Part I. Physiological Studies on the Pathogenicity of Fusarium lycopersici Sacc for the Tomato Plant

Part II. Responses of the Tomato in Solution Cultures with Deficiencies and Excesses of Certain Essential Elements

By

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INTRODUCTION

According to records of the Plant Disease Bureau of the United States Department of Agriculture, Fusarium wilt is distributed widely through the Middle Atlantic, Gulf and Mississippi Valley States with scattered areas in other States from the Atlantic to the Pacific. The wilt of Tomatoes (Lycopersicum esculentum Mill), caused by Fusarium lycopersici Sacc, is prevalent in most of the tomato sections of Maryland, particularly the Eastern Shore.

Two aspects must be distinguished in investigations of soil-borne parasites. The first has to do with the inception of the fungus into the plant and the second deals with its progress up the stem. They are two distinct phenomona. Many resistant plants will exhibit symptoms of the fungus in the stem at or near the root crown yet the fungus does not advance up the stem and therefore, externally, the crop is normal. Controlled experimentation should be directed primarily along four lines: soil and air temperatures, optium moisture, soil composition, and varietal responses. These factors are the determinants of the general distribution and severity of the disease.

Many of the factors underlying the resistance and susceptibility of tomato varieties to Fusarium lycopersici have been widely investigated. These studies, however, have dealt

principally with ecological factors of temperatures, moisture, evaporation and hydrogen-ion concentrations. Studies have been made by investigators of the Fusarium diseases of other crops which have shed light on the responses of the plants to the fungus under various nutrient treatments. They do not state, however, whether the treatements decreased the original infection of the plants or reduced the ultimate damage to the crop yield because of some metabolic change within the plant. It has been known for some time that deficiencies or excesses of mineral elements modify the organic composition of plants.

A deficiency of phosphorus, for example, will cause an increase of soluble forms of nitrogen and also an increase in the percentage of sugars in the plant. A deficiency of nitrogen will also increase the percentage of soluble carbohydrates in the plant. What effect would these metabolic changes have on the relative susceptibility of the crop to this disease? Will an excess of these soluble organic substances in the conductive vessels favor the growth of the fungus in the plant? What influence would excesses of these essential elements have on the severity of the wilt disease?

The author has made nutritional studies of the Fusarium wilt of tomatoes with these important questions in mind. The work was begun in the Fall of 1930 at the Maryland Agricultural Experiment Station. Two general phases of the nutritional relationships of this disease have been investigated. In the first phase of the work the effects of varying the amounts of the different elements on the susceptibility to the wilt disease

were studied for early and late growth stages of the plant.

In the second phase of the study, the elements were varied, by increments, from total absence up to an excess of the elements. The scope of the second phase of the work could not include all of the elements so calcium was chosen because preliminary work indicated that it exerted the greatest influence on the infection of tomatoes by the wilt fungus.

In addition, the effects of fertilizer applications on tomato wilt was investigatedfor greenhouse pot cultures.

During the past year, particular attention has been given to the toxic action of the fungus within the plant and to the inhibitory effect of extracts from a resistant variety to its growth in pure cultures.

REVIEW OF LITERATURE.

It is the intention in this paper not to present a complete review of the physiological studies that have been made on disease resistance but rather to refer to only a few of the outstanding contributions dealing directly with Fusarium investigations. Jones (36) sounded the keynote for such an investigation as this when he stated that "In plant pathological studies.....the time has come when we should put relatively less stress upon the parasite as an independent organism and relatively more on the disease." He suggested that workers give "increased attention to the relation of environment to disease inception and development".

Symptomology of Fusarium wilt of tomato - This disease

has been adequately described in earlier reports by McWhorter and Parker (48), Clayton (5,6), Norton (50,51), Edgerton (12), Edgerton and Moreland (13,14,15), Humphrey (31) and others. Whole fields may become infected or, even more frequently, spots of infection will occur throughout large plantings of tomatoes. The loss from this disease ranges from one to one hundred per cent and it has been estimated that the average annual decrease in marketable crop is 115,000 tons.

It is frequently found on bluff, prairie and sandy soils where the climate is dry and warm. The most prominent symptoms exhibited by the plants are yellowing, stunting and wilting, culminating in death. The fungus enters through the roots and grows up through the woody, conductive tissue of the stems to the leaves, fruit pedicals, fruit and sometimes the seeds. Fusarium lycopersici does not penetrate the root hairs of the tomato but infection takes place somewhere on the small roots.

The yellowing of the plants begins frequently, although not always, at the lower leaves. The leaves curl upward and inward. Stunting is noticed if infection occurs when the plants are young but wilting is a variable symptom. The disease may be apparent as a blight rather than a wilt under unfavorable environmental conditions or with resistant varieties.

The most important diagnostic symptom is the brown discoloration in the vascular tissue, making a ring between the pith and bark. This may be seen by making cross and longitudinal sections of the stem.

Immature fruits, one inch in diameter, become yellow and

then deep red, indicative of ripeness. Their seeds and pulp, however, fail to develop as normal fruits.

In the field, the death of the host does not usually occur until the first flowers and fruits are produced but in greenhouse cultures it sometimes takes place at a much earlier stage of development.

Description of Fusarium lycopersici Sacc. - Fusarium lycopersici was first described by Saccardo from Italy in 1882 as a variety of Fusarium oxysporum. It belongs to the sub-group "Elegans" of the genus "Fusarium". This sub-group contains all the Fusarium wilt organisms such as those causing wilt of cotton, cowpeas, melon, cabbage and potatoes.

A great deal of difficulty has been experienced by workers with Fusaria in identifying the organism because of the gradual growth of a voluminous mass of species, the polymorphism of the organisms in culture, the range of spore size and the kaleidoscopic range of colors. The monograph of Appel and Wollenweber (1) contained reliable criteria for the classification of this fungus. This was followed by the work of Wollenweber, et al, (53,67,68), Lewis, 1913 (43), Sherbakoff, 1915 (57), Haymaker, 1928 (28), Leonian, 1929 (41), Coons and Strong, 1931 (7) and others. All of these reports have led to a clarification in terms, standardization of media and a closer definition of the physiologic strains within the species.

Edgerton (12) describes the fungus as follows:

"Fusarium lycopersici produces two types of conidia, the small, singlecelled, hyaline microspores and the larger falcate,

hyaline, septate macrospores. In the macrospores there are usually three septae, though any number from one to five are not uncommon.

"The fungus grows well on almost all of the common culture media, producing spores in abundance in pure culture. There is some variation in different strains of the fungus on culture media. Some of the cultures make an almost flat growth, while others are covered with a rather heavy floccose growth of aerial mycellium. On some culture media some strains produce a rather deep pink or purple color, while other strains are colored only slightly. These varying strains are all able to infect young tomato plants, producing the typical wilt disease, although some are more pathogenic than others.

"Fusarium lycopersici grows best on a medium testing not higher than ≠ 15 of Fuller's scale. It will grow on a medium testing slightly above ≠ 30 but the growth is considerably retarded even at ≠ 20. Growth does not seem to be retarded in a medium testing slightly alkaline".

Cause of wilting and death of host -- The two theories that have been presented in the literature are (1) the clogging of the xylem tissues of the stem and limiting the passage of water and, (2) the production of a toxic substance in infected plants. This substance might be a specific secretion of the fungus itself or produced as a result of the interaction of the host and parasite. If the former theory is the correct one, it should be possible to reproduce the symptoms artificially by cutting away the xylem tissues on one side of the stem of a healthy plant. It has been

proved, however, that no wilting occurs if the xylem tissues are cut away, since there is sufficient lateral diffusion of water, to keep the plant turgid. (Clayton, 5) It is difficult, even, to find mycellium in some of the discolored vascular tissues even when some virulent spores might be present. Tomato wilt is also different from mechanical wilting in that there may be a complete killing of vascular tissues and yet new shoots may regenerate just above a wilting branch.

It appears that the injuries produced seem more comparable to the injection of toxic substances. Evidence presented in the literature by investigators of Fusarium diseases support this theory. Brandes (4) in observations of the Fusarium wilt of bananas, Haskell (27) studying the Fusarium wilt of potatoes and Goss (22) working with potato wilt and potato stem-end rot, conclude that death in these diseases is due to a toxic substance produced by the fungus. Brandes was one of the first to express the toxic theory of wilting. White (66) is one of the tomato wilt investigators who believes that the wilting is due to toxins. Haymaker (28) came to this same conclusion. Evidence presented by Bisby (2,3) suggests that the toxic substance produced is not specifically active in certain plants. Ludtke and Achmed (45) believe that the wilting caused by parasitic fungi is attributed to one or several substances of amine nature. Filtrates contain organically bound nitrogen. Synthetic amines caused similar wilting. They were not sure whether the amines present were the cause or effect of wilting, however.

The lateral diffusion of a toxic substance in tobacco was found by Gray (23) to be very slight and affected only the leaves directly in its path.

The conclusions reached by Clayton (5) indicate that this toxic substance exerts an influence on the permeability of the cell membranes. If the permeability were increased the loss of water from the cells would be facilitated. Similarly, if permeability of the cells were decreased, the movement of the water would be retarded. The cells, therefore, which are farthest from the veins might dry up, while those next to the veins remain turgid. No data has been found concerning the difference in transpiration per unit weight between healthy and diseased plants. The author has made the observation, however, that just before the wilting of infected plants transpiration is slightly increased but this is invariably followed by a marked decrease therein, after external symptoms are apparent.

Control of Tomato wilt -- The following general methods of control have been suggested by investigators with this disease: selection of high grade seed from uninfected fields; new plant beds every year; burning of diseased plants; crop rotation practice or planting on virgin soil; use of wilt resistant varieties; and fertilization to obtain appropriate alkalinity of the soil. Spraying is impractical because the internal infection occurs beneath the surface of the soil.

Many investigators have developed resistant strains of tomatoes. Foremost among these must be mentioned Pritchard (52).

The disease can be carried over in the seed but most commonly it occurs through infected seedlings. Rotation of crops reduces infection but it never entirely eradicates it since the fungus is viable for long periods in the soil (10 years). Over a period of many years Pritchard selected four resistant varieties - Greater Baltimore, Stone, Marvel (Merveille des Marchis) and Norton. Characterized by their work on this phase of the problem also are Edgerton (9,10,11), Edgerton and Moreland (13,14,15), Elliott and Crawford (16), Essary (17) and May (46). From the standpoint of resistance to Fusarium wilt, the Marglove and Invincible were superior to the Stone variety in tests made by McWhorter and Parker (48) in Virginia. However, resistance at best is but relative, for White (66), working in Kansas, reports heavy infection on two strains of Bonnie Best which had been selected for resistance in Ohio. This apparent "loss" of resistance may be caused by environmental changes, which affect either the host or the pathogene or both, introducing factors modifying the character of resistance, or by the presence of distinct physiological races of the pathogene in different localities. McClintock (47) has also made this same observation.

Edgerton (9,10,11,12) reported that an application of lime at the rate of ten tons per acre reduced the disease in the seed bed from 51.0 per cent to 4.4 per cent. In the field the wilt was reduced in the Acme variety from 77 per cent to 40

per cent and in the Earliana from about 75 per cent to 23 per cent.

Influence of temperature on tomato wilt -- Clayton (5), in a detailed study on the effects of temperature on Fusarium diseases of the United States, states that all occur in regions characterized by high temperature. He has ascertained the definite temperature limits of the Fusarium wilt disease, the fungus alone and the host alone. Tomatoes (Susceptible Chalk's Jewel variety and Mangus variety) develop most vigorously at temperatures ranging from 24° to 31°C. This range includes the optimum temperature for the fungus which is 28°C. He gives the following classification of the influence of temperature on external symptoms.

1. Temperature optimum for the disease, i.e., 25° to 31°C, a sudden wilting which appears first in the lower leaves, then in those progressively higher up, and is rarely accompanied by yellowing of the leaves affected.

2. Temperature just above or just below the optimum, i.e., 32° to 34°C or 20° to 24°C, wilting accompanied, and often preceded the yellowing of the leaves. The appearance may frequently be that of a slow blight rather than of a wilt, for there is more yellowing than actual wilting and the plants often show stunting of growth.

3. At temperatures above 34°C or below 20°C there is no external evidence of the disease.

4. In addition to the manifestations of disease mention-

ed above, the fungus may enter the host but penetrate the bundles in only the lower portions of the stem. This condition is often the result of a short exposure to temperatures favoring the disease, followed by a drop in temperature sufficient to check further development of disease. Plants thus infected are possibly lighter in weight than uninfected plants grown under similar conditions.

Air temperatures were as effective in controlling the disease as soil temperatures.

Edgerton (11) makes the statement that Fusarium wilt "was associated with high temperatures".

Investigations have been made by Johnson and Hartman (34) of Tomato wilt and Thielavia root rot of tobacco with special reference to temperature. Seasonal variations of resistant strains varied as much as 100% because of this one factor alone. These diseases are favored by high soil temperatures. Jones (36) believes that with all the Fusarium diseases the direct influence of temperature is upon the vegetative activities of the parasite. No definite conclusions to this were presented, however. The optimum temperature for the Tomato wilt disease and for the fungus alone is 28°C. Jones leaves this question in our minds for consideration. How far is the disease due primarily to the influence of temperature on the parasite, how far primarily to the influence on the host and how far to a combination of these?

Influence of moisture on Tomato wilt -- Humbert (30 made the statement that hot, dry weather favors the Fusarium wilt. This is true especially if preceded by a period of high rainfall. Clayton (6) also worked on the relations of soil moisture to this disease. He grew plants from soil moistures of thirteen to thirty five per cent, the higher value representing complete saturation. Low soil moistures, thirteen to nineteen per cent, favored resistance to the disease. Plants growing in soil kept saturated were immune from attack. Any moisture shortage sufficiently severe to check the vegetative growth of the host below 2/3 of the moisture holding capacity of the soil, also checked the disease. If rapidly growing plants at below 20°C were placed in a favorable temperature of 25° to 30°C they were soon attacked. However, if the soil became dry, wilting was delayed.

Influence of hydrogen-ion concentration on tomato wilt -- Sherwood (58) has investigated the influence of hydrogen-ion concentration of the soil on Fusarium lycopersici. He states that the highest percentage of wilt always occurred in the most acid soils of his series but no limiting degree of acidity or alkalinity could be found at which the disease would not develop.

He also investigated the pH range of the organism in pure culture. His series ran from pH of 1.8 to 8.4. No growth of the fungus was apparent at pH 1.8 but it grew well in the

range of p H 2.8 to 8.4. At all hydrogen-ion concentrations from pH 3.6 to 8.4 the growth of the organism was accompanied by a change towards greater acidity of the culture media.

Scott (56) found that in pure cultures of Fusarium lycopersici, maximum growth was attained at a pH of 4.5 to 5.3 and 5.85 to 6.85, with a decrease between 5.3 to 5.8. He also grew plants under varying pH values in the soil. Minimum wilt was obtained from soils with a pH value of approximately 7.0 with maximums on either side of this value, the higher maximum occurring at about a pH of 4.5 to 5.3.

Investigations of Fusarium wilt of other crops -- Reynolds (54) worked on the wilt of Flax (Fusarium lini). The problem was investigated from the standpoint of the substances present in the plant which are used by the fungus. Glucose produced more mycelliar growth than any other sugar, and potassium nitrate proved to be the best source of nitrogen.

He found that autoclaving caused some changes in the juice of flax which permitted growth of Fusarium lini. This was attributed to the destruction of an injurious material, the alteration of some enzyme which was in some manner effective in depressing growth, or to the changing of substances of doubtful food value to some more available form. The glucoside linimarin or phaseolunatin is present in flax plants. Hydrocyanic acid is produced from this glucoside upon hydrolysis. Reynolds found a greater quantity of this compound in resistant varieties.

In a later work (55), he gives evidence of the presence of a second toxic compound in the flax juice, which is relative-

ly thermostable, soluble in water, ether and alcohol and is essentially non-volatile. Its toxicity is low, however, and only in high concentrations inhibits growth completely.

Several workers have reported on investigations with the Fusarium which causes cabbage yellows. Gilman (20) found that Fusarium conglutinans Wollenw has a high optimum temperature and is very resistant to drying both in pure culture and in the soil. Tisdale (62) and Jones, et al (40) state that cabbage yellows is prevalent in Wisconsin during midsummer when the soil is dry and hot, resulting in infection of even small seedlings of high resistant varieties. Tims (61) studied Wisconsin varieties of cabbage and found temperatures of 27° to 33° favorable for disease inception correlated with low soil moisture.

With the scurf organism, Fusarium batatatis Wollenw and the stem-rot organism, Fusarium lyperoxysporum Wollenw of sweet potatoes, Harter and Whitney (25,26) have found that the optimum temperature is 30°C while infection occurs over a wide range of soil humidity. Goss (21) has studied the environmental conditions favorable to the potato wilt caused by Fusarium oxysporum.

The wide distribution of Fusarium moniliforme Sheldon and its role in the ear-rots of corn have been discussed by Leonian (41,42). There have been questions raised as to its pathogenicity in seedling blight diseases of this crop. Leonian believes that it does infect the corn plant at low

temperatures (20° to 23°C) and recommends as a control, late planting to avoid cool, wet soil conditions.

Garcia (18) found that the percentage of Chile wilt (Fusarium annuum) is greatest on heavy soils where water accumulates and remains for some time after irrigation.

Dickson (8) investigated the resistance of corn to seedling blight (Fusarium stage of Gibberella saubinettiim). He states that soil temperature, moisture and light intensity are the most influential factors in this disease, coupled, undoubtedly, with some effects of mal-nutrition due to soil deficiencies or excesses. The fungus attacks the plant when the metabolism thereof is sufficiently unbalanced to inhibit the normal production of protective tissue.

Taubenhaus, et al (60) reports that cotton wilt is more common in acid soils. Neal (49) presents a rather complete discussion of the literature pertaining to cotton wilt, caused by Fusarium vasinfectum Atk. He conducted physiological experiments on the fungus and found that the optimum temperature was 28° to 30°C and the optimum pH was 4.0 to 5.5. He presents the theory that this Fusarium disease is caused by toxic substances produced by the fungus, which cannot be destroyed but are reduced in dilutions. He found no correlation between the severity of the disease and iron accumulation, aluminum toxicity nor various nutritive treatments, with the exception of potassium and calcium. The results of both greenhouse and field experiments show that an excess of potassium salts decreased the wilt damage,

because of delayed infection. For control of cotton wilt he suggests the use of resistant varieties and fertilization, using a formulae of eight to 10 per cent phosphoric acid, four to five per cent potash and from four to six per cent nitrogen.

Similar types of investigations have been reported for the Fusarium wilt and root rot of peas. Foremost in this work are Gueguen (24), Turesson (65), Jones (35,37), Jones and Drechsler (38), Jones and Linford (39), Gilchrist (19), Togashi (64), and Linford (44).

EFFECTS OF NUTRIENT TREATMENT ON THE SUSCEPTIBILITY OF TOMATO TO FUSARIUM WILT

Water Cultures

Methods

Germination of seeds -- The seeds of the Marglobe (resistant) and Bonnie Best (susceptible) varieties of tomato were germinated between layers of moist filter paper in a moist chamber fitted with a glass cover. They were kept at room temperature until the roots were approximately one centimeter in length. They were then transferred to a germination net, similar to the one described by Johnston (32): two strips of paraffined cotton fly-netting were fastened over a circular glass dish. These pieces of netting were separated from each other by a piece of bent glass tubing about 0.5 centimeter thick. Tap water was kept continuously flowing through this germinator.

Distilled Water -- The distilled water for the culture solutions was obtained from a Barnstead steam heated still and stored in a 25 gallon tin-lined tank and 5 gallon glass bottles until used.

Solutions used -- The solutions used in the following experiments were devised by Johnston (33) and were composed of calcium nitrate, magnesium sulfate and potassium phosphate. The complete solutions contained all six of the elements in these three compounds.

A second series of solutions was used in which each of

the elements was removed from the culture medium. In this case, it was necessary to introduce a new compound containing the element with which the deficient element was associated in the original solution.

Table 1 shows the volume-molecular concentrations of chemically pure salts used in preparing the stock solutions for these deficient solutions. The stock solutions were diluted 100 times with distilled water for growing the plants. The approximate concentrations of these elements in the deficient solutions expressed as parts per million are presented in Table 2.

A third series of solutions was used in which the concentrations of the elements were double those of the normal complete solutions, with the exception of calcium, wherein the concentration was three-fourths greater than the normal concentration. These solutions will be referred to hereafter as "excess solutions". The volume-molecular concentrations of salts used in preparing the stock solutions for the excess solution cultures are given in Table 3. These stock solutions were also diluted 100 times with distilled water for growing the plants. Table 4 gives the approximate concentrations of the salts in these solutions expressed as parts per million.

A comparison is drawn in Table 5 between the calculated and actual osmotic pressures of all of the solutions used.

Traces of manganese (1.0 p.p.m.) as $MnSO_4$ and boron

(0.55 p.p.m) as H_3BO_3 were added to each of the three series of solutions. Iron as Ferric tartrate (0.5 percent) was added every day to each culture in two cubic centimeter aliquots, while the plants were young, and thereafter only three times a week.

The pH values of these solutions, as determined by the Youdon quinhydrone electrode method are listed in Table 6. They are all within the maximum hydrogen-ion concentration range for this disease.

Table 1 - Volume-molecular concentrations of chemically pure salts used in preparing stock solutions for deficient nutrient solutions.

Solutions Containing	C. P. Salts							
	$Ca(NO_3)_2$	$Ca(H_2PO_4)_2$	$CaSO_4$	$Mg(NO_3)_2$	$MgSO_4$	KH_2PO_4	K_2SO_4	NH_4NO_3
All Elements	.003 M	----	.001	----	.001	.002	----	.003
No calcium	----	----	----	.001	.002	.002	----	.005
No magnesium	.002	----	.002	----	----	.002	----	.004
No potassium	.002	.001	.001	.001	.001	----	----	.003
No nitrogen	----	.001	----	----	.001	----	.001	----
No phosphorus	.004	----	----	----	.001	----	.001	.002
No sulfur	.004	----	----	.001	----	.002	----	.001

Table 2 -- Approximate concentrations of elements in deficient nutrient solutions expressed as parts per million.*

Solutions containing	Elements					
	Ca	Mg	K	N	P	S
All elements	160	24	78	168	62	64
No calcium	---	72	78	168	62	64
No magnesium	160	--	78	168	62	64
No potassium	160	48	--	168	62	64
No nitrogen	40	24	78	---	62	64
No phosphorus	160	24	78	168	--	64
No sulfur	160	24	78	168	62	--

* Parts per million equals milligrams per liter.

Table 3 -- Volume-molecular concentrations of chemically pure salts used in preparing stock solutions for excess nutrient solutions.

Solutions containing	C. P. Salts								
	$Ca(NO_3)_2$	$Ca(H_2PO_4)_2$	$CaSO_4$	$Mg(H_2PO_4)_2$	$MgSO_4$	KH_2PO_4	K_2SO_4	KNO_3	NH_4N
All elements	.003	----	.001	----	.001	.002	----	---	.00
Excess calcium	.006	.001	----	----	.001	----	.001	---	--
Excess magnesium	.004	----	----	----	.002	.002	----	---	.00
Excess potassium	.002	.001	.001	----	.001	----	----	.004	.00
Excess nitrogen	.003	----	.001	----	.001	.002	----	---	.00
Excess phosphorus	.002	----	.002	.001	----	.002	----	---	.00
Excess sulfur	----	.001	.003	----	.001	----	----	.002	.0

Table 4 -- Approximate concentrations of elements in excess nutrient solutions expressed as parts per million. *

Solutions Containing	Elements					
	Ca	Mg	K	N	P	S
All elements	160	24	78	168	62	64
Excess calcium	280	24	78	168	62	64
Excess magnesium	160	48	78	168	62	64
Excess potassium	160	24	156	168	62	64
Excess nitrogen	160	24	78	336	62	64
Excess phosphorus	160	24	78	168	124	64
Excess sulfur	160	24	78	168	62	128

* Parts per million equals milligrams per liter.

Table 5 -- Calculated*and actual values of Osmotic Pressures of the Solutions.

Solution	Calculated Value		Actual Value
	V.M.C.	O. P.	O.P.
Complete	.010	0.50	0.57
Boron deficient	.010	0.50	0.57
Boron excess	.010	0.50	0.57
Calcium deficient	.010	0.50	0.47
Calcium excess	.009	0,45	0.46
Potassium deficient	.009	0.45	0.46
Potassium excess	.011	0.55	0.58
Nitrogen deficient	.003	0.15	0.25
Nitrogen excess	.015	0.75	0.69
Sulfur deficient	.008	0,40	0.60
Sulfur excess	.012	0,60	0,59
Magnesium deficient	.010	0.50	0.54
Magnesium excess	.010	0.50	0.56
Phosphorus deficient	.008	0.40	0.43
Phosphorus excess	.011	0.55	0.54

* Formulae

V.M.C.	O. P.
.040 M	2.00 Atm.
.030	1.50
.020	1.00
.010	0.50

Table 6 -- Hydrogen-ion Cencentrations of Solutions.

Solutions containing	pH Values	Solutions containing	pH Values
All elements	5.20	All elements	5.20
No boron	5.27	Excess boron	5.08
No calcium	5.28	Excess calcium	5.10
No magnesium	5.24	Ex. magnesium	5.33
No potassium	5.05	Ex. potassium	5.25
No nitrogen	4.60	Ex. nitrogen	5.33
No Phosphorus	5.91	Ex. phosphorus	4.60
No sulfur	4.96	Excess sulfur	5.30

Origin of fungi cultures -- Cultures of Fusarium lycopersici were obtained from the United States Department of Agriculture, the University of Chicago and pure cultures from transfers of infected plants on the Horticultural Farm of the Maryland Experiment Station at Beltsville, Maryland, and the greenhouses of Mr. T. H. White. Tests made with these four strains of the fungus proved the one from the greenhouses of Mr. T. H. White to be the most virulent and in all of the experiments reported this strain was used.

Media for fungi cultures -- Boiled rice and Coon's synthetic media for Fusaria are the best media for the Fusarium organism. The formulae for Coon's media is as follows: Saccharose, 7.2 grams; Dextrose, 3.6 grams; Magnesium sulfate, 1.33 grams; Potassium acid phosphate, 2.73 grams; Potassium nitrate, 2.02 grams; Water, 1000 cubic centimeters and Agar agar, 12 grams. Sterilized alsike clover and tomato stems can be used with satisfactory results.

Time of harvest and data recorded -- In the following greenhouse experiments the plants were always harvested when the fruits were beginning to set on the lowest flower clusters. This varied from nine to twelve weeks, depending on the season of the year, the kind of weather, and the solution treatment. The data recorded included the spread of the disease, the symptoms of disease and treatment, height of plants, number of flower clusters, weight of fruit, green weights and dry weights (100^{o} for 24 hours).

Environmental conditions -- Temperature records were kept during the course of the experiments. The optimum temperature for the disease is 82°F (28°C). The only greenhouse available for this work had a wide range of temperature, from 50°F (10°C) during the night to 86°F or more (30°C) in the afternoon. The average of this range is approximately 70°F (22°C). According to Clayton (5), as reported above, the disease, at this temperature, "may frequently be that of a slow blight rather than of a wilt".

The moisture of the water cultures appeared to be too high for the proper development of the fungus. This difficulty was eliminated when sand cultures were used, however, and the moisture content of the sand plots was about 20-23%.

Light intensity was fairly constant during the experiments, with occasional periods of cloudy weather. However, light does not seem to be an important factor in the development of this disease.

The hydrogen-ion concentrations of the solutions were within the maximum range for Fusarium wilt.

Culture jars and solution treatments -- When the seedlings were about a week old or when the cotyledons had become well developed, they were transferred to two-quart Mason jars, one plant per jar. These jars hold about two liters of nutrient solution. The plants were supported in paraffined perforated cork stoppers by a little cotton around their stems. Most of the light was excluded by wrapping them with heavy, brown, manila paper,

thereby preventing the growth of algae in the solutions. The jars were placed on tables (two feet wide and ten feet long), the tops of which are thirty -six inches above the floor of the greenhouse.

The plants were grown for thirty days in complete solutions. The jars were then thoroughly washed with tap and distilled water. The roots of the plants were rinsed with distilled water. The cultures were divided into groups of ten plants and each group was subjected to treatments with solutions deficient in one of the essential elements until the plants matured.

It has appeared desirable in salt nutrition work to maintain the initial proportions of salts during the course of experiments. Arrangements were made to actuate a continuous nutrient solution renewal in the cultures. Shive's "Drip Method" for constant solution renewal (59) was chosen as an easy yet efficient method for obtaining the desired results. (Fig. 1)

Description of Shive's Drip Methods for water cultures -- Shelves for the storing of the solution reservoirs were erected in the center of the greenhouse tables. These shelves are six inches wide, eighteen inches above the level of the tables and run their entire length. The solutions are stored in two-quart glass jars with a 0.5 millimeter bore capillary tube outlet delivering approximately 1000 cubic centimeters per day. This outlet leads to another tube (2.0 millimeter bore) which extends to the bottom of the culture jar and serves also for aerating the

culture solution. A 1.0 millimeter bore waste tube maintains the level of the solutions in the culture jars at a constant height. The waste solutions are gathered in a pan and thrown away daily.

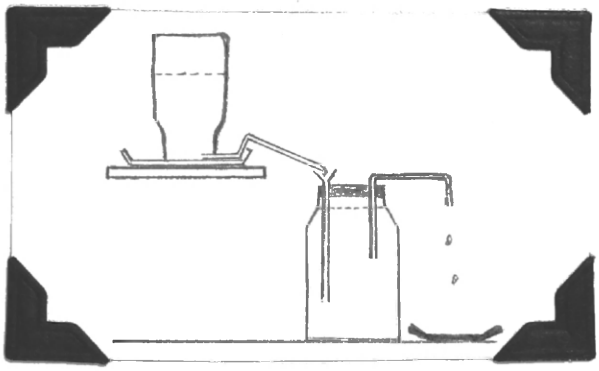


Fig. 1 -- Shive's Drip Apparatus for Constant Solution Renewal for Water Cultures

Methods of Inoculation -- Since the author does not know of any previous study of tomato wilt in water culture, preliminary experiments had to be undertaken in order to determine the best methods for artifically reproducing the normal infection of the tomato, which takes place through the roots. The following methods were utilized: (1) transference of fungi into a vertical slit cut in the stem at the root crown, (2) inoculation with a spore suspension in distilled water by the use of a hypodermic needle inserted in the stems and roots, (3) floating a mat of mycellium in the nutrient solution and (4) floating inoculated alsike clover and tomato stems in the culture solutions immediately surrounding the roots of the plants. Inoculations by each of these methods were made at three stages of growth: at the seedling stage when the plants were one week old, at five weeks of age, and at maturity, just before the fruit set.

Criteria of infection -- In this series of experiments satisfactory criteria for infection was difficult to ascertain because wilting and dwarfing rarely occurred, perhaps because of temperatures slightly below the optimum. The only constant symptom was the discoloration of the stem and this was used, therefore, to determine the progress of infection. To estimate the percentage of infected tissue, longitudinal and cross-section (at root crown) examinations of the discoloration of the stem tissues were made. The figures for the percentage of infection were determined by microscopic examination of cross and longitudinal sections of the stem tissue. Each plant was cut off at the root crown and examined. The percentage of infected tissue for the cross section indicates the amount of discolored areas of the whole conductive tissue. The percentage given is the average of the number of plants examined for each treatment. The stem was then slit from base to terminal and the height of the stem discoloration was measured. The percentage of the stem darkened was determined from the total heights of the stems. These percentages were averaged for the number of plants examined in each treatment.

Experimental Results

The best method for inoculating tomato plants with the Fusarium organism as shown by the results of the preliminary experiments is to float inoculated alsike clover stems in the culture media around the roots of the plants. When inoculated at the seedling state the maximum percentage of infection occurred,

as might be expected, regardless of the time of year and method of inoculation. At the second stage of development there was a decrease in the amount and rapidity of infection, governed to a marked degree by the environmental conditions. At maturity, infection was so slow, a good crop of fruit was set before the fungus penetrated the plants sufficiently to cause them injury.

Table 7 gives the percentages of infected tissue in one of the water culture experiments following the preliminary work. Later studies in sand are of more interest and no further data will be presented here. This table indicates the trend of subsequent results with sand.

Table 7 -- Percentages of infected tissue of inoculated plants in Experiment Three in deficient solutions as indicated by the microscopic examination of the discolored stem tissue. *

Solutions containing	Bonnie Best		Marglobe	
	Cross-section (at root crown)	Longitudinal	Cross-section (at root crown)	Longitudinal
	%	%	%	%
All elements	T	T	0.00	0.00
No boron	0.00	0.00	0.00	0.00
No calcium	60.00	60.00	40.00	40.00
No magnesium	T	T	0.00	0.00
No potassium	60.00	60.00	40.00	40.00
No nitrogen	0.00	0.00	0.00	0.00
No phosphorus	55.00	45.00	0.00	0.00
No sulfur	T	T	0.00	0.00
All elements	30.00	50.00	0.00	0.00

* T signifies a trace of discolored tissue.
Average infections of five plants per treatment.

Sand Cultures

Methods

The general proceedures described above were used in these experiments with certain necessary alterations.

Sand and culture jars -- The sand was obtained from the Berkeley Springs, W. Va. mine of the Pennsylvania Glass Sand Corporation of Lewistown, Pa. The factory analysis of this sand is given as follows: "Silica, 99.81%; alumina, 0.17%; oxide of iron, 0.014%; lime, none; magnesia, none; size, 20-mesh screen". An anlysis of the sand for size follows:

Particles passing through	Percent
20 mesh	51.71
40	34.18
60	5.14
80	3.54
100	5.39

It originates in a hard, quartzite rock and in addition to the commercial washings at the mine, it was further washed in tap water and flushed three times with distilled water before using.

Two-gallon cylindrical glazed earthenware crocks were obtained from the Red Wing Union Stone Works of Red Wing, Minnesota as "coffee urn linings". They are particularly adaptable to this work because they are "self draining". The bottoms of the crocks are convex with a flattened area at the center. The inside slopes on all sides to a one-inch hole.

The crocks were placed on the regulation greenhouse tables. Shive's "Drip Method" for sand cultures was used in

these experiments to obtain a constant renewal of solutions. (Fig.2)

Description of Shive's Drip Method for sand cultures -- Reservoirs and storage shelves similar to those used in the water culture experiments were employed in these experiments. The 0.5 millimeter bore capillary tubing drips directly on the sand and the solution spreads by capillarity throughout the culture crock. One thousand cubic centimeters or more of the solution were supplied to each crock daily. The speed of flow was gradually increased as the plants matured so that the hydrogen-ion concentration was kept as nearly constant as possible. The pH values of the waste solution were recorded frequently and renewal increased if these values went below 4.0 or above 6.0. The waste solution drips through the one-inch hole bored in the bottom of the crock into a waste pan which is emptied every day.

Fig. 2 -- Shive's Drip Method for Constant Solution Renewal for Sand Cultures

It is necessary to flush the sand at least once a week to prevent "concentration levels" and it is important that the nutrient solution be supplied in sufficient quantities to insure an excess constantly dripping into the waste pans. This enables the plants to obtain the necessary amounts of the elements and inhibits the accumulation of salts at any spot in the culture medium. In changing from complete solutions to deficiencies or excesses, the sand must be flushed at least six times with distilled water. This was found to be sufficient for removing most of the elements of the complete solution which might have accumulated around the sand particles.

Method of inoculation -- Inoculations were made by placing the fungus at the base of the roots through a hole bored with a wooden dibble. A liberal supply of the fungus mycellium was transferred from boiled rice and inserted in this hole and covered with sand. It was found desirable to add a one per cent sugar solution to the nutrient treatment to furnish carbohydrate supply for the fungus.

Criteria of infection -- Sand has advantages over water cultures in more nearly approximating soil conditions and it was found that these cultures reduced the difficulties experienced in inoculating and in establishing criteria for infection. Since the moisture content of the medium was decreased to the optimum of the fungus, wilting occurred more generally and other external symptoms of this disease were more frequently apparent.

For quantitative measurements, however, the heights of stem discoloration from microscopic examination and transfers of tissue from various levels of the plants to rice or agar slants were used for the determinations of the percentages of the plants infected.

Experimental Results

Experiment 1 -- The results of this experiment show the relative susceptibility of Bonnie Best and Marglobe plants to artificial inoculation in complete solutions. These data are presented in Tables 8 and 9.

Table 8 -- Average height and percentage of infection of Bonnie Best and Marglobe tomato plants grown in complete nutrient solutions and inoculated with Fusarium lycopersici Sacc.*

Series treatments	Bonnie Best		Marglobe	
	Stem Height cms.	Percentage of infection percent	Stem Height cms.	Percentage of infection percent
Uninoculated	92.40	0.00	92.60	0.00
Inoculated	64.60	100.00	82.10	25.00

* Averages of six plants for each treatment.

Table 9--Average green weights and dry weights of Bonnie Best and Marglobe tomato plants grown in complete solutions and inoculated with Fusarium lycopersici Sacc. *

Series treatments	Bonnie Best						Marglobe					
	Green weights			Dry weights			Green weights			Dry weights		
	Tops	Roots	Total	Tops	Roots	Total	Tops	Roots	Total	Tops	Roots	Total
	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.
Uninoculated	166.90	61.00	227.90	24.67	5.87	30.54	194.90	53.33	248.23	30.87	6.80	37.67
Inoculated	84.03	33.33	117.36	15.01	2.81	17.82	154.30	37.77	192.07	23.58	3.60	27.18

*Averages of six plants for each treatment

Externally, the roots appear normal. Adventitious roots occur on the stem which keep step with the yellowing of the leaves as the fungus spreads up the stem. (Figs. 3 and 4) This symptom has not been recorded by previous investigators.

Fig. 3

Fig. 4

Fig. 3 -- Tomato plants of the Bonnie Best variety. Left- Infected plants with adventive roots. Right - Uninfected plant with clean stem.

Fig. 4 -- Bonnie Best plant showing brown discoloration of vascular tissue, the dead leaves and a shriveled petiole.

Experiment 2 -- Preliminary experiments proved that infection was heavier when the plants were inoculated in the seedling stage and up to five weeks. Therefore, all inoculations were limited to these early stages of growth and were varied according to the time of changing solutions. All solutions were changed when the plants were four weeks old. In this experiment, the plants were inoculated one week before the solutions were changed from a complete solution to solutions with deficiencies and excesses of the essential elements. The percentages of infected tissue for the Bonnie Best variety are given in Table 10. The same data for the Marglobe variety are presented in Table 11.

Experiment 3 -- In this experiment, the plants of both varieties were inoculated at the time the complete solutions were replaced by solutions with deficiencies and excesses of the elements. The percentages of infected tissue for the Bonnie Best and Marglobe varieties are given in Tables 10 and 11 respectively.

Experiment 4 -- Inoculations were withheld in this experiment for one week. The solutions were changed when the plants were four weeks old and inoculations were made one week later. The influence of delayed inoculation on the percentage of infected tissue of the Bonnie Best plants is shown in Table 10. In Table 11 the same data for the Marglobe variety is given.

Table 10 -- Percentages of infected tissue of BONNIE BEST plants inoculated at different times and treated with deficient and excess nutrient solutions. *

Solutions containing	Inoculation before Solution Change		Inoculation at same time as Sol. Change		Inoculation after Solution Change	
	Cross Sec %	Longitud. %	Cross Sec %	Longitud. %	Cross Sec %	Longitud. %
All elements	100.00	100.00	25.00	10.00	25.00	8.00
No Boron	0.00	0.00	T	T	---*	---
No Calcium	100.00	100.00	25.00	25.00	20.00	58.00
No Magnesium	50.00	50.00	25.00	15.00	---	---
No Potassium	100.00	100.00	0.00	0.00	8.00	2.00
No Nitrogen	0.00	0.00	0.00	0.00	0.00	0.00
No Phosphorus	25.00	30.00	0.00	0.00	---	---
No Sulfur	0.00	0.00	0.00	0.00	0.00	0.00
Ex. Boron	90.00	100.00	---	---	---	---
Ex. Calcium	50.00	74.00	10.00	5.00	60.00	65.00
Ex. Magnesium	50.00	87.00	---	---	---	---
Ex. Potassium	100.00	100.00	75.00	85.00	31.00	34.25
Ex. Nitrogen	75.00	100.00	70.00	35.00	T	T
Ex. Phosphorus	60.00	60.00	---	---	---	---
Ex. Sulfur	50.00	60.00	100.00	100.00	0.00	0.00

* "T" denotes Trace.
"---" denotes No Treatment.
Figures represent averages of infected plants.

Table 11 -- Percentages of infected tissue of MARGLOBE plants inoculated at different times and treated with deficient and excess nutrient solutions. *

Solutions containing	Inoculation before Solution Change		Inoculation at same time as Sol. Change		Inoculation after Solution Change	
	Cross Sec %	Longitud %	Cross Sec %	Longitud. %	Cross Sec %	Longitud %
All elements	0.00	0.00	0.00	0.00	0.00	0.00
No Boron	0.00	0.00	0.00	0.00	---*	---
No Calcium	25.00	37.50	25.00	15.00	10.00	5.00
No Magnesium	0.00	0.00	0.00	0.00	---	---
No Potassium	50.00	63.00	25.00	20.00	25.00	20.00
No Nitrogen	0.00	0.00	0.00	0.00	15.00	8.00
No Phosphorus	0.00	0.00	0.00	0.00	---	---
No Sulfur	0.00	0.00	0.00	0.00	25.00	10.00
Ex. Boron	3.00	10.00	---	---	---	---
Ex. Calcium	3.00	8.25	0.00	0.00	0.00	0.00
Ex. Magnesium	0.00	0.00	---	---	---	---
Ex. Potassium	30.00	44.00	T	T	8.00	6.50
Ex. Nitrogen	30.00	29.00	T	T	T	T
Ex. Phosphorus	0.00	0.00	---	---	---	---
Ex. Sulfur	25.00	25.00	0.00	0.00	5.00	2.00

* "T" denotes Trace.
"---" denotes No Test.
Figures represent averages of infected plants.

An examination of these composite comparative tables shows clearly the influence of the time of inoculation and nutrient treatment on the susceptibility of the two varieties to the Fusarium organism.

Boron deficiency inhibited fungus infection in both varieties of tomatoes. Excesses of this element render them more susceptible to infection. A lack of calcium in the nutrient solution broke down the resistance of the Marglobe plants regardless of the time of inoculation. Calcium excesses reduced infection in Bonnie Best plants and from the reports of Edgerton (10) it appears probable that greater applications of lime would reduce this infection further.

Magnesium and phosphorus deficiencies and excesses reduced the infection of the Bonnie Best plants 50 percent and inhibited any infection of the Marglobe variety.

The resistance of the Marglobe variety is again destroyed when the plants are grown in solutions either deficient in or with excesses of potassium. The Bonnie Best variety does not lose its susceptibility when growing in excesses of potassium but infection is reduced when treated with solutions lacking in potassium, if inoculations are delayed.

The absence of nitrogen in the culture solutions is associated with practically no infection of either variety. However, when nitrogen is in excess, infection is increased in the resistant variety and appears normal in the susceptible

variety unless inoculations are not made until the plants are five weeks old. Sulfate deficiency and excess cause the same reactions on the two varieties as do treatments with nitrogen. Excesses of nitrogen and sulfur increased infection in both varieties as compared to their deficiencies while excess of calcium reduced it in comparison with its deficiency.

Experiment 5 -- Because calcium showed this interesting difference, a series of experiments was conducted in sand cultures wherein the concentration of this element was varied from total absence to 400 p.p.m. The data covering the percentages of infection for these experiments is given in Tables 12 and 13. With the Marglobe variety, an interesting trend is descernible. Below 80 p.p.m. over 50 per cent of the plants showed infection and the amount of darkened stem tissue was high. At 160 p.p.m., the normal concentration, and above, there was a marked decrease in the number of plants infected and in the discolored tissue.

Table 12 -- Number of infected plants grown in varying concentrations of Calcium as indicated by external symptoms of wilting or death and by examination of internal discoloration of the stem tissues.

Calcium Concentrations in the solutions	Bonnie Best			Marglobe		
	No. Inoc. Plants	No. Inf. Plants	% Inf. Plants	No Inoc. Plants	No. Inf. Plants	% Inf. Plants
p.p.m.	No.	No.	%	No.	No.	%
0	10	6	60.00	10	5	50.00
5	8	4	50.00	9	5	55.00
20	8	4	50.00	10	5	50.00
40	9	3	33.00	10	5	50.00
60	8	3	37.50	10	6	60.00
80	10	10	100.00	10	6	60.00
160	9	8	88.88	10	0	0.00
280	10	7	70.00	10	3	30.00
400	10	9	90.00	10	2	20.00

Table 13 -- Percentages of infected tissue of inoculated plants grown in solutions containing varying concentrations of calcium.

Calcium concentrations in the solutions	Bonnie Best		Marglobe	
	Cross Sec.	Longitud.	Cross Sec.	Longitud.
p.p.m.	%	%	%	%
0	60.00	80.00	45.00	42.00
5	55.00	61.03	55.00	40.30
20	60.00	70.00	50.00	40.00
40	60.00	58.00	60.00	63.00
60	70.00	63.00	40.00	46.60
80	85.00	86.00	55.00	31.00
160	25.00	25.00	0.00	0.00
280	50.00	45.00	10.00	6.00
400	80.00	75.00	10.00	11.00

Such a definite trend is not found in the data for the Bonnie Best variety, however, There seems to be a slight decrease in infection at a concentration of 160 p.p.m. but in other experiments 100 per cent infection has been obtained at this concentration so this decrease is not significant. It may be than the concentration of calcium in this series was not sufficiently high to bring about a reduction in infection in this variety as it did with the Marglobe variety.

Calcium, as Calcium nitrate, was added to Coon's media from 0 to 53,000 p.p.m. The higher the concentration, the slower the growth of the fungus but only at 53,000 p.p.m. was the ultimate growth decreased to any marked degree.

It would be interesting to increase the concentration of calcium still higher in sand culture work and to conduct a similar series of experiments with nitrogen and sulfur.

Effects of Fertilizer Treatements on Tomato Wilt in Greenhouse Pot Cultures

Methods

For several years field experiments with Fusarium wilt have been carried on by the Horticultural Department of this Station at the Beltsville Farm. Soil was obtained from these plots and to insure thorough infestation it was inoculated shortly after it was brought to the greenhouse. Three week old seedlings were potted out November 11, 1933. The plants were harvested April 2, 1934. Each treatment was duplicated, six plants to a pot. The plants were thinned out as they matured.

Experimental Results

An examination of Tables 14 and 15 shows the results of these experiments.

In comparing these tables with Tables 10 and 11, it will be noticed that increases and decreases in infection are quite similar for related treatments. Where the soil received no treatment or a 4-10-6 fertilizer, the Bonnie Best plants showed symptoms of infection, while the Marglobes are highly

Table 14 -- Effects of fertilizer treatments on Bonnie Best plants in soil infested with Fusarium lycopersici, rate of application, pH values, percentage of infected plants and discolored stem tissue.

Treatments	Rate per acre	pH values (Truog)	Infected Plants	Discolored Stem Tissue	
				Cross.Sec.	Longitud.
	lbs		%	%	%
No Fertilizer	---	6.5	80.00	75.00	59.00
Lime	4000	8.0	80.00	60.00	81.00
4-10-6	1000	6.0	100.00	40.00	37.00
4-10-6) Lime)	1000 4000	8.0	0.00	0.00	0.00
Blood	500	5.0	80.00	60.00	55.00
Manure	20000	5.5	100.00	75.00	67.75
Manure) Lime)	20000 4000	8.0	60.00	50.00	63.00
8-0-0) Lime)	1000 4000	8.5	100.00	75.00	72.30
0-10-6	1000	6.0	60.00	10.00	5.00
8-10-6	1000	6.5	100.00	75.00	84.08
4-0-6	1000	6.5	40.00	10.00	75.00
4-20-6	1000	6.0	60.00	45.00	42.70
4-10-0	1000	6.5	100.00	85.00	91.00
4-10-12	1000	6.0	100.00	70.00	56.00

Table 15 -- Effect of fertilizer treatments on Marglobe plants in soil infested with Fusarium lycopersici, on relative heights, percentage of infected plants and percentage of discolored stem tissue.

Treatments	Relative Hgts. cms.	Infected plants %	Discolored stem tissue Cross Sec. %	Discolored stem tissue Longitud. %
No Fertilizer	77.33	0.00	0.00	0.00
Lime	103.11	40.00	5.00	18.00
4-10-6	92.83	0.00	0.00	0.00
4-10-6) Lime)	92.27	0.00	0.00	0.00
Blood	65.17	0.00	0.00	0.00
Manure	92.71	20.00	10.00	30.25
Manure) Lime)	91.30	60.00	50.00	30.00
8-0-0) Lime)	94.40	80.00	55.00	33.33
0-10-6	83.93	0.00	0.00	0.00
8-10-6	88.90	0.00	0.00	0.00
4-0-6	79.80	0.00	0.00	0.00
4-20-6	83.32	40.00	25.00	18.00
4-10-0	78.10	80.00	60.00	71.50
4-10-12	74.35	0.00	0.00	0.00

resistant in both of these series. Lime appears to have an inhibitory effect on infection unless it is applied with manure or large amounts of nitrogen. Treatments with excesses of this element in sand cultures always results in decreased infection. Low nitrogen reduces the susceptibility of the Bonnie Best variety to a marked degree. High and low phosphorus treatments follow the same trend as they do in sand cultures for the Bonnie Best plant but high phosphorus applications to the soil reduces the resistance of the Marglobe variety. Potassium treatments show similar agreement.

The results with the above fertilizer treatments and the sand culture experiments suggest that heavy applications of lime would be of practical value in reducing the amount of wilt infection. If a fertilizer mixture is used, lime should be added, preferably, with a well balanced fertilizer, such as a 4-10-6.

STUDIES ON THE TOXIC ACTION OF THE FUNGUS

Recent evidence suggests that toxic substances are probably responsible for the wilting of plants. Two studies were made in order to obtain some information with regard to the probably toxic action of the fungus.

The experiment reported by Neal (49) in his work on cotton wilt was repeated for this study. The fungus was grown at room temperature for 18 days in 40, 125 cc Erlenmeyer flasks on 50 cc of Coon's nutrient media and treated as follows: In a series of 8 flasks, the mats were collected on a #50 filter paper,

by means of a Buchner suction filter and the filtrates boiled for 10 minutes. Another series of 8 flasks was treated similarly but not boiled. In another series of 8 flasks, the mycelial mats were collected on filter paper as described above, ground with fine quartz sand for several minutes in a mortar and the mixture extracted with 75 cc of sterile distilled water. Another series was prepared in the same way and the extract boiled for 10 minutes. A fifth series of 8 flasks, for controls, contained Coon's media. Each series was divided into two groups of four flasks each. Bonnie Best plants, which had been grown in complete nutrient solutions until they were 8 to 10 inches high, were placed in two flasks of each series with roots intact. Other plants were inserted in the remaining two flasks after the roots had been severed under water. This was repeated for Marglobe plants. All the flasks were placed in the greenhouse and examined until wilt symptoms appeared. They were then photographed. Figs. 5 to 8 show these plants at the end of 48 hours. Notes recorded as the wilting developed are given in Table 16.

Osmotic pressure, conductivity and pH values of Coon's media, the filtrate and mat extract are presented in Table 17.

Both the filtrates and extracts of the mats possess toxic properties and caused severe wilting after 48 hours. The reaction was the same regardless of whether or not the roots had been removed.

Table 16 -- Effects of Filtrates and Mat extracts of 18-day old cultures of Fusarium lycopersici on Tomato plants. *

Treatment	Wilt symptoms present after 12 hours	24 hours	48 hours	60 hours
Control:				
Coon's-Roots intact	None	None	None	None
Coon's-Roots off	None	None	None	None
Filtrate:				
Boiled-Roots intact	None	None	Slight	Positive
Unboiled-Roots intact	None	None	Slight	Positive
Boiled-Roots off	Slight	Slight	Positive	Pronounced
Unboiled-Roots off	Slight	Slight	Positive	Pronounced
Mat extract				
Boiled-roots intact	None	None	Slight	Positive
Unboiled-Roots intact	None	None	Slight	Positive
Boiled-Roots off	Slight	Slight	Positive	Pronounced
Unboiled-Roots off	Slight	Slight	Positive	Pronounced

* Bonnie Best and Marglobe showed the same reaction to these treatments.

Table 17 -- Osmotic pressure, conductivity and pH values of Coon's media, filtrate of 18-day old culture of Fusarium lycopersici and mat extract. of same cultures

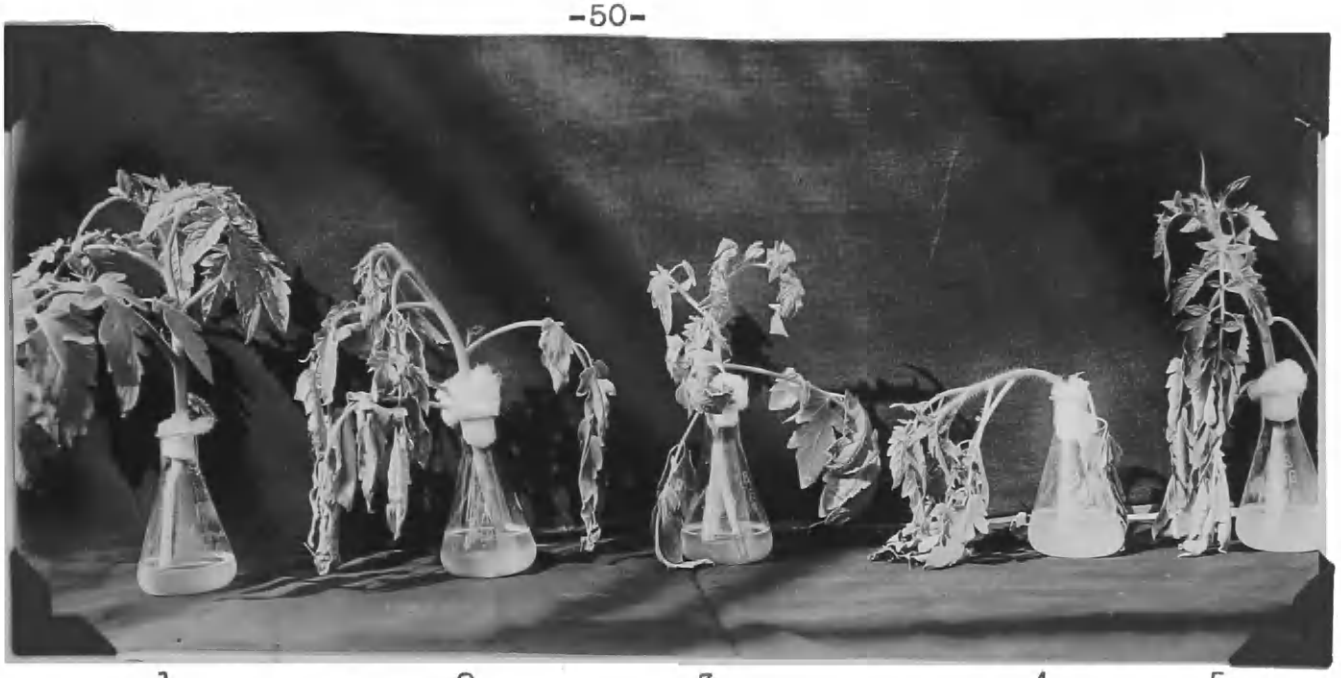
Media	pH	Conductivity	Osmotic Pressure atm.
Coon's	4.50	$5.13 \times 10^{3}_{3}$	3.496
Filtrates	7.29	$3.75 \times 10^{3}_{4}$	1.411
Mat extract	7.20	2.10×10^{4}	0.061

1 2 3 4 5

Fig. 5 -- Marglobe plants with roots cut off after 48 hours in flasks containing (1) Coon's media, (2) Filtrate of 18 day-old culture of Fusarium lycopersici, not boiled, (3) Filtrate boiled, (4) Extract of mycellium mat not boiled and (5) Extract of mycellium mat boiled.

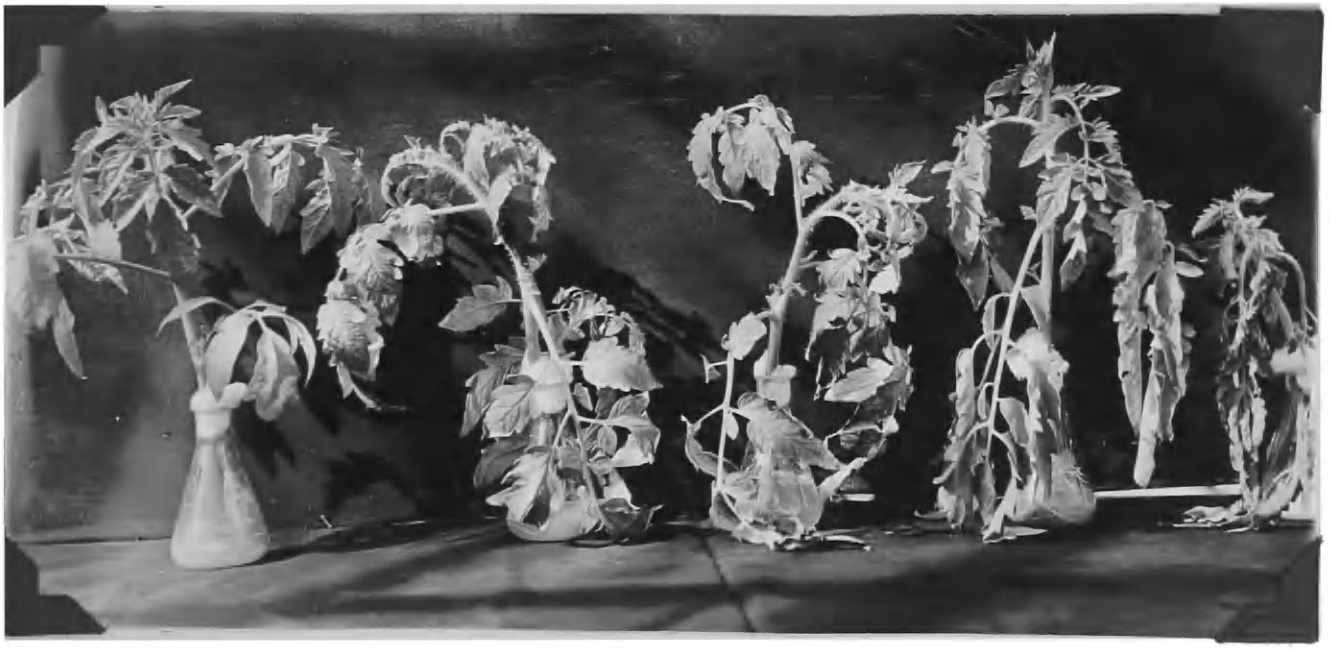
1 2 3 4 5

Fig. 6 -- Marglobe plants with roots intact after 48 hours in flasks containing (1) Coon's media, (2) Filtrate of 18-day old culture of Fusarium lycopersici not boiled, (3) Filtrate boiled, (4) Extract of mycellium mat not boiled and (5) Extract of mycellium mat boiled.



1 2 3 4 5

Fig. 7 -- Bonnie Best plants with roots cut off after 48 hours in flasks containing (1) Coon's media, (2) Filtrate of 18-day old culture of Fusarium lycopersici not boiled, (3) Filtrate boiled, (4) Extract of mycellium mat not boiled and (5) Extract of mycellium mat boiled.



1 2 3 4 5

Fig. 8 -- Bonnie Best plants with roots intact after 48 hours in flasks containing (1) Coon's media, (2) Filtrate of 18-day old culture of Fusarium lycopersici not boiled, (3) Filtrate boiled, (4) Extract of mycellium mat not boiled and (5) Extract of mycellium mat boiled.

Heating does not remove the toxic properties of either the filtrate or mat extract. The control plants remained turgid. Therefore the wilting must be caused by some specific secretion of the fungus. If this substance is of amine nature as reported by Ludtke and Achmed (45) it would be expected that they would be present in the filtrates and mat extracts even after heating for such substances are relatively thermostable. Both the filtrate and mat extract were clear fluids yet the plants wilted in each. This would definitely indicate that toxins cause the wilting and mechanical clogging is only an indirect factor.

The second study approached the subject from a different angle. The terminals of young plants 8 to 10 inches high were removed with a sharp scalpel. A V-shape was cut into the stem at this point. Two methods were used. In the first, a piece of rubber tubing about $1\frac{1}{2}$ inches long was inserted over the stem about $\frac{3}{4}$ of an inch and held in place by a small rubber band. A 2 mm bore glass tube of the same length was inserted in the rubber until it rested on the cut stem. The fungus, from a 7-day old rice culture was placed in the bottom of the glass against the cut surface. The glass tube was kept filled with Coon's media. The second method consisted of wrapping cellophane around the stem and tying it in place with thin cotton string. The cellophane cap extended about $\frac{1}{2}$ inch above the cut surface and rice grains covered with mycellium were placed in this cap. This served as a reservoir for Coon's solution.

The fungus grew down into the stems of both varieties tested. At the end of ten days, darkened tissue was visible from one to five inches. Transfers were made from a section of these stems to agar slants and the fungus was obtained from it again. As the fungus progresses down the stem it would seem that, if a toxic substance were present, the leaves would wilt or show wilt symptoms. This would be definite proof that the wilting phenomena is not caused by mechanical clogging, because the roots and stem below the wilted leaf would not be affected. The method for inoculating through the terminal is shown in Figs. 9 and 10. Note the wilted lateral shoot in Fig. 9. The fungus was obtained from this shoot on an agar slant.

Fig. 9

Fig. 10

Fig. 9 -- Glass and rubber tubing inserted over cut stem for terminal inoculation. Note wilted lateral shoot at base of tubing.

Fig. 10-- Cellophane wrapped around cut stem for terminal inoculation.

EFFECTS OF PLANT EXTRACTS ON GROWTH OF FUSARIUM LYCOPERSICI IN PURE CULTURE

Considering the possibility that the resistant Marglobe variety might contain some inhibitory substance for the growth of the fungus, tests were made on the expressed juices of the Bonnie Best and Marglobe tomatoes. Experiments were carried out using both solid and liquid media. The tops of the plants were cut off, ground in a Nixtamal mill and the juice pressed out under 2000 pounds per square inch in a Carver hydraulic press.

Juice that is to be neither ultrafiltered or autoclaved is immediately centrifuged and then transferred to flasks or test tubes for inoculation. If solid media is desired, agar is added.

For ultrafiltration tests, the juice is centrifuged to throw down all the particles that are in suspension and is then filtered through Coor's Filter candles or a Mandler Filter into sterilized flasks. Transfers are made from these with sterilized pipettes into test tubes, previously sterilized and ready for use. Agar may be added here, also, if solid media is desired.

The juice is taken immediately from the press to the autoclave. It is then centrifuged to throw down the heat coagulated material and reautoclaved. Agar should be added before the second sterilization for solid media.

If the Marglobe juice is autoclaved, Fusarium lycopersici will grow on it normally as it does on Coon's media. However, if

the juice is not heated, growth is greatly retarded and sometimes completely inhibited. The fungus will grow on the Bonnie Best juice under any treatment. This same condition occurs in solid media. The fungus growth on liquid cultures of the two juices, autoclaved and unautoclaved can be seen in Figs. 11 and 12.

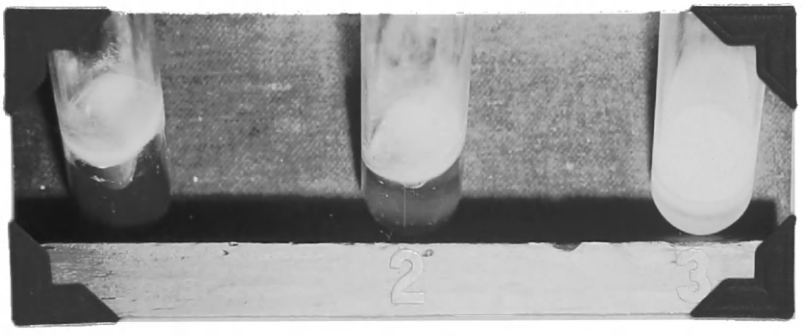


Fig. 11 -- Fifteen-day old cultures of Fusarium lycopersici growing on extract of Bonnie Best plants. (1) Extract autoclaved, (2) Extract not autoclaved and (3) Coon's media.

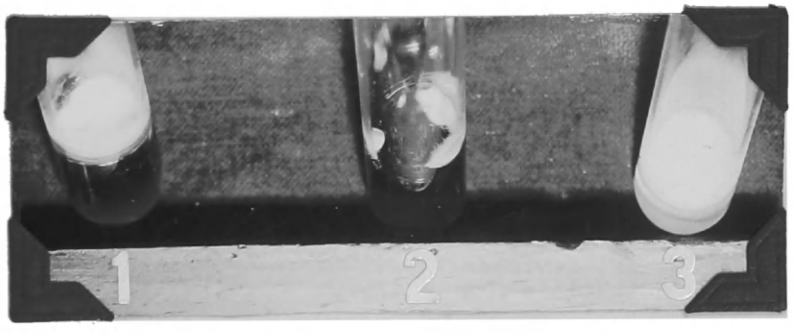


Fig. 12 -- Fifteen-day old cultures of Fusarium lycopersici growing on extract of Marglobe plants. (1) Extract autoclaved, (2) Extract not autoclaved and (3) Coon's media.

The mats of the fungus were dried between layers of filter paper and weighed. Data are given in Table 18. Note that the mats were much larger on autoclaved Marglobe juice than on Coon's or Bonnie Best juice, indicating that growth was accelerated in some way under these conditions. This confirms Reynolds work with flax. (55) It is apparent, therefore, that there is some inhibitory substance in the Marglobe juice and that this substance is destroyed by heating. It has also been observed that this substance is removed or undergoes alteration if the juice is permitted to stand for four to six days before inoculating.

Table 17 -- Weights of mycellium mats of 12-day old cultures of Fusarium lycopersici on juices of Bonnie Best and Marglobe plants autoclaved and unautoclaved.

Culture Number	Coon's Medias	Bonnie Best		Marglobe	
		Autoclaved	Unautoclaved	Autoclaved	Unautoclaved
	gms.	gms.	gms.	gms.	gms.
1	0.1298	0.1653	0.1704	0.2146	0.0733
2	0.1242	0.1852	0.1763	0.1876	0.1042
3	0.1324	0.1509	0.1718	0.2048	0.1126
Average	0.1288	0.1671	0.1728	0.2023	0.0967

The fungus will grown on the extracts of both varieties following ultrafiltration. (Figs. 13 and 14) The pores of the filter candles have negative charges. If this inhibitory substance should be charged positively it would be removed from the solution during the filtering process. It is undoubtedly an absorption phenomona. If the extracts are treated with charcoal for 24 hours, the fungus is able to grow on them.

No effort has been made to determine the exact nature of this substance. An interesting observation was made during the expression of these juices from the plants. The juice from the Bonnie Best plants came out much more readily and at a lower pressure than the Marglobe juice. This suggests that there may be some anatomical differences between the two varieties or some physical characteristic of the Marglobe variety, in addition to this chemical variation, not found in the Bonnie Best which resists invasion by the fungus. Observations were made of freehand sections of stems at various levels. Two general differences were noted. The xylem of the Marglobe variety was more compact than that of the Bonnie Best and the walls of the

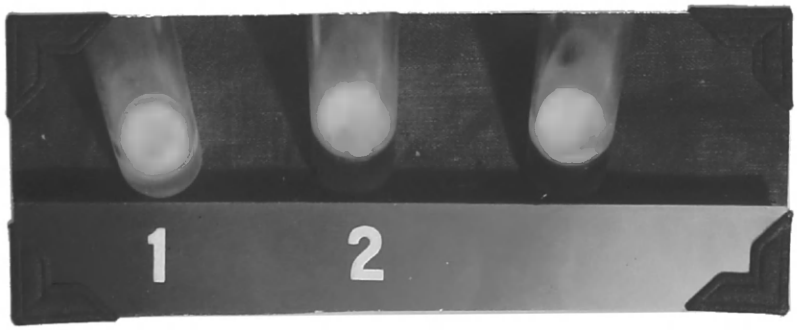


Fig. 13 -- Twelve-day old cultures of Fusarium lycopersici growing on extract of Bonnie Best plants. (1) Coon's media, (2) Extract not filtered and (3) Extract filtered.

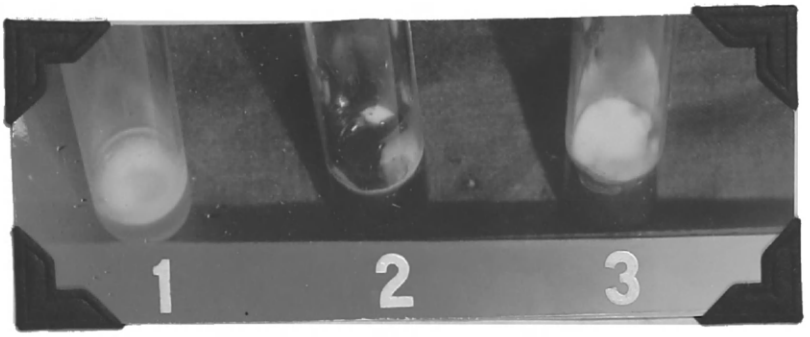


Fig. 14 -- Twelve-day old cultures of Fusarium lycopersici growing on extract of Marglobe plants. (1) Coon's media, (2) Extract not filtered and (3) Extract filtered.

pith cells of the former type appeared to be about twenty-five per cent thicker. Just what influence these variations might exert on the inception of the fungus would make an interesting study.

SUMMARY

1. Tomato plants of the Bonnie Best variety and Marglobe variety were grown in solutions deficient in, and in solutions with excesses of the following essential elements: boron, calcium, magnesium, potassium, nitrogen, phosphorus and sulfur. They were inoculated with Fusarium lycopersici Sacc., the fungus which causes Fusarium wilt. Bonnie Best plants are susceptible to this disease. The Marglobe variety is relatively resistant under normal growing conditions. This work was conducted with both water and sand cultures. The maximum percentage of infection occurred when the plants were inoculated before they were one month old. Boron and nitrogen deficiencies inhibited fungus infection in the Bonnie Best variety. The susceptibility of this variety was decreased when the plants were treated with excesses of calcium, magnesium and phosphorus and solutions lacking in magnesium and phosphorus. If inoculations are delayed until the plants are five weeks old, Bonnie Best plants are not as susceptible to infection in potassium deficient solutions as they are under normal conditions.

The resistance of the Marglobe variety is decreased when the plants are treated with solutions lacking in calcium and potassium and excesses of potassium, nitrogen and sulfur.

2. A study was made of the effect of varying calcium by increments on the infection of these plants. Concentrations were ranged from complete absence to 400 p.p.m. None of the concentrations of calcium used in this investigation caused significant de-

creases in the susceptible of the Bonnie Best plants. The Marglobe variety, however, was definitely susceptible at concentrations below 160 p.p.m.

3. Fertilizer plots in the greenhouse suggest that heavy applications of lime accompanying a well balanced fertilizer, such as a 4-10-6, would be of practical value in reducing the amount of wilt infection.

4. Filtrates from 18-day old cultures of Fusarium lycopersici and extracts from the mats of fungus the same age possess toxic properties to the same degree. Heating does not destroy these properties. The reaction was the same regardless of whether or not the roots of the plants were removed.

5. The wilting of the upper leaves following terminal inoculations would show that the wilting phenomena is not caused by mechanical clogging but by some toxic secretion of the fungus.

6. Untreated Marglobe juice inhibits the growth of the fungus in pure culture. If the juice is autoclaved or ultrafiltered, growth will be normal. The fungus will grow on untreated Bonnie Best juice.

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LITERATURE CITED

1. Appel, Otto and H. W. Wollenweber. Grundlagen einer monographie der gattung Fusarium (Link). Arb. K. Biol. Anst. Land. W. Forstw. Bd. 8, Heft. 1, 207 p. Verzeichnis der wicktigsten benutzten Schriften. 196-198. 1910.

2. Bisby, G. R. A Fusarium disease of garden peas in Minnesota. (Abstract) Phytopath. 8:77. 1918.

3. _____ Studies on Fusarium diseases of potatoes and truck crops in Minnesota. Minn. Agr. Exp. Sta. Bul. 181, 1919.

4. Brandes, E. W. Banana wilt. Phytopath. 9:339-389. 1919.

5. Clayton, Edward E. The relation of temperature to the Fusarium wilt of the tomato. Am.Jr. Bot. 10:71-88. 1923.

6. _____ The relation of soil moisture to the Fusarium wilt of the tomato. Am. Jr. Bot. 10:133-147. 1923.

7. Coons, G. H. and M. C. Strong. The diagnosis of species of Fusarium by use of growth-inhibiting substances in the culture medium, Mich. Agr. Sta. Tech. Bul. 115. 1931.

8. Dickson, J. G. Relation of plant physiology and chemistry to the study of disease resistance in plants. Jr.Am. Soc. Agron. 17:676-695. 1925.

9. Edgerton, C. W. Seed bed sanitation. La. Ext. Div. Cir. 9. 1915.

10. _____ A new method of selecting tomatoes for resistance to the wilt disease. Sci. (N.S.) 42:914-915. 1916.

11. _____ A study of wilt resistance in the seed bed. Phytopath. 8:5-24. 1918.

12. _____ Tomato wilt. La. Agr. Exp. Sta. Bul. 115. 1920.

13. _____ and C. C. Moreland. Diseases of the tomato in Louisiana. La. Agr. Exp. Sta. Bul. 142. 1913.

14. _____ Tomato wilt. La. Agr. Exp. Sta. Bul. 174. 1920.

15. _____ Tests of the wilt resistance of different tomato varieties. La. Agr. Exp. Sta. Bul. 184. 1921.

16. Elliott, J. A. and R. F. Crawford. The spread of tomato wilt by infected seed. Phytopath. 12:428-434. 1922.

17. Essary, S. H. Notes on tomato diseases with results of selection for resistance. Tenn. Agr. Exp. Sta. Bul. 95. 1912.

18. Garcia, Fabian. Reduction of Chile wilt by cultural methods. New Mex. Agr. Exp. Sta. Bul. 216. 1933.

19. Gilchrist, G. G. Nature of resistance to rootrot caused by ascochyta sp. and some other fungi to the epicotyl of the pea. Phytopath. 16:269-276. 1926.

20. Gilman, J. C. Cabbage yellows and the relation of temperature to its occurrence. Ann. Mo. Bot. Gard. 3:25-84. 1916.

21. Goss, R. W. Relation of environment and other factors to potato wilt caused by Fusarium oxysporum. Neb. Agr. Exp. Sta. Res. Bul. 23:1-84. 1923.

22. _____ Potato wilt and the stem-end rot caused by Fusarium eumartii. Neb. Agr. Exp. Sta. Res. Bul. 27. 1924.

23. Gray, J. A study of the non-parasitic leaf spot of tobacco. M. S. thesis deposited in the library of the University of Wisconsin, 1920.

24. Gueguen, Fernand. Sur eine maladie du Collet du pois. Ann. Serv. Epiphyties Mem. et Rap. t. 2(1913):302-309. 1915.

25. Harter, L. L. and W. A. Whitney. Relation of soil temperature and soil moisture to the infection of sweet potatoes by the stem-rot organism. Jr. Agr. Res. 34:435-441. 1927.

26. _____ The comparative susceptibility of sweet potato varieties to stem-rot. Jr. Agr. Res. 34:915-920. 1927.

27. Haskell, R. J. Fusarium wilt of potato in the Hudson River Valley, N. Y. Phytopath. 9:223-260. 1919.

28. Haymaker, H. H. Pathogenicity of two strains of the tomato wilt fungus, Fusarium lycopersici Sacc. Jr. Agr. Res. 36:675-695. 1928.

29. _____ Relation of toxic excretory products from two strains of Fusarium lycopersici Sacc. to tomato wilt. Jr. Agr. Res. 36:697-719. 1928.

30. Humbert, J. G. Tomato diseases in Ohio. Ohio Agr. Exp. Sta. Bul. 321. 1918.

31. Humphrey, H. B. Studies on the relation of certain species of Fusarium to the tomato blight of the Pacific Northwest. Wash. Agr. Exp. Sta. Bul. 115. 1914.

32. Johnston, E. S. Seasonal march of climatic conditions of a greenhouse as related to plant growth. Md. Agr. Exp. Sta. Bul. 245. 1921.

33. _____, _____ and W. H. Dore. The influence of boron on the chemical composition and growth of the tomato plant. Plt. Phys. 4:31-62. 1929.

34. Johnson, J. and R. E. Hartman. Influence of soil environment on the rootrot of tobacco. Jr. Agr. Res. 17:41-86. 1919.

35. Jones, L. R. Stem and rootrot of peas in the United States caused by species of Fusarium. Jr. Agr. Res. 26:459-475. 1923.

36. _____ The relation of environment to disease in plants. Am. Jr. Bot. 11:601-609. 1924.

37. _____ Resistance of peas to rootrot. Phytopath. 16:459-465. 1926.

38. _____ and C. Drechsler. Rootrot of peas in the United States caused by Aphanomyces euteiches. Jr. Agr. Res. 30:293-325. 1925.

39. _____ and M. B. Linford. Pea disease survey in Wisconsin. Wis. Agr. Exp. Sta. Res. Bul. 64. 1925.

40. _____, J. C. Walker and W. B. Tisdale. Fusarium resistant cabbage. Wis. Agr. Exp. Sta. Res. Bul. 48. 1920.

41. Leonian, L. H. Studies on the variability and dissociations in the genus Fusarium. Phytopath. 19:735-869. 1929.

42. _____ Pathogenicity and variability of Fusarium moniliforme from corn. W. Va. Agr. Exp. Sta. Bul. 248. 1932.

43. Lewis, C. E. Comparative studies of certain disease producing species of Fusarium. Maine Agr. Exp. Sta. Bul. 219. 1913.

44. Linford, M. B. A. Fusarium wilt of peas in Wisconsin. Wis. Agr. Exp. Sta. Res. Bul. 85. 1928.

45. Ludtke, M. and H. Achmed. Uber einen pflanzlichen Welkstoff. Biochem. Z. Bd. 257:256-266. 1933.

46. May, C. Effect of grafting on resistance and susceptibility of tomatoes to Fusarium wilt. Phytopath. 20:519-521. 1930.

47. McClintock, J. A. Tomato wilt. Ga. Agr. Exp. Sta. Bul. 138. 1920.

48. McWhorter, F. P. and M. M. Parker. Fusarium wilt of tomato in Virginia. Va. Truck Exp. Sta. Bul. 64. 1928.

49. Neal, D. C. Cotton wilt: a pathological and physiological investigation. Ann. Mo. Bot. Gard. 14:359-424. 1927.

50. Norton, J. B. S. Difference in varieties of fruit and truck crops with reference to disease. Md. State Hort. Soc. 15:62-67. 1912.

51. _____ Tomato diseases. Md. Agr. Exp. Sta. Bul. 180:102-114. 1914.

52. Pritchard, F. J. Development of wilt resistant tomatoes. U.S.D.A. Bul. 1015. 1922.

53. Reinking, O. A. and H. W. Wollenweber. Tropical Fusaria. Phillip. Jour. Soc. 32:103-253. 1927.

54. Reynolds, E. S. Nutritional studies on Fusarium lini. Plt. Phys.. 1:151-164. 1926.

55. _____ Studies on the Physiology of Plant disesse. Ann. Mo. Bot. Gard. 18:57-95. 1931.

56. Scott, I. T. The influence of hydrogen-ion concentration on the growth of Fusarium lycopersici and on tomato wilt. Mo. Agr. Exp. Sta. Res. Bul. 64. 1924.

57. Sherbakoff, C. D. Fusaria of potatoes. Cornell Agr. Exp. Sta. Mem. 6. 1915.

58. Sherwood, E. C. Hydrogen-ion concentration as related to the Fusarium wilt of tomato seedlings. Am. Jr. Bot. 10:537-553. 1923.

59. Shive, J. W. and A. L. Stahl. Constant rates of continuous solution renewal for plants in water culture. Bot. Gaz. 84. 1927.

60. Taubenhaus, J. J., W. N. Ezekiel and D. T. Killough. Relation of cotton root rot and Fusarium wilt to the acidity and alkalinity of the soil. Tex. Agr. Exp. Sta. Bul. 389. 1928.

61. Tims, E. C. Influence of soil temperature and soil moisture on the development of yellows in cabbage seedlings. Jr. Agr. Res. 33:971-992. 1926.

62. Tisdale, W. B. The influence of soil temperature and soil moisture upon the Fusarium disease in cabbage seedlings. Jr. Agr. Res. 24:55-86. 1923.

63. _____ Relation of temperature to the growth and infecting power of Fusarium lini. Phytopath. 7:356-360. 1917.

64. Togashi, K. On three species of Fusarium which cause the wilt disease of pea. Jr. Soc. Agr. and For. Sapporo, Japan. 18;149-154. 1926.

65. Turesson, G. Fusarium viticola Thum. infecting peas. Bot. Notiser 1920: 113-125. 1920.

66. White, P. P. Studies on Fusarium wilt caused by Fusarium lycopersici Sacc. Jr. Agr. Res. 34;197-239. 1927.

67. Wollenweber, H. W. Studies on the Fusarium problem. Phytopath. 3;24-50. 1913.

68. _____et al. Fundamentals for taxonomic studies of Fusarium. Jr. Agr. Res. 30;833-843. 1925.

Part II. Responses of the Tomato in Solution Cultures with Deficiencies and Excesses of Certain Essential Elements

Contents Page

Part II. Responses of the Tomato in Solution Cultures with Deficiences and Excesses of Certain Essential Elements.

INTRODUCTION

McMurtrey (12) investigated the effects of the deficiencies of certain elements on the growth of tobacco. In the introduction to this report he makes this statement: "Once the distinctive symptoms of a deficiency of each element are known for one plant species, such as the tobacco plant, their recognition in other plants will be relatively simple. Although there may be minor variations in the symptoms shown by different plant species, the characteristic effects are likely to be essentially the same."

It would seem upon first thought that this is a very broad statement to make and it is hardly conceivable that plants with entirely different types of vegetative growth from those of the tobacco, would react in the same way to a deficiency of the elements. The tomato might be expected to resemble the tobacco more closely than such crops as corn and wheat. The author experimented with wheat, treating with deficiencies of the main elements and found the symptoms to be somewhat different from those reported by McMurtrey. A general study of these symptoms as exhibited by the tomato has been carried on at this Station for several years. In the main, the work has been conducted in connection with the investigation of Fusarium wilt reported in Part I and includes notes on the effects of excesses of each element. In 1929 and 1930 a study of the effects of removing phosphorus at different stages of

growth was made. It is true that the needs of different plants for a given element vary widely and also that the needs of a definite plant vary at different stages of its growth.

The practical value of this work was demonstrated last year. E. H. Nicholson planted about 40 acres of tomatoes on land at Stemmers Run, Maryland, owned by H. L. Jacks of Crosse and Blackwell, Baltimore. In large areas the plants began to yellow on the lower leaves and turn dark at the terminals. Dead spots were abundant on the leaves. No parasitic organism could be detected as cuasing this condition. The symptoms seemed to indicate that the soil was deficient in phosphorus and the Plant Physiology Department recommended an application of superphosphate. A heavy rain followed this application and shortly thereafter, the plants showed signs of recovery. It is undoubtedly important, in addition to measuring the growth attained by each crop, under various nutrient treatments or fertilizer practices to recognize the external characteristics of plants resulting from the soil conditions under which it is grown.

REVIEW OF LITERATURE.

The literature dealing with the decreased growth made by plants grown in media deficient in each of the essential elements, is abundant. Most of the work, however, has been limited to the effects of a single element on the chemical

composition and anatomy of various plants. A key has been prepared by McMurtrey (12) which is a considerable value in recognizing the malnutrition diseases of tobacco. He states in his summary: "Typically, a deficiency of nitrogen is shown by the plant as a whole assuming a light-green color, with more or less yellowing and drying or firing of the lower leaves to a light-brown color. The roots are long, little branched and white. A shortage of phosphorus, on the contrary, produces a plant that is dark green in color and may show some yellowing and drying of the lower leaves to a greenish-brown color. The roots of such plants are long, little branched, and reddish brown in color, owing to the presence of iron compounds on their surface.

"Potassium and magnesium hunger, in contract to nitrogen and phosphorus hunger, show localized effects, of which chlorosis of the lower leaves is the dominant characteristic. Typical potassium hunger is distinguished from magnesium hunger by the small necrotic spots or specks at the tip and margins of the chlorotic leaves which usually do not occur in the case of the latter. The chlorotic areas in the case of potassium hunger are of a yellowish color, while with magnesium hunger they are pale green or white, with the principal veins tending to retain the green color in both cases. The leaves turn or tuck under at the tips and margins with potassium deficiency and tend to turn or cup up in the case of magnesium hunger.

"As contrasted with the deficiencies given above, which are general or become apparent on the older or lower leaves, those showing themselves typically on the new growth or bud leaves are iron, manganese, sulphur, calcium and boron. Deficiency of iron, manganese or sulfur does not result in the death of the terminal bud, while lack of calcium or boron produces death of the terminal bud as a final result. The first three deficiencies produce chlorosis of the younger leaves, each of a characteristic type. Iron chlorosis and manganese chlorosis resemble each other in that the veins tend to retain their green color, but in the case of manganese deficiency a necrotic spotting occurs scattered over the leaf, while no necrotic spots occur with iron deficiency. The chlorosis resulting from sulfur deficiency differs from that just described in that the veins are of a lighter green color than the tissue between the veins. In the case of sulfur hunger the roots are typical, being unusually abundant and white in color, while with iron and manganese shortage they are not so abundant nor so white.

"Calcium and boron deficiencies differ from each other in that the initial effects of their shortage produce different symptoms. A shortage of calcium first becomes apparent as a peculiar hooking downward of the tip of the young leaves of the bud, followed by the death of the young leaves at their tips and margins, and if later growth takes place the tips and margins show a cut-out appearance. In contrast with this effect,

shortage of boron is shown in a light-green color at the base of the young leaves of the bud, followed by their breakdown which, if not too severe, is followed by later growth, which causes the young leaves to become distorted or twisted at their bases. In most cases of boron deficiency the tip of the leaf remains alive for some time after the base has broken down."

No such composite review is available for the tomato plant and no reports have been encountered covering the effects of excesses of the elements on this crop.

Pember (15) studied the effects of potassium and phosphorus on barley at the Rhode Island Agricultural Experiment Station. The actual phosphorus absorbed was greatest during the first six weeks and even though an excess was present there was a limit to the amount the plant absorbed.

Brenchley (1), in a recent work, has upheld Pember's results that the greatest absorption of phosphorus by barley occurs during the sixth week. For this reason the necessity of fertilizing just before or just after sowing is emphasized. She found that if phosphorus was present for six weeks, tillering and ear and grain formation were as good, if not slightly better, than if it were present till harvest. The uptake of phosphorus continued steadily upward until just before harvest so that the percentage of phosphorus absorbed could be correlated with the length of time phosphorus was available. Enough

phosphorus was absorbed during the first six weeks, however, to make the maximum amount of dry weight.

The results of some work by Gericke of California (2,3,4,5,7) are well worth reviewing. All elements are not required by the plant to the same degree to produce normal growth. Generally more of the elements are absorbed than absolutely needed by the plant but some elements are needed at one time and in greater quantities in the life of the plant while others are needed at other times and in smaller amounts. Choosing wheat as his crop, he found that if plants were grown in deficient magnesium, phosphorus, sulfur and potassium solutions after six weeks, they developed more grain and straw than those which grew to maturity in the presence of these elements. These elements are either unnecessary for the plant or harmful to the plant in later stages of growth. Nitrogen is essential at all times for good, normal, vegetative growth. Calcium and iron must be present for at least twelve weeks.

Sorokin and Sommer (18) find that if calcium is absent from the culture media, plants die within one or two weeks.

METHODS

The Marglobe variety of tomato was used in these studies. The methods of seed germination, solution reservoirs, solutions used and Shive's "Drip Method" for continuous solution renewal have been adequately described under "Methods" of Part I. The

elements included in these studies were boron, calcium, potassium, nitrogen, magnesium, phosphorus and sulfur. In most of the experiments reported, the plants were treated with a solution containing all of the elements for the first thirty days. Transfers were then made to jars containing excesses of each particular element or to jars lacking in one of the essential elements. Symptoms of deficiencies and excesses, heights of plants, flower clusters, fruit, green weights and dry weights were recorded.

EXPERIMENTAL RESULTS

Deficiencies of Essential Elements in the Culture Media

The data for these experiments are given in Tables 1 and 2. The former table gives the average heights and green weights for the plants receiving these treatments and the latter table presents the number of flower clusters, number of fruit and weight of fruit.

Table 1 -- Average heights of Marglobe plants after growing 30 days in complete solutions, the heights at harvest (95 days), the growth made by these plants between the 30th and 95th day while receiving treatments with deficient nutrient solutions and the green weights.*

Solutions containing	Heights 30 days	Heights 95 days	Growth 30-95 days	Green Weights		
				Tops	Roots	Total
	cms.	cms.	cms.	gms.	gms.	gms.
All elements	10.5	112.9	102.4	156.4	60.7	217.1
No boron	14.7	53.7	39.0	55.7	15.9	71.6
No calcium	11.8	55.0	43.2	63.7	8.7	72.4
No magnesium	9.5	68.9	59.4	48.4	6.2	54.6
No potassium	13.1	68.9	55.8	57.7	9.8	67.5
No nitrogen	12.9	46.9	34.0	20.7	10.9	31.6
No phosphorus	10.9	83.8	72.9	60.3	23.9	84.2
No sulfur	12.1	93.5	81.4	128.6	28.6	157.2

* Averages for five plants.

Table 2 -- Number of flower clusters, number of fruit and weight of fruit of Marglobe plants treated with solutions deficient in one of the essential elements.*

Solutions containing	Number of Flowers	Number of Fruit	Weight of Fruit gms.
All elements	13	7	62.0
No boron	5	0	0.0
No calcium	4	0	0.0
No magnesium	6	0	0.0
No potassium	5	0	0.0
No nitrogen	4	0	0.0
No phosphorus	8	2	4.5
No sulfur	8	2	9.0

* Figures are totals for five plants.

There is a decrease in height and green weight whenever any one of the elements is removed from the cultural solutions. This decrease is of the smallest value with sulfur deficiency and with a lack of phosphorus is slightly larger. The greatest decrease follows nitrogen starvation. Generally, no fruit is produced in these cultures except on plants growing in solutions containing no phosphorus and no sulfur. The fruit that are produced are small and light.

Deficiencies of Phosphorus at Different Stages of Growth

In this part of the investigation, a study was made of the effect on growth when phosphorus was withdrawn from the culture media at intervals. A large number of plants were divided into groups of ten. One group was placed in phosphorus deficient solutions at the beginning of the experiment. A second group was grown in complete solutions for the duration of the experiment (100 days). At intervals of ten days, a series of ten plants were transferred from the complete solutions to solutions with phosphorus withdrawn. The average heights of these plants showing the effect of transfer into a solution lacking in this element are given in Table 3. (Fig. 1) The green weights, dry weights and fruit produced by these plants are presented in Table 4.

A second phase of this study consisted of transferring the plants to phosphorus deficient solutions for a short time

and then returning them to complete solutions. The data for this study are given in Tables 5 and 6. (Fig. 2)

Table 3 -- Average heights of Marglobe plants grown in complete then in permanently phosphorus deficient solutions. *

Phosphorus removed after	Ten-day period ending							
	January		February			March		
	19	29	8	18	28	10	20	30
	cms.	cms.	cms.	cms.	cms.	cms.	cms.	cms.
30 days	11.7	19.7	27.7	34.4	39.0	42.1	44.6	46.6
40 "	10.1	20.0	28.9	42.3	47.3	54.9	60.2	65.6
50 "	10.6	19.1	27.5	32.0	44.0	59.4	63.3	68.8
60 "	11.0	18.6	26.1	34.3	42.7	51.8	57.3	62.7
70 "	10.7	18.0	25.5	33.4	41.0	52.2	58.2	64.0
80 "	9.1	16.4	23.9	33.1	38.6	47.0	53.9	60.7
90 "	11.2	19.9	28.3	36.5	47.1	55.5	59.8	64.3
100 "	11.3	19.6	27.6	34.9	44.8	54.4	61.3	68.3

* First measurements taken Jan. 19 were taken after the plants had grown in complete solutions for thirty days.
The diagonal line indicates the heights at the end of each ten-day period in which they were grown in complete solutions and just before they were changed to the solutions deficient in phosphorus.

1 2 3 4 5

Fig. 1 -- Marglobe plants grown in permanently phosphorus deficient solutions. 1. Check; 2. - P after 60 days; 3 - 50 days; 4 - 40 days; and 5 - 30 days.

Table 4 -- Average green weights, dry weights and fruit production of Marglobe plants grown in complete then in permanently phosphorus deficient solutions.

Phosphorus removed after	Green Weights			Dry Weights			Fruit production
	Tops	Roots	Total	Tops	Roots	Total	
	gms.	gms.	gms.	gms.	gms.	gms.	gms.
30 days	19.2	12.3	31.5	3.2	1.0	4.2	0.0
40 "	46.7	34.6	81.3	6.9	3.2	10.1	0.0
50 "	64.0	49.1	113.1	10.9	4.5	15.4	0.0
60 "	57.1	37.7	94.8	10.7	3.6	14.3	0.0
70 "	65.0	42.3	107.3	11.5	3.9	15.4	0.0
80 "	53.6	21.3	74.9	10.5	4.4	14.9	1.6
90 "	61.0	33.8	94.8	11.2	5.6	16.8	3.9
100 "	59.8	33.0	92.8	11.5	5.2	16.7	4.1

Table 5--Average heights of Marglobe plants grown for thirty days in a complete nutrient solution and then in phosphorus deficient solutions for varying periods.*

Time in complete solution	Phosphorus removed after	Phosphorus returned after	Ten-day period ending							
			January		February			March		
			19	29	8	18	28	10	20	30
days	days	days	cms.	cms.	cms.	cms.	cms.	cms.	cms.	cms.
30	30	--	11.7	19.7	27.7	34.4	39.0	42.1	44.6	46.6
30	30	50	11.9	20.4	28.7	38.8	57.9	68.6	72.3	75.9
30	30	70	10.8	18.7	26.7	33.9	37.9	47.4	55.7	64.9
100	--	--	11.3	19.6	27.6	34.9	44.8	54.4	61.3	68.3

*Averages of ten plants
First measurements, January 19, were taken after the plants had grown in complete solutions for thirty days.

Table 6--Average green weights, dry weights and fruit production of Marglobe plants grown for thirty days in a complete nutrient solution and then in phosphorus deficient solutions for varying periods.*

Time in complete solution	Phosphorus removed after	Phosphorus returned after	Green weights			Dry weights			Fruit Production
			Tops	Roots	Total	Tops	Roots	Total	
days	days	days	gms.	gms.	gms.	gms.	gms.	gms.	gms.
30	30	--	19.2	12.3	31.5	3.2	1.0	4.2	--
30	30	50	62.0	31.7	92.7	12.0	3.8	15.8	15.3
30	30	70	48.0	20.9	68.9	7.9	2.6	10.5	--
100	--	--	59.8	33.0	92.8	11.5	5.2	16.7	4.1

*Averages of ten plants

1 2 3 4

Fig. 2 -- Marglobe plants grown in temporarily phosphorus deficient solutions. 1 Check; 2 - P for 40 days; 3 - P for 20 days; and 4 - P to harvest. All plants were grown for 30 days in complete solutions.

In preliminary experiments it was found that tomato plants do not grow normally when deprived of phosphorus during the first thirty days of their life. If this element is supplied for thirty to forty days, however, plants later deprived of phosphorus at successive ten-day interwals show an increase in height, green weights and dry weights. It will be noted that if phosphorus is lacking after seventy days (nine weeks) no definite injury becomes apparent for the length of the experiment and the total heights and weights do not vary markedly from those grown in complete solutions.

Phosphorus must be present for at least forty days before any flower buds will develop. Occasionally a few are found on plants deprived of phosphorus before this time but they soon dry up and drop off. It was noted, also, that even though flower buds form on plants receiving phosphorus for longer than six weeks, fruit does not set unless the element is present for at least seventy days. This parallels the time when injury from the lack of phosphorus does not become significant to the plants. There was an average of about six flower buds formed in these cultures, but only a small percentage of fruit was set when the experiment was concluded.

Gericke (6) has reported stimulation of growth in wheat when some of the elements were removed, temporarily, from the culture solution. There seemed to be a similar stimulation in tomato plants as shown by the above experiments when phos-

phorus was removed from the culture solution for twenty days. However, McMurtrey (12) believes that, under these conditions, the plants were retarded from starvation but just at the time when the elements were applied to the plants again the days were gradually lengthening and under the favorable light condition, growth was stimulated. He does not believe the same results would be obtained in the fall when the daily light periods are becoming shorter. Gericke increased the nitrate ion in his phosphorus deficient solutions. McMurtrey states "that the plants might have been stimulated in growth by the presence of more nitrate and that they were able to develop for the time under consideration without showing the effects of phosphorus deficiency".

Excesses of Essential Elements in Culture Media

Typical data on plants receiving excesses of the nutritive elements are given in Tables 7 and 8. An excess of nitrogen causes a decrease in height and green weight. A decrease in these growth data also occurs on plants treated with excesses of calcium. Potassium and sulfur in abundance increase these values. Fruit is produced on all cultures with the exception of nitrogen.

Varying Concentrations of Calcium in the Culture Media

In these experiments the concentrations of calcium were varied from its entire absence to 400 p.p.m. The growth resulting from these treatments is presented in Table 9. It will be noticed that there is a gradual increase in height and green

weight from 5 p.p.m. of calcium to 160 p.p.m., the normal concentration. However, after this optimum is reached, there is a dropping off in these values indicating that 160 p.p.m. of calcium is the optimum concentration of calcium for artificial culture work.

Table 7 -- Average heights of Marglobe plants after growing 30 days in complete solutions; the heights at harvest (78 days), the growth made by these plants between the 30th and 78th day while receiving treatments with excess nutrient solutions, the number of flower clusters and number and weight of the fruit.*

Solutions containing	Heights 30 days	Heights 78 days	Growth 30-78 days	Number of Flowers	Number of Fruit	Weight of Fruit
	cms.	cms.	cms.			gms.
All elements	6.5	113.5	107.0	9	4	74.10
Excess boron	6.9	113.2	106.6	10	3	27.85
Excess calcium	6.5	103.8	97.3	8	3	36.75
Excess magnesium	5.3	111.2	105.9	9	4	38.00
Excess potassium	5.7	118.7	113.0	9	6	50.00
Excess nitrogen	6.2	100.2	94.0	9	0	0.0
Excess phosphorus	6.0	111.2	105.2	5	4	67.50
Excess sulfur	5.9	121.0	115.1	5	3	14.35

* The heights represent averages of five plants. The flowers and fruit are totals for five plants.

Table 8 -- Average green weights and dry weights of Marglobe tomato plants treated with solutions containing an excess of the essential elements.*

Solutions containing	Green Weights			Dry Weights		
	Tops	Roots	Total	Tops	Roots	Total
	gms.	gms.	gms.	gms.	gms.	gms.
All elements	113.72	15.75	129.47	11.62	1.25	12.87
Excess boron	120.20	21.27	141.47	15.58	1.59	17.17
Excess calcium	98.12	29.47	127.59	13.08	4.56	17.64
Excess magnesium	138.75	26.75	165.50	11.69	2,52	14.21
Excess potassium	148.37	31.87	180.24	16.17	3.51	19.68
Excess nitrogen	87.70	14.62	101.32	8.25	1.21	9.46
Excess phosphorus	150.00	46.00	196.00	10.59	1.58	12.17
Excess sulfur	153.75	30.50	184.25	12.74	2.08	14.82

* Figures represent averages of five plants.

Table 9 -- Average height growth and green weights of Marglobe plants treated with varying concentrations of calcium. *

Calcium concentrations in the solutions p.p.m.	Heights cms.	Green Weights Tops gms.	Roots gms.	Total gms.
0	60.49	95.33	21.38	116.71
5	57.65	82.23	27.36	109.59
20	59.89	88.44	28.38	116.82
40	66.64	97.50	23.69	121.19
60	72.16	121.38	33.90	155.28
80	83.78	154.64	32.99	187.63
160	95.30	167.08	42.60	209.68
280	89.36	150.83	52.06	202.89
400	82.02	141.00	33.22	174.22

* Figures represent averages of ten plants.

COMPARISON OF EXTERNAL SYMPTOMS PRODUCED BY DEFICIENCIES AND EXCESSES OF CERTAIN ESSENTIAL ELEMENTS.

All comparisons of the growth produced when any of the elements were lacking or were in excess in the solutions were based on the growth obtained in a complete solution which has been found to give good results for the tomato plant. In general, plants grown in the control solution showed an increase in dry weight over plants grown in incomplete solutions. The same decrease in dry weights was not produced in every case by an excess of these elements in the solutions. The plants appeared normal in every respect, with an abundant vegetative growth, many flower buds, large fruit setting and many branched roots. The leaves showed a good healthy dark green color and the roots were white, abundant, well branched and averaged 73.2 centimeters in length. (Fig. 7)

BORON

A constant source of boron is necessary for normal growth, setting of fruit and development of fruit. (10) It functions as a simple nutrient element needed in extremely minute quantities. It is essential for all meristematic cell division.

Deficient cultures -- The effects of boron deficiency were usually apparent about nine days after the plants were transferred to a solution lacking this element. The cotyledons and leaves turned to a distinct purple color in a few days.

The stems became stunted and the terminal shoot curled inward, yellowed and died. The conductive tissue broke down. A striking characteristic was the extreme brittleness of the petioles and mid-ribs, described by Johnston and Dore (9) as similar to the breaking of a piece off cheese. The roots showed extremely poor growth and were yellow to brown in color. The fruits were covered with darkened or dried areas which were apparently due to the breaking down of the cells making up this tissue. These dead areas may be compared to the injury by blossom end rot with the difference that they do not always begin at the blossom end of the fruit but are scattered over the entire surface. (10) (Fig. 7)

Excess cultures -- The effects of an excess of boron were not apparent for several weeks after the plants were transferred to a solution with 1.10 p.p.m. The heights and color of these plants were quite normal in appearance. The only evidence of injury from an excess of this element was a "burning" around the margins of the lower leaves. In all other respects they were normal. Newell (13), however, using a concentration of 2.75 p.p.m., reported "a yellowing of the whole leaf with large brown spots giving them a curious mottled appearance" in addition to the death of the margins of the leaves. He found also a slight decrease in dry weights at this concentration. (Fig. 9)

CALCIUM

Calcium is necessary for normal root and leaf develop-

ment. Cell walls have been thought to contain calcium in some form in the middle lamellae. Cells formed in its absence, therefore, are likely to be weak and more easily injured. It favors the translocation of starch. Its exact function as a plant food is still debatable. (16,17)

Deficient cultures -- Three to five days in deficient calcium solutions were sufficient for plants to exhibit the symptoms denoting a lack of this element. Turgidity was immediately lost, the plants becoming weak and flabby. The terminal bud soon died. The stem, near the terminal, became spotted with dead areas and the upper leaves, first a darker green than normal, soon yellowed at the edges, dried up and dropped off. The lower leaves generally remained normal. If new leaves were produced, they also died in a short time. Since nuclear division may occur without the formation of cell walls, the entire plant is stunted in growth. The roots were short, numerous, much branched and dark brown in color, accompanied by some decomposition. The average length of the roots was 28.4 centimeters. (Figs. 3 and 7)

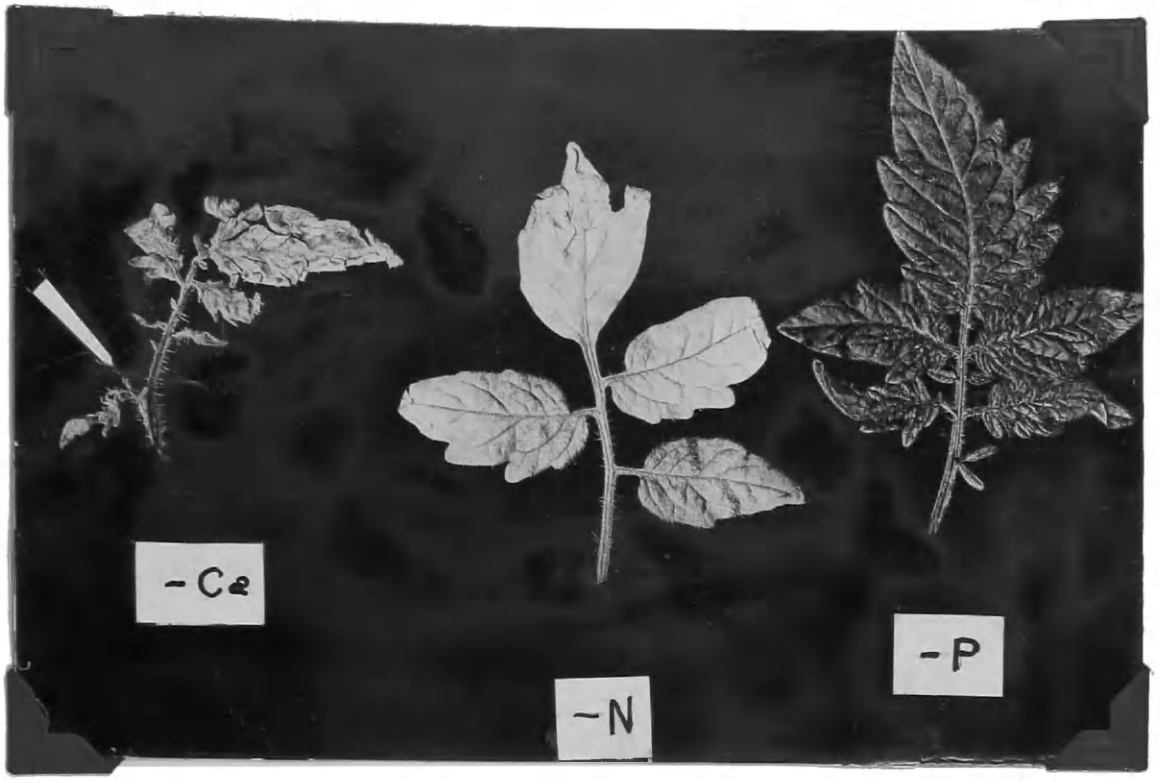

Fig. 3 -- Typical leaf symptoms of plants grown in solutions deficient in calcium, nitrogen and phosphorus.

Excess cultures -- Although the plants grew tall, they appeared weak and less turgid than plants in normal solutions. The leaves were long and slender with small, pointed leaflets. The terminal shoot was small and poorly developed. A small amount of fruit was set. The roots were apparently normal in color, bunched at the top with several longer, unbranched tap roots, averaging 44.4 centimeters in length. (Fig. 9)

MAGNESIUM

Magnesium is a component of chlorophyll. It has been thought to be necessary for the formation of nucleoproteins.

Raber (17) states that "it also seems to be necessary for the transportation of phosphorus, and , since this latter element is necessary for fat formation, perhaps the magnesium affects the formation of fats only indirectly."

Deficient cultures -- Chlorosis of the leaves of plants deprived of this element occurred in five to eight days. A mottling of the leaves to a light green and yellow began on the margins and gradually spread inward between the veins until the whole leaf was affected. The veins remained normal in color. Growth was stunted because of the inability of the plants to produce chlorophyll and the stem became abnormally slender. An etching of the petiole occurred simultaneously, weakening them and causing the leaves to drop. The roots were long, with few lateral branches. (Figs. 4 and 7)

Excess cultures -- There were no abnormal symptoms apparent from the excesses of this element employed in these experiments, except a series of light brown, pin spots up and down the stem but these evidently did not interfere with normal growth. The plants were tall, strong and healthy, with numerous flower clusters. The roots appeared somewhat shorter and there was no definite tap root. (Fig. 9)

Fig. 4 -- Typical leaf symptoms of plants grown in solutions deficient in magnesium.

POTASSIUM

Formation and translocation of carbohydrates is inhibited without the presence of potassium. (14) Potassium salts are radioactive and their effect upon the general health and nutrition of the plant has been attributed by Stoklasa to this property (17). Cessation of growth occurs because of decreased cell division. The cells elongate but fail to divide and the plants are very susceptible to disease. It is necessary for turgesence, increasing the pressure in the cells

and the water absorbing power of the plant as a whole.(19)

Deficient cultures -- Deficiency symptoms of this element appeared after seven to ten days as yellowish to brown spots on the leaves near the margin, which gradually spread until the whole leaf became yellow, and premature death occurred. Growth was general retarded. Etchings were found on the main stem, which was more slender than normal. The margins of the leaves turned under and the lower leaves were pimpled between the veins. The roots were 45.1 centimeters long and had few lateral branches. The color of the roots was a pale yellow. (Figs. 5 and 8)

Fig. 5 -- Typical leaf symptoms of plants grown in solutions deficient in potassium.

Excess cultures -- The excesses of potassium used exerted no abnormal influence on the plants. They remained strong and healthy with many flower clusters and a heavy setting of fruit. A slight tendency for a decrease in the hairs on the stem was noted. The roots were darker brown and bunched at the top. Their average length was 34.8 centimeters. (Fig. 10)

NITROGEN

Nitrogen enters into the composition of protein, nucleoproteins and other nitrogenous substances which are synthesized by plants. Because of its role in protein production, protoplasm development is closely correlated with the amount of nitrogen in the medium.

Deficient cultures -- A yellowing of the entire plant occurred in about six to eight days. The lower leaves slowly dried up and dropped off. The decreased protoplasm caused a general reduction in leaf and stem growth. The veins became pink in color. The roots were approximately 61.7 centimeters in length, unbranched and white. (Figs. 3 and 8)

Excess cultures -- An excess of nitrogen stimulated vegetative growth at the expense of flowers and fruit. The terminal shoot was depressed but lateral branches were numerous. Few fruits set. The leaves were spotted with dead areas, curled, roughly pimpled and yellowed interveinously. Maturity was greatly delayed and the resistance of the plant to disease was

reduced, probably because of "a change in the physiological resistance within the plant and also to a thinning of the cell wall, allowing a more ready infection from without". (8,16) The roots were light brown in color, with few branches and averaged 45.8 centimeters in length. (Fig. 10)

PHOSPHORUS

Phosphorous is generally supplied to the plant as phosphate and it enters into the formation of nucleic acid, nucleoproteins and lecithin. Photosynthesis may occur in its absence but the breaking down of the insoluble carbohydrates into a soluble form for translocation is inhibited. Root growth is stimulated by this element and ripening processes hastened. (16) Loew (11) stated that no cell division took place in the absence of phosphorus and attributed this to the absence of phospholipoids for the formation of which phosphorus is necessary. It is an essential constituent of the chloroplasts and these are necessary for carbohydrate synthesis. Phosphorus is most abundant in meristematic tissues. If the element is withdrawn from the plant and this supply is used up, it may be forcibly withdrawn from the lower leaves and roots (19).

Deficient cultures -- The deficiency symptoms appeared in seven to ten days. The plant took on a very dark blue-green or purplish color. The lower leaves gradually turned black. The stem was slender, stunted and covered with deep blue pin spots. The roots were long, with few lateral branches

and brown in color. McMurtrey (11) attributed a similar discoloration of tobacco roots to the deposits of iron compounds on their surface. (Figs. 3 and 8)

Excess cultures -- The stems appeared slightly reduced in size and vigor. Otherwise, this treatment did not seem to affect the normal growth and development of tops, flowers, fruit or roots. (Fig. 10)

SULFUR

Sulfur functions as a building material in the formation of proteins. It is needed in much smaller quantities than any of the ten major essential elements, with the exception of iron. It is necessary for cell division and fruit setting. (17).

Deficient cultures -- It took two weeks for symptoms to appear on these cultures. Because of the small amount necessary, the supply was exhausted slowly. The leaves became very thick and firm. The entire plant took on a pale green color and the under sides of the veins turned purple, but there were no necrotic spots. Cell division was not retarded to the same degree as with some of the other deficiencies, but there was a marked decrease in dry weight. The stem was stiff, woody and more slender than normal. It frequently was taller than plants grown in media containing this element. The roots were white, abundant and much branched. The roots of these plants averaged 28.7 centimeters in length. (Figs. 6 and 8)

Excess cultures -- The leaves of these plants were marked by a rough pimpling and curling inward as with nitrogen excess. No dark areas occurred interveinously, however. The lower leaves died on the margins. The terminals were a pale yellow. The hairiness of the stem was reduced. The roots appeared long, light and unbranched, averaging 61.2 centimeters in length. (Fig. 10)

Fig. 6 -- Typical leaf symptoms of plants grown in solutions deficient in sulfur.

SUMMARY

1. Tomato plants of the Marglobe variety were grown in solutions deficient in and in solutions with excesses of the following essential elements: boron, calcium, magnesium, potassium, nitrogen, phosphorus and sulfur. Data on the external symptoms exhibited by tomato plants under these various treatments were recorded.

2. Generally there was a decreased growth of plants grown in solutions deficient in each of the elements. This same decrease was not produced in every case by an excess of these elements in solutions. It is apparent that the plants can make relatively good growth in concentrations double that of the control cultures, with the exceptions of calcium, nitrogen and sulfur.

3. The distinctive symptoms of tomato plants grown in solutions deficient in each of the essential elements studied were strikingly similar to those reported by McMurtrey for tobacco. It was noticed, however, that these effects were generally manifested much earlier on this crop. However, this varies considerably according to the time of year and environmental conditions. Because of the ability of the tomato to withstand the presence of high concentrations of the elements in the nutrient media no such distinctive responses could be distinguished between the plants treated with excesses of the various elements.

4. When the concentration of calcium was varied from zero to four hundred parts per million, optimum heights and weights were obtained from plants treated with one hundred and sixty parts per million of this element. On either side of this concentration a decrease in growth data was produced.

5. Phosphorus must be present for the first forty days or no flower buds will be produced and growth will be retarded. Plants placed in permanent deficient solutions after seventy days (nine weeks) showed no appreciable injury from a lack of phosphorus. Greater dry weight was obtained from plants grown in a temporarily deficient phosphorus media for twenty days than that obtained by plants where phosphorus was present until maturity. This may be due, however, to the season of the year when the experiment was conducted or to the concentration of the other ions in the solutions rather than to the temporary absence of the element.

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Fig. 7 -- Growth attained by Marglobe plants in (1) a complete solution and in solutions deficient in (2) boron, (3) calcium and (4) magnesium.

1 2

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Fig. 8 -- Growth attained by Marglobe plants in solutions deficient in (1) potassium, (2) nitrogen, (3) phosphorus and (4) sulfur.

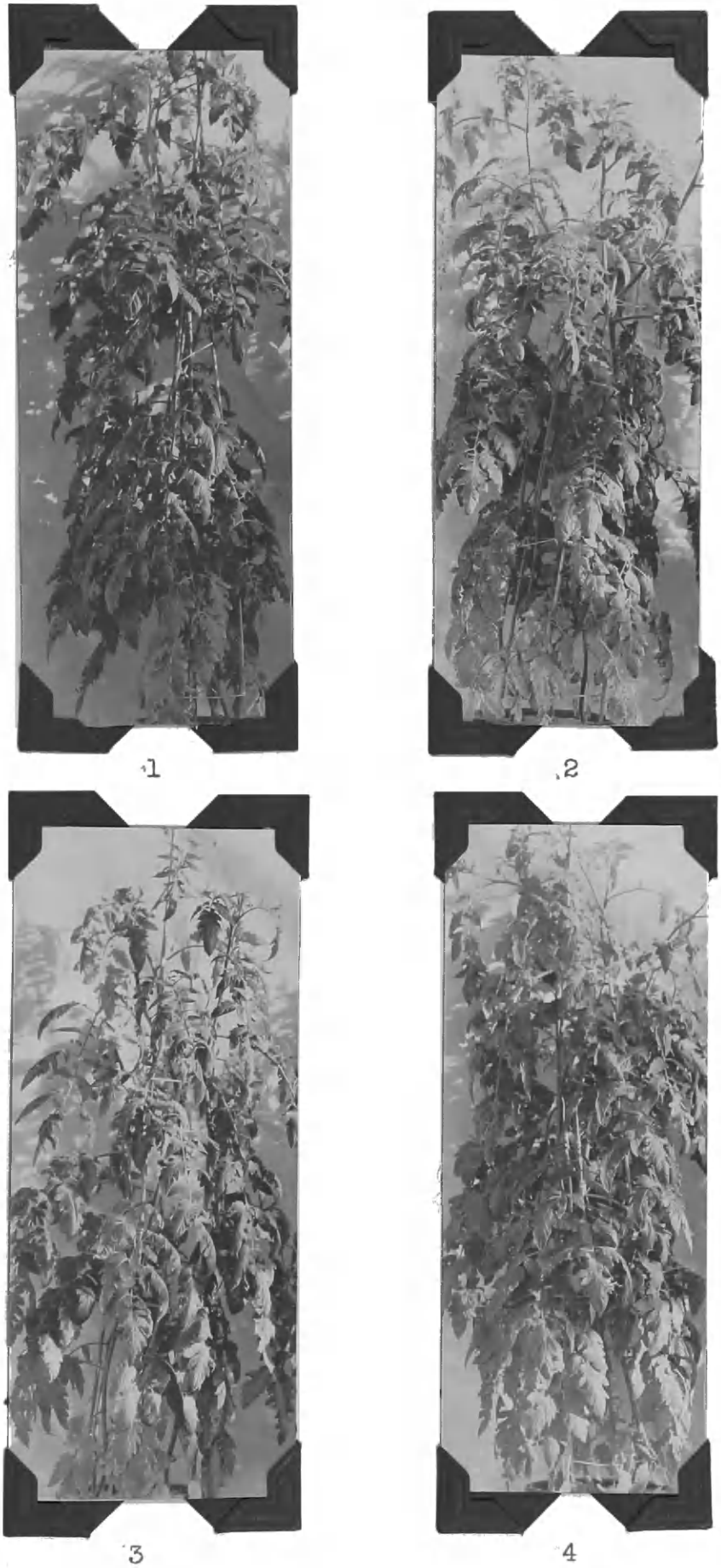

Fig.9--Growth attained by Marglobe plants in (1) a complete solution and in solutions with excesses of (2) boron, (3) calcium and (4) magnesium.

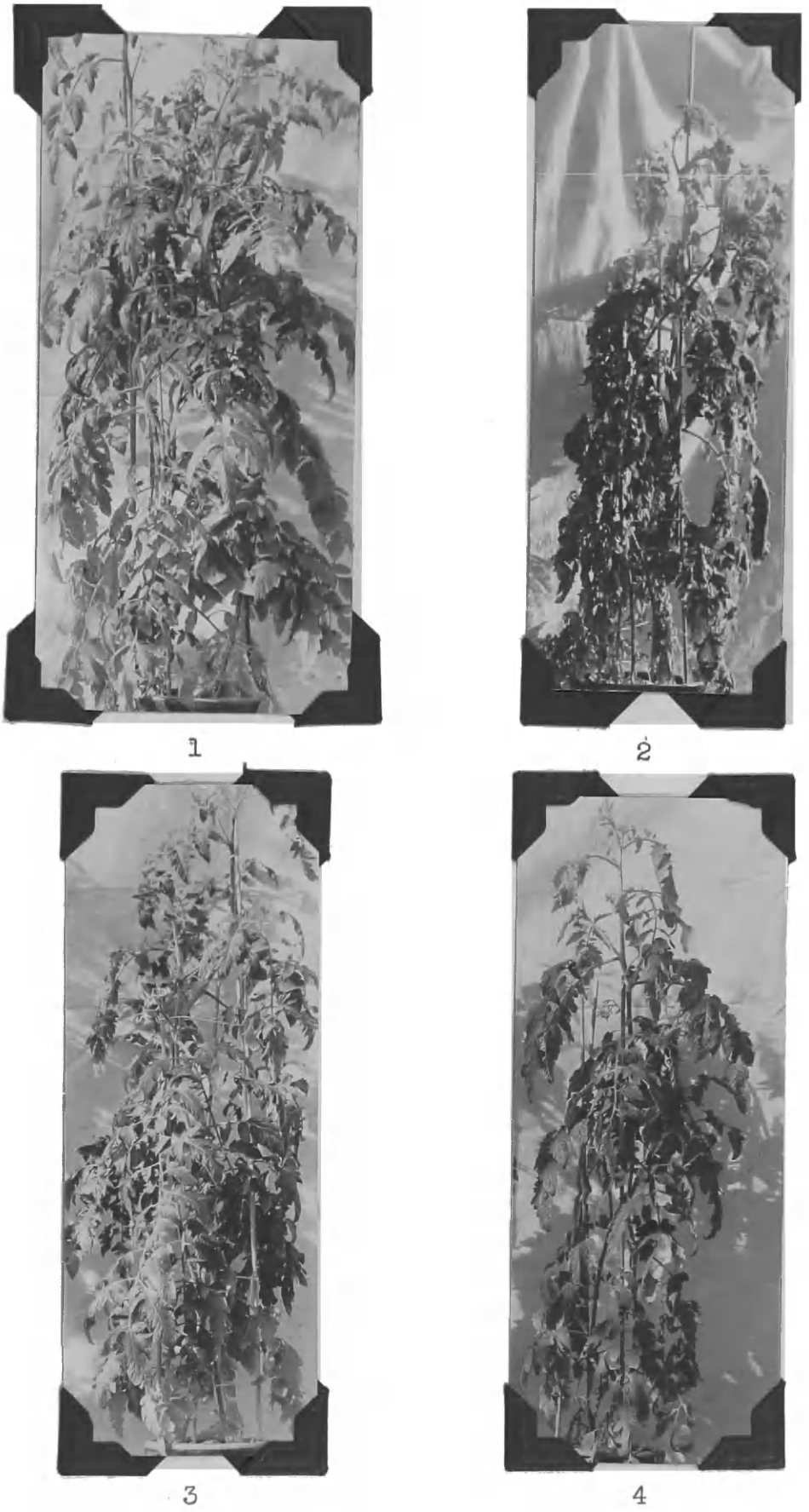

Fig. 10--Growth attained by Marglobe plants in solutions with excesses of (1) potassium, (2) nitrogen, (3) phosphorus and (4) sulfur.

LITERATURE CITED

1. Brenchley, W. F. Phosphate requirements of barley at different periods of growth. Am. Jr. of Bot. 43. 1929.

2. Gericke, W. F. Beneficial effect to plant growth of the temporary depletion of some of the essential elements in the soil. Sci. 59. 1924.

3. _____ Beneficial effects to wheat growth due to the depletion of available phosphorus in culture media. Sci. 60. 1924.

4. _____ On the adaptation of wheat to growth media deficient in nutrients. Sci. 58. 1923.

5. _____ On the physiological balance in nutrient solutions for plant cultures. Am. Jr. Bot. 9:180-182. 1922.

6. _____ Salt requirements of wheat at different phases of growth. Bot. Gaz. 80. 1925.

7. _____ Studies on the effect of nitrogen applied to oats at different periods of growth. Jr. Am. Soc. Agron. 14. 1922.

8. Heald, F. D. Manual of plant diseases. McGraw-Hill Company, New York. 1926.

9. Johnston, E. S. and W. H. Dore. The influence of boron on the chemical composition and growth of the tomato plant. Plt. Phys. 4:31-62. 1929.

10. _____ and P. L. Fisher. The essential nature of boron to the growth and fruiting of the tomato. Plt. Phys. 5:387-392. 1930.

11. Loew, Oscar The Physiological role of mineral nutrients. U.S.D.A. Div. Veg. Phys. and Path. Bul. 18. 1899.

12. McMurtrey, J. E. Distinctive effects of the deficiency of certain essential elements on the growth of tobacco plants in solution cultures. U.S.D.A. Tech. Bul. 340. 1933.

13. Newell, H. J. Range of boron concentrations in nutrient solutions for plants. Master's thesis deposited in library of University of Maryland. 1930.

14. Nightingale, Schermerhorn and Robbins. Some effects of potassium deficiency on the histological structure and nitrogenous and carbohydrate constituents of plants. N.J. Agr. Exp. Sta. Bul. 499. 1930.

15. Pember, F. R. Studies by means of both pot and solution cultures of the phosphorus and potassium requirements of the barley plant in different stages of growth. R. I. Agr. Exp. Sta. Bul. 169. 1917.

16. Russell, E. J. Soil Conditions and Plant Growth. Longmans, Green and Company, New York. 1915.

17. Raber, Oran. Textbook of general plant physiology. McMillan Company, New York. 1928.

18. Sorokin, H. and A. L. Sommer. Changes in the cells and tissues of root tips induced by the absence of calcium. Am. Jr. of Bot. 16. 1929.

19. Woods, A. F. Relation of nutrition to the health of the plants. U. S. D. A. Yearbook 1901:155-176. 1901.