Does the Ploidy Level of Kentucky Bluegrass Cultivars Affect Resistance to Black Cutworm?

By Tyler Eaton, Dr. R. Chris Williamson, Department of Entomology, University of Wisconsin—Madison, and Dr. Geunhwa Jung, Department of Plant Pathology, University of Wisconsin—Madison

Black cutworm (*Agrotis ipsilon*) is a severe pest of turfgrass. In fact, it has been called the most severe pest on golf course putting greens. Subsequently, much research has been dedicated to finding effective means of controlling black cutworm. Recent studies suggest that Kentucky bluegrass (*Poa pratensis L.*) is resistant to black cutworm. Our lab has been conducting studies to determine the potential mechanisms of that resistance. One possible factor that we wanted to investigate was the effect of polyploidy on resistance. It has been suggested that plants with higher ploidy levels will have a higher level of resistance to pests, while plants with lower ploidy levels will have a lower level of resistance (Levin, 1983). Kentucky bluegrass is known to have wide variations in ploidy level within and among cultivars. Research has demonstrated that chromosome numbers for Kentucky bluegrass can be as low as 28 or as high as 126. Therefore, any differences in resistance between cultivars might be attributed to differences in ploidy level.

In order to determine the effect of ploidy level on resistance, it was necessary to determine the ploidy level in each of many different Kentucky bluegrass cultivars. Counting chromosomes would have been a laborious process. Instead, we decided to use a much more simple technique called flow cytometry. Flow cytometry is an analytical technique that has many applications to research. For instance, information obtained from a flow cytometer can be used to study the evolution and physiology of plant species. Recently, flow cytometry has been applied to the field of turfgrass research. A flow cytometer has a number of useful functions that include the ability to sort chromosomes and to quantify the amount of DNA in cells. Our intent was to quantify the nuclear DNA content of Kentucky bluegrass cultivars and then correlate DNA content with ploidy level. It would then be possible to calculate ploidy level based on DNA content.

There are several hundred commercial Kentucky bluegrass cultivars out on the market. We chose the cultivars for our flow cytometry study based on two factors. The first factor was morphology. A classification system was recently developed in which many Kentucky bluegrass cultivars were sorted into twelve types based on morphological traits (Bonos et al, 2000). We took advantage of this system by using it as our source for morphological data and selected at least two cultivars from each of the twelve types. The second factor that we wanted to consider was genetic diversity. We wanted the cultivars in the study to represent a wide range of genetic diversity. To this end, we utilized a map of genetic distance that had been generated with RAPD molecular marker data. The cultivars that we chose were widely distributed across the map thus ensuring genetic diversity. Based on the two factors described above, we selected thirty cultivars of Kentucky bluegrass for our study. We selected one plant to represent each cultivar. We then isolated intact nuclei from the leaf tissue of each plant and ran the nuclei through a flow cytometer in order to determine nuclear DNA content (Arumuganathan and Earle, 1991). In order to find the correlation between DNA content and chromosome number, it was necessary to count chromosomes for some of the plants. The same plants were used to generate the RAPD molecular marker data, the flow cytom-

etry data, and the ploidy level data.

The various quantities for DNA content can be seen in Table 1. Our results conclude that it is possible to find a correlation between DNA content and chromosome number in Kentucky bluegrass. This correlation can be represented by an equation:

DNA content = 0.2273*(Chromosome Number) - 5.771.

Due to variations between flow cytometers, this equation may not be accurate for everyone. However, researchers could generate their own equation based on data from their flow cytometer. To date, we have conducted several feeding assays with the objective of correlating ploidy level with resistance to black cutworm. However, our results suggest that it is unlikely that ploidy level has an effect on resistance of Kentucky bluegrass to black cutworm.

Literature cited

Arumuganathan, K. and E. D. Earle, (1991). Estimation of nuclear DNA content of plants by flow cytometry. Plant Molecular Biology Reporter. 9(3) pp. 229-240.

Bonos, S. A., W. A. Meyer, J. A. Murphy, (2000). Kentucky bluegrasses make comeback on fairways, roughs. Golf Course Management. pp. 59-64.

Levin, D. A. (1983). Polyploidy and novelty in flowering plants. The American Naturalist. Vol 122. pp. 1-25.

Type	Cultivar	DNA Content (pg)	St Dev
Compact	Alpine	11.63	0.12
Compact	Blackstone	7.45	0.07
Compact	Glade	11.14	0.06
Midnight	Award	8.67	0.06
Midnight	RugbyII	8.89	0.08
America	Brilliant	6.79	0.03
America	Unique	7.08	0.04
Julia	Caliber	12.94	0.32
Julia	Julia	8.19	0.16
MidAtlantic	Monopoly	10.27	0.28
MidAtlantic	SR2000	12.61	0.17
MidAtlantic	Voyager	7.17	0.11
Bellevue	Classic	8.29	0.07
Bellevue	Suffolk	10.74	0.09
Aggressive	NorthStar	11.78	0.16
Aggressive	Touchdown	11.36	0.30
CELA	Challenger	12.44	0.21
CELA	Eclipse	5.32	0.16
Other	Ascot	12.23	0.17
Other	Coventry	9.92	0.24
Other	Washington	10.60	0.23
BVMG	Abbey	11.43	0.08
BVMG	Baron	10.83	0.10
BVMG	Crest	11.23	0.19
BVMG	Victa	10.84	0.17
BVMG	Viva	11.53	0.06
Shamrock	Shamrock	17.84	0.55
Shamrock	SR2100	11.80	0.17
Common	Kenblue	7.16	0.14
Common	South Dakota	9.36	0.02

Table 1. DNA contents of thirty cultivars of Kentucky bluegrass. All twelve morphological types are represented. Each sample was run through the flow cytometer four times for an accurate estimate of DNA content. Standard deviation for each sample was calculated.