STUDIES ON MUSKMELON WILT,
CAUSED BY <u>FUSARIUM BULBIGENUM</u>
CKE. AND MASS. VAR.
<u>NIVEUM</u> WR. F. 2.

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STUDIES ON MUSKMELON WILT, CAUSED BY *FUSARIUM BULBIGENUM* CKE. AND MASS. VAR. *NIVEUM* WR. F. 2.

by

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INTRODUCTION

Muskmelons have been produced for several years as a profitable crop in California, Arizona, Colorado, Texas, Michigan, Maryland, and many other states. Within recent years, the muskmelon acreage has increased somewhat rapidly and the season of consumption has become longer. The shipping season in commercial regions begins about the first of May with shipments from the Imperial Valley of California and some sections of Texas and Florida. The peak of carlot shipments is reached in June and July with late shipments from Michigan, Indiana, parts of Colorado and California. According to statistics compiled by the Bureau of Agricultural Economics the total acreage of commercial muskmelon production for the United States in 1938 was 123,250, with an approximate yield of 15,103,000 crates.

Michigan ranks sixth in production of muskmelons representing a crop income of approximately $717,000 annually. In the past the growing of muskmelons as a part of a systematic crop rotation tended to minimize the injurious effects of diseases. More recently, due to increased intensive cultivation, and a lack of available land for rotation, diseases have become limiting factors in muskmelon production. In the intensive muskmelon growing areas in southwestern Michigan losses of 25 to 65 per cent of the crop have been reported from leaf spot or blight. Anthracnose and mildews have been limiting factors in other production areas. Among these serious diseases is a comparatively new one known as Fusarium wilt. Because of its apparent rapid and wide dissemination, and a lack of effective control measures, other than by growing resistant varieties, its further investigation is desirable.

History of the Disease

Fusarium wilt of muskmelons is a relatively new disease. It was first described in detail by Leach and Currence (1) in 1938.

In 1898 Sturgis (2) observed a Fusarium fruiting on the stems of a wilted muskmelon in a field where bacterial wilt was prevalent. He made no inoculations, but suggested that it might be in part responsible for the very prevalent wilt diseases of muskmelons. He states that the organism was a species of Fusarium possibly the same as the one associated with wilt of watermelons.

In the same year Selby (3) reported a wilt of cucumbers and muskmelons and stated that it appeared "to be due to the same or to a similar fungus to that causing the southern watermelon wilt described by E. F. Smith." He made no inoculations and did not study it further.

Stone (4) in 1911 described a Fusarium stem rot of cucumbers and muskmelons in the greenhouse but did not identify the pathogen.

In 1920 Taubenhaus (5) published a report on "Wilts of the Watermelon and Related Crops," but no reference is made to a Fusarium wilt of muskmelons.

A serious disease of muskmelons in France was described by Dufrènoy (6) in 1921. According to the author the disease appears to be due to a Fusarium which he identifies as _Fusarium solani_ var. _cyanum_ sub. var. (?). His description of the symptoms and studies of the organism indicate that this disease was probably the same as the disease in question.

Cook (7) in 1923 called attention to a disease of muskmelons in the greenhouse. Symptoms appeared as brown streaks on the stems from which he isolated a pure culture of a Fusarium, "very similar to _Fusarium vasinfectum_." No inoculations were made to determine its pathogenicity.

Wollenweber (8) isolated a Fusarium from muskmelon fruits in Canada, but no wilting of plants was observed.

Fusarium wilt of muskmelons was reported from New York in 1930 and 1931 by Chupp (9), from Minnesota by Leach (10) in 1931 and was first found in Berrien County Michigan in 1936.

Distribution

The Fusarium wilt of muskmelons is reported as spreading and becoming increasingly destructive in several states. In 1937 the disease was reported from Connecticut and Washington (11). Chupp reported a gradual spread of the disease in Monroe and Niagara counties in New York, and Leach reported a 2 per cent loss in Minnesota (11). The same year *Fusarium bulbigenum niveum* wilt was reported from New York by O. A. Reinking, who stated that specimens had been sent in from Schenectady and Lockport (12).

In 1939 *Fusarium* sp. causing muskmelon wilt in New York was reported by Chupp as follows; "Gradually spreading in Ontario Lake District. Observed it in Schenectady county for the first time." (13)

Fusarium bulbigenum niveum f.2 in Minnesota was reported more destructive than for several previous years and was spreading and becoming more destructive each year, according to Carl J. Eidol (13).

The disease has also been reported from Maryland (14), and from Missouri (15).

Fusarium wilt is becoming particularly important in Michigan. It was found in Berrien County first in 1936 where approximately one to two per cent infected plants were found in one large field. Since that time isolations have been made by Muncie and KenKnight from Van Buren, Bay, and Wayne Counties. Reports from growers indicate that the disease has occurred in one or two other locations in the state.

Economic Importance

Fusarium wilt is capable of destroying 75 to 90 per cent of a crop during favorable seasons where the soil is heavily infested. Ninety per cent losses have been reported from Minnesota by Leach (1). KenKnight and Lewis, in a survey of the muskmelon areas of Berrien County Michigan in 1939 report the following observations: "In a small field, perhaps two or three acres, where Seaton's melons were, the vines were nearly all dead except for the Seaton selections. The five rows of selections were on the edge of the field. None of the vines were dead although some of them were badly spotted with Macrosporium. We found, upon carefully surveying the rest of the field, only five living plants. These were not especially badly blighted by Macrosporium. It was concluded that Fusarium wilt was the cause of the death of the other plants."

In the summer of 1940 the writer found five to ten per cent Fusarium wilt in several fields in Berrien County. In some of the fields bacterial wilt was prevalent and an exact estimate of Fusarium wilt could not be made.

An estimate of the actual annual loss, due to Fusarium wilt, in muskmelon production cannot be determined until a more extensive survey is made.

Symptoms

Six general symptoms of Fusarium wilt are known. These are: damping off, seedling wilt, seedling rot, wilt of more mature plants in the field, stunting, and root canker. In heavily infested soil seedlings may die before they emerge. These symptoms may be confused with damping off caused by other fungi. Seedling wilt is first observed as a wilting of the cotyledons, characterized by a loss of turgidity and a limp feeling when taken between the fingers. These cotyledonary leaves may wither and dry up before further wilting of the plant occurs. Later the entire seedling droops over, and if pulled the root appears brown with necrotic or rotted tip. On older seedlings especially in cool soils, the disease acts as a root rot, the parts below ground being completely necrotic. Small local lesions may be found on the hypocotyl, or the plant may wilt without any evidence of local necrosis. The seedling symptoms are found to be closely correlated with temperature and moisture factors.

The first symptom on older plants is nearly always a wilting of one or more runners. The wilt generally starts at the tip of the runner. In the early stages of wilting, plants wilt during the day and regain turgidity at night. Before the plant is completely wilted necrotic lesions may appear on the stem near the ground line. These lesions usually involve only one side of the stem extending for some distance as a long narrow brown streak, but sooner or later the whole plant wilts and dies. The necrotic lesions on old stems first appear as dark green, collapsed tissue that later turn brown. Droplets of red gum-like material are secreted from these stem lesions and harden on the surface. If moisture conditions are favorable necrotic lesions on plants become covered with salmon pink masses of fungus spores. The roots of infected plants appear browned or necrotic often cankered of split. The necrotic areas may involve only the hypocotyl but more generally the entire root system. If cross sections are cut through the stems of wilted plants the vascular bundles show brown streaks. This is a characteristic symptom that can be used to distinguish Fusarium wilt from bacterial wilt.

If the plants remain alive until the fruits are set, infection of the latter may occur. This infection appears as a rotted area at the stem end of the fruit which later becomes covered with pinkish-white mycelial growth.

Two additional symptoms have been observed on plants growing in the greenhouse and to a lesser extent in the field. The first is a chlorotic spotting of the leaves and stunting of the plants. Later these chlorotic leaves or spots appear as scorched areas. The second symptom is characterized by short internodes and an excessive number of male flowers near the ends of the runners. The flowers are smaller and usually paler in color than normal ones. These last symptoms have been observed repeatedly, and where they did occur, the plants were dead from Fusarium wilt later in the season.

Pathogenicity

Variations in Pathogenicity of Different Isolates

Cultures of the muskmelon wilt pathogen were obtained from diseased field material by surface sterilizing pieces of stems or roots with mercuric chloride and plating on potato dextrose agar. When the fungus grew out of the material transfers were made to tubes of potato agar. These were kept in stock culture. Three of the isolations used in this work were obtained from Leach in Minnesota. Transfers from these were made for the stock cultures. All cultures thus obtained were used in subsequent infection experiments.

A typical infection experiment consisted in growing the Fusarium isolate to be used in sterile oats contained in a wide mouth flask. After four or six weeks, when growth had spread throughout the oats, some of the material was mixed with sterile soil in which muskmelon seedlings were then grown either by planting seed directly in the inoculated soil or by transplanting seedlings that had been growing in sterile soil, to the inoculated soil. Infection and typical wilting of the seedlings resulted from many such experiments. Some of the isolates did not produce wilting, however. These were discarded as being of very low pathogenicity or else a saprophytic form of Fusarium that closely resembled the Fusarium bulbigenum Niveum causing muskmelon wilt.

In the infection experiments a wide variation was observed in the pathogenicity; namely, length of time for wilting and severity of infection, of the different isolates. Investigations were then conducted to describe variations of Fusarium bulbigenum niveum as to pathogenicity and cultural characteristics.

A limited number of isolates were used in these experiments. Selections were made on the basis of cultural characteristics and location from which the original isolation was made. Some of the isolates have been in culture for two or three years, but the greater number were isolated by the writer from diseased plant material. The sources of the isolates used in this investigation are shown in Table 1. Each isolate is designated by an Arabic numeral.

Table 1- Sources of isolates used in investigation

Isolate	Place of collection	Form of material	Contributor	Date of isolation
1	Minnesota	culture	Leach	1937
11	Minnesota	culture	Leach	1937
12	Ingham Co.Mich.	melon vines	Miller	1940
14	Minnesota	culture	Leach	1937
15	Bay Co.Mich.	culture	KenKnight	1939
16	Ingham Co.Mich.	melon vines	Miller	1940
21	Berrien Co.Mich	melon vines	Miller	1940
23	Wayne Co.Mich.	culture	Kenknight	1939
25	VanBuren Co. Mich	culture	Muncie	1938
26	Berrien Co.Mich	melon vines	Miller	1940
27	Berrien Co.Mich	melon vines	Miller	1940
30	Berrien Co.Mich	melon vines	Miller	1940
31	Berrien Co.Mich	melon vines	Miller	1940
50	Berrien Co.Mich	melon vines	Miller	1940

In order to obtain a pure strain for each of the isolates used, monoconidial cultures were made by using the micro-manipulator method. A spore suspension was made from each tube culture of the isolate. Single spores were picked from a drop of each suspension with the micro-needle and placed on agar drops suspended on cover glasses over Van Tieghem cells in sterile petri dishes. After the spores had germinated bits of the mycelial growth were transferred to sterile potato dextrose agar plates. Test tube slants were made from the agar plates by brushing spores from the plate with a fine needle and transferring to the tube slant.

The different isolates were tested in the greenhouse on muskmelon seedlings over a period of twelve months. The plants were grown in six inch pots filled to within one inch of the top with a mixture of well rotted compost and sand that had been steamed for twelve hours.

The inoculum used to infect the soil was obtained by growing the fungus in oat cultures as previously described. The contents of one flask containing a given isolate were thoroughly mixed with the soil in two pots using equal amounts in each pot. Fourteen sets of pots were thus prepared with the various strains. Two pots which received no inoculum were used as checks.

Several series of tests were made using Pride of Wisconsin variety of muskmelon for the host. This variety was used because of its comparatively high susceptibility. Usually six to eight seeds were planted in each pot. In some of the tests the seeds were germinated in sterile soil and four to six uniform seedlings were then transplanted to the inoculated pots. This latter method assured a more uniform stand and more unifrom wilting of the plants within each set of pots.

The observed symptoms of seedling infection in these tests were similar to those previously described. A number of seedlings grown in the infested soil was found to be stunted but otherwise normal. In most cases these plants later wilted from Fusarium infection.

The various isolates were found to differ in virulence. This condition is clearly shown in Table 2. In this set of experiments the seeds were planted directly in inoculated soil.

Table 2- Results of pathogenicity tests with various isolates of *Fusarium bulbigenum niveum* f.2.

Isolate	Number of plants	Number of plants wilted	Percentage of plants wilted
1	11	6	54.5
11	10	1	10.0
12	10	8	80.0
14	9	5	55.5
15	11	4	36.2
16	10	2	20.0
21	11	2	18.0
23	8	2	25.0
25	10	6	60.0
26	9	1	11.0
27	11	1	9.9
30	9	0	0
31	11	3	27.2
50	9	4	44.4
check	12	0	0

Plants were approximately 18 days old.

The length of time required to produce the first symptoms of infection varied also with the different strains as shown in Table 3.

Table 3- Virulence of 14 isolates expressed in percentage of plants wilted based on time required for wilting.

Isolate	Number of plants	Percentage of plants wilting			
		Days after planting			
		6	8	10	17
1	8	0	12.5	25.0	100.0
11	8	0	0	12.5	25.0
12	8	25	62.5	100.0	100.0
14	8	0	50.0	50.0	100.0
15	8	0	37.0	75.0	100.0
16	8	50	87.5	87.5	100.0
21	8	0	0	25.0	75.0
23	8	0	0	62.5	75.0
25	8	0	12.5	25.0	25.0
26	8	0	0	25.0	78.5
27	8	0	0	0	0
30	8	0	12.5	25.0	37.5
31	8	0	12.5	12.0	25.0
50	8	0	25.0	62.5	100.0
check	8	0	0	0	0

Strain 12 produced the greatest amount of wilt in a given period of time; strain 16 ranked second, and strain 15, third. However, in some instances other strains were more virulent in the early part of the experiments. These three strains were consistently pathogenic and highly virulent. Throughout the experiments several other strains produced an equal percentage of wilting but over a longer period of time.

The results of these experiments indicate the presence of strains of the muskmelon wilt organisms. However, some of the variations noted may be due to uncontrollable factors such as distribution and growth of the organism throughout the soil, root injury of the host, natural variations in susceptibility of individual plants and slight natural variations that might occur even within a single strain of the pathogen. Discrepancies also occur between recent isolations and those that have been kept in culture for some time. It was noted that a single passage of a Fusarium strain of the latter through the host markedly increased its virulence.

The stability of any isolate or strain cannot be considered permanently dependable. In several cases the reisolations from infected plants in the greenhouse were not macroscopically like the parent isolate. Nevertheless, all of this does not lessen the fact that in the field one may pick up strains with varying degrees of virulence, and a resistant or susceptible plant is conditioned by the virulence of the particular strain of the organism.

Cultural Characteristics of Isolates

Studies were made on the cultural characteristics of the fourteen isolates when grown on potato dextrose agar. Each of the isolates was reisolated from wilted seedlings from the pathogenicity tests and plated on agar. When growth had spread over the plate uniform disks were cut from each of these and transplanted to fresh agar plates. Cultures were classified according to macroscopic appearance of the colonies using criteria similar to those used by Wellman in describing cultural characteristics of strains of *Fusarium lycopersici* (16). Briefly described the cultural classes are:

Completely appressed, A; Mat slimy, appressed to the agar, no fluffy aerial mycelium; mat forming a tough film over the agar; color, vinaceous purple (17) but ranging to pink or buff.

Intermediate appressed, IA; Most of mat slimy, tough appressed, but small tufts or bunches of white cottony aerial mycelium; color, pale purple or salmon.

Intermediate raised, IR; Underneath mat appressed, slimy, vinaceous purple to pink, mostly covered with thin water-soaked, white aerial mycelium.

Fully raised, R; Mat raised, velvety, compact, white, with two or three purple or buff colored rings seen from the bottom of the plate.

The greatest difference in growth characteristics of *Fusarium bulbigenum niveum* strains were observed on ten day old growth on potato dextrose agar. Table 4. The cultures were held at room temperature and in diffused light.

Table 4- Macroscopic growth characters of 14 *Fusarium bulbigenum niveum* isolates grouped according to culture classes.

Culture group	Isolate number	Type of mat growth	Aerial mycelium	Color	Growth in Cm.
A	11	appressed slimy	none	buff with small red center	5.5
	14	do.	do.	purple and white streaked	5.0
IA	21	appressed dry	cottony white tuft in center	purple with white margin	5.0
	23	appressed slimy	white, scattered, water-soaked	white transparent	5.0
	26	appressed dry	scanty very small scattered white tufts	white to buff, light and darker rings	5.5
IR	16	slightly raised dry	scanty growth over all white woolly	purple and white streaked	4.5
	1	slightly raised dry	scanty growth over all white cottony	white with pink streaks	5.5
IR	15	slimy appressed margin	white water-soaked thick at center	pale pink to buff	5.0
	50	raised	white hair-like growth over all	yellow center, white margin	5.0

Table 4- continued:

Culture group	Isolate number	Type of mat growth	Aerial mycelium	Color	Growth in Cm.
	31	do.	white very tall hair-like threads	faintly pink	8.0
	27	do.	thick white cottony tuft	white, small pink blotches	4.0
R	12	do.	very thick white felt-like	white to slightly yellow	3.5
	30	do.	white thick velvet-like	faint pink to yellow	5.5
	25	do.	thick woolly	white	6.0

In spite of the marked difference in appearance of the strains of Fusarium bulbigenum niveum (Plates I and II) the writer found no close correlation between cultural characteristics and pathogenicity. Strain 12, the most highly virulent, occurred in cultural class R, with abundant, white, felt-like aerial mycelium. Strains 15 and 16, next in rank of virulence, occurred in class IR, with underneath mat appressed, vinaceous purple to pink and covered with thin white aerial mycelium. Strain 14, slightly less virulent, occurred in class A, with a tough appressed mat, vinaceous purple in color, slimy, and without aerial mycelium. On account of the relatively small number of cultures grown and with the fine distinctions between the rather numerous - 14 - pathogenicity classes, irregularities in the general trends are unavoidable. A closer relationship might be shown between cultural types and pathogenicity with more extensive experiments. It was noted in this work that a greater percentage of highly virulent strains did appear in the groups with light colored abundant aerial mycelial growth.

Relationship of the Parasite to Host

Pathological Histology

Mode of Infection

Histological studies were made by killing and fixing diseased material with chrom-acetic acid, washing and embedding in paraffin in the usual manner, and cutting with a microtome. Different stains and staining technic were employed depending upon the type of material and the sections. Sections were mounted in balsam. In some of the studies free hand sections were employed. In every case where sections were made for histological studies some of the same material was surface sterilized and plated on agar. From these plates reisolations of *Fusarium bulbigenum niveum* F2. were made and the pathogenicity of the organism determined by infection experiments. This procedure eliminated the possibility of dealing with a saprophytic fungus that might have been present in the sectioned material.

Observations and histological studies indicate that infection may occur through the root tip or hypocotyl. In wilted seedlings the hypocotyl often appears browned and cross sections show mycelial strands and necrotic areas in the tissue, the root showing no indication of infection. In some seedlings a necrosis of the tips of the roots is the first histological symptoms of the disease. In older plants infection may occur through the root tip or through small cracks or injuries at the base of the stem just at the top of the ground. The writer has observed that stems of rapidly growing succulent plants in the greenhouse often crack at the ground level. Infection may occur through these cracks.

It is not definitely known by what means the tip of the germ tube or mycelium penetrates the outer cell wall of the epidermal cells, where infection occurs through the root tip, but it is probably accomplished by the excretion of some dissolving enzyme. These cell walls in the young root tip are not especially thick and do not seem to be cutinized. In the staining they do not stain more deeply than do the other cell walls of the cortical tissue.

Growth of the parasite in the host

Immediately upon penetration of the epidermal cells, a food relationship is established with the host and the germ tube becomes rapidly growing mycelium. Growth is intracellular and the cell walls seem to offer little resistance to the passage of the hyphae. The fungus spreads rapidly in the vascular tissue, and growth may spread into the cortical cells. (Plate III). While the greatest amount of vegetative growth occurs in the vascular tissue near the point of infection it is not limited and may grow for great lengths through the vascular bundles. Cross sections of a fruit peduncle five feet from the point of infection showed hyphal strands in the vascular tissue. At this point the mycelium seems to be confined to the vessels. The fungus is probably not able to penetrate the walls of these old lignified vessels. It has been noted that the mycelium here is very scant. The explanation is probably to be found in the meager supply of organic food in the vessels.

Some disintegration of cortical and vascular cells along with a distinct browning occurs at the place of infection and where the vegetative growth of the fungus is abundant. Cross sections of stems at the point of infection when stained lightly retained this brown color, and often showed crystal-like darker stained inclusions in the disintegrated tissue.

Leach and Currence (1) report that long distance dissemination of muskmelon wilt is accomplished by means of infected seed. They observed the mycelial strands of wilt fungus under the seed coat in sectioned seed from rotted fruits.

Further investigations were made by the writer on this problem. Seeds were taken from infected portions of fruit, washed free of the placenta and killed in picric acid. The seeds were thoroughly washed and embedded in paraffin. Sections 14 microns thick were cut with a rotary microtome, stained several hours with safrinin, counterstained with light green and mounted in balsam. Upon examination, mycelium was found to be present in the thin walled tissue of the seed coat and in the remains of the nucellus. (Plate IV). This mycelium may possibly have entered through the funiculus or micropyle.

Further evidence of seed infection was obtained by surface sterilizing some of the seed from infected fruits with mercuric chloride and plating on sterile agar. When germination of the seed occurred the fungus mycelium grew out from the inside of the seed along with the developing embryo. It was also noted that the surface of seed from infected fruits carried the wilt pathogen.

Means by Which Fusarium bulbigenum niveum Induces Wilting

Two theories have been proposed to account for wilting in plants induced by different species of bacteria and fungi. The mechanical plugging theory was cited by Stewart (18) in 1897 and by Smith (19) in 1899. This theory suggests that the growth of the organism within the vessels and the formation of gums and tylosses cut off the water supply and consequently the plant wilts. More recently it has been shown that certain fungi and bacteria when grown on broth media produce substances that are toxic to plants and cause wilting in this way. This latter theory is more generally accepted now, and it is with this theory that the writer is concerned here.

One of the first to experiment on the factors involved in the wilting of a plant attacked by a microorganism was Hutchinson (20) of India. In 1913 working on a disease of tobacco which he considered to be caused by _Bacillus solanacearum_, he fed plants a substance which had been precipitated by alcohol from pure bouillon cultures of the pathogen. The plants which obtained this substance developed wilt while those fed a boiled solution of the same substance did not wilt. He considered the wilting as a consequence of protoplasmic intoxication.

Coons (21) reporting on the work of Goss in 1916 calls attention to the wilting of potato plants when placed in a filtrate representing a synthetic solution upon which a species of Fusarium had been grown. Control plants placed in an uninoculated medium remained healthy. It was concluded that wilting was due to poisons generated by the fungus.

In 1919 Brandes (22) published a report on Banana Wilt in which he included results from experiments conducted to show the relation between excretions of toxic substances by the parasite and the symptoms exhibited by the attacked host. He noted that the fungus was not present in quantities sufficient to cause serious obstruction to the passage of water, and concluded from his work that certain toxins were produced by the fungus that would produce typical symptoms of the disease.

White (23) working with _Fusarium lycopersici_ demonstrated the presence of toxic substances in the filtrate from broth cultures of the organism. He concluded that organic acids are present in the filtrate which are toxic to tomato plants. He also showed that the toxins are non-specific.

In 1923 Fahmy (24) studied the toxic substances produced by _Fusarium solani_ when the organism was grown on Richard's solution. He found that the toxic effect of the filtrate was not due to the change in pH and showed that the unknown toxic substance was thermo-stable and non-volatile.

Picado (25) 1923 attempted to determine the nature of the substance secreted by parasitic fungi in which the host tissues are either injured or killed at some distance from the point of attack. He worked with _Verticillium albo-atrum_, _V. duboys_ both parasites of the potato, _Fusarium solani_ from the potato; _F. cubense_, the pathogen of banana wilt; and with a saprophytic _Penicillium_. The results indicated that the withering of the plants, the browning of the vessles, and the destruction of the tissue were not all produced by the same causes, although in each instance evidence was seen of the action of substances having toxic properties, such action resulting in the symptoms produced by the different diseases. After extracting the albuminoids, enzymes, and toxins from the mycelium with alcohol and concentrating the liquid he obtained a waxy brownish residue. This proved to be thermo-stable and very toxic to plants. Picado's conclusions are that "the poisons produced by the fungi are not true toxins in the sense of toxins against which immunization is possible. They may be compared in action to poisons found in some mushrooms, such as Amanita toxins." He suggests that the action at a distance from certain parasitic fungi may be due to a chronic poisoning of the plant.

Rosen (26) in 1926 working with filtrates from broth cultures of _Fusarium_ _vasinfectum_ concludes that there are at least two toxic substances present in the filtrates. One is a volatile compound slightly alkaline, and the other is an organic salt in the form of a nitrite.

Ludthe and Ahmet (27) in 1933 attempted to isolate the toxin from culture fluid of _Fusarium_ _vasinfectum_ by drying in vaccum and extracting with methyl alcohol. This residue showed high wilting power. It was further purified until an 0.5 per cent solution killed plants in a short time. According to the writers the substance is a viscous hygroscopic brown amine.

The common method used in proving the presence of toxic substances is to grow the pathogen on some nutrient medium, filter the organism from the medium, and place the plants, usually with their roots cut off, into the filtrate. This method has been used by the writer. In Table 5 are given in brief outline form the experiments conducted and the results. In all the experiments vigorously growing seedlings were used. They were pulled, their roots cut off under water, and the plants transferred to test flasks or tubes containing the filtrate.

The filtrates used were obtained by growing the organism on Coons' synthetic medium for six weeks and then filtering the medium through a Berkefeld, type N filter to remove all the spores and bits of mycelium. The filtrations were made under aseptic conditions. The filtrate was then tested for sterility by plating on agar. No growth occurred, which indicated that results obtained from the ensuing experiments could not be due to infection of the plant with the fungus or other organisms.

Table 5- Action of various filtrates obtained from broth cultures of <u>Fusarium bulbigenum niveum</u>.

Exp. no.	Treatment of filtrate	Plants	No. of plants	No. wilted	Time for wilting
I(a)	filtered Berkefeld N.	musk-melon	5	5	15 hours
1(b)	medium un-inoculated	do.	5	0	
2	filtered Berkefeld N.	do.	5	5	15 hours
3(a)	do.	tomatoes	5	3	23 hours
3(b)	do.	beans	5	5	24 hours
4	autoclaved 15 pds.	musk-melon	5	5	15 hours
5(a)	Dil'n. 1-1	do.	3	3	24 hours
5(b)	Dil'n. 1-2	do.	3	3	33 hours
5(c)	Dil'n. 1-5	do.	3	2	33 hours
6	filtered Berkefeld N.	do. with roots	5	4	33 hours

Experiments 1(a) and 1(b). The results of these experiments demonstrate the presence of toxic substances in the filtrate from broth cultures of <u>Fusarium bulbigenum niveum</u>. Wilting of all the plants which were placed in the inoculated medium occurred in fifteen hours with no recovery when transferred to water. The plants which were placed in the uninoculated medium showed no wilting after twenty-four hours.

Experiment 2 was designed to show the effect of the pH change in the medium on the plant. A portion of the inoculated medium was adjusted to the original pH of the broth and again tested on plants. Wilting of the plants occurred after fifteen hours which indicates that the toxic effects of the filtrate are not due to the pH of the solution.

Experiments 3(a) and 3(b) show the non-specific properties of _Fusarium_ _bulbigenum_ _niveum_ toxin. When bean and tomato seedlings were cut off under water and placed in portions of the toxic filtrate typical wilting occurred.

Experiment 4 was designed to see if the toxic properties of the filtrate were inactivated by heating. A portion of the filtrate was autoclaved at fifteen pounds pressure for twenty minutes. It was then checked on cut stems of muskmelon seedlings for wilting. After fifteen hours all the plants showed typical wilting. The results indicate that the toxin produced is thermo-stable.

Experiment 5 shows the effect of diluting the filtrate. Dilutions 1-1, 1-2 and 1-5 were made using distilled water. Each dilution produced wilting of the plant but the higher the dilution the longer time required for wilting.

Experiment 6 shows that the toxic substance can be absorbed by the unbroken roots. Wilting occurred ten to twelve hours later in this case than when the roots were cut from the plants before placing them in the toxic filtrate.

In brief, the results of the foregoing experiments demonstrate the production of a toxic substance by Fusarium bulbigenum niveum which is capable of producing wilt in plants. The toxic substance is thermostable and non-specific. It is effective in comparatively weak concentrations, and can be absorbed by the roots of the plant.

To determine the chemical nature of the toxic materials produced by Fusarium bulbigenum niveum the following chemical studies were undertaken. The methods used were, in the main, standard procedure for the isolation and identification of organic compounds from fungus material or from solutions which contain the substances. The materials used were fungus mats which had been growing on Richard's solution for six to eight weeks, and the filtrates obtained from the solution.

The fungus mats were dried to constant weight in a 65 degree oven and ground to a fine powder with a mortar and pestle. The powder was shaken up with water, warmed over a low flame and filtered. The filtrate was tested for toxic properties on cut muskmelon stems. all plants placed in the filtrate showed distinct wilting at the end of two hours, and after twelve hours they were completely wilted and drying. No recovery occurred when they were transferred to water.

Some of the filtrate from the fungus mats containing the water soluble material was shaken up with 80% alcohol and filtered. The residue, a slight, brownish, gum-like mass on the filter paper, was but slightly soluble in ether, insoluble in 80% alcohol and soluble to slightly soluble in water. Minute traces in aqueous solution were highly toxic to muskmelon plants.

The flaky brown substance which contained the toxins was analyzed chemically according to the procedure given by Mann and Saunders (28). Nitrogen determinations were made using the Lassaigne Sodium Test. Some of the substance was heated with molten sodium, extracted with water, boiled with ferrous sulphate, and treated with ferric chloride. HCl was then added and a Prussian Blue precipitate settled out, thus giving a positive test for an organic nitrogen compound. The Lassaigne Sodium Test for sulphur was negative.

Ammonia was evolved on heating when the substance was treated with concentrated NaOH solution. Treatment with dilute sodium carbonate solution liberated carbon dioxide. In addition to these reactions the substance reacted with nitrous acid evolving nitrogen. The foregoing reactions indicate the presence of an acid amide, acid nitrile or imide of dibasic acids. Further attempts to isolate a definite compound were unsuccessful.

Attempts were made to isolate the toxins from the culture medium on which Fusarium bulbigenum niveum had been growing. The solution was filtered free of all the fungus material and shaken up with 80% alcohol. A heavy buff colored precipitate settled out, which when filtered, dried, and redissolved in water, produced typical wilting of plants in 12-24 hours. This material precipitated from the culture medium gave similar reactions to those given by the toxic material extracted from the mycelial mats. Some further tests were conducted. A benzoyl derivative was made from the toxic substance using the Schotten-Baumann reaction. A small amount of the precipitate was treated with NaOH. Two to three cubic centimeters benzoyl chloride were added and the mixture was shaken vigorously for five minutes. A thick suspension resulted. The solution was then filtered and the residue washed with water, transferred to about 15 cubic centimeters absolute alcohol in a small evaporating dish, mixed thoroughly, heated to boiling and again filtered. The alcoholic filtrate was cooled and a very fine white precipitate settled out. This benzoyl derivative was thoroughly dried on a slide in air and the melting point determined. This ranged from 79-86 degrees centigrade. Further attempts to purify and identify this compound have so far been unsuccessful. The melting point and other reactions of the derivative indicate the presence of an amine in the original substance.

All chemical tests were necessarily made with minute quantities of the material, and certain tests were not always clear cut. Purification and identification of the toxins produced by Fusarium bulbigenum niveum responsible for the wilting of muskmelon plants awaits further investigation.

Varietal resistance

Some progress has been made in developing muskmelon strains resistant to Fusarium bulbigenum niveum. Hybrid melons more or less resistant to the wilt disease have been developed at the Minnesota Experiment Station (29). Some work has also been done at the Michigan Experiment Station and similar work is in progress at the Maryland Station (14).

Since resistant varieties are necessary for control, and no entirely satisfactory ones have been developed that meet the requirements of the Michigan grower, further breeding and selection work was begun by the writer at the Michigan Experiment station in 1940. The data and observations that follow form a progress report of that work.

For parent stock use was made of two Minnesota hybrids, wilt resistant 10-38, and 11-38, several commercial varieties, and several varieties of Afghanistan muskmelons. The seed of the latter varieties were obtained through H. L. Seaton, Horticulture Department from W. E. Whitehouse, Senior Horticulturist of the United States Department of Agriculture. The Afghanistan varieties were used because they represented possibly more closely the wild strains of muskmelons in the hope that they would show more resistance to the wilt pathogen than the highly developed American commercial varieties.

The first plantings were made in the greenhouse in heavily infested soil. Crosses and self pollinations were made throughout the flowering season. A high percentage of the pollinations set under greenhouse conditions. From the resistance vines a great many selections of both the crosses and self-pollinations were obtained for further study.

In Table 6- a comparative index of resistance under greenhouse conditions for the commercial varieties used in the breeding work is given.

Table 6- Index of wilt resistance of seedlings of certain varieties of muskmelons planted in the greenhouse in 1940.

Variety #	Name of variety	Initial stand	Percentage of plants wilting			
			Days after planting			
			15	24	30	41
#45	Mildew Resistant	42	19	54	64	85
	Honey Rock	36	33	55	61	86
10-38	Minn. Wilt Resist.	36	8	12	16	22
26647M	T. Whittaker	36	11	33	50	75
10-25	Pollock	42	5.5	26	26	30
11-38	Minn. Wilt Resist.	34	2.7	8.3	8.3	8.3
*3-8(1)	Hearts of Gold	42	10	25	60	65
*2-18(4)	Hearts of Gold	42	12	32	54	70

* Field selected Hearts of Gold

The Afghanistan varieties are not included in this table, for most of the ones used showed a much higher degree of resistance under the conditions of the test. They did, however, show symptoms of infection, but the greater part of them survived until their fruits were mature. It was thought that a more accurate check on the resistance could be obtained by growing them under field conditions. Growth of the Afghanistan varieties in the greenhouse was much more vigorous than that of the commercial varieties. Fewer fruits were set and they were large and of very poor quality and flavor.

The breeding and selection work was continued in the field during the summer of 1940. Plantings consisted of previous greenhouse selections of both crosses and selfs based on degree of indicated resistance and commercial possibilities, and were made in heavily infested soil.

The method of hand pollinating in the field consisted in covering both staminate and pistillate flowers before they opened. At the time of covering, the pistillate flowers, that were to be used in the crosses, were opened and the anthers removed. This was necessary since some of the flowers on muskmelons are perfect. When the flowers opened the pollen was applied and the pistillate flower again covered until it closed. Considerable difficulty has been encountered in making hand-pollinations in the field. The pollination records show that only four per cent of the attempts were successful.

Minnesota varieties showed almost total resistance up until the melons begin ripening. However, before the fruits were gone some of the vines had succumbed to wilt. The ribbed Minnesota hybrid #10-38 showed greater possibilities than #11-38 under Michigan conditions. The Afghanistan varieties were much less resistant under field conditions than they had been in the greenhouse. However, several of the hybrid selections did show promise. Below is a short description of three selections from selfs made on these hybrids.

Selection 9-40 (hybrid-Pollock X Afg.126067)

This strain is highly resistant to Fusarium. The fruit is elongate to oval, slightly netted, large and somewhat variable in shape. The size of the seed cavity is rather large, quality of flesh poor. Plate V.

Selection 21-40 (hybrid-Honey Dew X Afg.126063)

The fruit of this strain is slightly oval to round, yellow when ripe. It is ribbed and distinctly netted. Flesh is yellow, seed cavity small, quality fair. The vine is coarse and vigorous, with moderate to high resistance. Plate VI.

Selection 17-40 (hybrid-Hales Best X Afg.126041)

This selection is approximately 50 per cent resistant. The fruits are oval, smooth, ribbed, yellow when ripe. Seed cavity is small and the quality of flesh good.

Some of the commercial hybrids showed varying degrees of resistance. Selections were also made during the summer of 1940 from commercial fields in different muskmelon areas of the state. On Kenneth Johnson's farm in Berrien County a single hill of Hearts of Gold was found growing at the end of the season in a four acre field when all the other plants had either died from Fusarium wilt or Macrosporium leaf spot. Two fruits were obtained from this plant for further work in the breeding and selecting program.

A further planting of hybrids and field selections was made in the greenhouse during the winter of 1940-1941. Additional crosses, selfs and close-pollinations have been made from which selections will be obtained for further field plantings. Table 7 gives the relative resistance of the 1941 greenhouse plantings.

Table 7- Index of wilt resistance of certain selections of muskmelons grown in the greenhouse during the winter of 1940-1941.

Variety or selection	Date of planting	Percentage of plants infected at different dates			
		Date observed			
	12-7-40	12-20-40	1-15-41	2-17-41	4-21-41
*Hearts of Gold		10	15	31	45
Minn. 10-38		0	5	20	25
Selection 21-40		0	5	5	10
Mildew Resist. #45		15	20	35	60
Selection 9-40		1	10	10	20
Selection 27-40		10	15	30	50

*Field selected from Berrien County - 1940

Selection 21-40 was about 90% resistant under the conditions of the test. Selection 9-40 ranked second with 80% resistance and the Minnesota hybrid showed 75% resistance. The field selected Hearts of Gold, while not as resistant showed a great deal of promise with a few very resistant individual plants.

Summary

Fusarium wilt is a comparatively new disease of muskmelons. It is caused by a specific fungus, *Fusarium bulbigenum niveum* F.2, which was first described in detail by Leach and Currence in 1938. The disease appears as a distinct wilting of the plants which may take place during the seedling stage or when the plants are more mature.

During hot, dry seasons, in soils that have become infested with the organism, the disease may become of great economic importance. Losses up to 90% of the crop have been reported.

The fungus enters its host through small root tips or through injuries on the roots or the hypocotyl by means of conidial germ tubes or through direct penetration by the mycelium. It grows intracellularly setting up a food relationship with the host. The most abundant growth occurs in the vascular tissue and may appear at great distance from the point of infection. Fruits may

become infected with the organism in which case wide spread dissemination is enhanced by transmission of the fungus on or in the seed.

Physiologic specialization exists within the species _Fusarium_ _bulbigenum_ _niveum_ F.2. Both pathogenic and cultural tests have shown distinct differences in the 14 isolates studied.

Wilting of muskmelon plants infected with _Fusarium_ _bulbigenum_ _niveum_ has been shown to be due to toxic substances produced by the organism. Chemical analysis of these substances indicate that the toxins are amines, amino acids, or other closely related compounds.

The most satisfactory control measure for Fusarium wilt has been in the production and use of wilt-resistant varieties. The relative resistance of certain varieties is shown in Table 6. It will be noted also that certain hybrids bred and selected by the writer during the course of these studies show promise of giving biotypes that will be much more highly resistant than the original parent commercial stock, and by a continued program of breeding and selection may be developed into pure strains with desirable commercial qualities.

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Description of Plates

Plates I and II.	Ten day old petri dish cultures of different strains of *Fusarium bulbigenum niveum*. Growth on potato dextrose agar. Isolates 1, 12, 15 and 50, intermediate raised, aerial mycelium. Isolates 11 and 14, mycelium completely appressed. Isolate 16, section of raised mycelium. Isolates 21 and 26 intermediate appressed. Isolates 25, 30 and 27 raised, thick aerial mycelium.
Plate III.	Cross section of a muskmelon stem from a Fusarium infected plant. From a paraffin section stained with Safranin and light green. (Note mycelium in the vessels.)
Plate IV.	Cross section of a muskmelon seed coat internally infected with the wilt fungus. (a) Thickened cells of the outer seed coat. (b) Parenchyma cells of inner seed coat. (c) Densely compressed cells of the remains of the nucellus. (d) Mycelium of the wilt pathogen..
Plate V.	Hybrid melon. Cross between Honey Dew and Afghanistan variety 126063. Picture - a selection from the 2nd generation.
Plate VI.	Hybrid melon. Cross between Pollock and Afghanistan variety 126067. Picture - a selection from the 2nd generation. (Note netting and size of seed cavity.)

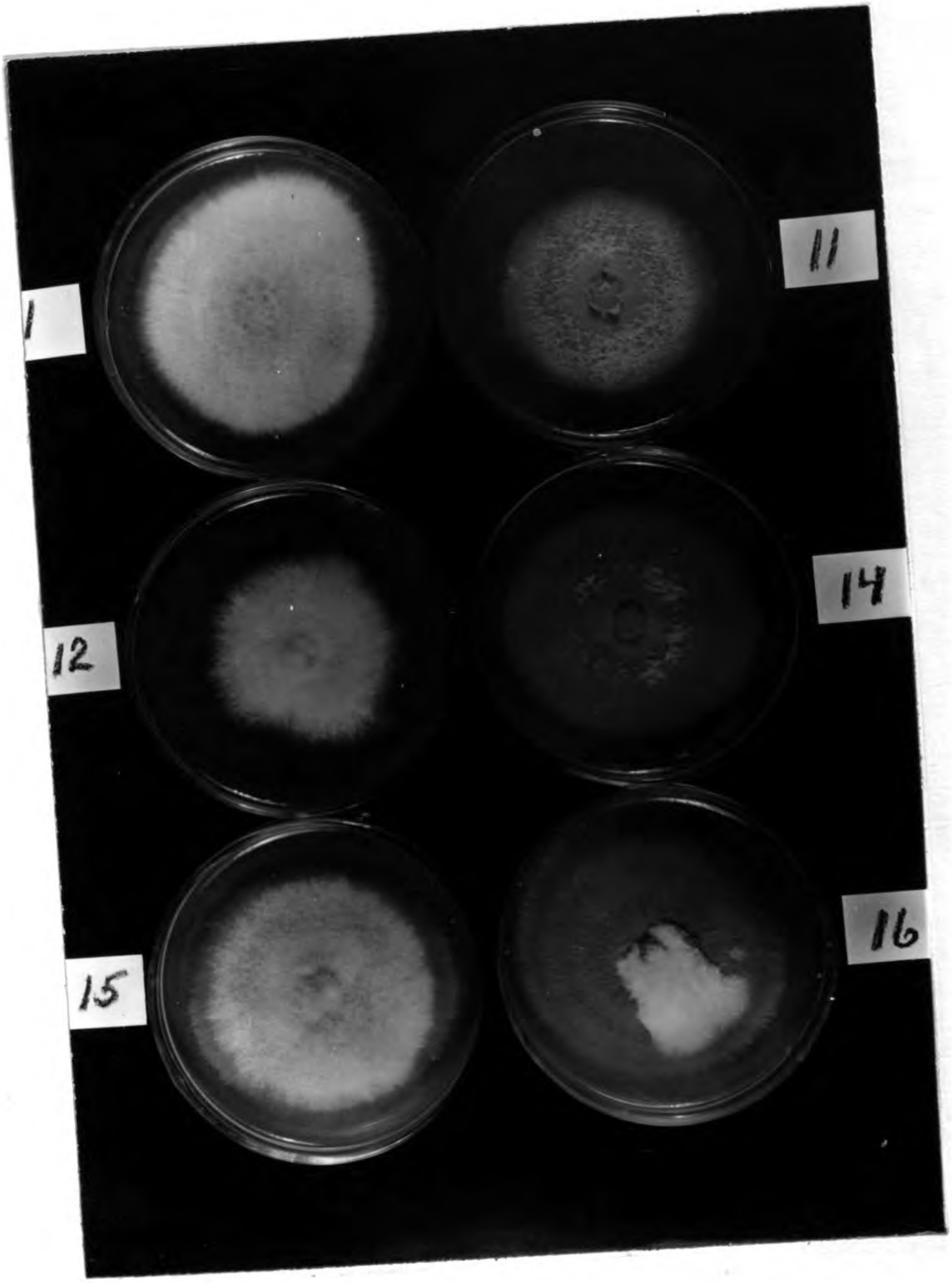
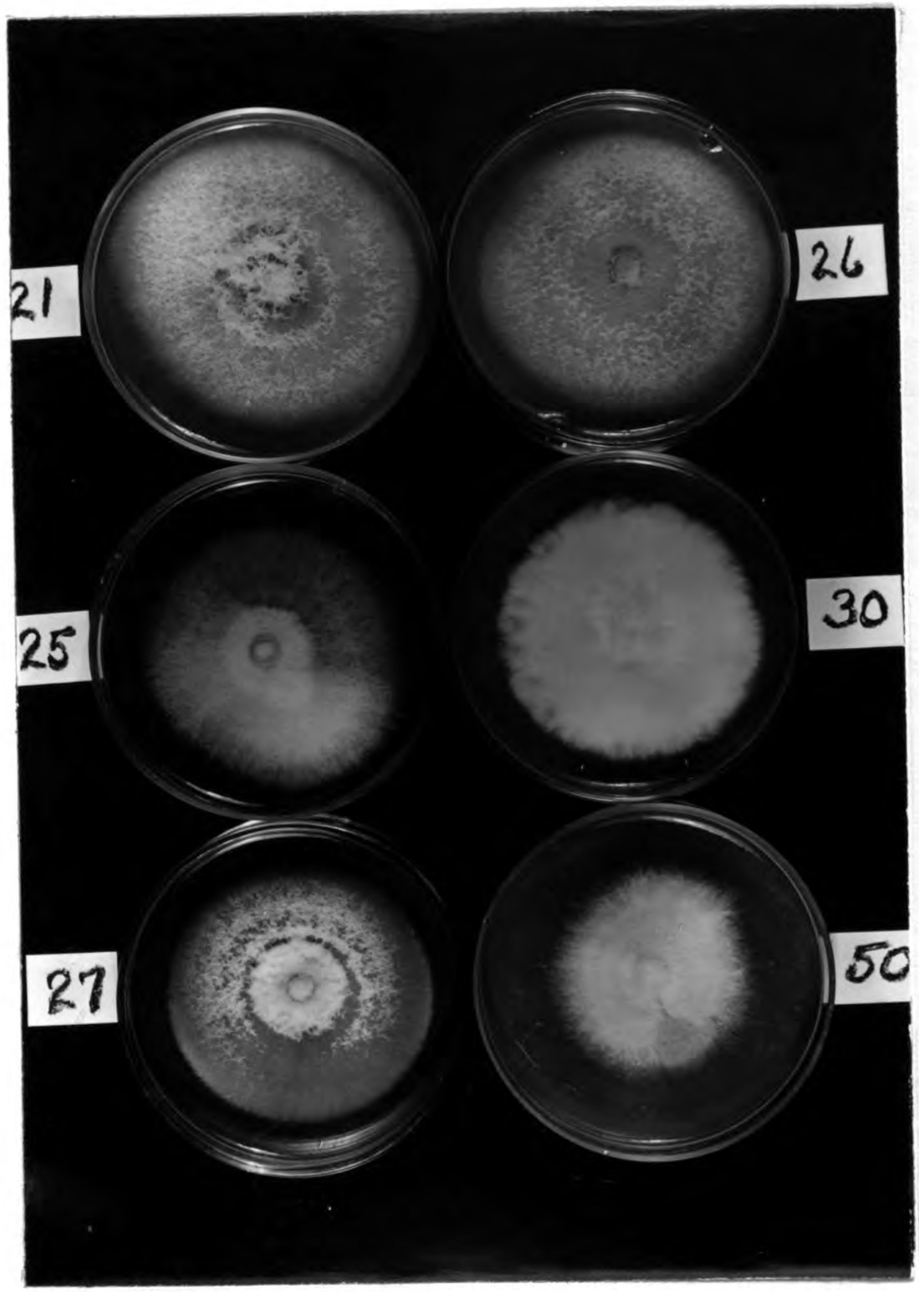


PLATE I



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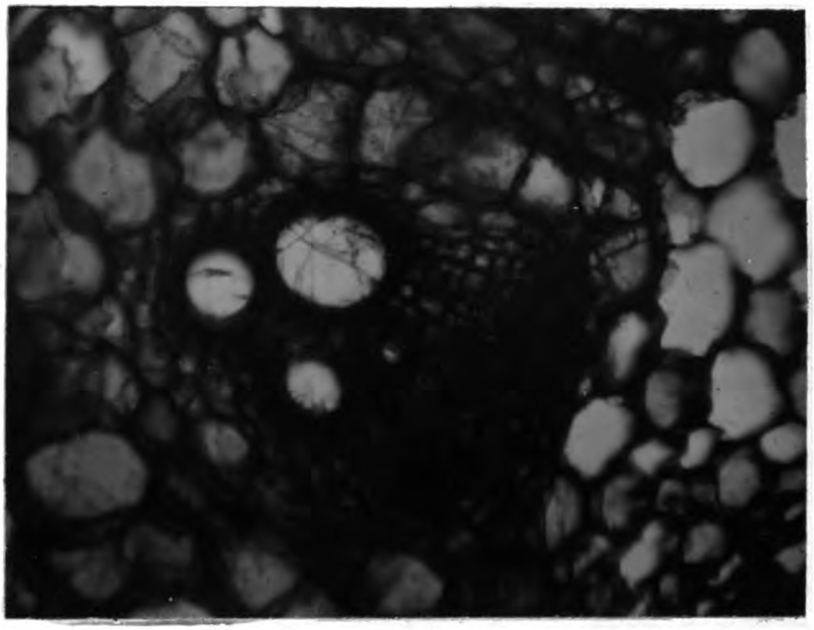

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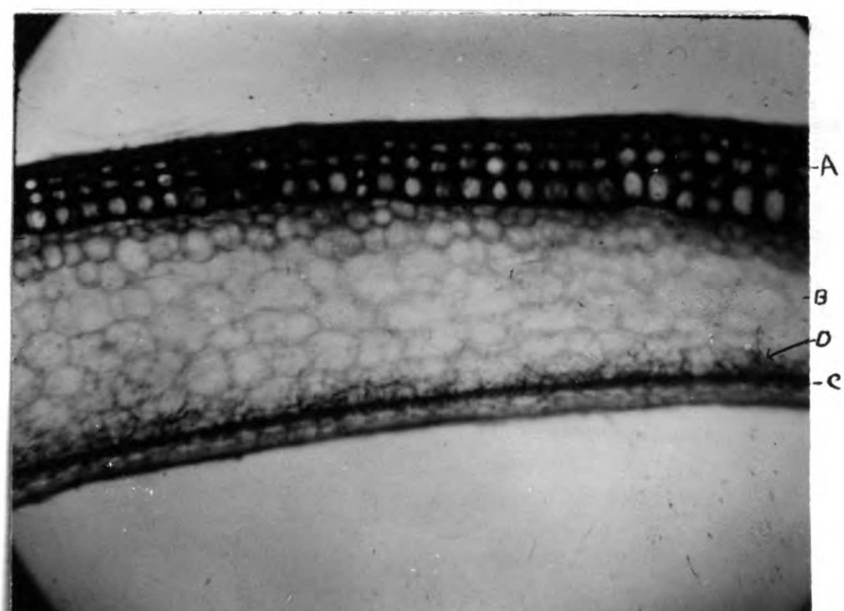

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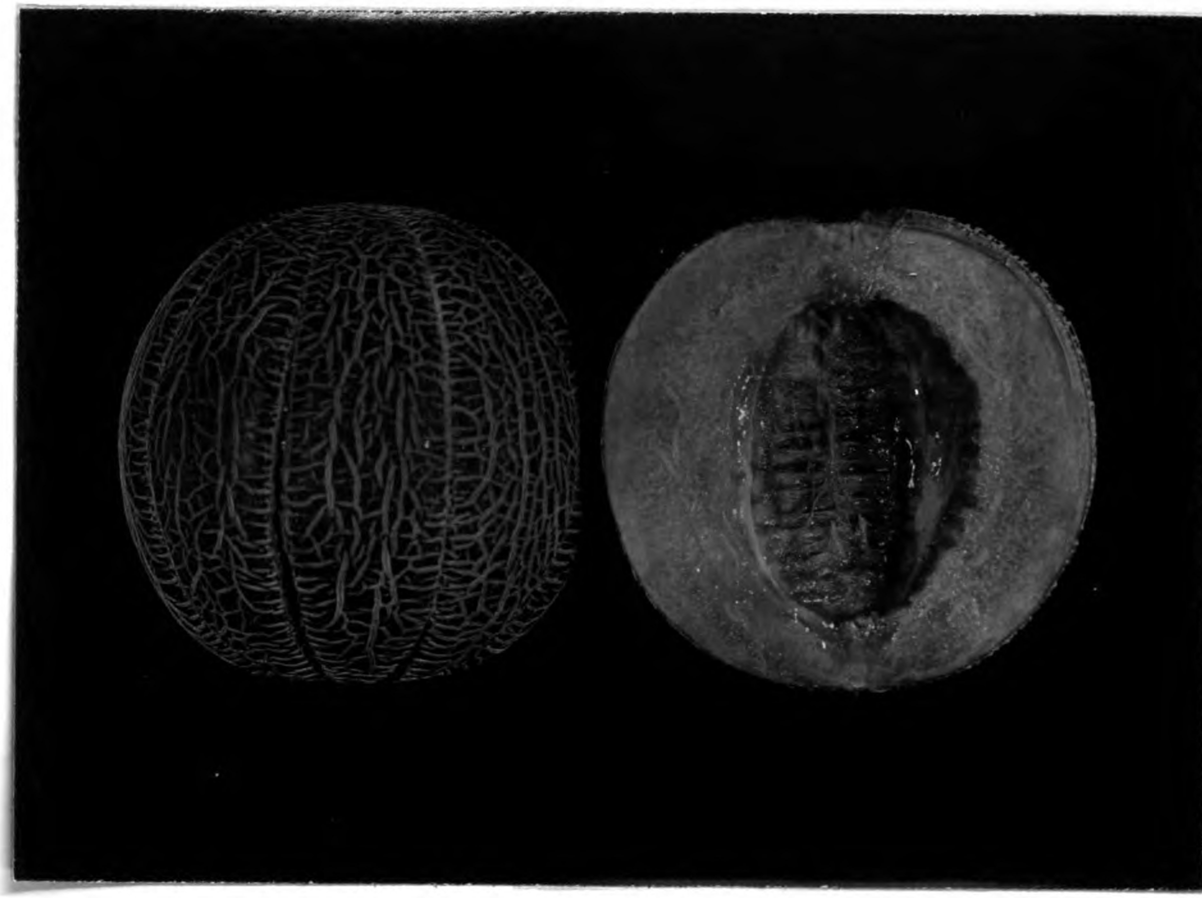

PLATE V

PLATE VI