# *European Commission*

**Combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009**
**and**
**Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008**

# Glyphosate

## Appendix A to Volume 3 – B.9
## (PPP) MON 52276
## Literature data on biodiversity

Rapporteur Member State: Assessment Group on Glyphosate (AGG) consisting of FR, HU, NL and SE

# Version History

| When | What |
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| 2021/06 | Initial RAR |
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The RMS is the author of the Assessment Report. The Assessment Report is based on the validation by the RMS, and the verification during the EFSA peer-review process, of the information submitted by the Applicant in the dossier, including the Applicant's assessments provided in the summary dossier. As a consequence, data and information including assessments and conclusions, validated and verified by the RMS experts, may be taken from the applicant's (summary) dossier and included as such or adapted/modified by the RMS in the Assessment Report. For reasons of efficiency, the Assessment Report should include the information validated/verified by the RMS, without detailing which elements have been taken or modified from the Applicant's assessment. As the Applicant's summary dossier is published, the experts, interested parties, and the public may compare both documents for getting details on which elements of the Applicant's dossier have been validated/verified and which ones have been modified by the RMS. Nevertheless, the views and conclusions of the RMS should always be clearly and transparently reported; the conclusions from the applicant should be included as an Applicant's statement for every single study reported at study level; and the RMS should justify the final assessment for each endpoint in all cases, indicating in a clear way the Applicant's assessment and the RMS reasons for supporting or not the view of the Applicant.

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# APPENDIX TO VOL. 3CP, SECTION 9 ECOTOXICOLOGY - LITERATURE ON BIODIVERSITY

Several literature articles are referenced in the Biodiversity Assessment report ([redacted] 20201) concerning the indirect effects via trophic interactions.

The study summaries submitted below were prepared by the applicant upon request from the AGG during the admissibility check phase (June-August 2020) and reviewed by the RMS.

The table below summarizes the literature review data provided by the applicant to address the assessment of biodiversity. It is presented by group of organisms.

| No. | Reference | Remarks |
|---|---|---|
| **Part 1 : References for assessment of indirect effects via trophic interaction for birds discussion** | | |
| 1 | Boatman N, Brickle N, Hart J, Milson. J, Morris A., Murray A, Murray K and Robertson P. 2004. Evidence for the indirect effects of pesticides on farmland birds. IBIS - International Journal of avian science Volume 146, Issue S2, November 2004 pages 131-143 | |
| 2 | Bright J, Morris A and Winspear R. 2008. A review of Indirect Effects of Pesticides on Birds and mitigating land-management practices. RSPB Research Report No. 28 | |
| 3 | Burfield, I., (Author of a Chapter within the following book edited by Bota, G., Morales M.B., Manosa, S. and Camprodon, J. 2005. Ecology and Conservation of Steppe-land birds. ISBN 978-84-87334-99-3 | |
| 4 | Campbell, L.H and Cooke A.S. (eds.). 1997. The Indirect effects of pesticides on birds. An official JNCC (Joint Nature Conservation Committee) extended summary of Campbell, L.H. RSPB., Avery, M.I., Donald, P., Evans, A.D., Green, R.E., and Wilson, J.D. 1997 A review of the indirect effects of pesticides on birds (JNCC Report No. 227) | |
| 5 | Cunningham H, Bradbury R, Chaney K and Wilcox A. 2005. Effect of non-inversion tillage on field usage by UK farmland birds in winter. Bird Study (2005) 52, 173–179 | |
| 6 | Department of the Environment, Food and Rural Affairs (DEFRA). 2006. Assessing the Indirect Effects of Pesticides on Birds. PN0925 | |
| 7 | Donald P, Sanderson F, Burfield I and van Bommel F. 2006. Further evidence of continent-wide impacts of agricultural intensification on European farmland birds, 1990–2000. Agriculture, Ecosystems & Environment, Volume 116, Issues 3–4, September 2006, Pages 189-196 | |
| 8 | Easton W. and Martin K. 1998. The effect of vegetation management on breeding bird communities in British Columbia. Ecological Applications 8 (4), 1092-1103 | |
| 9 | Kerry F.L. Guiseppe, Francis A. Drummond, Constance Stubbs, and Stephen Woods. 2006. TB192: The Use of Glyphosate Herbicides in Managed Forest Ecosystems and Their Effects on Non-target Organisms with Particular Reference to Ants as Bioindicators. University of Maine Agricultural and Forest Experiment Station Technical Bulletin 192 | |
| 10 | Guynn D, Guynn S, Wigley T and Miller D. 2004. Herbicides and forest biodiversity—what do we know and where do we go from here? Wildlife Society Bulletin 32(4):1085-1092 | |
| 11 | Jahn T and Höker H. 2014. Protection of biodiversity of free-living birds and mammals in respect of the effects of pesticides. Environmental Research of the Federal Ministry of the Environment, Nature Conservation and Nuclear Safety Project no. (FKZ) 3710 63 411; Report no. (UBA-fb) 001830. Report | |

| No. | Reference | Remarks |
|---|---|---|
| | available on the UBA (German Federal Environment Agency (Umweltbundesamt)) website | |
| 12 | Marshall J, Brown V, Boatman N, Lutman P and Squire G. 2001. The impact of herbicides on weed abundance and biodiversity. PN0940 | |
| 13 | McLaughlin A and Mineau P. 1995. The impact of agricultural practices on biodiversity. Agriculture, Ecosystems and Environment 55 (1995) 201-212 | |
| 14 | Santillo D, Leslie D and Brown P. 1989b. Responses of Songbirds to Glyphosate induced habitat changes on Clearcuts. The Journal of Wildlife Management, Vol. 53, No. 1 (Jan., 1989), pp. 64-71 | |
| 15 | Sullivan T and Sullivan S. 2003. Vegetation management and ecosystem disturbance: impact of glyphosate herbicide on plant and animal diversity in terrestrial systems. Environ. Rev. 11: 37–59 (2003) doi: 10.1139/A03-005 | |
| 16 | Traba J and Morales M. 2019. The decline of farmland birds in Spain is strongly associated to the loss of fallowland. Scientific Reports 9: 9473 | |
| **Part 2: <u>References for assessment of indirect effects via trophic interactions discussions related to terrestrial vertebrates and amphibians</u>** | | |
| 1 | Anthony R and Morrison M. 1985. Influence of glyphosate herbicide on small mammal populations in Western Oregon. Northwest Science, Volume 59, No. 3 | |
| 2 | D'Anieri P, Leslie D and McCormick L. 1987. Small mammals in glyphosate-treated Clear-cuts in northern Maine. Canadian Field Naturalist 101 (4): 54-550 | |
| 3 | Edge C, Gahl M, Pauli B, Thompson D and Houlahan J. 2011. Exposure of juvenile green frogs (*Lithobates clamitans*) in littoral enclosures to a glyphosate-based herbicide. Ecotoxicology and Environmental Safety 74 (2011) 1363-1369 | |
| 4 | Edge C, and Houlahan J. 2012. A silviculture application of the glyphosate-based herbicide VisionMAX™ to wetlands has limited direct effects on amphibian larvae. Environmental Toxicology and Chemistry · October 2012 DOI: 10.1002/etc.1956 · Source: PubMed | |
| 5 | Edge C, Gahl M, Thompson D and and Houlahan J. 2013. Laboratory and field exposure of two species of juvenile amphibians to a glyphosate-based herbicide and *Batrachochytrium dendrobatidis*. Science of The Total Environment Volume 444, 1 February 2013, Pages 145-152 | |
| 6 | Edge C, Thompson D, Hao C and Houlahan J. 2014. The response of amphibian larvae to exposure to a glyphosate-based herbicide (Roundup WeatherMax™) and nutrient enrichment in an ecosystem experiment. Ecotoxicology and Environmental Safety 109 (2014) 124 - 132 | |
| 7 | Edge C, Baker L, Lanctôt C, Melvin S, Gahl M, Kurband M, Navarro-Martín L, Kidd K, Trudeau V, Thompson D, Mudge J and Houlahan J. 2020. Compensatory indirect effects of an herbicide on wetland communities. Science of the Total Environmental 718 (2020) 137254 | |
| 8 | Gagne N, Belanger L and Huot J. 1999. Comparative responses of small mammals, vegetation, and food sources to natural regeneration and conifer release treatments in boreal balsam fir stands of Quebec. Canadian Journal of Forestry Research 29: 1128–1140 | |

| No. | Reference | Remarks |
|---|---|---|
| 9 | Guynn D, Guynn S, Wigley T and Miller D. 2004. Herbicides and forest biodiversity—what do we know and where do we go from here? Wildlife Society Bulletin 32(4):1085-1092 | Summary already submitted under Part 1, point 10 on this document. |
| 10 | Kerry F.L. Guiseppe, Francis A. Drummond, Constance Stubbs, and Stephen Woods. 2006. TB192: The Use of Glyphosate Herbicides in Managed Forest Ecosystems and Their Effects on Non-target Organisms with Particular Reference to Ants as Bioindicators. University of Maine Agricultural and Forest Experiment Station Technical Bulletin 192 | Summary already submitted under Part 1, point 9 on this document. |
| 11 | Santillo D, Leslie D and Brown P. 1989a. Responses of Small Mammals and Habitat to Glyphosate Application on Clearcuts. The Journal of Wildlife Management, Vol. 53, No. 1 (Jan., 1989), pp. 164-172 | |
| 12 | Santillo D, Leslie D and Brown P. 1989b. Responses of Songbirds to Glyphosate induced habitat changes on Clearcuts. The Journal of Wildlife Management, Vol. 53, No. 1 (Jan., 1989), pp. 64-71 | Summary already submitted under Part 1, point 14 on this document. |
| 13 | Sullivan, D.S. and Sullivan, T.P. 2000. Non-target impacts of the herbicide glyphosate: A compendium of references and abstracts. 5th Edition. Applied Mammal Research Institute, Summerland, British Columbia, Canada | |
| 14 | Sullivan T and Sullivan S. 2003. Vegetation management and ecosystem disturbance: impact of glyphosate herbicide on plant and animal diversity in terrestrial systems. Environ. Rev. 11: 37–59 (2003) doi: 10.1139/A03-005 | Summary already submitted under Part 1, point 15 on this document. |
| 15 | McLaughlin A and Mineau P. 1995. The impact of agricultural practices on biodiversity. Agriculture, Ecosystems and Environment 55 (1995) 201-212 | Summary already submitted under Part 1, point 13 on this document. |
| **Part 3:** **References for assessment of indirect effects via trophic interactions for aquatic organisms discussion** | | |
| 1 | Baker LF, Mudge JF, Thompson DG, Houlahan JE, Kidd KA. 2016. The combined influence of two agricultural contaminants on natural communities of phytoplankton and zooplankton. Ecotoxicology. 25:1021-32. | Study summary presented in the Appendix to Volume 3 CA on general literature review for ecotoxicology |
| 2 | Edge CB, Baker LF, Lanctôt CM, Melvin SD, Gahl MK, Kurban M, Navarro-Martín L, Kidd KA, Trudeau VL, Thompson DG, Mudge JF, Houlahan JE. 2020. Compensatory indirect effects of an herbicide on wetland communities. Sci Total Environ. 718:137254. | Summary already submitted under Part 2, point 7 on this document. |
| 3 | Rolando CA, Baillie BR, Thompson DG, K Little. 2017. The Risks Associated with Glyphosate-Based Herbicide Use in Planted Forests. Forests 8(6):208 | |
| **Part 4 :** **References for assessment of indirect effects via trophic interactions for bees discussion** | | |
| 1 | Burgett M, Fisher G. 1990. A review of the Belizean honey bee industry: Final report prepared at the request of The Belize Honey Producers Federation. Department of Entomology, Oregon State University, Corvallis, Oregon. | |
| 2 | Chamkasem N, JD Vargo. 2017. Development and independent laboratory validation of an analytical method for the direct determination of glyphosate, glufosinate, and aminomethylphosphonic acid in honey by liquid chromatography/tandem mass spectrometry. J Reg Sci 5:1-9. | |

| No. | Reference | Remarks |
|---|---|---|
| 3 | Ferguson F. 1987. Interim report. Long term effects of systemic pesticides on honey bees. The Australian Beekeeper (September issue). Pages: 49-53. | Interim report of Ferguson 1988. Study summary provided for the full report of Ferguson 1988 only. |
| | Ferguson F. 1988. Long term effects of systemic pesticides on honey bees. Bee keeping in the year 2000: Second Australian and International Beekeeping Congress, Surfers Paradise, Gold Coast, Queensland, Australia, July 21-26, 1988. Editor: John W. Rhodes. Pages: 137-141. | |
| 4 | Last G, Lewis G, G Pap. 2019. Regulatory report on the occurrence of flowering weeds in agricultural fields. Sponsored by the European Crop Protection Association. ERM report number (submitted upon request) | |
| 5 | Laberge L, Legris J, Couture G. 1997. Glyphosate residues in pollen and honey after applications in an agro-forest environment. Draft Report Ministere des Ressources naturelles du Quebec, Direction de lenvironement forestier Quebec. | The applicant indicated that this reference is an error. The correct reference should be : Laberge L, Couture G, Legris J, Langevin R. 1995. Evaluation des impacts du glyphosate utilisé dans le milieu forestier.<br>No summary was provided as not available at time of submission.<br>Data gap : Applicant to provide the full text paper and study summary of Laberge L, Couture G, Legris J, Langevin R. 1995 (Evaluation des impacts du glyphosate utiliséze dans le milieu forestier.) together with its english certified translation. |
| 6 | Motta EVS, Raymann K, Moran NA. 2018. Glyphosate perturbs the gut microbiota of honey bees. Proc Natl Acad Sci U S A. 115:10305-10310. | Study summary presented in the Appendix to Volume 3 CA on general literature review for ecotoxicology |
| 7 | [redacted]. 2020. Residues of Glyphosate in Food, Feed and Urine. Bayer Internal Report number TRR0000298. | |
| **Part 5 : <u>References for assessment of indirect effects via trophic interactions for non-target arthropods discussion</u>** | | |
| 1 | Guiseppe KFL, Drummond FA, Stubbs C, Woods S. 2006. The Use of Glyphosate Herbicides in Managed Forest Ecosystems and their Effects on Non-Target Organisms with Particular Reference to Ants as Bioindicators; Maine Agricultural and Forest Experiment Station Technical Bulletin 192; Maine Agricultural and Forest Experiment Station, University of Maine: Orono, ME, USA, p. 51. | Summary already submitted under Part 1, point 9 on this document. |
| 2 | Sullivan TP, Sullivan DS. 2003. Vegetation management and ecosystem disturbance: impact of glyphosate herbicides on plant and animal diversity in terrestrial systems. Env Rev 11:37-59. | Summary already submitted under Part 1, point 15 on this document. |

| No. | Reference | Remarks |
|---|---|---|
| 3 | Warburton DB, Klimstra WD. 1984. Wildlife use of no-till and conventionally tilled corn fields. Journal of Soil and Water Conservation. 39:327-330. | |
| **Part 6 : <u>References for assessment of indirect effects via trophic interactions for soil organisms discussion</u>** | | |
| 1 | Cerdeira A, Duke SO. 2010. Effects of glyphosate-resistant crop cultivation on soil and water quality. GM Crops 1:1-9. | |
| 2 | Duke SO, Lydon J, Koskinen WC, Moorman TB, Chaney RL, Hammerschmidt R. 2012. Glyphosate effects on plant mineral nutrition, crop rhizosphere microbiota, and plant disease in glyphosate-resistant crops. J Agric Food Chem. 2012 Oct 24;60(42):10375-97. | |
| 3 | Knox OCG, Nehl DB, Mor T, Ronerts GN, Gupta V. 2008. Genetically modified cotton has no effect on arbuscular mycorrhizal colonization of roots. Field Crops Res. 109:57–60. | |
| 4 | Silva V, Montanarella L, Jones A, Fernández-Ugalde O, Mol HGJ, Ritsema CJ, Giessen V. 2018. Distribution of glyphosate and aminomethylphosphonic acid (AMPA) in agricultural topsoils of the European Union. Sci Tot Environ. 621:1352-1359 | Study summary available in Volume 3 CA B.8.5.2. |
| 5 | Sullivan DS, TP Sullivan. 2000. Non-target impacts of the herbicide glyphosate: A compendium of references and abstracts. 5th Edition. Applied Mammal Research Institute, Summerland, British Columbia, Canada. | Summary already submitted under Part 2, point 13 on this document. |
| 6 | Powell JR, Campbell RG, Dunfield KE, Gulden, RH, Hart MM, Levy-Booth DJ, Klironomos JN, Pauls KP, Swanton CJ, Trevors JT, Antunes PM. 2009. Effect of glyphosate on the tripartite symbiosis formed by Glomus intraradices, Bradyrhizobium japonicum, and genetically modified soybean. Appl. Soil Ecol. 41:128–136. | |
| 7 | Lu GH, Hua XM, Cheng J, Zhu YL, Wang GH, Pang YJ, Yang RW, Zhang L, Shou H, Wang XM, Qi J, Yang YH. 2018. Impact of Glyphosate on the Rhizosphere Microbial Communities of An EPSPSTransgenic Soybean Line ZUTS31 by Metagenome Sequencing. Curr Genomics. 19:36-49. | |
| 8 | Savin MC, Purcell LC, Daigh A, Manfredini A. 2009. Response of mycorrhizal infection to glyphosate applications and P fertilization in glyphosate-tolerant soybean, maize and cotton. J. Plant Nutrit. 32:1702–1717. | |
| **Part 7 : <u>References for assessment of indirect effects via trophic interactions for non-target terrestrial plants organisms discussion</u>** | | |
| 1 | Koning LA, de Mol F, Gerowitt, B. 2019: Effects of management by glyphosate or tillage on the weed vegetation in a field experiment. Soil and Tillage Research 186: 79-86. | |
| 2 | Colbach N. et. al., 2018. Landsharing vs landsparing: how to reconcile crop production and biodiversity? A simulation study focusing on weed impacts. Agriculture, Ecosystems and Environment (2018), Vol. 251, pp. 203 217 | |

## PART 1: REFERENCES FOR ASSESSMENT OF INDIRECT EFFECTS VIA TROPHIC INTERACTION FOR BIRDS DISCUSSION

### 1. Boatman *et al.*, 2004

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Boatman N, Brickle N, Hart J, Milson. J, Morris A., Murray A, Murray K and Robertson P. |
| **Year** | 2004 |
| **Title** | Evidence for the indirect effects of pesticides on farmland birds |
| **Document No** | IBIS - International Journal of avian science<br>Volume 146, Issue S2, November 2004 pages 131-143 |
| **Short description of literature article** | Indirect effects of pesticides, operating through the food chain, have been proposed as a possible causal factor in the decline of farmland bird species. To demonstrate such a link, evidence is needed of (1) an effect of food abundance on breeding performance or survival; (2) an effect of breeding performance or survival on population change; and (3) pesticide effects on food resources, sufficient to reduce breeding performance or survival, and hence to affect the rate of population change. Evidence under all three categories is only available for one species, the Grey Partridge *Perdix perdix*, although data showing effects of pesticides on food resources and relationships between food resources and breeding performance are also available for some other species. This paper reports on recent work investigating the effects of pesticides on Yellowhammer *Emberiza citrinella* and Skylark *Alauda arvensis* during the breeding season. No significant effects of pesticides were recorded on Skylark chick condition or growth rate, but sample sizes were small. Invertebrate food abundance affected chick condition (Skylark) and the number of chicks fledging (Yellowhammer and Corn Bunting *Miliaria calandra*; relationship for the latter derived from re-analysis of data from an earlier study). |
| **Short description of findings** | The data presented in the article, provides evidence that indirect effects of pesticides on bird species do occur, although for some species, unequivocal evidence is only available for effects of insecticides. The authors state that it remains unclear how important indirect effects of pesticides are in relation to other factors affecting populations of farmland birds, suggesting further work is required to investigate the likely impact of the results presented here at the population level. The author also suggests that indirect effects of pesticides form part of a suite of causal factors likely to be implicated in the declines of farmland bird species. |
| **Relevance of this literature article to the submission** | This literature article is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | Indirect effects of pesticides in general on birds (e.g. brood reduction, chick condition and number of chicks fledging) are documented for several species, most comprehensive data being on the Grey Partridge. However, the importance of pesticides in relation to other stressors affecting farmland birds is still unclear.<br>The study does not include results specific for glyphosate and/or the representative uses for this risk assessment. |

## 2. Bright *et al*., 2008

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Bright J, Morris A and Winspear R |
| **Year** | 2008 |
| **Title** | A review of Indirect Effects of Pesticides on Birds and mitigating land-management practices |
| **Document No** | RSPB Research Report No. 28 |
| **Short description of literature article** | This report is a review of bird population status in the UK, conducted by the RSPB (Royal Society for the Protection of Birds) for the Pesticide Safety Directorate in the UK. The report reviews the status of bird species considered to be most at risk from exposure to pesticides in farmland environments.<br>The report - in addition to providing a status on bird populations in UK farmland, also discusses three ways in which pesticides may affect bird species; 1. Reduced invertebrate abundance due to direct effects of insecticides; 2. Reduced invertebrate abundance due to indirect effects of herbicides via loss of host plants and 3. Reduced abundance of weed seeds due to direct effects of herbicides. The article also discusses schemes used within the UK where farmers implement management techniques that are beneficial to biodiversity. |
| **Short description of findings** | A review of the population status of various UK bird species is presented in this report. This includes an assessment on the impact of land management schemes and provides recommendations that include a number of ways for further reducing the effects of pesticides on farmland birds, with many being examples of Integrated Crop Management.<br>Many species of farmland bird have shown huge declines in numbers and range over the past four decades. These have been linked to agricultural intensification, which has taken the form of a suite of changes in farmland practice. One of these is increased use of pesticides. Concern has switched from the direct lethal or sublethal effects of pesticides on birds, such as declines in sparrowhawks due to decreased eggshell thickness resulting from use of organochlorine insecticide seed treatments in the 1950s and 1960s, to the indirect effects of pesticides. These indirect effects act predominantly via reduction in food supplies. As well as reducing numbers of target invertebrates and weeds, insecticides and herbicides can reduce availability of non-target and beneficial species. This is predominantly due to insecticide use causing decreased abundance of insect food, and herbicide use causing decreased weed seed abundance, and decreased insect abundance, due to loss of host plants. |
| **Relevance of this literature article to the submission** | This report is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | Agricultural intensification has led to large declines in the abundance and range of farmland species over the last four decades. Three main |

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| | pathways leading to pesticide-induced indirect effects on birds are discussed: direct and indirect reduction of invertebrate abundance and direct reduction in weed seeds.<br>The study includes results from the impact on biodiversity from pesticide use in general, and not specifically from glyphosate and/or the representative uses for this risk assessment. |

### 3. Burfield, 2005

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| **Data point** | KCP 10.1.2 |
| **Author** | Burfield, I., (Author of a Chapter within the following book edited by Bota, G., Morales M.B., Manosa, S. and Camprodon, J.) |
| **Year** | 2005 |
| **Title** | Ecology and Conservation of Steppe-land birds |
| **Document No** | Published |
| **Short description of literature article** | The book contains a chapter discussing the distribution of farmland bird species. The link to the book is below.<br>https://www.lynxeds.com/product/ecology-and-conservation-of-steppe-land-birds/<br>The GRG have not been unable to obtain a copy of this document and secure the copyrights in time for this submission. The book will be obtained and the copyright resolved and will be available if requested.<br><br>The book was used to illustrate the distribution of birds on agricultural land within the biodiversity section of the risk assessment. |
| **Short description of findings** | There are no specific data or findings presented in the document with respect to effects of glyphosate on birds. This document is used to illustrate and support the distribution of bird species in the EU. |
| **Relevance of this literature article to the submission** | This literature article is cited in the indirect effects via trophic interaction and biodiversity section of the ecotoxicology dossier section (M-CP). |
| **RMS comments and conclusion** | This book illustrates the distribution of birds in steppic (agricultural) habitats. Of the 65 birds identified as priority species for these habitats, 83% had an unfavourable conservation status in Europe.<br>The study does not include results specific for glyphosate and/or the representative uses for this risk assessment. |

## 4. Campbell and Cooke, 1997

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Campbell, L.H and Cooke A.S. (eds.). |
| **Year** | 1997 |
| **Title** | The Indirect effects of pesticides on birds |
| **Document No** | This is an official JNCC (Joint Nature Conservation Committee) extended summary of Campbell, L.H. RSPB., Avery, M.I., Donald, P.,, Evans, A.D., Green, R.E., and Wilson, J.D. 1997 A review of the indirect effects of pesticides on birds (JNCC Report No. 227) |
| **Short description of literature article** | The article presents a review of the indirect effects of pesticides on farmland birds, commissioned by the Dept. of the Environment and English Nature in 1995. Based on reviewing the current knowledge of the indirect effects of pesticides on farmland birds in the UK and to establish the significance of their impacts and make recommendations for further work, to discuss ameliorative management and to outline policy options. The review focuses on cereal crops and on lowland farmland breeding birds in the UK. The review describes trends in farmland bird populations, the diet of farmland birds, including the trends in abundance of farmland bird dietary items; the trends in pesticide use and results of detailed studies into the ecology of farmland birds. Ultimately, the article draws conclusions on the indirect impact of pesticides on farmland bird species. |
| **Short description of findings** | The Authors conclude that there were insufficient data available to assess which features of agricultural activity or change are most important in determining the abundance of bird food and birds. Noted a critical lack of detailed data on the diet of the majority of farmland bird species. There was a shortage of data on trends on abundance of the range of invertebrate and plants species upon which farmland bird species feed.<br>The Authors do however conclude that there is convincing evidence that a range of both scarce and more widespread farmland breeding birds were in decline, with evidence of short-term declines in many invertebrate types and plants on which these bird species feed. The was also evidence to suggest that the declines are in part attributable to pesticides with temporal associations between pesticide (% cropped area sprayed) use and the periods of rapid decline of bird species.<br>The Authors also highlighted that organic farms hold higher densities of breeding and wintering birds, that grey partridge hick survival increases with insect abundance and selective pesticide use.<br>They also acknowledge that factors other than pesticides have also contributed to farmland bird species decline, with only the effects on one species being directly attributed to pesticide use (grey partridge). |
| **Relevance of this literature article to the submission** | This literature article is used to provide additional information on indirect effects and biodiversity. |

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| **RMS comments and conclusion** | Identifying the most important factor causing declines in the abundance of bird feed and birds in agricultural areas is not possible due to limited data, with the exception of the grey partridge, whose decline was directly attributed to pesticide use. Nonetheless, short-term declines in farmland bird feed (invertebrates and plants) were linked to pesticide use (% crop area sprayed).<br>The RMS notes that there are other statements in the paper that give a bit more weight to the role of pesticides (pg 9):<br>*"Although the observed long-term declines in invertebrates and plants have taken place during a period of considerable change in agricultural practices and could have been caused by a range of factors, the scale of the short-term effects of pesticides suggest that they are likely to be one of the more important factors influencing the gross abundance of potential bird food items."*<br>However, the study does not include results specific for glyphosate and/or the representative uses for this risk assessment. |

## 5. Cunningham *et al.*, 2005

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Cunningham H, Bradbury R, Chaney K and Wilcox A. |
| **Year** | 2005 |
| **Title** | Effect of non-inversion tillage on field usage by UK farmland birds in winter. |
| **Document No** | Bird Study (2005) 52, 173–179 |
| **Short description of literature article** | This paper describes a study where the cereal crop establishment methods of non-inversion tillage and ploughing, were compared by assessing their use by several guilds of wintering farmland bird species. Commercial cereal fields were censused over the winter months of 2000 to 2003, using whole field count methodologies. Multi-variate statistical methods were then applied to the count data to assess the difference in bird use between the field, whilst controlling for the effects of a variety of other variables. |
| **Short description of findings** | In late winter, Skylarks *Alauda arvensis*, granivorous passerines and gamebirds occupied a greater proportion of fields established by non-inversion tillage than conventional tillage. There were also more species of granivorous passerines in non-inversion tillage fields. As well as documented benefits for resource protection, such as soil and water conservation, non-inversion tillage methods (*i.e.*, methods which use more herbicides than conventional ploughing, thus getting rid of weeds and leaving more seeds available at the surface of the ground) appear to enhance suitability of winter cereal fields for foraging birds. |
| **Relevance of this literature article to the submission** | This paper is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | The study shows that skylarks, granivorous passerines and gamebirds occupied a greater proportion of fields established by non-inversion tillage methods than conventional tillage. Also, more species of granivorous passerines were found in non-inversion tillage fields. Hence, it can be concluded that the intended use of glyphosate in non-inversion tillage systems may influence the species composition in the field. |

## 6. DEFRA, 2005

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Department of the Environment, Food and Rural Affairs (DEFRA) |
| **Year** | 2006 |
| **Title** | Assessing the Indirect Effects of Pesticides on Birds |
| **Document No** | PN0925 |
| **Short description of literature article** | The aim of the study was to investigate the possible effects of pesticides on the food supply of farmland birds and their indirect effects on the demography of individual bird species, and to develop a framework for a risk assessment of the indirect effects of pesticides to aid the registration process. Three possible Type effects were established. Type 1 considered direct effects of insecticides on insect species; Type 2 considered effects of herbicides on insect plant hosts and Type 3 considers the impact of herbicides on weed species, which provide green matter or seeds for herbivorous and seed eating species. The overall project had four principal objectives:<br>1. To develop a causal framework for the assessment of indirect effects of pesticides on farmland birds. Also, to identify those species most at risk from indirect effects and the mechanisms by which they may be affected (Work undertaken by Royal Society for the Protection of Birds (RSPB), University of Oxford, GCT, CSL).<br>2. To conduct large-scale replicated field experiments to manipulate food resources available to farmland birds to demonstrate the magnitude of any indirect effects (Work undertaken by GCT, CSL).<br>3. To examine the current risk assessment based regulatory procedures in relation to indirect effects of pesticides (Work undertaken by CSL).<br>4. To propose suitable risk management practices that may reduce indirect effects of pesticides on birds (Work undertaken by CSL).<br><br>The overall report presents separate reports covering each of the principal objectives. |
| **Short description of findings** | A framework to determine the relationship between insect abundance and effects on bird populations was developed.<br>A large-scale field experiment was undertaken to assess the relative magnitude of the Type 1 and Type 3 class effects on bird populations.<br>The magnitude and impact of mitigation and compensation measures were considered including buffer zones, where pesticidal inputs are reduced. |
| **Relevance of this literature article to the submission** | This paper is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | A large-scale field experiment was carried out for testing Type 1 and 3 indirect effects by altering the food supplies directly (increasing seed densities) and by decreasing arthropod densities using an insecticide. The results show reductions in breeding productivity of yellowhammers due to depletion of arthropod food sources following the use of pesticides. In addition, a model was developed for assessing |

risks to yellowhammers and pesticide mitigation measures are discussed.

No specific results from the use of glyphosate were presented in the report, except for a statement; "… *most rotational set-aside is sprayed with glyphosate, in April or May, to prevent weeds from seeding and to clear the ground prior to cultivations for the following crop. The destruction of the vegetation early in the nesting season renders nesting birds vulnerable to predation, and also reduces the density of invertebrates by removing their habitat and food plants."*

### 7. Donald *et al*., 2006

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Donald P, Sanderson F, Burfield I and van Bommel F |
| **Year** | 2006 |
| **Title** | Further evidence of continent-wide impacts of agricultural intensification on European farmland birds, 1990–2000 |
| **Document No** | Agriculture, Ecosystems & Environment, Volume 116, Issues 3–4, September 2006, Pages 189-196 |
| **Short description of literature article** | Between 1990 and 2000, farmland birds showed a significant decline across Europe, a trend not shared by bird assemblages of other habitats over the same period. Mean trends for each farmland species in the period 1990–2000 were positively correlated with trends over the period 1970–1990, and there was little change in population trajectory for most species over the 30-year period. Of the 58 species classed by an independent assessment as being primarily birds of farmland, 41 showed negative overall mean trends across Europe in 1990–2000, 19 of them significant. There was a significant negative correlation between mean national trends of all farmland species and indices of national agricultural intensity. This relationship strengthened when the 19 declining species were considered alone and was not apparent when only non-declining species were considered. Population trends of terrestrial non-farmland bird species over the same period were unrelated to agricultural intensity. Trends in farmland bird populations were independent of the proportion of farmland under agricultural environment prescriptions. The results support earlier evidence that population trends of farmland birds across Europe can be predicted from gross national agricultural statistics. Substantial changes in agricultural policy, particularly the removal of economic incentives that lead to agricultural intensification, are required if 2010 targets for halting loss of biodiversity are to be met in an enlarged European Union. |
| **Short description of findings** | The results provide evidence that, previously documented declines in farmland bird populations across Europe for the period 1970–1990 continued between 1990 and 2000, so a pattern of decline is detectable in farmland bird populations for at least 30 years. This pattern of long-term decline was not apparent in bird assemblages of other habitats, suggesting that declines in farmland bird populations were driven by factors specific to that habitat, rather than being part of a general decline in bird populations across the continent. The strong correlation between population trends of declining farmland species and certain indices of agricultural intensity, and the lack of such a correlation with non-declining farmland species or non-farmland species, suggested agricultural intensification as a plausible and likely causal factor. However, not all farmland species exhibited patterns of population decline. Of the 58 species included in the analyses, 17 exhibited positive overall mean trends, eight of them significant. Mean country trends of the eight increasing species were positively correlated with a number of indices of agricultural intensity, suggesting that agricultural intensification is not |

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| | universally deleterious and that a small number of bird species might benefit. |
| **Relevance of this literature article to the submission** | This paper is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | A significant decline in 19 out of the 58 species of farmland birds occurred between 1990–2000, this trend being negatively correlated to indices of agricultural intensity. However, these indices were also positively correlated to 8 out of the 58 species that had positive trends, suggesting that these few species benefit from agricultural intensification.<br>The study does not include results specific for glyphosate and/or the representative uses for this risk assessment. |

## 8. Easton and Martin, 1998

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Easton W. and Martin K. |
| **Year** | 1998 |
| **Title** | The effect of vegetation management on breeding bird communities in British Columbia. |
| **Document No** | Ecological Applications 8 (4), 1092-1103 |
| **Short description of literature article** | This paper describes the impact of vegetation management on breeding bird communities in conifer plantations over a four-year period. The study compared manual thinning with manual thinning plus herbicide (glyphosate) application. The study was conducted in British Columbia in Canada. The control and two treatments were each replicated three times. Plot sizes ranged between 22 and 47 hectares. Pre-treatment data were collected during two months prior to treatment applications. The treatments reduced the volume of deciduous trees by 90-96%. Post treatment data were collected annually between May and July each year between 1993–1995. The herbicide treated sites remained depauperate of deciduous vegetation, while the manually thinned sites experienced regrowth of deciduous trees.<br>Birds were surveyed using point counts at permanently established stations, with the number of stations varying according to plot size. All stations were at least 75 meters from the edge of the plot and were at least 150 m apart. Observations were undertaken from each station at least twice at different times in the morning, with six different observed conducting the surveys across all sites. Nests were found by thorough searching of the plots in the final two post treatment years (1994 and 1995). Plots were monitored for nest every 3-4 d for the number of eggs and nestlings. Nests were considered successful if at least one offspring fledged.<br>Species richness, abundance, evenness, turnover, assemblages, species and nesting success were all statistically analysed. |
| **Short description of findings** | A number of bird species declined (up to 25% decrease for single species at 3 years after treatment), while the total number of individuals increased, and common species dominated after herbicide treatment. The chance of survival of an average nest was estimated at 17% in herbicide-treated areas (30% in control and 48% in manually thinned areas). Number of species, total number of individuals, and evenness increased after manual treatment. Turnover of bird species was highest in the herbicide-treated areas and lowest in control areas. Residents, short-distance migrants, ground gleaners, and conifer nesters increased significantly after herbicide treatment. Deciduous nesters and foliage gleaners increased in abundance (non-significantly) in control and manually thinned areas. Warbling Vireos (*Vireo gilvus*), which are deciduous specialists, declined in areas treated with herbicide and may be particularly susceptible to glyphosate application. (Nashville warblers (*Vermivora ruficapilla*) disappeared from glyphosate treated areas). Although treated areas exhibited similar increases in the total number of birds, nesting |

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| | success of open-cup nesting species was significantly lower in the herbicide-treated than in manually thinned areas. The author suggests that habitat variability may be a mechanism for producing nested subset structure of bird community composition. Overall, the composition of bird communities became more homogeneous after herbicide treatment, and it showed little change after manual thinning. |
| **Relevance of this literature article to the submission** | This paper is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | Common bird species increased in abundance, whereas deciduous specialists declined (Warbling vireos) or even disappeared (Nashville warblers) from glyphosate-treated areas. Here also the nesting success of open-cup nesting species was reduced.<br>The observation that the total number of individuals increased in the treated fields might appear as a positive effect, but see discussion on page 9:<br>"*the overall abundance of birds increased despite poor nesting success (only 8%). For example, Dusky Flycatchers increased although they had lower nest survival. This implies that trends in abundance may be decoupled from trends in productivity, a characteristic of ''source–sink'' population regulation (Brawn and Robinson 1996) (…)*<br>*As the numbers of Dusky Flycatchers was high in the study area, they may have opportunistically inhabited the poorer quality habitat of the herbicide-sprayed areas."*<br>Overall, the results from this study show that weed management with glyphosate may have a negative impact on bird biodiversity. |

## 9. Guiseppe *et al*., 2006

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Kerry F.L. Guiseppe, Francis A. Drummond, Constance Stubbs, and Stephen Woods |
| **Year** | 2006 |
| **Title** | TB192: The Use of Glyphosate Herbicides in Managed Forest Ecosystems and Their Effects on Non-target Organisms with Particular Reference to Ants as Bioindicators |
| **Document No** | University of Maine Agricultural and Forest Experiment Station Technical Bulletin 192 |
| **Short description of literature article** | This publication reviews and synthesizes the results of many research studies designed to elucidate the ecological effects of the herbicide glyphosate used in forested landscapes, focusing on studies that had relevance to north temperate forest ecosystems and selected published investigations that cover a wide range of faunal and floral taxa that might be exposed to herbicides during applications.<br>This review was initiated as part of a research project conducted by Drs. Woods, Drummond, and Stubbs, at the University of Maine, to elucidate the effects of the use of glyphosate on insect communities associated with the Maine forested landscape.<br>The review includes a considered review of the impacts of glyphosate on flora and fauna taxa groups, but focuses on the forest ant species as bioindicators.<br>The field research focused primarily on the Hymenoptera (ants, bees, and wasps). Where ants are one of the dominant groups of animals (with several taxa considered keystone species) associated with forested landscapes in North America, and they are associated with many important ecosystem processes such as soil nitrogen cycling, soil aeration, predation, and seed dispersal. Therefore, we incorporated into this general review of the impacts of glyphosate on resident fauna and flora the potential role that ants might play as bioindicators, that is, as measures of ecosystem disturbance, particularly of the effects of herbicide use in forested landscapes. |
| **Short description of findings** | In general, the application of glyphosate in forest landscapes to suppress the growth of non-crop deciduous shrubs and trees appears to have limited immediate direct effects on non-target fauna. Long-term negative effects of glyphosate also appear to be limited in scope, although some species are affected. These long-term effects on the animals are most likely caused indirectly by the altered plant community and levels of light penetration. Some of the documented effects of the use of herbicides for site preparation or release are contrary to any hypothesized negative effect of the herbicide on resident fauna. In fact, some studies have shown that the density of non-target species increases in herbicide-treated clearcuts relative to non-treated clearcuts. The potential use of ants as indicators of herbicide induced environmental change in forests is due to several characteristics, because ants are sensitive to soil, temperature and moisture changes so as potential indicators of environmental change in agroecosystems, they do have some value, however their |

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| | association with specific levels of disturbance in agroecosystems is unknown. |
| **Relevance of this literature article to the submission** | This paper is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | This review identified transient declines and changes in species composition of birds caused indirectly by herbicides. Similarly, indirect effects on mammals were generally short-term.<br>Studies on amphibians showed no effects of herbicides, with the exception of one study that reported 100% mortality of tadpoles from 3 species exposed to Roundup® (POEA formulation). This type of formulation is known to be very toxic to amphibians but is not part of the application for renewal of glyphosate. Therefore, these findings are not considered relevant for this evaluation. |

**10. Guynn *et al*., 2004**

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Guynn D, Guynn S, Wigley T and Miller D |
| **Year** | 2004 |
| **Title** | Herbicides and forest biodiversity—what do we know and where do we go from here? |
| **Document No** | Wildlife Society Bulletin 32(4):1085-1092 |
| **Short description of literature article** | This article is a short review of the different management practices use of herbicides in forestry. The direct and indirect impact of habitat alteration on biodiversity is discussed and how public opinion influences the use of herbicides in forestry applications, despite effects being varied and generally short-term. |
| **Short description of findings** | The author proposed approaches for future research in this area, that should address landscape and site-specific issues, be based on rigorous experimental approaches and be relevant to public concerns. |
| **Relevance of this literature article to the submission** | Within the Biodiversity Assessment Report (Document KCA 8.7_001) [cited in the risk assessments presented in 'M-CP Section 10 Ecotoxicology'] on page 36, there is a sentence cited as being attributable to this paper. This relates to changes in bird community composition following glyphosate application in forestry. The specific sentence reads: '…*Where changes were assessed against untreated control sites to differentiate the effects of glyphosate from other background environmental factors such as recovery trajectory following tree harvest and show similar responses to other herbicides commonly used in managed forests* (Guynn, 2004).' The Guynn (2004) paper is a secondary source of information, and therefore, for completeness, the original reference linked to information presented in this sentence is for a 5-year bird study conducted in Canada by Mackinnon & Freedman (1993). This study has now been summarised and the full text article has been submitted. The original source reference is:<br>Mackinnon D.S and Freedman B (1993) Effects of silvicultural use of the herbicide glyphosate on breeding birds of regenerating clearcuts in Nova Scotia, Canada. Journal of Applied Ecology 30:395–406.<br>The Journal paper and a summary of the article are submitted. |
| **RMS comments and conclusion** | The study generally concludes that use of herbicides (glyphosate) in forestry improves productivity, but nonetheless raises societal concerns. However, the response of wildlife to herbicide-induced habitat alteration is highly variable and mostly temporary. |

## 11. Jahn *et al*., 2013

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Jahn T and Höker H |
| **Year** | 2014 |
| **Title** | Protection of biodiversity of free-living birds and mammals in respect of the effects of pesticides |
| **Document No** | Environmental Research of the Federal Ministry of the Environment, Nature Conservation and Nuclear Safety<br>Project no. (FKZ) 3710 63 411; Report no. (UBA-fb) 001830<br>Report available on the UBA (German Federal Environment Agency (Umweltbundesamt)) website;<br>https://www.umweltbundesamt.de/sites/default/files/medien/378/publikationen/texte_30_2014_protection_of_biodiversity.pdf |
| **Short description of literature article** | The UBA present an analysis of risk management measures with respect to efficiency and acceptance by farmers and authorities of implementing measures to protect biodiversity on farmland.<br>The UBA state, that at present (2014 - date of publication) agri-environmental schemes aiming to compensate for the negative effects of modern agriculture cover only about 0.5 % of the arable land in Germany.<br>The UBA describe the development of an index of pesticide sensitivity for farmland birds and mammals in Germany. According to expert opinion pesticides are among the major causes for population declines of farmland birds and provide supporting evidence for this view.<br>The document describes a scheme of umbrella species to simplify risk management, outlining strategies for implementing an effective risk management and provide estimated costs associated with their implementation. A levy on PPPs targeted to the implementation of a region-specific risk management would be practicable at relatively low costs. |
| **Short description of findings** | The report by the UBA contains a review of habitat management proposals and anticipated costs associated with their implementation in Germany. The document also presents a review of population trends in agricultural landscapes, including specific information on habitat selection and crop-specific occurrence of farmland bird species. Whilst the report does not provide specific data that may inform on endpoint selection for the presented risk assessment, the document has been cited in the trophic interaction section of the bird risk assessment, as being a relevant review document that includes information relatable to the discussion on direct and indirect effects associated with herbicide use in agricultural landscapes. |
| **Relevance of this literature article to the submission** | This report is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | The report reviews many studies on birds, mammals, etc, including glyphosate-specific research. The RMS' review was limited to the sections dealing with effects of *herbicides* on biodiversity. Based on expert judgement, the authors classified 30% of the bird and 45% of the |

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| | mammal species included in this review as being highly negatively impacted by herbicides (including glyphosate). |

## 12. Marshall *et al*., 2001

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Marshall J, Brown V, Boatman N, Lutman P and Squire G. |
| **Year** | 2001 |
| **Title** | The impact of herbicides on weed abundance and biodiversity |
| **Document No** | PN0940 |
| **Short description of literature article** | This publication reviews existing data on farmland bird species, invertebrates, weed assemblages, weed seed banks in the agricultural environment and considers the influence of cropping practices, habitat fragmentation and loss and herbicide use in the UK over the 25-30 years prior to the date of publication.<br>The links between invertebrates and a range of weed species was also considered, with some species hosting rare and also pest species. |
| **Short description of findings** | The data indicate that herbicides, by controlling weeds and modifying abundance and species assemblages, have impacted on wildlife in arable land. These non-target effects need to be considered for regulatory reasons, particularly with the requirements under EU Regulation 91/414. With such dramatic changes in biodiversity, there are also calls for more sustainable production methods. The challenge will be to grow crops and maintain an appropriate population of weed species to support farmland wildlife. Under horticultural conditions, this may be difficult, in terms of crop quality protection. Nevertheless, under arable and horticultural production, there may be opportunities to develop sacrifice areas, such as conservation headlands, or to develop much greater selectivity of herbicide action, either through selective chemistry or application or a combination of these. |
| **Relevance of this literature article to the submission** | This publication is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | This is a review report, and the RMS has focused on the sections dealing with effects of herbicides on biodiversity. The review shows that herbicides cause changes in vegetation and thus indirectly impact birds and invertebrates.<br>The results are not specific to glyphosate, but to herbicides in general. |

**13. McLaughlin and Mineau, 1995**

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | McLaughlin A and Mineau P. |
| **Year** | 1995 |
| **Title** | The impact of agricultural practices on biodiversity |
| **Document No** | Agriculture, Ecosystems and Environment 55 (1995) 201-212 |
| **Short description of literature article** | Agricultural activities such as tillage, drainage, intercropping, rotation, grazing and extensive usage of pesticides and fertilizers have significant implications for wild species of flora and fauna. Species capable of adapting to the agricultural landscape may be limited directly by the disturbance regimes of grazing, planting and harvesting, and indirectly by the abundance of plant and insect foods available. Some management techniques, such as drainage, create such fundamental habitat changes that there are significant shifts in species composition. This paper considers the relative merits of conventional versus conservation tillage (reduced, or no-till) farming, and reviews the benefits of rest-rotation grazing, crop rotation and intercropping in terms of maintaining wild species populations.<br>There are several undesirable environmental impacts associated with fertilizer and pesticide usage, and this paper attempts to provide an account of the ways in which these inputs impact on biodiversity at various levels including plant, invertebrate, and vertebrate groups. Factors which are considered include the mobility, trophic interactions, persistence, and spectrum of toxicity for various pesticides. The ecological virtues of organic and inorganic fertilizers are compared, and the problems arising from excessive use of fertilizer are discussed. |
| **Short description of findings** | The findings in this review indicate that chemical fertilizer loadings must be better budgeted to not exceed local needs, and that pesticide inputs should be reduced to a minimum. The types and regimes of disturbance due to mechanical operations associated with agricultural activity may also be modified to help reduce negative impacts on groups of species, such as birds. For those plant and insect species which need to be controlled for agronomic reasons, the population decreases brought about by disturbance regimes may be desirable as a form of pest management. The prevalence of agriculture over such a large portion of the Canadian landscape means that it is important that we find solutions to conflicts that arise between agriculture and wild species. |
| **Relevance of this literature article to the submission** | This paper is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | This is a review paper, and the RMS has focused on the sections dealing with effects of herbicides on biodiversity. The results indicate that conservation tillage reduces the risk of accidental mortality of small mammals and promotes greater abundance of waterfowl, compared to ploughed fields.<br>The results are not specific to glyphosate, but to herbicides in general. |

**14. Santillo *et al*., 1989b**

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Santillo D, Leslie D and Brown P |
| **Year** | 1989b |
| **Title** | Responses of Songbirds to Glyphosate induced habitat changes on Clearcuts. |
| **Document No** | The Journal of Wildlife Management, Vol. 53, No. 1 (Jan., 1989), pp. 64-71 |
| **Short description of literature article** | The authors examined breeding bird populations and habitats on glyphosate (Roundup) treated and untreated clearcuts in Northcentral Maine.<br>Six study sites were used characteristic of areas of traditional herbicide use in the region, comprising 4-5 year-old clear cuts with suppressed softwood regeneration. The sites ranged between 31 and 62 ha in size. Two were previously treated with herbicide (glyphosate) and 4 were untreated clearcuts. Within 1 year of the study start, 2 untreated clearcut sites were treated with herbicide, effectively giving a 1, 2 and 3-year post treatment growth potential opportunity across the six sites.<br>Breeding birds were censused using the spot map method and delineated territories according to standards of the International Bird Census Committee (1970).<br>One 10-ha plot was established within all of the plots. Five were approximately square (300.3 x 333m), with the 6$^{th}$ being rectangular (250 x 400m). Each plot was censused more than 9 times from the last week May to the first week July in both 1985 and 1986 using the same observers on all occasions Census was conducted at the same time of day on each occasion with the same 2 observers used for both 2 years of the study. Census data were supplemented using nest location data and perching data.<br>Vegetation across all sites was monitored within each of the bird census plots within the clearcut sites, with all forb and shrub species recorded and the coverage visually estimated. The density and height of the vegetation was also established. Differences in vegetation between treated and untreated sites and across the years was analysed. The relationship between habitat parameters and densities of individual bird species, of all birds and of 2 distinct foraging groups (insectivores and omnivores) was examined.<br>The percent changes in bird densities on treated sites were standardized for general population fluctuations and other year to year biases by adjusting for changes in densities on controls. The ratio of treatment: control densities in 1985 was compared to the same ratio in 1986 to compute percent change. Before and after sites were compared to their corresponding controls |
| **Short description of findings** | Total numbers of birds, common yellowthroats (*Geothlypis trichas*), Lincoln's sparrows (*Melospiza lincolnii*), and alder flycatchers (*Empidonax alnorum*) were less abundant ($P < 0.05$) on treated clearcuts than on untreated clearcuts. |

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| | Songbird densities were correlated with habitat complexity, especially hardwood regeneration, foliage height diversity (FHD), and vegetation height.<br>Leaving untreated patches of vegetation and staggering herbicide treatments on large clearcuts will maintain bird populations similar to those on untreated clearcuts. |
| **Relevance of this literature article to the submission** | This paper is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | This study indicates that total number of birds, as well as the abundance of some bird species (common yellowthroats, Lincoln's sparrows and alder flycatchers) are reduced on glyphosate-treated clearcuts. It was also shown that some vegetation management options can compensate for the negative effects of the herbicide. |

## 15. Sullivan and Sullivan, 2003

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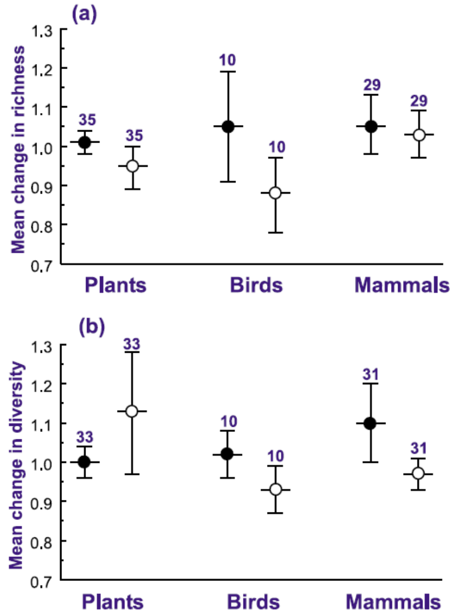
| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Sullivan T and Sullivan S. |
| **Year** | 2003 |
| **Title** | Vegetation management and ecosystem disturbance: impact of glyphosate herbicide on plant and animal diversity in terrestrial systems |
| **Document No** | Environ. Rev. 11: 37–59 (2003) doi: 10.1139/A03-005 |
| **Short description of literature article** | This paper presents a review of glyphosate's use in weed control, or vegetation management, to enhance crop production. Because of its widespread use and environmental compatibility, this review was designed to evaluate glyphosate herbicide in terms of: (i) its role as a disturbance agent and (ii) a measure of its impact on species diversity of terrestrial plants and animals. The analyses are based on 60 published studies of terrestrial plants and animals in temperate zone forest- and agricultural -ecosystems. Species richness and diversity of vascular plants was either unaffected or increased, particularly herbaceous species, in response to glyphosate. Responses of plant species in forest ecosystems differ from those in agricultural ecosystems where glyphosate is used to repeatedly reduce non-crop vegetation in most situations. Richness and diversity of songbirds appeared little affected by glyphosate-induced habitat alteration. In studies on small mammal communities and glyphosate use, none found significant reductions in species richness or diversity. As for avian responses, some small mammal species declined temporarily whereas others increased in abundance. The impact of glyphosate on large mammalian herbivores was measured by abundance of animals and food plants and by habitat use. Hares (*Lepus spp. L.*) and deer (*Odocoileus spp. Rafinesque* and *Capreolus capreolus L.*) were little affected, whereas reductions in plant biomass and related moose (*Alces alces L.*) forage and habitat use generally occur for 1–5 years after treatment. Studies on terrestrial invertebrates covered a wide range of taxa with variable responses in abundance to glyphosate treatments. The magnitude of observed changes in mean species richness and diversity of vascular plants, birds, and small mammals, from the effects of herbicide treatment, were within the mean values of natural fluctuations of these variables. The biological significance of this impact is limited to shifts in species composition based on changes in floral composition and structure of habitats. Management for a mosaic of habitats within forest and agricultural landscapes, which provide a range of conditions for plant and animal species, should help ameliorate the short-term changes in species composition accompanying vegetation management with glyphosate. |
| **Short description of findings** | The results of this review suggest that the role of glyphosate herbicide as a disturbance agent in vegetation management is tied to temporary reductions in relative abundance of herb and shrub species, at least in forest ecosystems. This ephemeral response contrasts sharply with that in most agroecosystems where non-crop vegetation is reduced or eliminated by use of glyphosate. The general lack of community-wide reductions in plant species diversity on herbicide-treated sites indicates |

that this exogenous agent of disturbance may have the least impact of all forestry and agricultural practices. Such practices range from

**Fig. 1.** Mean proportional change in (*a*) species richness and (*b*) species diversity for annual fluctuations in control units (●) and for the difference (○) between control and treatment units for communities of vascular plants (references 1, 4, 6, 7, 10, 11, and 12; see Table 1), song birds (references 13, 15, 16, 17, and 19; see Table 2), and small mammals (references 20, 21, 23, 24, 25, 10, 26, 27, 29, and 30; see Table 3). Bars represent 95% confidence interval. Sample size, the number of annual (controls) or control-treatment comparisons, is given above the upper bar of each interval.




harvesting through the many stages of regeneration, plant growth, and crop development. Changes in species richness and diversity of terrestrial plants and animals have served as our measure of the impact of glyphosate on biodiversity. The magnitude of observed changes in mean species richness and diversity of vascular plants, birds, and small mammals, from the effects of herbicide treatment in forestry, were within the mean values of natural fluctuations of these variables. The biological significance of this impact is limited to shifts in species composition based on changes in floral composition and structure of habitats and the degree to which that shift affects associated animals.

**Relevance of this literature article to the submission**

This paper is used to provide additional information on indirect effects and biodiversity.

**RMS comments and conclusion**

Overall, the abundance of songbirds which prefer deciduous cover decreased, whereas that of songbirds which prefer ‘open’ habitat or conifer cover increased after glyphosate treatment, and hence richness and diversity appeared little affected. No effect on the species diversity or richness of small mammals was identified, though reductions in abundance of specific species are documented. Larger mammals were generally less affected by glyphosate treatment; nonetheless, reduced moose activity due to decreased browse availability is reported to last 1-5 years post-treatment.

Although the overall the biological significance of the results was considered to be small (magnitude of effect within natural variation), there were several examples of negative effects on birds:

"*Of the seven published studies reported on avian responses to glyphosate treatment, three reported declines in densities of some songbird species in at least the first posttreatment year*."

"*Easton and Martin (1998) reported a decline in the number of bird species in herbicide-treated areas versus control areas immediately after treatment.*"

There are also several examples of negative effects of glyphosate on plants, briefly mentioned as 'ephemeral responses':

"*In a 7-year posttreatment study, glyphosate treatments were found to reduce significantly Vaccinium spp. but not species richness or diversity*"

"*herbicide treatments decreasing [woody] cover and affecting the floral community more than manual cutting treatments*"

"*species richness of shrubs and forbs was less on all treated clearcuts compared to untreated clearcuts*"

## 16. Traba and Morales, 2019

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Traba J and Morales M |
| **Year** | 2019 |
| **Title** | The decline of farmland birds in Spain is strongly associated to the loss of fallowland. |
| **Document No** | Scientific Reports 9: 9473 https://doi.org/10.1038/s41598-019-45854-0 |
| **Short description of literature article** | This paper analysed the change in the amount of available fallow land in Spain between 2002 and 2017, to establish the yearly rates of change in the fallow surface availability in Spain. The Authors also analysed changes in bird population numbers over the same time period, using bird yearly census data collected by volunteers and bird population abundance index for each species and year, using Trend and Indices Monitoring data (TRIM) software. From this index the annual change rate is estimated for each species. Data for the Farmland Bird Index (FBI) in Spain, a summary population index that includes information from the species classified as common farmland birds under the Spanish CBMP were used. The FBI is an official indicator of the quality of EU's agroecosystems for biodiversity, as well as of the effectiveness of agricultural environmental measures applied under European CAP.<br>The combined population index for the subset of common farmland birds that are particularly abundant in cereal farmland (Cereal Bird Index, CBI), provided also by SEO/Bird Life, was used to explore the relationship of cereal farmland specialists with the variation in fallow surface. Finally, the Authors used the little bustard population index, as an indicator of the response of fallow specialists to changes in fallow surface.<br>To estimate the relationship between bird trends (Little Bustard index, FBI and CBI) and fallow land, single linear regressions between change rates of bird population indices and the change rate in total fallow surface over the period considered were fitted. |
| **Short description of findings** | Fallow land in Spain, a country harboring the largest European populations of many endangered farmland birds, has decreased by 1.1 million ha in 15 years. The significant positive relationship between yearly change rates of the Spanish Farmland and Cereal Bird Indices (FBI and CBI) and fallow surface change highlights the adequacy of fallow land cover as an indicator of the state of farmland bird communities at country level. Moreover, the strong and positive association between the reduction in abundance of the fallow specialist little bustard and fallow surface suggests a potential causal link between these two factors.<br>These results highlight the need for a new CAP that guarantees the maintenance of fallow land in European agroecosystems if farmland bird populations are to be conserved. |

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| **Relevance of this literature article to the submission** | This paper is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | Annual change rates of bird population indices were positively correlated to the change in fallow surface. In particular, the reduction in the fallow specialist little bustard was strongly and positively related to the reduction in fallow surface.<br>The paper includes no results specific to glyphosate. |

## PART 2: REFERENCES FOR ASSESSMENT OF INDIRECT EFFECTS VIA TROPHIC INTERACTIONS DISCUSSIONS RELATED TO TERRESTRIAL VERTEBRATES AND AMPHIBIANS

### 1. Anthony & Morrison, 1985

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Anthony R and Morrison M |
| **Year** | 1985 |
| **Title** | Influence of glyphosate herbicide on small mammal populations in Western Oregon |
| **Document No** | Northwest Science, Volume 59, No. 3 |
| **Short description of literature article** | The effect of glyphosate application on vegetation and small mammal populations in the Coast Range of western Oregon was investigated. Diversity, abundance, and biomass of small mammal populations increased one-year post-spray on glyphosate-treated sites when compared to control sites. These changes were ephemeral, and the above parameters were similar to pre-spray values two years after glyphosate application. The changes in diversity, abundance, and biomass were primarily a result of the increase in numbers of *Microtus oregoni* following an increase in grass cover on treated grids one year post-spray, the transient effects of glyphosate treatment on vegetation had no detrimental effects on small mammal populations. |
| **Short description of findings** | The presented results indicated that changes in small mammal communities occur following herbicide modification of vegetation on early-growth clear-cuts in western Oregon. Such changes are most noticeable with species that use grassy habitats (particularly microtine rodents), and numbers of such species increase or decrease depending on the purpose and type of treatment. Given rapid regrowth of vegetation damaged by herbicides, small mammal communities rapidly return to pre-treatment numbers often within a two-year period. |
| **Relevance of this literature article to the submission** | This literature article is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | Overall, the results indicate that the abundance, diversity, and biomass of small mammals increased one year post-spray due to increased herbaceous cover and returned to pre-spray levels two years post-spray, possibly due to the recovery of shrubs. |

## 2. D'Anieri *et al.*, 1987

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | D'Anieri P, Leslie D and McCormick L |
| **Year** | 1987 |
| **Title** | Small mammals in glyphosate-treated Clear-cuts in northern Maine. |
| **Document No** | Canadian Field Naturalist 101 (4): 54-550 |
| **Short description of literature article** | Effects of glyphosate [N-(phosphonomethyl) glycine] on small mammals following helicopter application at 2.25 kg/ha, in four- to five-year-old clear-cuts were evaluated by snap- and pit-trapping one area one year after treatment, one area two months before and after treatment, and one untreated control. All areas were sampled simultaneously in four trapping periods from July to October 1984. Seven species were captured, but Masked Shrews (*Sorex cinereus*), Deer Mice (*Peromyscus maniculatus*), Southern Red-backed Voles (*Clethrionomys gapperi*), and Pygmy Shrews (*Microsorex hoyi*) comprised 97% of 290 captures. Only Southern Red-backed Voles were affected by glyphosate application, being significantly more abundant on the control and less numerous on the one-year-old spray area. No short-term changes in captures occurred after the 1984 herbicide application. |
| **Short description of findings** | The data presented in the article, compares trapping data for seven small mammal species in two forestry locations (one treated and one untreated with glyphosate) over consecutive years, following glyphosate application (at rates that are slightly higher than the maximum application rates proposed for the EU renewal) sampled during the same time periods each year. The findings highlighted that the abundance of one species was reduced as the herbicide treated site promoted a change in the habitat and plant cover, but the overall species richness of the small mammal community was not affected by herbicide treatment. |
| **Relevance of this literature article to the submission** | This literature article used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | The results indicate that Southern Red-backed Voles were less abundant in the one-year-old spray area than in the control area, whereas species richness was not affected by glyphosate treatment. |

**3. Edge *et al*., 2011**

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Edge C, Gahl M, Pauli B, Thompson D and Houlahan J |
| **Year** | 2011 |
| **Title** | Exposure of juvenile green frogs (*Lithobates clamitans*) in littoral enclosures to a glyphosate-based herbicide |
| **Document No** | Ecotoxicology and Environmental Safety 74 (2011) 1363-1369 |
| **Short description of literature article** | In this experiment, juvenile green frogs (*Lithobates clamitans*) were exposed to two concentrations of a glyphosate-based herbicide (2.16 and 4.27 kg a.e./ha) (VisionMax®, 540 g a.e./L, Monsanto, Winnipeg, MB,<br>CAN), which were based on typical application scenarios in Canadian forestry. The experimental design employed frogs inhabiting *in situ* enclosures established at the edge of small naturalized wetlands that were split in half using an impermeable plastic barrier.<br>The study was carried out at the Long-Term Experimental Wetlands Area (LEWA) on Canadian Forces Base Gagetown in New Brunswick, Canada. As part of a larger ongoing experiment, 10 permanent wetlands were divided in half using an impermeable high density polyethylene (HDPE) barrier. On each side of the divided wetland, one enclosure (0.8 m x 2 m, 1.6 $m^2$) was constructed from aluminum flashing and 3.2 mm HDPE mesh. The two long sides and one short side were constructed of aluminum and the remaining short side was constructed of mesh. Each enclosure had a removable mesh cover held in place with Velcro.<br>Enclosures were situated so that half of the enclosure was terrestrial and the other half aquatic, with the mesh side partially submerged in the water to allow for water circulation.<br>5 first year green frogs were randomly assigned and placed into each enclosure. Bodyweights and snout vent lengths were recorded.<br>Treatment sides of the split wetlands were randomly assigned to one of the two herbicidal application rates and sprayed on the same day as frogs were added to the enclosures. Animals were counted on 1, 4, 7 and 14 days after treatment. On DAT 14, snout vent lengths were measured, and frogs were weighed and then euthanized, livers removed, and hind feet removed and preserved in ethyl alcohol. Liver somatic index (LSI) was calculated by dividing the wet liver mass by wet weight body mass and multiplying by 100.<br>*Batrachochytrium dendrobatidis* fungal infection rates were established in frogs by examining the webbing of the hind feet under light microscope, looking for fungal thalli. |
| **Short description of findings** | When analyzed using nominal target application rates, exposure to the glyphosate-based herbicide had no significant effect on survival, body condition, liver somatic index or the observed rate of *Batrachochytrium dendrobatidis* infection. However, there were marginal trends in both ANOVA analysis and post-hoc regressions regarding *B. dendrobatidis* infection rates and liver somatic index in relation to measured exposure estimates. Results from this study highlight the importance of field research and the need to include multiple endpoints when examining potential effects of a contaminant on non-target organisms. |

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| | The authors found that direct overspray with the commercial glyphosate herbicide formulation had no significant effect on survival or body condition of juvenile green frogs under exposure regimes which span normal and worst-case scenarios in the Canadian forest use sector. |
| **Relevance of this literature article to the submission** | This paper is used to provide additional information on indirect effects and biodiversity.<br>The measured application rate of VisionMax® was negatively correlated to liver somatic index (significant, $p=0.0032$, $r^2=0.75$) and fungal infection rates in amphibians (borderline significant $p=0.052$, $r^2=0.41$). |
| **RMS comments and conclusion** | The measured application rate of VisionMax® was negatively correlated to liver somatic index (significant, $p=0.0032$, $r^2=0.75$) and fungal infection rates in amphibians (borderline significant $p=0.052$, $r^2=0.41$).<br>This study describes direct effects from the glyphosate formulation VisionMax to green frogs (*Lithobates clamitans*) rather than indirect effects on biodiversity. Hence, the results seem more relevant for the standard risk assessment for amphibians. |

## 4. Edge *et al*., 2012

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Edge C, and Houlahan J |
| **Year** | 2012 |
| **Title** | A silviculture application of the glyphosate-based herbicide VisionMAX™ to wetlands has limited direct effects on amphibian larvae |
| **Document No** | Environmental Toxicology and Chemistry · October 2012 DOI: 10.1002/etc.1956 · Source: PubMed |
| **Short description of literature article** | This paper describes the impact of a glyphosate-based herbicide on amphibian growth and development, when applied to naturalized wetlands.<br>A replicated, whole ecosystem experiment was conducted using 10 naturalized wetlands split in half with an impermeable barrier. Then one half of each wetland was exposed to the glyphosate-based herbicide, Roundup VisionMAX™.<br>The herbicide formulation was applied directly to the surface of one side of each wetland using a backpack sprayer, applied at one of two target aqueous exposure rates (high = 2,880, low = 550 µg acid equivalents [a.e.]/L), and the other side was left as an untreated control.<br>The amphibian population within the wetland systems were monitored for two years. |
| **Short description of findings** | The survival and growth of green frog larvae (*Lithobates clamitans*) were assessed for two years following herbicide treatment. The herbicide did not have a negative impact on survival or growth of *L. clamitans* larvae at either treatment level. In fact, mean larval abundance was typically greater in the treated sides than in control sides within the year of herbicide application. The effects of an increase in the abundance of a superior competitor species on the abundance of other species requires further investigation by monitoring contaminated compared to similar non-contaminated systems over long periods of time.<br>The results indicate that typical silviculture use of VisionMAX™ poses negligible risk to larval amphibians, likely because the combined effects of sorption and degradation in natural wetlands limit the exposure magnitude and duration. |
| **Relevance of this literature article to the submission** | This report is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | This study describes direct effects from the glyphosate formulation VisionMax to green frogs (*Lithobates clamitans*) rather than indirect effects and effects on biodiversity. VisionMAX™ had no negative impact on the survival and growth of green frog larvae, but generally increased the larval abundance. Further studies are needed to investigate the ecological consequences of this amphibian potentially outcompeting other species. |

## 5. Edge *et al*., 2013

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Edge C, Gahl M, Thompson D and and Houlahan J |
| **Year** | 2013 |
| **Title** | Laboratory and field exposure of two species of juvenile amphibians to a glyphosate-based herbicide and *Batrachochytrium dendrobatidis* |
| **Document No** | Science of The Total Environment Volume 444, 1 February 2013, Pages 145-152, Elsevier |
| **Short description of literature article** | This paper investigates the impact of glyphosate-based herbicide exposure interactions with other stressors in the field. In this case, juveniles of two amphibian species (*Lithobates clamitans* and *Lithobates pipiens*) were exposed in a 16-day field experiment, to the herbicide Roundup WeatherMax™ at four application rates (0, 2.16, 4.32 and 8.64 kg a.e./ha) to investigate effects on survival, liver somatic index (LSI), body condition, and incidence of disease caused by *Batrachochytrium dendrobatidis* (*Bd*). In a separate 16-day laboratory experiment, juvenile *L. clamitans* were exposed to both the herbicide and *Bd*. |
| **Short description of findings** | Results of the Authors' investigations showed that this particular herbicide formulation had no effect on juvenile survival, LSI, body condition, or disease incidence, nor was there an interaction between exposure to herbicide and exposure to the disease in tests which closely mimic real-world exposure scenarios. These experiments suggest that Roundup WeatherMax™ as typically used in agriculture is unlikely to cause significant deleterious effects on juvenile amphibians under real world exposure conditions. |
| **Relevance of this literature article to the submission** | This report is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | Roundup WeatherMax™ had no effect on juvenile survival, liver somatic index, body condition, or disease incidence in amphibians.<br>This study describes direct effects from the glyphosate formulation WeatherMax to amphibians rather than indirect effects and effects on biodiversity. Hence, the results seem more relevant for the standard risk assessment for amphibians. |

**6. Edge *et al*., 2014**

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Edge C, Thompson D, Hao C and Houlahan J |
| **Year** | 2014 |
| **Title** | The response of amphibian larvae to exposure to a glyphosate-based herbicide (Roundup WeatherMax™) and nutrient enrichment in an ecosystem experiment |
| **Document No** | Ecotoxicology and Environmental Safety 109 (2014) 124 - 132 http://dx.doi.org/10.1016/j.ecoenv.2014.07.040 |
| **Short description of literature article** | This report describes the impact of a glyphosate-based herbicide on amphibian growth and development, applied with and without nutrient enrichment, to simulate a real-world situation where herbicides are applied in combination with fertilizers.<br>A replicated, whole ecosystem experiment was conducted using 24 small wetlands split in half with an impermeable barrier. The wetlands were exposed to a glyphosate-based herbicide, Roundup WeatherMax™, alone or in combination with nutrient enrichment. The impact on survival, growth or development of amphibians was assessed. The herbicide was applied at one of two concentrations (low = 210 µg a.e./L, high = 2880 µg a.e./L) alone and in combination with nutrient enrichment to one side of wetlands and the other was left as an untreated control. Each treatment was replicated with six wetlands, and the experiment was repeated over two years. |
| **Short description of findings** | In the high glyphosate and nutrient enrichment treatment the survival of wood frog (*Lithobates sylvaticus*) larvae was lower in enclosures placed *in situ* on the treated sides than the control sides of wetlands. However, these results were not replicated in the second year of study and they were not observed in free swimming wood frog larvae in the wetlands. In all treatments, wood frog larvae on the treated sides of wetlands were slightly larger (< 10%) than those on the control side, but no effect on development was observed. The most dramatic finding was that the abundance of green frog larvae (*Lithobates clamitans*) was higher on the treated sides than the control sides of wetlands in the herbicide and nutrient treatments during the second year of the study. The results observed in this field study indicate that caution is necessary when extrapolating results from artificial systems to predict effects in natural systems. In this experiment, the lack of toxicity to amphibian larvae was probably due to the fact the pH of the wetlands was relatively low and the presence of sediments and organic surfaces which would have mitigated the exposure duration. |
| **Relevance of this literature article to the submission** | This report is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | The survival of the wood frog larvae was lower in *in situ* enclosures treated with high glyphosate concentrations and nutrient enrichment during the first year of the study, and the larvae from all treatments were larger than those in the controls. The abundance of green frog |

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| | larvae was larger on the treated sides than in the control sides, which may be a concern, since it may result in outcompeting other species (such as the wood frog). |

## 7. Edge *et al*., 2020

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Edge C, Baker L, Lanctôt C, Melvin S, Gahl M, Kurband M, Navarro-Martín L, Kidd K, Trudeau V, Thompson D, Mudge J and Houlahan J |
| **Year** | 2020 |
| **Title** | Compensatory indirect effects of an herbicide on wetland communities. |
| **Document No** | Science of the Total Environmental 718 (2020) 137254 |
| **Short description of literature article** | Using a replicated whole-ecosystem experiment and path analyses (assesses the effects of a set of variables on a specified outcome, similar to multiple regression), the Authors examined the direct and indirect effects of a glyphosate-based herbicide and nutrient enrichment on wetland communities. The latter did not impact any measured endpoints. The strongest drivers of macrophyte, benthic invertebrate, and amphibian assemblages were the ephemerality and the size of wetlands, factors which were not altered by herbicide applications. The herbicide had a direct negative effect on macrophyte cover, amphibian larval abundance, and the proportion of predatory benthic invertebrates. However, both amphibians and invertebrates were positively affected by the reduction in the macrophyte cover caused by the herbicide applications. The opposing directions of the direct and indirect effects lead to no net change in either group. The compensatory dynamics observed herein highlight the need for a better understanding of indirect effects pathways to determine whether common anthropogenic disturbances alter the ecological communities in small wetland ecosystems |
| **Short description of findings** | Nutrient enrichment did not impact any measured endpoints. The strongest drivers of macrophyte, benthic invertebrate, and amphibian assemblages were the ephemerality and the size of wetlands, factors which were not altered by herbicide applications.<br>The herbicide had a direct negative effect on macrophyte cover, amphibian larval abundance, and the proportion of predatory benthic invertebrates.<br>However, both amphibians and invertebrates were positively affected by the reduction in the macrophyte cover caused by the herbicide applications. The opposing directions of the direct and indirect effects lead to no net change in either group.<br>The compensatory dynamics observed herein highlight the need for a better understanding of indirect effects pathways to determine whether common anthropogenic disturbances alter the ecological communities in small wetland ecosystems.<br>Some conclusions from the paper include:<br>- Glyphosate application has a direct negative effect on wetland macrophyte cover.<br>- The indirect effect of macrophyte reduction on the amphibian and benthic invertebrate communities opposed the direct effect.<br>- Compensatory dynamics make it difficult to determine the overall effects of chemical contamination on wetland communities. |

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| | - Natural variation in abiotic environmental factors has a greater effect on wetland communities than herbicide application |
| **Relevance of this literature article to the submission** | This paper is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | The data presented here were derived from the study by Edge *et al*., 2014, summarised above.<br>The results show that glyphosate may have both a direct negative effect and an indirect positive effect on amphibians and invertebrates. |

## 8. Gagne *et al*., 1999

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Gagne N, Belanger L and Huot J |
| **Year** | 1999 |
| **Title** | Comparative responses of small mammals, vegetation, and food sources to natural regeneration and conifer release treatments in boreal balsam fir stands of Quebec |
| **Document No** | Canadian Journal of Forestry Research 29: 1128–1140 |
| **Short description of literature article** | Abundance and species diversity of small mammals were compared among three regeneration methods used in boreal balsam fir (*Abies balsamea (L.) Mill.)* forests. Those methods were natural regeneration after "careful logging" to retain advance regeneration and planting (black spruce, *Picea mariana* (Mill.) BSP) followed by herbicide (Vision®) release or brushsaw cutting release.<br>In total twelve sites were sampled, ranging from 6 to 9 ha in size, selected from within large clearcut areas. Prior to harvesting, spruce budworm had killed about 50% of the merchantable trees. Eight blocks were scarified in autumn 1989 and planted spring 1990 with black spruce. Four with successful natural regeneration were not treated. Two years after planting, conifer release was used to reduce competing vegetation, with four plantations released with Vision and four with brushsaw (all woody vegetation within 1-m of the seedling was removed.).<br>In July of each year 1992 - 1994, the vegetative cover was monitored across all sites, with woody regrowth mapped using transects within mammal trapping grids and grids. The relative abundance of small mammals was established by live and pit-fall trapping, performed in the brushsaw and Vision treated plantations and in four naturally regenerating cutblocks also in late summer months of 1992-1994.<br>The abundance of food e.g. ripe berries within a 5-m radius of mammal traps was also determined. Foliar arthropod species and abundance was sampled using plastic sheets ($1m^2$) placed 30 m apart at 3 locations within each mammal trapping grid. The foliage above the plastic sheets was beaten to release any arthropods. Pitfall traps were also used to collect arthropods. Fruiting bodies of fungi were also collected. Small mammals were also trapped to establish their stomach contents. |
| **Short description of findings** | Deciduous vegetation was reduced for two growing seasons in both plantation types after treatment (but more in the glyphosate treatment compared to brushsaw plantation), and foliar arthropods decreased for one growing season. In herbicide-treated plantations, red raspberry (*Rubus idaeus L.*) shrub cover, near-ground vegetation, and production of berries were reduced for two growing seasons (reduction was 70% in first season, 55% in second season). After herbicide release, the abundance of the red-backed vole (*Clethrionomys gapperi (Vigors*)) was significantly lowered for two growing seasons. This negative effect was associated with reduced cover during the first two post-treatment growing seasons. In the |

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| | short term, herbicide-treated plantations constitute poorer red-backed vole habitats than brushsaw plantations. In early successional boreal balsam fir stands, planting did not markedly affect small mammals probably because natural regeneration was common in these plantations. |
| **Relevance of this literature article to the submission** | This report is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | The abundance of the red-backed vole was significantly reduced by glyphosate treatment for two growing seasons. |

## 9. Guynn *et al*., 2004

Summary already submitted under Part 1, point 10 on this document.

### 10. Guiseppe *et al*., 2006

Summary already submitted under Part 1, point 9 on this document.

## 11. Santillo *et al*., 1989a

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| **Data point** | M-CP 10.1.1 & 10.1.2 |
| **Author** | Santillo D, Leslie D and Brown P |
| **Year** | 1989a |
| **Title** | Responses of Small Mammals and Habitat to Glyphosate Application on Clearcuts. |
| **Document No** | The Journal of Wildlife Management, Vol. 53, No. 1 (Jan., 1989), pp. 164-172 |
| **Short description of literature article** | The authors investigate the effects of herbicide-induced habitat changes on small mammals in clear cuts in Northcentral Maine.<br>Six study sites were used characteristic of areas of traditional herbicide use in the region, comprising 4-5 year-old clearcuts with suppressed softwood regeneration. The sites ranged between 31 and 62 ha in size. Two were previously treated with herbicide and 4 were untreated clearcuts. Within 1 year of the study start, 2 untreated clearcut sites were treated with herbicide, effectively giving a 1, 2 and 3-year post treatment growth potential opportunity across the six sites.<br>Small mammals were trapped using removal and pitfall traps. Hardwood and softwood stems were counted in each site. Vegetation across all sites was visually estimated. Surface temperatures were monitored, to determine effects of foliage removal on microclimate. Invertebrates were sampled using sweep netting along transects. Ground invertebrates were also sampled based on those that fell into small mammal pitfall traps. Analysis of quantified parameters was performed and differences across sites were established. |
| **Short description of findings** | Fewer small mammals were captured on glyphosate (nitrogen-phosphonomethyl glycine) (Roundup, Monsanto, St. Louis, Mo.)-treated clearcuts 1-3 years post-treatment compared to untreated clearcuts.<br>Insectivores (Soricidae) comprised 72% of small mammal captures and were less abundant ($P < 0.001$) for all 3 years post-treatment.<br>Herbivores (Microtinae) were less abundant 1 ($P < 0.01$) and 2 years ($P < 0.001$) post-treatment.<br>Omnivores (Cricetinae and Zapodidae) were equally abundant on treated and untreated clearcuts.<br>Differences in small mammal abundance paralleled herbicide-induced reductions in invertebrates and plant food and cover.<br>Patches of untreated vegetation within herbicide-treated clearcuts provided a source of invertebrates and plant food. |
| **Relevance of this literature article to the submission** | This paper is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | Overall fewer small mammals were found on glyphosate-treated than on untreated clearcuts, in particular insectivores and herbivores being less abundant at least two consecutive years post-treatment. |

## 12. Santillo *et al*., 1989b

Summary already submitted under Part 1, point 14 on this document.

## 13. Sullivan & Sullivan, 2000

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| **Data point** | KCP 10.1.2 |
| **Author** | Sullivan, D.S. and Sullivan, T.P. |
| **Year** | 2000 |
| **Title** | Non-target impacts of the herbicide glyphosate: A compendium of references and abstracts. 5th Edition |
| **Document No** | Applied Mammal Research Institute, Summerland, British Columbia, Canada |
| **Short description of literature article** | The book is a compendium of references and abstracts outlining the "non-target impacts of the herbicide glyphosate". This is a secondary source document that was prepared given the apparent incomplete and scattered sources of information on this subject.<br>The Authors identify that a common complaint from both lay and professional people is: "What research has been done on non-target impacts of glyphosate and how do we access this information?"<br>The book presents the results of a computerized literature search resulting in a 5$^{th}$ Edition of the compendium, that comprises several thousand references covering environmental impacts, toxicology, efficacy, and human health. Thus, the compendium has evolved as a means to provide, in as complete a manner as possible, a collection of titles and abstracts of articles reporting on the non-target impacts of this herbicide.<br>This book contains many titles and abstracts on the topic of non-target impacts on soil microbes / soil microorganisms. Specific reference to any single reference is not meant, with the book being cited to illustrate the amount of research on soil microorganisms that is available. |
| **Short description of findings** | Abstracts are presented from many different articles as secondary sources of information. No specific results or opinions by the Authors are presented in the book. The book is cited within the ecotoxicology section to illustrate the amount of data available in the literature. |
| **Relevance of this literature article to the submission** | This book was cited in the higher tier risk assessment to illustrate the available literature, within the indirect effects via trophic interactions and biodiversity section of the ecotoxicology dossier. |
| **RMS comments and conclusion** | The book is a compendium of references and abstracts illustrating the available literature on the impact of glyphosate on non-target organisms. No specific examples are discussed. |

## 14. Sullivan and Sullivan, 2003

Summary already submitted under Part 1, point 15 on this document.

## 15. McLaughlin and Mineau, 1995

Summary already submitted under Part 1, point 13 on this document.

## PART 3: REFERENCES FOR ASSESSMENT OF INDIRECT EFFECTS VIA TROPHIC INTERACTIONS FOR AQUATIC ORGANISMS DISCUSSION

### 1. Baker *et al.*, 2016

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| **Data point** | M-CP 10 |
| **Author** | Baker, L., Mudge, J., Thompson, D., Houlahan, J., Kidd, K. |
| **Year** | 2016 |
| **Title** | The combined influence of two agricultural contaminants on natural communities of phytoplankton and zooplankton. |
| **Document No** | Ecotoxicology (2016) 25: 1021 – 1032. |
| **Short description of literature article** | By examining changes in the phytoplankton and zooplankton communities of shallow, partitioned wetlands over a 5 month period, the potential for direct and indirect effects of the glyphosate-based herbicide, Roundup WeatherMax© applied at the maximum label rate was assessed, both in isolation and in a mixture with nutrients (from fertilizers). |
| **Short description of findings** | The presented results indicated that worst-case contamination of wetlands with the herbicide Roundup WeatherMax© in combination with fertilizer nutrients resulted in transient and relatively minor disruptions of plankton community structure.<br>Despite the identification of longer-term, indirect impacts on the zooplankton community, it would appear that the regulated use of this glyphosate-based herbicide, which prohibits direct application to wetlands such as those used in this study, is unlikely to result in the serious impairment of wetland plankton communities, as might have been predicted from the findings of laboratory based studies of similar glyphosate based herbicides. The findings of significant effects only in the treatment containing both the herbicide and fertilizers implies that effective ecotoxicological risk assessments should also consider scenarios in which other contaminants or stressors may co-occur in the receiving system, as the possibility exists for joint activity. Addressing the significance of complex ecosystem level responses to complex mixtures of contaminants, as was done in this study, will contribute to more ecologically relevant ecotoxicological risk assessments. |
| **Relevance of this literature article to the submission** | This literature article is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | See RMS comment in the Appendix to Volume 3 CA B.9 on General Literature review for ecotoxicology |

## 2. Edge et al 2020

Summary already submitted under Part 2, point 7 on this document.

**3. Rolando *et al*., 2017**

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| **Data point** | M-CP 10 |
| **Author** | Rolando, C., Baillie, B., Thompson, D., Little, K. |
| **Year** | 2017 |
| **Title** | The Risks Associated with Glyphosate-Based Herbicide Use in Planted Forests. |
| **Document No** | Forests 8 (6):208 |
| **Short description of literature article** | The paper provides a literature review of the current use of glyphosate-based herbicides in planted forests and the associated risks. |
| **Short description of findings** | Glyphosate-based herbicides are the dominant products used internationally for control of vegetation in planted forests. Few international, scientific syntheses on glyphosate, specific to its use in planted forests, are publically available. We provide an international overview of the current use of glyphosate-based herbicides in planted forests and the associated risks. Glyphosate is used infrequently in planted forests and at rates not exceeding 4 kg $ha^{-1}$. It is used within legal label recommendations and applied by trained applicators. While the highest risk of human exposure to glyphosate is during manual operational application, when applied according to label recommendations the risk of exposure to levels that exceed accepted toxicity standards is low. A review of the literature on the direct and indirect risks of operationally applied glyphosate-based herbicides indicated no significant adverse effects to terrestrial and aquatic fauna. While additional research in some areas is required, such as the use of glyphosate-based products in forests outside of North America, and the potential indirect effects of glyphosate stored in sediments, most of the priority questions have been addressed by scientific investigations. Based on the extensive available scientific evidence we conclude that glyphosate-based herbicides, as typically employed in planted forest management, do not pose a significant risk to humans and the terrestrial and aquatic environments. |
| **Relevance of this literature article to the submission** | This literature article is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | This paper presents an international overview of the current use of glyphosate-based herbicides in planted forests and the associated risks. It concludes that glyphosate-based herbicides, as typically employed in planted forest management, do not pose a significant risk to humans and the terrestrial and aquatic environments.<br>Sediment sorption and degradation of glyphosate have been identified as a primary removal mechanism for glyphosate from the water column in forested freshwater environments, a potential source of risk, particularly to sediment dwelling organisms. However, these risks are tempered by the strong ionic sorption mechanisms which are considered to limit leaching or diffusion into the water column and bioavailability of sediment-bound residues.<br>This paper also states that subtle, sub-lethal, long-term, indirect effects, or potential interactions of glyphosate-based herbicides with other environmentally relevant stressors (e.g., herbicide mixtures, |

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| | low dissolved oxygen, pH, excess nutrient inputs, other chemical pollutants) are less well understood as compared to simple direct acute or chronic effects. |

## PART 4: REFERENCES FOR ASSESSMENT OF INDIRECT EFFECTS VIA TROPHIC INTERACTIONS FOR BEES DISCUSSION

### 1. Burgett and Fischer, 1990

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| **Data point** | M-CP 10 |
| **Author** | Burgett, M. and Fisher, G. |
| **Year** | 1990 |
| **Title** | A review of the Belizean honey bee industry. |
| **Document No** | Final report prepared at the request of The Belize Honey Producers Federation. Department of Entomology, Oregon State University, Corvallis, Oregon. |
| **Short description of literature article** | A review of the beekeeping condition in Belize over a 6 week period; in January and February 1990. This was triggered by a concern of reported declines in honey production. The beekeeping industry was in a state of decline throughout the nation and was especially evident during the 1988 and 1989. Multiple potential causes in the decline were considered, included results from specific feeding and spray trials looking at specific herbicides including those containing glyphosate such as Roundup, and other herbicides such as paraquat, applied aerially for marijuana eradication. Other potential causes investigated included beekeeping practices / conditions; poor brood / hive nutrition, the impacts of diseases (such as sacbrood and bald brood), parasites (acarine parasites such as the tracheal mite, the Rennie – an endoparasite of adult bee and Varroa – an ectoparasite of both adult and pupal honey bees). Predation (wax moths), poor apiary management, insecticidal practices (sprays for wide-spread vector control e.g. mosquitos; control of exotic fruit flies using malathion) and the impact of invasive bee species (Africanised honey bee) were also considered. |
| **Short description of findings** | The results concluded that nearly all apiaries showed positive signs of mild to severe Africanisation, confirmed using the USDA designed morphometric test FABIS (Fast Africanised Bee Identification System) nrtive. Concerning nutrition, low food stores were observed in multiple colonies due to inadequet number of foraging bees, not to a lack of available flora. Concerning diseases, there was a low frequency of sacbrood, but a high frequency of bald brood caused by the early instar predatory wax moths larvae tunnelling across the face of capped brood, but was not considered to more than an annoyance to beekeepers. Concerning parasites, these were not considered to have had a great impact on colonies. Then concerning pesticides practice, despite some minor impacts for control of certain pests (froghoppers in sugar cane) changes in farming practice reduced the impact of chemicals) ing farmiong.<br><br>In the feeding trials using Roundup herbicide where multiple hives were exposed to herbicide via sucrose solution, at 100 to 1000 times greater than would be encountered by foraging bees in an open spray situation of ca. 1-2 acres of sprayed marijuana. There was no increase in adult honey bee mortality as a result of feeding a mixture of RoundupR plus Nalcotrol II (a drift control agent), or Nalcotrol II alone, compared to the control colonies. Statistical analysis was not |

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| | required for the daily adult honey bee mortality as the average number of dead bees/day was so far below the hazard threshold of more than 100 bees/day. The interpretation of the field trial testing is that no acute or chronic effects of Roundup Ready were shown on daily adult bee mortality, adult population growth or brood production.<br><br>In a spray trial, multiple colonies were positioned near to blooming vegetation and then the whole area including the hives, was aerially sprayed, and bee mortality and brood counts were conducted at 2, 4 and 6 weeks post spray. There was no elevation in adult bee mortality over that of the control colonies, either in an acute or chronic mode. The data for adult honey bees and brood production over time were analyzed in the same manner as the data for the feeding trial. There was no statistically significant difference.<br><br>On the claim that herbicidal sprays are removing large amounts of honey bee forage – quite simply the report identified the area of potential bee forage that is destroyed by aerial applications of herbicides as being so insignificant as to be meaningless as a valid claim for the following reasons: (1) honey bees are not known to utilize marijuana as a nectar or pollen source, (2) assuming a worse case drift effect, as much area of non-target vegetation would be affected, which may or may not be providing significant nectar and pollen to foraging honey bees at the time of application, and (3) relative to the amount of blooming forage available to a colony of bees, one or two acres sprayed within their foraging range of ca. 12,000 acres is a minuscule amount.<br><br>Conclusions: The review team identified that it was evident that the honey bee industry in Belize was in a serious state of decline. The primary cause of this situation, in the professional opinion of the VOCA (Volunteers in Overseas Cooperative Assistance) consultants and the other review team members, is the presence of Africanized honey bees in Belize. The "Africanization" syndrome is completely compatible with the current beekeeping situation. On the other hand there exists no irrefutable evidence that the aerial application of RoundupR is causing any harm to the beekeeping industry. Based on the known toxicological effects to honey bees and the results of our field trials, no scientific evidence supports a hypothesis of herbicidal toxicity or damage to the beekeeping industry of Belize. |
| **Relevance of this literature article to the submission** | This literature article is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | The authors conducted two types of field evaluations: (1) feeding trials whereby glyphosate with the anti-drift additive, and the additive alone, were fed directly to honey bee colonies using a 40% sugar solution as the toxicant vehicle and (2) a spray trial where ca. 1.5 acres of blooming vegetation containing 5 colonies were aerially sprayed with a 5% RoundupR plus 0.25% Nalcotrol II at a rate of 40 gallons of spray per acre (one gallon of RoundupR). |

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| | Based on these trials they concluded exposure to RoundupR produced no acute or chronic effects on adult honey bees or brood production. RMS considers these trials poorly described (study design, results) and not relevant/reliable to addresse indirect effects/biodiversity issues.<br><br>Concerning the removal of the honeybee forage, the potential bee forage that is destroyed by aerial applications of herbicides was considered insignificant by the study authors, this paper is not relevant to address indirect effects/biodiversity issues. |

## 2. Chamkasem and Vargo, 2017

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| **Data point** | M-CP 10 |
| **Author** | Chamkasem, N and Vargo, J. |
| **Year** | 2017 |
| **Title** | Development and independent laboratory validation of an analytical method for the direct determination of Glyphosate, Glufosinate and Aminomethylphosphonic acid in honey by liquid chromatography/tandem mass spectrometry. |
| **Document No** | Journal of Regulatory Science 5 (2) (2017) 1-9 |
| **Short description of literature article** | This paper described a liquid chromatography/tandem mass spectrometry (LC-MS/MS) method developed for the determination of glyphosate, glufosinate and aminomethylphosphonic acid (AMPA) in honey using a reversed-phase column with weak anion-exchange and cation-exchange mixed-mode. |
| **Short description of findings** | The in-house and the inter-laboratory validation studies, using spiked blank honey and honey with incurred residue of glyphosate, demonstrated that the method is quick, rugged, selective, and sensitive enough to determine glyphosate, glufosinate and AMPA in honey at or above the 25 ng/g level. It can be used as an alternative method to the ELISA technique as well as to the traditional FMOC derivatization methods which are tedious and time-consuming. |
| **Relevance of this literature article to the submission** | This literature article provided additional information on the analytical method for glyphosate in honey and was referenced in the biodiversity section of the dossier. |
| **RMS comments and conclusion** | This paper describes an inter-lab validation of an LCMS /MS method using a negative ion-spray ionization mode for the direct determination of glyphosate, glufosinate, and AMPA in honey.<br>Nineteen honey samples were collected from the local market and a private honey farm and analyzed by the proposed method. Nine samples (47%) contained glyphosate higher than 16 ng/g (estimated LOQ).<br>Max value was of 121 ng glyphosate/g honey. Glufosinate and AMPA were not detected in any of the samples. |

## 3. Ferguson, 1988

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| **Data point** | M-CP 10 |
| **Author** | Ferguson, F. |
| **Year** | 1988 |
| **Title** | Long term effects of systemic pesticides on honeybees. |
| **Document No** | Second Australian and International Beekeeping Congress, Surfers Paradise, Gold Coast, Queensland, Australia, July 21-26, 1988 |
| **Short description of literature article** | This paper evaluates the effects of several systemic pesticides (including glyphosate) on worker honeybees, particularly sublethal concentrations and effects on the life span of the bees.<br><br>Pesticides at concentrations ranging from 5ppm to 0.1 ppm were added to sugar solutions and offered to the bees of free-flying colonies. Brood area and bee numbers were measured. Sunflower and oil seed rape were treated with pesticides at recommended field rates. Pollen and nectar sacs were collected from foraging bees and analyzed for pesticide residues. |
| **Short description of findings** | Glyphosate did not significantly affect the brood and bees ($P<0.05$). In comparison, the other pesticides included in this study had significant impacts on the brood mortality, development and reproduction in the hives. |
| **Relevance of this literature article to the submission** | This literature article is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | Not relevant to address indirect effects/biodiversity issues (this feeding study only aims to investigate direct toxic effects).<br>Study poorly described (study design, environmental conditions, etc…), test item not identified, no results presented (only a statement that glyphosate did not significantly affect the brood and bees). |

**4. Last *et al*., 2019**

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| **Data point** | M-CP 10 |
| **Author** | Last, G., Lewis, G., Pap, G. |
| **Year** | 2019 |
| **Title** | Regulatory report on the occurrence of flowering weeds in agricultural fields. |
| **Document No** | European Crop Protection Association report by ERM (2019) |
| **Short description of literature article** | This report uses industry herbicide efficacy trial data performed on a variety of crops to enhance the Tier 1 bee risk assessment.<br><br>It was hypothesized that a significant exposure of bees is made via the 'weeds in the treated field'. It is suggested in the EFSA bee Guidance that if <10% of the area of use contains attractive flowering weeds then the exposure route is not relevant.<br><br>Relevant information was extracted from the efficacy data with the intention of demonstrating that, for some crops, the occurrence of attractive flowering weeds in treated fields is relatively rare and constitutes less than 10% of the area of use, thereby highlighting that the weeds in the treated field scenario is not applicable for many typical commercially grown crops.<br><br>The data were analysed and assessments made specifically on the presence of weed species during each trial, the growth stage of the weed species present, the attractiveness to bees of the weed species present, the ground coverage of the weed species present, the trial location, dates of the trial and the crop growth stage used in the trials. |
| **Short description of findings** | The data set has been used to demonstrate that the presence of attractive flowering weeds in arable fields, in terms of both incidence and percentage ground cover, is much lower than 10% and therefore, in accordance with the EFSA bee Guidance Document, the 'weeds in the treated field' exposure scenario is not considered relevant for many typical arable crops. For permanent crops it is considered that further data from additional efficacy trials should be gathered and analysed before drawing any firm conclusions on the relevance of this exposure scenario. |
| **Relevance of this literature article to the submission** | This literature article is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | In an attempt to demonstrate that the flowering weeds scenario should not be considered relevant (in the framework of the standard risk assessment of direct toxic effects as recommended in EFSA guidance on bees, 2013), the European Crop Protection Association (ECPA) launched a project to analyse the data on the presence of weeds in control plots of herbicide efficacy trials from different crops, supplied by a number of companies (Syngenta, Bayer Crop Science, BASF, Dow Agrosciences (now Corteva Agriscience) and Monsanto (now part of Bayer)). Within this project, data was available from eight arable crops (cereals, maize, oilseed rape, sunflower, potatoes, sugar |

beet, peas and beans) and three permanent crops (orchards, citrus and grapes).
The information provided by the different companies included the trial ID number, location (co-ordinates of the trial site, postal code, country), information on the plots (number of replicates, plot size), whether the trial was conducted to GEP, date of the trial, crop species, crop BBCH stage (min, max, majority), weed species, weed BBCH growth stage (min, max, majority), weed diameter and height, weed percentage ground cover, weed density, tillage practices.

RMS notes that:
- Data was not always available for one or more of these parameters. For example, information on the weed BBCH stage and ground cover was available for only a small part of the trials.
- The trials generally seem to be well spread over the different regulatory zones in Europe. However, trials performed in the Northern zone were a minority, and even lacking for some of the crops. Further, for some crops (e.g. citrus) the data was limited to trials performed in Southern Europe. However, this can generally be explained by the geographical spread of the regions where the crop is typically grown.
- The authors set a threshold of 10% weed ground cover within a single field (as “significant fraction”, referring to Appendix N of the Bee guidance (EFSA, 2013)). Specific data or an argumentation to underpin the assumption that a weed ground cover within a field of below 10% is not significant for bees has not been provided. It is therefore assumed that this threshold of 10% originates from a misinterpretation of the text in Appendix N of the Bee guidance (EFSA, 2013).
- Data on the ground coverage is not available for all weed recordings considered. Consequently, the authors assumed that for those recordings where no data on the ground coverage is available, the weed coverage was less than 10%. It is however not possible to prove that this assumption is correct based on this dataset (i.e. it might have been the case that although the % ground coverage was not recorded, it was > 10%). Therefore, the ‘percentage of attractive weeds > 10% ground coverage’ as presented in the tables in the report cannot be considered a reliable value. In addition, the value of 10% as a trigger to determine whether weed coverage within a field is significant is not supported by data.
- To determine whether a weed species was attractive to bees, a distinction was made between dicot and monocot species, with only dicot species considered to be attractive. As acknowledged by the study authors, there are exceptions to this rule.
- The monthly distribution of weed recordings was plotted for each crop, as was the monthly distribution of the weed BBCH growth stage. The latter plots indicated that, with the exception of oilseed rape, flowering weeds were generally only present in arable fields during the months of March, April, May, June and July. Based on these observations, the

authors concluded that the 'flowering weeds in the treated field exposure scenario' may only be relevant for many arable crops between the months of March and July. However, this observation is based on the available trial data which did not cover all months of the year for each crop (as also acknowledged by the authors). Therefore, this conclusion is of limited reliability.

- Histograms were plotted for each crop to present the distribution and frequency of the crop growth stage for the trials in the dataset. These plots show that the majority of the weed recordings were made at early crop BBCH stages only.
- Although data from trials where conservation tillage was applied is also available, intensive tillage operations were performed prior to sowing in the majority of cases.
- Attractive weeds have been defined as dicotyledonous species at a flowering growth stage (BBCH 60 - 69). BBCH growth stages of <60 or ≥70 have been assumed to not be flowering and therefore not attractive to bees. RMS believes this study may be relevant to address indirect effect issues via the reduction of food availability subsequent to herbicide use. In such purpose, even if it is likely that only a small proportion of weeds in the field will be flowering at the time of application and flowering weeds that are sprayed will rapidly wilt and their flowers will no longer be attractive to bees, flowering weeds only represent a portion of all weeds (including those not yet flowering). In agricultural landscapes, weeds may be the only permanent source of food. Removing the weeds at early development stage may deprive bees of the only source of food normally available later on. RMS then considers that (to address the relevance of weeds as food source), all weeds at BBCH 0 to 69 should be considered.

Overall, a powerful data set is available that has been used here to demonstrate that the presence of attractive flowering weeds in arable fields, in terms of both incidence and percentage ground cover, is not considered relevant for many typical arable crops. Due to the shortcomings listed above, RMS does not agree with the conclusion of the analysis.

Besides, if the analysis of Last et al (2019) is used in the purpose to address the impact of weed removal (using glyphosate) on food availability, the results shoults should be re-analysed (with different assumptions). However the percentages of weed occurrences reported in this analysis (not only considering those flowering), already breached the "threshold" of 10% in several crops or were around this value (in a lesser extent for sugar beet and pea). Besides, the drawbacks identified in this analysis may considerably underestimate the relevance of weeds.

The results from these efficacy trials may actually indicate that the weeds are present and relevant in more than 10% of cases.

## 5. Laberge L, Legris J, Couture G. 1997

The applicant indicated that this reference is an error. The correct reference should be :
Laberge L, Couture G, Legris J, Langevin R. 1995. Evaluation des impacts du glyphosate utilisé dans le milieu forestier.
No summary was provided as not available at time of submission.

**Data gap** : Applicant to provide the full text paper and study summary of Laberge L, Couture G, Legris J, Langevin R. 1995 (Evaluation des impacts du glyphosate utiliséze dans le milieu forestier.) together with its english certified translation.

## 6. Motta *et al.*, 2018

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| **Data point** | M-CP 10 |
| **Author** | Motta E. V. S. et al. |
| **Year** | 2018 |
| **Title** | Glyphosate perturbs the gut microbiota of honey bees. |
| **Document No** | Proceedings of the National Academy of Sciences of the United States of America (2018), Vol. 115, No. 41, pp. 10305-10310 |
| **Short description of literature article** | A study was conducted to demonstrate that the relative and absolute abundances of dominant gut microbiota species are decreased in bees exposed to glyphosate at concentrations documented in the environment. |
| **Short description of findings** | The authors found that glyphosate affects the bee gut microbiota composition and that bacterial species and strains within this community vary in susceptibility to glyphosate. The results also suggest that establishment of a normal microbial community is crucial for protection against opportunistic pathogens of honey bees. Furthermore, the results highlight one potential mechanism by which glyphosate affects bee health.<br><br>Since bee gut symbionts affect bee development, nutrition, and defence against natural enemies, perturbations of these gut communities may be a factor making bees more susceptible to environmental stressors including poor nutrition and pathogens. |
| **Relevance of this literature article to the submission** | This literature article is used to provide additional information on indirect effects and biodiversity. However, suitable scientific approaches to assess effects are not specified, thus relevance of the effects remained unclear. This paper was also covered by the LRR and classified as ‘c’ as the findings were not relatable to the EU level risk assessment and relevance to the renewal of glyphosate could not be determined. |
| **RMS comments and conclusion** | See RMS comment in the Appendix to Volume 3 CA on general literature review for ecotoxicology |

**7. [redacted]., 2020**

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| **Data point** | M-CP 10 |
| **Author** | [redacted] |
| **Year** | 2020 |
| **Title** | Residues of glyphosate in food, feed and urine. |
| **Document No** | Bayer Crop Science internal report – not published. |
| **Short description of literature article** | This report places social and traditional media reports about alleged glyphosate residue findings into perspective regarding analytical methods, reported findings and dietary risk assessment.<br><br>Social and traditional media reports of pesticide residues in food, specifically reports of residues in foods originating from agricultural practices growing conventional or GM crops, are becoming more common. Many of these reports list residue values while attempting to attribute exposure from these residues to a potential consumer health issue. This has created an unrealistic expectation or desire that food and beverages should have a zero-residue level. The topic of residues needs to be better understood by consumers and the people they look to for advice, including health care professionals, nutritionists and dietitians. As a start, it is important to convey that residues are to be expected regardless of agriculture practice, including both organic and conventional, and that the presence of residues does not equate with harm (Winter et al., 2019). A next step is to provide the necessary context around what a reported detection means, and what that value means in terms of current safety guidelines.<br><br>The objectives of the provided report are to: 1) review the assays available for measuring glyphosate in food, water and beverages, urine and other substances; 2) review reports of testing glyphosate in food or urine and other consumer items, assess the plausibility of these findings and convert these values to estimates of exposure; and 3) put these exposure estimates into context by comparing them to health-based guidance values. This paper indicates that 1) glyphosate residues are neither unexpected nor ubiquitous as media reports have implied; 2) residue information can be equivocal based on mostly media results from assays that were not validated and did not properly utilize experimentally-derived limits; 3) concentrations of residues in agricultural commodities/food demonstrate a high level of compliance because they are at or below expected amounts; and 4) modeled or empirical exposure of glyphosate to consumers is low compared to the allowable intakes. |
| **Short description of findings** | In general, residue date for glyphosate indicate that chronic residue exposure is well below established ADIs. |
| **Relevance of this literature article to the submission** | This literature article is used to provide additional information on indirect effects and the consumer risk assessment. |
| **RMS comments and conclusion** | In this paper, the only data of relevance for the environmental risk assessment is the glyphosate content in honey. This paper contains |

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| | actually a review of existing published papers related to glyphosate contamination in different matrices. It is RMS opinion that it is not relevant for indirect effects/biodiversity issues. Such data may potentially be used in a risk assessment (via honey consumption) even if honey is not currently considered the "more" relevant matrice. The maximum level of glyphosate in honey that was retrieved in this paper is 163 µg/kg from literature (source cited: Rubio et al, 2014) and 610 µg/kg in honey from market survey (source cited in the report: EFSA). |

## PART 5: REFERENCES FOR ASSESSMENT OF INDIRECT EFFECTS VIA TROPHIC INTERACTIONS FOR NON-TARGET ARTHROPODS DISCUSSION

### 1. Guiseppe *et al*., 2006

Summary already submitted under Part 1, point 9 on this document.

The analysis of RMS related to non-target arthropods is reported thereafter:
Guiseppe KFL et al, 2006 reviewed articles related to ecological effects of the herbicide glyphosate used in forested landscapes. Among these papers, some stated that homopteran densities were lower in herbicide-treated plots compared with brush-saw-treated plots and non-treated control plots. It was hypothesized that indirect effects of herbicide treatment altered the nutritional quality of tree and shrub species (as homoptera feed on either phloem or xylem). Also indirect effects of herbicides on communities of herbivorous arthropods, in most cases, were hypothesized to be a result of reduced floral resources and the effect that this reduction would have on arthropods that require them during at least one phase of their life cycle. Studies are referenced that stated that herbicides have indirect effects on beneficial wasp and bees. These studies present correlative relationships that suggest that decreases in flowering plants in agricultural fields results in decreases in the abundance of wasps and bees and often concomitant increases in the density of insect pests.

## 2. Sullivan and Sullivan, 2003

Summary already submitted under Part 1, point 15 on this document

Regarding non-target arthropods, the review of Sullivan TP, Sullivan DS. 2003 concluded that the diversity of terrestrial invertebrates in glyphosate-treated areas is variable. Abundance and diversity of invertebrates in a given treated area is principally a function of the degree of vegetation control and changes in vegetation structure

### 3. Warburton and Klimstra, 1984

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| **Data point** | M-CP 10 |
| **Author** | Warburton, D. and Klimstra, W. |
| **Year** | 1984 |
| **Title** | Wildlife use of no-till and conventionally tilled corn fields. |
| **Document No** | Journal of Soil and Water Conservation. 39 (5):327-330. |
| **Short description of literature article** | A study was conducted to compare a field of no-till corn with a field of conventionally tilled corn, on farms in Illinois and investigate the impact on wildlife. Numbers of birds, mammals and invertebrates were assessed via traps and observation over 6 months on the two study sites. |
| **Short description of findings** | The no-till cornfield provided more favorable wildlife habitat than the conventionally tilled cornfield. That was evident from the greater abundance of invertebrates, birds, and mammals in the no-till field. Conventional tillage creates a specialized habitat; there is less food and cover, which reduces wildlife carrying capacity. The no-till provides habitat that supports more abundant and stable animal communities. |
| **Relevance of this literature article to the submission** | This literature article is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | Invertebrate, avian, and small mammal populations in a no-till corn field and a conventionally tilled corn field were compared.<br>This study states (with data) that no-till provides habitat that supports more abundant and stable animal communities. The relative richness of the no-till field as wildlife habitat was investigated. Crop residue and other interrow cover increased habitat complexity in the no-till field (no quantitative habitat measures were made but weedy vegetation obviously provided greater niche variet in the no-till field). When compared with that in the conventional field, this cover resulted in greater diversity within the invertebrate community and a more stable small mammal population.<br>However, the study does not include results specific for glyphosate or herbicides in general.<br>It is hypothesized that reliance of no-till agriculture on pesticides may have fewer off-farm environmental impacts than conventional tillage, but the sublethal and long-term effects of pesticides on animal populations using no-till fields are not well understood and must be considered.<br>The authors also hypothesized that maintaining uncultivated areas in the field and between narrow crop rows may establish an equilibrium between predator and prey populations as they noted the absence of serious pest related problems during the study. |

## PART 6: REFERENCES FOR ASSESSMENT OF INDIRECT EFFECTS VIA TROPHIC INTERACTIONS FOR SOIL ORGANISMS DISCUSSION

### 1. Cerdeira and Duke, 2010

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| **Data point** | M-CP 10 |
| **Author** | Cerdeira, A. and Duke, S. |
| **Year** | 2010 |
| **Title** | Effects of glyphosate-resistance crop cultivation on soil and water quality. |
| **Document No** | GM Crops (2010) 1:1 16-24. |
| **Short description of literature article** | Potential risks and benefits of glyphosate on soil and water are assessed and compared to the effects of the herbicides that are replaced when glyphosate-resistant crops (GRCs) are adopted. |
| **Short description of findings** | The presented results indicated that perhaps the most important indirect effect is that GRCs promote the adoption of reduced or no-tillage agriculture, resulting in a significant reduction in soil erosion and water contamination.<br><br>Being a broad-spectrum, foliar applied herbicide, with little or no activity in soil, glyphosate is highly compatible with reduced- or no-tillage agriculture and has contributed to the adoption of these practices in the Western Hemisphere. This contribution to environmental quality by GRCs is perhaps the most significant one. Numerous regulatory tests of glyphosate and glyphosate products, using rigorous protocols meeting international standards, as well as product post-marketing surveillance, have failed to reveal any effects that could help substantiate any claims of adverse health and environmental outcomes.<br><br>Glyphosate and AMPA residues are not usually detected in high levels in ground or surface water in areas where glyphosate is used extensively. Furthermore, both glyphosate and AMPA are considered to be much more toxicologically and environmentally benign than most of the herbicides replaced by glyphosate. |
| **Relevance of this literature article to the submission** | This literature article is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | This paper provides an overview of GRC (Transgenic glyphosate-resistant crops) related studies and aim to contrast certain risks of GRCs with the risks that the GRCs displace.<br>It states that potential effects of glyphosate on soil and water are minimal, compared to the effects of the herbicides that are replaced when GRCs are adopted.<br>It states that GRCs crops promote the adoption of reduced- or no-tillage agriculture, resulting in a significant reduction in soil erosion and water contamination. Glyphosate and its degradation product, aminomethylphosphonate (AMPA), residues are not usually detected in high levels in ground or surface water in areas where glyphosate is |

used extensively. Furthermore, both glyphosate and AMPA are considered to be much more toxicologically and environmentally benign than most of the herbicides replaced by glyphosate.

Additionnal notes by RMS:
Other studies referenced in this paper concluded that there was still insufficient data to determine whether glyphosate application increases incidence of Fusarium spp. associated diseases in GR crops. Other stated that high doses of glyphosate in soil reduce colonization of pepper (Capsicum annuum) plant roots with mycorrhizae. Whether effects were due to a direct effect on the mycorrhizae or to effects on the plant is not known. The doses of glyphosate used also inhibited growth of pepper. However, plants with mycorrhizae were more resistant to the growth-inhibiting effects of glyphosate.

## 2. Duke *et al.*, 2012

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| **Data point** | M-CP 10 |
| **Author** | Duke, S., Lydon, J., Koskinen, W., Moorman, T., Chaney, R., Hammerschmidt, R. |
| **Year** | 2012 |
| **Title** | Glyphosate effects on plant mineral nutrition, crop rhizosphere microbiota and plant disease in glyphosate-resistant crops. |
| **Document No** | Journal of Agricultural and food chemistry 2012, 60, 10375-10397 |
| **Short description of literature article** | This paper evaluates literature on glyphosate-resistant (GR) crops, regarding impact of mineral deficiencies and increased plant disease. |
| **Short description of findings** | Although it is clear that glyphosate does increase severity of disease on glyphosate sensitive plants, the published evidence for its effects on GR plants presents a different story. Overall, it appears that in GR crops the baseline disease resistance or susceptibility of the host plant, not the presence of the glyphosate resistance gene or treatment with glyphosate, is the major contributor to susceptibility. The yield data for crops that are now predominantly GR cultivars do not support the view that there are significant mineral nutrition or disease problems with GR crops. There might be effects of glyphosate in GR crops on mineral nutrition and/or disease under particular but uncommon conditions (e.g., specific soil, environmental conditions, particular GR crop cultivars, and/or glyphosate formulations). |
| **Relevance of this literature article to the submission** | This literature article is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | This review concludes that:<br>- although there is conflicting literature on the effects of glyphosate on mineral nutrition on GR (glyphosate-resistant) crops, most of the literature indicates that mineral nutrition in GR crops is not affected by either the GR trait or by application of glyphosate;<br>- most of the available data support the view that neither the GR transgenes nor glyphosate use in GR crops increases crop disease;<br>- yield data on GR crops do not support the hypotheses that there are substantive mineral nutrition or disease problems that are specific to GR crops.<br><br>However, the finding that GR crops with only a change in their EPSPS are about 50-fold less sensitive to glyphosate than similar GS crops indicated that mineral nutrition is not involved in the mode of action of<br>glyphosate.<br><br>RMS considers that only data on GS (glyphosate-sensitive) crops are relevant for the purpose of risk assessment.<br><br>RMS notes that further statements are available in the review on GS crops, i.e. sensitive crops. Mijangos et al. examined glyphosate effects on GS plants (triticale and peas) and their rhizosphere microbial communities. Ammonia concentrations increased in |

rhizosphere soil after glyphosate treatment compared to the control. Functional diversity of the rhizosphere microbial community was examined. Community diversity and richness were reduced at the highest rate of glyphosate application in rhizospheres of killed GS pea and GS triticale, but not in soil from triticale grown alone.
(This study Mijangos et al was not assessed by RMS)

Also the review states that glyphosate can have effects on mineral nutrition of GS plants through its herbicidal effects on plant roots and other parts of the plant.

It also states that treatment of GS plants with glyphosate can result in increased susceptibility to pathogens.

RMS also notes the following information of interest: "*Metal cations are present in a tank mix solution, and pH is raised by addition of microelement fertilizer or by hard water, precipitation of glyphosate reduces the plant uptake of glyphosate, thereby significantly reducing its herbicidal effectiveness*".

### 3. Knoex *et al.*, 2008

| | |
|---|---|
| **Data point** | M-CP 10 |
| **Author** | Knox, O., Nehl, D., Mor, T., Roberts, G., Gupta, V. |
| **Year** | 2008 |
| **Title** | Genetically modified cotton has no effect on arbuscular mycorrhizal colonization of roots. |
| **Document No** | Field Crops Research 109 (2008) 57-60. |
| **Short description of literature article** | This paper investigates the conjecture that genetically modified plants, expressing insecticidal or herbicidal tolerance traits, do not form mycorrhizal symbioses. A comparison was made in the mycorrhizal development in commercial cultivars of cotton expressing genes for insect resistance, glyphosate tolerance or both and their conventional parent line. Shoots and roots were analyzed, cotton weights measured and soil cores taken from each field plot. |
| **Short description of findings** | The findings of the experiment reported in this paper clearly indicate that field grown cotton, regardless as to whether it is conventional or GM for either insecticidal or herbicide tolerance or both traits, is mycorrhizal. |
| **Relevance of this literature article to the submission** | This literature article is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | This study indicates that field grown cotton, regardless as to whether it is conventional or GM for either insecticidal or herbicide tolerance or both traits, is mycorrhizal.<br>It does not imply an application of glyphosate (only the genetically modified plant).<br>The paper is not relevant for the assessment of glyphosate. Besides, in Europe, cropping systems are not carried out with glyphosate resistant crops (GMO). |

**4. Silva e*t al*., 2018**

| | |
|---|---|
| **Data point** | M-CP 10 |
| **Author** | Silva, V., Montanarella, L., Jones, A., Fernández-Ugalde, O., Mol, H.,<br>Ritsema, C., Geissen, V. |
| **Year** | 2018 |
| **Title** | Distribution of glyphosate and aminomethylphosphonic acid (AMPA) in agricultural topsoils of the European Union |
| **Document No** | Science of the total environment (2018) 621:1352-1359 |
| **Short description of literature article** | The study describes the result from a field experiment with consecutive GIS analysis to estimate the distribution of glyphosate and AMPA in European topsoils and estimates their potential spreading by wind and water erosion. |
| **Short description of findings** | Glyphosate and/or AMPA were present in 45 % of the topsoils collected, originating from eleven countries and six crop systems, with a maximum concentration of 2 mg/kg. Several glyphosate and AMPA hotspots were identified across the EU. |
| **Relevance of this literature article to the submission** | This literature article is used to provide additional information on indirect effects and biodiversity.<br>This paper was also covered by the LRR (KCA 7.5 monitoring data). |
| **RMS comments and conclusion** | Study summary available in Volume 3 CA B.8.for fate section. |

## 5. Sullivan & Sullivan, 2000

Summary already submitted under Part 2, point 13 on this document.

## 6. Powell *et al.*, 2009

| | |
|---|---|
| **Data point** | M-CP 10 |
| **Author** | Powell, J., Campbell, R., Dunfield, K., Gulden, R., Hart, M., Levy-Booth, D., Kilronomos, J., Pauls, K., Swanton, C., Trevors, J., Antunes, P. |
| **Year** | 2009 |
| **Title** | Effect of glyphosate on the tripartite symbiosis formed by *Glomus intraradices*, *Bradyrhizobium japonicum*, and genetically modified soybean. |
| **Document No** | Appl. Soil Ecol. 41:128−136. |
| **Short description of literature article** | Studies were conducted to (1) estimate the effects of glyphosate on the establishment and functioning of AM and rhizobial symbioses with GM soybean, and (2) to estimate the interdependence of the symbioses in determining the response of each symbiosis to glyphosate. These objectives were addressed in two experiments; the first investigated the importance of the timing of glyphosate application in determining the responses of the symbionts and the second varied the rate of glyphosate application. |
| **Short description of findings** | The results concluded that Glyphosate applied at recommended field rates had no effect on *Glomus intraradices* or *Bradyrhizobium japonicum* colonization of soybean roots, or on soybean foliar tissue. $N_2$-fixation was greater for glyphosate-treated soybean plants than for untreated plants in both experiments, but only when glyphosate was applied at the first trifoliate soybean growth stage. These data deviate from previous studies estimating the effect of glyphosate on the rhizobial symbiosis, some of which observed negative effects on rhizobial colonization and/or $N_2$-fixation. We did observe evidence of the response of one symbiont (stimulation of $N_2$-fixation following glyphosate) being dependent on co-inoculation with the other; however, this interactive response appeared to be contextually dependent as it was not consistent between experiments. |
| **Relevance of this literature article to the submission** | This literature article is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | Glyphosate applied at recommended field rates had no effect on Glomus<br>intraradices or Bradyrhizobium japonicum colonization of soybean roots, or on soybean foliar tissue [P]. $N_2$-fixation was greater for glyphosate-treated soybean plants than for untreated plants in both experiments, but only when glyphosate was applied at the first trifoliate<br>soybean growth stage.<br>These data deviate from previous studies estimating the effect of glyphosate on the rhizobial symbiosis, some of which observed negative effects on rhizobial colonization and/or $N_2$-fixation.<br><br>GM soybean was used. |

**7. Lu *et al*., 2018**

| | |
|---|---|
| **Data point** | M-CP 10 |
| **Author** | Lu GH, Hua XM, Cheng J, Zhu YL, Wang GH, Pang YJ, Yang RW, Zhang L, Shou H, Wang XM, Qi J, Yang YH. |
| **Year** | 2018 |
| **Title** | Impact of Glyphosate on the Rhizosphere Microbial Communities of An EPSPS Transgenic Soybean Line ZUTS31 by Metagenome Sequencing. |
| **Document No** | Current Genomics. 19 (36-49) |
| **Short description of literature article** | Studies were conducted to clarify whether glyphosate has impact on nitrogen-fixation, pathogen or disease suppression, and rhizosphere microbial community of a soybean *EPSPS*-transgenic line ZUTS31 in one growth season. |
| **Short description of findings** | The results concluded that the formulation of glyphosate-isopropylamine salt did not significantly affect the alpha and beta diversity of the rhizobacterial community of the soybean line ZUTS31, whereas it significantly influenced some functional genes involved in nitrogen-fixation in rhizosphere soil during the single growth season after glyphosate treatment. |
| **Relevance of this literature article to the submission** | This literature article is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | Comparative analysis of the soil rhizosphere microbial communities was performed by 16S rRNA gene amplicons sequencing and shotgun metagenome sequencing analysis between the soybean line ZUTS31 foliar sprayed with diluted glyphosate solution and those sprayed with water only in seed-filling stage.<br>This study indicates that the formulation of glyphosate-isopropylamine salt did not significantly affect the alpha and beta diversity of the rhizobacterial community of the soybean line ZUTS31, whereas it significantly influenced some functional genes involved in PGPT (Plant Growth Promoting Traits) in the rhizosphere during the single growth season. |

**8. Savin *et al*., 2009**

| | |
|---|---|
| **Data point** | M-CP 10 |
| **Author** | Savin, M., Purcell, L., Daigh, A., Manfredini, A. |
| **Year** | 2009 |
| **Title** | Response of mycorrhizal infection to glyphosate applications and P fertilization in glyphosate-tolerant soybean, maize and cotton. |
| **Document No** | Journal of Plant Nutrition. 32:1702−1717. |
| **Short description of literature article** | Glasshouse studies were conducted to investigate the impact of glyphosate and phosphorus (P) fertilizer on mycorrhizal infection rates of glyphosate-tolerant cotton, maize, and soybean. |
| **Short description of findings** | Microbial biomass, water soluble P, Mehlich-3 P, and acid and alkaline phosphatase activities were not significantly impacted by glyphosate or P in the greenhouse. Phosphorus fertilization decreased mycorrhizal infection rates in cotton and maize and increased shoot biomass and shoot P in soybean in 2005, and decreased mycorrhizal infection in soybean and increased shoot biomass in cotton and maize and shoot P in all three crops in 2006. In pasteurized soil, glyphosate decreased percent mycorrhizal infection in maize, increased infection in cotton, and did not significantly affect infection in soybean. When soil was not pasteurized, glyphosate did not significantly alter mycorrhizal infection in any crop. It was concluded that the potential for glyphosate to alter AM fungal infection in glyphosate-tolerant plants may depend on whether soil microbial communities are compromised by other factors. |
| **Relevance of this literature article to the submission** | This literature article is used to provide additional information on indirect effects and biodiversity. |
| **RMS comments and conclusion** | The objective of this study was to determine if dynamics of the rhizosphere microbial community were altered by applications of glyphosate and P fertilizer to glyphosate-tolerant cotton, maize, and soybean growing in low-P soil in the greenhouse.<br>The hypothesis tested was that glyphosate application to glyphosate tolerant crops changes the rhizosphere microbial community such that plant growth and crop productivity may be hindered under conditions of low phosphorus nutrition.<br>In first experiment, soil was pasteurized, Roundup Weather Max, (1.1 kg ae/ha) was applied.<br>In second experiment, soil was not pasteurized, Roundup Original Max (same rate as in first experiment) was applied.<br><br>Overall, the study concludes that when the indigenous soil community and potential inoculum was not altered by pasteurization, glyphosate was not inhibitory nor stimulatory to mycorrhizal infection rates after six weeks of plant growth.<br>In contrast, pasteurization, while not reducing the total microbial biomass, did impose a stress on the microorganisms and likely inhibited particular microbes and biochemical functioning in the soil. The potential for glyphosate to alter arbuscular mycorrhizal fungal infection in glyphosate-tolerant plants may depend on whether soil microbial communities are compromised by other factors. |

## PART 7: REFERENCES FOR ASSESSMENT OF INDIRECT EFFECTS VIA TROPHIC INTERACTIONS FOR NON-TARGET TERRESTRIAL PLANTS DISCUSSION

### 1. Koning *et al*., 2019

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|---|---|
| **Data point** | M-CP 10 |
| **Author** | Koning, L., de Mol, F., Gerowitt, B. |
| **Year** | 2019 |
| **Title** | Effects of management by glyphosate or tillage on the weed vegetation in a field experiment. |
| **Document No** | Soil and Tillage Research 186 (2019) 79-86. |
| **Short description of literature article** | This paper investigates the effects of glyphosate applications versus tillage on the weed vegetation in a field experiment that focused solely on the treatments and the weeds without the complication of a crop or crop rotation.<br><br>Two different glyphosate doses were included in the experiment, 100% and 50% of the recommended dose on the product label, in order to assess the effect of both a normal frequent application as well as the effect of a frequently applied reduced dose. Two different tillage methods were investigated, chisel plow and mould board plow, to evaluate the influence of a minimal versus a fully soil turning approach to plowing. It was hypothesized that each of the treatments would lead to unique results in the factors of weed species number, density, true diversity and community composition due to the unique pressure exerted by each method. |
| **Short description of findings** | The findings of the experiment reported in this paper indicate that the hypothesis that each of the treatments would lead to unique results were confirmed. However, the main driving factor is whether and how the soil is disturbed, which was zero in the glyphosate treatments. In the experiments, applying glyphosate further selected for species being better protected against this herbicidal ingredient. |
| **Relevance of this literature article to the submission** | This literature article is used to provide additional information on indirect effects, best management practices, and biodiversity. |
| **RMS comments and conclusion** | Overall, any method employed influenced the weed composition in some way. Some species were favored over others depending on the weed management method, but the overall biodiversity of the weed community was not more negatively affected by one method compared to another.<br>Species which were distinctly more rare on plots treated with glyphosate than on tillaged plots belonged especially to two groups: root and rhizome propagating species (Cirsium arvense, Equisetum arvense, Elymus repens, Rumex acetosella) and annual agricultural weeds with no pronounced seasonality in their germination (Stellaria media, Matricaria chamomilla, Capsella bursa-pastoris, Lamium purpureum).<br>The weed community in the glyphosate treatments with differing doses grew apart over time. Particularly Chenopodium album and |

| | |
|---|---|
| | Epilobium tetragonum were spared by the 50% glyphosate dose compared to the 100% dose. |

## 2. Colbach N. et. al.

| Data point: | CA 9 |
|---|---|
| Report author | Colbach N. *et. al.* |
| Report year | 2018 |
| Report title | Landsharing vs landsparing: how to reconcile crop production and biodiversity? A simulation study focusing on weed impacts |
| Document No | Agriculture, Ecosystems and Environment (2018), Vol. 251, pp. 203-217 |
| Guidelines followed in study | None |
| Deviations from current test guideline | None |
| GLP/Officially recognised testing facilities | No, not conducted under GLP/Officially recognised testing facilities |
| Acceptability/Reliability: | Not relevant by title/abstract |

**Full summary of the study according to OECD format**

Weeds are harmful for crop production but are crucial for biodiversity in agricultural landscapes. Two contrasting strategies exist for reconciling these ecosystem services: landsharing, where crop production and biodiversity are maximised in individual fields, or landsparing, where some fields or habitats are assigned for biodiversity conservation while the remaining fields aim to maximise production. The objective of the present study was to evaluate these two strategies in silico, based on a case study with maize-based cropping systems including genetically modified varieties that allow the use of the highly efficient herbicide glyphosate in crops. The virtual-field model FLORSYS simulates multi-species weed floras and their impact on crop production and biodiversity depending on cropping systems and pedoclimate. It was scaled up to the landscape level by simulating several fields in parallel, including semi-natural habitats and integrating between-field seed dispersal depending on plant height, seed mass and dispersal mode. Three series of scenarios were simulated over 28 years and 10 weather repetitions in a small landscape consisting of four 3-ha fields in Aquitaine (South-Western France): (1) landsharing scenarios based on a single diverse rotation (soybean/maize/wheat/maize), with different crop patterns in the landscape, (2) landsparing scenarios with varying proportions (ranging from 0 to 100%) of contrasting cropping systems in the landscape, either cropping system aiming to maximise biodiversity or one aiming to maximise production, and (3) landsparing scenarios including permanent grass strips (10% of each field).

**Materials and methods**

*A short presentation of FLORSYS*

Weed and crop life-cycle

FLORSYS is a virtual field where cropping systems can be experimentally tested and a large range of crop, weed and environmental measures estimated.

The input variables of FLORSYS consist of (1) a description of the simulated field (daily weather, latitude and soil characteristics); (2) all the simulated cultural operations in the field, with dates, tools and options; and (3) the initial weed seed bank which is chosen to reflect the regional species pool. These input variables influence the annual life-cycle which applies to annual weeds and crops, with a daily timestep. Pre-emergent stages (surviving, dormant and germinating seeds, emerging seedlings) are driven by soil structure, temperature and water potential. Post-emergent processes (e.g. photosynthesis, respiration, growth, etiolation) are driven by light availability and air temperature. Crop:weed canopy is simulated with a 3D, individual-based representation. At plant maturity, weed seeds are added to the soil seed bank; crop grains are harvested to determine crop yield. Life-cycle processes also depend on management practices, in interaction with weather and soil conditions on the day the operations are carried out. To reduce the simulation time greatly lengthened by the 3D canopy representation, usually only a representative field sample (e.g. 6 m × 3 m) is simulated. Total seed and plant populations of the simulated field are then deduced by multiplying simulated densities by the ratio of the total vs. simulated field areas. FLORSYS parameters are currently available for 25 weed species. The advent of glyphosate resistance in HR systems was not considered here as a previous study showed that the impact of glyphosate resistance on biodiversity and crop production was negligible at the temporal scale (30 years) and in the cropping systems analysed here (Colbach *et al*., 2017a).

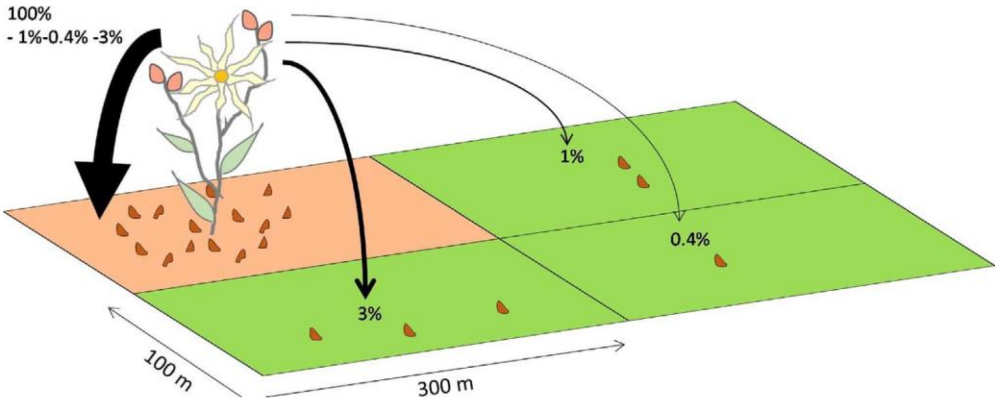
<u>Domain of validity</u>
FLORSYS was evaluated with independent field data, showing that daily weed species densities and, particularly, densities averaged over the years were generally well predicted and ranked in the model's original region, i.e. Burgundy (Colbach et al., 2016). At more southern latitudes, a corrective patch was necessary to keep weeds from flowering during winter. This correction was also used in the present simulations while awaiting the development of a better phenology submodel in FLORSYS. Crop densities and yields were also generally predicted satisfactorily.

<u>Assessing weed impacts on crop production and biodiversity</u>
To make yields of different crop species comparable, yields were transformed into energy production by multiplying them by their energy content (Lechenet *et al*., 2014). The weed densities simulated by FLORSYS were translated into nine weed-impact indicators depicting the weed flora impact on crop production and biodiversity (Mézière *et al*., 2015b). Two indicators assess the weed harmfulness for crop production, i.e. crop yield loss and harvest pollution by weed debris. A third indicator assesses harvesting problems due to green weed biomass blocking the combine. A fourth indicator, i.e. field infestation by weed biomass during crop growth, assesses sociological harmfulness and reflects the farmer’s worry of being thought incompetent by his peers even there is no effect on yield loss.

Five biodiversity indicators reflect the contribution weeds make to biodiversity. The two first focus on wild plant diversity, i.e. weed species richness, and weed species equitability. Three others assess the role of weeds as food resources for three types of major organisms in the agro-ecosystems, i.e. weed seeds on soil surface in autumn and winter to feed field birds, lipid-rich seeds on soil surface in summer to feed carabids, and weed flowers in spring and summer to feed domestic bees. The contribution of cropped plants to biodiversity was not included.

*The changes necessary for upscaling the model*
<u>Parallelize fields</u>
Instead of a single field, a cluster of neighbouring fields is simulated (Fig. 1). Each field is located on the simulated cluster via the coordinates of its vertices, V1(x1y1), …Vn(xn,yn). Any number of vertices and any field shape and area are accepted. Each field is simulated via parallel runs of FLORSYS, depending on its particular soil texture, cultural practices and initial weed flora. Only weather is common to all fields. The area and shape of the simulated field polygon determine the rate of seeds dispersed between fields but have no effect on how seeds and plants fare inside fields.

**Figure 1.** Example of field cluster of four contiguous fields simulated with FLORSYS, with seed dispersal among fields. Dispersal percentages are those of *Amaranthus retroflexus*.




Semi-natural habitats

The simulated polygon cluster can also include semi-natural habitats such as permanent grass strips required by EU legislation (EU Regulation No 1307/2013) to protect water courses from pesticide drifts, permanent flower strips to promote insect biodiversity or simply uncultivated field edges or road margins. Here, we focused on sown grass strips which are a few meters wide and follow the whole field edge. These strips are usually sown with a mixture of multi-annual grass and legume species after a few tillage operations (Cordeau *et al*., 2010). After that, only mowing is permitted in grass strips; tillage, pesticides or fertilizers are prohibited.

In FLORSYS, weeds and their contribution to biodiversity are simulated in these habitats, using the same formalisms as in arable fields. Three multi-annual species were parameterized for FLORSYS, i.e. two legumes (*Medicago sativa* and *Trifolium repens*) and one grass species (*Lolium perenne*). The FLORSYS life-cycle was modified to allow vegetative regrowth of multi-annual plants: when a multi-annual plant reaches its maximum possible plant height and width, an offspring is created and placed randomly at the outer rim of the parent plant.

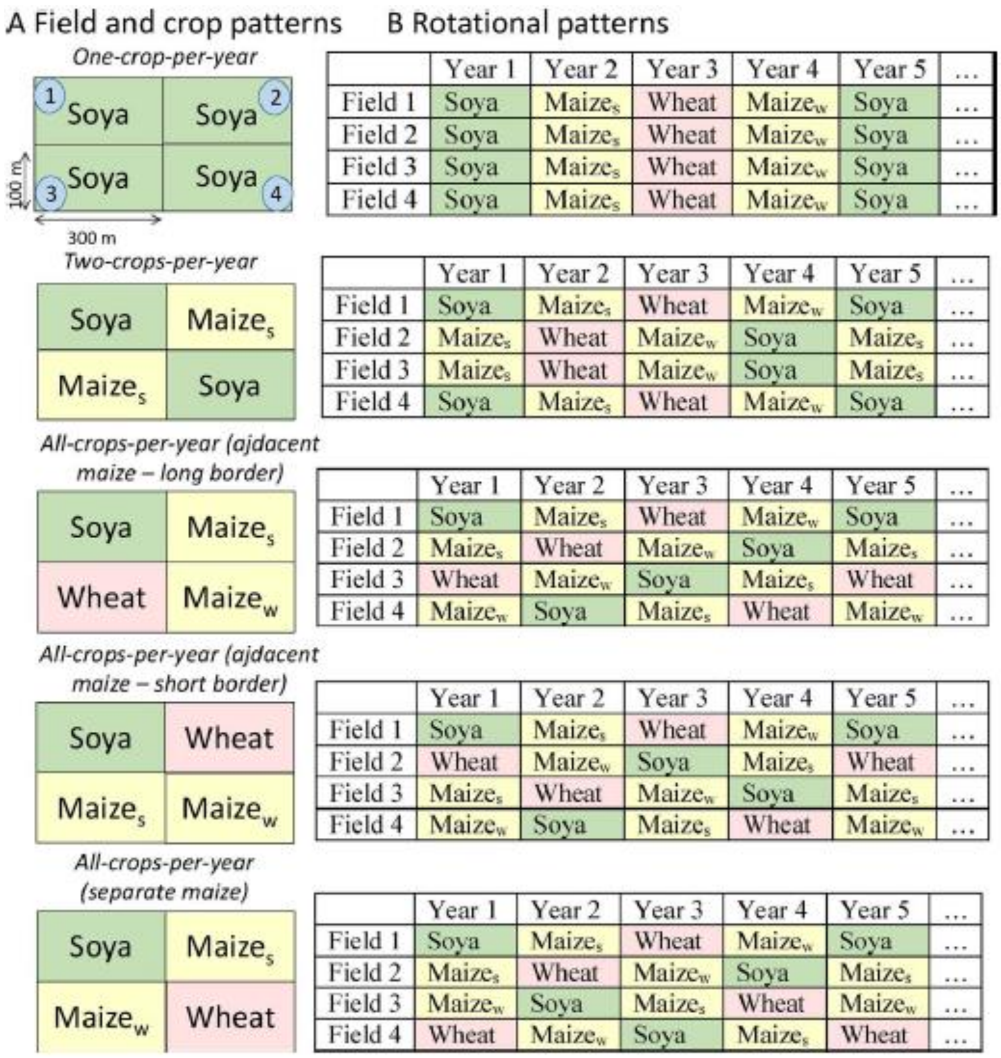
In addition, when a field or grass strip is mown, cut plants (whether annual or multi-annual) produce new shoots if they have not yet started to produce seeds. Their flowering and maturation are delayed compared to uncut plants, and their biomass accumulation through photosynthesis is reduced because of leaf-area loss.

Seed dispersal by natural vectors

Seed dispersal among pairs of plots (fields or semi-natural habitats) was simulated following the principle developed by Colbach *et al*. (Colbach *et al*., 2001; Colbach and Sache, 2001). First, data from Thomson *et al*. (2011) was used to predict mean and maximum dispersal distances as a function of species traits known to influence seed dispersal distances, i.e. height from which the plant releases its seed (or plant height if unknown), seed mass and dispersal mode.

Simulation study

Several simulation series were run: (1) landsharing scenarios based on a single diverse rotation, with different crop patterns in the landscape, (2) the same scenarios disregarding weed seed dispersal among fields, (3) landsparing scenarios, with varying proportions of contrasting cropping systems in the landscape, and (4) landsparing scenarios including permanent grass strips. All simulations were run with a simplistic field pattern, consisting of four rectangular fields, resulting in a total of 12 ha for the field cluster.




| | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | … |
|---|---|---|---|---|---|---|
| Field 1 | Soya | Maize$_s$ | Wheat | Maize$_w$ | Soya | … |
| Field 2 | Soya | Maize$_s$ | Wheat | Maize$_w$ | Soya | … |
| Field 3 | Soya | Maize$_s$ | Wheat | Maize$_w$ | Soya | … |
| Field 4 | Soya | Maize$_s$ | Wheat | Maize$_w$ | Soya | … |

| | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | … |
|---|---|---|---|---|---|---|
| Field 1 | Soya | Maize$_s$ | Wheat | Maize$_w$ | Soya | … |
| Field 2 | Maize$_s$ | Wheat | Maize$_w$ | Soya | Maize$_s$ | … |
| Field 3 | Maize$_s$ | Wheat | Maize$_w$ | Soya | Maize$_s$ | … |
| Field 4 | Soya | Maize$_s$ | Wheat | Maize$_w$ | Soya | … |

| | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | … |
|---|---|---|---|---|---|---|
| Field 1 | Soya | Maize$_s$ | Wheat | Maize$_w$ | Soya | … |
| Field 2 | Maize$_s$ | Wheat | Maize$_w$ | Soya | Maize$_s$ | … |
| Field 3 | Wheat | Maize$_w$ | Soya | Maize$_s$ | Wheat | … |
| Field 4 | Maize$_w$ | Soya | Maize$_s$ | Wheat | Maize$_w$ | … |

| | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | … |
|---|---|---|---|---|---|---|
| Field 1 | Soya | Maize$_s$ | Wheat | Maize$_w$ | Soya | … |
| Field 2 | Wheat | Maize$_w$ | Soya | Maize$_s$ | Wheat | … |
| Field 3 | Maize$_s$ | Wheat | Maize$_w$ | Soya | Maize$_s$ | … |
| Field 4 | Maize$_w$ | Soya | Maize$_s$ | Wheat | Maize$_w$ | … |

| | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | … |
|---|---|---|---|---|---|---|
| Field 1 | Soya | Maize$_s$ | Wheat | Maize$_w$ | Soya | … |
| Field 2 | Maize$_s$ | Wheat | Maize$_w$ | Soya | Maize$_s$ | … |
| Field 3 | Maize$_w$ | Soya | Maize$_s$ | Wheat | Maize$_w$ | … |
| Field 4 | Wheat | Maize$_w$ | Soya | Maize$_s$ | Wheat | … |

Fig. 2. Cluster of four contiguous fields simulated with FLORSYS showing the crop patterns of a soybean/maize/wheat/maize rotation at 1st year (A) and the rotational crop patterns over time (B) in the landsharing scenarios. Subscripts w and s indicate previous crops of maize (Nathalie Colbach ©2016).

Landsharing scenarios

Each field of the four-field cluster was managed with the same cropping system aiming to produce medium biodiversity and medium crop production, a soybean/maize/wheat/maize rotation with superficial tillage, glyphosate in maize and conventional herbicide treatments in other crops. This system had been identified in previous simulation studies as the best compromise for reconciling production and biodiversity in a given field. Five annual crop-patterns were tested, with one, two or all crops present each year. The three all-crops-per-year scenarios differed in the annual crop allocation in the field cluster. In two scenarios, the two maize crops of the rotation were always grown in adjacent fields but differed in the length of the common border. In the third scenario, maize was always grown in separate fields.

Landsparing vs. landsharing among fields

The field pattern was managed with a combination of cropping systems aiming either at high production or at high biodiversity. Five combinations were tested, growing (1) 100% and 0%, (2) 75% and 25%, (3) 50% and 50%, (4) 25% and 75%, and (5) 0% and 100% of fields with the high-production and the high-biodiversity cropping systems, respectively. The systems used here were both HR maize monocultures with glyphosate spraying in June, the high-production system with superficial tillage followed by sowing on 10 May, the high-biodiversity system with direct drilling on 1 May.

Landsparing with grass strips

The same four-field cluster was used, with 10% of each field grown with permanent grass strips and the remaining with the high-production cropping system. The grass strips were a mixture of *Lolium perenne* and *Trifolium repens* aiming at 95% of grass and 5% of legume plants. They were sown after rolling, following a wheat crop managed according to the medium cropping system. Three annual mowing frequencies in grass strips were tested, either none, one (mid May, with biomass export) or three operations (early May, late June, early Sept.) per year. Tillage, pre-sowing herbicide programmes and cut biomass management were kept constant as a preliminary sensitivity analysis had shown that the effect of these practices on weed-impact indicators was negligible.

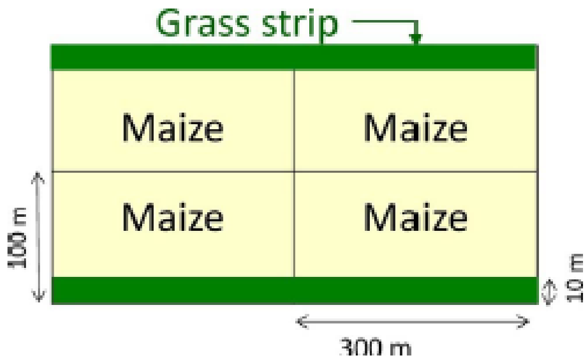



**Fig. 3.** Cluster of four contiguous fields including 10% permanent grass strips and 90% crop area grown with the high-production maize monoculture cropping system (Nathalie Colbach ©2016).

The effect of weed seed dispersal

The 13 scenarios were also simulated without any weed seed dispersal. The comparison of the two series, with or without seed dispersal, allowed us to quantify how much weed dynamics and impact in individual fields were changed when seed dispersal was included.

Statistics

The analysed output variables were the weed-impact indicators at the landscape scale. The indicator values weighted by the relative plot area were averaged over the field cluster (hence "landscape"). If the landscape included grass strips, biodiversity indicators and crop production were calculated over the four fields and four grass strips, while the harmfulness indicators were calculated only over the fields.

First, scenarios were assessed based on a multi-criteria evaluation, using a Principal Component Analysis on the weed-impact indicators (column) by scenarios (row) matrix, using the FactoMineR package (Lê *et al*., 2008) using the R software version 3.3.0 (R Development Core Team, 2016).

Then, scenarios were assessed using individual indicators. Analyses of variance were run with PROC GLM of SAS to explain indicator values as a function of scenario, weather repetition, time and interaction between cropping system and time. Least-square means of indicator values were compared for cropping systems, using least-significant difference tests (p =0.05).

Finally, the stability of performance over time was assessed. Standard-deviations of indicator values over time were calculated and analysed as a function of scenario and weather repetition.

## Results

*To share or to spare?*

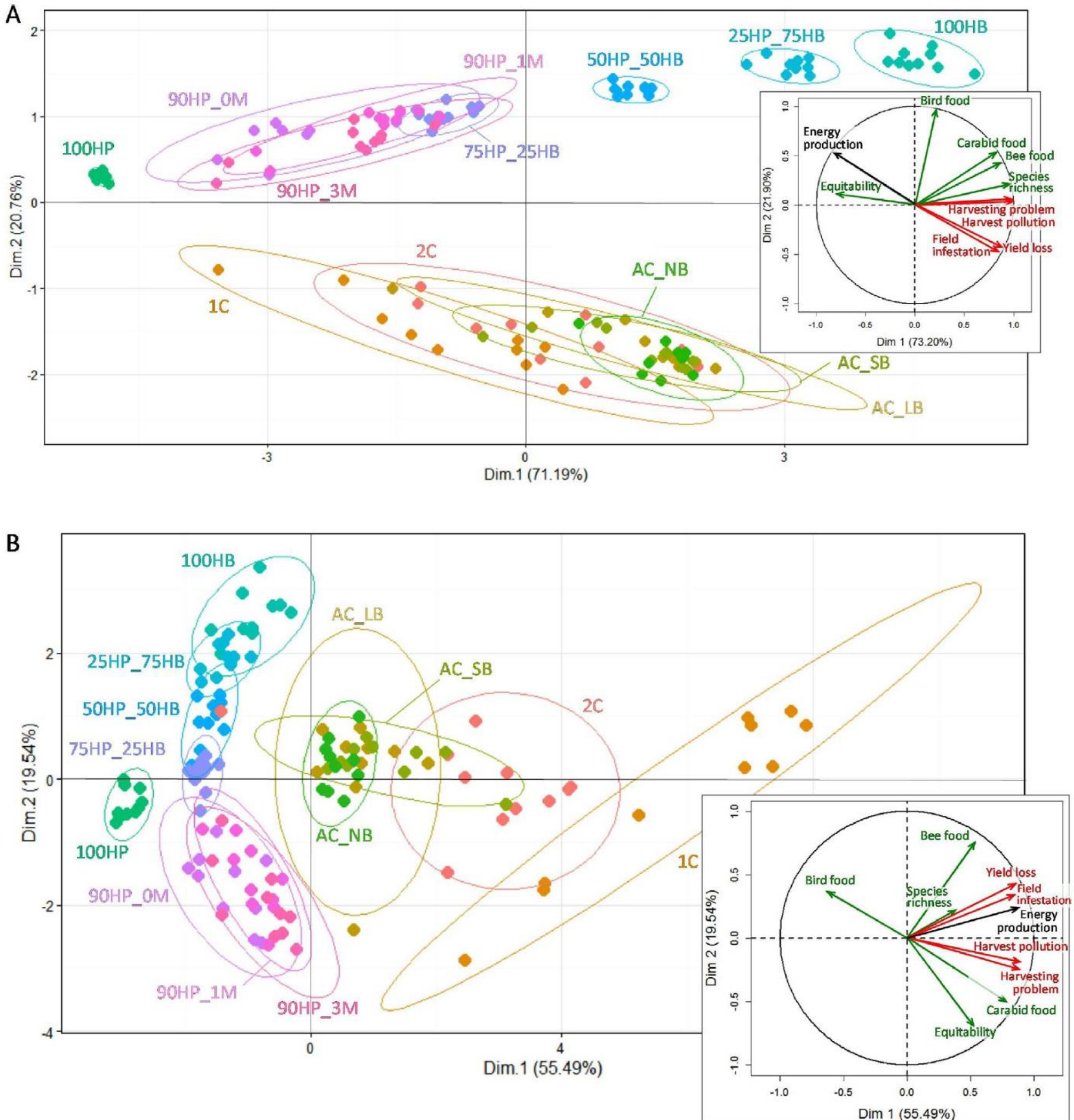
The first axis of the Principal Component Analysis diagram (Fig. 4A) accounted for 71.4% of the total variability where scenarios with high species equitability and crop energy production (left side) differed from scenarios with lower values of these indicators and higher values of biodiversity and weed harmfulness indicators, particularly crop yield loss (right side). The second axis accounted for 20.9% of the total variability where scenarios differed with respect to bird food provision.

The 100%-high-biodiversity scenario (100HB) was the scenario most closely associated with the FLORSYS biodiversity indicators (except species equitability) and the high-biodiversity cropping system had thus been judiciously chosen. The other landsharing scenarios were discriminated by axis 1 from the 100%-high-biodiversity (far right) to the 100%-high-production scenario (100HP, far left). The three scenarios with the 10% grass strips were located on axis 1 between the 75% and the 100%-high-production scenarios. The scenarios with a high percentage of high-production cropping systems were also those most closely associated with energy production and species equitability.

The landsharing scenarios with their diverse rotations (1C, 2C etc.) behaved quite differently and were discriminated by a yield loss vs. species equitability and energy production gradient. The more crops were grown each year in the landscape, the higher crop yield loss and the lower the production were. Moreover, the performance of the allcrops scenarios varied less among weather repetitions than the single-crop scenarios, i.e. the ellipses representing 95% confidence intervals were smaller.

Conversely, the more intensive the crop management was, the less the performance of the scenario varied, i.e. the 100%-high-production points were very close, in contrast to the 100%-high-biodiversity points. Including semi-natural habitats in a scenario (i.e. 90HP_0 M, 90HP_1 M, 90HP3 M) also increased the inter-repetition variability compared to the 100%-high-production scenarios.

We also analysed the scenarios based on the inter-annual variability in weed-impact indicator values (Fig. 4B). The two first axes accounted for only approximately 75% variability. Scenarios differed with respect to high bird-food variability (upper left side) and with high variability in carabid food and species equitability (lower right side) on one hand, with respect to high vs. low variability in bee food and crop yield loss on the other hand (upper right side vs. lower left side). Landsparing scenarios (based on monoculture) without grass strips were mostly associated with high bird-food variability. The more high-production cropping systems were included in a scenario (e.g. 100 HP), the less bird food and bee food varied over the years (i.e. the less they tended to be located toward the upper side). Landsparing scenarios including grass strips were those with the overall lowest inter-annual variability (located toward the lower left side). Landsharing scenarios (with diverse rotations) were all located toward the right side of the graph, indicating high interannual variability for all indicators, except bird food. The less crops were grown each year in the field cluster (e.g. 1C), the higher this variability was.




| Land-sharing | Crops per year | Maize fields |
|---|---|---|
| 1C | 1 | Cover whole landscape |
| 2C | 2 | Share no border |
| AC_NB | All | Share no border |
| AC_SB | All | Share short border |
| AC_LB | All | Share long border |

| Landsparing | % fields aiming to maximise | |
|---|---|---|
| | Crop production | Biodiversity |
| 100HP | 100 | 0 |
| 75HP_25HB | 75 | 25 |
| 50HP_50HB | 50 | 50 |
| 25HP_75HB | 25 | 75 |
| 100HB | 0 | 100 |

| Land-sparing with grass strips | % high-production fields | % grass strips | Grass strip mowing per year |
|---|---|---|---|
| 90HP_0M | 90% | 10% | 0 |
| 90HP_1M | 90% | 10% | 1 |
| 90HP_3M | 90% | 10% | 3 |

Fig. 4. Principal Component Analysis on the weed-impact indicators (column) by scenarios x weather repetition (row) matrix (with enlarged correlation circles as inserts). Contributions to axes can be found in Section D.5.4 online. Ellipses represent a 95% confidence interval. The longer the arrows are, the higher the correlations with each axis are. Landsharing scenarios used the same cropping system with a soya/maize/wheat/maize rotation and differed in the number of crops grown each year (see Fig. 2). Landsparing scenarios combined cropping systems aiming to maximise either crop production or biodiversity, and differed in the proportion of fields managed with each system. Landsparing scenarios with grass strips managed fields with a high-production system and differed in the mowing frequency of the grass strips (Nathalie Colbach ©2016).

A. Average over time and landscape for each scenario and weather repetition.

B. Inter-annual variability per scenario and weather repetition, i.e. standard-deviation of indicator values.

*To aggregate or segregate crops in time?*

The inter-annual variation in landscape weed-impact indicators depended on the annual crop-pattern in the landsharing scenarios. In the 1-crop-per-year scenario, the rotational patterns were clearly visible. In the 2-crops-per year scenario, the amplitude of the indicator variation was lower but there was still a crop-based pattern. In the three remaining scenarios where the same crops were grown each year, no temporal pattern was visible. Similar rotational patterns were observed for the other indicators. Generally, maize was the crop with the highest crop production and the lowest weed harmfulness, and wheat was usually the crop with the highest biodiversity.

Table 1: Effect of landsharing and landsparing scenarios on weed-impact indicators at the landscape scale. Comparison of means and partial $R^2$ after analyses of variance of indicators averaged over the field cluster as a function of scenario, weather repetition, time, and the interaction between scenario and time. All independent variables were qualitative ones. Means of a given column followed by the same letter are not significantly different at p = 0.05 (least significant difference test). Cells are coloured from red (lowest value) to green (highest value) for biodiversity indicators and crop production, from green (lowest) to red (highest value) for harmfulness indicators. (For interpretation of the references to color in this table legend, the reader is referred to the web version of this article.)

| | *Weed-related biodiversity in landscape* | | | | | *Crop* | *Weed harmfulness in crops (excluding grass strips)* | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Scenario | *Species richness* | *Species equitability* | *Bird food* | *Carabid food* | *Bee food* | *production (MJ/ha)* | *Yield loss* | *Harvest pollution* | *Harvesting problem* | *Field infestation* |
| **A. Landsharing scenarios: Annual crop pattern in landscape (crops per year) entirely grown with soybean/maize/wheat/maize** | | | | | | | | | | |
| One | 11.22 G | 0.35 E | 3.55 J | 2.54 I | 0.66 F | 68344 D | 22.68 E | 1.20 F | 1.47 F | 1.07 F |
| Two | 12.04 E | 0.33 FE | 4.16 HI | 3.39 G | 0.91 E | 60184 E | 33.92 C | 1.60 E | 1.89 E | 1.61 D |
| All (adjacent maize-long border) | 12.94 D | 0.31 G | 4.11 I | 3.94 F | 1.08 D | 55511 F | 40.20 B | 1.94 DC | 2.13 D | 1.98 B |
| All (adjacent maize- short border) | 12.82 D | 0.28 IH | 4.26 HG | 4.06 E | 1.07 D | 55807 F | 39.46 B | 1.92 DC | 2.21 DC | 1.92 CB |
| All (separate maize) | 12.94 D | 0.26 I | 4.31 G | 4.15 E | 1.11 D | 51920 G | 44.31 A | 2.04 C | 2.27 C | 2.15 A |
| **B. Landsparing scenarios: % fields with high-production vs. % fields with high-biodiversity cropping systems in *landscape*** | | | | | | | | | | |
| 0% - 100% | 15.72 A | 0.29 H | 9.58 A | 8.66 A | 2.78 A | 59257 E | 40.83 B | 2.78 A | 3.18 A | 1.83 C |
| 25% - 75% | 14.88 B | 0.31 G | 8.90 B | 7.53 B | 2.23 B | 70045 D | 30.30 D | 2.38 B | 2.75 B | 1.36 E |
| 50% - 50% | 13.26 C | 0.32 FG | 8.02 C | 6.13 C | 1.57 C | 80603 C | 19.69 F | 1.88 D | 2.16 DC | 0.87 G |
| 75% - 25% | 11.72 F | 0.40 C | 7.11 D | 4.43 D | 0.94 E | 90257 B | 9.93 G | 1.21 F | 1.43 F | 0.44 H |
| 100% - 0% | 8.44 J | 0.70 A | 5.36 F | 0.63 J | 0.18 I | 100452 A | -0.10 H | 0.00 J | 0.00 J | 0.00 I |
| **C. Landsparing scenarios with 10% grass strips and 90% high-production cropping systems in *landscape*: Mowing frequency in grass strips** | | | | | | | | | | |
| None | 10.81 H | 0.43 B | 6.83 E | 3.09 H | 0.47 H | 90295 B | 0.03 H | 0.52 I | 0.62 I | 0.01 I |
| 1 per year | 10.65 H | 0.37 D | 7.13 D | 3.49 G | 0.55 G | 90161 B | 0.14 H | 0.98 G | 1.13 G | 0.02 I |
| 3 per year | 10.18 I | 0.34 FE | 6.81 E | 3.16 H | 0.51 HG | 90272 B | 0.03 H | 0.52 I | 0.62 I | 0.01 I |
| **D. Partial R²** | | | | | | | | | | |
| Scenario | 0.34 | 0.39 | 0.76 | 0.83 | 0.70 | 0.50 | 0.54 | 0.46 | 0.46 | 0.46 |
| Weather repetition | 0.01 | 0.01 | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 | 0.00 | 0.00 | 0.01 |
| Time | 0.24 | 0.08 | 0.03 | 0.02 | 0.02 | 0.04 | 0.05 | 0.04 | 0.04 | 0.07 |
| Scenario*Time | 0.13 | 0.09 | 0.14 | 0.07 | 0.12 | 0.17 | 0.13 | 0.14 | 0.14 | 0.15 |

| | *Weed-related biodiversity in landscape* | | | | | *Crop* | *Weed harmfulness in crops (excluding grass strips)* | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Scenario | *Species richness* | *Species equitability* | *Bird food* | *Carabid food* | *Bee food* | *production (MJ/ha)* | *Yield loss* | *Harvest pollution* | *Harvesting problem* | *Field infestation* |
| ***A. Landsharing scenarios: Annual crop pattern in landscape (crops per year) entirely grown with soybean/maize/wheat/maize*** | | | | | | | | | | |
| Total | 0.72 | 0.57 | 0.93 | 0.92 | 0.83 | 0.72 | 0.72 | 0.64 | 0.63 | 0.69 |

All effects were significant at p = 0.0001.

The annual crop pattern also influenced the average performance of the landsharing scenarios. The more crops were present each year, the more biodiversity (except species equitability) and weed harmfulness increased, and the more production decreased. The increase was most important for crop yield loss and field infestation (approximately double in all-crop vs. one-crop scenarios) and lowest for wild plant diversity (+15% for species richness, −26% for equitability).

The location of the various crops in the field cluster also had an effect. Weed impact was highest in the all-crops scenario with separate maize fields and lowest in the one-crop scenario. The biggest increase occurred for yield loss and field infestation which approximately doubled; the lowest effect was observed for plant diversity, with only 15% increase for species richness and 25% decrease for species equitability. These differences were entirely due to weed seed dispersal between fields. Indeed, when the same scenarios were simulated again without seed dispersal, the differences between scenarios were negligible. The remaining differences were due to interactions with the year, i.e. depending on the scenario, a given crop could be grown in different years and thus under different weather conditions, depending on the annual crop mixture.

In the all-crops scenarios with separate maize, a field grown with winter wheat (which allows most weed development) in year N is always adjacent to a field grown with wheat in year N +1. This configuration allows the highest spatio-temporal weed dispersal, particularly when previous and next wheat crops share a long border (Fig. 6A vs. B). In the all-crops scenarios with adjacent maize, previous and next wheat crops would only link up in half of the years, via a long border when simultaneous maize shared a short border and vice versa. The latter case was less favourable to weed dynamics than the former (Fig. 6B vs. A). In the one-crop scenario, consecutive wheat crops were never adjacent, which resulted in the lowest dynamics (Fig. 6C). The two-crops scenario was intermediate: in one year out of four, spatio-temporal dispersal was very high, with two wheat crops in year N + 1 adjacent of wheat crops in year N resulting in a combination of long-border and short-border dispersal (Fig. 6A and B); during the remaining years, there was either no wheat or no previous wheat, and thus little spatio-temporal dispersal.

The lower average crop production performance in the all-crops scenarios was compensated by a greater stability, in terms of production, weed harmfulness and biodiversity (Fig. 4B). The variability was divided by approximately three for carabid food offer and crop production when comparing the one-crop and all-crops scenarios; it was only reduced by 30% for bee food offer and field infestation, and it increased by 20% for bird food offer.

*How much area for biodiversity?*
Almost all grass-strip-free landsparing scenarios performed better than any of the landsharing scenarios. The scenario associating 75% high-production and 25% high-biodiversity systems was best for reconciling production and biodiversity. It performed better than all landsharing scenarios, both in terms of production and biodiversity. The one exception was bee food for which 50% highbiodiversity systems or more were needed in the landscape to outperform the landsharing scenarios. Of course, when the objective was only crop production, than the scenario with 100% high production was the most adequate while the 100% high-biodiversity scenario was best when aiming at only biodiversity (except for species equitability).

Weed-impact indicators (except bird food) were also generally more stable over time in the landsparing scenarios than the landsharing scenarios (see Section of *to share or to spare?*). This is not surprising insofar as the landsparing scenarios were based on monoculture, and the landsharing scenarios on a succession of diverse crops.

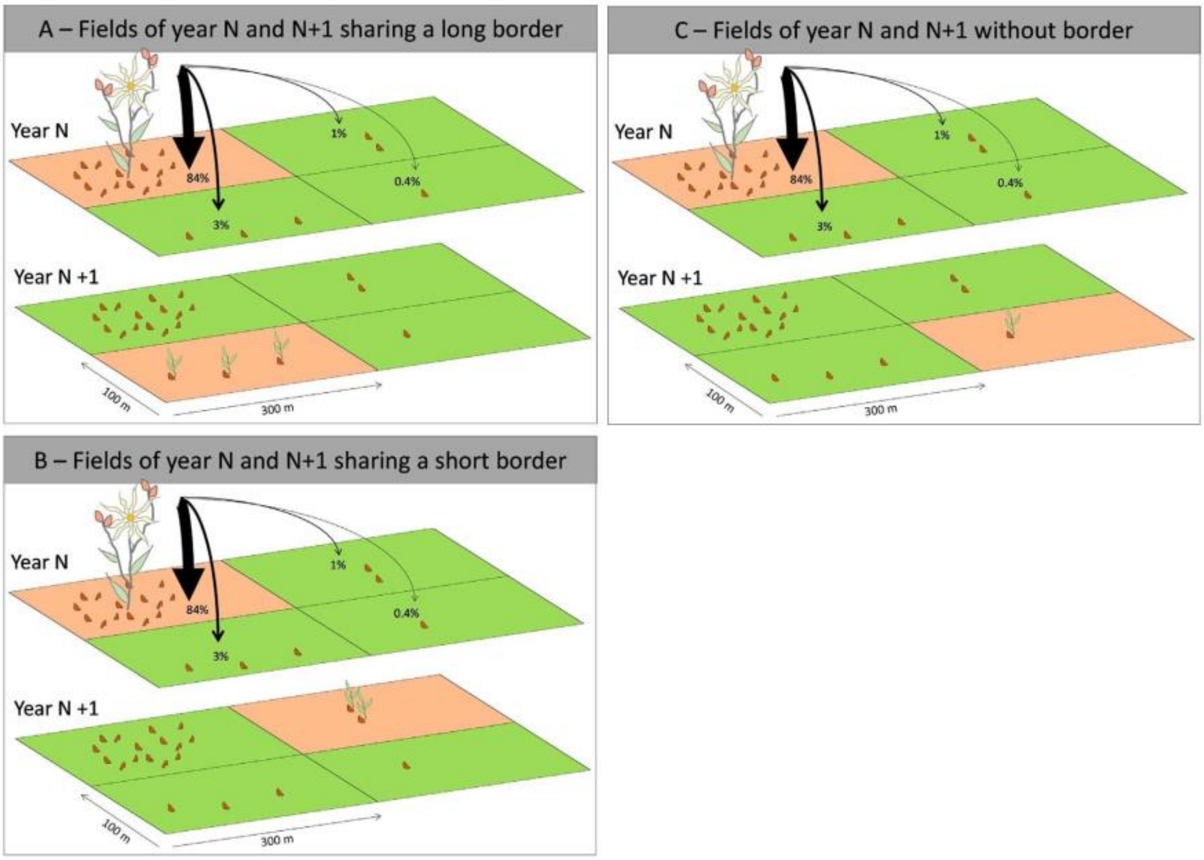
*The advantages of grass strips?*
At the landscape scale, landsparing scenarios with 10% of grass strips and 90% of the high-production system resulted in a performance similar to the cropping-system association scenario with 75% high production and 25% high biodiversity and better results than any landsharing scenarios. Total crop production in the landscape was the same, even though only 90% of the landscape was sown with crops in the scenarios with grass strips.

Scenarios with grass strips resulted in more inter-annual variability in a few weed impact indicators than the 75%–25% association scenario (Fig. 4B). This was particularly true for species equitability and carabid food, as well as harvest pollution and harvesting problems (i.e. 90HP_0 M, 90HP_1 M and 90HP_3 M were in the lower section of Fig. 4B, together with these indicators). The remaining indicators, particularly crop production, yield loss and field infestation varied less among years that in the 75%–25% association (i.e. 90HP_0 M, 90HP_1 M and 90HP_3 M were opposite these indicators in Fig. 4B).

The mowing frequency in the grass strip had little effect on either landscape weed impact or inter-annual variability (Fig. 4B). Plant diversity slightly decreased with mowing frequency; functional biodiversity was highest with a single annual mowing. Crop production and field infestation were not affected, though harvest pollution and harvesting problems were slightly higher with one annual mowing than for no or three annual mowing operations.

*How much landscape effect is due to seed dispersal?*
As mentioned in Section of "*to aggregate or segregate crops in time*?", most of the difference between landsharing scenarios were due to seed dispersal, and the effect of dispersal on weed-impact indicators increased with the number of crops present each year. In the landsparing scenarios, seed dispersal had no effect in the 100% high-production and 100% high-biodiversity scenarios. In the intermediate scenarios, the effect of seed dispersal depended on the indicator: the effects on crop production, yield loss and field infestation were not significant, and the effect on bee food was negligible. All other indicators were increased with the incorporation of seed dispersal in the model, except species equitability which decreased.

Figure 6. Schematic representation of spatial-temporal weed dispersal in the 4-field-cluster, depending on the proximity of crops favourable to weed reproduction in consecutive years, with favourable N and N+ 1 fields sharing a long (A), short (B) or no border (C). Dispersal percentages are those of *Amaranthus retroflexus* as an example.




## Conclusion

The present study developed an original method, combining a detailed mechanistic model for simulating the effects of cultural practices on weeds and crops with a spatially-explicit representation of small landscapes. This made it possible to draw new conclusions on how to associate different cropping systems and land-use types in order to reconcile contrasting objectives, in this case weed harmfulness control and weed-mediated biodiversity promotion. As a proof of concept, the method was applied to the particular case of maize-based cropping systems using highly efficient non-selective herbicides, in which case landsparing scenarios including either cropping systems aiming to maximise biodiversity or semi-natural grass strips performed best. In order to move further towards practical application, the present conclusions need to be confirmed with further simulation studies, assessing the sensitivity of the results to both modelling hypotheses (e.g. seed dispersal kernel) and landscape structure (e.g. field patterns). Moreover, the results are specific to the pedoclimate and cropping systems as well as the targeted ecosystem services, and further research is needed to design multifunctional cropping practices at the landscape scale adapted to each particular production context and set of objectives. Convincing farmers to change their practices based on simulations with a model can be difficult when they have not participated in the design of the model (Prost *et al*., 2012). Consequently, these new practices should be co-designed in workshops with farmers and extension services.

**Assessment and conclusion**

**Assessment and conclusion by applicant:**

Not relevant by title/abstract: The paper presents a mechanistic model for looking at cropped and non-cropped areas and their impact on biodiversity. No endpoint data are presented that could be used in the ecotoxicological regulatory risk assessment / glyphosate EU renewal, thus not relevant for the risk assessment.

**Assessment and conclusion by RMS:**

This is a simulation study to evaluate the contribution of crop management practices for reconciling crop production and weed-mediated biodiversity. In the particular case of maize-based cropping systems using non-selective herbicides, landsparing was shown to be generally more efficient than landsharing.

*Landsharing: production and biodiversity are maximised in individual fields*
*Landsparing: some fields or habitats are assigned for biodiversity conservation while the remaining fields aim to maximise production*

The landsparing scenario combining fields aiming to maximise crop production with either fields aiming to maximise biodiversity (25% of landscape) or grass strips (10% of landscape) were best, resulting in high crop production and medium biodiversity at the landscape scale. Landsharing scenarios always produced less biodiversity and less production.

Landsparing was achieved here by two contrasting options in terms of farm and landscape organization, either by combining complementary cropping systems aiming at different ecosystem services, or by including permanent grass strips in the landscape. Because of weed seed dispersal, the more diverse crops were grown each year in the landscape, the higher and the more stable the weed impact was, i.e. harmfulness for crop production and contribution to biodiversity.

When harvest quality was considered among the targets, including semi-natural habitats into the landscape was more efficient than associating contrasting cropping systems. According to the study authors, the advised strategy could be radically different when the priority among objectives changed. For instance, when the emphasis was on maximising production when attempting to reconcile production and a stable bird food offer, a landsparing strategy was advised; however, if the emphasis was on bird food stability over time rather than on production, a landsharing scenario growing a single crop each year was better.

The best strategy not only depends on the objective, the conclusions are also specific to the tested pedoclimate and cropping system types. Previous simulation studies at the field scale showed important
variations in weed impacts, even among maize-based cropping systems. The changes that here transformed a high-production system into a high-biodiversity one (i.e. delayed sowing and no till) will therefore not necessarily have the same effect in other production contexts. Conclusions also depend on the scale of the study which, for instance, determines the type of seed dispersal to consider. The study authors recommend caution when applying these results to other regions or cropping systems and recommend further simulation studies for different regions or systems. The study authors also recommend that the performance of the best solutions should be tested in field studies.

RMS notes that the sensitivity of weed dynamics to field shapes and areas was not studied here as the first aim to test the method for a multi-criteria evaluation of multi-specific weed impacts at the multi-field scale.

RMS further notes that according to the study authors, landsharing scenarios had been identified in previous simulation studies as the best compromise for reconciling production and biodiversity in a given field.

The present results were obtained with a single, small "landscape" with regular properties (i.e. four adjacent square fields).

Whether a landsharing or landsparing strategy is preferable will depend on the specific objectives, and may be case dependent.

Overall, RMS considers that there are uncertainties related to the intrinsic limitations of predictive models (e.g. simplification of complex natural processes), the relevance of some of the input values for the present analysis, and to the definition of the environmental scenarios.

This study is relevant for assessment of biodiversity and the definition of compensation measures in agricultural landscapes. However RMS considers the output of this simulation of low reliability.