This is to certify that the

dissertation entitled

Isozyme Genetics, Cultivar Relationships, and Disease Reaction of Creeping Bentgrass (Agrostis palustris Huds.)

presented by

Scott Eric Warnke

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Plant Breeding and Genetics

Dal S. Dale
Major professor

Date 7/21/95

REMOTE STORAGE RSF

PLACE IN RETURN BOX to remove this checkout from your record.
TO AVOID FINES return on or before date due.

DATE DUE	DATE DUE	DATE DUE
JAN 09 2018		

20# Blue 10/13 p:/CIRC/DateDueForms_2013.indd - pg.5

ISOZYME GENETICS, CULTIVAR RELATIONSHIPS, AND DISEASE REACTION OF CREEPING BENTGRASS (*Agrostis palustris* Huds.)

By

Scott Eric Warnke

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Crop and Soil Sciences

1995

ABSTRACT

ISOZYME GENETICS, CULTIVAR RELATIONSHIPS, AND DISEASE REACTION OF CREEPING BENTGRASS (*AGROSTIS PALUSTRIS* HUDS.)

By

Scott Eric Warnke

Creeping bentgrass is a cool-season turfgrass species primarily used for the establishment of golf course greens, tees, and fairways. Efficient breeding efforts with this species will require that the level of genetic understanding of creeping bentgrass be improved. Molecular markers such as isozymes can be an effective tool for studying the genetic behavior of an organism and can lead to more efficient breeding stratagies through marker assisted selection. The objectives of this research were to utilize isozymes to improve the level of genetic understanding of creeping bentgrass to assess the level of genetic diversity in the cultivated germplasm, and to determine the disease reaction of bentgrasses to *Sclerotinia homoeocarp* Bennett. the causal agent of dollar spot.

Inheritance data from tetraploid *Agrostis palustris* Huds. populations were collected. Segregation data at the *Tpi-1, Tpi-2, Got-2, Pgi-2* and *Pgm-2* loci were used to identify allozymes and distinguish between allo- or autotetraploid segregation. Fixed heterozygosity at the *Tpi-2* and *Got-2* loci and disomic segregation for a tetra-allelic individual at the *Pgi-2* locus provided strong genetic evidence to support allotetraploid inheritance in cultivated creeping bentgrass.

The unweighted pair group method with arithmetic averages (UPGMA) cluster analysis generated from the between cultivar genetic distance matrix divided the

cultivars into two clusters. One cluster contains ten cultivars all potentially related to the cultivar Seaside. The second group contains the tightly clusterd cultivars Pennlinks, Southshore, Pro/Cup and Lopez as well as a group of cultivars with some unique allozyme characteristics.

Thirty-one cultivars of bentgrass representing 2 species (*Agrostis palustris* Huds. and *Agrostis tenuis* Sibth.) were screened for their reaction to a single isolate of *Sclerotinia homoeocarpa* Bennett. Plants were rated on a scale of 1 (dead plant) to 9 (no disease damage). The overall disease means of cultivars ranged from 1.0 to 2.6. Sixty-three percent of the bentgrass population was killed by the pathogen and 96% received a score of 1 or 2 indicating that resistance to this pathogen is very low.

To Mary Ann, Erik, and Samantha
for all of your love, friendship, encouragement, and understanding

ACKNOWLEDGMENTS

I would like to express my gratitude to my advisors, Dr. Bruce Branham, and Dr. Dave Douches for their guidance and friendship during my studies. They both offered me tremendous support in the development of my academic and research skills. I would also like to thank the members of my committee, Dr. James Hancock, Dr. Brian Diers, and Dr. Joe Vargas. I learned much from each of them during the course of my studies. I would also like to thank all the members of the turf group and potato group for their support and encouragement. Thanks also to the Michigan Turfgrass Foundation for their financial support of this research. And last but not least I would like to thank my parents Ron, and Marilyn and my brother Kevin for their love encouragement, and financial support throughout my studies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The bentgrasses are native to W. Europe (Harlan, 1992) with the genus *Agrostis* consisting of approximately 200 species (Hitchcock, 1951). There are four species which are commonly accepted as turfgrass types. These are *Agrostis palustris* - creeping Bentgrass (2n=4x=28), *Agrostis canina* - velvet bentgrass (2n=2x=14), *Agrostis tenuis* - colonial bentgrass (2n=4x=28), and *Agrostis gigantea* - redtop bentgrass (2n=6x=42). Considerable variation exists in the literature concerning the naming of these species. For example, creeping bentgrass is referred to as *Agrostis palustris* Huds, *Agrostis stolonifera* L. and *Agrostis stolonifera* L. var. *palustris* (Huds.) Farw. Hitchcock (1951) considers *A. stolonifera* and *A. palustris* to be two distinct species with the primary difference being more prolific stolon production by *A. palustris*. Hitchcock lists the cultivated creeping bentgrass in the United States as examples of *A. palustris*.

According to Duich (1985), the first bentgrasses to be used for putting greens in the United States were known as South German Bentgrass. South German bentgrass was harvested from pastures in present day Austria and Hungary and later from other areas of Europe. South German bentgrass consisted of a mixture of the four bentgrasses mentioned above and over time would segregate into patches of predominatly creeping bentgrasses and to a lesser extent velvet bentgrass in favorable temperate climates. Many of the better appearing patches were selected and maintained vegetatively by the United States Golf Association Green Section at the Arlington Turf Gardens in Arlington, VA. Many hundreds of clones were eventually selected, some o

f whichbecame known as the C-series creeping bentgrasses. The best of the C-series bentgrasses were used for vegetative establishment of putting greens. The most widely used C-series bentgrasses were Toronto (C-15), Cohansey (C-7), Washington (C-50), Arlington (C-1), Congressinal (C-19), and Old Orchard (C-52) each of which was well adapted to specific regions of the United States.

The only available seeded creeping bentgrass from the late 1920's until the 1950's was the variety Seaside. Seaside was discovered growing in tidal flatlands near Coos Bay, Oregon. However, Seaside was much inferior in quality to the available vegetatively propagated material. Therefore, putting greens in the United States were primarily established vegetatively until the 1950's when the desire to utilize creeping bentgrass for fairways led breeders to begin developing an acceptable seeded creeping bentgrass. Holt and Payne (1951) studied the growth rate, texture, density, type of growth and drought tolerance of seedling progenies from 49 C-series clones of creeping bentgrass to determine the extent of variability within and among strains for these characteristics. The results indicated that seed propagation of most of the available C-series creeping bentgrass clones was not promising due to the high level of variability in the seeded progeny.

In 1955, the variety Penncross was released from Pennsylvania State University by Dr. H.B. Musser and established a new level of excellence for seeded creeping bentgrasses. Penncross is often referred to as the landmark variety (Rogers, 1991) of creeping bentgrasses. Penncross has much higher overall quality and is less likely to segregate into distinct patches than Seaside. Penncross creeping bentgrass is the first

generation (Syn-0) seed only, produced by random crossing of 3 vegetatively propagated clonal strains (Hein, 1958).

Creeping bentgrass has the strongest stoloniferous growth habit of any cool-season turfgrass species and it is also able to tolerate cutting heights of 1.25 cm or less making it the species of choice under highly managed golf course conditions. The increased popularity of golf and the resultant increase in golf course construction has led to the use of creeping bentgrass on a much wider scale. Creeping bentgrass is now commonly planted on golf course tees, greens, and fairways. The increased utilization of creeping bentgrass has resulted in an increase in breeding efforts, with the 1993 National Turfgrass Evaluation Programs bentgrass variety trial containing 27 varieties. However, the increase in creeping bentgrass varieties has not been accompanied by a similar increase in the level of scientific knowledge about the genetics of this species.

Jones (1956a), in a set of three papers, examined the cytological relationships in the key members of the *Agrostis* genus. The first paper examined *A. canina* L. subsp. canina (2n=2x=14) and *A. canina* L. subsp. montana Hartm. (2n=4x=28). Meiotic analysis of these two species indicated that the diploid regularly forms seven bivalents and the tetraploid behaves as an autopolyploid.

The second paper (Jones, 1956b) examined the significance of chromosome pairing in the tetraploid hybrids of *A. canina* subsp. montana. with *A. tenuis*. and *A. stolonifera*. *A. tenuis* and *A. stolonifera* formed only bivalents at meiosis with *A. tenuis* possibly being a segmental allotetraploid *and A. stolonifera* behaving as a strict allotetraploid with well differentiated genomes. Chromosome pairing evidence in

crosses between *A. tenuis* and *A. stolonifera* indicated that these two species might have one ancestral diploid type in common.

The third paper (Jones, 1956c) dealt with *Agrostis gigantea* Roth. and its hybrids with *A. tenuis* and *A. stolonifera*. *A. gigantea* regularly formed twenty-one bivalents at meiosis and the pentaploid hybrids of *A. gigantea* with *A. tenuis* and *A. stolonifera* all showed typical metaphase pairing of 14 bivalents and seven univalents. The genome constitutions assigned to these three species were *A. tenuis* $A_1A_1A_2A_2$, *A. stolonifera* $A_2A_2A_3A_3$ and *A. gigantea* $A_1A_1A_2A_2A_3A_3$. Meiotic pairing studies can provide useful information about genomic relationships, however, they are not a definitive indicator of polyploid type because random bivalent pairing during meiosis would result in allopolyploid chromosome pairing behavior with autopolyploid segregation ratios. The most definitive method of determining genomic relationships is through the use of genetic markers (Krebs & Hancock 1989).

Molecular markers are a fast, reliable, and readily available source of information that can be utilized to provide an increased genetic understanding of a species. Protein markers code for proteins that can be separated by electrophoresis to determine the presence or absence of specific alleles (Tanksley, 1983). The most widely used protein markers in plant breeding and genetics are isozymes. Isozymes are multiple molecular forms of an enzyme having the same catalytic activity. Isozyme analysis is fairly simple and inexpensive and has been used in a number of applications. Outcrossing rates have been estimated by Shaw et al.(1981) and Shaw and Brown (1982). Isozymes have been used to facilitate the introgression of genes from wild

species (Tanksley et al, 1980) as well as for measuring genetic variability (Brown and Weir, 1983), and for varietal patenting and protection (Bailey, 1983). Isozymes have also been utilized for gene-centromere mapping (Douches and Quiros, 1987) and quantitative trait locus mapping (Stuber, 1982). Additionally, isozymes have been very useful for the determination of the mode of inheritance (disomic or tetrasomic) in polyploid species (Quiros, 1982).

Creeping bentgrass is susceptible to a wide range of diseases including dollar spot, brown patch, *Fusarium* blight, *Pithium* blight, red thread, stripe smut and *Typhula* blight (Beard 1973), therefore, preventative fungicide programs are often required. On golf courses in the temperate and hot humid regions of the United States more money is spent to manage dollar spot than on any other turfgrass diseases (Vargas, 1994). *Sclerotinia homoeocarpa* Bennett. the causal agent of dollar spot has developed resistance to cadmium-containing fungicides (Cole et al. 1968), benzimidizol compounds (Warren et al. 1974) and recently the dimetheylase inhibitor fungicides (Vargas 1994). Therefore, the development of some level of genetic resistance to this important pathogen of creeping bentgrass is important from both an economic and fungicide management perspective.

The objectives of this research were to 1) improve the level of genetic understanding of the cultivated species of creeping bentgrass *Agrostis palustris* Hud., 2) assess the level of genetic diversity in the cultivated germplasm and determine the location of germplasm that may be useful for broadening the genetic base of the

cultivated species, and 3) determine the disease reaction of bentgrasses to *Sclerotinia homoeocarpa* Bennett. the causal agent of dollar spot. The information gathered from this research will provide the ground work for future breeding work aimed at improving this important turfgrass species.

LIST OF REFERENCES

Bailey, D.C. 1983. Isozymic variation and plant breeders' rights. In: Isozymes in Plant Genetics and Breeding, Part A. Eds. S.D. Tanksley and T.J. Orton. Elsevier Science Publishers, Amsterdam 425-440.

Beard, J.B. 1973. Turfgrass: Science and Culture. Prentice Hall. USA New Jersey pp. 73.

Brown, A.H.D., and B.S. Weir 1983. Measuring gentic variability in plant populations. In: S.D. Tanksley and T.J. Orton (eds.) Isozymes in Plant Genetics and Breeding. Elsevier Co., Amsterdam.

Cole, H., B. Taylor, and J. Duich. 1968. Evidence of differing tolerance to fungicides among isolates of *Sclerotinia homoeocarpa*. Phytopath. 58:683-686.

Douches, D.S., and C.F. Quiros. 1987. Use of 4x-2x crosses to determine gene-centromere map distances of isozyme loci in *Solanum* species. Genome, 29: 519-527.

Duich, J.M. 1985. The bent grasses. Weeds trees and turf. 72-78.

Harlan, J.R. 1992. Crops and Man. American Society of Agronomy, Madison, Wisc.

Hein, M.A. 1958. Registration of varieties and strains of grasses. Agron. Jour. 50:399.

Hitchcock, 1951. Manual of the grasses of the United States 2nd ed. Washington D.C.

Holt, E.C., and K.T. Payne. 1952. Variation in spreading rate and growth characteristics of creeping bentgrass seedlings. Agronomy Journal. 44:88-90.

Jones, K. 1956a. Species differentiation in *Agrostis* I. Cytological relationships in *Agrostis canina* L. J. Genet. 54:370-376.

Jones, K. 1956b. Species differentiation in *Agrostis* II. The significance of chromosome pairing in the tetraploid hybrids of *Agrostis canina* subsp. Montana Hartm., *A. tenuis* Sibth. and *A. stolonifera* L. J. Genet. 54:377-393.

Jones, K. 1956c. Species differentiation in *Agrostis* III. *Agrostis gigantea* Roth. and its hybrids with *A. tenuis* Sibth. and A. stolonifera L. J. Genet. 54:394-399.

Krebs, S., and J. Hancock. 1989. Tetrasomic inheritance of isozyme markers in the highbush blueberry, *Vaccinium corymbosum* L. Heredity 63:11-18.

Quiros, C.F. 1982. Tetrasomic inheritance for multiple alleles in alfalfa. Genetics 101:117-127.

Rogers M. 1991. Cool-season turfgrass varieties. Grounds Maintence. Sept pp. 22.

Shaw, D.V., A.L. Kahler and R.W. Allard. 1981. A multilocus estimator of mating parameters in plant plant populations. Proc. Nat. Acad. Sci. 78:1298-1302.

Shaw, D.V., and A.H.D. Brown 1982. Optimum number of marker loci for estimating outcrossing in plant populations. Theor. Appl. Genet. 61:321-325.

Stuber, C.W., M.M. Goodman and R.H. Moll. 1982. Improvement of yield and ear number resulting from selection at allozyme loci in a maize population. Crop Sci. 22:737-740.

Tanksley, S.D., and C.M. Rick 1980. Isozymic gene linkage map of the tomato: applications in genetics and breeding. Theor. Appl. Genet. 57: 161-170.

Tanksley, S. D. 1983. Molecular Markers in Plant Breeding. Plant Molecular Biology Reporter. 1:3-8.

Vargas, J.M. 1994. Management of turfgrass diseases. p. 23-26 2nd ed. CRC Press, Inc., Boca Raton Fl.

Warren, C.G., P. Sanders, and H. Cole. 1974. Sclerotinia homoeocarpa tolerance to benzimidazole configuration fungicides. Phytopathology 64:1139-1142.

CHAPTER I

ISOZYME ANALYSIS SUPPORTS ALLOTETRAPLOID INHERITANCE IN TETRAPLOID CREEPING BENTGRASS (*Agrostis palustris* Huds.)

ABSTRACT

Inheritance data from tetraploid *Agrostis palustris* Huds. populations produced by crossing selected plants from cultivated creeping bentgrass varieties is reported. Twelve enzyme systems were evaluated using the Histidine-Citrate pH 5.7 and Tris-citrate/Lithium-borate pH 8.3 buffer systems. Four enzyme systems exhibiting polymorphism in the Tris-citrate/Lithium-borate buffer system were selected for further genetics studies. Segregation data at the *Tpi-1, Tpi-2, Got-2, Pgi-2* and *Pgm-2* loci were used to identify allozymes and distinguish between allo- or autotetraploid segregation patterns. Two allozymes were identified at the *Pgm-2* and *Tpi-1*, loci while three allozymes were identified at the *Got-2* and *Tpi-2* loci. The *Pgi-2* locus was highly polymorphic with six different allozymes identified. Fixed heterozygosity at the *Tpi-2* and *Got-2* loci and disomic segregation for a tetra-allelic individual at the *Pgi-2* locus provided strong genetic evidence to support allotetraploid inheritance in creeping bentgrass.

INTRODUCTION

The bentgrasses are native to Western Europe (Harlan 1992) with the genus *Agrostis* consisting of approximately 200 species (Hitchcock 1951). The four species commonly accepted as turfgrass types are *Agrostis palustris* Huds. - creeping bentgrass (2n=4x=28), *Agrostis canina* - velvet bentgrass (2n=2x=14), *Agrostis tenuis* - colonial bentgrass (2n=4x=28), and *Agrostis gigantea* - redtop bentgrass (2n=6x=42). Creeping bentgrass is the most widely utilized of the turf-type bentgrasses because of its excellent tolerance of low mowing heights and a strong stoloniferous growth habit that makes it well suited for the establishment of golf course greens and fairways.

Limited genetic research has been conducted on the genus *Agrostis,* however, Jones (1955) examined the cytological relationships in the key members of the *Agrostis* genus. He examined the significance of chromosome pairing in the tetraploid species hybrids of *A. canina* subsp. montana Hartm. with *A. tenuis* Sibth. and *A. stolonifera* L. Jones concluded that *A. tenuis* and *A. stolonifera* form only bivalents at meiosis with *A. tenuis* possibly being a segmental allotetraploid and *A. stolonifera* behaving as a strict allotetraploid with well-differentiated genomes.

A. stolonifera and *A. palustris* are two separate species according to Hitchcock (1951) with the primary difference being more prolific stolon production by *A. palustris*. Hitchcock cites some early vegetatively-propagated creeping bentgrass varieties as well as naturalized populations in the pacific northwest as examples of *A. palustris*. The creeping bentgrass variety Seaside originates from a naturalized population in Coos Bay, Oregon (Duich 1985) and is likely to have provided much of

the initial germplasm for many of the current creeping bentgrass varieties (Warnke et al. 1995).

Previous isozyme analysis in creeping bentgrass have focused on varietal identification. Wilkinson and Beard (1972) used acrylamide gel disc electrophoresis to distinguish one seed-propagated and five vegetatively-propagated creeping bentgrass varieties. Yamomoto and Duich (1994) utilized bulk plant leaf samples to identify cross-pollinated bentgrass species and cultivars with starch gel electrophoresis. However, isozymes have not previously been used for genetic analysis or to determine polyploid type in creeping bentgrass. The objectives of this research were to study isozyme segragation patterns in controlled crosses of creeping bentgrass and used this segregation data to infer the type of polyploidy occurring in creeping bentgrass.

MATERIALS AND METHODS

Approximately 650 clones randomly selected from creeping bentgrass varieties included in the National Turfgrass Evaluation Program's 1989 bentgrass variety trial were screened for isozyme polymorphism. Progeny for isozyme analysis were developed from controlled crosses among highly fertile bentgrass clones selected for their differences in isozyme banding patterns. Crosses were made by placing reproductive tillers from field-grown plants in 30ml test tubes held upright in clay pots. Clear plastic bags supported by wooden stakes were placed over the tillers to maintain pollination isolation. The pots were placed in the greenhouse and the test tubes filled with water as needed. Seed harvested from the two parents of each cross was kept separate to examine whether any self-fertilization had occurred (Table 1.1).

Isozyme analysis was performed on individual creeping bentgrass plants that were at least 2 months old. Plants were analyzed 7 to 10 days after fertilization with 20-20-20 soluble fertilizer. A crude protein extract was obtained by macerating 4 to 5 newly-expanded leaves in 80ul of chilled extraction buffer (Weeden, 1989) composed of 75mM Tris-HCL buffer, pH 7.5, 5% PVP-40 (w/v), 14mM mercaptoethanol (0.2% v/v). The extraction was done in chilled, 12 sample, porcelain plates with a plexiglas rod rounded on one end. The crude extracts were absorbed onto 3 x 8 mm Whatman 3MM wicks and stored over night at
-20°C.

Phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), glutamate oxaloacetate transaminase (GOT), triosphosphate isomerase (TPI), anodal peroxidase (PRX), esterase (EST) and alcohol dehydrogenase (ADH) were resolved in Tris-citrate/Lithium borate pH 8.3 gels (Weeden,1989), after 4.5h of electric current at 50 to 60 mA. Acid phosphatase (APS), 6-phosphogluconate dehydrogenase (6-PGDH),

Table 1.1. Clones included in creeping bentgrass segregation study, the clone source variety, the crosses made, and the mating system.

Parental clones	Clones Source	Crosses Made	Mating System
PE 91-25	Penneagle	(PE91-25 X NA91-22)*	Cross
PE 91-24	Penneagle	(PV91-21 X PE91-24)	Cross
PV 91-21	Providence	(PV91-21 X NA91-22)	Cross
NA 91-22	National	(PE91-25 X PV91-21)	Cross
Syn 91-3	Syn 89-1	(Syn91-3 X EM91-6)	Self[a]
EM 91-6	Emerald	(Nor91-7 X SR91-30)	Cross
Nor 91-7	Normarc 101 (Regent)		
SR 91-30	SR 1020		

*Underlined clone in each cross is the female parent

[a]Was scored as a self-fertilization of Syn91-3

aconitase (ACO), malate dehydrogenase (MDH), and shikimic acid dehydrogenase (SDH) were resolved in a Histidine-Citrate pH 5.7 buffer system after 4.5h of electric current at 35 mA (Weeden, 1989). Gel slabs consisted of 10% potato starch (Starch Art, Smithville, TX). Gel trays which provided for a direct contact between the gel and tray buffers were used. Enzyme activity stains were prepared according Vallejos (1983). PGM activity was improved by the inclusion of 1.5 ml of 0.5% (w/v in H_2O) glucose 1-6 diphosphate to the staining solution.

In the anodal section of the gel, the fastest migrating zone of activity in each enzyme system was designated the first locus and the next zone the second locus. The fastest migrating allele at each locus was designated the first allele, the next allele was the second allele and, so on until all alleles were assigned a number. Loci designation in allopolyploids is complicated by the fact that each locus contains homoeoalleles that form heterodimers but the chromosomes they are located on do not pair during meiosis, therefore, two loci are present at each designated locus in this study. Allopolyploid wheat isozyme loci have been assigned genomic designations based on studies with monosomic lines (Hart, 1983). However, genomic designation is not currently possible in creeping bentgrass, therefore, all homoeoloci have been grouped together with no genomic designations made. Chi-square tests were used to test hypothesized genetic ratios. Whenever possible, plants homozygous for alternate alleles at a locus were selected for crossing to determine if self-fertility occurs. Plants appearing to exhibit a duplex allelic state at a putative locus were also selected since inheritance data from these plants provides the clearest differentiation between disomic and tetrasomic inheritance in self and testcross progenies (Table 1.2).

Table 1.2 Expected gamete and progeny class frequencies for disomic and tetrasomic inheritance in creeping bentgrass.[a]

Cross type	Parental genotype	Expected gamete ratios		Expected phenotypic ratios	
		Disomic	Tetrasomic	Disomic	Tetrasomic
1	*bbbb X bbbc	(bb) X (1bb:1bc)	(bb) X (1bb:1bc)	1:1	1:1
2	aaac X aaac	(1aa:1ac) X (1aa:1ac)	(1aa:1ac) X (1aa:1ac)	3:1	3:1
3	bbbb X cccc	(bb) X (cc)	(bb) X (cc)	0:1	0:1
4	dddd X dddf	(cc) X (1cc:1cf)	(cc) X (1cc:1cf)	1:1	1:1
5	aabb X aabb	(ab) X (ab)	(1aa:4ab:1bb) X (1aa:4ab:1bb)	0:1:0	1:34:1
6	dddf X dddg	(1dd:1df) X (1dd:1dg)	(1dd:1df) X (1dd:1dg)	1:1:1:1	1:1:1:1
7	abbc X aabb	(1ab:1ac:1bb:1bc) X (1ab)	(1cc:2ab:2bc:1bb) X (1aa:4ab:1bb)	1:1	1:17:14:3:1
8	aabb X aaaa	(ab) X (aa)	(1aa:4ab:1bb) X (aa)	0:1	5:1
9	bccc X bccc	(1bc:1cc) X (1bc:1cc)	(1bc:1cc) X (1bc:1cc)	1:3	1:3
10	bcde X dddf	(1bd:1be:1cd:1ce) X (1dd:1df)	(1bc:1bd:1be:1cd:1ce:1de) X (1dd:1df)	1:1:1:1:1:1:1:1	1:1:1:1:1:1:1:1:1
11	bcde X dddg	(1bd:1be:1cd:1ce) X (1dd:1dg)	(1bc:1bd:1be:1cd:1ce:1de) X (1dd:1dg)	1:1:1:1:1:1:1:1	1:1:1:1:1:1:1:1:1

*Letters represent alleles defined by comparative mobilities

aAddapted from Beaver and Iezzoni (1993)

RESULTS

A total of 12 enzyme systems were examined, however, only five showed excellent resolution and exhibited polymorphism in the anodal region of the gel using the tris-citrate, lithium borate pH 8.3 buffer system, therefore, *Pgi-2*, *Tpi-1*, *Tpi-2*, *Got-2*, and *Pgm-2* were selected for further study. *MDH, SDH, EST,* and *ACO* exhibited complex banding patterns; *6PGDH* and *APS* were poorly resolved with the buffer systems used; and PRX was monomorphic, so these enzyme systems were not studied further.

Phosphoglucomutase (PGM) In *A. palustris*, PGM is a monomeric enzyme, which encodes for two putative loci designated *Pgm-1* and *Pgm-2* (Figure1a). In this study, segregation data were not obtained for the *Pgm-1* locus. *Pgm-1* often exhibited fixed heterozygosity, therefore, segregating populations were difficult to find. Segregation data were available from one cross at the *Pgm-2* locus and this cross followed a test cross segregation pattern for the $Pgm\text{-}2^2$ and $Pgm\text{-}2^3$ alleles (Table 1.3).

Phosphoglucoisomerase (PGI). Two zones of activity were present in the four crosses scored for *PGI*. Zone 1 was poorly resolved and difficult to score reliably (Figure 1b). Zone 2 designated *Pgi-2* has a complex banding pattern which has been previously reported by Yamamoto and Duich (1994). Six alleles were scored in the four crosses we evaluated (Table 1.3). Data from an extensive evaluation of creeping bentgrass varieties indicates that one or two additional putative alleles may exist in the cultivated creeping bentgrass population (Warnke et al. 1995).

Pgi-2 is a dimeric enzyme and in many cases the alleles present at this locus have very similar mobilities, making allele designation difficult. However, the clone NA91-22 has one copy of the slow migrating $Pgi\text{-}2^7$ allele and the clone PV91-21 has

one copy of the slow migrating *Pgi-2*^{6} allele (Figure 1b.) making it possible to score allele presence or absence, in the segregating progeny, based on heterodimer location. The cross between these two clones produced four progeny classes indicating that PV91-21

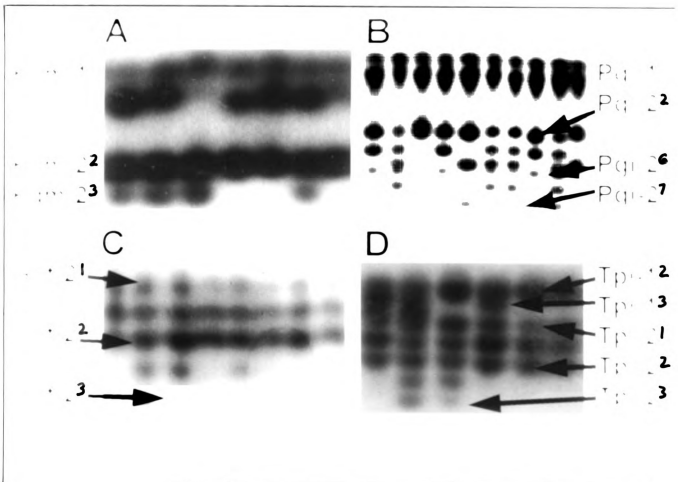

Figure1.1. Allozyme phenotypes of creeping bentgrass. **(A)** *Pgm-1* and *Pgm-2*, **(B)** *Pgi-1* and *Pgi-2*, **(C)** *Got-2* showing fixed heterozygosity for the *Got-2*^{1} and *Got-2*^{2} alleles and 1:1 segregation for the *Got-2*^{3} allele, **(D)** *Tpi-1* and *Tpi-2* showing fixed heterozygosity for the *Tpi-2*^{1} and *Tpi-2*^{2} alleles and 1:1 segregation for the *Tpi-2*^{3} allele.

Table1.3. Segregation and chi-square values at 5 polymorphic loci in creeping bentgrass

Locus	Cross	Cross type*	Observed progeny Phenotypes								Ratio Tested	(χ^2)	P
					dd[z]	dg	df	dfg					
Pgi-2	PV 91-21 X NA 91-22	6			63	70	58	65			1:1:1:1	(2.74)	0.46
			bd	bdg	bde	bdeg	cd	cdg	cde	cdeg			
	PE 91-25 X NA 91-22	11	19	18	24	21	20	19	22	18	1:1:1:1:1:1:1:1	(1.52)	0.98
			bd	bdf	bde	bdef	cd	cdf	cde	cdef			
	PE 91-25 X PV 91-21	10	13	23	11	17	18	24	12	14	1:1:1:1:1:1:1:1	(10.2)	0.23
						dd	df						
	Nor 91-7 X SR 91-30	4				85	106				1:1	(2.31)	0.17
						ab	abc						
Tpi-2	PE 91-25 X PV 91-21	7				73	74				1:1	(0.01)	0.95
	PV 91-21 X PE 91-24	7				120	115				1:1	(0.11)	0.81
	Nor 91-7 X SR 91-30	5				192					0:1:0	(0.00)	1.00
Got-2	PE 91-25 X NA 91-22	7				77	68				1:1	(0.56)	0.48
	PE 91-25 X PV 91-21	8				172					1:0	(0.00)	1.00
						aa	ac						
	Syn 91-3 ⊗	2				20	58				1:3	(0.13)	0.89
						bb	bc						
Pgm-2	Nor 91-7 X SR 91-30	1				100	87				1:1	(0.90)	0.42
						cc	bc						
Tpi-1	Syn 91-3 ⊗	9				9	34				1:3	(0.31)	0.57
	PV 91-21 X PE 91-24	3				205					0:1	(0.00)	1.00

*Cross types and expected phenotypic ratios are defined in Table 2. [z]**Pgi-2**: b=*Pgi-2*^{2}, c=*Pgi2*^{3},d=*Pgi2*^{4},e=*Pgi2*^{5},f=*Pgi-2*^{6}, g=*Pgi-2*^{7}; **Tpi-1**: b=*Tpi-1*^{2} c=*Tpi-1*^{3}; **Tpi-2**: a=*Tpi-2*^{1}, b=*Tpi-2*^{2}, c=*Tpi-2*^{3}; **Got-2**: a=*Got-2*^{1}, b=*Got-2*^{2}, c=*Got-2*^{3}; **Pgm-2**: b=*Pgm-2*^{2}, c=*Pgm-2*^{3}.

has one copy of the *Pgi-2*^{6} allele and three copies of the *Pgi-2*^{4} allele while the NA91-22 clone has one copy of the *Pgi-2*^{7} allele and three copies of the *Pgi-2*^{4} allele. PV91-21 and NA91-22 were both crossed to PE91-25 and eight progeny classes were detected from each cross indicating that the PE91-25 clone is tetra-allelic having the *Pgi-2*^{2}, *Pgi-2*^{3}, *Pgi-2*^{4} and *Pgi-2*^{5} alleles. The fourth cross at *Pgi-2* involves Nor91-7 X SR91-30 and exhibits test cross segregation for the *Pgi-2*^{4} and *Pgi-2*^{6} alleles indicating that Nor91-7 is homozygous for the *Pgi-2*^{4} allele and SR91-30 has three copies of the *Pgi-2*^{4} allele and one copy of the *Pgi-2*^{6} allele.

Glutamate oxaloacetate transaminase (GOT) In the three crosses studied, two zones of activity were detected in the GOT zymograms. The most anodal zone corresponds to *Got-1*, however, this locus was monomorphic in the crosses studied. The slower migrating zone corresponds to *Got-2*. Fixed heterozygosity for the *Got-2*^{1} and *Got-2*^{2} alleles was exhibited in the cross PE91-25 X PV91-21. Segregation at the *Got-2* locus was very similar to the *Tpi-2* locus in that three alleles were present in one of the parental clones and fixed heterozygosity was observed for the *Got-2*^{1} and *Got-2*^{2} alleles. The cross PE91-25 X NA91-22 was another cross between di-allelic and tri-allelic genotypes similar to the *Tpi-2* locus and again only two progeny classes were observed. One class had the *Got-2*^{1} and *Got-2*^{2} alleles and the other had the *Got-2*^{1}, *Got-2*^{2}, and *Got-2*^{3} alleles, indicating that the di-allelic clone exhibted fixed heterozygosity (Fig 1c). The final cross to examine the *Got-2* locus was the self of Syn 91-3 and a 3:1 segregation ratio for the *Got-2*^{1} and *Got-2*^{3} alleles was exhibited. (Table 3).

Triosphosphate isomerase (TPI). The *TPI* zymogram revealed two very closely migrating zones of activity, which are coded by two unlinked loci. The separation of these two loci can be seen more clearly in diploid *A. canina* populations (data not shown). At the *Tpi-1* locus we observed segregation data for two allozymes. The faster allozyme was assigned *Tpi-1*^{2} and the slower band was assigned *Tpi-1*^{3}. Two additional allozymes, one with

a faster migration than *Tpi-1*^{2} and the other having a slower migration than *Tpi-1*^{3} have been observed in other populations (Warnke et al. 1995).

The cross PV 91-21 X PE 91-24 is homozygous for alternate alleles at *Tpi-1* and all 205 progeny examined exhibited a three-banded heterozygous pattern indicating all progeny were of hybrid origin. The second cross segregating at the Tpi-1 locus, Syn 91-3 x Em 91-6, exhibited a high level of self-fertility based on homozygous alternate alleles at the Tpi-2 locus. The selfed progeny of Syn91-3 fit a 3:1 ratio of heterozygotes to homozygous individuals supporting a hypothesis that this clone has one copy of the *Tpi-1*^{1} allele and three copies of the *Tpi-1*^{2} allele.

The Tpi-2 locus has alleles which are quite easily scored. Three crosses were scored for Tpi-2 segregation and in all cases fixed heterozygosity was observed which again supports disomic inheritance in this species. The crosses PE91-25 X PV91-21 and PV91-21 X PE91-24 both involve a tri-allelic clone having the *Tpi-2*^{1}, *Tpi-2*^{2} and *Tpi-2*^{3} alleles crossed to a diallelic clones having the *Tpi-2*^{1} and *Tpi-2*^{2} alleles. Only two progeny classes were observed, one having the *Tpi-2*^{1} and *Tpi-2*^{2} alleles and the other having the *Tpi-2*^{1}, *Tpi-2*^{2}, and *Tpi-2*^{3} alleles indicating that the diallelic clones are exhibiting allotetraploid segregation (Fig 1d).

DISCUSSION

Genetic data from co-dominant molecular markers can clearly distingish between allo- and auto-polyploidy due to a difference in the progeny classes observed from crosses. A diploid organism with two different alleles at a locus (*A* and *a*) produces one heterozygote class (*Aa*). Selfing of this individual will produce a progeny array of 1*AA*:2*Aa*:1*aa*. However, with tetrasomic inheritance, in an autotetraploid, three different classes of heterozygotes can be produced (*AAaa*), (*Aaaa*) and (*AAAa*). Selfing of an individual having the (*AAaa*) genotype will result in a progeny array of 1*AAAA*:8*AAAa*:18*AAaa*:8*Aaaa*:1*aaaa* (Muller, 1914; Haldane, 1930). In contrast, preferential pairing of genomes in an allotetraploid having *AA* on a chromosome pair in one genome and *aa* on a chromosome pair from the other genome would produce only AAaa progeny. Therefore, allopolyploidy results in "fixed" heterozygosity which would not be expected in a autopolyploid.

Tetrasomic inheritance has been documented using codominant isozyme loci in a number of plant species including *Medicago sativa* (Quiros, 1982), *Solanum tuberosum* L. (Martinez- Zapater and Oliver, 1984; Quiros and McHale, 1985), *Haplopappus spinulosus* (Hauber, 1986), *Tolmiea menziesii* (Soltis and Soltis, 1988), *Heuchera micrantha* (Soltis and Soltis, 1989) and *Vaccinium corymbosum* L. (Krebs and Hancock, 1988). Disomic inheritance in allopolyploids has been documented in tetraploid *Tragopogon mirus* and *T. miscellus* (Roose and Gottleib, 1976) tetraploid *Prunus cerasus* L. (Beaver and Iezzoni, 1993) and for triplicated loci in hexaploid *Triticum aestivum* (Hart, 1983) and quadruplicated loci in octaploid *Fragaria* X *ananassa* (Arulsekar et. al., 1981)

A. palustris, which is the primary cultivated species of bentgrass in the United States, is not well-understood genetically. If genetic advancement is to be made, an understanding of the creeping bentgrass genome must be developed. Co-dominant molecular markers provide a tool for use in understanding polyploid inheritance

patterns. The segregation data from crosses that exhibit fixed heterozygosity as well as the tetra-allelic segregation, observed for the cross of PE91-25 with NA 91-22 and PV91-21, provides strong genetic evidence for disomic rather than tetrasomic inheritance in creeping bentgrass (Table 3). Tetrasomic inheritance, either by the formation of quadravalents or random bivalent pairing, would result in 12 different progeny classes rather than the 8 which are observed in these crosses and expected with disomic inheritance (Table 1.2).

Mating systems in allopolyploids and autopolyploids generally compliment the type of polyploidy exhibited. For example, all autopolyploids maintain heterozygosity via cross fertilization and exhibit low fertility in response to self-fertilization. However, most allopolyploids are self-compatible with several being highly self-pollinated (Mackey, 1970). Creeping bentgrass would appear to be an exception to this system in that it is a predominately outcrossing allotetraploid. The high level of outcrossing in creeping bentgrass results in some characteristics which are normally attributed to autopolyploids such as the many tri and tetra-allelic genotypes observed in creeping bentgrass.

In performing crosses with creeping bentgrass, it is not possible to emasculate flowers due to small floret size, and attempts at self fertilization were ineffective. Therefore when possible, crosses that had loci fixed for alternate alleles were made to allow for the establishment of the level of self-fertilization. The clones PV 91-21 and PE 91-24 are homozygous for alternate alleles at the *Tpi-1* locus and all 205 progeny exhibited a three-banded heterozygous pattern indicating that no self fertilization occurred. However, based on homozygous alternate alleles at the *Tpi-2* locus in the cross of Syn91-3 X Em91-6, only 17 out of 146 progeny were crosses. Interestingly, the clone Syn91-3 is much less polymorphic than other clones used in this study. Moreover, some of the selfed progeny of this clone were homozygous at all loci examined. Homozygous alternate alleles were not available for all crosses studied,

however, in all cases seed harvested from the two parents was kept separate and alleles from the proposed male parent segregated in expected ratios inferring little or no self-fertilization had occured. With all crosses except Syn91-3 X EM91-6, seed was present on the parent tillers of only one of the two clones placed in isolation, suggesting that a timing mechanism may be involved in reducing self-fertility. The data from this study indicates that both cross- and self-fertilization can occur in creeping bentgrass with outcrossing being the predominant mating system.

Dudeck and Duich (1967) studied the utility of a systematic program of inbreeding and selection for breeding colonial bentgrass, *Agrostis tenuis* Sibth. Some loss of vigor, increased plant mortality and an increase in self-sterility were reported as the degree of inbreeding increased. However, only two generations of selfing were reported, therefore, no conclusions can be drawn as to the long-term effects of selection for self-fertility in *A. tenuis*. Current bentgrass varieties are synthetics, therefore, segregation for important characteristics can occur during the seed production process. However, inbreeding might provide a method of reducing the segregation of desirable characteristics which would create more uniform varieties. The utilization of inbreeding in creeping bentgrass would require a more thorough understanding of its effects on fertility and plant vigor as well as the mechanisms of self incompatibility in this species.

The *Pgi-2* locus is very highly polymorphic in creeping bentgrass, with 7 or 8 scorable alleles present in the cultivated population, additionally, *Pgi-2* has the highest staining activity of the enzyme systems tested. The combination of these two factors indicates that this locus may have potential for the fingerprinting of creeping bentgrass varieties. PGI allozymes have been useful in the fingerprinting of ryegrass varieties (Gilliland et al. 1982) and Yamamoto and Duich (1994) were able to distinguish 12

creeping bentgrass varieties based on banding patterns at the *Pgi-2* locus. However, in an analysis of additional bentgrass varieties, Warnke et al. (1995) were not able to distinguish all creeping bentgrass varieties based on the *Pgi-2* locus.

Isozymes provide a relatively inexpensive, reliable, and informative source of genetic markers for creeping bentgrass. Isozymes may also have utility in establishing varietal and species relationships and estimating the genetic diversity of the cultivated and non-cultivated species (Warnke et al. 1995). Isozymes were chosen for the initial studies in creeping bentgrass because they are polymorphic and technically less complicated and less expensive than DNA markers such as RAPDs and RFLPs. The information provided by isozyme markers can be utilized to develop highly informative mapping populations for future studies with DNA-based markers. Genetic maps developed with RFLP and RAPD markers could aid in the dissection of quantitative traits through QTL analysis and provide a means of rapid and reliable selection for simply inherited traits in breeding populations.

LIST OF REFERENCES

Aruldekar, S., R.S. Bringhurst, and V. Voth. 1981. Inheritance of PGI and LAP isozymes in octaploid cultivated strawberries. J. Amer. Soc. Hort. Sci. 106:679-683.

Beaver, J.A., and A.F. Iezzoni. 1993. Allozyme inheritance in tetraploid sour cherry (*Prunus cerasus* L.) J. Amer. Soc. Hort. Sci. 118(6):873-877.

Dudeck, A.E., and J.M. Duich. 1967. Preliminary investigations on the reproductive and morphological behavior of several selections of colonial bentgrass, *Agrostis tenuis* Sibth.

Duich, J.M. 1985. The bent grasses. Weeds trees and turf. 72-78.

Gilliland, T.J., M.S. Camlin, and C.E. Wright. 1982. Evaluation of phosphoglucoisomerase allozyme electrophoresis for the identification and registration of cultivars of perennial ryegrass (*Lolium perenne*) Seed Sci. & Technol., 10:415-430.

Haldane, J.B.S. 1930. Theoretical genetics of autopolyploids. J. Genetics 22:359-372.

Harlan, J.R. 1992. Crops and Man. American Society of Agronomy, Madison, Wisc.

Hart, G.E. 1983. Genetics and evolution of multilocus isozymes in hexaploid wheat. In Isozymes: Current Topics in Biological and Medical Research 10:365-380.

Hauber, D.P. 1986. Autotetraploidy in Haplopappus spinulosus hybrids: evidence from natural and synthetic tetraploids. Amer. J. Bot. 73:1595-1606.

Hitchcock, 1951. Manual of the grasses of the United States.

Jones, K. 1956. Species differentiation in *Agrostis* I. Cytological relationships in *Agrostis canina* L. J. Genet. 54:370-376.

Jones, K. 1956. Species differentiation in *Agrostis* II. The significance of chromosome pairing in the tetraploid hybrids of *Agrostis canina* subsp. Montana Hartm., *A. tenuis* Sibth. and *A. stolonifera* L. J. Genet. 54:377-393.

Jones, K. 1956. Species differentiation in *Agrostis* III. *Agrostis gigantea* Roth. and its hybrids with *A. tenuis* Sibth. and A. stolonifera L. J. Genet. 54:394-399.

Krebs, S., and J. Hancock. 1989. Tetrasomic inheritance of isozyme markers in the highbush blueberry, *Vaccinium corymbosum* L. Heredity 63:11-18.

MacKey, J. 1970. Significance of mating systems for chromosomes and gametes in polyploids. Hereditas 66:165-176.

Martinez-Zapater, J.M. and J.L. Olivier. 1984. Genetic analysis of isozyme loci in tetraploid potatoes (Solanum tuberosum L.). Genetics 108:669-679.

Muller, H.J. 1914. A new mode of segregation in Gregory's tetraploid Primulas. Amer. Nat. XLVIII, 508-512.

Quiros, C.F. 1982. Tetrasomic inheritance for multiple alleles in alfalfa. Genetics 101:117-127.

Quiros, C.F., and N. McHale. 1985. Genetic analysis of isozyme variation in diploid and tetraploid potatoes. Genetics 111:131-145.

Roose, M.L., and L.D. Gottlieb. 1976. Genetic and biochemical consequences of polyploidy in *Tragopogon*. Evolution 30:818-830.

Soltis, D.E., and P.S. Soltis. 1988. Electrophoretic evidence for tetrasomic segregation in *Tolmiea menziesii* (Saxifragacea). Heredity 60: 375-382.

Soltis, D.E., and P.S. Soltis. 1989. Tetrasomic inheritance in Heuchera micrantha (Saxifragaceae). J. Hered. 80:123-126.

Vallejos, E. 1983. Enzyme activity staining. In S.D. Tanksley and T.J. Orton (eds.) Isozymes in plant genetics and breeding, Part A, 469-516, Elsevier, Amsterdam.

Weeden, N.F., and J.F. Wendel 1989. Visualization and interpretation of plant isozymes. In D.E. Soltis and P.S. Soltis (eds.) Isozymes in Plant Biology 5-45.

Wilkinson, J.F., and J.B. Beard. 1972. Electrophoretic identification of *Agrostis palustris* and *Poa pratensis* cultivars. Crop Sci. 12:833-834.

Yamamoto, I., and J.M. Duich. 1994. Electrophoretic identification of cross-pollinated bentgrass species and cultivars. Crop Sci. 34:792-798.

CHAPTER II

RELATIONSHIPS AMONG CREEPING BENTGRASS (*Agrostis palustris* Huds.) CULTIVARS BASED ON ISOZYME POLYMORPHISMS

ABSTRACT

An understanding of the genetic variability within a crop species is essential to its improvement. The objectives of this research were to study the utility of isozyme patterns for creeping bentgrass cultivar discrimination and to estimate the relationships between creeping bentgrass cultivars based on isozyme patterns. Seventy plants from each of 18 creeping bentgrass cutivars and 25 plants from one plant introduction were scored for 24 isozyme polymorphisms representing six loci. All cultivars except a small group containing the cultivars Pennlinks, Pro/Cup, Southshore and Lopez could be uniquely characterized based on a 20% or greater band frequency in one cultivar verses absence of the band in the most closely clustered cultivar. The isozyme patterns from each plant were used to generate a genetic distance matrix for the plants within a cultivar, and the average band frequency within a cultivar was utilized to create a genetic distance matrix between cultivars. The cultivars Pennlinks, Pro/Cup, Southshore and Lopez had the highest average within-cultivar genetic distances indicating that additional marker loci will be needed to distinguish these cultivars. The UPGMA cluster analysis generated from the between cultivar genetic distance matrix divided the cultivars into two clusters. One cluster contains the variety Seaside as its base suggesting that this variety may have provided some initial germplasm for this group. The second group contains the cultivars Pennlinks, Southshore, Pro/Cup and

Lopez as well as a group of cultivars with some unique allozyme characteristics. The plant introduction PI251945 was distantly related to the cultivated germplasm indicating that European material may be a source of genetic diversity to broaden U.S bentgrass germplasm.

INTRODUCTION

The bentgrasses are native to Western Europe (Harlan 1992) with the genus *Agrostis* consisting of approximately 200 species (Hitchcock 1951). The four species commonly accepted as turfgrasses are *Agrostis palustris* Huds - creeping bentgrass (2n=4x=28), *Agrostis canina* - velvet bentgrass (2n=2x=14), *Agrostis tenuis* - colonial bentgrass (2n=4x=28), and *Agrostis gigantea* - redtop bentgrass (2n=6x=42). Creeping bentgrass is the most widely utilized of the turf-type bentgrasses because it has excellent tolerance of low mowing heights and a strong stoloniferous growth habit making it ideally suited for the establishment of golf course greens and fairways in areas where cool-season turfgrasses are adapted.

An understanding of the genetic diversity present in the cultivated germplasm of a species as well as the location of new sources of genetic variability is important for the optimal utilization of genetic resources. Plant breeders must have an understanding of the genetic variability of elite germplasm because continued reselection within this germplasm can narrow the genetic base of elite material and ultimately increase the potential vulnerability to pests and abiotic stresses. Information about the location of new sources of genetic variability can help broaden the genetic base of elite material and maintain long-term improvement.

Information about the relatedness of creeping bentgrass cultivars is limited because it is an allogamous species and in many cases the parental clones used in synthetic cultivar development are of unknown origin making accurate estimates of relatedness based on coefficients of parentage impossible. However, in a number of species, estimates of genetic similarity between cultivars have been determined based

on molecular markers such as isozymes in soybeans (Cox et al., 1985) and potatoes (Douches and Ludlam, 1991), RFLP's in barley (Melchinger et al., 1994), maize (Messmer et al., 1993) and tall fescue (Xu et al. 1994) and RAPD markers in lima bean (Nienhuis et al., 1995), and olive (Fabbri et al., 1995). Isozyme markers are not as numerous as RFLP or RAPD markers, however, they are polymorphic in creeping bentgrass populations (Warnke et al., 1995) and technically simpler than RFLP or RAPD markers with large population sizes. The objectives of this research were to (1) assess isozyme patterns for creeping bentgrass cultivar identification, (2) determine the optimum sample size for estimating allozyme frequencies from creeping bentgrass populations, and (3) estimate the genetic relationship between creeping bentgrass cultivars based on these allozyme frequencies.

MATERIALS AND METHODS

Eighteen *Agrostis palustris* Huds. cultivars representing most of the named creeping bentgrass cultivars commercially available in the United States and one plant introduction were assayed for isozyme polymorphisms. Seed was obtained from the 1993 National Turfgrass Evaluation Programs (NTEP) bentgrass cultivar trial or directly from the seed company that produced the cultivar. In a few cases seed from the 1989 NTEP bentgrass cultivar trial was used (Table2.1).

The condition of plants for analysis is vital for successful isozyme resolution. It was determined that etiolated tissue from plants maintained in the laboratory for 1 week prior to analysis produced more consistant enzyme activity. Individual plants at least two months old were used for isozyme analysis. Plants were maintained in the greenhouse and fertilized every two weeks with a 20-20-20 water soluble fertilizer solution and trimmed regularly to promote tiller production. A 4 - 5 leaf sample of newly expanded leaves was collected from each plant and crushed in 80ul of an extraction buffer composed of 75mM Tris-HCL buffer, pH 7.5, 5% PVP-40 (w/v), and 14mM Mercaptoethanol (0.2% v/v). The extraction was done in chilled 12-sample porcelain color plates and macerated with a plexiglass rod rounded on one end. The crude extracts were absorbed onto 3 x 8 mm Whatman 3MM wicks and stored over night at -20°C, or at -80°C when longer term storage was needed.

Phosphoglucose isomerase (PGI, E.C.5.3.1.9), phosphoglucomutase (PGM, E.C.5.4.2.2), glutamate oxaloacetate transaminase (GOT, E.C.2.6.1.1), and triosphosphate isomerase (TPI, E.C.5.3.1.1) were resolved in tris-citrate/lithium borate

Table 2.1. Creeping bentgrass cultivars studied, the number of plants scored in each, and the average within cultivar genetic distance (AWGD) of each.

Cultivar	Year[a]	Source	Number Evaluated	AWGD[c]
1 Penneagle	1978	1993 NTEP bentgrass trial	72	0.233
2 Penncross	1955	1993 NTEP bentgrass trial	73	0.210
3 Trueline	1995	1993 NTEP bentgrass trial	73	0.267
4 Crenshaw	1993	1993 NTEP bentgrass trial	73	0.203
5 Southshore	1992	1993 NTEP bentgrass trial	73	0.326
6 Providence	1988	1993 NTEP bentgrass trial	70	0.230
7 National	1988	1989 NTEP bentgrass trial	70	0.312
8 Viper	1995	International Seeds	72	0.173
9 18th Green	1995	1993 NTEP bentgrass trial	73	0.257
10 Cobra	1988	1989 NTEP bentgrass trial	73	0.180
11 Emerald	1973	1989 NTEP bentgrass trial	73	0.138
12 Pennlinks	1987	1993 NTEP bentgrass trial	73	0.387
13 SR1020	1987	Seed Research	73	0.265
14 Putter	1988	Jacklin Seed	73	0.161
15 Seaside	1924	1993 NTEP bentgrass trial	73	0.258
16 Lopez	1994	1993 NTEP bentgrass trial	72	0.325
17 Pro/Cup	1994	1993 NTEP bentgrass trial	73	0.340
18 Cato	1993	1993 NTEP bentgrass trial	70	0.152
19 PI251945	1958[b]	Plant Intro. Station Pullman,WA	25	0.104

a year of release

b year collected

c Average within-cultivar genetic distance calculated using Nei's 1972 distance formula

pH 8.3 buffer system (Weeden, 1989), after 4h of electric current at 50 to 60 mA. Gel slabs consisted of 10% potato starch obtained from Starch Art, (Smithville, TX). Gel trays that provided for a direct contact between the gel tray and buffer were used. Enzyme activity stains were prepared according to the method of Vallejos, (1983).

Each gel contained 25 plants from a cultivar and 2 check plants, of known allozyme composition, to aid in band scoring. Allelic bands (Warnke et. al. 1995) were scored as 1 present or 0 absent for each plant. A total of 75 plants from each cultivar were analyzed, however, due to enzyme degradation in some samples, fewer plants were used to establish band frequencies (Table 2.1). The band frequency in the population was obtained by dividing the number of plants containing the band by the total plants examined. Genetic distances between and within each cultivar were established using Nei's 1972 distance formula. A dendrogram based on the distance matrix was constructed by applying the unweighted pair group method with arithmetic averages (UPGMA) cluster analysis. The distance matrix and dendrogram were both constructed using NTSYS-pc version 1.7 (Rohlf, 1992). Between-variety genetic distances were calculated using sample sizes of 4, 8, 12, 16, 20, 25 and 70 to establish the optimum population sample size for estimating the genetic distance between varieties.

RESULTS AND DISCUSSION

A total of 24 alleles were scored for 70 plants in each of the 18 cultivars. Segregation data is available for 14 of the 24 bands scored (Warnke et al. 1995). Six of the bands for which no segregation data are available come from the *Pgm-1* and *Pgm-2* loci. Genetic analysis (Warnke et al. 1995) has shown this to be a monomeric enzyme so assignment of additional alleles at these loci were based on band mobility differences compared to the check plants run with each gel. The *Pgi-2* locus in creeping bentgrass is highly variable with seven scorable alleles present, however, the dimeric structure of this enzyme and close mobilities of many alleles does not allow for the accurate classification of all alleles present in some genotypes without progeny testing. Therefore, only the fastest and slowest migrating alleles for each plant, i.e. those that were easily detected, were scored. Average band frequencies ranged from 0.01 for the *Pgm-1*^{4} and *Pgm-2*^{4} bands and 1.00 for the *Pgm-1*^{1} and *Got-2*^{1} bands (Table 2.2).

Distance values (Table 2.3) ranged from 0.007 for Southshore and Pennlinks to 0.277 for Cato and PI251945. The dendrogram resulting from the UPGMA cluster analysis is shown in Figure 2.1. The UPGMA cluster analysis separates the cultivars into two main groups. The first group includes 10 cultivars (Penneagle, Putter, Penncross, Trueline, Viper, Emerald, 18th Green, Cobra, Crenshaw and Seaside). With the exception of Crenshaw these are strongly creeping cultivars having a prostrate to semi-erect growth habit. The cultivar Seaside is at the base of this group and may

have provided much of the initial germplasm for creeping bentgrass cultivar development over the last 40 years. Seaside originated as a naturalized population growing in tidal flats near Coos Bay, Oregon and was the only widely availiable seeded bentgrass in

Table 2.2. Cultivar allele frequencies for loci that were polymorphic within *A. palustris*.

Locus-allele	Cultivars 1[a]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Average
Tpi-1[1]	0.01	0.29	0.16	0.01	0.28	0.07	0.04	0.00	0.00	0.03	0.00	0.41	0.03	0.00	0.20	0.09	0.35	0.04	0.08	0.11
Tpi-1[2]	0.96	0.92	0.99	0.95	0.97	0.98	1.00	1.00	0.96	1.00	1.00	1.00	1.00	0.99	0.95	1.00	1.00	1.00	0.84	0.97
Tpi-1[3]	0.77	0.83	0.87	0.80	0.72	0.85	0.81	1.00	0.96	1.00	0.95	0.71	0.72	0.92	0.87	0.77	0.77	0.85	1.00	0.85
Tpi-2[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.97	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	0.99
Tpi-2[2]	0.99	0.95	0.75	0.99	0.89	0.96	0.98	1.00	1.00	1.00	1.00	0.87	1.00	0.96	0.99	0.85	0.88	0.88	1.00	0.94
Tpi-2[3]	0.39	0.23	0.40	0.01	0.36	0.32	0.31	0.00	0.00	0.03	0.05	0.40	0.05	0.39	0.00	0.37	0.48	0.63	0.16	0.24
Got-2[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Got-2[2]	0.96	0.97	0.96	1.00	0.93	0.99	0.94	0.93	0.79	0.83	1.00	0.97	0.99	1.00	0.71	0.97	0.93	1.00	1.00	0.94
Got-2[3]	0.12	0.00	0.10	0.01	0.29	0.00	0.11	0.43	0.01	0.37	0.00	0.20	0.00	0.00	0.33	0.01	0.16	0.00	0.04	0.12
Pgm-1[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Pgm-1[2]	0.00	0.00	0.00	0.19	0.63	0.31	0.56	0.00	0.00	0.00	0.00	0.57	0.47	0.00	0.00	0.71	0.67	1.00	0.00	0.27
Pgm-1[3]	1.00	1.00	1.00	0.81	0.31	0.45	0.43	0.96	1.00	1.00	1.00	0.37	0.15	0.63	1.00	0.29	0.36	0.00	1.00	0.67
Pgm-1[4]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Pgm-2[1]	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.44	0.00	0.00	0.00	0.36	0.00	0.00	0.00	0.01	0.36	0.00	0.07
Pgm-2[2]	1.00	1.00	1.00	1.00	1.00	0.97	1.00	1.00	0.92	1.00	1.00	1.00	1.00	1.00	0.95	1.00	1.00	1.00	1.00	0.99
Pgm-2[3]	0.20	0.21	0.17	0.36	0.38	0.45	0.62	0.19	0.65	0.15	0.39	0.44	0.48	0.00	0.81	0.36	0.47	0.08	0.24	0.35
Pgm-2[4]	0.00	0.00	0.00	0.00	0.00	0.04	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Pgi-2[1]	0.08	0.00	0.44	0.20	0.09	0.00	0.01	0.83	0.61	0.21	0.60	0.33	0.23	0.00	0.00	0.40	0.14	0.00	0.00	0.22
Pgi-2[2]	0.32	0.00	0.00	0.48	0.09	0.17	0.08	0.00	0.04	0.24	0.25	0.09	0.03	0.15	0.49	0.37	0.31	0.00	1.00	0.22
Pgi-2[3]	0.57	0.63	0.32	0.23	0.60	0.41	0.10	0.13	0.31	0.19	0.07	0.49	0.54	0.65	0.39	0.08	0.33	0.37	0.00	0.34
Pgi-2[4]	0.34	0.48	0.47	0.24	0.48	0.79	0.79	0.31	0.43	0.63	0.31	0.36	0.85	0.62	0.31	0.36	0.40	0.96	0.04	0.48
Pgi-2[5]	0.42	0.81	0.57	0.60	0.52	0.37	0.19	0.64	0.60	0.29	0.71	0.60	0.00	0.50	0.51	0.69	0.60	0.05	0.04	0.46
Pgi-2[6]	0.26	0.00	0.11	0.00	0.15	0.09	0.06	0.08	0.00	0.29	0.07	0.11	0.23	0.00	0.28	0.07	0.14	0.03	0.76	0.14
Pgi-2[7]	0.05	0.00	0.00	0.24	0.01	0.00	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05

a Cultivar numbering described in table 2.1.

Table 2.3. Matrix of Nei's coefficients of genetic distance for 19 creeping bentgrass cultivars. [a]

	1[b]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	0.000																		
2	0.025	0.000																	
3	0.024	0.024	0.000																
4	0.030	0.043	0.043	0.000															
5	0.063	0.062	0.069	0.067	0.000														
6	0.044	0.048	0.052	0.048	0.026	0.000													
7	0.098	0.114	0.102	0.073	0.059	0.036	0.000												
8	0.065	0.070	0.030	0.053	0.108	0.099	0.137	0.000											
9	0.062	0.058	0.040	0.047	0.105	0.071	0.115	0.036	0.000										
10	0.029	0.052	0.032	0.042	0.084	0.051	0.090	0.037	0.054	0.000									
11	0.044	0.049	0.024	0.021	0.100	0.071	0.113	0.018	0.023	0.037	0.000								
12	0.064	0.059	0.054	0.059	0.007	0.036	0.069	0.085	0.085	0.087	0.076	0.000							
13	0.105	0.122	0.115	0.105	0.052	0.031	0.060	0.145	0.104	0.098	0.132	0.066	0.000						
14	0.022	0.023	0.033	0.050	0.051	0.026	0.090	0.084	0.081	0.046	0.067	0.060	0.080	0.000					
15	0.040	0.058	0.068	0.039	0.085	0.068	0.108	0.081	0.055	0.043	0.053	0.083	0.129	0.084	0.000				
16	0.086	0.096	0.074	0.048	0.035	0.046	0.069	0.091	0.096	0.098	0.066	0.024	0.084	0.081	0.107	0.000			
17	0.066	0.071	0.068	0.054	0.010	0.030	0.058	0.108	0.101	0.089	0.084	0.008	0.071	0.064	0.078	0.015	0.000		
18	0.164	0.178	0.164	0.174	0.065	0.061	0.078	0.232	0.208	0.169	0.219	0.088	0.052	0.109	0.241	0.086	0.075	0.000	
19	0.073	0.152	0.126	0.079	0.175	0.135	0.176	0.147	0.153	0.076	0.103	0.171	0.195	0.137	0.077	0.149	0.143	0.277	0.000

a genetic distance based upon Nei (1972).

b cultivar numbering described in table 2.1.

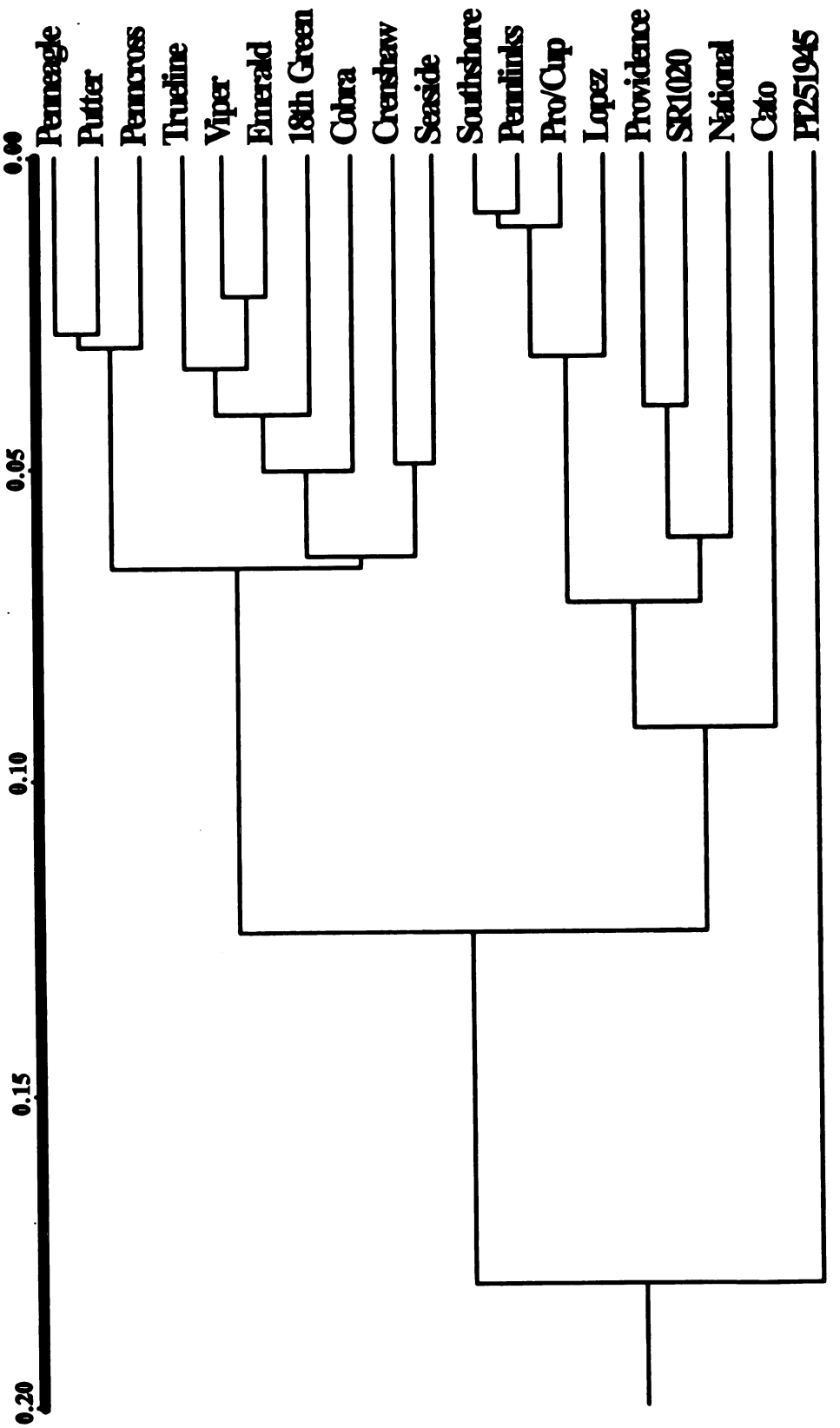

Figure 2.1. Dendrogram of 18 creeping bentgrass cultivars generated by UPGMA cluster analysis

theU.S. from the 1920's until 1955 when Penncross was released (Duich, 1985). The second cluster contains nine cultivars that can be divided into two groups. Four of these cultivars (Southshore, Pennlinks, Pro/Cup and Lopez) cluster quite closely and are difficult to distinguish from one another with the isozyme polymorphisms studied. One reason for the tight clustering of these four cultivars is that they possess most of the isozyme bands observed in this study. This isozyme diversity is evident in the fact that these four cultivars have the highest average within-cultivar genetic distances (AWGD) of any of the cultivars tested (Table 2.1). The cultivar Southshore is a very broad-based cultivar derived from the progenies of 203 selected clones (Hurley et al., 1994) which would explain its high AWGD The last four cultivars in the second group (Providence, SR1020, National and Cato) do not group closely with any of the other cultivars and, in fact, these cultivars all possess some unique isozyme characteristics. The plant introduction PI251945 was included in the study to gain some insight into the European bentgrass germplasm. PI251945 was collected in 1958 from Austria and, based upon these results, it is quite distantly related to the United States cultivars examined, suggesting that European germplasm may be a means to broaden the genetic diversity of U.S. germplasm. The cultivars SR1020 (5 clone synthetic) and Crenshaw (six clone synthetic) share three parental clones in common (Engelke et al., 1995). Despite this common genetic base they have very different isozyme profiles and do not cluster closely in the dendrogram. These cultivars are synthetic populations and differences in the initial parent clones that make up these cultivars can result in large genetic distances between the cultivars due to numerous isozyme band differences. For

example, the exclusion of two SR1020 parental clones from Crenshaw could result in the exclusion of isozyme bands present in SR1020 from Crenshaw. Furthermore, the addition of three new parental clones to the cultivar Crenshaw could result in bands being present in Crenshaw that are not in SR1020 leading to a large number of band differences between the two cultivars and a large genetic distance estimate.

Creeping bentgrass cultivar identification via isozymes is complicated by the fact that cultivars are synthetics and considerable within-cultivar variation exists. The establishment of isozymes as a reliable characteristic for fingerprinting requires that band frequencies in a given cultivar be stable in different environments and generations. In this study all cultivars except Southshore, Pennlinks, Pro/Cup and Lopez could be distinguished based on a band being present in one cultivar at a frequency greater than 20% and absent from the most closely-related cultivar. The cultivar Lopez is close to meeting this requirement at the *Got-2*^{3} allele. However, further research needs to be done on seed lot variability and post-establishment effects on isozyme band frequencies to determine the overall utility of isozymes for creeping bentgrass cultivar discrimination. Additionally, it appears that more enzyme systems will need to be evaluated to reliably distinguish Pennlinks, Pro/Cup and Southshore.

Yamamoto and Duich (1994) examined the utility of isozyme analysis with bulk plant leaf sampling and were able to distinguish twelve creeping bentgrass cultivars using only the *Pgi-2* locus. Østergaard and Nielson (1981) investigated the utility of using the *Pgi-2* locus for cultivar identification in tetraploid ryegrass and found that different seed lots did not differ with respect to frequencies of allele classes. Additionally, seed size differences and low germination frequencies did not lead to

significant within-cultivar allozyme frequency differences. Hayward et al. (1978) demonstrated Pgi-2 isozyme profiles in perennial ryegrass did not differ significantly between seed stocks and samples from three year old swards. Similar studies must be conducted for creeping bentgrass to establish the utility of isozyme analysis for creeping bentgrass variety discrimination in the turf industry.

Sample size is an important consideration in designing experiments for outcrossing species. One method for determining optimum sample sizes was discribed by Xu et al. (1994) in an RFLP analysis of tall fescue cultivars. Average genetic distances are calculated for different population sizes and the point at which there is no longer a significant difference between the average genetic distance of two population sizes is considered the most efficient sample size. In our study average genetic distances between cultivars were calculated using data from 4, 8, 12, 16, 20, 25, and 70 plants. The mean distance between cultivars decreased and became consistent as population size increased (Figure 2.2). The difference between means ($\alpha = 0.05$) was not significant for population sizes of 16 and 20 or 16 and 25, however, there was a significant difference between population sizes of 16 and 70 as well as 20 and 70 but not between 25 and 70 indicating that a random sample of 25 plants is the minimum number of plants needed to accurately estimate the isozyme variation within these creeping bentgrass cultivars. In the study by Xu et al. (1994) the largest population size examined was 20 and there was no significant difference between population sizes of 16 and 20 plants. These results indicate that 16 to 25 plants is an adequate population size for sampling the genetic variation within these two outcrossing species.

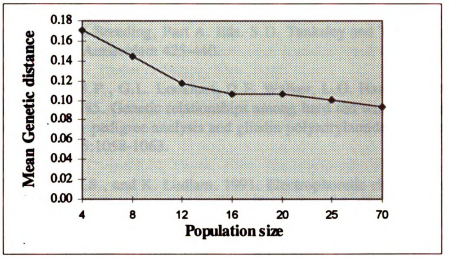

Figure 2.2. Effects of sample size on the mean genetic distances among creeping bentgrass cultivars. The difference between population sizes of 25 and 70 were not significant ($\alpha = 0.05$).

LIST OF REFERENCES

Bailey, D.C. 1983. Isozymic variation and plant breeders' rights. In: Isozymes in Plant Genetics and Breeding, Part A. Eds. S.D. Tanksley and T.J. Orton. Elsevier Science Publishers, Amsterdam 425-440.

Cox, T.S., J.P., G.L. Lookhart, D.E. Walker, L.G. Harrell, L.D. Albers, and D.M. Rodgers. 1985. Genetic relationships among hard red winter wheat cultivars as evaluated by pedigree analysis and gliadin polyacrylamide gel electrophoretic patterns. Crop Sci. 25:1058-1063.

Douches, D.S., and K. Ludlam. 1991. Electrophoretic characterization of North American potato cultivars. American Potato Journal. 68:767-780.

Duich, J.M. 1985. The bent grasses. Weeds trees and turf. 72-78.

Engelke, M.C., V.G. Lehman, W.R. Kneebone, P.F. Colbaugh, J.A. Reinert, and W.E. Knoop. 1995. Registration of 'Crenshaw' creeping bentgrass. Crop Sci. 35:589.

Fabbri, A., J.I. Hormaza, and V.S. Polito. 1995. Random amplified polymorphic DNA analysis of olive (*Olea europaea* L.) cultivars. J. Amer. Soc. Hort. Sci. 120(3):538-542.

Harlan, J.R. 1992. Crops and Man. American Society of Agronomy, Madison, Wisc.

Hayward, M.D., N.J. McAdam, T. Balls, and M. Zaruk. 1978. The use of isoenzymes as genetic markes. Report. Welsh Plant Breeding Station. 47.

Hurley, R.H., V.G. Lehman, J.A. Murphy and C.R. Funk. 1994. Registration of 'Southshore' creeping bentgrass. Crop Sci. 34:1124-1125.

Melchinger, A.E., A. Graner, M. Singh, and M.M. Messmer. 1994. Relationships among European barley germplasm: I. genetic diversity among winter and spring cultivars revealed by RFLPs. Crop Sci. 34:1191-1199.

Messmer, M.M., A.E. Melchinger, R.G. Herrmann, and J. Boppenmaier. 1993. Relationships among early European maize inbreds: II. Comparison of pedigree and RFLP data. Crop Sci. 33:944-950.

Nei, M. 1972. Genetic distance between populations. Amer. Natur. 106:283-292.

Nienhuis, J., J. Tivang, P. Skroch, and J.B. dos Santos. 1995. Genetic relationships among cultivars and landraces of lima bean (*Phaseolus lunatus* L.) as measured by RAPD markers. J. Amer. Soc. Hort. Sci. 120(2):300-306.

Østergaard, H. and G. Nielsen. 1981. Cultivar identification by means of isoenzymes I. Genotypic survey of the Pgi-2 locus in tetraploid ryegrass. Z. Pflanzenzüchtg. 87:121-132

Rohlf, F.J. 1992. NTSYS-pc. Numerical taxonomy and multivariate analysis system, version 1.70. Exeter Software, New York.

Xu, W.W., D.A. Sleper, and G.F. Krause. 1994. Genetic diversity of tall fescue germplasm based on RFLPs. Crop Sci. 34:246-252.

Yamamoto, I., and J.M. Duich. 1994. Electrophoretic identification of cross-pollinated bentgrass species and cultivars. Crop Sci. 34:792-798.

CHAPTER III

SCREENING CREEPING BENTGRASS (Agrostis palustris Huds.) AND COLONIAL BENTGRASS (Agrostis tenuis Sibth.) FOR RESISTANCE TO DOLLAR SPOT

ABSTRACT

Thirty-one cultivars of bentgrass representing 2 species (*Agrostis palustris* Huds. and *Agrostis tenuis* Sibth.) were screened for their reaction to a single isolate of *Sclerotinia homoeocarpa* Bennett. Two inoculations were conducted and plants were rated on a scale of 1 (dead plant) to 9 (no disease damage). The overall disease means of cultivars ranged from 1.0 to 2.6. Sixty-three percent of the bentgrass population was killed by the pathogen and 96% received a score of 1 or 2 indicating that resistance to this pathogen is very low. Those plants showing enhanced tolerance to *S. homoeocarpa* were selected and will be evaluated further in an effort to understand the mechanisms involved in there survival.

INTRODUCTION

Creeping bentgrass (*Agrostis palustris* Huds.) is a cool season turfgrass species primarily used for golf course putting greens and fairways. Dollar spot, caused by *Sclerotinia homoeocarpa* Bennett, is a major disease of creeping bentgrass in areas where high humidity, prolonged leaf wetness and temperatures greater than 20 C occur. *S. homoeocarpa* causes straw-colored, sunken spots approximately 5 cm in diameter on creeping bentgrass greens and fairways. Foliar lesions are typically straw colored with brown margins.

On golf courses in the temperate and hot humid regions of the United States more money is spent to manage dollar spot than on any other turfgrass disease (Vargas, 1994). In areas where frequent fungicide applications are practiced *S. homoeocarpa* has shown an ability to develop resistance to Cadmium-containing fungicides (Cole et al. 1968), Benzimidizol compounds (Warren et al. 1974) and recently the Dimethylase inhibitor fungicides (Vargas, 1994).

Endo et al. (1964) and Endo and Malca (1965) found a toxin associated with stunting and necrosis of bentgrass roots. The lack of pathogenicity at 10 and 32 C was associated with a lack of toxin production (Endo, 1963). The toxic substance was found to have chemical similarities to D-galactose (Malca and Endo 1965), however, plant response differences between the toxin and D-galactose lead them to conclude that D-galctose is not the toxic substance produced *in vivo* (Endo and Malca 1965).

A practical means of slowing resistance to these fungicides and reducing fungicide application costs is to develop host plant resistance to this disease. Research on the host pathogen interaction of *A. palustris* and *S. homoeocarpa* is limited. Cole et

al. (1969) in a study of the influence of fungal isolate and bentgrass variety on dollar spot development screened 18 different creeping bentgrass populations with 4 different dollar spot isolates and found a significant fungal isolate variety interaction. Reports from regional variety trials (Colbaugh & Engelke, 1993) (Hsiang & Cook, 1993) (Doney & Vincelli, 1993) provide somewhat conflicting information, however, the varieties SR1020 and Emerald are consistently highly susceptable, while the colonial bentgrasses have shown the highest levels of resistance.

The improvement of creeping bentgrass resistance to dollar spot will require a more thorough understanding of the methods of pathogenesis utilized by *S. homoeocarpa* as well as the plant defense mechanisms available in creeping bentgrass. Determinining plant resistance mechanisms under field conditions can be misleading, because it is difficult to determine if a plant without disease symptoms is resistant or was not exposed to disease pressure. This problem can be avoided by using artificial inoculations under controlled environmental conditions. The objectives of this research were to 1) establish a means of inoculating individual bentgrass plants under controled environment conditions, 2) establish the range of reaction among and within bentgrass populations to *S. homoeocarpa* and, 3) select the most resistant plant for further genetics studies.

MATERIALS AND METHODS

Seeds from 31 different bentgrass cultivars and plant introductions (Table3.1) were planted in flats containing 128 cells of 3.5 cm^2 volume filled with Bacto soil-less media. The flats were misted twice a day until plants reached the three-leaf stage. The healthiest 100 plants from each cultivar and plant introduction were selected and watered as needed, fertilized monthly with a 20-20-20 soluble fertilizer, and clipped twice monthly.

A *S. homoeocarpa* isolate obtained from dollar spot infected turf at the Hancock Turfgrass Research Center East Lansing, MI was used to prepare infested topdressing according to the method of Goodman and Burpee (1991). A mixture of sand and cornmeal (2:1) was placed in 30 x 40-cm aluminum baking pans lined with aluminum foil. The sand cornmeal mixture was smoothed to a depth of 1-cm and autoclaved at 121 C twice for 60 minutes then moistened to 6% (v/v) with 1% lactic acid in sterile deionized water. Three fungal colonies on 20ml of potato dextrose agar in 9-cm petri dishes were cut into 100-200 pieces and placed on the 1200cm^3 of sterile sand-cornmeal. After incubation for 2 weeks at room temperature the media was sliced and forced through a 2.5-mm mesh screen.

Five-month old creeping bentgrass plants from the 31 different bentgrass varieties and plant introductions were inoculated by top-dressing with 0.75g of *S. homoeocarpa* infested sand cornmeal and then placed in a growth chamber. Populations to be screened were split into two groups due to growth chamber space limitations. Controls of both uninoculated and inoculated plants with noninfested sand-cornmeal were included with each screening. The flats and growth chamber walls

Table 3.1. Results of artifical inoculation of 31 bentgrass populations with *S. homoeocarpa.*

Entry	Species	Origin	Mean	Number of Plants in Class							Number
			Rating	* 1	2	3	4	5	6	7	Scored
DF-1	Creeping	Tee-2-Green Corp.	2.6	13	60	3	6	12	4	2	100
A-1	Creeping	Tee-2-Green Corp.	2	27	61	3	4	2	3	0	100
Lopez	Creeping	Finelawn Research	1.9	24	71	1	3	1	0	0	100
Cobra	Creeping	International Seeds	1.7	42	52	2	4	0	0	0	100
Syn 92-1-93	Creeping	Texas A&M Univ.	1.7	52	40	0	6	2	0	0	100
Bar Ws 4210	Creeping	Barenbrug Holland	1.6	48	45	3	3	1	0	0	100
Pennlinks	Creeping	Tee-2-Green Corp.	1.6	58	33	1	7	1	0	0	100
88CBE	Creeping	International Seeds	1.5	42	32	5	0	0	0	0	79
18th Green	Creeping	Johnson Seeds	1.5	54	46	0	0	0	0	0	100
Southshore	Creeping	Loft's Seed, Inc	1.5	52	46	0	2	0	0	0	100
PI 235541	Creeping	Pullman WA.	1.5	65	20	2	6	1	0	0	94
Providence	Creeping	Seed Research	1.5	49	48	0	1	0	0	0	98
G-2	Creeping	Tee-2-Green Corp.	1.5	44	55	0	0	0	0	0	99
G-6	Creeping	Tee-2-Green Corp.	1.5	49	50	0	1	0	0	0	100
Penneagle	Creeping	Tee-2-Green Corp.	1.5	57	40	0	3	0	0	0	100
SR1020	Creeping	Seed Research	1.4	61	36	3	0	0	0	0	100
Penncross	Creeping	Tee-2-Green Corp	1.4	63	36	0	1	0	0	0	100
PI 235440	Creeping	Pullman WA.	1.3	70	20	0	2	0	0	0	92
PI 251945	Creeping	Pullman WA.	1.3	72	25	1	2	0	0	0	100
A-4	Creeping	Tee-2-Green Corp.	1.3	78	20	0	1	1	0	0	100
Syn 92-5-93	Creeping	Texas A&M Univ.	1.3	73	23	2	2	0	0	0	100
Syn 92-2-93	Creeping	Texas A&M Univ.	1.3	76	22	0	1	1	0	0	100
Pro/Cup	Creeping	Forbes Seed	1.2	81	19	0	0	0	0	0	100
Emerald	Creeping	International Seeds	1.2	81	18	0	1	0	0	0	100
Crenshaw	Creeping	Texas A&M Univ.	1.2	75	25	0	0	0	0	0	100
Cato	Creeping	Texas A&M Univ.	1.2	79	21	0	0	0	0	0	100
Trueline	Creeping	Turf Merchants	1.2	72	20	0	0	0	0	0	92
SR7100	Colonial	Seed Research	1.1	90	9	0	0	0	0	0	99
Seaside	Creeping	Standard	1.1	89	11	0	0	0	0	0	100
Tendez	Colonial	Finelawn Research	1	94	1	0	0	0	0	0	95
Syn 1-88	Creeping	Texas A&M Univ.	1	86	14	0	0	0	0	0	100
		Total or mean	1.4	1916	1019	26	56	22	7	2	3048
		% of total plants		63	33	0.8	1.8	0.7	0.2	0.1	
		% of plants selected								3.7	
				* Rating from 1-9 1=dead plant 9=no damage							

were misted daily at 1700 h to increase relative humidity. The growth chamber was shut down every night during the inoculation phase to eliminate air movement. The flats were maintained at 75 to 85% relative humidity and 25 to 30 C from 1700 h to 0900 h. Temperatures were 25 to 30 C and relative humidity 30-60% from 0900 to 1700. Disease pressure was maintined for seven days and each group of varieties received two inoculations to control for the possibility of escapes. A 1-9 rating scale was established (Table 3.1) to assess the level of damage to each plant. Disease ratings were taken two weeks after inoculation because this allowed for a more accurate assessment of plant damage.

Table 3.2. Visual rating criteria for bentgrass plants inoculated with *Sclerotinia homoeocarpa.*

Score	Visual criteria
1	dead plant
2	one or two surviving tillers at edge of plant
3	three or four surviving tillers
4	five or six surviving tillers
5	seven or eight surviving tillers
6	nine or ten surviving tillers
7	greater than 10 surviving tiller with leaf necrosis very visable
8	greater than 10 surviving tillers with some leaf necrosis visable
9	no plant damage visable

RESULTS AND DISCUSSION

The range of disease scores went from 1 to 7 with 96% of the plants receiving a score of 1 (dead plant) or 2 (1 or 2 surviving tillers) (Table 3.1). The average disease rating was a 1.4, indicating that overall disease pressure was very high. The inoculation procedure resulted in very uniform disease pressure based on visual observation of the level of mycelia growth on individual plants (Figure 3.1). One hundred thirteen plants (3.7% of the plants screened) received a score from 3 to 7 and these plants were selected for further study. The variety DF-1 had the highest level of resistance with a mean score of 2.6 and 6 plants with a score of 6 or better. However, this variety was still heavily damaged by the disease with 73 out 100 plants having a score of 1 or 2. The two colonial bentgrass varities SR7100 and Tendez showed high levels of susceptability under these inoculation conditions having mean scores of 1.1 and 1.0, respectively.

Plants employ a wide range of defense mechanisms to restrict damage from potential pathogens. Defense mechanisms can be classified as avoidance, resistance and tolerance (Parlevliet, 1981). Avoidance mechanisms are those that reduce the contact of potential pathogens with the host species. Resistance and tolerance mechanisms operate after pathogen contact has been established. Resistance mechanisms are mostly of a chemical nature and result in a reduction in the growth and development of the pathogen. Tolerance mechanisms generally do not reduce pathogen growth, however, the level of damage to the host is reduced.

Lumsden (1978) in a review of pathogenesis in plant diseases caused by *Sclerotinia* species states that the success of *Sclerotina* spp. as a pathogen appears to be

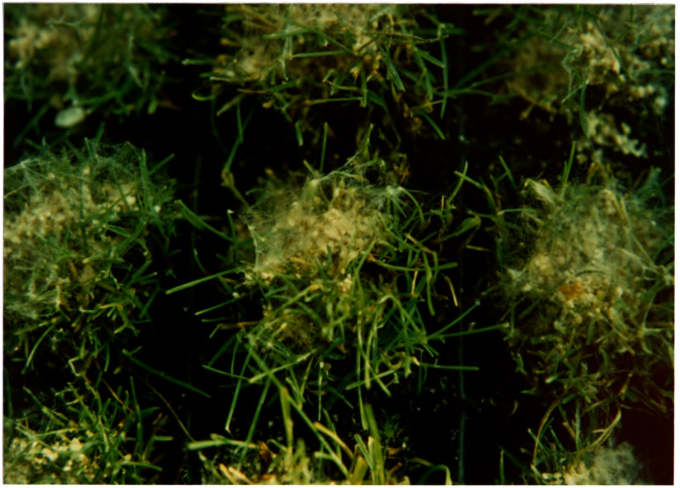

Figure 3.1. *Scleortinia homoeocarpa* mycelia development on creeping bentgrass plants 12 hours after inoculation.

dependent on a complex combination of factors that can overwhelm the host plant by rapidly acting before the host can respond. This type of pathogenesis would appear to occur with bentgrass as well because extensive fungal development can occur within 12 hours of inoculation. This type of pathogenesis is likely to circumvent plant resistance mechanisms such as appositional cell wall formation, which has been shown to be important for fungal resistance in the Gamineae (Sherwood and Vance, 1980).

Avoidance-type mechanisms such as open plant canopies that reduce plant moisture levels have been shown to reduce disease incidence in other plant species affected by *Sclerotina* spp. (Schwartz et al. 1978). Bentgrass plant canopy characteristics are not well understood so the importance of this factor in dollar spot resistance cannot be estimated. However, high levels of turf moisture are essential for dollar spot development and management factors which reduce turf moisture levels, such as early morning dew removal, have been shown to reduce the incidence of dollar spot under field conditions (Williams et al. 1993). Therefore, open canopy types that reduce turf moisture levels and plants with reduced levels of guttation fluid excretion might be expected to exhibit an avoidance type of defense mechanism under field conditons. Colonial bentgrass is a rhizomatous species with a more erect leaf orientation than creeping bentgrass which could result in a microclimate less condusive to high moisture levels and explain why this species shows lower levels of dollar spot than creeping bentgrass under field conditons. However, this type of plant defense mechanism would be eliminated from our growth chamber experiments because plants were misted daily to maintain leaf wetness which could explain why the colonial bentgrasses preformed so poorly in this study.

The fact that no completely resistant plants were observed in this experiment might indicate that a tolerance type of defense mechanism is being exhibited by the higher rated surviving plants. This type of mechanism would be important for bentgrass recovery from dollar spot injury, however, its importance in the reduction of disease incidence under field conditions needs further evaluation. A potential tolerance mechanism could be reduced susceptibility to the pathogen produced toxin or an ability to dispose of the toxin. The experimental variety DF-1 was developed by selecting salt tolerant clones from the variety Seaside, therefore, it is possible that this variety has an efficient mechanism of dealing with toxic substances.

Further research needs to be conducted to determine the importance of turf canopy characteristics and turf wetness levels on dollar spot development. Additionally, an understanding of the importance of disease tolerance under field conditions needs to be determined. If disease avoidance and tolerance mechanisms are shown to be important in disease reduction under field conditions then genetic information needs to be developed for these characteristics so that effective breeding strategies can be established. A more thorough understanding of the mechanism of *S. homoeocarpa* pathogenesis and elucidation of mechanisms by which bentgrass might resists infection should provide the scientific base from which cultivars with improved resistance to this important disease can be developed.

LIST OF REFERENCES

Colbaugh, P.F. and M.C. Engelke. 1993. Sclerotinia dollar spot on bentgrasses. Biological and cultural tests. 8:111.

Cole, H., J.M. Duich, L.B. Massie, and W.D. Barber. 1969. Influence of fungus isolate and grass variety on *Sclerotinia* dollarspot development. Crop Sci. 9:567-570.

Doney, J.C. Jr., and P.C. Vincelli. 1993. Reactions of bentgrasses to dollar spot and brown patch. Biological and Cultural Tests. 8:118.

Endo, R.M. 1963. Influence of temperature on rate of growth of five fungus pathogens of turfgrass and on rate of disease spread. Phytopathology 53:857-861.

Endo, R.M., L.Malca, and E.M. Krausman. 1964. Degeneration of the apical meristem and apex of bentgrass roots by a fungal toxin. Phytopathology 54:1175-1176.

Endo, R.M., and I. Malca. 1965. Morphological and cytohistological responses of primary roots of bentgrass to Sclerotinia homoeocarpa and D-Galactose. Phytopathology 55:781-789.

Goodman, D.M, and L.L. Burpee. 1991. Biological control of dollar spot disease of creeping bentgrass. Phytopathology. 81:1438-1446.

Hsiang, T., and S. Cook. 1993. Resistance of bentgrass cultivars to dollar spot disease. Biological and cultural tests. 8:110.

Lumsden, R.D. 1979. Histology and physiology of pathogenesis in plant diseases caused by *Sclerotinia* species. Phytopathology 69(8):890-895.

Malca, I., and R.M. Endo. 1965. Identification of galactose in cultures of *Sclerotinia homoeocarpa* as the factor toxic to bentgrass roots. Phytopathology 55:775-780.

Schwartz, H.F., J.R. Steadman, and D.P. Coyne. 1978. Influence of *Phaseolus vulgaris* blossoming characteristics and canopy structure upon reaction to *Sclerotinia sclerotiorum*. Phytopathology 68:465-470.

Sherwood, R.T., and C.P. Vance. 1980. Resistance to fungal penetration in Gramineae. Phytopathology 70:273-279.

Vargas, J.M. 1994. Management of turfgrass diseases. p. 23-26 2nd ed. CRC Press, Inc., Boca Raton Fl.

Williams D.W., A.J. Powell Jr. and P.C. Vincelli. 1993 Response of dollar spot to dew removal from creeping bentgrass. Agronomy Abstracts pp. 165.