THE INFLUENCE OF CHILLING, ABOVE THE FREEZING POINT, ON CERTAIN CROP PLANTS

by

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INTRODUCTION

It has been held that short periods of cold weather are sometimes responsible for injury to crop plants of a subtropical and tropical origin, aside from fungus organisms or frost. It is common knowledge that growth is retarded by such temperatures but it seems not to be generally conceded that definite lesions may result. It has, therefore, been the object of this study to investigate the effect of temperature near to but above the freezing point on certain crop plants and, if injury is found to occur, to learn the relative susceptibility of different crops and varieties and under what circumstances injury takes place.

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REVIEW OF LITERATURE

Very little has been contributed to this subject, especially in recent years. Molisch (1896)[1] critically reviewed the literature making special reference to the work of Bierkander (1778), Geoppert (1830), Hardy (1854), Sachs (1854), and Kunisch (1880), and conducted rather extensive experiments of his own. Some 58 plants from subtropical regions were examined and found to be injured by temperatures ranging from 1 to 7°C. Some showed characteristic black spots on their leaves, other rolled and ultimately dropped their leaves, some died and some survived without injury.

A microscopic examination of _Episcia_ leaves that had been killed showed that whereas most of the cells were dead, plasmolysis had not taken place. The plasma was dead but the veins and midribs were still alive, with the epidermis and mesophyll spread stiff between them.

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1. Reference is to literature cited.

Great resistance was shown by the guard cells of the stomata and only when the vein cells succumbed did the leaves drop. The short hairs on the leaves died before the long conical ones.

Sachs attbributed the injury to the inability of the roots to absorb and convey sufficient water from the cold soil to the leaves to make up for the transpiration deficit and hence the plants died of drought. But Molisch disproved this for Episcia, Sanchezia, and some 10 other plants, by showing that even when they were kept in air having a humidity of an average humidity of 98 percent, thus reducing transpiration to a minimum, the characteristic injury occurred. He also pointed out that plants differed decidedly in susceptibility. Experiments were conducted in darkness, in light, in normal air, in oxygen, and in hydrogen alone and in all cases similar results were obtained. Molisch attributed the injury to disturbances induced in the metabolism (Stoffwechtsell) of the plant. Since it is certain that with the lowering of temperature to slightly above the freezing point, certain functional processes are retarded, if not entirely inhibited, while others continue, that is, the harmonious functioning of the cell processes are upset. He suggested that in tropical plants which have not accustomed themselves to such circumstances harmful metabolism products are produced which accumulate in sufficient quantity to be harmful to the protoplasm.

The following is a list of the more common plants mentioned in Molisch's paper, the name of the investigator, the temperature, and period of exposure to which the plants were subjected and the results.

Plant	Temperature	Period of Exposure	Reaction
(From Bierkander)			
Cucumis melo	1 to 2°C.	5 days	Black spots
Cucumis pepo	"	"	" "
Mirabilis longiflora	"	"	" "
Portulaca aleracea	"	"	" "
Solanum tuberosum	"	" "	" "
Thumbergia capensis	"	"	Dropping of leaves
(From Kunisch)			
Coleus	2 to 7°C.	24 hrs.	Brown discoloration of leaf tips
(From Molisch)			
Nicotiana tabacum	2 to 5°C.	2.5 Mos.	Not injured
Cineraria rugosa	"	"	" "
Less Common Plants from Tropics:			
(From Molisch)			
Episcia	2.4 to 4.4°C.	24 hrs.	Brown spots
Sanchezia nobilis hook	1.5 to 4.2°C.	"	" "
Eranthemum tricolor Nichols	2° to 5°C.	5 days	Dead
Eranthemum igreum Linden	"	1 wk.	"
Anoectochilus setaceus Blume	"	3-4 wks.	"

Faris (1926), working on sugar cane in Cuba, reported the appearance of "Mancha blancas" or white bands two to four inches wide across the leaves in which the tissues seemed to have lost the power to form chlorophyll. He was able to produce similar bands artificially by tying a paper funnel around the plant shoot and filling it with ice for three consecutive nights. The white bands developed six days later. A study of the weather conditions showed that the bands occurred when low temperature followed periods of rainfall. He thought that rain stimulated growth and the cold water standing in the cornucopiae of the stalks chilled the newly exposed tender tissues and thus caused the chlorsis of the leaves. Cold weather not preceded by rainfall caused only slight chlorotic bands. Marked varietal differences were found and some twenty varieties were arranged on a scale of one to ten according to their resistance.

In the Habana Province where this study was made no freezing temperatures have ever been recorded. He concludes that a temperature from 5 to 10^{o}C. for two to three nights is sufficient to cause white bands on susceptible plants. According to Faris cold chlorosis has also been reported from Australia, Hawaii, and Louisiana.

Collins (1927) found that albinism in certain varieties of barley could be induced by growing the plants at temperatures below 45°F. (11.5°C.). When grown at temperatures above 65°F., chlorophyll developed normally.

Collins (1926) states that cool nights, but without frost injure, dwarf and delay growth of young cotton plants.

Marcarelli (1918) observed that yellowing of the young plants of late rice, irrigated with cold water in the upper Vercelli district, Piedmont, Italy, was caused by frequent early morning low temperatures accompanied by mist.

Pantanelli (1918) experimented with the relation between various salts and the injury to plants exposed to temperatures near the freezing point. Wheat, beets and sunflowers were exposed to freezing temperatures and maize and tomatoes to temperatures "a little above 0°C." Different lots in each case were supplied with sodium, potassium, ammonium, and magnesium salts. The same relations were observed whether death resulted from freezing or from cooling alone. The author concludes that "resistance to cold has no connection with the concentration of the cell sap, nor with its content in acids or salts, but with the amount of sugar retained by the cell during cooling." Certain relations, however, were observed between applications of nitrates, phosphoric acid and potassium and the injury sustained by the plants.

EXPERIMENTAL METHODS

The investigations reported herein were carried out during the summer of 1927 in the greenhouses of the Kansas State Agricultural College, Manhattan, Kansas. Good ventilation was afforded and excessive temperatures were prevented by covering the glass with a coating of white wash (Calcium-hydroxide) which, however, excluded very little light. The plants made a normal growth, those not subjected to chilling or other abnormal conditions maturing seed in the usual way. Even soybeans, which often grow thin-stemmed and decumbent in greenhouses, were in no way different from field-grown plants.

The plants were grown in wooden flats or boxes 24 by 24 by 4 inches and in some two thousand red clay 4-inch greenhouse pots. They stood for most of the time on the greenhouse floor. Their position was changed every third day to equalize conditions dependent upon location, since otherwise the outer ones often grew more rapidly than the inner ones. Thick plantings were made and later thinned to one sturdy plant to each pot or about 3 by 4 inches apart in the flats.

Soybeans and velvet beans were germinated in coarse quartz sand and the most vigorous seedlings transplanted to pots on account of the scarcity and low viability of the seed. Twenty-five to one hundred pots were planted at intervals throughout the time these experiments were in progress, with the object of having plants two to three weeks old whenever needed.

The Chilling Chamber. The chilling was effected in a chamber specially constructed in the greenhouse for low temperature investigations. (Fig. 1.) Its walls were constructed of sheet cork, protected on the outside by plaster and cement; the entire thickness of cork and plaster being 12 inches. The chamber was fitted with three doors, each 42 by 24 inches which fitted snugly into the openings they covered. These doors were closed during the night only. During the day they were replaced by double glass fitted into wooden frames, the glasses being separated by a space of three inches. The chamber was approximately 10 feet long, 4 feet wide, and 3 feet deep. The coils cooling the chamber were 6 inches away from the four walls and floor leaving a space of approximately 43 cubic feet between them. Wooden boards were placed lengthwise on cross pieces 6 inches from the floor. On these boards the pots were placed in juxtaposition and at least a pot diameter away from the coils. In no instance were the plants allowed to touch the coils. Plants from each set similarly treated were placed across the chamber so that each group had an equal number of plants in the middle and toward the


Fig

Figure 1. Cold chamber and refrigeration plant in the agronomy greenhouse.

sides. This precaution was hardly necessary since comparisons with three thermometers over a period of four days indicated that there was no appreciable difference in temperature for any length of time in different sections of the chamber.

Cooling was effected by a carbon dioxide refigeration plant thermostatically controlled. By this arrangement it was possible to maintain the desired temperatures within a range of about three centigrade degrees. The temperatures within the chamber and in the greenhouse were continuously recorded by two Tycos thermographs, manufactured by Sharp and Mason, London, registering in degrees Fahrenheit which were checked twice daily with mimimum, maximum, and laboratory thermometers suspended with them. The original temperature records have been included in the appendix of the original copy placed in the college library.

Watering. Watering of all the plants before and after chilling was done daily but at no regular time, approximately 40 to 50 cc. of water being applied to each plant. In all instances (except where quantity of water was the subject of investigation) water was applied immediately before the plants were placed in the chilling chamber. While the plants were being chilled they received no water. Those pots which were saturated prior to chilling were watered a sufficient time previous to chilling to allow all the free water to drain off.

Humidity. The humidity of the air within the chamber ranged, as measured with a Tycos hygrodeik (manufactured) by Lloyds, Rochester, N.Y.) between 95 and 98 percent. Sufficient moisture condensed on the refrigeration pipes to warrant a tri-weekly scooping out of the water from the chamber floor. It seems likely that from the plants very little, if any, transpiration took place. While the pots were in the chamber they remained so moist that watering was unnecessary even over a five day period.

Air Supply. The opening of the doors, replacing and lifting of the glass frames, together with fluctuating temperatures in the chamber, forced in and withdrew sufficient air to effect a regular exchange between the inside and the outside. Under such circumstances the air in which the plants stood was not very different from that in the greenhouse.

Method of Rating Injuries. The amount of injury, retardation of growth and other abnormalities induced by chilling were recorded, in most instances, within 24 hours after the plants were removed from the chamber, while the immediate effects were most clearly visible and again after two or three weeks. All records of injury were made on a percentage basis. As each species reacted in its own particular way, it was impossible to adopt a uniform system of estimating the amount of injury that had taken place. The first notes were based on the proportion of leaf and stem surface showing decided injury. Final notes were based on supposed permanent injury to the plant. Thus a few dry tips or drooping petioles, depending on the total number of leaves of the plant, was rated as a five to ten per cent injury; whereas a total loss of all leaves might be rated at 95 percent and of the stem and all leaves at 100 percent. After a little practice there appeared to be no difficulty in assigning approximately the same figure to plants which had been injured alike at different times. As a rule plants recorded soon after chilling as having suffered twenty-five percent injury or less recovered and were capable of seed production. Those injured more than twenty-five percent and less than fifty recovered occasionally. Plants recorded as fifty percent injured or more seldom recovered.

Scope of the Investigation. Preliminary experiments were first conducted to find what plants might be expected to suffer injury and what tmperatures and periods of exposure were necessary to induce such injury. More careful experiments were then inaugurated to determine not only the temperature and duration necessary to produce injury, but also the relative susceptibility of different crops and varieties as well as the effect of various salt solutions in influencing the degree of injury. The effect of varying amounts of water in the soil was also investigated. Summer crop plants, mostly of subtropical origin and all sensitive to the least degree of frost, were included in this study. Unless otherwise indicated they were grown from Kansas-produced seed. The following is a list of the various plants used:

Cowpeas. Vigna Catjang Var. sinensis
Early Buff variety
Whippoorwill variety from Georgia

Velvet Beans. Stizolobium deeringianum
Ordinary mottled variety from Georgia

Peanuts. Arachis hypogaea
Valentia and Virginia Bunch varieties, source of seed unknown.
Spanish from Georgia

Soybeans. Soya max
Manchu and Virginia varieties

Cotton. Gossipium hirsutum
Westex variety from Texas
Trice variety from North Carolina
Delfos variety from Mississippi
Oklahoma Triumph from Oklahoma

Sunflower. Helianthus annus
White seeded variety

Tomato. _Lycospersicum esculentum var. validum_

Potato. _Solanum tuberosum_

Flax. _Linum usitatissium_

Watermelons. _Citrullus sp._

Pumpkin. _Curcubita pepo._

Tepary Bean. _Phaseolus acutifolius (A. Gray) var. latifolius (Freeman)_

Buckwheat. _Fagopyrum esculentum_

Maize. _Zea mays_

Midland Dent and Colby Bloody Butcher varieties and F_1 Kansas Sunflower, Hybrid Number 5413-1 X 5412-1.

Sorghums. Blackhull kafir, _Andropogon sorghum var. vulgare._

Kansas Orange. _Andropogon sorghum var. saccharatum_

Rice. _Oryza Sativa_

Early Prolific and Honduras varieties from California

Sudan grass. _Androphogon Sudanense_

Teff grass. _Eragrostis Abyssinica_

From the Union of South Africa.

EXPERIMENTAL RESULTS

The outstanding result of the investigation was the very evident injurious effects of chilling[1] on certain plants and the high degree of resistance in others. A point of considerable interest was the reaction of different species and varieties not only with respect to differences in the degree but also with respect to the nature of the injury. It seems desirable therefore to describe briefly the nature of the injury in each case.

Nature of the Injuries. The most obvious effect of chilling on susceptible plants was the drying and falling off of the leaves or portions of leaves in a way very similar to that which follows frost damage. The effect, however, in all except velvet beans, took place much more slowly, being apparent only from 24 hours to several days after chilling. In velvet beans, the effect was visible an hour or two after chilling when the leaves of young plants showed light purplish or purplish to black discoloration. This condition was followed by the drying of the leaves in the course of two to three days. Where the exposure was brief there was no immediate effect but in the course of five to seven days light brown areas appeared on the leaves. Four to five-week-old plants showed no abnormalities at first but in a day or two a very pronounced chlorotic condition set in throughout the entire

1. Chilling in this study refers in all cases to temperatures above zero Centigrade. In no case was the temperature allowed to go below zero.

plant, from the top downward, some four to five days after chilling, accompanied by the dropping of the turgid leaves.

In most cases the effect on cowpeas was not immediately visible except that when they came out of the chilling chamber the leaves were partly folded. A chlorotic condition confined to the other leaves and more especially to leaf sections, similar to that of the older velvet bean plants, usually developed within 36 hours. The leaves dropped while turgid and still green for the greater part. Slight injuries were evident in the form of disrupted or blistered areas confined to the edges of the younger leaves (Fig. 2). These areas eventually dried, turned brown, and disintegrated, leaving a leaf with a jagged outline. Cowpea plants were seldom killed outright, although the leaves were easily injured and the growth of the roots was considerably retarded, (Fig. 4). New growth was generally produced from the uninjured stem buds. (Fig. 6).

The after effects of chilling manifested by cotton and soybeans were very similar, although as pointed out later, there was the most marked difference in the degree of injury. The most striking effect was the presence of spots or fringes of light green which later turned to a white coriacious condition. To the casual observer such leaves would seem to have been injured by frost except that with chilling the entire leaf was seldom affected. In severly injured leaves and occasionally in others, a narrow brick red band separated the living and dead portions of the leaves (Fig. 3).

Peanuts showed no obvious effects immediatly after chilling. After about three days the tops wilted more and more during the afternoons, recovering somewhat at night. Wilting and recovery alternated until the peanuts died or recovered completely. Later examination showed that the root systems had been seriously injured (Fig. 5). In the case of young Virginia Bunch peanut plants a few leaflets developed narrow, dry brittle borders. More often individual leaflets became much dwarfed in comparison with the normal ones next to them (Fig. 2, bottom). In general the tops were uninjured. Injuries to the root systems, however, as will be shown later, were sufficient to greatly retard growth and in some cases to cause the death of the plants.

In watermelons and pumpkins the margins of the leaves turned light green and ultimately brown. When severly chilled the plants were unable to remain upright, the leaves then turning slightly yellow and folding inward. Chilling very clearly inhibited the rate of growth but otherwise there was no marked effect either with respect

Fig. 2.

Figure 2. Types of injuries shown by various plants after chilling. Left to right.

Top row--Velvet bean basal leaves.

(1) leaf from a slightly injured plant; (2) and (3) from severely injured plants.

Middle row--Early Buff cowpeas.

(1) terminal leaf from slightly injured plant; (2) and (3) from severely injured plants.

Bottom row--Peanut leaves showing characteristic dwarfing of leaflets and fringing that occurs after chilling.

Side--Kansas Orange Sorghum.

Left--irregular Faris band.

Right--fringed tip.

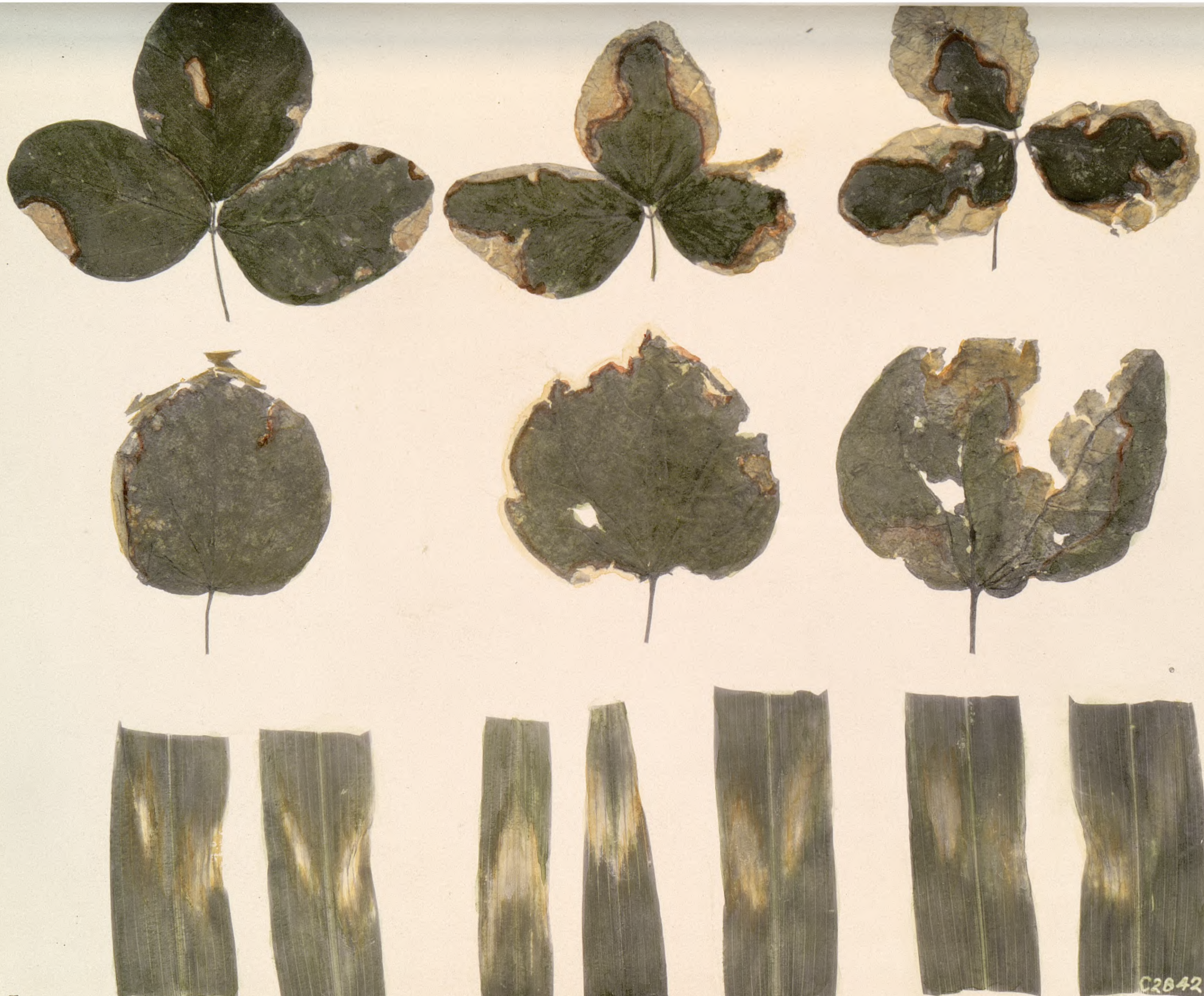

Fig. 3.

Figure 3. Types of injuries shown by various three-week-old plants after chilling. Left to right.

Top row--leaves from Virginia soy bean plants chilled at 0.5 to 5.0° C. for 72, 96 and 132 hours respectively.

Middle row--Delfos cotton leaves chilled 48, 72 and 96 hours respectively.

Bottom row--"Faris" or chill bands on maize leaves from plants chilled for 60 hours at 2 to 4° C.

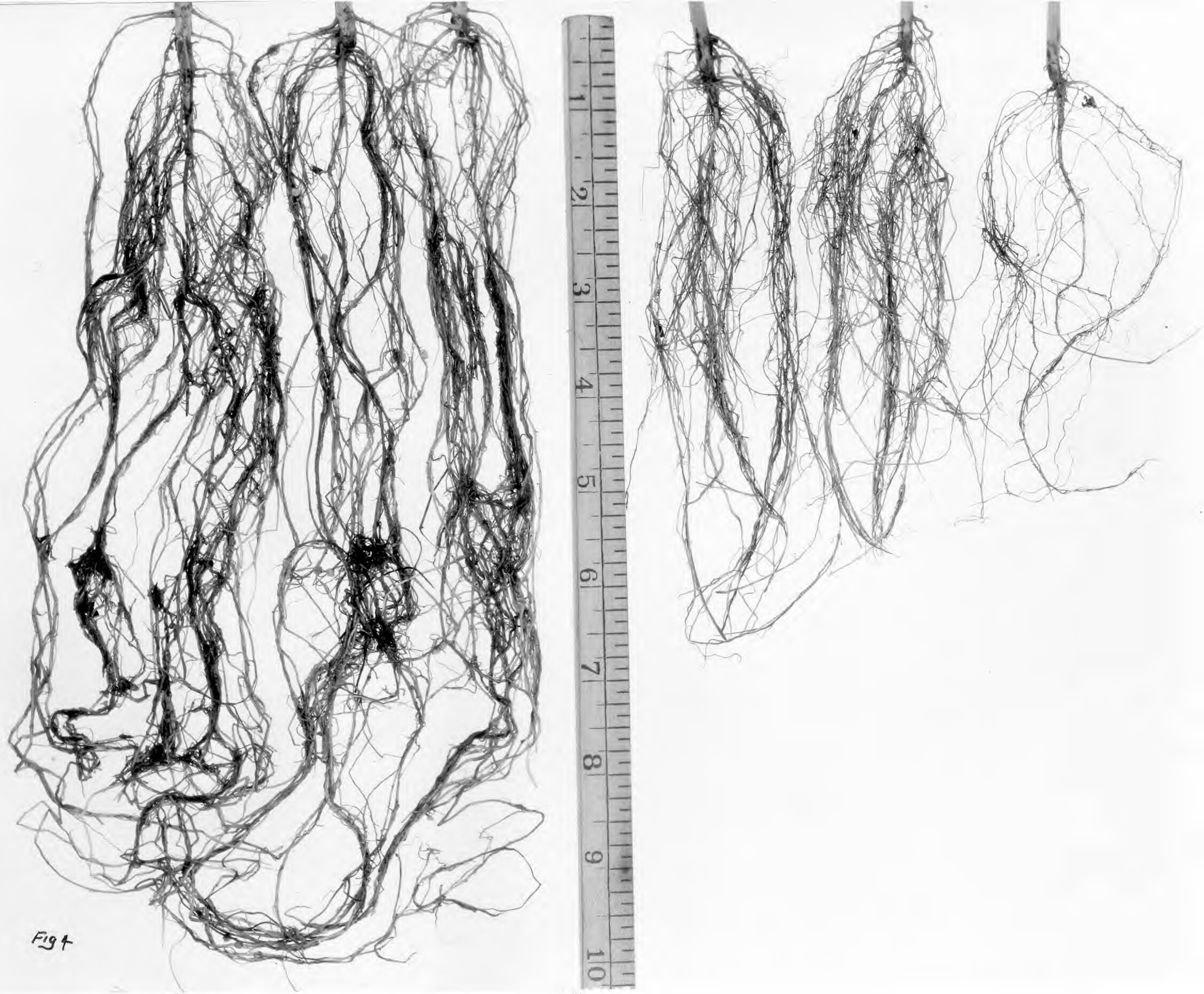

Fig 4

Figure 4. The effect of chilling on 3-week-old Whippoorwill cowpea plants. Left, not chilled. Right, chilled for 36 hours at 2 to 4^{o} C. Plants six weeks old when photographed.

Fig.5.

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Figure 5. Injuries to the roots and tops of one-month-old Spanish peanut plants when chilled at 2 to 4° C. Left to right. (1) and (2) chilled for 84 hours; (3) and (4) not chilled.

Fig. 6

Figure 6. Nature of growth and recovery made by Early Buff cowpeas one month after chilling. Chilled for various lengths of time when three weeks old. Left to right.

(1) not chilled.
(2) chilled 24 hours.
(3) chilled 60 hours.
(4) chilled 84 hours.
(5) chilled 90 hours.

to the roots or leaves.

Buckwheat and Tepary beans were severely wilted during and immediately after chilling appearing as though they had been frosted. They recovered later to the extent that after a week or two scarcely any injury was perceptible.

Potatoes, tomatoes, and flax showed no appreciable injury or retardation in growth in any part during or after chilling, even when chilled as long as 120 hours. In a few tomato plants some half-developed leaf tips lost their turgidity and became pale green. Recovery in every case was very rapid.

Chilling had no marked immediate effect on maize, sorghum, Sudan grass or Teff grass. From five to ten days after chilling there developed on the leaf blades light yellow transverse bands, varying in size and intensity of color, similar to those described by Faris (1926) in sugar cane. Their breadth and number per plant were proportional to the duration of the chilling. With advancing age these areas became transparent, filmy, and rust colored toward the edges (Fig. 3, bottom). It was evident that these bands occurred on those parts of the blades which formed the cornucopia of the plant at the time of chilling. In this region the most active growth takes place and incidentally here the youngest and most easily injured tissue is to be found. Faris (1926) suggests that the water held in the cornucopia chills the tender tissue in immediate contact with it. During the course of this investigation water was seen to have collected in the cornucopiae of the maize and sorghum plants used. Plants severely injured allowed their leaves to roll or droop as a browning of the entire plant set in and when dead appeared not unlike plants that had succumbed to drought or from intense heat.

Microscopic examination of maize root sections from chilled and unchilled plants showed brown lesions on the roots of chilled plants only. Below these the epidermal tissue had perished. Mycelia were found in apparently healthy root sections of chilled plants, while plants not chilled failed to reveal any abnormalities either internally or externally.

Unlike the foregoing plants, rice plants became yellow first in the leaf sheaths and then in the upper halves of the blades. When the chlorotic condition was confined to the sheaths, the plants recovered but when more extensive they perished. They did not develop Faris bands, nor did they show injuries on the roots. The yellowed sheaths invariably peeled away from the leaves they enveloped, becoming somewhat white, while their blades took on streaks of yellow and faint red areas.

Cessation of normal functioning and discoloration took place alike throughout the entire plant, without any one part showing especially characteristic markings of its own.

Relation of Period of Exposure to Injury

One of the first points to be investigated was the duration of exposure necessary to produce damage. For this study various plants were exposed to temperatures slightly above freezing for various periods of time ranging in general from 12 to 132 hours. Six to ten plants were used as a unit and the temperature in the chilling chamber was 0.5° to 5.0°C in every case except as otherwise stated. Tables I to III give the averages of the estimated percentage injury sustained by the individual plants, as well as the least and greatest percentage injury in each set.

TABLE I

EFFECT OF CHILLING ON LEGUMINOUS PLANTS

Length of time chilled at 0.5 to 5.0°C., Hours	Percentage injury to plants: 24 hrs. after chilling: Average per plant	Percentage injury to plants: 24 hrs. after chilling: Range	Percentage injury to plants: 2 weeks after chilling: Average per plant	Percentage injury to plants: 2 weeks after chilling: Range	Height of plants four weeks after chilling. Inches	
Velvet Beans- (Three weeks old).						
12	0.0	0-00	4.4	0-10	---	---
24	11.4	8.30	46.4	30-100	---	---
36	42.4	10.50	80.	60-100	---	---
48	60.3	10-90	98.	100-100	---	---
Early Buff Cowpeas- (Three weeks old).						
12	0.0	0-0	3.	3-3	---	11-13
24	3.0	0-9	10.	10-10	---	13-14
36	26.5	15-30	20.	20-20	---	12-14
60	45.0	44-55	40.	40-40	---	11-12
84	55.7	40-60	60.	60-60	---	9-10
96	77.0	70-90	96.	96-96	---	5-7
Spanish Peanuts- (Three weeks old.)						
0	---	---	---	---	6.1	5-7
36	0.7	0-3	5.3	0-8	5.2	3.5-6
48	5.0	4-9	70.0	35-90	3.6	3-45
84	10.0	5-12	70.5	15-90	3.3	3-4
96	4.0	4-5	88.5	70-100	3.5	2.5-4
120	7.8	6-12	95.8	90-100	2.8	2-4
Manchu Soybeans- (Four weeks old).						
84	0.0	0-0	0	0-0	---	---
120	0.0	0-0	0	0-0	---	---
Virginia Soybeans- (Three weeks old).						
48	6.2	0-10	4.8	0-10	20.1	12-26.5
72	44.	25-60	40.	20-100	17.7	10-23

Table I, continued.

96	45.0	15-70	39.0	20-80	16.0	10-25
132	51.3	0-70	60.0	20-100	14.4	9-17
Tepary Beans - (Two weeks old).						
48	5.	5-5	0.	0-0	---	---
84	80.	80-80	5.	5-5	---	---

TABLE II

EFFECT OF CHILLING ON SORGHUMS AND MAIZE

Length of time chilled at 0.5 to 5.0°C., Hours	Percentage injury to plants				Height of plants Four weeks after chilling. Inches.	
	24 Hrs. after chilling		2 weeks after chilling			
	Average per plant	Range	Average per plant	Range	Average Per plant	Range
Blackhull kafir- (Five weeks old).						
0	---	---	---	---	19.6	18-20
24(a)	0.0	0	3.0	0-5	19.1	18-19
48	0,0	0	4.0	3-5	16.5	15-18
72	0.0	0	4.3	2-6	17.2	15.5-18
Blackhull kafir- (Six weeks old).						
24(a)	0.0	0	3.0	5-10	---	----
48(a)	0.0	0	5.4	5-7	---	----
60	0.0	0	3.0	0-15	---	----
72	0.0	0	10.0	10-10	---	----
Kansas Orange Sorgo- (Six weeks old).						
0	---	---	---	---	18.0	16-21
24(a)	0.0	0	0	0	21.6	16-22
48(a)	0	0	2.2	3-5	19.1	16.5-22
60	0	0	5.9	0-10	18.8	16-22
72	0	0	5.1	0-12	16.2	15-18
Maize- Midland Dent- (Six weeks old).						
0	---	---	---	---	38.3	31-40
24	5	5-5	5	5-5	---	26-36
48(a)	10	10-10	15	15-15	---	22-33
60(a)	20	20-20	20	20-20	---	22-31
72	30	30-30	25	25-25	28.8	21-37

(a) Five plants only.

TABLE III

EFFECT OF CHILLING ON MISCELLANEOUS PLANTS

Number of plants	Age Wks.	Species	Time chilled. Hrs.	Temperature	* Injury	Hght. of plants three weeks after chilling. Inches
20	2	Early Prolific Rice	12	2 to 4°C.	20	8-12
20	2	" "	24	"	42	6
20	2	" "	36	"	96	d
20	2	" "	48	"	100	d
20	2	" "	72	"	100	d
20	2	Honduras Rice	12	"	12	8-12
20	2	" "	24	"	18	6
20	2	" "	36	"	25	d
20	2	" "	48	"	100	d
20	2	" "	72	"	100	d
20	3	Sudan Grass	12	"	10	11-16
20	3	" "	24	"	10	11-15
20	3	" "	36	"	20	11-15
20	3	" "	48	"	35	9-11
20	3	" "	72	"	50	7-9
20	3	Teff "	12	"	11	12-16
20	3	" "	24	"	20	12-16
20	3	" "	36	"	15	11-13
20	3	" "	48	"	35	9-10
20	3	" "	72	"	50	d
6	4	Watermelon	36	0.5 to 5.0°C	10	--
--	-	" "	60	"	60	--
6	4	Pumpkin	48	"	15	--
--	-	"	84	"	55	--
12	4	Buckwheat	84	"	5	--
6	3	Tomato	36	"	5	--
--	-	"	84	"	5	--
6	8	Tomato	36	"	5	--
--	-	"	120	"	5	--
12	4	Potato	84	"	0	--
6	4	Sunflower	84	"	0	--
--	-	"	120	"	0	--
30	3	Flax	84	2 to 4°C.	0	--
--	-	"	120	"	0	--

d--dead.

* Average percentage injury 48 hours after chilling.

From the foregoing tables it is clearly evident that some plants were exceptionally sensitive to the injurious influences of chilling, while others were exceedingly hardy. Thus an exposure of 12 hours produced noticeable injury in rice and velvet beans, and 24 hours or longer caused severe injury, (Fig. 7, top). Early Buff cowpeas were severely injured by an exposure of 60 hours or longer, (Fig. 8, top.). Peanuts, Sudan grass and Teff grass were slightly injured by a 24-hour exposure, severe injury occurring only when chilled for more than 36 hours. Peanuts reacted differently than the others in this group in that the effects were not apparent until several days after were chilled, a circumstance due to the fact that the roots and not the tops were injured, (Fig. 8, bottom). Maize, sorghums, watermelons, and pumpkins were hardier still as they showed after 48 hours exposure only about 10 to 15 percent injury and at that were very slow in showing abnormalities. The remainder of the plants included in this investigation can be considered as being highly resistant to chilling. Thus,buckwheat, Tepary beans and Virginia soybeans were injured only 10 percent when chilled for 60 hours and potatoes and sunflowers exhibited no injury when chilled for twice that period. The ability of many of these crops especially such as tomatoes to resist chilling is of special interest as

Fig. 7.

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Figure 7. Injuries to three-week-old Velvet beans (top row) and three-week-old Delfos cotton plants (bottom row) when chilled at 0.5 to 5.0° C., left to right chilled 12, 36, 60 and 84 hours, respectively.

Fig. 8.

Figure 8. Relation of temperature to injury.

Top row, left to right--injury to four-week-old Early Buff cowpeas when chilled at 0.5 to 5.0° C. for 0, 24, 36, 60 and 84 hours respectively.

Bottom row--injury to four-week-old Spanish peanuts when chilled at 0.5 to 5.0° C. for 0, 2, 36, 48, 84 and 96 hours respectively.

in view of their inability to survive subzero temperatures.

Relation of Temperature to Injury

Several of the plants which were found to be exceptionally sensitive, namely, Delfos cotton, velvet beans, and Whippoorwill cowpeas were subjected to a temperature range of 5 to 10°C. for various lengths of time. The injury sustained by each at the various intervals is given in Table IV. It is evident that severe injury occurred at the higher temperatures but as would be expected a longer period of exposure was necessary. Whippoorwill cowpeas, however, were very sensitive even at this temperature, being rather severely injured by an exposure of 48 hours. Delfos cotton and velvet beans survived exposures of the same period without severe injury. This is of special interest in view of the fact that velvet beans were very sensitive at the lower temperatures. As at the lower temperatures more damage was apparent two weeks after chilling than 24 hours after. Altogether, the results may be taken to show that injurious effects may be brought on with rather brief exposures at temperatures considerably above the freezing point.

TABLE IV

EFFECT OF CHILLING ON VELVET BEANS; COTTON AND COWPEAS

Length of time chilled at 5 to 10°C.	Percentage Injury to Plants			
	24 Hours after chilling		2 weeks after chilling	
	Average per plant	Range	Average per plant	Range
(Plants three weeks old).				
Delfos Cotton				
48	0	0	25	25-25
72	3.0	0-12	30	30-30
96	7.7	0-12	38	35-40
120	51.0	25-60	78	40-100
Velvet Beans				
48	1.8	0-8	1.6	0-6
72	12.2	0-30	9.0	0-30
96	40.0	35-50	42.5	10-100
120	73.0	35-90	86.0	50-100
Whippoorwill Cowpeas				
24	0.0	0-0	40.0	40-40
48	41.6	20-50	41.6	20-50
72	59.0	35-70	70.0	60-100
96	71.8	65-80	100.0	100-0
120	92.1	90-96	100.0	100-0

Varietal Differences

That there may be important varietal differences has been suggested by the data already presented. In view of the importance of this subject in relation to varietal adaptation and improvement, some tests were made to differentiate the extent of such differences. Three varieties of cotton and three of peanuts were therefore chilled for various periods of time with the results presented in Table V.

TABLE V

EFFECT OF CHILLING ON VARIETIES OF COTTON AND PEANUTS

Variety	24 Hrs. after chilling	2 Wks. after chilling	3 Wks. after chilling
Cotton Plants. (Three weeks old).			
Chilled 24 hours at 2 to 5°C.			
Trice	60	45	30
Delfos	75	56	40
Westex	65	60	45
Chilled 84 hours at 2 to 8°C.			
Trice	55	70	70
Delfos	80	85	80
Westex	90	90	95
Chilled 96 hours 5.0 to 10°C.			
Trice	40	70	55
Delfos	75	85	80
Westex	85	95	100
Chilled 108 hours at 5.0 to 10°C.			
Trice	60	75	30
Delfos	75	75	70
Westex	85	85	85
Average of 4 exposures.			
Trice	54	65	46
Delfos	76	75	67
Westex	81	82	81
Peanuts. (Plants five weeks old)			
Chilled 84 hours at 0.5 to 5.0°C.			
Virginia Bunch	15.5	25	12
Spanish	54.4	88	100
Valentia (a)	68.0	95	100
Peanuts. (Plants eight weeks old)			

Chilled 144 hours at 2 to 10°C.

Virginia Bunch (a)	43.3	90	--
Spanish (a)	60.0	95	--
Valentia (a)	71.0	100	--

(a) Five plants only.

That varieties of cotton and peanuts differ greatly in their ability to resist the influences of chilling is very evident. Trice cotton proved to be the most hardy followed by Delfos, while Westex was the most sensitive. Thus an average for all trials was 46 percent for Trice, 67 percent for Delfos, and 81 percent for Westex (Fig. 9, bottom).

But an even greater differences were exhibited between varieties of peanuts. Virginia Bunch was exceptionally hardy while Valentia and Spanish were very sensitive. An exposure of 84 hours was fatal to Valentia but scarcely injured the Virginia Bunch. Spanish was intermediate. The same relation obtained with an exposure of 144 hours (Fig. 9, top).

Attention has already been called (Tables I-IV) to the susceptibility of Whippoorwill cowpeas, a variety from Georgia which was injured far more than early Buff from Kansas, although exposed to a temperature approximately five degrees higher.

Important differences were also observed in rice, Honduras being injured decidedly less than Early Prolific (Table III). Similar differences were observed with soybeans. Virginia soybeans were severely injured with an exposure of 72 hours whereas Manchu survived an exposure of 120 hours without injury.

Fig. 9.

Figure 9. Effect of chilling on three-week-old varieties of peanuts (top) and cotton (bottom). Left to right: Top row -- peanut plants chilled at o.5 to 5.0^{o} C. for 120 hours. (1) Virginia Bunch peanuts, (2) Spanish peanuts, (3) Valentia peanuts, (4) Spanish peanuts not chilled. Bottom row -- Cotton chilled at 2 to 8^{o} C., for 84 hours. (1) Trice cotton, (2) Delfos cotton, (3) Westex cotton, (4) Delfos cotton not chilled.

Relative Injury to Different Plants

The plants included in this investigation can be conveniently arranged into five classes according to their manner of reacting to low temperatures. This classification is based on the degree of injury as well as on the particular mode of reaction exhibited by each species (Fig. 10). Such a classification must of course be considered as distinctly preliminary owing to the incompleteness of the investigation and is presented only for convenience.

In class I are included those plants which are killed by an exposure of 60 hours to temperatures from 0.5 to 5.0°C. They are rice, Velvet beans, cowpeas and cotton.

In class II are included those which are decidedly injured by such temperatures but which with favorable conditions will recover. This class includes Sudan grass, Teff grass, Spanish and Valentia peanuts.

Class III includes those which in general are not likely to suffer serious injury by the conditions specified above. They are Virginia Bunch peanuts, maize, sorghum, watermelons and pumpkins.

Class IV includes those likely to be injured less than 10 percent; namely, buckwheat, Tepary beans, and soybeans.

Fig10.

Figure 10. Injuries to different plants when chilled at 0.5 to 5.0° C., for 72 hours. Left to right: (1) Velvet bean one month old, (2) Cowpea 17 days old, (3) Westex cotton 10 days old, (4) Virginia Bunch peanuts one month old, (5) Manchu soybeans one month old.

Class V includes those plants which when exposed at 0.5 to 5.0°C. were not injured so far as could be observed. They are potatoes, sunflowers and flax.

The Relation of Moisture to Chilling Injury

In order to learn what effect the amount of moisture in the soil had on the percentage of injury from chilling varying amounts of water were added for five days the plants were then chilled. Water was withheld from them 24 hours prior to any differential treatment being applied. The normal amount of water the plants received each day was 35 to 40 cc. This was sufficient to leave the soil in each four-inch pot more or less saturated. The least amount applied was 10 cc. per pot per day, being the minimum at which plants could be kept alive. In all, the following amounts were applied in various experiments; namely, 10, 15, 20, 23, 40, 55, 90, 120 cc. daily and 90 cc. daily with six hours submersion immediately before chilling. The results a s given in Table VI show that the amount of moisture in the soil had a profound influence on the sensitivity of the plants.

TABLE VI

THE RELATION OF SOIL MOISTURE TO THE EFFECT OF CHILLING ON VARIOUS PLANTS

Amt. of water applied to plants Cu.cc.	Percentage Injury to Plants						Height of plants-Inches
	24Hrs. after chilling		2Wks. after chilling		Plants chilled		Plants not chilled
	Ave.p. plant	Range	Ave.p. plant	Range	Ave.p. plant	Range	Ave.p.plant or range
Velvet Beans. (Three weeks old)							
Chilled 84 hours at 0.5 to 5.0°C.							
c.c.							
15	44	20-60	d	d	--	--	--
25	58	20-75	d	d	--	--	--
55	68	25-95	d	d	--	--	--
90	85	70-94	d	d	--	--	--
Velvet Beans.							
Chilled 18 hours at 2 to 4°C.							
20	.75	0-20	14	5-20	--	--	--
40	32	10-85	38	15-100	--	--	--
120	82	65-100	80	60-100	--	--	--
Manchu Soybeans. (Four weeks old).							
Chilled 84 hours at 0.5 to 5.0°C.							
15	12.4	10-20	13.0	5-20	15.7	11-19	--
25	7.0	7-7	2.6	2-3	20.0	17-24	--
55	2.0	1-4	1.4	0-3	25.4	22-28	--
90	1.2	1-2	4.6	4-6	27.4	22-31	--
90s	0.0	0-0	0.0	0-0	24.0	17-22	--
Black Eye Cowpeas. (Four weeks old).							
Chilled 84 hours at 0.5 to 5.0°C.							

(Continued next page)

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15	46	40-50	49	45-50	6.8	6-7	--
25	50	40-60	80	80-80	6.6	5-8	--
55	60	25-80	86	75-90	9.2	10-11	--
90	80	75-90	80	80-80	10.4	10-11	--
90s	79	50-80	80	80-85	8.8	7-9	--

Early Buff Cowpeas. (Three weeks old).

Chilled 36 hours at 2 to 4°C.

10	30	30-30	20	---	8.2	6-9	10
20	40	40-40	30	---	8.5	7-10	10
40	40	40-40	40	---	8.5	7.5-10	13.2

Delfos Cotton. (Three weeks old).

Chilled 18 hours at 2 to 5.0°C.

10	2.2	0-5	5	5-5	6.1	6-7	5.5
20	3.0	0-10	10	10-10	5.6	5.5-6	5.7
40	11.8	0-25	20	20-20	6.3	5.5-7	6.1

Spanish Peanuts. (Three weeks old).

Chilled 60 hours at 2 to 4°C.

10	0	0	7	5-12	3.5	2-5	2.5-3.5
20	0	0	12	0-20	4.4	2-5	5-5
40	0	0	25	25-25	4.7	3-5	5-6

Spanish Peanuts.

Chilled 84 hours at 2 to 4°C.

10	0	0	20	20-20	--	--	--
20	0	0	60	60-60	--	--	--
40	0	0	75	75-75	--	--	--

Maize; (Kansas Sunflower Hybrid F_1). (Three weeks old)

Chilled 36 hours at 2 to 5°C.

10	0	0	6	5-10	9.5	9-10	8
20	0	0	35	35-35	10.3	9-12	9
40	0	0	50	50-50	9.5	9-10	11

(Continued next page)

Continued from page 45.

Maize, (Kansas Sunflower F_1 Hybrid)

Chilled 60 hours at 2 to 5°C.

10	0	0	7	5-15	--	--	--
20	0	0	42	40-45	--	--	--
40	0	0	50	50-50	--	--	--

(d) Died.
(s) Submersed for 6 hours prior to chilling.

In almost every instance plants were far more severely injured in saturated than in moderately wet soil. In very dry soil they suffered slightly more than in the latter, but considerably less than in saturated soil. Thus the injury two weeks after chilling on Velvet beans exposed for 18 hours was 14 percent in dry soil and 80 percent in wet soil. Corresponding figures for Early Buff cowpeas exposed for 36 hours were 20 percent and 40 percent for dry and wet soil and for Spanish peanuts exposed for 84 hours, 84 percent and 75 percent respectively. Manchu soybeans were injured slightly more in dry soil but this may possibly have been due to drought rather than too cold. Cowpea plants were very slow to recover when they were chilled in dry soil, even though they showed here the least reaction; when chilled in saturated soil they recovered very quickly considering the degree of injury.

The influence of various amounts of moisture in determining the nature of the reaction suggests that the contention often advanced that plants are injured by cold during wet spells is by no means amiss. Also, that when low temperatures, sufficiently low to induce chilling, occur chilling are apt to be more severe than during dry weather, or at least while the plants are standing in dry soil.

Influence of Chilling on Plants of Different Ages

In the greater part of this investigation plants were chilled when three weeks old. In order to determine the relative sensitiveness of plants at different ages, Velvet beans, Delfos cotton and Black Eye cowpeas of various ages were chilled with the results as given in Table VII.

The results of this experiment show that in general the younger the plants, the more they were injured. Velvet beans, 28 days old, chilled for 24 hours at 0.5 to 5.0°C, were injured only 9.4 percent while plants half this age showed double this amount, and nine-day-old plants were killed. Similar, though much less marked differences, were observed with the Black Eye cowpeas (Fig. 11). As for the cotton plants there was a tendency for them to be most hardy when 35 days old. Even as short a chill as 24 hours at 2 to 4°C proved fatal to many ten-day-old plants whereas 17 and 25-day-old ones succumbed only after

Fig. 11.

Figure 11. Injury to Black Eye cowpeas of various ages when chilled at 0.5 to 5.0° C. Left to right: (1) One month old not chilled, (2) one month old, chilled 96 hours -- partly killed, (3) three weeks old, chilled 72 hours -- killed outright.

after 48 hours (Fig. 12). However plants 58 days old withered, dropped their leaves and were injured in all cases more than those 35 days old.

That young plants and the newer parts of old plants suffered more than the full-grown seems not unnatural. It would follow from this that where the most vigorous growth is taking place, and where cell activity is most intense, there the changes are brought about which ultimately have a detrimental influence on parts or on the plant as a whole.

Fig. 12.

Figure 12. Injuries shown by Delfos cotton plants of various ages when chilled at 1.5 to 5.0° C, for 96 hours. Left to right: (1) Twenty-six days old not chilled, (2, 3, and **4** are 26, 19, and 10 days old, respectively. Photographed 24 hours after chilling.

TABLE VII

EFFECT OF CHILLING ON PLANTS OF DIFFERENT AGES

Age of plants days	Percentage of Injury			
	24 Hrs. after chilling		2 Wks. after chilling	
	Average per plant	Range	Average per plant	Range
Velvet Beans.				
Chilled 24 hours at 0.5 to 5.0°C.				
9	51	30-85	--	100
14	20	15-30	--	80-100
28	9.4	5-25	--	70-100
Chilled 36 hours.				
9	61	50-85	--	100
14	25	20-30	--	80-100
28	18	10-30	--	70-100
Black Eye Cowpeas.				
Chilled 72 hours at 0.5 to 5.0°C.				
21	65	--	--	--
28	50	--	--	--
Delfos Cotton.				
Chilled 24 hours 2 to 4°C.				
10	21	0-80	21	0-80
17	10	0-20	10	0-20
Chilled at 48 hours.				
10	75	60-90	95	90-100
17	62	10-90	86	40-100
25	47	10-92	53	5-100
Chilled 72 hours.				
10	97	85-100	100	100
17	96	89-97	100	100

Continued from page 50.

25	83	65-90	98	90-100
35	6.6	0-20	9.8	0-30
58	8.3	4-15	15.3	6-20

Chilled 96 hours.

25	88	70-96	100	100
35	18.2	0-35	18.1	0-35
58	62	50-80	71.2	30-100

Chilled 108 hours.

35	30.6	25-50	48	40-60
58	72.0	70-85	93.7	5-100

The Relation of Salt Solutions to Chilling Injury

To learn whether the presence of certain salt solutions in the soil would have an influence on the reaction of plants to chilling, the following experiment was conducted.

Forty cubic centimeters of one-twentieth molecular solutions of calcium chloride, calcium nitrate, potassium chloride, potassium nitrate, sodium chloride and sodium nitrate were applied to peanut, cowpea, maize and cotton plants for six consecutive days prior to chilling, previous to which they received no water for 48 hours. To the control plants an equal amount of tap water was applied for the same length of time. For the salt solutions tap water was also used. The plants were chilled when three weeks old unless otherwise stated. To find out whether base exchange had a possible bearing on the influence of these salts a part of the maize plants were grown in quartz sand with potassium nitrate, calcium nitrate and tap water. The influence of these solutions on the retardation or hastening of the ill effects of chilling, as the case may be, proved very interesting. The results are given in Tables VIII to XII.

TABLE VIII

EFFECT OF SALT SOLUTIONS ON THE REACTION OF MAIZE TO CHILLING

Solution	Percentage injury after chilling: after 48 hrs.: Av. per plant	Range	after 2 weeks: Av. per plant	Range	Height of plants--inches: plants chilled: Av. per plant	Range	not chilled: Av. per plant	Range
Bloody Butcher Corn (4 weeks old)								
chilled 72 hours 0.5 to 5.0° C.								
Water	51	45-60	26	20-30	18.4	18-19	--	--
KNO_3	12	10-15	0	0-0	22.2	22-23	--	--
$Ca(NO_3)_2$	4	3-8	11	0-20	25.0	25-25	--	--
$NaNO_3$	11	10-15	19	15-30	19.0	16-21	--	--
chilled 108 hours 0.5 to 5.0° C.								
Water	20	20-20	78	45-100	17.5	----	23.9	23.5-24
KNO_3	42	30-60	61	35-100	20.5	----	---	---
$Ca(NO_3)_2$	46	30-60	86	35-100	20.0	----	---	---
$NaNO_3$	40	40-40	95	75-100	11.0	----	---	---
Kansas Sunflower Corn F1 Hybrid (2 weeks old)								
chilled 36 hours 2 to 4° C.								
Water	--	---	50	50-50	10	10-10	11.0	11-11
KNO_3	--	---	36	30-40	7.9	7-8	11.5	11-12
KCl	--	---	45.5	45-48	8.9	8-9.5	12.0	12-12
$CaCl_2$	--	---	60	60-60	9.0	9-9	11.0	11-11
$Ca(NO_3)_2$	--	---	70	70-70	8.3	7.5-10	12.0	11-13
NaCl	--	---	65	65-65	9.7	9.5-10	10.0	8-12
$NaNO_3$	--	---	84	80-90	8.0	6-9	12.0	12-12

Continued from page 53.

chilled 60 hours 2 to 4° C

Water	--	---	50.0	50-50	--	---	--	---
KNO_3	--	---	29.0	25-45	--	---	--	---
KCl	--	---	40.0	35-45	--	---	--	---
$CaCl_2$	--	---	57.0	57-57	--	---	--	---
$Ca(NO_3)_2$	--	---	64.7	61-68	--	---	--	---
NaCl	--	---	77.8	77-90	--	---	--	---
$NaNO_3$	--	---	85.0	80-90	--	---	--	---

TABLE IX

EFFECT OF SALT SOLUTIONS ON THE REACTION OF MIDLAND DENT MAIZE TO CHILLING

Solution	Percentage injury after one week	
	Average	Range
(2-week-old plants grown in quartz sand, chilled 72 hours at 2 to 4° C.)		
Water	26.5	20-30
KNO_3	22.2	0-50
$Ca(NO_3)_2$	74.3	70-85

TABLE X

EFFECT OF SALT SOLUTIONS ON THE REACTION OF EARLY BUFF COWPEAS TO CHILLING

(plants 3 weeks old chilled 2 to 4^{o} C.)

	Percentage injury				Height of plants--inches 3 weeks after chilling			
	24 hrs. after chilling		2 weeks after chilling		plants chilled		not chilled	
Solution	Av. per plant	Range	Av. per plant	Range	Av. per plant	Range	Av. per plant	Range
chilled 24 hours								
Water	5	0-15	20	15-25	10.1	9-11	13.2	12-14
KNO_3	13	10-15	12	10-15	10.2	9-12	11.5	10-13
KCl	45	45-45	17	15-20	11.0	9-12	10.0	10-10
$CaCl_2$	32	10-40	37	35-40	10.0	9.5-10.5	12.4	10-14
$Ca(NO_3)_2$	49	35-55	32	30-35	10.2	9-13	12.2	11-13
NaCl	40	25-55	42	40-45	10.1	9.5-11	10.5	9.5-11
$NaNO_3$	50	50-50	50	45-55	10.1	9.5-11	12.2	11-13
chilled 36 hours								
Water	40	40-40	40	40-40	8.5	7-10	13.2	12-14
KNO_3	40	40-40	50	50-50	8.3	7.5-10	11.5	10-13
KCl	50	50-50	40	40-40	8.5	8-10	10	10-10
$CaCl_2$	56	50-60	70	70-70	8.4	7-10	12.4	10-14
$Ca(NO_3)_2$	50	50-50	60	60-60	9.0	8-10	12.2	11-13
NaCl	60	60-60	75	75-75	7.7	7-8.5	10.5	9.5-11
$NaNO_3$	65	65-65	85	85-85	7.9	7-8.5	12.2	11-13

TABLE XI

EFFECT OF SALT SOLUTION ON THE REACTION OF SPANISH PEANUTS TO CHILLING

Solution	Average percentage injury: 24 hrs after chilling: entire plant	Average percentage injury: one week after chilling: entire plant	Average percentage injury: two weeks after chilling: entire plant	Average percentage injury: two weeks after chilling: roots only	Average height of plants--inches: 2 weeks after chilling: chilled	Average height of plants--inches: 2 weeks after chilling: not chilled
chilled 60 hours 2 to 4° C.						
Water	0	25	40	60	4.5	5.5
KNO_3	0	50	20	30	4.0	7.0
KCl	0	18	30	40	3.5	7.5
$CaCl_2$	0	18	50	50	3.5	4.5
$Ca(NO_3)_2$	0	29	75	75	4.5	6.0
$NaCl$	0	65	60	55	3.5	7.0
$NaNO_3$	0	75	80	80	4.0	7.0
chilled 84 hours 2 to 4° C.						
Water	0	75	--	--	--	--
KNO_3	0	75	--	--	--	--
KCl	0	80	--	--	--	--
$CaCl_2$	0	90	--	--	--	--
$Ca(NO_3)_2$	0	90	--	--	--	--
$NaCl$	0	80	--	--	--	--
$NaNO_3$	0	90	--	--	--	--

TABLE XII

EFFECT OF SALT SOLUTIONS ON THE REACTION OF COTTON TO CHILLING

Solution	Percentage injury: 24 hrs. after chilling: Av. per plant	Percentage injury: 24 hrs. after chilling: Range	Percentage injury: 2 wks. after chilling: Av. and range	Height of plant--inches: Chilled: Av. per plant	Height of plant--inches: Chilled: Range	Height of plant--inches: Not chilled: Av.
Westex Cotton (4 weeks old)						
chilled 48 hours at 0.5 to 5.0° C.						
Water	17	15-40	20	11.4	10.5-12.5	---
KNO_3	24	10-35	10	11.6	11-12.5	---
$Ca(NO_3)_2$	35	5-70	20	11.8	11-11.5	---
$NaNO_3$	22	10-30	30	10.0	9-11	---
Delfos Cotton (3 weeks old)						
chilled 18 hours at 2 to 4° C.						
Water	11.8	0-25	20	6.3	---	6.5
KNO_3	17	0-25	10	6.3	---	6.6
$Ca(NO_3)_2$	37	30-50	20	6.0	---	6.0
$NaNO_3$	26	15-30	25	6.8	---	6.5

The least amount of injury occurred where potassium nitrate had been applied, followed by those treated with potassium chloride. The next in order were those that received an equal amount of water, calcium chloride, calcium nitrate, sodium chloride, while those that had sodium nitrate injured the most; in several instances fatally (Fig. 13).

It seems that the potassium cation assisted the plants to withstand chilling, whereas calcium appeared in several instances to cause more injury than that perceptible on the control plants. Sodium had a decided deleterious effect. As for the anions less injury occurred where chlorides had been used than with the corresponding nitrates.

The maize plants grown in quartz sand showed that the salt applied rather than a liberated base was responsible for the difference in injury. In this instance potassium nitrate treated plants showed slightly less injury than the control plants, while 2-week-old plants that had received calcium nitrate were nearly killed.

The differential reactions brought out by the salt solutions may have been due to the effect of the individual salts on the constitution of the plants. As far as is known, all the cations and anions used, including sodium,

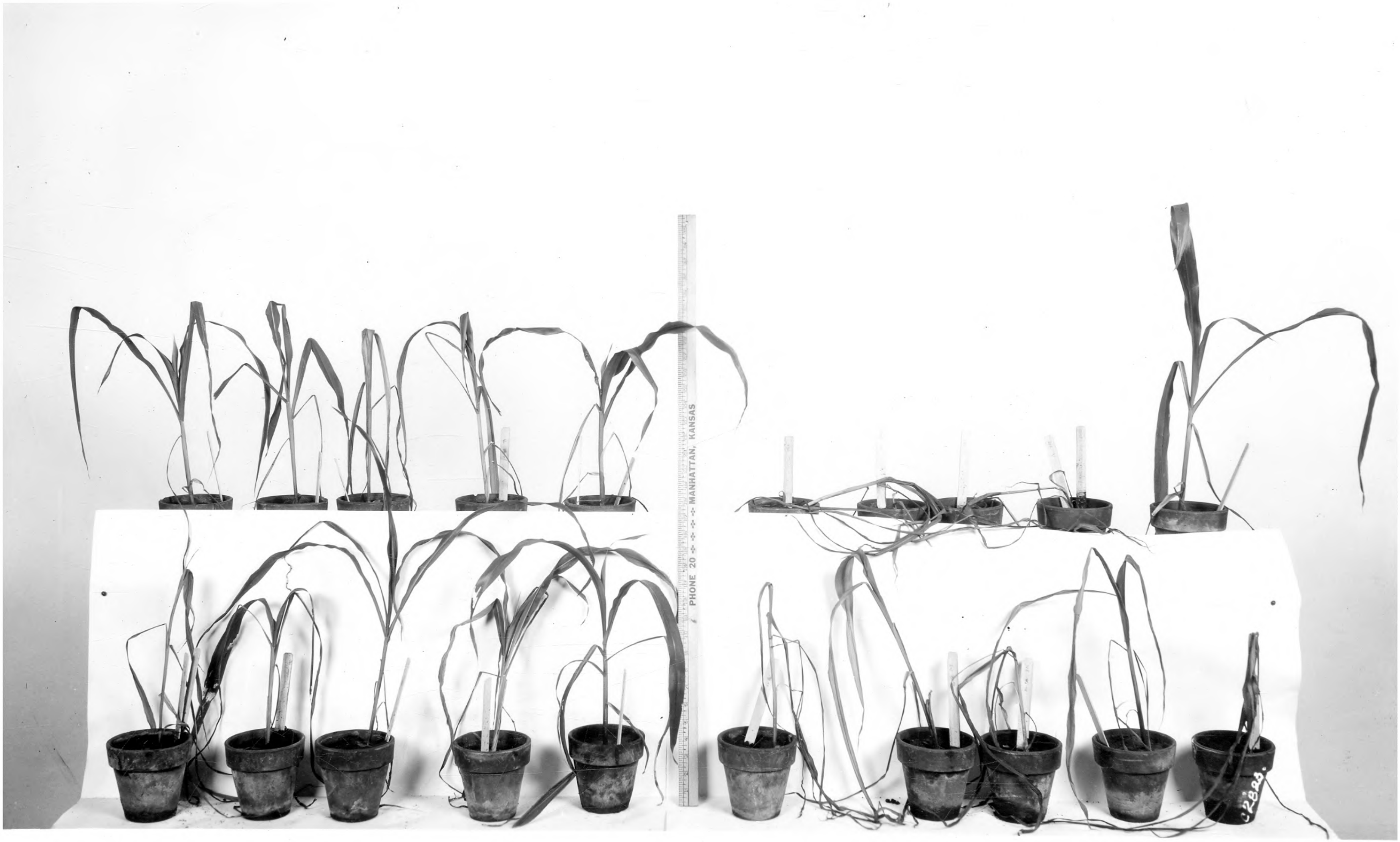

Fig. 13.

Figure 13. Injury to 4-week-old Midland Dent maize when chilled at 0.5 to 5.0° C., for 108 hours (severe) after being treated with one-twentieth molecular solution of $Ca(NO_3)_2$, KNO_3, and $NaNO_3$. Top row, left to right: KNO_3 and $Ca(NO_3)_2$. Bottom row, left to right: Control (tap water) and $NaNO_3$.

are taken up by plants. Especially interesting is the fact that potassium assisted the plants to endure chilling, when it is considered that potassium fertilizers are recommended for application to fields where maize suffers from root rot. In this experiment, those plants that were treated with sodium nitrate, and were incidentally the most severely injured, were the only ones to show outward indications of the presence of a pink fusarium similar to that causing maize root rot.

Another possibility is the effect of the salt solution on the temperature of the soil, if not also on flocculation. The work of Bouyoucos (1913) showed that salt solutions have a very predominant influence on the rise of the temperature in soils.

Chilling Injuries in Nature

Observations made at the Colby and Fort Hays Experiment Stations, in the greenhouse at Manhattan and in the Union of South Africa prove that the baneful influences of chilling occur in nature in no way different to those artificially produced.

On June 19th, 1927, at Colby, Kansas, cotton plants, one month old were observed to have been injured and many killed by "frost." But for at least four weeks prior to this date no frost had been recorded. The weather records, however, showed that the maximum temperatures for June 13th and 14th had been 49° and 50° Fahr., respectively, while the minimum for the 12th, 13, and 14th were 45°, 43° and 45° respectively. In terms of degrees centigrade there had been at least a 36 hour period during which the temperature did not go above 10 degrees. The last three days mentioned were cloudy and misty and there was a precipitation of 0.6, 0.2, and 0.1 inches respectively during that time. The cold and wet conditions during these three days then, have been responsible for the injury noted above.

At Fort Hays, Kansas, Faris bands in more exaggerated form than those artificially produced were observed on sorghums, maize and Sudan grass on October 1st, 1927. They were particularly noticeable on the leaves of young suckers or the secondary growth of exposed plat corners and on young volunteer plants. A cold and misty period from September 25th to 27th had been held responsible for the abnormalities observed. The maximum temperatures for the 26th and 27th were 43° and 45° Fahr., respectively, while the minimum for the 25th, 26th, and 27th, and 28th were 42°, 36°, 37°, and 34° Fahr., respectively. In terms of degrees centigrade, during the 26th and 27th there was a 36-hour period, when the temperature did not go above 7°.

After the experiments reported on here had been completed, the once chilled and other plants in the greenhouse reacted to a cold period during the 17th and 22nd of September. The cold that caused this reaction, according to a thermograph record, was that experienced when the temperature remained below 50° Fahr. (10° C.) for thirteen hours immediately before and after midnight of September 20th. The minimum reached was 39° Fahr. (3.89° C.). Normal three-week-old cowpea plants three days after this chilling behaved no different than those that had been artificially chilled for 12 hours at 5 to 10° C.

Mr. A. R. Saunders reported that during a cold spell in November 1927, at the Experiment Station at Potchefstroom, Union of South Africa, early planted maize and amber cane exhibited prominent Faris bands.[1]

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1. Private correspondence; letter of November 26, 1927.

GENERAL DISCUSSION

The investigation reported in this dissertation point definitely to the fact that chilling initiates certain changes in crop plants which have a detrimental influence on them. The results thus agree almost completely with those obtained by Molisch (1896), Faris (1926), and Pantanelli (1918, '19, '20). It is further to note that most of the characteristic abnormalities observed by Molisch were observed also in the present case for crop plants and described in more or less the same way as recorded by him.

The relative sensitiveness of the plants included in this investigation suggests that their distribution may be dependent quite as much upon their ability to endure chilling as upon other climatic factors such as total heat units, mean temperatures, forst-free periods, etc., which ordinarially receive much more attention. Thus plants that proved most sensitive to chilling are the staple crops of the subtropics such as rice, velvet beans, cotton and peanuts, while the hardier ones are extensively grown in temperate regions, as for example maize, sorghums, water-melons and pumpkins. The most hardy have a very wide distribution but are essentially northern crop plants, as soybeans, buckwheat, flax and sunflowers. That varietal distribution may also be dependent upon chilling injury is definitely suggested by the difference in hardiness of certain varieties of cotton, peanuts, cowpeas and soybeans. Trice cotton which is grown toward the very northern limits of the cotton belt in the Carolinas was found to be decidedly hardier than Delfos, a variety from Mississippi and Westex from Texas.

What the ultimate cause of the deleterious influences that follow chilling may be is a matter requiring further investigation. It is well known that physiological processes are definitely limited by temperature, some much more than others. With low temperatures it is conceivable or even probable that some are retarded more than others, upsetting possibly the sensitive balance between the various processes with the result that harmful products accumulate in the plant cells. This seems to have been Molisch's view.

Purewitsch, according to Palladin (1917), found that the respiratory ratio is at a minimum from 2 to 4° C.; that is, at this temperature oxygen absorption proceeds much faster than the elimination of carbon dioxide. Oxidation in the cells is therefore not complete and toxic substances may be formed.

In the investigations reported in this paper greater injury was observed on plants growing in saturated than in dry soil and therefore in an oxygen-deficient soil. This conceivably might accentuate the relation noted above.

The carbohydrate nitrogen ratio in plants may also be a very important factor in determining the resistance to chilling. Pantanelli concluded from his experiments that those plants which retained the most sugar are able to withstand cold best, because at low temperatures carbohydrates are used in transpiratory combustion. He pointed out further that as long as a cell has sugar at its disposal it does not consume albumens and when the sugar is exhausted autodigestion of the proteins takes place. Since nitrogen is a limiting factor in growth, it follows that more nitrogen there is present in the plant the more carbohydrates are used up in building new tissue. According to the work of Fischer (1916), Krause and Kraybill (1918) a rapid vegetative growth is characterized by a high nitrogen carbohydrate ratio. Hence those plants making the most active growth and thus using considerable carbohydrates for growth are likely to have the least in reserve for respiratory combustion during a cold period. This hypothesis is substantiated by the fact that in general where nitrates were applied the greatest amount of injury took place. The single exception, with potassium nitrate, is easily explained by the well known fact that potassium functions especially in photosynthesis, in the movement of carbohydrates within the plant and that it tends to counteract the ill effects of too much nitrogen.

Where injury occurred with normally watered plants as compared to those in dry soil, it is likely that prior to chilling the former plants made a more vigorous growth, using up carbohydrates, while those in dry soil making less growth had a greater carbohydrate reserve to meet the chilling period. It was clearly evident that as long as the plants received different amounts of water, the growth they made was in proportion to the relative amounts applied.

Nelson (1926) suggests from his own and the work of Armstrong that at low temperatures there is a liberation or accumulation of some toxic fragments resulting from the mixing of hydrolytic enzymes and glucoside. The protoplasm being unable to function properly at the low temperatures cannot throw off these toxic compounds in the normal manner.

That certain plants die from drought after their roots have been injured to a greater or lesser extent is most probable. This was the conclusion of Sachs as quoted by Molisch (1896). In the present study this was found to be the case with peanuts. The plants did not show injury or perish until the roots had been injured to such an extent as to be able to supply the top growth with an adequate amount of water.

In conclusion it must be pointed out that the chilling of plants should not be considered from the point of air temperatures alone; soil temperatures warrant as much consideration. In spring soil temperatures are considerably lower than air temperatures and the former fluctuate far less than the latter. The top growth of the plants may experience a chill first, and when the air has warmed up again the roots may still be in very cold soil. The chilling period to which the plant as a whole is subjected may be longer than that indicated by either soil or air temperatures separately.

Summary and Conclusions

The results of the experimental work reported in this paper supports the following conclusions.

In most summer crop plants constitutional disturbances having a serious influence on them are called into effect when they are subjected to chilling; even though the temperature does not go as low as zero degrees centigrade.

The outstanding result of the investigation was the very evident effects of chilling on certain plants and the high degree of resistance in others. The duration and the intensity of the cold were important factors determining the nature of the reactions. An exposure of 24 to 36 hours at

0.5 to 5.0° C., was fatal to rice, velvet beans and cotton. With the same exposure cowpeas were completely defoliated and only straggly plants were produced. Peanuts, Sudan grass and Teff grass exposed to chilling temperatures for 48 hours appeared at first to be uninjured but died in the course of about two weeks; at the same exposure maize, sorghums, watermelons and pumpkins were slightly injured. Soybeans, potatoes, buckwheat, Tepary beans and flax proved to be exceedingly hardy, it being possible to expose them to chilling temperatures for 84 to 96 hours without injury. At 5-10° C. velvet beans proved to be hardier than whip-poorwill cowpeas. At this temperature cowpeas were injured when exposed for 24 hours; velvet beans and cotton with an exposure of 60 hours.

Marked differences in relative hardiness between varieties was observed. Trice cotton which is grown near the northern limit of the cotton belt in North Carolina proved more hardy than Delfos from Mississippi, while Westex, a variety specially bred for Texas conditions, was considerably more tender than the foregoing. Whippoorwill cowpeas from Georgia became chlorotic when chilled for 12 hours at 5 to 10° C., whereas Early Buff, from Kansas, did not assume this condition until chilled for 36 hours at 0.5 to 3.0° C. Virginia Bunch peanuts were hardier than Spanish and the latter hardier than Valentia. Early Prolific rice was more sensitive than Honduras and Manchu soybeans were hardier than Virginia. The relative hardiness of the different plants indicate that their distribution from subtropical to temperate regions may be dependent to some extent upon their ability to endure chilling.

A point of considerable interest was the characteristic reactions of different species, not only with respect to differences in degree of injury, but also with respect to the nature of the injury. Cowpea and rice leaves became chlorotic 24 to 48 hours after chilling, while cotton and soybeans showed brown and white spots respectively and the grasses in general showed white bands across the leaves similar to those described by Faris in sugar cane. Lesions not artificially produced were observed on field crops at Hays and Colby and at Manhattan on greenhouse plants when the air temperature remained between 33 and 50^{o} Fahr., for one to three days.

Cowpeas, peanuts, maize and velvet beans were far more severely injured in wet than in dry soil. Soybeans behaved in the contrary manner, although the injury in the latter case was slight and may possibly have been due to drought.

In experiments with cowpeas, cotton and velvet beans, young plants suffered materially more than old plants treated in a similar manner.

The least amount of injury occurred where potassium nitrate had been applied, followed by those treated with potassium chloride. The next in order were those that received an equal amount of water, calcium chloride, calcium nitrate, sodium chloride, while those that had sodium nitrate were in several instances fatally injured.

There seemed every justification to say that the potassium cation assisted the plants to withstand chilling better than those that had received an equal amount of water. As for calcium there appeared as much, and in several instances more, injury than occurred with the control plants. Sodium had a decided deleterious effect.
As for the anions less injury occurred where chlorides had been used than with the corresponding nitrates, except for potassium nitrate.

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APPENDIX

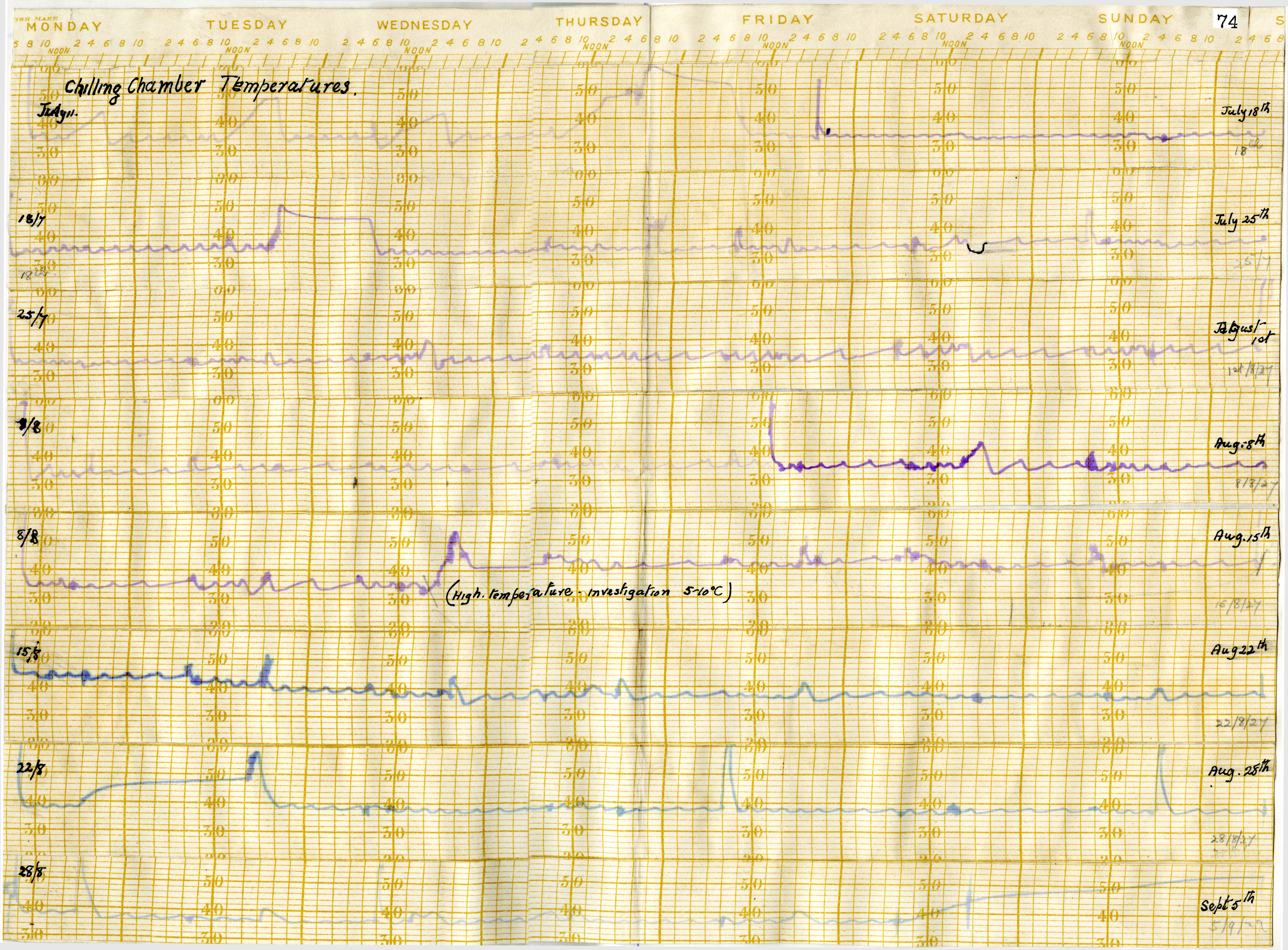
1. Eight weekly thermograph records from the thermograph kept in the chilling chamber. July 18th. to September 5th. 1927.

2. Thermograph records of the greenhouse temperatures for the same time as in 1.

3. Thermograph record of the greenhouse temperature. September 19th. to 24th.

4. Minimum and maximum temperatures at the Colby Experiment Station. May 19th to June 20th. 1927.

5. Minimum and maximum temperatures at the Fort Hays experiment station. September 1st. to October 6th. 1927.


MONDAY
TUESDAY
WEDNESDAY
THURSDAY
FRIDAY
SATURDAY
SUNDAY
NOON
Chilling Chamber Temperatures.
July 18th
18/7
July 25th
25/7
August 1st
1st/8/27
8/8
Aug. 8th
8/8/27
8/8
Aug. 15th
(High. temperature - investigation 5-10°C)
15/8/27
15/8
Aug 22nd
22/8/27
22/8
Aug. 28th
28/8/27
28/8
Sept 5th
5/9/27


MONDAY
TUESDAY
WEDNESDAY
THURSDAY
FRIDAY
SATURDAY
SUNDAY
NOON
Agronomy Greenhouse.
1927
11/7
July 18th
18/7
July 25th
25/7
August 1st
1/8
Aug 8th
8/8
Aug 15th
15/8
Aug. 22
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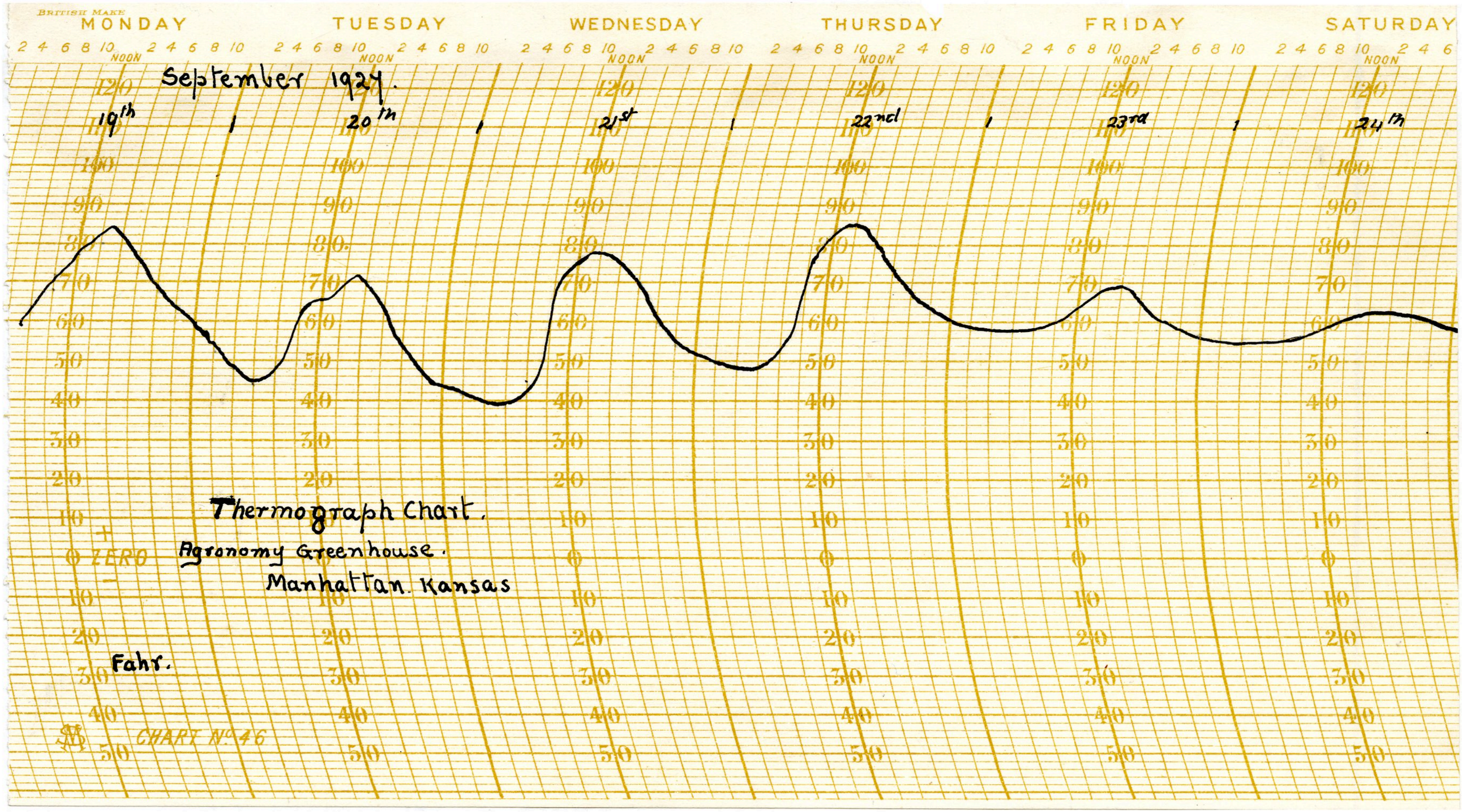
Aug 28th
28/8
Sept. 5th


BRITISH MAKE
MONDAY
TUESDAY
WEDNESDAY
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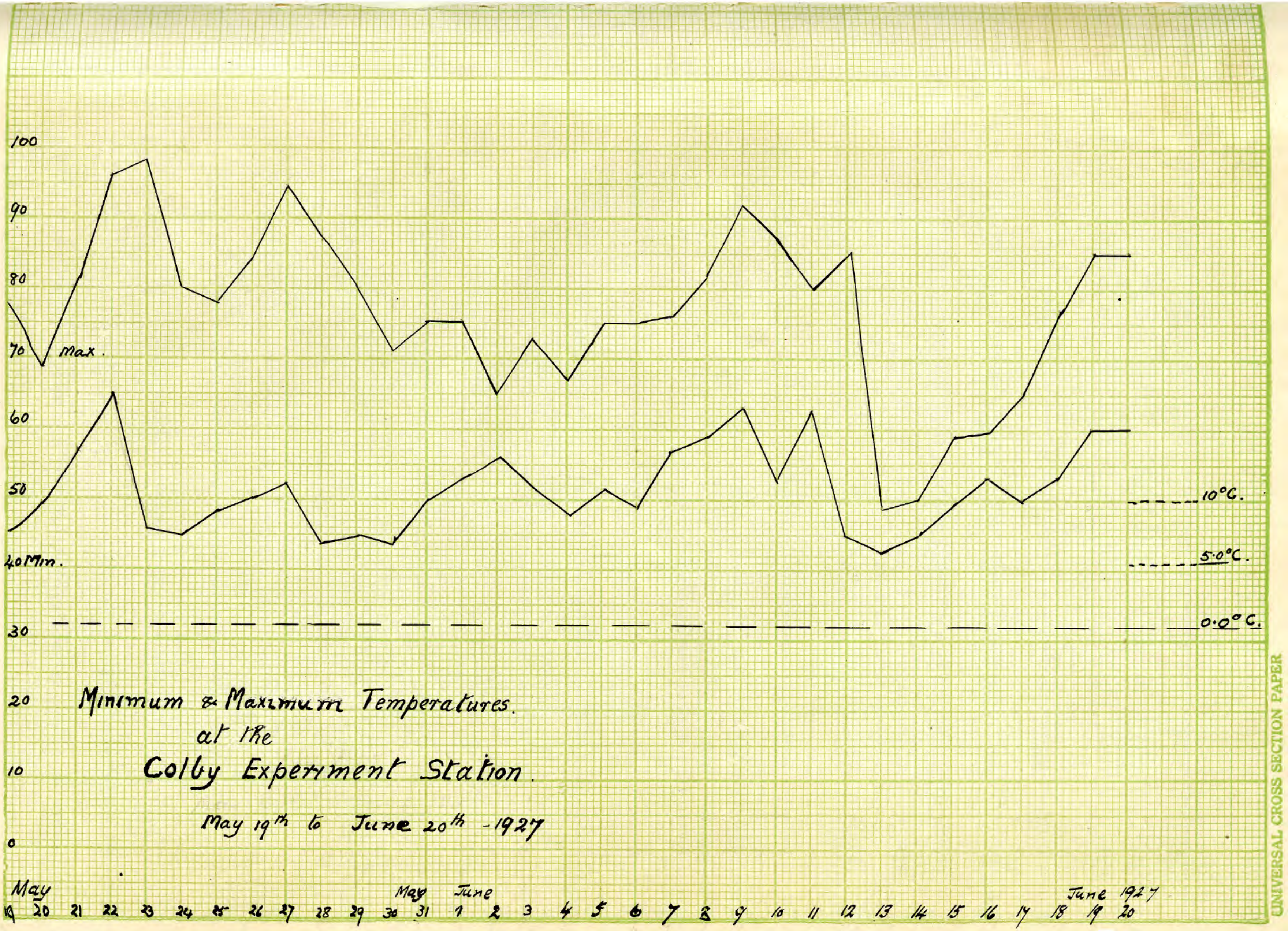
FRIDAY
SATURDAY
NOON
September 1924.
19th
20th
21st
22nd
23rd
24th
Thermograph Chart.
Agronomy Greenhouse.
Manhattan. Kansas
ZERO
Fahr.
CHART No. 46


100
90
80
70
60
50
40
30
20
10
0
Max.
Min.
10°C.
5.0°C.
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Minimum & Maximum Temperatures
at the
Colby Experiment Station.
May 19th to June 20th - 1927
May
May
June
June 1927
19 20 21 22 23 24 25 26 27 28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
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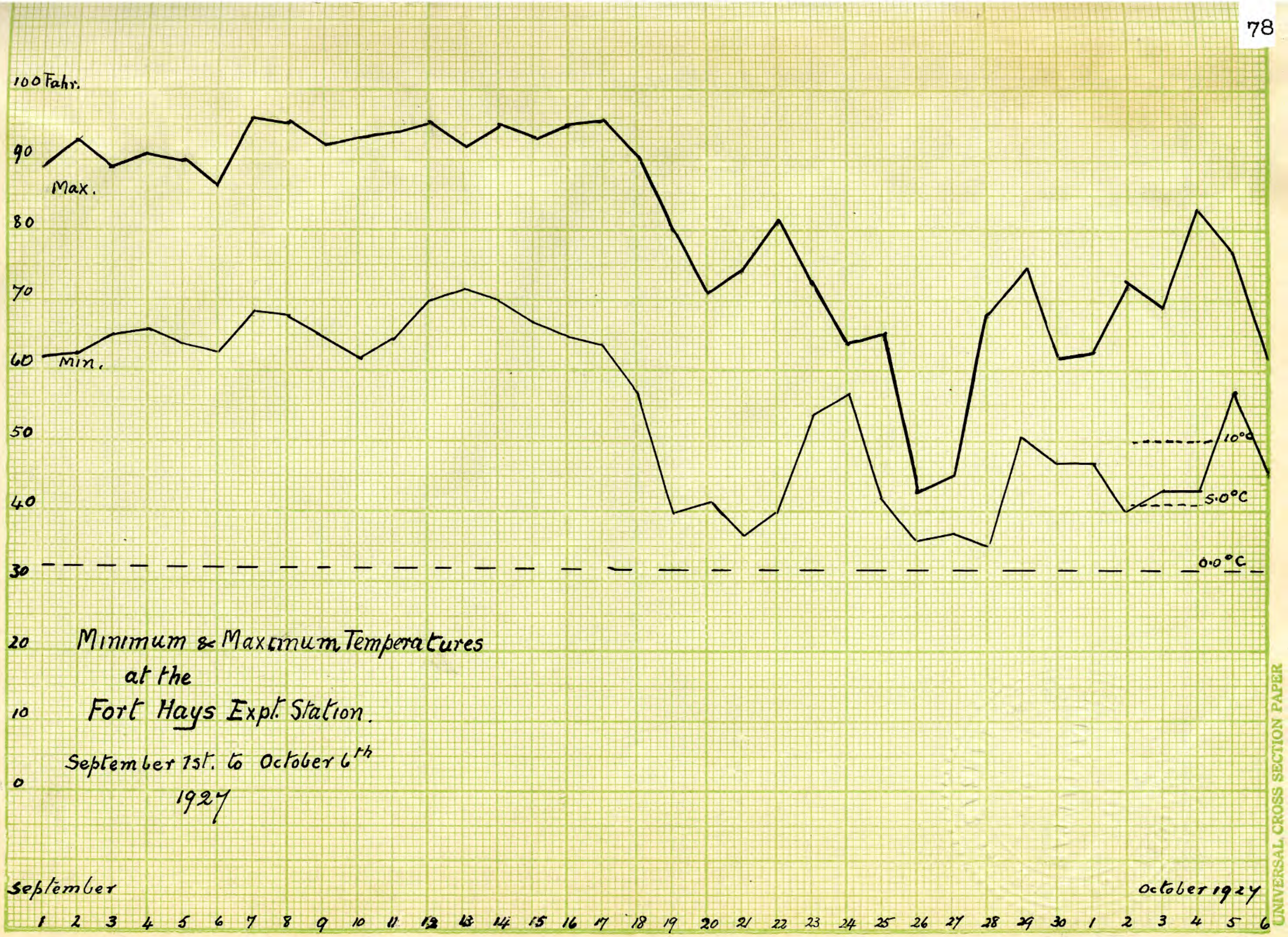
100 Fahr.
90
80
70
60
50
40
30
20
10
0
Max.
Min.
10°C
5.0°C
0.0°C
Minimum & Maximum Temperatures
at the
Fort Hays Expt. Station.
September 1st. to October 6th
1927
September
October 1927
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 1 2 3 4 5 6
UNIVERSAL CROSS SECTION PAPER