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Weed Risk Assessments for *Zea mays* L. subsp. *mays* (Poaceae) – Maize and Genetically Engineered Herbicide-Resistant Maize

Top: Volunteer maize in soybeans. Middle: Exceptional abundance of volunteer maize in soybeans in Illinois fields in 2010 [source: Aaron Hager (Hager, 2014)]. Bottom images: individual and clumped volunteer maize in maize [source: Lizabeth Stahl (Stahl, 2014)].

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Introduction Plant Protection and Quarantine (PPQ) regulates noxious weeds under the authority of the Plant Protection Act (7 U.S.C. § 7701-7786, 2000) and the Federal Seed Act (7 U.S.C. § 1581-1610, 1939). A noxious weed is defined as "any plant or plant product that can directly or indirectly injure or cause damage to crops (including nursery stock or plant products), livestock, poultry, or other interests of agriculture, irrigation, navigation, the natural resources of the United States, the public health, or the environment" (7 U.S.C. § 7701-7786, 2000). We use weed risk assessment (WRA)—specifically, the PPQ WRA model (Koop et al., 2012)—to evaluate the risk potential of plants, including those newly detected in the United States, those proposed for import, and those emerging as weeds elsewhere in the world.

Because the PPQ WRA model is geographically and climatically neutral, it can be used to evaluate the baseline invasive/weed potential of any plant species for the entire United States or for any area within it. As part of this analysis, we use a stochastic simulation to evaluate how much the uncertainty associated with the analysis affects the model outcomes. We also use GIS overlays to evaluate those areas of the United States that may be suitable for the establishment of the plant. For more information on the PPQ WRA process, please refer to the document, *Background information on the PPQ Weed Risk Assessment*, which is available upon request.

***Zea mays* L. subsp. *mays* – Maize and genetically engineered herbicide-resistant maize**

Species Information

Family: Poaceae

Synonyms: None that are relevant for the WRA.

Common names: Maize and corn (NGRP, 2014). For a larger list of common names see the Germplasm Resources Information Network, online database (NGRP, 2014). In this document, we used the term maize to refer to *Z. mays* subsp. *mays* because it is more broadly used than the name corn (Burrows and Tyrl, 2013).

Botanical description: The widely cultivated forms of maize are annual grasses with a single, unbranched stem (i.e., the stalk) that grow 1.5-4.0 meters tall (Burrows and Tyrl, 2013; Galinat, 1979; Nafziger and Bullock, 1999; Zhengyi et al., 2014), depending on the cultivar. Leaves are arranged in two ranks along the stem (distichous), and male and female flowers are borne on separate inflorescences called tassels (male) and cobs (female) (Galinat, 1979; Richardson et al., 2006).

Initiation and scope: On May 6, 2014, the Center for Food Safety (CFS) petitioned the Secretary of the United States Department of Agriculture to regulate genetically engineered (GE) multiple herbicide-resistant maize and soybean plants (DAS-40278-9, DAS-68416-4 and DAS-44406-6) as Federal noxious weeds (CFS, 2014). The CFS requested that "the agency should not make any decision until it has properly applied all of its full

[Plant Protection Act] PPA authority" by evaluating all direct and indirect impacts of these herbicide-resistant taxa (CFS, 2014). They maintain that because the PPA's definition of a noxious weed includes indirect impacts caused by plants, APHIS should evaluate and consider all of the following factors associated with cultivation of these GE taxa, including: 1) environmental impacts associated with chemical treatment of seeds; 2) herbicide drift on other crops; 3) impact of herbicides on non-target organisms; 4) impact of metabolites on non-target organisms; 5) transgenic contamination of non-GE crops; 6) impact to public health; 7) impact of increased herbicide use; 8) seed market concentration; 9) stacking of multiple GE traits; 10) trade impacts; and 11) impacts of these GE taxa as volunteers in other crops.

On July 2, 2014, the PPQ Deputy Administrator asked PERAL to conduct weed risk assessments for the GE maize and soybeans indicated in the petition using our standard weed risk assessment process. The PPQ WRA process uses a series of mostly yes and no questions about a plant species' biology and impacts to evaluate its risk potential (Koop et al., 2012). Although the risk model was developed and validated with a wide range of weeds and non-weeds from across the United States, it was not designed to evaluate the weed risks associated with GE organisms nor many of the impacts of concern to the CFS. The PPQ WRA, like other widely used weed risk assessment tools (e.g., Pheloung et al., 1999), evaluates a species' ability to establish, naturalize and spread, and to cause direct or indirect impacts. Many of the issues of concern to the CFS, while perhaps important to consider, relate to changes in herbicide use that may occur after cultivation of these herbicide-resistant plants. Thus we address the impacts of these GE taxa as volunteers in other crops (#11 above). We evaluate the impacts to trade (#10 above) in question Imp-P3 of our model (see Appendix B). The impact of GE herbicide-resistant maize on public health was evaluated by the Food and Drug Administration (McMahon, 2011) separately and is only referenced here.

In this document we evaluate the weed risk associated with the GE maize biotype DAS-40278-9 developed by Dow AgroSciences (Dow AgroSciences, 2011). That biotype produces a protein that inactivates two different kinds of herbicides: aryloxyalkanoate family herbicides, including 2,4-dichlorophenoxyacetic acid (2,4-D); and aryloxyphenoxypropionate (AOPP) acetyl coenzyme A carboxylase (ACCase) inhibitors ("fop" herbicides) (BRS, 2013; Dow AgroSciences, 2011; Heap, 2014). If APHIS deregulates this biotype of maize under Title 7 of the Code of Federal Regulations part 340, it will be the first commercially available maize biotype with resistance to both of these herbicide types. Furthermore, Dow AgroSciences states that through hybridization with other deregulated, GE herbicide-resistant biotypes, DAS-40278-9 can be stacked with resistance to glyphosate and glufosinate herbicides (Dow AgroSciences, 2011) resulting in maize biotypes resistant to herbicides with four different modes of action.

As part of our approach for this risk assessment, we evaluated and compared the weed risk potential for maize that has not been genetically engineered with herbicide resistance and maize genetically engineered for any type of herbicide resistance. Hereafter we use the terms non-GE maize and GE herbicide-resistant maize to distinguish between these two types of plants. Although the CFS requested the USDA to evaluate a specific type of GE herbicide-resistant maize (DAS-40278-9), we increased the scope of this assessment to ensure we consider all evidence related to the impact of herbicide resistance in maize. We recognize that some cultivars of maize have been genetically engineered with other types of traits not related to herbicide resistance. For the purpose of this weed risk assessment, we did not evaluate the risks associated with these other types of engineered traits.

Both DAS-40278-9 and a similar untransformed maize hybrid were grown at several sites to determine if they differed in agronomic traits such as plant vigor, height, germination, and yield; no significant differences in those traits were detected (Dow AgroSciences, 2011). However, those studies focused on agronomic traits and not the botanical traits evaluated by our WRA. Unless we found specific evidence to the contrary, we assumed that non-GE maize and GE herbicide-resistant maize did not differ for the other traits considered in the PPQ WRA (e.g., dispersal, seed production rates, tolerance to mutilation, breeding system).

Foreign distribution: Maize was domesticated in Mexico and Central America, and was later distributed to South and North America in pre-Columbian times (Bonavia, 2013; Galinat, 1979). Later, European explorers introduced it to other countries throughout the world (Bonavia, 2013; Galinat, 1979) where it is currently cultivated.

U.S. distribution and status: Maize is widely cultivated in the United States for a variety of purposes, including for human consumption and animal feed (USDA-NASS, 2013). The majority of production is centered in the U.S. maize belt located in the north-central region of the United States, south and west of the Great Lakes (USDA-NASS, 2013). Of the approximate 97 million acres of maize planted in 2012 and 2013 in the United States, 21 and 14 percent of that, respectively, represented GE herbicide-resistant biotypes (USDA-NASS, 2013). Maize is not regulated as a noxious weed by either APHIS or any state government (7 CFR § 360, 2014; NRCS, 2014).

WRA area[1]: Entire United States, including territories.

[1] "WRA area" is the area in relation to which the weed risk assessment is conducted [definition modified from that for "PRA area"] (IPPC, 2012).

1. *Zea mays* subsp. *mays* analysis for non-GE maize and GE herbicide-resistant maize

Establishment/Spread Potential

Non-GE maize. Maize is a highly domesticated crop species that does not exist outside of cultivation (Bonavia, 2013; Burrows and Tyrl, 2013; Galinat, 1979). Breeding over the last 7,000 years has transformed it radically (Bonavia, 2013). During that time, it has lost its ability to disperse on its own (i.e., the cob does not shatter; Fedoroff, 2003; van der Pijl, 1982). Casual or volunteer maize is only found in areas associated with the cultivation, storage, and transport of maize seeds and grain, and other products that may be contaminated with maize seed (Mühlenbach, 1979; PestID, 2014; Richardson et al., 2006). Maize does not appear to possess any seed dormancy, which limits its ability to form a persistent seed bank (Australian Government, 2008; Palaudelmàs et al., 2009; Stahl, 2014). Furthermore, it is not tolerant of mutilation. These factors and others have limited the ability of maize to establish, naturalize, and spread. We had very low uncertainty for this risk element.
Risk score = 1 Uncertainty index = 0.06

GE herbicide-resistant maize. Except for herbicide resistance, we found no evidence and think it unlikely that DAS-40278-9, or other biotypes of GE herbicide-resistant maize, will differ from non-GE maize with respect to Establishment/Spread Potential. GE herbicide-resistant maize is only likely to express a fitness advantage over non-GE herbicide-resistant maize in production systems in which herbicides are routinely applied (Crawley et al., 2001). We had very low uncertainty for this risk element.
Risk score = 2 Uncertainty index = 0.06

Impact Potential

Non-GE maize. Volunteer maize is a weed in other crops and in maize-maize rotation systems because it competes with other planted crops for resources (Stahl et al., 2013). In U.S. crop rotations, soybeans often follow maize (Davis et al., 2008). Volunteer maize in soybeans reduces soybean yield (Alms et al., 2008; Andersen et al., 1982; Beckett and Stoller, 1988), contaminates harvested soybeans (Andersen et al., 1982; Davis, 2009), increases costs of control (Andersen et al., 1982; Beckett and Stoller, 1988), and may sustain populations of maize root worm in the field until the next maize crop (Stahl et al., 2013). Volunteer maize can also be problematic in maize followed by maize cropping systems because volunteer plants compete with planted maize and reduce overall yield (Alms et al., 2008; Stahl et al., 2013). We found no evidence that maize is a weed in natural systems. Although maize temporarily appears along roadways and waste places where seeds are dropped (Burrows and Tyrl, 2013; Mühlenbach, 1979), it does not seem to be considered a weed in those habitats. We had very low uncertainty for this risk element.
Risk score = 2.2 Uncertainty index = 0.09

GE herbicide-resistant maize. GE herbicide-resistant maize has or will have impacts similar to unmodified maize in annual crops, except they may be a little harder to control. GE herbicide-resistant volunteer maize causes yield losses in crops such as sugar beets (Kniss et al., 2012), soybean (Marquardt et al., 2012), and cotton (Thomas et al., 2007). Volunteer offspring of herbicide-resistant crops are more difficult to control than nonresistant volunteers (Floate et al., 2002; Stahl et al., 2013). While other herbicides are available to control these new biotypes, management is usually more complex because growers will have to use either additional herbicides or different herbicide mixes (Hager, 2010). Furthermore, because some countries regulate and/or prohibit GE maize (ISAAA, 2014; USDA-FAS, 2012), market access for U.S. maize may be restricted, at least temporarily (Jie, 2014). We had very low uncertainty for this risk element.
Risk score = 2.4 Uncertainty index = 0.08

Geographic Potential

Because maize does not exist outside of cultivation (Bonavia, 2013; Burrows and Tyrl, 2013; Galinat, 1979), we were unable to determine its geographic potential using our standard methodology which uses climatic data from where a species is native and naturalized. Instead we provided a map of the current acreage of all commercially cultivated maize (both non-GE and GE) in the contiguous United States where the vast majority of corn is produced (Fig. 1). Because the impact of volunteer maize is confined to maize cropping systems, this map was the most relevant for this risk assessment. Maize is widely cultivated in the United States (Carpenter et al., 2002; Stahl et al., 2013; USDA-ERS, 2014) across a broad range of climates. It requires temperatures above 50 °F for proper development; serious loss of leaf function occurs if temperatures decrease to about 40 °F (Nafziger and Bullock, 1999). Maize requires about 560 mm (or 22 in.) of water during the growing season to produce a high yield, although water use depends on evaporative demand (Nafziger and Bullock, 1999).

The PPQ WRA uses the answers from the Geographic Potential questions to address three questions under the Establishment and Spread risk element about a species' adaptive potential. For the reason explained above, we were unable to do this for maize. Instead, we estimated adaptive potential for maize using information about its closest relative and most likely ancestor, Mexican teosinte (*Z. mays* subsp. *mexicana*) (Bonavia, 2013; Galinat, 1979). The two taxa are still close enough related that interspecific gene flow is possible (de Freitas Terra et al., 2011; Fukunaga et al., 2005; Warburton et al., 2011). If their adaptive potential differs, we expect the potential for maize to be greater, since cultivars have been developed for more tropical and temperature latitudes (Bonavia, 2013; Galinat, 1979). Mexican teosinte occurs in areas with Plant Hardiness Zones 8-12, areas with 20-100+ inches of annual precipitation, and the following Köppen-Geiger climate classes: tropical rainforest, tropical savanna, humid subtropical, steppe, desert, and marine west coast.

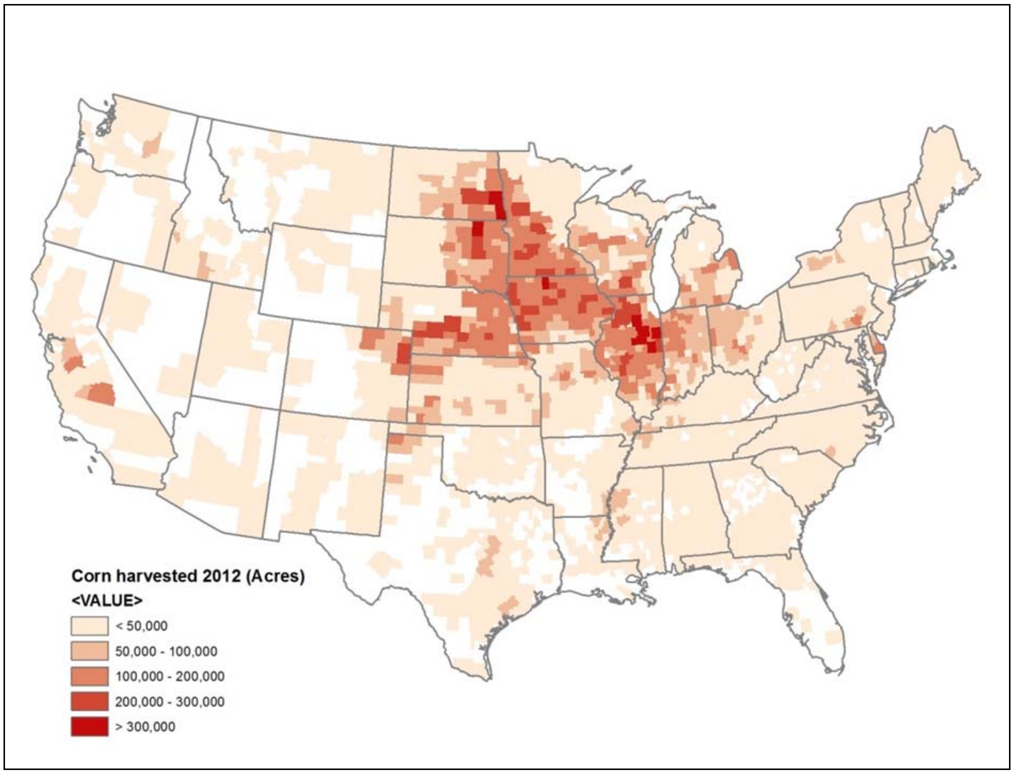

Figure 1. Distribution and acreage cultivated under commercial maize in the contiguous United States. Data obtained from the National Agricultural Statistics Service (USDA-NASS, 2014).

Entry Potential Both non-GE maize and other biotypes of GE herbicide-resistant maize are widely cultivated in the United States (Carpenter et al., 2002; Stahl et al., 2013; USDA-ERS, 2014). Dow AgroSciences petitioned APHIS Biotechnology Regulatory Services (BRS) to deregulate a new biotype of maize (DAS-40278-9) that has been genetically engineered for resistance to two different types of herbicides (Dow AgroSciences, 2011). Thus, because maize is already widely cultivated and because the entry of the new biotype is imminent, we did not evaluate the entry potential for maize.

<u>2. Results</u>

Unmodified maize:
Model Probabilities: P(Major Invader) = 7.1%
P(Minor Invader) = 64.5%
P(Non-Invader) = 28.4%
Risk Result = Evaluate Further
Secondary Screening = Evaluate Further

Herbicide-resistant maize:
Model Probabilities: P(Major Invader) = 9.8%
P(Minor Invader) = 68.4%
P(Non-Invader) = 21.8%
Risk Result = Evaluate Further
Secondary Screening = Evaluate Further

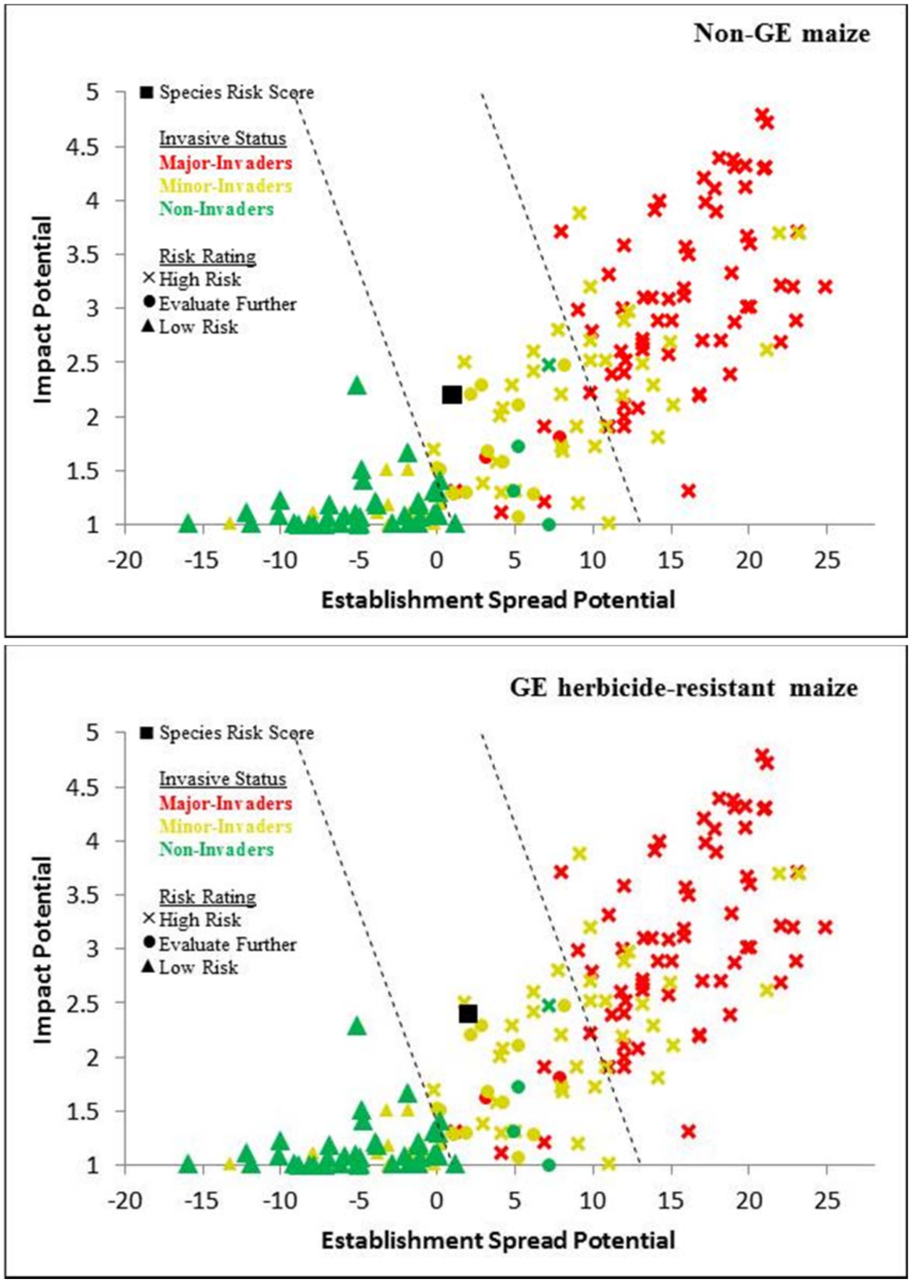

Figure 2. Risk scores (black box) for non-GE maize (top) and GE herbicide-resistant maize (bottom) relative to the risk scores of species used to develop and validate the PPQ WRA model (other symbols). See Appendix A and B for the assessments.

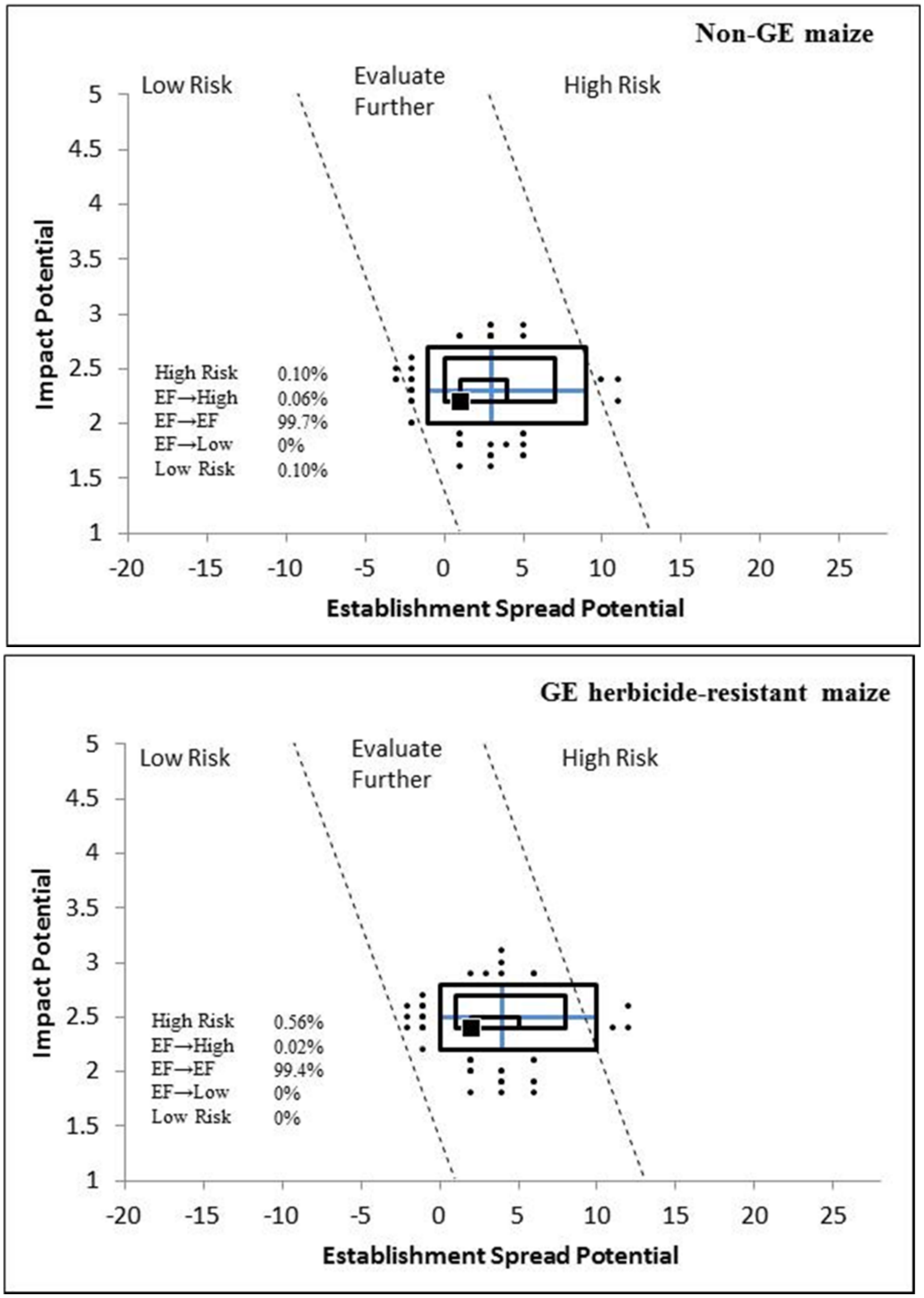

Figure 3. Model simulation results (N=5,000) for uncertainty around the risk score for non-GE maize (top) and GE herbicide-resistant maize (bottom). The blue "+" symbol represents the medians of the simulated outcomes. The smallest box contains 50 percent of the outcomes, the second 95 percent, and the largest 99 percent.

3. Discussion

The results of the weed risk assessments for non-GE and GE herbicide-resistant maize were both Evaluate Further (Fig. 2). This conclusion is applied to plant taxa with risk scores that are intermediate between non-invasive/non-weedy taxa, and major invaders and weeds (Fig. 2). Our logistic regression model, which was developed and validated using 204 U.S. weeds and non-weeds indicates that the set of traits categorizing both types of maize are similar to other minor-invaders in the United States. Because uncertainty was very low in these assessments, our conclusions are well supported by the uncertainty simulations (Fig. 3). Additionally, an earlier weed risk assessment of maize also classified it as evaluate further (Pheloung, 1995).

Given that non-GE maize does not exist outside of cultivation (Bonavia, 2013; Burrows and Tyrl, 2013; Galinat, 1979), we were somewhat surprised that it was not low risk, particularly because it has no natural dispersal vectors and is highly domesticated. The model classified it as a minor-invader because of a few reasons: it is an annual grass that produces viable seeds, is dispersed unintentionally by people, and impacts some crop production systems. If non-GE maize did not volunteer in crops and reduce yields, or if it were not unintentionally dispersed by people, our analysis would have concluded that it was Low Risk (data not shown).

The PPQ WRA is a qualitative WRA that was not designed to evaluate the risk associated with GE plants. Thus, we were not surprised that the risk scores for non-GE and GE herbicide-resistant maize differed by so little. Only two questions differed between the assessments: ES-20, which evaluates herbicide resistance, and Imp-P3, which evaluates impact on U.S. trade. Because GE herbicide-resistant maize may be more difficult to control than non-GE maize (Floate et al., 2002; Stahl et al., 2013), release of additional biotypes of herbicide-resistant maize, such as DAS-40278-9, will further complicate weed management and may reduce crop yields even further if these biotypes are not managed properly. Other herbicides are available to control 2,4-D and “fop” resistant maize plants (Dow AgroSciences, 2011). DAS-40278-9 maize is susceptible to the “dim” (cyclohexanedione) herbicides like clethodim and sethoxydim, plus some ALS inhibiting herbicides such as imazamox (Dow AgroSciences, 2011), but we do not know if those herbicides are commonly used to control volunteer maize. It is worth nothing that releasing DAS-40278-9 would allow crop breeders to use conventional breeding to create one plant biotype with resistance to four different herbicides (i.e., stacked resistance).

In their petition to the Secretary of Agriculture, the CFS raised concerns about a variety of indirect impacts that may be associated with the deregulation of DAS-40278-9 (CFS, 2014). It is beyond the scope of the PPQ WRA process to evaluate the impacts associated with changes in herbicide use patterns that are expected if DAS-40278-9 is commercially cultivated in the United States. However, within PPQ’s authority to safeguard U.S. plant resources from pests and noxious weeds, decision makers and risk managers should consider that a change in the pattern and types of herbicides used will likely lead to a shift in weed species communities, and may increase the likelihood that resistance to 2,4-D and "fop" herbicides develops in current weed populations (see discussion in Johnson et al., 2009). The development of herbicide resistance in crop weeds is important to many stakeholders, including the Weed Science Society of America, which maintains an extensive database of herbicide-resistant weeds in the United States and elsewhere (Heap, 2014).

One of the concerns generally associated with GE herbicide-resistant crops is that the genes coding for herbicide resistance will be transferred to weedy crop relatives through hybridization and introgression, making them more problematic in crops (Adugna and Bekele, 2013; Londo et al., 2011; Warwick et al., 2009). Some evidence indicates that gene flow between maize and its close relative Mexican teosinte may be occurring (de Freitas Terra et al., 2011; Fukunaga et al., 2005; Warburton et al., 2011). In one study, fertile hybrids between the two were produced (Doebley, 1990). Mexican teosinte is present in a few locations in the United States (Kartesz, 2014; NRCS, 2014; Wunderlin and Hansen, 2014), but it is not clear to what extent this taxon is naturalized in the United States. It is occasionally cultivated in the southern states as a green forage (cited in Weakley, 2012). In some regions of Mexico, teosinte is a well-established wild plant (Galinat, 1979) that occurs with maize and is a significant weed (Perdomo-Roldán et al., 2009; Villaseñor Ríos and Espinosa García, 1998). Introduction of herbicide resistance genes into teosinte may increase its fitness in croplands and other environments where herbicides are routinely used, but we do not know how likely gene flow would be in the United States.

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Appendix A. Weed risk assessment for non-GE *Zea mays* L. subsp. *mays* (Poaceace). The Microsoft Excel file where the risk assessment was conducted is available upon request.

Question ID	Answer - Uncertainty	Score	Notes (and references)
ESTABLISHMENT/SPREAD POTENTIAL			
ES-1 (Status/invasiveness outside its native range)	d - negl	0	Cultivated maize is highly domesticated, and as such does not have a native range per se. It was domesticated in Mexico and Central America, and was later distributed to South and North America in pre-Columbian times (Bonavia, 2013; Galinat, 1979). Later European explorers introduced it to other countries throughout the world (Bonavia, 2013; Galinat, 1979). Maize does not persist long after cultivation (Crawley et al., 2001; Starr et al., 2008), but does appear as a casual or waif species where seeds from agricultural activity drop on the ground, such as in farmlands, roadsides, railways, and grain/seed processing facilities (Brandes, 2003; Burrows and Tyrl, 2013; Dunn, 1905; Weakley, 2012). In our literature review of maize's ability to escape, naturalize, and spread, we found that maize is reported 1) to not escape (Gue´zou et al., 2007), 2) to be known only from cultivation (Jaryan et al., 2012; Khuroo et al., 2007; Tomson, 1922), and 3) as a casual or waif species in other countries (e.g., French and Murphy, 1994; Landcare Research, 2014; Pyšek et al., 2002; Reynolds, 2002; Richardson et al., 2006; Verloove, 2006) and in the United States (e.g., Dean et al., 2008; Liogier and Martorell, 2000; Mühlenbach, 1979). In one region of the U.S. maize is present Maize was reported as "naturalized" in Madagascar (Kull et al., 2012), but that study's definition of naturalized includes species that would be defined as casual by others. Without additional information, it is impossible to determine maize's true status in Madagascar. Maize is also reported as naturalized in Australia (Randall, 2007), but we could not verify its status with the original source. *Zea mays* is reported as introduced to Chile, and possibly naturalized (Marticorena and Quezada, 1985), but we could not discern which subspecies was being discussed in this reference. Maize is also reported as "possibly naturalized" in Taiwan (Wu et al., 2004). Thus, the evidence for naturalization is not very strong. Based on the overall weight of the evidence, we answered "d" with negligible uncertainty. The alternate answers for the Monte Carlo simulation were both "e."
ES-2 (Is the species highly domesticated)	y - negl	-3	Maize is widely cultivated around the world for a variety of different purposes including for consumption as a vegetable, animal feed, ethanol, maize oil, starch, and high fructose syrup (Carpenter et al., 2002; Simpson and Conner-Ogorzaly, 1986). It has been cultivated for at least 7,000 years and has been domesticated and bred for a variety of forms and uses (Simpson and Conner-Ogorzaly, 1986) to the point where it may no longer be able to exist without cultivation (Burrows and Tyrl, 2013; Galinat, 1979). During its domestication, several major ancestral traits were changed, including 1) shattering of infructescences, 2) internode length on the branches, 3) the number of rows and kernels in the ears, and 4) the exposure of the kernels (Bonavia, 2013). No wild form of maize is known to exist (Bonavia, 2013).
ES-3 (Weedy congeners)	y - low	1	*Zea* is a genus with about five species native to Central and South America (Mabberley, 2008). Randall's Global Compendium of Weeds lists two species that have been identified as weedy, but by

Question ID	Answer - Uncertainty	Score	Notes (and references)
			only one source each: *Zea luxurians* and *Z. perennis* (Randall, 2012). Because this assessment is focusing on *Z. mays* subsp. *mays*, it is prudent to examine other subspecies in *Z. mays* for their weed potential. *Zea mays* subsp. *mexicana*, also known as teosinte, is considered a weed (Vibrans and Flores, 1998; Villaseñor Ríos and Espinosa García, 1998). In some high elevation regions of Mexico, it is considered a significant weed because it occurs with cultivated maize and looks very similar to it in the vegetative state (Perdomo-Roldán et al., 2009). "Very high population densities of teosinte can be found in this region and maize cultivation sometimes has to be suspended because of the problem" (Perdomo-Roldán et al., 2009). "The Toluca teosinte retains the typical characteristics of an agrestal weed such as the ability to form fruit even on very small plants, large variations in size and biomass of fertile individuals, and fruit polymorphism" (Perdomo-Roldán et al., 2009).
ES-4 (Shade tolerant at some stage of its life cycle)	n - low	0	We did not find any direct evidence that maize is shade tolerant. However, given that maize is grown in high light environments, and has been domesticated to maximize production in these types of environments, it seems unlikely it is shade tolerant. This is supported by evidence that maize displays shade avoidance syndrome where shifts in the ratio of red to far red light (as happens between sunny and shaded habitats) promote changes in growth and morphology to avoid shade (Dubois and Brutnell, 2009; Page et al., 2011). Furthermore, maize is a C_4 plant (Dubois and Brutnell, 2009). "C_4 plants occur in hot and dry climatic conditions with usually high light intensity....C_4 plants have higher photosynthetic efficiency than C_3 plants, namely in arid, hot, and under high-light conditions..." (Ashraf and Harris, 2013). Because higher energy costs may only be met in well-lit environments, it seems unlikely that maize is well equipped to survive in shady environments. Maize requires lots of sunlight to thrive (Nafziger and Bullock, 1999). One study showed that maize plants growing near forest edges show lower biomass production than those in the middle of the crop field (Sklenicka and Salek, 2005).
ES-5 (Climbing or smothering growth form)	n - negl	0	Maize is an erect, single-stem annual herb (Burrows and Tyrl, 2013; Richardson et al., 2006; Zhengyi et al., 2014); it is neither a vine nor an herb with a basal rosette of leaves.
ES-6 (Forms dense thickets)	n - mod	0	Maize often grows as a volunteer in crops the year after it was grown (Hager, 2010) due to incomplete harvest of all cobs and maize seed. Ordinarily, plants occur as scattered individuals or clumps of plants across the field (see top image on WRA cover page; Hager, 2014). Under some circumstances, however, the density of volunteer maize can be high and in one exceptional year, it reached 500,000 per acre (123 juvenile plants per square meter) in some Illinois soybean fields in 2010 (Hager, 2010). Because these high densities are uncommon and are limited to where maize is cultivated, and because these densities are temporary, we answered no, but with moderate uncertainty.
ES-7 (Aquatic)	n - negl	0	Maize is a terrestrial herb (Burrows and Tyrl, 2013; Richardson et al., 2006; Zhengyi et al., 2014); it is not an aquatic plant.
ES-8 (Grass)	y - negl	1	Maize is a grass (NGRP, 2014).
ES-9 (Nitrogen-fixing woody plant)	n - negl	0	We found no evidence of nitrogen fixation. Furthermore, maize is not a member of a plant family known to fix nitrogen (Martin and

Question ID	Answer - Uncertainty	Score	Notes (and references)
			Dowd, 1990; Santi et al., 2013)
ES-10 (Does it produce viable seeds or spores)	y - negl	1	Maize reproduces exclusively by seed (Asli and Houshmandfar, 2011; Australian Government, 2008). Maize is cultivated by planting seeds in late spring in open fields (Pendleton, 1979).
ES-11 (Self-compatible or apomictic)	y - low	1	We found some conflicting evidence for this question. Each maize plant has separate male and female inflorescences (Paliwal, 2000). One source reports that maize is an outcrossing species with genetic self-incompatibility (Bonavia, 2013). However, another states that it has a selfing rate of about 5 percent (Sleper and Poehlman, 2006). "Modern maize can fertilize itself, but outcrossing is common..." (Carpenter et al., 2002). Part of the process of developing inbred lines for hybridizing involves controlled self-pollination where pollen from a plant is transferred to the female flowers of the same plant (Eberhart, 1979). Thus, although maize may be primarily an outcrossing species, it appears that selfing does occur and is an important process in crop breeding.
ES-12 (Requires special pollinators)	n - negl	0	Maize is wind-pollinated (Bonavia, 2013; Galinat, 1979; Paliwal, 2000). Insect pollination has not been observed in maize (cited in Australian Government, 2008).
ES-13 (Minimum generation time)	b - negl	1	Maize is an annual plant (Burrows and Tyrl, 2013; Galinat, 1979; Zhengyi et al., 2014). Alternate answers for the Monte Carlo simulation were both "c."
ES-14 (Prolific reproduction)	n - high	-1	Maize cobs have 4-30 rows of spikelets (Galinat, 1979; Zhengyi et al., 2014). The number of ovules that develop into kernels on a single cob ranges from 300-1000 (Australian Government, 2008). On average cobs can have about 1000 kernels (Galinat, 1979). Eight plants per square meter is the high end of plant cropping density (Australian Government, 2008). Some hybrids are planted at densities of 55,000-65,000 plants per hectare (5.5-6.5 plants per square meter) (Pendleton, 1979). Usually the uppermost ear on a plant develops fully (Nafziger and Bullock, 1999). Assuming 5.5–6.5 plants per square meter, one cob per plant, 1000 kernels per cob, and a seed establishment rate of 90 percent (Nafziger and Bullock, 1999), then we can expect 4950-5850 kernels per square meter, which meets this question’s threshold of 5000 per square meter. In one study, seed yield of maize ranged between 4,200 and 11,200 kg per hectare (0.42-1.12 kg per square meter) (Allen and Obura, 1983). If the average weight per maize grain is 0.266 g (Cummings et al., 2008), then about 1350 to 3780 viable seeds are being produced per square meter, which does not meet the threshold for a yes response. Both of these estimates apply to cultivated maize which generally uses hybrid seed and is grown in managed conditions favorable for maize. Because volunteer maize is produced through open pollination, plants are often not as vigorous as most hybrid varieties planted (Carpenter et al., 2002). Volunteer maize plants tend to produce smaller cobs or are barren at higher densities (Stahl et al., 2013). Thus seed production rates for volunteer maize are likely less than cultivated maize. Because ultimately we are concerned more with volunteer maize as a weed as opposed to cultivated maize, we answered no.
ES-15 (Propagules likely to be dispersed unintentionally by people)	y - low	1	Regular agricultural activities such as harvesting and plowing will likely result in the spread of unharvested maize seed over short distances within fields as it does for other plant species (Heijting et

Question ID	Answer - Uncertainty	Score	Notes (and references)
			al., 2009; McCanny and Cavers, 1988), particularly since some maize is not harvested due to stalk breakage, kernel loss, or dropped ears during harvest (Stahl et al., 2013).Maize is sometimes a component of bird seed and as such is unintentionally spread by people (Richardson et al., 2006). It is common along railroads in the St. Louis area particularly around grain elevators; "[h]eaps of maize seeds on the ground there, and along many classification tracks, were striking sights" (Mühlenbach, 1979). We considered this evidence here, and not in ES-16 because in this case maize is the commodity and not a contaminant or hitchhiker of another commodity or traded good.
ES-16 (Propagules likely to disperse in trade as contaminants or hitchhikers)	y - negl	2	The United States has intercepted maize as a contaminant or hitchhiker in a variety of goods including soybean shipments (PestID, 2014). "Because various transportation vehicles, temporary storage sites and port elevators are used commonly with all exported crops and it is difficult to remove all residues from them ..." seeds of other crops are likely to be present in grain and seed shipments (Shimono and Konuma, 2008). One study confirmed that "many farmers are unknowingly introducing substantial levels of weed and volunteer crop seeds into their farming systems at crop seeding, even though crop seed cleaning techniques are employed" (Michael et al., 2010). Seeds from the previous year's crop will often be a contaminant of next year's seed or grain crop despite seed cleaning practices (Salisbury and Frick, 2010).
ES-17 (Number of natural dispersal vectors)	0	-4	Fruit and seed description For ES-17a through ES-17e: The fruit of maize is a caryopsis (a grain), which is a dry, indehiscent single-seeded fruit (Australian Government, 2008). Maize grains are held tightly to the cobs and do not disperse individually, conferring a low establishment rate for maize plants in nature (Fedoroff, 2003). The cob itself remains on the plant until harvested, but if left on the plant or if damaged by pests, it will eventually fall to the ground (Australian Government, 2008). The leaves (husks) that subtend the cobs tightly clasp the cob, further limiting natural dispersal (Paliwal, 2000).
ES-17a (Wind dispersal)	n - negl		Because maize kernels are relatively large, have no morphological features associated with wind-dispersal, and remain attached to the cob (Fedoroff, 2003), wind dispersal is highly unlikely. "Cultivated *Zea* seems an 'impossible' plant in its domestication, devoid of all natural dispersal ability" (van der Pijl, 1982). Through domestication, maize has lost its ability to disperse its seeds (Bonavia, 2013).
ES-17b (Water dispersal)	n - low		We found no evidence that maize seeds or cobs regularly or naturally disperse by water. Given that maize is reported to lack any natural dispersal ability (see ES-17a), we answered no with low uncertainty.
ES-17c (Bird dispersal)	n - low		We found no direct evidence that maize is dispersed by birds. Maize kernels are generally well protected from birds by the closely overlapping leaf sheaths (i.e., the husk). However, after harvest, individual kernels and exposed cobs are likely to remain on the soil surface. One study fed maize to four different bird species that are likely to be associated with maize fields and that feed on maize (Cummings et al., 2008). The authors found that no seeds passed the digestive tracts intact, nor stuck to the outside of the birds on mud

Question ID	Answer - Uncertainty	Score	Notes (and references)
			(Cummings et al., 2008), suggesting that bird dispersal of maize is not likely. Dispersal of seeds retained in the crop or gizzard is possible, but is not likely (Cummings et al., 2008). A literature review by the Australian Government also concluded that bird dispersal is unlikely (Australian Government, 2008).
ES-17d (Animal external dispersal)	n - high		We found no evidence that maize is dispersed by adhering to animals.
ES-17e (Animal internal dispersal)	n - mod		We found no direct evidence of endozoochory (seed dispersal in animal guts). In one study of the foraging activity of field mice in U.S. corn fields, mice consumed the seed and help reduce the incidence of volunteer corn (Flick, 2013). One group of authors that fed maize to wild boar found that in a few extremely rare cases excreted maize seeds could germinate (Wiedemann et al., 2009). Thus although endozoochory is possible, it does not appear to be a significant mechanism for dispersal. An Australian Government report concluded that animal-dispersed maize seed is unlikely, although possible (Australian Government, 2008).
ES-18 (Evidence that a persistent (>1yr) propagule bank (seed bank) is formed)	n - mod	-1	We found no evidence that maize seeds can survive in the soil for more than one year. Seeds from one maize crop can survive over one winter and germinate the next year when conditions are favorable (Australian Government, 2008; Palaudelmàs et al., 2009), but this does not address long-term persistence in the field. The ability of maize kernels to germinate in late autumn after harvest suggests they don't have any seed dormancy (Palaudelmàs et al., 2009). Extension agents at the University of Minnesota have never observed volunteer maize returning in the second cropping year after it was initially planted, nor have they seen any studies reporting it (Stahl, 2014). The only circumstances that may favor prolonged persistence would be a severe and prolonged drought (Stahl, 2014), but this would seem unlikely, particularly if farmers irrigate their fields. Based on this information, we answered no.
ES-19 (Tolerates/benefits from mutilation, cultivation or fire)	n - low	-1	We found no evidence that maize is tolerant to or benefits from mutilation, cultivation, or fire. Because maize is so well studied, we answered no with low uncertainty.
ES-20 (Is resistant to some herbicides or has the potential to become resistant)	n - low	0	We found no evidence that unmodified maize is resistant to herbicides. It is not listed in the Weed Science Society of America's database of herbicide-resistant to herbicides (Heap, 2014). Maize is innately tolerant to many post-emergence herbicides (Carpenter et al., 2002); however, the scope of this question is restricted to herbicide resistance per se. Maize can be controlled effectively with various herbicides, including clethodim, diclofop, fluazifop, glufosinate, glyphosate, quizalofop, and sethoxydim (Andersen et al., 1982; Hager, 2010; Terry et al., 2012).
ES-21 (Number of cold hardiness zones suitable for its survival)	5	0	This question and the next two are measures of the adaptive potential of a species. Because maize does not exist outside of cultivation, we could not answer these questions directly for cultivated maize. Instead we used the distribution of its close relative *Z. mays* subsp. *mexicana* to answer them.
ES-22 (Number of climate types suitable for its survival)	6	2	See ES-21.
ES-23 (Number of precipitation bands suitable	9	1	See ES-21.

Question ID	Answer - Uncertainty	Score	Notes (and references)
for its survival)			
IMPACT POTENTIAL			
General Impacts			
Imp-G1 (Allelopathic)	? - max		Two laboratory studies showed that both maize pollen and extracts of maize pollen are allelopathic on germinating seeds, inhibiting growth of the hypocotyl and radicle (Jiménez et al., 1983; Ortega et al., 1988). One of these studies (Jiménez et al., 1983) reported that Mexican farmers have noted the inhibitory effects of maize pollen on weeds in the field. If maize allelopathy were biologically significant at field levels, we would have expected for there to be many more field-based reports of allelopathy given how widely cultivated maize is. Consequently, without further evidence we answered unknown.
Imp-G2 (Parasitic)	n - negl	0	We found no evidence that maize is parasitic. Furthermore, maize is not a member of a plant family known to contain parasitic plants (Heide-Jorgensen, 2008; Nickrent, 2009; Walker, 2014).
Impacts to Natural Systems			
Imp-N1 (Change ecosystem processes and parameters that affect other species)	n - negl	0	We found no evidence that maize can form persistent populations in natural areas or is even considered a weed in these systems. Because there is no evidence that maize can exist outside of cultivation (e.g., Galinat, 1979) and because it is so widely cultivated and studied, we answered no with negligible uncertainty for most of the questions pertaining to natural systems.
Imp-N2 (Change community structure)	n - negl	0	We found no evidence (see notes for Imp-N1).
Imp-N3 (Change community composition)	n - negl	0	We found no evidence (see notes for Imp-N1).
Imp-N4 (Is it likely to affect federal Threatened and Endangered species)	n - negl	0	We found no evidence (see notes for Imp-N1).
Imp-N5 (Is it likely to affect any globally outstanding ecoregions)	n - negl	0	We found no evidence (see notes for Imp-N1).
Imp-N6 (Weed status in natural systems)	a - low	0	Although reported as a waif in two different areas of California managed for biological diversity, maize "poses virtually no economic or ecological threat because it is barely capable of reproduction in the wild" (Dean et al., 2008). Alternate answers for the Monte Carlo simulation were both "b."
Impact to Anthropogenic Systems (e.g., cities, suburbs, roadways)			
Imp-A1 (Impacts human property, processes, civilization, or safety)	n - low	0	We found no evidence.
Imp-A2 (Changes or limits recreational use of an area)	n - low	0	We found no evidence.
Imp-A3 (Outcompetes, replaces, or otherwise affects desirable plants and vegetation)	n - low	0	We found no evidence.
Imp-A4 (Weed status in anthropogenic systems)	a - mod	0	Maize temporarily appears along roadways and waste places where seeds are dropped (Burrows and Tyrl, 2013). Present throughout the railway network of St. Louis, occurring more commonly by grain elevators (Mühlenbach, 1979). In southeastern Australia, maize and

Question ID	Answer - Uncertainty	Score	Notes (and references)
			other cereals "occasionally escape cultivation to become weeds along roadsides, irrigation channels and in disturbed sites" (Richardson et al., 2006). Present on tips and waste ground in the United Kingdom (Richardson et al., 2006). Because of its extensive use, maize occasionally appears on waste ground about towns in England (Dunn, 1905). There is no doubt that maize appears in anthropogenic areas associated with grain processing, storage, and transport. However, other than Richardson and Richardson (2006), we found no evidence it is considered a weed of these systems. Given how widely cultivated maize is, we answered "a" but with moderate uncertainty. Alternate answers for the Monte Carlo simulation were both "b."
Impact to Production Systems (agriculture, nurseries, forest plantations, orchards, etc.)			
Imp-P1 (Reduces crop/product yield)	y - negl	0.4	"Stalk lodging or breakage, dropped ears, and kernel loss during harvest can result in volunteer maize the following year" (Stahl et al., 2013). In one Spanish study, density of volunteer maize ranged from 0.0036 to 0.8728 plants per square meter (36 to 8728 plants per hectare; Palaudelmàs et al., 2009). Volunteer maize is a weed in crops that follow maize cultivation because it competes for resources with the next crop (i.e., weed interference) and reduces crop yield (Stahl et al., 2013). Several studies have shown that increasing densities of volunteer maize in soybeans result in greater reductions in soybean yield from 0 to 58 percent (Alms et al., 2008; Beckett and Stoller, 1988). Volunteer maize comes up as either individual plants or clumps of plants when entire cobs remain unharvested on the soil (Stahl et al., 2013). A clump of maize reduces soybean yield to a greater extent than individual plants, and affects soybean yield in a radius of up to 86 cm around the clump (Beckett and Stoller, 1988). Maize planted at a simulated density of 4040 clumps per hectare reduced soybean yield by an average of 31 percent (Andersen et al., 1982). Although these densities of maize are somewhat high, such densities do occur in patches (Andersen et al., 1982). Even maize that is partially controlled by herbicides reduces soybean yield (Alms et al., 2008). Volunteer maize can also be problematic in maize to maize cropping systems because it competes with planted maize (Alms et al., 2008; Stahl et al., 2013). Because volunteer maize is produced through open pollination, plants are often not as vigorous as most hybrid varieties that are planted (Carpenter et al., 2002) and consequently they lag behind in growth and development. Although volunteer maize in maize can contribute to total yield, it tends to have a greater negative impact on total yield (Alms et al., 2007), produces smaller cobs or is barren at higher densities (Stahl et al., 2013). One study showed that "uncontrolled, partially controlled, or late controlled volunteer maize caused maize yield loss" from 0-40 percent, depending on treatment and year (Alms et al., 2008). Trials by the University of Minnesota showed that at densities of two or more plants per square meter, volunteer maize begins to have a significant impact on maize yield (Stahl et al., 2013).
Imp-P2 (Lowers commodity value)	y - low	0.2	"Volunteer maize interferes with soybean harvest" and may reduce the value of the crop because of contamination of maize kernels in soybeans (Andersen et al., 1982; Davis, 2009). Because "[v]olunteer maize is not controlled by most soil-applied soybean herbicides"

Question ID	Answer - Uncertainty	Score	Notes (and references)
			(Beckett and Stoller, 1988), it will require treatment with selective post-emergence herbicides that won't damage the soybean crop. An alternative to herbicide sprayers are special rope-wick applicators that apply herbicides to maize plants that have emerged above the soybean canopy (Andersen et al., 1982). Additional herbicide treatments and use of specialized technology will increase the costs of production and lower the value of the commodity. In addition to these impacts, volunteer maize in soybean-maize crop rotation systems will help to sustain populations of maize pests such as maize root worm (CRW) in years when maize is not planted (Stahl et al., 2013). Even if volunteer maize plants possess the engineered trait for insect resistance (Bt), this trait is not being expressed as strongly in volunteer maize as it was in the hybrid generation, leading to feeding levels similar to those in maize plants not possessing the trait (Stahl et al., 2013). "Chronic exposure of CRW larvae in soybean and maize to sub-lethal doses of the Bt toxin also has the potential to hasten development of resistance to Bt-CRW traits" (Stahl et al., 2013). Thus, volunteer maize is likely to have indirect impacts on pest populations that will reduce the value of future crops.
Imp-P3 (Is it likely to impact trade)	n - mod	0	Maize is used for multiple purposes and as such is a valuable commodity. The United States is one of the world's leading exporters of maize (USDA-ERS, 2014). Although seeds of harvested crops are often contaminants of next year's seeds and grains (Salisbury and Frick, 2010; Shimono and Konuma, 2008), we found no evidence that seed or grain of other crops contaminated with non-GE maize is likely to impact trade (e.g., APHIS, 2014).
Imp-P4 (Reduces the quality or availability of irrigation, or strongly competes with plants for water)	n - low	0	While all plants compete with each other for moisture, we found no evidence that maize is particularly aggressive in this regard. We found no evidence that maize reduces the quality or availability of irrigation.
Imp-P5 (Toxic to animals, including livestock/range animals and poultry)	n - low	0	Maize is not an inherently toxic plant (Burrows and Tyrl, 2013). The majority of U.S. maize produced (including the kernels and leafy stalks) is processed into animal feed (Carpenter et al., 2002). However, under some circumstances, particularly under drought stress and/or high nitrogen fertilization, maize accumulates high concentrations of nitrates that when eaten will sicken and kill animals in a few days (Burrows and Tyrl, 2013). This disease is known as maizestalk disease (Burrows and Tyrl, 2013). Another disease associated with maize consumption occurs when maize is infected with *Aspergillus* and *Fusarium* fungal species. These taxa produce mycotoxins that lead to a serious neurological disease in animals that was originally called moldy maize poisoning, but later called equine leukoencephalomalacia (Burrows and Tyrl, 2013). Thus, because maize is not inherently toxic, and because it may only become toxic under improper management, we answered no.
Imp-P6 (Weed status in production systems)	c - negl	0.6	Volunteer maize is a weed (Andersen et al., 1982; Beckett and Stoller, 1988; Davis, 2009; Hager, 2010). A survey by the Weed Science Society of America of weeds in different crops across the United States classified maize as a common weed in potatoes in Washington and a troublesome weed in wheat in Tennessee (Bridges, 1992). Maize is a spontaneous weed in other crops as well, including rice (Galinato et al., 1999). Because of the direct and

Question ID	Answer - Uncertainty	Score	Notes (and references)
			indirect impacts of volunteer maize, it is targeted for control when it occurs in other crops, including maize (Hager, 2010; Ogg and Parker, 2000; Stahl et al., 2013). Volunteer maize is controlled in soybeans using a variety of techniques including herbicide application, cultivation, and hand-hoeing (Andersen et al., 1982; Beckett and Stoller, 1988).
GEOGRAPHIC POTENTIAL			Because maize does not exist outside of cultivation, the majority of records for maize in GBIF (2014) probably represent collections from cultivation where it is supplemented with irrigation water. Thus we did not use GBIF records to evaluate the geographic potential of maize. Instead we answered the questions below using maize's close relative, *Z. mays* subsp. *mexicana*. Unless otherwise indicated, the following evidence represents geographically referenced points obtained from the Global Biodiversity Information Facility (GBIF, 2014).
Plant hardiness zones			
Geo-Z1 (Zone 1)	n - negl	N/A	We found no evidence that it occurs in this hardiness zone.
Geo-Z2 (Zone 2)	n - negl	N/A	We found no evidence that it occurs in this hardiness zone.
Geo-Z3 (Zone 3)	n - negl	N/A	We found no evidence that it occurs in this hardiness zone.
Geo-Z4 (Zone 4)	n - negl	N/A	We found no evidence that it occurs in this hardiness zone.
Geo-Z5 (Zone 5)	n - negl	N/A	We found no evidence that it occurs in this hardiness zone.
Geo-Z6 (Zone 6)	n - low	N/A	We found no evidence that it occurs in this hardiness zone.
Geo-Z7 (Zone 7)	n - low	N/A	We found no evidence that it occurs in this hardiness zone.
Geo-Z8 (Zone 8)	y - negl	N/A	Mexico.
Geo-Z9 (Zone 9)	y - negl	N/A	Mexico.
Geo-Z10 (Zone 10)	y - negl	N/A	Mexico.
Geo-Z11 (Zone 11)	y - low	N/A	Mexico and Guatemala.
Geo-Z12 (Zone 12)	y - high	N/A	Mexico.
Geo-Z13 (Zone 13)	n - low	N/A	We found no evidence that it occurs in this hardiness zone.
Köppen -Geiger climate classes			
Geo-C1 (Tropical rainforest)	y - mod	N/A	Guatemala.
Geo-C2 (Tropical savanna)	y - mod	N/A	Mexico.
Geo-C3 (Steppe)	y - low	N/A	Mexico.
Geo-C4 (Desert)	y - high	N/A	Mexico.
Geo-C5 (Mediterranean)	n - low	N/A	We found no evidence it occurs in this climate class.
Geo-C6 (Humid subtropical)	y - negl	N/A	Mexico and Guatemala.
Geo-C7 (Marine west coast)	y - negl	N/A	Mexico.
Geo-C8 (Humid cont. warm sum.)	n - low	N/A	We found no evidence it occurs in this climate class.
Geo-C9 (Humid cont. cool sum.)	n - negl	N/A	We found no evidence it occurs in this climate class.
Geo-C10 (Subarctic)	n - negl	N/A	We found no evidence it occurs in this climate class.
Geo-C11 (Tundra)	n - negl	N/A	We found no evidence it occurs in this climate class.
Geo-C12 (Icecap)	n - negl	N/A	We found no evidence it occurs in this climate class.
10-inch precipitation bands			
Geo-R1 (0-10 inches; 0-25 cm)	n - negl	N/A	We found no evidence it occurs in this precipitation band.
Geo-R2 (10-20 inches; 25-51 cm)	n - low	N/A	We found no evidence it occurs in this precipitation band.
Geo-R3 (20-30 inches; 51-	y - negl	N/A	Mexico.

Question ID	Answer - Uncertainty	Score	Notes (and references)
76 cm)			
Geo-R4 (30-40 inches; 76-102 cm)	y - negl	N/A	Mexico.
Geo-R5 (40-50 inches; 102-127 cm)	y - low	N/A	Mexico.
Geo-R6 (50-60 inches; 127-152 cm)	y - low	N/A	Mexico.
Geo-R7 (60-70 inches; 152-178 cm)	y - negl	N/A	Mexico.
Geo-R8 (70-80 inches; 178-203 cm)	y - negl	N/A	Mexico.
Geo-R9 (80-90 inches; 203-229 cm)	y - negl	N/A	Mexico.
Geo-R10 (90-100 inches; 229-254 cm)	y - low	N/A	Mexico.
Geo-R11 (100+ inches; 254+ cm)	y - negl	N/A	Mexico and Guatemala.
ENTRY POTENTIAL			
Ent-1 (Plant already here)	y - negl	1	Maize is widely cultivated in the United States (e.g., Carpenter et al., 2002; Stahl et al., 2013).
Ent-2 (Plant proposed for entry, or entry is imminent)	-	N/A	
Ent-3 (Human value & cultivation/trade status)	-	N/A	
Ent-4 (Entry as a contaminant)			
Ent-4a (Plant present in Canada, Mexico, Central America, the Caribbean or China)	-	N/A	
Ent-4b (Contaminant of plant propagative material (except seeds))	-	N/A	
Ent-4c (Contaminant of seeds for planting)	-	N/A	
Ent-4d (Contaminant of ballast water)	-	N/A	
Ent-4e (Contaminant of aquarium plants or other aquarium products)	-	N/A	
Ent-4f (Contaminant of landscape products)	-	N/A	
Ent-4g (Contaminant of containers, packing materials, trade goods, equipment or conveyances)	-	N/A	
Ent-4h (Contaminants of fruit, vegetables, or other products for consumption or processing)	-	N/A	
Ent-4i (Contaminant of	-	N/A	

Question ID	**Answer - Uncertainty**	**Score**	**Notes (and references)**
some other pathway)			
Ent-5 (Likely to enter through natural dispersal)	-	N/A	

Appendix B. Weed risk assessment for GE herbicide-resistant maize, *Zea mays* L. subsp. *mays* (Poaceace). As explained above under "Initiation and Scope," unless we found evidence to the contrary, we assumed non-GE and GE herbicide-resistant maize will have similar, if not identical life history traits. Consequently, below we only show the questions and answers for those traits and impacts where we either found a difference, expect a difference to occur, or found additional or different evidence for the GE biotype. Otherwise, the answers and evidence for herbicide-resistant maize is the same as that shown in Appendix A. The Microsoft Excel file where the risk assessment was conducted is available upon request.

Question ID	**Answer - Uncertainty**	**Score**	**Notes (and references)**
ESTABLISHMENT/SPREAD POTENTIAL			
ES-20 (Is resistant to some herbicides or has the potential to become resistant)	y - negl	1	The maize biotype (DAS-40278-9) that is evaluated in this risk assessment was genetically engineered by Dow AgroSciences to resist two types of herbicides (Dow AgroSciences, 2011). This biotype contains a gene coding for the production of aryloxyalkanoate dioxygenase enzyme that inactivates herbicides of the aryloxyalkanoate family, including phenoxy auxins and aryloxyphenoxypropionate (AOPP) acetyl coenzyme inhibitors (Dow AgroSciences, 2011). This enzyme confers resistance to herbicides with one of either two types of modes of action, such as 2,4-D and "fop" herbicides (BRS, 2013; Dow AgroSciences, 2011; Heap, 2014). Dow AgroSciences states that through conventional breeding, DAS-40278-9 can be stacked with resistance to glyphosate and glufosinate herbicides (Dow AgroSciences, 2011) resulting in maize biotypes resistant to herbicides with four different modes of action.
IMPACT POTENTIAL			
Impacts to Natural Systems			
Imp-N1 (Change ecosystem processes and parameters that affect other species)	n - negl	0	In one study, four different crops that were genetically engineered for herbicide or insect resistance, including glufosinate-resistant maize, and their non-transformed counterparts were planted into 12 different habitats and monitored for recruitment and survival over 10 years (Crawley et al., 2001). "In no case did populations of either conventional or transgenic plants increase, and transgenic plants never persisted significantly longer than conventional plants. All populations of maize, rape and sugar beet were extinct at all sites within 4 years of sowing" (Crawley et al., 2001). Thus, we do not expect for any of the impacts evaluated under natural systems to differ from those of non-GE maize.
Impact to Production Systems (agriculture, nurseries, forest plantations, orchards, etc.)			
Imp-P1 (Reduces crop/product yield)	y - negl	0.4	As with non-GE maize, we expect GE herbicide-resistant maize to volunteer the season after its cultivation (Hager, 2009). "The frequency of volunteer maize in soybean fields in northern Indiana increased each year concurrently with the adoption of glyphosate-resistant maize" (Davis et al., 2008). GE herbicide-resistant volunteer maize has resulted in yield loss of other crops such as sugar beets (Kniss et al., 2012), soybean (Marquardt et al., 2012), and cotton (Thomas et al., 2007). Because herbicide-resistant maize will be more difficult to manage (see evidence under Imp-P2), it may result in greater yield loss than if it were non-GE maize.

Question ID	Answer - Uncertainty	Score	Notes (and references)
Imp-P2 (Lowers commodity value)	y - negl	0.2	We have already documented in Appendix A how non-GE maize lowers commodity value. Volunteer offspring of herbicide-resistant crops are more difficult to control than the nonresistant versions (Floate et al., 2002; Stahl et al., 2013). Many maize hybrids are now stacked with herbicide resistance to both glyphosate and glufosinate (Stahl et al., 2013). "[I]f you have to use additional herbicides to control volunteer (weedy) herbicide tolerant crop plants, in a crop grown with the same herbicide resistance, the increased cost is a direct result of using the first herbicide-tolerant crop. In contrast, the evolution of herbicide-resistant weeds is more of an indirect result" (Davis, 2009). Sometimes GE herbicide-resistant volunteer maize also expresses the engineered gene for insect resistance (Bt) (Davis, 2009), as hybrids stacked with these two resistances are grown in the United States (USDA-ERS, 2014). A recent study suggests that these volunteer plants, which won't be as robust as their parents, may facilitate more rapid evolution of Bt resistance in maize rootworm populations (Davis, 2009) or at the very least provide a host refuge for rootworm populations between maize crops in a rotation. Furthermore, "[d]epending on what trait(s) were in the hybrid planted the previous year and the current year, cultivation [i.e., tilling] may be the only viable option" to control maize volunteers (Stahl et al., 2013), which may lead to some soil erosion. Development and use of herbicide-resistant crops is making their control as volunteer plants more challenging (Owen and Zelaya, 2005). Although our answer for this question was already yes for conventional maize, the additional evidence available for herbicide-resistant maize allowed us to use negligible uncertainty.
Imp-P3 (Is it likely to impact trade)	y - negl	0.2	The United States is one of the world's leading exporters of maize (USDA-ERS, 2014) and generally exports non-segregated maize grain (i.e., non GE and GE maize grain are mixed; Dyer et al., 2009). An international database has been developed for tracking country approvals for specific GE crops (ISAAA, 2014). Not all countries accept GE products and some have banned GE maize (e.g., France; USDA-FAS, 2012). Countries regulating GE maize will likely reject our exports if they find them contaminated with GE biotypes that are not yet approved for entry or that are prohibited. For example, China recently rejected 1.25 million tons of U.S. maize after they detected it was contaminated with a GE type resistant for insect pests (Jie, 2014). Thus, wider use of GE maize in the United States may limit or further restrict foreign market access. If U.S. maize exporters distinguished between GE and non-GE maize for export, it is still possible that GE maize may contaminate shipments of non-GE maize. This may occur in several ways, including 1) the crop seed U.S producers plant is contaminated with GE maize (Jemison and Vayda, 2001), 2) GE volunteer maize volunteers in non-GE maize (Shimono et al., 2010), 3) seed/grain contamination from machinery and storage shelters that process both types of maize (Shimono and Konuma, 2008), and 4) gene flow through pollen (Jemison and Vayda, 2001; Palaudelmàs et al., 2009).

Question ID	Answer - Uncertainty	Score	Notes (and references)
Imp-P5 (Toxic to animals, including livestock/range animals and poultry)	n - low	0	Among its concerns for the weed risk potential associated with GE maize, the Center for Food Safety was concerned about animal and human safety issues stemming from the inserted gene, the enzyme, and the metabolites produced as a result of that new biochemical pathway (CFS, 2014). As part of its request to deregulate DAS-40278-9, Dow AgroSciences submitted to the US Food and Drug Administration (FDA) a Consultation of Bioengineered Foods. The FDA concluded that "[b]ased on the information provided by the company [Dow AgroSciences] and other information available to the agency, FDA did not identify any issues under the Federal Food, Drug and Cosmetic Act that would require further evaluation at this time" (McMahon, 2011). Based on the FDA's determination, we answered no with low uncertainty.
Imp-P6 (Weed status in production systems)	c - negl	0.6	Like conventional maize, herbicide-resistant volunteer maize is also considered a weed because it reduces crop yield and lowers the value of the commodity due to the indirect costs associated with treatment (see Imp-P1 and Imp-P2). Herbicide-resistant maize is more problematic than conventional maize as a volunteer, particularly in herbicide-resistant crops, and will need to be controlled in crop rotations (Davis, 2009; Hager, 2009; Kniss et al., 2012; Marquardt et al., 2012). When controlling volunteer maize, farmers need to consider several factors, the most important of which is whether the plants are herbicide resistant and which herbicides they are resistant to. This will determine which herbicide formulations and additives will be needed for control. "Several postemergence herbicides provide excellent control of volunteer glyphosate- or glufosinate-resistant maize" and herbicide formulation tables have been designed to help farmers chose the most effective control strategies (Hager, 2010). If DAS-40278-9 is deregulated by BRS and cultivated by farmers, it will introduce resistance to two additional herbicides in U.S. maize. Furthermore, if through conventional breeding all four resistances are stacked (Dow AgroSciences, 2011), controlling volunteer maize plants will be more challenging.
ENTRY POTENTIAL			
Ent-1 (Plant already here)	n - negl	0	Herbicide-resistant maize is cultivated throughout the United States (USDA-ERS, 2014); however, the particular biotype that initiated this assessment has not yet been released.
Ent-2 (Plant proposed for entry, or entry is imminent)	y - negl	1	Dow AgroSciences petitioned APHIS-BRS to deregulate a new biotype of maize (DAS-40278-9) that has been genetically engineered for resistance to two different types of herbicides (Dow AgroSciences, 2011). Thus, the entry of this biotype is imminent and we don't need to proceed further with the evaluation of entry potential.