

KS-01-32: Page-1

Sept. 20, 1992

SAMPLE REPORT: PINELANDIA BIOPHYSICAL LABORATORY

LABORATORY CODE: KS-01-32

PLANT MATERIAL: *Acorus calamus* (sweet flag) - aquatic plant with single leaf blades 5-7 ft. tall.

FORMATION: Circle of downed plants about 40 ft. diameter with about a 5 ft. dia. patch of standing plants at the center point. Located at Troy, Illinois; probably formed in early June.

COLLECTED BY: Mr. Peter A. Bostrom, Troy, Illinois.

COMMENTS ON SAMPLES: The plants were received in excellent shape and this was one of the most informative and completely documented sample sets submitted to this lab. - included were photographs, discussed in a 10-page report, a sampling map and a VCR of the formations last year. It is important to note that this circle was an almost exact repeat of one last year of 35 ft. diameter and again with the standing, upright cluster at the center. Mr. Bostrom's report also contains other interesting comments about these formations.

LABORATORY STUDIES:

1.) Cell Wall Pit Examination - in this species the cells were very large and the wall pits were not readily located. Considerable time was expended examining various leaf cell layers in an attempt to find a tissue type which could be used in a pit size analysis. Finally after this long, frustrating search a very thin layer of cells was located immediately beneath the epidermis of the very thick leaf mid-rib.

The pit diameters were compiled in the usual manner and the statistical analyses conducted. The mean cell wall pit diameters (in microns) and s.d. values have been superimposed on a photograph taken by Mr. Bolstrom, as shown in the attached figure. In parenthesis are the differences, expressed in percent, between the circle sample and the control value. In general there appears to be an increase in the pit expansion from the center to the edge of the formation. This would suggest a gradient of the transient energy across the formation.

II.) Examination leaf rippling effect - this was discussed by Mr. Bolstrom in his 1991 report and shown in the VCR tape. He describes this effect very well - "Many of the leaves (in the circle plants) exhibited an odd rippling effect. They looked shriveled as if they were in the process of drying up, but were as green as the surrounding leaves without this effect." The wavy leaves were not seen in the controls or normal upright plants outside the circle region.

In order to examine the various regions of interest on the leaves, with a minimum of disturbance to the tissue integrity, a surface replica technique was employed. Polystyrene in an acetone solvent was spread on the leaves and allowed to dry, after which it was peeled off and microscopically examined. If applied correctly this gives an accurate profile of the surface details.

All six of the sample groups were examined, within the rippled regions, outside, and on normal control leaves. The cell walls and stomate regions were seen in clear detail and there were no apparent differences between the surface characteristics of the circle samples (either within or without the wavy regions) and the control leaves, with the exception of one notable difference in the stomates on sample-#5. The stomates in the "corrugated" region on samp.#5 appeared to be filled with a crystalline deposit which was not seen to the same degree in the other samples or controls.

III.) Simulation of Leaf Ripples - examples of the rippling effect in the submitted circle samples, are shown in the attached photograph (tears in leaves occurred during shipping and storage). It is interesting to note that sample-#5 disclosed the most pronounced ripple effect and this was also the sample giving the maximum value of cell wall pit expansion (and heavy crystalline deposits within the stomates).

Attempts were made to mechanically reproduce this leaf wrinkling with no success. If however, transient heating was applied in the form of about a 10 sec. exposure in a microwave oven the ripple effect is immediately apparent along the leaf edge. In these experiments leaves were taken from the control (non-rippled) set. This readily simulated effect again suggests the presence of a very rapid, transient heating in the field formation process.

The physics of the ripple formations is relatively simple. First it only occurs along that portion of the circle formation leaves, where the leaf mid-rib is very pronounced (about half way up the plant). The bottom portion of these plants is cylindrical shape and the top is a flat leaf with

a far less pronounced mid-rib. When a heavy mid-rib section of a leaf expands during the rapid heating the central portion is constrained by the heavy mid-rib (dehydration would be slightly greater on the outer portion of the leaf). During the cool-down (immediately after the exposure) the outer leaf edge is free to contract whereas the inner tissue is held in position. This sets up stresses which are partially released through a wrinkling of the tissue on the outside edge of the leaf. It is like taking a strip of the old fashion crepe paper and periodically stretching one edge - one ends up with the same type of rippled effect. This heating under field conditions could be very brief and would not substantially effect the plant cell integrity and the normal appearing, external green color.

SUMMARY:

The pattern of the cell wall pit expansion across the circle formation and the presence of the leaf wrinkling or ripple effect indicates a very rapid, high heat content, transient energy involved in the circle formation. This type of energy is consistent with what has been indicated in other crop formations both in the U.S.A. and in the U.K. Why it occurred at the same location, two years in a row, remains an enigma.



Dr. W.C. Levengood



Pit. Diameter Control 2.19 ±0.34 (dia. in microns)					
2.67 ±0.54	3.30 ±0.76	3.09 ±.60	2.94 ±0.72	2.43 ±0.61	2.73 ±0.59
(+21.9%)	(+50.7%)	(+41.7%)	(+34.2%)	(+11.0%)	(+24.7%)
Samp. #6	Samp. #5	Samp. #4	Samp. #3	Samp. #2	Samp. #1

Code: KS-01-32 Mean cell wall pit diameters in Acorus calamus tissue taken at the locations of sampling (N=30 random pits per sample). Arrows are superimposed on a photograph of the 1991 circle - photo taken by Mr. Peter Bostrom. Locations are approximate, but Mr. Bostrom reported that the 1992 formation is almost identical in structure. Control sample taken approximately 50-ft. from circle

(all circle pit dia. values are statistically significant at P<0.05)

KS-01-32 Ripple effect in
leaves from circle samples.
Sweet Flag (Acorus calamus)

Samp. #5

Samp. #3

Samp. #4

