UNIVERSITY OF WESTERN ONTARIO
London, Ontario, Canada

1. <u>Further observations on an "opposite leaf" phenotype</u>.

In MGCNL <u>41</u>: 197 we described briefly a plant with opposite leaves. Premature comments were presented concerning the heritability of this phenotype; during the 1967 winter and summer crops, an additional 61 plants presenting the "opposite leaf" phenotype have been studied.

The progeny data include:

1967 planting	Number of plants	
	"Opposite leaf"	Normal
Original plant, ⊗ ear A winter	6	25
summer	6	38
ear B winter	2	29
summer	9	30
subtotal	23	122
Normal sibs X "opp. leaf", summer	25	166
Normal sibs X Normal sibs, summer	3	69
Open pollinated "opp. leaf", summer	10	16

Random distribution of the respective "opp. leaf" kernels on the ear has been observed in all cases.

Progeny from outcrosses to unrelated stocks, using "opposite leaf" plants as males and/or females have not yet been classified.

Sporocyte analysis of two "opposite leaf" plants has yielded:

Opposite leaf plant number	Number of meiocytes								% Abnormal meiocytes	% Sterile pollen
	Normal				with bridges		with laggards			
	M_I	A_I	M_{II}	A_{II}	A_I	A_{II}	M_I-A_I	M_{II}-A_{II}		
5402-45	91	45			12		30		29	26
5402-50			71	66	7			52	40	33
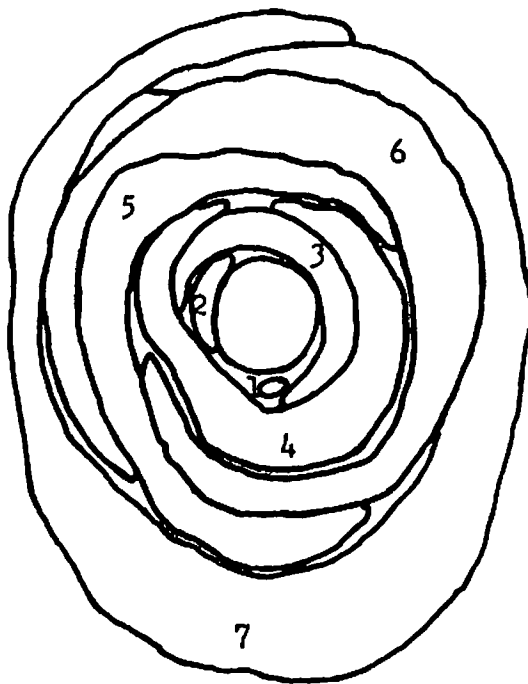
FIGURE 1
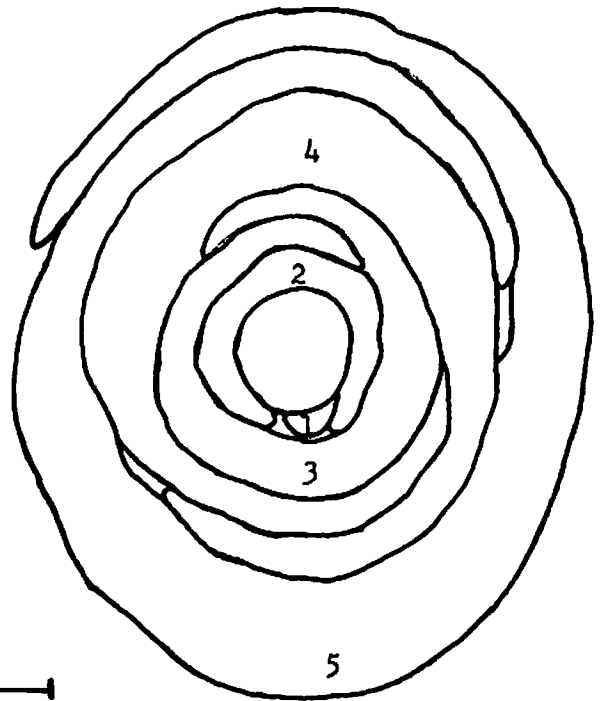
0.5 mm

FIGURE 2

LEAVES ARE NUMBERED BEGINNING WITH YOUNGEST PRIMORDIUM

NORMAL PLANTS

ABNORMAL PLANTS

BLADE WIDTH AT MID-POINT (cm)

NODE NUMBER FROM BASE
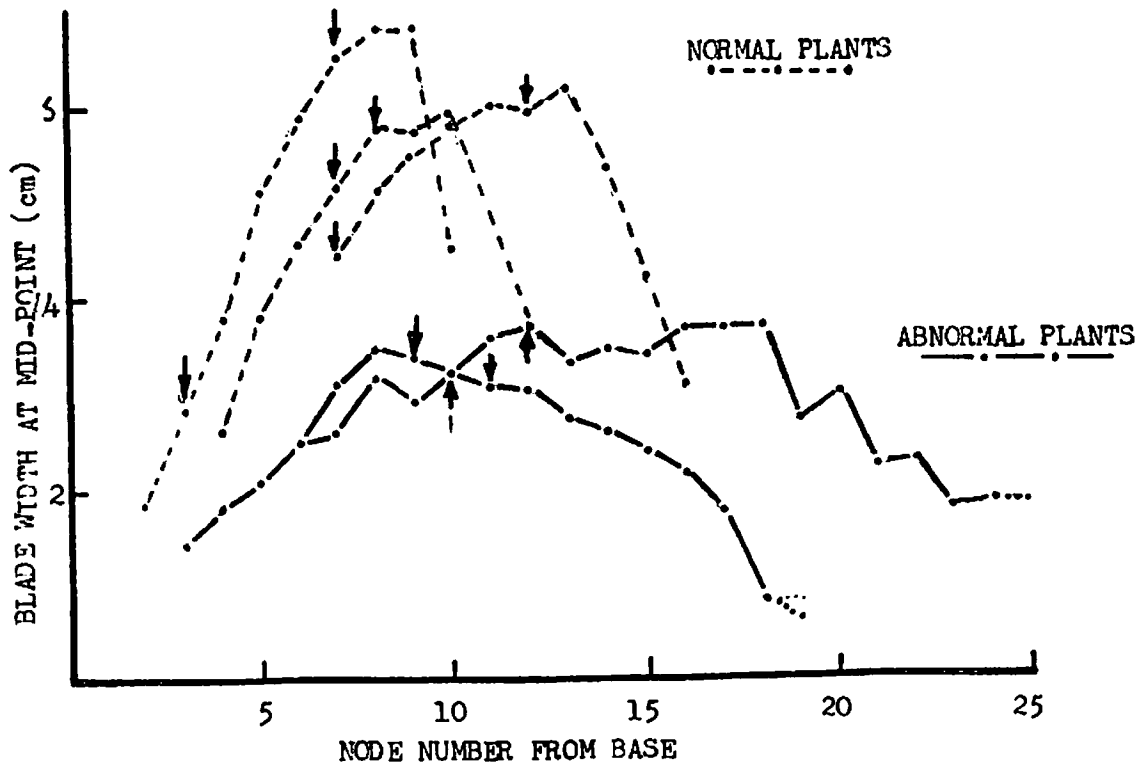
ARROWS INDICATE NODES BETWEEN WHICH EAR SHOOTS ARE LOCATED

FIGURE 3

Pachytene analysis has failed to reveal the chromosomes involved in a possible aberration and aneuploidy. Mitotic karyotyping has not been initiated. No B chromosomes were observed in pachytene and the parental stock had no previous history of any aneuploid condition.

In addition to the production of opposite leaves, these "opp. leaf" plants exhibited other modifications of leaf arrangement and structure. These include special arrangement of leaves usually accompanied with some internodal fusions, reduction in leaf width, and increased number of nodes. One plant produced a number of spirally arranged leaves with "between-leaf-fusions" prior to the production of pairs of opposite leaves above the ear shoot. Another, however, had opposite pairs inserted at nodes 7 and 8 followed by individual leaves, spirally arranged, to the base of the tassel. A number of plants produced a series of spirally arranged leaves with rather serious internodal fusions. The possibility that the varied phenotypic expressions were a product of environmental interactions seems worthwhile for future study.

Fig. 1 represents a cross section below the tip of the youngest leaf primordium (1) from an abnormal plant. This apex was collected prior to the tassel initiation. This section clearly illustrates the spiral arrangement of the leaves and can be compared with the distichous arrangement of a similar section from a normal plant (Fig. 2). Besides this spiral phylotaxy, the width of the sheath and the amount of sheath overlap seem considerably less in the "opp. leaf" plant than in the normal plant.

Leaf width of "opp. leaf" plants is generally considerably less than in normals and can be used to identify abnormal plants (at the 4-5 leaf stage) before the recognition of other characters is possible. In Fig. 3, blade width taken at the midpoint of the leaf blade is plotted at each node, for 3 normal and 2 abnormal plants.

Measurements were made on plants that had reached maturity under similar conditions and over the same period of time. Arrows indicate the nodes between which rudimentary or mature ears were located.

On all plants a certain number of the youngest leaves were dead and lost. These data suggest that in most cases leaves from "opp. leaf" plants were up to ½ the width of comparable normal leaves. Further it appears that the node number at which ears were produced is not altered appreciably in these abnormal plants.

The "opposite leaf" phenotype appears to include a broad spectrum from the largely spiral phylotaxy possessing a few nodes with true opposite leaves to an almost completely opposite leaf plant. The conversion which has been seen in almost every specimen from alternate to opposite leaf insertion may be influenced by environment as our winter grown (greenhouse) materials have thus far presented the more extreme spiralling. The availability of a large seed source will permit further study of these morphogenetic aspects.

The idea that specific genetic information controls leaf insertion and thus the phylotaxy of the plant is not new. However, we are presented with a system for the study of other problems which in turn may provide suggestions on how the architecture of the corn plant may be further modified. The applied applications of this project are being considered.

R. I. Greyson
D. B. Walden

2. <u>Acrylamide gel electrophoresis of maize pollen protein.</u>

Pollen proteins have been separated by electrophoresis on acrylamide gel columns in pairs of large polyethylene reservoirs holding fourteen tubes per pair. The gels were prepared according to the method of Ornstein and Davis; the best travel and separation was obtained using a tris-glycine buffer of pH 9 in both upper and lower reservoirs and a current of 5mA per tube. The gels were stained with amido black and destained electrophoretically in an apparatus which allowed the current to be applied across the gel column.

Several preparations, obtained by subjecting the extracted protein to such preparatory treatments as ammonium sulfate precipitation and dialysis gave poorer resolution than the crude extract. The latter was prepared by homogenizing the pollen in cold mannitol buffer, 0.5 M pH 8.0, centrifuging at 20,000 g for 10 minutes and adding the supernatant to the columns.

Pollen and other parts of several different genotypes are being examined by this method.

D. B. Hayden
F. S. Cook

3. <u>The separation and detection of some dehydrogenase isozymes in maize pollen.</u>

The electrophoretic separation on acrylamide gels described above was used to detect several dehydrogenases in the pollen of "Seneca 60" ($\underline{su}_1/\underline{su}_1$). Soluble protein extracted from fresh pollen in the mannitol buffer of pH 8.0 was separated and detected on the gels using a system containing nitroblue tetrazolium (Fine and Costello, Methods in Enzymology, Vol. VI).

The method used differs from that of Ornstein and Davis in that the protein sample was mixed with a solution of 50% sucrose and was added directly on top of the large pore gel without any further polymerization. When this method is used, care must be taken to add the buffer in such a way that there is a minimum amount of agitation.

Two or more isozymes of the following NAD-dependent dehydrogenases were detected: malic, glutamic, lactic and alcohol. Five distinct isozyme bands were found for malic dehydrogenase.

D. B. Hayden
F. S. Cook