposed to airborne pollen from an isolated field of oilseed rape. Timmons *et al.*, (1995) furthered the study referred to above to determine whether the levels of airborne pollen detected was enough to effect significant levels of gene flow. Emasculated and de-petalled oilseed rape plants were situated at set distances from oilseed rape fields and seed set was recorded. Gene flow frequencies of 0.8% at 2500m and 1.2% at 1500m from the source were recorded. Although the bait plants were depetalled this does not entirely prevent the possibility of insect mediated pollen transfer so gene flow may not have been entirely due to wind. Thompson *et al.*, (1999) conducted similar studies recorded higher levels of pollination using male sterile oilseed rape plants; at one of the bait plant sites with a cross pollination rate of 33%, the majority of the sample was shown to be from a field of rape 900m away. The maximum distance that cross-pollination was recorded was at 4km from the source crop. The patterns of pollination detected in this study suggested that insects had an

important role in pollination, high numbers of seeds were set per siliqua at distant sites, despite an overall low frequency of pollination events.

This work provides valuable, if somewhat variable information on the theoretical levels of pollen flow that can occur from fields of oilseed rape. When fertile receptor plants are used, levels of gene flow are considerably lower. Downey (1999) sampled seed from fields of conventional canola (spring oilseed rape) that were growing close to herbicide tolerant oilseed rape fields. Much lower levels of gene flow were recorded; 1.5% at 20m, 0.4% at 50m and 0.1% at 100m from the pollen source. The levels were considerably lower than those recorded when using male sterile or emasculated bait plants, and are also lower than previously reported work by Stringham and Downey (1978 and 1982) where seed was sampled from small plots of fertile receptor plants growing at different distances from large canola fields.

Evidence of gene flow from these experiments using both male sterile and fertile bait plants shows that pollen is dispersed for considerable distances and could contaminate other rape fields or allow transgenes to spread into feral and volunteer populations. There are very few reports of cross-pollination being measured between large agricultural field crops of GM and conventional oilseed rape in the UK where source and receptor crops are of equivalent size. Gene flow data from large field-scale experiments would provide realistic and invaluable information for risk assessment and isolation requirements for GM oilseed rape varieties.

There is presently much interest in applying data from outcrossing experiments and pollen movement from trials to the modelling and prediction of transgene escape from large field releases. Studies of dispersal have often described both pollen and seed dispersal from plants as being strongly leptokurtic (Levin and Kerster 1974), meaning that most pollen and seed are transported or fall close to their original sources, with occasional long distance transportation events.

These leptokurtic distribution patterns, which are the results of numerous factors including the foraging behaviours of insect pollinators, physical attributes of pollen, crop densities and field

shape, or local atmospheric conditions (Levin and Kerster 1974) have been fitted to an exponential power function or Weibull probability function (Bateman, 1947, Kareiva, Morris, Jacobi, 1994, Morris, Kareiva, Raymer, 1994). In general the exponential function has been applied successfully depicting dispersal and gene flow at the crop or farm scale, whereas the Weibull function appears more suited to data for short distance events. Alternative models utilised by Lavigne, Godelle., Reboud, Gouyon, (1996) and Tufto, Engen, Hindar, (1997) produced models of pollen dispersal based on a consideration of Brownian motion in 3 dimensions to describe pollen deposition. Under some conditions such as wind strength varying in direction during an experiment, this mechanistic method gives a better fit than the descriptive exponential power function (Tufto *et al.* 1997). McCartney and Lacey 1991 modelled pollen dispersal from oilseed rape using a steady state advection diffusion model (McCartney and Fitt, 1985) which had been used for modelling dispersal of fungal spores above cereal crops, the equation predicted the change in concentration with height and distance downwind of the pollen source. The development of models describing the dispersal of GM pollen from oilseed rape are clearly of value to risk assessment programmes. Risk assessment of GM crops aims to understand the consequences of the release of GMO's and to quantify the risks to the agricultural environment associated with such releases.

1.8.3 Gene flow to related species

The spread of transgenes by introgression has been identified as one of the risks of growing genetically modified plants. The concern is that introgression of transgenes into wild plants will make them more invasive or weedy (Raybould and Gray, 1993). The importance of gene flow between crops and their wild relatives has been widely researched in Europe and is a critical issue for the adoption of transgenic crops.

An essential part of risk assessment of genetically modified crops is assessment of the potential for transgene transfer and subsequent introgression from a GM crop to related wild

species. The impact a transgene has on a related species will depend on the trait coded for by the gene, and the biology of the plant i.e. the ability to survive and reproduce, and whether the transgene provides a selective advantage or is harmful to human health or the environment (Jorgensen and Andersen, 1994; Scheffler and Dale, 1994). Several factors have been identified as influencing the opportunity for hybridisation between species; physical distance between species, synchrony of flowering, method of pollen dissemination, specific parental genotypes, direction of cross, influence of male sterility and environmental factors (Scheffler and Dale, 1994). Scheffler and Dale (1994) reviewed the literature and discussed the relative ranking of species by their ability to form hybrid progeny when crossing with *B. napus*, this review is summarised in Table 6. Evidence of spontaneous hybridisation has been shown with species such as *Brassica rapa, Brassica juncea*, *Brassica oleracea* and *Hirschfieldia incana* (Table 6).

Brassica rapa is perhaps the most important species that *B. napus* can hybridise with spontaneously in the U.K. and other areas of Northern Europe. *Brassica napus* (genome AACC; 2n=38) and non-cultivated forms of *Brassica rapa* (genome AA; 2n=20) hybridise relatively easily and due to their close genomic relationship (Jorgensen and Andersen, 1994; Jorgensen, Andersen, Landbo, Mikkelsen, 1996 and Jorgensen, Andersen, Hauser, Landbo, Mikkelsen, Ostergard, 1998; Scott and Wilkinson, 1998).

Wild *B. rapa* is an economically important weed in temperate regions of Eurasia, North America, South Africa, Australia, New Zealand and some European countries such as Denmark (Jorgensen, 1999). Although it is found in the UK it is not of major agricultural significance, it is found sporadically in areas such as Humberside in the east of England, where it locally constitutes a significant agronomic problem in oilseed rape fields (Beeney, pers. comm. 2000). It is also found in semi-natural locations along the Thames valley (Scott and Wilkinson, 1999). However, in Scotland, N. Europe and Canada where a significant area of turnip rape (*Brassica rapa*) is grown, the problem

of transgene introgression and persistence may be more significant particularly as turnip rape volunteers are already a common problem in oilseed rape fields.

Spontaneous hybridisation and backcrossing between *Brassica napus* and weedy *Brassica rapa* has been shown to occur in the field (Jorgensen and Andersen, 1994; Jorgensen *et al.*, 1996). Hybrids have the full complement of the *B. rapa* genome, the fertility of some of the F_1 hybrids is nearly as high as that of pure *B. rapa* (Jorgensen *et al.*, 1996; Hauser, Shaw, Ostergard, 1998). Although in some cases the hybrids have poor fertility (Jorgensen and Andersen, 1994). In agricultural crop rotations the dormancy and germination of *B. napus* x *B.rapa* hybrid seeds could limit gene flow because dormancy within a population will disperse germination in time. The periodic emergence of seedlings from a seed bank results in flushes of plants, which will ensure some flowering and seeding adults in an arable field (Mortimer, 1990). Hybrid seeds are generally non-dormant (in crosses made in both directions) whereas *B. rapa* exhibits heteroblasty, however, dormancy can be restored in seeds from the first backcross to *B. rapa* (Landbo and Jorgensen 1997). Due to the cropping system of oilseed rape (normally 2-5 year rotations) the lack of dormancy in hybrid seeds means that it is less likely that hybrid plants will reach maturity as seeds may germinate under unfavourable conditions for survival (Jorgensen, 1999). However when hybrid germination coincides with germination of wild *B. rapa* there is certainly the potential for backcrossing to wild *B. rapa* to occur.

The high sexual compatibility between *B. napus* and weedy *B. rapa* implies that hybridisation between herbicide tolerant *B. napus* and cultivated *B. rapa* (turnip rape/bird rape) is inevitable where fields of these crops are grown in close proximity or where feral or volunteer populations exist in the same field. There are reports of spontaneous hybridisation between cultivated *B. rapa* and *B. napus* in the field e.g. Bing, Downey, Rakow, (1996) and Downey, (1999). The implications of this are significant, since there would potentially be a reduction in the efficacy of the herbicides used on HT oilseed rape (or other HT crop systems) in these fields. In common with

the weedy species, hybrids would however only become more competitive or invasive in both cultivated or natural habitats where there was a selection pressure imposed with the herbicide in question and they produced fertile progeny that expressed the transgene in question. Hybridisation between commercial, GMHT oilseed rape crops and non-tolerant turnip rape crops may enable hybrids to hybridise and backcross more freely with the cultivated and weedy form of *Brassica rapa* and other related species (Brown and Brown, 1996).

The introgression of a transgene such as a herbicide tolerance gene, conferring tolerance to a widely used broad spectrum herbicide into a related weed species, feral or volunteer population may result in altered fitness in an agricultural situation. The weed species, like the crop plant would only become invasive under the selective conditions of the specific herbicide where normal competition was eliminated, such as those in arable fields or field margins (Downey, 1999). It has been suggested that the introgression of transgenes conferring enhanced fitness characters such as pest or disease resistance into wild plants may make them more competitive or invasive in natural habitats. The fitness of wild relatives containing introgressed genes from oilseed rape will depend on both the genes introgressed and the recipient ecosystem (Jorgensen, 1999).

1.8.4 Population dynamics of volunteer oilseed rape

Large numbers of seeds are left in the field after harvest of oilseed rape crops, seed loss at harvest has been estimated at between 0.1 and 0.5 t/ha (Bowerman, 1984; Vera, McGregor, Downey, 1987; Lutman, 1993; Brown, Erickson, Davis, Brown, 1995; Price, Hobson, Neale, Bruce, 1996). Lutman (1993) concluded that this amount of seed shed would equate to approximately 10000 seeds/m^2 in the field. This amount of seed could clearly form a significant seed bank even if only a small proportion of the seeds persists.

Volunteer oilseed rape can cause significant problems, particularly in broad-leaved crops (Knott, 1993, 1995) as there are few selective herbicides that can be used effectively in these crops.

Oilseed rape volunteers tend to germinate over a long period which can make the optimal timing of herbicide application difficult (Lutman, 1993). Volunteer populations occurring in rape fields can also compete strongly with crop plants and could significantly reduce the overwintering potential as the small plants resulting from competition may fail to survive severe frosts and damage by vertebrate herbivores (Blanck, 1989). Additional problems may be encountered when a range of oilseed rape varieties are grown over several years, particularly with the development of varieties with different quality traits which could potentially cross pollinate and contaminate subsequent rape crops. The persistence of rapeseeds in soil also has implications for risk assessments of genetically modified varieties following commercial release by allowing transgenes to disperse both temporally and spatially. Oilseed rape has been shown to hybridise spontaneously with several related species such as *Brassica rapa* (Jorgensen and Andersen 1994; Jorgensen *et al.*, 1996, 1998; Landbo, Andersen, Jorgensen, 1996; Scott and Wilkinson 1998) and *Raphanus raphanistrum* (Chevre, Eber, Baranger, Renard, 1997). The persistence of rapeseed and development of volunteer or feral populations could potentially add further to the spread and persistence of transgenes by introgression to related species.

High population densities of herbicide tolerant oilseed rape volunteers may cause difficulties with weed and volunteer management in rape and other broad-leaved crops. If a crop of conventional rape becomes contaminated with glufosinate or glyphosate tolerant volunteers the efficacy of commonly used crop desiccants (such as glufosinate and glyphosate) could be compromised. HT volunteers may also cause management problems in rotations where sequences of HT crops are grown such as HT sugar beet or maize. Populations of herbicide tolerant volunteers could allow further spread and persistence of the transgene(s) outwith the original release site and increase numbers of tolerant seed returning to the soil due to cross-pollination with the crop.

There are a wide range of factors that may influence the survival, spread and establishment of volunteer populations, including pollen movement, seed dispersal, climatic conditions, crop

rotation sequences and turnover of the seed bank. Models of the population dynamics of oilseed rape volunteers have been developed (Colbach, Meynard, Clermont-Dauphin, Messean, 1999; Pekrun, Lane, Lutman, 1999) which encompass variables such as seed loss at harvest, crop rotation, soil cultivation, moisture distribution in the soil and the level of volunteer control in each crop in the rotation. Colbach *et al.*, (1999) developed a model to evaluate the influence of cropping systems on gene dispersal from transgenic crops to volunteers. The model incorporated several variables including regional cropping pattern and cultivation techniques. Simulations enabled the identification of cropping systems where there was low risk of transgene escape.

Table 6. Relative ranking of species by their ability to form hybrid progeny when crossed with *B. napus* (scale 1-9). (Adapted from Scheffler and Dale, 1994).

Species	Status and distribution	Hybridisation F2 progeny produced	Hybridisation Backcross progeny produced	Rank	Reference Examples
B. rapa / B.campestris (2*n* = 20) Wild turnip	Locally abundant on roadsides, arable fields, waste ground. Probably native in most of Europe.	Yes	Yes	1	Jorgensen and Andersen, 1994 Scott and Wilkinson, 1998
B. juncea (2*n* = 36) Brown mustard Indian mustard	A casual of fields, roadsides, tips and cities. Introduced to Europe.	Yes	Yes	2	Bing *et al*, 1996 Jorgensen *et al.*, 1996 Jorgensen, 1999
B. oleracea (2*n* = 18) Wild cabbage	Probably native on Mediterranean coasts from Spain to Greece. Widely introduced elsewhere, and naturalised on sea cliffs in France, UK and Germany.	Yes	Yes	3	Robbelen, 1966 Chiang *et al.*, 1977
B. nigra (2*n* = 16) Black mustard	Sea cliffs, roadsides, fields. Probably native through most of W. Europe to Turkey and C. Europe, southern Scandinavia.	?	Yes	5	Bing *et al.*, 1996
H. incanal B. adpressa (2*n* = 14) Hoary mustard	Common on waste ground, railways, sand dunes. Native around the Mediterranean to the Near East. Introduced to N. Europe.	No	Yes	6	Lefol *et al.*, 1996 Chevre *et al.*, 1999
R. raphanistrum (2*n* = 18) Wild radish, Runch	Casual of fields, gardens, docks etc. Probably a native of Europe.	No	Yes	6	Darmency *et al.*, 1995 Chevre *et al.*, 1999
S. arvensis (2*n* =18) Charlock	A very common weed of fields, riverbanks, roadsides, waste ground. Probably native in Europe.	No	No	8	Lefol *et al.*, 1996 Chevre *et al.*, 1996

1.8.4.1 Secondary dormancy characteristics of volunteer oilseed rape

In combination with high seed losses before harvest, secondary dormancy is a major contributing factor to the development of oilseed rape as a volunteer weed. Oilseed rape seeds have no innate dormancy at maturity (Lutman 1993) most seed will germinate given conducive environmental conditions, however, if seeds are buried by cultivation they can acquire secondary dormancy and can survive for several years in the seedbank (Pekrun, Potter, Lutman, 1997a, Pekrun and Lopez-Granados 1995). Schlink (1995) found that rape seed persistence is due to induced dormancy and that burial depth, duration of burial and choice of cultivar influence the number of persisting seeds.

Secondary dormancy can be induced under experimental conditions by exposing seeds to sub-optimal germination conditions in darkness (Pekrun and Lopez Granados 1995; Pekrun *et al.*, 1997a, Pekrun, Lutman, Baeumer, 1997b, Pekrun, Lopez-Granados, Lutman, 1997c). Seeds will also develop light sensitivity during prolonged exposure to darkness in a semi-imbibed state, this can been induced artificially by imbibition in an osmotic solution in the dark, exposure under light conditions results in no induced dormancy (Pekrun *et al.*, 1997b, Pekrun, Hewitt, Lutman, 1998). Temperature also appears to be significant in relation to dormancy of rapeseeds. Pekrun *et al*, (1997b) found that germination of dormant seeds was maximised when exposed to light and fluctuating temperatures and was prolonged by constant temperature and dark conditions. Enforced seed burial experiments have demonstrated a similar trend to tests carried out in the laboratory, when seeds are buried deeply in soil showed increased persistence (Schlink, 1995).

Varietal differences in the development of secondary dormancy have also been found in both winter and spring rape genotypes (Pekrun *et al.*, 1997a). Laboratory tests carried out on 47 cultivars showed a wide range of response ranging from 2% dormant seed in c.v. Falcon to over 50% in c.v. Apex. Although these tests were carried out under controlled conditions varietal choice could have a substantial effect on following volunteer populations.

1.8.4.2 Control of volunteer oilseed rape

The problems associated with volunteer oilseed rape populations occurring in following broad-leaved and other crops has been discussed in 1.8.4. There are two main reasons why relatively large seed banks of oilseed rape can occur. Seed losses before and during harvest can be very large with seed losses estimated at 200-500kg/ha (Lutman, 1993; Bowerman 1984, Price *et al.*, 1996). The second reason is that seeds can persist in the field due to the induction of secondary dormancy (Pekrun *et al.*, 1997a).

There are several cultural methods of control of volunteer oilseed rape, primarily based on preventative rather than curative measures. The choice of oilseed rape cultivar may have important implications for persistence, a number of cultivars have been studied, the ability to persist appears to vary significantly between genotypes (Pekrun *et al.*,1997a; Pekrun *et al.*, 1997c). Although, the harvesting technique does not appear to greatly influence seed losses, the optimum technique will depend on weather conditions at harvest (Bowerman, 1984; Brown *et al.*, 1995), the timing of harvest having the most significant impact on seed loss particularly if the crop is over ripe or harvest is delayed (Price *et al.*, 1996).

Chemical control of volunteer oilseed rape and other broadleaved weeds is relatively easy in cereal crops, there are a number of compounds that will provide effective control e.g. amidosulfuron, metasulfuron + thifensulfuron, tribenuron, carfentrazone (BCPC, 1999). In broad-leaved crops, rape volunteer control is more difficult and there are fewer chemicals for effective control. In potatoes for example, metibuzin and rimsulfuron are recommended (BCPC, 1999). In rape crops, volunteers cannot be controlled by chemicals, although the introduction of herbicide tolerance systems may provide an effective tool for the control of volunteer rape in HT rape crops. However, in the longer term, herbicide tolerant types may cause problems particularly if other herbicide tolerant crops are being utilised in rotations. Derksen *et al.*, (1999) have reported that in cropping systems in Canada

volunteer herbicide (glyphosate) tolerant oilseed rape caused problems particularly in reduced tillage systems where glyphosate is used pre drilling of following crops.

Soil cultivations following harvest are perhaps the most influential practices for controlling rape volunteers. Cultivation controls the amount of dormant rapeseed returning to the soil. In dry conditions seeds may become dormant when incorporated into the soil, the more deeply they are cultivated the more likely they are to persist (Pekrun, Lutman, Lopez-Granados, 1996; Pekrun *et al.*, 1998). Because seeds lying on the soil surface are less likely to acquire dormancy, post harvest cultivations should only begin after the conditions for germination are optimal so that most of the seeds will germinate thus reducing the amount of viable seed returned to the seed bank (Lutman, 1993).

1.8.5 Invasiveness of transgenic oilseed rape

The weediness or invasiveness of a herbicide tolerant plant depends largely on the interaction between the intrinsic characters of the plant, in combination with the specific habitat that the plant lives in (Keeler, 1989; Tiedje, Colwell, Grossman , Hodson, Lenski , Mack, Regal, 1989). The main concern related to herbicide tolerance is whether there is a non-specific enhancement of fitness of the plant due to the presence of the specific herbicide tolerance construct. For example, in the absence of spraying with glufosinate, glufosinate tolerance is unlikely to contribute to weediness. It is relatively unlikely that selective concentrations of glufosinate would be found outwith commercial fields, except perhaps in field margins.

Several comparative experiments have investigated the invasiveness of GM and non-GM lines of oilseed rape. The competitiveness of GM glufosinate tolerant rape was observed by Crawley, Hails, Rees, Kohn, Buxton, (1993) under a range of habitats and climatic conditions with no selective pressure from the herbicide. The differences observed in these particular experiments showed that GM lines were less invasive than non-GM lines.

A seed burial experiment comparing the persistence of seeds of GM spring oilseed rape lines modified for high laurate and high stearate production and their parent lines suggest that oil modified lines were no more likely to persist in the soil than conventional oilseed rape (Booth, Walker, Whytock, Sovero, 1996). Similar work designed to assess the comparative persistence of GM high stearate oilseed rape and non-GM parent lines suggest that there would be little increased risk of persistence of GM lines (Linder and Schmitt, 1995).

A recent study by Linder, (1998) determined the potential persistence of the seed performance of *Brassica napus* (genetically modified high stearate/high laurate types) wild B. *rapa* and *B. rapa* x *B. napus* hybrids (high laurate type). Results showed that high stearate *B. napus* expressed higher levels of induced dormancy than controls particularly under conditions of low nutrients. Under the same conditions high laurate *B. napus* also exhibited lower germination rates than parental controls. Further alteration of environmental factors (darkness + high nutrients) demonstrated that high laurate *B. napus* had higher overall dormancy than its control. As previously demonstrated, (Landbo and Jorgensen, 1997) *B. napus* x *B. rapa* hybrids exhibited low levels of dormancy in contrast to the *B. rapa* parent which showed low levels of germination and high levels of dormancy.

Sweet *et al.,* (1997) investigated the invasiveness of GM and non-GM lines of glufosinate tolerant oilseed rape by simulating seed shed into field margins. Predictably, neither the GM nor the non-GM rape established feral populations. Predation by molluscs and vertebrates were probably the principal limiting factor in establishment. Monitoring volunteer and feral populations in following crops and in field margins close to release sites where GM herbicide tolerant and high stearate lines have been grown have not detected an increase in volunteer management problems (Norris, Simpson, Sweet, Thomas, 1999). At several sites neither GM or non-GM volunteers have been detected. In certain cases where both GM and non-GM lines had been grown at the same site, lower numbers of GM volunteers were detected than non GM up to three years post-GM release.

The results of studies investigating the comparative persistence or invasiveness of GM and non-GM *B. napus* varieties predictably seem to be applicable only to the specific trait that is being examined. Certain novel traits will enhance particular fitness components of the crop plant or weedy hybrid in particular environments. It is also likely that the GM trait may have different effects depending on the genetic background with which it is associated, in the same way as non-GM varieties differ in their dormancy characteristics.

1.9 Objectives

Crop plants with herbicide tolerance constitute the first major introduction of GM plants. The long term benefits and risks of the deployment of GM crops in agricultural systems needs further investigation. The successful utilisation of herbicide tolerant crops in current farming systems in the U.K. will require an understanding of the agronomy and ecology of these crops.

Gene flow from GM HT oilseed rape has been widely researched, although there has been less focus on the agricultural consequences of gene flow. The scale of experimentation in gene flow research has been highlighted as a limiting factor when translating data from small plot trials to large farm scale situations. The persistence of transgenes in the environment via persisting rape seed in the seed bank, feral and volunteer populations also requires investigation. There is also concern about the indirect effects that GM HT crops may have on biodiversity due to the specific reliance of these cropping systems on broad-spectrum herbicides and the resulting shifts in weed species populations.

This research investigates some of the main areas of environmental and agricultural concern when growing herbicide tolerant oilseed rape; the work was undertaken with the following objectives:

To determine the:

1. Rates at which herbicide tolerance genes are likely to transfer from crops and plots to other oilseed rape varieties and crops.

2. Rates at which herbicide tolerance genes are likely to transfer from volunteer and feral populations of rape to non HT crops.

3. Effects of herbicides used on HT crops in terms of weed control and plant diversity in arable ecosystems.

4. The nature of weediness of HT or multiple herbicide tolerant rape in agricultural environments compared to non-HT rape.

2. MATERIALS AND METHODS

2.1 OUTCROSSING BETWEEN HERBICIDE TOLERANT AND CONVENTIONAL OILSEED RAPE CULTIVARS

2.1.1 Cross pollination between variety trial plots in National List genetically modified herbicide tolerant winter oilseed rape trials (harvested in 1997 and 1998)

2.1.1.1 Description of pollen and seed sample sources and location of trials

Levels of hybridisation and production of viable seed were assessed between trial plots of genetically modified herbicide tolerant (GMHT) winter and conventional oilseed rape varieties growing in National List GM (NLGM) trials in two harvest years (1997 and 1998). Three UK trial sites were selected in 1997 and two in 1998. The main objective of the study was to provide an indication of varietal differences in cross pollination rate.

Plots in all NLGM trials studied were approximately 40m^2 (2m x 20m) and were harvested after swathing by standard small plot combine harvesters.

a) NL trials sampled in 1997

National List (NL) trials of GM herbicide tolerant winter oilseed rape in 1996/97 consisted of three replicates containing two GM varieties tolerant to the broad-spectrum herbicides, glufosinate-ammonium or glyphosate. The trials also contained five non-tolerant conventional control varieties (Synergy, Express, Nickel, Falcon and Apex). The trial sites are shown in Table 7.

Table 7. Location of National List winter oilseed rape sites sampled 1996/97

Site	National List non-GM	National List GM
Caxton (Cambridgeshire)	*	*
CocklePark (Northumberland)	*	*
Bridgets (Hampshire)	*	*
Wye (Kent)	*	

b) NL trials sampled in 1998

National List (NL) trials of GM herbicide tolerant winter oilseed rape in 1997/98 consisted of three replicates containing 7 GM varieties tolerant to the broad-spectrum herbicides glufosinate-ammonium or glyphosate. Four varieties were tolerant to glufosinate ammonium and 3 varieties were tolerant to glyphosate. The trials also contained five non-tolerant conventional control varieties (Synergy, Alpine, Pronto, Falcon and Apex). The trial sites are shown in Table 8.

Table 8. Location of National List winter oiiseed rape sites sampled 1997/98

Site	National List non-GM	National List GM
	*	*
CocklePark (Northumberland)		
Bridgets (Hampshire)	*	*

2.1.1.2 Seed sampling - NLGM trials 1997 and 1998

The required weight of seed was sampled from a bulk of 200g. Seed samples were tested from all the varieties in the all NLGM trials to screen for single and double herbicide tolerance in a glasshouse test.

2.1.1.3 Growing on seed samples and herbicide tolerance testing of oilseed rape seedlings

a) Growing on seed samples (1997 seed samples)

Seed from NLGM trials grown in 1996/97 were tested by sowing two seeds per cell in 308 cell trays (Hassey) containing a multi-purpose peat based potting compost (Shamrock). Seeds were sown using a semi-automated sowing machine to give an average of 600 plants per tray. Two replicates of 600 plants per variety were randomly arranged on glasshouse benches.

b) Growing on seed samples (1998 seed samples)

Seed samples harvested from NLGM trials grown in 1997/98 were tested by hand sowing 1000 seeds in seed trays (40cm x 50cm, FYBA) containing a multi-purpose peat based potting compost

(Shamrock). Two replicates of 1000 plants per variety were randomly arranged on glasshouse benches. 1000 seed samples were counted using an automated digital seed counter (Pfeuffer, Contador)

c) Herbicide tolerance testing of oilseed rape seedlings

Seed samples of both GM and conventional varieties from the NLGM winter rape trials from both harvest years were grown in a glasshouse (18-22°C; Supplementary lighting 400w HPS 16hr photoperiod) with a non-herbicide tolerant control to check the efficacy of herbicide treatments (a conventional non-tolerant winter oilseed rape variety; cv. Falcon).

Plants were sprayed at growth stage 1,2 (Sylvester-Bradley and Makepeace, 1984) with either glufosinate-ammonium (200g/l) at 400g a.i./ha or glyphosate (360 g/l) at 720g a.i./ha both herbicides in the case of seed samples from GMHT varieties) using a hand sprayer (Hozelock, Polyspray P2)

The numbers of surviving plants were assessed approximately 7 and 14 days after treatment for glufosinate and glyphosate respectively. Survivors from each replicate were re-treated with the same dose rate of herbicide at growth stage 1,3. Surviving plants were counted approximately 7-14 days after the second treatment for glufosinate and glyphosate to confirm their tolerance.

2.1.1.4 Data analysis

The data from the herbicide screening tests of seedlings were converted into an outcrossing frequency by calculating the mean percentage of herbicide tolerant plants out of the total number of seedlings per glasshouse test. Outcrossing frequencies were then presented as a percentage on the original trial plan in the results. The GM herbicide tolerant pollen sources and the plots of the varietal association cv. Synergy are highlighted on the trial plans. A preliminary analysis of the testing system showed no statistical differences between replicates in the herbicide tests thus no formal analysis is presented.

2.1.2 Cross pollination between NLGM and non-GM National List trials (harvested in 1997 and 1998)

2.1.2.1 Description of pollen and seed sample sources and location of trials

Samples of seed were harvested from plots in National List conventional variety trials which were grown adjacent to NLGM trials in 1997 and 1998. Four UK trial sites were selected in 1997 and two in 1998. The objective of the study was to investigate varietal differences in outcrossing and also to determine the effects on distance on cross pollination rate.

NL trials were separated from NLGM trials by a 'pollen barrier' of a non-GM variety of oilseed rape (minimum of 6m wide). Seed samples taken in both years were taken by removing the required weight of seed from a bulk of approximately 200g which had been harvested previously by a small plot combine harvester.

a) NL trials sampled 1997

Samples of harvested seed were taken from four (non-GM) NL sites (Table 7) grown in 1996/97. Samples from non-GM varieties were selected from linear transects of plots nearest to the GM trial, 50m, and 100m or the furthest point from the GM trial.

b) NL trials sampled 1998

At both sites (Bridgets and Cockle Park), in order to test whether varietal associations or restored hybrid types showed different levels of cross pollination, samples of these varieties were selected in addition to the two adjacent conventional plots of rape for comparison. At Cockle Park, samples of seed from non-GM varieties were selected from all plots in the first 20m nearest to the GMNL trial and also from linear transects of plots at 10m intervals to the furthest edge of the trial.

2.1.2.2 Trial design and establishment: testing non-GM National List seed samples for herbicide tolerance

Seed samples were grown in field plots, drilled using a HEGE 90, 12 row plot drill at a depth of 2 cm. Plots were rolled after drilling using a 12m Cambridge roll. The final field trial test plot size in 1998 was 6m x 1.8m and 9m x 1.8m in 1999.

a) NL trials sampled 1997

Seed samples collected from the four (non-GM) NL trials harvested in 1997 (Table 7) were sown in field plots in a randomised block design replicated three times with herbicide susceptible control plots to indicate the efficacy of herbicide treatments (a conventional winter rape variety cv. Express). The seed rate was set to give an average of 600 plants per plot (trials were sown on two dates 23.4.98 and 20.5.98).

b) NL trials sampled 1998

Seed samples collected from the two NL trials harvested in 1998 (Table 8) were sown in field plots in a randomised block design replicated twice with herbicide susceptible control plots to indicate the efficacy of herbicide treatment (a conventional winter rape variety cv. Express). Seed rate was set to give an average of 1600 plants per plot (trials were sown on 30.4.99)

2.1.2.3 Growing on seed samples and herbicide tolerance testing of oilseed rape seedlings

Field trials were duplicated in both years in order to allow testing for both glufosinate and glyphosate tolerance. Each identical trial was sprayed with either glufosinate-ammonium (200 g/l) at 600g a.i./ha or glyphosate (360 g/l) at 1440g a.i./ha when plants were at the 3-5 leaf stage (GS 1,3-1,5) using a tractor mounted sprayer (Sprayranger 24). The numbers of surviving plants were assessed approximately 7 and 14 days after treatment for glufosinate and glyphosate treatments respectively. Surviving plants from each replicate were re-treated with herbicide at growth stage 1,5, surviving plants were re-counted approximately 7-14 days after the second treatment for glufosinate and glyphosate respectively to confirm their herbicide tolerance.

2.1.2.4 Data analysis

The data from the herbicide screening tests of seedlings were converted into an outcrossing frequency by calculating the mean percentage of herbicide tolerant plants out of the total number of seedlings per glasshouse test. A preliminary analysis of the testing system showed no statistical differences between replicates in the herbicide tests thus no formal analysis is presented.

Outcrossing frequencies were summarised for each site, as an example of the data, the results from Cockle Park from the two harvest years 1997 and 1998 are presented in the results.

2.1.3 Outcrossing between field scale areas of herbicide tolerant and other winter oilseed rape cultivars

2.1.3.1 Establishment of experimental plots and source of plant material

Blocks of herbicide tolerant and conventional winter oilseed rape varieties were established in adjacent areas of approximately 0.8ha (92m x 92m) in a 10 hectare field (Figure 2) at NIAB, Cambridge, U.K. The plant material used is described in Table 9 below. Conventional winter rape plots were split into two varieties (c.v. Apex and Synergy) to determine potential genotypic 'susceptibility' cross pollination (plot size 46m x 92m) (Figure 2). All Plots were drilled at normal recommended seed rates for restored hybrid, varietal associations and conventional types (70, 70, 120 seeds/m^2 respectively) using a standard 24 row farm drill (Amazone 24) at 2cm depth.

Table 9. Source of plant material for experiment 2.1.3 - winter oilseed rape herbicide tolerant (HT) and conventional varieties

Variety type	Herbicide tolerance	Variety - code/name	Herbicide tolerant system supplier
Transgenic restored hybrid (RH)	Glufosinate-ammonium (Liberty)	LL1	PGS/Aventis
Transgenic variety	Glyphosate (Roundup)	RR1	Monsanto
Non-transgenic variety	Imidazolinone (Imazamox)	IMI	Pioneer Hi-bred/ Cyanamid
Varietal association (VA)	Non-tolerant	Synergy	CPB Twyfords UK
Conventional variety	Non-tolerant	Apex	Advanta SeedsUK

2.1.3.2 Management of field experiment 2.1.3

The field experiment was maintained using standard agronomic practices described in Table 10. Appropriate herbicides were applied to the herbicide tolerant varieties and a standard herbicide programme was used for conventional variety plots (Table 16).

2.1.3.3 Additional records of weather conditions, crop growth stages and incidence of pollinating insects

a) Throughout the growing season assessments of growth stages of the individual varieties were taken to estimate synchrony of flowering between plots. Growth stages of oilseed rape are according to Sylvester-Bradley and Makepeace (1984). Weather conditions were recorded at the NIAB meteorological station on a daily basis from the onset to the end of the main flowering period (9.4.99-27.4.99).

a) Crop density was measured for each plot by counting twenty randomly selected 1m row lengths in November 1998, counts were converted into plants per m^2.

b) The heights of varieties were recorded when the majority of the rape plants were at growth stage 4.8 (27.4.99). Twenty plants from each plot were measured from soil level to the tip of the main raceme using a 2m steel rule.

c) The incidence of pollinating insects (honey bees/bumble bees) was recorded for a period during flowering. Transects were walked along the edges of plots on three occasions when the oilseed rape was in full flower (6.5.99, 12.5.99, 19.5.99). The number of bees in a 90m long, 1-2m wide strip was counted for each plot along 4 transects.

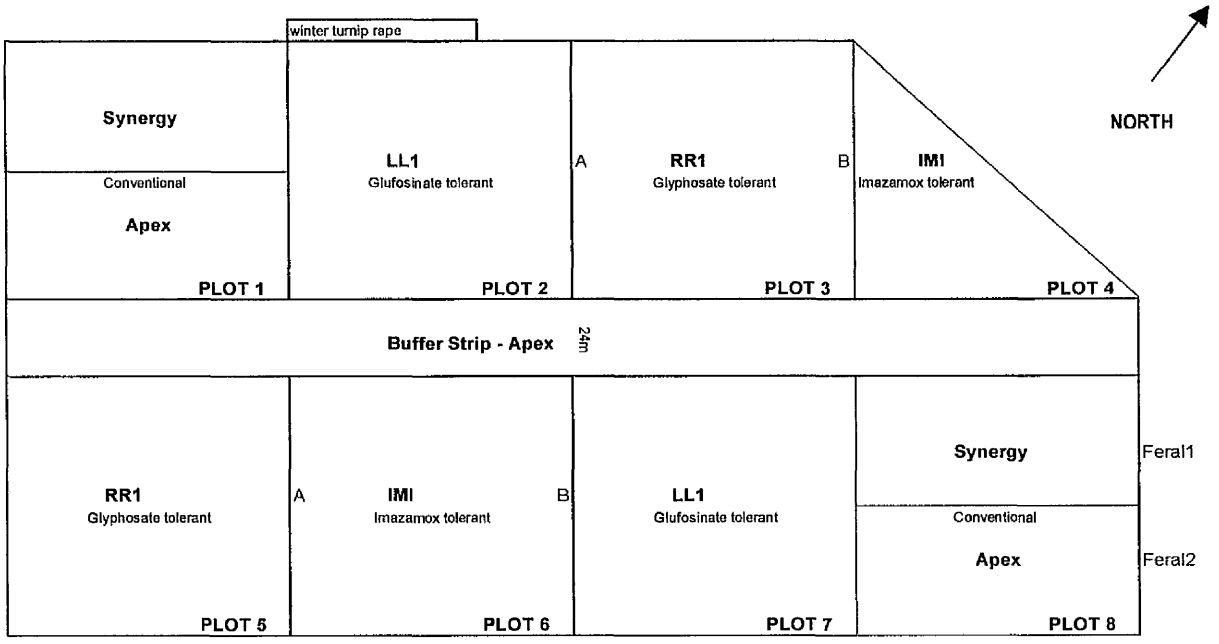


Figure 2. Field experiment (2.1.3) layout of herbicide tolerant and conventional winter oilseed rape variety plots, winter turnip rape and simulated feral populations at NiAB, Cambridge, U.K

Legend

1. Each main plot was 92m x 92m
2. Synergy and Apex plots were 46m x 92m
3. Winter turnip rape plot (c.v. Debut) was 24m x 50m (Section 2.1.4)
4. A and B were the sides of plots from which samples were taken in both directions
5. Feral 1 and 2 were feral rape populations consisting of approximately 100 flowering glyphosate tolerant winter oilseed rape plants (Section 2.2.2)

2.1.3.4 Seed sampling and harvesting procedure

a) Location of sampling points

Three transects across each 92 x 92m block were sampled at 1.5m, 6.5m, 11.5m, 16.5m, 21.5m, 41.5m, 61.5m, 81.5m from the adjacent oilseed rape variety. Conventional plots were divided into two winter rape varieties in equal areas, and were sampled at more frequent distances; 1.5m, 6.5m, 11.5m, 16.5m, 21.5m, 26.5m, 31.5m, 41.5m, 51.5m, 61.5m, 71.5m, 81.5m, 91.5m. To assess whether the late or early period of flowering was more sensitive to cross pollination, samples from conventional plots were also split into two halves, giving an upper and lower raceme seed sample.

b) Seed harvesting and sampling

The main raceme was removed from 20 plants within a $1m^2$ quadrat at each sample point by hand cutting with secateurs (Plate 1, Appendix 1). Racemes were collected in large cloth bags which were labelled and sealed with string. Samples were dried in cloth bags in the glasshouse on wooden slatted benches for 10-14 days (18-22°C). Seeds were removed from pods by crushing the racemes in cloth bags and hand sieving (1mm slot sieve) until the seed sample was free of debris. The bulk seed sample was thoroughly mixed by hand and sub-sampled to test two replicates of 1000 seeds per sample. Samples of 1000 seeds were prepared using a digital automated seed counter (Pfeuffer, Contador).

2.1.3.5 Growing on seed samples and herbicide tolerance testing of oilseed rape seedlings

Seed samples of GM herbicide tolerant and conventional winter oilseed rape varieties were grown in plastic seed trays (40cm x 50cm, FYBA) containing a multi-purpose peat based potting compost (Shamrock) in glasshouse conditions (18-22°C; Supplementary lighting 400w HPS 16hr photoperiod). A herbicide susceptible control was grown with each herbicide tolerance test to evaluate the efficacy of the herbicide treatments (a conventional non-tolerant winter oilseed rape variety; cv. Falcon). Plants were sprayed at growth stage 1,2 with either glufosinate-ammonium (200g/l) at 400g a.i./ha, glyphosate (360 g/l) at 720g a.i./ha or imazamox (40g/l + wetter) at 70g a.i./ha using a hand sprayer (Hozelock, Polyspray P2).

The numbers of surviving plants were assessed approximately 7 and 14 days after treatment for glufosinate, glyphosate and imazamox respectively. Survivors from each replicate of 1000 plants were re-treated with herbicide at growth stage 1,3. Surviving plants were counted approximately 7-14 days after the second treatment for glufosinate, glyphosate and imazamox respectively.

Table 10. Field experiment (2.1.3) establishment and farm operations details*

Date	Standard farm operations for all plots
1.09.98	Subsoiling
2.09.98	Plough (depth 30cm)
2.09.98	Power harrow (Dynadrive)
4.09.98	Cambridge roll (Cousins)
16.09.98	Drilling winter oilseed rape plots 1,5 and 6 (Amazone 24 drill)
17.09.98	Drilling winter oilseed rape plots 2,3,4,7 and 8 (Amazone 24 drill)
18.09.98	Cambridge roll (Cousins)
22.09.98	Apply slug pellets (Thiodocarb 4% w/w)
25.09.98	Applied irrigation (25mm)
20.03.99	Apply fungicide (carbendazim + flusilazole 125:250 g/l @ 0.8l/ha) (Sprayranger 24)
24.03.99	Apply fertiliser (26:13:0) (Nodet spreader)
9.07.99	Swath all rape plots
13.07.99	Combine harvest (Dominator 36)
27.08.99	Disced (Pettit discs)

*see Table 16 for details of herbicide applications and timings

2.1.3.6 Data analysis

Data from herbicide tolerance tests were used to estimate frequencies of outcrossing at each sample distance. The data has been presented as percentage out of the total number of herbicide tolerant seeds recovered at the range of sample distances in each plot. The mean data from individual transects from each plot have been presented to show the overall decline in herbicide tolerant seeds as a function of distance. The data was also used in to compare two dispersal models for their fit to describe the relationship between cross pollination and distance from source (Section 3.3).

2.1.4 Outcrossing between genetically modified glufosinate tolerant winter oilseed rape (*Brassica napus*) and a conventional winter turnip rape variety (*Brassica rapa* cv. Debut)

2.1.4.1 Establishment of experimental field plot winter turnip rape (cv. Debut)

An area (24m x 50m) of a common commercially grown variety of winter turnip rape (cv. Debut) was established adjacent to a large area (90m x 90m) of GM herbicide tolerant winter oilseed rape, tolerant to the broad-spectrum herbicide glufosinate ammonium (Liberty) in autumn 1998 at NIAB, Cambridge, U.K (Figure 2).

Plots were drilled at normal seed rates (180 seeds/m^2) using a standard 24 row farm drill (Amazone 24) at 2 cm depth, the trial was maintained using standard agronomic practices (Table 10 and 11) weather conditions were recorded at the NIAB meteorological station on a daily basis.

2.1.4.2 Seed sampling and harvesting procedure

Prior to harvest of the turnip rape plot, seed samples were removed by hand at set distances into the plot of turnip rape. Seeds were sampled at set distances (1m, 6m, 11m, 16m, 21m, 31m, 41m, 51m) along three linear transects from the interface between the turnip rape crop and the GM herbicide tolerant variety. Racemes were harvested dried and processed using the same procedure described in 2.1.3.4 (b)

2.1.4.3 Growing on seed samples and herbicide tolerance testing of oilseed rape seedlings

Seeds were tested for glufosinate tolerance using the same procedure described in 2.1.3.5 except that a total of 4000 seeds were tested.

2.1.4.4 Data analysis

Data from herbicide tolerance tests were used to estimate frequencies of outcrossing at each sample distance. The data has been presented as a percentage of herbicide tolerant seed out of the total number of herbicide tolerant seed tested from sample. The mean data from each seedling test was presented showing the decline in herbicide tolerant seeds as a function of distance.

Table 11. Field experiment (2.1.4) establishment and farm operations details

Date	Standard farm operations for all plots
1.09.98	Subsoiling
2.09.98	Plough (depth 30cm)
2.09.98	Power harrow (Dynadrive)
4.09.98	Cambridge roll (Cousins)
18.09.98	Drilling winter turnip rape plot (cv Debut) (Amazone 24)
18.09.98	Cambridge roll (Cousins)
25.09.98	Applied irrigation (25mm)
07.10.98	Apply slug pellets (Thiodocarb 4% w/w)
11.11.98	Apply herbicide (Metazachlor (500g/l) @ 2.5l/ha + fluazifop-P-butyl (250g/l) @ 0.75 l/ha + Partna adjuvant @ 1.2l/ha) (Sprayranger 24)
20.03.99	Apply fungicide (carbendazim + flusilazole 125:250 g/l @ 0.8l/ha) (Sprayranger 24)
24.03.99	Apply fertiliser (26:13:0) (Nodet spreader)
9.07.99	Swath all rape plots
14.07.99	Combine harvest
27.08.99	Disced (Pettit discs)

2.2 THE INFLUENCE OF POLLEN SOURCE SIZE AND FERTILITY OF RECIPIENT PLANTS ON OUTCROSSING IN OILSEED RAPE

2.2.1 Long distance cross pollination of isolated male sterile and male fertile receptor plots of oilseed rape positioned at a range of distances and directions from an 11.5 hectare area of herbicide tolerant winter oilseed rape

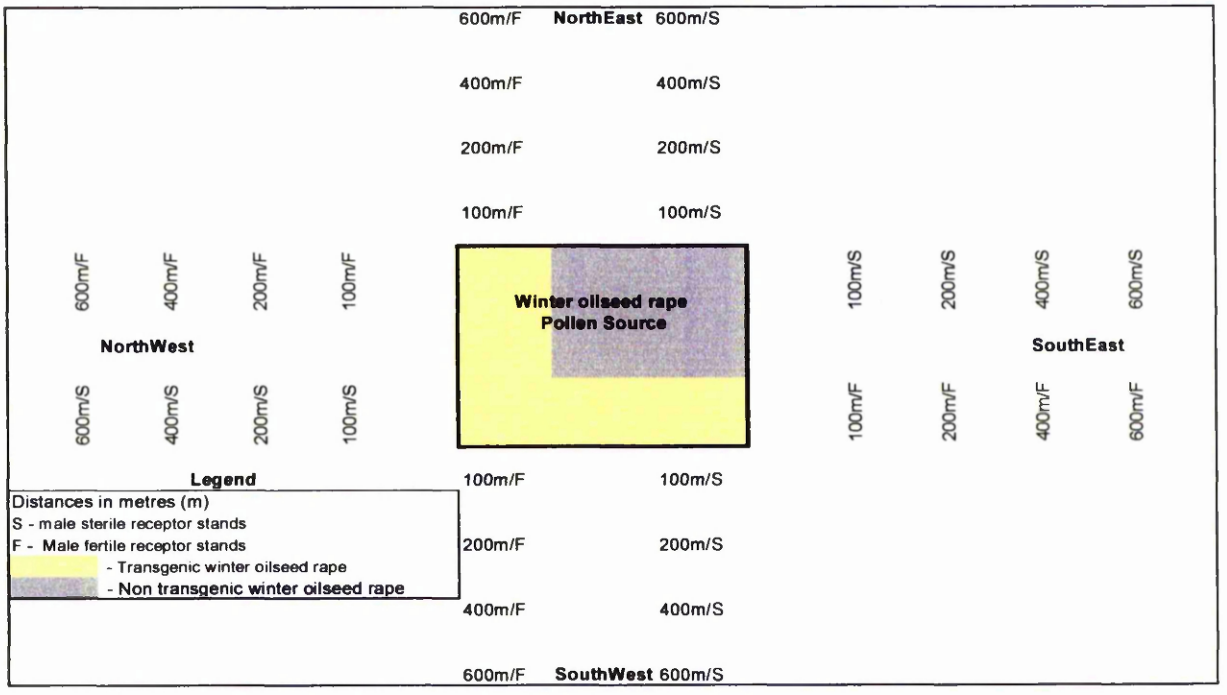
2.2.1.1 Location and description of transgenic pollen source

The field trial utilised as the transgenic pollen source was grown at a farm in Cambridgeshire by Plant Genetic Systems (PGS) in 1998-99. The trial consisted of an area of approximately 11.5 ha of winter oilseed rape with about 25% of male fertile plants in this area containing the BAR gene conferring tolerance to the herbicide glufosinate ammonium. A pollen barrier of non-GM oilseed rape (cv. Lipton) was grown around the perimeter of the trial; 10m wide on the north west and south east sides and 20m wide on the north east and south west sides. Figure 3 shows the layout of the receptor plant plots in relation to the GM and non-GM components of the pollen source.

2.2.1.2 Cultivation of male sterile receptor plants

The male sterile part of the spring oilseed rape varietal association cv. Concept was grown under glasshouse conditions (18-22°C; Supplementary lighting 400w HPS 16hr photoperiod) in 30cm diameter plastic pots containing multi-purpose peat based potting compost (Shamrock) and sand mixture 2:1.

Plants were grown so that flowering coincided as closely as possible with the onset of flowering of the field crop (bait plants were at GS 4.0 when placed in the field). Six male sterile plants in pots were positioned (0.5m apart) in linear plots at a range of distances (100m, 200m, 400m, 600m) and directions north east, south east, south west and north west from the transgenic pollen source. Detailed weather data was obtained from the meteorological station at the NIAB farm approximately 25km north of the release site.



*not to scale

Figure 3. Schematic layout of male sterile and fertile receptor plant plots in relation to transgenic herbicide tolerant and non transgenic winter oilseed rape pollen sources (measurements are in metres*)

2.2.1.3 Cultivation of fertile receptor plants

The fully self-fertile spring oilseed rape restored hybrid cv. Superol was used for comparison with the male sterile bait plants. Plants were grown in exactly the same way as the male sterile plants (see section 2.2.1.2) and were positioned in pots at the same distances and directions from the field of transgenic herbicide tolerant oilseed rape. In order to reduce the possibility of cross-contamination between fertile and sterile plants, plots were positioned 100m apart. Fertile plants were left in position for the same period of time as sterile plants.

2.2.1.4 Growing on seed samples and herbicide tolerance testing of oilseed rape seedlings

Seeds from each plot of male sterile bait plants were bulked sub sampled and tested in five replicates of 100 seeds where possible. Seeds from fertile plants were bulked and tested in five replicates of 1000 seeds. Seedlings were tested for glufosinate ammonium tolerance using the same procedure described in section 2.1.3.5.

2.2.1.5 Mean seed set per siliqua in male sterile receptor plants

Seed number per siliqua was assessed to determine whether seed set per pod decreased at further distances from the pollen source to give an indication of the potential involvement of insects or wind in the transfer of pollen, low seed set indicating greater wind than insect mediated pollination (in male sterile plants). The mean number of seed set per siliqua in 1999 was recorded by counting 10 randomly selected siliquae from the main stem of plants from each distance and directions.

2.2.1.6 Data analysis

Data from herbicide tolerance tests were used to estimate frequencies of outcrossing in each plot of receptor plants at each sample distance. The data has been presented as a percentage of herbicide tolerant seed out of the total number of herbicide tolerant seed tested from each receptor plot. Data from each seedling test for each direction from the pollen source was meaned and presented as the overall decline in herbicide tolerant seeds as a function of distance.

2.2.2 Outcrossing between artificial feral populations of genetically modified glyphosate tolerant winter oilseed rape and conventional varieties of winter oilseed rape

2.2.2.1 Establishment of artificial feral populations

Two populations of glyphosate tolerant winter oilseed rape (final density approximately 100 flowering plants) were sown within 2m from the edge of conventional winter oilseed rape crops of Apex and Synergy at NIAB, Cambridge, U.K (Figure 2). The plots were sown by hand at 2cm depth at the same time as the large ('receptor') areas of winter oilseed rape. The large plots of winter oilseed rape were established and maintained as described in Tables 10 and 16. The populations were observed for synchrony of flowering with the crop and activity of pollinating insects.

2.2.2.2 Seed sampling and harvesting procedure

Three transects spaced 4m apart were sampled at 0m, 2m, 5m, 10m, 15m, 20m and 25m from the interface between the feral populations and the conventional (receptor) plots of oilseed rape. Racemes were harvested dried and processed using the same procedure described in 2.1.3.4 (b)

2.2.2.3 Growing on seed samples and herbicide tolerance testing of oilseed rape seedlings

Seeds were tested for glyphosate tolerance using the same procedure described in 2.1.3.5

2.2.2.4 Data analysis

Data from herbicide tolerance tests were used to estimate frequencies of outcrossing in each conventional variety (receptor) plot at each sample distance. The data has been presented as a percentage of herbicide tolerant seed out of the total number of herbicide tolerant seed tested from each receptor plot sample. Data from each seedling test was meaned and presented as the overall decline in herbicide tolerant seeds as a function of distance. The cross pollination data was also used to compare two dispersal models for their fit to describe the relationship between cross pollination and distance from source (Section 3.3).

2.2.3 Cross pollination between artificial genetically modified herbicide tolerant volunteer populations and conventional varieties of winter oilseed rape

2.2.3.1 Establishment of experimental plots

Genetically modified glufosinate tolerant seed was incorporated into the seed of cv. Synergy (varietal association, VA), cv. Pronto (restored hybrid, RH) and cv. Apex to give the following densities of GMHT volunteers: 0, 1, 20, 40 plants/m^2. Plots (1.6 x 8m) were drilled using a standard plot drill (HEGE 90) at standard seed rates for conventional, varietal association and restored hybrid varieties (120, 70, 70 seeds/m^2 respectively), the trial was maintained using standard agronomic practices at NIAB farm , Cambridge, U.K (Table 12). The trial consisted of 4 treatments replicated 3 times, treatments were separated by 'pollen barrier' areas which consisted of 12m wide strips of a non-GM

variety of winter oilseed rape c.v. Apex (Figure 4). Synchrony of flowering of the barriers between treatments was observed and plant densities in plots were recorded. Detailed weather data during the flowering period was obtained from the meteorological station at the NIAB farm.

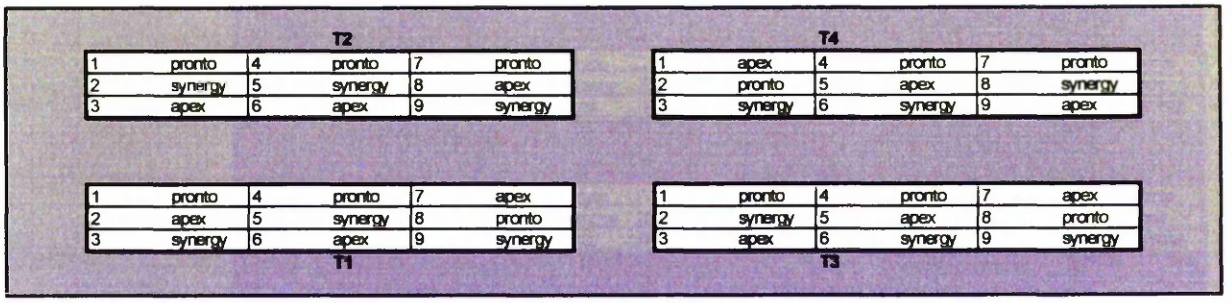


Figure 4. Plot and treatment layout of cross pollination experiment between simulated herbicide tolerant volunteer populations and conventional varieties of winter oilseed rape

Legend
T1=1 plant/m^2, T2= 20plants/m^2, T3 = 40plants/m^2, T4 =0 plants/m^2
The grey shading indicates non -GM oilseed rape 'pollen barrier'

2.2.3.2 Seed harvesting

Each plot was swathed at GS 6.5 and harvested 6 days later using a small plot combine (HEGE 80), Seed samples were harvested from only the central area of each plot to avoid cross-contamination between plots.

2.2.3.3 Growing on seed samples and herbicide tolerance testing of oilseed rape seedlings

The bulked seed from each plot was then sub-sampled and tested for glufosinate tolerance using the same procedure and dose rate described in 2.1.3.5

Table 12. Field experiment (2.2.1) establishment and farm operations details

Date	Standard farm operations for all plots
1.09.98	Subsoiling
2.09.98	Plough (depth 30cm)
2.09.98	Power harrow (Dynadrive)
4.09.98	Cambridge roll (Cousins)
18.09.98	Drilling winter oilseed rape plots (HEGE 80 plot drill)
18.09.98	Cambridge roll (Cousins)
25.09.98	Applied irrigation (25mm)
07.10.98	Apply slug pellets (Thiodocarb 4% w/w)
11.11.98	Apply herbicide (Metazachlor (500g/l) @ 2.5l/ha + fluazifop-P-butyl (250g/l) @ 0.75 l/ha + Partna adjuvant @ 1.2l/ha) (Sprayranger 24)
20.03.99	Apply fungicide (carbendazim + flusilazole 125:250 g/l @ 0.8l/ha) (Sprayranger 24)
24.03.99	Apply fertiliser (26:13:0) (Nodet spreader)
9.07.99	Swath all rape plots
14.07.99	Combine harvest
27.08.99	Disced (Pettit discs)

2.2.3.4 Data analysis

Data was initially presented as the mean number of herbicide tolerant seeds detected in harvested seed samples from each treatement. The data were the mean of both herbicide tolerance test trays and across replicates in the field.

Statistical Analysis of cross pollination data

The data were analysed using regression. Initial inspection of the data suggested that simple linear models might provide an adequate description of the relationship between percentage initial contamination and percentage final contamination. For analysis, the data for replicate seed trays were included separately (i.e. the raw data from each tray were used, not the mean of the trays). The explanatory regression model included the variate "percentage initial contamination", and the grouping factor, variety (with three levels; Apex, Pronto and Synergy). The full interaction between

the variate and factor was fitted. This type of model results in a "regression with groups" (Digby *et al.*, 1989).

During the analysis the model is fitted sequentially and the change in percentage variance accounted for by adding each component, together with a t-statistic for the significance of the added parameter. Initially, a linear function (with a constant) is fitted for the data for the first level of the grouping factor (Apex in this case). Next, a model with separate constants, but a single gradient (estimated from the Apex data) is fitted to test whether a model with separate intercepts, but a common gradient gives a significant improvement on the first model. Finally, the full interaction, in which separate constants and gradients are estimated for each variety, is fitted with similar reporting of any improvement in fit to the data.

2.3 WEED CONTROL IN HERBICIDE TOLERANT AND CONVENTIONAL OILSEED RAPE

2.3.1 Year one - Weed control in herbicide tolerant and conventional varieties of winter oilseed rape (1998-1999)

2.3.1.1 Establishment of experimental plots, source of plant material and previous cropping history

Blocks of herbicide tolerant and conventional winter oilseed rape varieties were established in adjacent areas of 0.8ha (92m x 92m) in a 10 hectare field at NIAB, Cambridge, U.K (Figure 2). Each $92m^2$ area was sub-divided into four smaller sub-plots of 18m x 92m to enable more uniform sampling of weed populations. An untreated 4m wide, 92m long strip was positioned at right angles to the sub-plots. Previous cropping history of the experimental field is shown in Table 13. Plant material used is described in Table 14.

Plots were drilled at normal recommended seed rates for restored hybrid and varietal association (70 seeds/m^2) and conventional types (120 seeds/m^2) using a standard 24 row farm drill (Amazone) at 2cm depth. Details of trial establishment and management procedures are described in Table 15.

Table 13. Previous cropping history of field area in experiment 2.3.1

Year	Crop/Area	Variety
1994	winter wheat / 7.8ha	Riband
1994	winter beans / 0.7ha	-
1994	combinable peas / 1.5ha	Guido
1995	winter wheat / 9.6ha	Riband
1995	winter beans / 0.4ha	-
1996	winter wheat / 10ha	Riband
1997	winter beans / 10ha	Punch
1998	winter wheat / 10ha	Soissons

- no data

Table 14. Source of plant material - winter oilseed rape herbicide tolerant and conventional varieties used in experiment 2.3.1

Herbicide tolerance	Variety type	Variety	Supplier/Breeder
Glufosinate-ammonium (Liberty)	Transgenic restored Hybrid (RH)	LL1	PGS/Aventis
Glyphosate (Roundup)	Transgenic variety	RR1	Monsanto
Imidazolinone (Imazamox)	Non transgenic variety	IMI	Pioneer Hi-Bred/ Cyanamid
Non-tolerant	Varietal association (VA)	Synergy	CPB Twyfords U.K.
Non-tolerant	Conventional variety	Apex	Advanta Seeds U.K.

Table 15. Field experiment (2.3.1) establishment and farm operations details*

Date	Standard farm operations for all plots
1.09.98	Subsoiling
2.09.98	Plough (depth 30cm)
2.09.98	Power harrow (Dynadrive)
4.09.98	Cambridge roll (Cousins)
16.09.98	Drilling winter oilseed rape plots 1,5 and 6 (Amazone 24 drill)
17.09.98	Drilling winter oilseed rape plots 2,3,4,7 and 8 (Amazone 24 drill)
18.09.98	Cambridge roll (Cousins)
22.09.98	Apply slug pellets (Thiodocarb 4% w/w)
25.09.98	Applied irrigation (25mm)
20.03.99	Apply fungicide (carbendazim + flusilazole 125:250 g/l @ 100:200 g a.i./ha) (Sprayranger 24)
24.03.99	Apply fertiliser (26:13:0) (Nodet spreader)
9.07.99	Swath all oilseed rape plots
13.07.99	Combine harvest (Dominator 36)
27.08.99	Disced (Pettit discs)

*see Table 16 for herbicide applications and timings

2.3.1.2 Field experiment establishment and maintenance

The trial was maintained using standard agronomic practices (Table 15). The appropriate herbicides were applied to the herbicide tolerant varieties, a standard herbicide programme was used for conventional winter variety plots (Table 16)

2.3.1.3 Herbicide applications to experimental plots of winter oilseed rape

Herbicides were applied by following consultation with the agrochemical companies involved and by following normal local farm practice for the conventional treatments. Applications of herbicides were made using a Sprayranger 24 self-propelled sprayer. Herbicide dose rates and timings of application are shown in Table 16.

Table 16. Herbicide dose rates, active ingredients and application timings to plots of conventional and herbicide tolerant winter oilseed rape in experiments 2.1.3 and 2.3.1

Variety	Herbicide	Active ingredient g/l	Dose rate g a.i./ha	Recommended Timing	Actual Timing
LL1	Liberty	Glufosinate ammonium: 200	600	Before 6 leaf stage of weeds	G.S.21 (wheat)
RR1	Roundup	Glyphosate: 360	720	4/6 leaf stage of crop	2-3 leaf stage of crop
IMI	Imazamox[1]	AC 299,263: 40	70	2/4 leaf stage of weeds	G.S. 21 (wheat)
Apex + Synergy	Butisan S	Metazachlor: 500	1250	Up to 2-4 leaf stage of weeds	G.S. 21 (wheat)
Apex + Synergy	Fusilade[2] EW	Fluazifop-P-butyl: 200	150	2 expanded leaf stage of weeds	G.S. 21 (wheat)

[1] Imazamox only applied to 24m wide strip in imazamox tolerant plots due to PSD restrictions
[2]Fusilade applied with Partna adjuvant (1.2l/ha)

2.3.1.4 Weed assessment methods: pre-herbicide application

a) Weed plant counts

Twenty 0.25m^2 quadrats were thrown in each sub-plot using a "W" sampling pattern, giving a total of 80 quadrats per 92m x 92m main plot (4 sub-plots x 20 quadrats). Five fixed quadrats were positioned randomly within a 4m wide untreated strip which were adjacent to each of the sub-plots. Total numbers of each weed species were recorded in each 0.25m^2 quadrat in all sub-plots and untreated fixed quadrats.

b) Quantitative weed measurements

Height and growth stage were recorded on twelve plants per main plot for both crop and weeds. Weed species were measured when they occurred at estimated densities in excess of approximately 10 plants per m^2. Measurements were taken from plants selected at random over the whole (92m x 92m) area of each main plot. Heights were recorded using a 50 cm steel rule by measuring from soil level to the upper leaf on each plant.

c) Crop and weed cover visual assessments

Percentage crop cover was visually estimated over each main plot. Weed cover was visually estimated over each main plot for species that occurred at densities in excess of 10 plants per m^2.

d) Crop density estimation

Twenty 1m randomly selected lengths of rows of plants in each main plot, counts were converted into plants/m^2.

2.3.1.5 Weed assessment methods: post-herbicide application

Post herbicide assessments were conducted when it was clear which weeds were surviving herbicide treatments. In most cases this was approximately 8 weeks after treatment.

a) Percentage weed control assessment

A visual assessment of the percentage control of the main weeds (occurring at densities in excess of 10/m^2) was made. The visual index scoring system is shown in Table 17.

b) Percentage crop damage assessment index

To determine whether any crop damage occurred as a result of herbicide applications to the oilseed rape varieties, a visual assessment was made based on an index system shown in Table 18.

c) Crop and weed cover assessments

Crop and weed cover assessments were repeated as previously described in pre-herbicide assessments in section 2.3.1.4 (c)

d) Weed Counts – early spring

Weed counts were made using the identical quadrat size and sampling pattern described in 2.3.1.4 but with reduced sampling intensity. Twelve quadrats were thrown per treated sub-plot and 3 quadrats were counted in untreated quadrats.

e) Weed plant counts – fixed quadrats

In order to estimate the late emergence of weeds and to make comparison with herbicide treated areas, total weed counts of each species were made in the fixed quadrats in each of the untreated strips adjacent to each sub-plot.

f) Assessment of weed biomass in plots of winter oilseed rape

In order to identify efficacy of weed control treatments, provide information on the potential weed seed production and thus seed return to the soil seedbank, a measurement of weed biomass was made in mid-June after the oilseed rape flowering period prior to harvest.

All weed species vegetation was removed by hand at ground level from eight 0.25m^2 quadrats randomly positioned at least 1.5m from tramlines in each sub-plot. Weed species were identified, counted, dried separately in ovens (105°C for 12hrs) and dry weights recorded for each species and treatment.

g) Estimation of numbers of seed shed at harvest and density of volunteers germinated after harvest

To estimate the potential for return of transgenic seed to the seedbank, counts of seed shed were carried out after harvesting operations were completed. Ten counts of oilseed rape seeds on the soil surface were made randomly across each main plot using a 15cm x 15cm quadrat. Counts of shed seeds were conducted 2 days after harvest. Ten counts of volunteer plants were made randomly across each main plot using a 15cm x 15cm quadrat.

h) Harvest and yield assessments

Plots were harvested individually in order to give an estimation of yield for each variety. The total fresh weight of grain from each plot was recorded. A sample of 500g was removed from the bulk of grain from each plot, samples were further analysed for dry matter content. The dry matter contents in conjunction with the fresh yield were used to calculate the dry matter yield at 9% moisture as follows:

$$\text{Dry matter yield at 9\% moisture} = \frac{(10\text{x plot fresh yield kg x plot dry matter \%})}{\text{Plot width x plot length x 91}}$$

Table 17. Percentage weed control visual assessment scale

% Control	Description of symptoms
100	Complete control
97-98	Control virtually complete. Isolated weed still visible
95	Very good control. A few weeds still viable but most killed or severely damaged
90	Good control. Some weeds still viable but most clearly damaged and unlikely to recover
85	Most weeds severely affected but some weeds may recover
75	Many weeds severely affected but control insufficient
65	Some weeds killed but many surviving after transient growth check
1-50	Some control, many weeds unaffected or likely to fully recover
0	No visible effect

Table 18. Percentage crop damage assessment scale

Damage Index	Description of crop damage symptoms
0	no visible crop effects
2-3	necrotic spotting on some leaves, light chlorosis or discolouration, leaf de-waxing apparent, slight stunting suspected
5	necrotic damage more obvious, moderate chlorosis or discolouration, slight stunting suspected
10	damage visible on most leaves, severe chlorosis or discolouration, moderate stunting apparent but crop recovers quickly
15	severe damage to leaves, some stunting, occasional plants (tillers) killed. Recovery slow or incomplete
25	severe leaf and stem damage, pronounced stunting, plot noticeably thinned by plant/tiller death or growth check
35	stunting more severe, increased plant/tiller death
50-99	previous effects progressively more pronounced, plot unlikely to recover
100	DEAD

2.3.2 Year two - Herbicide tolerant and conventional winter oilseed rape volunteer and weed control in winter wheat (1999-2000)

In order to determine whether herbicide tolerant and conventional rape volunteers differed in their susceptibility to commonly used herbicides in cereal crops, a winter wheat crop was drilled which was superimposed on the plots where herbicide tolerant oilseed rape had previously been grown in year one (Figure 2).

2.3.2.1 Establishment of experimental plots and source of plant material

The layout of blocks of winter wheat were the same as in year 1, although no untreated areas were maintained. Plots and surrounding areas were all drilled with winter wheat (cv. Soissons). The trial was maintained using standard agronomic practices described in Table 19.

2.3.2.2 Winter wheat herbicide programme

Herbicides were applied by following normal local farm practice for the conventional treatments. Applications were made using a Sprayranger 24 (24m boom). Diquat + paraquat 80:120g/l at 160:240g a.i./ha + Silwet L77 (adjuvant) at 0.05 l/ha respectively were applied prior to drilling to control emerged volunteer rape seedlings from the previous crop. Herbicide dose rates and timings of application are shown in Table 20.

2.3.2.3 Weed Assessment Methods: pre- and post-herbicide application

a) Weed assessment methods

Methods used to assess weeds both pre and post herbicide treatment were the same as the methods detailed in sections 2.3.1.4 - 2.3.1.5 in year one of the experiment. A specific assessment of oilseed rape volunteer incidence was made which is described below.

b) Volunteer oilseed rape assessment

The incidence of volunteers in the winter wheat crop in was recorded both pre- and post herbicide. Oilseed rape seedlings were counted in conjunction with weed species counts. Visual observations of the trial were made throughout April-June to check for any flowering volunteer rape plants.

2.3.3 Data analysis from year one and year two experiments

Pre- and post-herbicide weed counts and weed biomass data was statistically tested using analysis of variance (ANOVA) using GENSTAT 5 release 3.2. The most frequently occurring weeds were selected for further analysis by ANOVA, several commonly occurring weeds were carried through all analyses where possible. In both years as well as including individual species in the analysis of weed biomass, weed biomass data from all species was also pooled to give an analysis of total biomass per treatment. Low weed densities in year two did not allow a full analysis to be carried out on pre- and post-herbicide weed counts.

Table 19. Field experiment (2.3.2) establishment and farm operations details*

Date	Standard farm operations for all plots
26.08.99	Subsoiling
31.08.99	Plough (depth 30cm)
03.09.99	Power harrow (Dynadrive)
06.09.99	Cambridge roll (Cousins)
06.10.99	Power Harrow (Dynadrive) (shallow)
07.10.99	Combi-drilled (Amazone 24)
11.10.99	Cambridge roll (Cousins)
28.10.99	Apply insecticide Cypermethrin (0.25l/ha) (Sprayranger 24)
13.03.00	Apply growth regulator New 5C Cycocel (2.5l/ha) (Sprayranger 24)
15.03.00	Apply fertiliser Bunns 26:13:0
04.05.00	Apply fungicide Folicur (0.5l/ha) + Bravo (1l/ha) (Sprayranger 24)
05.05.00	Apply fertiliser Bunns 26:13:0
17.05.00	Apply fertiliser Nitram 34.5%N
20.05.00	Opus (1 l/ha) + Bravo (1l/ha) (Sprayranger 24)
17.07.00	Apply dessicant Roundup Biactive 2l/ha (Sprayranger 24)

*see Table 20 below for herbicide applications and timings

Table 20. Field experiment (2.3.2) herbicide applications

Variety	Herbicide product name	Active ingredient g/l	Dose rate g a.i./ha	Recommended Timing	Actual timing
Soissons	Stomp 400SC	Pendimethalin 400	1200	Early post-em or GS 23	GS13
	Isoproturon 500	Isoproturon 500	1500	Early post-em	GS13
	Mecoprop-P	Mecoprop-P 600	1200	GS 10-31	GS24

3. RESULTS AND DISCUSSION

3.1 OUTCROSSING BETWEEN HERBICIDE TOLERANT AND CONVENTIONAL OILSEED RAPE CULTIVARS

3.1.1 Cross pollination between variety trial plots in National List genetically modified (NLGM) herbicide tolerant winter oilseed rape trials (harvested in 1997 & 1998)

Herbicide tolerance and double herbicide tolerance was detected in seed samples taken from conventional and herbicide tolerant varieties harvested from National List Trials from both years. Cross pollination frequencies are expressed as the percentage of herbicide tolerant plants per test sample (600 plants in 1997 and 1000 plants in 1998). In Figures 5-14, the plot configuration of the trials are shown and each variety is represented by one rectangle. The dimensions of each trial plot was approximately 2m x 20m. Mean frequencies of glufosinate and glyphosate tolerance are shown for each plot. Overall, frequencies of glyphosate and glufosinate tolerance tended to decrease with increasing distance from the GM pollen source plots (e.g. from 1.33% in an adjacent plot to 0.25% at 2m, in open pollinated varieties, Figure 9) and varied between varieties, with cv. Synergy often being cross pollinated at higher levels (e.g. 16.91% in an adjacent plot compared to 0.91% in cv. Express in an adjacent plot, (Figure 12). Higher levels of herbicide tolerance were detected in 1998 compared to 1997 trials (e.g. Apex ranging from 0-1.83% at Cockle Park 1997, Figures 9 and 10 compared to a range of 0.6%-4.3% at Cockle Park 1998, Figures 11 and 12). Double tolerance to both glufosinate and glyphosate was also detected in seed samples collected from the herbicide tolerant varieties in the trials in both years (e.g. 0.1%-10.5%).

The control tests of non-tolerant rape seedlings (cv. Falcon) resulted in all plants killed by application of either glufosinate or glyphosate applied at the equivalent timings and dose rates. Plate 2 (Appendix 1) shows the symptoms of herbicide application on single and double herbicide tolerant plants grown from seeds harvested from a NLGM trial.

Figures 5-14 **Plot configuration of National List GM winter oilseed rape trials and the levels of glufosinate and glyphosate tolerance detected in seed samples from these plots**

Synergy 0	GLU1	GLU1	GLY1 0.41	Express 0.08	Falcon 1.08
Falcon 0.17	Nickel 8.75	Express 7.16	Falcon 0.33	Synergy 1.19	Nickel 0.25
GLY1 0	Express 0.66	Apex 1.17	Nickel 0.33	GLY1 1.75	Apex 0
Apex 0	Discard	Synergy 1.41	Discard	GLU1	Discard

Figure 5. **% Glufosinate tolerance - Caxton 1997**

Synergy 4.25	GLU1 0.16	GLU1 0.83	GLY1	Express 1.0	Falcon 0.16
Falcon 0.66	Nickel 0.58	Express 0.5	Falcon 7.75	Synergy 8.91	Nickel 0.25
GLY1	Express 0.33	Apex 0	Nickel 0.83	GLY1	Apex 0.33
Apex 9.25	Discard	Synergy 1.0	Discard	GLU1 2.0	Discard

Figure 6. % Glyphosate tolerance - Caxton 1997

Nickel 0.33	GLY1 0.33	Falcon 0.16
Synergy 1.66	Falcon 0.08	GLY1 0.25
GLY1 1.58	Express 0	Apex 1.25
GLU1	Apex 2.66	GLU1
Apex 11.25	GLU1	Express 10.66
Falcon 4.5	Nickel 13.8	Synergy 2.5
Express 1.58	Synergy 2.33	Nickel 1.66

Figure 7. % Glufosinate tolerance - Bridgets 1997

Nickel 1.83	GLY1	Falcon 1.16
Synergy 5.91	Falcon 9.16	GLY1 0.17
GLY1	Express 1.91	Apex 8.83
GLU1 1.16	Apex 0.33	GLU1 0.58
Apex 2.25	GLU1 0.16	Express 0
Falcon 1.08	Nickel 0.16	Synergy 0.83
Express 0.08	Synergy 0.75	Nickel 0.33

Figure 8. % Glyphosate tolerance - Bridgets 1997

Apex 0.83	GLU1	Falcon 0.16
Nickel 0.33	Falcon 0.16	Synergy 1.67
GLY1 0.08	Apex 0.33	Express 0.33
Falcon 0.5	Synergy 1.58	GLY1 1.33
Express 0.41	GLY1 0.16	GLU1
GLU1	Express 1.33	Nickel 17.58
Synergy 19.83	Nickel 0.25	Apex 0.91

Figure 9.%Glufosinate tolerance-Cockle Park 1997

Apex 0	GLU1 0	Falcon 0.58
Nickel 2.25	Falcon 1.25	Synergy 2.25
GLY1	Apex 1.83	Express 0.91
Falcon 17.08	Synergy 16.91	GLY1
Express 1.33	GLY1	GLU1 0.58
GLU1	Express 2.91	Nickel 3.16
Synergy 1.33	Nickel 0.33	Apex 1.25

Figure 10.%Glyphosate tolerance-Cockle Park 1997

GLY 1 1.1	GLU 1	Pronto 0.3
GLY 3 10.5	GLU 3	Falcon 0.45
GLU 2	Alpine 3.65	GLY 1 0.75
GLU 3	GLY 3 0	GLU 1
Pronto 0.95	Synergy 3.7	GLY 3 5.25
Alpine 1.15	GLU 4	GLY 2 1.75
Falcon 6.1	Apex 4.3	Synergy 4.05
GLU 1	GLU 2	GLU 4
GLY 2 7.4	Pronto 4.4	Apex 5.35
GLU 4	GLY 2 4.25	GLU 2
Apex 1.25	Falcon 0.35	Alpine 4.85
Synergy 2.1	GLY 1 0.4	GLU 3

Figure 11.%Glufosinate tolerance-Cockle Park 1998

GLY 1	GLU 1	Pronto 4.6
GLY 3	GLU 3 1.05	Falcon 0.8
GLU 2 0.9	Alpine 0.55	GLY 1
GLU 3 0.3	GLY 3	GLU 1 8.85
Pronto 0.5	Synergy 8.75	GLY 3
Alpine 0.5	GLU 4 1.15	GLY 2
Falcon 0.65	Apex 0.6	Synergy 12.35
GLU 1 3.05	GLU 2 0.3	GLU 4 2.0
GLY 2	Pronto 1.1	Apex 0.7
GLU 4 1.05	GLY 2	GLU 2 0.25
Apex 0.6	Falcon 2.5	Alpine 0.55
Synergy 1.65	GLY 1	GLU 3 0.25

Figure 12.%Glyphosate tolerance-Cockle Park 1998

GLY 2 0.05	Alpine 2.55	GLY 3 0.35
Alpine 0.3	GLU 2	Synergy 10.2
Apex 2.25	GLY 3 0.35	GLU 2
Falcon 1.45	Synergy 2.05	GLU 4
GLU 2	Apex 5.7	GLY 1 0.25
GLU 3	GLY 2 0.25	GLU 1
Pronto 7.75	GLU 4	Falcon 11.15
Synergy 6.15	Pronto 5.5	Alpine 2.45
GLY 3 0.25	GLU 1	GLY 2 0.15
GLU 1	Falcon 10.85	GLU 3
GLU 4	GLY 1 0.1	Apex 5.7
GLY 1 0.15	GLU 3	Pronto 1.2

Figure 13.% Glufosinate Tolerance -Bridgets 1998

GLY 2	Alpine 9.05	GLY 3
Alpine 6.75	GLU 2 0	Synergy 14.6
Apex 1.9	GLY 3	GLU 2 0
Falcon 0.7	Synergy 12.75	GLU 4 0
GLU 2 0	Apex 2.8	GLY 1
GLU 3 0	GLY 2	GLU 1 0
Pronto 0.15	GLU 4 0	Falcon 1.45
Synergy 4.65	Pronto 1.2	Alpine 1.4
GLY 3	GLU 1 0	GLY 2
GLU 1 0	Falcon 0.75	GLU 3 0
GLU 4 0.1	GLY 1	Apex 0.6
GLY 1	GLU 3 0	Pronto 0.6

Figure 14 .% Glyphosate Tolerance -Bridgets 1998

Legend (Figures 5-14)

1. GLU are glufosinate tolerant emitter plots
2. GLY are glyphosate tolerant pollen emitter plots.
3. Plots of the varietal association cv. Synergy are highlighted.

3.1.2 Transgene flow into non-GM National List (NL) variety trials (harvested in 1997 and 1998)

a) Transgene flow from NLGM to conventional NL trials in 1997

Herbicide tolerance to glufosinate and glyphosate was detected in seed samples harvested from three of the trials tested, Caxton, Cockle Park and Bridgets. No transgenic plants were found in seed samples from Wye Regional Trial Centre. As an example of the data recorded, Table 21 shows the frequency of outcrossing detected in each plot at the Cockle Park site in 1997, summarised data from the other trial sites is shown in Table 40-42 (Appendix 2). Seed samples from the varietal association cv. Synergy contained higher levels of herbicide tolerant seed indicating it was more frequently cross pollinated. There was no indication that restored hybrids were cross pollinated more frequently than other varieties e.g. cv. Pronto.

Typically, outcrossing frequencies were low and ranged from 0.1%-0. 4% in plots of open pollinated varieties nearest the transgenic pollen source. Levels of outcrossing in cv. Synergy ranged from 0.1%-2% in plots sampled nearest the GMHT pollen source. All of the seedlings grown in the non-herbicide tolerant control plots were killed by the applications of either glufosinate or glyphosate. The proximity of the conventional NL trial to the NLGM trial pollen source at Cockle Park is shown in

Figure 15. Plate 4 (Appendix1) shows an example of field testing procedure for the detection of herbicide tolerance in oilseed seedlings.

b) Transgene flow from GM NL to conventional NL trials in 1998

Using data from Cockle Park as an example of the data recorded, Table 22 and 23 shows the frequency of outcrossing detected in plots sampled at the Cockle Park site in 1998. Herbicide tolerance to glufosinate and glyphosate was detected in seed samples harvested from both Bridgets and Cockle Park trial sites. In common with data collected in 1997, the incidence of outcrossing declined steeply with increasing distance from the GM pollen source. Overall, fewer plots in samples from Bridgets contained herbicide tolerant seed (Table 42 Appendix 2). Seed samples from varietal associations such as cv. Synergy tended to be more frequently cross pollinated with GMHT varieties and often produced higher levels of herbicide tolerant seed.

Typically, outcrossing frequencies were low at both sites and ranged from 0.03%-0. 25% in plots of open pollinated varieties nearest the transgenic pollen source (approx. 6m). Outcrossing frequencies in varietal association cultivars ranged from 0.1%-0. 7% in plots sampled nearest the GM pollen source. There was no indication that restored hybrids were outcrossed more frequently than other varieties. All of the seedlings grown in the non-herbicide tolerant control plots were killed by the applications of either glufosinate or glyphosate. The proximity of the conventional NL trial to the NLGM trial at Cockle Park is shown in Figure 15.

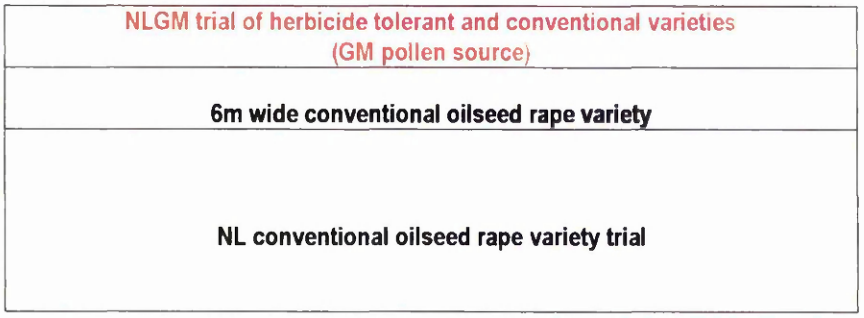


Figure 15. The proximity of National List trials of herbicide tolerant winter oilseed rape varieties in relation to National List trials of conventional winter oilseed rape at Cockle Park in 1997 and 1998

Table 21. Percentage glufosinate and glyphosate tolerance detected in seed samples from plots growing in a National List winter oilseed rape trial adjacent to a National List trial of genetically modified herbicide tolerant winter oilseed rape at Cockle Park, UK, 1997

Variety type	Plot distance from GM source (m)	Herbicide tolerance (%)
		Glufosinate tolerance
Open pollinated	6	0.05
Varietal association	44	0.05
Open pollinated	50	0.05
		Glyphosate tolerance
Open pollinated	6	0.05-0.16
Restored hybrid	34	0.05
Open pollinated	38	0.05
Varietal association	44	0.11
Open pollinated	50	0.05-0.16
Open pollinated (c.v. Apex)	68	0.05

Table 22. Percentage glufosinate tolerance detected in seed samples from plots growing in a National List winter oilseed rape trial adjacent to a National List trial of genetically modified herbicide tolerant winter oilseed rape at Cockle Park, UK, 1998

Distance from GM source	Range of herbicide tolerance detected in different variety types (%) Open pollinated	Varietal association	Restored hybrid
6-10	0.09-0.25	-	-
11-20	0.03-0.56	0.16-0.78	-
21-30	0.03-0.09	0.22	-
31-40	0.03	0.16	0
41-50	0.03-0.13	0.03	0
51-60	0.03-0.06	-	-
61-70	0.03	0.06	-
71-80	0.03-0.06	-	-
81-90	0	0.03	-
91-100	0	-	-
101-110	0	0	-
111-120	0.06	0.03	-
121-130	0.03	0.03	-

- zero plots sampled
0= herbicide tolerance detected

Table 23. Percentage glyphosate tolerance detected in seed samples from plots growing in a National List winter oilseed rape trial adjacent to a National List trial of genetically modified herbicide tolerant winter oilseed rape at Cockle Park, UK, 1998

Distance from GM source	Range of herbicide tolerance detected in different variety types (%) Open pollinated	Varietal association	Restored Hybrid
6-10	0.03-0.25	-	-
11-20	0.03-0.25	0.03-0.047	-
21-30	0.03-0.06	0.37-0.25	-
31-40	0.03-0.06	0.13	0.06
41-50	0.03-0.06	0.06	0.06
51-60	0.03	-	-
61-70	0.03	0.06-0.13	-
71-80	0.06	0.03-0.09	-
81-90	0	0.03-0.09	-
91-100	0	-	-
101-110	0.03	0.03	-
111-120	0.03-0.16	0.03	-
121-130	0.03	0.03-0.06	-

- zero plots sampled
0= herbicide tolerance detected

3.1.3 Outcrossing between field scale areas of herbicide tolerant and other winter oilseed rape cultivars

3.1.3.1 Crop density, crop height, growth stage measurements and pollinating insects

All plots and varieties were within the normal crop densities for oilseed rape restored hybrids, varietal association and normal open pollinated varieties. A variable seedbed across the field meant that there were some differences in crop establishment (Table 24). Mean crop height differed across the field and between varieties. Plots 1-4 tended to be shorter than plots 5-8 although there was considerable variation across all plots. Growth stages varied across varieties and within plots, particularly at the onset of flowering, Table 24. Plate 4 (Appendix 1) shows some of the differences in uniformity of flowering between and within plots in the experiment.

During flowering, observations were made of bee species visiting the crop to assess their involvement in cross pollination events. Observations were made when weather conditions were conducive to bee foraging. Three species were recorded, *Bombus terrestris*, *Bombus lapidarius* and *Apis melifera*. Results showed that only 4 bees were recorded on the 6th May, 25 on 12th of May and 39 on the 19th May (1999). Numbers of bees recorded were extremely low and low numbers of bees were also noted during general observations of the crop throughout the flowering period. Due to the high frequency of zeros, the data was not analysed. Over all sample days there were 1.42 bees recorded per 92m strip (16 x 92m strips sampled on each day). There was a slight increase in bee activity in later days probably due to fuller flowering of the crop and warmer air temperatures.

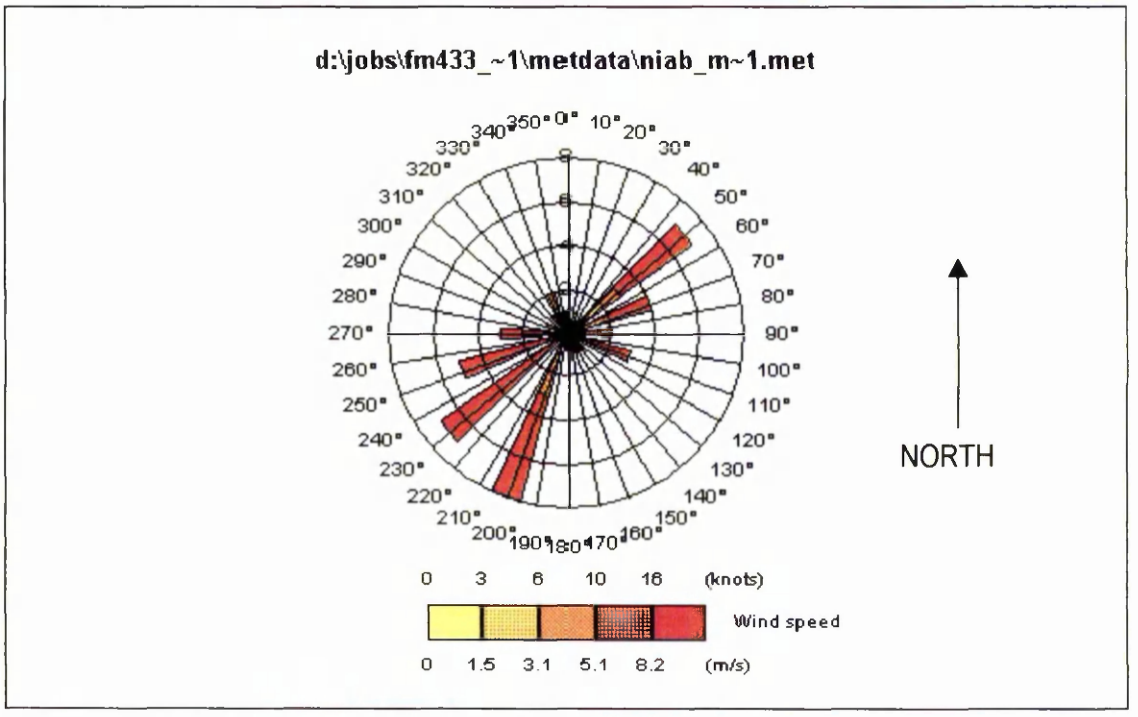
Table 24 Mean crop density, height and growth stage range during the main winter oilseed rape flowering period April-May 1999

Variety	Plot No.	Mean crop [1]density (Plants/m^2)	Mean crop [2]height (m)	Crop growth stage range during the main flowering period*				
				6.04.99	13.04.99	20.04.99	27.04.99	17.05.99
Apex	1	72.8 (6.793)	1.18 (0.011)	3,5-4,5	3,5-4,5	4,2-4,4	4,8-4,9	5,6-5,8
Synergy	1	67.6 (7.175)	1.06 (0.019)	3,5-4,5	4,0-4,5	4,1-4,7	4,8-5,0	5,7-5,9
LL1	2	70.8 (6.365)	1.21 (0.023)	3,5-4,3	3,6-4,0	4,2-4,5	4,8-5,0	5,6-5,9
RR1	3	82.0 (7.407)	1.14 (0.015)	3,1-3,5	4,0-4,7	4,7	4,9-5,0	5,7-5,8
IMI1	4	98.8 (8.315)	1.13 (0.016)	3,1-4,0	4,0-4,7	4,5-4,8	4,9	5,6-5,9
RR1	5	64.4 (5.549)	1.18 (0.016)	3,5-4,3	3,7-4,5	4.5-4,8	4,8-5,0	5,6-5,9
IMI1	6	79.6 (4.941)	1.12 (0.017)	3,1-4,5	3,7-4,7	4,7-4,9	4,8-5,0	5,7-5,9
LL1	7	82.8 (6.971)	1.25 (0.013)	3,1-4,5	3,7-4,5	4,3-4,7	4,9-5,0	5,6-5,9
Apex	8	86.4 (4.740)	1.25 (0.019)	3,5-4,5	4,1-4,7	4,9-5,0	4,8-5,0	5,8-5,9
Synergy	8	64.0 (7.729)	1.22 (0.012)	3,5-4,5	4,1-4,7	4,7-4,9	4,9	5,8-5,9

*Crop growth stages according to Sylvester-Bradley and Makepeace (1984)
[1]-(n=20),
[2]- (n=12)
Values in parentheses = Standard error for each mean

3.1.3.2 Meteorological measurements - summarised wind velocity and directional data for the main winter oilseed rape flowering period 9.04.99-25.05.99

The summarised wind velocity and wind directional data is graphically represented in Figure 16. The wind diagram shows the direction from which the wind was blowing and because only one reading was taken per day the wind diagram assumes that the wind varies over a 30 degree sector. There was a predominance of approximately south westerly and north easterly winds during the main flowering period. Detailed weather data are shown in Table 43, (Appendix 3).



*Wind diagram prepared and supplied by Cambridge Environmental Research Consultants

Figure 16. Summarised wind speed and wind direction data from the period 9.04.99 - 25.05.99 recorded at the NIAB meteorological station Cambridge, UK*

3.1.3.3 Outcrossing data from plots of conventional and herbicide tolerant winter oilseed rape

Outcrossing frequencies are expressed as a percentage of herbicide tolerant seedlings detected in seed samples from each sample point along each transect, the data presented is the mean of two tests of 1000 seeds per distance and over three transects per plot (each transect consisted of either 13 or 8 sample distances depending on variety). The schematic layout of the field plots in the experimental field is shown in Figure 2. Plates 5-7 (Appendix 1) show examples of the symptoms of herbicide treatments on oilseed rape seedlings. The results of outcrossing frequencies as a function of distance (Figures 17-21) have been separated into 3 main categories:

1. Glufosinate tolerant oilseed rape as a pollen source (Figures 17-19)
2. Glyphosate tolerant oilseed rape as a pollen source (Figure 20)
3. Imazamox tolerant oilseed rape as a pollen source (Figure 21)

1. Glufosinate tolerant oilseed rape as a pollen source

a) Outcrossing levels in cv. Synergy were the highest over all samples tested from plots growing adjacent to glufosinate tolerant oilseed rape (Figure 17). There was a large difference in the levels of outcrossing detected in plots 1 and 8. Samples nearest the interface with the pollinator in plot 8 were approximately twice the level of those in plot 1 (15.3% compared with 32.0% in plots 1 and 8 respectively). Although there were differences in outcrossing levels between the two plots of the same variety, there was a consistent pattern of decline with distance from the pollen source. The profile of the outcrossing decay curve showed a gradual decline, where outcrossing values declined less rapidly with increasing distance from the pollen source. Levels of outcrossing at all distances were considerably higher than in all other varieties. The level of outcrossing at the most extreme sample distance (91.5m) was nearly 50 times higher than mean outcrossing levels from all plots crossed with either glufosinate and glyphosate tolerant rape at 81.5m.

b) Levels of outcrossing in cv. Apex plot 1 were lower than in cv. Apex plot 8 at the interface with the glufosinate tolerant pollen source (Figure 18). This corresponds to the lower levels of outcrossing detected in Synergy plot 1 compared to Synergy plot 8. The steep decline in outcrossing level with distance in the plots of Apex was consistent in both plots and differed from the profile of the decline curve of cv. Synergy. The levels of outcrossing detected in plot 3a (RR1) and plot 6b (IMI) were higher over all distances compared to those detected in cv Apex (Figure 19) however the decline rate in outcrossing with distance in the GMHT plots was similar to that of cv Apex. Similar low levels of outcrossing were detected at the most extreme sampling point (81.5m), GMHT plots ranged from 0.06-0.1% and Apex 0.03%

2. Glyphosate tolerant oilseed rape as a pollen source

Outcrossing levels in plots 2, 4 and 6a which were cross pollinated with glyphosate tolerant oilseed rape were comparable to levels detected in plots cross pollinated with glufosinate

tolerant rape (Figure 20). The outcrossing rates at the interface between the pollen source and receptor plots were within the same range (1.6% -1.7% glyphosate tolerance compared with 1.5%-1.7% glufosinate tolerance) and the decline curve with distance was a similar profile to cv. Apex, with a sharp decline in outcrossing in the first 50m from the pollen source.

3. Imazamox tolerant oilseed rape as a pollen source

   Outcrossing levels in plots cross pollinated with the imazamox tolerant variety were lower than in crosses with GMHT varieties (Figure 21). Levels of outcrossing in plots 3b (RR1) and 7 (LL1) were considerably lower than all other outcrossing data e.g. 0.1% at 1.5m (plot 3b) and 0.4% at 1.5m (plot 7), and were lower than in plot 5 (RR1). Although the outcrossing levels declined with distance in plots 3b and 7, the extremely low levels detected at all distances meant that the decline profile was different to that of other varieties. Outcrossing data from plot 5 was more comparable to data from other plots crossed with GMHT varieties and followed a similar pattern of decline in outcrossing frequency with distance.

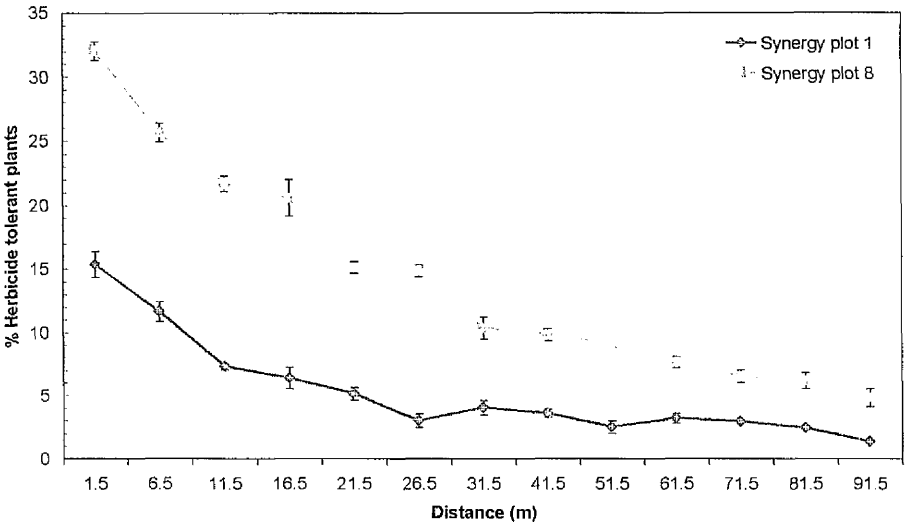


Figure 17. The percentage glufosinate tolerant seeds detected in seed samples from plots of conventional winter oilseed rape (cv. Synergy) growing adjacent to plots of glufosinate tolerant winter oilseed rape, for layout of plots refer to Figure 2, Note: difference in ordinate scale on Figure 18, error bars: +/- standard error, n=6

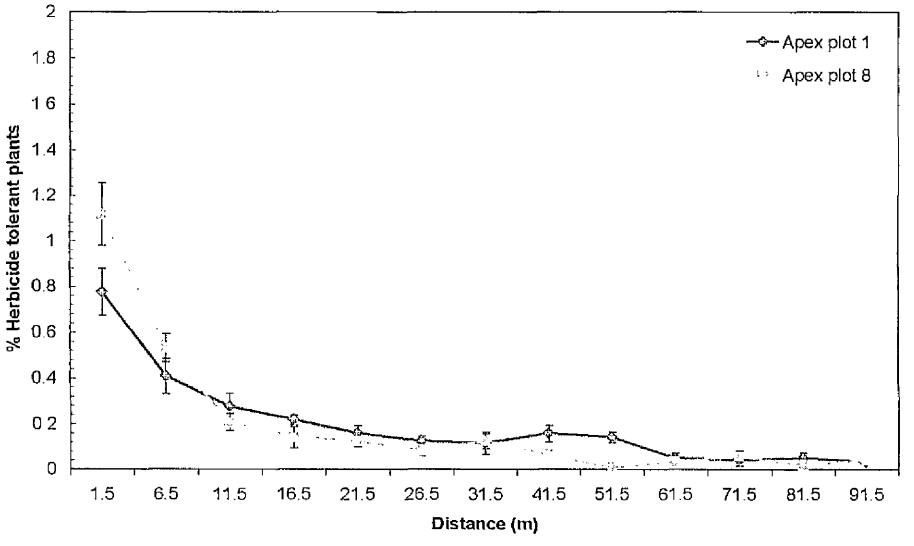


Figure 18. The percentage glufosinate tolerant seeds detected in seed samples from plots of conventional winter oilseed rape (cv. Apex) growing adjacent to plots of glufosinate tolerant winter oilseed rape

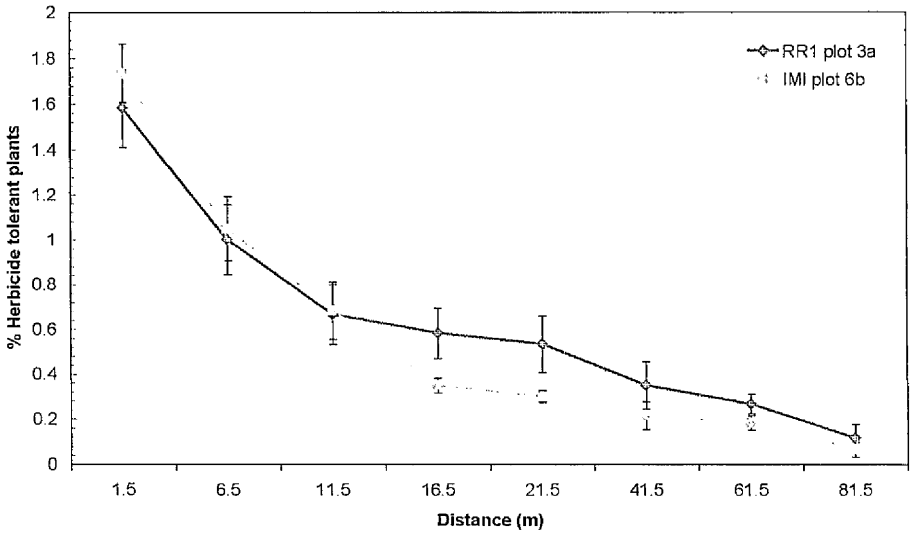


Figure 19. The percentage glufosinate tolerant seeds detected in seed samples from plots of glyphosate tolerant (cv. RR1) and imazamox tolerant (cv. IMI) winter oilseed rape growing adjacent to plots of glufosinate tolerant winter oilseed rape, for layout of plots refer to Figure 2, Note: difference in ordinate scale compared to Figure 17, plot numbers followed by a/b refer to the left or right hand side of the plot, error bars: +/- standard error, n=6

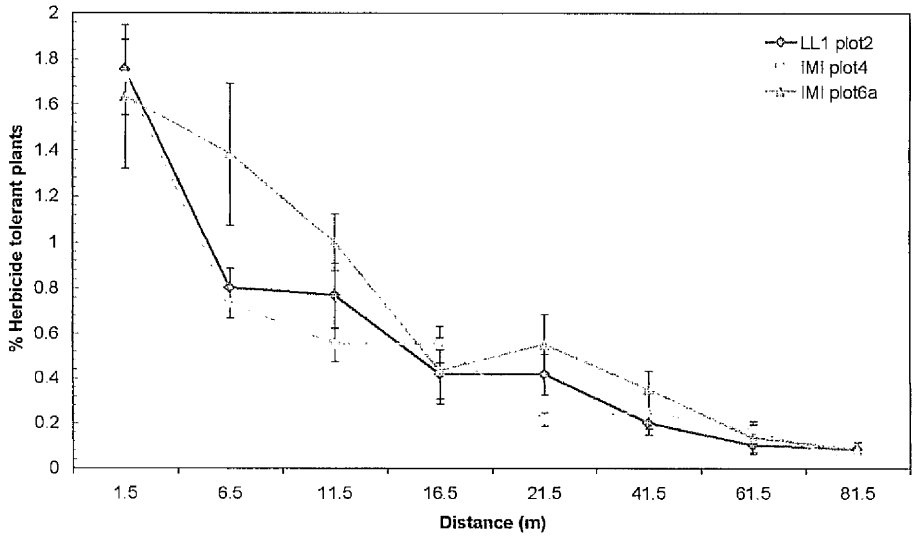


Figure 20. The percentage glyphosate tolerant seeds detected in seed samples from plots of glufosinate tolerant (cv. LL1) and imazamox tolerant (cv. IMI) winter oilseed rape oilseed rape growing adjacent to plots of glyphosate tolerant winter oilseed rape

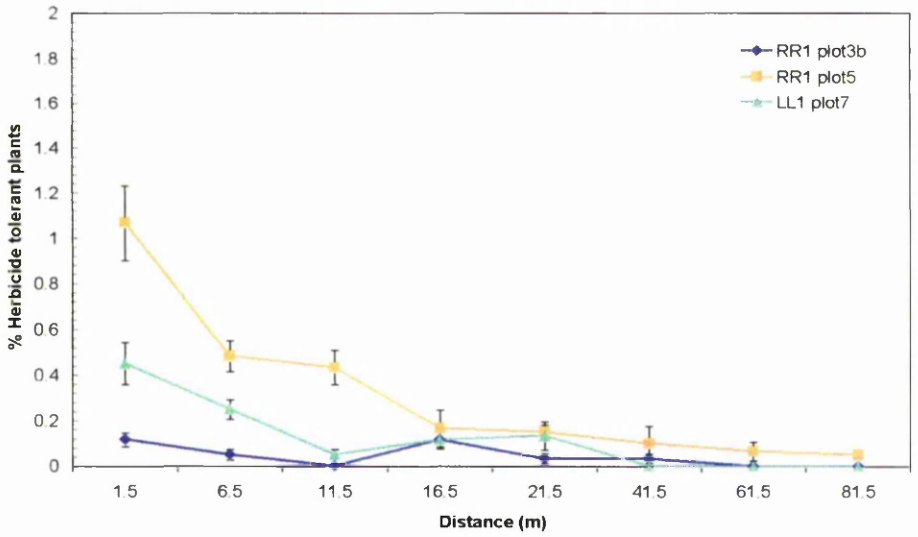


Figure 21. The percentage imazamox tolerant seeds detected in seed samples from plots of glyphosate tolerant (cv. RR1) and glufosinate tolerant (cv. LL1) winter oilseed rape oilseed rape growing adjacent to plots of imazamox tolerant winter oilseed rape, for layout of plots refer to Figure 2, note a: difference in ordinate scale compared to Figure 17, note b: source plot area (plot 4 - approx. half area of all other plots) adjacent to plot 3b, plot numbers followed by a/b refer to the left or right hand side of the plot, error bars: +/- standard error, n=6

3.1.4 Outcrossing between genetically modified herbicide tolerant glufosinate tolerant winter oilseed rape (*B. napus*) and a conventional winter turnip rape variety (*Brassica rapa* cv. Debut)

A plot of winter turnip rape (*B. rapa*) cv. Debut was established adjacent to a plot of glufosinate tolerant oilseed rape (Figure 22). Observations during the flowering period showed that the winter turnip rape was flowering in advance of the glufosinate tolerant oilseed rape. When the turnip rape was at an estimated growth stage of 4,5 the glufosinate tolerant oilseed rape pollinator was between growth stage 4,0-4,2. The prevailing wind conditions for the site are shown in Figure 16. There was a predominance of approximately south westerly and north easterly winds during the main flowering period.

Seeds sampled from the conventional turnip rape cv. Debut were tested for glufosinate tolerance, outcrossing frequencies presented are the mean of four tests of 1000 seeds across three sample transects and are expressed as a percentage of herbicide tolerant seedlings detected.

Outcrossing data is presented in Figure 22. The levels of outcrossing detected were low compared to those found in crosses between *Brassica napus* varieties e.g. 0.24% at 1m compared with an overall mean (excluding cv. Synergy) of 1.19% at 1.5m for *B. napus* varieties in Figures 18-21. Herbicide tolerant seeds were not detected in samples further than 41m away from the pollen source unlike cross pollination between varieties of *B. napus* where outcrossing was detected at distances up to 91.5m from the pollen source, despite the greater number of seeds tested at each distance. The decline in outcrossing frequency with distance in *B. rapa* samples followed the same trend, although at lower levels, found in outcrossing data in previous experiments between the same species.

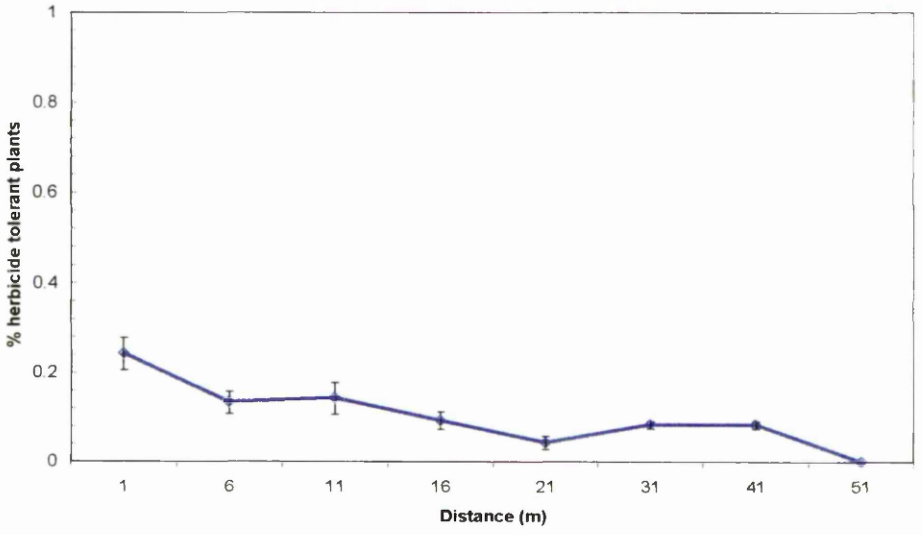


Figure 22. Percentage glufosinate tolerant seeds detected in seed samples from a plot of winter turnip rape *(Brassica rapa)* growing adjacent to a plot of glufosinate tolerant winter oilseed rape (*Brassica napus*), error bars: +/-standard error, n=12

3.1.5 DISCUSSION

3.1.5.1 Cross pollination between variety trial plots in National List genetically modified herbicide tolerant winter oilseed rape trials (harvested in 1997 & 1998)

The experiments showed that outcrossing frequencies declined with increasing distance from the pollen sources. Outcrossing levels were comparable over the different sites and sample years although seed tested from trials harvested in 1998 showed higher values overall; e.g. compare outcrossing levels in cv. Synergy from 1997 and 1998 at Cockle Park (Figures 8,9,10 and 11). This was most likely due to an increase in the number of GMHT varieties in the trials increasing the immediate and background levels of pollen. In 1997 there were two GMHT varieties in trial, and seven in 1998 (4 glufosinate tolerant and 3 glyphosate tolerant varieties). The higher outcrossing frequencies were particularly evident at the Cockle Park site in 1998 (Figures 10 and 11).

Levels of cross pollination were frequently higher in the seed samples tested from the varietal association cv. Synergy, in samples taken from all trials in both years, although this was not always the case. This inconsistency may have been due to the intervening plot(s) contributing competing pollen which was not recorded as outcrossing in cv. Synergy. The seed of the variety cv. Synergy consists of a mixture of 80% male sterile hybrid and 20% of a male fertile pollinator, this high proportion of male sterility implies that varietal associations such as cv. Synergy would be more receptive to pollen from external sources.

Levels of glufosinate tolerance detected in cv. Falcon were also often higher than in other fully self fertile varieties (e.g. at the Bridgets site 1997 and 1998). The explanation for this is not clear, it is possible that the receptive period of the gynaecium of cv. Falcon coincided well with the pollen release from the herbicide tolerant varieties or a majority of plots were situated downwind of the GMHT plots. A further explanation for the detection of unusually high values in apparently random plots may have been a result of cross contamination of seed during the harvesting process, thus it is possible that seed samples may have contained a mixture of two or more varieties.

The detection method for the herbicide tolerance transgenes in the glasshouse tests was robust, seedlings were sprayed twice before final surviving plant counts were made, which allowed easy identification of herbicide tolerant plants. Double tolerance was also readily identified when seedlings were sprayed twice with a sequence of glufosinate and glyphosate.

The levels of outcrossing reported in this study compared well with other studies. For example, Bilsborrow *et al.* (1998) measured pollen transfer between high and low erucic acid cultivars of oilseed rape. Their results showed that at two test sites erucic acid contents of the double low variety samples ranged from 0-6.3% and 0-4.3%. The experiment showed that cross pollination did not consistently decline with distance and high levels were detected randomly throughout the sample area. The results of monitoring pollen concentrations using rotorod traps showed that pollen concentrations decreased rapidly with distance downwind; the concentration at 2m was 53.5% of the level at the source. They suggest that because there was no clear decline gradient in outcrossing levels with distance from the source despite the declining levels of pollen concentration that insects may have been involved in pollen dispersal.

In this experiment, because the plots are laid out in a randomised block design, it is difficult to obtain balanced sets of data for each variety and some comparisons are disqualified by close proximity to two emitting plots. While this evidence of cross pollination is perhaps less robust because of the *ad hoc* nature of the experimental design, it provides valuable preliminary data of the influence genotype on outcrossing obtained.

3.1.5.2 Transgene flow into non-GM National List variety trials (harvested in 1997 and 1998)

Outcrossing frequencies declined rapidly and subsequently remained at levels between 0.03% and 0.05% at further distances from the pollen source, there were some fluctuations in plots sampled beyond those nearest the pollen source (Tables 21-23). It was only possible to detect minimum outcrossing levels of 0.05% in 1997 experiments and 0.03% in 1998 experiment, if more precise detection limits were used it may have been possible to detect rarer cross pollination events at larger

distances. It is possible that the low levels and less clear decline in cross pollination with increasing distance (at larger distances) may be associated with the pollen source size, the presence of non-GM barrier crops and the fact that the receptor plot(s) were non-uniform i.e. a trial of a large number of varieties randomised in the field with differing flowering times and growth habits etc. A similar effect, was shown by Beckie, Hall, Warwick, (2001) where outcrossing frequencies changed little with increasing distance (between 50m and 400m) from the pollen source after the first 50m (0.07%). Interestingly Beckie *et al.* (2001) were using much larger uniform pollen sources (32-64ha) and recorded outcrossing within the range of the frequencies presented here.

Seed samples from plots of cv. Synergy confirm this hybrid (varietal association) to be more receptive to pollen from external sources. The cv. Synergy was also often cross pollinated at greater distances than other varieties, for example, at Bridgets GMHT seed was detected in a plot of cv. Synergy at approximately 150m from the outer edge of the GMHT pollen source (0.11% glufosinate tolerance and 0.22% glyphosate tolerance) where no other outcrossing was detected. The data did not suggest that restored hybrids such as cv. Pronto were more "susceptible" to outcrossing than other conventional varieties.

Although the data from experiments conducted in both years is comparable, the location of these trials in the wider geographical context, proximity to the GMHT trial pollen source and randomisation of plots within the GMHT trials means that it is difficult to make direct comparisons. There is however commonality between sites in terms of the range of outcrossing values detected and the fact that cross pollination levels showed an overall decline with increasing distance from the GMHT pollen source.

Both experiments using small trial plots as pollen sources and receptors demonstrated; the influence of distance on outcrossing level, the potential influence of pollen source size and receptor and the influence of varietal type on outcrossing levels.

3.1.5.3 Outcrossing between field scale areas of herbicide tolerant and other winter oilseed rape cultivars and a winter turnip rape cultivar (*B.rapa*)

3.1.5.3.1 Outcrossing recorded in plots of oilseed rape (*Brassica napus)*

The large differences in outcrossing levels between the two conventional varieties (cv. Apex and cv. Synergy) in plots (1 and 8) in this experiment confirm previous results from tests carried out on seed samples from National List trials (Sections 3.1.1 and 3.1.2), where cv. Synergy and other varietal associations generally produced higher levels of outcrossing compared with either conventional or restored hybrid varieties. The differences are due to the high proportion of male sterile plants in varietal association cultivars such as Synergy, Gemini or Lipan and thus the reduced competition from self pollen. Outcrossing levels obtained from all varieties in the experiment followed the same exponential decline in cross pollination with distance. Both glufosinate and glyphosate were detected at similar levels and recipient varieties showed similar rates of decline with distance from the transgenic pollen sources.

Overall, the levels of imazamox tolerance detected were the lowest (Figure 21). The outcrossing levels detected in the plots 3b (RR1) and 7 (LL1) were considerably lower than in other plots. There are a combination of factors that may have caused this; waterlogged soil during the winter visually reduced crop vigour and the variety also began flowering the earliest which reduced the period over which pollen exchange could occur, most importantly, imazamox tolerant plot 4 (adjacent to plot 3, side b) was approximately half the area of all other plots; pollen source size has been considered as one of the major influences on cross pollination (Timmons *et al.*, 1995).

3.1.5.3.2 Outcrossing recorded in winter turnip rape *(Brassica rapa)*

The outcrossing frequencies recorded in the small plot (0.12ha) of turnip rape cv. Debut growing adjacent to a glufosinate tolerant plot of oilseed rape (0.8ha) were lower than those detected in plots of oilseed rape. Theoretically, the outcrossing level should have been higher because *Brassica rapa* is largely self-incompatible and requires cross pollination facilitated by insects or wind to set seed (Snow and Jorgensen, 1999). The average outcrossing level of 1.19% at 1.5m in oilseed rape was

nearly five times higher than the level recorded in the turnip rape plot at 1m (0.24%). It is likely that the large differences are due to synchrony of flowering times and less genetic compatibility between the two species. The winter turnip rape cultivar "Debut" began flowering earlier than the adjacent glufosinate tolerant crop; it was noted on the 19th of April that plots 1 and 2 had the least flowering plants (growth stage 3,7-4,0), the winter turnip rape was at growth stage 4,2. Other factors such as the unfavourable prevailing wind conditions (Figure 16) combined with the low numbers of bees recorded in the experimental area (see Section 3.1.3.1) may have reduced the amount of pollen dispersal from the GM source plot.

The results of this study are limited by the pollen source and recipient crops, their orientation and the genotypes involved, which may have considerable effect on the synchrony of flowering times and thus outcrossing levels.

3.1.5.3.3 Comparison of outcrossing results with previous studies

Recorded levels of outcrossing in oilseed rape are variable, being dependant on factors such as experimental design, relative size of pollen source and recipient, variety type, as well as site specific factors such as climatic conditions and topography. These factors combine to produce different results in terms of level of transgene dispersal from GM crops of oilseed rape.

The range of outcrossing levels recorded in cv. Apex and between adjacent plots of GMHT varieties in this study were compared with previous work carried out by Champolivier *et al.* (1999), Downey (1999), Beckie *et al.* (2001) in Figure 23. Although there are substantial differences in experimental design such as pollen source size (e.g. Beckie *et al* 2001 used pollen sources of between 32ha and 64ha), environmental conditions and genotypes all the data is broadly comparable and shows a similar decline in outcrossing with distance from pollen source.

There have been fewer studies of cross pollination between commercial *B. napus* and *B. rapa* crops. Data on geneflow from large commercial fields to small plots of *B. napus* (canola) and *B. rapa* were obtained by Stringham and Downey (1978). The outcrossing levels between fields of *B.*

rapa reported by Stringham and Downey (1978) were high e.g. 8.5% at 46m, 5.8% at 137m and 3.7% at 366m. This was compared with similar experiments on *B. napus* by Downey (1999) where the outcrossing rates were between one quarter and one sixth of that of *B. rapa*. Although these data sets do not investigate the interspecific outcrossing levels obtained, they do imply that the outcrossing levels in *B. rapa* are considerably higher than in *B napus*. An experiment conducted by Manasse and Kareiva (1991) reported outcrossing between a trial of transgenic *B. napus* and blocks of a commercial cultivar of *B. rapa*. Their experiment also showed higher levels of outcrossing than those reported here, results of 0.022% at 50m and 0.011% at 100m from the central block, in this study no outcrossing was detected at distances greater than 40m. Although data in previous work and in the current study are not directly comparable, both sets of studies showed a common decline gradient with distance from the pollen source.

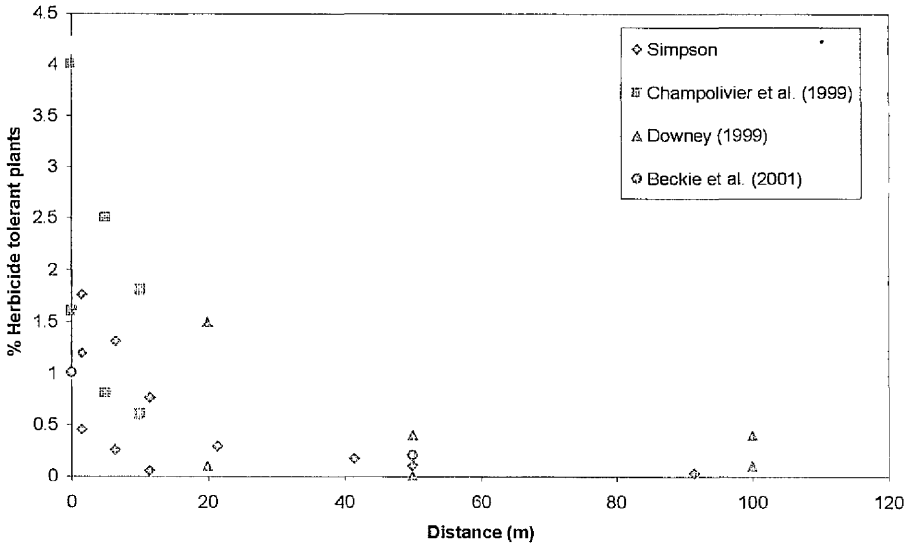


Figure 23. Comparison of oilseed rape outcrossing data from the range of results of experiment 3.1.3 (excluding data from cv. Synergy) with Champolivier *et al.* (1999) Downey (1999) and Beckie *et al.*, (2001)

3.1.5.3.4 Factors influencing outcrossing

Crop growth: The large differences in outcrossing values from the same variety in this experiment (plots 1 and 8) can be partially explained by the extensive bird grazing damage to plants and subsequent stunting of growth in plot 1 and the adjacent glufosinate tolerant plot 2 during the winter and early spring. General observation and the differences in mean heights of the varieties at flowering time indicated that many of the plants were stunted and less vigorous compared to those in plot 7 and 8 (Table 24). The non-uniform flowering in certain plots can be seen in Plate 4 (Appendix 1).

Synchrony of flowering: probably influenced the amount of cross pollination between the imazamox tolerant variety (cv. IMI) and other varieties. Although growth stages presented in Table 24 do not imply large differences in flowering times, from general observations, the cv. IMI flowered earlier than the other varieties. The GMHT varieties and the conventional varieties started flowering at approximately the same time and all varieties showed considerable overlap in their flowering periods. As previously discussed there was a similar lack of synchrony in flowering time observed in the plot of winter turnip rape. Differences in uniformity of flowering within plots may have also contributed to fluctuations in data more than initiation of flowering (Plate 4, Appendix 1). It may be possible that certain points during the flowering period in oilseed rape are more conducive to cross pollination due to insect activity or wind, this has been previously reported by Williams (1984) and is discussed under *dispersal mechanisms.*

The size of pollen source: has been associated with differences in cross pollination levels reported (Timmons *et al.*, 1995, Raybould and Gray, 1993, Levin and Kester, 1974). A theoretical study by Crawford *et al.* (1999) examined the effect of increasing emitter size on the levels of cross pollination which will occur. It was concluded that a square plot of 400m^2 would produce a "pollen dispersal characteristic" of about 3/4 of that of a field of 4ha (40000m^2), the indications were that the

effectiveness of pollen dispersal would decline markedly at plot sizes less than 400m^2. Experiments with small emitter crops may well underestimate the amount of cross pollination between whole fields, the size of the plots used in this study (8464m^2) should be sufficient to estimate "field scale" cross pollination according to Crawfords' calculations.

The effects of reduced pollen source size are evident in plot 3b (which had the lowest outcrossing levels, Figure 20) growing next to plot 4, which was approximately half the size of all other plots. The outcrossing levels at 1.5m detected in plot 5a were approximately ten times greater than those recorded in plot 3b (both receptor plots the same variety), demonstrating the large effect of pollen source size. The effects of different sizes of pollen source and recipient crop an outcrossing are investigated in Section 3.2

Dispersal mechanisms: Pollen dispersal and pollination is affected by a complex sequence of environmental and biological processes, each of which is extremely variable. These stages include the release of pollen, its transport, its deposition, and whether or not pollination occurs at the target. The biological factors of crop type and growth stage characterise the timing of pollen release. In addition meteorological factors will affect when the pollen is released within a given period, how it disperses in the atmosphere, the level of deposition and the pollen viability hence the resulting likelihood of pollination.

It is widely recognised that both insects and wind influence the transport of oilseed rape pollen. Oilseed rape pollen has frequently been detected above and downwind of different sized source crops during flowering (Landridge and Goodman, 1982; Mesquida and Renard, 1982; Williams 1984 McCartney and Lacey, 1991; Thompson *et al.,* 1999). Some studies have indicated that bees are an effective vector for long distance dispersal (Scheffler, Parkinson, Dale, 1995; Ramsay *et al.*,1999; Squire, Crawford, Ramsay, Thompson, Brown, 1999; Thompson *et al.,* 1999;) and short distance dispersal (Cresswell, 1994; Cresswell *et al.*, 1995; Bilborrow *et al.*, 1998). McCartney and Lacey, (1991) examined crops of oilseed rape over several seasons and measured

pollen dispersal, they identified a marked diurnal periodicity of pollen production in oilseed rape crops, with maximum concentrations occurring early in the afternoon. The highest concentrations of oilseed rape pollen were found on warm dry and windy days. McCartney and Lacey (1991) also calculated that pollen concentration 100m downwind would be between 2-10% of the values within the crop which can be extrapolated to a cross pollination rate from the upwind crop of 0.6-2%, these levels are of a similar range to the data presented here for open pollinated varieties.

Williams (1984) also investigated the density of pollen being emitted from oilseed rape crops. Their results also showed a diurnal periodicity with peaks occurring in the late morning and early afternoon. They concluded that wind might be an important agent in pollination of oilseed rape by effecting pollination by movement of flowers and cross pollination by carrying pollen through the crop. Williams (1984) also suggested that the different ages of flowers on an oilseed rape plant may also affect whether the flower is insect or wind pollinated. In the initial stages of flower development the stigma is level with the floral surface and is shielded by the four upright stamens which surround it, Williams (1984) suggested that at this stage the flower is more likely to be pollinated by an insect than wind. During the three days over which a flower is open, the gynaecium lengthens so that the stigma is raised above the floral surface and becomes exposed, at this stage the flower is more likely to be pollinated by wind blown pollen.

Counts of bee species during the main flowering period were carried out where possible at times that were conducive to bee flight and foraging. General observations were also made during routine visits to the experimental field. The extremely low numbers of all bee species recorded suggested that wind may have been the main pollen dispersal agent. However practicalities limited the amount of time and frequency of visits to the crop on days that there may have been significant flights of bees which may have strongly influenced the outcrossing levels recorded.

There does not appear to be a detectable relationship between the summarised wind data and the outcrossing levels detected. According to Figure 16, the wind direction was mainly along the

length of the experimental field approximately equally in both directions (Figure 2), which makes correlation of the outcrossing data with prevailing wind direction difficult, particularly without quantitative measurements of airborne pollen densities. Wind velocity and directional data recorded one occasion each day is of limited use when attempting to explain precise directional differences in outcrossing levels. Thus more detailed measurements of wind velocity and direction which could be related to pollen dehiscence and receptivity of gynaecia are required.

There are no known reports that have clearly separated the influence of wind and insects on cross pollination in oilseed rape most probably due to the technical difficulties involved. The relative importance of wind and insects as vectors for the dispersal of pollen and subsequent fertilisation in this study is uncertain.

Inheritance of HT trait: The hemizygous male and homozygous female parents of the hybrid glufosinate tolerant variety (LL1) contain the *Bar* gene at two loci so that the hybrid produces glufosinate tolerant and non-tolerant pollen in a 5:3 ratio. This means that using herbicide tolerance in seedlings to measure cross pollination from glufosinate tolerant varieties will only measure 5/8ths of the actual level (B. Uijtewaal, pers comm. 2000). This should theoretically have affected the levels of outcrossing detected in this experiment. However, the levels of glufosinate and glyphosate tolerance detected in plots in this study are approximately equivalent, other factors such as flowering time and weather conditions may have balanced out this predicted theoretical effect. It was not certain whether the low levels of cross pollination in plots crossed with the imazamox tolerant variety were due to a difference in the heritability of the herbicide tolerance trait. Related gene flow work in the UK does not suggest that the heritability in the non-GM imazamox tolerant variety is any different to that of the GMHT cultivars (G. Ramsay pers. comm. 2000). It is more likely that several factors such as flowering time, vigour of crop, wind direction, insect activity and pollen source size combined to strongly influence the outcrossing levels.

Sampling and herbicide tolerance testing: Herbicide tolerance testing using the direct screening method of F1 seedlings for the herbicide tolerance markers allowed easy identification of tolerant seedlings after two herbicide sprays. When tested using analysis of variance no significant differences were found between the numbers of surviving plants in the two replicates of test trays of oilseed rape seedlings.

The context and objectives of an experiment are clearly important when deciding on sampling effort. The experimental objectives of this study were to determine cross pollination frequencies between "field scale" areas of oilseed rape, and to simulate the likely levels of contamination that may result from neighbouring crops of GM and non-GM oilseed rape. This type of data can be used to quantify the risks to the agricultural environment associated with growing genetically modified oilseed rape and provide data for the calculation of isolation distances and prediction of transgene dispersal. Data from this experiment have been utilised in Section 3.3 to compare two dispersal models, the implications for sampling procedures and risk assessment of GMHT oilseed rape are discussed.

3.2 THE INFLUENCE OF POLLEN SOURCE SIZE AND FERTILITY OF RECIPIENT PLANTS ON OUTCROSSING IN OILSEED RAPE

3.2.1 Long distance cross pollination of isolated male sterile and male fertile receptor plots of oilseed rape positioned at a range of distances and directions from an 11.5 hectare area of herbicide tolerant oilseed rape

The receptor stands of male sterile and male fertile *B. napus* plants were successfully positioned in the field when the transgenic winter oilseed rape (pollen source) was at growth stage 4,5 (approximately 50% of flowers opened). The receptor plants were all at the same growth stage 4,0. Any opened or partially opened flowers were removed from plants prior to positioning in the field. Figure 3 shows the location of the receptor stands in relation to the transgenic pollen source.

3.2.1.1 Records of wind speed and direction during the period receptor plant stands were located in the field

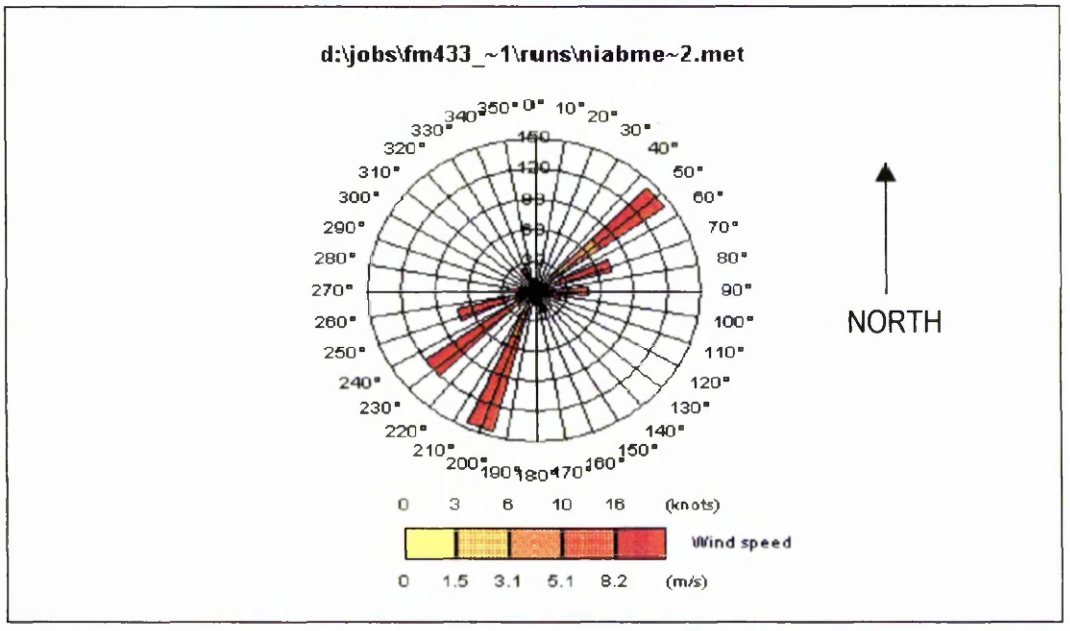
The summarised wind velocity and wind directional data is presented in Figure 24. The wind diagram shows the direction from which the wind was blowing and because only one reading was taken per day the wind diagram assumes that the wind varies over a 30 degree sector. There was a predominance of approximately south westerly and north easterly winds during the main flowering period. Detailed weather data are shown in Table 44 (Appendix 3).

3.2.1.2 Number of seeds per siliqua in pods sampled from male sterile receptor plants

Seed number per siliqua was assessed to determine whether seed set per pod decreased with distance from the pollen source to give an indication of the potential involvement of insects or wind in the transfer of pollen, decreasing seed set potentially indicating greater wind than insect mediated pollination. Seed set per siliqua in random samples of pods taken from each male sterile receptor stand of 6 plants is shown in Figure 25. There was no clear consistent trend of declining seed set with increasing distance in any direction from the pollen source, the fluctuations suggesting that pollen transfer to receptor plots is not solely wind mediated. Total seed set for male sterile and male fertile receptor plots is shown in Tables 25 and 26.

To test whether the number of seeds per pod changed with distance from the GM pollen source, linear regressions were fitted to the observed data. In the case of the fertile receptor plants a significant relationship between distance and numbers of seeds per pod was observed only in the case of the receptor plants to the north west. Here, the number of seeds per pod was found to be positively correlated with distance. The estimated gradient of the fitted line was (± s.e.) 0.01±0.004, ($P<0.001$) indicating that, on average, pods contained one extra seed for every 100m traveled from the pollen source. However, the overall fit of the regression to the data was poor ($R^2 = 26.9\%$). In all other cases there was no evidence of a systematic change in the number of seeds per pod with distance from the pollen source.

In the case of the male sterile receptor plants a significant relationship between distance and number of seeds per pod was found only in the case of receptor plants to the north east of the pollen source. In this case the number of seeds per pod decreased with distance from the pollen source. The estimated gradient of the fitted line was –0.02± 0.008 ($P = 0.01$) indicating that, on average, pods contained two fewer seeds for every 100m travelled from the pollen source. The overall fit of the regression to the data was poor ($R^2 = 14.9\%$). In all other cases there was no evidence of a systematic change in the number of seeds per pod with distance from pollen source.



**Wind diagram prepared and supplied by Cambridge Environmental Research Consultants

Figure 24. Summarised wind speed and wind direction data from the period 27.04.99 - 25.05.99 recorded at the NIAB meteorological station Cambridge, UK

Table 25. Total viable seed set per male sterile receptor plant stand positioned at different distances and directions from a crop of glufosinate tolerant winter oilseed rape

	Total seed set per receptor plant stand per distance*			
Direction/Distance	100m	200m	400m	600m
North West	500 (5)	500 (5)	500 (5)	500 (5)
North East	660 (7)	500 (5)	300 (3)	200 (2)
South East	580 (6)	400 (4)	200 (2)	475 (5)
South West	100 (1)	420 (5)	550 (6)	200 (2)

*Values rounded up to the nearest 5 seeds
Values in parentheses = number of tests of 100 seeds or fraction of 100

Table 26. Total viable seed set per male fertile receptor plant stand positioned at different distances and directions from a crop of glufosinate tolerant winter oilseed rape

Direction/Distance	Total seed set per receptor plant stand per distance* 100m	200m	400m	600m
North West	5500 (4)	9810 (8)	6580 (5)	8960 (8)
North East	3980 (3)	6400 (5)	5980 (4)	4160 (3)
South East	6220 (5)	4920 (3)	2000 (3)	5035 (4)
South West	3000 (3)	3580 (3)	3400 (3)	3260 (3)

*Values rounded up to the nearest 5 seeds
Values in parentheses = number of tests of 1000 seeds or fraction of 1000

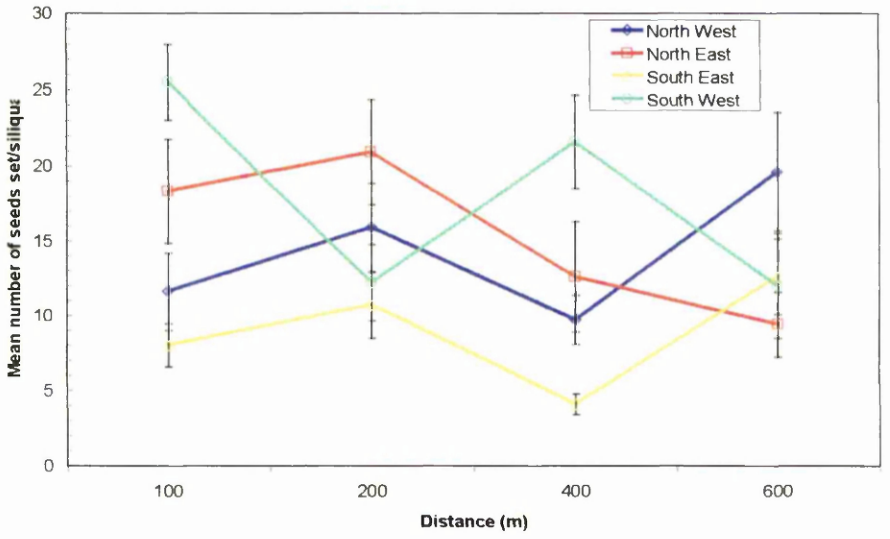


Figure 25. Mean number of seeds set per siliqua in random samples taken from male sterile receptor plants positioned at a range of distances and directions from a crop of herbicide tolerant winter oilseed rape, error bars: +/-standard error, n=10, see Figure 3 for layout of receptor plots, distances are from the edge of the non-transgenic 'pollen barrier' (Figure 3)

3.2.1.3 Cross pollination of male sterile and male fertile oilseed rape plants in receptor plots

Outcrossing frequencies are expressed as a percentage of herbicide tolerant seedlings detected in bulked seeds harvested from male sterile and fertile receptor plots at each distance/direction, the data presented are the means of between 3 and 7 tests of 100 seeds tested per distance for male sterile plants and between 3 and 8 tests of 1000 seeds for male fertile plants (Figures 26 and 27). Numbers of herbicide tolerance tests varied due to different total seed set at each distance (Tables 25 and 26). The total seed set for male sterile and male fertile receptor plots is also shown in Tables 25 and 26.

The herbicide screening results from both male sterile and fertile receptor plants showed a general decline in the amount of herbicide tolerant seeds detected with increasing isolation distance from the transgenic pollen source. The highest levels were recorded in samples from male sterile plants at 100m with plots to the north west and south east containing the highest levels of GMHT seeds. Relatively high levels of GMHT seeds were detected to the north west and south west in male fertile plants. An outlying result at 400m to the south west (male sterile plants) shows a slight increase in levels of herbicide tolerant plants detected before a steep decline at 600m from the pollen source.

There was a large difference in the levels of cross pollination detected in male sterile plants compared with fertile plants. The mean percentage of herbicide tolerant seeds detected at 100m in all directions from male sterile receptor plants was 13.52% compared with 0.042% from fully fertile plants. No herbicide tolerant seeds were detected in male fertile receptor plots at any distance to the north east of the pollen source or at 600m in any direction.

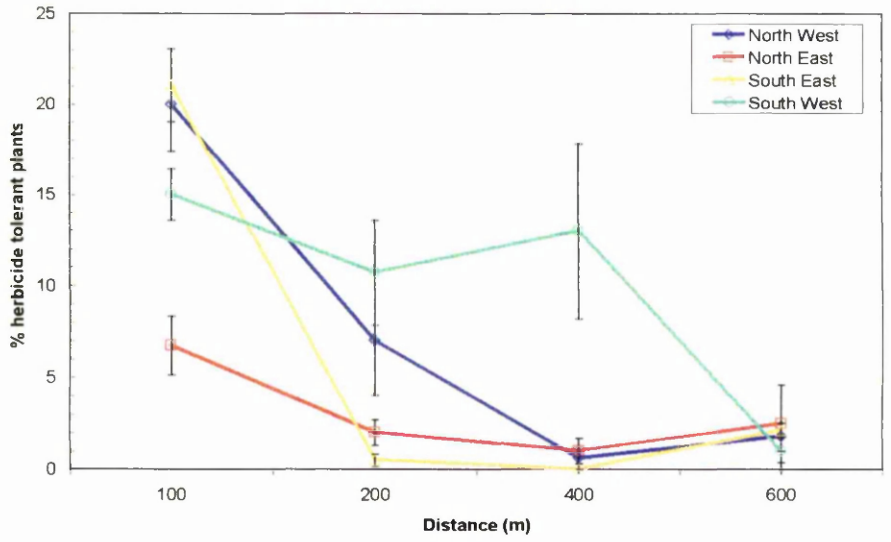


Figure 26. Percentage of herbicide tolerant plants detected in seed samples harvested from male sterile receptor plants growing at a range of distance and directions from a crop of glufosinate tolerant winter oilseed rape Note: difference in ordinate scale between Figures 26 and 27, error bars in Figure 26: +/- standard error, no error bars shown in Figure 26 due to extremely low number of positive observations in tests, see Tables 26 and 27 for number of observations (n), see Figure 3 for schematic layout of receptor plots, distances are from the edge of the non-transgenic 'pollen barrier'

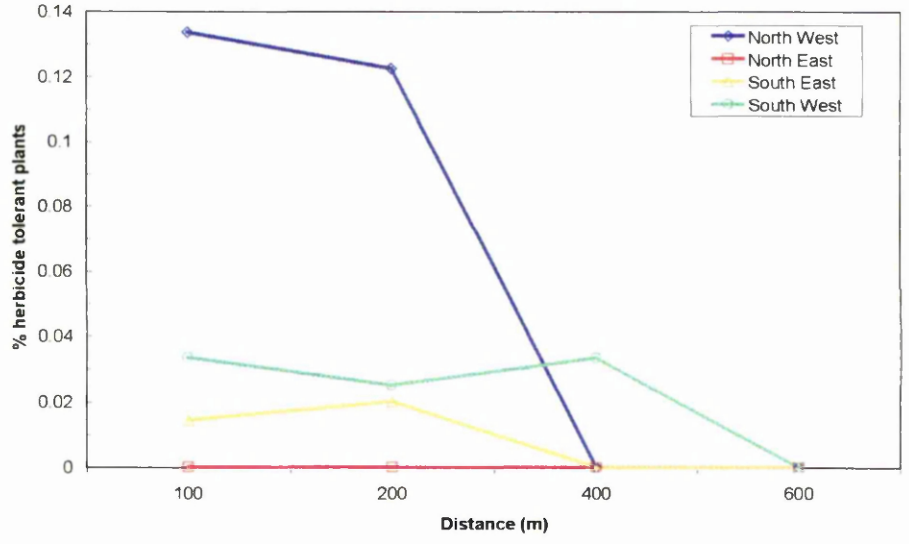


Figure 27. Percentage of herbicide tolerant plants detected in seed samples harvested from male fertile receptor plants growing at a range of distance and directions from a crop of glufosinate tolerant winter oilseed rape

3.2.2 Outcrossing between artificial feral populations of genetically modified glyphosate tolerant winter oilseed rape and conventional varieties of winter oilseed rape

Artificial feral populations of 100 plants were established adjacent to plots of conventional winter oilseed rape (cv. Apex and cv. Synergy, Figure 2). Flowering of the glyphosate tolerant plants coincided well with the conventional oilseed crops. Plate 8 (Appendix 1) shows the proximity of the flowering artificial feral populations to the recipient crops of cv. Apex and cv. Synergy.

3.2.2.1 Outcrossing data from plots of winter oilseed rape (cv. Synergy and cv. Apex)

Seeds sampled from the conventional oilseed rape varieties were tested for glyphosate tolerance, outcrossing frequencies are expressed as a percentage of herbicide tolerant seedlings detected in seed samples and are the mean of two tests of 1000 seeds across three sample transects. Outcrossing data recorded in cv. Apex and cv. Synergy are presented in Figure 28.

Outcrossing data in cv. Synergy (Figure 28) showed a decline with distance comparable with results in Section 3.1.3 and the data is further examined in Section 3.3. In this case, the GMHT pollen source area was very small ($2m^2$) in comparison with the receptor plot ($4232m^2$); and the results reflect this difference in source size when compared with results of Section 3.1.3 where the pollen source area was approximately twice the size of the receptor plot ($8464m^2$, Figure 2). For example, 3% outcrossing was recorded in plants immediately next to the small pollen source compared with a mean of 23.7% outcrossing in plants at 1.5m from a source larger than the receptor. Outcrossing levels detected in cv. Synergy at the nearest sample point to the pollen source were approximately 18 times greater than outcrossing levels in cv. Apex (Figure 28).

Outcrossing was only detected in 1 transect sampled in cv. Apex at the nearest point to the pollen source. The lack of outcrossing data recorded for cv. Apex clearly demonstrates the influence of pollen source size on the levels of outcrossing obtained and the influence of the fertility of the recipient crop. Three factors that are common to studies throughout this work are also demonstrated in this experiment; the influence of distance on outcrossing levels, the influence of fertility of a variety on outcrossing levels and the influence of the size of pollen source on outcrossing level.

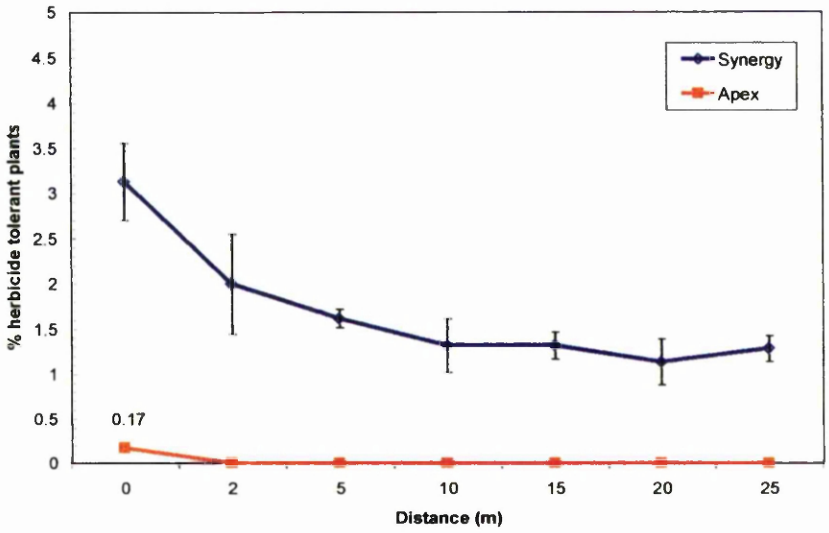


Figure 28. Percentage glyphosate tolerant seeds detected in seed samples from plots of conventional winter oilseed rape cv. Synergy and cv. Apex growing adjacent to a small artificial feral populations of 100 glyphosate tolerant winter oilseed rape plants, 0m distance is equivalent to plants touching, error bars: +/-standard error, n=6

3.2.3 Cross pollination between artificial genetically modified herbicide tolerant volunteer populations and conventional varieties of winter oilseed rape

Herbicide tolerance was detected in all of the treatments in the experiment including the control treatment (T4) where no contaminant GMHT seed had been added. The levels of herbicide tolerant seed detected increased with increasing initial contamination of varieties. The variety 'Synergy' produced the largest amount of herbicide tolerant seed out of the three varieties tested in each treatment. The restored hybrid cv. Pronto produced a higher level of herbicide tolerant seed compared with cv. Apex through all treatments although this trend was not significant (See 3.2.3.1).

3.2.3.1 Results from regression analysis of outcrossing data between conventional oilseed rape and populations of herbicide tolerant volunteers

The initial regression analysis explained a high percentage of the variance in final contamination levels (R^2 = 89.9%) and the overall fit of the model to the data was significant (P<0.001). Estimates of the individual parameters suggested that the constant did not differ significantly from 0 (t (66 d.f.) = 1.30, P=0.199), while the estimated gradient was significantly greater different from 0 (t (66 d.f.) = 24.48, P<0.001); the estimate (± s.e.) was 0.57±0.023. The estimated constant of zero suggested that seed return to the soil would not be contaminated if no contaminating GMHT plants were present in the initial plant population. The estimated gradient parameter of the fitted relationship between initial contamination rate and final contamination rate suggested that the percentage contamination by the GMHT construct would decrease by approximately 43% in a single season, over the range of plant densities examined. The fitted data and fitted model are shown in Figure 29a

When separate constants were fitted for each variety, the overall fit of the model increased (R^2 = 93.1%, P<0.001). The significance tests for the individual parameters suggested that the constants for Apex and Pronto did not differ significantly from 0 (t (64 d.f.) = -1.56, P=0.145, and t (64 d.f.) = 1.45, P=0.151, respectively), while the estimated constant for cv. Synergy was significantly

greater than 0 (t (64 d.f.) = 5.48, P<0.001); the estimated value was 3.24 ± 0.755. The precision of the estimate of the common gradient increased (0.55±0.020) and was significant (t (64 d.f. = 27.95, P<0.001). These results indicated that even in the case where no GMHT seed was incorporated into the initial population of the variety cv. Synergy approximately 3% of the seed shed onto the soil surface contained the GMHT construct. These results support other findings in this study which indicate that the varietal association cv. Synergy is more open to cross-pollination than standard varieties, such as cv. Apex and cv. Pronto. The estimated gradient in this case suggested a slightly smaller reduction in the proportion of GMHT seed in the population; in the order of 45% of the initial level of contamination being lost over the range of population densities examined. The data and fitted model, with separate constants, are shown in Figure 29b.

Fitting the full interaction between initial contamination level and variety further improved the overall fit of the model (R^2 = 94.7%, P<0.001). As before, except in the case of cv.Synergy for which there was some evidence that the constant was greater than 0 (t (62 d.f.) = 2.08, P = 0.042, estimate = 1.81± 0.872), there was no evidence that the constant was greater than 0. Thus, with the full interaction model fitted, the results suggested that approximately 2% of seed shed by cv. Synergy would contain the GMHT construct if a pollen source was available, even if the initial seedbank did not contain any GMHT seed.

The significance tests for the individual varieties suggested that the final contamination rate for Apex and Pronto could be explained by a single linear relationship with initial contamination rate. Thus, while there was evidence of a significant fit for a gradient greater than 0 for Apex (t (62 d.f.) = 14.45, P<0.001), there was no evidence that the gradient for Pronto was significantly different from this value (t (65 d.f.) = 1.41, P=0.161). The estimated common gradient was 0.45±0.039. There was evidence that the gradient for the variety Synergy was significantly different from the other varieties (t (62 d.f.) = 3.79, P<0.001). The estimated parameter value was 0.62± 0.045; slightly lower than the value suggested by the model in which a common intercept was fitted. Thus, the full

interaction model indicated that the contamination rate of seed shed by Synergy would be approximately 38% less than the level in the initial seedbank. The data and fitted full interaction model are shown in Figure 29c.

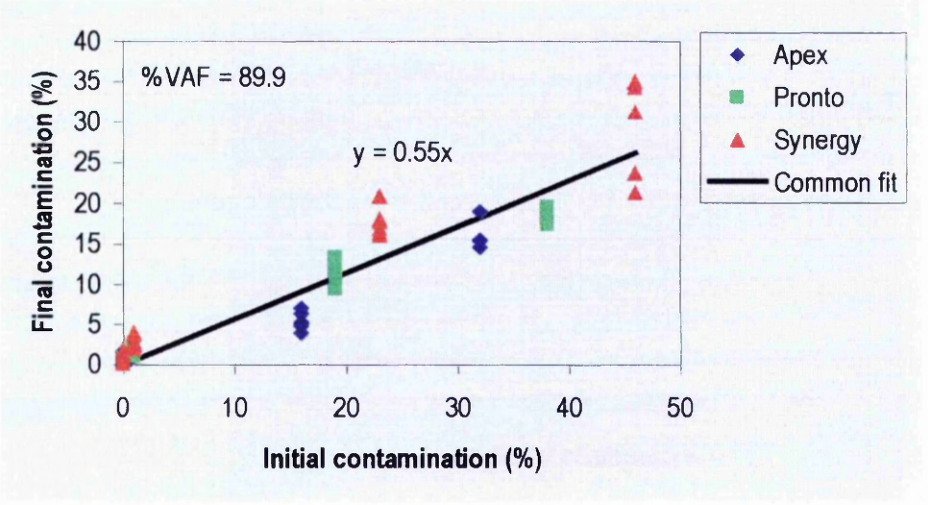


Figure 29 (a) The fitted relationship between intial GMHT volunteer contamination levels and final GMHT seed contamination levels in conventional varieties of oilseed rape

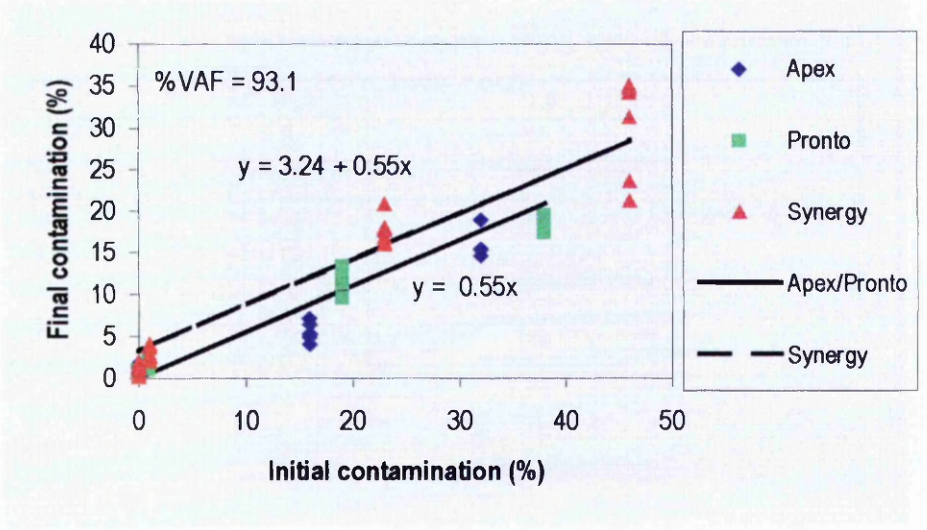


Figure 29 (b) The fitted relationship between initial GMHT volunteer contamination levels and final GMHT seed contamination levels in conventional oilseed rape varieties using separate constants for each variety

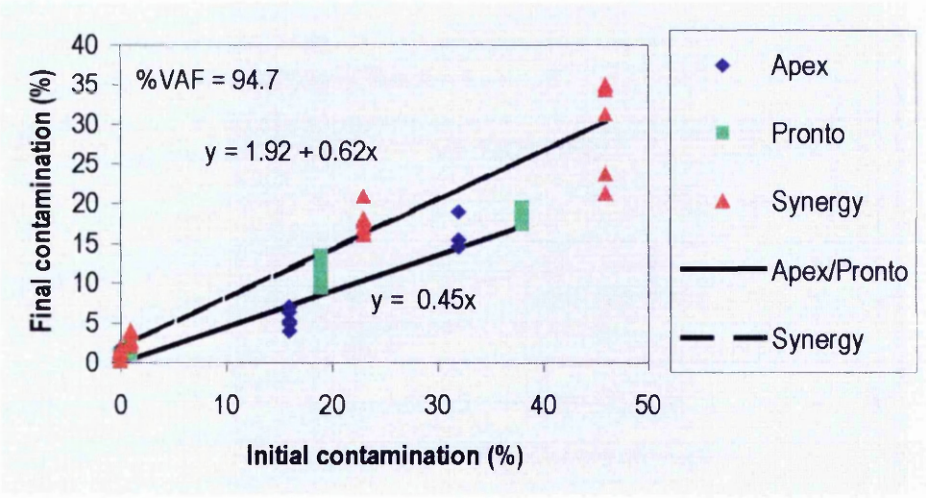


Figure 29 (c) The fitted full interaction between initial GMHT volunteer contamination levels and final GMHT seed contamination levels in conventional oilseed rape varieties

3.2.4 DISCUSSION

3.2.4.1 Long distance cross pollination from an 11.5 hectare area of herbicide tolerant oilseed rape

The decline in outcrossing recorded in plots of male sterile receptor plants was equivalent to dispersal gradients recorded in previous outcrossing experiments in this project. Fertile bait plants showed extremely low cross pollination levels with the GM pollen source at all distances (Figure 27). The highest levels of outcrossing in male sterile receptor plants were detected to the south west, which correspond to the highest levels recorded in male fertile plants (Figures 26 and 27). The lowest cross pollination levels were found in plants to the north east, which also corresponds to the data recorded in male fertile plants. Relatively high, unexpected levels of cross pollination were detected in male sterile plants at 600m from the source in all directions. Cross pollination frequencies at 600m (male sterile plants) to the south east, north east and north west were higher than those detected at 200m in the same directions.

It is possible that these variable cross pollination levels are a result of insect activity rather than wind mediated pollination. Bees have been associated with the dispersal of pollen over long distances (Osborne, Clark, Morris, Williams, Riley, Smith, Reynolds, Edwards, 1999; Ramsay *et al.*, 1999; Thompson *et al.*, 1999) and between small plots where random cross pollination events were associated with insect activity (Bilsborrow *et al.*, 1998). The prevailing wind directions recorded for the period the receptor plant stands were positioned in the field partially support the role of wind as a vector for pollen dispersal. The prevailing north easterly and south westerly winds correspond to the high level of herbicide tolerant seed detected to the south west of the pollen source, but does not correspond to the levels detected in the male sterile and fertile receptor plants positioned to the north east, where the lowest levels of herbicide tolerant seeds for both male sterile and fertile bait plant stands were detected.

The most likely explanation for the low levels of tolerant seed found in this direction is in the layout of the pollen source. Figure 3 shows the location of GMHT plant material within the pollen source, the north east side consisted of mainly non-GM winter rape lines and also a 20m wide surrounding (non-GMHT) 'pollen barrier' this would have diluted the concentration of GMHT pollen being dispersed. The lower levels may have also been a result of the sloping topography of the fields to the north east, whereas all other receptor plots were positioned on level areas of land relative to the pollen source. Zero GMHT seed was detected in male fertile plants at 600m from the GMHT trial in any direction and there was only one incidence of GMHT seeds being detected at 400m at very low levels (south west). The mean outcrossing level across all directions at 100m from the source in male sterile receptors was 392 times greater than in male fertile receptors (15.68% compared to 0.04%).

The variation in experimental designs has made direct comparison of results with other experiments difficult. Timmons *et al.* (1995) reported fertilisation frequencies of 0.08% and 1.2% in single emasculated plants located at 1.5 km from commercial fields. Thompson *et al.* (1999) used existing field crops of 55ha as pollen donors in their study. At one of the receptor plant sites, with a pollination rate of 33%, the majority of the sample (>80%) was shown to have been fertilised from the nearest crop 900m away. Levels of cross pollination were recorded at a maximum distance of 4km from the nearest pollen source in the same study. Simpson *et al.* (1999) noted up to 28% of seed from male sterile receptor plants at 100m from a 9 ha trial contained a herbicide tolerance marker, at 400m in the same study levels had declined to between 1 and 7%. These results broadly agree with this study where 6-21% herbicide tolerant seeds were detected at 100m and 0-13% at 400m from the pollen source.

Thompson *et al.* (1999) also recorded seed number per siliqua in male sterile receptor plants. It was reported that further than 100m from the pollen source, values of seed per siliqua changed relatively little. The large range of values at extreme distances were not wholly attributed to

wind dispersal because of a high seed set despite low overall number of pollination events and the absence of high levels of airborne pollen which suggested the involvement of insects. Thompson *et al.* (1999) also noted that numbers of seed per siliqua changed relatively little after 100m. A similar effect is evident in these results where in the case of the male sterile receptor plants a significant relationship between distance and number of seeds per pod was found only in the case of receptor plants to the NE of the pollen source. Also, the total quantity of seed set in male sterile plots to the north east showed a clear decline with distance (Table 25) suggesting that dispersal of pollen in this direction may have been largely wind mediated. The role of wind as a vector would have been supported if measurements of pollen concentrations at each sample station had been taken.

Squire *et al.* (1999) and Thompson *et al.* (1999) attributed variations of seed set at similar distances (500m) to differences in 'microgeographical habitat'. Although they reported that the relative importance of wind and insects in the pollination of their bait plants was unclear, they concluded that bee involvement might explain high seed set at very distant locations where airborne pollen concentrations were very low. This may have also been the case in male sterile receptor plots at 600m from the pollen source in this study where an increase in herbicide tolerance marker frequencies were noted in three directions (Figure 26).

Although these results are from only one season and at a single site using a non-uniform pollen source, they demonstrate the large difference in cross-pollination between male sterile and fertile plants. The results also clearly show the effect of cross pollination between a large pollen source and small recipient plot. Using male sterile plants to detect gene flow gives an indication of the highest theoretical levels of cross pollination that could be expected. The levels recorded in fertile receptor plants are more representative of a situation where gene flow may occur between a GMHT crop and feral or volunteer plant population.

There is evidence in the results (section 3.2.1.2) that the presence of 'pollen barriers' and also the location of the GMHT plants in the trial have affected the results by potentially diluting pollen

emissions (Morris *et al.*, 1994). The results also support both the role of wind and insects as pollen dispersal vectors.

3.2.4.2 Outcrossing between artificial feral populations of genetically modified glyphosate tolerant winter oilseed rape and conventional varieties of winter oilseed rape

The seed samples taken from cv. Synergy contained particularly high levels of GMHT seed (Figure 28). Even allowing for background levels of pollen contamination in the experimental field the amounts of GMHT seeds detected in samples from the varietal association cv. Synergy were remarkably high (over 15 times higher) in comparison to cv. Apex. GMHT seeds were only detected in one sample from Apex at very low levels (0.17%) immediately adjacent to the feral population of 100 plants (Figure 28).

There was a strong indication that there was a different varietal response to having small populations of GMHT feral plants growing in close proximity. This result reflected previous work in the project (Section 3.1) that showed outcrossing levels detected in varietal associations to be considerably higher than in open pollinated varieties such as cv. Apex. Although flowering time of the small feral plots and the two conventional varieties were approximately the same, small differences may have contributed to large differences in the levels of outcrossing detected. Other factors such as the prevailing wind direction, insect activity and background levels of GM pollen may have also contributed to variations in outcrossing levels between varieties. The data also clearly shows the effect of a small pollen source when compared to outcrossing results in Section 3.1.3, where outcrossing frequencies were much higher (and much larger than the source plots used in this experiment) and the pollen source was twice the size of the recipient plot of cv. Apex and cv. Synergy.

Although there is no dispersal gradient for cv. Apex, the decline in outcrossing recorded in the recipient plot of cv. Synergy is of a similar profile to data recorded in cv. Synergy in Section 3.1.3. There are no previous studies where direct comparison is possible, Scheffler *et al.* (1993) and Bilsborrow *et al.* (1998) both estimated low levels of gene flow from small plots to somewhat larger

areas of oilseed rape. Their experimental designs are representative of geneflow from a feral population to a nearby or surrounding crop. Work conducted by Scheffler *et al.* (1993) using a small 9m diameter circular plot of GM oilseed rape in the middle of a 1.1ha field of conventional rape to measure gene flow showed a very rapid decline in gene flow levels. A level of 1.6% crossing was detected at 1m however at 12m the levels had dropped to 0.016%. It is likely that this sharp drop in cross pollination can be partly attributed to the small size of the pollen source in relation to the size of the recipient plot. The area of transgenic pollinator crop in the study described above was considerably larger than the $2m^2$ area used in this study and the higher outcrossing levels reflect this. However, when compared with previous data such as in Section 3.1.3 where much larger plots were used, the data of Scheffler *et al.* (1993) suggested that pollen source size has a considerable effect. For example, Scheffler *et al.* (1993) recorded 1.6% at 1m compared with an average of 1.19% at 1.5m here, at 12m the levels had dropped to 0.016% which is considerably lower than the average value reported here of 0.46% at 11.5m.

Feral oilseed rape is a common weed of soil dumps, roadsides and field margins and generally at sites which are associated with disturbance due to human activity (Charters, Robertson, O'Brien, Squire, 1996; Squire *et al.*, 1999). The population dynamics of oilseed rape enable it to persist in soil seedbanks for several years (Lutman, 1993). This implies that if GMHT crops of oilseed rape are widely grown the development of persisting seedbanks of GMHT oilseed rape are inevitable. It is unlikely that herbicide tolerance will confer a selective advantage except in areas which are regularly sprayed with the specific herbicide in question. However, the persistence of oilseed rape in the seedbank will enable transgenes to survive through successive generations and assuming that populations are in close proximity to crops of oilseed rape it seems likely that cross pollination would occur. This may, for example, cause unexpected weed control problems in subsequent crop or have crop quality implications.

3.2.4.3 Cross pollination between artificial genetically modified herbicide tolerant volunteer populations and conventional varieties of oilseed rape

General discussion of cross pollination data

The large difference in HT seed return from plots of cv. Synergy compared with cv. Apex and cv. Pronto is due to the high proportion of male sterile plants in cv. Synergy. Indirect evidence also suggests that at high population densities the pollinator plants in cv. Synergy may be lost through competition in early growth stages (NIAB, 2001). The loss or stunting of the pollinator plants in cv. Synergy due to high densities of GMHT volunteers would mean that the volunteers act as pollinators, which would account for the extremely high levels of contamination detected in cv. Synergy. High outcrossing rates in cv. Synergy have been observed throughout the gene flow studies in oilseed rape in this and Section 3.1.3.

Discussion of regression analysis results

The results give an indication of the potential behaviour of populations of GMHT volunteers in different conventional varieties. In nearly all cases the final percentage contamination rate was lower than the initial contamination rate (except in cv. Apex and cv. Pronto at the lowest initial contamination rates).

Evidence gathered in the experiment suggested that, over a wide range of initial GMHT contamination rates, the final proportion of GMHT seed in the total population was a constant fraction of the initial contamination rate. The results indicate that in crops of standard varieties the reduction in GMHT volunteers as a proportion of total population is approximately 0.5 per generation through pollen competition alone. In the case of cv. Synergy, the reduction in GMHT volunteers is lower (approximately 0.38 per generation), probably as a result of higher cross-pollination rates (see section 3.1) and lower competition from the lower plant populations used in the case of cv. Synergy.

The results presented here give an indication of the combined effects of competition and cross-pollination on the longevity of GMHT traits in a mixed population of oilseed rape. In a practical situation, the results suggest that competition between the conventional and GMHT varieties tested

in this study, would reduce the levels of GMHT individuals in the population to low numbers in a few generations. However, in a rotation in which the GMHT trait would confer selective advantage because the GM-linked herbicide was used in other crops, the GMHT trait might persist for a considerable time. In addition, although it appears from the present study as though the level of the GMHT trait in the oilseed rape population might decline relatively quickly, it must be borne in mind that the processes of competition and population dynamics which will determine that decline are stochastic processes GMHT individuals might persist for considerable numbers of years as volunteers or in feral populations not subject to herbicide control. This may enable unwanted spread of the transgene through cross-pollination to subsequent conventional, HT crops or populations of oilseed rape outside agricultural fields. Some of these aspects are explained in a simple population projection model in section 3.5.

3.3 EMPIRICAL RELATIONSHIPS BETWEEN CROSS-POLLINATION RATE AND DISTANCE FROM SOURCE

In a situation in which GM pollen is dispersing into a crop of conventional oilseed rape, the observed rate of cross-pollination will depend on the source strength of the pollen source (i.e. the amount of GM pollen produced) and dispersal behaviour of the pollen with distance. Gliddon (1999) considered that most of the data reported for transgene dispersal experiments are inappropriately presented to be of use in risk assessment partly because of experimental design (e.g. using small pollen sources and large recipient populations) and most fail to fit a distribution to the data. It was considered worthwhile to further investigate the relationship between outcrossing and distance from pollen source using the data set from experiment 3.1.3.

Pollen dispersal is affected by a sequence of complex environmental and biological processes, each of which is extremely variable. The biological factors of crop type and growth stage characterise the timing of pollen release. In addition meteorological factors will affect when the pollen is released within a given period, how it disperses in the atmosphere, the level of deposition and the pollen viability, hence the resulting likelihood of pollination.

The dispersal behaviour of small particles such as pollen grains has been studied for many decades and models with various degrees of complexity have been developed. Dispersal models have been used to describe the dispersal of fungal spores (McCartney and Bainbridge,1984; Fitt and McCartney 1986) pollen (Raynor, Hayes, Ogden, 1974; McCartney and Lacey 1991; Lavigne, Klein , Vallee, Pierre, Godelle, Renard, 1998) and seeds (Colbach *et al.*, 1999). Of these models, the simplest are empirical dispersal curves which are derived by fitting pre-selected functions of a suitable general form to observed data.

Among the empirical dispersal curves the two which have received the greatest attention are the negative exponential (e.g. McCartney and Lacey, 1991; Manasse, 1992; Kareiva *et al.*, 1994) and the inverse power-law (e.g. Gregory 1968; McCartney and Bainbridge, 1984). The negative exponential model tends to underestimate deposition near the source whereas the inverse power law

model tends to overestimate deposition near the source (McCartney and Bainbridge, 1984). All empirical models have limitations as they are essentially descriptive, not interpretative and should not be extrapolated outside the observed range (McCartney and Fitt, 1985).

Simple versions of these models are given as, respectively, equations 1 and 2.

$$y = a \cdot \exp(-b \cdot x) \quad 1.$$

$$y = a \cdot x^{-b} \quad 2.$$

3.3.1 Materials and Methods

Data for experiment 3.1.3 (Outcrossing between field scale areas of herbicide tolerant and other winter oilseed rape cultivars) were used to compare negative exponential and inverse power-law models for their fit to describe the observed relationship between cross-pollination and distance from source. The data were discussed in section 3.1.5 above.

Estimates of the parameters in equations 1 and 2 were obtained by ordinary least-squares linear regression after suitable transformation of the original data. The linear forms of equations 1 and 2 are shown as equations 3 and 4, respectively, below.

$$\ln(y) = \ln(a) - b \cdot x \quad 3.$$

$$\ln(y) = \ln(a) - b \cdot \ln(x) \quad 4.$$

Thus, the parameters of the negative exponential model are obtained as the intercept and gradient of the fit of the logarithm of the number of cross-pollination events against the distance. The corresponding parameters for the power-law are obtained by fitting the logarithm of the number of cross-pollination events against the logarithm of distance. In both cases regression analyses were performed on $\ln(y+1)$ to avoid missing values when $y = 0$. The transformation affects the estimated constant (a) but not the gradient (b).

Five varieties acted as potential recipients of GM pollen in the outcrossing experiment 3.1.3; glufosinate tolerant cv.LL1, glyphosate tolerant cv. RR1, imidazolinone tolerant cv. IMI, and conventional cvs. Apex and Synergy. Although all three HT varieties acted as donors of GM pollen, as a result of the trial layout, and because recipient varieties were only tested for the presence of HT

traits from their immediate neighbours, only a sub-set of all possible donor*recipient combinations was tested. These combinations are shown in Table 27, below (Also see Figure 2 for field layout of plots).

Within each possible recipient-donor combination a separate regression analysis was carried out for each of the three replicate transects, resulting in a set of three estimates of goodness of fit for the different dispersal models. A formal comparison of the ability of the negative exponential and inverse power law models to describe the data was made by carrying out a paired t-test on the percentage variance accounted for (%vaf) from the regression analyses.

In order to investigate the influence of the recipient and donor varieties on the cross-pollination behaviour in the trial, variation in the parameters from the negative exponential and inverse power law models, and the %vaf, were examined using REML (Residual Estimates by Maximum Likelihood). For each parameter the data set available for REML analysis consisted of 36 values distributed over the recipient and donor combinations shown in Table 27. For the REML analysis individual replicates were assumed to be a random sample, and formed the residual term of the mixed model. The fixed effects model was specified as the interaction between donor and recipient varieties. Fitting the dispersal curves by regression and the REML analysis of the parameter values was carried out in Genstat 5.4 (for Windows NT).

Table 27. Combinations of recipient and donor variety tested for cross-pollination of HT traits in outcrossing experiment 3.1.3. Values in parentheses are the numbers of replicate transects of each combination examined

	Donor		
	LL	RR	IMI
Recipient			
LL	*	Yes (3)	Yes (3)
RR	Yes (3)	*	Yes (6)
IMI	Yes (3)	Yes (6)	*
Synergy	Yes (6)	No	No
Apex	Yes (6)	No	No

3.3.2 Results

Empirical dispersal curves

The observed cross pollination data could be fitted to the negative exponential and inverse power law models with varying degrees of success. The fitted models and observed data are shown in Figures 30-37. In each case the fitted model uses the mean of the parameter estimates (given in Table 29). The percentage variance accounted for (%vaf) data for the fitted models is given in Table 28. The parameter estimates for the fitted models for each recipient/donor combination are shown in Table 29. The paired t-test on the %vaf data suggested that the inverse power law model gave a better description of observed out-crossing than the negative exponential model ($P<0.001$). The %vaf for the inverse power law model was greater than that for the negative exponential model in 27 of the 36 transects examined. For the inverse power law model, %vaf ranged from 17.9 to 95.8, with a mean of 76.0, while for the negative exponential model the range was 0.3 to 92.6 with a mean of 63.8, while the standard errors for the %vaf data were generally higher for the negative exponential model than the inverse power law model (Table 28).

REML analysis of the variation in parameter estimates

The pattern of parameter estimates across recipient and donor combinations was similar for both models. The estimated constants were higher for Synergy as a recipient than for any other variety in both cases, with the imidazolinone tolerant variety (cv. IMI) showing lower values than the others. This pattern was repeated to some extent for the gradient parameter in the case of the inverse power law model, but not for the negative exponential model.

The REML analysis suggested that the effects of both recipient (χ^2 =112.2, (4 d.f.), $P<0.001$) and donor (χ^2 =77.9, (2 d.f.), $P<0.001$) on the estimated constant (a) of the inverse power law model were significant. However, in the case of the gradient parameter (b), only the effect of donor was significant (χ^2 =13.0, (2 d.f.), $P<0.01$). For the negative exponential model, the effects of both recipient (χ^2 =200.4, (4 d.f.), $P<0.001$) and donor (χ^2 =100.8, (2 d.f.), $P<0.001$) on the estimated constant (a) of the inverse power law model were significant. In case of the gradient parameter,

again, only the effect of donor (χ^2 =19.2, (2 d.f.), P<0.001) was significant. For both models, the imidazolinone tolerant variety as a donor (cv. IMI) gave rise to relatively flat dispersal gradients (Figures 36 and 37), while the glyphosate tolerant variety as a donor (cv. RR1) gave rise to relatively steep dispersal gradients (Figures 34 and 35).

Empirical dispersal curves were also fitted to the data for the crop of cv. Synergy cross pollinated with a GMHT feral rape population (Section 3.2.2). The results broadly agreed with those observed in the data for experiment 3.1.3, although the overall fits of the models to the data were poorer. In the case of the NE model, percentage variance accounted for was 22.6%. The estimated values for the ln (*a*) and *b* parameters were ((± s.e) 3.03± 0.18 and –0.03± 0.012 respectively. These values are comparable to those obtained for experiment 3.1.3 (Table 29). As with the data from experiment 3.1.3, the IPL model fitted the data for the feral populations better than the NE model (R^2 = 34.4%). The observed parameter values for the IPL model were (for ln(*a*) and *b* respectively) 3.3± 0.20 and –0.28± 0.088. As with the NE model these values were found to be similar to those obtained in the data for experiment 3.1.3 (Table 29).

Table 28. Percentage variance accounted for[1] for linear regression models fitted to observed out-crossing data for herbicide tolerance traits in several combinations of recipient and donor varieties of oilseed rape

	Dispersal model					
	Inverse power law			Negative exponential		
	Donor			Donor		
	LL1	RR1	IMI	LL1	RR1	IMI
Recipient						
LL1	*	87.0 (4.22)	70.90 (4.57)	*	73.7 (6.11)	50.6 (10.60)
RR1	79.2 (7.36)	*	49.8 (12.35)	73.4 (9.92)	*	36.5 (12.35)
IMI	88.7 (3.74)	78.0 (4.87)	*	71.3 (2.27)	72.9 (4.91)	*
Synergy	82.5 (3.48)	*	*	80.5 (8.94)	*	*
Apex	82.6 (2.38)	*	*	58.1 (6.50)	*	*

[1]figures in parentheses are the s.e.m.s for each mean

Table 29. Parameter estimates[1] for linear versions of two empirical dispersal functions fitted to observed out-crossing data for herbicide tolerance traits in several combinations of recipient and donor varieties of oilseed rape

	Dispersal model					
	Inverse power law			Negative exponential		
Parameter: Constant (*a*)	Donor			Donor		
Recipient	LL1	RR1	IMI	LL1	RR1	IMI
LL1	*	3.3 (0.13)	1.9 (0.18)	*	2.4 (0.10)	1.1 (0.12)
RR1	3.1 (0.25)	*	1.7 (0.45)	2.4 (0.18)	*	1.1 (0.14)
IMI	3.3 (0.05)	3.3 (0.19)	*	2.3 (0.04)	2.4 (0.16)	*
Synergy	5.9 (0.06)	*	*	5.0 (0.02)	*	*
Apex	2.5 (0.09)	*	*	1.5 (0.07)	*	*
Parameter: Gradient (*b*)	LL	RR	IMI	LL	RR	IMI
Recipient						
LL1	*	-0.60 (0.056)	-0.45 (0.056)	*	-0.03 (0.002)	-0.02 (0.002)
RR1	-0.46 (0.059)	*	-0.34 (0.097)	-0.02 (0.003)	*	-0.01 (0.004)
IMI	-0.59 (0.036)	-0.59 (0.041)	*	-0.02 (0.001)	-0.03 (0.002)	*
Synergy	-0.50 (0.036)	*	*	-0.02 (0.002)	*	*
Apex	-0.51 (0.029)	*	*	-0.02 (0.001)	*	*

[1]the values shown are the transformed values obtained by fitting equations 3 and 4 with $\ln(y+1)$ as the dependent variate. Estimated values for the fitted NE and IPL models can be obtained by substituting the values for *a* and *b*, above, into $y = [\exp(a)-1]\cdot[\exp(-b\cdot x)]$, and $y = [\exp(a)-1]\cdot[\exp(-b\cdot\ln(x))]$ respectively.

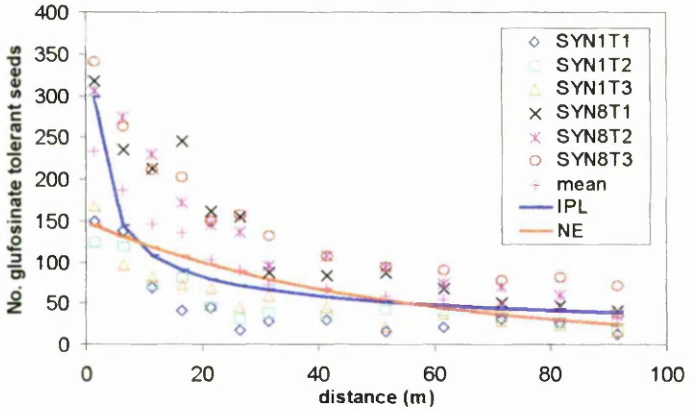


Figure 30. Fitted and observed outcrossing data where cv. Synergy is the recipient crop and cv. LL1 is the donor crop

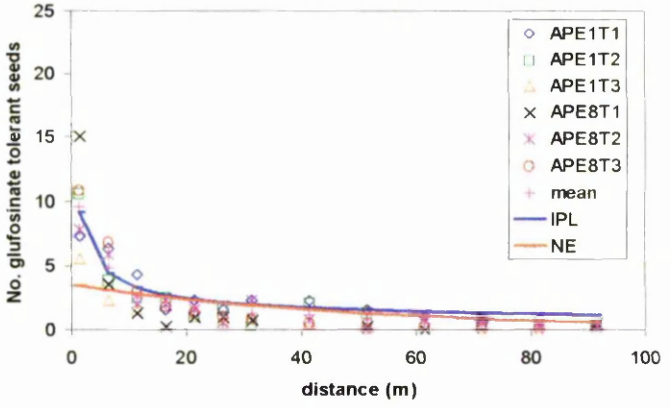


Figure 31. Fitted and observed outcrossing data where cv. Apex is the recipient crop and cv. LL1 is the donor crop

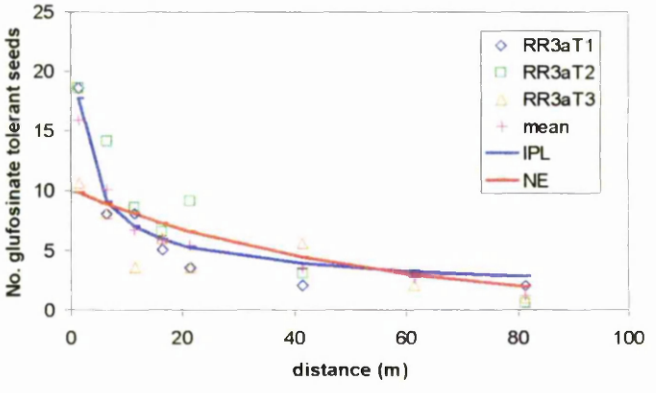


Figure 32. Fitted and observed outcrossing data where cv. RR1 is the recipient crop and cv. LL1 is the donor

Legend

IPL - inverse power law
NE- negative exponential
T1-3= Sample transects 1-3
NOTE: Difference in Y axis scale in Figure 30.

SYN1& 8= cv. Synergy plots 1&8
APE1& 8= cv. Apex plots 1&8
RR3a= cv.RR1 plot 3a

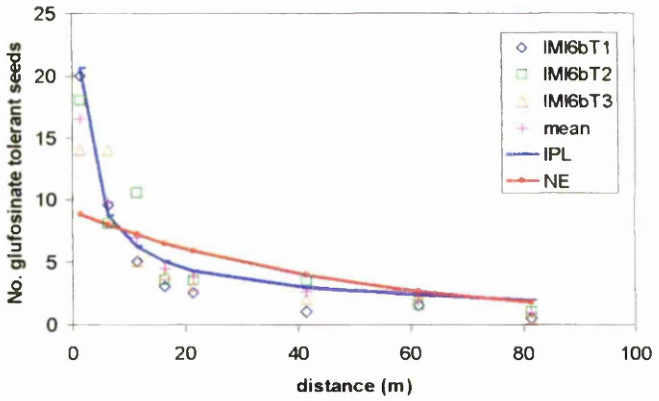


Figure 33. Fitted and observed outcrossing data where cv. IMI is the recipient crop and cv. LL1 is the donor

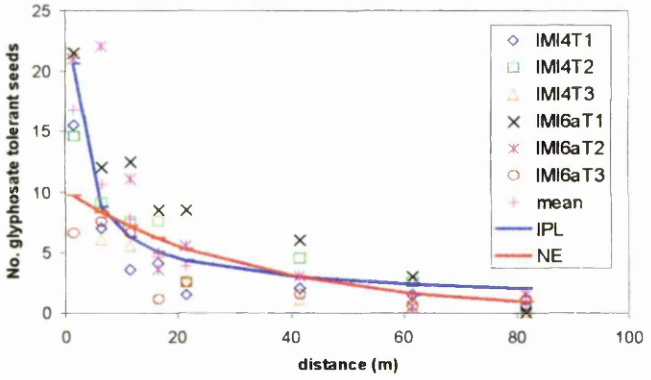


Figure 34. Fitted and observed outcrossing data where cv. IMI is the recipient crop and cv. RR1 is the donor

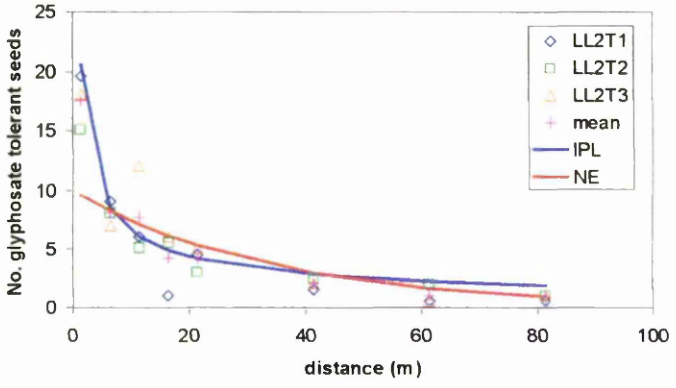


Figure 35. Fitted and observed outcrossing data where cv. LL1 is the recipient crop and cv. RR1 is the donor

Legend

IPL - inverse power law
NE- negative exponential
T1-3= Sample transects 1-3
IMI4, 6a&6b = cv. IMI plots 4& 6 sides a and b
LL2 = cv. LL1 plots 2

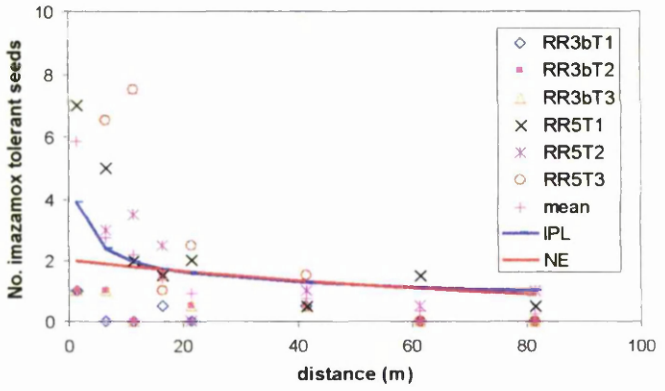


Figure 36. Fitted and observed outcrossing data where cv. RR1 is the recipient crop and cv. IMI is the donor

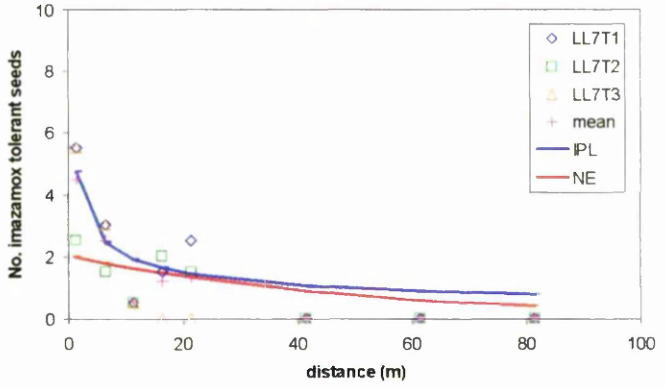


Figure 37. Fitted and observed outcrossing data where cv. LL1 is the recipient crop and cv. IMI is the donor

Legend

IPL - inverse power law
NE- negative exponential
T1-3= Sample transects 1-3
NOTE: difference in Y axis scale in Figures 36 and 37

LL7= cv. LL1 plot 7
RR3b and 5 = cv.RR1 plots 3b and 5

3.3.3 Discussion

The results presented here suggest that decline in out-crossing rate with distance for sources of GMHT pollen is better described by an inverse power-law function than an exponential function. One of the main consequences of this difference is that for a given source of GM pollen, dispersal behaviour which is described by a power-law is more likely to lead to cross-pollination at large distances than dispersal behaviour which is described by an exponential decay. Pollen dispersal described by the inverse power law function also showed higher estimates at short distances compared with the negative exponential function.

Kareiva *et al.* (1994) stressed the importance of the form of the dispersal curve in risk assessment for GM crops. Kareiva *et al.* (1994) examined best-fitting versions of the exponential and Weibull functions for data from GM cotton over a similar range of distances as used in this study. Examination of the data presented in Figure 3 of Kareiva *et al.* shows that both the exponential and Weibull functions underestimated observed data at the tail of the data. Further evidence that dispersal functions for pollen are relatively flat comes from studies on sterile and fertile receptor plants presented in section 3.2.1 this study. In this case the dispersal distances were extended to 600m and cross-pollination events were still readily detectable at the largest distance examined (in the order of 2 to 5% of seeds sampled at 600m in male sterile plants).

When considering the ecological behaviour of GMHT traits resulting from the dispersal curves obtained in this study, the likely consequences can be obtained from simulation studies carried out on air-borne fungal diseases (Minogue, 1989; Shaw, 1996). These studies have shown that populations with power-law dispersal functions have typically shown expansion which has a patchy spatial pattern over a large spatial scale. This type of patchy population expansion is likely to have three consequences for regulation and risk assessment for GM crops.

First, the patchy pattern of GM out-crossing will make sampling for detection of GM traits less efficient than would be the case if the dispersal behaviour showed exponential decay with distance.

Secondly, as a consequence of the first point, sampling costs to guarantee a given level of environmental contamination is not exceeded will be higher than they would be for a phenomenon with exponential decay with distance. Lastly, for a given source of GM pollen, the distance over which sampling will be required will be much larger than it would be if outcrossing showed an exponential decay with distance.

3.4 WEED CONTROL IN HERBICIDE TOLERANT AND CONVENTIONAL OILSEED RAPE

3.4.1 Year 1- Weed control in herbicide tolerant winter oilseed rape (1998-1999)

3.4.1.1 Pre-herbicide application assessments

Table 30 shows winter oilseed rape crop density, height and growth stage measurements recorded pre-herbicide application. The crop densities vary primarily due to a non-uniform seedbed/emergence across the experimental field and also because of initial seed rates used for varietal association (cv. Synergy) and restored hybrid types (cv. LL1)

Table 30. Mean crop density, crop height and growth stage assessed pre-herbicide application (28.10.98)

Plot No./Treatment	Mean crop density (plants/m^2)*	Crop growth stage range**	Mean crop height (cm)***
1 Apex (Conventional)	72.8 (6.793)	1,2-1,3	2.33 (0.211)
1 Synergy (Conventional)	67.6 (7.175)	1,0-1,3	3.33 (0.211)
2 Glufosinate (LL1)	70.8 (6.365)	1,2-1,4	2.92 (0.259)
3 Glyphosate (RR1)	82.0 (7.407)	1,2-1,3	3.08 (0.259)
4 Imazamox (IMI)	98.8 (8.315)	1,1-1,2	2.67 (0.188)
5 Glyphosate (RR1)	64.4 (5.549)	1,2-1,3	3.33 (0.355)
6 Imazamox (IMI)	79.6 (4.941)	1,2-1,3	3.67 (0.333)
7 Glufosinate (LL1)	82.8 (6.971)	1,2-1,3	3.58 (0.193)
8 Apex (Conventional)	86.4 (4.740)	1,2-1,3	4.33 (0.422)
8 Synergy (Conventional)	64.0 (7.729)	1,2-1,3	4.33 (0.558)

Values in parentheses = standard error of each mean, * n=20, ***n=12
**Growth stage according to Sylvester-Bradley and Makepeace (1984)

The results of crop and weed cover recorded pre-herbicide treatment in each plot is shown in Table 31, where values of crop cover tended to vary from low in replicate 1 (particularly plots 1 and 2) to high cover percentages in replicate 2. Weed cover tended to vary less between replicates, but varied between plots, with the highest value in plot 5 (cv. RR1, glyphosate treatment). Weed growth stage ranges and mean weed height were approximately uniform over both replicates.

Table 31. Mean crop cover, weed cover, weed growth stage range and weed height assessed pre-herbicide application (28.10.98)

Plot No./Treatment	Mean crop cover (%)	Mean weed cover (%)*	Weed growth stage range**	Mean weed height (cm)*[1]
1 Conventional***	5	19	11-13	13.1(0.773)
2 Glufosinate (LL1)	5	14	11-13	13.7 (0.916)
3 Glyphosate (RR1)	5	19	11-12	13.2 (0.601)
4 Imazamox (IMI)	19	15	11-13	14.3 (0.719)
5 Glyphosate (RR1)	16	20	11-12	15.2 (0.726)
6 Imazamox (IMI)	12.5	19	11-13	14.8 (0.489)
7 Glufosinate (LL1)	19	15	11-15	12.3 (0.829)
8 Conventional***	24	15	11-13	12.99 (1.246)

Values in parentheses = standard error of each mean, [1]n=12
* Measurements only taken for main weed present (TRIAE, c.10/m^2)
**Growth stage according to Tottman (1987)
***Measurements taken across both conventional varieties

3.4.1.2 Post-herbicide application assessments

Weed control was scored visually on a percentage scale, the results are shown in Table 32. All of the treatments controlled the main weed species present; winter wheat volunteer (*Triticum aestivum*). Overall, glyphosate treatment (RR1) produced the highest level of control (99%) and the imazamox treatment (IMI) the lowest (85%) (Also see Figures 37-40) for weed count numbers/m^2 pre and post herbicide treatment).

The percentage crop cover increased in the period between pre- and post-herbicide assessments. The percentage cover of the wheat volunteers (*Triticum aestivum*) was reduced to trace levels (0.1%) in most plots with the exception of the glufosinate treated plots where cover was reduced but some weeds survived.

Table 32. Post herbicide assessment of percentage control of winter wheat volunteers (*Triticum aestivum*) assessed on 23.02.99

Plot No./Treatment	% Control of winter wheat volunteers
1 Conventional***	95
2 Glufosinate (LL1)	90
3 Glyphosate (RR1)	99
4 Imazamox (IMI)	85
5 Glyphosate (RR1)	99
6 Imazamox (IMI)	85
7 Glufosinate (LL1)	90
8 Conventional***	90

***Measurement taken across both conventional varieties

Table 33. Crop growth stage range and percentage crop and main weed cover recorded post-herbicide application assessed on 23.02.99

Plot No./Treatment	Crop growth stage range*	Crop cover (%)	Weed cover (%)
1 Conventional***	1,4-1,6	7.5	T
2 Glufosinate (LL1)	1,4-1,6	10	2.5
3 Glyphosate (RR1)	1,4-1,6	30	T
4 Imazamox (IMI)	1,5-1,7	28	T
5 Glyphosate (RR1)	1,4-1,8	25	T
6 Imazamox (IMI)	1,5-1,7	34	T
7 Glufosinate (LL1)	1,4-1,6	40	5
8 Conventional***	1,5-1,7	50	T

T= ≤0.1%
*Growth stage according to Sylvester-Bradley and Makepeace (1984)
**Measurements taken across both conventional varieties

3.4.1.3 Results of weed counts pre- and post-herbicide treatment in herbicide treated plots

Four of the most abundant and consistently occurring weeds (*Triticum aestivum, Alopecurus myosuroides, Anagallis arvensis, Galium aparine*) were selected for statistical testing by analysis of variance (ANOVA). The output from ANOVA is shown in Appendix 5. Figures 37-40 show the mean number of plants/m^2 (of selected species) recorded pre- and post-herbicide by treatment. The full

range of species recorded in plots both pre- and post herbicide are shown in Tables 45 and 47 (Appendix 4). Weed numbers/m^2 were reduced in all herbicide treatments post-herbicide application, although some treatments were more effective than others and they differed in the spectrum of weeds they controlled (Figures 38-41) and Plates 9-13, Appendix). All treatments were effective in controlling the main weed present, wheat volunteers *(Triticum aestivum).* When compared, the population density recorded post-herbicide was significantly different ($P<0.01$) due to poorer control in imazamox (IMI) treated plots.

Numbers of *Alopecurus myosuroides*/m^2 were reduced post-herbicide in all treatments. Comparison between treatments showed significantly different levels were recorded ($P<0.05$), with the imazamox treatment (IMI) being least effective, while the conventional herbicide treatment (Conv) gave the most effective control. There were significant differences between levels of *Anagallis arvensis* recorded in treatments pre-herbicide treatment due to the high population density recorded in the conventional treatment ($P<0.05$). Numbers of *A. arvensis* per m^2 were reduced post herbicide in all treatments with the exception of conventional treatment where levels increased ($P<0.01$).

3.4.1.4 Results of weed counts recorded at the pre- and post-herbicide treatment assessment timing in untreated areas

The equivalent four weeds were selected for analysis in the untreated areas as for treated plots. The output from ANOVA is shown in Appendix 5. The full range of species recorded in plots at the pre- and post-herbicide timings in the untreated areas are shown in Tables 46 and 48 (Appendix 4). Weed numbers/m^2 tended to fluctuate in untreated areas, reflecting patchiness in the experimental field. Weed density was frequently reduced post-herbicide, although levels overall remained higher than in treated plots. When treatments were compared the were significant differences between the densities of *Triticum aestivum* and *Alopecurus myosuroides* ($P<0.01$) (at the pre and post-herbicide assessment timings).

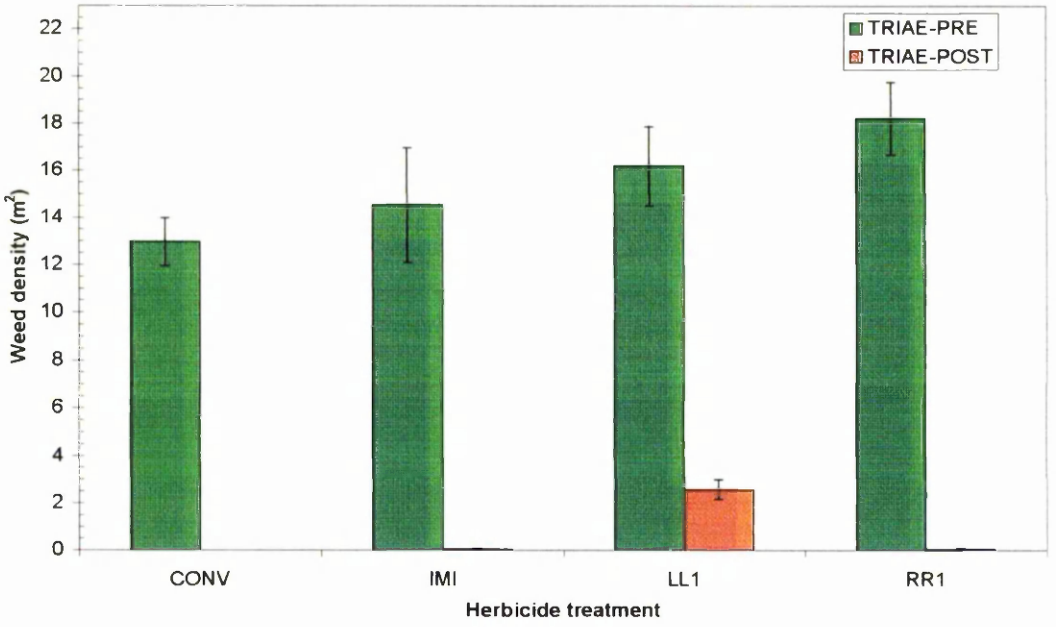


Figure 38. Density of volunteer winter wheat plants (*Triticum aestivum*) in herbicide tolerant and conventional crops of winter oilseed rape pre and post herbicide treatment in year 1, herbicide treatments: CONV - conventional, IMI - imazamox, LL1 - glufosinate, RR1 – glyphosate, error bars - +/- standard error, n=8

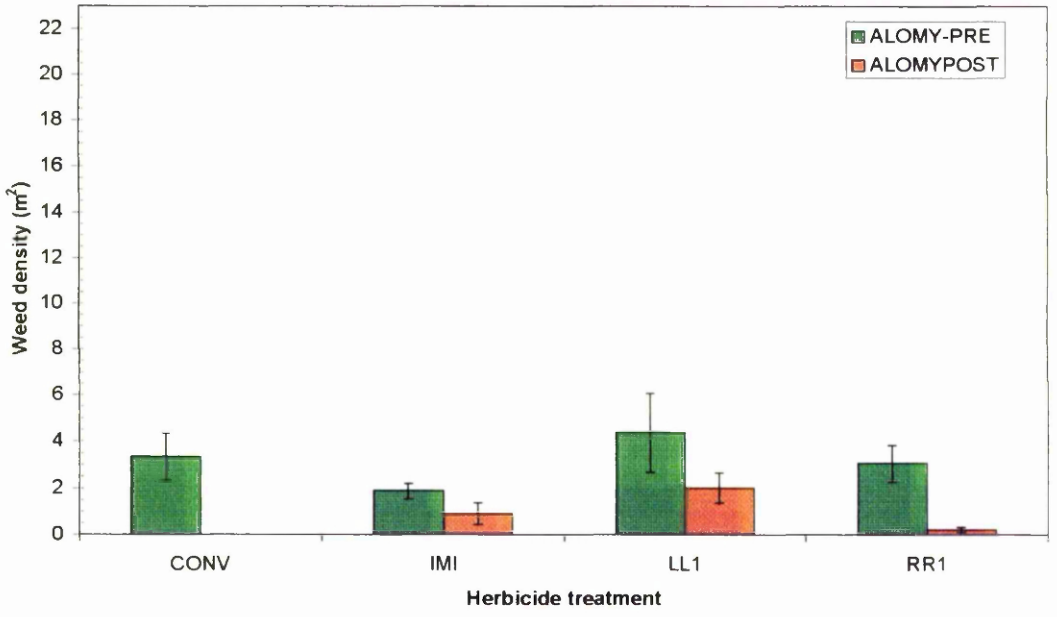


Figure 39. Density of blackgrass plants (*Alopecurus myosuroides*) in herbicide tolerant and conventional crops of winter oilseed rape pre and post herbicide treatment in year 1

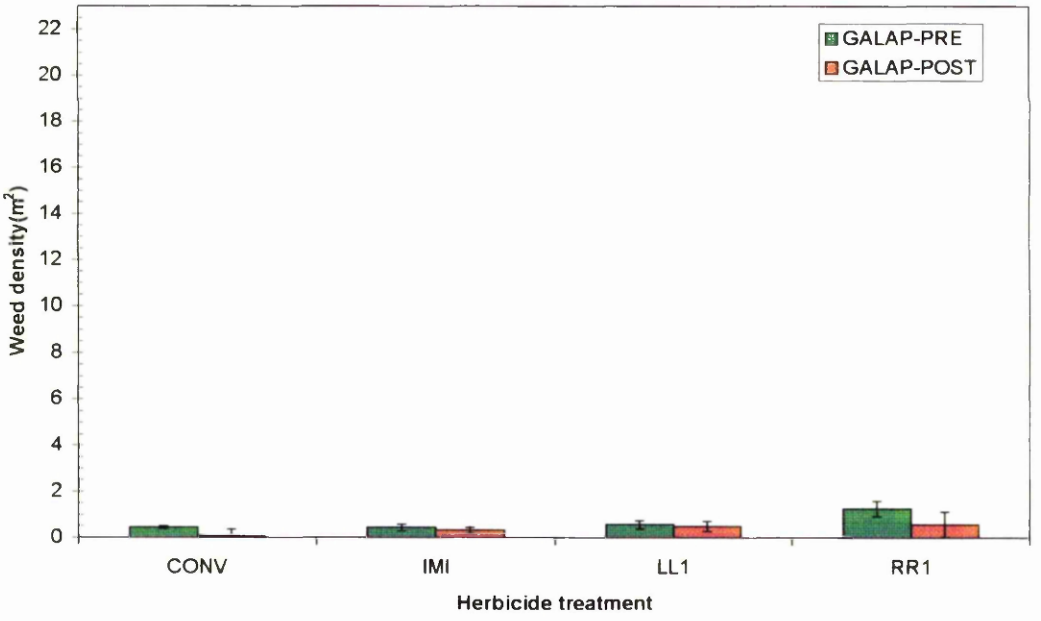


Figure 40. Density of cleavers plants (*Galium aparine*) in herbicide tolerant and conventional crops of winter oilseed rape pre and post herbicide treatment in year 1, herbicide treatments: CONV - conventional, IMI - imazamox, LL1 - glufosinate, RR1 – glyphosate, error bars - +/- standard error, n=8

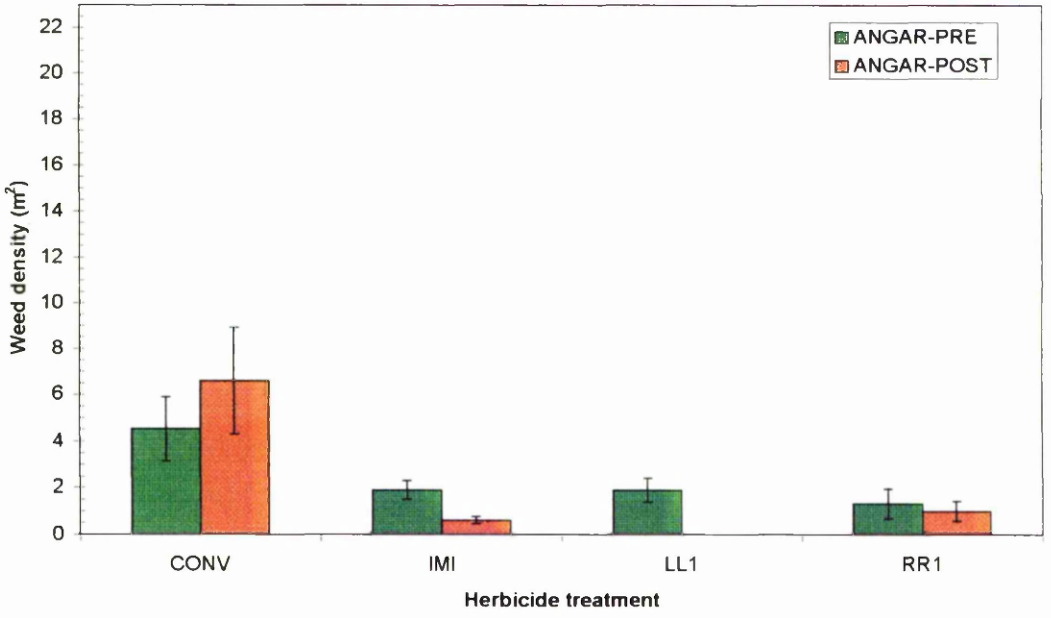


Figure 41. Density of scarlet pimpernel plants (*Anagallis arvensis*) in herbicide tolerant and conventional crops of winter oilseed rape pre and post herbicide treatment in year 1

3.4.1.5 Weed biomass recorded in treated plots June 1999

Weed biomass assessments showed that a range of weed species were present in all treatments prior to harvest. The mean dry weight (g/m^2) of the range of weed species sampled are shown in Table 49 (Appendix 4). The most frequently occurring and abundant weeds were selected for further statistical testing by ANOVA and are presented in Figures 42-45. The outputs from ANOVA are shown in Appendix 5. Overall, glyphosate treated plots (RR1) produced the lowest mean weed biomass and the imazamox treatment (IMI) produced the highest level.

Treatments differed in the spectrum of weed species they produced. Glufosinate treated plots (LL1) produced high levels of the two main graminaceous weeds present; equivalent levels of *Alopecurus myosuroides* to the imazamox (IMI) treated plots, levels of which were significantly higher than in the other treatments after herbicide application ($P<0.05$). Neither of the main graminaceous weeds were found in the weed biomass samples taken from the conventional treatment. When compared, there were significantly different levels between the treatments of both *Anagallis arvensis* and *Picris echiodes* ($P<0.05$), with the highest populations of these weed species found in the conventional and imazamox treatments.

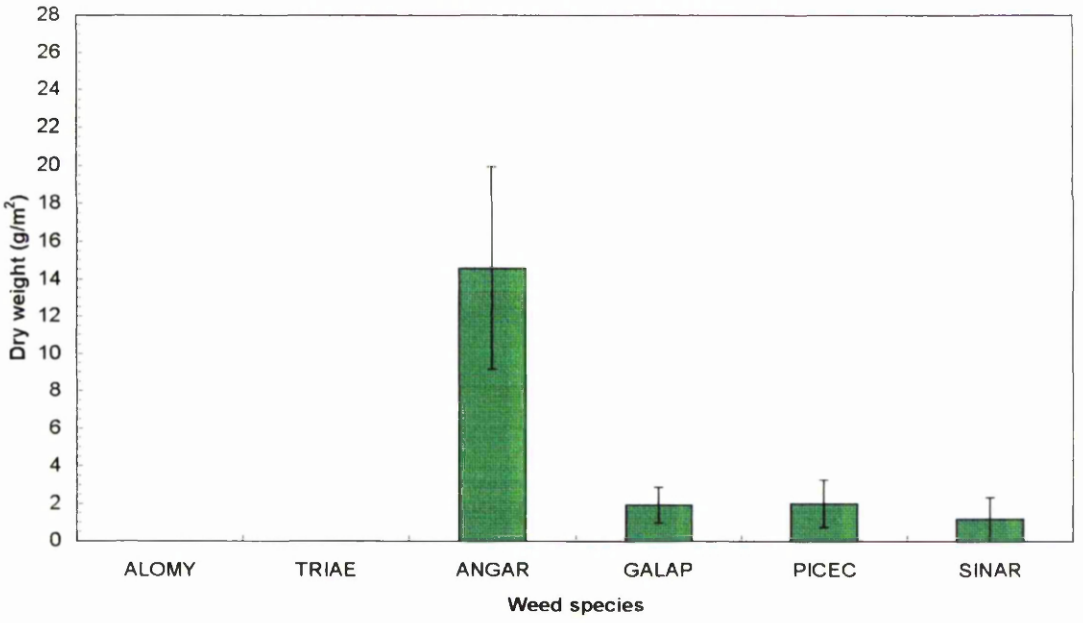


Figure 42. Dry weight of selected commonly occurring weed species sampled from herbicide treated plots of conventional winter oilseed rape in year 1 (July 1999), TRIAE-***Triticum aestivum***, ANGAR-***Anagallis arvensis***, GALAP-***Galium aparine***, PICEC-***Picris echiodes***, SINAR-***Sinapis arvensis***, **error bars - +/- standard error, n=8**

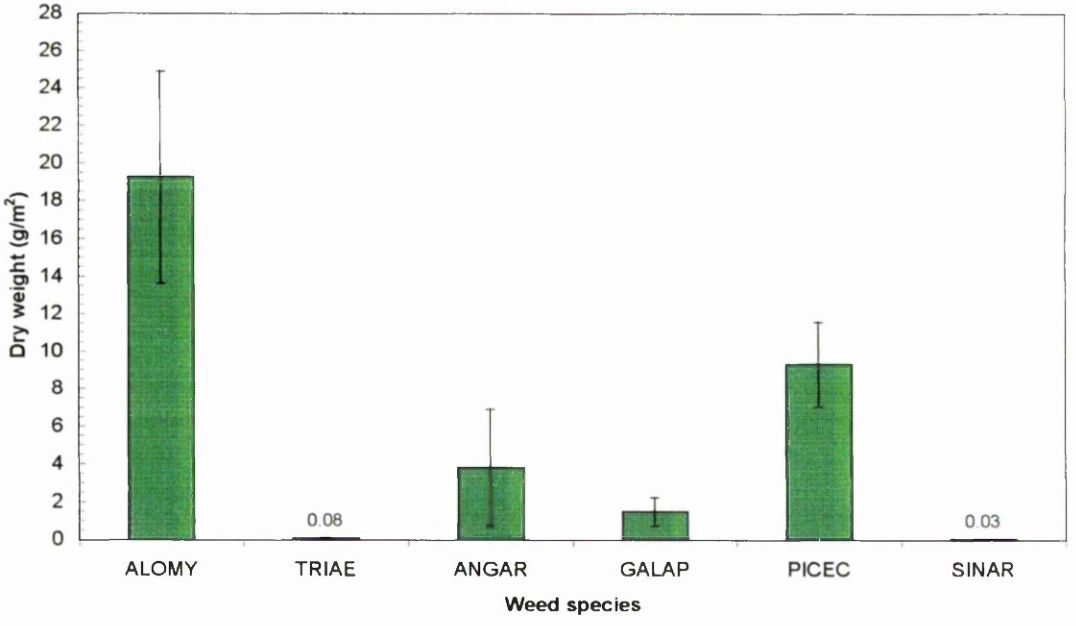


Figure 43. Dry weight of selected commonly occurring weed species sampled from herbicide treated plots of imazamox tolerant winter oilseed rape in year 1 (July 1999) TRIAE-***Triticum aestivum***, ANGAR-***Anagallis arvensis***, GALAP-***Galium aparine***, PICEC-***Picris echiodes***, SINAR-***Sinapis arvensis***, **error bars - +/- standard error, n=8**

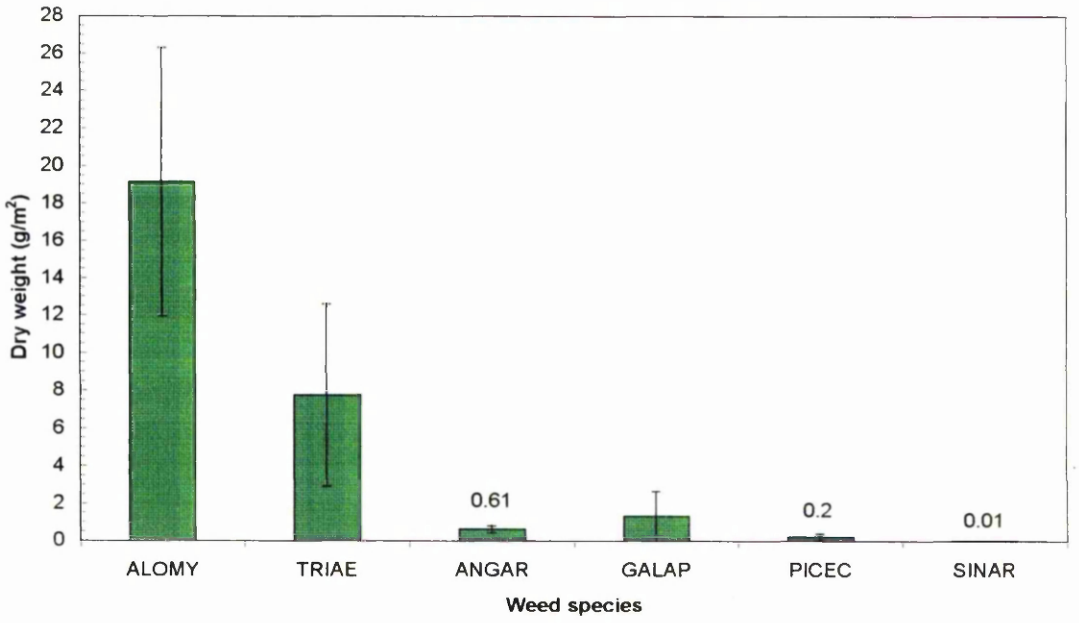


Figure 44. Dry weight of selected commonly occurring weed species sampled from herbicide treated plots of glufosinate tolerant winter oilseed rape in year 1 (July 1999). ALOMY-***Alopecurus myosuroides***, TRIAE-***Triticum aestivum***, ANGAR-***Anagallis arvensis***, GALAP-***Galium aparine***, PICEC-***Picris echiodes***, SINAR-***Sinapis arvensis***, error bars - +/- standard error n=8

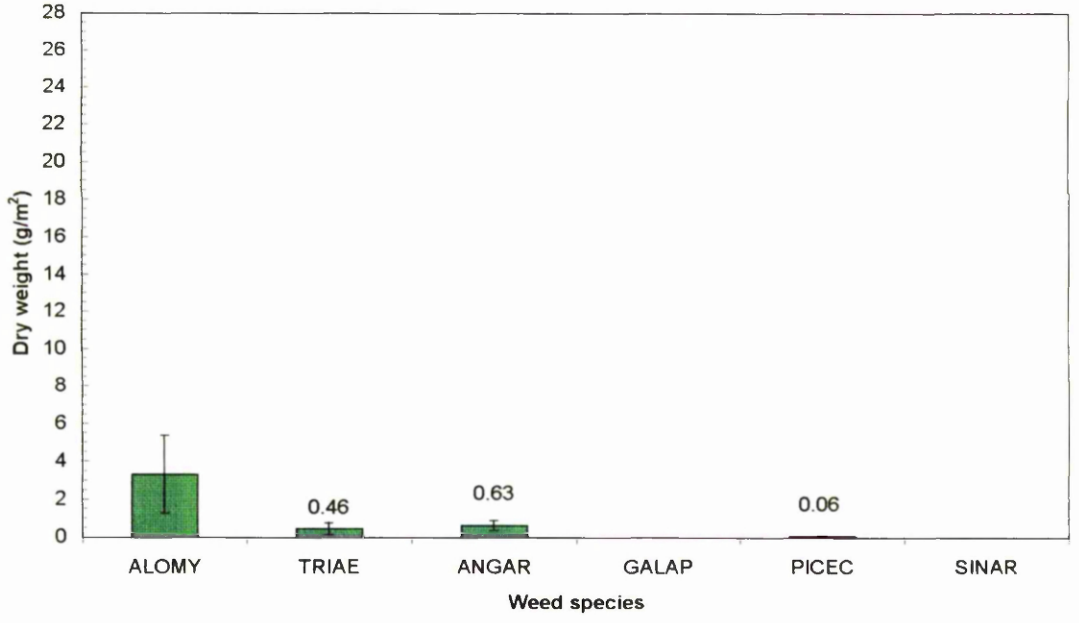


Figure 45. Dry weight of selected commonly occurring weed species sampled from herbicide treated plots of glyphosate tolerant winter oilseed rape in year 1 (July 1999) TRIAE-***Triticum aestivum***, ANGAR-***Anagallis arvensis***, GALAP-***Galium aparine***, PICEC-***Picris echiodes***, SINAR-***Sinapis arvensis***, error bars - +/- standard error, n=8

3.4.1.6 Fresh weight and dry matter yields of winter oilseed rape plots

Table 34 below shows total yields from plots of herbicide tolerant and conventional winter oilseed rape harvested in July 1999. Yields were low, partly due to the experimental varieties used in the study. The imazamox tolerant variety produced the lowest yield.

Table 35 shows the estimated mean seed losses immediately after harvest of herbicide tolerant and conventional winter oilseed rape plots in July 1999. The results showed that seed losses were within the normal range expected for a crop of oilseed rape (e.g. Lutman 1993) and that there was potential for development of a seedbank of volunteer rape seed.

Table 34. Fresh weight and dry matter yields of winter oilseed rape plots harvested 13.7.99

Treatment/Plot	Harvested seed* Plot wt. (kg)	Tonnes/ha	Dry matter yield** t / ha
1 Apex (Conventional)	506	2.50	2.33
1 Synergy (Conventional)	532	2.63	2.08
2 Glufosinate (LL1)	2257	2.97	2.84
3 Glyphosate (RR1)	2254	2.96	2.85
4 Imazamox (IMI)	256	1.78	1.76
5 Glyphosate (RR1)	2600	3.42	3.33
6 Imazamox (IMI)	379	1.84	1.83
7 Glufosinate (LL1)	2195	2.88	2.91
8 Apex (Conventional)	1085	2.99	2.93
8 Synergy (Conventional)	1485	3.73	3.37

*Total weight of plots
**Dry matter yield at 9% moisture

Table 35. Estimated seed losses from winter oilseed rape plots after harvest (14.07.99)

Treatment/plot	Mean seed shed/m^2	Mean wt. seed shed per m^2 (g)*	Seed loss/t/ha**
1 Apex (Conventional)	2266.6	11.3 (1.251)	0.11
1 Synergy (Conventional)	4782.2	23.9 (3.305)	0.23
2 Glufosinate (LL1)	5022.2	25.1 (2.410)	0.25
3 Glyphosate (RR1)	3346.6	16.7 (3.102)	0.16
4 Imazamox (IMI)	3711.1	18.5 (2.192)	0.18
5 Glyphosate (RR1)	2613.3	13.0 (2.503)	0.13
6 Imazamox (IMI)	5604.4	28.0 (3.988)	0.28
7 Glufosinate (LL1)	3017.7	15.0 (2.708)	0.15
8 Apex (Conventional)	4204.4	21.0 (2.220)	0.21
8 Synergy (Conventional)	4204.4	21.0 (2.219)	0.21

Values in parentheses = standard error of each mean (n=10) **assume 1000 seeds = 5g

3.4.2 Year 2 - Weed and oilseed rape volunteer control in winter wheat (1999-2000)

3.4.2.1 Results of pre-herbicide application assessments

Table 36 shows crop measurements recorded pre-herbicide application. Overall, the winter wheat crop was uniform in density and growth stage across the experimental field. Table 37 shows the growth stage of the main weeds present in all plots weed cover was at trace levels (0.1%) due to the small growth stage of weeds.

Table 36. Mean crop density, growth stage, crop cover and height recorded pre-herbicide application (29.10.99)

Plot No. (and previous years treatment)	Mean density plants/m^{2*}	Crop growth stage**	Crop cover (%)	Mean crop height (cm)***
1 Conventional	23.6 (1.618)	12	5	7.2 (0.423)
2 Glufosinate (LL1)	25.2 (1.229)	12	5	7.0 (0.426)
3 Glyphosate (RR1)	25.5 (1.432)	12	5	6.6 (0.287)
4 Imazamox (IMI1)	23.5 (1.337)	12	5	6.6 (0.357)
5 Glyphosate (RR1)	20.5 (1.373)	12	5	7.6 (0.416)
6 Imazamox (IMI1)	25.7 (1.409)	12	5	7.1 (0.484)
7 Glufosinate (LL1)	23.7 (1.154)	12	5	7.7 (0.372)
8 Conventional	24.7 (1.229)	12	5	6.7 (0.522)

Values in parentheses = standard error of each mean, *n=20, ***n=6
** Growth stage according to Tottman (1987)

Table 37. Mean growth stages and heights of the two main weeds present recorded pre-herbicide application in all plots (29.10.99)

Assessment	*Brassica napus**	*Galium aparine***
Weed cover (%)	0.1	0.1
Weed growth stage	1.0	1.0
Weed height (cm)	1	1

*Growth stage according to Sylvester Bradley and Makepeace (1984)
** Growth stage according to Lutman and Tucker (1987)

3.4.2.2 Results of post-herbicide application assessments

The effects of herbicide treatment in the winter wheat crop was consistent throughout the experimental area. The results of the assessment of percentage weed control are shown in Table 38. Oilseed rape (*Brassica napus*) volunteers were the most susceptible weed while volunteer beans (*Vicia faba*) and cleavers (*Galium aparine*) were the least susceptible. Crop growth stage (GS) measurements showed that all plots were at GS 22 (Tottman, 1987) and crop cover was at 65% in all plots. The weed cover assessment showed that the four main weed species present were at 0.5% cover (individual plot data not shown)

Table 38. Percentage control of main weeds present post herbicide application (4.01.00)

Treatment	BRANA	VICFA	GALAP	CIRVU
1 Conventional	98	20	20	85
2 Glufosinate (LL1)	98	20	20	85
3 Glyphosate (RR1)	98	20	20	85
4 Imazamox (IMI1)	98	20	20	85
5 Glyphosate (RR1)	98	20	20	85
6 Imazamox (IMI1)	98	20	20	85
7 Glufosinate (LL1)	98	20	20	85
8 Conventional	98	20	20	85

Key: BRANA = *Brassica napus;* VICFA = Vicia faba; GALAP = *Galium aparine* ; CIRVU= *Cirsium vulgare*

3.4.2.3 Weed count results pre- and post-herbicide treatment

The herbicide treatment reduced numbers of most weed species although in some cases weed numbers increased post-herbicide treatment e.g. *Galium aparine* (in glyphosate, glufosinate and conventional treated plots). Numbers of oilseed rape volunteers (*Brassica napus*) recorded during pre-herbicide weed counts were extremely low, there was no consistent pattern of distribution across previous years rape plots. Rape volunteer numbers were reduced to very low or zero levels post-herbicide treatment in all the previous years treatments (Figure 46) and no oilseed rape was recorded in weed biomass samples.

No formal statistical analysis is presented on pre- and post-herbicide weed counts carried out in year two because of extremely low and variable weed densities of approximately 0.1 plants/m^2. The full set of data recorded in the plots both pre- and post herbicide in year 2 (1999-00) are shown in Tables 51 and 52 (Appendix 6).

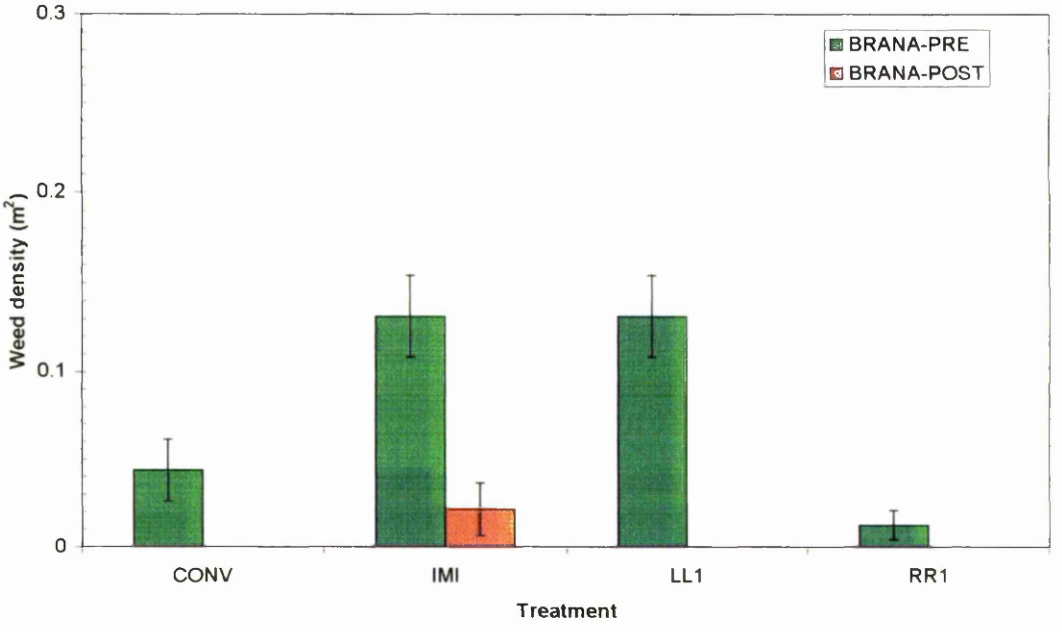


Figure 46. Density of volunteer winter oilseed rape plants (*Brassica napus*) recorded in a crop of winter wheat (cv. Soissons) in 2000 pre and post herbicide treatment following a crop of conventional winter oilseed rape grown in 1998-1999, herbicide treatments: CONV - conventional, IMI - imazamox, LL1 - glufosinate, RR1 – glyphosate, error bars - +/- standard error , n=8

3.4.2.4 Weed biomass recorded in treated plots June 2000

Weed biomass sampling showed that weeds were present in all plots prior to harvest of the winter wheat crop. All plots contained a range of weed species. Frequently occurring and abundant weeds were selected for further statistical analysis by ANOVA. The full output from ANOVA is shown in Appendix 5. As an example, four of the most commonly occurring weed species are presented in Figures 47-50. The mean dry weight (g/m^2) of the full range of weed species sampled are shown in Table 53 (Appendix 6)

Overall, glyphosate (RR1) and conventional (Conv) treated plots from the previous year contained the least weed biomass and the former IMI treated plots produced the highest total weed biomass, with *Alopecurus myosuroides* contributing to most of this (Table 39). The most abundant weed species across all the previous years treatments was *A. myosuroides*, when compared, the population density of this weed was significantly higher in (the former year 1) imazamox treated (IMI) plots than in other (previous years) treatments ($p<0.05$). There were also significant differences between treatments when the total pooled dry weight of all species present in each plot were combined, with the former imazamox treated plots producing the highest total weed biomass (Table 39).

Table 39. Combined weed dry weight of all species in present in plots of winter wheat 1999-2000

Previous years treatment (1998-99)	Total mean biomass of all species (g/m^2)
Conventional	4.4
RR1	6.6
IMI	29.1
LL1	13.5

(Lsd = 15.51 ; $p<0.05$)

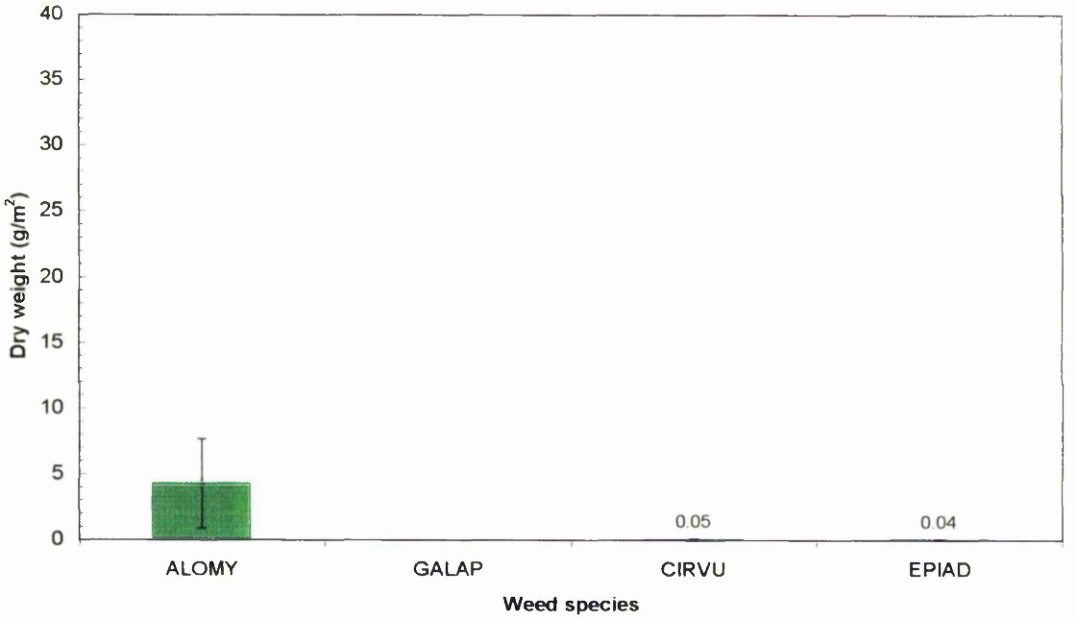


Figure 47. Dry weight of selected commonly occurring weed species sampled from a crop of winter wheat (cv. Soissons) following a crop of conventional winter oilseed rape (July 2000)

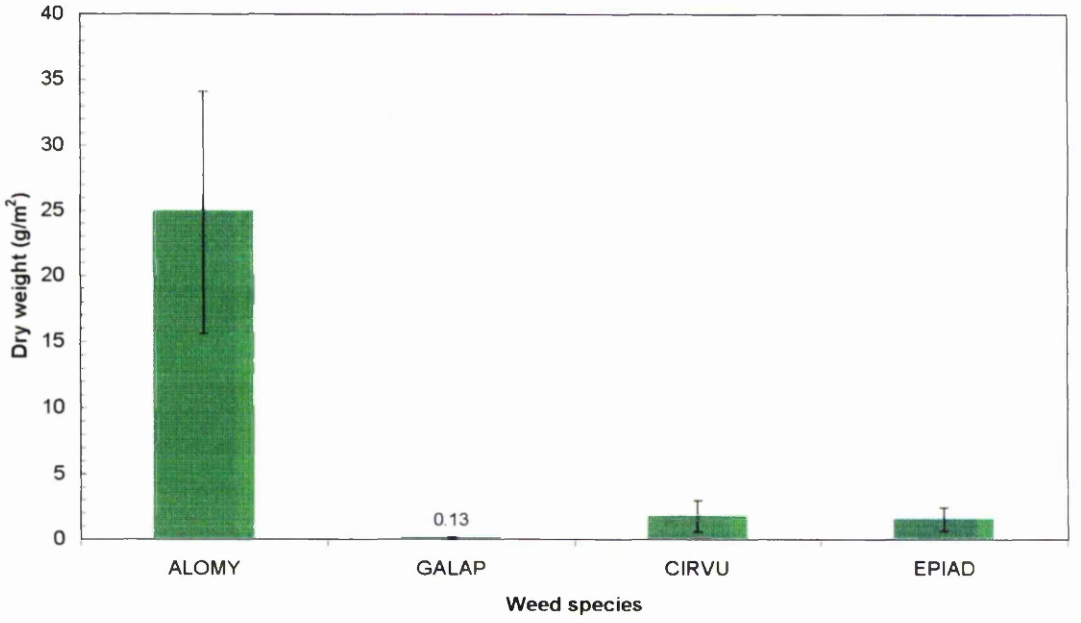


Figure 48. Dry weight of selected commonly occurring weed species sampled from a crop of winter wheat (cv. Soissons) in July 2000 following a crop of imazamox tolerant winter oilseed rape grown in 1998-1999

Legend
ALOMY-*Alopecurus myosuroides*, GALAP-*Galium aparine*, CIRVU-*Cirsium vulgare*, EPIAD-*Epilobium adenocaulon*
Error bars - +/- standard error , n=8

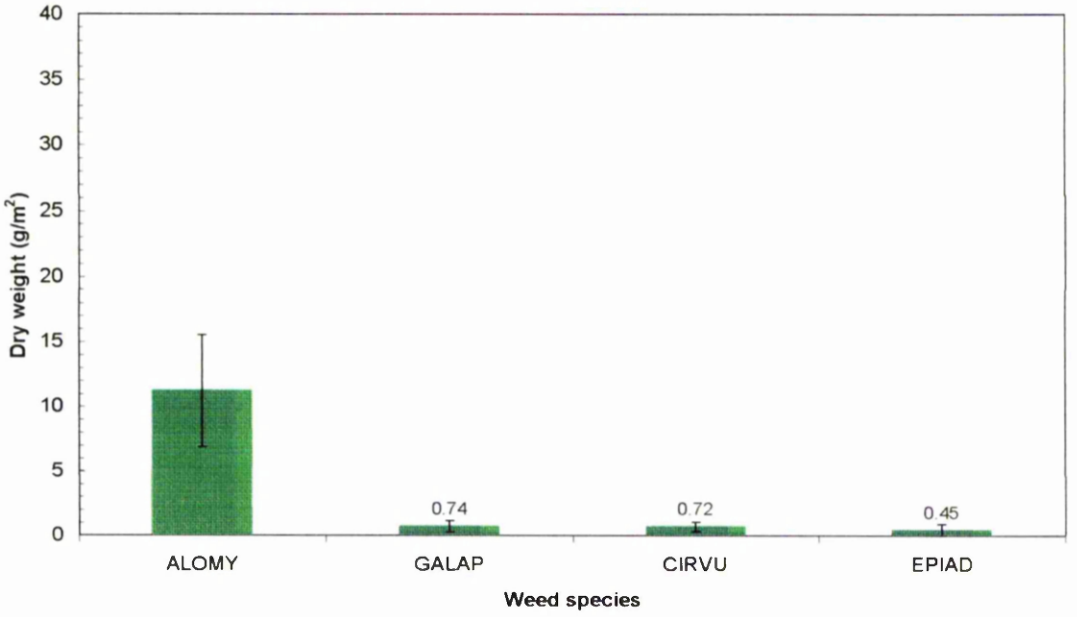


Figure 49. Dry weight of selected commonly occurring weed species sampled from a crop of winter wheat (cv. Soissons) in July 2000 following a crop of glufosinate tolerant winter oilseed rape grown in 1998-1999

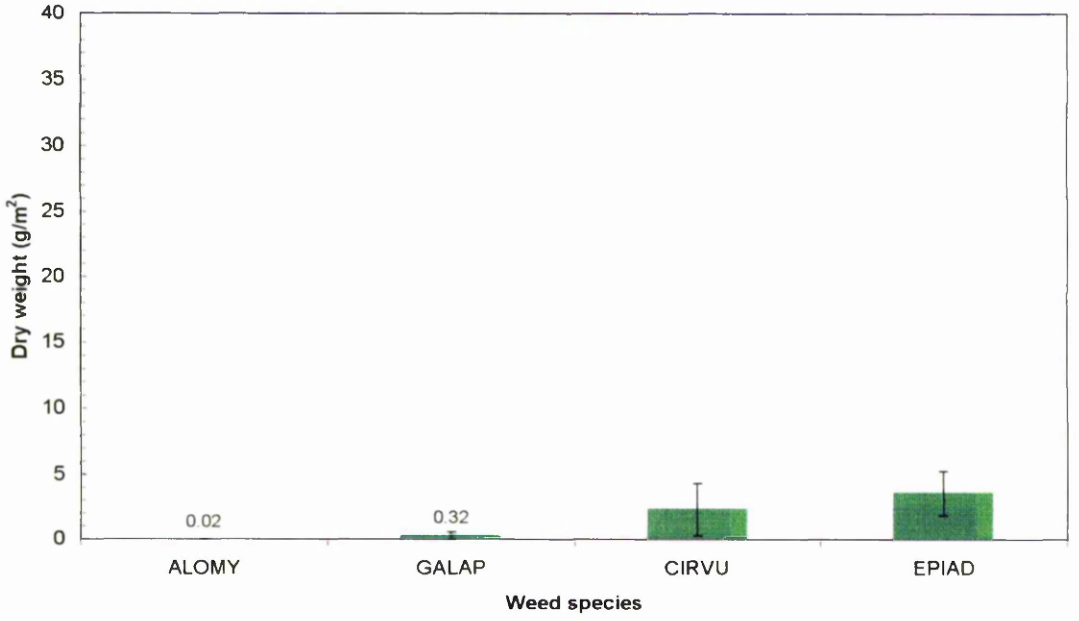


Figure 50. Dry weight of selected commonly occurring weed species sampled from a crop of winter wheat (cv. Soissons) in July 2000 following a crop of glyphosate tolerant winter oilseed rape grown in 1998-1999

Legend
ALOMY-*Alopecurus myosuroides*, GALAP-*Galium aparine*, CIRVU-*Cirsium vulgare*, EPIAD-*Epilobium adenocaulon*
Error bars - +/- standard error , n=8

3.4.3 DISCUSSION

3.4.3.1 Weed control in conventional and herbicide tolerant winter oilseed rape (1998-1999)

Crop and weed assessments made pre-herbicide application showed that all oilseed rape varieties and the main weed species, volunteer wheat (*Triticum aestivum*) were at similar growth stages, heights and densities. There were some differences in crop cover percentages between plots in replicate 1 (lower % cover) and plots in replicate 2 (Table 31). The more irregular crop cover in replicate 1 was attributed to grazing by birds during crop establishment. The distribution and population density of weed species across the experimental field varied. For example, the glyphosate treated plots (RR1) contained the highest density/m^2 of *Triticum aestivum* pre-herbicide (Figure 38). Other species such as mayweed (*Matricaria sp.*) and shepherds purse (*Capsella bursa-pastoris*) had highly variable distribution pattern across the field and low densities hence were not included in the analysis (Table 45, Appendix 4).

The results showed that all the herbicides controlled weeds by reducing their number/m^2 post-treatment, and that there were some differences in activity spectra (Figures 38-41). Imazamox treated plots (70g a.i./ha) tended to contain the highest levels of weeds/m^2 post herbicide treatment and contained significantly higher levels of the two main grass weeds (*T. aestivum* and *A. myosuroides)*. Both of these species tended to re-grow in the glufosinate treatment (600g a.i./ha) after initially being severely damaged (Table 32 and Plate 12, Appendix 1). The conventional treatment (Metazachlor + Fluazifop-P-butyl; 1250+150g a.i./ha) controlled *T. aestivum* the best overall (zero plants/m^2 recorded post herbicide, Figure 37). None of the treatments fully controlled cleavers (*Galium aparine*), the imazamox and glufosinate treatments performed the least well against this weed (Figure 40). Samples of weed biomass recorded in July 1999 showed conventional, imazamox and glufosinate treatments to have equivalent amounts of *G. aparine,* whereas samples from the glyphosate treatment (720g a.i./ha) contained none. Observation of the glyphosate treated plots showed that *G. aparine* growth was stunted after treatment and that the

crop plants eventually out-competed weed growth sufficiently. The weed biomass results provided a good indication of the overall efficacy of the different treatments and the potential seed return to the weed seedbank, although the results were extremely variable due to patchiness of weed pattern and the relatively low intensity sampling frequency.

The imazamox treatment did not perform as well as the other treatments. Higher densities of *T. aestivum* and *A. myosuroides* per m^2 were recorded post-herbicide and there were high biomass levels of many other weed species in imazamox treated plots, in particular, several broad-leaved weeds; *Picris echioides, Veronica hederifolia* and *Vicia faba*. The presence of volunteer beans was due to the selective activity of imazamox, a commonly used selective herbicide in soybean (Plate 10, Appendix 1). The reasons for the lack of adequate control of other weed species with imazamox at this site were not clear. There are no published reports of imazamox use in HT rape in the UK for comparison with this study. In a report from the US and Canada investigating HT canola systems, where 84% weed control was achieved with imazethapyr/imazamox, good control of less sensitive weeds such as cleavers was reported as a distinct advantage of the system (Anon[1], 2000). These agronomic benefits of the HT imazamox system were not evident in this study, in fact the conventional treatments performed better. Differences in the application timings in the US and Canada where spring rape types (canola) are grown may explain the low activity that imazamox had in this study due to different environmental conditions at herbicide application. Shaner (1989) reviewed the factors affecting soil and foliar availability of the imadazolinones. An important aspect relating to their herbicide activity were the environmental conditions relating to plant growth which affected absorption of the herbicide.

Green and Strek (2001) also investigated the performance of acetolactate synthase inhibiting herbicides and reported that; rainfall, humidity, temperature, light, soil moisture and wind all influence herbicidal activity and stated that weather conditions could influence herbicide performance before, during and after application. Green and Strek concluded that conditions that favour plant

growth would generally favour weed control. Plant growth/climatic conditions would have been different in a winter crop in the UK compared to the spring canola crops in the USA and Canada where weeds would be growing more vigorously and ambient temperatures and light levels would be higher.

Read and Ball (1999b) reported on a series of weed control trials carried out over several years with glufosinate tolerant spring and winter oilseed rape. Glufosinate applications were compared with post-emergence conventional treatments (broadleaf herbicide + graminicide). Their results suggest that a single glufosinate application out-performed the conventional two-spray system for control of the major grass and broad-leaved weeds. In common with their results, results of this experiment showed that there was less effective control of *G. aparine* with a single glufosinate application. This may be due to either the prolonged germination period of *G. aparine* through the winter (Williams and Morrison, 1987) or where a dense crop canopy has reduced spray penetration and coverage of the target weed. Read and Ball (1999b) also suggest that higher dose rates would be required when grass weeds are at larger growth stages.

Pilorge and Mircovich (1999) who reported on weed control strategies using glufosinate tolerant rape also showed that, at more advanced growth stages, wheat volunteers were less susceptible. Derksen *et al.* (1999) who reported on weed control in HT oilseed rape in Canada also stated that glufosinate generally did not give good control of grass weeds compared with glyphosate and only provided "top growth" control of perennials. This was reflected in this study where the glufosinate treatment initially severely scorched *Alopecurus myosuroides* and *Triticum aestivum,* but both had considerable re-growth and recovery (Plate 12, Appendix) as shown by the high biomass figures for these species in the summer prior to harvest (Figure 44). This evidence suggests that for optimal control graminaceous weeds must be treated at early growth stages.

The agronomic performance of the glufosinate treatment was average, a higher initial dose rate would have given more complete control of larger blackgrass and wheat volunteer plants and may

have compared better with the conventional two spray programme. A split, two spray treatment could have enabled control of some of the later germinating weeds and better overall control of graminaceous weeds, particularly *A. myosuroides*. The low activity of glufosinate in some species may be due to environmental conditions such as low temperatures and light levels (Kocher, 2001) which would have been present during the autumn herbicide application.

The glyphosate treatment had the lowest overall weed population recorded post-herbicide treatment and produced the lowest weed biomass/m^2 out of all the treatments in this experiment (Figure 43). Glyphosate did not provide complete control of all weed species, some of the less sensitive weeds such as *Alopecurus myosuroides* and *Galium aparine* at larger growth stages were not fully controlled by the dose rate used in this study (720g a.i./ha). The occurrence of these weeds in the biomass samples suggests that they were either only partially controlled to begin with or, in the case of *G. aparine,* prolonged emergence meant that many weeds escaped treatment.

Pilorge and Mircovich (1999) reported on the efficacy of a 2l/ha (720g a.i./ha) dose rate of glyphosate at a range of weed growth stages in oilseed rape, they found that some weeds such as *Capsella bursa-pastoris* were less sensitive at larger growth stages and that others were generally less sensitive to the herbicide e.g. *Viola arvensis*. Derksen *et al.* (1999) also reported on some of the limitations of glyphosate in minimal tillage HT rape crops in Canada, such as poor control of *Polygonum convolvulus* and *Taraxacum officinale*.

The glyphosate treatment in this study was very effective allowing the crop to establish well without competing weeds, as shown by the pre and post-herbicide weed counts (Figure 38-41). The very low weed biomass produced in the glyphosate treated plots suggests that weed seed return in these plots would be lower and that continued use of glyphosate tolerant crops in a rotation may eventually affect the species composition of the weed seedbank (Figure 45).

3.4.3.2 Herbicide tolerant and conventional oilseed rape volunteer and weed control in winter wheat (1999-2000)

All crop measurements and percentage crop cover pre-herbicide application in year two showed uniform growth across the previous years winter rape treatments (Table 36). Pre-herbicide counts of weeds showed very low densities of oilseed rape volunteers (*Brassica napus)* and *Galium aparine* which were considered the main weeds present. Nearly all oilseed rape volunteers were controlled by the standard herbicide programme used in the winter wheat crop (Figure 46). No volunteers were seen flowering during site visits later in the season and none were recorded during the weed biomass sampling in June 2000. The low initial volunteer population was probably due to the normal farm management of the field post-harvest in the previous year that was aimed at reducing the amount of seed return to the seedbank and thus subsequent volunteer populations. Post harvest cultivations in year one were not started until most of the seed on the soil surface had germinated, several weeks after harvest. The volunteer density represented a very small proportion of the potential population that may have germinated from the seedbank.

The estimated seed losses at harvest ranged from 0.11t/ha - 0.28t/ha (Table 35) and corresponded well to previous reports of seed loss estimates that range from 0.1t/ha-0.5t/ha (Bowerman, 1984, Vera *et al.*, 1987, Lutman, 1993, Brown *et al.*, 1995, Price *et al.*, 1996). The seedbank was also sampled at this site using a wet seiving method similar to Roberts and Rickets (1979) (full data set is not presented). Results showed that there was an average of 1094.4 seeds/m^2 in January 2000 declining to 797seeds/m^2 in January 2001. Seeds sampled from both years from each of the original oilseed rape plots were then tested for herbicide tolerance to glufosinate and glyphosate (using the method described in 3.1.3). Results showed that all seeds sampled from former glufosinate were tolerant to glufosinate, this was also the case for seeds sampled from glyphosate tolerant plots. No tolerant seeds were found in samples from conventional plots and no double tolerant seeds were detected. Although the volunteer oilseed rape plant

population was extremely low, the experiment demonstrated the equal susceptibility of herbicide tolerant and conventional volunteers to commonly used herbicides in winter wheat.

Norris *et al.*, (1999) monitored several sites around the U.K to assess the weediness and persistence of GMHT oilseed rape in following crops. Results showed variable population densities of volunteer plants, however at none of the sites were GMHT volunteers considered to be more difficult to control than conventional volunteers. Simpson and Sweet (2000) collected monitoring data from National List trial sites where GMHT oilseed rape had been grown, there were no instances of increased populations of volunteers at any of the sites in the U.K.

Although there was no suggestion that GMHT volunteers were weedier than their non-GM counterparts, there was no selection pressure imposed on the populations of GMHT volunteers described in the studies above. Careful rotation planning weed management would be required where HT oilseed rape crops have been grown to avoid selection with the specific herbicide(s). For example avoiding the use of glyphosate on fallow land following a glyphosate tolerant rape crop, avoiding the use of glyphosate / glufosinate as crop desiccants and avoiding combinations of two crops with the same tolerance in rotation. In the case of imidazolinone tolerant oilseed rape, sulphonylurea herbicides must be avoided in subsequent crops as there is the likelihood of cross-tolerance between the two ALS herbicide groups. Experience from Canada where volunteer rape is a common weed (Legere, Simard, Thomas, De Pageau, Warwick, Derksen, 2001) has shown that HT volunteers can be easily controlled by adjusting existing volunteer control programmes and using alternative auxinic herbicides (e.g. MCPA), or by using herbicide mixtures or using non-chemical control methods (Beckie *et al.*, 2001).

Control of herbicide tolerant volunteers is important because their presence enables persistence of the transgene in the agro-ecostystem via the spread of HT genes into neighbouring fields of oilseed rape, field margin populations of feral rape plants and potentially producing unexpected weed problems or have crop quality implications such as in organic production where

transgenic crops are prohibited. The dispersal of HT genes from HT oilseed rape volunteers and from feral populations of HT plants have been investigated in Section 3.2.

No *A. myosuroides* was recorded during pre- or post-herbicide weed counts in any of the plots in the second season which suggests that there was late or an extended period of emergence (Behrendt and Hanf, 1979) or that the plants were small and were missed at the first assessment, since *A. myosuroides* accounted for the highest weed biomass in June. Interestingly, the levels of total weed biomass recorded in the oilseed rape treatments in 1998-99 corresponded to the highest levels of weed biomass recorded in the winter wheat plots. In particular, the high levels of blackgrass recorded in both the former imazamox and glufosinate treated plots in the winter wheat correspond to the highest levels detected in biomass samples from the winter rape treatments in 1998-99. The former glyphosate plots produced the least (zero) biomass of blackgrass which also fitted the previous years weed control and weed biomass data.

It is possible that these effects were due to the higher (in the case of imazamox and glufosinate) or lower (glyphosate) seed return in these plots, but may also be confounded by a positional effect in the field where *A. myosuroides* was more abundant, since there were differing densities between treatments pre-herbicide application in year 1 and in untreated areas. It would be possible to counteract this effect by re-analysing the data and using the pre-herbicide counts in autumn 1998 as a covariate, i.e. to distinguish which are genuine differences caused by the use of the GM crop-herbicide system rather than patchy weed distribution. This method of analysis would be applicable if the cropping was continued on this experimental site and the effects of the GMHT crops were investigated through a complete rotation over several years.

3.4.3.3 General considerations - experimental design limitations and sampling procedure

It was anticipated that these results would provide information which further work could be based on and may provide a starting point for examination of the interactions of GMHT crops in rotation. The large plot size meant that the experiment was restricted to two replicates, although the plot size allowed the use of full size farm spray equipment that more closely represented actual spray applications in arable fields.

Statistical analysis of the data was limited due to the fact that the experiment consisted of only two replicates. The limited replication meant that background variation was high and the power of the experiment to detect small differences between herbicide treatments was low. The distribution of weeds within fields is not uniform and they are generally aggregated in patches (Marshall 1988; Rew, Cussans, Mugglestone, Miller, 1996) this variability means several replicates would be required to give a representative experimental area, reduce experimental error and increase the significance of results. Clearly, because of the low replication there may have been differences between treatments that could not be detected.

Finally, and most importantly, the results are site specific in terms of weed spectrum; soil type and climatic factors, changing these factors would inevitably alter the results of the herbicide treatments. However, the experiment does give a broad understanding of the effects of the herbicide tolerant oilseed rape systems and provides a basis for more specific studies. The effects of HT systems being integrated in farm rotations is one particular study that this work begins to address by monitoring weed populations in former HT or conventional oilseed rape plots and assessing HT oilseed rape volunteer persistence in following crops in the rotation and around field margins.

3.4.3.4 The economics and agronomic benefits and problems associated with the cultivation of herbicide tolerant oilseed rape

It is difficult to ascertain whether these systems will be more cost effective than conventional systems because the cost of seed and chemicals in the UK are currently not known. In the US and Canada, where these systems have been widely adopted there have been several reports on the comparative costs of the different growing systems.

In Lethbridge, Alberta (Western Canada) farmers have rapidly adopted the HT systems. A two year study of the weed control and economic advantages showed that although there were obvious weed control advantages, there was not a corresponding yield advantage over conventional systems (Anon[2] 2000). The study stated that one of the main factors that increased profitability was whether there were "difficult" weeds present that couldn't be controlled with conventional treatments e.g. *Sinapis arvensis*. However, if competitive weeds were controlled in conventional crops then yields were not much lower. The study also highlighted the fact that seed and chemical costs were higher than in conventional systems and this had an additional effect on profitability.

Another report by the Canola Council of Canada (2000) compared transgenic canola to conventional canola in terms of the costs associated with the different growing systems. One of the main factors that increased the cost of growing transgenic rape was the higher initial seed cost. Seed costs were calculated at around 50% higher than conventional seed. The herbicide input analysis for the two systems revealed that transgenic system costs were about 40% lower than for conventional systems. When all the variables surveyed in the report are taken into account (including fertiliser inputs and cultivation) the gross margin of the transgenic systems were 32% higher than the conventional production system. Unlike most UK farming systems, both the above reports were for U.S. and Canadian farms where spring canola is grown predominantly using minimal tillage.

A report of the FACTT (Familiarisation and Acceptance of Crops incorporating Transgenic Technology) project by Booth *et al.* (1999) suggested that the use of glufosinate may not always give a yield response in winter rape. These workers speculated on the costs of conventional vs. HT

systems and concluded that although herbicide costs may well be reduced, the seed costs may increase (as the above reports suggest) and impact on the profitability of the HT system. The results of FACTT project identified several benefits of the HT system relating to the more effective control of Brassica weeds and opportunity for adopting flexible weed management systems based on actual weed occurrence as opposed to current widespread application of pre-emergence herbicides.

In common with all crops, high yielding, robust cultivars suited to the growing conditions in the U.K will be required for herbicide tolerant oilseed rape to be successfully adopted in U.K. farming systems. Without a distinct yield advantage or utilising the system for control of a particular weed problem it is difficult to envisage high levels of adoption of the technology given the number of existing, effective herbicides on the market (Marshall, 1998). Yield is a complex of several factors in crop production, it may be less direct advantages or longer term effects of using HT crops that may enable greater net farm returns e.g. reduced weed pressure in following crops, or reduction in contamination in harvested grain.

Although not all the HT systems in year one in this experiment provided optimal control of weeds, some of the potential benefits and difficulties have been identified during the course of this work:

1. Herbicides can be applied in response to the weed populations present and the possibility of repeat applications if new infestations occur. Although post-emergence conventional treatment was used in this study, normally conventional treatments are applied pre-emergence, thus relying on good soil conditions and not taking into account weed population density or species present.
2. There were not high numbers of Brassica weeds (e.g. *Sinapis arvensis*) in this experimental field, however, there is clearly opportunity in HT systems for control of weeds that are closely related to rape or volunteer rape. HT systems enabled control of the more difficult weeds such as *G. aparine.*

3. Further weed control options can allow the development of improved weed control strategies and more sustainable systems. Including herbicide rotations helping to prevent the build up of herbicide resistant weed species.

Some of the potential difficulties associated with single applications of herbicides in GMHT systems have also been identified;

1. Weed growth stages - relating to timing of herbicide application: The GMHT systems use contact herbicides and thus optimum weed control is given when weeds have fully expanded cotyledons and some leaf growth. However, by leaving weeds growing for longer, some weeds will be more advanced and more difficult to control. In addition crop growth will provide shelter for some weeds so that they escape treatment.
2. Weed population dynamics: Depends on species present (some species germinate preferentially in the spring or autumn). Where there is high diversity of weed species it may be difficult to control them all in a single spray. In this study *T. aestivum* emerged first and plants were at advanced less susceptible growth stages before a decision was made to apply glufosinate and glyphosate to the later emerged broad-leaved species such as *G. aparine* and *A. arvensis*.
3. Weather conditions: glyphosate and glufosinate are both foliage applied contact herbicides, glyphosate having translocated activity and glufosinate some translaminar activity. Herbicide efficiency is affected by temperature, light levels and by the status of the weeds; growing or not. Applications of both treatments in autumn when temperatures and light levels are lower result in slower action. Weather conditions may also simply prevent spraying thus allowing weeds to keep growing past their most sensitive growth stages.

In order to maximise efficacy of the GMHT rape systems the above factors need to be taken into account. Efficient control of weeds that emerge over longer periods of time may require a split application spray programme if crop competition is not suppressing later germinating weeds. Where

there is a high diversity of species with different population dynamics two sprays may be more effective.

3.4.3.5 Weed control in herbicide tolerant oilseed rape and implications for botanical diversity

It is difficult to extrapolate the long term effects of the herbicide treatments on botanical diversity in the arable environment from a single two season field experiment. Information on the effects of herbicides on diversity can only reliably be generated from studies made over considerably longer periods of time that investigate the effects of the herbicides in a wide range of arable environments. Studies such as the government sponsored Farm Scale Evaluations and the BRIGHT project (Botanical and rotational implications of growing herbicide tolerant crops) are investigating some of the key effects of the herbicides and the interactions between different GMHT crops over several growing seasons.

The results showed that there were some differences in the relative efficacy of the herbicides and thus the potential for these herbicides to cause shifts in weed communities. The weed biomass assessment shows the relative abundance and number of species found in each treatment prior to harvest and thus the potential for differences in weed seed return to the seedbank.

The change in active ingredient and the timing of herbicide application in HT winter oilseed rape (i.e. from mainly early pre-weed emergence sprays to later post emergence sprays) will likely cause a change in the weed species that are being controlled or those that escape treatment, causing some species to become more abundant and reductions in populations of others. Derksen *et al* (1999) reported evidence of shifts in weed communities in Canada where GMHT oilseed rape is widely grown, indicating that some of the Cruciferous weeds such as *Capsella bursa-pastoris* may become less abundant in the future.

Arable weed flora represents both a component of biodiversity itself and a potential food source for other organisms, and the weed seedbank in an arable field represents the potential weed flora of an arable ecosystem. Most weed species common on arable land form persistent seed banks

and in a single year only a proportion of the seeds will germinate and grow (Jones and Maulden, 1999). Seedbanks thus represent a major component of botanical diversity in arable fields that reflect the management of arable fields over several years. Weeds represent an important element of the food chain for birds, both directly through providing seeds and indirectly by supporting insects. Thus a change in herbicide use may potentially cause a shift in certain weed species and may have a knock-on effect on invertebrates and farmland birds.

Watkinson, Freckleton, Robinson, Sutherland (2000) simulated the effect of the introduction of GMHT crops (sugar beet) on weed populations and the consequences for seed-eating birds, using *Chenopodium album* as the model weed. They predicted that weed populations might be reduced to low levels or nearly eradicated and the effects on birds (Skylarks) may be severe due to the major loss of a food source. Their model has several weaknesses, in that they assume complete control of the weed species during the intervening years between the GMHT crops, parameters such as what happens to the weed seeds after they have been shed have also been neglected. Watkinson *et al.* (2000) did not identify any benefits of the GMHT cropping system such as the more flexible, later application of post emergence herbicides thus favouring breeding birds (Firbank and Forcella, 2000; Pidgeon, Dewar, May, 2001).

Careful management and the utilisation of HT crops as 'tools' in farming systems may potentially benefit the agro-ecosystem. Changes in management of weeds in crops such as GMHT sugar beet have been demonstrated to be beneficial to both the farmer and biodiversity (Pidgeon *et al.*,2001). Pidgeon *et al.* (2001) found that leaving weeds in a GMHT sugar beet crop for up to 10 weeks after sowing gave no yield penalty, in some instances this reduced aphid colonisation of beet plants and increased beetle numbers. They did not detect any decrease in invertebrate biodiversity when conventional and GMHT crops were compared.

It is feasible that GMHT crops could compliment government policies for the enhancement of botanical and wildlife diversity and abundance such as the U.K. Biodiversity Action Plan (BAP). It

may be possible to integrate the use of GMHT crops with conservation headlands in a similar strategy to conventional crops promoting diverse flora and seeding weeds in less economically important parts of the field (Wilson and Aebischer, 1995).

The opponents of GMHT crops suggest that their commercial introduction may necessitate or encourage the use of management which could have long term adverse effects on arable-ecosystems. Clearly the change in use of any herbicide will affect an arable ecosystem. However, there is evidence from some studies that GMHT crops may be useful agronomic tools and may actually promote the development of sustainable farming systems. It is the long term benefits and risks of GMHT crops in agricultural systems that require further examination.

4. POPULATION PROJECTION MODEL FOR VOLUNTEER / FERAL OILSEED RAPE POPULATIONS CONTAINING GMHT TRAITS

4. POPULATION PROJECTION MODEL FOR VOLUNTEER / FERAL OILSEED RAPE POPULATIONS CONTAINING GENETICALLY MODIFIED HERBICIDE-TOLERANT TRAITS.

4.1 Introduction

The results reported in Section 3.1, 3.2 and 3.3 indicate the potential for the dispersal of GMHT traits by cross-pollination of conventional varieties with GM pollen. While an understanding of the dispersal behaviour of GM traits is important in devising strategies for the safe deployment of GM crops in agriculture, there is also a need to understand the behaviour of weedy and feral populations of GM oilseed rape in the case where cross-pollination occurs and a seed bank containing GM seed is allowed to establish. In an attempt to address this second issue, and to draw together results presented in Section 3.4, a simple population projection model is developed and analysed in this section.

Several simulation models, relating to various aspects of GM cropping systems, have been developed in recent years, including: (1) the effect of herbicide tolerance on the performance of oilseed rape as a weed (Squire, Burn and Crawford, 1997); (2) the impact of herbicide tolerant oilseed rape on long term herbicide use Madsen., *et al.* (1999); (3) the impact of GM oilseed rape on whole cropping systems Colbach., *et al.* (1999); (4) the potential for gene escape via the seed bank Pekrun, *et al.* (1999); and (5) the impact of GMHT sugar beet on bird and weed populations over large spatial scales Watkinson, *et al.* (2000). The publications of Squire *et al.* (1997) and Colbach *et al.* (1999) and Pekrun *et al.* (1999) are of particular relevance to this study, being concerned with herbicide tolerant oilseed rape. A major focus of the current study has been the potential for cross-pollination between conventional and GM oilseed rape for the spread of GMHT traits. Neither Squire *et al.* (1997) nor Pekrun *et al.* (1999) considered this aspect of behaviour of volunteer oilseed rape populations in their models. The GeneSys model described by Colbach *et al.* (1999) does consider cross-pollination between conventional ("classic") oilseed rape and GM oilseed rape, but is aimed at simulating the levels of the GM trait in *crops* of both types over a large spatial scale and is a relatively complex simulation with a large number of parameters. Data gathered in the present study

will allow the construction of a simple model which includes the effects of cross pollination and competition between conventional and GMHT varieties in *volunteer* and *feral* oilseed rape populations. The model which is developed here is, therefore, distinct from previous mathematical descriptions of the behaviour of GMHT oilseed rape populations.

4.2. Model Development

Caswell (2001) draws an important distinction between *projection* models and forecasting or *predictive* models. As Caswell notes, "A forecast predicts what *will* happen. A projection describes what *would* happen, given certain hypotheses." An important result of this is that, generally, projection models are conceptually simpler than simulations. Furthermore, as Caswell (2001) demonstrates, using the population projection approach leads naturally to development of models based around projection matrices and Markov processes to calculate population changes in discrete time steps. This approach is particularly relevant to annual plants such as oilseed rape.

Consider the situation in which cross pollination events have occurred in a conventional oilseed rape variety, or a seed source contaminated with a low percentage of GMHT seed has been sown. In either case, based on the results presented in section 3.4, a seed bank containing both conventional and GMHT seed is likely to be produced. The results presented in section 3.2.3.1 give an estimate of the net effect of competition and cross-pollination between the conventional and GMHT plants in the population over a single season. These results suggested that in combination with both varietal associations and standard varieties, the proportion of the seed bank carrying the GM trait showed a proportional decrease which was independent of the initial density of the GM seed in the population; the effect being greater in the case of standard varieties than the case of the varietal association examined (cv. Synergy). Assuming that plants of conventional and GM varieties produce similar number of seeds, it is assumed that the proportional reduction in the GM component of the seed bank is replaced directly by conventional seed. Thus the results of section 3.2.3.1. can be summarised in two projection matrices (**V** and **S**) which give the single generation mutual effects

of conventional and GM varieties in the case of, respectively, varietal associations and standard varieties.

$$\mathbf{V} = \begin{pmatrix} 0 & 1.38 \\ 0.62 & 0 \end{pmatrix} \quad \mathbf{S} = \begin{pmatrix} 0 & 1.55 \\ 0.45 & 0 \end{pmatrix} \qquad \textbf{1.}$$

In both matrices the first column summarises the effect of the conventional variety on the population of GM seed, while the second column summarises the effect of the GM variety on the population of conventional seed. The matrices **V** and **S**, can be used to generate simple population projections, given values for the initial population sizes. Assuming that the population at time t+1 is dependent only on the population at time t and the interactions captured in V and S, the populations at t+1 are calculated by multiplying the matrices to vectors (**Pv** and **Ps** respectively) giving the population sizes at time t, as shown in equation 2.

$$\mathbf{Pv}_{t+1} = \mathbf{V} \bullet \mathbf{Pv}_t$$

$$\mathbf{Ps}_{t+1} = \mathbf{S} \bullet \mathbf{Ps}_t \qquad \textbf{2.}$$

As a simple illustration of the effect of this operation, population projections arising from the equations 2 are shown in Figure 51 assuming starting populations of 1100 and 11 seeds for the conventional and GM varieties in both cases. Figure 51 bears comparison with Figure 3 in Colbach *et al.* (1999) and Figure 4 in Pekrun *et al.* (1999). Generally, without reproduction, the simple projection models represented in equations 2 predict an oscillating, but declining seed bank size over time, with the general trend showing an exponential decay in all cases.

As indicated above, the simple models given in equations 2 do not contain any reproduction. Inclusion of reproduction in the model demands consideration of several other factors which are of interest: the effect of the fraction of the seed bank which does not germinate, the impact of different control efficiencies, and the effect of seed mortality.

Consider a population of seeds, *P* in the soil seed bank. At the start of the growing season a given fraction, *g*, will germinate, so (assuming all germinated seeds produce a seedling) the number of

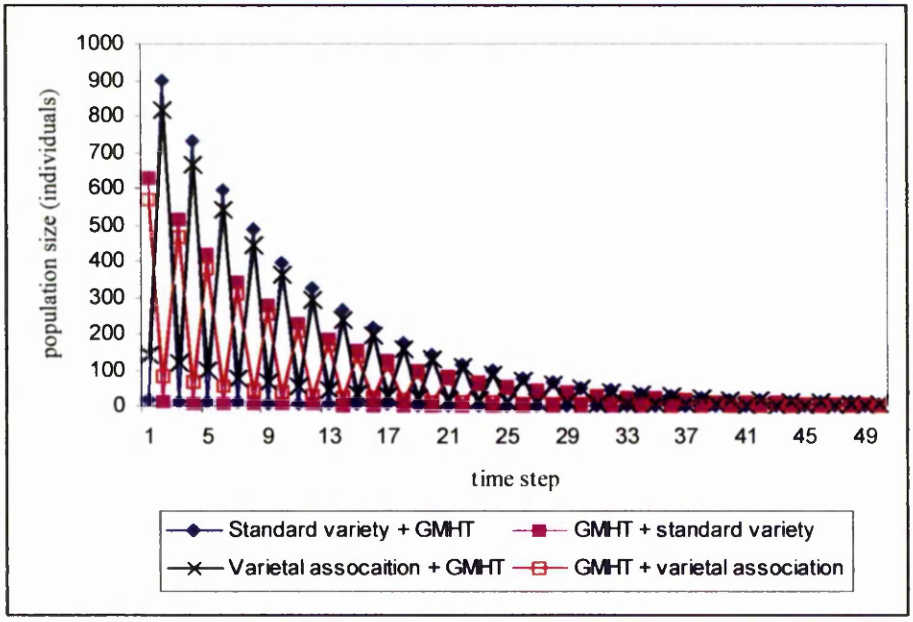


Figure 51. Population projections for the seed bank of a GMHT variety and either a standard or varietal association conventional variety assuming that the populations are described by equations 2. The initial population sizes in both cases were conventional (standard or varietal association) = 1100, GMHT = 11

seedlings produced is $g \cdot P$; note, this implies that $(1-g) \cdot P$ seeds remain in the seed bank. Estimates of the value for g were obtained from section 3.4. relating to the number of seeds detected in the soil and the subsequent number of emerged seedlings. Typically, weed control is applied early in the growing season when weed populations are at low density and prior to reproduction. If control results in the removal of a fraction, *c*, of the seedlings, the population remaining after control is $(1-c) \cdot g \cdot P$; this is the population of individuals available for reproduction. Values for c were obtained from the data presented in section 3.4 on weed control (see, for example, Figure 46).

A typical approach in modelling weed populations (Cousens and Mortimer , 1995) is to include reproduction as a single parameter, *R*, the per capita growth rate for the population at low density. This parameter describes the multiplication rate of single seed when allowed to reproduce

under conditions of no competition. Following reproduction the number of seeds in the population is given by $R\bullet(1\text{-}c)\bullet g\bullet P$. Data for R, estimated from plants growing at low density, are not available within this study, but data for seed production per plant for single varieties growing under typical conditions, can be estimated from the data presented in Table 26, Section 3.4.

To obtain the seed production in the population, adjusted to take account of the competition and cross-pollination between conventional and GM types, the potential seed production values for each type, given by $R\bullet(1\text{-}c)\bullet g\bullet P$, are combined in a vector (**Pv** or **Ps**, as described above) and the relevant projection matrix is multiplied to the vector.

The seed produced in the current generation is added to the non-germinated fraction $[(1\text{-}g)\bullet P]$ to give the total seed bank. Seed mortality was not assessed in the current study, and several other workers have noted that this is a difficult parameter to estimate accurately (Cousens and Mortimer, 1995; Squire *et al.*, 1997; Pekrun *et al.*, 1999). Data reported in Squire *et al.* (1999) and Madsen *et al.* (1999) suggest that the figure may be as high as 90%. In the current study a range of possible values for the seed mortality fraction were examined. In general, if a given fraction, m, of the non germinating seed bank subsequently dies, the net effect of the population processes described above, plus seed mortality in the non-germinated fraction of the seed bank, leads to an expression for the population projection over time given in equation 3./

$$\mathbf{Pv}_{t+1} = \mathbf{V}\bullet\mathbf{Pv}_t + [\,(1\text{-}m)\bullet(1\text{-}g)\bullet\mathbf{Pv}_t]$$

$$\mathbf{Ps}_{t+1} = \mathbf{S}\bullet\mathbf{Ps}_t + [\,(1\text{-}m)\bullet(1\text{-}g)\bullet\mathbf{Pv}_t] \qquad \textbf{3.}$$

Essentially the same model can be used to project the populations of feral oilseed rape not subject to control. However, in this case density-dependent regulation of seed production will become significant and it is necessary to replace the simple reproductive parameter R, by a suitable function describing the density-regulated reproduction of individuals under crowding (Cousens and

Mortimer, 1995). The model shown in equation 4 has been found to be suitable for a number of annual species.

$$R \bullet P / [1+(a \bullet P)^{-b}] \qquad \textbf{4.}$$

Values for the parameters *a* and *b*, describing the density-dependence of reproduction were selected on the basis of those which have been found to be typical in the literature. The values selected were $a = 0.5$, $b = 0.7$. Depending on the seed mortality rate, these values gave final projected seed bank populations of between 1000 and 10000 per square metre, which are within the range of typical values for oilseed rape (Squire *et al.*, 1997).

The model represented in equations 3 was implemented in an Excel™ workbook. Population projections were produced for combinations of varietal association and GMHT types, and standard variety and GMHT types under conditions of a four year rotation in which the control efficacy, *c*, varied among years in the rotation. Every third year a low value for *c* for the GMHT type was used to provide projections of populations when weed control was based on the use of the herbicide associated with the HT trait. The changes in feral populations were projected by assuming that control in all years for these projections was given by a value of $c = 0$. Four complete rotations were projected in each run of the model, giving population projections over a 16 year period. The values for *c* over the four years of the projected rotation were for conventional varieties: $c = 0.95$, 0.95, 0.98, 0.85, and for the GMHT varieties, $c = 0.95$, 0.95, 0.2, 0.85. Projections were made for populations with values of *m* of 0.9, 0.75, and 0.5. All of the projections were started with a conventional population of 1100 seeds per square metre and a GMHT population of 11 seeds per square metre (1% of the conventional population). The value for the conventional variety was obtained from the seed bank estimates made in Section 3.4. The contamination rate of 1% was selected as being representative of values which might occur either by cross pollination or by seed contamination.

4.3 Results and Discussion

Representative results from the model are presented in Figures 52a-c. Figure 52a shows the projected changes in the seed bank of a mixed population of conventional and GMHT oilseed rape when the conventional variety is a varietal association. The projections suggested that, seed mortality of either 0.9 or 0.75 will lead to a decline in the population, while a mortality of 0.5 will lead to an increase in the population. Under conditions where the population might increase (i.e. when m = 0.5), the number of GMHT seeds in the seed bank was projected to increase rapidly until it was approximately equal to, but never greater than, the number of conventional seeds in the population. Essentially similar results (Figure 52b) were produced in the case of a standard variety and a GMHT variety, although the numbers of individuals of both types of oilseed rape were only slightly but consistently lower than in the case of the varietal assocation/GMHT combination.

Within the model, these differences arise from the higher net reproductive rate of the GM trait in combination with a varietal association than with a standard variety. Since the structure of the projection model is essentially a competition model, it is to be expected that the changes in the population sizes of the two components will be correlated. One interpretation of the observed results is that in the case of the varietial association/GMHT combination the competition is weaker than in the standard variety/GMHT combination, leading to both types performing slightly better in the former case. Clarke & Beaumont (1991) noted that the type of correlations observed in the current study are a feature of other types of competition model, and suggested that competition may act to prevent genotypes from being lost from mixed populations. If that observation is correct, the implications for the persistence of GMHT traits in weedy populations are important since they suggest that competition and cross-pollination together may buffer low levels of GM traits from extinction in these populations. The empirical and modelling observations in this study support this hypothesis.

Considering the results of projections of feral populations, the model indicated that stable population sizes containing large numbers of both conventional and GMHT types could be produced

within a few years of the establishment of the population (Figure 52c). The projections are in agreement with observations of the relatively long persistence and stability of feral oilseed rape (Squire *et al.* 1997). However, it should be noted that the model parameters used in this study were selected as typical of those found in the literature, and might therefore, be expected to generate results that result in a population increase towards an upper asymptote.

While the model developed here is very simple, it has the capacity to make projections of populations of volunteer and feral populations of oilseed rape containing GM traits. Generally, despite its simplicity, the model produces results that are in agreement with those generated by more complex simulations (Squire *et al.*, 1997; Colbach *et al.*, 1999). Further elaboration of the model is possible, particularly with respect to parameters for seed mortality and density regulation of reproduction. However, the model has proved useful in utilising empirical observations made in the earlier parts of this study and in synthesising some useful ecological results from those observations.

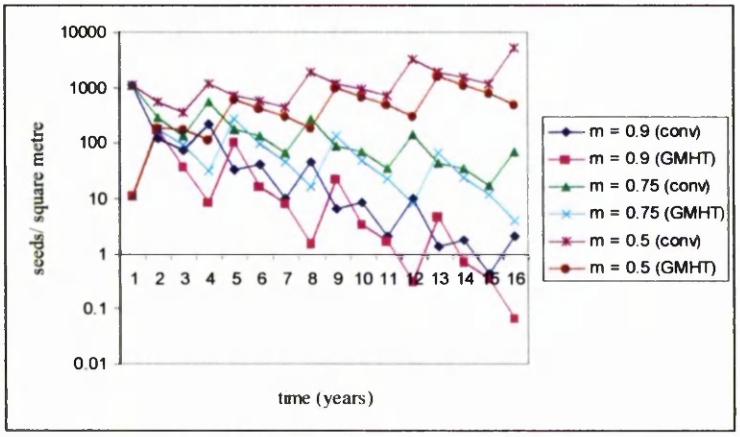


Figure 52a. Projected changes in seed bank populations for conventional and GMHT varieties in a mixed volunteer population subject to rotational control and three different levels of seed mortality. GMHT variety and varietal association

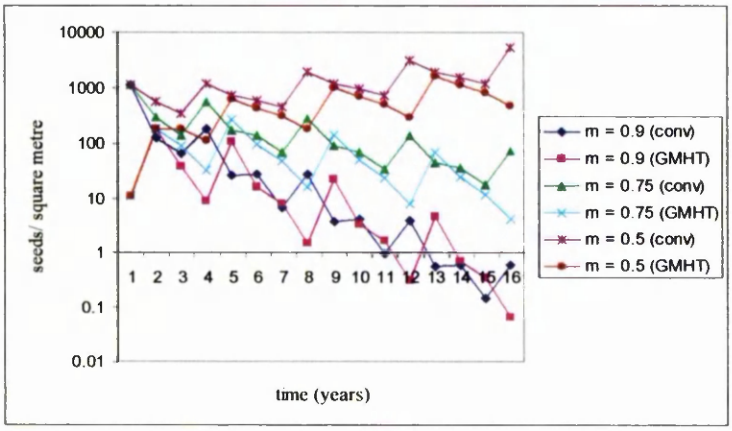


Figure 52b. Projected changes in seed bank populations for conventional and GMHT varieties in a mixed volunteer population subject to rotational control and three different levels of seed mortality. GMHT variety and standard variety

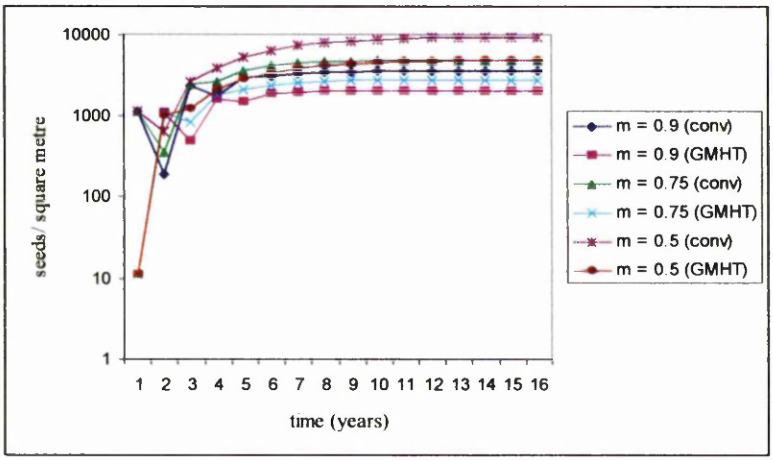


Figure 52c. Projected changes in seed bank populations for conventional and GMHT varieties in a mixed feral population subject to density-dependent regulation and three different levels of seed mortality. GMHT variety and varietal association

5. GENERAL DISCUSSION AND CONCLUSIONS

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The range of comparisons between different genotypes and varying pollen source and recipient populations of HT oilseed rape reported here have provided new data describing the dispersal of herbicide tolerance genes in agronomic environments. The experimental models simulated dispersal of HT genes between; field scale areas of HT oilseed rape, populations of GMHT feral rape/volunteers and crops of conventional oilseed rape and a crop of GMHT oilseed rape and a commercial crop of winter turnip rape (*B. rapa*). The experiments demonstrated the importance of the self-fertility of the recipient populations and the influence of the pollen source size on the levels of cross pollination, and the relationship between cross pollination and distance from pollen sources. The levels of cross pollination were considerably higher when the self fertility of the recipient crop was low, as was the case with the varietal association hybrid rape types containing high proportions of male sterile plants.

Cross pollination was measured between a range of genotypes grown in areas of approximately 0.8 ha. The data showed that at the sampling points nearest the pollen source levels of outcrossing were an average of 1.2% at 1.5m while at 81.5m outcrossing levels declined to an average of 0.05%. The comparable average outcrossing in cv. Synergy showed considerably higher average levels; 23.7% at 1.5m and 4.3% at 81.5m. The data clearly showed the influence of recipient crop genotype on the level and range of outcrossing levels observed. The dispersal gradients for outcrossing in the fully fertile oilseed rape plots showed a rapid decline with increasing distance from the pollen source.

Measurement of the highest theoretical levels of cross pollination using male sterile receptor plants positioned around a 11 ha area of GMHT oilseed rape showed a similar pattern of decline in cross pollination with distance as the study described above, and that cross pollination events were still detectable at 600m from the pollen source. There was some evidence of wind and insect

involvement in the dispersal of pollen from the GMHT source field, and also evidence of a dilution effect of conventional "pollen barrier" crops on transgene dispersal.

Simulated populations of GMHT feral populations of oilseed rape, growing in the field margin of a conventional crop, showed there was the potential for contamination of conventional crops to occur from these populations, and that the levels of cross pollination observed were also higher when the recipient population was a varietal association hybrid rape type.

Two simple dispersal models were compared, the inverse power law (IPL) and the negative exponential function (NE) to investigate the empirical relationships between cross pollination and distance from pollen source. The data were used to compare these models for their ability to describe the observed relationship between outcrossing and distance from source. The IPL model described the decline in outcrossing rate with distance better than the NE function. The consequences of this are that dispersal described by the IPL is more likely to lead to cross pollination at both large and short distances from the pollen source. The results obtained in this study, which assessed dispersal indirectly by examining the phenotype of seed set in pollinated plants, broadly agree with previous studies in which pollen dispersal has been examined directly (Thomson *et al.*, 1999). Where dispersal of pollen follows an inverse power law there are implications for risk assessment of GM crops of oilseed rape; the ecological behaviour of GMHT traits resulting from the dispersal curves obeying an inverse power law are likely to result in patchy population expansion. This will likely have an impact on the efficiency of sampling techniques for GM traits. Furthermore, establishing effective isolation distances for GM and non-GM crops when grown at a large scale becomes more problematic because of the long probability tail of the dispersal curve when the IPL describes dispersal. Linear regression carried out on data collected in this study using male sterile and fertile receptor plants indicated that over distances of 100m to 600m the dispersal curve for pollen is essentially flat, over these distances. Thus "safe" isolation distances may need to be considerably greater than suggested by dispersal levels indicated by the negative exponential

function. Indeed, the results gathered in this study suggest that, in considering the development of sampling methods for regulatory control of GMHT traits, a concentration on dispersal distances from known *sources* of pollen is unlikely to be effective. Rather, methods focussed on detecting whether contamination has, in fact, occurred in known *target* populations and crops. In this context, a combination of the methods proposed by Wilkinson, Davenport, Charters, Jones, Allainguillaume, Butler, Mason and Raybould (2000) and sampling protocols which have been developed recently in the context of regulatory control of plant diseases (Madden and Hughes, 1999; Hughes, Gottwald and Levy, 2002).

The experimental arrangement adopted in the gene flow studies indicated that cross pollination between fields, feral and volunteer populations of GMHT oilseed rape are likely to occur if these crops are incorporated into U.K. farming systems. More data are required from larger scale studies in order to predict likely cross pollination levels occurring when GM oilseed rape is commonly grown in arable rotations over large areas. In addition, data are needed in order to determine isolation required for seed crops, particularly of hybrid varieties since, in these crops, parent lines are male sterile and thus very susceptible to unintended outcrossing.

Mixed populations of GMHT and conventional oilseed rape genotypes were studied in order to simulate different levels of GMHT volunteer infestation. The results indicated that the final percentage contamination rate was also dependent on the degree of self-fertility in the contaminated crop. Contamination of harvested seed was higher in varieties with low self fertility compared with fully self fertile varieties. However, in both cases the contamination rate in the harvested seed was lower than in the initial population. The evidence gathered in the experiment suggested that over a wide range of initial GMHT contamination rates, the final proportion of GMHT seed in the total population was a constant fraction of the initial contamination rate. The results suggested that in an agronomic situation, competition between the conventional variety and GMHT volunteers would reduce the levels of GMHT individuals in the population to low numbers within a few generations.

Data arising from the contamination studies, weed control experiments and seed bank sampling was used to develop a simple population projection model which can be used to examine the potential for persistence of volunteer and feral oilseed rape populations containing a mixture of conventional and GMHT types. Broadly, the model generated population projections that were in agreement with more complex simulations published elsewhere. The model suggested that control efficacy and seed mortality were important in determining the fate of the population over time. Forcella (1999) has previously highlighted the importance of control rate in preventing the build up of a large seed bank of velvet leaf (*Abutilon theophrasti*) in simulated populations of GMHT soybean. Seed mortality is widely recognised as a key variable in seed bank dynamics, and also one that is difficult to assess. An important result to emerge from the model is the key role which cross pollination and competition might play in maintenance of GMHT traits in volunteer and feral populations. Competing populations are, essentially, linked and changes in the population of one competitor have direct consequences for the other. The possibility of mutual competition for pollination sites in the mixed population appears to act as a buffer to either component being driven to extinction in the model. Similar results have been reported in more general competition models examined by Clarke & Beaumont (1992). A general conclusion from this study is that GMHT traits may persist in volunteer and feral populations over many years, even if the numbers of individuals is rather low. Similar results were obtained in the more complex GeneSys model (Colbach *et al.*, 1999) providing further support for this general conclusion.

A long-term consequence of the adoption of GMHT oilseed is the potential for a change in the species of arable weeds due to the activity of the new herbicides used. Although the time scale of this study was not suitable for longer-term investigation of changes in species diversity; the results provided some valuable information on the activity spectra of the herbicides. Weed biomass measurements taken from plots of herbicide tolerant oilseed rape showed that shifts in arable weed populations may be possible with continued use of a specific herbicide due to differences in weed

seed return to the seed bank. Whether these shifts in botanical diversity are significant and have impacts on seed banks are currently being studied in the BRIGHT project and in the Farm Scale Evaluation (Lutman and Sweet, 2000).

Limited data on the behaviour of oilseed rape volunteers showed that herbicide tolerant and putative double tolerant winter oilseed rape plants were as susceptible as conventional oilseed rape volunteers to the normal broad-leaf selective herbicides used in cereal crops. Evidence from this and other studies (Sweet *et al.*, 1997; Norris *et al.*, 1999; Simpson and Sweet, 2000) has shown that GMHT volunteers were not weedier or more invasive in the absence of selection pressure than their non-GM counterparts. Some Canadian research has suggested that multiple herbicide tolerance in oilseed rape would be a potential problem to manage in the U.K. (Orson, 2002). The findings from this project and other studies suggest that conventional management of other crops in arable rotations can adequately control GM volunteers. However, farmers do need to be fully informed about potential outcrossing and be able to develop appropriate strategies for managing multiple tolerance.

This research project has shown that HT oilseed rape will have a range of impacts on arable farming systems so that new practices and management will be required for them. The impacts on non-HT oilseed rape crops mean that farmers will need to adopt practices familiar to seed producers, which involve consideration of crops growing outside their own field boundaries. The impacts on botanical diversity will depend on the management of the new herbicides applied to HT oilseed rape more than on their activity. Thus, there is scope to manipulate the use of potentially more powerful herbicides in ways which can enhance diversity especially at times when crops are less vulnerable to weed competition.

6. FUTURE WORK

6. FUTURE WORK

Research into the biodiversity and botanical diversity impacts of herbicide tolerant crops are currently underway and should result in a clearer impression of how they will impact on the diversity in arable farming systems. However, there remain other research areas with scope for increasing our understanding of the likely implications of herbicide tolerant oilseed rape. These include:

- Further large scale studies of gene flow in oilseed rape - make comparisons between hybrid oilseed rape types including three-way hybrids and varietal associations. Incorporate direct measurements of pollen concentration and local meteorological data to assist with interpretation of data.
- Investigations of the transfer of HT genes between GMHT feral populations and conventional oilseed rape crops to aid the prediction of gene flow and transgene spread in the environment. Investigate the population dynamics of feral oilseed rape and its association with agriculture.
- Investigate cross pollination levels between GMHT oilseed rape volunteers and different genotypes of conventional oilseed rape. Make detailed measurements of fecundity of competing volunteer and crop plants to determine the effects of competition between crop and volunteer plants
- More extensive studies of cross pollination between commercial varieties *of B. rapa* and *B. napus*.
- Investigation of the management of the herbicide programmes used in herbicide tolerant oilseed rape in order to protect crop performance while encouraging non-competitive weed growth and diversity. This would include studies of the timing of application of herbicides and also differential applications to different parts of fields e.g. investigate management of field margins and headland set aside in conjunction with herbicide tolerant oilseed rape and any environmental benefits.

- The further development of the population projection model developed in this project. This development could have several aspects but two technical suggestions and one area of application are given here. First, quantification of seed mortality is required to more accurately parameterise the model. Secondly, the model is completely deterministic in its current form and some of the single-value parameters should be replaced by random numbers, drawn from suitable probability distributions, to implement stochastic population projections. This is an important point to consider if the model is to be used to project the persistence of small populations (which have a large probability of random, local extinction) of GMHT individuals (Caswell, 2001). Finally, the model could be used as demonstration tool to stimulate discussion among groups of stake-holders in discussion on the impact and management of GMHT crops. A suggested first task toward this objective would be to hold a focus group of weed ecologists to make suggestions for the improvement of the model.

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APPENDICES

APPENDIX 1.

Plate 1. An example of a main raceme sample taken from plot 7 cv. LL1 (June 1999) for testing for herbicide tolerance

Foreground: a single main raceme sample of winter oilseed rape from plot 7.

Background: complete sample of 20 main oilseed rape racemes taken from $1m^2$.

Plate 2. Single and multiple tolerant winter oilseed rape plants grown from seeds harvested from National List GM winter oilseed rape trial at Caxton, Cambridgeshire harvested in 1998

A - glufosinate tolerant plant from plot 16 (cv. Synergy) treated with glyphosate (360g/l) at 720g a.i./ha (15 days after treatment)

B - glyphosate tolerant plant from plot 16 (cv. Synergy) treated with glufosinate (200g/l) at 400g a.i./ha (10 days after treatment)

C - plant tolerant to both glufosinate and glyphosate grown from seed harvested from plot 18 (cv. GLU1) 15 days after treatment with glyphosate and 12 days after treatment with glufosinate

Plate 3. Herbicide tolerance testing (1999) of seed samples harvested in 1998 from National List trials growing adjacent to GM National List trials

Plate 3. shows the very low numbers of oilseed rape seedlings surviving herbicide treatment, indicating low levels of outcrossing with GMHT oilseed rape. The black arrows indicate surviving plants 14 days after treatment with glufosinate. Plots in the background were treated with glyphosate (360g/l) at 1440g a.i./ha when plants were at the 3-5 leaf stage using atractor mounted sprayer. Surviving plants were re-treated and a final count made of surviving plants approximately 14days after the second treatment.

Plate 4. Large scale geneflow field experiment of herbicide tolerant and conventional winter oilseed rape in flower (April 1999)

1 - Plot 1 cv.Apex

2 - 24m wide buffer strip of non-GM winter oilseed rape (cv. Apex) between plots 1-4 and plots 5-8

3 - Plot 5 (cv. RR1) glyphosate tolerant winter oilseed rape

4 - Plot 6 (cv. IMI1) imidazolinone tolerant winter oilseed rape

5 - Plot 7 (cv. LL1) glufosinate tolerant winter oilseed rape. Note the non-uniform flowering of this plot relative to the adjacent plot of Synergy (8).

6 - Plot 8 (cv. Apex) conventional winter oilseed rape

7 - Plot 8 (cv. Synergy) winter oilseed rape varietal association

Refer to Figure 2 for schematic layout of plots.

Plate 5. Herbicide tolerance testing winter oilseed rape seed samples for imazamox tolerance

A tray containing 1000 seedlings grown from a winter oilseed rape seed sample from plot 3b experiment 3.1.3. Seedlings have been sprayed twice with Imazamox (40g/l) at 70g a.i./ha., surviving seedlings are clearly visible.

Plate 6

Herbicide tolerance testing winter oilseed rape seed samples for glyphosate tolerance

Tray originally containing 600 seedlings grown from a winter oilseed rape seed sample from experiment 3.1.1. Seedlings have been sprayed twice with glyphosate (360g/l) at 720g a.i./ha, surviving seedlings are clearly visible.

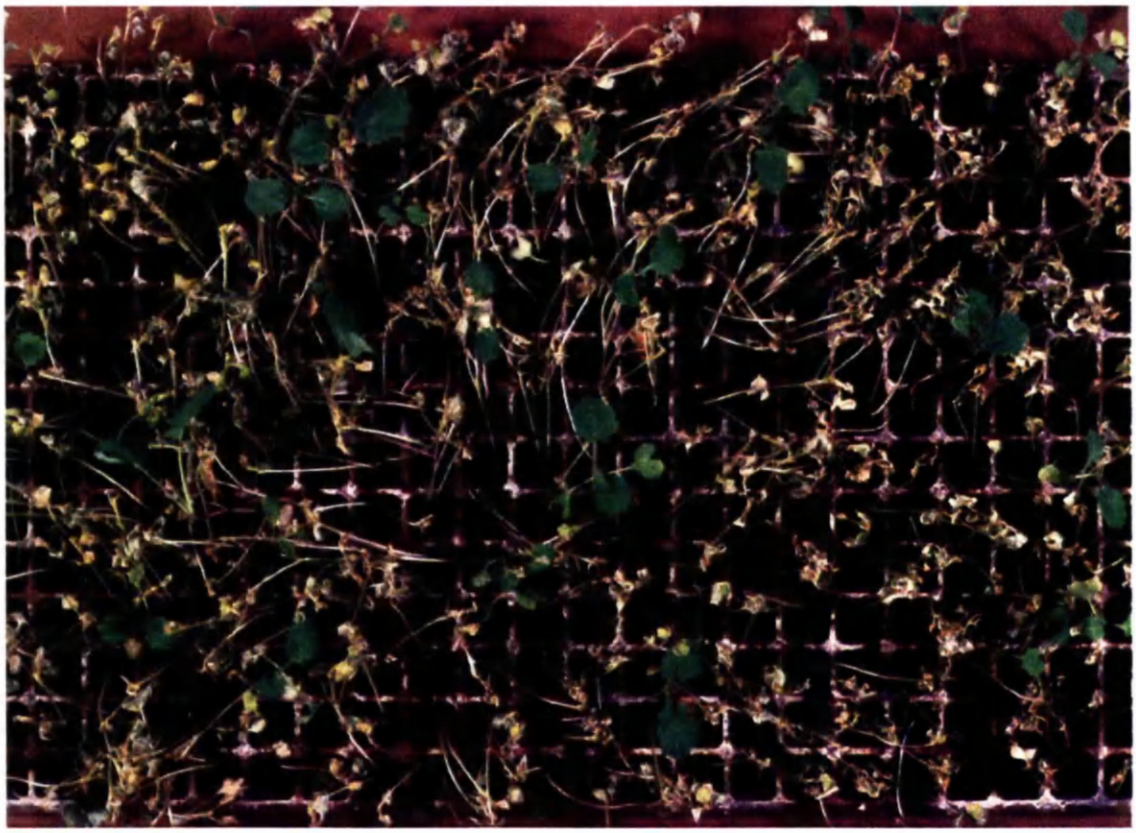

Plate 7

Herbicide tolerance testing winter oilseed rape seed samples for glufosinate tolerance

Tray originally containing 600 seedlings grown from a winter oilseed rape seed sample from experiment 3.1.1. Seedlings have been sprayed twice with glufosiante ammonium (200g/l) at 400g a.i./ha, surviving seedlings are clearly visible.

Plate 8. Artificial glyphosate tolerant feral winter oilseed rape populations flowering next to conventional winter oilseed rape plot 8

Artificial feral rape populations flowering next to plot 8 cv.Apex in the foreground and next to plot 8 cv. Synergy in the background.

Plate 9. An example of weed infestation in winter oilseed rape (cv. Apex) pre-herbicide treatment (November 1998)

Photograph shows infestation of mainly volunteer wheat plants (*Triticum aestivum*) at approximately growth stage 21 and oilseed rape at growth stage 1.4

Plate 10. An example of weed control in conventional winter oilseed rape (cv. Apex)

Winter oilseed rape cv. Apex treated with Metazachlor (500g/l) at 1250g a.i./ha and Fluazifop-P-butyl (200g/l0 at 150g a.i./ha. The photograph taken post-herbicide treatment in January 1999 and shows effective control of large winter wheat volunteers, large field bean volunteer surviving (centre-left).

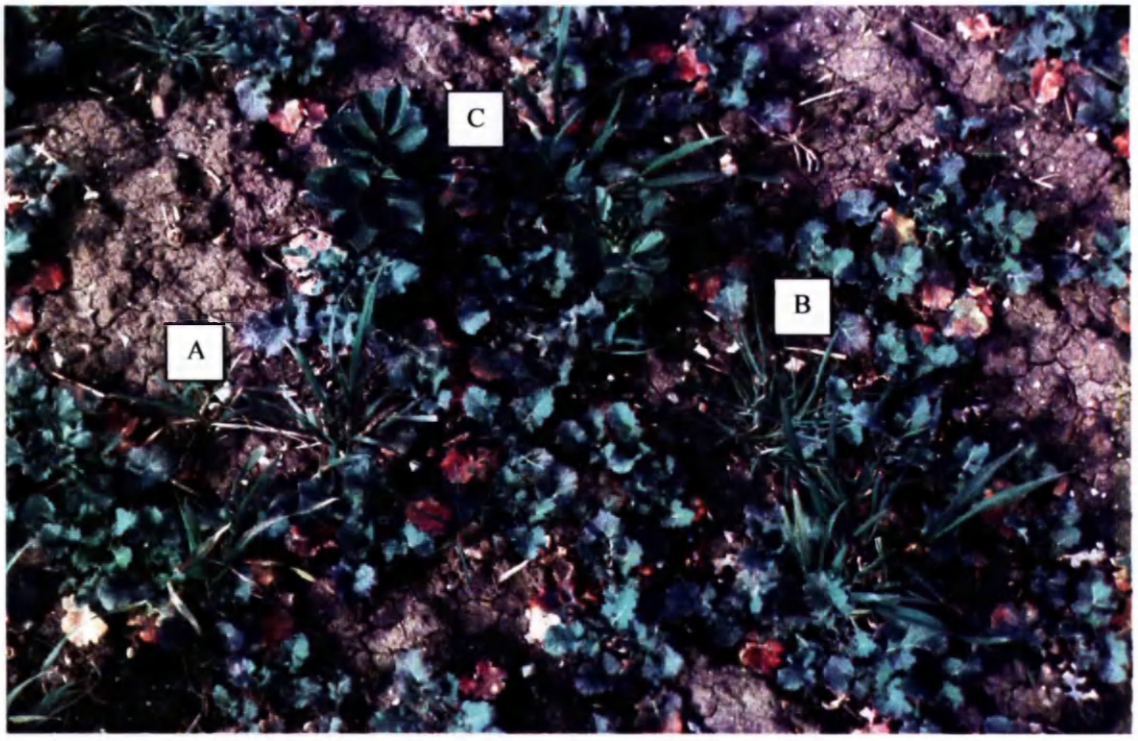

Plate 11. An example of weed control in Imazamox tolerant winter oilseed rape (cv. IMI)

Winter oilseed rape cv. IMI treated with Imazamox (40g/l) at 70gai/ha. The photograph was taken post-herbicide treatment in January 1999. The photograph shows ineffective control of large winter wheat volunteers (*Triticum aestivum*) (A), blackgrass (*Alopecurus myosuroides*) (B) and volunteer field beans (*Vicia faba*) (C).

Plate 12. An example of weed control in glufosinate tolerant winter oilseed rape (cv. LL1)

Winter oilseed rape cv. LL1 treated with glufosinate ammonium (200g/l) at 600g a.i./ha. The photograph was taken post-herbicide treatment in January 1999, and shows ineffective control (re-growth) of large winter wheat volunteers.

Plate 13. An example of weed control in glyphosate tolerant winter oilseed rape (cv. RR1)

Winter oilseed rape cv. RR1 treated with glyphosate (360g/l) at 720g a.i./ha. The photograph was taken post-herbicide treatment in January 1999, and shows effective control of large winter wheat volunteers and broad leaved weeds.

APPENDIX 2

Table 40. Percentage glufosinate and glyphosate tolerance detected in seed samples from plots growing in a National List winter oilseed rape trial adjacent to a National List trial of genetically modified herbicide tolerant winter oilseed rape at Caxton, UK, 1997

Variety type	Plot sample distance from GM source (m)	% Herbicide tolerance
		Glufosinate tolerance
Varietal association	6	2
Open pollinated	10	0.11
Restored hybrid	16	0.05
Varietal association	22	0.22
Varietal association	34	0.05
Varietal association	50	0.05
Varietal association	56	0.05
		Glyphosate tolerance
Varietal association	6	0.16
Open pollinated	10	0.05-0.33
Varietal association	22	0.16
Open pollinated (cv. Apex)	36	0.16
Open pollinated	50	0.05
Varietal association	56	0.11

Table 41 Percentage glufosinate and glyphosate tolerance detected in seed samples from plots growing in a National List winter oilseed rape trial adjacent to a National List trial of genetically modified herbicide tolerant winter oilseed rape at Bridgets, UK, 1997

Variety type	Plot sample distance from GM source (m)	% Herbicide tolerance
		Glufosinate tolerance
Open pollinated	16	0.05
Restored hybrid	16	0.05
Varietal association	150	0.11
		Glyphosate tolerance
Open pollinated	16	0.05-0.44
Restored hybrid	16	0.16
Varietal association	28	0.05
Varietal association	150	0.22

Table 42 Percentage glufosinate and glyphosate tolerance detected in seed samples from plots growing in a National List winter oilseed rape trial adjacent to a National List trial of genetically modified herbicide tolerant winter oilseed rape at Bridgets, UK, 1998

Variety type	Plot sample distance from GM source (m)	% Herbicide tolerance
		Glufosinate tolerance
Varietal association	12	0.03-0.06
Open pollinated	12	0.03
Varietal association	24	0.03-0.09
Varietal association	36	0.03
Open pollinated	36	0.03
Varietal association	48	0.03
Varietal association	60	0.03
Open pollinated	72	0.03
		Glyphosate tolerance
Open pollinated	12	0.03
Varietal association	12	0.03-0.31
Varietal association	24	0.03-0.09
Varietal association	36	0.03
Open pollinated	36	0.03
Varietal association	48	0.03
Varietal association	60	0.03
Open pollinated	72	0.03

APPENDIX 3.

Table 43 Weather data from the NIAB Meterological station during the main oilseed rape flowering period of experiment - Outcrossing between field scale areas of herbicide tolerant and other winter oilseed rape cultivars

Month	Date	Cloud	Wdir	WSpd	Dry	Wet	Max	Min	Sun
April	9	8	200	5	13.2	11.8	15.7	10.5	2.9
	10	8	230	9	12.2	11	15.2	7.2	3.8
	11	6	270	9	9.4	7	13.1	3.7	8.5
	12	7	250	13	8.9	7.3	12.7	6	4
	13	7	270	13	7.9	5.4	8.7	2.8	6.2
	14	2	340	5	4.9	2.9	8.7	-2.2	8.1
	15	3	250	9	5.4	3.4	10.1	-1.2	5.2
	16	6	290	5	7.7	5.6	11.2	0	6.6
	17	3	360	5	7.8	6.1	11.1	-1	4.9
	18	8	20	2	4.8	4.4	10.6	2.9	4
	19	7	70	5	7.4	5.9	10.6	3.2	5.5
	20	8	110	13	8.2	7.2	13.3	4.4	0
	21	6	200	19	13.3	11.1	14.6	7.6	3.4
	22	7	230	19	12.3	10.5	15.4	8.8	7
	23	8	110	9	11.9	10.3	12.9	5.7	0.1
	24	8	320	5	10.7	8.9	14.6	6	1
	25	3	140	9	12.9	10	16.2	5.5	6.8
	26	7	50	9	13.6	12.2	18	8.3	2
	27	3	50	9	14.2	12.6	17.8	8.6	10.9
	28	1	50	13	14.5	12.4	17.1	4.9	11.8
	29	5	50	9	11.2	9.1	15.9	6.2	9
	30	1	50	5	12.6	8.7	18.7	1.7	13.1
May	1	5	200	2	13.7	11.5	17	5.5	2
	2	8	50	2	10.9	10	17.9	6.5	3.2
	3	7	160	5	15.2	13.5	20.4	7.5	5.9
	4	8	70	5	10.7	9.3	16.3	6.8	2.7
	5	7	70	9	13	11.9	17.4	6.2	1.6
	6	8	200	5	14.4	13.1	18.1	9.4	1.5
	7	6	110	9	17.2	16.2	19.6	5.2	2.8
	8	7	230	13	14.3	13.5	17	7.6	5.9
	9	8	230	9	15.3	15	20.4	11.5	6.1
	10	7	230	13	15.7	15.4	17.9	11.8	9.1
	11	7	200	9	15.9	15.6	18.1	9.1	4.5
	12	7	200	13	15.3	15	18.1	9.5	5.6
	13	7	230	19	13.5	13.2	16.5	8.9	6
	14	7	230	5	13.3	12.9	16.7	9.1	4.1
	15	7	90	5	13	12	15.5	8.1	1.7
	16	3	90	5	13.1	12.3	15.3	4.2	6.1
	17	8	200	19	11	10.7	15.3	5.5	7.8
	18	7	70	13	12.6	12.3	16.7	7.8	8.6
	19	5	50	5	16.6	15.1	21.9	7.4	7.3
	20	4	340	5	15.4	13.6	21.1	9.2	4.7
	21	6	250	13	17.9	17.6	18.1	8	10.6
	22	6	250	9	12.2	11.9	16.8	7.4	4.3
	23	8	270	13	15	14.2	19.5	9.5	3.2
	24	8	200	9	13.3	13.1	19.4	11.7	12.7
	25	6	250	5	13.9	13.6	17.1	7.6	4.8

Table 44 Weather data from the NIAB Meterological station during the main oilseed rape flowering period of field experiment - Long distance cross pollination from an 11.5 hectare area of herbicide tolerant oilseed rape

Month	Date	Cloud	Wdir	WSpd	Dry	Wet	Max	Min	Sun
April	27	3	50	9	14.2	12.6	17.8	8.6	10.9
	28	1	50	13	14.5	12.4	17.1	4.9	11.8
	29	5	50	9	11.2	9.1	15.9	6.2	9
	30	1	50	5	12.6	8.7	18.7	1.7	13.1
May	1	5	200	2	13.7	11.5	17	5.5	2
	2	8	50	2	10.9	10	17.9	6.5	3.2
	3	7	160	5	15.2	13.5	20.4	7.5	5.9
	4	8	70	5	10.7	9.3	16.3	6.8	2.7
	5	7	70	9	13	11.9	17.4	6.2	1.6
	6	8	200	5	14.4	13.1	18.1	9.4	1.5
	7	6	110	9	17.2	16.2	19.6	5.2	2.8
	8	7	230	13	14.3	13.5	17	7.6	5.9
	9	8	230	9	15.3	15	20.4	11.5	6.1
	10	7	230	13	15.7	15.4	17.9	11.8	9.1
	11	7	200	9	15.9	15.6	18.1	9.1	4.5
	12	7	200	13	15.3	15	18.1	9.5	5.6
	13	7	230	19	13.5	13.2	16.5	8.9	6
	14	7	230	5	13.3	12.9	16.7	9.1	4.1
	15	7	90	5	13	12	15.5	8.1	1.7
	16	3	90	5	13.1	12.3	15.3	4.2	6.1
	17	8	200	19	11	10.7	15.3	5.5	7.8
	18	7	70	13	12.6	12.3	16.7	7.8	8.6
	19	5	50	5	16.6	15.1	21.9	7.4	7.3
	20	4	340	5	15.4	13.6	21.1	9.2	4.7
	21	6	250	13	17.9	17.6	18.1	8	10.6
	22	6	250	9	12.2	11.9	16.8	7.4	4.3
	23	8	270	13	15	14.2	19.5	9.5	3.2
	24	8	200	9	13.3	13.1	19.4	11.7	12.7
	25	6	250	5	13.9	13.6	17.1	7.6	4.8

APPENDIX 4.

Table 45. Pre herbicide weed counts 26.10.98 - Mean weed numbers/sub-plot/m2 in treated plots

									Species code						Total	Plot	Treat
Treatment	Replicate	Plot	Sub-plot	TRIAE	ALOMY	GALAP	ANGAR	VICFA	SONsp.	MATsp.	SENVU	STEME	CAPBP	CIRAV		mean	mear
CONV	1	1	1	16.8	4.8	2.4	1.8	0.2	0.6	0	0	0	0	0	27		
			2	14.2	0	0	12.2	0.2	0.2	0	0.2	0	0	0	27		
			3	9.6	0.2	0.4	7.8	0	0.4	0	0	0	0	0	18		
			4	14.2	0.4	0.2	5.6	0	0	0	0	0	0	0	20	23	
	2	8	1	10	6.2	0.4	2.6	0.4	0	2.4	0	0	0	0	22		
			2	16.2	7.2	0	1.4	0	0.4	0.6	0	0	0	0	26		
			3	12.8	4.8	0.2	4	0	0	0.8	0	0	0	0.4	23		
			4	10	3.2	0	0.8	0	0.4	0.2	0	0	0	0	15	21	22
LL1	1	2	1	17	0.6	0	1.6	0.6	0.8	0	0	0	0	0	21		
			2	12.6	2.4	1	2.2	2.6	0.2	0	0	0	0	0	21		
			3	21.4	1	1.2	3	0	0.4	0	0	0	0	0	27		
			4	15.8	0.4	1.6	4.8	0.4	0	0	0	0	0.2	0	23	23	
	2	7	1	20.8	11.6	0.6	1.2	0.4	0.2	1.2	0	0	0	0	36		
			2	10.2	4.6	0	0.8	0.2	0	0.8	0	0	0.2	0	17		
			3	10.4	12	0	0.8	0	0	0.8	0	0	0.8	0	25		
			4	21.4	2.4	0.2	0.8	0.4	0	0	0	0	0	0	25	26	24
RR1	1	3	1	21.2	2.8	0.4	1	0	0.4	0.2	0	0	0	0	26		
			2	17.4	2	0.6	0.4	0.4	0	0	0	0.2	0.4	0	21		
			3	21.6	0.8	1.8	5.2	0.8	1	0	0	0	0.2	0	31		
			4	15.6	1.2	0.2	2.8	0.2	0.6	0	0	0	0.2	0	21	25	
	2	5	1	26.2	5.6	4.8	0.4	0.2	0	1.2	0	0	0.2	0	39		
			2	13.4	6.4	0.2	0	0.2	0	0	0	0	0	0	20		
			3	15.6	4.6	1.4	0.4	0	0	0	0	0	0	0.6	23		
			4	14.8	1	0.8	0.4	0.4	0	0	0	0	0	0	17	25	25
IMI	1	4	1	9.8	1.2	1	2.8	0	0	0	0	0	0.2	0	15		
			2	20	2.2	0.2	3	0.2	0	0	0	0.2	0	0.6	26		
			3	28.8	1.8	0.6	0.6	0	0.6	0	0.2	0.2	0	0	33		
			4	7.2	1	0.4	0.8	0	0	0	0	0.2	0	0	10	21	
	2	6	1	12.2	3	0	0.8	0.2	0.2	0.4	0	0	0	0	17		
			2	15.4	3.2	0.4	1.4	0.4	0.2	0.2	0	0	0	0	21		
			3	10.8	0.6	0.8	3.2	0	2	0	0	0	0	0	17		
			4	12	2	0.2	2.6	0	0.4	0	0	0	0	0	17	18	20

Table 46.Weed counts (pre herbicide timing) Autumn 29.10.98 - Mean weed numbers/sub-plot/m2 in untreated plots

				Species code										
Treatment	Replicate	Plot	Sub-plot	TRIAE	ALOMY	GALAP	ANDAR	VICFA	SONsp.	MATsp.	SENVU	STEME	CAPBP	CIRAV
CONV	1	1	1	8	1.6	0	0	0	0	0	0	0	0	0
			2	8	0	0	0.8	0	0	0	0	0	0	0
			3	6.4	0	0	2.4	0.8	0	0	0	0	0	0
			4	8.8	0	0	1.6	0.8	0	0	0	0	0	0
	2	8	1	12.8	5.6	0	4.8	0	0	0	0	0	0	0
			2	3.2	2.4	0	0.8	0	0	0	0	0	0	0
			3	8.8	0.8	0	4	0	0	0.8	0	0	0	0
			4	4	3.2	0	0	0	0	0	0	0	0	0
LL1	1	2	1	12.8	2.4	0	0	0.8	0	0	0	0	0	0
			2	22.4	1.6	0	0	0.8	0	0	0	0	0	0
			3	10.4	0.8	0	0.8	0.8	1.6	0	0	0	0	0
			4	11.2	0.8	0	1.6	0	0	0	0	0	0	0
	2	7	1	18.4	8	0.8	0.8	0	0	1.6	0	0	3.2	0
			2	10.4	12	0	0.8	0	0	0.8	0	0	0.8	0
			3	6.4	1.6	0.8	1.6	0	0	0	0	0	0	0
			4	18.4	3.2	0	0.8	0	0	0	0	0	0	0
RR1	1	3	1	12	0.8	0	0	0	0	0	0	0	0	0
			2	9.6	2.4	0.8	0	0	0	0	0	0	0	0
			3	14.4	4	0	0.8	0	0	0	0	0	0	0
			4	13.6	2.4	0	0	0	0.8	0	0	0	0	0
	2	5	1	15.2	4.8	0	0.8	0	0.8	0	0	0	0	0
			2	4	1.6	0	0.8	0	0	0	0	0	0	0
			3	8	0.8	1.6	2.4	0	0	0	0	0	0	1.6
			4	7.2	0.8	0	0	0	0	0	0	0	0	0
IMI	1	4	1	15.2	0	0.8	0.8	0	0	0	0	0	0	0
			2	11.2	0	0	0.8	0.8	0	0	0	0	0	0
			3	9.6	0.8	0	0	0	0.8	0	0	0	0	0
			4	0	0	0	0	0	0	0	0	0	0	0
	2	6	1	10.4	0	0	0	0	0	0.8	0	0	0.8	0
			2	7.2	0	0	0.8	0	0	0	0	0	0	0
			3	7.2	0	0	0	0	0	5.6	0	0	0	0
			4	12	1.6	0	1.6	0	0	0	0	0	0	0

Table 47. Post herbicide weed counts Spring 10.03.99 - Mean numbers/sub-plot/m2 in treated plots

Treatment	Rep	Plot	Sub-plot	TRIAE	ALOMY	GALAP	ANGAR	POLAV	SINAR	VERHE	ALLsp.	MYOAR	VICFA	SONsp.	MATsp.	SENVU	STEME	APHAR	CAPBP	Total	Plot mean	T m
CONV	1	1	1	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	11		
			2	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	15		
			3	0	0	0	13	0	0	0	0	0	1	1	0	0	0	0	0	15		
			4	0	0	0	13	0	0	0	0	0	1	0	0	0	0	0	0	14	14	
	2	8	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3		
			2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2		
			3	0	0	0	2	0	0	0	0	0	0	1	1	0	0	0	0	5		
			4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	
LL1	1	2	1	2	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	6		
			2	4	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	6		
			3	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3		
			4	3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	5	5	
	2	7	1	4	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10		
			2	3	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	7		
			3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2		
			4	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	5	
RR1	1	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
			2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1		
			3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2		
			4	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	3	1	
	2	5	1	0	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0	5		
			2	0	1	1	4	0	0	0	0	0	0	0	0	0	0	0	0	5		
			3	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	2		
			4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	
IMI	1	4	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2		
			2	0	0	0	1	0	0	3	0	0	0	0	0	0	0	0	0	4		
			3	0	1	1	0	0	0	3	0	0	1	0	0	0	0	0	0	6		
			4	ABANDONED																Abandoned	4	
	2	6	1	0	3	1	1	0	0	0	0	0	0	0	0	0	0	0	1	6		
				0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	2		
				0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	4		
				0	0	0	1	0	2	0	0	0	1	0	0	0	0	0	0	4	4	

Table 48. Weed counts (post herbicide timing) Spring 10.03.99 - Mean weed numbers/sub-plot/m2 in untreated plots

				Species code														
Treatment	Rep	Plot	Sub-plot	TRIAE	ALOMY	GALAP	ANGAR	POLAV	SINAR	VERHE	ALLsp.	MYOAR	VICFA	SONsp.	MATsp.	SENVU	STEME	APHAR	CAPBP
CONV	1	1	1	8	7	0	3	0	0	0	0	0	0	0	0	0	0	0	0
			2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			3	8	0	1	7	0	0	0	0	0	0	0	0	0	0	0	0
			4	8	1	0	11	0	0	0	0	0	0	0	0	0	0	0	0
	2	8	1	11	8	0	0	0	0	0	0	0	0	0	0	0	0	0	1
			2	5	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			3	11	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0
			4	8	1	0	3	0	0	0	0	0	0	0	0	0	0	0	1
LL1	1	2	1	21	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			2	28	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0
			3	21	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			4	16	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	7	1	19	36	3	0	0	0	0	0	0	0	0	0	0	0	0	0
			2	12	27	0	3	0	0	0	0	0	0	0	0	0	0	0	0
			3	9	5	0	3	0	0	0	0	0	0	0	0	0	0	0	0
			4	16	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RR1	1	3	1	11	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			2	7	7	1	0	0	0	4	0	0	0	0	0	0	0	0	0
			3	4	5	0	1	0	0	0	0	0	0	1	0	0	0	0	0
			4	4	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	5	1	13	17	1	3	0	0	0	0	0	0	0	0	0	0	0	0
			2	5	3	1	0	0	1	0	0	0	0	0	0	0	0	0	1
			3	11	0	1	1	0	0	0	0	0	0	4	0	0	0	0	5
			4	9	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IMI	1	4	1	8	1	4	0	0	0	0	0	0	0	0	0	5	0	0	0
			2	12	0	7	0	0	0	7	0	0	0	0	0	1	0	0	0
			3	ABANDONED															
			4	ABANDONED															
	2	6	1	19	0	0	0	0	0	1	0	0	0	0	0	1	0	0	3
			2	7	1	0	1	0	0	1	0	0	0	0	0	0	0	0	1
			3	7	0	0	1	0	0	0	0	0	0	0	5	0	0	0	0
			4	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Table 49. Post herbicide weed biomass Summer 24.06.99 - Mean weed dry wieght per species/sub-plot in treated plots (g/m2) contd overleaf

Treatment	Rotation	Replicate	Plot	Sub plot	ALOMY	ANGAR	KICEL	PICEC	Dry wt (g/m2) SONsp.	TRIAE	VICFA	VERHE	ODOVE	CHYSE	POLAV	EPIAD	KICSP	GALAP	MATsp.
CONV	1	1	1	1	0.0	10.6	0.1	10.7	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0	5.2	0.2
				2	0.0	18.3	0.9	0.4	0.1	0.0	87.5	0.6	0.0	1.3	0.0	0.1	0.0	0.4	0.0
				3	0.0	38.7	2.8	0.2	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.2	0.0	0.0
				4	0.0	36.0	1.3	0.9	0.2	0.0	4.8	0.7	0.0	0.3	0.0	0.0	0.0	7.0	0.0
		2	8	1	0.0	8.5	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
				2	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.8	2.9
				3	0.0	2.3	1.6	0.2	7.7	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
				4	0.0	2.0	0.0	2.5	0.0	0.0	107.1	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0
LL1		1	2	1	32.6	0.3	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
				2	6.3	1.3	0.3	0.0	0.0	0.4	0.0	0.0	0.1	0.1	0.0	0.0	0.1	10.6	0.0
				3	4.0	1.3	0.0	0.0	2.4	1.7	0.0	0.0	0.0	0.7	0.0	0.1	0.1	0.0	0.0
				4	20.2	1.0	0.0	1.5	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
		2	7	1	32.9	0.0	0.1	0.1	0.0	29.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5
				2	56.7	0.0	0.1	0.0	0.0	29.9	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
				3	0.3	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0
				4	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0
RR1		1	3	1	1.2	1.2	0.1	0.0	0.0	2.3	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
				2	2.8	0.3	0.0	0.0	0.0	1.4	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
				3	1.1	0.2	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
				4	0.1	2.2	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.8	0.0	0.0
			5	1	17.2	0.6	0.0	0.4	0.0	0.0	0.0	0.0	0.4	0.1	0.0	1.7	0.0	0.0	0.3
				2	3.8	0.2	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
				3	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.6	0.0	0.0	0.0
				4	0.0	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
IMI		1	4	1	34.6	3.3	0.0	10.4	0.0	0.0	18.1	0.3	0.0	0.2	0.0	0.0	0.1	1.7	0.3
				2	4.8	0.2	0.0	15.1	0.0	0.0	0.0	10.0	0.0	0.2	0.1	0.0	0.0	2.4	0.1
				3	23.6	22.2	0.8	3.5	4.5	0.5	52.9	16.2	0.0	0.1	0.0	0.8	0.0	5.4	1.7
				4	missing sub-plot														
		2	6	1	38.2	0.0	0.0	11.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.9	0.0
				2	25.8	0.0	0.0	16.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
				3	5.0	0.2	1.8	8.5	0.0	0.0	0.0	0.0	1.2	0.0	0.0	0.0	0.0	0.1	0.0
				4	2.9	0.8	0.1	0.0	2.6	0.1	38.8	0.2	0.8	0.0	0.0	0.0	0.1	0.0	0.0

Table 49. contd/ Post herbicide weed biomass Summer 24.06.99 - Mean weed dry wieght per species/sub-plot in treated plots (g/m2)

Treatment	Rotation	Replicate	Plot	Sub plot	VIOAR	CAPBP	EPHEX	LACSE	Dry wt (g/m2) EPHPL	SINAR	SSYOF	CIRVU	STEME	COPSQ	ATXPA	CONAR	AETCY	SONOL	CHEAL	Sub plot Total	Plot Mean
CONV	1	1	1	1	0	0	0.035	9.1	0	0	0	0	0	0	0	0.21	0	0	0	36.945	
				2	0	0	0.05	0	0	0	0	0	0	0	0	0	0	1.965	0	111.425	
				3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	42.71	
				4	0	0	0.165	5.45	0	9.4	0	0	0	0	0	0	0	0	0	66.13	64.30
		2	8	1	0	0.915	0	0	0	0	0.064	0	0	0	0	0	0.24	0	0	10.534	
				2	0	1.935	0	0	0	0	0.395	0	0.29	0	0	0	4.45	0	0	11.365	
				3	0	0.4105	0	0	0	0	0	0	0	0	0	0	0	0	0	12.4355	
				4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	113.65	36.996
LL1		1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33.94	
				2	0	0	0	0	0	0	0	0	0	0	0.045	0	0	0	0	19.355	
				3	0	0	0.145	0	0	0.07	0	0	0	0	0.05	0	0	0	0	10.58	
				4	0	0	0	0	0	0	0	0	0	0	0.03	0	0	0	0	23.065	21.7
		2	7	1	0.57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	64.085	
				2	0	0	0	0	0	0	0	0	0	0	0	0	0.31	0	0	87.06	
				3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.13	
				4	0	0	0	0	0	0	0	0	0	0.21	0	0	0	0	0	2.625	38.7
RR1		1	3	1	0	0	0	0	0.275	0	0	0	0	0	0	0	0	0	0	5.125	
				2	0	0	0.005	0	0	0	0	0	0	0	0	0	0	0	0	5.175	
				3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.795	
				4	0	0	0.085	0	0	0	0	0	0	0	0.335	0	0	0	0	4.715	4.20
			5	1	0	0	0	0	0	0	0	0	0	0	0	0	0.08	0	0	20.83	
				2	0	0	0.06	0	0	0	0	0	0	0	0.255	0	0.255	0	0	4.915	
				3	0	0	0	0	0	0	0	0	0	0.02	0.155	0	0	0	0	1.37	
				4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.56	6.918
IMI		1	4	1	0	0	0.03	0	0.63	0.235	0	0	0	0	0	0	0	0	0	69.92	
				2	0	0	0.13	0	0	0	0	0	0	0	0.075	0	0	0	0	33.18	
				3	0	0	0.225	0	9.6	0	0.23	15.25	0	0	0	0	0	0	0	157.445	
				4	Abando															0	65.136
		2	6	1	0	0.125	0	0	0	0	0	0	0	0	0	0	0	0	0	50.595	
				2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.285	42.925	
				3	0	0	0.05	0	0	0	0	0	0	0	0	0	0	0	0	16.825	
				4	0	0	0	0	0	0	0	0	0	0	0.04	0	0	0	0	46.395	39.1

Table 50. Key for weed species abbreviations used in years 1 and 2 of experiment 3.4

Code	Scientific name	Common name
AETCY	*Aethusa cynapium*	Fools parsley
ALLsp.	*Allium sp.*	Onion
ALOMY	*Alopecurus myosuroides*	Black grass
ANGAR	*Anagallis arvensis*	Scarlet pimpernel
APHAR	*Aphanes arvensis*	Parsley piert
ATXPA	*Atriplex patula*	Orache
BRANA	*Brassica napus*	Oilseed rape
CAPBP	*Capsella bursa-pastoris*	Shepherds purse
CHEAL	*Chenopodium album*	Fat hen
CHYSE	*Chrysanthemum segetum*	Corn marigold
CIRVU	*Cirsium vulare*	Spear thistle
CONAR	*Convulvulus arvensis*	Field bindweed
COPSQ	*Coronopus squamatus*	Swine cress
EPHEX	*Euphorbia exigua*	Dwarf spurge
EPHPL	*Euphorbia platyphyllos*	Broad spurge
EPIAD	*Epilobium adenocaulon*	American willowherb
GALAP	*Galium aparine*	Cleavers
KICEL	*Kickia elatine*	Sharp leafed fluellen
KICSP	*Kickia spuria*	Round leafed fluellen
LACSE	*Lactuca serriola*	Prickly lettuce
MATsp.	*Matricaria sp.*	Mayweed
MYOAR	*Myosotis arvensis*	Common Forget-me-not
ODOVE	*Odonites verna*	Red bartsia
PICEC	*Picris echoiodes*	Bristly ox tongue
POAAN	*Poa annua*	Meadow grass
POLAV	*Polygonum aviculare*	Knotgrass
RANRE	*Ranunculus repens*	Creeping buttercup
SENVU	*Senecio vulgaris*	Groundsel
SINAR	*Sinapis arvensis*	Charlock
SONOL	*Sonchus oleraceus*	Smooth sowthistle
SONsp.	*Sonchus sp.*	Sow thistle
SSYOF	*Sysimbrium officionale*	Hedge mustard
STEME	*Stellaria media*	Chickweed
TRIAE	*Triticum aestivum*	Wheat volunteer
VERHE	*Veronica hederifolia*	Ivy leaved speedwell
VICFA	*Vicia faba*	Volunteer beans
VIOAR	*Veronica arvensis*	Field pansy

APPENDIX 5.

Statistical testing of pre and post herbicide counts and biomass assessmentsYear 1- outputs from ANOVA using GENSTAT release 3.2

Abbreviations used in tables

d.f. - degrees of freedom
s.s. - sum of squares
m.s. - mean squares
v.r. - variance ratio
Fpr - F-probability

CONV – conventional
IMI – Imazamox
LL1 – Glufosinate
RR1 - Glyphosate

PRE HERBICIDE Treated plots

Weed species: *Triticum aestivum*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	30.03	30.03	1.24	
Treatments	3	121.92	40.64	1.67	0.196 NS
Residual	27	655.23	24.27		
Total	31	807.19			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean weeds/m^2	12.98	18.23	14.53	16.20
L.s.d.	5.054			

Weed species: *Alopecurus myosuroides*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	96.605	96.605	16.30	
Treatments	3	25.405	8.468	1.43	0.256 NS
Residual	27	160.025	5.927		
Total	31	282.035			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean weeds/m^2	3.35	3.05	1.87	4.37
L.s.d.	2.498			

Weed species: *Galium aparine*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	0.1250	0.1250	0.14	
Treatments	3	3.7650	1.2550	1.39	0.268NS
Residual	27	24.4250	0.9046		
Total	31	28.3150			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean weeds/m^2	0.45	1.27	0.45	0.57
L.s.d.	0.976			

Weed species: *Anagallis arvensis*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	36.147	36.147	8.50	
Treatments	3	49.409	16.470	3.87	0.020*
Residual	27	114.804	4.252		
Total	31	200.361			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean weeds/m^2	4.53	1.32	1.90	1.90
L.s.d.	2.115			

PRE HERBICIDE Un-treated plots (weed counts recorded at the pre herbicide timing)

Weed species: *Triticum aestivum*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	12.50	12.50	0.67	
Treatments	3	172.38	57.46	3.07	0.044*
Residual	27	504.70	18.69		
Total	31	689.58			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean weeds/m^2	7.50	10.50	9.10	13.80
L.s.d.	4.436			

Weed species: *Alopecurus myosuroides*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	25.920	25.920	5.18	
Treatments	3	50.080	16.693	3.33	0.034*
Residual	27	135.200	5.007		
Total	31	211.200			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean weeds/m^2	1.70	2.20	0.30	3.80
L.s.d.	2.296			

Weed species: *Galium aparine*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	0.0800	0.0800	0.55	
Treatments	3	0.4000	0.1333	0.92	0.445 NS
Residual	27	3.9200	0.1452		
Total	31	4.4000			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean weeds/m^2	0.000	0.300	0.100	0.200
L.s.d.	0.3909			

Weed species: *Anagallis arvensis*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	3.380	3.380	3.13	
Treatments	3	8.540	2.847	2.63	0.070 NS
Residual	27	29.180	1.081		
Total	31	41.100			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean weeds/m^2	1.80	0.60	0.50	0.80
L.s.d.	1.067			

POST HERBICIDE Treated plots

Weed species: *Triticum aestivum*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	0.1409	0.1409	0.38	
Treatments	3	39.0416	13.0139	34.41	<0.001***
Residual	26(1)	9.5566	0.3676		
Total	30(1)	48.4301			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean weeds/m^2	0.00	0.04	0.06	2.58
L.s.d.	0.623			

Weed species: *Alopecurus myosuroides*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	0.873	0.873	0.71	
Treatments	3	19.469	6.490	5.31	0.005**
Residual	26(1)	31.748	1.221		
Total	30(1)	52.086			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean weeds/m^2	0.00	0.21	0.88	2.00
L.s.d.	0.553			

Weed species: *Galium aparine*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	0.3734	0.3734	1.18	
Treatments	3	1.1784	0.3928	1.25	0.313 NS
Residual	26(1)	8.1955	0.3152		
Total	30(1)	9.7204			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean weeds/m^2	0.083	0.583	0.318	0.500
L.s.d.	0.5770			

Weed species: *Anagallis arvensis*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	59.150	59.150	6.04	
Treatments	3	222.007	74.002	7.56	<0.001***
Residual	26(1)	254.489	9.788		
Total	30(1)	535.642			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean weeds/m^2	6.63	1.00	0.81	0.00
L.s.d.	3.215			

POST HERBICIDE Untreated plots (Weed counts made at the post herbicide timing)

Weed species: *Triticum aestivum*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	1.24	1.24	0.06	
Treatments	3	556.57	185.52	9.04	<0.001***
Residual	25(2)	513.01	20.52		
Total	29(2)	1070.52			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean weeds/m^2	7.33	8.00	10.29	17.83
L.s.d.	4.665			

Weed species: *Alopecurus myosuroides*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	0.117	0.117	0.02	
Treatments	3	75.235	25.078	3.75	0.024*
Residual	25(2)	167.152	6.686		
Total	29(2)	239.644			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean weeds/m^2	4.17	0.67	0.42	0.83
L.s.d.	2.663			

Weed species: *Galium aparine*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	3.884	3.884	1.97	
Treatments	3	14.620	4.873	2.47	0.085 NS
Residual	25(2)	49.253	1.970		
Total	29(2)	63.111			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean weeds/m^2	0.17	0.67	1.89	0.33
L.s.d.	1.445			

Weed species: *Anagallis arvensis*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	0.117	0.117	0.02	
Treatments	3	75.235	25.078	3.75	0.024*
Residual	25(2)	167.152	6.686		
Total	29(2)	239.644			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean weeds/m^2	4.17	0.67	0.42	0.83
L.s.d.	2.663			

YEAR ONE POST HERBICIDE - WEED BIOMASS ASSESSMENT (treated plots)

Weed species: *Tritcum aestivum*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	95.65	95.65	2.03	
Treatments	3	353.82	117.94	2.51	0.081 NS
Residual	26(1)	1223.01	47.04		
Total	30(1)	1656.67			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean g/m^2	0.0	0.5	-0.2*	7.8
L.s.d.	7.05			

*missing value estimate

Weed species: *Alopecurus myosuroides*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	35.2	35.2	0.21	
Treatments	3	2484.1	828.0	4.87	0.008**
Residual	26(1)	4424.8	170.2		
Total	30(1)	6883.2			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean g/m^2	0.0	3.3	19.1	19.1
L.s.d.	13.41			

Weed species: *Galium aparine*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	30.901	30.901	5.65	
Treatments	3	17.320	5.773	1.06	0.384 NS
Residual	26(1)	142.090	5.465		
Total	30(1)	188.276			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean g/m^2	1.92	0.00	1.64	1.32
L.s.d.	2.403			

Weed species: *Anagallis arvensis*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	524.31	524.31	8.90	
Treatments	3	1038.00	346.00	5.88	0.003**
Residual	26(1)	1530.91	58.88		
Total	30(1)	3081.27			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean g/m^2	14.5	0.6	4.4	0.6
L.s.d.	7.89			

Weed species: *Picris echioides*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	4.27	4.27	0.37	
Treatments	3	462.10	154.03	13.25	<0.001***
Residual	26(1)	302.20	11.62		
Total	30(1)	720.69			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean g/m^2	2.02	0.06	9.36	0.20
L.s.d.	3.504			

Weed species: *Vicia faba*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	36.7	36.7	0.06	
Treatments	3	3648.2	1216.1	1.86	0.162 NS
Residual	26(1)	17034.6	655.2		
Total	30(1)	20672.9			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean g/m^2	24.9	0.0	15.8	0.0
L.s.d.	26.31			

Weed species: *Veronica hederifolia*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	33.725	33.725	3.82	
Treatments	3	90.232	30.077	3.41	0.032*
Residual	26(1)	229.256	8.818		
Total	30(1)	337.243			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean g/m^2	0.16	0.12	3.97	0.00
L.s.d.	3.052			

Weed species: *Lactuca serriola*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	7.115	7.115	2.34	
Treatment	3	19.384	6.461	2.12	0.122 NS
Residual	26(1)	79.189	3.046		
Total	30(1)	105.683			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean g/m^2	1.82	0.00	0.07	0.00
L.s.d.	1.794			

Weed species: *Cirsium vulgare*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	10.209	10.209	1.40	
Treatments	3	30.626	10.209	1.40	0.265 NS
Residual	26(1)	189.495	7.288		
Total	30(1)	225.060			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean g/m^2	0.00	0.00	2.26	0.00
L.s.d.	2.775			

Weed species: *Sinapis arvensis*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	3.187	3.187	1.12	
Treatments	3	7.908	2.636	0.92	0.444 NS
Residual	26(1)	74.293	2.857		
Total	30(1)	85.382			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean g/m^2	1.18	0.00	0.08	0.01
L.s.d.	1.737			

Weed species: *Euphorbia platyphyllos*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	4.812	4.812	1.71	
Treatments	3	13.603	4.534	1.61	0.210 NS
Residual	26(1)	73.032	2.809		
Total	30(1)	89.073			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean g/m^2	0.00	0.03	1.52	0.00
L.s.d.	1.723			

APPENDIX 6.

Table 51.Pre herbicide weed counts 29.11.99 - Mean weed numbers per species/sub-plot in treated plots

Treatment	Rep	Plot	Sub-plot	Species (plants/m^2)					Total no. weeds present	Weed no. present per plot
				BRANA	GALAP	VICFA	CIRsp.	ANGAR		
CONV	1	1	1	0.00	0.00	0.05	0.00	0.00	0.1	
			2	0.00	0.05	0.00	0.10	0.00	0.2	
			3	0.10	0.05	0.45	0.05	0.00	0.7	
			4	0.00	0.00	0.00	0.00	0.00	0.0	0.2
	2	8	1	0.10	0.00	0.10	0.00	0.00	0.2	
			2	0.10	0.00	0.05	0.05	0.00	0.2	
			3	0.05	0.00	0.00	0.00	0.00	0.1	
			4	0.00	0.00	0.15	0.00	0.00	0.2	0.2
IMI	1	4	1	0.05	0.15	0.05	0.00	0.00	0.3	
			2	0.05	0.00	0.00	0.00	0.05	0.1	
			3	0.05	0.20	0.10	0.00	0.00	0.4	
			4	0.00	0.20	0.00	0.00	0.00	0.2	0.2
	2	6	1	0.10	0.05	0.25	0.00	0.15	0.6	
			2	0.15	0.25	0.00	0.05	0.00	0.5	
			3	0.05	0.20	0.00	0.50	0.00	0.8	
			4	0.20	0.00	0.15	0.00	0.00	0.4	0.5
LL	1	2	1	0.05	0.10	0.00	0.00	0.00	0.2	
			2	0.10	0.15	0.05	0.15	0.00	0.5	
			3	0.20	0.20	0.00	0.00	0.00	0.4	
			4	0.25	0.10	0.00	0.05	0.05	0.5	0.4
	2	7	1	0.00	0.00	0.05	0.00	0.00	0.1	
			2	0.05	0.00	0.00	0.00	0.00	0.1	
			3	0.20	0.00	0.00	0.05	0.00	0.3	
			4	0.20	0.00	0.00	0.00	0.00	0.2	0.1
RR	1	3	1	0.00	0.00	0.05	0.00	0.00	0.1	
			2	0.00	0.05	0.05	0.00	0.00	0.1	
			3	0.00	0.00	0.00	0.05	0.00	0.1	
			4	0.05	0.00	0.05	0.00	0.00	0.1	0.1
	2	5	1	0.00	0.90	0.00	0.00	0.00	0.9	
			2	0.05	0.00	0.05	0.00	0.00	0.1	
			3	0.00	0.05	0.00	0.10	0.00	0.2	
			4	0.00	0.00	0.00	0.05	0.00	0.1	0.3

Table 52. Post herbicide weed counts Spring 1.03.00 - Mean weed numbers/sub-plot/m2 in treated plots

Treatment	Replicate	Plot	Sub-plot	Species (plants/m^2)									Total no.	Weed no.
				BRANA	GALAP	VICFA	CIRsp.	ANGAR	EPIAD	POLAV	LACSE	AETCY	weeds present	present per plot
CONV	1	1	1	0.00	0.05	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.1	
			2	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.1	
			3	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.2	
			4	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.1	0.1
	2	8	1	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.1	
			2	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.1	
			3	0.00	0.00	0.05	0.00	0.00	0.00	0.20	0.00	0.00	0.3	
			4	0.00	0.05	0.10	0.00	0.00	0.00	0.00	0.30	0.00	0.5	0.2
IMI	1	4	1	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.1	
			2	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.2	
			3	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.1	
			4	FLOODED	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.1
	2	6	1	0.00	0.20	0.05	0.00	0.00	0.15	0.00	0.00	0.00	0.4	
			2	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.45	0.00	0.5	
			3	0.05	0.00	0.10	0.35	0.00	0.00	0.00	0.00	0.00	0.5	
			4	0.00	0.00	0.20	0.00	0.00	0.35	0.00	0.15	0.00	0.7	0.5
LL	1	2	1	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.1	
			2	0.00	0.20	0.00	0.10	0.00	0.25	0.00	0.00	0.00	0.6	
			3	0.00	0.40	0.00	0.00	0.00	0.25	0.00	0.00	0.05	0.7	
			4	0.00	0.10	0.00	0.00	0.00	0.10	0.00	0.00	0.05	0.3	0.4
	2	7	1	0.00	0.05	0.10	0.00	0.00	0.05	0.00	0.00	0.00	0.2	
			2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0	
			3	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.1	
			4	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.2	0.1
RR	1	3	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0	
			2	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.1	
			3	0.00	0.15	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.3	
			4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.1
	2	5	1	0.00	1.30	0.00	0.00	0.00	0.05	0.00	0.00	0.00	1.4	
			2	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.1	
			3	0.00	0.05	0.00	0.15	0.00	0.10	0.00	0.00	0.00	0.3	
			4	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.1	0.5

Table 53. Post herbicide weed biomass Summer 24.06.00 - Mean weed dry weight per species/sub-plot in treated plots (g/m²)

Treatment	Rep	Plot	Sub-plot	Ave. dw/sub-plot (g/m²)													Weed biomass present per plot
				ALOMY	CIRsp	CHYSE	EPIAD	POAAN	ANGAR	VICFA	GALAP	CONAR	EPHEX	PICEC	RANRE	Total dw weeds present	
CONV	1	1	1		0	0.28	0	0	0	0	0	0	0	0	0	0.3	
			2	0.87	0	0	0	0	0	0	0	0	0	0	0	0.9	
			3	0	0.11	0	0	0	0.06	0.83	0	0	0	0	0	1.0	
			4	5.14	0	0.15	0	0	0	0	0	2.58	0	0	0	7.9	2.5
	2	8	1	24.04	0	0	0	0	0	0	0	0.26	0	0	0	24.3	
			2	0	0	0.5	0	0	0	0	0	0	0	0	0	0.5	
			3	0	0.34	0	0	0	0	0	0	0	0	0	0	0.3	
			4	0	0	0	0.34	0	0	0	0	0	0	0	0	0.3	6.4
IMI	1	4	1	15.63	0	0	0	0	0	0	0.64	0	0.01	0	0	16.3	
			2	0	0.01	0	1.95	0	0	0	0.03	0.75	0	0	0	2.7	
			3	75.31	0	0	0	0	0	0	0	0	0.69	3.49	0	79.5	
			4	0	0	0	0	0	0	0	0	0	0	0	0	0.0	24.6
	2	6	1	51.57	0	0.74	0	0	0	0	0	0.62	0	0	0	52.9	
			2	13.94	5.87	0	4.03	0	0	0	0.27	0	0	0	0	24.1	
			3	16.7	8.34	0	6.52	0	0	0	0	0	0	0	0	31.6	
			4	25.79	0	0	0	0	0	0	0.13	0	0	0	0	25.9	33.6
LL	1	2	1	5.88	0.52	0	0	0	0	0	0	0	0	0	0	6.4	
			2	0.87	0.77	0	0	0	0	0	2.81	0	0	0	0	4.5	
			3	0	2.04	0	0	0	0	0	2.6	0	0	1.93	0.24	6.8	
			4	5.14	0	0	0	0	0	0	0.5	0	0	0	0	5.6	5.8
	2	7	1	28.29	0	0	0	0	0	0	0	0.86	0	0	0	29.2	
			2	6.04	0	0	0	0	0	0	0	0	0	0	0	6.0	
			3	31.66	2.43	0	0	0.01	0	0	0	0	0	0	0	34.1	
			4	12.14	0	0	3.66	0	0	0	0	0	0	0	0	15.8	21.3
RR	1	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0.0	
			2	0.22	0	0	0	0	0	0	0	0	0	0	0	0.2	
			3	0	2.42	0	7.8	0	0	0	0	0	0	0	0	10.2	
			4	0	0	0	10.96	0	0	0	0	0	0	0	0	11.0	5.4
	2	5	1	0	0	0	9.26	0	0	0.5	0.42	0	0	0	0	10.2	
			2	0	0	2.27	0.75	0	0	0	0	0	0	0	0	3.0	
			3	0	16.28	0	0	0	0	0	2.17	0	0	0	0	18.5	
			4	0	0	0	0.11	0	0	0	0	0	0	0	0	0.1	7.9

APPENDIX 7.

Statistical testing of biomass assessment data from 1999-2000 Year 2 - output from ANOVA using GENSTAT 3.2

Abbreviations used in tables

d.f. - degrees of freedom
s.s. - sum of squares
m.s. - mean squares
v.r. - variance ratio
Fpr - F-probability
Lsd - least significant difference

CONV – conventional
IMI – Imazamox
LL1 – Glufosinate
RR1 - Glyphosate

POST HERBICIDE - WEED BIOMASS (treated piots)

Weed species: *Alopecurus myosuroides*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	315.0	315.0	1.37	
Treatment	3	2879.6	959.9	4.18	0.015*
Residual	26(1)	5974.3	229.8		
Total	30(1)	9079.8			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean g/m^2	3.8	0.0	24.9	11.3
L.s.d.	15.58			

Weed species: *Galium aparine*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	0.4028	0.4028	0.75	
Treatment	3	2.4856	0.8285	1.54	0.227NS
Residual	27	14.5413	0.5386		
Total	31	17.4297			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean g/m^2	0.00	0.32	0.13	0.32
L.s.d.	0.753			

Weed species: *Anagallis arvensis*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	0.00001125	0.00001125	1.00	
Treatments	3	0.0003375	0.0001125	1.00	0.408NS
Residual	27	0.0030375	0.0001125		
Total	31	0.0034875			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean g/m^2	0.0075	0.0000	0.0000	0.0000
L.s.d.	0.01088			

Weed species: *Cirsium sp.*

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	23.44	23.44	2.19	
Treatments	3	25.31	8.44	0.79	0.512NS
Residual	27	289.36	10.72		
Total	31	338.12			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean g/m^2	0.06	2.34	1.78	0.72
L.s.d.	1.157			

Weed species: Epilobium adenocaulon

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	0.490	0.490	0.06	
Treatments	3	61.122	20.374	2.53	0.078NS
Residual	27	217.382	8.051		
Total	31	278.994			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean g/m^2	0.04	3.61	1.56	0.46
L.s.d.	2.911			

Total weed biomass (all species pooled, treated plots)

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr
Replicates	1	477.6	477.6	2.09	
Treatments	3	2969.9	995.6	4.36	0.013*
Residual	27	6167.3	228.4		
Total	31	9631.8			

Table of means

Treatment	Conv.	RR1	IMI	LL1
Mean g/m^2	4.4	6.6	29.1	13.5
L.s.d.	15.51			