PROJECT REPORT No. 266

**DEVELOPING STRATEGIES FOR
REDUCING THE RISK FROM
HERBICIDE-RESISTANT
WILD-OATS (*Avena* spp.)**

DECEMBER 2001

Price £8.00

DEVELOPING STRATEGIES FOR REDUCING THE RISK FROM HERBICIDE-RESISTANT WILD-OATS (*Avena* spp.)

by

S R MOSS[1], S E HUGHES[1], A M BLAIR[2] AND J H CLARKE[2]

[1] IACR-Rothamsted, Harpenden, Hertfordshire AL5 2JQ

[2] ADAS Boxworth, Boxworth, Cambridgeshire CB3 8NN

CONTENTS **Page Number**

ABSTRACT

The objective was to characterise resistance in wild-oats (*Avena* spp.) and to develop strategies for the prevention, containment and control of herbicide-resistant populations. Laboratory, glasshouse, outdoor container and field experiments were conducted and this integrated approach proved very successful.

Resistance was confirmed on 65 farms in 19 counties of England, showing that resistance is widespread geographically. Resistant wild-oats have not yet been recorded in Wales, Scotland or Northern Ireland. Resistance was shown to occur in both species of wild-oats - *Avena fatua* (common wild-oats) and *Avena sterilis* ssp. *ludoviciana* (winter wild-oats).

Cross-resistance studies showed that some wild-oat populations were only resistant to "fops" (aryloxyphenoxypropionates, e.g. fenoxaprop, fluazifop-P-butyl) but not to "dims" (cyclohexanediones, e.g. tralkoxydim, cycloxydim) or any other herbicide group. Other "fop" - resistant populations showed cross-resistance to the "dim" tralkoxydim and to other herbicides, such as imazamethabenz-methyl and flamprop-M-isopropyl. No resistance was detected to tri-allate, isoproturon, difenzoquat or cycloxydim.

Associated MAFF funded research identified two mechanisms of resistance. *Enhanced metabolism* results in herbicide detoxification and tends to confer partial resistance, but plants may be cross-resistant to several herbicide groups. Surprisingly, five annual applications of herbicides to which partial resistance already existed, did not result in any increase in level of resistance in a population (T/11) with enhanced metabolism. However, these studies also showed that resistance did not decline when herbicide use was reduced. *Target site resistance* blocks the site of herbicide activity and only affects "fop" herbicides in populations studied to date. Resistance tends to be absolute, and may develop faster than enhanced metabolism.

Correct timing and dose is critical to maximise control of partially resistant wild-oats. Full rates applied early (2-3 leaves) were shown to be capable of good control. Later applications gave poorer control, especially when reduced rates were used. As dose was reduced and timing delayed, the risk of inadequate control increased, especially on resistant populations. Susceptible populations were well controlled, but highly-resistant populations were poorly controlled regardless of dose or timing.

The key "take home" messages are: monitor wild-oat herbicide performance and identify causes of poor activity; if resistance is suspected, have seed samples tested to establish the type and severity of resistance; contain the resistance problem and prevent it spreading; do not use "fop" or "dim" herbicides as the only means of grass-weed control in consecutive crops; consider herbicides with alternative modes of action; if resistance is suspected or confirmed, apply post-emergence herbicides when weeds are small (1-3 leaves) to maximise control of partially resistant wild-oats.

SUMMARY

The overall objective was to conduct research on the characterisation of herbicide resistance in wild-oats leading to the development of strategies for the prevention, containment and control of herbicide-resistant populations. The experimental programme comprised the following topics, each with its own sub-objective:

1.   **Population dynamics of resistant populations.**
       Objective: To determine the effects of cultivation type, herbicide dose rate, mixtures/sequences and herbicide rotations on the rate of development of resistance in wild-oats.

       (a)   Interactions of cultivations and herbicide rate on resistance in wild-oats.

       (b)   Influence of dose rate, herbicide rotations, mixtures and sequences on resistance in wild-oats.

       (c)   Influence of resistance on variation in herbicide performance in different years.

       (d)   Selection for resistance in wild-oats: Glasshouse studies.

       (e)   Deselection in resistant wild-oats: Glasshouse studies.

2.   **Cross-resistance patterns.**
       Objective: To determine the cross-resistance patterns in a range of wild-oat populations.

3.   **Occurrence and distribution of resistant populations.**
       Objective: To determine the distribution and relative frequency of resistance in the two wild-oat species.

4.   **Influence of resistance on wild-oat control at field recommended doses.**
       Objective: To determine to what extent the control of wild-oats is reduced at field recommended rates.

       (a)   Field experiments.

       (b)   Container experiments.

This summary includes an outline of the methods used, key results and conclusions for each of the above topics. A final section presents overall conclusions and implications for preventing and managing herbicide resistant wild oats.

1. **Population dynamics of resistant populations.**

(a) **Interactions of cultivations and herbicide rate on resistance in wild-oats.**

The aim was to determine the effect of cultivation type on the rate of development of resistance in wild-oats, as on theoretical grounds resistance is expected to develop faster where non-inversion tillage systems are practised. An experiment was conducted in outdoor containers sown with a mixture of wheat and either a partially-resistant wild-oat population (T/11 1995 from Essex) or a standard susceptible population (LLUD 95). Both were *Avena sterilis* ssp. *ludoviciana.* Fenoxaprop-P-ethyl was applied each year and two cultivation systems, ploughing (inversion) and tine cultivation (non-inversion) simulated. Seeds from surviving plants were collected each year and resown. After five years seeds were evaluated in a glasshouse dose response assay to determine more critically whether any change in degree of resistance had occurred.

The excellent level of control of the LLUD susceptible population every year by full and half rate fenoxaprop confirmed the susceptibility of this population. The resistance index value of 2.8 for the T/11 1995 population in the glasshouse dose response evaluation confirmed the partial resistance status of this population. Such a resistance index is relatively modest, especially when compared with some other populations which have resistance indices of over 15. Resistance indices are the ratios of the ED_{50} values (herbicide rate required to reduce foliage fresh weight by 50% relative to the no-herbicide controls), and provide a simple measure of the degree of resistance in comparison with the response of a susceptible standard.

In outdoor containers, control of the T/11 population was poorer than the susceptible standard, confirming that resistance in this population does impact on herbicide activity in outdoor conditions. Resistance was clearly only partial, as some control was achieved and this varied considerably from year to year, probably due to the effects of environmental conditions.

The overall level of control of the T/11 population increased with increasing dose rate, as would be expected. However, even at the highest rate, the level of control of T/11 was clearly poorer than for the susceptible LLUD and the difference between the two populations tended to increase as dose rate was reduced. These results support the view that the container system provides a good simulation of true field conditions. These results also indicate that while reduced rates can achieve excellent control of susceptible wild-oats, there is less consistency than at higher rates.

Major differences between cultivations in terms of herbicide efficacy were not recorded. A significant difference between cultivations in herbicide activity was detected in the third year but this

was not reproduced in subsequent years. There was no clear evidence that resistance to fenoxaprop had increased during the five years either. The glasshouse dose response assay showed very clearly that there had been no selection for resistance following herbicide use, or deselection where no herbicides were used. Resistance was maintained at the same level as at the start of the experiment.

(b)   **Influence of dose rate, herbicide rotations, mixtures and sequences on resistance in wild-oats.**

The aim was to determine whether these factors affect the rate of evolution of resistance in wild-oats. Experiments were conducted in outdoor containers at two sites, Rothamsted (Roth.) and Boxworth (Box.), sown with a mixture of wheat and either a partially-resistant wild-oat population (T/11 1995 from Essex) or a standard susceptible population (LLUD 95). A range of herbicide treatments was applied each year and seeds from surviving plants were collected each year and resown each autumn. After five years seeds were evaluated in a glasshouse dose response assay to determine more critically whether any change in degree of resistance had occurred.

Consistently excellent (96 - 100%) control of the LLUD susceptible standard was achieved at both sites by fenoxaprop and tralkoxydim, showing that the experimental system, application method and environmental conditions were conducive to good control of susceptible wild-oats in every year. Difenzoquat and imazamethabenz gave more variable control averaging 84 - 88%.

In comparison with the good control of the susceptible LLUD, the generally moderate control of the partially resistant T/11 population by most herbicides could only be due to resistance. Although resistance of the T/11 population to fenoxaprop, tralkoxydim and imazamethabenz was clearly demonstrated, the actual level of control achieved varied considerably between sites and years. Thus reduction in seed return achieved by full rate fenoxaprop varied from 39% (Box. yr 1) to 92% (Box. yr 3 & Roth. yr 5), for tralkoxydim from 23% (Box. yr 1) to 94% (Roth. yr 5), and for imazamethabenz from 22% (Box. yr 4) to 88% (Roth. yr 5). Year to year and site to site differences would probably be even larger in actual fields and are most likely caused by uncontrollable climatic/environmental differences whose effects on herbicide activity are poorly understood. This highlights the difficulty of detecting relatively small shifts in sensitivity to herbicides on a year to year basis, as such differences are likely to be overshadowed by environmental effects on herbicide activity.

For single herbicide treatments, the best control in terms of reductions in seed return of the partially resistant T/11 population was achieved by full rate difenzoquat at both sites, with an average 89% seed reduction. Difenzoquat was the most consistent herbicide at both sites against T/11 over the five years, although it was usually less effective than fenoxaprop and tralkoxydim on the susceptible

LLUD population. Difenzoquat achieved a similar level of control of the susceptible LLUD (84%).
Consequently there was no evidence of resistance to difenzoquat in the T/11 population, in contrast
to fenoxaprop, tralkoxydim and imazamethabenz. Triallate gave generally poor control of both
LLUD and T/11, but there was no evidence that its activity was affected by resistance.

Although the experiments were not specifically designed to study antagonism and synergy between
herbicides, some effects were evident. There was an antagonistic effect between fenoxaprop and
imazamethabenz, a generally neutral (purely additive) effect between fenoxaprop and difenzoquat
and also a generally neutral, but sometimes synergistic effect, between fenoxaprop and tralkoxydim.

Increasing levels of resistance should be associated with declining levels of herbicide activity.
However, there was no clear trend for overall declining herbicide performance at either site during
the five years of the experiments. The results for fenoxaprop, tralkoxydim and imazamethabenz for
T/11, compared with the susceptible LLUD standard, confirm that resistance occurs in T/11, but
there was no clear evidence for increasing levels of resistance despite repeated annual herbicide
applications and an apparently appreciable degree of selection in terms of plant kill each year.

Resistance is an evolutionary process, resulting from a gradual change in sensitivity at the population
level. This highlights the need to make detailed comparisons under more controlled conditions.
Consequently seeds collected from different treatments after five years, were used in a glasshouse
dose response assay to determine more critically whether there had been any change in response to
herbicides. Seeds of the original T/11 population used to sow up the containers in 1995 were used as
a baseline. The glasshouse assay confirmed that there had been no change in level of resistance after
five years annual use of fenoxaprop, tralkoxydim and imazamethabenz. There was also no
difference between annual use of full as against half rate. Nor was there any evidence for
deselection in the absence of herbicides for five years. Surprisingly, resistance was maintained at the
same level as at the start of the experiment, as also occurred in the container experiment in section 1
(a).

It is important to note that the population used, T/11, is now known to be resistant due to an
enhanced ability to metabolise herbicides. The resistance mechanism was not known when the
experiment commenced. Other resistance mechanisms exist in wild-oats, notably target site
resistance (insensitive ACCase). It is possible that resistance will build up faster in populations
which possess other resistance mechanisms, and this is currently under investigation.

(c)   **Influence of resistance on variation in herbicide performance in different years.**

The aim was to determine how the performance of a herbicide varied from year to year under standardised conditions when used on a partially resistant and a susceptible wild-oat population. Experiments were conducted in outdoor trays at two sites, Rothamsted and Boxworth, sown with either a partially-resistant wild-oat population (T/11 1995 from Essex) or a standard susceptible population (LLUD 95). In contrast to the previous experiments, trays were sown with the identical seed populations in each of four years. The herbicide treatments comprised four rates of fenoxaprop-P-ethyl: 6.875, 13.75, 27.5, 55 g a.i./ha, representing 1/8, ¼, ½ and full field rate. The following assessments were made on the wild-oats: number of plants before spraying; number of surviving plants and total foliage fresh and dry weight (mid April - early June).

Responses to fenoxaprop differed between years and sites, despite exactly the same seed populations being sown each year (LLUD 95 and T/11 1995). Over the four years, control of the susceptible LLUD by full rate fenoxaprop ranged from 94 - 98% at Boxworth and 94 - 97% at Rothamsted, and at half rate from 60 - 98% at Boxworth and 90 - 96% at Rothamsted, based on foliage fresh weight reductions. In comparison, control of the partially resistant T/11 by full rate fenoxaprop ranged from 27 - 76% at Boxworth and 51 - 77% at Rothamsted; and at half rate from 18 - 43% at Boxworth and 20 - 66% at Rothamsted.

The full rate of fenoxaprop gave consistently good control of the susceptible LLUD population in all years at both sites. Half rate (27.5 g a.i./ha) also gave good control at both sites in most cases, although control was poorer (60%) in year one at Boxworth, demonstrating that reduced rates may be less consistent even on susceptible populations.

It was evident that on the partially resistant T/11, not only was control always poorer at comparable rates, but that performance of fenoxaprop tended to fall more rapidly as dose rate was reduced from full to half rate, than was the case with the susceptible LLUD population. The varying level of control of the identical populations in different years is probably due to environmental variables, although the precise reasons are hard to define.

(d)   **Selection for resistance in wild-oats: Glasshouse studies.**

The scale of the container and tray experiments detailed in the experiments above meant that only a single resistant population, T/11, could be included. In order to put the results in a wider context simpler selection experiments were conducted over a five year period in the glasshouse using a wider range of populations. The four populations used in this experiment comprised one susceptible (LLUD 1994) and one partially resistant (T/11 1994) population of *Avena sterilis* ssp. *ludoviciana*,

and one susceptible (LFAT 1994) and one partially resistant (ESSEX 2A/F 1994) population of *Avena fatua*. The T/11 population was the same as that used initially in the experiments detailed in sections 1(a to c) above. The objective was to impose a known degree of selection on these populations for five generations in order to determine whether, and to what degree, resistance increased. For the two partially resistant populations, two levels of selection were imposed ("Low" and "High"). Fenoxaprop-P-ethyl was applied at discriminating doses in the range 13.75 - 125 g a.i./ha depending on the initial degree of resistance. When herbicidal symptoms were evident, pots were sorted visually and the plants least affected by the herbicide were retained. Visual selection of plants was generally satisfactory as there was a wide range of responses from completely dead through to minor symptoms. All the LLUD plants died in year 4 so none could be retained for growing on for seed production. The aim was that "high selection" would be achieved by growing on about 2.5 % (equivalent to a 97.5% plant kill) and the "low selection" about 10% (equivalent to a 90% kill) from an initial population of about 500 sprayed plants. Populations were isolated to prevent cross-pollination and seeds collected each year were used to sow up pots in the subsequent year.

After five generations of selection, populations were evaluated in a glasshouse dose response assay to determine whether any change in degree of resistance had occurred. The original populations and seed samples from the same populations after five generations of selection were used.

The approach has worked well but choosing appropriate discriminating doses, which give sufficient effects without killing plants, is not always easy. The dose used on the LLUD population was too high in year 4, so all plants were killed and this population lost. The two levels of selection mimicked the effect of full and reduced doses of herbicide quite well.

The LFAT and T/11 populations showed no evidence of selection for resistance at all. The T/11 results supported those found in the outdoor container experiments detailed in sections 1 (a and b) very well. The glasshouse assay confirmed that there had been no increase in level of resistance after five years annual use of fenoxaprop. There was also no difference between the two levels of selection. However, the intrinsic resistance in the T/11 population to fenoxaprop was confirmed.

In contrast, with the Essex 2A/F population, there was evidence of an increased level of resistance in the population subjected to a lower level of selection, but not with the higher selection. The reasons for this difference is not easy to explain. While the T/11 population is known to possess an enhanced ability to metabolise fenoxaprop, the mechanism in the original Essex 2A/F population, which had a very marginal level of resistance, is unknown.

The results do show that continued use of a herbicide to which there is already resistance does not inevitably result in a rapid increase in resistance over a five year period in a population with enhanced metabolism. However, the results also show that different populations may respond differently to selection, and that generalisations based on research conducted on only one or a small number of populations, may be misleading.

(e) **Deselection in resistant wild-oats: Glasshouse studies.**

An important aspect in long term resistance management is what happens when selection pressure from herbicides ceases, either because herbicides are no longer used or herbicides are used which are equally active on resistant and susceptible plants (neutral selection pressure). Consequently a study was undertaken to establish whether resistance level declined when four resistant populations were grown in the absence of herbicides for five consecutive years.

The four populations used in this experiment comprised one highly resistant (ESSEX 1A/L 1994) and one partially resistant (T/11 1994) population of *Avena sterilis* ssp. *ludoviciana*, and one highly resistant (KENT 1A/F 1994) and one partially resistant (ESSEX 2A/F 1994) population of *Avena fatua*. The partially resistant populations were the same as those used in the 1(d) selection experiment. The aim was to grow these on without any herbicide application for up to five generations in order to determine the degree of reversion towards susceptibility to fenoxaprop-P-ethyl, using a dose response assay.

There was no evidence of deselection for resistance to fenoxaprop in three of the four populations grown on for five generations without further herbicide treatment, (Essex 2A/F, T/11 and Kent 1A/F). However, in contrast to the other three populations, there was evidence of deselection for resistance to fenoxaprop in the Essex 1A/L population. This population had a high initial level of resistance and while the level of resistance in the Essex 1A/L population had decreased after five years without herbicide, it was still substantial, and certainly reversion to full susceptibility had not been achieved. The results showed that stopping using herbicides does not automatically mean that there will be any decline in level of resistance over a five year period.

The results of the selection (1 d) and deselection (1 e) studies taken together, show that continuing or ceasing to use the herbicide to which there is already resistance, does not inevitably result in a change in resistance over a five year period, at least in a population with enhanced metabolism. However, the results also show that different populations may respond differently to selection and deselection, and that generalisations based on research conducted on only one or a small number of

populations, may be misleading. Other resistance mechanisms exist in wild-oats, notably target site resistance (insensitive ACCase). It is possible that resistance will build up or decline faster, in populations which possess other resistance mechanisms, and this is currently under investigation.

2.   Cross-resistance patterns.

The aim was to determine the cross-resistance patterns in a range of wild-oat populations of both species of wild-oats. Five glasshouse/laboratory dose response experiments were undertaken: The first utilized "fop" (aryloxyphenoxypropionate) and "dim" (cyclohexanedione) herbicides; the second a range of non - "fop" and "dim" herbicides; the third, fourth and fifth, conducted in petri-dishes, triallate. Ten populations of wild-oats of both species (7 *A. fatua* (F), 3 *A. ludoviciana* (L)) from seven different counties of England were used in this series of experiments. Most of them had showed evidence of resistance in preliminary single dose screening experiments.

The results demonstrate clearly that there is continuum of response to fenoxaprop, from susceptible through to highly resistant. Resistance indices (RI) varied from 1.0 - 13.1. Populations cannot simply be placed into arbitrary susceptible and resistant categories. The variability in response was not due simply to a difference in the proportion of highly resistant individuals within the population. Virtually all plants of some partially resistant populations would survive an intermediate rate of herbicide that would kill all susceptible plants. Higher rates would kill all plants of the partially resistant population but allow highly resistant plants to survive. Subsequent work has helped to explain this. Some populations (e.g. T/41 and Suffolk 1A/F) have target site (ACCase) insensitivity, which confers a high level of resistance, whereas other populations (e.g. T/11) possess an enhanced ability to metabolise herbicides which normally confers partial resistance.

Populations with resistance to one "fop" herbicide (fenoxaprop) also tended to show resistance to another "fop" (fluazifop), but not always to the same degree. However, resistance to "fops" was not directly correlated with resistance to "dims", despite these having the same mode of action. The two populations (Kent 1A/F and Suffolk 1A/F) with the highest level of resistance to "fops", showed no evidence of resistance to tralkoxydim. However, five other populations showed very clear evidence of resistance to both tralkoxydim and fenoxaprop, although again the degree of resistance varied. However, despite clear evidence of resistance to tralkoxydim in several populations, there was no evidence of resistance to cycloxydim in any population. The results demonstrate that with wild-oats, as with black-grass, the degree of resistance to herbicides within the same chemical group can vary. Relating resistance to chemical group can be misleading, and there is a need to consider herbicides

individually as resistance may occur to some (e.g tralkoxydim), but not all (e.g. cycloxydim), of the herbicides within a chemical group.

Studies with *non* "fop" and "dim" herbicides demonstrated clearly that with wild-oats the degree of cross-resistance to herbicides with different modes of action can vary considerably. Two populations which showed the greatest level of resistance to the "fop" fenoxaprop, (Kent 1A/F RI = 6.8, Suffolk 1A/F RI = 13.1) showed no clear evidence of resistance to any of the non - "fop" and "dim" herbicides tested, nor to the "dims" tralkoxydim or cycloxydim, or to tri-allate in the petri dish experiments. In those two populations, resistance was confined to "fops" only. In contrast, four populations which showed partial resistance to both fenoxaprop and tralkoxydim, (Lincs 7A/F, Oxford 5A/F, T/11 and Dorset 1A/F), all showed evidence of cross-resistance to imazamethabenz and flamprop, despite these herbicides having different modes of action. This was probably due to the presence of an enhanced metabolism mechanism, which has been confirmed in T/11 and Dorset 1A/F in associated MAFF funded biochemical studies. In contrast Suffolk 1A/F has been shown to possess target site resistance to fenoxaprop, which appears to be specific to "fops" and not affect "dims". This contrasts with black-grass, where target site resistance affects both "fops" and "dims".

There was no clear evidence of resistance to difenzoquat, isoproturon or tri-allate in any population. One population (Essex 2 A/F) did show some insensitivity to tri-allate in two petri-dish experiments and also showed the second highest level of insensitivity to difenzoquat of the ten populations evaluated. This may be coincidence, but in North America some populations of wild-oats show resistance to both triallate and difenzoquat, despite these herbicide having different modes of action. This population did not show a high degree of resistance to other herbicides, but warrants further investigation.

3.   **Occurrence and distribution of resistant populations.**

The aim was to collect and evaluate populations of wild-oats with suspected resistance to determine the distribution, degree of resistance and relative frequency of resistance in the two wild-oat species. The scope of the project did not include random surveys. Wild-oat populations were collected from a range of sources each year, and over five years 121 populations were evaluated for resistance to fenoxaprop-P-ethyl, tralkoxydim and imazamethabenz in glasshouse pot assays.

Several organisations conduct screening tests for resistant wild-oats in the UK. A compilation exercise was conducted for all wild-oat populations found to be resistant to fenoxaprop-P-ethyl both

within this project and by other organisations/companies in order to determine how many individual farms had been identified as containing resistant wild-oats. These results are summarised here. By 1999 resistance to fenoxaprop-P-ethyl had been detected on **65 farms** distributed over **19 counties** of England. The counties with the greatest recorded number of farms with confirmed resistance were: Essex - 17; Lincolnshire – 7; Norfolk – 7; Cambridgeshire – 6; Somerset – 6. Resistant wild-oats have not yet been detected in Wales, Scotland or Northern Ireland. Although fenoxaprop was used as the standard herbicide for screening for resistance, cross-resistance to herbicides with the same, and different mode of action, often occurs.

Populations showing the greatest resistance to fenoxaprop tended *not* to show resistance to tralkoxydim or imazamethabenz. This is probably due to the presence of target site resistance affecting only "fop" herbicides. In contrast, some partially fenoxaprop resistant populations showed cross-resistance to tralkoxydim and imazamethabenz, probably due to the presence of an enhanced metabolism mechanism. Associated MAFF funded work on the biochemical basis of resistance supports these conclusions.

Sampling and testing wild oat samples collected from different patches within the same field, and from several different fields within the same farm, confirmed that resistant wild-oats may occur quite widely across farms, and not be confined to a single patch (Essex 10, Essex 12, Dorset 1, Kent 1). However on other farms (Essex 1A, Cambs. 3A, Wilts 1A) there was clear evidence for resistance being confined to localised patches which were often relatively small (< 0.25 ha). It was evident from field observations that these patches were often elongated in the direction of cultivations/combining, indicating that resistance had probably originated some years previously.

Resistant wild-oats were also detected on three neighbouring farms in Essex, which raises issues about the spread of resistant wild-oats. There was no common link between the farms which operated independently. Wild-oats are self pollinating so spread of resistance genes in pollen is unlikely. Spread of resistant seeds is a more likely reason although the method of transport is unknown.

For the 60 populations for which there was a positive species identification, 45 (75%) were *Avena fatua* and 15 (25%) *Avena sterilis* ssp. *ludoviciana*. Thus resistance occurs in both species of wild-oats, but relatively more commonly in *A. sterilis* ssp. *ludoviciana* than would be predicted from the frequency of occurrence of the two species. It is generally estimated that *A. sterilis* ssp. *ludoviciana* comprises less than 20% of wild-oat infestations in the UK, although no random survey has been conducted for many years.

The pot assay technique worked well and gave consistent results. The results clearly identified highly fenoxaprop-resistant wild-oats. However, many populations showed partial resistance and interpreting the significance of these more marginal levels of insensitivity is more difficult. This highlights the fact that resistance in wild-oats is not always absolute and a wide range of responses is possible. As it is difficult to define a fixed threshold for resistance the use of the * or "R" rating system, as used for black-grass, for categorising different degrees of resistance in screening assays is recommended.

The correlation with results from the cross-resistance dose response assays (see Section 2) for the 10 populations included in both series of experiments was very good and confirms clearly that cross-resistance patterns are complex, especially in terms of quantitative differences.

One population was identified which had resistance to fenoxaprop and tralkoxydim (Cambs. 3A/L) but no resistance to imazamethabenz. This may be due to the presence of target site resistance affecting both "fops" and "dims", and biochemical studies are in progress to confirm this. This demonstrates that different cross-resistance patterns are possible and the risks involved in making generalised statements about cross-resistance.

4.   Influence of resistance on wild-oat control at field recommended doses.

(a)   Field experiments.

A series of field experiments was conducted in fields where the presence of resistant wild-oats had been confirmed, to help determine to what extent the control of wild-oats was reduced at field recommended rates. Two field experiments were conducted each year, making a total of 10 experiments over the five years. An experiment was conducted for three successive years in the Essex 10/L (= T/41) field in order to study the year to year variability in herbicide response at one site.

The following herbicides were applied at full and half recommended rates: fenoxaprop-P-ethyl, difenzoquat, imazamethabenz, tralkoxydim, flamprop-M-isopropyl and clodinafop-propargyl. In addition mixtures of the lower rate of fenoxaprop-P-ethyl plus the lower rate of either difenzoquat, imazamethabenz or tralkoxydim were also applied at all sites. Herbicides were applied mainly in March or April when wild-oats were at growth stage 12 - 32. Panicle numbers were assessed as a measure of herbicide efficacy.

Field experiments were conducted at the Essex 10A/L site for three successive years in adjacent areas. Resistance to fenoxaprop resulted in consistently poor activity with this herbicide. Associated MAFF funded work has shown that this population has target site resistance specific to "fop" herbicides. In contrast, tralkoxydim worked consistently well each year. This supports the glasshouse screening studies that showed a high level of resistance to fenoxaprop, but no resistance to tralkoxydim or imazamethabenz. However, the glasshouse screening experiments conducted in 1995 included samples from three widely distributed areas within this field, as well as other fields on this farm (Essex 10). There was evidence of considerable variation in susceptibility to tralkoxydim and imazamethabenz both between fields and between samples from within the trial site field. Thus it should not be too readily assumed that there is not the potential for evolution of resistance to tralkoxydim in this field, despite the consistently good control achieved in these trial.

The results for this site also highlight the problem of identifying resistance purely on the basis of field experience. Do the consistent, but generally only modest results for difenzoquat (72 – 86%) indicate partial resistance or no resistance? Almost certainly the latter – but one has no way of knowing purely from the field results. Flamprop, imazamethabenz and clodinafop gave variable results over the three years, and control was generally, but not always, mediocre. Partial resistance may have been involved, but this cannot be confirmed purely by the field experience. This highlights the problem of detecting partial resistance and the need for tests under more controlled conditions, such as glasshouse pot assays.

The results for the Essex 2A/F and Oxford 5A/F populations were generally consistent with the more detailed glasshouse cross-resistance studies conducted with these two populations. Marginal levels of resistance did not always result in poor control in the field when full rates were applied, but there was evidence that performance was reduced, often substantially, at reduced rates. There was evidence for poorer control with tralkoxydim and imazamethabenz at the Oxford 5A/F site, which was consistent with the results of the cross-resistance studies and screening assays. The results for the Dorset 1A/F population, also included in the glasshouse cross-resistance studies, were disappointing as this population had shown an interesting cross-resistance profile in glasshouse tests. It was not possible to relate field results to glasshouse studies due to the variability in distribution of wild-oats in the field.

The results for the Cambs. 3A/L population were erratic and overall poor control of the wild-oats was achieved, probably because the population was very high. In the glasshouse screening experiment, there was evidence of resistance to fenoxaprop and tralkoxydim, but not to

imazamethabenz. Although control was poor, the field experiment results do support the view that this population shows resistance to the former two herbicides, but not to the latter, or to difenzoquat. This site also demonstrated the difficulty of controlling high populations.

As in the container experiments (see above), there was evidence of antagonism between fenoxaprop and imazamethabenz. In five out of six comparisons (omitting Essex 2B/F, Dorset 1A/F, Cambs. 3A/L, Essex 7A/L, sites where populations were excessively high or gave variable or very high control) fenoxaprop + imazamethabenz gave levels of control less than would have been predicted from the purely additive effects of the individual half rate applications. Mean control was 8% less than predicted (range -38% to +31%). In a total of 14 comparisons (8 container and 6 field) control by the fenoxaprop + imazamethabenz mixture was less than predicted on 12 occasions.

With the mixtures of fenoxaprop + tralkoxydim control was better than predicted in four out of six field comparisons (mean +9%, range -2% to +30%). In a total of 14 comparisons (8 container and 6 field) control by the fenoxaprop + tralkoxydim was better than predicted on 10 occasions. Thus, there was some evidence of synergy between these two herbicides, but this was not consistent.

With the mixtures of fenoxaprop + difenzoquat control was less than predicted in five out of six field comparisons (mean -19%, range -78% to +18%). In a total of 14 comparisons (8 container and 6 field) control by the fenoxaprop + difenzoquat was less than predicted on 10 occasions. Thus, there was some evidence of antagonism between these two herbicides, but the effects were generally less pronounced and less consistent than for imazamethabenz.

(b)   **Container experiments.**
Outdoor container studies can simulate field conditions and permit comparison of herbicide performance on several populations under identical soil and climatic conditions, which is difficult to achieve in true field experiments. Two outdoor container experiments were conducted using some of the populations evaluated in the glasshouse cross-resistance studies. Two of the populations (Essex 2A/F and Dorset 1A/F) had also been sites of field experiments. Using the same populations in each of the experimental situations allows a much more comprehensive appraisal of the impact of resistance to be made.

The first container experiment involved two "fop" (fenoxaprop, fluazifop) and two "dim" (tralkoxydim, cycloxydim) herbicides applied at the recommended rates at two timings. The second container experiment included a range of other herbicides with different modes of action

(imazamethabenz, flamprop, difenzoquat, isoproturon, tri-allate). Imazamethabenz, was applied at two timings but the others were applied on a single date.

The results showed that resistance in wild-oats could substantially reduce the efficacy of "fop" and "dim" herbicides, and some herbicides with different modes of action. The results generally agreed very well with predictions based on the glasshouse dose response studies. Cross-resistance patterns are not simple, as resistance even within the "fop" and "dim" classes was not directly correlated in different populations. Some populations (Suffolk 1A/F, Kent 1A/F) showed high resistance to specific "fops", but no resistance to any "dim" or other herbicides. Other populations (Wilts. 1A/L, Dorset 1A/F) showed some resistance to both "fops", to the "dim" tralkoxydim and also to flamprop and imazamethabenz (Dorset 1A/F only). No population showed resistance to cycloxydim.

Responses to difenzoquat, isoproturon and especially tri-allate were rather variable, but there was no definitive evidence for resistance in most populations. One population, Essex 2A/F, gave consistently poor control with all three herbicides indicating that this population does possess mechanisms which confer partial resistance to several herbicides with different modes of action. However further studies would be needed to confirm this. Isoproturon and difenzoquat gave excellent control of some populations which were highly resistant to other herbicides.

It was clear that the impact of resistance on herbicide efficacy is closely related to herbicide timing in many populations. With the susceptible standard, LFAT, timing, or size of plant, had no effect on efficacy – excellent control was achieved consistently with all four "fop" and "dim" herbicides and with imazamethabenz. Where there was a high degree of resistance (e.g. Suffolk 1A/F to fenoxaprop; Kent 1A/F to fluazifop; Dorset 1A/F to tralkoxydim) control was poor to mediocre regardless of timing. However with partially resistant populations, timing had a large effect. Earlier applications of fenoxaprop, tralkoxydim and imazamethabenz at the three leaf stage were often highly effective, whereas later applications at the 2-3 tiller stage gave poorer control.

The conclusions were clear. With susceptible wild-oats, good control can be achieved regardless of timing. With highly resistant wild-oats, poor control is likely regardless of timing. With partially resistant wild-oats, as application is delayed, the chance of achieving adequate control decreases. Thus a knowledge of the degree of resistance and cross-resistance patterns in any population is essential to ensure good control.

Overall conclusions and implications for preventing and managing herbicide resistant wild oats.

- Herbicide-resistant wild-oats, first identified in 1993, have now been found on 65 farms in 19 counties of England and are geographically widespread. As no random surveys have been conducted, the full extent of resistance is unknown. Resistant wild-oats have not yet been recorded in Wales, Scotland or Northern Ireland.

- Resistance occurs in both species of wild-oats - *Avena fatua* (common wild-oats) and *Avena sterilis* ssp. *ludoviciana* (winter wild-oats). Most cases have arisen separately on individual farms rather than spread from a single source. Wild-oats self-pollinate, so any spread occurs by seed movement between farms, not via pollen.

- Resistance often develops in patches which may be mistaken for spray misses. Patches may be less than 100 m^2 initially, but often lengthen in the direction of combining.

- Some wild-oat populations are only resistant to "fops" (e.g. fenoxaprop, fluazifop-P-butyl) but not to "dims" (e.g. tralkoxydim, cycloxydim) or any other herbicide group. Other "fop" - resistant populations show cross-resistance to the "dim" tralkoxydim and to other herbicides, e.g. imazamethabenz-methyl, flamprop-M-isopropyl. To date, no resistance has been found in the UK to tri-allate, isoproturon, difenzoquat or cycloxydim (N.B. resistance to some of these does occur elsewhere in the world).

- Herbicide strategy should be tailored to the resistance mechanism(s) present. Associated MAFF funded research has shown that at least two mechanisms of resistance exist in UK wild-oats: enhanced metabolism and target site (insensitive ACCase) resistance. **Enhanced metabolism** resistance appears to be more common and results in herbicide detoxification. Resistance is partial but plants may be cross-resistant to several different herbicide groups. Resistance does not necessarily increase rapidly, but does not decline if herbicide use is reduced. **Target site resistance** blocks the site of herbicide activity. In UK populations studied so far, it only affects "fop" herbicides (and not "dims" as is the case with target site resistant black-grass). Resistance tends to be absolute, and may develop faster than enhanced metabolism, but this has yet to be confirmed.

- Correct timing is critical to maximise control of partially resistant wild-oats. Full rates applied early (2-3 leaves) can give good control. Later applications may give poor control, especially if reduced rates are used. As dose is reduced and timing delayed, the risk of inadequate control increases, especially if resistance is present. Susceptible populations should be well controlled regardless of timing or dose. Herbicides will not control highly-resistant populations at any timing.

TECHNICAL DETAILS

Introduction

Wild-oats (*Avena fatua* and *Avena sterlis* ssp. *ludoviciana*) are a major grass-weed of cereal crops in the UK. HRAC (Herbicide Resistance Action Committee) funded work, conducted jointly by IACR-Rothamsted and ADAS Boxworth, identified herbicide resistance in three populations of wild-oats in 1993. These were the first recorded cases in the UK. Herbicide resistant wild-oats have also been identified in 8 other countries world-wide: Australia, Belgium, Canada, Chile, France, Italy, South Africa, USA. Consequently there is a risk from herbicide resistant wild-oats in the UK and the research project was initiated in order to gain a better understanding of resistance in this weed.

Objectives of the research project

To conduct research on the characterisation of herbicide resistance in wild-oats leading to the development of strategies for the prevention, containment and control of herbicide-resistant populations.

This was a totally integrated project, conducted collaboratively by IACR-Rothamsted and ADAS Boxworth, funded jointly by HGCA, MAFF, Aventis, Cyanamid (now BASF), Monsanto, Novartis and Zeneca (now Syngenta).

The experimental programme comprised the following topics:

1.   **Population dynamics of resistant populations.**

      Objective: To determine the effects of cultivation type, herbicide dose rate, mixtures/sequences and herbicide rotations on the rate of development of resistance in wild-oats.

      (a)   **Interactions of cultivations and herbicide rate on resistance in wild-oats.**

      (b)   **Influence of dose rate, herbicide rotations, mixtures and sequences on resistance in wild-oats.**

      (c)   **Influence of resistance on variation in herbicide performance in different years.**

      (d)   **Selection for resistance in wild-oats: Glasshouse studies.**

      (e)   **Deselection in resistant wild-oats: Glasshouse studies.**

2.   Cross-resistance patterns.

Objective: To determine the cross-resistance patterns in a range of wild-oat populations.

3.   Occurrence and distribution of resistant populations.

Objective: To determine the distribution and relative frequency of resistance in the two wild-oat species.

4.   Influence of resistance on wild-oat control at field recommended doses.

Objective: To determine to what extent the control of wild-oats is reduced at field recommended rates.

(a)   Field experiments.

(b)   Container experiments.

Each of the above topics is covered in a separate section below, which includes materials and methods, results and conclusions for each section. The final discussion aims to put the results from all the sections into context and relate these to other relevant research, leading to recommendations for prevention and management of herbicide resistant wild-oats.

SECTION 1

Population dynamics of resistant populations

1 (a). Interactions of cultivations and herbicide rate on resistance in wild-oats

Introduction

The aim of this experiment was to determine the effect of cultivation type on the rate of development of resistance in wild-oats. The rationale behind this was that cultivation records for fields where there were resistant wild-oats (*Avena* spp.) and black-grass (*Alopecurus myosuroides*) indicated that resistance tended to be more prevalent on fields where non-inversion tillage was used routinely. There are theoretical reasons why this is so, namely that with minimum tillage most plants are derived from recently shed seeds retained close to the soil surface, whereas with ploughing, older, less selected seeds are brought up to the surface and these have a buffering effect slowing up the development of resistance. There is little scientific evidence to support these observations, so an experiment was conducted in large outdoor containers in which different cultivation systems were simulated.

Materials and Methods

Container experiment

This experiment was established at Rothamsted in 44 plastic containers in October 1995 and ran for five consecutive years. Each container (39.5 x 32.5 x 23.0 cm deep) had 13 x 6.5 mm drainage holes drilled in the bottom, a layer of 5 L of 'Hydroleca' lightweight aggregate added to improve drainage and then a Kettering loam soil + grit (5:1) + slow release fertilizer ('Osmacote mini' 18% N, 6% P_2O_5, 12% K_2O at 2.4 kg/tonne) mix added to fill the box within 5 cm of the top. Three rows, each of 10 wheat seeds cv. Hereward were sown lengthways in each container, down the centre line and at each side so that there was 13.75 cm between rows. Between each pair of rows of wheat, three rows each of 20 wild-oat seeds were sown. Thus a total of 30 wheat seeds (= 234 seeds/m^2) in three rows and 120 wild-oat seeds (= 936 seeds/m^2) in six rows were sown in each container. The same soil was then added to cover seeds to 2.5 cm depth and containers were then placed outside on a sandbed.

Twelve containers were sown with a standard susceptible population of *Avena sterilis* ssp. *ludoviciana* (LLUD95) and 32 containers were sown with a partially resistant population of the same species (T/11 1995). This population, from Essex, had shown partial resistance to fenoxaprop in previous glasshouse studies having a resistance index (ratio of ED_{50} values relative to a susceptible standard) of 2.3 and also showing some cross-resistance to tralkoxydim, imazamethabenz and flamprop-M-isopropyl. Subsequent MAFF funded studies showed that the T/11 population was resistant due to an enhanced ability to metabolise herbicides (Cocker *et al*., 2000).

The two cultivation treatments simulated in this experiment were plough (inversion) and tine (non-inversion tillage). Containers were resown each autumn, either with T/11 seed collected from the same treatment that summer (simulating tine cultivations) or with T/11 seed collected from the same treatment in the preceding year (simulating ploughing). The scheme for T/11 is summarised below where t0 is the seed sown at the start of the experiment in autumn 1995 producing t1 seed in the following July/August.

Year	"Plough"	"Tine"
1	t0--->t1a	t0--->t1
2	t0--->t1b	t1--->t2
3	t1a--->t2a	t2--->t3
4	t1b--->t2b	t3--->t4
5	t2a--->t3	t4--->t5

Note that with simulated ploughing, seed collected in each summer was stored for one year before sowing thus simulating ploughing up seeds after burial for a year, whereas with simulated tine cultivation seeds were sown in the autumn following the summer of collection. Containers with susceptible LLUD seed were sown each year with seed from the same original sample to act as a reference.

The treatments comprised: 2 cultivations x 1 population (T/11) x (untreated + 3 herbicide rates) x 4 replicates = 32 containers **plus** 1 population (LLUD) x 3 herbicide doses x 4 replicates = 12 containers. The herbicide treatments comprised three rates of fenoxaprop-P-ethyl ("Cheetah Super"): 13.75, 27.5 and 55 g a.i./ha, representing ¼ , ½ and full field rate.

Seeds were sown in early October each year and herbicide treatments were applied between mid February and mid April when wild-oat plants were at the 1-3 tiller stage and 8 – 18 cm high, and wheat plants had 2-3 tillers. A laboratory pot sprayer was used delivering 220 - 273 litres water/ha at 210 kPa through a single 'Teejet' 110015 VK nozzle 50 cm above the plants.

The following assessments were made on the wild-oats each year: number of plants before spraying; number of surviving plants (April/May); panicles per container (mid June); spikelets per panicle on a random 10 panicles/container (late June/early July). The containers were moved into sets comprising the same population and treatment in May each year to minimize any potential cross pollination. A minimum of 1m was maintained between sets - wild-oats are a self-pollinating species so cross-pollination was highly unlikely. Spikelets (the dispersal unit of wild-oats) were collected on several occasions during July each year from each container, air dried and stored in envelopes. The number of viable seeds per spikelet was assessed in August on a random 25 spikelets per container taken from the collected samples. The panicle, spikelets per panicle and seeds per spikelet data was used to determine the seed production per container.

Results obtained were analysed using two-way analysis of variance in randomised blocks using GENSTAT statistical package.

Glasshouse evaluation

After the completion of the container experiment described above, seeds from that experiment were evaluated in a glasshouse dose response assay to determine more critically whether any change in degree of resistance had occurred. The following six populations were used: LLUD 95 (susceptible standard); T/11 1995 (original population used at start of the container experiment); Plough F/3 1999; Tine F/3 1999; Plough Nil 1999; Tine Nil 1999 (seeds collected from simulated plough or tine containers in 1999 either treated annually with the highest rate of fenoxaprop (F/3 = 55 g a.i./ha) or left untreated (Nil). By 1999 seeds from the simulated tine cultivations had received four years of selection whereas seeds from simulated plough had received only two years selection.

Approximately 80 seeds were placed in each petri-dish containing three normal filter papers and one glass fibre paper. Eight dishes were prepared for each population. The seeds were treated with 9 ml of deionised water on 31 January 2001 and incubated at 17°C 14-hour day, 11°C 10-hour night. On 5 February 2001 germinated seeds were sown in 5cm square pots containing compost (1 seed per pot, 208 pots per population).

The experiment comprised a fully randomised design with 16 replicate pots per herbicide dose and 40 untreated pots per population. Fenoxaprop-P-ethyl was applied at seven doses (6.87 - 440 g a.i./ha) at the 3 leaf stage on 26 February 2001, 21 days after sowing, using a laboratory sprayer delivering 278 litres water/ha at 210kPa through a single "Teejet" 110015VK ceramic nozzle. Herbicide activity was recorded on 26 February 2001, 28 days after spraying, by assessing foliage fresh weight for each individual pot.

Dose response data was analysed using a logistic relationship between foliage fresh weight and $\log_{10}$ dose and $\log_{10}ED_{50}$ values were determined (Ross 1987). The ED_{50} values (herbicide rate required to reduce fresh weight by 50% relative to the no-herbicide controls) referred to in the text and tables have been detransformed from the $\log_{10}$ values.

Results (Tables 1& 2; Figure 1 & 2)

Container experiment

- The mean number of wild-oat plants emerged per container prior to spraying was 67 for T/11 (range 54 - 82 over the five years) and 65 for LLUD (range 61 - 69) representing a 56% and 54% emergence respectively. On average 98 panicles were produced in each untreated container.

- The % reduction in plant numbers (Table 1; Figure 1) are based on the numbers surviving herbicide treatment (post-spray) compared with those present pre-spraying for the same container. The % reduction in seed return values (Table 2; Figure 2) are based on herbicide treatment figures compared with untreated containers.

- In every year, the highest rate (=field rate) of fenoxaprop achieved excellent control (100 %) of susceptible LLUD plants. Half rate also gave good control (86 - 100% reduction in plant numbers) whereas the lower rate - equivalent to ¼ field rate - gave more variable control, (56 - 90% reduction). This confirms the susceptible status of the LLUD population and demonstrates clearly that the wild-oat growth stage, application methods and conditions were conducive to achieving excellent control of susceptible wild-oats at the full rate and half rate.

- At the highest rate (55 g a.i./ha) the level of control of T/11 plants on simulated tine cultivations varied between years, but was always lower than the susceptible standard, LLUD (Table 1; Figure 1). Control of plants tended to increase during the first three years, decrease in the fourth year, and then increase again in the fifth year. Thus, despite sowing seeds from plants surviving herbicide treatment in the previous crop each year, there was no evidence that resistance was increasing. The % reduction figures for plant numbers were broadly similar for the two cultivations, the biggest difference (13% for fenoxaprop at 55 g a.i./ha) occurring in year 4. Lower rates of herbicide gave poor control of T/11 plants on both cultivations in every year (< 67 %).

- The highest dose of fenoxaprop achieved a 97% (plough) and 100% (tine) reduction in seed return of T/11 in the year 5, although in years 1 - 4 control had been more variable and substantially poorer than for the LLUD susceptible standard (100% every year) (Table 2; Figure 2). Reduction in seed return was poor every year at the lowest herbicide rate, (<48%) and in most years at half field rate (<56 %) except in year 5 (74 - 83%). Activity of fenoxaprop was high on other experiments conducted in year 5, which suggests that the environmental conditions were particularly favourable for herbicide activity in that year

(2000). For the LLUD population, a consistently high degree of suppression of seeding was achieved with the highest two doses (93-100%), with more variable control at the lowest dose (37-96%).

- There were no consistent differences between cultivations in terms of reduction in seed return during the five years. A statistically significant difference (P≤0.05) was recorded in year 3 between the two cultivations in terms of the % reduction in seed return at the highest rate of fenoxaprop (55 g a.i./ha). The value for the tine treatment (56%) was lower than the plough value (88%). However, this difference was not apparent in the % reduction in plant numbers data and was not reproduced in years 4 or 5.

Glasshouse evaluation (Table 3)

- The mean foliage weights for the untreated pots were in the range 3.69 - 4.91 g indicating similar growth in the absence of herbicides.

- In Table 3, the $\log_{10}ED_{50}$ values are presented together with individual and pooled standard errors. The detransformed ED_{50} values are also presented with the ratio of these values to the LLUD 1995 susceptible standard. These ratios provide a simple form of "Resistance Index" for comparing responses. The greater the value, the higher the degree of resistance.

- The ED_{50} value for the T/11 1995 population used at the start of the container experiment was significantly (P<0.05) higher than the LLUD susceptible standard. The Resistance Index was 2.8 confirming that the T/11 population showed partial resistance to fenoxaprop.

- The ED_{50} values for the other four populations tested did not differ significantly (P≥0.05) from the original T/11 1995 population. The Resistance Indices were very similar, from 2.7 to 3.1. There was no indication that further selection by fenoxaprop for four years (Tine F/3 1999) or absence of selection for four years (Tine NIL 1999) had changed the sensitivity of the population to fenoxaprop relative to the starting population, T/11 1995. Likewise there was no evidence that the simulated cultivations, which had resulted in different degrees of selection pressure (four applications of fenoxaprop to Tine F/3 1999; two applications to Plough F/3 1999) had affected the level of resistance in any way.

Conclusions

The excellent level of control of the LLUD susceptible population every year by full and half rate fenoxaprop confirmed the susceptibility of this population. The resistance index of 2.8 for the T/11 1995 population in the glasshouse dose response evaluation confirmed the partial resistance status of this population. Such a resistance index is relatively modest, especially when compared with some other populations which have resistance indices of over 15.

In outdoor containers, control of the T/11 population was poorer than the susceptible standard, confirming that resistance in this population does impact on herbicide activity in outdoor conditions. Resistance was clearly only partial, as some control was achieved and this varied considerably from year to year, probably due to the effects of environmental conditions.

The overall level of control of the T/11 population increased with increasing dose rate, as would be expected. However, even at the highest rate, the level of control of T/11 was clearly poorer than for the susceptible LLUD and the difference between the two populations tended to increase as dose rate was reduced. These results support the view that the container system provides a good simulation of true field conditions. These results also indicate that while reduced rates can achieve excellent control of susceptible wild-oats, there is less consistency than at higher rates.

Major differences between cultivations in terms of herbicide efficacy were not recorded. A significant difference between cultivations in herbicide activity was detected in the third year but this was not reproduced in subsequent years. There was no clear evidence that resistance to fenoxaprop had increased during the five years either. The glasshouse dose response assay showed very clearly that there had been no selection for resistance following herbicide use, or deselection where no herbicides were used. Resistance was maintained at the same level throughout the experiment.

Interaction of cultivations and herbicide rate on resistant wild oats

1 (a). Container experiments

Table 1. Percentage reduction in plant numbers

T/11 results

Fenoxaprop dose g/ha	Year one 1995/96		Year two 1996/97		Year three 1997/98		Year four 1998/99		Year five 1999/2000	
	Plough	Tine	Plough	Tine	Plough	Tine	Plough	Tine	Plough	Tine
Nil	8.8	8.9	26.2	31.9	11.1	2.8	25.5	22.6	6.7	16.0
13.75	10.3	10.9	34.5	29.1	25.6	14.7	28.7	32.3	45.2	38.8
27.5	16.6	20.4	42.3	46.0	21.8	28.1	46.8	32.6	66.7	62.9
55	29.8	29.7	57.3	60.6	81.1	80.8	51.2	64.3	83.2	86.1
S.E.M.	2.94		6.14		6.19		4.9		4.92	
d.f.	21		21		21		21		21	

LLUD results

Fenoxaprop dose g./ha	Year one 1995/96	Year two 1996/97	Year three 1997/98	Year four 1998/99	Year five 1999/2000
13.75	63.0	89.8	55.7	77.9	77.8
27.50	100.0	98.3	86.4	99.6	98.8
55.00	100.0	100.0	99.6	99.6	99.6

Table 2. Percentage reduction in seed return

T/11 results

Fenoxaprop dose g./ha	Year one 1995/96		Year two 1996/97		Year three 1997/98		Year four 1998/99		Year five 1999/2000	
	Plough	Tine	Plough	Tine	Plough	Tine	Plough	Tine	Plough	Tine
Nil	0	0	0	0	0	0	0.0	0.0	0.0	0.0
13.75	12.4	25.7	9.9	20.3	14.3	-18.2	13.1	6.0	48.3	33.0
27.5	10.0	32.0	55.7	52.2	26.4	0.5	4.3	16.7	74.3	83.0
55	59.1	51.1	83.4	87.5	87.9	56.1	57.2	61.0	97.4	99.5
S.E.M.	6.56		6.52		9.29		11.27		3.60	
d.f.	15		15		15		15		15	

LLUD results

Fenoxaprop dose g./ha	Year one 1995/96	Year two 1996/97	Year three 1997/98	Year four 1998/99	Year five 1999/2000
13.75	76*	96.0	37*	46*	67*
27.50	100	100	93*	98*	100
55.00	100	100	100*	100*	100

* = based on the mean of untreated T/11 plants

Figure 1. Influence of simulated cultivations on activity of fenoxaprop on a partially resistant wild-oat population over a five year period

1 (a). Container experiments

Percentage reduction in T/11 plant numbers.
55g fenoxaprop/ha
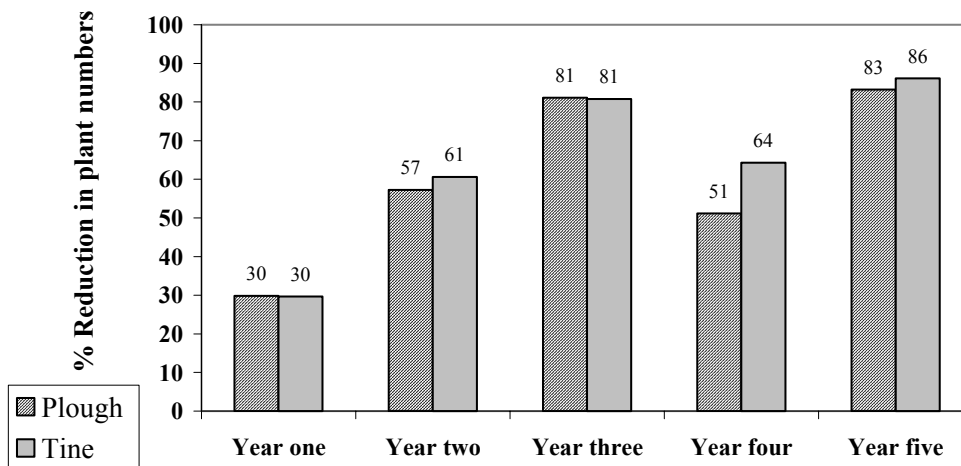
Figure 2. Influence of simulated cultivations on activity of fenoxaprop on a partially resistant wild-oat population over a five year period

Percentage reduction in T/11 seed return.
55g fenoxaprop/ ha
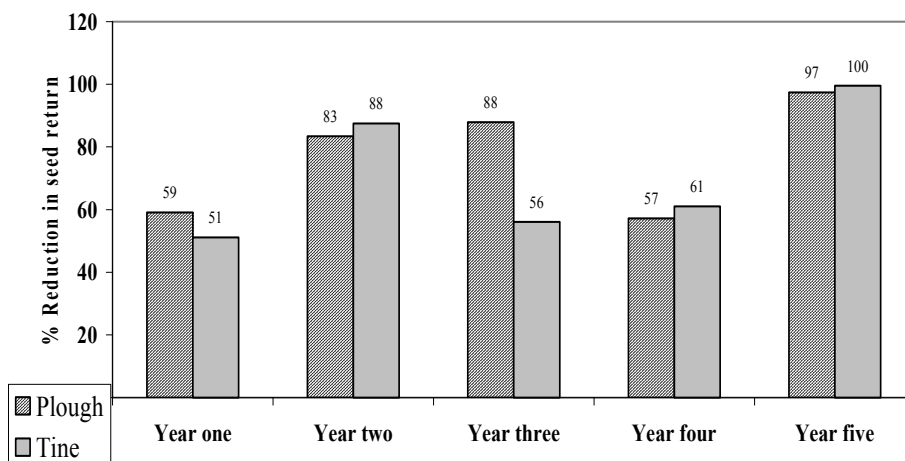
Table 3. Interaction of cultivations and herbicide on resistant wild oats

1 (a). Glasshouse dose response evaluation

Population	LOG$_{10}$ ED$_{50}$ Values [S.E. in Brackets]	Detransformed ED$_{50}$ values [Resistance index*]
LLUD 1995	**1.4649** [0.0778]	29.17 **[1.0]**
T/11 1995 (original population)	**1.9035** [0.0973]	80.08 **[2.8]**
"Tine" NIL 1999	**1.9194** [0.1004]	83.07 **[2.9]**
"Plough" NIL 1999	**1.9608** [0.0946]	91.38 **[3.1]**
"Tine" F/3 1999	**1.9546** [0.1554]	90.08 **[3.1]**
"Plough" F/3 1999	**1.8904** [0.0835]	77.70 **[2.7]**
COMBINED S.E. ±	**0.1046**	
L.S.D.	**0.3054**	

* = Ratio to LLUD susceptible standard

1 (b). Influence of dose rate, herbicide rotations, mixtures and sequences on resistance in wild-oats

Introduction

The aim of this experiment was to determine whether these factors affect the rate of evolution of resistance in wild-oats. There are different opinions as to whether reduced herbicide rates favour the development of resistance and to what extent mixtures and sequences affect this process. There is little scientific evidence available but the rate of development of resistance is clearly of critical importance in the long-term prevention and management of resistant populations. This experiment was conducted in large outdoor containers in which a range of different herbicide treatments were applied to partially resistant wild-oats.

Materials and Methods

Container experiments

These experiments were established at ADAS Boxworth and IACR-Rothamsted in 100 large plastic containers at each site and ran for five years. The containers, soil used and method of sowing were the same as described in section 1 (a) above. Seeds were sown between 6-11 October each year.

For each site, five containers were sown with a standard susceptible population of *Avena sterilis* ssp. *ludoviciana* (LLUD95) per replicate and 20 containers per replicate were sown with a partially resistant population of the same species (T/11 1995). This was the same population described in section 1 (a) above. There were four replicates in a randomised block design at both Boxworth and Rothamsted. At Rothamsted containers were kept outside on the surface of a sandbed and at Boxworth boxes were kept on an outdoor hardstanding area. The containers were moved into sets comprising the same population and treatment in May each year to minimize any potential cross pollination. A minimum of 1m was maintained between sets - wild-oats are a self-pollinating species so cross-pollination was highly unlikely.

Containers were resown each autumn with T/11 seed collected from the same treatment that summer. Containers with LLUD susceptible seed were sown each year with seed from the same original sample to act as reference.

The following 20 treatments were applied to the partially resistant T/11 population:

Code.

1 Nil Untreated control

2. T   Tri-allate (2.25 kg a.i./ha) as Avadex Excel 15G granules @ 15 kg/ha (15% w/w a.i.)

3. t   Tri-allate (1.125 kg a.i./ha) as Avadex Excel 15G granules @ 7.5 kg/ha

4. F   Fenoxaprop-P-ethyl (55 g a.i./ha) as Cheetah Super @ 1 l/ha (55 g/l a.i.)

5. f   Fenoxaprop-P-ethyl (27.5 g a.i./ha) as Cheetah Super @ 0.5 l/ha

6. D   Difenzoquat (750 g a.i./ha) as Avenge 2 @ 5 l/ha (150 g/l a.i.)

7. d   Difenzoquat (375 g a.i./ha) as Avenge 2 @ 2.5 l/ha

8. I   Imazamethabenz-methyl (600 g a.i./ha) as Dagger @ 2 l/ha (300 g/l a.i.) plus "Agral"

9. i   Imazamethabenz-methyl (300 g a.i./ha) as Dagger @ 1 l/ha plus "Agral"

10. X   Tralkoxydim (350 g a.i./ha) as Grasp @ 1.4 l/ha (250 g/l a.i.) plus "Output" adjuvant

11. x   Tralkoxydim (175 g a.i./ha) as Grasp @ 0.7 l/ha plus "Output" adjuvant

12. X**R**D Tralkoxydim (350 g a.i./ha) or difenzoquat(750 g a.i./ha)

13. x**R**d Tralkoxydim (175 g a.i./ha) or difenzoquat (375 g a.i./ha)

14. F**R**D Fenoxaprop-P-ethyl (55 g a.i./ha) or difenzoquat (750 g a.i./ha)

15. f**R**d   Fenoxaprop-P-ethyl (27.5 g a.i./ha) or difenzoquat (375 g a.i./ha)

16. f+x Fenoxaprop-P-ethyl (27.5 g a.i./ha) as Cheetah Super @ 0.5 l/ha + tralkoxydim
         (175 g a.i./ha) as Grasp @ 0.7 l/ha plus "Output" adjuvant

17. f+i Fenoxaprop-P-ethyl (27.5 g a.i./ha) as Cheetah Super @ 0.5 l/ha + imazamethabenz-methyl (300 g
         a.i./ha) as Dagger @ 1 l/ha plus "Agral"

18. f+d Fenoxaprop-P-ethyl (27.5 g a.i./ha) as Cheetah Super @ 0.5 l/ha + difenzoquat (375
         g a.i./ha) as Avenge 2 @ 2.5 l/ha

19 . T+f Tri-allate (2.25 kg a.i./ha) as Avadex Excel 15G granules @ 15 kg/ha + fenoxaprop-P-
         ethyl (27.5 g a.i./ha) as Cheetah Super @ 0.5 l/ha

20. t+f Tri-allate (1.125 kg a.i./ha) as Avadex Excel 15G granules @ 7.5 kg/ha + fenoxaprop-P-ethyl (27.5
         g a.i./ha) as Cheetah Super @ 0.5 l/ha

Note: Treatments 12-15: R indicates a herbicide in a rotation with a different herbicide treatment in alternate years. The treatment in bold and underlined denotes that which was applied in this fourth year, 1998/99.

The five containers of the LLUD population per replicate were treated with the full rate of either triallate, fenoxaprop, difenzoquat, imazamethabenz and tralkoxydim (treatment numbers 2, 4, 6, 8, 10).

Tri-allate granules were applied to appropriate boxes using a "pepperpot" in October or November each year (except the first year - January). Other herbicide treatments were applied between mid February and mid April each year (usually in mid March) when wild-oat plants were at the 1 - 4 tiller stage, and wheat plants had 2 - 4 tillers. At both sites a laboratory pot sprayer was used delivering 220 - 273 litres water/ha at 210 kPa through a single flat fan nozzle.

The following assessments were made on the wild-oats each year: number of plants before spraying; number of surviving plants (May); panicles per container (June); spikelets per panicle on a random 10 panicles/container (late June/early July). Spikelets (the dispersal unit of wild-oats) were collected from each container on several occasions during July each year, air dried and stored in envelopes. The number of viable seeds per spikelet was assessed on a random 25 spikelets per box taken from the bulked samples by squeezing seeds to determine whether they contained a caryopsis. The panicle, spikelets per panicle and seeds per spikelet data was used to determine the seed production per container.

Results obtained were analysed using two-way analysis of variance in randomised blocks using the GENSTAT statistical package.

Glasshouse evaluation

After the completion of the container experiments described above, seeds from both container experiments were evaluated in a single glasshouse dose response assay to determine more critically whether any change in degree of resistance had occurred. The following 18 populations were used: LLUD 1995 (susceptible standard); T/11 1995 (original population used at the start of the container experiment at both sites); the T/11 Nil, f, F, x, X, f+x, i, I samples collected in 2000 at both sites (see herbicide list in previous section for codes). By 2000 herbicide treated T/11 seeds samples had received five years of further selection with annual applications of herbicide.

Approximately 80 seeds were placed in each petri-dish containing three normal filter papers and one glass fibre paper; 8 - 20 dishes were prepared for each population. The seeds were treated with 9ml of deionised water on 27 October 2000 and incubated at $17^{\circ}C$ 14-hour day, $11^{\circ}C$ 10-hour night. On 1 - 3 November 2000 germinated seeds were sown in 5cm square pots containing compost (1 seed per pot, 208 - 520 pots per population).

The experiment comprised a fully randomised design with 16 replicate pots per herbicide dose and 32 untreated pots per population. Fenoxaprop-P-ethyl was applied at eight doses (3.44 - 440 g a.i./ha) to LLUD 1995, T/11 1995, T/11 2000 Nil, f, F and f+x; tralkoxydim (+"Output") at nine dose (5.47 - 1400 g a.i./ha) to LLUD 1995, T/11 1995, T/11 2000 Nil, x, X and f+x; imazamethabenz (+ "Agral") at 10 doses (18.75 - 9600

g a.i./ha) to LLUD 1995, T/11 1995, T/11 2000 Nil, i, and I. Herbicides were applied at the 3 leaf stage on 22 November 2000 using a laboratory sprayer delivering 265 litres water/ha at 210kPa through a single "Teejet" 110015VK ceramic nozzle. Herbicide activity was recorded on 14 - 18 December 2000, 22 - 26 days after spraying, by assessing foliage fresh weight for each individual pot.

Dose response data was analysed using a logistic relationship between foliage fresh weight and log_{10} dose and $log_{10}ED_{50}$ values were determined (Ross, 1987). The ED_{50} values (herbicide rate required to reduce fresh weight by 50% relative to the no-herbicide controls) referred to in the text and tables have been detransformed from the log_{10} values.

Results

Container experiments

- On average over 90% wheat plants emerged at both sites and there was no obvious evidence of crop damage from any herbicide treatment.

- There were usually 45 - 80 wild-oat plants per container at time of spraying. On average 69 (Boxworth) or 98 (Rothamsted) panicles were produced in each untreated container. The mean emergence and establishment of T/11 wild-oat plants was 49% at Boxworth and 56% at Rothamsted, based on 120 seeds sown per container. This slightly lower emergence and panicle number at Boxworth was probably a result of the greater exposure of the containers to the elements. At both sites the % emergence of seeds tended to decline over the five year period from 57 - 67% in the first year to 43 - 51% in the fifth year. This decline may be linked to seed born fungal infection, *Pyrenophora avenae* (seedling blight). The emergence of LLUD 95 seeds averaged 53% of seeds sown.

- The % reduction in plant numbers (Table 4) are based on the numbers surviving herbicide treatment (post-spray) compared with those present pre-spraying for the same container. The % reduction in seed return values (Table 5; Figure 3) are based on herbicide treatment figures compared with untreated containers.

- The full rate treatments of fenoxaprop and tralkoxydim gave excellent control of the susceptible LLUD population in terms of seed return – a consistent result at both sites in virtually every year. With one exception, 96 - 100% control of seed return was achieved with the both herbicides at both sites. Very good control in terms of reduction in plant numbers was also achieved consistently. This demonstrates that application and environmental conditions were conducive to good herbicide activity. Control of LLUD by difenzoquat (average 84%, omitting one negative value) and imazamethabenz (88%) was more variable, but control was noticeably better in terms of seed return than control of plants. Tri-allate gave generally poor to mediocre control at both sites.

- The higher rates of fenoxaprop and tralkoxydim gave poorer control of the T/11 population than the LLUD susceptible standard, both in terms of plants and seed return, in every year at both sites. The level of control of seed return has been: fenoxaprop 46%, 87%, 49%, 67% 92% (Rothamsted); 39%, 83%, 92%, 81%, 77% (Boxworth); tralkoxydim 62%, 93%, 46%, 58%, 94% (Rothamsted), 23%, 63%, 81%, 87%, 77% (Boxworth). These levels are consistently poorer than for the susceptible LLUD but there has been no clear evidence for any decline in herbicide performance on the T/11 population. This confirms not only that T/11 shows resistance to both herbicides, but that the presence of resistance is likely to increase the variability in herbicide activity between years.

- Imazamethabenz at full rate gave mediocre control of T/11 seed return at both sites: 35 - 88% at Rothamsted, 22 - 75% at Boxworth. This confirms that T/11 shows resistance to imazamethabenz but with no clear trend for rapidly increasing level of resistance. In most years, imazamethabenz gave the poorest control of T/11 of all the four post-emergence herbicide treatments.

- The most effective treatment on T/11 was full rate difenzoquat (D) which gave an average of 89% reduction in seed return (range = 63 - 100%). This was similar to the control of the LLUD susceptible standard (84%), indicating that the T/11 population does not show resistance to this herbicide, unlike all the other post-emergence treatments. Full rate difenzoquat gave the best control of any single herbicide treatment in every year at both sites, but half rate difenzoquat has been less consistent than the full rate, sometimes giving appreciably poorer level of control.

- Levels of control were not consistently higher or lower at one site or the other. For example in year 4, in terms of reduction in seed return, levels of control were higher at Rothamsted than at Boxworth for 13 of the 19 T/11 herbicide treatments. In the previous year, (year 3), the opposite trend occurred with 14 treatments giving higher control at Boxworth. At Rothamsted fenoxaprop at full rate gave the highest level of control of T/11 seed return in year 5, as also occurred on the other container experiment described in section 1(a). In contrast, at Boxworth, fenoxaprop gave the second lowest control of seed return in year 5. This indicates that site to site differences do occur, perhaps due to environmental conditions, but not necessarily in a consistent manner.

- The control of T/11 achieved by the rotational difenzoquat treatments, applied in years 2 and 4, and the rotational fenoxaprop and tralkoxydim treatments, applied in years 1, 3 & 5, was broadly similar to the corresponding rates applied alone. Control tended to be better in years when difenzoquat was used, especially at the full rate.

- Tri-allate at both rates gave poor control of T/11 at both sites. However, there was no evidence that it was affected by resistance as control of T/11 plants (mean 35%) was similar to control of the susceptible LLUD (37%). Control of T/11 seed return by the sequence of tri-allate followed by half rate fenoxaprop (T+f) was appreciably better than triallate alone, but still generally poorer than full rate fenoxaprop alone. Half rate triallate followed by half rate fenoxaprop gave mediocre control, 18 - 67% reduction in seed return.

- The higher rates of tri-allate, fenoxaprop, difenzoquat, imazamethabenz and tralkoxydim generally gave higher levels of control than half rates, as expected. However, there was no consistent trend for specific herbicides to be relatively more affected by resistance at reduced doses. Activity of fenoxaprop and difenzoquat was sometimes markedly poorer at half than at full rate. The smallest differences between full and half rates have occurred with tralkoxydim - a consistent finding at both sites.

- Mixtures of low rate fenoxaprop plus low rate imazamethabenz (f+i) showed clear evidence of antagonism at both sites. In the eight comparisons, this mixture gave an average 36% reduction in seed return, which was 19% less (range -37 to + 3%) than would be predicted from the purely additive effects of the efficacies of the individual components, f and i (55%). Control was poorer than expected in seven of the eight comparisons.

- Mixtures of low rate fenoxaprop plus low rate tralkoxydim (f+x) sometimes showed evidence of synergistic interaction, but this was not consistent. For example in year 4 this mixture gave appreciably higher levels of control of seed return (Rothamsted 79%; Boxworth 90%) than would be predicted from the purely additive effects of the efficacies of the individual components (56%, 43%). In the 10 comparisons, this mixture (f+x) gave an average 75% reduction in seed return, which was 4% more (range -37 to + 47%) than would be predicted from the purely additive effects of the efficacies of the individual components, f and x (71%). Control was better than expected in six of the ten comparisons.

- Mixtures of low rate fenoxaprop and low rate difenzoquat appeared to give a mainly additive effect in most cases. In the eight comparisons, this mixture gave an average 60% reduction in seed return, which was 8% less (range -30 to + 3%) than would be predicted from the purely additive effects of the efficacies of the individual components, f and d (68%). In six of the eight comparisons, the actual efficacy was within 3% of that predicted. Control was poorer than expected in five of the eight comparisons.

- There was no clear trend for overall declining herbicide performance during the five years of the experiment at either site. The results for fenoxaprop, tralkoxydim and imazamethabenz for T/11 compared with the susceptible LLUD standard confirm that resistance occurs in T/11, but there was no clear evidence for an increasing level of resistance despite repeated annual applications of herbicides.

Glasshouse evaluation

- The mean foliage weights for the untreated pots were in the range 2.92 - 4.01 g for the Rothamsted samples and 3.58 - 5.06 g for the Boxworth samples, which were harvested slightly later. This indicates broadly similar growth in the absence of herbicides.

- In Table 6, the $\log_{10}ED_{50}$ values are presented together with individual and pooled standard errors. The detransformed ED_{50} values are also presented with the ratio of these values to the LLUD 1995

susceptible standard. These ratios provide a simple form of "Resistance Index" for comparing responses. The greater the value, the higher the degree of resistance. Dose responses are shown in Figures 4 & 5.

- The ED_{50} value for the T/11 1995 population used at the start of the container experiment was significantly (P<0.05) higher than the LLUD susceptible standard for all three herbicides, fenoxaprop, tralkoxydim and imazamethabenz. The Resistance Indices were 2.0, 91.5 and 28.1 respectively confirming that the T/11 1995 initial population showed partial resistance to all three herbicides.

- The ED_{50} values for the T/11 population treated with both rates of fenoxaprop (f and F), tralkoxydim (x and X) and imazamethabenz (i and I) were not significantly higher than the T/11 1995 initial population at either site. Indeed, the Resistance Indices were similar to the T/11 1995 population: fenoxaprop 2.1 - 2.5; tralkoxydim 57 -117; imazamethabenz 15.6 - 26.5. This indicates that there had been no further evolution of resistance despite five years annual applications of herbicide. There was also no difference between annual use of full rate as against half rate.

- Similarly the half rate mixture of fenoxaprop and tralkoxydim (f + x) had also not resulted in any increase in resistance to either component for either the Rothamsted or Boxworth T/11 selected populations: fenoxaprop 2.0 and 1.9; tralkoxydim 73 and 89.

- The T/11 Nil populations had been grown on for five years without receiving any herbicide, seeds being resown each year in new containers which did not get treated. There was no evidence of deselection, or decline in resistance level at either site with any herbicide. The ED_{50} values were not statistically different (P≥0.05) to the T/11 1995 initial population for either site or to any of the populations treated with fenoxaprop, tralkoxydim or imazamethabenz for five years. The Resistance Indices were: fenoxaprop (2.2 and 1.7); tralkoxydim (84.7 and 86.4); imazamethabenz 26.2 and 17.6 for Rothamsted and Boxworth T/11 Nil samples respectively.

- The resistance indices for all the T/11 populations were broadly similar for each herbicide, but there were large difference between herbicides: fenoxaprop 1.7 to 2.5; tralkoxydim 57.1 - 117.3; imazamethabenz 17.6 - 28.1. However, in the container experiment (see previous section), full rate fenoxaprop and tralkoxydim gave broadly similar levels of control of T/11, with each herbicide giving better control than the other in five of the 10 comparisons. Imazamethabenz usually gave poorer control, with both fenoxaprop and tralkoxydim giving better control in seven of the 10 comparisons. Consequently the resistance indices do not give a good indication of the likely impact of resistance on herbicide performance with tralkoxydim, in particular, giving very high indices which exaggerate the likely loss of efficacy. Comparisons of ED_{50} values and resistance indices between populations treated with the same herbicide should be valid, but care is needed in using these parameters to compare herbicides.

Conclusions

The container system worked well and appears to provide a good model system to simulate field conditions. The establishment of wild-oats declined at both sites during the five years of the experiment, and this seems to be associated with an increase in seed born fungal pathogens, especially *Pyrenophora avenae* (seedling blight). However, there was no evidence that this affected relative herbicide efficacy.

Consistently excellent (96 - 100%) control of the LLUD susceptible standard was achieved at both sites by fenoxaprop and tralkoxydim, showing that the experimental system, application method and environmental conditions were conducive to good control of susceptible wild-oats in every year. Difenzoquat and imazamethabenz gave more variable control averaging 84 - 88%.

In comparison with the good control of the susceptible LLUD, the generally moderate control of the partially resistant T/11 population by most herbicides could only be due to resistance. Although resistance of the T/11 population to fenoxaprop, tralkoxydim and imazamethabenz was clearly demonstrated, the actual level of control achieved varied considerably between sites and years. Thus reduction in seed return achieved by full rate fenoxaprop varied from 39% (Box. yr 1) to 92% (Box. yr 3 & Roth. yr 5), for tralkoxydim from 23% (Box. yr 1) to 94% (Roth. yr 5), and for imazamethabenz from 22% (Box. yr 4) to 88% (Roth. yr 5). Year to year and site to site differences would probably be even larger in actual fields and are most likely caused by uncontrollable climatic/environmental differences whose effects on herbicide activity are poorly understood. This highlights the difficulty of detecting relatively small shifts in sensitivity to herbicides on a year to year basis, as such differences are likely to be overshadowed by environmental effects on herbicide activity.

For single herbicide treatments, the best control in terms of reductions in seed return of the partially resistant T/11 population was achieved by full rate difenzoquat at both sites, with an average 89% seed reduction. Difenzoquat was the most consistent herbicide at both sites against T/11 over the five years, although it was usually less effective than fenoxaprop and tralkoxydim on the susceptible LLUD population. Difenzoquat achieved a similar level of control of the susceptible LLUD (84%). Consequently there was no evidence of resistance to difenzoquat in the T/11 population, in contrast to fenoxaprop, tralkoxydim and imazamethabenz. Triallate gave generally poor control of both LLUD and T/11, but there was no evidence that its activity was affected by resistance.

Although the experiments were not specifically designed to study antagonism and synergy between herbicides, some effects were evident. There was an antagonistic effect between fenoxaprop and imazamethabenz, a generally neutral (purely additive effect) between fenoxaprop and difenzoquat and also a generally neutral, but sometimes synergistic effect, between fenoxaprop and tralkoxydim.

Increasing levels of resistance should be associated with declining levels of herbicide activity. However, there was no clear trend for overall declining herbicide performance at either site during the five years of the experiments. The results for fenoxaprop, tralkoxydim and imazamethabenz for T/11, compared with the susceptible LLUD standard, confirm that resistance occurs in T/11, but there was no clear evidence for increasing levels of resistance despite repeated annual herbicide applications and an apparently appreciable degree of selection in terms of plant kill each year.

Resistance is an evolutionary process, resulting from a gradual change in sensitivity at the population level. This highlights the need to make detailed comparisons under more controlled conditions. Consequently seeds collected from different treatments after five years, were used in a glasshouse dose response assay to determine more critically whether there had been any change in response to herbicides. Seed of the original T/11 population used to sow up the containers in 1995 were used as a baseline. The glasshouse assay confirmed that there had been no change in level of resistance after five years annual use of fenoxaprop, tralkoxydim and imazamethabenz. There was also no difference between annual use of full as against half rate. Nor was there any evidence for deselection in the absence of herbicides for five years. Surprisingly, resistance was maintained at the same level as at the start of the experiment, as also occurred in the container experiment in section 1 (a).

It is important to note that the population used, T/11 is now known to be resistant due to an enhanced ability to metabolise herbicides (Cocker *et al*. 2000). The resistance mechanism was not known when the experiment commenced. Other resistance mechanisms exist in wild-oats, notably target site resistance (insensitive ACCase). It is possible that resistance will build up faster in populations which possess other resistance mechanisms, and this is currently under investigation.

Influence of dose rate, herbicide rotations, mixtures and sequences on resistance in wild-oats

1 (b). Container experiments

Table 4. Percentage reduction in plant numbers

T/11 results

Treatment	Year one 1995/96		Year two 1996/97		Year three 1997/98		Year four 1998/99		Year five 1999/2000	
	Roth.	Box.	Roth.	Box.	Roth.	Box.	Roth.	Box.	Roth.	Box.
Nil	0.3	38.9	27.0	21.5	10.6	48.2	19.3	Not	17.1	37.6
T	6.3	38.6	9.6	25.3	22.3	60.2	42.4	counted	42.7	67.1
t	10.5	39.2	6.4	43.7	14.7	63.3	24.0		-	-
F	24.5	58.9	55.9	46.5	40.8	82.0	48.2		90.8	85.0
f	15.6	41.5	51.5	30.2	21.9	51.2	45.6		65.1	67.8
D	60.0	68.9	58.8	47.2	49.6	88.2	84.3		70.2	-
d	31.7	42.5	46.7	24.7	5.4	68.3	64.9		73.8	85.2
I	20.1	51.4	45.4	25.1	24.0	69.2	49.6		70.4	80.3
i	20.5	43.2	43.2	32.8	6.9	66.7	48.1		44.8	69.7
X	19.0	45.8	49.9	57.1	32.4	71.8	54.1		63.7	78.7
x	30.0	34.9	49.9	43.1	6.0	62.5	57.8		55.5	74.6
XRD	17.4	40.5	57.3	20.2	(31.6)	72.4	92.0		-	-
xRd	12.3	46.1	40.0	15.6	28.9	68.9	76.1		66.9	86.4
FRD	18.2	57.5	59.2	27.4	(82.3)	86.5	89.7		-	-
fRd	16.1	47.3	43.9	22.6	27.8	66.6	71.9		65.4	75.9
f + x	40.5	50.8	53.4	51.5	17.1	71.6	67.9		88.8	69.2
f + i	16.3	45.3	39.8	30.4	14.6	54.1	45.8		-	-
f + d	30.5	40.4	47.7	32.7	31.3	78.0	79.1		-	-
T + f	32.7	57.9	27.9	55.0	15.0	61.2	53.9		65.6	82.2
t + f	14.3	43.6	41.8	51.5	10.1	60.4	49.8		-	-
S.E.M.	2.644	5.64	5.06	5.56	7.39	5.63	5.06		4.47	5.36
d.f.	57	57	55	57	51	47	53		37	36

LLUD results

Treatment	Year one 1995/96		Year two 1996/97		Year three 1997/98		Year four 1998/99		Year five 1999/2000	
	Roth.	Box.	Roth.	Box.	Roth.	Box.	Roth.	Box.	Roth.	Box.
T	10.6	55.8	19.9	31.9	42.2	56.6	25.4	Not	30.1	60.0
F	99.6	100.0	99.6	75.4	98.2	99.4	99.6	Counted	100.0	100.0
D	52.9	53.6	49.5	17.8	36.9	69.1	64.8		53.7	72.1
I	83.1	100.0	54.8	9.8	18.9	76.9	41.9		96.8	48.3
X	100.0	91.9	99.2	98.1	98.4	99.6	99.1		100.0	100.0

Influence of dose rate, herbicide rotations, mixtures and sequences on resistance in wild-oats

1 (b). Container experiments

Table 5. Percentage reduction in seed return

T/11 results

Treatment	Year one 1995/96		Year two 1996/97		Year three 1997/98		Year four 1998/99		Year five 1999/2000	
	Roth.	Box.	Roth.	Box.	Roth.	Box.	Roth.	Box.	Roth.	Box.
Nil	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0
T	23.1	31.2	52.0	6.8	43.1	14.0	30.3	-14.8	30.7	23.8
t	17.2	34.6	15.0	36.8	29.1	14.9	4.5	2.7	-	-
F	45.5	38.8	87.2	82.6	49.3	92.1	66.8	80.9	92.4	77.0
f	14.0	26.6	60.2	23.8	29.2	59.4	29.3	15.4	73.8	68.6
D	96.2	62.9	100.0	88.8	71.0	97.9	99.7	88.0	99.3	-
d	34.6	37.8	98.1	80.8	51.1	44.9	83.3	13.8	89.6	46.1
I	54.8	55.3	67.2	46.6	35.1	73.8	40.2	21.6	88.2	75.0
i	20.1	28.8	53.8	44.2	14.7	50.7	37.3	35.6	76.2	27.6
X	62.2	23.0	93.4	63.3	46.1	81.0	58.2	86.9	93.5	77.4
x	58.6	34.5	80.2	63.8	36.2	61.7	38.0	32.1	91.1	73.0
XRD	68.3	44.2	99.8	97.0	(61.7)	81.5	100.0	100.0	-	-
xRd	55.4	39.2	95.0	73.0	45.1	61.6	78.5	-5.9	86.2	76.2
FRD	55.8	32.7	100.0	97.3	(94.6)	94.3	97.8	90.1	-	-
fRd	23.7	34.3	90.5	89.5	53.5	58.5	94.1	73.8	70.6	61.7
f + x	70.5	50.8	85.6	73.8	65.2	85.6	78.5	90.0	94.3	55.3
f + i	21.9	10.5	61.4	40.3	43.3	46.0	43.4	22.5	-	-
f + d	43.8	24.6	97.6	59.7	62.5	73.9	88.6	30.0	-	-
T + f	21.0	46.7	81.9	86.6	74.8	72.8	48.4	66.1	90.6	75.5
t + f	21.8	18.0	65.1	66.5	44.6	65.0	29.9	43.6	-	-
S.E.M.	5.16	8.23	5.07	8.60	6.57	4.90	7.62	16.65	3.98	14.126
d.f.	54	54	52	54	48	44	50	49	34	33

LLUD results

Treatment	Year one 1995/96		Year two 1996/97		Year three 1997/98		Year four 1998/99		Year five 1999/2000	
	Roth.	Box.	Roth.	Box.	Roth.	Box.	Roth.	Box.	Roth.	Box.
T	38.0	18.0	56.0	8.1	73.6	28.3	15.7	-112.0	18.3	-89.7
F	100.0	100.0	100.0	96.3	99.6	99.9	100.0	96.9	97.9	100.0
D	99.0	78.3	100.0	91.2	72.0	82.1	90.1	-14.2	91.6	49.5
I	100.0	100.0	100.0	97.9	96.0	94.8	78.2	35.9	96.7	77.9
X	100.0	98.0	100.0	100.0	99.8	100.0	97.4	66.3	100.0	100.0

Figure 3. Influence of dose rate, herbicide rotations, mixtures and sequences on resistance in wild-oats

1 (b). Container experiments

Percentage reduction in T/11 seed return for fenoxaprop

Percentage reduction in T/11 seed return for tralkoxydim

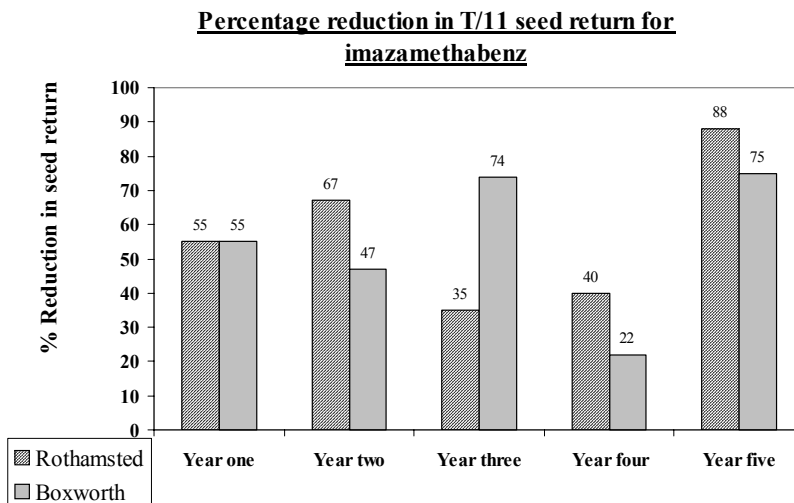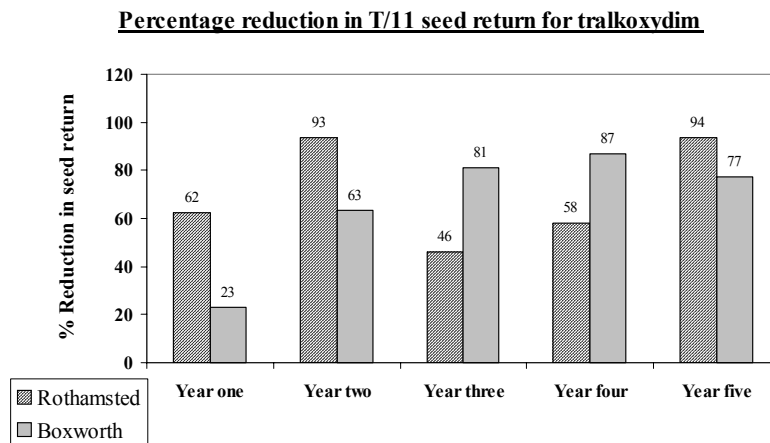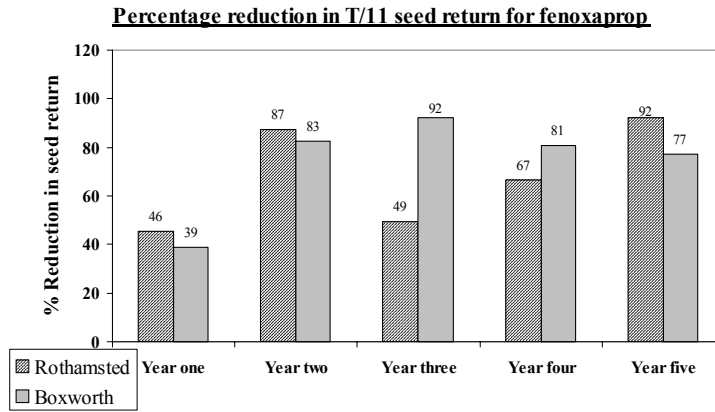**Percentage reduction in T/11 seed return for imazamethabenz**

Table 6. Influence of dose rate, herbicide rotations, mixtures and sequences on resistance in wild-oats

1 (b). Glasshouse dose response evaluation

Fenoxaprop

Population	Log_{10} ED$_{50}$ values		Detransformed ED$_{50}$ values [Resistance index*]	
	Rothamsted	Boxworth	Boxworth	Boxworth
LLUD 1995 Susceptible	1.2930	1.2930	19.63 [1.0]	19.63 [1.0]
T/11 1995 Original	1.5835	1.5835	38.32 [2.0]	38.32 [2.0]
T/11 f 2000	1.6167	1.6195	41.37 [2.1]	41.64 [2.1]
T/11 F 2000	1.6421	1.6891	43.87 [2.2]	48.87 [2.5]
T/11 f+x 2000	1.6014	1.5645	39.94 [2.0]	36.68 [1.9]
T/11 Nil 2000	1.6256	1.5210	42.22 [2.2]	33.19 [1.7]
Combined S.E.+	0.1038	0.1052	-	-
L.S.D. (p$\leq$0.05)	0.2877	0.2916	-	-

* = Ratio to LLUD susceptible standard

Tralkoxydim

Population	Log_{10} ED$_{50}$ values		Detransformed ED$_{50}$ values [Resistance index*]	
	Rothamsted	Boxworth	Boxworth	Boxworth
LLUD 1995 Susceptible	0.1752	0.1752	1.5 [1.0]	1.5 [1.0]
T/11 1995 Original	2.1375	2.1375	137.2 [91.5]	137.2 [91.5]
T/11 x 2000	2.1538	1.9325	142.5 [95.0]	85.6 [57.1]
T/11 X 2000	2.2455	2.1219	176.0 [117.3]	132.4 [88.3]
T/11 f+x 2000	2.0392	2.1263	109.5 [73.0]	133.7 [89.2]
T/11 Nil 2000	2.1039	2.1127	127.0 [84.7]	129.6 [86.4]
Combined S.E.+	0.1538	0.1443	-	-
L.S.D. (p$\leq$0.05)	0.4262	0.4001	-	-

* = Ratio to LLUD susceptible standard

Table 6 (continued). Influence of dose rate, herbicide rotations, mixtures and sequences on resistance in wild-oats

1 (b). Glasshouse dose response evaluation

Imazamethabenz

Population	Log_{10} ED$_{50}$ values		Detransformed ED$_{50}$ values [Resistance index*]	
	Rothamsted	Boxworth	Boxworth	Boxworth
LLUD 1995 Susceptible	2.0076	2.0076	101.8 [1.0]	101.8 [1.0]
T/11 1995 Original	3.4568	3.4568	2863.0 [28.1]	2863.0 [28.1]
T/11 i 2000	3.3916	3.2006	2463.9 [24.2]	1586.9 [15.6]
T/11 I 2000	3.4311	3.2746	2698.1 [26.5]	1881.9 [18.5]
T/11 Nil 2000	3.4262	3.2539	2668. [26.2]	1794.4 [17.6]
Combined S.E.+	0.0981	0.0838	-	-
L.S.D. (p<0.05)	0.2720	0.2323	-	-

* = Ratio to LLUD susceptible standard

Figure 4. Influence of dose rate, herbicide rotations, mixtures and sequences on resistance in wild-oats

1 (b). Glasshouse dose response evaluation: Rothamsted Site

Response to Fenoxaprop

Response to Tralkoxydim
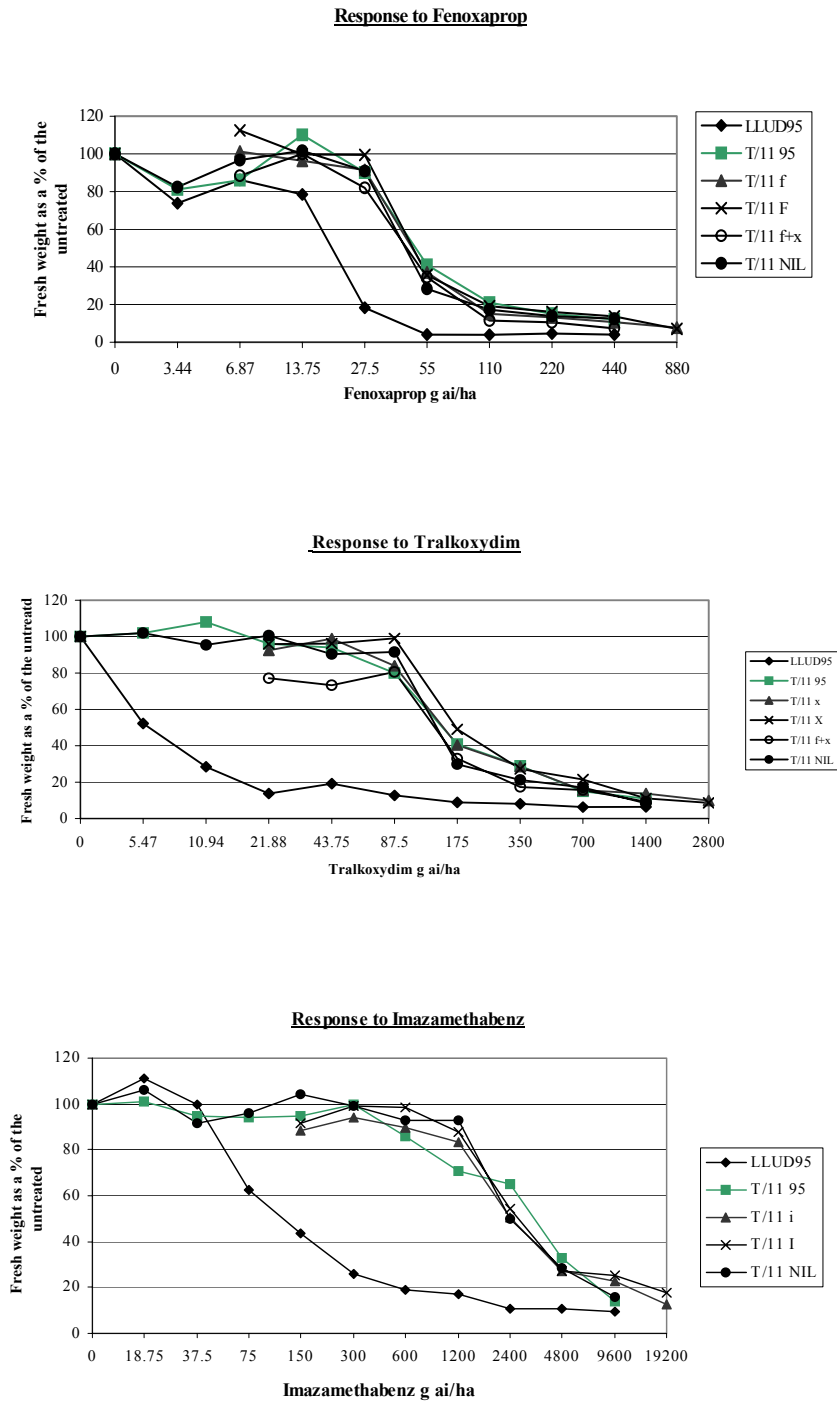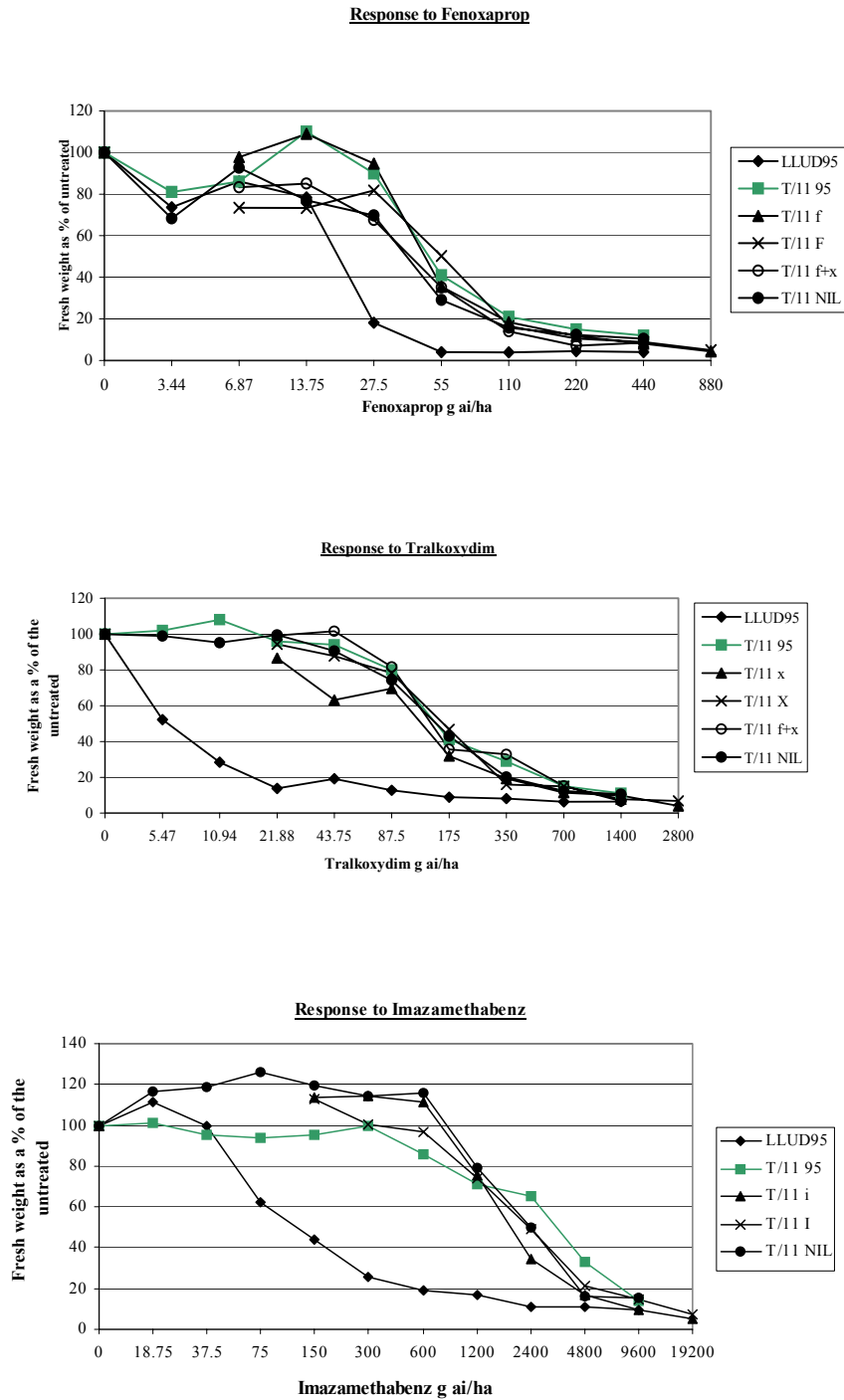

Response to Imazamethabenz

Figure 5.   Influence of dose rate, herbicide rotations, mixtures and sequences on resistance in wild-oats

1 (b). Glasshouse dose response evaluation: Boxworth Site

Response to Fenoxaprop

Response to Tralkoxydim

Response to Imazamethabenz

1 (c). Influence of resistance on variation in herbicide performance
  in different years

Introduction

Many factors affect herbicide activity, apart from resistance. It can be difficult to determine to what degree poor control from a herbicide is due to resistance as compared with environmental and other factors which vary from one year to the next. Herbicide resistance may often only result in reduced activity, rather than no activity from herbicides.   Consequently experiments were conducted in outdoor containers under standardised conditions in order to see how the performance of a herbicide varied from year to year when used on a partially resistant and a susceptible population. Soil and wild-oat population were identical each year, so these factors were kept constant.

Materials and Methods

This experiment was set up between 1 - 13 October each year for four successive years, 1995 - 1998 at both Rothamsted and Boxworth using 40 plastic trays per site. Each tray (27 x 18 x 10 cm deep) was filled to within 3cm of the top using the same soil mix as detailed in sections 1 (a and b) above. Four rows, each of 12 wild-oat seeds, were sown in each tray. Twenty trays were sown with a partially resistant population of wild-oats (T/11 1995) and 20 trays with a standard susceptible population of the same species (LLUD 1995). These were the same populations as used in sections 1 (a and b) above, but exactly the same populations were sown each year, using the original 1995 seed samples. No wheat seed was sown. Soil was then added to cover seeds to 2.5 cm depth. The trays were then sunk into an outdoor sandbed at Rothamsted or placed on a concrete hardstanding area at Boxworth.

The treatments comprised: two populations (T/11, LLUD) x (untreated + 4 herbicide rates) x 4 replicates = 40 trays. The herbicide treatments comprised four rates of fenoxaprop-P-ethyl: 6.875, 13.75, 27.5, 55 g a.i./ha, representing 1/8, ¼, ½ and full field rate.

Herbicide treatments were applied between mid February and mid April at both locations when wild-oat plants were at the 2 - 3 tiller stage and mainly 10 - 15 cm tall. Laboratory pot sprayers were used delivering 220 - 275 litres water/ha at 210 kPa through single flat fan nozzles. The following assessments were made on the wild-oats: number of plants before spraying; number of surviving plants and total foliage fresh and dry weight (mid April - early June).

Results (Tables 7 & 8; Figures 6 & 7)

- The emergence/survival over winter was better for T/11 than LLUD at both sites in all four years. The mean number of wild-oat plants emerged per tray prior to spraying was 35 (R) and 29 (B) for T/11 and 28 (R) and 23 (B) for LLUD. This represents an emergence/survival rate of 73% (R) or 61% (B) of sown T/11 seeds and 59% (R) or 48% (B) of LLUD seeds. The emergence rates at both sites have shown no evidence of a decline in emergence as seed ages. Emergence at Boxworth has tended to be lower than at Rothamsted, probably due to the more exposed location of the site.

- The % reduction in plant numbers is based on the numbers surviving herbicide treatment (post-spray) compared with those present pre-spraying for the same tray. The % reduction in foliage weight values are based on herbicide treatment figures compared with untreated trays.

- The full rate (55 g a.i./ha) of fenoxaprop-P-ethyl gave very good control of plants of the susceptible LLUD population at both sites in all years (98 - 100%). This was a consistent result at both sites in all four years. The half rate (27.5 g a.i./ha) also gave very good control of LLUD plants at both sites in most years (mainly > 94%), but has occasionally given poorer control, giving only 61% reduction in plant numbers in the first year at Boxworth.

- The 1/8 (6.875 g a.i./ha) field rate always gave poor to mediocre control of the LLUD susceptible standard, (maximum 42% reduction in plant numbers). However, the control achieved by the ¼ rate (13.75 g a.i./ha) has been variable from year to year, ranging from zero reduction in plant numbers in year one at Boxworth, up to 98% reduction at Rothamsted in year four.

- Effects on the vigour of wild-oat plants, as measured by foliage weight, were often greater than the effects on plant number would indicate. This shows that many of the plants surviving herbicide were damaged. The assessment of fresh and dry foliage weights gave generally similar results in terms of % reduction relative to untreated trays, so only fresh weight data is presented here.

- The foliage weight assessments confirmed that the susceptible LLUD was generally well controlled by full or half rate fenoxaprop-P-ethyl at both sites whereas control of the partially resistant T/11 was poor to moderate at both sites at all rates. Control of T/11 by the full rate of fenoxaprop was roughly equivalent to that achieved by ⅛ to ¼ rate on the susceptible LLUD. This has been a consistent finding at both sites in all four years.

- In all years at both sites, full rate fenoxaprop-p-ethyl (55g a.i./ha) gave good (>94%) control of the susceptible standard, LLUD, based on foliage fresh weight data (Rothamsted: 97%, 97%, 94%, 97%; Boxworth: 94%, 95%, 98%, 97%). Half rates also gave very good control in most instances: (Rothamsted: 95%, 96%, 90%, 96%; Boxworth: 60%, 95%, 98%, 96%). Reducing the rate from 55 to 27.5 g a.i./ha resulted in only a modest loss of control of the LLUD susceptible population at Rothamsted and Boxworth. Over the four years, half rate (27.5 g a.i./ha) has, with one exception, has given levels of

control of LLUD within 4% of full rate (55 g a.i./ha). On one occasion there was a 34% loss of control (Boxworth, first year).

- With the partially resistant T/11 population, control was always reduced compared to the susceptible standard, but some control was always achieved at the full dose - up to 77% (Rothamsted, second year). Reducing the rate from 55 to 27.5 g a.i./ha caused a much bigger loss of efficacy than with the susceptible LLUD. Results based on foliage fresh weight reductions for T/11 at full/half rate were : Rothamsted 51%/20%, 77%/66%, 65%/35%, 70%/38%; Boxworth 27%/18%, 56%/29%, 76%/43%, 60%/43%.

- The relative efficacy of full rate fenoxaprop over the four years was similar in the section 1(b) and 1(c) experiments at both sites. At Rothamsted, the highest levels of control of T/11 by full rate fenoxaprop occurred in year 2 and the lowest control in year 1 in both 1 (b) and (c) experiments. At Boxworth, the highest control occurred in year 3, and the lowest control in year 1.

Conclusions/Comments

Responses to fenoxaprop differed between years and sites, despite exactly the same seed populations being sown (LLUD 95 and T/11 1995). Over the four years, control of the susceptible LLUD by full rate fenoxaprop has ranged from 94% - 98% at Boxworth and 94% - 97% at Rothamsted; and at half rate from 60% - 98% at Boxworth and 90% - 96% at Rothamsted, based on foliage fresh weight reductions. In comparison, control of the partially resistant T/11 by full rate fenoxaprop has ranged from 27% - 76% at Boxworth and 51% - 77% at Rothamsted; and at half rate from 18% - 43% at Boxworth and 20% - 66% at Rothamsted.

The full rate of fenoxaprop gave consistently good control of the susceptible LLUD population in all years at both sites. Half rate (27.5 g a.i./ha) also gave good control at both sites in most cases, although control was poorer (60%) in year one at Boxworth, demonstrating that reduced rates may be less consistent even on susceptible populations.

It was evident that on the partially resistant T/11, not only was control always poorer at comparable rates, but that performance of fenoxaprop tended to fall more rapidly as dose rate was reduced from full to half rate, than was the case with the susceptible LLUD population.

The varying response between the same populations annually is probably due to environmental variables, although the precise reasons are hard to define.

Influence of resistance on variation in herbicide performance in different years

1 (c). Container experiments

Table 7. Percentage reduction in plant numbers

Fenoxaprop g/ha	Population	Year one 1995/96		Year two 1996/97		Year three 1997/98		Year four 1998/99	
		Roth.	Box.	Roth.	Box.	Roth.	Box.	Roth.	Box.
Nil	T/11	2.3	0.0	33.2	20.5	10.3	16.4	2.4	0.0
6.875	T/11	2.6	0.0	30.4	15.6	9.7	8.2	13.8	2.0
13.75	T/11	3.1	0.0	20.4	28.5	11.5	1.1	21.9	4.1
27.50	T/11	-1.2	0.0	20.5	25.3	3.5	4.8	29.7	17.8
55.00	T/11	12.8	47.9	41.9	26.7	59.0	32.1	34.4	52.5
Nil	LLUD	-0.8	0.0	17.4	12.8	2.9	1.9	11.9	2.5
6.875	LLUD	1.8	0.0	33.9	12.0	7.6	10.6	41.8	9.0
13.75	LLUD	81.4	0.0	88.3	23.7	66.6	81.1	98.3	57.1
27.50	LLUD	99.2	61.4	97.6	93.6	98.3	99.0	100.0	98.6
55.00	LLUD	100.0	100.0	99.1	98.2	100.0	99.2	100.0	100.0
S.E.M.		2.292	11.26	6.75	7.67	5.98	6.49	4.87	?
d.f.		27	18	27	27	27	27	27	27

Table 8. Percentage reduction in foliage fresh weight

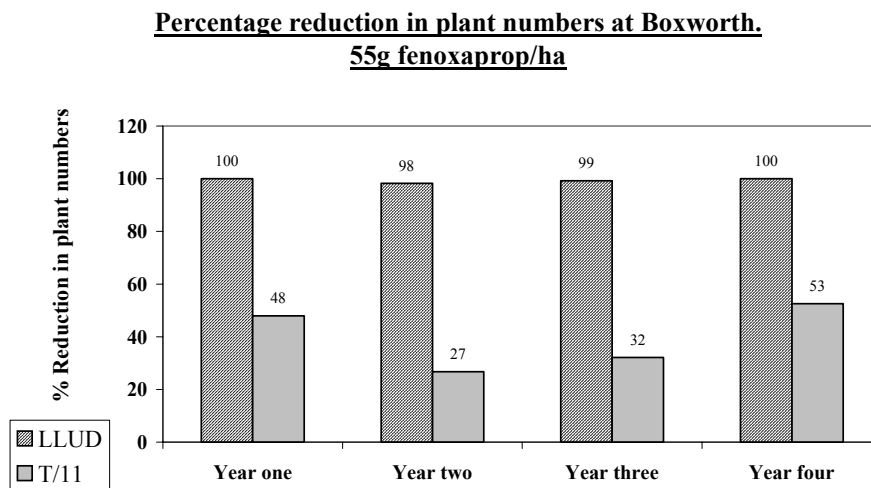| Fenoxaprop g./ha	Population	Year one 1995/96		Year two 1996/97		Year three 1997/98		Year four 1998/99	
		Roth.	Box.	Roth.	Box.	Roth.	Box.	Roth.	Box.
Nil	T/11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6.875	T/11	6.1	-0.6	35.7	22.9	11.5	7.1	15.1	35.1
13.75	T/11	-1.9	-22.7	41.4	26.4	6.6	6.1	28.6	30.7
27.50	T/11	20.0	17.6	66.3	28.7	34.6	42.9	37.6	42.5
55.00	T/11	50.7	26.7	76.7	56.0	64.8	75.7	70.0	59.7
Nil	LLUD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6.875	LLUD	-4.2	-7.8	54.7	25.0	41.6	16.8	63.1	62.6
13.75	LLUD	81.2	31.3	91.0	61.6	78.6	86.6	93.0	85.9
27.50	LLUD	94.6	60.2	95.9	95.2	89.5	98.1	96.0	95.7
55.00	LLUD	97.0	93.8	96.9	95.3	93.6	98.2	97.1	96.9
S.E.M.		5.70	13.15	3.22	9.54	5.18	8.01	4.70	5.33
d.f.		21	14	21	21	21	21	20	21

Figure 6. Influence of resistance on variation in herbicide performance in different years

1 (c). Container experiments: plant assessments

Percentage reduction in plant numbers at Rothamsted.
55g fenoxaprop/ ha

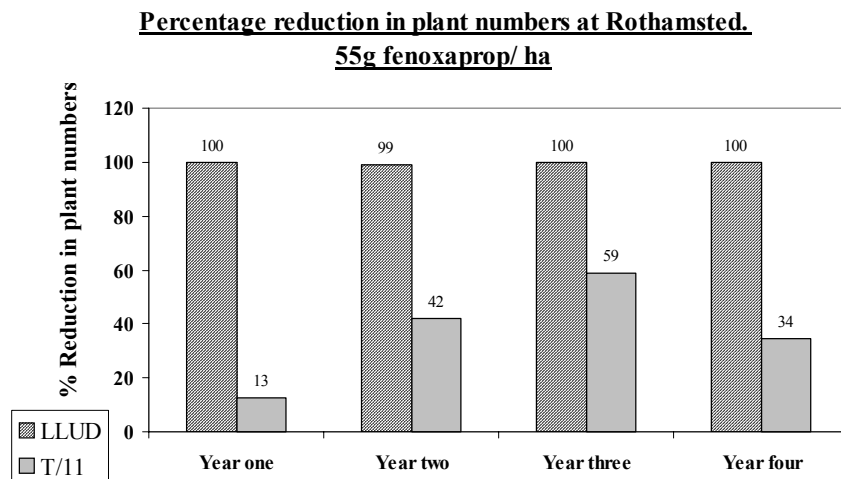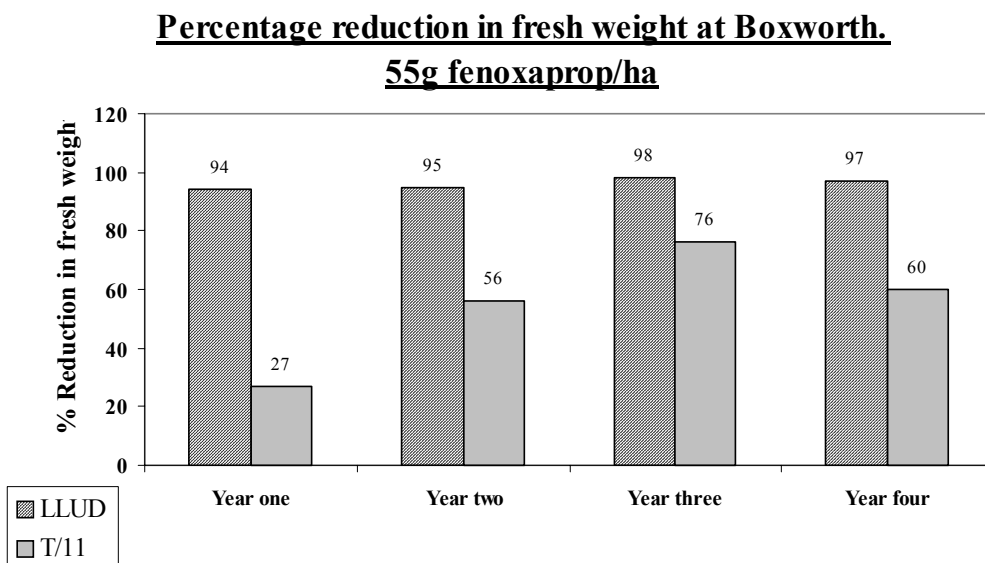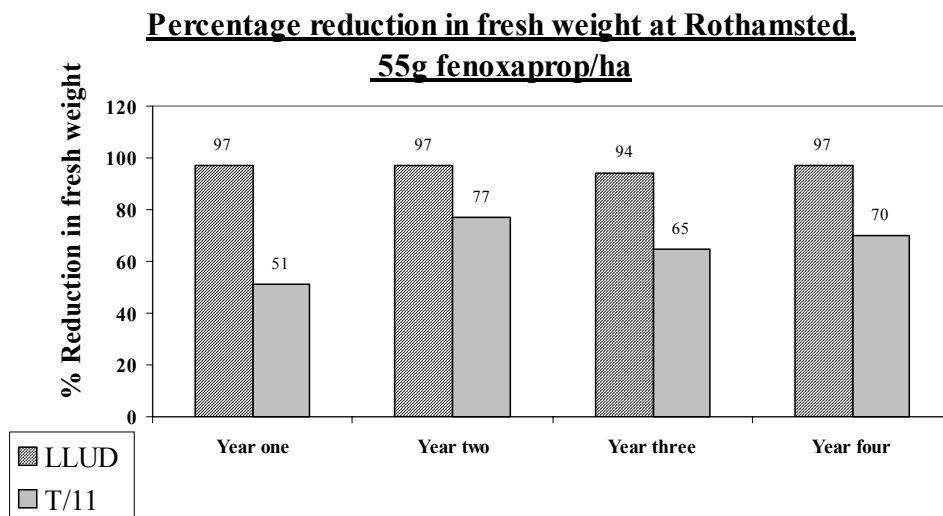**Percentage reduction in plant numbers at Boxworth.**
55g fenoxaprop/ha

Figure 7. Influence of resistance on variation in herbicide performance in different years

1 (c). Container experiments: seed return assessments

Percentage reduction in fresh weight at Rothamsted. 55g fenoxaprop/ha

Percentage reduction in fresh weight at Boxworth. 55g fenoxaprop/ha

1 (d) Selection for resistance in wild-oats: glasshouse studies.

Introduction

The scale of the container experiments detailed in sections 1 (a to c) meant that only a single resistant population, T/11, could be included. To put the results in a wider context it is important that other populations are studied in relation to selection for resistance. Consequently, simpler selection experiments were conducted over a five year period in the glasshouse using a wider range of populations.

Materials and Methods

The four populations used in this experiment comprised one susceptible (LLUD 1994) and one partially resistant (T/11 1994) population of *Avena sterilis* ssp. *ludoviciana*, and one susceptible (LFAT 1994) and one partially resistant (ESSEX 2A/F 1994) population of *Avena fatua*. The T/11 population was the same as that used initially in the experiments detailed in sections 1(a to c) above. The objective was to impose a known degree of selection on these populations for five generations (1996 - 2000) in order to determine whether, and to what degree, resistance increased. For the two partially resistant populations, two levels of selection were imposed ("Low" and "High"). Seeds collected each year were used to sow up pots in the subsequent year.

Dry seeds (50-80/dish) were placed in petri-dishes (25-50 dishes/population) containing four filter papers and left in an incubator at 30 - 35°C for one month in order to help break dormancy. Each seed was then pricked through the middle with a needle and 9 ml water added to each dish. After 5 - 7 days, single germinated seeds were sown in individual 5cm square pots containing compost. Pots were placed in a glasshouse and after plant emergence pots were sorted to achieve uniform plant growth stage for each population. Approximately three weeks after sowing, fenoxaprop-P-ethyl was applied at the following discriminating doses listed for each of the five years for each population:

T/11 High - 55, 55, 110, 110, 124 g a.i./ha; T/11 Low - 13.75, 55, 68.75, 82.5, 96 g a.i./ha.
Essex High - 55, 55, 55, 55, 55; Essex Low - 13.75, 55, 55, 55, 55 g a.i./ha.
LLUD - 13.75, 22, 27.5, 55, - g a.i./ha.
LFAT - 13.75, 22, 27.5, 41.3, 41.3 g a.i./ha.

Fenoxaprop-P-ethyl was applied using a laboratory pot sprayer delivering 208 - 284 L/ha water at 210 kPa through a single Teejet 110015 VK nozzle. After 3 - 5 weeks, pots were sorted visually and the plants least affected by the herbicide were retained. All the LLUD plants died in year 4 so none could be retained for growing on for seed production. The aim was that "high selection" would be achieved by growing on about 2.5% (equivalent to a 97.5% plant kill) and the "low selection" about 10% (equivalent to a 90% kill) from an initial population of about 500 sprayed plants. The actual numbers of plants sprayed and the numbers and percentage retained for growing on to produce seed were:

Population	Number of plants treated each generation (range)	Number of plants retained for seed production	% selection (range and **mean**)
T/11 High selection	392 - 516	12 - 16	2.5 - 4.1 % **(3.1 %)**
T/11 Low selection	231 - 578	24 - 50	7.1 - 11.2 % **(9.6 %)**
Essex 2A High selection	425 - 570	12 - 16	2.5 - 3.8 % **(3.0 %)**
Essex 2A Low selection	440 - 588	46 - 60	9.9 - 11.3 **(10.4 %)**
LFAT	440 - 536	6 - 16	1.12 - 3.6 **(2.7 %)**
LLUD	264 - 578	2 - 16*	0.35 - 6.1 % **(3.0 %)**

* = all plants died in year 4

Visual selection of plants was generally satisfactory as there was a wide range of responses from completely dead through to minor symptoms. The retained plants were transplanted into 25 cm diameter pots of standard compost at a density of 4-6 plants per pot. There were 10 pots for the two low selection pressure sets and four pots for each of the other four sets. Each set of pots was isolated in a glasshouse or outdoor polythene tunnel to minimize any chance of cross-pollination. Seed samples were collected from each set of pots on several occasions during the summer (July and August), air dried and stored in envelopes. These seeds were then used for sowing up pots in the subsequent year.

After five generations of selection, populations were evaluated in a glasshouse dose response assay to determine whether any change in degree of resistance had occurred. The original populations (LFAT 1994, T/11 1994, Essex 2A/F 1994; = /ORIGINAL) and samples from the same populations after five generations of selection (= /SEL, collected in 2000) were used.

Approximately 80 seeds were placed in each petri-dish containing three normal filter papers and one glass fibre paper (8 dishes per population). The seeds were pricked to break dormancy then treated with 9ml of deionised water on 21 September 2000 and incubated at 17°C 14-hour day, 11°C 10-hour night. On 28 September 2000 germinated seeds were sown in 5cm square pots containing compost (1 seed per pot, 208 pots per population).

The experiment comprised a fully randomised design with 16 replicate pots per herbicide dose and 40 untreated pots per population. Fenoxaprop-P-ethyl was applied in a staggered range of seven doses from 3.44 - 1760 g a.i./ha) depending on the degree of resistance expected. Herbicides were applied at the 3 leaf stage on 18 October 2000 20 days after sowing using a laboratory sprayer delivering 257 litres water/ha at 210kPa through a single 'Teejet' 110015VK ceramic nozzle. Herbicide activity was recorded on 8 - 10 November 2000, 21 - 23 days after spraying, by assessing foliage fresh weight for each individual pot.

Dose response data was analysed using a logistic relationship between foliage fresh weight and log10 dose and $\log_{10}$ ED_{50} values were determined (Ross 1987). The ED_{50} values (herbicide rate required to reduce fresh weight by 50% relative to the no-herbicide controls) referred to in the text and tables have been detransformed from the $\log_{10}$ values.

Results (Table 9)

- There was no evidence of selection for increased resistance to fenoxaprop in the LFAT population. The $\log_{10}ED_{50}$ value for the population selected for five years (LFAT/SEL) was not statistically different to that for the original population (LFAT/ORIGINAL), and the resistance index was only 1.2.

- There was no evidence of selection for increased resistance to fenoxaprop in the Essex 2A High selection population. The $\log_{10}ED_{50}$ value for the population selected for five years (ESSEX 2A/HIGH/SEL) was not statistically different to that of the original population, (ESSEX 2A/ORIGINAL) although the resistance index was 1.9. In contrast, there was evidence of selection for increased resistance to fenoxaprop in the Essex 2A Low selection population. The $\log_{10}ED_{50}$ value for the population selected for five years (ESSEX 2A/LOW/SEL) was statistically different (P≤0.05) to that of the original population, (ESSEX 2A /ORIGINAL) and the resistance index (relative to original population) was 7.5.

- There was no evidence of selection for increased resistance to fenoxaprop in the T/11 populations at either level of selection. The $\log_{10}ED_{50}$ value for the populations selected for five years (T/11 LOW/SEL and T/!! HIGH/SEL) were not statistically different to that of the original population, (T/11 1995) and the resistance indices (relative to original population) were almost identical (1.0 - 1.1).

- The partial level of resistance in the T/11 original population was confirmed. The ED_{50} value for the T/11/ORIGINAL population was 3.1 x greater than that for the LFAT/ORIGINAL 1994 value. This resistance index was similar to those recorded in the glasshouse dose response assays detailed in sections 1 (a and b), (2.0 - 2.8), although in those comparisons a different susceptible standard was used (LLUD 1995).

Conclusions

The approach has worked well but choosing appropriate discriminating doses, which give sufficient effects without killing plants, is not always easy. The dose used on the LLUD population was too high in year 4, so all plants were killed and this population lost. Achieving an appropriate discriminating dose of herbicide requires care. The two levels of selection mimicked the effect of full and reduced doses of herbicide quite well.

The LFAT and T/11 populations showed no evidence of selection for resistance at all. The T/11 results supported those found in the outdoor container experiments detailed in sections 1 (a and b) very well. The glasshouse assay confirmed that there had been no increase in level of resistance after five years annual use of fenoxaprop. There was also no difference between the two levels of selection. The intrinsic resistance in the T/11 population to fenoxaprop was confirmed.

In contrast, with the Essex 2A/F population, there was evidence of an increased level of resistance in the population subjected to a lower level of selection, but not with the higher selection. The reasons for this difference is not easy to explain. While the T/11 population is known to possess an enhanced ability to metabolise fenoxaprop, the mechanism in the original Essex 2A/F population, which had a very marginal level of resistance, is unknown.

The results do show that continued use of a herbicide to which there is already resistance does not inevitably result in a rapid increase in resistance over a five year period in a population with enhanced metabolism. However, the results also show that different populations may respond differently to selection, and that generalisations based on research conducted on only one or a small number of populations, may be misleading.

1 (e). Deselection in resistant wild-oats: glasshouse studies.

Introduction

An important aspect in long term resistance management is what happens when selection pressure from herbicides ceases, either because herbicides are no longer used or herbicides are used which are equally active on resistant and susceptible plants (neutral selection pressure). Consequently a study was undertaken to establish whether resistance level declined when four resistant populations were grown in the absence of herbicides for 5 consecutive years.

Materials and Methods

The four populations used in this experiment comprised one highly resistant (ESSEX 1A/L 1994) and one partially resistant (T/11 1994) population of *Avena sterilis* ssp. *ludoviciana*, and one highly resistant (KENT 1A/F 1994) and one partially resistant (ESSEX 2A/F 1994) population of *Avena fatua*. The partially resistant populations are the same as those used in the 1(d) selection experiment. The aim is to grow these on without any herbicide application for up to 5 generations in order to determine the degree of reversion towards susceptibility, if any.

Seeds were pre-germinated as described in section 1(d) above and sown in individual 5cm pots. 50 plants of each population were transplanted into 25 cm diameter pots of compost (5 plants per pot) on 10 May 1999. The four sets of 10 pots per population were isolated in separate areas of a sandbed and seed was collected during July to September each year. Seeds collected each year were used to sow up pots in the subsequent year.

After five generations of deselection, populations were evaluated in a glasshouse dose response assay to determine whether any change in degree of resistance to fenoxaprop had occurred. This was the same assay as described in section 1 (d) above. The original populations (= /ORIGINAL) samples from the same populations after five generations of deselection (= /DESEL, collected in 2000) were used.

Results (Table 9 & 10)

- There was no evidence of deselection for resistance to fenoxaprop in the Essex 2A/F population (Table 9). The $\log_{10}$ ED_{50} value for the population deselected for five years (ESSEX 2A/DESEL) was not statistically different to that of the original population, (ESSEX 2A/ORIGINAL) and the resistance index (relative to original population) was 1.1.

- There was also no evidence of deselection for resistance to fenoxaprop in the T/11 population (Table 9). The $\log_{10}$ ED_{50} value for the populations deselected for five years (T/11 /DESEL) was not statistically different to that of the original population, (T/11 /ORIGINAL) and the resistance index (relative to original population) was identical (1.0).

- There was no evidence of deselection for resistance to fenoxaprop in the Kent 1A/F population (Table 10). The $\log_{10}$ ED_{50} value for the population deselected for five years (KENT 1A/DESEL) was not statistically different to that of the original population, (KENT 1A/ORIGINAL) and the resistance index (relative to original population) was 1.1.

- However, in contrast to the other three populations, there was evidence of deselection for resistance to fenoxaprop in the Essex 1A/L population (Table 10). The $\log_{10}$ ED_{50} value for the population deselected for five years (ESSEX 1A/DESEL) statistically different to that of the original population, (ESSEX 1A/ORIGINAL) and the resistance index (relative to original population) was 0.08.

- The high and very high level of resistance in the Kent 1A/F and Essex 1A/L original populations was confirmed. The ED_{50} value for the KENT 1A/ORIGINAL population was 5.5 x greater, and the ESSEX 1A/ORIGINAL 69.1 x greater, than that for the susceptible standard, LFAT/ORIGINAL (Tables 9 and 10).   The ED_{50} for ESSEX 1A/DESEL, although substantially lower than for the original ESSEX 1A/ORIGINAL, was still 5.3 x greater than for the susceptible standard, LFAT/ORIGINAL.   This showed that while the level of resistance had decreased after five years without herbicide, it was still substantial, and certainly reversion to full susceptibility had not been achieved.

Conclusions

The results showed that stopping using herbicides does not automatically mean that there will be any decline in level of resistance over a five year period.   There was no evidence of deselection for resistance to fenoxaprop in three of the four populations grown on for five generations without further herbicide treatment, (Essex 2A/F, T/11 and Kent 1A/F). However, in contrast to the other three populations, there was evidence of deselection for resistance to fenoxaprop in the Essex 1A/L population. This population had a high initial level of resistance and the dose response curves did not give as good a fit as with the other populations. There were certainly differences between the original and deselected population, but the ED_{50} values and resistance indices tend to exaggerate the effect of deselection. While the level of resistance in the

Essex 1A/L population had decreased after five years without herbicide, it was still substantial, and certainly reversion to full susceptibility had not been achieved.

The results of the selection (1 d) and deselection (1 e) studies taken together, show that continuing or ceasing to use the herbicide to which there is already resistance, does not inevitably result in a change in resistance over a five year period, at least in a population with enhanced metabolism. However, the results also show that different populations may respond differently to selection and deselection, and that generalisations based on research conducted on only one or a small number of populations, may be misleading. Other resistance mechanisms exist in wild-oats, notably target site resistance (insensitive ACCase). It is possible that resistance will build up or decline faster, in populations which possess other resistance mechanisms, and this is currently under investigation.

Table 9. Selection and deselection for resistance in wild-oats

1 (d & e). Glasshouse dose response evaluation

FENOXAPROP ED$_{50}$ VALUES

Population	LOG$_{10}$ ED$_{50}$	Detransformed ED$_{50}$ values [Ratio to original population]
LFAT/ORIGINAL	1.3468	22.23 [1.0]
LFAT/SEL	1.4352	27.24 [1.2]
Combined S.E. ±	0.1017	-
L.S.D. (P< 0.05)	0.3134	-

Population	LOG$_{10}$ ED$_{50}$	Detransformed ED$_{50}$ values [Ratio to original population]
ESSEX 2A/ORIGINAL	1.3610	22.96 [1.0]
ESSEX 2A/LOW/SEL	2.2382	173.08 [7.5]
ESSEX 2A/HIGH/SEL	1.6370	43.35 [1.9]
ESSEX 2A/DESEL	1.4093	25.67 [1.1]
Combined S.E. ±	0.2000	-
L.S.D. (P< 0.05)	0.5828	-

Population	LOG$_{10}$ ED$_{50}$	Detransformed ED$_{50}$values [Ratio to original population]
T/11 /ORIGINAL	1.8421	69.51 [1.0]
T/11 LOW/SEL	1.8707	74.25 [1.1]
T/11 HIGH/SEL	1.8242	66.71 [1.0]
T/11 /DESEL	1.8397	69.13 [1.0]
Combined S.E. ±	0.0987	-
L.S.D.	0.2880	-

Table 10. Deselection in resistant wild-oats

1 (e). Glasshouse dose response evaluation

FENOXAPROP ED$_{50}$ VALUES

Population	LOG$_{10}$ ED$_{50}$	Detransformed ED$_{50}$ values [Ratio to original population]
ESSEX 1A/ORIGINAL	3.1861	1535.01 [1.0]
ESSEX 1A/DESEL	2.0706	117.65 [0.08]
Combined S.E. ±	0.3317	-
L.S.D. (P< 0.05)	1.0222	-

Population	LOG$_{10}$ ED$_{50}$	Detransformed ED$_{50}$ values [ratio to original population]
KENT 1A/ORIGINAL	2.0898	122.97 [1.0]
KENT 1A/DESEL	2.1191	131.54 [1.1]
COMBINED S.E. ±	0.0940	-
L.S.D. (P<0.05)	0.2897	-

SECTION 2

Cross-resistance patterns in a range of wild-oat populations

Introduction

Most of the initial screening studies for resistance in wild-oats have involved the use of fenoxaprop-P-ethyl. However, it is important that cross resistance to other herbicides, of both the same and different herbicide classes, is determined. Previous experience with black-grass (*Alopecurus myosuroides*) showed that cross-resistance patterns are complex and difficult to predict (Moss and Clarke, 1992; 1995). There is a need to consider herbicides individually as resistance may occur to some, but not all, of the herbicides within the same herbicide class. It is also important that a number of different populations from a wide geographical area are used so as to achieve a broader perspective. The objective was to determine the cross-resistance patterns in a range of populations of both wild-oat species, *Avena sterilis* ssp. *ludoviciana* and *Avena fatua*. It is essential that the populations studied reflect the range of resistance found in the UK, otherwise potentially misleading conclusions could be made regarding the best herbicide strategy to adopt as part of a prevention or control strategy.

Materials and Methods

Five glasshouse/laboratory experiments were undertaken: Experiment 1 involved "fop" (aryloxyphenoxypropionate) and "dim" (cyclohexanedione) herbicides; Experiment 2 a range of non - "fop" and "dim" herbicides; Experiments 3, 4 & 5 conducted in petri-dishes, triallate.

Ten populations of wild-oats of both species (7 *A. fatua* (F), 3 *A. ludoviciana* (L)) from seven different counties were used in this series of experiments. Most of them had showed evidence of resistance in preliminary single dose screening experiments. The 10 wild-oats populations used were as follows:

LLUD 1995: a susceptible *Avena ludoviciana* population (as used in section 1 (a to c) above.

LFAT 1994 or 1996: a susceptible *Avena fatua* population (as used in section 1 (d) above.

T/11 1995 (Essex): a partially fenoxaprop resistant *A. ludoviciana* population, first found in 1993, showing some degree of cross-resistance to other herbicides (as used in section 1 (a to e) above).

ESSEX 2A/F 1994 (Worm): a marginally fenoxaprop-resistant population in 1994 screen (as used in section 1 (d and e) above.

LINCS 7A/F 1996 or 1998 (Ciba 1/F Frisk): a partially fenoxaprop-resistant population which showed evidence of cross-resistance to tralkoxydim in the 1996 screening experiment.

WILTS 1A/L 1997 (Scot): a partially fenoxaprop-resistant population which showed evidence of cross-resistance to tralkoxydim in the 1995 screening experiment.

DORSET 1A/F 1996 (Down): a partially fenoxaprop-resistant population showing cross-resistance to tralkoxydim and imazamethabenz in 1995/1996 screen.

OXFORD 5A/F 1997 (New): a partially fenoxaprop-resistant population showing cross-resistance to tralkoxydim and imazamethabenz in 1996 screen.

KENT 1A/F 1994: a highly resistant highly fenoxaprop-resistant population showing no cross-resistance to other herbicides in 1994/95 screen (as used in section 1 (e) above).

SUFFOLK 1A/F 1996 (Ciba X/F): a highly fenoxaprop-resistant population showing no cross-resistance to other herbicides in 1996 screen.

Experiments 1 & 2: Glasshouse dose response assays

The ten populations were used in two glasshouse dose response assay to determine cross-resistance patterns. The following herbicides were applied;

Experiment 1	**Herbicide**	**Dose range**
	Fenoxaprop-P-ethyl	6.875 - 880 g a.i./ha
	Fluazifop-P-butyl (+ "Agral")	7.813 – 1,000 g a.i./ha
	Tralkoxydim (+ "Output")	5.47 – 350 g a.i./ha
	Cycloxydim (+ "Actipron")	9.38 – 300 g a.i./ha
Experiment 2	Imazamethabenz (+ "Agral")	18.75 – 2,400 g a.i./ha
	Flamprop-M-isopropyl	87.5 – 11,200 g a.i./ha
	Difenzoquat	123.75 – 3,960 g a.i./ha
	Isoproturon	78.1 – 2,500 g a.i./ha

The herbicides were applied in a staggered range of six - eight doses within the ranges given above. Each dose was twice the preceding dose in the range. Recommended adjuvants were used: ("Agral" at 0.1% with fluazifop; "Agral" at 500ml/ha with imazamethabenz; "Output" at 0.375% with tralkoxydim; "Actipron" at 0.8% with cycloxydim).

Single plants were grown from pre-germinated seeds in individual 5 cm square pots containing compost in a glasshouse. In both experiments, herbicides were applied at the 2-3 leaf stage using a laboratory sprayer delivering 244 - 270 litres water/ha at 210 kPa through a single 'Teejet' 110015VK ceramic flat fan nozzle. There were 10 - 12 replicate pots per herbicide dose for each population and 32 - 40 untreated pots per population. Foliage fresh weight per pot was recorded 25-31 days after spraying as a measure of herbicide activity.

Foliage fresh weight data were analysed using a Maximum Likelihood Programme (Ross, 1987) and $\log_{10}ED_{50}$ values calculated. ED_{50} values were detransformed from $\log_{10}$ data and represent the herbicide dose required to reduce foliage fresh weight by 50%, relative to the untreated controls. Resistance index (RI) is a measure of degree of resistance and is the ratio of the ED_{50} value relative to the susceptible standard, LLUD 95. Comparisons of the degree of resistance between populations and herbicides can be made using these values.

Experiments 3,4 & 5: Petri-dish dose response assays
Petri-dish assays were used to determine the response of wild-oat populations to tri-allate. Experiment 3 was conducted to determine the response of three wild-oat populations (LLUD 1995; T/11 1995; T/41 1994 (= ESSEX 10A/L)) in order to establish a technique which could then be used to investigate other populations. Experiment 4 included the same ten populations used in the two glasshouse dose assays (Experiments 1 & 2), and Experiment 5 included six of those populations plus the T/41 1994 population used in Experiment 3.

Approximately 50 wild-oat seeds were put in each 9 cm petri-dish containing three Whatman cellulose filter papers covered by one glass fibre filter paper. There were 12 - 20 dishes per population. The seeds were pricked and 9mls of water added to each dish. The dishes were then placed in an incubator at 17°C/11°C with a 14 hour light and a 10 hour dark phase.

After 5 - 7 days, seeds with a shoot growth of 3 – 10 mm were transferred to new petri dishes (25 seeds per dish and 18 dishes per populations). A range of eight concentrations of tri-allate (Experiments 3 & 4: Nil, 0.156, 0.313, 0.625, 1.25, 2.5, 5, 10 ppm; Experiment 5: Nil 0.041, 0.123, 0.37, 1.11, 3.33, 10, 30 ppm), prepared from a liquid formulation of "Avadex" (480 g tri-allate/litre) was then added to these dishes (7 mls solution per dish). The nil dishes were given 7ml of distilled water and all the dishes were then sealed with 'Parafilm' and stored in the same incubator.

Fourteen days after the addition of herbicide, the dishes were assessed by measuring the shoot length of each germinated seed. Shoot length data (mean shoot length per dish) was analysed in the same manner as the foliage fresh weight data in Experiments 1 & 2.

Results

Experiment 1 (Table 11; Figures 8, 9, 10, 11)

- To make comparisons easier, histograms (Figures 8 - 11) based on the resistance indices are given with the populations presented in order of increasing resistance to fenoxaprop. The same order has been used for each herbicide so that similarities and differences betwen resistance patterns can be observed.

- The results demonstrate very clearly that there is a continuum of response to fenoxaprop, from susceptible to highly resistant, with resistance indices ranging from 1.0 to 13.1. The two populations which had shown the greatest resistance in single dose screening tests (Suffolk 1A/F and Kent 1A/F) were also the most resistant in this assay.

- The T/11 population acts as a good standard for interpretative purposes as it has been used in the container/tray experiments detailed in sections 1 (a, b & c). In those outdoor container studies there was ample evidence that performance of fenoxaprop is substantially reduced by the levels of resistance found in T/11 (Resistance Index = 5.4), although some control was always achieved. It is harder to predict what impact the partial resistance recorded in the Essex 2A/F, Essex 2A/F and Oxford 5A/F populations, with resistance indices of 1.7 – 2.6, might have under field conditions. One of the field trials conducted in 1996/1997 was at the Essex 2A/F site (see later section). Full rate fenoxaprop gave good control (97%) whereas half rate gave only 53% control of panicles. It appears probable that this loss of efficacy is due, at least in part, to the presence of marginal resistance. It may be noted that in the section 1(c) container experiments, using half-rate compared to full rate fenoxaprop, generally caused only a small loss of efficacy on a susceptible population (LLUD) but a much larger loss on the partially resistant T/11.

- The histograms for fluazifop show that while sites showing resistance to fenoxaprop also tended to show resistance to fluazifop, there was not a direct correlation. In particular the relative resistance to these two herbicides was reversed in the Kent 1A/F and Suffolk 1A/F populations. Thus the Kent population, which was first detected due to failure of fluazifop in the field, showed a very high level of resistance and much greater resistance to this herbicide than the Suffolk population. Whereas Suffolk 1A/F was the population most resistant to fenoxaprop, it was only the sixth most resistant population to fluazifop.

- The results for tralkoxydim showed even less correlation with resistance to fenoxaprop. The Kent 1A/F and Suffolk 1A/F, which showed the greatest level of resistance to fenoxaprop, showed either no or only marginal levels of resistance to tralkoxydim. However five of the other populations showed very clear evidence of resistance to tralkoxydim and with these the relative degree of resistance to fenoxaprop and tralkoxydim showed no obvious correlation.

- Despite clear evidence of resistance to tralkoxydim in several populations, there was no evidence of resistance to another cyclohexanedione herbicide, cycloxydim, in any population, with the possible exception of Wilts 1A/L. The significance of the apparent partial resistance of the Wilts 1A/L population is unclear and whether it has significance in the field is uncertain at present.

- The two susceptible standards were well controlled and responded similarly to the four herbicides giving resistance indices between 1.0 and 1.9 for both populations. The LLUD population was used as the susceptible baseline in these studies as the LFAT plants showed evidence of disease and grew relatively poorly even in the absence of herbicide. There was concern that this might make them more sensitive to the herbicides, but in fact there was no evidence for this.

- Care is needed in interpreting these results in terms of impact on field performance of these herbicides. In particular the very high resistance indices recorded for tralkoxydim in some populations, almost certainly overstate the degree of resistance seen in the field. This has also been observed with *Lolium multiflorum* (Cocker *et al.*, 2001). Tralkoxydim tends to be much more active under glasshouse conditions and indeed gave a high level of control of the susceptible LLUD and LFAT at about 10% of field rate. In contrast, the response to fenoxaprop and fluazifop in the glasshouse tends to be closer to field rates with good control being achieved at typically 50 – 100% of field rate.

- The resistance index for T/11 was 31.0 for tralkoxydim and 5.4 for fenoxaprop. In the section 1(b) container experiments, control of seed return of T/11 by tralkoxydim at the field rate has ranged from 23 to 94% during the five years, with a mean of 68%. Control of seed return of T/11 by fenoxaprop has ranged from 39 – 92%, with a mean of 71% over the five years. In contrast, control of the susceptible LLUD has always been over 96% for both herbicides (with one exception). Thus, despite the large difference in resistance indices, resistance appears to reduce activity of fenoxaprop and tralkoxydim against T/11 to an approximately equal degree under outdoor conditions. There is no doubt that resistance to tralkoxydim and fenoxaprop exists, but more information is needed to help interpret glasshouse results in terms of likely impact on performance in the field.

Table 11. Experiment 1: Cross-resistance to "fop" and "dim" herbicides

Log$_{10}$ ED$_{50}$ values

Population	Fenoxaprop	Fluazifop	Tralkoxydim	Cycloxydim
LLUD 95	1.2285	0.9361	0.7084	0.8452
LFAT 96	1.3537	1.2077	0.9844	<0.9720
Wilts 1A/L	2.0570	1.8494	1.9308	1.3926
T/11	1.9629	1.6207	2.1997	1.0266
Oxford 5A/F	1.6395	1.6690	2.3491	1.0754
Essex 2A/F	1.4676	1.3605	1.1577	1.0509
Dorset 1A/F	2.0358	1.3216	2.5169	0.8228
Lincs. 7A/F	1.6402	1.6627	2.0762	0.8742
Suffolf 1A/F	2.3445	1.4077	1.0826	1.0769
Kent 1A/F	2.0591	2.8101	0.8823	0.9349
Combined S.E. ±	0.0673	0.1370	0.1499	0.0821
L.S.D. (P<0.05)	0.1924	0.3915	0.4286	0.2347

Detransformed ED$_{50}$ values [Ratio to LLUD = Resistance Index]

Population	Fenoxaprop	Fluazifop	Tralkoxydim	Cycloxydim
LLUD 95	16.9 **[1]**	8.6 **[1]**	5.1 **[1]**	7.0 **[1]**
LFAT 96	22.6 **[1.3]**	16.1 **[1.9]**	9.7 **[1.9]**	<9.4 **[<1.3]**
Wilts 1A/L	114.0 **[6.7]**	70.7 **[8.2]**	85.3 **[16.7]**	24.7 **[3.5]**
T/11	91.8 **[5.4]**	41.8 **[4.8]**	158.4 **[31.0]**	10.6 **[1.5]**
Oxford 5A/F	43.6 **[2.6]**	46.7 **[5.4]**	223.4 **[43.7]**	11.9 **[1.7]**
Essex 2A/F	29.4 **[1.7]**	22.9 **[2.7]**	14.4 **[2.8]**	11.2 **[1.6]**
Dorset 1A/F	108.6 **[6.4]**	21.0 **[2.4]**	328.8 **[64.3]**	6.7 **[1.0]**
Lincs 7A/F	43.7 **[2.6]**	46.0 **[5.3]**	119.2 **[23.3]**	7.5 **[1.1]**
Suffolk 1A/F	221.0 **[13.1]**	25.6 **[3.0]**	12.1 **[2.4]**	11.9 **[1.7]**
Kent 1A/F	114.6 **[6.8]**	645.8 **[74.8]**	7.6 **[1.5]**	8.6 **[1.2]**

Figure 8.   Experiment 1: Response to "fop" and "dim" herbicides: fenoxaprop

RESPONSE OF 10 WILD-OAT POPULATIONS
fenoxaprop - P - ethyl
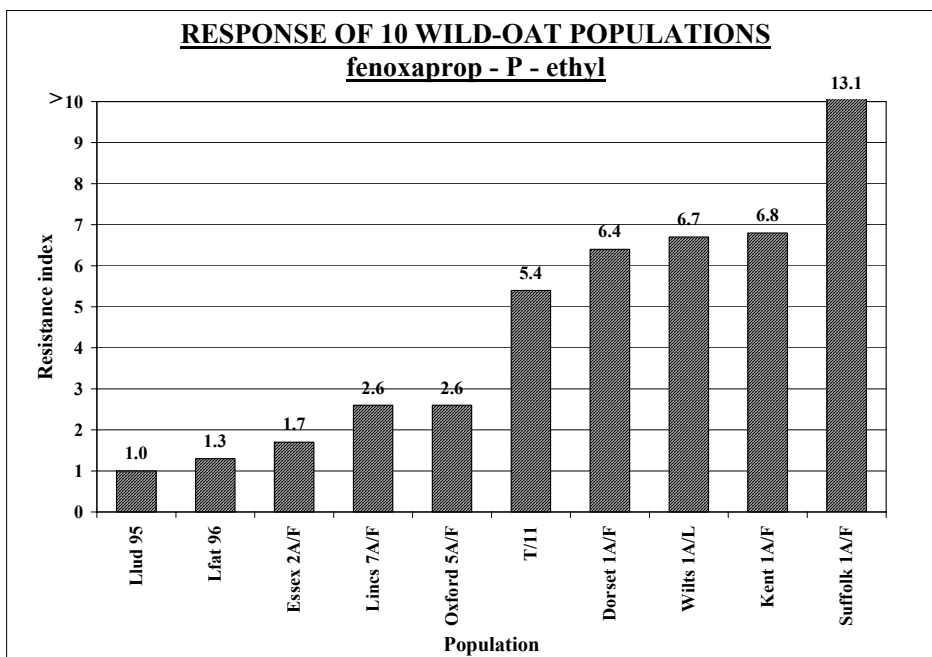
Figure 9.   Experiment 1: Response to "fop" and "dim" herbicides: fluazifop

RESPONSE OF 10 WILD-OAT POPULATIONS
fluazifop - P - butyl
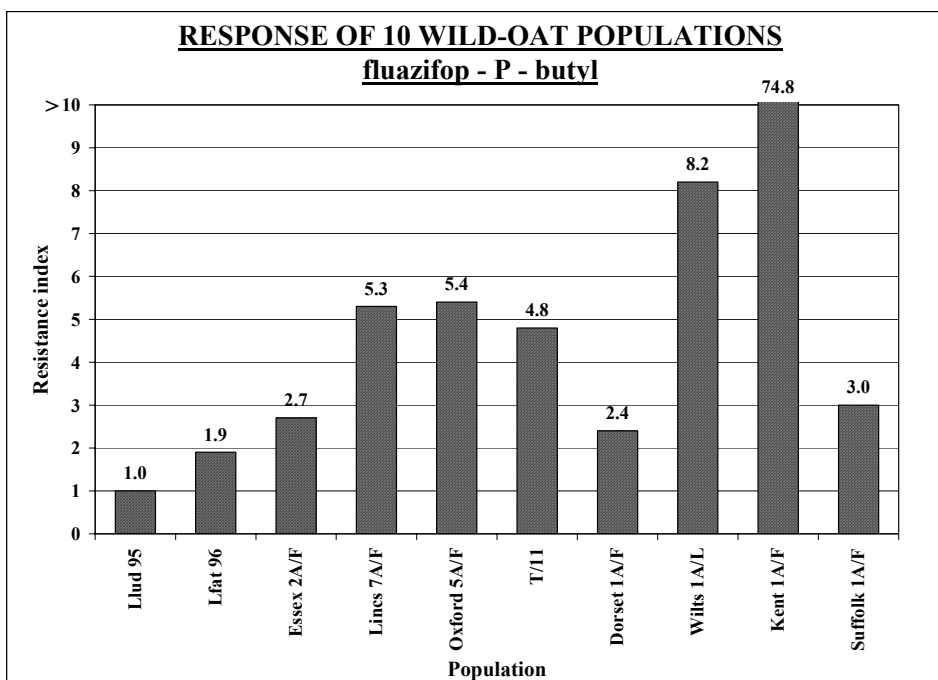
Figure 10.   Experiment 1: Response to "fop" and "dim" herbicides: tralkoxydim

RESPONSE OF 10 WILD-OAT POPULATIONS
tralkoxydim
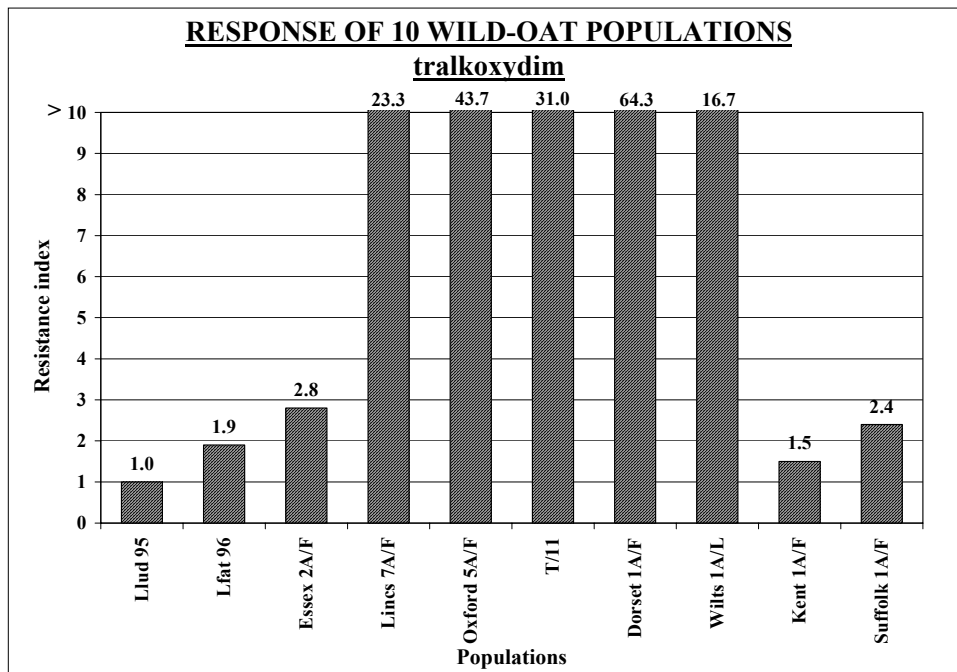
Figure 11.   Experiment 1: Response to "fop" and "dim" herbicides: cycloxydim

RESPONSE OF 10 WILD-OAT POPULATIONS
cycloxydim
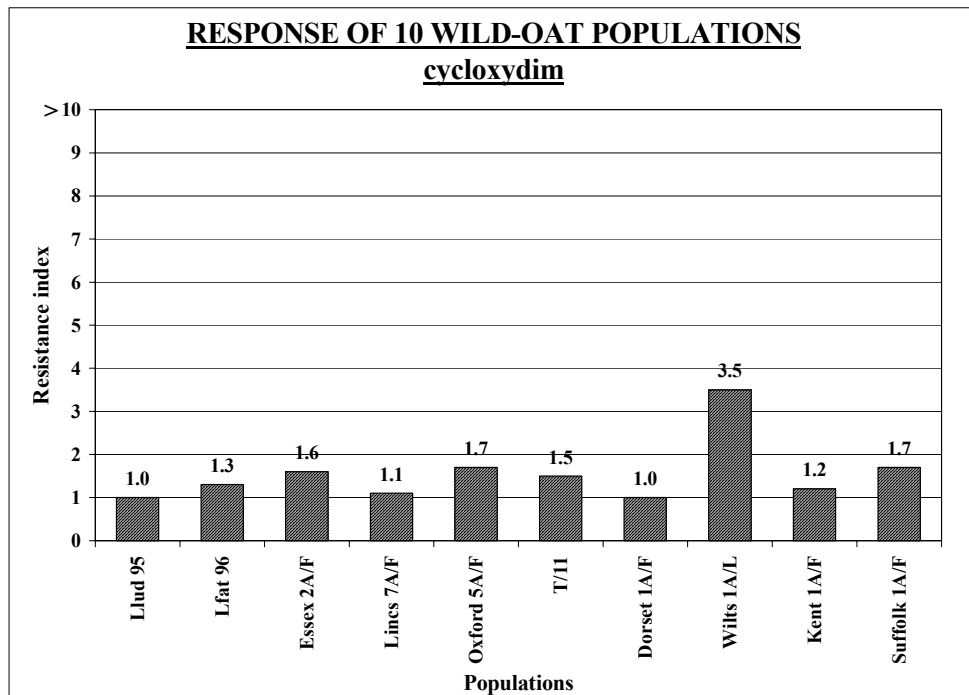
Experiment 2 (Table 12; Figures 12, 13, 14, 15)

- To make comparisons easier, histograms (Figures 12 - 15) based on the resistance indices (R.I.) are given with the populations presented in order of increasing resistance to fenoxaprop. The same order has been used for each herbicide in both Experiments 1 & 2 so that similarities and differences between resistance patterns can be observed.

- The results demonstrate very clearly that some populations show resistance to imazamethabenz, with resistance indices of up to 19.5, but this is not directly correlated with fenoxaprop resistance. The two populations with the greatest resistance to fenoxaprop (Kent 1A/F and Suffolk 1A/F) showed no, or only marginal, resistance to imazamethabenz. However there did appear to be an association between imazamethabenz and tralkoxydim resistance (see Experiment 1 results). The four populations showing the greatest resistance to tralkoxydim (Lincs. 7A/F, Oxford 5A/F, T/11 and Dorset 1A/F) also showed the greatest resistance to imazamethabenz, although the scale of the resistance indices varied. Dorset 1A/F showed the greatest resistance to both herbicides.

- Clear evidence of resistance to flamprop-M-isopropyl was demonstrated. The four populations showing the greatest resistance to this herbicide were the same four which showed the greatest resistance to imazamethabenz and tralkoxydim (Lincs 7A/F, Oxford 5A/F, T/11 and Dorset 1A/F). However, of these four populations, the one showing the highest level of resistance to flamprop (Lincs 7A/F, R.I. = 71.8) showed the lowest level of resistance to tralkoxydim and second lowest to imazamethabenz. The two populations with the greatest resistance to fenoxaprop (Kent 1A/f and Suffolk 1A/F) showed no resistance to flamprop.

- There was no clear evidence of resistance to difenzoquat in any population, although two populations (Essex 2A/F and Lincs 7A/F) had resistance indices of 4 to 5. One of these (Lincs 7A/F) had shown the highest level of resistance to flamprop but the other one (Essex 2A/F) had shown only marginal resistance to other herbicides.

- There was no evidence of resistance to isoproturon in any population. The highest resistance index was 2.1 for Oxford 5A/F, but most populations had resistance indices very similar to the susceptible standards, LLUD and LFAT.

- The Essex 11A/L (T/11) population acts as a good standard for interpretative purposes as it has been used in the container/ tray experiments detailed in section 1(a, b & c) for the past four years. In the 1(b) outdoor container studies there was ample evidence that performance of imazamethabenz was substantially reduced by the levels of resistance found in T/11 (RI = 12.3), although some control was achieved. Control of seed return by imazamethabenz at field rate ranged from 22 – 88 % with a mean of 56 %. In contrast, control of the LLUD susceptible standard has generally been good in the containers, averaging 88% control (range 36 - 100%).

- In the outdoor containers in the 1(b) experiment, difenzoquat has given the most consistent control of the T/11 population. Control of seed return at the field rate has ranged from 63 - 100 % with a mean of 89 %. On the susceptible standard, LLUD, difenzoquat has averaged 84 % control (range 50 - 100 %, excluding year 4 at Boxworth, where zero (-14%) control was achieved). Thus control of the T/11 and LLUD has been similar overall, confirming that there is no evidence of resistance to difenzoquat in T/11 population and that the resistance indices of 2.6 in the dose response experiment does not indicate resistance impacting on herbicide performance.

- The two susceptible standards, LLUD and LFAT, were well controlled and responded similarly to the four herbicides giving resistance indices between 0.6 and 1.4 for both populations. The LLUD population was used as the susceptible baseline in these studies as the same population has been used in the outdoor container experiments detailed in sections 1 (a, b and c).

- Care is needed in interpreting these results in terms of impact on field performance of these herbicides. Some herbicides work better in glasshouse experiments than in the field which can cause a misinterpretation of results, although the results in comparison with the 1(b) outdoor container experiments on T/11 agree with each other quite well.

Table 12. Experiment 2: Cross-resistance to *non* "fop" and "dim" herbicides

Log_{10} ED_{50} values

Population	Difenzoquat	Imazamethabenz	Isoproturon	Flamprop
LLUD 95	2.3992	1.3118	-0.7951	2.7639
LFAT 94	2.4119	1.3423	-0.6470	2.5447
Wilts 1A/L	2.6717	1.6366	-0.8836	3.2984
T/11	2.8085	2.4032	-0.6251	3.8968
Oxford 5A/F	2.6509	1.9761	-0.4755	3.8394
Essex 2A/F	3.0283	1.8068	-1.0084	3.2511
Dorset 1A/F	2.7695	2.6014	-0.5540	4.1371
Lincs 7A/F	3.0602	2.0200	-0.8738	4.6201
Suffolk 1A/F	2.7467	1.7426	-0.7204	2.7651
Kent 1A/F	2.3907	1.4570	-0.6705	2.5566
Combined S.E.±	0.1440	0.1268	0.1582	0.2771
L.S.D. (P<0.05)	0.4114	0.3623	0.4520	0.7920

Detransformed ED_{50} values   [Ratio to LLUD = Resistance Index]

Population	Difenzoquat	Imazamethabenz	Isoproturon	Flamprop
LLUD 95	246.7 [1]	20.5 [1]	160.3 [1]	580.6 [1]
LFAT 94	258.2 [1.0]	22.0 [1.1]	225.4 [1.4]	350.5 [0.6]
Wilts 1A/L	469.6 [1.9]	43.3 [2.1]	130.7 [0.8]	1971.7 [3.4]
T/11	643.5 [2.6]	253.0 [12.3]	237.1 [1.5]	7884.8 [13.6]
Oxford 5A/F	447.6 [1.8]	94.6 [4.6]	334.6 [2.1]	6908.9 [11.9]
Essex 2A/F	1067.4 [4.3]	64.1 [3.1]	98.1 [0.6]	1783.0 [3.1]
Dorset 1A/F	588.2 [2.4]	399.4 [19.5]	279.3 [1.7]	13710.4 [23.6]
Lincs. 7A/F	1148.7 [4.7]	104.7 [5.1]	133.7 [0.8]	41697.5 [71.8]
Suffolk 1A/F	558.1 [2.3]	55.3 [2.7]	190.4 [1.2]	582.2 [1.0]
Kent 1A/F	245.9 [1.0]	28.6 [1.4]	213.6 [1.3]	360.3 [0.6]

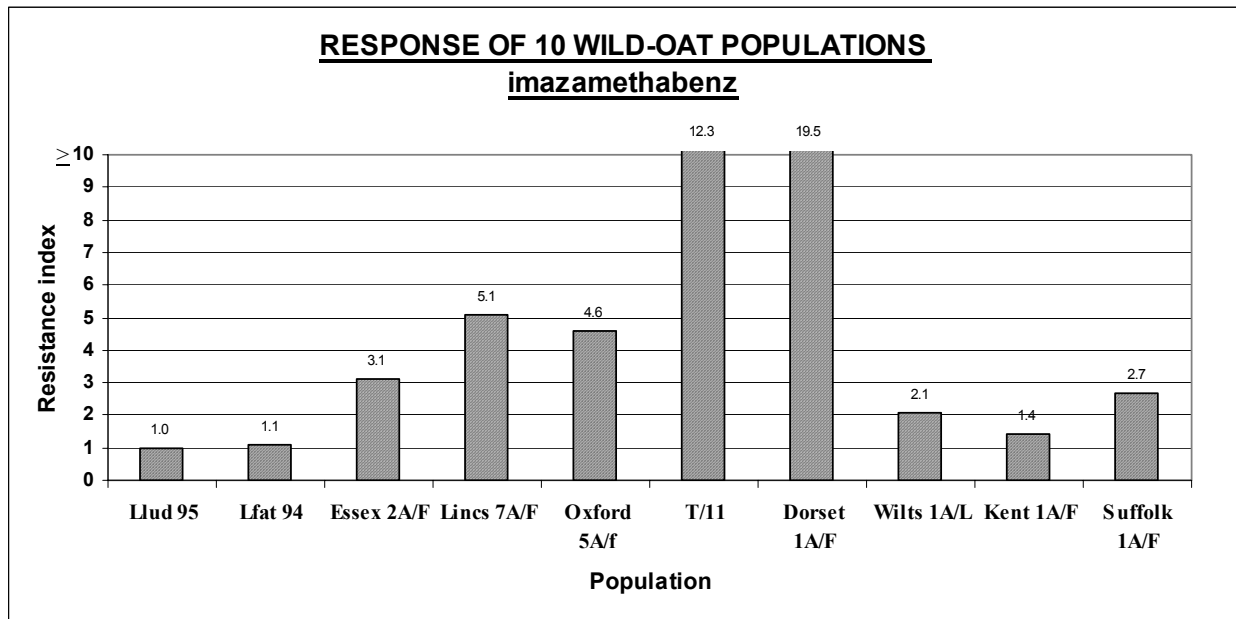**Figure 12. Experiment 2: Response to *non* - "fop" and "dim" herbicides: imazamethabenz**

RESPONSE OF 10 WILD-OAT POPULATIONS
imazamethabenz

Figure 13. Experiment 2: Response to *non* - "fop" and "dim" herbicides: flamprop

RESPONSE OF 10 WILD-OAT POPULATIONS
flamprop
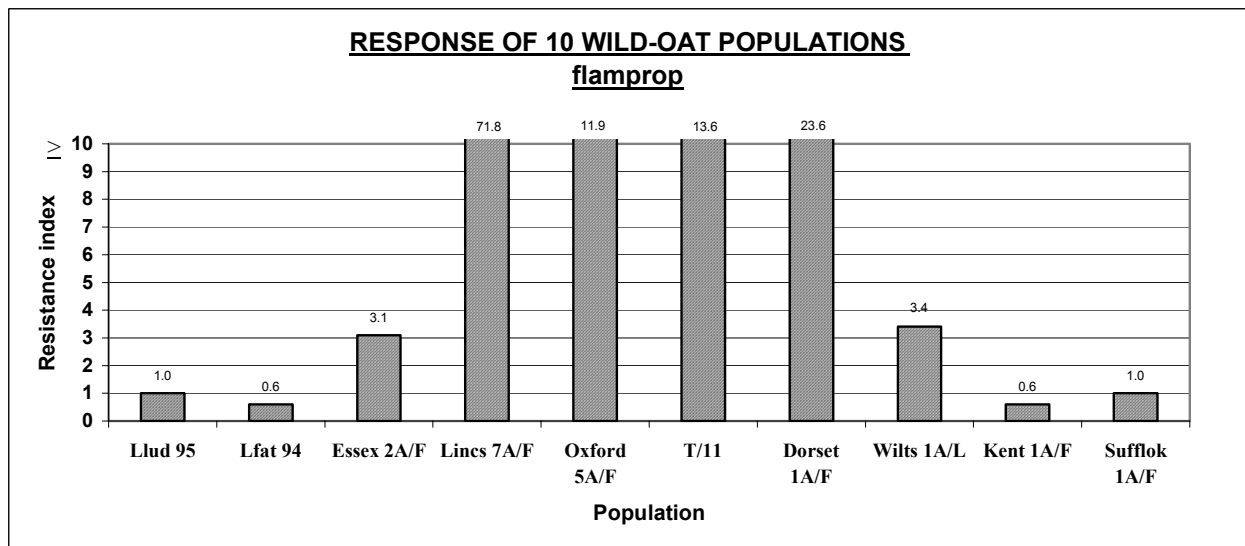
Figure 14. Experiment 2: Response to *non* - "fop" and "dim" herbicides: difenzoquat

RESPONSE OF 10 WILD-OAT POPULATIONS
difenzoquat
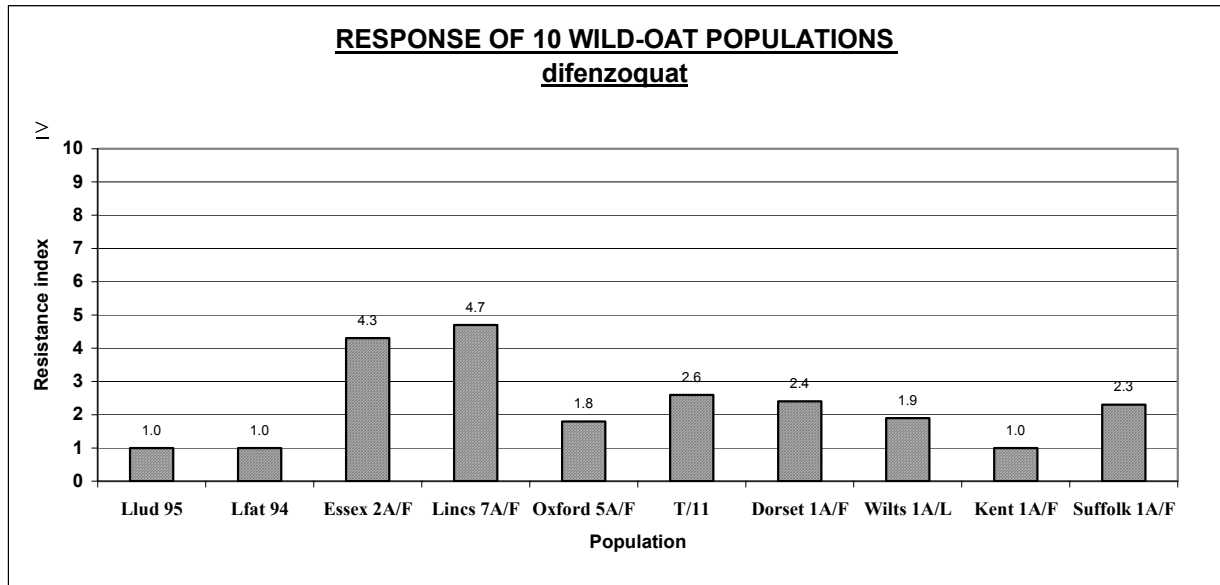
Figure 15. Experiment 2: Response to *non* - "fop" and "dim" herbicides: isoproturon

**RESPONSE OF 10 WILD-OAT POPULATIONS**
isoproturon
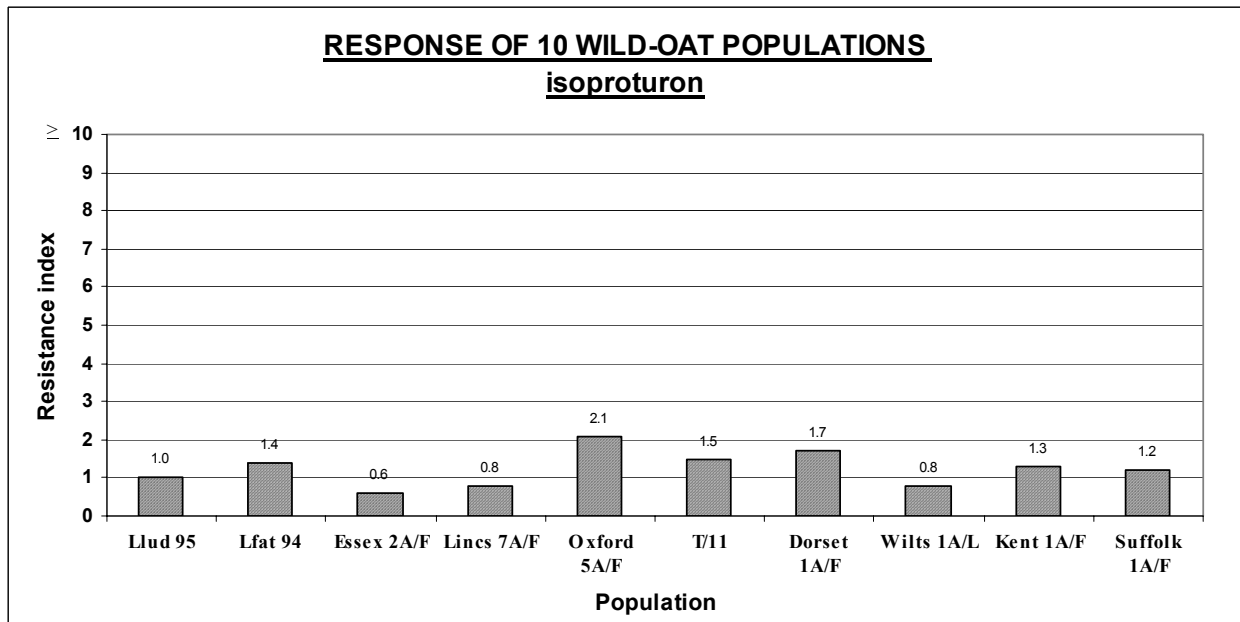
Experiment 3 (Table 13; Figure 16)

- In order to see differences and similarities more clearly and make comparisons easier, a graph has been created (Figure 16) showing shoot length in comparison to the dose in ppm. The dose range used seemed ideal, with slight reductions at the lowest concentration and large reductions at the highest concentration for all populations.

- There was no evidence of a difference in response between the three populations. Although the resistance indices for the two Essex populations were higher (1.9, 2.8) than for the susceptible standard, (1.0), the differences were not (quite) statistically significant.

- The T/41 (Essex 10 A/L) population is highly resistant to fenoxaprop due to an insensitive target site (ACCase) (Cocker *et al*., 2000). The T/11 population has been used in the container experiments and cross-resistance studies and shows cross-resistance to tralkoxydim, imazamethabenz and flamprop-M-isopropyl (see previous sections). The mechanism of resistance in T/11 is enhanced metabolism (Cocker *et al*., 2000). Despite this extensive pattern of cross-resistance, it shows no resistance to tri-allate.

- In the container studies (section 1 (b)) triallate alone has not performed well against the T/11 population. However, there was no evidence of resistance to tri-allate in the container studies which supports the results from this petri-dish study.

Table 13. Experiment 3: Petri-dish assay with tri-allate

Population	Log_{10} ED_{50} Values	Detransformed ED_{50} values (Ratio to LLUD 95 in brackets = Resistance index)
LLUD 95	-0.5947	0.25 (1.0)
T/41 1994	-0.1632	0.69 (2.8)
T/11 1995	-0.3258	0.47 (1.9)
COMBINED S.E. ±	0.1470	-
LSD (P≤ 0.05)	0.4367	-

Figure 16. Experiment 3: Petri-dish assay with tri-allate
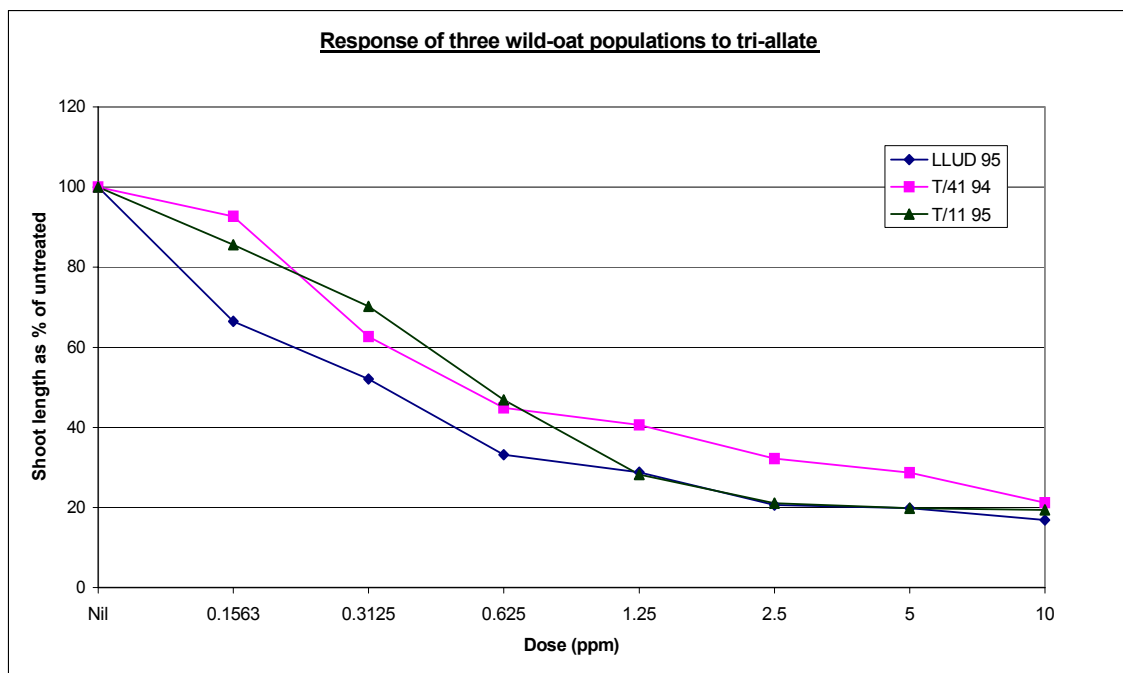
Experiment 4 & 5 (Table 14)

- In Experiment 4, only one of the ten populations had an ED_{50} value significantly higher than the LLUD susceptible standard (Table 14). This was Essex 2A/F which had a resistance index of 7.1. This was not a population that had shown high resistance to other herbicides in the cross-resistance studies detailed in Experiments 1 & 2 above. Some populations appeared to be more sensitive to tri-allate than the LLUD susceptible standard, with resistance indices of less than 1. However, this may have been due to less than ideal curve fitting and this was why some populations were included in Experiment 5 at a wider range of concentrations of tri-allate.

- In Experiment 5, Essex 2A/F was again the only population to have an an ED_{50} value significantly higher than the LLUD susceptible standard (Table 14), thus supporting the results of Experiment 4. There was generally good agreement between the results for Experiments 3, 4 & 5 with all the other populations showing no clear evidence of resistance to tri-allate. Both the T/11 and Dorset 1A/F populations have been shown to have an enhanced ability to metabolise herbicides (Cocker *et. al.,* 2000) but there was no evidence that this had a significant impact on tri-allate activity, although the resistance indices were always above 1 for both populations in all three petri-dish experiments (T/11 - 1.9, 2.1, 2.3; Dorset 1A/F 1.2, 2.3).

Table 14. Experiment 4 & 5: Petri-dish assay with tri-allate

Population	Experiment 4 LOG$_{10}$ ED$_{50}$ Values	Experiment 5 LOG$_{10}$ ED$_{50}$ Values
LLUD 1995	-0.3604	-0.6639
LFAT 1994	-1.4734	-1.0025
Wilts 1A/L	-1.1788	-0.8329
T/11 1995	-0.0481	-0.2969
Oxford 5A/F	-0.5568	-
Essex 2A/F	0.4911	0.1532
Dorset 1A/F	-0.2907	-0.2952
Lincs. 7A/F	-0.2957	-
Suffolk 1A/F	-0.1959	-
Kent 1A/F	-0.9730	-
T/41 1994	-	-0.6082
Combined S.E. ±	0.1615	0.1322
L.S.D. (≤ 0.05)	0.4568	0.3778

Population	Experiment 4 Detransformed ED$_{50}$ Values [Ratio to LLUD = R.I.]	Experiment 5 Detransformed ED$_{50}$ values [Ratio to LLUD = R.I.]
LLUD 1995	0.436 [1.0]	0.217 [1.0]
LFAT 1994	0.034 [0.08]	0.099 [0.46]
Wilts 1A/L	0.066 [0.15]	0.147 [0.68]
T/11 1995	0.895 [2.05]	0.505 [2.33]
Oxford 5A/F	0.277 [0.64]	-
Essex 2A/F	3.098 [7.11]	1.423 [6.56]
Dorset 1A/F	0.512 [1.17]	0.507 [2.34]
Lincs. 7A/F	0.506 [1.16]	-
Suffolk 1A/F	0.637 [1.46]	-
Kent 1A/F	0.106 [0.24]	-
T/41 1994	-	0.246 [1.13]

R.I. = Resistance Index

Conclusions

The results demonstrate clearly that there is continuum of response to fenoxaprop, from susceptible through to highly resistant. Populations cannot simply be placed into arbitrary susceptible and resistant categories. The variability in response was not due simply to a difference in the proportion of highly resistant individuals within the population. Virtually all plants of some partially resistant populations would survive an intermediate rate of herbicide that would kill all susceptible plants. Higher rates would kill all plants of

the partially resistant population but allow highly resistant plants to survive. Subsequent work (Cocker *et al*., 2000) has helped to explain this. Some populations (e.g. T/41 and Suffolk 1A/F) have target site (ACCase) insensitivity, which confers a high level of resistance, whereas other populations (e.g. T/11) possess an enhanced ability to metabolise herbicides which normally confers partial resistance.

Populations with resistance to one "fop" herbicide (fenoxaprop) also tended to show resistance to another "fop" (fluazifop), but not always to the same degree. However, resistance to "fops" was not directly correlated with resistance to "dims", despite these having the same mode of action. The two populations (Kent 1A/F and Suffolk 1A/F) with the highest level of resistance to "fops", showed no evidence of resistance to tralkoxydim. However, five other populations showed very clear evidence of resistance to both tralkoxydim and fenoxaprop, although again the degree of resistance varied. However, despite clear evidence of resistance to tralkoxydim in several populations, there was no evidence of resistance to cycloxydim in any population. The results demonstrate that with wild-oats, as with black-grass, the degree of resistance to herbicides within the same chemical group can vary. Relating resistance to chemical group can be misleading, and there is a need to consider herbicides individually as resistance may occur to some (e.g tralkoxydim), but not all (e.g. cycloxydim), of the herbicides within a chemical group.

Studies with *non* "fop" and "dim" herbicides demonstrated clearly that with wild-oats the degree of cross-resistance to herbicides with different modes of action can vary considerably. Two populations which showed the greatest level of resistance to the "fop" fenoxaprop, (Kent 1A/F RI = 6.8, Suffolk 1A/F RI = 13.1) showed no clear evidence of resistance to any of the non - "fop" and "dim" herbicides tested, nor to the "dims" tralkoxydim or cycloxydim, or to tri-allate in the petri dish experiments. In those two populations, resistance was confined to "fops" only. In contrast, four populations which showed partial resistance to both fenoxaprop and tralkoxydim, (Lincs 7A/F, Oxford 5A/F, T/11 and Dorset 1A/F), all showed evidence of cross-resistance to imazamethabenz and flamprop, despite these herbicides having different modes of action. This was probably due to the presence of an enhanced metabolism mechanism, which has been confirmed in T/11 and Dorset 1A/F in associated MAFF funded biochemical studies. In contrast Suffolk 1A/F has been shown to possess target site resistance to fenoxaprop, which appears to be specific to "fops" and not affect "dims". This contrasts with black-grass, where target site resistance affects both "fops'" and "dims".

There was no clear evidence of resistance to difenzoquat, isoproturon or tri-allate in any population. One population (Essex 2A/F) did show some insensitivity to tri-allate in two petri-dish experiments and also showed the second highest level of insensitivity to difenzoquat of the ten populations evaluated. This may be coincidence, but in North America some populations of wild-oats show resistance to both triallate and difenzoquat, despite these herbicide having different modes of action. This population did not show a high degree of resistance to other herbicides, but warrants further investigation.

SECTION 3

Occurrence and distribution of resistant populations

Introduction

Herbicide resistant wild-oats were first detected in the UK in 1993 and by 1995, at the start of this project, only a few cases (about 6 - 8) had been confirmed. Most of these were in Essex but resistant wild-oats had also been found in Dorset and Kent. Resistance had been detected in both species of wild-oats, although relatively more commonly in *Avena sterilis* ssp. *ludoviciana*, which was somewhat surprising as this species is less common than *Avena fatua*. Thus one aim of the project was to collect and evaluate populations of wild-oats with suspected resistance to determine the distribution, degree of resistance and relative frequency of resistance in the two wild-oat species. No random surveys were planned.

Materials and Methods

Wild-oat populations were collected from a range of sources each year, including direct farmer contacts, ADAS, consultants, agrochemical companies and distributors. They were not collected at random but mainly in response to reports of inadequate control of wild-oats by herbicides. The details of the numbers of populations tested each year are summarised below:

Number of samples evaluated for resistance each year in terms of farms, populations and species.

	1995	1996	1997	1998	1999
Farms	17	17	19	13	10
Populations	30	30	25	16	20
A. fatua	14	20	18	10	9
A. sterilis ssp. *ludoviciana*	14	10	7	6	11
Mixed species	2	0	0	0	0

Susceptible standards for both species of wild-oats (LFAT = *Avena fatua*, LLUD = *Avena sterilis* ssp. *ludoviciana*) are included in this total. Some of the populations comprised either separate fields on single farms or sub-samples from within the same field in order to determine the distribution of resistance. Some farms were sampled in more than one year.

A separate glasshouse assay was conducted each year using the sample methodology. Dry seeds (50-80/dish) were placed in petri-dishes (6-8 dishes/population) containing four filter papers. Seeds had been previously stored dry at 30-35ºC for two weeks to four months in order to help break dormancy. Each seed was then pricked through the middle with a needle and 9 ml water added to each dish. The seeds were then put in an incubator set at 17°C 14 hour day and 10°C 10 hour night. After about 7 days, 25 pots were prepared for each population by sowing 6 germinated seeds in each 9.0 cm diameter pot containing a Kettering loam/grit mix. Soil was added to cover seeds to 5mm depth. Emerging seedlings were thinned to leave five plants per pot.

Approximately 3 weeks after sowing, when plants were at the 2 - 3 leaf stage, herbicide treatments were applied using a laboratory sprayer delivering 222- 280 L water/ha at 210 kPa through a single Teejet 110015 VK nozzle. The herbicide treatments consisted of fenoxaprop-P-ethyl applied at 27.5 and 55 g a.i./ha, tralkoxydim (+ "Output" adjuvant) at 43.75 g a.i./ha and imazamethabenz (+ "Agral") at 150 g a.i./ha. The rates of tralkoxydim and imazamethabenz are ⅛ and ¼ of the recommended field rates respectively and were used as previous studies had shown these herbicides to be much more active in glasshouse conditions than in the field. There were five untreated pots for each population arranged in five replicate randomized blocks.

The number of surviving plants and foliage fresh weight were recorded for each pot 3 - 4 weeks after spraying as a measure of herbicide activity. Assessing the numbers of surviving plants was imprecise because some plants showed severe symptoms, but were not actually dead. Some severely affected plants showed signs of recovery. Measurement of fresh foliage weight appeared to be the most reliable assessment method as it incorporated both plant kill and effects on plant growth in a single assessment. The mean foliage fresh weights for untreated pots ranged averaged about 14 g/pot. Percentage reductions in foliage fresh weight were calculated for each pot in relation to the untreated controls for the same population, and analysed by analysis of variance.

Results

- Full results for the response of each of the 121 populations to the three herbicides tested are not presented here for reasons of space. Results are presented for 15 populations, including the populations

used subsequently in the cross-resistance studies (see section 2), some populations evaluated in more than one year and the standard reference populations (Tables 15, 16, 17). Populations are identified by county and numbers refer to different farms. Letters differentiate fields on the same farm. The code /F (*Avena fatua*) or /L (*Avena sterilis* ssp. *ludoviciana*) indicates the species of wild-oats.

- The susceptible standards LFAT and LLUD 95 were well controlled (84 - 96 %) by the higher rate of fenoxaprop in all years (Table 15). The two species appeared equally sensitive to fenoxaprop. Control of several other populations was similar to, or greater than that achieved on these susceptible standards e.g. Herts. 1B. This demonstrates that LFAT and LLUD are good standards for susceptible populations in general, and are not atypically sensitive.

- In contrast, consistently poor control (<50%) of several populations was achieved by fenoxaprop at full rate (55 g a.i./ha) including both species of wild-oats. These populations came from a wide geographical area and this demonstrates that the highly resistant wild-oat are not confined to a localised area and also exists in both species of wild-oats.

- In each year, there was a continuum of response to fenoxaprop (higher rate) from low (<25%) to high (>85%) % reduction values. Figure 17 presents the 1997 results for all populations tested in that year arranged in order of increasing % reductions. The continuum of response was not simply a reflection of different proportions of plants surviving for different populations. All plants of each population tended to respond to a similar degree. This highlights the fact that resistance in wild-oats is not absolute and a wide range of responses is possible, so it is difficult to define a fixed threshold for resistance. Figure 17 also demonstrates that several populations showed a similar response to the LFAT and LLUD susceptible standards, confirming that these are good standard reference populations for susceptibility.

- One solution is to use a modification of the star rating system devised for resistant black-grass (see Clarke, Blair & Moss, 1994). This can be used to assign resistance rankings for fenoxaprop. This system involves using the mean % reduction value of the two susceptible standards, LLUD and LFAT as a reference point for susceptibility, dividing this by **five**, so that the % reduction values between the susceptible standard and zero are separated into five equal categories. One of these categories, at the susceptible end of the range, is sub-divided about its mid point into two smaller categories, S and 1*. For example, if the mean of LLUD and LFAT for the higher rate of fenoxaprop was 92.4% (as in 1998), each category = 18.5%. Thus 5* = 0 – 18.5%; 4* = 18.6% – 37%; 3* = 37.1% – 55.4%; 2* = 55.5% – 73.9%; 1* = 74 – 83.2; Susceptible = 83.3% – 92.4% (and over). In practice the six categories calculated above are more than are needed for screening purposes and some can be combined. The following four 'R' category system has been suggested: 5/4* = RRR, 3/2* = RR, 1* = R? (needs further evaluation), S = susceptible (see Moss *et al.*, 1999 for further details).

- The ten populations used in the more detailed cross-resistance studies (see section 2) had all been previously included in the screening experiments and are included in Table 15. The results for the glasshouse screening experiments (mean % reductions) and the resistance indices (from the cross-

resistance studies) for fenoxaprop were: (Suffolk 1A - 11%, 13.1; Kent 1A - 3%, 6.8; Wilts. 1A - 26%, 6.7; Dorset 1A - 52%, 6.4; T/11 - 41%, 5.4; Oxford 5A - 75%, 2.6; Lincs. 7A - 69%, 2.6; Essex 2A - 52%, 1.7; LFAT - 90%, 1.3; LLUD - 92%, 1.0.

- There was a clear inverse correlation in that, as resistance index for fenoxaprop declined (indicating greater susceptibilty), the % reduction in the single dose screening test increased (also indicating greater susceptibility). The Essex 2A population with a resistance index of only 1.7 would have been expected to show only marginal insensitivity in the glasshouse screen, and thus a higher % reduction value than that actually recorded. The explanation for this discrepancy is almost certainly due to the fact that the seed sample used in the cross-resistance test was collected in 1994 when the field was heavily infested with wild-oats, whereas for the screening experiment a 1997 sample was collected from the few wild-oats surviving herbicide treatment in the plots of the field experiment conducted that year. Thus there had been the potential for three years further selection.

- Overall, the correlation between the fenoxaprop results from the cross-resistance experiment and this single dose screening experiment was very good. There was also good consistency in the results for populations tested in several years (Table 15). In many cases these were for separate samples collected in the different years. This demonstrates that an accurate determination of the degree of resistance to fenoxaprop can be obtained from a single dose assay, provided that the dose used is sufficiently discriminating.

- The lower rate of fenoxaprop (27.5 g a.i./ha) gave, as expected, generally poorer control than the higher rate (55 g a.i./ha) (data not presented). Some populations which were well controlled by the higher rate, were poorly controlled by the lower rate. It appears that the use of this lower rate can amplify differences which may be of marginal importance in the field. It was concluded that for screening purposes the use of fenoxaprop at 55 g a.i./ha is recommended as the best single dose for discriminating between populations.

- Differences between populations in the percentage reductions values for tralkoxydim were generally lower than for fenoxaprop (Table 16). The susceptible standards LFAT and LLUD, and also Herts 1B, were well controlled (87 - 96%). Many populations (e.g. Essex 1A, Essex 10A, Oxford 3A, Suffolk 1A, Kent 1A) which showed high resistance to fenoxaprop were as well controlled by tralkoxydim as the susceptible standards.

- The tralkoxydim results for the 10 populations used in the glasshouse screening experiments (mean % reductions) and the more detailed cross-resistance studies (resistance indices in section 2) were: (Suffolk 1A - 83%, 2.4; Kent 1A - 88%, 1.5; Wilts. 1A - 34%, 16.7; Dorset 1A - 41%, 64.3; T/11 - 17%, 31.0; Oxford 5A - 53%, 43.7; Lincs. 7A - 38%, 23.3; Essex 2A - 64%, 2.8; LFAT - 91%, 1.9; LLUD - 91%, 1.0. Both tests confirm resistance to tralkoxydim in the Wilts 1A, Dorset 1A, T/11, Oxford 5A and Lincs. 7A populations, but the resistance indices are considerably higher than for fenoxaprop and tend

- to exaggerate the level of resistance. Interestingly, these five populations showed generally only *partial* resistance to fenoxaprop.

- The Cambridge 3A population showed clear evidence of resistance to tralkoxydim and also showed high resistance to fenoxaprop. This demonstrates that a high degree of resistance to both herbicides can occur in some populations, although this is the only population found so far with this characteristic.

- Differences between populations in the percentage reductions values for imazamethabenz (Table 17) were, as with tralkoxydim, generally lower than for fenoxaprop. The susceptible standards LFAT and LLUD were fairly well controlled (66 - 96%). As with tralkoxydim, many populations (e.g. Essex 1A, Essex 10A, Oxford 3A, Suffolk 1A, Kent 1A), which showed high resistance to fenoxaprop, were as well controlled by imazamethabenz as the susceptible standards.

- The imazamethabenz results for the 10 populations used in the glasshouse screening experiments (mean % reductions) and the more detailed cross-resistance studies (resistance indices in section 2) were: (Suffolk 1A - 75%, 2.7; Kent 1A - 90%, 1.4; Wilts. 1A - 76%, 2.1; Dorset 1A - 42%, 19.5; T/11 - 27%, 12.3; Oxford 5A - 47%, 4.6; Lincs. 7A - 63%, 5.1; Essex 2A - 52%, 3.1; LFAT - 85%, 1.1; LLUD - 81%, 1.0. Both tests confirm resistance to imazamethabenz in Dorset 1A and T/11 populations and partial resistance in the Oxford 5A and Lincs. 7A populations, although the control of Dorset 1A in the glasshouse screening test was rather better than the resistance index figure would predict.. These four populations all showed resistance to tralkoxydim and *partial* resistance to fenoxaprop.

- The Cambs. 3A populations did not show evidence of resistance to imazamethabenz, despite showing resistance to tralkoxydim. This demonstrates that resistance to these two herbicides is not always correlated. This is the only population known so far which shows this characteristic and demonstrates that different cross-resistance patterns can occur. Interpretation of these results in terms of cross-resistance patterns needs to be made with care as only single doses of tralkoxydim and imazamethabenz were used.

- Sampling and testing wild oat samples collected from different patches within the same field, and from several different fields within the same farm, confirmed that resistant wild-oats may occur quite widely across farms, and not be confined to a single patch (Essex 10, Essex 12, Dorset 1, Kent 1). However on other farms (Essex 1A, Cambs. 3A, Wilts 1A) there was clear evidence for resistance being confined to localised patches which were often relatively small (< 0.25 ha). It was evident from field observations that these patches were often elongated in the direction of cultivations/combining, indicating that resistance had probably originated some years previously. Some of the wild-oat populations collected in these studies have been used in more detailed research on characterising wild-oats in different patches and investigating gene flow (Cavan, Biss & Moss, 1998).

- Resistant wild-oats were also detected on three neighbouring farms in Essex, which raises issues about the spread of resistant wild-oats. In that case (Essex 10) there was no common link between the farms which operated independently. Wild-oats are self pollinating so spread of resistance genes in pollen is unlikely. Spread of resistant seeds is a more likely reason although the method of transport is unknown.

- Several organisations conduct screening tests for resistant wild-oats in the UK. A compilation exercise was conducted for all wild-oat populations found to be resistant both within this project and by other organisations/companies in order to determine how many individual farms had been identified as containing resistant wild-oats. These results have been published (Moss *et al.*, 1999) and are summarised here. By 1999 resistance to fenoxaprop-P-ethyl had been detected on **65 farms** distributed over **19 counties** of England (Table 18). The counties with the greatest recorded number of farms with confirmed resistance were: Essex - 17; Lincolnshire – 7; Norfolk – 7; Cambridgeshire – 6; Somerset – 6. Resistant wild-oats have not yet been detected in Wales, Scotland or Northern Ireland. Although fenoxaprop was used as the standard herbicide for screening for resistance, cross-resistance to herbicides with the same, and different mode of action, often occurs.

- For the 60 populations for which there is a positive species identification, 45 (75%) are *Avena fatua* and 15 (25%) *Avena sterilis* ssp. *ludoviciana.* Thus resistance occurs in both species of wild-oats, but relatively more commonly in *A. sterilis* ssp. *ludoviciana* than would be predicted from the frequency of occurrence of the two species. It is generally estimated that *A. sterilis* ssp. *ludoviciana* comprises less than 20% of wild-oat infestations in the UK, although no random survey has been conducted for many years.

Conclusions

The pot assay technique worked well and gave consistent results. The results clearly identified highly fenoxaprop-resistant wild-oats. However, many populations showed partial resistance and interpreting the significance of these more marginal levels of insensitivity is more difficult. This highlights the fact that resistance in wild-oats is not always absolute and a wide range of responses is possible. As it is difficult to define a fixed threshold for resistance the use of the * or 'R' rating system, as used for black-grass, for categorising different degrees of resistance in screening assays is recommended.

The correlation with results from the cross-resistance dose response assays (see Section 2) for the 10 populations included in both series of experiments was very good and confirms clearly that cross-resistance patterns are complex, especially in terms of quantitative differences.

Populations showing the greatest resistance to fenoxaprop tended *not* to show resistance to tralkoxydim or imazamethabenz. This is probably due to the presence of target site resistance affecting only "fop" herbicides. In contrast, some partially fenoxaprop resistant populations showed cross-resistance to tralkoxydim and imazamethabenz, probably due to the presence of an enhanced metabolism mechanism. Associated work on the biochemical basis of resistance supports these conclusions (Cocker *et al.*, 2000).

One population was identified which had resistance to fenoxaprop and tralkoxydim (Cambs. 3A/L) but no resistance to imazamethabenz. This may be due to the presence of target site resistance affecting both "fops" and "dims", and biochemical studies are in progress to confirm this. This demonstrates that different cross-resistance patterns are possible and the risks involved in making generalised statements about cross-resistance.

The compilation exercise showed that by 1999, wild-oats with resistance to fenoxaprop-P-ethyl had been detected on **65 farms** distributed over **19 counties** of England. Resistance occurred over a wide geographical area and this demonstrates that the highly resistant wild-oat are not confined to a localised area. Resistance also exists in both species of wild-oats. Resistant wild-oats have not yet been detected in Wales, Scotland or Northern Ireland.

Table 15. Results of five glasshouse screening evaluations of 15 populations for resistance to:

Fenoxaprop-P-ethyl 55 g a.i./ha

% Reduction in foliage fresh weight

Population	Species	1995	1996	1997	1998	1999
Essex 1A (Straw S/B)	*A. ludo.*	1	-	27	-	9
Essex 10A (T/41)	*A. ludo.*	-	0	-	21	19
Oxford 3A (Hk)	*A. ludo.*	0	0	-	-	-
Cambridge 3A (Arr)	*A. ludo.*	-	-	-	28	9
Suffolk 1A (Lav)	*A. fatua*	-	1	20	-	-
Kent 1A (OSR)	*A. ludo.*	3	-	-	-	-
Wilts. 1A/L (Scot)	*A. ludo.*	22	-	30	-	-
Dorset 1A (Down)	*A. fatua*	36	50	-	69	-
T/11 95 (SB)	*A. ludo.*	19	-	41	62	40
Oxford 5A (New)	*A. fatua*	-	75	74	-	-
Lincs. 7A (Frisk)	*A. fatua*	-	67	57	84	-
Essex 2A (Worm)	*A. fatua*	-	-	52	-	-
Herts. 1B (Clay)	*A. fatua*	-	-	82	-	87
LFAT (SB)	*A. fatua*	93	88	84	95	-
LLUD (SB)	*A. ludo*	96	88	88	92	95

Table 16. Results of five glasshouse screening evaluations of 15 populations for resistance to:

Tralkoxydim 43.75 g a.i./ha

% Reduction in foliage fresh weight

Population	Species	1995	1996	1997	1998	1999
Essex 1A (Straw S/B)	*A. ludo.*	93	-	85	-	86
Essex 10A (T/41)	*A. ludo.*	-	91	-	87	91
Oxford 3A (Hk)	*A. ludo.*	77	91	-	-	-
Cambridge 3A (Arr)	*A. ludo.*	-	-	-	41	16
Suffolk 1A (Lav)	*A. fatua*	-	91	75	-	-
Kent 1A (OSR)	*A. ludo.*	88	-	-	-	-
Wilts. 1A/L (Scot)	*A. ludo.*	24	-	43	-	-
Dorset 1A (Down)	*A. fatua*	29	56	-	37	-
T/11 95 (SB)	*A. ludo.*	20	-	38	7	4
Oxford 5A (New)	*A. fatua*	-	65	41	-	-
Lincs. 7A (Frisk)	*A. fatua*	-	30	51	34	-
Essex 2A (Worm)	*A. fatua*	-	-	64	-	-
Herts. 1B (Clay)	*A. fatua*	-	-	89	-	89
LFAT (SB)	*A. fatua*	93	89	90	91	-
LLUD (SB)	*A. ludo*	96	89	90	91	87

Table 17. Results of five glasshouse screening evaluations of 15 populations for resistance to:

Imazamethabenz 150 g a.i./ha

% Reduction in foliage fresh weight

Population	Species	1995	1996	1997	1998	1999
Essex 1A (Straw S/B)	*A. ludo.*	89	-	73	-	42
Essex 10A (T/41)	*A. ludo.*	-	83	-	84	67
Oxford 3A (Hk)	*A. ludo.*	84	86	-	-	-
Cambridge 3A (Arr)	*A. ludo.*	-	-	-	85	69
Suffolk 1A (Lav)	*A. fatua*	-	88	62	-	-
Kent 1A (OSR)	*A. ludo.*	90	-	-	-	-
Wilts. 1A/L (Scot)	*A. ludo.*	80	-	71	-	-
Dorset 1A (Down)	*A. fatua*	38	32	-	57	-
T/11 95 (SB)	*A. ludo.*	26	-	33	29	19
Oxford 5A (New)	*A. fatua*	-	50	43	-	-
Lincs. 7A (Frisk)	*A. fatua*	-	68	77	45	-
Essex 2A (Worm)	*A. fatua*	-	-	52	-	-
Herts. 1B (Clay)	*A. fatua*	-	-	77	-	50
LFAT (SB)	*A. fatua*	92	83	78	87	-
LLUD (SB)	*A. ludo*	96	83	74	87	66

Figure 17. Wild-oat glasshouse screening experiment 1997:   % reduction in foliage fresh weight relative to untreated controls after treatment with fenoxaprop-P-ethyl at 55 g a.i./ha
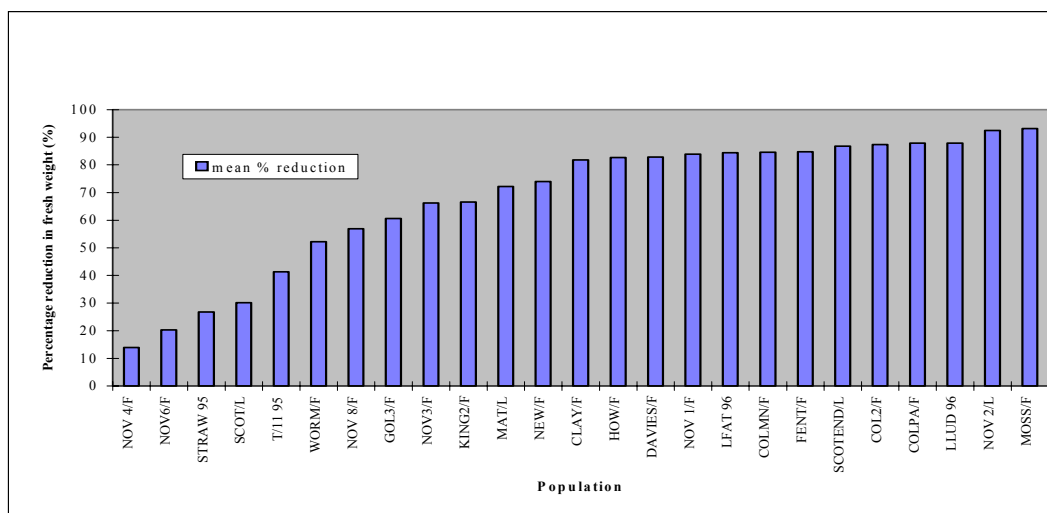

Table 18.   The distribution of farms in England, by county, where wild-oats resistant to fenoxaprop-P-ethyl had been detected by 1999 (from Moss *et al*. 1999).

County	Number of farms
Bedfordshire	1
Berkshire	1
Cambridgeshire	6
Devon	1
Dorset	1
Essex	17
Kent	2
Gloucestershire	2
Humberside	1
Leicestershire	1
Lincolnshire	7
Norfolk	7
Northamptonshire	1
Nottinghamshire	2
Oxfordshire	1
Somerset	6
Suffolk	3
Warwickshire	3
Wiltshire	2
TOTAL	**65 farms in 19 counties**

SECTION 4

Influence of resistance on wild-oat control at field recommended doses

4 (a). Field experiments

Introduction

Care is needed in interpreting results from glasshouse experiments in terms of impact on field performance. Consequently a series of field experiments was planned in fields where the presence of resistant wild-oats had been confirmed, to help determine to what extent the control of wild-oats was reduced at field recommended rates. An additional objective was to gain information on the relationship between the degree of resistance detected in the glasshouse experiments and the impact of resistance on herbicide performance in outdoor conditions.

Materials and Methods

Two field experiments were conducted each year, making a total of 10 experiments over the 5 years. The limited number of winter cereal fields available with confirmed resistance in wild-oats meant that some sites were not ideal in terms of population uniformity and density. The location and resistance ratings ("R" values) for fenoxaprop and tralkoxydim, based on pot screening assays (see Section 3) for seeds collected prior to the initiation of the experiments, are given below. An experiment was conducted for three successive years in the Essex 10/L (= T/41) field in order to study the year to year variability in herbicide response at one site.

	1995 - 1996	1996 - 1997	1997 - 1998	1998 - 1999	1999 - 2000
Site 1.	Essex 10A/L	Essex 10A/L	Essex 10A/L	Cambs. 3A/L	Oxford 5A/F
Fenoxaprop	RRR	RRR	RRR	RRR	R?
Tralkoxydim	S	S	S	RR	RR
Site 2.	Essex 2B/F	Essex 2A/F	Dorset 1A/F	Lincs. 9A/F	Essex 7A/L
Fenoxaprop	RR	R?	RR	R?	R?
Tralkoxydim	R?	not tested	RR	RR	not tested

Resistance ratings (See Moss *et al.*, 1999):

R? = Early indications that resistance may be developing, possibly reducing herbicide performance

RR = Resistance confirmed, probably reducing herbicide performance

RRR = resistance confirmed, highly likely to reduce herbicide performance.

Associated studies of the biochemical basis of resistance showed that the Essex 10A/L population had an insensitive form of the target enzyme, ACCase (= target site resistance), while the Dorset 1A population had an enhanced ability to metabolise herbicides (= enhanced metabolism resistance), (Cocker *et al.*, 2000).

The following treatments were applied at every site (except Essex 2B/F):

1. Untreated
2. Fenoxaprop-P-ethyl (82.5 g a.i./ha) as "Cheetah Super" @ 1.5 l/ha (55 g/l a.i.)
3. Fenoxaprop-P-ethyl (41.25 g a.i./ha) as "Cheetah Super" @ 0.75 l/ha
4. Difenzoquat (990 g a.i./ha) as "Avenge 2" @ 6.6 l/ha (150 g/l a.i.)
5. Difenzoquat (495 g a.i./ha) as "Avenge 2" @ 3.3 l/ha
6. Imazamethabenz-methyl (600 g a.i./ha) as "Dagger" @ 2 l/ha (300 g/l a.i.) plus "Agral"
7. Imazamethabenz-methyl (300 g a.i./ha) as "Dagger" @ 1 l/ha plus "Agral"
8. Tralkoxydim (350 g a.i./ha) as "Grasp" @ 1.4 l/ha (250 g/l a.i.) plus "Output" adjuvant
9. Tralkoxydim (175 g a.i./ha) as "Grasp" @ 0.7 l/ha plus "Output"
10. Flamprop-M-isopropyl (600 g a.i./ha) as "Commando" @ 3 l/ha (200 g/l a.i.)
11. Clodinafop-propargyl (60 g a.i./ha) as "Topik 240EC" @ 0.25 l/ha (240 g/l a.i.)

In addition, mixtures of the lower rate fenoxaprop-p-ethyl plus the lower rate of either difenzoquat, imazamethabenz-methyl or tralkoxydim were applied at all sites.

Experiments comprised a randomised block design with 6 x 3 m plots in four replicates and were established in wheat crops in areas expected to have even populations of wild-oats. Treatments were applied using an Oxford Precision backpack sprayer calibrated to give 225 l/ha spray volume. Sites were sprayed mainly in March or April (range January to June) each year when the wheat was at growth stages 21 - 32 (range 13 - 57) and wild-oat plants were at growth stages 12 - 32 (range 12 - 51).

Wild-oat plants were assessed on the untreated plots prior to spraying. Wild-oat panicles were assessed in July or August each year using 10 random quadrats per plot, the size dependent on the population density.

Results

The panicle counts and % reduction relative to the untreated controls are presented in Tables 19, 20, 21, 22 & 23 with the appraisal of the results for each year immediately after each table.

Table 19. Field experiments: Year 1 1995 - 1996

Herbicide	g a.i./ha	Essex 10A/L		Essex 2B/F	
		Panicles per m^2	% Reduction	Panicles per m^2	% Reduction
Untreated	-	179	-	90	-
Fenoxaprop	82.5	155	13	84	7
Fenoxaprop	41.3	157	12	-	-
Difenzoquat	990	24	86	59	35
Difenzoquat	495	64	64	-	-
Imazamethabenz	600	90	49	70	23
Imazamethabenz	300	114	36	-	-
Tralkoxydim	350	15	92	80	11
Tralkoxydim	175	41	77	-	-
Flamprop	600	36	80	-	-
Clodinafop	60	124	30	70	22
Fenoxaprop + tralkoxydim	41.3 + 175	8	96	-	-
Fenoxaprop + imazamethabenz	41.3 + 300	102	43	-	-
Fenoxaprop + difenzoquat	41.3 + 495	70	61	-	-
S.E. ±		18.4	-	10.2	-

Essex 10A/L

- When the plots were sprayed (13 May 1996) there were on average 37 wild-oat plants/m^2.

- Although most treatments reduced panicle numbers above the crop (good from cosmetic point of view) some, particularly the imazamethabenz treatments, still had high numbers of panicles below the top of the wheat canopy.

- Fenoxaprop, at both rates, was the only herbicide which visually did not appear to have any effect at reducing the number of wild-oat panicles. Clodinafop also gave poor control (30%).

- Tralkoxydim (92%) and difenzoquat (86%) at the higher rates gave the best control of the single herbicide treatments.

- The mixture of half rate fenoxaprop plus half rate tralkoxydim was the most effective treatment (96%), being slightly better than the full rate of tralkoxydim alone (92%). The predicted level of control, based on levels of control by each component applied alone, was 80%. Thus the actual level of control was 16% greater providing some evidence for synergism.

- Fenoxaprop plus low rates of imazamethabenz or difenzoquat gave levels of control equivalent to low rates of imazamethabenz and difenzoquat alone. Thus there was no benefit from the addition of fenoxaprop and the mixture with imazamethabenz was 1% and for difenzoquat 8% less than would have been predicted from the simple additive effects of the individual components.

- The results were generally in agreement with expectations based on resistance ratings. Thus the "fop" herbicides gave poor control whereas other herbicides, especially the "dim" tralkoxydim, worked well showing that resistance to the "fops" and "dims" is not always linked.

Essex 2B/F

- There were an average of 20 wild-oat plants/m^2 when plots were treated on 6 June 1996 at a very advanced stage of growth. This was later than the recommended timing for most herbicides to be applied.

- Levels of control were poor for all herbicides. There was a difference in the growth of the wild-oats with panicles tending to be below the top of the wheat canopy on those plots which were treated.

- Fenoxaprop, imazamethabenz and clodinafop tended to hold the plants at the growth stage at which they were treated (growth stage 51) and did not allow the panicles to fully emerge.

- On the untreated plots most of the panicles were above the wheat crop.

- Interpreting the results in relation to resistance ratings at this site is difficult due to the confounding effects of late application, although both "fop" herbicides performed poorly and the best treatment, albeit still poor, was difenzoquat..

Table 20. Field experiments: Year 2 1996 - 1997

Herbicide	g a.i./ha	Essex 10A/L		Essex 2A/F	
		Panicles per m^2	% Reduction	Panicles per m^2	% Reduction
Untreated	-	10.4	-	4.09	-
Fenoxaprop	82.5	8.6	17	0.11	97
Fenoxaprop	41.3	9.8	6	1.93	53
Difenzoquat	990	1.7	83	1.52	63
Difenzoquat	495	5.6	46	1.87	54
Imazamethabenz	600	0.3	98	1.26	69
Imazamethabenz	300	2.5	76	2.94	28
Tralkoxydim	350	0.1	99	0	100
Tralkoxydim	175	0.3	97	0.36	91
Flamprop	600	6.2	40	0.61	85
Clodinafop	60	3.8	63	0.06	99
Fenoxaprop + tralkoxydim	41.3 + 175	0.3	97	0.02	100
Fenoxaprop + imazamethabenz	41.3 + 300	4.1	60	1.56	62
Fenoxaprop + difenzoquat	41.3 + 495	3.4	67	1.09	73
S.E. ±		2.71	-	0.867	-

Essex 10A/L

- When the plots were sprayed (27 January 1997) there were on average 9.7 wild-oat plants per m^2. The lower than expected numbers of wild-oat plants on this site appeared to be due to an overspray of the site by the farmer with isoproturon in the autumn.

- Fenoxaprop-p-ethyl gave poor control (<17%) as in the previous year in this field. Difenzoquat showed similar levels of control to 1996 (83 v 86%) whereas imazamethabenz-methyl was far more effective (98 v 49%). Tralkoxydim was again very effective when used alone at both full and half rate, achieving over 97% control. Flamprop was much less effective than in 1996 (40 v 80%) whereas clodinafop-propargyl showed improved levels of control over 1996 (63 v 30%).

- Again, the tralkoxydim plus fenoxaprop-p-ethyl mixture was the most effective mixture (97%) but the control was no better than half rate tralkoxydim alone (97%).   The fenoxaprop-p-ethyl plus

imazamethabenz-methyl mixture gave levels of control 17% less than would have been predicted from the simple additive effects of the individual components, so there was some evidence for antagonism. The mixture of fenoxaprop plus difenzoquat gave 18% better control than would be predicted (67 vs 49%), but control was still inadequate.

- In contrast to last year, there was no evidence for any synergistic effects from mixing half rates of fenoxaprop-p-ethyl and tralkoxydim (actual value 97%; predicted value 98%).

Essex 2A/F

- There were on average 2 wild-oat plants per m^2 when the plots were treated (21 March 1997).

- Fenoxaprop and clodinafop-propargyl gave levels of control (>97%) well above those seen at the other site. Tralkoxydim was again the best herbicide treatment (100%) although it was closely matched by fenoxaprop-p-ethyl (97%) and clodinafop-propargyl (99%) at this site. Half rate fenoxaprop-p-ethyl was appreciably poorer (53%) than full rate (97%).

- Of the other herbicides, difenzoquat (63%) and imazamethabenz-methyl (69%) at the full rate gave poorer levels of control than at the other site. Flamprop gave better control at this site (85%) than at the other site (40%) but control was inferior to the "fops" and "dims".

- The mixture of half rates fenoxaprop and tralkoxydim gave slightly better control (100%) than would have been predicted from the purely additive effect of the individual half rate applications (96%).

- Mixtures of fenoxaprop-p-ethyl and imazamethabenz-methyl and difenzoquat were 4% and 5% poorer than would have been predicted (66% and 78% respectively), although better than either herbicide at half rate alone.

- The resistance indices in glasshouse dose response studies using this population (see Section 2) were 1.7 for fenoxaprop and 2.8 for tralkoxydim, indicating very marginal levels of resistance. Consequently the good control achieved by full rate fenoxaprop (97%) and tralkoxydim (100%) supports the view that these marginal levels of resistance do not necessarily impact severely on herbicide efficacy in the field. However, it should be noted that half rate fenoxaprop gave mediocre control (53%) and this might be due to the impact of marginal levels of resistance.

Table 21. Field experiments: Year 3 1997 - 1998

Herbicide	g a.i./ha	Essex 10A/L Panicles per m^2	% Reduction	Dorset 1A/F Panicles per m^2	% Reduction
Untreated	-	44.8	-	37.2	-
Fenoxaprop	82.5	55.6	-24	27.8	25
Fenoxaprop	41.3	17.7	60	15.9	57
Difenzoquat	990	12.5	72	28.3	24
Difenzoquat	495	17.3	61	0	100
Imazamethabenz	600	20.1	55	7.2	81
Imazamethabenz	300	18.7	58	0.2	100
Tralkoxydim	350	2.4	95	35.1	6
Tralkoxydim	175	1.9	96	55.5	-49
Flamprop	600	67.6	-51	57.6	-54
Clodinafop	60	4.8	89	28.3	24
Fenoxaprop + tralkoxydim	41.3 + 175	1.9	96	18.7	50
Fenoxaprop + imazamethabenz	41.3 + 300	16.3	64	4.3	89
Fenoxaprop + difenzoquat	41.3 + 495	7.7	83	10.1	73
S.E. ±		12.9	-	-	

Essex 10A/L

- When the plots were sprayed (16 March 1998) there were on average 39 wild-oat plants per m^2.

- Fenoxaprop-p-ethyl gave erratic, but poor control of wild-oats this year, as it has done in each of the three years of experiments in this field (mean of both rates = 14% over 3 years).

- Difenzoquat at full rate has given a similar moderate level of control in each of the three year (86%, 83%, 72% for years 1,2 & 3). Half rate difenzoquat has averaged 57%.

- Imazamethabenz at full rate has given variable results over the three years (49%, 98%, 55%) and control by half rate imazamethabenz has ranged from 36% – 76%.

- Flamprop was ineffective in 1998 and has given generally variable control over the three years (80%, 40%, -51%).

- Clodinafop-propargyl gave better control this year (89%) but has given variable results over the three years (30%, 63%, 89%).

- These results highlight the problem of identifying resistance purely on the basis of field experience. Do the consistent, but generally only modest results for difenzoquat (72 – 86%) indicate partial resistance or no resistance? Almost certainly the latter – but one has no way of knowing purely from the field results. Flamprop, imazamethabenz and clodinafop gave variable results over the three years, and control was generally, but not always, mediocre. Partial resistance could have been involved, but there is absolutely no way of knowing purely from the field experience. This highlights the problem of detecting partial resistance and the need for tests under more controlled conditions, such as glasshouse pot assays.

- Tralkoxydim was very effective when used alone at both full and half rate, achieving over 94% control. Tralkoxydim has given the most consistently good control of any single herbicide treatment over the three years (92%, 99%, 95% at full rate). Half rate gave reduced levels of control in the first year, but levels of control similar to the full rate in the last two years 77%, 97%, 96% at half rate.

- The tralkoxydim plus fenoxaprop-p-ethyl mixture was the most effective mixture but the control was no better than half rate tralkoxydim alone. The actual level of control (96%) was 2% less than that predicted (98%) from the purely additive effects of the individual half rate applications. In the first year of the field experiment there was some evidence of synergy with this mixture but not in the second and third years. Control from this mixture has been consistently good over the three years (96%, 97%, 96%), but not appreciably better than full rate tralkoxydim alone.

- The fenoxaprop-p-ethyl plus imazamethabenz-methyl mixture only gave only a slight benefit over imazamethabenz-methyl alone. There was evidence of antagonism between fenoxaprop and imazamethabenz (expect value = 84%; actual value 64%). Although in some instances antagonism has been marginal, this has been a consistent trend in each of the three years of the field experiment at this site. Control by this mixture has been mediocre over the three years (43%, 60%, 64%).

- The mixture of fenoxaprop plus difenzoquat gave control similar to that predicted from the purely additive effects of the individual components (83% v 85%). In the first year control from the mixture was slightly poorer than expected, in the second year better than predicted and in this third year similar to that predicted. Overall the mixture appeared to give purely additive effects with no clear evidence of synergy or antagonism. However, control over the three years was generally mediocre (61%, 67%, 83%).

- There was less of a trend for reduced control with half rates this year compared with the two previous years.

Dorset 1A/F

- There were on average 33 wild-oat plants per m^2 when the plots were treated (20 March 1998). The distribution of wild-oats was very uneven over the trial, despite it being sited in an area historically known to be severely infested with wild-oats. This resulted in large differences in populations between

plots making interpretation of the results difficult. There also appeared to be a lot of secondary growth of wild-oats late on in the season which compounded the problem of interpretation.

- Fenoxaprop, clodinafop, tralkoxydim and flamprop gave poor control and this is consistent with resistance screening and cross-resistance experiments with this population (see section 2).

- Imazamethabenz appeared to give generally the best overall control, when both dose rates are considered. This conflicts with results from the cross-resistance studies with this population (see section 2) which indicated resistance to imazamethabenz at this site.

- Difenzoquat gave confusing results, achieving 100% at half rate but only 24% at full rate.

- The mixtures gave moderate control with the fenoxaprop/imazamethabenz mixture giving the best result (89%).

- Because of the uneven distribution of wild-oats at this site, no definitive conclusions should be drawn from the results from this site.

Table 22. Field experiments: Year 4 1998 - 1999

		Cambs. 3A/L		Lincs. 9A/F	
Herbicide	g a.i./ha	Panicles per m^2	% Reduction	Panicles per m^2	% Reduction
Untreated	-	262	-	1.49	-
Fenoxaprop	82.5	244	8	0.10	93
Fenoxaprop	41.3	288	-12	0.88	41
Difenzoquat	990	71	73	0.78	47
Difenzoquat	495	193	24	1.17	22
Imazamethabenz	600	202	21	0.92	39
Imazamethabenz	300	293	-18	1.70	-14
Tralkoxydim	350	274	-4	0.52	65
Tralkoxydim	175	291	-13	0.82	45
Flamprop	600	234	8	1.32	12
Clodinafop	60	242	7	0.67	55
Fenoxaprop + tralkoxydim	41.3 + 175	303	-16	0.04	98
Fenoxaprop + imazamethabenz	41.3 + 300	331	-30	0.42	72
Fenoxaprop + difenzoquat	41.3 + 495	141	45	1.23	17
S.E. ±		35.0	-	0.436	-

Cambs. 3A/L

- When plots were sprayed (8 April 1999) there were 50 wild-oat plants/m^2.

- The level of control achieved was generally poor with virtually all herbicides. Results of percentage reduction in panicle numbers ranged from 73% to -30% and were much lower than the results of the second field trial. This was a very heavily infested site with 262 panicles/m^2 on untreated plots.

- Difenzoquat at full rate gave the best control of the wild-oats in this experiment (73%). Half rate was poorer with 24% control. Difenzoquat is known to be a consistent herbicide in many trials and experiments as there has been no evidence for resistance. The field trial that ran for three years at Roding, finishing last year, found difenzoquat to give consistent control averaging 80% over the three years (range 72% - 86%).

- Imazamethabenz was the herbicide giving the next best level of control although at full rate this was only a 21% reduction.

- All other herbicide treatments gave very poor, or negative, control.

- The results were erratic and, overall, poor control of the wild-oats was achieved, probably because the population was very high. In the glasshouse screening experiment, there was evidence of resistance to fenoxaprop and tralkoxydim, but not to imazamethabenz. Although control was poor, the field experiment results do support the view that this population shows resistance to the former two herbicides, but not to the latter, or to difenzoquat. This site does demonstrate the difficulty of controlling high populations.

Lincs. 9A/F

- When plots were sprayed (19 April 1999) there were 3.3 wild-oat plants/m^2.

- The treatments gave generally better control than at the other site. This may have been due at least in part to the lower density of wild-oats, with less than 2 panicles/ m^2 compared with over 250 / m^2 at the first site.

- As a single treatment fenoxaprop achieved the highest level of control at 93%. Half rate was appreciably poorer than full rate, achieving 41% control. The fenoxaprop resistance rating for this population was R?, so these results are consistent with this. Similarly in the second year (see above), on a site with partial resistance (Essex 2A/F), full rate fenoxaprop gave 97% control but only 53% at half rate.

- Tralkoxydim and imazamethabenz gave only moderate control at full rate (39%, 65%). The resistance screening tests (see previous section), indicated partial resistance to both herbicides, so this may explain the poorer performance in the field.

- Difenzoquat achieved 47% and 22% reduction respectively for the full and half rates. This was a lower percentage reduction than for the other site and is difficult to explain in terms of resistance as there is no evidence of resistance to difenzoquat, although this population has not been tested in a screening assay.

- Clodinafop gave only a 55% and flamprop a 12% reduction in panicle numbers. Better results would have been expected from clodinafop, especially as fenoxaprop at the full rate gave good control.

- The mixture of half rates fenoxaprop and tralkoxydim gave 30% better control (98%) than would have been predicted from the purely additive effect of the individual half rate applications (68%). This synergistic effect has been noted in several other field experiments and in some container experiments.

- Mixtures of fenoxaprop and imazamethabenz (72%) and difenzoquat (17%) gave either 31% better (imazamethabenz) or 37% poorer (difenzoquat) results than would have been predicted (41% and 54% respectively). There was no evidence of antagonism between fenoxaprop and imazamethabenz in this experiment, although this has occurred in several of the other field and container experiments (see Section 1).

- The results were generally consistent with glasshouse resistance screening tests. Full rate fenoxaprop gave good control but half rate was appreciably poorer. Poorer results for imazamethabenz and tralkoxydim are probably also linked with partial resistance. However, it is harder to explain the relatively poor results for difenzoquat and clodinafop, which would have been expected to give better control than that recorded.

- The results highlight the problem of identifying resistance purely on the basis of field experience. Wild-oat control was generally better at this site than at Cambs. 3A/L, but was this due to lower levels of resistance or lower infestation level? Also, while some of the levels of control could be explained in terms of known resistance level, other results could not. However, wild-oats tend not to be uniform in distribution and this makes assessment particularly difficult on sites with low infestations.

Table 23. Field experiments: Year 5 1999 - 2000

Herbicide	g a.i./ha	Oxford 5A/F		Essex 7A/L	
		Panicles per m^2	% Reduction	Panicles per m^2	% Reduction
Untreated	-	149	-	17.4	-
Fenoxaprop	82.5	5	97	0	100
Fenoxaprop	41.3	31	80	0	100
Difenzoquat	990	10	93	0	100
Difenzoquat	495	120	19	0	100
Imazamethabenz	600	37	75	0.5	97.1
Imazamethabenz	300	77	48	1.0	94.3
Tralkoxydim	350	62	58	0	100
Tralkoxydim	175	58	61	0	100
Flamprop	600	144	3	0	100
Clodinafop	60	39	74	0	100
Fenoxaprop + tralkoxydim	41.3 + 175	4	98	0	100
Fenoxaprop + imazamethabenz	41.3 + 300	73	51	0	100
Fenoxaprop + difenzoquat	41.3 + 495	141	5	0	100
S.E. ±		24.8	-	-	-

Oxford 5A/F

- When plots were sprayed (30 March 2000) there were 11 wild-oat plants/m^2.

- Fenoxaprop at full rate gave good control (97%) but half rate was poorer (80%). The screening experiment and cross-resistance studies with this population (see Section 2) indicated marginal resistance to fenoxaprop (resistance index = 2.6), which is supported by these field results. As with most other populations rated R?, good control was achieved at full rate but poorer control at the half rate.

- Clodinafop gave 74% control, appreciably poorer than for full rate fenoxaprop (97%).

- The cross-resistance studies (Section 2) indicated higher resistance to tralkoxydim (resistance index = 43.7), and in this field experiment control by tralkoxydim was mediocre at both full and half rates (58 - 61%). This supports the findings of the glasshouse dose response experiments and the screening assays in which this population was rated RR.

- Control by full rate imazamethabenz (75%) and flamprop (3%) was also mediocre or poor. The cross-resistance studies indicated resistance to both these herbicides (resistance indices of 4.6 and 11.9 respectively). Thus the field experiment results support the view that resistance was responsible for the reduced control recorded.

- Difenzoquat at full rate gave good control (93%), which was consistent with the results of the cross-resistance experiments (see Section 2) in which there was no evidence of resistance to difenzoquat in this population (resistance index = 1.8), or indeed in any other population. However, half rate difenzoquat gave poor control (19%).

- The mixture of half rates fenoxaprop and tralkoxydim gave 6% better control (98%) than would have been predicted from the purely additive effect of the individual half rate applications (92%). Mixtures of fenoxaprop and imazamethabenz (51%) and difenzoquat (5%) gave poorer control than would have been predicted (89% and 84% respectively). Thus there was evidence of antagonism between fenoxaprop and imazamethabenz in this experiment, but the performance of the fenoxaprop+difenzoquat mixture was particularly poor for unknown reasons.

Essex 7A/L

- This was the only field experiment conducted in spring, rather than winter wheat. When plots were sprayed (15 May 2000) there were 29 wild-oat plants/m^2.

- All treatments gave excellent control of wild-oats showing that populations rated as R? can be well controlled by a wide range of different herbicides.

- There was no evidence at this site that half rates of fenoxaprop gave appreciably poorer control than full rates - both gave 100% control.

- It is possible that as this site was in spring wheat, the wild oats were at a earlier stage of growth when sprayed, and hence control was better.

Conclusions

Field experiments were conducted at the Essex 10A/L site for three successive years in adjacent areas. Resistance to fenoxaprop resulted in consistently poor activity with this herbicide. Associated work has shown that this population has target site resistance specific to "fop" herbicides. In contrast, tralkoxydim worked consistently well each year. This supports earlier glasshouse screening studies that showed a high level of resistance to fenoxaprop, but no resistance to tralkoxydim or imazamethabenz (Section 3). However, the glasshouse screening experiments conducted in 1995 included samples from three widely distributed areas within this field, as well as other fields on this farm (Essex 10). There was evidence of

considerable variation in susceptibility to tralkoxydim and imazamethabenz both between fields and between samples from within the trial site field. Thus it should not be too readily assumed that there is not the potential for evolution of resistance to tralkoxydim in this field, despite the consistently good control achieved in these trials.

The results for this site also highlight the problem of identifying resistance purely on the basis of field experience. Do the consistent, but generally only modest results for difenzoquat (72% – 86%) indicate partial resistance or no resistance? Almost certainly the latter – but one has no way of knowing purely from the field results. Flamprop, imazamethabenz and clodinafop gave variable results over the three years, and control was generally, but not always, mediocre. Partial resistance may have been involved, but this cannot be confirmed purely by the field experience. This highlights the problem of detecting partial resistance and the need for tests under more controlled conditions, such as glasshouse pot assays.

The results for the Essex 2A/F and Oxford 5A/F populations were generally consistent with the more detailed cross-resistance studies conducted with these two populations (Section 2). Marginal levels of resistance did not always result in poor control in the field when full rates were applied, but there was evidence that performance was reduced, often substantially, at reduced rates. There was evidence for poorer control with tralkoxydim and imazamethabenz at the Oxford 5A/F site, which was consistent with the results of the cross-resistance studies and screening assays. The results for the Dorset 1A/F population, also included in the cross-resistance studies in Section 2, were disappointing as this population had shown an interesting cross-resistance profile in glasshouse tests. It was not possible to relate field results to glasshouse studies due to the variability in distribution of wild-oats in the field.

The results for the Cambs. 3A/L population were erratic and overall poor control of the wild-oats was achieved, probably because the population was very high. In the glasshouse screening experiment, there was evidence of resistance to fenoxaprop and tralkoxydim, but not to imazamethabenz. Although control was poor, the field experiment results do support the view that this population shows resistance to the former two herbicides, but not to the latter, or to difenzoquat. This site also demonstrated the difficulty of controlling high populations.

As in the container experiments (See Section 1) there was evidence of antagonism between fenoxaprop and imazamethabenz. In five out of six comparisons (omitting Essex 2B/F, Dorset 1A/F, Cambs. 3A/L, Essex 7A/L, sites where populations were excessively high or gave variable or very high control) fenoxaprop + imazamethabenz gave levels of control less than would have been predicted from the purely additive effects of the individual half rate applications. Mean control was 8% less than predicted (range -38% to +31%). In

a total of 14 comparisons (8 container and 6 field) control by the fenoxaprop + imazamethabenz mixture was less than predicted on 12 occasions.

With the mixtures of fenoxaprop + tralkoxydim control was better than predicted in four out of six field comparisons (mean +9%, range -2% to +30%). In a total of 14 comparisons (8 container and 6 field) control by the fenoxaprop + tralkoxydim was better than predicted on 10 occasions. Thus, there was some evidence of synergy between these two herbicides, but this was not consistent.

With the mixtures of fenoxaprop + difenzoquat control was less than predicted in five out of six field comparisons (mean -19%, range -78% to +18%). In a total of 14 comparisons (8 container and 6 field) control by the fenoxaprop + difenzoquat was less than predicted on 10 occasions. Thus, there was some evidence of antagonism between these two herbicides, but the effects were generally less pronounced and less consistent than for imazamethabenz.

4 (b). Outdoor container experiments

Introduction

Glasshouse experiments allow differences in response between many populations to be determined rapidly but the differences found may not relate directly to the field. Field experiments (which must of necessity involve only a single weed population unless seeds are sown artificially) are essential, but it is impossible to entirely separate the effects of climate, environment and resistance on herbicide performance, especially when resistance is partial rather than absolute. Outdoor container studies can simulate field conditions and permit comparison of herbicide performance on several populations under identical soil and climatic conditions. Two outdoor container experiments were conducted using some of the populations evaluated in the glasshouse cross-resistance studies (see Section 2). Two of the populations (Essex 2A/F and Dorset 1A/F) had also been sites of field experiments (see previous section). Using the same populations in each of the experimental situations allows a much more comprehensive appraisal of the impact of resistance to be made.

The first container experiment involved two "fop" and two "dim" herbicides applied at two timings. The second container experiment included a range of other herbicides with different modes of action. One of these herbicides, imazamethabenz, was applied at two timings but the others were applied on a single date.

Materials and Methods

Experiment 1 (Response to "fop" and "dim" herbicides)

This experiment was established in a sandbed at Rothamsted in 180 plastic trays (27 x 18 x 10 cm deep) on the 28th September 1998. The six wild-oat populations used were as follows, and all had been used in the glasshouse cross-resistance evaluations (see Section 2): LFAT 1994, Essex 2A/F 1994, Dorset 1A/F 1996, Suffolk 1A/F 1996, Wilts. 1A/L 1997 and Kent 1A/F 1994.

Trays were filled with Kettering loam and grit (5:1) and "Osmacote mini", a slow release fertilizer. Four rows of wild-oat seeds were sown in the respective boxes with 12 seeds per row (48 seeds per box). Soil was then added to cover seeds to a depth of 2.5 cm and labelled. The Kent 1A/F population had four rows of 30 seeds sown due to poor emergence in previous experiments. The trays were sunk into an outdoor sandbed on the 1 October 1998. By the 15 December 1998, most wild-oat populations were at the 3 leaf stage, but Suffolk 1A/F plants were still emerging.

The first spraying date was 16 December 1998, except for Suffolk 1A/F. The number of wild-oat plants per tray was recorded before spraying. A laboratory pot sprayer was used delivering 265L/ha water at 210 kPa through a single Teejet 110015 VK nozzle 50cm above the plants. The four herbicides applied were: fenoxaprop-P-ethyl (55 g a.i./ha); fluazifop-P-butyl (125 g a.i./ha) + 0.1% "Agral"; tralkoxydim (250 g a.i./ha) + 0.375% "Output"; cycloxydim (100 g a.i./ha) + 0.8% "Actipron". There were three replicates and two untreated containers per population per replicate. The trays were returned to the sandbed after spraying. The Suffolk 1A/F population (first spray date) was sprayed on the 8 February 1999 when the majority of plants had reached the 3 leaf stage (volume rate = 271 l/ha).

Wild-oat plants sprayed at the 3 leaf stage were assessed on 3 and 16 March 1999 (Suffolk 1A/F on 25 March 1999) by counting the number of surviving plants and determining foliage fresh weights for each container.

The second spraying date was the 8 March 1999 for all populations (including Suffolk 1A/F). The number of wild-oat plants per tray was recorded before spraying. Herbicides were applied at the same doses and in the same manner as for the first date (volume rate = 270 l/ha). Most wild-oat plants were at the 2-3 tiller stage. Wild-oat plants sprayed at this stage were assessed on the 28 and 29 April 1999.

Experiment 2 (Response to non - "fop" and "dim" herbicides)
This experiment was established in a sandbed at Rothamsted in 144 plastic trays (27 x 18 x 10 cm deep) on the 1 October 1999. The six wild-oat populations used were the same ones as used in Container Experiment 1: LFAT 1994, Essex 2A/F 1994, Dorset 1A/F 1996, Suffolk 1A/F 1996, Wilts. 1A/L 1997 and Kent 1A/F 1994. Trays were prepared and wild-oat seeds sown in the same manner as Container Experiment 1, except that the Kent 1A/F population had four rows of 30 seeds sown and Lfat 1994 four rows of 15 seeds due to poor emergence in previous experiments. The trays were sunk into an outdoor sandbed on the 4 October 1999.

The five herbicides applied were: tri-allate granules (2.25 kg a.i./ha), imazamethabenz + 500 ml "Agral"/ha (600 g a.i./ha), difenzoquat (750 g a.i./ha), flamprop-M-isopropyl (600 g a.i./ha) and isoproturon (2 kg a.i./ha). Tri-allate was applied pre-emergence on 7 October 1999 using a hand held applicator. Imazamethabenz was applied on two dates, on 12 January 2000 when wild-oat plants were at the 3 leaf stage and on 7 March 2000 at the 2 tiller stage. The other three herbicides were applied on 7 March 2000 only. The number of wild-oat plants per tray was recorded before spraying. A laboratory pot sprayer was used delivering 245-265L/ha water at 210 kPa through a single Teejet 110015 VK nozzle 50cm above the plants. There were three replicates and two untreated containers per population per replicate.

The tri-allate, the first imazamethabenz treatment and one untreated were assessed on 28 March 2000 by counting the number of surviving plants and determining foliage fresh weights for each container. The remaining treatments were assessed in the same manner on 10 May 2000.

Results

Experiment 1 (Response to "fop" and "dim" herbicides) (Table 24; Figures 18 to 25)

- All the herbicide treatments, irrespective of timing, achieved excellent control (> 90%) of the susceptible standard, LFAT. This shows that the herbicide applications and growth stage of the wild-oats were conducive to achieving very good control of susceptible wild-oats.

- Fenoxaprop gave poor control of the Suffolk 1A/F population at both timings. This was consistent with the high level of resistance recorded in the glasshouse dose response experiments – this population showed the greatest resistance to fenoxaprop (Resistance Index, RI = 13.1) of the 10 populations evaluated (see Section 2). With the four populations, Wilts. 1A/L, Essex 2A/F, Dorset 1A/F and Kent 1A/F, control was better at the earlier than the later timing. The decline in fenoxaprop activity with delayed application was related to the degree of resistance to fenoxaprop recorded in the glasshouse dose response experiments. Thus the decline for Essex 2A/F (RI = 1.7) was much lower (11%) than for the other three populations (RIs 6.7, 6.4, 6.8, and % decline 33% - 53%) on a plant numbers basis. For three of these populations, Wilts. 1A/L, Essex 2A/F, Dorset 1A/F, control at the earlier timing was as good as for the susceptible standard.

- Fluazifop gave excellent control of the Suffolk 1A/F population at both timings, despite very poor control with fenoxaprop. The RI in the dose response experiment was 3.0 – much lower than for fenoxaprop – so the container results support this rating. In contrast, control of Kent 1A/F was poor, especially at the latter timing. The RI in the dose response experiment was 74.8 – the highest for any population, so again the container results support this. It is notable that the relative degree of resistance to fenoxaprop and fluazifop is reversed for the Suffolk 1A/F and Kent 1A/F populations, despite both herbicides being "fops". The Essex 2A/F and Dorset 1A/F populations were well controlled by fluazifop, especially at the early timing, again consistent with low RIs (2.7, 2.4). Wilts. 1A/L showed slightly reduced control, especially on a plant number basis, and again this was consistent with an RI of 8.2, which was the second highest recorded.

- Tralkoxydim gave excellent control of all populations, except for Wilts. 1A/L at the later timing and Dorset 1A/F at both timings, on a plant number basis. Dorset 1A/F had a RI of 64.3 and Wilts. 1A/L 16.7 in the glasshouse dose response experiments, higher than for the other populations included in the container experiment (1.5 – 2.8). So again the results from both experiments were in agreement. The Kent 1A/F and Suffolk 1A/F populations were both very well controlled by tralkoxydim, showing that

their high resistance to fluazifop and fenoxaprop ("fops") respectively is not correlated with resistance to the "dim" tralkoxydim.

- Cycloxydim gave complete control of all populations at both timings. This again supports the results of the dose response experiments in which no clear evidence of resistance was found. Wilts. 1A/L had a RI of 3.5 but there was not even a hint of reduced activity in the containers.

- Some of the RIs in the dose response experiments were very high e.g. 74.8 for Kent 1A/F for fluazifop, 64.3 for Dorset 1A/F for tralkoxydim. However, this did not result in an absence of control in the container experiment, rather there were reductions in activity. Generally there was an excellent agreement between the RI's and activity in containers. However, while the RIs were generally good indicators of resistance, absolute values for different herbicides were not always related to the degree of reductions in activity. Thus control of Suffolk 1A/F by fenoxaprop (RI = 13.1) was much poorer than control of Dorset 1A/L by tralkoxydim (RI = 64.3).

Table 24. Container experiment 1: Effect of herbicide timing (at 3 leaved and 2-3 tillers) on activity of "fop" and "dim" herbicides against six populations of wild-oats

% Reduction in *foliage fresh weight*								
	Fenoxaprop 55g/ha		Fluazifop 125g/ha		Tralkoxydim 250g/ha		Cycloxydim 100g/ha	
Population	3 lvs	2-3 tiller	3 lvs	2-3 tiller	3 lvs	2-3 tiller	3 lvs	2-3 tiller
LFAT 94	94.6	96.5	92.1	95.5	94.2	95.3	91.7	96.4
Wilts 1A/L	96.5	80.1	94.8	83.3	96.0	69.2	96.0	96.1
Essex 2A/F	93.9	86.7	95.7	94.7	94.3	89.9	92.7	95.5
Dorset 1A/F	94.5	64.5	94.9	87.2	87.0	64.2	95.5	95.7
Kent 1A/F	94.3	22.7	41.0	43.6	96.4	75.4	97.6	96.8
Suffolk 1A/F	38.8	32.0	90.6	97.9	85.7	97.6	95.1	98.0
S.E. ±	6.82							
LSD (P≤ 0.05)	19.14							
C.V%	13.8%							

% Reduction in *plant numbers*								
	Fenoxaprop 55g/ha		Fluazifop 125g/ha		Tralkoxydim 250g/ha		Cycloxydim 100g/ha	
Population	3 lvs	2-3 tiller	3 lvs	2-3 tiller	3 lvs	2-3 tiller	3 lvs	2-3 tiller
LFAT 94	100	100	98.1	100	100	100	100	100
Wilts 1A/L	98.5	65.5	86.2	76.2	100	65.5	100	100
Essex 2A/F	100	88.5	100	100	100	96.4	100	100
Dorset 1A/F	100	47.1	100	86.3	58.1	27.4	100	100
Kent 1A/F	86.6	33.3	64.6	5.1	97.6	100	100	100
Suffolk 1A/F	-15.5	-2.3	100	100	100	100	100	100
S.E. ±	6.09							
LSD (P≤ 0.05)	17.09							
C.V. %	12.5%							

Figure 18. Container Experiment 1: Response to fenoxaprop - effects on foliage fresh weights

WILD-OAT CONTAINERS 1998/99
Fenoxaprop 55g.a.i/ha
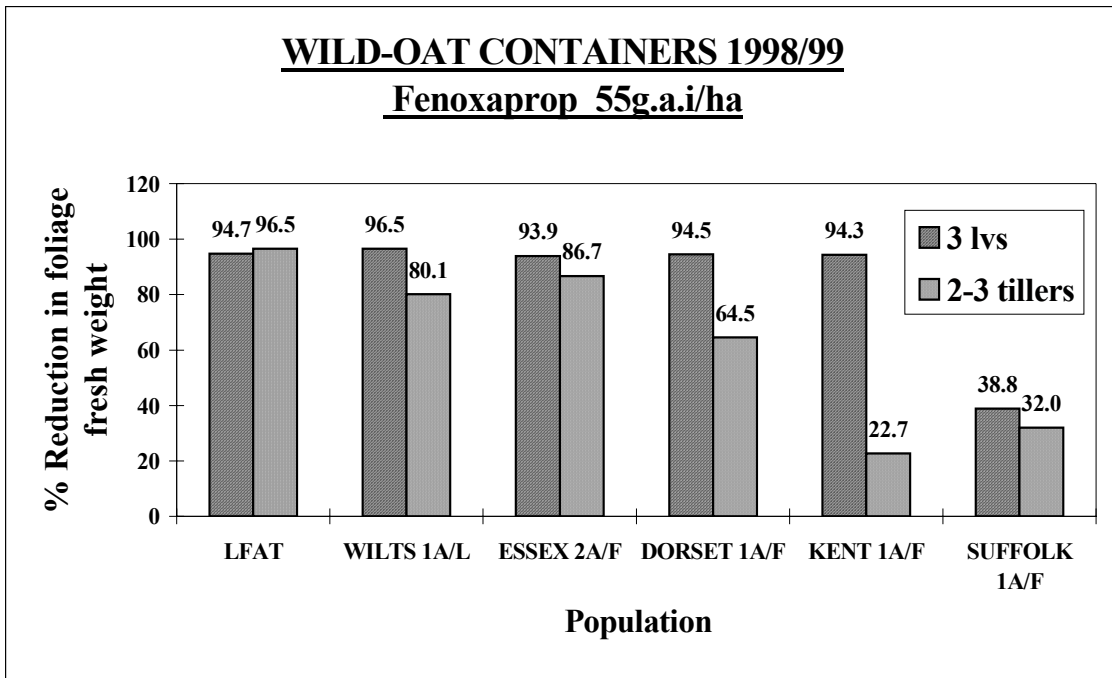
Figure 19. Container Experiment 1: Response to fluazifop - effects on foliage fresh weights

WILD-OAT CONTAINERS 1998/99
Fluazifop 125g.a.i/ha
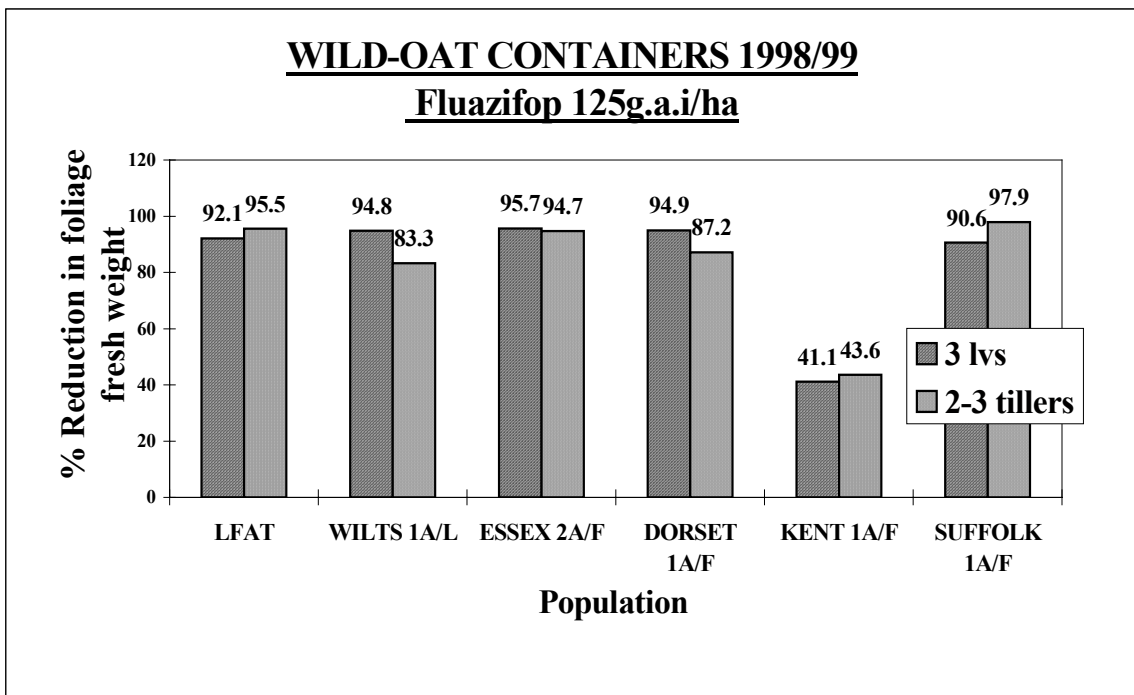
Figure 20. Container Experiment 1: Response to tralkoxydim - effects on foliage fresh weights

WILD-OAT CONTAINERS 1998/99
Tralkoxydim 250g.a.i/ha
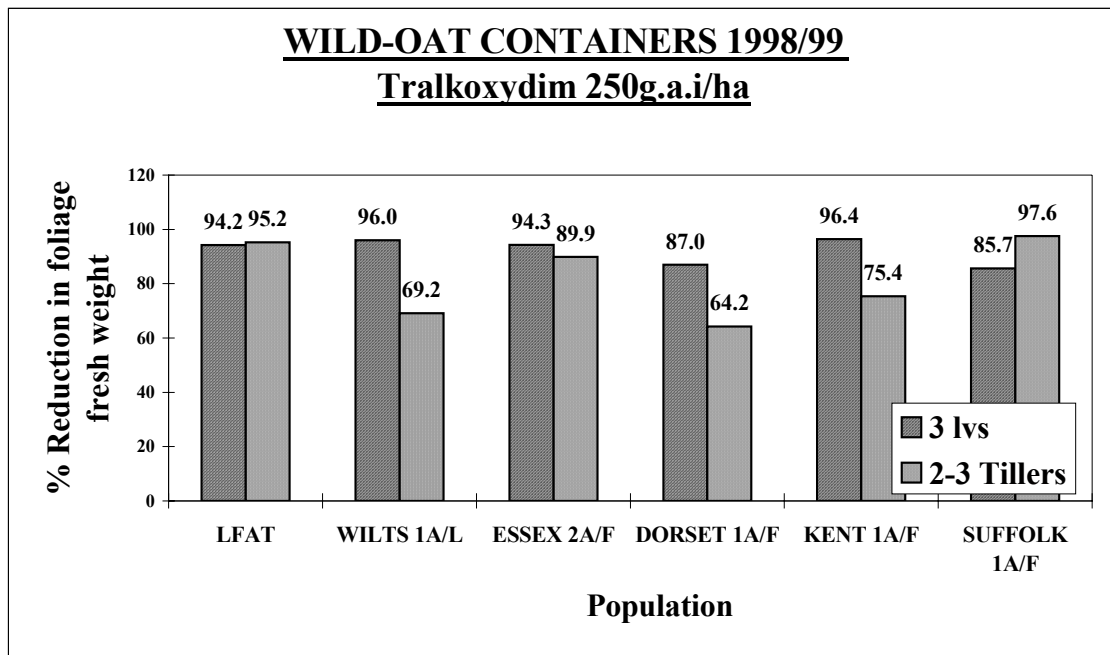
Figure 21. Container Experiment 1: Response to cycloxydim - effects on foliage fresh weights

WILD-OAT CONTAINERS 1998/99
Cycloxydim 100g.a.i/ha
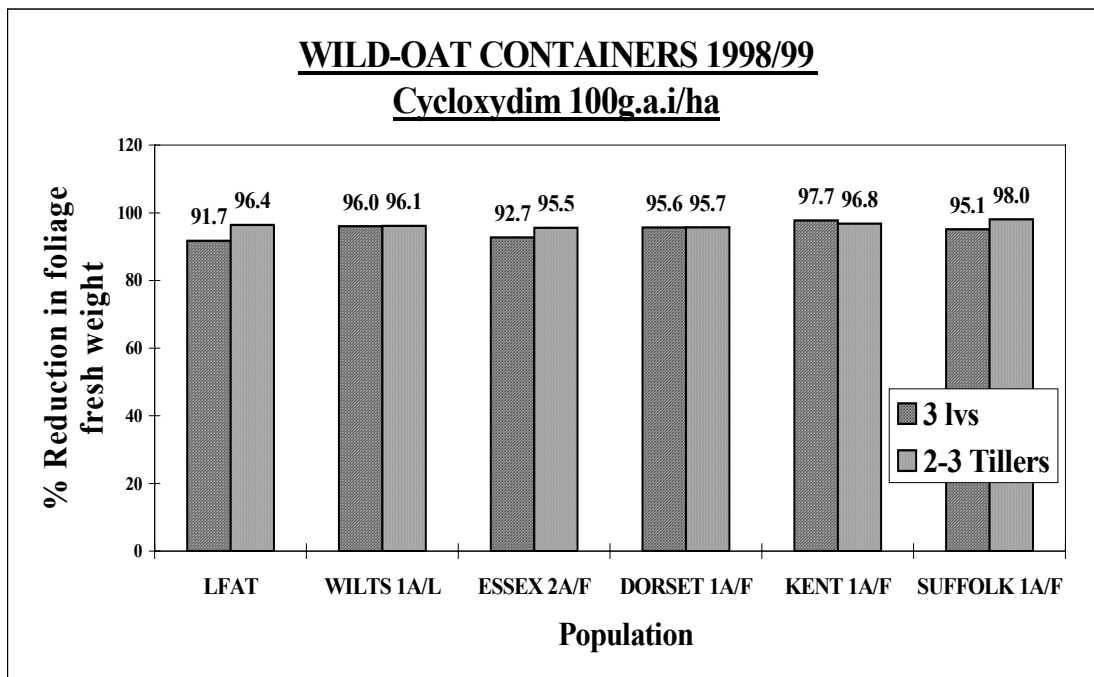
Figure 22. Container Experiment 1: Response to fenoxaprop - effects on plant numbers

WILD-OAT CONTAINERS 1998/99 - Fenoxaprop 55g.a.i/ha
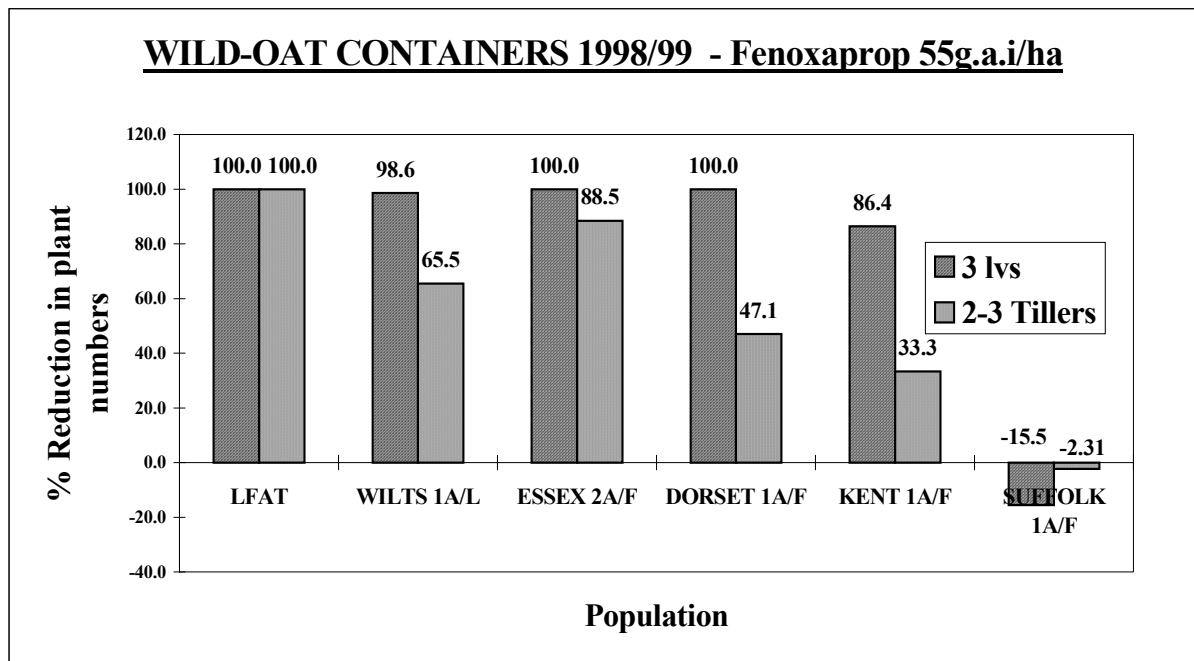
Figure 23. Container Experiment 1: Response to fluazifop - effects on plant numbers
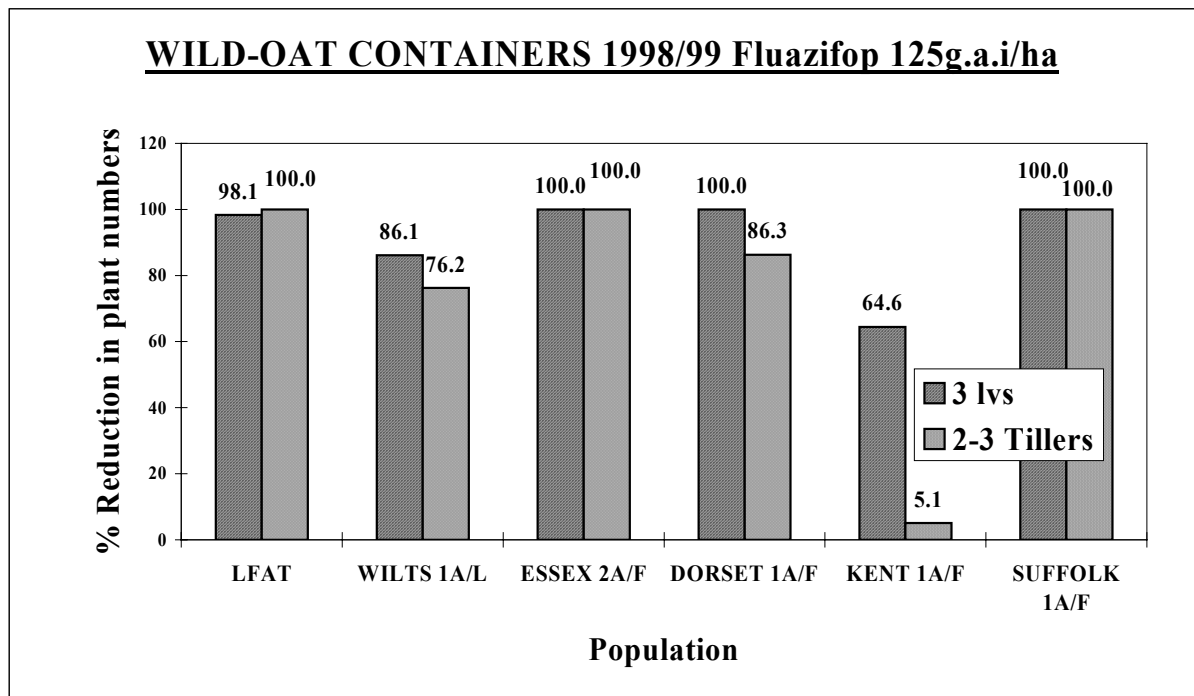
WILD-OAT CONTAINERS 1998/99 Fluazifop 125g.a.i/ha

Figure 24. Container Experiment 1: Response to tralkoxydim - effects on plant numbers

WILD-OAT CONTAINERS 1998/99 Tralkoxydim 250g.a.i/ha

Figure 25. Container Experiment 1: Response to cycloxydim - effects on plant numbers

WILD-OAT CONTAINERS 1998/99 - Cycloxydim 100g.a.i/ha
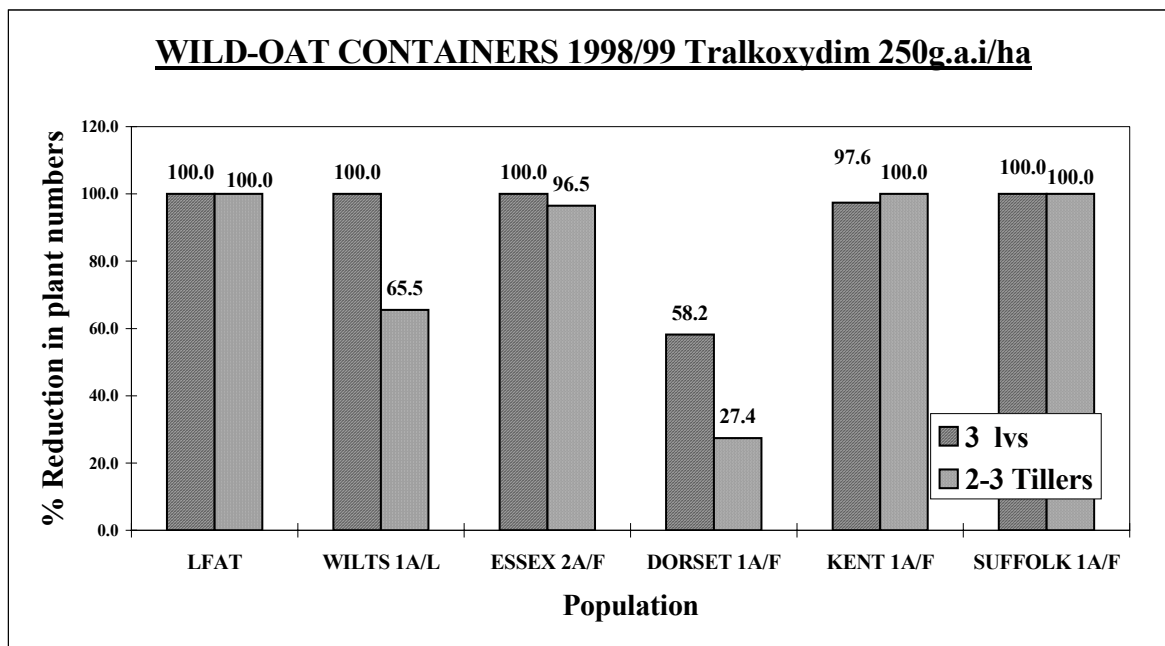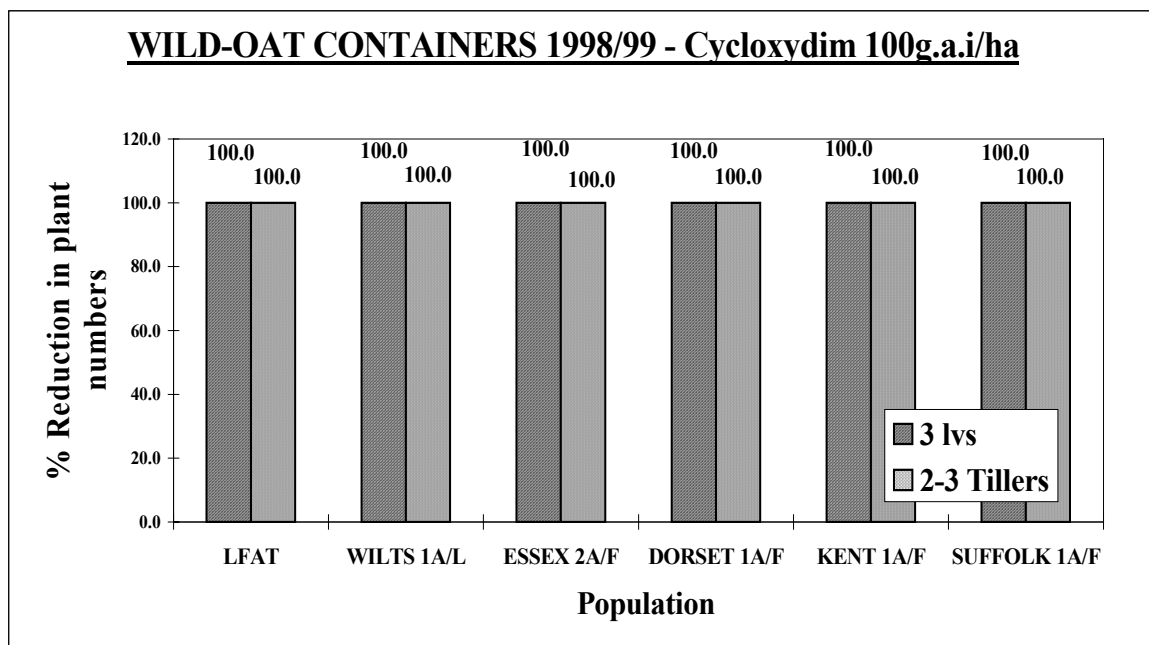
Experiment 2 (Response to non - "fop" and "dim" herbicides) (Table 25; Figures 26 to 35)

- Imazamethabenz at both timings, difenzoquat and flamprop achieved good control (> 90% on a foliage fresh weight basis) of the susceptible standard, LFAT. This shows that the herbicide applications and growth stage of the wild-oats were conducive to achieving good control of susceptible wild-oats. Levels of control by tri-allate and isoproturon were also fairly good on a foliage weight basis (83 - 89%) but poorer on a plant basis (61 - 78%).

- Imazamethabenz gave poor control of the Dorset 1A/F population at both timings. This was consistent with the high level of resistance recorded in the glasshouse dose response experiments – this population showed the greatest resistance to imazamethabenz (Resistance Index, RI = 19.5) of the 10 populations evaluated (see Section 2).   Control of Dorset 1A/F was particularly poor at the later timing. Control of the Wilts. 1A/L, Kent 1A/F and Suffolk 1A/F populations by imazamethabenz was excellent (> 93%) at both timings. The decline in imazamethabenz activity on a foliage fresh weight basis with delayed application was related to the degree of resistance to imazamethabenz recorded in the glasshouse dose response experiments. Thus the decline for Kent 1A/F, Wilts 1A/L and Suffolk 1A/F (RIs = 1.4 - 2.7) was much lower (0 - 4%) than for Essex 2A/F (RI = 3.1, 19% decline) and Dorset 1A/F (RI = 19.5, 35% decline on a foliage fresh weight basis. Consequently the container results were consistent with those from the glasshouse dose response assays. The Essex 2A/F population was well controlled (>94%) when treated at the 3 leaf stage, but poorly controlled (21- 75%) at the 2 tiller stage. The RI in the glasshouse dose response assay was 3.1, indicating a marginal level of resistance. This population was the most vigorous growing of all the populations with a tendency to be the tallest and be at a slightly more advanced growth stage than the other populations. This may have contributed to the poorer control at the later timing.

- Difenzoquat gave rather variable control. On a foliage weight basis, control of Kent 1A/F and Suffolk 1A/F was good. These populations had shown the highest resistance to "fop" herbicides in Container Experiment 1 and in glasshouse dose response assays. This shows that resistance to "fops" is not necessarily related to resistance to difenzoquat. Control of the Dorset 1A/F and Wilts 1A/L populations was moderately good on a fresh weight basis, but poorer in terms of control of plant numbers. This implies that many of the plants that survived difenzoquat application were severely affected by the herbicide. Control of the Essex 2A/F population was poorest, both in terms of foliage weight and plant numbers.   Interestingly, this population was also one least well controlled in the glasshouse dose response assays, where a RI of 4.3 was recorded.

- Flamprop gave good control of Kent 1A/F and Suffolk 1A/F, but poorer control of Essex 2A/F, Wilts 1A/L, and especially Dorset 1A/F. Again these results were consistent with the RI values obtained in the glasshouse dose response assays, which were 0.6, 1.0, 3.1, 3.4 and 23.6 respectively. The Dorset 1A/F

111

population clearly showed cross-resistance to both imazamethabenz and flamprop, as well as being resistant to fenoxaprop and tralkoxydim.

- Isoproturon gave rather variable results with good control of some populations which had shown resistance to other herbicides (Wilts 1A/L, Dorset 1A/F, Suffolk 1A/F). Control of some other populations was poorer (Essex 2A/F and Kent 1A/F). In the glasshouse dose response assay, all populations were equally susceptible to isoproturon, with no evidence of resistance. Consequently differences in isoproturon activity between populations in the container experiment do not appear to be related to resistance and may be due to slight differences in growth stage or plant vigour. It may be significant that the poorest control was of Essex 2A/F, which was the most vigorously growing population. This may have contributed to the poorer control, as may also have been the case with imazamethabenz.

- Tri-allate gave variable control. The best control was of the susceptible standard, LFAT. Control of all the other populations was mediocre to poor, with no clear pattern emerging. The poorest control was of the Essex 2A/F population, which also showed the poorest control with difenzoquat and isoproturon, and relatively poor control with the later application of imazamethabenz. This population showed the least sensitivity to triallate in both the petri-dish assays described in the cross-resistance studies detailed in Section 2. Imazamethabenz and difenzoquat also gave mediocre control in a field experiment at this site, in contrast to fenoxaprop and tralkoxydim at full rate which gave excellent control (see Section 4). As stated above, this population tends to show a high level of vigour in terms of speed of emergence and growth, which may contribute to its insensitivity to some herbicides. However the consistency of results from glasshouse, container and field experiments indicate this population does possess mechanisms which confer partial resistance to several herbicides with different modes of action.

Table 25. Container Experiment 2: Effect of herbicide timing (at 3 lvs and 2 tillers) on activity of
non -"fop" and "dim" herbicides against six populations of wild-oats

% Reduction in _foliage fresh weight_					
Population	**Imaz. Early** **600 g/ha**	**Imaz. Late** **600 g/ha**	**Difenzoquat** **750 g/ha**	**Flamprop** **600 g/ha**	**Isoproturon** **2.0 kg/ha**	**Triallate** **2.25 kg/ha**
	3 Leaves	**2 Tillers**	**2 Tillers**	**2 Tillers**	**2 Tillers**	**Pre-emergence**
LFAT 94	98.2	95.3	90.1	93.4	89.4	82.5
Wilts 1A/L	97.1	93.1	84.2	64.0	93.1	43.5
Essex 2A/F	94.0	74.7	60.3	75.7	61.0	-24.3
Dorset 1A/F	77.1	41.9	81.1	33.3	91.4	37.9
Kent 1A/F	98.1	96.5	91.6	91.6	73.0	73.7
Suffolk 1A/F	99.0	99.0	94.7	96.7	97.4	58.7
S.E. ±	4.67					
LSD (P≤ 0.05)	13.18					
C.V.%	10.4 %					

% Reduction in _plant numbers_					
Population	**Imaz. Early** **600 g/ha**	**Imaz. Late** **600 g/ha**	**Difenzoquat** **750 g/ha**	**Flamprop** **600 g/ha**	**Isoproturon** **2.0 kg/ha**	**Triallate** **2.25 kg/ha**
	3 Leaves	**2 Tillers**	**2 Tillers**	**2 Tillers**	**2 Tillers**	**Pre-emergence**
LFAT 94	100.0	98.6	81.9	94.4	61.1	77.5
Wilts 1A/L	100.0	99.0	37.8	23.5	74.5	47.6
Essex 2A/F	98.5	20.9	16.3	39.5	17.8	14.7
Dorset 1A/F	45.9	18.8	19.7	16.2	87.2	40.0
Kent 1A/F	100.0	100.0	74.6	97.3	40.0	60.0
Suffolk 1A/F	100.0	100.0	81.9	98.9	91.5	40.4
S.E. ±	5.08					
LSD (P≤ 0.05)	14.32					
C.V.%	13.7 %					

Figure 26. Container Experiment 2: Response to imazamethabenz - effects on foliage fresh weight

WILD OAT CONTAINERS 1999/2000
Imazamethabenz 600g.a.i/ha
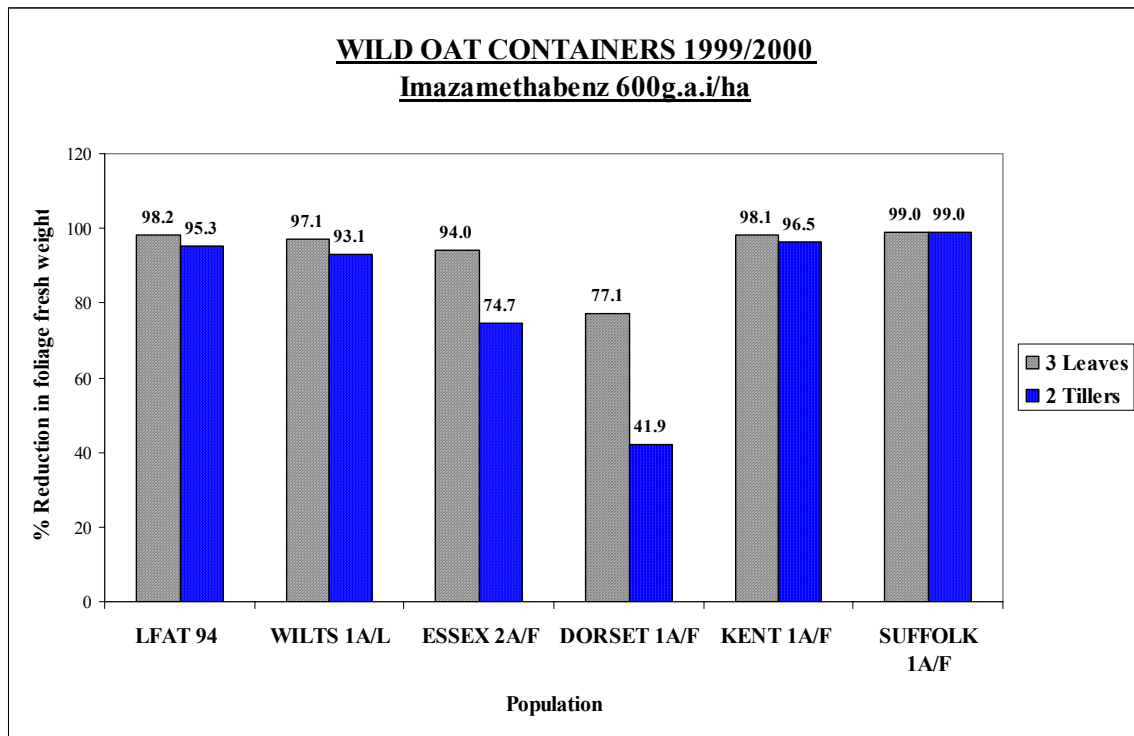
Figure 27. Container Experiment 2: Response to difenzoquat - effects on foliage fresh weight

WILD-OAT CONTAINERS 1999/2000
Difenzoquat 750g.a.i/ha
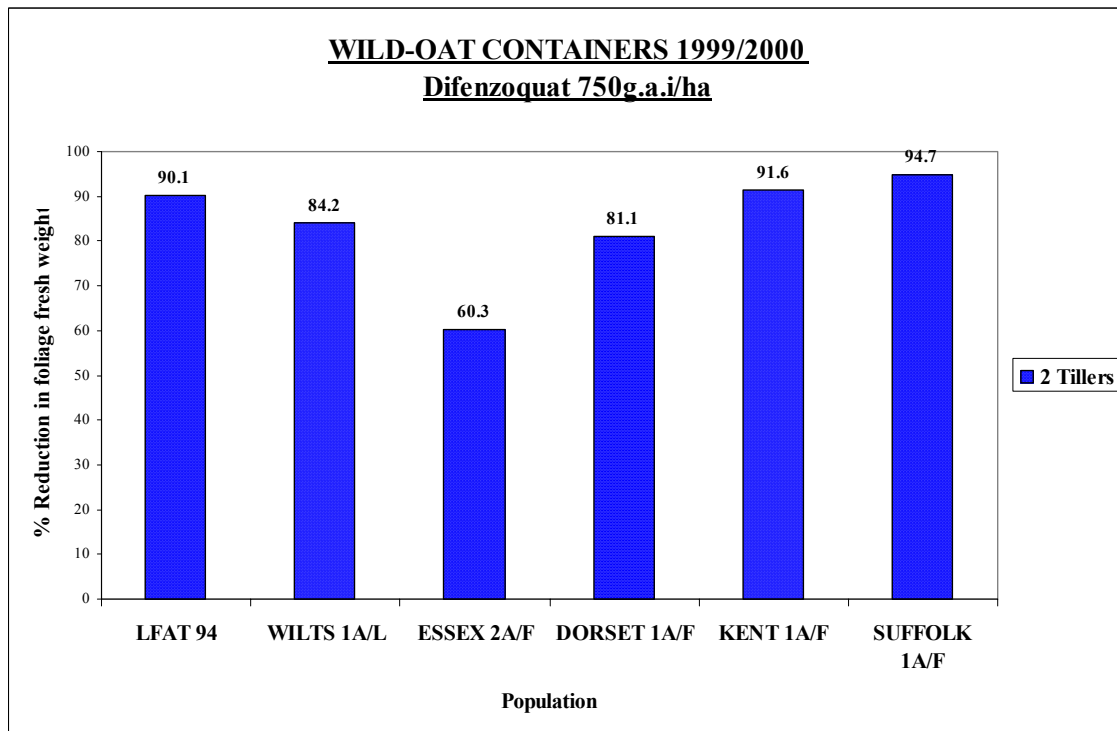
Figure 28. Container Experiment 2: Response to flamprop - effects on foliage fresh weight

WILD-OAT CONTAINERS 1999/2000
Flamprop 600g.a.i/ha
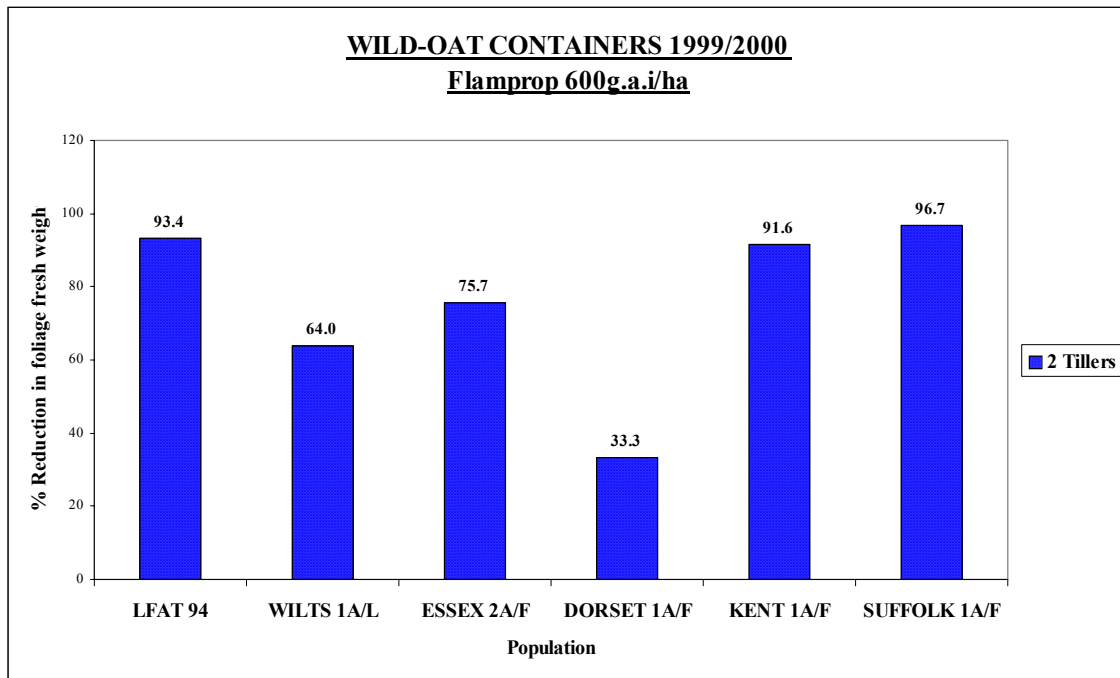
Figure 29. Container Experiment 2: Response to isoproturon - effects on foliage fresh weight

WILD OAT CONTAINERS 1999/2000
Isoproturon 2.0kg.a.i/ha

115
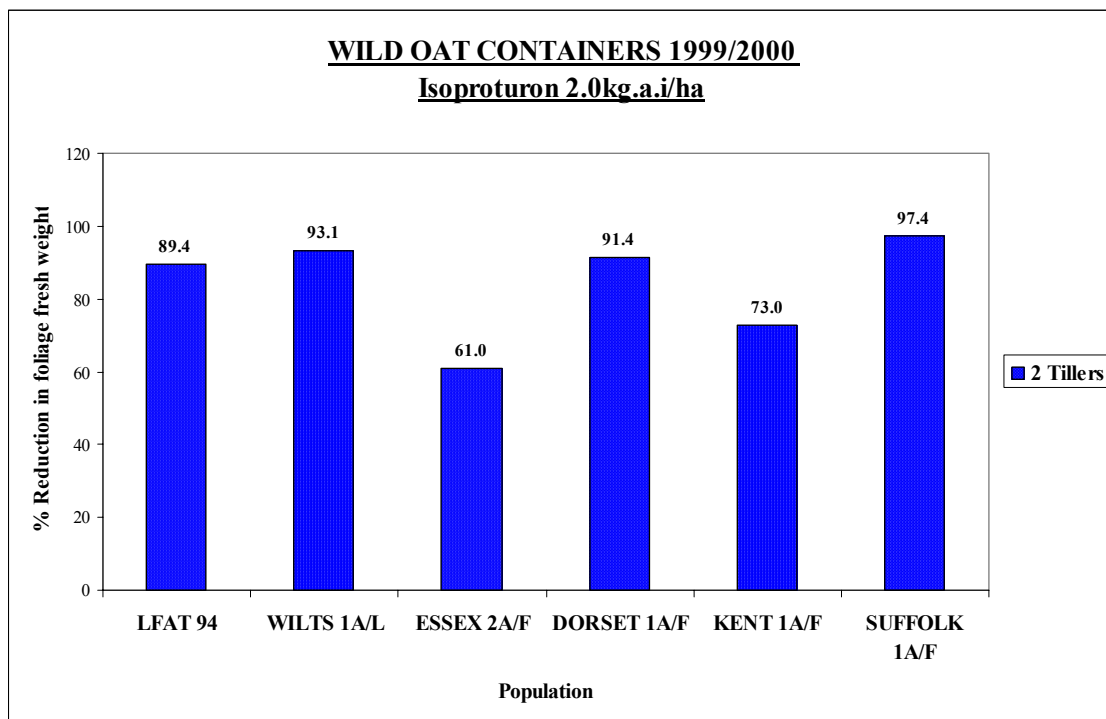
Figure 30. Container Experiment 2: Response to triallate - effects on foliage fresh weight

WILD-OAT CONTAINERS 1999/2000
Triallate 2.25kg.a.i/ha

Y-axis: % Reduction in foliage fresh weight

X-axis: Population

Legend: Pre-em

Values:
- LFAT 94: 82.5
- WILTS 1A/L: 43.5
- ESSEX 2A/F: -24.3
- DORSET 1A/F: 37.9
- KENT 1A/F: 73.7
- SUFFOLK 1A/F: 58.7
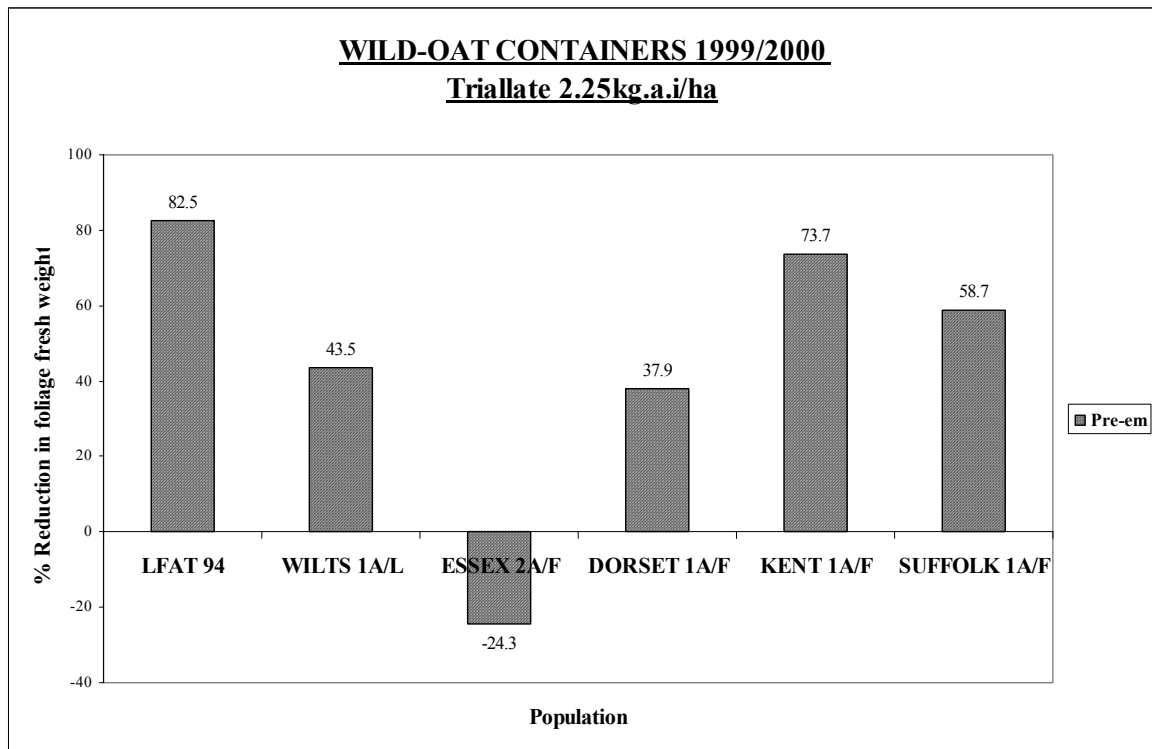
Figure 31. Container Experiment 2: Response to imazamethabenz - effects on plant numbers

WILD-OAT CONTAINERS 1999/2000
Imazamethabenz 600g.a.i/ha
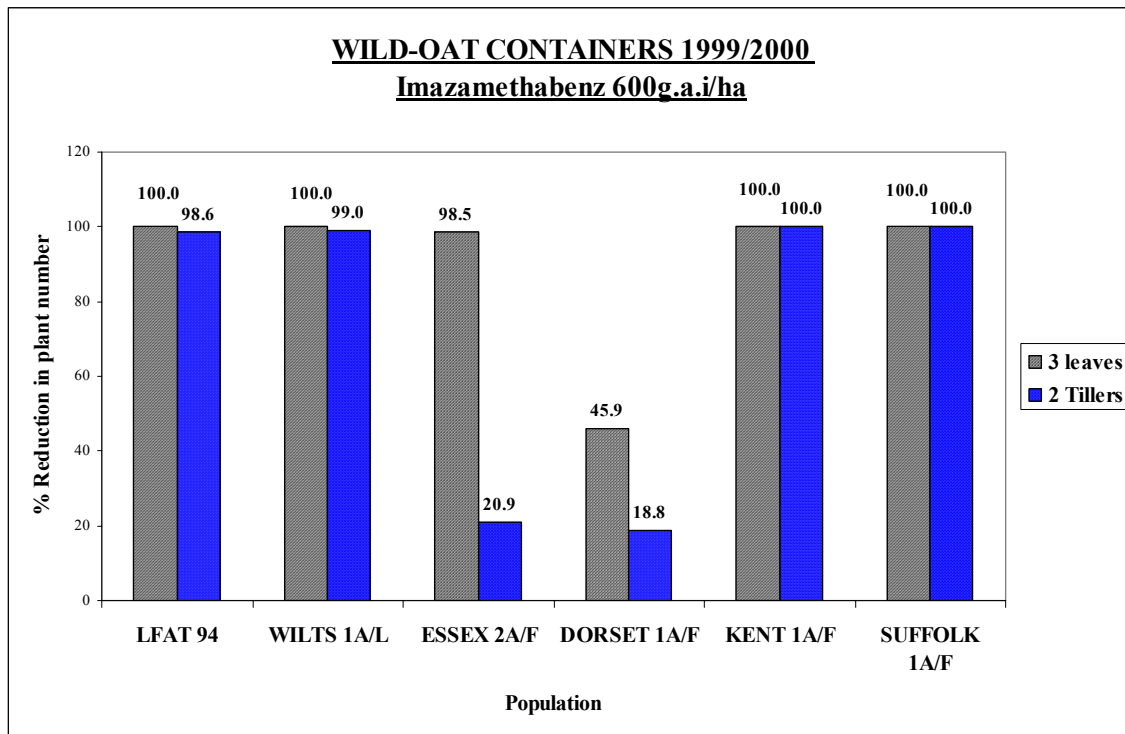
Figure 32. Container Experiment 2: Response to difenzoquat - effects on plant numbers

WILD-OAT CONTAINERS 1999/2000
Difenzoquat 750g.a.i/ha
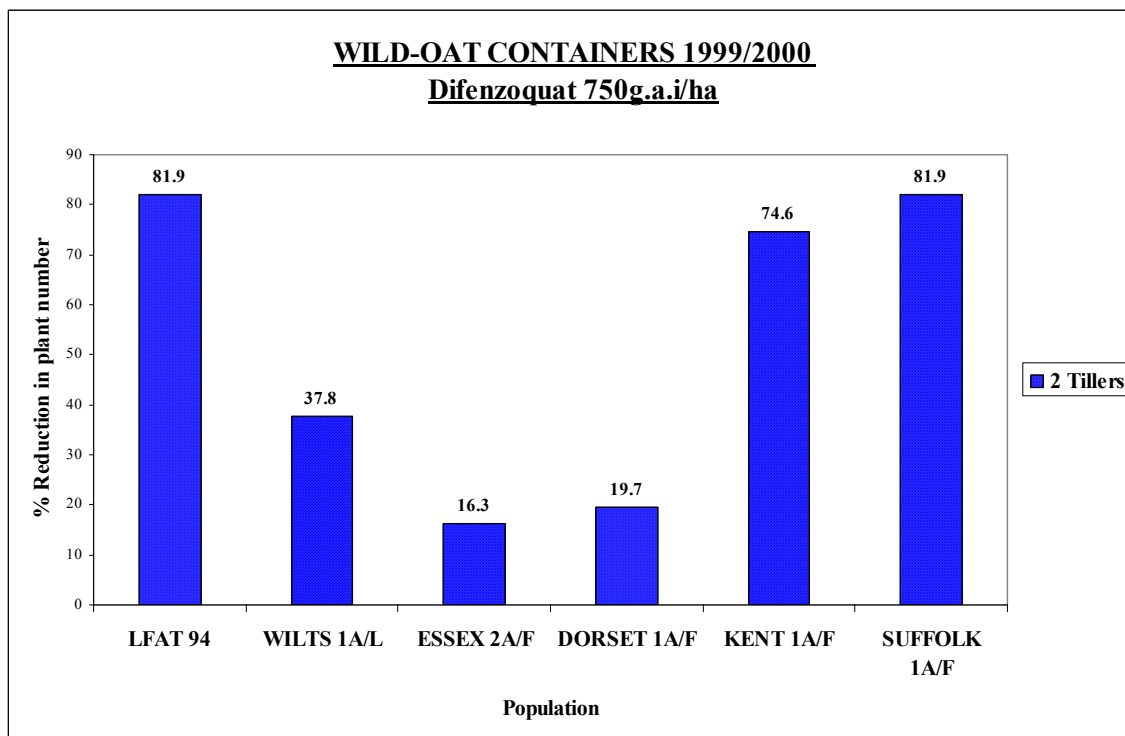
Figure 33. Container Experiment 2: Response to flamprop - effects on plant numbers

WILD-OAT CONTAINERS 1999/2000
Flamprop 600g.a.i/ha
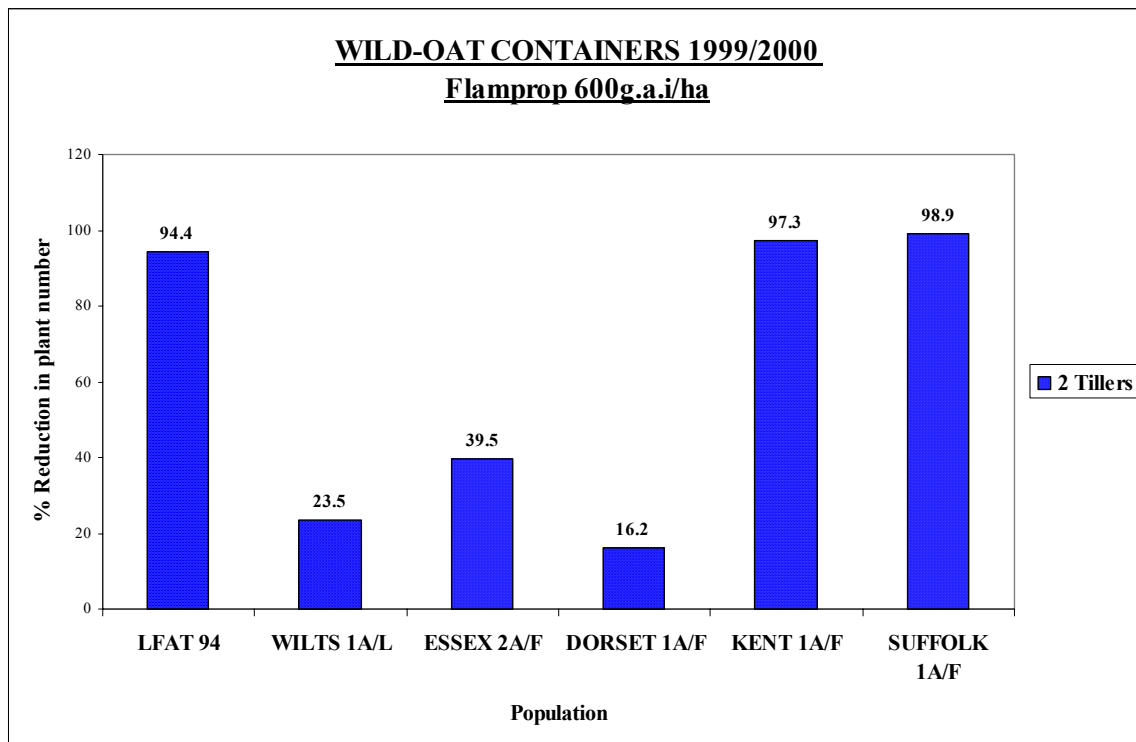
Figure 34. Container Experiment 2: Response to isoproturon - effects on plant numbers

WILD OAT CONTAINERS 1999/2000
Isoproturon 2.0kg.a.i/ha
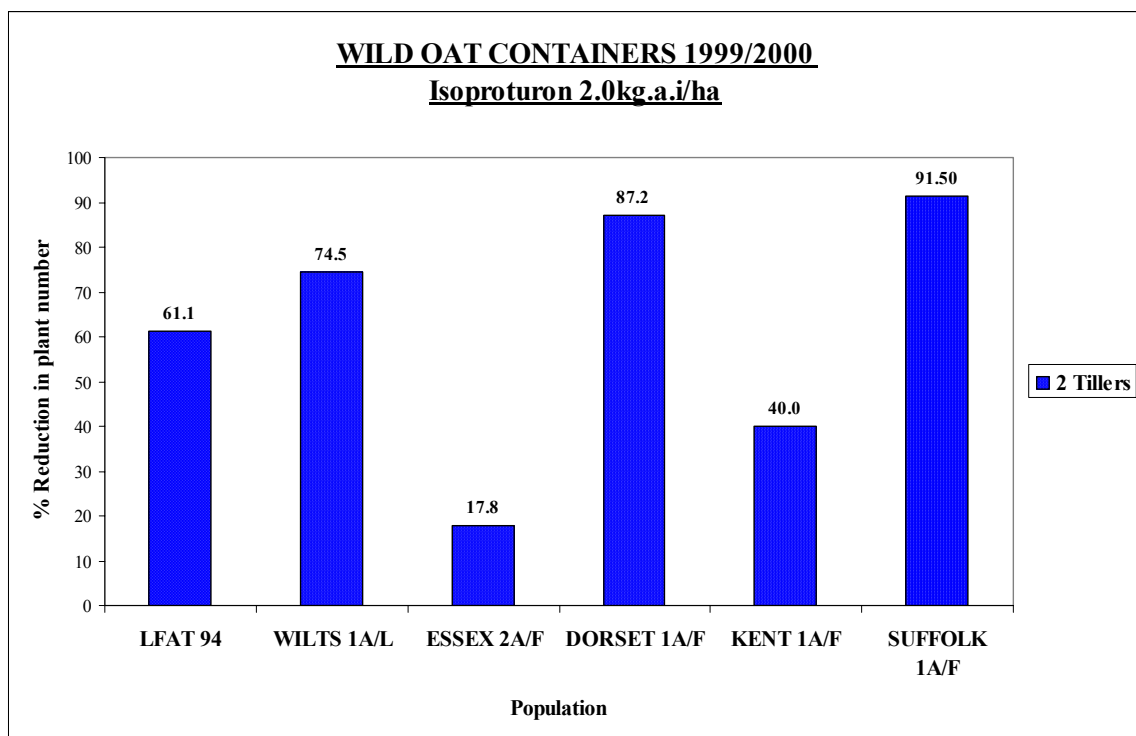
Figure 35. Container Experiment 2: Response to triallate - effects on plant numbers

WILD OAT CONTAINERS 1999/2000
Triallate 2.25kg.a.i/ha
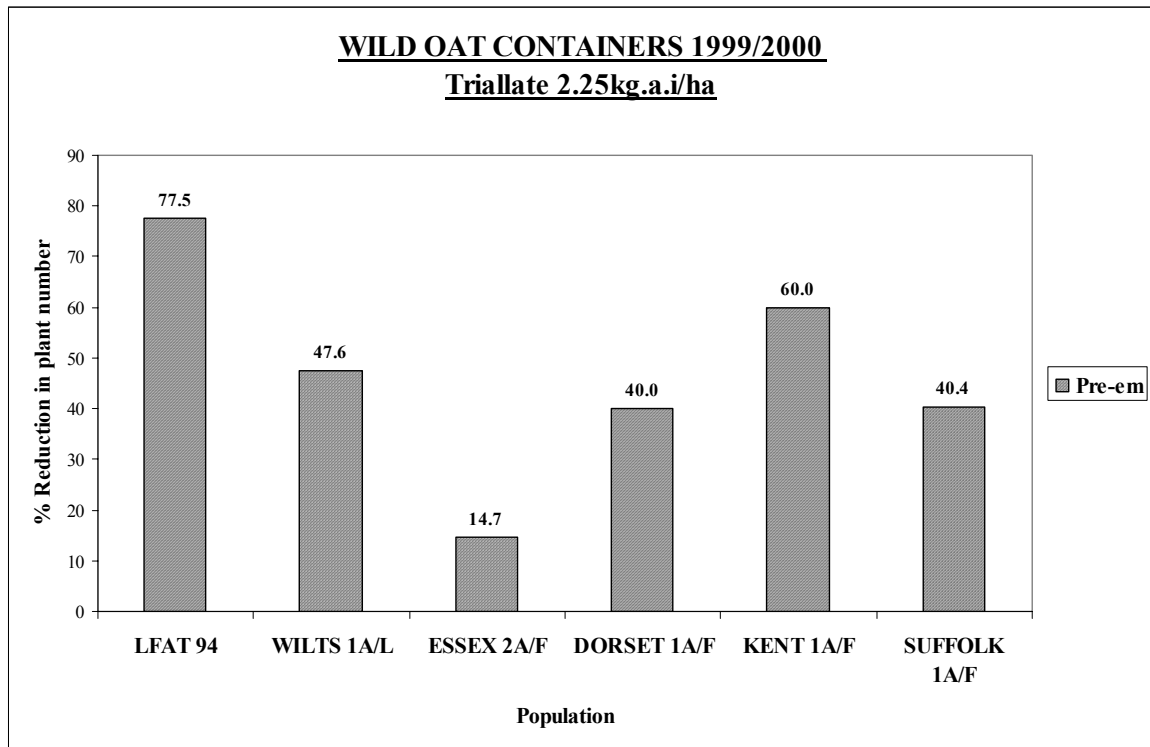
Conclusions

The container results showed that resistance in wild-oats could substantially reduce the efficacy of "fop" and "dim" herbicides, and some other herbicides with different modes of action. The results generally agreed very well with predictions based on the glasshouse dose response studies. Cross-resistance patterns are not simple, as resistance even within the "fop" and "dim" classes was not directly correlated in different populations. Some populations (Suffolk 1A/F, Kent 1A/F) showed high resistance to specific "fops", but no resistance to "dim" or other herbicides. Other populations (Wilts. 1A/L, Dorset 1A/F) showed some resistance to both "fops", to the "dim" tralkoxydim and also to flamprop and imazamethabenz (Dorset 1A/F only). No population showed resistance to cycloxydim. Responses to difenzoquat, isoproturon and especially tri-allate were rather variable, but there was no definitive evidence for resistance in most populations. One population, Essex 2A/F was consistently poorly controlled by all three herbicides indicating that this population does possess mechanisms which confer partial resistance to several herbicides with different modes of action. However further studies would be needed to confirm this. Isoproturon and difenzoquat gave excellent control of some populations which were highly resistant to other herbicides.

It was clear that the impact of resistance on herbicide efficacy is closely related to herbicide timing in many populations. With the susceptible standard, LFAT, timing or size of plant, had no effect on efficacy – excellent control was achieved consistently with all four herbicides in experiment 1 and imazamethabenz in Experiment 2. Where there was a high degree of resistance (e.g. Suffolk 1A/F to fenoxaprop; Kent 1A/F to fluazifop; Dorset 1A/F to tralkoxydim), control was poor to mediocre regardless of timing. However, with partially resistant populations, timing had a large effect. Earlier applications of fenoxaprop, tralkoxydim and imazamethabenz at the three leaf stage were often highly effective, whereas later applications at the 2-3 tiller stage gave poorer control.

The conclusions are clear. With susceptible wild-oats, good control can be achieved regardless of timing. With highly resistant wild-oats, poor control is likely regardless of timing. With partially resistant wild-oats, as application is delayed, the chance of achieving adequate control decreases. Thus a knowledge of the degree of resistance and cross-resistance patterns in any population is essential to ensure good control.

Acknowledgements

References and further reading

CAVAN, G., BISS, P. & MOSS, S. (1998). Herbicide resistance and gene flow in wild-oats (*Avena fatua* and *Avena sterilis* ssp. *ludoviciana). Annals of Applied Biology* **133**, 207-217.

CAVAN, G., CUSSANS, J. & MOSS, S. (2001). Managing the risks of herbicide resistance in wild oat. Weed Science **49,** 236-240.

CLARKE, J.H., BLAIR, A.M. & MOSS, S.R. (1994). The testing and classification of herbicide resistant *Alopecurus myosuroides* (black-grass). In: *Proceedings of the Association of Applied Biology Aspects of Applied Biology* **37**: *Conference on Sampling to Make Decisions,* 181-188.

CLARKE, J.H. & MOSS, S.R. (1999). Weed control update and the impact of herbicide resistance. *HGCA Roadshow - 1999*

CLARKE, J.H., ORSON, J. & MOSS, S.R. (2000). The future for grass weed management in the UK. *Pesticide Outlook – April 2000*, 59-63.

CLARKE, J.H., MOSS, S.R. & ORSON, J.H. (2000). Grass-weed management in an arable rotation. *HGCA conference: Crop Management into the new Millennium*, 15.1 – 15.14.

COCKER, K.M., MOSS, S.R. & COLEMAN, J.O.D. (1999). Multiple mechanisms of resistance to fenoxaprop-P-ethyl in United Kingdom and other European populations of herbicide-resistant *Alopecurus myosuroides* (black-grass). *Pesticide Biochemistry and Physiology* **65**, 169-180.

COCKER, K.M., COLEMAN, J.O.D., BLAIR, A.M., CLARKE, J.H. & MOSS, S.R. (2000). Biochemical mechanisms of cross-resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides in populations of *Avena* spp. *Weed Research* **40**, 323-334.

COCKER, K.M., NORTHCROFT, D. S., COLEMAN, J.O.D., & MOSS, S.R. (2001). Resistance to ACCase-inhibiting herbicides and isoproturon in UK populations of *Lolium multiflorum*: mechanisms of resistance and implications for control. *Pest Management Science* **57**, 587-597.

MOSS, S.R., CLARKE, J. & RICHARDSON, A. (1994). Variation in sensitivity to herbicides in UK wild-oat populations (*Avena fatua*) and (*Avena sterilis* spp. *ludoviciana*). *Project report for HRAC.* 119pp.

MOSS, S.R. & CLARKE, J. (1992). Herbicide resistance in black-grass (*Alopecurus myosuroides). Final report of 4 year project 0047/87 and 0070/87. HGCA Report No.* **62**. 75pp.

MOSS, S.R. & CLARKE, J. (1995). Inheritance of herbicide resistance in black-grass (*Alopecurus myosuroides*) and response of the weed to a range of herbicides. *HGCA Project Report No.* **116.** 100 pp.

MOSS, S.R., CLARKE, J.H., BLAIR, A.M., CULLEY, T.N., READ, M.A., RYAN, P.J. & TURNER, M. (1999). The occurrence of herbicide-resistant grass-weeds in the United Kingdom and a new system for designating resistance in screening assays. In*: Proceedings 1999 Brighton Conference - Weeds*, 179-184.

MOSS, S.R. & CLARKE, J. (2001). Dealing with herbicide-resistant wild-oats. HGCA Topic Sheet No. 46.

ROSS, G.J.S. (1987). *Maximum Likelihood Programme User Manual (Version 3.08).* Numerical Algorithms Group Ltd., Oxford, England.