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LAB REPORT #18: AN INTRODUCTION

As you know , Dr. W.C. Levengood has generated a series of lab reports related to his study of crop circles. Beginning with this report, the reader will find introductory highlights presented in non-technical terminology. It is our hope that this may help facilitate communication regarding the findings to readers of a non-scientific background.

Report #18 further defines the oscillation method currently being used to analyze formation and control samples from crop circles, etc. The method was introduced in Reports #16 and #17. After months of hard work this method has been refined as a valuable tool, not least because the technique considerably automates this time-consuming work and eliminates the "subjective" choice of objects to measure (i.e. cell wall pits), previously viewed as an objection by some.

The seeds of wheat, oats, and barley are surrounded by a thin tissue, or membrane, called the bract. The energies which create crop circles somehow alter the structure of this membrane in such a way that (in comparison to controls) it changes the rate at which ions (electrically-charged particles) are transported across it.

The methodology is as follows. The bract tissue is removed from the seed and attached to a positive and negative electrode at either end (Fig.1). A small, low-power current is run through the tissue. While this is happening the bract is subjected to internal mechanical stress by means of the changing water pressure induced by short, scheduled periods of evaporation. This method was adapted from a 1985 study by Levengood, one page of which is excerpted in Figure 3. Bracts which have been subjected to crop circle energies are permanently changed so as to transport more ions across the bract membrane. This means that more electric current can cross the membrane. It is the differences or changes in this current which are measured while internal water pressure is applied and then released (Fig.2). The key factor measured is the"amplitude coefficient" (hereinafter called "alpha"). Alpha is equal to the minimum current recorded divided by the maximum recorded as it crosses the membrane during periods of stress or rest. Obviously what we are looking at here is proportional change. It is not the amount of current,(which varies by plant and setup) that matters but the proportional current change. This reveals how much the membrane has been altered.

A new method has also been adopted for statistical use of controls. Now, for every measurement taken on a circle sample, a measurement is taken on a control and the two are compared. The odds that a circle sample's alpha value is higher than a control's by pure chance is 1 in 2, or 0.5. The odds that alpha values would be higher by pure chance in 6 samples vs. 6 controls is 0.5×10^{-6} -- less than one chance in a million!

Correlation of the oscillation method with the previous method can be seen in Figure 4. In Lab Reports #1-14 a method was developed using a factor that combined: 1) the change in rate of seedling development from circle seeds vs. controls, and 2) the percentage increase in cell wall pits (not to be confused with stomata). The oscillation method dispenses with cell wall pit measurement. As Fig.4 shows, seedling development rates generally match the alpha values determined by the oscillation method, demonstrating the accuracy of this new technique. Samples and controls that had been examined with the old method over the past few years have been laboriously re-examined with the oscillation technique and the results agree. Thus this is a further confirmation of the earlier work and an indicator of continuity.

Note that crop circle energies are not neatly predictable in a linear fashion but exhibit some characteristics of deterministic chaos. In Figure 2, sample "Ring-5" has only slightly higher alpha value and greatly higher growth rate. Nevertheless ring-5's alpha value is above any control, and such a result is to be expected occasionally from any event involving turbulent, chaotic energies.

Note that differences in energy levels within the formation can be traced by comparing the alpha values of the samples. And perhaps most exciting, a "proximity effect" has been found (see sample "Prox-10" in Fig.4). Standing crop up to a short distance from a circle shows the effects of circle energies. Control samples are taken at least 100 meters away and show no effects.

A final, important note. In 1993 numerous samples were examined that came from deliberately "hoaxed" wheat, flattened by us and sampled at varying time intervals. In none of these were higher alpha values or growth rates observed. It is strongly felt that this and previous work now places the "burden of proof" squarely on the shoulders of alleged hoaxers. If they are to have us believe their claims, then they must show us how they changed the alpha factors and seedling growth rates in the crop they flattened. Further, theyymust show how they produced this effect in standing crop at the edge of the formation. If they cannot show this, why should we waste more valuable time listening to them?

October 12, 1993

RESEARCH REPORT: PINELANDIA BIOPHYSICAL LAB.

Technique for Examining Crop Circle Energetics

I. Introduction

In a monograph issued last March (Report No. 15) data were presented which related to the very early investigative phases in a research program directed toward establishing a method which would clearly distinguish between crop circle formations and normal plant growth (including of course man produced plant crushing). In general this research was based on the hypothesis that, since very well defined, significant differences have been observed in the cell wall pit size data from bract tissue obtained from crop formations, one might also anticipate physical alterations in the cellulosic microfibrils comprising the cell walls of the bracts and seeds. This report discusses the confirmation of this concept and its use as a verification method for examining the distribution of energies within crop formations.

The main purpose here is to present the reader with a general understanding of how this method is applied to the crop formation problem and to introduce data from a typical sampling problem. This is not intended as a "how to - procedure manual"; to do this would entail considerable technical details not really of interest to most readers concerned with the crop circle phenomena. In the future this quantitative technique for examining crop circle formations will be referred to as the "OSC" (induced oscillation) method.

Following the months of work in the early exploratory phases it became quite apparent that there were a number of issues which had to be examined before embarking on any scheduled program of direct application to the crop formation problems. These early data clearly suggested that the cell wall microfibril configurations were being altered by the crop formation energies; however, a number of critical questions remained as to the function of the parameters within the experimental arrangement and their influence on the variabilities within the OSC method. The following is a list of the most important issues.

1) It was important to understand the basic mechanisms involved in the OSC method and the recorded responses from the hydrating tissues.

2) Conduct apparatus modifications until one approached a minimum in the experimental errors relative to the natural variations occurring in the plant tissues.

3) Completely understand the nature of the responses and be able to precisely control the periods of the induced oscillations.

4) Conduct a large statistically based study to determine the levels of significance between the alpha values obtained in crop formation tissues compared with those from normal, upright plants (controls), lodged and man crushed material.

5) Examine the question - is there any relationship between the OSC data (mean alpha values) and the crop formation energy-induced transitions in the stem nodes, cell wall pit sizes and enhanced seed development (or embryo termination in the early, immature plant material)?

All of these issues have been critically examined and there appears to be no problems or barriers preventing the use of the OSC method as a verification tool. As a final comment it should be again pointed out that this is an automated system and the final data are presented on a chart recorder. In other words no observations or measurements are made by the experimenter until after the test run, at which time the alpha values are taken from the recorder chart and entered into the computer for analyses.

II. Details of Verification Method

The experimental procedure is based on the automated monitoring of controlled, induced oscillations in the transport of ions through the bract tissues of plants. The magnitude of these oscillations relate to the structure of ion channels or submicroscopic, molecular pores (not to be confused with cell wall pits) in the cell membranes of the plant. In general the tissue is placed under conditions of non-linear oscillation kinetics. Within the bract membrane system there is a coupling between electrical and mechanical forces.

The mechanics of creating this electrokinetic situation is summarized in Fig.1, in a highly schematic form. The wheat bract is used as an example; however, the method has also been successfully extended to *Avena sativa* (oats), *Hordeum vulgare* (barley) and *Zea mays* (corn).The bract is removed from the floret and placed across the surrogate electrodes (cotton swabs) saturated with deionized water. The dc voltage applied across the electrodes produces electrophoretic transport through the bract tissue (see comment at bottom of Fig.1). The controlled oscillations are induced through the application of hydrostatic pressure pulses (P) within the microfibril network of the plant cell walls. The experimental details of producing P will not be discussed here.

This general type of mechanoelectrical transduction methodology has been presented under the framework of "The Teorell Oscillator Membrane Model". Teorell[1] developed this concept as a suggested model for explaining electrical oscillations in animal nerve fibers, and it was later pointed out by Page and Maeres[2] as being implicated in electro-osmotic-ion-water flux coupling in plant cells; however, these workers used a synthetic membrane in their experimental system. The existence of an induced electro-mechanical oscillatory transduction in the cellulosic microfibrils of seed testa was shown by Levengood[3] in 1985. Reproduced in Fig.2 is the page from this ion transport paper showing the electrical response to the mechanical stimulations.

References:

(1) Teorell, T. (1966) Electrokinetic Considerations of Mechanoelectrical Transduction. Annals New York Acad. Sciences, pp. 950-966.

(2) Page, K.R. and Meares, P. (1973) Solute-Water Interactions in the Teorell Oscillator Membrane Model. in Ion Transport in Plants, ed P.W. Anderson, Academic Press, N.Y., pp.65-75.

(3) Levengood, W.C. (1985) Ion Transport in the Testa of Germinating Seeds. J. Exp. Botany, 36, pp. 1053-1063.

The main purpose of presenting this historical perspective is to provide the reader with references if further reading is desired, and to

demonstrate that the procedure, although complex is based on sound scientific principals.

Pressure pulses (P) are controlled to provide 12 min. periods (6 min. on-off) in the induced oscillation within the ionophoretic transport. The chart response curves in Fig.3 provide an example of the large amplitude (alpha level) differences which are often obtained between crop formation and control samples. From these chart recorder traces, detailed statistical analyses of the oscillation patterns have demonstrated that the important factor (in relation to the crop circle verification procedure) is what has been termed the "amplitude coefficient" or alpha (α) for brevity purposes, and is given by;

$$\alpha = (A_m / i_m)$$

where i_m is the peak or maximum ion current for that cycle and A_m the amplitude of the current (determined by the base line level of the minima), as indicated by the arrow in Fig.3, pointing to the first cycle of the formation test.

Each sample run provides five alpha values per trace. The current procedure involves six replicate tests on individual bracts (each selected from a different plant if available). Controls and crop circle samples are ran in alternate tests. The 30 data points (alphas) are entered into a computer program ("Statview") which provides a convenient means of statistically analyzing many aspects of the data population. The most reliable, consistent information from the alpha values is based on a statistical analysis of the paired, thirty data point alpha populations.

For the reader to understand the time expended on a verification test the following table was prepared from an actual test run (to be discussed in the following section) performed on oat samples submitted by Mr. Chad Deetken in 1992. The sample group included seven sets (6-8 plants per set) from the formation and three controls - typical of a good sample collection.

a) For a 10 sample set with three replicated pairs this gives 30 test runs total:

b) Operator time:

Sample preparation and electrode cleaning -
about 10 min./test run (300 min. total) ---------- 5 hrs.

Verification Method

Alpha calculations from raw data taken from
chart recorder and entering into computer ---------- 5 hrs.

Total 10 hrs.

c) Apparatus running time-
at 1.5 hrs./test run ----------Total 45 hrs.

It should be pointed out that verification tests become more informative the greater the number of samples and controls submitted from a particular formation.

III. Crop Formation-Application of Method

In Report No. 16, issued June 23, 1993, are results from studies conducted on two crop formations formed at Albertsville, Canada, one in a wheat field and one in oats. Both formations have been verified with the new technique. The results from oats (KS-01-94) were selected here to demonstrate the application of the method. The diagram of this formation as shown in Report No. 16, locates three samples within an inner circle, three in an outer ring, one "proximity control" taken 10 ft. radially from the outer ring and three controls taken 250 ft. from the formation.

The mean amplitude coefficients or alpha values from all ten samples are summarized in the upper bar chart in Fig.4, with the sample designation below each bar ("Cir-1" means sample #1 from the circle, etc.). The horizontal dashed-line shows that all seven formation samples have mean alpha values (four with $P<0.05$) which lie above the highest mean in the controls (Cont-7). Let us now consider the possibility that this happened by chance. If we select one formation sample, there is a 0.5 probability that it will lie above a control sample (neglecting the rare chance of identical values). The probability that this isolated sample will lie above all three control samples is $(0.5)^3$ or 0.125, which is not of high significance. If we now consider the probability of the mean value in all seven samples being higher than all three controls we obtain $(0.125)^7$ or about 0.5×10^{-6}, that is, less than one chance in a million for this distribution of the alpha values in the formation samples being totally random.

The lower bar chart in Fig.4 summarizes the seedling development factor (also discussed in Rep. No.16) obtained from seed germination data within this same sample group. The dashed line serves the same purpose as discussed above, and here again we find all seven formation bars lying above this line (although Ring-4 sample is only slightly above). Applying the same arguments of probability we again find a less then one in a million chance of this distribution being a simple random pattern.

IV Comments

Implications arising from the data presented extend considerably beyond the simple procedure of determining the "authenticity" of a crop formation. Below are a number of comments which may help to illustrate this point.

a) The two sets of bar chart data in Fig.4 not only have roughly similar patterns, but they also clearly suggest that the crop formation energies are influencing both the macromolecular structure of somatic tissues (bract) but also the biosynthetic pathways in the germinating seed tissue.

b) Variations in the mean alpha values are quite apparent throughout a crop formation and if sufficient samples are available it not only establishes the fact that the transient energies produce phenomenalistically consistent residual energy signatures, but may also provide a means of mapping the energy gradients within a formation.

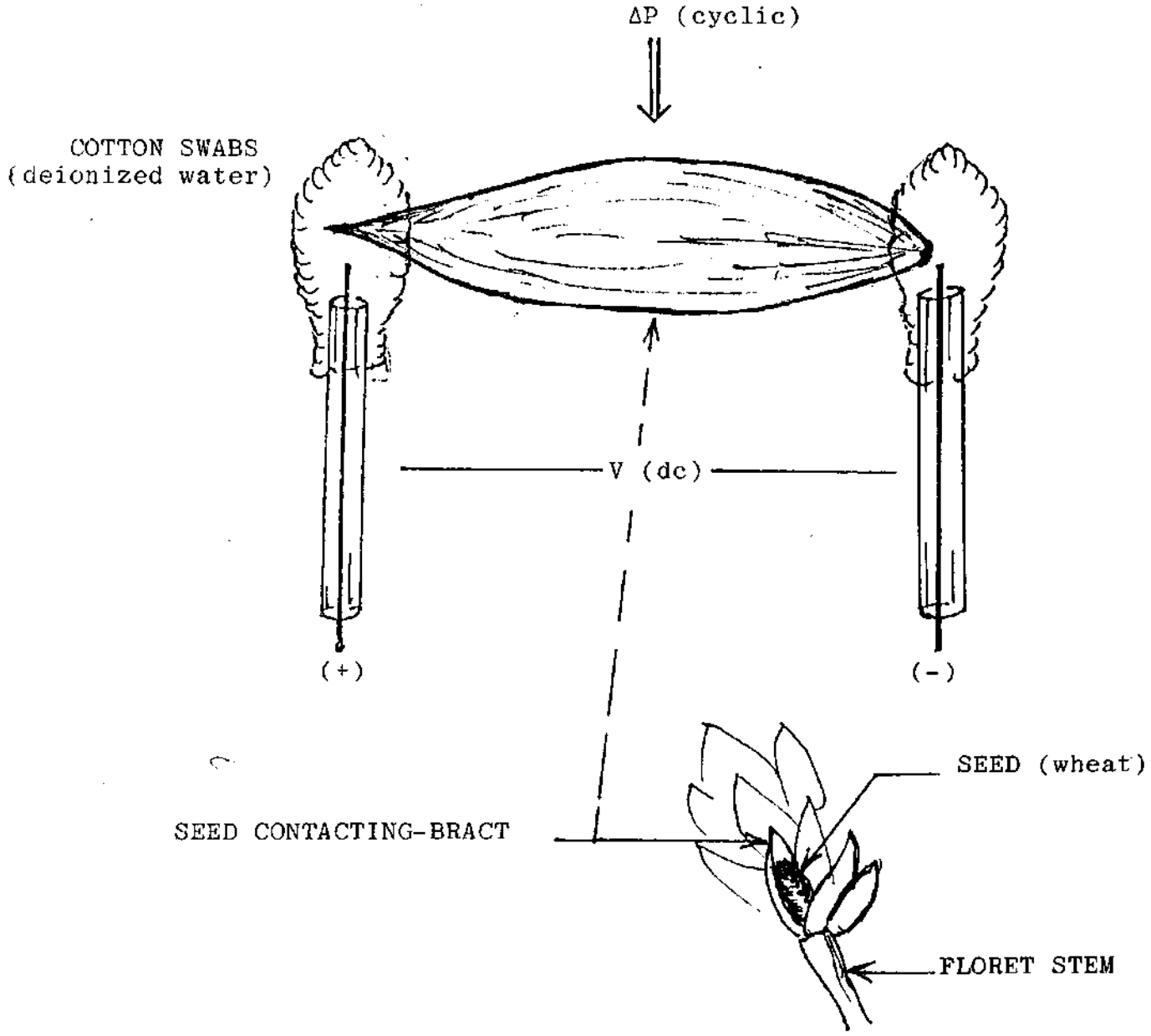
c) The high alpha mean for the "Prox-10" sample (far right in the bar charts of Fig.4) is way out of line with the "Cont.-7-8-9" samples and again confirms the fact that the energies creating the seed and bract tissue alterations can "spill over" and extend beyond the formation (also observed in the Kennewick formation, Rep. No.17).

d) During the course of establishing this procedure, numerous test series were conducted on reported lodged plants and man produced plant crushing. Most of these were from 1993 test series sampled by Nancy Talbott, John Burke, Chad Deetken, et al. In none of these tests has there been a statistically significant increase in the OSC values from the lodged or downed plants. Most of these data will be discussed in future reports where they apply to the overall sample survey.

Dr. W.C. Levengood

Fig.1

SCHEMATIC DIAGRAM OF CROP CIRCLE VARIFICATION SYSTEM



Ionophoresis under conditions of controlled oscillations of hydrostatic pressure (ΔP) within the microfibril network of the bract tissue. System modified from; Levengood,W.C., "Electrophoresis in plant cell organelles", Bioelectrochemistry and Bioenergetics, 25, pp.225-239 (1991), to produce electro-osmotic flux coupling.

Fig.2 Example of elctro-mechanically induced oscillations.

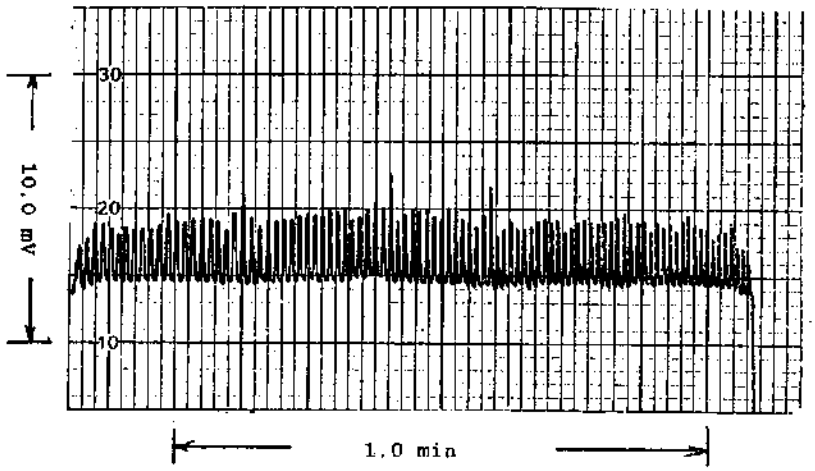


FIG. 5. Electrical conductivity oscillations in a partially hydrated *Helianthus annuus* pericarp section given mechanical flexure. Potential of 1·5 V applied across sections about 3 × 10 mm with mechanical bending stresses applied at about 1 s intervals.

cells exposed to light (and the accompanying heat). For example, Zeiger, Moody, Hepler, and Varela (1977) report an increase in membrane potential difference with light exposure (thermal energy) in *Allium cepa* guard cells. Bentrup, Gratz, and Unbehauen (1973) in addition to showing a general increase in membrane potential with light-on in leaf cells from *Vallisneria spiralis* also observed a transient hyperpolarization with light-off and a transient de-polarization with light-on; exactly opposite the responses shown in Fig. 1 with the seed coat hydration. One important difference between these fully hydrated cell walls and the seed coats is the state of cell wall flexibility. In the seeds the cell walls are still very rigid and moisture is at a low level. The factor controlling ion movement in the seed coat tissues is micropore alterations. In the fully hydrated cells the situation is more complex with photosynthesis and electrogenic ion transfer occurring in the more hydrated, flexible membranes.

As rapid, transient changes in ionic concentrations and pressure differences are imposed on the cell walls, very pronounced and rapid changes in electrical conductivity may occur. That such rapid changes are possible under conditions of varying cell wall extensions was clearly demonstrated in the preceding section dealing with mechanically stressed microfibril networks. This rise and fall in potential with applied stress (Fig. 5) may depend on how the spatial pattern changes in the cellulosic microfibril network. The direction of the bending in the seed coat sections is approximately at right angles to the lateral microfibrils in the cell walls, extension of which could have a profound influence on the molecular bonding and surface configuration of the media supporting ionic conductivity (Green, 1962). A simple two dimensional analogue of these spatial changes may be seen by holding a piece of cheese-cloth up to a light source then stretching along one or both axes. Quite marked changes are observed in the pore sizes and shapes due to mechanically induced alterations in the macrofibre network. It has also been suggested by Steudle and Zimmermann (1974) that cell wall extensibilities and pressure effects may account for observed oscillatory patterns in the electrical responses of plant cell membranes. Page and Meares (1973) were able to produce electrical potential oscillations by creating pressure differences across artificial membranes.

Fig.3 Chart recorder traces showing induced oscillations in a formation and control sample set.

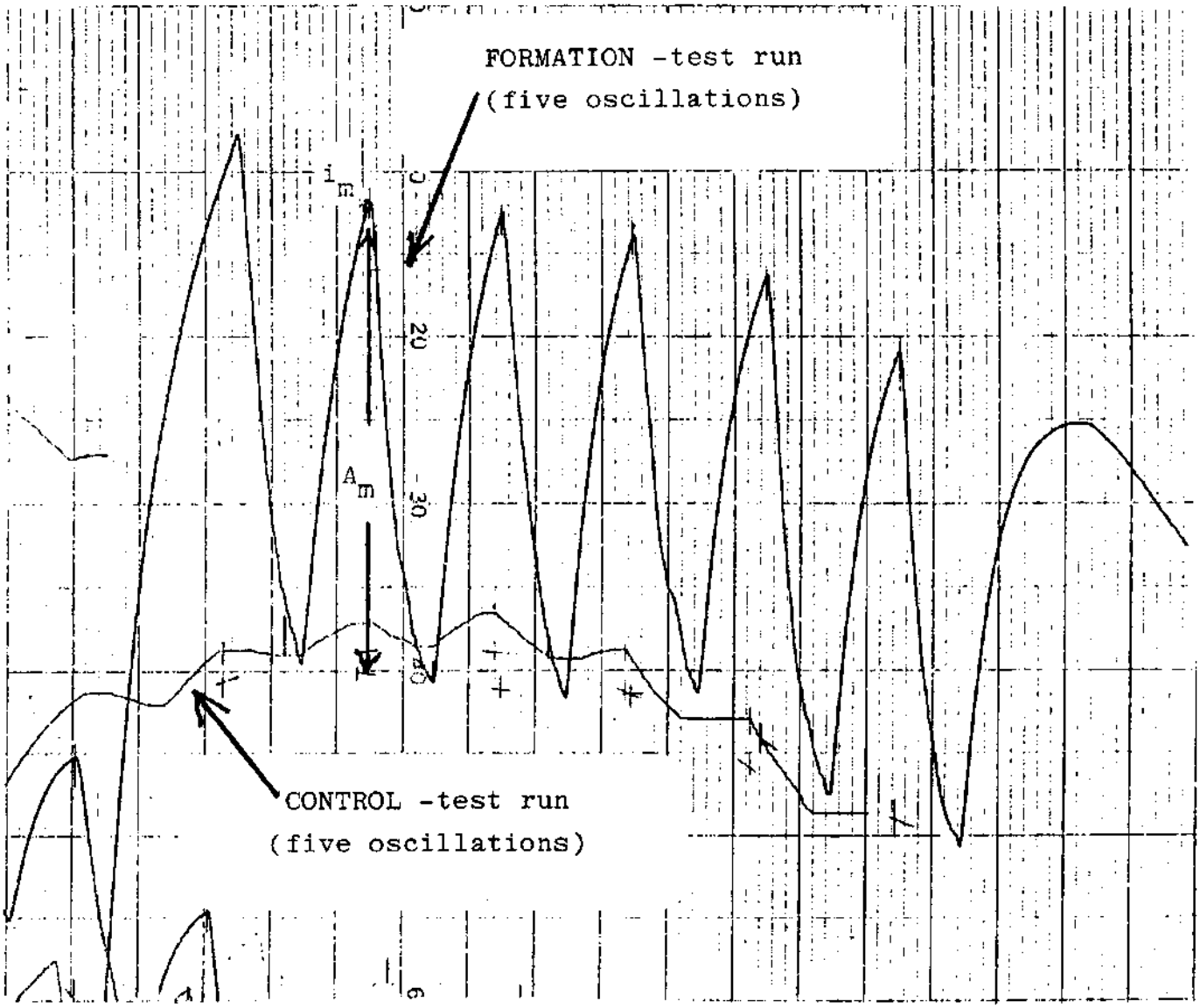
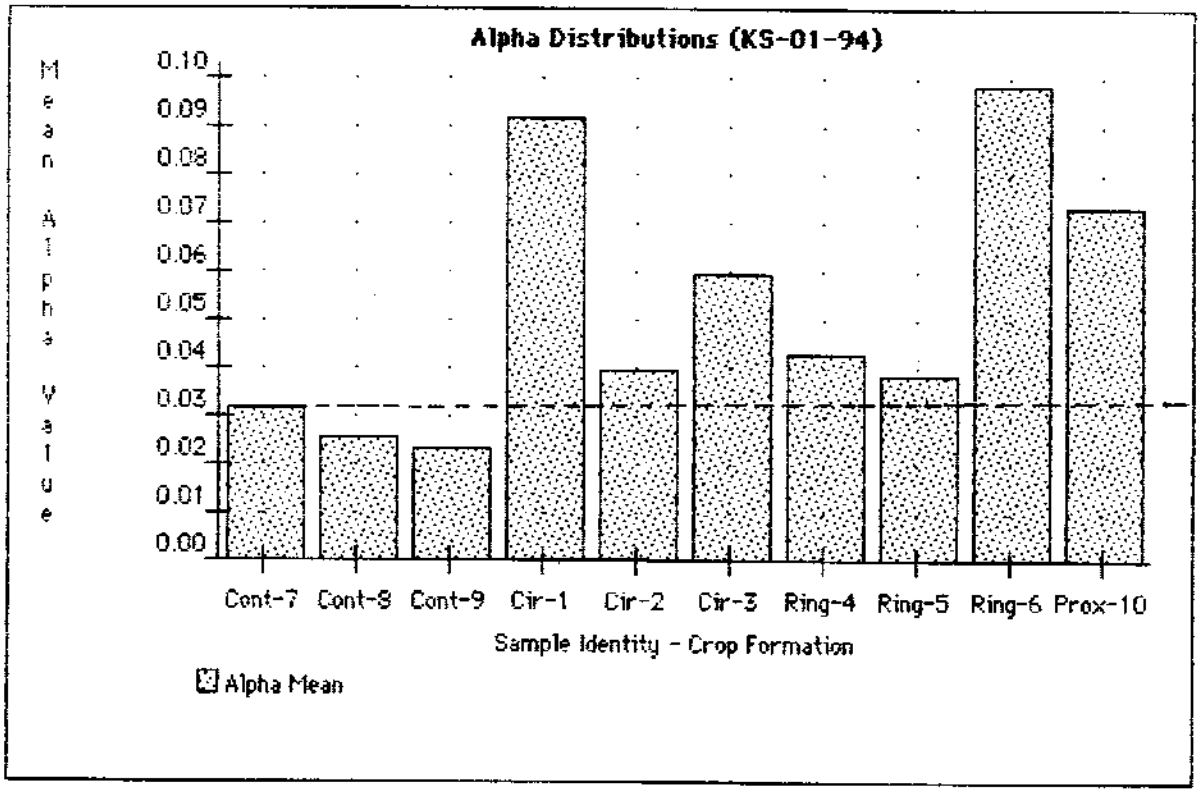
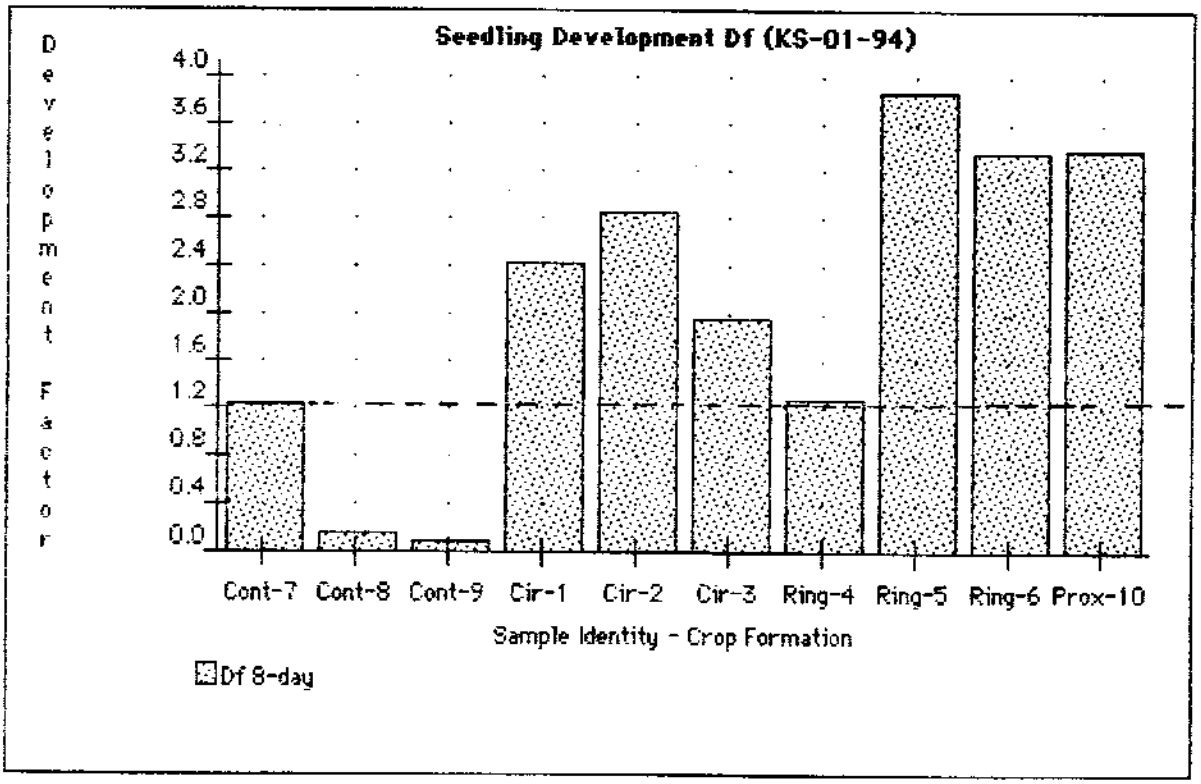


Fig.4 Alpha values compared with seed growth data from a Canadian crop formation.





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