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Weed Risk Assessments for non-herbicide resistant and herbicide resistant types of *Agrostis stolonifera* L.

Agrostis stolonifera **prepared by J. M. DiTomaso, University of California – Davis**

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Executive Summary

We assessed the weed risk potentials of herbicide resistant and non-herbicide resistant types of *Agrostis stolonifera* L., creeping bentgrass, using our Plant Protection and Quarantine (PPQ) weed risk assessment guidelines. These guidelines are consistent with the general guidance provided by international and North American standards for risk assessment. We evaluated each species using a model tested with 204 plants with known weed/invasive behavior in the United States. The risk ratings presented in the table below are based on the species' ability to establish, spread, and cause impact. They do not consider the potential geographic area of the United States suitable for species establishment.

We found that both resistant and non-resistant types of creeping bentgrass had High weed risk potentials. High risk species have risk scores and traits consistent with highly invasive and weedy U.S. species. Our uncertainty about the basic risk scores was relatively small because of the wealth of information about the species and its behavior and performance in the United States.

Scores for the two resistance types were quantitatively but not qualitatively different from each other, which is perhaps not surprising because this model was created to predict the overall invasive potential of a species, and not to evaluate differences in invasiveness between plants with different genotypes.

Risk managers are encouraged to review the potential and realized geographic area, as well as other information in the assessments, when evaluating risk management options.

Type	**Weed Risk Potential Rating**	**Establishment/ Spread Potential[a]**	**Impact Potential**
Non-Herbicide resistant	High	23 (0.09)	3.7 (0.05)
Herbicide resistant	High	24 (0.09)	3.7 (0.05)

[a] Score (Uncertainty). The uncertainty estimate is a proportion of total potential uncertainty (mean for all evaluated species = 0.17).

Table of Contents

1. Introduction to the PPQ Weed Risk Assessment Process

1.1. Background

In this document, we assess the weed risk potential of several plant species using Plant Protection and Quarantine's (PPQ) weed risk assessment guidelines (PPQ, 2009). The weed risk assessment (WRA) process and the predictive model utilized are consistent with the general guidance provided by international and North American standards for risk assessment (IPPC, 2009: ISPM Nos. 2 & 11; NAPPO, 2008: RSPM No. 32). The weed risk assessments below contain information relevant for the initiation, pest categorization, and risk assessment phases. These phases correspond to Stage 1 (initiation) and Stage 2 (pest risk assessment) of pest risk analysis (IPPC, 2009: ISPM No. 2).

PPQ WRA is consistent with international guidelines

A weed risk assessment can be initiated for any number of reasons, including, but not limited to, evaluation for listing or delisting Federal Noxious Weeds or plants for propagation which are designated as "Not Allowed Pending Pest Risk Assessment" (NAPPRA) (PPQ, 2009). We note the reason for initiation, along with other background information, in each assessment.

We combine pest categorization and pest risk assessment

One of the phases of pest risk analysis is pest categorization, in which the pest is evaluated to determine whether it has the characteristics of a quarantine pest or a regulated non-quarantine pest (IPPC, 2009: ISPM No. 2). The intent of this phase is to identify (i.e., screen out) pests that clearly do not meet these definitions before subjecting them to a potentially lengthy risk assessment process. However, because some plants that do not have evidence of spread or impact elsewhere later become weeds (IPPC, 2009: ISPM No. 2; Whitney and Gabler, 2008), PPQ subjects most plants to the full weed risk assessment process to evaluate their pest potential based on their inherent biological traits (e.g., Mack, 1996; Reichard, 2001). Essentially, we combine the pest categorization and risk assessment phases, and use the risk assessment as a screening tool to categorize the potential risk and pest status of the plant.

1.2. Terminology

Confounded weed terminology

Terminology in the weed/invasive plant literature is confounded, as words such as "weed" and "invasive" have variable and subjective meanings (Richardson et al., 2000). Development and validation of the PPQ model required some flexibility in terminology, particularly at different phases of the work. As with other studies that have developed and/or tested WRA systems (e.g., Gordon et al., 2008; Pheloung et al., 1999), we relied on information available in the literature to identify plants belonging to three categories of invasiveness: non-invaders, minor-invaders, and major-invaders. In this usage, invader broadly refers to a plant's overall ability to spread and cause negative impacts, and reflects two components of risk (IPPC, 2009: ISPM No. 11).

In the PPQ WRA system, we evaluate the establishment/spread potential and impact potential of a species as two separate risk elements. Under establishment/spread we adopt a stricter definition of invasive that refers to a species' capacity to establish and spread throughout a landscape (*sensu* Richardson et al., 2000). However, at the end of the PPQ WRA process, we return to the broad usage of the term invader because we relate a species' risk scores back to the dataset that was used to develop and test the WRA model. If introduced into the United States, Low risk plants are likely to become non-invaders, while High risk plants are likely to become major invaders.

1.3. Risk assessment overview

Model based on U.S. plants with known behavior

We developed and validated the WRA process (Stage 2) using 204 plants with known weed/invasive behavior in the United States (non-invaders, minor-invaders, and major-invaders) (*manuscript in review*). The process consists of a weed risk model as well as a secondary screening tool developed to further evaluate plants with intermediate risk scores.

WRA process does not make policy recommendations

We do not use the PPQ WRA process to make policy recommendations. Instead, we categorize weed risk and relate a species' risk scores to the reference dataset of species with known invasiveness in the United States. This process results in one of three possible conclusions: "Low risk," "Evaluate further," and "High risk." While these conclusions are not official policy recommendations, the analytical and statistical methodologies behind them support management decisions of allowing entry for Low risk species, denying entry for High risk species, and evaluating further other species as appropriate. This yields results similar to outcomes reached using other weed risk assessment systems (e.g., Pheloung et al., 1999; Reichard and Hamilton, 1997).

Agency does risk management separately

PPQ program managers use weed risk assessments to evaluate what Federal action may be appropriate. If regulatory action is prudent, program managers evaluate which risk mitigation options would reduce risk to an acceptable level. This risk management process corresponds to Stage 3 of pest risk analysis (IPPC, 2009). For cultivated plants not yet present in the United States, most management decisions will be to either allow or exclude entry.

1.4. Authority

PPQ regulates plants 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 Federal noxious weed is "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). Plants meeting that definition are generally prohibited or restricted from entering the United States or moving through it (interstate). For transparency with stakeholders, these species are listed under the Federal Noxious Weed regulations (7 CFR § 360, 2010). Except for plant species unlikely to contaminate import or export pathways, most Federal noxious weeds are co-listed as noxious weed seeds (see 7 CFR § 361, 2010).

2. Guide for Interpretation of WRA Results

In this document, we summarize the results for several weed risk assessments. For a description of the WRA process and model, or a guide on answering questions used in the assessment, see the PPQ WRA Guidelines (PPQ, 2009).

Establishment/spread and Impact risk elements

Below, we present risk scores for the establishment/spread and impact risk elements, along with their mean uncertainty. Risk scores can range from -25 to 32 and 1.0 to 5.1, respectively, with greater scores indicating greater risk. Descriptions with each risk element highlight the risk factors that contributed to that score. We used the scores from these two risk elements to characterize the overall risk potential of the species and estimate the likelihood that it will be a non-invader, minor-invader, or major-invader (see below).

Geographic and entry potential are separate

Although we do not use the geographic and entry potentials of a species to estimate the overall invasive potential of a plant, these elements are none-the-less important components of risk. We report these elements separately so that regional and national managers can make appropriate decisions for their jurisdictions. Under geographic potential, we report the percent of the United States suitable for species establishment based on three climate variables: USDA cold plant hardiness zones, Köppen-Geiger climate classes, and ten-inch precipitation bands. Under entry potential we evaluate the likelihood of species entry into the United States. All four scores can range from 0 to 1, with higher scores indicating higher risk.

Uncertainty

For each of the risk scores described above, we report an index of uncertainty that describes the overall level of uncertainty associated with that risk element. The index ranges from zero to one, where a one corresponds to maximum uncertainty (i.e., all questions answered as unknown). The index considers the uncertainty rating given by the analyst to each question (negligible, low, moderate, high, or maximum) and the relative weight of each question in the risk element.

WRA model In the next section of each assessment, we present the results from the WRA model and secondary screening (2° screening). The core of the WRA model is a logistic regression model (Appendix A) that uses the scores from the establishment/spread and impact risk elements to determine the probabilities that a species will be a major-, minor-, and non-invader (*sensu lato*). Because most management decisions for plants will be to either allow or exclude entry, we used cutoff scores determined by Receiver Operating Characteristic (ROC) curve analysis (Appendix B) to categorize the overall risk of plant introduction (i.e., "low risk" or "high risk") and facilitate management decisions. ROC curve analysis is an analytical tool used in decision making that maximizes the predictive ability of a model while minimizing false-positive and false-negative errors (Caley and Kuhnert, 2006; Metz, 1978).

Secondary screening of species classified as "evaluate further" Species classified as "evaluate further" are species with intermediate risk scores, and are subjected to a secondary screening tool (Appendix C). With this tool, we examine specific traits that by our analysis were highly associated with plant invasive status in the United States. This approach is designed to help resolve the risk potential of the species. However, even after secondary screening, some species may remain in the "evaluate further" category.

In the discussion section of each assessment below, we briefly review the available evidence and report our final conclusion. We also introduce additional information that may be relevant to managers in decision-making.

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4. Weed Risk Assessments

4.1. Non-Herbicide Resistant *Agrostis stolonifera* L., Creeping Bentgrass

Background Information

Family: Poaceae

Initiation: On March 11, 2011, APHIS BRS requested that the Animal and Plant Health Inspection Service assess the weed risk of creeping bentgrass for both non-resistant and herbicide-resistant types (Huberty, 2011). These assessments should help BRS assess the risk of genetically modified creeping bentgrass.

Foreign distribution: Creeping bentgrass is native to Portugal, Africa, temperate and tropical Asia, Europe, and Greenland (NGRP, 2009). Some of the areas to which it has been introduced include Canada, (Darbyshire, 2003), Australia (Weber, 2003), Marion Island (Gremmen et al., 1998), New Zealand (Pheloung et al., 1999), Costa Rica, Tanzania, and Iceland (GBIF, 2011).

U.S. distribution & status: Creeping bentgrass is present in all states of the United States. According to some, it was probably introduced into North America prior to 1750 (Hannaway and Larson, 2004). There is controversy regarding its nativity within the United States (Nature Serve, 2011). More current data has revealed that creeping bentgrass is a highly variable complex of polyploid biotypes mostly exotic to the United States (DiTomaso and Healy, 2007). Some Northern, mesic populations are considered native (Harvey, 2007), and turfgrass types may also be native (Beard, 2011). Creeping bentgrass is a cool-season turfgrass, mainly used on golf courses and other playing fields (Mallory-Smith and Zapiola, 2008). It is also used for erosion control, cover, and food for wildlife and forage (Hannaway and Larson, 2004). When creeping bentgrass invades a lawn, non-selective contact herbicides like glyphosate can be used to remove it (Bigelow and Reicher, 2011).

WRA area: The weed risk assessment area under consideration is the United States and its territories and possessions.

4.1.1. Analysis of Non-Herbicide Resistant Creeping Bentgrass

Establishment/Spread Potential

Having been in the United States for over 250 years and occurring in every state, creeping bentgrass' distribution appears to correspond to its abiotic limits (Nature Serve, 2011) (Fig. 1). Characteristics which contributed to its relatively high establishment and spread risk score include: withstands immersion for extended periods; adapts to a wide range of soil and climate conditions; completes its life cycle in less than a year; disperses by wind, water, and animals; forms a seed bank; and has stolons that withstand mutilation. We had

low uncertainty with this risk element.
Risk score = 23 Mean uncertainty = 0.09

Impact Potential The thick mats formed at times by creeping bentgrass changes community composition and to a lesser extent changes the structure of ecosystems (Nature Serve, 200911). After invasion by creeping bentgrass on Marion Island, the native herb- and moss-dominated vegetation of the drainage lines changed into a dense grassland (Gremmen et al., 1998). A restoration ecologist communicated the need to remove creeping bentgrass, an exotic, from meadows using Round-Up (Tangren, 2004). National Park personnel have expressed concern about the establishment of creeping bentgrass within park boundaries (Nature Serve, 2011; USGS, 2011). It is reported as a weed in agricultural systems in Australia (Randall, 2007) and Germany (Holm et al., 1979), but with no further information about the level of damages. In the United States, the primary agriculture-related impacts of creeping bentgrass are likely to occur in grass seed crops, with some marginal impacts in fruit and nut orchards (Banks et al., 2004). We had low uncertainty for this risk element.
Risk score = 3.7 Mean uncertainty = 0.05

Geographic Potential Creeping bentgrass occurs in all states of the United States (Kartesz, 2010).

Entry Potential Because, creeping bentgrass is already present in United States (Kartesz, 2010), we did not need to evaluate its entry potential.

Figure 1. Expected distribution of *Agrostis stolonifera* in the entire United States. Alaska, and Hawaii are shown on the left, Puerto Rico on the right; none are drawn to scale.

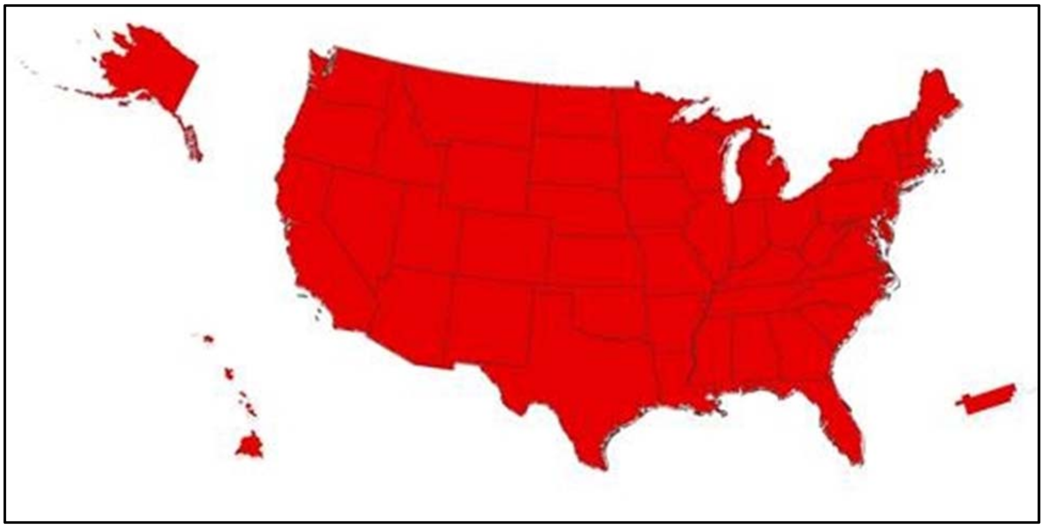
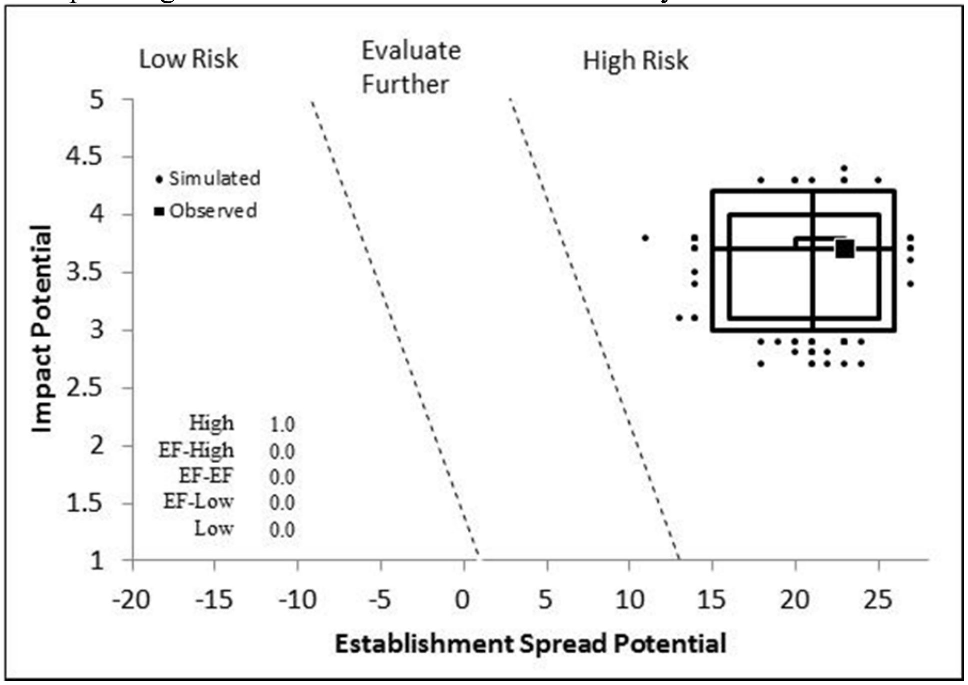
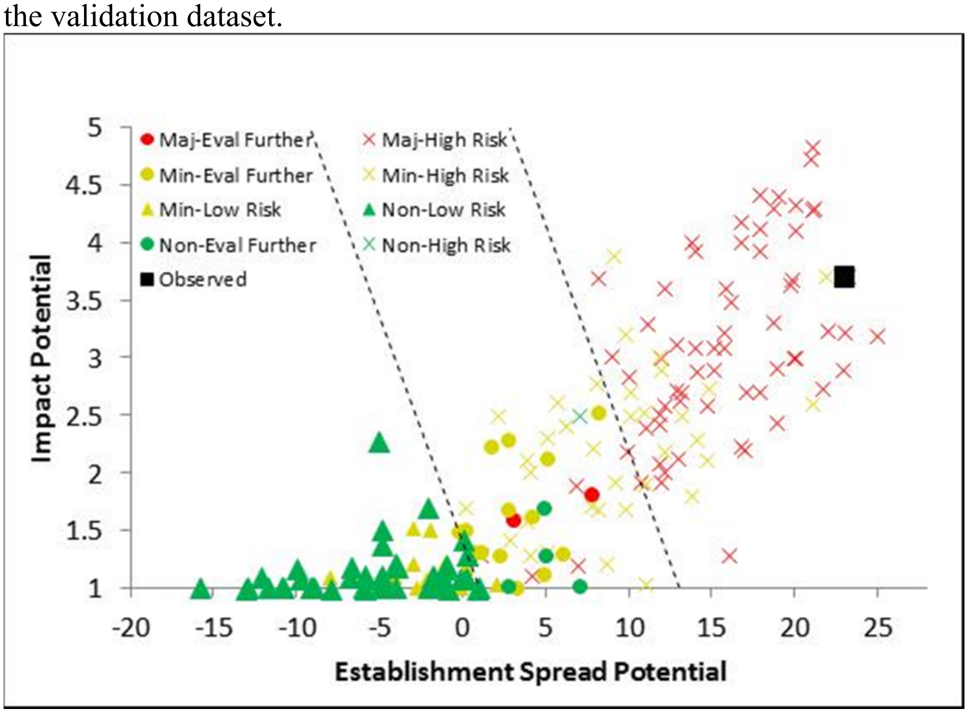


Figure 2. Risk score of non-herbicide resistant creeping bentgrass, incorporating Monte Carlo simulation of uncertainty.[a]



[a] Vertical and horizontal lines indicates means of the simulated outcomes. The first box contains 50 percent of the outcomes, the second 95 percent, and the third 99 percent.

Figure 3. Risk score of non-herbicide resistant creeping bentgrass relative to the validation dataset.



4.1.2. Results & Conclusion

Model Probabilities: P(Major Invader) = 0.971
P(Minor Invader) = 0.028
P(Non-Invader) = 0.001
Risk Result = High Risk
Secondary Screening = Not Applicable

The result of the weed risk assessment for non-herbicide resistant creeping bentgrass is **High Risk**.

4.1.3. Discussion

Creeping bentgrass is a high risk species (Figs. 2 and 3). When compared with other United States major-invaders examined in the validation study (Koop et al., 2012), it amassed one of the highest observed risk scores based upon its ability to establish and spread. Its impact score was about average, compared to other high risk species (Figs. 2 and 3). It has rapidly spread on Marion Island, displacing native vegetation and dominating a range of habitats (Gremmen et al., 1998). In Hawaii, it was assessed as High Risk (PIER, 2011). In California it is described as having limited impact; that is, it is invasive (spreading) but is considered a minor invader on a statewide level (Cal-IPC, 2006). It has established in national parks as an exotic (undesirable) plant species (USGS, 2011). New Zealand risk assessors consider creeping bentgrass invasive (establishes and spreads), yet useful (Pheloung et al., 1999). Creeping bentgrass is a serious weed in meadows where Round-Up is used to restore infested areas (Tangren, 2004). When creeping bentgrass invades a lawn, non-selective contact herbicides like glyphosate can be used to remove it (Bigelow and Reicher, 2011). It is reported as a weed in agricultural systems in Australia (Randall, 2007) and Germany (Holm et al., 1979). In the United States it could affect yields and quality of grass seed crops, but otherwise might only be a sanitary issue (i.e., affects costs but not productivity) as an orchard floor weed in some fruit and nut crops (Banks et al., 2004).

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4.2. Herbicide Resistant *Agrostis stolonifera* L., Creeping Bentgrass

Background Information

Note: nearly all of the information used in the analysis of both types of creeping bentgrass is exactly the same, regardless of type.

Family: Poaceae

Foreign distribution: Creeping bentgrass is native to Portugal, Africa, temperate and tropical Asia, Europe, and Greenland (NGRP, 2009). Some of the areas to which it has been introduced include Canada, (Darbyshire, 2003), Australia (Weber, 2003), Marion Island (Gremmen et al., 1998), New Zealand (Pheloung et al., 1999), Costa Rica, Tanzania, and Iceland (GBIF, 2011).

U.S. distribution & status: Non-resistant creeping bentgrass is present in all states of the United States. It is not anticipated that the distribution for resistant creeping bentgrass will be significantly different than that for non-resistant creeping bentgrass (4.1).

WRA area: The weed risk assessment area considered here is the United States and its territories and possessions.

4.2.1. Analysis of Herbicide Resistant Creeping Bentgrass

Establishment/Spread Potential

Having been in the United States for over 250 years and occurring in every state, creeping bentgrass' distribution appears to correspond to its abiotic limits (Nature Serve, 2011) (Fig. 1). Characteristics which contributed to a relatively high establishment and spread risk score included the same factors as for non-resistant creeping bentgrass (4.1.1): withstands immersion for extended periods; adapts to a wide range of soil and climate conditions; completes its life cycle in less than a year; disperses by wind, water, and animals; forms a seed bank; and has stolons that withstand mutilation. We have no reason to expect these factors would be significantly different (greater or lesser) for the resistant type.

Research has demonstrated that the resistance gene can escape into grass populations in the field. If trait persistence depends upon continued glyphosate application, that may not create much environmental risk (Zapiola et al., 2008). Despite that, the trait so far seems to be resilient in the environment (e.g., Bollman et al., 2012), perhaps because of the ability of the species to reproduce vegetatively.

Risk score = 24 Mean uncertainty = 0.09

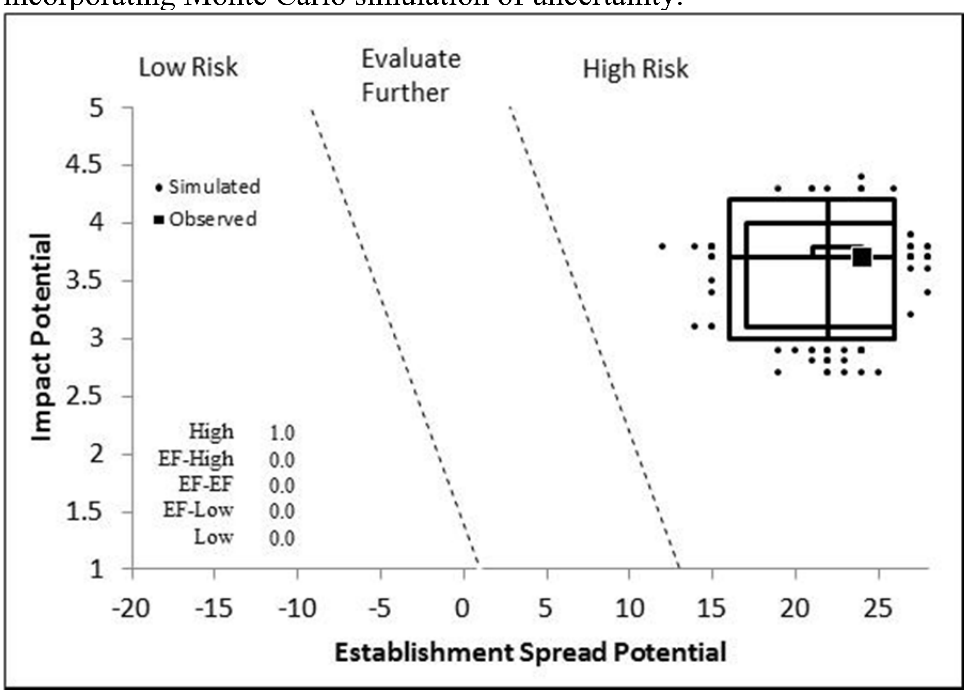
Impact Potential Reasonably, the impact potential of herbicide resistant creeping bentgrass should be a little greater than that for the non-herbicide resistant type, because control may be more difficult, requiring different but still common herbicides for control (e.g., Bollman et al., 2012). Based on our assessment questions, however, the resistant type had the same risk score as the non-resistant type.

Risk score = 3.7 Mean uncertainty = 0.05

Geographic Potential We have no reason to believe that the geographic potential of herbicide resistant creeping bentgrass would be significantly different than that for non-resistant creeping bentgrass (Fig. 1), which is nationwide.

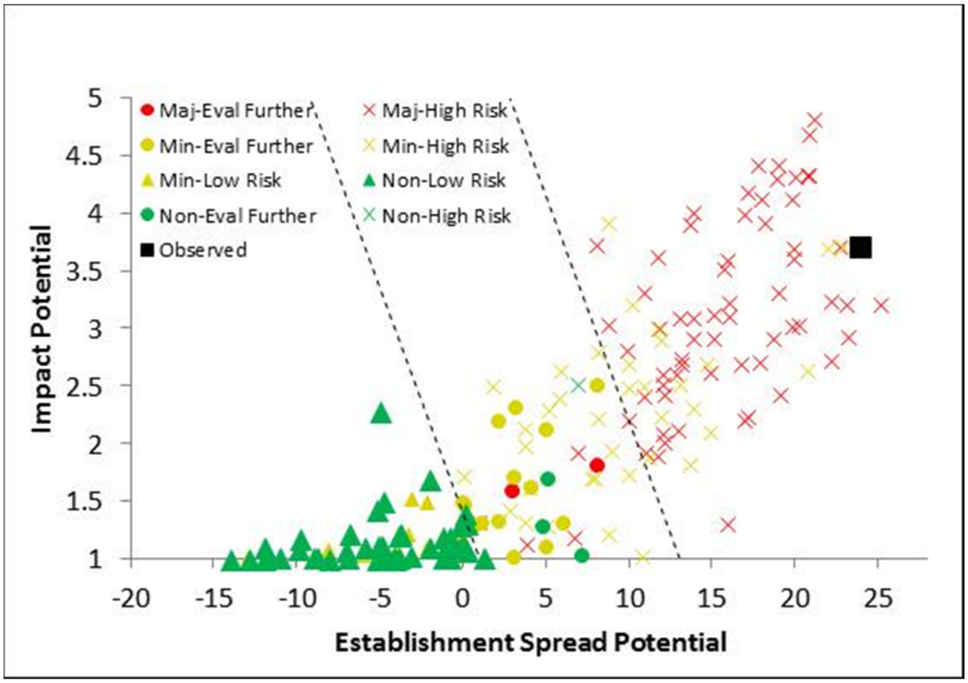
Entry Potential If approved for release, introduction of herbicide resistant creeping bentgrass is certain.

Figure 4. Risk score of herbicide resistant creeping bentgrass, incorporating Monte Carlo simulation of uncertainty.[a]



[a] Vertical and horizontal lines indicates means of the simulated outcomes. The first box contains 50 percent of the outcomes, the second 95 percent, and the third 99 percent.

Figure 5. Risk score of herbicide resistant creeping bentgrass relative to the validation dataset.



4.2.2. Results & Conclusion

Model Probabilities: P(Major Invader) = 0.977
P(Minor Invader) = 0.022
P(Non-Invader) = 0.001

Risk Result = High Risk

Secondary Screening = Not Applicable

The result of the weed risk assessment for herbicide resistant creeping bentgrass is **High Risk**.

4.2.3. Discussion

Herbicide resistant creeping bentgrass was High risk (Figs. 4 and 5). The only change in this analysis from above (non-herbicide resistant type) was answering "Yes" to the following question: "Is resistant to some herbicides or has potential to acquire herbicide resistance?" which increased the risk score by 1 point. While quantitatively different (Figs. 2 and 4), the difference is not qualitatively different.

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5. Appendices

Appendix A. Logistic regression model formulas

Below are the formulas for the logistic regression model of the probabilities of being a major-, minor-, and non-invader. E/S and Imp refer to the risk scores from the Establishment/Spread and Impact risk elements. All three probabilities sum to 1 for each plant.

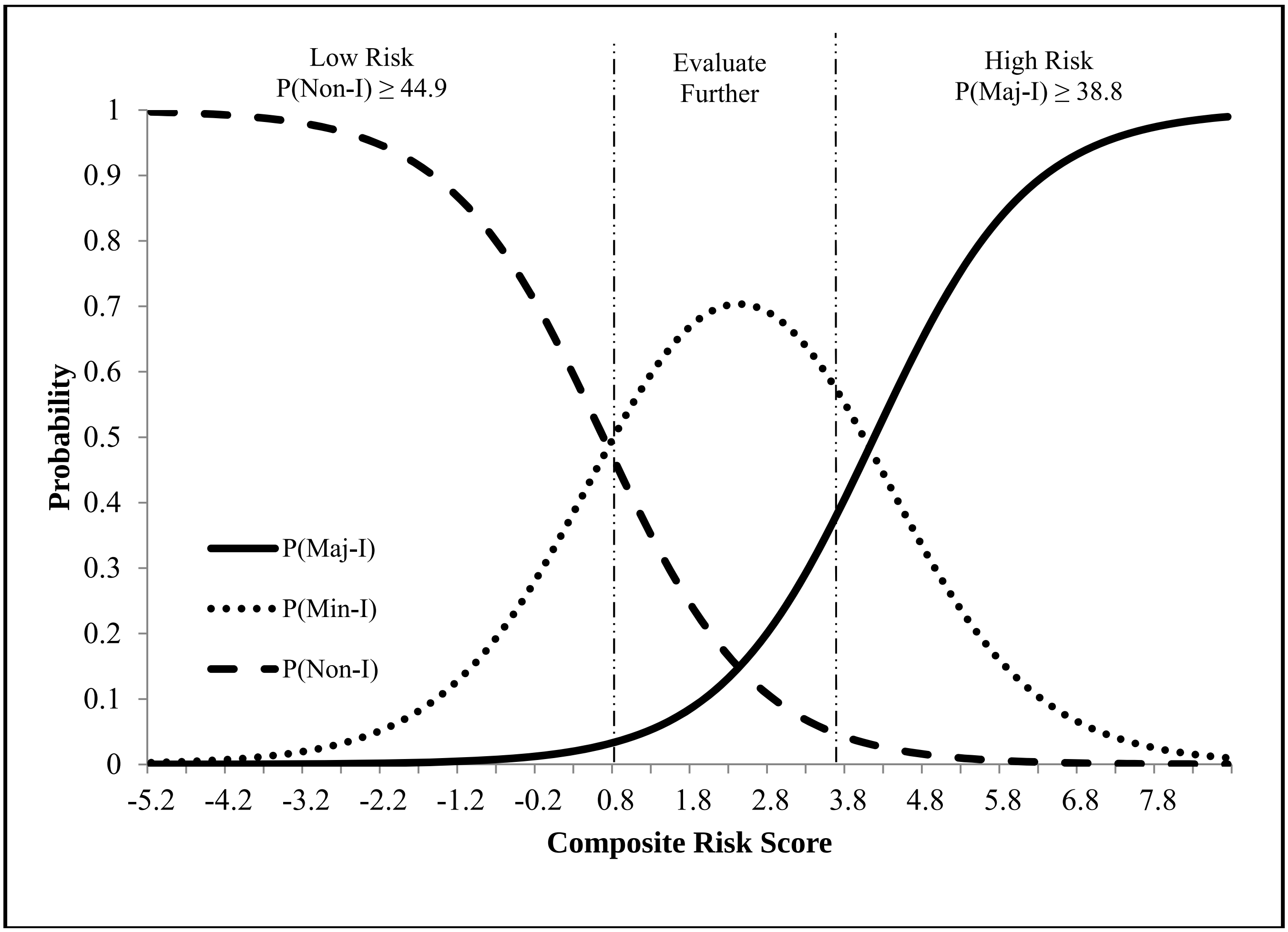
$$P(Maj - I) = \frac{1}{1 + e^{(4.1348 - (0.2356 \times E/S) - (0.6019 \times Imp))}}$$

$$P(Min - I) = \frac{1}{1 + e^{(0.6366 - (0.2356 \times E/S) - (0.6019 \times Imp))}} - P(Maj - I)$$

$$P(Non - I) = 1 - \left(\frac{1}{1 + e^{(0.6366 - (0.2356 \times E/S) - (0.6019 \times Imp))}}\right)$$

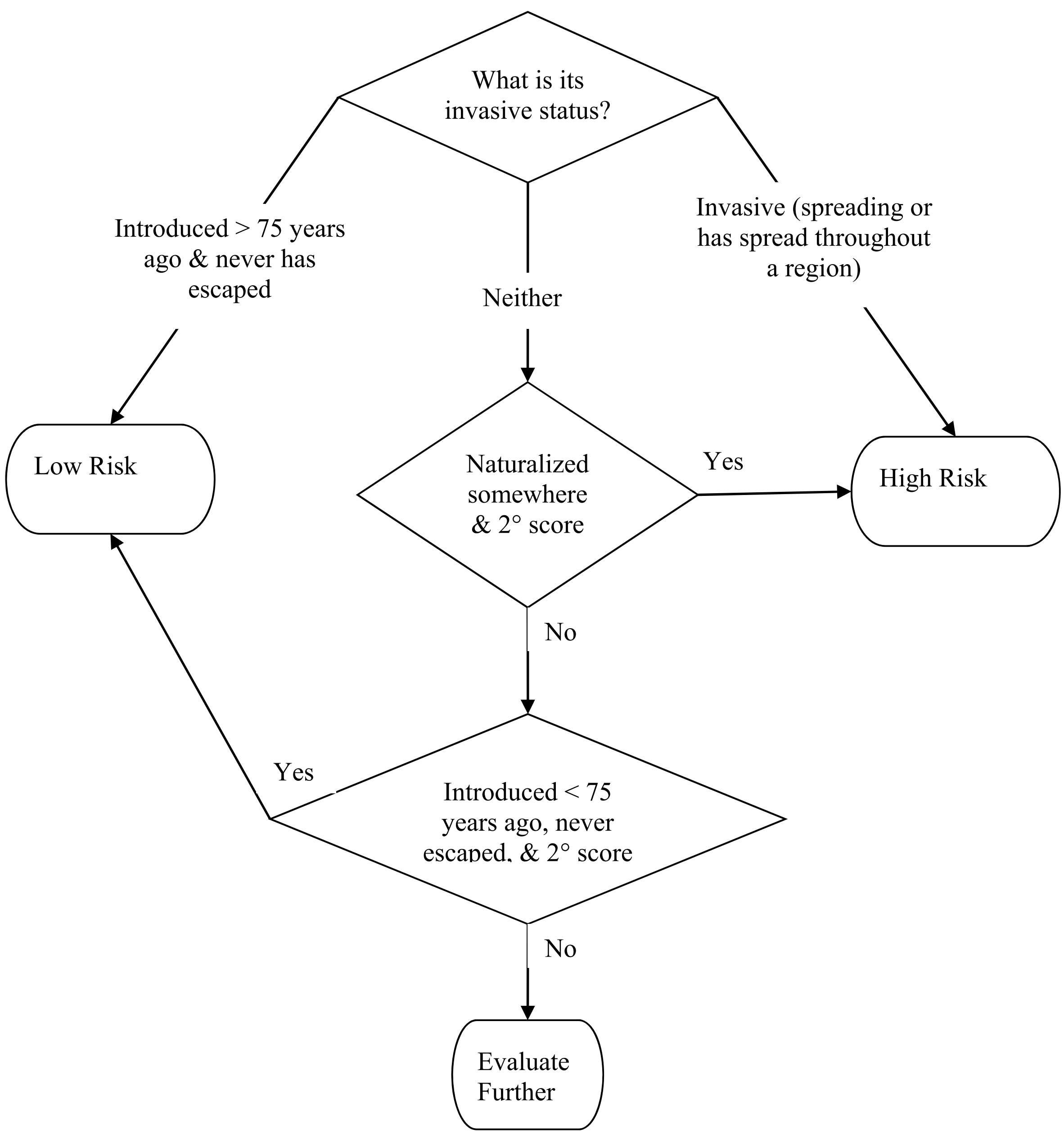
Appendix B. Model cut-off scores

In the diagram below we present the cut-off scores for the model probabilities for non-, minor-, and major-invaders. Composite Risk Score refers to a linear combination of the risk scores for the establishment/spread and impact risk elements. It is used in determining the probabilities and is calculated as (0.2356 × E/S) + (0.6019 × Imp). The cutoff scores below were determined by Receiver Operating Characteristic (ROC) curve analysis. This analysis maximizes the probabilities of accurately identifying non- and major-invaders, while minimizing errors. In the analysis, we assumed that the cost of a false-positive and false-negative error were equal.



Appendix C. Secondary screening system.

This system uses key questions that were strongly associated with invasive status in the United States. The first is question E/S-1 from the WRA model, and refers to the species invasive status anywhere in the world, including in the United States if recently established. The first part of the questions in the next two diamonds represents choices from E/S-1. The secondary score is the sum of the scores for six questions from the WRA model: 1) prolific reproduction; 2) minimum generation time; 3) shade adapted; 4) commodity contaminant; 5) number of natural dispersal vectors; and 6) forms dense thickets.



Appendix D. Risk score reference dataset.

Risk score distribution for the 204 species used to develop (N=102) and test (N=102) the PPQ WRA model. Marker color corresponds to the a priori classification for a species (major-, minor-, and non-invader). Marker type (triangle, circle, and x) corresponds to the conclusion following use of the model and secondary screening, if applicable.