POSTER PAPERS

Monitoring weediness and persistence of genetically modified oilseed rape (*Brassica napus*) in the UK

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ABSTRACT

Results from monitoring the incidence and persistence of genetically modified (GM) oilseed rape volunteers at National List sites and at eight large scale release sites throughout the UK show that volunteer numbers in subsequent crops can vary considerably from site to site. At some sites volunteers were fully controlled in subsequent crops. At other sites viable GM seeds persisted in the soil, producing volunteer populations for up to three years after the release. Where GM and non GM varieties were grown together, the proportion of transgenic volunteers in subsequent crops appears to be lower than the original percentage grown. Initial results from this study suggest that weediness and invasiveness of oilseed rape volunteers is not enhanced by the specific genetic modifications studied.

INTRODUCTION

Some of the first large scale releases of genetically modified (GM) oilseed rape in the UK have been monitored by NIAB since 1995. The work has been commissioned by the Department of the Environment, Transport and the Regions (DETR) and by the Ministry of Agriculture, Fisheries and Food (MAFF) to provide more data on the agricultural and environmental consequences of growing GM oilseed rape.

This study investigates whether genetically modified (GM) oilseed rape is likely to become more persistent in agricultural environments and more invasive of natural habitats, by studying the frequencies and consequences of intra-specific gene flow and seed dispersal.

Oilseed rape seeds from conventionally bred varieties have been shown to persist in the soil for several years (Lutman & Lopez-Granados, 1998) and can cause significant volunteer problems in broadleaved crops (Knott, 1995). Cross-pollination between oilseed rape varieties occurs readily at short distances and infrequently at long range (Simpson *et al.*, in press). The persistence and 'escape' of transgenes could arise by pollen flow and seed dispersal, however several comparative experiments have provided no conclusive evidence to suggest that transgenic rape is any more persistent or invasive than conventional types (Sweet *et al.,* 1997, Booth *et al.,* 1996, Crawley *et al.,* 1993).

The incidence and persistence of volunteers and feral populations following GM releases has been examined at a range of sites across the UK. These sites have grown either herbicide tolerant oilseed rape (glufosinate tolerant or glyphosate tolerant), or oilseed rape with a seed oil modification for high lauric acid.

MATERIALS AND METHODS

A total of eight sites with areas of between 1ha and 38ha were monitored for this study, three of which had their first GM releases in 1995. Monitoring of high lauric acid oilseed rape release sites commenced in 1998. All sites will continue to be studied for a minimum of two years.

Each site was visited monthly from April to November. The incidence of volunteers and feral rape plants in these fields and in the surrounding field margins and roadsides was recorded. Volunteers were counted in the cereal crops following the various releases and where possible after the harvest of following cereal crops. Volunteer and feral plants were tested for herbicide tolerance using a non-destructive assay disk test (Sweet *et al.,* 1997). Leaf tissue samples were also taken from plants to confirm presence or absence of the transgenes by polymerase chain reaction (PCR) using transgene specific primers. DNA was isolated from plant tissue using a DNeasy extraction kit obtained from Qiagen Ltd. UK. PCR reaction conditions were supplied by the biotechnology companies responsible for the production of each transgenic variety grown at the sites. Samples collected in 1998 are currently being tested by PCR.

Seeds were taken from volunteers or feral plants flowering concurrently with, and within 400m of the herbicide tolerant GM crop. Seedlings were screened for tolerance to either glyphosate or glufosinate (Simpson *et al.*, in press).

Post release monitoring of National List (NL) GM oilseed rape trials was conducted on two occasions, the first in November and a second in April to determine volunteer survival.

RESULTS

Numbers of volunteers recorded at National List sites were low, although only one year of post release monitoring has been conducted. Most sites recorded no volunteers and only one site recorded more than one plant per square metre. Numbers of volunteers generally decreased between autumn and spring monitoring visits.

No volunteers were seen throughout the 1998 growing season at the three sites where high laurate GM oilseed rape was grown in 1997.

Numbers of volunteers found at sites where herbicide tolerant GM oilseed rape was grown (Tables 1-4) varied from zero to several thousand. Numbers tended to be low in the crop following the GM rape, and to be more prevalent in the second crop post GM release. At the Cambridgeshire site 77 volunteers were recorded in 1997, in the winter wheat crop following the release. In 1998 over 2400 volunteers were counted in the same field, two years after the GM trial had been harvested. In contrast, in the cereal crops following the GM release no volunteers were recorded in two other fields where GM trials had been grown. Between harvest of the cereal crop and the drilling of the following crop of peas (approximately six months), an estimate of over 15000 volunteers was made in one of these fields. At the Devon site over 600 volunteers were counted in the release field three years post release.

At the Cambridgeshire site herbicide tolerant plants were found both in following crops and in semi-natural locations. (Tables 1-4). Phenotypic testing with glufosinate consistently detected lower numbers of herbicide tolerant plants compared to non-tolerant plants at this site, although the proportions at each release field had originally all grown between 45% and 100% GM plants. Testing of seeds from a roadside population of feral rape at this site at 150m distance from the flowering GM trial (Table 4) showed no tolerant plants, although an experiment using male sterile bait plants at 100m distance in the same location gave 8% tolerant seedlings (Simpson *et al.*, 1999).

Table 1. Testing of volunteers within GM crop field.

Site	Area of rape (ha)	Approx % GM	Year tested	Crop	Number tested	%tolerant
Cambs 1995/96	5.2	48%	1996/97	w.wheat	77	6.5 #
			1997/98	w.barley	90	23.3
			1998	post harvest	142	16.9
Cambs 1996	1.0	100%	1997/98	w.oats	0	0.0
			1998	post harvest	87	42.5
Cambs 1996/97	7.2	69%	1997/98	w.wheat	0	0.0
			1998	post harvest	19	10.5
N.I.A.B. 1996/97	2.8	49%	1997/98	w.wheat	16	0.0
			1998	post harvest	0	0.0

Results confirmed by PCR

Table 2. Herbicide screening of seeds taken from volunteers within GM crop area.

Site	Area of rape (ha)	Approx % GM	Year Tested	Crop	Number tested	%tolerant
Cambs 1995/96	5.2	48%	1998	w.wheat	3650	6.0
Cambs 1996/97	7.2	69%	1997	w.wheat	3696	7.6
Devon 1995	1.0	100%	1997	forage rape	615	100.0

Table 3. Testing of volunteers outside GM crop fields.

Site	Location	Year Tested	Number tested	% tolerant
Cambs	adjacent field	1997	541	0.0
	roadside	1998	92	16.3
	adjacent field	1998	135	40.0
Norfolk	adjacent field	1998	160	0.0

Table 4. Herbicide screening of rape seeds taken from outside GM crop area.

Site	Location	Distance from GM rape	Year tested	Number tested	% tolerant
Cambs	adjacent field	20m	1997	7998	0.0*
	roadside	20m	1997	6758	0.1
	roadside	150m	1998	3947	0.0
Norfolk	adjacent field	50m	1998	2004	0.0
Berkshire	adjacent crop	10m	1998	14421	0.4

DISCUSSION

The very low incidence of volunteers found at National List sites shows that GM herbicide tolerant oilseed rape does not appear to increase problems of volunteer management in subsequent crops. Furthermore, the gene flow data from the NL GM winter rape trials (Simpson *et al.*, 1998) showed that a proportion of plants grown from seed sampled from GM plots are hybrids expressing tolerance to both glufosinate and glyphosate. There is no indication at present that these multiple tolerant hybrids are more difficult to control in following crops than conventional or single tolerant rape varieties.

The absence of oilseed rape volunteers at the high laurate sites shows that at these sites weed management was extremely good in the following wheat crop, and the GM oilseed rape presented no more of a problem than conventional volunteers. Post harvest conditions in 1997 were ideal for fast germination of oilseed rape seeds prior to cultivation which reduced numbers of seeds returning to the soil seed bank. Post harvest cultivation and weather conditions affect seed return to the soil and may influence the numbers of volunteers seen one year after harvest of the rape crop (Pekrun & Lutman, 1998; Bowerman,1993). These sites will continue to be monitored as different post rape harvest conditions at different sites may produce more volunteers in subsequent crops. However these results indicate that the high laurate trait does not appear to cause problems in control.

Monitoring volunteer incidence indicates that large numbers of oilseed rape seeds are persisting in the soil for up to three years post GM release at some sites (Cambs and Devon). Previous studies show that rape seeds can survive in soil for several years (Lutman & Pekrun 1998, Lutman 1993) due to environmentally induced secondary dormancy.

Plant Genetic Systems releases of glufosinate tolerant winter and spring rape at the Cambridgeshire site show that the numbers of GM compared to non-GM volunteer plants found both in following crops and in semi-natural situations were low. Previous work looking at the survival and persistence of GM rape lines reflects the situation reported here (Sweet *et al.,* 1997, Booth *et al.,* 1996, *Crawley et al.*, 1993). The differences in proportions of GM to non-GM plants detected could however be due to a number of factors. Loss of expression of the transgene in subsequent volunteer populations may occur (Metz *et al.,* 1997). Results of

PCR tests on samples from susceptible volunteers will determine whether the transgene is present. There is also the possibility that some GM seed was shed at a different time to non GM seed prior to harvest, affecting the ratio of transgenic seed returning to the soil seed bank. Different proportions of GM to non GM seed may have germinated immediately after harvest, or the GM volunteers may be less fit or viable than their non transgenic counterparts. Incidence of GM herbicide tolerant rape plants in these volunteer populations suggest that weediness and invasiveness is not enhanced by this specific genetic modification. Although some transgenic plants were found outside the initial GM area at one site, these were all eliminated by normal cultivation methods. Spread of the transgene outside the crop area was probably due to seed spillage in this case. Pollen flow can occur as indicated by the bait plant experiment (Simpson *et al*, in press), but monitoring detected no cross-pollination of feral plants. This may have been due to pollen competition from neighbouring plants, and from self-fertilization.

In conclusion, results of monitoring to date do not indicate that herbicide tolerant and high laurate GM oilseed rape varieties are likely to be any more weedy in an agricultural environment than conventionally bred oilseed rape. Transgenic volunteers with these traits do not appear to be more invasive of habitats outside the crop than their non transgenic counterparts. Future monitoring of these sites will give an indication of the longevity and viability of transgenic seeds in the soil several years after the initial release. This information will be useful for predicting effects of future GM traits on persistence of oilseed rape volunteers.

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Millet as a model-crop to assess the impact of gene flow toward weed populations

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ABSTRACT

Data on pollen dispersal and interspecific crosses between foxtail millet, an autogamous summer cereal, and the green foxtail, are reported. Progeny of hybrids are identified as the giant green foxtail, a botanical variety of green foxtail called "major". Growth and reproduction of the two wild types are compared as well as their competition effect against maize. The fitness associated with a herbicide resistance gene transferred to millet is studied. The results are used to make inferences about the impact of gene flow from the crop to its wild relative.

INTRODUCTION

Biotechnologically derived herbicide-resistant crops are purported to be major steps in weed control. However, one of the main scientific and public concerns is that recombinant genes may escape from agronomic species and enter wild plants, via spontaneous hybridization between crops and wild plants, and create "super weeds". The literature provides little information on the probability of such hybridizations, and even less on the survival and behaviour of hybrids and descendants. Indeed, the basic question is not only the possible occurrence of hybrids, but the conditions that could lead to the spread of their descendants in arable fields and wild habitats, and the final impact of these plants for farmers and the environment.

A well-documented case is that of foxtail millet (*Setaria italica*), an autogamous summer cereal, which can intercross spontaneously with the green foxtail (*S. viridis*). Resulting hybrids and descendants are identified as the already known giant green foxtail, a botanical variety of the wild species (*S. viridis* var. *major*). These plants have a bigger vegetative development than green foxtail and reproduce abundantly. They can be found as weeds in cultivated fields as well as in waste places. Surveys show that they are currently released in fields of foxtail millet, for instance in the area of Angers (France), or in the northern half of China where more than three million hectares are still grown. However, in spite of the recurrent gene flow working in these areas from antiquity times, the frequency of the "*major*" variety is not so high as it would be expected on the basis of their apparent developmental advantage. This suggests that "*major*" plants could be unadapted. Since herbicide resistant varieties of millet are highly desired and available now, this must be quantified in order to predict the spread of herbicide resistance genes in the wild species.

The paper summarizes the main biological features regulating the appearance of the hybrid "*major*" plants. Then growth, reproductive and competitive ability of typical green foxtail and giant green foxtail are compared to estimate their relative weediness. The effects on growth and reproduction of a herbicide resistance gene transferred to the crop is also investigated on nearly isogenic lines in order to check if it is a neutral change in the absence of herbicide. The results are used to indicate the likely impact of gene flow from the crop to its wild relative.

POLLEN FLOW AND HYBRIDIZATION

Foxtail millet and green foxtail are two autogamous species. Outcrossing rate between plants growing 20-40 cm apart ranges from 0 to 2.2 % in both species (Till-Bottraud *et al.*, 1992). We carried out an experiment to study the effect of the distance between a pollen donor and a target plant. One m^2 plots of a green-pigmented variety were sown at different distances along eight lines radiating from a 20 m^2 plot of a red-pigmented variety. The total area of the green-pigmented plants was 120 m^2 spaced over a 1.1 ha circular field. Red pigmentation of seedlings was used as the dominant marker to identify hybrids among the progeny of green-pigmented target plants.

The farthest hybrids were detected at 24 m (Table 1). Of course, a larger size pollen donor plot should improve the chances of observing pollen migrating farther, and also higher hybrid frequency at each distance. We conclude that, even with an autogamous crop, risks of long distance pollination cannot be excluded. More data on pollen flow, as obtained with male sterile plants as target, are reported in Wang *et al.* (1997).

Table 1. Percentage of hybrids between two varieties of foxtail millet according to distance (mean, and maximum value at the risk P=0.05).

Hybrid (%)	Distance (m) 0.5	1	2	4	8	12	18	24	>30
Mean	0.71	0.57	0.32	0.10	0.033	0.009	0.005	0.001	0
Max	0.74	0.60	0.34	0.12	0.039	0.012	0.009	0.004	0.001

The above results can apply to interspecific crosses as no peculiar barrier to interspecific hybridization seems to occur. Plants of green foxtail within a millet field (inter-rows=0.75 m; 10 plants per linear m) produce on average 0.2% of hybrids (Darmency *et al.*, 1987; Till-Bottraud *et al.*, 1992). Reciprocal crosses occur at a lower rate when both species are grown at the same density, 0.002%, and in addition most of the seeds are harvested and exported out of the field, which finally reduces again the actual hybrid output on the field.

Interspecific hybrids are 76% sterile, but further selfed generations behave normally (Darmency & Pernès, 1985). Multivariate analysis of morphological and reproductive traits of the F2 generation of hybrids and various "*major*" plants collected in the wild showed overlapping of the two groups and clear-cut difference from green foxtail and millet. The similarity of hybrid descendants with the "*major*" variety was confirmed by the finding, in "*major*" plants collected in France, of some segregation for characters that make the difference between green foxtail and millet (Darmency & Pernès, 1987, Till-Bottraud *et al.*, 1992).

COMPARISON OF WILD TYPE TO "*MAJOR*"

Seedlings development in growth cabinet

Emergence and growth of young seedlings is a key period that often determines the intensity of weediness of a species against crops. Two populations of green foxtail and two of giant green foxtail, in both cases one population belonging to China and the other from France, were germinated and grown in a growth cabinet at 25°C during 16 h day (230 µmol.photon.$m^{-2}.s^{-2}$) and 20°C during 8 h in the dark (30 plants each population). A cultivar of foxtail millet was also used. The development (height and weight) of the "*major*" was intermediate between that of the green foxtail and that of the millet (Table 2). This suggests higher potential for competition of the "*major*" type.

Table 2. Characteristics of seedlings of a cultivar of millet, two samples of green foxtail (minor) and two of giant green foxtail (*major*).

Type	No. of days to 3rd leaf	Height at 19 days (cm)	Height at 31 days (cm)	Weight at 31 days (g)
Millet	15.7 d	4.4 a	9.0 b	14.0 a
Major 1	16.7 c	3.2 b	8.1 b	8.1 b
Major 2	16.7 c	3.5 b	10.2 a	7.8 b
Minor 1	17.2 bc	2.0 c	5.9 c	4.5 c
Minor 2	18.4 a	1.6 c	5.4 c	4.4 c

a-d values different at P = 0.05

Experiment in a maize field

A field competition experiment was conducted at the INRA experimental farm to compare the growth of green foxtail and giant green foxtail in a maize field. As the soil must be kept weed free to protect the experiment from interferences with other weeds, and additional weed control techniques such as hoeing disturb the soil too much, atrazine-resistant assessions of the weeds were used, and the maize sprayed with atrazine. The two weeds (origin France) and maize (cv. Dea) were grown pure or with a second species. Maize was sown at 7.4 plants/m^2 with an inter-row of 0.75 m. The same day, plots 3.75 m x 0.75 m were sown with *Setaria* seeds and the mean emergence density was 47 plants/plot for the "minor" green foxtail and 184 for the giant, "*major*" type. The time of emergence was recorded for each plant and all plants were located on a map. The size of 33 days old seedlings of maize, 40 days for *Setaria*, were measured. Each plant was harvested separately, observed for morphological traits, then dried and weighed, four months after sowing for *Setaria*, five for maize. Both design and result interpretation were performed as in Assémat & Allirand (1995) and Assémat *et al.* (1995).

Table 3. Early size (L, cm) and reproductive dry weight (DW, g/plant) for small, medium and big plants in pure and mixture plots (see text).

Species		Small plants		Medium plants		Big plants	
		L	DW	L	DW	L	DW
Maize	Pure	35.6	130.3	38.4	160.8	40.0	176.8
	+ *Major*	34.8	122.0	38.8	163.2	40.8	181.5
	+ Minor	33.1	120.7	39.6	163.4	44.7	186.8
Major	Pure	5.3	0.15	6.9	1.66	7.9	5.95
	+ Maize	3.3	0.08	5.7	0.38	7.0	0.88
Minor	Pure	3.8	0.20	4.1	1.47	5.3	5.96
	+ Maize	2.6	0.04	4.2	0.22	5.7	0.83

Main results concerning plant reproductive output are listed in Table 3. Within each plot, plants were split in 3 groups according to total dry weight (small plants less the 1st quartile, then medium plants, and big plants higher than 3rd quartile). Both size at 33 days and reproductive dry weight increased with total biomass. A large variability was found in both *Setaria* for the reproductive output, up to a ratio of 1 for small plants to 20 for big plants, much less for the size at 33 days. In this experiment, the competitive effects of "*major*" and "minor" types on crop yield was low and significant against small plants only. The competitive effect of maize on both *Setaria* was very important for all types of plants. A higher reproductive investment for "minor" was suspected for most plants, especially because vegetative biomass was much lower than for "*major*". The strong link between results of competition and early size (which, in fact, is measuring emergence earliness within the population) has to be inferred in order to make clear ecological differences between "*major*" and "minor" types.

EFFECTS OF A RESISTANCE GENE

Three different herbicide resistances have been recently obtained in foxtail millet through classical breeding. Atrazine- (Darmency & Pernès, 1985), trifluralin- (Wang *et al.*, 1996) and sethoxydim- (Wang & Darmency, 1997) resistant lines will solve the weed control problem in millet fields. However, secondary effects of the resistance genes are possible. For instance, atrazine resistance resulted in millet varieties having lower potential yield, but yield penalty was compensated by better weed control (Darmency & Pernès, 1989). In contrast, results on BC2 descendants of sethoxydim resistant and susceptible lines showed higher grain yield with the resistant type in the absence of herbicide (Wang & Darmency, 1996).

We show here data on the growth and yield of more isogenic materials, BC7, resistant and susceptible to sethoxydim. Millet was sown as pure lines in a randomized block design (5 replicates) in the field. No significant differences were recorded for vegetative characters and most reproductive ones (Table 4). The weight of 1000 grains was lower for the resistant plants. Since grain weight per plant seemed to be higher, this made higher number of grains produced by the resistant plants. These results confirm previous records on BC2. Apart from a possible pleiotropic effect of the resistance gene on seed production, this would suggest a close linkage with a gene controlling seed output in the original resistant parent. Therefore, transfer of this group of linked genes to wild plants, even in the absence of herbicide use, could be advantageous and enhance fitness and weediness of green foxtail populations.

Table 4. Characteristics of sethoxydim resistant and susceptible BC7 lines of millet (Per plant values, Test of Tuckey at P=0.05).

Trait	Resistant	Susceptible	Difference
Flowering time (day)	83.1	83.4	NS
Height (cm)	156.0	167.0	NS
Flag length (cm)	28.8	31.8	NS
Tiller number	3.6	3.4	NS
Spike length (cm)	22.6	21.3	NS
Vegetative weight (g)	34.6	34.3	NS
Grain weight (g)	37.9	32.2	NS
Weight of 1000 grains (g)	2.9	3.2	0.03

CONCLUSIONS AND PERSPECTIVES

The case of millet illustrates the ancient and repeated occurrence of hybrids between a crop and a wild relative. Gene flow is continuous and leads to hybrid descendants that could establish in the field and waste habitats long before the use of transgenes. However, their apparent more vigorous morphology does not make them more competitive weeds against maize. The reproductive to vegetative dry weight ratio is higher for the common green foxtail, which could explain the low frequency of "*major*" in field populations. In contrast, when "*major*" plants will originate in crosses with a herbicide resistant millet, they could pick up the herbicide resistance that provides a new adaptive value when sprayed with the herbicide. Therefore, it is likely that the dispersal of herbicide resistance genes would change the present situation. In addition, the resistance genes transferred to millet can also have side effects, such as that observed above on the seed output, which can enhance fitness of the weeds.

All these features show that accurate investigations on the biology of the target weeds and wild plants are needed in order to predict the impact of gene flow between transgenic crops and their wild relatives. The case of herbicide resistance is easy to study because the selection pressure, the herbicide is under the control of the researcher or the farmer. More complex are the cases of stress and pest resistances. As well as knowledge on weed biology and actual selection pressures in the fields, there are working plans to reduce gene flow. Transformation and breeding strategies of the crop could be improved. For instance, reciprocal crosses in millet have not the same chance, so that a resistance gene located on the chloroplast DNA of the crop would be safer. Linkage to genes unadaptive or detrimental to wild plants, such as those encoding for less seed produced and no seed shedding, or multigenic and recessive traits that would not be expressed in hybrids, could delay the spread of transgenes.

Further studies are carried out in the framework of a EC-China project involving both millet and wheat. These aim to provide guidelines for both the breeders and the farmers in order to use safely and durably herbicide resistant crops. As for the work on millet, they focus on the presence of the "*major*" type (i.e. past introgression) versus the epidemiology of the spread of resistance genes in the vicinity of fields where resistant millet is grown now in China (i.e. current gene flow). In addition, molecular markers are being developed to study the genetic structure of wild populations as well as cytological investigation using *in situ* chromosome painting to check the presence in wild plants of chromosomes of the crop.

Foxtail millet is also a good model to study the role of the mode of inheritance of the resistance gene, and the effect of the location of the gene on chromosomes to prevent or delay such a gene escape. For instance, the three resistances obtained in millet are differently inherited:- cytoplasmic (atrazine), nuclear recessive (trifluralin) and nuclear dominant (sethoxydim), which provides the opportunity to validate computer models with data from artificial populations involving green foxtail and hybrids submitted to various treatments. This approach is expected to show which kind of gene or population management is best suited to delay the establishment of the resistance genes within a population.

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