Aspects of environmental risk assessment for genetically modified plants with special reference to oilseed rape

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Intensive research over the past 10 years has resulted in the production of genetically modified lines of several important crop species including oilseed rape (*Brassica napus*), potato (*Solanum tuberosum*) and raspberry (*Rubus idaeus*). Many of these lines have increased market potential which has led to commercial pressure for their release as new cultivars although any release must be preceded by a rigorous risk assessment in statutory controlled field trials. However, concern that any release into the environment may allow transfer of the inserted genes to neighbouring commercial fields, feral (escapes from cultivation) and volunteer populations or natural populations of wild relatives has aroused intense debate. Paucity of information on pollen and seed dispersal ranges, the geneflow dynamics of agricultural fields, the distribution of cultivated and feral populations and on the ecological status of potential feral populations has done little to alleviate these concerns and could lead to a polarization of views based on conjecture rather than fact. If the advances in biotechnology are to be applied, then any risks, real or perceived, must be quantified.

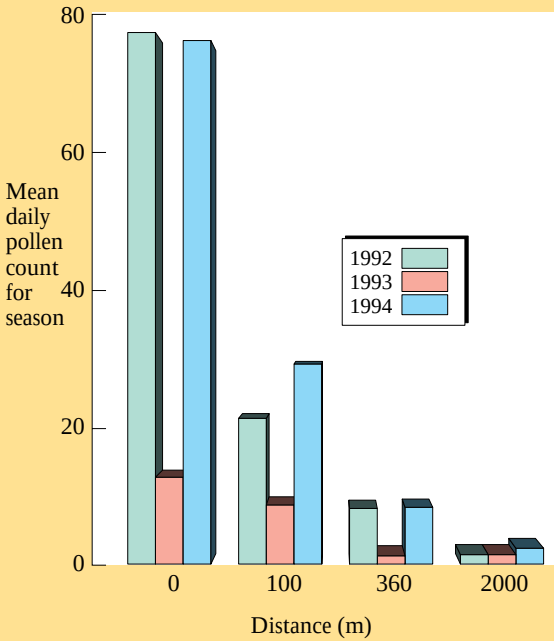

Figure 1 Mean daily pollen counts for the flowering season of oilseed rape in 1992, '93 and '94 (54, 47 and 42 days respectively) recorded at four distances from a field margin (0, 100 and 360 m and 2.5 km in 1992, 2.0 km in 1993 and 1.5 km in 1994).

Large-scale commercial fields of genetically modified crops were not available in the UK and work at SCRI has used fields of non-genetically modified oilseed rape to estimate pollen movement and geneflow by using naturally occurring genetic variation between cultivars of the same species. The results can then be used to assess the likely movement of introduced genes from fields of genetically modified crops.

Pollen movement Detailed pollen profiles were constructed along a linear transect from an oilseed rape field covering a distance of up to 2.5 km. Airborne pollen densities were found to decline with distance although there was considerable variation between years in both pollen concentrations and in the rate of decline (Fig. 1). In all years, the pollen concentrations detected at 360 m were 10-11% of that recorded at the field margin. Low pollen densities were consistently recorded at 1.5-2.5 km from the source which did not differ significantly between years and so probably represents background levels. The most striking feature of these data was the large disparity between the high densities of pollen recorded from large-scale agricultural fields and the lower levels reported by other workers from small-scale trial plots. Oilseed rape pollen seems to be released in large quantities and dispersed further than had been predicted from small-scale trial plots. Caution should be exercised, therefore, in the interpretation of small scale experiments when trying to predict the levels of geneflow likely to occur under standard agricultural conditions.

Long-range geneflow It was not known if the low levels of pollen consistently detected at 2 km were sufficient to effect significant levels of geneflow. Emasculated (and depetalled) oilseed rape plants placed at increasing distances from an oilseed rape field were used as bait plants for airborne pollen.

Figure 2 Aerial view of small section of area surveyed in Tayside region of Scotland (May, 1993).

Some seed set was observed on all of the bait plants. The percentage seed set declined with distance and correlated well with the decline in airborne pollen concentrations. Seeds produced on plants 2 km away from the source gave rise to plants with 38 chromosomes that were phenotypically normal *B. napus*, thereby demonstrating the capacity for long-range geneflow up to this distance.

Distribution of cultivated oilseed rape fields and potential feral populations A ground survey was conducted over two years, covering 70 km^2 in the North East Fife and Tayside regions of Scotland to investigate the distribution of cultivated oilseed rape and non-cultivated, potentially feral populations (Fig. 2). The mean distance between cultivated winter and spring sown oilseed rape fields and feral populations identified within the survey area was 1.0 km and 1.2 km respectively. Approximately 10% of the feral populations occurred within 100 m of cultivated fields.

Characterization of feral oilseed rape populations Feral populations of oilseed rape varied in size from individuals to populations exceeding 1000 plants (Fig. 3a, b). The seed return per plant and germination frequency varied greatly between sites. Many of the sites mapped were subject to weed management practices (cutting, spraying or rotovating) which prevented many plants in a population reaching maturity. However, 24% of the populations surveyed successfully set seed although relatively few populations were located at the same site for more than one year.

Geneflow between agricultural fields The mean distance between cultivated oilseed rape fields was 0.9 km and approximately 10% were situated within 100 m of one another. These distributions suggest that

Figure 3 (a) Population of oilseed rape in field margin (North East Fife, April 1993); (b) Population of oilseed rape occurring on ex-agricultural soil (Tayside, June 1993).

the potential exists for geneflow to occur between agricultural fields under current farming practices. The main problem associated with attempts to deter-

Figure 4 Large-scale screening of seed progeny collected from measured intervals within a commercial field of a winter sown oilseed rape cultivar (Falcon) situated adjacent to a field of spring sown cultivar (Comet). Flowering 'hybrid' seed (produced as a result of inter-cultivar crosses) can easily be identified amongst large numbers of non-flowering intra-cultivar crosses and selfs.

mine the frequency of geneflow occurring between adjacent agricultural fields is detecting potentially very low frequencies of hybrid seed (produced by cross pollination between donor and recipient cultivars) among large numbers of seed produced by intra-cultivar crosses and selfing. To overcome the problem of scale, detection of hybrids was approached using a 2-phase screening system which involved morphological markers to carry out the initial large-scale screening of seed progeny (approx. 350,000) (Fig. 4) followed by a second phase using molecular markers (RAPDs) to confirm the reliability of the initial screening. In oilseed rape there are few morphological marker genes suitable for efficient large-scale screening of commercial cultivars. However, the vernalization requirement of winter oilseed rape varieties was found to be a suitable marker for large-scale screening in the field to test for cross-pollination between cultivated fields of winter and spring varieties. Using this approach, geneflow between fields was detected at a distance of 100 m from the source field margin. A similar approach is being applied to adjacent fields of raspberry and potato and, in the case of raspberry, geneflow has already been detected at 120 m.

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Genetic improvement of trees

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Improved tree breeding to meet the needs of world energy, building and fibre (paper-making) requirements, in an environmentally sustainable manner, requires a major advance in the understanding of the nature, source and interaction of the genes controlling commercially important traits in trees. A firm basis is being established in genome analysis in legumes and brassicas; however, woody and technically difficult and long-term crops like trees have been largely ignored. Sitka spruce accounts for about 60% of the timber production in the UK and yet the breeding of the crop is at a relatively under-developed stage, despite it being introduced into the UK in the 1920's. This is largely because it has a long juvenile stage which makes each breeding cycle a protracted affair. It takes about 15 years for a germinating seedling tree to start flowering, and perhaps a further 20 years before mature aspects of such a tree can be fully assessed to permit the selection of superior genotypes.

Recent advances in molecular techniques being used at SCRI, eg RFLPs and PCR based techniques such as RAPDs, offer the very real prospect of identifying molecular markers that can be linked to important traits and hence permit the early selection of potentially desirable genotypes prior to field planting. The prospect for greatly increasing the efficiency and speed of the breeding and selection processes in tree genetics programmes is therefore very good.

The programme at SCRI has aimed to combine existing expertise in genetics, breeding, wood chemistry and molecular skills of SCRI, and the germplasm and

Figure 1 Mature trees.