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The objective of this study was to investigate ryegrass seedling characteristics that, by themselves, or combined with presently known characters, could improve varietal identification tests.

Thirteen characters were studied on 30 varieties of Lolium multiflorum Lam., L. perenne L., L. rigidum Gaud, and L. hybridum Hausskn., including leaf size and area, epidermal characteristics, structural elements, and leaf blade vernation.

Mean differences among species occurred in the following characteristics:

Lolium multiflorum and L. perenne: leaf width, leaf length, leaf area, minor and major vascular bundles, lower and upper sclerenchyma fiber groups, hair density, number of vascular bundles, and leaf blade vernation.

Lolium multiflorum and L. hybridum: leaf width, leaf length,

leaf area and leaf vernation.

Lolium perenne and L. hybridum: leaf width, leaf length, leaf area, and total of sclerenchyma fiber groups, and leaf vernation.

Lolium perenne and L. rigidum: leaf width, leaf length, leaf area, and leaf vernation.

Lolium multiflorum and L. rigidum: leaf blade vernation.

Lolium hybridum and L. rigidum: leaf blade vernation.

Tetraploids and diploids were significantly different on the average, within their respective species, as follows:

Lolium multiflorum: leaf width, leaf length, leaf area, stomata density.

Lolium perenne: leaf width, leaf length, leaf area, stomata density.

Lolium hybridum: stomata density, and number of major vascular bundles.

Some significant differences among varieties within species and ploidy levels were observed in the following characteristics:

Lolium multiflorum tetraploids: leaf width, leaf length, leaf area, minor vascular bundles.

Lolium multiflorum diploids: leaf width, leaf length, leaf area, minor vascular bundles, total vascular bundles.

Lolium perenne tetraploids: leaf area.

Lolium perenne diploids: leaf length, leaf area, minor vascular bundles.

Although varietal means differed for many of the characters, individual plants could not be identified on the basis of a single character.

An illustration was prepared to show how a combination of morphological characters might be used in varietal identification. With a table of varietal means for nine characters, only two (Jolanda and Tetrone--both tetraploids of L. multiflorum) of the 30 varieties studied were indistinguishable.

The leaf blade vernation of Wimmera (L. rigidum) and Tetrelite (L. hybridum) appeared to be intermediate between that of L. multiflorum and L. perenne. The vernation was folded from the midrib, like in L. perenne, while the edges of the leaf were inrolled and overlapping, somewhat like L. multiflorum. This type of vernation is being called semi-rolled, to conveniently distinguish from "rolled" and "folded".

Identification of Species and Varieties of Ryegrass (*Lolium* spp.)

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an unforgettable and forever-loved friend,

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

IDENTIFICATION OF SPECIES AND VARIETIES OF RYEGRASS (Lolium spp.).

INTRODUCTION

Of the eight species of Lolium, varieties of four are in the commercial seed trade. Some of these varieties are annual, some are perennial. Most are diploid, but some are tetraploid. Some are used for forage, some are used for turf.

Effective merchandizing of ryegrass seed requires accurate methods of identification of varieties because of their varied uses and quality characteristics. Previous work has identified a number of characteristics of a physiological, physical, chemical, or morphological nature by which ryegrass varieties may differ.

The fluorescence test is a standard laboratory test for distinguishing annual from perennial seed. Unfortunately, varieties are not all pure for this characteristic and the test is not biologically sound for all varieties. Chromosome counts may be used to distinguish between tetraploid and diploid varieties, but this procedure is laborious and costly, making it difficult to do on a large scale.

Considerable plant-to-plant variation exists in a cross-pollinated species such as ryegrass, so that no two plants within a variety are exactly alike. It is therefore not possible for diagnostic techniques to be as precise as in self-pollinated crops where plants

within a variety are more uniform. It will probably be necessary to use a combination of characteristics, rather than a single character, to positively identify individual seeds or seedlings.

The objectives of this research were to investigate characteristics of ryegrass seedlings that might be combined with presently known characters to develop more positive varietal identification tests. Thirteen morphological characteristics of seedling leaves, including size, epidermal characteristics and structural elements were studied. Observations were made on 30 varieties representing the species Lolium multiflorum Lam., L. perenne L., L. rigidum Gaud, and L. hybridum Hausskn.

LITERATURE REVIEW

Taxonomy of the Genus Lolium

A certain controversy has existed for a long time about the genus Lolium. Authors disagree on the number of species, characters for separation, and on fusion or separation from Festuca. Rouville (1853) recognized three species. Hitchcock (1950), in his Manual of the Grasses of the U.S.A., recognized six species: L. perenne, L. multiflorum, L. temulentum, L. persicum, L. subulatum, and L. strictum. Essad (1954) recognized five different species. The allogamous group, including L. perenne, L. multiflorum and L. rigidum can be clearly separated from the autogamous group, L. temulentum and L. remotum. Lolium multiflorum and L. rigidum are like L. temulentum and L. remotum in being separable from each other only with difficulty. L. perenne differs more from L. rigidum than it does from L. multiflorum. Hubbard (1956) stated that "characters such as awn length, size of spikelets, number of florets, height of plant and duration (annual, biennial), are of little value for specific or even varietal separation, between L. multiflorum, L. aristatum and L. italicum as individuals hybridize freely, yielding a wide range of intermediates."

Crosses made by geneticists provided more information about the biosystematics of Lolium. Terrel (1966) summarized the data from this source as follows: (1) all taxa are diploid with 2n = 14; (2) L. multiflorum, L. perenne, and perhaps L. rigidum are self-incompatible and cross-pollinated; (3) L. remotum, L. temulentum, and perhaps L. rigidum var. rottbollioides (L. loliaceum) are self-compatible and self-pollinated; (4) certain Lolium and Festuca species can cross and exchange genes.

Several natural hybrids of various Lolium species and inter-generic hybrids with Festuca were found, chiefly in Europe, by Terrel (1966), mainly hybrids of L. perenne x F. pratensis. This ability of certain Lolium species to cross with Festuca section Bovinae suggested to certain authors that the genera Lolium and Festuca should be united, although species of Lolium are more closely related to each other than to Festuca. Terrel disagreed, being "in favor of, at least in the present stage of knowledge, the retention of Lolium and Festuca as two distinct genera."

The most complete, and up to date revision of the genus was made by Terrell (1968). He presented an "alpha taxonomy" dividing the genus into eight species, based on the following key:

Key to Mature and Complete Plants

A. Plants either perennial with spikelets 2- to 10- flowered or annual (or biennial) with spikelets 11- to 22-flowered; glumes less than 15 mm. long.

B. Perennial; spikelets 2- to 10-flowered; glumes one-third to equaling or slightly exceeding spikelet, but usually about one-half to three-fourths as long; rachis glabrous or scaberulous only on angles; lemmas awnless (or awns to 8 mm. long at least in hybrids with L. multiflorum); leaf blades 1-6 (usually 2-4) mm. wide; immature leaves folded in young shoots ---------- L. perenne

BB. Annual or biennial (or perennial at least in hybrids with L. perenne); spikelets (11- to 22-flowered; glumes typically one-fourth to one-half as long as spikelets; rachis scaberulous; lemmas usually with awns to 15 mm. long (or sometimes awnless at least in hybrids with L. perenne); leaf blades 2-8 (usually 4-7) mm. wide; immature leaves rolled in young shoots ----------L. multiflorum

AA. Plants annual; spikelets 2- to 11-flowered; glumes of various lengths.

C. Mature cariopses plump and thick, only 2-3 times longer than wide; lemma apices usually rounded or blunt, if awned then awns attached 0.5-2.0 mm. below apices; typically weeds of grain crops or flax.

D. Lower florets in a spikelet 5.2-8.5 mm. long, 1.5-3.0 mm. wide; mature cariopses usually 4.2-7.0 mm. long, 1.6-3.0 mm. wide; glumes 7-30 mm. long; spikes 5-40 cm. long; leaf blades usually 3-10 mm. wide, awns present or absent; rachis 0.5-3.5 mm. thick ----------L. temulentum

DD. Lower florets in a spikelet 3.5-5.4 mm. long, 1.2-1.8 mm. wide, mature cariopses usually 3.2-4.5 mm. long, 1.2-1.8 mm. wide. glumes 5-16 mm. long; spikes 2-23 cm. long; leaf blades usually 1-6 mm. wide; usually awnless (or rarely awns to 10 mm. long); rachis slender, 0.5-1.5 mm. thick---L. remotum

CC. Mature cariopses more than 3 times longer than wide; lemma apices various, if awned then awns attached 0.2-1.0 mm. below apices, habitats various.

E. Lemmas awnless or awns less than 3 mm. long (rarely longer in L. rigidum from southwest Asia); rachis 0.5-3.0 mm. thick.

F. Florets large, 6.3-12.0 mm. long, 1.0-2.4 mm. wide; spikelets only 2- to 4-flowered, with long (2.2-6.3 mm.) rachilla segments; glumes usually acute or acuminate, 14-25 mm. long ------- ---------- L. sublatum

FF. Florets smaller, 3.2-8.5 (rarely to 9.8) mm. long, 0.9-2.0 mm. wide; spikelets 2- to 11-flowered, with short (1.0-3.5 mm.) rachilla segments; glumes obtuse to acute, 4-20 mm. long (to 30 mm. in Madeira Islands)------- ---------- L. rigidum (two varieties)

EE. Lemmas with awns more than 3 mm. long; rachis slender, 0.5-1.5 mm. thick.

G. Lower florets in a spikelet 8-12 mm. long, 1.5-2.7 mm. wide (5-6.5 times longer than wide); paleas often 0.5-1.8 mm. longer than their lemmas or equal to them; spikelets 1.5-7.0 mm. wide; restricted to southwest Asia and Middle East ----------L. persicum

GG. Lower florets in a spikelet 3-10 mm. long, 0.7-1.5 mm. wide (4-10 times longer than wide); paleas equal to lemmas in length or to 0.5 mm. longer; spikelets 1-4 mm. wide; restricted to Canary, Cape Verde, and Madeira Islands ---------- L. canariense

He added that the

chaotic state of the nomenclature of Lolium considerably hampered efforts to devise a better classification; some order had to be made of the approximately 480 published names. One of the major sources of taxonomic difficulties

> in the genus is the intergradation among L. multiflorum, L. perene, and the polymorphic, L. rigidum. It appears that repeated hybridization and introgression occurred during their evolution specially during the past several thousand years of man's disturbance of habitats in the Mediterranean and Southwest Asia.

He reported that

> many characteristics have been used to separate L. multiflorum and L. perenne, for instance, differences in the marginal teeth of the lemma and palea (Lakon, 1919; Helbo, 1926), and shoot-bud prophyll (Beddows, 1937). However, these and a number of other differences strongly overlap.

The authors diverged about the importance of certain characters to separate L. multiflorum and L. perenne. A great number emphasized the presence or absence of awns. For Hubbard (in correspondence cited by Terrel, 1968) the single most reliable characteristic is the leaf vernation. Terrel (1968) believed that the number of florets per spikelet is a more reliable character for separation than awns, since that is determined by a more complex genetic mechanism. Terrel finally listed the main distinguishing characteristics to separate L. multiflorum and L. perenne as follows:

	L. multiflorum	L. perenne
Duration	annual or biennial (or shortlived perennial, at least in hybrids)	perennial

	L. multiflorum	L. perenne
Leaf Vernation	rolled	folded
Blade Width	usually 3 to 8 mm	Usually 2 to 4 mm
Rachis Indument	scabrous	glabrous or scaberulous only on rachis angles
Spikelets	with 12 to 22 florets	with 2 to 10 florets
Awns	typically present to 15 mm long (or absent, at least in hybrids).	typically absent (but to 8 mm long, at least in hybrids.

Fluorescence Test

The discovery by Gentner (1929) that seedlings of L. multiflorum, germinated on filter paper, secreted a substance (since 1957 known as annuloline) which showed fluorescence under ultraviolet light, whereas L. perenne did not, led to the simplest test to separate these two species. Certainly at Gentner's time the number of varieties of each species would have been very small, so the crossings and hybridizations were limited.

Several authors emphasized the importance and usefulness of Gentner's discovery to identify the two species. These included Linehan and Mercer (1931), Foy (1931), Chmelar (1934), and Dorph-Petersen (1934).

The fluorescence test soon received a great amount of criticism. As early as 1930, Nilsson reported results disproving Gentner's

assertion that perennial ryegrass did now show fluorescence. Later, Gentner himself recognized that some exceptions can occur in 10 to 20% of the cases in perennial rye grass.

Genetic studies by Corkill (1932), Linehan and Mercer (1933) and Woodforde (1935) brought more light on the inheritance of the character. Fluorescence was controlled by a single, dominant gene. Normally L. perenne was pure for non-fluorescence and L. multiflorum for fluorescence. The F_1 generation of interspecific crosses of L. multiflorum x L. perenne gave a rate of three fluorescent to two non-fluorescent. No genetic linkage was found between fluorescence and awns, shoot vernation, longevity or vigor. Munn (1937) disagreed with this last statement. He considered fluorescence as a useful indicator of plant longevity, but which must be interpreted with care. Although so subjected to strong criticism, the fluorescence test continued to be used and even its use increased more and more. But the more it was used, the more it was criticized. Rampton (1938) made nursery trials and fluorescence tests with several lots of ryegrass and found that the fluorescence test could be misleading, since the fluorescence test indicated that the ryegrass lots checked were approximately 5% perennial, whereas the nursery behavior indicated less than 1% of perennial-like plants. Therefore he concluded that the fluorescence test cannot be used as an infallible guide to classifying questionable lots of Oregon-grown

domestic ryegrass seed. Baekgaard (1955) reported fluorescence ranging from 1% to 11% among 16 varieties of perennial ryegrass, without any association of the annual characters with the fluorescence. Sacks and Simon (1960) arrived at the same conclusion related to winter-killing, awn development, and growth type.

The lack of uniform results from the fluorescence test led to some suggestions of levels of tolerance. So, Tapp (1945) suggested that ryegrass which fluoresces no more than 10% might be considered as perennial, that which fluoresces 90% to 100% as Italian ryegrass, and that which fluoresces from 11% to 89% as ryegrass. This tendency still prevails today in certification programs, where different tolerances are set for the great number of varieties, thus making the situation more confused, and the test some kind of "gentlemen's agreement" or mere compromise.

Nyquist (1960) obtained homozygous 100% fluorescent clones of perennial ryegrass and did not find morphological differences between those and non-fluorescent perennial ryegrass. So, he stated that the fluorescent test use in the seed trade is justified only in situations where a good association of fluorescence and annual characteristics exists. "For those varieties, which are known to be pure, regardless of the fluorescent percentage, and are grown under rigid certification standards for the maintenance of genetic purity, the test is a false indicator and serves no useful purpose." An

adequate certification program, with standards for previous cropping history, field inspection, and isolation is a better replacement for the fluorescent test. But even in those cases where the association exists, breeders world-wide probably should release only non-fluorescent perennial ryegrass, to maintain the usefulness of the fluorescence test. If this practice is not followed, perennial ryegrass varieties high in fluorescence presumably will become mixed with the original non-fluorescent perennial ryegrass, and the fluorescence test then will be meaningless. If the fluorescence test is not strengthened in this manner, it is better to eliminate its use, replacing it by good certification standards.

It seemed that Nyquist's advice came too late or was not heard, since Copeland (1962) reported that the samples of ryegrass represented to be perennial varieties fluoresced from 0 to 18.7%, and a good correlation was found between perennial samples with high percentage of non-fluorescence and persistence through the winter at Ames, Iowa.

Although these data and opinions show that the fluorescence character is not always related to the other annual characters, the fluorescence test has been used world-wide to determine the purity of seed samples with respect to L. multiflorum and L. perenne. In the U.S.A. the Federal Seed Act prescribed (1939) the use of the fluorescence test to separate annual from perennial ryegrasses, by

using two formulas. Maximum percent of fluorescence contamination is 9.75%, which yields the permitted maximum mixture of 5% annual ryegrass (Manual for Testing Agricultural and Vegetable Seeds, U.S.D.A. Handbook No. 30, 1952).

Special emphasis on problems caused by the lack of confidence in the fluorescence test was shown by Jensen (1963) who reported that the

> introduction of various varieties of ryegrass into Oregon during the past years and the regulation under the Federal Seed Act to label all ryegrasses on the basis of the 'fluorescence test' have introduced problems in labeling annual ryegrass. Formerly only two kinds, annual and perennial ryegrasses were grown, with different dates of pollination. However, new varieties introduced had pollination dates that coincide with one or both of the strains. Production of biennial and tetraploid varieties further confuse the issues.

She concluded that the

> fluorescence test, useful for determining contamination of perennial by annual or hybrid is invalid as a 'non-fluorescence' test for annual ryegrass when genetic mixtures occur even though it is a useful tool to detect mechanical mixtures of perennial and annual. Identification by other means such as seeds or seedlings might be more accurate.

Labeling based on fluorescence of annual ryegrass presents several problems because of lack of laboratory testing methods which give reproducible results, further complicated by the presence of dormancy in annual ryegrass seeds. Factors affecting the formation and detection of annuloline in the roots of annual ryegrass needs

to be determined. Formulas used to determine pure seed percent imply greater accuracy than exists, and do not recognize the existence of biennial, polyploid or hybrid seed.

Leaf Blade Vernation

Description of perennial and annual ryegrasses were made by Breakwell (1918), Jepson (1925) and Levy and Davies (1930). Among the most important characters listed were folded and rolled leaves, respectively, for perennial and annual ryegrasses. Other authors who used this character for identification of annual and perennial ryegrass include Schoth and Hein (1940), Thomas and Davies (1946), Burton (1950), and Burger (1962), who used this character in an analytical key.

Knowledge about the genetic transmission of this character was obtained by Rebischung (1952) who proposed genes for two interspecific differences: rolled vs. folded leaves, and absence vs. presence of auricles. Each character was respectively controlled by three allelic pairs, $P_1\ p_1$, $P_2\ p_2$, $P_3\ p_3$, and $0_1\ o_1$, $0_2\ o_2$, and $0_3\ o_3$, with complex intercellular interactions as well as change in the dominance at the 3rd locus. Furthermore the corresponding genes were linked with the respective values, p_1=0.39, p_2=0.25, p_3=0.33 or 0.48. According to Nyquist and Schulke (1961) this hypothesis needs verification.

Some relationship between leaf blade vernation and leaf shape was found by Metcalfe (1960) who stated that leaf bud vernation profoundly influenced the structure of the blade, for in those that are conduplicated (folded) in the bud it is usual to find that the midrib project abaxially to form a keel.

What seems to be a better terminology for leaf bud was used by Terrel (1968) who described L. perenne as having "leaf blades folded in young shoots," and L. multiflorum as having "leaf blades rolled in young shoots,"

The value of this character as a complementary identification tool was shown by Nittler and Kenny (1972) who used rolled or folded leaf bud to complete the separation of Italian ryegrass that had not developed leaves or stems, since Italian ryegrass had rolled leaf buds and perennial ryegrass seedlings had folded leaf buds.

Identifications of Tetraploids and Diploids

The discovery by Blakeslee and Avery (1937) that colchicine was an effective agent for inducing chromosome duplication in plants was the "open sesame" for a tremendous increase in number of polyploids of many different plants, grasses, or dicotyledons.

The great desire for polyploid plants emerged a long time ago, with the information from different authors on the increased virtues of those plants over their diploid counterparts, such as large seed,

larger size, greater protein content, more vigor, greater yield, longer-lived, etc. These authors include Wilson (1925), Müntzing (1936), Myers (1947), Rooney and Sullins (1970).

The presence of polyploids in a great number of species elicited a multitude of studies on chromosome counts and relationship of ploidy level and number or size of several characteristics useful in identification of plants. Sax and Sax (1927), Karpechenko (1928) and Randolph (1932) reported correlations between chromosome number and size of pollen mother cells, microspores, stomata, chloroplasts, and stomata frequency.

Chromosome Counting

With the increasing number of polyploids, the methods of chromosome counting became more and more important. Therefore a great number of studies were made and several new techniques or improvements of methods were suggested by numerous authors. McKay and Clarke (1946) suggested the use of pectinase for ease of maceration of root tips of barley for chromosome counting. Wolff and Luippold (1956) improved the squash technique for barley root tips. Latour (1960) adapted Wolff's squash technique for chromosome counting in grasses, using young leaves, and found better results also in ryegrass, since this new technique provided more dividing cells than root tips. Bennet (1964) adapted Latour's

technique, using grass shoot tissues, and found several advantages, such as no chromatid separation or fragmentation, and continuous supply of suitable cells in division not dependent on the season.

Also some specific methods, adaptations or improvements of earlier methods were made for chromosome counting of ryegrass, after the increasing number of tetraploids induced by colchicine. Essad (1962), Ahloovalia (1965) Schoorel and Radersma (1965), and Will, Kronstad and TeKrony (1967) can be listed here for their contributions.

The methods of chromosome counting in dicotyledons or monocotyledons (mainly grasses) received a great deal of criticism. This can be easily seen by the number of different new, adapted, or improved methods suggested by different authors. Fürste (1962), Bennet (1964), Speckman, Post and Dijkstra (1965), Nüesch (1966), Kranski and Bula (1970, and Tan and Dunn (1973) presented several disadvantages of the chromosome counting methods, such as chromosome fragmentation, that disturbed the correct counting as it simulated a higher chromosome number; chromatid separation and fragmentation caused by pretreatment with α-bromonaphtalene; time-consuming and requiring very well-trained personnel; the necessity of limiting the identification to a smaller number of seeds representing a seed lot or cultivar population.

Other plant characteristics have been investigated for possible values in distinguishing tetraploids from diploids.

Stomata size and number. Stomata size and number were emphasized by a number of authors and suggested as a powerful tool for ploidy separation of different species. Sax and Sax (1927), Karpechenko (1929), Randolph (1932), Evans (1955), Ormrod and Rennen (1967), Tan and Dunn (1973) deserve to be listed here for their valuable contributions in this field.

Some criticisms were made by Schwanitz (1952) on the reliability of distinction between diploid and tetraploid plants of several genera using stomata length, due to variation in size within the ploidy levels.

For specific studies in ryegrass, very good contributions were made by Speckman, Post and Dijkstra (1965), who reported that diploid and tetraploids could be separated with a large degree of certainty if the selection was based on the stomata length. They stated that tetraploid plants can be separated from the diploid ones with a large degree of certainty by determining the average stomata length of 10 measurements per plant. Because of the great variation there was an overlapping in the L. multiflorum material, which resulted in an error of about 18%.

Seed size and weight. Barclay and Armstrong (1966) found that in tetraploid Westerwolds seed size is closely related to chromosome

number; the larger seed size of tetraploids results in bigger seedlings, and more dry weight per plant, but the relative growth rate of the diploid is greater than that of the tetraploid.

However, Schoorel and Radersma (1965) had a different opinion, since they observed that although it was relatively easy to distinguish a lot of tetraploid Lolium seeds from a lot of diploid seeds, the size difference proved to be insufficient for identification of individual seeds when data obtained by sieving with split sieves were plotted against frequency.

The use of seed weight to separate ploidy levels in ryegrass was reported by Kranski and Bula (1970). They could separate statistically four of the five tetraploid cultivars from the two diploid cultivars and the tetraploid Petra.

Pollen size and/or density. References to pollen size and/or density related to ploidy level and/or as a tool for separation of tetraploids and diploids in different species were made by Sax and Sax (1927) for Tradescantia caniculata, Evans (1955), in red clover, white clover and lucerne, Funke (1956), in clover, for pollen density, Gould (1957), in the genus Andropogon, Speckmann, Dijkstra, and Kate (1967), in Brassica campestris, and Tan and Dunn (1973), in Bromus inermis.

Specific references on pollen size and/or density related to ploidy level and/or used as identification character for ploidy level

in ryegrass were made by Myers (1939), who used colchicine to induce tetraploids and found that the plants which produced tetraploid root tips also produced giant pollen grains, and Speckman, Post and Dijkstra (1965) who stated that in grasses there is no connection between the number of germinal pores in the pollen and ploidy.

Number and Size of Stomatal Chloroplasts. Number and size of stomatal chloroplasts were related to ploidy level in several species by Sax and Sax (1927), in Tradescantia caniculata, Mochizuki and Suaka (1955) and Dudley (1958) both in sugar beet, Butterfass (1958) and Nüesch (1966) in red clover, Frandsen (1967) in potato, Bingham (1968) in alfalfa.

The only specific reference to ryegrass relating to stomatal chloroplasts was made by Speckman, Post and Dijkstra (1965) who stated that no connection exists between this character and ploidy in grasses. This was confirmed by Tan and Dunn (1973) who stated that chloroplast counts are not feasible in bromegrass. The infeasibility of this method for grasses seems to be related to the clumped distribution of the chloroplasts, making the count impossible.

Leaf Dimensions

Leaf dimension as related to ploidy level and used as a character for distinguishing tetraploid and diploid plants was referred

to only on recent work done by Kranski and Bula (1970). They used several vegetative characteristics to separate diploid and tetraploid cultivars of L. perenne and L. multiflorum and found that leaf width measurements after two weeks of growth at 20° C and 20 h. photoperiods provided the most definitive statistical separation of the cultivars. They used also leaf length, getting some separation, but less effectively than leaf width. No reference was found on the use of leaf area as a complementary or independent tool for ploidy separation in ryegrass or any other species.

Leaf and Seedling Characteristics

The use of leaf buds to separate L. multiflorum from L. perenne was first reported by Bedows (1937), who observed that annual ryegrass normally had hairs right on the apex of the bud, besides more tapering sides, whereas hairs in perennial ryegrass were chiefly on the shoulders, where they can be fairly dense.

A detailed study of the young vegetative plant of L. perenne was made by Soper and Mitchell (1956). They grew plants in full daylight at 58° F. and made a thorough description of the anatomical structure of the leaf, dimensions of the cells, and proportions of the various tissues in the leaf blade, besides the development of the leaf from its initiation to maturity.

The influence of different factors (mainly light and temperature)

was studied by different authors, furnishing good information on how to get a certain result (more length or more width, for example) by the use of proper environments. Mitchell and Soper (1958) found that under shading at high temperature (24° C), leaf blades were almost twice the length and half the width reached in full daylight at lower temperature. Forde (1966) reported differences of 20 and 40 fold in leaf length and area by contrasting light and temperature treatments.

The detailed study of Soper and Mitchell with L. perenne was completed by Evans (1964), who made a complete comparison between the anatomy and morphology of L. multiflorum and L. perenne, grown under two different light levels, concluding that the two species are similar morphologically. The main anatomical differences were those of leaf-blade tissue proportions, particularly sclerenchyma and apex characters. Both species reacted similarly to shading, that is, the number of tillers and the rate of growth were reduced and the leaf and cell lengths were increased. He reported that most of the stomata were situated on the upper side of the blade in rows opposite to air spaces. Unicellular hairs occurred in rows on the margins of the blade and at the apex of each ridge on the upper side.

The great importance of epidermal characteristics for the identification of grasses was emphasized by Davies (1959). He

studied the microscopic characteristics of the leaf of several grasses, and he found that the internerve epidermis of the leaf blade was generally the least affected by growth stage, therefore it was the most useful location for identification purposes. He reported L. multiflorum and L. perenne as species in which the internerve epidermis of the leaf blade was not materially affected by gradation, having neither cork cells nor silico-suberose couples, and without asperities (except at the leaf-tip and margins).

He finally stated that "no epidermal features were found which would serve to distinguish between L. multiflorum, L. perenne, and Festuca pratensis, lowland grasses which present the greatest difficulty in identification by morphological characters."

The use of epidermal characteristics (stomata, hairs, silica cells, buliform cells, etc.) for identification was difficult; use of strips was troublesome and disappointing, since only a small and not exactly defined area of the epidermis could be observed at the same time. Therefore, the new technique of epidermal imprints, devised by Stoddard (1965) brought a new, easy and very useful tool for the identification of plants, based on epidermal characteristics.

He spread a thin film of clear nail polish on the leaf of alfalfa; after it dried it was stripped off of the leaf with forceps. He found that the cell pattern of the upper surface was consistently characteristic of the species or variety.

Differences in maturity of the leaves of ryegrass were a problem for uniformity and comparison of different experimental results. This difficulty was overcome by two detailed genetic works of Edwards (1967 and 1969). His findings can be summarized as follows: (a) response in rate of leaf elongation independently of duration of elongation occurs between L. multiflorum and L. perenne and the basic pattern of leaf formation is very similar in both species; (b) the time of unfolding of a leaf is very closely associated with the time of maturity of the next older leaf on the same side of the apex. Thus a leaf ceases growth when the next younger leaf immediately above it starts elongating rapidly, though which was cause and which was effect it was not possible to say; (c) basic association between the time of maturation of leaf (n) on a shoot and the time of unfolding of leaf (n+2) has not been greatly disturbed by selection; (d) finally he concluded that in all lines studied only two leaves (one on each side of the apex) were elongating rapidly at any one time, and an increase in the rate of unfolding was associated with a decrease in the duration of elongation and vice-versa, and this was the basis of the observed negative correlated response between leaf size and rate of leaf appearance.

The important work of Evans (1964), with emphasis on overall dimensions, tissue proportions and mesophyl and epidermal cells of the leaf blade was partly repeated, in a broader way, and

completed in other aspects by the splendid work of Sant (1969). He made a detailed comparison of the leaf dimensions, number of structural elements, tissue proportions, stomatal and unicellular hair densities, and mesophyll cell number and size in one variety of L. multiflorum and one of L. perenne. He found large differences between these species in leaf dimensions, number of structural elements, and in stomatal and unicellular hair counts, but little difference in the relative proportion of different tissues and in cell size.

He finally stated that

> leaves of L. multiflorum were wider, longer, and had more schlerenchyma fibre groups and vascular bundles than leaves of L. perenne from similar stages. These differences also extended to the frequency of stomata and unicellular hairs in the epidermis. In most characters examined, excluding the tissue proportions and mesophyll cell dimensions, L. multiflorum appeared to be one stage of leaf development ahead of L. perenne up to the third leaf and this gap widened in later leaves.

A paramount proof of the great utility of leaf characteristics for ryegrass identification was given by Kranski and Bula (1970), who reported leaf width and length the best characters (among five different characters) for separation of diploids and tetraploids of L. perenne and L. multiflorum.

MATERIALS AND METHODS

Seed Lots

Seeds of the following species and varieties were used in this study: Lolium multiflorum (diploid: Gulf, Magnolia, Florida, Italian, St. Totori, Ooba-hikari; tetraploid: Barmultra, Tewera, Jolanda, Biliken, Aubade, Tetrone); Lolium perenne (diploid: Viris, Splendor, Linn, Manhattan, Pennfine, Pelo, Game, NK 100, NK 200, Barenza; tetraploid: Massa, Agresso, Reveille, Taptoe); Lolium rigidum (Wimmera 62); Lolium hybridum: (Astor, diploid and Tetrelite, tetraploid.) Seeds were of the certified generation or latter.

Seedlings were produced by germinating seeds on blotter paper at 20° C and 8 h light for 2 days. Seedlings were transferred to peat pellets (6 to 10/pellet), moved to growth chambers at 20° C 20 h light (given by eight fluorescent bulbs, 50 cm above the plants), and randomly distributed. Plants were watered twice a day.

Leaf Measurements

When the third leaf was emerged, the first leaf of 25 plants/variety was cut 5 mm below the ligule top with a sharp scissor. Leaf length was measured with a rule (in mm) and each leaf was cut in half. Width was measured in the middle under 10x magnification, using an ocular reticle. Leaf area was calculated by the formula:

A=. 905 LW (Kemp, 1960), where A=area, L=length, and W=Width. When the fourth leaf was emerged, the second leaf was cut the same way as above.

Leaf Epidermal Characteristics

Epidermal imprints of the upper (adaxial) and lower (abaxial) sides were taken from a 25 mm section of the base of the blade, using nail polish (Revlon 61-clear or Cutex-creme) according to the technique devised by Stoddard (1965), with some adaptations for better and faster imprints with ryegrass.

The 25 mm of leaf was laid on a microscope slide (with upper or lower side of leaf up, for upper or lower side imprints) and fastened at both ends with masking tape strips. Care was taken to keep the leaf evenly flat. With a smooth brush, three to six strokes (depending on greater or smaller thickness of nail polish) were given to cover the epidermis; all strokes were given always in the same direction, to give an even coverage of the leaf epidermis, and to avoid air bubbles. Excess nail polish extending past the leaf edges were removed with a razor blade to avoid later sticking and tearing of imprint. After about 45 minutes drying, the top end of the film was lifted with a thin dissecting needle, the sides were loosened (by sliding the needle underneath the film, from top toward base) and the film was peeled off by grasping the top end with a sharp tweezers

and pulling slowly. The imprint was laid on the same microscope slide with the leaf evenly flattened, fastened with tape strips, and identified by number.

Ten imprints of the upper side and five of the lower side were made for each variety. They were stored in slide boxes and lasted indefinitely. For examination under a microscope, they were observed dry without a cover slide.

Stomata and unicellular hair densities were measured, under 100x, in the imprints of the upper side of leaf 2 (10 imprints/variety). The area of observation was set by focusing under 100x, on the first stomata row of the upper edge, close to the leaf sheath. The microscope slide was then moved two ocular reticles (about 2 mm) from the first stomata (the closest to the sheath). The ocular (10x) was changed to 20x, thus the countings were made under 200x. (It was impossible to make the counting under 100x because the leaves of perennial varieties were too narrow to fill the total field of the microscope.) The area under 200x had as a reference starting point the upper edge of the leaf and was calculated as equal to about 1.13 mm^2.

Stomata lengths were measured (5/leaf, 50/variety) by focusing the same way as above, under 100x, the first row of stomata, close to the upper edge of the leaf; the magnification was then changed to 450x and measurements made from the sequence of five stomata of

the leaf, from base to tip. Care was taken to measure only the very clear stomata in the film. When it was not possible (in a very few cases) to measure adequately the stomata of the first row in that area, the second row was used. Pictures were taken of the first row of stomata, close to the upper edge, to show the different densities of stomata and unicellular hairs.

Leaf Blade Vernation

Plants at the third or fourth leaf stage (about 14 and 21 days, respectively, after sowing) were cut with a scissors or razor blade as close as possible to the ground.

On a piece of soft wood, a transverse cut was made, 5 mm from the base avoiding distortion, and the slices were observed under 45x. Twenty-five plants/variety were cut and the leaf bud vernation (rolled or folded) was recorded.

Vascular Bundles and Sclerenchyma Fiber Groups

When the fourth leaf was emerged, leaf 2 was separated from the plant by cutting its sheath with a scissors (10 leaves/variety). By using a very sharp razor blade, each blade was transversely cut, as close to the sheath as possible, stained for 30 to 60 seconds in a weak safranin solution, rinsed in water, and placed, with a small drop of water, on a microscope slide. Sclerenchyma and vascular

bundles were observed under 45x or 100x and counted. Special care was taken when counting. Only bundles completely formed and very distinct were counted; the vascular bundles were classified as major when they had the three distinct xylem vessels. The presence of even a single sclerenchyma cell was considered as a sclerenchyma fiber group.

Statistical Analysis

A Tukey's test, also known, according to Steel and Torrie (1960), as the "honestly significant difference (hsd) procedure", was used for the data to determine statistical differences between the 4 species, the 7 ploidy levels, and the 30 varieties.

RESULTS

Identification by Leaf Dimensions

First Leaf Width

Species. Table 1 shows that first leaves of L. multiflorum seedlings (1.6 mm) were wider than those of L. perenne (1.21 mm) and L. hybridum (1.46 mm), but approximately equal to those of L. rigidum (1.61 mm). The widest average was shown by Wimmera 62 (L. rigidum) and the narrowest average was shown by L. perenne.

The individual plant measurements of L. multiflorum and L. perenne, showed a great deal of overlap (Fig. 1).

Ploidy level. Table 1 shows that tetraploid seedlings of L. multiflorum and L. perenne had wider leaves, on the average, than those of diploid seedlings. The single tetraploid and diploid varieties of L. hybridum were not significantly different in leaf width (Table 1).

Although mean leaf width differed significantly at the ploidy level (Fig. 2) individual leaf widths of L. multiflorum vary a great deal so that a positive identification of ploidy level was not always possible when based only on this character. This result confirms, in a broader way, the results of Kranski and Bula (1970), who found statistically significant differences between leaf widths of seven varieties of L. perenne and L. multiflorum of different ploidy levels.

Table 1. Width of first leaf of 30 ryegrass varieties. Figures are means of 25 observations per variety

Species and varieties	Ploidy level	Leaf width (mm)	
Lolium multiflorum			
Billiken	Tetraploid	1.91	
Aubade	"	1.86	
Jolanda	"	1.78	
Tewera	"	1.74	
Barmultra	"	1.72	
Tetrone	"	1.64	
		1.77	
St. Tottori	Diploid	1.73	
Ooba-hikari	"	1.45	
Magnolia	"	1.40	St. Tottori*
Florida Rust-resistant	"	1.36	
Italian	"	1.34	
Gulf	"	1.22	
		1.42	L. multiflorum tetraploids**
Overall mean		1.60	
Lolium perenne			
Reveille	Tetraploid	1.54	
Taptoe	"	1.41	
Massa	"	1.40	
Agresso	"	1.27	
		1.40	
Linn	Diploid	1.41	
Viris	"	1.29	
Norlea	"	1.28	
Pelo	"	1.20	
Game	"	1, 17	
Manhattan	"	1. 16	
NK 100	"	1. 16	
Pennfine	"	1. 16	
Barenza	"	1. 14	
Splendor	"	1. 13	
NK 200	"	1.09	
		1.20	L. perenne tetraploids**
Overall mean		1.25	L. multiflorum**
Lolium hybridum			
Tetrelite	Tetraploid	1.44	
Astor	Diploid	1.48	
		1.46	L. multiflorum** L. perenne**
Lolium rigidum			
Wimmera 62	Diploid	1.61	L. perenne**

** significant at 1% level * significant at 5% level

Each variety or species or ploidy level, listed at right, differs significantly from those of the same group on the same horizontal line or lower.

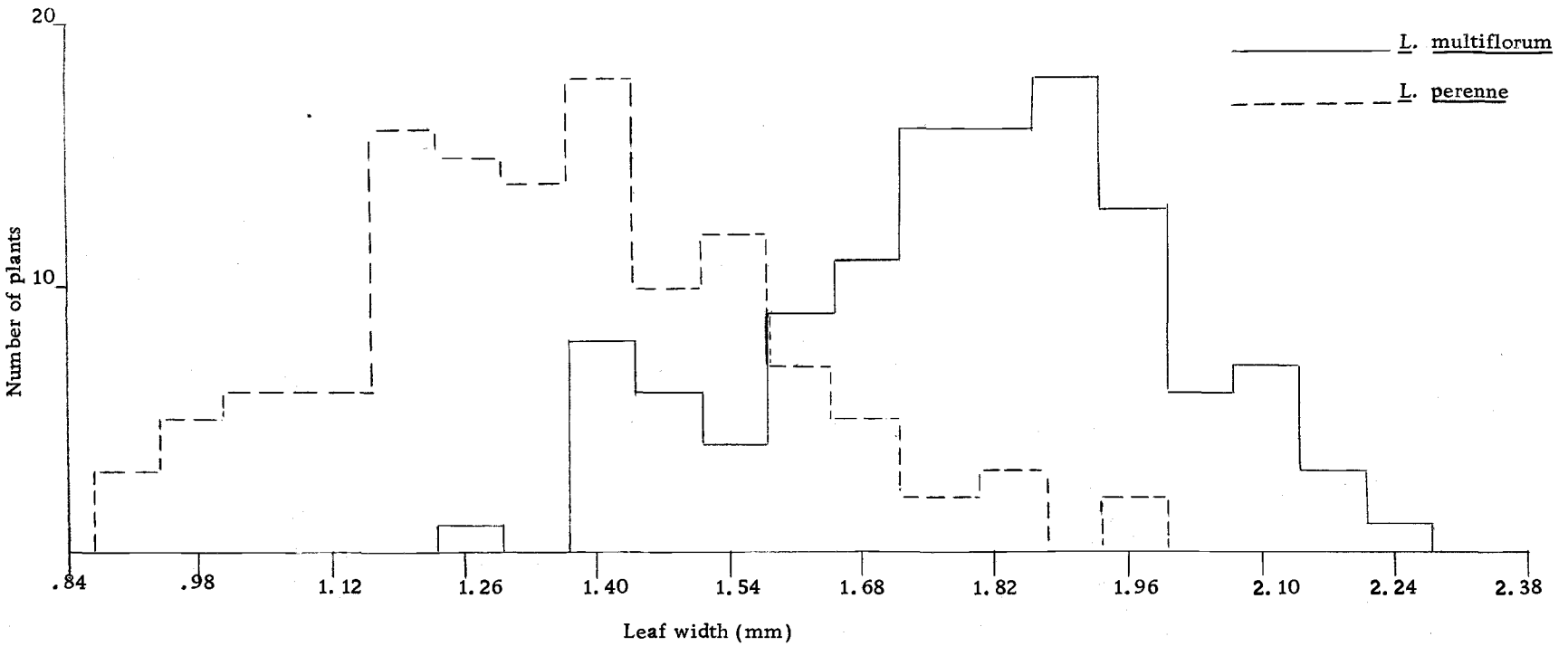


Figure 1. Frequency distribution of first leaf width of 25 plants each of 12 varieties of Lolium multiflorum and 12 varieties of Lolium perenne.

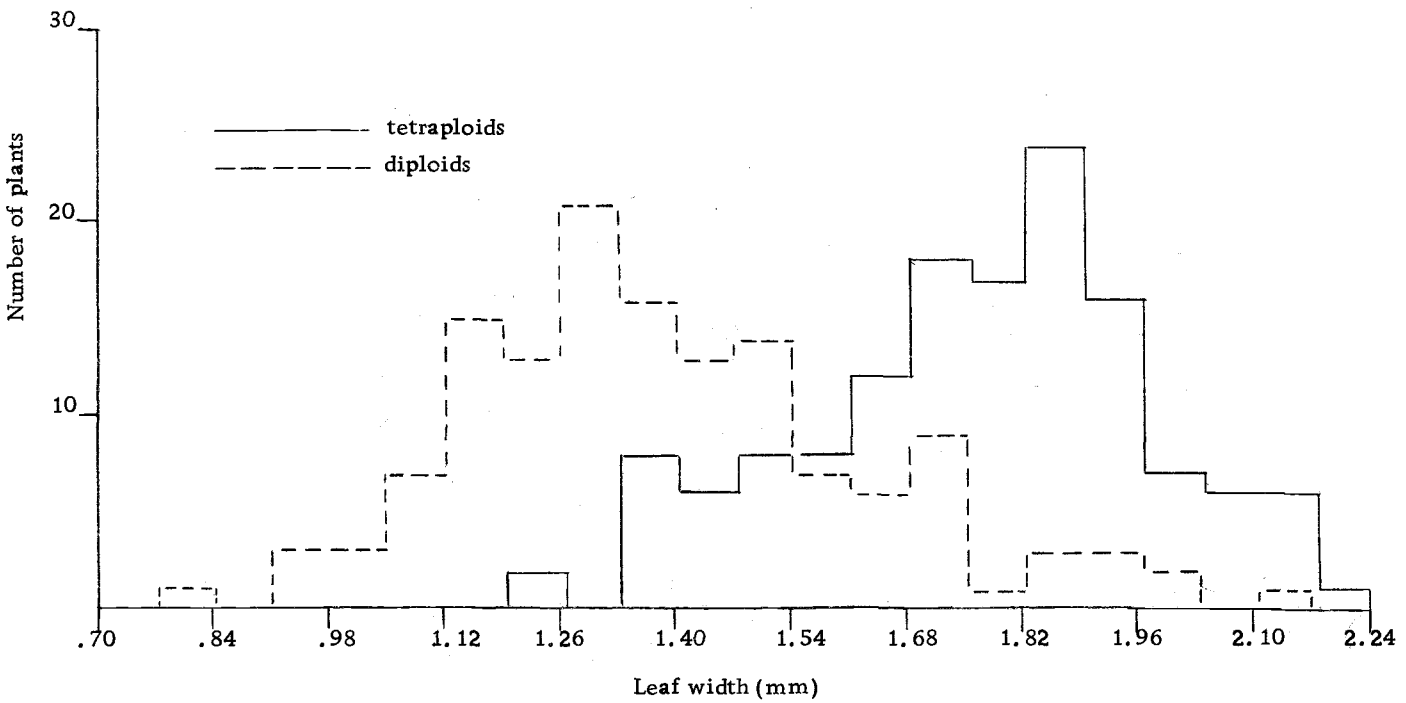


Figure 2. Frequency distribution of first leaf width of 25 plants each of six tetraploid and six diploid varieties of Lolium multiflorum.

Varieties. The diploid variety of L. multiflorum with the widest leaves was St. Tottori, which had a leaf width significantly different from four of the five other varieties in this group. Among L. perenne varieties, Reveille had the widest leaf but was significantly different from only one variety. No significant difference occurred in leaf width among L. perenne tetraploid or diploid varieties (Table 1).

First Leaf Length

Species. L. multiflorum, as shown in Table 2, had longer first leaves (98.57 mm) on the average, than L. perenne (71.72 mm) and L. hybridum (90.50 mm). Leaves of L. perenne were significantly shorter than the leaves of L. rigidum seedlings. The average leaf length of L. multiflorum and L. rigidum were not significantly different; neither was the average leaf length of L. hybridum and L. rigidum seedlings.

Ploidy level. Leaves of tetraploid seedlings of L. multiflorum and L. perenne, as shown in Table 2, were significantly larger than those of diploids within the same species. However, the single diploid and tetraploid varieties of L. hybridum did not show a significant difference in leaf length (Table 2).

Although mean lengths of tetraploid and diploid leaves varied, individual plant measurements overlapped so that individual plants

Table 2. Length of first leaf of 30 ryegrass varieties. Figures are means of 25 observations per variety

Species and varieties	Ploidy level	Leaf length (mm)	
Lolium multiflorum			
Barmultra	Tetraploid	122.08	
Aubade	"	120.56	
Tetrone	"	111.48	
Jolanda	"	107.60	
Billiken	"	99.26	Barmultra*
Tewera	"	88.72	Aubade* Tetrone *
		108.37	
St. Tottori	Diploid	106.84	
Ooba-hikari	"	97.44	
Gulf	"	96.88	
Florida Rust-resistant	"	80.64	St. Tottori*
Magnolia	"	75.92	
Italian	"	74.88	Ooba-hikari* Gulf*
		88.77	L. multiflorum tetraploids**
Overall mean		98.57	
Lolium perenne			
Reveille	Tetraploid	91.36	
Massa	"	80.04	
Taptoe	"	79.92	
Agresso	"	78.96	
		82.57	
Norlea	Diploid	98.64	
Viris	"	72.04	Norlea*
Barenza	"	70.12	
NK 200	"	68.80	
Linn	"	67.82	
Game	"	65.76	
NK 100	"	64.80	
Splendor	"	64.60	
Manhattan	"	58.98	
Pennfine	"	57.68	
Pelo	"	56.68	
		67.99	L. perenne tetraploids**
Overall mean		71.72	L. multiflorum**
Lolium hybridum			
Tetrelite	Tetraploid	92.16	
Astor	Diploid	88.84	
Overall mean		90.50	L. multiflorum**
			L. perenne**
Lolium rigidum			
Wimmera 62	Diploid	91.12	L. perenne**

* significant at 5% level ** significant at 1% level

Each variety or species or ploidy level, listed at right, differs significantly from those of the same group on the same horizontal line or lower.

could not be identified by this character alone (Fig. 3).

Varieties. Among tetraploid varieties of L. multiflorum, Barmultra had the longest first leaf on the average, differing significantly from Billiken and Tewera. Among diploids of L. multiflorum, St. Tottori showed the longest leaf, significantly longer than those of three other diploid varieties. Ooba-hikari and Gulf had longer first leaves than those of Italian. Among diploids of L. perenne, Norlea had the longest first leaves, significantly different from those of all other diploid varieties.

Leaf Area

Species, ploidy levels and varieties varied more in leaf area than in any of the other characteristics studied.

Species. As shown in Table 3, the first leaves of L. multiflorum (145.15 mm^2) had the largest area, followed by L. rigidum (133.30 mm^2) and L. hybridum (121.42 mm^2). L. perenne leaves had the smallest area (82.85 mm^2), significantly smaller than the area of the first leaf of seedlings of the other three species.

Ploidy level. Table 3 shows that leaves of tetraploid seedlings were larger in area than those of diploids in both L. multiflorum and L. perenne; however, the single tetraploid and diploid varieties of L. hybridum did not show a significant difference in leaf area. Individual leaves, however, vary widely (Fig. 4), thus no complete

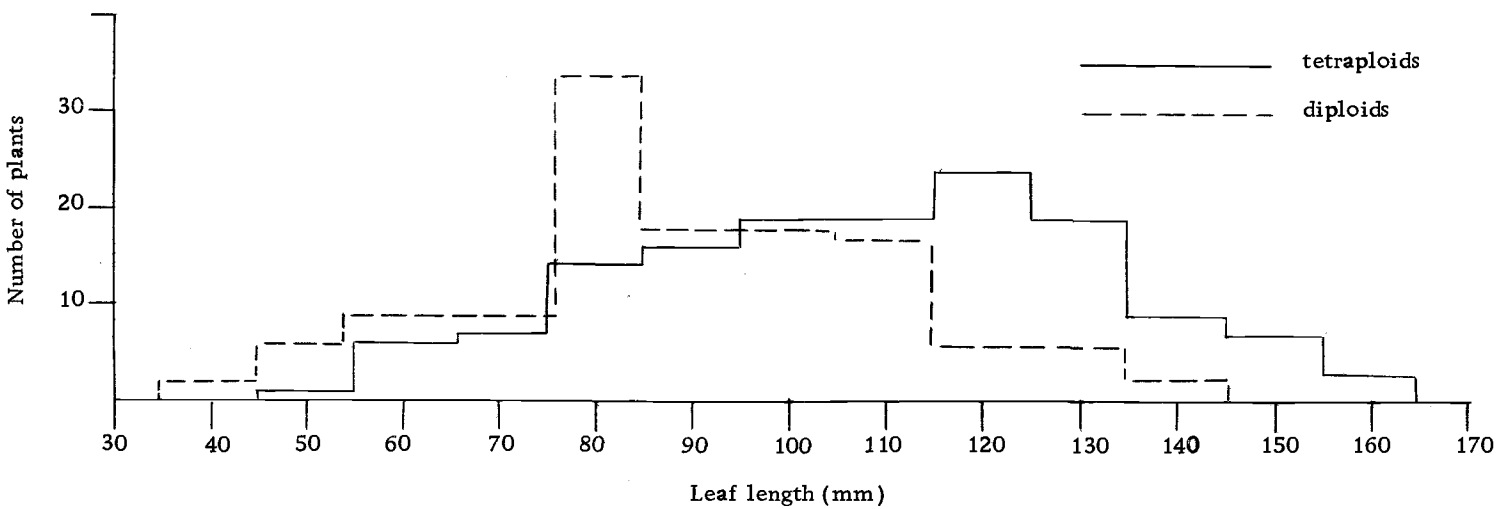


Figure 3. Frequency distribution of first leaf length of 25 plants each of six tetraploid and six diploid varieties of Lolium multiflorum.

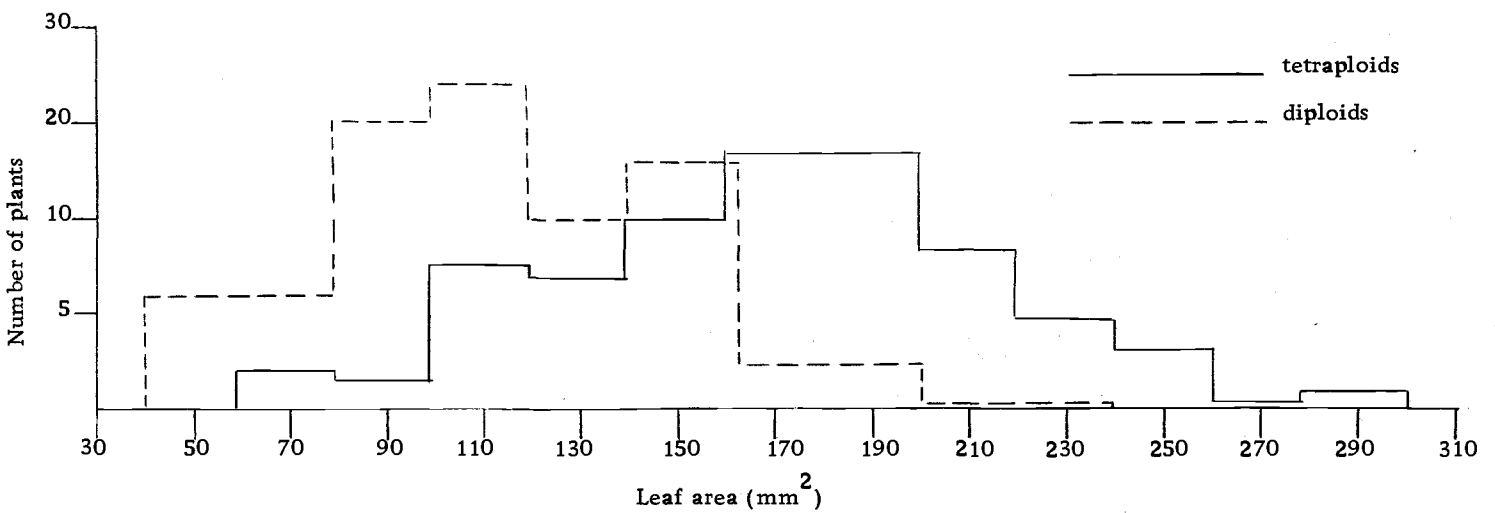


Figure 4. Frequency distribution of first leaf area of 25 plants each of six tetraploid and six diploid varieties of Lolium multiflorum.

Table 3. Area of first leaf of 30 ryegrass varieties. Figures, calculated by the formula, Area=.905 width x length, are means of 25 observations per variety.

Species and varieties	Ploidy level	Area (mm^2)
Lolium multiflorum		
Aubade	Tetraploid	202.62
Barmultra	"	191.10
Billiken	"	172.83 Aubade** Barmultra**
Jolanda	"	172.58
Tetrone	"	166.92
Tewera	"	140.80 Billiken** Jolanda** Tetrone**
		174.48
St. Tottori	Diploid	166.29
Ooba-hikari	"	127.51 St. Tottori**
Gulf	"	113.13
Florida Rust-resistant	"	99.31 Ooba-hikari**
Magnolia	"	96.44 Gulf**
Italian	"	92.31
		115.83 L. multiflorum tetraploids**
Overall mean		145.15
Lolium perenne		
Reveille	Tetraploid	122.92
Taptoe	"	103.07 Reveille**
Massa	"	102.06
Agresso	"	91.18
		104.80
Norlea	Diploid	115.01
Linn	"	87.12 Norlea**
Viris	"	84.81
Barenza	"	72.92
Game	"	70.82 Linn**
NK 200	"	68.54 Viris**
NK 100	"	62.94
Splendor	"	66.39
Manhattan	"	62.30
Pelo	"	61.74
Pennfine	"	60.85
		73.95 L. perenne tetraploids**
Overall mean		82.85 L. multiflorum**
Lolium hybridum		
Tetrelite	Tetraploid	122.60
Astor	Diploid	120.25
		121.42 L. multiflorum**
		L. perenne**
Lolium rigidum		
Wimmera 62	Diploid	133.30 L. perenne**

** significant at 1% level

Each species, ploidy level or variety, listed at right, differs significantly from other species, ploidy level or varieties of the same group on the same horizontal line or lower.

and distinctive identification could be accomplished by this characteristic alone.

Varieties. Within the tetraploid varieties of L. multiflorum, Aubade (202.62 mm^2) and Barmultra (191.10 mm^2) had leaf areas significantly larger than those of the other varieties, while Tewera (140.80 mm^2) had the smallest first leaf area, significantly smaller than those of the other five tetraploid varieties. Within the diploids of L. multiflorum, St. Tottori (166.29 mm^2) had the largest area, significantly larger than those of all other diploid varieties. Italian (92.32 mm^2) and Magnolia (96.44 mm^2) showed the smallest first leaf area, significantly smaller than all the other diploids of L. multiflorum.

Within the tetraploids of L. perenne (Table 3) Reveille (122.92 mm^2) had the largest area, significantly larger than all the other tetraploid varieties. Within the diploids, Norlea leaves had the largest area, significantly larger than all the other diploid varieties. The turf type varieties were smallest in first leaf area (Table 3).

Microscopical Characteristics of the Leaf Epidermis

Unicellular Hair Density

Species. As seen in Table 4, the average number of unicellular hairs in an area of 1.13 mm^2 was significantly larger (13.82 with

Table 4. Number of unicellular hairs per 1.13 mm^2 of second leaf of 30 ryegrass varieties. Figures are means of 10 observations per variety

Species and varieties	Ploidy level	Number	
Lolium multiflorum			
Billiken	Tetraploid	18.1	
Tewera	"	16.7	
Jolanda	"	14.0	
Aubade	"	13.7	
Tetrone	"	12.2	
Barmultra	"	11.1	
		14.4	
Italian	Diploid	18.0	
Gulf	"	14.1	
Ooba-hikari	"	13.2	
Florida Rust-resistant	"	12.2	
Magnolia	"	12.1	
St. Tottori	"	10.2	
		13.3	
Overall mean		13.8	
Lolium perenne			
Taptoe	Tetraploid	6.7	
Reveille	"	3.4	
Massa	"	2.6	
Agresso	"	2.1	
		3.9	
Pennfine	Diploid	9.9	
NK 200	"	8.5	
Norlea	"	8.3	
NK 100	"	4.1	
Barenza	"	4.0	
Viris	"	3.7	
Game	"	3.3	
Linn	"	3.3	
Manhattan	"	3.3	
Pelo	"	2.4	
Splendor	"	2.3	
		4.8	
		4.5	L. multiflorum**
Lolium hybridum			
Tetrelite	Tetraploid	5.6	
Astor	Diploid	12.4	
Overall mean		9.0	
Lolium rigidum			
Wimmera 62	Diploid	10.3	

** significant at 1% level.

Species listed at right differ significantly from the other species on the same line.

range of 18.1 to 10.2) in L. multiflorum than in L. perenne (4.53, with range of 9.9 to 2.1). An enormous variation occurred among individual plant measurements, reducing the usefulness of this character for separating L. multiflorum and L. perenne easily and accurately (Fig. 5 and Pl. 1 and 2).

No significant differences occurred in unicellular hair density of the second leaf among diploids and tetraploids of any of the three species, or among varieties within those groups (Table 4).

Stomata Density

Species: Table 5 shows that the four species do not differ significantly in number of stomata in an area of 1.13 mm^2 of the second leaf.

Ploidy level. As shown in Table 5, the second leaves of tetraploid seedlings had, on the average, fewer stomata per area of 1.13 mm^2 than the diploids in L. multiflorum, L. perenne, and L. hybridum. Among the 12 characteristics studied, stomata density was most closely related to ploidy level within all three species. However, on an individual plant basis, a great overlap occurred (Fig. 6), thus reducing the feasibility of using this character alone for ploidy level identification (Pl. 3, 4, and 5).

Varieties. No significant differences were found between varieties of the three species, although differences between some

Table 5. Number of stomata per 1.13 mm^2 of second leaf of 30 ryegrass varieties. Figures are means of 10 observations per variety

Species and varieties	Ploidy level	Number
Lolium multiflorum		
Tetrone	Tetraploid	34.5
Billiken	"	34.3
Tewera	"	34.1
Barmultra	"	33.9
Aubade	"	32.5
Jolanda	"	31.8
		32.5
Italian	Diploid	52.4
Magnolia	"	47.4
Florida Rust-resistant	"	46.0
St. Tottori	"	37.1
Ooba-hikari	"	35.9
Gulf	"	34.0
		42.1 L. multiflorum tetraploids**
Overall mean		37.8
Lolium perenne		
Reveille	Tetraploid	30.1
Massa	"	28.0
Taptoe	"	27.7
Agresso	"	24.7
		27.6
Pennfine	Diploid	46.0
Norlea	"	44.4
Game	"	43.8
Manhattan	"	42.1
Viris	"	41.4
Pelo	"	41.2
NK 200	"	39.6
Linn	"	38.6
Barenza	"	38.0
Splendor	"	37.2
NK 100	"	35.4
		40.7 L. perenne tetraploids**
Overall mean		37.2
Lolium hybridum		
Tetrelite	Tetraploid	27.7
Astor	Diploid	46.6 Astor**
Overall mean		37.1
Lolium rigidum		
Wimmera 62	Diploid	30.5

** significant at 1% level. Ploidy level or variety listed at right differs significantly from ploidy level or varieties on the same line.

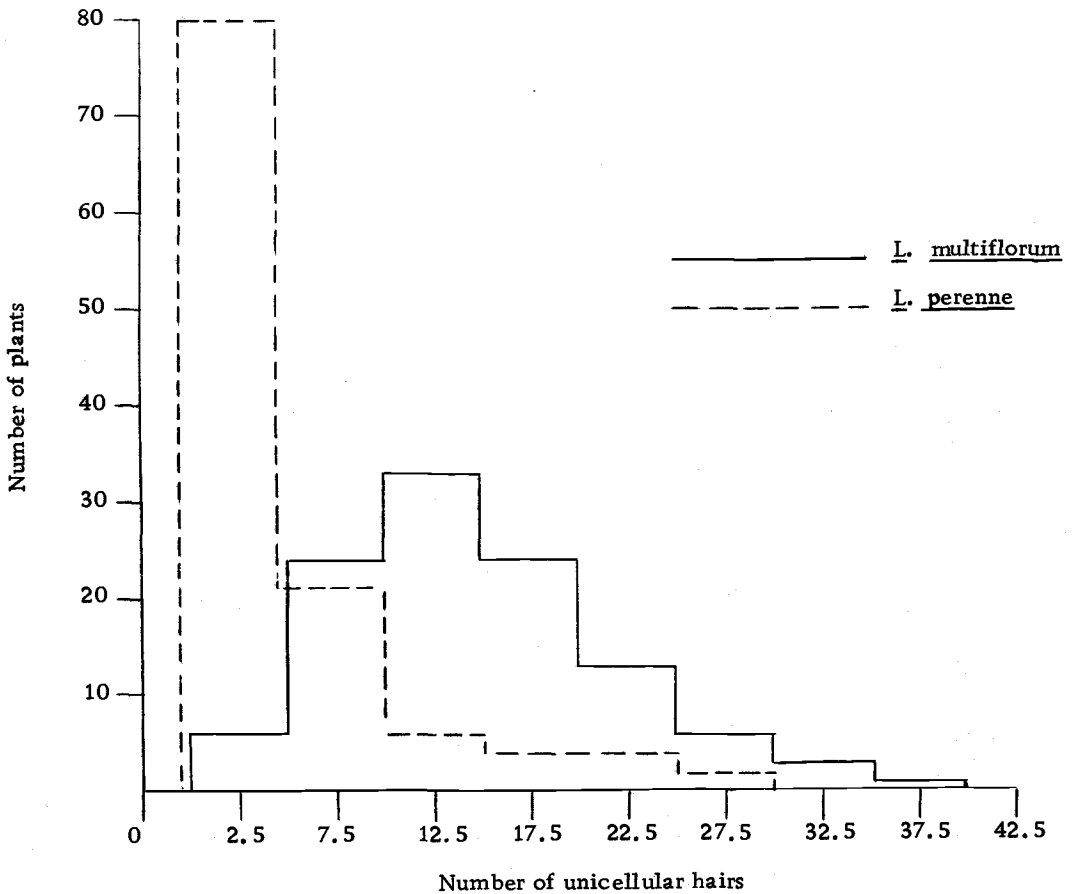


Figure 5. Frequency distribution of number of unicellular hairs per 1.13 mm^2 of 10 second leaves each of 12 varieties of Lolium multiflorum and 12 varieties of Lolium perenne.

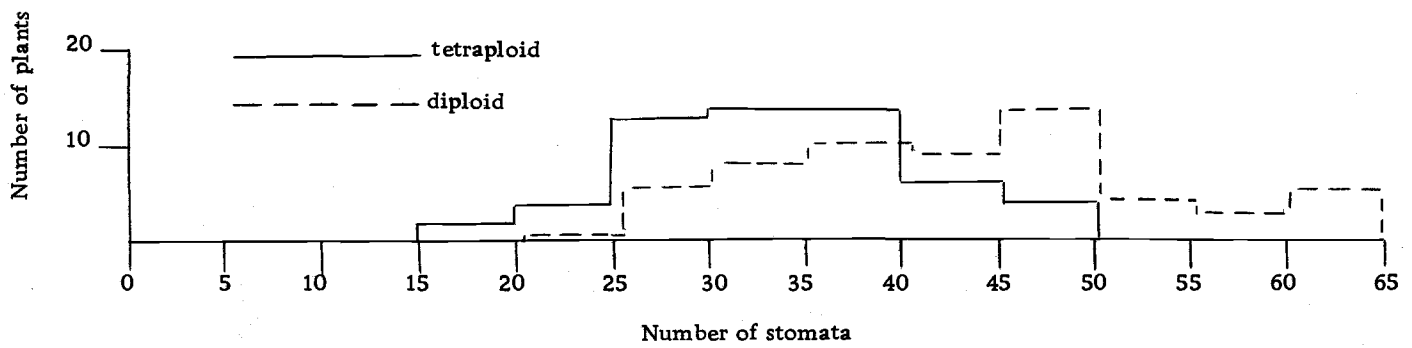


Figure 6. Frequency distribution of number of stomata per 1.13 mm^2 of ten second leaves each of six tetraploid and six diploid varieties of Lolium multiflorum.

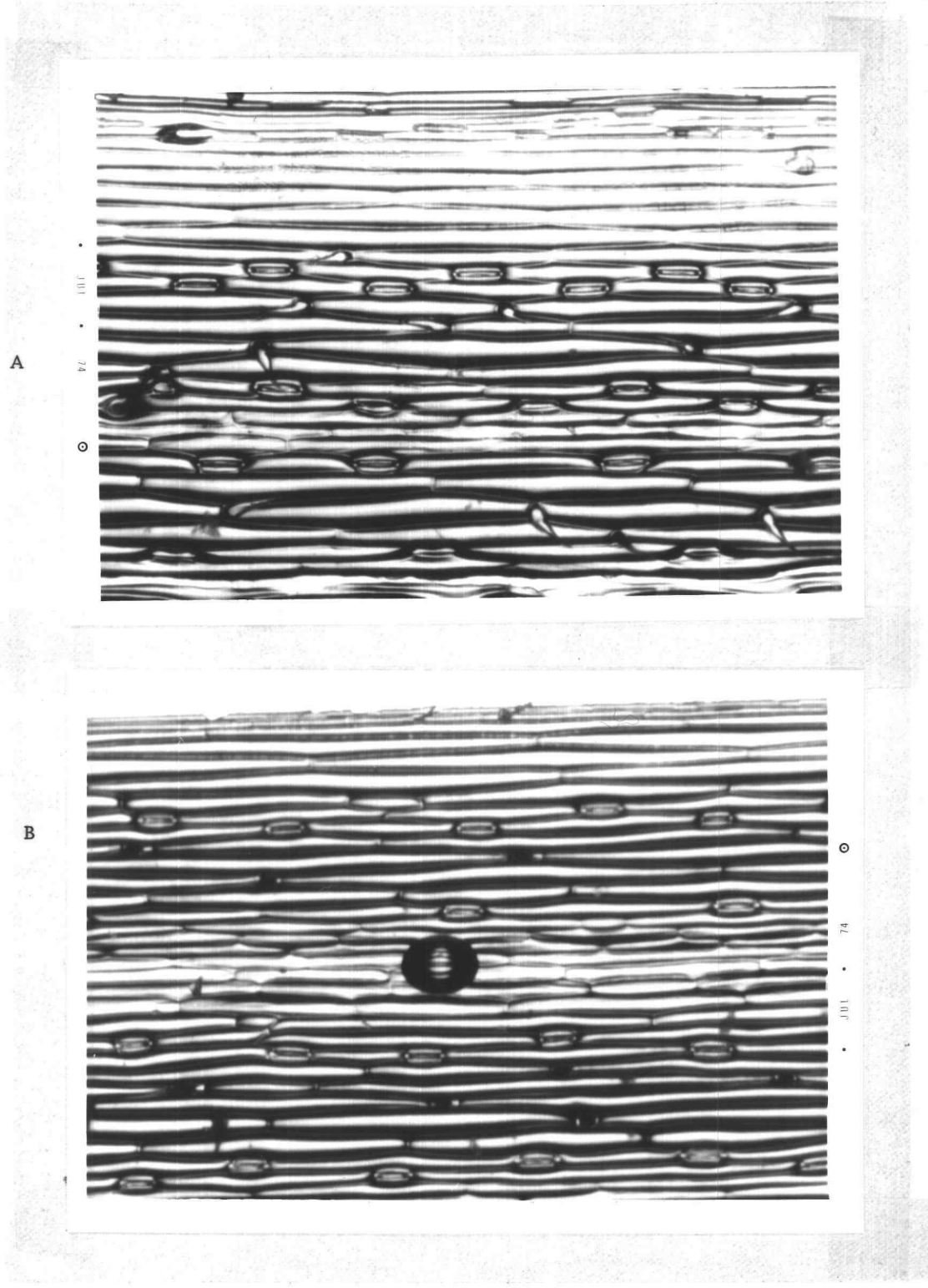
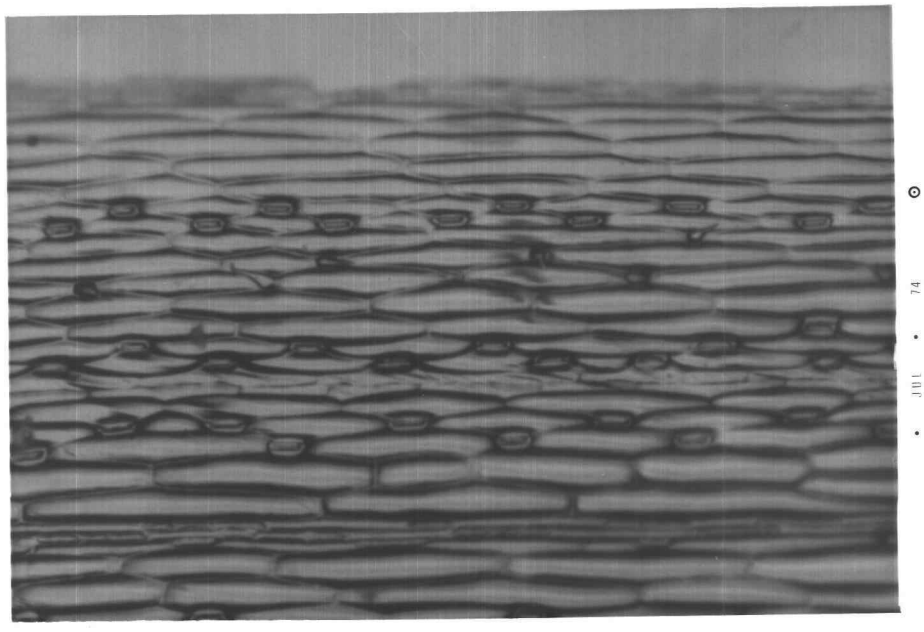


Plate 1. Number of hairs per area in two species of ryegrass
A Lolium multiflorum (Aubade)

B Lolium perenne (Pennfine)

A



B

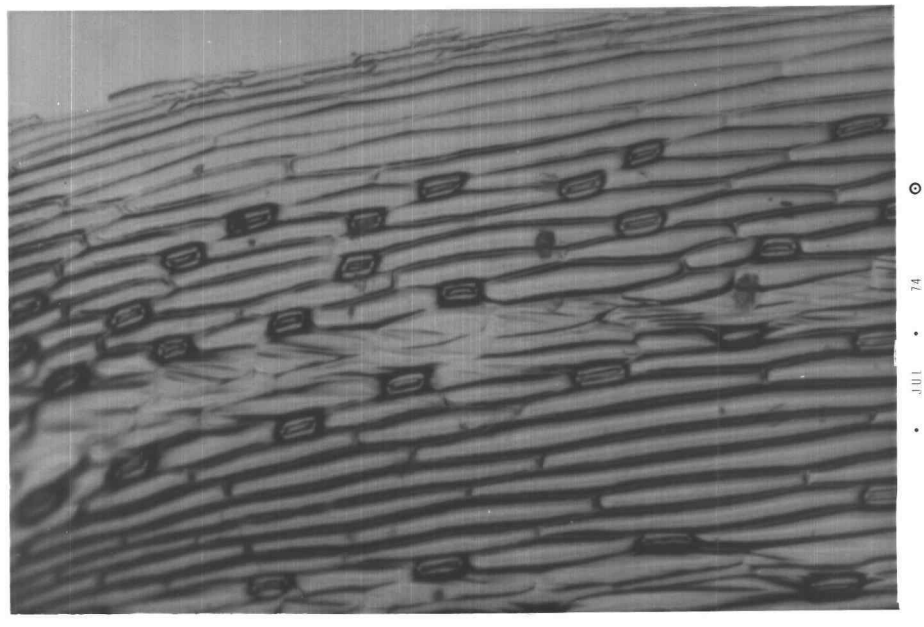
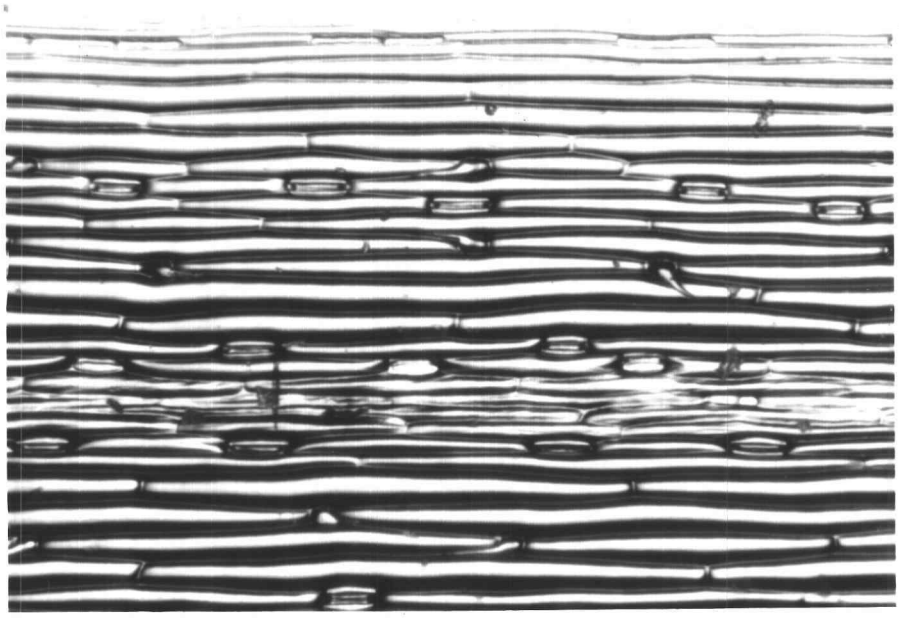


Plate 2. Number of hairs per area in two species of ryegrass

A Lolium multiflorum (Magnolia)

B Lolium perenne (Massa)

A



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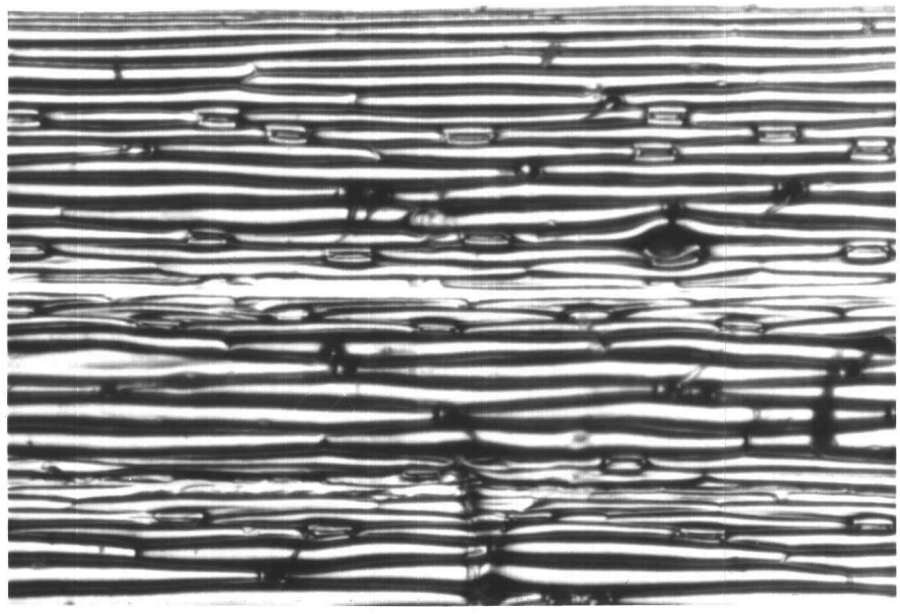


Plate 3. Number of stomata per area in tetraploid and diploid of two species of ryegrass.

A Lolium multiflorum tetraploid (Barmultra)

B Lolium multiflorum diploid (Florida)

A

B

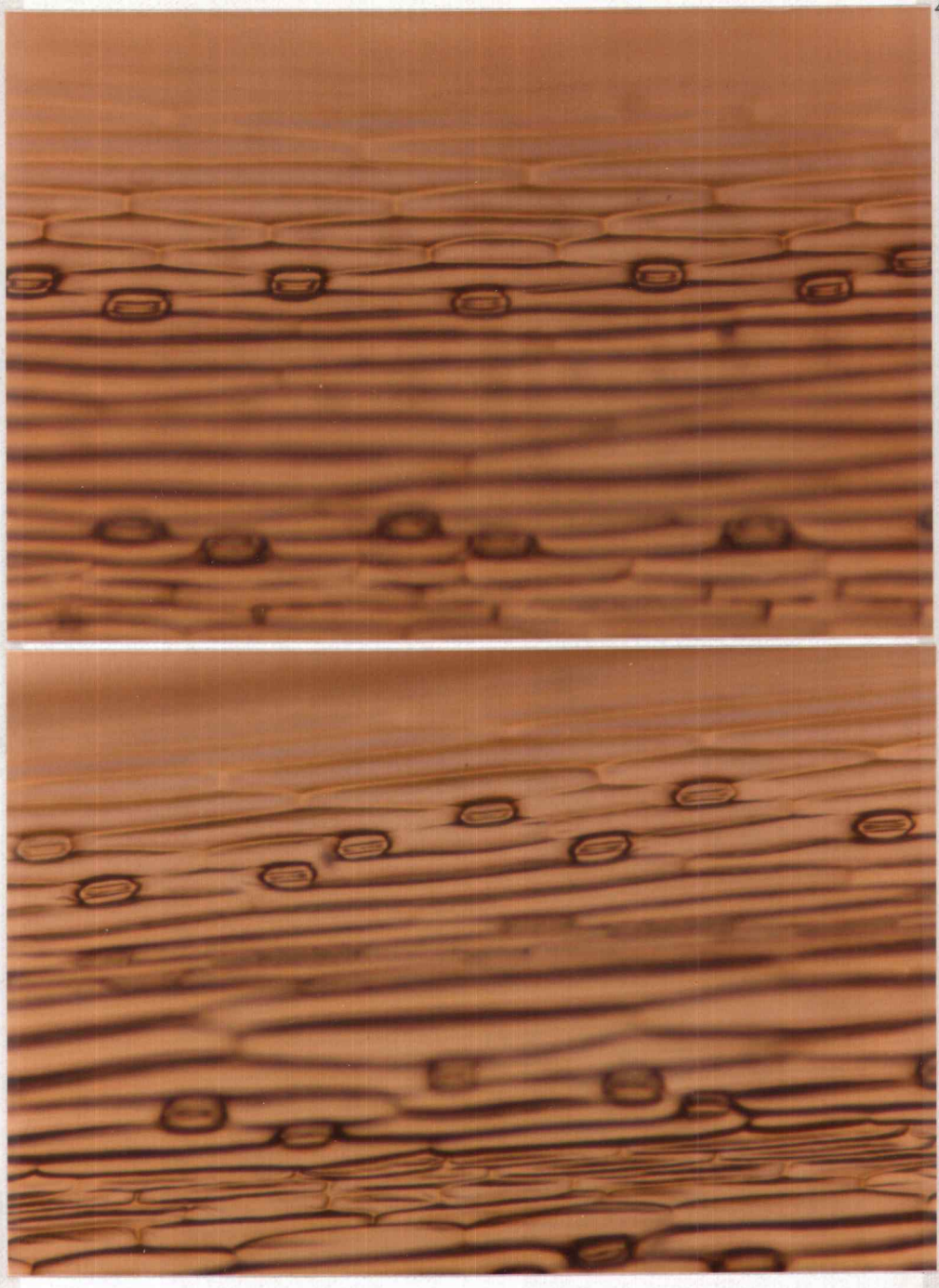
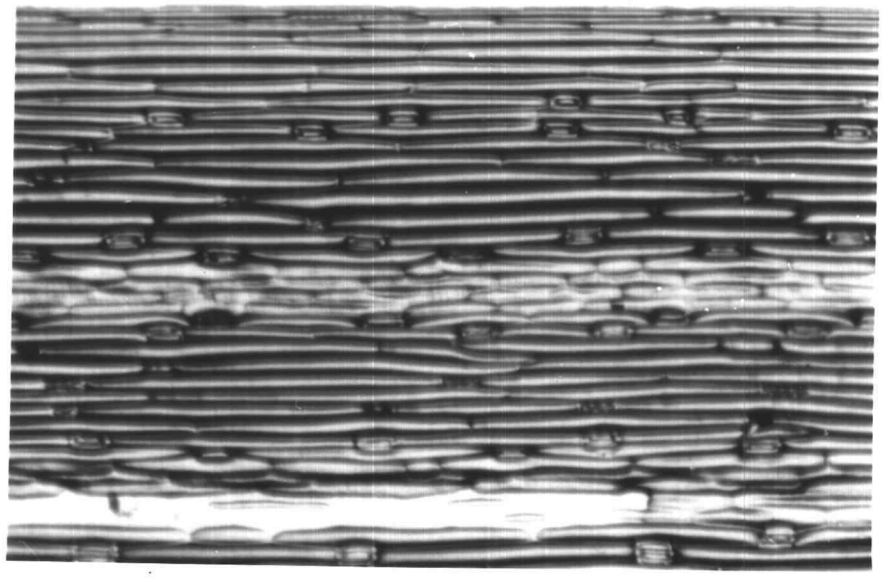


Plate 4. Number of stomata per area in tetraploid and diploid of two species of ryegrass.

A Lolium perenne tetraploid (Reveille)

B Lolium perenne diploid (Linn)

A



B

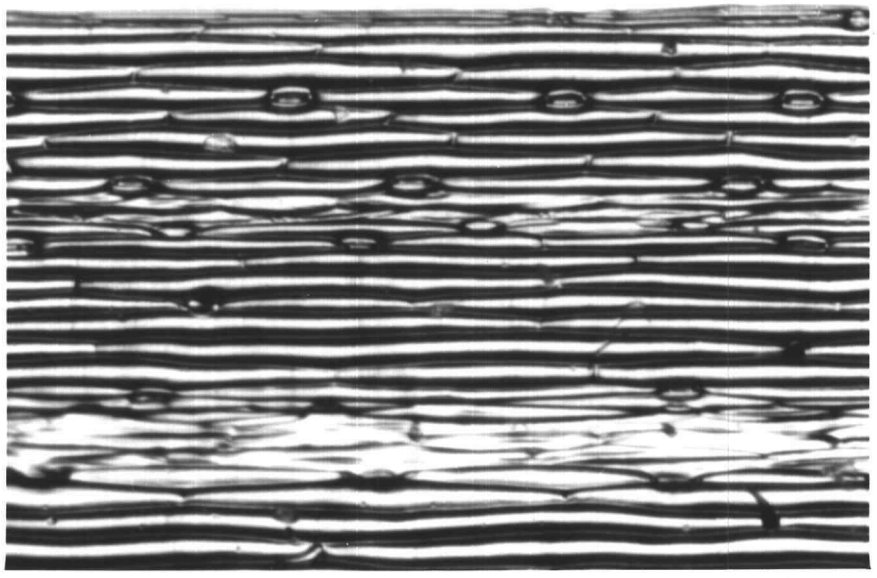


Plate 5. Epidermal characteristics of two ryegrass species.

A Lolium perenne (Manhattan)
B Lolium hybridum (Tetrelite)

varieties within L. multiflorum and L. perenne were considerable (Table 5).

Stomata Length

Species. The average stomata length did not vary significantly among the species, although L. rigidum and L. perenne had a large average difference (. 152 mm. for L. rigidum against . 109 mm for L. perenne) (Table 6).

Ploidy level. No relationship was found between stomata length and ploidy, diploid and tetraploid means for L. multiflorum and L. perenne being similar. Astor (diploid) and Tetrelite (tetraploid), the varieties of L. hybridum, showed differences in mean stomata length but the small number of observations and the great plant-to-plant variation increased the Least Difference, requiring large values for significance. These data do not agree with results reported by Speckman, Post and Dijkstra (1965), who found significant differences between stomata length of diploids and tetraploids in two varieties of L. multiflorum and two of L. perenne. Perhaps the disagreement of results are due to (a) different statistical tests, the L. S. D. test compared with the more conservative Tukey's test used here; (b) the kind and number of varieties used: they worked with only two varieties of each species, whereas 12 of L. multiflorum and 15 of L. perenne were used here; (c) number of measurements: they made

Table 6. Stomata length in the second leaf of 30 ryegrass varieties. Figures are means of five measurements per plant and 10 plants per variety

Species and varieties	Ploidy level	Length (mm)
Lolium multiflorum		
Billiken	Tetraploid	.142
Barmultra	"	.133
Tetrone	"	.130
Jolanda	"	.130
Tewera	"	.116
Aubade	"	.099
		.125
Florida Rust-resistant	Diploid	.146
Gulf	"	.134
Italian	"	.128
St. Tottori	"	.112
Magnolia	"	.106
Ooba-hikari	"	.101
		.121
Overall mean		.123
Lolium perenne		
Taptoe	Tetraploid	.115
Massa	"	.114
Reveille	"	.099
Agresso	"	.098
		.106
Linn	Diploid	.130
Pelo	"	.129
Splendor	"	.126
NK 100	"	.123
Manhattan	"	.121
Game	"	.120
Barenza	"	.109
Viris	"	.100
Norlea	"	.094
Pennfine	"	.077
NK 200	"	.074
		.109
Overall mean		.109
Lolium hybridum		
Tetrelite	Tetraploid	.114
Astor	Diploid	.150
Overal mean		.131
Lolium rigidum		
Wimmera 62	Diploid	.152

25 measurements (5/each 5 leaves of each plant), whereas five (only 1 leaf/plant) were made here.

Varieties. No significant difference was observed in stomata length among diploid or tetraploid varieties of L. multiflorum, L. perenne, and L. hybridum (Table 6).

Vascular Bundles and Sclerenchyma Fiber Groups

Major Vascular Bundles

Species. The average number of major vascular bundles in the second leaf was significantly higher (3.82 with range of 5.8 to 3.1) in L. multiflorum than in L. perenne (3.26 with range of 4.5 to 2.8). No significant difference was found between average number of vascular bundles among other species (Table 7).

Ploidy level. The second leaves of tetraploid seedlings had significantly more major vascular bundles than leaves of diploid in L. hybridum.

Varieties. No significant differences were found in number of major vascular bundles in L. multiflorum, L. perenne, and L. hybridum varieties (Table 7).

Minor Vascular Bundles

Species. As seen in Table 8, the second leaves of L. multiflorum showed the highest number (4.23) of minor vascular bundles,

Table 7. Number of major vascular bundles in second leaf of 30 ryegrass varieties. Figures are means of 10 observations per variety

Species and varieties	Ploidy level	Number
Lolium multiflorum		
Tewera	Tetraploid	5.1
Jolanda	"	4.0
Billiken	"	3.9
Barmultra	"	3.6
Aubade	"	3.6
Tetrone	"	3.4
		3.9
Magnolia	Diploid	4.8
Gulf	"	4.1
Ooba-hikari	"	3.5
St. Tottori	"	3.4
Italian	"	3.3
Florida Rust-resistant	"	3.1
		3.7
Overall mean		3.8
Lolium perenne		
Reveille	Tetraploid	4.5
Taptoe	"	3.9
Massa	"	3.1
Agresso	"	3.0
		3.6
Game	Diploid	4.0
NK 200	"	3.3
Pennfine	"	3.2
NK 100	"	3.2
Viris	"	3.1
Linn	"	3.0
Splendor	"	3.0
Manhattan	"	3.0
Norlea	"	2.9
Pelo	"	2.8
Barenza	"	2.8
		3.1
Overall mean		3.3 L. multiflorum**
Lolium hybridum		
Tetrelite	Tetraploid	4.5
Astor	Diploid	3.0 Tetrelite*
Overall mean		3.7
Lolium rigidum		
Wimmera 62	Diploid	3.8

* significant at 5% level ** significant at 1% level

Species or varieties listed at right differ significantly from species or varieties on the same line.

Table 8. Number of minor vascular bundles in the second leaf of 30 ryegrass varieties. Figures are means of 10 observations per variety

Species and varieties	Ploidy level	Number
Lolium multiflorum		
Barmultra	Tetraploid	5.4
Tetrone	"	5.0
Aubade	"	4.9
Billiken	"	4.7
Jolanda	"	4.5
Tewera	"	2.2 Barmultra* Tetrone* Aubade*
		4.5
St. Tottori	Diploid	5.9
Ooba-hikari	"	4.5
Italian	"	4.1
Florida Rust-resistant	"	3.6 St. Tottori*
Gulf	"	3.4
Magnolia	"	2.1
		3.9
Overall mean		4.2
Lolium perenne		
Agresso	Tetraploid	4.9
Massa	"	4.1
Taptoe	"	3.9
Reveille	"	3.0
		4.0
Viris	Diploid	4.5
Linn	"	4.2
Splendor	"	4.0
Manhattan	"	3.9
Barenza	"	3.9
Pennfine	"	3.7
Norlea	"	3.6
Pelo	"	3.6
NK 100	"	3.1
NK 200	"	3.0
Game	"	2.1 Viris*
		3.6
Overall mean		3.7 L. multiflorum**
Lolium hybridum		
Tetrelite	Tetraploid	2.8
Astor	Diploid	3.9 L. multiflorum*
Overall mean		3.3
Lolium rigidum		
Wimmera 62	Diploid	3.7

* significant at 5% level ** significant at 1% level

Species and varieties listed at right differ significantly from species or varieties within the same group on the same line or lower.

significantly more than those of L. perenne (3.70) and L. hybridum (3.35). However, individual plant measurements within species vary, decreasing the potential of this character for separation of individual plants of the species.

Ploidy level. No significant differences were found in minor vascular bundles among diploid and tetraploids of L. multiflorum, L. perenne and L. hybridum.

Varieties. Among diploids of L. multiflorum, St. Tottori showed the highest number (5.9) of minor vascular bundles, significantly different from those of three other diploid varieties. Among diploids of L. perenne, Viris had the highest number of minor vascular bundles, significantly different from Game.

Total Vascular Bundles

Species. Table 9 shows that the second leaves of seedlings of L. multiflorum had a higher number (8.05 and range of 9.3 to 6.2) of total vascular bundles than L. perenne (6.9, with range of 7.9-6.1). Individual plants of tetraploids and diploids varied in number of vascular bundles (Fig. 7), so a reliable identification of species could not be made on the basis of this characteristic alone.

Ploidy level. Second leaves of tetraploid plants showed a higher number of total vascular bundles than diploid seedlings in L. multiflorum, L. perenne, and L. hybridum.

Table 9. Number of major and minor vascular bundles in second leaf of 30 ryegrass varieties. Figures are means of 10 observations per variety

Species and variety	Ploidy level	Number
Lolium multiflorum		
Barmultra	Tetraploid	9.0
Billiken	"	8.6
Aubade	"	8.5
Jolanda	"	8.5
Tetrone	"	8.4
Tewera	"	7.8
		8.5
St. Tottori	Diploid	9.3
Ooba-hikari	"	8.0
Gulf	"	7.5
Italian	"	7.4
Magnolia	"	6.9 St. Tottori*
Florida Rust-resistant	"	6.2
		7.6 L. multiflorum tetraploids**
Overall mean		8.0
Lolium perenne		
Agresso	Tetraploid	7.9
Taptoe	"	7.8
Reveille	"	7.5
Massa	"	7.2
		7.6
Viris	Diploid	7.6
Linn	"	7.2
Splendor	"	7.0
Manhattan	"	6.9
Pennfine	"	6.9
Barenza	"	6.7
Norlea	"	6.5
Pelo	"	6.5
NK 100	"	6.3
NK 200	"	6.3
Game	"	6.1
		6.7 L. perenne tetraploids**
Overall mean		7.0 L. multiflorum*
Lolium hybridum		
Tetrelite	Tetraploid	7.3
Astor	Diploid	6.9
Overall mean		7.1
Lolium rigidum		
Wimmera 62	Diploid	7.5

* significant at 5% level ** significant at 1% level

Each species, ploidy level or variety listed at right differs significantly from species, ploidy level or variety, of the same group, at the same line.

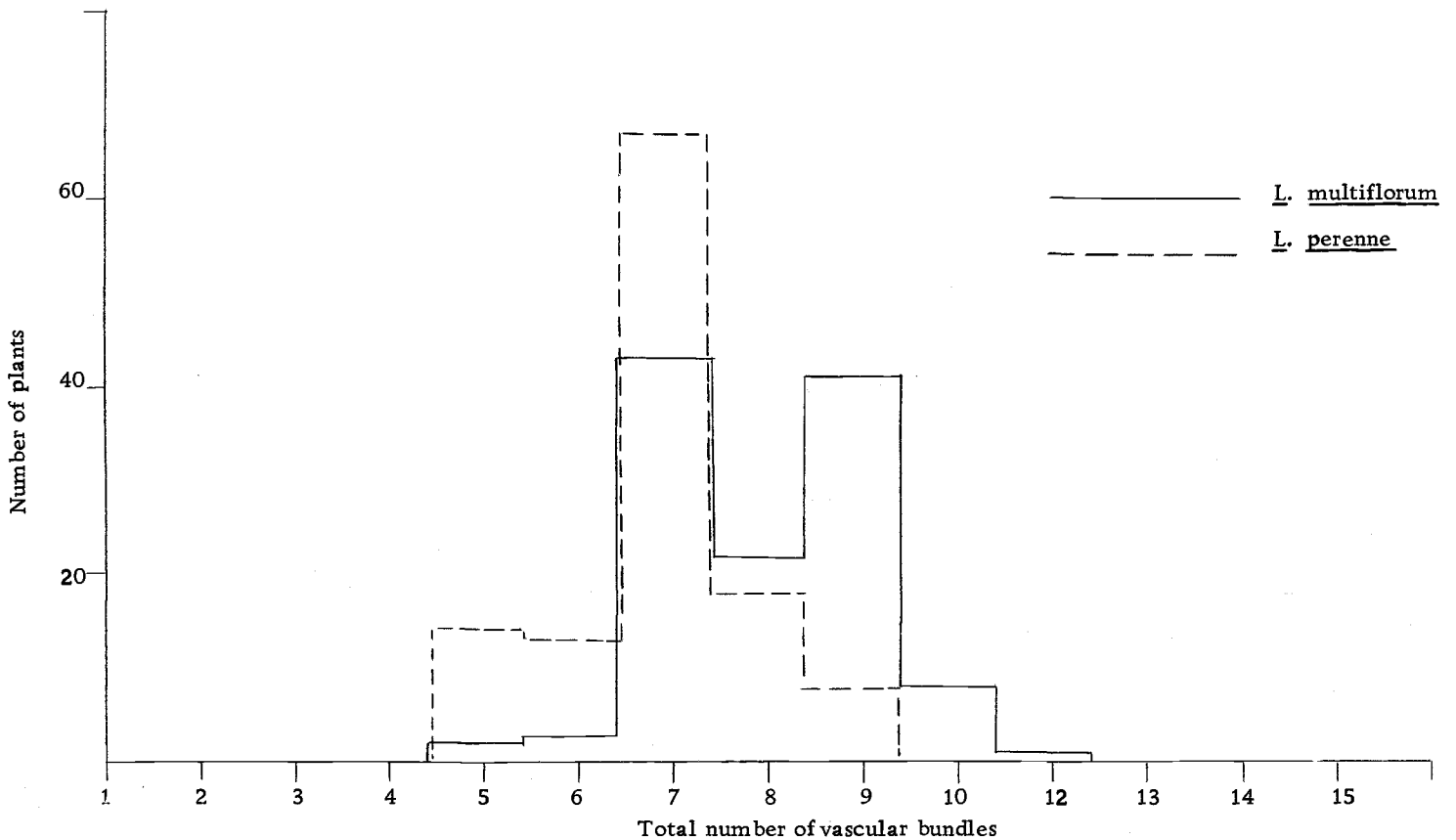


Figure 7. Frequency distribution of total vascular bundles in the second leaf of 10 plants each of 12 varieties of Lolium multiflorum and 12 varieties of Lolium perenne.

Varieties. St. Tottori, a diploid of L. multiflorum had the highest number (9.3) of vascular bundles, significantly higher than the average of two other diploid varieties (Table 9).

Lower Sclerenchyma Fiber Groups

Species. As seen in Table 10, the second leaves of seedlings of L. multiflorum had an average number (2.29) of lower sclerenchyma fiber groups higher than that for L. perenne (1.17). These results did not agree with those presented by Sant (1969), who found statistically significant differences at .001% level for the average number of lower sclerenchyma fiber groups for the third, fourth, etc., leaves, but not for the second one. This was possibly due to (a) number of varieties used--one variety of each species compared to 12 of L. multiflorum and 15 of L. perenne used here; or (b) to the location of the leaf section--a transverse cut at the middle of the leaf, compared to a cut at the base, close to the sheath. When individual plant observations were made, some overlap was seen (Fig. 8), thus limiting the use of this character, alone, for identification.

Ploidy level. No significant difference was found for the number of lower sclerenchyma fiber groups in tetraploid and diploid varieties.

Varieties. Although substantial differences exist between

Table 10. Number of lower sclerenchyma fiber groups in second leaf of 30 ryegrass varieties. Numbers are means of 10 observations per variety

Species and varieties	Ploidy level	Number
Lolium multiflorum		
Aubade	Tentraploid	2.5
Barmultra	"	2.5
Tetrone	"	2.1
Jolanda	"	1.9
Billiken	"	1.9
Tewera	"	1.4
		2.0
Italian	Diploid	2.9
St. Tottori	"	2.8
Gulf	"	2.7
Florida Rust-resistant	"	2.5
Ooba-hikari	"	2.3
Magnolia	"	2.0
		2.5
Overall mean		2.3
Lolium perenne		
Taptoe	Tetraploid	1.3
Reveille	"	1.2
Agresso	"	1.0
Massa	"	1.0
		1.1
NK 100	Diploid	1.6
Pelo	"	1.4
NK 200	"	1.3
Viris	"	1.2
Norlea	"	1.2
Game	"	1.1
Linn	"	1.1
Barenza	"	1.0
Pennfine	"	1.0
Manhattan	"	1.0
Splendor	"	1.0
		1.2
Overall mean		1.2 L. multiflorum**
Lolium hybridum		
Tetrelite	Tetraploid	1.3
Astor	Diploid	2.5
Overall mean		1.9
Lolium rigidum		
Wimmera 62	Diploid	2.0

** significant at 1% level

Species listed at the right differs significantly from the species on the same line.

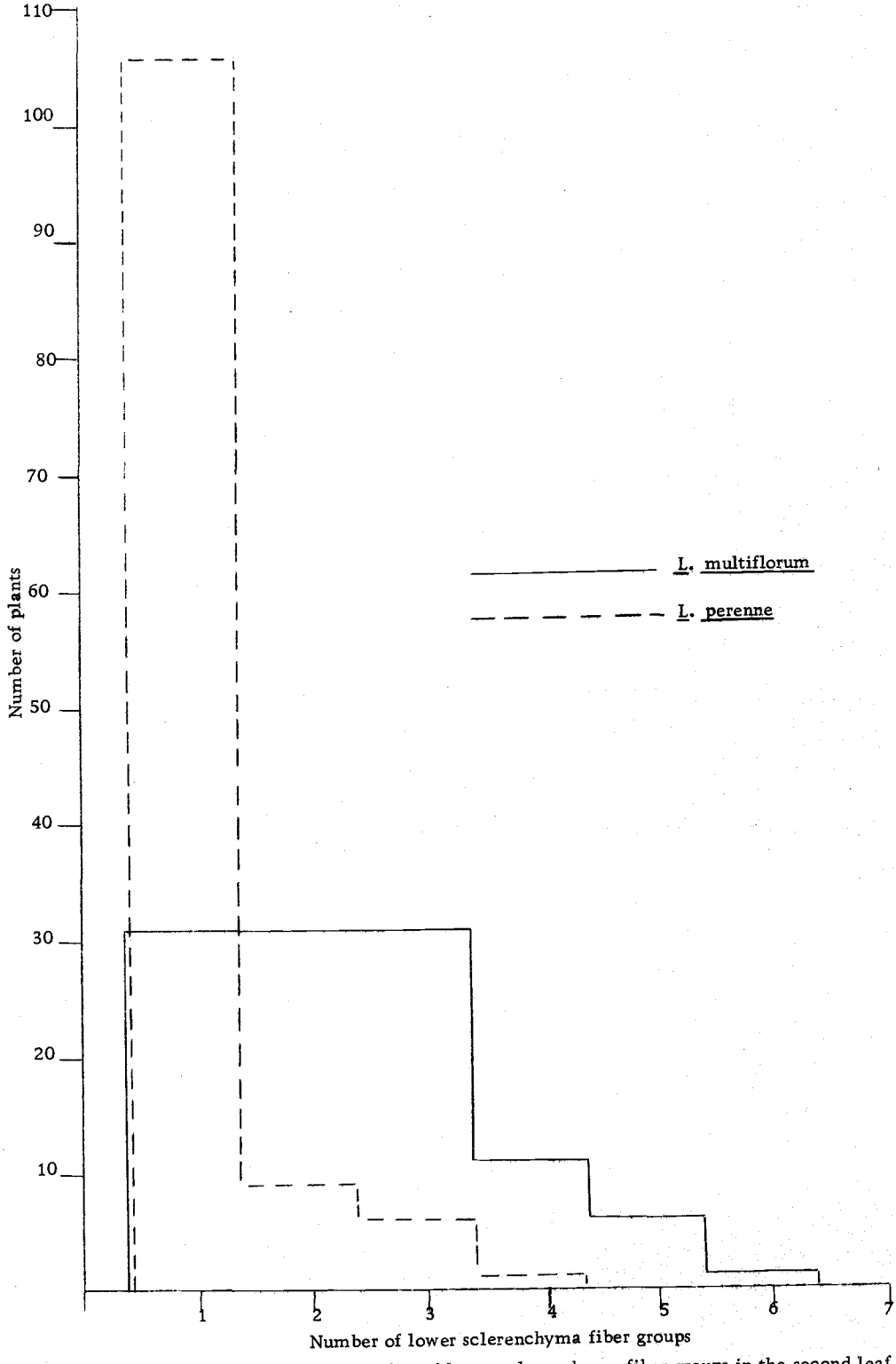


Figure 8. Frequency distribution of number of lower sclerenchyma fiber groups in the second leaf of 10 plants each of 12 varieties of Lolium multiflorum and 12 varieties of Lolium perenne.

variety means, no significant difference in number of lower sclerenchyma fiber groups was found between the varieties of the three species. These results agreed with the work of Sant (1969), who found no statistical significance for lower sclerenchyma fiber groups between one variety of L. multiflorum and one of L. perenne (Table 10).

Upper Sclerenchyma Fiber Groups

Species. Data presented in Table 11 show that the second leaves of seedlings of L. multiflorum had a significantly higher number (5.025) of upper sclerenchyma fiber groups than leaves of L. perenne (1.82) and L. hybridum (3.15). Leaves of seedlings of L. perenne had a smaller average number of upper sclerenchyma fiber groups than those of L. rigidum. These results agree with those presented by Sant (1969) who found that the number of upper sclerenchyma fiber groups in one variety of L. multiflorum was significantly different from that of one variety of L. perenne. However, individual leaves of plants within species showed a great variation, thus limiting the usefulness of this character for use in identification of species (Fig. 9).

Ploidy level. Tetraploid and diploid seedlings did not significantly differ in the number of upper sclerenchyma fiber groups in the second leaves.

Table 11. Number of upper sclerenchyma fiber groups in second leaf of 30 ryegrass varieties. Numbers are means of 10 observations per variety

Species and varieties	Ploidy level	Number
Lolium multiflorum		
Aubade	Tetraploid	6.3
Barmultra	"	6.3
Billiken	"	5.9
Tetrone	"	5.1
Jolanda	"	4.8
Tewera	"	3.7
		5.3
St. Tottori	Diploid	6.1
Ooba-hikari	"	5.2
Florida Rust-resistant	"	4.4
Gulf	"	4.4
Italian	"	4.4
Magnolia	"	3.2
		4.7
Overall mean		5.0
Lolium perenne		
Taptoe	Tetraploid	1.6
Reveille	"	1.5
Massa	"	1.1
Agresso	"	.9
		1.3
Linn	Diploid	3.9
Pennfine	"	2.7
Pelo	"	2.5
NK 200	"	2.2
Splendor	"	2.1
NK 100	"	2.0
Game	"	1.8
Viris	"	1.8
Norlea	"	1.3
Manhattan	"	1.1
Barenza	"	.8
		2.0
Overall mean		1.8 L. multiflorum**
Lolium hybridum		
Tetrelite	Tetraploid	3.0
Astor	Diploid	3.3
Overall mean		3.1 L. multiflorum**
Lolium rigidum		
Wimmera 62	Diploid	4.3 L. perenne**

** significant at 1% level.

Species listed at right differ significantly from species on the same line.

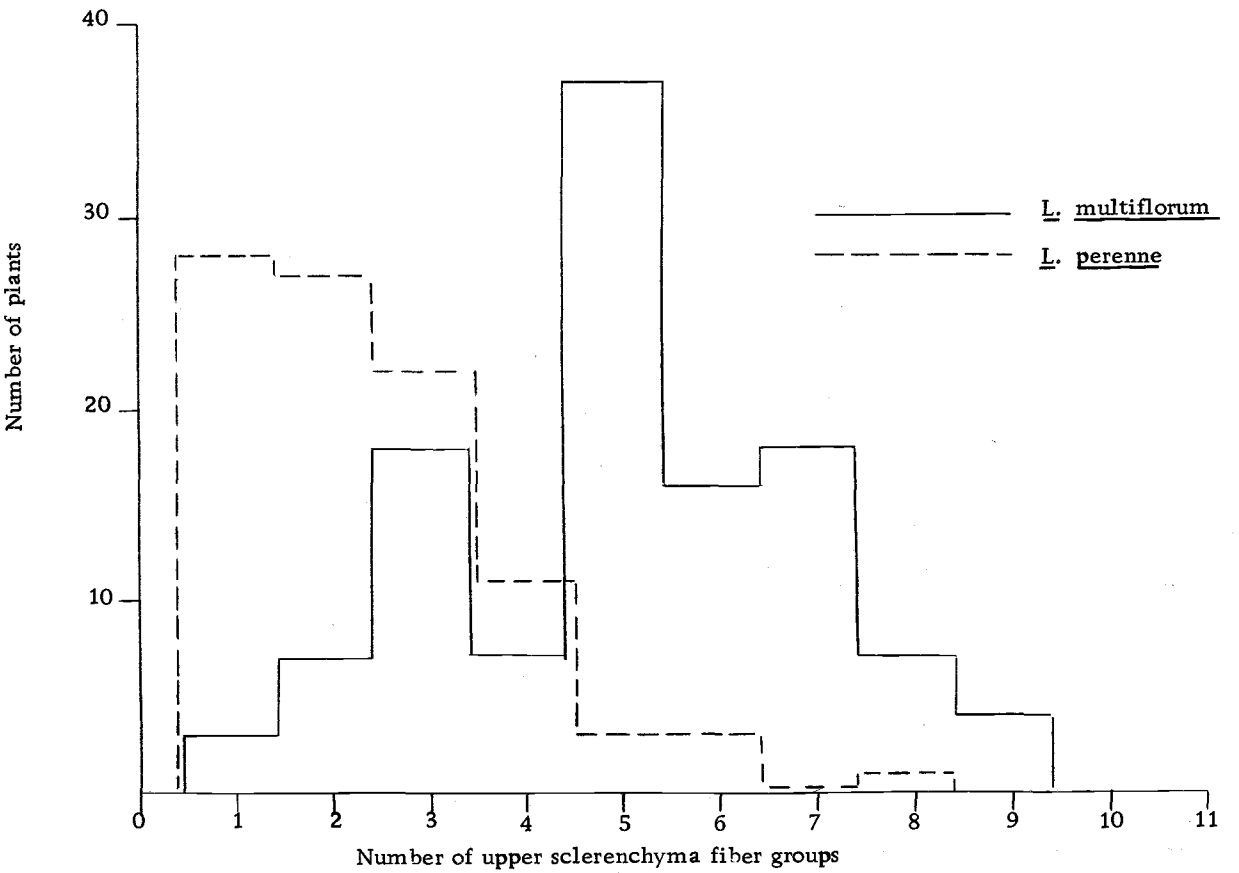


Figure 9. Frequency distribution of number of upper sclerenchyma fiber groups in the second leaf of 10 plants each of 12 varieties of Lolium multiflorum and 12 varieties of Lolium perenne.

Varieties. Second leaves of seedlings of different varieties did not significantly differ in the average numbers of upper sclerenchyma fiber groups.

Total Sclerenchyma Fiber Groups

Species. The second leaves of seedlings of L. multiflorum, as presented in Table 12, had a significantly higher number (7.32) of total sclerenchyma fiber groups than those of L. perenne (2.99) and L. hybridum (5.05). Individual leaves of plants within the first two species varied in number of total sclerenchyma fiber groups so that species could not be clearly differentiated on a single plant basis (Fig. 10).

L. perenne had fewer total sclerenchyma fiber groups than L. rigidum.

Ploidy level. Leaves of tetraploids and diploid seedlings did not differ significantly in average number within the three species (Table 12).

Varieties. No significant difference occurred in the total number of sclerenchyma fiber groups among different varieties of L. multiflorum, L. perenne, and L. hybridum.

Table 12. Number of lower and upper sclerenchyma fiber groups in second leaf of 30 ryegrass varieties. Figures are means of 10 observations per variety

Species and varieties	Ploidy level	Number
Lolium multiflorum		
Aubade	Tetraploid	8.80
Barmultra	"	8.80
Billiken	"	7.80
Tetrone	"	7.20
Jolanda	"	6.77
Tewera	"	5.10
		7.40
St. Tottori	Diploid	8.80
Ooba-hikari	"	7.50
Florida Rust-resistant	"	7.40
Italian	"	7.30
Gulf	"	7.10
Magnolia	"	5.20
		7.23
Overall mean		7.32
Lolium perenne		
Taptoe	Tetraploid	2.90
Reveille	"	2.70
Massa	"	2.00
Agresso	"	1.90
		2.37
Linn	Diploid	5.00
Pelo	"	3.90
Pennfine	"	3.70
NK 100	"	3.60
NK 200	"	3.50
Game	"	3.10
Splendor	"	3.10
Viris	"	3.00
Norlea	"	2.50
Manhattan	"	2.10
Barenza	"	1.80
		3.21
Overall mean		2.99 L. multiflorum**
Lolium hybridum		
Tetrelite	Tetraploid	4.30
Astor	Diploid	5.80
Overall mean		5.05 L. multiflorum** L. perenne**
Lolium rigidum		
Wimmera 62	Diploid	6.30 L. perenne**

** significant at 1% level

Species listed at right differ significantly from species on the same line.

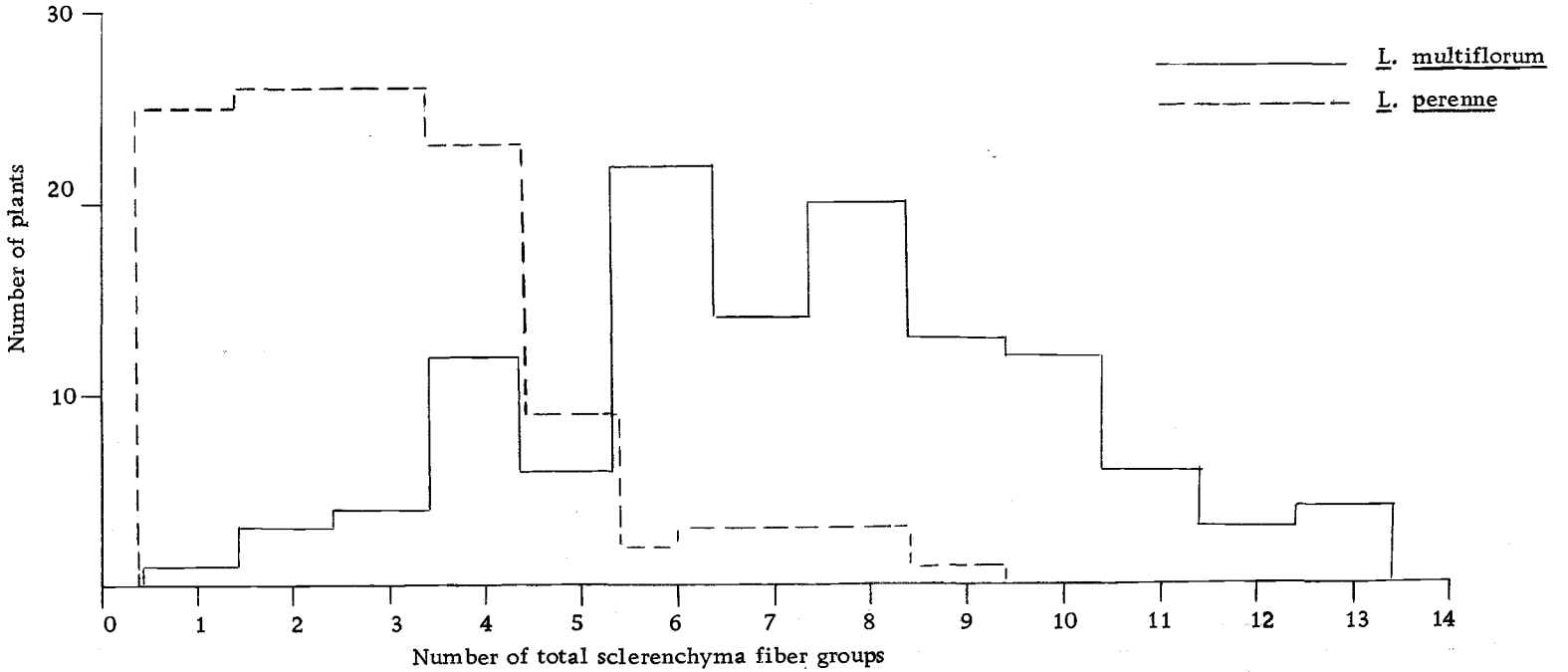


Figure 10. Frequency distribution of total sclerenchyma fiber groups in the second leaf of 10 plants each of 12 varieties of Lolium multiflorum and 12 varieties of Lolium perenne.

Leaf Blade Vernation

Three types of leaf blade vernation were observed among varieties of the four species (Table 13 and Pl. 6 and 7).

All L. multiflorum varieties showed the typical rolled pattern while all the L. perenne varieties showed the typical folded pattern that has been described. A few atypical plants occurred in some of the L. perenne varieties, but it is not known if these were due to seed lot contamination or due to hybridization or other causes.

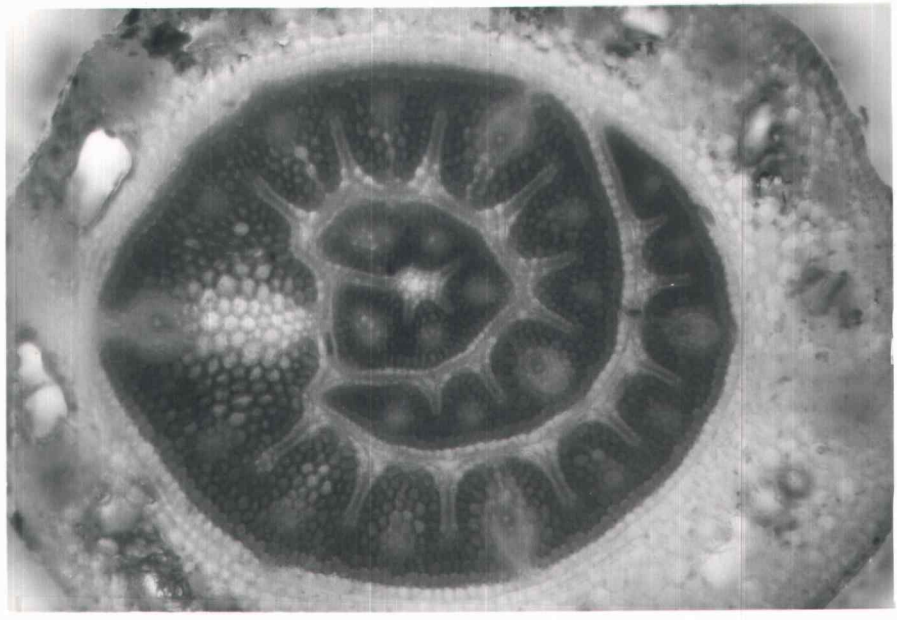
The leaf blade vernation of Wimmera 62 (L. rigidum) and of Tetrelite (L. hybridum) appeared to be intermediate between that of L. multiflorum and L. perenne in being folded from the midrib, while the edges of the leaf were in-rolled and overlapping, somewhat like L. multiflorum. This type of vernation is being called "semi-rolled" to conveniently distinguish it from "rolled" and "folded".

Although Tetralite, the tetraploid variety of L. hybridum, possessed the semi-rolled type of vernation, Astor, the diploid variety of L. hybridum, possessed the rolled type of vernation.

Table 13. Leaf blade vernation of 30 ryegrass varieties. Observations made on 25 plants per variety, 14 or 21 days after seeding

Species and Varieties	Perenniality	Type of vernation
Lolium multiflorum		
Aubade	Annual	Rolled
Barmultra	"	"
Billiken	"	"
Jolanda	"	"
Tetrone	"	"
Tewera	"	"
Florida Rust-resistant	"	"
Gulf	"	"
Italian	"	"
Magnolia	"	"
Ooba-hikari	"	"
St. Tottori	"	"
Lolium perenne		
Agresso	Perennial	Folded
Massa	"	"
Reveille	"	"
Taptoe	"	"
Barenza	"	"
Game	"	"
Linn	"	"
Manhattan	"	"
NK 100	"	"
NK 200	"	"
Norlea	"	"
Pelo	"	"
Pennfine	"	"
Splendor	"	"
Viris	"	"
Lolium hybridum		
Tetrelite	Perennial	Semi-rolled
Astor	"	Rolled
Lolium rigidum		
Wimmera 62	"	Semi-rolled

A



B

Plate 6. Typical leaf blade vernation of two species of ryegrass.
A Lolium multiflorum (Magnolia) showing rolled vernation
B Lolium hybridum (Tetrelite) showing some rolled vernation

A

B

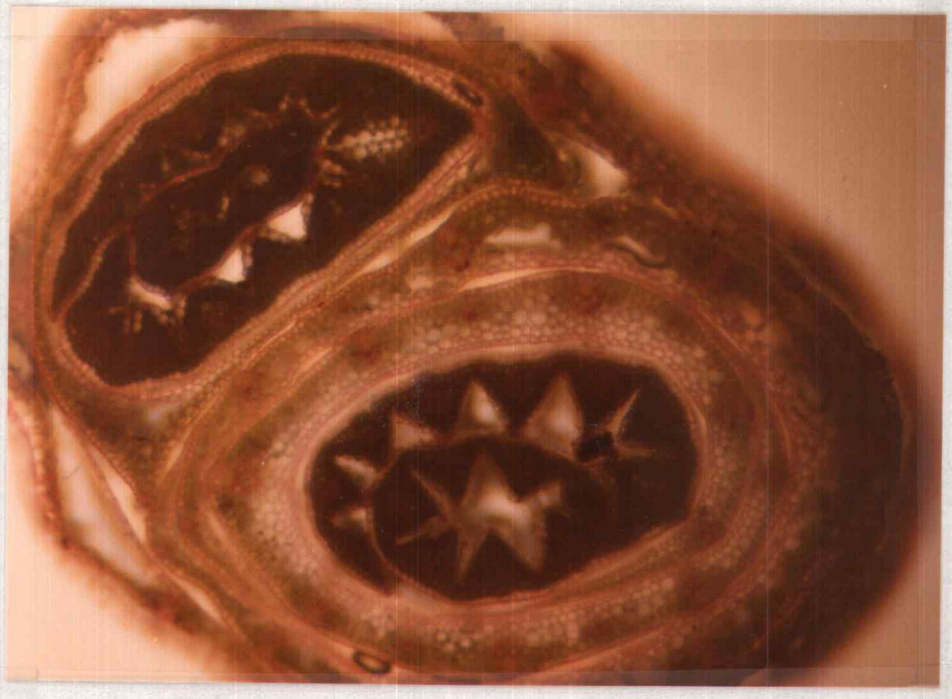


Plate 7. Typical leaf blade vernation of two species of ryegrass.
A Lolium perenne (Manhattan) showing folded vernation
B Lolium rigidum (Wimmera 62) showing semi-rolled

DISCUSSION

The Organization for Economic Cooperation and Development scheme for seed certification lists more than 100 varieties of perennial and almost 100 of annual ryegrasses, and those numbers are growing very fast with the new varieties produced for different uses, as turf, lawn, pasture, etc.

Laboratory techniques now used for identifying ryegrass varieties are limited mainly to the fluorescence test for distinguishing between annuals and perennials. The validity of the fluorescence test is being questioned as newly developed varieties increasingly fail to meet the traditional standards of annuals being fluorescent and perennials being non-fluorescent.

The introduction of tetraploid varieties has increased the interest in developing identification tests to distinguish tetraploids from diploids. The two ploidy levels can be positively distinguished by making chromosome counts, but only a few seed testing laboratories in the world, and probably only one in the United States, are currently performing this difficult and expensive procedure.

Thus the need for additional procedures to supplement or replace the traditional fluorescence test and chromosome count for identification of ryegrass species, ploidy levels and varieties, is apparent.

Of the 12 morphological characteristics of seedling leaves investigated for varietal differentiation possibilities, the mean values of several varied among species, ploidy levels and/or varieties.

Previously reported differences in leaf vernation of annuals and perennials were substantiated for all 27 varieties of L. multiflorum and L. perenne. In addition to the rolled character of L. multiflorum and the folded character of L. perenne leaves, a third type of vernation present in L. hybridum and L. rigidum has been reported for the first time. This type of vernation appears intermediate between rolled and folded and is termed semi-rolled for a convenient term of reference.

The most useful characteristics for species differentiation appear to be leaf blade vernation, leaf dimensions and area, number of vascular bundles and unicellular hair density.

For differentiation of ploidy levels, leaf dimensions and area, stomata density and vascular bundles provided the most useful information.

Leaf dimensions, and area, and number of vascular bundles gave the most useful information for differentiation of individual varieties.

Ryegrass is open-pollinated and plants within a variety would be expected to vary in their individual characteristics. This is

evident in the morphological characteristics of seedling leaves. Because of this variation and overlapping of characteristics between varieties, individual plants cannot usually be positively identified as to variety or ploidy level on the basis of any one of the leaf characteristics included in this study. A possible solution lies in observing seedlings for several characters and rating them according to their reactions over a range of characteristics. An illustration of how such a system would result in differentiation of the 30 varieties is given in Table 14. This table was set taking the overall mean and range for the four species of each of the characters and dividing the total range in three equal parts around the mean.

To make the division of each character in three grades, the overall range for the four species was divided in three equal parts. The highest third was designated a, the middle was called b, and the lowest third was designated c. Since the overall mean for all species was used, this table will perhaps be useful for evaluation and distinction of other ryegrass varieties not included in this study.

With this table we could separate all 30 varieties, except Jolanda and Tetrone (both diploids of L. multiflorum). Separation was obtained between the four species, which differed in several characters. Also the ploidy levels presented some difference.

Better distinction was obtained between diploids of Lolium

Table 14. Morphological characteristics of 30 ryegrass varieties

Species and varieties	Rolled or folded	Leaf width	Leaf length	Leaf area	Hair density	Stomata density	Stomata length	Vasc. bundles	Scle. fiber
Lolium multiflorum									
Aubade	r	a	a	a	a	c	c	a	a
Barmultra	r	a	a	a	b	c	a	a	a
Billiken	r	a	a	a	c	c	a	a	a
Jolanda	r	a	a	a	a	c	b	a	a
Tetrone	r	a	a	a	a	c	b	a	a
Tewera	r	a	b	a	a	c	b	b	b
Florida r.r.	r	b	b	b	a	a	a	c	a
Gulf	r	b	a	b	a	c	a	b	a
Italian	r	b	b	b	a	a	b	b	a
Magnolia	r	b	b	b	a	a	b	b	b
Ooba-hikari	r	b	a	b	a	b	c	a	a
St. Tottori	r	a	a	a	b	b	b	a	a
Lolium perenne									
Agresso	f	b	b	c	c	c	c	b	c
Massa	f	b	b	b	c	c	b	b	c
Reveille	f	a	b	b	c	c	c	b	c
Taptoe	f	b	b	b	b	c	b	b	c
Barenza	f	c	c	c	c	b	b	b	c
Game	f	c	c	c	c	a	b	c	c
Linn	f	b	c	c	c	b	b	b	b
Manhattan	f	c	c	c	c	a	b	b	c
NK 100	f	c	c	c	c	b	b	c	c
NK 200	f	c	c	c	b	b	c	c	c
Norlea	f	b	a	b	b	a	c	b	c
Pelo	f	c	c	c	c	a	b	b	b
Pennfine	f	c	c	c	b	a	c	b	c
Splendor	f	c	c	c	c	b	b	b	c
Viris	f	b	c	c	c	a	c	b	c
Lolium hybridum									
Tetrelite	s.r.	b	b	b	c	c	b	b	b
Astor	r	b	b	b	a	a	a	b	b
Lolium rigidum									
Wimmera 62	s.r.	a	b	b	b	c	a	b	a

a = highest values
b = intermediate values
c = lowest values
s.r. = semi-rolled

multiflorum than the tetraploids. In Lolium perenne the distinction was good for both ploidy levels. Fair difference was determined also for the two varieties of L. hybridum.

The reliability of morphological measurements may be influenced by variations in the environmental conditions under which the seedlings are grown. Temperature, light and moisture may vary somewhat, even in growth chambers. Seed size and vigor may also influence seedling size in the one and two-leaf stages. Better differentiation may be obtained in later growth stage, but efforts were made here to develop methods that could be used within two to three weeks. Small seedlings also require less growth chamber space and can be handled with more precision than older plants. Although morphological characters are subject to some variation, the same can be said for fluorescence test.

The cellulose acetate imprint method was very useful for studying microscopical characteristics of the leaf epidermis. The imprints provide a permanent record of leaf characters and are available for re-study at any time. Epidermal characteristics were easier to view in the imprints than on the leaf. Epidermal characteristics characters were not too useful for distinguishing varieties, although species differed in epidermal hair density and stomata density. Since other characters, particularly leaf blade vernation, are available for species differentiation, the imprint method may not be of practical value for ryegrass

identification.

Measurement of leaf width, length and area is an easy and fast procedure and has value for distinguishing tetraploids and diploids. A trained person can measure more than 100 plants an hour.

Several additional possibilities exist for future research and development of varietal identification methods for ryegrass. There is an indication that tetraploids and diploids may differ in the number of chloroplasts in the outer sheath of the vascular bundles. Pathological tests hold promise for distinguishing varieties. Rust resistance can be used to differentiate cereal varieties and may be useful for grasses as well. Wilkins (1973) has shown that rye grass varieties differ in reaction to Drechslera siccans and D. caternaria.

SUMMARY

Among the 13 morphological characteristics of ryegrass seedling leaves that were investigated, the characters showing the most promise for varietal identification were leaf dimension and area, unicellular hair density, stomata density, and leaf vernation.

Mean differences among species occurred in the following characteristics:

Lolium multiflorum and L. perenne: leaf width, leaf length, leaf area, minor and major vascular bundles, lower and upper sclerenchyma fiber groups, hair density, and number of vascular bundles.

Lolium multiflorum and L. hybridum: leaf width, leaf length, leaf area, and leaf vernation.

Lolium perenne and L. hybridum: leaf width, leaf length, leaf area, and total of sclerenchyma fiber groups, and leaf vernation.

Lolium perenne and L. rigidum: leaf width, leaf length, leaf area, and leaf vernation

Lolium multiflorum and L. rigidum: leaf blade vernation.

Lolium hybridum and L. rigidum: leaf blade vernation.

Tetraploids and diploids were significantly different on the

average, within their respective species, as follows:

Lolium multiflorum: leaf width, leaf length, leaf area, stomata density.

Lolium perenne: leaf width, leaf length, leaf area, stomata density.

Lolium hybridum: stomata density, and number of major vascular bundles.

Some significant differences among varieties within species and ploidy levels were observed in the following characteristics:

Lolium multiflorum tetraploids: leaf width, leaf length, leaf area, minor vascular bundles.

Lolium multiflorum diploids: leaf width, leaf length, leaf area, minor vascular bundles, total vascular bundles.

Lolium perenne tetraploids: leaf area.

Lolium perenne diploids: leaf length, leaf area, minor vascular bundles.

Although varietal means differed for many of the characters, individual plants could not be identified on the basis of a single character.

An illustration was prepared to show how a combination of morphological characters might be used in varietal identification. With a table of varietal means for nine characters, only two (Jolanda and Tetrone--both tetraploids of L. multiflorum) of the 30

varieties studied were indistinguishable.

The leaf blade vernation of Wimmera (L. rigidum) and Tetrelite (L. hybridum) appeared to be intermediate between that of L. multiflorum and L. perenne. The vernation was folded from the midrib, like in L. perenne, while the edges of the leaf were in-rolled and overlapping, somewhat like L. multiflorum. This type of vernation is being called "semi-rolled," to conveniently distinguish from "rolled" and "folded".

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APPENDIX

Appendix Table 1. Analysis of variance for width of first leaf as affected by variety, species, and ploidy level

Source	DF	MS	F
Variety	29	1.39918	38.29**
Error	720	.0365392	
Total	749		

C. V. 3.80%

Appendix Table 2. Analysis of variance for length of first leaf as affected by variety, species, and ploidy level

Source	DF	MS	F
Variety	29	8485.02	29.21**
Error	720	290.482	
Total	749		

C. V. 5.71%

Appendix Table 3. Analysis of variance for area of first leave as affected by variety, species, and ploidy level

Source	DF	MS	F
Variety	29	42478.1	46.82**
Error	729	907.217	
Total	749		

C. V. 7.60%

Appendix Table 4. Analysis of variance for hair number counted under 200X in area of imprints of second leaf, as affected by variety, species, and ploidy level

Source	DF	MS	F
Variety	29	265.136	6.74**
Error	270	39.3122	
Total	299		

C. V. 32.01%

Appendix Table 5. Analysis of variance for stomata number counted under 200X, in area of imprints of second leaf, as affected by variety, species, and ploidy level

Source	DF	MS	F
Variety	29	466.914	7.66**
Error	270	60.9693	
Total	299		

C. V. 9.37%

Appendix Table 6. Analysis of variance for length of stomata from imprints of second leaf measured under 450X, as influenced by variety, species, and ploidy level

Source	DF	MS	F
Variety	29	423.510	2.43**
Error	270	134.442	
Total	299		

C. V. 15.06%

Appendix Table 7. Analysis of variance for major vascular bundles of second leaf as influenced by variety, species, and ploidy level

Source	DF	MS	F
Variety	29	3.69885	7.49**
Error	270	.494074	
Total	299		

C. V. 8.89%

Appendix Table 8. Analysis of variance for minor vascular bundles of second leaf as influenced by variety, species, and ploidy level

Source	DF	MS	F
Variety	29	8.05069	10.28**
Error	270	.784815	
Total	299		

C. V. 10.18%

Appendix Table 9. Analysis of variance for total of vascular bundles of second leaf as influenced by variety, species, and ploidy level

Source	DF	MS	F
Variety	29	6.88742	8.10**
Error	270	.85	
Total	299		

C. V. 5.55%

Appendix Table 10. Analysis of variance for lower sclerenchyma fibers of second leaf as affected by variety, species, and ploidy level

Source	DF	MS	F
Variety	29	4.12368	5.20**
Error	270	.793333	
Total	299		

C. V. 23.59%

Appendix Table 11. Analysis of variance for upper sclerenchyma fibers of second leaf as affected by variety, species, and ploidy level

Source	DF	MS	F
Variety	29	31.0685	12.32**
Error	270	2.5207	
Total	299		

C. V. 21.69%

Appendix Table 12. Analysis of variance for total of sclerenchyma fibers of second leaf as affected by variety, species, and ploidy level

Source	DF	MS	F
Variety	29	53.7057	12.85**
Error	270	4.17852	
Total	299		

C. V. 18.39%