SOME PUBLISHED SCIENTIFIC LITERATURE RELATED TO GM CANOLA THAT IS RELEVANT IN THE CONTEXT OF THE CURRENT DEBATE IN INDIA ABOUT TRANSGENIC MUSTARD

GENE FLOW & CONTAMINATION STUDIES

Schulze J, Frauenknecht T, Brodmann P, Bagutti C (2014). Unexpected diversity of feral genetically modified oilseed rape (Brassica napus L.) despite a cultivation and import ban in Switzerland. PLoS One. 2014 Dec 2;9(12):e114477. doi: 10.1371/journal.pone.0114477. eCollection 2014.

http://www.ncbi.nlm.nih.gov/pubmed/25464509

Abstract

Despite cultivation and seed import bans of genetically modified (GM) oilseed rape (Brassica napus L.), feral GM plants were found growing along railway lines and in port areas at four sites in Switzerland in 2011 and 2012. All GM plants were identified as glyphosate-resistant GM event GT73 (Roundup Ready, Monsanto). The most affected sites were the Rhine port of Basel and the St. Johann freight railway station in Basel. To assess the distribution and intra- and interspecific outcrossing of GM oilseed rape in more detail, we monitored these two sites in 2013. Leaves and seed pods of feral oilseed rape plants, their possible hybridization partners and putative hybrid plants were sampled in monthly intervals and analysed for the presence of transgenes by real-time PCR. Using flow cytometry, we measured DNA contents of cell nuclei to confirm putative hybrids. In total, 2787 plants were sampled. The presence of GT73 oilseed rape could be confirmed at all previously documented sampling locations and was additionally detected at one new sampling location within the Rhine port. Furthermore, we found the glufosinate-resistant GM events MS8xRF3, MS8 and RF3 (all traded as InVigor, Bayer) at five sampling locations in the Rhine port. To our knowledge, this is the first time that feral MS8xRF3, MS8 or RF3 plants were detected in Europe. Real-time PCR analyses of seeds showed outcrossing of GT73 into two non-GM oilseed rape plants, but no outcrossing of transgenes into related wild species was observed. We found no hybrids between oilseed rape and related species. GM plants most frequently occurred at unloading sites for ships, indicating that ship cargo traffic is the main entry pathway for GM oilseed rape. In the future, it will be of major interest to determine the source of GM oilseed rape seeds.

Laura A. Mesa, Bradley G. Howlett, Jan E. Grant, Raphael K. Didham (2013). Changes in the relative abundance and movement of insect pollinators during the flowering cycle of *Brassica rapa* crops: implications for gene flow. Journal of Insect Science: Vol. 13 | Article 13

Abstract

The potential movement of transgenes from genetically modified crops to non-genetically modified crops via insect-mediated pollen dispersal has been highlighted as one of the areas of greatest concern in regards to genetically modified crops. Pollen movement depends sensitively on spatial and temporal variation in the movement of insect pollinators between crop fields. This study tested the degree of variation in the diversity and relative abundance of flower-visiting insects entering versus leaving pak choi, *Brassica rapa* var. *chinensis* L. (Brassicales: Brassicaceae), crops throughout different stages of the flowering cycle. The relative abundance of flower-visiting insects varied significantly with *Brassica* crop phenology. Greater numbers of flower-visiting insects were captured inside rather than outside the crop fields, with the highest capture rates of flower-visitors coinciding with the peak of flowering in both spring-flowering and summer-flowering crops. Moreover, the ratio of flower-visiting insects entering versus leaving crop fields also varied considerably with changing crop phenology. Despite high variation in relative capture rates, the data strongly indicate non-random patterns of variation in insect movement in relation to crop phenology, with early-season aggregation of flower-visiting insects entering and remaining in the crop, and then mass emigration of flower-visiting insects leaving the crop late in the flowering season. Although pollen movement late in the flowering cycle might contribute relatively little to total seed set (and hence crop production), the findings here suggest that extensive late-season pollinator redistribution in the landscape could contribute disproportionately to long-distance gene movement between crops.

Munier DJ, Brittan KL, Lanini WT (2012). Seed bank persistence of genetically modified canola in California. Environ Sci Pollut Res Int. 2012 Jul;19(6):2281-4. doi: 10.1007/s11356-011-0733-8. Epub 2012 Jan 19.

http://www.ncbi.nlm.nih.gov/pubmed/22258428

Abstract

Introduction:
Canola, which is genetically modified (GM) for tolerance to glyphosate, has the potential to become established as a new glyphosate resistant weed, thus reducing the effectiveness of glyphosate.

Methods:
Volunteer from dormant canola seeds produced thousands of plants per hectare in the fourth year (2011) following a 2007 crop harvest. This occurred with no additional canola seed production since the 2007 harvest.

Results:
Volunteer plants following harvests of annual crops are typically only a problem for the first year after harvest. In California, glyphosate is the core herbicide on over a million hectares of high value row, tree, and vine crops and new glyphosate resistant weeds reduce the effectiveness of glyphosate.

Conclusions:
The combination of dormant seed and herbicide resistance makes GM glyphosate-resistant canola a new and difficult California weed which was first observed in the winter of 2009.

Ellstrand, N. C. (2012). Over a decade of crop transgenes out-of-place. In: Regulation of Agricultural Biotechnology: The United States and Canada (pp. 123-135). Edited by Chris A. Wozniak and Alan McHughen. Springer Netherlands.

http://www.ask-force.org/web/Regulation/Wozniak-McHughen-Regulation-Ag-Biotech-US-Canada-2012.pdf#page=136

Abstract
An early concern about genetically engineered crops was that transgenes might have undesirable consequences if they ended up in plants and places for which they were not intended. It has been over two decades since the first transgenic plants were environmentally released and over a decade since they have been commercialized. It is time to take stock of crop transgenes out of place. I have assembled a selected compilation of 22 incidents of in which crop transgenes were detected in living organisms (seeds or plants) in situations for which they were not intended. Contrary to initial concerns, crop transgenes have rarely introgressed into wild populations, but rather have often ended up in a different variety of the same species. Evolving regulatory policy should heed the large (and increasing) number of well-documented cases of transgenes out-of-place. In particular, it should treat the processed

products of transgenic crops differently from living organisms, such as grain, that, if planted, are capable of multiplying their genes.

Meredith G. Schafer, Andrew A. Ross, Jason P. Londo, Connie A. Burdick, E. Henry Lee, Steven E. Travers, Peter K. Van de Water, Cynthia L. Sagers (2011). The Establishment of Genetically Engineered Canola Populations in the U.S. PLOS One.
http://dx.doi.org/10.1371/journal.pone.0025736

http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0025736

Abstract
Concerns regarding the commercial release of genetically engineered (GE) crops include naturalization, introgression to sexually compatible relatives and the transfer of beneficial traits to native and weedy species through hybridization. To date there have been few documented reports of escape leading some researchers to question the environmental risks of biotech products. In this study we conducted a systematic roadside survey of canola (*Brassica napus)* populations growing outside of cultivation in North Dakota, USA, the dominant canola growing region in the U.S. We document the presence of two escaped, transgenic genotypes, as well as non-GE canola, and provide evidence of novel combinations of transgenic forms in the wild. Our results demonstrate that feral populations are large and widespread. Moreover, flowering times of escaped populations, as well as the fertile condition of the majority of collections suggest that these populations are established and persistent outside of cultivation.

Remy Chifflet, Etienne K. Klein, Claire Lavigne, Violette Le Feon, Agnes E. Ricroch, Jane Lecomte and Bernard E. Vaissiere (2011). Spatial scale of insect-mediated pollen dispersal in oilseed rape in an open agricultural landscape. Journal of Applied Ecology 2011, 48, 689–696. doi: 10.1111/j.1365-2664.2010.01904.x

http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2664.2010.01904.x/pdf

Summary
Interest in pollen-borne gene dispersal has grown with the cultivation of genetically modified plants. To date, both experimental data and models of oilseed rape (OSR) Brassica napus pollen movement at the landscape scale do not clearly differentiate between wind- and insect-mediated dispersal. Estimations of insect-mediated gene dispersal would be valuable for managing potential escapes of transgenes. 2. To quantify the intensity and spatial scale of pollen dispersal by insect pollinators in an agricultural landscape, bait points made of flowering male-sterile OSR that attract OSR pollinators were located at six distances (10–1100 m) from the closest OSR fields and feral populations. Flower-visiting insects were caught by net on these male-sterile flowers and were rubbed onto the stigmas of male-sterile OSR plants grown in a pollen-proof greenhouse to do a manual pollination of their flowers. In this way we were able to assess the insects' OSR pollen load and seed production at each of the six distances. 3. A large diversity of insects carried OSR pollen and contributed to seed production, but not pollen beetles Meligethes aeneus. Logistic regression analyses of the seed-set success from the manual pollination demonstrated that seed set significantly increased with the proximity of OSR fields, the size of the pollinating insect, and the main daily temperature. Seed set was not affected by the pollinating insect's order or genus. Seed set, both observed and predicted by the model, was above zero for flowers pollinated with large bees caught at >1100 m from the nearest OSR field. 4. Synthesis and applications. Our study provides clear evidence that a large variety of insect species can transfer viable pollen between OSR plants over considerable distances. However, only 39Æ4% of the insects caught on male-sterile flowers carried OSR pollen. Our results provide valuable data to improve models of pollen dispersal for entomophilous crops at the landscape scale. These models are essential to help land-managers reduce pollen-borne gene dispersal from genetically modified plants to wild and cultivated relatives.

Londo JP, Bautista NS, Sagers CL, Lee EH, Watrud LS (2010). Glyphosate drift promotes changes in fitness and transgene gene flow in canola (Brassica napus) and hybrids. Ann Bot. 2010 Dec; 106(6):957-65. doi: 10.1093/aob/mcq190. Epub 2010 Sep 18.

http://www.ncbi.nlm.nih.gov/pubmed/20852306

Abstract

Background And Aims:
With the advent of transgenic crops, genetically modified, herbicide-resistant Brassica napus has become a model system for examining the risks and potential ecological consequences of escape of transgenes from cultivation into wild compatible species. Escaped transgenic feral B. napus and hybrids with compatible weedy species have been identified outside of agriculture and without the apparent selection for herbicide resistance. However, herbicide (glyphosate) exposure can extend beyond crop field boundaries, and a drift-level of herbicide could function as a selective agent contributing to increased persistence of transgenes in the environment.

Methods:
The effects of a drift level (0·1 × the field application rate) of glyphosate herbicide and varied levels of plant competition were examined on plant fitness-associated traits and gene flow in a simulated field plot, common garden experiment. Plants included transgenic, glyphosate-resistant B. napus, its weedy ancestor B. rapa, and hybrid and advanced generations derived from them.

Key Results:
The results of this experiment demonstrate reductions in reproductive fitness for non-transgenic genotypes and a contrasting increase in plant fitness for transgenic genotypes as a result of glyphosate-drift treatments. Results also suggest that a drift level of glyphosate spray may influence the movement of transgenes among transgenic crops and weeds and alter the processes of hybridization and introgression in non-agronomic habitats by impacting flowering phenology and pollen availability within the community.

Conclusions:
The results of this study demonstrate the potential for persistence of glyphosate resistance transgenes in weedy plant communities due to the effect of glyphosate spray drift on plant fitness. Additionally, glyphosate drift has the potential to change the gene-flow dynamics between compatible transgenic crops and weeds, simultaneously reducing direct introgression into weedy species while contributing to an increase in the transgenic seed bank.

Knispel, A.L., & McLachlan, S.M. (2010) Landscape-scale distribution and persistence of genetically modified oilseed rape (Brassica napus) in Manitoba, Canada. Environmental Science and Pollution Research, 17(1): 13-25. doi: 10.1007/s11356-009-0219-0. Epub 2009 Jul 9.

http://www.ncbi.nlm.nih.gov/pubmed/19588180

Background, Aim And Scope:
Genetically modified herbicide-tolerant (GMHT) oilseed rape (OSR; Brassica napus L.) was approved for commercial cultivation in Canada in 1995 and currently represents over 95% of the OSR grown in western Canada. After a decade of widespread cultivation, GMHT volunteers represent an increasing management problem in cultivated fields and are ubiquitous in adjacent ruderal habitats, where they contribute to the spread of transgenes. However, few studies have considered escaped GMHT OSR populations in North America, and even fewer have been conducted at large spatial scales (i.e. landscape scales). In particular, the contribution of landscape structure and large-scale anthropogenic dispersal

processes to the persistence and spread of escaped GMHT OSR remains poorly understood. We conducted a multi-year survey of the landscape-scale distribution of escaped OSR plants adjacent to roads and cultivated fields. Our objective was to examine the long-term dynamics of escaped OSR at large spatial scales and to assess the relative importance of landscape and localised factors to the persistence and spread of these plants outside of cultivation.

Materials And Methods:

From 2005 to 2007, we surveyed escaped OSR plants along roadsides and field edges at 12 locations in three agricultural landscapes in southern Manitoba where GMHT OSR is widely grown. Data were analysed to examine temporal changes at large spatial scales and to determine factors affecting the distribution of escaped OSR plants in roadside and field edge habitats within agricultural landscapes. Additionally, we assessed the potential for seed dispersal between escaped populations by comparing the relative spatial distribution of roadside and field edge OSR.

Results:

Densities of escaped OSR fluctuated over space and time in both roadside and field edge habitats, though the proportion of GMHT plants was high (93-100%). Escaped OSR was positively affected by agricultural landscape (indicative of cropping intensity) and by the presence of an adjacent field planted to OSR. Within roadside habitats, escaped OSR was also strongly associated with large-scale variables, including road surface (indicative of traffic intensity) and distance to the nearest grain elevator. Conversely, within field edges, OSR density was affected by localised crop management practices such as mowing, soil disturbance and herbicide application. Despite the proximity of roadsides and field edges, there was little evidence of spatial aggregation among escaped OSR populations in these two habitats, especially at very fine spatial scales (i.e. <100 m), suggesting that natural propagule exchange is infrequent.

Discussion:

Escaped OSR populations were persistent at large spatial and temporal scales, and low density in a given landscape or year was not indicative of overall extinction. As a result of ongoing cultivation and transport of OSR crops, escaped GMHT traits will likely remain predominant in agricultural landscapes. While escaped OSR in field edge habitats generally results from local seeding and management activities occurring at the field-scale, distribution patterns within roadside habitats are determined in large part by seed transport occurring at the landscape scale and at even larger regional scales. Our findings suggest that these large-scale anthropogenic dispersal processes are sufficient to enable persistence despite limited natural seed dispersal. This widespread dispersal is likely to undermine field-scale management practices aimed at eliminating escaped and in-field GMHT OSR populations.

Conclusions:

Agricultural transport and landscape-scale cropping patterns are important determinants of the distribution of escaped GM crops. At the regional level, these factors ensure ongoing establishment and spread of escaped GMHT OSR despite limited local seed dispersal. Escaped populations thus play an important role in the spread of transgenes and have substantial implications for the coexistence of GM and non-GM production systems.

Recommendations And Perspectives:

Given the large-scale factors driving the spread of escaped transgenes, localised co-existence measures may be impracticable where they are not commensurate with regional dispersal mechanisms. To be effective, strategies aimed at reducing contamination from GM crops should be multi-scale in approach and be developed and implemented at both farm and landscape levels of organisation. Multiple stakeholders should thus be consulted, including both GM and non-GM farmers, as well as seed developers, processors, transporters and suppliers. Decisions to adopt GM crops require thoughtful and inclusive consideration of the risks and responsibilities inherent in this new technology.

A. Snow, T. M. Culley, L. G. Campbell, P. M. Sweeney, S. G. Hegde and N. C. Ellstrand (2010). Long-term persistence of crop alleles in weedy populations of wild radish (Raphanus raphanistrum). New Phytologist (2010) 186: 537–548 doi: 10.1111/j.1469-8137.2009.03172.x

http://onlinelibrary.wiley.com/doi/10.1111/j.1469-8137.2009.03172.x/pdf

Summary

• Hybridization allows transgenes and other crop alleles to spread to wild / weedy populations of related taxa. Researchers have debated whether such alleles will persist because low hybrid fitness and linkage to domestication traits could severely impede introgression. • To examine variation in the fates of three unlinked crop alleles, we monitored four experimental, self-seeding, hybrid populations of Raphanus raphanistrum · Raphanus sativus (radish) in Michigan, USA, over a decade. We also compared the fecundity of advanced-generation hybrid plants with wild plants in a common garden experiment. • Initially, F1 hybrids had reduced fitness, but the populations quickly evolved wild-type pollen fertility. In Year 10, the fecundity of plants from the experimental populations was similar to that of wild genotypes. Crop-specific alleles at the three loci persisted for 10 yr in all populations, and their frequencies varied among loci, populations and years. • This research provides a unique case study of substantial variation in the rates and patterns of crop allele introgression after a single hybridization event. Our findings demonstrate that certain crop alleles can introgress easily while others remain rare, supporting the assumption that neutral or beneficial transgenes that are not linked to maladaptive traits can persist in the wild.

Devos, Y., De Schrijver, A., Reheul, D. (2009). Quantifying the introgressive hybridisation propensity between transgenic oilseed rape and its wild/weedy relatives. Environmental Monitoring and Assessment, 149: 303–322

https://www.researchgate.net/publication/5598095_Quantifying_the_introgressive_hybridisation_propensity_between_transgenic_oilseed_rape_and_its_wildweedy_relatives

Abstract

In order to estimate the introgressive hybridisation propensity (IHP) between genetically modified (GM) oilseed rape (Brassica napus) and certain of its cross-compatible wild/weedy relatives at the landscape level, a conceptual approach was developed. A gene flow index was established enclosing the successive steps to successfully achieve introgressive hybridisation: wild/weedy relatives and oilseed rape should co-occur, have overlapping flowering periods, be compatible, produce viable and fertile progeny, and the transgenes should persist in natural/weedy populations. Each step was described and scored, resulting in an IHP value for each cross-compatible oilseed rape wild/weedy relative. The gene flow index revealed that Brassica rapa has the highest introgressive hybridisation propensity (IHP value = 11.5), followed by Hirschfeldia incana and Raphanus raphanistrum (IHP = 6.7), Brassica juncea (IHP = 5.1), Diplotaxis tenuifolia and Sinapis arvensis (IHP = 4.5) in Flanders. Based on the IHP values, monitoring priorities can be defined within the pool of cross-compatible wild/weedy oilseed rape relatives. Moreover, the developed approach enables to select areas where case-specific monitoring of GM oilseed rape could be done in order to detect potential adverse effects on cross-compatible wild/weedy relatives resulting from vertical gene flow. The implementation of the proposed oilseed rape-wild relative gene flow index revealed that the survey design of existing botanical survey networks does not suit general surveillance needs of GM crops in Belgium. The encountered hurdles to implement the gene flow index and proposals to acquire the missing data are discussed.

Nishizawa, T., Nakajima, N., Aono, M., Tamaoki, M., Kubo, A., Saji, H. (2009) Monitoring the occurrence of genetically modified oilseed rape growing along a Japanese roadside: 3- year observations. Environmental Biosafety Research, 8: 33-44

http://journals.cambridge.org/download.php?file=%2FEBS%2FEBS8_01%2FS1635792209000013a.pdf&code=718362f04278e8ba3a840b894cced959

Abstract
Monitoring for escape of genetically modified (GM) oilseed rape (Brassica napus) during transport can be performed by means of roadside evaluations in areas where cultivation of this GM crop is not conducted, such as in Japan. We performed a survey of oilseed rape plants growing along a 20-km section of Japan's Route 51, one of the main land transportation routes in central Japan for imports of GM oilseed rape from the Port of Kashima into Keiyo District. Oilseed rape plants were found each year, but the number of plants varied substantially during the three years of our study: 2162 plants in 2005, 4066 in 2006, and only 278 in 2007. The low number in 2007 was probably caused by roadwork. Herbicide-resistant individuals were detected in the three consecutive years (26, 8, and 5 individuals with glyphosate resistance), but glufosinate-resistant plants (9 individuals) were detected only in 2005. The roadside plants occurred mainly along the inbound lane from Kashima to Narita. These plants are likely to have their origin in seeds spilled during transportation of cargo from the port, since there are no potential natural seed source plants for B. napus near Route 51. This is the first detailed report on the transition and distribution of herbicide-resistant oilseed rape plants following loss and spillage along Japanese roads.

Warwick, S. I., Beckie, H. J. and Hall, L. M. (2009), Gene Flow, Invasiveness, and Ecological Impact of Genetically Modified Crops. Annals of the New York Academy of Sciences, 1168: 72–99. doi: 10.1111/j.1749-6632.2009.04576.x

http://onlinelibrary.wiley.com/doi/10.1111/j.1749-6632.2009.04576.x/abstract?userIsAuthenticated=false&deniedAccessCustomisedMessage=

The main environmental concerns about genetically modified (GM) crops are the potential weediness or invasiveness in the crop itself or in its wild or weedy relatives as a result of transgene movement. Here we briefly review evidence for pollen- and seed-mediated gene flow from GM crops to non-GM or other GM crops and to wild relatives. The report focuses on the effect of abiotic and biotic stress-tolerance traits on plant fitness and their potential to increase weedy or invasive tendencies. An evaluation of weediness and invasive traits that contribute to the success of agricultural weeds and invasive plants was of limited value in predicting the effect of biotic and abiotic stress-tolerance GM traits, suggesting context-specific evaluation rather than generalizations. Fitness data on herbicide, insect, and disease resistance, as well as cold-, drought-, and salinity-tolerance traits, are reviewed. We describe useful ecological models predicting the effects of gene flow and altered fitness in GM crops and wild/weedy relatives, as well as suitable mitigation measures. A better understanding of factors controlling population size, dynamics, and range limits in weedy volunteer GM crop and related host or target weed populations is necessary before the effect of biotic and abiotic stress-tolerance GM traits can be fully assessed.

Warwick SI, Legere A, Simard MJ, James T (2008). Do escaped transgenes persist in nature? The case of an herbicide resistance transgene in a weedy Brassica rapa population. Mol Ecol. 2008 Mar; 17(5):1387-95. Epub 2007 Oct 29.

http://www.ncbi.nlm.nih.gov/pubmed/17971090

Abstract

The existence of transgenic hybrids resulting from transgene escape from genetically modified (GM) crops to wild or weedy relatives is well documented but the fate of the transgene over time in recipient wild species populations is still relatively unknown. This is the first report of the persistence and apparent introgression, i.e. stable incorporation of genes from one differentiated gene pool into another, of an herbicide resistance transgene from Brassica napus into the gene pool of its weedy relative, Brassica rapa, monitored under natural commercial field conditions. Hybridization between glyphosate-resistant [herbicide resistance (HR)] B. napus and B. rapa was first observed at two Québec sites, Ste Agathe and St Henri, in 2001. B. rapa populations at these two locations were monitored in 2002, 2003 and 2005 for the presence of hybrids and transgene persistence. Hybrid numbers decreased over the 3-year period, from 85 out of approximately 200 plants surveyed in 2002 to only five out of 200 plants in 2005 (St Henri site). Most hybrids had the HR trait, reduced male fertility, intermediate genome structure, and presence of both species-specific amplified fragment length polymorphism markers. Both F(1) and backcross hybrid generations were detected. One introgressed individual, i.e. with the HR trait and diploid ploidy level of B. rapa, was observed in 2005. The latter had reduced pollen viability but produced approximately 480 seeds. Forty-eight of the 50 progeny grown from this plant were diploid with high pollen viability and 22 had the transgene (1:1 segregation). These observations confirm the persistence of the HR trait over time. Persistence occurred over a 6-year period, in the absence of herbicide selection pressure (with the exception of possible exposure to glyphosate in 2002), and in spite of the fitness cost associated with hybridization.

Comment in

Dlugosch KM, Whitton J (2008): Can we stop transgenes from taking a walk on the wild side? [Mol Ecol. 2008]. Mol Ecol. 2008 Mar;17(5):1167-9. doi: 10.1111/j.1365-294X.2008.03663.x.

Abstract

Whether the potential costs associated with broad-scale use of genetically modified organisms (GMOs) outweigh possible benefits is highly contentious, including within the scientific community. Even among those generally in favour of commercialization of GM crops, there is nonetheless broad recognition that transgene escape into the wild should be minimized. But is it possible to achieve containment of engineered genetic elements in the context of large scale agricultural production? In a previous study, Warwick et al. (2003) documented transgene escape via gene flow from herbicide resistant (HR) canola (Brassica napus) into neighbouring weedy B. rapa populations (Fig. 1) in two agricultural fields in Quebec, Canada. In a follow-up study in this issue of Molecular Ecology, Warwick et al. (2008) show that the transgene has persisted and spread within the weedy population in the absence of selection for herbicide resistance. Certainly a trait like herbicide resistance is expected to spread when selected through the use of the herbicide, despite potentially negative epistatic effects on fitness. However, Warwick et al.'s findings suggest that direct selection favouring the transgene is not required for its persistence. So is there any hope of preventing transgene escape into the wild?

D'Hertefeldt T, Jørgensen RB, Pettersson LB. (2008). Long-term persistence of GM oilseed rape in the seedbank. Biology Letters. June 23 2008; 4: 314–317.

http://rsbl.royalsocietypublishing.org/content/4/3/314

Abstract

Coexistence between genetically modified (GM) and non-GM plants is a field of rapid development and considerable controversy. In crops, it is increasingly important to understand and predict the GM volunteer emergence in subsequent non-GM crops. Theoretical models suggest recruitment from the

seedbank over extended periods, but empirical evidence matching these predictions has been scarce. Here, we provide evidence of long-term GM seed persistence in conventional agriculture. Ten years after a trial of GM herbicide-tolerant oilseed rape, emergent seedlings were collected and tested for herbicide tolerance. Seedlings that survived the glufosinate herbicide (15 out of 38 volunteers) tested positive for at least one GM insert. The resulting density was equivalent to 0.01 plantsm^{-2}, despite complying with volunteer reduction recommendations. These results are important in relation to debating and regulating coexistence of GM and non-GM crops, particularly for planting non-GM crops after GM crops in the same field.

Knispel AL, McLachlan SM, Van Acker RC, Friesen LF. Gene flow and multiple herbicide resistance in escaped canola populations. Weed Science. 2008; 56: 72–80

http://www.bioone.org/doi/abs/10.1614/WS-07-097.1

Abstract

Gene flow among herbicide-resistant (HR) canola varieties can lead to the development of multiple HR canola plants, creating volunteer canola management challenges for producers. In western Canada, escaped populations of HR canola are ubiquitous outside of cultivated fields, yet the extent of gene flow resulting in herbicide resistance trait stacking in individuals within these populations remains unknown. The objectives of this study were to document the presence of single and multiple herbicide resistance traits and assess the extent of gene flow within escaped canola populations. Seed was collected from 16 escaped canola populations along the verges of fields and roadways in four agricultural regions in southern Manitoba from 2004 to 2006. Glyphosate resistance was found in 14 (88%) of these populations, glufosinate resistance in 13 (81%) populations, and imidazolinone resistance in five (31%) populations. Multiple herbicide resistance was observed at levels consistent with previously published canola outcrossing rates in 10 (62%) of the tested populations. In 2005 and 2006, maternal plants from two escaped populations were tested using trait indicator test strips for glyphosate and glufosinate resistance to confirm outcrossing events. In 2005, two of 13 tested maternal plants with single herbicide resistance traits produced progeny with both glyphosate and glufosinate resistance. In 2006, of 21 tested plants, 10 single HR maternal plants produced multiple HR progeny, and five nonresistant maternal plants produced resistant offspring. This is the first report indicating that intraspecific gene flow results in stacking of herbicide resistance traits in individuals within escaped canola populations, confirming that multiple HR canola volunteers are not confined to agricultural fields. Results of this study suggest that escaped populations of crop plants can contribute to the spread of genetically engineered novel traits, which has important implications for containment, especially for highly controversial pharmaceutical and industrial traits in crop plants.

Franzaring J., Holz I., Fangmeier A., & Zipperle J. (2008). Monitoring the absence of glyphosate and glufosinate resistance traits in feral oilseed rape and wild crucifer populations. In: Implications of GM crop cultivation at large spatial scales. Theorie in der Ökologie, 14: 90-92. Frankfurt, Peter Lang.

Pivard, S., Adamczyk, K., Lecomte, J., Lavigne, C., Bouvier, A., Deville, A., Huet, S. (2007). Where do the feral oilseed rape populations come from? A large-scale study of their possible origin in a farmland area. Journal of Applied Ecology, 45(2): 476-485

1. Many cultivated species can escape from fields and colonize seminatural habitats as feral populations. Of these, feral oilseed rape is a widespread feature of field margins and roadside verges. Although considered in several studies, the general processes leading to the escape and persistence of feral oilseed rape are still poorly known. Notably, it remains unclear whether these annuals form transient

populations resulting mainly from seed immigration (either from neighbouring fields or during seed transport), or whether they show real ability to persist (either through self-recruitment or seed banks).
2. We conducted a 4-year large-scale study of factors involved in the presence of feral oilseed rape populations in a typical open-field area of France. The results were subjected to statistical methods suitable for analysing large data sets, based on a regression approach. We subsequently addressed the relative contribution of the ecological processes identified as being involved in the presence of feral populations.
3. Many feral oilseed rape populations resulted from seed immigration from neighbouring fields (about 35–40% of the observed feral populations). Immigration occurred at harvest time rather than at sowing. Around 15% of such populations were attributed to immigration through seed transport.
4. The other half resulted from processes of persistence, mainly through persistent seed banks (35–40% of the observed feral populations). This was all the more unexpected because seed banks have not yet been documented on road verges (despite being frequent within fields). Local recruitment was rare, accounting for no more than 10% of the feral populations.

***Synthesis and applications*.**
Understanding the dynamics of feral oilseed rape populations is crucial for evaluating gene flow over an agro-ecosystem. Our results show that, while many feral populations do come from annual seed dispersal, a significant number also result from seeds stored in the soil for several years. In the current context of coexistence and management of transgenic with non-transgenic crops, feral persistence and, especially, the seed bank contribution to the dynamics of feral populations need to be considered seriously. The latter, combined with self-recruitment, indicates a high potential for the persistence of transgenes and the possible emergence of gene-stacking.

Richard G FitzJohn, Tristan T Armstrong, Linda E. Newstrom-Lloyd, Aaron D Wilton, Michael Cochrane (2007). Hybridisation within *Brassica* and allied genera: evaluation of potential for transgene escape. Euphytica. November 2007. Vol. 158. Issue 1. PP 209-230.

http://link.springer.com/article/10.1007/s10681-007-9444-0

Abstract

Determining the potential for hybridisation between transgenic crops and their relatives is a major component of risk assessment. Recent assessments of the extent of reproductive compatibility between crops and their relatives draw heavily on existing data from experimental crosses to infer the likelihood of hybridisation and introgression. Since the literature in this area continues to grow at a rapid pace, it is essential that such analyses can be easily updated. We used a database approach to assemble data on reproductive compatibility for eight crop species in *Brassica*,*Raphanus* and *Sinapis*, using data from hand pollination, spontaneous (unassisted) pollination and trials using in vitro techniques (e.g. embryo rescue), incorporating 326 studies and 216 species combinations. We found many reports for major crop species (*B. juncea*, *B. napus*, *B. oleracea* and*B. rapa*), but fewer for minor crops *(B. carinata*, *B. nigra*, *Raphanus sativus* and *Sinapis alba*). Many species combinations remain untested, and we highlight these information gaps. While reproductively incompatible species can be discounted as targets for transgene escape, compatible species must be evaluated further in the particular context where transgenic crops are grown. Because the data is retained in a database in a relatively unmodified form, multiple views of the data can be generated; this review represents one possible view of this data. Our approach also allows new data to be easily incorporated into future reanalyses and can be extended to other crop groups, and as such is a useful method of assembling, analysing and sharing data for risk assessment.

A Messean, C Sausse, J Gasquez, H Darmency (2007). Occurrence of genetically modified oilseed rape seeds in the harvest of subsequent conventional oilseed rape over time. European Journal of Agronomy. Vol. 27, Issue 1. July 2007. Pages 115-122.

http://www.sciencedirect.com/science/article/pii/S1161030107000263

Abstract
Is returning to a conventional crop possible after growing a genetically modified (GM) variety? Oilseed rape volunteers in oilseed rape crops can cause seed admixture in the harvest. Farmers who desire to go back to conventional varieties after growing genetically modified varieties must comply with the 0.9% threshold of GM material in the harvest if they are to declare it as non-GM for food and feed use in Europe. The farm-scale GM platforms set up in France in 1995 were used to estimate the amount of GM seeds in the harvest of a conventional variety grown 3–8 years later in the same field. We show that over time the rate of GM seeds admixture in the harvest largely exceeded the European threshold in 6 out of 18 cases. In one case, the GM level was as high as 18%. However, it varied considerably according to the varieties used. These findings are discussed in the light of studies about seed shedding, seed dormancy and survival in the soil which are suggested to be the main source of large differences among GM varieties, which ultimately determines the frequency of volunteer plants growing amongst and harvested in admixture with the sown crop. These findings are equally discussed in light of differences of growth habit and potential competitiveness between conventional varieties responsible for different seed output by volunteers. To help cope with coexistence over time, we discuss appropriate uses of varieties and crop management in order to prevent seed admixture.

Simard, M.J., Légère, A., Warwick, S.I. (2006) Transgenic Brassica napus fields and Brassica rapa weeds in Québec: sympatry and weedcrop in situ hybridization. Canadian Journal of Botany, 84: 1842-1851

http://www.nrcresearchpress.com/doi/abs/10.1139/b06-135#.V4Cdd_l97IU

Hybridization between the herbicide-resistant transgenic crop Brassica napus L. (canola) and its weedy relative Brassica rapa L. (bird rape) has been documented in Quebec. Our goal was to evaluate the actual hybridization potential based on range overlap and actual in situ hybridization rates. This was done by mapping *B. napus* canola fields, comparing them with the sampling locations of *B. rapa* herbarium specimens from Quebec, gathering information on the presence of *B. rapa* in certified canola seed production fields, and surveying for *B. rapa* populations located in, or close to *B. napus* field margins. Progeny from these populations were screened for herbicide resistance (HR) and for the presence of the HR transgene. Two fields were also selected to evaluate *B. rapa* density effects on hybridization rates. Significant sympatry was observed in several areas of the province; hybridization occurred in all eight populations (1.1% to 17.5% hybrid seed) located in field margins and in one (1.1%) out of three populations located less than 10 m from a *B. napus* field. Hybridization rates decreased exponentially as*B. rapa* density increased, but interplant rates (0% to 68%) were highly variable. Environmental problems could be generated by the release of *B. napus* crops with traits conferring fitness benefits in non-managed areas.

Allainguillaume J, Alexander M, Bullock JM, Saunders M, Allender CJ, King G, Ford CS, Wilkinson MJ (2006). Fitness of hybrids between rapeseed (Brassica napus) and wild Brassica rapa in natural habitats. Mol Ecol. 2006 Apr; 15 (4): 1175-84.
http://www.ncbi.nlm.nih.gov/pubmed/16599976

Abstract

Fitness of hybrids between genetically modified (GM) crops and wild relatives influences the likelihood of ecological harm. We measured fitness components in spontaneous (non-GM) rapeseed x Brassica rapa hybrids in natural populations. The F1 hybrids yielded 46.9% seed output of B. rapa, were 16.9% as effective as males on B. rapa and exhibited increased self-pollination. Assuming 100% GM rapeseed cultivation, we conservatively predict < 7000 second-generation transgenic hybrids annually in the United Kingdom (i.e. approximately 20% of F1 hybrids). Conversely, whilst reduced hybrid fitness improves feasibility of bio-containment, stage projection matrices suggests broad scope for some transgenes to offset this effect by enhancing fitness.

Ford CS, Allainguillaume J, Grilli-Chantler P, Cuccato G, Allender CJ, Wilkinson MJ (2006). Spontaneous gene flow from rapeseed (Brassica napus) to wild Brassica oleracea. Proc Biol Sci. 2006 Dec 22; 273(1605):3111-5.

http://www.ncbi.nlm.nih.gov/pubmed/17015343

Abstract

Research on the environmental risks of gene flow from genetically modified (GM) crops to wild relatives has traditionally emphasized recipients yielding most hybrids. For GM rapeseed (Brassica napus), interest has centred on the 'frequently hybridizing' Brassica rapa over relatives such as Brassica oleracea, where spontaneous hybrids are unreported in the wild. In two sites, where rapeseed and wild B. oleracea grow together, we used flow cytometry and crop-specific microsatellite markers to identify one triploid F1 hybrid, together with nine diploid and two near triploid introgressants. Given the newly discovered capacity for spontaneous introgression into B. oleracea, we then surveyed associated flora and fauna to evaluate the capacity of both recipients to harm cohabitant species with acknowledged conservational importance. Only B. oleracea occupies rich communities containing species afforded legislative protection; these include one rare micromoth species that feeds on B. oleracea and warrants further assessment. We conclude that increased attention should now focus on B. oleracea and similar species that yield few crop-hybrids, but possess scope to affect rare or endangered associates.

Christian Damgaard, Gosta Kjellsson (2005). Gene flow of oilseed rape (Brassica napus) according to isolation distance and buffer zone. Agriculture, Ecosystems and Environment 108 (2005) 291–301.

Abstract

The introduction of genetically modified (GM) crops in the EU has raised questions concerning gene dispersal and coexistence with non-GM-farming. Quantitative estimates of the gene dispersal from fields with GM-crops to fields with conspecific non-GM-crops (conventional or organic) are therefore needed in order to suggest isolation distances and other management strategies to keep GM-pollination below acceptable threshold values. A meta-analysis of available gene-flow data for oilseed rape (Brassica napus) was performed. The probability distribution that seeds of non-GM-oilseed rape are fertilised by foreign pollen grains from a neighbouring field of GM-oilseed rape is modelled as functions of the width of the recipient (i.e. pollen receiving) field and the distance to the pollen donor fields. Furthermore, the significance of using a buffer zone (removal of a 1–5 m border of a recipient field parallel to the pollen donor field) to reduce GM-pollination of the crop, is quantified and discussed. The predicted median and 95% credibility level of the probability of foreign pollination is calculated as a function of the width of the recipient field and the buffer zone, as well as the distance between fields. Analysis of different management strategies shows that an increasing isolation distance is more effective to reduce GM-pollen dispersal than the use of a buffer zone, especially for small recipient fields. The analysis shows that increasing the width of a recipient oilseed rape field, relative to the pollen donor field, will have a large effect on reducing the average level of fertilisation by foreign pollen within the recipient field. The results indicate that a GM-pollination percentage <0.1% will be possible if the isolation distance exceeds 100 m and the width of the non-GM-field is larger than 200 m. If a threshold value of 0.3% is acceptable, an isolation distance of 50 m should be sufficient even for smaller fields. The use of a 5 m discarded buffer zone surrounding the non-GM-field is expected to reduce GM-pollination by about a third. The implications of the results for field management in conventional and organic farming are discussed.

Simard, M.J., Légère, A., Séguin-Swartz, G., Nair, H., Warwick, S.I. (2005). Fitness of double vs. single herbicide-resistant canola. WeedScience, 53: 489-498

http://www.bioone.org/doi/abs/10.1614/WS-04-207R1

Since 1995, canola cultivars with herbicide resistance (HR) have been readily adopted by Canadian producers. Gene flow between these cultivars with different HR traits has led to the occurrence of double herbicide–resistant (2HR) volunteers. To evaluate the fitness of canola volunteers with double HR, we compared three 2HR combinations to each of their parent single-HR plants (1HR: glufosinate-R, imidazolinone-R, glyphosate-R) commercial canola lines in separate greenhouse experiments. The replacement series design included five ratios of 2HR vs. 1HR plants at a single density of 129 plants m^{-2} and three stress treatments: herbicide application with either glufosinate, imazethapyr, or glyphosate; competition with a wheat crop; and a control without herbicide or wheat competition. Fitness indicators included aboveground biomass at 5 and 12 to 16 wk, seed production, and reproductive allocation. The 2HR plants showed delayed reproductive growth but were generally as competitive as 1HR commercial lines. Plant biomass of 2HR canola was comparable to or greater than 1HR canola, whereas seed biomass of 2HR canola was less than that of 1HR canola in half of the cases, likely because of delayed reproductive growth and early harvesting. Glufosinate–glyphosate 2HR was the fittest combination. Herbicide application had little effect on 2HR biomass at harvest, except for imazethapyr, which reduced the biomass and seed production of 2HR plants with imidazolinone-glyphosate resistance by 30%. The latter effect could have been from the unsuspected presence of 2HR plants with only one of the two acetolactate synthase mutations conferring resistance to imidazolinones. Wheat competition reduced fitness values of both 2HR and 1HR canola similarly, but seed production was still 64% that of the controls. Overall, there was little indication of reduced fitness in 2HR canola compared with commercial 1HR varieties.

Lutman PJ, Berry K, Payne RW, Simpson E, Sweet JB, Champion GT, May MJ, Wightman P, Walker K, Lainsbury M (2005). Persistence of seeds from crops of conventional and herbicide tolerant oilseed rape (Brassica napus). Proc Biol Sci. 2005 Sep 22; 272(1575):1909-15.

http://www.ncbi.nlm.nih.gov/pubmed/16191596

Abstract

A series of rotation experiments at five sites over four years has explored the environmental and agronomic implications of growing herbicide tolerant oilseed rape and sugar beet. This paper reports on the population dynamics of volunteer rape (Brassica napus). The experiments compared four winter oilseed rape (WOSR) cultivars: a conventional cultivar (Apex) and three developmental cultivars either genetically modified (GM) to be tolerant to glyphosate or glufosinate, or conventionally bred to be tolerant to herbicides of the imidazolinone group. Seed losses at harvest averaged 3575 seeds m(-2) but ranged from less than 2000 up to more than 10000 seeds m(-2). There was a rapid decline in seed numbers during the first few months after harvest, resulting in a mean loss of seeds of 60%. In subsequent seasons, the seed bank declined much more slowly at four of the five sites (ca 20% per year) and the models predicted 95% seed loss *after approximately 9 years*. Seed decline was much faster at the fifth site. There were no clear differences between the four cultivars in either the numbers of seeds shed at harvest or in their subsequent persistence. The importance of the persistence of GM rape seeds, in the context of the coexistence of GM and non-GM crops and the role of good management practices that minimize seed persistence, are discussed.

Halfhill MD, Zhu B, Warwick SI, Raymer PL, Millwood RJ, Weissinger AK, Stewart CN Jr. (2004). Hybridization and backcrossing between transgenic oilseed rape and two related weed species under field conditions. Environ. Biosafety Res. 3: 73-81

http://www.ncbi.nlm.nih.gov/pubmed/15612504

Determining the frequency of crop-wild transgene flow under field conditions is a necessity for the development of regulatory strategies to manage transgenic hybrids. Gene flow of green fluorescent protein (GFP) and Bacillus thuringiensis (Bt) transgenes was quantified in three field experiments using eleven independent transformed Brassica napus L. lines and the wild relatives, B. rapa L. and Raphanus raphanistrum L. Under a high crop to wild relative ratio (600:1), hybridization frequency with B. rapa differed among the individual transformed B. napus lines (ranging from ca. 4% to 22%), however, this difference could be caused by the insertion events or other factors, e.g., differences in the hybridization frequencies among the B. rapa plants. The average hybridization frequency over all transformed lines was close to 10%. No hybridization with R. raphanistrum was detected. Under a lower crop to wild relative ratio (180:1), hybridization frequency with B. rapa was consistent among the transformed B. napus lines at ca. 2%. Interspecific hybridization was higher when B. rapa occurred within the B. napus plot (ca. 37.2%) compared with plot margins (ca. 5.2%). No significant differences were detected among marginal plants grown at 1, 2, and 3 m from the field plot. Transgene backcrossing frequency between B. rapa and transgenic hybrids was determined in two field experiments in which the wild relative to transgenic hybrid ratio was 5-15 plants of B. rapa to 1 transgenic hybrid. As expected, ca. 50% of the seeds produced were transgenic backcrosses when the transgenic hybrid plants served as the maternal parent. When B. rapa plants served as the maternal parent, transgene backcrossing frequencies were 0.088% and 0.060%. Results show that transgene flow from many independent transformed lines of B. napus to B. rapa can occur under a range of field conditions, and that transgenic hybrids have a high potential to produce transgenic seeds in backcrosses.

Friesen, L.F., Nelson, A.G., Van Acker, R.C. (2003). Evidence of contamination of IR (Brassica napus) seedlots in western Canada with genetically engineered herbicide resistance traits. Agronomy Journal, 95: 1342–1347

http://kurt-schweizer.ch/home/gentech/RapsKanadaVanAcker.pdf

The objective of this study was to survey pedigreed canola (Brassica napus L.) seedlots for contaminating herbicide resistance traits because of complaints from farmers regarding glyphosate [N-(phosphonomethyl)glycine]-resistant canola volunteers occurring unexpectedly in their fields at densities and in patterns that suggested that pollen-mediated gene flow from neighboring fields in previous years was not the source of contamination. Twenty-seven unique, commercial certified canola seedlot samples were collected. Glyphosate-resistant seedlot samples were not collected. Canola samples were planted in the field, and when the canola had two to four true leaves, glyphosate, glufosinate [2-amino-4-(hydroxymethylphosphinyl)butanoic acid], and thifensulfuron {methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2- yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylate} herbicides were applied. Surviving canola plants were counted. Of the 27 seedlots, 14 had contamination levels above 0.25% and therefore failed the 99.75% cultivar purity guideline for certified canola seed. Three seed-lots had glyphosate resistance contamination levels in excess of 2.0%. Unexpected contamination (even at 0.25%) can cause problems for producers that practice direct seeding and depend on glyphosate for nonselective, broad-spectrum weed control. To avoid unexpected problems and costs, it is important that farmers are cognizant of the high probability that pedigreed canola seedlots are cross-contaminated with the various herbicide resistance traits.

Hugh J Beckie, Suzanne I Warwick, Harikumar Nair, Ginette Seguin-Swartz (2003). Gene Flow in Commercial Fields of Herbicide Resistant Canola (Brassica napus). Ecological Applications. Vol. 13, Issue 5. October 2003. Pages 1276-1294. DOI: **10.1890/02-5231**

http://onlinelibrary.wiley.com/doi/10.1890/02-5231/full

Abstract

Multiple herbicide resistance to glyphosate, glufosinate, bromoxynil, or imidazolinone in volunteer plants of canola (Brassica napus) has been attributed to pollen flow among cultivars with different resistance traits. A study was conducted in Saskatchewan, Canada, in 1999 and 2000 to assess gene flow in space and time in adjacent commercial fields of glyphosate- and glufosinate-resistant canola, including (1) estimation of gene flow with distance; (2) frequency and distribution of volunteers, and effect on gene flow; (3) effect of adventitious double herbicide-resistant seed presence in seedlots planted; and (4) a comparison of various marker systems to track gene flow events. At 11 sites in 1999, gene flow was determined by sampling seeds from plants located at 0, 50, 100, 200, 400, 600, or 800 m along a transect perpendicular to the common border in the paired fields, spraying seedlings with glyphosate and glufosinate, and confirming the presence of the transgenes using commercial test strips and PCR analysis. In the spring of 2000, putative double herbicide-resistant volunteers that survived sequential herbicide applications were mapped at three of the sites using GPS and resistance in sampled plants was characterized. In 1999, gene flow between the paired fields was detected to a maximum distance of 400 m. Values ranged from 1.4% outcrossing at the border common to the paired fields to 0.04% at 400 m. In 2000, gene flow as a result of pollen flow in 1999 was detected to the limits of the study areas (800 m). Large variation in gene flow levels and patterns among the three sites was evident. Adventitious presence of double herbicide-resistant seed in glyphosate-resistant seedlots planted at two of the sites in 1999 contributed to the occurrence of double herbicide-resistant volunteers in 2000. The results of this study suggest that gene stacking inB. napus canola volunteers in western Canada may be common, and reflects pollen flow between different herbicide-resistant canola, presence of double herbicide-resistant off-types in seedlots, and/or agronomic practices typically employed by Canadian growers.

Lutman P.J.W., Freeman S.E., Pekrun C. (2003). The long-term persistence of seeds of oilseed rape (Brassica napus) in arable fields. The Journal of Agricultural Science, 141:231–240. http://dx.doi.org/10.1017/S0021859603003575

http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=197001&fileId=S0021859603003575

The present paper reports on three sets of experiments exploring the persistence of seeds of oilseed rape (*Brassica napus*). The first, where known numbers of seeds were buried in September 1991 in two field experiments, demonstrated substantial initial losses of seeds, such that only 0·2 and 3·8% of seeds were still present after 4 months. In these experiments, which were not disturbed by mechanical cultivation, there was little evidence of further decline over the following 13 months. In the second of the two experiments, seeds were then left undisturbed for a further 136 months. A mean of 1·8% of seeds were still present after this period, providing further confirmation of the lack of decline in seed numbers in these undisturbed conditions. In the second pair of experiments, known numbers of seeds of three rape cultivars were broadcast onto plots and then either ploughed into the soil immediately after the start of the experiments, or were exposed to weekly shallow tine cultivation followed by ploughing after 4 weeks. The former created a larger seedbank than the latter. The experiments were then ploughed, annually (Expt 1) or at less frequent intervals (Expt 2); appreciable numbers of seeds survived for 65 months in both. Calculations based on exponential decline curves indicated that 95% seed loss would take 15–39 months, depending on the site, cultivar and initial post-harvest stubble treatment. The third part of the paper is based on more detailed studies of persistence of seeds of six cultivars in Petri dishes and buried in 25 cm pots. This work confirmed that cultivars differed in their persistence, as Apex was confirmed as highly persistent, whereas Rebel was short-lived. There were inconsistencies in the response of cultivar Synergy between the Petri-dish and pot experiment, which need further study. This experiment also reinforced the conclusion of the initial field experiments that little seed loss occurs in the absence of cultivations. Appreciable numbers of rape seeds will persist up to 4 years, in normal cropping conditions and in the absence of cultivation one experiment has confirmed persistence for over 11 years.

Ramsay, G., Thompson, C., Squire, G. (2003) Quantifying landscape-scale gene flow in oilseed rape. Final Report of DEFRA Project RG0216: An experimental and mathematical study of the local and regional scale movement of an oilseed rape transgene.

https://www.researchgate.net/publication/228581735_Quantifying_Landscape-Scale_Gene_Flow_in_Oilseed_Rape

Executive Summary

1. All the methods required for this study were adapted and put into place. These consisted of protocols for the use of male-sterile plants to estimate potential crosspollination, recording airborne pollen using suction traps and impaction on static surfaces, recovering pollen from bees, microscopic identification of pollen types, DNA extraction and fingerprinting methods for pollen and vegetative plant material, PCR detection of transgenes, and screens for marker genes giving resistance to antibiotics and sulphonylurea herbicides.
2. Male-sterile plants were used to generate large data sets of maximum landscape-scale gene flow. This method over-estimates gene flow found in more normal situations due to the lack of competing self-pollen. In one season, direct comparisons were made of frequencies of fertilisation of male-sterile recipients and the movement of a marker gene into the seeds produced by male-fertile recipients. The data from malesterile plants over-estimated the flow of a marker gene into a normal male-fertile population by about one order of magnitude.
3. Gene flow into larger populations of male-fertile oilseed rape is less than that into smaller populations. In one experiment to investigate this effect, increasing recipient populations from 10 plants to areas of approximately 0.1 ha reduced the inflow of genes about 4-fold. This reduction was greater in internal parts of these larger blocks.
4. Rates of cross-pollination, measured either by pollination of male-steriles or by tracking a marker gene in the progeny of male-fertile plants, dropped rapidly over the first few tens of metres from the edge of a field, but beyond that the decline with distance was slight over long distances. The exact shape of the decline varied between seasons and did not follow one mathematical description. However, declines with increasing distance are best described by functions giving a sharp initial decline then a long tail.
5. Cross-pollination was highly variable from site to site and dependent on additional factors than simply distance to the nearest source. This applied whether gene flow was measured by fertilisation of male-sterile plants, or the presence of marker genes in seeds from male-fertile plants.
6. Gene flow was detected over long distances. To facilitate a mathematical description of the decline in fertilisation and to characterise better the long tail in the distribution, sites with male-sterile plants were set out at 5 and 26 km from the nearest known source with little expectation of finding pollination. Low levels of fertilisation occurred at both sites. Although the origin of such events is hard to ascribe with absolute certainty, it appears that they were due to normal natural pollination via vectors which operate over long distances. A critical appraisal of this long-distance gene flow is presented in an appendix.
7. Gene flow over the landscape involving a specific gene reflects the proportion of competing donor fields carrying that gene within a radius of a few kilometres.

8. Pollen movement and fertilisation in oilseed rape have been assumed by some researchers to be due only or mainly to wind transport. Our data indicated that insects were predominantly responsible for pollination in the areas and years examined, even close to a source field. In one experiment, gene flow took place several km upwind of the source as effectively as downwind. Evidence was gathered which is suggestive of bee-to-bee contact in the hive being a major means of effective pollen dispersal through the foraging area of the colony. However, pollination of male-sterile plants took place over longer distances than those flown by worker honeybees. Of the many dispersive insects visiting OSR flowers, the pollen beetle was found on flowers at the most distant site and is already known to move over long distances.
9. Patterns of pollination in these experiments were relatively insensitive to airborne pollen deposition and therefore should not be modelled on that basis. Factors such as the numbers of fields being worked simultaneously by single bee colonies, the degree of mixing achieved, other insect activity, and the relative areas of sources and sinks will partially govern cross-pollination rates. 10. The data generated in this project permitted estimates to be made of cross-pollination into different types of recipient population over widely different distances. These are presented along with a note of the assumptions made and uncertainties requiring consideration.
10. Increasing separation distance is an inefficient means of maintaining stringent crop purity, and complete freedom from impurity is unlikely to be guaranteed by geographical separation. However, even relatively small separation distances reduce impurity through cross-pollination in fields of fully-fertile oilseed rape varieties to a low level, around 0.1% or below.

Marie-Josée Simard, Anne Légère, Denis Pageau, Julie Lajeunesse, Suzanne Warwick (2002). Frequency and Persistence of Volunteer Canola (*Brassica napus*) in Québec Cropping Systems. Weed Technology 16(2):433-439. 2002. http://dx.doi.org/10.1614/0890-037X(2002)016[0433:TFAPOV]2.0.CO;2

http://www.bioone.org/doi/abs/10.1614/0890-037X(2002)016%5B0433%3ATFAPOV%5D2.0.CO%3B2

Abstract

The presence of volunteer canola is becoming a significant agro-ecological concern, given the large-scale use of herbicide-tolerant varieties in some areas. Our goal was to estimate the frequency and persistence of volunteer canola in Québec cropping systems by surveying fields that included a single canola crop since 1995. A survey was conducted in 131 fields in the main canola-growing areas of Québec: in the Saguenay-Lac Saint-Jean region and the Québec City–La Pocatière area. Volunteer canola plants were counted in 0.25-m^2 quadrats every 10 m along a W pattern, and every 15 m along the margins of 88 fields. Volunteer canola plants were found in 90% of the fields surveyed and in a wide range of crops, including cereal, corn, and soybean. Average densities of 4.9 and 3.9 plants/m^2were found 1 yr after canola production in fields and field margins, respectively. Volunteer canola densities decreased significantly over time. However, volunteer plants were still present at low densities 4 and 5 yr after production. Dense stands of volunteer canola were found before postemergence herbicide application in no-till fields (9.8 ± 4.1 plants/m^2), suggesting that, contrary to what was suggested in the literature, seeds could become dormant in no-till as well as in tilled systems. A small proportion of the volunteer canola plants observed in no-till fields near Québec City and Ottawa included plants that had overwintered, either originating from fall-germinated seedlings, harvested adult plants that had grown new leaves before the onset of winter, or spring regrowth from the base of unharvested adult plants from experimental plots. The presence and persistence of low densities of volunteer canola may not have been a cause of concern until now. However, producers should be made more aware of the potential short-and long-term problems associated with potential gene flow between different herbicide-tolerant canola (HT canola) varieties and also between HT canola and related weed species.

Mary A. Rieger, Michael Lamond, Christopher Preston, Stephen B. Powles, Richard T. Roush (2002). Pollen-Mediated Movement of Herbicide Resistance Between Commercial Canola Fields. 28 June 2002 Vol 296 Science. PP. 2386-88.

There is considerable public and scientific debate for and against genetically modified (GM) crops. One of the first GM crops, *Brassica napus* (oilseed rape or canola) is now widely grown in North America, with proposed commercial release into Australia and Europe. Among concerns of opponents to these crops are claims that pollen movement will cause unacceptable levels of gene flow from GM to non-GM crops or to related weedy species, resulting in genetic pollution of the environment. Therefore, quantifying pollen-mediated gene flow is vital for assessing the environmental impact of GM crops. This study quantifies at a landscape level the gene flow that occurs from herbicide-resistant canola crops to nearby crops not containing herbicide resistance genes. Gene transfer is a complex process and is dependent on many factors including environmental conditions, plant variety, insect behavior, and plant density. These observations, coupled with our data on long distance pollen movement, indicate that laboratory and small-scale experiments may not necessarily predict pollination under commercial conditions. This study demonstrates that cross-pollination between commercial canola fields occurs at low frequencies but to considerable distance.
Comment in *Agbiotech. A little pollen goes a long way*. **[Science. 2002]**

Snow AA, Andersen B, Jørgensen R. 1999. Costs of transgenic herbicide resistance introgressed from *Brassica napus* into weedy *B. rapa.* Mol. Ecol. 8: 605-615

http://onlinelibrary.wiley.com/doi/10.1046/j.1365-294x.1999.00596.x/abstract

Wild relatives of genetically engineered crops can acquire transgenic traits such as herbicide resistance via spontaneous crop–wild hybridization. In agricultural weeds, resistance to herbicides is often a beneficial trait, but little is known about possible costs that could affect the persistence of this trait when herbicides are not used. We tested for costs associated with transgenic resistance to glufosinate when introgressed into weedy *Brassica rapa*. Crosses were made between transgenic *B. napus* and wild *B. rapa* from Denmark. F_1 progeny were backcrossed to *B. rapa* and BC_1 plants were selected for chromosome numbers similar to *B. rapa*. Further backcrossing resulted in a BC_2generation that was hemizygous for herbicide resistance. We quantified the reproductive success of 457 BC_3 progeny representing six full-sib families raised in growth rooms (plants were pollinated by captive bumblebees). Pollen fertility and seed production of BC_3 plants were as great as those of *B. rapa* raised in the same growth rooms. Segregation for herbicide resistance in BC_3 plants was 1:1 overall, but the frequency of resistant progeny was lower than expected in one family and higher than expected in another. There were no significant differences between transgenic and nontransgenic plants in survival or the number of seeds per plant, indicating that costs associated with the transgene are probably negligible. Results from this growth-chamber study suggest that transgenic resistance to glufosinate is capable of introgressing into populations of *B. rapa* and persisting, even in the absence of selection due to herbicide application.

Downey RK. Gene flow and rape – the Canadian experience. In: Lutman PJW, ed. Gene Flow and Agriculture: Relevance for Transgenic Crops. Vol 72: British Crop Protection Council Symposium Proceedings; 1999:109–116.

SOIL IMPACTS

Yongbo Liu, Junsheng Li, C Neal Stewart Jr, Zunlan Luo, Nengwen Xiao (2015). The effects of the presence of *Bt*-transgenic oilseed rape in wild mustard populations on the rhizosphere nematode and microbial communities. Science of The Total Environment. Volumes 530–531, 15 October 2015, Pages 263–270. doi:10.1016/j.scitotenv.2015.05.073

Abstract

The adventitious presence of transgenic crops in wild plant populations is of ecological and regulatory concern. In this context, their effects on non-target, below-ground organisms are not well understood. Here, we introduced, at various frequencies, *Bt*-transgenic oilseed rape (OSR, *Brassica napus*) into wild mustard (*Brassica juncea*) populations in the presence and absence of the target herbivore (*Plutella xylostella*). The impacts on soil nematode and microbial communities were assessed in this system. There were no significant changes on the number of nematode genera and abundance in proportions of OSR with mustard. Nonetheless, the Shannon–Wiener and Pielou evenness index was lowest in plant stands containing 50% of Bt-transgenic OSR. Among treatments, there was no significant variation for culturable soil microbes. There was a positive association between foliar herbivory and the abundance of plant parasitic (PP) and *cp*-3 nematodes, whereas there was no association between herbivory and soil microbial populations. There was no direct effects of the presence of Bt-transgenic OSR in wild mustard populations on the rhizosphere nematode and microbial communities, whereas its indirect effects via aboveground herbivory might be important to consider for biosafety assessments.

SE Hannula, W de Boer, JA van Veen. (2014). Do genetic modifications in crops affect soil fungi? A review. Biology and Fertility of Soils. Vol. 50, Issue 3, PP 433-446.

http://link.springer.com/article/10.1007/s00374-014-0895-x

Abstract

The use of genetically modified (GM) plants in agriculture has been a topic in public debate for over a decade. Despite their potential to increase yields, there may be unintended negative side-effects of GM plants on soil micro-organisms that are essential for functioning of agro-ecosystems. Fungi are important soil organisms and can have beneficial or harmful effects on plants. Their benefits to agro-ecosystems come from their activities as free-living saprobes breaking down soil organic matter thereby releasing nutrients to the crops, as well as from mutualistic interactions. On the other hand, soil-borne plant pathogenic fungi can cause severe damage in crops. Understanding of the impact of GM plants on the dynamics and functioning of soil fungi is essential to evaluate the possible risks of introduction of GM plants for ecosystem functioning. In recent years, over 50 studies have addressed the effects of various GM traits in crops on soil fungal community structure and function. These studies showed that GM crops can have positive, negative, or neutral effects on both free-living and plant-associated soil fungi. The observed discrepancy in results of these studies is discussed. This is done by highlighting a number of case studies. New methods developed in recent years have enabled microbial ecologists to get a better picture on the functioning and assembly of soil fungal communities. This review presents and discusses two of the most promising methods which are also readily usable in risk assessment of GM plants on soil fungi and that could help answer remaining key questions in the field.

Robert J. Kremer, Nathan E. Means (2009). Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. Europ. J. Agronomy 31 (2009) 153–161

http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.661.3265&rep=rep1&type=pdf

Abstract

Current crop production relies heavily on transgenic, glyphosate-resistant (GR) cultivars. Widespread cultivation of transgenic crops has received considerable attention. Impacts of glyphosate on rhizosphere microorganisms and activities are reviewed based on published and new data from long-term field projects documenting effects of glyphosate applied to GR soybean and maize. Field studies conducted in Missouri, U.S.A. during 1997–2007 assessed effects of glyphosate applied to GR soybean and maize on root colonization and soil populations of Fusarium and selected rhizosphere bacteria. Frequency of root-colonizing Fusarium increased significantly after glyphosate application during growing seasons in each year at all sites. Roots of GR soybean and maize treated with glyphosate were heavily colonized by Fusarium compared to non-GR or GR cultivars not treated with glyphosate. Microbial groups and functions affected by glyphosate included Mn transformation and plant availability; phytopathogen–antagonistic bacterial interactions; and reduction in nodulation. Root-exuded glyphosate may serve as a nutrient source for fungi and stimulate propagule germination. The specific microbial indicator groups and processes were sensitive to impacts of GR crops and are part of an evolving framework in developing polyphasic microbial analyses for complete assessment of GR technology that is more reliable than single techniques or general microbial assays.

Jeff R Powell, David J Levy-Booth, Robert H Gulden, Wendy L Asbil, Rachel G Campbell, Kari E. Dunfield, Allan S Hamill, Miranda M Hart, Sylvain Lerat, Robert E Nurse, K Peter Pauls, Peter H Sikkema, Clarence J Swanton, Jack T Trevors, John N Dlironomos (2009). Effects of genetically modified, herbicide-tolerant crops and their management on soil food web properties and crop litter decomposition. Journal of Applied Ecology. Vol. 46. Issue 2. April 2009. PP 388-396. DOI: 10.1111/j.1365-2664.2009.01617.x

http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2664.2009.01617.x/full

Summary

1. Genetically modified (GM), herbicide-tolerant crops have been adopted extensively worldwide, resulting in increased homogenization of agricultural practices. However, several countries still view GM crops with trepidation, citing potential risks to human health and the environment. We currently know little about how non-target biota responds to cultivation of GM crops under field conditions.
2. This study describes a series of microcosm and field experiments in Ontario, Canada, that estimated the effects of transgenic, glyphosate-tolerant (GT) crops and their management on the abundances of detritivorous soil biota and crop litter decomposition.
3. Absolute abundance of few of the measured biotic groups were affected by either the herbicide or variety treatments and, where significant effects were observed, the responses were not consistent across all years or for all sample dates within a year. More frequently, but not consistently, the GT herbicide system was associated with increased fungal : bacterial biomass ratios, suggesting a state of reduced enrichment.
4. Although the conventional and GT varieties studied differed in composition, we observed few effects of the modification for glyphosate-tolerance on maize *Zea mays* and soybean *Glycine max* litter decomposition. Overall, the herbicide system associated with GT crops reduced soybean- and corn-litter decomposition, but responses were inconsistent across Ontario, with many trials demonstrating no effect. Effects were probably underrepresented in this study as average daily precipitation was positively correlated with the magnitude of this system effect and many sites received well-below average levels of precipitation.

Synthesis and applications. Most concerns regarding the potential impacts of GM crops on non-target biota have targeted traits associated with the biotechnology itself. However, shifts in management practices associated with biotechnology are also widespread and have the same, if not greater, potential to alter the structure and functioning of agroecosystem biodiversity. The lack of observed permanent negative effects on soil biota in this study is heartening; however, more research is required to determine the functional consequences of observed transient effects and effects on other biota, as well as how altered crop litter decomposition affects agroecosystem nutrient cycling and carbon sequestration.

Lupwayi NZ, Harker KN, Clayton GW, O'Donovan JT, Blackshaw RE (2009). Soil microbial response to herbicides applied to glyphosate-resistant canola. Agriculture, Ecosystems & Environment. Vol. 129. Issues 1-3. PP 171-176. doi:10.1016/j.agee.2008.08.007

http://www.sciencedirect.com/science/article/pii/S0167880908002429

Abstract

Adoption of glyphosate-resistant canola (*Brassica napus* L.) has increased glyphosate applications to this crop, and concerns have been raised about unintended consequences of these multiple applications. A field trial was conducted to evaluate the effects of pre-seed and in-crop glyphosate and alternative herbicides on soil microbial community functional structure, diversity and biomass. Pre-seed treatments were 2,4-D,glyphosate and 2,4-D + glyphosate, and in-crop treatments were glyphosate applied once, glyphosate applied twice, ethalfluralin, ethalfluralin + sethoxydim + ethametsulfuron + clopyralid, and sethoxydim + ethametsulfuron. Rhizosphere and bulk soil was collected at flowering stage of canola and analyzed for bacterial community-level substrate utilization patterns and microbial biomass C (MBC). Where differences were significant, pre-seed application of both 2,4-D and glyphosate altered the functional structure and reduced the functional diversity of soil bacteria, but increased MBC. These effects were not necessarily concurrent. The reduction in functional diversity was due to reduction in evenness, which means that the soil where both pre-seed herbicides had been applied was dominated by only few functional groups. In 1 year, two in-crop applications of glyphosate also reduced the functional diversity of soil bacteria when applied after pre-seed 2,4-D, as did in-crop sethoxydim + ethametsulfuron following pre-seed glyphosate. Even though significant differences between herbicides were fewer than non-significant differences, i.e., there were no changes in soil microbial community structure, diversity or biomass in response to glyphosate or alternative herbicides applied to glyphosate-resistant canola in most cases, the observed changes in soil microbial communities could affect soil food webs and biological processes.

Giovannetti M, Sbrana C, Turrini A (2005). The impact of genetically modified crops on soil microbial communities. Riv Biol. 2005 Sep-Dec; 98 (3):393-417.

http://www.ncbi.nlm.nih.gov/pubmed/16440278

Abstract

Genetically modified (GM) plants represent a potential benefit for environmentally friendly agriculture and human health. Though, poor knowledge is available on potential hazards posed by unintended modifications occurring during genetic manipulation. The increasing amount of reports on ecological risks and benefits of GM plants stresses the need for experimental works aimed at evaluating the impact of GM crops on natural and agro-ecosystems. Major environmental risks associated with GM crops include their potential impact on non-target soil microorganisms playing a fundamental role in crop residues degradation and in biogeochemical cycles. Recent works assessed the effects of GM crops on soil microbial communities on the basis of case-by-case studies, using multimodal experimental approaches involving different target and non-target organisms. Experimental evidences discussed in this review confirm that a precautionary approach should be adopted, by taking into account the risks associated with the unpredictability of transformation events, of their pleiotropic effects and of the fate of transgenes in natural and agro-ecosystems, weighing benefits against costs.

Kari E. Dunfield and James J Germida (2003). Impact of Genetically Modified Crops on Soil- and Plant-Associated Microbial Communities. Journal of Environmental Quality. American Society of Agronomy. Vol. 33. No.3. PP 806-815. doi:10.2134/jeq2004.0806

https://dl.sciencesocieties.org/publications/jeq/abstracts/33/3/0806

Abstract

Transgenic or genetically modified plants possess novel genes that impart beneficial characteristics such as herbicide resistance. One of the least understood areas in the environmental risk assessment of genetically modified crops is their impact on soil- and plant-associated microbial communities. The potential for interaction between transgenic plants and plant residues and the soil microbial community is not well understood. The recognition that these interactions could change microbial biodiversity and affect ecosystem functioning has initiated a limited number of studies in the area. At this time, studies have shown the possibility that transgenes can be transferred to native soil microorganisms through horizontal gene transfer, although there is not evidence of this occurring in the soil. Furthermore, novel proteins have been shown to be released from transgenic plants into the soil ecosystem, and their presence can influence the biodiversity of the microbial community by selectively stimulating the growth of organisms that can use them. Microbial diversity can be altered when associated with transgenic plants; however, these effects are both variable and transient. Soil- and plant-associated microbial communities are influenced not only by plant species and transgene insertion but also by environmental factors such as field site and sampling date. Minor alterations in the diversity of the microbial community could affect soil health and ecosystem functioning, and therefore, the impact that plant variety may have on the dynamics of the rhizosphere microbial populations and in turn plant growth and health and ecosystem sustainability, requires further study.

Kari E. Dunfield, James J. Germida. (2001). Diversity of bacterial communities in the rhizosphere and root interior of field-grown genetically modified *Brassica napus.* FEMS Microbiology Ecology. Vol. 38, Issue 1. Pp1-9. http://dx.doi.org/10.1111/j.1574-6941.2001.tb00876.x

http://femsec.oxfordjournals.org/content/38/1/1.abstract

Abstract

Plant roots significantly affect microbial diversity in soil, but little is known on how genetically modified plants influence soil microbial communities. We conducted a 2-year field study to assess the effects of herbicide-tolerant genetically modified canola (oilseed rape, *Brassica* sp.) on microbial biodiversity in the rhizosphere. During the 1998 and 1999 field seasons, four genetically modified and four conventional canola varieties were grown at four different field locations across Saskatchewan, Canada. The rhizosphere and root interior microbial communities were characterized through fatty acid methyl ester analysis and community level physiological profiles. Principal component analysis indicated that the root interior and rhizosphere bacterial community associated with the genetically modified variety Quest (*Brassica napus*) was different from conventional varieties Excel (*B. napus*) and Fairview (*Brassica rapa*), based on both fatty acid composition and carbon substrate utilization. In addition, all root-associated microbial communities associated with genetically modified canola varieties had significantly higher levels of 10:02OH, 12:02OH, 12:03OH, a15:0, 15:1ω5c, cy17:0, 18:3ω6,9,12c, 19:0ω8c and Sum in Feature 3, suggesting alterations in the composition of the microbial community associated with plants. This study indicates that the composition and functional diversity and the microbial community were influenced by plant variety.

SD Siciliano, JJ Germida (1999). Taxonomic diversity of bacteria associated with the roots of field-grown transgenic *Brassica napus* cv. Quest, compared to the non-transgenic *B. napus* cv. Excel and *B. rapa* cv. Parkland. FEMS Microbiology Ecology. Vol. 29. Issue 3. PP 263-272.

http://femsec.oxfordjournals.org/content/29/3/263.abstract

Abstract

The composition and diversity of the bacterial community associated with plant roots is influenced by a variety of plant factors such as root density and exudation. In turn, these factors are influenced by plant breeding programs. This study assessed the diversity of root-endophytic and rhizosphere bacterial communities associated with three canola cultivars (Parkland, *Brassica rapa*; Excel, *B. napus*; and Quest, *B. napus*) grown at two field sites. Quest, a derivative of Excel developed by the Alberta Wheat Pool, has been genetically engineered to tolerate the herbicide glyphosate. Approximately 2300 bacteria were isolated from roots of plants and identified based on fatty acid methyl ester (FAME) profiles. One third of the isolates were positively identified by FAME analysis (i.e. SIM index ≥0.3) with another third assigned tentative identifications (SIM index <0.3). Fewer *Bacillus, Micrococcus* and *Variovorax* isolates, and more *Flavobacterium* and *Pseudomonas* isolates were found in the root interior of Quest compared to Excel or Parkland. Furthermore, fewer *Arthrobacter* and *Bacillus* isolates were recovered from the rhizosphere of Quest compared to Excel or Parkland. The bacterial root-endophytic community of the transgenic cultivar, Quest, was separated by principal component analysis from the other cultivars, and exhibited a lower diversity compared to Excel or Parkland. The rhizosphere of all cultivars yielded more *Arthrobacter, Aureobacterium*, and *Bacillus* isolates, but fewer *Micrococcus, Variovorax* and *Xanthomonas* isolates compared to the root interior. The results from this study indicate that the composition of the root-endophytic bacterial community of canola differs between cultivars.

IMPACT ON NON-TARGET ORGANISMS INCLUDING BENEFICIAL ORGANISMS

Lora A Morandin, Mark L Winston (2005). Wild bee abundance and seed production in conventional, organic, and genetically modified canola. Ecological Applications. Vol.15. Issue 3. June 2005. Pages 871-881. DOI: **10.1890/03-5271**

The ecological impacts of agriculture are of concern, especially with genetically modified and other intensive, modern cropping systems, yet little is known about effects on wild bee populations and subsequent implications for pollination. Pollination deficit (the difference between potential and actual pollination) and bee abundance were measured in organic, conventional, and herbicide-resistant, genetically modified (GM) canola fields (Brassica napus and B. rapa) in northern Alberta, Canada, in the summer of 2002. Bee abundance data were collected using pan traps and standardized sweep netting, and pollination deficit was assessed by comparing the number of seeds per fruit from open-pollinated and supplementally pollinated flowers. There was no pollination deficit in organic fields, a moderate pollination deficit in conventional fields, and the greatest pollination deficit in GM fields. Bee abundance was greatest in organic fields, followed by conventional fields, and lowest in GM fields. Overall, there was a strong, positive relationship between bee abundance at sampling locations and reduced pollination deficits. Seed set in B. napus increased with greater bee abundance. Because B. rapa is an obligate outcrossing species, the lack of pollination deficit in the organic (B. rapa) fields likely was due to the high bee abundance rather than a lower dependence ofB. rapa on pollinators than B. napus canola. Our study illustrates the importance of wild bees to agricultural production and suggests that some agroecosystems may better sustain wild bee abundance, resulting in greater seed production. Further research on why some cropping systems, such as genetically modified, herbicide-resistant canola, have low wild bee abundance would be useful for management of agroecosystems to promote sustainability of food production.

Riccardo Bommarco, Lorenzo Marini, Bernard E. Vaissière (2012). Insect pollination enhances seed yield, quality, and market value in oilseed rape. Oecologia. Vol. 169. Issue 4. PP 1025-1032.

Abstract

The relationships between landscape intensification, the abundance and diversity of pollinating insects, and their contributions to crop yield, quality, and market value are poorly studied, despite observed declines in wild and domesticated pollinators. Abundance and species richness of pollinating insects were estimated in ten fields of spring oilseed rape, *Brassica napus* var. SW Stratos™, located along a gradient of landscape compositions ranging from simple landscapes dominated by arable land to heterogeneous landscapes with extensive cover of semi-natural habitats. In each field, we assessed the contribution of wind and insect pollination to seed yield, seed quality (individual seed weight and oil and chlorophyll contents), and market value in a block experiment with four replicates and two treatments: (1) all flowers were accessible to insects, self and wind pollination, and (2) flowers enclosed in tulle net bags (mesh: 1 × 1 mm) were accessible only to wind and self pollination. Complex landscapes enhanced the overall abundance of wild insects as well as the abundance and species richness of hoverflies. This did not translate to a higher yield, probably due to consistent pollination by honey bees across all fields. However, the pollination experiment showed that insects increased seed weight per plant by 18% and market value by 20%. Seed quality was enhanced by insect pollination, rendering heavier seeds as well as higher oil and lower chlorophyll contents, clearly showing that insect pollination is required to reach high seed yield and quality in oilseed rape. Our study demonstrates considerable and previously underestimated contributions from pollinating insects to both the yield and the market value of oilseed rape.

Ximena Araneda Durán; Rodrigo Breve Ulloa; José Aguilera Carrillo; Jorge Lavín Contreras; Marcelo Toneatti Bastidas (2010). Evaluation of yield component traits of honeybee-pollinated (Apis mellifera L.) rapeseed canola (Brassica napus L.). Chilean Journal of Agricultural Research, Vol. 70, No. 2, 2010, pp. 309-314

http://www.bioline.org.br/abstract?id=cj10033

Description

Recent introduction of hybrid varieties raises the question if bees (Apis mellifera L.) contribute as pollinator agents in developing the full yield potential of rapeseed (Brassica napus L.). In order to evaluate the yield achieved by B. napus cv. Artus pollinated by A. mellifera testing was carried out in the district of Freire, La Araucania Region, Chile. This consisted in isolating or excluding rapeseed plants from pollinators with exclusion cages. Treatments applied were total exclusion (Tl), partial exclusion (T2) and free pollination (TO) with a density of 6.5 hives ha -1, in order to determine the following yield components traits: grains per silique, siliques per plant, 1000 grain weight and yield. The experimental design used was randomized complete blocks with three treatments and three replicates. Results obtained show that the parameter least affected by bee intervention was the grains per silique variable. In contrast, siliques per plant and 1000 grain weight parameters presented significant differences, contributing to a yield greater than 5 t ha -1; which represented a figure 50.34% higher than in the treatment without bees. It may be concluded that the inclusion of bees in crops is fully justified as a production tool.

Muhammad Sidddique Munawar, Shazia Raja, Mahjabeen Siddique, Shahid Niaz and Muhammad Amjad (2009). The pollination by honeybee (Apis mellifera l.) increases yield of canola (Brassica napus l.). Pak. Entomol. Vol. 31, No.2, 2009 103

http://www.pakentomol.com/Downloads/Issues/2009-2/ent4-paper%20canola%20paperfinal[ms%20munawar.pdf

Abstract

In order to quantify the response of honeybee on canola seed yield, an experiment was conducted at National Agriculture Research Center, Islamabad, during 2007-08, in complete randomized block design with two treatments (i. Plants caged with honeybees ii. Plants caged without honeybees) with four replications each. Number of pods set, pods weight, seed count in hundred pods and yield per plant were measured in twenty randomly harvested plants. The results showed significant increase in all the plant parameters caged with bees as compared to the plants without bees (control). Number of pods and number of seeds pod-1 with pollination were 815 and 20 while without pollination was 349 and 15. The seed weight and yield (gm) with pollination was 26 and 7.6 (gm) and without pollination was 9.3 and 1.51 (gm), respectively. It is concluded from the experiment that honeybee visitation to the canola flowers is important for pollination and increasing seed yield.

DP Abrol (2007). Honeybees and Rapeseed: A Pollinator–Plant Interaction. Advances in Botanical Research. Vol. 45. Pages 337-367. doi:10.1016/S0065-2296(07)45012-1

http://www.sciencedirect.com/science/article/pii/S0065229607450121

Abstract

Bees and *Brassica* plants have mutualistic relationship and coevolved during the long course of their evolutionary history. Since most of the oilseed crops are cross-pollinated, adequate pollination is vital for any significant seed production. Among the various pollinating agents, honeybees play a predominant role in increasing the yield of rapeseed. Almost one-third of total cropped area under oilseeds has been reported to be entomophilous. The yield of rapeseed and mustard can be doubled through pollination by insects. Pollinators not only enhance the yield of the crop but also contribute to uniform and early pod setting. Therefore, planned honeybee pollination could result in increased productivity and improvement in other parameters through the process of heterosis. Both protective applications of pesticides against pests and use of bees should be integrated in a manner to boost oilseed production and honey production. The relevant information on the mutualistic interactions between honeybees and rapeseed is discussed in this chapter to stimulate further research efforts to enhance productivity of oilseed crops.

A J Haughton, G T Champion, C Hawes, M S Heard, D R Brooks, D A Bohan, S J Clark, A M Dewar, L G Firbank, J L Osborne, J N Perry, P Rothery, D B Roy, R J Scott, I P Woiwod, C Birchall, M P Skellern, J H Walker, P Baker, E L Browne, A J G Dewar, B H Garner, L A Haylock, S L Horne, N S Mason, R J N Sands, and M J Walker (2003). Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. II. Within-field epigeal and aerial arthropods. Philos Trans R Soc Lond B Biol Sci. 2003 Nov 29; 358(1439): 1863–1877. doi: 10.1098/rstb.2003.1408

http://www.ncbi.nlm.nih.gov/pmc/articles/pmc1693277/pdf/14561319.pdf

The effects of the management of genetically modified herbicide-tolerant (GMHT) crops on the abundances of aerial and epigeal arthropods were assessed in 66 beet, 68 maize and 67 spring oilseed rape sites as part of the Farm Scale Evaluations of GMHT crops. Most higher taxa were insensitive to differences between GMHT and conventional weed management, but significant effects were found on

the abundance of at least one group within each taxon studied. Numbers of butterflies in beet and spring oilseed rape and of Heteroptera and bees in beet were smaller under the relevant GMHT crop management, whereas the abundance of Collembola was consistently greater in all GMHT crops. Generally, these effects were specific to each crop type, reflected the phenology and ecology of the arthropod taxa, were indirect and related to herbicide management. These results apply generally to agriculture across Britain, and could be used in mathematical models to predict the possible long-term effects of the widespread adoption of GMHT technology. The results for bees and butterflies relate to foraging preferences and might or might not translate into effects on population densities, depending on whether adoption leads to forage reductions over large areas. These species, and the detritivore Collembola, may be useful indicator species for future studies of GMHT management.

COMPOSITIONAL ANALYSIS

Song Chen, Qi Peng, Jiangin Gao, Xiaoying Zhour, Jiefu Zhang, Huiming Pu, Cunkou Qi (2015). Fatty Acid Composition and Seed Quality Traits of the Transgenic Rapeseed W-4 (Brassica napus L.) with Down-regulated Expression of fad2 Gene. Agricultural Science & Technology 2015, Vol. 16 Issue 8, p1769-1774.

Abstract

To clarify the effect of down-regulated expression of fad2 gene on the seed nutritional quality of rapeseed, the fatty acid composition, amino acid composition, oil content, protein content, crude fiber content and glucosinolate content in the seeds of both transgenic line W-4 and its control Westar were compared. The results showed that the oleic acid content in W-4 was 86.03% ±0.20%, which was 29.36% higher than that in the control (P<0.01); the linoleic acid content was 2.86%± 0.01%, which was reduced by 84.03% compared with that in the control (P<0.01); the linolenic acid content in W-4 was 3.040/。 ±0.040/，reduced by 57.60%。 (P<0.01); the palmitic acid content in W-4 was 3.23%±0.07%, reduced by 18.63% (P<0.01); the eicosenoic acid content in W-4 was increased by 18.46% compared with that in the control (P<0.01); the erucic acid content in W-4 was increased by 13.15% (P< 0.05); there was no significant difference in stearic acid content between the treatment and control groups (P>0.05). The amino acid composition analysis showed that total 18 amino acids, including 8 essential amino acids, were detected in both W-4 and Westar; there were no significant differences in contents of the 18 amino acids between the treatment and control groups except that of tyrosine (P>0.05); the contents of oil, proteins, glucosinolates and crude fiber in W-4 were 45.40% ± 0.17% (P>0.05), （P>0.05), 18.20%±1.21% （$>0.05) and 12.29% ± 0.04% (P>0.05), respectively. All the results above showed that the down-regulated expression of fad2 had great effects on fatty acid composition and accumulation in rapeseed seeds, but had no significant effects on other seed quality traits, such as oil content, protein content, crude fiber content and glucosinolate content.

Syed K, Shinwari ZK (2013). Allelopathic effect of methanolic extracts of genetically modified and non-genetically modified canola on soybean. Toxicol Ind Health. 2016 Mar; 32(3):564-75. doi: 10.1177/0748233713501366. Epub 2013 Oct 8.

http://www.ncbi.nlm.nih.gov/pubmed/24105070

Abstract

This study on the effect of genetically modified (GM) and non-GM canola on soybean was carried out for physiological and biochemical biosafety assessment of GM canola. Methanolic extracts of GM and non-GM canola were assessed on seed germination and growth of soybean (Glycine max L.) under sterilized conditions. The extracts applied were of 3, 5, and 10% concentrations. The results showed that methanolic extracts of both GM and non-GM canola improved the germination percentage. However, germination rate index was significantly decreased with concomitant increase in mean germination time of soybean. A significant rate of decrease was observed in root fresh weight while increase in shoot

length took place; when treatment of GM canola extracts were applied, however, no effect was observed in shoot fresh weight. A significant increase in protein contents, as well as phenolic, carotenoids, proline, and chlorophyll a content, was observed when different GM canola treatments (3, 5, and 10%) were applied to soybean; however, a significant rate of reduction in chlorophyll b content was observed by the application of GM canola treatment. Similar results were observed for superoxide dismutase, peroxidase, and catalase activities. A significant increase in the sugar content levels was observed when GM canola treatments (3, 5, and 10%) were applied to soybean.

Shewmaker C, Sheehy JA, Daley M, Colburn S, Ke DY. Seed-specific overexpression of phytoene synthase: Increase in carotenoids and other metabolic effects. Plant J. 1999; 20(4): 401–412

Abstract

A bacterial phytoene synthase (crtB) gene was overexpressed in a seed-specific manner and the protein product targeted to the plastid in Brassica napus (canola). The resultant embryos from these transgenic plants were visibly orange and the mature seed contained up to a 50-fold increase in carotenoids. The predominant carotenoids accumulating in the seeds of the transgenic plants were alpha and beta-carotene. Other precursors such as phytoene were also detected. Lutein, the predominant carotenoid in control seeds, was not substantially increased in the transgenics. The total amount of carotenoids in these seeds is now equivalent to or greater than those seen in the mesocarp of oil palm. Other metabolites in the isoprenoid pathway were examined in these seeds. Sterol levels remained essentially the same, while tocopherol levels decreased significantly as compared to non-transgenic controls. Chlorophyll levels were also reduced in developing transgenic seed. Additionally, the fatty acyl composition was altered with the transgenic seeds having a relatively higher percentage of the 18 : 1 (oleic acid) component and a decreased percentage of the 18 : 2 (linoleic acid) and 18 : 3 (linolenic acid) components. This dramatic increase in flux through the carotenoid pathway and the other metabolic effects are discussed.

PERSISTENCE OF TRANSGENE

Sharma R, Damgaar D, Alexander TW, Dugan ME, Aalhus JL, Stanford K, McAllister TA (2006). Detection of transgenic and endogenous plant DNA in digesta and tissues of sheep and pigs fed Roundup Ready canola meal. J Agric Food Chem. 2006 Mar 8; 54(5):1699-709.

http://www.ncbi.nlm.nih.gov/pubmed/16506822

Abstract

The persistence of plant-derived recombinant DNA in sheep and pigs fed genetically modified (Roundup Ready) canola was assessed by PCR and Southern hybridization analysis of DNA extracted from digesta, gastrointestinal (GI) tract tissues, and visceral organs. Sheep (n = 11) and pigs (n = 36) were fed to slaughter on diets containing 6.5 or 15% Roundup Ready canola. Native plant DNA (high- and low-copy-number gene fragments) and the cp4 epsps transgene that encodes 5-enolpyruvyl shikimate-3-phosphate synthase were tracked in ruminal, abomasal, and large intestinal digesta and in tissue from the esophagus, rumen, abomasum, small and large intestine, liver, and kidney of sheep and in cecal content and tissue from the duodenum, cecum, liver, spleen, and kidney of pigs. High-copy chloroplast-specific DNA (a 520-bp fragment) was detected in all digesta samples, the majority (89-100%) of intestinal tissues, and at least one of each visceral organ sample (frequencies of 3-27%) from sheep and swine. Low-copy rubisco fragments (186- and 540-bp sequences from the small subunit) were present at slightly lower, variable frequencies in digesta (18-82%) and intestinal tissues (9-27% of ovine and 17-25% of porcine samples) and infrequently in visceral organs (1 of 88 ovine samples; 3 of 216 porcine samples). Each of the five cp4 epsps transgene fragments (179-527 bp) surveyed was present in at least 27% of ovine large intestinal content samples (maximum = 64%) and at least 33% of porcine cecal content samples (maximum = 75%). In sheep, transgene fragments were more common in intestinal digesta than in ruminal or abomasal content. Transgene fragments were detected in 0 (esophagus) to 3 (large intestine) GI tract tissues from the 11 sheep and in 0-10 of the duodenal and cecal tissues collected from 36 pigs. The feed-ingested recombinant DNA was not detected in visceral tissues (liver, kidney) of lambs or in the spleen from pigs. Of note, however, one liver and one kidney sample from the pigs (different animals) were positive for a 278-bp fragment of the transgenic cp4 epsps (denoted F3). Examination of genomic libraries from these tissues yielded no conclusive information regarding integration of the fragment into porcine DNA. This study confirms that feed-ingested DNA fragments (endogenous and transgenic) do survive to the terminal GI tract and that uptake into gut epithelial tissues does occur. A very low frequency of transmittance to visceral tissue was confirmed in pigs, but not in sheep. It is recognized that the low copy number of transgenes in GM feeds is a challenge to their detection in tissues, but there was no evidence to suggest that recombinant DNA would be processed in the gut in any manner different from endogenous feed-ingested genetic material.

TOXICOLOGY STUDIES

IM Zdziarski, JW Edwards, JA Carman, JI Haynes (2014): GM crops and the rat digestive tract: A critical review. Environment International. Vol. 73, December 2014. Pages 423-433. doi:10.1016/j.envint.2014.08.018

http://www.sciencedirect.com/science/article/pii/S0160412014002669

Highlights

- Histopathology studies on rats eating approved GM crops were reviewed.
- Only crops containing any of three widely-eaten GM genes were reviewed.
- Published studies could be found for only 19% of these crops.
- Most histopathology studies were published years after approval.
- All reviewed papers were found to be significantly inadequate or flawed.

Abstract

The aim of this review is to examine the relationship between genetically modified (GM) crops and health, based on histopathological investigations of the digestive tract in rats. We reviewed published long-term feeding studies of crops containing one or more of three specific traits: herbicide tolerance via the *EPSPS* gene and insect resistance via *cry1Ab* or *cry3Bb1* genes. These genes are commonly found in commercialised GM crops. Our search found 21 studies for nine (19%) out of the 47 crops approved for human and/or animal consumption. We could find no studies on the other 38 (81%) approved crops. Fourteen out of the 21 studies (67%) were general health assessments of the GM crop on rat health. Most of these studies (76%) were performed after the crop had been approved for human and/or animal consumption, with half of these being published at least nine years after approval. Our review also discovered an inconsistency in methodology and a lack of defined criteria for outcomes that would be considered toxicologically or pathologically significant. In addition, there was a lack of transparency in the methods and results, which made comparisons between the studies difficult. The evidence reviewed here demonstrates an incomplete picture regarding the toxicity (and safety) of GM products consumed by humans and animals. Therefore, each GM product should be assessed on merit, with appropriate studies performed to indicate the level of safety associated with them. Detailed guidelines should be developed which will allow for the generation of comparable and reproducible studies. This will establish a foundation for evidence-based guidelines, to better determine if GM food is safe for human and animal consumption.

STABILITY OF MALE STERILITY

Martine Denis, Regine Delourme, Jean-Pierre Courret, Celestina Mariani, and Michel Renard (1993). Expression of Engineered Nuclear Male Sterility in Brassica *napus* - Genetics, Morphology, Cytology, and Sensitivity to Temperature. Plant Physiol. (1993) 101: 1295-1304

A dominant genetic male sterility trait obtained through transformation in rapeseed (Brassica napus) was studied in the progenies of 11 transformed plants. The gene conferring the male sterility consists of a ribonuclease gene under the control of a tapetum specific promoter. Two ribonuclease genes, RNase *T1* and barnase, were used. The chimaeric ribonuclease gene was linked to the bialophos-resistance gene, which confers resistance to the herbicide phosphinotricine (PPT). The resistance to the herbicide was used as a dominant marker for the male sterility trait. lhe study presented here concerns three aspects of this engineered male sterility: genetics correlated with the segregation of the 1-DNA in the progenies; expression of the male sterility in relation to the morphology and cytology of the androecium; and stability of the engineered male sterility under different culture conditions. Correct segregation, 50% male-sterile, PPT-resistant plants, and 50% male-fertile, susceptible plants were observed in the progeny of seven transformants. The most prominent morphological change in the male-sterile flowers was a noticeable reduction in the length of the stamen filament. The first disturbances of microsporogenesis were observed from the free microspore stage and were followed by a simultaneous degeneration of microspore and tapetal cell content. At anthesis, the sterile anthers contained only empty exines. In some cases, reversion to fertility of male-sterile plants has been observed. Both ribonuclease genes are susceptible to instability. lnstability of the RNase TI-male sterility trait increased at temperatures higher than 25°C. Our results do not allow us to confirm this observation for the barnase male-sterile plants. However, the male-sterile plants of the progeny of two independent RNase *TI* transformants were stably male sterile under all conditions studied.

VEGETABLE OIL CONSUMPTION VS. NUTRITIONAL NEEDS

DM Hegde (2012). Carrying Capacity of Indian Agriculture-Oilseeds. Current Science. Vol.102, No.6, March 2012.

The carrying capacity of Indian agriculture to support oilseeds production to meet the vegetable oil needs of the Indian population has been considered in the context of available sources of oil from oilseed and non-oilseed origins. India needs to produce 17.84 Mt of vegetable oils to meet the nutritional fat needs of projected population of 1685 million by 2050. This can be easily achieved from various sources like annual oilseeds and also from supplementary sources of oil like rice bran, cottonseed, coconut, oil palm, corn, etc. However, the actual vegetable oil consumption has already exceeded the nutritional needs by a large margin and is likely to further go up sharply in the years to come in response to income growth. This requirement will be difficult to meet by the Indian agriculture given the current status of resources, technology and management.

Summary

An average fat intake of 29 g per head per day is adequate to meet the nutritional needs of Indians, which translates into annual vegetable oil needs of 10.585 kg per person. For a projected Indian population of 1685 million by 2050, 17.84 Mt of vegetable oils is required to meet the fat nutrition. This is equivalent to roughly 59.41 Mt of oilseeds. If one assumes 25% of vegetable oils from crops other than annual oilseeds, then the country needs to produce just 44.56 Mt of oilseeds by 2050 to meet fat nutrition of the projected population. With full adoption of currently available oilseed technologies, this level of production could easily be achieved. There is tremendous scope to increase vegetable oils production from supplementary sources like rice bran, cotton seed, oil palm, corn, TBOs, coconut, etc. This along with bridging the yield gap in annual oilseeds by effective technology transfer can easily meet the vegetable oil needs of the country in the foreseeable future. However, the present level of vegetable oils consumption has already exceeded the nutritional needs by a large margin and is likely to go up further in response to income growth and lower oil prices. Any unbridled increase in per capita vegetable oil consumption in future will be a formidable challenge on the carrying capacity of Indian agriculture to support oilseed production.

INTELLECTUAL PROPERTY RIGHTS & PATENTS

Jorge Mayer, Jade Sharples, and Carol Nottenburg (2004). Resistance to Phosphinothricin. Cambia Intellectual Property Resource.

This white paper focuses primarily on the patent landscape surrounding the *bar* gene which confers resistance to the broad-spectrum herbicide, glufosinate.

simple intellectual property ownership situation. Essentially all key patents are held by Bayer Crop Science, although assignees listed on the patent documents include Plant Genetic Systems, Hoechst, AgrEvo and Aventis. To understand why the *bar* gene patent portfolio is now in the hands of Bayer Crop Science, a schematic overview of the corporate consolidation history which led to the creation of one of the major players in the agrichemicals business worldwide is included in the analysis.

CENTRE OF DIVERSITY OF B.JUNCEA

Sheng Chen, Zhenjie Wan, Matthew N. Nelson, Jitendra S. Chauhan, Ping Lin, Bob Redden, Wayne A. Burton, Surinder Banga, Yuehua Chen, Phillip A. Salisbury, Tingdong Fu and Wallace A. Cowlin. (2011). Two distinct genetic diversity groups of oilseed *Brassica juncea* in both China and India. 17th Australian Research Assembly on Brassicas (ARAB) Wagga Wagga NSW August 2011

ABSTRACT

Oilseed *Brassica juncea* is an agriculturally and economically important crop with a long history of cultivation in India and China, and increasingly in Australia. However, the centre of origin and diversity of oilseed *B. juncea* has been a controversial issue in the last century. In this study, allelic diversity of oilseed *B. juncea* predominantly from China and India was evaluated using simple sequence repeat (SSR) markers. Two major groups were identified and each included both Chinese and Indian accessions. Diversity groups 1 and 2 were also observed when A- and B-genome markers were analysed separately. The Shannon diversity index, based on analysis of geographical distribution, also revealed a diversity hotspot in central and western China. In this study, SSR allelic diversity in the A genome and B genome supported a polyphyletic origin and secondary centres of genetic diversity of oilseed *B. juncea* in China and India.

ADVANCES IN AGRONOMIC MANAGEMENT OF INDIAN MUSTARD

Kapila Shekhawat, S. S. Rathore, O. P. Premi, B. K. Kandpal, and J. S. Chauhan (2012). Advances in Agronomic Management of Indian Mustard (Brassica juncea (L.) Czernj. Cosson): An Overview. International Journal of Agronomy Volume 2012, Article ID 408284, 14 pages doi:10.1155/2012/408284

India is the fourth largest oilseed economy in the world. Among the seven edible oilseeds cultivated in India, rapeseed-mustard contributes 28.6% in the total oilseeds production and ranks second after groundnut sharing 27.8% in the India's oilseed economy. The mustard growing areas in India are experiencing the vast diversity in the agro climatic conditions and different species of rapeseed-mustard are grown in some or other part of the country. Under marginal resource situation, cultivation of rapeseedmustard becomes less remunerative to the farmers. This results in a big gap between requirement and production of mustard in India. Therefore site-specific nutrient management through soil-test recommendation based should be adopted to improve upon the existing yield levels obtained at farmers field. Effective management of natural resources, integrated approach to plant-water, nutrient and pest management and extension of rapeseed-mustard cultivation to newer areas under different cropping systems will play a key role in further increasing and stabilizing the productivity and production of rapeseed-mustard. The paper reviews the advances in proper land and seedbed preparation, optimum seed and sowing, planting technique, crop geometry, plant canopy, appropriate cropping system, integrated nutrient management and so forth to meet the ever growing demand of oil in the country and to realize the goal of production of 24 million tonnes of oilseed by 2020 AD through these advanced management techniques.

EVIDENCE OF FASTER YIELD GROWTH IN NON-GM AREAS

Jack A. Heinemann, Melanie Massaro, Dorien S. Coray, Sarah Zanon Agapito-Tenfen & Jiajun Dale Wen (2014) Sustainability and innovation in staple crop production in the US Midwest, International Journal of Agricultural Sustainability, 12:1, 71-88, DOI: 10.1080/14735903.2013.806408 &

Reply to comment on sustainability and innovation in staple crop production in the US Midwest. International Journal of Agricultural Sustainability. Volume 12, Issue 4, 2014

http://www.tandfonline.com/doi/full/10.1080/14735903.2014.939843

An agroecosystem is constrained by environmental possibility and social choices, mainly in the form of government policies. To be sustainable, an agroecosystem requires production systems that are resilient to natural stressors such as disease, pests, drought, wind and salinity, and to human constructed stressors such as economic cycles and trade barriers. The world is becoming increasingly reliant on concentrated exporting agroecosystems for staple crops, and vulnerable to national and local decisions that affect resilience of these production systems. We chronicle the history of the United States staple crop agroecosystem of the Midwest region to determine whether sustainability is part of its design, or could be a likely outcome of existing policies particularly on innovation and intellectual property. Relative to other food secure and exporting countries (e.g. Western Europe), the US agroecosystem is not exceptional in yields or conservative on environmental impact. This has not been a trade-off for sustainability, as annual fluctuations in maize yield alone dwarf the loss of caloric energy from extreme historic blights. We suggest strategies for innovation that are responsive to more stakeholders and build resilience into industrialized staple crop production.

First the yields of maize and rapeseed were compared in North American and Western European (W. European) agroecosystems because these agroecosystems are of equal maturity and have similar access to sophisticated biotechnological and IP options, and are constrained by a similar latitude and operate in the same growing season (Licker et al. 2010). We mainly 74 J.A. Heinemann et al. Downloaded by [223.227.126.136] at 04:28 09 July 2016 focused on where different choices in biotechnologies were made. A significant difference between the two agroecosystems is the virtual absence of GM crops in our group of six W. European countries. In contrast, the adoption of GM soybeans, maize, rapeseed and cotton in the North American agroecosystem has reached near saturation. According to the industry site GMO Compass (Anon 2011), the proportion of GM rapeseed reached 82% in the United States by 2007 and 95% in Canada by 2009. In the United States, GM maize reached a reported 88% by 2011, GM soybeans 94% by 2011 and GM cotton 94% by 2012 (USDA 2012a).

Consistent with what is observed for maize, the yield gap appears to be increasing for Canada, the other earliest adopter of GM crops, for rapeseed (Table 1). The average yields of rapeseed for Canada have always been lower than W. Europe's, by an average of 11,000 hg/ha between 1961 and 1985, and an even larger average difference of 17,300 hg/ha between 1986 and 2010, the period when Canada moved to GM and Europe did not.

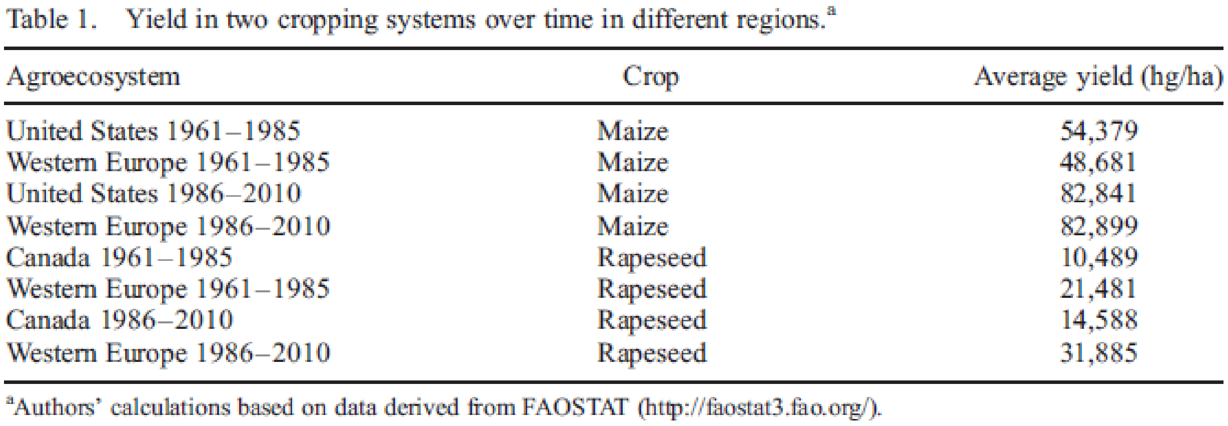

Table 1. Yield in two cropping systems over time in different regions.[a]

Agroecosystem	Crop	Average yield (hg/ha)
United States 1961–1985	Maize	54,379
Western Europe 1961–1985	Maize	48,681
United States 1986–2010	Maize	82,841
Western Europe 1986–2010	Maize	82,899
Canada 1961–1985	Rapeseed	10,489
Western Europe 1961–1985	Rapeseed	21,481
Canada 1986–2010	Rapeseed	14,588
Western Europe 1986–2010	Rapeseed	31,885

[a] Authors' calculations based on data derived from FAOSTAT (http://faostat3.fao.org/).

GLUFOSINATE TOXICITY

Anthony Laugeray, Ameziane Herzine, Olivier Perche, Betty Hébert, Marine Aguillon-Naury, Olivier Richard, Arnaud Menuet, Séverine Mazaud-Guittot, Laurianne Lesné, Sylvain Briault, Bernard Jegou, Jacques Pichon, Céline Montécot-Dubourg and Stéphane Mortaud (2014). Pre- and postnatal exposure to low dose glufosinate ammonium induces autism-like phenotypes in mice. Frontiers in Behavioural Neurosciences. Vol.8. Article 390 published: 20 November 2014 doi: 10.3389/fnbeh.2014.00390

Glufosinate ammonium (GLA) is one of the most widely used herbicides in agriculture. As is the case for most pesticides, potential adverse effects of GLA have not been studied from the perspective of developmental neurotoxicity. Early pesticides exposure may weaken the basic structure of the developing brain and cause permanent changes leading to a wide range of lifelong effects on health and/or behavior. Here, we addressed the developmental impact of GLA by exposing female mice to low dose GLA during both pre-and postnatal periods and analyzed potential developmental and behavioral changes of the offspring during infancy and adulthood. A neurobehavioral test battery revealed significant effects of GLA maternal exposure on early reflex development, pup communication, affiliative behaviors, and preference for social olfactory cues, but emotional reactivity and emotional memory remained unaltered. These behavioral alterations showed a striking resemblance to changes seen in animal models of Autistic Spectrum Disorders. At the brain level, GLA maternal exposure caused some increase in relative brain weight of the offspring. In addition, reduced expression of *Pten* and *Peg3* – two genes implicated in autism-like deficits – was observed in the brain of GLA-exposed pups at postnatal day 15. Our work thus provides new data on the link between pre- and postnatal exposure to the herbicide GLA and the onset of autism-like symptoms later in life. It also raises fundamental concerns about the ability of current safety testing to assess risks of pesticide exposure during critical developmental periods.

We also attach three reports by Pesticides Action Network on Glufosinate ammonium, from 2001, 2008 and 2012 with many scientific studies cited for the adverse health and environmental impacts of Glufosinate.

TOXICITY OF BARNASE

Barnase Ribonuclease is toxic to Humans and other Mammals: (Late) Prof Joseph Cummins, Geneticist

Barnase ribonuclease is an enzyme toxic to cells, it is produced in nature by the bacterium, Bacillus amyloliquefaciens, which synthesizes and secretes the enzyme . The enzyme is not toxic to the bacterium that produces it because it is inhibited by another protein, barstar, produced within that bacterium. As the enzyme is secreted from the bacterial cell barstar is removed from its complex with barstar. The bacterial gene for barnase and the gene for barstar have been employed extensively in transgenic plants and animals to set up regulatorysystems that are capable of ablating (killing) specific organs or tissues of the organism by the interplay of barnase and barstar. Mustard (Brassica Juncea) was modified with the barnase gene driven by a pollen specific (tapetum) promoter that produced male sterile lines of the mustard plants by ablating pollen cells (1) Male sterile plants can be used to limit pollen release from a crop or to produce hybrids. United States Patent 6,509,516 deals with producing male sterile Brassica employing barnase (2), while United States patent 6,969.786 deals with producing hybrid canola (Brassica napa or B.napus) using a male sterile line created using barnase and barstar genes (3).The production of Brassica hybrids using the barnase-barstar system has been illustrated and explained (4).

Barnase, unaccompanied by its specific inhibitor barstar, is known to be a potent cell poison [5]. Traces of barnase are toxic to the rat kidney [6] and to human cell lines[7]. Barnase is actually being exploited as a conditional 'suicide gene' to cause celldeath in mammalian [8] and human [9] cells when it is induced. Cell toxicity caused by barnase may be affected by RNA interference (RNAi) (10).The hazard associated with RNAi is shown by experiments over saturating small RNA pathways leading to the death of many experimental mice (11).RNAi is known to circulate among the cells of plants (12) so the ablation of anther cells may be accompanied by RNAi spread throughout the entire plant.

The pollen produced by the hybrid mustard actually contains the barnase gene combined with the barstar. Is barnase expressed at low basal levels in the plant tissues when it is not in the induced state (is the regulatory transgene leaky)? Are the constructs sufficiently stable to ensure that the barnase is only active in the anther? How much RNAi is translocated throughout the plant?As indicated earlier, Barnase, even if expressed at low levels could prove toxic to a wide range of animals that interact with the plant, including not only humans, but also rodents and bees. It could also enter the human food chain in bee honey. As pointed out earlier, in bacteria, barnase is synthesized but immediately joined to barstar to avoid cell death. The newly synthesized barnase (combined with barstar) has a short leader sequence at the N-terminus, that sequence directs t the barnase –barstar complex to the cell membrane. As barnase is secreted from the cell membrane barstar is separated from barnase and left behind in the cell interior (13). If barnase can be separated from barstar at the cell membrane of bacteria there are likely to be situations where plant cells separate barnase from barstar.

The pathway for folding and the stability of barnase has been studied extensively (14). It seems likely that the barstar-barnase complex is very stable and likely to persist in the grain of food crops such as mustard. However, in spite of the extensive use of the barnase-barstar systems in Brassica such as mustard and oilseed rape (canola), there does not seem to have been any effort to measure the quantity of the barnase-barstar complex in the grain of the food crops. The barnase-barstar complex in transgenic mustard is likely to be produce a strong immunological response such as inflammation or it may even be an allergen. However, these aspects to not appear to have been studied. There is evidence that barstar induces a strong immune response (15). However, the barnase-barstart complex needs fuller study in terms of immune responses, including allergy.

The barnase-barstar system for pollen control has been used with both mustard and with canola. The canola label is used with two species Brassica rapa (Polish rapeseed) and Brassic napus (Argentine rapeseed) (16) Mustard (Brassica juncea) may produce inter specific hybrids with canola (B. rapa) (17) but such hybrids are not yet grown commercially. It is wisest to consider the hazards of barnase-barstar transgenic crops as being separate but related , not as being identical as has been done in some safety evaluations.

In conclusion, the actual toxicity of barnase and the toxicity of the barnase-barstar complex in food and feed cries out for fuller study regarding the impact on humans and animals. The main areas requiring fuller study prior to the exposure of millions of people and millions of animals to the toxins are the exposure to the highly toxic barnase ribonuclease on consuming transgenic food and the potential toxic immune responses to the bartnase-barstar complex in the transgenic crops.

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