A STUDY OF THE NATURE OF RESISTANCE OF DENT CORN

TO DIPLODIA ZEAE (SCHW.) LEV. STALK ROT

by

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INTRODUCTION

In the United States, 'Diplodia disease', caused by _Diplodia zeae_ (Schw.) Lev., is one of the most prevalent diseases of dent corn. The corn belt from Nebraska eastward seems to offer conditions best suited to its development, but it has been reported as far south as Florida and Texas, and has occasionally been observed in the Pacific Northwest. For the year 1938, the estimated annual reduction in yield from _D. zeae_ in Illinois alone, was 60,777,000 bushels or twelve per cent. During this same period a loss of 102,000 bushels or 0.5 per cent was reported for Maryland (34).

'Diplodia disease', though non-systemic, can under optimum growth conditions, attack practically every portion of the maturing corn plant. It also assumes the form of a seedling blight. The most evident and destructive form of the 'Diplodia disease' is the molding or rotting of the ears. Root rot also is quite prevalent and destructive. Reddish or purplish spots of varying size and shape on the leaf sheath are caused in part by this fungus. A less evident, but destructive form of injury due to _D. zeae_ results from its attack upon stalk tissue of the maturing corn plant. This stalk rot reduces the yield through premature death and frequently causes lodging of the plant.

The principal method of stalk infection according to Durrell (8) is as follows:

Prior to flowering, the ligules of the leaf sheath clasp the stalk very tightly. After the flowers have matured and stalk elongation has ceased, the action of the wind on the leaves causes the ligules to loosen and expose the sheath cavity, thus making it possible for masses

of pollen together with such spores as may be present in the air, to collect here. This mass of moist pollen forms an excellent medium for fungus growth and from this source of inoculum hyphae invade the stalk tissue.

The principal method by which the pathogen is carried over winter is undoubtedly in either the vegetative or fruiting condition on old stalks or discarded ears. It has been shown, however, that the fungus can grow in ordinary field soil containing organic matter, so it is possible that it can live in soil in a purely saprophytic manner, independent of old host remains. Wind is believed to be the principal agent in dissemination of the spores.

D. zeae is an active feeder and is reported to produce numerous enzymes by which it digests the constituents of the host tissue that it permeates. Durrell (8) states, "....utilization of cellulose by the fungus is of particular interest, as the penetration and growth in the stalk and weakening of the nodes are thus more readily understood".

Measures for control of D. zeae have in the past, been: sanitation, crop rotation, care in curing and storing the harvested crop, seed disinfection, and care in the selection, storing and testing of seed corn. In more recent years, with the advent of hybrid corn, a more effective control has been introduced: the breeding and selection of D. zeae resistant inbred lines of dent corn combined with subsequent hybridization and testing for resistance of the hybrids.

MATERIALS AND METHODS

In order to grow corn under conditions of controlled nutrition, twelve nutrient solution tanks, similar to those used by Shear (42) were constructed. These tanks were made of 24 gauge galvanized iron, bound with one inch angle iron and measured approximately 60x4x9 inches. The capacity of the tanks was approximately 270 liters. Tops for the tanks were constructed of one inch pine shelving. Baskets, measuring 4x4x9 inches were made from 1/4 inch mesh galvanized wire and fastened below openings in the wooden tops. Each tank accomodated twelve plants. (Fig.I)

All galvanized metal surfaces exposed to solutions in the tanks were coated with pure asphalt to prevent zinc toxicity.

The solutions were aerated by means of a small paint sprayer air compressor, for thirty minutes at intervals of two hours. An electric time clock provided automatic control. A half inch black iron pipe line to each tank and an 'aerator', consisting of a 58 inch length of black iron pipe, drilled at eight places with 1/32 to 1/64 inch holes, capped at one end, and with an elbow and 14 inch length of pipe at the other, completed the aeration system. Rubber garden hose was utilized as connection between air line and 'aerator'.

A means of supplying iron solution to the tanks at a uniform rate throughout the day was devised by the author. An automatic dispatcher (Plate 3E), consisting of a separatory funnel with a ground glass valve at the top as well as the bottom, was operated by means of an electro-magnet, through the air compressor time clock. The two valves were

connected by a lever arm in such a way that when one was closed the other was open. Thus, when the electro-magnet operated, the valve at the bottom of the separatory funnel closed, and the valve at the top opened. This adjustment enabled the solution from the supple bottle to flow into the separatory funnel until full. An air vent tube, running above the supply bottle provided for the escape of air in the separatory funnel. At the end of the thirty minute period, as the current was cut off, the electro-magnet dropped back to its original position, opening the lower valve and closing the upper. A glass tubing pipe line supplied each tank with iron solution as it came from the dispatching separatory funnel. The individual flow to each tank was regulated to a uniform drop by means of screw clamps.

A solution level indicator for each tank was made of a cork float attached to a stiff rod which ran up through a hole in the tank top, past a marker. This indicator made lifting of the tops unnecessary.

In the greenhouse, supplementary lights were used. One 75 watt bulb in a fourteen inch reflector for each tank was sufficient for normal growth and flowering. The height of bulb above the tank was adjustable. An electric time clock operated the lights to give daily, the equivalent of fifteen hours of light.

A red cellophane covered wooden frame, large enough to accomodate full grown plants permitted the growing of corn plants under light conditions where all wave lengths shorter than approximately 529 $m\mu$ were eliminated. This frame had an air vent at the top and a door in the side to facilitate addition of water and chemicals to the tank. (Plate 3 B)

Germinated seeds were planted in the tanks to insure a uniform stand.
The seeds were treated with Semesan Jr. and placed at two inch intervals
on a piece of closely woven cloth. The cloth and seeds were placed
on wet sterilized sand and covered to a depth of about one half inch
with sand. When the coleoptile length was approximately one inch, the
cloth was lifted out of the sand and the seedlings carefully removed
from the cloth. This procedure reduced the root hair damage to a
minimum. The plants showed very few effects of transplanting.

The wire baskets of the tank tops were filled to the level of the
solution with sterilized pea-size gravel. Two seedlings were planted
in each basket, in such a manner that their roots dipped into the solu-
tion, but the seed itself was above the solution. As the plants grew,
gravel was filled in around them to provide support. When it became
evident that the plants were well established they were thinned to one
plant per basket.

Solutions used in the tanks during the summer of 1940 are presented
in Table I. All tanks received a uniform addition of 0.25 p.p.m. each
of boron as boric acid (H_3BO_3) and manganese as manganous sulphate
($MnSO_4.2H_2O$). A daily addition of 0.5 p.p.m. of iron as ferric citrate
($FeC_6H_5O_7$) was added to each phosphorus deficient tank. The remaining
tanks each received a daily addition of 1 p.p.m. of iron as ferric
citrate. The pH of the solutions was maintained between 5.0 and 5.5.
There were four plants from each of three seed sources to a tank.
Tanks 1 to 6 inclusive contained plants chosen on the basis of their
relative susceptibility to _E. zeae_ stalk rot as indicated in an Illinois
mimeographed report. (20) These were : susceptible--R4 x 4-3, fairly
susceptible--66 x 90, and resistant Hy x 38-11. Tanks 7 to 12 inclusive

contained plants of each of three inbred strains chosen on a corresponding basis. (20) These were: susceptible--R4, fairly susceptible--90, and resistant--88-11.

The same set of six treatments was applied to both crosses and inbred strains.

Tanks 1 and 7 had the full nutrient solution and were considered as check tanks, because they received no additional treatment.

Tanks 2 and 8 received a solution deficient in nitrogen.

Tanks 3 and 9 received a solution deficient in phosphorus.

Tanks 4 and 10 received a solution deficient in potassium.

Tanks 5 and 11 received a full nutrient solution, and the plants were kept clipped back to two-thirds their normal leaf area.

Tanks 6 and 12 received a full nutrient solution and the ears bagged to prevent kernel formation.

Solutions in the tanks were changed three times during the growing season at such times as were required for best plant growth. One half the plants in each tank were inoculated with a single spore isolation strain of D. zeae (Lz 9) two weeks (Aug.4) after flowering, and readings made twenty nine days later (Sept.1).

For the winter of 1940-41 six tanks were set up in the greenhouse. Three tanks were planted to single crosses: R4 x 4-8, 66 x 90 and Hy x 38-11. The remaining three tanks were planted to three F3 lines of dent corn which had shown a tendency toward susceptibility to D. zeae in the 1940 field inoculations. These lines were designated as D1, D2 and D3.

Attempts were made to alter the carbohydrate content of the stalk. The F3 lines were grown at low, normal and high nitrogen levels in

TABLE I

Constitution of solutions used in study of the effects of nutrition on the reaction of corn to _Diplodia zeae_ for the summer of 1940. (17)

Salt	ml. molar solution per tank	P.P.M.						
		Ca	Mg	K	N NO3	N NH3	S	PO4
Full Nutrient Solution								
KH2PO4	145			21				50
Ca(NO3)2	286	42			30			
KNO3	853			123	44			
MgSO4	262		25				31	
Mg(NO3)2	108		13		11			
NH4NO3	1510				68	68		
Totals		42	36	144	153	68	31	50
Low Nitrogen								
KH2PO4	145			21				50
Ca(NO3)2	68	10			7			
CaCl2	213	32						
K2SO4	427			123			51	
MgSO4	570		33				44	
NH4NO3	132				7	7		
Totals		42	33	144	14	7	95	50
Low Phosphorus								
KH2PO4	27			4				10
Ca(NO3)2	286	42			30			
KNO3	853			123	44			
MgSO4	262		23				31	
Mg(NO3)2	108		13		11			
NH4NO3	1510				68	68		
K2SO4	59			17			70	
Totals		42	36	144	153	68	101	10
Low Potassium								
KH2PO4	11			1.6				4
Ca(NO3)2	286	42			30			
NaNO3	853				44			
MgSO4	262		23				31	
Mg(NO3)2	108		13		11			
NH4NO3	1510				68	68		
Na2HPO4	130							46
Totals		42	36	1.6	153	68	31	50

tanks 7, 8 and 9 respectively. The single crosses were grown at high
nitrogen (tank 10) and normal nitrogen levels (tanks 11 and 12).
Tank 12 was fitted with the previously mentioned red cellophane chamber.

One half of the plants in each tank were inoculated with D. zeae
strain F-1 two weeks after flowering, and readings were made three
weeks later.

Solutions used in the greenhouse (Table II) were suggested by
Dr. J. D. Sayre, U.S.D.A., Ohio Agricultural Experiment Station,
Wooster, Ohio. In addition to the salts mentioned in the above table,
boron, manganese and iron were added at twice the rate used in the
summer of 1940.

In the summer of 1941, some of the treatments carried on in tanks
the previous summer were repeated under field conditions. One hundred
plants of each of two inbred lines, K4 and D5, were planted in the field
corn nursery. Twenty plants of each line were bagged to prevent the
formation of kernels. Twenty plants, at the time of flowering, were
clipped to two thirds their leaf area. Twenty plants were marked as
checks and received no treatment. At a different location these same
lines were planted under conditions of high nitrogen, two top dressings
of Chilean nitrate being added during the early growth of the plants.
All plants were inoculated with D. zeae, Strain F-1, two weeks after
flowering. Readings were made three weeks later.

Ten single spore isolations of D. zeae taken from a naturally
infected corn stalk, were made and tested in the field in 1939. Due,
probably to unfavorable environmental conditions for the growth of
the organism, no perceptible difference in virulence was observed,
the infection being confined in most cases, to the inoculated internode.

TABLE II

Constitution of solutions used in study of the effects of nutrition under greenhouse conditions on the reaction of corn to _Diplodia zeae_ for the winter of 1940-41. (38)

Salt	Ml. molar solution per tank	P.P.M.								
		Ca	Mg	K	N NO_3	N NH_3	S	P	Cl	Na
Full Nutrient Solution										
KH_2PO_4	190			27				22		
KNO_3	1299			188	67					
$Ca(NO_3)_2$	1500	192			135					
$MgSO_4$	539		48				64			
NH_4NO_3	90				5	5				
Totals		192	48	215	207	5	64	22		
Low Nitrogen										
KH_2PO_4	190			27				22		
KCl	1298			188					168	
$Ca(NO_3)_2$	258	38			27					
$CaCl_2$	1045	244							426	
$MgSO_4$	539		48				64			
NH_4NO_3	53				3	3				
Totals		282	48	215	30	5	64	22	594	
High Nitrogen										
KH_2PO_4	190			27				22		
KNO_3	1298			138					168	
$Ca(NO_3)_2$	1859	287			201					
$NaNO_3$	7685				398					655
$MgSO_4$	539		48				64			
NH_4NO_3	175				10	10				
Totals		287	48	215	609	10	64	22	168	655

Consequently, a strain, _D._ _zeae_ F-1, highly pathogenic under environmental conditions at the Iowa Agricultural Experiment Station was obtained by Dr. G. C. Kent. This check strain, _D._ _zeae_ F-1, under Maryland conditions for the years 1940 and 1941 has proven to be no more pathogenic than those isolated locally.

Cultures of the various strains of _D._ _zeae_ were maintained on potato-dextrose agar and for the production of spores transferred to dilute corn meal extract agar. Four to five weeks were required for the production of spores.

The plants were inoculated approximately two weeks after the time of flowering by introducing a spore suspension into the middle of the internode by means of a modified inoculating needle. The modified inoculating needle consisted of a four inch length of small brass tube, 3/32 of an inch outside diameter, closed and drawn to a point at one end, with a hole cut just back of the point, and a small rubber bulb at the other end. A puncturing needle was made by driving a finishing nail through a 2"x1¼"x1" piece of wood and filing the nail to a sharp point. A hole was first made with the puncturing needle, held in the palm of the hand, and then a drop of inoculum was introduced in the stalk by inserting the inoculating needle and pressing the rubber bulb.

Readings of infection were made three weeks after inoculation. The stalks were split and the discoloration and rotting of the tissue caused by the organism was recorded in number of internodes passed. (Plate 6)

Diplodia zeae was reisolated and plated out from artificially infected material after surface sterilization of the stalk tissue with a saturated solution of calcium hypochlorate. Areas of suspected

secondary invasion by other organisms were surface sterilized and the organism isolated. In all cases the spread of B. zeae was restricted to the discolored area.

Stalk tissue for sectioning was fixed in Kelly's mixture for twenty-four hours, a change to fresh solution being made once during this period. The fixed material was then washed in running water for twenty-four hours and run through the ethyl alchol-butyl alcohol series and finally imbedded in Tissuemat.

Kelly's mixture has the following composition:

Stock solution

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Potassium dichromate.............................2.5 gm.
Mercury dichloride...............................5.0 gm.
Water...........................................100 cc.
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At the time of using, 9 cc. of the stock solution is mixed with 1 cc. of neutralized formalin. (6)

Sections of fifteen to twenty $\mu\mu$ in thickness were made on a rotary microtome. The abundant sclerenchyma tissue of the mature corn stalk made sectioning difficult. A triple stain of safranin, gentian violet, and orange "G" was found to be the most satisfactory for staining the fungus hyphae within the stalk tissue. Vital staining of the hyphae in the living tissue was also used. Neutral red was found to be the most satisfactory for this type of staining.

Photomicrographs of B. zeae hyphae within the plant cells were made with equipment involving the use of a 2_4 x 3_4 inch Graflex camera.

To study the possibility that substances, toxic to the fungus, might be present in the stalk tissue of resistant plants, two inbred lines of dent corn were selected: one, R4, reportedly resistant, (20) and the other, L7, an #5 inbred line selected in 1940 from the dent

corn nursery for susceptibility to D. zeae stalk rot. These two field grown lines were treated identically as follows:

1. The lower four nodes and internodes of the stalk were selected, wiped clean and cut into cross-sectional pieces about one-fourth of an inch thick. This material was immediately placed in a container immersed in a freezing mixture of solid carbon dioxide and absolute alcohol.

After the material had completely frozen it was allowed to thaw. Immediately after thawing it was run through a small food grinder and then placed in a Carver laboratory press (Fig. 2). A pressure of 10,000 pounds per square inch was maintained for a period of three minutes. The expressed sap was collected and kept frozen until used.

Upon thawing, the sap was diluted with an equal amount of distilled water, and a modified Czapek's solution was made, using this diluted sap. This solution was first filtered through an asbestos suction filter and then through a sterile Berkfeld filter. The solution was then transferred with sterile 25 ml. pipette into four sterile 250 ml. erlenmeyer flasks. The flasks were inoculated with a culture of D. zeae strain F-1, which had been grown on corn meal extract agar. A uniform inoculum for each flask was obtained by plugging out the inoculum from the same sector of the culture with a sterilized cork borer. The cultures were kept at a temperature of approximately $30^{\circ}C$.

2. The lower four nodes and internodes of the stalks were selected, wiped clean and cut at the internode into lengths. These pieces were weighed and placed in an electric oven at $95^{\circ}C$. When dry they were weighed and then ground in a Wiley Mill. Distilled water was added to the ground material to bring it up to the original green weight. This material was then divided into four 25 ml. samples and autoclaved at fifteen pounds pressure for twenty minutes.

When cool the samples were inoculated uniformly with inoculum taken from the same sector of the culture.

3. The lower four nodes and internodes of the stalk were selected , wiped clean and weighed. The samples were then cut into one-fourth inch cross-sectional pieces and immediately ground in a small food grinder and extracted in a Carver laboratory press at a hydraulic pressure of 10,000 pounds per square inch, maintained for three minutes.

The expressed sap was collected and immediately divided into two equal portions, 3A and 3B. Sample 3A was brought to a boil as quickly as possible after extraction. Sample 3B was quickly frozen in a mixture of solid carbon dioxide and absolute alcohol.

Sample 3A was diluted with an equal amount of distilled water. Nutrients were added to make Czepek's agar and four 25ml. samples of the media were sterilized at fifteen pounds pressure for twenty minutes. These samples were then poured into sterile petri dishes, cooled, and inoculated with uniform samples of inoculum obtained from the same sector of the culture. These cultures were maintained at a temperature. of approximately 30°C.

Sample 3B was thawed and diluted with an equal amount of distilled water. Nutrients to make Czepek's solution were added and the solution filtered through an asbestos suction filter and then through a sterile Berkfeld filter. Four samples were drawn in sterile 25 ml. pipetts and transferred to sterile 250 ml. Erlenmeyer flasks. The flasks were then inoculated with uniform samples of inoculum obtained from the same sector of the culture. The cultures were maintained at a temperature of approximately 30°C.

RESULTS

Plant growth in the tanks for the summer of 1940 was satisfactory. The check plants of the single cross series were, to all appearances, normal. Good sized, well developed ears were formed. Ear formation in the inbred series was not so good as it was under field conditions.

Plants in the nitrogen deficient tanks, 2 and 5, gave the characteristic nitrogen deficiency symptoms, interveinal yellowing followed by a general lighter, yellowish-green coloration. The maturing lower leaves became 'fired', having a brown, dried up appearance. The silking date was delayed from one and one half to two weeks as compared to the check plants in the single cross series. The roots were thinner than the checks and took on a brownish cream coloration.

Plants in the low phosphorus tanks, 2 and 5, showed striking differences in their phosphorus requirements. Of the single crosses R4 x 4-8 was the first to show phosphorus deficiency symptoms, the symptoms being quite severe before Hy x 38-11 showed any deficiency symptoms at all. Although intermediate in its phosphorus requirements, 66 x 90 more nearly approached R4 x 4-8 than Hy x 38-11 in this respect. The inbred lines showed phosphorus deficiency symptoms much later than did the single crosses. The relative phosphorus requirements of the inbred lines were: R4—highest, 38-11—intermediate, but approaching those of R4, and 90 lowest.

Phosphorus deficiency symptoms were as follows: interveinal necrotic spots occurred on the leaf; most abundantly from the tip to a midway point on the blade. These necrotic areas varied from 1/32 to 1/8 inches across, according to their relative maturity and degree

of deficiency. These purplish margined areas were oblong and ran parallel to the vein. The entire leaf margin also took on a purplish coloration, the extent of which increased with the severity of the deficiency. Older leaves were the first to show these symptoms. The whole plant was stunted. Roots of the phosphorus deficient plants were purplish in color and were fewer in number than those of the check plants. Silking occurred from two to three weeks later than on the checks.

Plants in the deficient potassium tanks, 4 and 10, probably received too severe a deficiency solution, for the plants became infected with a root rot and died prematurely. Differences in potassium requirements were in evidence. The first to show deficiency symptoms was 66 x 90, Hy x 38-11 was the next and R4 x 4-8, the last, being two weeks later than 66 x 90 in showing these symptoms. It is interesting to note that R4 x 4-8 was the first to show phosphorus deficiency symptoms while it was the last to show potassium deficiency symptoms. Of the inbred lines, 38-11 was the first to show potassium deficiency symptoms. The last was R4. Potassium deficiency is indicated by the dying of the tissue in the leaf margins, starting at the tip and progressing toward the base. The entire tip of the leaf becomes brownish, wrinkled and finally dies. The older leaves are the first to show these symptoms.

The clipping of the leaves in tank 5 and 11 was a little too severe, or was begun too far in advance of flowering, for the ears failed to develope normally.

Condensed inoculation data of the 1940 tank experiment is presented in Table III. In each case, comparison is made with the

inoculated plants of the full nutrient solution tanks, 1 or 7, and expressed as, more susceptible, more resistant, or the same degree of infection as the check plants. Data are presented in this manner to facilitate comparison with the results of Shear (42) and Holbert, Hoppe and Smith (16).

Photographs of the split stalks from each tank are shown in Plates 7 to 16. The stalk to the left in each group of three is an uninoculated stalk from that group and is to be considered a check for that treatment. Secondary invasion and insect injury is evident in some of the stalks. Isolation of organisms within similar stalk tissue confirmed the presence of secondary invasion in areas of suspected secondary invasion.

In general, under conditions of deficient available nitrogen or phosphorus, the plants were more resistant to the spread of D. zeae than when grown under conditions of full nutrient. This observation is in agreement with Shear's finding. (42)

Under conditions of partial defoliation for the 1940 tank experiment the plants were in general more resistant to D. zeae than the check plants. This observation is not in agreement with Holbert, Hoppe and Smith (16), but may have been due to poor ear formation. Poor ear formation probably offset the effect of reduced leaf area on the relative amount of carbohydrate in the stalk

Prevention of ear formation, tanks 6 and 12, in general increased the relative resistance to the spread of D. zeae in the stalk tissue. This observation is in agreement with the findings of Holbert, Hoppe and Smith. (16)

Plant growth of single crosses in the greenhouse tank experiment

TABLE III

Relative susceptibility to Diplodia zeae of dent corn stalks grown
during the summer of 1940 in nutrient solutions under various
conditions of nutrition and treatment.

Tank number and treatment	Relative susceptibility[1].	Stalks in each group		
		single crosses	inbred lines	Total
Tanks 2 and 8 Deficient nitrogen				
	More susceptible	0	1	1
	More resistant	2	4	6
	Unchanged	4	0	4
Tanks 3 and 9 Deficient phosphorus				
	More susceptible	1	2	3
	More resistant	3	4	7
	Unchanged	1	0	1
Tanks 5 and 11 Partial defoliation				
	More susceptible	2	0	2
	More resistant	2	5	7
	Unchanged	2	1	3
Tanks 6 and 12 Prevention of ear formation				
	More susceptible	2	2	4
	More resistant	2	4	6
	Unchanged	2	0	2

1. Susceptibility relative to the genitically identical plants grown
under normal conditions of nutrition: full nutrient tanks 1 and 7.
Unchanged susceptibility indicates that the stalk infection readings
were the same as for the check plants.

for the winter of 1940-41 was normal with exception of the formation
of numerous rudimentary ears. Inoculation with b. zeae resulted in
practically no infection. Plates 13 to 20 show the extent of this
infection. The stalk at the left, designated by a white dot, is a
check stalk for that tank and was inoculated only with sterile water.
Tank 10, receiving a high nitrogen solution, showed very slight spread
of disease within the stalk. Tank 11, receiving a normal supply
of nitrogen also showed practically no infection after inoculation.
Tank 12, under light conditions where approximately all wave lengths shor-
ter than 529 μμ were eliminated, likewise showed no infection.

The inbred lines showed practically no infection in both the nor-
mal and low nitrogen tanks. The high nitrogen tank, however, gave
more indication of infection. Plants of inbred line D1 were infected
for one and one half internodes. All plants of this line, including
the check plants were dead when harvested. One plant of inbred line
D2 was rotted and discolored to three internodes and the other plant
to two internodes. Both inoculated plants were dead while the check
plants still retained their green coloration. One plant of the inbred
line D3 had one internode discolored while the other plant had two
internodes discolored. One check plant was in good condition while
the other showed root and stalk rot.

Condensed data on the results of field inoculation of plants
subjected to previously mentioned treatments is presented in Table IV.

The susceptible inbred line D3 responded in a greater degree to
treatment designed to influence its relative susceptibility than did
the line 84. Prevention of ear formation resulted in increased resis-
tance to spread of b. zeae within the plant. Reduction of leaf area

tended to increase the susceptibility of inbred line L8 to D. zeae.

The susceptibility of K4 was unchanged when grown under conditions of reduced leaf area. A high rate of nitrogen application had no effect on the relative susceptibility to D. zeae in either line.

The seasons of 1959, 1940 and 1941 for Maryland have apparently not been entirely favorable to the development of D. zeae as compared to the reported degree of infection in the corn belt.

TABLE IV

Diplodia zeae inoculation readings of two inbred lines subjected to various treatments in the field corn nursery for the summer of 1941

Treatment	Average number of internodes invaded	
	Inbred line K4	Inbred line L5
No treatment-check plants	1	3/4
Prevention of ear formation	7/8	1/8
Partial defoliation	1	1
High nitrogen level	1	3/4

Results obtained from the plant extraction experiment are shown in Plate 5. In each picture R4 is on the left and inbred line D5 on the right. In every case but one, D. zeae grew profusely.

Anatomical studies of stalk tissue grown under various conditions of nutrition and treatment suggest that mechanical barriers within the stalk tissue were not instrumental in bringing about resistance to D. zeae. Hyphae seemed to enter the cellular and intercellular spaces at will, and gave no indication that mechanical pressure was exerted. Plate 4 shows four photomicrographs of D. zeae hyphae within dent corn stalk tissue. Plate 4A shows hyphae within an intercellular space of parenchyma tissue. Plate 4B shows hyphae passing through a seive tube cell wall. Plate 4D shows a characteristic constriction of the hyphae at the middle lamella as the hypha passes from one sclerenchymatous cell to another. A general view of D. zeae hyphae within a parenchymatous cell is shown in Plate 4C.

DISCUSSION

It is generally agreed that resistance to D. zeae can be established in inbred lines of dent corn, through breeding and selection for resistance (15,43,44,45). Smith, Hoppe and Holbert (44) have presented evidence of the positive influence of inbreds on hybrid reaction to D. zeae. They present an instance where resistance was manifested by one hybrid involving a highly resistant, and a very susceptible inbred, indicating a dominance of resistance in this cross.

A rapid and convenient inoculation technique, consisting of needle puncture injection of the lower internode with a pycnidiospore suspension of a pure culture of D. zeae, was developed by Smith, Hoppe and

Holbert (44). This inoculation approached natural infection.

Resistance of one set of tissue of the corn plant to D. zeae does not necessarily insure resistance of other parts of the same plant. Moreover, conditions that predispose stalk tissues to invasion by D. zeae and those that predispose the ear and grain to invasion by the fungus are not always the same (18).

By means of artificial inoculation, Holbert, Hoppe and Smith (18) came to the following conclusions concerning conditions affecting the relative susceptibility of dent corn to D. zeae:

1. When grain set was prevented the stalk tissue was invaded less rapidly and to a less extent.

2. When leaf area was reduced during the latter half of August either by artificial defoliation (25-35 per cent of the leaf area removed) or from natural causes such as bacterial leaf blight or an attack of second brood chinch bugs, the stalk tissue was invaded more rapidly and to a greater extent.

3. Artificial or natural chilling and freezing temperatures increased the rate and extent of stalk tissue invasion.

In each case cited, increased susceptibility of the stalk tissue to the fungus is associated with conditions that involve a reduction of the carbohydrate reserve of the plants, and conversely, increased resistance is associated with conditions that involve an increase in the carbohydrate reserve of the plant.

Shear (42) found that the extent of infection of dent corn with D. zeae is influenced markedly by variations in the nutrition of the corn plant. Plants grown with a limited supply of nitrogen or phosphorus were more resistant than plants receiving a normal supply of nutrient. These results tend to support the theory advanced by Holbert,

Hoppe and Smith (16), that resistance to D. zeae stalk rot is corre-
lated with a high concentration of reserve carbohydrate in the stalk
of the dent corn plant.

ANATOMICAL ASPECT OF THE PROBLEM. It was decided at the start of
this investigation that it would be well to consider the problem of
corn stalk resistance to D. zeae, first, from an anatomical standpoint.
Variations in the amount of mechanical tissue, thickness of cell wall,
size of intercellular spaces, etc. could conceivably be responsible,
at least in part, for variations in resistance.

It is well known that environmental conditions govern to a cer-
tain extent the anatomical composition of the plant. Holbert, Hoppe
and Smith (16) state, "....comparisons between strains on an anatomical
basis are reliable only when the strains have been grown under prac-
tically identical conditions".

The nutrient supply has a very definite influence on the plant.
The lack of an element in a nutrient solution may induce early maturity
and senescence, while its presence in excess may delay maturity and
senescence (24). The effect of the relative supply of an element is
not only reflected in the plant as a whole but also in the finer de-
tail of the structure of the cells themselves.

Petri (35) states that the conditions of nutrition of a plant
determine in large measure the degree of its resistance or suscepti-
bility to a pathogene. The histological or morphological structure
or properties and the function of the organs and tissues may be modi-
fied in such a way as to slow or hasten the attacks of the pathogene.

Observation by several investigators indicates that plants grown
under conditions of deficient available nitrogen exhibit structural

characteristics typical of xeromorphy. Nitrogen deficient plants are stiff and woody owing to thick cell walls and formation of mechanical fibers, sclerenchymatous tissue, etc. (30,42). Schneider (41) has described and illustrated certain anatomical features related to different conditions of mineral supply, emphasizing the small vascular bundles of plants grown in solutions deficient in phosphorus and nitrogen.

Lignification and hardening of cell walls is reduced by large amounts of available nitrogen, because under such circumstances carbohydrate is utilized in processes concerned with syntheses of protein (28,30,41).

Nausbaum (27), in his cytological study of the resistance of apple varieties to Gymnosporangium juniperi virginianae, states that the facts that there is no apparent alteration of the cuticle or epidermal cell wall at the point of entry and that the cuticle is often indented, indicate mechanical pressure as the principal factor involved in penetration.

Nausbaum and Keitt (28) on the other hand, indicate that resistance of young apple leaves to Venturia inaequalis is attributed primarily to relation of the fungus and material emanating from the host tissue, rather than to mechanical barriers. This is in agreement with Johnstone (17), and Schmidt (40).

Nausbaum (27) states further, "The fact that there was no splitting apart of the walls in advance of the hyphal tips preclude the view that the fungus might have some solvent action upon the middle lamella."

From anatomical studies of dent corn stalk tissue invaded by D. zeae it is concluded, in the investigation reported in this thesis, that mechanical barriers are not instrumental in effecting resistance

to D. zeae stalk rot.

CHEMICAL ASPECT OF THE PROBLEM. Holbert, Hoppe and Smith (16) as previously noted, have attributed increased susceptibility of stalk tissue to the fungus, to a reduction of the carbohydrate reserve of the plant.

In this investigation it was decided to grow plants under conditions that would presumably alter the carbohydrate content of the stalk and by means of artificial inoculation determine the effect upon the relative susceptability of the stalk to D. zeae.

There is abundant evidence in literature to indicate that plants grown with a limited supply of either nitrogen or phosphorus accumulate reserve carbohydrate in their stalks (2,25,42).

In support of the theory advanced by Holbert, Hoppe and Smith, inoculation experiments of dent corn plants deficient in either nitrogen or phosphorus, reported in this thesis, showed increased resistance to the spread of D. zeae.

Plants grown with an amount of nitrogen well in excess of that required for normal growth accumulate nitrogen in the form of nitrate, ammonium, basic-free a -amino, amide, basic, and protein nitrogen in the tissue. Under the same conditions, the tissue content of reducing sugars, starch and dextrin, and hemicellulose is reduced (2).

Murneek and Gildehous (26) reporting an investigation by Wallace say that the omission of potassium from a complete fertilizer results in an increased absorption of nitrogen and phosphorus and when either nitrogen or phosphorus is omitted from the fertilizer there is a decreased absorption of the other two elements.

Gassner and Franke (12) report that an increase in the CO_2 supply to wheat leaves resulted in an increased rust attack, which is explained by the increased nitrogen content.

Inoculation of plants grown under conditions of high amounts of available nitrogen reported in this experiment, failed to support the theory advanced by Holbert, Hoppe and Smith (16), that resistance is associated with high amounts of reserve carbohydrate in the stalk. Conditions of reduced carbohydrate failed to increase the degree of resistance. It should be noted, however, that environmental conditions were in general not conducive to the spread of the disease.

In addition to previously mentioned means of affecting the carbohydrate content of the stalk tissue, it was decided to grow plants under light conditions where all wave lengths shorter than approximately 529 $\mu\mu$ were eliminated. Popp (35) found that plants grown under these light conditions had considerably less starch and total carbohydrate and generally an increase in total nitrogen.

This experiment failed to support the theory advanced by Holbert, Hoppe and Smith (16). Greenhouse conditions were unfavorable to the spread of _D. zeae_ and so this experiment does not present conclusive proof of what effect such a light wave length has on the fungus growth under more favorable conditions.

In common with most of the grasses, the vegetative parts of corn do not contain starch, and its nearest equivalent, an amylodextrin soluble in warm water and showing a lavender color with iodine, is only a minor constituent of the plant. Sucrose is the characteristic carbohydrate of the vegetative plant and accounts for approximately half of the total carbohydrate of the stalk (5).

Sayre, Morris and Richy (39) found that sucrose was 51 per cent
and total sugar 36 per cent higher in drought-injured, barren plants
than in adjacent check stalks. Loomis (25) found sucrose increases
of nearly 100 per cent in defruited stalks. Colin and de Gugnac (5)
state that carbohydrate accumulations above the normal level, as a
result of drought injury, were about equally distributed between
dextrin and sucrose with other fractions playing a minor role.

Kemp and Henson (19) working with sugar corn plants found that
stalks of barren plants contained 22.28 per cent total carbohydrates
whereas stalks of plants having a normal set of grain contained 11.55
per cent total carbohydrate. Sugar corn plants pollinated with dent
corn pollen and having dent grain had a stalk content of 7.20 per cent
total carbohydrate.

Brunson and Latshaw (3) have found that when set of grain in
corn plants is prevented or reduced by prevention of normal pollina-
tion, protein and to a less extent nitrogen-free extract tend to
accumulate in greater than normal proportions in other organs of the
plant, and the proportion of fiber is considerably reduced, particular-
ly in the cobs and stem.

As reported in this thesis, prevention of ear formation in the
tank experiment and under field conditions effected increased resis-
tance of the dent corn stalk to *D. zeae*. This observation is in
agreement with the theory that an accumulation of reserve carbohydrate
in the stalk increases resistance to *D. zeae* (16).

Sayre, Morris and Richey (39) found that when the leaf area was
reduced artificially at the time of tasseling the total sugar content
of the stems was lowered. The removal of the four leaves about the

ear decreased the total sugar content 2.8 per cent on the average as compared to normal plants. The removal of the upper and lower leaves decreased the total sugar 2.5 per cent.

Reduction of leaf area in the experiments reported in this thesis was not effective in increasing the susceptibility of the stalks of dent corn to D. zeae. This lack of increased susceptibility may have been due in part to the generally unfavorable conditions for the spread of the disease.

THEORIES OF RESISTANCE. In lieu of an actual solution to the problem of the nature of resistance, various theories have been suggested. Ward (47) in his work on the relations between host and parasite in brome grasses and their brown rust, Puccinia dispersa, proposed that immunity was due to antagonistic reactions between host and parasite of the nature of the formation of toxins and antitoxins. Gibson (13), Allen (1) and others are generally in agreement with Ward. Leach (21) has suggested an hypothesis based on a possible specific food requirement on the part of the fungus and the corresponding lack of this food substance within the host. Edgecombe (9) performed serological studies of wheats, susceptible and resistant to Puccinia rubigo-vera tritici. Purified globulin extracts of the grain were used as immunizing agents and the resulting antisera were given the precipitin test using the original extracts as test antigens. The results showed a relationship between precipitin reaction and rust resistance. Since the globulins used were believed to have been a part of the cytoplasm, it was reasoned that the genetic factors responsible for resistance were in some way associated with the factors producing the specific globulins.

Leeman (22) proposed the theory that the holistic quality of the cell may play an important role in active plant immunity. This theory was based upon the assumption that the protoplasm of an immune host may not contain any specific substance detrimental to the invading organism, but that the living protoplasm as a whole, built up into a complex system, may present an unfavorable substrate. The phenol hypothesis of Newton and Anderson (29) suggests that rust resistance in wheat may be caused by the liberation of toxic phenolic compounds in the host cell upon the entrance of the fungus. It is suggested that this mechanism may be responsible for different types of rust reactions when one considers the possible conditions following the action of fungal enzymes upon the phenolic compounds in the host-cell protoplasts.

Mausbaum (27) states that cytological evidence has not settled the question as to whether the antagonism against the apple rust fungus is already present in the make-up of the host-cell protoplasts or is induced by the parasite and presents a counter-reaction against the intruder. The substance or substances antagonistic to the fungus seems, however, to be specific, because he found many cases where the haustorium occupying the host cell had died with the cell itself showing no visible change.

As early as 1915, Reed (36) noted that _Gymnosporangium juniperi virginianae_ killed infected cells of resistant apples quickly, but stimulated those of susceptible varieties into higher metabolic activity. Allen (1) has shown the _Puccinia triticina_ causes practically no pathological changes in the infected cell.

Stakman (46) demonstrated that strains of _Puccinia graminis_ which kill infected cells very rapidly result in hypersensitive spots which

are restricted to a few necrotic cells, whereas strains which do not kill infected cells can develope large leaf spots. The more virulent a parasite is to the cell it infects, the less virulent it is on the host as a whole.

Most parasites actually thrive only in meristematic tissue, or in meristematic cells, or in cells which have reverted, at least locally, to the embryonic stage. In the "host-parasite" system, the affected parts of the host cell revert to an embryonic condition. The starch-building capacity of plasts is inhibited; starch being utilized by the microorganism more rapidly than it can be replaced, the plasts are soon deprived of their starch contents; oil droplets become more numerous and enlarged between starch grains (7).

Dufrenoy(7) in discussing the "host-parasite" system states:

Two cases of inter-relation between parasite and host have been distinguished:

1. "Pertophytes" kill the living cells before penetrating them to make use of the contents of the dead cells.

2. "Eu-parasites", obtaining their food directly from living cells, can thrive as such but only so far as the infected living cells outline the infection.

Each component of the system, either the host or the parasite, must be identified not only from the botanical point of view as to species, but from the genetic point of view as to the strains involved; the genetic constituents of both determine the potentiality of interrelation under given ecological condition.

Immunity of plants to fungi, bacteria, or viruses can be studied from two points of view; first, the nutritional relation between the host and parasite, the parasite being considered ad deriving its food from the host and second, the toxic effect of the host cell contents on the pathogen.

NUTRITIONAL RELATIONSHIP. Dufrenoy (7) states that the food a parasite needs may be mainly polypeptids and carbohydrates in a water-soluble form. The parasite must either induce the host cells

to alter their metabolic activity in a way which will make available
for the parasite the nutrients it needs, or the parasite itself must,
by enzymatic action, attack the material which it contacts in the
infected cells.

A nutritional relationship may be expected to develop between a
susceptible host and a parasite mainly when the two following conditions
occur: 1. the water-soluble constituents in the host cell remain water-
soluble during the early stages of infection, so that they are easily
translocated out for the benefit of the parasite, 2. the highly meta-
bolized products become water-soluble, as infection enhances proteolytic
and amylolytic activity.

CHEMICAL RELATIONSHIP. Ezekiel and Fudge (11) have suggested that
the general immunity of monocots to root rot is due at least in part to
the presence in the roots of these plants of minute quantities of acidic,
ether-soluble substances, possible organic acids of esters.

Kargopoleva (14) has found that the cell sap of wheat varieties
immune or highly resistant to Puccinia triticina is characterized by
a high content of phenols similar to protocatechuic acid while that
of susceptible varieties is low or entirely lacking in these compounds.
Lefrancy (7) has shown that in resistant plants the vacuolar sap of
cells adjacent to those killed by the invading pathogen become rich
in phenolic compounds: he suggests that such elaboration of toxic
materials is an important role in the resistance of plants to
Rhysatotrichum omnivorum.

TOXIC SUBSTANCES. Attempts have been made to obtain information
on the mode of parasitism of various fungi by a study of their reac-
tions to solutions obtained from appropriate and in appropriate host
plants.

One of the earliest experiments was that carried on by Ward (47) who found that the uredospores of *P. dispersa* (*P. bromina* Eriks.) would germinate equally well in extracts of the host leaves, whether of resistant or susceptible varieties, as in distilled water.

Ezekiel (10) claims to have established a correlation between the susceptibility of a host variety, and the effect of its extract upon rust spores. He obtained his extracts by simple pressure, and found that they required considerable dilution before germination could be obtained. Extracts prepared with the use of heat were found to be more toxic than those which had not been heated.

Johnstone (17) carried out experiments on the germination of conidia of *Venturia inaequalis* in various extracts of apple leaves. She reported a clear correlation between their toxicity, and the resistance of the respective varieties to infection. In order to preserve thermostable components, she did not heat her extracts, but, by using them immediately after preparation, minimized enzymatic activity as much as possible. It seems probable, however, that enzymatic activity could have taken place during the period following inoculation.

In contrast to the findings of Ezekiel (10) and Johnstone (17), Nobecourt (31), who tried the effect of extracts from a number of unsuitable hosts on various fungi incapable of attacking them, found that many allowed good germination of the spores. Parker-Rhodes (32) found that a substance toxic to the germination of the uredospores of the rusts *Puccinia glumarum tritici*, and *P. triticina*, obtained from triturated leaves was produced in the course of autolyis. He emphasized the fact that extraction method and subsequent treatment may

influence the effect of an extract upon spore germination and fungus growth.

Since method of extraction and subsequent treatment may affect the nature of plant extracts, several methods of extraction and treatment were used in the experiment to determine the effect of corn stalk extracts, from resistant and susceptible plants, upon growth of _D. zeae_. As has been previously noted, all treatments were ineffective in preventing fungus growth.

It is possible that staling products may be produced in the stalks, however, it is scarcely likely that such substances would accumulate without metabolic changes in the tissue. Since _D. zeae_ causes practically no pathological changes in the cells of the corn stalk, it is not likely that staling products are instrumental in causing resistance.

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Plate 1.
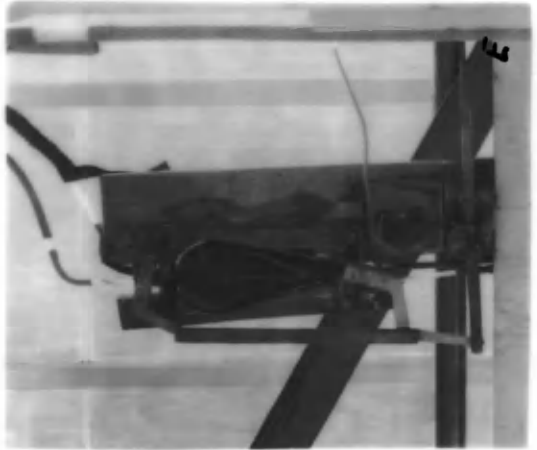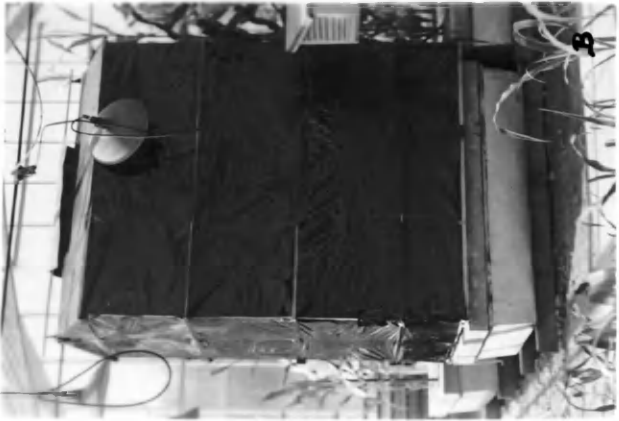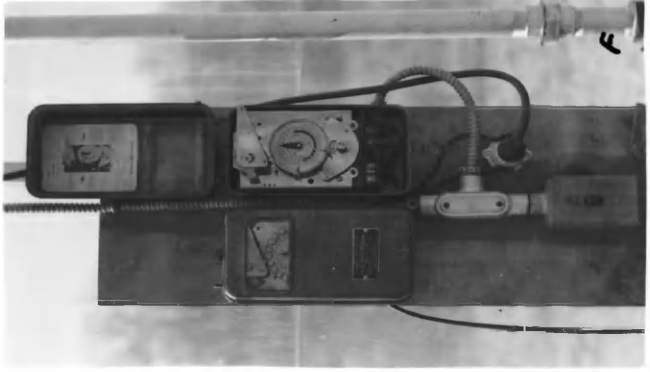
Plate 2.

Plate 3.

Plate 4

Plate 5

Plate 6.

Plate 7.

$R_4 \times 4$-8

66×90

$8/30/40$

$H_y \times 38$-11

48
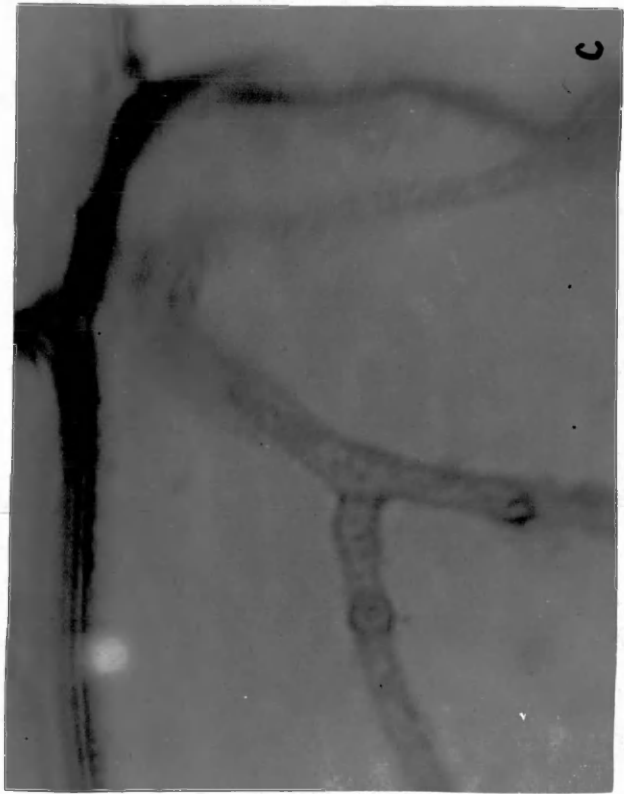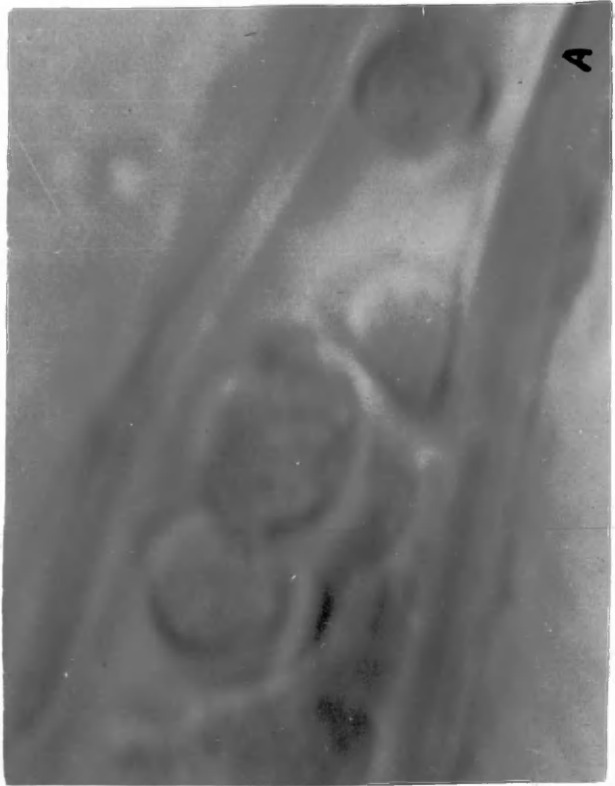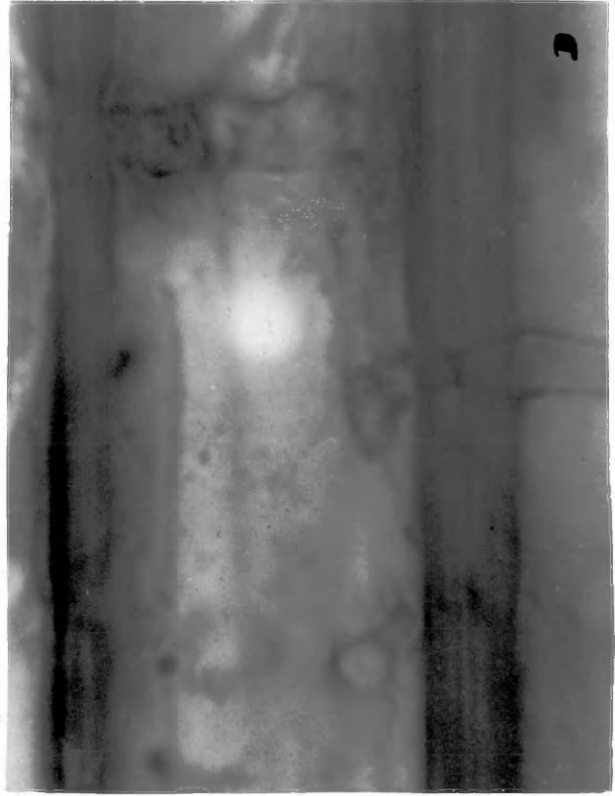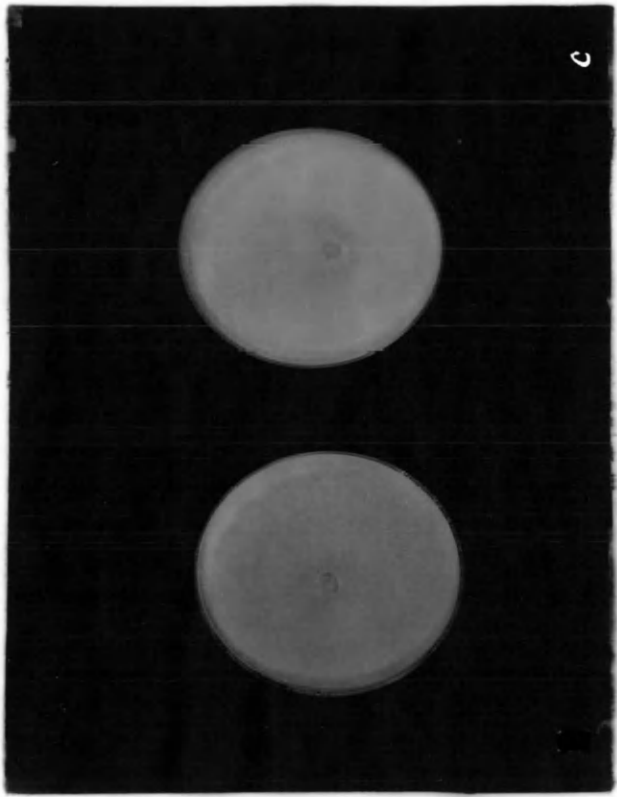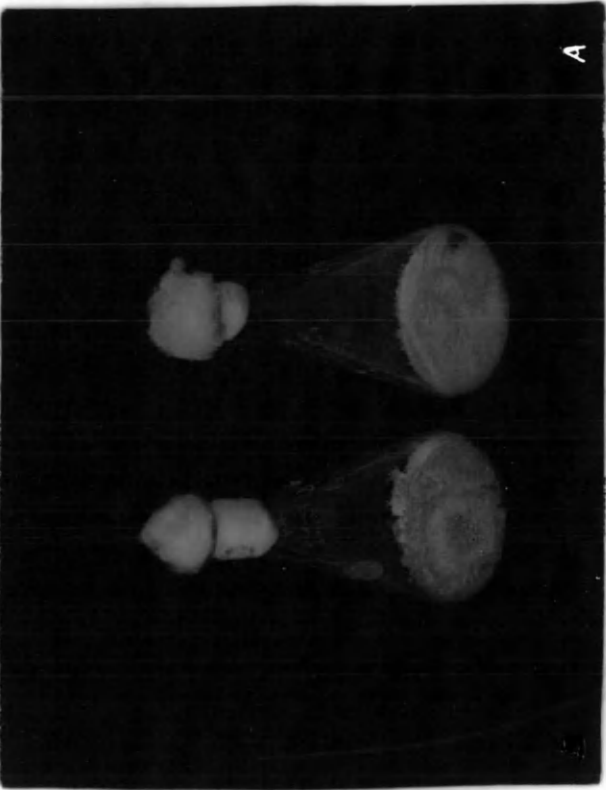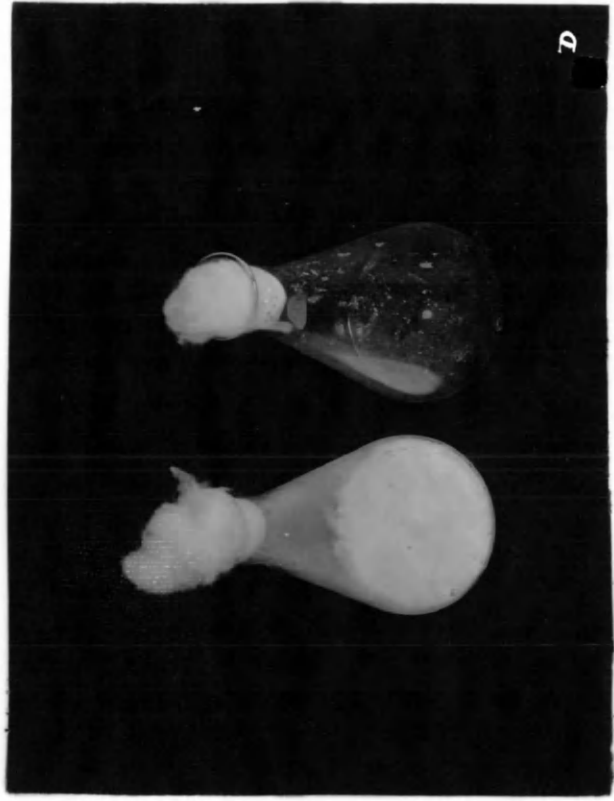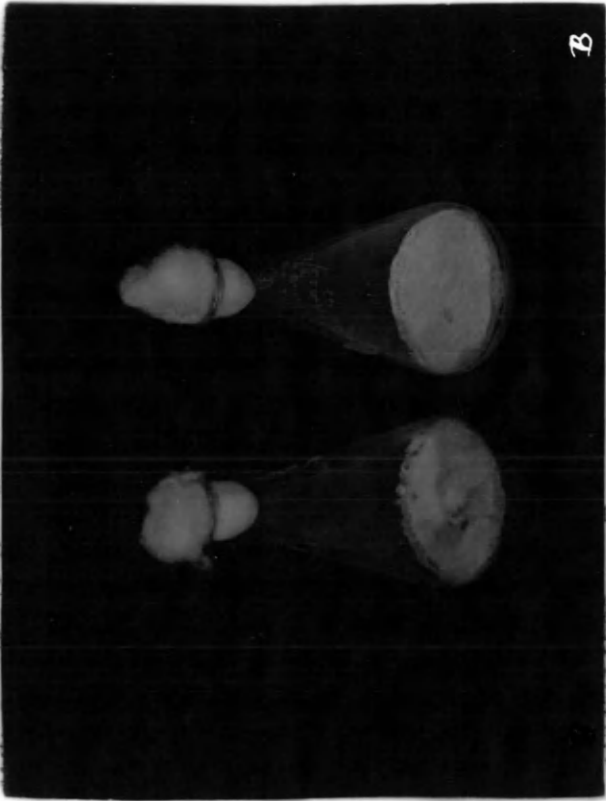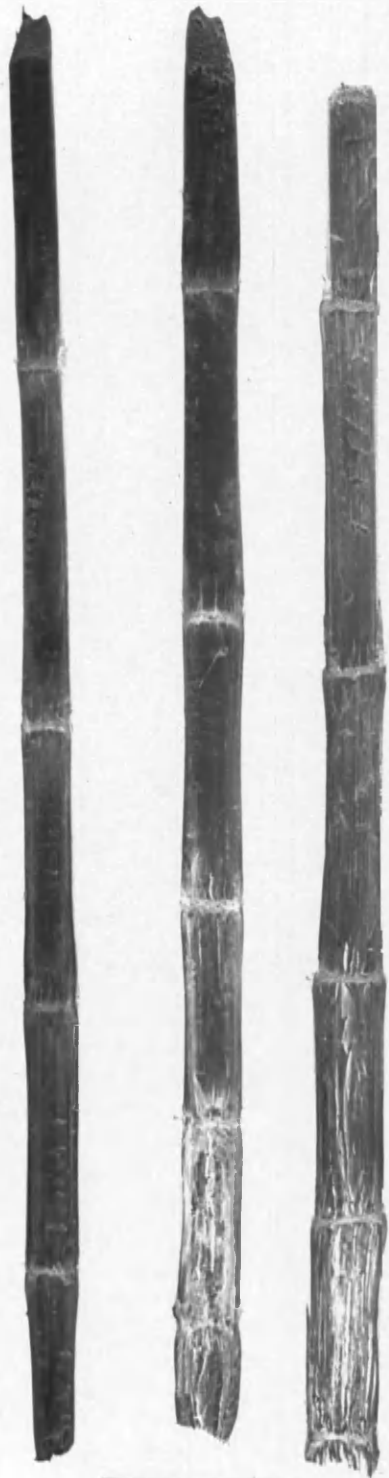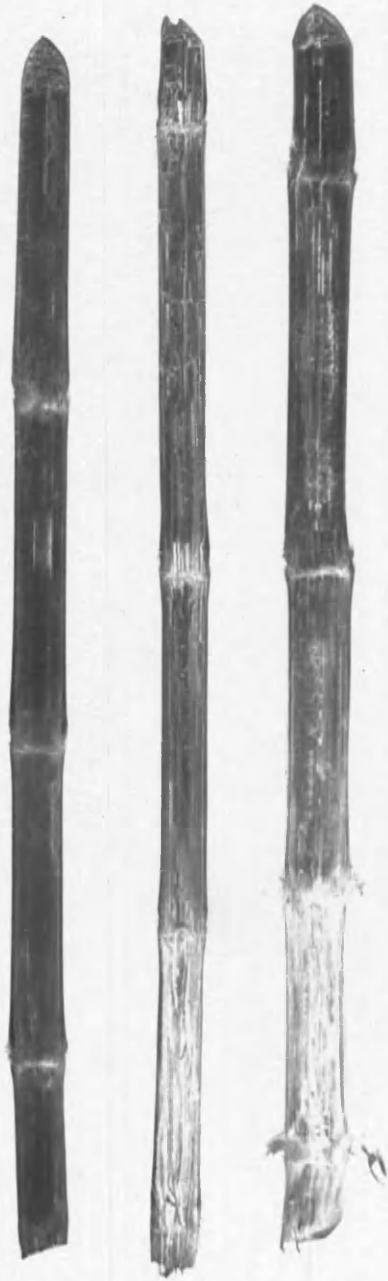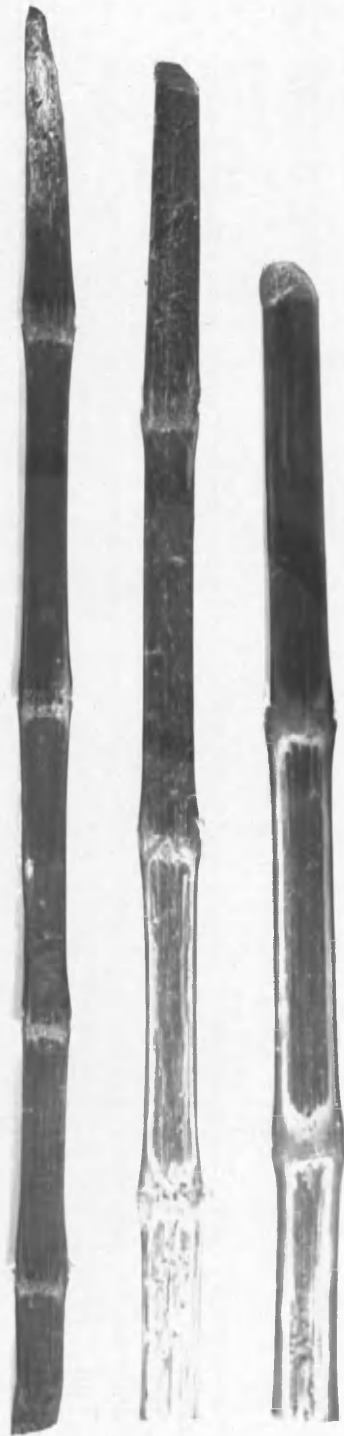
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R 4

90
8/30/40

Insect-Injury

38-11
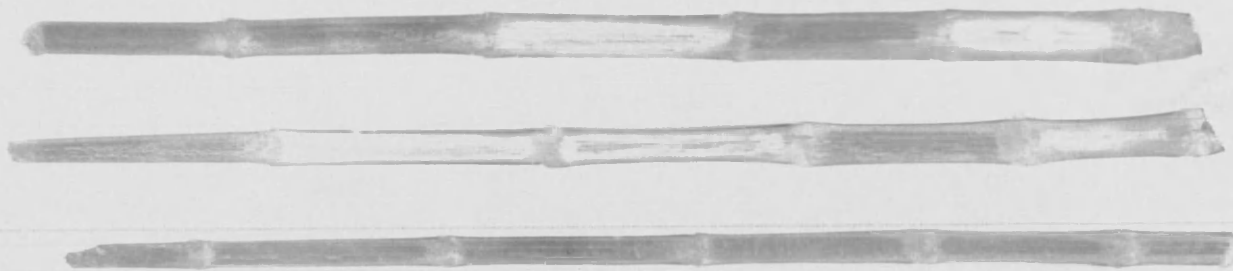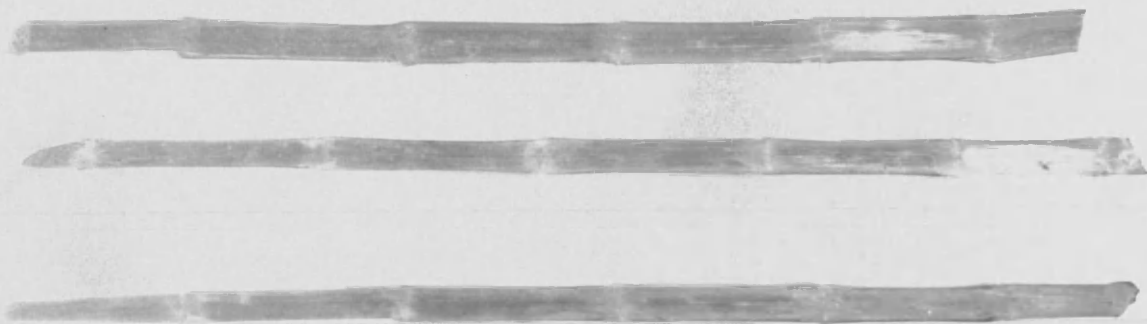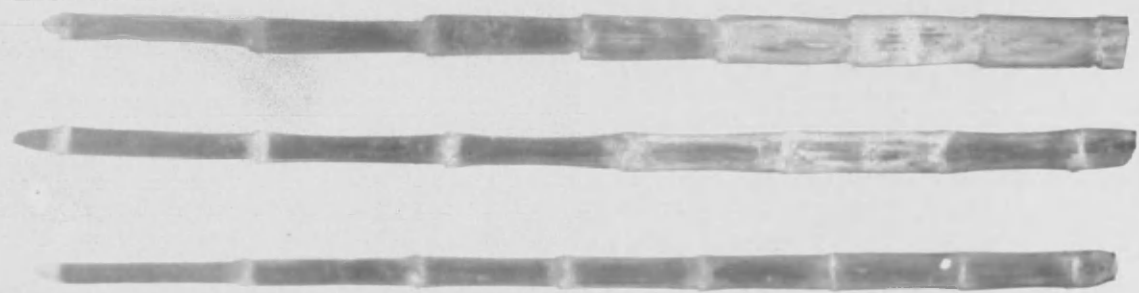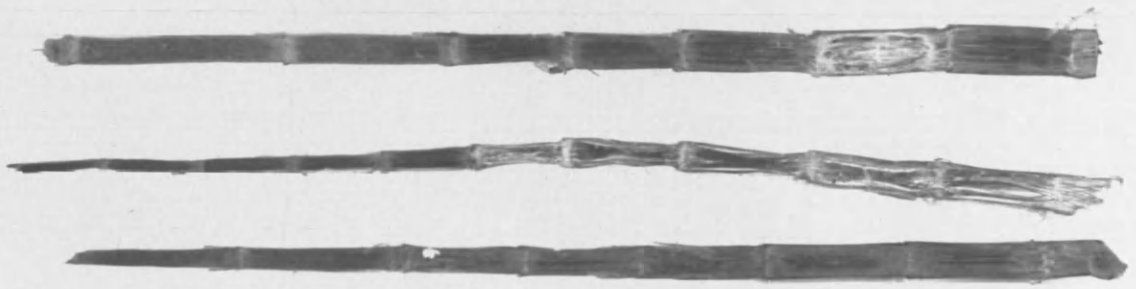
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Plate 9.

Plate 9.

Plate 10.

Plate 11.

Plate 12.

Plate 13

Plate 14.

55
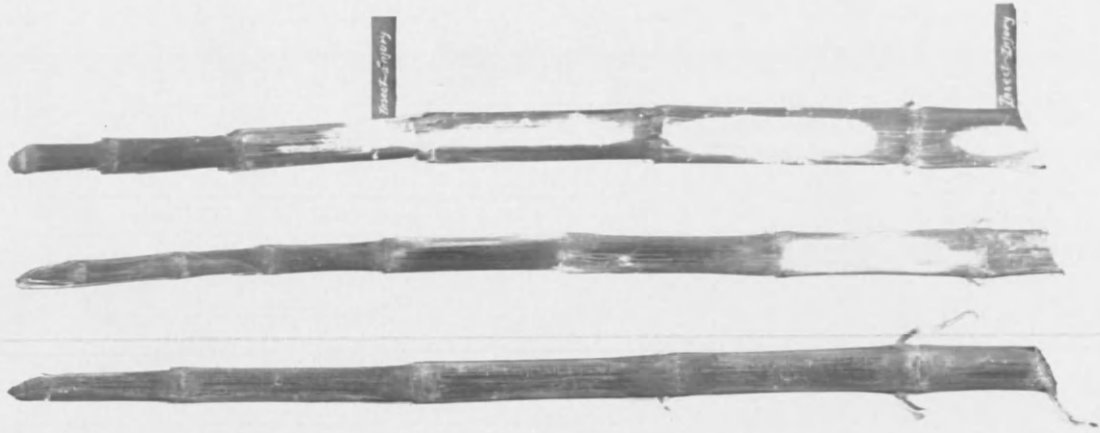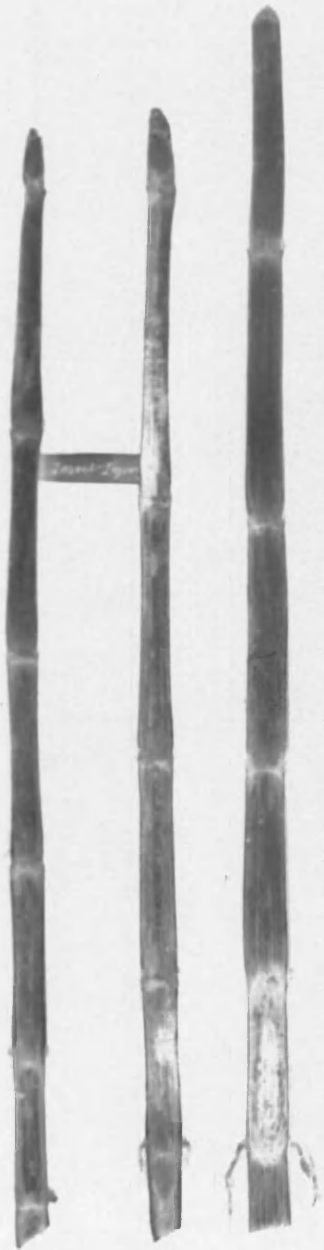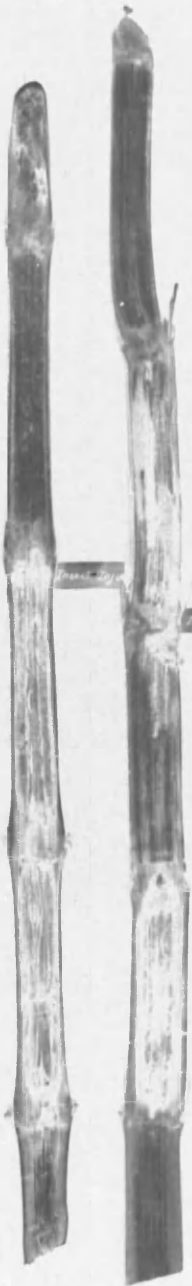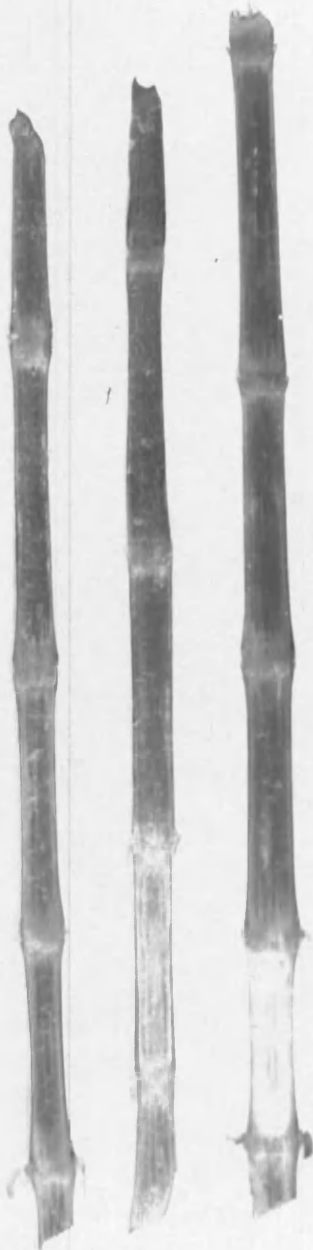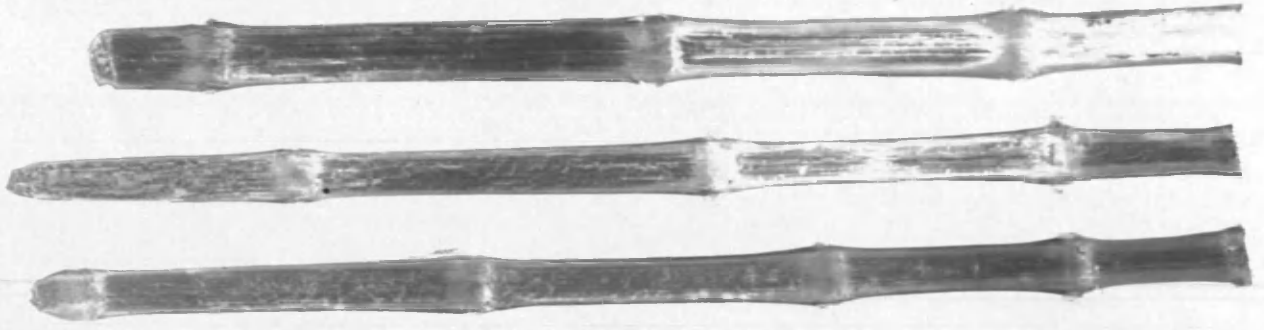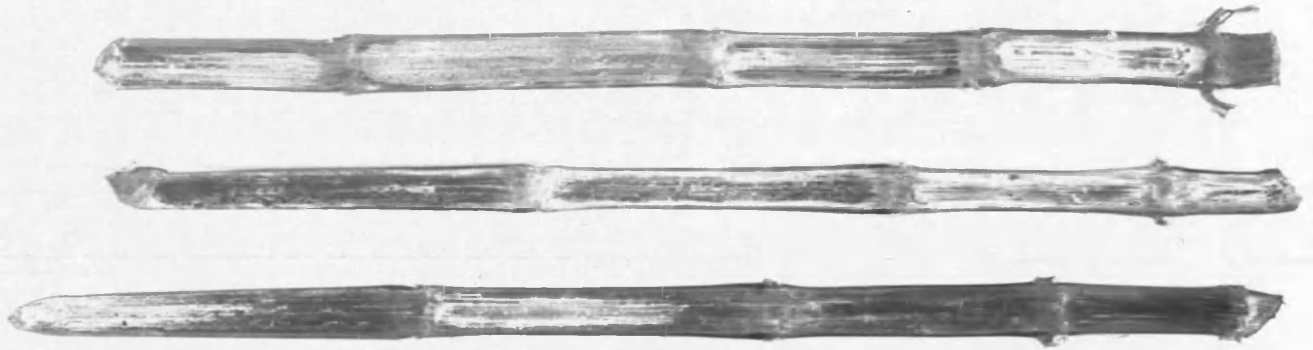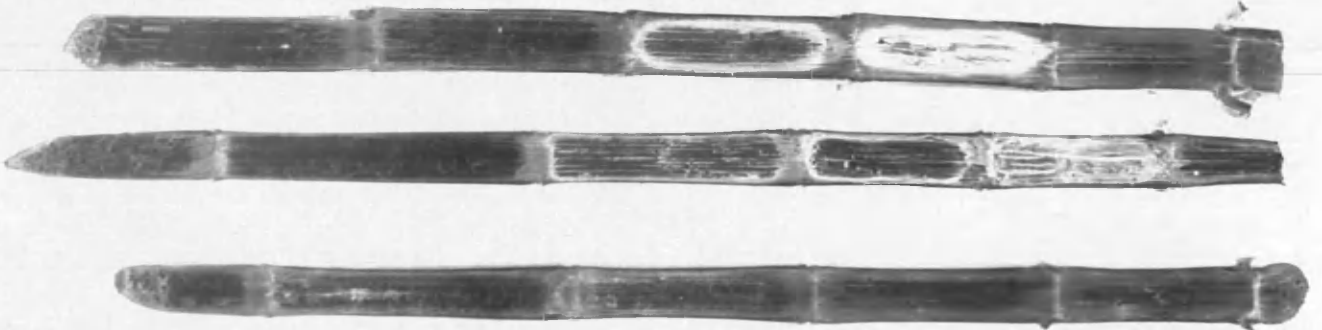
Plate 15.

Plate 16.

Tank 3

Tank 6

Tank 1

Tank 2

Tank 5

Plate 17.

59
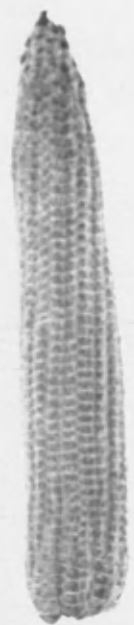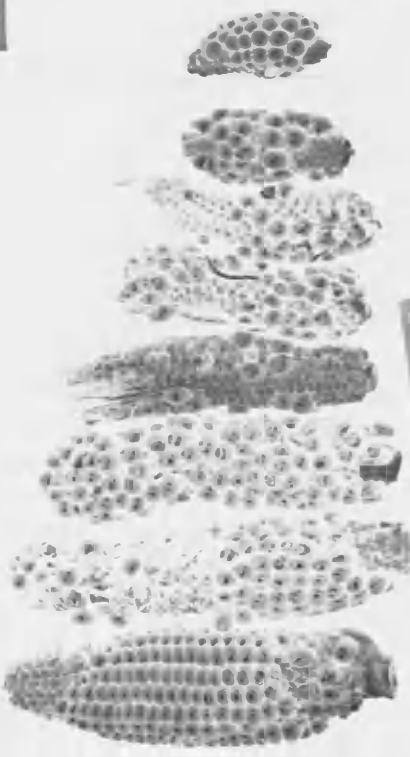
HY X 38-11

TANK 10

4·17·41

Plate 18
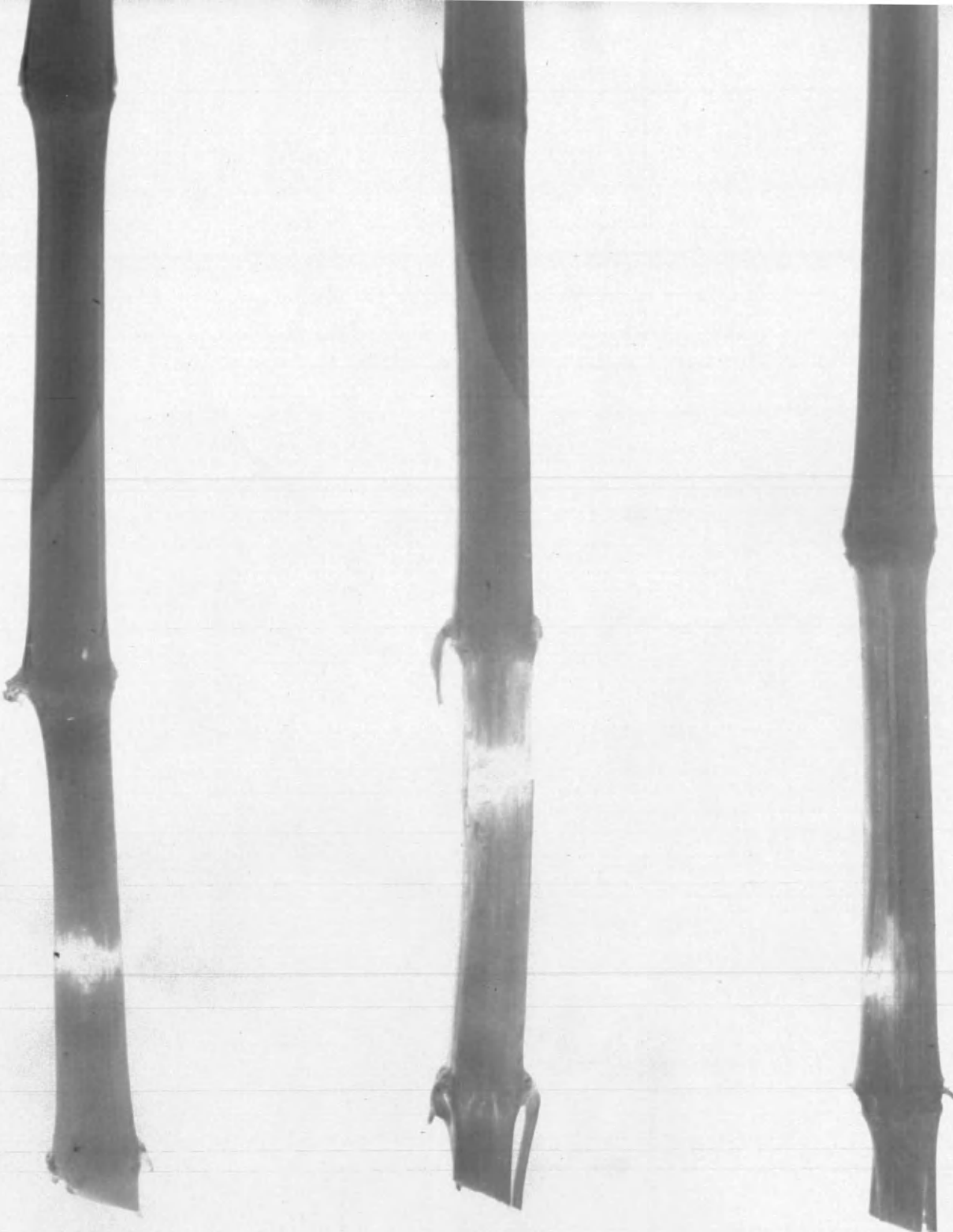
TANK 11

HY X 38-11   4·17·41

Plate 19.

HY X 38-11   TANK 12   4·17·41
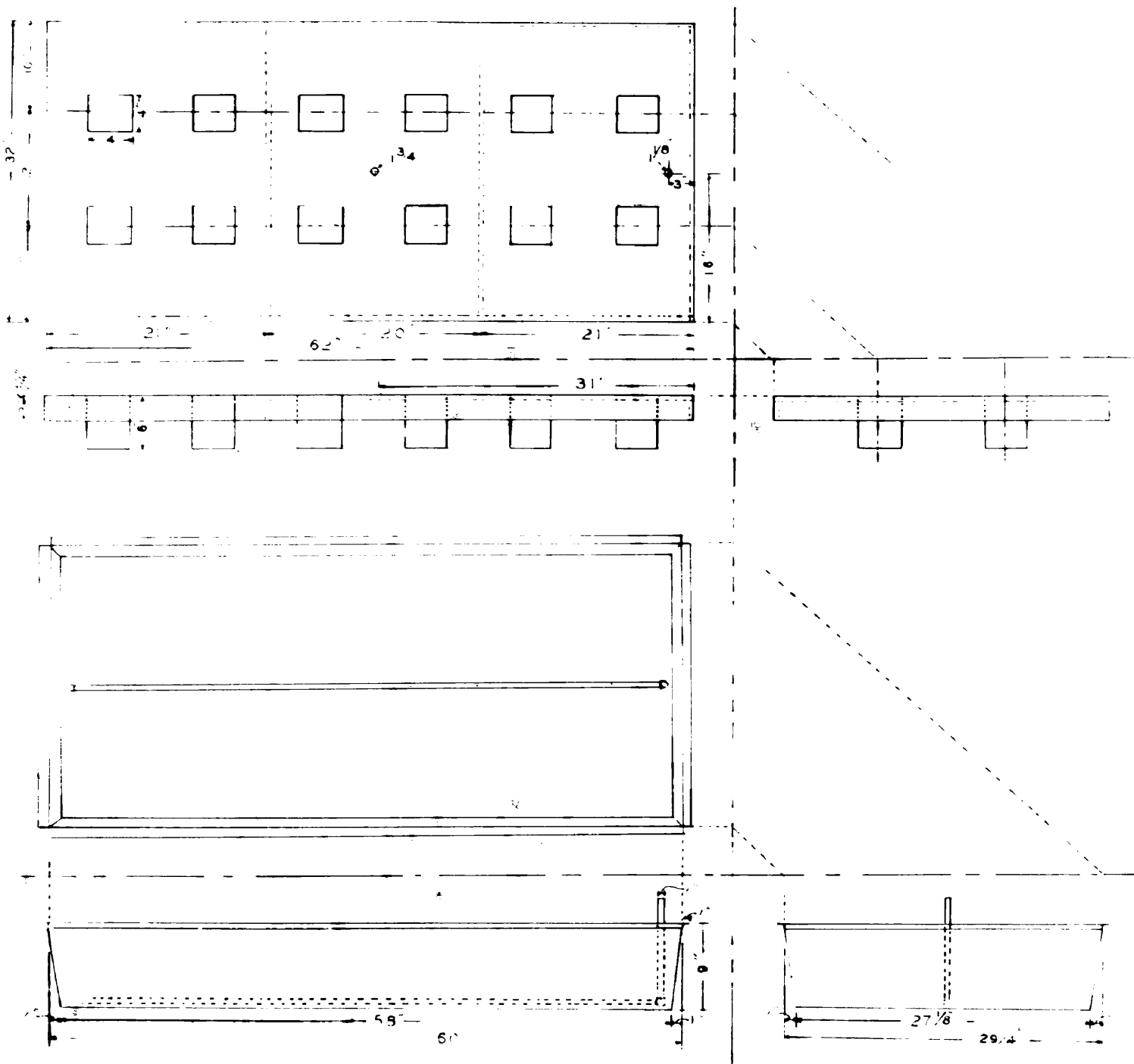
Plate 20.

NUTRIENT SOLUTION TANK
AND TOP

SCALE - 1"=10"   A D HOADLEY

Figure 1.

Figure 2.