NITROGEN, POTASSIUM AND CALCIUM IN RELATION TO FUSARIUM WILT OF MUSKMELON

By

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INTRODUCTION AND REVIEW OF LITERATURE

Fusarium wilt of muskmelon (*Cucumis melo* L.) caused by *Fusarium bulbigenum* (Cke. and Mass.) var. *niveum* Wr. f. 2 was first reported in 1930 by Chupp (3) in several fields in New York. Two years later it was reported by Leach (11) in Minnesota. The disease has been fully described by Leach and Currence (12) who indicate that it also occurs in Michigan, Arizona and Canada.

This disease was first discovered in Maryland about fifteen years ago, although it was not officially reported until 1940. At the present time it is the limiting factor in muskmelon production in Anne Arundel County. Wilt is also present on the Eastern Shore of Maryland, but a serious outbreak has not been noted.

The relation of nutrition to disease resistance has been the subject of extensive study, and there have been numerous instances in the literature where plant diseases have been partially controlled by the modification of fertilizer practices. A comprehensive review of this subject has been written by Wingard (26). Of particular interest are those papers pertaining to the relation of nutrition to the control of diseases caused by *Fusarium* species.

Fisher (7), in his study of Fusarium wilt of tomato, found that a heavy application of lime plus low nitrogen reduced the amount of invasion by the pathogen. Cook (4), working with the same disease, obtained similar results when a low nitrogen nutrient was supplied. Thomas and Mack (20) analyzed, by the foliar diagnosis technique, tomato plants grown on wilt-infested soil. Sampling before symptoms of the disease appeared, they found that those plants which remained healthy contained more potassium than did those plants which became diseased. They found, also, that plants which became diseased had a low calcium content. Sherwood (18), working with the same disease, observed that the highest percentage of wilt always occurred in the most acid soils in his experiments. He found that, in general, the percentage of wilt decreased as the H-ion concentration decreased until a pH of 7.4 was reached. Scott (17) noted that less Fusarium wilt of tomato occurred when the plants were grown at a pH of 6.4 to 7.0 and that more wilt appeared at more acid or more alkaline conditions.

Neal (15), Smith (19), Tisdale and Dick (21), and Young and Tharp (27) have reported that the application of high potash fertilizers, particularly to soils deficient in potassium, reduced the severity of wilt in cotton caused by _Fusarium vasinfectum_ Atk. The effect of various nitrogen levels was not significant (35, 21) although Young and Tharp (27) felt that the application of sufficient amounts of potash to balance the available nitrogen and phosphorus was highly important in controlling the cotton wilt disease.

Cabbage yellows caused by _Fusarium conglutinans_ Woll. has been studied by Walker and Hooker (22). These workers found that, when plants were grown in sand culture, reduction of the potassium level consistently increased the severity of the disease.

Various workers have demonstrated the fact that fungi which invade vascular tissue will produce phytotoxic substances, when grown _in vitro_ and presumably when growing in the host plant (1, 6, 9, 12, 24). Recently Gottlieb (8) has demonstrated the presence of a toxin in the trachea of tomato plants infected with _Fusarium bulbigenum_ var. _lycopersici_ (Brushi) Wr. and Wr. and R.).

The present study was undertaken to determine what influence various levels of nitrogen, potassium, and calcium nutrition have on the resistance of the muskmelon to Fusarium wilt, as well as to determine the relation of these nutritional levels to the host-parasite relationship. Certain relevant studies of possible toxin formation by the fungus *in vitro* have been included.

MATERIALS AND METHODS

Source of Seed.

The variety Bender's Surprise was used in all greenhouse experiments, and the Clarke variety, commonly planted in Anne Arundel County, in the field experiment.

Inoculum and Inoculation Technique.

The culture of Fusarium, A122-1-1A, used throughout the greenhouse and laboratory experiments, was the third successive single-spore isolate from a culture isolated by Dr. Mark W. Woods in July, 1938, from wilted muskmelon plants in Anne Arundel County. In the field experiment ground was selected which was naturally infested with the organism. Isolates from diseased plants obtained from the experimental plot closely resembled A122-1-1A in morphological and cultural characteristics.

The inoculum used in this study was prepared by growing the fungus on 100 c.c. of liquid Leonian's medium[1] in 250 c.c. Erlenmeyer flasks. When the fungus mat had covered the surface of the medium (approximately 15 days at 27° C.), the cultures were filtered, and the fungus mat was ground for one minute with 250 c.c. of tap water in a Waring Blendor. The suspension of spores and mycelial fragments was used without further treatment.

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[1]Leonian's medium: potassium monobasic phosphate 1.20 g., magnesium sulphate 0.60 g., peptone 0.60 g., maltose 6.25 g., malt extract 6.25 g., and water 1000.00 ml.

Culture and Inoculation of Plants in Greenhouse.

All greenhouse experiments were planned as randomized block designs. Spergon-treated seeds were sown directly into 2-gallon crocks of steam sterilized, quartz sand. In the first two experiments fine sand (predominantly of 40 mesh grade) was used. When it was proved that a coarse grade of sand (predominantly of 20 mesh grade) was superior for muskmelons (16), this grade was used in all subsequent experiments. After germination, the stand was thinned to the desired number of uniformly-sized plants. Nutrient solutions were started after the first true leaves appeared.

Nutrient solutions were made from molar concentrations of the salts indicated in Table I. Throughout this study the following elements were supplied at the constant rates indicated: calcium at 240 p.p.m., magnesium at 48 p.p.m., and the phosphate ion at 190 p.p.m.

TABLE I

Salts Used in Preparing Nutrient Solutions Supplied Muskmelon Plants in Sand Culture Experiments

Salt	Parts per million furnished by one c.c. of molar solution per liter of nutrient solution. Cation	Anion
$Ca(NO_3)_2 \cdot 4H_2O$	40.0	124.0
KH_2PO_4	39.0	95.0
$MgSO_4 \cdot 7H_2O$	24.0	96.0
$CaCl_2$	40.0	71.0
KCl	39.0	35.5
KNO_3	39.0	62.0
$Mg(NO_3)_2 \cdot 6H_2O$	24.0	124.0
$NaNO_3$	23.0	62.0

The solutions were supplemented by the addition of approximately 0.11 p.p.m. manganese, 0.02 p.p.m. zinc, 0.02 p.p.m. copper, and 0.12 p.p.m. boron. Iron was supplied as ferric tartrate at the rate of 1 c. c. of a 0.5% solution per liter of nutrient solution. All solutions were made fresh daily in tap water. They were applied to the crocks at the rate of 500 c.c. once a day. Every week crocks were thoroughly flushed with tap water to insure against building up a concentration of any element.

During periods of short day length (October to March), additional light was furnished by 300 watt bulbs suspended about two feet above the plants.

Inoculation of plants was accomplished by making a dibble hole in the sand adjacent to the tap root and pouring 15 c.c. of inoculum on the roots thus exposed.

Culture and Inoculation of Plants in Field.

The field experiment was conducted on the farm of Mr. Charles Pumphrey, Millersville, Maryland. Three years before its use in this experiment, the field was planted with muskmelons. According to the farmer, the crop had been a total loss because of wilt. After the crop failure, the field lay fallow for one year and then was sowed with vetch. The cover crop was turned under in March, and in mid-June the land was prepared for muskmelons. Soil samples were taken subsequent to the second plowing (Table II).

The design of this experiment was that of a split block with lime vs. no lime as the whole plot. Whole plots were replicated 5 times. Within each sub-plot were 2-row fertilizer plots, 30 feet long, allowing eight hills per plot, each hill containing two plants. Lime was applied broadcast prior to the final harrowing at the rate of 1500 pounds per acre. This

TABLE II

Results of Tests on Soil Taken from Field Plots*

Element	Amount Present - Pounds per Acre
Calcium	0-300
Magnesium	10-30
Aluminum	0-50
Iron	0-10
Nitrogen, nitrate	0-5
Nitrogen, ammonia	0-5
Phosphorus	50-100
Potassium	0-100
Manganese	0-3

pH value - 5.1 Per cent organic matter - 0.4

Soil type - sandy loam

*Analysis made by the Soils Department, University of Maryland.

amount was calculated to raise the pH to 6.0. The fertilizer formulas applied were 6-6-5 (normally used for muskmelons by the majority of farmers in Anne Arundel County), 4-8-8, 4-8-12, 4-8-16 and 4-8-20. The materials used in the mixtures appear in Table III. Fertilizer was applied in the bottom of the row at the rate of 800 pounds per acre.

Number 2 tin cans were used for the propagation of the muskmelon plants. This is common practice in the county when an early crop is desired. These cans were segregated into groups, each group receiving in the bottom of the can a small amount (approximately one-half teaspoon) of the fertilizer mixtures. The cans were then filled with a mixture of half bank sand and half compost, placed in a cold frame and seeded with four seeds. After germination, the stand of plants was thinned to two seedlings per can. After approximately 20 days, all of the young plants had at least two true leaves and were transplanted to the field. Fifty-four

TABLE III

Materials from Which the Fertilizer Formulas Used in the Field Experiment Were Derived

	Pounds Used per Ton of Mixture				
Material	6-6-5	4-8-8	4-8-12	4-8-16	4-8-20
Sterilized Tobacco Stems	50	50	50	50	50
Nitrogen Tankage	40	25	25	25	25
Sulfate of Ammonia	570	380	380	380	380
Superphosphate	600	800	800	470	235
Triple Superphosphate	—	—	—	140	240
H. G. Muriate Potash	170	270	400	535	670
Magnesium Limestone	570	475	345	400	400

days after transplanting, all plants were examined and rated for wilt. Representative samples of diseased and healthy plants were cultured for the causal agent.

Disease Criteria.

In the first greenhouse experiments the plants were rated as either dead or healthy. In subsequent experiments plants were rated according to a system developed for the field experiment. A description of the classes in this system appears in Table IV and diagrams of typical plants in some of the classes are shown in Figures 1, 2, 3 and 4. Within each classification consideration was given to the total amount of the plant affected including the extent to which fruit-bearing was prevented. Thus, a diseased plant which matured a marketable fruit on an apparently healthy runner received a slightly lower rating than a similarly diseased plant which matured no fruit. In the last greenhouse experiment the plants were cut at the ground line, and free hand sections were made one inch below the cotyledonary node. Disease ratings were made on the basis of the percentage of the cross section of the stem invaded by the fungus hyphae.

TABLE IV

System Used for Rating Plants Infected With the Fusarium Wilt Disease

Rating	Description
0 - 4	Healthy or showing slight to moderate discoloration of vascular system when stem is cut at the soil line.
5 - 9	One branch root necrotic and showing a reddish-brown discoloration. Discoloration extends as a streak up the tap root. Several drops of reddish-brown exudate on stem at first or second internode. Tip leaves of one runner slightly yellowed and cupped or runner nearly dead. Rest of plant of normal appearance.
10 - 14	One branch root necrotic and showing a reddish-brown discoloration. Discoloration extends as a streak up the tap root. Brown discolored streak on approximately half of main stem. Streak may also extend part way along branch stem. Leaves of stems showing this streak more or less yellowed and cupped. Several branch stems of normal appearance.
15 - 19	One or more branch roots necrotic and showing reddish-brown discoloration which extends up the tap root. Several brown discolored streaks on main stem and all branch stems except one. Leaves on remaining stems more or less yellowed and cupped.
20	Entire plant dead.

Chemical Analysis of Plants.

Analysis for nitrate content of the main stem of muskmelon plants was done according to the rapid method of Carolus (2).

Nutrition of the Fungus.

The fungus was grown in 100 c.c. Erlenmeyer flasks on potato dextrose agar at room temperature. After the fungus had covered the surface of the agar, a spore suspension was made with sterile tap water. One c.c. of this suspension was inoculated into 250 c.c. Erlenmeyer flasks containing autoclaved nutrient solutions. The nutrient solutions, which were the same as those applied to the muskmelon plants, comprised all combinations of nitrate at the following levels: 447 p.p.m., 620 p.p.m., 868 p.p.m., and 994 p.p.m.; and potassium at the following levels: 195 p.p.m., 273 p.p.m., 351 p.p.m., 429 p.p.m., and 507 p.p.m. Four replications were prepared. After ten days growth at 27° C., the fungus mats were filtered off, oven dried and weighed.

In the second experiment, a spore suspension was prepared as previously described. Nutrient solutions were prepared as before comprising all combinations of nitrate at levels of 100 p.p.m., 273 p.p.m., and 447 p.p.m., and potassium at levels of 78 p.p.m., 156 p.p.m., and 195 p.p.m. Three series of four replications each were prepared: Series 1, pH 3.3; Series 2, pH 6.0; and Series 3, pH 8.0. One extra flask was prepared for each series for initial pH determination. On the basis of the initial determination, Series 1 was adjusted to its proper pH with molar HCl; Series 2, with 0.5 molar HCl or 0.5 molar NaOH as necessary; and Series 3, with 0.5 molar NaOH. Series 1 was buffered with 0.8 c.c. molar NaH_2PO_4 and 0.2 c.c. molar NaCl; Series 2, with 0.4 c.c. molar NaH_2PO_4, 0.4 c.c. molar Na_2HPO_4, and 0.2 c.c. molar NaCl; Series 3, with 0.8 c.c. molar Na_2HPO_4 and 0.2 c.c. NaCl. After preparation of the solutions, each flask was inoculated with 1 c.c. of spore suspension and incubated for three days at 27° C. Then the first replication in each series was titrated, and the pH of all replications was adjusted to the initial pH level. At the end of six days total incubation, the second replication was titrated, and all replications were adjusted for pH. At the end of nine days incubation, the third replication was titrated, and all replications were adjusted for pH. After ten days total incubation, the fungus mats were filtered off, oven dried, and weighed.

Attempts to Demonstrate a Toxin.

300 c.c. Erlenmeyer flasks were filled with approximately 125 c.c. of Leonian's solution and inoculated with 3 c.c. of spore suspension. Three flasks were shaken continuously in a shaker for 96 hours; three, for 72 hours; and three, for 48 hours. Comparable sets of three flasks were incubated on the shelf for the same periods of time. All incubation took place at room temperature. Uninoculated checks were run in both shake and still culture for 96 hours. Following incubation, the fungus mats were filtered off, and the solutions were centrifuged at approximately 3500 r.p.m. for 5 minutes to remove any spores and mycelial fragments. Part of the solution from each of the 96-hour shake, 96-hour still, and check still flasks was given the following treatment to extract certain proteins that might be present: (1) add 30%, by weight, solid ammonium sulfate and filter off on celite, wash with 15 c.c. 30% ammonium sulfate; (2) elute with tap water and adjust to pH 7.0 with 0.1 normal KOH; (3) dialyze both eluent and filtrate against tap water for 12 hours. Fifteen c.c. from all the solutions at full and half strength plus the dialyzed eluents and filtrates from the above were put into small shell vials. Young muskmelon plants having three true leaves were separated under water from their root systems and placed in the vials for 48 hours.

In the next experiment, inoculated flasks of Leonian's solution were incubated at room temperature for 96 hours in shake and in still culture. After the fungus had been filtered off, the solutions were centrifuged for 10 minutes and dialyzed for 12 hours. Extractions of the dialyzed solutions with 95% ethyl alcohol and ethyl ether were made. Young plants were tested in full and half strength dialyzed solutions as before.

In another experiment, inoculated flasks of Wellman's Differential Solution[1], Tochinai's Solution[2], and Leonian's Solution were prepared and inoculated with 3 c.c. of spore suspension. 125 grams of healthy young muskmelon tissue were washed thoroughly in sterile water and ground in a Waring Blendor with 500 c.c. of sterile water. The resulting suspension was filtered through paper, put in flasks, and immediately inoculated with 9 c.c. of spore suspension. A regular nutrient solution containing 447 p.p.m. of nitrate and 195 p.p.m. of potassium plus 5% sucrose was prepared and inoculated with 3 c.c. of spore suspension. All of the foregoing solutions were incubated in both shake and still culture at room temperature for 96 hours. Uninoculated check solutions were incubated in the same manner except for the muskmelon tissue extract which was made at the end of the 96 hour incubation period. After incubation, all solutions were filtered and centrifuged at approximately 3500 r.p.m. for 10 minutes and put into shell vials at full and half strength. Young plants from which the roots had been excised under water were placed in the vials for 48 hours.

The Fusarium was incubated in Leonian's Solution in still culture at room temperature for thirty days. The filtrate was prepared and tested in the manner previously described in dilutions of one-half, one-fourth and one-eighth original strength. Comparable uninoculated check solutions were included in the experiment.

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[1]Wellman's Differential Solution: proteose peptone 5.0 g., dihydrogen potassium phosphate 0.5 g., magnesium sulphate 0.5 g., maltose 15.0 g., ferrous sulphate 0.03 g., water 1000 c.c.

[2]Tochinai Solution: peptone 10.0 g., monopotassium phosphate 0.5 g., magnesium sulphate 0.25 g., maltose 20.0 g., and water 1000.0 c.c.

RESULTS

Relation of Nutrition to Susceptibility

Greenhouse Studies

The purpose of the preliminary tests was to find out the nutrient balance necessary for optimal growth of muskmelon plants in quartz sand and to ascertain the differences in nitrate content of main stems of plants grown on varying levels of nitrogen and potassium. The growth measurements have been recorded in Table V. It is apparent from the totals that there was no significant increase in growth after a level of 447 p.p.m. of nitrate was reached. Potassium alone had no effect on total growth.

TABLE V

Total Growth in Inches of Muskmelon Plants Grown in Quartz Sand for 60 Days and Supplied With Nutrient Solutions Containing Various Levels of Nitrate and Potassium.

P.P.M. Nitrate	P.P.M. Potassium 78	117	156	195	Total Growth
100	185a/	192	189	196	762
273	234	263	253	235	985
447	273	336	346	272	1227
620	276	383	332	250	1241
744	335	366	355	323	1379
Total Growth	1303	1540	1475	1276	

L.S.D. at 1% point between nitrate totals - 292
L.S.D. at 5% point between nitrate totals - 214
L.S.D. at 5% point between basic figures - 135

a/ Basic figures in table represent total growth of 4 plants.

Within the 117 and 156 p.p.m. levels of nitrate, plants receiving 447, 620, and 744 p.p.m. of nitrate made significantly more growth than did those receiving 100 p.p.m. of nitrate. At 78 p.p.m. of potassium no significant increase in growth occurred until the 744 p.p.m. of nitrate was reached. No significant increases occurred in the 195 p.p.m. of potassium series. At 447 p.p.m. of nitrate and above, regardless of the level of potassium, the plants had a healthy green appearance. At concentrations of nitrate below 447 p.p.m., the plants appeared to be nitrogen deficient because of their light green appearance.

The nitrate analyses are shown in Table VI. The amount of nitrate in the stem tissue analyzed was highly correlated with the amount of nitrate present in the nutrient solution. At the lowest level of potassium there was significantly more nitrate than at any of the higher levels. Between the potassium levels of 117, 156, and 195 p.p.m. there was no significance.

TABLE VI

Average Amount of Nitrate in Stem Tissue of Muskmelon Plants Grown in Quartz Sand for 60 Days and Supplied with Nutrient Solutions Containing Various Levels Nitrate and Potassium.

P.P.M. Nitrate	P.P.M. Potassium 78	117	156	195	Mean P.P.M. Nitrate Per Plant
273	21.5a/	22.3	21.4	23.3	22.1
447	39.0	28.0	39.0	32.8	34.7
620	38.6	38.0	37.4	40.3	38.6
744	51.1	38.9	38.5	40.3	42.2
Mean P.P.M. nitrate per plant	37.6	31.8	34.1	34.2	

L.S.D. at 1% point between means - 4.5
L.S.D. at 5% point between means - 3.4
L.S.D. at 5% point for basic figures - 7.0

a/ Basic figures in table represent average of 8 plants. Original reading for each plant was coded before analysis to represent 0.01 of actual p.p.m. present.

TABLE VII

Correlation Between the Amount of Nitrate in the Nutrient Solution and the Number of Muskmelon Plants Killed by Fusarium Wilt.

P.P.M. Nitrate	Number of Plants Killed
100	10
275	12
447	17
620	25
744	26

r - 0.977**

** Significant at the 1% point.

Figure 5 shows the results of the first experiment in which the plants were inoculated with the *Fusarium*. At the lower levels of nitrate, potassium exerted a pronounced effect on the number of plants which were killed by the fungus. Nitrate levels of 620 and 744 p.p.m. were apparently too high to be influenced by potassium. It was found that if nitrate was considered alone, there was a highly significant correlation (Table VII) between the amount of nitrate furnished and the total number of plants killed by the fungus. Such a correlation was significant at the 5% point when potassium was similarly considered (Table VIII). The symptoms displayed by the diseased plants in this experiment were typical of Fusarium wilt in all respects.

When the preceding experiment was duplicated, the lower levels of nitrate (100 and 272 p.p.m.) and of potassium (78 and 117 p.p.m.) were omitted since experience aad shown that plants grown under these

TABLE VIII

Correlation Between the Amount of Nitrate in the Nutrient Solution and the Number of Muskmelon Plants Killed by Fusarium Wilt.

P.P.M. Potassium	Number of Plants Killed
78	28
117	24
156	19
195	19
r - .948*	

* Significant at the 5% point.

nutritional conditions did not make normal growth. Two new levels of nitrate were added, 868 and 992 p.p.m., and four new levels of potassium, 273, 351, 429, and 507 p.p.m. The new potassium series was used in the hope that results comparable to the first experiment might be obtained at levels of nitrate where the plants made normal growth. Table IX shows the count of dead plants 30 days after inoculation. There was no correlation between either nitrate or potassium levels and the total mortality.

TABLE IX

Number of Plants Killed by Fusarium Wilt 30 Days After Inoculation. Plants Grown in Quartz Sand Under Various Levels of Nitrate and Potassium.

P.P.M. Nitrate	P.P.M. Potassium					Total Plants Dead
	195	273	351	429	507	
447	4	5	2	4	5	20
620	8	4	3	7	5	27
744	4	7	5	3	4	23
868	5	4	4	5	2	20
992	5	6	6	6	5	28
Total Plants Dead	26	26	20	25	21	

It might be significant to note that this experiment was performed during the winter, whereas all other similar experiments were done during the spring and summer months.

The following summer another experiment was started. This time levels of nitrate and potassium were used which covered the ranges established by the preceding experiments (nitrate - 100, 447, 744, and 992 p.p.m.; potassium - 78, 195, 351, and 507 p.p.m.). Thirty days after inoculation the plants were pulled from the sand and rated according to the system developed for the field experiment (Table IV and Figures 1 to 4).. The results which appear in Table X show a highly significant difference existing between the 100 p.p.m. and the 447, 744, 992 p.p.m. nitrate levels but no significance for potassium effects or for the interaction between nitrate and

TABLE X

Effect of Inoculation With Fusarium Wilt Organism on Plants Grown in Quartz Sand Under Various Levels of Nitrate and Potassium.

P.P.M. Nitrate	P.P.M. Potassium 78	195	351	507	Mean Disease Rating Per Plant
100	7a/	60	80	85	5.8
447	115	97	118	127	11.4
744	136	132	77	122	11.7
992	112	100	80	140	10.8
Mean Disease Rating per Plant	9.3	9.7	8.9	11.9	

L.S.D. at 1% point between means - 4.2
L.S.D. at 5% point between means - 2.2

a/ Basic figures represent cumulative rating for 10 plants.

potassium. Cultures were made from all plants in the experiment irrespective of disease rating. *Fusarium* was isolated from every plant. The cultures obtained were classified into eight different groups according to gross morphological differences. Representative strains from each group were increased and tested for pathogenicity on young muskmelon seedlings. All strains were pathogenic except one group which was isolated from two plants out of the 160 plants cultured.

Since it had been shown in the previous experiment that even apparently healthy plants were invaded by the Fusarium wilt fungus, an experiment was set up to determine the extent to which plants would be invaded when grown under different nutritional levels. The data (Table XI) shows a highly significant difference between the 100 p.p.m. level of nitrate and the 447 and 744 p.p.m. levels. There was no significance among any of the levels of potassium nor were there any significant effects arising from the interaction of nitrate and potassium.

TABLE XI

Cumulative Percent Invasion by the Fusarium Wilt Fungus of the Hypocotyl of Plants Grown on Various Levels of Nitrate and Potassium.

P.P.M. Nitrate	P.P.M. Potassium 78	195	273	Total Percent[a] Invasion
100	23	44	116	183
447	139	843	725	1707
744	731	723	858	2312
Total Percent[a] Invasion	893	1610	1699	

L.S.D. at 1% point between totals - 1468
L.S.D. at 5% point between totals - 1074

a/ Represents total of 54 plants.

Field Experiment

Preparatory to inaugurating field experiments, most of Anne Arundel County was surveyed to determine the extent of the disease, interview prospective cooperators, and ascertain the cultural procedures common to the farms growing muskmelons. The fertilizer ratio most commonly used by the eleven growers interviewed was 6-6-5. On the majority of the farms, lime was seldom used and most of the soil tested was in the pH range of 4.5 to 5.5. In one field visited the apparent result of soil acidity was striking (Figure 6). This field was divided into three definite sections: (1) all plants healthy, soil pH of 5.5, (2) 95% of the plants dead from Fusarium wilt, soil pH 4.1, and (3) 50% of the plants dead or severely affected with Fusarium wilt, soil pH of 4.8. In many other fields visited, calcium and magnesium deficiency symptoms were noted. Because of these observations, it was decided to include in the field experiment limed plots in which the pH would be raised to 6.0, a level consistent with that recommended for muskmelon culture (7, 23).

The data obtained (Table XII) showed highly significant differences between the limed and unlimed plots. There were no interacting affects between lime and any of the fertilizer ratios used. The difference between

TABLE XII

Amount of Fusarium Wilt in Muskmelon Plants Grown in Wilt-Infested Soil Modified by Lime and Various Fertilizer Ratios.

	Fertilizer					Mean Disease Rating per Plant
	6-6-5	4-8-8	4-8-12	4-8-16	4-8-20	
Lime	29.4[a]	21.1	16.8	16.7	14.8	3.95
No lime	43.8	25.8	24.7	29.8	16.7	5.63
Mean Disease Rating per Plant	7.32	4.69	4.15	4.65	3.15	

L.S.D. at 1% point between means for lime - 0.83
L.S.D. at 1% point between means for fertilizers - 1.65
L.S.D. at 5% point between means for fertilizers - 1.25

a/ Basic figures in table are cumulative averages of 5 plots.

the check fertilizer, 6-6-5, and the other ratios was highly significant; there was no significance within the 4% nitrogen series except that the 4-8-20 ratio was significantly better at the 5% point than the ratios 4-8-8 and 4-8-16. Since this is based on only one year's results, the superiority of the 4-8-20 ratio may be more apparent than real.

Effect of Nutrient Solutions on Growth of Fungus in Vitro.

In the nutrient solutions, the growth of the *Fusarium* was chiefly correlated with the amount of nitrate present. The differences over the nitrate range were highly significant (Table XIII). Within the levels of potassium, 351 and 429 p.p.m. of potassium produced significantly (at 1% point) less growth than did the 273 p.p.m. level. There was no difference in growth among solutions containing 195, 273, and 507 p.p.m. of potassium.

TABLE XIII

Weights of Fusarium Wilt Fungus Mats After 10 Days Growth in Nutrient Solutions Containing Various Amounts of Nitrate and Potassium.

P.P.M. Nitrate	P.P.M. Potassium					Mean Weight (mg.) Per Flask
	195	273	351	429	507	
447	345a/	405	343	388	413	94.7
620	494	560	524	559	499	131.8
744	629	672	558	527	580	148.3
868	684	721	628	624	686	167.1
994	765	745	789	672	810	189.0
Mean Weight (mg.) Per Flask	145.8	155.1	142.1	138.5	149.4	

L.S.D. at 1% point between means - 12.1
L.S.D. at 5% point between means - 9.4

a/ Basic figures in table represent total weight of 4 flasks.

The total weights in milligrams of the Fusarium grown at three levels of pH was 1244 mg. at pH 3.3, 274 mg. at pH 6.0, and 585 mg. at pH 8.0. The L.S.D. at the 1% point for these total weights was calculated to be 124.4 mg. Thus, the difference in weights between all three levels was highly significant. The complete analysis of variance of this experiment appears in Table XIV. It should be noted that the variance for pH levels is very large. In order to more fully evaluate the effects of pH on the growth of the fungus in the various levels of nitrate and potassium, the figures within each pH series were analyzed separately. The data are presented in Tables XV, XVI, AND XVII. In these tables the effects of pH on growth at different levels of nitrate and potassium become clear. At pH 3.3 there was a highly significant increase in growth of the fungus with each increase in nitrate levels. At pH 6.0 no significant increase in growth occurred until the highest level of nitrate was attained, whereas at pH 8.0 there were no significant differences in total growth between

TABLE XIV

Analysis of Variance of Experiment in Which the Fusarium Wilt Organism was Grown for Ten Days Under Various Levels of Nitrate and Potassium Within Each of Three pH Series.

Source of Variation	Degrees of Freedom	Variance	"F" Value
Total	107		
Nitrate	2	1714.75	52.940**
Potassium	2	5.50	0.170
pH	2	6814.35	210.384**
NO_3 x K	4	52.62	1.613
NO_3 x pH	4	164.02	5.064**
K x pH	4	129.07	3.985**
NO_3 x K x pH	8	241.24	7.448**
Error	81	32.39	

** Denotes significance at the 1% point.

TABLE XV

Growth of the Fusarium Wilt Fungus at pH 3.3 Under Various Levels of Nitrate and Potassium.

P.P.M. Nitrate	P.P.M. Potassium			Mean Weight (mg.) per Flask
	78	156	195	
100	99a/	103	78	23.3
273	160	159	122	36.7
447	185	167	171	43.6
Mean Weight (mg.) per flask	37.0	35.7	30.9	

L.S.D. at 1% point between means - 6.8
L.S.D. at 5% point between means - 5.0

a/ Basic figures in table represent total weight from 4 flasks.

TABLE XVI

Growth of the Fusarium Wilt Fungus at pH 6.0 Under Various Levels of Nitrate and Potassium.

P.P.M. Nitrate	P.P.M. Potassium			Mean Weight (mg.) per Flask
	78	156	195	
100	-5a/	9	17	1.8
273	10	11	37	4.8
447	56	94	45	16.3
Mean Weight (mg.) per flask	5.1	9.5	8.3	

L.S.D. at 1% point between means - 9.2
L.S.D. at 5% point between means - 6.8

a/ Basic figures represent total weight from 4 flasks.

any of the nitrate levels. When the pH was held at 3.3, solutions containing 78 p.p.m. of potassium produced significantly (at 5% point) more growth than did solutions containing 195 p.p.m. of potassium. When the fungus was grown at pH 6.0 and at pH 8.0, potassium levels had no effect on total growth.

TABLE XVII

Growth of the Fusarium Wilt Fungus at pH 8.0 Under Various Levels of Nitrate and Potassium.

P.P.M. Nitrate	P.P.M. Potassium 78	156	196	Mean Weight (mg.) per Flask
100	57a/	26	79	13.5
273	74	61	73	17.3
447	78	73	64	17.9
Mean Weight (mg.) per flask	17.4	13.3	18.0	

a/ Basic figures represent total weights from 4 flasks.

Toxin Production by the Fusarium Wilt Fungus in Vitro.

All of the experiments failed to demonstrate the formation of a toxin by the Fusarium in vitro. The localized chlorosis and necrosis which sometimes appeared on leaves in filtrates from fungus solutions was no worse than on leaves in check solutions. There were instances when the fungus apparently reduced the inherent toxicity of the culture solution. This is clearly shown in Table XVIII and Figures 7 and 8. Extraction with 95% ethyl alcohol and with ethyl ether failed to reveal any substance not present in the check solutions.

TABLE XVIII

Effect on Excised Tops of Young Muskmelon Plants After 24 Hours in Filtrate from 30-Day Old Culture of Fusarium bulbigenum var. niveum f. 2

Solution	Dilution	Number Plants Turgid	Number Plants Wilting
Culture filtrate	1/2	7	3
Sterile filtrate	1/2	2	8
Culture filtrate	1/4	8	2
Sterile filtrate	1/4	5	5
Culture filtrate	1/8	10	0
Sterile filtrate	1/8	5	5
Tap water		9	1

DISCUSSION

Throughout the course of this study, a striking relationship between nitrogen nutrition and the behavior of Fusarium bulbigenum var. niveum f. 2 has been observed. In all of the greenhouse inoculation experiments, except one, an increase of nitrogen in the nutrient solution resulted in a significantly greater invasion of the plant by the fungus and a large number of plants killed. There is little evidence that potassium plays any part in inducing resistance to the disease when nitrogen and potassium are balanced and in amounts sufficient for normal growth of the muskmelon plant. In the field experiment the highly significant reduction in the amount of disease occurred between the 6-6-5 and the 4-8-8, 4-8-12, 4-8-16, and 4-8-20 fertilizer ratios. Within the 4% nitrogen ratios there were no highly significant differences. It seems probable, therefore, that the field results can be attributed mainly to the reduction of the percentage of nitrogen in the ratios rather than to an increased percentage of potassium.

The nutrition studies of the Fusarium in vitro indicate that nitrogen is the important element governing growth of the fungus. Cox (5) in his studies on the host-parasite relationship of muskmelon wilt found that the fungus enters the roots and inhabits the xylem primarily during the early stages of the disease, although, other tissues are invaded later. It appears, then, that the Fusarium must depend largely, at least in the early stages of infection, on elements in the xylem stream for its nutrition. Thus, it is possible that an abundance of soluble forms of nitrogen in the xylem could influence the extent of invasion.

The possibility that an abundance of nitrogen makes host cells more susceptible to invasion by the parasite cannot be overlooked. Host cells might also be rendered more susceptible to toxic substances or by-products of metabolism which might be produced by the fungus or by an interaction between the fungal hyphae and the host cells.

During the course of this study, responses in wilt-diseased plants indicated that toxins were produced. Figure 9 shows evidence of this particularly in the severely affected leaf where microscopic investigation of the veins, mesophyll, and petiole showed no fungal hyphae or spores present. Originally, it was planned to test the effect of toxin produced by the fungus *in vitro* on excised plants which had been grown on different levels of nitrogen and potassium. Since, under the experimental conditions recorded here, a detectable toxin was not obtained it is felt that the initiation of toxin formation may depend on some substance found only in living plant cells; or the fungus may produce in the plant a non-toxic principle which in turn initiates an irreversable toxic reaction in the plant cells. Whatever the end product is, it must be diffusable through the plant in order to account for the leaf symptoms which are characteristic of the disease.

The effects of pH on the amount of disease present in the field experiment parallels the work of Sherwood (18) and Scott (17) who obtained similar results working with tomato wilt caused by *Fusarium lycopersici*. The field results with lime are also correlated with the data obtained in the laboratory where the fungus *in vitro* made the poorest growth when held at a pH of 6.0. Since there was no significant interaction between lime and any of the fertilizer ratios, it is possible that the lowered disease index in the limed plots resulted from inhibiting the growth of the fungus in the soil or in lowering its virulence. It is recognized, however, that muskmelons grow best when the soil reaction is slightly acid or neutral (10,23). That muskmelons have a high calcium requirement is also well known (14, 25). Some of the beneficial effects of lime, therefore, may have come from providing the plants with soil conditions that were more nearly optimum.

SUMMARY

The observations obtained from greenhouse and field experiments indicate that high levels of nitrogen result in a significantly greater invasion of muskmelon plants by the pathogen (*Fusarium bulbigenum* (Cke. and Mass.) var. *niveum* Wr. f. 2) and a larger number of plants killed. There was little evidence that potassium plays any part in inducing resistance to the disease when nitrogen and potassium are balanced and in amounts sufficient for normal growth of the muskmelon plant. *In vitro* the pathogen made more total growth as the nitrogen content of the medium was increased; however, little correlation was observed between total growth and potassium supply.

The addition of lime to the soil in an amount sufficient to raise the soil pH to 6.0 significantly reduced the amount of disease in the field plots. Part of the reduction may be attributable to the change in pH since, *in vitro*, the growth of the pathogen was drastically inhibited when the pH of the medium was held at 6.0.

The experimental procedures followed in this investigation failed to demonstrate the formation of a toxin by the pathogen *in vitro*.

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Figure 1

Diagram of a muskmelon plant infected with Fusarium wilt and given a disease rating of 7.

Figure 2

Diagram of a muskmelon plant infected with Fusarium wilt and given a disease rating of 13.

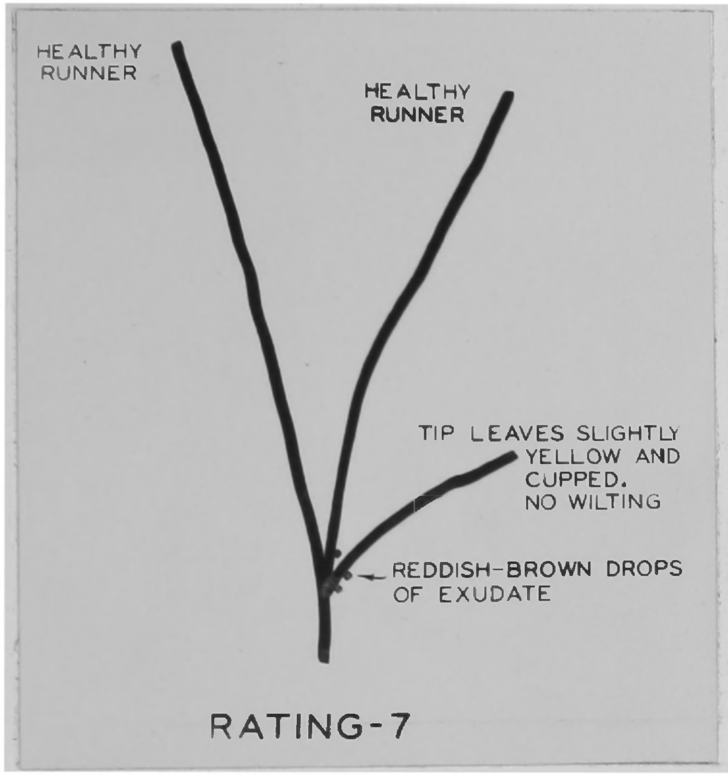

FIGURE 1

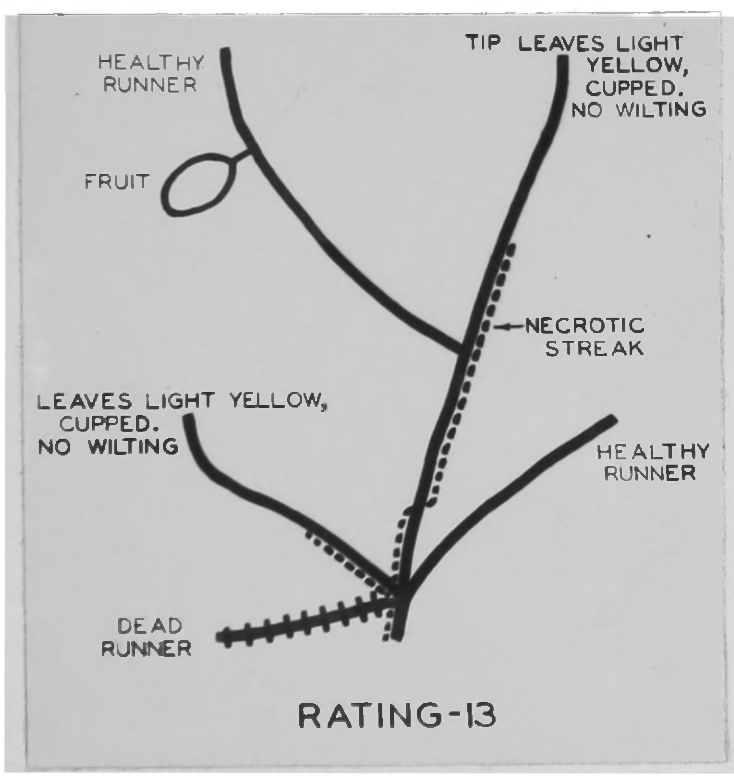

FIGURE 2

Figure 3

Diagram of a muskmelon plant infected with Fusarium wilt and given a disease rating of 17.

Figure 4

Diagram of a muskmelon plant infected with Fusarium wilt and given a disease rating of 19.

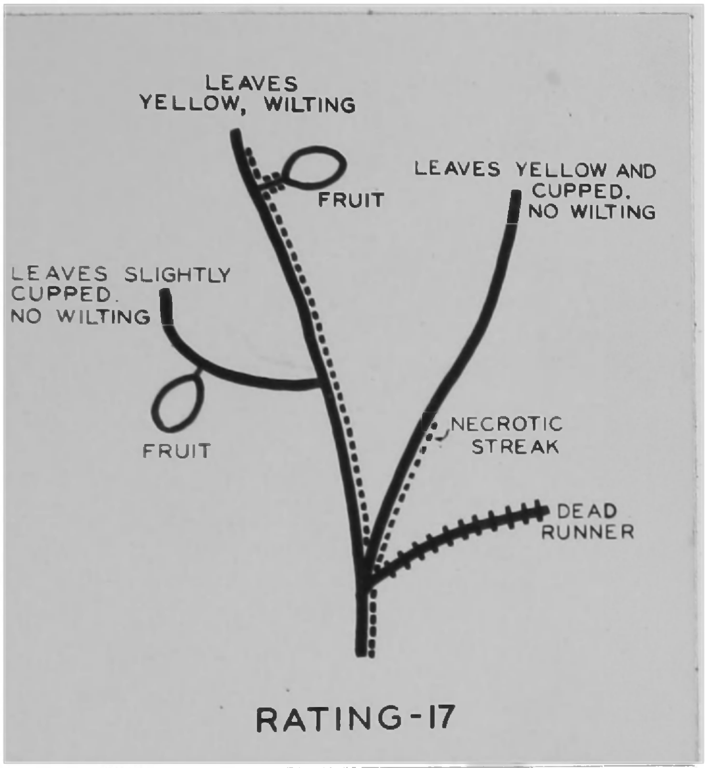

FIGURE 3

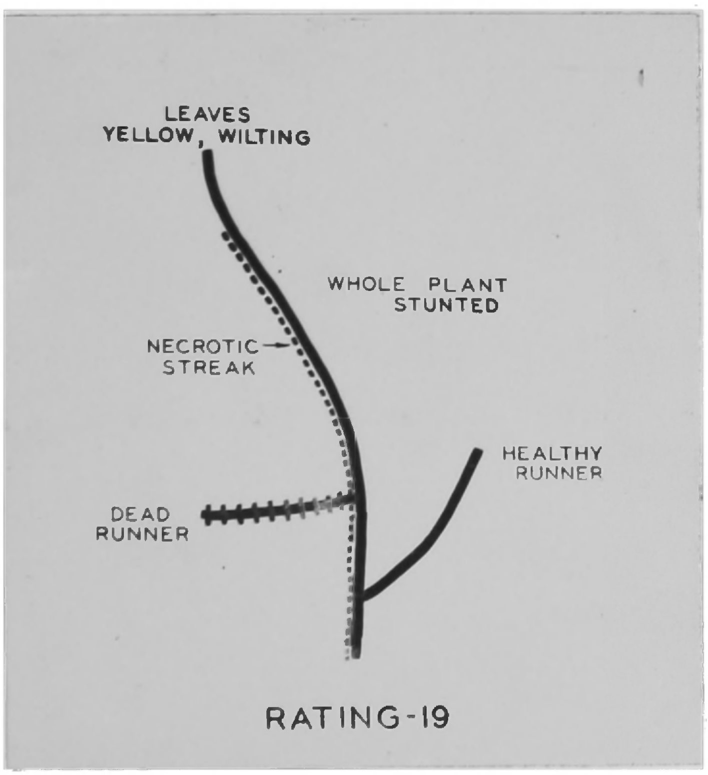

FIGURE 4

Figure 5

Results of greenhouse experiment in which muskmelon plants, grown on various nitrate and potassium levels, were inoculated with *Fusarium bulbigenum* var. *niveum* f. 2.

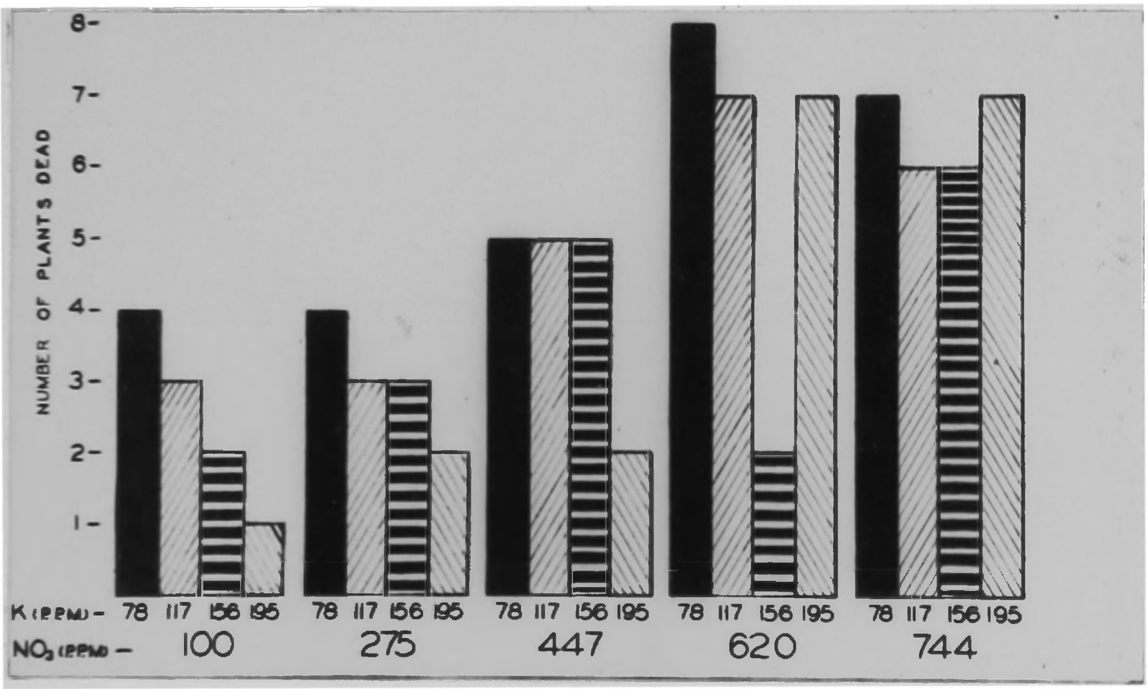
8-
7-
6-
5-
4-
3-
2-
1-
NUMBER OF PLANTS DEAD
K (PPM) - 78 117 156 195 78 117 156 195 78 117 156 195 78 117 156 195 78 117 156 195
NO_3 (PPM) - 100 275 447 620 744

FIGURE 5

Figure 6

Muskmelon field in which Fusarium wilt was present. The soil in the left foreground where 50% of the plants were wilt-infected had a pH of 4.8. In the center where 95% of the plants were dead from wilt the soil pH was 4.1. In the right background where all plants were apparently healthy the soil pH was 5.5.

FIGURE 6

Figure 7

Leaves from excised muskmelon plants which had been held in Leonian's solution for 72 hours. Leaf on right from tap water check plant.

Figure 8

Leaves from excised muskmelon plants which had been held for 72 hours in a filtrate from a *Fusarium* culture grown in Leonian's solution for 96 hours in shake culture. Leaf on right from tap water check.

FIGURE 7

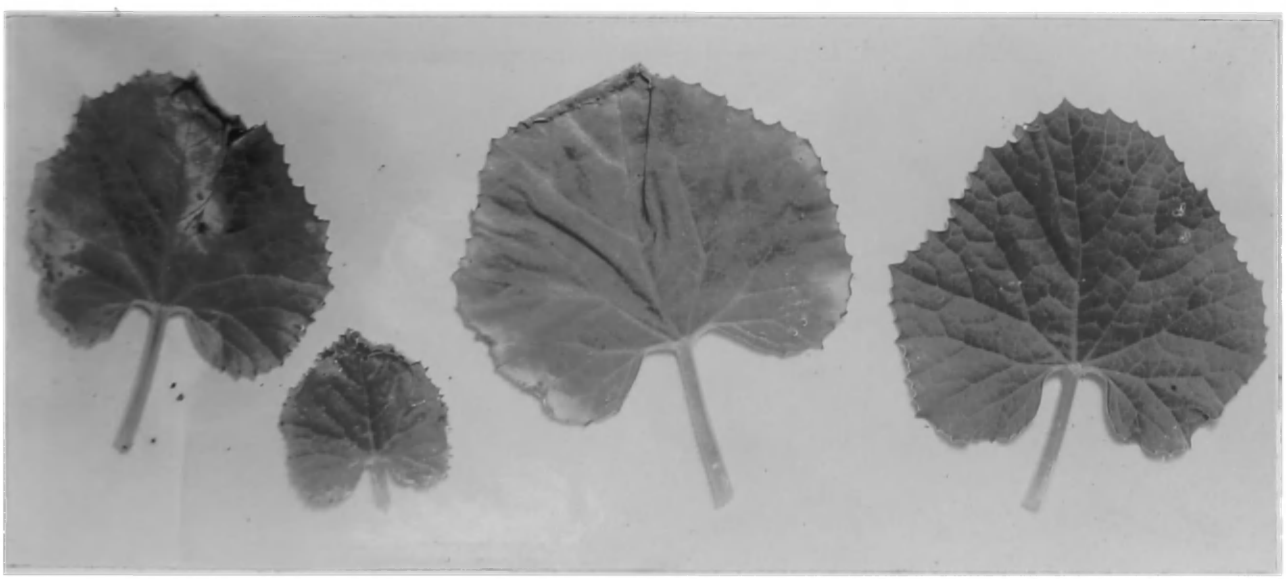

FIGURE 8

Figure 9

Leaves from muskmelon plant invaded by *Fusarium bulbigenum* var. *niveum* f. 2. Leaf at lower right was closest to base of plant. Microscopic examination of this leaf showed no hyphae or spores of the pathogen present. Leaf from healthy plant shown in upper left corner. Photograph taken by transmitted light after all leaves had been soaked in distilled water for 12 hours.

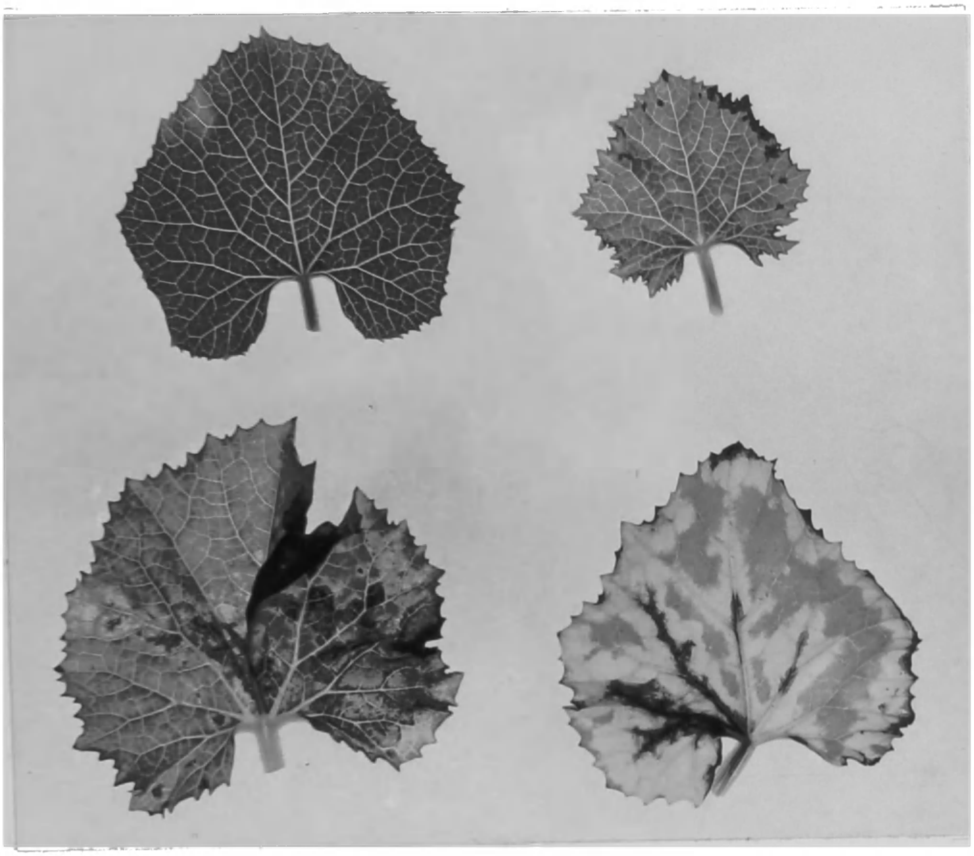

FIGURE 9