THE EFFECT OF FERTILIZERS ON THE CHEMICAL COMPOSITION AND PHYSICAL PROPERTIES OF TOBACCO

by

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TABLE OF CONTENTS

TABLE OF CONTENTS CONT'D

The Effect of Fertilizers on the Chemical Composition And Physical Properties of Tobacco

by

Ho Liu

SUMMARY

I. The effect of three forms of lime and five different combinations of fertilizers on the chemical composition and the physical properties of tobacco was studied in field plot experiments. The effect of various nutrient salts on the chemical composition and physical properties of tobacco was also studied in sand cultures using some solutions of Type I of the three salt solutions recommended by the Division of Biology and Agriculture, National Research Council.

II. Increased yield of air-cured leaves from the field plots was obtained wherever dolomite lime was applied and also where cotton seed meal was applied as a source of ammonia.

III. Increased yield of dry matter in the sand cultures was always obtained from solutions of high $Ca(NO_3)_2$ concentration, while high concentrations of $MgSO_4$ decreased the dry weights.

IV. The nicotine content of leaves from field plots increased with the application of lime, and also where the source of ammonia was nitrate of soda or cotton seed meal.

V. Nicotine content of plants was high whenever the proportion of $Ca(NO_3)_2$ was high, but not in direct ratio. A high

proportion of KH_2PO_4 also favored nicotine synthesis.

VI. The chlorine content of the ash decreased with the application of lime. Application of muriate of potash increased the chlorine content of ash about three-fold as compared with the plants grown on plots receiving potash in the sulphate form.

VII. Application of lime and cotton seed meal improved the color of ash. Muriate of potash used as a fertilizer produced a greenish ash with high degree of firmness.

VIII. A high proportion of $Ca(NO_3)_2$ in sand cultures decreased the ash content of plants and also produced a white and loose ash. Magnesium sulphate increased ash content and improved firmness.

IX. A large supply of nitrate produced rough leaves of a dark color and of poor burning quality.

X. The presence of available potassium appears to be essential to good burning quality. Chlorine is detrimental to a good burn, but chlorine free leaves did not always possess a good burning quality especially when the plants were grown in the presence of a liberal supply of nitrates.

XI. It is suggested that further studies of the comparative value of dolomite lime and calcite lime on tobacco should be made. Cotton seed meal might find justification for use as fertilizer for tobacco when a combination of high yield and good quality are desired. Our results confirm previous prevailing opinion that muriate of potash is not desirable as a source of potassium for tobacco.

XII. No one particular sand culture solution could be selected to indicate a fertilizer formula for tobacco, since none of the solutions studied supported the plant to maturity.

INTRODUCTION

Tobacco is an exhausting crop and liberal applications of commercial fertilizers are necessary. In applying fertilizers to tobacco, the selection of form and amount of plant food as well as their relations to the soil reaction has to be considered, so that not only there will be an increase in yield but also an improvement in quality. Numerous studies of the effect of fertilizers on the chemical composition and physical properties of tobacco have been made and several factors have been well established, nevertheless, the more we study, the more complicated is the problem since the variation of fertilizer combinations, soil conditions and tobacco types are of a complicated nature. It has been reported that dolomitic lime can be used to prevent "Sand-Drown", a chlorosis caused by magnesium deficiency. This makes it evident that magnesium from dolomite lime serves as plant food. Therefore, it was thought that a comparative study of several different forms of lime varying in their ratio of calcium to magnesium on tobacco would be of both practical and scientific interest.

Methods perfected by means of researches on salt requirements of plants has suggested a new method of attack on the problem of the effect of plant food ingredients on the chemical composition and physical properties of tobacco. Garner (8) well says that there are two methods of attacking the problem of the relation of the composition to the burn, one of which may be called the analytical and the other, the synthetical. Investigations on this subject mostly, if not all together have fallen under the head of the analytical method, which consists simply in making comparative analyses of samples of tobacco having good and poor burning qualities and attempting to trace the relation between the differences in composition and the good and bad burning qualities. In solution and sand cultures, we have plants grown under controlled external conditions and regulated plant food supply. Thus we may consider the properties of these plants as synthetical, by which is meant that instead of fixing the burn first and trace back to the chemical composition, we fix approximately the chemical composition and trace out the burning qualities. In an attempt to find the possibility of a synthetic method of attack on the relation of chemical composition and physical properties, sand cultures, with several solutions of Type I, as outlined by the Division of Biology and Agriculture of the National Research Council were conducted.

HISTORICAL

The effect of lime on the soil has been studied extensively and in general when applied in correct amounts and at correct intervals, it improves the physical, biological, and chemical properties of the soil, and increases its producing power. Application of lime to tobacco has been found to increase the yield in many cases. As the results of lime studies in the flue-cured district Mathewson (18) reports an increase in production both on good and on poor soils. Fletcher (6) studied the effect of lime on many different varities of tobacco in Virginia and reported an increase in production. In some cases the increase was nearly fifty percent. Anderson (1) has reported a study made in the flue-cured district with the dark tobacco, in which the use of lime not only increased the yield on all the plots but also increased the value of tobacco.

However, tobacco is marketed not only for its bulkiness, but also for its quality, therefore quality is equally as important as production. The effect of lime on the quality of tobacco has been the subject of controversy and should be given careful study. Mathewson (18) reports that the direct application of lime to tobacco may be somewhat injurious to the quality of the plant by hastening the decay of the vegetable matter in the soil thus increasing the ammonia supply, and thereby producing a dark and coarse tobacco. On some poor soils, however, lime might produce both a larger yield and better quality because of the increase of food supply rendered available. Fletcher (6) reported that when lime was used on heavily fertilized tobacco it appeared to have the tendency to make the leaves and plant rather coarse. The question seems to be concerned with the proper regulation of plant food supply rather than an argument against a direct application of lime to tobacco. Garner (7), in a study of the "sand-drown", a chlorosis of tobacco caused by a deficiency of magnesium, has found that it can be controlled by the application of dolomite lime. Magnesium deficiency not only effects the weight of the plant, but also the quality, color, and elasticity. Thus in some cases the use of dolomitic lime may be necessary in order to secure good quality.

In view of the fact that in most cases applications of lime have increased the yield of tobacco and that the injurious effect may be prevented by regulating the plant food supply, a study of the kind of lime and rate of application when incorporated with a proper amount and suitable kind of fertilizer ingredients may be of some practical value to the former.

Field experiments concerning fertilizer requirements of tobacco do not give conclusive results as there are a great variety of factors involved. A study in which all other factors are eliminated, or at least controlled, except the variation of the amount and kind of plant food was thought to be of interest to determine both the yield and the quality of tobacco as influenced by such variations.

Solution cultures have been used quite extensively for the determination of different combinations and concentrations of elements for the best growth of plants. The one which is most generally used is that of Knop (14) which contains 0.1 per cent $Ca(NO_3)_2$, 0.025 per cent KNO_3, 0.025 per cent KH_2PO_4, 0.025 or 0.0125 per cent $MgSO_4$ and a small quantity of $FePO_4$. Shive (25) simplified Knop's solution by eliminating potassium nitrate, thus making a solution containing only three salts, namely, mono-potassium phosphate, calcium nitrate, and magnesium sulphate, with a trace of iron. These three salts supply the ions: K, Ca, Mg, PO_4, NO_3, and SO_4, which, with a trace of iron, form the essential plant food elements, except those obtained from water and the atmosphere. Livingston (16) and Tottingham (16) suggested six possible types of solutions, all containing the same six ions but derived from different compounds as shown by the following table. These developments, together with the sand culture methods of McCall (21) laid a basis for a

I	II	III	K_2SO_4 $Mg(NO_3)_2$	KNO_3 $Mg(H_2PO_4)_2$	KH_2PO_4 $Mg(NO_3)_2$
$Ca(NO_3)_2$	$Ca(NO_3)_2$	$Ca(H_2PO_4)_2$	$Ca(H_2PO_4)_2$	$CaSO_4$	$CaSO_4$
KH_2PO_4	K_2SO_4	KNO_3	K_2SO_4	KNO_3	KH_2PO_4
$MgSO_4$	$Mg(H_2PO_4)_2$	$MgSO_4$	$Mg(NO_3)_2$	$Mg(H_2PO_4)_2$	$Mg(NO_3)_2$

systematic and uniform research of plant nutrition. The subsequent plan formulated by the Division of Biology and Agriculture, of the National Research Council (15) is the one upon which the present work is based.

Chemical composition has been shown to reflect directly the burning quality and aroma of tobacco. The earlier works of Nessler (22), Mayer (19), Barth (2), Patterson (23) and the more recent work of Garner (8), have shown that a high content of potash improves the burning quality, while high content of chlorine works in the opposite direction. High content of phosphoric acid has been shown to be injurious to burning quality, while high content of sulphate is either beneficial or exerts little effect.

Concerning aroma, many have attributed this quality to nicotine content. Thus Fasca and Imai (5) stated that tobacco should contain a certain per cent. of nicotine. The most favorable quantity, however, was not determined. The work of Garner (10) indicates that it is rather the quality of nicotine than the quantity that determines the aroma. Thus he states that fine tobacco contains only moderate amounts of nicotine and aroma is due to its decomposing products during the fermentation process.

The quantity of nicotine has been found to vary under different chemical and physical influences. Garner (10) concludes that very rich heavy soils and excessive quantity of nitrogenous fertilizers which tend to produce a coarse rank growth produce a high percentage of nicotine. Mayer (20) finds that the nicotine content of tobacco increases with the increase of light and temperature. These variations induced Garner (10) to conclude that the relative importance of any one factor in its effect to production of nicotine can be determined only by experiments so planned and conducted as to exclude or at least to control, the effect of all others.

In the experiments reported, it is aimed to study the influence of different combinations of fertilizers with and without lime on the nicotine contents of tobacco in the field and also on the effect of certain plant food elements on the nicotine content of tobacco grown in sand cultures with external factors controlled. The chlorine content of tobacco has been reported to increase when chloride salts are applied. Little study, however, has been made on the effect of other plant food elements and lime on the chlorine content of tobacco. With the same samples collected for nicotine analyses, chlorine content of ash were determined for the purpose of throwing some light on the availability of chlorine with respect to different soil treatments.

Besides making chemical analyses of nicotine and chlorine, physical properties were studied. The only quantitative study on the physical properties made was the percentage of ash. Due to the lack of sufficient samples as well as the lack of methods and apparatus, the other studies of physical properties were made only qualitatively.

EXPERIMENTAL

FIELD EXPERIMENTS.

Method of Experimentation.

Field tests on the rate of application of different forms of lime to tobacco, when applied to a slightly acid soil.

Field plots were located on a field of Leonardtown loam at La Plata, Maryland. The soil is rich and the lime requirement according to the Veitch (29) test was only about a ton to the acre. One acre of the land was selected and divided into ten parts. Each of the ten plots received different fertilizer treatments. The tenth acre plots were subdivided into four plots thus making forty plots to the acre. Each forteith acre plot received a different lime treatment. The three forms of lime used were ground limestone, whose chemical composition varied with respect to the ratio of CaO to MgO. Lime was spread broadcast and harrowed under. Five different fertilizer combinations, all having the formula 6-8-4 but varying in the sources of individual ingredient were applied, broadcast at the rate of 800 pounds to the acre. Table III gives the various treatments.

Seedlings of the Maryland Broad Leaf type of tobacco were secured from a commercial plant bed for use in these plots. This type of tobacco is prefered because it is commonly recommended for Maryland conditions owing to its wide and large leaves of less body with a tendency towards lighter color and possessing good burning quality (9).

Table I. Fertilizer and Lime Treatments of the Field Plots

La Plata Field.

Plot No.	Fertilizer treatments (800# of 6-8-4 per acre)	Lime Treatments
I A_1	50% of the NH_3 derived	1500# $CaCO_3$ (ground limestone)
B_1	from $NaNO_3$ and 50% from	" $CaCO_3$ $MgCO_3$ (10:1)
C_1	$(NH_4)_2SO_4$; P_2O_5 from acid	" $CaCO_3$ $MgCO_3$ (2:1)
Check	phosphate and K_2O from K_2SO_4.	No Lime
II A_2	NH_3 derived from $(NH_4)_2SO_4$.	1000# $CaCO_3$ (ground limestone)
B_2	P_2O_5 from acid phosphate,	" $CaCO_3$ $MgCO_3$ (10:1)
C_2	and K_2O from muriate of	" $CaCO_3$ $MgCO_3$ (2:1)
Check	potash.	No Lime
III A_1	NH_3 derived from $NaNO_3$,	Same as A_1 under I.
B_1	P_2O_5 from acid phosphate,	" " B_1 " I.
C_1	and K_2O from sulphate of	" " C_1 " I.
Check	potash.	No Lime
IV A_2	NH_3 derived from $(NH_4)_2SO_4$.	Same as A_2 under II.
B_2	P_2O_5 from acid phosphate,	" " B_2 " II.
C_2	and K_2O from sulphate of	" " C_2 " II.
Check	potash.	No Lime
V A_1	50% NH_3 derived from $(NH_4)_2$	Same as A_1 under I.
B_1	$(NH_4)_2SO_4$ and 50% from cotton	" " B_1 " I.
C_1	seed meal, P_2O_5 from acid phos-	" " C_1 " I.
Check	phate, and K_2O from K_2SO_4.	No Lime.
VI A_2	Repeat I.	Same as A_2 under II.
B_2		" " B_2 " II.
C_2		" " C_2 " II.
Check		No Lime.

(Table I continued)

VII	A_1	Repeat II	Same as A_1 under I.
	B_1		" " B_1 " I.
	C_1		" " C_1 " I.
Check			No Lime
VIII	A_2	Repeat III.	Same as A_2 under II.
	B_2		" " B_2 " II.
	C_2		" " C_2 " II.
Check			No Lime.
IX	A_1	Repeat IV.	Same as A_1 under I.
	B_1		" " B_1 " I.
	C_1		" " C_1 " I.
Check			No Lime
X	A_2	Repeat V.	Same as A_2 under II.
	B_2		" " B_2 " II.
	C_2		" " C_2 " II.
Check			No Lime.

Presentation of Results.

Yield - Weights obtained from field plots represent the weights in pounds of air-cured leaves. Table II gives the various weights.

Chemical Composition and Physical Properties - Nicotine content of tobacco was determined by the Kissling method (13). Percentage of ash (24) and chlorine (30) content of ash was determined by the official method of A.O.A.C. Table III gives the results of the various determinations and qualitative studies of the plant ash.

Discussion.

Apparent Condition of Plants - Little difference in the condition of plants in the field plots was apparent. Lime treatment showed practically no visible difference. Fertilizer treatments showed that in plots V and X where cotton seed meal was applied, growth was more vigorous and leaves were smooth and thin. In plots I and VI where all of the nitrogen was applied in the form of nitrate of soda, the leaves were dark in color and coarse in texture.

Yield - The yield of all of the field plots was low due to the fact that the wet spring of 1924 delayed the planting and the dry summer following checked the growth so that when har-

Table II. The Effect of Lime and Fertilizer Treatments in the Yield of Tobacco.

La Plata Field.

Plot No.	Yield in lbs. per plot.	Corresponding to yield in lbs per acre	Increase or decrease over check, per acre
I A_1	11.66	466.	-4.
B_1	13.00	520.	50
C_1	12.25	490	20
Check	11.75	470	
II A_2	10.50	420	-150
B_2	11.00	440	-130
C_2	13.00	520	- 40
Check	14.00	560	
III A1	13.75	550	180
B_1	13.75	550	180
C_1	13.25	530	160
Check	9.25	370	
IV A_2	9.75	390	-130
B_2	8.50	340	-180
C_2	13.00	520	0
Check	13.00	520	
V A_1	13.00	520	- 60
B_1	17.25	690	110
C_1	17.00	680	100
Check	14.50	580	
VI A_2	19.75	470	110
B_2	17.50	700	450.

(Table II continued)

C_2	11.50	460	100
Check	9.00	360	
VII A_1	14.50	580	180
B_1	14.25	570	170
C_1	10.75	430	30
Check	10.00	400	
VIII A_2	12.75	510	- 10
B_2	10.25	410	-110
C_2	13.00	520	0
Check	13.00	520	
IX A_1	14.00	560	-280
B_1	16.25	650	-190
C_1	14.50	580	-260
Check	21.00	840	
X A_2	21.75	870	150
B_2	22.25	890	170
C_2	21.25	850	130
Check	18.00	680	

vested the plants were somewhat immature.

In comparing the different forms of lime we find in Table IV that the highest yield was obtained from plots receiving dolomite lime whose ratio of CaO:MgO was 10:1. The next highest yield was obtained from plots to which was applied dolomitic lime whose ratio of CaO:MgO was 2:1. Calcium carbonate treatment gave a yield a little lower than that of check. Since the applications were low and the difference of yield was small, the favorable effect of dolomite over calcite lime was not conclusive but suggests a field for further study.

Table V shows that when the yield of the different fertilizer treatments are compared, little difference can be found when the plant ingredients were all inorganic, but where the cotton seed meal was substituted for 50% of the total nitrogen the yield was about 30% higher. Cotton seed meal usually contains about 6% of magnesium, however, no indications could be found to account for the increase in yield as due to the magnesium content.

Chemical Composition and Physical Properties.- From a study of Table III we find that a slight variation of nicotine content due to fertilizer treatments was obtained. While nitrate of soda and cotton seed meal plots produced a higher percentage of nicotine, the other treatments did not seem to show any difference.

Lime treatments decidedly increased the nicotine content

Table IV The Effect of Lime Treatments on the Yield of Tobacco

La Plata Field Tests, 1924

Plot No.	Lime Treatments	Weight of Tobacco in Lbs. Per Acre	Average for each form of Lime Treatment	Increase or decrease over check per acre
A_1	1500# $CaCO_3$ (Ground Limestone)	642.1		
A_2	1000# $CaCO_3$ " "	665.0	653.5	-11.5
B_1	1500# $CaCO_3$ $MgCO_3$ (10:1)	745.0		
B_2	1000# " " "	695.0	720.0	55.0
C_1	1500# $CaCO_3$ $MgCO_3$ (2:1)	677.5		
C_2	1000# " " "	717.5	687.5	22.5
Check	No Lime	665.0	665.0	

Table V. The Effect of Fertilizer Treatments on the Yield of Tobacco.

La Plata Field Tests 1924.

Plot No.	Fertilizer Treatments. (800# 6-8-4 per acre)	Weight of Tobacco in lbs. per acre.	Average for each treatment.
I.	50% NH_3 from $(NH_4)_2SO_4$ 50%	596.6	
VI.	from $NaNO_3$. P_2O_5 from Acid	497.5	546.9
	Phosphate and K_2O from K_2SO_4.		
II.	NH_3 from $(NH_4)_2SO_4$;P_2O_5 from Acid	485.0	
VII.	Phosphate; K_2O from KCl.	495.0	490.0
III.	NH_3 from $NaNO_3$; P_2O_5 from Acid	500.0	
VIII.	Phosphate; K_2O from K_2SO_4.	490.0	450.0
IV.	NH_3 from $(NH_4)_2SO_4$; P_2O_5 from Acid	442.5	
& IX.	Phosphate; K_2O from K_2SO_4	652.5	547.5
V.	50% NH_3 from $NaNO_3$; & 50% from	617.5	
X.	Cotton Seed Meal; P_2O_5 from Acid	832.5	725.0
	Phosphate; and K_2O from K_2SO_4.		

Table III. Effect of Lime and Fertilizer Treatments on the Nicotine and Chlorine Content and the Ash of Leaves.

La Plata Field.

Plot No.	Percentage of Nicotine of Leaves	Percentage of Chlorine of ash	Percentage of ash of Leaves	Color of Ash	Character of Ash
I a	2.755	0.334	18.22	White	Firm
b	2.779	0.228	20.48	Whitish green	"
c	2.486	0.356	22.68	" "	"
Check	2.749	0.343	21.67	Greenish gray	"
II a	2.768	1.190	21.45	" "	Very firm
b	2.355	1.290	20.77	" "	" "
c	3.355	1.018	21.74	" "	" "
Check	2.536	1.297	20.54	Gray	" "
III a	2.725	0.886	20.05	White	Flaky, loose
b	2.905	0.323	17.64	"	" "
c	2.345	0.306	18.11	"	" "
Check	2.400	0.238	19.46	Whitish gray	" "
IV a	2.369	0.202	18.27	" "	Flaky, firm
b	2.907	0.181	18.33	" "	" "
c	2.351	0.119	21.73	" "	" "
Check	2.075	0.207	18.08	Gray	" "
V a	2.303	0.319	19.19	White	" "
b	2.677	0.318	21.08	"	" "
c	2.729	0.266	20.39	"	" "
Check	2.146	0.340	19.12	"	" "

(Table III continued)

VI a	3.087	0.264	19.05	White	Flaky, loose
b	3.456	0.322	19.85	"	" "
c	3.096	0.311	18.63	"	" "
Check	1.819	0.673	21.43	Whitish gray	" "
VII a	2.782	0.986	17.16	" brown	Firm
b	3.164	0.940	20.83	" "	"
c	2.301	0.886	14.76	" "	"
Check	2.254	1.312	14.42	" "	"
VIII a	2.524	0.263	16.82	Gray	Flaky, loose
b	2.590	0.267	15.96	"	" "
c	2.241	0.257	16.72	"	" "
Check	1.998	0.403	16.80	Whitish gray	" "
IX a	2.598	0.100	17.15	" "	Flaky, firm
b	2.451	0.103	18.09	" "	" "
c	4.096	0.194	17.66	" "	" "
Check	2.613	0.144	17.30	Whitish, brown	" "
X a	2.988	0.419	17.37	White	" "
b	3.456	0.267	16.97	"	" "
c	2.951	0.213	15.68	"	" "
Check	2.304	0.323	17.21	"	" "

especially when dolomitic lime was applied. Of the two dolomitic limes, the one with a ratio of CaO:MgO as 10:1 produced a higher percentage of nicotine. The difference, however, was only indicative.

With few exceptions lime treatment has decreased chlorine content. It is interesting to note also that as the percentage of MgO in the lime increased, the chlorine content of the plant decreased.

Fertilizer treatments influenced decidedly the chlorine content of tobacco. By substituting muriate of potash for sulphate of potash, the chlorine content of ash increased three times. In treatments where the source of nitrogen was from sulphate of ammonia and potassium was from potassium sulphate, the chlorine content showed a slight decrease over the other treatments.

Different forms of lime and fertilizers failed to show any effect upon the total percentage of ash in the leaves. The color and character of ash, however, were determined largely by the supply of lime and by certain forms of fertilizers. Wherever lime was not applied, the ash was darker than where lime was used. No difference, however, could be distinguished between the effect of the three forms of limes, Muriate of potash gave a firm and greenish ash.

The texture, color, and burn of the leaves were studied by a practical tobacco judge. Lime failed to exert any effect on the three above mentioned properties. Muriate of potash was detrimental to the burning quality. Nitrate of soda produced a dark leaf with heavy veins. It is estimated that the use of cotton seed meal improved the texture and the burn about 30%.

Sand Culture Experiments.

For the purpose of studying the effect of various molecular and ionic ratios, sand cultures were employed, using solutions of Type I and a sub-strata of pure quartz sand. The type of tobacco used was also Maryland Broad Leaf the same as that for the field experiments. Sand cultures are preferred over solution cultures as McCall (21) has found that the average dry weights of both tops and roots of winter wheat seedlings were decidedly greater for the plants grown in sand than for those grown in the solutions.

Method of Experimentation - GERMINATION - In order to eliminate as many heterogeneous factors as possible in the way of plant food absorption the tobacco stock was grown in quartz sand with Shive's solution R_5C_2 (25) instead of in soil. Owing to the small size of the tobacco seed and the slow growth of the tobacco seedlings, considerable difficulty was encountered in the raising of a healthy stock on pure sand for transplanting. Preliminary tests indicated that germination on sand was better than on mosquito net since the holes in the net must be small enough to catch the seed and large enough not to be clogged when dipped in parafine. For moistening the sand tap water was found to be better than distilled water. However, a nutrient solution R_5C_2 (25) gave the best result since the seeds of tobacco are small and the reserve plant food is scanty.

Having selected the proper materials with which to work, the question arose, as to the method to be followed for feeding and for checking rapid evaporation. To insure an equitable distribution of the solution several were set around the crock so that the distance between any two tubes was not more than three inches. Equal amounts of solution being added through each tube whenever new solution was introduced.

Rapid evaporation and successive additions of nutrient resulted into a hard crust on top of the sand much like that formed on an alkali soil. This difficulty was avoided by sealing the crock with a wax (3) before seeding small holes being cut through the wax by means of a cork borer, at equal intervals all over the surface of the sand. The seed was first mixed with about 50 times as much pure sand and a gram or so of the mixture which should carry about 5 seeds is poured into each hole. The solution was introduced and renewed in the same way as for the regular crocks.

Preparation of Crocks. - Into each one-gallon glazed earthenware crock was poured 4000 grams of air-dry quartz sand which had been previously washed with tap and distilled water. A glass feeding tube, approximately 1 cm. in bore, was placed close to the inside of the crock at one side, and a suction tube, essentially a glass tube having a 5 mm. bore, was placed close to the inside at the opposite side. The lower end of the suction tube reached down to the bottom of the crock and carried a tuft of glass wool caught by a narrow neck to prevent the sand from coming up when suction was applied. The weight of the arrangement (including the crock, the sand and the tubes) is obtained, and just for convenience we designate this as "weight I". Enough of a certain nutrient solution, corresponding to what the crock should receive during the course of the experiment is added to flood the sand. After having saturated the sand, the excess is sucked out through the glass tube, this manipulation serving to set the tubes into a permanent position and at the same time firming and smoothing the surface of the sand. The weight of this system was obtained, which we will call "weight II". The crocks are then sealed with a wax seal similar to that used by Briggs and Shantz (3). A hole was then bored through at the centre of the seal with a 1½ cm. cork borer, to the depth of approximately 3 centimeters. The plants were pulled out of the bed after having flooded the bed with enough water to loosen the roots from the sand. The roots are then washed and loosely suspended in the hole, while dry quartz sand is introduced into the hole through a funnel until the latter is filled. By this means the root was least disturbed and the danger of injury to the plant by the hot paraffin wax was avoided.

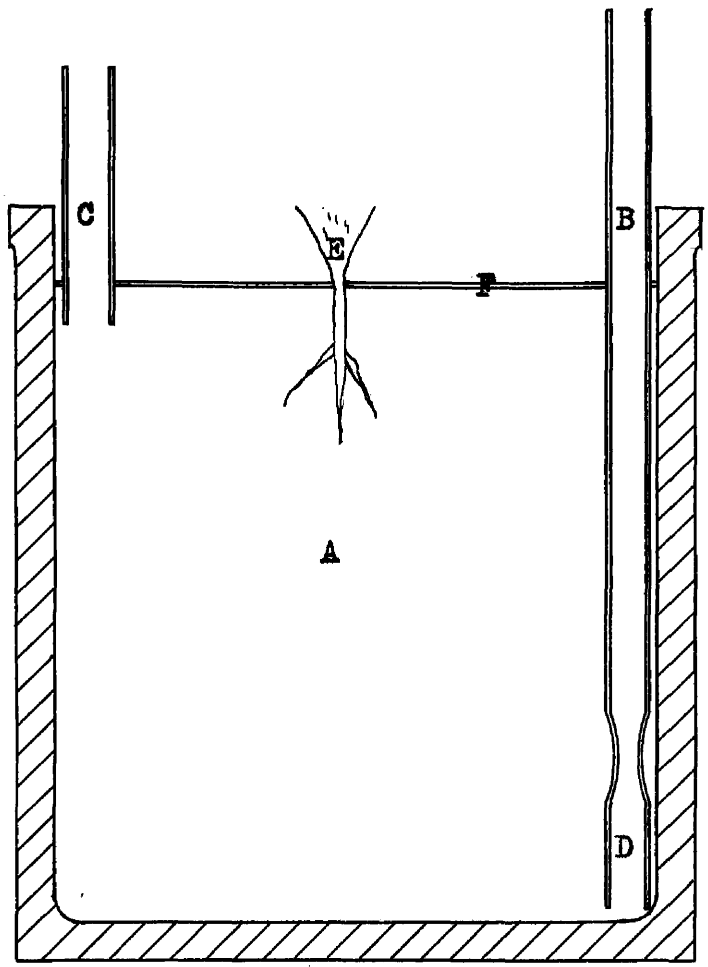


Fig. I.

Arrangement of Crock.

A-Quartz sand.
B-Suction tube.
C-Feeding tube.
D-Glass wool.
E-Plant.
F-Wax seal.

The weight of this final arrangement is obtained which we will call weight III.

If from weight III we subtract weight II, we obtain the addition of weight due to parafin and other minor additions. Add to weight I, the grams of water necessary to give a moisture content of 16% and we get the final weight of the crocks. A sufficient amount of solution is then removed or added as may be required to bring the system to the final weight. Figure I shows a diagramatic cross section of the crocks.

Renewing of Solutions.

The method of renewing solutions differed somewhat from that of McCall (21), but followed closely to that of Johnston (12). Whenever the solutions were renewed each container with its plants was weighed and enough distilled water was added to bring its total weight back to its original weight. Enough solution (300c.c.) was then added to flood the sand by siphoning over from Erlenmyer flask. After a few minutes the excess solution was withdrawn through the small suction tube and the culture again brought back to its original weight. Solution was renewed every $3\frac{1}{2}$ days.

The arrangement of the siphoning apparatus is shown in Figure II.

Solutions Studied.

It is a common observation that plant individuals vary

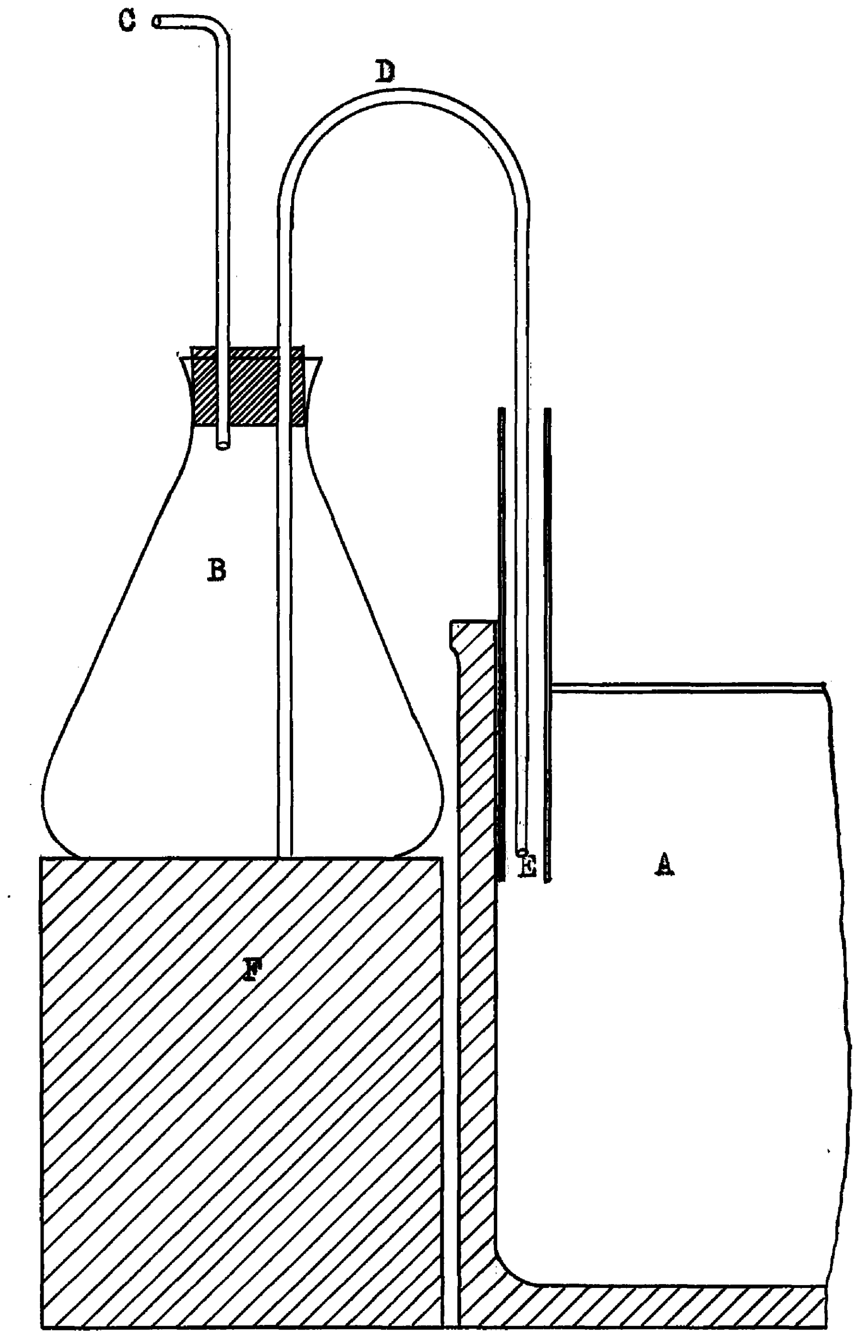


Fig. II.

Arrangement of Feeding Apparatus.

A-Quartz sand.
B-Flask containing nutrient solution.
C-Blowing tube.
D-Siphon tube.
E-Feeding tube.
F-Support for flask.

although the seeds may have originated from the same mother plant and the plants may have received equal treatments. For this reason six duplicates were used for each culture. Owing to this fact, time was not available to cover all the solutions of Type I. Reports of various authors have shown that wherever nitrate is low, growth is poor, therefore several solutions which had low proportions of calcium nitrate were omitted. Tables VI and VII give the solutions studied.

Presentation of Results.

Yield - (Series I) The transplanting for the first series was made April 26, 1924. The plants of this series were grown for 6½ weeks when the plants were cut before maturity on account of the appearance of a disease which will be discussed later. Dry weight of plant was obtained by desicating the air-cured tobacco over sulphuric acid for a week. The method for obtaining the other weights followed precisely the directions given by McCall (15). Table VIII gives the dry weights of tops, roots and entire plant.

(Series II) Transplanting on the second series was made December 9, 1924. The plants of this series were grown for 10 weeks when the plants were cut before maturity on account of the appearance of a disease similar to that which developed in Series I. The methods of obtaining the different weights were the same as those for Series I. The weights are recorded in Table IX.

Table VI.

Molecular Properties of the Solutions Employed in Series I.

Lab No.	Solution No.	Molecular proportions			Partial Volume - Molecular concentrations		
		KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
I	R_2S_2	2	2	4	.0049	.0049	.0099
II	R_2S_3	2	3	3	.0047	.0071	.0071
III	R_2S_4	2	4	2	.0045	.0090	.0045
IV	R_3S_1	3	1	4	.0076	.0025	.0101
V	R_3S_2	3	2	3	.0072	..0048	.0072

Table V.

Molecular Properties of the Solutions Employed in Series II.

Lab No.	Solution No.	Molecular proportions			Partial Volume - Molecular concentrations		
		KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
I	R_1S_1	1	1	6	.0027	.0027	.0161
2	R_1S_6	1	6	1	.0020	.0122	.0020
3	R_2S_4	2	4	2	.0045	.0090	.0045
4	R_3S_4	3	4	1	.0065	.0086	.0021
5	R_4S_3	4	3	1	.0090	.0068	.0022
6	R_5S_2	5	2	1	.0118	.0047	.0023
7	R_5C_2	4	1	3	.0180	.0052	.0150

TABLE VIII.

Green weight of Plants, Dry weight of Tops, of Roots, and of Entire Plant.

(Series I.)

Crock No.	Green wt. of plants	Dry wt. of tops	Dry wt. of roots	Total dry wt. of plants
	Gms.	Gms.	Gms.	Gms.
I. 1	60	4.005	1.249	5.254
2	50	5.817	lost	5.817
3	55	5.072	0.969	6.041
4	70	6.048	0.985	7.033
5	80	6.501	0.679	7.180
6	75	5.725	1.133	6.858
Total	390	33.168	5.015	58.183
Average	65	5.528	1.003	6.531
II. 1	85	7.526	1.331	8.857
2	75	5.610	0.690	6.300
3	85	6.625	1.178	7.803
4	lost			
5	60	6.940	1.215	8.155
6	54	2.980	0.346	3.326
Total	359	29.681	4.760	34.441

TABLE VIII (Continued.)

Crock N No.	Green wt. of plants	Dry wt. of tops	Dry wt. of roots	Total dry wt. of plants
Average	69.7	5.936	0.952	6.888
III.1	83	6.304	0.871	7.175
2	104	8.525	1.278	9.803
3	105	7.993	1.394	9.387
4	100	10.016	1.783	11.799
5	80	6.993	0.876	7.869
6	50	3.038	0.514	3.552
Total	522	42.869	6.716	49.585
Average	87	7.145	1.119	8.264
IV. 1	59	5.672	0.895	6.567
2	46	5.243	0.996	6.239
3	55	4.351	0.924	5.275
4	40	4.985	0.884	5.869
5	45	3.970	0.952	4.922
6	50	3.751	0.873	4.624
Total	295	27.972	5.524	33.496
Average	49	4.662	0.920	5.582
V. 1	75	6.970	1.217	8.187
2	30	3.805	0.767	4.572
3	85	7.757	1.125	8.882

TABLE VIII (Continued.)

Crock No.	Green wt. of plants	Dry wt. of tops	Dry wt. of roots	Total dry wt. of plants
4	80	6.673	1.032	7.705
5	70	6.086	1.043	7.129
6	70	5.373	0.570	5.943
Total	410	36.664	5.754	42.418
Average	70	6.111	0.959	7.069

TABLE IX.

Green Weight of Plants, Dry Weight of Tops, Dry Weight of Roots, and of Entire Plant.

(SeriesII)

Crock No.	Green Wt. of Tops	Dry Wt. of Tops	Dry Wt. of Roots	Total Dry Wt. of Plants
	Grms	Grms	Grms	Grms
I. 1	19	1.452	0.57	2.022
2	13	1.448	0.29	1.738
3	38	3.125	0.89	4.015
4	37	3.076	0.98	4.056
5	30	2.265	0.31	2.575
6	21	2.097	0.93	3.027
Total	158	13.463	4.	17.433
Average	26.33	2.244	0.666	2.910
II. 1	65	6.372	1.47	7.842
2	70	8.782	2.03	10.812
3	50	4.825	0.87	5.695
4	66	6.713	1.37	8.083
5	33	3.250	0.65	3.900
6	62	4.507	1.36	5.867
Total	346	34.449	7.76	42.209
Average	57.67	5.741	1.29	7.033
III.1	49	3.400	1.07	4.470
2	31	3.208	0.80	4.008
3	71	7.343	1.69	9.033
4	71	7.240	1.82	9.06
5	57	5.337	1.47	6.807

Crock No.	Green Wt. of Tops	Dry Wt. of Tops	Dry. Wt. of Roots	Total Dry Wt. of Plants
Grms	Grms	Grms	Grms	Grms
6	69	6.593	1.51	8.103
Total	348	33.121	8.36	41.481
Average	58	5.521	1.39	6.914
IV. 1	54	3.295	1.22	4.515
2	26	3.515	0.85	4.365
3	81	5.674	2.28	7.954
4	69	6.598	2.44	9.038
5	30	4.305	0.56	4.865
6	69	6.413	1.30	7.713
Total	329	29.800	8.65	38.450
Average	54.83	4.967	1.44	6.409
V. 1	47	4.889	1.37	6.259
2	- - -	- - -	- - -	Lost
3	41	4.473	1.43	5.903
4	38	3.994	1.28	5.274
5	26	2.626	0.29	2.916
6	53	5.353	1.29	6.643
Total	205	21.335	5.66	26.995
Average	41.25	4.267	1.13	5.399
VI. 1.	37	3.487	0.970	4.467
2	21	3.452	0.800	4.252
3	38	3.592	1.130	3.722
4	43	4.650	1.550	6.200
5	96	6.215	1.420	7.635
6	40	3.850	1.090	4.940

Continued.

	Grms	Grms	Grms	Grms
Total	275	25.246	6.96	32.206
Average	45.83	4.208	1.16	5.368
VII. 1	30	2.383	1.010	3.393
2	32	3.518	.940	4.458
3	31	3.112	1.600	4.712
4	33	3.855	1.510	5.365
5	38	3.958	1.190	5.148
6	10	1.442	0.430	1.872
Total	174	18.268	6.680	24.948
Average	29.00	3.045	1.113	4.158

Chemical Composition and Physical Properties-The same methods used for analysis of samples from the field plots were used for analysis of samples from sand cultures, except that the whole plant instead of the leaves only was used. Slight modifications in the weight of samples ans strength of reagents had to be made as the total weight of samples was small. Table XII gives the nicotine and chlorine contents and the ash of plants.

Table XII. The Nicotine and Chlorine Contents and the Total Ash of Plants.

Series I.

Lab. No.	Percentage o of Nicotine of Plants	Percentage of of Ash of Plants	Color of Ash	Character of Ash
I.	0.695	22.84	Whitish gray	FLaky, firm
II.	0.849	22.86	Gray	" "
III.	0.957	I9.70	White	Loose
IV.	0.663	24.00	Gray	Firm
V.	0.992	2I.36	White	Loose

Table XII (Continued)

Series II.

I.	0.687	I9.64	Gray	Flaky, firm
II.	0.942	20.98	White	Loose
III.	0.7IO	I7.IO	"	"
IV.	I.040	I9.48	"	"
V.	0.753	2I.34	Gray	Flaky, loose
VI.	0.672	2I.34	Light Gray	" "
VII.	0.455	2I.82	Gray	Flaky, firm

DISCUSSION

Apparent Condition of Plants. - The general apparent condition of plants were quite similar to those observed in the field. The effect of calcium nitrate was quite pronounced. Where the proportion of this salt was high, the color of the leaf was dark green and usually lacking in smoothness. (Plate I) Low concentrations of calcium nitrate resulted in small plants with smooth and pale colored leaves. Variation in the potash supply failed to show any apparent effect on the leaves. "Potash-hunger" a common observation in the field was not produced even with culture R_1S_6 which had the lowest concentration of KH_2PO_4. High concentration of $MgSO_4$ did not show any injury, but with low concentrations the lower leaves showed a characteristic bleaching especially at the tip and near the margin, suggesting symptoms of so called "sand-drown". The following is a detailed account of the bleaching. (Plate II).

I. Bleaching of leaves - Bleaching of tissue between the veins at the tip and near the margin of lower leaves was observed in all six duplicate plants of culture R_1S_6. This was not observed in any of the other plants of the other cultures. The same condition was reproduced in solution cultures with R_1S_6.

Tottingham (27) has reported the bleaching of tissue between veins of wheat leaves as occurring in regions of the triangle where the proportion of KNO_3 was high and also where the proportion of $MgSO_4$ was low.

Plate I.

Treatment

Plant	Solution number.	Molecular Porportions KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	Partial volume.- Molecular concentrations. KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
A	R_1S_1	1	1	6	.0027	.0027	.0161
B	R_1S_6	1	6	1	.0020	.0122	.0020
C	R_2S_4	2	4	2	.0045	.0090	.0045
D	R_3S_4	3	4	1	.0065	.0086	.0021
E	R_4S_3	4	3	1	.0090	.0068	.0022
F	R_5S_2	5	2	1	.0118	.0047	.0023
G	R_5C_2 ( )	4	1	3	.0180	.0052	.0150

Plate I Characteristic Appearance of Tobacco Plants Grown in Sand Culture Solutions. - Plate shows the vigorous growth of plants and the lack of smmothness of leaves where the proportion of calcium nitrate is high.

Plate I.

A B C D

E F G

Plate II

Treatment.

Plant	Solution No.	Molecular Proportions			Partial Volume - molecular concentration.		
		KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
A	R_1S_6	1	6	1	.0020	.0122	.0020
B	R_5C_2	4	1	3	.0180	.0052	.0150

Plate II Characteristic Appearance of Tobacco Plants Grown in Sand Culture Solutions. - Plant A has spotted leaves especially at the margin, while the color of the leaves of B is rather uniform.

Plate II.

A B

Garner (7) reports that a disease of tobacco having the same symptoms as that commonly called "sand-drown", was found to be due to the lack of magnesium. Applications of magnesium salts and dolomitic lime prevented the appearance of the disease. He thinks that the quantity of magnesia required to prevent this "sand-drown" is small, probably less than 20 pounds per acre.

Of all the solutions studied, culture R_1S_6 has the lowest proportion of magnesium sulphate, therefore this bleaching similar to "sand-drown" may be attributed to the lack of magnesia. However, the above result raises a question as to the <u>amount</u> of magnesia required to prevent "sand-drown". Calculated on the basis of two million pounds of surface soil per acre and assuming that the moisture content is 16%, 20 pounds of magnesia would give a concentration in the soil solution of 31.25 parts per million, while culture R_1S_6 has a concentration of magnesia a little more than 4500 parts per million, or 155 times as high as that indicated by Garner. The total magnesium supply of a normal productive soil is about 45,000 pounds to the acre, but the amount available for plants at any period probably is much less than 4500 parts per million. The above results together with Tottingham's observations, would indicate that the proportion of the other nutrients, especially nitrates, may play an important part in the production of "sand-drown" formerly associated with magnesium deficiency.

A Method for Reproducing "Sand-Drown".

In an earlier experiment, effort was made to reporduce "sand-drown" in pot cultures with soil obtained from the field where the disease appeared during the previous season. Garner reports a new method for producing "sand-drown" under artificial conditions by leaching the soil with nutrient solutions lacking in magnesium. Since this method can be used only under conditions of high moisture content of the soil and high concentrations of other salts, producing "sand-drown" in sand cultures with culture solution R_1S_6 is suggested as a promising method when studies of "sand-drown" are desired. With sand cultures, the moisture content, the osmotic concentration of the solution as well as the proportion of nutrients can be controlled. When culture R_1S_6 is used, however, there is one objection and that is the blackening of leaf-stems and the death of buds which result in the disfiguring of the plant as a whole.

A pronounced injury of this type was observed in all cultures studied. After the plant has grown to a certain size, a black spot is first observed at the point where the yound leaf is emerging from the bud. This spot will grow along the veins until the whole leaf is darkened and gives the appearance of being dried out. The darkening was not limited to the single leaf but attacked the next the bud and also the lower leaves.

The time of the first appearance of the black spot could be correlated roughly with two factors, (1) the amount of calcium nitrate supplied and (2) the rate of transpiration. The latter may be the consequence of the former since an increase in the supply of calcium nitrate produces a faster growth rate which is accompanied by an increase in the transpiration rate. With cultures which have lower proportions of calcium nitrate the disease appears but at a later date. Table X shows the number of days after transplanting when the black spots appeared and the quantity of water transpired before the appearance of the disease.

Soil analyses have shown that the concentration of any plant food elements present in the soil is never at a state of equilibrium, but fluctuates from season to season. (31) (11). Studies of Burd (4) on absorption of plant food elements by barley at successive states of growth have shown that it is progressive during the growing stages, but a sudden drop occurs at maturity, with a practically complete cessation at the time of ripening. This has led him to point out that such fluctuations in the rate of absorption might be reflected in the nutritional peculiarities of the crop grown in sand and water cultures.

If quantity of transpiration be used as an indices of stages of growth, we may conclude that when the tobacco plant comes to a stage of growth when transpiration reaches about

Plate III.

Plate III shows the successive stages of bud infury. Plant A shows a normal bud. Plant B shows the bud injured and had stopped to develop. Plant C shows the bud entirely dried out and the leaves are badly wrinkled near the basal stem.

Plate III.

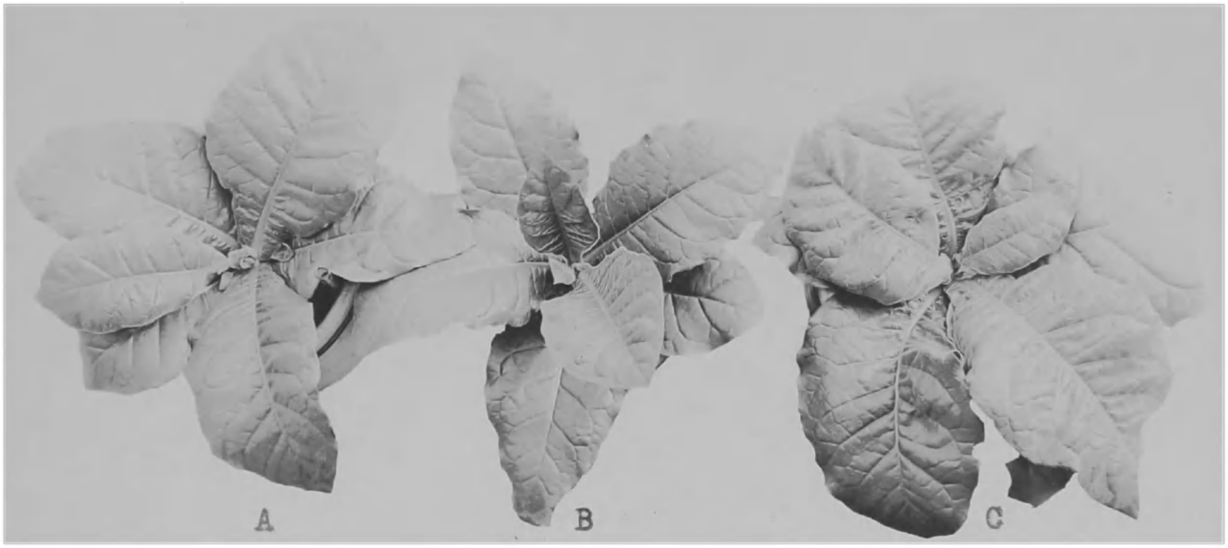


TABLE X.

The number of days after transplanting when injury first appeared, the total transpiration of plants before the first indication of injury, and the total transpiration of plants at the time of cutting. (Data from Series II.)

Lab. No.	No. of days after transplanting when injury appeared	C.C. of H_2O transpired before the appearance of injury	C.C. of H_2O transpired at the time of cutting.
I. 1	53	211	401
2	Free		215
3	53	260	565
4	65	983	983
5	Free		645
6	Free		671
II. 1	43	780	1549
2	51	840	1720
3	53	475	1035
4	45	693	1848
5	51	642	1097
6	46	558	1723
III. 1	51	621	1288
2	Free		580
3	67	800	1350
4	43	495	1965
5	51	585	1190

6.	46	652	1500
IV. 1	48	635	1270
2	44	368	713
3	40	701	2275
4	41	612	1691
5	Free		601
6	53	620	1442
V. 1	Free		1000
2	- - - - - - - - -	Lost	- - - - - - - - -
3	64	1210	1320
4	49	747	1226
5	49	554	794
6	51	640	1106
VI. 1	Free		665
2	51	757	847
3	51	780	1135
4	53	675	1060
5	Free		1121
6	64	850	935
VII.1	64	875	940
2	59	815	920
3	64	830	1010
4	67	750	1105
5	59	801	1306
6	Free		225

600 to 800 c.c. of water, it is passing from growing stage to maturing stage. At this stage of growth, either a decrease in the osmotic concentration or a change of plant food proportion is necessary to produce successfully the next stage of growth. When this change is not made physiological disturbance occurs and Burd suggests that high concentration of soil solution at certain stages of growth are probably not only unnecessary but may be undesirable. If the above is correct, then we may conclude that no individual solution studied in this experiment was adequate for all stages of growth of tobacco and the superiority of one solution over the other can be measured only by specifically designated purposes such as their influence on the nicotine content, or the smoothness of leaves.

The same disease was later reproduced in solution cultures using R_3S_6 and Histological studies of cross sections were made of sections of the leaf-stems 50 microns thick cut by means of a freezing microtone. The results of several studies are tabulated in Table XI.

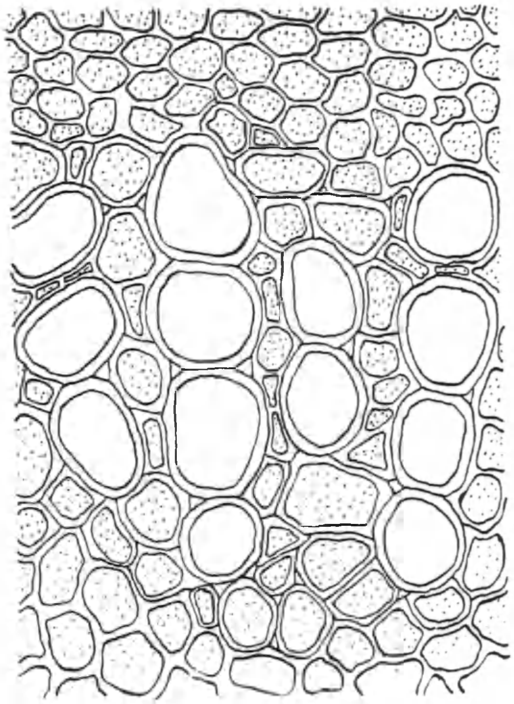
Table XI. Showing the Comparative Study of the Tissues of a Plant.

Condition of tissues	Normal	Slightly injured	Badly injured
Starch content	Plenty	Few grains	None
Oxalate crystal-filled cells	Few	More	Much more
Easily oxidizable substance	Browned after 5 min.	Browned after 3 min.	Browned immediately
Size of ducts	Large	Small	Smaller
Content of cells in general	Normal	Granular	Brown & granular

Plate IV.

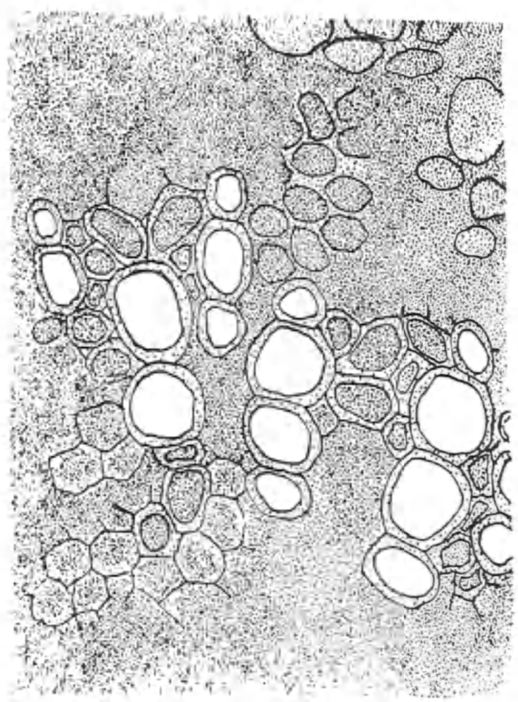
A-Section of vascualr bundle showing the normal tissue.

B-Section of vascular bundle showing the injured tissue. The ducts are smaller indicating infiltration. The adjacent cells are filled with brownish granules and much distorted.



(x433)

A.



(x433)

B.

Explanation of methods employed.

(a) Starch test was made by staining the sections with iodine solution and observing the comparative number of starch grains under the microscope.

(b) Oxalate crystal-filled cells were counted under the microscope after which the crystals were dissolved out by the addition of a drop of normal HCl.

(c) A comparison of the difference in the easily oxidizable substances in the tissue was made by slicing a normal tissue, a slightly injured tissue and a badly injured tissue, at the same time, and observing the time taken for each tissue to show a brown discoloration. The browning started from the vescular bundles and spread gradually throughout the entire tissue.

(d) The size of ducts and granulation of tissue are merely a qualitative comparison as viewed under the microscope.

In a study of the injury due to excessive nitrogen supply, Sorauer (26) reported that when vegetables were excessively fertilized, especially with sewage, there was a perceptible increase of the easily oxidizable substance which turned brown in the air, and not infrequently some of the ducts were filled with an inky fluid.

The same author also reported that when an excessive ammonium sulphate was given to Begonia Semperhorene, four days after fertilization, the young shoots became discolored at the base and began to drop. The pith and the bark were found to be filled with masses of calcium oxalate, the individual crystals were not as sharp as those in healthy specimens, but more rounded like tubers. No starch was present in the diseased tissue, the ducts being frequently filled with a brown, granular content, and the cell wall of all tissues were brown.

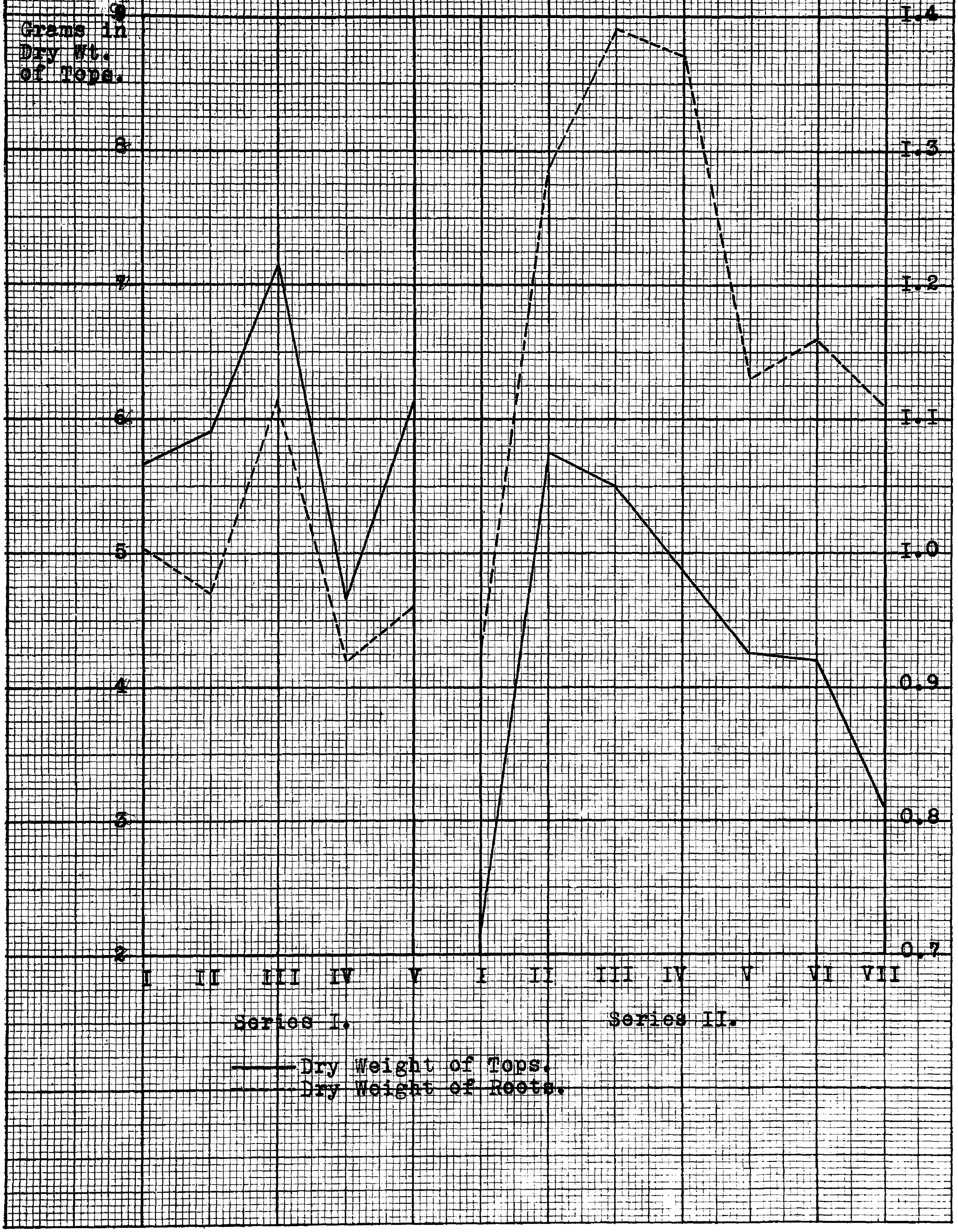
From the microscopic studies, it appears that the injury is typical of that caused by excess of nitrogen. Whether the other cultures which had apparently identical symptoms were due to excessive nitrogen was not certain, as reproduction of the injury in solution cultures with the other solutions was not attempted.

Yield - In general the yield of tops and roots in sand cultures shows a close correlation as shown by graph in figure III. However, the ratio of tops to roots in the two series shows a considerable variation. The dry weight of tops in the first series is roughly about six times that of the roots, while the dry weight of tops of the second series is about four times that of the roots. Very little correlation between the green weight of tops and dry weight of tops or dry weight of plants could be made.

Since there is a close correlation between the dry weight of tops and that of the roots, the discussions which follow will treat the total dry weight as a whole instead of considering the tops and roots separately.

Fig. III.

Graph Showing the Correlation between
the Dry Weights of Tops and Roots.



The yield of dry matter follows closely the supply of $Ca(NO_3)_2$.q Highest yield was obtained in solution R_1S_6 which has the highest proportion of $Ca(NO_3)_2$ and lowest proportion of $MgSO_4$. Lowest yield was obtained in solution R_1S_1 which has the lowest proportion of $Ca(NO_3)_2$ but high in $MgSO_4$. Solution R_3S_1 having the same proportion of $Ca(NO_3)_2$ as that of R_1S_1 but out-yielded the latter because in R_3S_1 the proportion of KH_2PO_4 comes next to $Ca(NO_3)_2$ as an important factor concerned in high yields.

It has been suggested that solution or sand culture studies would serve as indicative of suitable fertlizer formulas for field experiments. In considering the individual solutions studied, it is hard to select any one solution which has a plant food proportion best suited for tobacco based upon the yield of dry matter, because of all the solutions studied, none of them was able to support the plant to maturity. A pronounced injury which checked the normal development of the plant by killing off the terminal bud occurred in all solutions studied. Furthermore, solution R_1S_6 which gave the highest yield showed the worst injury and produced very coarse leaves.

Chemical Composition and Physical Properties-

From analysis of plants raised in sand cultures, as shown on Table XII we find that solutions high in proportions of $Ca(NO_3)_2$ produced plants having a high content of nicotine, however, this was not in direct ratio. Increasing the proportion of KH_2PO_4 also seemed to increase nicotine content.

Plants raised in sand cultures where chlorides were not used as a plant food, contained a trace of chlorine not enough to permit quantitive analysis. The trace of chlorine may come from the seed or have been introduced as an impurity of the salts.

Solutions having high proportions of $Ca(NO_3)_2$ produced plants having a low content of ash, possessing a white color and exhibiting a loose consistency. A high ash content seemed to be correlated with a high proportion of $MgSO_4$. This salt also darkened the color of ash and increased its firmness. The above results confirm those of Patterson (23) and Garner (8) in that a certain amount of calcium is necessary to produce a good ash.

The physical properties of leaves from sand cultures were similar to the general observations of those of the leaves raised in the field plots. High proportion of $Ca(NO_3)_2$ produced thick and dark red colored leaves of a rough texture. A study of the burn of the leaves revealed the fact that chlorine was not the only inhibition factor against good burning quality as these samples low in chlorine in general did not burn any better than ordinary field grown tobacco which usually would contain about 0.2% of chlorine in the ash. A general relationship between the potash supply and the burning quality was observed. Whenever the supply of KH_2PO_4 was high, the burning quality was good. High proportion of $Ca(NO_3)_2$ produced leaves which would burn with a flame instead of holding a glow. Garner (8) has reported that if the mineral substances of a leaf were extracted the leaf would loose its glow, but would burn in flames as the result of the presence of cellulose, organic acids, pectin, nicotine and other organic nitrogenous compounds. A large supply of nitrate probably would increase the cellulose content and certainly would increase the organic nitrogenous compounds.

GENERAL CONCLUSION

Yield Data

The results from a single season of field experiments shows an increase in yield from the application of dolomite lime but not from calcite lime. Where the ratio of CaO:MgO was wide, the yield was higher than where it was narrow. Four different combinations of inorganic fertilizers gave no marked difference in the yield, but where 50% of the total ammonia was supplemented by cotton seed meal, the increase in yield was about 30%.

In sand cultures, it was found that the yield of the dry tops was closely correlated with the yield of dry weight of roots. High proportion of $Ca(NO_3)_2$ gave high yields of dry matter, and high proportions of $MgSO_4$ gave low yields of dry matter. In cultures receiving the same proportion of $Ca(NO_3)_2$, the higher the proportion of KH_2PO_4 over $MgSO_4$ the better the yield. No single solution, however, could be selected as preferable for tobacco, which would serve as a guide to the selection of a favorable fertilizer fromula for the field because of the appearance of an injury in the plants of all cultures. The cultures which gave high yields not only showed prominent injury, but also produced coarse leaves of poor quality.

The above mentioned injury seemed to appear when the plant had attained a certain amount of growth. The external symptoms started with a black spot at the stem of a young leaf just emerging from the bud, spread along the veins next to the bud, and finally the lower leaves. Anatomical studies of the injured tissue produced in solution cultures using R_3S_4 gave indications of injury typical of that caused by excess of nitrogen.

Symptoms of "sand-drown" produced in sand cultures in all six duplicate plants of culture R_1S_6 was later reproduced in solution cultures using the same molecular proportions of salts.

Nicotine Content

Nicotine content of tobacco leaves from the field plots increased where nitrogen was applied in the forms of nitrate of soda and cotton seed meal. Lime applications increased nicotine content with slightly greater increase when the lime contained a ratio of CaO:MgO as 10:1. Plants raised in sand cultures showed that high proportions of $Ca(NO_3)_2$ produced high content of nicotine, though not in direct ratio. KH_2PO_4 seemed to influence favorably too.

Chlorine Content

Chlorine content varied largely under the influence of fertilizers. Substituting muriate of potash for sulphate of potash, chlorine content increased three times. Lime applications decreased chlorine content and increasing the ratio of MgO to CaO decreasing the chlorine content. Plants raised in sand cultures contained only a trace of chlorine, the chlorine content seldom was being over 0.4% of ash where no chloride was applied to the cultures.

Ash Content

Analyses of leaves from field plots showed that neither the lime nor the fertilizers had any appreciable effect on the percentage of ash. Where lime was not applied, however, the ash was gray, and where muriate of potash was applied, the ash was greenish or brownish with very firm consistency. Sulphate salts also darkened the color of ash and increased firmness though to a lesser degree.

Color and Burning Qualities.

Heavy applications of available nitrogen in the form of nitrate was found to produce coarse leaves with dark color both in the field and in sand cultures. The coarseness of the leaves was generally associated with poor burning quality. High chlorine content was found to be not the only factor inhibiting good burning quality, the presence of available potassium being essential to good burn. Where the proportion of $Ca(NO_3)_2$ was high in sand cultures, the leaves failed to maintain a glow but would burn in flames. This behavior is probably due to the high content of organic nitrogenous compounds.

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