THE VIABILITY OF GRASS SEED UNDER WET TROPICAL CONDITIONS
WITH SPECIAL REFERENCE TO STORAGE, HARVESTING PERIOD
AND GENETICAL INHIBITION.

by

M.J. Russell, B.Sc. (Agric.), M.D.A.

Submitted in part fulfilment of the requirements
for the Diploma in Tropical Agriculture.

The Imperial College of Tropical Agriculture

TRINIDAD, B.W.I.

June, 1956.
ACKNOWLEDGEMENTS:

INTRODUCTION:

The viability of grass seed under wet tropical conditions .............................................. 1

SECTION I:

An experiment to measure the effect of moisture and humidity on the viability of Ixophorus unigetus seeds in storage ................................................................. 8

Results and Analysis ........................................................................................................... 11

Summary ............................................................................................................................... 18

SECTION II(A):

An investigation into the variation in viability of the seeds of some Trinidad grasses taken at intervals through the dry season ......................................................... 21

GRAPH: Germination of Mexican Grass seed harvested at monthly intervals ............ facing 22

GRAPH: Germination of Mexican Grass seed against period of storage ................. facing 22

SECTION II(B):

Germination tests on some of the seed samples in store in the lime box, Old Farm, I.C.T.A. .... 28

SECTION III:

Some cytogenetical investigations on certain Trinidad grasses .............................. 29

REFERENCES:

Introduction and Sections I & II ..................................................................................... 49

Section III ......................................................................................................................... 51

SECTION IV: (Not submitted for D.T.A.)

The use of tri-phenyl-tetrazolium chloride as a measure of grass seed viability .......... 53

References for Section IV ............................................................................................... 58.
ACKNOWLEDGMENTS

I am most grateful to the following for co-operation in the execution of my project and the preparation of my thesis:

To Mr. W. J. Badcock, my supervisor, for his constant help and encouragement.

To members of the Botany and Plant Pathology departments for their information and forbearance.

To Mr. Darsan of the Agriculture Department for his advice and co-operation.

To Driva Ali at Old Farm who collected all the monthly seed samples for me.

To Mr. G. Smith and the Meteorological Department for data used in Section II A, and to Mr. M. Breese and the Entomological Department for data used in Section III.
INTRODUCTION

THE VIABILITY OF GRASS SEED UNDER WET TROPICAL CONDITIONS

The whole question of the viability of grass, vegetable, tree and other commercially important seeds in the wet tropics has been under investigation for many years, at first sporadically as the need arose, but during recent years intensively, especially in certain centres such as Trinidad, Hawaii, South Africa, etcetera. In the Imperial College of Tropical Agriculture several post-graduate theses of recent years have included work on grass seed viability. (Tuley, 1955; Evans 1955; and Davies 1955).

Much of the earlier work and hence references refer to experiments with temperate grasses and American and Australian experiments deal with both temperate and tropical species. It is necessary to refer to this basic work as it often has considerable application to our specific problems. An important source of information in this category is "Twenty years of Seed Research" by Barton and Crocker.

In all the centres where research on seed viability in the wet tropics has been undertaken there is an ill-defined dry season, in Trinidad of 4-5 months duration, during which grasses set most or all of their seed and in the middle of which, generally, seed viability of samples is highest. (Dadey 1955).

However, seed is rarely used for propagation in the wet tropics and although this may be a reflection on the ubiquitous system of using silage grasses for fodder which are planted from sets, rather than leys or pastures planted from seed, it is more likely to be because few grasses do in fact set apparently viable seed in the wet tropics. If they do it is often impossible to harvest them satisfactorily in the field, and to store them without rapid deterioration. The regular supply of cheap, dependable seed of various major economic grasses, especially of low-growing, grazing types might well revolutionize farming practice in the tropics, often a most important policy in view of present world trends. It is therefore
of the utmost importance to tackle this problem objectively and systematically so that by presenting the difficulties and possibilities in a clear light practical solutions may be found.

The first limitations on flowering and seed production may well be said to be nutrition and environment. These are the concern of the agronomist and the farmer. The next limiting factor however, is genetics. In many grasses the genetical make up is such that no seed or seed which is sterile, is set due to homozygosity in a self-sterile community, e.g. Echinochloa pyramidalis in Trinidad (Davies 1955), or certain types of polyploidy, e.g. aneuploidy. This side of the problem is dealt with in detail in Section III of this thesis.

In many plants and especially in hybridisation the physiology of reproduction and embryo development is a limiting factor in seed production. It is known, for instance, that the length of the pollen tube is genetically defined in a species. In the case of hybridisation the length of the style in the female parent may be longer than the maximum length of the pollen tube of the male and so fertilisation will not be effected. In cross fertilised species pollinating agents may be absent. The occurrence of genetical incompatibility between strains or ecotypes often leads to the formation of empty or aborted seed. This is, strictly, a genetical problem. There are many physiological processes in the formation of a viable seed from an unfertilised ovule which can be upset thus resulting in nonviable seed. This is a field which has hardly been touched in grasses and might prove rewarding in later studies.

If the grass manages to set viable seed the next problem is to harvest it. Natural hazards such as weather and pests are too well appreciated to need mentioning here. Certain standard behaviour such as premature shedding of seed needs special attention. It has been found in Trinidad with Botriochloa pertusa, and Setaria sphacelata that cutting and sheaving the culms and allowing them to mature in the stock gives a far greater yield of mature seed and results in very little premature shedding in the case of B. pertusa.
S. sphacelata ripens unevenly so that this method allows all seed to come to maturity (Davies 1955).

Paspalum dilatatum has been shown to shed its viable seed early but retain on the panicale empty florets. Harvesting with a shaker or shaker-suction harvester collects a high percentage of sound seed. The cause of the poor seed setting in Dallis grass is due to photoperiodicity. W. E. Knight in 1955 found that a 14-hour day with temperatures at 70-80°F. and night temperature of 65-70°F. gave a larger number of panicles and a high percentage of viable seed, while a day length of 12 hours or a lower night temperature considerably increased the percentage of light seed produced and decreased the number of panicles formed.

The use of defoliants to induce the seed to ripen evenly has been tried (Dadey 1955) but its application in the field is rather specific.

The period at which flowering grasses set most viable seed has already been mentioned. It is necessary to determine this period for each species of grass, and its application in conjunction with seed dormancy will be important to the farmer growing and using his own seed.

Many successfully harvested seeds, especially those harvested primitively by hand-threshing, etcetera, present a considerable problem in cleaning. Although it has been established that to store seeds for maximum viability and longevity they must be well matured, carefully harvested, thoroughly dried and stored in an unthreshed condition under cool, dry conditions, (Haferkamp et al 1953), nevertheless many grasses such as Andropogon gayanus and Hyperrhena rufa must be cleaned of a large excess of trash which would otherwise waste storage space, encourage pests and interfere with sowing as well as making accurate preliminary germination counts impossible. The use of a hammer mill with appropriate screens is described as effective for many grasses by Schwim 1952 (Davies 1955). Primitive cleaning methods such as winnowing are hardly applicable to grass seeds because of their small size and light weight, while
similar considerations often apply to threshing machines of the conventional type.

Storage of grass seed in the wet tropics is an enormous problem. In the first place full investigations into the ideal conditions of storage for grass seeds of the wet tropics, must be completed. Recommendations, especially to small farmers, can then be made on the type of stores to be used, the maximum period of storage of different species, pretreatments and other factors. It is known generally that the ideal conditions for storage of grass seed are coolness and dryness (Haferkamp et. al 1953, Barton and Crocker, and many others) and that moisture has a more detrimental effect than temperature under the normal range of conditions (Akamine, E.K. 1943).

Experiments on the effect of humidity and temperature in storage on grass seeds have been carried out by several workers notably Akamine 1943, Bass 1953, and the conditions just stated apply generally but more work needs to be done on individual species. An account of such an experiment on *Ixophorus unisetus* continued from that laid down by Davies in 1955 is given in Section I, together with results to date.

The storage of seed under hermetically sealed conditions, which is being investigated for grain in France at the present time, seems to depend in efficacy entirely on the species concerned. *Kochia indica* at 30°C keeps better under hermetically sealed conditions, whereas *Phleum pratense* loses germination, compared with the control, under these conditions (Shenberger 1952). It seems that there is a minimum moisture content limiting retention of viability in sealed chamber storage of seeds, as well as a maximum.

Finally the germination capacity of grass seed is very considerably affected by dormancy. Grass seeds in most cases show at least a weak dormancy period, viz. *Ixophorus unisetus*, while others such as the *Pennisetum* will only germinate under certain conditions of environment, and *Paspalum dilatatum* and *P. notatum* rarely germinate under laboratory conditions and only very slowly under field conditions.
There are a variety of reasons for grass seed dormancy, at least it would be better to say that grass seeds achieve dormancy, which under natural conditions will tend to establish and spread the species, by a variety of means. Crocker has listed these mechanisms under eight general headings (Akamine 194h).

(1) The presence of enclosing structures that hinder maximum expansion of the seed.

(2) The presence of structures that interfere with gaseous exchange between the embryo and the environment.

(3) Dormancy of the embryo (Physiological).

(4) Need within the seed for stimulators of respiratory and nutritive activity.

(5) The presence of inhibitors produced by the seed hulls.

(6) Immaturity of the embryos.

(7) Inability of the seed to absorb water.

(8) Secondary dormancy.

A large number of experiments and tests involving many techniques have been applied to the breaking of seed dormancy, but unless these mechanisms are borne clearly in mind, such investigations will be fruitless or at the best give an answer which is not fully understood.

In Hawaii Akamine selected twelve native grasses whose normal germination capacity in the laboratory was very low and investigated them in order to describe their dormancy under Crocker's categories (194h). He was able to show that several of these categories applied to the dormancy of his twelve grasses.

Methods which have been used to break dormancy in various seeds include the use of alternating temperatures before germination (Harrington 1923, Morinaga 1926, Akamine 194h, Drake 1951). This affects categories 2, 3, 4 and 8, and 1 to a certain extent, which accounts for its wide application. Categories 1, 2, 5 and 7 are obviously broken by scarifying or dehulling the seed, a method very commonly used. Categories 3, 4, 6 and 8 are often affected by
reducing or increasing the oxygen or the carbon dioxide potential about the seed (Morinaga 1926). Category 5 can be dealt with by providing the germinating seeds with an absorptive medium which absorbs the inhibitor produced.

Under category 6, etc., the use of nutritive solutions to initiate germination is most efficacious. Those used generally contain nitrogen and dilute solutions of potassium nitrate are the most usual (Toole, E.H. and Toole, V.K., 1939-h1), although other nitrogen-bearing chemicals such as NaNO₃, NaNΟ₂, and HNO₃ in dilute concentrations have been used to induce germination (Morinaga 1926). Thiourea has been used to break secondary dormancy in pulses (Phillips, et al. 1951). Concentrated sulphuric acid is often used as a scarifier, more especially on certain vegetable seeds, and to destroy the fascicles of *Pennisetum* spp. which secrete germination inhibitors. The length of time over which the treatment is applied is closely defined for each species. A minute's over-treatment may destroy the sample. Simple soaking in water is often effective in breaking dormancy. The length of time involved and the temperature vary from species to species but 2 days at 30°C is a useful average. The process of after-ripening in warm conditions may often break secondary dormancy. The treatment is sometimes applied after hulling, e.g. *Urochloa pullulans* (Akamine 1941) but may also be applied direct to some seeds for shorter times and at higher temperatures, e.g. *Chloris gayana* at 85°C (Dadey 1955).

Many of these techniques and many more of use on occasion or undeveloped as yet, are of general application only in the laboratory but the most important can be easily adapted to field conditions.

In the laboratory the system of germination of seeds in an optimum medium, e.g. moist filter-paper, moist sand, etc., is often ineffective as a gauge of seed viability. Germination capacity is perhaps the correct term for results obtained for a sample at any given time by this method. The germination capacity of a given sample at a given time is its viability less its dormancy. The viability is determined by a few methods, the most promising of which at the moment are the embryo staining techniques based on
enzyme activity within the living embryos. The particular enzyme system which reacts most favourably with certain chemicals to indicate those parts of the embryo which are alive is the dehydrase system. Lakon in 1927 employed this using sodium biselenite as a stain. In 1941 Kuhn and Jerchel used triphenyl tetrazolium chloride which is reduced by dehydrase to the red formosan. This was found to have distinct advantages over the latter substance and in 1951 Lakon used it and evolved a topographical staining method for testing the viability of various seeds, chiefly cereals and pulses. The method as applied to grasses presents certain handling difficulties but is well worth investigation. Davies worked on it in 1955 chiefly on _Ixophorus unisetus_ with some success.
SECTION I.

EXPERIMENT TO MEASURE THE EFFECT OF MOISTURE AND HUMIDITY ON THE VIABILITY OF IXOPHORUS UNISESUS SEEDS IN STORAGE.

The difficulties of storing grass seed in the wet tropics were pointed out in the introduction. It is generally assumed, as Haferkamp et al. assumed in a paper on "The Relation of Age of Seed to Germination and Longevity", that the best method of storage of most seeds includes low and constant temperature and moisture and a lack of oxygen, similar to the conditions pertaining to seeds deep in the soil.

In 1943 E.K. Akamine in Hawaii carried out an experiment on the changing viability of some legumes and cereals and the grass Pennisetum ciliare stored under eight different humidities at room temperature. Relative humidities of 15, 30, 45, 60, 75 and 90% were achieved by enclosing the seeds over sulphuric acid of varying concentrations. The other two treatments were to enclose the seed in an air-tight container and to store the seeds in the open in the laboratory.

Germination tests, in the soil because of an inhibitor in the fascicles, were made every two months for a period of 18 months and subsequently every six months for 7 years. The grass seed maintained its viability for 2½ years at 15% R.H., 2 years at 30% R.H., and 1½ years at 45% R.H. and only dropped to half that value by the end of the seven years. At humidities above this the germination maintained its initial level for only 3 to 5 months and then dropped off rapidly to zero before the end of the experiment. The treatments in air-tight and open conditions maintained their germination capacity a little longer than the 60, 75 and 90% treatments, but not as well as the first three treatments and there was little difference between the two treatments.

Davies set up the following experiment in 1955. The seeds were harvested on the 29th January and their germination capacity determined. It was found to be 85%. They were then stored, as described
below, on 15th February. The first test, it must be assumed, for Davies mentions no date, was on the 20th April. Davies gives the results of this test and their statistical analysis in his thesis.

The next test was carried out by Mr. Darsan on the 20th July. The results of this were handed to the author who did the third and fourth tests on October 18th and January 19th 1956, respectively.

Complete results of the second, third and fourth tests are appended. The fifth test will take place in May and the sixth in September, and the seventh in January 1957, making three 4 month tests for the second year.

**Experimental Procedure**

The experiment was laid out as a factorial design in which seeds at six different moisture contents are stored under four temperature treatments. The moisture conditions are 0.5%, 2%, 6%, 12%, 18.5% and no control. The temperature treatments are 20°F., 45°F., 60°F., and room temperature which varies between 68°F and 86°F., with an average of 80°F. There are two replications.

The seeds were weighed out in one gramme samples and then put in test tubes over the following mixtures to maintain the required moisture contents.

- 0.5% 10 gms. CaCl₂ passing a 10-20 mesh sieve.
- 2.0% 2 gms. CaCl₂, 8 gms. Na₂CO₃ anhydrous
- 6.0% 8 gms. Na₂CO₃ anhydrous, 2 gms. Na₂CO₃·10H₂O
- 12.0% 1 gms. Na₂CO₃ anhydrous, 6 gms. Na₂CO₃·10H₂O
- 18.5% As at harvest stored in the tube.

On the appropriate date the specimens are brought out from the Port-of-Spain cold store to the laboratory. The seeds are counted out onto blotting paper in butter dishes, fifty to a dish, and as there are twenty-four treatments replicated twice, there are forty-eight dishes. The seeds are covered with a second piece of filter paper on which is written in pencil an identification number and letter. The blotting paper is then soaked in water drawn freshly from the tap and pipetted on until the papers are thoroughly wet but not inundated. The whole is
then covered by an inverted butter dish whose serrated edges interlock fairly closely with the basal one and thus inhibit the entry of dust and spores. The dishes need to be watered roughly every forty-eight hours. A simple test of water requirement is to hold the dish on its side. If water drips out it is saturated, but if not a short squirt from the pipette is given. Under these conditions there was not at any time any trouble with mould.

Germination counts were made on the sixth day, the tenth day and the twentieth day and the remainder then discarded. Seedlings showing a green first leaf as well as a root were removed and counted. At the end of the period those seedlings which had aborted, i.e., produced a root but no green leaf, were noted down but not counted in with the germinated seed numbers.

In counting out seeds into the germination dishes care was taken to exclude all weed seeds and all Ixophorus unisetus seed which were judged not to be good seed, i.e., would not have been potentially viable before storage. Since the purpose of the experiment is to measure the effect of different storage humidities and temperatures on the viability of the grass seeds, an error of dead seeds should not be introduced, especially as the numbers of such seeds in each dish may vary from five to twenty seeds on average. A more accurate method of eliminating this error would have been, of course, to have had many replications and thus standardise the error. Like most ideals, however, this could not be achieved. The alternative, using two replications of only fifty seeds each, is to apply a personal method to reduce this error, which by its severity, may achieve a fairly high degree of standardisation.

However, Davies probably did not apply this refinement - he makes no mention of it - which would account for the latter two sets of results being higher than the preceding set. If this is so then it may be necessary to apply a correction factor to his figures to achieve a correlation of the results in order to assess the effects of humidity and temperature during storage on viable grass seeds.
This correction factor might be obtained by using my selection method on 1000 grass seeds taken at random from a large sample of *Echophorus unisetus* (there being none of the original sample left).

It had been hoped to check the humidity of seed in the various tubes but this is now impossible as there are not enough seeds left. In fact, there may not be enough to complete the third and fourth year trials (three 6-month trials for the second year, two 6-month trials for the third year and a final one at the end of the fourth year).

The results to date, their statistical analyses, and a summary of trends, follow herewith:

**EXPERIMENT ON 20TH JULY, 1955**

**REPLICATION 1.**

<table>
<thead>
<tr>
<th>Moistures</th>
<th>Temperatures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>m₁</td>
<td>43.9</td>
</tr>
<tr>
<td>m₂</td>
<td>40.4</td>
</tr>
<tr>
<td>m₃</td>
<td>39.2</td>
</tr>
<tr>
<td>m₄</td>
<td>46.1</td>
</tr>
<tr>
<td>m₅</td>
<td>39.2</td>
</tr>
<tr>
<td>m₆</td>
<td>23.6</td>
</tr>
</tbody>
</table>

**REPLICATION 2.**

<table>
<thead>
<tr>
<th>Moistures</th>
<th>Temperatures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>m₁</td>
<td>35.7</td>
</tr>
<tr>
<td>m₂</td>
<td>35.7</td>
</tr>
<tr>
<td>m₃</td>
<td>45.0</td>
</tr>
<tr>
<td>m₄</td>
<td>40.4</td>
</tr>
<tr>
<td>m₅</td>
<td>38.1</td>
</tr>
<tr>
<td>m₆</td>
<td>20.3</td>
</tr>
</tbody>
</table>

Total \(1010^2 = 1020100\)
REPLICATION 1~2

Moistures | Temperatures
--- | ---
| t1 | t2 | t3 | t4 |
m1 | 8.2 | 0 | 3.5 | 5.8 |
m2 | 4.7 | 2.4 | 1.1 | 3.5 |
m3 | 5.8 | 4.6 | 2.3 | 1.2 |
m4 | 5.7 | 1.2 | 3.5 | 1.2 |
m5 | 1.1 | 13.2 | 3.5 | 7.3 |
m6 | 3.3 | 11.1 | 0 | 8.1 |

G = 1989.5 Correction Factor = $\frac{\sigma^2}{\mu}$ = 821.60,62

REPLICATION 1+2

Moistures | Temperatures
--- | ---
<table>
<thead>
<tr>
<th>t1</th>
<th>t2</th>
<th>t3</th>
<th>t4</th>
<th>Totals</th>
<th>Means</th>
</tr>
</thead>
</table>
m1 | 79.6 | 73.8 | 79.7 | 95.8 | 328.9 | 111.1 |
m2 | 76.1 | 80.8 | 77.3 | 98.1 | 332.3 | 111.5 |
m3 | 84.2 | 85.4 | 87.7 | 100.4 | 357.7 | 119.7 |
m4 | 36.5 | 75.0 | 74.9 | 79.6 | 326.0 | 108.5 |
m5 | 77.3 | 74.6 | 79.7 | 108.4 | 340.5 | 113.6 |
m6 | 43.9 | 69.7 | 116.8 | 81.9 | 314.1 | 104.7 |
Totals | 447.6 | 459.3 | 517.9 | 564.7 | 1989.5 |
Means | 37.3 | 38.3 | 43.2 | 47.1 |

ANALYSIS OF VARIANCE:

<table>
<thead>
<tr>
<th>Source</th>
<th>D.F.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>1</td>
<td>19.38</td>
<td>19.38</td>
<td>1.34</td>
</tr>
<tr>
<td>Temperature</td>
<td>3</td>
<td>740.10</td>
<td>246.70</td>
<td>17.05 ***</td>
</tr>
<tr>
<td>Moisture</td>
<td>5</td>
<td>164.73</td>
<td>32.95</td>
<td>2.28</td>
</tr>
<tr>
<td>Interaction</td>
<td>15</td>
<td>1509.23</td>
<td>100.62</td>
<td>6.95 ***</td>
</tr>
<tr>
<td>Error</td>
<td>23</td>
<td>332.77</td>
<td>14.47</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>2766.21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The Standard Error of each block is $1h.h7$

$$= \frac{100 \times 3.80k \times h8}{1989.5} = 9.18\%$$

of the General Mean

S.E. of a single dish = $1h.h7$

S.E. of the mean of 12 dishes = $\frac{1h.h7}{12}$

S.E. of the difference of two means of 12 dishes = $\sqrt{\frac{1h.h7 \times 2}{12}} = 1.55$

When $n = 23$, $t$ at 5% = 2.069, $t$ at 1% = 2.807, $t$ at 0.1% = 3.767

'. The Significant Difference between two means of 12 dishes

at 5% = 1.55 x 2.069 = 3.21

at 1% = 1.55 x 2.807 = 4.45

at 0.1% = 1.55 x 3.767 = 5.84

From this:-

$t_4 > t_1 \& t_2 \text{xxx}, t_3 > t_1 \text{xxx}$

$t_3 > t_2 \text{xx}$

$t_4 > t_3 \text{x}$

S.E. of the Difference of 2 means of 8 dishes = $\sqrt{\frac{1h.h7 \times 2}{8}} = 1.90$

'. The Significant Difference between two means of 8 dishes

at 5% = 1.90 x 2.069 = 3.93

at 1% = 1.90 x 2.807 = 5.33

at 0.1% = 1.90 x 3.767 = 7.16

From this:-

$m_3 > m_6 \text{xx}$

$m_3 > m_4 \text{x}$
EXPERIMENT ON 18TH OCTOBER, 1955

**REPLICATION 1**

<table>
<thead>
<tr>
<th>Moisutes</th>
<th>$t_1$</th>
<th>$t_2$</th>
<th>$t_3$</th>
<th>$t_4$</th>
<th>$t_5$</th>
<th>$t_6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m_1$</td>
<td>8.4</td>
<td>36.9</td>
<td>51.9</td>
<td>63.4</td>
<td>47.3</td>
<td>36.9</td>
</tr>
<tr>
<td>$m_2$</td>
<td>42.7</td>
<td>40.4</td>
<td>47.3</td>
<td>47.3</td>
<td>39.2</td>
<td>42.7</td>
</tr>
<tr>
<td>$m_3$</td>
<td>42.7</td>
<td>49.6</td>
<td>53.1</td>
<td>54.3</td>
<td>36.9</td>
<td>50.8</td>
</tr>
<tr>
<td>$m_4$</td>
<td>55.0</td>
<td>55.0</td>
<td>55.0</td>
<td>55.0</td>
<td>55.0</td>
<td>55.0</td>
</tr>
<tr>
<td>$m_5$</td>
<td>53.9</td>
<td>52.7</td>
<td>51.9</td>
<td>51.9</td>
<td>55.6</td>
<td>54.4</td>
</tr>
<tr>
<td>$m_6$</td>
<td>0</td>
<td>66.1</td>
<td>33.2</td>
<td>0</td>
<td>0</td>
<td>47.3</td>
</tr>
</tbody>
</table>

Total $981.72^2 = 969631.09$

**REPLICATION 2**

<table>
<thead>
<tr>
<th>Moisutes</th>
<th>$t_1$</th>
<th>$t_2$</th>
<th>$t_3$</th>
<th>$t_4$</th>
<th>$t_5$</th>
<th>$t_6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m_1$</td>
<td>1.1</td>
<td>0</td>
<td>5.8</td>
<td>5.3</td>
<td>5.3</td>
<td>5.3</td>
</tr>
<tr>
<td>$m_2$</td>
<td>3.5</td>
<td>2.3</td>
<td>0</td>
<td>9.5</td>
<td>9.5</td>
<td>9.5</td>
</tr>
<tr>
<td>$m_3$</td>
<td>5.8</td>
<td>1.2</td>
<td>7.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$m_4$</td>
<td>3.4</td>
<td>3.4</td>
<td>8.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$m_5$</td>
<td>1.1</td>
<td>12.9</td>
<td>3.5</td>
<td>23.8</td>
<td>23.8</td>
<td>23.8</td>
</tr>
<tr>
<td>$m_6$</td>
<td>0</td>
<td>1.2</td>
<td>4.9</td>
<td>11.5</td>
<td>11.5</td>
<td>11.5</td>
</tr>
</tbody>
</table>

Total $981.62^2 = 963538.56$

**REPLICATION 1 + 2 = INTERACTION TABLE**

<table>
<thead>
<tr>
<th>Moisutes</th>
<th>$t_1$</th>
<th>$t_2$</th>
<th>$t_3$</th>
<th>$t_4$</th>
<th>Totals</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m_1$</td>
<td>95.7</td>
<td>73.8</td>
<td>98.0</td>
<td>121.5</td>
<td>389.0</td>
<td>84.6</td>
</tr>
<tr>
<td>$m_2$</td>
<td>81.9</td>
<td>83.1</td>
<td>94.6</td>
<td>104.1</td>
<td>363.7</td>
<td>85.4</td>
</tr>
<tr>
<td>$m_3$</td>
<td>79.6</td>
<td>100.4</td>
<td>99.2</td>
<td>108.6</td>
<td>387.8</td>
<td>85.8</td>
</tr>
<tr>
<td>$m_4$</td>
<td>93.4</td>
<td>86.6</td>
<td>98.1</td>
<td>111.0</td>
<td>322.1</td>
<td>85.5</td>
</tr>
<tr>
<td>$m_5$</td>
<td>88.9</td>
<td>98.3</td>
<td>100.3</td>
<td>104.0</td>
<td>327.5</td>
<td>83.0</td>
</tr>
<tr>
<td>$m_6$</td>
<td>0</td>
<td>93.4</td>
<td>71.3</td>
<td>11.5</td>
<td>176.2</td>
<td>29.0</td>
</tr>
</tbody>
</table>

Total $439.5$ $561.5$ $561.5$ $429.7$ $1966.3$ $329.0$

Means $36.6$ $64.6$ $66.8$ $35.8$
## Analysis of Variance

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replications</td>
<td>1</td>
<td>0.20</td>
<td>0.20</td>
<td>13.7</td>
</tr>
<tr>
<td>Temperatures</td>
<td>3</td>
<td>111.33</td>
<td>371.33</td>
<td>21.19</td>
</tr>
<tr>
<td>Moistures</td>
<td>5</td>
<td>3956.17</td>
<td>791.23</td>
<td>12.14</td>
</tr>
<tr>
<td>Interaction</td>
<td>15</td>
<td>5066.03</td>
<td>337.74</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>23</td>
<td>623.57</td>
<td>27.11</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>47</td>
<td>10759.97</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The Standard Error of each dish is 27.11.

\[
\text{Coefficient of Variation} = \frac{100 \times 5.207 \times 4.8}{1966.3} = 12.71\% \text{ of the General Mean}
\]

The Standard Difference of 2 means of 12 dishes is:

\[
\sqrt{\frac{27.11 \times 2}{12}} = 2.13
\]

When \( n = 23 \) at 5\% = 2.069, at 1\% = 2.807, at 0.1\% = 3.767.

- The Significant Difference between 2 means of 12 dishes
  - at 5\% = 2.13 \times 2.069 = 4.41
  - at 1\% = 2.13 \times 2.807 = 5.98
  - at 0.1\% = 2.13 \times 3.767 = 8.02

From this:

\[
t_3 > t_1 \text{ and } t_4 \text{ XXX, } t_2 > t_4 \text{ XXX }
\]

S.E. of the difference of 2 means of 8 dishes is:

\[
\sqrt{\frac{27.11 \times 2}{8}} = 2.60
\]

- The Significant Difference between 2 means of 8 dishes
  - at 5\% = 2.60 \times 2.069 = 5.38
  - at 1\% = 2.60 \times 2.807 = 7.30
  - at 0.1\% = 2.60 \times 3.767 = 9.79

From this:

\[
m_6 < m_1, m_2, m_3, m_4, m_5 \text{ XXX, } m_1 > m_4, m_5 \text{ XX, } m_3 > m_4 \text{ & } m_5 \text{ XX}
\]
EXPERIMENT ON 19TH JANUARY, 1956.

### Replication 1

<table>
<thead>
<tr>
<th>Moistures</th>
<th>( t_1 )</th>
<th>( t_2 )</th>
<th>( t_3 )</th>
<th>( t_4 )</th>
<th>( t_1 )</th>
<th>( t_2 )</th>
<th>( t_3 )</th>
<th>( t_4 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( m_1 )</td>
<td>90.6</td>
<td>54.9</td>
<td>55.6</td>
<td>59.3</td>
<td>92.7</td>
<td>43.9</td>
<td>54.3</td>
<td>60.7</td>
</tr>
<tr>
<td>( m_2 )</td>
<td>47.3</td>
<td>42.0</td>
<td>49.6</td>
<td>34.4</td>
<td>49.6</td>
<td>51.9</td>
<td>54.3</td>
<td>63.4</td>
</tr>
<tr>
<td>( m_3 )</td>
<td>38.1</td>
<td>47.3</td>
<td>56.8</td>
<td>61.9</td>
<td>40.4</td>
<td>51.3</td>
<td>62.0</td>
<td>55.6</td>
</tr>
<tr>
<td>( m_4 )</td>
<td>53.9</td>
<td>47.3</td>
<td>54.3</td>
<td>11.5</td>
<td>43.9</td>
<td>51.3</td>
<td>55.6</td>
<td>0</td>
</tr>
<tr>
<td>( m_5 )</td>
<td>53.1</td>
<td>49.6</td>
<td>55.6</td>
<td>0</td>
<td>60.7</td>
<td>51.3</td>
<td>35.7</td>
<td>0</td>
</tr>
<tr>
<td>( m_6 )</td>
<td>0</td>
<td>53.1</td>
<td>38.1</td>
<td>0</td>
<td>0</td>
<td>56.8</td>
<td>28.0</td>
<td>0</td>
</tr>
</tbody>
</table>

Total: 993.7

### Replication 2

<table>
<thead>
<tr>
<th>Moistures</th>
<th>( t_1 )</th>
<th>( t_2 )</th>
<th>( t_3 )</th>
<th>( t_4 )</th>
<th>( t_1 )</th>
<th>( t_2 )</th>
<th>( t_3 )</th>
<th>( t_4 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( m_1 )</td>
<td>2.3</td>
<td>8.0</td>
<td>1.3</td>
<td>1.0</td>
<td>2.3</td>
<td>10.3</td>
<td>4.7</td>
<td>29.0</td>
</tr>
<tr>
<td>( m_2 )</td>
<td>2.3</td>
<td>10.3</td>
<td>4.7</td>
<td>29.0</td>
<td>2.3</td>
<td>7.0</td>
<td>5.2</td>
<td>9.3</td>
</tr>
<tr>
<td>( m_3 )</td>
<td>0</td>
<td>7.0</td>
<td>1.3</td>
<td>11.5</td>
<td>0</td>
<td>3.7</td>
<td>10.1</td>
<td>0</td>
</tr>
</tbody>
</table>

Total: 898.0

### Interaction Table

<table>
<thead>
<tr>
<th>Moistures</th>
<th>( t_1 )</th>
<th>( t_2 )</th>
<th>( t_3 )</th>
<th>( t_4 )</th>
<th>Totals</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>( m_1 )</td>
<td>83.1</td>
<td>95.8</td>
<td>109.9</td>
<td>120.0</td>
<td>408.8</td>
<td>51.1</td>
</tr>
<tr>
<td>( m_2 )</td>
<td>96.9</td>
<td>93.5</td>
<td>103.9</td>
<td>97.8</td>
<td>392.1</td>
<td>49.0</td>
</tr>
<tr>
<td>( m_3 )</td>
<td>78.5</td>
<td>101.6</td>
<td>118.8</td>
<td>120.5</td>
<td>318.8</td>
<td>52.4</td>
</tr>
<tr>
<td>( m_4 )</td>
<td>87.8</td>
<td>101.6</td>
<td>109.9</td>
<td>11.5</td>
<td>310.8</td>
<td>38.9</td>
</tr>
<tr>
<td>( m_5 )</td>
<td>113.8</td>
<td>103.9</td>
<td>91.3</td>
<td>0</td>
<td>309.0</td>
<td>38.6</td>
</tr>
<tr>
<td>( m_6 )</td>
<td>0</td>
<td>109.9</td>
<td>66.4</td>
<td>0</td>
<td>176.0</td>
<td>22.0</td>
</tr>
</tbody>
</table>

Total: 2016.1

Means: 38.3 50.5 50.0 29.2 168.0

G = 2016.1

C.F. = \( \frac{2016.1^2}{18} \) = 88680.4

\( \text{Total} = 1022.4 \)
### Analysis of Variance

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replications</td>
<td>1</td>
<td>17.16</td>
<td>17.16</td>
<td>29.63 XXX</td>
</tr>
<tr>
<td>Temperatures</td>
<td>3</td>
<td>3780.58</td>
<td>1293.59</td>
<td>21.93 XXX</td>
</tr>
<tr>
<td>Moistures</td>
<td>5</td>
<td>5295.83</td>
<td>1059.17</td>
<td>13.66 XXX</td>
</tr>
<tr>
<td>Interaction</td>
<td>15</td>
<td>871.14</td>
<td>58.079</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>23</td>
<td>977.70</td>
<td>h2.51</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>18785.11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The Standard Error of each dish is **h2.51**

\[ \text{Coefficient of Variation} = \frac{100 \times 6.52 \times h2.51}{2016.1} = 15.5\% \]

The S.E. of the difference of 2 means of 12 dishes = \[ \frac{h2.51 \times 2}{\sqrt{12}} \]

When \( n = 23 \), \( t \) at 5\% = 2.069, \( t \) at 1\% = 2.807, \( t \) at 0.1\% = 3.767

\[ \text{The Significant Difference between 2 means of 12 dishes} \]

- at 5\% = 2.66 \times 2.069 = 5.50
- at 1\% = 2.66 \times 2.807 = 7.47
- at 0.1\% = 2.66 \times 3.767 = 10.02

From this:
\[ t_3 > t_1 & t_2 \text{ XXX, } t_2 > t_1 & t_3 \text{ XXX} \]
\[ t_1 > t_3 \text{ XX} \]

The S.E. of the difference of 2 means of 8 dishes = \[ \frac{h2.51 \times 2}{\sqrt{8}} \]

\[ = \frac{3.26}{\sqrt{8}} \]

\[ \text{The Significant Difference between two means of 8 dishes} \]

- at 5\% = 3.26 \times 2.069 = 6.74
- at 1\% = 3.26 \times 2.807 = 9.15
- at 0.1\% = 3.26 \times 3.767 = 12.28

From this:
\[ m_6 < m_1 & m_2 \text{ & } m_3 \text{ & } m_4 \text{ & } m_5 \text{ XXX} \]
\[ m_3 > m_4 \text{ & } m_5 \text{ XXX, } m_1 > m_5 \text{ XXX} \]
\[ m_1 > m_4 \text{ XX, } m_2 > m_4 \text{ & } m_5 \text{ XX} \]
SUMMARY OF RESULTS

Moistures

<table>
<thead>
<tr>
<th>Moisture</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>m₁</td>
<td>0.5%</td>
</tr>
<tr>
<td>m₂</td>
<td>2.0%</td>
</tr>
<tr>
<td>m₃</td>
<td>6.0%</td>
</tr>
<tr>
<td>m₄</td>
<td>12.0%</td>
</tr>
<tr>
<td>m₅</td>
<td>18.5%</td>
</tr>
<tr>
<td>m₆</td>
<td>No control viz. 70 - 100% R.H.</td>
</tr>
</tbody>
</table>

Temperatures

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>t₁</td>
<td>20°F</td>
</tr>
<tr>
<td>t₂</td>
<td>45°F</td>
</tr>
<tr>
<td>t₃</td>
<td>60°F</td>
</tr>
<tr>
<td>t₄</td>
<td>Room temperature viz. 70-85°F.</td>
</tr>
</tbody>
</table>

20TH APRIL, 1955 - 1ST EXPERIMENT (DAVIES 1955).

Moisture and Interaction treatments both gave highly significant differences, but the temperature treatments gave no significant differences.

In the moisture totals the 6%, 2% and 0.5% treatments were significantly better (higher germination) than the 12% treatment at the 1% level, while the 12% treatment was significantly better at the 1% level than the 18.5% and no Control treatments between which there was no significant difference. There was also no significant difference between the 0.5%, 2.0% and 6.0% treatments.

20TH JULY, 1955 - 2ND EXPERIMENT *

While interaction remained highly significant, there was an interesting reversal between temperature treatment which is now highly significant, and moisture treatment which no longer had a significant effect.

In the temperature means the room temperature treatment was significantly better than the 20°F and the 45°F at the 5% level and better than the 60°F treatment at the 1% level. The 60°F treatment was better than the 20°F at the 5% level and better than the 45°F treatment at the 0.1% level. There was therefore a clear and not unexpected gradation from the highest to the lowest temperature treatment.

In the moisture means the 6% treatment was significantly better than the 'no control' treatment at the 1% level, and the 12% treatment at the 0.1% level, but there were no other significant differences.

* Experiment undertaken by Mr. Darsan, Agriculture Department.
18TH OCTOBER, 1955 - 3RD EXPERIMENT

All three treatments, temperature, moisture and interaction are now highly significant.

In the temperature means the 60°F treatment gave the highest germination, being significantly better than the 20°F and the room temperature treatments at the 5% level. The 45°F treatment gave the second highest results and was significantly better than the room temperature treatment, also at the 5% level, and better than the 20°F treatment at the 1% level. This shift in germinability at 6 months from the room temperature to 60°F - 45°F range is most important.

In the moisture means the highest treatment 'no control' showed a complete collapse, there being several complete failures in germination, and this mean was significantly poorer than all the other treatments at the 5% level. Apart from this the 0.5% and 6.0% treatments were significantly better than the 1% and 18.5% treatments, and there was no significant difference between the 0.5%, 2.0% and 6.0% treatments, although the mean for 2.0% was lower and showed an insignificantly higher value than the two high moisture treatments. In comparison with the previous experiment the trend of maintained germinability is moving more definitely into the low moisture treatments area.

19TH JANUARY, 1956 - 4TH EXPERIMENT

Moisture, temperature and interaction treatments were still all highly significant.

In the temperature means the 45°F - 60°F range was still optimum for germination and the 60°F and 45°F treatments were still significantly better than the 20°F and room temperature treatments at the 5% level. The 20°F treatment, however, was now significantly better at the 1% level than the room temperature treatment - a most interesting development.

In the moisture means the 'no control' treatment maintained its low position as expected, being significantly poorer than all
the other treatments at the 5% level. The 6% treatment now showed the best germinability, being significantly better than the 12% and 18.5% treatments at the 5% level, while the 0.5% treatment was significantly better than the 18.5% treatment at the 5% level and better than the 12.0% treatment at the 1% level, and the 2.0% treatment was better than the 12.0% and 18.5% treatments at the 1% level. These three low moisture treatments have maintained their germinability over the course of the year as the higher moisture treatments have lost their germinability. The trend shows clearly and it is perhaps a little surprising that the 2% treatment continues to lag a little behind the other two. It may be that this treatment at 45°F - 60°F most closely resembles the conditions met within the soil if the seed falls in the natural state, and the lag may therefore represent a dormancy.

It will be interesting to see when the 12.0% and 18.5% treatments show some significant difference since in temperate regions 12.0% moisture is considered to be just possible for storing grain and other seeds for 6 months, while it is considered that 18.5% of moisture in stored seeds is court ing disaster.

**SOME GENERAL INTERPRETATIONS**

The most important thing to note from the figures is that despite the 0.5 - 6% moisture and 45°F - 60°F temperature block being significantly the optimum for seed storage, the 0.5 - 6% moisture treatments at room temperature give the highest germination on average throughout the experiment. This is of considerable practical importance. It means that under Trinidad conditions the practical farmer can store Mexican grass seed without lowering the temperature for at least a year as long as he dries the sample to below 6% moisture. It is of interest to compare the total number of seeds germinated in each experiment. By using G it is seen that

\[
\begin{align*}
G \text{ in Experiment 1} &= 191.9 \quad 47.6 \\
G \text{ in Experiment 2} &= 1989.5 \quad -23.2 \quad \text{Difference} \\
G \text{ in Experiment 3} &= 1966.3 \quad 49.8 \\
G \text{ in Experiment 4} &= 2016.1
\end{align*}
\]

there is a tendency for the germination to rise but no marked flush. This can probably be attributed to the progressive breakdown of some mechanical dormancy.
AN INVESTIGATION INTO THE VARIATION IN VIABILITY OF THE SEEDS OF SOME TRINIDAD GRASSES TAKEN AT INTERVALS THROUGH THE DRY SEASON.

Of those grasses which flower and set seed in Trinidad most do so almost continuously throughout the season only a few flowering at specific periods, such as *Echinochloa pyramidalis* in May.

In temperate regions grasses show a more definite seasonal cycle of functions so that seed is produced largely at those times when it will stand most chance of reproducing the species. Very little seed is set in winter, for instance, because its chance of germinating to produce a plant or of surviving as a dormant seed are slim. In some of the areas of the wet tropics the seasons are less well defined and if conditions are not ideal for the germination of a seed it will lie dormant in the soil litter generally without harm. Nevertheless, there are obviously certain seasons which are more favourable for flowering and setting seed or for germinating and producing flushes of new growth. It would be interesting to investigate whether the increased numbers of seed produced during such a period were related to a future growth flush by way, perhaps, of dormancy. It is generally recognized in Trinidad that there is a high degree of flowering and seed-setting in grasses in March to April in the middle of the dry season, and it may be that these lie dormant for a month or six weeks until the rainy season provides ideal conditions not only for germination but for a rapid flush of growth which will perhaps re-establish a much depleted population.

There seems no reason why the viability of seed should vary through the season therefore the germination capacity through the season should be some measure of the dormancy of seeds set at different times.

If no separation of light seeds is made then germination capacity will be correspondingly lowered and will become a measure
of the agricultural value of the seed. The failure of pollination or of fertilised ovules setting seed is considerable in adverse climatic conditions.

Tuley in 1951 germinated seven grasses at monthly intervals through the dry season and attempted to correlate germinability with rainfall, afternoon humidity and sunlight hours per day. February and March were the driest months with the highest mean diurnal sunlight exposed and they showed the highest germinability. The experiment which follows was designed to test the germination of seeds of some other grasses taken at monthly intervals through the dry season, and by retesting them at three month intervals to determine whether there were any maturation flushes.

It would have been ideal to have made, in conjunction with the monthly germination tests, counts of empty florets, and viability checks on good seed using the tetrazolium test. This would have given a much clearer picture both of the effect of climatic conditions on seed setting and viability and of dormancy and its decline with seed storing period. However, due to the large amount of time involved in applying the tetrazolium test so frequently, and the additional time necessary to separate sound seed from light seed in samples these aspects of the experiment could not be undertaken.

**Experimental Procedure**

The germination capacity of each sample was tested immediately after harvest and again at three monthly intervals, by the usual technique of putting fifty seeds between filter paper in butter dishes and keeping them moist for fifteen days.

The seed, after collection, was kept in paper bags stored in reasonably air-tight tins over a mixture containing two parts of anhydrous calcium chloride and eight parts of anhydrous sodium carbonate to give a controlled humidity of 2% R.H.

The following grasses were selected for testing:
Mexican grass
Jeragua grass
Toco grass
Dallis grass
Kyaswa grass
Rhodes grass
Gambia grass (Nigeria)

* Grasses included in P. Tuley's tests in 1954.

Results

Andropogon gayanus

Samples harvested in November were examined under a lens and were found to be in full flower. Those samples harvested in December, January, and February all showed no viability and appeared, on examination, not to have set seed. The wilted remains of the gynaeicum or an aborted embryo were found within the hermaphrodite spikelet.

The reasons for this would seem to be either a genetical incompatibility or some interference in pollination, fertilisation, or development of the fertilised ovule.

Ischaemum aristatum

Only small amounts of the spikelets of this grass were obtained in December, January, February and March. Most spikelets, consisting of a sessile, awned fertile floret and a sessile sterile floret, were blind or in flower, and a germination test showed no germinability.

Paspalum dilatatum

Those seeds harvested in December, January and February showed no germination despite many of the seeds containing plump caryopses. This was as expected from past attempts by many workers to germinate the seed of both P. dilatatum and P. notatum without removing the interlocking palea and lemma or otherwise pretreating the seed.

100 seeds germinated in April gave two seedlings. Of these seeds some had been attacked by a fungus or an insect and had an extruded, cankerous appearance.
It was also interesting to note that the seeds probably release a cellulose-dissolving enzyme which leaves the filter papers peppy and rather sticky.

_Hyparrhenia rufa_

Samples harvested in November were examined and found to be in flower. Samples harvested in December and January did not germinate, but the February sample showed a 4% germination. In April two dishes of _H. rufa_ were prepared.

From the January sample which showed no germination on harvesting, a sample of fifty seeds was taken by rubbing in the palm of the hand, winnowing, and picking out from this selection only firm seeds. The selection germinated 54% in 7 days, but there was no further germination in the following eight days.

The April harvested seed showed 10% germination in a sample of 50 seeds.

The harvesting and cleaning of seed samples of _H. rufa_ is most important in obtaining a good germination capacity.

_Chloris gayana_

<table>
<thead>
<tr>
<th>Germination Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Harvested</strong></td>
</tr>
<tr>
<td>22nd Nov.</td>
</tr>
<tr>
<td>4th Jan.</td>
</tr>
<tr>
<td>20th Jan.</td>
</tr>
<tr>
<td>16th Feb.</td>
</tr>
<tr>
<td>2nd April</td>
</tr>
</tbody>
</table>

The seed of _Chloris gayana_ showed a very low germination throughout but a tendency to improve after storing for three or four months. The highest first test figure was in February.

The replications are numbers of dishes of 50 seeds and they were adjusted according to the previous result.
Pennisetum polystachyon

<table>
<thead>
<tr>
<th>Harvested</th>
<th>First Test</th>
<th>Second Test</th>
<th>Third Test</th>
<th>No of Replications</th>
</tr>
</thead>
<tbody>
<tr>
<td>11th Oct.</td>
<td>22nd Nov.</td>
<td>0.33%</td>
<td>21st Jan.</td>
<td>12th April 12%</td>
</tr>
<tr>
<td>21st Nov.</td>
<td>25th Nov.</td>
<td>Nil</td>
<td>21st Feb.</td>
<td>Nil</td>
</tr>
<tr>
<td>1st Jan.</td>
<td>7th Jan.</td>
<td>Nil</td>
<td>12th April</td>
<td>Nil</td>
</tr>
<tr>
<td>20th Jan.</td>
<td>21st Jan.</td>
<td>Nil</td>
<td>12th April</td>
<td>Nil</td>
</tr>
<tr>
<td>16th Feb.</td>
<td>21st Feb.</td>
<td>Nil</td>
<td>12th April</td>
<td>Nil</td>
</tr>
<tr>
<td>2nd April</td>
<td>12th April</td>
<td>0.5%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The germination of *P. polystachyon* was generally nil or very low as was to be expected since the fascicles contain an inhibitor which in germination dishes builds up a local concentration which is sufficient to inhibit the germination of the seeds. It appears that with age less of this inhibitor is produced since the seed harvested in October showed 12% germination after 6 months storage.

**Ixocarpus unisetus**

<table>
<thead>
<tr>
<th>Harvested</th>
<th>First Test</th>
<th>Second Test</th>
<th>Third Test</th>
<th>No of Replications</th>
</tr>
</thead>
<tbody>
<tr>
<td>12th Oct.</td>
<td>24th Nov.</td>
<td>6.33%</td>
<td>21st Jan.</td>
<td>12th April 55%</td>
</tr>
<tr>
<td>17th Nov.</td>
<td>25th Nov.</td>
<td>8.0%</td>
<td>21st Feb.</td>
<td>36.3%</td>
</tr>
<tr>
<td>1st Jan.</td>
<td>7th Jan.</td>
<td>15.3%</td>
<td>12th April</td>
<td>6.0%</td>
</tr>
<tr>
<td>20th Jan.</td>
<td>21st Jan.</td>
<td>1.0%</td>
<td>12th April</td>
<td>26.0%</td>
</tr>
<tr>
<td>16th Feb.</td>
<td>21st Feb.</td>
<td>3.5%</td>
<td>12th April</td>
<td>9.0%</td>
</tr>
<tr>
<td>2nd April</td>
<td>12th April</td>
<td>12.0%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The seed of *Ixocarpus unisetus* showed a fairly low germination after harvest but germination increased considerably in all cases with age, reaching 55% for the October seed after 6 months storage.

A graph of variation of germination with date of harvest is given against rainfall, and hours of sunshine curves. A graph of age of storage and germination capacity is also given as not all the samples were tested at the same period after harvest.
Meteorological Data

Mean Daily Hours of Sunshine

<table>
<thead>
<tr>
<th>Month</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Jan</th>
<th>Feb</th>
<th>March</th>
<th>April</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hours</td>
<td>5.3</td>
<td>6.1</td>
<td>7.2</td>
<td>6.7</td>
<td>7.2</td>
<td>8.0</td>
<td></td>
</tr>
</tbody>
</table>

Total Rainfall in inches

<table>
<thead>
<tr>
<th>Month</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Jan</th>
<th>Feb</th>
<th>March</th>
<th>April</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ins</td>
<td>9.7</td>
<td>9.8</td>
<td>6.9</td>
<td>4.6</td>
<td>3.0</td>
<td>1.8</td>
<td></td>
</tr>
</tbody>
</table>

Mean Relative Humidity at 1:00 p.m.

<table>
<thead>
<tr>
<th>Month</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Jan</th>
<th>Feb</th>
<th>March</th>
<th>April</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>84</td>
<td>82</td>
<td>74</td>
<td>71</td>
<td>69</td>
<td>67</td>
<td></td>
</tr>
</tbody>
</table>

Results

There seems to be little correlation between month of harvest and weather conditions, although both the post-harvest and the three month curves rise to a peak in December.

In Chloris gayana there was a slight rise to a maximum for the February value, but such results can hardly be considered significant in view of the very few replications, irregularity of germination and low germination values.

The graph of germination of Erophorus unisetus after accurate storage periods is most interesting. The lines for November, December (Jan. 14th) and January are almost parallel. The curve for October involving three readings would also be a parallel line if the middle reading had been ignored. The curve for February is not as steep as the others, probably because there was only 7 weeks difference between tests. This suggests that, in fact, if tests had been done, say, at fortnightly intervals, a batch of very similar curves would have been produced with increasing gradients.

Summary

Seven grasses were used to collect seed from monthly. This was tested almost immediately and then at about 3 months and at 6 months, the seeds being stored meanwhile at 2% R.H. and about 78°F.

Of these seven grasses Andropogon gayanus and Ischæmmum aristatum set no viable seed, the former because of interference in pollination or seed setting, and the latter because the florets were harvested immature or in flower.
Paspalum dilatatum and Hyparrhenia rufa showed very little germination, the former because of mechanical dormancy and the latter because of a large proportion of blind seed and trash.

Chloris gayana and Pennisetum polystachyon showed generally poor germination due to dormancy which was alleviated in the latter case by storage.

Ixophorus unisetus gave much better germination generally. The germination of monthly samples showed little correlation with meteorological data, but in every case increased with period of storage and in each month from October to January this increase was remarkably similar, thus indicating the breaking-down of dormancy.
Graph of Germination of Mexican Grass Seed
Harvested at Monthly Intervals
Including Mean Sunshine & Rainfall Curves Over That Area

Key:
1 = 1st Test
2 = 2nd Test (3 Months)
3 = 3rd Test (6 Months)
4 = Sunshine (Hours per Day - Mean Monthly)
5 = Rainfall (Inches per Month)

Graph:
Germination:
- Per Cent

Meteorological Data:
- Sunshine (Hours)
- Rainfall (Inches)

Graph of Germination of Mexican Grass Seed Against Period of Storage

Key:
1 = October 12th
2 = November 17th
3 = January 4th
4 = January 20th
5 = February 16th
6 = April 2nd
C = Comparison Line
GERMINATION TESTS ON SOME OF THE SEED SAMPLES IN STORE
IN THE LIME-BOX, OLD FARM, I.C.T.A.

Tests on October 17th 1955

<table>
<thead>
<tr>
<th>Grass</th>
<th>Date Harvested</th>
<th>Treatment</th>
<th>Germination Nos. at. Replications x 50 seeds</th>
<th>6 Days</th>
<th>8 Days</th>
<th>10 Days</th>
<th>Total</th>
<th>% by Jan. 1955</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andropogon gayanus</td>
<td>8.11.55</td>
<td>A B</td>
<td>0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2%</td>
</tr>
<tr>
<td>Ixophorus undulatus</td>
<td>23.9.55</td>
<td>A B</td>
<td>12 0 0</td>
<td></td>
<td></td>
<td></td>
<td>12%</td>
<td>6%</td>
</tr>
<tr>
<td>Paspalum dilatatum</td>
<td>18.11.55</td>
<td>A B</td>
<td>0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4%</td>
</tr>
<tr>
<td>Pennisetum polystachyon</td>
<td>3.7.55</td>
<td>A B</td>
<td>6 0 0</td>
<td></td>
<td></td>
<td></td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>Pennisetum pedicellatum</td>
<td>26.11.53</td>
<td>A B</td>
<td>1 1 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2%</td>
</tr>
<tr>
<td>Panicum maximum</td>
<td>2.10.55</td>
<td>A B</td>
<td>0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grass</th>
<th>Date Harvested</th>
<th>Treatment</th>
<th>Germination Nos. at. Replications x 50 seeds</th>
<th>8 Days</th>
<th>12 Days</th>
<th>19 Days</th>
<th>Total</th>
<th>% by Previous Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pennisetum polystachyon</td>
<td>10.8.55</td>
<td>A</td>
<td>1 1 2</td>
<td></td>
<td></td>
<td></td>
<td>14%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>19.11.55</td>
<td>A</td>
<td>2 0 0</td>
<td></td>
<td></td>
<td></td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>Pennisetum pedicellatum</td>
<td>22.11.54</td>
<td>A</td>
<td>8 0 0</td>
<td></td>
<td></td>
<td></td>
<td>16%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.2.54</td>
<td>A</td>
<td>0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the first batch treatment A involved tap water as the germination medium while that in treatment B was a 0.2% potassium nitrate solution. Since in the three sets of results A and B were almost identical, their figures were combined in the table.

On November 11th, 1955, 6 sets of Setaria sphacelata seed were examined microscopically and found to be blind. It is assumed from these results that S. sphacelata does not normally set viable seed in Trinidad. However, C.R. Horrell in 1952 found in a sample of Tananyika S. sphacelata 11% fully formed seed, but in a Uganda sample none.

It seems likely that the use of a lime box for storing grass seed is limited. Seed so stored may maintain its viability for 12 to 18 months, but then quickly loses it.
SECTION I
SOLS CYTOTOLOGICAL INVESTIGATIONS ON TRINIDAD GRASSES

In the course of experiments on the viability of grass seed attention was turned to those grasses in Trinidad which set no seed at all. Among these are such important grasses as Lucunlta grass - \textit{Ischaemum timorense}, Pangola - \textit{Digitaria decumbens}, and \textit{Echinochloa pyramidalis}. In addition there are many grasses in which very little seed is set, e.g. Toco grass - \textit{Ischaemum aristatum}, Bahia grass - \textit{Paspalum notatum}, Brachiaria decumbens, \textit{B. Brizantha}, Jaragua - \textit{Hyparrhenia rufa}, and others which, while setting plenty of seed, show a very low viability e.g., \textit{Pennisetum polystachyon} and \textit{P. pedicellatum}, Dallis grass - \textit{Paspalum dilatatum}, \textit{Andropogon gayanus}.

Of the three grasses in the first category, it was known that Pangola is triploid, from work at Tifton, Georgia. The \textit{Echinochloa pyramidalis} in Trinidad has all arisen by vegetative propagation from one introduction (Davies 1955). It is thus clonal and since no fertile seed is set, must be self sterile due to polyploidy.

It was therefore decided to make some chromosome counts on certain of Trinidad's more economic but sterile grasses and cytogenetical work was commenced on \textit{Erophorus unisetus} and \textit{Paspalum dilatatum} in a preliminary attempt to select a rapid but effective method for making chromosome examinations.

It is perhaps pertinent at this stage to mention briefly the phenomenon of polyploidy and describe its genetic consequences. Within the Spermatophyta the plant is typically diploid its somatic cells containing two homologous sets of chromosomes and at cell division these chromosomes divided mitotically to produce two identical sets of chromosomes exactly similar to the parent. In the formation of the germ cells within the ovaries and the anthers there is a meiotic or reduction division followed closely by a mitotic division to produce four haploid cells, the double division being known as meiosis. The reduction division involves a
separation of the chromosomes and their rearrangement to lie opposite their homologues on a diametric plane within the nucleus. From this point the homologues attracted by their centromeres come to lie in juxtaposition and actually appear to coalesce at certain points along their length. These are known as chiasmata. During this time there is a thickening and twisting of the chromosomes which can all be seen to be double, thus showing four chromatids in each double homologue or bivalent. At this stage there may be an interchange of material between two homologous chromatids. This is known as crossing over and is the basis of genetical reassortment resulting from sexual reproduction on which depends evolution. The chiasmata then pass along the chromosomes towards the opposite ends of the bivalent to those of the centromeres and the distal ends of the bivalent separate. A final separation is effected and the chromosomes move apart and withdraw to the poles and a mitotic division follows to form four haploid groups of chromosomes different in genetic constitution to the parents which gave rise to them. The haploid group is known as the genome. Certain discrepancies may arise in this process resulting in the production of germ cells with more than one genome. This is polyploidy and it is usually divided into two groups, the definition of which is still controversial. Autopolyploidy is generally considered to be the condition in which the genome number becomes doubled in the germ cell and each genome or group of genomes is exactly identical to the other. Autopolyploids usually arise as a result of the suppression of cyto-kinesis in somatic mitosis. A cell results with two diploid nuclei which fuse to form a tetraploid nucleus, or the chromatids fail to separate completely in mitosis and a single nucleus is formed where there should have been two and hence a tetraploid cell arises. If this occurs in the germinal tissue diploid germ cells will arise which, if fusing with normal haploid cells, will give rise to triploid progeny. The triploids at meiosis will do one of two things. Either they will, if homologous, form a haploid number of trivalents and these will divide, similarly to bivalents, after crossing over to attempt to form three gametes. This does not occur and consequently
such triploids do not produce fertile seed. Some may, however, form bivalents and monovalents and fertile haploid gametes arise and there is the abortion of one genome, the incompatible one. This rarely occurs in autotetraploidy. Another cause of autotetraploidy may be the failure of the mitotic division in meiosis. This will result in gametes containing two sets of chromosomes which are not the same because of crossing over in meiosis. This is not therefore strictly autopolyploidy. The fusion of such a gamete with a normal haploid gamete will result in the union of two genomes. The third which has undergone crossing over in the previous mitosis will abort as it is incompatible. Normal diploid progeny will arise from such a fusion. When two such tetraploids fuse a certain number of the progeny will be fertile, about 50%, and tetraploid.

In these cases of autopolyploidy the chromosome number is always a fixed multiple of the haploid number (euploidy), since autopolyploids always contain complete genomes. Thus a chromosome count revealing 48 chromosomes may indicate that the plant is diploid with 24 chromosomes as a genome or tetraploid with a 12 genome, or hexaploid with an 8 genome, or triploid with a 19 genome. If it were a grass the likelihood would be a hexaploid with 8 chromosomes in the genome.

It can be seen, therefore, that autopolyploids, while partially self-fertile, will, if crossed with normal diploids, produce generally sterile triploids. Similarly auto octaploids and diploids will produce pentaploids which tend to be sterile. Pentaploids are also produced when a hexaploid and a tetraploid are crossed and are generally sterile for the same reasons.

The partial sterility of autopolyploids is due to the multivalent associations formed and their occasional irregular segregation. Thus in the meiosis of an autotetraploid with forty chromosomes it is unlikely that ten tetravalent chromosomes will be formed as short
chromosomes move faster than long ones and once chiasmata are formed between two or three conjugating chromosomes a further chromosome does not generally link up. A mixture, therefore, of tetravalents, trivalents, bivalents and monovalents arises and the gametes formed will consequently be sterile.

Allopolyplody is generally considered to be a case of polyplody in which the genomes of a gamete are dissimilar. Allopolyploids may arise in a variety of ways. The usual cause is in the formation of a hybrid. When two genetically different gametes are sufficiently compatible to fuse a hybrid is formed. When the hybrid comes to form gametes the two genomes are not always entirely homologous with the result that a certain number of bivalents are formed in meiosis but also some monovalents. Only the bivalents transmit their chromatids to the gametes with a consequent reduction in chromosome number. As a result of continued interbreeding between such hybrids an aneuploid series arises. Allopolyplody will result when hybrids undergo a somatic mitosis without cytokinesis. The resultant polyplod will result in two genomes of one type and two genomes of the other type (Amphidiploid). If such a tetraploid is crossed with a diploid there will be three different genomes at fertilisation. These will come together as homologues at meiosis and will segregate as univalents, bivalents and trivalents. The gametes will therefore contain incomplete genomes and random fertilisation will produce in the F2 a mixed population containing different chromosome numbers. These are known as an aneuploid series.

Anomalies in chromosome numbers may also arise as a result of atypical behaviour at meiosis, e.g. lagging of homologues on the metaphase plate so that they get left out of the final gametes. Again an aneuploid series arises.

Allopolyploids may also arise by the crossing of two individuals within a genus with different chromosome numbers, e.g. a hexaploid tobacco variety(g) with 72 chromosomes crossed with a
tetraploid variety with $48$ chromosomes ($t$) produced an allohexaploid with two ($g$) genomes and four ($t$) genomes, e.g. $(g + 2t) \times 2 = 6gt = 72$ chromosomes.

Varieties containing aneuploid series of chromosomes will intercross with variable results depending on the partial affinity of chromosomes of different genomes. A classification of such individuals can be measured only by observation of multi-valent frequencies in meiosis, and by careful cytological and genetical analysis of the parent species and the hybrids, and the polyploids and the mullo- and poly-sonics. A polysomic organism is one in which there has been irregularity at meiosis resulting in two homologues becoming incorporated in the same gamete. If this gamete fuses and gives rise to an individual the chromosome will be duplicated throughout that individual. The complementary gamete will contain neither of the homologues and the individual arising from it will be mullo-sonic.

In aneuploids the chromosome formula of a plant with more than $11$ bivalents is $(11 + x)$ bivalents & $(7 - x)$ univalents, e.g. a plant with 38 chromosomes would have $17\,_{II}$ and $1\,_{I}$, i.e. $(11 + 3)\,_{II}$ and $(7 - 3)\,_{I}$, and a plant with 39 chromosomes would have $18\,_{II}$ and $3\,_{I}$, and with $10\, - 19\,_{II}$ and $2\,_{I}$, and $1\,_{I} - 20\,_{II}$ and $1\,_{I}$ and $2\,_{II}$ which is the standard vulgare make up in Triticum, i.e. diploid with a genome of 21. Plants not conforming to this, e.g. $20\,_{II} + 0\,_{I}$ are generally sterile. Kihara (see R. Nerton Love 1940).

Morphological and physiological differences between diploids and polyploids are attributed to the increased nucleus and cell size since chromosome recombination from genomes of different species (in allelopolyploids) will not be a constant factor and therefore in themselves will not be responsible for such differences. This complication does not arise in autopolyploidy and therefore polyploids from diploid parents can be expected to show only those morphological and physiological variations from the parents which can be directly attributable to larger cell and nucleus size.
Polypliods in nature are often adaptations of the original diploids to new ecological conditions. Thus *Festuca cambes-biana* (2n = 20) is an annual grass found commonly in Timbuktu but limited to the moist swamp-shore, whereas *E. albida* (2n = 40), although similar morphologically, is a perennial living in drier conditions, while *E. pallescens* (2n = 60) is a complete xerophyte. Ecological differences also occur between intraspecific chromosomal races (Myers).

Frequent examples of different ecotypes being polypliods, one of the other occur, in the Gramineae and generally the degree of xerophytism increases with the chromosome number.

R. Merton Love working in California (1953) has studied the many species and their hybrids of the graminaceous genus Stipa and from a thorough evaluation of morphological differences correlated them with chromosome make up. Both the genuses Stipa and Digitaria show aneuploid series (Myers), while Nielsen made an intensive study of *Panicum virgatum* and found in a collection of 59 plants 2n numbers of 18, 36, 51, 72, 90, 108. This, of course, is a euploid series. In this case too, what was said above about morphological and physiological differences between diploids and their autoploids was borne out. In an attempt to correlate these intraspecific chromosome races with morphological differences, he found statistically that: In all possible comparisons of isolates with different chromosome numbers, 66.4% showed significant morphological differences, while among isolates of similar chromosomes numbers 58.6% of the differences were significant on average. This meant that differences were found among isolates of the same chromosome number almost as frequently as among isolates with different chromosome numbers and thus points to the fact that intraspecific variation due to polypliody does not produce significantly different morphological variants. Myers concludes his paper by giving a list of chromosome numbers of many tropical grasses with their references.

It is hoped that this short and rather sketchy introduction to polypliody and its effect on plant sterility will serve to explain
the basis of the work which follows. It is realised, of course, that the full cytogenetical investigation of a grass is a major project, and all that can be done here is to probe the cytological techniques which might be applicable to such studies since, to date, comparatively little has been done on the cytogenetics of tropical grasses.

The first preliminaries to making chromosome counts of grasses was to find suitable material. Chromosome counts are generally made from smears, crushes or squashes and the material used is generally pollen mother cells or root tips. The ideal method is probably to make smears of pollen mother cells (P.M.C.s). However, it was necessary in this instance to use root tips since the plants being dealt with generally did not produce pollen and if they did, only at certain times. The crush method for root tips using microtomed sections is too slow for quick chromosome counts. The squash method of examining root tips was therefore chosen.

It was then necessary to select an applicable technique from those in general use for root tip squashes. The essential requirements of a good squash are as follows. First the material must be satisfactorily fixed so that there is a minimum further disturbance of the cell morphology, so that the cell stains well, and with due regard to the clarity of the preparation since grass chromosomes are not only very small but also closely clumped. Although such fixatives as osmium tetroxide vapour are sometimes used in the Crystal Violet and Iodine Technique, acetic acid is the best fixative for chromosome work. It is not a general precipitant but is a strong precipitant of nucleo-proteins while dissolving out the histones. Nucleo-proteins precipitated by acetic acid are insoluble in alcohol or water. Acetic acid is also a good carrier for several important chromosome stains. In practice, glacial acetic acid is used with absolute alcohol as a fixative. While the acetic acid fixes the heterochromatin, the alcohol dehydrates the tissue, fixes the cytoplasm and dissolves out certain cell inclusions, thus clearing the preparation. A further modification
of this fixing solution was made by Carnoy who included chloroform with aqueous acetic acid and alcohol. This dissolves out certain cell inclusions and lipids; the aqueous alcohol fixes the cytoplasm more gently, and the acetic acid precipitates the hetero-chromatin. Nevertheless acetic alcohol was consistently better than Carnoy’s fluids in giving a clear, well fixed cell preparation. Other fixing mixtures which are sometimes used are Bouin’s fluid, Farmer’s fluid, Craf, La Coeur 2 BE and chromacetic.

The next stage is generally staining although mordanting to make the tissue take the stain better, is done in some techniques. Chromatin stains include among the best Basic fuschin (fuchsin) used in the Feulgen method, Crystal Violet and Acetic Carmine, Acetic Orcein, and Acetic Laecomid more recently. Both fuchsin and crystal Violet owe their acidophylic staining properties to pararosanilin which is an organic chloride containing a quinonoid linkage which when oxidised by acid chromatin confers colour to the tissue in which it is lodged. Crystal Violet stains chromatin strongly and darkly but is very soluble in alcohol. It is therefore fixed by a solution of KI/I₂ complex carried in 80% alcohol so that differentiation occurs at the same time (Newton’s method) and then dehydrated in clove oil to prevent further loss of the stain, cleared in xylene and mounted in Canada Balsam or Buparal.

The standard stain for chromatin is acetic carmine which is basic and thus acidophylic. It is prepared by saturating boiling 45% acetic acid with carmine, cooling and filtering. For rapid counts on some plants it may be used without a fixative directly on a squash as the acid acts as the fixative, but this is not applicable to grass root tips. Acetic carmine stains cytoplasm to a lesser extent, especially if acetic alcohol is used as the fixative. Acetic carmine is often used with a mordant to produce a dye lake which is highly basic. The mordants used are salts of aluminium or ferric iron or alums which serve to combine the carmine with the acidophylic chromatin. Lakes are insoluble in water and in neutral alcohol, hence after differentiation, the depth of staining
will remain unaltered by washing as by dehydration.

In these investigations acetic orcein was used. It is made by saturating boiling glacial acetic with orcein, cooling and diluting to a 45% acetic acid solution. Its action is similar to that of acetic carmine. Its big disadvantage is that at saturation, which is about 2% orcein, there is continuous precipitation of orcein from solution. The stain cannot be used effectively at weaker dilutions and must therefore always be filtered before use. Even then it is not as good as acetic carmine being neither as strong nor giving such a clear differentiation between cytoplasm and chromosomes. Acetic lacmoid was not tried as it was not available.

The value of making semi-permanent preparations was investigated but in the interests of speed, and microscopical resolution these were discontinued.

EXPERIMENTAL PROCEDURE:

Preliminary experiments were undertaken with five grasses:

- *Setaria sphacelata* (Kenya) StapF. - African Thimothy
- *Digitaria decumbens* Stent. - Pangola grass
- *Ischaemum timorense* Kunth. - Incuntu grass
- *Paspalum dilatatum* Poir. - Dallis grass
- *Melinis minutiflora* Beauv. - Molasses grass

Of these the first three set no viable seed in Trinidad and Dallis grass but a little.

Sods of these grasses were brought in from the trial plots on December 30th 1955 and kept in the laboratory in a large, shallow, enamel tray well watered, so well, in fact, that the Setaria sphacelata made much superior growth to the other grasses. Whenever root tip material was required a stool of each grass was separated and washed free of soil and put in a beaker of water for 1 or 2 days. This induced prolific root growth in *S. sphacelata* but the responses in the other grasses were poor though adequate. However, because of this most of the preliminary slides were of *S. sphacelata.*
Specimens of root tips for smears were taken by snipping off the last millimeter of the lateral root hairs on a white, rapidly growing root with scissors. The root tips were transferred to acetic alcohol and stayed there fixing just as long as it took to finish snipping and set up the rest of the dishes, usually about five minutes. The root tips were transferred from the acetic alcohol to a watch glass containing five drops of acetic orcein and one drop of normal hydrochloric acid as a macerator. The transference of the minute material was done most satisfactorily with a camel hair brush which, teased out onto a slide, released most of the tips which were then transferred with a needle en bloc to the stain.

The tips in the stain were warmed gently by taking the watch glass through a spirit burner flame until acetic acid could be seen volatilising. The watch glass was then allowed to cool and the tips kept out of the evaporating edges of the stain.

When cool as many tips as possible were transferred to a drop of acetic orcein on a slide and teased out to lie individually. During these operations about 50% of the specimens were lost.

A cover slip was then placed carefully on the material, pressed down gently under a filter paper, and tapped out using the wooden handle of a mounted needle merely dropped onto the surface, the tapping should be light but sharp so that the maximum spreading of cells occurs without breaking the cover slip. The cover slip was then pressed down firmly under several layers of filter paper.

The slide was inverted over acetic alcohol so that the cover slip was just under the surface. Separation occurred within three minutes. The slide and cover slip were then taken through two changes of absolute alcohol for two minutes each. The cover slip and slide were recombined in a large drop of Buparal and allowed to settle out. Care had to be taken not to trap air bubbles. Generally a cloudiness arose in the preparation due
probably to the alcohol containing some water, it being impossible to keep alcohol absolute in this humidity.

Clearer slides showed many young cells and occasional recently divided cells in the meristems but there was a general lack of clarity and under high magnifications it was seen that there was not enough differentiation between cytoplasm and nuclear material all being stained a rather uniform pink.

Much of the difficulty lay at first with mere technique, but as this improved it became clear that either the method was poor or the material was not pre-treated correctly for obtaining mitosis.

Cedar wood oil and glycerine were tried as temporary mounting agents and the former found to be better than Buparal – there was less cloudiness. Weaknesses in the method lay with the short period of fixation which could with advantage be much longer, e.g., 2h hours or more at a lower temperature. The use of steel needles introduced too much ferric iron into the stain. The stain had been filtered and the precipitate washed with acetic acid (15%) and the filtrate was probably too dilute. The alcohol was too aqueous. With heavier staining the slide could have been kept longer in the alcohols to get a better differential destaining.

It seemed obvious that further work using the above technique would be fruitless. More reading was therefore done, and a lot of references were checked, in order to find a technique which might be more applicable to the problem, and there was found in the course of reading Plant Breeding Extracts for 1954 a modification of the acetic carmine technique by Hyde and Cardella in Stain Technique 1953, 28, p.305. However, this was not used because on Thursday, February 23rd, the author had the pleasure and good fortune to meet Dr. S.L. Emsheller of the U.S. Department of Agriculture who was visiting the Imperial College from Washington as a guest of Dr. Harlote, to observe and give advice as a plant physiologist and cytogeneticist.

Dr. Emsheller made several helpful suggestions regarding this work:
1. The material as prepared was probably not ideal for showing active mitosis in any quantity in the squashes. He suggested that sods should be dug, and stools divided and potted in the laboratory so that when material was wanted actively growing plants could be removed, washed and snipped. He felt that this should give a better chance of finding satisfactory mitotic cells in root tip smears. He mentioned that at Beltsville they water the plants with warm water a few hours prior to depotting to get a growth flush. He also mentioned that they were working then on the occurrence of division at different times of the day to see whether at certain times there was a higher incidence of mitoses in representative root tips, representing periodic growth flushes.

2. The fixation should be rather longer at lower temperatures. R. Merton Love, 1953, suggests that such material be left in fixative at 5°C for several months.

3. Acetic carmine should be used.

4. Pre-treatment of the root tips with colchicine induces heteropyknosis.

Sods of *Paspalum dilatatum*, *Ixophorus undisetus* and *Melinis minutiflora* were left in soak overnight to soften their earth and then hosed on the afternoon of February 21th. The *Paspalum dilatatum* sod was very rich in creamy new roots. Dr. Hunsweiler removed these root tips with forceps and they were put in a phial of acetic alcohol. The other two sods were treated similarly but the *Melinis minutiflora* gave little material partly because the new roots were smaller and browner and thus harder to find. The three phials of root tips in fixative were put in the refrigerator.*

The remaining soil-free sods were clipped of most of their roots and left in a shallow enamel tray containing a little water.

Meanwhile further investigation of literature on the pretreatment of root tips with colchicine was made. Other substances bring about a similar effect of heteropyknosis and tend to spread chromosomes which are closely clumped (Sharma and Mookerjea, 1954). Notable among these is paradichlorobenzene which has the considerable advantage of the temperature of the refrigerator and hence the fixations was 15°C.
over colchicine, of being very much cheaper. J.R. Meyer in 1945 produced a paper on the use of paradichlorobenzene to facilitate chromosome study and gave the following schedule.

Prefixation: 5 or 10 grammes of paradichlorobenzene are held overnight in 500 mls. of distilled water in a stoppered bottle at 60°C. Root tips are immersed in the cold sol for one to four hours.

Fixation: In 65% acetic acid, acetic alcohol or Carnoy's fluid for 12 to 24 hours.

Hydrolysis: Concentrated hydrochloric acid added to nine times its volume of water (i.e. 10%) and used at 60°C. Immerse the root tips in the acid for 10 to 30 minutes and then wash thoroughly in water. Dissect out the meristems in 45% acetic acid.

Staining and Smearing: The usual acetic orcein technique.

To Make Permanent: Take through acetic alcohol, 90% alcohol, absolute alcohol (two changes) and mount in Euparal (aged preferably).

On 27th February Dr. Emsweller attempted to use the root tips which had been put in fixative on the 21st. They were found to need a considerable period of maceration in a mixture of N hydrochloric acid and absolute alcohol about half and half. In the first attempts to make squashes with this material there was no separation of the cells indicating that the middle lamellae had not yet broken down. Also the cells took up too much stain and there was consequently no differential effect.

The sods which had been lying partially immersed in a shallow tray of water over the weekend had put out a profusion of new roots with very promising looking tips. In the Melinis minutiflora sod the response had been slower. The tips of the other two were removed to fixative. The sods were taken to the glass houses on the roof and there divided into stools which were severely trimmed and potted in a coarse potting medium. They were subsequently watered regularly twice a day.
As a result of his participation in the experiment Dr. Emsweller was able to make the following statements:

(a) Maceration seems most effective in a 50:50 mixture of concentrated hydrochloric acid and absolute alcohol for about five minutes, followed by thorough washing.

(b) Fixation in Carnoy's Fluid (Glacial acetic acid - 6 parts; absolute alcohol-3 parts; chloroform - 1-1/3 parts) is effective in clearing the cells since the chloroform dissolves out all the fatty cell inclusions.

(c) Quick mounts showed chromosomes generally in early prophase. They were small and clumped together.

He made the following recommendations:

(1) Use root tips taken in the morning in order to be more likely of getting a good proportion of cells in metaphase.

(2) Pre-treat with para-dichlor-benzene to disperse the chromosomes and cause heteropyknosis.

(3) Fix in Carnoy's fluid.

(4) Macerate in concentrated hydrochloric acid and absolute alcohol (50:50) for five minutes followed by washing.

(5) Dissect out the meristem onto a slide and crush it in a drop of acetic carmine. Heat gently over a spirit flame after application of a flat cover slip.

(6) Press out with blotting paper and seal the preparation with gum-mastic around the edges of the cover slip. Gum-mastic is made by heating paraffin wax and gum together, but paraffin wax melted round the edge of the cover slip is equally effective for temporary slides and more easily dislodged.

I therefore proceeded with the work with these recommendations as a guide.

On the 2nd of March a plant each of *Eucnephorus unisetus* and *Paspalum dilatatum* were taken from the pots and washed. There had been a prolific formation of active white shoots. Root tips were taken and put in para-dichlor benzene prepared the previous night
(see above) with 5 grammes in 200 mls. of distilled water. They were put in at 11.15 a.m. and removed to four phials at 2.45 p.m. (A), two containing acetic alcohol and two containing Carnoy's fluid made up with 45% acetic acid, and put in the refrigerator. Meanwhile two more plants were removed from the roof and root tips taken and put in P.D.B. at 3.15 p.m. (B) and removed to the fixative at 8.45 p.m.

On the sixth of March, 4 days later, several slides were made. The first batch was to investigate some root tips of *Ixophorus unisetus* which had been in P.D.B. since the 2nd March. They were brown and hard and did not soften up much in concentrated hydrochloric acid. One large root tip however, gave a good crush. The chromatin appeared strong and dark but there had been widespread dispersal and it was impossible to distinguish chromosomes.

The second batch was of root tips previously treated with P.D.B. and kept in fixatives for four days at 15°C. Two or three tips were taken from each phial and examined.

(a) The *Ixophorus unisetus* (A) in Carnoy's fluid were macerated for seven minutes. This was found to be not enough.

(b) The *Paspalum dilatatum* (A) in Carnoy's were macerated for fifteen to twenty minutes until the root tips were translucent. This was still not long enough. The tissue took stain rather poorly and although the nuclei could be seen they were not very clearly differentiated. Subsequently a larger root tip which was better macerated showed no chromosomes.

(c) The *Ixophorus unisetus* (A) in acetic alcohol were macerated for 25 minutes and then double washed for ten minutes. A convenient and safe method of washing root tips is to put them in a filter papered funnel and allow water to drip through so that they are continuously washed. An alternative method is to enclose them in a short length of glass tube (20 cm x 5 mm) open at both ends but with one end covered by a small cheese-cloth bag. Water is then passed through gently and continuously from the other end. Squashes were made of three larger root tips, and one especially showed chromosomes in
Most of the meristematic cells. They were very small under ordinary high power magnification (x 800), however they seemed to be well dispersed and may have been in late anaphase.

(d) *Faspalum dilatatum* (B) in Carnoy's was macerated for three hours. The resulting tissue was translucent and flaccid. The tissue then stained heavily and appeared almost lignified!

(e) *Ixophorus unisetus* (B) in acetic alcohol:

Dr. Emsweller prepared a good slide after maceration in 60:40 concentrated hydrochloric acid and absolute alcohol, squashing in Carnoy's and staining in acetic carmine. Nuclei showed mostly interphase but an early prophase was found with very approximately 30-45 chromosomes.

Further sets of root tips were then taken in the early mornings to try and locate the active stage of mitosis. The material used for the slide just mentioned was taken at 3.15 p.m. (see above) and may have been taken just prior to the onset of mitosis.

Root tips of *Faspalum dilatatum*, *Ixophorus unisetus*, and *Melinis minutiflora* (C) were taken and put in P.D.B. at 5.35 a.m. On the 9th March root tips of *P. dilatatum* and *I. unisetus* (D) were taken at 2.45 a.m. and put in P.D.B. and removed to acetic alcohol at 7.45 a.m.

Group C was in P.D.B. for about 8 hours.

Using both acetic carmine and acetic orcein as stains results on all this material were generally disappointing. The root tips taken at 2.45 a.m. showed no heterochromatin. The acetic orcein was found to be too heavy a stain as well as suffering, as already mentioned, from the disadvantage of continuously precipitating so that it has always to be filtered before using. With acetic carmine it was latterly found difficult to get any staining - perhaps due to the prolonged effect of concentrated hydrochloric acid on the cell contents. Dr. Emsweller had already tentatively suggested the use of cytase as a macerating agent and it was decided to use this.

A general reference on the use of enzymes to improve cytological techniques by Stuart and Emsweller in 1943 was read. Inorganic cell wall solvents were reported to be ineffective. Various fungal extracts
notably from Aspergillus niger, Chaetomium globosum, and Meta-
trhizium spp. had proved more useful as macerators. A proprietary
cellulose called 'Clarase' is obtainable in the U.S.A.

The most useful biological source of cell macerating enzymes
is the water snail, Helix pomatia, and two papers describe the

The use of an extract of stomach contents of these snails is
very feasible since it contains a complex of carbohydrate and
cellulose breaking enzymes including cytase and there is a complete
absence of proteolytic enzymes which would interfere with chromo-
somes. Faberge says "Many workers consider it impossible to make
consistently good squash preparations of certain material, such as
root tips of grasses" - he goes on to describe the problem of
grass root tips and describes how cytase maceration proved effective
when due to the failure of all else, the experiment was about to be
abandoned. There is no doubt that concentrated hydrochloric acid is
useless as a grass root tip macerator since by the time it has done
its job of maceration it has destroyed the cell contents also.

Faberge described the extraction of snail cytase from Helix
pomatia as follows. The snails are killed in chloroform, the shells
removed and the mantle cavities opened, whereupon the stomach is
evident. It is pierced to release its contents which are collected.
They may be preserved with toluene. If the root tips are macerated
in small phials of this liquid, little is required at each operation.
The normal stain technique pertains to the rest of the preparation.

Chambers working in 1955 with ferns of the genus Blechnum,
had notable success using snail cytase and describes its extrac-
tion and preparation in more detail. The stomachs of Helix pomatia
and H. asperser were removed and pulverised in a little water.
The liquid is then centrifuged for five minutes at 1000 r.p.m.
He says: "The root tip material is prefixed in a saturated
aqueous solution of para-dichlor-benzene and then fixed in alcohol.

* I am indebted to Mr. K. Shepherd, B.Sc., for these two references.
acetic acid (3:1 mixture). Before being placed in the enzyme, the root tips are given two changes of a few minutes each in water. Blotting between changes is essential. Three or four hours treatment at 25°C is satisfactory. A rinse in tap water, followed by at least a minute in 4% acetic acid assists the aceto-carmine staining. Storage under refrigeration maintains the enzyme activity of the stomach contents extract.

(1) On the 16th March root tips of *Ixophorus unisetus, Brachiaria brizantha* and *Setaria sphenolota* were taken at 1.15 a.m. and put in P.D.B. They were removed to acetic alcohol and put in the refrigerator at 8.30 a.m.

(2) Root tips of *I. unisetus, Paspalam dilatatum, Ischaemum aristatum* and *Ischaemum timorense* were taken at 11.30 a.m. and put in P.D.B. They were removed to acetic alcohol at 15°C at 6.45 p.m.

(3) Root tips of *P. dilatatum* which had been preserved in acetic alcohol at 15°C. since 7.45 a.m. 9th March (see above) were removed, washed twice in tap water and transferred at 10.55 a.m. to cytase prepared as follows:

About a dozen /snails/ were collected from the College pond, put in a jar with chloroform and then removed as needed. Later the same day the shells were cracked off - a difficult process - and the first parts of the alimentary canal removed, probably the stomach, crop and gizzard and some of the small intestine. The snails still showed some signs of life hours after partial immersion in chloroform. A better method* which results in extrusion of the snail from its shell is apparently to put the snails in a sealed vessel completely full of air-free water at about 50°C and thus asphyxiate them.

The alimentary canals were crushed up with distilled water and the murky liquid was then poured off and centrifuged. A clear solution was obtained in 20 minutes at 5000 r.p.m. (c.F. Chambers), these being minimum requirements. The cytase-containing liquid was stored in the refrigerator.

* I am indebted to Mr. Breese, M.A. of the Entomological Department for these pieces of information.
The P. dilatatum root tips were removed from the cytase at 6.15 p.m. after 7 hours 20 minutes maceration. They were washed, put in acetic acid for a couple of minutes, and then squashed in acetic carmine. The two tips squashed well and under the microscope it could be seen that many of the younger cells had been dissolved, at least partially. However, very little stain had been taken up and no nuclear material was found.

1. Root tips of I. unisetus, Brachiaria brizantha, and Setaria sphacelata were removed from fixative, washed and put in cytase at 7.45 a.m., 8.35 a.m. and 8.50 a.m. respectively (17th March).

Slides made in aceto-carmine between 1.00 and 3.00 p.m. showed nuclei in I. unisetus and B. brizantha, but all in interphase except for a solitary cell in I. unisetus removed from cytase after 2 3/4 hours immersion, which seemed to be at metaphase with the plane of view cutting across the metaphase plate. A slide of S. sphacelata at 3.00 p.m. (i.e. 6 hours 20 minutes immersion) showed cells to be insufficiently macerated.

It seems that at least six hours in this source of cytase is necessary to macerate root tips of these three species. However, it may well be that the extraction of the cytase material could be considerably refined.

The experiments were discontinued at this point as there was no more time to be spared.

**Summary:**

The paper describes a series of trial experiments to discover a rapid, workable, laboratory technique for making chromosome counts of grass using root tip squashes. The material used was initially Setaria sphacelata, Isophorus unisetus, Paspalum dilatatum, and Melinis minutiflora, and subsequently Brachiaria brizantha, Ischaemum aristatum and Ischaemum timorense as well.

The final technique to be recommended is as follows:

(1) Prefixation in a sol of para-dichlor-benzene for three hours.

This causes spreading of the clumped chromosomes and some heteropyknosis.
(2) Fixation in acetic-alcohol (3:1 mixture) at 15°C for up to four days. This may be superfluous.

(3) Double washing which must be thorough and interrupted by blotting.

(4) Maceration in a cytase solution for 3 - 6 hours according to the material. The cytase should preferably be a reliable proprietary product to achieve uniformity.

(5) Washing in 45% acetic acid and dissection.

(6) Squashing in a drop of acetic carmine on a slide, applying a flat cover slip and tapping for dispersal. Then heating gently to remove air bubbles, introducing another drop of stain under the cover slip if necessary and pressing out under several filter papers. It is here that corrugated cover slips become apparent.

(7) Put molten paraffin wax round the edge of the cover slip to seal off the preparation, if it is worth keeping and is required for further reference.

(8) Examine under a power of x1000. A green filter in the light source has been found to be an advantage in showing up chromatin material.

It is impossible to say when mitotic flushes occur during the day. They may occur at irregular times or at various times according to the variety, but it seems likely that mitosis does occur in diurnal flushes and not irregularly.

The mapping of chromosomes of tropical grasses is inevitably a major task and not within the scope of a non-specific department.
REFERENCES

Introduction

Akamine, E.K. 1943.
The effect of temperature and humidity on viability of stored seeds in Hawaii.

Akamine, E.K. 1944.
Germination of Hawaiian range grass seeds.

Barton, Lela V. and Crocker, W.
Twenty Years of Seed Research - Faber & Faber Ltd., London.

Bass, L.N. 1953.
Relationship of temperature, time and moisture content to the viability of seeds of Kentucky blue grass.
Proc. Iowa Acad. Sci. 60

A resume of experiments and trials with tropical grasses.
Postgraduate Thesis, I.C.T.A.

A developmental study of seven introduced tropical grasses, with special reference to seed viability and storage tests, under Trinidad conditions.
Postgraduate Thesis, I.C.T.A.

Drake, Vera C. 1951.
Some factors influencing the germination of Dallis grass seed.
From Herb. Abstr. 22, 1213.

Evans, D.C.F. 1954.
Preliminary investigations on seed viability, storage and growth of potentially useful grasses under Trinidad conditions.
Postgraduate Thesis, I.C.T.A.

Haferkamp, et al., 1953.
Relation of age of seed to germination and longevity.
Agron. Journ. h5, No. 9, l3h.

Harrington, 1923.
Use of alternating temperatures in seed germination.
Knight, W.E.; 1955.
The effects of photoperiods and temperature of growth, flowering and seed production of *Paspalum dilatatum*, Poir.

Morinaga, T., 1926.
The effect of alternating temperatures on the germination of seeds.

Phillips, et. al. 1951.
Preliminary investigations on the use of thiourea for breaking secondary dormancy in alyce clover seed.

The use of 2:3:5-triphenyl tetrazolium chloride as a measure of seed germinability.
*Plant Physiol.* 22, 151.

Preliminary observations on viability of hulled Timothy seed.

Toole, E.H. and Toole, V.K. 1939-41.
Investigations on the use of 0.2% (KNO₃) as a germinating medium.

Tuley, F., 1951.
Germination tests of seven tropical grass seeds.
*Postgraduate Thesis. I.C.T.A.*
REFERENCES FOR SECTION III

BOOKS
Babcock and Clausen - Genetics in Relation to Agriculture, McGraw Hill Book Co. 1927.
Robertis, Nowinski and Saez - General Cytology - Saunders 1950.

LITERATURE
Belling, J., 1921.
On counting chromosomes in pollen mother cells.
Amer. Nat. 55, 573.
Bradley, 1948.
A method for making aceto carmine squashes permanent without removal of the cover slip.
Stain. Tech. 23, 141-4
Chambers, T.C., 1955.
The use of snail stomach cytase in plant cytology.
Nature 175, 215.
Tetrazolium salt as a seed germination indicator.
Faberge, A.C. 1945.
Snail stomach cytase, A new reagent for plant cytology.
Stain. Tech. 20, 1.
Greathouse, Klemme and Barker, 1942.
Hyde and Gardelle, 1953.
A mordanting fixation for intense staining of small chromosomes.
Stain. Tech. 28, 305.
Jones, K., 1952.
Autotetraploidy in Agrostis canina.
Nature 169, 159.
La Cour, L.F. 1952.
Smears and squashes review.
Lab. Pracce. 3, 326.


Love, R. Merton, 1940.

Chromosome number and behaviour in a plant breeder's sample of pentaploid wheat hybrid derivatives.

McCintock, 1929.

Modified iron aceto-carmine smear technique.
Stain. Tech. 4, 53.

Myers, W.M.

Cytology and genetics of forage grasses.

Myers, J.R. 1945.

Prefixing with para-dichlor-benzene to facilitate chromosome study.
Stain. Tech. 20, 121.


Cytological disturbances influencing fertility in Bromus inermus.
Bot. Gaz. 116, 293.

Sharma and Mookerjea, 1954.

Para-dichlor-benzene and other chemicals in chromosome work.
Stain. Tech. 20, No.1.

Stuart, N.W. and Haswell, S.L., 1943.

The use of enzymes to improve cytological techniques.
Science 98, 569.

Stebbins and Love.

A cytological study of California forage grasses.

Stebbins, G.L., Jr., 1940.

The significance of polyploidy in plant evolution.
The American Naturalist 74, 51.
THE USE OF TRI-PHENYL-TETRAZOLIUM CHLORIDE AS A MEASURE OF GRASS SEED VIABILITY.

As was mentioned in the introduction the use of triphenyl-tetrazolium chloride (T.T.C.) as a measure of seed viability was first made by Kuhn and Jerkel in 1941 and then used by Lakon in 1951 to establish his topographical staining method.

Lakon worked mostly with cereals and especially with maize. He assumed at first that in monocotyledons the whole embryo must be alive in order to take nutriment from the endosperm and germinate. He found however that certain parts of the scutellum need not stain for the seed still to germinate. In fact as long as the live embryo is in connection with the live endosperm through the live internodal part of the scutellum the rest of the scutellum need not stain. In addition, in maize and many other monocotyledons, adventitious roots arise very early from primordia in the epicotyl region behind the radicle at the base of the coleoptile. Since, if the plumule and coleorhiza are quite capable of growth, and the plant is independent of its radicle and coleorhizal, embryos stained only in the regions of the plumule and coleoptile, internodal scutellum and epicotyl region are viable. On this basis Lakon classified a number of staining patterns for maize dividing them into those which indicated viability and those which did not. He continued this work with other monocotyledons and some dicotyledons, mostly pulses. (Helen J. Cottrell 1948).

In certain cases care must be taken not to confuse necrotic areas with stained areas as patterns may be similar (Hyde 1952).

Many workers have used tri-phenyl-tetrazolium chloride and bromide tests in recent years and several modifications of Lakon’s technique have been evolved. Some workers consider only
staining of the embryo to indicate viability and in certain seeds this gives a close correlation with germination tests.

The general technique is to soak the seeds in water overnight (about 16 hours), section through the region of the embryo, immerse in a 1% solution of tetrazolium with cut surfaces uppermost (although staining with surfaces downward makes little difference in results, the former method makes observation simpler - Cottrell '48) and allow to stain for a variable period according to the species, e.g. for Bahia grass about 24 hours (Porter et al.), for perennial rye grass 5 hours at 30°C (Hyde 1952), for rice Miles in 1954 showed 4 hours to be ideal with concentrations of T.T.B. of 0.1%, 0.5%, and 1.0% but obtained almost as good results using 1.0% solution for one hour.

The use of tetrazolium for testing grass seed viability is limited by the hardness and very small size of grass seed which make them very difficult to section and handle in the laboratory. Also they are frequently invested with a complex hull or fascicle which must be separately removed to reveal the caryopsis e.g. Mexican grass (Ixophorus unisetus), Pennisetum, Jaragua grass (Hyparrhenia rufa) etcetera.

Hyde in 1952 working on the seed of perennial rye-grass (Lolium perenne) sectioned the caryopsis through about 2/3 its length so that a sagittal section of the embryo was exposed. This he did, after soaking for 16 hours, with a bent scalpel under a dissecting microscope at X 10 magnification. The seeds were examined after staining (as mentioned above) at X 20 magnification and classified according to their stain pattern. Hyde adds that 'the staining tests were completed within 24 hours and occupied a total working time of approximately 70 minutes for each sample of 400 seeds'.

Porter et alia in 1947 working on Bahia grass (Paspalum notatum) not only presoaked the seeds for 16 hours, but put them in a germinator for three days at alternating temperatures of
20°C for 16 hours and 35°C for 24 hours to achieve optimum results.

Davies in 1955 working at I.C.T.A. on Mexican grass seed (Ixophorus unisetus) released the caryopsis by removing palea and lemma, soaked the caryopses over-night, and then stained them for four hours in 1% T.T.C. He found it unnecessary to section the seed and obtained good results, when correlated with germination, by assuming the seed to be visible only if the embryo was completely stained. The results were not statistically correlated with germination figures, and the test appeared to be rather more accurate in the case of the samples with a high percentage of viable seeds than in the case of the samples with low viability. Nevertheless Davies was very optimistic about the method as applied to Ixophorus unisetus seed.

The author carried out some preliminary tests on rice to gain some idea of the technique. It was established that the pericarp should be removed and the naked caryopsis presoaked. The best staining was then obtained with 0.5% tetrazolium solution, since 1% solution gave very intense and rapid staining in 15 minutes. Dehusked but unsoaked seeds in 1% solution gave almost complete staining in 20 minutes, but 0.5% solution was less satisfactory. Husked rice showed 18% staining after soaking in tap water for 19 hours and the stained embryo was visible through the pericarp. This was not so with presoaked millet as the pericarp was thicker and more heavily pigmented. The germination capacity of the rice samples was almost 100%.

Tests were then continued on Mexican grass (Ixophorus unisetus) seed. The palea and lemma must first be removed to find the small caryopsis. This must then be presoaked and since the brown pericarp completely occludes the embryo, the caryopsis must be bisected longitudinally through the embryo.
The sectioned seeds were immersed in tetrazolium in a watch glass and observed under a dissecting lens. Staining had generally commenced after five minutes using 0.5% tetrazolium solution, which had been found to be the most satisfactory strength. The criterion of viability was a complete staining of the embryo, and staining seemed to be generally complete in half an hour.

The second tests were done with seed of *Pennisetum polystachyon*. These are invested with a fascicle which must be removed before the caryops is found. Methods used were first light burning. This gave a good recovery of seeds but some appeared scorched and a germination test on such a sample showed no germination. Immersion in concentrated sulphuric acid was then tried. This was also effective in recovering caryopses but the action of the acid is fast and the seed may easily be left in too long and lost. 10-12 minutes seems to be the optimum immersion period in industrial concentrated sulphuric acid.

The seeds were soaked in water for eight hours (16 hours is too long), sectioned through the embryo and immersed in a 0.5% tetrazolium solution for 25 minutes. Only one seed showed any staining after this time. It seems likely that 24 hours immersion would have given better results.

The outstanding disadvantage in these tests was the time consumed and the strain involved in sectioning minute caryopses. The time involved per seed was certainly in excess of Hyde's total of 70 minutes for 400 seeds. Laboratory workers trained in handling small seeds and in this technique might well succeed in getting down to such speeds. The method then becomes much quicker than a standard germination test.

However the tetrazolium test serves to indicate viability and as such is unique. Therefore unless an alternative method presents itself the technique must be adapted to grass seed.
It could be much improved by some mechanical method of bisecting the caryopses perhaps with a modified microtome.
REFERENCES

SECTION IIA.

Cottrell, Helen J. 1948.
Tetrazolium salt as a seed germination indicator.
*Annals, App. Biol.* 25, 123.

Hyde, E.O.C. 1952.
Methods for determining the viability of various seeds by tetrazolium staining. 2. Perennial Rye grass.

Lakon 1951.
Further studies of the topographical tetrazolium colour test and the determination of seedling vigour.
From :-- *Herb. Abstr.* 22, 1220.

Miles, J.J. 1954.
The use of triphenyl-tetrazolium bromide in viability tests of rice seed.

The use of 2,3,5 triphenyl-tetrazolium chloride as a measure of seed germinability.

Ritvaneer, T. 1953.
The application of tetrazolium chloride for rapid determination of germination capacity in seed of Phleum pretense.
From :-- *Herb. Abstr.* 24, 477.