AN ABSTRACT OF THE THESIS OF

Ethan Benjamin for the degree of Master of Science in Crop Science presented on November 25, 1986

Title: Development of Oven and Karl Fischer Techniques for Moisture Testing of Grass Seeds

Abstract approved: Don F. Grabe

Seed moisture is a primary factor influencing seed quality during harvesting, storage, and marketing of grass seed. However, the Association of Official Seed Analysts' Rules for Testing Seeds do not contain methods for moisture testing. The oven methods in use by seed testing laboratories in the U.S. are diverse and many produce erroneous results. The International Seed Testing Association Rules for Seed Testing contain methods for 95 kinds of seeds, but many of the methods are empirical in nature and lacking in accuracy. The objective of this research was to develop accurate oven moisture testing methods for seeds of temperate grass species.

The test variables investigated were oven temperature, time of drying, grinding the seed, and original moisture level of the seed. Chi-square analysis was used to determine if the results of the oven methods were within $\pm$ 0.5% of those obtained by the Karl Fischer reference method. The species included were perennial ryegrass (*Lolium perenne* L.), orchardgrass (*Dactylis glomerata* L.), bentgrass

(*Agrostis tenuis* L.), Kentucky bluegrass (*Poa pratensis* Huds.), tall fescue (*Festuca arundinacea* Schreb.), and red fescue (*Festuca rubra* L.).

Drying to constant weight at temperatures of 90, 100, and 105°C gave moisture percentages lower than the true value. Drying periods of 6 h or less at 130°C gave moisture percentages in agreement with Karl Fischer results. Ground and whole seed gave similar moisture percentages after drying to constant weight, but moisture was removed more rapidly from ground seeds. The required drying time for greatest accuracy depended on the original moisture content of the seed. Moisture was removed most rapidly from the highest moisture seed; thus, it is not possible to select one drying period that will provide the same degree of accuracy on seed with different moisture levels.

Seed moisture tests on these six temperate-climate grass species should be conducted on whole seeds at 130°C. The drying periods should be 3 h for perennial ryegrass, Kentucky bluegrass, tall fescue, and red fescue, 1.5 h for orchardgrass, and 1 h for bentgrass.

DEVELOPMENT OF OVEN AND KARL FISCHER TECHNIQUES FOR MOISTURE TESTING OF GRASS SEEDS

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DEVELOPMENT OF OVEN AND KARL FISCHER TECHNIQUES FOR MOISTURE TESTING OF GRASS SEEDS

INTRODUCTION

The development of standardized moisture testing techniques for grass seeds is a primary requirement for the grass seed industry throughout the world, since no other factor affects the quality of grass seed so immensely as moisture content (Hunt and Pixton, 1974). Methods and standards of moisture testing are not the same for every country, hence there is a need for agreement on accuracy and standardization of methods to be applied to seed moving in international trade. Extensive work has been done on moisture determination for seeds that are of great commercial importance in the Poaceae, Compositae, and Leguminosae families, but research on grasses is limited.

Methods of measuring moisture content are generally of two types: basic and practical (Mathews, 1962). Basic methods measure loss of water upon heating or measure water directly by chemical means. Oven methods and the Karl Fischer chemical method are examples. Practical methods such as electric moisture meters are rapid and specifically designed for routine use. Oven methods, probably the oldest method, have been the most widely used techniques for moisture determination of seeds and agricultural products. In performing the air-oven moisture test, a weighed quantity of seeds is heated at a certain temperature for a specified period of time. The loss in weight during heating is considered to be the moisture content of the sample (Pande, 1974).

Van Wyk (1978) conducted a survey of members of the International Seed Testing Association (ISTA) and found that many species, methods, and equipment were being used for moisture testing that were not included in the ISTA Rules. Grabe (unpublished) found 35 combinations of time and temperature for oven methods used in the United States and Canada. Many of these oven methods have been adopted as official procedures by several government and technical agencies. Since the apparent moisture content varies with time and temperature of drying, these laboratories would obviously obtain different results. Because of the empirical nature of results produced by oven testing techniques, there was a need for the development of a universally accepted reference method measuring true moisture content of grass seeds.

The Karl Fischer method for moisture determination is ideally suited for this purpose since it measures total water in the seed samples and results are independent of time and temperature. It is based on titration of the sample containing water with the Karl Fischer reagent. The procedure depends upon the reaction of iodine with water in the presence of sulphur dioxide and pyridine to form hydriodic acid and sulphuric acid. The endpoint of the reaction is indicated by the liberation of free iodine which is consumed as long as there is any water present in the sample (Pande, 1974).

Studies in this research were concentrated on (1) the development of a standardized Karl Fischer technique that can be used as a reference system for moisture determination of grass seed, and (2) the development of oven methods for grass seeds that would provide similar results with this reference method. The grass species selected for

this study were perennial ryegrass (Lolium perenne L.), orchardgrass (Dactylis glomerata L.), Kentucky bluegrass (Poa pratensis Huds.), bentgrass (Agrostis tenuis L.), tall fescue (Festuca arundinacea Schreb.), and red fescue (Festuca rubra L.).

The results of this research were presented in the form of a manuscript.

LITERATURE REVIEW

Need for Moisture Testing Methods

The development of moisture testing methods for grass seeds is an essential prerequisite since no other factor affects the quality of seeds so profoundly as moisture content. Dry, sound seeds can ordinarily be stored for long periods with no material loss of viability but wet seeds are certain to deteriorate completely in a short time. Moisture content is one of the most important characteristics of seeds. Excessive moisture sharply reduces storability by determining the length of time before mold and insect invasion (Hunt and Pixton, 1974).

Harvestability of seeds is also related to moisture content. There is a direct relationship between kernel damage during harvest and moisture content. Combine harvesting at high moisture levels leads to incomplete shelling, threshing, broken kernel tips, and kernel crackage. Safe limits of moisture content vary with the kind of grass seed and have to be determined experimentally for each kind; near this critical moisture level, moisture content must be determined accurately. Incorrect moisture values can introduce difficulties in buying and selling seed on a dry basis. The exact dry weight of seed must be known, for example, in calculating the yield of cleaned seed from wetter uncleaned seed. Accurate methods of moisture determination are therefore of vital importance to the seed marketing industry.

Methods and standards for seed moisture testing are not the same in every country, hence there is need for agreement among countries on

accuracy of methods and on standardization of methods to be applied to grass seed moving in international trade. Extensive work has been done in developing methods for seeds that are of great commercial importance in the Poaceae, Compositae, and Leguminosae families such as the cereal grains, oil-bearing seeds, and legumes. Many of these methods are empirical in nature but give reproducible results within acceptable limits. They are widely used and have been adopted as "official" methods by government agencies or certain technical organizations. Relatively little work has been done on grass seeds, the assumption often being made that these can be analyzed by the methods used for other commercially important seeds.

Van Wyk (1978) conducted a survey of members of the International Seed Testing Association (ISTA) to determine various methods of moisture testing in seeds. This survey revealed that many species were being tested throughout the world that were not yet included in the ISTA Rules; that many researchers were using methods other than those prescribed by the ISTA Rules; and there was a wide variety of equipment being used for moisture testing. Grabe (unpublished) also conducted a survey of all seed testing laboratories in the United States and Canada and found thirty-five combinations of time and temperature presently in use in oven testing procedures. Since the apparent seed moisture content varies with time and temperature of drying, these laboratories would obviously obtain different moisture percentages. For these reasons there was a dire need for the development of standard oven testing procedures calibrated against an accepted basic

reference method to be followed by all seed testing laboratories worldwide, thus assuring more uniform and accurate results.

Seed-Moisture Interaction

In any evaluation and development of moisture testing methods, a lucid understanding of what these methods are measuring is important. Therefore, a knowledge of seed structure and chemical composition, of moisture and water properties, and the interaction of these components will provide the necessary background information needed to make the evaluations and develop the techniques. Seeds are inherently hygroscopic in nature, their moisture content therefore tends to achieve a state of dynamic equilibrium with the relative humidity of the atmosphere around them (Harrington, 1972). Relative humidity is a measure of water vapor in air relative to the amount that air can hold at saturation. As air is heated or warmed up, the amount of water vapor it can hold increases rapidly. Harrington (1972) demonstrated that the amount of water a kilogram of air can hold at saturation approximately doubles for each $10^{o}C$ rise in temperature. As long as the moisture content of seeds is below equilibrium with the relative humidity, a water potential gradient will exist, with water vapor moving from the atmosphere to the seeds, lowering the relative humidity of the atmosphere and raising the seed moisture content.

Conversely, when moist seeds are placed in a dry atmosphere, moisture will flow from the seeds into the atmosphere (Justice and Bass, 1978). Because the air cannot hold nearly all the moisture held in the seed, the air will soon become saturated and unless new dry air

is provided, drying of the seeds will stop. Since seed deterioration is affected by moisture content, knowing what factors affect water absorption and retention as well as their effects is important. Thickness, structure, and chemical composition of the seed coat and seed itself affect the rate of water absorption and retention by seed. Of the various seed constituents, proteins are the most hygroscopic, carbohydrates are slightly less so, and lipids are hydrophobic (Justice and Bass, 1978). Therefore, seeds high in protein or carbohydrates or both, can hold more moisture at a given temperature and relative humidity than could seeds high in oil or lipids. There could possibly be variations of seed moisture among different seed lots of the same variety due to difference of chemical composition brought about by varying cultural practices during seed maturation.

Before exploring methods and techniques for the determination of moisture in a particular sample, it is worthwhile to understand the forces involved and consider several distinct phenomena in water adsorption. A moisture-containing material is not simply a juxtaposition of dry substance and water; the absorbed water is always bound to the seed with a definite amount of binding energy (Pande, 1974). Water exists in a biological system, such as seed, in two states. "Free" water is held by capillary forces in the pores and interstitial spaces, it exhibits the normal characteristics of water and may be removed by relatively mild drying (Hart and Golumbic, 1962). "Bound water" on the other hand, is adsorbed on surfaces of the seed structure and is held more firmly. There is chemical and physical interaction between the water molecules and the molecular constituents of the

seed, particularly those containing polar groups. This bound water or water of constitution is held in the seed by very strong chemical forces (Hunt and Pixton, 1974).

Many molecules, although electrically neutral as a whole, contain atoms on which there is a higher concentration of positive or negative charge than on other atoms. These are called polar molecules. Water, ammonia, and alcohols are typical examples of polar molecules. The negative pole of one polar molecule is attracted electrostatically to the positive pole of another. Grass seeds contain high molecular weight compounds, such as carbohydrates and proteins, which have many polar groups. Hunt and Pixton (1974) described the nature of water adsorption by starch and protein. Starch is a very large molecule; being a natural polymer it is made up of glucose ring units connected to each other through oxygen atoms and characterized by hydroxyl groups on the ring, ring oxygen, and bridge oxygen. These are all centers of polarity or suitable points for interaction with water molecules through hydrogen bonding.

Proteins, on the other hand, carry a wider variety of polar and ionic groups (NH_2, NH, OH, and COOH) in the side chains of their amino-acid units. These groups have residual pairs of valence electrons which tend to form covalent bonds (hydrogen bonds) with the hydrogen atoms of water molecules (Lloyd, 1938; Pauling, 1945). Of these the carboxyl group can coordinate or bind 4 or 5 molecules of water; the amino group, 3 molecules; hydroxyl group, 3 molecules; the imino and carbonyl groups, 2 molecules each. Molecules with easily mobile charges become polar by induction when a strong polar molecule

like water approaches them. Physical forces of attraction, sometimes called Van der Waal's forces, also serve to hold water molecules to surfaces.

As a result of all these attractive forces, water is held in seeds with varying degrees of strength, ranging from the relatively weak capillary attraction to a force equal to that of a covalent chemical bond. For a given set of external conditions of temperature, pressure, and relative humidity, a condition of dynamic equilibrium must exist between the free water and the various types of bound water, and between the total amount of water present in the seed and moisture in the surrounding atmosphere. The total quantity of water is considered as seed moisture content and is a constant under one set of conditions. If conditions are changed so that some of the free water is removed new equilibria are established in which the total amount of water will have a different constant value. Thus, in drying seeds at a given temperature, pressure and relative humidity, it may not be assumed that, because the seeds have attained constant weight, all of the water has been removed (Hart et al., 1959).

An isotherm curve describing the amount of water adsorbed by seeds at a particular temperature as a function of the equilibrium vapor pressure or relative humidity has been useful in the study of moisture relations of seeds (Hunt and Pixton, 1974). This isotherm or moisture equilibrium curve is a typical sigmoid curve. Hunt and Pixton describe the first portion of the isotherm where the curve is concave to the humidity axis, as representing the absorption of the first layer of water vapor onto the surface of the absorbing material

(mono layer or bound layer as described by Harrington, 1972). The region of inflection represents the deposition of a second layer of water molecules (multilayer water); and the final curved portion represents the continued adsorption of additional layers (mobile or free water).

If a sample of seed comes to equilibrium with an atmosphere (having a given temperature and relative humidity) while losing moisture [desorption], the moisture content of the sample will be higher than if it reached equilibrium with the same atmosphere while gaining moisture (adsorption). This influence of a sample's previous history on its moisture content is called hysteresis. Hypotheses involving both chemical and physical factors have been advanced to explain this phenomenon (McBain, 1935). The hysteresis effect presumably does not produce actual errors in a given moisture determination, but frequently causes non-uniformity in a lot of seeds which, in turn, makes sampling errors more likely. It can produce confusing results and apparent errors on lots of seed stored in the same atmosphere but having different previous histories.

Moisture Testing Methods

Methods of measuring moisture are classified into two categories, basic (primary) and practical or secondary (Mathews, 1962). Basic methods measure moisture directly by determining weight loss from heating due to removal of moisture or from a chemical reaction. That is, there is direct measurement of water, whether this is by weight loss (oven methods), absorbance of light (spectrophotometric methods),

or chemical reaction (Karl Fischer). Secondary methods such as electric moisture meters determine quantitatively some physical or chemical property or characteristic of seeds which is related or correlated with their moisture content. Percentage moisture is determined from charts that relate the measurement of the property to the moisture content as determined by a primary method. Some of the primary methods that have been widely accepted and are considered relatively accurate are termed basic or reference methods. They do not require calibration against some other method and are themselves used in calibrations. Several basic methods have been used for moisture testing in cereals, oil seeds, and edible legumes, but there is no general agreement among countries as to which method is best.

Oven Methods

Oven drying, probably the oldest method, has been the most widely used technique for the determination of moisture in seeds and agricultural products. Removal of moisture from the seed sample requires that the partial pressure of moisture in the vapor phase be lower than that of water in the sample (Pande, 1974). In most cases temperature and time of heating are established empirically, based on the attainment of "constant" weight (Mitchell and Smith, 1977). Accuracy then would depend on the requirement that (1) constant weight be due to complete removal of water, and (2) total weight loss be due only to water. Mitchell and Smith conclude that because of these requirements, some procedures for analysis give good precision with unknown accuracy.

Among the factors that influence results from oven-drying methods are the state of divisions (the finer the particles, the greater the diffusing surface), pressure, time, and temperature. The size, shape, and material of the sample dish are also significant as well as position of the dish in the oven, and the closeness of its contact with the oven bottom (Pande, 1974).

Air-Oven Methods

Air oven methods because of their widespread use have been officially adopted by numerous governmental agencies and international organizations. They are relatively simple and inexpensive to conduct and give reproducible results between laboratories. In performing the air-oven moisture test a weighed quantity of seeds is heated at a certain temperature for a specified period of time. The loss in weight during heating is considered to be the moisture content of the sample. However, seeds and other natural organic substances with cellular structure usually contain organic matter which is easily decomposed (Mitchell and Smith, 1977). In experiments using several kinds of seeds, Hart and Neustadt (1957) found and oily liquid condensed in a tube outside the oven while heating samples of soybean (Glycine max L.) and flax seed (Linum usitatissimum L.). Pande (1974) defined this reaction as a permanent loss, where the heating of the specimen may cause substances other than water to be driven off. This causes the measured loss of weight to be greater than it should be, therefore, giving an erroneously high value for moisture content.

ISTA lists for several crops specific drying temperatures

(frequently 103^{0} or 130^{0} C), times, and grinding requirements (ISTA, 1976). Most of the newer techniques for moisture testing developed by various seed testing organizations use these rules to compare to their results. Therefore, it is of interest to trace the evolution and development of air-oven moisture testing methods in the ISTA Rules.

Karl Fischer Method

It must be acknowledged that the determination of moisture, while a fundamental part of any analytical scheme, remains one of the most empirical of procedures under oven testing methods. The reproducible estimation of water by such elementary physical change as a loss of weight, logically necessitates a strict adherence to predetermined conditions of temperature, pressure, and physical state of seeds. Even with such conditions accurately controlled, the values thus obtained may or may not represent "true" moisture content, depending on the chemical composition of the sample and the presence or absence of volatile compounds other than water (Fosnot and Haman, 1945).

The development of a chemical method for the determination of moisture in seeds was greatly retarded owing to the lack of specificity of any reagents for water. However, Fischer (1935) introduced a titration method involving a complex reagent consisting of pyridine, methanol, sulphur dioxide, and iodine. This reagent has a definite specificity for water, is one of the most theoretically sound methods for determining moisture content and allows the determination of water in a wide variety of substances.

The Karl Fischer method for determining moisture content is based

on titration of the sample containing water with the reagent; the method depends upon the reaction of iodine with water in the presence of sulphur dioxide and pyridine (an organic base serving as a buffer) to form hydriodic acid and sulphuric acid. The endpoint of this reaction is indicated by the liberation of free iodine which is consumed as long as there is any water present in the sample (Pande, 1974). Originally visual detection of the endpoint was done by observing the brown color of free iodine, but with the introduction of automatic titration equipment, endpoint detection is ascertained by electrochemical methods. That is, by passing a small constant current between a pair of indicator electrodes immersed in a sample solution and monitoring the corresponding voltage by means of a microammeter and dc voltage source. A polarizing voltage is applied during the addition of the Karl Fischer reagent to the sample solution, until eventually all sample water has been consumed in the reduction of iodine by sulphur dioxide. In the absence of water, the free iodine in the Karl Fischer reagent is no longer reduced to iodide and is therefore present as an efficient current carrier. The resulting large standing current is then observed by the microammeter as a sharp endpoint indication (Schalch, 1984).

The Karl Fischer titration procedure is frequently used as a basic reference method for other procedures. It has been successfully adapted to all cereal grains and is used to test the accuracy of official oven methods. The U.S. National Bureau of Standards asserts that the Fischer method is the only valid method for measuring water, however, their results were based on limited sampling (Jones and

Brickenkamp, 1981). It is useful in measuring moisture content of solids, liquids, and gases, and is appropriate for use on seeds, since it gives total water, that is "free" plus "bound" water (Pande, 1974). A major advantage is that results are not dependent on temperature or duration of drying and prolonged heating at high temperature is avoided. Some of the disadvantages of the Karl Fischer method, such as length of reaction time and considerable technical skill has been overcome by the automation of the procedure (Jones and Brickenkamp, 1981). Frediani (1952) also devised an ingenious electrically operated, automatic titration device for determining moisture by the Karl Fischer method in order to obtain higher precision and greater rapidity.

Fosnot and Haman (1945) used the Karl Fischer titration method in a preliminary investigation of its application to the determination of moisture in cereals including wheat (Triticum aestivum L.), and barley (Hordeum vulgare L.). They concluded that the Fischer reagent can be used for the determination of moisture in cereals with necessary modifications of treatment for various materials, including accurate verification of fineness of grind and contact time with the Fischer reagent. Hart and Neustadt (1957) successfully adapted the method to all cereal grains and used it to test the accuracy of official oven methods. They found that several grains gave favorable comparisons except for peas (Pisum sativum L.), corn (Zea mays L.), and flaxseed (Linum usitatissimum L.). Low results for peas and corn were explained by incomplete removal of "bound" water by the oven while high results on flaxseed from the oven method were attributed to some

decomposition and loss of volatile oils at 130° C. Besides Hart and Neustadt, Kostyrko and Plebansko (1965) also used a modified Stein Laboratory Mill to extract water from grain using methanol as a solvent.

Weise et al. (1965) in testing both the Karl Fischer and gas chromatography techniques, extracted samples from 1 to 5 days by soaking in methanol at ambient temperature. Blank determinations by both procedures indicated that no interfering substances were extracted along with water. Makower (1950) noted the rapidity with which methanol extracts water from finely divided materials. The affinity of methanol for water also aids extraction. With cereal grains, oil-bearing seeds, and grasses extraction is complete. Hart et al. (1959) reported on the standardization of the ASAE air-oven methods for whole seeds of 32 common agricultural and vegetable seeds including eight grass species. All were calibrated against the Karl Fischer method with which they were in very close agreement. USDA oven methods have been designed to give results which agree with those obtained by the Karl Fischer method, although a satisfactory method has not been developed for soybeans (Glycine max L.) (Hunt and Pixton, 1974).

Jones and Brickenkamp (1981) applied the automatic Karl Fischer titration to the determination of the moisture content of corn, wheat, soybeans (Glycine max L.), rice (Oryza sativa L.), and oats (Avena sativa L.). Their results showed that the method was precise (with a standard deviation of 0.07%), easy to apply, applicable to a wide range of moisture content, relatively rapid, and complete extraction of water was obtained. Duval (1954) determined moisture content of

several grains by oven analysis and results checked within 1.01% of those obtained by titration techniques. Bolling (1960) used desiccant absorption for water extraction of wheat and results compared favorably with the Fischer titration. Hart et al. (1962) used a methanol extraction procedure combined with infrared spectrophotometry for measuring water in seeds, grains, and grasses and also obtained favorable results with the Fischer method.

Grabe (1984) compared ISTA methods for testing seed moisture with the Karl Fischer method and several oven methods. Moisture content of seven representative species (including legumes and grasses) ranged from 1.97% lower to 1.13% higher than the Karl Fischer technique. Grinding the seeds shortened the drying period, but introduced errors because of moisture loss during grinding. It was reported that a lot of work had been done on cereal moisture, but not on legumes, grasses, vegetables, and flower seeds. There was thus a great need for moisture testing research in these crops, especially grasses (ISTA, 1984).

MANUSCRIPT

DEVELOPMENT OF OVEN AND KARL FISCHER TECHNIQUES FOR MOISTURE TESTING OF GRASS SEEDS

ABSTRACT

Seed moisture is a primary factor influencing seed quality during harvesting, storage, and marketing of grass seed. However, The Association of Official Seed Analysts' Rules for Testing Seeds do not contain methods for moisture testing. The oven methods in use by seed testing laboratories in the U.S. are diverse and many produce erroneous results. The International Seed Testing Association Rules for Seed Testing contain methods for 95 kinds of seeds, but many of the methods are empirical in nature and lacking in accuracy. The objective of this research was to develop accurate oven moisture testing methods for seeds of temperate grass species.

The test variables investigated were oven temperature, time of drying, seed grinding, and original moisture level of the seed. Chi-square analysis was used to determine if the results of the oven methods were within $\pm$ 0.5% of those obtained by the Karl Fischer reference method. The species included were perennial ryegrass (Lolium perenne L.), orchardgrass (Dactylis glomerata L.), bentgrass (Agrostis tenuis L.), Kentucky bluegrass (Poa pratensis Huds.), tall fescue (Festuca arundinacea Schreb.), and red fescue (Festuca rubra L.).

Drying to constant weight at temperatures of 90, 100, and 105^{o}C gave moisture percentages lower than the true value. Drying periods of 6 h or less at 130^{o}C gave moisture percentages in agreement with Karl Fischer results. Ground and whole seed gave similar moisture percentages after drying to constant weight, but moisture was removed more rapidly from ground seeds. The required drying time for greatest

accuracy depended on the original moisture content of the seed. Moisture was removed most rapidly from the highest moisture seed; thus, it is not possible to select one drying period that will provide the same degree of accuracy on seed with with different moisture levels.

Seed moisture tests on these six temperate-climate grass species should be conducted on whole seeds at 130°C. The drying periods should be 3 h for perennial ryegrass, Kentucky bluegrass, tall fescue and red fescue, 1.5 h for orchardgrass, and 1 h for bentgrass.

Additional Index Words: *Lolium perenne* L., *Dactylis glomerata* L., *Agrostis tenuis* L., *Poa pratensis* Huds., *Festuca arundinacea* Schreb., *Festuca rubra* L.

DEVELOPMENT OF OVEN AND KARL FISCHER TECHNIQUES FOR MOISTURE TESTING OF GRASS SEEDS

INTRODUCTION

Accurate oven methods for testing seed moisture content are essential for seed moving in domestic and international trade, for research on seed quality, and for other purposes.

Oven methods for 95 kinds of seeds are presently prescribed in the International Rules for Seed Testing of the International Seed Testing Association (ISTA, 1985). For cereals, ISTA methods follow the methods of The International Organization for Standardization (ISO, 1985) and The International Association for Cereal Chemistry (ICC, 1976). These methods, in turn, are based on the basic vacuum oven-phosphorus pentoxide method. The basis of methods prescribed for seeds other than cereals is not clear, but the methods appear to have been established empirically without comparison with basic reference methods. For these species, repeatability of results is good, but their accuracy is unknown. Moisture content of seven representative species determined by ISTA oven methods ranged from 1.97% lower to 1.13% higher than those obtained by the Karl Fischer method (Grabe, 1984).

The Rules for Testing Seeds of The Association of Official Seed Analysts (AOSA, 1981) do not include methods for testing seed moisture. A survey of AOSA laboratories showed that at least 35 combinations of time and temperature are presently used in oven testing procedures (Grabe, unpublished). This situation would contribute to

discrepancies in moisture percentages obtained by different laboratories since apparent seed moisture content varies with the time and temperature of drying.

Selection of accurate oven drying schedules must be based on results obtained by standard reference methods that are independent of drying time and temperature. Of several basic methods available, the Karl Fischer titration procedure appears to be the method of choice for seeds and grains (Fischer, 1935; Mitchell and Smith, 1980). This procedure was introduced in 1935 and has been used frequently on cereals (Fosnot and Haman, 1945; Makower, 1950; Hart and Neustadt, 1957; and others). Jones and Brickenkamp (1981) applied the automatic Karl Fischer titration to the determination of the moisture content of corn (Zea mays L.), wheat (Triticum aestivum L.), and rice (Oryza sativa L.). Their results indicated that the method was precise (with a standard deviation of 0.07%), easy to apply, applicable to a wide range of moisture content, and completely extracted water. Hart, et al. (1959) developed oven methods for 32 crops using time and temperature regimes that gave similar results to those of the Karl Fischer method.

In view of the deficiencies discussed in ISTA methods, this research was initiated to develop more accurate oven methods for testing moisture content of seeds of temperate-climate grass species. Selection of proper temperatures and drying periods was based on moisture contents determined by the Karl Fischer method. To do so, it was first necessary to adapt the Karl Fischer method to grass seeds.

MATERIALS AND METHODS

Seedlots of six temperate grass species were included in this study: perennial ryegrass (Lolium perenne L.), orchardgrass (Dactylis glomerata L.), Kentucky bluegrass (Poa pratensis Huds.), bentgrass (Agrostis tenuis L.), tall fescue (Festuca arundinacea Schreb.), and red fescue (Festuca rubra L.). Each seedlot was divided into three sublots which were adjusted to low, medium, and high moisture levels. Low (4-7%) and high (13-16%) moisture levels were achieved by equilibrating the sublots over saturated solutions of lithium chloride and ammonium sulfate for 3 weeks in humidity chambers. Medium moisture levels (8-11%) consisted of the original seed that was not adjusted for moisture content. The sublots were then stored in air-tight containers for the duration of the studies.

Oven Moisture Testing Methods

Duplicate 3-g samples of whole or ground seed were weighed into previously tared aluminum drying dishes. Dishes were 55 mm diameter x 15 mm high. The uncovered dishes were placed in a Thelco forced-draft oven at the desired temperature controlled to $\pm$ 1^{o}C. After the specified drying period, the dishes were covered, placed in desiccators for 1 h, and weighed. Samples were weighed to the nearest 0.001 g and moisture loss was calculated on a wet weight basis. For comparison of whole and ground seed, samples were ground in a Wiley laboratory mill through a 20-mesh screen.

The Karl Fischer Moisture Testing Procedures

Seed samples were ground in a Wiley laboratory mill through a 20-mesh screen. One-g portions were placed in 50-mL flasks with 50 mL reagent grade methanol. The stoppered flasks were left for 24 h at room temperature without stirring. Preliminary tests indicated these procedures allowed for complete extraction of moisture from the seeds.

The entire sample (methanol plus ground seed) was then transferred to the reaction vessel through the sample inlet port. Moisture in the sample was titrated with Hydranal Composite No. 5 reagent. Titration was carried out with a Fischer Automatic K-F Titrimeter System Model 392 and Fischer Scientific Digital/Dispenser Burette Model 395 (Fischer Scientific Co.). The Karl Fischer titration technique followed the procedures of Mitchell and Smith (1980) and the instruction manual supplied by the manufacturer.

Determination of Oven-drying Temperature and Time for Perennial Ryegrass

A series of oven tests was conducted with perennial ryegrass to determine the drying temperature and time required to give moisture percentages in agreement with the Karl Fischer technique. Whole and ground seed samples at three moisture levels were dried at 90, 100, 105, and 130^{o}C. Moisture percentages were calculated after 1, 4, 17, 24, 48, and 72 h. Moisture content was determined by the Karl Fischer technique at the beginning of each series of oven tests.

Determination of Drying Times at 130°C for Five Grass Species

For the other five grass species, whole and ground seed samples at three moisture levels were dried at 130°C for 0.5, 1, 2, 3, 4, 5, and 7 h. Moisture percentages were calculated after each drying period and compared to the Karl Fischer determinations.

Comparison of Oven and Karl Fischer Techniques on Ten Samples from Each Sublot

The oven drying periods at 130°C that provided the closest approximation to the Karl Fischer technique in the two previous experiments were selected for further testing of the six grass species by paired comparisons between the two methods. Ten samples of whole and ground seed from each moisture level of each species were tested by the appropriate oven method and the Karl Fischer method. Results of these paired measurements were tested by chi-square analysis to determine if the oven results were within the desired accuracy range of $\pm$ 0.5% of the Karl Fischer values at the 95% level of probability (Freese, 1960).

Paired Comparisons between Oven and Karl Fischer Techniques on Ten Unrelated Seedlots

Ten additional seedlots of each grass species produced in 1983, 1984, or 1985 were obtained from The Oregon State University Seed Laboratory. A range of unknown moisture contents was prepared by placing the seedlots in short-term storage under different relative humidity conditions. The moisture contents of the samples were then

tested by the Karl Fischer and oven methods in paired comparisons. For the oven method, whole and ground seeds were tested at 130^{o}C for the drying period that provided the most accurate results for the medium moisture seed in the previous experiment. Results of these tests were analyzed by chi-square analysis to determine if the oven moisture percentages were within the chosen accuracy level of $\pm$ 0.5% of the Karl Fischer values.

RESULTS AND DISCUSSION

Determination of Drying Temperature and Time for Perennial Ryegrass

Drying curves were developed for perennial ryegrass seed to determine the effects of temperature, time of drying, grinding, and original moisture level on the apparent moisture percentage obtained by the oven method of moisture determination. The drying curves are shown in Figures 1-4. The true moisture content as determined by the Karl Fischer method is indicated by the broken lines on the graphs (Bonner, 1972).

Moisture percentages at 90, 100, and 105°C generally were lower than the Karl Fischer value. The exceptions were the high moisture sublots in which the Karl Fischer values were reached only after extended periods of drying at 100 and 105°C (Fig. 2, 3).

At 130°C (Fig. 4) constant weights were attained at apparent moisture levels exceeding the Karl Fischer value. The higher values are apparently due to release of volatiles other than water and possibly to decomposition of the sample forming additional water (Pande, 1974).

The temperature of 130°C, then was the only temperature providing moisture percentages equal to or greater than the Karl Fischer value. The proper drying time at 130°C would be the time required for the moisture curve to intersect the Karl Fischer line. Based on this criterion, the most accurate moisture percentages for whole seed were obtained after 6, 3 and 2 h for the low, medium, and high moisture

samples, respectively. The required drying times for ground seed samples were 2 and 1 h for low and medium moisture levels, respectively.

After constant weight was attained, the moisture percentages for ground and whole seed were very similar. Moisture was removed at a faster rate from ground seed, however (Fig. 1-4). This is attributed to the greater diffusing surface in ground seed and the faster rate of evaporation from these surfaces (Pande, 1974).

Moisture was removed most rapidly from high moisture samples. This occurred at all temperatures (Fig. 1-4). The difference in drying rates are directly related to the tenacity with which water is held by the seed. Pande (1974) reports that water is held in seeds with an energy that increases with dryness. It is thus not possible to select one oven drying period that is equally accurate at all moisture levels. Selection of an appropriate temperature will necessarily be a compromise that will result in the fewest inaccurate results over all samples tested.

Determination of Drying Times at 130 C for Five Grass Species

The effects of drying temperature on moisture testing of the other five grass species would be expected to be consistent with the effects observed in perennial ryegrass. Therefore, the effects of drying time, grinding, and moisture level were studied only at 130^{o}C in the other species. The results are shown in Fig. 5-9.

The drying curves for the five grass species were similar to those for perennial ryegrass. In each species, drying of ground seed

was more rapid than of whole seed and drying of high moisture seed was more rapid than of low moisture seed.

Accurate moisture percentages for whole seed were obtained after 3, 1.5, and 0.7 h for low, medium, and high moisture samples of orchardgrass (Fig. 5); 2, 1, and 0.5 h in bentgrass (Fig. 6); 3, 3, and 0.5 h in Kentucky bluegrass (Fig. 7); 7, 3, and 2 h in tall fescue (Fig. 8); and 6, 3, and 2 h in red fescue (Fig. 9).

Comparison of Oven and Karl Fischer Methods on Ten Samples from Each Sublot

To test the repeatability of the oven methods, oven moisture tests were conducted on ten samples from each sublot and moisture percentages were compared with Karl Fischer values for the same samples. The results of the 300 paired comparisons are summarized in Table 1. The mean deviations between the oven and Karl Fischer methods for the samples tested ranged from 0.01% for orchardgrass to -0.28% for Kentucky bluegrass. Standard deviations varied from 0.06% for perennial ryegrass to 0.19% for Kentucky bluegrass. The minimum average difference for ten samples was 0.0% while the maximum difference was -0.64% for Kentucky bluegrass. The calculated chi-square values were all lower than the tabulated chi-square value of 18.3 at 10 df, indicating that the oven moisture measurements were well within $\pm$ 0.5% of the Karl Fischer values at the 95% level of probability. The maximum difference of -0.64% observed for Kentucky bluegrass was the only deviation which was greater than the $\pm$ 0.5% accuracy level.

Comparison of Oven and Karl Fischer Methods on Ten Unrelated Seedlots

Further comparisons of the oven and Karl Fischer methods were made on ten additional seedlots of each species to test the validity of the oven methods on seedlots that were not involved in the development of the methods. The seedlots varied in age and moisture content. The drying periods were those deemed most accurate for the medium-moisture samples.

Mean deviations over 120 samples ranged from 0.05% for perennial ryegrass to 0.29% for Kentucky bluegrass. Standard deviations varied from 0.15% for tall fescue to 0.27% for orchardgrass. The minimum average difference observed between the two methods was -0.01% for red fescue and the maximum was 0.67% for tall fescue. Hart et al. (1959) compared the oven and Karl Fischer techniques on these grass species and reported average deviations ranging from 0.11 to 0.02% and standard deviations from 0.14 to 0.20%. Calculated chi-square for all samples tested in our laboratory were lower than the tabular value of 18.3 at 10 df (Table 2). This meant that the oven measurements were well within the desired accuracy level of $\pm$ 0.5% of the Karl Fischer values at the 95% level of probability. Deviations greater than $\pm$ 0.5% occurred seven times over the 120 samples tested.

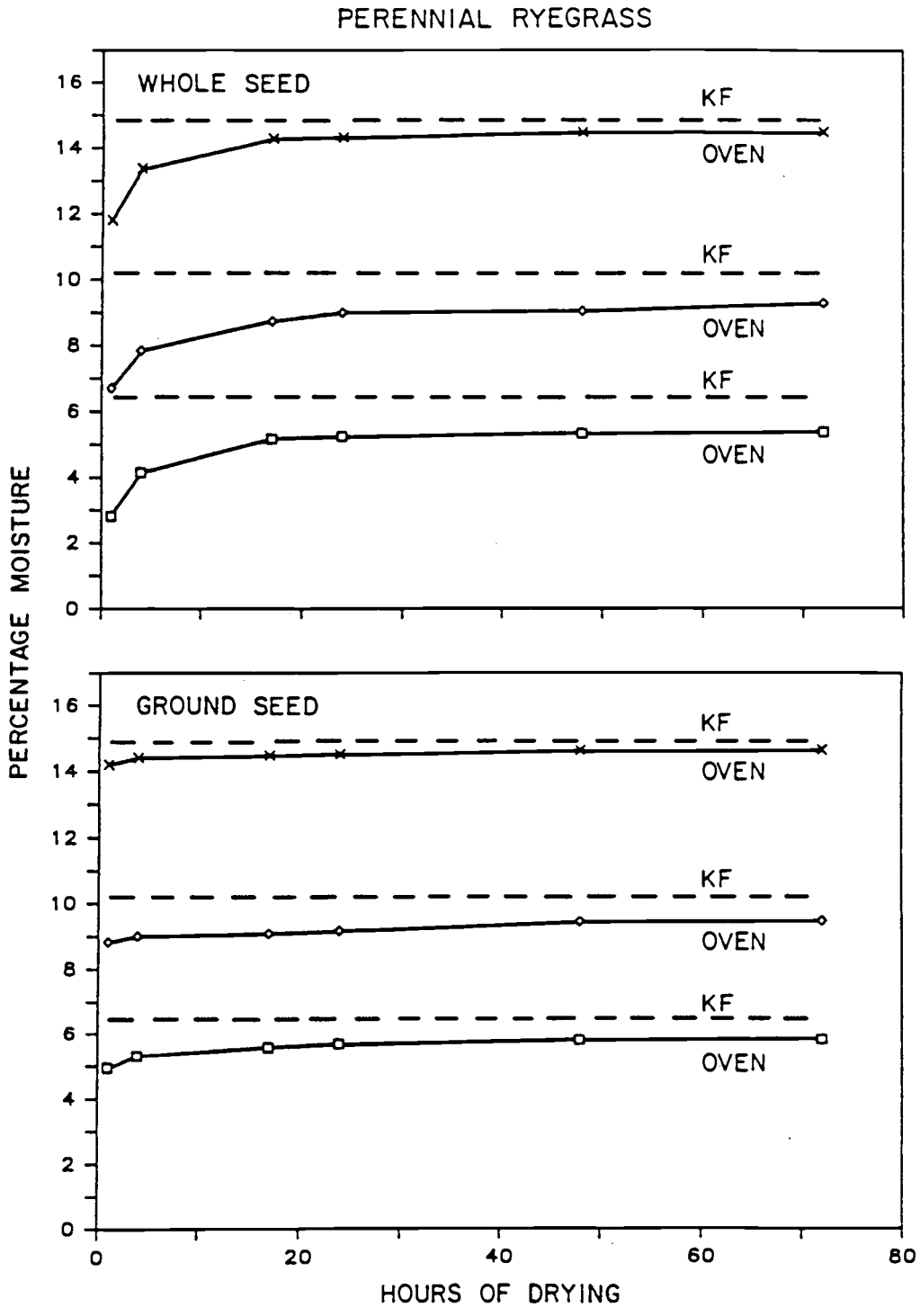

Figure 1. Comparison of perennial ryegrass moisture percentage obtained by oven drying and Karl Fischer techniques. Whole and ground seed samples at three moisture levels dried at 90°C up to 72 h.

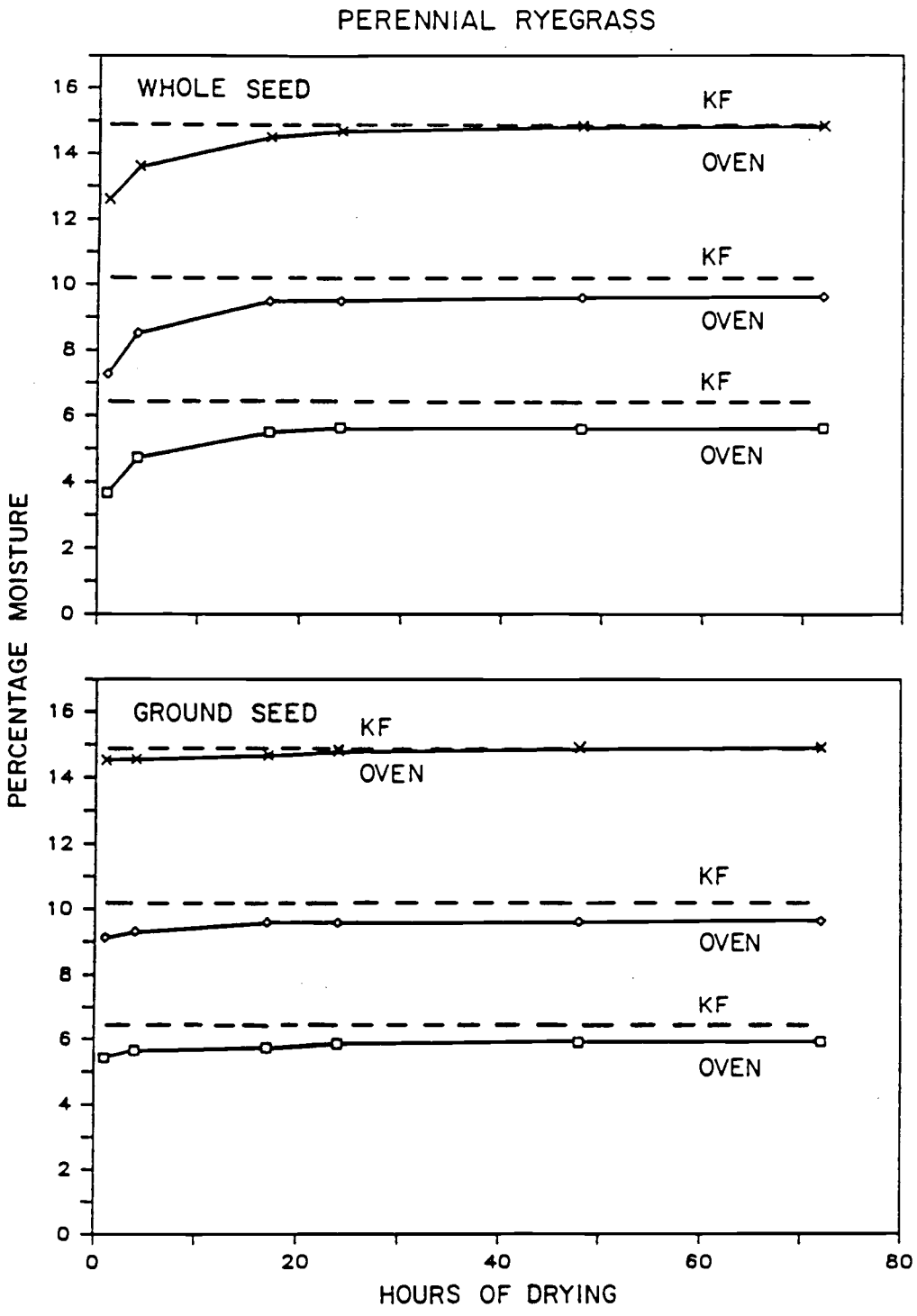

Figure 2. Comparison of perennial ryegrass moisture percentage obtained by oven drying and Karl Fischer techniques. Whole and ground seed samples at three moisture levels dried at 100^{0}C up to 72 h.

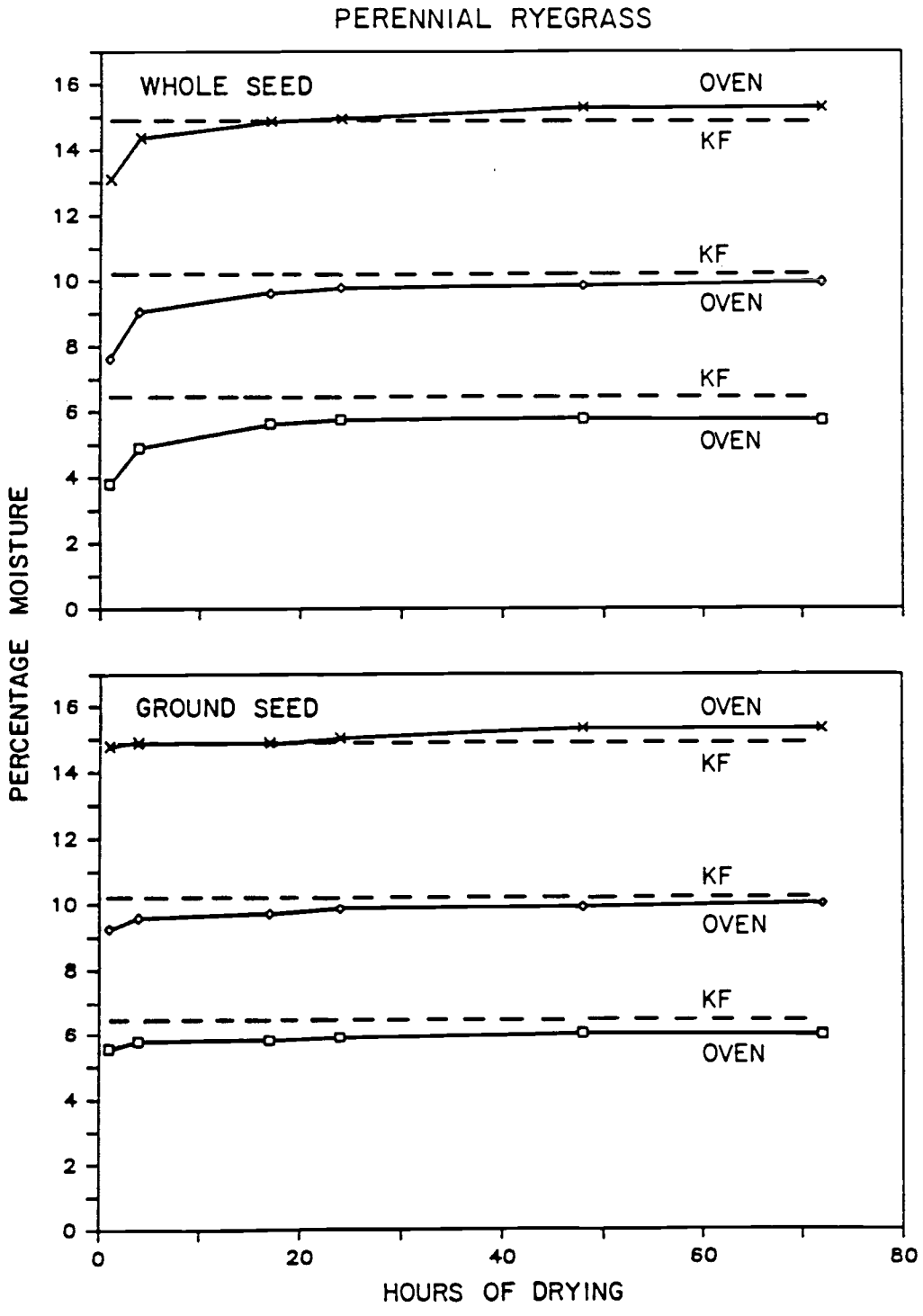

Figure 3. Comparison of perennial ryegrass moisture percentage obtained by oven drying and Karl Fischer techniques. Whole and ground seed samples at three moisture levels dried at 105°C up to 72 h.

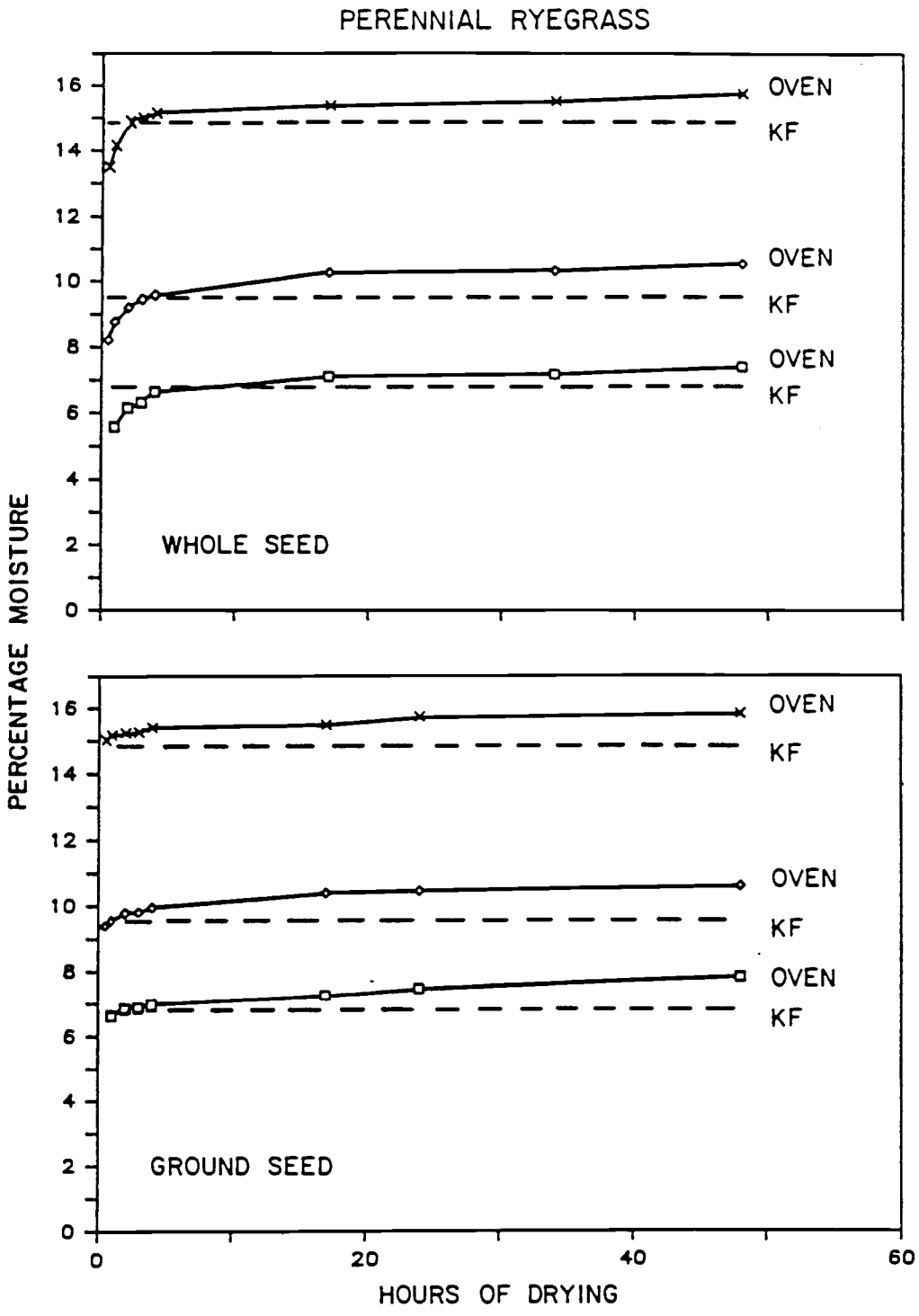

Figure 4. Comparison of perennial ryegrass moisture percentage obtained by oven drying and Karl Fischer techniques. Whole and ground seed samples at three moisture levels dried at 130°C up to 48 h.

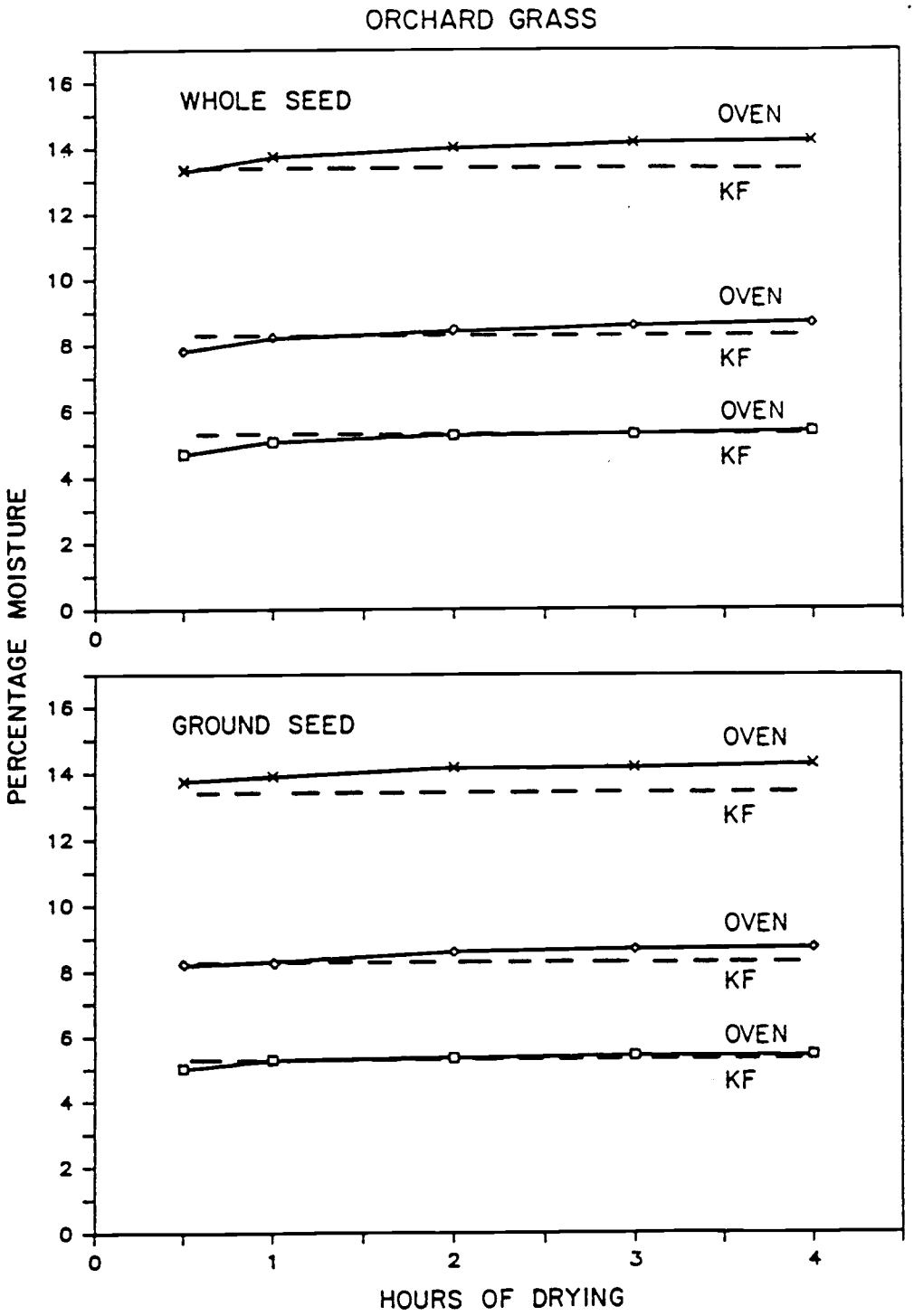

Figure 5. Comparison of orchard grass moisture percentage obtained by oven drying and Karl Fischer techniques. Whole and ground seed samples at three moisture levels dried at 130°C up to 4 h.

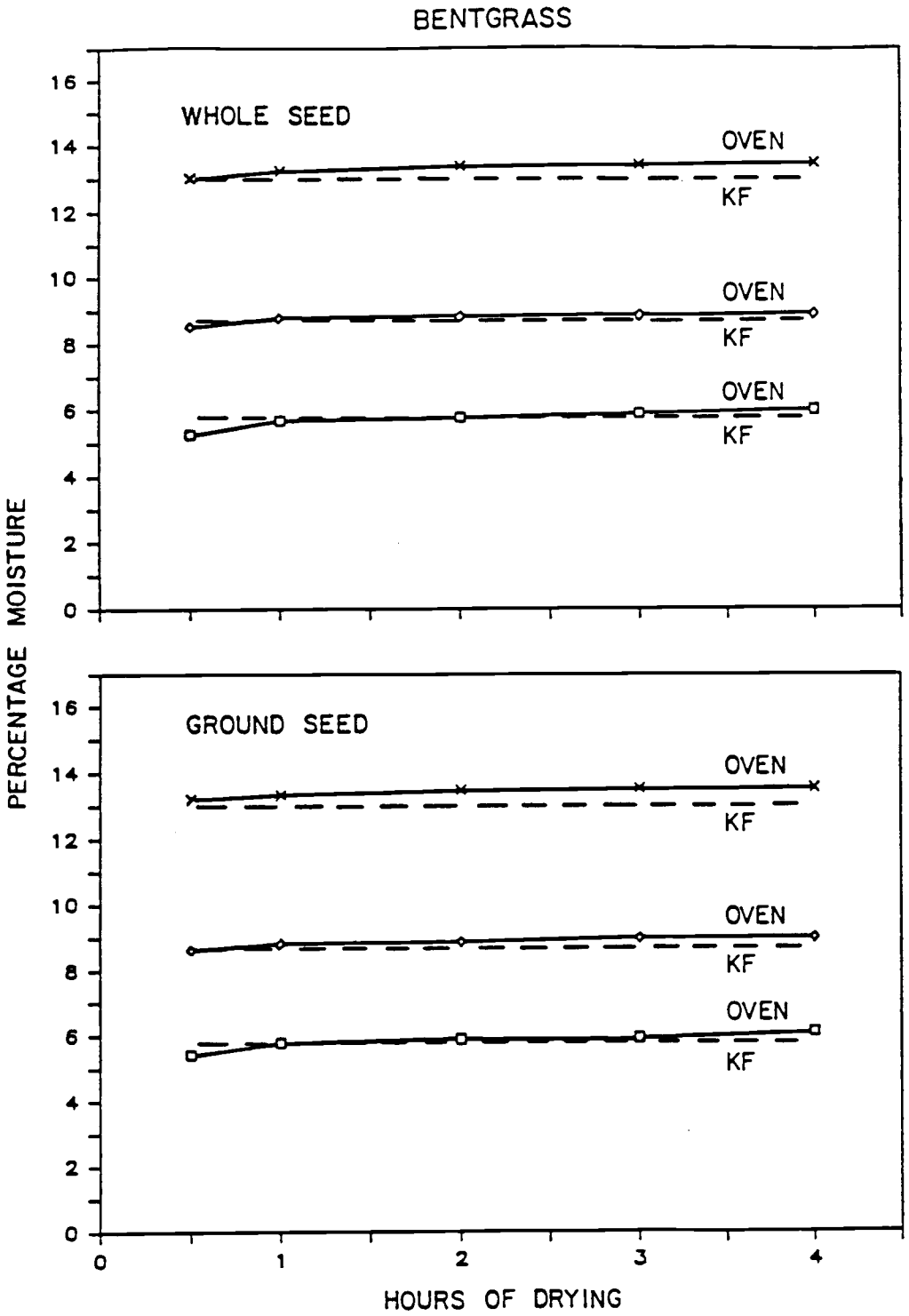

Figure 6. Comparison of bentgrass moisture percentage obtained by oven drying and Karl Fischer techniques. Whole and ground seed samples at three moisture levels dried at 130°C up to 4 h.

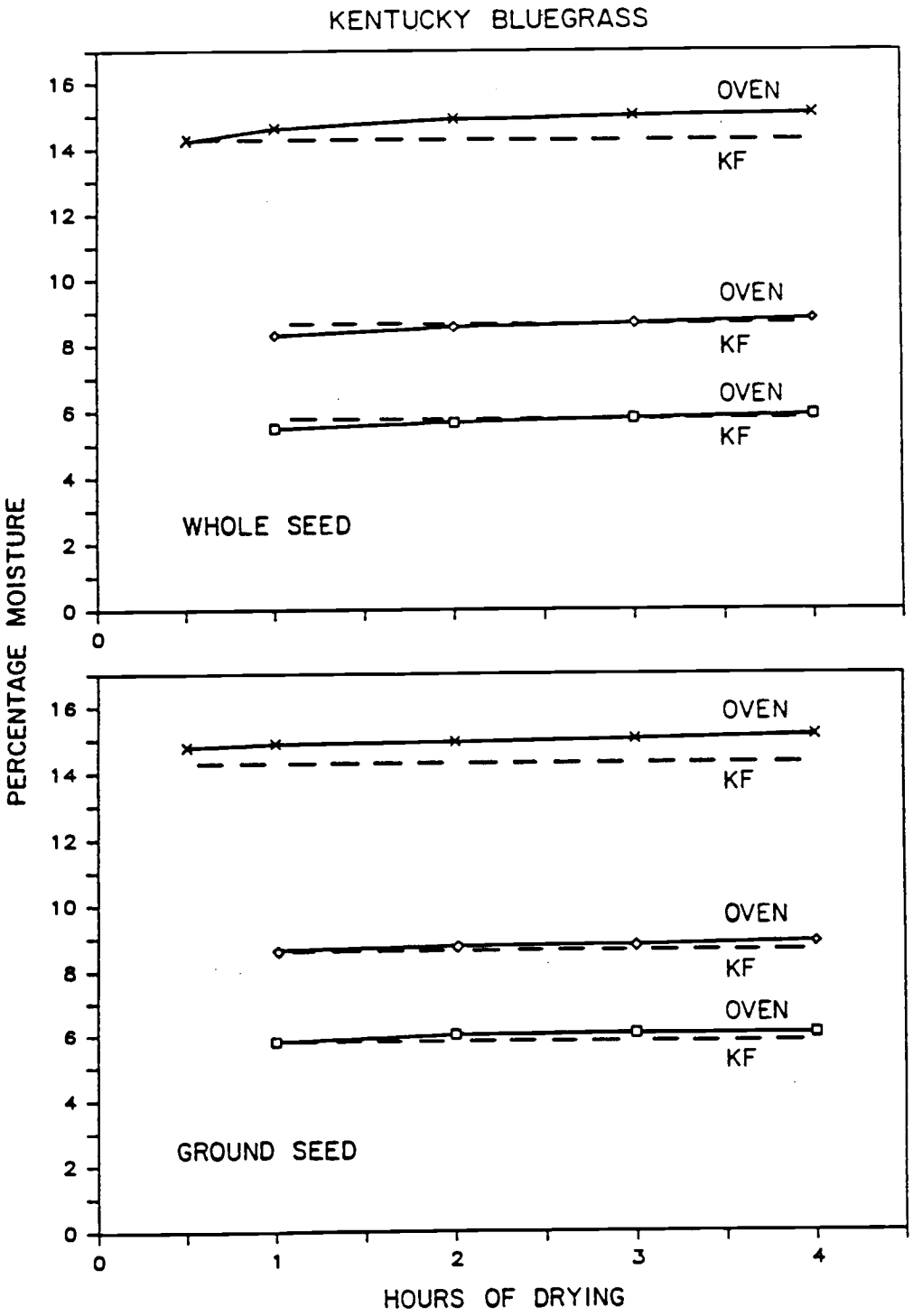

Figure 7. Comparison of Kentucky bluegrass moisture percentage obtained by oven drying and Karl Fischer techniques. Whole and ground seed samples at three moisture levels dried at 130°C up to 4h.

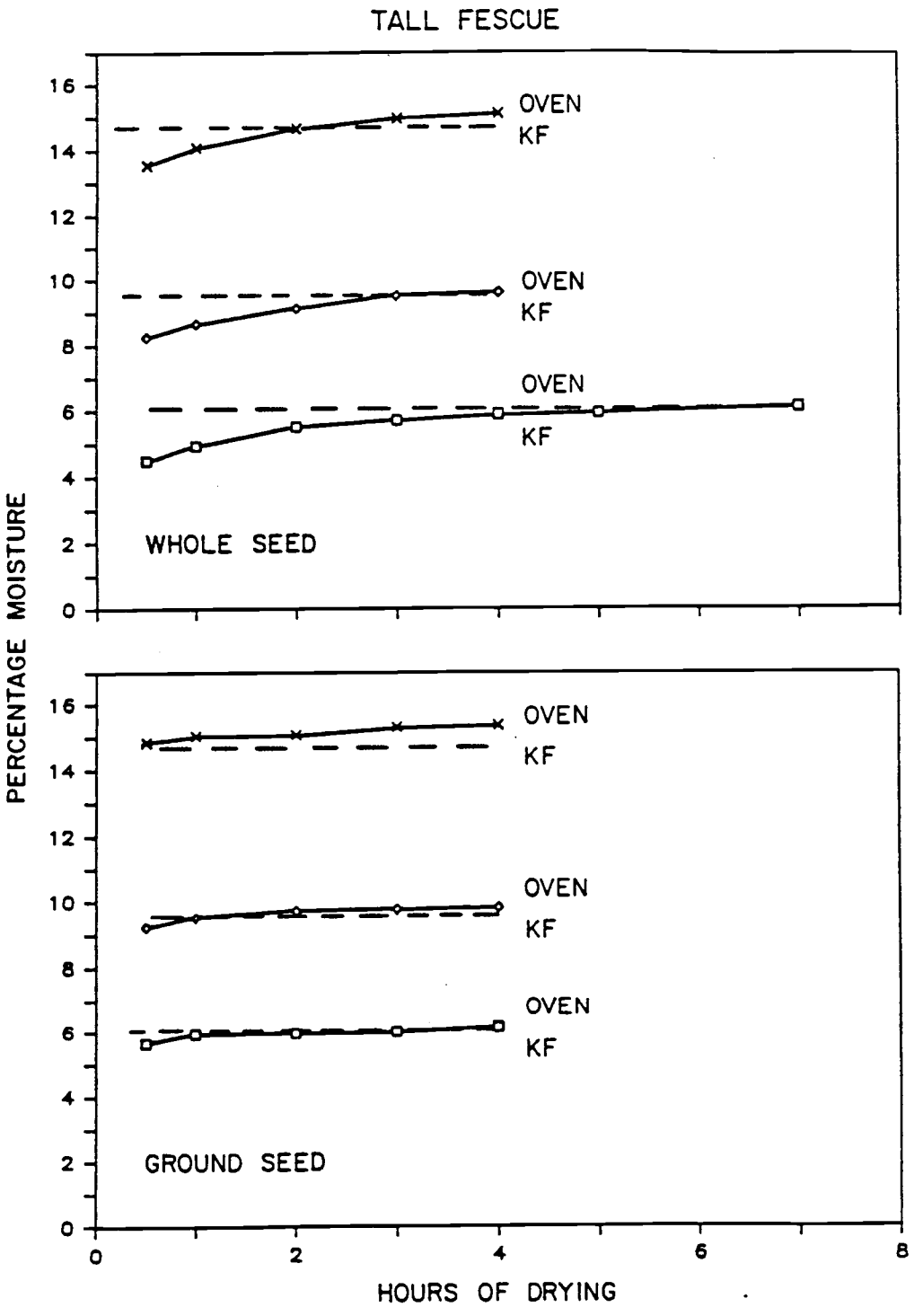

Figure 8. Comparison of tall fescue moisture percentage obtained by oven drying and Karl Fischer techniques. Whole and ground seed samples at three moisture levels dried at 130°C up to 7 h.

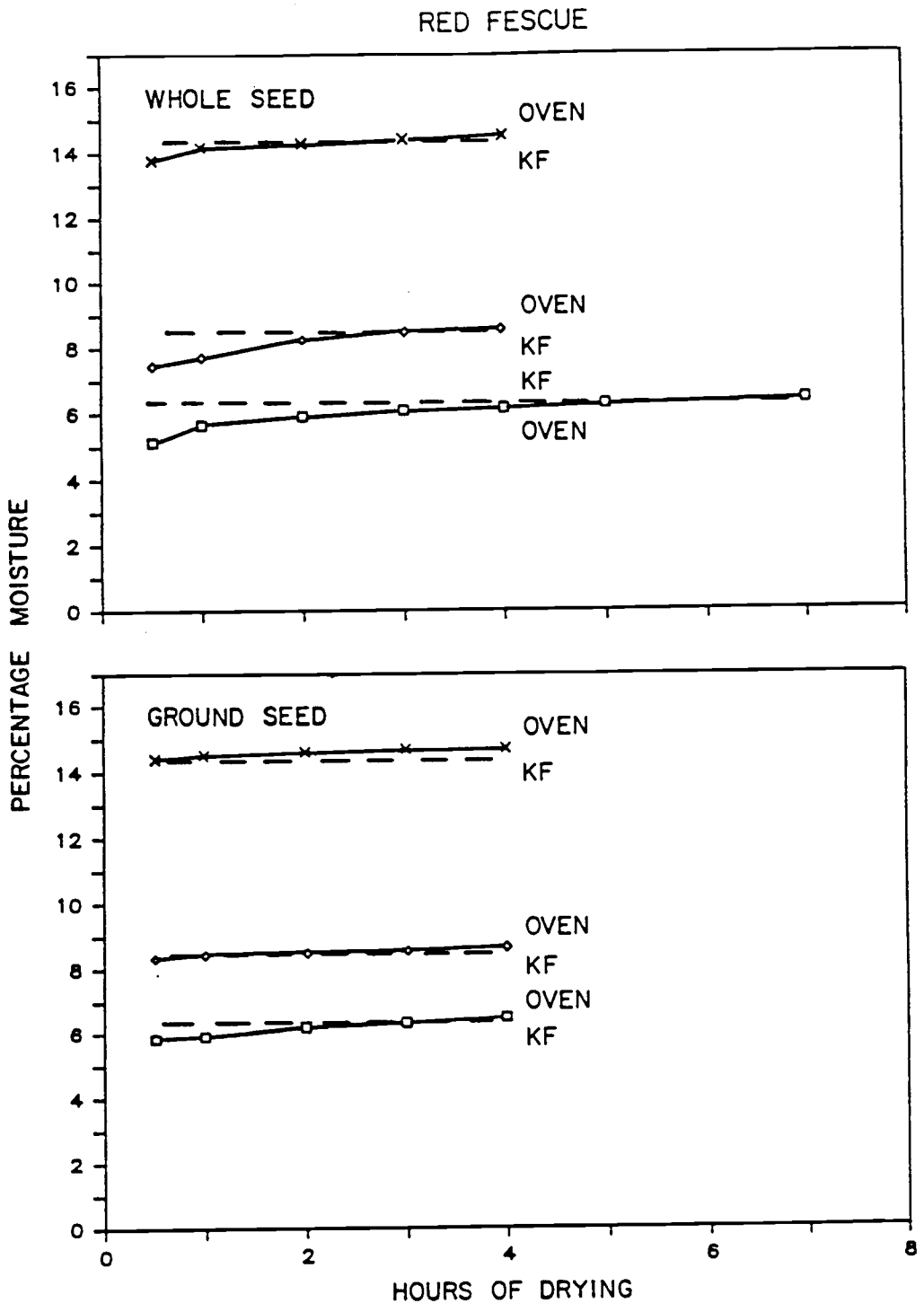

Figure 9. Comparison of red fescue moisture percentage obtained by oven drying and Karl Fischer techniques. Whole and ground seed samples at three moisture levels dried at 130°C up to 7 h.

Table 1. Moisture content of grass seed samples determined by Oven and Karl Fischer methods. Each figure is the average of ten samples from each sublot.

Species	Moisture Level	Sample Preparation	Drying Time	Moisture content Oven	Moisture content K.F.	Min. dev.	Max. dev.	Mean dev.	Std. dev.	Cal.† x^2
			h	%	%					
Perennial ryegrass	low	whole	6	6.40	6.34	0.03	0.28	0.06	0.13	2.72
	low	ground	2	6.38	6.34	0.01	0.16	0.04	0.09	1.32
	medium	whole	3	9.27	9.37	-0.03	-0.22	-0.09	0.08	2.21
	medium	ground	1	9.44	9.37	0.01	0.20	0.07	0.06	1.26
	high	whole	2	14.79	14.72	-0.02	0.23	0.07	0.12	2.47
Orchard-grass	low	whole	3	5.38	5.37	0.00	-0.09	0.01	0.06	0.54
	low	ground	1	5.36	5.37	-0.09	0.20	-0.01	0.10	1.33
	medium	whole	1.5	8.42	8.40	-0.02	0.13	0.02	0.08	0.99
	medium	ground	1	8.49	8.40	0.00	0.22	0.09	0.10	2.43
	high	whole	0.7	12.78	12.79	-0.01	-0.14	0.01	0.08	0.77
Bentgrass	low	whole	2	5.80	5.86	0.01	0.35	-0.06	0.17	4.12
	low	ground	1	5.66	5.86	-0.04	-0.33	-0.20	0.10	6.87
	medium	whole	1	8.76	8.86	-0.01	-0.26	0.09	0.09	2.42
	medium	ground	1	8.82	8.86	-0.02	-0.29	-0.04	0.16	3.49
	high	whole	0.5	12.97	12.77	-0.07	0.41	0.20	0.14	8.08
Kentucky bluegrass	low	whole	3	6.13	5.98	0.00	0.35	0.15	0.16	6.53
	low	ground	1	5.97	5.98	-0.01	0.22	0.01	0.13	2.30
	medium	whole	3	8.74	8.70	0.00	0.22	0.04	0.10	1.51
	medium	ground	1	8.54	8.70	-0.06	0.37	-0.16	0.14	6.50
	high	whole	0.5	13.93	14.21	0.03	-0.64	-0.28	0.19	15.95
Tall fescue	low	whole	7	6.17	6.23	0.03	-0.18	0.07	0.09	1.61
	low	ground	3.5	6.34	6.23	0.03	0.24	0.11	0.10	3.05
	medium	whole	3	9.46	9.58	-0.02	-0.25	-0.12	0.08	2.90
	medium	ground	1	9.54	9.58	0.02	-0.23	-0.04	0.13	2.33
	high	whole	2	14.54	14.57	0.00	-0.31	-0.03	0.17	3.94
Red fescue	low	whole	6	6.24	6.35	0.00	-0.24	0.11	0.07	2.43
	low	ground	3	6.42	6.35	0.01	0.20	0.07	0.08	1.45
	medium	whole	3	8.64	8.50	0.03	0.35	0.14	0.10	4.27
	medium	ground	1.5	8.50	8.50	0.01	-0.25	0.00	0.11	1.66
	high	whole	2	14.29	14.15	0.06	0.27	0.14	0.07	3.51

Oven measurements will be within ± 0.5% of the Karl Fischer values at the 95% level of probability.

† Tabulated x^2 for all species was 18.3

Table 2. Moisture content of grass seed samples determined by Oven and Karl Fischer methods. Each figure is the average of ten unrelated samples from each species.

Species	Moisture Range	Sample Preparation	Drying Time	Moisture Content		Min. dev.	Max. dev.	Mean dev.	Std. dev.	Cal. χ^2 †
				Oven	K.F.					
	%		h	%----------		---------%				
Perennial ryegrass	9-16	whole	3	12.40	12.35	-0.03	0.56	0.05	0.22	6.50
	9-16	ground	1	12.46	12.35	0.02	0.58	0.11	0.23	8.40
Orchardgrass	7-14	whole	1.5	10.85	10.70	0.05	0.48	0.15	0.27	12.73
	7-14	ground	1	10.84	10.70	-0.08	0.59	0.14	0.26	11.76
Bentgrass	8-13	whole	1	11.00	10.89	0.03	0.39	0.11	0.16	5.15
	8-13	ground	1	11.07	10.89	0.03	0.39	0.18	0.16	8.11
Kentucky bluegrass	8-15	whole	3	11.55	11.35	0.11	0.45	0.20	0.18	10.19
	8-15	ground	1	11.64	11.35	-0.09	0.56	0.29	0.19	16.80
Tall fescue	8-16	whole	3	12.17	12.07	-0.04	0.34	0.09	0.15	3.97
	8-16	ground	1	12.32	12.07	0.02	0.67	0.24	0.21	14.31
Red fescue	8-15	whole	3	12.13	11.98	-0.04	0.65	0.15	0.23	10.08
	8-15	ground	1	12.16	11.98	-0.01	0.38	0.18	0.19	9.07

Oven measurements will be within $\pm$ 0.5% of the Karl Fischer values at the 95% level of probability.
† Tabulated χ^2 for all species was 18.3.

CONCLUSIONS

Of the four drying temperatures investigated, 130°C is the only practical alternative. Although moisture tests are commonly conducted at temperatures between 100 and 105°C for 24 h, the drying curves show that moisture content would be underestimated by about 1% at these temperatures. The seeds are nearly at constant weight after 24 h drying and the true moisture value is not obtained after 72 h.

It is probable that other temperatures between 110 and 130°C would give moisture values matching the Karl Fischer values. Theoretically, a temperature could be found for each species that, when seeds were dried to constant weight, the moisture curves would coincide with the Karl Fischer value. This would be more desirable than choosing a drying time at which the moisture curve crosses the Karl Fischer line at a sharp angle. However, it would not be convenient for seed testing laboratories to maintain ovens at more temperatures than the 103 and 130°C now required. Rather, it is more practical to maintain an oven at a temperature of 130°C and regulate the length of the drying period for each species.

Another advantage of the 130°C drying temperature is that the oven can be loaded and unloaded several times a day to increase the number of samples tested. A 1-h oven moisture test also becomes a practical quick test for species such as chaffy grasses that cannot be tested accurately with electronic moisture testers.

Grinding seeds shortens the time required for drying to the true moisture value, and ground and whole seeds gave equivalent values when

dried to constant weight. There are disadvantages connected with grinding such as the expense of the grinder and the cost of time and labor for grinding. There are also possibilities of introducing errors in the determinations because ground seed can gain or lose moisture rapidly when exposed to air. Grinding would still be recommended, however, for species in which whole seeds require extended drying periods.

This investigation demonstrates a shortcoming inherent in all oven methods of moisture testing. It is not possible to select a single temperature and drying time that will provide the same degree of accuracy for seed at different moisture levels. When exposed to the same drying temperature water is removed more rapidly from high moisture seeds because it is less tightly held than in drier seeds. The best that can be done is to select a drying time that is accurate for the moisture level expected in the majority of seed samples. In this study, drying times that were accurate for air-dry seeds were selected since the majority of seed moving in the seed trade industry would be expected to be reasonably close to this moisture content.

While the methods described here are satisfactory for testing seed in the 7-16% moisture range, it does not follow that the same degree of accuracy would exist for testing seed with higher or lower moisture - this needs to be explored further. Where knowledge of seed moisture is extremely critical (such as in storage at extremely low moisture levels) the oven method may lack the necessary accuracy, and the Karl Fischer or other basic method should be utilized.

Seed moisture tests on these six temperate-climate grass species

should be conducted on whole seeds at 130°C. The drying period should be 3 h for perennial ryegrass, Kentucky bluegrass, tall fescue and red fescue; 1.5 h for orchardgrass; and 1 h for bentgrass. Hart et al. (1959) recommended the same drying times for bentgrass, the fescues and perennial ryegrass, but suggested 1 h for orchardgrass and Kentucky bluegrass. In contrast, the present ISTA Rules prescribe 1 h for all six species. The ISTA methods for five of the six species would have underestimated the true moisture content of air-dry seeds as determined by the Karl Fischer method in these studies.

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APPENDICES

APPENDIX

Table 1. Effect of grinding to various mesh sizes compared with whole seed on the moisture content of annual ryegrass dried at 130°C for 1 h.

Mesh size	Moisture content
	%
20	9.76
40	9.47
60	9.44
Coffee mill	9.83
Whole seed	8.44

$LSD_{.05} = 0.41$

APPENDIX

Table 2. Effect of grinding to various mesh sizes compared with whole seed on the moisture content of annual ryegrass dried at 100°C for 24 h.

Mesh Size	Moisture content
	%
20	9.44
40	9.39
60	9.11
Coffee mill	9.31
Whole seed	9.26

$LSD_{.05} = 0.21$

APPENDIX

Table 3. Effect of drying at 130°C for 1 h. in different types of containers on the moisture content of annual ryegrass.

Drying container	Moisture content
	%
Large aluminium	10.74
Large glass	10.80
Large plastic	10.91
Standard aluminium	10.73

$LSD_{.05}$ = 0.09

APPENDIX

Table 4. Comparison of moisture content obtained by drying annual ryegrass at 100°C for 24 h. on top and bottom oven shelves.

Oven shelf	Moisture content
	%
Top	9.23
Bottom	9.17

F value not significant at the 5% level of probability.

APPENDIX

Table 5. Effect of time of weighing on the moisture content of perennial ryegrass dried at 130°C for 1 h.

Time of weighing	Moisture content
	%
Weighed hot	9.11
After 1 h	8.74
After 2 h	8.74

$LSD_{.05} = 0.08$

APPENDIX

Table 6. Effect of sample size on the moisture content of annual ryegrass dried at 100°C for 24 h.

Sample size	Moisture content
g	%
3	8.89
5	9.93

F value not significant at the 5% level of probability.

APPENDIX

Table 7. Effect of quantity of methanol on moisture extraction from 1g ground annual ryegrass seed.

mL methanol	Moisture content
	%
15	8.63
25	9.18
50	9.63
75	9.62
100	9.64

$LSD_{.05} = 0.37$

APPENDIX

Table 8. Moisture extraction for 24 h. on a 1 g sample of perennial ryegrass using 50 vs 75 mL of methanol. Moisture content obtained by the Karl Fischer technique.

Quantity of methanol	Moisture content
50 mL	8.44
75 mL	8.56

F value not significant at the 5% level of probability.

APPENDIX

Table 9. Effect of extraction time in methanol on the moisture content of annual ryegrass. Moisture content obtained by the Karl Fischer technique.

Extraction time	Moisture content
h	%
1	10.02
2	10.44
4	10.77
8	10.72
16	10.89
24	11.10
48	11.08

$LSD_{.05} = 0.33$

APPENDIX

Table 10. Comparison of extraction time, 24 vs 48 h on the moisture content of perennial ryegrass soaked in 50 mL of methanol. Moisture content obtained by the Karl Fischer technique.

Extraction time	Moisture content
	%
24 h	8.60
48 h	8.61

F value not significant at the 5% level of probability.

APPENDIX

Table 11. Effect of sample size on the moisture content of perennial ryegrass soaked in 50 mL methanol for 24 h. Moisture content obtained by the Karl Fischer technique.

Sample size	Moisture content
	%
1 g	8.48
2 g	8.53

F value not significant at the 5% level of probability

APPENDIX

Table 12. Effect of mesh size on the moisture content of annual ryegrass soaked in 50 mL of methanol for 24 h. Moisture content obtained by the Karl Fischer technique.

Mesh size	Moisture content
	%
20	10.80
40	10.73
Coffee	10.79

F value not significant at the 5% level of probability.

APPENDIX

Table 13. Comparison of stirring (at 1000 rpm.) vs non stirring on the moisture content of annual ryegrass soaked for 24 h in methanol. Moisture content obtained by the Karl Fischer technique.

Treatment	Moisture content
	%
Stirring	10.92
Non stirring	11.10

F value not significant at the 5% level of probability.

The Karl Fischer technique used in our laboratory for moisture determination of grass seeds

The Fischer equipment consisted of the Automatic K-F Titrimeter system model 392 and Fischer Scientific Digital/ Dispenser burette model 395.

Filling and purging the system

The first requirement in operating the Automatic titrimeter titration apparatus is to fill the system with Karl Fischer reagent (Hydranal) and purge it of any entrapped moisture by the following procedure.

1. Remove the cap from a bottle of Fischer reagent and quickly replace it with the cap of the connector tubing assembly attached to the digital burette.
2. Depress the red Power button to On position.
3. Set dispense rate mode selector on the digital burette to the 100% position.
4. Depress Start button on digital burette and allow syringe to move a full discharge stroke.
5. Press Fill button and allow piston to return to zero position.
6. Unloop metal springs from adaptor assembly on top of reagent bottle connected to the model 392 accessory system, and fill with reagent grade methanol.
7. Replace adaptor assembly on top of reagent bottle and secure with metal springs.
8. Depress Power button on model 392 K-F accessory to On position.

9. Depress and hold the Solvent switch until about 50 mL of methanol has been pumped into the reaction vessel.
10. Depress the Waste switch on the model 392 to drain the reaction vessel.
11. Repeat steps 9 and 10 to ensure that all moisture has been driven from the system.

Setting the front panel controls on the model 392.

To properly set the front panel controls for normal operation, perform the following steps.

1. Remove one electrode from its input jack.
2. While pressing the Reference push button, rotate the Current Adjust knob to cause the panel meter to read approximately 10 microamps.
3. Return the electrode lead to its input jack.
4. Adjust the endpoint set -uA knob to a setting of 20.
5. Set the Proportioning band and Minimum delivery controls to approximately 6.
6. Adjust the Endpoint time-sec to 40 seconds.

Sample preparation.

All grass seed samples used for moisture testing should preferably be stored in sealed containers to maintain original moisture content.

1. Remove approximately 3 g of seed from the storage container and grind in a Wiley laboratory mill through a 20-mesh screen.

The mill hopper should be covered during grinding to minimize exposure of seed to the atmosphere.

2. Using Wartman weighing paper quickly weigh 1 g of this ground seed on an analytical balance and transfer to a 50 mL Erlenmeyer flask.
3. Immediately introduce 50 mL of reagent grade methanol into this flask, stopper securely and allow extraction of moisture to occur for 24 h.

Sample analysis.

To determine the moisture content of the samples, proceed as follows:

1. Depress and hold the solvent switch on the model 392 until the platinum wires of the electrodes are fully immersed in the methanol.
2. Loosen knurled plastic nut which secures waste outlet tubing (medium-sized port at right rear) on reaction vessel cover, and pull tubing upward until the end of the tubing is at least one inch above the level of methanol. Finger tighten the nut to secure tubing. **Note**. Whenever it is desired to drain the reaction vessel, return the end of the waste outlet tubing to the bottom of the vessel and press the waste button.
3. Adjust Stirrer control for desired speed. This should be the fastest speed producing a small vortex without air bubbles.
4. With the digital burette in the Remote dispense mode, press the

Start button; then press the Reset button on the model 392. This titrates any water in the reagent grade methanol.

5. When a stable endpoint has been reached and held, as indicated by the illumination of the endlamp on the model 392, press the Fill button on digital burette.
6. Using a funnel, transfer the sample (which was left for 24 h) into the reaction vessel through the sample inlet port and re-tighten stopper.
7. Press the Start button on the digital burette.
8. Press the Reset button on the model 392 to initiate automatic titration.
9. When the Endlamp again illumines, record the volume of reagent used (Hydranal) as V.
10. Unscrew reaction vessel, rinse with methanol, and repeat steps 9 and 10 under filling and purging section and steps 1 to 9 under sample analysis section for the other seed samples.
11. Calculate percent water as:

$$\%H_2O = \frac{(V-B) \times F \times 100}{1000 \times W} = \frac{(V-B) \times F \times 0.1}{W}$$

where V = volume of Hydranal used in the titration of the seed sample (mL)

* F = titre value of Hydranal. ($mg.H_2O/mL$)

W = sample weight (g)

1000 = conversion factor to milligrams.

100 = conversion factor to percent.

** B = vol. of Hydranal used in titration of methanol blank.

** Each day a methanol blank titration must be done to account for any water found in the methanol used in the present extraction. This is accomplished by the following procedure.

1. Fill three 50 mL Erlenmeyer flasks with 50 mL of reagent grade methanol, stopper securely and leave for 24 h.
2. Repeat steps 1 to 9 as was done for sample analysis.
3. Record the amount of Hydranal used to titrate the blank.
4. Repeat the procedure for the other two blanks.
5. Calculate the average of the three blanks and use as 'B' in the formula.

* Hydranal must be standardized each time a new bottle is opened. This reagent tends to deteriorate in storage, therefore it is advisable to do daily standardizations to ensure accurate results.

This titre value is established using the following steps:

1. Syringe 0.05 mL of distilled water.
2. Follow steps 1 to 9 for sample analysis.
3. Repeat 5 to 10 times depending on the experience of the operator.
4. Calculate the titre value of Hydranal as:

$$F = \frac{1000 \times G}{\text{mL Hydranal used}}$$

where 1000 = milligram conversion factor.

G = grams of distilled water.

Note. 1 mL H_2O = 1 g = 1000 mgs.

5. Find average of titre values and use in main formula.