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Report on the separation distances required to ensure cross pollination is below specified limits in non-seed crops of sugar beet, maize and oilseed rape.

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Report on separation distances required to ensure cross-pollination is below specified limits in non-seed crops of sugar beet, maize and oilseed rape

EXECUTIVE SUMMARY

MAFF is reviewing guidelines on the separation distances used to ensure that harvested crops reach certain levels of genetic purity. To support this, MAFF requires a desk study of the existing knowledge, particularly on levels of purity of certified seed stocks as affected by separation distances. This study will report on the extent to which the relationship between distance from pollen sources and the cross-pollination of adjacent crops can be quantified. MAFF is particularly interested in the predicted initial rate of decline in the cross-pollination at short distances, and in the rates of cross-pollination at relatively long distances. MAFF is also interested in identifying the

distance beyond which further increases in separation distance has minimal effects on cross-pollination levels.

The study should cover all production and breeding systems, including hybrid systems, exploited by oilseed rape, maize, and beet and advise on the distances needed to achieve off-type thresholds of 0.1, 0.5 and 1% in commercial non-seed crops.

Those experiments and practical experience which have assessed the degree of cross-pollination which takes place at various separation distances have been reviewed. The information has been assessed for its validity for whole field to field situations in the UK. After considering all the results available, a robust, representative set of data has been identified. These results have been applied to typical farm situations in order to assess the level of impurities which would have resulted from cross-pollination over the whole field. Recommendations for separation distances are presented which take into account the more important factors which influence the levels of cross-pollination.

Maize

It is concluded that the levels at which wind-borne maize pollen fertilises adjacent crops is strongly affected by the size of the emitting crop, the strength of the wind and any barriers that intervene. Hence the separation distances have been derived from experiments where these factors seem to have been very favourable to cross-pollination.

The maize grain is affected by cross-pollination and not the rest of the plant. Because the grain only forms a proportion of the total crop, the separation of receptor crops intended for silage can be reduced and still maintain the proportion of the produce affected by cross-pollination at below the threshold.

The separation distances advised between maize fields to meet specified threshold levels of cross-pollination are presented in the table below.

Oilseed rape

It is concluded that while long distance pollination has been recorded, in practice the level of cross-pollination between fields declines rapidly with distance. Where the receptor crop is of a variety which contains male sterile plants, the levels of cross-pollination seem to be significantly increased compared to conventional varieties.

The oilseed separation distances advised are presented in the table below. Winter and spring types

of *B rapa* and *B napus* should be treated as one crop for separation purposes.

Beet crops

As only the maternal plant tissues are used, cross-pollination will not affect the produce of non-seed crops. In any case bolters can and should be removed from the receptor crops.

Summary of separation distances (m) required to maintain cross-pollination of the whole fields at below specified levels for fields of 2ha or more

CROP	THRESHOLD LEVELS OF CROSS- POLLINATION		
	1%	0.5%	0.1%
Oilseed rape (B. napus and rapa)			
Conventional varieties and restored hybrids	1.5m	10m	100m*
Varietal associations and partially restored hybrids	100m	n/a	n/a
Maize/sweetcorn			
For grain	200m	300m	n/a
For silage	130m	200m	420m

n/a – insufficient information to produce a recommendation (see report for more detail)

* – see Section 5.6.2.

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Report on separation distances required to ensure cross-pollination is below specified limits in non-seed crops of sugar beet, maize and oilseed rape

1. OBJECTIVE

MAFF is reviewing guidelines on the separation distances used to ensure that harvested crops reach certain levels of genetic purity. To support this, MAFF requires a desk study of the existing knowledge, particularly on levels of purity of certified seed stocks as affected by separation distances. This study will report on the extent to which the relationship between distance from pollen sources and the cross-pollination of adjacent crops can be quantified. MAFF is particularly interested in the predicted initial rate of decline in the cross-pollination at short distances, and in the rates of cross-pollination at relatively long distances. MAFF is also interested in identifying the distance beyond which further increases in separation distance has minimal effects on cross-pollination levels.

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Those experiments and practical experience which have assessed the degree of cross-pollination which takes place at various separation distances have been reviewed. The information has been assessed for its validity for whole field to field situations in the UK. After considering all the results available, a robust, representative set of data has been identified. These results have been applied to typical farm situations in order to assess the level of impurities which would have resulted from cross-pollination over the whole field. Recommendations for separation distances are presented which take into account the more important factors which influence the levels of cross-pollination.

2. SOURCES OF INFORMATION

2.1 Previous practical experience

2.1.1 Certification schemes

Certification schemes carry no records of the separation distance that was actually achieved. The only thing known about the separation is that it was found to comply with the minimum distance required by the regulations. Furthermore, while post-control plots are grown, there is a body of evidence to show that visually identifying off-types can underestimate the level of genetic impurity. Where off-types are particularly obvious, one gets nearer to a measure of the true cross-pollination levels, but in these cases it is not clear whether the contamination is through cross-pollination resulting from lack of separation or other causes.

2.1.2 Field crops that already require separation to maintain product purity

Currently there are few examples of crops where there is a requirement to maintain a separation distance between commercial fields of the same species in order to avoid cross-pollination. Two situations may offer some practical experience of maintaining separation distances for commercial production:

- a. Low erucic acid from high erucic acid oilseed rape (see section 5.2.2).**
- b. Sweetcorn from other types of maize (see section 4.2.2).**

2.2 Reports of experiments

Reports in journals have been scrutinised by referees but many are from countries where the environmental conditions may not be the same as in the UK. Usually the experiments described are detailed and the detail is sufficient to come to some conclusions about their relevance to UK conditions and the issues being discussed in the report.

Conference papers are not refereed but do come under the scrutiny of the participants in the conference. However, the level of detail presented is less.

Relevance of small scale experiments:

Experiments on cross-pollination have been aimed at different objectives and therefore the design and interpretation may not be relevant to the consideration of cross-pollination between relatively large areas of non-seed crops. The objectives of these experiments fall into three main groups:

1. To assess the separation needed for seed production crops. Purity in seed production crops has to be high, particularly for the higher grades of seed. Furthermore, seed production by female parents which are either self incompatible or male sterile, as in the production of F1 hybrids, may leave the plants much more liable to pollination by foreign pollen than a self fertile production generation.

2. Attempts to assess the role of honeybees in the pollination process. Most of these do not assess the amount of cross-pollination that takes place.

3. Estimation of the likelihood of gene flow into wild or feral populations of the same or related species. Many of these look at the distances travelled by pollen, or at cross-pollination of isolated plants which may be male sterile or self-incompatible. They are likely to overestimate the degree of cross-pollination which would result in a farm crop, where there are numerous plants of the same variety and competing pollen is being produced by all these neighbouring plants. This type of experiment is frequently a very poor guide to the amount of cross-pollination which will occur between crops on a whole field basis.

2.3 Other sources of information

There is a large body of unpublished information. Some of this is from investigations in progress which will be published. Other information comes from small investigations or for other reasons is not published. This kind of data can be useful in confirming the results of more precise experiments over a wider range of environmental conditions.

2.4 Interpretation of experiments

Successful cross-pollination depends upon a number of circumstances coinciding. For example, the foreign pollen has to be available at the right time, it has to be conveyed in the direction of the receiving plants and the competing pollen produced by receptor plants themselves has to be at a low level. Hence there are a number of reasons why cross-pollination may not take place between fields which, had the prevailing conditions been different, would have cross-pollinated. The highest levels of cross-pollination occur when favourable circumstances coincide for the majority of these factors. Similarly, some experiments are conducted in conditions which are more favourable than others. Hence it is not just the average value that occurs across a number of experiments that matters, but also the maximum value because it may be an indication of the cross-pollination levels that could occur when favourable conditions for all or most of the factors affecting it coincide. However, by contrast the effects of experimental error also have to be taken into account. There is a probability that the maximum value is one where the experimental error has caused an over-estimation of the level of cross-

pollination.

3. FACTORS AFFECTING THE DEGREE OF CROSS-POLLINATION

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This section considers the factors which affect levels of cross-pollination without reference to specific crops, in order to provide a rationale for the interpretation of data presented in the sections on maize, oilseed rape and beet.

3.1 Pollen source - (emitter)

Size of pollen source. Some experiments are performed with a pollen source of very restricted size. There does seem to be an *a priori* case for increasing emitter size to produce a larger pollen cloud and thus all other factors being equal to lead to increased pollination. While this seems likely for wind pollination it may not be true for insect cross-pollination if the numbers of insects are a limiting factor. The size of the emitter needs to be considered against the likely average size of fields in the UK. A theoretical study by Crawford (1999) has examined the effect of increasing emitter size on the levels of cross-pollination which will occur. He concluded that a square plot of 400m² would produce a 'pollen dispersal characteristic' of about 3/4 of that of a field of 4ha (40,000m²), but the indications were that the effectiveness of dispersal would decline markedly at plot sizes less than 400m². This means that experiments with small emitter plots may under-estimate the amount of cross-pollination taking place between whole fields and this has to be taken into account when making recommendations.

Attractiveness to insects. Attractive crops are more likely to be visited and therefore numbers of insects available for cross-pollination are less likely to be limiting.

3.2 Transmission to receptor crop

Vectors

Crops are often designated wind or insect pollinated. In practice for some crop species it

would appear that a combination of wind and insect pollination may be involved, in which case the proportion may vary from crop to crop and even day to day. The actual vector may vary with the country being considered and this could be one of the factors which affects the relevance of experiments performed in non-UK environmental conditions. For example, Chamberlain and Chadwick (1972) showed that spores will bounce off dry surfaces increasing the distance of spread and therefore the wetness of foliage and other surfaces will have an influence on the distance which pollen will travel.

Barriers

a. **Woods and hedges.** Woods and hedges serve as a potential barrier both to wind-borne pollen and to flying insects.

b. **Topography.** It is well known that wind velocity and airflow are affected by topography and this could influence the amount of pollen delivered to the receptor field.

c. Barrier crops of the same species as the crop

i. **Around emitter crop.** There are theoretical reasons why a barrier crop of another variety around an emitter might reduce cross-pollination by the emitter to other crops. It makes the distance the pollen has to travel greater; competing pollen is introduced which is more likely to reach the receptor crop than that from the emitter area, and insects are less likely to leave the crop and move directly to the receptor area.

ii. **Around receptor crop.** Barriers around the receptor crop also increase the distance between crops, introduce competing pollen and may serve as a physical barrier both to wind borne pollen and insects. Insects may be more likely to make their first visit in the field to a plant in the barrier.

3.3 Factors affecting degree of cross-pollination once pollen has arrived at the receptor crop

Synchronisation of its arrival with the receptivity of the flowers. For an experiment to be a true test of the likely maximum potential for cross-pollination between farm crops there has to be coincidence of the time when the pollen is emitted and the flowers of the receptor are receptive. In particular a receptor that is partially self-pollinating and starts

flowering before the emitter, may under estimate the degree of cross-pollination which would occur if the two crops were perfectly synchronised (Bateman 1947b).

Viability and competitive ability after transport to receptor crop. If the period between emission and arriving at the stigma is long and involves exposure to adverse conditions the pollen may lose both its viability and its capacity to compete with fresher pollen produced in the vicinity of the receptor plants. All the indications are that under normal UK atmospheric conditions at flowering, pollen will remain viable for long enough to make it unlikely that this is a factor limiting cross-pollination between adjacent fields.

Amount of competing pollen produced by the receptor crop could have a major effect on the success rate of the incoming pollen. Conditions of least competition, are in experiments where male sterile plants producing no pollen, are used to detect pollen in the air. This situation also occurs in F1 hybrid seed crops, where male sterile plants are fertilised by emitters a number of rows away; and in those crops which are a mixture of male sterile plants and pollinators such as varietal associations in oilseed rape.

3.4 Factors affecting the degree of gene expression in the harvested crop after pollination by foreign pollen

Part of the plant being harvested. After cross-pollination, the genetic material is incorporated into the seed and may influence the characters manifest by the seed. The degree of influence will depend on the form of inheritance. However the genetic composition of the maternal plant (other than the seed) will not be affected by the pollination and it will therefore not manifest any of the characteristics transferred by the pollen.

Inheritance. Most important characters are dominantly inherited and for the characters which have been sought by the breeders, are mostly homozygous. This is the most potent form of emitter as far as the manifestation of cross-pollination is concerned. When the emitter plants are heterozygous and the character is dominant, only half the pollinations in the receptor crop are manifest. In the rather less common case, where the character is manifest as the recessive form, no cross-pollinations are apparent even though genes from the emitter have been incorporated into the seed of the receptor. While this outlines the general principle, many traits have more complicated systems of inheritance controlled by a number of genes.

Furthermore there are a few characteristics such as low glucosinolate content in oilseed rape, for which the characteristics of the seed are controlled by the maternal genotype. In this case, seed created by cross-pollination with high glucosinolate pollen is not itself high glucosinolate but the seed of the progeny are high glucosinolate. For traits with this type of inheritance, cross-pollination is not important to commercial crops.

4. MAIZE AND SWEETCORN

4.1 Brief description of relevant morphology and crop use

Maize is typically 95% cross-pollinated (Poehlman 1959) although the plants are completely self-compatible. Pollen is emitted from the tassels at the top of the plant 2-3m above the ground and received by the female flowers about 1.5m up the stem which after fertilisation develop into the cob. The whole of the aerial part of the crop is used, usually for cattle feed in the form of silage. The proportion of grain is normally 20-40% by weight of the whole crop.

Sweetcorn is rather shorter than maize, typically only 1.5 to 2m tall. The grain portion of sweetcorn is consumed by humans, mostly being sold 'on-the-cob' as a vegetable. It is the same species as maize and crosses freely with it.

4.2 Practical experience of separation distances in maize

4.2.1 Certification scheme

No maize seed is currently grown in the UK. Probably all the seed used in the UK is F1 hybrid varieties, produced by pollinating male-sterile female parents from nearby rows of male-fertile plants. Hence the capacity for cross-pollination in the seed production process is considerably more than that in commercial crops where all the adjacent plants are fertile. Minimum separation distance in the EU is 200m for all categories of seed production and this is reckoned sufficient to maintain inbred lines at 99.9% purity.

Seed production in the USA is now to OECD standards with a 200m separation distance. In the past, a minimum separation of 660ft (198m) was used between different types such as maize and sweetcorn and for producing high grade seed lots, but this was reduced to 410 ft (123m) for certified seed lots of the same texture and colour. These separation distances aimed to limit cross-pollination of small groups of male sterile rows interplanted with pollinating rows by other crops to 0.5%. The above separation distances could be progressively reduced as barrier rows were added down to a minimum of only 85ft (25m) or even to zero where the crop was 20 acres (8ha) or more and had 10 barrier rows around it (Jugenheimer 1976). Tables of certification regulations produced in Jugenheimer and in Poehlman (1959) suggest that one row of maize crop separation is

equivalent to about 40ft (12m) of open field separation for distances in the range 0-660ft (0-200m)

4.2.2 Separation distances used by sweetcorn producers

Cross-pollination of sweetcorn by maize produces grain with less sweetness. In order to avoid this, growers of sweetcorn try to maintain separation from maize fields. In the USA, Gerber (1983) suggested 250ft (80m) is sufficient but anecdotal evidence suggests that UK growers attempt to maintain up to 200m separation (Day, personal communication.)

4.3 Results of maize experiments

4.3.1 Field experiments which measure the effect of separation distance on levels of cross-pollination

With no maize seed being produced in the UK there has been little or no incentive to look at cross-pollination in maize in the UK. Bateman (1947b) reported that in an experiment which used a 3m square of emitter plants to pollinate isolated plants, the level of cross-pollination dropped from 40% at 2.5m to about 1% at 20m, with some difference in the degree of pollination in the two directions assessed. In another experiment measuring rate of decline in pollen concentration with distance, the movement of pollen was strongly affected by the direction of the wind.

In the US, Jones and Brooks (1950) conducted three years of experiments in Oklahoma which used large emitting crops and relatively large receptor blocks of c.900m², placed down wind of the emitters. Both emitter and receptor were open pollinated varieties. These showed that crossing occurred at high rates with the open fronts of the receptor blocks, so that in the first five rows of a block at 25m crossing was 32% and was still 7% in the five rows at the front of a block at 125m. Twenty rows back into the receptor blocks, the level of cross-pollination had dropped to 6 and 2% respectively (see section 4.3.2.2). There was considerable difference in the crossing between years, one wet season with less wind than usual, roughly halving the crossing rate. Jones and Brooks compared their results with others from Nebraska and concluded that they were broadly comparable. He also cited Russian work which showed lower levels of cross-pollination but concluded that the emitter was downwind of the receptor crop. However Airy is cited by Jugenheimer (1976) to have found crossing rates of only 4.5% at 10ft(3m), falling to 0.9% at 53ft (16m) and 0.2% at 170ft (52m). The experimental details of this experiment are not stated. Raynor *et al* (1972) compared their pollen flow experiments with the cross-pollination results of Jones

and Brooks and concluded that their figures for pollen transmission were lower. However, Raynor used an emitter of only 18m in diameter and when the sideways dispersion of pollen shown in their paper is taken into account, it may well be that their results are not inconsistent with those of Jones and Brooks.

Messean (1999) has presented a figure from France for cross-pollination of 1% at a separation distance of 25-40m and concluded that wind direction had an important influence. In this experiment, the maize crop filled the separation distance and may have acted as a barrier.

There are other experiments which show much lower crossing rates such as that of Paterniani and Stort (1974) in Brazil but these have considered the levels of cross-pollination caused by a few plants, or a small square of crop in a much larger surrounding area of receptor crop. They can therefore be discounted as estimates of the likely crossing rates between adjacent fields.

4.3.2 Measurements of individual factors which influence cross-pollination

4.3.2.1 Pollen source - emitter crop

Theoretical calculations have been done which suggest that cross-pollination will be underestimated by using small emitters but this does not appear to have been tested in experiments for maize (section 3.1).

A number of workers have suggested that the distance the pollen is dispersed is the result of the balance between its horizontal speed largely determined by the wind, and its vertical speed which is a balance between gravity and any upward air current. It would follow from this that the vertical distance between adjacent crops might effect the amount of pollen that passes to the receptor crop. However this factor does not seem to have been considered.

4.3.2.2 Transmission to recipient crop

Emberlin *et al.* (1999) recorded that collection of maize pollen by bees from the

tassels held at the top of the plants has been observed by a number of workers. However they concluded that insects have very little or no role in cross-pollination of maize because there is no incentive for pollen collecting insects to visit the female flowers half way up the stem (Treu and Emberlin 2000).

Experiments which monitor the movement of pollen (Raynor *et al* 1972; Bateman 1947b) or record levels of crossing (Messean 1999) have both shown that pollination can be highly directionally-orientated with a much higher incidence downwind of the emitting crop.

Role of barriers. The layout of the Jones and Brooks (1952) experiment enabled comparisons of the effectiveness of bare ground, barrier crops of maize and trees in reducing cross-pollination. Thirty foot trees close to the emitter (occupying space equivalent to 25 maize crop rows) did reduce the cross-pollination compared to bare ground, but it appeared that the reduction was considerably less than if the space occupied by the trees had been filled with intervening crop. This was probably because while the trees provided a larger obstacle, they did not introduce any competing pollen as did the intervening crop. However, the trees did seem to have a beneficial effect compared to bare ground right back to 300ft (91m), cutting the cross-pollination to about 50%. More interestingly with the first five rows as a barrier, the cross-pollination in the next five rows was only 50% of the first five rows. Increasing the barrier rows to fifteen had a further beneficial effect reducing the level to approximately 25% of that in the first five rows.

Further indication of the effectiveness of barriers can be deduced from the work of Guberac *et al.* (1999) who showed in Croatia that compared to the row adjacent to the pollinators, the third male sterile female parent row set only 35% to 49% of seeds. In this case there was no competing pollen so the absolute levels of cross-pollination are high but it does appear that in the relatively light winds prevailing, the intervening two rows intercepted more than half of the pollen, a rather greater reduction than recorded by Jones and Brooks.

4.3.2.3 Factors affecting degree of cross-pollination once pollen has arrived at the receptor crop

Amount of competing pollen. A number of authors have suggested that for maize, pollen concentration (and/or the relative concentration of emitter and receptor pollen) should be a good indication of the potential cross-pollination which will take place (Jones and Brooks 1950; Bateman 1947b; Treu and Emberlin 2000). Hence the amount of pollen produced

by the receptor crop will influence the success of foreign pollen in achieving cross-pollination.

Synchronisation of its arrival with the receptivity of the flowers. In his experiment with an open flowering variety, in which the plants apparently had a wide range of flowering dates, Bateman showed the importance of synchrony between emitter and receptor. Indeed the lack of it, almost ruined his experiment. Results of variety trials suggests that the differences in tasselling time between the F1 hybrid varieties currently being used in the UK is small (NIAB 1999a). With tasselling continuing for some 14 days and stigmas receptive for a large part of that period, it appears that there is potential for all varieties to cross-pollinate. The flowering period for sweetcorn in the UK is similar to that of the maize varieties.

4.4 Factors affecting the degree of gene expression in the harvested crop after pollination by foreign pollen

Part of the plant being harvested. Pollination will affect the composition of the grain, but will not affect the composition of the stover and leaves. The grain content in maize grown for silage in the UK would commonly be about 40%; levels of 50% may sometimes occur in crops harvested at a relatively late maturity.

Inheritance. Some F1 hybrids are heterozygous for some traits. In this case, only half the pollen will carry the trait and this may result in only half the cross-pollinated plants showing the trait in the seed or in the next generation of plants.

4.5 Recommendation on separation distances for maize and sweetcorn

The following only considers the levels of cross-pollination by adjacent crops. No allowance has been made for impurities introduced by other means, such as admixture in the seed sown or in the harvested crop or for any contribution made by groundkeepers growing in the receptor crop. Recommendations have been made on the assumption that the rectangular field will be at least 2ha.

When crops are isolated by open ground or low growing crops, it appears that the first few maize rows intercept a high proportion of the cross-pollination and it then decreases exponentially with distance. Therefore the level of cross-pollination at the average distance of the whole field from the edge of the emitter is not a perfect guide to the cross-pollination in the whole field. In some cases up to 40% of the cross-pollination within

the whole field will occur in the nearest 10m.

The effect of field alignment has been investigated by assuming a field of rectangularity 4 in which the long side is four the times the length of the short side. The degree of cross-pollination occurring when the long or the short side is aligned towards the emitter crop is compared.

The best body of data for estimating levels of cross-pollination in maize is that of Jones and Brooks but it represents a worst case scenario because of the high winds and the dry conditions prevailing during the experiment. Recent unpublished data also suggests that the American study showed more extreme pollination than is normal in France. The table below gives the cross-pollination levels predicted at various separation distances for 2 and 5 ha fields.

Effect of separation distance, field alignment and barrier rows on the cross-pollination percentage of the grain in 2 and 5ha maize fields calculated using the data of Jones and Brooks.

Separation distance	No barrier				5m of barrier			
	Receptor side adjacent to contaminating crop							
	Long side		Short side		Long side		Short side	
Field size	2 ha	5ha	2 ha	5ha	2 ha	5ha	2 ha	5ha
0m	14.0	10.7	5.2	3.4	10.8	8.8	4.3	2.8
25	8.3	6.4	3.3	2.2	6.5	5.2	2.8	1.8
76	4.1	3.2	1.7	1.2	3.2	2.6	1.5	1.0
126	2.1	1.8	1.0	0.70	1.6	1.5	1.0	0.62
200	1.0	0.88	0.5	0.37	0.73	0.71	0.42	0.32
300	0.39	0.33	0.23	0.19	0.28	0.26	0.20	0.17
400	0.21	0.21	0.16	0.14	0.19	0.19	0.16	0.14
500	0.15	0.14	-	-	0.13	0.13	-	-

Recommendation for separation distances. There is no evidence to suggest that the levels of cross-pollination in the grain are any different for sweetcorn and the separation distances recommended are therefore the same. However, because the maize crop harvested for silage in the UK seldom exceeds 50% grain, the separation distances can be reduced, yet still maintain the levels of impurities introduced by cross-pollination below the limits. It can be seen from the table that field orientation makes a considerable difference to the average level of cross-pollination occurring over the whole field.

Recommendation to limit cross-pollination to 1% or less on a whole field basis. The table above shows that this can be achieved for grain by a separation of 200m, while a crop barrier on the receptor crop will reduce the separation distance, the effect will be small. In the case of silage where no more than 50% of the crop is grain, the impurity level can be restricted to 1% by a separation distance of 130m.

Recommendation to limit cross-pollination to 0.5% or less on a whole field basis.

The table above shows that this can be met for the grain by a 300m separation. However in the case of silage where no more than 50% of the crop is grain, the impurity level can be restricted to 0.5% by a separation distance of 200m.

Recommendation to limit by cross-pollination to 0.1% or less on a whole field basis.

The table above shows that even at 500m this limit would not be consistently achieved for the grain. However in the case of silage, the impurity level can be restricted to 0.1% by a separation distance of 420m.

Multiple adjacent fields. The separation distances specified above are derived for adjacent fields with long sides abutting, where the field is down wind of the emitter and a strong wind prevails. In this situation there would be negligible cross-pollination in the upwind direction, and another field abutting against the down wind long side would not add significantly to the level of cross-pollination. The evidence suggests that where the winds are light, cross-pollination levels are much reduced. Although there might then be some cross-pollination from both directions, the cross-pollination over the whole field is unlikely to be greater than the threshold level.

5. OILSEED RAPE

5.1 Brief description of relevant morphology and crop use

The oilseed rape crop in the UK consists of two species, *Brassica napus* (amphidiploid) and *Brassica rapa* (diploid), of which there are both spring and winter forms. The majority of the crop grown in the UK is of the *B. napus* type of which some 90% are autumn sown winter types (Nix 1998). The *B. rapa* area declined sharply to 4000ha in 1999 (NIAB 1999b).

Brassica napus. Plants of the conventional varieties of *B. napus* are entirely self-fertile, however considerable cross-pollination between plants within the field takes place. Scheffler *et al.* (1993) summarises work from a number of sources which suggests the crossing rate varies from 22% to 36%, while Lavigne *et al.* (1998) deduced a rate of 41%. A review by Timmons *et al* (1995) concluded that it could vary from 5 to 55%. *B. napus* can be pollinated by *rapa* but crossing rates have been low except where there has been a large excess of male *B. rapa* plants (Gray and Raybould 1998). These rates of cross-pollination will not apply where male sterile female parents are used in the seed

production process. In seed crops to produce F1 hybrids, female plants rely for pollination on pollen from neighbouring rows and consequently the opportunity for foreign pollen to fertilise them is greater. In some F1 hybrid varieties, fertility is restored so that in the commercial crops, the foreign pollen competes with the crop's own pollen on the same basis as for conventional varieties although this restoration is not always complete (White D, personal communication). Breeding systems are becoming more complicated and a number of hybrid systems now exist where the commercial crop contains a proportion of male sterile plants by design (NIAB 1999b). In varieties classed as varietal associations, the commercial crop consists of a mixture of male sterile plants pollinated by a proportion of fertile plants, which may be as low as 20%.

Brassica rapa. Varieties are generally self-incompatible and are pollinated by surrounding plants within the crop. *B. rapa* also produces seed as a result of cross-pollination with *B. napus* (Gray and Raybould 1998) but the cross-pollination rate varies with the circumstances. Where an isolated plant is exposed solely to a *B. napus* pollen, for example as a single plant within a *napus* crop, crossing rates can be high, however where other *B. rapa* plants provide compatible *rapa* pollen the crossing rates with *napus* pollen have been low.

The part of the crop utilised is the seed. This is crushed to extract the oil and the residue is then used as a high protein cattle feed.

5.2 Practical experience of separation distances in oilseed rape

5.2.1 Certification scheme

The separation requirements for certified seed production

Species	Crops to produce	Minimum separation distance	Minimum separation distance	Minimum purity level
		(metres) UK	(metres) EU	UK and EU (%)
<i>Brassica napus</i> oilseed rape				
Conventional varieties	Basic seed	400	200	99.9
	Certified seed	200	100	99.7
Hybrids	Certified seed (F1)	300	300	90.0
<i>Brassica rapa</i> L.var. <i>silvestris</i> (turnip rape)				
Conventional varieties	Basic seed	400	400	99.9
	Certified seed	200	200	99.7
Hybrids	AS FOR <i>BRASSICA NAPUS</i>			

Failures of seed lots in the certification system due to varietal impurity have been rare. Of the 647 check samples grown in post control plots in the last five years only two have failed to meet the varietal purity standard of 99.7% and these failures were thought to be due to factors other than cross-pollination.

5.2.2 Separation distances for high erucic acid (HEAR) crops

HEAR crops are subject to a separation distance requirement from other crops of 50m. The EU limit for the level of erucic acid in low erucic acid crops is 2% (Bilsborrow *et al.* 1998). Low erucic varieties contain close to 0% erucic acid and the high ones are usually about 50%. The heterozygote produced by crossing does not fully express the erucic acid content of a homozygous individual. Consequently crossing has to have been at more than 4% before a crop would fail the 2% limit. The level of checking on the produce is low and for these two reasons the field experience of this scheme is not a sensitive test of the limits to be considered in this report.

5.3 Results of experiments

5.3.1 Field experiments which measure the effect of separation distance on levels of cross-pollination

5.3.1.1 *B napus*. Field experiments show widely varying results which seem to be partially caused by the methodology of the experiments themselves, some of which were conducted in conditions which were significantly different to those that occur in fields.

Downey (1999) has cited the results of experiments conducted in the 1970s on the Canadian prairie, in which very large blocks of emitter crop (c65ha), produced 2.1% crossing at a distance of 46m in isolated small receptor blocks (46m²). More recently, two tests of cross-pollination using large fields as both emitter and receiver showed contrasting results with 1.5% crossing at 20m in one and only 0.1% in the other. While at 100m the crossing was 0.1% and 0.4 % respectively.

Champolivier *et al* (1999) have published a graph of the decline in crossing with distance in *B. napus*. This field scale study suggested that at zero distance, cross-pollination varied with site from 1.6 to 4%; falling to 0.8 to 2.5% at 5m; while at 10m it had declined to 0.6-1.8% and to 0.2-0.6% at 30m. These figures from France are higher than observed in two

experiments performed in the USA, where both the receiving and emitting crops were relatively small, and in which cross-pollination at 0.9m was 1% and at 5m, 0.55% (Morris *et al* 1994).

The levels measured by Simpson (2000) at Cambridge in 1999 are comparable with the above trials. With low levels of bees, and emitter and receptor plots of 0.8ha, cross-pollination averaged 1.6% at 1.5m, 0.86% at 5m, 0.68% at 11.5m, 0.23% at 41m and 0.12% at 81m. The results are derived from a large number of comparisons all made in one year and omit some transects with low values where the ability of the varieties to cross-pollinate might have been limited; they therefore represent conservatively high figures, bearing in mind that the high figures are likely to be ones where experimental error has also operated to inflate the cross-pollination level measured. It is interesting to note that the worst transects are not substantially higher than the mean values, at 2.15% at 1.5m, and 0.6% at 40m. Nevertheless the fact that both the top values tend to fall in one transect suggests that there are some circumstances where the mean may underestimate the potential for cross-pollination if all the right circumstances coincide.

Scheffler *et al.* (1993) used a relatively small 9m diameter emitter circle in the centre of a 1.1ha field of receptors and in a field well provided with bees showed a more rapid decline in the level of cross-pollination with distance. While the 1.6% crossing at 1m was comparable with the experiments mentioned above, it had fallen to 0.016% at 12m. Such a large decline in cross-pollination with distance would be partly explained by the small size of the emitter crop. Scheffler records that similar experiments were also done in Belgium and France and showed slightly less distant cross-pollination than the UK experiment, but presumably they also suffered from the drawback of the small pollen source.

Synergy is a varietal association with only 20% fertile plants included to provide pollen for the other 80% male sterile F1 hybrid plants. A direct comparison has been made by Simpson (2000) of the cross-pollination in Synergy and in the conventional variety Apex. At 1.5m there was 15.5% cross-pollination in Synergy, 18 times that in Apex at 0.82%. At 21m the cross-pollination fell to 5.1% in Synergy, 27 times that in Apex and at 91m, Synergy cross-pollination was 1.36%, 45 times the level in Apex.

5.3.1.2 *B rapa*. Conventional varieties of turnip rape have certain similarities to varietal associations in that the majority of seed is produced by crossing because the plants are largely self-incompatible. The early experiments of Bateman (1947a), using radishes (*Raphanus raphanistrum*) and turnips (*Brassica rapa*), produced high rates of crossing in experiments

conducted with a small area of crop as an emitter. This was surrounded by 'stringers' of relatively isolated plants reaching out at increasing distance from the emitter. The most extreme in terms of the small numbers of receptor plants used, showed 50% crossing at 20 ft (6m) falling to 31% at 40ft(12m). A similar experiment with larger plant numbers showed 4.5% at 10ft(3m), 1.5% at 20ft(6m) and nil at 40ft (12m), while a further experiment looking at cross-pollination within a small square of plants showed a much more rapid decline with cross-pollination falling to 1.2% only 6.4 ft (2m) away from the source plants. This latter experiment is probably the most relevant to field scale cross-pollination.

Experiments done over two years (Stringham and Downey 1978) in the 1970s with very large source crops and small receptors produced high outcrossing rates; 8.5% at 46m, 5.8% at 137m and 3.7% at 366m. This was compared with similar experiments on *napus* by Downey (1999) where the *napus* rates were between one quarter and one sixth that of the *rapa* crossing at 2.1%, 1.1% and 0.6% respectively. Three subsequent tests using large fields showed that at one site an average cross-pollination level of 0.28% was not obviously altered by increase in distance from 4 to 100m while at the other two sites cross-pollination averaged 0.02% for separation distances of 250 and 600m.

5.3.2 Measurements of individual factors influencing cross-pollination

5.3.2.1 Emitter crop - Pollen source

Size of pollen source. Crawford *et al.* (1999) have conducted theoretical calculations showing the influence of field size on the likely dispersal of pollen. See section 3.1.

Attractiveness to insects. Oilseed rape is highly attractive to honey bees which appear to work it preferentially. It has been shown that bees tend to work one particular crop per visit and tend to continue to work the same crop in successive visits. (Osbourne 1999). However, crops that are immediately adjacent might well be perceived by bees as being one continuous crop. It is not clear how far apart the areas have to be before loyalty to a particular collecting area over a number of visits becomes apparent.

5.3.2.2 Transmission to recipient crop

Role of vectors. Treu and Emberlin (2000) describe oilseed pollen as being typical of insect pollinated crops. However there seems to be no studies that positively differentiate between the proportion of cross-pollination in field crops which is due to insect pollination, and that which is due to wind-borne pollen. Winter oilseed rape flowers in April and into early May, while the majority of spring oilseed rape flowering takes place in June. Consequently it is widely believed by workers with the crop, that insects are more important to cross-pollination in spring sown than in autumn sown crops. Scheffler (1993 and 1995), in experiments well provided with bees, showed no obvious effect of the prevailing wind direction on the degree of cross-pollination, and concluded that bees were the main agent of cross-pollination in these spring sown crops. For winter sown crops, Simpson *et al* (1999) showed more pollination of male steriles in the direction towards which the prevailing wind blew at distances of 100m or more. Two variety trials reported in the same paper, show evidence of pollination being greater in one direction, and particularly at distances of less than 2m. Attempts to correlate wind direction averaged over the flowering period, with the degree of cross-pollination in different directions are complicated by the fact that oilseed rape frequently flowers for a period of a month (Scheffler 1995). Peak flowering covers a more limited period, hence it could be the wind direction on a small number of critical days which is important, and

might give a guide to what would happen if the wind flow between two crops was maintained in a constant direction over a long period of time.

Role of barriers: Actual evidence for the effectiveness of crop barriers in oilseed rape is not easily obtained. Morris *et al.* (1999) have compared the effects of bare ground, intervening oilseed rape crop, or a partial filling of the intervening space with a barrier oilseed crop close to the emitting crop, for *B. napus* crops well provided with bees at two sites in the USA. The distance between emitter and receptor was less than 8m. Their conclusion was that it was best to fill the whole gap with a trap crop but the differences between the treatments were not large. In Canada, Stringham and Downey (1978) sampled receptor blocks separated from the emitter by fallow or barley. They found that the edge towards the emitter did not have any greater level of cross-pollination than the rest of the plot and concluded that barrier rows on a receptor crop would not reduce the transmission into more distant parts of the crop. However Bateman's evidence did suggest that barriers might be effective (see next section).

5.3.2.3 Factors affecting degree of cross-pollination once pollen has arrived at the receptor crop

Pollen viability. Viability of pollen with time seems to depend heavily on species (Pacini *et al.* 1997). It has not been possible to find information on the viability of rape pollen held under natural conditions. The capacity of pollen to pollinate flowers when carried by insects, seems to decline quite rapidly with the number of flowers visited. Cresswell (1994) concluded that 91% of cross-pollination occurred on the next four flowers visited by bumble bees and that none occurred after the fourteenth flower. A similar study on white clover showed that bumble bees pollinated more successive flowers than honey bees, which deposited the majority of pollen at the next 15-20 flowers visited (IGER, 1999). This, coupled with the work of Osbourne (1999), suggests that while bees may travel long distances from the hive (up to 2km and sometimes more) and that the pollen may remain viable on bees after they have revisited the hive (Ramsay *et al.* 1999), long distance cross-pollination, by bees between fields is not likely to occur at anything but trace levels.

The area of the receiving crop. The evidence of experiments by Bateman (1947a) has shown that the mass of the receiving crop influences the degree of cross-pollination. The mechanism for this is unclear but as outside rows of plants were more heavily crossed than the inside ones, it suggests that giving insects a greater mass of plants to visit, either impedes their progress or dilutes the effect of their cross-pollination.

Synchrony of flowering. While varieties of oilseed rape do flower at slightly different times, the long period of flowering means that all varieties show considerable overlap in their flowering periods so that separation by varieties of different flowering dates is impracticable. However the bulk of the winter crop has normally completed flowering well before the spring crop (see section 5.3.2.2).

5.4 Factors effecting the degree of gene expression in the harvested crop after pollination by foreign pollen

Part of the plant being harvested. Pollination will normally affect the composition of the seeds (see section 3.4). It will not affect the composition of the straw.

Inheritance. Some F1 hybrids are heterozygous for some traits. In this case, only half the pollen emitted will carry the trait and this may result in only half the cross-pollinated plants showing the trait in the seed or in the next generation of plants.

5.5 Other evidence on oilseed rape cross-pollination

NIAB has made measurements of cross-pollination between plots in variety trials where the spread of genes from varieties with herbicide resistance or high erucic acid content has been recorded (Simpson *et al.* 1999). Because the plots are laid out in a randomised block design, it is difficult to obtain balanced sets of data and some comparisons are disqualified by close proximity to two emitting plots, etc. The plots are relatively small, typically about 40m² however their long-sides are about 20m and therefore they are a rather better test of cross-pollination than by square plots of the same area. Those receptor plots where a single plot lies between it and the emitter plot, have a near edge about 2.5m away from emitting plants and a far edge about 4.3m away. The maximum percentage cross-pollination values between conventional varieties where one plot lies between the emitter and receptor plots is given below for trials at three locations in 1997. (The figures in brackets are for the varietal association Synergy.):

	Cambs	Northumberland	Hampshire
Glufosinate Res	1.0 (2.2)	0.7	0.2 (2.7)
Glyphosate Res	1.2 (4.3)	3.0 (1.8)	1.8

The cross-pollination of conventional varieties at a distance which averaged 3.4m, but which, because of the exponential nature of the pollen fallout process is effectively rather less, are comparable to the values quoted for Simpson in section 5.3.1.1. They have to be qualified with a proviso as to their being overestimates because of the incorporation of an error margin. The increased susceptibility of Synergy with its 80% male sterile plants, to cross-pollination is also apparent, although in this case the difference between Synergy and conventional varieties is not as great as measured in the experiment of Simpson (section 5.3.1.1). This may be because the intervening plot contributes competing pollen which was not recorded as cross-pollination to the synergy. While this evidence is less robust because of the *ad hoc* nature of the experimental design, it does give some confidence to the repeatability of the data of Champolivier and Simpson over more sites and years.

Similar evidence has been collected from 1997 spring sown variety trials (Simpson, 2000) where four transects produced a crossing figure of 2.3% at 1m, falling to 1.1% at 5m which compares with Simpson's 1.1% between autumn sown plots at 3.4m in the same district in the same year, and with 0.86% at 5m in his 1999 winter experiment.

5.6 Recommendations on separation distances for oilseed rape

The following only considers the levels of cross-pollination by adjacent crops. No allowance has been made for impurities introduced by other means, such as admixture in the seed sown or in the harvested crop or for any contribution made by groundkeepers growing in the receptor crop. Recommendations have been made on the assumption that the rectangular field will be at least 2ha.

Because cross-pollination declines exponentially with distance, the level of cross-pollination at the average distance of the whole field from the edge of the emitter is not a good guide to the cross-pollination in the whole field. A large proportion of the cross-pollination within the whole field will occur in the nearest 10m. Calculations using Simpson's data for conventional varieties, suggests that in the relatively extreme case of a 5 ha field with the long side adjacent to the contaminating field, a third of the cross-pollination of the whole field will be found in the first 10m from the boundary.

The following table, achieved by applying Simpson's data to notional strips of increasing distance from the edge closest to the contaminating field, gives a good estimate of the importance that field size, field alignment and separation distance will have. The effect of field alignment has been investigated by assuming a field of rectangularity 4 in which the long side is four the times the length of the short side. The degree of cross-pollination occurring when the long or the short side is aligned towards the emitter crop is compared.

Effect of field size, alignment, and separation distance on the percentage cross-pollination of whole fields, calculated using the data of Simpson in which the cross-pollination level was 1.6% at 1.5m, 0.68% at 11.5m and 0.12% at 81.5m from the contaminating crop.

Field area	Crop separation 1.5m		Crop separation 11.5m	
	Receptor side adjacent to contaminating crop			
	Long	Short	Long	Short
2ha	0.36	0.18	0.24	0.15
5 ha	0.27	0.16	0.19	0.14
10ha	0.23	0.15	0.17	0.13

The above table shows that an 11.5m separation substantially reduces the level of cross-pollination when the alignment or field size are such that cross-pollination is high. Furthermore with fields of only 2ha the rate of cross-pollination can be much greater than in 5ha, if the emitter and receptor fields have their long sides abutting.

5.6.1 Recommendations on separation distances for conventional varieties

To limit cross-pollination to 1% or less on a whole field basis. The above calculations suggest cross-pollination levels of less than 1% on a whole field basis, should be achievable with a 1.5m separation distance. With a 5ha field and the two boundaries relatively adversely aligned, the safety margin is nearly four times, which would allow for smaller fields or other unusual environmental conditions. For a 2ha field, the margin is just under three times. Such a margin would encompass the most extreme case published by Champolivier for France.

To limit cross-pollination to 0.5% or less on a whole field basis. An 11.5m separation between fields provides a safety margin at the 0.5% level. In fact 10m would be sufficient separation to provide a safety margin of at least two times.

To limit cross-pollination to 0.1% or less on a whole field basis. A high proportion of experiments showed cross-pollination at above this level even at distances as great as 60m. At this distance the decline in % cross-pollination with distance is very slow. This means that any distance deduced from interpolation from graphs is subject to high errors. The certification standard for basic seed apparently achieves this level of freedom from cross-pollination, by a separation distance of 400m. It is suggested that while a distance of 100m would probably normally lead to cross-pollination of less than 0.1%, the degree of certainty in achieving this level is poor.

5.6.2 Recommendations on separation distances for hybrids

Varietal associations. The only results available are for the varietal association Synergy as the receptor of incoming pollen. The one full experiment with Synergy suggests that it would be difficult to achieve less than 1% cross-pollination even at 100m. There appears to be no data available for greater separation distances. However measurements in variety trials suggest that the proclivity of Synergy to cross-pollination is less extreme than measured in the experiment. The recommendation for varietal associations at this time is that to achieve less than 1% cross-pollination requires a large separation distance. These cannot be reliably quantified at this time but less than 1% cross-pollination between fields is probably achieved by a 100m separation distance.

Fully restored hybrids. Varieties designated as fully 'restored' may have small proportions of self-sterile plants amongst them but can be considered to be substantially the same as conventional varieties. Only one comparison of a restored hybrid with a conventional variety is known. The cross-pollination in these two spring types was comparable.

Partially restored hybrids. There are also varieties on the market with up to 50% male sterility in the farm crop. This raises the question, at what proportion of male sterility does the risk of cross-pollination increase to such an extent, that a variety can no longer be considered to be the same as conventional varieties and requires a greater separation distance. The answer to this question is apparently not yet available and any demarcation line would have to be set conservatively, at say 10% male sterility.

5.6.3 Separation of spring and winter types

While from a normal sowing, the flowering periods of spring and winter crops are quite different; flowering times can be heavily modified by sowing date and other husbandry or environmental factors. It is therefore suggested that spring and winter types are considered as one crop for separation purposes.

5.6.4 Separation between *rapa* crops

Experiments from Canada have given widely varying results but suggest that the crossing rate between *rapa* crops may be rather higher than between *napus* crops. However because of the safety margin included in the *napus* distances it is appropriate that the same recommendations should apply to separation between *rapa* crops.

5.6.5 Separation between *rapa* and *napus* crops

Experiments have shown the degree of crossing which can occur between *rapa* and *napus* plants to be highly dependent on the circumstances. While it is less than between plants of the same species, it is probably safest to treat the two species as one for separation purposes. This is already the policy for certification and removes the need to identify which species is being grown on a field by field basis.

5.6.6 Multiple adjacent fields

The separation distances specified above are derived for adjacent fields of 2ha or more with long sides abutting. However, where there are emitting fields on more than one side of the receptor crop, the level of cross-pollination is increased. For a field of 5ha or more, the margins allowed are sufficient for the recommendations to be robust even when the fields are surrounded on two sides. However, smaller fields surrounded on more than one side, may in some circumstances, show levels of cross-pollination which exceed the limits set. This might be addressed by other approaches to defining separation which might be applied to smaller fields alone.

6.0 BEET

6.1 Brief description of relevant morphology and crop use

Beet are plants of the species *Beta vulgaris*, they occur in diploid, triploid and tetraploid forms. Varieties are grouped in a number of forms grown for specific uses; sugar beet, fodder beet, red beet and leaf beet, all of which are normally biennial.

In sugar beet, the root is harvested at the end of the first growing season from plants that have not flowered. In some cases, the residual roots and tops are fed to stock. Incoming pollen will only affect the composition of the seed produced on those plants that bolt in the first year. The proportion bolting depends on the earliness of sowing and the variety, but is typically less than 1% of the roots. Pollination will have no influence on the composition of the roots and foliage. The occasional bolting stem gets loaded onto a lorry, but any seed that has remained attached is very unlikely to make its way into the processing plant.

The roots of fodder beet are consumed by stock and some of the tops are also utilised, and again these portions of the crop are unaffected by the source of pollination of the seed. It is possible that occasional seeds which have not been shed from bolters are consumed by animals but they will tend to avoid the seed heads because of their fibrosity. The percentage of plants that bolt is typically around 1%.

Red beet and leaf beet are considered to be vegetables rather than field crops in which the root and leaves respectively are eaten. As far as is known, in none of these crops is the seed consumed by humans or stock.

6.2 Recommendation

As it is only the vegetative portions of the crops of all the forms of beet which are harvested, the risk of the utilised portion of the crop being affected by cross-pollination is exceedingly small.

Growers who are worried about the exceedingly small risk of incorporating seed into their utilised crop, can take the precaution of destroying their bolters during the growing

season. It is recognised good agricultural practice to cut the bolters before flowering to prevent the formation of ground keepers.

7. CONCLUSION

Summary of separation distances (m) required to maintain cross-pollination of the whole fields at below specified levels for fields of 2ha or more

CROP	THRESHOLD LEVELS OF CROSS-POLLINATION		
	1%	0.5%	0.1%
Oilseed rape (<i>B. napus</i> and <i>rapa</i>)			
Conventional varieties and restored hybrids	1.5m	10m	100m*
Varietal associations and partially restored hybrids	100m	n/a	n/a
Maize/sweetcorn			
For grain	200m	300m	n/a
For silage	130m	200m	420m

n/a – insufficient information to produce a recommendation (see report for more detail)

* – see Section 5.6.2.

These recommendations on separation distances are derived from information from experiments on levels of cross-pollination and take into account factors which operate when this information is applied to whole fields. While the information can be used to produce robust recommendations, which will ensure pollination levels at less than the threshold level in almost all cases; it must be recognised that it is impossible to give an absolute guarantee that pollination levels will never exceed that level.

The recommendations on separation distance are designed to limit impurities when cross-pollination occurs at the highest levels recorded in the experiments. In some cases these experiments were outside the UK where environmental conditions may be different. On-going work on separation distances being carried out in the UK and Europe may produce more estimates of the cross-pollination taking place in conditions relevant to the UK at some time in the future. These recommendations could then be reviewed.

There may be more sophisticated ways of defining separation distances than the distance between the nearest borders of the two crops and this is likely to be particularly important for small fields. However, the usefulness of such methods will depend on whether they can be easily applied to real 'on farm' situations.

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