SESSION 4A
TRENDS IN WEED POPULATIONS

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Papers

4A-1 to 4A-4

MODELING   POTENTIAL   SPREAD   OF   *ABUTILON   THEOPHRASTI* (VELVETLEAF)

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ABSTRACT

Abutilon theophrasti is one of the worst agricultural weeds in the United States, yet to date it has not reached that status in California. We collected herbaria records, weed literature, and surveys to determine its historical and current distribution in the state. When results were mapped, 38 out of 58 counties in California had *A. theophrasti* reported in herbaria records and 19 had *A. theophrasti* reported in surveys. The combined results showed 42 counties with *A. theophrasti* present historically and/or currently. A plot of cumulative number of counties containing *A. theophrasti* by decade demonstrated that the distribution of *A. theophrasti* has increased exponentially over the past 40 years. The climate matching/mapping software CLIMEX® was used with real and estimated data on environmental requirements of *A. theophrasti* to map its potential distribution in California. The resulting map showed the species was unlikely to spread into areas without irrigation. Experiments to quantify environmental limits of growth, reproduction, and seed germination and overwintering were used to refine estimates of input parameters for CLIMEX. Results indicate that based on environmental requirements, *A. theophrasti* has the potential to become a serious, invasive weed in irrigated agricultural regions of California. Furthermore, its current wide distribution indicates that a source of seeds is available from which this species can spread.

INTRODUCTION

The introduction and naturalization of alien weeds into new regions is widespread in many parts of the world (Forcella, 1985). In the United States, despite the presence of federal and state seed laws, most alien weeds are not considered significant until after they become economic pests, thus introductions continue to occur. Very little research has been conducted on weed epidemiology, yet application of this field to weed science might provide important insights into the mechanisms and characteristics of weed introductions (Forcella, 1985). Several recent books highlight the importance of biological invasions as an emerging focus of ecology and conservation biology (di Castri *et al.*, 1990; Drake *et al.*, 1989).

Currently the literature regarding what constitutes a potentially invasive weed is limited and not conclusive. A review of these works suggests that certain genetic and ecophysiological characteristics might indicate whether a species is likely to be a successful invader. These include rapid seed germination, especially at low temperatures; high rates of spread (Forcella, 1985); longevity (Barrett and Richardson, 1986); effective dispersal (Howe and Smallwood,

1982); a high level of genetic variation; ability to self-fertilize; phenotypic plasticity (Newsome and Noble, 1986); and ability to tolerate a wide range of variability in climate and habitat (Swincer, 1986). These references disagree on the important criterion of whether a successful invader should be a generalist or a specialist (as defined by Baker, 1965).

Abutilon theophrasti (velvetleaf) is ranked as one of the most troublesome weeds in the midwestern United States (Stoller *et al.*, 1993), and increasingly in other parts of North America (Andersen *et al.*, 1985; Warwick and Black, 1986) and in Europe (Sattin *et al.*, 1992). *A. theophrasti* was originally introduced from China into North America in the 1700s as a potential fiber crop (Spencer, 1984). In the last 40 years this species has undergone rapid range extension northward and southward in North America. Many studies have reported decreases in crop yield due to competition from *A. theophrasti* (reviewed in Warwick and Black, 1988).

A. theophrasti is a rapidly growing, summer annual, C_3 weed that overwinters in the seed stage. A typical pioneer or colonizing species, it has rapid growth and high rates of photosynthesis that contribute to its success in disturbed habitats (Regnier *et al.*, 1988; Warwick and Black, 1988). While *A. theophrasti* has not been observed to invade natural (non-agricultural) ecosystems, within agroecosystems it is a serious problem that continues to spread. Characteristics that render it a particularly troublesome weed include hardseededness (Horowitz and Taylorson, 1984; Lueschen and Andersen, 1980), extremely long-lived seeds (Egley and Chandler, 1983; Lueschen *et al.*, 1993), production of allelopathic chemicals (Sterling and Putnam, 1987), and serving as host of several insects and diseases of crops (reviewed in Warwick and Black, 1988).

Warwick and Black (1986) examined genecological variation in *A. theophrasti* populations from Canada. Significant intrapopulation differences in growth, germination, and morphological characteristics were found, which were correlated with latitude and climate. These workers found that *A. theophrasti* exhibited several genetic features characteristic of successful colonizers, including polyploidy, self-fertilization, and high levels of population differentiation (Warwick, 1990; Warwick and Black, 1986). *A. theophrasti* accessions collected from around the United States grew and reproduced at latitudes to the north and south of their current range, demonstrating potential to expand into a wider area (Andersen *et al.*, 1985).

A. theophrasti has been documented in California only since the mid-1900's. Throughout this century to the present, various floras and weed bulletins have listed the species only sparingly in the state. Thus, following its introduction into the California flora, *A. theophrasti* initially increased slowly. In contrast, current weed records document the widespread occurrence of *A. theophrasti* in a number of California counties and in several different cropping situations. Lack of a detailed collection history precludes determination of the factors responsible for the recent increase in occurrence of this weed in California. Regardless of the cause, the seriousness of *A. theophrasti* in most of the country and its increasing presence in California strongly suggest that it could become one of the worst weeds in the state.

One possible reason why *A. theophrasti* is not more serious in California could be that since its introduction was recent, its maximum rate of spread and final distribution have not yet been

reached. To address this question, a detailed collection history of the past and current distribution of *A. theophrasti* in the state was conducted, which allowed calculation of the rate of spread since its introduction. Alternatively, it is possible that even though *A. theophrasti* is now found widely, it is spreading slowly due to lack of adaptation to some aspect of the climate in California. California has a Mediterranean climate, with hot, dry summers and wet, moderately cold winters. *A. theophrasti* seeds and plants are very responsive to temperature, moisture, and temperature x moisture interactions. To address the question of adaptation, a computer model was used to predict potential distribution of *A. theophrasti* based on its climatic requirements.

MATERIALS AND METHODS

Distribution of *A. theophrasti* in California

In 1994, six herbaria throughout the state of California were visited to gather collection information about *Abutilon theophrasti*. Data collected included the date, location, and county in which the specimen was collected as well as any notes regarding the crop or area where the specimen was growing. Notes were also made of specimens collected elsewhere in the United States. To add to the historical data collected from the herbaria, University of California Cooperative Extension Farm Advisors in 52 counties were surveyed in 1995 about the current status of *A. theophrasti* in those counties.

The herbarium data and survey results were used to determine the decade during which *A. theophrasti* first occurred, if at all, in each of California's 58 counties. These data were then used to determine the rate of spread of *A. theophrasti* in California. Starting in the decade from 1910-1919, the first decade in which *A. theophrasti* was recorded in California, the cumulative number of counties in which *A. theophrasti* occurred was plotted by decade. To provide comparisons to the rates of spread of other introduced weed species, the equations of Forcella (1985) were fitted to the *A. theophrasti* data using SigmaPlot 3.0 (SPSS, Inc., Chicago, IL, USA). The decade of first occurrence was also mapped by county using ArcView 3.0 (ESRI, Inc., Redlands, CA, USA).

Potential spread of *A. theophrasti* in California

CLIMEX, a computer model developed by Sutherst and Maywald (1985), was used to determine the potential spread of *A. theophrasti* in California based on the climatic requirements of populations of *A. theophrasti* growing elsewhere. The CLIMEX model assigns an "ecoclimatic index" (EI), scaled from 0 (poor match) to 100 (strong match), to specified locations from which meteorological data are available (Maywald and Sutherst, 1985). The EI encompasses measures of how favorable annual temperatures (temperature index, TI) and rainfall (moisture index, MI) are to the species of interest, as well as how strongly temperature or moisture stresses may affect the species. For a plant that overwinters as seed, plant population growth can be modeled adequately in CLIMEX using only the effect of temperature and moisture, expressed as the growth index (GI, calculated from TI and MI), because the plant is assumed to have avoided stressful conditions (Dr. R.W. Sutherst, personal communication). Without any stress parameters, GI=EI. CLIMEX generates maps with dots

proportional to the value of EI. These maps represent the modeled species potential distribution because larger dots (larger EIs) represent environments more favorable to the species.

The EI is computed based on parameter values set for the species by the user. The parameter values can be determined by fitting a species' known geographical distribution or by using laboratory or field data regarding the temperature and moisture requirements of the species. The moisture and temperature parameters for *A. theophrasti* were determined in an iterative manner by starting first with temperature and moisture requirements obtained from the literature (Andersen *et al.*, 1985; Warwick and Black, 1988). These parameters were adjusted to fit the known distribution of *A. theophrasti* in China (where *A. theophrasti* is native) and India (Spencer and Sankaran, 1985). The parameters were then tested and adjusted on known distributions from North America, Australia, and Europe.

The original CLIMEX meteorological database contained data for only seven locations in California, most of them coastal. Data collected from the herbaria and survey indicated that most *A. theophrasti* occurred in areas of irrigated agriculture in California's Central Valley and deserts. Thus, to determine the potential spread of *A. theophrasti* in California, meteorological data from the University of California UCIPM Project IMPACT database (Statewide IPM Project, University of California, Davis, CA, USA) was used to create 40 additional CLIMEX sites representing a greater diversity of California's climates (UCIPM, 1993). These new sites contained data for the average monthly temperature and rainfall for the five years from January 1989 to December 1993. The moisture, temperature, and stress parameters for *A. theophrasti* that had been set using the other known distributions were then used to generate a predicted distribution within California. Further adjustments to the predicted distribution were made using the "Irrigation" feature in CLIMEX which allows the addition of known amounts of moisture to the meteorological database during either "winter" or "summer."

RESULTS AND DISCUSSION

Distribution of *A. theophrasti* in California

A. theophrasti has been recorded in 42 of 58 counties in California. The first specimen recorded in California came from a citrus grove in the city of Riverside, Riverside County, in the southern portion of the state in 1917. The next earliest records were in the 1920's and came from four counties in the northern portion of the state. In the north, *A. theophrasti* spread to two new counties in the 1930's, while in the south, the 1930's brought no new records of *A. theophrasti*. Starting in the 1950's *A. theophrasti* began to disperse throughout the state, with seven new counties recorded. The most rapid spread of *A. theophrasti* into new counties occurred in the 1960's and 1970's when it was found in eight new counties each decade. By the 1980's, records from previously uninfested counties began to decline as *A. theophrasti* had already been found in most of the agricultural counties in the state.

The rate of spread of *A. theophrasti* within California, calculated as the cumulative number of counties with *A. theophrasti* versus decade, fits well the logistic equation of Forcella (1985)

(R^2=0.997, P<0.0001). The maximum number of counties into which *A. theophrasti* was predicted to spread was 49, with the point at which the rate of spread was predicted to decrease occurring after the sixth decade (1960-1969). The initial rate of spread, computed as in Forcella (1985), was 11.11 (R^2=0.78).

Potential spread of *A. theophrasti* in California

The CLIMEX parameters given in Table 1 produced the best fit for the distribution of *A. theophrasti* in China, where it native, and in India. The resulting map matches the known historical distribution in these two countries, described by Spencer and Sankaran (1985). When the parameters in Table 1 were then used to model *A. theophrasti* distribution on the North American continent, the predicted distribution matched the distributions reported by Warwick and Black (1988) with the exception of the desert southwestern United States. An examination of indices (GI, TI, and MI) for several sites in California where *A. theophrasti* has been collected this decade (1990-1999) shows that the growth index (GI) is less than 33. This means that the climate in these sites matches the parameters for *A. theophrasti* for only one-third of the year. The primary limitation to matching the given parameters imposed by the climate in California is a lack of moisture (low MIs), because the TIs all approach or equal 100 (100 is a perfect fit).

Table 1. Parameters used in the CLIMEX model[1].

Temperature Parameter	Temperature (°C)	Moisture Parameter	Moisture (% soil moisture)
DV0	6	SM0	0.1
DV1	10	SM1	0.5
DV2	40	SM2	2.0
DV3	45	SM3	3.0
PDD[2]	1670		

[1] Parameters are defined in Maywald and Sutherst (1985).
[2] Minimum degree days required for development.

Without extensive irrigation, California lacks water to support agriculture. To account for the possible role of irrigation in the ability of *A. theophrasti* to persist in California, 70 mm per week of water were added to the rainfall totals in CLIMEX for the six summer months from April to September. Seventy mm per week of irrigation is a rough estimate of an average weekly value, based on amounts of water applied in Riverside County, California, in the summer. The actual amounts of irrigation used vary throughout the state based on the evaporative demand at any location, in any season (Dave Cudney, personal communication). The effect of this added irrigation in the model was to raise MI, and thus GI, to near 90 in areas that previously had low values for these indices. This adjustment demonstrated that irrigation could improve the match between climatic parameters based on *A. theophrasti*'s native habitat in China and the climate of California. The predicted distributions of *A. theophrasti* in California without and with irrigation added to the model are shown in Figure 1.
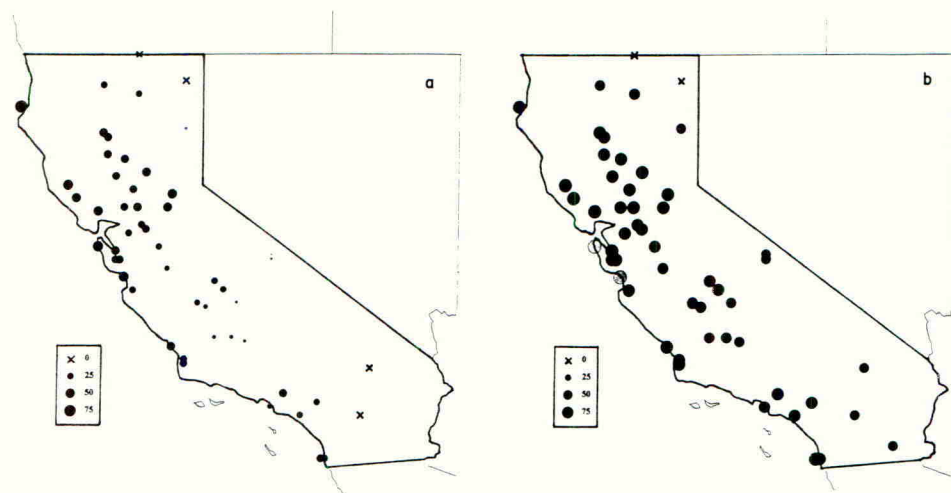
Figure 1. EI (ecoclimatic index), which is proportional to the size of dots (100 is maximum), for *A. theophrasti* in California from the CLIMEX model run a) without added irrigation, and b) with added irrigation.

CONCLUSION

A. theophrasti currently occurs in most of the counties in California and in all counties with a significant amount of agricultural production. However, until recently it spread only gradually without becoming a serious weed in most areas. It appears that although *A. theophrasti* is tolerant of the temperatures experienced in California's Mediterranean climate, its presence may be closely linked to use of irrigation in agriculture, which is typically applied during the hot, dry summer months. Predictions of potential spread of this weed in California agricultural regions were greatly enhanced by the simulated addition of irrigation water. This suggests that this weed has the potential to become a serious, invasive weed in irrigated agricultural regions of California. However, potential problems with it will be dependent on the crop, soil, and management methods specific for each location. Furthermore, its dependence on irrigation indicates that possible strategies for its management may be found in management of irrigation water.

ACKNOWLEDGMENTS

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GENOTYPIC VARIATION IN THE DEVELOPMENT OF SECONDARY DORMANCY IN OILSEED RAPE AND ITS IMPACT ON THE PERSISTENCE OF VOLUNTEER RAPE

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ABSTRACT

Oilseed rape seeds can develop secondary dormancy and as a result of this remain ungerminated in the soil for many years. We have investigated inter- and intravarietal variation in secondary dormancy in oilseed rape seeds by repeatedly testing 47 cultivars in a standard Petri dish test, using an osmotic solution and darkness to impose dormancy. Cultivars showed a wide range of response, ranging from below 2 %, as in for example, Falcon, Acrobat, Industry, to over 50 % dormant seed in Apex, Nimbus and Mars. The problem of varying results from test to test is discussed. The experiments demonstrate that varietal choice can have a substantial effect on subsequent volunteer rape populations and should be taken into consideration by growers when selecting oilseed rape cultivars.

INTRODUCTION

Oilseed rape seeds can persist in the soil, due to induced secondary dormancy, for at least five years (Schlink, 1994, 1995) and very likely for ten years (Sauermann, 1993, Kohout & Soukup, 1996). Volunteers can emerge from the soil seedbank and cause weed problems in future crops. At the moment volunteer oilseed rape mainly causes problems in broad-leaved crops. Oilseed rape volunteers tend to emerge in several flushes which makes timing of herbicide application difficult (Garrett & Orson, 1989, Knott, 1995, Lutman, 1993).
There are currently between 10 and 20 types of oilseed rape in the breeding process, conferring traits for specific oil qualities (Carruthers, 1995). Changing from one type of oilseed rape to another will not always be possible without reductions in quality due to volunteers of a different oil quality. Also, persistence of oilseed rape seeds needs to be considered in risk assessments of genetically manipulated rape. Whereas pollen and seed movement causes dispersal in space, the soil seedbank causes dispersal in time (Linder & Schmitt, 1994).

One way of influencing the population dynamics of volunteer oilseed rape may be to grow genotypes that have a low potential to develop secondary dormancy. Earlier experiments on a small number of German and British cultivars showed that there is considerable genotypic variation in the development of secondary dormancy (Schlink, 1994, 1995, Pekrun *et al.*, 1997a, Pekrun *et al.*, 1997b, Pekrun *et al.*, 1998). Thus, it appeared important to test a larger range of genotypes. The aim of the experiments presented here is to give some information about the potential to develop secondary dormancy within the British oilseed rape cultivars.

MATERIALS AND METHODS

Seeds of 26 spring and 21 winter rape cultivars were provided by the National Institute of Agricultural Botany of the UK (NIAB). Seeds were air dried and stored dry in a seed store until used. The experiments consisted of a 4 week incubation period, with the seeds under osmotic stress, followed by a 2 week germination test. The seeds were kept in an incubator at $20 \pm 1°C$ in darkness throughout this period, except for the germination assessments which were done in a dark room under green safety light ($500 < \lambda < 600$ nm). Four dishes of 100 seeds were tested for each cultivar. For the incubation seeds were spread into 9 cm-Petri dishes containing two layers of Whatman No. 1 filter paper and 8 ml of a polyethylene glycol 6000-solution generating a water potential of - 15 bars at 20°C (Michel & Kaufmann, 1973). This treatment was known to induce secondary dormancy (Pekrun et al., 1997a). In the subsequent germination test, seeds were transferred onto filter papers in fresh Petri dishes. These were moistened with 7 ml of demineralized water. One week after the start of the germination test, seeds were checked for the first time. All non-germinated seeds were transferred into fresh Petri dishes for another week's testing. Germinated seeds were discarded. After the second week the Petri dishes were unwrapped and all remaining seeds were checked. The number of firm, viable seeds was counted. These surviving seeds were considered to be dormant. Previous work had shown that seeds that remained firm and healthy looking after the two week germination test were dormant and could only be stimulated to germinate by some other dormancy breaking treatment (see Schlink, 1994, Pekrun et al., 1997a, Pekrun et al.,1997b).

Each cultivar was tested three times. It was not possible to test all 47 genotypes at the same time, so each of the three experiments was split into 5 - 6 sub-groups of c. 9 cultivars. Seeds of Starlight were included as a control in each sub-test to monitor their specific environmental conditions. The combination of cultivars per sub-test was randomised, to exclude systematic errors. The proportion of dormant seeds was analysed using regression techniques based on a Generalised Linear Model (GLIM). This analysis standardised the results in relation to the dormancy level exhibited by the Starlight seeds in each sub-test. In the tables the mean percentage of dormant seeds is shown together with the standard error of mean.

RESULTS

Results differed greatly between cultivars and in some cases also between tests. Tables 1 and 2 present results for spring and winter rape cultivars, respectively. The percentages of dormant seeds for the three experiments are given separately together with the average of the experiments. The results for both, winter and spring cultivars, is very similar. Almost 50% of cultivars exhibited a relatively low level of dormancy (<10%) but a substantial minority showed much higher levels, reaching over 50% in extreme cases. There was no clear separation between low, medium and high dormancy types. The results of some cultivars differed between experiments, particularly in the cultivars showing the largest percentage dormancy. This effect is clearly seen in the Starlight data, Experiment 3 having fewer dormant seeds (Table 3).

Table 1. Percentage of dormant seeds of 25 spring rape cultivars in three separate incubation experiments and average of dormant seeds in Experiment 1-3. s.e. = standard error

Cultivar	Experiment 1		Experiment 2		Experiment 3		Average (1-3)	
	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
Acrobat	0.1	0.20	1.1	1.14	1.7	1.87	1.0	0.73
Aries	15.3	3.43	32.3	5.74	4.4	3.20	17.3	2.47
Ester	10.7	3.68	16.3	4.09	17.7	5.95	14.9	2.70
Global	10.6	2.83	7.5	2.89	2.6	2.28	6.9	1.55
Industry	1.2	1.23	1.3	1.23	0	0.02	0.8	0.58
Liaison	2.5	1.12	6.8	2.79	7.0	4.76	5.4	1.9
Licosmos	1.7	0.96	17.4	4.28	11.6	4.89	10.2	2.18
Liga	2.2	1.10	11.7	3.60	0	0.04	4.6	1.25
Longbow	7.0	2.12	5.2	2.46	6.4	3.56	6.2	1.60
Marinka	1.3	1.37	4.2	2.21	1.7	1.87	2.4	1.06
Mars	25.0	4.65	71.7	5.05	55.9	7.28	50.7	3.33
Maskot	19.2	4.60	22.3	4.72	2.5	2.40	14.7	2.34
Melodi	36.5	5.45	39.6	5.61	3.5	2.62	26.6	2.76
Nimbus	63.9	5.04	71.8	5.26	23.9	6.42	53.3	3.23
Pisces	30.7	4.85	15.6	4.22	0.9	1.32	15.8	2.20
Plumbshot	10.7	3.68	30.8	5.53	9.7	4.33	17.1	2.64
Rebel	15.5	4.12	11.9	3.73	3.0	2.46	10.2	2.03
Solar	0.9	0.63	1.9	1.50	0.7	1.42	1.1	0.72
Spok	4.2	2.41	-	-	1.9	1.99	3.0	1.56
Sprinter	24.5	4.55	34.4	5.71	14.9	5.29	24.6	3.00
Star	16.1	3.47	33.1	5.07	6.0	3.44	18.4	2.34
Summit	33.7	5.31	73.1	4.75	25.2	8.92	43.9	3.80
Superol	0.3	0.69	2.3	1.61	0	0.04	0.9	0.58
Triolo	0	0	0.8	0.98	1.4	1.72	0.7	0.66
Trophy	1.7	1.07	9.1	3.32	4.7	3.07	5.1	1.54

Table 2. Percentage of dormant seeds of 21 winter rape cultivars in three separate incubation experiments and average of dormant seeds in Experiment 1 - 3. s.e. = standard error

Cultivar	Experiment 1		Experiment 2		Experiment 3		Average (1-3)	
	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
Alpine	12.2	3.05	12.9	3.70	25.0	6.85	16.7	2.78
Amber	4.2	1.73	9.6	3.41	2.6	2.86	5.5	1.59
Apex	60.2	6.58	81.4	4.49	86.9	3.98	76.1	2.98
Arietta	0.9	0.63	10.1	3.50	8.5	4.07	6.5	1.79
Askari	23.2	4.45	25.8	5.32	7.7	3.87	18.9	2.65
Bristol	18.7	3.79	38.9	6.03	20.6	6.80	26.0	3.27
Capitol	48.6	6.36	70.3	5.35	26.7	6.68	48.5	3.56
Cobra	4.3	2.33	5.9	2.68	0.4	0.94	3.6	1.23
Commanche	2.6	1.82	4.0	2.22	0	0.03	2.2	0.96
Corniche	45.1	5.57	69.5	5.53	1.3	2.01	38.7	2.71
Envol	46.5	6.27	36.6	5.40	2.6	2.28	28.7	2.87
Express	0.6	0.97	3.4	1.97	0	0.04	1.3	0.7
Falcon	0.3	0.42	5.2	2.43	0	0.03	1.8	0.82
Gazelle	9.3	2.49	6.0	2.59	0	0.04	5.1	1.20
Inca	4.6	1.59	0	0.01	-	-	2.3	0.80
Jazz	5.6	1.79	27.5	4.99	6.8	3.79	13.3	2.16
Lizard	22.3	4.72	6.3	2.68	20.1	6.28	16.3	2.77
Mandarin	18.4	4.40	23.3	4.80	3.9	3.02	15.2	2.40
Nickel	1.2	1.23	2.1	1.60	0	0.04	1.1	0.67
Rapier	3.1	1.26	1.1	1.14	0	0.02	1.4	0.57
Synergy	1.2	0.90	17.2	4.06	0	0.03	6.1	1.38

Table 3. Percentage of dormant seeds from three experiments with seeds of cultivar Starlight. Date = start of incubation in each sub-experiment, s.e. = standard error

	Experiment 1			Experiment 2			Experiment 3	
Date	mean	s.e.	Date	mean	s.e.	Date	mean	s.e.
30.4.96	38.5	5.25	08.10.96	69.5	5.07	09.6.97	16.5	6.40
14.5.96	37.5	0.87	14.10.96	57.5	6.29	11.6.97	17.5	4.70
30.5.96	60.5	4.92	18.10.96	60.8	6.07	19.6.97	19.3	6.30
20.6.96	64.8	4.31	23.10.96	66.8	3.73	19.6.97	14.8	3.01
12.7.96	63.5	7.05	31.10.96	49.3	7.33	20.6.97	3.7	0.95
15.7.96	53.8	5.14						

DISCUSSION

The potential of oilseed rape seeds to develop secondary dormancy clearly has a genetic component. Results ranged from 0.7 % dormant seeds on average of the three experiments for some cultivars, to almost 80 % for others. However, results varied not only between cultivars but also in a number of cases between the repeated experiments. This was shown particularly

clearly by Starlight, which was included in each sub-experiment as the standard. Thus, the question needs to be posed whether the test procedure, as described in this paper, may not always give reliable information about a cultivar's potential to become dormant.

Large variation between experiments seems to be a typical feature of dormancy studies with oilseed rape. It has been observed not only in experiments using osmotic solutions to impose dormancy (Pekrun *et al.*, 1997a) but also in work investigating the persistence of oilseed rape seed in soil (Lutman, 1993, Schlink, 1994). The different levels of response in our experiments may be either caused by varying environmental conditions, varying responsiveness of the seeds or both. It is not clear which factors may be causing the variation but we believe that by repeating the experiments several times an average response can be quoted with confidence. Further work is planned to confirm the responses of those cultivars exhibiting the greatest variation between experiments. It was a clear feature of the work that only those cultivars showing high levels of dormancy showed high levels of variation. Thus one can be more confident in stating which cultivars have a low potential to persist.

Despite the problems of reproducibility, previous research has shown that genotypic differences assessed in experiments like the ones presented here can generate sound results. For example, nine repeated tests with cultivars Jet Neuf and Liglandor, although showing a very variable response in the general level of dormancy induction, generated a constant relationship between the two cultivars (Pekrun, 1994). The same can was true for experiments testing a number of cultivars under a range of environmental conditions, e.g. at various water potentials to induce dormancy (Pekrun *et al.*, 1998), or various temperatures (Pekrun *et al.*, 1997b).

Two issues remain to be resolved. Firstly, are the responses seen in these tests using only one sample of seeds from each cultivar, representative of the behaviour of the cultivars, wherever they are grown? Secondly, are the differences in dormancy potential shown in these Petri-dish tests reflected in the persistence of seeds in the field? Earlier work indicates that there are good correlations between behaviour in laboratory and field tests (Pekrun, 1994, Schlink, 1994, 1995), but further work is planned to address these issues.

The tests described in this paper, although requiring further confirmatory studies, do clearly demonstrate that rape cultivars differ in their potential to persist. Where volunteer rape plants cause serious problems growers should consider selecting cultivars that do not have a high potential to persist. Such information should be considered for inclusion in data sheets generated on the attributes of recommended cultivars.

ACKNOWLEDGEMENTS

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CHANGES IN FIELD WEEDS IN HUNGARY DURING THE LAST 46 YEARS

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ABSTRACT

In Hungary two changes of great importance have been made to the production and land tenure conditions in the last 46 years. From the early 1950s, private ownership and small-scale farming systems were gradually replaced by state and cooperative ownership with large-scale production systems. The reverse took place in 1990 so that at present large-scale farming systems prevails on 65-70 % of the agricultural area, but on some 30-35 % the small-scale systems have been reestablished. About 2-3 % of the total area remains uncultivated. National weed surveys have shown that these structural changes have caused significant changes in the weed cover of the arable land and in the weed dominance, influencing their extent and rate of spread. The surveys showed that up to 1988, the number of weed species significantly decreased but the survey in 1996 showed an increase in the number of species. Up to 1988, the coverage of perennial monocots and dicots greatly decreased with an increase in annual grasses and broad-leaved weeds. The survey of 1996, however, showed that coverage of the woolly thistle (*Cirsium arvense*), couch (*Agropyron repens*) and of other perennial species had significantly increased. Of the 25 major weeds encountered in 1987/88, the occurrence of 6 has diminished, and that of 8 has increased, while 3 has become predominant. Sunflower (*Helianthus annuus*), the so-called cultivated weed has spread significantly.

INTRODUCTION

Plant protection specialists agree that efficiently pest control requires knowledge of the pest biology. Identification of the predominantly weed species on cultivated areas is an important prerequisite.The first survey of weeds in Hungary was carried out by Dr. Ujvárosi Miklós and co-workers between 1947 and 1950.

The primary objective of the research reported here is to improve our knowledge of the weed populations of cultivated areas of Hungary in order to develop effective weed

control/weed regulation technologies. Similarly to Dr. Ujvárosi, we believe that we do not have sufficient

current information on the extent, composition, of field weeds particularly following changes in the agricultural system. Without this information no advance can be made in weed management.

The extreme variability of the relief of Hungary, influences both the weed cover and the species composition of weed populations, and this was demonstrated in the first survey. Changes in the agricultural system from the late 1950s to the late 1980s involved the development of large-scale farming system with extensive use of chemical weed control justified further surveys in 1969-70 and in 1987-88. In order to follow the great changes from 1988, another survey was conducted in 1996-97. These three surveys were sponsored by the Department for Plant Protection and Agro-environment of the Ministry of Agriculture, and its predecessors. The actual survey work was personally supervised by Dr. Ujvárosi in 1969-70, with assessments made for all the surveys by the weed specialists working at the regional plant health and soil conservation stations and trained by himself. The method used was uniform. It is intended to repeat these national surveys at no more than 20 year intervals.

MATERIALS AND METHODS

Two main types of the weed populations establish in areas with regular soil cultivation: the weeds of cereals and hoed crops. In Hungary there are typified by weeds of winter wheat and of maize respectively. Survey sites and numbers were chosen to represent the landscapes and soil types important for arable growing according to their national significance. .
A total of 202 survey sites were assessed in 17 different types of soils and sub-soils (Figure 1). At each site there were five wheat fields and five maize fields next to each other. Then, 2 detection points/field were designated, without herbicide application. Assessments were made with the Balázs-Ujvárosi method on 5x5 m squares and around them, twice in a growing period (in wheat the second survey was made on stubble).
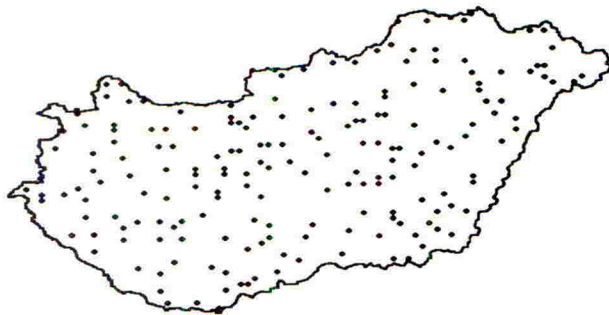

Figure 1. Locations of the national weed survey in Hungary

RESULTS

Data of the first two surveys was processed by hand, while those of the third and fourth ones by computer. In each case, 25 summary tables were first made, followed by detailed analysis. For the latest survey only preliminary results are available.

Up to survey 3 in 1987-88, the trend was for fewer weed species to be detected but in 1996, an increase was observed (Table 1).

Table 1.   Number of weed species in wheat found at five different localities in survey at different times

Locality	Number of species			
	1949-50	1969-70	1987-88	1996
UJRÓNAFŐ	66	55	28	29
JÁSZAPÁTI	82	78	54	73
HOTTÓ	75	75	53	111
ŐRISZENTPÉTER	114	92	30	77
JÁSZBERÉNY	95	101	24	56

In the most recent survey, there are 8 weed species among the first 25 species which significantly increased in importance, and 6 species the predominance of which decreased (Table 2).

The proportion of perennial monocots and dicots in the total weed cover decreased greatly up to 1969-70, while since 1988 the proportion increased near to that observed in the first survey (Table 3).

Table 3. Proportion of wheat fields containing particular weeds or any weeds at different times

1949-50	1969-70	1987-88	1996
1. P 48.9 %	A 75.15 %	A 80.2%	A 78.28%
2. A 48.0 %	P 21.45 %	P 16.8%	P 20.62%
Fields containing weeds 96.9 %	96.6 %	97.0 %	99.0%

where   A   are annual weeds
          P   are perennial weeds

Table 2. Weed species in wheat fields in Hungary in order of their occurence in 1987-88

Weed (species)	1949-50		1969-70		1987-88		1996	
	rank	occurence %	rank	occurence %	rank	occurence %	rank	occurence %
Ambrosia elatior	21	0.39	8	0.87	4	2.57	1	5.37
Echinchloa crus-galli	9	0.86	1	3.73	1	4.42	2	3.9
Amaranthus retroflexus	17	0.51	5	1.47	3	3.06	3	3.63
Chenopodium album	3	1.53	3	2.06	2	3.08	4	2.8
Cirsium arvense	2	2.00	7	1.12	8	0.71	5	1.81
Convolvulus arvensis	1	7.93	2	2.51	5	1.94	6	1.66
Matricaria inodora	66	0.07	26	0.23	6	1.30	7	1.49
Datura stramonium	179	0.01	59	0.06	19	0.38	8	1.10
Amaranthus chlorostachys	105	0.02	18	0.39	13	0.57	9	0.94
Sorghum halepense			94	0.03	18	0.40	10	0.78
Galium aparine	138	0.01	50	0.09	12	0.59	11	0.73
Elymus repens	27	0.28	12	0.51	20	0.38	12	0.65
Helianthus annuus	206	0.00	119	0.01	16	0.42	13	0.59
Bilderdykia convolvulus	14	0.71	6	1.14	11	0.60	14	0.58
Panicum miliaceum	199	0.00	192	0.00	23	0.29	15	0.56
Xanthium strumarium	130	0.01	113	0.01	24	0.27	16	0.55
Polygonum lapathifolium	29	0.25	16	0.40	10	0.60	17	0.53
Setaria glauca	7	1.11	4	1.95	7	0.72	18	0.50
Sinapis arvensis	22	0.37	13	0.48	9	0.63	19	0.47
Apera spica-venti	56	0.08	36	0.14	14	0.46	20	0.47
Hibiscus trionum	25	0.30	11	0.51	17	0.41	21	0.41
Papaver rhoeas	24	0.35	21	0.32	15	0.43	22	0.32
Chenopodium hybridum	60	0.07	53	0.08	33	0.18	23	0.32
Stachys annua	13	0.74	9	0.82	31	0.19	24	0.32

From the early 1970s up to the end of the 1980s, chemical weed control had been very intensive on arable crops, and it might have been expected that total weed cover would decrease throughout Hungary. However, no such decline was observed (Table 2). This value was 4.5 between 1988 and 1996. The ground cover of weeds appears also to have changed little over time (Table 4).

Table 4. Weed ground cover in wheat fields at different times

Years of survey	Total weed cover %
1949-1950	24.9
1969-1970	25.4
1987-1988	24.5
1996	29.1

It was found that volunteer rye (*Secale* cereale) has totally disappeared from the wheat fields, while volunter sunflower (*Helianthus annuus*) has spread considerably (Fig. 2.).

Figure 2.
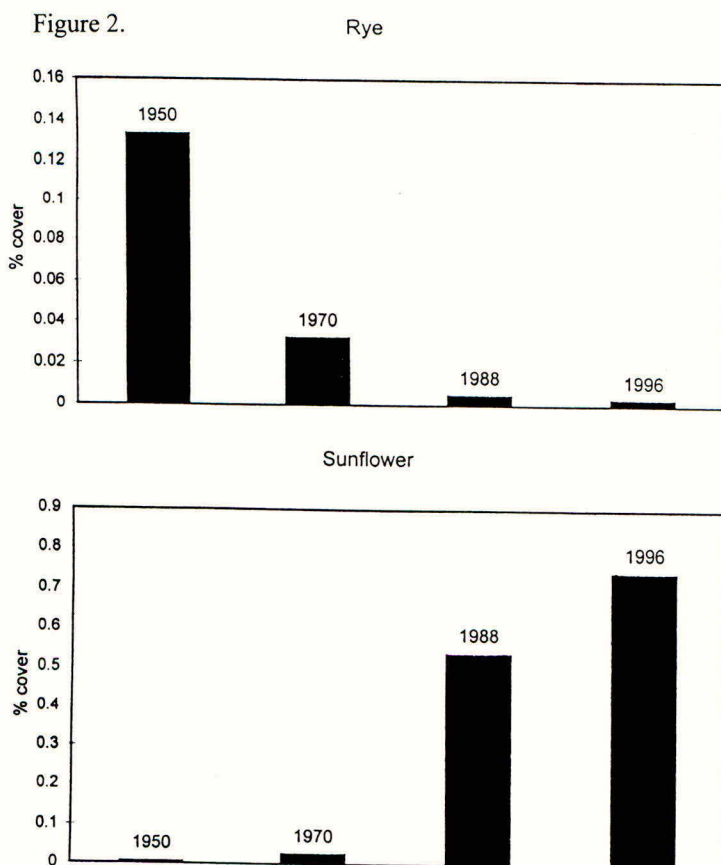
Briefly it can be summarized that the obtained data and results are valuable and can be directly and safely used by the scientists, the specialists and the practical plant protection experts for their work.

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WEED SEED CONTAMINANTS IN CEREAL SEED

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ABSTRACT

The incidence of weed seeds in samples of certified cereal seed tested at the Official Seed Testing Station for Scotland over the last decade is discussed and compared with data from previous surveys and those from tests of pre-certification seed in 1996/7. Contamination of certified seed is now at very low levels (0.38 seeds per kilogram of certified barley in 1996/7). Legislation and seed cleaning have had a major influence on the overall size of weed seed populations in cereal seed and on the component species of these populations. Significant numbers of weed seeds are however present in pre-certified seed, and the use of uncleaned farm-saved seed could be an important agency for the spread of weeds.

INTRODUCTION

In the UK, laws to protect the users of seed date back to the *Adulteration of Seeds Act* of 1869. This early legislation was inspired by investigations that showed that much of the seed sold to farmers was of poor quality, either fraudulently or due to ignorance. The testing of seed and the control of its trade was not compulsory until the introduction of the 1917 *Testing of Seeds Order* and many of its provisions were embodied in the 1920 *Seeds Act* and 1922 *Seeds Regulations*. The main objective of this legislation was to prevent the sale of seed of low viability. Its secondary aim was to prevent the sale of seeds containing large quantities of injurious weed seeds (more than 5%). The 1922 Regulations did not require a declaration of analytical purity to be made for seed of cereals. In a review of the legislation in 1949/50, by the Horne Committee (Anon., 1950), weeds were considered from three points of view: (i) those which are particularly noxious and might be prohibited entirely; (ii) other noxious weeds more generally distributed, the presence of which should be declared to the purchaser; and (iii) common but not particularly noxious weeds, difficult in some cases to clean out.

Horne report recommendations were the framework for the 1961 *Seeds Regulations* which for for cereals required a declaration of: analytical purity; the percentage by weight of all weed seeds when this exceeded 0.5%; and the number of the injurious weeds (*Elytrigia repens*, *Alopecurus myosuroides*, wild oat (*Avena fatua, A. ludoviciana* and *A. sterilis*) and *R. crispus*), in a 200g sample. UK seeds legislation required seed to be officially tested and the buyer given written information on its quality. There were few limits on the quality that could not be sold but, on entry to the EC in 1973, a more protective system was adopted through the 1964 *Plant Varieties and Seeds Act* - implemented with respect to seed in the 1973 *Seeds Regulations*. These regulations set standards for various aspects of seed quality and made it an offence to sell seed that did not meet them. For other seed contaminants the standards required

were much more stringent than those of pre-EC legislation and current standards as detailed in the *Cereal Seed Regulations 1993 & Amendment Regulations 1995* are outlined in Table 1.

Table 1: Maximum number of other seeds permitted by current Seed Regulations

Seed Category	Sample size	All other species	Other cultivated cereal species	All species other than cultivated cereals	*Avena fatua Avena sterilis, Avena ludoviciana* (wild oats) or *Lolium temulentum*	*Raphanus raphanistrum* or *Agrostemma githago*	*Raphanus raphanistrum* or *Agrostemma githago* or *Bromus sterilis* or *Elytrigia repens*
Minimum Standard							
Basic Seed	500g	4	1	3	0	1	not applicable
1st Generation	500g	10	7	7	0	3	not applicable
2nd Generation	500g	10	7	7	0	3	not applicable
Higher Voluntary Standard							
Basic Seed	1000g	1	0	1	0	0	not applicable
1st Generation	1000g	2	1	1	0	not applicable	1
2nd Generation	1000g	4	3	2	0	not applicable	1

Whereas there have been a number of studies on the effect of the 1920 *Seeds Act*, and its regulations of 1922 and 1961, on the quantities of weeds in seeds samples (Broad, 1952; Gooch, 1963; MacKay, 1964; Elliott and Attwood, 1970), there is little information available on the impact of EC legislation.

MATERIALS AND METHODS

The present survey gives details of the occurrence of other seeds in samples of certified cereal seed, tested at the Official Seed Testing Station for Scotland, over the last decade. In addition results from testing barley seed samples taken before and after seed cleaning and certification in 1996/7 are presented. Seed samples were tested in accordance with the International Seed Testing Association Rules (Anon., 1997). The findings are compared to those of previous surveys and evidence provided to the Horne committee (unpublished).

RESULTS

A total of 44 other species were found in samples of certified cereal seed tested in the three seasons: 1986/7; 1991/2; and 1996/7 (Table 2). Overall the levels of contamination were very low with an average of less than one other seed found in each sample search of at least 25,000 seed. The number of species found was greatest in barley (43) and lowest in oats (13) even although oats had the lowest proportion of samples that were free of other species. "Other cereal seed" was the most frequent contaminant (figure 1), particularly in oat samples where the proportion containing "other cereal seed" was greater than 70%. Of species other than cereals, *E. repens* was most common in barley and oats, *P. convolvulus* in oats and *G. aparine* in wheat. Overall the proportion of cereal samples contaminated with *E. repens* increased over the survey period.
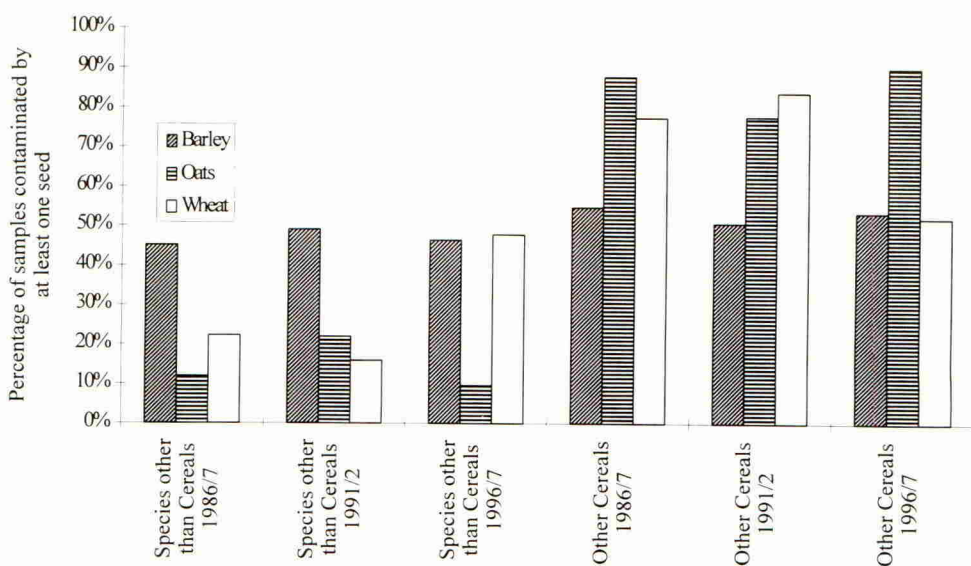
Figure 1 Relative proportions of seeds of "other cereals" and seeds of "species other than cereals" in samples of certified cereal seeds tested during 1986/7, 1991/2 and 1996/7.
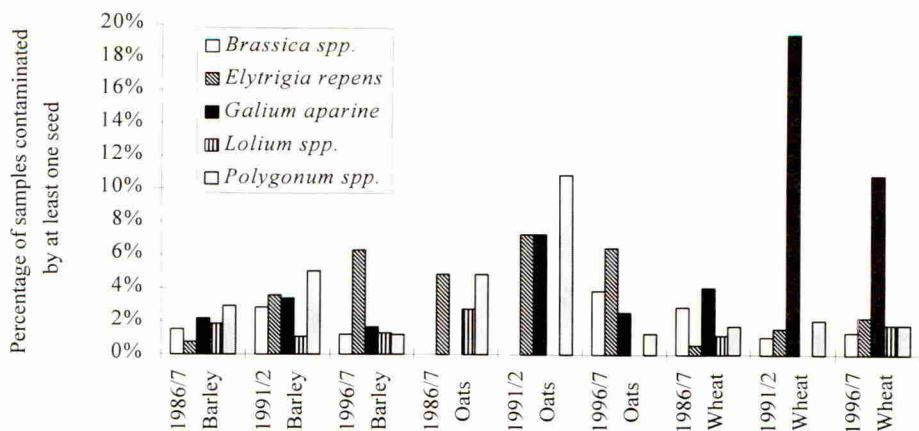

Figure 2 The occurrence of the most common weed seeds in samples of certified cereal seed

Whereas certification seed samples were relatively free of seed of other species, the same was not true of samples submitted for analytical purity testing before seed cleaning and certification (pre-certification samples) (table 3). Although the weight of pre-certification samples examined is 880g less than that of certification samples the average content of other seeds was about 20 times greater. Except for *Anchusa arvensis*, *Festuca* spp., *Raphanus raphanistrum*, *Sinapis arvensis*, *Veronica persica* and *Vicia sativa*, all species found in 1996/7 certification barley samples were found in 1996/7 pre-certification samples. Seventeen species

not found in certified seed were contaminants of pre-certification samples including: *Agrostis* spp., *Anthriscus sylvestris, Apera spica-venti, Atriplex patula, Avena ludoviciana, Bromus mollis, Bromus sterilis, Cerastium vulgatum, Chamomilla* spp., *Dactylis glomerata, Erodium cicutarium, Plantago major, Prunella* spp., *Senecio jacobea, Sonchus arvensis, Sonchus asper* and *Veronica hederifolia.*

Table 2 Frequency of occurrence of one or more seed of other species in 100g certified cereal seed samples.

OTHER SEED	BARLEY			OATS			WHEAT		
	1986/7	*1991/2*	*1996/7*	*1986/7*	*1991/2*	*1996/7*	*1986/7*	*1991/2*	*1996/7*
Anchusa arvensis	0.08%	-	0.09%	0.69%	-	-	-	-	-
Arrhenatherum elatius	-	0.18%	0.09%	-	-	-	-	-	-
Atriplex patula	-	0.18%	-	-	-	-	-	-	-
Avena fatua	0.17%	-	-	-	-	1.28%	-	-	-
Avena sativa	1.78%	1.07%	1.56%	n/a	n/a	n/a	1.16%	0.53%	0.45%
Brassica spp.	1.53%	2.86%	1.20%	-	-	3.85%	2.89%	1.05%	1.36%
Bromus sterilis	-	0.18%	0.18%	-	-	-	-	-	-
Capsella bursa-pastoris	0.08%	-	-	-	-	-	-	-	0.45%
Carex spp.	0.08%	-	-	-	-	-	-	-	-
Cerastium tomentosum	-	0.18%	-	-	-	-	-	-	-
Chenopodium album	-	1.07%	0.18%	-	-	-	-	0.53%	2.71%
Cirsium vulgare	-	-	0.09%	-	-	-	-	-	-
Cynosurus cristatus	-	-	0.09%	-	-	-	-	-	-
Dactylis glomerata	0.34%	-	-	-	-	-	-	-	-
Daucus carota	0.08%	-	-	-	-	-	-	-	-
Echium vulgare	0.08%	-	-	-	-	-	-	-	-
Elytrigia repens	0.76%	3.58%	6.26%	4.83%	7.23%	6.41%	0.58%	1.58%	2.26%
Festuca spp.	-	-	0.18%	-	-	-	-	-	-
Fumaria officinalis	0.25%	1.07%	-	-	-	-	-	-	-
Galeopsis tetrahit	0.17%	0.36%	0.37%	-	-	-	-	-	-
Galium aparine	2.20%	3.40%	1.66%	-	7.23%	2.56%	4.05%	19.47%	10.86%
Juncus spp.	0.08%	-	-	-	-	-	-	-	-
Hordeum vulgare	n/a	n/a	n/a	60.69%	46.99%	50.00%	27.17%	36.84%	26.24%
Lolium spp.	1.86%	1.07%	1.29%	2.76%	-	-	1.16%	-	1.81%
Matricaria perforata	2.12%	0.18%	-	-	-	-	-	0.53%	-
Myosotis arvensis	-	0.18%	-	-	-	-	-	-	-
Phleum spp.	0.17%	0.36%	-	-	1.20%	-	-	-	-
Pisum sativum	0.51%	-	-	-	-	-	-	0.53%	-
Poa annua	-	-	0.09%	-	-	-	-	-	-
Polygonum aviculare	1.61%	1.61%	0.83%	2.76%	-	-	0.58%	1.05%	1.36%
Polygonum convolvulus	0.59%	3.04%	0.37%	0.69%	9.64%	1.28%	1.16%	0.53%	0.45%
Polygonum persicaria	0.76%	0.36%	-	1.38%	1.20%	-	-	0.53%	-
Prunella vulgaris	0.08%	-	-	-	-	-	-	-	-
Raphanus raphanistrum	-	-	0.09%	-	-	-	-	-	-
Rumex obtusifolius	-	-	0.09%	-	-	-	-	-	-
Sinapis alba	0.08%	-	-	-	-	-	-	-	-
Sinapis arvensis	-	-	0.09%	-	-	-	0.58%	-	-
Spergula arvensis	0.08%	-	-	-	-	-	0.58%	-	-
Stellaria media	0.59%	0.36%	0.18%	-	-	-	0.58%	-	0.45%
Trifolium repens	0.08%	-	-	-	-	-	-	-	-
Triticum aestivum	19.66%	18.60%	17.11%	19.31%	10.84%	25.64%	n/a	n/a	n/a
Veronica hederifolia	0.08%	-	-	-	-	-	-	-	-
Vicia sativa	-	0.18%	0.09%	0.69%	-	-	-	-	-
Viola spp.	0.08%	0.36%	0.18%	-	-	-	-	-	-
No of samples tested	1180	559	1087	145	83	78	173	190	221
Total number of seeds found	974	543	648	276	168	117	130	502	161
Percentage of clean samples	71%	67%	72%	30%	40%	27%	64%	49%	62%
Samples with other seeds	345	186	304	102	49	57	62	96	84

- Species not found

Table 3 Number and frequency of occurrence of seeds of other species in certification and pre-certification samples of barley seed in 1996/7

Species	1087 x 1000 g Certified Seed Samples							254 x 120 g Pre-Certification Seed Samples						
	Total	Mean	Min.	Max.	Range	sd	% Occurrence	Total	Mean	Min.	Max.	Range	sd	% Occurrence
Alopecurus geniculatus	0	0	0	0	0	0	0%	8	0.0315	0	3	3	0.2649	1.57%
Arrhenatherum elatius	1	0.0009	0	1	1	0.0303	0.09%	12	0.0472	0	8	8	0.5163	1.97%
Avena fatua	0	0	0	0	0	0	0%	14	0.0551	0	10	10	0.6571	1.18%
Avena sativa	32	0.0294	0	12	12	0.3992	1.56%	12	0.0472	0	4	4	0.3411	2.76%
Brassica spp.	28	0.0258	0	6	6	0.3024	1.20%	36	0.1417	0	7	7	0.7026	6.69%
Capsella bursa-pastoris	0	0	0	0	0	0	0%	11	0.0433	0	5	5	0.3902	1.57%
Chenopodium album	5	0.0046	0	4	4	0.1250	0.18%	72	0.2835	0	43	43	2.7343	7.09%
Chrysanthemum segetum	0	0	0	0	0	0	0%	123	0.4843	0	120	120	7.5295	1.57%
Cirsium arvense	0	0	0	0	0	0	0%	7	0.0276	0	2	2	0.1866	2.36%
Elytrigia repens	134	0.1233	1	10	9	0.6505	6.26%	516	2.0315	0	61	61	6.5293	31.50%
Fumaria officinalis	0	0	0	0	0	0	0%	43	0.1693	0	19	19	1.3655	3.94%
Galeopsis tetrahit	5	0.0046	0	2	2	0.0802	0.37%	73	0.2874	0	32	32	2.3066	5.12%
Galium aparine	24	0.0221	0	4	4	0.1954	1.66%	38	0.1496	0	6	6	0.6722	7.09%
Holcus lanatus	0	0	0	0	0	0	0%	6	0.0236	0	4	4	0.2657	1.18%
Lolium spp.	14	0.0129	0	1	1	0.1128	1.29%	500	1.9685	0	146	146	12.2906	15.75%
Matricaria perforata	0	0	0	0	0	0	0%	11	0.0433	0	4	4	0.3694	1.97%
Myosotis arvensis	0	0	0	0	0	0	0%	8	0.0315	0	3	3	0.2495	1.97%
Phleum pratense	0	0	0	0	0	0	0%	19	0.0748	0	4	4	0.4150	3.94%
Poa annua	1	0.0009	0	1	1	0.0303	0.09%	473	1.8622	0	147	147	12.8605	16.14%
Poa trivialis	0	0	0	0	0	0	0%	12	0.0472	0	7	7	0.4847	1.57%
Polygonum aviculare	21	0.0193	0	6	6	0.2585	0.83%	324	1.2756	0	120	120	7.8947	24.02%
Polygonum convolvulus	6	0.0055	0	2	2	0.0958	0.37%	151	0.5945	0	134	134	8.4295	2.76%
Polygonum lapathifolium	0	0	0	0	0	0	0%	7	0.0276	0	3	3	0.2577	1.18%
Polygonum persicaria	0	0	0	0	0	0	0%	70	0.2756	0	21	21	1.6878	7.87%
Rumex crispus	0	0	0	0	0	0	0%	9	0.0354	0	1	1	0.1852	3.54%
Stellaria media	2	0.0018	0	1	1	0.0429	0.18%	104	0.4094	0	22	22	1.8750	11.42%
Triticum aestivum	336	0.3091	0	22	22	1.0477	17.11%	59	0.2323	0	14	14	1.3735	7.09%
Viola spp.	33	0.0304	0	31	31	0.9422	0.18%	61	0.2402	0	24	24	1.9114	3.94%
Other species*	13	0.0120	0	1	1	n/a	1.20%	168	0.6614	0	120	120	n/a	10.24%
Total seeds of other species	648	0.5961	-	-	-	-	-	2947	11.6024	-	-	-	-	-
Clean samples	783	-	-	-	-	-	72.03%	82	-	-	-	-	-	32.28%

* The occurrence of individual species that make up this group was less than 1% in pre-certification samples
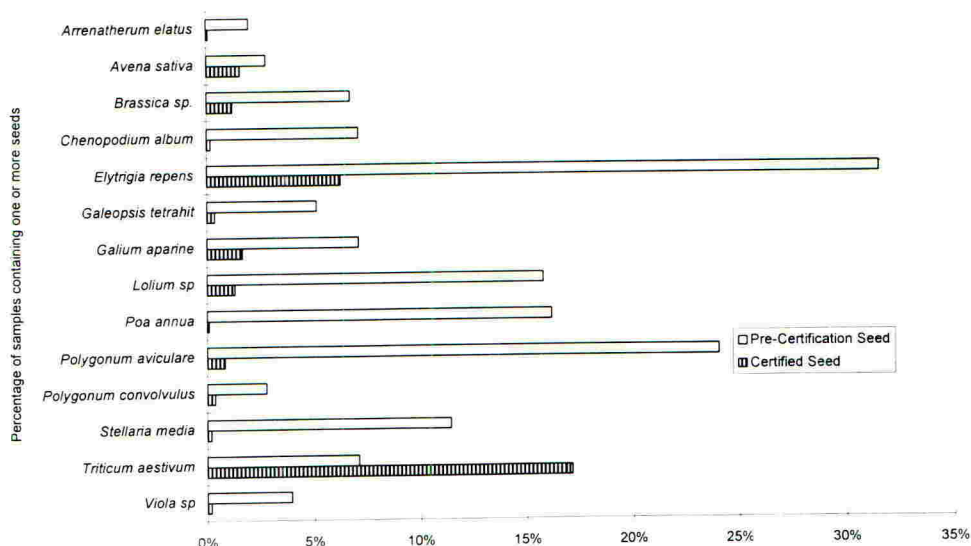
Figure 3 The occurrence of the most common weed seeds in certification and pre-certification samples of barley seeds in 1996/7.

Whereas over 70% of certification samples were free from contamination by seed of other species, 68% of pre-certification samples were contaminated. Except for *Triticum aestivum* the occurrence of individual species was higher in pre-certification samples (figure 3). The difference in occurrence varied and was least in *A. sativa* and *E. repens* (two and five times less respectively) and greatest in *Poa annua* (175 times less).

DISCUSSION

Comparison of the results of this survey with previous surveys is difficult. Previous surveys did not distinguish between different categories of certified, pre-certified and farm-saved seed. Sample sizes varied from as low as 50g, (samples tested before the introduction of the 1961 *Seeds Regulations*), to 3000g, (samples tested in a survey of wild oat contamination of cereal seed in 1970). Furthermore, most of the results of previous surveys are expressed in terms of frequency of occurrence of individual species, a measure that does not permit a quantitative assessment of the overall levels of contamination. Nevertheless, clearly, weed contamination of cereal seed lots has steadily decreased since the 1920's. Weed seed levels in today's certified cereal seed are at much lower level than those reported in the surveys of Broad (1952) and Gooch (1963). Except for *E. repens* the occurrence of weed species in barley seeds is at its lowest in 1996/7 certified seed (figure 4) and overall contamination with weeds was less than 0.38 seeds per sample. On the other hand the frequency of weed occurrence in 1996/7 pre-certification seed is similar to that found in previous surveys.

There have been major changes in the species composition of the weed seed population. Besides the increase in *E.repens*, there is the appearance of *P.annua* and *Stellaria media* as significant components of the weed seed population in this survey. In previous surveys

P. annua was not recorded as a weed of cereals and the only report of *S. media* was at levels of occurrence of less than 1% in 1960/1 cereal seed. *Polygonum* spp. were recorded in all surveys but since 1960/1 there has been a dramatic change in component species. *P. convolvulus*, with an occurrence greater than 20%, was the major component in previous surveys with other *Polygonum* species present at levels less than 5%. In the present survey, the level of occurrence of *P. convolvulus* was only 2.8%, in pre-certification barley seed, with *P. aviculare* being the most frequently occurring species at a level of 24%.
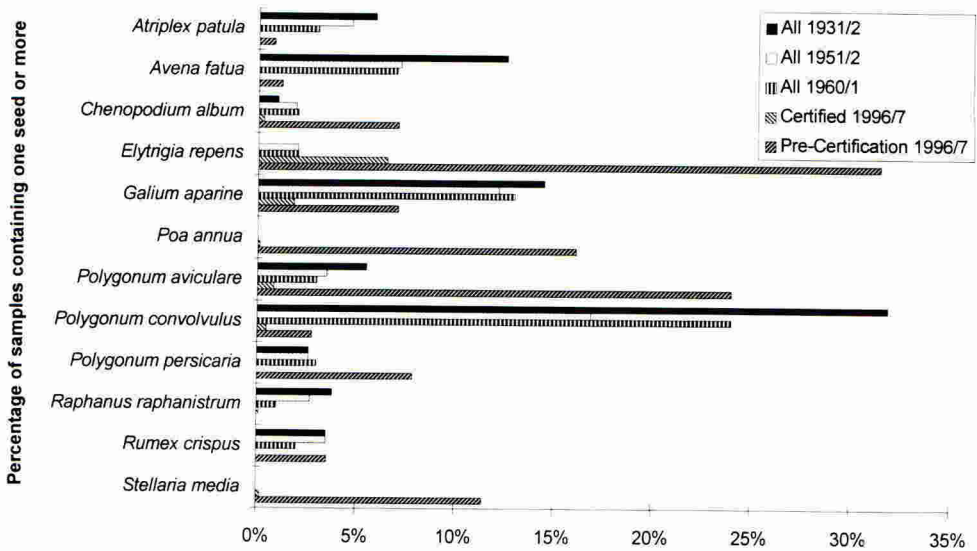
Figure 4 The occurrence of the most common weed seeds in samples of barley seed

Changes in the seeds' legislation have driven the improvement in seed quality. The exceptionally low level of weeds in today's certified cereal seed is a result of the standards set in the Seeds Regulations. These regulations prohibit the sale of seed with even comparatively low levels of weeds (see table 1). The success of legislative control is clear when on examines the level of wild oat contamination (table 4). Before the 1961 Seeds Regulations, which required a statutory declaration of wild oat levels in cereal seed, contamination of seed was widespread and thought to be the most common method of spread of these weeds. The introduction of the 1961 *Seeds Regulations* was marked by a reduction in levels of contamination. The 1974 *Seeds Regulations*, which prevented the sale of cereal seed with more than one wild oat in 500g, has contributed to the further lowering of levels of this weeds group (*A. fatua*, *A. ludoviciana* and *A. sterilis*).

Legislation has not been as successful in the control of *E. repens*, whose incidence has increased over recent years. The main reason is that this weed is perennial and once established the production of rhizomes is prolific. The sowing of cereal seed contaminated with *E. repens* may lead to the introduction of the weed to new areas and, once established, seed production is prolific and cultural control under continuous cereals is difficult.

The difference in occurrence of weeds between pre-certification and certification seed is a measure of the effectiveness of modern seed cleaning equipment. This equipment effectively reduces weed contamination to negligible levels. The prevalence of other cereal seed as the major contaminant of certified cereal seed is a result of difficulties in removing seed of similar physical characteristics.

Table 4   Frequency of occurrence of wild oats in cereal samples

	Percentage containing at least one seed in 50g						
	1928/9	1930/1	1934/5	1943/4	1947/8	1951/52	1960/61
Wheat	5	3	2	1	2	2	4
Barley	9	13	4	3	4	7	7
Oats	5	4	3	3	3	2	4

	Percentage containing at least one seed in 200g			Percentage containing at least one seed in 3,000g
	1961/2	1962/3	1963/4	1969/70
Wheat	2	4	5	9
Barley	7	8	10	18
Oats	5	5	6	9

	Percentage of certified seed samples containing at least one seed in 1,000g		
	1986/7	1991/2	1996/7
Wheat	0.2	0	0
Barley	0	0	0
Oats	0	0	1.3

Whereas seed was considered the most important agency for the introduction of weeds in the 1960's (Salisbury, 1961; Wellington, 1960) today's certified seed gives growers a guarantee of high quality and low levels of contamination. Weeds are however present in pre-certified seed and the use of uncleaned farm-saved seed could be an important agency for the spread of weeds within a farm enterprise. More data is required on the occurrence of weeds in seed drills before definitive conclusions can be draw on the significance of weed seed as a contaminant of today's cereal seed.

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SESSION 4B

ENVIRONMENTAL IMPACT OF GENETICALLY MODIFIED CROPS

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Session Organiser DR J B SWEET
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Papers   4B-1 to 4B-4

TRANSGENES FOR STRESS TOLERANCE: CONSEQUENCES FOR WEED EVOLUTION

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ABSTRACT

Stress is often considered only in terms of physical pressures such as drought or heat. In reality, parasites, pests or pathogens are also natural constraints on plant performance. The commercialisation of transgenic stress tolerance genes may add fuel to natural processes of plant evolution with outcomes that are difficult to predict. This paper discusses some of the factors which direct the structure of natural plant communities and assesses the role which stress tolerance plays in the survival of individual plant genotypes in competition. A case is made for increased collaboration between "biotechnologists" and ecologists to more effectively assemble qualitative data on the genetic diversity and density of crop relatives in anticipation of transgene introgression from cultivated varieties containing traits for stress tolerance.

INTRODUCTION

Commercialisation of crops containing transgenes for stress tolerance will inevitably lead to generalised dispersal of these genes *via* crop-wild relative hybrids or crop escapes. It is unlikely that escape of stress tolerance transgenes will produce new pernicious weeds with an immediately obvious impact on natural or semi-natural habitats. However it is possible that stress tolerance genes may release plants from environmental constraints, resulting in genotypes that out compete less competitive conspecifics. While resultant changes in community structure may be subtle, there is an increasing recognition that our ignorance of ecosystem function requires that current levels of diversity should not be thoughtlessly altered. As genetically modified (GM) stress tolerant crops approach commercialisation, there is heightened interest in the role of stresses in plant population dynamics. In this review we have drawn upon scattered experience to highlight some salient issues. Consequences of increased stress tolerance in natural populations cannot readily be predicted because of complex, largely uncharacterised, but nevertheless crucial interactions between plants and their neighbours. This paper outlines the extent to which stresses limit crop production and the genetic modification strategies available to reduce such yield losses. We also examine the limits of our knowledge of how these stresses drive the population dynamics of natural communities. We place particular emphasis on the effects of viruses, because the use of viral transgenes for virus tolerance has great potential to trigger rapid and significant co-evolutionary change.

CONSTRAINTS ON PLANT PERFORMANCE: POTENTIAL, ATTAINABLE AND ACTUAL YIELDS

For a given crop, <u>potential</u> plant production is the productivity of the most efficient conceivable genotype under (theoretically) optimal environmental conditions. <u>Attainable</u> production, which approximates to record crop yields is limited by environmental reallities such as soil structure and nutriment availability. Fertiliser "out of the bag" has been a routine yield-increasing measure in intensive farming systems, that is now being seen by many to be unsustainable with a tendency to cause environmental harm such as eutrophication of surface waters. With this realisation, plant breeding is tending to focus more on the development of crops with improved tolerance of yield-constraining abiotic stresses rather than the fertiliser responsiveness which drove "The Green Revolution". <u>Actual</u> production is generally about one quarter of the "attainable " values (Boyer, 1982). The shortfall is a result of biotic stresses (competition with other plants, pests and pathogens), plus a component attributable to non-optimal physical conditions (for example, drought/ inundation, salinity, pollution/ toxicity) that can be defined as abiotic stresses. The primary and secondary gene pools of some crop species naturally possess a diversity of stress tolerance traits (see Raybould, 1995 for a review) but this "natural" diversity is assumed to be limited and decreasing as populations of land races and wild relatives become extinct. *In vitro* genetic "engineering", on the other hand, offers access to an almost unlimited pool of genes for stress tolerance from other plant species and organisms.

GM ABIOTIC STRESS TOLERANCE

In OECD countries before 3/12/96, there had been about 50 controlled (experimental) field releases of GM crop varieties with transgenes for some kind of abiotic stress tolerance. A diverse range of mechanisms has been assessed, but it is appropriate here only to highlight a few of these. One example is the development of cold-tolerant / frost-hardy plants that have the potential to grow in regions from which they are presently excluded by the climate. GM plants with enhanced osmotolerance (including drought tolerance) potentially have the ability to establish in soil poisoned with sodium chloride (a common side-effect of irrigation or deforestation). It is also noteworthy that the ultimate effect of many abiotic stresses is oxidative damage to plant cells. Therefore modifications for oxidative stress tolerance have the potential for a wide range of pleiotropic effects. Metal tolerance is a potentially lucrative market for GM plants. Phytoremediation is a relatively cheap and publicly acceptable means of cleaning land contaminated with heavy metals. A number of species, especially crucifers such as *Brassica juncea* and *Thlapsi caerulescens*, naturally hyperaccumulate heavy metals. Crops such as oilseed rape have been modified with mammalian metallothionine genes to the same purpose (e.g. Salt *et al.*, 1995). The metals can be recovered by incinerating the plants after harvest. Aluminium tolerance is also an important attribute as about one third of the world's agricultural soil contains phytotoxic concentrations of aluminium. Transgenic plants expressing bacterial genes for citrate production have recently been shown to enhance aluminium tolerance (De la Fuente *et al.* 1997).

GM HERBICIDE TOLERANCE

Weeds are a major source of biotic stress to crops and herbicide tolerant plants offer novel strategies for weed control. In OECD countries up to December, 1996, there had been nearly 1,800 (experimental) releases of herbicide tolerant crops and a few of these genotypes have

entered commerce [eg.RoundUp Ready ® soybean (*Glycine max*)]. There is a growing dependence on herbicides in arable crop rotations and this pressure has inevitably selected plant biotypes with resistance to more than one herbicide class. The process is now being fuelled because a number of crops are being tailored to specific niches. For example, herbicide tolerant GM lupins permit the use of non-selective herbicides in herbicide-tolerant grass communities of Western Australia, GM upland cotton (*Gossypium hisutum*) is used in rotations with maize (*Zea mays*) and/or soybean in the USA and, in the Canadian prairies, GM herbicide tolerant oilseed rape (*Brassica spp*) and flax (*Linum usitatissimum*) have niches in rotations with cereals. It can, of course, be argued that herbicide tolerant plants will not have any competitive advantage in natural communities not exposed to the specific herbicide, and there is no doubt that usage of herbicides can be regulated and (by agreement) restricted to specific farming systems. As a consequence harm may be manageable. Nevertheless, herbicide tolerance may have potentially undesirable effects in non-agricultural habitats. In Western Australia simazine-resistant blue lupins (*Lupinus angustifolius*) are well established along the edges of roads and in disturbed sites adjoining rivers. Currently, phosphinothricin is used to control these ruderals but that option will be unavailable if the transgene for phosphinotricin tolerance (*bar*), currently in GM sweet lupin cultivars, spreads to the feral *L. angustifolius* populations by hybridisation. The species has several properties (bee pollinators - excellent fliers, sticky pollen, 10% outcrossing potential) to facilitate hybridisation. The crop is also likely to be dispersed to road-sides (including areas of natural beauty and in national parks) by seed spillage during transport; much as feral oilseed rape populations became established and are maintained on the edges of motorways around London (Crawley & Brown, 1995).

GM PEST RESISTANCE/ TOLERANCE

Plants grown in dense monocultures are subject to fluctuating but frequently heavy pest pressures (particularly from insects). There are several reasons for this including selective crop breeding for the elimination of natural compounds toxic or distasteful to insects (to make crops palatable as human food); the cultivation of crops in regions where they are not native and where they are exposed to insects against which there has been no opportunity to develop defences; and the use of non-specific pesticides that kill predators of pest species. Up to December 1996, genes coding for toxins derived from *Bacillus thuringiensis* (Bt) were the source for 97% of the 700 releases of GM crops with a specified "obtained" insect tolerance (information on the remaining 403 releases is not available presumably because of commercial sensitivities). Commercialisation is proceeding rapidly. Bt cotton or maize designed to tolerate lepidopterans and Bt potato protected against coleopterans are commercially available in the USA. In 1996, there were 1.8 million ha of Bt cotton in the USA and a further 30,000 ha in Australia. *B. thuringiensis* (as bacteria and spores) had been used for many years in the form of sprays. There are a few other GM approaches which have some promise for specific pest targets (e.g. Schroeder *et* al., 1995, Gatehouse *et al.*, 1996, Gheysen *et al.*, 1996). However it is not possible to generalise about the durability of these approaches in the face of target evolution. Selection for tolerance can be rapid, especially when pest populations have high initial frequencies of resistance alleles (as has recently been shown to be the case for Bt resistance in tobacco budworm; Gould *et al.*, 1997).

GM FUNGAL, BACTERIAL AND VIRAL RESISTANCE

Despite the undoubted economic losses attributable to fungi in crops, there have been only a modest number (142) of releases of GM crops with resistance specifically targeted on these

parasites. Notwithstanding the diverse range of antifungal chemicals which are in the marketplace, none provides a panacea, and fungicide tolerance can evolve quickly. Similarly, there is compelling evidence for rapid turnover of resistance breaking pathotypes/ pathovars which nullify the efforts of traditional plant breeding for resistance to some fungal pathogens. As a consequence, genetic modification may yet have a potentially important role in the management of some fungi [e.g. the pathogen (*Spongospora subterranea f.s. nasturtii*) causing crook root in watercress] for which no effective (non-polluting) treatment is available. Transgenes coding for enzymes catalysing the hydrolysis of major structural components in filamentous fungi seem promising, although one or two alternative approaches are also under test. Presumably because of their perceived relative unimportance in field crops of the affluent "North", bacterial plant parasites have been targets for only 17 experimental releases.

By contrast, viruses are internal parasites which are inaccessible to chemicals and invisible to an unaided eye, although their impact is frequently catastrophic. Conventional breeding and selection has had a few notable and durable successes in the struggle with viruses, but is constrained by the paucity of resistance genes available for use in any species given the burgeoning capacity of resistance-breaking viruses to evolve. Effects of viruses spread between plants by vector fungi or invertebrates are often minimised by targeting the vectors with chemicals, despite the cost and the potential consequences for beneficial non-target species. However, because of anticipated speed to market (given the ease of *in vitro* manipulation and the apparent simplicity and small size of viral genomes), transformation for pathogen-derived virus tolerance was an early focus for research. Over the past decade major structural proteins of a virion's coat have become the standard transgenes for virus tolerance. To December, 1996, there had been 446 R&D releases of genetically modified plants containing one or more capsid coding sequence, each targeting a narrowly defined virus "species". Cucurbits, sugar beet, solanaceous crops and maize have been the most commonly released virus-tolerant GM crops (brassicas and cereals have been assessed on a smaller scale) and potyvirus-tolerant squash (Freedom2 ® *Cucurbita pepo*) has been commercialised in the USA. When deregulating this transgenic squash, the USDA explicitly based its decision on the apparent absence from natural populations of related species of the viruses against which the transgenes "protect", thereby making it unlikely that a "superweed" would be created by hybridisation with a weedy relative currently suppressed by specific pathogen pressure (Kling, 1996). In reality, the absence of presence does not equate with presence of absence and, in any event, the information is both "investigator" and geography specific.

ECOLOGICAL FITNESS; EVOLUTION-COEVOLUTION OF PLANT VIRUSES AND THEIR HOSTS

Compared with the effects of herbivory (e.g. Fritz & Simms, 1992) and fungal disease (e.g. Jarosz & Davelos, 1995), there are very few data on the effects of viruses on natural populations [a rare example is a study of the effects of cucumber mosaic virus on purslane; *Portulaca oleracea*: Friess & Maillet, 1996)], although there is much data from agricultural systems where viruses can have devastating effects (e.g. Cooper, 1995).

The patchiness of natural communities is presumed to facilitate stable coexistence of hosts and viruses which routinely circulate among multiple species/ genera (although, in reality, there are few relevant data to set against the plethora of theoretical arguments). It might be argued that viruses are irrelevant to the wild species which they infect but, extrapolating from sparse data, it is a plausible hypothesis that viruses do eliminate host populations directly or as a result of

synergy or chance. In any event, it is appropriate to address the areas of uncertainty and to review some of the factors which modify plant communities.

Viruses have been associated with a bewildering variety of pathogenic effects. Nevertheless, their stable coexistence in natural communities is thought to depend fundamentally upon the benefits (not the harm) they do to their hosts. Theoreticians have proposed that parasites are substantial selective forces favouring the maintenance of genetic variation by sexual reproduction. Of course, for these effects to be important selectively, viruses must lessen the performance of some infected individuals and should also be a prevalent although not necessarily a uniform force in the natural environment. Data on virus impact on wild plants are not very numerous but it has been recorded that at least some plant genotypes measurably suffer when infected (e.g. *Anthoxanthum* and brome mosaic bromovirus: *Betula* and cherry leaf roll nepovirus: *Eupatorium* and tobacco leaf curl geminivirus: Cooper, 1997, Friess & Maillet, 1996).

Genetic variation affecting biotic interactions is often not considered in studies of plant dynamics. However, plant genes which (naturally) modify the rate of virus invasion and the outcome of infections in plants are known (indeed one has been cloned and sequenced). Similarly, plant genes for fungicidal chemicals (e.g. stilbenes) or for traits conferring tolerance to specific herbicides or insect feeding (e.g. lectins and protease inhibitors) are being exploited for the protection of transgenic crops. There are essentially no numerical data on the prevalence of these or similar genes in wild populations. Consequently, it is not possible to infer their impact on the structure of natural communities, although it is widely accepted that insect dynamics are modulated by a very subtle interaction of attractants/ repellent/ antifeedant compounds (e.g. Fritz and Simms, 1992).

Even though direct evidence from field studies is only available in one instance (Friess & Maillet, 1996), the experience of plant breeders seeking to exploit natural biodiversity in wild relatives of crop plants suggests that viruses probably do direct changes in plant populations. In regions where specific viruses have been endemic for centuries, the local crop cultivars tend to show much less severe symptoms when infected than more modern cultivars selected elsewhere. Two factors are worth mentioning in this context; one general and the other virus-specific. A variety of simply inherited "major" genes and a few multigene families have been brought into commercial cultivars from wild species and the scale of searches for these traits gives a crude measure of their abundance in natural communities. As outlined by Cooper (1997), virus resistance/ tolerance traits are very rare or absent in the wild relatives of the economic crops. For example, more than 300 tobacco varieties and *Nicotiana* species were tested in a vain search for resistance to cucumber mosaic cucumovirus and, in spite of an extensive search within the genus *Beta*, no source of inherited immunity to beet luteoviruses was found. Such experiences are difficult to put into perspective. Numerical data relating to field exposure of wild plants to viruses are not widely reported and information concerning, for example, the reaction of one cereal species to barley yellow dwarf luteovirus (which is in any event a very heterogeneous and plastic genetic entity) might not reflect the situation in another grass species.

Furthermore, with viruses, there is an additional "background" factor that may be important in contributing to the prevalence of specific virus tolerance. This is not attributable to plant genotypes, but to background levels of infection by specific viruses singly or in combination. When plants are infected by viruses, some individuals are protected against later infection by

more severely damaging isolates of the same virus. The epidemiological consequences have not been the subject of detailed investigation, but circumstantial evidence suggests that in at least one instance a "cross protecting" virus has escaped from plants into which it is deliberately introduced and that such "mild" isolates are now tending to diminish disease severity in surrounding crops.

It is important to realise that cross protection characteristically diminishes severity of disease but does not prevent virus replication and may indeed facilitate virus evolution. Significantly, field use of "mild" isolates near genetically resistant plants has facilitated virus evolution towards enhanced virulence on these resistant products of traditional breeding (Pelham et al., 1970). This lack of durable protection, which is a regrettable feature from a commercial viewpoint, might be encouraging when considering the durability of impact on components of the natural environment. In any event, these background constraints on the progress of viruses within natural communities are liable to be augmented substantially with the deployment of transgenic crop cultivars with viral transgenes. The genetics and physiology of virus tolerance is undoubtedly diverse. Furthermore, there are few data on the durability of stress tolerance traits; in crops, experience suggests that resistance-breaking virus genotypes evolve constantly but do not always spread, despite the selection imposed by a novel crop genotype and its environment.

VIRUSES AND WILD CABBAGES - AN EVOLVING CASE STUDY

To investigate some of the issues discussed above, scientists at Oxford and Furzebrook from NERC's Centre for Ecology and Hydrology are studying the growth and reproduction of natural populations of wild cabbage (Brassica oleracea) in relation to viral infection. The study populations are on the sea cliffs of Dorset, which are remote from horticultural brassica production. Two hundred and eleven plants were assayed among five geographically separated populations. Bioassays revealed the presence of about a dozen virus pathotypes from which four were selected for more detailed study because each is a plausible target for transgenic management in horticultural brassicas. ELISA revealed wide variation among sites in the incidence of all four pathotypes; all four occurred together in five individuals, 159 of the plants contained either three, two or one of the viruses and 47 plants were virus-free. Strikingly, more than 60% of the turnip yellow mosaic virus (TYMV) occurrences were in individual cabbage plants at one site (A.J. Gray, M.L. Edwards, J.I. Cooper & A.F. Raybould, unpublished data), an observation reminiscent of that made by Skotnicki et al. (1993) on TYMV in Cardamine lilacina of Australia's Mt. Kosciusko alpine region. In addition, a field experiment suggests that TYMV infection has significant negative effects on growth of wild cabbage (L.C. Maskell, unpublished data) and greenhouse experiments show genetic variation among seedling progeny from wild parents for ability to limit virus multiplication (M.L. Edwards & J.I. Cooper, unpublished data). Taken at face value, these data suggest that viruses are stress factors capable of constraining the performance and fecundity of infected individuals and thereby effecting genetic change for tolerance of these parasites. To understand the mechanisms and the complexity attributable to interactions among host genotypes (particularly those determining herbivore interactions [Mithen et al., 1995]), viruses and herbivores (including virus vectors) we have embarked on a broad-ranging and long-term analysis of the population dynamics and the genetics of virus resistance in these brassica communities.

CONCLUSION

In natural environments biotic and abiotic stress factors are assumed to decrease plant

performance. Individual plants will suffer and populations may be eliminated by demographic processes after stress-driven debilitation, diminished fecundity, or reduced life span. Being both fecund and mutable, it is likely that parasites and invertebrate pests will force the pace of wild plant evolution, and the outcome of the anticipated change resulting from transgene spread to periagricultural environments cannot presently be predicted. Commercial field crops currently contain plant-derived "resistance" genes. Almost certainly, these genes are more dispersed and prevalent than they were in their wild source plants and no harm to the diversity of natural communities has been recognised to result from their deployment. However, it is not possible to extrapolate and to quantify the consequences which might result from the commercial use of GM stress-tolerance traits. When the transgenes derive from viruses, they are substantially different from the current "natural" resistance / tolerance traits in terms of context and ubiquity. Their presence introduces a substantially new dimension into the dynamics of plant/virus coevolution, even though virus-derived nucleic acids are normal constituents of natural plant populations where they undoubtedly also contribute to the evolution of viruses. Hitherto, virus evolution has been affected by multiple infections constrained, at least in part, by the serendipitous behaviour of vectors. There is a risk that the spread of virus-derived transgenes will eliminate this element of chance as the presence of viral nucleic acid becomes uncoupled from vector behaviour. However, there are few data on the normal levels of multiple infection in any plant.

An observer of natural communities expects to see variation in species abundance. The impact of parasites on plant biodiversity and on the outcome of evolution in natural populations now needs to be addressed; the role played by stress-tolerance in wild plants studied by ecologists must not be neglected. Collaborations between field ecologists and biotechnologists have been lamentably few but there is a now a clear need to survey wild species using the most sensitive molecular tools and to assemble data on the density of susceptible populations, prevalence/ diversity of locally available parasites (big and very small) plus the abundance and activities of bacteria, fungi and invertebrates. Climate and edaphic factors relevant to abiotic stress also need to be assessed on a case by case basis at least for a few "representative" plant species, particularly relatives of crops which are now showing greatest commercial promise. Worldwide, the families which need most immediate consideration include the Brassicaceae (with particular respect to oilseed rape), the Leguminoseae (clover, lupins etc.), the Gramineae, and the Chenopodiacae (sugar beet). In Australia and the USA, the Cucurbitaceae (melons, squashes etc.) must be added but there is a dirth of information concerning a diverse range of woody perennials (e.g. rose relatives and poplars). National and even local differences must be recognised. For example, wild *Solanum* species are abundant and diverse in Australia (57 weedy species), whereas the UK has only 2 (both relatively uncommon). Such factors need to be considered when selecting subjects for investigation.

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THE POSSIBLE IMPACTS OF MULTIPLE TRANSGENES

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ABSTRACT

Multiple transgenes can be introduced intentionally by plant breeding, or unintentionally by pollen transfer from transgenic crops to closely related plant species. A potential consequence of the presence of regions of genetic homology between different transgene constructs is genetic instability. In determining the possible impact of this form of instability, an important research objective is to seek to understand the underlying genetic mechanism. With the deliberate introduction of several transgenes through plant breeding, any transgene instability should be identified during the extensive evaluation and testing that is necessary for the breeding and production of new plant varieties. Genetic instabilities resulting from the transfer of transgenes by pollen into wild and weedy species are most likely to lead to down regulation of particular transgenes or to complete silencing of expression. This would result in the plants reverting to their wild-type phenotype. However, transgene interactions of this kind should be considered carefully during the biosafety assessment process. The introduction of several herbicide tolerance genes into crops is possible, but there are at present commercial and patenting constraints on their development. In the future, when herbicide tolerance genes are free from patent protection, there is a greater likelihood of breeders combining tolerance to different herbicides. Any regulatory approval needs to consider the consequences and possible impacts on the agricultural and wider environment from the presence of several herbicide tolerance genes. It is important now and in the future for the chemical, breeding and agricultural industries to develop strategies for the control of plants containing one or several herbicide tolerance transgenes.

INTRODUCTION

Over the past decade there have been dramatic advances in the production of transgenic plants. Millions of different transgenic lines have been created and thousands of field trials have been carried out to evaluate a wide range of genetically modified crops (Dale, 1995). We are now seeing the commercialisation of crops in North America, Europe and in other parts of the world. Initially transgene constructs were simple, but there is an increasing tendency to use several transgene constructs to modify plant phenotypes in more complex ways. Two or more transgene constructs (multiple transgenes) may become combined in plants for two main reasons: (1) intentionally, as a product of plant breeding; and (2) unintentionally, as a result of sexual hybridization and gene flow from transgenic crops to related crop, feral or weed species.

The possible impacts of the presence of multiple transgenes, whether by design through breeding or by chance through cross pollination, is an important consideration in biosafety assessments leading to experimental field releases and to commercial releases of transgenic crops for widespread use in agriculture. The aim in this paper is to consider some of the salient issues relevant to assessing the likelihood of the production of plants with multiple transgenes, along with some of the consequences and responsibilities they present for agriculture and the environment.

1. THE LIKELIHOOD OF MULTIPLE TRANSGENES.

Plant breeding

Where it is necessary to combine several different transgene constructs into a plant, it is generally not achieved by multiple transformations of the same plant line. It is most often carried out by combining constructs through sexual hybridisation between lines containing one transgene construct. Their are two principal reasons for this. The first is that multiple transformations of the same plant line would require the use of a different selectable marker gene for each transformation. It would be undesirable to have several marker genes (eg. antibiotic resistance, herbicide tolerance) in a line being developed for commercial use. The second is that regulatory approval is likely to be simplest to achieve for transgenic plant lines containing one transgene construct. A single transgene construct may contain several genes, but because they will be inherited as a block, are easier to assess and evaluate.

In theory, it is possible to combine a wide range of transgenes into the same plant. But there are some constraints over what is likely to be done in practice, and this subject will be returned to later.

Gene flow

Over the past decade, we have learnt a considerable amount about the possibility of gene flow from transgenic crops to related plant species. There are two main components affecting this: the distance of pollination and sexual compatibility. As examples, data on potato and oilseed rape are summarised in Tables 1 and 2.

2. GENETIC INTERACTIONS BETWEEN MULTIPLE TRANSGENES

Genetic homology between parts of transgene constructs can cause instability of gene expression. This homology can be for the promoter or the protein coding regions. Instability most often results in transgene or homologous endogenous genes being down regulated or silenced. There are various hypotheses of how this works and it may involve pairing of homologous sequences, methylation and/or of the production of aberrant or abnormal RNA. The transgene instability phenomenon is also influenced by position of the transgene within the genome, by transgene copy number, by arrangement of transgenes, by sexual generation and, in some instances, by environmental conditions. The phenomenon of gene silencing does also have important applications for regulating the expression of

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resident genes (cosuppression) in many useful transgenic plant lines, including the tomatoes with modified ripening qualities that are in commercial production. In the present context, however, the genetic consequences of interactions between multiple transgenes are that genes could be unstable, silenced or switched off (Senior & Dale, 1996).

Table 1. The frequency of pollination in potato and oilseed rape at different distances under field conditions.

Crop / Distance (metres)	% cross pollination with plants of the same species	Number of seeds screened
Potato[a] 0m (leaves touching)		
3m (up to this distance)	24%	Total number screened =
10 m	2%	30 K
20 m	0.02%	
Oilseed rape[b]	0%	
0 m		
1 m	5%	7 K
12 m	1.6%	12 K
24 m	0.016%	110 K
36 m	0.0041%	117 K
48 m	0.0011%	180 K
	0.00034%	295 K

References: [a] Dale et al., 1992; McPartlan & Dale 1994; See also Conner & Dale 1996
[b] Scheffler et al., 1993; Scheffler et al. 1995; Dale & Scheffler 1996

Table 2. Sexual compatibility of potato and oilseed rape with related feral and weed species

Crop	Related species / ease of hybridization under field conditions
Potato [a]	*Solanum nigrum* Not able to hybridize naturally under field conditions. Hybrids from forced pollinations display poor fertility. *Solanum dulcamara* No evidence of hybridization under field conditions or forced pollinations
Oilseed rape [b]	*Brassica rapa & B. juncea* Can cross pollinate under field conditions *B. adpressa & Raphanus raphanistrum* Cross pollination has been observed under field conditions with male sterile oilseed rape *Sinapis arvensis* Negligible evidence of hybridization under field conditions. Hybrids are possible by forced pollination. Studies are continuing.

References: [a] McPartlan & Dale 1994.   [b] Scheffler & Dale, 1994; see also Dale, 1994.

Since 1989 various silencing phenomena have been identified which control (trans)gene expression in plants. The first was identified after analysis of a sequential transformation experiment (Matzke et al. 1989). Two partially homologous transgenes were introduced sequentially into tobacco. The transgenic plants obtained did not show the expected antibiotic resistant phenotype. Instead a large proportion of progeny were sensitive even though they contained complete constructs. Analysis showed this effect was due to promoter methylation and transcriptional inactivation. Silencing was also obtained when the transgene constructs were brought together following sexual hybridization (Matzke *et al.*, 1993).

A second, and somewhat surprising gene silencing mechanism (co-suppression), was identified in 1990 (Napoli *et al.*, 1990; van der Krol *et al.*, 1990). Here resident genes were silenced by the introduction of homologous transgenes in a sense orientation. Previously it was known that reversing gene coding regions (antisense orientation) between normal control sequences could result in gene suppression (Rothstein *et al.*, 1987), whereas genes introduced in a sense orientation would be over-expressed. In many instances this is correct, but it is now well established that a sense transgene can also silence a homologous resident gene. This has now been utilised as an alternative method for switching off unwanted genes in plants.

Considerable effort has been expended to determine how (trans)gene silencing works. A more complete picture is now emerging showing that there are two basic types of silencing (Baulcombe and English 1996). These operate either at gene transcription by methylation of the promoter or post-transcriptionally via an RNA based mechanism. Both appear to function most effectively when multiple copies of transgenes are present at a single insertion site (or locus) rather than in plants containing single copies, or multiple insertions at different loci in the genome. Gene silencing can occur in these latter instances but are comparatively rare.

Transcriptional gene silencing occurs when transgenes are driven by homologous promoters. It results in heritable alterations in methylation and gene activity. Recent evidence suggests transcriptional silencing is dependent on copy number, where single copies do not induce silencing while multicopies at a single locus can silence introduced genes (Matzke *et al.*, 1994). This affects genes even after separation of the inducing locus from the affected locus.

Cosuppression of genes occurs post-transcriptionally where two genes have a high similarity in coding sequence (70 % +). Current models suggest a link between the production of aberrant RNA from multicopy loci, and subsequent down regulation of gene expression (Metzlaff *et al.*, 1997). Single copies of transgenes do not generally induce silencing. By altering the coding sequence it is possible to reduce sequence similarity but still produce the same product. This allows over-expression of genes without the risk of unwanted silencing. Similarly if the objective is to silence a endogenous gene, using the native sequence will ensure the tightest down-regulation.

3. CONSEQUENCES OF TRANSGENE INSTABILITY

Plant breeding

Plant breeding involves extensive testing of plant lines. This testing is carried out over several years, over different sexual generations and in a range of environments. Any gene instability resulting from the presence of multiple transgenes should therefore be identified during this evaluation and selection process. It will be important to identify any significant environmental interactions, with the presence of several transgenes, but this is not a phenomenon restricted to genetically modified crops; it is also a feature of conventionally bred plant varieties (Qian *et al.*, 1986).

There are a few instances where cosuppression is initiated by developmental or environmental influences. Some transgenes are known to be silenced after an initial lag period, either at a defined (de Carvalho *et al.*, 1992) or at different (Hart *et al.*, 1992; Dorlhac de Borne et al., 1994) stages of development. Environmental conditions can influence transgene expression. High light intensity has been implicated in the supression of chalcone synthase (CHS) in *Petunia* (van der Krol *et al.*, 1990) and 1,3-glucanase genes in tobacco (de Carvalho *et al.*, 1992). Cultural conditions caused silencing of an acetolactate synthase gene (ALS) in tobacco when seedlings were transplanted to the field, but not when they were sown directly (Brandel *et al.*, 1995). Germination and growth conditions could induce silencing of chitinase (Hart *et al.*, 1992) and nitrate reductase (Dorlhac de Borne *et al.*, 1994) genes in tobacco spp. These types of gene silencing are uncommon and their cause is uncertain. However, there is no reason to believe that plant lines containing single or multiple transgenes are any more vulnerable to environmental variation than conventionally bred plant varieties.

Gene flow

The presence of multiple transgenes in a crop, feral or weed species resulting from cross pollination with transgenic crops, may potentially lead to instability in transgene expression. Because instability generally means that transgene expression will be switched down or switched off, these plants would revert to their wild-type phenotype. In the case of herbicide tolerance, this would mean that herbicide tolerant plants would become susceptible to the particular herbicide.

It is likely, therefore, that transgene instability in these cases will have a negligible impact on plants in natural habitats. In considering the potential impacts of transgene instability it is important to recognise that a wide range of mechanisms will be used to modify plants in the future, including the down regulation of undesirable substances in plants (eg allergens and toxins). Instability in these processes could potentially result in enhancement of these substances in the plant lines involved.

4. CONSEQUENCES OF MULTIPLE HERBICIDE TOLERANCE TRANSGENES

From plant breeding
In theory, many combinations of herbicide tolerance transgenes are possible in plant

breeding programmes. In practice, however, there are commercialisation and patenting constraints that inhibit this. Most companies developing and using herbicide tolerance transgenes have clear development plans for their use and are unlikely to combine different herbicide tolerance transgenes. Eventually, as patent protection for these genes runs out, there will be fewer constraints on breeders wanting to introduce several different herbicide tolerance transgenes. In some cases, herbicide tolerance transgenes are used as selectable markers, without the intention to use the corresponding herbicide in agriculture. In these instances, an additional herbicide tolerance transgene might be combined into crop plants. Another potential reason why more than one herbicide tolerance transgene may be present is if the crop is required to be grown on soils that have high residues of a particular herbicide. In these instances, breeders may wish to incorporate another herbicide tolerance, to provide selective weed control.

Flax is severely inhibited by residues of chlorosulfuron or metsulfuron methyl which are used to control broadleaf weeds in cereal crops. This means farmers have continuously to crop with cereals or have a summer fallow period (McHughen and Holm 1991). Introduction of a mutant ALS gene into flax conferred sulfonyl urea herbicide tolerance (McHughen 1989) to allow the cropping of flax on residue-containing land. Such transgenic lines have been extensively field tested, and found to be effective, and thus provide farmers with a more flexible crop rotation option (McHughen and Holm 1991).

From gene flow

Certain interest groups who are against the use of genetic modification in crop plants argue that gene flow of any kind into related plant species is undesirable; and often, they use the term genetic pollution for this phenomenon. There is no scientific justification for arguing that gene flow of this kind is, in principal, undesirable and potentially harmful. An assessment of its impact can only be made by considering each crop and transgene case by case.

The issue of impact of gene flow is a particularly challenging area for regulatory decision making (Dale & Irwin, 1995). Companies wishing to register herbicide tolerant transgenic varieties are usually required to describe strategies for the control of weeds and volunteer plants that may be, or become, resistant to a particular herbicide. This will also be true if companies wish to introduce more than one herbicide tolerance transgene into a plant variety. It is possible in the future that regulatory approval may become conditional on providing comprehensive monitory oversight, and reporting back to the regulatory authorities.

5. STRATEGIES FOR HANDLING MULTIPLE HERBICIDE TOLERANCE

Regulatory approval for environmental release and change of pesticide use requires proposers to outline their strategies for handling multiple herbicide tolerance, whether this is by the deliberate production of multiple herbicide tolerance in plant breeding, or the development of multiple herbicide tolerance though gene flow. It is important for industry to propose and develop strategies for the long term use of different herbicide tolerance transgenes. It is also important for the agricultural industry to develop strategies for the

use of herbicide tolerant transgenic varieties, and it is encouraging to see activity in this direction. The Codes of Practice (BSPB, NFU and UKASTA), the LEAF (Linking Environment And Farming) Guidelines for Integrated Crop Management and the Pesticides Forum may provide valuable channels to facilitate this process in the future.

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THE AGRICULTURAL IMPLICATIONS OF GENETICALLY MODIFIED PLANTS

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ABSTRACT

Whilst plant improvement has generated considerably improved cultivars of crop plants, the availability of recently developed transgenic techniques has opened up new avenues for progress. Environment and human health are protcted by regulation. There is a need to assess the impact of transgenic crops at farm and industry level. A wide range of new traits have or are being introduced; these will need to be assessed one by one and, where necessary, amendments to agricultural practice made. Also, a number of generic impacts justify examination. Potentially large benefits for agriculture could accrue from GM plants.

INTRODUCTION

The improvement of plant species through deliberate selection, crossing of closely related species and use of earlier novel improvement technologies like colchicine doubling of chromosomes to produce dihaploids or γ radiation-induced mutation to produce new genotypes of malting barley during 1960's and 1970's have contributed to quantum steps forward in production.

More recently, the development of novel genetic transformation technologies has allowed the transfer of genetic material between unrelated species. Hence a new range of opportunities to adapt plants has been made available.

Understandably, the introduction of such novel plant types has required control. In the European Union (EU) controls are laid down in Directive 90/220. Within member states national authorities operate regulations which comply with it, for example, Environmental Protection Act 1990 and the Genetically Modified Organism (Deliberate Release) Regulations 1992 in England.

For the United Kingdom the competent national authority is the Secretary of State for the Environment, Transport and the Regions(DETR) acting jointly with the Agricultural Ministers on agricultural matters. They are advised by the Advisory Committee for Releases to the Environment (ACRE). The role of ACRE/DETR is to ensure the human and environmental safety aspects of deliberate releases of genetically modified organisms, whilst pesticide use and introductions of plant varieties are regulated independently, in UK by Pesticides Safety Directorate (Control of Pesticides Regulations, 1986; Plant Protection Products Regulations 1995) and Plant Variety Rights Office (Council Directives 40/457/EEC on Common Catalogue of Varieties of Agricultural Plant Species and 70/458/EEC on Vegetable Seed).

However, there is currently no method of ensuring the agricultural suitability or the impact on the farm, farming system or farm business of genetically modified organisms, in the context of this paper, genetically modified (GM) higher plants.

First commercial releases of GM higher plants in UK will be herbicide tolerant arable crops and it is important in this context to note initiatives from Ministry of Agriculture, Fisheries and Food which were launched to the industry during July 1997 and which sought to stimulate discussion on the introduction of GM herbicide tolerant crops and on possible strategies to tackle any agricultural problems that may arise (MAFF, 1997). Comments on this discussion document were due by 13 September 1997 and are currently under consideration.

GM PLANTS: ACHIEVEMENTS TO DATE

According to Raybould and Gray (1993) most common crop plants grown in the UK have been genetically modified, at least in experimental conditions and with varying percentages of success. See table 1.

Table 1: Genetic Transformation of Crop Plants in the UK (modified from Raybould & Grey, 1993)

CROP	TRAITS
Sugar Beet (*Beta vulgaris*)	1, 2, 3
Oilseed Rape (*Brassica napus* ssp *oleifera, biennis,* and *annua*)	1, 2, 3, 4, 5, 6
Cabbage, cauliflower and close relations (*Brassica oleracea*)	2, 3
Potato (*Solanum tuberosum*)	1, 2, 3, 4
Tomato (*Lycopersicum esculentum*)	1, 2, 3, 4
Maize (*Zea mays*)	1, 3, 4
Wheat (*Triticum aestivum*)	1, 2, 3, 4, 6
Barley (*Hordeum vulgare*)	4, 6
Rye (*Secale cereale*)	Probably 1, 2, 3, 4, 6
Ryegrasses (*Lolium perenne; L. multiflorum*)	1, 4, 5, 6
Clover (*Trifolium repens*)	1, 3, 4
Lucerne (*Medicago sativa*)	1, 2, 4
Field/Broad Bean (*Vicia faba*)	2, 3
Runner Bean (*Phaseolus vulgaris*)	2, 3
Pea (*Pisum sativum*)	2, 3
Carrot (*Daucus carota*)	Unknown
Lettuce (*Lactuca sativa*)	2
Sunflower (*Helianthus annus*)	1, 4
Flax/Linseed (*Linum usitatissimum*)	1
Conifer (e.g. *Picea* spp, *Pseudotsuga* spp)	1, 3, 4
Apple (*Malus pumita*)	3
Strawberry (*Fragaria x ananessa*)	3
Raspberry (*Rulus idaens*)	3

Key to Traits: 1 - Herbicide tolerance; 2 - disease resistance; 3 - pest resistance; 4 - quality characteristics; 5 - male sterility; 6 - adaption to environment

Commercial interests would be expected to drive the exploitation of new genotypes and it is not surprising therefore that those crops with largest area and/or highest value are being exploited to the greatest extent to begin with. These include sugar beet, maize, oilseed rape and to a lesser extent potato. Details of permitted releases in EU, primarily for research, are given at Table 2 (EC, 1997). It should be noted that the nomenclature in Table 2 is slightly different from that in Table 1, beet including both fodder and sugar beet.

Table 2: GM Plant Releases per Country as at 28 February 1997

COMMON NAME	AT	BE	DE	DK	ES	FI	FR	GB	GR	IE	IT	NL	PT	SE	Total
African Violet												1			1
Alfalfa		1			1										2
Apple								1							1
Barley						1									1
Beet		6	19	22	5	1	39	18		2	8	11		5	136
Carnation												3			3
Carrot												1			1
Cauliflower		5													5
Chicory		12					4	1			10	6			36
Chrysanthemum												1			1
Cotton					1				3						4
Eucalyptus					1			2							3
European plum					1										1
Grape							2								2
Lettuce							4								4
Maize	1	18	15		14		83	5	1		56	10			203
Marigold											8				8
Melon					1		3								4
Oilseed rape		32	13	2	1	1	77	52				5		6	189
Petunia		2													2
Poplar		1					4	2							7
Potato	2	1	10	7	1		5	18			9	31	3	9	96
Rape		11										1			12
Silver Birch						1									1
Soybean							3				3				6
Squash							1				1				2
Strawberry								1							1
Sunflower					1		3					2			6
Sweet Orange					1										1
Thale Cress														1	1
Tobacco		1					25	4							30
Tomato					12		4	1	1		32	2	2		54
Wheat					1		5								6
Total Number of Plants Released															830

Source: EC Working Documents

GM PLANTS IN AGRICULTURE: CHALLENGES AND OPPORTUNITIES

The foregoing comments suggest that there is likely to be a continuum of novel GM plants coming into practical agriculture, beginning with the crops with largest area or

value and carrying more simple, monogenic, traits particularly herbicide tolerance. Thereafter a range of traits will be introduced, with no common specific characters. These will be primarily monogenic traits. In the future, speculatively, within 10 years, polygenic GM plants with tolerance to adverse environmental factors in particular, will be introduced.

Herbicide Tolerance

Herbicide tolerance traits may be carried in plants as primary traits or as markers but in the practical situation there is no difference at present. In the longer term however there could be agricultural and market benefits if specific herbicide tolerance traits were to be linked to specific quality characteristics. These benefits would be especially valuable where one crop, for example oilseed rape, had been developed into a range of cultivars with totally different quality characteristics, for example, high laurate or high erucic acid oil contents.

To date a number of herbicide tolerances have been developed. These include tolerance to glyphosate, glufosinate, sulfonyl ureas, the hydroxybenzonitrile herbicides, and are in addition to resistance to triazines and substituted-urea herbicides which have developed by non-transgenic means.

The benefits of herbicide tolerance, especially with the otherwise non-selective active ingredients like glyphosate and glufosinate are in yield, through good weed control; in quality, through removal of existing volunteers of the same species and in economic terms and therefore unit cost of production.

Lutman (1984) reported grass weeds, especially volunteer cereals, to have potential to reduce yield of winter oilseed rape but effects were sometimes variable; Ward and Askew (1984) showed similar results with mixed populations of mono- and dicotyledonous species whilst Bowerman (Pers. Comm) showed some species, for example, *Galium aparine* to be particularly aggressive and therefore to reduce yields. Data from Nix (1996) reported that an average budgeted cost of herbicides for winter rapeseed was approximately £65 per ha and that for spring rapeseed £40 per ha. It is expected that herbicides for use on tolerant crops will be competitive with these prices.

Against the benefits of herbicide tolerance must be balanced potential disadvantages. These include cross-pollination and consequent stacking (pyramiding) of genes; the potential for herbicide tolerant volunteers to develop and the potential for a number of companies to maintain viable research and development programmes from which diverse herbicides could be developed.

Regrettably, there is no universally applicable response to the risks listed, since problems, if any, vary with crop and situation.

Whilst it is well proven that a number of common crops produce volunteers, for example:

Oilseed rape c.25kg/ha (equivalent to 400 seeds/m2 -informal author's estimate)

Potatoes	370,000 tubers/ha (Lutman, 1977)
	460,000 tubers/ha (Lumkes, 1974)

and weed beet, either from regenerated beet tops post-harvesting or from seed formed from bolters, depending upon season and conditions (Longden, 1993). The significance and impact of such volunteers is variable.

The removal of volunteer rapeseed from winter wheat is easily practicable currently whilst such volunteers in dried peas (Knott, 1993) or sugar beet (May and Hilton, 1993) would pose problems. The position could be further compounded if the volunteer was tolerant to the same herbicides to which the crop had been made tolerant. A particularly difficult position could occur if glyphosate tolerant potatoes were introduced: volunteer potatoes are a major weed in UK and elsewhere and are arguably best controlled by glyphosate. Clearly glyphosate, within PSD cleared normal application dose, could not control such herbicide tolerant volunteers.

Much discussion has taken place on cross-pollination as a major source of gene leakage. In oilseed rape experience shows this to be negligible in practice, provided isolation occurs. For example, the MAFF "regulation" of 50m between low and high erucic rapeseeds has proven totally successful in commercial practice. Cross-pollination in commercial maize seems possible, but since volunteers do not normally occur and commercial crops are not home-saved for seed, it seems such leakage would be unimportant. In anemophilous species, particularly those which flower in their first year of growth, like ryegrasses, then some challenges on gene dispersal remain unsolved. Also, the need to control bolting in sugar beet is increased and further encouragement of growers to prevent flowering and further seed formation using current knowledge and expertise is essential.

Disease Resistance

Currently the focus in releases in EU is upon viruses especially cucumber mosaic virus, potato viruses X and Y, PLRV and tomato yellow leaf curl viruses, tomato spotted wilt virus, BNYVV in sugar beet, viruses of melon and squash; fungi in rapeseed, potato, sugar beet, carrot and bacteria, especially *Erwinia*, in potato, *Pseudomonas* in tomato. All will have beneficial effects in terms of enhancing saleable yield of produce, reducing current costs of control (where these exist) and in the process, of reducing pesticide use (especially insecticides) and subsequent environmental contamination and residues in food. Additionally, control of BNYVV in sugar beet or *Erwinia* in potato would have beneficial side effects in terms of reducing constraints upon trade, especially internationally.

In terms of disadvantages in practical agriculture, from disease control there would appear to be none, upon the proviso that the disease resistances remained stable. However, this seems an unlikely proposition overall and monitoring of disease strains may need intensifying. Additionally, with viruses there is always the potential for recombination between viruses and of transcapsidation. The issue of variability has already been alluded to but with transcapsidation the epidemiology of viruses could change such that growers of non-virus resistant crops would be disadvantaged.

Pest Resistance

Pest resistance embraces pests which cause direct losses to crops as well as those which carry diseases, like viruses, which in turn have detrimental effects upon crop production.

Aphids are major vectors of a number of viruses (e.g. *Myzus persicae* transmits a number of viruses in potato and sugar beet) and if virus tolerance is introduced into plants then their significance as disease vectors would be diminished. Presuming that such vectors did not transmit other diseases and did not cause direct economic damage by their own presence then need for controls would cease. That, in turn, would reduce insecticide use and selection pressure exerted upon aphids. Estimates suggest use of aphicides on potatoes and sugar been for virus vector (i.e. aphid) control to amount to between 42 and 67% of all foliar insecticides used in the UK (Barker *et al*, 1992).

In terms of treatment of crops for direct effects of pests, evidence shows considerable economic and environmental benefits could accrue from GM plants. For example, nematodes in potato can reduce yield up to 40% where severe. Cost of nematicide is on average £350/ha. Approximately 25,000 ha receive nematicide treatment.

Quality and Agronomic Characteristics

Transgenic technologies allow introduction of novel genes, enhanced promotion of genes and the potential to down-regulate or silence genes. This capability gives rise to an enormous and varied range of opportunities in terms of quality of produce from plants and enhanced agronomic and storage potential. Examples are discussed later.

Virtually all quality and agronomic traits in GM plants increase profitability to individual businesses and the industry as a whole through offering new products and/or reducing unit costs of production. Examples of new or enhanced products from existing crops are most readily exemplified in oilseed rape where a range of novel fatty acid compositions in the oil have been produced. These include high laurate rape (C12); high oleate rape (C18.1); ultra-high erucic acid rape (C22.1). The first is a completely novel product and will therefore offer UK agriculture a totally new opportunity; high oleate rapeseed should offer lower costs of processing/refining than traditional 'double low' rapeseed and should be more market-competitive whilst ultra-high erucic acid rapeseed will produce more of an already higher value product, namely erucic acid. Moreover extraction/refining costs are likely to be reduced.

In sugar beet there is the potential to enhance sugar yield or to produce novel products, for use in added-value health markets.

The potato has proven particularly suited to novel technologies and is being developed for specialist starch content, an added-value product with easier starch extraction than cultivars with mixed starch-type content. Also transgenic technologies are developing potato cultivars for the food processing industry, which will not be adversely affected by cold storage (e.g. 4°C). Currently, potatoes for processing are stored at temperatures between 7°C and 10°C according to their intended end use. At those

temperatures the production of reducing sugars, which produce unacceptably dark brown pigments when cooked, is inhibited but unfortunately the development of sprouts on tubers is promoted. Sprouting creates rapid deterioration in store. The usual agricultural practice is to treat potato stores with sprout suppressants, predominantly CIPC. This causes added cost (approximately £0.80 per tonne stored) and inevitably there are residues from CIPC on the stored produce. These novel cold storing types will therefore produce higher quality potatoes without use of agrochemical sprout suppressants. Presuming 20% of UK production to be stored for processing, then cost saved would be approximately £1 million per annum.

In terms of storage, enhanced shelf life and therefore value to producer and end-user the well established tomato product FLAVR SAVR is an ideal extant commercial example.

Field agronomic characters have potential to alter costs of production and therefore unit cost. Presuming the loss of seed from shattering in winter rapeseed to be 20kg/ha on average then the introduction of shatter-resistant rapeseeds to growers would be approximately £5 per ha or approximately £2.0 million pa to the UK industry.

Male Sterility

The introduction of male sterility ensures hybridity in progeny; hybrids have heterotic vigour. Whilst maize and sugar beet cultivars are already predominantly hybrids, rapeseed and many other cereals are not.

Evidence from breeders' trials suggests that hybrid rapeseed has yield advantages of up to 25%. Experience would suggest that in large scale practical agriculture such added potential might not be totally exploited but nonetheless 10% yield enhancement should be easily possible. To the individual winter rapeseed grower, 10% yield enhancement would produce approximately 0.32 t/ha extra rapeseed which at £150/tonne has a value of £48/ha, or over UK, approximately £19 million per annum.

Adaption to Environment

Current concensus suggests that many primary agricultural areas will be subject to higher temperatures and, perhaps, variations in weather patterns, particularly rainfall. Also, many areas worldwide are already subject to aridity, drought, salts or cold temperatures. Hence, there will be an increasing emphasis upon genes which will confer tolerance or adaption to such environmental stresses.

Notwithstanding these issues, there are already limitations on water availability for irrigation and, water application equipment is also limited by economic constraints.

It is already known that some species are more adapted to salinity (e.g., barley); drought (e.g., xerophytes) or heat (C4 plants) stresses. Also some plants are able to tolerate high levels of specific molecules (e.g. some *Brassicaceae*) and have potential for use as bioremediators.

Genes conferring adaption to environmental stresses will permit agricultural production to continue at reasonable levels in future on land areas that might otherwise cease to be productive. The net value of this is unknown.

GM PLANTS IN AGRICULTURE: GENERIC ISSUES

Farm Practice

There is a need to maintain the identity and integrity of specific GM crops. This could entail investment in extra storage facilities both on farms and with end users. New grain storage capacity costs approximately £80-100 per tonne of storage space.

In field practice, GM releases will entail enhanced controls of volunteers where these have been identified as problematical. Additionally, increased segregation of oilseeds, pulses and cereals will probably be needed. However, judging from experience with oilseed rape it is unlikely that isolation distance as great as those for certified seed production will be required; grass/hay crops may be an exception.

Home-Saved Seed (H-SS)

The trends in H-SS could be variable. If virus resistance was to be introduced to potatoes then one of the commonest sources of degeneration in stocks would be removed and use of H-SS could increase. Such a trend could have disastrous effects upon seed tuber sales and through that the viability of commercial potato breeding enterprises. These could be overcome through restrictive contracts on initial seed supply. Conversely, the introduction of hybridity in rapeseed would reduce H-SS substantially since hybrid F1 seed would segregate if grown-on. Provided the extra cost of hybrid seed was less than potential yield enhancement, growers are likely to be enthusiastic about growing new hybrid cultivars. Hence use of H-SS in rapeseed could decline rapidly.

In terms of cultivars with herbicide tolerance or enhanced agronomic characteristics, prediction is difficult. It would seem likely that growers may wish to save seed from such crops but equally likely that breeders will want to achieve a return on their enhanced investment in transgenics. Moreover, for the individual grower it may be difficult to ascertain that all seed maintained its specific GM attributes. This would be especially so where various types of a crop, GM and non-GM were grown on the same holding.

Due Diligence

In most UK agricultural situations there is every likelihood that there will be a large number of field boundaries abutting neighbours. The scenarios whereby a chance sample of a crop is failed, having been taken from plants grown near a common boundary and partially cross-pollinated by another crop, have yet to be addressed. The problem of "look-alike" agrochemicals requires elucidation too: where would liability lie if a herbicide tolerant crop was to be damaged by an active ingredient akin

to that to which it is tolerant but applied in another product from that originally intended by breeders?

Diversity

It appears likely that continuous use of total herbicides to a given field would, over time, reduce diversity. Evidence already exists to show that some formerly widespread weeds are declining (Fryer and Chancellor, 1970). From a purist arable agricultural standpoint that may appear irrelevant. However, clearly, disappearance of species is undesirable overall. Moreover, experience shows that with selective herbicides, whilst some species have declined, more pernicious species seem to have increased and the spectre of herbicide resistant weeds needs consideration.

Economic Competitiveness

Whilst the capability to introduce novel products to temperate crops in UK is an enormous scientific success it must be remembered that trade is increasingly world-based and increasingly derestricted. Two points arise, namely, will temperate countries with high cost agricultural industries be able to compete with areas of the world where production costs are low or with areas which have higher potential yields for producing products in question, for example, rapeseed oil and tropical oils? Secondly, should the emphasis be on improving achievement of highest potential yielding areas rather than temperate agriculture alone?

CONCLUSIONS

The introduction of GM plants to agriculture offers great potential both for food and non-food crops. Within agriculture itself practices will need to be adapted to obviate pitfalls, most of which are already identified. Some issues remain unresolved, particularly at the business and international levels.

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THE IMPACT OF RELEASES OF GENETICALLY MODIFIED HERBICIDE TOLERANT OILSEED RAPE IN THE UK

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ABSTRACT

The impacts of the cultivation of genetically modified herbicide tolerant oilseed rape (*Brassica napus)* on agriculture and the environment are being studied. This paper reports results of monitoring sites for up to three years where commercial seed crops and variety trials had been grown. The studies include evaluations of the impacts of the agricultural management of the GM crops as well as the direct effects of the releases of the GM crop on the environment.

INTRODUCTION

Herbicide tolerant oilseed rape

Risk assessments for the release of GM oilseed rape have been largely based on studies of non-transgenic rape or of small scale releases of GM oilseed rape (Harding and Harris, 1995). In 1995 seed production of GM glufosinate ammonium tolerant spring oilseed rape (GMSOSR) commenced in UK at 3 locations ranging in size from 1 - 3.5 ha. In addition Plant Genetic Systems established a 5 ha trial site for testing breeding lines of winter oilseed rape, of which approximately half were genetically modified. In 1996 another GMSOSR seed crop and a further trial area of 7 ha of mostly GM winter oilseed rape (GMWOSR) were established.

In addition in 1995 and 1996 GMSOSR was grown in UK National List Trials at 4 sites and in 1997 both glufosinate and glyphosate tolerant spring rape was trialled at 4 sites. In 1996/7 both glufosinate tolerant and glyphosate tolerant winter oilseed rape was grown in 10 trials at various locations in UK.

These crops and trials have provided an opportunity to examine the characteristics of these GM crops and to determine whether they have any impacts that are different from those of non-transgenic crops. They are enabling the risk assessments conducted originally by Plant Genetic Systems, Monsanto and AgrEvo to be verified by studies of several trials at a range of geographic sites grown under normal farming conditions.

Herbicides

The cultivation of herbicide tolerant crops will result in changes in herbicide usage from the currently used selective herbicides to the broad spectrum herbicides (eg glufosinate and glyphosate) which the varieties tolerate. In addition, the subsequent volunteers generated by these crops may need different herbicides to eradicate them. These changes in herbicide programmes may have different effects on plant and animal biodiversity in fields and field margins. Trials conducted by NIAB are examining impacts on field margins of glufosinate and glyphosate compared with currently used herbicides. In addition, the establishment of

volunteer herbicide tolerant oilseed rape in herbicide treated field margins is also being examined to determine whether feral populations are likely to establish adjacent to herbicide tolerant crops.

Studies of plant diversity in crops subjected to the new herbicide programmes are also planned for the future.

Research objectives

The objective of the NIAB research is to determine the likely agricultural and environmental consequences of the cultivation of herbicide tolerant oilseed rape by studying both the direct impact of large scale releases and the impact of any associated changes in agronomic practices.

MATERIALS AND METHODS

The oilseed rape used in this study consisted of transformed breeding lines, parent lines and F1 varieties developed by Plant Genetic systems, (Rudelsheim & Huybrechts, 1995); the transformations consisted of the introduction of a Kanamycin resistance gene, a male sterility gene, a male fertility restorer gene and a glufosinate-ammonium tolerance gene (*Bar* gene).

Comparisons were made with the performance and behaviour of the wide range of non-transformed oilseed rape varieties grown in National List trials.

The sites of the crops were monitored using the methods described by Sweet and Shepperson (1996 & 1997).

Monitoring Sites

Monitoring sites were established at 4 locations in Devon, Lincolnshire and Yorkshire where glufosinate tolerant SOSR seed production crops were grown in 1995 and 1996. A site in Cambridgeshire which has grown successive crops of glufosinate tolerant winter oilseed rape since 1995 is also being monitored. National List trial sites of both spring and winter oilseed rape varieties including glufosinate and glyphosate tolerant varieties in Cambridgeshire are also being monitored. At each site a range of monitoring studies are being conducted (Sweet & Shepperson, 1996 and 1997) and this paper considers the preliminary results of certain studies.

Cross Pollination

At each site, pollination of the nearest oilseed rape crop (usually between 0.5 - 1 km in distance), oilseed rape volunteers and related cruciferous weeds and hedgerow plants up to a distance of 200 m was studied. Seed samples were collected from plants flowering synchronously with the GM rape and tested for the presence of the herbicide tolerance gene using PCR (see below) or by growing the seed and testing the resultant seedlings for herbicide tolerance.

Detection of Herbicide Tolerance

Plants were tested destructively for herbicide tolerance by spraying with a 1% dilution in water of Challenge or Liberty herbicide consisting of 150 g/l of glufosinate ammonium. Non-

destructive testing was done by placing 1 cm diameter filter paper discs soaked in a 1% solution in water of Challenge or Liberty on to leaves of test plants. Sensitivity in both instances was recorded after 5-6 days.

The presence of the *Bar* gene, which is responsible for the oxidation of the phosphinothrycin produced by the glufosinate ammonium herbicide, was also conducted by amplification by PCR of the products of specific primers for the *Bar* gene (Rogers *et al.* 1996). The test was sensitive to a dilution of 1 GM plant sample in 100 non-GM plant samples of equal size. Testing was conducted on samples ranging from single plant samples to samples diluted 1:50. PCR tests were conducted jointly by the Laboratory of the Government Chemist and NIAB.

Seed Dispersal

Seed dispersal was recorded at each site by testing volunteers that arose in the field margins, neighbouring fields, tracks and roads traversed by farm machinery associated with the GM crop. The seed bank of GM seed remaining in the soil at each site was assessed after 3 seasons.

Weediness and Invasiveness of GM Oilseed Rape

Oilseed rape volunteers can be serious weeds of subsequent crops but are normally controlled by post harvest cultivations and applications of herbicides to emerged volunteers either pre- or post-drilling of the following crop. At each monitoring site the numbers of volunteer rape plants were measured post harvest and in the subsequent crops (mostly wheat). Assessments were made of whether numbers were higher or lower than those of non GM oilseed rape, grown under comparable conditions.

In addition, seed of a breeding line of spring oilseed rape was mixed in equal portions with seed of the same line transformed with the *Bar* gene. In August 1996 the seed was broadcast by hand at a rate of 10 kg/ha into two field margins that had previously received the herbicide treatments described in below. The numbers of GM and non-GM oilseed rape plants was assessed over a two year period.

Herbicides used on tolerant oilseed rape : Effect on field margins

A study commenced in April 1996 to study the effects of glufosinate, glyphosate and a standard spring oilseed rape herbicide programme on plant populations in two field margin sites, one on the NIAB farm and the other on a farm in Grantchester, Cambridgeshire. Both were adjacent to fields in arable rotations but with 3m buffer areas to reduce the likelihood of farm crop agrochemicals being applied to them that might interfere with the study. The sites were managed as though adjacent to spring oilseed rape crops of both herbicide tolerant and non-tolerant crops. Each site was subjected to a series of herbicide treatments to simulate drift or overspraying of field margins as follows:-

Treatments per site	Number	Replication	Total Plots
Glufosinate - N, 0.1N, 0.01N	3	3	9
Glyphosate - N, 0.1N, 0.01N	3	3	9
Standard - N, 0.1N, 0.01N	3	3	9
Unsprayed	1	3	3
TOTAL	10		30

N = normal dose rate
Standard programme = cycloxydim (Laser) and benazolin + clopyralid (Benazolox).
Glufosinate = "Liberty"
Glyphosate = "Roundup"

Seed of a similar line of oilseed rape (*Brassica napus*) with and without glufosinate tolerance was scattered onto the field margin treatments in August 1996 to simulate seed dispersed at harvest.

The effects of the herbicide treatments on botanical composition and establishment of feral rape are being assessed over two seasons after the herbicide treatments. This report describes the results of experiments and assessments made up to June 1997, i.e. one year after the herbicide treatments.

RESULTS

Cross Pollination

The seed crops were isolated from other rape crops by at least 0.5 km and were grown on farms or areas of farm that did not normally cultivate oilseed rape. However, oilseed rape volunteers were detected in close proximity to some seed crops and their seed tested for the presence of the transgene. In addition the nearest margin of the nearest oilseed rape crops was sampled for seed which was tested for the presence of the *Bar* gene. To date, no cross pollination with other oilseed rape crops has been detected in the several thousand seed samples tested, though tests are still being conducted on samples, and molecular tests are incomplete.

Similarly, tests of volunteer and feral rape growing within 200m of the GM crops have not detected any cross pollination to date, though tests are not yet complete.

Cruciferous weeds were fairly common at most sites but were successfully eradicated from the GM crops by the glufosinate treatments. Field margin populations of *Sinapis arvensis, Raphanus raphanistrum, Brassica napus, Capsella bursa-pastoris, Alliaria petiolata, Hirschfeldia incana* and *Rorippa nasturtium-aquaticum* (one site) were recorded as flowering at the same time as the GM crops and seed collected from them. Seed was grown and tested for herbicide tolerance or subjected to molecular tests for the *Bar* gene. No herbicide tolerant seed has yet been detected. Populations of cruciferous weeds growing near to the GM crops were revisited in 1996 and 1997 and tested for herbicide tolerance. None has been found yet.

Seed Dispersal

Most seed of the GM oilseed rape (OSR) crops was transferred to bags from the combine harvesters and transported in this way, so that spillage was minimised. However, at some sites the harvesters travelled up to 4 km on roads post harvest, shedding seed. Populations of OSR volunteers emerged alongside farm tracks leading from the GMOSR crop fields, in the autumn of 1995 and 1996. However, these populations were very transient and gradually declined during the winter until no plants were detectable in the spring.

The GM spring OSR crops were the last crops to be harvested at all sites in 1995 and 1996. Subsequently, the harvesters remained uncleaned until they harvested winter barley crops the following year. In these barley fields patches of GM oilseed rape volunteers emerged after harvest where the barley seed had flushed out GMOSR remaining in the harvesters and deposited it on the ground. These populations were subsequently eradicated by cultivations and herbicides.

To date no feral populations of GMOSR have established at the sites where GM seed crops and trials were grown in 1995 and 1996. However, the soil seed banks at each site will be tested in 1997 as populations could still establish from residual seed.

Weediness and Invasiveness

Volunteer populations of GMOSR were assessed in the crops following all the spring oilseed rape seed crops, two National List spring OSR trials containing GM varieties and the winter GM oilseed rape trials sown in 1995. The results are shown in Table 1.

At the Cambridgeshire site 48% of the area was sown with GMWOSR, however only 5 of 77 volunteers (6.5%) were tolerant to glufosinate. At the Lincs 96 and York sites volunteer numbers were associated with seed spillage and unsprayed areas. At the other sites volunteer numbers were very low or non-existent.

The numbers of rape plants that established in the two field margin sites treated with various herbicide treatments were assessed in 1997 and the results from the NIAB site are shown in Table 2.

At the second site at Grantchester there was considerable pigeon damage and other grazing of the field margin trial area. Numbers of rape plants establishing were very low and plants were destroyed before herbicide sensitivity tests could be completed.

Table 1. Oilseed rape volunteers occurring in crops following GM oilseed rape crops and trials

Site	Crop and Area	Following Crop	No. of Volunteers	No. Herbicide Tolerant
Lincs 1995	GMSOSR 1 ha	Sugar beet	4	4
York 1995	GMSOSR 3.5 ha	Winter wheat	43*	43
Devon 1995	GMSOSR 1 ha	Winter wheat and winter barley	0	0
Lincs 1996	GMSOSR 1 ha	Winter wheat	120**	120
Cambs 1995	GMWOSR 2.5 ha WOSR 2.7 ha in mixed plots	Winter wheat	77	5
NIAB 1995	SOSR 2 ha 0.03 ha GMSOSR in trial plots	Winter wheat	0	0

SOSR = Spring oilseed rape   WOSR = Winter oilseed rape

GM = genetically modified for glufosinate tolerance

*   35 of these plants were in one area where broad-leaved weed herbicide had not been applied.

**   Clump of plants due to spillage of seed when bagging from combine harvester.

Table 2. The number of oilseed rape plants that established in the NIAB field margin site treated with various herbicide treatments.

Treatment	Rate	Total No. of oilseed rape plants*	No. of GM oilseed rape plants*
Glufosinate	N	3	0
Glufosinate	0.1N	0	0
Glufosinate	0.01N	0	0
Glyphosate	N	6	1
Glyphosate	0.1N	12	3
Glyphosate	0.01N	1	0
Standard	N	0	0
Standard	0.1N	0	0
Standard	0.01N	0	0
Untreated		1	0

*   Number of plants established in 3 x 5m plots of approximately 1m width, sown with OSR seed at 10kg/ha rate.

Herbicide Effects

The phytotoxic effects of the herbicides applied to field margin and hedgerow plants were assessed at specific times as follows:

One month post spraying: *NIAB and Grantchester*

Glyphosate caused considerable chlorosis and necrosis of woody hedgerow, herbaceous and graminaceous species at N and a little at 0.1N rates.

Glufosinate caused considerable necrosis at N rate and less at 0.1N in herbaceous and graminaceous species and some brown spotting on woody hedgerow species.

Standard: considerable necrosis observed at N rate and less at 0.1N in herbaceous and graminaceous species and some brown spotting on woody hedgerow species. Some cruciferous weeds little affected.

3 months after herbicide treatment: *NIAB*:

Untreated plots: the most common species was couch (*Elymus repens*), followed by bindweed (*Convolvulus arvensis*) and *Poa annua* (annual meadow-grass), then the following species were found at lower frequencies; *Dactylis glomerata* (cocksfoot), yarrow (*Achillea millefolium*), creeping thistle (*Cirsium arvense*), nettles *(Urtica diocia)*, cow parsley (*Anthriscus sylvestris)* and hogweed (*Heracleum spondylium*).

Glufosinate treatments: Some areas of bare ground were observed at the N rate, and cow parsley, yarrow, bindweed, nettles, Poa, creeping thistle and hogweed were less common than in the untreated plots. Dactylis and couch were found in similar frequencies to the untreated plots.

Glyphosate treatments: bare ground was common on the N and 0.1N rate plots, but uncommon on the 0.01N rate plots. Couch was found to be reduced at the N rate but not at lower rates. The other common species found in the untreated plots were found on these plots at much reduced frequencies, but at the N and 0.1N rate many other species not found in the untreated plots were found at low frequencies also, indicating an increase in species diversity. Diversity was not increased on the 0.01N plots. N rate caused defoliation of exposed branches of hawthorn and rose.

Standard treatments: matted grasses were found in high frequencies on the N rate plots, but not at the lower treatment rates. Yarrow was found in higher frequencies at the N and 0.1N rates, and Poa was found at higher frequencies at the 0.1N and 0.01N rates than on the untreated plots. The other common species found in the untreated plots were found at similar frequencies at all the treatment rates.

Grantchester

Untreated plots: areas of dead grass were the most frequent ground cover. The most frequent species found were couch and nettles, followed by cow parsley, creeping thistle and barren brome (*Bromus sterilis*) at lower frequencies.

Glufosinate treatments: areas of dead grass were less common than in the untreated plots, and creeping thistle and barren brome were less common at all treatment rates. Couch was found at higher frequencies at the 0.1N and 0.01N rates. *Lolium perenne* (perennial ryegrass) was found to be more common that in the untreated plots on the N rate plots. Nettles and cow parsley were found at similar frequencies to the untreated plots at all treatment rates. No damage to the hedgerow caused by application of the herbicide was visible.

Glyphosate treatments: areas of bare ground rather than dead grass were found at all treatment rates. Barren brome was found to be much less common, being found on one 0.01N rate plot only. Couch and nettles were found to be less common on the N and 0.1N rate plots, but more common on the 0.01N rate plots. Cow parsley and creeping thistle were found at the same frequencies as on the untreated plots. In the N rate plots there was no increase in species diversity and some defoliation of exposed branches of woody hedgerow species.

Standard treatments: bare ground rather than dead grass was found, although only on the N rate plots. Dead grass was found at the same frequency as the untreated plots on the 0.1N and 0.01N rate plots. Couch was found to be more frequent than on the untreated plots at all treatment rates. Creeping thistle and barren brome were found to be less frequent at all treatment rates. Garlic mustard (*Alliaria petiolata*), hedge mustard (*Sisymbrium officinale*), scentless mayweed (*Tripleurospermum maritimum*), Lolium, bindweed and prickly sow thistle (*Sonchus asper*) were found to be more frequent than on the untreated plots at all treatment rates. No damage to the hedgerow caused by application of the herbicide was visible.

One year after herbicide treatment: *NIAB*

Untreated plots: Couch was found to be the most common species, followed by cow parsley, and the following species at lower frequencies: *Holcus lanatus* (Yorkshire fog), bindweed, cleavers (*Galium aparine*), hogweed, yarrow, nettles and creeping thistle.

Glufosinate treatments: couch was found to be much reduced in frequency at all treatment rates. Yarrow, creeping thistle, nettles and cleavers were found in lower frequencies on the 0.1N and 0.01N rate plots. Holcus was found in higher frequencies on the N and 0.01N rate plots. Cow parsley, bindweed and hogweed were found at similar frequencies to the untreated plots.

Glyphosate treatments: damage to the hedgerow was found on all N rate plots, the lower branches of hawthorn (*Crataegus monogyna*) being set back in growth or dead. Bare ground was also frequent on the N rate plots. Couch was found to be much less common on all treatment rate plots, however Holcus was more common on the 0.1N and 0.01N rate plots. Bindweed and cleavers were found to be more common on the N and 0.01N rate plots, whereas creeping thistle was found to be more common on the N and 0.1N rate plots. Oilseed rape plants established on plots of all treatment rates. The other common species found on the untreated plots were found on these plots at similar frequencies. Many other annual species not found on the untreated plots were found at low frequencies, indicating an increase in species diversity.

Standard treatment: Holcus was found at higher frequencies on all treatment rate plots. Nettles and creeping thistle were less common at all treatment rates. Yarrow was found at a higher frequency on the N rate plots, but at a lower frequency on the 0.1N and 0.01N plots. Couch was found to be less frequent on the N and 0.01N rate plots. The other common species found on the untreated plots were found on these plots at similar frequencies.

Grantchester

Untreated plots: barren brome was the most frequent species found, followed by nettles, cow parsley, Lolium and cleavers at lower frequencies.

Glufosinate treatment: barren brome was found to be much less common at all treatment rates. Cleavers and nettles were also found at lower frequencies at all treatment rates. Lolium was found to be more common at all treatment rates. Cow parsley was found at similar frequencies to the untreated plots. Many other species not found on the untreated plots were found on all treatment rate plots, indicating an increase in species diversity.

Glyphosate treatments: damage to woody hedgerow species was found on 2 out of the 3 N rate plots, the lower branches being dead. Barren brome was less frequent at all treatment rates. Bare ground was common on N and 0.1N rate plots, uncommon on 0.01N plots. Nettles and cleavers were found at lower frequencies on all treatment rate plots. Lolium was found at higher frequencies on the 0.1N and 0.01N rate plots. Oilseed rape has established on some plots. More species diversity was found on the N and 0.1N rate plots.

Standard treatment: barren brome and cleavers were found at lower frequencies on all treatment rate plots. Bare ground was frequent on the N rate plots. Oilseed rape established on some of the plots. Nettles were found to be less common at the 0.1N and 0.01N treatment rates, and Lolium to be more common at the same rates. More species diversity was found compared to the untreated plots.

DISCUSSION

No pollination of oilseed rape and other crucifers has so far been detected in this study. It is assumed that the GM oilseed rape plants found outside the release field sites arose from dispersed seed, either at drilling or, more likely, post harvest. Given the nature of the releases, ie their comparatively small size, their isolation from other crops and use of land that had no record of growing oilseed rape or that rape had not been grown for at least ten years previously, it is not surprising that the local incidence of cruciferous weeds was low and that few opportunities for cross pollination occurred.

Seed dispersal was also restricted by the size of the crops and the nature of the handling post harvest, though spillages and distribution of seed occurred via the harvesters at some sites. However, where GM seedlings did occur from dispersed seed their survival was very low due to farm operations on cultivated land and various environmental stresses on uncultivated land eg. predation, frost etc. No feral/volunteer populations of GM rape have been observed to establish outside the release sites, though areas of seed spillage continue to be monitored.

Volunteer numbers of GMSOSR in the crops which followed were generally low, and usually associated with failures in volunteer control. The incidence in a following crop of sugar beet (4 plants/ha) was surprisingly low. Numbers of SOSR volunteers appearing in wheat crops following National List trials recorded at NIAB and elsewhere have also been very low and this generally indicates that both GM and non-GMSOSR are readily controlled by current farm practices.

Generally winter oilseed rape (WOSR) is more widely grown in the UK and its winter hardiness and biennial character enhance its weediness compared with SOSR. At the Cambridge GMWOSR site 77 WOSR plants were counted in 5 ha. Their distribution pattern suggesting that they had not been controlled by the normal arable management of the winter wheat crop that followed. However, the low incidence of GMWOSR in this volunteer population suggested that its weediness was not enhanced by the genetic modifications.

The lower establishment rate of GMSOSR in the field margin trials also suggests that GMSOSR does not have enhanced colonising characters. However, these trials generally had low levels of SOSR establishment and testing for the herbicide tolerance gene was seriously affected by heavy predation at one site, so that little weight should be attached to these results. Trials to study establishment of GMWOSR in field margins are currently underway at NIAB.

The effects of the herbicides on the field margins showed, as anticipated, that the broad spectrum systemic fungicide glyphosate showed the highest levels of phytotoxicity removing perennial species and allowing colonisation by annuals, while glufosinate appeared no more phytotoxic than the currently used herbicides. Establishment of oilseed rape volunteers appeared to be enhanced by the glyphosate treatments, though these results need to be treated with extreme caution because of the generally low levels of establishment. However, it may be prudent to advise farmers to avoid allowing drifts of broad spectrum herbicides into field margins, since they may allow volunteer herbicide tolerant and other GM rape to establish which can then provide sources of contaminant seed and pollen for subsequent rape crops.

The effects of the herbicides used on herbicide tolerant WOSR on field margins is currently being studied at NIAB. In addition, the rate of evolution of multiple herbicide tolerance in adjacent crops and plots is being studied and the weediness, invasiveness and herbicide sensitivity of multiple tolerant plants is also being investigated by workers in UK and France. (Champolivier et al. 1997 and Dale, 1997). From these studies it will be possible to determine both the agronomic and environmental impacts of herbicide tolerant oilseed rape. NIAB is studying the effects on farming operations and management and the longer term consequences for agriculture.

Future Research and Monitoring

In the future, crops containing a range of transgenes will be grown on European farms in various rotations. Multiple stress tolerance genes will be introduced or transfer naturally into cultivated crops and closely related weed/wild species. The interactions and impacts of these gene combinations will be increasingly difficult to predict and risk assessments more difficult to perform. Research into these interactions is a high priority but, because of the magnitude of the problem, it should focus on crop and trait combinations considered to be of high risk.

As GM crops become more widely grown, monitoring of all commercial releases becomes impractical. Thus monitorng should also place emphasis on selected crop/trait/environment combinations considered to be of the highest risk. However additional studies should be carried out to determine whether data from crop records, crop assurance programmes and seed sales of GM crops can be used to catalogue all GM crops on farms annually and provide a database for investigations if unpredicted incidences occur.

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