



Review

Teosinte and maize × teosinte hybrid plants in Europe – Environmental risk assessment and management implications for genetically modified maize

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ABSTRACT

The reporting of teosinte and maize × teosinte hybrid plants in maize fields in Spain and France has fuelled the continuing debate on the environmental risks and benefits of genetically modified (GM) crops in Europe. Concern has been expressed that GM maize may hybridise with teosinte or maize × teosinte hybrids, leading to the development of invasive weeds that pose unconsidered risks to the environment. In order to assess these risks, we hypothesised plausible pathways to harm from the cultivation and import of GM maize events MON810, Bt11, 1507 and GA21 for situations where GM maize plants and teosinte/maize × teosinte hybrids are sympatric. This enabled identification of events that must occur for harm to occur, and derivation of risk hypotheses about the likelihood and severity of these events. We tested these risk hypotheses using relevant available information. Overall, we conclude that the envisaged harmful effects to the environment arising from gene flow from GM maize to teosinte/maize × teosinte hybrids when cultivating or importing current commercial varieties of GM insect-resistant and herbicide-tolerant maize would be no greater than those from conventional maize: neither trait is likely to increase the abundance of teosinte or maize × teosinte progeny. Regardless of the likelihood of gene flow to teosinte or maize × teosinte hybrids, continuous cultivation of herbicide-tolerant maize, along with the repeated and exclusive application of the relevant herbicide, should be avoided in order to maintain the effectiveness of weed management. While scientific uncertainties about certain steps in the pathways remain, the risk assessment can be completed, using worst-case assumptions to handle these uncertainties.

1. Introduction

Teosinte is the common name for a group of annual and perennial grass species (Poaceae) of the genus *Zea* of which the subspecies maize (*Zea mays* subsp. *mays*) is the main domesticated taxon. Teosinte includes highly variable species and subspecies that are native to Mexico and Central America (OECD, 2003; Andersson and de Vicente, 2010). The taxonomy of teosinte has not been easy to establish. However, based on its distribution, morphology, cytology and genetics, the genus *Zea* is currently classified into nine taxa within six species in two sections (*Zea* and *Luxuriantes*) (Wilkes, 1967; Iltis and Doebley, 1980; Fukunaga et al., 2005; Warburton et al., 2017). There is only one species (*Z. mays*) in the section *Zea*, which includes four subspecies (*Z. mays* subsp. *mays*, *mexicana*, *parviglumis*, and *huehuetenangensis*). Five more species make up the section *Luxuriantes*, including three recently

identified taxa from Mexico in Nayarit, Michoacan and Oaxaca (Sánchez et al., 2011; Warburton et al., 2017). The use of the term ‘teosinte’ generally refers to all of these taxa collectively, other than cultivated maize (*Z. mays* subsp. *mays*).

In Mexico and Central America, most teosinte species and subspecies have very narrow geographic distributions consisting of only few local populations (Fukunaga et al., 2005), and are endangered requiring conservation. *Z. mays* subsp. *mexicana* and *parviglumis* (referred to hereafter as *mexicana* and *parviglumis*, respectively) are widely distributed, mostly in agricultural fields, where they are considered non-aggressive weeds (Andersson and de Vicente, 2010). These two subspecies are occasionally cultivated for forage. Some teosinte taxa have also become established or even naturalised outside their centre of origin, and are considered weeds that can compete with cultivated maize (Sánchez et al., 2011; Pardo et al., 2016). Densities of teosinte

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can be high in fields with continuous maize cropping, and may cause severe loss of crop yield and quality; therefore, teosinte is subject to control or eradication measures (Balbuena et al., 2011; EFSA, 2016a; Pardo et al., 2016).

Teosinte – presumably *Z. mays* subsp. *parviglumis* – has been detected in the Poitou-Charentes region of France since 1990 (Arvalis, 2013). Teosinte was also reported from maize fields in Spain (in the Ebro Valley (Aragón) and, to a lesser extent, in the region of Cataluña in the summer of 2014), though it was first observed in 2009 (Pardo et al., 2016). Teosinte found in Spain was assumed to be *mexicana*, but Trtikova et al. (2017) recently demonstrated that the teosinte found in Spain is of admixed origin, most likely involving *mexicana* as one parental taxon and an unidentified cultivated maize variety as the other. The origin of these plants remains unknown (Trtikova et al., 2017). Throughout this paper, we use the term teosinte to refer to *mexicana* and *parviglumis*, which are the presumed taxa detected in maize fields in Spain and France, respectively (also covering maize × teosinte hybrids).

The recent reporting of maize × teosinte hybrid plants in maize fields in Spain led some non-governmental organisations to claim that GM maize may hybridise with weedy teosinte relatives in Europe, leading to the development of invasive weeds that pose previously unconsidered risks to the environment (e.g., Testbiotech, 2016a). They also argued that more data are needed on the identity of observed teosinte and maize × teosinte hybrids, the biological activity of transgenes in teosinte, and the efficacy of methods used to control teosinte as weeds before any conclusions can be drawn on actual risks. They therefore recommended that the European Commission halts the cultivation of maize MON810 in Spain and postpone the voting on the authorisation of three GM maize events for cultivation (e.g., Testbiotech, 2016b). In contrast, the European Food Safety Authority (EFSA), which was mandated by the European Commission to look into the issue, concluded that there are no data that invalidate the previous environmental risk assessment (ERA) conclusions and risk management recommendations on the cultivation of the GM maize events MON810, Bt11, 1507 and GA21 made by its GMO Panel (EFSA, 2016a).

In this paper, we use problem formulation to develop plausible pathways to harm from cultivating and importing GM maize for situations where GM maize plants and teosinte would co-exist in Europe, focusing on specific topics typically considered in the ERA of GM crops. From these pathways, we identify events that must occur for the risk to be realised, and derive testable risk hypotheses for each step. At their most conservative, each hypothesis presumes that the step in the pathway will not occur, and therefore that harm will not arise. If a conservative hypothesis is falsified, a new hypothesis that the step is unlikely is tested. We use relevant available information to test these risk hypotheses. Corroboration of these risk hypotheses would strengthen the conclusion of negligible risk via the pathway in question, whereas finding that all the hypotheses on a particular pathway were false would indicate non-negligible risk (Raybould, 2006). We focus on maize MON810, Bt11, 1507 and GA21, because these events are currently in the authorisation pipeline for cultivation in Europe (in the case of maize MON810, the market application covers the renewal of authorisation).

Maize MON810 and Bt11 express a Cry1Ab insecticidal protein derived from *Bacillus thuringiensis* subsp. *kurstaki*, and maize 1507 expresses a truncated Cry1F protein from *B. thuringiensis* subsp. *aizawai*, both conferring protection against lepidopteran target pests such as the European corn borer (ECB, *Ostrinia nubilalis*) and species belonging to the genus *Sesamia*. Maize Bt11 and 1507 also express phosphinothricin-N-acetyltransferase (PAT) from *Streptomyces viridochromogenes*, providing tolerance to herbicides based on glufosinate-ammonium, but are not intended to be marketed as herbicide-tolerant crops and should therefore not be treated with glufosinate-ammonium herbicides. Maize GA21 expresses a modified version of 5-enolpyruvylshikimate-3-phosphate synthase (mEPSPS), conferring tolerance to herbicides based on

glyphosate (EFSA, 2016a).

2. Protection goals and harm

The cultivation and importation of GM crops is subject to a risk assessment and regulatory approval before entering the market in Europe, as in most jurisdictions (Craig et al., 2008; Devos et al., 2014a). Pre-market ERA addresses the question to which extent the use of GM crops poses risks to the environment (EFSA, 2010). Robust ERAs begin with an explicit problem formulation where protection goals, plausible and relevant exposure scenarios and the potential adverse effects from those exposures are identified. Risk is then characterised by testing specific hypotheses about the probability that harm (= an adverse effect on something of value) will occur and severity of that harm should it occur. The decision on the level of acceptable risk is taken by risk managers who weigh policy options to accept, minimise or reduce characterised risks with other relevant information such as the economic, social or political implications of the proposed activity.

A crucial step of problem formulation for an ERA is to identify what qualifies as harm under the relevant regulations (Sanvido et al., 2012). Identification of these harms to those components of the environment (e.g., species, ecosystem services, habitats) that are valued and/or protected by relevant existing laws or policies can be referred to as setting operational protection goals for ERA. Operational protection goals are derived from more broadly defined policy protection goals, as ones that can be more clearly predicted or measured (Garcia-Alonso and Raybould, 2014; Devos et al., 2015, 2016a; Layton et al., 2015; EFSA, 2016b). This focuses the assessment on the phenomena that are important for decision-makers (Evans et al., 2006), and away from the multitude of other changes that may interest scientists, but which are irrelevant for ERA (Raybould, 2006, 2007, 2010; Gray, 2014; Devos et al., 2016a).

When defining harm, an important consideration is whether the proposed activity may lead to new harms, or only to different ways of causing harm that already result from current practice. In most cases, if not all, the envisaged harmful effects to the environment from cultivating or importing GM crops are of the same kind as those from conventional crops (Tiedje et al., 1989; Boulter, 1995; NRC, 2000, 2002; Connor et al., 2003; Lemaux, 2009; Mannion and Morse, 2012; Knox et al., 2013; NAS, 2016). Hence, definitions of harm for ERAs for GM crops are really statements about what would be considered unacceptable increases in the frequency or severity, or both, of harmful effects if a particular GM crop was to be used instead of a similar conventional crop. In this paper, we use the phrase “cause harm” in this relative sense, rather than to imply that growing or importing conventional crops is harmless to the environment (Sanvido et al., 2012; Devos et al., 2014b).

The cultivation of conventional crops is not subject to pre-market regulatory scrutiny in most jurisdictions, with the notable exception of Canada (Smyth and McHughen, 2008). New conventional crop varieties, including those produced by mutagenesis, also do not require pre-market approvals for importation, although there are numerous post-market regulations concerning food safety. From the lack of pre-market regulation, we may infer that the environmental effects of using conventionally-bred crops are acceptable to society. Therefore, risks posed by a GM crop can be considered acceptable, provided that the likely effects of its cultivation or import are within the legally permitted effects of cultivation or import of the conventional crop.

A typically assessed concern in ERAs of GM crops is that the acquisition of transgenes through gene flow by cross-compatible wild or weedy relatives could increase their persistence and abundance compared with gene flow from conventional counterparts (Ellstrand, 2003; Hokanson et al., 2010, 2016; Huesing et al., 2011; Macdonald, 2012). If these plants become more persistent or abundant in agricultural land, they may exacerbate weed problems, thereby causing or increasing economic harm by reducing yield or the quality of the crops they infest,

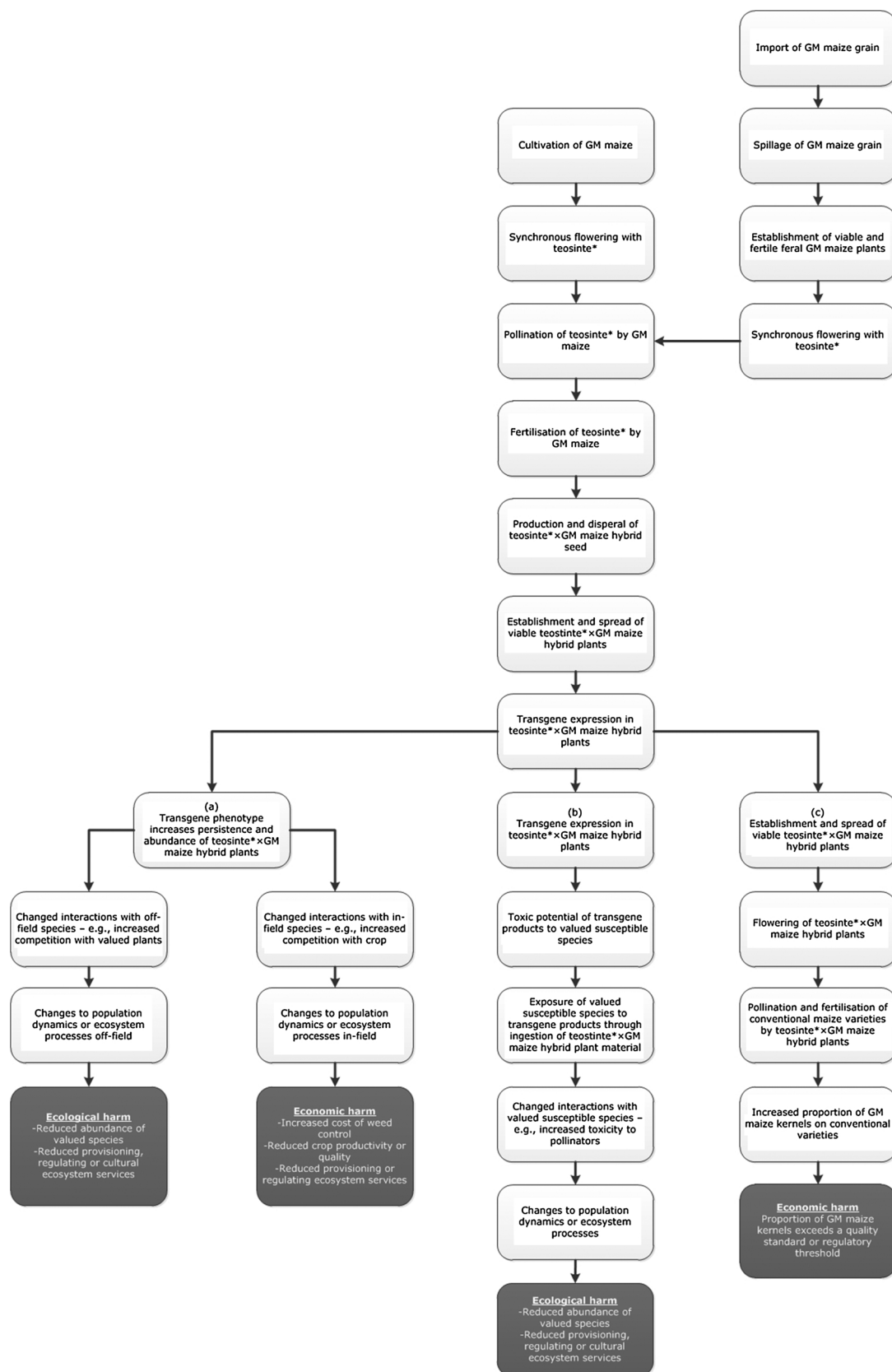


Fig. 1. Steps in a generic pathway to harm resulting from hybridisation between GM maize (pollen parent) and teosinte (seed parent), including maize × teosinte hybrid plants. Part (a) represents the final steps to ecological and economic harm resulting from greater persistence and abundance of teosinte × GM maize hybrids compared with their conventional counterparts (* including maize × teosinte hybrid plants) part (b) represents the final steps to ecological harm resulting from the toxicity of transgene products to valued susceptible species exposed to teosinte × GM maize hybrid plant material compared with their conventional counterparts (* including maize × teosinte hybrid plants) and part (c) represents the final steps to economic harm resulting from gene flow from teosinte × GM maize hybrids to conventional maize (* including maize × teosinte hybrid plants).

or by increasing costs of weed control. If transgenes acquired by cross-compatible relatives result in increased persistence and abundance of those plants outside cultivated land and beyond the geographical range of their conventional counterpart, they may cause ecological harm by reducing ecosystem services, including the cultural services provided by biodiversity (Sanvido et al., 2012; Devos et al., 2015). These harms could also be caused by the novel traits conferred by the transgenes, in cases where the newly expressed proteins in the cross-compatible relative are toxic to non-target organisms at concentrations present in the field (Romeis et al., 2008; Devos et al., 2012b, 2016b; De Schrijver et al., 2016).

Hybrid plants derived from crosses between the GM crop and a cross-compatible relative may facilitate the potential for gene flow by forming populations that harbour a transgene and pass them to other cross-compatible plants (including cultivated plants) through cross-pollination. This could contribute to admixtures and result in economic harm. In Europe, an adventitious or technically unavoidable presence of approved GM material in non-GM food/feed products is accepted up to a level of 0.9%. If the content of GM material in a non-GM product exceeds this threshold, the product has to be labelled as containing GM material, which may affect its market acceptability (Devos et al., 2008, 2009).

3. Pathway to harm concepts

To further frame the ERA, plausible pathways are devised in the problem formulation to describe how the proposed activity – in this case cultivating or importing GM maize in Europe – could lead to possible harm to operational protection goals (Raybould, 2006, 2007, 2010; Johnson et al., 2007; Nickson, 2008; Wolt et al., 2010; Gray, 2012; Tepfer et al., 2013; Layton et al., 2015). A pathway to harm is a conceptual model outlining events that must occur for an activity to cause a specified harm. As described below, the steps in the pathway enable the formulation of hypotheses that can be tested in order to characterise risk. A risk assessment may include many pathways because the proposed activity could lead to different harms, or because a particular harm could arise in different ways, or both. Each step in the pathway leads to a hypothesis that harm will not arise. Consider a pathway in which Event A must lead to Event B in order for harm to occur. A conservative hypothesis would be that Event A never leads to Event B. Less conservative hypotheses might be that Event B does not occur at a certain time or place, or does not occur above a certain frequency or magnitude (Raybould, 2010). The precise form of the risk hypothesis will depend on how harm is defined and how decisions on the acceptability of risk will be made.

Testing and corroboration of a risk hypothesis builds confidence that risk is negligible via the pathway in question; and corroboration following a rigorous test gives greater confidence than does a weak test (Raybould, 2006). Falsification of all of the risk hypotheses in a pathway would indicate high probability of harm, and high risk if the harm was severe enough. Hypotheses may be tested with existing data; if these tests are inconclusive for regulatory decision-making, new studies may be undertaken. Enabling the testing of risk hypotheses makes the pathway to harm approach very powerful for ERA: harm is agreed explicitly from the start; existing information is used effectively; new data are collected with a clear purpose; risk is characterised against well-defined criteria of hypothesis corroboration or falsification. Furthermore, although pathways to harm are not decision trees, they can be adapted to allow clear communication of risk assessment conclusions.

4. Pathways to harm

In the hypothesised pathways to environmental and economic harm, a distinction can be made between the cultivating and importing of GM crops, because the risk quantified as a combination of the level of

exposure and potential impacts are expected to be higher under cultivation conditions than import conditions (EFSA, 2010; Devos et al., 2012a; Roberts et al., 2014).

4.1. Cultivation conditions

The production and establishment of viable and fertile hybrid plants harbouring the acquired transgenes form the early steps in various putative pathways to harm (Fig. 1). From there, a pathway can lead to hybrids becoming more persistent or abundant than their conventional counterparts owing to the presence and expression of the transgenes, such as those conferring abiotic or biotic stress tolerance (Fig. 1a). For example, presence and expression of a transgene for herbicide tolerance in hybrid plants could lead to in-field economic harm if the hybrids become a troublesome weed that can no longer be controlled by the herbicide. The greater persistence or abundance of the transgenic hybrids may adversely affect other organisms, ecosystem services or the abiotic environment (Craig et al., 2008; EFSA, 2010; Macdonald, 2012). These adverse effects must be greater than those caused by growing conventional plants in order for them to be considered harmful. Such effects may occur outside and inside agricultural fields; harm outside fields will usually be regarded as ecological, whereas harm inside fields may be regarded principally as economic (Sanvido et al., 2012; Garcia-Alonso and Raybould, 2014).

Other pathways to harm arise from the properties of the novel traits, independent of any effect that they have on persistence or abundance of the hybrid plants (Craig et al., 2008; EFSA, 2010; Macdonald, 2012) (Fig. 1b). For example, if hybrid plants derived from spontaneous crosses between the GM crop and a cross-compatible relative are a food plant for a valued lepidopteran, presence and expression in a hybrid population of a transgene for control through toxicity to a pest Lepidoptera could lead to ecological harm even though the persistence or the abundance of the relative is unchanged. Likewise, ingestion of potentially harmful amounts of GM hybrid pollen deposited on host plants of valued lepidopterans could be a hazard to the larvae of these butterflies feeding on these host plants during pollen dehiscence (Lang and Otto, 2010; Perry et al., 2010, 2011, 2012, 2013).

Another pathway to harm occurs if hybrid plants derived from spontaneous crosses between the GM crop and a cross-compatible relative, backcross with either parent, serving as a local genetic bridge. In this case, they may extend the potential for transgene flow by forming populations harbouring transgenes that are passed on to other, sympatric cross-compatible plants. If this facilitates the passing of the transgene into conventional (non-GM) plantings of the crop, this could contribute to admixtures with potential for economic harm (Devos et al., 2008, 2009) (Fig. 1c).

4.2. Import conditions

In addition to cultivation, the previously formulated pathways can be initiated by the importation of GM seeds/grains to Europe. Imported seeds/grains can be released accidentally during handling, transportation, storage and processing. Spillage may occur near shipping centres such as ports or rail-freight depots, or near milling and processing plants, or alongside transport routes. Depending on which plant species and which genes are involved, and on the characteristics of the receiving environment, spilled seeds/grains may grow and establish transient or self-perpetuating populations (Crawley and Brown, 1995; Hodkinson and Thompson, 1997; Mack et al., 2000; Bagavathiannan and Van Acker, 2008). These feral plants may mediate gene flow among cross-compatible plants in the landscape, and may adversely affect other organisms, ecosystem services or the abiotic environment (Devos et al., 2012a).

5. Hypothesis testing

Relevant information available in the scientific literature used to test the risk hypotheses derived for each step of the hypothesised pathways is summarised narratively below. In cases where no information or insufficient information is available to test a risk hypothesis, conservative (worst-case) assumptions were made, assuming progression in the pathway for that specific step.

5.1. Pathways from cultivation of GM maize

5.1.1. Testable hypothesis: the cross-fertilisation of teosinte by (GM) maize does not lead to the production of viable and fertile progeny

For GM maize to cause ecological or economic harm through the hypothesised pathways, teosinte must first successfully acquire the transgenes via gene flow, and produce viable and fertile progeny that stably express the transgenes. This requires the species to be sympatric, flower synchronously and have sufficient level of genetic and structural relatedness.

Most teosinte relatives flower synchronously with maize, although *parviglumis* can flower 2–3 weeks later than maize (Rodríguez et al., 2006). In Mexico, maize typically flowers earlier than *mexicana*. Therefore, maize tassels are likely to be senescent when *mexicana* silks are receptive.

Data from controlled field experiments show that maize can spontaneously hybridise to various degrees with most teosinte relatives when grown together (reviewed by Andersson and de Vicente, 2010). Hybridisation rates between maize and *mexicana* are low (less than 1% per generation) due to pre-zygotic barriers that render *mexicana* unreceptive to maize pollen (Evans and Kermicle, 2001; Kermicle and Evans, 2005), and much higher between maize and *parviglumis* where this incompatibility is absent (> 50%) (Ellstrand et al., 2007). The resultant teosinte (seed parent; female) × maize (pollen parent; male) hybrids are fertile, and can produce more seeds than teosinte (Guadagnuolo et al., 2006; Ellstrand et al., 2007). Moreover, Chavez et al. (2012) found that the force required to detach grains from the cob of F₁ hybrids is substantially less than that for maize, suggesting that progeny dispersal and introgression will not be prevented by the teosinte × maize cob infructescence. Thus, where GM maize plants and teosinte plants are sympatric and flower synchronously, maize alleles, transgenic or not, could move into teosinte populations at rates that depend on the teosinte (sub)species (Doebly et al., 1987; Ellstrand et al., 2007; Andersson and de Vicente, 2010; Trtikova et al., 2017).

Subspecies *mexicana* has co-existed and co-evolved in close proximity to maize in the Americas over thousands of years, and maintained distinct genetic constitutions (Smith et al., 1985; Doebly, 1990a; Fukunaga et al., 2005; Ross-Ibarra et al., 2009; van Heerwaarden et al., 2011; Warburton et al., 2011). Hufford et al. (2013) observed very little evidence for adaptive introgression from maize into *mexicana*. This observation suggests that introgression from maize (male) to *mexicana* (female) is very limited due to the “teosinte crossing barrier”, phenological differences and other biological factors that restrict crossability with maize (Wilkes, 1977; Doebly, 1990a,b; Evans and Kermicle, 2001; Stewart et al., 2003; Baltazar et al., 2005; Kermicle and Evans, 2005; Rodríguez et al., 2006). Similarly, in the case of maize × teosinte hybrids, their hand pollination with maize pollen resulted only rarely in viable progeny in exploratory crossing experiments (Trtikova et al., 2017).

While the crossing barrier may reduce the frequency with which hybrids are formed in the field, Trtikova et al. (2017) noted that it does not preclude their formation, even though at low rates. Studies have estimated that 4–8% of the *mexicana* genome is derived from maize (Fukunaga et al., 2005; Hufford et al., 2013). Gene flow and subsequent introgression of maize alleles into *mexicana* populations most probably results from crosses where *mexicana* first pollinates maize. The resultant hybrids then backcross with *mexicana* to introgress the maize alleles

into the teosinte genome (Aylor et al., 2005; Baltazar et al., 2005; Ellstrand et al., 2007; Hufford et al., 2013).

The extent of hybridisation and introgression between maize and *parviglumis* is still unknown (reviewed by Andersson and de Vicente, 2010). It is also unclear whether *parviglumis* presents genetic barriers to hybridisation with maize, as does *mexicana* (Kermicle and Allen, 1990; Doebly and Stec, 1993; Kermicle, 2006). Hand pollination generally yields high frequencies of hybrids, and hybrids often occur spontaneously in and near Mexican maize fields (Ellstrand et al., 2007; Andersson and de Vicente, 2010). However, using SSR markers, Fukunaga et al. (2005) identified *parviglumis* plants containing 20% or more of maize germplasm, but attributed the not fully differentiated gene pools to the recent origin of maize from *parviglumis*, instead of an admixture through introgression.

While gene flow from maize to teosinte can be observed in field trials designed to maximise the probability of hybridisation, actual rates of gene flow from maize to *mexicana* are probably quite low, even when teosinte is abundant (Baltazar et al., 2005). In contrast, hybridisation appears to be more common between maize and *parviglumis*. Overall, this suggests that the hybridisation potential between maize and *parviglumis* is substantially higher than that between maize and *mexicana*.

5.1.2. Testable hypothesis: teosinte × GM maize hybrid plants do not stably express the transgenes

There is limited information about the inheritance and expression of transgenes in teosinte × GM maize hybrid plants. Ellstrand et al. (2007) observed occasional glyphosate-tolerant progeny of teosinte that had been pollinated naturally by glyphosate-tolerant maize; however, the presence of the transgene was not tested genetically. We know of no other observations of transgene expression in maize relatives in the literature. No direct positive or negative impact of the transgene on the vegetative vigour and reproductive fitness of herbicide-tolerant teosinte × GM maize hybrids were observed in the absence of selective pressure by the intended herbicides (Guadagnuolo et al., 2006). Hence, we are conservative and assume that transgenes will be stably expressed without physiological costs in the progeny derived from crosses between GM maize and teosinte.

5.1.3. Testable hypothesis: transgenes do not increase the persistence and abundance of teosinte × GM maize hybrid plants compared to conventional maize

All teosintes are generally tropical: *mexicana* is adapted to the drier and cooler elevations of northern and central Mexico (1600–2700m), while *parviglumis* is adapted to the warmer, mesic middle elevations of southwestern Mexico (< 1800m) (Hufford et al., 2012). Since *mexicana* is adapted to high elevations, it might make it more likely to survive in Europe than other low elevation tropical teosinte.

Whether maize transgenes will enable sympatric teosinte populations to increase in size is dependent on the nature of the acquired transgenes and selective pressures. Theoretically, introduced traits providing resistance or tolerance to potentially population size-limiting biological and abiotic stressors (e.g., drought, temperature, salt, disease, pest, competition with other species) may allow plants to overcome some of these population size-limiting factors, and become more persistent or abundant (Warwick et al., 2009).

In the case of maize MON810 and Bt11, and 1507, teosinte progeny may acquire and harbour *cry1Ab* and *cry1F* genes, respectively, conferring protection against lepidopteran pests such as ECB. The expression of these transgenes may increase the survival or seed production of teosinte × GM maize hybrids under high infestation of target insect pests. However, this outcome is unlikely as teosinte already has high levels of pest resistance/tolerance (de Lange et al., 2014). De la Paz-Gutiérrez et al. (2010) found teosinte to be more resistant to 66 genera of insects than maize. Insect herbivory is not likely to be the critical factor controlling the abundance of teosinte in agricultural fields, nor in non-agricultural habitats. Teosinte reported in cultivated areas does not

appear to occur in areas outside of cultivation (Pardo et al., 2016), and it is possible that hybridisation with GM maize, which is highly domesticated (Matsuoka et al., 2002; OECD, 2003), could reduce the ability of teosinte to survive and reproduce outside agriculture. Hufford et al. (2013) found less evidence of introgression around loci thought to be linked to domestication in maize by teosinte, which might suggest that these domestication loci decreased the fitness of teosinte hybrids. It is therefore considered unlikely that insect resistance acquisition would change the population dynamics of teosinte in ways that could be ecologically harmful (EFSA, 2016a).

In the case of maize GA21, if teosintes were to acquire tolerance to glyphosate, then exposure to glyphosate may enhance their persistence in agricultural fields where the relevant herbicides are used. Since these plants would no longer be controlled by applications of glyphosate-containing herbicides, they could exacerbate weed problems, especially if maize GA21 is grown continuously and the relevant herbicides applied repeatedly and exclusively (Dewar, 2009). However, such practice is not recommended, even for conventional crops, and could be controlled by conditions of use.

Other, often less convenient, weed management measures would control glyphosate-tolerant teosinte × GM maize hybrids in subsequent crops (except in maize and sorghum) (Pardo et al., 2016). For example, rotating maize GA21 with non-glyphosate-tolerant broadleaved crops (such as soybean, oilseed rape, sugar beet and sunflower) would enable the control of teosinte with selective graminicides.

5.1.4. Testable hypothesis: teosinte × GM maize hybrid plants do not lead to additional impacts on other organisms, ecosystem services or the abiotic environment than conventional maize

Teosinte × GM maize hybrids produced under field conditions are expected to remain rare in Europe, especially if effective measures to control teosinte are employed in infested agricultural areas. Therefore, environmental exposure to these occasional plants would be negligible compared to exposure via the corresponding cultivated GM maize. Moreover, the insecticidal activity of the novel traits providing insect resistance (i.e., Cry1Ab, Cry1F) has been shown to be specific and limited to particular Lepidoptera (Marvier et al., 2007; Wolfenbarger et al., 2008; Naranjo, 2009; Duan et al., 2010; CERA, 2011b, 2013; Romeis et al., 2013; Comas et al., 2014; Koch et al., 2015; Baktavachalam et al., 2015). Teosinte is not expected to be an important resource of food for indigenous Lepidoptera with the exception of few pest species; therefore, exposure to potentially harmful amounts of hybrid pollen deposited on host plants is expected to be the main hazard to the larvae of valued Lepidoptera feeding on these host plants during maize pollen dehiscence. However, the amounts of pollen produced by occasional teosinte × GM maize hybrids are unlikely to adversely affect a significant proportion of valued lepidopteran larvae. For the herbicide tolerance conferring proteins PAT and mEPSPS, no adverse effects on other organisms are expected due to their biological function (Nair et al., 2002; Hérouet et al., 2005; CERA, 2011a,c). Consequently, these plants are not expected to create additional impacts on other organisms, ecosystem services or the abiotic environment, compared to conventional maize.

5.1.5. Testable hypothesis: teosinte × GM maize hybrid plants do not contribute to increased proportion of GM maize kernels on conventional maize

Because gene flow between maize and teosinte is mainly from teosinte (male) to maize (female), occasional teosinte × GM maize hybrid plants may cross-pollinate sympatric maize plants. However, the resulting grains do not easily shatter, and the majority of them will be harvested with the maize crop and thus largely removed from the field (Trtikova et al., 2017). Grain spillage during harvesting and post-harvesting activities will have limited implications in terms of volunteer and feral plants (Gruber et al., 2008; Palau delmàs et al., 2009; Pascher, 2016). Occasional teosinte × GM maize hybrid plants would therefore

cross-fertilise maize plants at levels well below the labelling threshold of 0.9% in Europe. If the corresponding GM maize is grown, the occurrence of occasional teosinte × GM maize hybrid plants would contribute trivial, and probably undetectable, additional amounts of GM pollen with the potential to cross-fertilise conventional maize.

5.2. Pathways from import of GM maize

5.2.1. Testable hypothesis: transgenes do not increase the potential of GM maize resulting from grain import spills to establish and flower compared to conventional maize

The potential of maize grains spilled during transport and processing to establish, flower and to produce pollen is extremely low and transient (Palau delmàs et al., 2009; Pascher, 2016). Maize is highly domesticated, not winter hardy in colder regions of Europe, and generally unable to survive in the environment without appropriate management (Raybould et al., 2012). While occasional feral maize plants may occur outside cultivation areas in Europe (Pascher, 2016), their survival is limited mainly by a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant pathogens, herbivores and cold climate conditions (Raybould et al., 2012). The fitness advantages provided by the transgenes of the GM maize events MON810, Bt11, 1507 and GA21 will not allow occasional feral GM maize plants to overcome some of these population size-limiting factors. Moreover, since these transgenes are not designed to alter flowering and pollen characteristics, GM maize plants do not have a relevant changed phenotype compared to conventional maize for these characteristics.

6. Risk characterisation and management

For gene flow between GM maize and teosinte to pose significant environmental risk, two essential criteria must be met. First, gene flow must occur; and secondly, the flow of transgenes must lead to adverse effects that are greater than those caused by gene flow from conventional maize varieties. Analysis of relevant available information using pathways to harm shows that transgene flow from GM maize to teosinte cannot be ruled out for situations where GM maize plants and teosinte would co-exist, but that adverse consequences from any acquisition of transgenes – conferring insect resistance and herbicide tolerance – by teosinte are unlikely to exceed those arising from fertilisation of teosinte by conventional maize.

The probable rarity of transgene flow from GM maize to *mexicana* is indicated by the low frequency of cross-fertilisation of teosinte by maize in field trials designed to maximise its occurrence, and by the genetic isolation of *mexicana* from maize in areas where they co-exist. In contrast, hybridisation appears to be more common between maize and *parviglumis*. There is no information on the expression of transgenes in teosinte × GM maize hybrid plants; therefore, to be conservative, the worst-case assumption is that any teosinte × GM maize hybrids will express/manifest the traits that the transgenes confer.

Information on the ecology of teosinte suggests that acquisition of insect resistance is unlikely to increase hybrid abundance or population dynamics in ways that could be ecologically harmful. Glyphosate tolerance could exacerbate weed problems if recommendations not to grow glyphosate-tolerant maize continuously and to apply the relevant herbicides repeatedly or exclusively are not followed. However, any increase in persistence of glyphosate-tolerant teosinte × GM maize hybrid plants would remain confined to agricultural fields, and could be managed, minimising the harm that could result from these effects. The adoption of integrated weed management reliant on multiple tactics and product stewardship is advocated. This would contribute to maintaining effective long-term management of teosinte (EFSA, 2016a) and the sustainable use of herbicide-tolerant cropping systems (Lamichhane et al., 2017; Svobodová et al., 2018). Since herbicide-tolerant cropping systems allow extending the use of existing non-

selective herbicides into crops, they provide an additional tool for weed management (Dewar, 2009), including the control of teosinte.

Overall, it may not be possible to corroborate any risk hypothesis along the pathway to harm under cultivation conditions to show that the pathway is blocked at any step; that is, hypotheses of the form “GM maize cannot hybridise with sympatric teosinte” are false, meaning ‘not corroborated’. However, at each step in the pathway to harm, a hypothesis that the event is rare can be corroborated to a greater or lesser extent; the only two exceptions to this are the more common hybridisation potential between maize and *parviglumis*, and the lack of testing of hypotheses about transgene expression in teosinte. Hence, while we cannot say that the pathways are blocked at any point, we can say that completion of a pathway to harm requires a succession of rare events. Since the combined probabilities of these events are low, we can regard this as showing low risk based on a weight of evidence rather than the result of a definitive test.

The likelihood of hybridisation between occasional feral GM maize plants resulting from grain import spills and teosinte occurring in agricultural fields is considered extremely low. Consequently, the realisation of any hazards via that pathway would be negligible under import conditions.

7. Concluding remarks

Some stakeholders have argued that much more data are needed before any conclusions can be drawn on the actual risks associated with the potential gene flow from GM maize to teosinte in Spain. However, formulation of plausible pathways to harm allowed the identification of events that must occur for the risk to be realised, and derive testable risk hypotheses for each step. This approach also enabled to propose risk mitigation measures for those risks that could be realised. While scientific uncertainties about certain steps in the pathways remain – indeed there can never be complete certainty about the occurrence of any natural phenomenon – this does not preclude completing the risk assessment because these uncertainties can be handled by making worst-case assumptions (Raybould and Cooper, 2005). On-going research projects will provide more information about the presence and abundance of teosinte in Spain, their genetics, morphology, biological and ecological behaviour, factors contributing to their dispersal, hybridisation rates, and the efficacy of methods used for their control (e.g., Pardo et al., 2016; Trtikova et al., 2017). While these data may be useful for basic research into the genetics and ecology of teosinte in Europe, they are unlikely to significantly contribute to ERA as acceptable risk from gene flow from GM maize events MON810, Bt11, 1507 and GA21 to teosinte can be shown using worst-case assumptions.

Disclaimer

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Conflict of interest

Alan Raybould is employed by Syngenta that develops GM crops and markets GM seed including maize products containing events Bt11, 1507 and GA21.

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