

Review

Evaluation of the Risks of Contaminating Low Erucic Acid Rapeseed with High Erucic Rapeseed and Identification of Mitigation Strategies

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Abstract: High erucic acid rapeseed (HEAR) oil is under increasing demand for various industrial applications. However, many growers are concerned that if they grow the crop, they will not be able to revert to other rapeseed varieties in the future due to the risk of erucic acid (EA) contamination of the harvested seed and inability to maintain acceptable erucic acid thresholds. This review considered published literature and, using the same criteria as that used to contain transgenic crops, aimed to identify the key risks of erucic acid contamination, broadly prioritise them and identify pragmatic mitigation options. Oilseed rape has a number of traits that increase the risk of low erucic acid rapeseed (LEAR) crops being contaminated with EA from HEAR varieties. The quantity of seed produced and the potential for seed dormancy coupled with partial autogamy (self-fertilisation) facilitate the establishment and persistence of volunteer and feral populations. The large quantities of pollen produced when the crop is in flower mean there is also a high potential for cross-pollination. Self-sown volunteer plants represent the highest potential contamination risk, followed by the presence of arable weeds (e.g., wild mustard) whose seeds are also high in EA. Other risks arise from the cross-pollination of compatible wild relatives and the mixing of seed prior to sowing. It is important that both HEAR and LEAR varieties are appropriately managed since risks and their potential for mitigation arise throughout the entire LEAR crop production process. The length of rotation, type of tillage, cultivar choice, buffer zones, effective weed management and basic machinery hygiene are all factors that can reduce the risk of erucic acid contamination of LEAR crops and maintain the required thresholds.

Keywords: HEAR crops; LEAR crops; *Brassica napus*; volunteers; adventitious mixing; cross-pollination; seed dormancy

1. Introduction

The global popularity of rapeseed or oilseed rape (*Brassica napus* var. *oleifera*) has grown steadily since World War II, with a significant upward trend seen over the last 30 years. This has been due to a number of factors, including improved breeding, the introduction of price incentives under the EU's Common Agricultural Policy, greater consumer interest in vegetable oils rather than hard fats, and rapeseed oil being granted Substances Generally Recognized as Safe (GRAS) status in the USA. The introduction of genetically engineered rapeseed offering herbicide resistance and the use of rapeseed for biofuel production has also significantly increased production such that it is now the most produced oilseed in the world [1,2]. The annual global seed harvest was around 70 million metric tons in 2015/2016, of which 22 million metric tons (31.4%) originated from the EU [3,4].

Erucic acid (EA) is the common name for the omega-9 fatty acid Z-13-docosenoic or cis-13-docosenoic acid. Using EA content as the classifier, there are three broad categories of

rapeseed. High Erucic Acid Rapeseed (HEAR) contains 45%–60% erucic acid compared to unimproved varieties that have ‘moderate’ content (35–40%) coupled with high ($>150 \mu\text{mol g}^{-1}$ seed) glucosinolate concentrations. Low Erucic Acid Rapeseed (LEAR), which includes double-low ‘00’ varieties (i.e., those low in EA and glucosinolate), has approximately 0.03% erucic acid [5–7].

Whilst EA is found naturally in some vegetable oils, the effects on human health have been questioned. In a recent review, tentative but inconclusive findings of an association between high EA dietary intake and a higher incidence of congestive heart failure in humans were reported [7]. Other effects observed in animals include changes in the weight of the liver, kidney and skeletal muscle. There have also been concerns that high concentrations of EA in feed may present a risk to poultry. The authors calculated a tolerable daily intake (TDI) of EA of 7 mg kg^{-1} body weight per day and concluded that this could be exceeded by some consumers, for example, children and infants with diets high in vegetable oils [7]. EU legislation requires oils and fats used in foodstuffs to contain less than 5% EA, expressed on a fat basis, and there are more stringent levels for infant foods (EC Regulation No. 2006/141/EC). To help deliver these standards, the EU threshold for HEAR in LEAR oil is 2% [8]. The Federation of Oils, Seeds and Fats Associations Ltd. (FOSFA) requires rapeseed crushers to comply with a quality standard (FOSFA 20A) that includes this 2% threshold and, consequently, crushers will reject harvests that exceed it.

Demand for both HEAR and LEAR oil is growing rapidly. Europe is currently the principal consumer of both oils, but increased demand is also being seen from emerging economies in Asia, particularly China and India, due to their population growth and increasing prosperity [4]. The markets for HEAR and LEAR can be quite different. LEAR oil tends to be used for products destined for human consumption (e.g., cooking and salad oils, margarines), for high protein animal feeds and for biodiesel. Whereas the unique properties of HEAR oil make it valued for various industrial applications, including high temperature lubricants, paints, inks and the anti-slip properties of HEAR oil are exploited in cling-film and polythene products [2].

HEAR crops are widely grown in Europe and although precise data is lacking, a production area of around 110,000 hectares yr^{-1} equivalent to approximately 1.6% of the total EU rapeseed crop is estimated [9]. HEAR varieties have the same general agronomy and produce similar yields to LEAR varieties but significant price premiums are offered in comparison. However, due to rapeseeds ecology and production methods, these crops have a high EA contamination potential [10,11] and consequently, despite high demand, there are a number of concerns regarding the risks producers may face if they chose to grow a HEAR crop. These include fears that land might become ‘contaminated’ with HEAR seeds and volunteer plants preventing growers from reverting to low EA varieties in the future. LEAR crops grown close to HEAR varieties could be subject to adventitious mixing via unintended cross-fertilisation resulting in an increased EA content within the seed. This would then exceed EA thresholds and be rejected for human consumption. There are also worries that unacceptable levels of EA could be introduced into the food chain causing health impacts and damaging the industry.

In response to concerns that the production of HEAR crops on a farm will prevent the growing of LEAR crops in the future and a lack of overall guidance, the purpose of this review was to evaluate two key research questions. Firstly, ‘What are the main factors and risk levels affecting the potential of LEAR crops to be contaminated with EA from HEAR varieties?’. This considered crop ecology, environmental issues and farming practices. The second question ‘What measures can be adopted to mitigate these risks and how effective are they?’, considered what effective, practicable and pragmatic options on farm could be adopted to reduce risks and ensure that any mixing is below the 2% threshold.

2. Methods

The methodology adopted for this study was not strictly that of a systematic review, however, it did utilise a similar systematic approach in that it used a pre-defined plan that identified the literature databases to be searched, the search terms and quality criteria. Database searches were supplemented with techniques including the snowballing of references located within manuscripts

identified during the literature searches. The literature databases used were Scopus, Web of Science, Google Scholar and Science Direct. Search terms included (but were not limited to): ‘high erucic acid rapeseed’ in combination with ‘low erucic acid rapeseed’, ‘volunteer’, ‘gene transfer’, ‘pollen transfer’, ‘cross-fertilisation’, ‘weed species’, ‘mitigation’, ‘buffer zone’, ‘seed spillage’, ‘pod shatter’, ‘hygiene’ and ‘seed dormancy’. Reviewed publications were limited to those written in English but were not restricted by any geographical boundary. Each published work identified was evaluated and critiqued before being summarised in terms of the two review questions. For a document to be accepted for inclusion in the review, a number of criteria needed to be satisfied. These included (i) ‘aims, objectives and context’ had to be clearly stated and appropriate, (ii) the article must contain a clear and detailed methodology which itself should include a description of the sampling approach utilized that is justifiable, representative and appropriate, (iii) the statistical analysis employed must be appropriate and (iv) the conclusions should be fully supported by the data. After application of these criteria, a total of 166 peer reviewed published documents considered to be of relevance and which met the inclusion criteria for the period from 1996 to 2019 were identified.

The mitigation measures adopted to avoid EA contamination of LEAR crops by HEAR are comparable to those used to maintain gene transfer below acceptable thresholds for transgenic rapeseed crops. The labelling threshold for the presence of EU authorised transgene material in non-genetically modified (GM) crops is 0.9% (EC Regulation No. 1829/2003, revised April 2008). The EC Scientific Committee on Plants [12] state that strict mitigation measures are required to reduce EA contamination via cross-pollination (outcrossing), seed and volunteer plants. Cross-pollination is defined as ‘the introduction of genetic material into a breeding line’ [13]. Volunteer plants refer to self-sown seeds that then germinate in subsequent crops. The review distinguishes between volunteers (plants identical to the parent plants) and hybrid volunteers (plants where there has been outcrossing between HEAR and LEAR cultivars). Essentially, mitigation measures considered appropriate to maintain gene transfer in transgenic rapeseed to below acceptable thresholds are in excess of those for HEAR rapeseed [5,14]. They can therefore be considered for the purposes of this review as adequate methods, despite their original context being for transgenic crops. The review was structured according to the risks posed at each stage in the production cycle of *B. napus* (Figure 1) and the associated mitigation options (adapted from [15]).

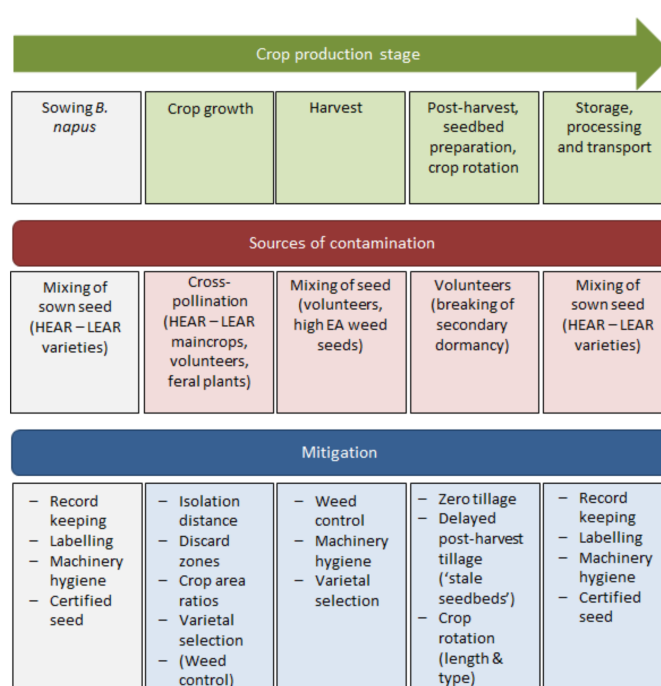


Figure 1. Critical control points for avoiding adventitious mixing (adapted from [15]).

3. Review Findings

Raised concentrations of EA in seed may result from cross-pollination, HEAR impurities in sown seed, volunteer or wild *B. rapa* (noted as occurring infrequently but capable of crossing with *B. napus*) and contamination of harvested rapeseed with the seeds of arable weeds such as wild mustard (*Brassica kaber*) or charlock (*Sinapis arvensis*) [5,16].

3.1. Sowing

Rapeseed is usually grown in rotation with early maturing spring or winter barley and often precedes winter wheat. Within the United Kingdom (UK), and indeed most of northern Europe, optimal sowing dates for winter oilseed rape are late August to early September. The crop has a long growth cycle being harvested in early summer the following year. Seeds typically have an oil content of around 40%. The spring sown crop has a shorter season, being sown in early spring and harvested late summer the same year. It has a lower oil content compared to a winter crop [17]. The risks of LEAR crop EA contamination at this stage in the crop growth cycle are considered low and arise from the sowing of LEAR seed contaminated with HEAR hybrid seed. Seed impurity is pertinent to both the sowing and the storage and processing phases of the production cycle in Figure 1. It is addressed in the post-harvest section later in this review.

3.2. Crop Growth

While the crop is growing (Figure 1) there are two main risks of LEAR crop EA contamination from HEAR cultivars: (i) cross-pollination from neighbouring HEAR crops, HEAR volunteers or HEAR hybrid volunteers when the crop is flowering; and (ii) premature seed shed from HEAR crops and the creation of HEAR volunteer populations within the cropped area, or feral populations outside the cropped area. Messean et al. [18] prioritised the risk (high to low) of the adventitious mixing of genetic material (in this case GM and non-GM crops) as: volunteers from seedbanks (high) > cross pollination (moderate) = wild relatives (moderate) > feral plants (low), with the caveat that all are subject to local variability in conditions. Kightley [19] dispute the risk posed by wild relatives, considering it to be low. *Brassica napus* has a number of traits that increase the risk of LEAR crops being contaminated with EA from HEAR varieties. The quantity of seed produced and the potential for seed dormancy coupled with partial autogamy (self-fertilisation) facilitate the establishment and persistence of volunteer and feral populations.

The large quantities of pollen produced when the crop is in flower means there is also a high potential for cross-pollination. The control of hybrid volunteers is undoubtedly important as a mechanism to reduce the risk of adventitious mixing of rapeseed types. The estimated contribution of volunteers to adventitious mixing varies considerably from around 0.1 to 10%. The variability is due in part to whether it is a pure HEAR-LEAR cross or a hybrid-pure LEAR cross [19]. It also depends on management practices, soil and climate [5,20–25]. Failure to control volunteers sufficiently will increase gene flow to the extent that coexistence would be difficult to achieve [13,18,26,27]. Squire and Begg [28] concluded that the influx of genotypes from *B. napus* in surrounding fields would maintain adventitious mixing below 1% only if *B. napus* had not been grown in the field previously where there was an absence of volunteers. *Brassica napus* has a number of traits that increase the risk of volunteer populations becoming both established and surviving for several years.

The first generation of volunteers following a HEAR *B. napus* crop in the rotation will not typically be hybrids. Hybridisation has the potential to result from cross-pollination with neighbouring LEAR crops or if a LEAR crop is grown later in the rotation. The latter will most likely be a few years (typically 3 or 4) later. According to Kightley et al. [19] EA levels result from the expression of dominant and recessive alleles, which have an additive effect. This results in a range of EA levels depending on the generation and the number of previous outcrosses. If cross-fertilisation has occurred via a neighbouring LEAR crop or a subsequent LEAR crop in the rotation, the persistence of the hybrid

first generation (F1) volunteer population requires surviving F1 hybrid volunteer plants to return seed to the soil seedbank. In order for this to occur, the hybrid F1 generation must have sufficient ‘fitness’ to permit reproduction and compete with the sown crop cultivars. The quantity of pollen and seeds produced by volunteers depends on what Cuthbert et al. [29] termed ‘growth vigour’ (plant heterosis) in combination with local climate and field specific factors such as soil type and underlying soil nutrient status [30]. A summary of these traits is given in Table 1.

Table 1. Potential morphological traits conferred to hybrid volunteers.

Trait	Description	Advantage/Disadvantage	Reference
Flowering time	HEAR F1 hybrids flower at time similar to that of the earliest parent. Growth vigour is not associated with flowering time.	Strong competition for nutrients, water & light. Where flowering with the crop is simultaneous outcrossing is likely.	[29]
Plant maturity	HEAR F1 hybrids mature at time similar to that of the earliest parent. Growth vigour is not associated with maturing time.	Strong competition for nutrients, water & light.	[29]
Growth rate & biomass production	HEAR—LEAR F1 plants often demonstrate superior growth rates and biomass accumulation compared to their parents.	The period between sowing & volunteer emergence may be critical regarding the intensity of competition.	[30]
Plant height & stem elongation	Hybrid plant height tends to be intermediate falling between that of each parent.	If the crop is shorter than the hybrid especially if hybrid has increased vigour, the volunteer has a selective advantage. However, this additional height allows volunteers to be more easily identified and so removed.	[29–31]
Lodging resistance	No evidence of high parent heterosis for lodging resistance in any HEAR hybrids.	No advantage conferred.	[31]
Seed yield	HEAR F1 hybrid may produce seed yields of up to 140% more than the highest yielding parent.	High potential for seed and so volunteer occurrence and persistence.	[29]
EA content	F1 hybrids may display greater EA content, although screening has identified this potential in only 6% of cultivars.	Enhanced potential for HEAR EA contamination in the crop.	[29]

The study by Cuthbert et al. [29] reports on hybrids deliberately created for the purpose of field trials and was a single assessment based on these field trials rather than widely grown commercial crops. Whether it reflects what would occur in reality is questionable. Hybrid vigour is usually generated if two (or more) specifically selected inbred lines are combined, which is unlikely in this context. That is not to say that advantages will not be conferred on the hybrid population, but it will be due to differences in traits rather than hybrid vigour. For example, where there is a difference in plant height due to the volunteer or hybrid volunteer being taller than the sown crop if the crop is a semi-dwarf variety. Furthermore, should hybrid vigour result from, for example, crosses between second generation (F2) HEAR volunteers and a LEAR crop, the traits are not likely to be prominent or

consistent. Field specific spatial factors can be important in determining the likelihood of a volunteer population becoming established. Gruber et al. [32] noted reduced growth in some hybrid F1 volunteer plants due to competition from the crop and/or germination in less favourable areas of the field. In contrast, hybrid F1 volunteers germinating in crops with a low plant density or in the crop headlands may achieve increased growth and fecundity due to decreased plant competition. The authors suggest establishing increased crop density as a mechanism to outcompete volunteers and reduce the risk of volunteer population establishment. Any mitigation strategy adopted should seek to prevent the ability of hybrid volunteers to exploit any potential ecological advantages. However, this will be site-specific and require a mitigation strategy for each farm individually [27]. The precautionary screening of seed and volunteers before flowering, coupled with knowledge of the occurrence of *B. rapa* and compatible relatives at the farm level has been suggested [5]. Volunteers were identified as high risk by Messéan et al. [18]. A hybrid volunteer population of 8% is calculated to increase seed EA content above the 2% threshold, with over 80% of the EA in the harvested seed derived from harvested HEAR hybrid volunteers and the remaining EA content from volunteer outcrossing or cross-pollination [33].

Cross-pollination is considered to be of medium risk by Messéan et al. [18] for causing LEAR crop EA contamination. A number of factors must be satisfied for its successful completion. It requires compatibility between the donor and receptor plant, sufficient proximity between the two plants, that they flower simultaneously and that the receptor crop has not already self-pollinated [13]. Successful HEAR pollen transfer may occur by several possible routes: (i) directly from persisting HEAR volunteer or hybrid HEAR–LEAR volunteer populations to a subsequent LEAR crop grown in the same field later in the rotation, (ii) from persisting HEAR volunteer or hybrid HEAR–LEAR volunteer populations to a LEAR crop in another field in close proximity, or (iii) to compatible wild relatives or feral populations.

Rapeseed has partial autogamy, i.e., is partially self-fertilising (to 70%) and partially cross-pollinating (to 47%) [34,35]. Where self-pollination has not occurred, pollen can be dispersed either via wind or insect vectors [18], although no correlation between outcrossing rates and prevailing wind direction has been identified [35]. Bilsborrow et al. [33] concluded that insects were mainly responsible for pollen transfer between adjacent field trial plots, although Kightley et al. [19] regard most pollen transfer via bees as being contained within the same crop. Due to partial self-fertilisation, *B. napus* has the capacity for self-recruitment, i.e., it can produce seed despite not being fertilised by a second donor plant. Even in locations where cross pollination may be difficult, small isolated and fragmented populations of HEAR volunteers (weeds in the crop) and feral plants (those growing outside the cropped area), have the potential to set seed. Volunteer HEAR populations may therefore persist for a number of years. There is also the potential for plants to cross-fertilise with other compatible plants, either with those in the host crop or with wild relatives. In ecological terms, Knispel and McLachlan [36] distinguished between self-replacing plant populations and those dependent on seed immigration, the former being able to sustain numbers despite being isolated from other *B. napus* seed sources. Volunteer plant populations isolated in large fields, for example, may persist for several years without any additional input of seeds from neighbouring crops. Where these are HEAR or hybrid volunteers, there is the risk of cross-fertilisation with a LEAR crop grown in the same field, or the production of seed that will be mixed with LEAR at harvest.

Outcrossing with neighbouring plants within the same field through direct physical contact is estimated at between 3% and 47% [34,37]. Gruber and Claupein [38] evaluated two risk scenarios. Under a low risk scenario (defined as low volunteer density, low seed persistence, low outcrossing rate) they concluded that < 0.1 volunteer seeds m^{-2} will be returned to the seedbank with an additional 0.4 seeds added via outcrossing with a neighbouring *B. napus* main crop. Under a high-risk scenario (high volunteer density, high persistence, high cross-pollination rate), seed return may increase to 519 seeds m^{-2} with a further 339 seeds returned via outcrossing. Both the low- and high-risk scenarios exceeded the 0.9% GM threshold. Only the low risk scenario represents acceptable levels of seed return to ensure compliance with the 2% EA threshold. The study by Gruber and Claupein [38] represents one cropping year, although there exists uncertainty regarding the longer-term impact of volunteers on

the adventitious mixing of genetic material. There is potential for the control of volunteers while the crop is actively growing using herbicides as part of the 'Clearfield' system [19]. This and other weed control strategies are discussed in the post-harvest section of this review.

In terms of mitigating cross-pollination, the distance between the donor and receptor plants is also seen as important by many authors. Outcrossing is typically <0.1% at distances of 100 m from the edge of the donor crop to that of the receptor crop [18,39,40]. Rates increased to 0.5% and 1% at distances of 10 m and 1.5 m respectively [39]. Cases of outcrossing exceeding 0.1% at distances of 100 to 1000 m have been documented but are less frequent [18,41,42]. Screening or the presence of barriers between potential donor and receptor plants may also reduce cross-pollination but evidence is slim. An EA content in LEAR crops of 0 to 0.8% when separated by a hedgerow and distance of <10 m was identified by [33]. However, a precise distance was not reported, nor did the authors discuss the physical dimensions or potential impact of the hedgerow. Outcrossing with neighbouring plants through direct contact physically, as may happen with different cultivars in the same field, has been estimated at between 12 and 47% [34]. One issue to note is that the published literature dealing with buffer distances is mostly in excess of 10 years old and, although not subject to regulation, the adoption of 50-m buffer zones between HEAR and LEAR crops is now widespread [19]. In reference to the findings of Ingram [39], this distance of 50 m would be expected to maintain cross pollination levels below the 0.5% stated for 10 m.

A further strategy is to rigorously enforce recommended separation zones, these being described as 'standard coexistence measures' [18]. Colbach et al. [43] used probability analysis to determine separation distances that would deliver an acceptable risk and concluded that a 'zero risk strategy' is not possible. Several studies, albeit those conducted over a small spatial scale, concluded that the percentage cross pollination decreases with increased distance between the donor and receptor crops, typically being less than 1% where there is a distance of 100 m between crops [10,18,40,42,44–46]. Irrespective of separation distance, cross-pollination will not be eliminated in its entirety, as adventitious mixing (in this instance GM and non-GM pollen) have been recorded at distances of 800 m [47], 1100 m [48] and 3000 m [49]. Nevertheless, separation distances of at least 100 m will reduce the problem significantly.

Messéan et al. [18] propose 'flexible coexistence measures' that permit two different crop varieties to be grown adjacent to one-another without the physical separation distance of isolation zones. They include non-GM buffer zones sufficient in size to prevent cross-pollination or discard zones in, for example, a non-GM crop where a zone of the side nearest the GM crop is not marketed as a non-GM crop. They are promoted in this context more for mitigating risk between two growers, one producing GM the other non-GM with emphasis on the coordination of activities.

At the landscape scale the presence of mixed farming or an increase in landscape heterogeneity (the presence of non-cultivated land, e.g., permanent grassland) has been identified to act as a buffer and reduce the risk [43,50]. Field area ratio and field shape are also factors that may contribute to the risk of adventitious mixing to a moderate degree [27,28]. A landscape scale analysis of cross-pollination between HEAR and LEAR cultivars concluded that cross-pollination alone would not result in exceedance of the 0.9% adventitious mixing threshold [51]. An exception, however, is where a large area of the donor crop is present in combination with a receptor crop at distances of less than 50 m. This may be increased for example, where there is a high ratio of crop edge to crop center, as encountered in smaller fields. In an assessment of multiple source HEAR crops adjacent to a single LEAR receptor crop, the recorded mean outcrossing rates were 3.1 and 3.5% for the outer 10 m of two field trials [35]. This was well above the mean 0.9% across multiple varieties established in an earlier literature review by the same authors [40]. The difference is explained as a result of elevated pollen levels originating from the multiple receptor crops and the four-fold magnitude in area of the donor crop located within 50 m of the receptor crop, compared to the equal size of donor and receptor crops in the other studies reviewed. Another important aspect is that insect vectors may find it easier to switch between small crops compared to larger ones. This may result in a higher than typical pollen transfer rate between donor and receptor plants [35] meaning that results derived from field plot recipient plants may

overestimate pollen transfer and cross-fertilisation. In the Dietz-Pfeilstetter et al. study [35] at the trial plot centre and 22 m from the donor crop, outcrossing was 1.8% to 1.9%. The authors concluded that a 10-m isolation distance may be sufficient to maintain thresholds at or below 0.9% over larger areas but would require revision for smaller fields. Sausse et al. [27] acknowledged the need to consider the area ratio between the donor and the receptor cultivars in contiguous (adjoining) fields, with an increasing risk of adventitious mixing occurring as the ratio between the donor and receptor crop increases.

There are also a number of lower risk routes by which LEAR crops could become contaminated by HEAR, with feral plant populations being the main concern. An early study Adler et al. [52] evaluated mechanisms that influence the potential for transgenic plants to persist within the environment. They concluded that these include dormancy, the parental contribution to endosperm formation (proportion of male versus female), the direction of the cross (which genotype provides the source of pollen), and how these factors interact with light, nutrient and cold period. For a feral plant to survive in, for example, a grass verge it must compete within a perennial plant community. This ability is affected by the sensitivity of the seed to factors such as soil nutrient content, being sensitised by cold stratification (vernalisation) to promote spring germination, and the response to light after sensitisation, coupled with the germination or seed survival [53]. Typically, crop seeds show no sensitivity to germination cues due to human selection [52]. *Brassica napus* would appear to be an exception to this general rule as secondary dormancy is induced by unsuitable germination cues such as light, soil moisture content and temperature [22,23]. One early study showed that sensitivity to environmental factors was present in wild annual plant populations due to competition with other plant species, and the narrow window of opportunity to successfully germinate and compete with the perennial plant species that dominate [53]. Feral *B. napus* plants present within a perennial plant dominated community located outside of the cropped area, such as within a permanent field boundary or hedgerow, are exposed to greater competition. Volunteer *B. napus* need only compete with the crop and, according to Cullen et al. [5], may have a further selective advantage in the form of enhanced plant vigour to enable it to do so. Evidence of the impact of feral populations is somewhat contradictory, partly due to their fragmented and site-specific nature [54]. In an assessment of five study areas and 20 growing seasons, it was concluded that the distribution and persistence of feral *B. napus* is possible but the proportion of the population overall and their contribution to adventitious mixing is negligible [18]. Two studies both noted that feral populations of cultivars were not of the dominant genotype currently being grown in adjacent fields [55,56]. They showed instead to have greatest similarity with the cultivar grown the previous year. Several studies have suggested that feral plants often survive for just one year even when self-seeding the previous year was prolific [54,55]. For example, it has been found that, in a given year, 35%–40% of feral *B. napus* plants resulted from the movement of seed from the adjacent field during the previous year [57]. One potentially contradictory observation was that there was no sufficient distinction of a proportion of feral genotypes to allocate a specific cultivar to them, which would be expected if derived from a single cultivar grown in an adjacent field the previous year [55].

The cultivar diversity of feral populations was observed to be greatest along roadside verges compared to footpaths, suggesting dispersal via farm vehicles over a period of time [56]. Verges adjacent to routes near storage areas where traffic frequency was greater tended to increase in feral *B. napus* cultivar diversity. Bailleul et al. [55] also concluded that traits expressed in feral populations may not be solely due to physical movement of seed from the cropped area via, for example, accidental spillage as there was potential persistence via cross-pollination between the main crop and feral plants in the boundary areas each year. In contrast to Messéan et al. [18] they concluded that feral plants indeed do persist and potentially may result in the adventitious mixing of traits with cultivars grown in neighbouring fields. The Bailleul et al. [55] study is unique in that it is the culmination of 4 years data over a larger spatial scale of 41 km². This conclusion is in agreement in part with Banks [54] who considered that despite the abundance of feral plants increasing in certain years, the risk of gene transfer from the feral population is minimal. Their study observed a maximum of three individual feral plants present within 10 m of flowering spring *B. napus*. They concluded that it would be highly

unlikely that these individual plants would be harvested with the main crop because flowering time is not typically synchronised with that of sown *B. napus* crops. If they were, the seed impurity would remain between 10 and 100 times below the threshold, in this case, the 0.9% for GM and non-GM crops and so far less than the 2% HEAR threshold. A caveat is the highly site-specific nature of the impact of feral plants, which must be taken into consideration on a site by site basis.

Cross-pollination between LEAR and high EA wild relatives represents a further potential cross-pollination pathway. Many authors dismiss this, however, as not being viable. Although *B. napus* can outcross with *B. campestris* or *B. rapa* (the parent of *B. napus*), cross-compatibility with wild plant species is usually low, and additionally, the offspring are typically sterile [38]. *Brassica napus* is allotetraploid. Outcrossing with a diploid wild species results in triploid offspring, which tend to be sterile [38]. In terms of seedling survival, the proportion of F1 hybrid seedlings noted by [58,59] was <1.5%. The overall seed abundance from *B. rapa* populations within 10 m of *B. napus* crops averaged 0.56% and plant abundance averaged 0.45%. The pollination of *B. rapa* by F1 hybrids is reported as 2.5% by [60]. The number of triploid F1 hybrid plants reaching maturity assessed one year later ranged from 0%–1.579% [61] and 0%–0.769% [62]. Kightley et al. [19] regard the hybridisation of wild brassicas and *B. napus* as unlikely. Wild turnip rape is cited as an exception where an elevated EA content in hybrid volunteers was reported by [63] but this is limited to a single study. Furthermore, the potential for cross-pollination between LEAR and high EA wild relatives will also be highly localised due to variation in spatial distribution and abundance in combination with the characteristics of the cultivar of origin and local site conditions [18,59,62].

3.3. Harvest

Of moderate priority to reduce the risk of LEAR crops being contaminated with HEAR is the management of arable weeds in the crop. The findings of [16] showed that volunteers are the most likely source if EA contamination levels are very high but low levels of EA contamination most likely result from arable weeds high in EA [16]. Such weeds include wild mustard (*Brassica kaber*, 31.5% EA), charlock (*Sinapis arvensis*, 31.7% EA), wild radish (*Raphanus raphanistrum* subsp. *Raphanistrum*, 26.7% EA), hedge mustard (*Erysimum officinale*, 20.9% EA) and cut-leaved cranesbill (*Geranium dissectum*, 9.9% EA) [64]. Wild radish has been noted as a particular problem. Another study identified that failing to separate wild radish seed from harvested *B. napus* seed increased EA levels above acceptable thresholds [65]. Where weeds are present, it has been suggested that separating parts of the crop where EA-rich weeds are present in abundance from the remaining LEAR *B. napus* harvested is a means to avoid EA contamination of the whole crop [66]. Weed management is achieved through an appropriate rotation or using novel weed control measures post-harvest (Section 3.4).

A further consideration is the capacity for rapeseed to disperse seeds into the soil both before and during harvesting [13] which is in stark contrast to crops such as maize. It will produce high numbers of seeds, a proportion of which may be shed prior to harvest as either whole pods or via pod shatter (dehiscence). Premature dehiscence as a frequent event may return up to 8000 seeds m⁻², a factor up to 50-times the number of seeds originally drilled [24]. Seed loss may be further exacerbated by season-specific factors. Hailstorms for example may cause severe pod shatter [67]. Varieties with enhanced shatter resistance (e.g., [68,69]) represent a valuable mechanism by which to prevent the establishment of volunteer *B. napus* populations. Methods to mitigate secondary dormancy are discussed in the following section.

3.4. Post-Harvest

The seeds of *B. napus* are small, spherical and have a smooth coat, all which facilitate passive burial. Burial depth is also a factor in germination success with the highest level of emergence occurring when a seed is buried between 1 and 5 cm. Seeds buried below 10 cm are much less likely to emerge [70–72]. Zero tillage as a means to prevent seed burial and secondary dormancy is cited by Kightley et al. [19] as a potential mitigation strategy and this is discussed later in this review. The seed exhibits Type 1

non-deep physiological dormancy, including germination at and tolerance of low temperatures with the capacity for germination declining from spring through to summer [71,73]. It has been found that seedling emergence during the spring was poor, decreasing further as the season progressed and soil temperatures increased [74]. At this point in the growing season, the likelihood of seeds entering secondary dormancy increases. *Brassica napus* seed is able to enter into secondary dormancy when conditions are unsuitable for germination. Secondary dormancy enables persistence beyond the following crop, potentially enabling germination in a *B. napus* crop grown years later. In Europe, seedbank persistence, demonstrated by the identification of genotypes matching HEAR crops, has been noted for periods up to 17 years [31,75–78]. It has been noted that volunteers and feral plants were persistent even though the cultivar in question had only been grown for one year [76]. Volunteers have been identified from varieties grown between 7 and 11 years previously that were no longer on commercial varieties lists [76]. Perhaps more intriguing was the presence of varieties that had apparently not been grown previously within the field under analysis, dating back as far as 19 years since entering varietal lists. Kightley et al. [19] believe such incidences to be the result of poor record keeping, also identified as a key mitigation strategy later in the review. Evidently, volunteers and feral populations arise early in the life cycle of a cultivar and may persist for many years after the cultivar is removed from commercial seed stocks. Secondary dormancy may be broken several years later. Many factors have been shown to affect the risk of secondary dormancy being broken, including the specific crop cultivar [32], soil cultivation practices [38,79], unsuitable temperature [20,23,24], light intensity [20,23,24], and soil moisture content or water-logged soils and hypoxia [20–22,25].

The control of volunteers is a critical issue and this is important at all crop production stages. A number of strategies to mitigate volunteers exist, which ideally should be applied in combination. Colbach et al. [43] identified the cropping system as the greatest single factor in determining seed impurity at harvest. Work has mainly focused on the period between *B. napus* crops in the rotation with the general agreement that the period should be maximised with no sequential cropping, ideally extending the rotation frequency beyond one year in four. An exponential decline in the persistence of the volunteer population after five years in the absence of additional seeds from feral plants was identified by [80]. Oilseed rape grown one year in four is likely to equate to the highest risk of cross-contamination [18,26,67,81,82]. It has been suggested that the reason for this is that a 3-year gap would see a 95% decline in seedbank viability under UK conditions, with 6 to 7 years being required for all seeds to lose viability [83]. However, this is a conservative estimate compared to the 13 to 17 years suggested by others, although the period may be less on lighter soils [31,75,76]. It is also based on the assumption that dormancy is maintained and there is no reseeded from volunteers during that time. The surviving 5% of viable seeds will still represent potential to produce a significant infestation in the following crop. While extending the rotation will evidently reduce the risk, the duration would need to be many years. Modelling exercises have found that a period of 16 years is required after harvest of a GM crop to reduce impurity to below 1% and that this could only be reduced to within five years (later revised to 3 years [84]), if mitigation strategies were applied ‘rigorously’ [28].

The crops within a rotation can also be used to mitigate risk, although an element of dispute exists between studies with respect to precisely which crops. The unsuitability of field conditions for the survival of *B. napus* volunteers associated with crops such as potato (*Solanum tuberosum*) or sugar beet (*Beta vulgaris*) was highlighted by [85]. The study found that the abundance of certain weeds that have similar life-cycles to *B. napus* (e.g., ‘slow determinate’ species such as shepherd’s purse (*Capsella bursa-pastoris*) and wild chamomile (*Matricaria chamomilla*)) declined significantly with the inclusion of both *S. tuberosum* and *B. vulgaris* crops in a rotation, therefore, the authors recommended their inclusion in a LEAR rotation as a potential mitigation strategy. The inclusion of *B. vulgaris* within a rotation is, however, in contrast to the more recent recommendations [86]. They specify avoiding *B. vulgaris* for the very purpose of reducing volunteer risk. A further issue that Debeljak et al. [85] do not appear to take into consideration is that growing *B. vulgaris* and *B. napus* in the same rotation may enhance the populations of pests such as beet cyst nematode or diseases including alternaria [87].

Similarly, it has been emphasised that the crop following *B. napus* should be selected to enable the effective control of broadleaved weeds, however, they cite winter wheat as an example [64]. Winter wheat would appear to be a more preferable option. Further, it is typically found within rotation with *B. napus* already and so will not require modification to existing crop cycles. An increase in spring crop frequency reduces volunteer emergence and reproduction rates relative to winter sown crops [71,73] enabling more effective implementation of the ‘stale seedbed’ technique [19] discussed later.

Leaper and Melloul [64] considered the efficacy of broadleaved weed control products available for wheat crops to be adequate in the removal of volunteer *B. napus* plants. The use of herbicides and the adoption of the ‘Clearfield’ system (imazamox herbicide-tolerant *B. napus* varieties) was cited by Kightley et al. [19] as one of three key potential mitigation strategies of value due to the efficacy of imazamox in the control of brassica weeds. Leaper and Melloul [64] also considered the control of broadleaved weeds to be crucial in order to prevent a further increase in abundance the following year and recommended the use of post-emergence herbicides. The ‘Clearfield’ system allows the control of weeds during crop growth whereas post-harvest weed control relies on an appropriate crop such as winter wheat following *B. napus* in the rotation. The other two strategies suggested by Kightley et al. [19] are dependent on appropriate tillage.

Recommended post-harvest field management strategies to reduce volunteer presence include delayed post-harvest tillage [26,31]. Encouraging the germination of dormant *B. napus* seeds (the breaking of secondary dormancy) by shallow tillage or delayed sowing to maximise ‘fatal pre-sowing volunteer emergence’ should be utilised where possible. This is the ‘stale seedbed’ technique also considered effective by Kightley et al. [19]. Delaying cultivation post-harvest allows the germination of seeds present on the soil surface, these can then be removed [13,18,26,83]. If the soil is dry however, light cultivation should be avoided post *B. napus* harvest due to the lack of soil moisture inducing secondary dormancy [82]. The ‘stale seedbed’ technique is potentially hindered by the lack of time, particularly after crops such as winter wheat, to permit volunteer plants to germinate and be removed with a herbicide [19] although this can be addressed by the inclusion of spring sown crops in the rotation. Gruber et al. [72] recommend either delaying or avoiding post-harvest stubble-tillage to prevent inducing secondary dormancy through seed burial. Kightley et al. [19] consider direct drilling or zero tillage as one of the three main methods to reduce volunteers in crops following oilseed rape. One potential weakness in this approach arises from a study in Canada where it was observed that crop residues in non-inversion tillage systems were sufficient to replicate the burial process [79] although this has not been reported elsewhere. The authors of this study also report that in fields where zero tillage was implemented and the winter was mild, there was the potential for harvested plants to overwinter and continue growth the following year [79]. A key concern is the ecological advantage conferred to overwintering volunteers, namely earlier flowering and potential earlier pod dehiscence contributing further seeds to the soil seedbank. Earlier dehiscence would render them less likely to be harvested with the main *B. napus* crop, although the larger size makes them potentially more difficult to remove with herbicide [79]. The authors hypothesise that this may be a more prevalent phenomena in areas with a warmer climate, a variable to be considered in a site-specific risk assessment. Where the following crop is sown in rows inter-row hoeing has also been recommended [78]. Ideally tillage post-harvest should be avoided altogether in order to prevent inducing secondary dormancy by seed burial. Focus purely on the control of volunteers takes a somewhat curative approach, that is, the shedding of seed is allowed to occur. Another critical issue is the poor shatter resistance of *B. napus* pods, which greatly increases the risk of volunteers. The selection of varieties with a lower risk of premature pod shatter is another important mitigation approach [71].

3.5. Storage, Processing and Transport

Contamination can result from the accidental commingling of crops via machinery and processing [88–90]. Impurities in seed batches have also caused problems [35] and some authors have suggested that the farm saving of seed is partly responsible for the diversity in genotypes observed

in crops of *B. napus* [55]. These issues can be dealt with through high levels of machinery hygiene, the prevention of seed spillage and ensuring concise records and labelling is maintained. Kightley et al. [19] highlight the importance of good record keeping and the issues that may arise when not adequately implemented. Mitigation strategies are summarised in Table 2.

Table 2. Post-harvest storage, transport and processing mitigation options.

Activity	The Issue	Mitigation Options	References
Varietal choice	Varietal choice will affect the strength of the traits most likely to affect the occurrence of volunteer and feral plants.	<ul style="list-style-type: none"> Select varieties offering cleistogamous flowering, these self-pollinating, closed flowering varieties reduce pollen transfer to non-cleistogamous varieties; Consider plant height. Dwarf varieties of HEAR will produce shorter F1 hybrids that are less competitive with LEAR crops; At present only semi-dwarf LEAR varieties are available commercially and the detection and removal of taller HEAR volunteers via rogueing or weed wipe would represent a potential alternative; Select varieties with a lower risk of premature pod shatter Select varieties with a lower disposition to secondary dormancy. This will reduce the risk of establishing volunteer populations. 	[29–31,69,71,91,92]
Machinery hygiene	<i>B. napus</i> seeds are small & difficult to remove from machinery.	<ul style="list-style-type: none"> Clean drill, tyres & other machinery parts in the field, not the yard; Minimise seed loss during harvest via swathing, desiccation, optimum harvest timing & careful setting of the combine. 	[18,26,93]
Seed storage	Seed contamination is unlikely to be the source if low levels of EA contamination are detected in LEAR seed but will be a potential source if levels are very high	<ul style="list-style-type: none"> Seed should be transported in its original packaging in the field & opened on site; A documented seed spillage procedure should exist; All seed should be clearly labelled & this should be sufficiently robust to remain attached & legible during storage; If seed is stored on farm, before sowing, physical separation should be maintained (e.g., separate storage building). 	[16,26,94]
Seed quality	Seed batch contamination and potentially farm-saved seed can introduce EA contamination.	<ul style="list-style-type: none"> Purchase only high quality, assured seed. Only use farm-saved seed if quality can be guaranteed. 	[35]
Transport	The cultivar diversity of feral populations is greatest along roadside verges suggesting dispersal via farm vehicles.	<ul style="list-style-type: none"> Avoid spillage from trailer by covering top & sealing gaps; Do not overfill. 	[26,55]

Friesen et al. [95] note a risk of low-level contamination and cross pollination during breeding and seed multiplication as a potential source of adventitious mixing. In response, Kightley et al. [19] recommend EA tests as standard for all seed producers.

4. Discussion

The information gathered by the review has been used to formulate a risk, mitigation and priority scoring matrix (Table 3). There are three levels of risk: 1 (low), 2 (moderate) and 3 (high) and mitigation 1 (low), 2 (moderate) and 3 (high). A simple overall ‘priority’ score is returned by multiplying the risk by the mitigation score (1–9) to give five classifications: very high (VH), high (H), moderate (M), low (L) and very low (VL).

Table 3. HEAR–LEAR risk and mitigation matrix.

Crop Production Stage and Sources of Contamination	Risk	Score	Mitigation	Score	Priority Score	Priority
Crop growth						
Cross-pollination: from volunteers in OSR crop	H	3	Varietal selection (cleistogamous varieties)	2	6	H
	H	3	Weed control using the ‘Clearfield’ system	3	9	VH
	H	3	Increased crop plant density	1	3	M
Cross-pollination: between HEAR–LEAR maincrops	M	2	Isolation distance of 100 m	3	6	H
	M	2	Isolation distance of 10 m	2	4	M
	M	2	Isolation distance of 1 m	1	2	L
	M	2	Discard zones in the outer 10 m	2	4	M
	M	2	Low crop edge to crop area ratio	2	4	M
	M	2	Increased landscape heterogeneity	2	4	M
	M	2	Varietal selection (cleistogamous varieties)	2	4	M
Cross-pollination: feral plants outside crop	L	1	Varietal selection (cleistogamous varieties)	2	2	L
	L	1	Weed control with conventional herbicide	2	2	L
Cross-pollination: compatible wild relatives (inside crop)	L	1	Varietal selection (cleistogamous varieties)	2	2	L
	L	1	Weed control using the ‘Clearfield’ system	3	3	M
Cross-pollination: compatible wild relatives (outside crop)	L	1	Varietal selection (cleistogamous varieties)	2	2	L
	L	1	Weed control with conventional herbicide	2	2	L
Harvest						
Mixing of seed: weeds with high EA content	M	2	Weed control using the ‘Clearfield’ system	3	6	H
Mixing of seed: volunteer HEAR/hybrid HEAR	H	3	Weed control using the ‘Clearfield’ system	3	9	VH
	H	3	Varietal selection (varieties with enhanced shatter resistance)	2	6	H
	H	3	Farm machinery hygiene	2	6	H
Post-harvest						
Volunteers (preventing secondary dormancy)	H	3	Zero tillage	3	9	VH
	H	3	Delayed post-harvest tillage (‘stale seedbeds’) with following winter crops	2	6	H
	H	3	Delayed post-harvest tillage (‘stale seedbeds’) with following spring crops	3	9	VH
	H	3	Varietal selection (varieties less susceptible to secondary dormancy)	2	6	H
Volunteers in subsequent crops	H	3	Crop rotation: increase the time between OSR crops (>5 years)	2	6	H
	H	3	Crop rotation: increase the time between OSR crops (>10 years)	3	9	VH
Storage, processing & transport						
Impurities in seed batches	H	3	Seed testing	3	9	VH
	H	3	Use certified seed	3	9	VH
	H	3	Concise record keeping and labelling	3	9	VH
Accidental commingling of crops via machinery and processing	H	3	Farm machinery hygiene	3	9	VH
Seed spillage (feral plants)	L	1	Cover trailer, do not overfill	3	3	M

In reference to experience gained through the Farm Scale Evaluations (FSE) [18], volunteer HEAR/HEAR hybrids growing in the cropped area pose the greatest risk of LEAR contamination with HEAR. As a result, in the crop growth part of the production stage, cross-pollination from volunteers has been assigned as being high risk (Table 3). The risk matrix also assigns aspects of other parts of the crop production stage that potentially result in HEAR or HEAR hybrid volunteer presence in the cropped area as high risk. This includes secondary dormancy post-harvest or impurities in seed batches during storage and processing. High mitigation potential occurs where the risk for that particular aspect is almost entirely eliminated. If a certified seed is used, for example, the risk of sowing seed contaminated with HEAR and the subsequent growth of HEAR/HEAR hybrids is, from this particular pathway, removed. The strategy is assigned a score of 3. The risk and mitigation strategies with the highest scores represent components of the crop growth cycle where the risk is high but there is a mitigation strategy that is also effective at that given stage that is also practical to implement. The ‘very high’ priority strategies will ideally already be in place or targeted for adoption first.

The implications of Table 3 are discussed in the context of the risks and their mitigation for a given stage in the crop cycle (Figure 1). Risks that arise during the storage and transport stage (and seed management and sowing stage) may be significantly curtailed purely through adherence to ‘good practice’. Seed testing, the use of certified seed and good record keeping are all sound methods to prevent HEAR material from contaminating a LEAR crop [16,19,26,93]. The risk score of 3 assumes a worse-case scenario where good practice is not followed but that this can be mitigated effectively and, if not currently implemented, should be undertaken as a priority. Stringent farm hygiene (Table 2) to prevent accidental mixing of seed [18,26,93] is a further priority which again, if ‘good practice’ is adhered to, will have already been adopted as part of the farm management system.

The post-harvest stage offers opportunities to reduce volunteer presence through three key methods [13,18,19,26,83]: (1) zero tillage to prevent the burial of seeds and prevent inducing secondary dormancy, (2) allowing volunteers to germinate and treating with a broad-spectrum herbicide pre-crop emergence (‘stale seedbed’ technique), and (3) including fewer oilseed crops in the rotation. In relation to (1) above, zero tillage is considered a highly effective method [19] and assigned as ‘very high’ priority (Table 3), subject to site-specific caveats. Zero tillage as a means of crop establishment is less effective on soils with poor structure, namely those with limited organic matter content (e.g., light sandy soils) or compacted soils with poor drainage [96]. Approach (2) above, the ‘stale seedbed’ approach, aims to allow volunteers to germinate immediately post-harvest before treatment with a non-selective herbicide [19]. Secondary dormancy is a greater risk where dry soils persist during the autumn [22,23], namely light sandy soils with a low water holding capacity in a low rainfall area, for example in the east of England. It is also exacerbated in anaerobic soil conditions subject to compaction and waterlogging. The effective implementation of both zero tillage and the ‘stale seedbed’ approach may be reduced where these environmental parameters exist. Light sandy soils, for example, may present challenges in the effective adoption of both strategies, with the importance of method (3) above becoming greater. The ‘sterile seedbed’ technique may also be restricted where the following crop is winter sown due to insufficient time for the volunteer plants to germinate [19]. The presence of spring sown crops in the rotation allows this technique to be implemented more effectively.

In summary, at the farm level, there is, mainly for economic reasons, the need to minimise the unwanted mixing of genotypes either to facilitate co-existence or to avoid increasing the risk of EA contamination of future LEAR crops. However, the general consensus is that it is impossible to produce 100% purity for a given market, hence the use of thresholds [13]. Begg et al. [84], using a modelling approach, concluded that ‘dedicated production of single varieties may be the only way that the co-existence of these crop types can be achieved’ and that ‘the intermittent use of unique varieties of oilseed rape, including GM, within an otherwise conventional rotation will not be feasible’. However, in contrast to Begg et al. [84], Supply Chain Initiative on Modified Agricultural Crops (SCIMAC) [13] cite the application of measures used during the GM Farm Scale Evaluations (FSE) [97,98] to be sufficiently robust to permit co-existence between different cultivars. Experience from the FSE has

implied that the guidelines were practical to implement in addition to being an effective approach [14]. Sausse et al. [27] recommend implementing flexibility in managing risk and tailoring any strategy to farm specific conditions through recording agricultural practices, assessing volunteer abundance, adapting the field management to its previous management history and environmental conditions. Begg et al. [84] added that although the maintenance at thresholds of 1% impurity is possible, it is not achievable consistently under all circumstances. With this in mind and considering the findings of the FSE audits [26] and this review, it is clear that there are risks at all crop production stages. Table 3 also identifies that the potential for risk mitigation is far greater in certain stages, with the seed management and storage/processing stages achieving high levels of mitigation purely through adherence to good practice.

5. Conclusions

Reviewing the work of the authors cited herein and on the approach adopted by ADAS [26] and the FSEs, it is evident that there are risks of various degrees throughout the entire LEAR crop production process. These arise both from the morphological traits of rapeseed and farm production methods as summarised in Figure 1. Mitigation strategies exist at each stage in the crop production cycle which, if used in combination, offer the potential for HEAR and LEAR crops to co-exist while maintaining acceptable EA thresholds in LEAR crops. As outlined above, good practice alone provides a strong element of mitigation at the seed management and storage/processing stages. Whilst every site and situation is different and should be evaluated individually considering localised issues such as soil type and rainfall, it does seem that with care and attention to detail, following good practice advice, that growers should not be unduly deterred from growing both HEAR and LEAR crops or reverting to LEAR after previously growing HEAR. The key is to understand the risks and adopt appropriate mitigation measures.

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