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	Ryegrass, Lolium perenne	L.	
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Studies were conducted to establish some characters which could be used, either in the field or in the laboratory, to differentiate between annual ryegrass (Oregon), <u>Lolium multiflorum Lam.</u>, and Linn perennial ryegrass, <u>Lolium perenne</u> L.

Vernation, percentage of glumes covering the spikelets, and height of seedlings seem to be fully reliable characters to differentiate the plants under study.

Texture of the tillers, blade width, spikes length, spikelets length, glume length, callus shape of the rachilla, and rachilla length, seem to be relatively good characters for differentiating these species.

Number of tillers, embracement of the auricles, number of

spikelets per spike, teeth on the lemma, awns on the lemma, and weight of the seedlings are inadequate characters to differentiate between the species studied.

Leaf color might be a useful guide to detect contamination, when the species are grown under identical conditions. A Comparison of Plant Characteristics of Annual Ryegrass (Oregon), <u>Lolium multiflorum</u> Lam., and Linn Perennial Ryegrass, <u>Lolium perenne</u> L.

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A Comparison of Plant Characteristics of Annual Ryegrass (Oregon), <u>Lolium multiflorum</u> Lam., and Linn Perennial Ryegrass, Lolium perenne L.

#### INTRODUCTION

Both, annual and perennial ryegrass, are important in the temperate zones of the world, where they are widely used for forage and turf purposes. They are very important in Oregon, which produces about 98 percent of the ryegrass seed in the United States (16). During the period 1964-65, about 40 million pounds of perennial ryegrass were produced in Oregon, with a total cash value of \$1,250,000(15).

There is a lack of clearly defined characteristics that can be used to distinguish between Oregon Annual Ryegrass (Lolium multiflorum Lam.) and Linn Perennial Ryegrass (Lolium perenne L.), during field inspections of ryegrass seed crops or in the seed purity analysis for the Oregon Certification Program. The Certification Inspector uses only a rough criterion for evaluating volunteer plants of one species in a crop of the other. The criterion most widely used during seed inspection is the presence of plants growing between the drill rows. Such plants are assumed to be contaminants by the Seed Certification Inspector because these seedlings are of unknown origin. This procedure does not give the best results, since there are many sources of contamination that need not necessarily fall from the drill between the rows, and subsequently be moved by wind, insects, animals, or other factors. The inspector also superficially tries to determine some obviously off-type plants, but plant seed characteristics of these species are very similar and adequate criteria for differentiation do not exist.

For a long time, the fluorescence test, described by Gentner (5) in 1929, has been used as a laboratory procedure to differentiate between annual and perennial ryegrass. But, today, with the advent of new varieties, the application of this test is less effective to determine species, since Nyquist (14) was able to demonstrate the existence of homozygous fluorescent perennial ryegrass.

The purpose of this study was to establish some practical, rapid, and accurate means which could be used, either in the field or in the laboratory, to differentiate between annual and perennial ryegrass.

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#### REVIEW OF LITERATURE

Both, annual and perennial ryegrass, belong to the family GRAMINEAE, subfamily FESTUCOIDEAE, tribe HORDEA, genus LOLIUM (6).

Linnaei (9) early in 1753 was the first to describe the genus <u>Lolium</u> and two species in it: <u>Lolium perenne</u> and <u>Lolium multi-</u> <u>florum</u>. The descriptions were based mainly on the characteristics of the spikes and on the perenniality of the plants. Linnaei stated that awnless seeds and perennial plants belong to the same species, and that awned seeds and annual plants make up other species.

Later on, many workers (8, 14, 19, 20) have differentiated these species by stating that <u>Lolium perenne</u> is a perennial or at least a short-lived perennial, and that <u>Lolium multiflorum</u> is an annual or short-lived species. But this definition shows the possibility of an overlapping life period.

The taxonomic characters of the genus are described by Hitchcock (6) as follows:

> "Spikelets several flowered, solitary, placed edgewise to the continuous rachis, on edge fitting to the alternate concavities, the rachilla disarticulating above the glumes and between florets; first glume wanting (except on the terminal spikelet and rarely in 1 or 2 spikelets in a spike), the second outward, strongly 3 to 5 nerved, equaling or exceeding the second floret; lemmas rounded on the back, 5- to 7-nerved, obtuse, acute or awned. Annual or perennials, with flat blades and slender usually flat spikes."

The same author (6) then describes the species within this genus, by stating that lemmas nearly or quite awnless make up <u>Lolium perenne</u>, and lemmas, at least the upper-awned, make up Lolium multiflorum. The description of each species is as follows:

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"Lolium perenne L. Perennial ryegrass. Shortlived perennial; culms erect or decumbent at base, 30 to 60 cm. tall; foliage glossy, the blades 2 to 4 mm. wide; spike often subpaleate, mostly 15 to 25 cm. long; spikelets mostly 6- to 10-flowered; lemmas 5 to 7 mm. long, at least the upper awned."

"Lolium multiflorum Lam. Italian ryegrass. Differing from L. perenne in the more robust habit, larger spikelets, and awned lemmas; spikelets 10to 20-flowered, 1.5 to 2.5 cm. long; lemmas 7 to 8 mm. long, at least the upper awned."

Lolium multiflorum Lam., commonly known as Italian ryegrass, is native in the Mediterranean regions of southern Europe, northern Africa, and Asia Minor. History indicates that it was first cultivated in northern Italy. It is not definitely known when it was first introduced in the United States, but probably in Colonial days. According to meager information available, its introduction to the Pacific Northwest, where it is most extensively grown at present for seed production, apparently was late in the nineteenth century (19).

Lolium perenne L., commonly known as perennial ryegrass, is native of all temperate Asia and North Africa. According to history, it was the first perennial grass to be grown in pure culture for forage in northern Europe. Its date of introduction into the United States probably was about the same time as for Italian ryegrass (19). It appears that for many years after the introduction of these two ryegrasses, their use was limited, but there has been a rapid increase in use throughout the country during the past 20 years (19).

In 1962, Schoth and Weihing (20) noted that due to free crosspollination between both species, in Oregon, numerous intermediate types developed naturally. The strain of <u>L</u>. <u>multiflorum</u> most frequently seeded in the United States was the one grown for seed in Oregon and was generally designated as Domestic ryegrass, although marketed under the name of Common ryegrass. The seed and plant characteristics indicate a combination of characters of both <u>L</u>. <u>multiflorum</u> and <u>L</u>. <u>perenne</u>. The plants are mostly annual. The seeds are short awned, in comparison to the true Italian which has rather long awns.

An amendment to the Rules and Regulations under the Federal Seed Act (21) in 1963 indicated that the correct labeling for marketing ryegrass should be based on the results of the fluorescence test showing the percentage of both annual and perennial ryegrass.

A major problem in seed production and certification is to define the main differentiating characteristics between both species in an accurate way. The most commonly used characteristics for

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distinguishing between those species are: A. fluorescence; B. tillers; C. leaves; D. inflorescence; E. seeds; and F. vigor.

#### Fluorescence

In 1929, Gentner (5) reported that the seedling roots of Italian ryegrass, <u>Lolium multiflorum</u> Lam., produced a fluorescent compound, and that the roots of perennial ryegrass, <u>Lolium perenne</u> L., do not.

Mercer and Linehan (11) found that the fluorescent character developed chiefly in the short-lived species and was frequently, but not always, accompanied by the presence of an awn.

In 1937, Munn (13) stated that the separation of the species and strains of the ryegrasses into non-fluorescent (long-lived) and fluorescent (short-lived) forms, by their capacity to show fluorescence under ultraviolet radiation, had proved entirely practical and, in many cases, extremely useful. He added that Italian ryegrass is normally highly or completely fluorescent and perennial ryegrass is normally highly or completely non-fluorescent; others may show as much as five percent fluorescent individuals and yet be completely awnless.

Rampton (18) pointed out that "Domestic" <u>Lolium multiflorum</u> had fluorescent seedlings varying from 83.0 percent up to 100 percent. He also reported in the same paper that the fluorescence analysis of 15 lots of domestic and imported ryegrass ranged between 78.0 and 100 percent. For perennial ryegrass, in the same study, the fluorescence analysis varied between complete non-fluorescence in one lot to 86.0 percent fluorescence in the highest lot. He concluded that the fluorescence test cannot be used as an infallible guide in classifying questionable lots of Oregon-grown domestic ryegrass seed, and that some of the domestic ryegrass seedlings used in his trials showed no fluorescence. He remarked that an experienced seed analyst, thoroughly familiar with the seed characters of the various ryegrass types, can, in most cases, quite accurately evaluate such seed samples, but the fluorescence test is useful for classification of most domestic ryegrass seed for making approximate determinations. It is also useful in serving as a check on the work of the seed analyst.

Justice (8) later noted that a comprehensive review of the literature pertaining to the use of the fluorescence test for <u>Lolium</u> spp. indicated that practically all the data which can be traced back to seed of known genetical constitution support the use of the fluorescence test in determining the percentages of annual ryegrass and the short-lived forms of perennial ryegrass in <u>Lolium perenne</u>. But later on, Nyquist (14) demonstrated the existence of fluorescent perennial ryegrass.

Axelrod and Belzile (2) determined the chemical composition

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of the substance that fluoresces under ultraviolet light and named it annuloline, and they indicated that they were unable to detect any annuloline in plants of Lolium perenne.

Baekgaard (3) arrived at the conclusion that there is no basis for questioning the trueness to species of samples from varieties with a considerable fluorescence, unless it can be proved that the fluorescence is due to intermingling of Italian ryegrass, or due to cross fertilization with Italian ryegrass.

Jensen and Langkilde (7) stated that seeds of <u>Lolium multi-florum</u> deviate in general from seeds of <u>Lolium perenne</u> by some characters, and fluorescence is included among them. They mentioned that <u>L</u>. <u>multiflorum</u> produced fluorescent lines along the roots due to the secretion of annuloline and <u>L</u>. <u>perenne</u> did not. But, they also found in <u>L</u>. <u>perenne</u> seeds which exhibit one or more of these characters, fluorescence included. They also were able to find some seedlings that did not show fluorescence. From their work, they concluded that the presence or absence of fluorescent lines along the roots of germinating seeds on filter paper is a character not fully reliable to distinguish between <u>L</u>. <u>perenne</u> and <u>L</u>. <u>multiflorum</u>.

Nyquist (14) suggested the possibility of a mixture of the species occurring in the seed fields, due to the conditions under which the two species are grown for seed production. He considered

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these conditions very poor in respect to maintaining genetic purity, specifically resulting in fluorescent perennial ryegrass.

#### Tillers

Several people observed different characteristics in the tillers. Rampton (18) reported that in perennial ryegrass the tillers and convex side of the rachis were often rough.

Other workers (19) stated that the culms of Italian ryegrass (annual) are cylindrical, whereas those of perennial ryegrass are slightly flattened.

Jensen and Langkilde (7) studied these species and found no significant differences in number or thickness of tillers.

#### Leaves

After a careful study of the leaf characteristics, Rampton (18) reported that the leaves of perennial ryegrass are mostly basal, numerous, comparatively narrow, folded in the bud, and dark green in color, whereas those of annual ryegrass were mostly borne in the stems, rolled in the bud, wider and less numerous, and the color of the foliage was lighter green than the perennial type.

Schoth and Hein (19) mentioned that the leaves of Italian ryegrass are rolled in the bud, whereas those of perennial are folded.

Jensen and Langkilde (7) also studied leaf characteristics

and tried to distinguish differences in the following characters: leaf in shoot, embracement of the auricles, width of midrib, percentages of leaf edges rough, blades color, and width. They found that perennial ryegrass was rolled in the bud, rougher in the leaf edge, lighter in color, and had narrower blades than annual ryegrass.

#### Inflorescence

Rampton (18) also studied characteristics of the spikes and spikelets and reported that, in perennial ryegrass, the spikelets were barely longer than the outer glume, whereas in annual ryegrass the spikelets were much longer than the outer glume.

#### Seeds

Some authors (1, 17, 18) have emphasized the presence of awns in annual ryegrass and its absence in perennial ryegrass and indicated that this difference is very definite, while others (7, 13) considered this characteristic unreliable to distinguish <u>Lolium</u> perenne from Lolium multiflorum.

Other seed characteristics were also studied by Pierpoint (17) who indicated that many small differences are found that can be detected in the laboratory such as size, color, sheen, shape of the lemma and palea, callus, rachilla size and shape.

MacKay (10) compared samples of French Mayenne Italian

ryegrass, Danish Italian ryegrass and Oregon Common ryegrass (annual). He stated that for the last variety mentioned, two main morphological types were present, one bearing a translucent lemma and the other with lemma opaque. These three varieties produced plants with a short vegetative growth period, and low persistence during the second year.

Among the characteristics studied by Jensen and Langkilde (7) are also the number of teeth on the central part of marginal nerves of lemma, but they concluded that this was not a fully reliable character to distinguish between <u>Lolium perenne</u> from <u>Lolium</u> <u>multiflorum</u>.

#### Vigor

Another possible differentiation between these species may be the relative vigor.

References studying specifically differences in vigor between these two species are rather poor. Several authors (19, 20) have made general observations on the most vigorous habit of growth of annual ryegrass as compared to perennial ryegrass, but no definite studies were found. Nyquist (14) stated that more seedling vigor, earlier, and more vigorous spring growth were possible to be found in annual ryegrass when compared with perennial ryegrass.

Whalley, McKell and Green (22) mentioned that, in general,

within seed lots, there is a close relationship between seedling vigor and seed size in grasses; between strains this relationship is less close, and among species little relationship exists. The factors which may be of importance in seedling vigor are rapidity of germination, rate of extension of both the root and shoot, and the overall efficiency of conversion of endosperm reserves to seedling length.

#### MATERIAL AND METHODS

All plants and seeds used in this study were derived from seeds whose parent plants had shown true characteristics of the species in question for several successive generations. Oregon annual ryegrass plants were derived from seeds whose parent plants had died after completing their reproductive cycle within the same growing season after being planted. The seed was typical of ryegrass grown in Oregon under the common designation of Oregon annual ryegrass. Although not a designated variety, it is recognized as an ecotype, which has become adapted to western Oregon conditions. The seeds that generated the plants of Linn perennial ryegrass were harvested from parent plants that were truly perennials, having survived more than three years in well isolated plots. The parent plants were randomly selected and transplanted from the original registered field of Linn perennial ryegrass.

Four hundred seeds of both annual and perennial ryegrass were germinated in plastic boxes according to the official rules for seed testing recommended by the Association of Official Seed Analysts (1). After ten days of germination, the seedlings were analyzed under the ultraviolet lamp for their fluorescence response. One hundred fluorescent seedlings of Oregon annual ryegrass and 100 non-fluorescent seedlings of Linn perennial ryegrass were selected

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for transplanting. After removal from the germination media, the seedlings were transferred into individual plant bands in the greenhouse, and kept for seven days, at which time they were transferred to cold frames. Fifty seedlings of each species were retained in these cold frames for studies on their relative vigor. The remaining 50 seedlings, after adaptation to the environmental conditions were transplanted to the field, in a systemized design for subsequent morphological studies.

#### Morphological Studies

#### Tillers

1. <u>Number</u>. This character was studied and recorded at three, 2-week intervals until the plants were 43 days old.

2. <u>Texture</u>. The texture of the tillers was determined in a subjective manner and was given an arbitrary classification as follows: very rough, rough, smooth, and very smooth. This determination was made when the plants were 52 days old.

#### Leaves

1. <u>Embracement of the Auricles</u>. The amount of overlapping or the distance between the tips of the auricles was measured in five different randomly selected leaves in each of the 50 plants of each species. This measurement was made when the plants were 85 days old.

2. <u>Color of the Leaves</u>. Color determinations were made on the blades of the leaves and were given a personal and arbitrary scale, as follows: very dark green, dark green, green, and light green. The observations were made when the plants were 52 days old.

3. <u>Blade Width</u>. The width of the blades was measured in their central part, in five randomly selected leaves in each plant, when they were 85 days old.

4. <u>Vernation</u>. The position of the blades within the new buds was observed in five different buds per plant, selected at random, and classified as rolled or folded, when the plants were 25 days old.

#### Inflorescence

A total of 250 spikes of each species was used to study these characters, and a total of 750 spikelets was studied in each species. These characters were studied in the annual plants transplanted into the field, and in perennial plants that were planted three years before, and which seed had the same origin of the seeds used in this study. The characters studied were the following:

1. <u>Spikes Size</u>. At maturity, 5 different spikes were taken at random from 50 plants of each species, totaling 250 spikes for each species. Their length was measured from the base of the lower spikelet up to the tip of the superior one.

2. <u>Number of Spikelets per Spike</u>. In the same spikes used before, the number of spikelets in each spike were counted.

<u>Spikelets Size</u>. From each of the spikes chosen in study
 3 spikelets (750 per species) were taken at random from the
 central part of the spike, and the length was measured.

4. Outer Glume Length. This character was studied in the same spikelets used in study 1 above. The outer glume was removed and measured.

#### Seeds

Five seeds were taken from the middle of each of the 750 spikelets observed in study 1 for a total of 3,750 seeds per species. These seeds were studied for the following characters:

1. <u>Shape of the Distal Rachilla Callus</u>. Classified as flattened or rounded.

2. Rachilla Length.

3. Teeth on the Lemma. Presence or absence.

4. Awns on the Lemma. Presence or absence.

#### Vigor Studies

Fifty seedlings of each species were retained in cold frames

for a study of their relative vigor. The following components were observed.

#### Height

The epigeous part of every seedling was measured at three, 4-week intervals, since the seedlings were 55 days old.

#### Weight

Immediately after measuring the seedlings length, all seedlings were cut at ground level and weight determinations were made for each seedling. This was repeated at each height determination described above.

#### Statistical Analysis

A t-test (4) was used to test for significant differences between the means of the characters compared in these species. When the characters were measured by a personal and arbitrary scale, the results are expressed as a percentage of each type.

#### RESULTS

#### Morphological Studies

#### Tillers

The number of tillers per plant was significantly higher in perennial plants for the first and second observations, but no significant differences were found at the third observation.

The texture of the tillers was considered rough or very rough in 98 percent of the annual plants and smooth or very smooth in 100 percent of the perennial ones. Only 2 percent of the annual plants showed this last characteristic.

#### Leaves

The average measurement of the embracement of the auricles for annual plants was 4.32 mm. and for perennial plants was 4.38 mm. These values were not statistically different.

It was found that 86 percent of the annual plants and 6 percent of the perennial ones were green or light green in color; and 14 percent of the annual plants and 94 percent of the perennial ones were very dark green or dark green in color.

Oregon annual ryegrass showed blades wider than those of Linn perennial ryegrass, at a one percent level of probability. Vernation seemed to be a very good character to differentiate between the two species, since they followed a completely different pattern. All annual plants were found to be rolled in the bud while all perennial plants were folded in the bud.

#### Inflorescence

Summarized data on the spikes length indicated that annual plants had larger spikes than perennial ones, and the difference was significant at a one percent level of probability.

No significant difference in number of spikelets per spike occurred between annual and perennial ryegrass.

The spikelet length of annual plants was larger than in perennial ones, and this difference was significant at a one percent level of probability.

Glumes of annual plants were found larger than those of perennial ones, and the differences were significant at a one percent level of probability.

#### Seeds

The callus shape of the rachilla was clearly defined, since all seeds of annual plants had rounded callus, whereas all seeds from perennial ones had flattened callus.

Seeds from perennial plants had a larger rachilla than those

from annual plants. The difference was significant at one percent level of probability.

No differences were found in the number of teeth on the lemma between annual and perennial plants.

Awns were present on about 98 percent of the lemmas from annual plants, whereas only about 73.5 percent of the lemmas from perennial plants were awned.

### Vigor Studies

Three measurements of height of the seedlings indicated that annual seedlings were significantly taller than perennial ones, at each of the three observations, at one percent level of probability.

Three weights of the seedlings showed that annual seedlings were heavier than perennial ones at the first observation; at the second observation, there was no significant difference between the species; and at the third observation, seedlings of perennial plants were heavier than those of annual plants.

More detailed information of the results may be found in the Appendix, Tables 1 to 16.

#### DISCUSSION

In the literature review, several possible ways were mentioned to differentiate between these species, but the characters used for these purposes have not been adequate for the fast and accurate distinction needed to determine contaminations in the field or in the laboratory. Results of this study indicate varying degrees of variability in characteristics used for differentiation of the two species.

#### Tillers

Three observations made at different times on the number of tillers showed contrasting results. In the first observation, perennial plants had a significantly higher number of tillers than annual plants, at a five percent level of probability; at the second observation, the same trend occurred but was significant at a one percent level; and at the third observation, the trend was reversed but no statistical differences were found. These figures seem to indicate a faster initial tiller production in perennial ryegrass, which is equaled later by annual. Since this characteristic was not consistent throughout the growth of the plants, it was not adequate to differentiate between these species under conditions of this study. It would be interesting to observe this character directly under crop conditions, when competition between plants is important and several other environmental factors may influence tiller development.

Texture seems to be a good character to differentiate these species since 98 percent of the annual plants had culms classified as rough or very rough and 100 percent of the perennial plants had culms considered as smooth or very smooth. The degree of overlapping was only 2 percent which may have been caused by an error of personal appreciation. These results would indicate that a more detailed microscopic study of the factors composing the texture, such as pubescence, spines, etc., should be conducted on plants at an earlier stage of growth.

#### Leaves

The auricles though widely used by taxonomists in the classification of the Hordeae tribe (6) showed no significant differences in this study, and consequently would not seem to be valuable for distinguishing between these two species. This is in sharp contrast with the description of Hitchcock (6) who stated that perennial ryegrass has minute or obsolete auricles and that annual ryegrass has prominent auricles. In this study, both species showed an average auricle length of 4.3 mm. that agrees with the definition made by Hitchcock for annual ryegrass but not for perennial. Some reasons to explain the divergence between the definition of the taxonomists and these should be investigated in more detail.

It was interesting to note that 94 percent of the perennial plants were considered within the range of very dark green and dark green, and 86 percent of the annual plants were classified as green or light green. But certain degree of overlapping was found: 6 percent of the perennial plants were within the range considered common for perennial plants. This relatively high degree of overlapping detracts from the value of leaf color as a basis of distinction. Also, this character is influenced by many environmental factors such as nutrients availability, moisture availability, temperature, etc. (12). It does not mean that this character becomes useless, because one of the objectives of the field crop inspector is to find a basis to distinguish contaminants in the crop; under these conditions, all plants are under very similar environment, and consequently, color differences between these two species might become perceptible. Further study of this character, using reliable devices as a colorimeter, might be valuable to establish differences between these species.

Blade width could provide a good basis on which to differentiate between these species, since the annual leaves were significantly wider than those of perennial. The differences observed were statistically significant at one percent level. Some overlapping was found, since the range in perennial blades was from 3 mm. to 6.5

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mm. and in annual blades it was from 4 mm. to 10 mm. This degree of overlapping makes consideration of this character difficult by itself, but if considered with other characters, it becomes valuable.

These observations agree with those discussed by several authors (14, 18) and with what is generally accepted.

Vernation may be one of the most useful characters to differentiate between these species. All of the perennial plants were folded in the bud (conduplicated), and all of the annual plants were rolled in the bud (convoluted). These results agree completely with the information found in the literature (7, 14, 19, 20). This character is perhaps the most accurate in an early inspection of the field crop.

#### Inflorescence

Annual plants had significantly longer spikes than perennial ones, at a one percent level of probability. Such a characteristic provides a very useful and practical means for differentiating these species, and can be complemented, for more accuracy and precision, with other characters such as emergence date of the spikes and length of the anthesis period. For this information to be more valuable, this study should be broadened and carried out under field conditions, observing carefully variation with environmental conditions such as fertility level, time of planting, etc.

The number of spikelets per spike would appear to be of little value for distinguishing the two species since no statistical differences were found. If some differences were found by additional studies, the character would need to be consistently different in order to satisfy the objective of this study.

There was a highly significant difference between the longer spikelets of annual plants and the shorter spikelets of perennial plants. These results agree with the description of the species made by Hitchcock (6), and indicated that spikelet size might be a valuable tool to differentiate between these two types of ryegrass, since the mean spikelet size of annual plants was almost twice that of perennial plants. Observations under crop conditions would be of value to confirm or deny the accurateness of this character.

The glumes of annual spikelets were longer than those of perennial. This difference was significant at the one percent level of probability. These results agree with the description made for annual and perennial ryegrass species by Hitchcock (6), but conflicts with that reported by Nyquist (14) who stated that annual ryegrass had shorter glumes than perennial ryegrass.

One of the most practical means to differentiate both types, at this state of development, may be a combination of two characters: spikelets size and glume length. In the annual ryegrass, the glume covered approximately 50 percent of the spikelet, whereas in perennial ryegrass the glume coverage is approximately 90 percent. A consideration of these characters might be of very practical use to the inspector.

#### Seeds

Careful study of the shape of the rachilla callus showed a significant difference between seeds of annual and perennial ryegrass. The results found agree with the observations made by Pierpoint (17) who reported that seeds of annual ryegrass had a rounded callus and those of perennial ryegrass had a flattened callus. This character might be useful in the seed testing laboratory, if the analysis is carried out by a skilled analyst, since some experience is necessary to discern the type of callus.

The rachillas of annual seeds were shorter than those of perennial seeds. Again, this agrees with the observations of Pierpoint (17). More observation, involving a larger number of seeds per spikelet, to observe its variation within the spikelets might lead to a clearer definition of this character, since the differences in this study were small and some degree of overlapping was found. The usefulness of this character is limited to the laboratory, where precise measurements can be taken.

All seeds of both types showed teeth on the lemma. It is

necessary to remark that only its absence or presence was observed; further research on the particular shape, number, distribution or position of the servations on the lemma might be valuable for further differentiation.

The seeds of perennial ryegrass were 98.8 percent awnless, and annual ryegrass seeds were 73.5 percent awned and 26.5 percent awnless. These results conflict with the general opinion that annual ryegrass has awned seeds and perennial ryegrass has awnless seeds (6, 14, 17, 18, 19, 20). It does not appear to be very reliable character since 1.2 percent of the perennial seeds were awned. In additional to the natural absence of awns in seeds, they are very fragile and may be knocked off by insects, animals, strong winds, etc. Agitation during cleaning and processing will especially cause their detachment. The presence or absence of awns could be used to alert an inspector in the field that he should check further using other characteristics of more reliability.

#### Vigor

In early stages of development, annual seedlings tended to be taller than perennial seedlings. These observations agree with those of several authors (7, 14, 17, 19, 20) and with the botanical description of Hitchcock (6).

The observation of this character may provide a very useful

and accurate differentiation between both species in the early stage of development. Conditions of observation would need to be under growing out trials where seedling age and environmental conditions can be controlled.

Annual seedlings were heavier than perennial ones in the first stages of development, but this fact is reversed with time. The explanation for this difference might be found in the loss of foliage observed in annual plants. The usefulness of this character as a basis for distinction is doubtful, since this type of information requires considerable time, equipment, and precision, and then it is not available for a rapid information. Its use would be of value only if the differences were consistent and widely different between the two types.

#### CONCLUSIONS

Detailed studies were made of the seed and morphological features as well as relative vigor of Linn perennial ryegrass (Lolium perenne L.) and Oregon annual ryegrass (Lolium multiflorum Lam.) to obtain information on ways to distinguish these two types under field and seed laboratory conditions. Fifty plants of Oregon annual ryegrass and 50 plants of Linn perennial ryegrass were used to compare the characteristics, and the following conclusions were drawn:

Vernation, percentage of glumes covering the spikelets, and height of seedlings seem to be fully reliable characters to differentiate the plants under study.

Texture of the tillers, blade width, spikes size, spikelets size, glume length, callus shape of the rachilla, and rachilla length seem to be relatively good characters for differentiating between these species, but not with complete accurateness. Further study under direct crop conditions and more detailed study on the laboratory are needed.

Number of tillers, embracement of the auricles, number of spikelets per spike, teeth on the lemma, awns on the lemma, and weight of the seedlings are not adequate characters to differentiate between the species studied. Leaf color may deserve special consideration, since it is affected by many environmental factors. The author feels that color observation might be a useful guide to detect contaminations when the species are grown under identical conditions.

Some useful conclusions can be drawn from this study to provide valuable information for the Seed Certification Program and for the Seed Testing Laboratory, fulfilling the primary objective of this study. These results are not intended to apply for all annual and perennial ryegrass, but only these two varieties included in the studies. The results obtained are believed helpful in that these two varieties comprise the major portion of all ryegrass grown in western Oregon. It is also expected that this study might serve as a basis for additional experimentation to determine if the difference found here might be consistent for all varieties of annual and perennial ryegrass.

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APPENDIX

	July 2		July 16		July 30	
	Ann.	Per.	Ann.	Per.	Ann.	Per.
Mean tillers per plant	6.34	7.52	9.64	12.04	52.40	49.68
t-value	*	*	*	:*	ſ	١S

Appendix Table 1. Average number of tillers per plant at three different stages of development.

\* Significant at 5% level of probability.
\*\*Significant at 1% level of probability.
NS No significant difference.

Appendix Table 2.	Texture at the base of the tillers, in percentage
	of each type.

	Annual	Perennial
Very smooth	-	54
Smooth	2	46
Rough	32	_
Very rough	66	

	Annual	Perennial
Mean mm. embracement auricles	4.32	4.38
t-value	Ĩ	١S

## Appendix Table 3. Average amount of the embracement of the auricles, in millimeters.

Appendix Table 4.	Color of the blades, in percentage of each
	type.

	Annual	Perennial
Very dark green	-	86
Dark green	14	8
Green	80	4
Light green	6	2

	Annual	Perennial
Mean mm. blade width	6.55	4.59
t-value	*	<*

# Appendix Table 5. Average blade width of the leaves in their median part, in millimeters.

Appendix Table 6. Vernation of the leaves, in percentage of each type.

	Annual	Perennial
Rolled	100	-
Folded	-	100

Appendix Table 7. Average length of the spikes, in millimeters.

	Annual	Perennial
Mean mm. spikes length per plant	209.9	153.7
t-value	k	<*

	Annual	Perennial
Mean number spikelets per spike	17.6	18.8
t-value	N	IS

Appendix Table 8. Average number of spikelets per spike.

Appendix Table 9. Average length of the spikelets, in millimeters.

	Annual	Perennial
Mean mm. per spikelet	17.5	9.1
t-value	*	*

Appendix Table 10. Average length of the glumes, in millimeters.

	Annual	Perennial
Mean mm. per glume	9.0	8.3
t-value	*:	*

		Annual	Perennial	
- <u></u>	Rounded	100	-	
Flattened	-	100		

Appendix Table 11. Shape of the callus of the rachilla, in percentage of each type.

Appendix Table 12. Average length of the rachilla, in millimeters.

	Annual	Perennial
Mean mm rachilla le	h 0.95	1.26
t-value		**

Appendix Table 13. Presence or absence of teeth on the lemma, in percentage of each type.

Annual	Perennial
100	100
_	-
	100

	Annual	Perennial
Awned	73.5	1.2
Awnless	26.5	98.8

Appendix Table 14. Presence or absence of awns on the lemma, in percentage of each type.

Appendix Table 15. Average height of the seedling at three different stages of development, in millimeters.

	July 13		Aug. 12		Sept. 11	
	Ann.	Per.	Ann.	Per.	Ann.	Per.
Mean mm. seedlings height	256.1	178.1	225.1	120.5	112.8	76.2
t-value	*	*	*	*	*	*

Appendix Table 16. Average weight of the seedlings, in grams, at three different stages of development.

	July 23		Aug. 12		Sep	t. 11
	Ann.	Per.	Ann.	Per.	Ann.	Per.
Mean gr. seedlings height	2.40	1.99	0.87	0.78	0.21	0.28
t-value	*	: ;;:	N	S	><>	\$