SESSION 8A

THE ENVIRONMENTAL IMPACT OF GM CROPS: COSTS AND BENEFITS A DECADE AFTER COMMERCIALISATION

Chairman and Session Organiser:	Dr Alan Raybould *Syngenta, Bracknell, UK*
Papers:	8A-1 to 8A-4

An Assessment of the Environmental Impact of Genetically Modified Crops in the US

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ABSTRACT

Genetically modified (GM) crops have become important components of modern agriculture in the past ten years. The United States had over 39 million hectares of GM crops grown in 2002 with much of these hectares comprised of Bt corn, Bt cotton, and herbicide tolerant (HT) soybeans. Commercialization of GM crops has been more limited in other areas, such as the EU. This paper describes the information collected to address ecological risks in the US as part of the initial regulatory process and provides a review of new information derived since commercialization, with a focus on field data. Field studies for Bt corn, Bt cotton, and HT crops in the US are reviewed specifically for impacts on arthropod populations and results show no consistent unexpected findings in abundance. Indeed, the adoption of these products can have direct and indirect benefits for the environment by the replacement of broad-spectrum insecticides and the facilitation of reduced tillage. The results of the field studies are discussed within the context of overall biodiversity in agroecosystems.

INTRODUCTION

Genetically modified (GM) crops have become an important tool in agriculture over the past ten years. The initial products have been mainly crops protected from insect damage using insecticidal proteins from the soil bacterium, *Bacillus thuringeinesis* (Bt), and crops made resistant to selected herbicides. GM crops have been commercialized more rapidly in some world areas that others (James, 2002). For example, in Europe, a moratorium has existed for several years on new registrations of GM crops, whereas, GM crops were planted on over 39 million hectares in the US in 2002 (James, 2002). The rapid rate of adoption in the US is likely due to various factors including grower satisfaction with the benefits of using GM crops (Gianessi *et al.*, 2002), consumer confidence relative to food safety regulation, and the suitability of these products with environmentally improved management practices (e.g., conservation tillage and reduced risk to nontarget organisms).

GM soybeans tolerant to Roundup herbicide account for the majority of the GM crop acres in the US. Most of the US GM corn acres were planted in corn expressing the Cry1Ab Bt protein providing European corn borer control. GM cotton expressing the Cry1Ac Bt protein providing tobacco budworm, pink bollworm and cotton bollworm control is also an important part of the U.S. GM crop acres. These products were initially registered in the mid-1990s. During the registration process, the regulating agencies concluded that there were no unreasonable adverse effects on the environment (FR, 1994; US EPA 2003). This paper provides an overview of the type of information collected to address ecological risks in the US for these products and reviews new information derived since commercialization, with a focus on field data and potential implications relative to biodiversity.

US REGULATORY FRAMEWORK

Responsibility for the regulation of GM crops in the US is divided among three federal agencies: the Department of Agriculture (USDA), the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA). Roles and specific elements of oversight were established in 1986 under the Coordinated Framework for the Regulation of Biotechnology (Federal Register, 1986)

Ecological risk assessment of GM crops falls within the responsibility of one or more US agencies depending on whether the introduced trait contains a plant-incorporated protectant (PIP). GM crops are, at least initially, separated into plant and animal (e.g. insects, birds, fish, wildlife, domestic animals and humans) assessment components. The plant assessment is reviewed primarily by the USDA (Animal Plant Health Inspection Service or APHIS), which focuses on the pest potential of the GM crop and gene flow. The animal assessment is governed by both USDA APHIS regulation and, especially in the case of insecticidal proteins (a specific type of PIP), EPA Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) regulation under Subdivision M (OPPTS, 1996).

Plant Assessment:

The GM plant is assessed to determine which of its characteristics are "familiar" (OECD, 1993; Hokanson *et al.*, 1999), relative to the parental or control lines. Familiarity enables regulators to focus on those characteristics/properties that require detailed risk assessment. As such familiarity is a characterization endpoint and not a risk conclusion. The basic approach to establish familiarity focuses on phenotypic measurements including germination, growth, time to flowering, pollen morphology, composition and reproduction. In addition to the phenotypic assessment, the potential for altered interactions with known pests (insects and diseases) and biologically meaningful consequences associated with gene flow from the GM crop to other plants is assessed.

Animal Assessment:

Initially the animal assessment focuses on identifying the hazard potential for the introduced trait. Proteins introduced to confer herbicide tolerance may have a history of exposure and/or lack a plausible toxicity mechanism. For these products, the lack of hazard potential may eliminate the need for extensive testing and risk assessment. Other proteins, such as Bt proteins, could potentially be toxic to insect species closely related to the target insect pest species. Therefore, a more comprehensive hazard and exposure assessment may be warranted. For pesticidal traits, the US EPA's Subdivision M (OPPTS, 1996) provides for a series of standard laboratory tests to assess hazard (Table 1).

Using the above-mentioned regulatory framework, the first Bt and herbicide tolerant (HT) crops were found to cause no unreasonable adverse effects to non-target organisms (FR, 1994; US EPA, 2003). No observed effect concentration (NOEC) values for Bt proteins were in excess of expected levels of exposure in the field (Table 1). After a product is registered for commercial use in the US, the ecological risk assessment is updated as new information becomes available. For example, when new information became available in the scientific literature on potential effects of Bt proteins on monarch butterflies (Losey *et al.*, 1999), the ecological risk assessment was re-evaluated and a data call-in was issued by the EPA for additional information. Independent scientists evaluated the information and concluded that Bt pollen had negligible impact on monarch butterfly populations (Sears *et al.*, 2001). Therefore, there was no need to initiate additional regulatory action. This process of updating the ecological risk assessment serves to maintain a robust and transparent technical foundation for GM products.

Table 1. Typical hazard assessment data collected for pesticidal GM products in accordance with US EPA guidelines with NOEC examples for Cry1Ab corn and Cry1Ac cotton.

Specific taxa/species	Test material	Cry1Ab*	Cry1Ac*
Beneficial insects			
Honey bee larva	Pure protein	NOEC>20 ppm	NOEC>20 ppm
Honey bee adult	Pure protein	Not reported	NOEC>20 ppm
Ladybird beetle	Pure protein	NOEC>20 ppm	NOEC>20 ppm
Parasitoid wasp	Pure protein	NOEC>20 ppm	NOEC>20 ppm
Lacewing	Pure protein	NOEC>17 ppm	NOEC>17 ppm
Soil organisms			
Earthworm	Pure protein	NOEC>20 ppm	Not reported
Collembola	Pure protein	NOEC>200 ppm	NOEC>20 ppm
Aquatic animals			
Daphnia	Pollen (only corn)	NOEC >100 mg /L	Not reported
Catfish	Grain/meal	NOEC >35% in diet	Not reported
Birds			
Bobwhite Quail	Grain/seed	NOEC > 100 g/kg diet	Not reported

* Data from from Betz et al. (2000) and Sims (1995). No unreasonable adverse effects are predicted because NOECs are in excess of potential environmental exposures.

Recently, the re-registration process was completed for Bt corn and Bt cotton. The products were again found to cause no unreasonable adverse effects on non-target organisms (US EPA, 2001). The regulators requested that, during the continued growing of these crops, additional data be submitted to confirm these findings, specifically for field arthropod data. Since the initial registrations of these products, a number of field studies have been conducted on the Bt crops and on HT soybeans. The following sections summarize field data currently available for non-target arthropods for Bt corn, Bt cotton and RR soybeans in the US that can confirm the results of the initial assessment. In addition, these data are discussed within a context of biodiversity of agricultural ecosystems.

FIELD EXPERIENCE WITH GMO CROPS IN THE US

Industry and academic scientists have used two basic approaches to field assessment: a plot-based approach where measurements are made on plots, typically smaller than standard fields but replicated for additional statistical power, and a monitoring approach where measurements are made on farm-scale fields under actual use conditions.

Bt cotton

Where the product is used, Bt cotton replaces most insecticide use for tobacco budworm, pink bollworm and bollworm control. If these pests are common, the use of Bt cotton results in a substantial reduction in overall insecticide use. For example, Roof & DuRant (1997) studied 10 pairs of farms with Bt cotton and conventional cotton and found that pesticide applications averaged 1.2 for Bt cotton and 4.8 for conventional cotton. Field studies of the non-target impact of Bt cotton have included comparisons with unsprayed conventional cotton and comparisons with conventional cotton treated as needed with insecticides. The former comparison, while not being agronomically realistic, allows the absolute environmental impact of the plant-expressed Bt protein to be assessed. The latter comparison involves the relative environmental impact of the alternative cropping systems, which is the most relevant comparison. Most studies have focused on measuring generalist predator populations because of their acknowledged importance as biological control agents within cotton agroecosystems.

Researchers working in the field have concluded that there are no significant adverse effects on non-pest arthropods in Bt cotton when compared to non-Bt cotton (Table 2) (Armstrong *et al.*, 2000; Hagerty *et al.*, 2001; Head *et al.*, 2001; Naranjo & Ellsworth, 2003). Importantly, significantly larger arthropod predator populations have been observed in Bt cotton fields than in conventionally managed cotton fields as would have been predicted from what is known of the insecticidal spectrum of the commonly used insecticides compared to Bt proteins (Roof & DuRant, 1997; Head et al., 2001; Naranjo & Ellsworth, 2003). For example, in a replicated farm scale study where Bt cotton was paired with conventional cotton production systems, abundance of *Geocoris*, *Orius* and spiders, three generalist predators was found to be significantly higher in Bt cotton (Head *et al.*, 2001). This has important consequences for secondary pest control; more outbreaks of secondary pests such as cotton aphids and beet armyworms are observed in sprayed conventional cotton fields than in Bt cotton field. Comparable results have been seen in a multiple year field studies in China where Wu and Guo (2003) found generalist predator populations to be higher in Bt cotton than in appropriately managed conventional cotton which led to fewer outbreaks of cotton aphids in the Bt cotton fields. Presumably consumer taxa other than predatory insects, including insectivorous birds and mammals, also may be affected by these differences in non-target arthropod populations (Firbank *et al.*, 2003; Watkinson *et al.*, 2000). In addition, the reduced insecticide use will have benefits to wildlife outside of the cotton fields because of reduced drift, lower insecticide levels in water, and lower potential for secondary poisoning of birds. Long-term suppression of target lepidopteran pests through the use of Bt crops may lead to even greater reductions in insecticide use in both Bt cotton and conventional cotton fields, with concomitant benefits for non-target populations (Carriere *et al.*, 2003).

Bt Corn

Comparable studies have been performed with Bt corn to those with Bt cotton with generally similar results (Table 2). Generalist predator populations are not significantly different in Bt corn and unsprayed conventional cornfields (Orr & Landis, 1997; Pilcher *et al.*, 1997; Wold *et al.*, 2001; Jasinski *et al.*, 2003). When Bt cornfields are compared with sprayed corn fields, many groups of non-targets are more common in the Bt fields (Orr & Landis, 1997; Dively & Rose, 2003). For example, Orr & Landis (1997) observed that in a

Table 2: Non-target arthropod field studies conducted on Bt cotton expressing Cry1Ac and Bt corn expressing the Cry1Ab corn in the US.

Type of Assessment	No. years/ No. sites/ Sample method	Results/Conclusions	Reference
Cotton			
Plot-based (16 rows x 27 m)	1 year/1 site/beat-net	No differences in key insect and spider predators inhabiting Bt vs non-Bt cotton	Armstrong *et al.*, 2000
Plot-based (24-40 rows x 18-46 m)	2 years/1 site/visual observations and beat cloth	No difference in predaceous arthropods between GM and non-GM crops	Hagerty *et al.*, 2001
Plot-based (0.03-0.15 ha)	2 years/1 site/beat sampling, sweep nets, whole plants and pitfalls	No negative impacts of *Bt*-cotton but strong effects of spraying non *Bt*-cotton	Naranjo & Ellsworth, 2002
Farm-scale	1 year/10 sites (paired fields)/visual observations	Population of beneficial arthropods slightly greater in *Bt*-cotton	Roof & DuRant, 1997
Farm-scale	1 year/5 sites/beat net	No differences in key insect and spider predators inhabiting Bt vs non-Bt cotton	Armstrong *et al.*, 2000
Farm-scale	1 year/3 sites (paired fields)/visual observations beat sheets	Bollgard preserves natural enemies populations more effectively than broad spectrum insecticides	Head *et al.*, 2001
Corn			
Plot-based (4 rows x 7.6 m)	2 years/1 site/visual observations	No detrimental effects on abundance of the insects observed	Pilcher *et al.*, 1997
Plot-Based (64 x 63 m)	1 year/1 site/visual observations	No significant differences in pest egg populations or its predators and parasitoids	Orr & Landis, 1997
Plot based (4 rows x 9.14 m and 30 x 24.6 m)	2 years/1 site/visual observations	No significant differences were detected for total predator density, or species diversity, of immature beneficial insects	Wold *et al.*, 2001
Farm-scale	1 year/5 sites (paired fields)/visual observations, sticky traps, soil samples, sweep net samples	No consistent significant effect on NTO populations	Jasinski *et al.*, 2003

couple of sampling periods, abundance of Coccinellid adults and lacewing larvae were significantly higher in Bt corn compared to conventional corn. In the US, the insecticide use associated with lepidopteran pests is much lower in corn than in cotton, so, although the use of Bt corn reduced insecticide use, Bt corn is associated with lower insecticide use reduction than Bt cotton. Thus, comparisons of Bt corn fields with sprayed conventional corn fields only are relevant to corn growing areas with relative high pest infestations.

Impacts of Bt corn on specialist parasitoids also have been quantified. The hymenopteran *Macrocentrus grandii* is a parasitoid of the European corn borer. Not surprisingly, in Bt corn fields where the European corn borer is completely controlled, this parasitoid is rare (Venditti & Steffey, 2003). Presumably any effective control tactic for corn borers would have a similar indirect effect on populations of this parasitoid. At a landscape level, the areas of non-Bt corn that are planted as part of the insect resistance management program for European corn borer also will serve as a refuge for *M. grandii*.

Comparable results have been observed in European studies. For example, Bourguet *et al.* (2002) found no significant effects of Bt corn on a variety of non-target predators and secondary pests. However, as in the US studies and presumably for the same reasons, they did observe some impact on specialist parasitoids.

Herbicide Tolerant Crops

Herbicide tolerant crops are not expected to directly affect animals because of the nature of the protein that is expressed in the plant to confer herbicide tolerance. Nonetheless, several field studies have been conducted on Roundup Ready (RR) and other herbicide tolerant soybean varieties in the US (Table 3). Two plot-based studies indicated no impact of soybeans with the RR trait (Buckelew *et al.*, 2000; McPherson *et al.*, 2003). Variation in insect abundance was noted among varieties, however, the differences were correlated to either plant height (Buckelew *et al.*, 2000) or to maturity (McPherson *et al.*, 2003). Jazinski *et al.* (2003) reported on a farm-scale study comparing paired fields of HT soybeans with non-GM soybeans. Farm-scale monitoring systems compare all aspects of the RR system and separation of effects due to weed control efficiency, varietal differences and weather can be difficult. Results reported by Jazinski *et al.* (2003) indicated that in most of the 6 paired soybean fields observed, no difference in arthropod abundance was noted between conventional and RR soybean fields. Decreased abundance of certain predatory species were associated with the HT fields if data were pooled from all sites, however, as discussed below, these differences could reflect phenotypic differences (height or maturity), or in timing of herbicide application, changes in tillage practices or other differences. Overall these reports indicate no adverse impact of herbicide tolerance trait on arthropods compared to conventional non-transgenic soybeans.

The plot-based studies mentioned above compared soybeans grown under similar tillage systems. An important aspect of the RR system is that producers plant a significantly higher percentage of acres using no-till or reduced tillage systems compared to producers using conventional soybean (American Soybean Association, 2001). A survey conducted by the American Soybean Association (2001) revealed that 54% of farmers interviewed credited RR soybeans as a factor that had the greatest impact in their adoption of reduced tillage or no-tillage in soybean production. Arthropods have been shown to be more abundant and diverse in minimal tillage crop fields compared to crops grown under conventional tillage (Warburton & Klimstra, 1984; Steffey, 1995) likely due to the increased ground cover (Witmer *et al.*, 2003). Although the effect of tillage was not specifically investigated in the soybean studies discussed above, the increase in productivity of soil and arthropods is likely to be associated with increased ground cover from the decreased tillage in RR soybeans.

Table 3: Arthropod field studies conducted on herbicide tolerant soybeans in the US.

Type of Assessment	Number of years/ Number of sites/ Sample method	Results/Conclusions	Reference
Plot-based (6-8 rows x 15.1 m)	2 years/ 5 sites/sweepnet	No differences in seasonal abundance of arthropod pests.	McPherson *et al.*, 2003
Plot-based (6 x 7.3m and 6 x 6 m)	1 year/2 sites/sweep net	No apparent direct effect of RR soybeans on arthropod populations although weed management can affect insect populations.	Buckelew *et al.*, 2000
Farm scale	1 year/6 sites (paired field)/yellow sticky traps and sweep net	No differences in arthropod populations. Reanalysis of data pooled across sites noted lower population of one insect predator in GM soybeans.	Jazinski *et al.*, 2003

Buckelew *et al.* (2000) and McPherson *et al.* (2003) demonstrated that the degree of weed control can have significant effects on arthropod abundance. Other studies with RR crops have shown that modification of the timing of herbicide application can lead to different levels of weed biomass and of ground cover (Dewar *et al.*, 2002; Witmer *et al.*, 2003). These studies indicate that herbicide tolerant crops can provide an important tool to manage weed biomass which can lead to in-field increases in invertebrate biodiversity for production systems where that is an objective compatible with crop production.

BIODIVERSITY IN THE AGRICULTURAL LANDSCAPE

The purpose of collecting data on in-field abundance of arthropods for Bt and HT crops is twofold. First, to determine if the genetically modified plant itself or the introduced trait has a direct adverse effect on arthropod abundance. As discussed above, studies to date in the US show no consistent adverse effects. A second purpose is to ascertain if the long-term use of the GM crops will result in changes in biodiversity in the agroecosystem. Recent studies, mainly in the UK, have indicated that the intensification of agriculture, through a variety of mechanisms, can lead to decreases in biodiversity (Robinson & Sutherland, 2002; Weibull *et al.*, 2003). GM crops can potentially mitigate some of the concerns of agricultural intensification providing a highly targeted, highly productive set of pest control solutions that can replace less environmentally compatible pesticide alternatives while, at the same time, increasing agricultural productivity and potentially improving wildlife habitat. In addition, GM crops can provide farmers with greater flexibility in their management operations and can provide opportunities to increase biodiversity within agricultural fields. For example, HT crops facilitate reductions in tillage, as has been observed with RR soybeans in North America which can increase invertebrate productivity (see above). A more innovative example comes from Brooms Barn in the UK where researchers showed that RR sugar beets enabled farmers to delay and reduce herbicide applications, thereby increasing the biomass of weed in sugar beet fields and providing food and shelter for non-target arthropods and other wildlife (Dewar *et al.*, 2002). Of course providing improved tools to farmers is only part of the solution if the aim is to preserve and increase biodiversity in agricultural landscapes. Agricultural policies also must encourage farmers to adopt appropriate agricultural practices. Nevertheless, the demonstrated and potential benefits of GM crops are such that their use can be expected to continue to grow. One of the responsibilities of scientists and policy makers will be to ensure that GM crops are used in ways that maximize their value.

CONCLUSIONS

- No unacceptable adverse effects are predicted for non-target organisms including beneficial insects, soil invertebrates, aquatic organisms, birds and mammals exposed to Bt corn, Bt cotton, and HT soybeans based on regulatory evaluation in the US.
- Many field studies have been conducted and the results to date for specific field tests, as well as some monitoring studies, for both Bt crops and herbicide tolerant crops suggest no consistent unexpected or adverse changes in non-target arthropod abundance relative to direct effects of the introduced trait.
- Both Bt and herbicide tolerant crops have been shown to have direct and indirect benefits for agroecosystems in the US through the replacement of broad-spectrum pesticides and the facilitation of reduce tillage which can lead to increased local biodiversity
- GM crops can potentially mitigate some concerns associated with agricultural intensification by providing a highly targeted, highly productive set of pest control solutions that can replace less environmentally compatible alternatives while, at the same time, increasing agricultural productivity and potentially improving wildlife habitat.

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The environmental impact of controlling weeds using broad spectrum herbicides in genetically modified herbicide tolerant crops: the farm scale evaluations explained

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Evaluation of transgenic herbicide-resistant oilseed rape and maize with respect to integrated pest management strategies

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ABSTRACT

In the present study, weed control in glufosinate-resistant maize and rape was comparable to that in conventional crops but was associated with fewer biological risks. New weeds emerged after glufosinate treatment. These plants increased the in-field biodiversity during vegetation but deposited their seeds in the soil, which can lead to later weed control problems. In maize, dead floral mulch and new weeds that emerged after treatment provided only a small degree of protection against soil erosion. Out-crossing of transgenic rape into neighbouring non-transgenic rape was far below the proposed thresholds of 0.5 % and 0.9 %. We believe that conservation tillage systems with herbicide-resistant crops have a greater potential in promoting integrated pest management than same systems that do not use transgenic herbicide-resistant varieties.

INTRODUCTION

Of all the options available for indirect and direct plant protection in integrated pest management (IPM), the application of pesticides should be used as the last choice and should be minimised to reduce the risk of harm to non-target organisms (Burth & Freier, 1996). Farmers growing transgenic herbicide-resistant (HR) crops commit themselves at a very early stage to use a specific herbicide. Alternative weed control measures then play a subordinate role. Without herbicides, many crops cannot be grown economically in accordance with the principles of IPM. This is especially relevant to countries like Germany, where an increasing proportion of farming land is managed by conservation tillage. Hence, the negative and positive economic and ecological effects of HR crops must be carefully considered when attempting to implement IPM strategies in agricultural practice.

Since 1996, the Biologische Bundesanstalt (BBA) in Kleinmachnow has been conducting a long-term field trial in conventional and HR maize and rape to elucidate the following key issues:

1. Effects of frequent glufosinate use on field flora after several crop rotations;
2. Consequences of volunteer HR rape in maize resistant to the same herbicide;
3. Ecological effects of new weeds that emerge in maize fields after glufosinate treatment;
4. Ecotoxicological differences in herbicide treatment strategies in conventional and herbicide-resistant maize and oilseed rape;
5. Potential impact of out-crossing of different rape varieties on the coexistence of farms cultivating transgenic and non-transgenic oilseed rape.

MATERIALS AND METHODS

The BBA trial of transgenic HR rape and maize was performed in fields in Dahnsdorf, Brandenburg, a site characterised by silty sandy soil with 1.42 % organic matter, an annual mean temperature of 8.4 °C, an average annual precipitation of 536 mm, and a pronounced pre-summer dryness period. The study, which was initiated in 1996, was designed as a randomised block field trial with four replicates and four courses of crop rotation, from winter rape to winter rye to maize to winter wheat (figure 1). Each crop rotation field (18 m x 20 m = 360 m²) was divided into three plots of equal size, where the following variants of oilseed rape and maize were grown:

Variant 1: Conventional rape treated as needed with metazachlor, quinmerac, carbetamide, dimefuron or fluazifop-P, and conventional maize treated as needed with metolachlor, pyridate or terbuthylazin.
Variant 2: Glufosinate-resistant rape (event GS 40/90) and maize (event T 25) with intensive glufosinate treatment, i.e. high-dose or two applications.
Variant 3: Glufosinate-resistant rape (event GS 40/90) and maize (event T 25) with extensive glufosinate treatment, i.e. low-dose or one application.

All three variants were subjected to uniform tillage, fertilisation and other plant protection measures. The kind of weed species and their abundance (weed-coverage) were determined before and after herbicide treatment. The yield of oilseed rape, maize, rye and wheat, and the feed values of maize were also determined. An ecotoxicological analysis based on the SYNOPS model (Gutsche & Roßberg, 1997), which considers the biological risk potentials of herbicides for fish, daphnia, earthworm and alga, was also performed in all three variants of maize and rape.

In order to reduce the transmission of transgenic rape pollen to fields with conventional rape, ruderal rape or wild relatives, the 16 test fields were surrounded by an unbroken strip of isogenic rape (7.5 m in width) positioned 15 to 35 m away (figure 1). To calculate the proportion of out-crossing, the rape seedlings yielded in the catch crop strip were treated with glufosinate under greenhouse conditions, and the survivors were genetically tested by PCR.

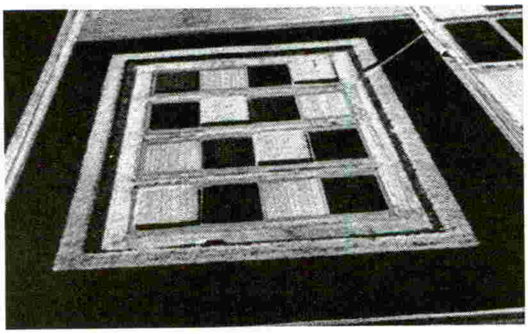

Figure 1. Aerial view showing the 4 fields of transgenic herbicide-resistant winter rape and maize surrounded by a strip of isogenic rape positioned a variable distance away; here, one crop rotation with rye and wheat has been completed. (Photo: Baier, 05/2001)

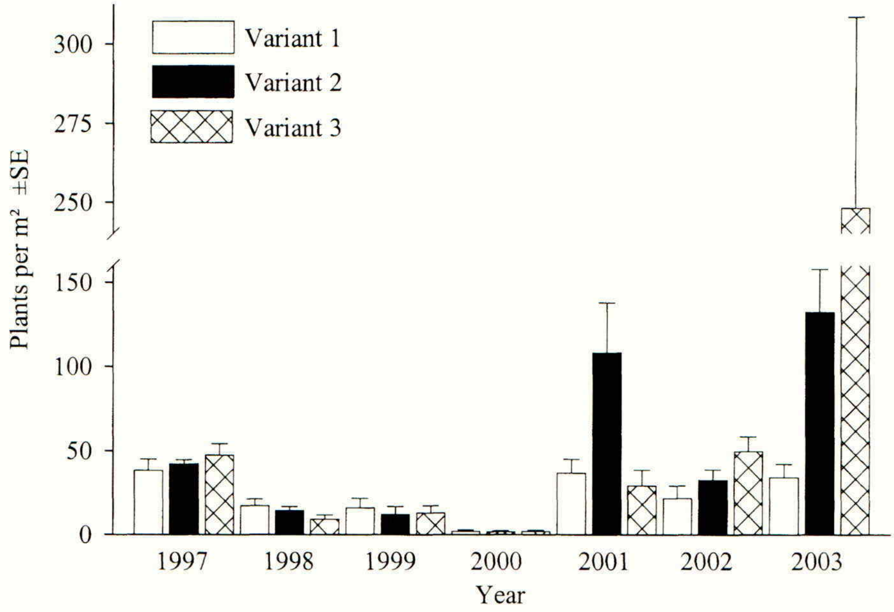

Figure 2. Annual abundance of *Chenopodium album* in maize before herbicide treatment (first crop rotation 1997 – 2000).

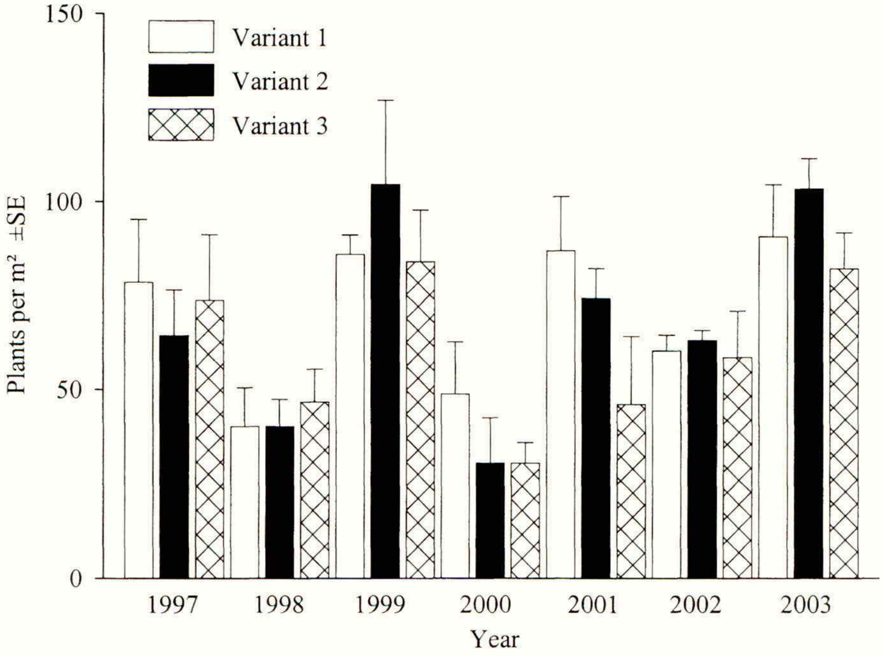

Figure 3. Annual abundance of *Viola arvensis* in maize before herbicide treatment (first crop rotation 1997 – 2000).

RESULTS

Chenopodium album and *Viola arvensis* were the most commonly observed weed species in the maize fields. *C. album* had similar abundance in all variants during the first crop rotation (1997 – 2000), but a marked annual increase has been observed in variants 2 and 3 since the second crop rotation in 2001 (figure 2). In the case of *V. arvensis*, the abundance levels registered during the first rotation persisted during the second crop rotation in all variants (figure 3). *Polygonum spp.*, another frequent weed genus, showed a trend similar to that of *C. album*.

In maize variant 1, *C. album* and *V. arvensis* coverage has remained very low 4 weeks after herbicide treatment since 1997 (table 1), and a small degree of diversity and abundance of associated floral species has persisted until the September harvest each year. New *C. album* emerged after glufosinate application in maize variants 2 and 3. In 2 of 3 years, *C. album* was almost as abundant 4 weeks after treatment as before treatment (table 1). In most cases, *V. arvensis* was not satisfactory controlled by glufosinate (table 1). The high degree of coverage by *V. arvensis* 4 weeks after treatment was usually attributable to growing plants which had already emerged before glufosinate treatment. The degree of coverage of *Polygonum* spp. in all maize variants did not exceed 0.2 % until 4 weeks after treatment.

Beginning with the second rotation in 2001, glufosinate-resistant volunteer oilseed rape emerged in maize variants 2 and 3; these plants predictably survived glufosinate treatment. Glufosinate was successfully supplemented with nicosulfuron or rimsulfuron in 2001 and 2002, respectively. Both of these herbicides and variable doses of glufosinate have adequately controlled weed growth in the HR crops during last 7 years. Yields of rape and maize variants 2 and 3 were comparable to those of the respective standard crop (variant 1), i.e. no significant differences observed. The pretty higher percentage of crude fibre observed in maize variants 2 and 3 in certain years was not significant.

Table 1. Effect of herbicide on *Chenopodium album* and *Viola arvensis* coverage (% ± SE) in maize 0 and 28 days after treatment (DAT).

Variant	*Chenopodium album*		*Viola arvensis*	
	0 DAT	28 DAT	0 DAT	28 DAT
Variant_1_2001	1.00 ± 0.204	0.08 ± 0.025	0.75 ± 0.144	0.08 ± 0.025
Variant_2_2001	1.25 ± 0.323	0.88 ± 0.125	0.50 ± 0.000	1.13 ± 0.125
Variant_3_2001	0.68 ± 0.197	0.75 ± 0.166	0.50 ± 0.000	1.00 ± 0.000
Variant_1_2002	1.13 ± 0.375	0.00	1.00 ± 0.204	0.03 ± 0.025
Variant_2_2002	0.88 ± 0.125	0.10 ± 0.000	1.00 ± 0.204	1.88 ± 0.315
Variant_3_2002	1.50 ± 0.204	0.20 ± 0.100	0.88 ± 0.125	1.50 ± 0.204
Variant_1_2003	0.30 ± 0.000	0.00	0.30 ± 0.000	0.00
Variant_2_2003	0.53 ± 0.165	0.40 ± 0.058	0.40 ± 0.058	0.53 ± 0.165
Variant_3_2003	1.15 ± 0.202	0.58 ± 0.149	0.53 ± 0.103	0.45 ± 0.050

The proportion of out-crossing of HR transgenic rape into the conventional rape cultivated as a catch crop strip around the experimental fields ranged from a mean 0.026 % to 0.13 % (table 2).

The distance between the transgenic pollen donor and the nearest recipient was roughly 25 m, 35 m and 15 m in 1998, 1999 and 2001, respectively. In the isogenic rape (variant 1), the percentage of out-crossing ranged between 1 % and 3 %.

Table 2. Occurrence of transgenic HR rape seeds in isogenic rape harvested in a pollen catch crop strip (approx. 0.5 ha) in 1998, 1999 and 2001.

Season	Number of sampling points	Seeds per sample	Plants treated (total)	Surviving plants (total)	Out-crossing rate [%]
1997/98	187	200	35,599	31	0.090
1997/98	14	1,000	13,396	18	0.130
1998/99	222	ca. 200	ca. 42,000	11	0.026
1998/99	14	1,000	13,641	10	0.070
2000/01	116	1,000	107,957	37	0.034

DISCUSSION

Allowing a pretty wide period for herbicide application in all HR crops increases the flexibility of weed control and enables better compliance with herbicide thresholds (Hommel & Pallutt, 2000). The addition of glufosinate to the list of post-emergence herbicides for oilseed rape and maize is therefore in the interest of IPM. Nevertheless, a second application of glufosinate is often required about 2 to 3 weeks after initial treatment for adequate weed control in maize. This increase in treatment intensity prevents the excessive accumulation of *C. album* seeds in the soil. The unsatisfactory effect of glufosinate on *V. arvensis* was reflected by a moderate to high abundance of this weed species in oilseed rape and maize. However, this gap in glufosinate activity in these crops is ecologically desirable because of the low competitive ability of *V. arvensis* (Schulte, 1999).

Except for the occurrence of HR volunteer rape in maize as a new competitive weed, the different glufosinate treatment intensities (from 0 litre/ha to 14.5 litres/ha) in the three rape and maize variants did not lead to differences in weed species diversity in any of the 7 years studied (Hommel & Pallutt, 2002).

The volunteer rape made it necessary to supplement glufosinate with another herbicide (Stelling, et al., 2000), which naturally offsets the economic and ecological advantages of HR crops. Therefore, when cultivating HR rape according to the principles of IPM, another crop resistant to the same herbicide should not be used within a given crop rotation.

Neither the dead mulch in maize nor the newly emerged weeds that survived 1 or 2 glufosinate treatments reached coverage levels capable of protecting the soil from wind or water erosion. None the less, the diversity and abundance of epigeous fauna in HR maize fields was probably still greater than that in conventional maize. The use of HR crops in special erosion-prevention systems with conservation tillage has some advantages over the use of conventional crops. For example, the intensity of herbicide use (e.g. dose, number of active compounds) is often lower, and the wider spraying window gives the farmer increased flexibility in selecting an optimum spraying date.

The out-crossing of transgenic HR rape to conventional rape can not be entirely prevented (Dietz-Pfeilstetter, et al., 2003). Therefore, practicable thresholds like 0.5 % for non-commercialised and 0.9 % for commercialised transgenic varieties, as recommended by the EC, are absolutely necessary. Abstention from transgenic rape growing in certain regions also seems to be an acceptable solution. Because of the long dormancy of oilseed rape (Pekrun, et al., 1997), the occurrence of volunteer transgenic rape in conventional rape fields poses an important obstacle to their coexistence. However, this problem is still less often investigated because the pollen donor plants can probably cause higher proportions of out-crossing than transgenic pollen originating from outside the field.
Compared to conventional oilseed rape and maize managed with herbicides such as metazachlor, quinmerac, carbetamide, dimefuron, fluazifop-P, metolachlor, pyridate, and terbuthylazin, the use of glufosinate in herbicide-resistant transgenic rape and maize has distinct ecotoxicological advantages because of glufosinate's lack of effect in the soil, the often later need for herbicide application, and the subsequently higher degree of crop and weed coverage, which reduces the biological risks of chemical weed control (Hommel & Pallutt, 2000).

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Life cycle and gene dispersal of oilseed rape volunteers (*Brassica napus* L.)

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ABSTRACT

To assess gene dispersal from oilseed rape volunteers, the whole life cycle of deliberately broadcast seeds and seed losses during harvest was observed in four different tillage operations. Treatments 1 and 3 were immediate stubble tillage, later followed by primary tillage with a plough or cultivator. In treatment 2, the stubble tillage was delayed for four weeks and later followed by primary tillage ploughing. Treatment 4 was zero tillage. The following crop in all treatments was winter wheat. In autumn, between about 7 and mostly less than 50% of the initial rape seeds emerged, whereas volunteer emergence was less than 0.01% in spring. Depending on the treatment, a seed bank was built up reaching up to 30% of the initial number of seeds. The seed bank was largest when seeds were immediately incorporated into the soil by stubble tillage. Up to 85% of seeds could not be found; the heaviest losses occurring when the seeds remained on the soil surface for a while in treatments 2 and 4. When volunteers flowered at the same time as an oilseed rape crop, gene dispersal in space by pollen transfer was possible. Flowering volunteer plants could be observed mainly in treatments 3 and 4, at a population density of up to 0.78 plants m^{-2} in treatment 3. A new generation of seeds could be produced by the volunteers that may found a further cohort of volunteers and enable gene dispersal in time or, in case of volunteers emerging in a rape crop, be harvested with the crop.

INTRODUCTION

With regard to a future labelling threshold of genetically modified (GM) food or feed in the EU, possible mixing of transgenic and conventionally bred crops is gaining in importance. Mixing of GM and conventional crops could occur by pollen-mediated gene flow between fields or by GM volunteers appearing within a conventional crop and then outcrossing within the field or being harvested. Since oilseed rape pods are not completely resistant to shattering, considerable seed losses can occur before or during harvest. Additionally, these seeds can become secondarily dormant under particular environmental conditions (Pekrun et al., 1998), persist for years in the soil (Roller, 2002) and emerge from this soil seed bank years later. Although volunteers are quite easily controlled by mechanical or chemical operations, control of volunteer rape in a rape crop is not possible. Soil tillage after oilseed rape harvest can affect whether spilled seeds become secondarily dormant or persistent (Gruber et al., 2003) by shifting the seeds into particular soil layers with different light, water or gaseous conditions. Therefore, the number of volunteer plants can be expected to vary depending on the method of tillage. Since it is known from laboratory experiments (Pekrun et al., 1997; Gruber et al., 2002) that genotypic differences exist in the level of dormancy, this capacity could additionally affect the number of emerging volunteers. The aim of this study was to observe the life cycle of volunteers from rape seed artificially broadcast on the soil in a defined number and from seed losses obtained under normal conditions during harvest. Different rape seed genotypes and tillage operations after harvest were factors to be examined for their effects on the number and time of emergence of volunteers, their flowering and seed production and subsequently their potential for gene flow.

MATERIALS AND METHODS

The experiments were set up 2001 and 2002 on the experimental station of the University of Hohenheim 'Ihinger Hof' near Stuttgart in south-west Germany (N 48° 44'/ E 8° 55'; altitude 450 m a. s. l., 689 mm mean annual precipitation, 8.0 °C mean annual temperature) on a loamy soil. In the first experiment (E1, artificial seed losses, established 2001 and 2002), 10,000 rape seeds were broadcast in July on a clean cereal stubble simulating harvest losses of a rape crop. The rape seed cultivars tested were Liberator and Artus, both near-isogenic to the transgenic, herbicide-tolerant cultivars Lilly^{LL} and Avalon^{LL}. In the second experiment (E2, practical seed losses, established 2002) the oilseed rape cultivar Liberator was grown and harvested normally. The experimental design was a split plot design with four replications in E1 and a block design with four replications in E2. The extent of seed loss was determined by catching the seeds on cloths sized 50 x 70 cm that were placed under the standing rape crop shortly before harvesting. After seeds had arrived at the soil surface either by broadcasting or by practical shedding, four different treatments of soil cultivation were performed (Table 1).

Table 1. Tillage treatments, implements and cultivation depth used in the experiments

Treatment	Stubble tillage (rotary tiller, 10 cm)	Primary tillage in autumn
T 1	Immediately	Plough (25 cm)
T 2	4 weeks delayed	Plough (25 cm)
T 3	Immediately	Cultivator (15 cm)
T 4	None	None

Stubble tillage followed immediately (within 24 hrs) after seeds had dropped to the soil in T1 and T3, or with four weeks delay in T2. No tillage was performed in the zero tillage treatment T4. The primary tillage in T1 and T2 was ploughing, and cultivating with a rigid tine cultivator in T3, all shortly before sowing the following crop winter wheat. Sowing in T4 was performed by direct drilling. A germination test determined the viability of the seeds used in the field.

Because of wet weather in autumn 2002, primary tillage and subsequent sowing of winter wheat was done with about two months delay (E1: January 9th 2002; E2: December 9th 2003) compared to the previous year (E1: October 31st 2001) and to the common sowing date for this region. Before drilling, a non-selective herbicide was applied in all treatments. No further herbicides were used after drilling to enable rape volunteers to grow.

In autumn and spring following the harvest of oilseed rape or the broadcast of seeds the mean emergence of rape volunteer seedlings was recorded on 0.25 cm^{-2} (total of 10 single positions) per plot shortly before stubble tillage (only T2) and/or primary tillage or direct drilling (all treatments). For determination of the soil seed bank, 40 soil samples were taken in a depth of 0-30 cm in spring in each plot. In 2002, soil sampling took place when the seed germination had already started, and shortly before this date in the year 2003. During the following vegetation period, the number and date of flowering and fruiting rape seed volunteers in the winter wheat was determined until harvest. In experiment 1 set up in 2001, seedlings emerging from the soil seed bank in the second spring (2003) after broadcast of seeds were also counted.

The statistical analysis was performed in SAS with the MIXED procedure and Satterthwaite's test. To meet the standards of the ANOVA, data were transformed ln (x + 0.1) if necessary.

RESULTS AND DISCUSSION

Seed outcome experiment 1 (artificial seed losses)

In experiment 1, about 50 to 85% of the seeds could not be registered in one of the surveys in both experimental years (Tables 2 and 3). The highest unknown losses occurred in T2 and T4 in both years, when seeds laid on the soil surface for a while.

Table 2. Outcome (mean) of broadcast rape seeds from two cultivars in E1 affected by tillage treatments from 2001 until 2002 (according to Gruber et al., 2003)

	% of 10,000 seeds m^{-2} initially broadcast			
Survey	T 1	T 2	T 3	T 4
		Liberator		
Autumn emergence 2001	12.3	12.0	12.4	7.1
Spring emergence 2002	0.0	0.0	5^{-3}	0.0
Seed bank spring 2002	0.8	0.0	1.9	0.0
Non viable	18.7	18.7	18.7	18.7
Unregistered loss	68.2	69.3	67.0	74.2
Spring emergence 2003	9^{-3}	8.8^{-4}	3.13^{-3}	1.81^{-3}
		Artus		
Autumn emergence 2001	25.9	12.6	19.1	11.3
Spring emergence 2002	1.0^{-2}	0.0	9.0^{-2}	8.8^{-4}
Seed bank spring 2002	4.1	1.5	9.8	0.0
Non viable	3.5	3.5	3.5	3.5
Unregistered loss	66.5	82.4	67.5	85.2
Spring emergence 2003	5.0^{-2}	1.1^{-3}	8.9^{-3}	3.1^{-4}

Table 3. Outcome (mean) of broadcast rape seeds from two cultivars in E1 affected by tillage treatments from 2002 until 2003

	% of 10,000 seeds m^{-2} initially broadcast			
Survey	T 1	T 2	T 3	T 4
		Liberator		
Autumn emergence 2002	26.0	34.4	26.4	22.4
Spring emergence 2002	1.1^{-3}	0.0	1.3^{-2}	4.0^{-4}
Seed bank spring 2003	9.8	0.0	14.0	0.8
Non viable	13.0	13.0	13.0	13.0
Unregistered loss	51.1	52.6	46.6	63.8
		Artus		
Autumn emergence 2002	31.1	35.3	32.7	22.1
Spring emergence 2002	7.0^{-4}	0.0	6.4^{-3}	2.0^{-4}
Seed bank spring 2003	7.9	0.4	3.8	1.5
Non viable	1.7	1.7	1.7	1.7
Unregistered loss	59.3	62.6	61.8	74.7

The second highest sink of the seeds was the autumn emergence ranging from about 7 to 35% of all initial seeds depending on cultivar and treatment. The smallest emergence was observed in the direct drilling treatment T4.

A maximum of 0.09% of the broadcast seeds was recorded as seedlings by the spring counting. The soil seed bank consisted of up to 14% of all broadcast seeds, depending on treatment and year. The highest levels resulted T1 and T3 with an immediate incorporation of the seeds in the soil after broadcasting. Differences observed in 2001 were only significant between the treatments within the cultivar Artus. In 2002 significant differences in the soil seed bank occurred between T1 and T3 on the one hand and T2 and T4 on the other hand within both cultivars (Table 4).

Table 4. Soil seed bank of oilseed rape seeds affected by tillage operations derived from harvest seed losses or deliberately broadcast seeds. In italics: *transformed data*, E1 2002 *ln (x + 0.1)*; SEM E1 2001: 141.0, E1 2002 (*transformed*): *1.30*, E2: 63.0; no significant differences (Satterthwaite's formula, α=0.05) between values with same letters; comparison within one experiment (E2) or within the same cultivar and year (E1) only.

Experiment	Cultivar	Soil seed bank (mean number of seeds m^{-2}, *transformed data*)				Soil seed bank (mean number of seeds m^{-2}, absolute data)			
		T1	T2	T3	T4	T1	T2	T3	T4
2001									
E1	Liberator	-	-	-	-	76^{A}	0^{A}	189^{A}	0^{A}
	Artus	-	-	-	-	416^{B}	$151^{B,C}$	982^{A}	0^{C}
2002									
E1	Liberator	*6,81*^{A}	*-2,30*^{B}	*7,05*^{A}	*-0.30*^{B}	983	0	1399	75
	Artus	*6,59*^{A}	*-0.47*^{B}	*5.70*^{A}	*-0.13*^{B}	794	38	378	151
E2	Liberator	-	-	-	-	378^{A}	38^{B}	$189^{A,B}$	$227^{A,B}$

These results can be attributed to the tillage operations that shifted the seeds in deeper, dry and dark soil layers in T1 and T3 where secondary dormancy can be induced. When lying on the soil surface for a longer period, seeds cannot become secondarily dormant and can be destroyed or damaged by seed predators and other environmental factors. If the seeds germinated, soil cultivation, herbicides and herbivores would also lead to a reduction of the plants. In spring 2003, a maximum of 0.05% of the seed bank recorded in E1 in the previous year emerged in the second following crop (maize). Overall, most plants were found in T1 with both cultivars tested. In the second experimental year, the autumn emergence was higher than 2001, probably due to the wet weather conditions. Nevertheless, a similar or higher level of persistent seeds was found in the soil seed bank in 2002 compared to 2001. Due to the increase of autumn emergence and the size of the soil seed bank, fewer seeds vanished without leaving recorded traces in the second experimental year.

Seed outcome experiment 2 (practical seed losses)

The harvest losses in experiment 2 were 1324 seeds m^{-2} on average, about 1.5% of the yield. Except T4, the main outcome of the seeds was the autumn emergence 2002 (Table 5).

Table 5. Outcome (mean) of seed losses during harvest from the cultivar Liberator in E2 affected by tillage treatments from 2002 until 2003

	(% of 1324 initial seeds lost m^{-2})			
Survey	T 1	T 2	T 3	T 4
	Liberator			
Autumn emergence 2002	49.0	90.4	61.1	32.2
Spring emergence 2002	0.0	0.0	0.04	0.07
Seed bank spring 2003	28.5	2.9	14.3	17.1
Non viable	0.3	0.3	0.3	0.3
Unregistered loss	22.1	6.4	24.3	50.3

Nearly 90% of all seeds emerged after delayed stubble tillage and primary tillage plough (T2), and about 60% and 50% in T3 and T1 respectively. A third of all lost seeds germinated without any tillage (T4). The high autumn emergence in T2 may be a result of the delayed stubble tillage that enabled ungerminated seeds to emerge after being triggered by soil cultivation. Because of high autumn germination, numbers entering the seed bank and unregistered losses were low. Maybe this mechanism particularly worked in the wet autumn 2002 and under the straw mulch in experiment 2, since it is not so apparent in experiment 1.

Between about 3 and 30% of the seeds were incorporated into the soil seed bank, with the smallest contribution to the seed bank occurred in T2 and the highest in T1. Only the differences between T1 and T2 were significant (Table 4, last line). Also in T4, without any soil movement by tillage, a considerable soil seed bank was built up by 17% of the seed losses. The level of germinated or otherwise registered outcome of seeds was higher in experiment 2 than in experiment 1. Seeds may have had better protection from predators or better germination conditions under the straw mulch in experiment 2. Also the comparatively high soil seed bank – even in T4 without any soil movement – may be a result of the covering straw mulch that kept the seeds in darkness and led to a better induction of secondary dormancy.

Flowering volunteers

Most flowering volunteers were observed in T3 and T4 (Table 6) with up to almost 0.8 plants

Table 6. Flowering oilseed rape volunteers of two cultivars affected by different tillage treatments T1-T4 in two experiments and years; results E1 2001 according to Gruber et al. 2003, May 2002, E1 and E2 2002: June 2003. No significant differences (Satterthwaite's formula, $\alpha=0.05$) between treatments with same letters; comparison within the same cultivar and year only

	T1	T2	T3	T4	SEM
		Mean of plants m^{-2}			
E1 2001					0.02
Liberator	0.00^{A}	0.00^{A}	0.03^{A}	0.05^{A}	
Artus	0.00^{C}	0.00^{C}	0.09^{B}	0.33^{A}	
E1 2002					0.05
Liberator	0.11^{B}	0.01^{B}	0.78^{A}	0.07^{B}	
Artus	$0.03^{A,B}$	0.01^{B}	0.15^{A}	$0.02^{A,B}$	
E2 2002					0.04
Liberator	0.13^{B}	0.11^{B}	0.59^{A}	0.55^{A}	

m^{-2} in experiment 1 from 2002. The highest number of flowering volunteers in experiment 1 established 2001 was observed in T4 with a volunteer density of about 0.3 plants m^{-2}, and in experiment 2 in T3 and T4, both with nearly 0.6 plants m^{-2}. In contrast to the first experimental year when volunteers and sown rape crop flowered simultaneously, the volunteers from harvest 2002 started flowering at the end of the common flowering period of oilseed rape in the region. Gene transfer by pollen consequently was possible in the 2001 experiments, though not likely in the 2002 experiments. It has to be proved whether the volunteers of the current year will produce ripe and viable seeds as they did the year before.

CONCLUSIONS

In terms of the whole life cycle, autumn emergence could contribute to the rapid removal of all spilled seeds, maybe especially in wet years. Hence stubble cultivation for inducing maximal emergence seems to be useful, although the time of the tillage operation is critical to prevent the build up of a soil seed bank. Since the persistence was higher when seeds were immediately incorporated into the soil compared to (temporarily) uncovered seeds, the cultivation system 'immediately incorporated' would entail the highest risk of gene flow in time. To minimise the soil seed bank and subsequent gene dispersal, the first soil cultivation after seed shedding should be delayed. A zero tillage system can result in many flowering volunteers. Therefore gene transfer by pollen and newly produced seeds can be minimised according to this study with delayed stubble tillage and subsequent use of plough for primary tillage. Although no reproducible differences could be observed between the cultivars in both experimental years, an influence of genotype on seed persistence generally seems to exist.

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