Volunteer potential of genetically modified oilseed rape with altered fatty acid content


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Abstract

Two experiments were undertaken to compare seed survival and potential persistence problems of transgenic oilseed rape modified to contain either, stearic acid, or lauric acid, as well as their parental lines. Two conventional varieties (Mars and Starlight) as well as two weed species (Sinapis arvensis and Brassica nigra) were used in the experiments. The first experiment simulated seed loss to the environment at both planting and harvest time with germination characteristics of the lines/species considered. The second experiment investigated seed loss to the environment on either cultivated or uncultivated land and simulated losses to field margins or roadside verges during transit. None of the line/species used was able to reach sexual maturity due to the competitive effect of the vegetation in the plots, and after 7 years, there was no evidence that any of the line/species had survived. Seed persistence varied between both line/species and according to when seed were deposited. The trials provide evidence to suggest that the conventional varieties and the weed species used were more likely to persist in the environment than either of the modified lines or their parents.

Keywords: Transgenic; Genetically modified; Oilseed rape; Brassica napus; Lauric acid; Stearic acid

1. Introduction

Major concerns have been expressed regarding the release of genetically modified crop species into the environment (Linder and Schmitt, 1995). The main environmental concerns are that the transgenic crop will either establish a feral population, or will cross-pollinate with one of its weedy relatives and subsequently establish a population with similar characteristics to the transgenic crop (Darmency, 1994; Raybould and Gray, 1994; Davenport et al., 2000; Wilkinson et al., 2000). If a competitive advantage over the existing species within that habitat is shown, the field ecology may be permanently affected (Glidson, 1994). Ecological problems associated with such a release are, as yet, not fully understood. The probability of transgene escape depends on the type of crop that is being grown (Linder and Schmitt, 1995). Crops such as sugar beet or turnips whose commercial production does not normally produce seed or asexual propagules are less likely to be the cause of transgene escape than crops in which the harvestable portion is the seed (e.g. oilseed or grain crops). However, there is always a chance that seed may be split accidentally on either the roadside verges leading to or from the field or on the field margins themselves prior to sowing. Additionally, seed dormant in the soil or dispersed prior to harvest may lead to establishment of feral or hybrid populations of the crop containing the transgene (Linder and Schmitt, 1994).
The probability that some transgenic crops or their progeny will grow where they are not intended appears to be high and it is necessary to quantify the likelihood of such populations becoming established and the impact they are likely to have on the field ecology and the environment (Crawley et al., 1993). It will be necessary to develop cultural practices that reduce the probability of the transgenes escaping and minimise the effect of the transgenic populations on the environment in the event of an escape.

Conventional oilseed rape can persist in fields after harvest as volunteers in subsequent crops. Genetic modification itself does not alter the fitness of a plant to become established (Crawley et al., 1993), but it has been suggested by Linder and Schmitt (1995) that modifications of seed oils may influence seed longevity and seedling establishment, because seed oils play a part during dormancy and are the primary carbon source utilised before photosynthesis (Levin, 1974; Mayer and Poljakoff-Mayber, 1982).

Genetic engineering of oilseed rape (Brassica napus L.) is relatively advanced compared to other crop species. Various traits have already been genetically modified, including alterations to the range of fatty acids produced in rapeseed. Two such modifications are the amplification of stearic acid, a fatty acid with industrial and food uses, normally found at low levels (Knutson et al., 1992) and the introduction of lauric acid, a fatty acid not normally found in rapeseed but with commercial value in the detergent and food industries. The latter was introduced from the Californian bay plant (Umbellularia californica Hout. & Arn. Nutt.) (Voelker et al., 1992), traditional sources of lauric acid being coconut and palm kernal oils (Mithen and Murphy, 1995).

Two experiments were used to assess the ecological consequences of introducing transgenic rapeseed into the environment. The aims were to determine whether rape lines with modified oil types differed in terms of invasiveness and persistence from conventional lines and weedy Brassica species under northern European growing conditions.

2. Materials and methods

A seed burial experiment was used to evaluate the volunteer potential of seed of various oilseed rape lines and weed species, and a seed scattering experiment was used to evaluate the likelihood of seed inadvertently dispersed in the field margins germinating and establishing viable populations. A high stearate (cv. 3242) and a high laurate line (LA002) were the transgenic lines assessed. These were compared with two parent lines of the transgenics (cv. A112 and 212/86), two conventional oilseed rape varieties (cv. Mars and Starlight) grown commercially in Scotland and two native weeds Sinapis arvensis L. (charlock) and Brassica nigra L. (black mustard).

2.1. Seed burial

Air and water permeable nylon bags were prepared for seed containment. Two burial dates were utilised, i.e. at “planting” (May 1995) and at “harvesting” (October 1995), with three seed exhumations. From each of the eight seed line/species, five replicates of 500 seed were sealed separately in a nylon bag. The bags were buried some 4 cm deep. A split–split-plot design was used, with the burial date being the main-plot, the exhumation date the sub-plot and a sub-sub-plot consisting of the eight line/species randomly arranged within each exhumation date.

Buried seeds were extracted at 6-month interval (December 1995, May 1996 and January 1997 for “planting” date and May 1996, January and July 1997 for “harvesting” date). On exhumation, each bag was opened and the number of ungerminated seed determined by visual examination and probing for soundness with a dissection needle. The data were transformed to a log10 scale before analysis of variance was performed using Genstat 5 (Release 3.1).

Germination assays were conducted using the seed remaining in the bags at the final exhumation. Whole seed from each bag were washed with distilled water and placed on filter paper moistened with deionised water in germination dishes. These were then placed for 5–7 days at 25 °C in the dark. The number of germinated seed was counted in each dish and the percentage of germination calculated. Analysis of variance was performed on the transformed data using Genstat 5 (Release 3.1) to establish any significant differences between line/species or between burial dates.
2.2. Seed scattering

Two treatments were made in a long-term grass ley site, i.e. cultivated and uncultivated. The cultivated treatment consisted of a single rototiller pass prior to seed scattering. Fifty seeds of each line/species were scattered on two dates; i.e. in May 1995 to simulate spillage at “planting” time, and October 1995 to simulate spillage at “harvest”. Scattering occurred in two distinct blocks, with six replicates of each treatment placed at random within the block. Each replicate was further divided into eight plots in a $4 \times 2$ matrix. Each of the eight line/species was allocated at random to one of these plots. Each plot consisted of a 0.5 m $\times$ 0.5 m area with the edges between plots marked by a grid constructed from 0.025 m diameter PVC pipe.

No additional agricultural input was performed. Each plot was surveyed at regular interval over the 6 months after scattering and the number of germinated seedlings per line/species was counted. Analysis of variance was performed using Genstat 5 (Release 3.1) and transformation of the data, as previously described for the seed burial experiment.

3. Results and discussion

3.1. Seed burial

More than 80% of the seed buried at “planting” germinated over the first 6 months as shown in Fig. 1a. Further germination over the following 12 months only occurred in B. nigra. Secondary dormancy might have been induced in all the line/species during the colder weather between the first and second exhumation (December 1995 to May 1996), similar to the findings of López-Grandos and Lutman (1998), with dormancy continuing to be expressed by the seed, even during the warmer months between the second and third exhumation (May 1996 to January 1997). The exception to this trend was the seed of B. nigra, with the results suggesting that dormancy had been broken to a certain degree as a significant amount of germination took place over the final 6 months of their burial. It is known that the seed from different wild species often show seasonal variation in germination and dormancy behaviour which is thought to be associated with temperature fluctuations (Pekrun et al., 1997). It is possible that similar variations were being expressed by the line/species tested in this experiment. Schlink (1994) found that rapeseed was able to persist in the field due to the induction of secondary dormancy, with seed remaining viable for at least five and possibly up to 10 years (Sauermann, 1993), so it could be argued that increased dormancy could lead to increased persistence of a particular line/species in the seedbank.

Variation between the initial germination rates of the different line/species was observed from the “planting” burial dates (Fig. 1a). Line 3242 (stearate) proved to have the highest germination rate with all the 500 buried seed germinating over the first 6 months of burial. This is in contrast to the findings of Thomson and Li (1997), who observed reduced seed germination and vigour in the seed of high stearate oilseed rape, which they attributed to a range of factors. They noted the accumulation of stearic acid in the seed membrane manifested itself as damaged cotyledon tissue and decreased levels of germination.

The high laurate parent 212/86 showed the next highest germination rate over the same period, closely followed by the weed B. nigra and line LA002 (laurate). It was not clear if the relatively high germination rate shown by the laurate line was due to the parental line’s genetic influence, as this line also showed very high levels of germination, or due to the raised levels of lauric acid as a consequence of the transgenic inserts. It is also possible that the high levels of germination shown in this line could be due to the genetic modification process itself, irrespective of the trait being engineered. The high stearate parent A112 and the two conventional varieties showed relatively low germination rates over the initial 6-month period of burial, with S. arvensis having the lowest germination rate. These results were consistent with those of Booth et al. (1996) and were thought to be due to the differing abilities of the line/species under test to show secondary dormancy. Pekrun et al. (1998) found that the six cultivars of oilseed rape utilised in their experiments differed in their ability to show dormancy, similar results being reported by Schlink (1994, 1995). Starlight was the only cultivar common to both the study described here and that of Pekrun et al. (1998). The results of both studies confirmed that Starlight was readily able to express secondary dormancy under certain environmental conditions.
conditions and was therefore able to persist in the seedbank. The line/species, which exhibited higher germination rates and low levels of dormancy, as shown by the high stearate and high laurate modified seed, were consequently less likely to be persistent when subjected to the conditions described. Linder and Schmitt (1995) found that on balance, there was little increased risk of persistence for escaped high stearate *B. napus* oilseed rape compared to controls due to increased germination and decreased dormancy characteristics of the modified line. The ability of the laurate line LA002 to express greater seed dormancy, and consequently greater potential persistence, than its parental line 212/86 has been observed by Linder
and Schmitt (1995), although in this latest experiment, both these lines showed relatively low dormancy levels, and therefore low potential persistence, in comparison with the conventional rapeseed lines.

Seed buried at “harvest” also tended to have fairly high levels of germination over the first 6 months after burial (Fig. 1b). Dormancy appeared to be expressed to a lesser extent over the 18-month burial period than by the seed buried at “planting”, as the seed of all the line/species continued to germinate between the second and final dates (January 1997 to July 1997). There was some variation between line/species regarding their ability to show dormancy between the first and second exhumations (May 1996 to January 1997) as shown by Pekrun et al. (1998) and Schlink (1994, 1995). The high stearate line 3242, similar to the results of Linder and Schmitt (1995), and the weed S. arvensis showed the least dormancy over this period, suggesting that persistence might be low under such conditions. The remainder of the line/species under test showed no significant germination, presumably because of induced dormancy, suggesting their persistence may be greater under such conditions. Between the second and final date the high stearate line 3242 and B. nigra appeared to show dormancy whilst all the other line/species showed significant germination, presumably because dormancy had been broken.

The high laurate line LA002 showed more likely to persist than the high laurate parent as indicated by Linder and Schmitt (1995). Certain line/species were able to break seed dormancy more effectively than others as reported by Schlink (1994, 1995) and Pekrun et al. (1998). B. nigra showed the greatest proportional increase in germination over this period, closely followed by the laurate line LA002, the other line/species except for the stearate line 3242 and S. arvensis, showed similar, but lesser, increases in the proportion of germination over the last 6 months of the burial period.

Comparisons between the “planting” and “harvest” dates (Fig. 2a–h) showed no significant difference between the mean number of seed ungerminated for the high stearate parent or cv. Mars. However, significant differences occurred between the mean number of seed ungerminated at the two dates for the other six line/species, in particular for the high stearate line 3242. In this line all seed germinated in the first 6 months after the “planting” date where 90 seeds remained from the initial 500 buried seeds 6 months after the “harvest” date (Fig. 2c), suggesting that seed dispersal at “harvest” was more likely to produce persistence than dispersal at “planting”.

Significant differences between the numbers of ungerminated seed were also observed for S. arvensis and B. nigra. The second and third exhumations of S. arvensis were significantly lower at “harvest” than at “planting” (Fig. 2g). The first and third exhumations of B. nigra showed the number of ungerminated seed at “harvest” to be significantly lower than those of the “planting” burial (Fig. 2h).

The results of the high laurate parent 212/86, the high laurate line LA002 and cv. Starlight, showed significant differences between the number of seed ungerminated between burial dates at the final extraction when the number of ungerminated seed from the “harvest” burial remained significantly lower than those from the “planting” one (Fig. 2b, d and f), suggesting that persistence was more likely if seed dispersal took place at planting than at harvest. Careful seed handling during planting is likely to reduce the persistence of these particular lines, assuming any seedlings germinating from seed shed at harvest are controlled prior to flowering and seed-set.

After the final exhumations, the ability of ungerminated seed to germinate under optimal conditions depended on the date of burial (Fig. 3). Most line/species had seed capable of germinating with those buried at “planting” having much higher percentages of germination than those buried at “harvest”. This was presumably because ungerminated seed from the final exhumation buried at “harvest” had more induced dormancy than those buried at “planting”.

It is possible that seasonal differences such as rainfall and temperature fluctuations could result in different germination characteristics to those found here for the line/species concerned. For example, very dry weather at harvest, or extreme temperature fluctuations as embodied by freeze–thaw cycles could reduce or increase the germination of some or all of the line/species.

A number of the line/species used in this experiment are currently being investigated in experiments encompassing “harvest” burials over a 4-year period.
Fig. 2. Mean number of ungerminated seed at each exhumation date for each burial: (a) Stearate parent A112, (b) Laurate parent 212/86, (c) Stearate 3242, (d) Laurate LA002, (e) cv. Mars, (f) cv. Starlight, (g) Sinapis arvensis, (h) Brassica nigra. Error bars (P ≤ 0.05): LSD$_1$ between burials at the same date, LSD$_2$ between dates at the same burial.
Fig. 3. Ungerminated still viable seed at the final exhumation. Error bars \( P \leq 0.05 \): LSD 1 between burials of the same line/species, LSD 2 between line/species at the same burial.

with exhumations 2 weeks and 1, 2, 6 and 9 months after burial. Preliminary data suggests that the high laurate line germinates readily at around harvest time compared to the other oilseed rape lines and the weed species, despite seasonal climatic differences. Using appropriate and timely management techniques (Pekrun et al., 1998), persistence of the laurate seed and its transgene is hence likely to be lower than in the conventional oilseed rape lines.

3.2. Seed scattering

The mean number of plants observed per plot 6 weeks after scattering at “planting” is given in Fig. 4. Irrespective of the cultivation treatment used, none of the seed scattered at “harvest” had emerged 6 weeks after scattering, or during subsequent surveys of the plots, the dense vegetation observed presumably having out-competed the line/species allocated to each particular plot.

The allocated line/species that emerged during the first 6 weeks after being scattered at “planting” differed in population densities and plant maturities between treatments. In all cases the allocated line/species had higher population densities in the cultivated than non-cultivated treatments and maturity was more advanced in the cultivated treatments than in the non-cultivated ones. Subsequent observations showed that in all cases, the allocated species never developed fully, but were out-competed by the vegetation growing in the plots, and never produced any seed.

The scattering experiment showed some differences between line/species in terms of their ability to compete against the surrounding vegetation (Fig. 4). Whichever treatment was used, B. nigra never became established, S. arvensis, also showing poor establishment in comparison to rapeseed lines. The two conventional cultivars were the least likely to become established in the uncultivated plots, although establishment of all the lines was shown to be poor in this treatment. There was little difference between oilseed rape lines, their establishment being only slightly better in cultivated than in uncultivated plots.

A survey of the area used for this experiment has been made some 7 years after seed were initially scattered. The area is currently under grass and clover, and no sign of Brassica weeds, or volunteer oilseed rape plants was found, adding further weight to the arguments described previously relating to the persistence of the varieties tested.
4. Conclusions

The ability of line/species to exhibit dormancy and to break it once it had been induced differed between both line/species and according to date of burial. The conventional rapeseed cultivars and S. arvensis were more likely to persist in the seedbank than any of the other lines. Seed of the oil modified and their parent lines showed extremely high levels of germination when placed under optimal germination conditions, suggesting that dormancy was easily broken. This characteristic would also reduce their persistence in the seedbank. These assumptions are made on the basis that appropriate control measures are taken to destroy volunteers before they set seed (Pekrun et al., 1998). All the line/species tested were unable to compete with the natural vegetation when seed were scattered on both cultivated and uncultivated plots. The seed scattered at “planting” were only marginally more successful than those scattered at “harvest” in the initial stages of the trial, but after 6 months, none of the allocated line/species had survived irrespective of treatment. No seed was ever produced, as the plants never reached sexual maturity, suggesting that even if the tested seed were released into the field they would be unlikely to establish because of their limited ability to compete with the natural vegetation.

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