COMPARISON OF ROOTING MEDIA AND METHODS IN ASSESSING SURVIVAL OF PROPAGULES OF TALL FESCUE (FESTUCA ARUNDINACEA, SCHREB.)

by

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COMPARISON OF ROOTING MEDIA AND METHODS IN ASSESSING SURVIVAL OF PROPAGULES OF TALL FESCUE (*FESTUCA ARUNDINACEA*, SCHREB.)

INTRODUCTION

The plant breeder often requires large quantities of seed from a genotype in order to obtain a progeny population of sufficient size that the complete genetic range will be expressed. If the breeder had to rely on a single plant it would take several years to obtain this much seed. However, through vegetative propagation of a single genotype it is possible for the breeder to obtain the seed in one growing season. Thus the time required to breed a new variety is reduced. A breeder wishing to examine the phenotype of a promising genotype would only obtain sufficient forage from one plant for chemical analysis, whereas by means of vegetative propagation enough plant material could be obtained for ruminant digestion studies. When parental genotypes are chosen for a new variety it is important that there should be as little delay as possible, in providing a large supply of seed of the variety for commercial use. By means of vegetative propagation of the parental genotypes much larger quantities of breeder's seed are made available for seed multiplication. As a result, the farmer can obtain seed of the new variety one or two years earlier. It has been proposed that vegetative

propagation could be used to increase the plants of genotypes with high specific combining ability so that large quantities of hybrid forage seed could be produced (21, p. 35).

Breeders of corn have been able to stabilize desired genotypes by inducing homozygosity through inbreeding. Multiplication of homozygous lines is widely used for producing hybrid corn seed. Grass breeders cannot readily use inbreeding owing to polyploidy and a high frequency of near self-sterility. Breeders of tree fruits have been able to propagate desirable genotypes vegetatively owing to the ease of propagation. However, essentially only one forage variety has been commercially propagated by this method. Ornamental plants have been extensively propagated by vegetative means and Watkins (23, p. 1-48) has written a popular bulletin on the uses of the method.

It has been observed that survival rates following vegetative propagation vary between genotypes and by seasons. This is a serious problem to the breeder because failure or partial failure of propagules in the greenhouse or nursery could result in the loss of seed or data for that growing season. Since vegetative propagation is an essential method for the breeder a knowledge of the factors influencing survival of grasses to

propagation, would greatly assist the full exploitation of the method. Recently, limited studies, particularly with legumes, have been undertaken to discover some of the principles involved in vegetative propagation of forages (17, p. 58-67). It is important that a thorough understanding be developed of the optimum methods for propagating grasses.

This study was undertaken to investigate methods for studying the survival of tall fescue after vegetative propagation. Various media were compared as possible substitutes for top soil. The conventional method of propagation had a number of disadvantages for research such as, quantities of rooting media, greenhouse space and labor required. Sample sizes convenient for the mechanics of propagation were compared for efficiency of design. In a study investigating methods of propagation economics of facilities and supplies would help to prevent limitations of either, from reducing the scope and adequacy of experiments.

Survival rates could be directly determined by counting the number of living propagules. A propagule could be considered as living if it had either viable leaves or roots, or both. However, some estimate of survival would be required to measure graded responses because if the treatments were mild, all the propagules

might live and no treatment variation would be observed, whereas one treatment might produce vigorous, flourishing propagules and another treatment might only allow the propagules to keep alive. For purposes of making observations a propagule would be hard to classify as dead or alive when at an intermediate stage. The relationship between a number of criteria and survival was studied.

REVIEW OF LITERATURE

In a study of vegetative propagation the rooting media are an important aspect and the methods of determining the relative effects of environments on the response to propagation are vital. The criteria used to evaluate success or failure of propagation may be relatively sensitive to environmental effects or may be largely under genetic control.

Bermuda grass is extensively planted by vegetative stolons and Burton (4, p. 551) estimated that more Bermuda grass is planted by this method than by seed. The development of the superior sterile variety, Coastal Bermuda grass, has resulted in studies of vegetative establishment of this improved variety in established stands of less desirable species by Newton et al. (19, p. 751-752).

Hanson and Carnahan (9, p. 45, 56, 71) indicated that vegetative propagation, by the separated tillers of an individual grass plant from the source nursery, could be used to establish tiller rows. These replicated rows permitted more accurate measurements of phenotypic characters than could be obtained from the original single plant in the source nursery. For instance tiller rows could be used for sheep grazing comparisons. The supply of available F_1 single cross

seed for testing purposes were limited. Valuable germ plasm could be maintained or increased by vegetative propagation.

Cowan (7, p. 305) in a comprehensive report on tall fescue pointed out that clonal lines varied in relative protein and "chromogen" content and reference was also made to differences in palatability between clonal lines. Cowan described propagules used to propagate clonal lines as a small portion of the basal stem of an individual tiller which had only one node and a portion of two internodes. One plant could be multiplied to 8000 plants in two years. Plants lifted from the nursery in February produced many more tillers than plants lifted in September or December.

Tysdal et al. (22, p. 35) successfully reproduced alfalfa by vegetative cuttings consisting of a node and an internode. These workers proposed a forage breeding method comparable to the method employed in producing hybrid corn. Instead of inbreeding to maintain desirable genotypes, parents with high specific combining ability would be vegetatively propagated. These vegetatively propagated parents would then be planted in isolated crossing blocks.

Grandfield et al. (8, p. 804-808) stated that vegetative propagation reduced the time and labor

involved in breeding alfalfa. Clonal lines could be vegetatively increased rapidly to supply enough seed in one season for field tests. This group found that Rhizoctonia sp. was a serious root and stem parasite, if alfalfa cuttings became infected, and only 25 percent of the cuttings survived if the sand was infested. The stem and leaf parasite (Ascochyta imperfecta) reduced the percentage of cuttings that rooted to 15 percent. Sterilization of the soil and rooting medium was recommended with additional sanitary measures to be taken in the field where old crop residues would be removed and destroyed.

Kernkamp et al. (14, p. 928-936) studied the effects of Rhizoctonia solani on alfalfa cuttings and generally confirmed the findings of Grandfield et al. (8, p. 804-808). The damping off condition was especially serious at high temperatures but was less noticeable at temperatures below 75° F. The infection spread from the sides of the flat towards the center indicating that the boards of the flats harbored the causal organism. Subsequently isolations of the fungus were made from the flats and the rooting medium. Fungicides did not effectively reduce the incidence of the fungus though Phygon applied dry to cuttings before planting proved the most

beneficial of the fungicides tested. As a result of this research, autoclaving of the rooting medium and flats was recommended. This asceptic procedure permitted growth of 12,000 cuttings with very few losses.

Hanson (10, p. 58) stated that rooting of stem cuttings was commonly employed in the breeding of alfalfa to obtain additional information on specific genotypes of alfalfa and to maintain those selected. The ease of clonal propagation obviated the need for inbreeding for the sole purpose of developing true breeding lines for maintaining the parental stocks of a variety. A low degree of self-fertility had also reduced the use of inbreeding and inbred lines in alfalfa. Vegetatively established alfalfa plants were compared with closely related alfalfa plants established as seedlings. Significant differences in agronomic performance had largely disappeared by the end of the second year. No way had been discovered to test a genotype as a seedling and as a vegetatively propagated clone at the same time.

Rooting Media

Tysdal et al. (22, p. 35-36) reported successfully making cuttings of alfalfa using a node and an internode when the cuttings were placed in sand. Improvement in rooting of cuttings treated with indole-butyric acid

was reported. However, since untreated cuttings rooted satisfactorily, the hormone treatment was not considered necessary.

White (24, p. 194-197) in 1946 devised a system of placing alfalfa cuttings in slowly running water. With this system 85 to 97 percent of the cuttings rooted, while the method previously employed, using moist sand, only 30 to 40 percent of the cuttings rooted.

Hanson (11, p. 614-615) reported successful rooting of red clover in running water, vermiculite, peat and sand. Increases in temperature increased the rate of rooting but at 95° F. rooting decreased. Relative humidity did not influence rooting unless it was very low and the temperatures were very high.

Hanson and Carnahan (9, p. 71) described propagules as a tiller separated from the plant with the old leaf sheath removed and roots trimmed away from the cluster of nodes at the base. The propagules may be planted directly in soil, sand, or vermiculite but to hasten establishment and to reduce labor and possible losses it was suggested that the tillers be planted in beakers or cans of water. A group of tillers from the same plant were identified with a tag or label and held together with a heavy elastic band. The water had to be changed

two or three times daily which was conveniently accomplished through provision for a limited continuous overflow of water. It was suggested that the propagules should be planted when the newly formed roots were between 1/4 and 1/2 of an inch in length.

Burton (2, p. 704-705) in 1936, increased development of adventitious roots on alfalfa propagules by treatment with hormones. Hairy Peruvian responded less to these treatments than Hardigan, while the Hairy Peruvian controls rooted more readily than the controls of the Hardigan variety.

Nowosad (20, p. 497-503) attempted to induce rooting in alfalfa and red clover by treatment of cuttings with various hormone concentrations. Soaking the basal ends of the cuttings in a solution containing 50 ppm of the hormone for a period up to 12 hours long, or mixing 10 ppm in the nutrient solution gave the greatest response. However, temperature, humidity, light conditions, maturity of propagule, the pH and chemical concentration of the growth media were cited as possibly influencing the success of propagation.

Nowosad (20, p. 481-496) treated timothy cuttings with a variety of concentrations of hormones in talc, charcoal and nutrient solutions. One hundred ppm in a charcoal carrier appeared to stimulate rooting when a

temperature of 50° F. was maintained. The timothy cuttings appeared to have been the basal vegetative shoot, cut at the surface of the ground and trimmed to leave three blades and a fully enclosed sheath about 2½ inches in length. This cutting was different from the propagules described by Cowan (7, p. 305) and Hanson and Carnahan (9, p. 71).

Barralles and Ludwig (1, p. 462) found that hormone treatments did not improve rooting of red clover cuttings. Scholz and Smidrkal (21, p. 176-180) improved the rooting of alfalfa, sainfoin and red clover cuttings with hormones. Lesins (17, p. 60-61) found that cuttings made from healthy plants in spring and summer rooted well but that devastating losses could occur in cuttings taken from greenhouse plants. Increases were observed in the number of roots as a result of application of a hormone preparation to alfalfa cuttings. In three separate trials the average percentage of propagules rooting without hormone treatment was 26.3 whereas 67.5 percent of the treated propagules rooted. In this research the running water technique of White (24, p. 194-197) did not give satisfactory rooting of cuttings.

In a study to determine whether or not rooting of tall fescue could be improved, Cowan (6, p. 78-86)

treated propagules from one clonal line with seven growth promoting hormones and chemicals. Propagules were prepared (a) with at least 1 inch roots and 1 inch of green leaf, (b) with less than 1 inch roots and less than 1 inch of green leaf and (c) cut about 1/16 of an inch above the crown and 1/4 of an inch below the leaf sheath or collar. Treatment concentrations ranged from 0 to 1000 ppm. Propagules were soaked for 20-21 hours in the treatment solution and placed on damp peat moss and wrapped with an inert plastic sheet. It was concluded that chemicals and hormones had no beneficial effect on the rapidity of root growth as in all cases root growth was retarded proportionally to the concentrations used.

Methods of Assessing Growth

Cowan (6, p. 78) used a series of visual classes to assess graded responses to propagation because methods of quantitatively measuring the relative success of propagation were not available. Langer (16, p. 197-198) measured the influence of management on sustained leaf and dry matter production of grasses. Methods developed for seedling plants growing in the field might be adaptable to a study of propagule development. Langer (16, p. 197-198) proposed a method, of measuring leaf growth, that would be reasonably reliable and suitably

rapid for field use. The total area of a population of leaves was determined from the means of linear measurements of the individual leaves. Some of the loss of accuracy, incurred by not measuring leaf widths, could be corrected by including a term obtained from the product of the covariance of length on mean breadth, and the number of leaves divided by K, where K is a constant obtained by dividing the actual area into the length by the width measurement of the leaf. These methods were only considered suitable for small samples within physiologically and genetically homogenous populations.

In a similar study on the pattern of seedling development in short-rotation and perennial ryegrass, Mitchell (18, p. 195-196) determined relative responses to temperature, shading, and defoliation by (a) count of total number of tillers, (b) number of leaves on main stem and on each tiller, (c) measurement of the rate of elongation of successive leaves on the main stem, and (d) leaf dimensions. The leaf length and median width was used to determine leaf area. Green weights and dry weights were determined for the tillers before and after treatment. The difference between the dry weights of

entire plants less roots, before and after treatment, was used to measure treatment effect. Full light produced the greatest increase in dry matter. Defoliation and shading reduced the dry matter increase by approximately 75 percent.

Mitchell (18, p. 195-196) worked with a surplus of seedlings which were selected for uniformity at an early age when they were transplanted. Final comparisons between treatments were based on groups of ten plants.

Jenkins (12, p. 532-536) summarized the four commonly used methods of measuring leaf area:

1. A system of blueprinting and planimetering whereby a record of the shape of the leaf was printed and the area was measured.

2. Counting of squares on a grid which was best adapted to rectangular types of leaves such as the turnip leaf.

3. The principle involved was that the intercepted light as measured by the photronic cell was proportional to the leaf area. Leaves had to be flattened exactly at right angles to the light and this was very difficult to accomplish with grass leaves.

4. Measuring parameters whereby the shape of the leaf was closely related to a geometric pattern and so the area of the leaf was calculated in the same manner

as the pattern. This method was not applicable to grass species.

Similarly, methods have been developed whereby the leaf area has been empirically related to linear measurements of some part of the leaf. These methods involved considerable preliminary work for each species and stage of growth thereof, for which measurements were desired and hence were not readily applicable to a heterogenous mixture of grass leaves.

The methods previously used were considered unsatisfactory by Jenkins (12, p. 532-536) who described a new method. An air flow planimeter was used to measure the area of detached leaves. Basically the apparatus consisted of two identically perforated plates mounted on an airtight drum which was connected to a constant speed rotary pump. The rate of air flow was noted with one plate covered (the measuring grid) and with the other plate open (the specimen grid); this gave a datum pressure. Leaves were mounted on the specimen grid and were held flat by suction pressure. The area of the leaves was determined by exposing part of the measuring grid to bring the air flow back to that of the datum pressure. The exposed area on the measuring grid was then equal to the area of leaves on

the specimen grid. From a vernier scale on the measuring grid a direct, accurate reading of area was quickly obtained.

Burton (3, p. 33) in an extensive study of the inheritance of morphological characters in alfalfa plants and their relation to plant yields, found a close association between green weights and dry weights. The correlation was very high both in the greenhouse and in the field with r values of .99 and .97 respectively. This indicated that green weights were adequate for determination of the relative yielding capacity when comparable environmental conditions prevailed.

MATERIALS AND METHODS

Introduction

Ten genotypes were selected for this study on the basis of generally observed differences in their response to vegetative propagation. Some variability was assured because the selections from the nursery were originally obtained as seed from widely divergent points in the United States and from Africa. The variability of morphology and growth habit observed in field and greenhouse plantings indicated wide genetic diversity.

Selections were lifted from a nursery at the Hyslop Agronomy Farm, Corvallis, Oregon, in 1956 and clonal material was transferred to the Research Station at Summerland, British Columbia, where field and greenhouse plantings were made. Some of the genotypes winter killed so thereafter clones were maintained in the greenhouse. Whenever possible throughout the study clones from the field were utilized. The nursery was irrigated approximately every ten days and cultivated as needed. Inflorescences were removed prior to seed setting to prevent volunteer seedlings from establishing.

For this study the clonal lines were considered as experimental material. Data, however, were obtained

on each genotype in order to measure treatment interactions with genotypes in Experiments I and II. Genotype 1 was selected as the check because it was of average size and the propagules were average in ease of preparation.

A clone or a clonal line was considered to be a plant or plants of one genotype. A propagule consisted of the basal portion of an individual tiller that had been broken away from the clone. The old sheaths were removed and the top was cut just above the sheath of the basal leaf unless otherwise stated. Roots were either trimmed to a stated length or removed completely.

Flats used measured approximately 2 feet long by 1 foot wide and were 3½ inches deep. Vitabands 2 x 2 x 3 inches were used for the solid rooting media, to delimit the location of each propagule. Rooting media were not sterilized in this study. The study was conducted in greenhouses routinely used for horticultural projects which determined the temperatures and space available. Temperatures were not precisely controlled because the cooling methods were not adequate for very hot days. In the afternoons the temperature rose to 85° - 90° F. for periods of approximately one hour. Lighting in the various greenhouses was considered comparable. There was no artificial lighting and essentially there was no shading of the greenhouse by buildings or trees.

This research was undertaken to discover techniques which would make efficient use of space and labor while obtaining data on factors affecting the response to vegetative propagation of tall fescue. Table 1 outlines the experiments and their objectives.

Table 1. OUTLINE OF EXPERIMENTS, OBJECTIVES AND THE CRITERIA MEASURED

Experiment Number	Objectives	Criteria Measured
I	a. To determine suitability of various rooting media b. To relate growth criteria to survival	Number of: leaves, roots, leafy propagules, rooted propagules
II	a. To compare methods of supporting propagules b. To determine relation between oven-dry and green weights	Weight: planting, harvest, increment
III	a. To determine sample size b. To relate growth criteria to survival	Number of: leaves, leafy propagules; Length of: largest leaf
IV	a. To relate growth criteria to survival	Number of: leaves, roots, leafy propagules
V	a. To relate growth criteria to survival	Number of: leaves, roots, leafy propagules Weight: planting, harvest, increment, leaves and roots
VI	a. To relate growth criteria to survival	Number of: leafy propagules Weight: planting, harvest, increment

Developmental stages of the propagules are illustrated in Figure 1 where propagules are shown: A two days old, B two weeks old, and C, D and E four weeks old. C shows a weak propagule with a relatively short leaf and some roots, D demonstrates vigorous growth, and E shows propagules which have no green turgid leaves and are considered dead.

The number of living propagules would provide data on survival though graded response would not be determined. Accordingly, other measurements were made. There follows a description of these criteria:

Leafy propagules -- each propagule was inspected and if it had one green turgid leaf it was counted as a living propagule.

Rooted propagules -- each propagule was examined and if it had one new main adventitious root two inches long or longer it was counted as a living propagule.

Leaf count -- each propagule was inspected and the number of green turgid leaves was counted and recorded.

Leaf length -- the longest green turgid leaf per propagule was measured to the nearest whole inch.

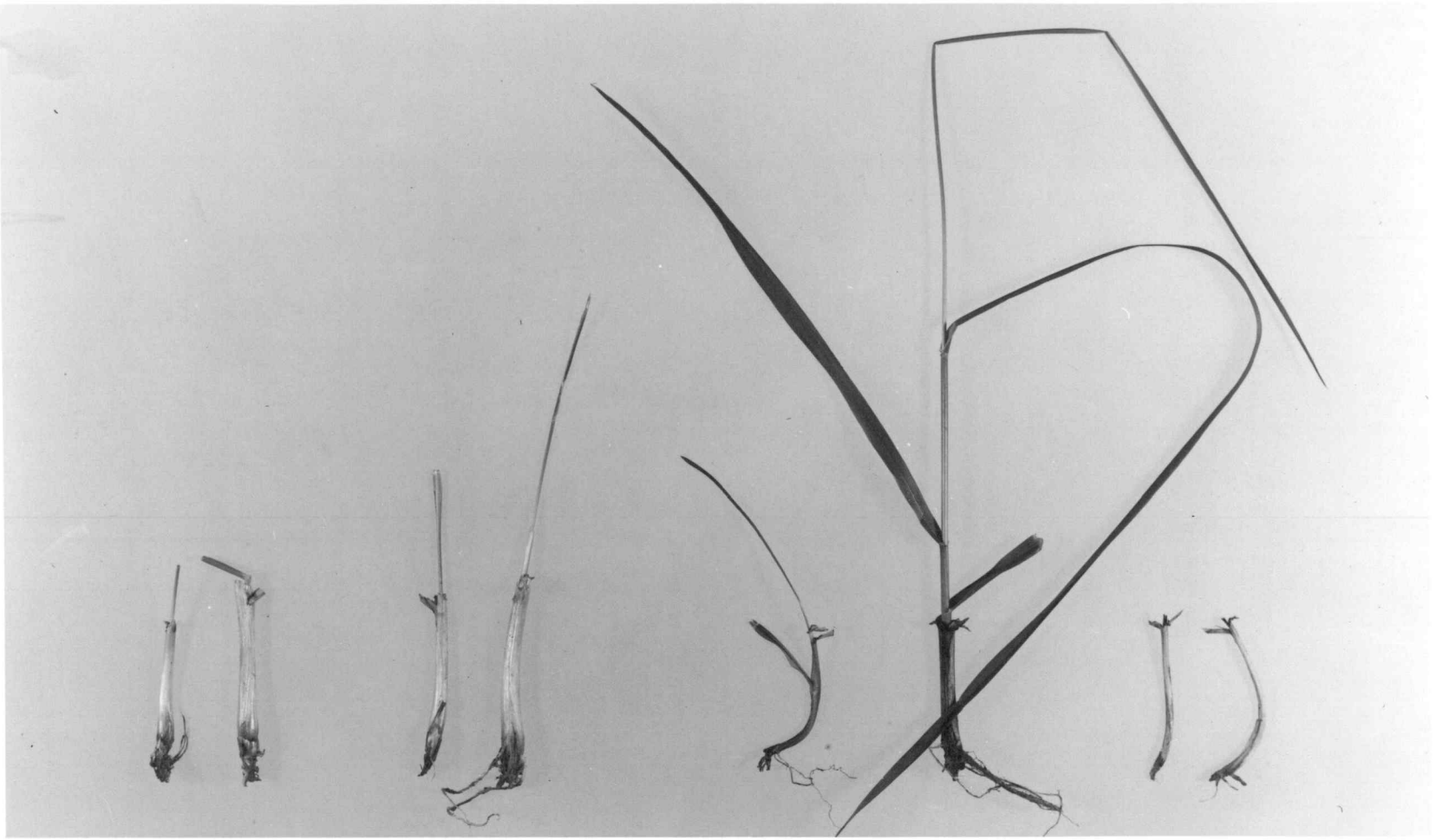

Figure 1. PROGRESSIVE DEVELOPMENT OF PROPAGULES AND VARIATION IN SUCCESS OF ESTABLISHMENT: (A) TWO DAYS OLD, (B) TWO WEEKS OLD, (C, D and E) FOUR WEEKS OLD: (C) WEAK, (D) VIGOROUS, (E) DEAD

Root count -- the number of new main adventitious roots two inches long or longer was counted for each propagule.

Planting weight -- the propagules of a sample were weighed as a group at the conclusion of an experiment.

Weight increment -- the harvest weight less the planting weight of that sample.

Leaf weight -- the new leaves were trimmed from the propagules and a sample weight of leaves produced was obtained in grams.

Root weight -- the new adventitious roots were trimmed from the propagules and a sample weight of new roots was obtained in grams.

Propagules were obtained in this manner. Clones were lifted from the nursery row or from pots in the greenhouse. The roots were cut to approximately two inches in length and washed free of soil. The tillers were then individually broken away from the clone and placed in cold water. Care was taken to prevent drying of the tillers. One plant of a genotype was used for one replicate and then discarded.

Propagules were prepared by either removing the roots from the basal node or by trimming the roots to one-half inch in length. The tiller was cut either just above the sheath of the basal leaf or the cut was made slightly above or slightly below this point to obtain uniform lengths of propagules of different genotypes.

Throughout the study the randomized block design was used. Genotypes were assigned at random to locations within the replicates. Except as indicated in Experiment III the sample size, throughout the study, consisted of ten propagules. Sample totals are reported though for some criteria data was recorded for each propagule and these values were summated to obtain sample totals. Gravimetric measurements, to the nearest one-tenth gram, were made on the sample. Green weights were obtained by shaking the ten propagules of a sample, before collectively weighing them, in order to remove excess water.

Experiment I - Rooting Media

Silt, a mixture of silt, sand and peat, coarse sand, vermiculite and distilled water were compared as media for growing propagules of tall fescue in the greenhouse. Ten genotypes were compared with three replications in September, 1957. A final evaluation, of leafy propagules, rooted propagules,

leaf counts, and root counts was made eight weeks after planting.

Several clones of each genotype were lifted from a nursery which had been established one year previously for this and similar experiments. The roots were uniformly trimmed from the propagules so that approximately one half inch remained. An attempt was made to cut off the culm or stem just above the sheath of the basal leaf. Due to the variations between and within genotypes, complete morphological uniformity was not obtained. The taller growing genotypes were cut at or just below the leaf sheath and the shortest growing genotypes were left with approximately one inch of leaf blade on the propagule. By this compromise, propagules of the various genotypes were similar in length.

The silt media was taken from a soil classified as Penticton silt. It was obtained from the top five inches of an undisturbed roadside where seepage, irrigation and cultivation had not affected the top soil.

The mixture of silt, sand and peat was mixed in a volume ratio of 7:2:3 respectively. The sand was taken from the supply obtained for the sand media which is described in the following paragraph. The peat was commercial garden peat. The ingredients were measured

and mixed for five minutes in a large cement mixer.

The sand media consisted of sand having large particles with sharp edges. The vermiculite used was the commercially available horticultural grade. The particles were smaller in size than the particles of vermiculite used in construction. Distilled water of high purity was used.

A polyethylene bag was used to line the flats for the distilled water media to make the flats water tight. The propagules were held in position in the distilled water by paper towel bands rolled into a ball. These bands were prepared from paper towels cut into four foot lengths and folded double lengthwise to a width of two and one-half inches. The folded towel was laid on the bench and the propagules placed along on top of it at intervals of approximately four inches. The root end was placed flush with one edge of the towel. The towel was then rolled into a ball held loosely by a rubber band and placed in position with the fold in a vertical plane and the propagules upright in the distilled water flat. The prepared flat and the propagules planted in this manner are illustrated in Figure 2. The shape of the plots in the distilled water media differed from the row plots of the solid media treatments. The water media plots were circular. However, care was taken to keep the plots

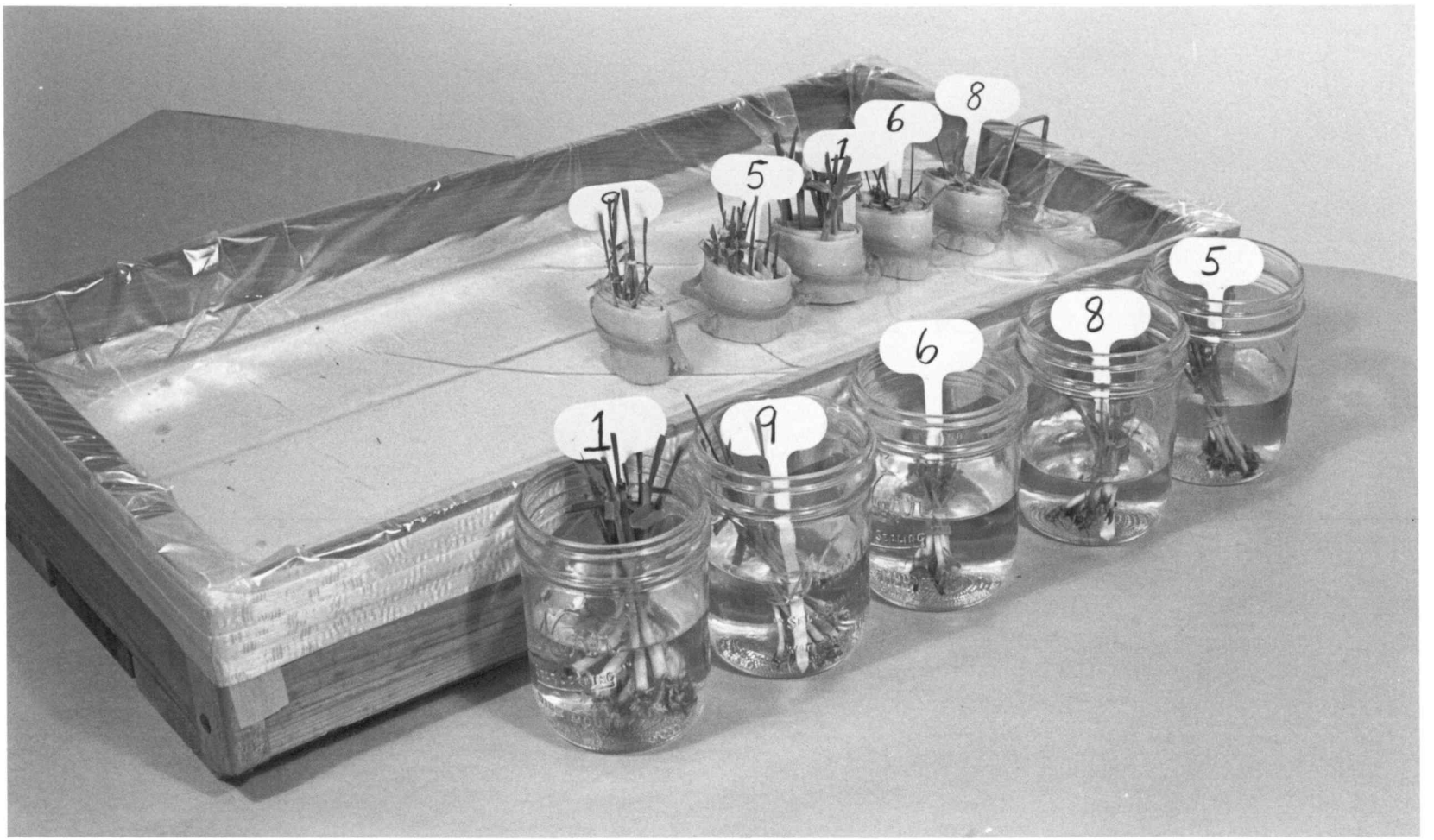

Figure 2. PROPAGULES SUPPORTED BY PAPER TOWELS, AND STANDING UPRIGHT IN PINT MASON JARS IN EXPERIMENT II

correctly oriented so that the experimental design was not invalidated.

Maximum-minimum Taylor thermometers were used to record daily temperatures in the greenhouses. The average maximum and minimum temperatures as recorded for replicates one, two and three were 62° F. and 45° F., 73° F. and 50° F., 96° F. and 49° F. respectively.

The solid media were watered with tap water. Distilled water was poured into the flats to restore the water level to a depth of 2½ inches. Care was taken to maintain the distilled water at no less than approximately two inches in depth. The objective in watering was to assure that water would always be plentiful and hence availability of water would not be a limiting factor to propagule development.

At the end of the eight weeks the propagules were evaluated. The solid media were gently washed from each propagule to expose the old roots and the main newly developed roots which were counted. The number of green turgid leaves per propagule were similarly recorded and totalled for the leaf count. Leafy and rooted propagules were counted. The check treatment for media was predetermined to be the silt media.

The data from these criteria were separately analyzed by the analysis of variance, and "F" tests of significance.

Correlation coefficients, based on genotype means, were computed between leafy propagules; rooted propagules, leaf counts and root counts. The expected mean squares for the analysis of variance are shown in Table 2.

Table 2. EXPECTED MEAN SQUARES FOR THE ANALYSIS OF VARIANCE IN EXPERIMENT I, 1957

Sources of Variation	D.F.	Expected Mean Square
Replications	2	Ve + 50 Vr + 5 Vrg + 10 Vrm
Media	4	Ve + 30 Vm + 10 Vrm + 3 Vmg
Genotypes	9	Ve + 15 Vg + 3 Vmg + 5 Vrg
Media x Genotypes	36	Ve + 3 Vmg
Replications x Media	8	Ve + 10 Vrm
Replications x Genotypes	18	Ve + 5 Vrg
Error	72	Ve
Total	149	

Experiment II - Propagule support and relationship between green and oven-dry weights

In May, 1958, one clone of each of five genotypes was divided into 40 propagules as described in Experiment I except that all the roots were removed from these propagules. The 40 propagules of each genotype were divided into four similar samples of

10 propagules each. Green weights were recorded. Two samples of each genotype were placed in a 95° C. oven for 24 hours and the oven-dry weights were recorded. The remaining two samples of each genotype were planted in distilled water as described in Experiment I, and were placed in a greenhouse where the temperature was controlled at 70° F. by day and 50° F. at night.

The propagules were weighed after a four week period and the propagules were then placed in a 95° C. oven and oven dried. Correlation coefficients were calculated between the green and oven-dry weights.

In August, 1958, a comparison was made between propagules wrapped in paper towels to hold them erect and between unwrapped propagules bound loosely together. Clones of five genotypes were lifted from the nursery and were grown in distilled water media with four replications. The treatments are illustrated in Figure 2.

The propagules were prepared as described in Experiment I except for two details; (a) the roots were clipped entirely away instead of leaving 1/2 inch of the old roots attached to the propagule, and (b) the genotypes were cut to different lengths depending on the morphology of the particular genotype. All propagules were cut off just above the sheath of the basal leaf.

As a result propagules of the tallest growing genotype were six to seven inches long in contrast to the shorter growing genotypes whose propagules were approximately four inches long.

The propagules were held in place by paper towels as described in Experiment I for one treatment, and for the other treatment the propagules of a given sample were gathered into a bundle and held together by a rubber band. They were then placed in pint Mason jars. Samples were weighed before planting.

The experiment was conducted in a greenhouse where the day temperature was set at 70° F. and the night temperature was kept at 50° F.

The propagules were weighed after a five week interval and then oven dried. "F" tests of significance were computed. The expected mean squares for the analysis of variance are shown in Table 3. Correlation coefficients were calculated between the green and oven-dry weights.

Table 3. EXPECTED MEAN SQUARES FOR THE ANALYSIS OF VARIANCE IN EXPERIMENT II, 1958

Sources of Variation	D.F.	Expected Mean Square
Genotypes	4	Ve + 8 Vg + 2 Vgr + 4 Vtg
Treatments	1	Ve + 20 Vt + 5 Vtr + 4 Vtg
Replications	3	Ve + 10 Vr + 2 Vgr + 5 Vtr
Genotypes x Treatments	4	Ve + 4 Vtg
Replications x Treatments	3	Ve + 5 Vtr
Replications x Genotypes	12	Ve + 2 Vgr
Error	12	Ve
Total	39	

Experiment III - Sample Size

Leaf length and leaf counts were compared as criteria for estimating the success of vegetative propagation of tall fescue in December, 1956. Optimum sample size was determined using the criteria leafy propagules, leaf counts and lengths. Ten genotypes were replicated twice in a silt medium.

Six months old clones were obtained from the greenhouse. Propagules were prepared as described in Experiment I. Twenty propagules of each genotype were selected in turn and planted for the first replicate. When this replicate was finished it was moved to the

greenhouse and the next replicate was planted in a similar manner. Genotype plots were randomly assigned in each replicate.

The flats were placed on greenhouse benches so that some distance separated replicates. One replicate was placed at the south side and the other was placed at the north side of the greenhouse. Temperatures were controlled at 70° F. by day and 50° F. at night.

Four weeks later the individual propagules were evaluated by counting the number of leafy propagules, leaves and roots. It was difficult to find the top of the propagule for a base from which to measure leaf length.

The method of determining an optimum sample size consisted of comparing the coefficients of variation of sample sizes of 5, 10 and 20 propagules. These sizes were chosen because under normal propagating procedures a flat could readily hold 10 propagules lengthwise and 5 propagules across the width. If these sample sizes were suitable for statistical purposes then they were ideally suited to the mechanical requirements of identifying rows and hence samples. Sufficient propagules were planted in rows five propagules long for sample sizes of twenty with the two replicates.

The first analysis was made by selecting at random one sample row of five propagules for each genotype, from each replicate. Analysis of variance, and "F" tests were calculated for the three criteria. The second analysis was made by randomly selecting two rows containing five propagules each for a sample size of ten, for each genotype from each replicate. The third analysis utilized the twenty propagules of each genotype as a sample size and the same analyses were calculated. The expected mean squares are shown in Table 4.

Table 4. EXPECTED MEAN SQUARES FOR THE ANALYSIS VARIANCE IN EXPERIMENT III, 1956

Sources of Variation	D.F.	Expected Mean Square
Genotypes	9	Ve + 2 Vg
Replications	1	Ve + 10 Vr
Replications x Genotypes	9	Ve
Total	19	

Correlation coefficients were calculated between leafy propagules, leaf lengths and leaf counts.

Experiment IV - Leaf and root counts to assess survival

In December, 1957, propagules were grown to determine if survival was associated with leaf and root counts. Ten genotypes were grown in distilled water media with

six replications. Clones were obtained from the greenhouse. Propagules were prepared as described in Experiment II (August) and they were planted in paper towels as described in Experiment I.

After three weeks the propagules were evaluated by counting the number of leafy propagules, leaves and roots. Correlation coefficients were calculated between leafy propagules, leaf and root counts.

Experiment V - Leaf and root counts and weight criteria to assess survival

Some changes from Experiment IV were made in the criteria employed to evaluate propagation response for Experiment V in January, 1958. Clones were obtained from the greenhouse. Ten genotypes were grown with three replications. Propagules were prepared as described in Experiment II (August), and planted in paper towels in distilled water media as described in Experiment I. In this experiment each sample was weighed before planting.

The samples were evaluated after three weeks growth by counts of leafy propagules, leaf and root counts. In addition, samples were weighed to determine harvest weights for each sample and new roots and green turgid leaves, were carefully cut away from the original propagule and each weighed separately on a sample basis. Correlation coefficients were calculated between leafy propagules;

leaf and root counts; leaf and root weights; and planting, harvest, and increment weights.

Experiment VI - Weight criteria to assess survival

In December, 1958, propagules were grown to permit observations on possible associations between leafy propagules and harvest and increment weights. Propagules of five genotypes were grown in distilled water media with ten replications. Propagules were prepared as described in Experiment II (August).

Clones were obtained from the greenhouse. The propagules were weighed and planted in pint glass jars containing 2½ inches of distilled water. The jars were placed at intervals along a greenhouse bench. The temperature was controlled at approximately 70° F. in the daytime and at 50° F. at night.

Four weeks later, harvest and increment weights were determined. A count of live propagules was recorded for each plot. Correlation coefficients between leafy propagules and the weight criteria were calculated.

EXPERIMENTAL RESULTS

Experiment I - Rooting Media

Mean squares for the analysis of variance for this experiment are reported in Table 5. Genotype interactions with media were not significant for any criteria used to measure survival though differences between media and between genotypes were significant for all criteria.

Sample treatment means for growth response in the different media as measured by the number of leafy and rooted propagules, and leaf and root counts, are reported in Table 6. Silt was the predetermined check.

Survival data (leafy propagules) showed that all media were significantly superior to silt. Means of the other four media showed no significant differences in survival. Rooted propagule data supported the same interpretation of media effects.

When survival was measured by leaf counts the mixture of silt, sand and peat was superior to silt at the 5 percent level of significance. However, propagules in the check media, silt, produced a significantly greater number of leaves than propagules grown in coarse sand, vermiculite and distilled water. Root counts were significantly lower for the propagules

Table 5. MEAN SQUARES[1] FROM THE ANALYSIS OF VARIANCE FOR THE CRITERIA; LEAFY PROPAGULES, LEAF AND ROOT COUNTS IN EXPERIMENT I, 1957

Source Variation	D.F.	Leafy Propagules	Rooted Propagules	Leaf Count	Root Count
Replications	8	1.02*	1.43**	3.75	403.25**
Media	4	0.66*	0.69**	143.13**	235.49**
Genotypes	9	0.58*	0.42**	112.33**	818.69**
Media x Genotypes	36	0.24	0.18	6.06	28.45
Replications x Media	8	0.16	0.16	17.81**	136.19**
Replications x Genotypes	18	0.01	0.20	7.09	122.38**
Replications x Media x Genotypes	72	0.25	0.15	5.01	25.43

* Significant at the 5 percent level.

** Significant at the 1 percent level.

1 Interaction mean squares involving replications, not shown as significant, were combined with third order interaction mean square for tests of significance.

Table 6. SAMPLE TREATMENT MEANS FOR PROPAGULE SURVIVAL IN FIVE MEDIA FOR EXPERIMENT I, 1957

Media	Leafy Propagules	Rooted Propagules	Leaf Count	Root Count
Silt	9.40	9.45	24.4	38.2
Silt, sand, peat	9.75	9.75	25.7	40.8
Coarse sand	9.70	9.75	22.9	43.6
Vermiculite	9.80	9.80	21.9	44.9
Distilled water	9.70	9.80	20.0	44.4
LSD at 5 percent level of significance	0.25	0.25	1.2	2.6

grown in silt than for comparable propagules grown in coarse sand, vermiculite or distilled water.

Individual genotype sample means for the four criteria are shown in Table 7. Genotypes differed significantly, with respect to the four criteria, from genotype number 1 the predetermined check.

The association between leafy propagules and the other criteria are reported in Table 14 later in this section under the heading "Associations of Criteria with Survival."

Table 7. GENOTYPE SAMPLE MEANS IN EXPERIMENT I, 1957

Genotypes	Leafy Propagules	Rooted Propagules	Leaf Count	Root Count
1	9.5	9.6	19.9	47.8
2	9.4	9.4	18.5	40.6
3	9.6	9.6	22.1	50.1
4	9.6	10.0	23.1	35.3
5	10.0	9.8	22.2	54.6
6	9.8	9.7	27.8	31.8
7	9.7	9.7	22.9	33.6
8	9.7	9.7	26.1	41.2
9	9.9	9.9	24.7	44.0
10	9.8	9.8	22.4	44.7
LSD at the 5 percent level of significance	0.4	0.3	1.7	3.7

Experiment II - Propagule supports and relationship between green and oven-dry weights

The mean squares for the analysis of variance for this experiment are reported in Table 8.

Differences due to treatments were highly significant at the conclusion of the experiment for both harvest and increment weight criteria. Genotypes differed significantly with respect to planting, harvest and increment weights. Genotypes interaction with treatments

Table 8. MEAN SQUARES[1] FROM THE ANALYSIS OF VARIANCE FOR WEIGHT CRITERIA IN GRAMS IN EXPERIMENT II, 1958

Sources of Variation	D.F.	Planting	Harvest	Increment
Replications	3	17.07**	205.34**	295.51**
Treatments	1	0.01	599.08**	594.44**
Genotypes	4	210.21**	559.53**	57.34**
Genotypes x Treatments	4	1.03	26.99	17.88
Replications x Treatments	3	1.59	12.95	9.60
Replications x Genotypes	12	17.08**	78.07**	26.22*
Replications x Genotypes x Treatments	12	1.39	8.54	7.65

* Significant at the 5 percent level.

** Significant at the 1 percent level.

1 Non-significant second order interaction mean squares involving replications were combined with third order interaction mean square for tests of significance.

was not significant at the 5 percent level of significance.

Sample treatment means for propagule growth are shown in Table 9. The glass jar treatment exceeded the paper towel treatment considerably in excess of the required difference for significance at the 1 percent level. The mean planting weights were the same for the two treatments.

Table 9. TREATMENT SAMPLE MEANS IN GRAMS FOR WEIGHT CRITERIA IN EXPERIMENT II, 1958

Support	Planting	Harvest	Increment
Glass jars	15.5	31.8	16.3
Paper towels	15.5	24.0	8.5
LSD at 1 percent level of significance--		3.3	2.5

Genotype sample means of planting, harvest and increment weights are reported in Table 10. Significant differences were found between genotypes and the check genotype (1) for all criteria.

There was a close and highly significant relationship between green and oven-dry weights as shown in Table 11.

Table 10. GENOTYPE SAMPLE MEANS IN GRAMS FOR WEIGHT CRITERIA IN EXPERIMENT II, 1958

Genotypes	Planting	Harvest	Increment
1	24.5	39.3	14.8
5	16.9	30.3	13.4
6	8.3	16.1	7.8
8	13.7	26.8	13.2
9	14.0	26.8	12.8
LSD at the 5 percent level of significance	1.8	4.4	4.2

Table 11. SIMPLE CORRELATION COEFFICIENTS (n=10) BETWEEN THE GREEN AND THE OVEN-DRY WEIGHTS IN EXPERIMENT II, 1958

Variables associated	May	August
	r	r
Green planting with oven-dry planting weight	.969**	- - -
Green harvest with oven-dry harvest weight	.868**	.963**

** Significant at the 1 percent level.

Experiment III - Sample size

Mean squares for genotypes and error for three plot sizes and three criteria are shown in Table 12. With a sample size of five propagules only leafy propagules showed significant difference between genotypes and that difference was only significant at the 5 percent level. However, genotype differences were significant at the 1 percent level of significance for all criteria with sample sizes of 10 and 20 propagules.

Table 12. GENOTYPES AND ERROR MEAN SQUARES WITH DIFFERENT SAMPLE SIZE IN EXPERIMENT III, 1956

Criteria	Number of Propagules Per Sample		
	5	10	20
Leafy propagules			
Genotype mean square	0.91*	3.58**	14.20**
Error mean square	0.20	0.24	0.64
Leaf count			
Genotype mean square	11.47	42.56**	220.01**
Error mean square	3.44	6.38	10.72
Leaf length			
Genotype mean square	176.49	667.56**	2779.99**
Error mean square	60.45	108.14	289.09

* Significant at the 5 percent level.

** Significant at the 1 percent level.

Coefficients of variation and sample means for three criteria with changes in sample size are shown in Table 13. Coefficients of variation decreased with increased sample

size. The leafy propagules coefficient of variation decreased from 10 percent at the sample size of 5 propagules to 5 and 4 percent for sample sizes of 10 and 20 respectively.

Coefficients of variation for leaf count were in a higher range going from 21 percent for a sample size of 5 to 15 percent for a sample size of 10 and decreasing to 10 percent for a sample size of 20 propagules. The coefficients of variation for leaf lengths were generally higher and ranged from 28 to 19 percent for the smallest and largest samples.

Table 13. COEFFICIENTS OF VARIATION (C.V.) AND SAMPLE MEANS FOR DIFFERENT NUMBERS OF PROPAGULES PER SAMPLE IN EXPERIMENT III, 1956

Criteria	Number of Propagules		
	5	10	20
Leafy propagules			
C.V. %	10	5	4
Sample mean	4.7	9.2	18.1
Leaf Count			
C.V. %	21	15	10
Sample mean	8.7	16.9	33.7
Leaf length			
C.V. %	28	20	17
Sample mean	28.0	52.7	102.6

The association between the three criteria recorded for this experiment are reported in Table 14, in this

section under the heading "Association of Criteria with Survival."

Association of Criteria with Survival - Experiments I, III, IV, V, VI

The correlations between leafy propagules (survival) and other criteria used in this study to evaluate survival are shown in Table 14. Leaf length was the only criteria with a negative value at -.093. Rooted propagules was closely associated with survival. Leaf counts were associated at the 10 and 1 percent levels of significance with high and low r values of .797 and .568. When all data for leaf counts were combined in one analysis the highly significant r value was .639. Root counts were more variable in association with leafy propagules with r values ranging from .235 to .726. The .458 value of r for root counts when experiments were combined was significant at the 5 percent level. Neither leaf nor root weights were significantly associated with survival.

Planting, harvest and increment weights, in increasing order of magnitude, were similar in association with survival. In Experiment V these criteria were not significantly associated with survival. In Experiment VI the increment weight r value of .979 was significant at the 1 percent level and planting and harvest weight r values

of .864 and .893 were significant at the 5 percent level. The combined data from Experiments V and VI for these criteria showed r values significant at the 10 percent level only and ranging from .461 for planting weight to .595 for increment weight.

Table 14. SUMMARY OF SIMPLE CORRELATION COEFFICIENTS BETWEEN LEAFY PROPAGULES AND CRITERIA FOR ASSESSING SURVIVAL FOR ALL EXPERIMENTS, 1956-1958

Criteria Associated with Leafy Propagules (Survival)	Experiments					
	I (n=10)	III (n=10)	IV (n=10)	V (n=5)	VI (n=5)	Total
Rooted propagules	.969**	---	---	---	---	.969**
Leaf length	---	-.093	---	---	---	-.093
Leaf count	.568	.779**	.797**	.593	---	.639**
Root count	.235	---	.726*	.476	---	.458*
Leaf weight	---	---	---	.537	---	.537
Root weight	---	---	---	.410	---	.410
Planting weight	---	---	---	.358	.864	.461
Harvest weight	---	---	---	.442	.898*	.593
Increment weight	---	---	---	.497	.979**	.595

* Significant at the 5 percent level.

** Significant at the 1 percent level.

DISCUSSION

Rooting Media and Propagule Support

The various media tested produced significantly different responses by the propagules irrespective of how the response was measured. The control rooting medium, silt, produced propagules having significantly fewer leafy and rooted propagules, than the other media. The other media did not appear to differentially affect the number of leafy and rooted propagules. However, leaf counts and root counts were affected by the media in which the propagules had been planted.

Leaf counts were significantly higher when propagules were grown in the mixture of silt, sand and peat, and were significantly lower for propagules grown in coarse sand, vermiculite and distilled water than leaf counts of comparable propagules grown in silt. Root counts were lowest for propagules grown in silt with the root counts of propagules grown in coarse sand, vermiculite and water being significantly higher.

There were no significant interactions between the various media and the growth response of the propagules. This information would suggest that an investigator could use whatever media was most suitable from an operational view point when making comparisons between genotypes and between treatments. Inferences from one media should

apply equally to any of the other four tested in the experiment. The interaction was not significant, though there was some interaction between media and growth responses of genotypes. However, in this experiment the interactions were not large in relation to environmental variation. Therefore, while other media could be substituted for top-soil for methodology experiments in this type of environment, before new practices were adopted from results of such experiments, these practices would need to be reassessed with the top-soil to be used in normal procedures.

During the media experiment, it became evident that the distilled water was the most convenient medium to use. This medium could be obtained readily, without labor, from a still. Distilled water did not require expensive storage and could be readily disposed of at the conclusion of an experiment. This medium did not require sterilization as there were neither weed seeds nor disease organisms in it. Recovery of roots was more easily and completely accomplished than from solid media. Propagules could be removed for observations at intervals during an experiment, and then the propagules could be returned to the media without apparent injury. Essentially, there was no variation in chemical, physical

or biological characteristics, on a daily, seasonal or geographical basis. Pertinent comparisons could be made between experimental results concerning vegetative propagation, when the experiments were conducted at widely separated locations if distilled water was used as a rooting medium. The other media tested did not have these attributes.

The comparison between glass jars and paper towels as supports for the propagules show very clearly that the propagules grew more vigorously in the glass jars. The harvest weight and total growth treatment means for propagules in glass jars was nearly double the treatment means for propagules grown in paper towel supports. This conclusion is strengthened by the fact that the treatment mean weights of the propagules at the start of the experiment were equal and so there was no bias due to planting weight differences. Genotype interaction with the treatments was not significant.

The use of glass jars provides compact samples with high propagule density per unit bench space and would permit a great diversity of treatment additions to the distilled water. The method requires less labor and paper towels are not needed which represents a saving over the other method. In addition variation, which might be introduced by changes in the chemical composition of different lots of paper towels, would be eliminated.

Sterilization of glass jars could be conveniently accomplished with disinfectants whereas flats must be autoclaved to obtain suitable sanitation.

The paper towel supports did not appear to physically handicap the propagules in the development of roots and leaves. Complete recovery of the entire propagule was obtained at harvest. It is unlikely that the paper towels supplied factors deleterious to propagule development because of the favorable propagule development in comparison to other media when propagules were grown in paper towels as shown in Table 6. The apparent explanation for the greater growth in glass jars, is that the entire propagule is exposed to light whereas the paper towels shaded the lower portion of the propagule. Mitchell (18, p. 195-196) found that the greatest increase in dry matter occurred in field light.

The reasons for the differential response of the propagules to the rooting media tested are somewhat obscure. One possible explanation for the low survival of propagules grown in silt might be due to differences in planting though conscious efforts were exerted to avoid such an event. It might be that silt absorbed water more slowly when the flats were being watered and consequently the propagules growing in silt were subjected to some moisture stress due to less adequate watering.

This particular silt is very infertile in the native state but even so the nutrients supplied to the propagules should have exceeded the supply available to propagules growing in distilled water and so lack of fertility is not likely to be responsible. Aeration may have influenced survival because silt is not as well aerated as for example, coarse sand. However, the explanation would not apply to the better survival in the distilled water medium where aeration would also be low.

The leaf counts were highest for propagules grown in the mixture of silt, sand and peat. This might result from the presence of the peat which might make some additional nutrients available to the propagules. Water would have been more readily absorbed during watering in this mixture than in the silt medium but moisture supply could not have been the sole cause because water would have been as readily absorbed by the coarse sand medium. Again in the distilled water there would have been no moisture stress and yet propagules growing in that medium produced the lowest number of leaves.

Root counts were significantly lower in the silt media and this might be explained by a lower recovery of roots due to the greater difficulty encountered in washing this medium from the roots. This explanation is supported by the high root counts on propagules grown in distilled

water, vermiculite and sand which did not cling to the roots. The mixture of silt, sand and peat was intermediate in the ease of freeing roots and the root counts on propagules grown in this medium were intermediate between the two extremes. However, only the larger roots were counted and there would be less likelihood of losing this larger root in the removal of the propagules than if smaller roots and root hairs had been counted.

The solid media tested were generally believed to be infertile and certainly the distilled water did not contribute nutrients. Differences between the media might have been reduced by addition of elemental growth factors. Genotype interaction with media might have occurred under conditions of higher fertility.

The highly significant differences between genotypes observed under rather uniform and infertile conditions and the ease of collecting data in the greenhouse raised the question of possible adaptation of this method to the screening of seedlings for yield. This approach would be more useful if growth behavior in the greenhouse could be related to field production.

The suitability of distilled water as a rooting medium might be expected in view of the successful use of water as a medium for rooting alfalfa and grasses as reported by White (24, p. 194-197) and Hanson and

Carnahan (9, p. 71) respectively. However, White required that the tap water be kept running whereas Hanson and Carnahan recommended two or three changes of water each day or preferably a continuous slow exchange of the rooting medium. In the experiment reported here the water was not changed and only sufficient water was added to maintain a high level around the propagules. The two published reports did not discuss the reasons for changing the medium or the specific effects that would result if the medium was not changed frequently. It is possible that the dissolved oxygen content was increased by changing the water. Another departure from the methods described by these workers involved the use of distilled water rather than tap water which might contain harmful organisms. These organisms might multiply if allowed to remain at room temperature and in this way set up an infection. Frequent changes of water might prevent a build up of these organisms. By contrast, the distilled water medium would contain only air borne organisms which would have some opportunity to attack the propagules whatever media was used. Distilled water had the advantage of uniformity over time and geographic locations whereas tap water varied during the season and from location to location. The chlorine content of tap water might in some places be high enough to interfere with propagule growth.

Moisture Content

Traditionally, in field plot forage research the variability due to relative moisture content is removed by obtaining oven dry weights. Since the environment is more uniform in a greenhouse experiment it was necessary to determine if this technique was required in this study which was conducted in the greenhouse.

In this study a number of green weights were compared to oven dry weights and high correlations and highly significant values ranging from .864 to .969 were obtained as shown in Table 11. This indicated that green weights were satisfactory and that little variability existed due to differences in moisture content. This variation due to variable moisture contents would reduce the precision of weight data, and when it was desired to detect small dry matter differences, oven dry weights would be required. This lack of variation in moisture content would save labor in research on vegetative propagation of grasses since time, skill and equipment are required to determine oven dry weights. These results are in agreement with those reported by Burton (3, p. 33) with alfalfa where the correlation between green and oven dry weights in the greenhouse was positive with an r value of .99.

There is another advantage to the close association between oven dry and green weights beyond the saving in

labor and equipment needed to determine oven dry weights. Harvest weights and hence total growth can be obtained for samples of propagules without undue damage to them when they are grown in water. A curve could be obtained to show the rate of growth. At the end of an experimental period, harvest and increment weights could be determined, and the propagules could be used for further experiments or could even be planted for propagation. However, if oven dry weights were required the propagules would have to be killed.

The lack of variation between genotypes in moisture content may be due to either genetic or environmental factors. The genotypes chosen for this particular study may have been genetically similar in the ratio of dry matter to moisture content, but since the genotypes were chosen at random with respect to this characteristic it is unlikely that this was so, unless the clonal lines in the nursery did not differ in this respect. However, the nursery was established with seed of a cross-pollinated crop obtained from widely separated geographical areas and so the genotypes must have been somewhat genetically heterogenous and heterozygous.

Accordingly the environment must be such that the various genotypes do not have an opportunity to express widely different genetic capacities with respect to

moisture content. Since the propagules were grown standing in water, maintained at a level well above the roots, it is unlikely that any moisture stress occurred. Therefore, it is reasonable to assume that an opportunity to manifest large differences in moisture content did not exist whereas in most forage experiments the available moisture supply is variable and differences in dry matter content do occur.

The lower associations between green harvest and oven dry harvest weights in May as compared to August might indicate a seasonal effect on this characteristic. Since the propagules were maintained in the greenhouse under the same temperature and day length conditions this lower association may have resulted from differences in the developmental stage of the tillers used to prepare the propagules.

Sample Size

The size and arrangements of samples and field plots must suit statistical and operational requirements. In field experiments the size and shape of the plot must be such that it covers the known gradient of heterogeneity of the site and accordingly plots are laid out lengthwise up and down hill because normally the soil variation is on the same gradient as the terrain. Plots must be large enough to obtain a uniform sampling of the environment so

that treatment effects and not local environmental differences are measured. Within limits with larger plot sizes there is less pretreatment variation between plots. The upper limit occurs as a result of soil heterogeneity and if the experimental site becomes too large due to large individual plots then the increasing variation due to site size reduces the benefits of larger individual plots.

In greenhouse experiments samples size is not restricted by soil heterogeneity. In general the environment is controlled in comparison to the environments under which field experiments are conducted. However, space itself is a more limiting factor in the greenhouse and it is essential that samples be as small as is consistent with significant relationships between the pertinent sources of variation.

Both field and greenhouse experiments require that plot shapes conform to a pattern which provides for ready identification and individual treatments. Research and maintenance operations can be carried out without errors and in less time when the experimental design has been planned for operational convenience. In this experiment the trial sample sizes were selected because of the ease of identifying and managing individual complete rows in a flat.

A sample size of five was not large enough for significant differences to be detected between genotypes when survival was measured by leaf counts and leaf lengths. A sample size of 10 was large enough so that differences between genotypes were detected by the three methods of assessing response.

It is generally agreed that the coefficient of variation should not exceed 20 percent and that 10 percent is a more desirable figure. In this experiment the coefficients of variation decreased as sample size increased. With a sample size of 10 the coefficients of variation for live propagules and leaf counts were both below 20 percent but the coefficient of variation for leaf length was 20 percent.

Mitchell (18, p. 195-196) used a sample size of ten plants but these were seedlings of a cross pollinated grass and therefore must have been genetically different. In this study the samples consisted of clonal lines which would be more uniform. It is assumed that a sample size of 10 was suitable for the more variable experimental material used by Mitchell.

Criteria for Measuring Propagule Growth

For the purpose of studying propagation, more experiments could be conducted if the interval between

planting and harvest could be reduced to four weeks as was done in this study. Such an approach eliminates field planting and subsequent harvesting if gravimetric data were required. With this method data concerning survival would be just an estimate of the survival that would have resulted from a field planting. This estimate would be biased upwards only, because propagules considered dead at three weeks would not be taken to the field and planted. The upward bias of the survival estimate is not likely large because once a propagule gets established by producing new leaves and roots it has overcome the most serious obstacle to survival. Comparisons could be made within propagules used in a shorter greenhouse experiment and some generalizations about field survival might be permitted.

In this study more than one type of observation was made on the same experimental propagules. If one criteria or method of measuring propagule response could be found that would describe changes in the propagules and at the same time provide data on probable survival of propagules this would be very desirable.

The criteria leafy propagules was considered to be the most reliable estimate of survival and accordingly data from the other criteria were correlated to data collected

by this method. Leafy propagules as a method of assessing the growth response of propagules had a number of advantages. The leafy propagule criteria measured significant differences between genotypes and treatments in all but one experiment where genotype differences were not significant. The data were relatively easy to collect and repeated observations could be made on a given population without any effect on the propagules. One of the disadvantages of these types of data was that no response gradient was recorded. The propagules were either dead or alive and no further information was obtained. Another disadvantage arose whenever a propagule was dying. The intermediate stages were difficult to classify as either dead or alive. Judgment was required and bias and error may have resulted.

The rooted propagules criteria had some of the advantages of leafy propagules. It was recorded in just one experiment but in that experiment it measured treatment and genotype differences in the same way as leafy propagules. Observations on genotypes made by this method were highly correlated with data from live propagules with r equal to .969. This association was so close that both types of data would not be required under the conditions of this study. Rooted propagules were not descriptive of the changes taking place in the

propagule. Propagules growing in solid opaque media would have to be seriously disturbed to make this kind of observation. Experimental errors were incurred in collecting the data because of the relative sizes and number of roots. It was not attempted here but it would be very difficult to differentiate between live and dead roots.

Leaf lengths were determined in one experiment. This type of data had some desirable features. The recording of the data did not appreciably disturb the propagules so that repeated observations could be made during any given experiment. Genotype differences were detected at a significant level. However, the data were very tedious to obtain and subject to a rather serious possibility of error. Measurements were made from the top of the original propagule but this point was often difficult to establish. Consequently, judgment was frequently required as to the appropriate point from which to measure. Differences of opinion could occur and an error in measurement would result. The correlation with survival which was almost zero might have been increased had leaf areas been measured. However, this would have considerably reduced the scope of the study due to the extra time required to make area measurements. The use of this method was not repeated due to the lack of agreement with survival data.

The leaf counts data collected detected significant differences between treatments and between genotypes. The information derived from these data was significantly and positively associated with survival data when the data from all experiments were pooled. However, the r value of .639 was too low to have a predictive value. Leaf counts were a suitable criteria in that propagules were not disturbed by the collection of the data so that repeated observations could readily be made during an experiment. The data were moderately easy to collect though compiling sample totals consumed appreciable amounts of time. The chief disadvantage of the data arose from the difficulty involved in making pertinent inferences about the causes of differences recorded. This type of data has an additional drawback in that the leaves must be judged as either alive or dead. This same disadvantage is encountered in determining leafy propagules and leaf lengths data. In counting live propagules if a weak propagule had a dying leaf and the observer classified it as dead that would reduce the sample yield by one tenth the number of live propagules. However, the sample total of leaves counted would be influenced by only one fiftieth if the sample total for leaf counts was 50, which was close to the average. This criteria was not the most useful one studied because the data was tedious to obtain and did not agree closely with survival data.

The weights of leaves and the weights of roots were not significantly associated with survival in Experiment V. However, in that experiment data from the other criteria were not significantly associated with survival. Despite the lack of association with survival, leaf and root weights had desirable features. The information was gravimetric, the yields could be subjected to chemical analysis and only a moderate amount of labor was involved in obtaining sample yields. Provided that the propagules had been trimmed free of roots and leaves at the beginning of the experiment errors involved in clipping the new leaves and new roots from the propagules were not serious. But collection of the data would seriously disturb the propagules and it is debatable as to whether or not the propagules would live after repeated defoliation and/or root removal. In any event this type of data could not be collected as frequently as live propagules, or leaf and root counts. In view of the disadvantages of these criteria their general use could not be recommended. However, these two criteria might be very useful in finding the answer to a specific problem where production of leaves or roots *per se* was being investigated.

Harvest and increment weights were easily collected. These criteria showed that significant differences existed between genotypes and treatments. In Experiment VI

there was a close and highly significant association with survival though when the data were pooled for the appropriate experiments these criteria were not significantly associated with survival at the 5 percent level. Harvest weight had to be determined in order to determine increment weight and so the operational features did not differ appreciably from those involved in obtaining weight increment data. This data required that the samples be weighed before planting and this weight had to be subtracted from the harvest weight for each sample which required an additional calculation.

Weight increment had a number of important advantages over the other criteria. It produced gravimetric data concerning changes that occurred in the propagules during an experiment. This advantage also applied to leaf weights and root weights except that with these criteria no information was obtained, about changes that may have occurred during the experiment, in the culm or stem from which the propagule originated. Increment weight data were determined quickly and accurately on a sample basis. Since it was not concerned with viability as such and since the entire sample was weighed there was no opportunity for the types of bias to occur that might have occurred in a determination of the other criteria. The

propagules grown in water could be weighed repeatedly without undue interference to development. The labor involved in obtaining increment weight data was considerably less than the labor required for obtaining the other types of data with the exception of harvest weights and leafy propagules.

From an examination of the kinds of information which the various criteria provide there was no substitute for leafy propagules if an estimate of actual survival was required. This was indicated in Table 14 where the correlations from the pooled experiments for the other criteria with live propagules were all too low to have any predictive value.

There are several possible explanations for the wide differences in degree of association between leafy propagules and the criteria used to measure propagule growth. The individual experiments were carried out at different times of the year which would indicate the influence of season on this association. The propagules were prepared differently in the various experiments. Propagule lengths were varied and roots were either left on or cut off entirely. Propagules varied within and between genotypes in length, circumference and weight. Different growth media were used which might cause the associations to change. Since some of the

criteria were inter-related it may be that indirect association also changed.

It appeared that genotypes differed with respect to the various criteria and yet environment also influenced the propagules. Knowledge of the relative magnitude of genetic and environmental effects on propagule survival would permit an increase in efficiency of experimental design and more pertinent interpretation of experimental results when studying vegetative propagation.

Future Research

Through vegetative propagation phenotypic studies are made possible on individual genotypes. Large quantities of seed from different pollen sources can be obtained from a genotype for determination of the potential value of that genotype as a parent. Seed, in the quantities required for commercial use, could be obtained from two outstanding parents by this method. Greater use will be made of vegetative propagation of grasses and it is important that the method be made as efficient as possible.

This study has shown the effects of various media on propagule growth. To determine survival it was necessary to make counts of the living propagules. Increment weight provided convenient data on gravimetric changes within the propagules. However, during the course of the study

it became apparent that additional information would help to make more efficient use of the method. The influence of length, weight and age on propagule survival needs to be determined. A knowledge of the effects of temperatures, seasons, hormones and nutrients might reduce losses after propagation. An estimate of the heritability of survival would help to develop a greater understanding of the principles involved when propagules survive.

SUMMARY

A mixture of silt, sand and peat, vermiculite, distilled water and sharp sand were compared with silt as rooting media for propagation of tall fescue in a greenhouse study involving ten genotypes. Leaf lengths, leaf and root counts, leaf and root weights and harvest and increment weights were compared for association with survival. Propagules were grown standing in glass jars and compared with propagules held upright by paper towel supports. A sample size of ten propagules was used. Green weights were related to oven dry weights.

A sample size of ten propagules was found to be appropriate for the requirements of both statistical and experimental designs. The close association between green and oven dry weights showed that the variation arising from differences in dry matter content did not contribute appreciably to genotype and treatment effects.

Distilled water, without frequent or continual changing, was found to be satisfactory as a rooting medium. Rooting media interactions with genotypes were not detected. However, differences in the leaf and root counts produced by propagules grown in the various media, indicated that new procedures or treatments evolved in experiments using distilled water as the rooting

medium, should first be tested on an experimental scale with the rooting medium to be used in practice.

Leafy propagules were the most sensitive criteria for measuring survival. Other criteria were not correlated closely enough with leafy propagules to predict survival in all experiments. However, in experiments where propagules were markedly affected by treatments, increment weight was significantly correlated with live propagules at a level high enough for predictive purposes.

Increment weight data were very convenient to obtain. Gravimetric information was provided on changes that occurred in propagules. Repeated observations could be made on propagules growing in distilled water. The method used to obtain this data offered few opportunities for experimental error or personal bias. Additional details could be obtained by trimming the new growth from the propagules and weighing the sample yield of leaves or roots.

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