



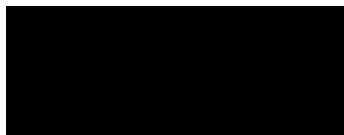
**Review of Scientific Literature Relevant to the  
Food/Feed and Environmental Risk Assessment of  
Bt11 × 59122 × MIR604 × 1507 × GA21 Maize**

**Literature Review**

**TEST GUIDELINE(S):**

Not Applicable

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**PERFORMING LABORATORY:**

Syngenta Crop Protection, LLC  
410 Swing Road  
Post Office Box 18300  
Greensboro, NC 27419-8300 USA

**LABORATORY PROJECT ID:**

Report Number: SSB-124-20

**SPONSOR(S):**

Syngenta Crop Protection, LLC  
410 Swing Road  
Post Office Box 18300  
Greensboro, NC 27419-8300 USA

## TABLE OF CONTENTS

<b>TABLE OF CONTENTS</b>	<b>2</b>
<b>LIST OF TABLES</b>	<b>3</b>
<b>LIST OF FIGURES</b>	<b>3</b>
<b>LIST OF ACRONYMS AND ABBREVIATIONS</b>	<b>4</b>
<b>1.0 OBJECTIVE</b>	<b>5</b>
<b>2.0 FORMULATING REVIEW QUESTIONS AND CLARIFYING THEIR PURPOSE</b>	<b>6</b>
2.1 Review Question .....	6
2.2 Eligibility/Inclusion Criteria .....	6
<b>3.0 SEARCHING FOR/IDENTIFYING RELEVANT PUBLICATIONS</b>	<b>14</b>
3.1 Electronic Bibliographic Databases .....	14
3.2 Internet Searches .....	14
3.2.1 Key organizations.....	14
3.2.2 Web-based search engines and databases .....	15
3.2.3 Manual searches .....	15
3.2.3.1 Checking reference lists .....	15
3.2.3.2 Hand searching.....	15
3.2.3.3 Citation searching.....	15
3.3 Constructing the Search Strategy .....	15
3.3.1 Database searching.....	15
3.3.1.1 Approaches to develop searches .....	15
3.3.1.2 Search terms .....	15
3.3.1.3 Free-text searching functions .....	17
3.3.1.4 Search strings .....	17
3.3.1.5 Key elements of review questions to use for best result .....	17
3.3.1.6 Use of multiple languages .....	21
3.3.1.7 Time period .....	21
3.3.1.8 Internet searching of regulatory agency webpages .....	22
3.4 Reference Publications.....	22
<b>4.0 Summarizing and Reporting the Data</b>	<b>22</b>
4.1 Selecting Publications .....	22
4.1.1 Database records .....	22
4.1.2 Records from key organizations.....	23
4.2 Results of the Publication Selection Process .....	24

4.3	Relevant Publications .....	29
4.4	Excluded Publications After Detailed Assessment of Full-Text Documents .....	30
4.5	Unobtainable Publications .....	32
4.6	Unclear Publications .....	32
4.7	Full-Text Documents .....	32
4.8	Narrative Synthesis/Summary of Relevant Publications .....	32
4.9	Implications of Relevant Publications on Risk Assessment .....	32
<b>5.0</b>	<b>RECORDS TO BE MAINTAINED</b>	<b>32</b>
<b>6.0</b>	<b>ARCHIVING OF RECORDS</b>	<b>32</b>
<b>7.0</b>	<b>REFERENCES</b>	<b>33</b>
	<b>APPENDIX SECTION</b>	<b>34</b>
APPENDIX A	Search history and subject indexing.....	35

## LIST OF TABLES

TABLE 1	Review question in PICO/PECO structure .....	6
TABLE 2	List of sub-combinations in scope for Bt11 × 59122 × MIR604 × 1507 × GA21 maize application .....	7
TABLE 3	Eligibility/inclusion criteria to establish relevance .....	8
TABLE 4	Overview of main categories of information/data requirements .....	11
TABLE 5	Key organization pages included in the search .....	14
TABLE 6	Search string strategy .....	18
TABLE 7	Matrix for the event terms in scope for Bt11 × 59122 × MIR604 × 1507 × GA21 maize .....	21
TABLE 8	Nomenclature for the single event and newly expressed proteins from the ISAAA database for use in searching regulatory agency web pages ....	22
TABLE 9	Electronic bibliographic database search results.....	25
TABLE 10	Regulatory agency webpage search details.....	26
TABLE 11	Results of the publication selection process for each review question and or group of information/data requirements searched .....	27
TABLE 12	Report of all publications excluded after detailed assessment of full-text documents .....	30
TABLE 13	Report of all publications excluded after assessment of internet documents .....	31

## LIST OF FIGURES

FIGURE 1	Flow chart of the publication selection process .....	28
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## **LIST OF ACRONYMS AND ABBREVIATIONS**

CAB	Commonwealth Agricultural Bureaux
EFSA	European Food Safety Authority
EPSPS	5-enol pyruvylshikimate-3-phosphate synthase
ERA	Environmental Risk Assessment
EU	European Union
GMO	Genetically Modified Organism
ISAAA	International Service for the Acquisition of Agri-Biotech Applications
MEDLINE	MEDical Literature Analysis and Retrieval System (online version)
mEPSPS	double-mutated 5-enol pyruvylshikimate-3-phosphate synthase
NTO	Non-target organisms
PAT	Phosphinothricin acetyltransferase
PICO/PECO	Population, Intervention/Exposure, Comparator, Outcomes
PMI	Phosphomannose isomerase

## 1.0 OBJECTIVE

The purpose of this systematic literature search was to identify literature and/or information on Bt11 × 59122 × MIR604 × 1507 × GA21 maize and all sub-combinations in scope that is relevant to the risk assessment of genetically modified organisms.

Bt11 × 59122 × MIR604 × 1507 × GA21 is a combined trait maize line developed by Syngenta using conventional breeding techniques. The Bt11 component of this breeding stack includes the *cry1Ab* gene which encodes Cry1Ab, a truncated (615 amino acids) version of the native, full length Cry1Ab protein produced by certain bacterial subspecies of *Bacillus thuringiensis*. Cry1Ab confers resistance to certain lepidopteran pests. Bt11 also includes the *pat* gene which encodes the enzyme phosphinothricin acetyltransferase (PAT), which confers tolerance to glufosinate herbicides. The *pat* gene in Bt11 maize was derived from *Streptomyces viridochromogenes*.

Maize event 59122 was developed by Dow AgroSciences using *Agrobacterium*-mediated transformation to insert a DNA fragment from plasmid PHP17662 into the maize genome. Event 59122 expresses the *Bacillus thuringiensis* strain PS149B1's Cry34Ab1 and Cry35Ab1 (Cry34/35Ab1) binary insecticidal proteins which are active against Corn Rootworm (*Diabrotica* spp). This event (also designated as DAS-59122-7) also produces the PAT protein from *Streptomyces viridochromogenes* which provides tolerance to glufosinate herbicides.

Maize plants derived from the transformation Event MIR604 express a modified Cry3A (mCry3A) protein for control of certain coleopteran pests (Chen and Stacy 2003) and a phosphomannose isomerase (MIR604 PMI) protein which acts as a selectable marker enabling transformed plant cells to utilize mannose as a primary carbon source (Negrotto *et al.* 2000).

Maize event 1507 was developed by insertion of a DNA fragment, PHI8999, into the maize genome using microprojectile bombardment. Event 1507 expresses the *Bacillus thuringiensis* var. *aizawai* Cry1F insecticidal protein, which confers protection against certain lepidopteran pests such as European Corn borer (*Ostrinia nubilalis*) and *Sesamia* spp. This event (also designated as TC1507) also produces the PAT protein from *Streptomyces viridochromogenes* which provides tolerance to glufosinate herbicides.

Maize plants derived from the transformation Event GA21 produce a double-mutated 5-enol pyruvylshikimate-3-phosphate synthase (mEPSPS) protein that confers tolerance to glyphosate in herbicide products (Lebrun *et al.* 2003).

Accordingly, this breeding stack hybrid expresses the introduced transgenes, producing the corresponding proteins present in Bt11, 59122, MIR604, 1507, and GA21 maize plants. Records must be specific to Bt11 × 59122 × MIR604 × 1507 × GA21 maize, or any of the in-scope sub-combinations to be considered relevant.

This report defines the 1) review question; 2) the search strategy; and 3) the explicit methods for selecting and categorizing the records. The results of the selection process are reported including consideration of the implications of any findings. This report aims to comply with the European Food Safety Authority (EFSA) explanatory note on literature searching for Genetically Modified Organism (GMO) applications (EFSA 2019).

## 2.0 FORMULATING REVIEW QUESTIONS AND CLARIFYING THEIR PURPOSE

### 2.1 Review Question

The review question associated with this literature search is:

Do either food/feed products derived from Bt11 × 59122 × MIR604 × 1507 × GA21 maize including all sub-combinations in scope or the combined intended traits have adverse effects on human and animal health and/or the environment?

This review question follows the PICO/PECO structure with key elements “Population, Intervention/Exposure, Comparator, Outcomes” (Table 1).

**TABLE 1** Review question in PICO/PECO structure

Element	Components of review question
<u>P</u> opulation	human and animal health and the environment
<u>I</u> ntervention/ <u>E</u> xposure	Bt11 × 59122 × MIR604 × 1507 × GA21 maize and all sub-combinations in scope, derived food/feed products, and/or relevant combinations of Cry1Ab, PAT, Cry34Ab1, Cry35Ab1, mCry3A, MIR604 PMI, Cry1F and mEPSPS and closely related variants
<u>C</u> omparator	conventional counterpart (if applicable)
<u>O</u> utcome	adverse effects

Because the search approach was not exclusionary (i.e., did not exclude the presence of any other events) searching the name of any of the single events comprising the Bt11 × 59122 × MIR604 × 1507 × GA21 maize stack had the possibility to return literature relevant to the stack of interest and all sub-combinations. A binary search term approach was developed to return literature on those titles related to all the possible stack combinations of this application. The interventions/exposures that appear in bold in Table 2 were used to develop the search terms. The boxes were used to identify the sub-combinations that would be captured by each search strategy. A similar approach was used for the proteins associated with the events.

### 2.2 Eligibility/Inclusion Criteria

Tables 3 and 4 summarize the eligibility/inclusion criteria for establishing relevance of retrieved records. Table 3 provides high level key concepts for inclusion/exclusion and Table 4 provides more explicit information on the information/data requirements concept. The eligibility/inclusion criteria are provided in the order of importance or ease of finding information on the criteria within a publication. The first failed eligibility/inclusion criterion was used as the primary reason for exclusion, and the remaining criteria were not assessed. Internet pages results were screened by date to remove those published prior to 2019. Pages without dates were evaluated further using the criteria in Tables 3 and 4.

**TABLE 2**      **List of sub-combinations in scope for**  
**Bt11 × 59122 × MIR604 × 1507 × GA21 maize application**

<b>Bt11 × 59122</b> Bt11 × 59122 × MIR604 Bt11 × 59122 × 1507 Bt11 × 59122 × GA21 Bt11 × 59122 × MIR604 × 1507 Bt11 × 59122 × MIR604 × GA21 Bt11 × 59122 × 1507 × GA21	<b>59122 × MIR604</b> 59122 × MIR604 × GA21 59122 × MIR604 × 1507 59122 × MIR604 × 1507 × GA21
<b>Bt11 × 1507</b> Bt11 × MIR604 × 1507 Bt11 × 1507 × GA21 Bt11 × MIR604 × 1507 × GA21	<b>MIR604 × 1507</b> MIR604 × 1507 × GA21
	<b>1507 × GA21</b>
	<b>59122 × GA21</b> 59122 × 1507 × GA21

**TABLE 3**      **Eligibility/inclusion criteria to establish relevance**

Concepts	Criteria	Comment
Stacked events obtained by conventional crosses/sub-combinations	This publication addresses the highest order breeding stack and/or a sub-combination or sub-combinations of the single events of the highest order breeding stack, independently of its/their origin.	This permits the selection of publications on the higher order breeding stack and/or sub-combinations of the single events of the higher order stack that are in scope of the Bt11 × 59122 × MIR604 × 1507 × GA21 application, independently of their origin. This permits the exclusion of publications on the single events of the higher stacked event, because the risk assessment of GMO applications for stacks covers only products in the scope of the GMO application – i.e., the higher order stack and sub-combinations of the singles involved, independently of their origin.
Intervention/exposure	Bt11 × 59122 × MIR604 × 1507 × GA21 maize and all sub-combinations in scope, derived food/feed products, and/or the intended trait(s)	Intended traits include glufosinate herbicide tolerance, glyphosate herbicide tolerance, coleopteran insect resistance, lepidopteran insect resistance, and mannose metabolism. Closely related variants of the insecticidal proteins included as relevant were those that shared the same tertiary level of Crickmore nomenclature for Cry1Ab, Cry34Ab, and Cry35Ab; and those that shared the same secondary level of Crickmore nomenclature for mCry3A and Cry1F. Any enzyme classified as a 5-enol pyruvylshikimate-3-phosphate synthase was considered relevant if the EPSPS protein imparts the glyphosate tolerance trait. Any enzyme classified as a phosphinothricin acetyl transferase was considered relevant. Any enzyme classified as a phosphomannose isomerase was considered relevant. Only unique protein combinations relevant to the stack and sub-combinations were in scope.
Information/data requirements	Data inform one or more information/data requirement(s) for the GMO and derived food/feed products under consideration, including the intended trait(s)	Publications that potentially contribute to the knowledge informing the risk assessment of Bt11 × 59122 × MIR604 × 1507 × GA21 maize (information/data requirements provided in Table 4) and all sub-combinations in scope were considered relevant. Based on the scope of the application certain information/data requirements were excluded. These are also detailed in Table 4. Publications addressing issues such as benefits, socioeconomics, ethics, crop protection, detection methods, efficacy, public perception and risk communication were excluded using this criterion, as they are not relevant to the risk assessment as defined in this document.



Scope of GMO application	The pathways and level of exposure to the GMO, derived food/feed products, and the intended trait(s) addressed in the publication are relevant for the intended uses of the GMO and derived food/feed products under regulatory review	Publications must address pathways and levels of exposure relevant to the scope of the application: import and processing of Bt11 × 59122 × MIR604 × 1507 × GA21 maize and all sub-combinations in scope for food/feed uses.
Reporting format	Original/primary data are presented in the publication or it is a risk assessment from a relevant key organization (such as regulatory agencies and risk assessment bodies involved in the risk assessment of GMOs)	Records that do not present original/primary data (e.g., reviews, editorials, position papers) were excluded. Risk assessments performed and reported by relevant key organizations were included as relevant if they address Bt11 × 59122 × MIR604 × 1507 × GA21 maize, any of the relevant sub-combinations, and unique combinations of Cry1Ab, PAT, Cry34Ab1, Cry35Ab1, mCry3A, MIR604 PMI, Cry1F, and mEPSPS and closely related variants.
Previously risk assessed publications	As indicated by EFSA, a publication should be included if it has not been previously risk assessed by EFSA and/or its GMO Panel and is not cited/referenced in an EFSA/GMO Panel output	If a publication has previously been considered by EFSA it was excluded. EFSA's scientific opinions were excluded. Any cited/referenced publications contained within documents produced by EFSA and/or its GMO Panel were also excluded.
Access	Full-text document is accessible	If potentially relevant full-text documents cannot be obtained, then they were listed in a table with a description of the (unsuccessful) methods that have been used to try to obtain a copy.
Population	Human and animal health, and/or the environment are addressed as general protection goals	All of the information/data requirements categories described in Table 4 are thought to inform the risk assessment related to human and animal health, and/or the environment. Therefore, if a publication meets the inclusion criteria described in this Table and is relevant to the information/data requirements in Table 4 it was considered relevant.
Outcomes	Effects/impacts on human and animal health, and/or the environment are addressed	Publications that address Bt11 × 59122 × MIR604 × 1507 × GA21 maize, or sub-combinations in scope also need to address effects/impacts on entities of concern, and potential determinants of exposure that place these entities at risk in order to be relevant to the risk assessment of Bt11 × 59122 × MIR604 × 1507 × GA21 maize, or sub-combinations in scope.

Comparator	If the publication is a comparative study that uses plant material as a test material, eligible publications must report a non-GM variety.	Publications that address Bt11 × 59122 × MIR604 × 1507 × GA21 maize or sub-combinations in scope, must also include a conventional counterpart as a comparator in those cases where comparative analysis is conducted, and plant material is used as test material. Any uncertainties about the appropriateness of the comparator were addressed in the assessment of the publication.
Plant Species	The publication may address the same plant species as the GMO under consideration, but could also address any plant species producing unique combinations of Cry1Ab, PAT, Cry34Ab1, Cry35Ab1, mCry3A, MIR604 PMI, Cry1F, and mEPSPS proteins in the stack or sub-combinations in scope of the application.	The review question addresses the safe use of the intended trait(s) of Bt11 × 59122 × MIR604 × 1507 × GA21 maize and all sub-combinations in scope. Therefore, GMOs that contain combinations of Cry1Ab, PAT, Cry34Ab1, Cry35Ab1, mCry3A, MIR604 PMI, Cry1F and mEPSPS or closely related variants unique to the product and sub-combinations in scope, but are introduced into another plant species may be included. For certain types of data, the presence of the transgenic proteins in a different plant species does not impact the assessment of Bt11 × 59122 × MIR604 × 1507 × GA21 maize or sub-combinations in scope. Those types of data are identified as event-specific in Table 4.
Target pest/organisms	Target pests/organisms addressed in the study are established in the European Union (EU).	Records related to the intervention/exposure and target pests/organisms were excluded because the scope of the application is import for food/feed uses and this is relevant for cultivation applications only.
Reporting format	A study should only be presented once, but if it is presented in more than one publication, all publications should be listed and grouped.	Duplicate publications were excluded at the initial screening stage. Only one copy of a study is presented even if it is reported in different publications.

**TABLE 4 Overview of main categories of information/data requirements**

Expert knowledge on data used in the risk assessment of the GMO is required but the list below provides some examples of relevant data/information. If certain data are considered stack/subcombination-specific or specific to unique combinations of the transgenic proteins expressed in the stack/subcombinations in scope then it is noted.

Information/data requirement	Non-exhaustive list of specific information/data requirements
Molecular characterization of the genetic modification of Bt11 × 59122 × MIR604 × 1507 × GA21 maize and any sub-combinations in scope	<ul style="list-style-type: none"> <li>Information on the insert including sequence, size, copy number, genetic element arrangement, deletions, location, sequence similarity searches, analysis of open reading frames (stack/sub-combination specific)</li> <li>Expression data of inserted/modified sequences (stack/sub-combination specific)</li> <li>Genetic stability (stack/sub-combination specific)</li> <li>Data on the equivalence between plant-produced and microbially-produced proteins (stack/sub-combination specific)</li> </ul>
Agronomic, phenotypic and compositional characterization of the Bt11 × 59122 × MIR604 × 1507 × GA21 maize and any sub-combinations in scope	<ul style="list-style-type: none"> <li>Comparative assessment of agronomic and phenotypic characteristics under field or controlled conditions (stack/sub-combination specific)</li> <li>Comparative analysis of key nutritional constituents (stack/sub-combination specific)</li> </ul>
Toxicological assessment of newly expressed protein(s), new constituents other than proteins, and the whole GM food/feed	<ul style="list-style-type: none"> <li>Toxicity studies (protein combinations in scope)</li> <li>Feeding studies (stack/sub-combination specific)</li> </ul>
Allergenicity assessment of the newly expressed protein and the GM food/feed, and adjuvanticity	<ul style="list-style-type: none"> <li>Serum screening (protein combinations in scope)</li> <li><i>In vivo</i> tests in animal models</li> <li>Expression data for endogenous allergens in maize (stack/sub-combination specific)</li> </ul>
Nutritional assessment of the newly expressed protein(s), other new constituents, as well as potential alterations in the total diet of the consumer or the animal	<ul style="list-style-type: none"> <li>Anticipated dietary intake of food/feed and the resulting nutritional impact (stack/sub-combination specific)</li> <li>Comparative growth performance studies with young rapidly growing animal species (stack/sub-combination specific)</li> </ul>
Post-market monitoring	<ul style="list-style-type: none"> <li>Description of mechanisms for determining actual changes to overall dietary intake patterns of Bt11 × 59122 × MIR604 × 1507 × GA21 maize and any sub-combinations in scope, to what extent this has occurred, and whether or not the product induces known (side) effects or unexpected side effects</li> <li>Information on the reliability, sensitivity and specificity of the post market monitoring</li> </ul>
Persistence and invasiveness assessment, including plant-to-plant gene transfer	<ul style="list-style-type: none"> <li>Measurements of volunteer occurrence and establishment (stack/sub-combination specific)</li> <li>Replacement capacity (stack/sub-combination specific)</li> <li>Fitness of the Bt11 × 59122 × MIR604 × 1507 × GA21 plant in various environmental conditions – if the relevant combinations or sub-combinations of newly expressed proteins are expressed in a different plant species then the publication may be considered relevant.</li> </ul>

Information/data requirement	Non-exhaustive list of specific information/data requirements
Assessment of plant to micro-organism gene transfer	<ul style="list-style-type: none"> <li>This type of data is covered in the scope of the single event literature review.</li> </ul>
Assessment of interactions with target organisms	<ul style="list-style-type: none"> <li>Excluded based on the scope of the application. The scope of this application covers the import, processing and food and feed use of Bt11 × 59122 × MIR604 × 1507 × GA21 maize and any sub-combinations in scope in the EU. According to the EFSA ERA Guidance (EFSA, 2010): “<i>resistance development is only relevant for applications with scope cultivation of GM plants and not for applications restricted to import and processing of GM plants and their products</i>” (EFSA, 2010). Therefore, an assessment of the potential resistance development in target organisms resulting from the import, processing and food and feed use Bt11 × 59122 × MIR604 × 1507 × GA21 maize is not relevant for this application.</li> </ul>
Assessment of interactions with nontarget organisms	<ul style="list-style-type: none"> <li>The EFSA ERA Guidance (EFSA 2010) states that: “in cases where the application does not include cultivation in the EU, direct environmental exposure of NTOs to the GM plant is via accidental release into the environment of seeds or propagules during transportation and processing. This may result in sporadic occurrence of feral plants and therefore exposure of NTO populations is likely to be negligible. The ERA will then focus on indirect exposure to products of the GM plant (e.g., through manure and faeces from animals fed the GM plant, and other by-products of industrial processes)”. Therefore, any publications that discuss direct exposure in test protein and laboratory studies or field survey data was considered not relevant based on scope of application.</li> </ul>
Assessment of interactions with biogeochemical and abiotic processes	<ul style="list-style-type: none"> <li>Excluded based on the scope of the application. The scope of this application covers the import, processing and food and feed use of Bt11 × 59122 × MIR604 × 1507 × GA21 maize and any sub-combinations in scope in the EU. According to the EFSA ERA Guidance (EFSA, 2010): “<i>applications concerning food/feed uses and import and processing do not require scientific information on possible environmental effects associated with the cultivation of the plant</i>” therefore, an assessment of the impacts of Bt11 × 59122 × MIR604 × 1507 × GA21 maize and any sub-combinations in scope on biogeochemical processes resulting from specific cultivation, management and harvesting techniques is not relevant given the scope of this application.</li> </ul>
Assessment of impact of specific cultivation, management and harvesting techniques	<ul style="list-style-type: none"> <li>Excluded based on the scope of the application. The scope of this application covers the import, processing and food and feed use of Bt11 × 59122 × MIR604 × 1507 × GA21 maize in the EU. Cultivation of Bt11 × 59122 × MIR604 × 1507 × GA21 maize in the EU is not included in the scope. According to the EFSA ERA guidance (EFSA 2010): “<i>for GM plants for import and processing that are not intended for cultivation in the EU, there is no need for an ERA for altered cultivation, management and harvesting techniques</i>”. Therefore, an assessment of impact of specific cultivation, management and harvesting techniques of Bt11 × 59122 × MIR604 × 1507 × GA21 maize and any sub-combinations in scope is not relevant for this application.</li> </ul>

Information/data requirement	Non-exhaustive list of specific information/data requirements
Risk mitigation	<ul style="list-style-type: none"><li>Excluded based on the scope of the application. Risk mitigation measures such as high dose/refuge strategy, isolation distance from protected habitats hosting species of conservation concern that are at risk, integrated pest/weed management are only relevant to cultivation. The scope of this application covers the import, processing and food and feed use of Bt11 × 59122 × MIR604 × 1507 × GA21 maize and any sub-combinations in scope.</li></ul>
Post-market environmental monitoring	<ul style="list-style-type: none"><li>Excluded based on the scope of the application. Monitoring such as insect resistance is relevant only to cultivation. The scope of this application covers the import, processing and food and feed use of Bt11 × 59122 × MIR604 × 1507 × GA21 maize and any sub-combinations in scope.</li></ul>

## 3.0 SEARCHING FOR/IDENTIFYING RELEVANT PUBLICATIONS

### 3.1 Electronic Bibliographic Databases

To search for different types of publications and unpublished work that could provide information on the review question, multidisciplinary citation databases which include grey literature (i.e., not peer reviewed) were used. Medline, Agricola, Commonwealth Agricultural Bureaux (CAB) Abstracts, and BIOSIS Previews (provided by Ovid Technologies) were searched. Each of the databases has a thesaurus. Searching these databases fulfills the requirement to search a minimum of at least two multi-disciplinary/large databases.

These databases were selected based on their coverage of scientific literature for relevant subjects including, but not limited to, biomedicine, plant diseases, agriculture, life sciences, pesticides, human health and nutrition, animal health, plant science, biotechnology and environmental studies. Detailed information (e.g., list of subjects covered, coverage dates, update schedule, and sources for data) regarding each of the databases searched can be obtained upon request. The document types in these databases include journal articles, technical letters and notes, conference proceedings, book chapters, reports, and articles in press.

### 3.2 Internet Searches

#### 3.2.1 Key organizations

The regulatory agencies that post their risk assessments on their websites (Table 5) were searched. The internet pages of regulatory agencies and risk assessment bodies that regulate stacks and post their risk assessments on their websites were searched for documents related to Bt11 × 59122 × MIR604 × 1507 × GA21 maize and the subcombinations in scope of the application.

**TABLE 5 Key organization pages included in the search**

Regulatory agency/risk assessment body	Web address
US Environmental Protection Agency	<a href="https://www.epa.gov/ingredients-used-pesticide-products/current-and-previously-registered-section-3-plant-incorporated">https://www.epa.gov/ingredients-used-pesticide-products/current-and-previously-registered-section-3-plant-incorporated</a>
Health Canada <sup>a</sup>	<a href="https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-products.html">https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-products.html</a>
Office of the Gene Technology Regulator	<a href="http://www.ogtr.gov.au/">http://www.ogtr.gov.au/</a>
National Technical Commission on Biosafety <sup>b</sup>	<a href="http://ctnbio.mctic.gov.br/inicio">http://ctnbio.mctic.gov.br/inicio</a>
Ministry of Agriculture, Forestry and Fisheries	<a href="http://www.maff.go.jp/e/">http://www.maff.go.jp/e/</a>

<sup>a</sup>Also searches Environment and Climate Change Canada (<https://www.ec.gc.ca/cc/>) and Canadian Food Inspection Agency (<http://www.inspection.gc.ca/plants/plants-with-novel-traits/notices-of-submission/eng/1300143491851/1300143550790>).

<sup>b</sup>Partial reports are excluded from review since there is no new information contained in these reports and they are not the official final opinion of the agency.

### **3.2.2 Web-based search engines and databases**

General search engines such as GOOGLE Scholar and web-based databases known to contain information specifically on effects of GMOs were not searched. The search of the databases and key organization websites is considered to provide an adequately comprehensive search of literature.

### **3.2.3 Manual searches**

#### **3.2.3.1 Checking reference lists**

For any reviews, methodological publications, guidelines and scientific opinions from regulatory agencies retrieved using the search strategy and classified as relevant to the review question, the reference list(s) from those records were manually searched for new records (2019 through the date the search was conducted) that met the eligibility/inclusion criteria.

#### **3.2.3.2 Hand searching**

Hand searching was not conducted. The search of the databases and key organization websites is considered to provide an adequately comprehensive search of literature.

#### **3.2.3.3 Citation searching**

Citation searching was not conducted. The search of the databases and key organization websites is considered to provide an adequately comprehensive search of literature.

## **3.3 Constructing the Search Strategy**

### **3.3.1 Database searching**

#### **3.3.1.1 Approaches to develop searches**

The “lumping” approach was utilized. A single search strategy was developed to capture all categories of information of interest in one search. This strategy was used because previous experience indicates that a manageable number of studies was returned.

#### **3.3.1.2 Search terms**

##### **Identifying search terms**

Search terms were identified by:

- Assessing subject indexing terms of relevant publications recorded in those electronic bibliographic databases that use thesauri
  - All publications returned from literature search reports that aim to comply with the EFSA explanatory note and deemed relevant to the review questions were examined to determine the subject indexing terms associated with it.

- Seeking suggestions from experts and stakeholders
  - The search terms were developed using a multi-disciplinary team (i.e., risk assessors, information specialists, regulatory affairs managers).

### **Free-text terms and subject indexing terms**

The searches with the Ovid platform utilized the keyword search in the advanced search window. The keyword search uses a default set of fields designated .mp, which vary by database. Therefore, Ovid uses the term “keyword” to indicate that it is executing a multi-field search. In each database the specific fields searched are a different combination of free-text and controlled vocabulary fields, with Ovid switching automatically to the appropriate fields when a database is selected.<sup>1</sup>

In Ovid, the fields used in the .mp keyword search are word searchable, therefore any search only has to find a single word in a controlled vocabulary field that contains phrases to return as search results all references indexed to that subject heading. Thus, a search strategy which includes “genetic\*” will return the following (highlighted below):

- **Genetically modified** foods or **genetic engineering** in the Subject Headings field in Agricola,
- Zea mays: species, maize, common, **genetically modified**, strain-Bt10 [Gramineae] in the Organism field in BIOSIS Previews,
- **Genetically engineered** organisms in the Subject Headings field in CAB Abstracts,
- Plants, **Genetically Modified** / ge [Genetics] or **Genetic Engineering** in MeSH Subject Headings in Medline

Subsequent combining of terms, (genetic\* AND (modif\* OR engineer\*)) (in bold), yields all references with these headings to be in the final results for that search set. Therefore, it is not necessary to search each exact controlled phrase in order to return all references for each of the specific headings.

<sup>1</sup> In Agricola the .mp fields are: free-text—abstract; geographic area; identifier; meeting information; map information; note; original title; personal name as subject; title—and controlled vocabulary—category code; subject heading.

In BIOSIS Previews the .mp fields are: free-text—abstract; book title; gene name; miscellaneous descriptors; methods & equipment; original language book title; title—and controlled vocabulary—biosystematic codes; chemicals & biochemicals; concept codes; diseases; geopolitical locations; major concepts; organisms; parts, structure & systems of organisms; sequence data; super taxa; taxa notes; time.

In CAB Abstracts the .mp fields are: free-text—abstract; identifiers; original title; title—and controlled vocabulary—broad terms; geographic location; organism descriptors; subject headings.

In Medline the .mp fields are: free-text—abstract; keyword heading word; original title; synonyms; title; unique identifier—and controlled vocabulary—floating sub-heading word; name of substance word; organism supplementary concept word; protocol supplementary concept word; rare disease supplementary concept word; subject heading word.



Appendix A provides 1) the search history (including the full strategy used and fields searched as run in the database) and number of publications identified (line by line) for each bibliographic database prior to de-duplication and 2) the subject indexing used by each database as shown within the brackets after each search term.

### **3.3.1.3 Free-text searching functions**

The search terms were selected to incorporate a wide variety of synonymous and related terms. Truncation and wildcards were used where appropriate to capture different conventions in spelling and variation in the endings of terms.

### **3.3.1.4 Search strings**

Search strings were combined with Boolean and proximity operators appropriate for the scope of the review.

### **3.3.1.5 Key elements of review questions to use for best result**

A very large number of publications were returned using only the four key elements of Event, Intended trait, newly expressed protein(s), and Trade Name. To prevent a very large number of publications from being returned while still achieving sensitivity, additional key elements were added to the search strategy. Sensitivity was defined as the ability to return the previously deemed relevant articles with the new search string. ‘A very large number’ is not defined in the Explanatory Note (EFSA 2019); however, the number returned with other search strategies (e.g., (Event OR Intended Trait OR Newly Expressed Protein(s) OR Trade Name) or (Event OR Trade name OR ((Intended Trait OR Newly Expressed Protein(s)) AND (Plant Species or GMO)))) was so large that it could not be de-duplicated by the search platform.

Therefore, the search structure included the following search concepts/key elements; Event, Trade Name, Newly Expressed Protein(s), or Intended Trait in the same publications as terms describing plant species and/or GMO general terms. The search strategy employed was:

- Event OR Trade name OR (Newly Expressed Protein(s) AND (GMO general OR Plant Species)) OR (Intended Trait – Insecticidal AND (GMO general AND Plant Species)) OR GMO general × Intended Traits

The search strategy employed captured literature relevant to Bt11 × 59122 × MIR604 × 1507 × GA21 maize and is provided in Table 6. The same search string was used in all databases. Because the binary search strategy is not exclusionary it allows for the possibility of returning records on all of the subcombinations in scope of the application without searching all of the subcombinations separately (see Table 7 for an example matrix for the event terms).

Since the Ovid search platform simultaneously searches free-text and subject headings there is no disadvantage to using all search terms in all databases. For example, if ‘Genetically engineered organisms’ is a subject heading in CAB Abstracts but not in Agricola including this term in the search of the Agricola databases still allows for free-text searching of this term.

**TABLE 6 Search string strategy**

Set	Field	Search string	Concepts/Key elements
1	Topic	Bt11 OR Bt 11 OR SYN-BT?11-1	Event Bt11
2	Topic	DAS59122* OR DAS 59122* OR 59122* OR DAS59I22* OR DAS 59I22* OR 59I22*	Event 59122
3	Topic	MIR604 OR MIR 604 OR SYN-IR6?4-5	Event MIR604
4	Topic	DAS15?7 OR DAS 15?7 OR DASI5?7 OR DAS I5?7 OR 15?7 OR I5?7 OR TC 15?7 OR TC15?7 OR TC I5?7 OR TCI5?7 OR DAS-?15?7-1 OR DAS?15?71	Event 1507
5	Topic	GA21 OR GA 21 OR GA2I OR GA 2I OR MON ØØØ21* OR MON ØØØ21* OR MON 00021* OR MON empty setempty setempty set21* OR MONØØØ21* OR MONØØØ21* OR MON00021* OR MONempty setempty setempty set21* OR M0N ØØØ21* OR M0N ØØØ21* OR M0N 00021* OR M0N empty setempty setempty set21* OR M0NØØØ21* OR M0NØØØ21* OR M0N00021* OR M0Nempty setempty setempty set21*	Event GA21
6		#1 AND (#2 OR #4)	Relevant event combinations
7		#2 AND (#3 OR #5)	Relevant event combinations
8		#3 AND #4	Relevant event combinations
9		#4 AND #5	Relevant event combinations
10		#6 OR #7 OR #8 OR #9	All relevant single event combinations
11	Topic	Agrisure <sup>a</sup>	Trade names of Bt11, MIR604, and GA21, highest order stack and subcombinations <sup>a</sup>
12	Topic	Herculex* ADJ3 (RW* OR I* OR 1* OR CB* OR XTRA*)	Trade names of single Events 59122 and 1507
13		#11 AND #12	Relevant combinations of trade names
14	Topic	Cry1Ab* OR Cry 1Ab* OR Cry1 Ab* OR Cry 1 Ab* OR CryIAb* OR Cry IAb* OR CryI Ab* OR Cry I Ab*	Newly expressed protein in Bt11
15	Topic	Phosphinothricin N acetyltransferase OR Phosphinothricin N acetyl transferase OR Phosphinothricin acetyltransferase OR Phosphinothricin acetyl transferase OR PPT acetyltransferase OR PPT acetyl transferase OR PT N acetyltransferase OR PT N acetyl transferase OR Glufosinate acetyltransferase OR Glufosinate acetyl transferase OR Glufosinate acetyltransferase OR Glufosinate acetyl transferase OR pat OR 111069-93-3 OR EC 2.3.1.183 OR E.C. 2.3.1.183	Newly expressed protein in Bt11, 59122 and 1507
16	Topic	Cry34Ab* OR Cry34 Ab* OR Cry 34Ab* OR Cry 34 Ab* OR Cry35Ab* OR Cry35 Ab* OR Cry 35Ab* OR Cry 35 Ab*	Newly expressed protein in 59122
17	Topic	mCry3A* OR mCry 3A* OR mCry 3 A* OR Cry3A* OR Cry 3A* OR Cry 3 A*	Newly expressed protein in MIR604

Set	Field	Search string	Concepts/Key elements
18	Topic	Phosphomannoisomerase OR Mannose 6-phosphate isomerase OR Phosphomannoseisomerase OR Phosphomannose isomerase OR 9023-88-5 OR AAA24109 OR EC 5.3.1.8 OR E.C. 5.3.1.8	Newly expressed protein in MIR604
19	Topic	Cry1F* OR Cry 1F* OR Cry 1 F* OR Cry1 F* OR CryIF* OR Cry IF* OR Cry I F* OR CryI F*	Newly expressed protein in TC1507
20	Topic	5 enol pyruvyl shikimate 3 phosphate synthase OR 5 enolpyruvyl shikimate 3 phosphate synthase OR 5 enolpyruvylshikimate 3 phosphate synthase OR EPSP synthase OR MEPSP synthase OR EPSPS OR MEPSPS OR EC 2.5.1.19 OR E.C. 2.5.1.19	Newly expressed protein in GA21 maize
21		#14 AND (#16 OR #19)	Relevant protein combinations
22		#16 AND (#17 OR #18 OR #20)	Relevant protein combinations
23		#15 AND (#17 OR #18 OR #20)	Relevant protein combinations
24		#19 AND (#17 OR #18 OR #20)	Relevant protein combinations
25		#21 OR #22 OR #23 OR #24	All combinations of newly expressed proteins
26	Topic	((Insect OR Insects OR coleoptera* OR lepidoptera* OR pest OR pests OR stalkborer* OR stalk borer* OR borer* OR cornborer* OR corn borer* OR noctuidae OR Crambidae OR Chrysomelidae OR earworm* OR ear worm* OR armyworm* OR army worm* OR cutworm* OR cut worm* OR rootworm* OR root worm* OR Ostrinia OR O nubilalis OR Diatraea OR D grandiosella OR D crambidoides OR Helicoverpa OR H zea OR Spodoptera OR S frugiperda OR S exigua OR Papaipema OR P nebris OR Elasmopalpus OR E lignosellus OR D saccharalis OR Striacosta OR S albicosta OR Agrotis OR A ipsilon OR Feltia OR F jaculifera OR Pseudaletia OR P unipuncta OR Diabrotica OR D virgifera OR D barberi OR ECB OR SWCB OR SCSB OR CEW OR FAW OR SCB OR WBC OR WCRW OR WCR OR NCRW OR MCR OR MCRW) ADJ2 (toleran* OR resistan* OR protect* OR control*)) OR Bacillus thuringiensis OR B thuringiensis OR ((glufosinate* OR gluphosinate* OR Basta* OR Liberty* OR Ignite* OR Rely* OR Finale* OR Challenge* OR gl?phosate OR gl?fosate OR roundup* OR round up* OR herbicide* OR pesticide*) ADJ2 (toleran* OR resistan* OR protect*))	Intended traits
27	Topic	GMO* OR LMO* OR GM OR GE OR transgen* OR ((genetic* OR living OR biotech*) ADJ3 (modif* OR transform* OR manipulat* OR improv* OR engineer* OR deriv*)) OR stack*	GMO general
28		#26 AND #27	Intended traits AND GMO general
29	Topic	GMHT OR GEHT OR GMHR or GEHR OR GMHTs OR GEHTs OR GMHRs or GEHRs	GMO general x intended traits - herbicidal
30		#28 OR #29	(Intended traits AND GMO general) OR GM general x intended traits - herbicidal

Set	Field	Search string	Concepts/Key elements
31	Topic	Maize* OR corn* OR Zea mays OR Z mays	Plant species
32		#30 AND #31	((Intended trait AND GMO general) OR GMO general × intended trait - herbicidal)) AND Plant species
33	Topic	((Bt OR Bacillus thuringiensis OR B thuringiensis) ADJ5 (maize* OR corn* OR mays)) OR Btmaize* OR Btcorn*	GMO general x intended traits - insecticidal
34		#10 OR #13 OR #25 OR #32 OR #33	(Combinations of Single event) OR (Trade names) OR (Newly expressed protein) OR  (((Intended traits AND GMO general) OR GMO general × intended traits – herbicidal) AND Plant species) OR  (GMO general x intended traits – insecticidal)

\*Trade names for all of the single events, the highest order stack and commercial sub-combinations all have Agrisure in their names. Therefore, by simply searching Agrisure it is possible to capture all relevant literature.

Single event and sub-combinations within scope of this literature review include:

Agrisure designation	Product
Agrisure 3122, Agrisure 3122A, Agrisure 3122 E-Z Refuge, Agrisure Artesian 3122A E-Z Refuge, Agrisure 3122 Refuge Renew	Bt11 × 59122 × MIR604 × 1507 × GA21 maize
Agrisure 3120, Agrisure 3120A, Agrisure 3120 E-Z Refuge, Agrisure Artesian 3120A E-Z Refuge, Agrisure EZ-Refuge,	Bt11 × 1507 × GA21 maize
Agrisure TL, Agrisure TD, Agrisure CB/LL, Agrisure CB	Bt11 maize
Agrisure RW	MIR604 maize
Agrisure GT, Agrisure TG, Agrisure Artesian GTA	GA21 maize

**TABLE 7**      **Matrix for the event terms in scope for Bt11 × 59122 × MIR604 × 1507 × GA21 maize**

Stacks in scope of the application	Binary Search Terms					
	Bt11 × 59122	Bt11 × 1507	59122 × MIR604	59122 × GA21	MIR604 × 1507	1507 × GA21
Bt11 × 59122 × MIR604 × 1507 × GA21	X	X	X	X	X	X
Bt11 × MIR604 × 1507 × GA21		X			X	X
Bt11 × 59122 × 1507 × GA21	X	X		X		X
Bt11 × 59122 × MIR604 × GA21	X		X	X		
Bt11 × 59122 × MIR604 × 1507	X	X	X		X	
59122 × MIR604 × 1507 × GA21			X	X	X	X
Bt11 × 59122 × MIR604	X		X			
Bt11 × 59122 × 1507	X	X				
Bt11 × 59122 × GA21	X			X		
Bt11 × MIR604 × 1507		X			X	
Bt11 × 1507 × GA21		X				X
59122 × MIR604 × 1507			X		X	
59122 × MIR604 × GA21						
59122 × 1507 × GA21				X		X
MIR604 × 1507 × GA21					X	X
Bt11 × 59122	X					
Bt11 × 1507		X				
59122 × MIR604			X			
59122 × GA21				X		
MIR604 × 1507					X	
1507 × GA21						X

### 3.3.1.6 Use of multiple languages

The search terms used were in the English language or utilized the Roman alphabet. For the event name and trade names that do not use words in the English language, translations are unlikely to exist.

### 3.3.1.7 Time period

Due to the use of multiple (i.e., 4) multi-disciplinary databases and redundancy in coverage it is unlikely that late addition of a publication would be missed. Therefore, the returned literature was limited to that which was published between January 1, 2019 and the date of the last database update prior to the search (see Table 9). Ovid only allows for limiting search by year, so the results were deduplicated against the prior year's results (██████████ 2019).

The records returned from the search of the regulatory agency webpages were manually excluded if they were dated prior to 2019. If a date could not be determined for the record, then the record was reviewed for relevance using the criteria in Tables 3 and 4.

### 3.3.1.8 Internet searching of regulatory agency webpages

The search terms selected are the event and protein names from the International Service for the Acquisition of Agri-Biotech Applications (ISAAA) (Table 8). The descriptions and information for the top 50 hits or 10% of the total hits (whichever is greater) for each search term/web page was collected.

**TABLE 8**            **Nomenclature for the single event and newly expressed proteins from the ISAAA database for use in searching regulatory agency web pages**

Event	Search term	Concepts/Key Elements
Bt11	Bt11	Event name
Bt11	Cry1Ab	Newly expressed protein
Bt11	Phosphinothricin N-acetyltransferase	Newly expressed protein
MIR604	MIR604	Event name
MIR604	mCry3A	Newly expressed protein
MIR604	Phosphomannose isomerase	Newly expressed protein
GA21	GA21	Event name
GA21	Modified 5-enolpyruvylshikimate-3-phosphate synthase enzyme	Newly expressed protein

## 3.4 Reference Publications

The search strategy is the same as the one that was previously validated with reference publications (██████████ 2019).

## 4.0 SUMMARIZING AND REPORTING THE DATA

### 4.1 Selecting Publications

#### 4.1.1 Database records

The process for selecting relevant publications was conducted in two stages. The first stage required a preliminary assessment of titles and abstracts. Those records that are clearly not relevant from reviewing the title only were excluded from further review. For those records that appeared relevant or have unclear relevance the abstract was reviewed. Those records that are clearly not relevant from reviewing the abstract were excluded from further review, while records that are relevant or have unclear relevance were reviewed in Stage 2.

Full-length articles were reviewed in Stage 2. An explanation of exclusion is provided for any full-length records that are deemed not relevant in Stage 2. Any relevant records identified in Stage 2 were subjected to reliability assessment and evaluation of the implications of the record on the food and feed or environmental risk assessments.

Two independent reviewers examined the records for inclusion/exclusion for each eligible information/data requirement at all stages of review. Reviews and selections were conducted independently. During the rapid assessment process (Stage 1), only records that were deemed clearly not relevant by all reviewers were excluded from further review. This conservative

approach ensures that all potentially relevant records are evaluated until they are deemed to be either relevant or clearly not relevant in Stage 2. Following the Stage 1 reviews, reviewers scored the records as either 1) relevant or unclear relevance, 2) clearly not relevant.

A kappa test was performed after the Stage 1 review and before any discussion of abstracts over which there was disagreement by the reviewers. Of the 286 records reviewed from the databases at Stage 1 there were 280 agreements to exclude, 2 records where both reviewers agreed to include it for Stage 2 review, and 4 disagreements where one reviewer selected to include while the other selected exclude. This yielded a kappa test score of .49.

Subsequently, the reviewers met to discuss the abstracts in which they disagreed and moved the 1 record over which there was disagreement forward to full-length review for a total of 3 records that were reviewed in Stage 2. There was no disagreement among the reviewers after Stage 2 therefore no tie breaker review was needed.

Because of the format of documents retrieved from internet searching of key organizations (i.e., title and abstract is not often provided) the kappa test was conducted only on the output of the database search.

#### **4.1.2 Records from key organizations**

The records returned from searching the websites of key organizations were considered relevant if they are risk assessments, scientific opinions/reports concerning the commercial release of GMO being examined or documents on the biology of the crop of interest. The regulatory agency webpages that are searched do not post primary data; therefore, all other document types were not considered relevant.

The format of records returned from regulatory agency websites does not often meet the format required to assess them using the 2-stage process followed for the database records. Those websites at which the records are published in English were assessed by two independent reviewers. Due to format, full-text documents were assessed to determine relevance. For those websites where the records are not published in English, the results were reviewed by a native speaker. If the document was deemed to be a relevant document type then it was translated into English and two independent reviewers determined if it met the criteria for inclusion. The rationale for record exclusion is provided only for those records classified as one of the relevant document types and if excluded based on other eligibility criteria.

Regulatory agencies in Mexico (Intersecretarial Commission on Biosafety of GMOs) and Argentina (National Advisory Commission on Agriculture Biotechnology) do not post the relevant document types on their respective agency websites and were not searched.

For the purposes of generating the statistics related to the records returned from the search of the regulatory agency websites certain assumptions were made. A unique internet record was defined as a unique URL. If the URLs for two documents were identical except for the file format (e.g., pdf versus .doc or .docx), one of the documents was considered a duplicate and excluded from statistical accounting. Documents that were classified as relevant were manually examined to determine if there were any duplicates among them. If a duplicate was identified then it was excluded.

Documents that are clearly labeled as draft or with a line for a signature that is blank were not reviewed.

## **4.2 Results of the Publication Selection Process**

For electronic bibliographic databases, the date on which the search was conducted, the date of the most recent update of the database, the service provider used, date span of the search, any limits applied to the search (e.g., study types, dates, languages), and the total number of records retrieved before and after removing duplicates was recorded (Table 9).

Additionally, the line-by-line strategy with the number of publications identified per line is presented (See Appendix A).

For records from websites, the following was recorded (if available): the website name and service publisher used, justification for choosing the source, the URL, the date on which the search was conducted, the date of the most recent website update at the time it was searched, the date span of the search, the search terms used, any limits to the search, and the number of relevant records retrieved.

There were no relevant publications so no manual references were searched as presented in the flowchart of the publication selection process (Figure 1).



**TABLE 9      Electronic bibliographic database search results**

<b>Database</b>	<b>Search Date dd/mm/yyyy</b>	<b>Service provider</b>	<b>Date span of the search dd/mm/yyyy<sup>a</sup></b>	<b>Any limits applied to the search</b>	<b>Total number of records retrieved after removing duplicates</b>
Agricola	03/09/2020	Ovid Technologies	01/01/2019 – 01/09/2020	Dates	39
BIOSIS Previews	03/09/2020	Ovid Technologies	01/01/2019 – 31/08/2020	Dates	83
CAB Abstracts	03/09/2020	Ovid Technologies	01/01/2019 – 31/08/2020	Dates	61
Medline	03/09/2020	Ovid Technologies	01/01/2019 – 02/09/2020	Dates	103

<sup>a</sup>Ovid only allows results to be limited by year. The results were de-duplicated across databases and then de-duplicated against the prior year's returned records. The frequency of database update varies. Ovid has provided us with the following update information: Agricola updated monthly on the 1<sup>st</sup> of the month, BIOSIS Previews updated weekly on Mondays, CAB Abstracts updated weekly on Mondays, and Medline updated daily.

**TABLE 10 Regulatory agency webpage search details**

Regulatory agency name	URL	Date of search dd/mm/yyyy	Date of most recent website update dd/mm/yyyy	Date span of search <sup>a</sup>	Total number of records retrieved after removing duplicates	Number of relevant records
Health Canada <sup>b</sup>	<a href="https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-products.html">https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-products.html</a>	29/07/2020	28/05/2020	No limitations	6	0
Ministry of Agriculture, Forestry and Fisheries	<a href="http://www.maff.go.jp/e/">http://www.maff.go.jp/e/</a>	20/07/2020	No update information provided	No limitations	3	0
National Technical Commission on Biosafety <sup>c</sup>	<a href="http://ctnbio.mcti.gov.br/">http://ctnbio.mcti.gov.br/</a>	15/07/2020	No update information provided	No limitations	1	0
Office of the Gene Technology Regulator	<a href="http://www.ogtr.gov.au/">http://www.ogtr.gov.au/</a>	23/07/2020	23/07/2020	No limitations	2	0
US Environmental Protection Agency	<a href="https://www.epa.gov/ingredients-used-pesticide-products/current-and-previously-registered-section-3-plant-incorporated">https://www.epa.gov/ingredients-used-pesticide-products/current-and-previously-registered-section-3-plant-incorporated</a>	05/08/2020	14/07/2020	No limitations	0	0

<sup>a</sup>Records published prior to 2019 were manually excluded (if any).

<sup>b</sup>Also searches Environment and Climate Change Canada (<https://www.ec.gc.ca/cc/>) and Canadian Food Inspection Agency (<http://www.inspection.gc.ca/plants/plants-with-novel-traits/notices-of-submission/eng/1300143491851/1300143550790>).

<sup>c</sup>Partial reports are excluded from review and statistics since there is no new information contained in these reports and they are not the official final opinion of the agency.

The results of the selection process are recorded in Table 11.

**TABLE 11 Results of the publication selection process for each review question and or group of information/data requirements searched**

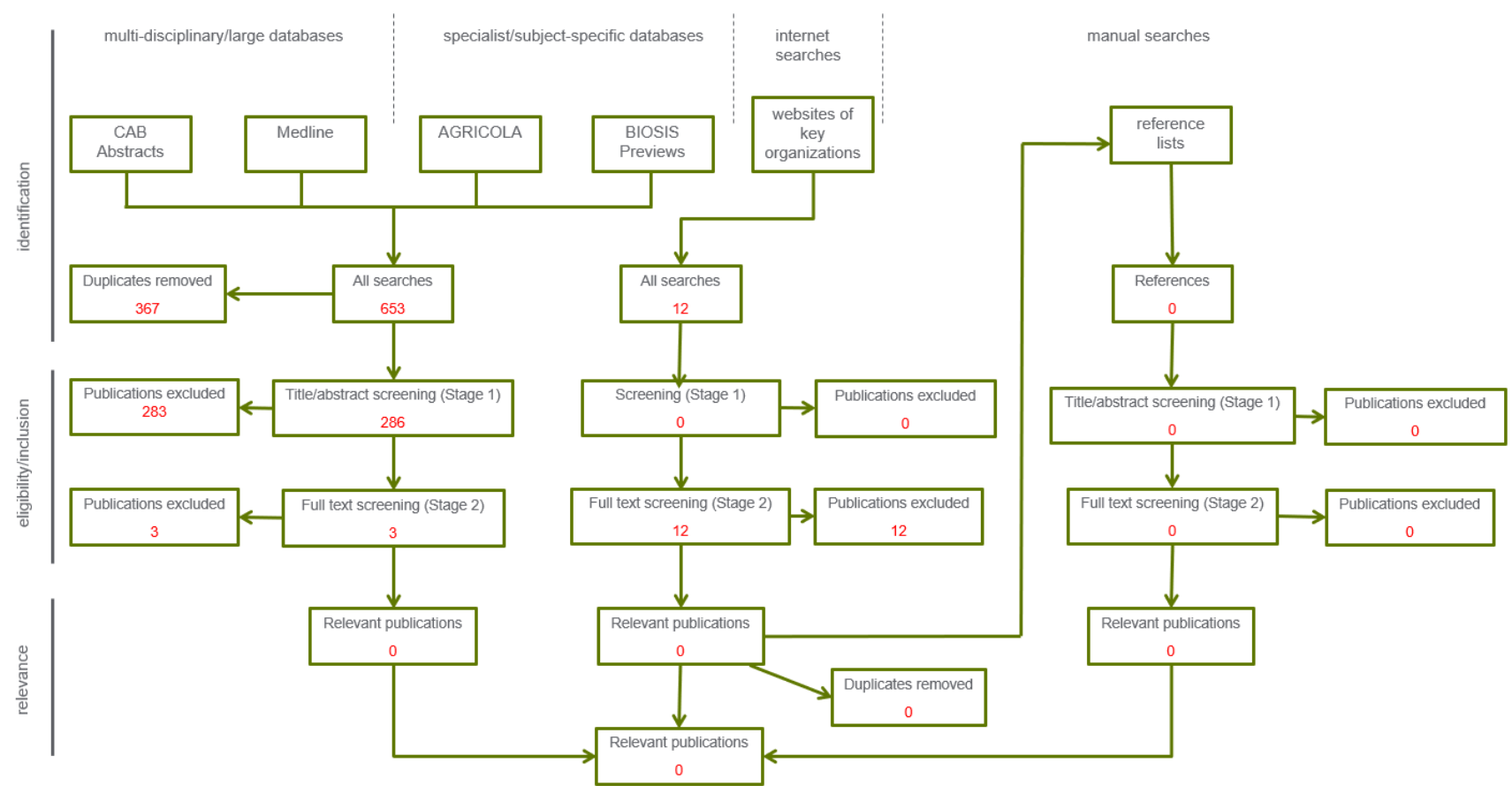
<b>Review question and/or category of information/data requirement(s) captured in the search</b>	<b>Number of publications</b>
Publications identified after all <sup>a</sup> searches of the scientific literature (excluding duplicates)	298
Database results identified	286
Internet results identified	12
Manual searching – checking reference lists <sup>b</sup>	0
Publications excluded from the search results after screening of title and abstracts <sup>c</sup> (stage 1)	283
Database results excluded	283
Internet results excluded	0
Manual searching - reference results excluded	0
Publications screened using full-text (stage 2)	15
Database results screened	3
Internet results screened	12
Manual searching - reference results screened	0
Publications excluded after full-text screening	15
Database results full-text excluded	3
Internet results full-text excluded	12
Manual search – references excluded	0
Unobtainable publications	0
Unclear publications	0
Publications considered relevant	0
Database results relevant	0
Internet results relevant	0
References from relevant internet documents	0

<sup>a</sup>Both from electronic bibliographic databases and other sources of scientific literature.

<sup>b</sup>No relevant records were returned from the searching of regulatory agency websites therefore there are no records reported as a result of manual searching.

<sup>c</sup>Due to the formatting of records from the websites of key organizations (i.e., a lack of abstracts and in some cases titles) these records were reviewed in a single stage in which the full-text document was reviewed.

**FIGURE 1**      **Flow chart of the publication selection process**



### **4.3 Relevant Publications**

No publications were considered relevant after detailed assessment of full-text documents.

No publications were considered relevant after detailed assessment of internet documents.

4.4 Excluded Publications After Detailed Assessment of Full-Text Documents

A list of the full bibliographic references for all excluded studies retrieved from database searching after detailed assessment of full-text documents for relevance (i.e., stage 2), with justification for their exclusion, is recorded in Table 12 and 13.

TABLE 12 Report of all publications excluded after detailed assessment of full-text documents

List of bibliographic references for all relevant publications, classified by category of information/data requirements			
Study author(s) and year	Title	Source	Reason(s) for exclusion based on eligibility/inclusion criteria table
Fast <i>et.al.</i> , 2020	Transgene expression in sprayed and non-sprayed herbicide-tolerant genetically engineered crops is equivalent	Regulatory Toxicology & Pharmacology	Intervention/exposure – The stacks examined in the publication are not those that are in scope.
Alves <i>et.al.</i> , 2020	Dung beetles and the conservation of diversity in an agricultural landscape with maize fields and Atlantic Forest remnants	Acta Oecologica	Intervention/exposure – The publication examined dung beetle populations adjacent to cultivated fields (i.e., the fields were not planted by the researchers). The authors asked the farmers to qualify their inputs in a survey where one of the options was “transgenic maize” and the results were compiled based on survey results. Therefore, while it is not know whether any of the maize products planted are those within the scope of this literature review because the data were combined it is not relevant.
Ramos <i>et.al.</i> , 2020	Agronomic parameters and morpho-agronomic characteristics of genetically modified maize hybrids compared to conventional maize hybrids	Bioscience Journal	Intervention/exposure – The stacks examined in the publication are not those that are in scope.

**TABLE 13      Report of all publications excluded after assessment of internet documents**

List of bibliographic references for all relevant publications, classified by category of information/data requirements <sup>a</sup>			
Study Author(s) and year	Title	Source	Reason(s) for exclusion based on eligibility/inclusion criteria table
Office of the Gene Technology Regulator (OGTR no date)	Application for a licence for DIR of GM plants into the environment - limited and controlled release - example answers	<a href="http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dirapps-limitedandcontrolledanswers-toc">http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dirapps-limitedandcontrolledanswers-toc</a>	Intervention/exposure

<sup>a</sup>The other 11 records excluded and not presented in this table did not fulfill the eligibility criteria for report format.

#### **4.5 Unobtainable Publications**

No publications were considered unobtainable.

#### **4.6 Unclear Publications**

No publications were considered unclear.

#### **4.7 Full-Text Documents**

Full text documents for all relevant publications were compiled using a reference management software (.RIS format) and accompany this final report.

#### **4.8 Narrative Synthesis/Summary of Relevant Publications**

A narrative synthesis/summary of the relevant studies describing their overall volume, strength, and direction per main category of information/data requirements was not reported because this literature review was conducted for annual PMEM reports on GMOs authorized in the EU market and therefore it is not required.

#### **4.9 Implications of Relevant Publications on Risk Assessment**

There were no relevant publications.

### **5.0 RECORDS TO BE MAINTAINED**

Records maintained include, but are not limited to, documentation of database search dates, database update dates, resolution of differences of opinion on records, the report, and any amendments or deviations.

### **6.0 ARCHIVING OF RECORDS**

The protocol, protocol amendments or deviations, raw data, related documentation, and the final report are archived at Syngenta in Research Triangle Park NC, USA.



## 7.0 REFERENCES

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## **APPENDIX SECTION**

## **APPENDIX A   Search history and subject indexing**

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rootworm\* or root worm\* or Ostrinia or O nubilalis or Diatraea or D grandiosella or D crambidoides or Helicoverpa or H zea or Spodoptera or S frugiperda or S exigua or Papaipema or P nebris or Elasmopalpus or E ignosellus or D saccharalis or Striacosta or S albicosta or Agrotis or A ipsilon or Feltia or F jaculifera or Pseudaleia or P unipuncta or Diabrotica or D virgifera or D barberi or ECB or SWCB or SCSB or CEW or FAW or SCB or WBC or WCRW or WCR or NCRW or MCR or MCRW) adj2 (toleran\* or resistan\* or protect\* or control\*) or Bacillus thuringiensis or B thuringiensis or ((glufosinate\* or glufosinate\* or Basta\* or Liberty\* or Ignite\* or Rely\* or Finale\* or Challenge\* or gl?phosate or gl?fosate or roundup\* or round up\* or herbicide\* or pesticide\*) adj2 (toleran\* or resistan\* or protect\*)))).mp. [mp=meeting information, title, original title, map information, note, abstract, heading words]

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#### 1. Efficacy of Bacillus thuringiensis (Bt) maize expressing Cry1F, Cry1A.105, Cry2Ab2 and Vip3Aa20 proteins to manage the fall armyworm (Lepidoptera: Noctuidae) in Brazil

Moscardini, ValAcra F. Marques, Luiz H. Santos, AntA'nio C. Rossetto, JaAcidino Silva, Oscar A.B.N. Rampazzo, Pedro E. Castro, Boris A.

Crop protection. 2020 Nov. 137(137)

[Journal Article, Government Publication]

AN: IND606978243.

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AB Recommendations to manage the impact of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to maize production include the use of Bt crops where approved, combined with the adoption of integrated pest management (IPM) and insect resistance management (IRM) practices. This study evaluated the efficacy of Bt maize expressing proteins Cry1FA "A" "Cry 1A,105A" "A" "Cry2Ab2A" "A" "Vip3Aa20 to control *S. frugiperda* under field conditions in Brazil. This Bt maize technology effectively managed seedling cutting injury, significantly reduced foliar feeding and achieved near 100% protection from kernel-feeding caused by both a Cry1F-resistant strain and a field-collected population of *S. frugiperda*. Consistent field-collected data across several years and planting regions suggest that this Bt maize technology will be an important tool for the effective management of all important feeding injury types caused by *S. frugiperda* in Brazil. As with any Bt maize hybrid, its implementation within IPM and IRM practices should help growers maintain maize crop sustainability in Brazil.

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<input type="checkbox"/>	18	(Phosphomannoisomerase or Mannose 6-phosphate isomerase or Phosphomannoisomerase or Phosphomannose isomerase or 9023-88-5 or AAA24109 or "EC 5.3.1.8" or "E.C. 5.3.1.8").mp. [mp=abstract, original language book title (non-english), book title (english), title, heading words]	821	Advanced	<a href="#">Display Results</a>   <a href="#">More</a>		
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<input type="checkbox"/>	26	((Insect or Insects or coleoptera* or lepidoptera* or pest or pests or stalkborer* or stalk	584578	Advanced	<a href="#">Display Results</a>   <a href="#">More</a>		

borer\* or borer\* or cornborer\* or corn borer\* or noctuidae or Crambidae or Chrysomelidae or earworm\* or ear worm\* or armyworm\* or army worm\* or cutworm\* or cut worm\* or rootworm\* or root worm\* or Ostrinia or O nubilalis or Diatraea or D grandiosella or D cramboides or Helicoverpa or H zea or Spodoptera or S frugiperda or S exigua or Papaipema or P nebris or Elasmopalpus or E lignosellus or D saccharalis or Striacosta or S albicosta or Agrotis or A ipsilon or Feltia or F jaculifera or Pseudaletia or P unipuncta or Diabrotica or D virgifera or D barberi or ECB or SWCB or SCSB or CEW or FAW or SCB or WBC or WCRW or WCR or NCRW or MCR or MCRW) adj2 (toleran\* or resistan\* or protect\* or control\*) or Bacillus thuringiensis or B thuringiensis or ((glufosinate\* or glufosinate\* or Basta\* or Liberty\* or Ignite\* or Rely\* or Finale\* or Challenge\* or gl?phosate or gl?fosate or roundup\* or round up\* or herbicide\* or pesticide\*) adj2 (toleran\* or resistan\* or protect\*)))).mp. [mp=abstract, original language book title (non-english), book title (english), title, heading words]

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# 1. Fluorescence of chlorophyll a in transgenic maize with herbicide application and attacked by Spodoptera frugiperda (Lepidoptera: Noctuidae)

Rocha de Souza, Michael William [Author, Reprint Author; E-mail: michael12011@hotmail.com]; Ferreira, Evander Alves [Author]; dos Santos, Jose Barbosa [Author]; Soares, Marcus Alvarenga [Author]; de Castro e Castro, Barbara Monteiro [Author]; Zanuncio, Jose Cola [Author].

Phytoparasitica. 48(4). SEP 2020.

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AB Physiological changes in maize plants, submitted to herbicides and pests, should be studied for integrated management in this crop. The objective was to evaluate the chlorophyll a fluorescence in maize genotypes damaged or not by *Spodoptera frugiperda*, and sprayed with herbicides. The herbicides atrazine and nicosulfuron were applied to the plants at doses of 3 kg ha<sup>-1</sup> and 0.06 kg ha<sup>-1</sup>, respectively. A randomized block design in a factorial scheme with an additional treatment 3 (2 x 2 + 1) was used, being the factor A: maize leaves exposed or not to the attack of *S. frugiperda*; factor B: Herculex (R) (transgenic maize encoding the Cry1F protein) and Isohybrid (non-transgenic maize) genotypes and an additional treatment represented by the PowerCore (R) genotype (stacked transgenic maize encoding the Cry1F, Cry1A.105 and Cry2Ab2 proteins). The initial fluorescence of chlorophyll a (F0 - electron quantum(- 1)), maximum fluorescence (Fm - electron quantum(- 1)), ratio between variable fluorescence and maximum fluorescence of chlorophyll a (Fv/Fm) and electron

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- ☐ 1. [Efficiency of \*Bacillus thuringiensis\* strains and their cry proteins against the red flour beetle, \*Tribolium castaneum\* \(Herbst.\) \(Coleoptera: Tenebrionidae\).](#)

Elgizawy, K. K. Ashry, N. M.

*Egyptian Journal of Biological Pest Control*; 2019. 29(94);(30 December 2019). 32 ref.

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AB *Bacillus thuringiensis* (*Bt*) is one of the used bioagents in insect pest control. Its toxicity is largely due to the insecticide endotoxins (crystalline (Cry) proteins) that act selectively on insects and nematodes. The efficiency of 20 of the most common Coleopteran-specific Cry proteins of *Bt* strains was tested against third instar-larvae of the red flour beetle *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae). The primary screening results revealed that 11 Cry proteins (Cry8Ea, Cry8Fa, Cry1Ba, Cry8Ca, Cry1Fb, Cry1Ea, Cry1Ca, Cry55Aa, Cry9Da, Cry1Da, and Cry1Ia) were not toxic at all, 4 Cry proteins (Cry1Aa, Cry14Aa, Cry8Aa, and Cry7Ab) did not cause mortality but caused significant inhibition of growth, and 5 Cry proteins (Cry3Aa, Cry37Aa, Cry22Aa, Cry51Aa, and Cry3Ba) were toxic to *T. castaneum* larvae. The active 5 Cry proteins were used in the subsequent experiments. Five concentrations, being 0.25, 0.5, 1.0, 1.5, and 2.0 g Cry protein/10 g diet were used against the third instar larvae, and their mortalities were estimated. The LC<sub>50</sub> values of Cry3Aa, Cry37Aa, Cry22Aa, Cry51Aa, and Cry3Ba were 0.46, 0.77, 1.25, 1.45, and 1.60 g/10 g, respectively. While the LT<sub>50</sub> values of the same Cry proteins (for the concentration 2 g/10 g diet) were 1.50, 1.93, 2.29, 2.23, and 4.22 days, respectively. The results indicated that Cry3Aa was the most active one against *T. castaneum* larvae. The results of the sublethal study showed that the application of LC<sub>30</sub> value of the active 5 Cry proteins reduced total eggs laid daily per female within 2 weeks, where, the percent decrease in egg numbers

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1. Performance of Sesamia nonagrioides on cultivated and wild host plants: Implications for Bt maize resistance management.

M Camargo A; Arias-Martin M; Castanera P; P Farinos G.

Pest Management Science. 2020 May 17.

[Journal Article]

UI: 32418304

Authors Full Name

M Camargo, Ana; Arias-Martin, Maria; Castanera, Pedro; P Farinos, Gema.

Abstract

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AB BACKGROUND: **Sesamia nonagrioides is an important maize pest in the Mediterranean basin that is effectively controlled by Cry1Ab-expressing maize (Bt maize).** The continued cultivation of **Bt maize in Spain** exerts high selection pressure on the target **pests**, which could lead to the development of **resistance**. Provision of refuges of non-Bt plants **is an essential component in the high-dose/refuge (HDR) strategy to delay resistance evolution**. Here we **analyze** the suitability of cultivated (rice and sorghum) **and** wild (Johnsongrass, cattail, common reed **and** giant reed) plants, reported **as** hosts of **S. nonagrioides, for larval development and oviposition of this pest compared to maize, and** we evaluate their potential role **in delaying resistance development to Bt maize**. RESULTS: Bioassays conducted with plant pieces or whole plants showed that the larval cycle could only be completed **in the three cultivated plants and in Johnsongrass. Females showed a strong preference for ovipositing on maize in comparison with sorghum or rice. Although** young larvae consumed more sorghum than **maize in two-choice bioassays, both larvae and adults had a better performance (shorter larval period and higher pupal weight, fecundity and fertility) when larvae fed on maize throughout their larval stage than when they fed on sorghum or rice.** CONCLUSION: None of the **alternative** hosts of **S. nonagrioides tested here should be considered as natural unstructured refuges within the HDR strategy for Bt maize and this pest in Spain, as** some of the necessary requirements to **fulfill** this strategy would not be met.

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2. Can the growing of transgenic maize threaten protected Lepidoptera in Europe?.

Loveli GL; Lang A; Ferrante M; Bacle V.

Insect Science. 2020 Jul 16.

[Journal Article]

UI: 32672413

Authors Full Name

Loveli, Gabor L; Lang, Andreas; Ferrante, Marco; Bacle, Victor.

Abstract

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AB We evaluated whether **protected** European butterflies can potentially be **at risk if transgenic maize is** extensively grown **in Central Europe**. We explored potential consequences of both **insect resistant (IR) and herbicide resistant (HR) transgenic maize. IR maize** can produce pollen that **is toxic to lepidopteran larvae, and** this puts butterfly species **at possible risk if the presence of young larvae coincides with maize flowering, during which large quantities of maize pollen can be deposited on vegetation. By considering the timing of maize flowering in Europe and the phenology of the protected Lepidoptera species, we found that 31 species had at least one generation where 50% of the larval stage overlapped with maize flowering, and 69 species for which first instar larvae were present during maize pollen shedding. HR maize allows high concentration herbicide treatments on fields without seasonal limitation, which can drastically reduce weed densities. In cases where such weed species are host plants for protected butterflies, reduced host plant/food availability can result, causing population decreases. By using published information, we first identified the important weed**

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