AN ABSTRACT OF THE THESIS OF

Donald J. Floyd for the degree of Doctor of Philosophy in Crop Science presented on April 28, 2000. Title: Ryegrass Fluorescence Expression during Three Generations of Seed Increase and its Heritability among Half-sib Families.

Abstract approved:

Redacted for Privacy

Reed E. Barker

Seedling root fluorescence (SRF) has been used as a test in the USA since 1941 to discriminate Italian ryegrass (*Lolium multiflorum* Lam.) from perennial ryegrass (*L. perenne* L.). Generally, roots of Italian ryegrass fluorescece when exposed to ultra-violet light, while roots of perennial ryegrass do not. Presence of fluorescent seedlings in lots of perennial ryegrass can cause concern among buyers who fear contamination of Italian ryegrass. The SRF trait has introgressed between the two species of ryegrass, and since 1991 breeders have documented inherent varietal fluorescence levels (VFL) of ryegrass cultivars before multiplication of seed under seed certification programs. Studies were conducted to further elucidate plant growing condition aspects of SRF in ryegrass. Four populations with different fluorescence levels were assembled from among ryegrass half-sib families. The four populations were increased independently for three generations at each of three Oregon locations. There was considerable variation for SRF among locations within populations, and for generations within location. Generally, each population increased in SRF each generation of

multiplication at Corvallis, but did not increase at the Aurora and Madras sites. Significant location X population and location X generation interactions indicated a strong location effect on SRF. Heritabilities of SRF were estimated using realized response from 26 half-sib families at the three locations. Heritability estimates ranged from 97 to 99 %. Although the heritability of SRF was high, there was considerable genotype X environment interaction for trait expression. Evidence of high variability for SRF expression should be considered as plant breeders describe VFL of cultivars, and multiple seed production locations and generations should be used in descriptions. Based on results of these studies, SRF level for a cultivar developed from a single location cannot be used to predict SRF expression at other locations. The SRF trait alone should not be used as a discriminator of ryegrass cultivars, and should be discontinued for use in measuring genetic contamination between Italian and perennial ryegrasses.

Ryegrass Fluorescence Expression during Three Generations of Seed Increase and its Heritability among Half-sib Families.

by

Donald J. Floyd

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented April 28, 2000
Commencement June, 2001

Doctor of Philosophy thesis of Donald J. Floyd presented on April 28, 2000

APPROVED:

Redacted for Privacy

Major Professor, representing Crop Science

Redacted for Privacy

Head of Department of Crop and Soil Science

Redacted for Privacy

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Redacted for Privacy

Donald J. Floyd, Author

ACKNOWLEDGMENTS

I thank my major professor, Dr. Reed Barker, for his guidance, support, and patience throughout this study. He not only has been a prized mentor, but a trusted colleague throughout my academic and professional career.

Secondly, I appreciate my family, Carol, Daniel and Marc for their sacrifice of time and fellowship during countless occasions when I needed to focus on completion of this research. I pray that someday they will come to understand why this course of study was so important to me.

I am grateful for the encouragement of my graduate committee members, Drs. Pat Hayes, Don Grabe, Tom Chastain, Steve Griffith, Tom Adams, and especially Dr. Jerry Pepin. He helped me understand the importance of this research.

I value the financial/technical support provided me by my employer, Pickseed West, Inc. It enabled me to pursue this research while still making a living. I feel very fortunate for that arrangement.

Lastly, but by no means least, I am indebted to two good friends and colleagues, Drs. Virginia Lehman and Leah Brilman. They have lent their ears over the years listening to my questions and concerns regarding agricultural research in general, and specifically to my program at Oregon State University. They instilled in me confidence when I needed it the most.

Donald J. Floyd

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF APPENDIX TABLES

Ryegrass Fluorescence Expression during Three Generations of Seed Increase and its Heritability among Half-sib Families.

INTRODUCTION

Ryegrass (*Lolium* sp.) seed that meets certified standards often provides an excellent sowing option for turfgrass managers and agronomists of turf and pasture swards. World-wide, the ryegrasses are probably the most widely used of all grasses (Barker and Kalton, 1989). Seed grown in Oregon supplies a major part of the world demand for ryegrass seed. In 1995, total perennial ryegrass (*L. perenne* L.) seed production in Oregon was over 80 M kg. Of this, eight percent was exported, primarily to Australia, Canada, and Japan. In the same year, 112 M kg of Italian ryegrass (*L. multiflorum* Lam.) seed was produced with 13% exported, primarily to Australia, Japan, Mexico, and Saudi Arabia (Oregon Ryegrass Growers Seed Commission, 1996; U.S. Planting Seed Trade, 1996; W.C.Young III, personal communication, 1997). The U.S. Crop Germplasm Committee for Forage and Turf Grasses ranked ryegrass high to medium in importance of all major cool-season grasses for preservation and enhancement (Floyd, 1997). The majority of perennial ryegrass seed production is sold to turfgrass markets while Italian and hybrid (*L. hybridum* Hausskin) ryegrasses are primarily used for forage purposes and temporary turf markets such as winter overseeding of golf courses.

Five counties accounted for 88% of all Oregon ryegrass certified seed production in 1998. All five counties are located in a region designated as the Willamette Valley. Certified ryegrass seed production in Oregon has occupied an

average of over 28,000 ha since 1990 (Oregon Seed Cert. Service, 1996). Perennial ryegrass has the largest number of cultivars eligible for certification of any other Oregon produced grass, followed by tall fescue (*Festuca arundinacea* Schreb.). The number of ryegrass cultivars formally accepted for eligibility in the Oregon seed certification system in 1970 was 28, 53 in 1980, 104 in 1990, and 288 by 1999 (Oregon Cert. and Foundation Seed and Plant Materials Board, 1970, 1980; Oregon Seed Cert. Service, 1990, 1999). The rapid increase was primarily the result of new turf or amenity type perennial ryegrass. More USA companies are investing in ryegrass breeding and cultivar development than any other grass for turf use (Frey, 1996). A report published in 1992 revealed that there were 85 named turf-type perennial ryegrass cultivars for commercial sale in the USA (Rose-Fricker et al., 1992). As of 1997, just five years later, 158 cultivars were sold commercially (Morris and Gao, U.S., 1997).

Prior to the mid-1960s, short-lived stemmy plant types generally were the only kind of perennial ryegrasses available to the turfgrass industry in North America (Meyer and Funk, 1989). Extensive breeding efforts were initiated in the late 1960s to improve perennial ryegrasses for permanent turf in the USA This led to the release of several cultivars; 'Manhattan', 'NK-100', and 'Pennfine' are examples. Perennial ryegrass was also promoted by commercial companies as a viable aesthetic turf and playing surface for southern USA turf areas, overseeded on dormant warm season species (Meyer and Funk, 1989). Perennial ryegrass is desired by turfgrass managers, primarily for its dark green, fine textured, and dense foliage. This is contrasted to Italian (or annual in USA terminology) ryegrass which exhibits light colored, coarse

textured, sparse foliage, and rapid growth elongation in turf applications. Thus, Italian ryegrass contamination detracts from the otherwise high quality of a monostand of perennial ryegrass.

Since its discovery as a phenotypic marker, seedling root fluorescence (SRF) has been used to separate Italian from perennial ryegrass (Gentner, 1929). Generally, roots of Italian ryegrass, growing on white filter paper, secrete annuloline, an alkaloid, that fluoresces under ultraviolet light (Axelrod and Belzile, 1958). Cultured seedlings of perennial ryegrass typically do not. Fluorescent perennial ryegrass and non-fluorescent Italian ryegrass, however, do exist (Nilsson, 1930 ; Nyquist, 1963; Nitzsche, 1963; Okora et al., 1999). In side by side comparisons of fluorescent and non-fluorescent plants from the same cultivars, fluorescence cannot be consistently associated with any of the generally accepted morphological differences between Italian and perennial ryegrasses (J.A. Scott, unpublished, 1981). The traditional application of fluorescence formulas has created problems for seed analysts and enforcement agencies testing these cultivars (J.A. Scott, unpublished, 1981). Further, it has been difficult to support a seed test that is highly variable from sample to sample within a seed lot.

In the European Economic Community (EEC), a new cultivar of ryegrass can be placed on a National List of Varieties when it has satisfied compliance with accepted standards for distinctness, uniformity, and stability (DUS). The required DUS testing program is very comprehensive (Nielsen et al., 1985). With increasing numbers of candidate cultivars, as well as the existing list of those already registered, distinctness is increasingly difficult to establish with present morphological characteristic tests. Lack

of morphological-genetic markers is accentuated by the cross pollinating nature of ryegrass. Perennial and Italian ryegrasses hybridize easily resulting in an integradation of plant types between the two species. Hybridization and contamination results in relatively large phenotypic and genotypic variability within a cultivar (Nielsen et al., 1985). Often the variation within a cultivar is greater than the variation seen among cultivars. Identification of cultivars could be improved by applying biochemical characters for differentiation in addition to morphological trait separation. However, since the fluorescence test is not always associated with the annual form of ryegrass, it cannot be used as a reliable biochemical test solely to identify a cultivar; at least not until there is better understanding of the trait.

Ryegrass seed is certified for commercial marketing in the USA based on strict standards. One standard is compliance with a SRF test. If a ryegrass cultivar is not documented as having an inherent varietal fluorescent level (VFL), the cultivar's VFL is considered 0% for perennial ryegrass and 100% for Italian ryegrass. In Oregon, perennial ryegrass cultivars are allowed SRF tolerance amounts of 0.1, 1, and 3 percentage points above the VFL, or above 0% if VFL is not established for foundation, registered, and certified seed production classes, respectively (Oregon Seed Cert. Service, 1995).

After nearly 50 years of debate over concerns about variability of SRF tests, the Association of Official Seed Analysts (AOSA) commented in their 1988 progress report that in recent years ryegrass breeding has resulted in varieties with atypical fluorescence patterns (AOSA, 1988). Shortly after, the Association of Official Seed

Certifying Agencies (AOSCA), adopted a rule change allowing plant breeders the opportunity to describe fluorescence levels in perennial and non-fluorescence levels in Italian ryegrass cultivars (AOSCA, 1991; USDA, 1995). Plant breeders began documenting inherent fluorescence for new and existing ryegrass cultivars in 1991 for seed certification purposes. When a certificate for U.S. Plant Variety Protection (PVP) is sought, a fluorescence value is also required from the plant breeder.

Because of current practices and breeding methods used to develop new ryegrass cultivars, especially for turf, it is inconceivable that one biological test could be used to separate all cultivars. Many cultivars are closely related, having originated from common ancestry. The SRF trait, if shown to be stable for a cultivar, could be used to demonstrate differences among cultivars, complimentary to morphological or other biochemical character differences.

Several concerns led to the initiation of my research. Can SRF be considered stable through generations of seed increase? Is the trait altered by genotype X environment interaction? What is the inheritance of this trait? How should new cultivars best be described for SRF? A recommendation was made in 1980 to conduct research on the stability of the fluorescence factor (Clark, 1980). It was also suggested that existing information regarding the trait be re-evaluated, and new procedures for reporting pure seed in ryegrass should be developed (Clark, 1980). By October 1992, 29 cultivars of perennial ryegrass had documented fluorescence percentages (USDA, 1992). In March of 1999, 152 cultivars of perennial ryegrass had fluorescence amounts recorded (AOSCA, 1999). My study was initiated in 1991, anticipating the trend for

breeders to document inherent values of fluorescence on newly developed cultivars. In addition, it was realized that there was limited information on whether or not conditions of a seed production environment could affect fluorescence expression. The only previous research on monitoring fluorescence expression through seed multiplication showed a 1% generation^{-1} increase for a hybrid ryegrass cultivar in New Zealand (Rumball, 1970).

The objectives of this study were to: 1. gain a better understanding of how ryegrasses are tested and evaluated for SRF, 2. determine if the level of fluorescence expression for a ryegrass cultivar remains unchanged over generations of seed increase and among locations of seed increase, and 3. to determine appropriateness of using SRF as a phenotypic marker to characterize ryegrass cultivars.

LITERATURE REVIEW

Ryegrass Biology

Eight species of the genus *Lolium* have been described: *L. perenne* L., *L. multiflorum* Lam., L. *rigidum* Gaud., *L. remotum* Schrank, *L. temulentum* L., *L. persicum* Boissi. & Hoh., *L. subulatum Vis.*, and *L. canariense* Stevd. (Terrell, 1968). The first three species are cross-pollinated by wind. The breeding behavior of *L. canariense* is unknown (Loos, 1994); with the remaining four considered autogamous. All but *L. perenne* have an annual life cycle, or at least are very short-lived perennials. The genus *Lolium* is not complicated with naturally occurring polyploids; all species are diploid (Jenkin and Thomas, 1938; Loos, 1993). My research concentrated on *L. perenne* and *L. multiflorum*. Worldwide, these two are appreciably the most important species of the genus. Common names are perennial ryegrass for *L. perenne*, and Italian (or annual in the USA) ryegrass for *L. multiflorum*. Early work described morphological differences between Italian and perennial ryegrasses (Breakwell, 1918; Jepson, 1925; and Levy and Davies, 1930). These are summarized in the Appendix. Jung et al., (1996) speculated that the best morphological characters for separating the two species were: presence or absence of awns, folding or rolling of leaves in young shoots (vernation), or the number of florets per spikelet.

L. perenne and *L. multiflorum* hybridize readily (Jenkin, 1924, 1931; Woodforde, 1935; Hubbard, 1968; Jung et al., 1996). In fact, natural and bred hybrids among the three outcrossing ryegrass species, *L. multiflorum* X *L. perenne* (=*L.* X

hybridum); *L. multiflorum* X *L. rigidum*; and *L. perenne* X *L. rigidum,* have been reported in Australia and Europe (Kloot, 1983; Humphries, 1980). Sufficient crossing occurs between the allogamous *Lolium* species to form a wide range of fertile hybrids. These cross and backcross readily, yielding progeny of continual variation in every morphological character used to separate them. Jenkin (1931) demonstrated the breeding compatibility between Italian and perennial ryegrass. Based on his results, he regarded Italian ryegrass as *L. perenne* var. *multiflorum.* Seed formation and germination appeared normal, without any significant differences related to the direction of the cross. Progeny seedlings were of normal vigor and development. Literature has emphasized the morphological or morphospecies concept, rather than a biological or genetic species concept for perennial/Italian ryegrasses (Jenkin, 1955; Loos, 1994; Stuessy, 1990). Tyler et al., (1987) concluded that European populations of ryegrass represent a "huge hybrid swarm"of plant types. Perennial ryegrass and Italian ryegrass demonstrate opposite extremes of adaptation to grazing versus infrequent hay harvesting.

Perennial ryegrass grows until vegetative tillers pass through a required combination of temperature and day length changes (Riewe and Mondart, 1985; Cooper and Calder, 1964). The life cycle of Italian ryegrass is intermediate between perennial ryegrass and plant forms having no vernalization and/or day length requirements for floral initiation. Variation in reproductive requirements appears to be population, or cultivar dependent. Generally, life form (cycle) has also been used to discriminate between the species, but this physiological trait is not as easily

recognizable as it might seem. There appears to be a continuum from very short-lived types to very persistent perennials. Westerwold ryegrass *(L. westerwoldicum)* is a subspecies of *L. multiflorum* which was identified in Holland. It has no specific requirements for flowering, and can form an inflorescence shortly after germination (Easton, 1983; Cooper and Calder, 1964). Perennial ryegrass has a floral induction requirement that can be satisfied by exposure to cold (2 to 4° C), or to short days. However, exposure to cold temperatures has the more pronounced effect on inducing flowering in these plants (Easton, 1983). Life cycle duration in *Lolium* shows a close association with floral inductive requirements, with the longer-lived perennial forms needing more environmental stimulus than the shortest life form, Westerwold.

Naylor (1960) emphasized the unsatisfactory reasoning for considering *L. perenne* and *L. multiflorum* as distinct species. Crosses between the two grass forms were made and resulting F_1 and F_2 hybrids were compared with the parents for cytology and six external morphological characters. Cytologically, the two species appeared to be structurally alike with no chromosome structural differences seen among any of the hybrids. There was no evidence of reduction in effective pairing in the first generation when the two parental populations were crossed. Because of the lack of chromosomal organizational differences in progeny resulting from crosses of the two forms, it was concluded that the two types may not have become separated until recently. Thomas (1981) revealed that even though gross chromosome morphology of Italian and perennial ryegrasses were very similar, there were distinct differences in banding patterns for the arbitrarily assigned chromosome numbers 5, 6, and 7. It was

unfortunate that only one accession representing each species was screened for karyotyping. Loos (1993) concluded that pooling all of the cross breeding forms into one species would be rash; especially since all three show morphological and chromosomal differences. For this reason, these ryegrasses are considered separate species.

Terrell (1968) hypothesized that *L. multiflorum* originated in southern Europe; noting that *L. perenne* is indigenous to southern and central Europe, as well as areas of northern Africa, the Middle East, and southwest Asia. It appears that repeated hybridizations and introgression occurred during their evolution, especially during the past several thousand years of domestication in the Mediterranean and southwest Asia. Further intervention through controlled crosses in modern plant breeding programs could also be responsible for introgressing alleles among species.

Using trisomic plants of perennial ryegrass crossed with Italian ryegrass, Ahloowalia (1981) found that the meiotic behavior, fertility, and seedling viability of the hybrid further confirmed close relatedness of the two taxa. However, occasional univalents in the diploids recovered from the perennial trisomic X annual cross indicated chromosomal differentiation between the genomes of the two taxa. It was suggested that the taxa may differ in cytoplasmic backgrounds as seen by seedling lethality in F_2 populations and by differential viability of the reciprocal crosses. Hybrids between the two species yield desirable features of both species, and hybrid ryegrass derivatives have been advocated as a means of attaining cultivars suited for short rotations (Corkill, 1949).

Both perennial and Italian ryegrasses possess 2n=14 chromosomes. Nevertheless, plant breeding efforts have developed tetraploid cultivars of each species, especially for forage use (Loos, 1994). Both species are self-incompatible to a high degree (Terrell, 1968). Jenkin (1927) reported a self-incompatibility mechanism in perennial ryegrass, and McCraw and Spoor (1983a) reported it for Italian ryegrass. McCraw and Spoor (1983b) evaluated one F_1 family of perennial ryegrass for the genetic control of self-incompatibility using the pollen-stigma reaction. Based on their research, they regarded the self- incompatibility system in the outcrossing *Lolium* species to be controlled by at least three multi-allelic loci. Gametophytic determination of pollen behavior was the rule for their tested species, and this confirmed earlier work (Spoor, 1976). In families of ryegrasses studied, seed set from self-fertilizations was about 0.25% (Cornish et al., 1980). Arcioni and Mariotti (1983) estimated self-fertilization rates of 7.72 and 7.76%, respectively for perennial and Italian ryegrass.

Fluorescence Test

The seedling root fluorescence (SRF) test is used to aid in distinguishing Italian ryegrass from perennial ryegrass. Gentner (1929) demonstrated that roots of Italian ryegrass seedlings produced bluish fluorescent tracks when grown on white filter paper and viewed under ultra-violet light. Roots of perennial ryegrass did not produce fluorescent tracks. Roots that fluoresce on filter paper do so because of a reaction of compounds from growing roots with the paper. Fluorescence was not seen when roots were grown on glass or unglazed pottery (Gentner, 1928). The methodology

developed by Gentner was implemented in 1930-31 at various centers of ryegrass production, e.g. New Zealand and Scotland. Exceptions to Gentner's observations were soon shown, leading to the conclusion that the separation of species using a fluorescence test alone is simply a generalization. From a simple evaluation of other ryegrass species obtained from the U.S. National Plant Germplasm System, most accessions of annual species express some fluorescence in their roots (D.J. Floyd, unpublished, 1991).

Some perennial ryegrass plants produce fluorescent progeny seedlings, and conversely, some Italian ryegrass mother plants can produce non-fluorescent progeny (Nilsson, 1930; Nyquist, 1963; Nitzsche, 1963). Foy (1931) attested that fluorescence in *Lolium* appears to be constantly associated with the short-lived nature or habit. The report indicated that complete fluorescence is exhibited by all the annual species of *Lolium*. The author indicated that faint blue fluorescence was observed on the rootlets of newly germinated seedlings of all forms of *Lolium*, but it disappeared in a few days. Mature plants of Italian and false (short-lived) perennial, if allowed to root on filter paper, showed the fluorescence character in the same way as freshly germinated seedlings.

Root fluorescence responses have been demonstrated in other crops including soybean (*Glysine max* L.), subterranean clover (*Trifolium subterraneum* L.), oat (*Avena sativa* L.), and *Bromus unioloides* H.B.K, (Delannay and Palmer, 1982; Foy, 1931; Finkner et al., 1954). Additionally, Goodwin and Kavanagh (1948) demonstrated that all but six of 135 species of vascular plants, representing 69 families,

showed root fluorescence. Even though many plant species, both dicots and monocots, express root fluorescence, a fluorescence test has not been widely applied to any seed crops except for the ryegrasses.

The routine use of the SRF test in the USA started approximately 1941 (AOSA, 1941). A report which referenced "The use of Ultra-Violet light in the Identification of *Lolium perenne* L. and *Lolium multiflorum* Lam." (Wright, 1941) officially recognized the test by AOSA at their annual meeting in 1944 (AOSA, 1944). This policy provided that a SRF test shall be made on all ryegrass samples for which the species is to be determined. After experimenting with various light sources, test length, and the lifting of seedlings, standard procedures for conducting fluorescence tests were proposed (Justice, 1949). A proposal was filed by AOSA recommending the test be part of the Federal Seed Act in autumn of 1949. The fluorescence test was placed in the Federal Registry of the Federal Seed Act on April 28, 1950 (J.P. Triplett, personal communication, 1997). It has been the official method used by the International Seed Testing Association (ISTA) since 1953 to separate kinds of ryegrass (Jones, 1983; ISTA, 1966).

From the discovery of SRF to the present day, there has been debate over how a fluorescence test is to be conducted. Topics involved in the discussion have been various ultra-violet light sources (Justice, 1949), differences in germination paper (medium) (Schmidt, 1953; H.R. Danielson, personal communication, 1992), moisture and temperature regimes of the germination part of the test (Justice, 1949; Nitzsche, 1960; Copeland and Hardin, 1967), and the actual time at which fluorescence is

examined after seed germination (Rampton, 1938; Nitzsche, 1960; Barker et al., 1997). Barker et al. (1997) additionally showed variation for SRF levels over test runs when the same seed lots of three perennial ryegrass cultivars were consecutively evaluated. The conditions for conducting a fluorescence test have not changed significantly since 1963, when rather rigid standards were implemented (Colbry, 1963).

Fluorescence is calculated as a percent of germinable seed that fluoresces for a given seed lot. Optimizing the detection of total fluorescence percentage for a given test involves evaluating all fluorescing roots, regardless of the intensity variation from root to root. Intensity refers to the brilliance of the fluorescing streak surrounding the seedling root. Commercial seed labs use descriptive terms as "bright" or "obvious", to "dull" or "hidden". Until recently, fluorescence testing rules for AOSA required that final fluorescence values be calculated only after roots were physically removed from the filter paper and all fluorescing streaks were counted (Colbry, 1963). Removal of seedlings is called lifting and was done because often the fluorescing streak is so faint that it is evident only after the root is removed from the paper while the test sample is under the ultra-violet light source. The practice of lifting was an attempt to limit errors in human judgement as analysts scored roots of variable fluorescent intensity. The procedure, however, was laborious and time consuming. The AOSA requirement for lifting was discontinued in June 1998 (AOSA, 1998a; AOSA/SCST, 1998). Evidence had been presented demonstrating that roots scored as fluorescent after lifting insignificantly contributed to Italian ryegrass detection in perennial ryegrass seed lots (Garbacik and Grabe, 1991; Barker et al., 2000).

Laboratory and seed conditions during the fluorescence test have been shown to cause variation in SRF results. Munn (1935) observed brighter intensity of fluorescence when seeds were germinated in the dark rather than in daylight. Harrison (1954) indicated higher fluorescence readings when seed samples were germinated at a constant temperature of 25 to 30°C rather than an alternating regime of 18 to 25°C. Nitzsche (1960) showed that fluorescence can increase the longer seed lots are stored. Ogburn and McNamara (1972) also hypothesized that differences in fluorescence intensity may simply be due to variation in age between seed lots.

The intensity of root fluorescence has been quantified under varying laboratory culture practices (D.F. Grabe and C. Garbacik, unpublished, 1990). A light spectrum analyzer was used to measure the relative emission of the fluorescence from a root against a reference filter paper background. They concluded that several factors can influence results of individual fluorescence tests. These influences included: 1. brightness of fluorescence decreases significantly with drying of the filter paper; 2. quality of light during seed germination made no difference in emission of fluorescence; and 3. light intensity during germination proved to be a significant factor altering brightness. Brightest fluorescence was shown for seedlings which were germinated in dark conditions, confirming the work by Radersma and Perdok (1965). As intensity of light increased, fluorescence faded. Duration of light exposure also proved to be a significant factor to brightness or fluorescence intensity. During the germination period, seedlings exposed to 24 h light had lower fluorescence than those exposed to

only 8 h per day. More intense fluorescence was observed at higher temperatures during the germination phase.

Radersma and Perdok (1965) used alternating germination temperatures of 20 to 30° C in their studies, and showed that waiting until the 18th day to make a fluorescence count maximized numbers of fluorescent seedlings. Conducting the test for 18 d was also supported by Nitzsche (personal communication, 1997). This length of test is inconsistent with current AOSA rules, which allow for 14 d completion of fluorescence tests.

Munn (1937) reported that a fluorescence test must be interpreted with care, and the final results should be weighed along with other supporting evidence regarding the classification of seed lots. Fluorescence readings made on suspicious appearing seed lots or those of unknown origins have proved to be useful as evidence upon which to make further inquiry. These comments were supported by Rampton (1938); and based on considerable published evidence, these comments are still prudent today.

Copeland (1966) examined other sources of variation involved in fluorescence testing. He considered within-sample error, among-sample error, among-germinator error, and among-laboratory error. Each source of error was studied independently, and the variation in each was compared to theoretical variability due to random sampling error from a model population. He concluded that other sources of error beyond that due to random sampling alone were occurring in "routine" fluorescence testing. He further found that in laboratories where several analysts are involved in interpreting test results, greater variability might be expected. Variability based on the

statistical model alone were considered insufficient to account for all the variation in fluorescence test results, and that tolerances needed to be developed for the test. However, his literature review indicated that a similar situation existed for test variability associated for "routine" germination tests. Using 100 seed tests, Cook (1980) found fluorescence values ranged from 6 to 24% among three laboratory examinations of the same lot of 'Linn' perennial ryegrass. Germination only ranged from 93 to 95% on the same tests.

Up to the time of Copeland's work, AOSA accepted tolerances for the root fluorescence test based on work done by Leggatt (1939). Leggatt advised AOSA that SRF test results should follow a binomial distribution similar to germination testing. Thus, tolerances based on a binomial distribution were accepted to account for variability in fluorescence test results without experimental evidence supporting this action. However, at the time of Copeland writing, AOSA rules allowed germination tolerances that were wider than those applied to fluorescence tests. During the same time, ISTA stated that their germination tolerances would be applicable to cover variation among fluorescence tests.

Copeland (1966) recommended that new confidence limits should be calculated for ryegrass fluorescence tests to account for not only variation due to random sampling, but also to account for experimental error due to the lack of complete test standardization. The test appears to be very sensitive to varying environmental (germinator) conditions along with variation in interpretation of results by analysts. He recommended that AOSA broaden their fluorescence tolerances to account for

variation that exists in current testing procedures. My literature search did not reveal evidence that his recommendation was ever applied.

Annuloline

The extracted pigment responsible for SRF in Italian ryegrass is a weak, basic alkaloid named annuloline that is only slightly soluble in water (Axelrod and Belzile, 1958). The structure of annuloline was determined as 2-(*trans* 3,4-dimethoxystyryl)-5-(4 methoxyphenyl)-oxazol (Karimoto et al., 1964).

Speaking generally for alkaloids, Dawson (1948) reported that not only is the ability of a plant to synthesize an alkaloid controlled genetically, so is its ability to synthesize it at a given rate. Dawson further stated that no concise function of alkaloids in plants has been found. He cited references hypothesizing that alkaloids tend to accumulate in bark, seeds, and other terminal structures, therefore, they may essentially be waste products of metabolism formed as a result of irreversible reactions, and are usually unavailable for further exploitation by plant cells.

Alkaloids are separated from most other plant components by their cationic nature existing in plants as basic salts of organic acids. Well known examples are nicotine, morphine, strychnine, colchicine, mescaline, cocaine, and caffeine. Alkaloids may function in protection against herbivore attack and in serving as growth regulators (Robinson, 1983). Robinson (1983) stated that alkaloids may serve the plant (being basic) by replacing mineral bases that help maintain ionic balance. Laroze and Alves da Silva (1952) postulated that alkaloids function by exchanging with soil cations, and

found alkaloids excreted by roots of several plants. Working with an Italian ryegrass breeding population, Schunack and Rochelmeyer (1965) found that there was no translocation of annuloline from the root to the shoot. They hypothesized that this was due to the poor solubility of the annuloline salts under physiological conditions that make transport of a water insoluble substance difficult. The start of alkaloid formation coincided with the ability of the grass plant to carry out autotrophic nutrition. From their cultured plants, alkaloid root content reached a maximum between the 13th and 15th day after initiating germination. The concentration dropped slowly until about 50 d, at which time only trace amounts could be detected. Thus, alkaloid formation was demonstrated in the juvenile stage only, and alkaloid production was independent of photosynthesis. Droughty conditions of seedling growing media tended to increase the development of alkaloid deposition.

Nitzsche (1966) explained that the annuloline molecule is large and of complicated structure. It is undoubtedly synthesized during different phases of plant growth. Each enzyme that contributes to its synthesis is formed through the activity of another gene. Consequently, synthesis can be blocked at several places by removing or hindering the responsible gene product. It is important to keep in mind that the SRF test only identifies annuloline as the end product of synthesis processes under polygenic control. It is thus logical to expect polygenic inheritance of its intensity as well. Based on field testing of F_2 progenies, Nitzsche (1966) concluded that because SRF is polygenic, it was not surprising that no indication has been shown for a correlation

between fluorescence and plant persistence, or between fluorescence and other measured morphological characters.

There does not appear to be any difference between otherwise similar plants (of a species) with and without alkaloid content on morphological features effecting growth rate, or on the ability to reproduce. For example, Dawson (1948) was able to produce tobacco (*Nicotiana tabacum* L.) plants that had nicotine contents of up to 12% on a dry weight basis. There were no externally visible differences between these plants and others that had zero nicotine. Similarly, where fluorescent (annuloline expressing) and non-fluorescent (annuloline not expressed) plants of the same cultivar have been compared, in both Italian and perennial ryegrasses, the two types appear phenotypically identical (D.J Floyd and G.W. Pepin, unpublished, 1996).

Robinson (1979) suggested that many more plants have the ability to synthesize alkaloids than have the ability to accumulate alkaloids. Accumulation of alkaloids is of prime interest in the interaction of plants and herbivores. He also indicated the biochemical basis for differing patterns of alkaloids within a related taxonomic group is presumably found in subtle differences in enzyme activity specificity, or compartmentalization. Methylation processes are likely to be significant variables, for example, a specific methylation event occurring early in the pathway can determine the subsequent direction of the pathway. The sites of accumulation may not be the sites of synthesis.

Lack of fluorescence expression in ryegrass suggests that non-fluorescent parents may lack the genes for the complete sequential development of annuloline. The

unexpected appearance of seedling fluorescence in perennial ryegrass may occur in non-fluorescent cultivars whenever the proper alleles are recombined.

It has been observed that a greater proportion of alkaloid-producing plants can be found in the tropics and at low altitudes. In other words, the most agronomically productive environments have the highest percentage of alkaloidal plants. Robinson (1979) hypothesized this relationship with environment may demonstrate that plants growing under the most favorable conditions can accumulate excessive amounts of alkaloids, whereas plants growing in more unfavorable habitats cannot afford the luxury. Levin and York (1978) presented the argument that alkaloid accumulation is in response to pest pressure. Thus, since the most favorable plant growing habitat is also the best for their pests, alkaloid storage confers a selective advantage to plant populations.

My literature search did not uncover any research suggesting a direct metabolic role or purpose of annuloline in ryegrass. The reason why it is generally produced in Italian ryegrass and not perennial ryegrass is still a physiological mystery. The two growth habits may have evolved separately; the former of short duration and the latter of a more persistent, perennial duration. Others believe that perennial ryegrass evolved from Italian ryegrass (S.E. Warnke and R.E. Barker, personal communication, 2000). Research work demonstrating genetic linkage of the SRF trait with ryegrass growth habit was not found. In other words, no studies have shown that fluorescence is genetically linked to annual growth habit, or for that matter, non-fluorescence linked to perennial growth habit even though there is a strong association or correlation with the

two forms. The term linkage has been used in past literature describing SRF in ryegrass. However, a strict definition of linkage is considered to be the tendency of certain genes to be inherited together due to their physical proximity on a chromosome (Barnes and Beard, 1992). This being the case, it is not surprising research has not clearly shown SRF to be linked with any other trait in ryegrass. Studies within this literature review only indicated positive correlations or associations for the expression of fluorescence with Italian ryegrass morphological and physiological characteristics.

Inheritance of Fluorescence

Beginning in the 1930s, selfing, hybridizations, and backcrosses were done between perennial and Italian ryegrass (Corkill, 1932; Linehan and Mercer, 1933; Woodforde, 1935). A summary of these researchers' results include: 1. The SRF trait is heritable. 2. Fluorescence is dominant in the F_1 generation of the cross of pure fluorescent X pure non-fluorescent ryegrass. 3. The F_2 generation in small-sized populations gives a ratio not significantly different from 3 fluorescent :1 non-fluorescent, suggesting that fluorescence may be inherited as a simple Mendelian dominant trait. 4. The backcross F_1 X pure fluorescent gives all fluorescent progeny, and the backcross F_1 X pure non-fluorescent gives a ratio approximately 1 fluorescent : 1 non-fluorescent. 5. No "genetic linkage", i.e. trait correlation is found between SRF and a) the possession of awns, b) shoot vernation, or c) seasonal growth longevity. Later work done in Oregon also added evidence to the theory of simple dominant gene

action for SRF (L.O. Copeland, personal communication, 1990; Copeland and Hardin, 1970).

Nitzsche (1966) claimed that the inheritance of alkaloid production was dominant in hybrids of *L. perenne* and *L. multiflorum*, but he demonstrated that two independent segregating genes for fluorescence and two genes for its intensity could be shown. His conclusion was based on the segregation performance of one Italian ryegrass cultivar crossed with one perennial ryegrass cultivar. Only two fluorescent and two non-fluorescent plants were selected from the two cultivars and used for diallel and selfed crosses. No reciprocal differences were detected in any of the crossed progenies.

In all of the inheritance studies previously conducted, none of the researchers mentioned fluorescence intensity except for Nitzsche. I assumed that seedling roots were not lifted when earlier experiments were conducted.

Except for Nitzsche's work, there has not been any mention of maternal cytoplasmic inheritance in the early research on SRF. One study evaluating hybrids made from interspecific crosses of tetraploid Italian and perennial ryegrasses, however, did reveal the occurrence of maternal effects affecting seed size and vigor (Stuczynski et al., 1969). Seeds of F_1 hybrids from a tetraploid Italian X perennial ryegrass cross usually proved to be well developed and germinated more rapidly than seeds of hybrids derived reciprocally. Moreover, seedling withering due to chlorophyll aberrations was less in hybrids derived from the cross of tetraploid Italian X perennial, than by hybrids

produced from the reciprocal. There was not any mention of SRF evaluation in this research.

The review of literature has not revealed complete agreement amongst researchers regarding simple inheritance of SRF. Most researchers used small populations of progeny and limited parentage that may not have contained the full range of genotypic classes. There still appears to be a need for understanding the genetic control of the trait's expression.

Genetic Shift of Fluorescence Trait in Seed Multiplication

Literature referring to the effect of seed multiplication generation on SRF expression of a ryegrass population is scant. Baekgaard (1962) demonstrated that some Danish cultivars of perennial ryegrass produced up to 20% fluorescent seedlings. He noted that late maturing cultivars tended to produce higher SRF percentages of fluorescence than early maturing cultivars. His work, however, did not include seed increase over production generations.

In out-breeding grass species, genetic variation in the breeder seed class cannot be eliminated; nor is it desirable to limit genetic variation contributing to heterosis once acceptable phenotypic uniformity has been attained. Seed certification schemes, however, are designed to minimize change between the foundation seed class and certified seed production generations. A shift toward earlier maturity (heading date) in perennial ryegrass has been observed in increasing generations of multiplication (Cooper, 1959; Griffiths, 1951). No changes, however, were reported by these authors

in SRF for a given cultivar over generations of seed increase. Munn (1937) reported a survey for perennial ryegrass with SRF ranging from of 5 to 50% among various locations of seed production, but gave no indication of generational relationship.

In the only study I found that addressed change of SRF through seed increase, Rumball (1970) in New Zealand monitored fluorescence expression through seven generations. He cited a nearly one percent per generation increase in fluorescence for a ryegrass cultivar.

Alternative Tests to Fluorescence

Other tests have been used in an attempt to more conclusively separate the two important ryegrass species. Morphologically, leaf vernation differences between perennial and Italian ryegrass have been used (Grabe, 1998; Okora et al., 1999). Generally, perennial types have folded leaves at the base, but annual types are rounded. Some plants, however, especially for hybrids, produce both vernation forms on the same plant. Other methods applying biochemical characters, such as electrophoresis of general seed proteins and isozymes have yielded good results (Larsen, 1966; Ferguson, 1984; Ferguson and Grabe, 1984). Like the SRF test, however, such alternative tests have not been supported by genetic linkage studies, and additionally, all biochemical methods have utilized bulk seed samples. In order for alternative tests to be developed, and applied, they must be accurate and cost effective on a single seed basis so percent seed lot contamination can be detected.

Several alternative tests to SRF have been used during the past two decades in attempts to find replacements to the fluorescence test. None have been adopted on a

wide scale, or have been relied upon as a replacement to SRF testing. Alternative tests need to be equal to, or hopefully, more accurate and reliable than the current SRF test for the separation of crop kinds. These tests would have to be economical and more rapid than SRF.

Payne et al., (1980) subjected 40 lots of 16 annual ryegrasses and 101 lots of 35 perennial ryegrasses to electrophoresis of seed proteins. Esterase enzyme activity was measured by appropriate staining procedures. They found that a darkly stained band displaying heavy esterase activity was present in all lots of the annual ryegrasses. The band was present, but only faintly stained from all 101 perennial ryegrass seed lots. They purposely assembled mixtures of seed lots varying in percentages of annual and perennial ryegrass composition. Samples of the mixtures were then electrophoresed and stained for esterase. Results suggested staining intensity of esterase was independent of SRF, and esterase activity appeared to be associated with characteristics of growth habit, but individual plants were not examined.

The electrophoresis test used by Payne et al., (1980) has the potential to be more sensitive than the fluorescence test for detecting annual ryegrass contamination in samples of perennial ryegrass. The procedure, however, may not be as sensitive as the fluorescence test in detecting small amounts (less than 5%) of perennial ryegrass contaminations in annual ryegrass samples. Some cultivar differences were observed in other esterase bands. Similar variations in these bands, however, were also found among replicates of the same seed lot. Thus, it was concluded that extracts stained for esterase are unsuitable for distinguishing between ryegrass cultivars. Griffith and

Banowetz (1992), however, were able to separate 18 cultivars of Italian from 74 cultivars of perennial ryegrass by electrophoresing protein extracts from bulk seed samples of each cultivar using isoelectric focusing gels. The gels were stained for esterase (EST 1, isoforms a and b) activity. The Italian ryegrass cultivars showed an esterase protein, unique in mobility, that was not seen in similarly treated perennial ryegrass proteins. They initiated work to find an antibody probe to use in identification of EST 1 in routine purity seed tests (Griffith and Banowetz, 1994). Upon extensive screening, they came to the conclusion that the structural differences between EST 1 and other esterase proteins present in the extracts of both ryegrass species were not sufficient to permit differentiation of the two ryegrass species by an anti-EST-1 antibody-based test.

Ferguson (1984) separated cultivars of perennial ryegrass based on differences in seed protein extractions. The extracts were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Densitometer scans of stained gels from each of 28 cultivars were conducted. Seed produced of each cultivar from different locations were also analyzed in an attempt to detect environmental effects on digested proteins during seed multiplication. A zone in the electrophoretic gels that had rather high variation was identified. Using differences in band number, location, and intensity for this zone of variation, most of the 28 varieties could be uniquely identified. Additionally, with the exception of only one cultivar, differences in cultivar banding patterns (based on location of seed production) were not evident. Further, to determine the effect of seed generation on subsequent banding pattern, she chose bulk

seed samples of two cultivars that had foundation and certified classes represented. No differences were seen for the different generations of either cultivar. Hayward and McAdam (1977) did detect a change in allelic frequency when staining for phosphoglucoisomerase, specifically PGI-2, during seed multiplication of two cultivars.

Nielsen et al. (1985) used four enzyme loci to separate 15 perennial ryegrass cultivars and six Italian ryegrass cultivars from each other. Stability of allelic frequencies was also studied by examining different seed lots of three of the cultivars. Two of the four enzyme loci moderately distinguished the perennial ryegrasses. However, all loci had to be considered in order to separate most of the Italian ryegrass cultivars. They concluded that genetic variation in enzyme loci may be suitable when used to measure distinctness of cultivars, but that more thorough research was needed. The loci observed in their study were not consistently stable through generations of seed multiplication representing different environments of production for three ryegrass cultivars. The authors cautioned that a prerequisite for being able to assign allelic frequency data from enzyme loci to a given cultivar, was that such data needed to be repeatable as the cultivar is grown in different environments. Stability of the marker system requires that loci are selectively neutral; i.e. there is no differential survival of any one genotype over another in a cultivar. Secondly, a tested cultivar must have been reproduced for enough generations to ensure that the amount of linkage disequilibrium between marker loci under selection has become negligible.

In the late 1960s confusion surrounded the separation of common Italian ryegrass and perennial ryegrass by field inspections of seed crops in state seed

certification programs. A study conducted at the time tried to differentiate between Oregon Annual Ryegrass (a common or ecotype cultivar) and 'Linn' perennial ryegrass based on morphological differences (Rosell, 1967). He concluded that because of the development of several new varieties, fluorescence tests were less effective at determining species than at the time of its discovery. Morphological tests were conducted on young and on mature plants, along with harvested seed samples. Leaf vernation, percentage of glumes covering the spikelets, and height of seedlings were reliable characters for differentiating plants of each species within the study. Callus shape of the rachilla attached to the seed along with the rachilla length were considered to be relatively good discriminating characters, but not as dependable as vernation, percentage of glumes covering the spikelets, and height of seedlings.

On a morphological test basis, leaf vernation is considered to be the most accurate seedling characteristic used for distinguishing Italian from perennial ryegrass (D.F. Grabe, personal communication, 1996). The Oregon Seed Trade Association (OSTA) considered using this character as a supplement to the fluorescence test when monitoring seed lots for certified status (OSTA seed certification subcommittee, personal communication, 1997). In a recent thesis, it was shown that vernation scores made on 3 to 4 week old seedlings would be a better test than SRF for separating the two ryegrass species (Okora, 1995). There are drawbacks to using vernation as a replacement test to SRF. It can involve considerable greenhouse space and can take up to 4 wk growth before measurements can be made. Additionally, the leaf vernation of

hybrid progeny resulting from field crossings can involve considerable subjective judgement on the part of the evaluator (personal observations, 1995).

Seed certification officials still depend on morphological differences plus a fluorescence test to separate the ryegrass crop kinds. Combining the fluorescence test with observations for leaf vernation (Grabe, 1998) seems to be the best contemporary way to describe foundation seed lots of cultivars. No other testing combination available today can profess the same field applicability, ease of use, low cost, and improved accuracy. It is again noted, however, that leaf vernation evaluations take greenhouse or growth chamber space and up to 4 wk for rearing plantlets.

Documentation of Varietal Fluorescence

Starting in the summer of 1987, meetings were held through the OSTA, the Association of Official Seed Certifying Agencies (AOSCA), AOSA, and the Society of Commercial Seed Technologists (SCST) for the purpose of developing procedures for documenting inherent fluorescence levels in ryegrass cultivars. In the autumn of 1990, AOSCA notified AOSA and SCST that procedures had been established allowing plant breeders the option of describing fluorescence levels for their cultivars. The National Grass Variety Review Board (NGVRB) was mandated in the Rules of Testing Seed, as part of the Federal Seed Act, to review and maintain a list of such cultivar fluorescence levels (VFL) (AOSCA, 1990). Thus, in 1991, the first cultivars were being reviewed and recommend by the NGVRB as having inherent fluorescence values. These cultivars were placed on a list published by the NGVRB through AOSCA (AOSCA,

1991). On 13 January 1995, a provision was included in the Federal Registry allowing plant breeders an opportunity to describe ryegrass cultivars with inherent fluorescence levels (J.P. Triplett, personal communication, 1997; USDA, 1995).

The documentation procedure first stipulates that cultivars of perennial and annual ryegrass will be considered 0% and 100% fluorescent, respectively, unless the VFL is described by the sponsoring plant breeder. Cultivars of ryegrass can be described and their fluorescence values published, even if such cultivars are not multiplied under recognized certification programs. The sponsoring breeder of a ryegrass cultivar is required to collect the necessary information and to report the findings for each cultivar on a form described as "Varietal Fluorescence Description Documentation" (F. W. McLaughlin, personal communication, 1990). Briefly, the procedures that the plant breeder must follow are:

1. A minimum 1,000 seed fluorescence test will be conducted on each of at least three different seed lots (of the application cultivar), representing at least two generations, one of which must be breeder class seed. These fluorescence tests follow AOSA rules.

2. The fluorescent seedlings in perennial ryegrass cultivars or the non-fluorescent seedlings in Italian ryegrass cultivars are grown out in a growth chamber, greenhouse, or in a field nursery to determine annuality characteristics. The breeder is instructed to follow standard accepted grow-out procedures similar to those suggested by Nittler and Kenny (1972). As the plants mature during the grow-out, they are compared phenotypically with plants from an Italian ryegrass check cultivar and with

plants from non-fluorescent seedlings of the test cultivar (for perennial ryegrass cultivar applications). Variety fluorescent descriptions (VFL), for perennial ryegrass cultivars, are calculated as

$$VFL\ (\%) = \frac{FL - A}{G} \times 100$$

Where FL is the number of germinated normal seedlings that fluoresce, A is the number of plants with annual-like characteristics, and G is the total number of germinated normal seedlings.

3. Results are recorded and then sent to AOSCA - NGVRB for review, recommendation, and publication. Requirements for documenting inherent fluorescence values do not consider fluorescence intensity.

Under current AOSCA guidelines, any fluorescence detected in a tested lot of perennial ryegrass above the level documented by the breeder is automatically considered annual ryegrass contamination. Correction tolerances are not recognized in a fluorescence test applied in this way.

Despite numerous papers which have cautioned about the interpretations of fluorescence tests (Munn, 1937; Rampton, 1938; Nyquist, 1963; Copeland and Hardin, 1967; Barker et al., 2000), the test is widely used for seed marketing in the USA My literature review has not revealed research that shows an agronomic benefit for a cultivar having an inherent fluorescence level. Fluorescence levels and its lack of exact association with annual-like growth habit, is a phenomenon that has perplexed seed analysts for over 60 years. In my short career in the grass seed business, I have heard

several pleas from seed trade people requesting abolishment of the SRF test for certifying ryegrass seed lots. There are also those in the seed trade, however, that contend SRF is still a valuable test as a separation of ryegrass kind. They support the idea that if the trait was further researched, seed marketing could benefit from a more complete understanding of the implications of the trait in producing a high quality seed product. An alternative test to SRF is still being sought. There is no doubt that one will be found as more knowledge is gained regarding the genetic differences among ryegrass species. In the meantime, the ryegrass marketing effort in the USA will continue making the best application of the SRF test results based on current standards.

My research was initiated to provide another stepping stone for improved knowledge of how fluorescence is implicated in ryegrass seed multiplication in different locations and years. If fluorescence testing continues, its application should be changed based on current research (Barker et al., unpublished, 1998). For example, when a cultivar is described for VFL, the amount of variation among tested lots and within limits of experimental error for the test should be considered along with the mean SRF level (Barker et al., 1997).

GENETIC SHIFT OF FLUORESCENCE EXPRESSION IN RYEGRASS DURING THREE GENERATIONS OF SEED INCREASE (MANUSCRIPT 1)

Abstract

Seedling root fluorescence (SRF) has been used since the early 1940s to discriminate Italian ryegrass (*Lolium multiflorum* Lam.) from perennial ryegrass (*L. perenne* L.). Generally, roots of Italian (annual) ryegrass fluoresce under ultraviolet light, while those of perennial do not. The trait, however, has readily introgressed from Italian to perennial and breeders determine fluorescence levels of new ryegrass cultivars before they enter seed certification programs. The objective of this study was to ascertain genetic change for the expression of SRF during generations of seed multiplication. Four ryegrass populations, constructed to have low, medium, and high fluorescence levels, were increased independently for three generations at each of three Oregon locations (Aurora, Corvallis, and Madras). Fluorescence levels were measured initially, and for each generation cycle at each location. There were significant differences in SRF among locations within populations and for seed production generation within locations. One population, for example, initially at 11% SRF increased to 36% over the three generations of seed multiplication at Corvallis, but decreased to 8 and 2% at the other two locations. The other three populations responded differently, showing large population X location and population X generation interactions for SRF expression. The location of increase and the seed production generation must be examined and carefully considered when describing fluorescence levels of cultivars for seed certification purposes. The large

amount of variation associated with environmental influences indicates SRF is a poor characteristic to describe and predict ryegrass cultivar genetic purity.

Introduction

World-wide, the ryegrasses (*Lolium* sp.) are probably the most widely used of all grasses (Barker and Kalton, 1989). The U.S. Crop Germplasm Committee for Forage and Turf Grasses ranked ryegrass high to medium in importance of all major cool-season grasses for preservation and enhancement (Floyd, 1997). Seed grown in Oregon supplies a major part of the world demand for ryegrass seed. The majority of perennial ryegrass (*L. perenne* L.) seed production is sold to turfgrass markets while Italian (*L. multiflorum* Lam.) and hybrid (*L. hybridum* Hausskin) ryegrasses are primarily used for forage purposes and temporary turf markets such as winter overseeding of golf courses.

Since its discovery as a phenotypic marker, seedling root fluorescence (SRF) has been used to separate Italian ryegrass from perennial ryegrass (Gentner, 1929). Generally, seedling roots of Italian ryegrass growing on white filter paper secrete an alkaloid (annuloline) that fluoresces under ultraviolet light. Similar cultured seedlings of perennial ryegrass do not. However, fluorescent perennial ryegrass lines exist as well as non-fluorescent Italian ryegrass (Nyquist, 1963; Nitzsche, 1963; Okora et al., 1999).

It is important to have a test that can detect Italian ryegrass physical and genetic contamination in perennial ryegrass cultivars, especially if the seed is to be used in permanent turf applications. The phenotypic differences between the two species in foliage color and leaf width can have significant impact in high quality perennial ryegrass turf. Italian ryegrass often exhibits more rapid vertical growth, less tillering,

with coarser and lighter colored foliage than perennial ryegrass. A contamination of this type of ryegrass would detract from the otherwise aesthetically pleasing appearance of the dark green color of perennial ryegrass. Short of a physical plant grow-out test for each seed lot, the fluorescence test is the only accepted test for detecting the presence of Italian ryegrass in seed lots of perennial ryegrass. However, this test is not exact, and false positives can easily occur due to a lack of complete test standardization, age of seed, and seed analyst subjectivity in scoring results. Additionally, tolerances (unlike for germination tests) are not applied to any suspect test results.

The SRF trait has introgressed from Italian to perennial ryegrass and plant breeders determine fluorescence levels of new ryegrass cultivars before they enter the seed certification system. Breeders began documenting inherent fluorescence levels for new and existing ryegrass cultivars in 1991 (AOSCA, 1991). By winter 1999, fluorescence values of 148 perennial ryegrass cultivars and 9 Italian ryegrasses were documented (USDA, 1999).

This study was initiated to obtain information that would assist ryegrass breeders in documenting fluorescence levels in developed cultivars. Objectives were: 1) to determine if fluorescence expression of a cultivar (population) remains stable through generations of seed increase, and 2) to determine if the geographical location or year of seed multiplication affects fluorescence expression of ryegrass cultivars. Wide variation caused by environmental influences where seed is grown would reduce dependability of the SRF test which is so extensively used in the USA.

Materials and Methods

Parental Sources and Population Development. Seven stock plants used as source material were obtained from the USDA-ARS, National Forage Seed Production Research Center (NFSPRC), Corvallis, OR. Five had a perennial ryegrass phenotype. These were progeny plants derived from open pollinations between 'Palmer' and clones collected from old turf areas in St. Louis, MO, and Washington D.C. The other two clones were selected from Italian ryegrass cultivars, one from 'Gulf' and the other from 'Marshall'. Parents were transplanted 31 January 1990, to a field at the research farm of Daehnfeldt, Inc., Corvallis, OR. Four ramets of each were spaced 25cm apart within three rows spaced 1 m apart. Ramets were arranged to facilitate all possible pair crossing among stock plants. Granular fertilizer of ammonium sulfate (21-0-0-24, N-P-K-S) was applied at a 56 kg N ha^{-1} rate prior to transplanting, and again 8 March 1990. The nursery was overhead irrigated with approximately 4cm of water in mid May prior to anthesis. Additional artificial irrigation was not supplied throughout the seed development season.

All possible pair cross combinations were made among stock plants in late May and early June 1990 by mutual bagging of inflorescences in 9.5 x 40 cm glycine bags. Supports were assembled to keep bags erect and intact under field conditions until seed maturation. Mature spikes from each pair cross were harvested in early July, and kept separate by maternal parent. Seed from each maternal parent of each cross were hand threshed and conditioned using hand screens and a commercial laboratory seed blower. Seed was stored in a cool (5°C), dry room until needed for germination.

Seed from several pair crosses were sown on white filter paper during October 1990. Rules from the Association of Official Seed Analysts (AOSA) were followed for germination and SRF tests (AOSA, 1994). Seedling roots were scored for the presence or absence of fluorescence. Only roots that had brilliant fluorescence without lifting from the filter paper and those that did not show evidence of SRF after lifting from the filter paper, were used in population development. Selected seedlings were transplanted to 4.0 x 19.5 cm plastic cone pots (conetainers) filled with commercial potting soil mix. Seedlings were cultured in a greenhouse at a daily temperature of 20°C with a natural 10h daylight period and ambient humidity. Artificial night heating was not supplied, but the temperature was prevented from dropping below 2°C. As progeny seedlings developed into well tillered plants, each was divided into about 20 ramets and placed together within a conetainer rack.

When inflorescence emergence initiated in early May 1991, heading dates, vegetative leaf vernation, presence or absence of floral awns, and a relative assessment of glume length were recorded for each progeny. These characteristics have been used to separate Italian and perennial ryegrass (Nittler and Kenny, 1972; Humphries, 1980; Jung et al., 1996). Maternal progenies were discarded if ramets were weak or failed to express reproductive spikes.

Thirty-five clonal maternal progenies were assembled into three synthetic populations based on previously defined foliage phenotype, similar heading date, and fluorescence expression (Table I-1). Populations 1 and 2 phenotypically resembled perennial ryegrasses, and Population 3 was a mixed phenotype of Italian and

Table I-1. Parental description of four ryegrass populations.

Original Cross†	Parental designation	Leaf vernation‡	Fluorescence expression§	Greenhouse heading date¶	Presence of awns	Glume length#
Population 1 (Constructed population base SRF = 10.00%)						
89V-2 x 89V-12	94-1	F	-	May 20	no	long
89V-2 x 89V12	99-1	F	-	May 23	no	long
89V-14 x 89V-16	123-7‡‡	F	-	June 01	no	long
89V-16 x M	142-2	I	+	May 22	yes	med.
89V-12 x 89V-16	152-1	F	-	May 20	no	long
89V-12 x 89V-16	155-6	F	-	May 23	no	long
89V-12 x 89V-3	160-15††	F	-	May 24	no	long
89V-16 x 89V-2	171-5	F	-	May 20	no	long
89V-2 x 89V-16	176-2	F	-	May 24	no	long
89V-2 x 89V-16	179-8	F	-	June 01	no	long
Population 2 (Constructed population base SRF = 22.22%)						
89V-2 x 89V-12	94-2‡‡	F	+	May 28	no	long
89V-2 x 89V-12	94-4††	F	-	June 01	no	long
89V-14 x 89V-16	123-3	F	+	May 28	no	long
89V-12 x 89V-16	152-8	F	-	May 31	no	long
89V-12 x 89V-16	155-2	F	-	May 25	no	long
89V-12 x 89V-3	160-4	F	-	May 22	no	long
89V-16 x 89V-3	168-5	F	-	May 28	no	long
89V-2 x 89V-16	176-1	F	-	May 28	no	long
89V-2 x 89V-16	179-2	F	-	June 01	no	long
Population 3 (Constructed population base SRF = 70.00%)						
M x 89V-14	14-4††	R	+	May 23	yes	short
M x 89V-12	26-8††	I	+	May 15	yes	short
M x 89V-12	34-2‡‡	F	-	May 21	no	long
M x 89V-3	43-2	I	+	May 14	yes	short
M x 89V-12	59-5	I	+	May 13	yes	short
G x 89V-12	106-4	I	-	May 12	yes	med.
G x 89V-12	106-13	R	-	May 13	yes	long
G x 89V-3	112-9	I	+	May 13	yes	med.
89V-16 x M	126-14††	R	+	May 28	yes	med.
89V-16 x M	142-10‡‡	F	+	May 20	yes	long

Table I-1 continued.

Population 4 (Constructed population base SRF = 16.67%)

VNS-Deriv.	228	F	+	May 28	yes	long
VNS-Deriv.	4867	R	-	May 13	yes	short
VNS-Deriv.	4948	R	-	May 31	yes	short
G-Deriv.	5149	R	-	May 31	yes	med.
VNS-Deriv.	5182	R	-	May 29	yes	short
G-Deriv.	5304	R	-	May 31	yes	short

† Original cross from which parental stock were produced. Original parents with 89 coding were obtained from Pure Seed Testing Inc., Hubbard, Oregon. They were phenotypically perennial ryegrass. Those coded as G, M, or VNS were derivatives from the cultivars Gulf, Marshall, or diploid Italian ryegrass, cultivar not stated.

‡ Leaf vernation was visually scored on expanding vegetative leaves: F = folded, R = rolled, I = intermediate.

§ + = SRF visible on filter paper, - = SRF not visible on filter paper.

¶ Heading date was determined when one spike of the parental plant was emerged from boot.

Glume length was subjectively evaluated relative to the total length of spikelet.

†† Half-sib family lines dropped from further increase for each population at all sites because of poor seed production in 1992.

‡‡ Additional half-sib lines discarded from each population at the Madras location because of poor seed production in 1992.

perennial ryegrass. Population 1 had ten maternal progenies, Population 2 had nine, and Population 3 had ten. Selected progeny constructing a fourth Population were derived from Gulf and other diploid accessions of unknown Italian ryegrass; i.e. variety not stated (VNS) classification. These were obtained from Agri-Seed testing and Tangent Seed Lab, International, two private seed testing laboratories. These laboratories donated the seedlings after fluorescence tests had been completed February 1991. Population 4 was composed of six clonal progeny parents possessing Italian ryegrass phenotype, however, five of the parents were non-fluorescent variants (Table I-1). Populations were assembled to represent low, medium, and high fluorescence levels based on parental SRF expression, and to represent both perennial and annual growth habit characteristics.

Seed Increase Locations and Syn 1 Generation Increase. Three Oregon locations (Table I-2) were chosen for seed increase of each population over three seasons (Syn 1 to Syn 3 generations). Sites at Corvallis and Aurora were chosen because they are located in ryegrass growing areas of western Oregon in the south and north Willamette Valley, respectively, and Madras is located in an area where ryegrass is not commonly grown. The Syn O generation was developed using ramets from each maternal parent of each population, transplanted to each field site in randomized complete block (RCB) designs with six replications. Each replication consisted of one ramet from each maternal parent of a population. Individual ramets within blocks were spaced on 50 cm grids.

Table I-2. Description of three Oregon locations where ryegrass seed was increased.

Location latitude/ longitude	Land form; elevation above sea level (m)	Soil series family, sub grouping, pH	1960-1990 Average annual ppt. (cm)	Cropping history of trial area for growing seasons, 1989-91
Aurora, OR 45°N 17'/ 122°45'	Willamette Valley; 49	Willamette silt loam; Fine-silty, mixed mesic; Pachic Ultic Agrixerolls; 6.2; high P, K levels; medium Ca, Mg levels	104.3	Strawberries (*Fragaria* x *ananassa* Duch); strawberries; fallow
Corvallis, OR 44°N 38' 123°12'	Willamette Valley; 69	Woodburn silt loam; fine-silty, mixed mesic; Aquiltic Agrixerolls; 6.0; high P, K levels; medium Ca, Mg levels	108.5	Oat (*Avena sativa* L.) and rapeseed (*Brassica napus* L.); oat and rapeseed; winter wheat (*Triticum aestivum* L.)
Madras, OR 44°N 38' 121°8'	Columbia plateau; 701	Madras loam; fine-loamy, mixed, superactive, mesic; Aridic Argixerolls; 7.1; high K levels; medium Ca, Mg levels	27.9	Dryland cereal trials; fallow; winter wheat

The four populations were transplanted at Corvallis on 5 November 1991, and at Aurora on 7 November. Populations were isolated from each other by crop barriers or physical distance throughout the study based on distances or pollen barriers that would prevent pollination between populations. Cereal rye (*Secale cereale* L.) was used as a pollen barrier at Corvallis and wheat (*Triticum aestivum* L.) or oat (*Avena sativa* L.) was used at Aurora. Barrier distances between populations were 14.4 m at Corvallis and 30.5 m at Aurora. Wheat, at a distance of 30.5 m, was used as a barrier at Madras. Granular fertilizer of ammonium sulfate + urea (33.5-0-0-12, N-P-K-S) was applied at 45 kg N ha^{-1} to both western sites each autumn. Split spring fertilizer applications were applied to western Oregon plots each year. Granular 33.5-0-0-12 (90 kg N ha^{-1}) was applied in March and an additional 34 kg N ha^{-1} was applied in April.

To avoid possible winter kill in central Oregon, parental clones of the four populations to be established at Madras, were maintained at the Pickseed West, Inc. (PSW) research farm, Albany, OR, during autumn 1991. Starting February 1992, ramets of these parents were transplanted to larger conetainers (7 x 25 cm) and kept at a daylight exposure of 9 h accomplished by covering plants with 6 mm black plastic covers at 1630 h PST daily. The plants were uncovered at 0730 h each morning. Populations were transplanted to field nurseries at Madras on 10 April 1992. Plots at Madras were only fertilized at transplanting time with a broadcast application of granular formulation of ammonium sulfate (21-0-0-24, N-P-K-S) at 45 kg N ha^{-1}. This fertility practice was done each season of the test. From the time of transplanting until

approximately one week before seed harvest, plots at Madras were irrigated weekly to maintain plant turgor and overall vigor for the test duration. Supplemental irrigation was not necessary at the other sites during the years of seed increase.

The first spring flush of weeds was controlled at the western Oregon locations by interrow applications of Paraquat 37% EC {1:1-dimethyl-4, 4'-bipyridinium dichloride} herbicide, at a rate of 0.24 L ha^{-1}. Treatment was conducted with a knapsack sprayer. Subsequent weed control was done by hand hoeing. Weed control at the Madras site was done by hand hoeing, after a pre-plant broadcast application of glyphosate {N-(phosphonomethyl) glycine} herbicide at the rate of 4.09 L ha^{-1} throughout the entire test.

Seed was harvested with a hand held serrated knife in late June to early July at all three locations by cutting each parental line when seed began to shatter (natural disarticulation). As seed was harvested, it was dried at ambient field conditions in a covered shed. Seed was threshed, conditioned and weighed for each ramet, and then bulked by maternal line to create half-sib families. For the following two growing seasons, half-sib family progeny were raised, allowed to cross pollinate, and allowed to set seed. Seed was harvested by separate maternal family at each location (Fig., page 46).

Syn 2 and Syn 3 Generation Increases. Five replicates of 100 random seeds for each maternal family were counted from the Syn 1 or Syn 2 increase of each population using a commercial, lighted counter board. Selection for caryopsis size was avoided. Since it was necessary to retain at least 100 viable seeds of each family line for a final

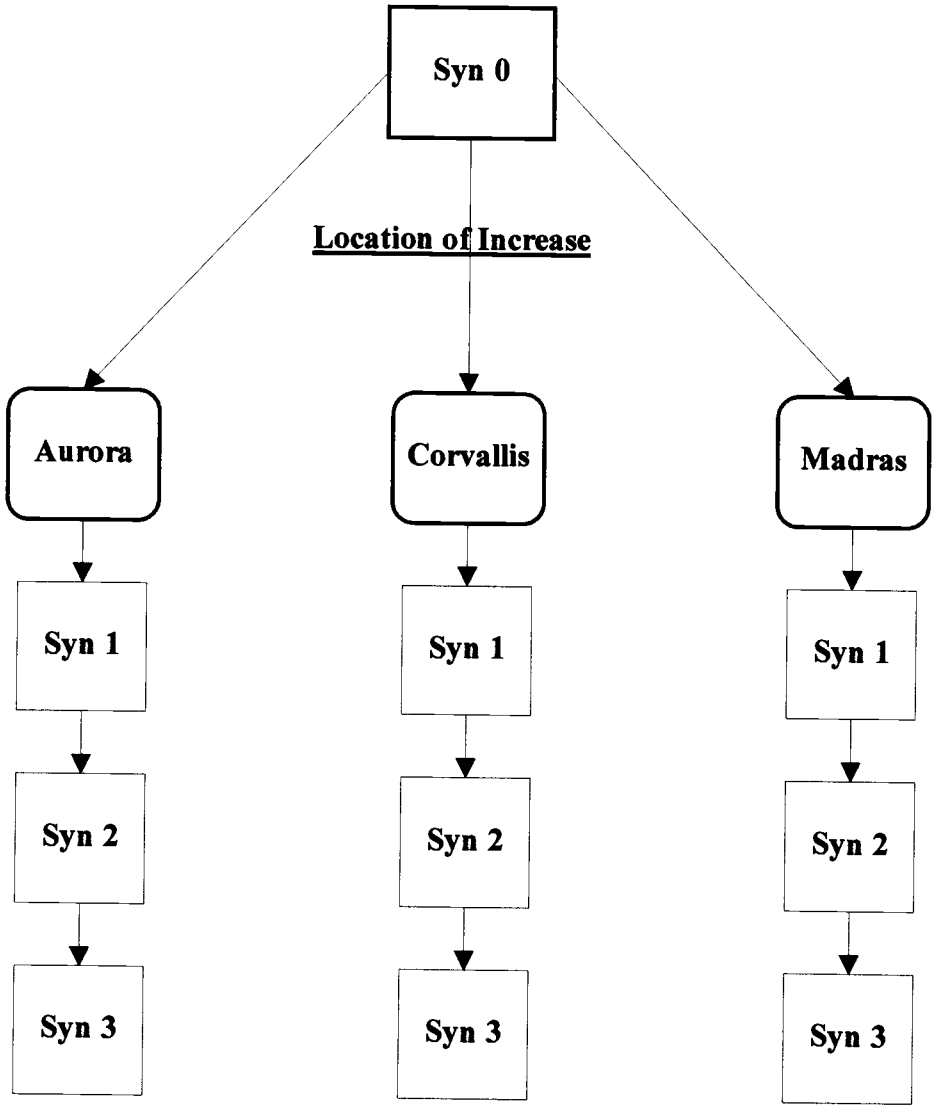

Figure. Conceptual diagram of population distribution and seed increase generations representative of the four populations developed. The Syn 0 generation was assembled as ramets of the same cross combinations and distributed to each of three locations, then seed was increased for three generations within each location.

fluorescence test, any family which did not produce a bulked amount of seed ≥600 in 1992, was discarded from future increase (Table I-1).

Direct seeded rows were established in RCB experimental designs with five replications at the western Oregon locations. Rows consisted of progeny seeds for each half-sib family of all populations. Rows were 1 m and spaced 61 cm apart. Seed was planted 8 mm deep by hand. Syn 1 seeds were sown on 18 October 1992 at Corvallis, and during the first week of November 1992 at Aurora. Syn 2 seed produced was sown at the two western sites on 20 October 1993 and 28 October 1993, respectively, in order to produce the Syn 3 generation. Identical family lines were sown at both western locations. Plots were planted using carbon banding (PNW Weed Control Handbook, 1995). Diuron {3 - (3, 4 dichlorophenyl)-1, 1- dimethylurea} was applied pre-emergence to control weeds. Spring weeds were controlled as noted above.

For the Madras location, if any maternal family did not produce at least 25 progeny seeds from the 1992 bulk harvest, it was dropped from succeeding increase generations. Thus, future increases at Madras did not quite parallel the family constitution of the Aurora and Corvallis locations. This was because of differential survival and recombination of parental stocks in 1992 relative in the higher growth stress environment at Madras compared to the other two locations (Table I-1). Syn 1 seeds were germinated, evaluated for fluorescence expression, transplanted to conetainers, and kept at PSW until transplanted to the field on 20 April 1993. Syn 2 progeny were transplanted 13-14 April 1994. Cereal barriers were not possible for

increase seasons 2 and 3 at Madras, so only distance was used. In these seasons, distance between populations was never less than 76 m.

Seed harvest consisted of cutting progeny plants in each maternal row at each location during July. Seed was naturally dried, threshed, conditioned, weighed for each maternal family row (or plant), and then bulked by maternal origin among replications for each half-sib family generated for each generation of open pollinated increase.

Seed Testing. Seed from both 1992 and 1993 harvests were kept in cool (5°C), dry storage from the time of conditioning until the beginning of seed testing. Germination and SRF tests were conducted during winter 1994-95 at the Oregon State University seed testing laboratory. Seed produced in 1994 was given a 7 d pre-chill treatment (stratification) at 5°C, as specified by AOSA rules, to allow breakage of latent dormancy. Dormancy was not a consideration for the older harvested seed (AOSA, 1994). Seed tests were conducted for each population/generation/location combination in 16.5 x 24.5 cm acrylic germination boxes with about 100 seeds in each box. Germination substrate was Whatman® white filter paper moistened by 2.1 g L^{-1} potassium nitrate solution.

Fluorescence tests were conducted with equal amounts of progeny represented from each half-sib family within a population to provide about 100 total seeds from a population in each of four replications of the test. The SRF test, then, closely approximated the 400 seed standard test conducted on commercial seed lots. For example, population 4 was constructed of six half-sib families; thus sixteen progeny seeds from each family for a given generation at a location were tested in four

replications; (16 x 6 x 4) = 384 seeds tested for one population/generation/location. Progeny seed of each family were hand sown and arranged randomly within a germination box. Germination boxes were arranged in the germinator in a completely randomized design. All boxes were confined to the same shelf in the same germinator for the duration of the experiment. Germinator lighting ($89\mu mol^{-2}s^{-1}$), temperature (alternating 15-25°C with light during the 8 h 25°C period), and relative humidity (approx. 95%) followed standard protocols (AOSA, 1994, 1998b). Each "400" seed test was conducted at two different times and data presented were pooled for the two runs. Seedling root fluorescence determinations were made in a darkroom, using a near ultra-violet two bulb lamp to visualize fluorescence at 7 d and 14 d after germination (AOSA, 1994). Fluorescent root streaks, detected after all seedlings were lifted from the filter paper at 14 d, were included in test fluorescent values.

Statistical Analyses. Data produced were analyzed using the GLM procedure (SAS Institute, 1994) to conduct an analysis of variance. Mean separation was by Fisher's protected least significant difference (LSD). The statistical model considered populations, generations, and locations as fixed effects. Results presented pertain to gross population changes by location and generation of seed multiplication.

Results and Discussion

The two largest mean squares for SRF were among populations and among location of seed increase (Table I-3). Differences among populations were expected because each population was purposely assembled to provide different base levels of SRF. Except for population 3, mean SRF levels among populations were highest for seed produced at Corvallis (Table I-4). Mean SRF at Corvallis increased over generations of seed increase, and was highest in the third generation. Mean SRF over three generations from seed produced for each population at Aurora were not different, and were significantly different at Madras in three populations for one generation each. Population and location were also the largest mean squares for germination percentage (Table I-3), but mean germination over the three generations across locations was only different for population 3 (Table I-5). Within locations, when germination was different from one generation to another, the differing generation was always Syn 1 (the earliest seed produced in the experiment), indicating the effects of seed age on germination. Variation in germination did not correspond to differences in SRF, and thus, differences in germination could not explain variation in SRF.

Rumball (1970) also showed significant among region effects for SRF when an intermediate (*L.* X *hybridum* Hausskin) cultivar was increased for seven generations. He found SRF levels ranging from 83 to 91%, but he did not track the same population harvested, multiplied, and re-sown at the same locations. Population 3 in the present study most closely resembled an intermediate ryegrass with high SRF. The Syn 3 generation of this population increased in SRF over its base level after it was increased

Table I-3. Mean squares from an analysis of variance for seedling root fluorescence (SRF) and germination of four ryegrass populations increased three generations at three locations.

Source of Variation	df	Mean squares	
		SRF	Germination
		----------%	----------
Population (P)	3	83,703.27**	2,006.57**
Generation (G)	2	938.51**	283.17
P X G	6	89.91	95.25
Location (L)	2	8,778.75**	322.94*
L X P	6	923.06**	159.25
L X G	4	724.56**	57.40
L X P X G	12	68.86	76.00
Residual	252	40.35	47.45

*,** Significant at the 0.05 and 0.01 probability levels, respectively.

Table I-4. SRF of four ryegrass populations increased three generations at three locations.

Population	Generation	Location		
		Aurora	Corvallis	Madras
		----------%----------		
1 (SRF = 10.00%)				
	Syn 1	3.98a†	15.69b	14.74a
	Syn 2	3.47a	27.47a	10.25b
	Syn 3	2.04a	36.42a	8.44b
	Mean	3.16a‡	26.53c	11.15b
2 (SRF = 22.22%)				
	Syn 1	29.24a	34.07c	17.91b
	Syn 2	26.61a	46.98b	24.27a
	Syn 3	27.10a	58.36a	27.74a
	Mean	27.65b	46.47c	23.31a
3 (SRF = 70.00%)				
	Syn 1	76.32a	78.62b	83.59b
	Syn 2	77.84a	83.57b	87.09b
	Syn 3	79.95a	92.75a	90.03a
	Mean	78.04a	84.98b	86.90b
4 (SRF = 16.67%)				
	Syn 1	1.52a	18.91b	2.55a
	Syn 2	0.13a	23.57ab	2.38a
	Syn 3	0.80a	28.36a	0.14a
	Mean	0.82a	23.70b	1.69a

† Population means with the same letter in a column representing values within a location and population are not different using GLM LS Means with $P \leq 0.05$.

‡ Location means with the same letter in a row are not significantly different using least significant difference test with $P \leq 0.05$.

Table I-5. Germination percentage (%) of four ryegrass populations increased three generations at three locations.

Population	Generation	Location		
		Aurora	Corvallis	Madras
		--------------- % ---------------		
1				
	Syn 1	95.46a†	88.77b	87.39b
	Syn 2	94.82a	91.92a	94.14a
	Syn 3	93.31a	90.15ab	90.24ab
	Mean	94.53a‡	90.28a	90.59a
2				
	Syn 1	97.14a	92.71a	90.37a
	Syn 2	94.14a	93.10a	92.47a
	Syn 3	92.19a	92.71a	89.90a
	Mean	94.49a	92.84a	90.91a
3				
	Syn 1	74.75b	84.18b	80.50a
	Syn 2	81.89a	89.17a	80.38a
	Syn 3	83.56a	89.16a	80.13a
	Mean	80.07a	87.50b	80.34a
4				
	Syn 1	93.88a	95.58a	81.19b
	Syn 2	96.36a	95.70a	98.18a
	Syn 3	97.40a	96.23a	95.58a
	Mean	95.88a	95.84a	91.65a

† Population means with the same letter in a column representing values within a location and population are not different using GLM LS Means with $P \leq 0.05$.

‡ Location means with the same letter in a row are not significantly different using least significant difference test $P \leq 0.05$.

three generations at Corvallis and Madras, but remained unchanged at Aurora. Mean SRF (over generations) was lower at Aurora than seed increased at the other two sites (Table I-4). These observations are indicative of the significant L x P and L x G interactions for SRF (Table I-3).

It is not clear why location of seed increase had such a large impact on SRF level. Generation response was similar at Aurora and Madras with little or no change in SRF, but always increased at Corvallis. Populations would not be expected to change unless there was a selection pressure or contamination from an outside source either from seed or pollen. Foreign pollen may have been a source of contamination because of high concentrations of commercial seed production fields of Italian ryegrass near Corvallis in the southern Willamette Valley (Young and Griffith, 1996). Even though populations at Corvallis were isolated by cereal rye, possible contamination sources may have been higher than suspected. Had Italian ryegrass pollen contamination been a factor for SRF increase, it seems unlikely that each population would have responded similarly because mean flowering time of the populations was different relative to each other (Table I-6). Italian ryegrass as a crop in neighboring fields, or as volunteers in land adjacent to the test plots, would have flowered a week to 10 d earlier than perennial ryegrass. Populations 1 and 2 phenotypically resembled true breeding perennial ryegrass. Perennial and Italian ryegrass overlap in flowering time during the day (Rampton, 1966), but may differ greatly in date of anthesis. The possibility of inadvertent contamination of pollen flow from outside sources, however, cannot be overlooked.

Table I-6. Mean flowering dates for four ryegrass populations increased for three generations at two locations.†

Population	Generation	Location	
		Aurora	Corvallis
1			
	Syn 1	149	148
	Syn 2	158	155
	Syn 3	152	146
	Mean	153	150
2			
	Syn 1	149	149
	Syn 2	159	157
	Syn 3	150	149
	Mean	153	152
3			
	Syn 1	148	150
	Syn 2	157	154
	Syn 3	147	144
	Mean	151	149
4			
	Syn 1	146	140
	Syn 2	154	149
	Syn 3	148	142
	Mean	148	144

† Date reported as calendar day of year (DOY).

The cereal grain isolation barriers were routinely scouted at both Aurora and Corvallis during each production season to remove any trace of volunteer ryegrass plants prior to flowering of the populations. Cereals were well headed, and substantially taller than all ryegrass populations at their respective flowering times. Similar scouting at the Madras location never detected volunteer ryegrass plants.

Of the four populations constructed, number 4 was phenotypically and physiologically most similar to volunteer Italian ryegrass. It would have been the only population likely to have flowering coinciding with commercial Italian ryegrass seed production fields. Mean flowering date of families within population 4 at Corvallis was 8 and 9 d earlier than mean flowering of families in populations 1 and 2 in 1992, 6 and 8 d earlier in 1993, and 4 and 7 d earlier in 1994 (Table I-6). Physical contamination with foreign Italian ryegrass pollen on populations 1 and 2 would have been of low probability because mean flowering time of each ryegrass form was separated by approximately one week each year of increase. Flowering time differences, the presence of a cereal crop physical barrier, and an absence of significant other Italian ryegrass characteristics in subsequent generations or grow-out tests, indicated that pollen contamination, from either Italian ryegrass commercial fields or among the populations themselves, was unlikely the cause of increased SRF at Corvallis.

For the present study, according to migration (contamination) theory (Falconer and MacKay, 1996), from 11 to 42% immigration from Italian ryegrass to the perennial-type populations would be needed to account for the generation to generation change of the reported magnitude. This amount of contamination from

pollen load was highly unlikely considering the wide variation in flowering dates and the duration of flowering in perennial ryegrass.

Results reported herein are supported by Copeland and Hardin (1970) who used SRF as a measure to detect pollen contamination in commercial ryegrass seed production fields in western Oregon. They found little contaminant Italian ryegrass pollen into perennial ryegrass beyond 6 m from the edge of ryegrass fields, even with large pollen loads from an adjacent Italian ryegrass field. Beyond 12 m from the border, there was no evidence of contamination from Italian ryegrass.

Johnson et al. (1996) studied the potential of uncontrolled hybridization among smooth bromegrass (*Bromus inermis* Leyss) accessions. They determined that cross pollination among accessions was 17% at 1.5 m from a centrally planted contaminant pollen source. Crossing to foreign pollen decreased to 0.5% at 9 m. Also in bromegrass, but using a different genetic marker than Johnson et al. (1996), Knowles and Ghosh (1968) showed average contamination of plots was 9.6%, 1.0%, and 0.2% for isolation distances of 1, 61, and 183 m from a contaminant source. Griffiths (1951) pointed out that for perennial ryegrass, as the abundance of non-contaminating pollen increases, in large production fields, fertilization by contaminating sources is reduced. Griffiths (1951) showed that border rows of plants were highly effective in reducing contamination among perennial ryegrass accessions, and suggested individual ryegrass plants were fertilized primarily by pollen from neighboring plants in their immediate vicinity.

Barker et al. (1997), and R.E. Barker, personal communication (1997) examined SRF levels for three cultivars increased four years from fields of the same cultivars located in both north and south sections of the Willamette Valley. Three laboratory SRF test runs were made on each seed lot. Besides the obvious difference among cultivars for SRF, they found SRF varied within cultivars across years and over test runs. However, they did not show differences across seed production environments indicating that pollen contamination from higher Italian ryegrass pollen loads in the south Valley was a contamination factor. Seed sampled by Barker et al. (1997) and other SRF studies came from perennial cropped fields of established cultivars. Thus, each cultivars' frequency of SRF expression should have been stable through years of certified seed production.

It is common cultural practice for growers of certified ryegrass seed to rogue from their fields individuals that they deem to be "off-type" prior to pollination of the crop. Every individual within the populations reported herein were allowed equal chance for interpollination, and none were artificially selected nor rogued to attain uniform floral nicking, plant growth type, or height. Seed sampled from populations reported here were from plots treated as annual crops in that they were destroyed after each harvest and volunteer seedlings were never a factor. Additionally, the variety fluorescent levels (VFL) for the cultivars used by Barker et al. (1997) were of relatively low value, i.e. 0.34 to 2.25%. Lowest base fluorescence level among my populations was 10% for population 1. Because of low levels and initially small population sizes,

(VFL) levels less than 5% may take longer than three generations to show appreciable increase within a population.

Selection and drift are two other forces that could have affected the distribution of alleles for SRF of the small population sizes used in this study. There was no evidence, but it is possible different locations provided a greater selection advantage for dominant SRF genotypes. Selective advantage between genotypes among varying environments could happen depending on differential tillering capacity, differences in plant height, or flowering duration among half-sib families. Over the three generations, one or two families in a population were shorter in plant height than were other families, and thus could have been less competitive during pollination than others (Appendix). Evidence of differential performance would probably be reflected in family seed yields. In general, seed yields and 1000 seed weights were largest for each year of increase at the Corvallis site versus Aurora (Appendix). It appeared that growth conditions were better in Corvallis than at Aurora. Favorable growing conditions could have contributed to enhanced SRF levels at the Corvallis site by Italian-like genotypes. No conclusive inferences, however, can be made claiming seed yield superiority of populations increased at one location relative to another because insufficient data to document these changes were collected. A separate experiment using clonal genotypes established at least at Corvallis and Aurora would be needed to collect data to measure environmental conditions causing seed production change. Representative samples of

each population raised from each generation among locations could be planted, and detailed agronomic characters measured for two or more seasons.

The two western sites had soils with similar pH and general physical properties. The Madras site had drier soils with higher pH, and of substantially different physical structure than the Willamette Valley soils. All soils of the study received the same level of supplemental nutrients. Each soil, however, had its own adsorptive properties, i.e. cation exchange capacities, and total nutrient availability. Each had its own water holding and retention capacity. Populations at the Madras site were irrigated throughout the duration of the test, while the two other locations relied only on precipitation. Soil temperatures were not monitored during the study. Future research on this topic should include more thorough classification of soil properties, soil water potentials, and initial soil fertility exchange capacity and soluble anion levels.

More thorough classification of parental stocks' floral induction and initiation requirements should be conducted before constructing future test populations. For example, it would have been beneficial to know *a priori* what parental clones required an inductive vernalization and which ones were vernalization insensitive. Additionally, parental clones should be evaluated for photoperiod sensitivity. The rationale here would be that natural selection may have occurred for specific individual plant types based on flowering habits across an array of environments.

Weather records for the three locations showed similar mean bi-weekly high and low temperatures during flowering and seed maturation periods (Appendix). There were no trend differences among the locations that would indicate differential

flowering, pollination, and fertilization activities amongst genotypes across locations based on temperature (Appendix).

It is not known to what extent self-fertility may have existed among parental stocks, nor if environment conditioned self-fertility. McVeigh (1975) reported that self-sterility is very pronounced in perennial ryegrass, but occasionally genotypes are found which are highly self-fertile. Others have shown similar results (Cornish et al., 1980; Scarrott, 1981; Jenkin, 1931; Beddows et al., 1962; Foster and Wright, 1970; Jones and Jenabzadeh, 1981; and Hayward and Wright, 1971). Foster and Wright (1970) discovered levels of self-fertility were similar in bagged ryegrass selfing units in and outside of a greenhouse in average years. However, more selfed seed was obtained outside in years that were drier and warmer for a given environment. They also found in a highly humid environment, mean self-fertility was reduced by a factor of three. Daily evaporation rates for the Aurora and Corvallis location during this study were similar (Appendix). Thus, the degree of selfing was assumed to be the same for populations at both locations, but the percent for each location was not measured by bagging heads or other methods. High self-fertility, or differential fertility could change allelic frequencies and thus affect SRF results. If families with high self-fertility were also high in SRF expression, they could compete more favorably in succeeding generations of increase.

Plot seed yields from each population were numerically higher at the Corvallis site than at Aurora (Appendix). As noted earlier, no direct comparisons were made between the two western Oregon locations and the Madras location because population

composition and planting arrangement were slightly different at Madras relative to the other locations.

Maternal control was artificially supported by sowing of the same number of progeny seeds per family component of each population. However, this control was maintained at each location during increase generations. This was essential because of the wide variation in seed production among parent clonal material in 1992 (Appendix). A future study could be conducted that simply bulks all seed produced, irrespective of individual half-sib family seed yield, and multiplies a representative sample of the bulk through several generations. It is my opinion this type of study would accentuate the appearance of a selection advantage being shown for phenotypes favored in different locations of seed production. Undoubtedly, families producing the largest and most abundant amount of seed would dominate any sample drawn from the bulk.

Genetic drift can also be an argument why SRF was expressed differently among locations. Size of initial populations were small in terms of number of parents. Whatever happened in the 1992 seed multiplication season in reference to biological sampling error from one location versus another could have allowed drift that was cumulative in the direction established in the first generation of increase. Alterations of allele frequency for SRF toward fixation in one cycle of sampling could increase the chances of fixation in the next cycle of sampling. Variance in parental fertility could help explain the possible incidence of genetic drift if data addressing such were gathered. Part of the rationale for establishing increase generations at all locations using equal numbers of progeny from each family of each population was to alleviate

potential drift from a physical sampling standpoint. Future research in SRF should incorporate much larger initial population sizes to hedge against effects of random drift.

It is emphasized that larger population sizes could have been constructed, and more thorough characterization of parents could have allowed better randomization of mating. It is common practice, however, for ryegrass cultivars to be based on low numbers of unrelated parents. This research focused at simulating such a cultivar development procedure.

Ryegrass SRF has long been used as a determinant trait for varietal integrity in seed certification schemes in the USA. In commercial applications, it is still assumed by many that variation in SRF test results for individual cultivars is entirely genetic in origin. Tolerances are not applied to test results based on environmental and management factors imposed on a cultivar during the multiplication process from breeder to certified seed production. Results reported here should inspire future experimentation designed to achieve a clearer understanding of how the environment where ryegrass plants are grown can affect the expression of SRF. Seed certification personnel have the responsibility of ensuring that consumers receive the advertised product, and they also have the charge to provide safeguards for seed growers to ensure they are not economically penalized for a crop of perennial ryegrass seed that may not meet certification standards based on false-positive SRF test results. In other words, actual contamination from Italian ryegrass may be misrepresented by variable SRF test results of certain seed production lots (personal communication with ryegrass seed growers, 1995). If causes of SRF variability are not better understood, the test

should not be used as a trait to describe cultivars, unless the results of the a laboratory test are reinforced with evidence of "off-type" appearance in production fields. We need to reconsider conclusions of some of the early researchers involved with the fluorescence test. Linehan and Mercer (1933) suggested that fluorescence testing should be used only for making approximate determinations in classifying ryegrass seeds. Rampton (1938) stated that fluorescence tests cannot be used as infallible guides to classification of questionable lots of Oregon grown domestic ryegrass seed. My results confirm conclusions drawn by the early researchers.

As long as our Federal Seed Act allows plant breeders the right to describe a cultivar's inherent fluorescence value, the test will continue to be used as a descriptive cultivar trait. Results of this research make it clear that plant breeders should maintain wide geographic seed lot sampling for applications to document fluorescence levels of new ryegrass cultivars. Since the VFL for a cultivar is used as a baseline for marketing, the plant breeder must be certain he actually reports an accurate estimate of the mean for the cultivar's fluorescence percentage.

References

Association of Official Seed Analysts. 1994. Rules for testing seeds. J. Seed Technol. 16:14-15, 19.

Association of Official Seed Analysts. 1998b. Rules for testing seeds. AOSA. Beltsville, MD.

Association of Official Seed Certifying Agencies. 1991. Annual report of the AOSCA. p. 126-127. Salt Lake City, UT.

Barker, R.E., R.L. Cook, and D.J. Floyd. 1997. Variation of seedling root fluorescence in perennial ryegrass. p. 133. *In* Agronomy abstracts. ASA, Madison, WI.

Barker, R.E., and R.R. Kalton. 1989. Cool season forage grass breeding : progress, potentials, and benefits. p. 5-20. *In* D.A. Sleper, K.H. Asay, and J.F. Pedersen (ed.). Contributions from breeding forage and turfgrasses. CSSA Spec. Publ. 15. CSSA, Madison, WI.

Beddows, A.R., E.L. Breese, and B. Lewis. 1962. The genetic assessment of heterozygous breeding material by means of a diallel cross. I. Description of parents, self- and cross-fertility and early seedling vigour. Heredity 17:501-512.

Copeland, L.O., and E.E. Hardin. 1970. Outcrossing in the ryegrasses (*Lolium* spp.) as determined by fluorescence tests. Crop Sci. 10:254-257.

Cornish, M.A., M.D. Hayward, and M.J. Lawrence. 1980. Self-incompatibility in ryegrass. IV. Seed set in diploid *Lolium perenne* L. Heredity 44:333-340.

Falconer, D.S., and T.F.C. MacKay. 1996. Introduction to quantitative genetics. 4th ed. Longman Group, Ltd, Essex, England.

Floyd, D.J. 1997. Ryegrass for turf (*Lolium* spp.). *In* R.E. Barker (ed.) Grass germplasm in the USA: a status report. U.S. Crop Germplasm Committee for Forage and Turf Grasses. USDA-ARS-NFSPRC, Corvallis, OR.

Foster, C.A., and C.E. Wright. 1970. Variation in the expression of self-fertility in *Lolium perenne* L. Euphytica 9:61-70.

Gentner, G. 1929. Über die verwendbarkeit von ultra-violetten strahlen bei der samenprüfung. Praktische Blätter für Pflanzenbau und Pflanzenschutz 6:166-172.

Griffiths, D.J. 1951. The liability of seed crops of perennial ryegrass (*Lolium perenne*) to contamination by wind-borne pollen. J. Agric. Sci. 40:19-38.

Hayward, M.D., and A.J. Wright. 1971. The genetic control of incompatibility in *Lolium perenne* L. Genetica 42:414-421.

Humphries, C.J. 1980. *Lolium*. p. 153-154. In Flora Europea. Vol. 5, ed. T.G. Tutin, et al. Cambridge Univ. Press.

Jenkin, T.J. 1931. Self fertility in perennial rye-grass (*Lolium perenne* L.). Bull. Welsh Pl. Breed. Sta. Serv. H. 12:100-119.

Johnson, R.C., V.L. Bradley, and R.P. Knowles. 1996. Genetic contamination by windborne pollen in germplasm-regeneration plots of smooth bromegrass. Plant Genetic Res. Newsletter 106:30-34.

Jones, R.N., and P. Jenabzadeh. 1981. Variation in self-fertility, flowering time and inflorescence production in inbred *Lolium perenne* L. J. Agric. Sci. 96:521-537.

Jung, G.A., A.J.P. VanWijk, W.F. Hunt, and C.E. Watson. 1996. Ryegrasses. p. 605-641. *In* L.E. Moser, D.R. Buxton, and M.D. Casler (ed.). Cool-season forage grasses. Agron. Monogr. 34. ASA, Madison, WI.

Knowles, R.P., and A.N. Ghosh. 1968. Isolation requirements for smooth bromegrass, *Bromus inermis* Leyss., as determined by a genetic marker. Agron. J. 60:371-374.

Linehan, P.A., and S.P. Mercer. 1933. Fluorescence of *Lolium* seedlings in ultra-violet light. Nature (London) 131:202-203.

McVeigh, K.J. 1975. Breeding for resistance to crown rust (*Puccina coronata corda* var. *lolii* Brown) in turf-type perennial ryegrass (*Lolium perenne* L.). PhD. diss. Rutgers, The State Univ. of NJ, New Brunswick (Diss. Abstr. AAG7517460).

Nittler, L.W., and T.J. Kenny. 1972. Distinguishing annual from perennial ryegrass. Agron. J. 64:767-768.

Nitzsche, V.W. 1963. Nichtfluoreszierendes Welsches weidelgras (*Lolium multiflorum* Lam.). Der Züchter 33:281-282.

Nyquist, W.E. 1963. Fluorescent perennial ryegrass. Crop Sci. 3:223-226.

Okora, J.O., C.E. Watson, L.M. Gourley, B.C. Keith, and C.E. Vaughn. 1999. Comparison of botanical characters and seedling root fluorescence for distinguishing Italian and perennial ryegrass. Seed Sci. & Technol. 27: 721-730.

Pacific Northwest Weed Control Handbook. 1995. Extension Services of Oregon State Univ., Washington State Univ., and the Univ. of Idaho. Publication orders, OR State Univ., Corvallis.

Rampton, H.H. 1938. The use of morphological characters as compared with fluorescence tests with ultra-violet light in classifying the ryegrasses (*Lolium* spp.) of western Oregon. J. Amer. Soc. Agron. 30:915-922.

Rampton, H.H. 1966. Time isolation as a safeguard to varietal purity in perennial ryegrass, annual ryegrass, and orchardgrass. Corvallis, OR. Agric. Exp. Sta. Oregon State Univ. Circular no. 623.

Rumball, W. 1970. Changes in mean character and uniformity of *Lolium* (*perenne* x *multiflorum*) var. 'Grasslands Manawa' during seed increase. N.Z. J. Agric. Res. 13:605-615.

SAS Institute. 1994. SAS/STAT guide for personal computers. Version 6.10 ed. SAS Inst., Inc., Cary, NC.

Scarrott, C.H. 1981. Self-incompatibility in diploid and tetraploid *Lolium* species. PhD thesis. Univ. of Birmingham. England.

U.S. Department of Agriculture. 1999. Items of interest in seed control. USDA Winter 1999. U.S. Gov. Print. Office, Washington DC.

Young, W.C. III, and S. Griffith. 1996. Extension estimates for Oregon forage and turfgrass seed crop acreage, 1996. Oregon State Univ. Exten. Ser., OR State Univ., Corvallis. (Available on-line with updates at http://www.css.orst.edu/seed-ext/Agronomy/99ftarc.html) (verified 17 April 1997).

HERITABLITY OF SEEDLING ROOT FLUORESCENCE AMONG HALF-SIB RYEGRASS FAMILIES (MANUSCRIPT 2)

Abstract

Seedling root fluorescence (SRF) has long been used in the USA to separate Italian ryegrass (*Lolium multiflorum* Lam.) from perennial ryegrass (*L. perenne* L.). Generally, roots of Italian (annual) ryegrass fluoresce under ultraviolet light, while those of perennial do not. The trait is significant in marketing high quality perennial ryegrass seed for use as turf. Contamination by Italian ryegrass in perennial ryegrass seed lots is not acceptable because of differential growth rate of these two species. SRF is not solely species specific, however, and the trait is known to exist in many physically uncontaminated perennial ryegrass seed lots by introgression. Ryegrass breeders can document inherent variety fluorescent levels (VFL) on cultivars submitted for Plant Variety Protection, and for seed certification programs in the USA. The objectives of this research were: 1) to estimate heritability of SRF among 26 half-sib families of ryegrass, and 2) to measure the variability of other Italian ryegrass associated morphological traits in half-sib family lines over generations and locations of seed increase. Families were increased three generations in each of three Oregon locations. Heritability estimates for SRF ranged from 97 to 99%, but there was a significant family X year interaction for the trait. Thus, although heritability of the trait is high, VFL cannot be precisely predicted for half-sib ryegrass family lines in cultivar development with data generated from only one location. Variation among families was also the largest source of variation for leaf vernation and reproductive heading without

vernalization. A significant interaction existed for replication X family for these two morphological traits and reflects the difficulty in scoring leaf vernation. Standardized grow-out conditions should be emphasized if these traits are used to supplement SRF in detection of Italian ryegrass contamination in perennial ryegrass seed lots, and when used to document a cultivar's inherent fluorescence level.

Introduction

World-wide, ryegrasses (*Lolium* sp.) are probably the most widely used of all grasses (Barker and Kalton, 1989). The USA Crop Germplasm Committee for Forage and Turf Grasses ranked ryegrass as high to medium in importance for preservation and enhancement of all major cool-season grasses (Floyd, 1997). More USA companies are investing in ryegrass breeding and cultivar development for turf use than any other grass (Frey, 1996).

Prior to the mid-1960s, short lived stemmy phenotypes were generally the only kind of perennial ryegrasses (*L . perenne* L.) available to the North American turfgrass industry (Meyer and Funk, 1989). Extensive breeding efforts were initiated in the late 1960s with the goal of improving the permanent turf application of perennial ryegrass. Such efforts led to release of several cultivars; 'Manhattan', 'NK-100', and 'Pennfine' are examples. During the early 1960s perennial ryegrass was also promoted as a viable aesthetic turf and playing surface for southern USA turf areas, overseeded on dormant warm-season species (Meyer and Funk, 1989).

Current perennial ryegrass and Italian ryegrass (*L . multiflorum* Lam.) cultivars are generally derived as synthetics. The two species can hybridize easily, creating hybrid or intermediate ryegrass, *L.* X *hybridum* Hausskn. (Jenkin, 1924; Woodforde, 1935; Hubbard, 1968). Often positive attributes of both species are recovered in cultivars created from compatible crosses. Synthetic cultivar development from these ryegrass species involves maximizing cross-fertilization among selected parental clones

via an efficient self-incompatibility mechanism (Jenkin, 1927; Terrell, 1968; McCraw and Spoor, 1983a,b).

Delivering perennial ryegrass seed lots free of Italian ryegrass contamination is a challenge for growers. The contamination is most significant for lots destined for permanent turf use. Italian ryegrass often exhibits more rapid vertical growth, less tillering with wider and lighter colored foliage than perennial ryegrass. Vegetative leaf vernation of Italian ryegrass is generally rolled, while being folded for perennial ryegrass. On a generative basis, Italian ryegrass floret lemmas have awns, while lemmas are awnless for perennial ryegrass. Perennial ryegrass grows vegetatively until tillers pass through a required combination of temperature and day length changes (Riewe and Mondart, 1985; Cooper and Calder, 1964). The life cycle of Italian ryegrass is less sensitive to seasonal temperature changes and its reproduction is most influenced by day length. Seed lots of Italian and hybrid ryegrasses, primarily used for forage purposes and temporary turf markets, are not so critically scrutinized for content of "other" ryegrasses as is perennial ryegrass.

Historically, the seedling root fluorescence (SRF) test has been used to separate the two ryegrass crop types. The test is often done in conjunction with routine germination tests. However, the utility of SRF as a discriminator of the ryegrasses has been debated since its discovery in 1929 (Gentner, 1929; Rampton, 1938; Nyquist, 1963; Nitzche, 1960). Seeds are sown on moist, white filter paper, and seedling roots are examined while exposed to an ultraviolet light source. Generally, roots of Italian ryegrass fluorescence, and those of perennial ryegrass do not. The pigment responsible

for the fluorescence is an alkaloid called annuloline (Axelrod and Belzile, 1958). However, SRF is not a definitive test because genetic admixtures are common, either occurring naturally, or generated artificially by grass breeders.

Inheritance of the SRF trait has not been thoroughly studied. Previous research on inheritance focused on greenhouse or otherwise controlled crosses, conducted with offspring grown in single environments using small population sizes (Nitzsche, 1966; Nyquist, 1963; Corkill, 1932; Linehan and Mercer, 1933; Woodforde, 1935). Summarizing previous researchers' results: 1. The capacity for SRF is heritable. 2. SRF is dominant in the F_1 generation (pure SRF X pure non-SRF ryegrass). 3. The F_2 generation gives a ratio not significantly different from 3 SRF : 1 non-SRF, suggesting that SRF is inherited as a simple Mendelian dominant trait. 4. The backcross F_1 X pure SRF gives all SRF progeny, and the backcross F_1 X pure non-SRF gives a ratio of approximately 1 SRF : 1 non-SRF. 5. No "genetic association", i.e. trait correlation, was found between SRF and a) the possession of awns, b) shoot vernation, or c) seasonal growth longevity. The nature of the genetic stocks used in these former studies, however, is not clear. Further, Nyquist (1963) only evaluated progeny from perennial X perennial crosses.

Because of the heterogeneous genetic nature of Italian and perennial ryegrasses, it is difficult to find pure line (homozygous) fluorescent and non-fluorescent individuals. However, Nyquist (1963) claimed to have created a 100% fluorescent perennial ryegrass population. There is no molecular test at present that allows an investigator the opportunity to classify, via allele differences, individual plants

according to their fluorescence expression. Because of this, we focused on determining the heritability of the fluorescence trait rather than using segregation analysis.

The main objective of this study was to determine the heritability of SRF among 26 ryegrass half-sib families. A secondary objective was to ascertain if the morphological characters commonly associated with SRF, leaf vernation and reproductive heading without vernalization, change between environments and generations of seed increase.

Materials and Methods

Parental Source Material. Pedigrees of half-sib families, locations of seed increase including cultural procedures followed for each generation of seed multiplication, and seed testing methods were reported earlier (Manuscript 1). Families were allowed to interpollinate within a population structure for three generations at three field locations, but the population structure was not considered in this analysis. The sites of increase were Aurora, Corvallis, and Madras, OR and site characteristics were reported earlier (Manuscript 1). In the previous report, SRF values were presented on a population basis. This present experiment examines SRF values of individual half-sib families. Additionally, morphological and physiological traits including vegetative shoot vernation and reproductive heading without vernalization were evaluated on seedlings grown out after being examined for SRF.

Seeds of each half-sib family from each location X generation combination were evaluated for SRF in each of two replicate runs. Run 1 was initiated October 1994, and Run 2 was initiated December 1994. Twelve to 20 seedlings of each family were established in a greenhouse grow-out test at the end of each run of SRF evaluation. Seedlings were transplanted singly into 3 x 12 cm cone shaped, plastic containers, choosing seedlings randomly from previously described germination/SRF tests (Manuscript 1). Abnormal seedlings from these germination/SRF tests were not considered nor transplanted.

After transplanting, seedlings were placed in a mist chamber located in the greenhouse facility of the USDA-ARS National Forage Seed Production Research

Center, Corvallis, OR. The mist chamber was constructed of clear plexiglass measuring 1.2 x 1.2 x 2.4 m and irrigation water was propelled through a fog-jet nozzle. Fogging occurred every hour for 10 s. Temperature in the chamber was 22°C for 12 h and 13°C for 12 h. Seedlings were cultured in this chamber for 18 d under natural day length and light intensity. In the mist chamber, as well as in the second step of the grow out procedure, plants were fertilized twice weekly with Peter's® liquid fertilizer, 20-20-20 (8% NH_4^+-N, 12% NO_3^- -N+P_2O_5+K_2O), at the dilution rate of 200:1 with tap water. Each plant (within the container racks) received 2 to 3 ml fertilizer solution application^{-1}.

After conditioning seedlings in the mist chamber, young plants were transferred to an open greenhouse growth room in a randomized complete block design with each SRF test run equivalent to a replication. Growth conditions for the second step of the grow-out were comparable to those described by Nittler and Kenny (1972). The room was set at 27°C for 13 h and 16°C for 11 h. Supplemental lighting was supplied by two 175w GE Lucalux® halogen high pressure sodium bulbs that provided a 20 h daylength. Plants were watered from above twice daily. Plants were assessed for leaf vernation as being folded or rolled in the bud 14 d after placement in the open greenhouse (Nittler and Kenny, 1972).

Each of the two grow-out runs (replications) were cultured until two plants flowered (from any family). Once flowers opened and anthers had extruded of these two plants, all other plants in the run were allowed to develop an additional 5 d. After 5 d all progeny expressing a floral spike were noted. Thus, grow-outs ran 34/37 d

beyond removing plantlets from the mist chamber for the two runs, respectively.

Statistical Analysis. Reported results represent the average of the two SRF tests and grow-out runs. The variance component method was used to estimate heritabilities among half-sib families for SRF. Data produced were analyzed using the GLM procedure (SAS Institute, 1994) to conduct an analysis of variance in order to calculate heritabilities among half-sib families at each location. The statistical model considered families and generations as random effects. The expected mean squares used for the calculations of heritabilities are presented in the Appendix.

Heritability for SRF among half-sib families at a location was estimated as:

$$h^2 = \frac{\hat{\sigma}^2_F}{\hat{\sigma}^2_P} = \frac{M_F - M_{FY} - M_{FR} + M_e}{M_F} \qquad [1]$$

where

h^2 = narrow sense heritablity of half-sib family means,

$\hat{\sigma}^2_F$ and $\hat{\sigma}^2_P$ = genotypic and phenotypic variance estimates among half-sib family means, respectively,

M_F = mean square associated with half-sib families,

M_{FY} = mean square associated with the interaction of half-sib families and generation of seed increase,

M_{FR} = mean square associated with the interaction of half-sib families and replications, and

M_e = residual mean square, or mean square error.

Morphological data were subjected to an analysis of variance using the GLM procedure (SAS Institute, 1994). Mean separation was conducted using Fisher's protected least significant difference (LSD).

Results and Discussion

Seedling root fluorescence evaluations. The largest source of variation in the analysis of variance for SRF was among half-sib families, followed by a highly significant mean square for generation of seed increase (Table II-1). These two sources of variation accounted for 93% of the variation associated with SRF. There were also significant family within location, generation X family, and generation X family within location interactions (Table II-1). Family mean SRF over locations ranged from 1.77% to 95.27% (Table II-2). In all but two families, SRF values for families multiplied at Corvallis were numerically greater than the same families increased at the other locations (Table II-2). Levels of SRF were highest for families 43-2, 59-5, 106-4, 106-13, and 112-9. These values were quite similar among locations of seed multiplication. Ranges for SRF expression for other families were larger across locations (Table II-2).

If SRF expression increased over three generations of seed multiplication at a location for a family, SRF for that same family at another location might decrease over generations. This demonstrates a strong G X E interaction for SRF expression as noted earlier for the trait (Manuscript I), and by the large significant mean square value for family with location [F (location)] (Table II-1). It is not obvious what environmental effects could have contributed to the interaction, nor is it evident what plant growth characteristics could lead to such variation. Individual family score data for the last two seasons of increase (the season for which families were increased in rows versus individual clonal material) for the Aurora and Corvallis sites, were either the same or earlier in flowering times, and always taller in mature plant height at the

Table II-1. Mean squares from an analysis of variance for SRF for each of 26 ryegrass half-sib families increased three generations at three locations.

Source	df	Mean Squares
		%
Replication (R)	1	76.93 NS
Family (F)	25	15,788.83**
R x F	25	19.52 NS
F (Location)	52	858.48**
F x R (Location)	52	29.54 NS
Generation (G)	2	2,308.90**
G x F	50	242.61**
G x R	2	31.84 NS
G x F x R	50	24.22 NS
G x F (Location)	104	118.36**
Residual	104	27.62

** Significant at 0.01 probability level.
NS = Not Significant

Table II-2. Mean SRF expression over three seed production generations for 26 ryegrass half-sib families at three locations.

Family	Location			Mean
	Aurora	Corvallis	Madras	
	----------%----------			
94-1	5.50 kl†	49.41 d	30.10 e	28.34
99-1	2.37 lm	30.44 ij	1.50 kl	11.44
142-2	0.38 m	15.72 mn	5.36 jkl	7.15
152-1	0.39 m	19.03 lm	6.34 jkl	8.59
155-6	2.74 lm	27.11 jk	9.99 ij	13.28
171-5	8.49 jk	38.76 fgh	20.76 fg	22.67
176-2	3.31 lm	10.98 no	8.36 jk	7.55
179-8	3.64 lm	27.47 jk	5.59 jkl	12.23
123-3	45.69 e	63.80 c	49.83 d	53.11
152-8	21.63 fg	45.39 de	21.71 fg	29.58
155-2	8.15 gh	41.10 efg	18.00 fgh	25.75
160-4	23.00 f	41.44 ef	16.34 ghi	26.93
168-5	13.80 hi	30.97 ij	17.28 fgh	20.68
176-1	12.36 ij	34.88 hi	15.44 ghi	20.89
179-2	14.32 hi	35.03 ghi	23.39 ef	24.25
43-2	70.31 d	81.19 b	84.43 b	78.64
59-5	88.31 b	94.00 a	91.15 ab	91.15
106-4	85.79 b	90.37 a	97.46 ab	88.54
106-13	75.95 c	79.92 b	72.06 c	75.98
112-9	95.09 a	95.57 a	95.14 a	95.27
228	4.92 kl	23.41 kl	11.00 hij	13.11
4867	0.00 m	5.31 o	0.00 l	1.77
4948	0.26 m	14.57 mn	0.00 l	4.94
5149	0.00 m	34.89 hi	0.00 l	11.63
5182	0.27 m	31.07 ij	4.04 jkl	11.79
5304	0.26 m	32.57 ij	0.00 l	10.94

† Means followed by the same letter within a column are not significantly different at $P \leq 0.05$.

Corvallis location relative to the Aurora site (Appendix). Generally, earlier flowering in ryegrass positively correlates with higher seed yield (under non-irrigated western Willamette Valley conditions) than ryegrass of later flowering. This is because the earlier types take advantage of stored soil water reserves before the onset of drought symptoms. Further, earlier maturing ryegrass often escapes the effects of stem rust (caused by *Puccinia graminis* Pers.) which can be highly detrimental to seed fill of late flowering accessions.

Thus, because of subtle differences in climatic conditions (Appendix) over the term of the experiment, especially for the two western Valley sites, and soil variation among locations, half-sib families may have responded differently in morphological growth characteristics. More often than not, seed yield (again, for the last two generations of seed multiplication from the two western Valley locations) among half-sib families was numerically greater for those families increased at Corvallis (Appendix). The increase in seed yields at Corvallis versus Aurora is partly the result of 1000 seed weights which, when harvested from half-sib families at Corvallis, were never less than those from half-sib families increased at Aurora, and were often higher (Appendix).

The above comments on plant growth characteristics and site climatic conditions do not explain why SRF expression for half-sib families would be higher for those increased at one location relative to another, but may indicate that ryegrass accessions increased at locations most conducive to optimal growth conditions for the grass plants themselves have a greater propensity to express the SRF trait. This effect

was reported previously for other plant species in regard to alkaloid production and accumulation (Robinson, 1979; Levin and York, 1978). Obviously, more research would be needed to specifically address this hypothesis in ryegrass.

Heritability estimates for SRF calculated using mean squares from Table II-4, were high (Table II-3), and indicated that SRF can be altered by selection, either naturally or artificially. Heritability is defined as the portion of phenotypic variation among individuals that is due to genetic differences. Total genetic variance can be sub-divided into additive genetic variance, dominance genetic variance, and epistatic variance (Dudley and Moll, 1969). Consequently, h^2 gives an indication to what extent variability in SRF among individuals in a population is due to environmental conditions. If genetic variation among progeny lines is large relative to environmental variation then heritability is high, and selection is more effective (Poehlman, 1979). The genotype X environment interaction variance is that part of the phenotypic variance attributable to the failure of differences between genotypes to be the same in different environments.

Heritability values for given traits are not absolute. They must be interpreted with caution considering the environmental conditions in which they were obtained and the genetic populations sampled. For example, the genetic variance among half-sib families within the current study could have been increased simply by a different choice of source parents, or a different family increase scheme, because the parents were purposely chosen to represent a very broad range of SRF.

Table II-3. Heritability estimates for SRF among 26 ryegrass half-sib families increased three generations at three locations.

Location	Heritability Estimate (h^2)
Aurora, OR	99
Corvallis, OR	97
Madras, OR	98

Table II-4. Mean squares from an analysis of variance for SRF for 26 ryegrass half-sib families increased three generations at three locations.

Source	df	Location		
		Aurora	Corvallis	Madras
		Mean squares		
		----------%----------		
Replication (R)	1	5.50 NS	76.89 NS	76.90 NS
Family (F)	25	6,115.01**	4,106.57**	6,083.20**
R x F	25	18.85 NS	28.39 NS	30.41 NS
Generation (G)	2	53.42*	4,928.29**	35.10 NS
F x G	50	67.66**	149.45**	163.37**
G x R	2	19.54 NS	70.25 NS	11.92 NS
Residual	50	14.56	1,381.55	36.67

*, ** Significant at 0.05 and 0.01 probability levels, respectively.
NS = Not Significant

When considering the overall ANOVA for each family X location combination over all three generations of increase, family was the largest source of variation in two of the three combinations, i.e. Aurora and Madras. Family was also a highly significant source of variation at the Corvallis site, but it was not as large as variation due to the main effect of generation. These data indicate that most of the phenotypic variation in SRF was genetic (Table II-4). The SRF trait varied significantly among the generations of family seed increase at two of three locations. Further, there was a highly significant interaction of family X generation. Thus, it appeared that SRF expression for a family also depended upon the generation of seed increase at each location.

Reliable comparisons of heritabilities between populations (or families) require rather large samples of progeny from each population being compared (Dudley and Moll, 1969). This was not necessarily the case for the number of progeny seedlings tested for SRF in these studies. While number of seedlings tested on a constructed population basis may have been sufficiently large (Manuscript 1), it was relatively small for individual half-sib families in this study, and genetic drift could have occurred.

Morphological trait evaluations. Means for frequency of plants with folded leaf vernation and reproductive heading ranged from 0 to 99% and 0 to 74%, respectively (Table II-5, II-6). These data were generated from the greenhouse grow-out of individual plantlets from families increased within the three locations.

Interpreting the mean squares for the two morphological traits is difficult since there was considerable variation associated with replication (Table II-7, II-8). Replication was accomplished by time (runs). Replication within runs may have shown

Table II-5. Mean leaf vernation expression (% folded) over three seed production generations for progeny of 26 ryegrass half-sib families at three locations when reared in a greenhouse environment.

	Location			
Family	Aurora	Corvallis	Madras	Mean
	----------%----------			
94-1	97 a†	100 a	100 a	99
99-1	100 a	93 bcd	100 a	98
142-2	94 a	100 a	100 a	98
152-1	98 a	97 abc	100 a	98
155-6	98 a	97 abc	100 a	98
171-5	100 a	91 cd	100 a	97
176-2	97 a	97 abc	100 a	98
179-8	97 a	98 ab	100 a	98
123-3	100 a	98 ab	100 a	99
152-8	100 a	89 d	99 a	96
155-2	100 a	95 abcd	100 a	98
160-4	100 a	93 abcd	100 a	98
168-5	100 a	94 abcd	100 a	98
176-1	100 a	94 abcd	100 a	98
179-2	97 a	100 a	100 a	99
43-2	27 bc	6 ef	47 b	27
59-5	21 c	5 ef	28 cd	18
106-4	26 bc	9 e	41 b	25
106-13	37 b	8 e	38 bc	28
112-9	26 bc	2 ef	26 de	18
228	22 c	8 e	17 ef	16
4867	0 d	0 f	1 g	0
4948	1 d	0 f	8 fg	3
5149	2 d	0 f	2 g	1
5182	1 d	0 f	1 g	1
5304	2 d	0 f	1 g	1

† Means followed by the same letter within a column are not significantly different at $P \leq 0.05$.

Table II-6. Mean reproductive heading percentage over three seed production generations for progeny of 26 ryegrass half-sib families at three locations when reared in a greenhouse environment.

	Location			
Family	Aurora	Corvallis	Madras	Mean
	----------%----------			
94-1	2 e†	2 g	0 f	1
99-1	0 e	3 g	0 f	1
142-2	0 e	7 g	0 f	2
152-1	0 e	2 g	0 f	1
155-6	0 e	0 g	0 f	0
171-5	0 e	9 fg	3 f	4
176-2	0 e	2 g	0 f	1
179-8	0 e	7 g	0 f	2
123-3	0 e	0 g	0 f	0
152-8	0 e	4 g	0 f	1
155-2	0 e	3 g	0 f	1
160-4	0 e	9 fg	0 f	3
168-5	0 e	3 g	0 f	1
176-1	0 e	0 g	0 f	0
179-2	0 e	4 g	0 f	1
43-2	0 e	29 de	0 f	10
59-5	3 e	25 e	2 f	10
106-4	1 e	28 e	1 f	10
106-13	3 e	27 e	3 f	11
112-9	0 e	23 ef	3 f	9
228	38 d	43 cd	29 e	37
4867	59 c	66 a	68 b	64
4948	59 c	49 bc	51 d	53
5149	73 b	58 ab	58 c	63
5182	81 a	50 bc	92 a	74
5304	76 ab	50 bc	54 cd	60

† Means followed by the same letter within a column are not significantly different at $P \leq 0.05$.

Table II-7. Mean squares from an analysis of variance for expression of folded leaf vernation from a greenhouse grow-out for 26 ryegrass half-sib families increased three generations at three locations.

Source	df	Location		
		Aurora	Corvallis	Madras
		Mean squares		
		----------%----------		
Replication (R)	1	3,510.26**	318.78**	1,000.16**
Family (F)	25	11,090.11**	13,044.37**	10,696.69**
R x F	25	338.08**	76.46**	190.19**
Generation (G)	2	333.81 NS	323.56**	1,155.71**
F x G	50	85.97 NS	38.64 NS	271.18**
G x R	2	809.78**	106.49 NS	264.58 NS
Residual	50	125.22	39.99	89.01

*, ** Significant at 0.05 and 0.01 probability levels, respectively.
NS = Not Significant

Table II-8. Mean squares from an analysis of variance for expression of reproductive heading from a greenhouse grow-out for 26 ryegrass half-sib families increased three generations at three locations.

Source	df	Location		
		Aurora	Corvallis	Madras
		Mean squares		
		----------%----------		
Replication (R)	1	18.01 NS	2,172.31**	7.85 NS
Family (F)	25	4,242.71**	2,683.14**	4,813.87**
R x F	25	61.57**	240.35*	27.32 NS
Generation (G)	2	1,236.52**	24.92**	69.89 NS
F x G	50	216.85**	315.47**	125.72**
G x R	2	32.81 NS	185.62 NS	18.70 NS
Residual	50	29.33	136.60	32.45

*, ** Significant at 0.05 and 0.01 probability levels, respectively.
NS = Not Significant

a different result for the significant variation in this main effect. Morphological growth comparisons can be greatly influenced by the time of year when tests are conducted because of day length alone. Regardless, variation among families remained the largest source of variation across all locations for each of the two traits. A significant interaction was shown in most instances for replication X family. Variability associated with replication and its interaction showed the high influence of growing condition when measuring these morphological traits. Altering heading response would be expected from differing day lengths, but it was not expected to affect leaf vernation. The leaf vernation response, however, reflects the difficulty of scoring this trait. There appeared to be a great variation in folded leaf vernation at the Corvallis and Madras sites among generations of family seed increase. This variability was similar to heading expression without vernalization treatment for families increased at the Aurora and Corvallis sites. Significant family X generation interaction was shown for heading at all three locations.

It was evident that SRF is not necessarily associated with other morphological traits normally considered to be exclusive to Italian ryegrass, e.g. rolled leaf vernation and heading without vernalization. If an association was the case, it would be expected that as SRF values increased, folded leaf vernation would have decreased, along with an increase of heading without a vernalization.

Ryegrass leaf vernation is controlled by multiple genes, and gene expression can be altered by environmental factors during seed production increase (Rebischung, 1951). Most likely reproductive heading would be as genetically complicated, or more

so. Families increased at Corvallis had higher incidence of rolled leaves and precocious heading of individuals than families increased at Aurora and Madras. It is unknown if particular environments provide a selective advantage based on heading or vernation for genotypes that are anatomically similar to true Italian ryegrass.

Breese and Hayward (1972) argued that longer lived (perennial) ryegrass genotypes are capable of asexual reproduction (vegetative growth and regrowth) in addition to normal sexual reproduction. At the other extreme, however, the shorter lived (Italian) ryegrass emphasizes almost exclusively sexual reproduction. The importance of vegetative growth was not a consideration in this research because family multiplication was not uniformly applicable and seed was not increased more than one year for each generation. Each cycle of increase was accomplished by sowing a new generation of progeny seeds annually and there may have been a selective advantage for vigorous tillering genotypes immediately following each sowing event, but not from vegetative spread of a plant. Genotypes that produced a larger number of tillers, however, could have been more competitive, and capable of producing more reproductive units (spikes) and consequently more pollen than the more vegetative genotypes. Larger numbers of tillers would increase the frequency of annual or Italian ryegrass growth forms during succeeding generations of regrowth.

The results of this study demonstrate high levels of heritability for SRF. Thus, for purposes of maintaining low to nil levels of SRF in cultivar development of perennial ryegrasses, the plant breeder needs to practice selection against the trait during cultivar development and during the maintenance of foundation seed supplies.

High heritability of SRF, and variation associated with other morphological traits demonstrated that plant breeders should be able to control the expression of SRF in ryegrass cultivars, particularly perennial ryegrasses cultivars. Selection for or against rolled leaf vernation and reproductive heading without vernalization is not likely to affect the level of SRF expression in cultivar development. These traits appear to be independently expressed relative to SRF, affected by environmental conditions of seed increase, and especially leaf vernation, are difficult to measure on a subjective basis. These traits along with SRF may not be the best discriminators of the two species. Other traits should be investigated which would more accurately separate ryegrass kinds and allelic frequency differences within cultivars.

References

Axelrod, B., and J.R. Belzile. 1958. Isolation of an alkaloid, annuloline, from the roots of *Lolium multiflorum*. J. Organ. Chem. 23:919-920.

Barker, R.E., and R.R. Kalton. 1989. Cool season forage grass breeding : progress, potentials, and benefits. p. 5-20. *In* D.A. Sleper, K.H. Asay, and J.F. Pedersen (ed.). Contributions from breeding forage and turfgrasses. CSSA Spec. Publ. 15. CSSA, Madison, WI.

Breese, E.L., and M.D. Hayward. 1972. The genetic basis of present breeding methods in forage crops. Euphytica 21:324-336.

Cooper, J.P., and D.M. Calder. 1964. The inductive requirements for flowering of temperate grasses. J. Br. Grassl. Soc. 19:6-14.

Corkill, L. 1932. Inheritance of fluorescence in rye-grass. Nature (London) 130:134.

Dudley, J.W., and R.H. Moll. 1969. Interpretation and use of estimates of heritability and genetic variances in plant breeding. Crop Sci. 9:257-267.

Floyd, D.J. 1997. Ryegrass for turf (*Lolium* spp.). *In* R.E. Barker (ed.) Grass germplasm in the USA: a status report. U.S. Crop Germplasm Committee for Forage and Turf Grasses. USDA-ARS-NFSPRC, Corvallis, OR.

Frey, K.J. 1996. National Plant Breeding Study-1. p. 38. Spec. Rep. 98. Iowa State Univ., Ames, IA.

Gentner, G. 1929. Über die verwendbarkeit von ultra-violetten strahlen bei der samenprüfung. Praktische Blätter für Pflanzenbau und Pflanzenschutz. 6:166-172.

Hubbard, C.E. 1968. Grasses. 2nd ed. Penguin Books, England.

Jenkin, T.J. 1924. The artificial hybridization of grasses. Welsh Plant Breeding Stn. Bull. Ser. H, no. 2.

Jenkin, T.J. 1927. Self and cross-fertilisation in *Lolium perenne* L. J. Genet. 17:11-17.

Levin, D., and B.M. York, Jr. 1978. The toxicity of plant alkaloids: an ecogeographic perspective. Biochem. Syst. Ecol. 6:61-76.

Linehan, P.A., and S.P. Mercer. 1933. Fluorescence of *Lolium* seedlings in ultra-violet light. Nature (London) 131:202-203.

McCraw, J.M., and W. Spoor. 1983a. Self incompatibility in *Lolium* species. 1. *Lolium rigidum* Gaud. and *L. multiflorum* L. Heredity 50:21-27.

McCraw, J.M., and W. Spoor. 1983b. Self-incompatibility in *Lolium* species. 2. *Lolium perenne* L. Heredity 50:29-33.

Meyer, W.A., and C.R. Funk. 1989. Progress and benefits to humanity from breeding cool-season grasses for turf. p. 31-48. *In* D.A. Sleper, K.H. Asay, and J.F. Pedersen (ed.). Contributions from breeding forage and turf grasses. CSSA Spec. Publ. 15. CSSA, Madison, WI.

Nittler, L.W., and T.J. Kenny. 1972. Distinguishing annual from perennial ryegrass. Agron. J. 64:767-768.

Nitzsche, V.W. 1960. Über die inkonstanz der fluoreszenz bei weidelgräsern. Zeitschrift für Acker und Pflanzenbau. 110:267-288.

Nitzsche, V.W. 1966. Die vererbung der fluoreszenz bei weidelgras (*Lolium* spp.). Zeitschrift für Pflanzenzücht. 56:88-95.

Nyquist, W.E. 1963. Fluorescent perennial ryegrass. Crop Sci. 3:223-226.

Poehlman, J.M. 1979. Breeding field crops. 2nd ed. AVI Publ. Co., Inc., Westport, CT.

Rampton, H.H. 1938. The use of morphological characters as compared with fluorescence tests with ultra-violet light in classifying the ryegrasses (*Lolium* spp.) of western Oregon. J. Amer. Soc. Agron. 30:915-922.

Rebischung, J. 1951. Étude de populations de ray-grass et du mode de transmission de deux caractères dans le genre *Lolium*. Annales Institut National Recherche Agronomique, Series B. 1:497-547.

Riewe, M.E., and C.L. Mondart, Jr. 1985. The ryegrasses. p. 241-246. *In* M.E. Heath, R.F. Barnes, D.S. Metcalf (eds.). Forages, the science of grassland agriculture. 4th ed. Iowa State Univ. Press, Ames, IA. Chapt 26.

Robinson, T. 1979. The evolutionary ecology of alkaloids. p. 413-448. *In* G.A. Rosenthal and D.H Janzen (eds.) Herbivores: their interaction with secondary plant metabolites. Academic Press, Inc. New York, NY.

SAS Institute. 1994. SAS/STAT guide for personal computers. Version 6.10 ed. SAS Inst., Inc., Cary, NC.

Terrell, E.E. 1968. A taxonomic revision of the genus *Lolium*. USDA Tech. Bul. 1392. U.S. Gov. Print. Office, Washington, DC.

Woodforde, A.H. 1935. The inheritance of a substance in the roots of seedling hybrid derivatives of *Lolium perenne* L. x *Lolium multiflorum* Lam., causing a fluorescence reaction visible in filter paper by screened ultra-violet light. J. Linnean Soc. London 50:141-150.

SUMMARY AND CONCLUSIONS

The seedling root fluorescence (SRF) test has long been used as a determinate for ryegrass seed marketing in the USA. Generally, seed lots of perennial ryegrass that have a level of SRF above zero are considered contaminated with Italian ryegrass. Since the growth habit and foliage color/texture of the two ryegrass species are vastly different, admixtures of the two are not acceptable in turfgrass markets.

More recently, SRF has been viewed as an exact test measurement for which plant breeders are allowed to describe their cultivars. If a perennial ryegrass cultivar expresses a level of fluorescence above zero, and the level is not attributable to any physical or genetic contamination of Italian ryegrass, a breeder can establish an inherent value of cultivar SRF. Under current USA seed labeling law, this documented value is viewed as stable during seed production.

Leaf vernation and heading without vernalization are two other traits used to separate the two ryegrass species. In the strict sense, Italian ryegrass expresses rolled leaf vernation when emerging from tiller buds, while the vernation of perennial ryegrass is folded. Genotypes of Italian ryegrass usually do not need to pass through a vernalization treatment before heading. On the other hand, perennial ryegrass individuals generally need to be vernalized before heading.

Research was conducted in western and central Oregon to determine the fate of SRF expression in four ryegrass populations increased for three generations at three locations. The two largest sources of variation for SRF were among populations and

among locations of seed increase. The study showed the existence of a significant genotype X environment interaction among populations for SRF. It is possible that changing the location of seed increase could provide a selective advantage for a preponderance of SRF genotypes from within ryegrass populations, but this response was not tested in these studies. Future research focused on SRF should include thorough response classification of floral induction/initiation requirements, plus degree of self fertility within populations. Complete characterization of soil properties among sites of increase, and detailed weather data during flowering and seed maturation periods could help quantify location differences.

Heritability of SRF was investigated for individual half-sib families across three seed multiplication environments without grouping in the population structure. Leaf vernation and reproductive heading were also measured in a greenhouse grow-out from field-grown progeny of each family.

Heritability of SRF on a half-sib family basis was high, ranging from 97 to 99% among the three locations of seed multiplication, indicating little environmental influence for SRF on a family mean basis. This is in agreement to earlier work reported in literature that showed SRF is controlled by a single gene, leading to the belief that environmental conditions would not alter trait expression. Further investigation of the data presented herein, however, showed significant location and generation effects on SRF as well as interactions with families. Even with high heritability, there was also large environmental influence on the trait.

Half-sib family seedlings were reared in a greenhouse and evaluated for leaf vernation and heading without vernalization. There was significant effect for the expression of leaf vernation among family lines. As scores were analyzed over the three increase generations across three environments, families with seed multiplied at two of three sites had a significant generation effect for leaf vernation expression. Further, as heading percentage without vernalization was measured and analyzed using the same statistical model, a significant main effect was found for families. A significant generation effect for heading without vernalization was indicated for two of the three locations, and a significant family X generation interaction at all three sites for heading was shown.

Results of this research once again raise questions about the value of SRF as a seed test used for marketing ryegrass seed in the USA. The evidence presented showed significant genotype X environment interaction for SRF as ryegrass populations are multiplied in seed production areas. The trait appears to be simply inherited on an individual half-sib family basis, but expression of the trait was different among three locations of seed increase. These results, coupled with voluminous data which have shown existence of laboratory, analyst evaluation, and seasonal variation of the test conductance itself, should easily show the fallacy of relying on SRF for marketing purposes. If the test is not eliminated, certainly interpretation and application should be modified. The test could be strengthened if biological tolerances were adapted and applied to test values. Utilization of seed production field

observations which notes the frequency of "off-type" ryegrasses being expressed could reinforce higher than expected SRF test values of seed lots.

My opinion is that the SRF test should only continue to be used as it was originally intended, that is as a measure of general ryegrass seed lot integrity. The test is not an absolute characteristic, and seed marketers should stop applying it in an exacting manner. As long as the Federal Seed Act allows plant breeders the right to describe a cultivar's inherent fluorescence value, the test will continue to be used as a definitive cultivar descriptive trait. Limitations of the test need to be recognized and considered as test results are interpreted.

The following recommendations are made:

1. Plant breeders should maintain seed lot sampling over a large geographic region when establishing inherent fluorescence values on new ryegrass cultivars. Cultivars should be described as having a mean fluorescence expression with a measure of biological variability associated with that mean.

2. A conservative view of the SRF test should be taken, and it should only be used for assessing general discrepancies in ryegrass seed lot integrity. Seed labeling laws need to be altered to put more reliance on seed field inspections or at least providing for seedling grow-outs to uncover "off-type" contamination in questionable seed lots identified through SRF tests. Re-examination of the proper interpretation and application of SRF testing in the seed trade is sorely needed.

3. Finally, the grass seed industry of the USA should continue to support the development of DNA based, or other alternative tests, which can supplement or replace SRF testing requirements.

BIBLIOGRAPHY

Ahloowalia, B.S. 1981. Use of partially male-sterile perennial ryegrass for hybrid cultivar production. Crop Sci. 21:415-418.

Arcioni, S., and D. Mariotti. 1983. Selfing and interspecific hybridization in *Lolium perenne* L. and *Lolium multiflorum* Lam. evaluated by phosphoglucoisomerase as isozyme marker. Euphytica 32:33-40.

Association of Official Seed Analysts. 1941. Report of editor of handbook. p. 9. *In* M.T. Munn (ed.). Proc. Assoc. Off. Seed Anal. Ames, IA. 8-11 Jul.

Association of Official Seed Analysts. 1944. Examinations. p. 25. *In* M.T. Munn (ed.). Proc. Assoc. Off. Seed Anal. Columbus, OH. 17-21 Jul.

Association of Official Seed Analysts. 1988. Progress report on the AOSA cultivar purity testing handbook. p. 20-21. AOSA, Lincoln, NE.

Association of Official Seed Analysts. 1994. Rules for testing seeds. J. Seed Technol. 16:14-15, 19.

Association of Official Seed Analysts. 1998a. Cultivar purity and testing handbook. AOSA. Beltsville, MD.

Association of Official Seed Analysts. 1998b. Rules for testing seeds. AOSA. Beltsville, MD.

Association of Official Seed Analysts, and Society of Commercial Seed Technologists. 1998. The seed technologist newsletter. p. 88. AOSA. Beltsville, MD.

Association of Official Seed Certifying Agencies. 1990. Annual report of the AOSCA. p. 128-29. Richmond, VA.

Association of Official Seed Certifying Agencies. 1991. Annual report of the AOSCA. p. 126-127. Salt Lake City, UT.

Association of Official Seed Certifying Agencies. 1999. A report of the National Grass Variety Review Board. Varietal Publ. 181. AOSCA. Meridian, ID.

Axelrod, B., and J.R. Belzile. 1958. Isolation of an alkaloid, annuloline, from the roots of *Lolium multiflorum*. J. Organ. Chem. 23:919-920.

Baekgaard, H.C. 1962. Continued examinations of the content of fluorescent seeds in Danish varieties of perennial ryegrass (*Lolium perenne*). Proc. Int. Seed Test. Assoc. 27:562-572.

Barker, R.E., R.L. Cook, and D.J. Floyd. 1997. Variation of seedling root fluorescence in perennial ryegrass. p. 133. *In* Agronomy abstracts. ASA, Madison, WI.

Barker, R.E., and R.R. Kalton. 1989. Cool season forage grass breeding: progress, potentials, and benefits. p. 5-20. *In* D.A. Sleper, K.H. Asay, and J.F. Pedersen (ed.). Contributions from breeding forage and turfgrasses. CSSA Spec. Publ. 15. CSSA, Madison, WI.

Barker, R.E., S.K. Davidson, R.L. Cook, J.B. Burr, L.A. Brilman, M.J. McCarthy, A.E. Guray, and W.D. Brown. 2000. Hidden fluorescence in the seedling root fluorescence test of ryegrass. Seed Technol. 22: 15-22.

Barnes, R.F., and J.B. Beard (ed.). 1992. A glossary of crop science terms. CSSA, Madison, WI.

Beddows, A.R., E.L. Breese, and B. Lewis. 1962. The genetic assessment of heterozygous breeding material by means of a diallel cross. I. Description of parents, self-and cross-fertility and early seedling vigour. Heredity 17:501-512.

Breakwell, E. 1918. Popular descriptions of grasses: The rye or *Lolium* grasses. Agric. Gaz. N.S. Wales 29:274-281.

Breese, E.L., and M.D. Hayward. 1972. The genetic basis of present breeding methods in forage crops. Euphytica 21:324-336.

Clark, B.E. 1980. Ryegrass fluorescence committee. J. Seed Technol. 5:91-93.

Colbry, V.L. 1963. Testing annual (Italian) and perennial ryegrass seed. AOSA Newslet. 37:20-27.

Cook, R.L. 1980. Effect of lot size on lot uniformity in *Lolium* spp. seed. M.S. thesis. Oregon State Univ. Corvallis, OR.

Cooper, J.P. 1959. The stability of S23 perennial ryegrass during seed multiplication. 1. Flowering behavior and early seedling growth. J. Br. Grassld. Soc. 14:183-190.

Cooper, J.P., and D.M. Calder. 1964. The inductive requirements for flowering of temperate grasses. J. Br. Grassl. Soc. 19:6-14.

Copeland, L.O. 1966. Confidence limits for fluorescence tests of ryegrass (*Lolium* sp.). M.S. thesis. Oregon State Univ. Corvallis, OR.

Copeland, L.O., and E.E. Hardin. 1967. Evaluation of techniques for testing fluorescence of annual ryegrass (*Lolium multiflorum* Lam.) seedlings. Proc. Assoc. Off. Seed Anal. 57:112-116.

Copeland, L.O., and E.E. Hardin. 1970. Outcrossing in the ryegrasses (*Lolium* spp.) as determined by fluorescence tests. Crop Sci. 10:254-257.

Corkill, L. 1932. Inheritance of fluorescence in rye-grass. Nature (London) 130:134.

Corkill, L. 1949. Pasture improvement in New Zealand. Empire J. Exper. Agric. 17:157-169.

Cornish, M.A., M.D. Hayward, and M.J. Lawrence. 1980. Self-incompatibility in ryegrass. IV. Seed set in diploid *Lolium perenne* L. Heredity 44:333-340.

Dawson, R.F. 1948. Alkaloid biosynthesis. p. 203-251. *In* F.F. Nord (ed.). Advances in enzymology and related subjects of biochemistry. Vol. 8. Interscience Publ., New York, NY.

Delannay, X., and R.G. Palmer. 1982. Four genes controlling root fluorescence in soybean. Crop Sci. 22:278-281.

Dudley, J.W., and R.H. Moll. 1969. Interpretation and use of estimates of heritability and genetic variances in plant breeding. Crop Sci. 9:257-267.

Easton, H.S. 1983. Ryegrasses. p. 229-236. *In* G.S. Wratt and H.C. Smith (ed.). Plant breeding in New Zealand. Butterworths of NZ, Wellington, NZ.

Falconer, D.S., and T.F.C. MacKay. 1996. Introduction to quantitative genetics. 4th ed. Longman Group, Ltd, Essex, England.

Ferguson, J.M. 1984. SDS-PAGE of seed proteins for identification of varieties and species of ryegrass (*Lolium* spp.). M.S. thesis. Oregon State Univ., Corvallis, OR.

Ferguson, J.M., and D.F. Grabe. 1984. Separation of annual and perennial species of ryegrass by gel electrophoresis of seed proteins. J. Seed Technol. 9:137-149.

Finkner, R.E., H.C. Murphy, R.E. Atkins, and D.W. West. 1954. Varietal reaction and inheritance of fluorescence in oats. Agron. J. 46:270-274.

Floyd, D.J. 1997. Ryegrass for turf (*Lolium* spp.). *In* R.E. Barker (ed.) Grass germplasm in the USA: a status report. U.S. Crop Germplasm Committee for Forage and Turf Grasses. USDA-ARS-NFSPRC, Corvallis, OR.

Foster, C.A., and C.E. Wright. 1970. Variation in the expression of self-fertility in *Lolium perenne* L. Euphytica 19:61-70.

Foy, N.R. 1931. Use of filtered ultra-violet light in diagnosis of the various types of rye-grass in New Zealand. N.Z. J. Agric. 43:389-400.

Frey, K.J. 1996. National Plant Breeding Study-1. p. 38. Spec. Rep. 98. Iowa State Univ., Ames, IA.

Garbacik, C., and D.F. Grabe. 1991. Fluorescence test in ryegrass. p. 38-39. *In* Young, W.C. III (ed.) 1991 Seed Production Research. Dep. of Crop and Soil Science Ext/CrS89, 4/92. Oregon State University, Corvallis, OR.

Gentner, G. 1928. Die verwendbarkeit von ultravioletten stahlen bei der samenprufung. Angew. Botanik. 10:471-472.

Gentner, G. 1929. Über die verwendbarkeit von ultra-violetten strahlen bei der samenprüfung. Praktische Blätter für Pflanzenbau und Pflanzenschutz. 6:166-172.

Goodwin, R.H., and F. Kavanagh. 1948. Fluorescing substance in roots. Bull. Torrey Club 75:1-7.

Grabe, D.F. 1998. Leaf vernation test for perennial ryegrass. Seed Technol. 20:85-90.

Griffith, S.M., and G.M. Banowetz. 1992. Differentiation of *Lolium perenne* L. and *L. multiflorum* Lam. seed by two esterase isoforms. Seed Sci. Technol. 20:343-348.

Griffith, S.M., and G.M Banowetz. 1994. Development of ryegrass identification methodology to replace fluorescence test. p. 38-39. *In* Young, W.C. III (ed.). 1994 Seed Production Research. Dep. of Crop and Soil Science Ext/CrS102,4/95. Oregon State University, Corvallis, OR.

Griffiths, D.J. 1951. The liability of seed crops of perennial ryegrass (*Lolium perenne*) to contamination by wind-borne pollen. J. Agric. Sci. 40:19-38.

Harrison, C.S. 1954. The technique of ultra-violet testing in New Zealand. Proc. Int. Seed Test. Assoc. 19:44-49.

Hayward, M.D., and A.J. Wright. 1971. The genetic control of incompatibility in *Lolium perenne* L. Genetica 42:414-421.

Hayward, M.D., and N.J. McAdam. 1977. Isoenzyme polymorphism as a measure of distinctiveness and stability in cultivars of *Lolium perenne*. Pflanzenzuchtg. 79:59-68.

Hubbard, C.E. 1968. Grasses. 2nd ed. Penguin Books, England.

Humphries, C.J. 1980. *Lolium*. p. 153-154. *In* Flora Europea. Vol. 5, ed. T.G. Tutin, et al. Cambridge Univ. Press.

International Seed Testing Association. 1966. International rules for testing seed. 1967. 31:1-300.

Jenkin, T.J. 1924. The artificial hybridization of grasses. Welsh Plant Breeding Stn. Bull. Ser. H no. 2.

Jenkin, T.J. 1927. Self and cross-fertilisation in *Lolium perenne* L. J. Genet. 17:11-17.

Jenkin, T.J. 1931. Self fertility in perennial rye-grass (*Lolium perenne* L.). Bull. Welsh Pl. Breed. Sta. Serv. H. 12:100-119.

Jenkin, T.J. 1955. Interspecific and intergeneric hybrids in herbage grasses XVII. Further crosses involving *L. perenne*. J. Genet. 53:442-466.

Jenkin, T.J., and P.T. Thomas. 1938. The breeding affinities and cytology of *Lolium* species. Tour of Botany 24:10-12.

Jepson, W.L. 1925. Manual of the flowering plants of California. Univ. Of California, Berkeley: Assoc. Students Store.

Johnson, R.C., V.L. Bradley, and R.P. Knowles. 1996. Genetic contamination by windborne pollen in germplasm-regeneration plots of smooth bromegrass. Plant Genetic Res. Newsletter 106:30-34.

Jones, R.N., and P. Jenabzadeh. 1981. Variation in self-fertility, flowering time and inflorescence production in inbred *Lolium perenne* L. J. Agric. Sci. 96:521-537.

Jones, T.W. 1983. Effects of temperature of seed set upon isoenzymes and fluorescence in tetraploid hybrid ryegrasses. Z. Pflanzenzüchtg. 90:136-144.

Jung, G.A., A.J.P. VanWijk, W.F. Hunt, and C.E. Watson. 1996. Ryegrasses. p. 605-641. *In* L.E. Moser, D.R. Buxton, and M.D. Casler (ed.). Cool-season forage grasses. Agron. Monogr. 34. ASA, Madison, WI.

Justice, O.L. 1949. A comparison of some ultraviolet lamps in testing ryegrass for fluorescence. p. 89-42. *In* M.T. Munn (ed.). Proc. Assoc. Off. Seed Anal. Corvallis, OR. 27 Jun.-1 Jul.

Karimoto, R.S., B. Axelrod, J. Wolinsky, and E.D. Schall. 1964. The structure and synthesis of annuloline, an oxazole alkaloid occurring in annual rye grass. Phytochemistry 3:349-355.

Kloot, P.M. 1983. The genus *Lolium* in Australia. Austral. J. Bot. 31:421-435.

Knowles, R.P., and A.N. Ghosh. 1968. Isolation requirements for smooth bromegrass, *Bromus inermis* Leyss., as determined by a genetic marker. Agron. J. 60:371-374.

Laroze, A., and J. Alves da Silva. 1952. The base-exchange potency of alkaloids. Congr. Luso-Espan. farm. 2 Porto. 267-269.

Larsen, A.L. 1966. A distinction between proteins of annual and perennial ryegrass seeds. Proc. Assoc. Off. Seed Anal. 56:47-51.

Leggatt, C.W. 1939. Statistical aspects of seed analysis. Botan. Rev. 5:505-529.

Levin, D., and B.M. York, Jr. 1978. The toxicity of plant alkaloids: an ecogeographic perspective. Biochem. Syst. Ecol. 6:61-76.

Levy, E.B., and W. Davies. 1930. Perennial ryegrass strain investigation. Single plant studies at the plant research station. N.Z. J. Agric. 41:147-163.

Linehan, P.A., and S.P. Mercer. 1933. Fluorescence of *Lolium* seedlings in ultra-violet light. Nature (London) 131:202-203.

Loos, B.P. 1993. Morphological variation in *Lolium* (Poaceae) as a measure of species relationships. Pl. Syst. Evol. 188:87-99.

Loos, B.P. 1994. The genus *Lolium* taxonomy and genetic resources. PhD diss. Wageningen: CPRO-DLO. Wageningen, Netherlands.

McCraw, J.M., and W. Spoor. 1983a. Self incompatibility in *Lolium* species. 1. *Lolium rigidum* Gaud. and *L. multiflorum* L. Heredity 50:21-27.

McCraw, J.M., and W. Spoor. 1983b. Self-incompatibility in *Lolium* species. 2. *Lolium perenne* L. Heredity 50:29-33.

McVeigh, K.J. 1975. Breeding for resistance to crown rust (*Puccina coronata corda* var. *lolii* Brown) in turf-type perennial ryegrass (*Lolium perenne* L.). PhD. diss. Rutgers, The State Univ. of NJ, New Brunswick. (Diss. Abstr. AAG7517460).

Meyer, W.A., and C.R. Funk. 1989. Progress and benefits to humanity from breeding cool-season grasses for turf. p. 31-48. *In* D.A. Sleper, K.H. Asay, and J.F. Pedersen (ed.). Contributions from breeding forage and turf grasses. CSSA Spec. Publ. 15. CSSA, Madison, WI.

Morris, K.N., and G. Gao. 1997. U.S. turfgrass variety list, 1997. NTEP no. 97-1. Natl. Turfgrass Fed., Inc. and Beltsville Ag. Res. Ctr., Beltsville, MD.

Munn, M.T. 1935. Under what conditions is the ultra-violet light reliable for detecting various types of ryegrass? News Letter Assoc. Off. Seed Anal. 9:2-4.

Munn, M.T. 1937. Fluorescence readings of the strains of the species of *Lolium*. *In* W.O. Whitcomb, C.W. Leggatt, and M.T. Munn (eds.). Proc. Assoc. Off. Seed Anal. Washington D.C. 23-26, Aug.

Naylor, B. 1960. Species differentiation in the genus *Lolium*. Heredity 15:219-233.

Nielsen, G., H. Østergaard, and H. Johansen. 1985. Cultivar identification by means of isozymes. II. Genetic variation at four enzyme loci in diploid ryegrass. Z. Pflanzenzüchtung. 94:74-86.

Nilsson, F. 1930. Einige resultate von isolations- und bastardierungsversuchen mit *Lolium multiflorum* Lam. und *Lolium perenne* L. Botaniska Notiser. 3-4:161-166.

Nittler, L.W., and T.J. Kenny. 1972. Distinguishing annual from perennial ryegrass. Agron. J. 64:767-768.

Nitzsche, V.W. 1960. Über die inkonstanz der fluoreszenz bei weidelgräsern. Zeitschrift für Acker und Pflanzenbau. 110:267-288.

Nitzsche, V.W. 1963. Nichtfluoreszierendes Welsches weidelgras (*Lolium multiflorum* Lam.). Der Züchter 33:281-282.

Nitzsche, V.W. 1966. Die vererbung der fluoreszenz bei weidelgras (*Lolium* spp.) Zeitschrift für Pflanzenzücht. 56:88-95.

Nyquist, W.E. 1963. Fluorescent perennial ryegrass. Crop Sci. 3:223-226.

Ogburn, C.S., and J. McNamara. 1972. The effects of test duration, root disturbance, and seedling transfer on fluorescence of *Lolium multiflorum* Lam. *In* D.F. Grabe (ed.). Assoc. Off. Seed Anal. p. 149-153. Salt Lake City, UT. 18-22, Jun.

Okora, J.O. 1995. Inheritance of the seedling root fluorescence trait and its relationship to botanical traits of annual and perennial ryegrass. M.S. thesis. Mississippi State Univ., Mississippi State, MS.

Okora, J.O., C.E. Watson, L.M. Gourley, B.C. Keith, and C.E. Vaughn. 1999. Comparison of botanical characters and seedling root fluorescence for distinguishing Italian and perennial ryegrass. Seed Sci. & Technol. 27:721-730.

OR Cert. and Fnd. Seed and Plant Mat. Board. 1970. Oregon Certified Seed Standards. OR State Univ., Corvallis.

OR Cert. and Fnd. Seed and Plant Mat. Board. 1980. Oregon Certified Seed Handbook. OR State Univ., Corvallis.

Oregon Ryegrass Growers Seed Commission. 1996. Assessment reports. Salem, OR.

Oregon Seed Certification Service. 1990. 1990 Oregon Certified Seed Handbook.. Oregon State Univ. Exten. Ser., OR State Univ., Corvallis.

Oregon Seed Certification Service. 1995. 1995 Oregon Certified Seed Handbook. Oregon State Univ. Exten. Ser., OR State Univ., Corvallis.

Oregon Seed Certification Service. 1996. 1996 Oregon Certification Activity Summary. Spec. report 96-009. Oregon State Univ. Exten. Ser., Corvallis.

Oregon Seed Certification Service. 1999. 1999 Oregon Certified Seed Handbook. Oregon State Univ. Exten. Ser., OR State Univ., Corvallis.

Pacific Northwest Weed Control Handbook. 1995. Extension Services of Oregon State Univ., Washington State Univ., and the Univ. of Idaho. Publication orders, OR State Univ., Corvallis.

Payne, R.C., J.A. Scott, and T.J. Koszykowski. 1980. An esterase isoenzyme difference in seed extracts of annual and perennial ryegrass. J. Seed Technol. 5:14-22.

Poehlman, J.M. 1979. Breeding field crops. 2nd ed. AVI Publ. Co., Inc., Westport, CT.

Radersma, S.C., and E.A. Perdok. 1965. How to obtain optimal results with the Gentner fluorescence test. Proc. Int. Seed Test. Ass. 30:637-645.

Rampton, H.H. 1938. The use of morphological characters as compared with fluorescence tests with ultra-violet light in classifying the ryegrasses (*Lolium* spp.) of western Oregon. J. Amer. Soc. Agron. 30:915-922.

Rampton, H.H. 1966. Time isolation as a safeguard to varietal purity in perennial ryegrass, annual ryegrass, and orchardgrass . Circular no. 623. Agric. Exp. Sta. Oregon State Univ. Corvallis, OR.

Rebischung, J. 1951. Étude de populations de ray-grass et du mode de transmission de deux caractères dans le genre *Lolium*. Annales Institut National Recherche Agronomique, Series B. 1:497-547.

Riewe, M.E., and C.L. Mondart, Jr. 1985. The ryegrasses. p. 241-246. *In* M.E. Heath, R.F. Barnes, D.S. Metcalf (eds.). Forages, the science of grassland agriculture. 4th ed. Iowa State Univ. Press, Ames, IA.

Robinson, T. 1979. The evolutionary ecology of alkaloids. p. 413-448. *In* G.A. Rosenthal and D.H Janzen (eds.) Herbivores: their interaction with secondary plant metabolites. Academic Press, Inc. New York, NY.

Robinson, T. 1983. Alkaloids. p. 281-294. *In* The organic constituents of higher plants, their chemistry and interrelationships. 4th ed. Cordus Press, North Amherst, MA.

Rose-Fricker, C., W.A. Meyer, and K.N. Morris. 1992. U.S. turfgrass variety list, 1992. NTEP no. 92-11. West. Reg. Coord. Com.-II and Beltsville Ag. Res. Ctr., Beltsville, MD.

Rosell, C. 1967. A comparison of plant characteristics of annual ryegrass (Oregon), *Lolium multiflorum* Lam. and *Linn* perennial ryegrass, *Lolium perenne* L. M.S. thesis, Oregon State Univ., Corvallis, OR.

Rumball, W. 1970. Changes in mean character and uniformity of *Lolium* (*perenne* x *multiflorum*) var. 'Grasslands Manawa' during seed increase. N.Z. J. Agric. Res. 13:605-615.

SAS Institute. 1994. SAS/STAT guide for personal computers. Version 6.10 ed. SAS Inst., Inc., Cary, NC.

Scarrott, C.H. 1981. Self-incompatibility in diploid and tetraploid *Lolium* species. PhD thesis. Univ. of Birmingham. England.

Schmidt, H.H. 1953. Untersuchungen zu den fluoreszenzerscheinungen der keimpflanzen von *Lolium* spp. im ultravioletten licht. II. Die bedeutung der filterpapiere für die ausbildung der fluoreszenzbahnen. Berichte der Deutschen Botanischen Gesellschaft. 66:420-426.

Schunack, W., and H. Rochelmeyer. 1965. Über die bildung von annulolin in *Lolium multiflorum* Lam. und seine ausscheidung aus den keimwurzeln. Planta Medica. 13: 1-10.

Spoor, W. 1976. Self-incompatibility in *Lolium perenne* L. Heredity 37:417-421.

Stuczynski, E., J. Stuczynska, W. Mazgalska, and B. Jasinska. 1969. Results of preliminary investigations on tetraploid interspecific hybrids *Lolium multiflorum* Lam. var. *westerwoldicum* x *Lolium perenne* L. Genetica Polonica 10:124-129.

Stuessy, T.F. 1990. Plant taxonomy. Columbia Univ. Press, New York.

Terrell, E.E. 1968. A taxonomic revision of the genus *Lolium*. USDA Tech. Bul. 1392. U.S. Gov. Print. Office, Washington, DC.

Thomas, H.M. 1981. The giemsa C-band karyotypes of six *Lolium* species. Heredity 46:263-267.

Tyler, B.F., K.H. Chorlton, and I.D. Thomas. 1987. Collection and field-sampling techniques for forages. p. 3-10. *In* B.F. Tyler (ed.). Collection, characterization and utilization of genetic resources of temperate forage grass and clover. IBPGR Train. Courses: Lect. Ser. 1. Int. Bd. Plant Gen. Resour., Rome.

U.S. Department of Agriculture. 1992. Items of interest in seed control. USDA October 1992. U.S. Gov. Print. Office, Washington DC.

U.S. Department of Agriculture. 1995. Federal seed act regulations part 201. USDA August 1995. U.S. Gov. Print. Office, Washington DC.

U.S. Department of Agriculture. 1999. Items of interest in seed control. USDA Winter 1999. U.S. Gov. Print. Office, Washington DC.

U.S. Planting Seed Trade. 1996. U.S.D.A.-Foreign Agricultural Service. Circular Series FFVS 13-96.

Woodforde, A.H. 1935. The inheritance of a substance in the roots of seedling hybrid derivatives of *Lolium perenne* L. x *Lolium multiflorum* Lam., causing a fluorescence reaction visible in filter paper by screened ultra-violet light. J. Linnean Soc. London 50:141-150.

Wright, W.H. 1941. The use of ultra-violet light in the identification of *Lolium perenne* L. and *Lolium multiflorum* Lam. Assoc. Off. Seed Anal. Handbook. AOSA, Lincoln, NE.

Young, W.C. III, and S. Griffith. 1996. Extension estimates for Oregon forage and turfgrass seed crop acreage, 1996. Oregon State Univ. Exten. Ser., OR State Univ., Corvallis. (Available on-line with updates at http://www.css.orst.edu/seed-ext/Agronomy/99ftarc.html) (verified 17 April 1997).

APPENDIX

Appendix 1. Morphological and physiological character differences between Italian and perennial ryegrasses.

Character	Italian Ryegrass (IR)	Perennial Ryegrass (PRG)
Life form	short lived	long lived
Plant height	taller than PRG	shorter than IR
Foliage arrangement	born mostly on stems	mostly basal
Foliage texture	wider and less numerous than PRG	more narrow than IR
Foliage color	lighter green than PRG	darker green than IR
Spikes	longer than PRG	spikes shorter than IR
Spikelets	longer than outer glume	< or = outer glume
Awns	lemmas awned	lemmas awnless
Seedling growth	rapid	slower than IR

Appendix 2. Mean flowering dates for ryegrass half-sib families within four populations increased three generations at two locations.†

Population 1	Location					
Family	Aurora Year			Corvallis Year		
	1992	1993	1994	1992	1993	1994
	DOY†					
94-1	148	156	151	149	155	147
99-1	147	157	150	146	155	145
123-7	156	160	152	152	156	148
142-2	148	161	152	149	153	147
152-1	147	158	152	147	155	147
155-6	148	158	152	147	155	145
160-15‡	149	—	—	149	----	-----
171-5	147	159	152	146	152	145
176-2	147	160	152	148	157	145
179-8	148	157	152	149	157	148
Population mean	149	158	152	148	155	146
LSD	1	3	1	1	2	2
Population 2						
94-2	154	161	150	152	158	150
94-4‡	150	----	-----	149	----	-----
123-3	148	159	151	149	157	148
152-8	148	159	151	148	158	151
155-2	147	157	149	147	155	147
160-4	147	159	151	147	155	149
168-5	150	158	150	150	157	150
176-1	148	160	151	149	157	149
179-2	147	159	150	148	157	149
Population mean	149	159	150	149	157	149
LSD	2	2	2	1	2	3

Appendix 2 continued.

Population 3	Location					
Family	Aurora Year			Corvallis Year		
	1992	1993	1994	1992	1993	1994
	DOY†					
14-4‡	147	-----	-----	151	-----	-----
26-8‡	150	-----	-----	150	-----	-----
34-2	151	160	150	150	155	145
43-2	148	154	146	151	153	143
59-5	147	156	147	150	154	144
106-4	147	156	146	147	153	143
106-13	146	155	146	148	153	143
112-9	147	156	147	148	154	144
126-14‡	151	----	----	153	----	----
142-10	147	160	147	147	157	145
Population mean	148	157	147	150	154	144
LSD	2	3	2	3	2	2
Population 4						
228	147	154	147	140	150	144
4867	146	154	145	140	149	143
4948	147	154	146	141	148	143
5149	146	154	144	140	148	140
5182	146	154	145	142	149	141
5304	146	154	145	139	148	138
Population mean	146	154	145	140	149	142
LSD	1	1	1	1	2	2

† Flowering data were only gathered from the two western Oregon sites. Date reported as calendar day of the year (DOY).

Appendix 3. Mean mature plant height for ryegrass half-sib families within four populations increased three generations at two locations.†

Population 1	Location					
Family	Aurora Year			Corvallis Year		
	1992	1993	1994	1992	1993	1994
	----------cm----------			----------cm----------		
94-1	45.8	78.4	69.6	49.5	90.2	88.6
99-1	57.7	79.6	67.2	60.8	97.0	88.0
123-7	22.8	78.8	68.8	23.3	95.4	85.4
142-2	67.7	80.4	67.8	63.5	92.6	88.8
152-1	45.0	80.0	70.2	41.8	99.6	88.2
155-6	53.0	79.6	65.0	53.5	96.0	88.8
160-15‡	36.2	—	—	40.0	—	—
171-5	56.0	81.4	70.2	49.0	89.4	84.8
176-2	60.8	80.4	70.2	62.5	103.2	86.6
179-8	61.7	83.6	71.0	59.7	106.2	100.0
Population mean	50.7	80.2	68.9	50.4	96.6	88.8
LSD	5.6	4.7	4.0	6.2	9.2	7.9
Population 2						
94-2	50.7	79.6	71.6	46.5	87.4	86.2
94-4‡	23.2	—	—	25.8	—	—
123-3	70.0	78.0	71.0	56.0	91.8	95.6
152-8	56.3	75.8	69.8	51.8	87.8	92.0
155-2	57.2	82.4	70.2	56.7	87.8	89.0
160-4	55.0	80.4	69.6	51.0	88.6	90.6
168-5	48.7	80.4	73.2	39.2	94.6	90.6
176-1	68.8	81.0	72.6	66.5	92.0	94.0
179-2	63.3	80.0	72.8	50.2	92.0	100.0
Population mean	54.8	79.7	71.4	49.3	90.3	92.3
LSD	7.7	5.0	3.4	9.6	11.6	9.3

Appendix 3 continued.

Population 3	Location					
Family	Aurora Year			Corvallis Year		
	1992	1993	1994	1992	1993	1994
	----------cm----------			----------cm---------		
14-4‡	92.0	—	—	86.2	—	—
26-8‡	68.0	—	—	69.2	—	—
34-2	36.0	85.0	80.6	28.8	94.6	115.4
43-2	91.2	118.8	108.2	95.2	120.8	131.0
59-5	109.2	100.6	99.6	88.2	123.8	123.6
106-4	89.2	102.2	98.6	83.2	117.8	122.0
106-13	90.2	106.0	98.6	83.7	118.4	119.8
112-9	92.5	98.8	101.8	79.5	122.6	125.4
126-14‡	67.0	—	—	44.3	—	—
142-10	85.2	83.4	81.2	78.0	88.2	104.2
Population mean	82.1	99.3	95.5	73.6	112.3	120.2
LSD	15.3	8.2	7.7	11.9	8.9	7.7
Population 4						
228	55.5	98.4	96.8	49.7	111.8	115.4
4867	95.2	123.6	117.0	102.8	151.6	132.0
4948	73.8	119.2	114.0	67.5	139.2	127.2
5149	61.5	126.6	116.2	55.2	148.0	128.0
5182	59.5	123.4	117.6	52.8	144.8	127.2
5304	65.5	116.4	114.0	58.7	146.6	129.0
Population mean	68.5	117.9	112.6	64.5	140.3	126.5
LSD	15.6	9.4	7.8	10.6	14.2	9.3

† Height reported as mean cm ramet^{-1} in 1992, and mean mid-row cm measurement in 1993 and 994.

‡ Half-sib family lines dropped from further increase at both sites because of poor seed production in 1992.

Appendix 4. Mean seed yield for ryegrass half-sib families within four populations increased three generations at two locations.†

Population 1	Location					
	Aurora			Corvallis		
Family	Year			Year		
	1992	1993	1994	1992	1993	1994
	g			g		
94-1	2.8	30.6	52.9	4.0	49.4	39.7
99-1	14.9	15.6	47.7	12.6	46.5	36.1
123-7	0.5	13.4	38.0	0.4	44.2	36.7
142-2	15.9	22.4	48.6	4.3	42.5	27.2
152-1	7.1	21.7	37.8	6.1	63.8	39.4
155-6	14.6	18.9	31.8	16.4	44.0	37.2
160-15‡	0.7	—	—	0.4	—	—
171-5	38.7	22.4	43.3	24.4	54.4	48.3
176-2	35.4	27.8	46.5	38.6	43.2	44.0
179-8	16.5	23.1	51.0	14.3	47.9	42.5
Population mean	14.7	21.8	44.2	12.2	48.4	39.0
LSD	9.2	10.5	11.6	4.8	14.8	10.2
Population 2						
94-2	2.6	29.6	40.2	0.8	40.4	31.9
94-4‡	0.4	—	—	0.1	—	—
123-3	30.9	26.4	35.8	14.7	32.2	30.3
152-8	38.7	33.2	42.2	21.4	51.5	38.6
155-2	23.5	24.0	41.1	22.5	40.7	27.5
160-4	16.3	21.9	31.8	11.8	33.6	35.5
168-5	8.8	27.7	45.1	2.2	36.7	36.5
176-1	36.1	25.3	51.5	24.5	40.9	32.1
179-2	31.0	28.1	44.3	16.6	34.8	44.6
Population mean	20.9	27.0	41.5	12.7	38.9	34.6
LSD	12.6	14.3	9.1	6.1	20.5	11.1

Appendix 4 continued.

Population 3	Location					
Family	Aurora Year			Corvallis Year		
	1992	1993	1994	1992	1993	1994
	g			g		
14-4††	0.5	—	—	0.9	—	—
26-8††	0.1	—	—	0.9	—	—
34-2	0.9	6.3	17.7	0.5	19.8	27.0
43-2	3.4	61.4	60.8	12.7	114.5	114.1
59-5	16.6	61.3	61.4	21.8	52.8	83.4
106-4	55.7	55.0	53.0	39.7	99.6	129.9
106-13	11.5	63.6	54.9	5.9	105.0	113.9
112-9	31.1	45.9	43.4	10.7	79.2	96.6
126-14††	0.2	—	—	0.1	—	—
142-10	2.8	5.8	21.2	5.7	16.1	30.4
Population mean	12.3	42.8	44.6	9.9	69.6	85.0
LSD	11.8	20.2	11.7	8.4	22.9	34.5
Population 4						
228	16.5	27.5	55.2	2.6	15.2	75.6
4867	86.1	126.3	178.0	52.1	62.2	128.6
4948	63.4	139.7	164.0	25.4	81.4	137.4
5149	34.2	158.9	167.8	17.1	103.2	164.9
5182	4.5	124.2	152.2	9.0	86.7	165.1
5304	15.2	113.6	134.3	6.0	40.0	134.0
Population mean	36.7	115.0	141.9	18.7	64.8	134.3
LSD	27.3	41.6	51.1	10.2	39.2	38.0

† Seed yields reported as mean grams ramet^{-1} in 1992, and mean grams meter^{-1} row in 1993 and 1994.

‡ Half-sib family lines dropped from further increase at both sites because of poor seed production in 1992.

Appendix 5. Mean one-thousand seed weight for ryegrass half-sib families within four populations increased three generations at two locations.

Population 1	Location					
Family	Aurora Year			Corvallis Year		
	1992	1993	1994	1992	1993	1994
	g			g		
94-1	1.7	2.0	2.1	1.5	2.2	2.1
99-1	2.2	1.8	2.1	1.9	2.5	2.5
123-7	1.3	1.8	2.1	1.2	2.3	2.2
142-2	2.2	2.1	2.2	1.8	2.6	2.4
152-1	2.0	2.0	2.2	1.9	2.4	2.3
155-6	2.1	1.9	2.1	1.8	2.5	2.2
160-15†	2.7	—	—	1.5	—	—
171-5	2.2	1.9	2.1	1.8	2.4	2.3
176-2	2.3	2.0	2.3	2.0	2.5	2.3
179-8	1.8	2.1	2.2	1.3	2.5	2.4
Population mean	2.1	2.0	2.2	1.7	2.4	2.3
LSD	0.1	0.1	0.2	0.2	0.2	0.2
Population 2						
94-2	1.4	1.9	1.9	1.2	2.1	2.0
94-4†	1.7	—	—	0.9	—	—
123-3	2.1	2.0	2.0	1.9	2.4	2.3
152-8	2.1	2.0	2.1	1.9	2.4	2.2
155-2	1.8	1.9	2.0	1.8	2.2	2.2
160-4	2.2	2.0	2.1	1.9	2.3	2.3
168-15	1.8	2.0	2.2	1.7	2.3	2.3
176-1	2.2	2.2	2.4	2.0	2.7	2.6
179-2	2.0	2.1	2.2	1.9	2.7	2.6
Population mean	1.9	2.0	2.1	1.7	2.4	2.3
LSD	0.2	0.1	0.2	0.1	0.2	0.1

Appendix 5 continued.

Population 3 Family	Location: Aurora Year			Location: Corvallis Year		
	1992	1993	1994	1992	1993	1994
	g			g		
14-4†	1.6	—	—	1.1	—	—
26-8†	3.0	—	—	3.2	—	—
34-2	2.5	1.9	2.2	3.0	2.5	2.5
43-2	1.8	3.3	3.1	2.3	3.8	3.6
59-5	2.3	2.9	3.1	2.8	3.6	3.5
106-4	2.3	3.1	3.1	3.1	3.5	3.4
106-13	2.2	3.2	3.2	2.2	3.4	3.6
112-9	2.3	2.8	2.9	2.6	3.4	3.3
126-14†	1.7	—	—	1.7	—	—
142-10	2.9	2.0	2.4	3.3	2.5	2.5
Population mean	2.3	2.7	2.9	2.5	3.2	3.2
LSD	0.1	0.1	0.1	0.3	0.3	0.1
Population 4						
228	2.1	2.8	2.9	1.9	2.9	3.1
4867	2.7	3.4	3.3	2.7	3.4	3.5
4948	2.6	3.2	3.2	2.5	3.6	3.5
5149	3.3	3.5	3.3	2.9	3.8	3.6
5182	1.7	3.1	3.0	2.2	3.4	3.4
5304	2.4	3.5	3.5	2.0	3.5	3.7
Population mean	2.5	3.3	3.2	2.4	3.4	3.5
LSD	0.2	0.1	0.1	0.1	0.1	0.2

† Half-sib family lines dropped from further increase for each population at both sites because of poor seed production in 1992.

Appendix 6. Expected mean squares (EMS) for an analysis of variance of SRF for 26 ryegrass half-sib families, increased for three generations within a location.

Source	df	EMS
Replication (R)	1	$\sigma_e^2 + f\sigma_{GR}^2 + g\sigma_{RF}^2 + fg\sigma_R^2$
Family (F)	25	$\sigma_e^2 + r\sigma_{FG}^2 + g\sigma_{RF}^2 + rg\sigma_F^2$
R x F	25	$\sigma_e^2 + g\sigma_{RF}^2$
Generation (G)	2	$\sigma_e^2 + f\sigma_{GR}^2 + r\sigma_{FG}^2 + rf\sigma_G^2$
F x G	50	$\sigma_e^2 + r\sigma_{FG}^2$
G x R	2	$\sigma_e^2 + f\sigma_{GR}^2$
Residual	50	σ_e^2

Appendix 7. Mean bi-weekly minimum and maximum air temperature at three locations in each of three growing seasons.

Season and Standard week†	Location								
	Aurora			Corvallis			Madras		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
	°C								
1992									
17	6	19	13	7	19	13	3	18	11
19	8	23	16	8	22	15	5	24	15
21	8	25	17	7	24	16	7	26	17
23	9	26	18	9	26	18	8	27	18
25	11	25	18	11	25	18	9	27	18
27	13	27	20	12	27	20	11	28	20
29	13	29	21	13	29	21	11	30	21
31	13	29	21	13	29	21	11	32	22
1993									
17	6	16	11	6	15	11	2	15	9
19	7	18	13	7	18	13	4	19	12
21	11	24	18	10	23	17	8	26	17
23	11	21	16	11	20	16	7	20	14
25	10	22	16	10	22	16	7	24	16
27	11	24	18	11	24	18	7	25	16
29	11	23	17	11	23	17	7	23	15
31	13	26	20	13	26	20	9	27	18
1994									
17	6	19	13	6	19	13	4	20	12
19	8	22	15	8	21	15	5	23	14
21	9	21	15	8	20	14	5	21	13
23	8	19	14	7	19	13	5	20	13
25	10	23	17	9	22	16	8	25	17
27	10	24	17	9	24	17	8	27	18
29	11	32	22	10	31	21	11	34	23
31	13	31	22	12	31	22	13	35	24

† Standard week 1 = January 1 to January 7.

Appendix 8. Daily minimum, maximum, and mean air temperature at three locations in each of three flowering seasons for ryegrass populations.

Year and Calendar day†	Location								
	Aurora			Corvallis			Madras		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
	°C								
1992									
135	6	26	16	4	26	15	8	28	18
136	7	24	16	6	23	15	3	26	15
137	8	30	19	9	27	18	8	27	18
138	10	28	19	6	27	17	10	31	21
139	10	24	17	9	24	17	11	27	19
140	9	21	15	4	21	13	6	18	12
141	2	18	10	3	17	10	1	18	10
142	3	21	12	4	20	12	2	21	12
143	5	28	17	7	26	17	7	26	17
144	11	30	21	10	29	20	10	31	21
145	10	34	22	13	32	23	12	33	23
146	13	25	19	13	25	19	11	31	21
147	6	24	15	8	23	16	4	22	13
148	9	23	16	11	23	17	7	25	16
149	12	24	18	11	25	18	6	23	15
150	11	25	18	12	26	19	9	27	18
151	11	28	20	12	28	20	10	29	20
152	12	32	22	10	32	21	12	32	22
153	8	27	18	7	27	17	7	29	18
154	8	26	17	10	26	18	6	28	17
155	7	30	19	5	28	17	8	28	18
156	8	25	17	8	25	17	5	26	16
157	8	27	18	7	26	17	8	27	18
158	9	28	19	7	28	18	8	29	19
159	9	26	18	6	25	16	6	28	17
160	11	26	19	11	26	19	9	30	20
161	10	23	17	6	22	14	7	31	19
162	12	24	18	13	24	19	11	27	19
163	11	18	15	9	17	13	9	28	19
164	10	19	15	9	21	15	4	17	11
165	10	19	15	10	18	14	4	18	11
166	8	23	16	8	21	15	6	18	12
Mean	9	25		8	25		7	26	

Appendix 8 continued.

Year and Calendar day†	Location								
	Aurora			Corvallis			Madras		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
	°C								
1993									
135	11	22	17	11	19	15	6	27	17
136	8	23	16	7	22	15	8	28	18
137	8	26	17	7	24	16	8	28	18
138	13	28	21	10	28	19	10	28	19
139	12	28	20	12	26	19	13	30	22
140	10	30	20	9	24	17	11	28	20
141	12	22	17	12	22	17	8	22	15
142	12	18	15	11	19	15	5	18	12
143	9	21	15	11	20	16	4	22	13
144	11	29	20	8	29	19	7	24	16
145	13	31	22	13	28	21	12	26	19
146	12	17	15	11	18	15	6	17	12
147	13	26	20	13	24	19	9	23	16
148	10	22	16	11	21	16	6	21	14
149	9	19	14	11	18	15	4	18	11
150	13	26	20	13	24	19	5	23	14
151	14	22	18	13	21	17	12	22	17
152	10	20	15	11	19	15	9	17	13
153	8	16	12	10	17	14	4	16	10
154	8	19	14	7	19	13	4	18	11
155	10	21	16	11	21	16	6	21	14
156	12	21	17	12	21	17	9	19	14
157	13	19	16	12	19	16	9	20	15
158	12	17	15	11	17	14	7	20	14
159	11	20	16	8	19	14	4	18	11
160	11	20	16	12	20	16	7	22	15
161	10	20	15	8	19	14	6	23	15
162	8	18	13	8	19	14	6	22	14
163	9	17	13	7	16	12	2	16	9
164	7	21	14	6	21	14	2	22	12
165	7	24	16	12	23	18	13	27	20
166	13	25	19	12	24	18	12	26	19
Mean	11	22		10	21		7	22	

Appendix 8 continued.

Year and Calendar day†	Location								
	Aurora			Corvallis			Madras		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
	°C								
1994									
135	9	21	15	9	19	14	6	21	14
136	9	17	13	8	16	12	3	16	10
137	8	16	12	9	16	13	1	16	9
138	9	16	13	9	16	13	4	16	10
139	9	19	14	7	19	13	5	21	13
140	11	18	15	11	16	14	6	17	12
141	11	16	14	9	16	13	3	18	11
142	11	20	16	8	19	14	6	21	14
143	9	24	17	7	23	15	6	24	15
144	10	28	19	7	26	17	9	28	19
145	10	29	20	9	27	18	12	31	22
146	8	23	16	6	22	14	8	26	17
147	6	18	12	3	18	11	3	20	12
148	8	18	13	8	19	14	6	19	13
149	9	18	14	8	14	11	9	16	13
150	6	17	12	3	17	10	1	18	10
151	10	24	17	9	23	16	5	24	15
152	7	19	13	7	19	13	7	22	15
153	9	20	15	8	20	14	4	21	13
154	6	21	14	3	21	12	6	25	16
155	6	21	14	12	21	17	8	25	17
156	10	20	15	9	20	15	9	22	16
157	11	17	14	9	18	14	6	13	10
158	9	16	13	7	17	12	5	16	11
159	6	18	12	6	19	13	3	17	10
160	9	23	16	6	23	15	2	23	13
161	9	26	18	9	26	18	9	26	18
162	12	29	21	12	28	20	11	30	21
163	12	26	19	9	25	17	11	28	20
164	11	22	17	11	22	17	13	27	20
165	9	16	13	8	16	12	4	18	11
166	8	17	13	8	16	12	2	16	9
Mean	9	20		8	20		6	21	

Appendix 9. Daily evaporation at three locations in each of three flowering seasons for ryegrass populations.

	Location								
	Aurora			Corvallis			Madras		
	Year								
Calendar day	1992	1993	1994	1992	1993	1994	1992	1993	1994
	mm								
135	6	5	3	4	3	3	8	6	4
136	5	4	5	6	4	2	8	7	9
137	8	5	4	6	5	3	7	7	5
138	8	8	3	6	5	1	6	8	7
139	4	6	2	4	5	5	4	9	4
140	3	3	3	5	4	3	5	10	3
141	4	5	3	4	5	1	7	8	4
142	8	5	2	7	5	4	7	7	5
143	4	4	5	5	6	6	7	5	5
144	8	8	5	7	1	6	8	8	8
145	4	7	8	8	6	7	9	7	9
146	4	2	6	5	1	6	6	8	9
147	5	5	4	6	6	5	12	15	9
148	4	4	4	6	3	4	6	6	7
149	7	3	2	5	2	2	8	8	6
150	7	4	2	7	5	4	8	8	8
151	8	4	6	8	1	6	10	10	+
152	8	3	2	8	3	2	10	7	3
153	+	2	4	7	2	6	10	8	6
154	4	4	4	9	3	4	9	8	6
155	8	4	5	8	4	2	9	8	5
156	7	4	4	8	4	4	13	7	8
157	8	+	2	7	3	2	8	9	1
158	6	2	5	7	3	3	9	8	8
159	7	5	3	7	5	3	9	6	5
160	6	5	4	6	5	4	9	9	4
161	5	+	6	5	3	6	11	11	8
162	5	0	8	6	4	7	7	8	8
163	4	5	+	1	3	5	9	8	6
164	4	4	5	4	6	2	4	8	9
165	5	4	4	3	4	1	6	6	9
166	4	5	3	5	5	2	5	6	7
Mean	6	4	4	6	4	4	8	8	6

+ zero or missing value