

Biology Matters: Seed- and Pollen-Mediated Gene Flow in Three Oilseed Crops, Safflower, Flax and Oilseed Rape

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Abstract

*Quantification of seed- and pollen-mediated gene flow (SMGF and PMGF) was conducted in two oilseed crops: flax (*Linum usitatissimum* L.) and safflower (*Carthamus tinctorius* L.) and compared to published studies in oilseed rape (*Brassica napus* L.). For all three species, pollen-mediated gene flow decreased rapidly with distance. Oilseed rape averaged <2.0 % outcrossing within 10 m of the source plot, while safflower and flax had more limited dispersal immediately adjacent to source plots, with outcrossing in the area of 0 to 3 m ranging from 1.67 to 0.48% and 1.50 to 0.14%, respectively. With mitigation, PMGF in safflower and flax is unlikely to hinder coexistence. Harvest losses in all three crops were significant and highly variable between fields and growers, with losses in oilseed rape averaging 3,600 seeds m⁻², safflower between 231 and 1,069 seeds m⁻², and flax between 53 and 1,986 seeds m⁻². In all three oilseed crops, SMGF was a source of adventitious presence (AP) in subsequent crops and challenges the ability of genetically modified (GM) crop growers to produce conventional crops, or to revert to conventional crops on the same land base. Vigilant herbicide control of crop volunteers is required in the year following GM crops to prevent AP and reduce seed bank replenishment. In safflower and flax, low PMGF limits the impact of volunteers and ruderal populations. Knowledge of the biological and agronomic factors contributing to SMGF and PMGF can inform coexistence and mitigation practices.*

Introduction

In Canada, coexistence of GM (genetically modified) and conventional crops should allow growers in adjacent and neighboring fields to independently choose the crop type (GM, conventional or organic) and also to allow different crop species to be grown in the same field within a rotation. Stewardship programs and mitigation measures therefore need to be developed along with GM crops to anticipate and prevent market harm. Mitigation measures are dependent on an understanding of seed- and pollen-mediated gene flow (SMGF and PMGF) as influenced by the biology of the crop, agronomic practices and spatial and temporal distribution of crops in each agro-ecosystem.

Western Canada is suitable for growing several oilseed crops including flax, safflower and oilseed rape. The novel traits being developed and their potential to cause market harm differ as does the intensity of production on the landscape. While Canada is the major source of flax world wide, it is a minor crop in Canada, with approximately 600,000 ha grown annually. Flax seed is marketed primarily to the European Union (EU) where it is used for manufacturing oil-based flooring and several industrial products including paints, varnishes, printer's ink and other coatings (Rowland et al. 1995). Transgenic techniques have been investigated to increase the level of very long chain polyunsaturated fatty acids (VLCPUFA) in oilseed species to supplement fish oils in the diet (Jhala, Weselake & Hall L.M. 2009). It is critical that GM flax can co-exist with conventional flax without affecting the current Canadian flax seed export market.

Safflower is produced in Canada as a specialty crop, for the birdseed market and is being investigated as a platform for plant-made pharmaceuticals (PMP) (McPherson, Weselake & Hall 2008). Conventional safflower acreages rarely exceed 1,000 ha annually in Canada. The production of PMP in Canada, while technically possible has been stalled by the lack of a regulatory policy for their large field scale release. PMP safflower can be isolated from conventional crops and mitigation measures enforced because of the value of the crop.

Conversely, oilseed rape (or canola) was grown annually on an average of 4.6 million ha over the last decade in Canada. GM canola has been produced in Canada since 1996, and varieties with tolerance to abiotic stress are currently being examined in field trials (Warwick, Beckie & Hall 2009). GM canola has been widely adopted and is not segregated from the small amount (ca. 2%) of conventional crop grown. Special gene flow mitigation measures for GM canola have not been implemented, except by seed growers to maintain varietal purity. Pollen- and seed-mediated gene flow has been extensively measured in canola and provides a comparator for the other crops.

Pollen-Mediated Gene Flow

Adventitious presence (AP) may occur through PMGF where transgenes may disseminate from GM crops to conventional crops by successful cross-fertilization. PMGF is influenced by the frequency of outcrossing inherent to the crops or cultivar, pollination mechanism, the size of the pollen source, weather events and several other factors (Matus-Cadiz, Hucl & Dupuis 2007, Hanson et al. 2005). The leptokurtic

distribution of pollen is similar between species although frequency of gene flow between adjacent plants and the maximum distance of gene flow differ (Beckie, Hall 2008). Comparison of PMGF between species is complicated by experimental protocols, including the size of trials/fields. Although PMGF is relatively limited in duration and distance (especially in self-pollinated crop species), receptor populations are frequently outside the control of the transgenic crop growers.

Flax is primarily a self-pollinating crop. At a mean distance of 10 cm, pollen-mediated gene flow was <2% and extensive seed screening was unable to locate marker genes further than 35 meters from the edge of the donor fields in small-scale multi-location-year trials (Jhala et al. in prep). Similarly, PMGF of safflower was a maximum of 1.67% adjacent to the pollen source, under good growing conditions. The maximum distance transgenes were found from the source was up to 100 meters. Despite the long flowering period and presence of bees and other insects, the extent of outcrossing was surprisingly low.

Oilseed rape PMGF has been extensively modeled and measured around the world (Beckie, Hall 2008). Adjacent plants can have outcrossing rates up to 20% but it diminishes quickly over short distances. In adjacent fields in western Canada, gene flow did not exceed 2% on field borders (Beckie et al. 2003). While long-distance gene flow events in canola have been recorded up to 3000 m at a landscape level in Australia (Rieger et al. 2002), gene flow was not observed beyond 400 meters from the source in western Canada (Hall et al. 2003).

The sacrifice of a trap strip in adjacent fields could be effectively used to reduce the frequency of outcrossing, and along with harvest blending of large fields, outcrossing alone for the three species is unlikely to exceed 1.0%. However, mitigation measures, such as trap crops, become the responsibility of the conventional grower who does not receive the benefit of the novel trait.

Seed-Mediated Gene Flow

For primarily self-pollinating species, SMGF is the most likely means of GM seeds being disseminated in non-GM crop seeds. There are a number of different potential sources of seed movement that may lead to AP. This includes seed shattering, harvest loss, transportation or loss of GM crop seeds during international trade. SMGF may also occur through seed movement by birds, animals, farm equipment, wind and water. SMGF is usually initiated with seed loss prior to and during harvest. All the oilseed crops discussed here were harvested with similar combine harvesters. Seed losses were highly variable because of crop maturity, weather, time of harvest and variability in combine efficiency. Because most of these factors are not within the control of the grower, harvest losses are difficult to mitigate.

Seed losses varied from 53 to 1986; 231 to 1069 and 1530 to 7131 seed m⁻², in flax, safflower and canola, respectively (McPherson et al. 2009, Dexter 2009, Gulden, Shirliffe & Thomas 2003). Small-seeded crops tend to have higher seed losses, due to mechanical difficulties in collecting and separating small seed (Table 1). All of these seed losses exceed the normal seeding rates in these crops, but not all seeds lost at harvest were viable. Immature or damaged seeds do not contribute to the seed bank, but the viability of crop seed lost at harvest is not commonly measured.

Seeds lost at harvest can remain on the soil surface, or they can be buried by tillage in the fall or spring, depending on the tillage practices of the grower. The depth of burial, especially for small seeds such as flax or canola, influences their fate. Seeds on the surface are subject to predation and extremes of temperature and moisture. Seeds that are buried experience reduced temperature and moisture extremes, and are protected from most predation, but may be more subject to diseases. Most crops lack primary dormancy and, depending on weather and burial, may germinate in the fall but are unable to survive the winter. Seeds from some crops including canola may have dormancy induced by burial, increasing the persistence of seed in the seed bank.

Seed bank dynamics of all three crops was examined in artificial seed banks at different depths by inserting naked, untreated seeds into packages permeable to soil and water, but protected from mammalian predators. At all burial depths, flax seed persisted less than two years in the seed bank. The first spring following burial a maximum of 23% of flax seeds were viable, but by the second spring no seeds were viable (Dexter 2009). There was no evidence of increased persistence with burial depth.

In similar experiments, safflower seeds persisted longer on the soil surface, with up to 80% viability in the spring following deployment of seed packages. Persistence was reduced in buried seeds, (McPherson et al. 2009), suggesting that burial enhanced germination and therefore depletion of the seedbank.

Canola exhibits complex seedbank persistence, influenced by genotypes and seed size (Gulden, Thomas & Shirliffe 2004a, Gulden, Thomas & Shirliffe 2004b). A proportion of canola seed can be induced into secondary dormancy and that proportion increases with seed burial. As such, the response of canola seed to burial was opposite that of safflower. Buried rapeseed tends to persist longer, while seed at the surface is more transient. Thus, mitigation measures to reduce seed persistence must also differ between the three oilseed species studied.

Volunteer Density and Emergence

GM crop volunteers that emerge and flower in subsequent years may set seeds and contribute to SMGF and PMGF (Hall et al. 2000, Jhala et al. 2009). The time of volunteer emergence influences their exposure to volunteer control practices including pre-seeding tillage or herbicide applications, and in-crop herbicide applications. Time of emergence also influences the relative competitive ability of the volunteer and the crop grown in rotation. Volunteers that emerge prior to a crop are generally more competitive (for example see O'Donovan et al. 2007) and have a higher fecundity than those that emerge after the crop. However, early emerging weeds can be subject to more control interventions than those that emerge later. Time of emergence, expressed as (E_{50}), the number of growing degree days (GDD) required for 50% emergence is affected by agronomic practices. Volunteer flax, compared to canola, emerges over a longer period of time (E_{50} 228 to 340 vs 90 to 132, respectively). Emergence in canola happens in a short period prior to in-crop herbicide application, while flax emergence occurs over an extended period throughout the growing period of the crop (data not shown). A similar comparison is

not available for safflower. However, observations suggest that safflower volunteers emerge primarily in late spring to early summer.

Volunteer emergence over years is usually examined in fields by survey methods, establishing an average volunteer density in the field. In the year after the commercial production of flax, volunteer densities were variable but were recorded up to 570 plants m^{-2} . Flax volunteers, similar to canola, continued to emerge over three years in some fields but at a much diminished rate, while safflower volunteers emerged only one year after the crop. The continued emergence of flax contradicted artificial seed bank studies which showed very limited seed persistence of naked seeds. In a subsequent study, we identified that flax seed in bolls are protected from moisture, delaying and extending emergence (data not shown). Thus, flax's physical mechanism of lengthening volunteer seed emergence is different than that of canola, which is based on secondary seed dormancy.

Safflower emergence occurred only for one year at a maximum of 17 plants m^{-2} . Safflower volunteers were uncompetitive and had reduced fecundity compared to those grown as a crop (McPherson et al. 2009). Canola volunteers occurred over three to five years and at initial average densities of 7.2 plant m^{-2} (Legere 2005). However, like seed distributions, volunteer canola populations were highly variable and have been recorded >200 plants m^{-2} in the spring following a canola crop. The fecundity of volunteers and the contribution of volunteer seed to seed bank and population persistence have not been well documented under field conditions.

Mitigation measures to facilitate co-existence

Evidence from gene flow studies of these oilseed crops indicate that PMGF leads to low levels of AP, and minimal isolation distances or trap crops would be required to meet reasonable thresholds. An understanding of pollination biology is critical, but mitigation measures must also depend on the traits involved. For example, PMGF from transgenic safflower intended for PMP cannot be allowed due to food and feed concerns, so an isolation distance of at least 1 km is recommended. Because of the small number of safflower growers in western Canada, this is readily achievable. For commodity crops grown in large fields, the proportion of a field subject to outcrossing at field boundaries is reduced, thus harvest blending with the rest of the field more effectively lowers AP levels in the harvested crop [for example see (Gustafson et al. 2005)]. In flax, an isolation distance of 3.0 m between GM and conventional flax fields would likely reduce PMGF to $< 0.14\%$ at the field boundary and AP in harvest grain would be very low, depending on the width of the field and other factors. In canola, the removal of 2 to 10 meters of crop would be more effective than isolation distances to reduce PMGF below 1.0% of a compatible adjacent crop.

To reduce AP in subsequent years, the control of SMGF via volunteers is critical for both flax and canola, but agronomic practices to achieve mitigation differ. Flax seed, lacking primary or secondary dormancy, can best be removed from the seed bank through germination. Tillage in the fall is likely to disrupt and bury seed and seed bolls, enhancing germination and removal from the seed bank. Densities of volunteers in the first year following commercial production will be high, however, herbicides have been identified for control of volunteer flax in canola and cereal crops (Jhala et al. 2009, Dexter et al. 2009) that also limit seed return.

Canola fields should not be tilled following harvest to allow seeds to germinate on the surface and minimize the induction of secondary seed dormancy (Gulden, Thomas & Shirliffe 2004a, Gulden, Thomas & Shirliffe 2004b). Densities of volunteer canola are expected to be highest the year following the crop, but effective herbicides for control of volunteers have been identified (Legere et al. 2006). The rapid diminishment of the seed bank is the desired outcome for both flax and canola.

For safflower, SMGF can be controlled by fall tillage and careful herbicide application in the year following the crop; however, given the concern with PMP safflower, a fallow year after safflower where volunteers can be effectively controlled in the absence of the crops is recommended.

The combination of sound agronomic and weed management practices and isolation distances and buffer zones between GM and conventional crops may provide a basis for western Canadian growers to meet the current market and regulatory requirements for the co-existence of GM, conventional and organic cropping systems.

Table 1. Physical parameters of three oilseed crops grown in western Canada

Physical parameter	Flax	Safflower	Oilseed Rape
Seed weight (1000 kwt) (g)	5 – 6.5	36	2-3
Seed/seed head	seed/boll	achene/capitulum	seed/silique
Harvest losses (seeds m ⁻²)	53 – 1986	231 – 1069	1530 - 7131

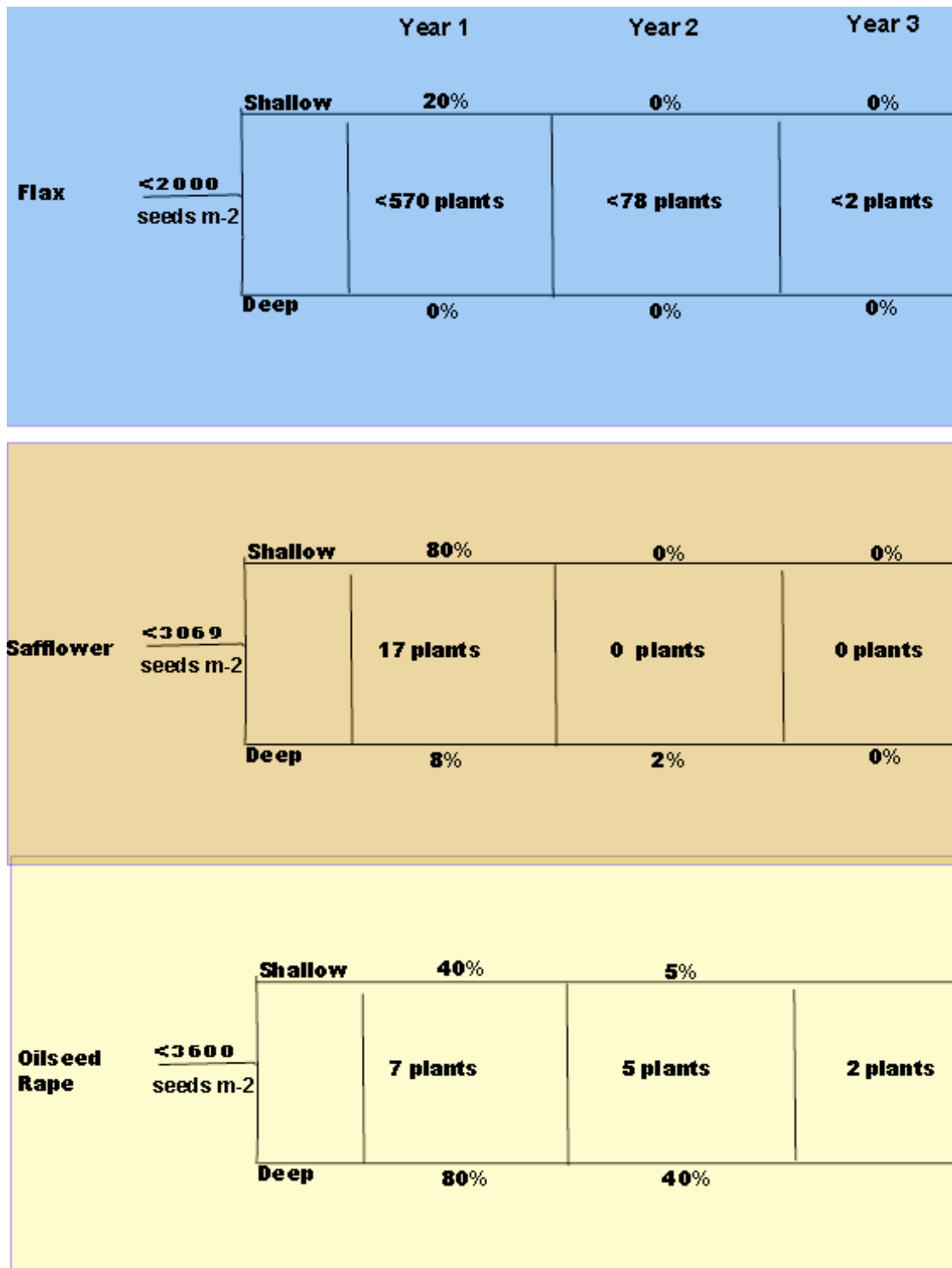


Figure 1. Summary of SMGF in flax, safflower and oilseed rape. The maximum inputs of seed prior to or during harvest is shown, with their subsequent decrease in viability over the next 3 years when buried shallow or deep, and the reduction in volunteer plant densities from field surveys. Data is compiled from several sources with details in text.

References

- Beckie, H.J. & Hall, L.M. 2008, "Simple to complex: Modelling crop pollen-mediated gene flow", *Plant Science*, vol. 175, no. 5, pp. 615-628.
- Beckie, H.J., Warwick, S.I., Nair, H. & Seguin-Swartz, G. 2003, "Gene flow in commercial fields of herbicide-resistant canola (*Brassica napus*)", *Ecological Applications*, vol. 13, no. 5, pp. 1276-1294.
- Dexter, J.E. 2009, "Quantification and risk assessment of seed-mediated gene flow with flax as a platform crop for bioproducts", *Ph.D. thesis*, University of Alberta, Canada.
- Dexter, J.E., Jhala, A.J., Hills, M.J., Yang, R.C., Topinka, K.C., Weselake, R.J. & Hall, L.M. 2009, "Quantification and mitigation of volunteer flax (*Linum usitatissimum* L.) in wheat (*Triticum aestivum* L.) (Accepted)", *Weed Science*.
- Gulden, R.H., Shirliffe, S.J. & Thomas, A.G. 2003, "Harvest losses of canola (*Brassica napus*) cause large seedbank inputs", *Weed Science*, vol. 51, no. 1, pp. 83-86.
- Gulden, R.H., Thomas, A.G. & Shirliffe, S.J. 2004a, "Relative contribution of genotype, seed size and environment to secondary seed dormancy potential in Canadian spring oilseed rape (*Brassica napus*)", *Weed Research*, vol. 44, pp. 97-106.
- Gulden, R.H., Thomas, A.G. & Shirliffe, S.J. 2004b, "Secondary dormancy, temperature, and burial depth regulate seedbank dynamics in canola", *Weed Science*, vol. 52, pp. 382-388.
- Gustafson, D.I., Horak, M.J., Rempel, C.B., Metz, S.G., Gigax, D.R. & Hucl, P. 2005, "An empirical model for pollen-mediated gene flow in wheat", *Crop Science*, vol. 45, no. 4, pp. 1286-1294.
- Hall, L., Topinka, K., Huffman, J., Davis, L. & Good, A. 2000, "Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple-resistant *B. napus* volunteers", *Weed Science*, vol. 48, no. 6, pp. 688-694.
- Hall, L.M., Good, A.G., Beckie, H.J. & Warwick, S.I. 2003, "Gene flow in herbicide resistance canola (*Brassica napus*): the Canadian experience" in *Ecological impact of GMO dissemination in agroecosystems*, eds. T. Lelly, E. Balazs & M. Tepfer, Facultas Verlags-und Buchhandels, AG, Austria, pp. 57-66.
- Hanson, B.D., Mallory-Smith, C.A., Shafii, B., Thill, D.C. & Zemetra, R.S. 2005, "Pollen-mediated gene flow from blue aleurone wheat to other wheat cultivars", *Crop Science*. 2005 July Aug; 45(4): 1610-1617.

Jhala, A.J., Raatz L., Dexter, J.E. & Hall, L.M. 2009, "Adventitious presence: Volunteer flax (*Linum usitatissimum* L) in herbicide resistant canola (*Brassica napus* L.) (Submitted)", *Weed Technology*.

Jhala, A.J., Weselake, R.J. & Hall L.M. 2009, "Genetically engineered flax (*Linum usitatissimum* L.): Potential benefits, risks, regulations and mitigation of transgene movement (In Press)", *Crop Science*.

Legere, A. 2005, "Risks and consequences of gene flow from herbicide-resistant crops: canola (*Brassica napus* L.) as a case study", *Pest Management Science*, vol. 61, pp. 292-300.

Legere, A., Simard, M.J., Johnson, E., Stevenson, F.C., Beckie, H.J. & Blackshaw, R.E. 2006, "Control of volunteer canola with herbicides: effects of plant growth stage and cold acclimation", *Weed Technology*. 2006 Apr; 20(2): 485-493.

Matus-Cadiz, M.A., Hucl, P. & Dupuis, B. 2007, "Pollen-mediated gene flow in wheat at the commercial scale", *Crop Science*, vol. 47, pp. 573-581.

McPherson, M.A., Yang, R.C., Good, A.G., Nielson, R.L. & Hall, L.M. 2009, "Potential for seed-mediated gene flow in agroecosystems from transgenic safflower (*Carthamus tinctorius* L.) intended for plant molecular farming", *Transgenic Research*, vol. 18, pp. 281-299.

McPherson, M.A., Weselake, R.J. & Hall, L.M. 2008, "Evaluation of gene flow in a minor crop: Safflower for the production of plant made pharmaceuticals in Canada" in *Biocatalysis and Agriculture Biotechnology*, eds. C. Hou & Shaw, Taylor and Francis Group, U.S.A.

O'Donovan, J.T., Harker, K.N., Clayton, G.W., Hall, L.M., Cathcart, J., Sapsford, K.L., Holm, F.A. & Hacault, K. 2007, "Volunteer barley interference in spring wheat grown in a zero-tillage system", *Weed Science*, vol. 55, no. 1, pp. 70-74.

Rieger, M.A., Lamond, M., Preston, C., Powles, S.B. & Roush, R.T. 2002, "Pollen-mediated movement of herbicide resistance between commercial canola fields", *Science (Washington)*, vol. 296, no. 5577, pp. 2386-2388.

Rowland, G.G., McHughen, A., Bhatti, R.S., Mackenzie, S.L. & Taylor, D.C. 1995, "The application of chemical mutagenesis and biotechnology to the modification of linseed (*Linum usitatissimum* L.)", *Euphytica*, vol. 85, no. 1-3, pp. 317-321.

Warwick, S.I., Beckie, H.J. & Hall, L.M. 2009, "Gene flow, invasiveness, and ecological impact of genetically modified crops", *Annals of the New York Academy of Sciences*, vol. 1168, no. 1, pp. 72-99.