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Review

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

Yann Devos[a,*], Sol Ortiz-García[b], Karen E. Hokanson[c], Alan Raybould[d]

[a] European Food Safety Authority (EFSA), GMO Unit, Via Carlo Magno 1a, IT-43126 Parma, Italy
[b] Inter-Secretarial Commission on Biosafety for Genetically Modified Organisms (CIBIOGEM), Colonia del Valle, Delegación Benito Juárez, CP 03100, Mexico City, Mexico
[c] University of Minnesota, Department of Horticultural Science, St. Paul, MN, USA
[d] Syngenta Crop Protection AG, CH-4058 Basel, Switzerland

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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

* Corresponding author.
E-mail address: Yann.Devos@efsa.europa.eu (Y. Devos).

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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,