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Weed Risk Assessments for *Glycine max* (L.) Merr. (Fabaceae) – Soybean and Genetically Engineered Herbicide-Resistant Soybean

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Glyphosate resistant volunteer soybeans in corn (source: Amitkumar Jhala, University of Nebraska-Lincoln, Jhala et al., 2013).

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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.

***Glycine max* (L.) Merr. – Soybean and genetically engineered herbicide-resistant soybean**

Species Family: Fabaceae

Information Synonyms: *Phaseolus max* L., *Soja max* (L.) Piper (NGRP, 2014).

Common names: Soybean, edamame, soya, soya-bean (NGRP, 2014).

Botanical description: *Glycine max* (soybean) is an erect annual herb with pinnate, 3-foliolate leaves. It produces 2-5 round seeds in pendulous pods and is cultivated as a protein source throughout temperate and tropical regions (Boerma and Specht, 2004; Singh, 2010; Zhengyi et al., 2014).

Initiation and scope: On May 6, 2014, the Center for Food Safety (CFS) petitioned the Secretary of the U.S. Department of Agriculture to regulate genetically engineered (GE) multiple herbicide-resistant corn 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 PPA [Plant Protection Act] authority” by evaluating all direct and indirect impacts of these herbicide-resistant taxa (CFS, 2014). They maintain that because the PPA definition of a noxious weed includes indirect impacts caused by plants, APHIS should evaluate and consider all of the following factors associated with cultivating these GE taxa when making its determination: 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 corn and soybeans indicated in the petition using its standard weed risk assessment process. The PPQ WRA uses a series of mostly yes and no questions about a 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 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 important to consider, relate to changes in herbicide use that will be expected to occur with the cultivation of these biotypes of corn and soybeans. 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 soybean to public health was evaluated by the Food and Drug Administration (FDA, 2011) separately and is only referenced here.

In this document we evaluate the weed risk associated with GE soybean biotypes DAS-68416-4 and DAS-44406-6 developed by Dow AgroSciences (Dow AgroSciences, 2010; Dow AgroSciences, 2011). DAS-68416-4 produces proteins that inactivate aryloxyalkanoate family herbicides and phosphinothricin, providing resistance to the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and glufosinate (Dow AgroSciences, 2010). DAS-44406-6 soybeans are resistant to 2,4-D and glufosinate, and also proteins that make the plants resistant to glyphosate (Dow AgroSciences, 2011).

As part of our approach for this risk assessment, we evaluated and compared the weed risk potential for soybean that has not been genetically engineered with herbicide resistance and soybean genetically engineered for any type of herbicide resistance. Hereafter we use the terms non-GE soybean and GE herbicide-resistant soybean to distinguish between these two types of plants. Although the CFS requested the USDA to evaluate specific biotypes of GE herbicide-resistant soybean (DAS-68416-4 and DAS-44406-6), we increased the scope of this assessment to ensure we consider all evidence related to the impact of herbicide resistance in soybean. We recognize that soybean has been genetically engineered with other types of traits. For the purpose of this weed risk assessment, we did

not evaluate the risks associated with these other types of genetically-engineered traits.

DAS-68416-4 and DAS-44406-6 were grown with similar non-GE soybean hybrids in a variety of sites to determine if they differed for a variety of agronomic traits such as plant vigor, height, germination, and yield; no significant differences in those traits were detected (Dow AgroSciences, 2010; 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: Soybean is native to Asia (NGRP, 2014) and cultivated in over 50 countries, including Canada, Brazil, Bolivia, Paraguay, Argentina, Italy, Yugoslavia, Nigeria, Uganda, South Africa, Russia, India, China, Myanmar, Thailand, Vietnam, Indonesia, Korea, and Japan (Boerma and Specht, 2004). Genetically engineered soybeans are grown in Brazil, Argentina, Canada, Paraguay, South Africa, Uruguay, Bolivia, Mexico, Chile, and Costa Rica (Clive, 2013).

U.S. distribution and status: Soybean is widely grown in the United States and is one of the country's most economically important crops (Bailey and Bailey, 1976; Boerma and Specht, 2004). Soybean cultivars that are genetically engineered to be resistant to the herbicides glyphosate and glufosinate have been rapidly adopted by growers in the United States (Boerma and Specht, 2004). In 2014, herbicide-resistant soybeans made up 94 percent of the soybean acreage planted in the United States (USDA ERS, 2014).

WRA area¹: Entire United States, including territories.

1. Non-GE and GE herbicide-resistant soybean analysis

Establishment/Spread Potential *Non-GE soybean*. Soybean is a herbaceous, nitrogen-fixing plant in the family Fabaceae (Bailey and Bailey, 1976; NGRP, 2014). It is highly domesticated and grown in over 50 countries (Boerma and Specht, 2004). Soybean has been recorded as a casual escape (Clement and Foster, 2000; Pysek et al., 2002; Weakley, 2010) because soybean seed left in the field after harvest will volunteer by germinating with the following crop (Fett, 1978; Staff, 2013). Soybeans do not occur outside of cultivation (Boerma and Specht, 2004). Soybean flowers are predominately self-pollinated (Boerma and Specht, 2004; Singh, 2010). The seeds are mainly dispersed through shattering, but seeds of

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).

wild soybean relatives are rarely dispersed longer distances (up to 400 meters away) (Kuroda et al., 2008) by birds (Norman, 1978; Singh, 2010). Seeds of wild soybean relatives might also disperse longer distances by water (Kuroda et al., 2008) or mammals (Kuroda et al., 2008; Norman, 1978). Soybean seeds can also move to new areas as contaminants of oil seed and bird seed (Clement and Foster, 2000). We had a low amount of uncertainty for this risk element.

Risk score = 2 Uncertainty index = 0.12

GE herbicide-resistant soybean. Except for herbicide resistance, which increased the score by one point, we found no evidence that DAS-68416-4 and DAS-44406-6 differ from conventional soybean with respect to agronomic traits (Dow AgroSciences, 2010; Dow AgroSciences, 2011) or the botanical traits evaluated under this risk element. We had a low amount of uncertainty for this risk element.

Risk score = 3 Uncertainty index = 0.12

Impact Potential *Non-GE soybean.* Soybean does not impact natural or anthropogenic systems, and does not compete well with other cultivated plants (CFIA, 2012).

Volunteer soybeans occasionally grow in other crops if soybean seed remains in the field from the previous season (Fehr et al., 1971), but volunteer soybeans do not economically impact production systems because they are easily controlled mechanically or with herbicide applications (CFIA, 2012; Staff, 2013). We had a low amount of uncertainty for this risk element.

Risk score = 1.6 Uncertainty index = 0.10

GE herbicide-resistant soybean. As glyphosate-resistant soybeans have become more widely grown in the United States, growers have begun to experience problems with volunteer glyphosate-resistant soybeans in other glyphosate-resistant crops. Glyphosate-resistant volunteer soybeans can be a problem in corn (Deneke, 2013; Jhala et al., 2013), and herbicide trials have been conducted for controlling volunteer glyphosate-resistant soybeans in fields of glyphosate-resistant cotton (York et al., 2005). Growers must apply alternate, non-glyphosate herbicides to control glyphosate-resistant volunteer soybeans. Additionally, GE soybeans can impact trade activities by contaminating non-GE crops. For example, the European Union will reject honey exports from Mexico if they are contaminated with pollen from GE soybeans (Villanueva-Gutierrez et al., 2014). Soybean seed from the United States was historically banned for import into the European Union due to concerns that GE soybean seed would not be readily detected if it became mixed with other soybeans (Boerma and Specht, 2004). The European Union has since lifted its ban on U.S. soybeans, but complex regulations and an onerous approval process have severely limited U.S. soybean exports into Europe (FAS, 2013). Our impact risk score for herbicide-resistant soybeans was greater than the score for non-GE soybeans, due to the impacts that glyphosate-resistant soybeans have had in crop rotation systems. We had a

low amount of uncertainty for this risk element.

Risk score = 2.4

Uncertainty index = 0.09

Geographic Potential Soybean is widely cultivated in the United States (Boerma and Specht, 2004). Figure 1 is a map of the current acreage of all soybean production (both non-GE and GE herbicide-resistant) in the contiguous United States (NASS, 2014). Soybeans are normally grown where growing season temperatures are between 50 and 104 °F (10 to 40 °C) (Singh, 2010). Soybean seeds require soil temperatures of at least 50 °F (10 °C) to germinate (CFIA, 2012; Singh, 2010); 79 to 93 °F (34 to 36 °C) is the optimum temperature range for germination (Norman, 1978). Soybeans require 19.7 to 29.5 inches (500 to 750 mm) of rainfall over the growing season to produce a good crop and must be irrigated in areas that receive less rain (Norman, 1978).

The PPQ WRA contains 36 questions about a species' climatic tolerances that are answered based on the native and naturalized distribution of the species. The answers are used to address three questions under the Establishment and Spread risk element about the species' adaptive potential. However, because soybean has not naturalized and does not occur outside of cultivation, we were unable to answer these questions using data for the species *G. max* and had to answer these questions using an alternative approach. Thus, we answered the geographic potential questions using data on naturalized occurrences of *G. soja*, the closest relative and wild ancestor of soybean (Boerma and Specht, 2004; Singh, 2010; Wang et al., 2011). *Glycine soja* occurs in areas with the temperatures of Plant Hardiness Zones 6-13, areas with 40-100+ inches of annual precipitation, and the following Köppen-Geiger climate classes: tropical savanna, humid subtropical, humid continental warm summers, and humid continental cool summers.

Entry Potential We did not assess entry potential because soybeans are already present and widely cultivated in the United States (Boerma and Specht, 2004).

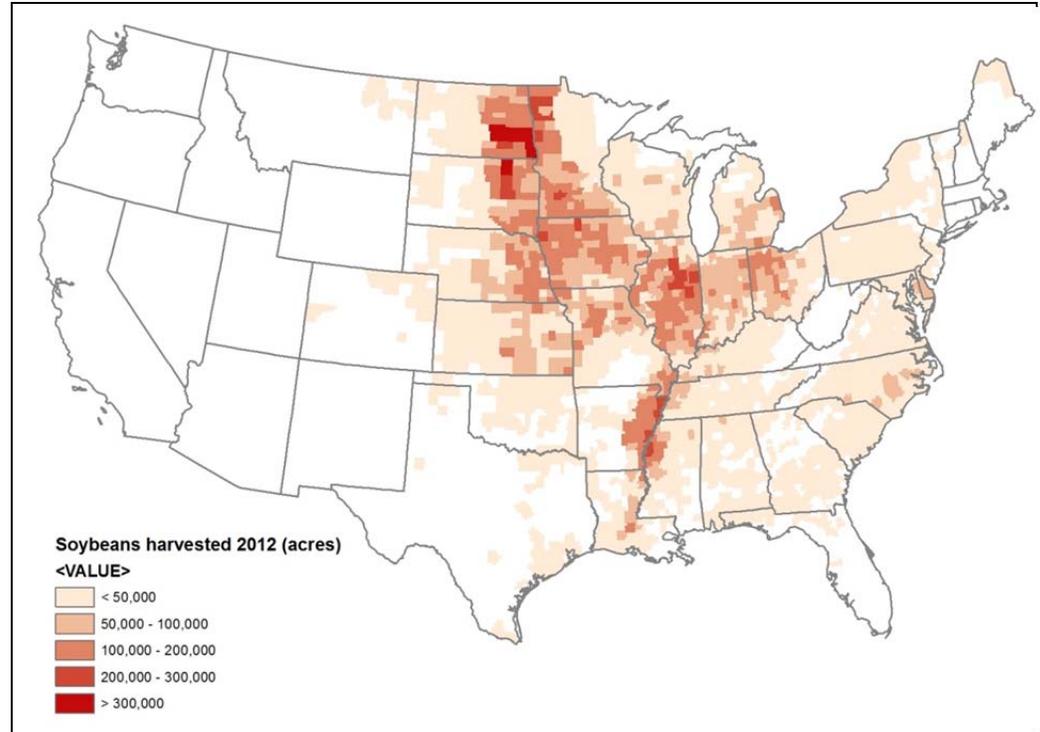


Figure 1. 2012 acreage of all soybeans (both non-GE and GE herbicide-resistant) harvested in the contiguous United States (NASS, 2014).

2. Results

Non-GE Soybean

Model Probabilities: P(Major Invader) = 6.3%
P(Minor Invader) = 62.7%
P(Non-Invader) = 31.1%

Risk Result = Evaluate Further

Secondary Screening = Evaluate Further

GE herbicide-resistant soybean

Model Probabilities: P(Major Invader) = 12.1%
P(Minor Invader) = 69.9%
P(Non-Invader) = 18%

Risk Result = Evaluate Further

Secondary Screening = Evaluate Further

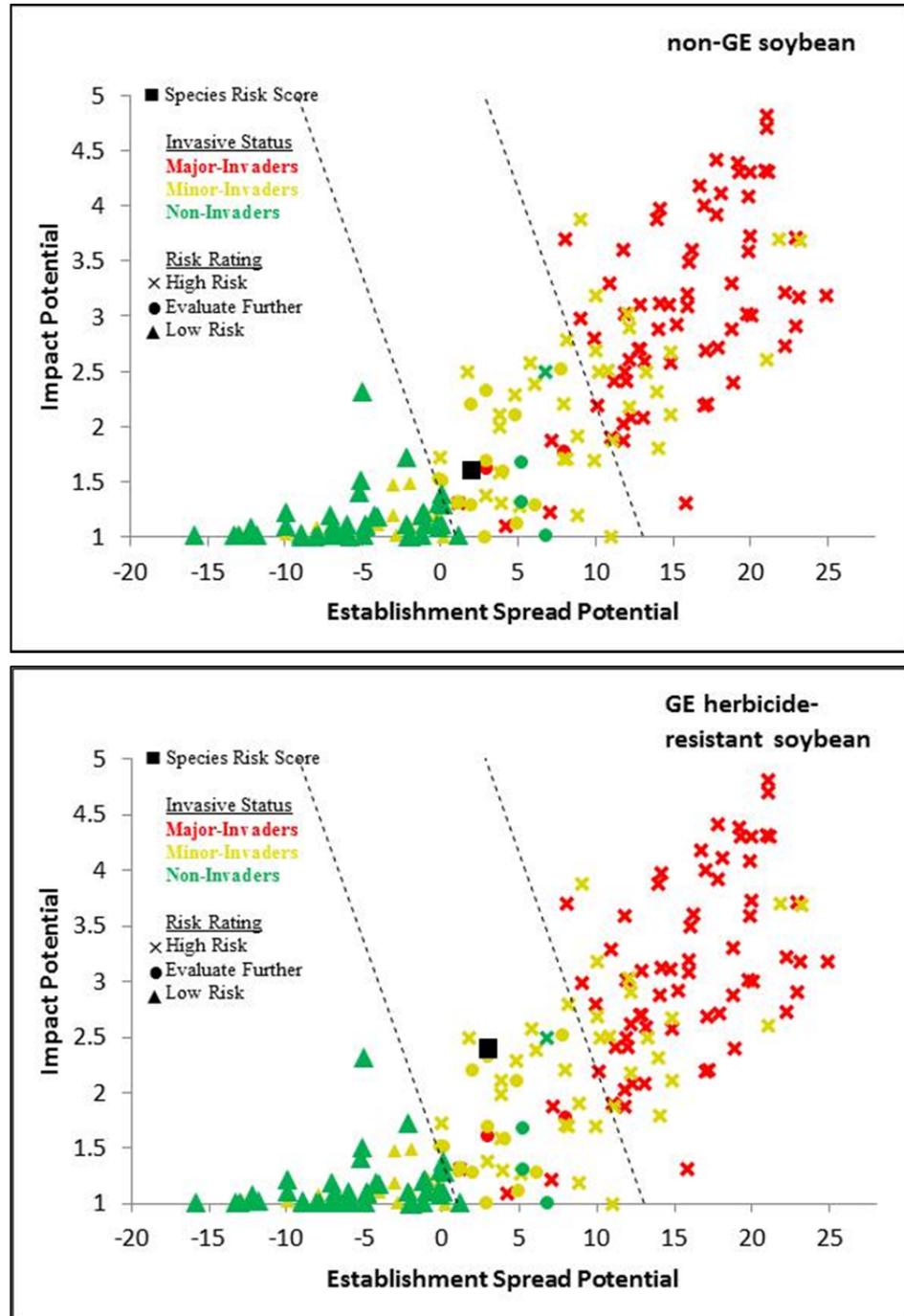


Figure 2. Non-GE soybean (top) and GE herbicide-resistant soybean (bottom) risk scores (black box) relative to the risk scores of species used to develop and validate the PPQ WRA model (other symbols). See Appendix A for the complete assessment.

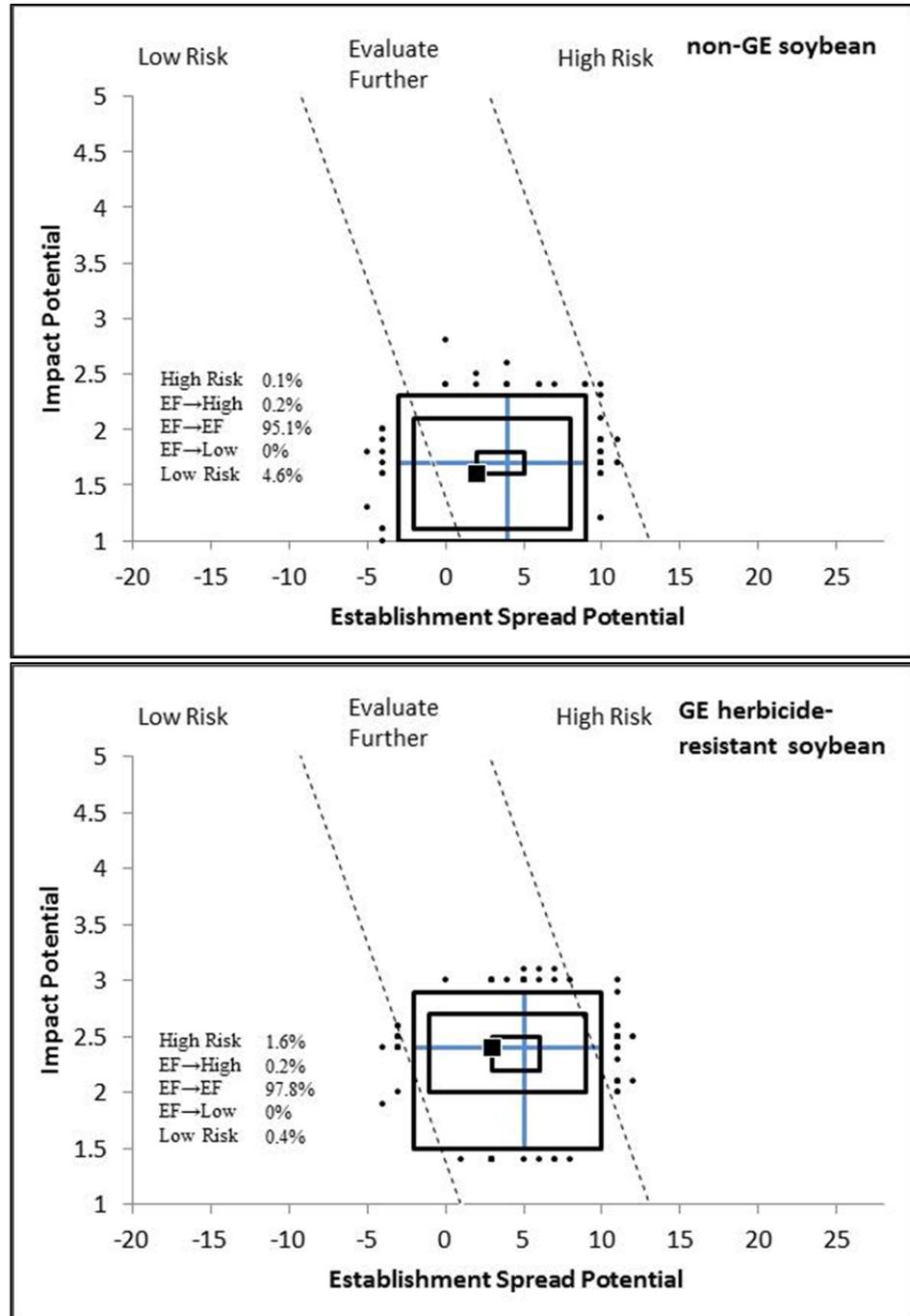


Figure 3. Model simulation results (N=5,000) for uncertainty around the risk score for non GE soybean (top) and GE herbicide-resistant soybean (bottom). The blue “+” symbol represents the medians of the simulated outcomes. The smallest box contains 50 percent of the outcomes, the second 95 percent, and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

3. Discussion

The result of the weed risk assessment for both conventional soybean and herbicide-resistant soybean is Evaluate Further. When compared to the 204 plant species used to develop and validate the PPQ WRA model, both types of soybean have traits in common with minor invaders (Fig. 2). These traits include being self-pollinated (Boerma and Specht, 2004; Singh, 2010), producing viable seeds (Bailey and Bailey, 1976; Boerma and Specht, 2004), and moving as a contaminant in other commodities (Clement and Foster, 2000). The results of our model were fairly robust; 96.3 percent of the simulated risk scores for conventional soybean received a result of Evaluate Further and 94.6 percent of the simulated risk scores for herbicide-resistant soybean were Evaluate Further (Fig. 3).

The PPQ WRA is a qualitative WRA that was not designed to evaluate the risk associated with GE species. However, in our model GE herbicide-resistant soybean had a significantly higher impact risk score than conventional soybean. This was mainly due to impacts that volunteer glyphosate-resistant soybeans have recently had in production systems in the United States. Growers have to apply alternate (non-glyphosate) herbicides to control volunteer glyphosate-resistant soybeans in glyphosate-resistant corn (Deneke, 2013; Jhala et al., 2013; Staff, 2013) and glyphosate-resistant cotton (York et al., 2005). Additionally, areas such as the European Union have rejected entry to conventional agricultural goods that may be contaminated by genetically engineered crops (FAS, 2013; Villanueva-Gutierrez et al., 2014).

While impacts of glyphosate-resistant volunteer soybeans in production systems have been documented, it is unclear if DAS-68416-4 and DAS-44406-6 soybeans, which are resistant to 2,4-D, glufosinate, and glyphosate, would have similar agricultural impacts. Soybeans are less sensitive to 2,4-D than to other herbicides, which is why 2,4-D is not recommended to control glyphosate-resistant volunteer soybeans (Jhala et al., 2013). However, soybeans have also been genetically-engineered to be resistant to herbicides such as 3,6-dichloro-2-methoxybenzoic acid (dicamba) (Monsanto, 2012), which is used to control glyphosate-resistant volunteer soybeans (Jhala et al., 2013). Soybeans genetically-engineered to be resistant to dicamba and/or other herbicides currently used to control glyphosate-resistant volunteer soybeans could reduce the herbicide options available to growers to control GE herbicide-resistant volunteer soybeans in the future.

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-68416-4 and DAS-44406-6 (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-68416-4 and DAS-44406-6 are commercially cultivated in the United States. However,

falling within PPQ's authority to safeguard U.S. plant resources from pests and noxious weeds, it is important to note that changing the patterns and types of herbicides used could shift weed species communities and may increase the chance of weed populations developing resistance to 2,4-D and other herbicides (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). Nakayama and Yamaguchi (2002) examined hybridization rates between cultivated soybean and *G. soja* plants in Japan. In their study the incidence of hybridization was 17.4 percent. However, because wild soybean species are not naturalized in North America (CFIA, 2012; Kartesz, 2014), the risk of naturalization with wild soybean relatives in the United States is low.

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Appendix A. Weed risk assessment for Non-GE soybean, *Glycine max* (L.) Merr. (Fabaceae). 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	Native to Asia and widely cultivated (NGRP, 2014). Grown in over 50 countries (Boerma and Specht, 2004) on 90.19 million hectares (Singh, 2010). Randall (2007) lists this species as escaping from cultivation. Clement and Foster (2000) list <i>G. max</i> as a casual escape. Listed as a casual escape by (Pysek et al., 2002). In the southern United States, "abundantly cultivated, rarely persisting as a waif" (Weakley, 2010). Not found outside of cultivation in Canada (CFIA, 2012). In agricultural fields, unharvested soybean seed can grow into volunteer plants the following season (Fett, 1978). In this context, the term "volunteer" refers to plants derived from seed prematurely dropped by agricultural crop plants prior to harvest in the previous growing season (Gressel, 2005). We consider volunteer plants to be escaped/casual. Based on this evidence, we answered "d" for escaped/casual with negligible uncertainty. The alternate answers used for the Monte Carlo simulation were both "e."
ES-2 (Is the species highly domesticated)	y - negl	-3	Soybean plants were first domesticated in China (Mabberley, 2008) between 1500-1100 B.C. (Boerma and Specht, 2004). During domestication, soybean plants were selected to have larger but fewer seeds than their wild relatives. Cultivated soybean plants have 8.24 times fewer seeds than wild relatives. Humans also selected for smaller plants with a vertical type growing habit rather than a vine type growing habit (Singh, 2010). <i>Glycine max</i> does not occur outside of cultivation; "[it] is a true domesticate. In the absence of human intervention, the species would not exist" (Boerma and Specht, 2004).
ES-3 (Weedy congeners)	n - mod	0	There are about 24 species in the genus <i>Glycine</i> , only two of which (<i>G. max</i> and <i>G. soja</i>) are annuals (Boerma and Specht, 2004). Holm et al. (1979) do not list any species of <i>Glycine</i> as Serious or Principal weeds. <i>Glycine gracilis</i> is considered a "weedy" form of soybean and this species is included in the subgenus <i>Soja</i> , but the validity of this species name is under debate by botanists (Boerma and Specht, 2004; Gressel, 2005); <i>Glycine gracilis</i> may be a hybrid between <i>G. max</i> and <i>G. soja</i> (Gressel, 2005; Wang et al., 2011). Although <i>G. gracilis</i> is called "weedy" (Boerma and Specht, 2004), we found no evidence of <i>G. gracilis</i> having significant impacts. Thus, we answered this question no with moderate uncertainty.

Question ID	Answer - Uncertainty	Score	Notes (and references)
ES-4 (Shade tolerant at some stage of its life cycle)	n - low	0	In soybean, "extreme shade conditions can induce rapid senescence of lower canopy leaves several weeks in advance of monocarpic senescence of the whole plant" (Burkey and Wells, 1996). Shaded plants have significantly lower yields than plants grown in full sun. Soybean plants that are shaded during part of the flowering and pod set period have a reduced number of seeds and are unable to recover from shade treatments even when the shade is removed midway through the reproductive period. However, relatively short periods of shade (7–10 days) have only limited effects on seed number (Singh, 2010). Soybeans grown in partial shade have lower yields and are more susceptible to white mold (Allman, 2014). Based on this evidence, we answered no with low uncertainty.
ES-5 (Climbing or smothering growth form)	n - negl	0	No evidence. <i>Glycine max</i> is a sparsely or densely branched 75-125 cm tall herbaceous plant with a bush-type growth habit (Boerma and Specht, 2004).
ES-6 (Forms dense thickets)	n - negl	0	<i>Glycine max</i> is a bushy, herbaceous plant (Zhengyi et al., 2014; Singh, 2010), but we found no evidence that soybean plants form dense thickets or populations under natural conditions. We used negligible uncertainty because this plant is well studied.
ES-7 (Aquatic)	n - negl	0	Not aquatic; terrestrial plant in the family Fabaceae (NGRP, 2014).
ES-8 (Grass)	n - negl	0	Not a grass; herbaceous plant in the family Fabaceae (Bailey and Bailey, 1976; NGRP, 2014).
ES-9 (Nitrogen-fixing woody plant)	n - low	0	Although its roots contain nitrogen-fixing nodules inhabited by <i>Bradyrhizobium japonicum</i> bacteria (Boerma and Specht, 2004; Singh, 2010), <i>G. max</i> is a herbaceous and not woody plant (Bailey and Bailey, 1976; NGRP, 2014; Zhengyi et al., 2014).
ES-10 (Does it produce viable seeds or spores)	y - negl	1	Grows from seed (Bailey and Bailey, 1976; Boerma and Specht, 2004). Produces seeds (Mabberley, 2008).
ES-11 (Self-compatible or apomictic)	y - negl	1	The flowers have diadelphous stamens that shed pollen directly on the stigma, resulting in a high percentage of self-fertilization. Only <0.5 percent to 1 percent of flowers are naturally cross-pollinated (Boerma and Specht, 2004; Singh, 2010).
ES-12 (Requires special pollinators)	n - negl	0	<i>Glycine max</i> flowers are predominately self-pollinated (Boerma and Specht, 2004; Singh, 2010); specialist pollinators are not required for seed set. The flowers are visited by generalist pollinators such as honeybees (<i>Apis mellifera</i>) but it is unclear if bees contribute to the pollination process (Chiari et al., 2005). Based on this evidence, we answered no with negligible uncertainty.
ES-13 (Minimum generation time)	b - negl	1	<i>Glycine max</i> and <i>G. soja</i> are grouped together in the subgenus <i>Soja</i> because they are both annuals; perennial species of <i>Glycine</i> are grouped under the subgenus <i>Glycine</i> (Boerma and Specht, 2004). The total growth cycle for <i>Glycine max</i> is 100 to 150 days (Singh, 2010). Based on this information, we answered "b." The alternate answers used for the Monte Carlo simulation were both "c."

Question ID	Answer - Uncertainty	Score	Notes (and references)
ES-14 (Prolific reproduction)	y - high	1	Because we did not have information about the number of seeds produced by volunteer soybeans, we used production data to answer this question and raised our uncertainty to high. In the United States, optimum yields in wide row spacing systems result from growing approximately 250,000 plants per hectare. Depending on the cultivar, <i>G. max</i> plants may have as many as 400 pods on a single plant, with two to three seeds per pod (Boerma and Specht, 2004). Each plant produces approximately 280 seeds per plant (Singh, 2010); edamame cultivars yield 40 to 50 pods per plant with 2 to 3 seeds per pod (Boerma and Specht, 2004). In India, soybeans planted at a density of 0.666 million plants per hectare produce approximately 47 seeds per plant, while soybeans planted at 0.148 million plants per hectare produce 154 seeds per plant (Singh, 2010). The world average soybean yield for 1997-1999 was 2.26 megagrams (Mg) per hectare from managed agricultural fields (Boerma and Specht, 2004). Using these numbers, soybean yields range from 3,000 seeds per square meter to 25,000 seeds per square meter. Based on this evidence, we answered yes with high uncertainty.
ES-15 (Propagules likely to be dispersed unintentionally by people)	y - low	1	Clement and Foster (2000) list <i>G. max</i> as a casual escape from food refuse. Grows "where seed is spilled on tips, waste land and near docks and factories" (Stace, 2010).
ES-16 (Propagules likely to disperse in trade as contaminants or hitchhikers)	y - low	2	Clement and Foster (2000) list <i>G. max</i> as spreading in oil seed and bird seed. Listed as a potential seed contaminant (NGRP, 2014). Listed in guide of seed impurities of grain (GTA, 1998). Based on this evidence, we answered yes with low uncertainty.
ES-17 (Number of natural dispersal vectors)	1	-2	Pod and seed descriptions used to answer ES-17a through ES-17e: "The pods are either straight or slightly curved, usually hirsute. The one to three seeds per pod are usually ovoid to subspherical" (Boerma and Specht, 2004). "Legume succulent, oblong, slightly curved, pendulous, 40-75 × 8-15 mm, densely silky hairy. Seeds 2-5, elliptic, suborbicular, or ovate to oblong, ca. 10 × 5-8 mm, many colored; testa smooth; hilum obvious, elliptic" (Zhengyi et al., 2014).
ES-17a (Wind dispersal)	n - negl		We found no evidence of wind dispersal; the seeds do not have any obvious adaptations for wind dispersal. Because their size and shape make this dispersal strategy highly unlikely, we used negligible uncertainty.
ES-17b (Water dispersal)	? - max		We found no information for <i>G. max</i> . <i>Glycine soja</i> seeds are rarely dispersed long distances (up to 400 meters), but the exact means of this long-distance dispersal is unknown. These seeds may be dispersed by water because <i>G. soja</i> plants are found alongside rivers and irrigation channels (Kuroda et al., 2008). Wild soybean species have been collected from river banks in China (Singh, 2010). Based on this evidence, we answered unknown.

Question ID	Answer - Uncertainty	Score	Notes (and references)
ES-17c (Bird dispersal)	y - high		We found no information for <i>G. max</i> . Other species of <i>Glycine</i> are dispersed by birds (Boerma and Specht, 2004). "The wild perennial <i>Glycine</i> species found outside of Australia were taken to other neighbouring regions by migratory birds via long distance dispersal" (Singh, 2010). "An examination of dove populations in Colombia revealed that about 14% of the birds had soybean seed...in their guts...Individual birds had consumed as many as 55 soybean seeds. Examination of the feeding area exposed piles of up to 30 cotyledons which had been regurgitated by doves after overfeeding" (Norman, 1978). Based on this evidence we answered yes but used high uncertainty.
ES-17d (Animal external dispersal)	n - low		Soybean seeds are smooth and round with no obvious adaptations to attach to animals. Thus, we answered no with low uncertainty.
ES-17e (Animal internal dispersal)	? - max		Whole soybeans can pass through cattle undigested (Lalman et al., 2007), but it is not known whether they remain viable. Kuroda et al. (2008) suggest that animals might rarely move <i>G. soja</i> seeds long distances. Rats and other rodents consume soybean seed (Norman, 1978). Eaten by deer (Seedland, 2014). However, because we did not have any direct evidence that animals disperse soybeans by consuming seeds, we answered unknown.
ES-18 (Evidence that a persistent (>1yr) propagule bank (seed bank) is formed)	n - high	-1	"Soybean seed rarely displays any dormancy characteristics" (CFIA, 2012). "Soybean seeds that are lost during harvest do not overwinter particularly well in the Midwestern U.S....In warmer climates, it is likely that seeds would imbibe and rot quickly, thus precluding the chances for volunteerism in the following growing season" (Gressel, 2005). Depending on the cultivar, soybean seeds can either be "soft" or "hard," with hard seeds surviving longer and contributing to a seed bank. The majority of cultivated soybean plants have been selected to have a soft seed coat so seeds readily imbibe water and can be easily processed (Keim et al., 1990; Shao et al., 2007). Soft-seeded cultivars may occasionally produce some seeds with hard seeds (Shao et al., 2007). "Soybeans with 14.0–14.3% moisture content and maintained at 5–8°C can be stored for >2 years without mould damage, whereas those kept at 30°C are susceptible to mould growth in few weeks and severely damaged in 6 months" (Singh, 2010). Soaking soybean seeds in water can speed up emergence rates (Singh, 2010). Based on this evidence, we answered no, but with high uncertainty because plants may produce some hard seeds that can last longer in the soil.

Question ID	Answer - Uncertainty	Score	Notes (and references)
ES-19 (Tolerates/benefits from mutilation, cultivation or fire)	n - mod	-1	Soybean plants will usually regrow when the main stem has been cut off, unless they are cut off below the cotyledons; soybean plants cut off or seriously injured below the cotyledons will not recover and should be considered dead (Hicks and Naeve, 2013; Pioneer, 2014). "If no leaves remain, regrowth will be very slow, even if growing points remain intact" (Pioneer, 2014). Because soybeans do not seem to have a vigorous growth response to mutilation in comparison to other plant species, we answered this question no. However, we used moderate uncertainty because soybeans can tolerate some mutilation under certain conditions.
ES-20 (Is resistant to some herbicides or has the potential to become resistant)	n - low	0	We found no evidence of natural herbicide resistance in non-GE <i>G. max</i> plants; soybean plants are sensitive to and can be damaged by herbicides residues in the soil (Boerma and Specht, 2004). Thus, we answered no with low uncertainty.
ES-21 (Number of cold hardiness zones suitable for its survival)	8	0	This and the next two questions measure the adaptive potential of a species. Because soybeans do not exist outside of cultivation, we could not answer these questions directly for cultivated soybeans. Instead we used the distribution of its close relative <i>G. soja</i> to answer them.
ES-22 (Number of climate types suitable for its survival)	4	2	
ES-23 (Number of precipitation bands suitable for its survival)	7	0	
IMPACT POTENTIAL			
General Impacts			
Imp-G1 (Allelopathic)	? - max		The allelopathic effects of <i>Glycine max</i> are unclear. Continuous cropping of soybeans often yields to decreased soybean yields, but a 30-year study of continuously cropped soybean plants in India showed no allelopathic-related yield reductions. Additionally, soybean root exudates have been shown to reduce velvetleaf weed populations, but not foxtail millet (Singh, 2010). Due to the conflicting information, we answered unknown.
Imp-G2 (Parasitic)	n - negl	0	<i>Glycine max</i> is a well-studied species, and we found no evidence that this plant is parasitic. <i>Glycine max</i> is a herbaceous plant in the family Fabaceae (Bailey and Bailey, 1976; NGRP, 2014), a family not known to contain parasitic plants (Heide-Jørgensen, 2008; Nickrent, 2009).
Impacts to Natural Systems			
Imp-N1 (Change ecosystem processes and parameters that affect other species)	n - low	0	In agricultural settings, soybeans increase the nitrogen and organic carbon content of the soil (Singh, 2010). However, we did not find any evidence of <i>G. max</i> occurring in natural ecosystems. Thus, we answered no, but used low uncertainty because <i>G. max</i> is a nitrogen-fixing plant.
Imp-N2 (Change community structure)	n - negl	0	We found no evidence that <i>G. max</i> grows in natural areas. We used negligible uncertainty for questions N2-N6 because <i>G. max</i> is not known to occur in natural ecosystems and it is well studied.

Question ID	Answer - Uncertainty	Score	Notes (and references)
Imp-N3 (Change community composition)	n - negl	0	We found no evidence that <i>G. max</i> grows in natural areas. "[Soybean] suffers heavily due to weed competition and losses due to weeds have been one of the major limiting factors in soybean production" (Singh, 2010).
Imp-N4 (Is it likely to affect federal Threatened and Endangered species)	n - negl	0	We found no evidence of <i>G. max</i> impacting threatened and endangered species.
Imp-N5 (Is it likely to affect any globally outstanding ecoregions)	n - negl	0	We found no evidence of <i>G. max</i> impacting globally outstanding ecoregions.
Imp-N6 (Weed status in natural systems)	a - negl	0	"The soybean plant has no weedy tendencies and is non-invasive in natural habitats in Canada. It does not grow in unmanaged habitats" (CFIA, 2012). Randall (2012) lists this species as a weed of the natural environment but the reference cited lists <i>G. max</i> as a pasture crop and does not include any information about <i>G. max</i> harming or having impacts in natural ecosystems (Werren, 2001). Because we did not find any evidence that <i>G. max</i> occurs in natural ecosystems, we answered "a" with negligible uncertainty. The alternate answers for the Monte Carlo simulation were both "b."
Impact to Anthropogenic Systems (cities, suburbs, roadways)			
Imp-A1 (Impacts human property, processes, civilization, or safety)	n - low	0	We found no evidence of this impact and used low uncertainty because this plant is well studied.
Imp-A2 (Changes or limits recreational use of an area)	n - low	0	We found no evidence of this impact and used low uncertainty because this plant is well studied.
Imp-A3 (Outcompetes, replaces, or otherwise affects desirable plants and vegetation)	n - low	0	"In managed ecosystems, soybean does not effectively compete with cultivated plants or primary colonizers" (CFIA, 2012).
Imp-A4 (Weed status in anthropogenic systems)	a - low	0	Listed as occurring in urban and industrial landscapes by Pysek et al. (2002) but no information is given about impacts. Thus, we answered "a" with low uncertainty. The 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)	n - low	0	In agricultural fields, unharvested soybean seed left in the field can grow into volunteer plants the following growing season (Fett, 1978; Gressel, 2005). "[I]f volunteer [soybean] plants develop in the rotational crop, losses attributable to interference are minimal" (Gressel, 2005). We found no evidence of these volunteer soybeans causing yield losses in production settings and soybean has been well studied. Thus, we answered no with low uncertainty.
Imp-P2 (Lowers commodity value)	n - high	0	"There is no information in the literature that describes any issue with volunteerism in soybeans...volunteerism of soybeans is not an economic problem" (Gressel, 2005). Volunteer soybeans serve as a reservoir for plant pathogens to survive between cropping seasons and increase inoculum of soil borne pathogens (Fett, 1978). This may cause growers to increase fungicide applications. However, because this is speculation, we answered no, but with high uncertainty.

Question ID	Answer - Uncertainty	Score	Notes (and references)
Imp-P3 (Is it likely to impact trade)	n - low	0	Soybeans can spread as a contaminant (Clement and Foster, 2000; NGRP, 2014), but we found no evidence that non-GE soybean seeds would impact trade activities. Thus, we answered no with low uncertainty.
Imp-P4 (Reduces the quality or availability of irrigation, or strongly competes with plants for water)	n - low	0	We found no evidence that volunteer soybeans reduce water available for irrigation to other crops, more so than other weeds. We answered no with low uncertainty because soybeans are well-studied.
Imp-P5 (Toxic to animals, including livestock/range animals and poultry)	n - high	0	The protein-rich seeds are used for animal feed and consumed by humans in a range of different cooked foods (Mabberley, 2008; Singh, 2010). Considered an excellent source of energy and protein when fed to cattle (Lalman et al., 2007). Not listed by Burrows and Tyrll (2001). Listed as toxic by Randall (2012). "Raw peanuts and other legumes contain a trypsin inhibitor or substance that inhibits or prevents the pancreas from producing trypsin, an enzyme essential for the absorption of protein by the intestine. Squirrels fed a steady diet of raw peanuts, soybeans, other legumes, and sweet potatoes could easily develop severe malnutrition" (Johnson, 2011). Eating raw soybeans can cause digestive problems and medical issues in humans (Robin, 2014). "Raw soybeans contain trypsin inhibitor. This is a major concern in monogastric animals, but not a factor in ruminants due to the rapid rate of protein degradation" (Willms, 2012). Because soybeans are commonly consumed by animals and humans with no adverse health effects, we answered no, but we used high uncertainty because soybeans can cause digestive problems in certain circumstances (e.g., when raw beans are consumed by monogastric animals).
Imp-P6 (Weed status in production systems)	c - mod	0.6	"Historically, soybeans are not considered a serious volunteer weed problem in corn because they are not very competitive and several herbicide options are available to control them in corn" (Staff, 2013). "[O]nly under certain environmental conditions [soybean] grows as a volunteer....If this should occur, volunteers do not compete well with the succeeding crop, and can easily be controlled mechanically or chemically." (CFIA, 2012). "Soybeans are not generally considered a serious volunteer weed problem as exemplified by the lack of published research" and volunteer soybeans are effectively controlled by herbicides and management practices used to control other weeds (Gressel, 2005). Listed as a casual escape occurring in agricultural habitats by Pysek et al. (2002), but this reference does not provide any information about impacts. Because volunteer soybeans are controlled in agricultural systems, we answered "c" with moderate uncertainty. The alternate answers for the Monte Carlo simulation were both "b."

Question ID	Answer - Uncertainty	Score	Notes (and references)
GEOGRAPHIC POTENTIAL			Because soybean does not exist outside of cultivation, most records for <i>G. max</i> in GBIF (2014) probably represent collections from irrigated cultivation. Consequently, we did not use GBIF records on <i>G. max</i> to evaluate geographic potential. Instead we answered the questions below using soybean's closest ancestor, <i>G. soja</i> . 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 <i>G. soja</i> occurs in this zone.
Geo-Z2 (Zone 2)	n - negl	N/A	We found no evidence that <i>G. soja</i> occurs in this zone.
Geo-Z3 (Zone 3)	n - negl	N/A	We found no evidence that <i>G. soja</i> occurs in this zone.
Geo-Z4 (Zone 4)	n - negl	N/A	We found no evidence that <i>G. soja</i> occurs in this zone.
Geo-Z5 (Zone 5)	n - low	N/A	We found no evidence that <i>G. soja</i> occurs in this zone.
Geo-Z6 (Zone 6)	y - negl	N/A	Japan.
Geo-Z7 (Zone 7)	y - negl	N/A	Japan.
Geo-Z8 (Zone 8)	y - negl	N/A	South Korea and Japan.
Geo-Z9 (Zone 9)	y - negl	N/A	South Korea and Japan.
Geo-Z10 (Zone 10)	y - negl	N/A	Japan.
Geo-Z11 (Zone 11)	y - low	N/A	Taiwan.
Geo-Z12 (Zone 12)	y - low	N/A	Taiwan.
Geo-Z13 (Zone 13)	y - mod	N/A	Taiwan.
Köppen -Geiger climate classes			
Geo-C1 (Tropical rainforest)	n - low	N/A	We found no evidence that <i>G. soja</i> occurs in this climate class.
Geo-C2 (Tropical savanna)	y - mod	N/A	Taiwan.
Geo-C3 (Steppe)	n - negl	N/A	We found no evidence that <i>G. soja</i> occurs in this climate class.
Geo-C4 (Desert)	n - negl	N/A	We found no evidence that <i>G. soja</i> occurs in this climate class.
Geo-C5 (Mediterranean)	n - negl	N/A	We found no evidence that <i>G. soja</i> occurs in this climate class.
Geo-C6 (Humid subtropical)	y - negl	N/A	Taiwan and Japan.
Geo-C7 (Marine west coast)	n - negl	N/A	We found no evidence that <i>G. soja</i> occurs in this climate class.
Geo-C8 (Humid cont. warm sum.)	y - negl	N/A	South Korea and Japan.
Geo-C9 (Humid cont. cool sum.)	y - low	N/A	Japan.
Geo-C10 (Subarctic)	n - negl	N/A	We found no evidence that <i>G. soja</i> occurs in this climate class.
Geo-C11 (Tundra)	n - negl	N/A	We found no evidence that <i>G. soja</i> occurs in this climate class.
Geo-C12 (Icecap)	n - negl	N/A	We found no evidence that <i>G. soja</i> 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 that <i>G. soja</i> occurs in this precipitation band.
Geo-R2 (10-20 inches; 25-51 cm)	n - negl	N/A	We found no evidence that <i>G. soja</i> occurs in this precipitation band.

Question ID	Answer - Uncertainty	Score	Notes (and references)
Geo-R3 (20-30 inches; 51-76 cm)	n - negl	N/A	We found no evidence that <i>G. soja</i> occurs in this precipitation band.
Geo-R4 (30-40 inches; 76-102 cm)	n - low	N/A	We found no evidence that <i>G. soja</i> occurs in this precipitation band.
Geo-R5 (40-50 inches; 102-127 cm)	y - negl	N/A	South Korea and Japan.
Geo-R6 (50-60 inches; 127-152 cm)	y - negl	N/A	South Korea and Japan.
Geo-R7 (60-70 inches; 152-178 cm)	y - negl	N/A	Taiwan.
Geo-R8 (70-80 inches; 178-203 cm)	y - negl	N/A	Japan.
Geo-R9 (80-90 inches; 203-229 cm)	y - negl	N/A	Taiwan and Japan.
Geo-R10 (90-100 inches; 229-254 cm)	y - negl	N/A	Taiwan and Japan.
Geo-R11 (100+ inches; 254+ cm)	y - negl	N/A	Taiwan and Japan.
ENTRY POTENTIAL			
Ent-1 (Plant already here)	y - negl	1	<i>Glycine max</i> is widely grown and is one of the most economically important crops in the United States (Bailey and Bailey, 1976).
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 some other pathway)		N/A	

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

Appendix B. Weed risk assessment for GE herbicide-resistant soybean, *Glycine max* (L.) Merr. (Fabaceae). As explained above under “Initiation and Scope,” unless we found evidence to the contrary, we assumed non-GE and GE herbicide-resistant soybean 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 soybean 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	This weed risk assessment is for soybean plants that have been genetically engineered to be resistant to the herbicides 2,4-D, glufosinate, and glyphosate (Dow AgroSciences, 2010; Dow AgroSciences, 2011; Johnson et al., 2012). Thus, we answered yes with negligible uncertainty.
IMPACT POTENTIAL			
Impact to Production Systems (agriculture, nurseries, forest plantations, orchards, etc.)			
Imp-P1 (Reduces crop/product yield)	y - high	0.4	"If [glyphosate-resistant volunteer soybean] populations are high and left uncontrolled, they may cause yield loss in corn" (Jhala et al., 2013). "Data on corn yield loss potential as a function of volunteer soybean density is not available" (Gunsolus, 2010). "Volunteer crop plants [in general] are considered to be weeds because they can reduce crop yield and quality and reduce harvesting efficiency" (York et al., 2005). Based on this evidence, we answered yes but with high uncertainty.
Imp-P2 (Lowers commodity value)	y - negl	0.2	Growers must apply alternate or additional non-glyphosate herbicides to achieve effective control of volunteer glyphosate-resistant soybean plants in glyphosate-resistant corn fields (Deneke, 2013; Jhala et al., 2013; Staff, 2013) and in glyphosate-resistant cotton fields (York et al., 2005). "[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" (Davis, 2009). Volunteer soybeans serve as a reservoir for plant pathogens to survive between cropping seasons and increase inoculum of soil borne pathogens (Fett, 1978). Thus, we answered yes, with low uncertainty.

Question ID	Answer - Uncertainty	Score	Notes (and references)
Imp-P3 (Is it likely to impact trade)	y - negl	0.2	Honey exports from Mexico can be rejected by the European Union (EU) if contaminated with pollen from GE soybeans (Villanueva-Gutierrez et al., 2014). Soybean seed from the United States was historically banned for import into the EU due to concerns that GE soybean seed would not be readily detected if it became mixed with other soybeans (Boerma and Specht, 2004). The EU has since lifted its ban on U.S. soybeans, but "[t]he EU's cautious approach to biotech crops remains in place with complex regulations and an onerous approval process. In 2009, the EU's inability to approve a biotech soybean variety led to a halt in U.S. shipments. Labeling requirements for products originating from biotech soybeans have further eroded demand for U.S. sales" (FAS, 2013). Soybean seeds can contaminate agricultural products (Clement and Foster, 2000). While regulation of GE crops may change in the future, we are answering this question based on current market conditions and regulations. Based on the evidence we found and current global regulations of GE crops, we answered yes with negligible uncertainty.
Imp-P5 (Toxic to animals, including livestock/range animals and poultry)	n - high	0	The FDA evaluated the toxicity trials conducted by Dow AgroSciences and concluded soybean plants genetically-engineered to be resistant to 2,4-D were not materially different in composition from non-GE soybeans and that these GE soybeans did not raise any safety or regulatory issues (FDA, 2011). For this reason our answer to this question for GE herbicide-resistant soybean is the same as our answer for non-GE soybean. Because soybeans are commonly consumed by animals and humans with no adverse health effects, we answered no, but we used high uncertainty because soybeans can cause digestive problems in certain circumstances (e.g., when raw beans are consumed by monogastric animals).
Imp-P6 (Weed status in production systems)	c - low	0.6	In 2003, there were "substantial populations of volunteer [glyphosate-resistant] soybeans in some corn fields" in the United States (Staff, 2013). Herbicide trials have been conducted for control of glyphosate-resistant volunteer soybeans growing in glyphosate-resistant cotton fields (York et al., 2005), and glyphosate-resistant volunteer soybeans have been a problem and targeted for control in corn (Deneke, 2013; Jhala et al., 2013). "Volunteer [glyphosate-resistant] soybean in cotton is normally not a major concern, but in years following hurricanes that damage the preceding soybean crop, volunteer plants from unharvested soybean seed can be a problem" (York et al., 2005). Because other herbicide-resistant soybeans have impacts in production settings, we answered "c" with low uncertainty. The alternate answered for the Monte Carlo simulation were both "b."

Question ID	Answer - Uncertainty	Score	Notes (and references)
ENTRY POTENTIAL			
Ent-1 (Plant already here)	n - negl	0	Soybean is widely grown in the United States and is one of the country's most economically important crops (Bailey and Bailey, 1976; Boerma and Specht, 2004). Soybean cultivars that are genetically engineered to be resistant to the herbicides glyphosate and glufosinate have been rapidly adopted by growers in the United States (Boerma and Specht, 2004); herbicide-resistant soybeans made up 94 percent of the soybean acreage planted in the United States in 2014 (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 two new biotypes of soybeans (DAS-68416-4 and DAS-44406-6) that have been genetically engineered for resistance to the herbicides 2,4-D, glufosinate, and glyphosate (Dow AgroSciences, 2010; Dow AgroSciences, 2011). Thus, the entry of these biotypes is imminent and we do not need to further evaluate entry potential.