USE OF A RANDOMIZED NESTED BLOCK DESIGN IN GENETICALLY MODIFIED, NON-SELECTIVE HERBICIDE RESISTANT SUGARBEET HYBRID TESTING

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Introduction

Field and small plot testing of genetically modified, non-selective herbicide resistant (GMNSR) sugarbeet, such as glyphosate- or glufosinate-resistant hybrids has been conducted since the early 1990s. This technology is expected to obtain a substantial share of the sugar beet seed market in the next couple of years. However, non-GMNSR varieties will continue to have utility within some sugarbeet markets until seed production levels can meet market demand and/or until GMNSR varieties obtain a disease resistance package capable of handling disease situations that currently require varieties with a specialty designation for a specific disease. Until all sugarbeet production areas have converted to 100% GMNSR, testing of GMNSR lines with non-GMNSR lines in the same experiments will be necessitated.

Objectives

Small plot research with experimental GMNSR sugarbeet hybrids can be problematic. Current GMNSR sugarbeet hybrid testing requires that the GMNSR sugarbeet trial be separated from the non-GMNSR commercial and semi-commercial trials to avoid non-target unintended exposure to herbicide. Non-GMNSR commercial hybrids are typically required within the GMNSR sugarbeet hybrid trial as a means to facilitate comparisons of GMNSR sugarbeet production data to the currently approved commercial non-GMNSR hybrids for determination of potential market approval at Southern Minnesota Beet Sugar Cooperative (SMBSC) and other sugarbeet processing facilities. SMBSC selects three to four commercial hybrid checks that are currently approved for use for the cooperative and are being tested in an adjacent commercial hybrid trial. These varieties are placed within the GMNSR sugarbeet hybrid trial to convert GMNSR hybrid performance data to commercial trial status. However, when commercial non-GMNSR check hybrids are randomized within the GMNSR sugarbeet trial in a typical experimental design such as a Lattice or Randomized Complete Block Design, non-target movement of herbicide sprays can occur and cause injury to non-resistant hybrids even when extraordinary measures are utilized in the application. This may prevent reliable comparisons of GMNSR hybrids to conventional commercial hybrids. Further, when commercial check hybrids are randomized within a GMNSR sugarbeet trial, planting of additional GMNSR hybrid border rows on either side of a GMNSR hybrid experimental unit is required to reduce herbicide drift to adjacent non-GMNSR hybrids and requires small plot spray equipment to maneuver within the plot area. Although the greatest concern is the movement of a non-selective herbicide onto susceptible commercial checks; non-target herbicide movement will impact the quality of the trial data whether the non-selective herbicide moves to a commercial hybrid or if a conventional sugarbeet herbicide moves to a GMNSR hybrid. Therefore, a design was needed for GMNSR hybrid testing that would allow spray techniques that were more typical of commercial applications but would also allow inclusion of non-GMNSR check hybrids into the randomization while preventing injury from non-target spray drift.

Statistical Methods

Design of valid yield trial experiments that allows for comparison of GMNSR versus non-GMNSR hybrids or treatments is made difficult by the type of treatments that are applied. To illustrate this dilemma, consider yield trial experiments for the comparison of glyphosate resistant versus conventional type sugarbeet hybrids. A design that may appear suitable for this type of experiment is a split plot design, with herbicide treatment (no herbicide versus herbicide) as the whole plot and hybrids as the subplot. However, this design is not appropriate because it requires that all subplot treatments in each of the whole plots be the same. In the trials comparing glyphosate resistant versus conventional sugarbeet hybrids, this would require that all hybrids in the trial be sprayed with glyphosate, resulting in death of the non-resistant hybrids. Thus, statistically valid comparisons between genotypes within a class (e.g., GMNSR or non-GMNSR) or between the mean of GMNSR versus non-GMNSR hybrids or treatments averaged across hybrids within a class would be impossible.

A design that allows for comparisons to be made for the situation described above is the two-stage nested design (Montgomery, 2005). A way to envision this design is to think of subplots consisting of hybrids, but herbicide resistant hybrids would be in the whole plot that receives herbicide and non-resistant hybrids would be in the whole plot that receives no herbicide. This design and the analysis would allow statistically valid comparisons among genotypes within a herbicide treatment class and between herbicide treatments, averaged across hybrids within a class. The sources of variation, degrees of freedom, and methods for calculating the *F*-statistics for the two-stage nested design are presented in Table 1.

Table 1. Sources of variation, degrees of freedom, and method for calculating the *F*-statistic for the two-stage nested design.

Sources of variation	Degrees of freedom†	Method for calculating the *F*-statistic
Replicate	r-1	Replicate MS/Error MS
Herbicide	h-1	Herbicide MS/Error MS
Genotypes(Herbicide)	(b - 1) + (nb - 1)	Genotype(Herbicide) MS/Error MS
Error	(r - 1) x (G – 1)	
Total	(r x G) - 1	

†r = number of replicates, h = number of herbicide treatments, b = number of biotech hybrids, nb = number of non-biotech hybrids, and G = total number of hybrids in the experiment (i.e., b + nb).

Mean separation between hybrids within a class (i.e., GMNSR or non-GMNSR) or between the mean of the classes, averaged across genotypes within a class can be done. The formula for calculating the least significant difference (LSD) between genotypes within a class at p=0.05 is:

$$t_{0.05/2,\text{Error df}}\sqrt{\frac{2 \text{ x Error MS}}{r}}$$

where r = the number of replicates.

Materials and Methods

Randomized Nested Block (RNB) design is the term that this paper will use to describe the variant of the two-stage nested design that SMBSC uses to group GMNSR and non-GMNSR lines within nests by replicate. The design was first used in 2004. A short range of filler sugarbeet is utilized between the GMNSR and non-GMNSR sugarbeet nests as a drift buffer strip to assure that non-target movement does not occur (Figure 1). In this way, the GMNSR or non-GMNSR hybrid nests within replicates can be sprayed with the appropriate herbicide product by traveling down the alleys between the plot ranges with a commercial size boom sprayer. This method is more similar to sprayers used in commercial applications as compared to spraying individual experimental units with small-plot equipment.

Figure 1. Map of GMNSR and Commercial/Semi-Commercial trials at one location.

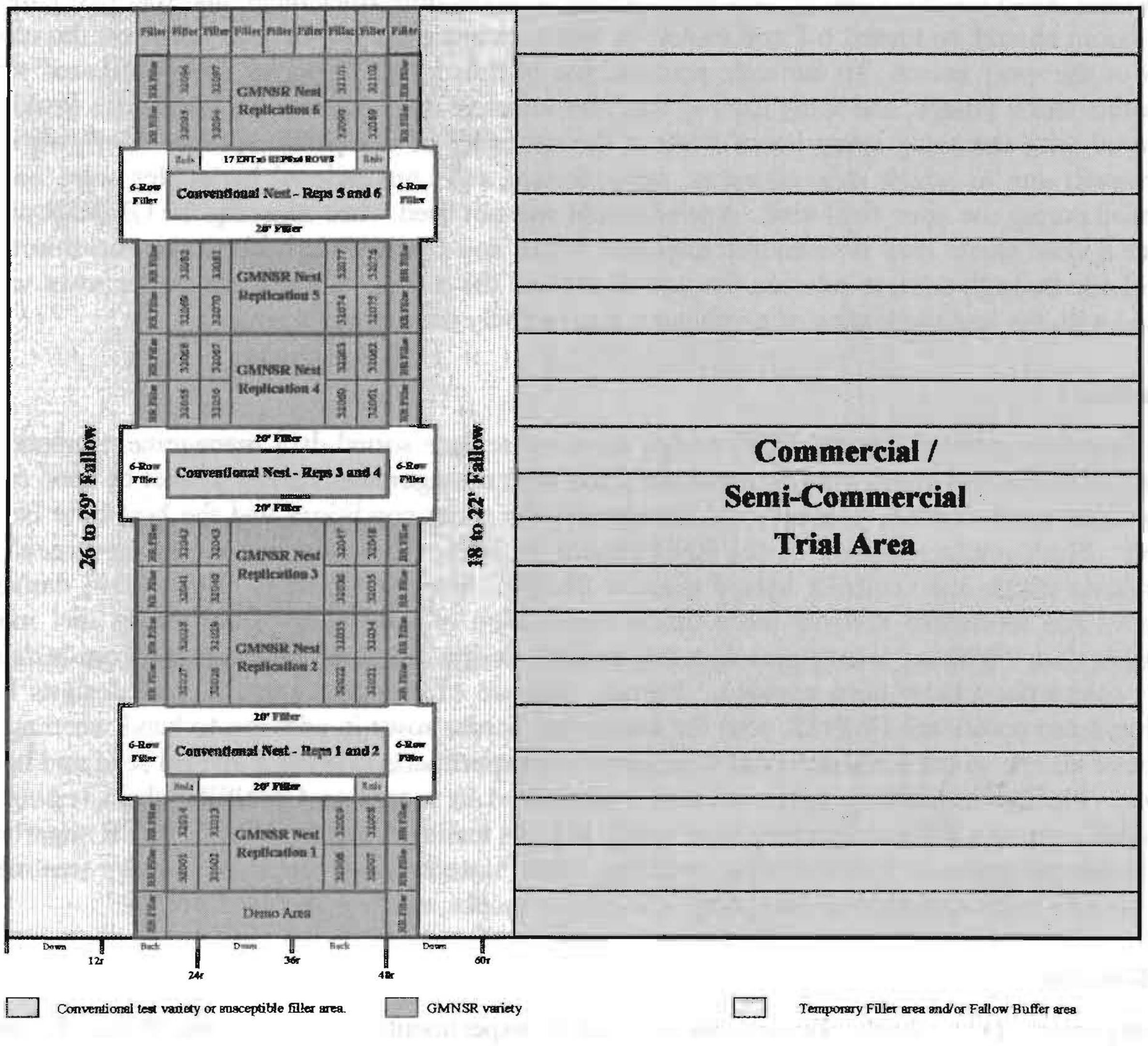

Figure 1 illustrates the GMNSR hybrid trial in relationship to the Commercial/Semi-Commercial variety trial and identifies GMNSR and non-GMNSR nests within the plot area. Experimental units were four rows spaced 22 inches apart by forty feet in length. The susceptible filler variety ranges were used as buffers to allow for herbicide spray drift without movement onto the non-GMNSR replicate nests. Buffer ranges were twenty feet in length or one half the length of an experimental unit. Herbicide treatments were applied down alley ways, perpendicular to the direction of the rows. The boom sprayer was forty feet wide, allowing for one half of each range to be sprayed on either side of the sprayer while traveling down the alleys. Susceptible buffer rows were planted along either side of the trial in addition to the use of a fallow border to assure that drift would not move sideways onto the cooperator field on one side or into the conventional trial on the other side. The area between the GMNSR trial and the Commercial/Semi-Commercial trial was also used as a turn area when spraying conventional herbicides on the Commercial/Semi-Commercial trial. Susceptible sugarbeet used as filler were purposely planted to extend beyond the main plot area in ranges that contained susceptible non-GMNSR hybrids. The beets that grew in these extension areas provided visual notification to the operator while traveling down the alley that he was approaching a susceptible filler range and that one half of the boom should be turned off and folded in when spraying the glyphosate range on the other half of the spray boom. To facilitate practical use of this design a sprayer was configured with separate tanks, pumps, and spray lines so that non-selective and conventional herbicides could be sprayed with the same spray boom without the necessity of a separate sprayer configuration. However, due to nozzle drip concerns, non-selective and conventional herbicides were never applied during the same field visit. A wind shield was not used when spraying the GMNSR plots since a wind shield may have caused exposure of the non-GMNSR hybrids to the non-selective herbicide through contact between the wet shield and the plants. Susceptible buffer rows were killed with the last application of glyphosate, leaving only the main plot area.

Summary

An important point is that the RNB design does not replace sound drift management practices. Wind direction and speed must be considered and drift management strategies must be used such as proper spray volume, pressure, and nozzles for the spray conditions and the herbicide being used. Since implementation of the RNB design in 2004, damaging non-target movement of herbicide within the GMNSR hybrid trials at SMBSC has not occurred. Better drift damage control has ultimately allowed more timely application of non-selective herbicides and more reliable data. With no loss of plot data due to drift, design analysis inefficiencies from missing plot calculations have been avoided. Further, the use of standard experimental designs had necessitated additional GMNSR seed for unsprayed border rows in addition to hand weeding to remove weeds, so the RNB design also requires less experimental GMNSR hybrid seed and hand labor. Finally, in addition to the success experienced in regard to GMNSR hybrid testing at SMBSC, use of a RNB design may have utility in trials testing the efficacy of GMNSR sugarbeet herbicide programs or hybrid testing involving other biotechnology traits that require treatment exclusivity and conversion of data using susceptible checks, as they are developed.

References

Montgomery, D.C. 2005. Design and analysis of experiments – 6th ed. John Wiley & Sons, New Jersey.