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TAXONOMY AND NOMENCLATURE OF THE CAUSAL ORGANISM OF THE COMMON SCAB OF THE POTATO

NOMENCLATURE OF THE POTATO SCAB ORGANISM

THE INFLUENCE OF SOIL TEMPERATURE UPON THE OCCURRENCE OF THE COMMON SCAB OF THE POTATO

by

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TAXONOMY AND NOMENCLATURE OF THE CAUSAL ORGANISM OF THE COMMON SCAB OF THE POTATO

Contents

Taxonomy and Nomenclature of the Causal Organism of the Common Scab of the Potato

The taxonomy and nomenclature, as well as the ideas concerning the natural distribution of the causal organism of the potato scab, seem to be in an unsettled state at the present time.

Thaxter (28), in 1890, interpreted the scab organism as a true fungus, and this idea was later confirmed by Arthur and Golden (1), and Bolley (5). While Thaxter (29), in 1891, expressed some uncertainty as to the correctness of the binomial Oospora scabies Thax., it was not until 1911, when Cunningham (12) announced that the organism was a filamentous bacterium, that its fungous nature was seriously questioned.

The group of congeneric species to which the scab organism belongs has been studied chiefly by medical men and soil bacteriologists for the reasons that it makes up a large percentage of the soil micro-flora, and certain species are pathogenic on man and lower animals. The group seems to have been misinterpreted, chiefly because the methods employed by bacteriologists make it difficult or impossible to study the morphological aspects of the organism, and while the majority of bacteriologists have heretofore considered this group to be of bacterial nature, there are certain workers who hold different views concerning its position.

Review of Literature

Bolley (4), in 1890, was the first worker to establish

the relationship between potato scab and a causal organism. He found a bacterium associated with the scab lesions with which he succeeded in producing the disease upon growing tubers.

Thaxter (28), a few months after Bolley's observation, described a fungous-like organism which he found associated with scab lesions. Pure cultures of this organism readily produced scab upon tubers growing in the field, also in the greenhouse. In 1891, Thaxter (29) applied the binomial Oospora scabies to the organism, but he expressed uncertainty as to the correctness of his decision on the grounds that the genus Oospora Wall., as described by Saccardo (25), had little or no scientific value. Thaxter's (29) description of the organism is rather brief, but as far as it goes it coincides with the description of the organism studied by the author. Thaxter's observations were confirmed by Bolley (5), who was unable to repeat his Indiana results with the bacterium, but was successful in isolating the organism described by Thaxter.

Cunningham (12), in 1911, after making a study of the organism, concluded that it was a higher bacterium because of the fact that it answered the description of the genus Streptothrix Cohn.

Güssow (15), in 1914, also assigned the organism to the higher bacteria because he thought that it answered the description of the genus Nocardia Trev., as described by Saccardo (26). He saw fit, however, to change the generic name Nocardia Trev. to Actinomyces Harz. Trevisan (30), in 1889,

held that the generic name Actinomyces Harz, 1877, was untenable for the reason that Meyen (20) had used the binomial Actinomyces horkelii in 1827 to designate a fungus. Güssow contended that, on a basis of Article 57 of the International Rules of Vienna, the generic name Actinomyce is different and distinct from Actinomyces and, therefore, the latter name should be applied to the genus. Accordingly, he designated the scab organism Actinomyces scabies (Thax.) Güssow.

Lutman and Cunningham (19), in 1914, maintained Cunningham's (12) idea concerning the bacterial nature of the organism. They maintained that the organism belonged to the genus Streptothrix Cohn, as described by Chester (7), and they identified it as Streptothrix chromogena Gasp. However, since the generic term, Streptothrix Cohn, was untenable on account of the priority of Streptothrix Corda (11), Gasperini (14), in 1892, applied the generic term Actinomyces Harz, and Lutman and Cunningham designated the scab organism Actinomyces chromogenus Gasp.

Discussion

While there may be little question in the minds of those who have studied the scab organism recently as to its being congeneric with the members of genus Actinomyces Harz, there is considerable doubt concerning the bacterial nature of this group. Cohn (9), Rosi-Doria (24), Krainsky (17), Beijerink (2), Migula (21), Saccardo (26), Waksman and Curtis (31 and 32), and Buchanan (6) all considered the members of

this group among the Schizomycetes. However, there are different views concerning this point.

Claypole (8) looked upon the Streptothrix Cohn group "as representing the ancestral type that gave both the higher fungi and the true bacteria, and not as being themselves higher bacteria."

Lehman and Neuman (18), in 1899, seemed to be undecided concerning the position of the group, but they favored the idea that it formed a connecting link between the Hyphomycetes and the Schizomycetes.

Sauvageau and Radais (27), in 1892, held that the group belonged with the Hyphomycetes and they included it in the genus Oospora.

Besson (4) agreed with Sauvageau and Radais, but he considered the group under the generic term Discomyces Rivolta, which is closely related to the genus Oospora Wall.

Drechsler (13) made a detailed study of the morphology of the genus Actinomyces. He isolated some 1400 organisms belonging to this genus from the air, water, soil, manure, etc., many individuals being derived from soils collected in Porto Rico, Cuba, Panama, Montana, Wisconsin, Kansas, New York and Massachusetts. Out of this number of organisms about 300, representing probably over 100 species, were selected for morphological examination. This selection included the causal organism of potato scab.

Drechsler's work brings out the morphological structure of these organisms so clearly that there can be little

doubt as to their true fungous nature and it seems to be quite evident that the species of this group may be differentiated upon a purely morphological basis.

Morphology of the Scab Organism

The author studied an organism isolated from scabby potatoes grown in Door county, Wisconsin, which gave positive results when tested for pathogenicity upon growing tubers.

For making microscopic studies, the organism was cultured upon short lengths of barley straw which had been sterilized in test tubes containing small amounts of water. The organism does well upon many sorts of vegetable matter as well as upon potato glucose agar, etc., but the straw lends itself very readily to the method of technique used and the fungus guttates very little, if any, on this medium, thus increasing the chances for getting good mounts. The method of preparing mounts used by Drechsler (13) makes it possible to study all parts of the fungus in their true relationship.

The vegetative filaments of the organism studied by the author range from .56 to .63 μ in diameter. They are freely branched and, so far as observed, they are non-septate. From four to six days after inoculating straw at a growing temperature of 27°C., the fungus commences to develop sporogenous hyphae in much the same manner as vegetative branches are developed. When the sporogenous hyphae have attained one-fourth their final length, they begin to assume a dextrorse spiral form which increases in definition until they have reached their final

length. After a spiral has reached its limit of growth, it develops into a definite spiral chain of spores. Drechsler's detailed study of this organism indicates that sporulation commences at the tip of these spirals, progressing downwards. He found this process to be the result of definite septum formation and not a mere fragmentation process, as many would have us believe.

The spores are from .7 to .77 by .98 u in size and hyaline. Upon germination they develop from one to four typical fungous germ tubes, which develop rapidy into a branched mycelial structure. In studying spore germination, the spores were sown upon hanging drops of a vegetable decoction and allowed to develop in Van Tiegham cells. At a growing temperature of 35°C. material was ready for study in about twelve hours.

If germinated spores are stained, it is necessary that the material be properly killed. If this is not done, the protoplasm in the young mycelium and in the germ tubes will separate at intervals and, when the stain is applied, the filaments may resemble chains of spores, especially when the separation happens to be quite regular.

Two other organisms, one isolated from scabby potatoes grown in Barron county, Wisconsin, and the other from scabby potatoes grown in Dane county, Wisconsin, were identified and found to be identical with the one described above. Both of these organisms were found to be pathogenic upon growing potato tubers.

It is quite evident from the studies of these organisms that they are identical with the one studied by Drechsler,

which he designates Actinomyces XVII.*

Distribution of the Organism

It is well known that certain members of the genus Actinomyces make up a large percentage of the organisms in the soil.

According to Waksman and Curtis (31), Hiltner and Störmer, in 1903, found that this group made up 20 per cent of the spring, 30 percent of the fall and 13 percent of the winter soil micro-flora.

Conn(10), in 1916, found that the group may make up as much as 38 per cent of the soil micro-flora, and Waksman and Curtis (32), in 1918, found that it made up from 3.5 per cent to 46 per cent of the total micro-flora (exclusive of fungi) of 25 soils collected in different parts of the country.

Beijerink (2), Krainsky (17), Waksman and Curtis (31 and 32), and others have found that a large number of the soil inhabiting members of this genus answered the description for Actinomyces chromogenus Gasp.

Lutman and Cunningham (19), in 1914, after doing some work with the potato scab organism, concluded that it answered Gasperini's description of Actinomyces chromogenus Gasp., as given by Chester (7), under Streptothrix chromogena Gasp.(syn.).

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*Drechsler procured the scab species which he designates Actinomyces XVII from Mr. M. Shapovalov of the Bureau of Plant Industry, U. S. Department of Agriculture, who has demonstrated its pathogenicity upon potato tubers.

Consequently, they considered the scab organism to be a widely distributed soil organism regardless of the distribution of the potato. Further than this, these men did not present any convincing evidence to uphold this theory.

Gasperini (14), in 1892, described the species Streptothrix chromogena, upon the basis of the production of a brown to black pigment which is readily diffusible throughout gelatin and agar culture media, a character which is possessed by the scab organism.

Krainsky (17), in 1914, previous to Lutman and Cunningham's publication (19), demonstrated that Actinomyces chromogenus Gasp. is made up of at least four distinct species, which he designated:

Actinomyces erythrochromogenes

Actinomyces diastatochromogenes

Actinomyces viridochromogenes

Actinomyces flavochromogenus

Waksman and Curtis (32), in 1918, found the species to consist of at least five different species, and Drechsler (13) found that at least three of the species which he described produce brown to black pigment when cultures upon various agar substrates.

In view of these facts, it is evident that Actinomyces chromogenus Gasp. is merely an aggregation of species, as pointed out by Conn (10), in 1916, and facts concerning the natural distribution of such a group do not necessarily apply to the distribution of the scab fungus.

Pratt (22) made a microbiological analysis of 109 samples of soil collected from desert, dry farming and reclaimed lands in Idaho and in no case did he succeed in isolating the scab fungus. His results from growing potatoes upon virgin and dry farming soils do not indicate a widespread occurrance of the scab fungus. Certain of Pratt's plots in 1915 upon raw desert land did produce considerable scab, but his method of seed treatment is not mentioned and all plots were irrigated with water of unknown history. In 1916, one of his dry farm plots developed 3.3 per cent scab, but this land had been farmed previously, affording opportunity for the introduction of the organism. Another plot produced .33 per cent scab, which is certainly within the range of experimental error.

Since Lutman and Cunningham (19) do not present conclusive experimental evidence in support of their idea concerning the wide distribution of the organism, it must be considered that their theory is without foundation.

Nomenclature

The binomial, Actinomyces chromogenus Gasp., applied to the scab fungus by Lutman and Cunningham (19), in 1914, has been accepted by many plant pathologists and bacteriologists, but, as pointed out above, the works of Krainsky (17), 1914, Waksman and Curtis (31 and 32), in 1916 and 1918, and Drechsler (13), in 1919, show that this species is made up of numerous

species, and consequently the binomial has no scientific value.

The question naturally arises as to the application of an appropriate binomial to designate the scab organism.

The group of congeneric species to which the scab organism belongs has been designated in whole or in part at different times by various names. Buchanan (6), in 1917, and The Committee of the Society of the American Bacteriologists on Characterization and Nomenclature (23), in 1917, have divided this group into two genera: Actinomyces Harz , 1877, usually parasitic, clubbed ends conspicuous in lesions, no aërial hyphae or conidia, some species anaërobic; and Nocardia Trevisan, 1899, usually saprophytic, aërial hyphae and conidia produced, usually aërobic.

Drechsler's work demonstrates quite clearly that the genus Actinomyces Harz must be classed with the Hyphomycetes among the Mucedineae and it is maintained by the author that the binomial, Actinomyces scabies (Thax.) Güssow, should be accepted as the name of the scab organism.

Summary

The taxonomy and nomenclature, as well as the ideas concerning the natural distribution of the causal organism of potato scab, have been in a rather unsettled state.

It is generally conceded by workers who have studied the scab organism recently that it is congeneric with the species included in the genus Actinomyces Harz, as described by

Chester under the generic term Streptothrix Cohn and by Saccardo (26) under the generic term Nocardia Trev.

Recent mycological studies of the genus indicate that it must be considered among the true fungi.

The author obtained the causal organism of potato scab from three lots of scabby potatoes grown in different localities. Studies made by the writer indicate quite clearly that the organism possesses true fungous characteristics and that the organisms from the three sources are identical morphologically with the scab organism studied by Drechsler.

The works of Krainsky, Waksman and Curtis, and Drechsler have demonstrated that Actinomyces chromogenus Gasp. is virtually a group made up of a number of species. Consequently, facts concerning the natural distribution of such a group do not necessarily apply to the scab fungus, and due to the lack of supporting evidence, it must be considered that Lutman and Cunningham's theory concerning the distribution of the scab fungus is without foundation.

In consideration of the facts concerning the nomenclature of the scab fungus, it is maintained by the writer that the binomial, Actinomyces scabies (Thax.) Güssow, should be accepted as the name of the organism which causes the common scab of the potato tuber.

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Nomenclature of the Potato Scab Organism

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NOMENCLATURE OF THE POTATO SCAB ORGANISM

H. H. McKINNEY

The taxonomy and nomenclature, as well as the ideas concerning the natural distribution of the causal organism of potato scab, were upset when Cunningham (3), 1912, placed the organism among the higher bacteria. Güssow (5), 1914, accepted this classification and considered Actinomyces the proper generic name. Later Lutman and Cunningham (7), 1914, identified it as *Actinomyces chromogenus* Gasp. and advanced the theory that it "is a widespread organism, which is found in practically all soils, so far as known. . . . "

Workers who have studied the scab organism recently seem to agree that it is congeneric with the species included in the genus Actinomyces Harz, as described by Chester (1), 1900, under the generic term Streptothrix Cohn and by Saccardo (8), 1889, as Nocardia Trev. However, the taxonomic position of this genus has for some time been a matter of considerable doubt. Sauvageau and Radais (9), 1892, considered the genus among the Hyphomycetes and this view was accepted by a few bacteriol-

ogists, but the majority of bacteriologists have classed it among the bacteria or assigned it to a position between the bacteria and the fungi.

Much work has been published relative to the phylogenetic position of the genus Actinomyces, but in practically all cases there seems to be a lack of thoroughness in the work done on this subject, as evidenced by the indefinite illustrations which are supposed to characterize the morphology of the various members of this genus.

Drechsler (4), 1919, made detailed morphological studies of eighteen members of the genus and he has supported his descriptions with highly detailed drawings. He has demonstrated quite clearly that the genus does not belong with the Schizomycetes, but rather with the Hyphomycetes in the family Mucedinaceae.

The writer, working in the plant pathology laboratory of the University of Wisconsin, has studied three strains of the scab organism isolated from scabby potatoes grown in three localities in this state, all of which are pathogenic upon growing tubers and produce the brown diffusible pigment upon gelatin and agar media. While there is some slight variation in the virulence and in pigment formation, the morphology of these organisms is identical with that of the scab fungus as described by Drechsler.

It is known that brown pigment forming Actinomyces species are quite widely distributed throughout soil, and in the past these forms were identified with *Actinomyces chromogenus* Gasp. on account of the pigment character. When we consider the works of Krainsky (6), 1914, Waksman and Curtis (10), 1916, and Drechsler (4), 1919, it is quite evident that this character cannot be considered specific. These workers have demonstrated that *Actinomyces chromogenus* is merely a group of numerous species differing in physiology and morphology. Therefore, data concerning the natural distribution of such a group do not necessarily apply to the scab fungus or to any other single species within the group, and consequently, due to the lack of supporting evidence, Lutman and Cunningham's theory concerning the universal distribution of the scab fungus must be abandoned.

In view of the above works it is plainly impossible, as pointed out by Conn (2), 1916, and Drechsler (4), 1919, to apply the binomial *Actinomyces chromogenus* Gasp. to the scab organism, and in consideration of all these facts the writer maintains with Drechsler that the binomial *Actinomyces scabies* (Thax.) Güssow should be accepted as the name of the organism which causes common scab of the potato tuber.

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THE INFLUENCE OF SOIL TEMPERATURE UPON THE OCCURRENCE OF THE COMMON SCAB OF THE POTATO

CONTENTS

INTRODUCTION

Previous to the conception of the germ theory many observing husbandmen considered temperature and moisture separately or in combination as the direct causes of many plant diseases, and we find today that the untrained observer very often attributes his rusts, mildews and blights entirely to the weather.

Students of plant pathology have for some time recognized the influence of climatic and weather conditions upon the development of disease. Massee (6), 1910, cites an experiment with potatoes infected with the late blight fungus (Phytophthora infestans) which bears out this observation. He found that infected plants grown in a greenhouse under warm moist conditions showed signs of the disease six weeks after planting, whereas infected plants grown under cold dry conditions did not show signs of the disease eleven weeks after planting. Some of the plants of the cold dry series were transferred at intervals to warm moist conditions after the sixth week and in from six to nine days after the transfers these plants showed definite signs of blight, while those plants left under cold dry conditions showed no signs of disease.

Field observations recorded by Jones (5a) in Wisconsin during 1915 and 1916, and by Coons (3) in Michigan during 1912 and 1915, indicate that the late blight fungus is more aggressive during cool, moist seasons.

Massee (7) observed that peach leaf curl was more

prevalent during cool, damp weather. Pierce (12) found the same to be true in California and he advanced the theory that the increased attack of the disease was due to a weakened condition of the host.

Balls (2), working in Egypt, found that Rhizoctonia spp. caused the "sore shin" disease of cotton at 20°C., but not at 33°C. He found that the growth of the fungus was retarded due to autointoxication at high temperatures in pure culture, while at lower temperatures no such effect was noted. Balls questioned whether or not this toxic substance prevented the attack of the host by the fungus.

GENERAL OBSERVATIONS CONCERNING SOIL TEMPERATURE AND POTATO SCAB

In reviewing the literature on the common scab disease of the potato tuber caused by Actinomyces scabies (Thax.) Güssow, we find numerous references to the influence of soil moisture and various organic and inorganic substances upon the development of this disease, but no mention seems to have been made relative to the influence of temperature upon scab further than a suggestion by Jones (5b) in way of a possible explanation of the fact that this disease is less prevalent in the chief European potato districts than in those of America.

While common scab is reported from the United States, Canada, Bermuda, the British Isles, Cuba, Jamaica, Germany, France, Tasmania, Australia and Africa, there is considerable

variation in regard to the prevalence of the disease in certain of the countries and provinces mentioned. Common scab is considered the most serious disease of the potato tuber in Wisconsin and also in many other states in this country, but in the chief potato districts of Europe, Bermuda and certain localities in the United States, the disease is not considered important.

Cumulative observations made by Mr. John Brann of the Wisconsin Experiment Station indicate that potatoes grown in Door county, Wisconsin, are unusually free from scab, as compared with those grown in other potato districts of Wisconsin. This county has a very mild equable temperature during the summer months, the mean temperature for Sturgeon Bay, the county seat, being 18.6°C. and 18.5°C. for the months of July and August respectively. These temperatures are from 2° to 6° lower than the mean temperatures for the principal recording stations of the U. S. Weather Bureau located in other Wisconsin potato districts. This temperature relationship is especially significant when we consider that most of the soil in Door county is of limestone origin, a condition which is considered quite favorable for the development of scab.

Prof. L. R. Jones, while travelling through European potato fields, found that scab was not considered a serious disease. Practically no seed is treated to prevent scab in Europe and, while the disease is present in all potato dis-

tricts, it does not seem to increase to any extent, even upon fields where potatoes have been the only crop for ten to forty successive years. According to Orton (11), 40 per cent of Germany's potato crop is fed to live stock. Since Morse (8) has shown that the scab organism will survive passage through the digestive tracts of cattle and horses, it would seem that all soil in German potato districts must be thoroughly infested with the scab organism. Certainly no such wholesale process of soil inoculation goes on in the United States, yet according to Anon. (1), scab is undoubtedly the most important disease of the potato tuber in this country.

Reference to temperature data for northern Europe, where the chief potato districts are located, reveals the fact that the mean temperature for the growing season is lower than that for the principal potato districts in the United States. According to Orton (9), the July isotherm of 65°F. runs south of the principal potato districts of Great Britain and northern Germany and only in Aroostock county, Maine, and parts of northern New York do we have extensive potato culture north of this isotherm. "The isotherm of 70°F.(21°C.) for June, July and August nearly marks the southern boundary of successful main crop potato production in the United States."

Orton (10) reports that scab is not a serious disease in the Bermuda Islands, and in correspondence with the author , Prof. E. J. Wortley of the Bermuda Agricultural Sta-

tion states that the islands are comparatively free from scab, in spite of the fact that the soils are highly calcareous. Upon consulting the temperature records for the Bermudas, Verrill(15), it is found that the mean monthly temperature during their potato growing season, October to February, is from 24°C. to 18°C. After planting, the temperature goes down rapidly and at the period of tuber development the temperature is 20°C., or below, and steadily on the downward trend, whereas, in the United States we find a reversal of the temperature curve with respect to the potato growing season with many short periods of extremely high temperature.

Mr. John Brann of the Wisconsin Experiment Station in conference with the author stated that more scabby potatoes were produced in Wisconsin during 1916 than during any other year in his experience in potato inspection work. Upon consulting the records of the U. S. Weather Bureau for 1916, it is found that in Wisconsin, July was one of the warmest Julys on record and August of the same year was one of the warmest Augusts on record.

Soil temperature records, kept at Madison, Wisconsin, by Johnson and Hartman (4), show that the temperature of the soil four inches under the surface averaged 28.1°C. during July, 1916, that during the same period in 1915 the same soil at the same depth averaged about 20.57°C., and during 1917, about 24.46°C. These data show that the soil temperature averaged very high during the growing season of 1916, and,

according to Weather Bureau records, this season stands out as an exceptionally warm one.

EXPERIMENTAL WORK IN LABORATORY AND GREENHOUSES

In order that the influence of soil temperature upon the development of scab might be determined under controlled conditions, a series of laboratory investigations were carried out, but before such work could be started it was necessary to carry on preliminary studies with respect to methods of inoculation.

SOURCE OF ORGANISM AND ISOLATION

Scabby tubers were obtained from three sources in Wisconsin and one organism was isolated from each lot.

The tubers were thoroughly scrubbed and rinsed in sterile water. A suitable scab spot was located on a tuber and a section cut out of same which included the scab tissue, the periderm and the starch cells. In one case, only starch tissue from beneath a scab spot was used. These sections were then passed through 95 per cent alcohol and disinfected in 1:1000 $HgCl_2$ for one minute, then thoroughly rinsed in sterile water to remove all $HgCl_2$. The tissue was then macerated in 10cc. of sterile water, suitable dilutions were made, and petri dishes poured in the usual manner. Potato glucose agar, unadjusted for reaction, was the standard medium used for isolating and growing the fungus except where otherwise mentioned.

At a temperature of 25°C. to 30°C. the organism became evident in from 1.5 to 3 days. The organism produced a decided tyrosinase reaction which was indicated by a dark brown to black margin of diffused pigment around the small colony. As soon as these brown areas appeared, the colonies were transferred to agar slants.

PATHOGENICITY TESTS

Since many of the species of Actinomyces produce a dark pigment, it was not safe to proceed with extensive inoculation work until the isolated organismshad demonstrated their pathogenicity in pure culture.

Young tubers from disinfected seed, growing in pots of disinfected soil, were inoculated with a sterile water suspension of the organisms from the three sources. At the end of 13 days the tubers were examined. It was found that out of nine tubers inoculated only four continued growth after inoculation and two of these tubers which had been inoculated with the same organism were decidedly scabbed. The positive culture was increased and used in all of the temperature work.

METHOD OF CONTROLLING SOIL TEMPERATURE

In the case of the controlled temperature studies, all plants were grown in round galvanized iron pots six inches in diameter and nine and one half inches deep. These pots were placed in water contained in insulated tanks. The appa-

ratus used was fully described by Jones (5c) a few years ago. High temperatures were maintained by introducing live steam into the water three times a day. In the case of the tanks running at 21°C. and above, it was necessary to operate partial vacuum electric heaters, so as to prevent excessive fall in temperature during the eight hour periods. In the case of the low temperatures, cold water or ice was used, depending upon the conditions.

At the end of each eight hour period the water temperature of each tank was recorded, after which the temperatures were adjusted.

The water in tanks operated at temperatures above room temperature was always raised one degree above the running temperature three times daily. The water in tanks operated at temperatures below room temperatures was lowered a degree below the running temperature three times daily. This method allowed for a drop of one degree below and a rise of one degree above running temperatures.

The mean temperature for any given tank during an eight hour period was obtained by taking the average of the water temperature after adjustment at the beginning of the period and the temperature at the end of the period. The daily mean was obtained by determining the average of the means for the three periods during the day. Plate I illustrates the temperature range in a general way during a 24 hour period.

FIRST TEMPERATURE EXPERIMENT

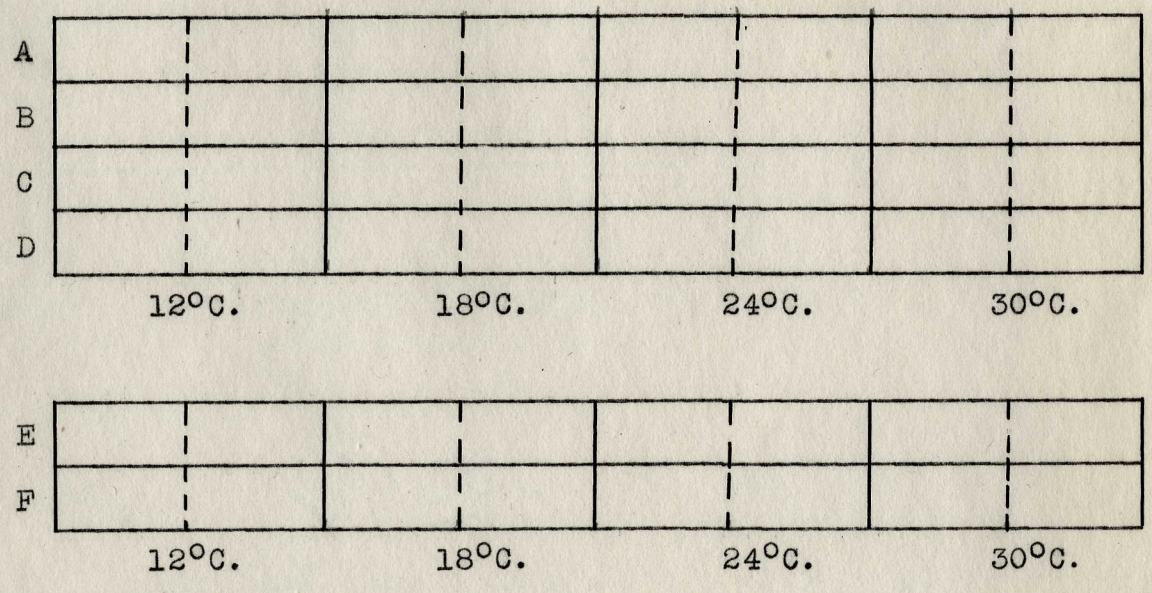
The main object of the first experiment was to determine a suitable method of inoculation for further work.

This experiment was started late in the fall of 1918 and at this period of the year there was some difficulty in getting suitable potato seed. The new crop of northern potatoes was not ready for planting on account of the fact that such stock had not passed through the rest period, and seed from the 1917 crop was rather old. Upon planting Wisconsin Irish Cobbler seed of the 1917 crop, it was found that a large percentage of the seed did not produce tops. Examination showed that such seed developed young tubers almost immediately after planting. With this in mind, it was decided to use such seed for part of the first experiment in order that results might possibly be obtained in a shorter time. As a safeguard against mishap, however, Irish Cobbler seed, 1918 crop, was obtained from North Carolina and used for part of the experiment. This seed was in prime condition and fine plants were obtained, considering the time of year when they were grown.

All of the seed was planted on November 8, 1918. Series A, B, C and D were all placed in the tanks November 8. They remained in water at room temperature (about 18°C.) until November 15, when all of the temperatures were adjusted as follows:

Two tanks were held at 12°C.-, two at 18°C.-, two at 24°C.-, and two at 30°C.-.

General plan of tanks



Legend:

A = Carolina Irish Cobblers. Soil inoculated at time seed was planted with 50 cc. of suspension of pure culture of organism. Checks not inoculated.

B = Wisconsin Irish Cobblers, 1917 crop. Soil inoculated at time seed was planted same as A.

C = Wisconsin Irish Cobblers, 1917 crop. Planted in field soil known to produce scabby potatoes. Check soil was steam sterilized.

D = Wisconsin Irish Cobblers, 1917 crop. Soil inoculated same as A, but pure precipitated CaCO was added to the soil.

E = Carolina Irish Cobblers planted in sterilized soil for purpose of direct tuber inoculation.

F = Wisconsin Irish Cobblers planted in sterilized soil for purpose of direct tuber inoculation.

Series E and F were placed in a greenhouse at a temperature ranging from 15°C.- to 20°C. until young tubers had developed to a size sufficient to be inoculated.

All pots used for growing potatoes for inoculation purposes were steam sterilized under a pressure of about four pounds for four to five hours, or at fifteen pounds pressure for thirty minutes to one hour. All soil, except where otherwise stated, was sterilized under a pressure of approximately four pounds for four to five hours. All seed was disinfected a considerable time before planting with a solution of mercuric chloride and water in the proportion of 1:1000. All plants were watered every other day throughout the experiment with tap water.

The fungus was increased upon potato glucose agar in 300 cc., 500 cc, and 750 cc. Erlenmeyer flasks. The agar was poured about seven millimeters thick on the bottom of the flasks. The surface of the agar was inoculated with the organism while the condensation water was still present so as to facilitate a better distribution over the surface of the medium. On a basis of Shapovalov's (13) work, cultures were incubated at a temperature of 27°C. for a period of three weeks. At the end of this period the fungus had sporulated abundantly and it was possible to get a good suspension of the spores.

General Technique and Methods of Inoculation

Before going into the temperature studies proper,

it was decided to determine the relative value of direct tuber inoculation, artificial soil inoculation and naturally infected field soil inoculation. While this preliminary work was planned with the above object in view, it was so planned that temperature data might also be obtained in case any of the methods gave positive results.

Soil Inoculation

Disinfected soil was inoculated with a spore suspension prepared in the following manner:

The tough, leathery surface growth of the fungus was scraped from the agar contained in six 750 cc. Erlenmeyer flasks. This material was thoroughly macerated in a sterile petri dish in order that the greatest number of spores might be dispersed. The inoculum was put into two liters of sterile water and thoroughly agitated. A pipette was used to distribute 50 cc. of the suspension through the soil as it was being placed over the seed piece.

Direct Inoculation of Tubers

This method consisted in growing the plants in disinfected soil contained in the metal pots described above (Series E and F). The pots were placed in the greenhouse at a temperature ranging from 15°C. to 20°C. until tuber formation. At this time (seven weeks after planting) the soil was carefully removed from the upper roots and tubers of the plants by means of a probe. When the tubers were located

they were carefully washed by means of a small stream of sterile water from a wash bottle. The tubers were then inoculated with a spore suspension or with spore material taken directly from the growing mass of fungus.

After inoculation, pots were placed in the temperature tanks until the end of the period of growth of the potato plants.

Infected Field Soil Inoculation

This method consisted in the use of soil collected from potato hills in which scabby potatoes were produced during the season of 1918. The seed was planted in this soil and pots were placed in the temperature tanks immediately.

Results

Table 1 and plates II and III present the results of this experiment. Plates IV and V give some idea as to the appearance of the tubers grown in the inoculated soil.

On December 2 (three and one half weeks after planting), the pots in series B, C, D and F were opened and it was found that practically all of the seed had rotted, thus throwing these series out of the experiment.

On December 26 (seven weeks after planting), the pots in series E were opened and all plants were found to be developing tubers which ranged in size from swollen stolons up to one inch in diameter. All of the tubers found (except in the case of check pots) were inoculated with a water sus-

Table 1.- Results of First Temperature Experiment.

	Soil Inoculation Series A				Tuber Inoculation Series E			
Soil temperature	12°C.	18°C.	24°C.	30°C.	12°C.	18°C.	24°C.	30°C.
Number of tubers in check pots	3	3	3	0	3	5	1	2
Number of tubers in inoculated pots	8	8	7	2	14	8	9	2
Total number of tubers in check and inoculated pots	11	11	10	2	17	13	10	4
Total number of inoculated tubers					5	3	8	9
Total number of inoculated tubers which developed after inoculation					5	3	3	2
Percentage of inoculated tubers which developed after inoculation					100	100	37.5	22.0
Number of scabby tubers	0	1	3	1	2	2	2	1
Percentage of tubers showing scab inf.	0	12.5	43.0	50.0	40.0*	66.0*	66.0*	50.0*
Sq. mm. of scab surface on inf. tubers	0	48	1260	12	20	22	336	63
Sq. mm. of surface of all tubers in inoculated pots	2587	17700	10686	900	1925*	25100*	15750*	2924*
Percentage of total tuber surface scabbed	0	.31	11.7	1.3	1.0*	.09*	2.13*	2.15*
Number of scab infections	0	8	169	1	5	1	22	20

*These figures are based upon the number of inoculated tubers which developed after inoculation and not upon the total number of tubers which were inoculated.

pension of the same strain of scab organism used in the other series. Check pots were handled in exactly the same manner as those pots in which tubers were inoculated, except that no inoculum was used in the distilled water. After inoculation, all the pots were placed in the temperature tanks, where they remained throughout the experiment.

On January 26 (eleven weeks after planting), the plants in both series A and E were examined and data on the development of scab were obtained.

Methods of Inoculation

While the tuber population of this experiment was exceedingly small, the experience gained in carrying out the inoculation work leaves little doubt as to the best general method of inoculation. If, on the basis of the data obtained, we consider the percentage of potato hills (pots of plants) or the percentage of the numbers of tubers as indices of the best method of inoculation, we would have to favor direct tuber inoculation, but if we consider the percentage of scab surface on all tubers grown in inoculated pots as the index, the data at hand show soil inoculation to be a better method than that of direct tuber inoculation. Thaxter (14) in his first work with the scab organism, uncovered tubers growing in a garden and merely scratched his initials on their surface. He applied the organism to the scratched surface, covered the tubers with soil and awaited developments.

While this method of inoculation served very well to demonstrate the pathogenicity of the causal organism, it is not a satisfactory method to use when working with large populations. Any method of inoculation which necessitates disturbing the growing tubers or other underground parts of the plant must be considered crude at best. Even when the greatest care is taken, fully thirty per cent of the young tubers are broken from the stolons or injured. The root systems of the plants are severely injured and reduced, and the whole plant is given a general set-back, from which it does not recover during the remaining short period of development. Many tubers which are seemingly uninjured do not develop after the disturbance.

Soil inoculation makes it possible for all tubers in a pot to become infected and the host plant is not hampered in any way during its critical period of development, thus insuring greater uniformity of results and a larger population from which to obtain data.

Influence of Temperature upon the Development of Scab

Since the population of this experiment was very small, only a general analysis of the results seems advisable.

In the case of both methods of inoculation, high soil temperature increased the amount of scab. This is especially true in the case of series A, which in the opinion of the author is the most significant series on account of the more nearly normal conditions under which it was handled.

There was some question as to the most appropriate method of determining an index to the amount of scab produced in the various lots of tubers involved in the comparison. It did not seem fair to base comparison upon the number of tubers showing scab, as by this method no account would be taken of the degree to which the tubers were infected. The number of individual infections on tubers was considered as a possible index, but in the case of this method no account would be taken of the degree of scabbiness, and, aside from this objection, in many cases it was not possible to decide whether a given scab spot was the result of one or of many infections which might have merged into one large scab area.

Taking all things into consideration, it seemed logical to express the amount of scab in terms of relative surface area. This method allowed for the consideration of the amount of tuber surface available for infection and it also took into consideration the number of tubers involved.

All tubers were sorted into the following diameters expressed in millimeters: 2.5, 5, 7.5, 10, 15, 20, 25, 30, 35, 40. Diameters were determined by taking an average of the dimensions of the major, minor and medium axes. This figure was taken as the mean diameter and from it was computed the area of a sphere of the same diameter. This area was taken as the theoretical area of the potato tuber.

While this method was far from being exact when one tuber was considered, it seemed quite likely that it was suit-

able for the purpose of comparison, especially when a considerable number of tubers were measured to allow for an equal distribution of error throughout the lots.

On a basis of the percentage of tuber surface scabbed, the optimum soil temperature for scab development seemed to be somewhere near 24°C. Aside from the influence of soil temperature upon the disease, this factor had a marked influence upon the development of the potato tuber. The optimum temperature for tuber development, however, seemed to be considerably lower than that for the development of the disease.

SECOND EXPERIMENT

This experiment was planned on a basis of the results obtained in experiment 1. A general summary of the methods employed follows.

Soil

The soil used in this experiment consisted of two parts leaf mold and five parts of garden loam which contained a slight amount of sand.

This mixture was put into the metal pots described under experiment 1 and steam sterilized for four hours under a pressure of about four pounds. Two days after sterilization the soil was emptied out of the pots on a clean concrete floor which had been disinfected immediately before with mer-

curic chloride and water in the proportion of 1:1000. The soil was well mixed by means of a sterile shovel and enough soil was removed from the lot to fill fourteen pots for the check plants. To this portion of the soil was added an additional amount of sterilized leaf mold and tap water, both of which were proportional to the amounts of these materials added to the inoculated soil. (This point will be taken up later under inoculation.) The check pots were prepared and the seed planted immediately.

Inoculation

The remainder of the soil for the remaining 42 pots was immediately inoculated with 4 liters of a leaf mold culture of the scab organism and in addition to this, 4 liters of a water suspension of spores and mycelium of the organism were added to the soil and the whole mass mixed as thoroughly as possible by means of a shovel. A proportional amount of sterilized leaf mold and water was added to the check soil previous to the preparation of the inoculated soil.

Soil Moisture and H. Ion Concentration

Soil samples of the final mixture were reserved for the purpose of determining moisture content, hydrogen ion concentration and lime requirement.

The moisture content was found to be 18 per cent.

The hydrogen ion concentration was determined by the

Department of Bacteriology of the University of Wisconsin and found to have a pH value of 7 by the colorometric method.

The lime requirement was determined by Truog's lime requirement test and the soil was found to be neutral.

Seed and Planting

Southern Irish Cobbler seed was used for this experiment. The seed was treated with mercuric chloride and water in the proportion of 1:1000. This was done early in the winter so as to prevent sprout injury. The seed was quite shriveled, but many short thick sprouts had developed on most of the tubers. Tubers ranged in size from 2 1/2" to 3" in diameter. They were cut once through the stem and seed end. One seed piece was planted 5" deep in each pot.

Soil inoculation and planting was done on February 3, 1919, and the 56 pots were immediately placed in seven temperature tanks (described previously). Five days later the temperatures were adjusted to 12°C., 15°C., 18°C., 21°C., 24°C., 27°C., and 27/30°C., and maintained within narrow limits throughout the period. The method of temperature regulation used in experiment 1 was employed in this experiment. (Plate I)

During the five days before adjustment all tanks were kept at a uniform temperature of about 18°C.

In the previous experiment it was found that the potato plant does practically nothing when submitted to a continuous temperature of 30°C., and for this reason it was de-

cided to crowd the temperature of one of the 27° tanks up to 30° two days out of seven. This tank was designated as the 27/30° tank.

Results

Table 2 and platesVI and VII present the results of this experiment. Plates VIII, IX and X give some idea as to the appearance of the tubers grown in the inoculated soil. These photographs include one sixth of the total tuber population. The tubers were selected in such a manner that the relative amount of scab and the relative size of the tubers would be as nearly representative of the whole population as possible.

Two of the seed pieces planted in the 24° series failed to sprout, thus the population of this series was cut down considerably.

On March 24 all plants were removed from the pots in the 18°, 21°, 24°, 27°, and 27/30° tanks. Two plants were removed from the 15° tank and one from the 12° tank. It was found that tubers were not as far advanced in the 12° and 15° tanks as were those growing at higher temperatures, and it was considered advisable to leave the remaining plants in the 12° and 15° tanks so as to allow for further development of the tubers. Eighteen days later these plants were taken out. Reference to table 2 will show that the development (total weight) of the tubers taken out of the 12° and the 15° tanks at the later date exceeded that of the tubers taken out of

Table 2.- Results of the Second Experiment.

Soil temperature	12°C.	15°C.	18°C.	21°C.	24°C.	27°C.	27/30°C.
No. of pots in tanks containing inoculated soil	5	4	6	6	4	6	6
Total No. of tubers in inoculated soil	90	67	120	146	66	180	128
Average No. of tubers per pot in inoculated soil	18	16.7	20	24.3	16.5	30	21.3
Total No. of scabby tubers in inoculated soil	9	17	65	80	63	122	54
Percentage of scabby tubers in inoculated soil	10.0	25.3	54.1	54.8	97.0	67.7	42.1
Sq. cm. of surface area of all tubers in inoc. soil	432.8	485.2	549.0	482.3	324.9	343.8	190.9
Sq. cm. of scab surface of all tubers in inoc. soil	.30	4.52	80.7	132.5	150.7	53.47	3.1
Sq. cm. of scab surface per pot	.15	.23	13.4	22.1	37.7	8.91	.5
Percentage of total tuber surface scabbed	.06	.92	14.5	27.4	46.3	15.0	1.62
Total weight in grams of all tubers in inoc. soil	176.0	229.5	217.0	164.4	124.0	100.3	28.5
Average weight per tuber in grams.	1.95	3.42	1.80	1.12	1.87	.55	.22

Data on the 12° and on the 15° series are based upon plants removed from the tanks 18 days after the removal of the plants grown at the high temperatures.

any of the tanks eighteen days before, and it will be noted that these later tubers showed only a trace of scab.

The check plants were examined and in the case of three pots slightly scabbed tubers were found. In all cases the infected tubers were next to the seed piece, which suggested that the seed was infected. It is hardly probable that the treatment was at fault, as all seed was carefully selected free from scab lesions. It is possible that infection might have taken place at planting time, since some of the inoculum had been handled a few hours before. The hands of the operator were thoroughly disinfected, but there is the possibility that the operator's clothing had become contaminated.

The results of this experiment are in line with the results of experiment 1. The curves on plate III give a clear idea as to the relative amount of scab produced on the different lots of tubers grown at the various temperatures.

As in the case of experiment 1, it is of interest to note the influence of temperature upon the development of the potato tuber. By referring to table 2 and plate VII, it will be seen that early tuber development at least is quite slow at temperatures below 18°C. and above 24°C. While tubers set very early at the high temperatures, they do not develop to the same extent as tubers produced at the lower temperatures.

When we consider that the scab organism develops best at temperatures ranging from 25°C. to 30°C. when growing in pure culture as shown by Shapovalov (13), it is not surprising

that we should get a great amount of scab at 24°C. If the causal organism only is considered in this relation, we might have reason to expect a greater percentage of scab on tubers grown at 27°C. and 30°C., but we must consider the host, as well as the parasite when thinking of the influence of any factor upon disease. The work carried on by the author and also that of others seem to indicate that scab lesions develop only on growing tubers. If this is true, it would not be reasonable to get as large a percentage of tuber surface scabbed on slow growing or stunted tubers such as those produced in the 27° and 27/30° tanks as upon tubers grown near the optimum temperature for the potato. It seems reasonable then that the optimum temperature for the development of the disease would be between the optimums for the host and fungus.

CONCLUSIONS

The results of the experimental work carried on indicate that high soil temperatures favor the development of common scab on the Irish potato tuber. This seems to explain in a large measure why this disease gives little trouble in potato districts having low temperatures during the growing season.

The optimum temperature for scab development lies somewhere near 24°C. and the optimum temperature for the development of the potato tuber is somewhat below that for scab development. The exact optimum temperature for tuber development cannot be determined from the results obtained in this work,

since it was not possible to bring the tubers to a sufficiently advanced stage of development to warrant definite conclusions. It does seem safe, however, to consider that this optimum is at least five or six degrees below the scab optimum.

Soil inoculation seems to be the most practicable method to use when working with this disease on a large scale. The direct inoculation of growing tubers is not conducive to satisfactory results, since many of the tubers are broken from the stolons or injured during the process.

Pure cultures of the scab fungus increased upon sterile leaf mold gave better results for soil inoculation than water suspensions of the organism.

While it is not possible to draw definite conclusions relative to the correlation of tuber growth and scab development, the general idea that the disease develops only on growing tubers is borne out by the results of this work.

SUMMARY

Seasonal and climatic factors have long been known to influence the development of many of our common plant diseases.

Many early observers did not pretend to consider the temperature factor independent of moisture, but during recent years numerous experiments have been carried on under sufficiently well controlled conditions to enable workers to formulate definite conclusions with respect to the influence of temperature alone.

Weather records and crop disease records show a marked correlation in many cases between temperature and the prevalence of potato scab.

Northern European potato districts, the Bermuda Islands and Door county, Wisconsin, have lower temperatures during the growing season, and also less scab, than the majority of the potato growing regions in this country.

In Wisconsin it has been observed that scab is more abundant during hot seasons.

The results of two experiments indicate that the optimum temperature for scab development lies somewhere near 24°C. and that the optimum temperature for the development of potato tubers is somewhat lower than that for scab development.

The handling of the scab fungus and the procuring of infection on a large scale were not limiting factors in the work.

Soil inoculation at planting time gave better results than the inoculation of developing tubers.

The inoculation of the soil with a water suspension of the spores and mycelium of the fungus did not seem to give as good results as the use of pure cultures of the fungus, which were developed upon leaf mold and mixed with the soil at planting time.

The chief limiting factor in the work was the matter of culturing the potato plant under artificial conditions.

The work carried on by the author and observations made by other workers indicate that scab develops only on growing tubers.

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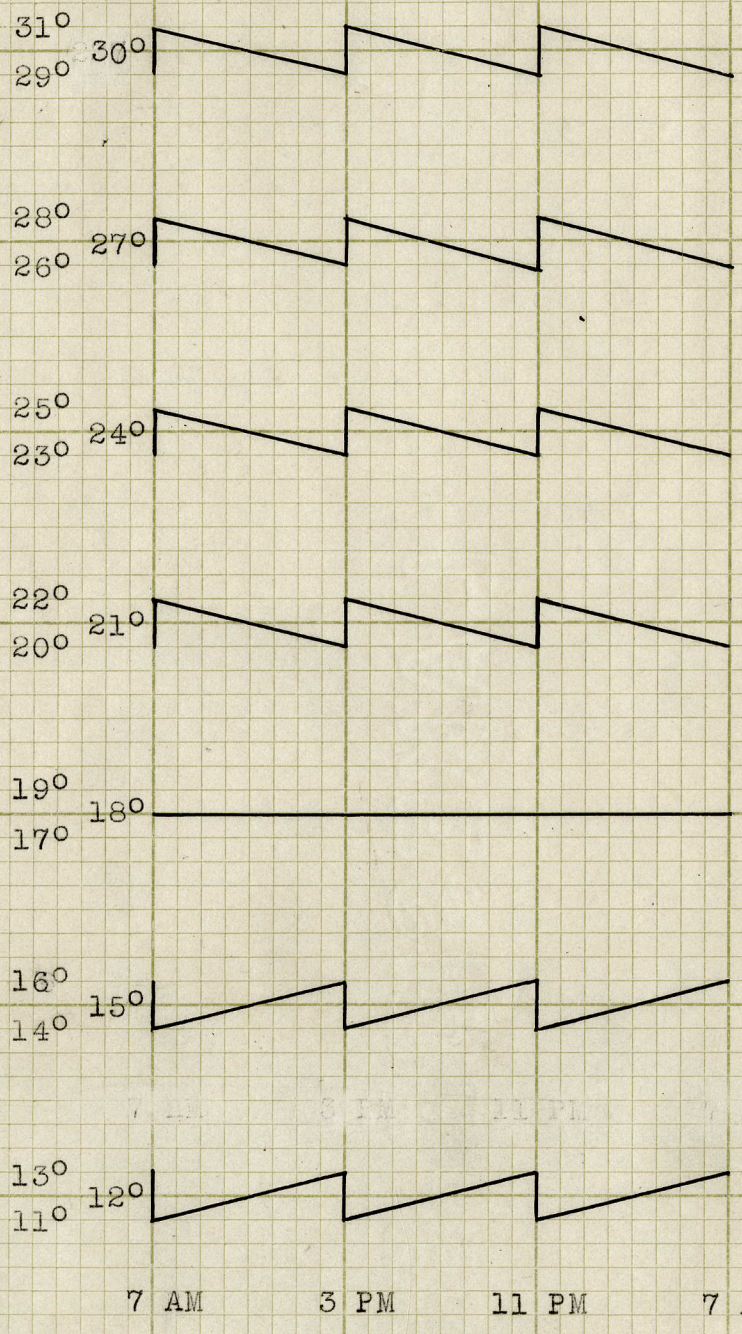
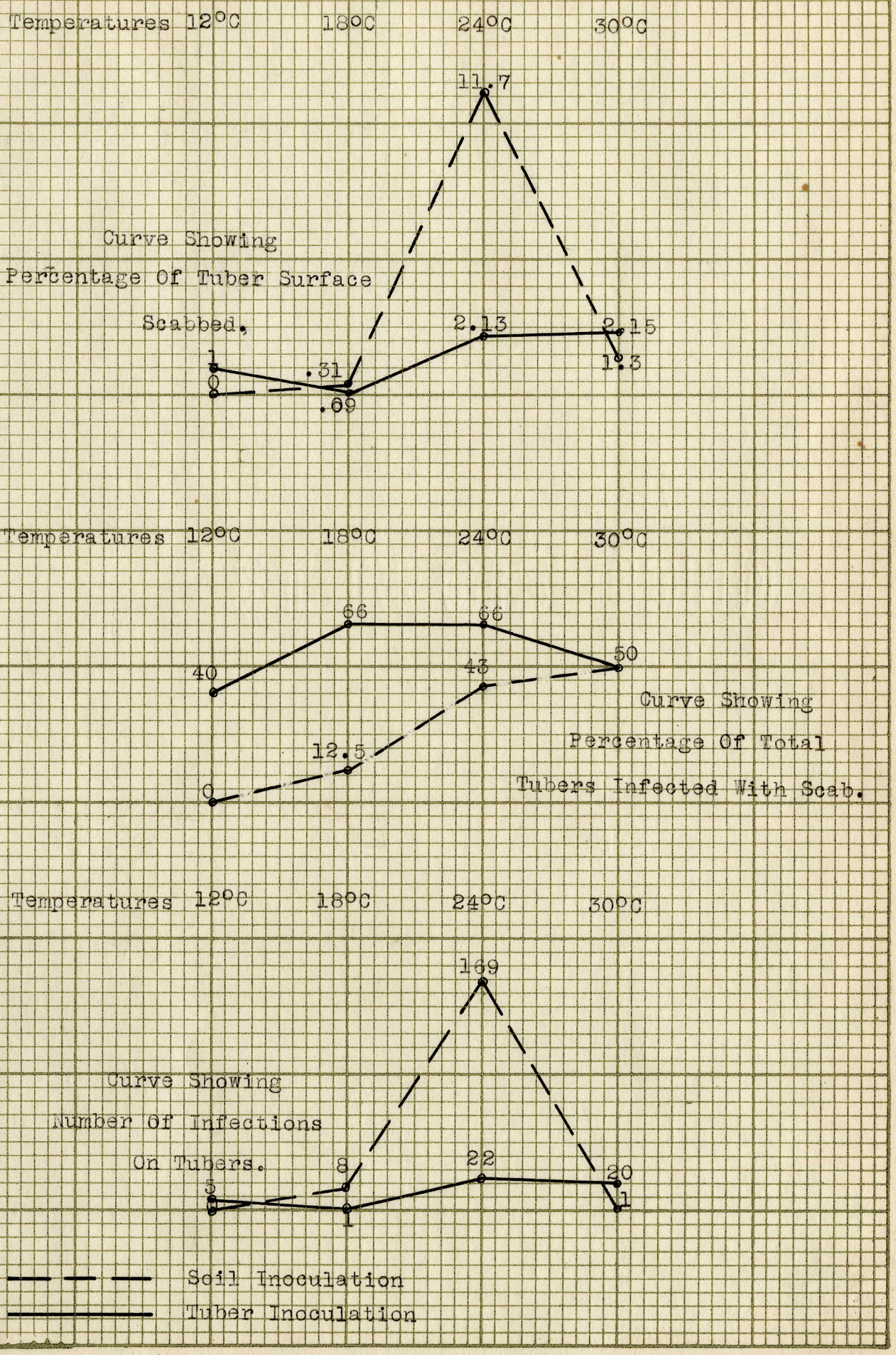
Plate I.

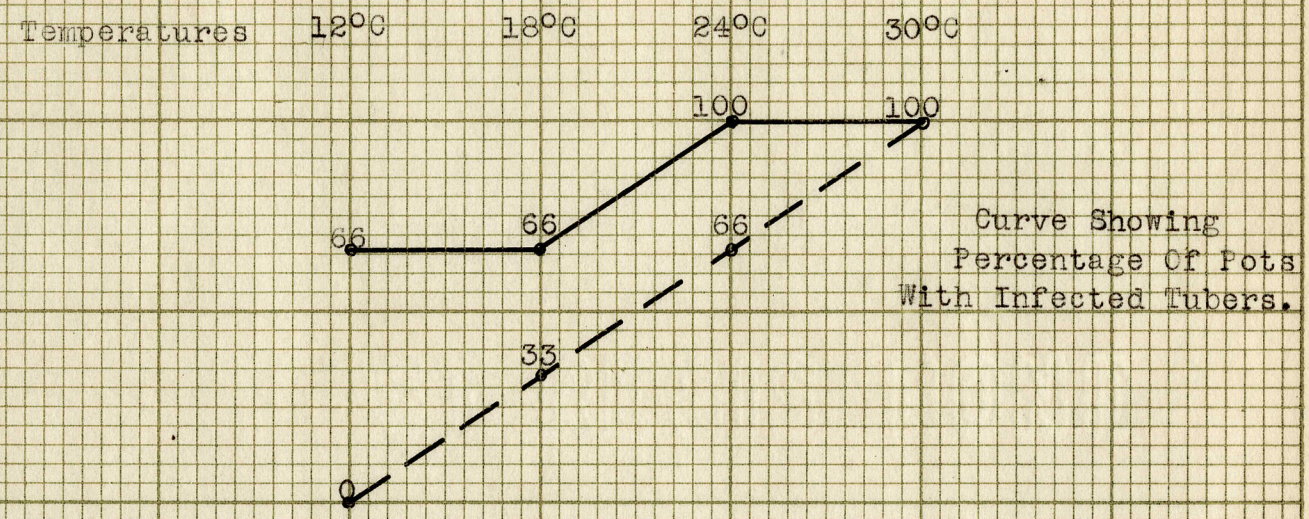
Curves Showing General Fluctuation In Tank Temperatures During A 24 Hour Period.



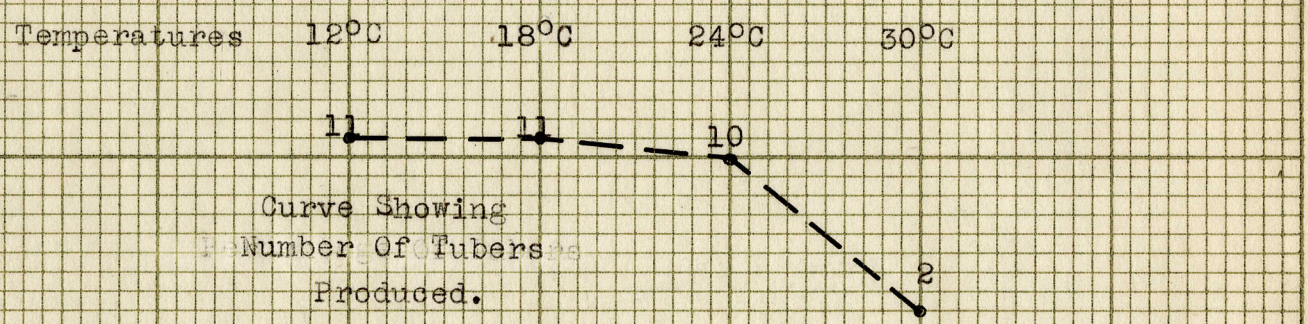
Experiment 1. Plate II.



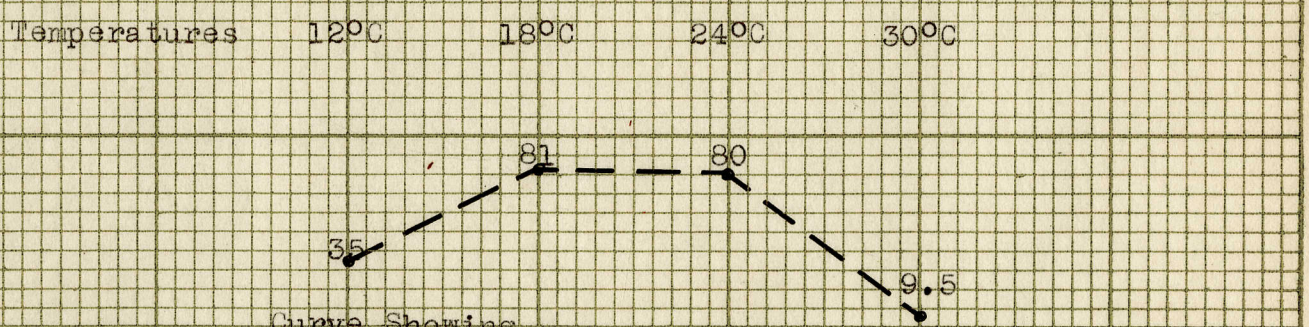
Experiment 1. Plate III.



Curve Showing
Percentage Of Pots
With Infected Tubers.



Curve Showing
Number Of Tubers
Produced.



Curve Showing
Weight Of Tubers
Produced.

Soil Inoc.

Tuber Inoc.

Photographs of scabby tubers from the soil and tuber inoculation series.

Experiment 1.

12°C.

18°C.

Plate V.

Photographs of scabby tubers from the soil and tuber inoculation series.

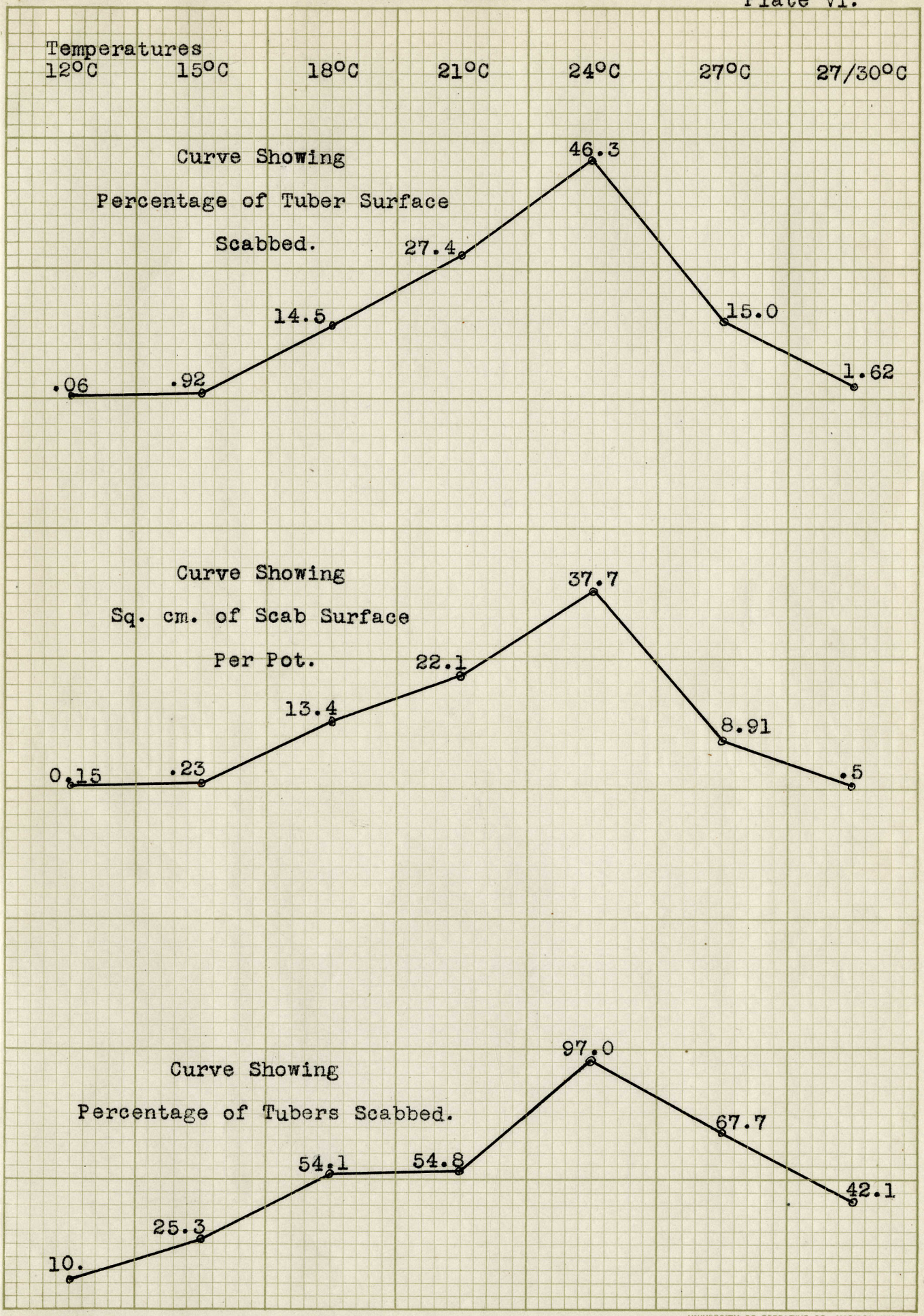
Experiment 1.

24°C.

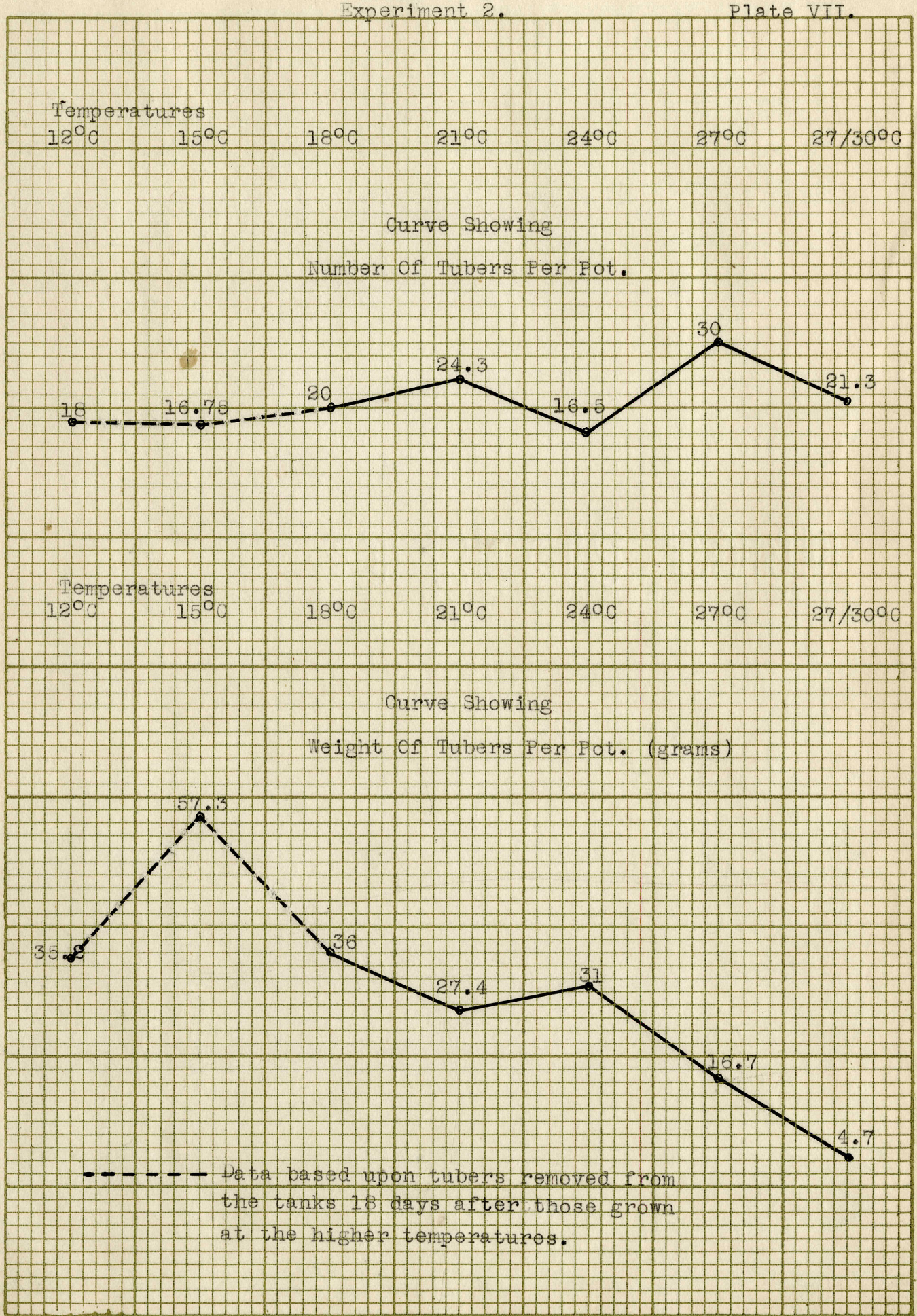
30°C.

Experiment 2.

Plate VI.

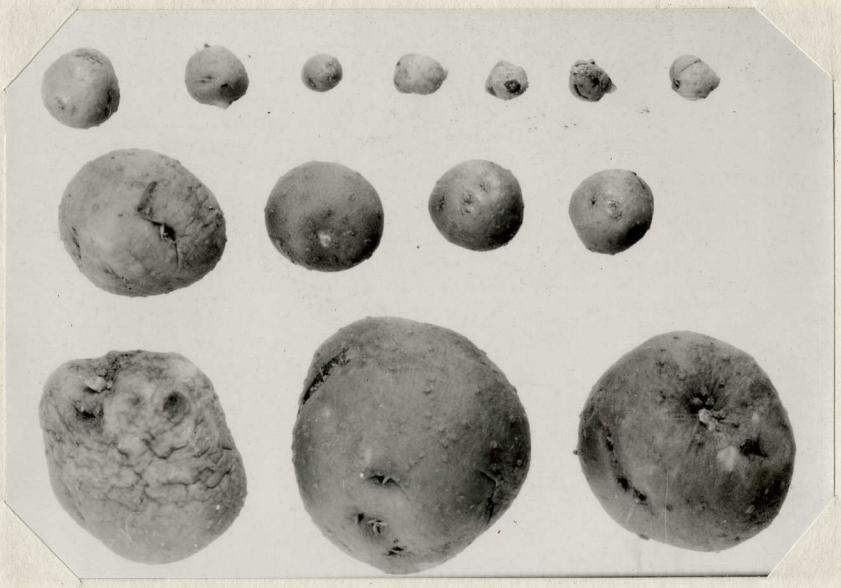


Experiment 2. Plate VII.



Photographs of scabby tubers.

Experiment 2.

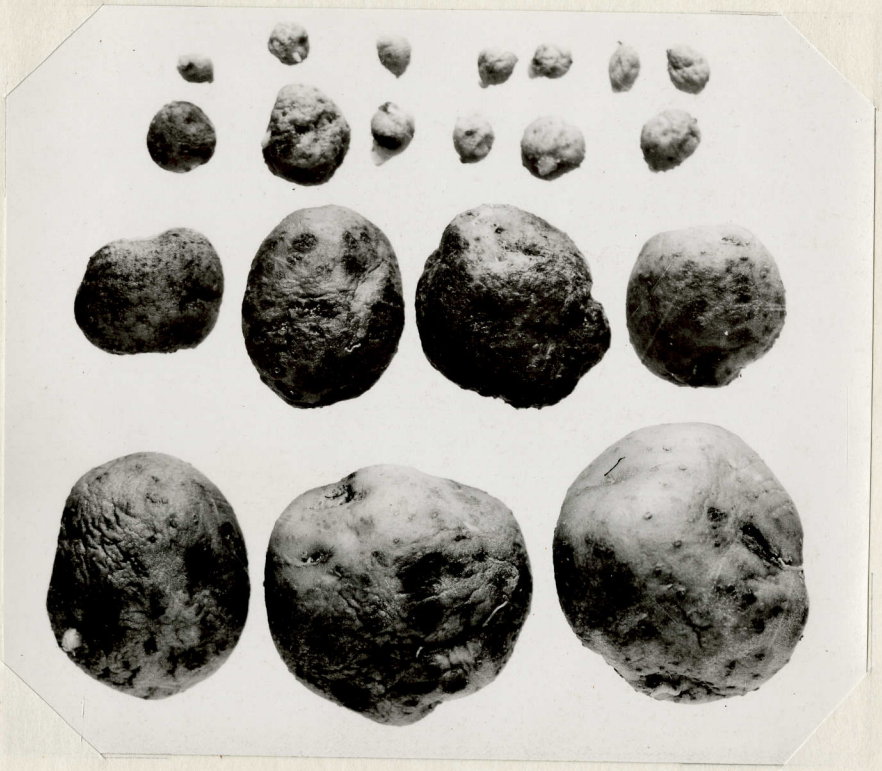


12°C.

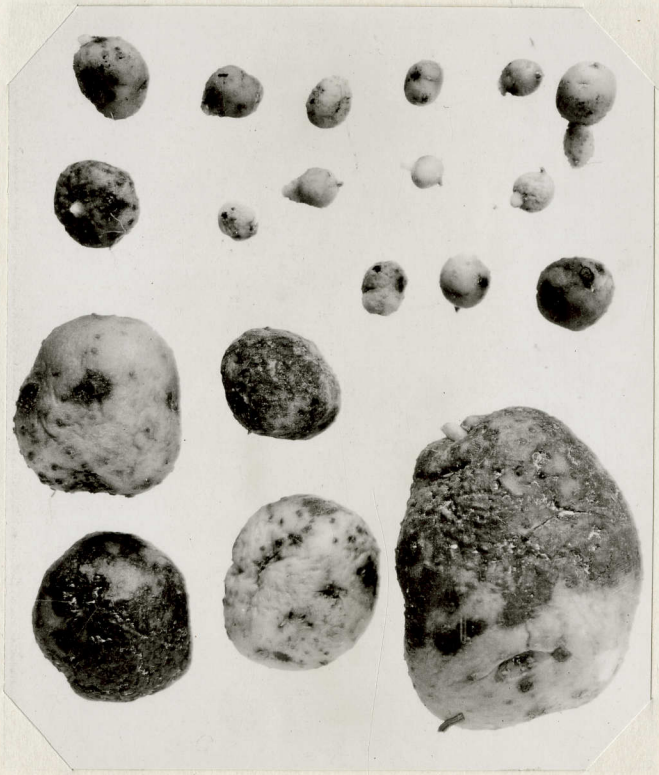
15°C.

Photographs of scabby tubers.

Experiment 2.



18°C.



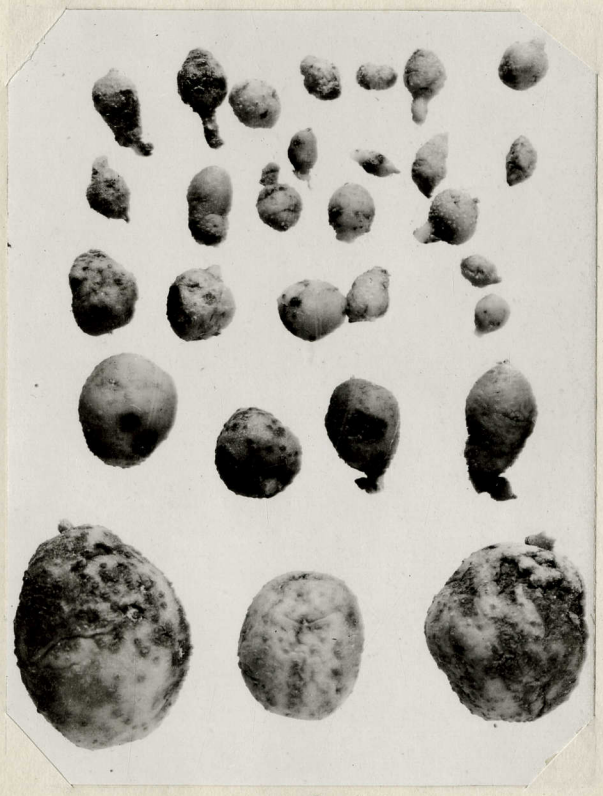
21°C.

Plate X.

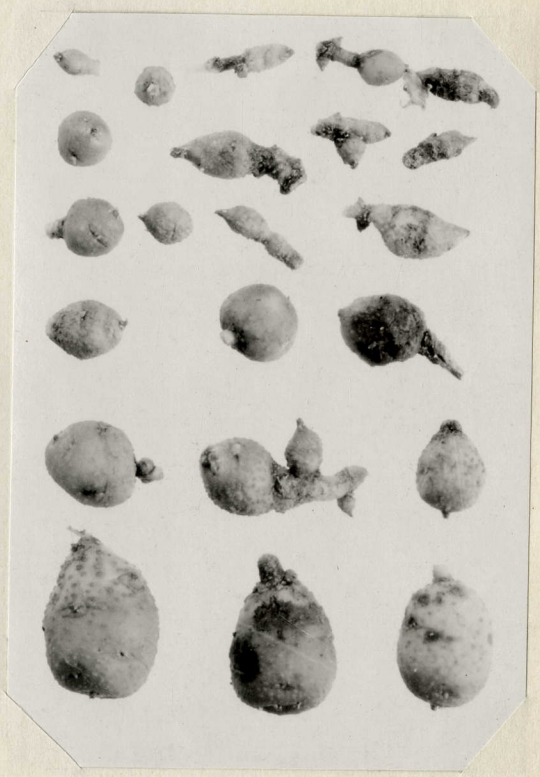
Photographs of scabby tubers.

Experiment 2.

24°C.



27°C.



27/30°C.

APPROVED: L. T. Jones

DATE: June 20, 1920