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Evaluating Annual Bluegrass Herbicide Resistance Evolution in Golf Course Fairways

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Annual bluegrass is one of the most diverse plant species in the world and is the most problematic winter annual weed in commercial turfgrass. Continuous application of the same herbicide mechanism of action for annual bluegrass control on golf courses has increased herbicide-resistant populations. The purpose of this research was to simulate six herbicide-use strategies to evaluate the risk of annual bluegrass resistance evolution to glyphosate. In a worst-case scenario of yearly glyphosate applications at dormancy, resistance evolved within 10 yr and was predicted to evolve in > 90% of populations by yr 20. When glyphosate was rotated on alternate years with a unique mechanism of action, resistance was delayed for 12 to 15 yr. Season-long control of annual bluegrass often requires multiple herbicide applications. Therefore, additional strategies were simulated in which glyphosate was applied at dormancy with combinations of PRE and/or POST herbicides at various timings. Resistance was most effectively delayed with a PRE application in late summer, a POST application in fall, and alternating glyphosate with a different POST option at dormancy. This delayed resistance by 25 yr and a 35% risk was predicted after 50 yr. Strategies utilizing three annual herbicide applications with unique mechanisms of action were more effective for controlling population growth compared to other strategies. Resistance was predicted to evolve within 35 yr for each of the strategies simulated. However, these results indicate annual bluegrass herbicide resistance can be managed by using an integrated herbicide program, rotating unique mechanisms of action as frequently as possible.

Nomenclature: Glyphosate; Annual bluegrass, *Poa annua* L.
Key words: Golf course, herbicide resistance, simulation modeling, turfgrass.

Poa annua es una de las especies de plantas más diversas del mundo y es la maleza de invierno más problemática en los céspedes comerciales. La aplicación continua del mismo mecanismo de acción de herbicidas para el control de *P. annua* en campos de golf ha incrementado las poblaciones resistentes a herbicidas. El propósito de esta investigación fue simular seis estrategias de uso de herbicidas para evaluar el riesgo de evolución de resistencia a glyphosate para *P. annua*. En el peor de los escenarios, usando aplicaciones de glyphosate anualmente durante el período de latencia del césped, la resistencia evolucionó en 10 años y se predijo que se daría en > 90% de las poblaciones dentro de 20 años. Cuando glyphosate se rotó en años alternos con un mecanismo de acción único, la resistencia se retrasó 12 a 15 años. El control de *P. annua* durante toda la temporada de crecimiento frecuentemente requirió múltiples aplicaciones de herbicidas. Así pues, se simularon estrategias adicionales en las cuales glyphosate fue aplicado durante la latencia con combinaciones de herbicidas PRE y/o POST en varios momentos. La resistencia fue retrasada en forma más efectiva con aplicaciones PRE tarde en el verano, una aplicación POST en el otoño, y alternando glyphosate con una opción POST diferente durante la latencia. Estas prácticas retrasaron la resistencia en 25 años y se predijo un 35% de riesgo después de 50 años. Estrategias utilizando tres aplicaciones de herbicidas anuales con mecanismos de acción únicos fueron más efectivas para el control del crecimiento de la población al compararse con otras estrategias. La evolución de resistencia se predijo dentro de 35 años para cada una de las estrategias simuladas. Sin embargo, estos resultados indican que *P. annua* con resistencia a herbicidas puede ser manejada al usar un programa integrado de herbicidas, rotando con mecanismos de acción únicos tan frecuentemente como sea posible.

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Annual bluegrass is one of the most pervasive, adaptable, and diverse plant species in the world. This is illustrated by its widespread distribution across the planet; it is one of the few species of nonnative higher plants to colonize Antarctica (Molina-Montenegro et al. 2012), and it also occurs in subtropical climates of Florida and at latitudes near the equator at higher elevations (Beard et al.

1978; Hemp 2008). Annual bluegrass is also the most problematic weed species invading managed turfgrass stands, requiring intensive herbicide programs for adequate control.

Many turfgrass managers apply a single herbicide mechanism of action at the same general time each year to control annual bluegrass. This is a result of economic considerations, past success with one product or program, more limited options for nondormant warm-season turfgrasses (e.g., use of sulfonylureas in warm climates), or ease of use with other maintenance practices (e.g., tank mixing postemergence herbicides during spring preemergence applications). Repeated herbicide applications of the same mechanism creates a shift in the genetic composition of weed populations as intense selection removes susceptible individuals and increases the frequency of resistant individuals over time (Jasieniuk et al. 1996). This has recently been observed on golf courses in the southeastern United States where 7 to 10 yr of continuous application of the same herbicide has resulted in herbicide-resistant annual bluegrass populations (McCarty 2011).

Resistance has been confirmed to nine mechanisms of action worldwide, including photosystem II inhibitors (e.g., simazine), photosystem I inhibitors (e.g., diquat), very-long-chain fatty-acid inhibitors (e.g., ethofumesate), and mitosis inhibitors (e.g., prodiamine) (Heap 2014; Isgrigg et al. 2001; Kelly et al. 1999). In addition, annual bluegrass populations resistant to glyphosate (inhibition of 5-enolpyruvyl shikimate-3-phosphate synthase) and acetolactate synthase (ALS) inhibitors (e.g., trifloxysulfuron, foramsulfuron) have been identified (Binkholder et al. 2011; Brosnan et al. 2012b; Cross et al. 2013; McElroy et al. 2013). This leaves only three mechanisms of action currently labeled for annual bluegrass control in commercial turfgrass in which resistance has not been reported (McElroy et al. 2013).

Selection pressure exerted by the nonselective herbicide glyphosate is lower in turfgrass systems compared to other herbicides because its use is limited to warm-season (C_4) grasses during complete dormancy. In most years, winter turf dormancy in the southeastern United States occurs in a relatively short time period, allowing for only one annual glyphosate application. This is in contrast with glyphosate-resistant crops [e.g., cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), and soybean (*Glycine max* [L.] Merr.)], in which as many as five glyphosate applications are made annually for broad-spectrum weed control (Norsworthy et al. 2007).

Simulation modeling has many practical applications, including predicting and assessing risks of herbicide resistance. Such models have been produced for troublesome weed species such as Palmer amaranth (*Amaranthus palmeri* S. Wats.), blackgrass (*Alopecurus myosuroides* Huds.), and rigid ryegrass (*Lolium rigidum* Gaudin) to evaluate the effects of cropping practices and herbicide use on the evolution of herbicide resistance (Cavan et al. 2000; Gustafson 2008; Neve et al. 2003). Simulations provide a method of evaluating resistance risks without the need for field trials, which are costly, highly variable, and require large-scale resources and long-term evaluation (Neve et al. 2010; Renton et al. 2014) that are generally unavailable for turfgrass research applications. Although simulation models cannot solve herbicide resistance issues, they can provide valuable information for reducing risks via implementing alternative herbicide-use strategies and/or cultural practices and can reveal directions for future research.

The recent increase in herbicide-resistant weed populations in golf course turf, specifically annual bluegrass, requires an immediate response to understand and mitigate risks and develop proactive approaches to manage resistance. Because turfgrass weed dynamics are vastly different from typical cropping systems, a basic simulation model was developed to evaluate annual bluegrass herbicide-resistance evolution. This model is an initial attempt to explore factors associated with annual bluegrass herbicide resistance in turfgrass systems. In particular, the relative risks of resistance for six glyphosate-use strategies are investigated as well as their respective ability to manage annual bluegrass population growth over time.

Materials and Methods

Model Introduction. The model presented is based upon annual bluegrass biology, general turfgrass weed management strategies, and some genetic parameters that are required to produce estimates of resistance evolution. Other herbicide resistance-simulation models were referenced during development (Cavan et al. 2000; Diggle et al. 2003; Neve et

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Table 1. Description of parameters used in the annual bluegrass herbicide resistance simulation models.

Parameter	Symbol	Value description[a]	Reference
Turf area exposed to herbicide		12 ha	GCSAA 2007
Initial seed bank density[b]	I	0–20,000 seed m^{-2}	Beard et al. 1978; Lush 1988; Watschke et al. 1979
Annual germination	GP_y	0.1 ± 0.025 (0.01–0.2)	Lush 1988; Naylor and Abdalla 1982; Wu et al. 1987
Cohort germination	GP_c		Kaminski and Dernoeden 2007;
Cohort 1		0.75 ± 0.05 (0.6–0.9)	McCarty 2011
Cohort 2		0.2 ± 0.025 (0.15–0.25)	
Cohort 3		$1 - (GP_1 + GP_2)$	
Natural mortality	MP_c	See Equation 1	Expert opinion
Established turf competition	ET_y		Expert opinion
Cohort 1		0.1 ± 0.025 (> 0.01)	
Cohort 2		0.05 ± 0.01 (> 0.01)	
Cohort 3		0.1 ± 0.025 (>0 .01)	
Seed production	SP_c	See Equation 2	Beard et al. 1978; Holm et al. 1997
Viability of new seed	NSV	0.9	Expert opinion
Seed removal	PSR_y	0.5 ± 0.05	Expert opinion
Loss of seed viability	LV_y	0.25 ± 0.05	Roberts and Feast 1973
Mutation rate[c]	MR_b	$1\times10^{-9} \pm 5\times10^{-8}$	Harms and DiMaio 1991; Jander et al. 2003; Saari et al. 1994
Initial resistance allele frequency	IF_R	$1\times10^{-8} \pm 5\times10^{-7}$	Jasieniuk et al. 1996; Neve et al. 2010

[a] Values for random parameters are means selected from a normal distribution with standard deviations in parentheses unless otherwise specified.

[b] The value for initial seed bank density was randomly selected from a uniform distribution with the specified range.

[c] Values for mutation rate and initial resistance allele frequency parameters were randomly selected from log-normal distributions.

al. 2003, 2010). Here, the parameters considered to have primary influence on evolution of annual bluegrass resistance were soil seed bank density, germination, natural mortality, seed production, seed removal, loss of seed viability, rate of mutation to produce herbicide resistance alleles, and initial frequency of herbicide-resistance alleles.

Parameter values or ranges used in the model were determined from a review of the literature and/or field observations, and are presented in Table 1. These parameters and methods of their incorporation into the simulations are discussed in detail below. Some of these parameters have not been estimated specifically for annual bluegrass in commercial turf scenarios. Where these estimates are unavailable, similar studies (i.e., herbicide resistance simulations) or expert opinion are used to establish estimates. Given the nature of annual bluegrass, most of these parameters are highly variable in the field, and a review of the literature supports this variability. In general, a mean value of each parameter is established, and where parameter values are uncertain or naturally vary, a distribution of values is used.

Annual Bluegrass Soil Seed Bank. Areas with colonized populations of annual bluegrass have large and persistent seed banks (Lush 1988). Estimates of viable annual bluegrass seed banks in previous literature have been wide-ranging. Beard et al. (1978) reported approximately 7,500 annual bluegrass seed m^{-2}, but infested golf course fairways can contain up to 185,000 seed m^{-2} (Watschke et al. 1979). To encompass the large variability associated with this parameter, a value between 0 and 20,000 seed m^{-2} was randomly chosen from a uniform distribution to represent initial seed bank density prior to herbicide selection. The total seed bank subjected to selection from herbicide applications is the product of seed bank density (seed m^{-2}) and 120,000 m^2, the average turfgrass area of golf course fairways in the United States (GCSAA 2007).

Seed Germination. Large annual bluegrass seed banks and extended seed viability contribute to the difficulty of predicting seed germination patterns. Lush (1988) reported an annual germination proportion of approximately 0.85 in a creeping bentgrass (*Agrostis stolonifera* L.) golf green in Australia, but seed germination is often higher in golf greens because of more intensive management

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compared to lesser maintained areas such as fairways and roughs (Naylor and Abdalla 1982; Wu et al. 1987). A data set to model this parameter was not available, but it was assumed that competition with established turfgrasses and variability in the depth of burial in the seed bank results in significantly reduced germination in fairways compared to greens. Because the true value of this parameter is unknown and is likely to vary among years and locations, a value was chosen from a normal distribution with a mean of 0.1 and a standard deviation of 0.025. Upper and lower bounds on this parameter were set to 0.2 and 0.01, respectively.

Annual bluegrass seed has the ability to germinate year-round under optimal conditions, but in the southeastern United States, germination typically occurs from late August to early May. The majority of annual germination occurs during an initial flush when day/night temperatures are consistently around 25/12 C. This usually happens in late September or early October in this region (McCarty 2011). A second flush of germination occurs in early winter when alternating high/low temperatures scarifies additional seed (McCarty 2011). Kaminski and Dernoeden (2007) investigated seasonal annual bluegrass germination patterns in Maryland and determined that 75% of germination occurred from late September to mid-November with approximately 25% occurring from November to May. Based on these data, three annual bluegrass cohorts were established.

Annual Bluegrass Cohorts. Because the true distribution among cohorts of total germinated seed is unknown and varies among years and locations, proportions of germination among cohorts were established using normal distributions. The first cohort consisted of annual bluegrass seed that germinated in fall (e.g., late August to early December). A value between 0.6 and 0.9 was chosen from a distribution with a mean of 0.75 and a standard deviation of 0.05. The second cohort comprised the winter germination flush (e.g., early December to mid-February). The value for cohort 2 was between 0.15 and 0.25 and chosen from a distribution with mean a mean of 0.2 and a standard deviation of 0.025. If the proportion of cohort 1 and cohort 2 was > 1, the value for cohort 2 became $(1 - GP_1)$ where GP_1 was the proportion of germinated seed for cohort 1. The proportion of the third cohort was $(1 - [GP_1 + GP_2])$ and represented seed germinating in early spring (e.g., after mid-February) after all herbicide applications had been made. An analysis of 10,000 runs of this submodel revealed the mean germination proportion of cohort 3 was 0.055.

Seedling Survival. In these simulations, plant survival to maturity (i.e., reproductive stage) is dependent upon herbicide applications and natural mortality. The effect of herbicide applications is discussed below. An artificial data set was developed based on expert opinion of expected survival of annual bluegrass seedlings considering intraspecific competition and competition with established turfgrasses. A nonlinear function was fit to the data (Equation 1):

$$MP_c = (0.0015 \times TG^{0.6}) + ET_y \qquad [1]$$

where MP_c is the predicted natural mortality proportion in cohort c, TG is the total number of germinated seed for the current year, and ET_y is the increase in natural mortality due to competition with established tufgrasses in year y. The value for ET_y was chosen from a normal distribution. Cohorts 1 and 3 were associated with nondormant established turf (i.e., more competitive); therefore, the distribution had a mean of 10% and a standard deviation of 2.5%. For cohort 2, established turfgrasses were assumed to be dormant (i.e., less competitive) and the mean of this distribution was 5% with a standard deviation of 1%.

Seed Production. Annual bluegrass is capable of producing seed at all mowing heights typically practiced on golf courses, although plants maintained at higher mowing heights produce more seed. This allows the species to persist in the seed bank and continually regenerate itself. Beard et al. (1978) reported that a single annual bluegrass plant could produce more than 360 seed plant^{-1}, but plants maintained at higher mowing heights are capable of producing from 1,000 to 2,250 seed plant^{-1} (Holm et al. 1997). A data set was not available to estimate this parameter, thus, a density-dependent competition function for seed production was implemented similar to that of Neve et al. (2010) (Equation 2):

$$SP_c = sd/[1 + (sd/m)] \qquad [2]$$

where SP_c is the number of seed produced per plant in cohort c, s is the number of seed produced per

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plant as d approaches 0, d is the number of plants m^{-2} surviving to seed production, and m is the maximum number of seed that can be produced m^{-2}. The s parameter was set to 2,000, 1,000, and 250 for cohorts 1, 2, and 3, respectively, and the m parameter was set to 200,000, 100,000, and 25,000, for cohorts 1, 2, and 3, respectively. These parameters allowed for realistic seed productivity per plant in simulations based on expert opinion and previous literature reports.

Seed Removal. Weed seed predation is an important ecological factor that can affect plant population dynamics. Insects, birds, and small mammals are responsible for most seed predation, and many turf weeds provide important food sources for arthropods (Blubaugh et al. 2011). Furthermore, mowing, irrigation, and other cultural practices in turfgrass systems can also contribute to weed seed removal prior to incorporation into the seed bank. A value for this parameter was based on expert opinion and selected from a normal distribution with a mean of 0.5 and a standard deviation of 0.05.

Loss of Seed Viability. A number of variables are involved in the maintenance of weed seed viability in soil including moisture, temperature, and exposure to light (Taylorson 1970). Because these factors vary depending upon seed location in the soil profile, a proportion of seed in the soil seed bank lose viability each year. This parameter is influenced by the lack of soil disturbance in perennial turfgrass stands. Roberts and Feast (1973) reported annual bluegrass seed can remain viable in the soil for $>$ 6 yr, and estimated a 17 to 21% annual decrease in the number of viable annual bluegrass seed in an undisturbed soil. Thus, a value was randomly selected from a normal distribution with a mean of 0.25 and a standard deviation 0.05. This value represented mean viability loss across all soil profile depths and encompassed loss through microbial decay and seed aging. The simulations assumed a proportion of 0.9 of newly produced seed was viable.

Genetic Parameters. Genetic parameters are difficult to estimate in general, but specifically for annual bluegrass because it is an allotetraploid species. Several of these parameters, however, are required for the evaluation of resistance evolution. These simulations consider three genetic parameters, including resistance inheritance, mutation rate, and initial resistance allele frequency. Relatively little information investigating these parameters is available for annual bluegrass. Glyphosate resistance inheritance in these simulations is considered to occur in Mendelian fashion via a single, incompletely dominant nuclear gene (Lorraine-Colwill et al. 2001).

Rates of gene mutation conferring herbicide resistance are difficult to quantify. Specific estimates for mutation rates for resistance to ALS-inhibitors in mouse-ear cress (*Arabadopsis thaliana* L.) and tobacco (*Nicotiana tabacum* L.) were reported as 1×10^{-9} and 2.7×10^{-8}, respectively (Harms and DiMaio 1991; Saari et al. 1994). In comparisons of mouse-ear cress mutants selected for herbicide resistance to ALS-inhibitors and glyphosate, Jander et al. (2003) reported mutations conferring glyphosate resistance occurred less frequently than those conferring ALS resistance. Therefore, the rate of mutation to produce glyphosate-resistant alleles was randomly selected from a log-normal distribution with a mean of 1×10^{-9} and a standard deviation of 5×10^{-8}.

Initial frequency of herbicide-resistance alleles is equally difficult to quantify. Even for relatively high mutation rates, millions of plants would need to be screened to have a reasonable level of confidence of finding one resistant individual (Jasieniuk et al. 1996). It is expected the initial frequencies of resistance will be an order of magnitude higher than mutation rates in the absence of selection, according to mutation-selection equilibrium (Jasieniuk et al. 1996; Neve et al. 2010). Therefore, initial resistance frequencies were established by random selection from a log-normal distribution with a mean of 1×10^{-8} and a standard deviation of 5×10^{-7}.

Herbicide Applications. In these simulations, herbicide applications occurred at different timings prior to the emergence of cohort 3 (i.e., after cohort 1 or cohort 2). No annual bluegrass seed was produced until after herbicide applications were made. Herbicide options, timings, and efficacy on genotypes are presented in Table 2. Residual activity of POST herbicides was not considered. When herbicides were applied at later timings, control of earlier cohorts was assumed to be reduced. Resistance to glyphosate is conferred in an incompletely dominant fashion; thus, control of heterozygous resistant plants is increased compared to

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Table 2. Potential management options, timings, and expected levels of annual bluegrass control in golf course fairways. Annual bluegrass control is dependent upon cohort and genotype. Simulations consider different combinations of these management options, results of which are discussed in the text and presented in accompanying figures.

		Annual bluegrass control (%)								
		Cohort 1			Cohort 2			Cohort 3		
Management option	Timing	SS[a–c]	SR	RR	SS	SR	RR	SS	SR	RR
PRE[d]	Late summer	95	95	95	75	75	75	0	0	0
POST alternate[e]	Fall	95	95	95	0	0	0	0	0	0
POST alternate	Winter dormancy	90	90	90	95	95	95	0	0	0
Glyphosate	Winter dormancy	80	10	5	95	25	5	0	0	0

[a] Abbreviations: S, herbicide-susceptible allele; R, herbicide-resistant allele.

[b] Genotypes pertain only to glyphosate resistance; resistance to alternate herbicides was not considered in simulations.

[c] Glyphosate resistance is conferred in incompletely dominant fashion; thus, control of SR is increased compared to RR.

[d] PRE herbicide options include indaziflam and prodiamine (Brosnan et al. 2012a).

[e] POST alternate herbicide options include simazine, pronamide, flumioxazin, glufosinate, and sulfonylureas (Flessner et al. 2013; Toler et al. 2007).

homozygous resistant plants. Resistance to alternate herbicides in these simulations was not considered.

Model Simulations. Simulations were developed and implemented using SAS version 9.3 (SAS Institute, Inc., Cary, NC) and are represented in the simplified flow chart in Figure 1. In the first year of each simulated location, a value for initial seed bank density (I seed m^{-2}) was generated and multiplied by 12 ha to produce a total viable seed bank (TSB) across the fairways of a golf course. A value for initial resistance allele frequency (F_{Ri}) was generated and used to produce an initial number of viable seed of three genotypes (SS, SR, RR; S, susceptible and R, resistant alleles) present in the seed bank during the first year of the simulation. A proportion of these viable seed of each genotype g (V_g) germinated each year (y) according to the germination proportion value (GP_y) (Equation 3):

$$G_g = V_g \times GP_y \quad [3]$$

where G_g is the total number of germinated seed of genotype g in year y. Germinated seed belong to one of three cohorts (c) according to the cohort germination proportion (GP_c) (Equation 4):

$$G_{gc} = G_g \times GP_c \quad [4]$$

where G_{gc} is the number of germinated seed of genotype g in cohort c. The survivability to reach reproductive stage was different for each cohort and depended upon natural mortality and subjection to herbicide application. For cohorts 1 and 2, this is given by Equation 5:

$$S_{gc} = G_{gc} \times (1 - MP_c) \times (1 - E_{hg}) \quad [5]$$

where S_{gc} is the number of treated plants of genotype g in cohort c reaching reproductive stage, MP_c is the expected proportion of natural mortality of plants in cohort c (see Equation 1 above), and E_{hg} is the efficacy of herbicide h on genotype g. Cohort 3 seed germinated after herbicide application and S_{gc} was obtained using Equation 5 without the E_{hg} term. Seed production per plant for each genotype and cohort was determined using a competition

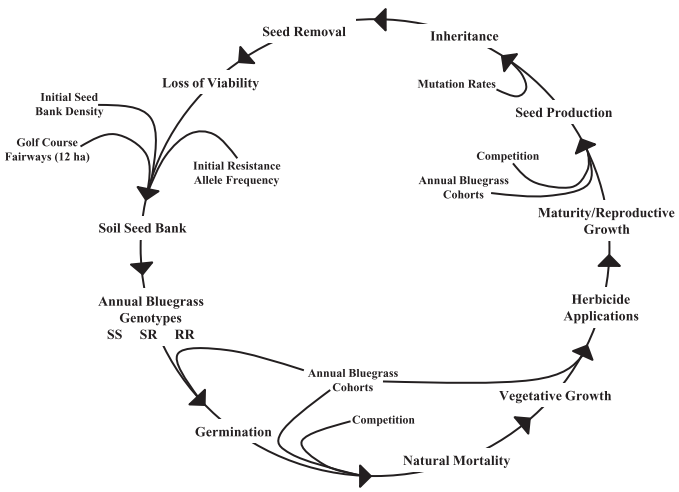

Figure 1. A simplified flow chart representing simulations of annual bluegrass herbicide resistance to glyphosate in golf course fairways. Details of each parameter and their incorporation into simulations are discussed in the accompanying text.

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function (see Equation 2 above) and calculated by Equation 6:

$$TS_{gc} = SP_c \times S_{gc} \quad [6]$$

where TS_{gc} is the total seed produced by genotype g in cohort c and SP_c is the seed production per plant for cohort c. Resistance inheritance was assumed to occur in Mendelian fashion and genotypic proportions of new seed were determined considering rates of mutation to produce alleles resistant to glyphosate (MR_g) by Equations 7, 8, and 9:

$$SS_{New} = (SS_c \times p^2) + (SR_c \times 0.25) + (RR_c \times q^2) \quad [7]$$

$$SR_{New} = (SS_c \times 2 \times p \times q) + (SR_c \times 0.5) + (RR_c \times 2 \times p \times q) \quad [8]$$

$$RR_{New} = (SS_c \times q^2) + (SR_c \times 0.25) + (RR_c \times p^2) \quad [9]$$

where SS_{New}, SR_{New}, and RR_{New} are the number of new seed of genotype SS, SR, and RR, respectively, SS_c, SR_c, and RR_c are the number of seed of SS, SR, and RR genotypes, respectively, from cohort c, p is 1 − MR_g, and q is MR_g. Although annual bluegrass is capable of cross pollinating, studies suggest the majority of annual bluegrass pollination occurs by selfing, and simulations consider only self-pollination when calculating frequencies of genotypes in new seed production (Darmency and Gasquez 1983). The number of viable seed of each genotype incorporated into the seed bank depended upon the proportion of seed removed prior to incorporation in year y (PSR_y) and the proportion of new viable seed (0.9) (Equation 10):

$$T_{Ng} = N_g \times (1 - PSR_y) \times 0.9 \quad [10]$$

where T_{Ng} is the total new seed of genotype g incorporated into the seed bank and N_g is the new seed produced of genotype g. A proportion of the remaining seed lying dormant in the seed bank over the simulated year lost viability and was used to calculate the remaining viable seed bank (N_{Vg}) by Equation 11:

$$N_{Vg} = I_{Vg} \times (1 - LV_y) \quad [11]$$

where I_{Vg} is the initial viable seed bank of genotype g. The new viable seed bank of each genotype thus became the sum of T_{Ng} and N_{Vg}. This value was used in the following simulated year. Resistance was considered to have evolved at a location when the resistant individuals (SR and RR) comprised > 20% of the seed bank. At this point, enough resistant individuals are present in the seed bank such that a turf manager could realize that a herbicide for annual bluegrass control had failed. Further, most previous simulation models for herbicide resistance consider this value as evolution of resistance (e.g., Neve et al. 2010). To prevent over-prediction of resistance, an integer was drawn from a Poisson distribution with mean equal to the predicted value in calculations where partial plants might have been predicted.

Upon establishment of parameter values and ranges, six glyphosate-use strategies for annual bluegrass control were simulated over a period of 50 yr and are discussed further below (Table 3). Each simulated strategy contained 10,000 runs representing a different location (i.e., golf course). The probability of resistance evolution for each strategy was determined by the proportion of simulation runs (out of 10,000 total) which evolved resistance after a period of time of implementing that particular strategy.

Upon evaluating resistance evolution for each glyphosate-use strategy, biological parameters that were considered to influence resistance development were subjected to sensitivity analysis by fixing the parameter of interest at a range of values, and other parameters were employed as described above. Effect of the parameter on predicted resistance risk was evaluated by the proportion of model runs (out of 10,000 total) evolving resistance after 15 yr of consecutive annual applications of glyphosate.

Results and Discussion

A worst-case scenario of single annual glyphosate applications at winter dormancy was considered. This glyphosate-use strategy is common at lower-budgeted golf courses in the transition zone because glyphosate is an economical option providing excellent broad-spectrum control of winter annual weeds common to turfgrass systems (B McCarty, personal observation). As a result of these use patterns, glyphosate-resistant annual bluegrass populations have increased in recent years. In these analyses, the first population evolving resistance was

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Table 3. Herbicide-use strategies that were simulated to evaluate their effects on evolution of glyphosate resistance in annual bluegrass.

Strategy	Herbicide	Application timing[a]	Notes
1	Glyphosate	Dormancy	Worst-case scenario of single annual applications
2	Glyphosate/POST alternate[b]	Dormancy Late summer	Herbicides rotated on alternate years
3	PRE[c] + glyphosate	Dormancy	A PRE added to single annual glyphosate applications
4	POST alternate + glyphosate	Fall Dormancy	A POST alternate added to single annual glyphosate applications
5	PRE + POST alternate + glyphosate	Late summer Fall Dormancy	A PRE and POST alternate added to single annual glyphosate applications
6	PRE + POST alternate + glyphosate/POST alternate	Late summer Fall Dormancy	A PRE and POST alternate with herbicides applied at dormancy rotated on alternate years

[a] Late summer herbicide applications were made prior to cohort 1 emergence. Fall applications were made after cohort 1 had emerged but prior to cohort 2 emergence. Dormancy applications were made after cohort 2 had emerged but prior to cohort 3 emergence.

[b] POST alternate herbicide options include simazine, pronamide, flumioxazin, glufosinate, and sulfonylureas (Flessner et al. 2013; Toler et al. 2007).

[c] PRE herbicide options include indaziflam and prodiamine (Brosnan et al. 2012a).

predicted to occur within 10 yr following annual glyphosate applications (Figure 2a). Risk of resistance remained low (< 10%) until yr 15 in which 30% of populations were predicted to evolve resistance. Beyond this point, risk of resistance increased rapidly, and by yr 20, 99% of populations were predicted to have evolved resistance. This, along with annual bluegrass evolving resistance to many other mechanisms of action, highlights the need for an integrated herbicide program to manage this weed.

Herbicide rotations are the foundation for a herbicide-resistance management program (Beckie and Reboud 2009). This has been well established in previous simulation studies for other weeds (Cavan et al. 2000; Neve et al. 2003). For weeds demonstrating a propensity to evolve resistance, such as annual bluegrass, the importance of alternating mechanisms of action cannot be understated. This was displayed in these simulations when glyphosate was rotated on alternate years with a unique mechanism of action and resistance evolution was delayed 12 to 15 yr (Figure 2b). Despite this delay, the total number of populations expected to evolve resistance still reached 99% by yr 40. The trend of a delay in resistance evolution continued as alternate herbicides were integrated more frequently than every other year (data not shown). For annual bluegrass populations that have yet to evolve resistance, it is conceivable to rotate mechanisms of action in greater frequencies with the number of labeled options available for dormant turfgrass (Flessner et al. 2013; Toler et al. 2007).

Considering the previous simulations, the problem remains that acceptable (i.e., season-long) annual bluegrass control is generally not attainable with a single herbicide application each season. Therefore, the effects of adding herbicide applications to an annual bluegrass management program with glyphosate were considered. The addition of a single herbicide application (PRE or POST) to an annual glyphosate application regime resulted in faster evolution of resistance compared to the worst-case scenario. Resistance was initially predicted in yr 8 when applying a PRE herbicide in late summer and glyphosate at dormancy (Figure 2c). A 30% risk was observed after 11 yr and a 90% risk was observed after 13 yr. The introduction of an alternate POST herbicide application in fall paired with a glyphosate application at dormancy also increased risk of resistance evolution compared to annual glyphosate applications alone. Resistance evolved in yr 9, and risk of resistance reached 60% by yr 15 and 99% by yr 20 (Figure 2d).

The decrease in time to evolve resistance with the aforementioned strategies is related to the population dynamics in these simulations. The majority (mean of 75%) of annual bluegrass germination

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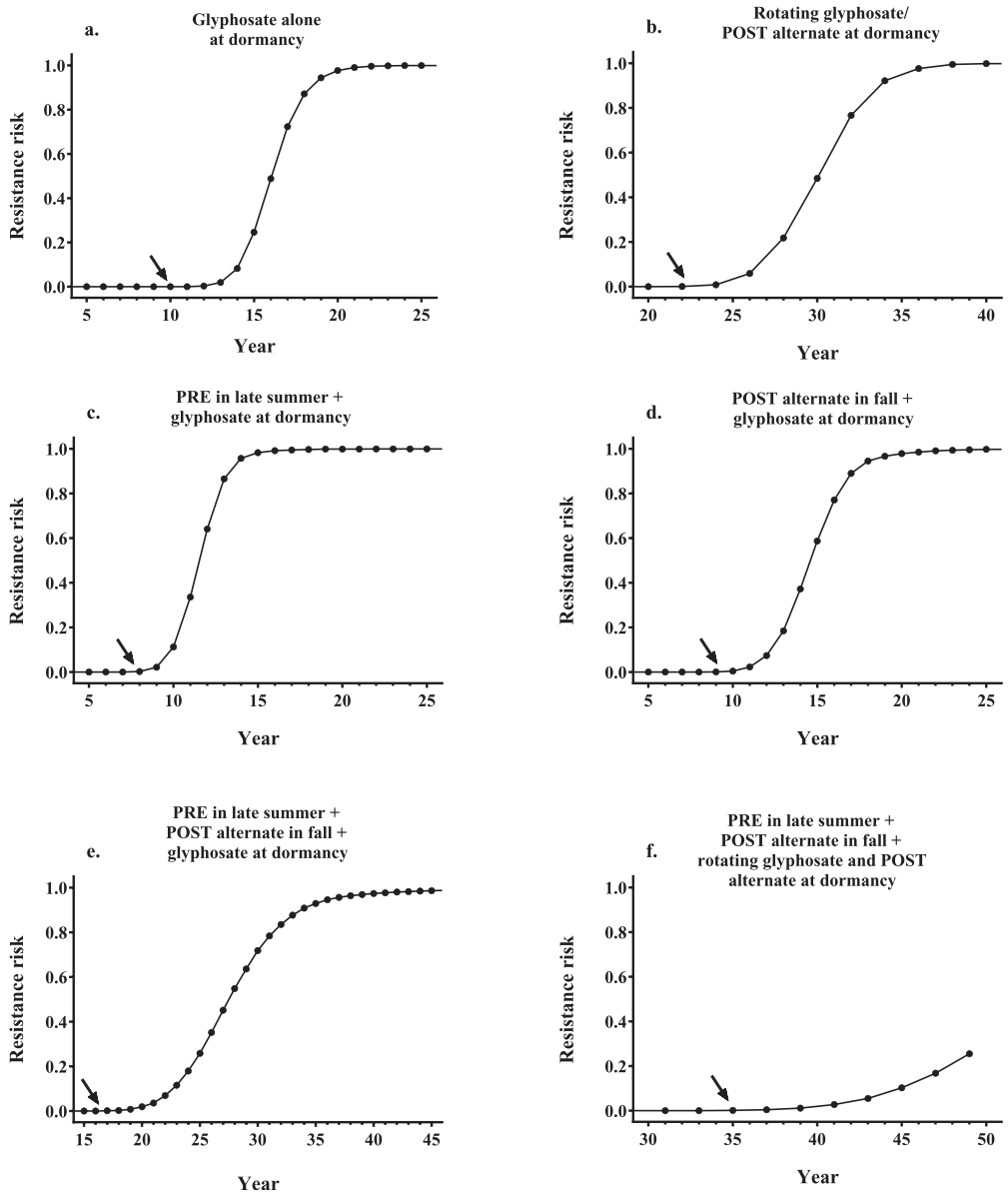

Figure 2. Predicted probability of annual bluegrass resistance to glyphosate for (a–f) six glyphosate-use strategies on golf course fairways. Probability of resistance is represented by the proportion of simulation runs (out of 10,000) that evolved resistance (resistant individuals comprising > 20% of seed bank) in a particular year. Arrows indicate the year when the first population evolved glyphosate resistance. The scale of the horizontal axis should be noted for each strategy.

occurred in cohort 1. PRE and POST herbicides were expected to provide 95% control of all genotypes in cohort 1 (Table 2). When only glyphosate was applied, 80% of the susceptible genotype was controlled in cohort 1 (Table 2). Therefore, a higher number of susceptible individuals survived to produce seed when glyphosate was applied alone, extending the buffer to resistance in the seed bank provided by susceptible seed. In reality, this effect depends on the interaction between the true proportion of seed germination and the actual timing of each herbicide application. This is difficult to simulate because timing of application and germination patterns would be variable among years and locations. This effect does, however, illustrate the importance of reducing selection from applying the same mechanism of action during subsequent growing seasons.

A more integrated approach involves making more than two herbicide applications each season.

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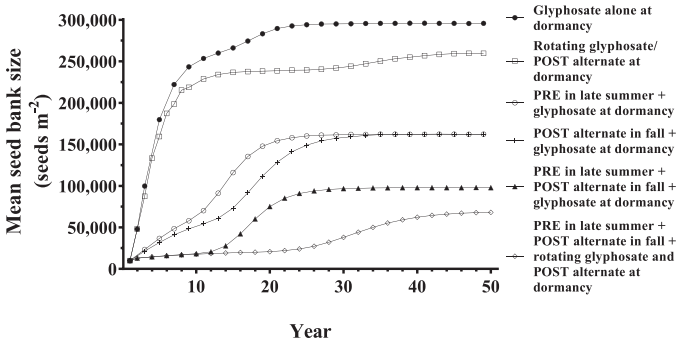

Figure 3. Predicted mean annual bluegrass seed bank size (seed m^{-2}) for six glyphosate-use strategies on golf course fairways. Each data point represents the mean of 10,000 simulated locations.

A strategy utilizing a PRE herbicide in late summer, a POST herbicide in fall, and glyphosate at dormancy was simulated. This both delayed the onset of resistance evolution and reduced the rate at which resistance risk increased compared to previously simulated strategies (Figure 2e). Resistance was initially predicted in yr 16 (6-yr delay) using this strategy, and risks remained < 10% until yr 23. After resistance was predicted to evolve, risk of resistance increased by approximately 5 to 8% each year compared to increases of 25 to 30% with other strategies.

Rotating an alternate mechanism of action with glyphosate at dormancy with a PRE herbicide in late summer and POST herbicide in fall further illustrates the benefits of an integrated weed management program. This was the most effective strategy because resistance was not predicted to evolve until yr 35 (Figure 2f). Furthermore, only 35% of populations were predicted to have evolved resistance after the 50-yr simulation period. In reality, the effectiveness of such a herbicide program to manage resistance would rely on using unique mechanisms of action at each application timing. Resistance to herbicides other than glyphosate were not considered in these simulations, but annual bluegrass has evolved resistance to nine herbicide mechanisms of action worldwide (Heap 2014). Therefore, turfgrass managers must ensure that their herbicide-use programs do not increase selection pressure exerted by herbicides other than glyphosate.

Controlling population size is an important factor in resistance management (Neve et al. 2011). Jasieniuk et al. (1996) demonstrated the effect of increasing weed populations on the probability of a resistant individual occurring in an area. For example, even at a relatively low mutation rate (e.g., 1×10^{-8}), the probability of a resistant individual occurring in a 30-ha field is 0.81 for a selfing weed with a density of 500 m^{-2}. This probability decreases to 0.15 if weed density is reduced to 50 m^{-2} (Jasieniuk et al. 1996). Annual bluegrass seed banks can be immense in golf course fairways where it has colonized (185,000 seed m^{-2}; Watschke et al. 1979); therefore, the importance of controlling population size has significant implications for annual bluegrass resistance management.

Herbicide-use strategies influenced annual bluegrass population growth. Single herbicide applications for annual bluegrass control each year resulted in rapid population increases, with mean seed bank size reaching a plateau of 295,000 seed m^{-2} for single annual glyphosate applications within 20 yr (Figure 3). Although rotating glyphosate with a POST alternate at dormancy delayed evolution of resistance, population size still expanded quickly and plateaued at 250,000 seed m^{-2}. Because larger populations are associated with fewer herbicide applications, rotating herbicides as frequently as possible becomes more important for resistance management when only single herbicide applications are made.

Population sizes were more effectively managed as additional herbicide applications were included in a control program. When a summer PRE or fall POST herbicide application was added to a glyphosate application at dormancy, populations increased slower compared to single applications and plateaued at approximately half that of the annual glyphosate applications alone (Figure 3). This effect was more drastic with three herbicide applications (PRE + POST + glyphosate). When a rotational strategy was included with this application program, mean seed bank size after the 50-yr simulation period was 60,000 seed m^{-2}. Therefore, the benefit of an integrated herbicide program is not only a delay of resistance evolution (Figure 2f), but also season-long control of annual bluegrass and its ability to reproduce and regenerate itself.

Although parameter interactions are certain to influence predicted resistance evolution in these simulations, certain biological parameters were influential on model output (Figure 4). These also highlight potential cultural strategies for managing annual bluegrass resistance. Initial seed bank density

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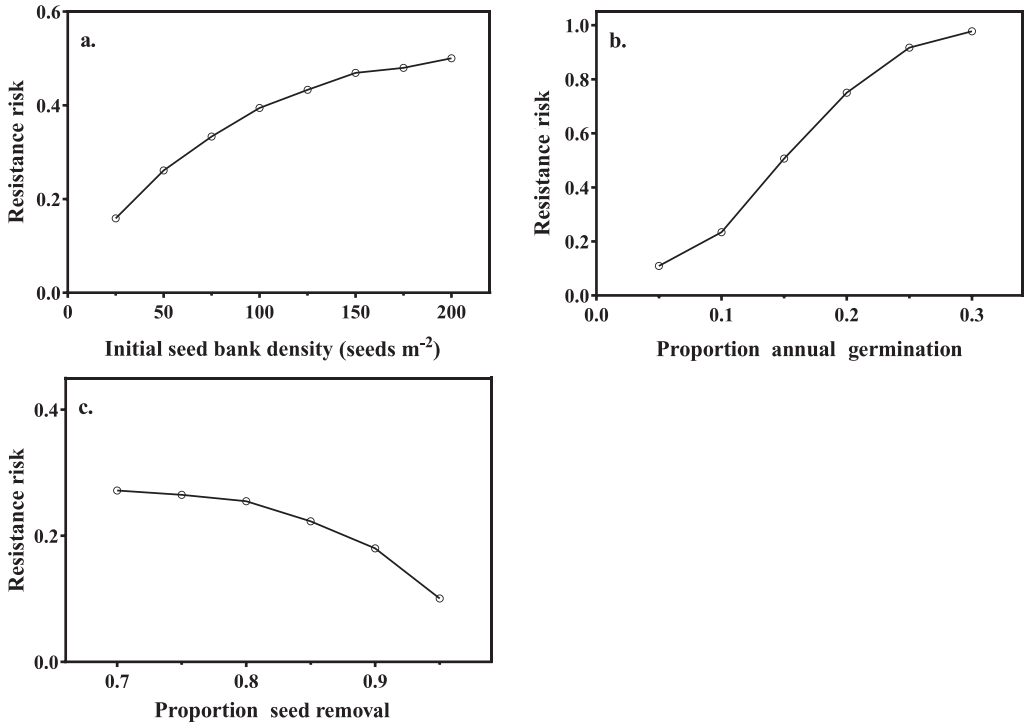

Figure 4. Sensitivity analyses showing main effects of (a) initial seed bank density, (b) germination proportion, and (c) seed removal on predicted probability of annual bluegrass resistance to glyphosate. Probability of resistance is represented by the proportion of simulation runs (out of 10,000) which evolved resistance (resistant individuals comprising > 20% of seed bank) after 15 yr of annual glyphosate applications at dormancy.

(Figure 4a) and germination proportion (Figure 4b) influence resistance risk particularly at lower parameter values, whereas proportion of seed removal (Figure 4c) was influential at high parameter values. The stronger influence of these parameters at more extreme values might have been observed due to the spatially implicit nature of this model. However, these clearly demonstrate the negative effect of increasing population size on resistance. As such, controlling population size with integrated herbicide programs, reducing annual bluegrass germination with healthy and competitive established turfgrass stands, and limiting replenishment of the seed bank by collecting clippings during mowing are all potential approaches to be employed in a resistance-management program.

These basic simulations serve to expand the understanding of annual bluegrass herbicide resistance evolution on golf courses and demonstrate the variables that influence its development in the field. It should be noted the practical application of these simulations are with the relative effectiveness of the herbicide-use strategies for delaying resistance as opposed to predicting actual time for resistance to evolve. For example, a program integrating three annual herbicide applications (i.e., PRE in summer + POST in fall + POST at dormancy) delayed glyphosate resistance 25 yr compared to annual glyphosate applications at dormancy and was effective for managing population growth. The ultimate success of this type of program in the field would rely on the turfgrass manager's use of unique mechanisms of action at each application timing and the ability to rotate these as frequently as possible.

A working knowledge of the biological and ecological characteristics of annual bluegrass populations at different locations should be obtained to make educated decisions on herbicide use and cultural practices for management of resistance. This becomes more important when considering herbicides such as ALS-inhibitors that have a greater propensity for selecting resistant individuals. Further improvements to these simulations could be made with studies specific to annual bluegrass on competition-based seed production, emergence patterns under different environmental conditions, and investigations into genetics associated with annual bluegrass resistance. Herbicide-resistant

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annual bluegrass populations pose a serious risk to the future of weed management in commercial turfgrass. Integrated annual bluegrass management programs are essential to prevent further resistance development to additional mechanisms of action.

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