Establishment and Lateral Growth of Glyphosate-resistant Creeping Bentgrass in Bare Soil

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SUMMARY. Plant establishment and lateral growth of glyphosate-resistant creeping bentgrass [*Agrostis stolonifera* (synonym *A. palustris*)] were assessed to determine if the insertion of the construct conferring herbicide tolerance affected establishment rate or aggressiveness characteristics in unmowed situations. Field studies were carried out in Michigan, Illinois, Ohio, and Oregon in 2000 and 2001 to examine the relative lateral growth of several transformed lines of creeping bentgrass, non-transformed controls, and cultivar standards. Vegetative plugs of creeping bentgrass were transplanted into replicated bare-soil plots and irrigated as needed to prevent moisture stress for an initial 6-week period. Measurements of maximum and minimum stolon spread, percent cover, and stand density for each entry were made in the field at all locations during 2000 and 2001. Few statistical differences ($P = 0.05$) in establishment and lateral growth were observed between individual lines of transgenic creeping bentgrass, non-transformed control lines, and standard cultivars and over a 15- to 18-month period. Overall, lateral growth and establishment rate of transgenic lines were similar to their non-transformed parent and the standard cultivars tested. Transgenic creeping bentgrass lines should have no greater potential for lateral growth than conventional creeping bentgrass cultivars currently in use.

Creeping bentgrass is a perennial, stoloniferous cool season grass predominantly used for recreational turfgrass on golf course greens, tees, and fairways in cool season turfgrass growing regions of the United States (Bradshaw, 1958; Hitchcock, 1950). Various weedy grasses, including annual bluegrass (*Poa annua*), are serious management problems in creeping bentgrass fairways and putting greens (Turgeon, 2002). Current potential controls for annual bluegrass in creeping bentgrass greens require multiple treatments and have not been completely effective in practice (Turgeon, 2002). Creeping bentgrass is sensitive to many herbicides used in an attempt to control weeds in creeping bentgrass (Dernoeden, 2000; Johnson et al., 1989). Genetically engineered

creeping bentgrass cultivars have been developed that are resistant to glyphosate, a non-selective herbicide, via the heterologous expression of a gene from the CP4 strain of *Agrobacterium* sp. encoding for a glyphosate-resistant form of 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS). EPSPS catalyzes the formation of EPSP from shikimate 3-phosphate and phosphoenolpyruvate. Glyphosate inhibits this reaction by interfering with binding of PEP (Thompson et al., 1987). The gene and construct are similar to that used to modify other glyphosate-resistant crops, such as soybean (*Glycine max*) (Padgett et al., 1995). This same gene has been used in the production of glyphosate-resistant corn (*Zea mays*), soybean, and several other crops (James, 1997, 1998).

Creeping bentgrass is a native turfgrass species, as well as introduced from Europe (Hitchcock, 1950; U.S.

Department of Agriculture, 2004). Many introduced turfgrass species have become naturalized along fence rows and roadsides adjacent to production fields. However, most of these turfgrass species, including creeping bentgrass, are not considered significant weed problems in production agriculture because they are relatively slow growing, small in stature, and quickly out-competed by most crop plants (Johnson and Riordan, 1999), and other competitive conditions. Creeping bentgrass has limited ability to establish from seed under competitive conditions. (Kendrick and Danneberger, 2002; Jonsdottir, 1991). Adoption of glyphosate-resistant creeping bentgrass could potentially simplify and improve the control of grass and broadleaf weeds that invade golf course turfgrass. However, there are concerns that glyphosate-resistant creeping bentgrass might become a weed in glyphosate-resistant crop fields or natural areas. However, glyphosate (Roundup Pro; Monsanto Co., St. Louis) promises only partial control of non-transgenic bentgrass, and several glyphosate alternatives for control of bentgrasses are available (Colquhoun et al., 2001; Dant, 2005; Hart et al., 2005). Creeping bentgrass, when managed at the low mowing heights typical of golf course maintenance, does not possess many characteristics of an ideal weed (Baker, 1965). Weediness is usually considered a multigenic trait and, therefore, it is unlikely that the introduction of a herbicide resistance gene into turfgrass could result in a new problem weed (Banks et al., 2003; Johnson and Riordan, 1999). Previous research has suggested that the introduction of an herbicide resistance gene may result in a competitive disadvantage in the absence of the herbicide (Bergelson et al., 1996).

Previous research has estimated the comparative development and potential weediness of transgenic vs. conventional creeping bentgrass when introduced into turfgrass grown in a variety of habitats and under a range of climatic conditions (Gardner et al.,

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.3048	ft	m	3.2808
2.54	inch(es)	cm	0.3937
48.8243	lb/1000 ft ²	kg·ha ⁻¹	0.0205
1.1209	lb/acre	kg·ha ⁻¹	0.8922
1.1692	pt/acre	L·ha ⁻¹	0.8553

2003). The objective of this study, however, was to determine if glyphosate-resistant creeping bentgrass differed from conventional creeping bentgrass in its relative rates of establishment and vegetative development as a colonizer in a non-competitive environment.

Materials and methods

Glyphosate-resistant creeping bentgrass plant material (ASR368) was a cloned R0 (originally transformed) plant established from stolon nodes and grown in 1-2/3-inch-diameter peat pellets (Jiffy Products of America Inc., Batavia, Ill.) in a cold frame in Oregon. All plants were cloned at the same time to maintain age equivalence. Three plants were established on each plot. The non-transformed tissue culture control (B99061R) plants were cloned from a single genotype. The conventional creeping bentgrass cultivars ('Penncross', 'Crenshaw', and 'Penn A-4') were propagated from single stolon nodes and for each cultivar there were three genotypes planted on a plot. The plants were irrigated to prevent drought stress and fertilized to eliminate visible nutrient deficiencies. Plants were maintained at a height of 1/2 inch.

Field studies were established on bare-soil plots in July 2000 at four locations: Marysville, Ohio (lat. 83°22'N, long. 40°16'W); Conklin, Mich. (lat. 85°52'N, long. 43°07'W); Carlyle, Ill. (lat. 89°22'N, long. 38°36'W); and Gervais, Ore. (lat. 122°56'N, long. 45°7'W). Each field location had three replicate plots for each entry in a randomized complete-block design. Each plot consisted of a 5 × 3-ft area populated with three plants of a single entry. Each plot was separated from other plots by 15-ft borders within replications and 4.6-m borders between replications. Soil cores (1-1/3 inches in diameter and 1 to 1-1/2 inches deep) were removed from the original area on 2- to 3-ft centers with an auger bit. Creeping bentgrass plugs of 2-inch diameter were transplanted directly into the core holes so that the plant crowns were at or slightly below the soil surface. The duration of the study was based on previously published reports concerning creeping bentgrass population dynamics which allowed for data collection for two complete growing seasons (Cattani et al., 1991; Jonsdottir, 1991).

Plots were irrigated as needed to

prevent drought stress during an initial 6-week establishment phase. Plots were monitored and individual seed heads were manually clipped to preclude any pollen development. Plants were not mowed for the duration of these experiments. Nitrogen (0.5–0.75 lb/1000 ft² per month during active growth), potassium, and phosphorus were applied as needed to compensate for visual deficiency symptoms. Constant pressure of weed invasion in the plots was reduced with chemical and mechanical management. The amine salts of 2,4-D, MCPP, and dicamba (3 pt/acre, Trimec Bentgrass Formula; PBI Gordon Inc., Kansas City, Mo.) were applied as necessary at label rates to control broadleaf weeds. Pendi-methalin (0.75–1.5 lb/acre a.i., Scott's Weed Grass Control 60WP; The Scotts Co., Marysville, Ohio) and dithiopyr (0.25 lb/acre a.i., Dimension 1 EC; Dow Agrosciences LLC, Indianapolis) were applied as necessary at label rates for control of grassy weeds. These herbicides may have affected stolon establishment rate, but they likely affected all lines equally. Plots were also hand cultivated as necessary to control weeds.

Evaluations during 2000 and early 2001 consisted of measuring the diameter of each transplanted plant. The longest stolon emerging (max) as well as the smallest stolon emerging (min) was measured once per month. Additional evaluations during 2001 were made by visually observing the percent groundcover and turfgrass density of each entry in each plot. This change facilitated a more rapid and direct assessment of development as plants within each plot had grown

into one another, precluding stolon length measure.

Studies were conducted at four locations. The data were analyzed using the general linear model procedure in SAS (SAS Institute Inc., Cary, N.C.). The mean length of the longest stolons (max) and shortest stolons (min) within each plot for each entry were calculated at each observation date. Means were based on nine observations per genotype (mean of two measurements on each of three plants of each line or cultivar per plot in three replications). Also recorded were the range of values observed for each mean. Stolon vigor was defined as the rate of lateral growth since the previous month. Later season evaluations for percent groundcover and turfgrass density (1–9 scale, 9 = greatest shoots per unit area) were averaged for each creeping bentgrass entry per observation date per location. Means were separated using Fisher's protected least significant difference test at $P = 0.05$ (SAS Institute Inc.).

Results and discussion

Analysis of variance with all locations combined revealed statistically significant location effects. Data were analyzed separately for each location. In most cases, glyphosate-resistant line ASR368 displayed no increase in lateral growth rate compared to traditional creeping bentgrass varieties or the non-transformed control.

In Illinois, significant differences between genotypes when measuring stolon extension were detected only during Sept. 2000 ($P = 0.023$) and Apr. 2001 ($P = 0.019$) when 'Penn A-4' was significantly smaller than all other plant genotypes (Table 1).

Table 1. Stolon length of a glyphosate-resistant line of creeping bentgrass (ASR368), its non-transformed control (B99061R), and creeping bentgrass reference cultivars in Illinois from Aug. 2000 to Apr. 2001. Means were calculated from the maximum and minimum values of three plugs per replicate (n = 9).

Cultivar or line	2000				2001
	August	September	October	November	April
	-----Stolon length (cm) ^z -----				
Crenshaw	29.7	39.0a ^y	39.7	43.0	35.3a
Penn A-4	24.7	22.3b	30.3	39.7	24.7b
Penncross	33.7	42.0a	44.0	50.0	42.0a
B99061R	30.0	38.0a	36.7	37.7	35.0a
ASR368	30.3	35.0a	35.3	37.3	33.7a
LSD _(0.05)	NS	10.9	NS	NS	8.6
CV	13.9	16.4	17.9	20.6	13.3

^z1 cm = 0.3937 inch.

^yMeans followed by the same letter are not significantly different according to Fisher's protected least significant difference test at $P = 0.05$ LSD_(0.05).

^{NS}Non-significant according to LSD_(0.05); CV = coefficient of variation.

Table 2. Percent ground cover and turfgrass density of a glyphosate-resistant line of creeping bentgrass (ASR368), its non-transformed control (B99061R), and creeping bentgrass reference cultivars in Illinois from May to Aug. 2001 (n = 3).

Cultivar or line	May		June		July		August	
	Cover (%) ^z	Density ^z	Cover (%)	Density	Cover (%)	Density	Cover (%)	Density
Crenshaw	58.3	7.0	71.7 a ^y	6.5	58.3 ab	8.0	66.7 ab	7.7
Penn A-4	35.0	5.8	31.7 b	6.7	40.0 c	7.0	43.3 c	6.8
Penncross	71.7	8.0	76.7 a	6.0	66.7 a	8.3	75.0 a	7.7
B99061R	51.7	7.8	65.0 a	8.3	60.0 ab	8.3	63.3 ab	7.8
ASR368	53.3	6.7	55.0 a	7.7	45.0 bc	7.7	55.0 bc	7.2
LSD _(0.05)	NS	NS	22.0	NS	18.4	NS	19.1	NS
CV	25.2	21.8	19.4	13.6	18.1	9.4	16.8	9.3

^zCover (%) = visual estimate of percent ground cover within a plot. Density determined on a 1–9 scale with 1 = low shoot number per unit area and 9 = high shoot number per unit area.

^yMeans followed by the same letter are not significantly different according to Fisher's protected least significant difference test at $P = 0.05$ [$LSD_{(0.05)}$].

^{NS}Nonsignificant according to $LSD_{(0.05)}$; CV = coefficient of variation.

Stolon length for ASR368 was not significantly different from B99061R or 'Penn A-4' and 'Penncross' reference cultivars at any measurement date. ASR368 stolon length was always within the range of measured values on non-transformed reference cultivars and B99061R. Significant differences between genotypes were detected in Illinois for percentage of plot cover during June, July, and Aug. 2001 ($P = 0.010, 0.049, 0.040$, respectively; Table 2). Plot coverage of 'Penn A-4' was significantly less than that of the other genotypes that were similar to each other during June, but was not significantly different from ASR368 on any other date. Plot coverage of 'Penncross' was higher than ASR368 during July and Aug. 2001. ASR368 was not significantly different from other genotypes and was intermediate to non-transformed genotypes for percentage of plot cover and shoot density during all measurement dates.

In Michigan, no differences in relative growth were observed among any of the lines in 2000 and 2001

(Tables 3 and 4). Maximum stolon length, percentage of plot coverage, and plant shoot density for ASR368 were numerically less than that of control plant genotypes measured during two growing seasons from Aug. 2000 through Oct. 2001.

In Ohio, relative growth of ASR368 as measured by percent cover

was similar to its non-transformed parent and cultivar Penn A-4 and significantly lower than that of 'Crenshaw' or 'Penncross' in July, Aug., and Sept. 2001. Estimates of turfgrass stand density were also significantly lower for ASR368 compared to 'Crenshaw' and 'Penncross' in Aug. and Sept. 2001. Transgenic event ASR368 did not

Table 3. Stolon length of a glyphosate-resistant line of creeping bentgrass (ASR368), its non-transformed control (B99061R), and creeping bentgrass reference cultivars in Michigan from Aug. 2000 to Mar. 2001. Means were calculated from the maximum and minimum values of three plugs per replicate (n = 9).

Cultivar or line	2000				2001	
	August	September	October	November	February	March
	----- Stolon length (cm) ^z -----					
Crenshaw	42.3	53.0	59.0	61.3	57.0	61.3
Penn A-4	34.7	51.3	63.7	65.0	58.7	63.3
Penncross	46.7	61.0	65.0	66.3	63.0	67.0
B99061R	40.3	54.7	63.3	64.3	57.3	62.3
ASR368	42.7	57.7	67.0	69.0	64.3	67.7
LSD _(0.05) ^y	NS	NS	NS	NS	NS	NS
CV	12.6	10.3	9.4	8.3	12.0	10.1

^z1 cm = 0.3937 inch.

^y $LSD_{(0.05)}$ = Fisher's protected least significant difference test at $P = 0.05$.

^{NS}Nonsignificant according to $LSD_{(0.05)}$; CV = coefficient of variation.

Table 4. Relative growth as measured by percentage cover and turfgrass density of a glyphosate-resistant line of creeping bentgrass (ASR368), its non-transformed control (B99061R), and creeping bentgrass reference cultivars in Michigan from Apr. 2001 to Oct. 2001 (n = 3).

Cultivar or line	April		May		June		July		August		September		October	
	Cover (%) ^z	Density ^z	Cover (%)	Density	Cover (%)	Density	Cover (%)	Density	Cover (%)	Density	Cover (%)	Density	Cover (%)	Density
Crenshaw	48.3	8.0	58.3	8.7	66.7	8.0	78.3	9.0	90.0	9.0	94.7	9.0	96.7	9.0
Penn A-4	51.7	7.7	65.0	8.3	75.0	8.7	85.0	9.0	92.0	9.0	96.3	9.0	97.3	9.0
Penncross	66.7	8.0	78.3	8.3	83.3	7.7	95.0	8.3	97.0	8.7	98.3	8.7	98.7	8.7
B99061R	45.0	6.3	50.0	7.7	60.0	8.0	78.3	8.7	86.7	9.0	90.7	9.0	92.3	9.0
ASR368	50.0	7.3	66.7	8.3	76.7	8.3	85.7	8.7	91.7	9.0	94.7	9.0	96.7	9.0
LSD _(0.05) ^y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV	20.1	11.9	18.1	6.0	17.6	5.9	13.0	4.2	7.7	2.9	4.7	2.9	3.4	2.9

^zCover (%) = visual estimate of percent ground cover within a plot. Density determined on a 1–9 scale with 1 = low shoot number per unit area and 9 = high shoot number per unit area.

^y $LSD_{(0.05)}$ = Fisher's protected least significant difference test at $P = 0.05$.

^{NS}Nonsignificant according to $LSD_{(0.05)}$; CV = coefficient of variation.

Table 5. Percentage ground cover and turfgrass density of a glyphosate-resistant line of creeping bentgrass (ASR368), its non-transformed control (B99061R), and creeping bentgrass reference cultivars in Ohio from May to Sept. 2001.

Cultivar or line	May		June		July		August		September	
	Cover (%) ^z	Density ^z	Cover (%)	Density	Cover (%)	Density	Cover (%)	Density	Cover (%)	Density
Crenshaw	36.7 a ^y	5.3	43.3 a	6.3	53.3 a	6.7	36.7 a	5.0 a	58.3 a	5.7 a
Penn A-4	17.7 b	3.7	13.3 c	5.7	23.3 bc	5.0	13.3 b	1.7 c	18.3 b	3.3 b
Penncross	37.7 a	5.3	33.3 ab	6.3	33.3 b	6.7	36.7 a	4.0 ab	46.7 a	4.3 ab
B99061R	27.0 ab	4.7	11.7 c	7.0	13.3 c	4.3	15.0 b	2.7 bc	26.7 b	3.3 b
ASR368	26.7 ab	5.0	20.0 bc	7.0	23.3 bc	5.3	15.0 b	2.0 c	26.7 b	3.3 b
LSD _(0.05)	13.2	NS	15.7	NS	19.7	NS	19.7	1.8	16.0	1.6
CV	24.2	14.5	34.2	9.6	35.7	21.4	44.9	30.9	24.1	21.3

^zCover (%) = visual estimate of percent ground cover within a plot. Density determined on a 1–9 scale with 1 = low shoot number per unit area and 9 = high shoot number per unit area.

^yMeans followed by the same letter are not significantly different according to Fisher's protected least significant difference test at $P = 0.05$ [LSD_(0.05)].

^{NS}Nonsignificant according to LSD_(0.05); CV = coefficient of variation.

Table 6. Stolon length of a glyphosate-resistant line of creeping bentgrass (ASR368), its non-transformed control (B99061R), and creeping bentgrass reference cultivars in Oregon in Aug. and Nov. 2000. Means were calculated from the maximum and minimum values of three plugs per replicate (n = 9).

Cultivar or line	August	November
--- Stolon length (cm ²)---		
Crenshaw	39.7	103.0
Penn A-4	40.7	101.7
Penncross	49.7	111.3
B99061R	42.7	106.3
ASR368	41.0	99.3
LSD _(0.05) ^y	NS	NS
CV	9.2	12.8

^z1 cm = 0.3937 inch.

^yLSD_(0.05) = Fisher's protected significant difference test at $P = 0.05$ [LSD_(0.05)].

^{NS}Nonsignificant according to LSD_(0.05); CV = coefficient of variation.

display a distinct advantage in establishment, stolon vigor, plot colonization, plant development, or relative shoot density over its corresponding non-transformed tissue culture control, B99061R, or other reference cultivars (Table 5).

Table 7. Percent cover of a glyphosate-resistant line of creeping bentgrass (ASR368), its non-transformed control (B99061R), and creeping bentgrass reference cultivars in Oregon from Sept. 2000 to Mar. 2001 (n=3).

Cultivar or line	2000		2001	
	September	October	February	March
Crenshaw	61.0	80.0	84.3	78.3
Penn A-4	63.3	76.7	88.7	82.7
Penncross	74.3	94.3	92.3	90.7
B99061R	54.3	76.7	87.0	88.3
ASR368	51.7	75.0	83.7	80.0
LSD _(0.05) ^z	NS	NS	NS	NS
CV	21.4	18.3	9.6	9.4

^zLSD_(0.05) = Fisher's protected least significant difference test east squares difference test at $P = 0.05$.

^{NS}Nonsignificant according to LSD_(0.05); CV = coefficient of variation.

In Oregon, there were no significant differences in stolon vigor among creeping bentgrass lines on any date (Table 6). Percentage of cover was estimated after Aug. 2000 when plants within individual plots began growing together. No significant differences were detected in relative ability to colonize the plots during any of the eight observation dates from Sept. 2000 through Aug. 2001 (Tables 7 and 8). Significant differences in shoot density were only detected during July when 'Penn A-4' exhibited significantly

higher shoot density than 'Crenshaw' and 'Penncross'. However, ASR368 was not significantly different from either the non-transformed tissue culture control or any of the commercial cultivars for relative shoot density at any measurement date.

Conclusion

Currently, creeping bentgrass is not considered an important weed of production agriculture and is considered a minor weed of pastures and naturalized areas (Banks et al., 2003;

Table 8. Percent cover and turfgrass density of a glyphosate-resistant line of creeping bentgrass (ASR368), its non-transformed control (B99061R), and creeping bentgrass reference cultivars in Oregon from May to Aug. 2001 (n = 3).

Cultivar or line	May		June		July		August	
	Cover (%) ^z	Density ^z	Cover (%)	Density	Cover (%)	Density	Cover (%)	Density
Crenshaw	96.7	6.3	96.7	7.0	100.0	6.0bc ^y	100.0	6.3
Penn A-4	75.0	7.0	78.3	6.3	90.0	8.0a	91.7	6.7
Penncross	100.0	6.3	100.0	6.0	100.0	6.7bc	100.0	6.3
B99061R	99.3	7.0	99.3	7.3	100.0	7.7a	100.0	8.0
ASR368	99.3	7.0	98.3	7.3	100.0	7.3ab	100.0	6.7
LSD _(0.05)	NS	NS	NS	NS	NS	0.9	NS	NS
CV	19.3	14.1	16.4	8.1	7.9	6.8	6.6	10.9

^zCover (%) = visual estimate of percent ground cover within a plot. Density determined on a 1–9 scale with 1 = low shoot number per unit area and 9 = high shoot number per unit area.

^yMeans followed by the same letter are not significantly different according to Fisher's protected least significant difference test at $P = 0.05$ [LSD_(0.05)].

^{NS}Nonsignificant according to LSD_(0.05); CV = coefficient of variation.

Shulte and Neuteboom, 2002). Several herbicides in addition to glyphosate are registered for control of creeping bentgrass in these situations (Butler, 2003; Colquhoun et al., 2001; Dant, 2005; Mueller-Warrant, 2003); however, the Roundup Pro label and other glyphosate-based herbicides promise only partial control of non-transgenic creeping bentgrass. In this research, plant establishment, stolon vigor, plot colonization, plant development, and turfgrass density of event ASR368 was not materially distinct from its non-transformed tissue culture control or from commercial non-transgenic cultivars under non-competitive unmanaged conditions at any location. The observed variability in creeping bentgrass lateral growth was less than that typically observed due to differences in climate and cultural practices, such as mowing, irrigation, and fertilization (Holt and Payne, 1951; Turgeon, 2002). These results agree with previous research conducted in turfgrass (Gardner et al., 2003, 2004) and for establishment of stolon nodes in non-competitive conditions (Williams et al., 2005) that demonstrate that glyphosate-resistant creeping bentgrass does not exhibit a novel phenotype that would make it more fit to occupy or become more invasive in unmanaged ecosystems or production agriculture than traditional creeping bentgrass.

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