2010 Purdue University
Turfgrass Research Summary

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Influence of Application Timing on Efficacy of Acelepryn and Merit Against Japanese Beetle Larvae in Kentucky Bluegrass Turf 2010

Doug Richmond, Tim Gibb, Walter Baldauf, Victoria Caceres, Terri Hoctor & Autumn Nance

Objective:

The primary objective of this study was to describe the efficacy of Acelepryn and DPX-HGW86 applied during mid-May and mid-July against Japanese beetle larvae in turfgrass

Materials and methods:

The experiment was located at the Nursery Complex at Purdue University (West Lafayette, IN) on a stand of turfgrass consisting primarily of Kentucky bluegrass maintained at 7.6 cm. Plots measuring 1.5 x 1.5 meters were arranged in a randomized complete-block design with 0.3 meter alleys between plots. Each treatment was replicated 4 times. All materials were applied using a hand-held CO_2 boom sprayer configured with four 8010 nozzles operating at 30 psi and calibrated to deliver a spray volume of 2 gal/1000ft^2. Immediately after products were applied, plots were irrigated (2 cm).

Field conditions on the May 15 treatment date were:
(1) Soil Temp.: 14°C
(2) Air Temp: 15°C
(3) Weather: Overcast, wind 0-5 mph
(4) Thatch: 1.0 cm

Field conditions on the July 23 treatment date were:
(1) Soil Temp.: 25 °C
(2) Air Temp: 27 °C
(3) Weather: Clear, wind 5-9 mph
(4) Thatch: 1.0 cm

Japanese beetle larval infestations were created by driving three, 8” diameter pvc cylinders into each plot along its mid-line and caging two separate groups of 40 Japanese beetle adults (50:50 sex ratio) within each cylinder at two week intervals during July. Larval populations were assessed September 15, 2010 using a sod cutter to remove a strip of sod lying directly beneath the caging area of each plot and examining the soil to a depth of 3 inches. The number of Japanese beetle larvae were counted and recorded. Variation in Japanese beetle larval populations was examined using main effects ANOVA and treatment means were compared using Fisher’s LSD test (α=0.05).

Results to date:

Table 1. Influence of application date and rate, and post-application irrigation on Japanese beetle larval densities and percent control following applications of Acelepryn 1.67 SC, DPX-HGW86 and Merit 75 WP in Kentucky bluegrass turf. Japanese larval populations were assessed on September 15, 2010.

Product	Formulation	Rate 1 (lbs ai/A)	Application Date	JB/ft^2	% Control
Acelepryn	1.67 SC	0.104	15-May-10	1.5±1.5 a	88
Acelepryn	1.67 SC	0.157	15-May-10	0.0±0.0 a	100
Merit	75 WP	0.3	15-May-10	0.5±0.5 a	96
Acelepryn	1.67 SC	0.104	23-Jul-10	0.0±0.0 a	100
Acelepryn	1.67 SC	0.157	23-Jul-10	2.3±0.9 a	81
Merit	75 WP	0.3	23-Jul-10	0.0±0.0 a	100
Untreated				12.0±2.9 b	---

Japanese beetle larval densities followed by the same letter are not significantly different (α=0.05).

All treatments significantly reduced white grub populations compared to untreated controls and all treatments provided acceptable levels of control. Application timing appeared to have no consistent influence on the efficacy of any of the materials used in this study.

Influence of QP Imidacloprid, Novaluron and Break-Thru on Japanese Beetle and Masked Chafer Larval Populations in Kentucky Bluegrass Turf

Doug Richmond, Tim Gibb, Walter Baldauf, Victoria Caceres, Terri Hoctor & Autumn Nance

Objective:

The primary objective of this study was to determine how combinations of QP Imidacloprid, Novaluron and Break-Thru (soil surfactant) applied during late-July influence Japanese beetle and masked chafer larval populations in Kentucky bluegrass turf.

Materials and methods:

The experiment was located at the Nursery Complex at Purdue University (West Lafayette, IN) on a stand of turfgrass consisting primarily of Kentucky bluegrass maintained at 7.6 cm. Plots measuring 1.5 x 1.5 meters were arranged in a randomized complete-block design with 0.3 meter alleys between plots. Each treatment was replicated 4 times. All materials were applied using a hand-held CO_2 boom sprayer configured with four 8010 nozzles operating at 30 psi and calibrated to deliver a spray volume of 2 gal/1000ft^2. Immediately after products were applied, plots were irrigated (2 cm).

<u>Field conditions on the July 23 treatment date were:</u>

(1) Soil Temp.: 25 °C
(2) Air Temp: 27 °C
(3) Weather: Clear, wind 5-9 mph
(4) Thatch: 1.0 cm

Japanese beetle larval infestations were created by driving three, 8” diameter pvc cylinders into each plot along its mid-line and caging two separate groups of 75 Japanese beetle adults within each cylinder at two week intervals during July. Masked chafer populations were naturally occurring. Larval populations were assessed September 15, 2010 using a sod cutter to remove a strip of sod lying directly beneath the caging area of each plot and examining the soil to a depth of 3 inches. The number of Japanese beetle and masked chafer larvae were counted and recorded. Variation in Japanese beetle and masked chafer larval populations was examined using main effects ANOVA and treatment means were compared using Fisher’s LSD test (α=0.05).

Results

Table 1. Influence of QP Imidacloprid, Novaluron and Break-Thru on Japanese beetle and masked chafer larval populations in Kentucky bluegrass turf. Larval populations were assessed on September 15, 2010.

Product	Formulation	Rate (g ai/A)	JB/ft^2	% Control	MC/ft^2	% Control
Untreated	1.67 SC	0.104	13.5±2.9 a	---	3.5±1.0 a	---
QP Imidacloprid	2F	450	0.0±0.0 b	100	0.0±0.0 b	100
Novaluron	0.83EC	102	3.7±2.1 bc	72	0.8±0.8 ab	79
Break-Thru	S233	365	4.7±3.0 bc	64	3.5±2.8 a	0
Novaluron+ Break-Thru	0.83EC S233	102+ 365	5.2±1.2 c	61	0.5±0.5 ab	85
QP Imidacloprid+ Break-Thru	2F S233	450+ 365	0.0±0.0 b	100	0.0±0.0 b	100
Novaluron+ QP Imidacloprid	0.83EC 2F	102+ 450	0.0±0.0 b	100	0.0±0.0 b	100
Novaluron+ QP Imidacloprid+ Break-Thru	0.83EC 2F 0.83EC	102+ 450+ 365	0.0±0.0 b	100	0.0±0.0 b	100
Acelepryn	1.67SC	71.2	2.2±0.8 bc	83	0.8±0.5 ab	78

Japanese beetle (JB) and masked chafer (MC) larval densities followed by the same letter are not significantly different (α=0.05).

All treatments significantly reduced Japanese beetle larval populations compared to untreated controls. All mixtures containing QP Imidacloprid provided 100% control of Japanese beetle and masked chafer larvae. Although Novaluron alone provided acceptable levels of Japanese beetle larval control, the mixture containing Novaluron and Break-Thru did not. All other mixtures provided acceptable levels of control against masked chafer.

Curative Efficacy of Meridian in Combination with the Sufactant Tween Against Billbuts in Kentucky Bluegrass Turf 2010

Doug Richmond, Tim Gibb, Walter Baldauf, Victoria Caceres, Terri Hoctor & Autumn Nance

Objective:

The primary objective of this study was to determine if the surfactant TWEEN provides enhanced activity of the insecticide MERIDIAN applied curatively against billbug larvae.

Materials and methods:

The experiment was located at the Nursery Complex at Purdue University (West Lafayette, IN) on a stand of turfgrass consisting primarily of Kentucky bluegrass maintained at 7.6 cm. Plots measuring 1.5 x 1.5 meters were arranged in a randomized complete-block design with 0.3 meter alleys between plots. Each treatment was replicated 4 times. All materials were applied using a hand-held CO_2 boom sprayer configured with four 8010 nozzles operating at 30 psi and calibrated to deliver a spray volume of 2 gal/1000ft^2. Immediately after products were applied, plots were irrigated (2 cm).

Field conditions on the June 22nd treatment date were:
(1) Air Temp: 26.1 °C
(2) Weather – clear 0-5 mph

Larval populations were measured on July 9th by extracting 4 turf and soil cores (10.8 cm diameter) from each plot and counting the number of billbug larvae in each core. Samples were taken at least 0.25 m inside the border of each plot. Variation in larval populations was examined using main effects ANOVA and treatment means were compared using Fisher's LSD test (α=0.05).

Results

Table 1. Billbug larval population densities (per 0.4 ft^2) and percent control resulting from applications of MERIDIAN in combination with TWEEN in Kentucky bluegrass turf. Applications were made on 5/17/2010 and larval populations were was assessed on 7/9/2010.

		Billbug Larvae	
TRT#	Treatment	Total	% Control
1	Untreated Control	14 a	--
2	Meridian 25WG@17 oz/a	2 bc	85.7
3	Meridian 25 WG@8.5 oz/a Tween@27.8 oz/a	4 bc	71.4
4	Meridian 25 WG@6.3 oz/a Tween@27.8 oz/a	2 bc	85.7

Numbers followed by same letters are not significantly different (Fisher LSD, α=0.05)

Towards Association Mapping of Drought Tolerance in Perennial Ryegrass

Xiaoqing Yu and Yiwei Jiang

Objective:

To identify candidate genes associated with drought tolerance traits in perennial ryegrass (*Lolium perenne* L.)

Rationale:

Association mapping, also known as linkage disequilibrium (LD) mapping, has recently been developed as an alternative and powerful tool for dissecting complex traits and identifying functional markers. Compared to quantitative trait loci (QTL) mapping in traditional bi-parental populations, association mapping offers fine mapping through exploitation of historical recombination events at the population level and tests large numbers of alleles rapidly. The genome wide-association mapping is feasible when LD declines slowly as distance increases, a low marker density is sufficient to detect an association between phenotype and genotype. When LD declines rapidly between loci with distance, a much greater density of marker is required to identify an association between phenotype and genotype. In this case, the candidate gene-association approach can be used to relate to polymorphisms in selected candidate genes that have purported roles in controlling phenotypic variation.

Perennial ryegrass is a self-incompatible species. Out-crossing species such as perennial ryegrass tends to have a rapid LD decay, and the potential for high resolution mapping of candidate genes is expected. The use of diverse populations may be particularly beneficial in perennial ryegrass since many potentially useful variations are still unexplored. Research on association mapping not only benefits grass breeders by providing molecular markers, but also improves our understanding of the genes underlying complex genetic traits.

Materials and methods:

The 192 diverse accessions were established in three locations varying in climate and soil textures in Indiana: Turf Center in West Lafayette, Pinney Agricultural Research Farm (North) in Wanatah, and Southwest Agricultural Research Farm in Vincennes. Phenotypic characterization of perennial ryegrass populations in different locations over two years will generate more reliable data for evaluating drought response and tolerance. Whole-plant responses to drought stress were examined for the populations. Samples were collected before and at the end of drought stress. A total of 109 microsatellite (SSR) markers available in perennial ryegrass were used to determine genetic diversity, population structure (Q) and relative kinship (K) of the populations.

Results to date:

Under drought stress, leaf wilting ranged from 1 (no wilting) to 9 (severely wilted), leaf water content ranged from 60 % to 82 %, canopy temperature ranged from 29°C to 38°C, and chlorophyll fluorescence ranged from 0.69 to 0.85 across all population, respectively.

Five genetic structural groups were identified in the population (Fig.1). No obvious relative kinship (K) was found in the population. An approximate 90% of the pairwise kinship estimates were from 0 to 0.05. Less than 10% of pairwise kinship estimates were around 0.1 to 0.15, representing some familial relationships. For model testing, the Q model (based on population structure) resulted in the smallest Bayesian Information Criterion (BIC) values for fitting different traits, compared to other models. It was considered as the best model in explaining phenotypic trait variations related to whole-plant tolerance under drought stress. The Q model will be used for association analysis to reduce false correlations between genes and traits. Simple linear and K models had the biggest BIC values that were not good choices. The mixed Q + K model was in between and was not the best choice. We are sequencing the candidate genes related to drought tolerance, and LD decay of these genes will be analyzed. Single nucleotide polymorphisms from different genes will be identified and tested in the whole population to obtain true associations of genes with drought tolerance traits, with consideration of model testing.

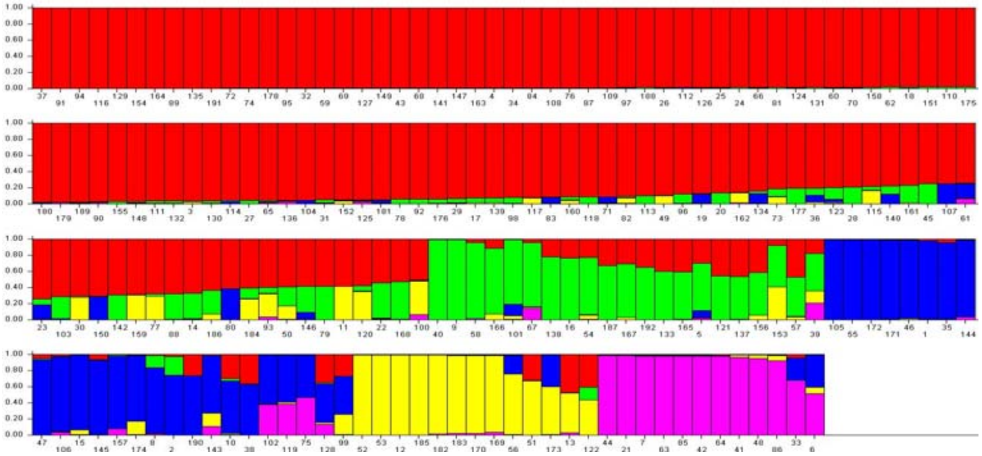

Fig. 1. Hierarchical organization of genetic relatedness of 192 accessions of perennial ryegrass. Numbers in the *x*-axis represent each individual accession. Numbers in the *y*-axis indicate the membership coefficient for each individual accession. The color of the bar indicates the five groups identified through the STRUCTURE program (G1 = red, G2 = green, G3 = blue, G4 = yellow, G5 = pink)

Acknowledgements

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Association of Simple Sequence Repeat (SSR) Markers with Submergence Tolerance in Diverse Populations of Perennial Ryegrass

Xiaoqing Yu and Yiwei Jiang

Objective:

To identify SSR markers associated with submergence tolerance in perennial ryegrass (*Lolium perenne* L.)

Rationale:

Turfgrass management and production is largely influenced by climate variability and weather extremes. The increased frequency of flooding negatively affects grass quality. Excess water in the soil reduces oxygen availability to the plant. The extended deep submersion can cause death in plants. Thus, development of more flood-tolerant cultivars is critical for enhancing sustainable management of turfgrass and other perennial grass species. A better understanding of plant physiological responses to flooding stress and identification of molecular markers associated with submergence tolerance will assist grass breeders in developing flood-tolerant cultivars. The variable growth and physiological responses of different perennial grass species and ecotypes to water excess stress provide an important basis for identifying molecular mechanisms of submergence tolerance and molecular markers linked to stress tolerance.

Perennial ryegrass is one of the most important cool-season grasses widely used in the turf and forage industry. Perennial ryegrass provides a good model for studying marker-trait association in perennial species due to its diploid genetics, existing resources of molecular markers, rapid stress responses, and wide distribution. The association results from perennial ryegrass may provide guideline for studying other popular perennial grass species that have more complex genomes. To the best of our knowledge, this study is the first report on association mapping of submergence tolerance traits in perennial grass species. This research has revealed the phenotypic diversity of submergence tolerance and identified marker-trait association, which will benefit the development of resistant cultivars of perennial ryegrass and other important grass species.

Materials and methods:

A total of 99 perennial ryegrass accessions were used in this study. Each accession was sown in a plastic pot (4-cm diameter, 9-cm deep) containing a sandy-loam soil with a pH of 6.9 in a greenhouse. All the accessions were confirmed as diploid by flow cytometry. The plants were watered daily and fertilized once a week with a soluble fertilizer (N- P_2O_5-K_2O, 24-8-16) (Scotts Inc., Marysville, OH, USA) to provide 240 kg N ha^{-1}, 33 kg P ha^{-1}, 132 kg K ha^{-1} and micronutrients. The duration of Exp 1 was from Oct. 6 to Nov. 21, 2009 and of Exp 2, from Dec. 23, 2009 to Feb. 5, 2010. Prior to submergence treatment, all the plants were cut to from 5 to 7 cm above the soil surface, then the plant height of each pot was recorded.

Submergence stress was imposed by submerging the grass pots in plastic containers (86 cm length × 38cm width × 30 cm height) with tap water (pH of 6.6). The water level was kept at 10 cm above the grass canopy. The control pots were placed in the same size containers without water. The stress treatments began on Nov. 14 of 2009 for Exp 1 and Jan. 29 of 2010 for Exp 2 and lasted 7 d for both experiments. Nutrients were not supplied to the plants and water was not changed during the treatment. During the periods of submergence stress, the air and water temperatures were 19.8 ± 1.0°C and 17.5 ± 0.8°C for Exp1 and 19.9 ± 1.0°C and 18.1 ± 1.4°C for Exp 2, respectively.

The leaf color was rated visually on a scale of 1 (yellow) to 9 (dark green) for both the control and submerged plants. Leaf photochemical efficiency was determined by measuring the chlorophyll fluorescence (Fv/Fm) at dark on randomly selected leaves in each pot using a fluorescent meter (OS-30P, OPTI-Sciences, Hudson, NH, USA). At the end of the 7 d treatments, plant height was measured by recording the longest leaf blade. The maximum plant height (HT) increasing during the treatment was obtained by subtracting the plant height prior to stress for each pot. The leaves corresponding to this HT were cut and the tissues were dried in an oven at 80°C for 3 d. The relative growth rate (RGR) was calculated as dry weight per growing day (total 7d) for both the control and the submerged plants. The percentage of reduction of all traits was used to indicate the plant submergence tolerance, calculated as [(control-submergence)/control] ×100.

Genomic DNA was extracted from the young leaves of each accession using a cetyltrimethyl ammonium bromide (CTAB) method. A total of 109 published genome-wide SSR markers in perennial ryegrass were screened in all accessions. All confirmed polymorphic alleles were used for population structure (Q) and relative kinship (K) analysis. The Q was determined by using STRUCTURE 2.3.2 software. The K was calculated using SPAGeDi software. The Loiselle coefficient was used to create the pair-wise kinship matrix. The simple linear, Q, K, and Q + K models were tested to identify the best model fitting each individual trait for association mapping. Both experiments were designed in a split plot with two treatments (control and submergence). Each experiment had three replicates arranged in three different benches in a greenhouse. Statistical analysis was performed using SAS Proc Mix with both accessions and submergence as a fixed effect. The treatment effects and accession differences for each trait were tested using the Least Significant Difference (LSD) test at $P < 0.05$.

Results to date:

Across all accessions, the mean leaf color dropped from 6.0 to 4.0 in Exp1 and from 6.1 to 2.9 in Exp 2, respectively, under submerged conditions. Submergence significantly reduced HT and RGR by 16% and 35%, respectively, in Exp 1, and 24% and 26%, respectively, in Exp 2 compared to their relative controls. Fv/Fm was also significantly reduced by submergence. Variations in color, Fv/Fm, HT, and RGR values were observed in response to the flooding treatments among the accessions in both experiments.

A total of 23 associations were identified between the SSR markers and the four calculated traits (percentage of reduction) using a simple linear model, while only 15 associations were found using the Q model (Table 1).

Table 1. Association of SSR markers with percentage of reduction of leaf color (Color), chlorophyll fluorescence (Fv/Fm), maximum plant height (HT), and relative growth rate (RGR) of perennial ryegrass accessions

Trait	Locus	Chromosome No.	Position (cM)	Simple Linear Model	Structure Q Model
Color	PRG	4	119	** [a]	
Color	PR10	NA[b]	NA	**	
Color	B2F1	5	28		**
Fv/Fm	B2G6a	1	NA	**	
Fv/Fm	B3D4	1	NA	**	
Fv/Fm	LPSSRH02C1	3	NA	**	
Fv/Fm	PR14	4	116	**	
Fv/Fm	PRG	4	119	**	
Fv/Fm	LpACT13H2	6	26	**	**
Fv/Fm	LpACT14C9	6	NA	**	**
Fv/Fm	B3C11	7	57	**	**
RGR	PR39	1	54	**	**
RGR	B1A2	3	68	**	
RGR	rv0992	3	NA	**	**
RGR	LpACT13H2	6	26	**	
RGR	B3C10	7	80	**	**
RGR	LPSSRH01A0	NA	NA	**	**
HT	LpSSR085	1	47	**	**
HT	PRE	1	52	**	**
HT	LpHCA17C11	2	49.3	**	**
HT	LpSSR021	2	43	**	**
HT	rye014	6	NA	**	**
HT	LpACT43C6	7	NA	**	**
HT	B4C4	7	94	**	**

[a] Significant association at $P < 0.01$
[b] Not known

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Natural Variation of Drought Response in *Brachypodium Distachyon*

Na Luo, Xiaoqing Yu and Yiwei Jiang

Objective:

To characterize drought response of natural population of *Brachypodium Distachyon*

Rationale:

The severity and frequency of drought stress is expected to increase due to climate change, which can significantly affect plant growth and development, particularly in some cool-season species. Drought stress is highly variable in nature, with frequent changes in intensity and duration. Plant species or genotypes may employ different strategies to adapt and survive drought stress. In addition, phenotypes of drought tolerance are controlled by complex gene networks, causing potential challenges to the study of drought mechanisms. Although responses and physiological mechanisms to drought stress have been extensively studied in different plant species, research exploring natural diversity in drought tolerance is still limited in many plant species.

Brachypodium distachyon (*Brachypodium*) is a temperate wild grass species and an excellent model system for studying other grasses. *Brachypodium* is a monocot plant and has all attributes that make it suitable for modern model organisms: small genome size (~ 355 Mbp), self-fertility, small physical status, a short-life cycle, diploidy in some ecotypes, and simple growth requirements. Therefore, *Brachypodium* provides a powerful tool to facilitate research aimed at improving the crops that are used for grain, forage, turfgrass and bioenergy production. To date, genetic and genomic research resources for *Brachypodium* have been developed as a new model system for grass crop research, which will facilitate experimental applications of *Brachypodium* and functional genomics research on other grass crops.

Materials and methods:

Fifty-seven accessions *Brachypodium* were used for the experiment. The selection of these accessions was based on maximization of the geographic and genetic diversity available in natural collections of *Brachypodium*. Each accession was sown in a plastic pot (4-cm diameter, 9-cm deep) containing a sandy-loam soil with a pH of 6.9 in a greenhouse. Plants were watered every two days and fertilized once a week with a soluble fertilizer (N- P_2O_5-K_2O, 24-8-16) (Scotts Inc., Marysville, OH) and micronutrients. The duration of the experiment was from Nov. 13, 2009 to Jan. 8, 2010 for Exp1 and from Dec. 26, 2009 to Feb. 24, 2010 for Exp 2. During the growing periods, the average air temperatures were 22ºC/20ºC (day/night) for Exp 1 and 21ºC/20ºC for Exp 2. The average photosynthetically active radiation (PAR) intensity was approximately 325 µmol m^{-2} s^{-1} for both experiments, with 10 hrs light period of both natural and artificial light.

Drought stress treatment began on Dec. 28, 2009 and ended on Jan. 8, 2010 for Exp1; this treatment began on Feb. 16, 2009 and ended on Feb. 24, 2010 for Exp 2. Drought stress was imposed by withholding water from the grasses until permanent wilting occurred (the leaves were no longer rehydrated at night and in the morning). The controlled pots received regular irrigation during the treatment. During periods of drought stress, air temperatures and PAR intensity were similar to the conditions prior to stress for both experiments.

Leaf wilting was visually rated on a scale of 0 (no observable wilting) to 3 (severely wilted or almost dead). Leaf photochemical efficiency was determined by measuring the chlorophyll fluorescence (Fv/Fm) after dark on randomly selected leaves in each pot using a fluorescent meter (OS-30P, OPTI-Sciences, Hudson, NH, USA). At the end of drought stress, plants were cut 2.5 cm above the soil surface and dried in an oven at 75°C for 72 h. Plant dry weight (DW) was recorded. Leaf water content (LWC) was measured according to equation: LWC = (FW-DW)/FW × 100, where FW is leaf fresh weight, DW is dry weight. The numbers of leaves and tillers were counted before and after the treatment for each pot. The percentage of reduction of parameters was used to indicate the plant drought response, calculated as [(control-drought)/control] ×100. The total water soluble sugar (WSS) was also measured in the leaf tissues.

The experiment was arranged in a split-plot design with 3 replications in the control and drought stress treatments for both experiments. Analysis of variance (ANOVA) was performed to investigate the effects of the treatment on the accessions. The analysis of variance and Fisher’s protected LSD mean were performed using PROC MIXED (SAS Institute, Version 9.1, Cary, NC. USA). The data on Fv/Fm, LWC, DW, and leaf and tiller numbers from both the control and drought treatment as well as from the percentage of reductions in these parameters was used for principal component analysis (PCA) using MVSP 32 software. The data on LWC and Fv/Fm reductions from the two experiments were combined for cluster analysis using Origin 8.0 software to separate the groups of accessions based on the results of PCA. Since the most susceptible accessions were almost dead in Exp1 after drought stress, WSS was only analyzed using a sample collected from Exp 2.

Results to date:

Significant treatment and accession effects were generally observed for traits, with two exceptions in both experiments. Under drought stress, Fv/Fm decreased from 0.82 to 0.23 and from 0.82 to 0.56 for Exp1 and Exp2, respectively. Under the same stress condition, LWC dropped from 77.1% to 18.2% for Exp1 and from 71.2% to 19.8% for Exp 2, while DW decreased by 47.4% in Exp 1. The WSS concentration increased 1.1 fold under drought stress (Table 3). Leaf and tiller numbers were also significantly reduced by drought stress.

Principle component analysis (PCA) revealed that reductions in chlorophyll fluorescence (Fv/Fm) and leaf water content (LWC) under drought stress explained most of the phenotypic variation, which was used to classify the tolerant and susceptible accessions. Four groups of accessions differing in drought tolerance were identified, with 3 tolerant, 16 moderately tolerant, 32 susceptible, and 6 most susceptible accessions. The tolerant group had little leaf wilting and fewer reductions in Fv/Fm and LWC, while the most susceptible groups showed severe leaf wilting and more reductions in Fv/Fm and LWC (Fig. 1). Drought stress increased total water soluble sugar concentration (TSS), but no differences in the increased TSS were found among different groups of accessions. The large phenotypic variation of

Brachypodium in response to drought stress can be used to identify genes and alleles important for the complex trait of drought tolerance.

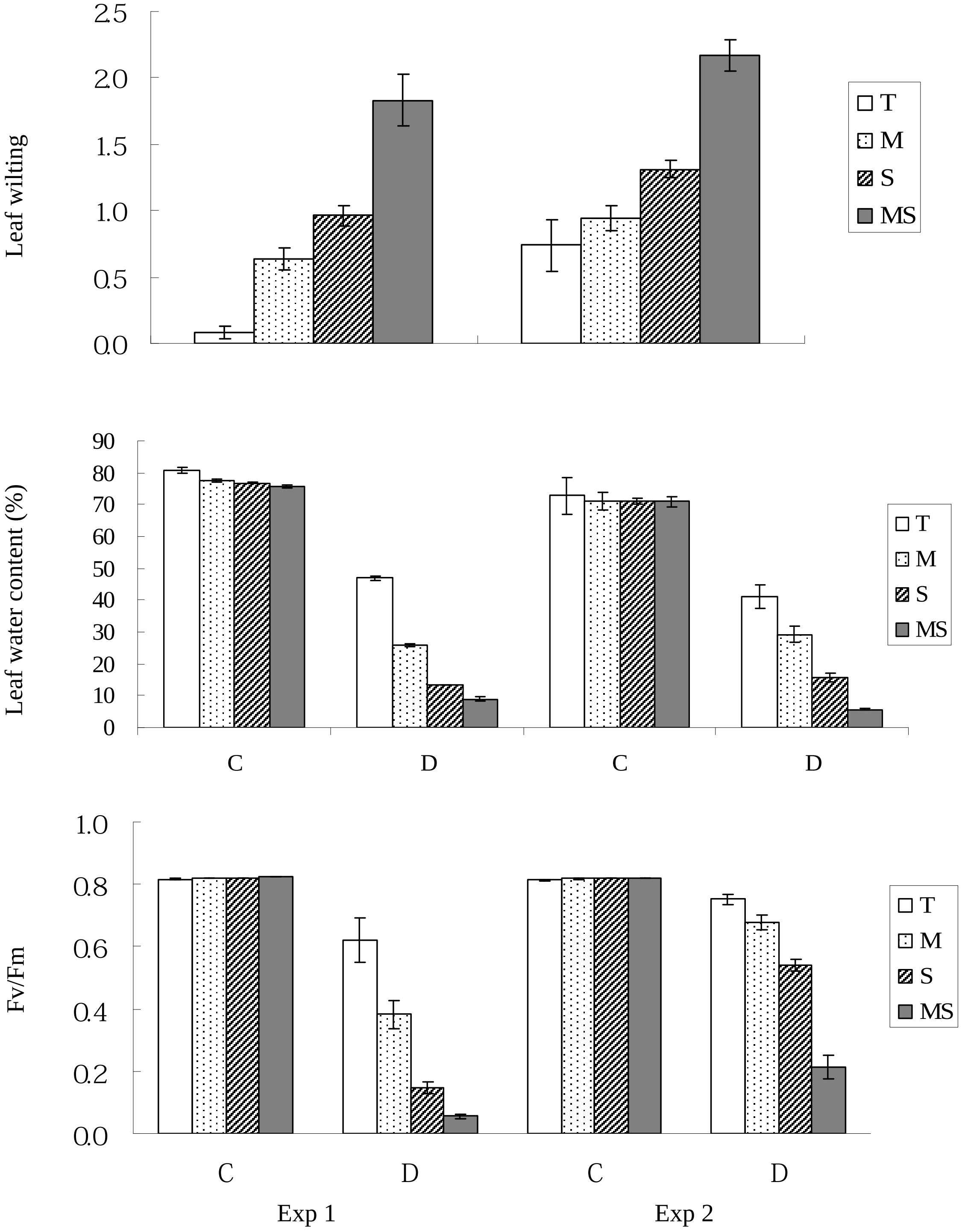

Fig.1. Leaf wilting, photochemical efficiency of PSII (Fv/Fm) and leaf water content (LWC) of *Brachypodium distachyon* under control (C) and drought stress (D) for experiment 1 (Exp1) and experiment 2 (Exp 2). T, M, S, and MS represent the tolerant, moderately tolerant, susceptible, and the most susceptible accessions. Vertical bars show ± S.E.

Removing perennial ryegrass from Kentucky bluegrass sports fields.

Aaron Patton, Assistant Professor – Turfgrass Extension Specialist, Purdue University
Dan Weisenberger, Research Technician, Purdue University
Zac J. Reicher, Professor - University of Nebraska Lincoln

Objective:

1. How do Corsair and Certainty perform removing perennial ryegrass from a stand of Kentucky bluegrass when applied in November, April, or June?
2. What efficacy can be expected with Corsair and Certainty at low, medium and high application rates?
3. Will Corsair or Certainty cause phytotoxicity to Kentucky bluegrass at these application timings and/or rates?

Rationale:

Sports fields managers often overseed Kentucky bluegrass (*Poa pratensis* L.) fields with perennial ryegrass (*Lolium perenne* L.) during the playing season. Perennial ryegrass germinates more quickly than other species thus helping with stand density and aesthetics. During non-playing times of the year when the manager is trying to encourage the field to fill-in and recover from use, perennial ryegrass does not spread and fill-in thin areas like Kentucky bluegrass. Also, perennial ryegrass is more prone to disease during summer than Kentucky bluegrass. This study was initiated to find a herbicide and timing of application that would remove the perennial ryegrass from the playing surface and allow Kentucky bluegrass to fill-in bare areas so that disease can be decreased on athletic fields and fields will be more capable of recovery.

Materials and methods:

The experiment was conducted at the W.H. Daniel Research and Diagnostic Center in West Lafayette, IN. An area was seeded to a four way blend of perennial ryegrass September 16, 2009 to simulate overseeding a sports field in the fall. A second area was an establish Kentucky bluegrass blend.

Experimental design was a randomized complete block with three replications and an individual plot size of 25 ft^2. Plots were mown twice a week at 2.5 inches. Plots were treated with herbicides on October 29, 2009, April 2, 2010, or June 4, 2010. Herbicides were applied in 87 gpa water with a CO_2-pressurized sprayer at 30 psi.

Perennial ryegrass coverage was collected by counting the intersections of a transect and then converted to percent cover. Injury was visually rated on a 1 to 9 scale with 7 or greater being acceptable injury. All data were analyzed using SAS

(SAS Institute, Inc). Data were analyzed as a 3 X 2 X 3 factorial with 3 timings of application (Nov. April, June), 2 herbicides [Corsair (chlorsulfuron), Certainty (sulfosulfuron)], and three rates of application. The untreated check was excluded during analysis.

Results to date:

- Although some injury to Kentucky bluegrass was observed it was not rated as unacceptable on any of the rating dates (data not shown).
- Certainty was only among the top treatments for the 6/4/10 application at 3 oz prod/A rate (Table 1).
- Corsair was part of the top treatments on all dates and rates with the exception of 10/29/09 and 4/2/10 at 2 oz prod/A.
- Corsair reduced ryegrass cover more than Certainty in this study.
- Corsair reduced ryegrass cover more on all three application dates at the 4.5 and 7 oz prod/A rates.
- The label maximum use rate for Corsair is 5.33 ounce of product per acre, and the rate for removing perennial ryegrass is 1 to 2 ounces product per acre.
- The label maximum use rate for Certainty is 2 ounces product per acre, and the rate for removing perennial ryegrass is 2 ounces product per acre.
- Thus, managers interested in reducing perennial ryegrass in Kentucky bluegrass swords could make a single application of Corsair at 4.5 oz/A in October or April or 2.0 oz/A in early June. The earlier the perennial ryegrass is removed the more recovery time there will be for Kentucky bluegrass.

Table. 1. Perennial ryegrass cover following applications of Corsair and Certainty.

Application timing	Herbicide	Rate of application	Cover[a] ryegrass July
		oz prod/A	
10/29/09	Corsair	7	4 a[b]
10/29/09	Corsair	4.5	3 a
10/29/09	Corsair	2	53 b
4/2/10	Corsair	7	3 a
4/2/10	Corsair	4.5	17 a
4/2/10	Corsair	2	57 b
6/4/10	Corsair	7	0 a
6/4/10	Corsair	4.5	0 a
6/4/10	Corsair	2	5 a
10/29/09	Certainty	3	64 bc
10/29/09	Certainty	2	84 cd
10/29/09	Certainty	1	78 cd
4/2/10	Certainty	3	86 cd
4/2/10	Certainty	2	94 d
4/2/10	Certainty	1	91 d
6/4/10	Certainty	3	21 a
6/4/10	Certainty	2	82 cd
6/4/10	Certainty	1	94 d
Untreated for comparision			96

[a] Cover was calculated from intersection count of transect. There were 49 possible intersections. The formula was (number of intersections with rye/49)*100 .

[b] Numbers followed by the same letter are not significantly different.

Effect of Tenacity on Annual Bluegrass Control in Kentucky Bluegrass

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Objective:

1. To evaluate multiple application scenarios of Tenacity for control of annual bluegrass in Kentucky bluegrass.
2. To evaluate two application start dates for the control of annual bluegrass with Tenacity.

Rationale:

Annual bluegrass (*Poa annua* L.) is a problem weedy grass in all areas of turf management. Annual bluegrass completes its life cycle and dies in summer leaving thin open areas for the invasion of other weeds. Tenacity (mesotrione) has shown promise in removing annual bluegrass from perennial grasses. This research was done to look at multiple applications in a number of different scenarios and two initial timings of application for annual bluegrass removal from a Kentucky bluegrass (*Poa pratensis* L.) stand.

Materials and methods:

The experiment was conducted on a mixed stand of Kentucky bluegrass and annual bluegrass at the W.H. Daniel Research and Diagnostic Center in West Lafayette, IN.

The experimental design was randomized complete block with three replications and an individualized plot size of 25 ft^2. Plots were mown as needed at 1.5 inches. Herbicides were applied in 87 gpa water with a CO_2-pressurized sprayer at 30 psi. Application rates and intervals are listed in Table 1.

Annual bluegrass coverage was visually rated. All data were analyzed using SAS (SAS Insitute, Inc). Data were analyzed as a 2 X 9 factorial with 2 initial application dates (Sept. 2, 2009 and Sept. 28, 2009) and 9 herbicide treatments.

Results to date:

- A similar response was seen for both start dates (Sept. 2, 2009 and Sept. 28, 2009) and so data was pooled across start dates and the results described below should be expected with either start date.
- Annual bluegrass was reduced by all treatments.

- There was no advantage to applying 3 applications per week vs. 2 applications per week.
- Mesotrione at 0.1 lb ai/A applied 2 times per week for 5 applications, mesotrione at 0.05 lb ai/A applied 2 times per week for 5 applications, and mesotrione at 0.05 lb ai/A applied 3 times per week for 5 applications were best treatments on all three ratings.
- Annual bluegrass coverage was reduced up to 35% in June and up to 60% in May for the best treatments on each date when compared to the untreated check.

Table 1. Annual bluegrass coverage pooled over two initial application dates following applications of Tenacity using eight different control strategies.

Herbicide	Application rate		Total application	Application Timing[a]	annual bluegrass coverage		
					April	May	June
	lb ai/A	oz prod/A					
Tenacity[b]	0.166	5.3	3	every 2 wks	34[c]b	41 b	33 bc
Tenacity	0.1	3.2	5	every 2 wks	21 cde	37 b	32 bcd
Tenacity	0.1	3.2	5	1 time/wk	25 bc	33 bc	39 ab
Tenacity	0.1	3.2	5	2 times/wk	17 cde	21 cd	28 bcd
Tenacity	0.1	3.2	5	3 times/wk	26 bc	33 bc	23 cd
Tenacity	0.05	1.6	10	2 times/wk	14 de	13 d	19 cd
Tenacity	0.05	1.6	10	3 times/wk	13 e	18 d	18 d
Tenacity[d]	0.15	4.8	5	1 time/wk	24 bcd	37 b	20 cd
Tenacity	0.08	2.6					
Untreated					65 a	73 a	53 a
P-value					<0.0001	<0.0001	0.0005

[a] Initial applications were Sept. 2, 2009 and Sept. 28, 2009

[b] All applications of Tenacity (mesotrione) included a NIS at the rate of 0.25% v/v.

[c] Numbers followed by the same letter are not significantly different.

[d] Initial application was 0.15 lb ai/A and the four following applications were 0.08 lb ai/A.

Controlling *Poa annua* on putting green height turf in Indiana, Kentucky, Michigan, and Nebraska
December 30, 2010

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Annual bluegrass *(Poa annua)* is the most troublesome and probably the most studied weed on golf courses throughout the United States. A number of herbicides and growth regulators are labeled and effective for *Poa annua* control on fairway height turf including bispyribac (Velocity), ethofumesate (Prograss), flurprimidol (Cutless) and paclobutrazol (Trimmit, TGR). As turfgrass extension specialists, we often enter discussions about how to limit or control *Poa annua* on putting greens. Outside of the typical cultural methods for exclusion on new putting greens, we have little confidence in growth regulators or herbicides because of labeling issues and the following three reasons:

1. Most of the previous research was done on fairways. For instance, Woosley et al. (2003) showed multiple applications of paclobutrazol to effectively control *Poa annua* at fairway height in Kentucky. Bigelow et al (2007) reported excellent suppression of *Poa annua* at fairway height turf in Indiana with flurprimidol. Type II plant growth regulators such as paclobutrazol or flurprimidol inhibit growth of the *Poa annua* more than creeping bentgrass, thus giving the bentgrass a competitive advantage to overtake the weakened *Poa*. This works well at fairway height as the creeping bentgrass can effectively and aggressively outgrow the over-regulated *Poa annua*. However, this approach is not well documented on greens height turf. The ability for creeping bentgrass maintained under the stressful putting greens environment to outgrow *Poa annua* will be greatly reduced, regardless of differential growth regulation. This same theory may also apply to herbicides that would control the *Poa annua* more quickly than growth regulators, but we are unsure if greens-height creeping bentgrass can fill in voids left by the dying *Poa annua* and if potential reductions in bentgrass quality following applications of herbicides would be acceptable.

2. Most of the previous PGR research was done with monthly applications and/or either summer or fall applications. Johnson and Murphy (1995 and 1996) reduced *Poa annua* by 20% or more in creeping bentgrass putting greens in GA with either spring or fall applications. Isgrigg et al. (1999a and b) used paclobutrazol or flurprimodol to reduce *Poa annua* populations by up to 80% in near Pinehurst, NC. Since fungicides are now applied every two weeks to most putting greens throughout the season, we can easily include growth regulators or herbicides in the tankmix with very little extra labor involved. Furthermore, recent research and practical experience suggests frequent applications at lower rates of growth regulators or herbicides are often more consistent and effective than infrequent applications at higher rates.

3. Most of the putting greens-height research was done in the southeast United States. For instance, Teuton et al (2007) showed bispyribac to be up to 99% effective on controlling putting greens-height *Poa annua* in Tennessee. Jackson and Murphy (1995 and 1996)

reduced *Poa annua* by 20% or more in creeping bentgrass greens in Georgia with either spring or fall applications. Work from Isgrigg et al. (1999a and b) mentioned previously was done near Pinehurst, NC. Growth regulators minimally increase the competitive ability of creeping bentgrass over *Poa annua*. The extended summer environmental pressures of southern sites likely favor the creeping bentgrass even more over annual bluegrass. A longer summer could weaken *Poa annua* more than creeping bentgrass and the additional stress of an applied growth regulator may tip the balance in favor of creeping bentgrass. However, in the northern regions of the US, summer stresses may not be enough to tip the balance significantly.

Because of these issues, we are evaluating seven season-long treatments of growth regulators or herbicides to control *Poa annua* on putting greens. By completing identical studies at four locations that differ widely geographically, we are able to extrapolate our results to a large portion of the United States.

How it's being done

Plots of green-height annual bluegrass/creeping bentgrass are already established on putting greens that are mowed daily at 0.125" and sand-topdressed regularly. The areas receive 2.5 to 3.0 lbs N/1000 sq ft/yr with 1.0 lb N in Sep and Nov, 0.5 lb N in late May and late June. Treatments are applied in 2 gals water/1000 sq ft and include:

1. Velocity 17.6WSP @ 1 oz product/acre on 14-day interval from mid-May to 2nd week September (8 apps)
2. Velocity 17.6WSP @ 2 oz product/acre on 7-day interval from mid-Aug to 2nd week September (4 apps)
3. Helena HM9930 @ 131 oz product/ acre applied mid-April and mid-August
4. Trimmit 2SC @ 8 oz product/acre on a 14-day interval from mid-April through mid-May, 16 oz/acre Mid-May to end of July, and 8 oz product/acre on a 14-day interval from August through mid-September (12 apps)
5. Cutless @ 8 oz product/acre on 14-day interval from mid-April to mid-May, 16 oz/acre mid-May through August, and 8 oz product/acre in September (12 apps)
6. Legacy (Primo/Cutless) @ 10 oz/acre mid-May to end of Sept (12 apps)
7. Primo Maxx @ 11 oz/acre on a 14-day interval from Mid-May to end of September (12 apps)
8. Untreated check

Most of these treatments are within label limits, and are based on superintendents and label recommendations as well as previous research experience. The Velocity treatments (2 and 3) may be added to the label in the future. Treatment 3 is an experimental herbicide with potential for *Poa annua* control (Askew et al., 2009) .

Visual quality and percent cover of creeping bentgrass and *Poa annua* are recorded monthly and transect counts are taken in mid-May and mid-August, the expected high and low points for *Poa annua* populations, respectively. The transect counts minimize subjectiveness between rates and will allow reliable comparisions between years within locations and across locations. This study has been underway since May 2009 in West Lafayette, IN; Lexington, KY; and East Lansing, MI, and since May 2010 in Lincoln, NE. We expect it to last a minimum of two more years at each location. Table 1 highlights applications and data recording for this study.

Results to date

- *Poa annua* populations naturally are at a seasonal high in April or May, drop to a seasonal low in August and then return to a seasonal high the following spring. Our data show that regardless of treatment, *Poa* cover dropped dramatically over the summer to almost insignificant populations (Figs. 1 and 2). Therefore, one could deduce incorrectly that their strategy is working if no untreated area for comparison is included on the course.
- *Poa annua* control was highly variable from location to location and among years (Figs. 3-6). Though data were recorded on 38 dates over the four locations, treatment differences were only evident on 0 to 4 dates in each location/year or on a total of 18 data recording dates so far.
- The best treatments improved *Poa annua* control vs the untreated check on 11 of the 18 (61%) dates. These results help explain the highly variable anecdotal results from superintendents across the country (Table 2).
- After two years of treatments, even the best performing treatments are not decreasing *Poa annua* cover significantly over the long term (Fig 7).

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Table 1. Dates of application and data recording in the greens-height *Poa annua* control study in Indiana, Kentucky, Michigan, and Nebrasaka. Actual dates are +/- 1 week depending on location.

Month	Week	Trt 1 Velocity	Trt 2 Velocity	Trt 3 HM9930	Trt 4 Trimmit	Trt 5 Cutless	Trt 6 Legacy	Trt 7 Primo	Trt 8 UTC	Percent cover	Transect counts
Apr	2			131 oz/A	8oz/A	8oz/A	10 oz/A	11 oz/A			
Apr	3									X	
Apr	4				8oz/A	8oz/A	10 oz/A	11 oz/A			
May	1										
May	2				8oz/A	8oz/A	10 oz/A	11 oz/A			
May	3									X	X
May	4	1 oz/A			8 oz/A	16 oz/A	10 oz/A	11 oz/A			
June	1										
June	2	1 oz/A			16 oz/A	16 oz/A	10 oz/A	11 oz/A			
June	3									X	
June	4	1 oz/A			16 oz/A	16 oz/A	10 oz/A	11 oz/A			
July	1										
July	2	1 oz/A			16 oz/A	16 oz/A	10 oz/A	11 oz/A			
July	3									X	
July	4	1 oz/A			16 oz/A	16 oz/A	10 oz/A	11 oz/A			
Aug	1										
Aug	2	1 oz/A		131 oz/A	8oz/A	16 oz/A	10 oz/A	11 oz/A			
Aug	3		2 oz/A							X	X
Aug	4	1 oz/A	2 oz/A		8 oz/A	16 oz/A	10 oz/A	11 oz/A			
Sep	1		2 oz/A								
Sep	2	1 oz/A	2 oz/A		8 oz/A	8 oz/A	10 oz/A	11 oz/A			
Sep	3									X	
Sep	4				8 oz/A	8 oz/A	10 oz/A	11 oz/A			
Oct	1										
Oct	2										
Oct	3									X	

Table 2. Summary of *Poa annua* control from season-long treatments on putting green height turf. Though *Poa* cover was rated a total of 38 dates among the locations, differences in *Poa* control was detected on only 18 dates.

Trt #	Product	Application Rate	Application Frequency	Application Dates	Total number of applications	Percentage of dates where *Poa annua* control was greater than the untreated check
		oz/Acre				%
1	Velocity WSP	1	2 wks	May-Sep	8	55
2	Velocity WSP	2	2 wks	Aug-Sep	4	44
3	HM9530	130	5 mo	Apr, Aug	2	50
4	Trimmit	8; 16	2 wks; 2 wks	Apr-May, Aug-Sep; June-July	8 4	61
5	Cutless	8; 16	2 wks; 2 wks	Apr-May; May-Aug	5 7	61
6	Legacy	10	2 wks	Apr-Sep	12	33
7	Primo	11	2 wks	Apr-Sep	12	28

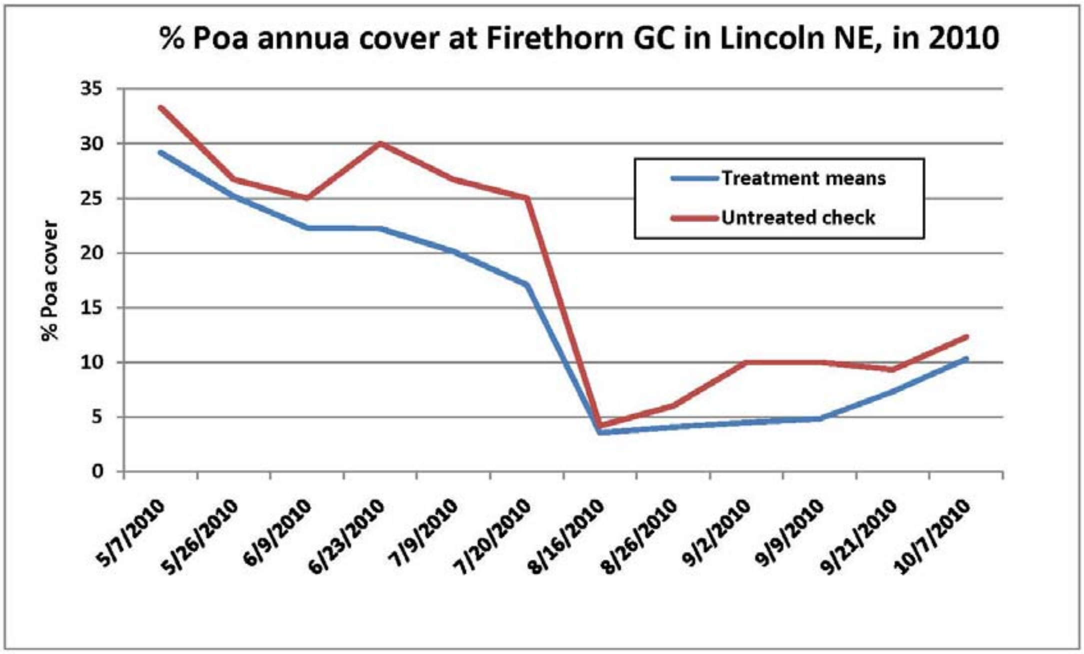

Figure 1. Percent cover of *Poa annua* visually rated in 2010 at the Nebraska site. Cover of the treatments was averaged over the 7 treatments. Regardless of treatment, *Poa annua* cover decreases naturally in August. This suggests that success of *Poa annua* control strategies could be misinterpreted as successful if untreated areas are not included for comparison.

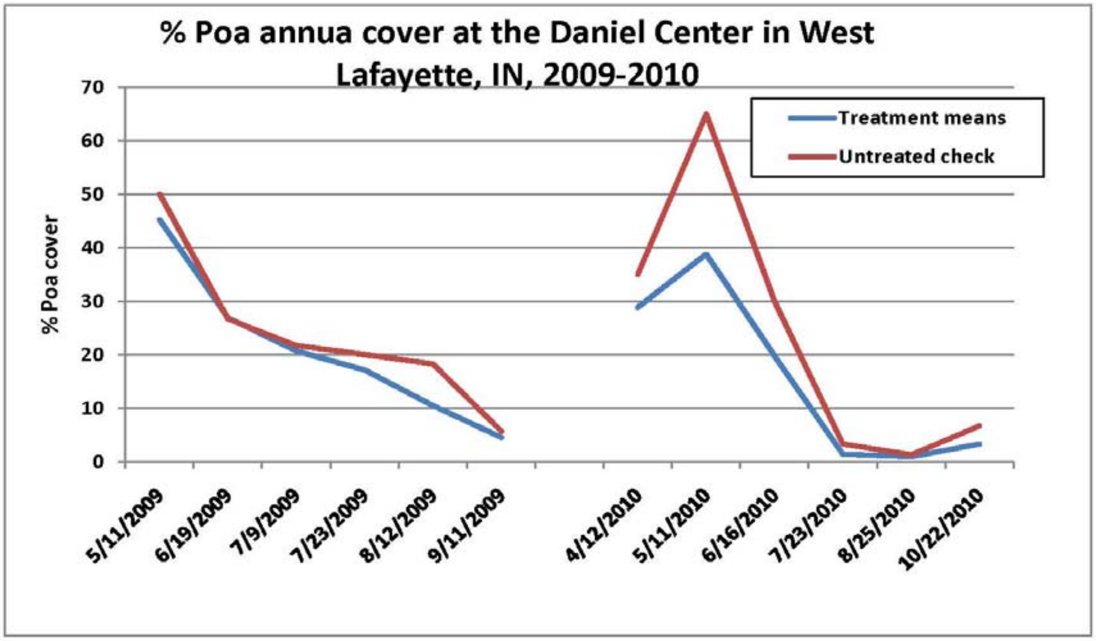

Figure 2. Percent cover of *Poa annua* visually rated in 2009-2010 in Indiana. Cover of the treatments was averaged over the 7 treatments. Regardless of treatment, *Poa annua* cover decreases naturally in August. This suggests that success of *Poa annua* control strategies could be misinterpreted as successful if untreated areas are not included for comparison.

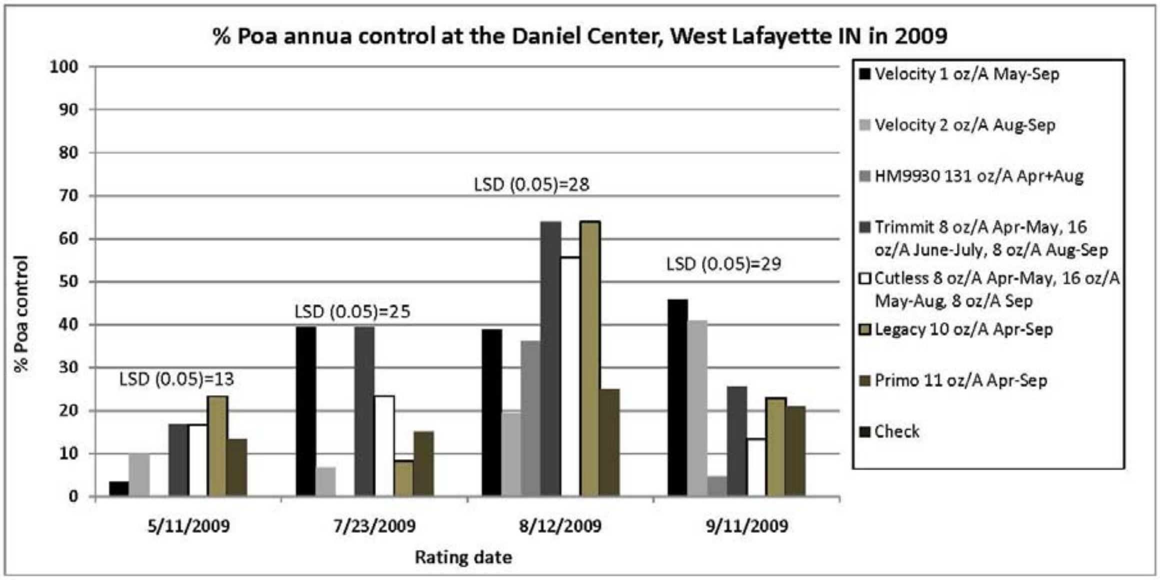

Figure 3. Percent *Poa annua* control in 2009 in Indiana. *Poa annua* cover was visually rated and then converted to percent control.

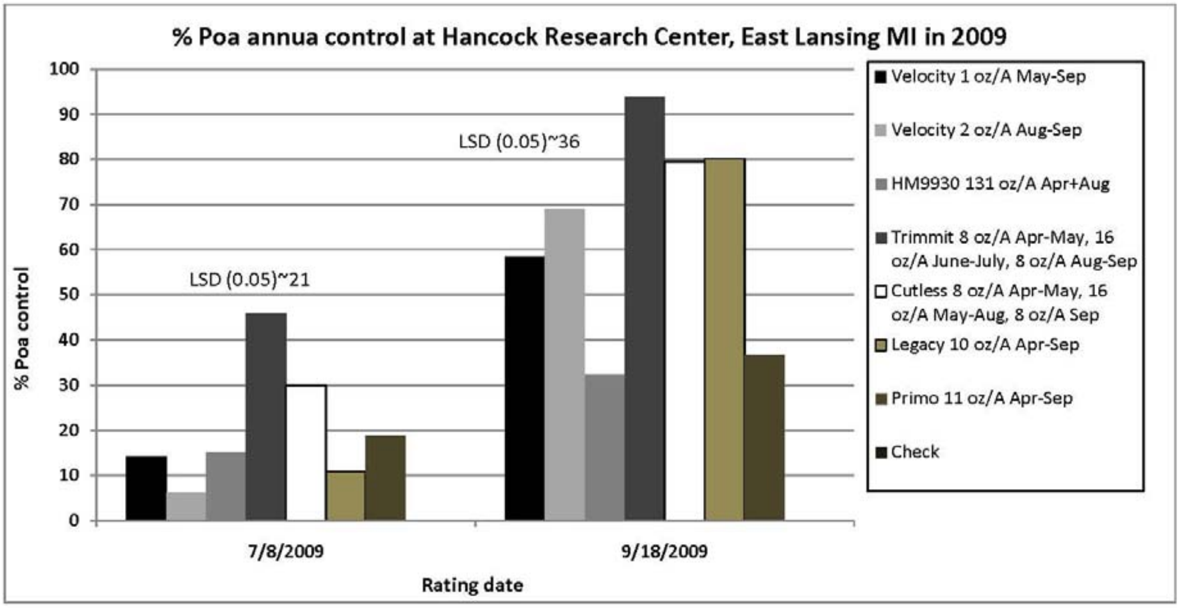

Figure 4. Percent *Poa annua* control in 2009 in Michigan. *Poa annua* cover was counted using a transect and then converted to percent control.

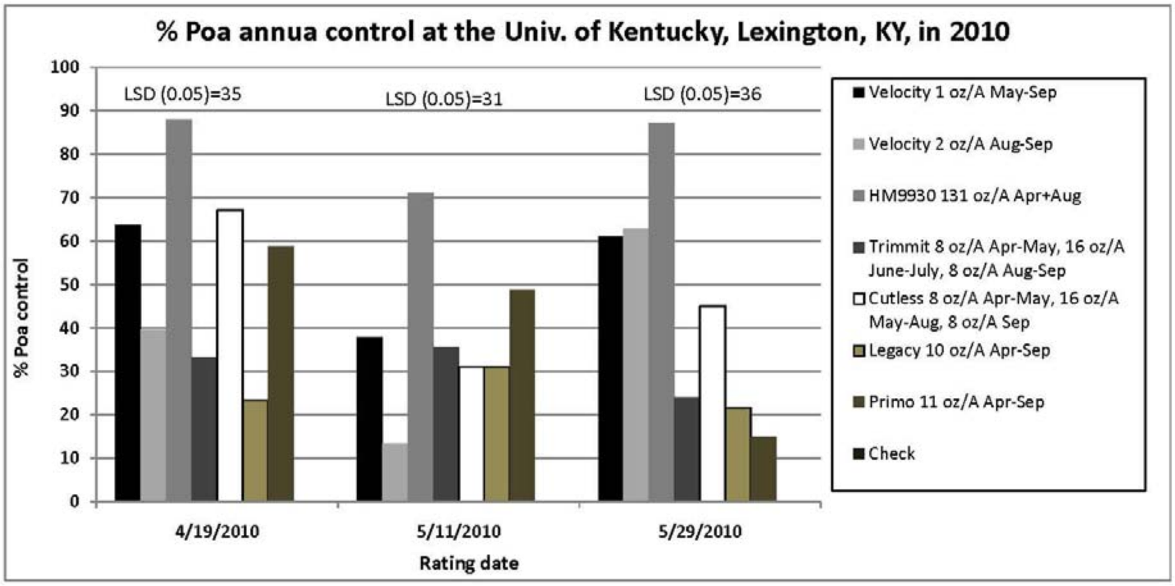

Figure 5. Percent *Poa annua* control in 2010 in Kentucky. *Poa annua* cover was visually rated and then converted to percent control.

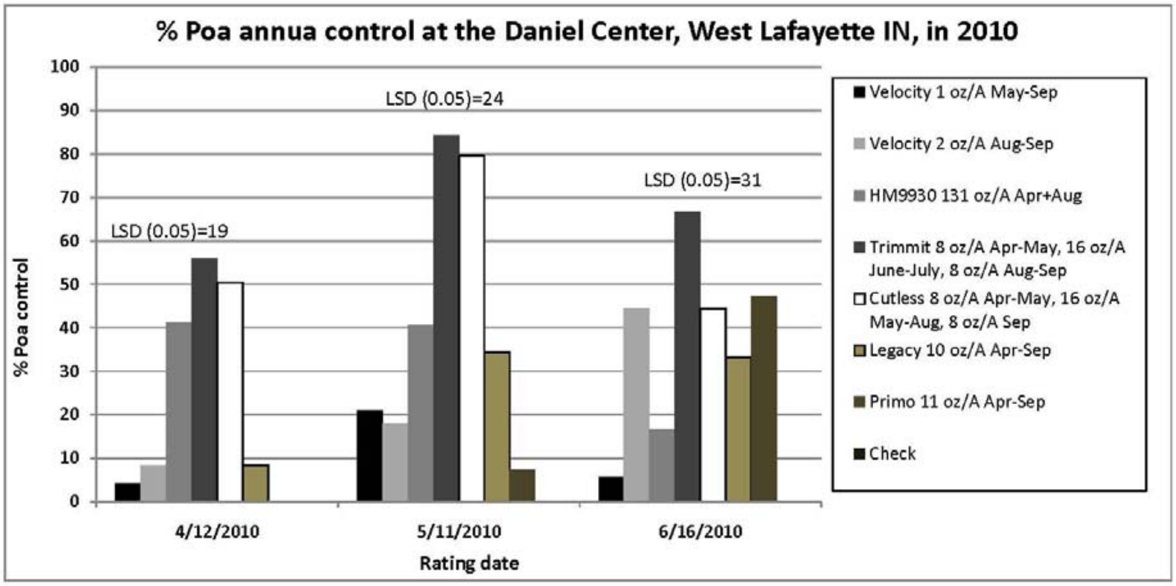

Figure 6. Percent *Poa annua* control in 2010 in Indiana. *Poa annua* cover was visually rated and then converted to percent control.

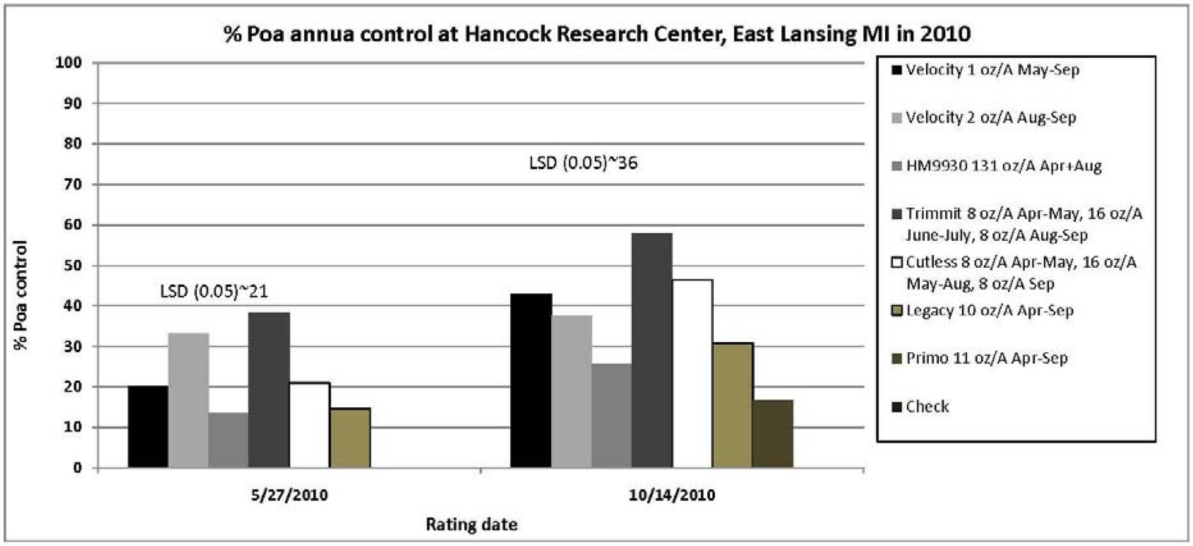

Figure 7. Percent *Poa annua* control in 2010 in Michigan. *Poa annua* cover was counted using a transect and then converted to percent control.

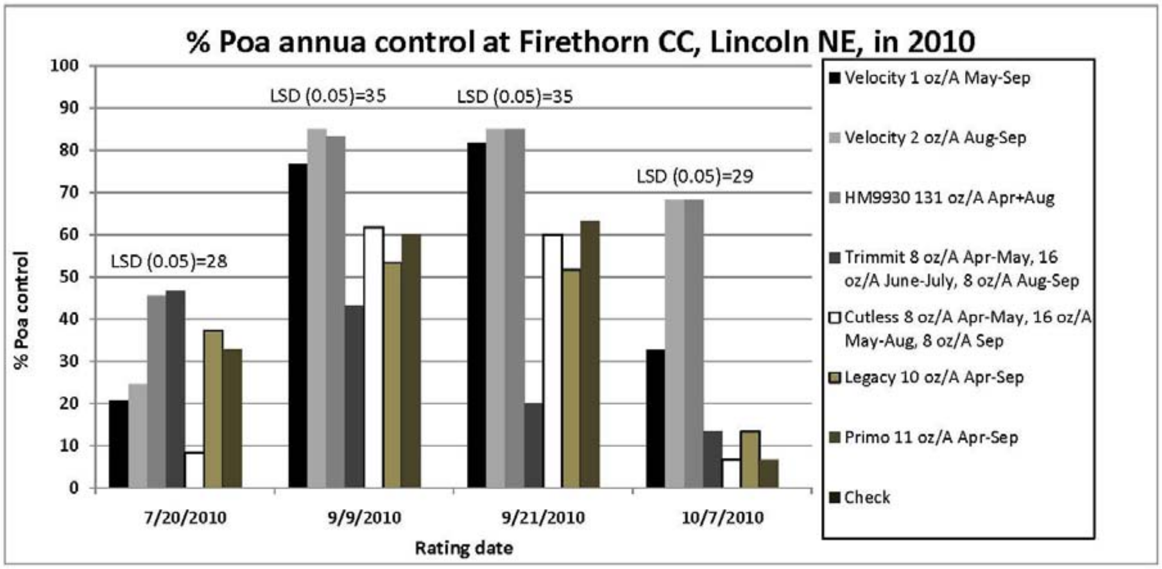

Figure 8. Percent *Poa annua* control in 2010 in Nebraska. *Poa annua* cover was visually rated and then converted to percent control.

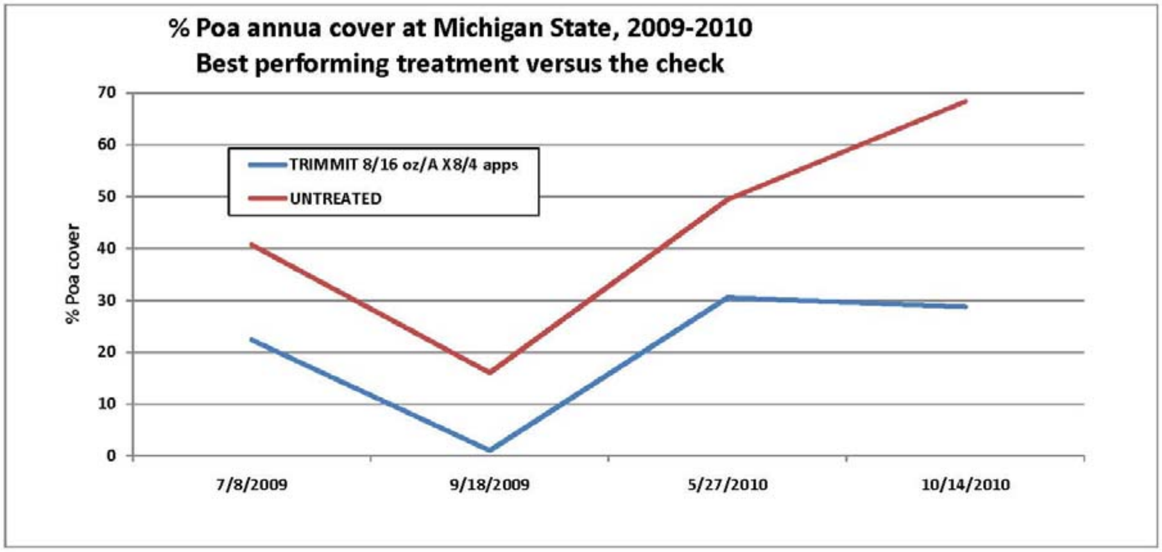

Figure 9. Percent *Poa annua* cover in 2009 through 2010 in Michigan. *Poa annua* cover was counted using a transect and thus comparisons over time are valid. Trimmit was the best performing treatments at this site and even though 24 applications were made over two year, little significant long term reduction in *Poa* coverage is observed.

Tenacity Application for the Control of Annual bluegrass

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Objectives:

1. Determine if there is a difference between using 5 oz prod/A or 8 oz prod/A of Tenacity for controlling annual bluegrass.
2. Determine if an application of Tenacity in the spring following fall applications increases efficacy.
3. Determine what timing of the first application of Tenacity in the fall gives the best efficacy.

Rational:

Annual bluegrass (*Poa annua* L.) is a problem weedy grass species in all turf systems. Annual bluegrass completes its life cycle and dies in summer leaving thin open areas for the invasion of other weeds. Tenacity (mesotrione) has shown promise in removing annual bluegrass from perennial grasses. This research was done to look at rates of application and timing of applications for annual bluegrass removal from a Kentucky bluegrass (*Poa pratensis* L.) stand.

How it was done:

The experiment was conducted at the Ackerman Golf Course, Purdue University, West Lafayette, IN during 2008 and 2009. The area was a mixed stand of Kentucky bluegrass and annual bluegrass.

Experimental design was a randomized complete block with three replications and an individual plot size of 25 ft^2. Plots were mown at 0.7 inches 2 or 3 times per week with clippings retuned. Herbicides were applied in 87 gpa water with a CO_2-pressurized sprayer at 30 psi. Application dates and rates are listed in Table 1.

Annual bluegrass was visually rated for percent coverage. The main effect of year was tested by Bartlett's test for homogeneity and it was determined they could be combined for analysis. All data were analyzed using SAS (SAS institute, Inc).

Results and Discussion:

- When rated in May, all the treatments with Tenacity at 5 oz prod/A, the three treatments of Tenacity at 8 oz prod/A with applications starting in September, and the Prograss treatment with three fall applications gave the best results (Table 1). But for the June rating, all the treatments with Tenacity at 5 oz prod/A and one treatment of Tenacity at 8 oz prod/A applied mid Sept, end Sept, mid April gave the best results. From this we would conclude that three applications of Tenacity at 5 oz prod/A in the fall would be the best strategy.

- Although the treatments with Tenacity at 5 oz prod/A with three applications in the fall followed by an application in the spring were among the best they did not out perform three applications of Tenacity at 5 oz prod/A in the fall only. Therefore the spring application did not increase efficacy.
- Of the treatments that performed best during the June rating, it did not seem to make a difference when the first application was made. Therefore starting in mid-September, end of September, or mid October all worked equally well.
- These results were obtained under the conditions of our experiment. Annual bluegrass is known to have tremendous genetic and phenotypic diversity and it is not clear whether our findings might be consistent with those of other regions.

Table 1. Annual bluegrass (*Poa annua* L.) coverage as affected by applications of Tenacity (mesotrione).

Herbicide	Rate	Number of applications	Application timing[a]	annual bluegrass coverage (%) May	annual bluegrass coverage (%) June
	oz prod/A				
Tenacity[b]	5	3	mid Sept, end Sept, mid Oct	46 bcd[c]	37 cde
Tenacity	5	3	end Sept, mid Oct, end Oct	31 cd	27 de
Tenacity	5	3	mid Oct, end Oct, mid Nov	31 cd	27 de
Tenacity	8	2	mid Sept, end Sept	55 bc	50 abc
Tenacity	8	3	mid Sept, end Sept, mid April	43 bcd	33 cde
Tenacity	8	2	end Sept, mid Oct	48 bcd	43 bcd
Tenacity	8	3	end Sept, mid Oct, mid April	50 bcd	41 cd
Tenacity	8	2	end Oct, mid Nov	58 b	52 abc
Tenacity	8	3	mid Oct, mid Nov, mid April	68 ab	49 abc
Tenacity	5	4	mid Sept, end Sept, mid Oct, mid April	50 bcd	38 cde
Tenacity	5	4	end Sept, mid Oct, end Oct, mid April	43 bcd	22 e
Tenacity	5	4	mid Oct, end Oct, mid Nov, mid April	25 d	22 e
Prograss	64	3	mid Sept, early Oct, late, Oct	51 bcd	49 abc
Prograss	64	3	mid Sept, early Oct, mid April	62 b	60 ab
Untreated				89 a	67 a
P-value				<0.0001	<0.0001

[a] Fall applications were made on a two week split.

[b] All applications of Tenacity included a NIS at the rate of 0.25%v/v.

[c] Numbers followed by the same letter are not significantly different.