THESIS

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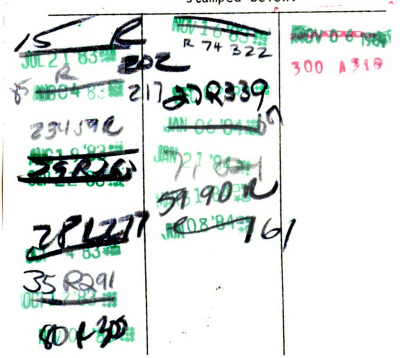

STUDIES ON THE PHYSIOLOGY AND PATHOGENICITY OF ACTINOMYCES OCCURRING ON POTATOES AND IN POTATO SOIL

by

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A THESIS

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A C K N O W L E D G E M E N T

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STUDIES ON THE PHYSIOLOGY AND PATHOGENICITY OF ACTINOMYCES CAUSING POTATO SCAB

Introduction

Conflicting reports have attended the use of mercurials as a soil disinfectant for the control of potato scab. As early as 1893 Beach (4) found that out of seven soil disinfectants mercuric chloride gave best results, effecting a decrease in scabby tubers of 27.4 per cent over untreated rows when the fungicide was applied as a 0.1 per cent aqueous spray to the furrows. Halsted (26), in 1899, after six years of field experimentation, concluded that of 16 soil disinfectants, mercuric chloride and several others reduced scab but not enough to render them of practical value. In 1901 Jones and Edson (31), working on Vermont soils, obtained no control of scab through the use of mercuric chloride.

During the late 1920's another period of intensive investigation began concerning the use of mercurials for soil treatment. In 1927, after three years of experimentation by the New Jersey Agricultural Experiment Station, Martin (44, 45) reported encouraging results from the use of Semesan (hydroxymercurichlorophenol sulfate 35 per cent). By the application of 30, 60, and 90 pounds per acre, he reported 41.5, 62.7, and 69.3 per cent scab-free tubers respectively, as compared with 5.6 per cent from untreated soil. However, in the case of 60- and 90-pound applications,

yield was decreased by 29.3 and 46.1 bushels per acre respecitvely, as compared with the check and the 30-pound application plots. In a subsequent report (1), experiments with several other organic compounds showed an increase in clean tubers of as much as 44.1 per cent over those from untreated check plots, and increases in yield were also secured. In another subsequent report (43), the use was reported of 15 pounds of mercurous chloride applied to the acre with a consequent yield of 92.1 per cent clean tubers as compared with 46.7 per cent on the plots not receiving the fungicide. In a paper (42) delivered before the Twenty-second Annual Meeting of the American Phytopathological Society, further promising results with the use of mercurials were indicated, as shown in the following table from this report:

Treatment	Yield per Acre (Bu.)	Free from Scab (%)	Severe Rhizoctonia (%)
Fertilizer alone	143.3	65.8	51.7
Fertilizer and 20 lbs. calomel/acre	176.0	93.9	2.7
Fertilizer and 10 lbs. yellow oxide of mercury/ acre	147.9	97.4	3.1
Fertilizer and 45 lbs. Semesan/acre	170.7	93.7	1.8

At the conclusion of two years' experimentation on soils in up-state New York, Taylor (64) in 1933 reported a marked increase of scabby tubers following the use of calomel (20 pounds per acre) and yellow oxide of mercury (10 pounds per acre). Organic mercury

compounds while causing more scab than the checks yielded potatoes with less scab than with the use of inorganic mercurials. In 1934 Taylor (63) reported the use of additional mercurials (Calogreen, DuBay "738", and corrosive sublimate), but continued to obtain tubers with significantly more scab than the checks.

The problem in New York was again taken up by Taylor and Blodgett (62) who reported in 1936 that mercurials increased scab not only when applied to the soil but when tubers were planted that had received seed treatment with mercurials.

On the other hand in Eastern New York State, on Long Island, Cunningham (11) reported as the result of two years' trials that there may be a marked decrease in the scab infestation following the addition of either yellow oxide of mercury or calomel to the fertilizer. With the use of four pounds of calomel and with four pounds of yellow oxide, the percentages of clean tubers were 57.14 and 85.61 per cent as compared with 38.23 per cent clean tubers as checks.

Results of trials in Ohio by Pierstorff (54) showed that mercurials had no appreciable effect on scabbing.

In 1934 MacLeod and Howatt (40) published conclusions based on six years' trials with mercurials on New Brunswick soils. Mercurous or mercuric chloride when applied at the rate of 10 or 15 pounds per acre were reported to be dependable for the control of scab and black scurf in heavily infested soils.

In the Netherlands, Slikke (60) treated the planting holes with 250 cc. of an 0.1 per cent solution of mercuric chloride, and

in 1935 reported good results in the control of scab as well as rhizoctoniose. More recently (1940), Meyer (47), also in Holland, obtained good results with calomel, yellow oxide of mercury, and corrosive sublimate. The latter was considered the most promising for practical control.

In Germany, Störmer (61) reported the efficacy of one per cent mercuric chloride when applied with superphosphates at 357 pounds per acre to sandy soil, but cited failure under other soil conditions.

Johnson (30) in 1936 reported that in England the use of 230 and 300 pounds of mercuric chloride per acre produced a crop of scab-free potatoes as compared to scabby potatoes grown on untreated plots.

The problem of mercurials for soil treatment of scab has been under consideration at this Station for a number of years. Frutchey and Muncie (17) used calomel at the rate of 20 pounds per acre, and yellow oxide of mercury and an organic mercurial compound, both at the rate of 10 pounds per acre. Not only did none of these materials cause a suppression of scab in comparison with the scab in check rows, but they were actually instrumental in producing an increase in scabbiness. Further trials along these lines were conducted by KenKnight (33, 34), who applied mercury compounds to the soil at rates of application from six to one hundred pounds per acre, but who also came to the conclusion that these mercurials were not only ineffectual in controlling scab but actually increased the trouble, at times three-fold.

Various explanations have been made to account for the varying reactions obtained from the use of mercurials. Daines (12) holds that the effectiveness of mercury compounds resides in their ability to be reduced to the elemental vapor in which form they are able to migrate in the soil and produce their fungicidal action. Any property that prevents the conversion of a mercury salt to mercury vapor destroys the fungicidal effects of the mercurial. Such limiting factors may be 1) the presence of mercury-precipitating ions; 2) a soil with a high mercury-binding capacity; and 3) a soil having a low reducing potential. Stormer (61) concluded that an acid medium is essential for the release of the fungicidal properties of the mercurial salt, which in the presence of alkaline fertilizers would be immobilized. MacLeod and Howatt (40) believed that the discrepancy in the reports is due to a failure to incorporate the fungicide into the soil evenly, and they concluded that the efficacy of mercurial treatments depended largely upon the thoroughness with which the space to be occupied by the tubers is impregnated with the fungicide. Frutchey (18) was of the opinion that the fluctuation in results had a more biological basis, and that in the increase of scab several factors were operative: 1) a reduction in microbial competition, whereby the bacteria killed by the mercurial allowed Actinomycetes to multiply; and 2) the presence of a mercury-resistant scab organism, whose virulence would be enhanced because of inhibited competition.

KenKnight (33) came to the conclusion that calomel is not rendered ineffective as an antiseptic when used as a soil treatment

in Michigan soil, but that differences in the effect of calomel are correlated with different strains or species of pathogenic Actinomyces.

In the light of prior investigations under Michigan conditions, and the probabilities to which they lead, the writer has been prompted to continue the scab control program by a study of the local Actinomycetal flora and its biology. If, as has been indicated by some of the workers mentioned above, the effect of mercurials on the soil is a function of the particular Actinomycetal flora, then knowledge of such a flora becomes necessary before effective control measures can be taken. The substance of this study, then, has assumed two forms: one, the isolation of local Actinomycetes from soil and scabby potatoes, and by means of their physiological characteristics a description of their identities; and two, the development of methods which will facilitate a determination of an isolate's pathogenicity toward the potato.

The necessity of accomplishing the first objective before adequate interpretation of results can be made is implied above. In connection with the second objective, Goss (23) has said: "The development of an experimental technique which will permit the study of the pathogene in the soil immediately surrounding the susceptible plant tissues, as contrasted with the present gross methods, is essential for the determination of the effect of many of these soil factors upon the disease."

PART I

Isolation and Differentiation

Historically, the systematic position of the Actinomycetes as a whole has often been questioned. Many bacterial systematists have included the group in the Schizomycetes because of 1) the slenderness of the mycelium; 2) the absence of discrete nuclei; 3) the bacterial nature of the arthrospores; 4) the non-chitinous nature of the cell walls; and 5) the occurrence of acid-fast staining reactions. On the other hand, mycologists such as Vuillemin (66), deBary (3), Gasperini (19), and Drechsler (15) have held that spore production of Actinomycetes is essentially similar to that of conidial production in the Fungi Imperfecti. Others, such as Lieske (38), Ørskov (50), and Jensen (29) preferred to consider the Actinomycetes as an independent group of plant life, placing them between the molds and the bacteria. Waksman (67) is of the opinion that they should be regarded as a group of fungi "to be classified separately from other groups till their exact systematic position has been definitely established."

If confusion exists regarding the systematics of Actinomycetes as a whole, then an even more chaotic condition prevails within the alliance itself. According to Waksman (67) more names have been applied to the genus Actinomyces than to any other group of microorganisms, and among these, in addition to the proper designation "Actinomyces", there have been such denominations as Leptothrix, Cladothrix, Oospora, Oidium, Discomyces, Nocardia, Streptothrix, Malbranchea, Micromyces, Cohnistreptothrix, and Microsiphonales. Further indication of the confusion

which exists lies in the number of species given for Actinomycetes by various workers. Waksman states more than 50 species have been isolated from the soil. Brumpt (8) lists some 70 species that have been found in the lesions of mammals, including man. Lieske (38) lists 76, and C. W. Dodge (14) in his compilation in "Medical Mycology" reports 108 species.

As an example of the imperfect taxonomic structure may be cited the condition which exists with regard to but one species, viz., Actinomyces scabies. Thaxter (65) in first describing potato scab named the etiologic agent Oospora (Actinomyces) scabies. Lutman and Cunningham (39) believed the organism to be identical with Actinomyces chromogenus Gasperini. Later, however, the studies of Krainsky (36), Conn (10), and Waksman and Curtis (71), showed that the characters of Actinomyces chromogenus were those of a group rather than of a single species. In 1914 Lutman and Cunningham (39) isolated "a large number of organisms" causing scab. In 1919 McKinney (46) described three strains of Actinomyces causing scab and Shapovalov (58) dealt with several strongly pathogenic forms isolated from scabby tubers originating in Maine, Vermont, and Wisconsin. Millard (49) described five strains in which "from the first, considerable variation was observed." Waksman (70) in 1919 described one strain of Actinomyces as the primary cause of scab, which he called Actinomyces scabies, but when compared with isolates from other workers, he found very considerable differences between them. Wollenweber (73) in 1920 created a number of new Actinomyces species, and attributed the different types of scab to these separate species. The causal agent of umbonate scab was said to be Actinomyces aerugineus, those of surface scab A. tri-

color and A. intermedius, that of deep scab A. incanescens, and those producing a variable type of scab A. xanthostroma and A. albus var. ochroleucus. These findings were corroborated by Janchen (29) who also added A. nigrificans as another causator of surface scab.

In England Millard and Burr (48), after a study of 24 strains, also came to the conclusion that there was no specific species responsible for the disease on potatoes, and that different species were responsible for different types of lesions. For example, A. clavifer and A. viridis caused an "intermediate" type of lesion, A. setonii, a"pitted"type, and A. flavus, a "tumulus" type.

Subsequent investigations by Goss (23), Afanasiev (2), and others have shown that the different types of scab are but progressive stages of the same disease caused by but one species. However, European workers, for instance De Bruyn (6, 7) still hold (as late as 1939) to the multiple origin interpretation of different types of scab.

Further, three strains attacking potatoes were isolated by Cocchi (9) from Italian soils. Kiessling (35) isolated a number of strains from German soils, and Wingerberg (72) isolated 21 different pathogenic strains. The latter, however, when compared to four strains obtained from the Centraalbureau in Baarn, Netherlands, proved to be quite diverse in their cultural characteristics. Seven pathogenic strains were isolated by Afanasiev (2) from scabby potatoes grown in Nebraska, and of nine reportedly pathogenic cultures received from other workers, he found none to be infectious. Leach, Decker and Becker (37) interpreted the differences in pathogenicity inherent in

two A. scabies isolates to be due to varying physiologic form, and they introduce the concept of races into the taxonomic problem.

Undoubtedly much of the confusion arises as a result of the intensely pleomorphic nature of the Actinomyces. Many workers have reported the fluctuations in growth of a single isolate as the result of varying environmental conditions, including moisture, temperature, hydrogen-ion concentration, constitution of the media, vapor pressure, etc. In addition, as pointed out by the studies of Schaal (57) the species A. scabies is decidedly unstable. When grown from monosporous lines, the colonies showed considerable sectoring, and some of these variants continued to sector, yielding a variety of distinctly different cultural types.

The cultural aspects of the problem, however, promise to becomes more orderly as increasingly greater use is made of synthetic media of precise composition, hydrogen ion concentration, sterilization, etc., and of standard conditions of incubation. The newer techniques will also bring about the invalidation of many inadequate descriptions which abound in the literature. According to Waksman (70), much of the variability mentioned by Lieske (38) was due to the use of non-synthetic nutrient agars which are variable in composition.

Various characters have been proposed by different authors as a basis for species differentiation. Petruschky (53) based a system upon the characteristics of club formation. Drechsler (15) used the differential characters of conidiophore coiling and branching and the mode of septation in the process of spore formation. Waksman (70), however, discovered these characteristics to be variable and the result

of varying composition of media. In addition, Drechsler's characters would have no importance in dealing with many of the pathogenic forms which do not produce conidiophores. Ørskov (50), also using morphological characters, divided the group according to the ability or inability of the mycelium to produce fragmentation. Waksman (70) has proposed a system of differentiating soil Actinomycetes based chiefly on physiological characteristics, and since in the problem of the writer there is no occasion to deal with a system encumbered by a consideration of species pathogenic to animals, it has been decided in this paper to make use of the physiological approach to classification based on the ideas of Conn and of Waksman. Both of these workers recognize that in a final classification the broadest divisions would perhaps be based on differences in morphology, but as Conn (10) pointed out, the constancy of morphological characteristics is a function of media constitution and conditions of growth, and as such, Actinomycetes must await first a better physiological understanding of their nature.

Methods:

Isolation: In this partial survey of the Actinomycetal flora of Michigan potato soils, isolations were made from soil and tubers collected from Field 19 (Michigan State College Campus, East Lansing) and from the Potato Experimental Farm at Lake City, Michigan. The method of isolating Actinomyces from potato tubers was that developed at this Station by KenKnight and Muncie (32), which consisted of disinfecting lesions in a 1:1000 mercuric chloride solution for one minute, rinsing in water, triturating in a sterile test tube containing water, and

using as a source of plating-out inoculum, the material contained in the tube. The plating medium which was partially selective for Actinomycetes was composed of one gram of glucose and one cc. of a 10 per cent solution of each of the following salts: KH_2PO_4, $NaNO_3$, KCl, and $MgSO_4 \cdot 7\ H_2O$; and 15 grams of agar per liter of distilled water, with the pH adjusted to 7.0. Incubation was carried out for at least seven days at a temperature of 25° C., though the thermometer often rose to 32° C. during the summer when the thermostatic control of the incubator was superseded by the degree of room temperature.

Random samples of the soil in Field 19 and samples of soil in the Sulfur Treatment Plots at Lake City were brought to the Station laboratory, each sample was well mixed, and from each lot platings were obtained by dipping a sterilized camel's hair brush into the soil and then gently flicking its adherent particles onto a Petri dish covered by a layer of the medium of above mentioned constitution. The same period and conditions of incubation were then used as for platings from lesions as previously described.

Differentiation: Conn (10) found it impossible to predict the growth of a certain Actinomycete upon a new medium from the knowledge of its growth upon other previously investigated media. As a consequence, each new medium tested serves to break up still further the types already recognized by their growth on other media. Therefore, to obtain as complete a characterization of an isolate as possible, a rather large variety of media have been employed, including a modified Czapek's Agar, Krainsky's Glucose Agar, Conn's Malate-Glycerin Agar, Conn's Citrate-Glycerin Agar, 15 per cent Gelatin, Sterile Skimmed Milk,

Potato Plug, Starch Agar, Nutrient Peptone Agar, Creatinin Solution, Carrot Agar, and Tyrosin Agar. The composition and physiologic importance of each of these media are given below. In all cases the purest obtainable chemicals, sugars, proteins, and biologicals were used. In the case of chemicals, chemically pure ingredients were employed wherever possible. Sugars, proteins and agar were of Bacto quality. All water used for media was distilled. Test tubes of as nearly the same diameter as possible were used and filled to a uniform height of two inches to facilitate comparative readings, particularly of proteolytic reactions. The length of time which a medium boiled during its preparation, and the period and pressure with which it was autoclaved had an ultimate effect upon physiological reactions; therefore, all heating and sterilizing were executed with as uniform a procedure as possible.

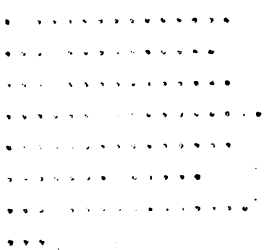
A. Modified Czapek's Agar (70)

$NaNO_3$	2 grams
K_2HPO_4	1 gram
$MgSO_4$	0.5 gram
KCl	0.5 gram
$FeSO_4$	0.01gram
Sucrose	30 grams
Agar	15 grams
Water (distilled)	1000 cc.

******

Incubation temperature..... 25° C.
Readings.....At intervals from 7 to 15 days.

This medium proved particularly useful in the study of cultural characteristics. The vegetative growth was rather scant due to the impoverished composition of the medium, and as this brought forth a good



development of aerial mycelium, the medium was particularly adapted for that aspect of culture.

B. Krainsky's Glucose Agar (36)

Glucose	10 grams
K_2HPO_4	0.5 gram
Asparagine	0.5 gram
Agar	15 grams
Water (distilled)	1000 cc.

*****

Incubation temperature..... 25° C.
Readings.....At intervals from 7 to 15 days

This medium, containing a more favorable nitrogen supply, was found to permit a heavier growth of organisms, and in some cases gave a characteristic growth reaction for certain species. A few organisms are chromogenic on this medium, and so make it possible to distinguish two types, one from the other, by means of their reactions on this agar.

C. Conn's Modified Malate-Glycerin Agar (10)

Calcium malate	10 grams
NH_4Cl	0.5 gram
K_2HPO_4	0.5 gram
Glycerin	10 grams
Agar	15 grams
Water (Distilled)	1000 cc.

*****

Reaction adjusted to pH 7.0 by use of NaOH
Incubation temperature..... 25° C.
Readings.....At intervals from 7 to 15 days

Krainsky, who developed the basic malate-glycerin agar used the medium in a study of Actinomycetal chromogenesis. Such pigmentation may occur in any one, two, or three regions: 1) in the aerial mycelium; 2) in the vegetative growth; 3) in the medium surrounding a colony - and the diversity of colors is considerable. By the addition of one per cent glycerin, Conn obtained also a stimulation

of growth. Since pigment production by any one strain is fairly constant, this medium is of particular differentiating value.

D. Conn's Citrate-Glycerin Agar (10)

Calcium citrate	10 grams
NH_4Cl	0.5 gram
K_2HPO_4	0.5 gram
Glycerin	10 grams
Agar	15 grams
Water (distilled)	1000 cc.

*****
Reaction adjusted to pH 7.0 by use of NaOH
Incubation temperature.....25° C.
Readings.....At intervals from 7 to 15 days

The uses of this medium are similar to those cited under Malate-Glycerin Agar, the only difference being that this medium produced more characteristic, and at times different growth and pigmentation of certain species. Difficulty was experienced in bringing the calcium citrate into solution, so that finally the expedient was employed of agitating the sediment before pouring a tube into a plate, and in that way a Petri dish of agar was obtained in which the particles of calcium citrate were more evenly distributed throughout the medium.

E. 15 per cent Gelatin

Gelatin (Bacto)..... 150 grams
Water (distilled).... 1000 cc.
*****
Unadjusted
Incubation temperature.....25° C.
Readings.....At intervals to 30 days.

Nearly all Actinomycetes liquify gelatin, but the rapidity with which different species accomplish proteolysis at a given hydrogen-ion concentration varies considerably. Soluble pigments are also produced, some of them diffusing into the liquified portion, others into the solid gelatin, and the constancy of the chromogenic reaction makes

this a valuable medium. A species is characterized as strongly proteolytic if the gelatin is rapidly peptonized, or as weakly proteolytic if the gelatin becomes gradually softened, resulting in a thickly viscid substrate. A quinone reaction is produced by some species, which leads to a re-gelatification of a previously peptonized gelatin.

F. Sterile Skimmed Milk

Reaction unadjusted
Incubation temperature.....25° and 37° C.
Readings..... Daily to 30 days

Milk manifests a wide range of reactions when saprophytized by Actinomycetes, including hydrolysis, coagulation or non-coagulation, subsequent peptonization, and color changes. The rapidity with which these reactions take place form another basis of differentiation. Brom cresol purple was added to the milk during the earlier part of this investigation but was later omitted because it interfered with color reactions.

G. Potato Plug

Treated for 30 minutes in 1:500 solution of
sodium carbonate in water
Incubation temperature.....25° C.
Readings.....7 days

This medium is particularly useful in demonstrating members of the old A. chromogenus group, since all of them produce a dark brown or black color on potato. That this dark reaction, however, is not always produced by the same principle has been shown by Waksman (70) who incubated a dozen potato-blackening species on tyrosin agar and found melanin production in the case of only a few. Implicated were strains of A. scabies, some of which formed no pigment on tyrosin

agar.

H. Waksman's Starch Agar (70)

Starch..... 10 grams
K_2HPO_4..... 1 gram
Mg SO_4..... 1 gram
NaCl..... 1 gram
$(NH_4)_2SO_4$..... 2 grams
$CaCO_3$..... 3 grams
Agar ..... 10 grams
Water (distilled)... 1000 cc.
*****
Incubation temperature.....25° C.
Readings.....At intervals from 10 to 15 days

The medium was prepared by adding the 10 grams of starch to 800 cc. of distilled water and boiling down to 500 cc. This was then in turn added to a solution of the above salts dissolved in another 500 cc. of distilled water. At the end of the incubation period the surface of each Petri dish culture was covered with a solution of iodine and potassium iodide. A clear zone encircling colonies indicated a diastatic action exerted by the Actinomycete. According to Waksman (69), most all Actinomycetes hydrolize starch, which is one of the best sources of carbon for these organisms.

I. Nutrient Peptone Agar

Beef Extract.....3 grams
Peptone.....5 grams
Agar.....15 grams
Water (distilled).....1000 cc.
******
Reaction adjusted to pH 7.0 with NaOH
Incubation temperature.....25° C.
Readings.....At intervals from 7 to 15 days

This is another medium elucidating the chromogenic nature of Actinomycetes.

J. Tyrosin Agar (68)

Tyrosin.....1 gram
K_2HPO_4.....1 gram
$MgSO_4$.....0.5 gram
KCl.....0.5 gram
$FeSO_4$.....0.01gram
Sucrose.....30 grams
Agar.....15 grams
Water (distilled).1000 cc.
******
Reaction unadjusted
Incubation.....25° C.
Readings.....At end of 15 days

As will be noted, the above formula is the same as Czapek's Agar cited under Medium A, with the exception that 0.1 per cent tyrosin has taken the place of the previous nitrogen source. As has been pointed out above, the browning which characterized the old chromogenus group of Actinomyces is not always caused by the same agent. In Waksman's studies (68) of a number of species producing the chromogenus reaction on organic media, only A. scabies and another species produced a brown soluble pigment on tyrosin agar. However, tyrosin agar is not the last word in the characterization of A. scabies since the ability to form the complex tyrosinase is not extant among A. scabies as a whole, and further may be lost in those that have the ability through frequent subculturing.

K. Creatinin Solution

Creatinin.....2 grams
K_2HPO_4.....1 gram
$MgSO_4$.....0.5 gram
KCl.....0.5 gram
$FeSO_4$.....0.01gram
Sucrose.....30 grams
Water (distilled).1000 cc.
******
Reaction unadjusted
Incubation temperature.....25° C.
Readings.....15 days

This formula again duplicates Czapek's Agar with the exception of $NaNO_3$ which has been replaced by creatinin. This medium has been found useful in separating species giving the tyrosinase reaction but which are not A. scabies, for the latter is not capable of producing a yellow soluble pigment in this solution.

L. Carrot Agar:

Carrots.....20 grams
Agar.....17 grams
Water (distilled)..1000 cc.
******
Reaction adjusted to pH 7.0
Incubation temperature...25° C.
Readings.....At intervals to 15 days

The use of carrot as a substrate proved useful in its ability to induce characteristic growth of some Actinomyces, particularly A. griseolus in this study, and in Waksman's researches also A. hominis.

Descriptions of the Species

1.

ISOLATE NO. 484

SOURCE OF ISOLATE: Same as for Isolate No. 649 (tuber) but from a different tuber.

1. Czapek's Agar: Aerial mycelium present, in color ranging from white to violaceous gray; vegetative growth from white to dull gray; soluble pigment absent.

2. Glucose Agar: Aerial mycelium absent; vegetative growth from pallid to faint yellow; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, dark gray, pulvinate; vegetative growth dull gray-green, giving rise to brown on 15th day; soluble pigment absent.

4. Citrate-Glycerin Agar: Aerial mycelium present, colored concentrically light gray and white; vegetative growth dull brown; soluble pigment absent. Wide borders of cleared agar around colonies.

5. Gelatin: Aerial mycelium absent; liquefication $\frac{1}{2}$ inch of gelatin in 30 days; no soluble pigment.

6. Milk: Aerial mycelium present, white; vegetative growth yellowish; top $\frac{1}{4}$ of milk colorless whey in 15 days turning to orange whey throughout in 30 days.

7. Potato Plug: Aerial mycelium absent; vegetative growth slimy and flesh-colored in 7 days and persisting to 30 days; potato plug not colored.

8. Starch Agar: Aerial mycelium absent; vegetative growth faint yellow; soluble pigment absent.

9. Nutrient Peptone Agar: Aerial mycelium present, scant, white; vegetative growth yellow; soluble pigment absent.

10. Tyrosin Agar: Aerial mycelium present, light gray; vegetative growth dark brown; soluble pigment present, dark amber brown.

Conclusions regarding identity: The reactions shown by this organism do

not coincide with any species described by Waksman and consequently it is being designated Actinomyces K484*.

2.

ISOLATE NO. 636

SOURCE OF ISOLATE: Same as for Isolate No. 638 (soil).

1. Czapek's Agar: Aerial mycelium present, white; vegetative mycelium buff; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, white; vegetative growth yellowish; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, white; vegetative growth brownish; soluble pigment absent.

4. Citrate-Glycerin Agar: Aerial mycelium present, white; vegetative growth yellowish; soluble pigment absent; halo of cleared medium around each colony.

5. Gelatin: Aerial mycelium absent; vegetative growth white; soluble pigment faint yellow; liquefaction complete in 20 days.

6. Milk at 25° C.: Aerial mycelium present, scant, whitish; vegetative growth ranging from buff to yellow; soluble pigment pale orange after 23 days; hydrolysis of milk (no curd) from $\frac{1}{4}$ inch after 7 days to 2 inches (entire tube) in 23 days.

7. Potato Plug: Aerial mycelium present as a small patch, for most part absent, even after 30 days, white; vegetative growth potato-colored, moist; potato plug not colored.

8. Starch Agar: Aerial mycelium present, white; vegetative growth pale light yellow; soluble pigment absent.

9. Nutrient Peptone Agar: Aerial mycelium present, white; vegetative growth yellowish; soluble pigment absent.

10. Tyrosin Agar: Aerial mycelium present, gray; vegetative growth reddish brown; soluble pigment present, brown.

*Isolates which heretofore have not been described are designated by a number preceded by the initial "K".

Conclusions regarding identity: The above reactions bear a close resemblance to those described by Waksman for A. poolensis Taubenhaus. The poolensis organism was originally isolated from diseased sweet potatoes by Taubenhaus and was later again isolated by Waksman and by Lipman. The only serious difference between these two descriptions lies in the reaction on potato, which in the case of A. poolensis consists of thin reddish-brown growth sinking into the plug, the potato becoming purplish with age, and in the case of the above shows a buff colored growth with the plug remaining uncolored. Agreement on other points is satisfactory enough for the writer to call this organism A. poolensis Taubenhaus.

3.

ISOLATE NO. 638

SOURCE OF ISOLATE: Soil from Sulfur Plots (Series III, #7, Potato Experimental Farm, Lake City, Michigan.) Soil had a pH as of August 21, 1940 (the date collected) of 4.8. Date of isolation August 30, 1940.

1. Czapek's Agar: Aerial mycelium present, concentrically white and gray; vegetative mycelium ranging from white to light gray; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, gray outlined by white; vegetative mycelium ranging from white to pale yellow; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium absent; vegetative mycelium pale yellow, some colonies becoming pinkish; soluble pigment absent.

4. Citrate-Glycerin Agar: Aerial mycelium present, white; vegetative mycelium white to pale yellow; soluble pigment absent.

5. Gelatin: Aerial mycelium absent; vegetative mycelium completely embedded, white; complete liquefaction in 23 days; soluble pigment absent.

6. Milk at 25° C.: Aerial mycelium absent; vegetative growth white; milk partially hydrolyzed (no prior formation of curd) to a pearly white; soluble pigment absent.

7. Potato Plug: Aerial mycelium present, scant, white; vegetative growth restrained, potato-colored; potato plug not colored.

8. Starch Agar: Aerial mycelium present, white in 15 days tending toward gray with age; vegetative growth ranging from pallid to pale yellow; soluble pigment absent.

9. Nutrient Peptone Agar: Aerial mycelium present, white; vegetative growth white to pale yellow; soluble pigment absent.

10. Tyrosin Agar: Aerial mycelium present, flaky white; vegetative growth brown; soluble pigment absent.

Conclusions regarding identity: No description recorded by Waksman agrees with the above reactions. The organism is being designated Actinomyces K638.

4.

ISOLATE NO. 640

SOURCE OF ISOLATE: Same as for Isolate No. 1239 (soil).

1. Czapek's Agar: Aerial mycelium present, ranging from light to dark gray; vegetative growth olive green with some colonies black centered; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, light gray green; vegetative growth chrome-yellow green; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, whitish; vegetative growth chrome-yellow green; soluble pigment present, yellow.

4. Citrate Glycerin Agar: Aerial mycelium present, gray green; vegetative growth pale grass green; soluble pigment absent.

5. Gelatin: Aerial mycelium present, light gray; vegetative growth yellow; soluble pigment present, light yellow; liquefaction slow, $\frac{1}{4}$ in 30 days.

6. Milk, 25° C.: Aerial mycelium present, scant, white; vegetative growth whitish; entire 2" of tube hydrolized (without prior curd formation) to a colorless whey in 10 days, later becoming orange-colored.

7. Potato Plug: Aerial mycelium present, ranging from white to mouse gray becoming violaceous with age; vegetative growth heavy and much convoluted; potato plug unaffected at first but with age turning black green; water below plug light brown; in age growth exudes black drops of fluid.

8. Starch Agar: Aerial mycelium present, greenish gray; vegetative growth dull light green; soluble pigment absent.

9. Nutrient Peptone Agar: Aerial mycelium present, greenish gray, vegetative growth dull light green; soluble pigment absent.

10. Tyrosin Agar: Aerial mycelium present, greenish-gray; vegetative growth yellow; soluble pigment present, light yellow brown.

Conclusions regarding identity: The above reactions are sufficiently like those of A. flavovirens Waksman to warrant, in the writer's mind, designating this organism by that name. The organism was first isolated by Waksman from soil.

5.

ISOLATE NO. 649

SOURCE OF ISOLATE: Scab lesion on a Chippewa potato tuber grown in the Botany Department greenhouse, Michigan State College, on soil obtained from compost heap, Field 19 (MSC Campus). Tubers lifted June 1940. Isolated August 2, 1940.

1. Czapek's Agar: Aerial mycelium present, whitish; vegetative growth pallid to yellow buff; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, hoary pale gray, pulvinate; vegetative growth grading from white into a faint olive brown; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, white; vegetative growth ranging from pale yellow buff to dark violaceous brown; soluble pigment encircling some colonies, purple.

4. Citrate-Glycerin Agar: Aerial mycelium present, hoary, dingy white; vegetative growth ranging from white to mostly khaki-brown; soluble pigment absent.

5. Gelatin: Aerial mycelium present, hoary, light gray; vegetative growth encircled with white; soluble pigment present, dark brown; liquefaction.

6. Milk, 25° C.: Aerial mycelium present, white; vegetative growth dark brown. Soluble brown pigment beginning seventh day and progressively spreading to entire extent of milk in 30 days. Coagulation and peptonization not observed.

7. Potato Plug: Aerial mycelium present, scant, white; vegetative growth brown; color of plug black; water below plug brown.

8. Starch Agar: Aerial mycelium present, scant, white; vegetative growth ranging from pallid to pale brown buff.

9. Nutrient Peptone Agar: Aerial mycelium present, concentrically ringed with gray and white; vegetative growth pallid gray to dull yellow brown; soluble pigment present, dark brown.

10. Tyrosin Agar: Aerial mycelium present, white; vegetative growth yellow; soluble pigment present, yellow brown.

Conclusions regarding determination: Reactions as a whole agree satisfactorily with those that Waksman found to hold for A. scabies, and the writer considers the isolate to be this species.

6.

ISOLATE NO. 651

SOURCE OF ISOLATE: Scab lesion on a Chippewa potato tuber grown in the Botany Department greenhouse, Michigan State College, on soil obtained from compost heap, Field 19 (MSC Campus). Tubers lifted June 1940. Isolated August 2, 1940.

1. Czapek's Agar: Aerial mycelium present, white to gray; vegetative growth ranging from pallid through pastal yellow to medium brown, soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, white to gray vegetative growth ranging from pallid to pastel yellow; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, white; vegetative growth white to yellow buff; soluble pigment absent.

4. Citrate-Glycerin Agar: Aerial mycelium present, white to gray; vegetative growth ranging from pallid through pastel yellow to medium brown; soluble pigment absent.

5. Gelatin: Aerial mycelium present, light gray ringed by white; vegetative growth yellowish; soluble pigment present, dark brown. Liquefaction not observed.

6. Milk 25° C.: Aerial mycelium present, white; vegetative growth dark brown; soluble pigment present with top 4/5ths of tube gray-brown, and bottom 1/5th peach on 30th day. Coagulation and peptonization not observed.

7. Potato Plug: Aerial mycelium present, scant, white; vegetative growth ranging from moist dirty gray to dry black; potato plug black; water below plug medium brown.

8. Starch Agar: Aerial mycelium present, white; vegetative growth ranging from white to yellow buff; soluble pigment absent.

9. Nutrient Peptone Agar: Aerial mycelium present, white; vegetative growth ranging from yellow to brown; soluble pigment present, dark brown.

10. Tyrosin Agar: Aerial mycelium present, white; vegetative growth yellow; soluble pigment present, amber brown.

Conclusions regarding determination: The reactions as a whole agree satisfactorily with those that Waksman found to hold for A. scabies. They also are similar to Isolate No. 649, except for the non-liquefaction of gelatin.

7.

ISOLATE NO. 673

SOURCE OF ISOLATE: Same tuber as for Isolate No. 1432.

1. Czapek's Agar: Aerial mycelium present, light gray; vegetative growth cream colored; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, light gray; vegetative growth cream colored; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, light greenish gray; vegetative growth light chocolate colored; soluble pigment present, brown.

4. Citrate Glycerin Agar: Aerial mycelium present, light gray; vegetative mycelium cream colored; soluble pigment present, faint brown.

5. Gelatin: Complete liquefaction in 10 days, soluble brown pigment in 15 days, turning somewhat olivaceous with age.

6. Milk, 25° C.: Partial peptonization of curd in 10 days, complete peptonization in 25 days with all of whey colored orange.

7. Milk, 37° C.: Complete peptonization of curd in 14 days, whey yellow-orange colored, later becoming orange-brown.

8. Potato: Aerial mycelium present, greenish white; plug turned pink at first, then with age deep reddish brown.

9. Starch Agar: Colonies puntiform, white; soluble pigment absent.

10. Nutrient Peptone Agar: Aerial mycelium present, velvety white; vegetative growth, yellow buff; soluble pigment present, faint brown.

Conclusions regarding identity: This isolate is considered to be A. griseolus Waksman, since there is very good agreement between the description given above and those listed by Waksman.

8.

ISOLATE NO. 676

SOURCE OF ISOLATE: Same as for Isolate No. 1432, but from a different tuber.

1. Czapek's Agar: Aerial mycelium present, gray; surface poroid; vegetative mycelium dull green; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, gray; surface poroid,

vegetative mycelium dull green; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, scant, grayish; vegetative mycelium dull dark green; soluble pigment present, green.

4. Citrate-Glycerin Agar: Aerial mycelium present, dark gray; surface poroid, exuding droplets of water at first; vegetative mycelium dull dark green.

5. Gelatin: Aerial mycelium present, white to gray; vegetative growth blue green, later changing to pallid yellow; complete liquefaction after 10 days; soluble pigment present, faintly oil yellow after 25 days.

6. Milk, 25° C.: Vegetative growth white on top with some colonies green and gray below; after 10 days entire contents of tube turned colorless whey. After 20 days colored orange.

7. Potato Plug: At first (7 days) aerial mycelium absent; vegetative growth dirty yellow green; plug uncolored. Later (30 days) aerial mycelium present, dark violaceous gray with some patches of white; vegetative growth convoluted; plug turned black green.

8. Starch Agar: Aerial mycelium absent; vegetative growth pallid.

9. Nutrient Peptone Agar: At first no aerial mycelium; vegetative mycelium light mustard-yellow. Later scant white aerial mycelium.

10. Tyrosin Agar: Aerial mycelium present, dark gray; surface of growth poroid to cokey; vegetative mycelium lightly olivaceous; soluble pigment present, amber brown.

Conclusions regarding identity: Waksman does not describe a species which approximates the above reactions. The organism is, therefore, placed in a new category, viz. A. K676.

9.

ISOLATE NO. 678

SOURCE OF ISOLATE: Same as for Isolate No. 638 (soil) save from Series I, #5 with a pH of 3.7.

1. Czapek's Agar: Aerial mycelium present, white; vegetative growth white; soluble pigment absent.

2. Glucose Agar: At first (7 days) subsurface colonies covered with white aerial mycelium, and vegetatively are colored buff orange; after 12 days aerial mycelium hoary white; vegetative growth from a pale violaceous brown to a milk chocolate brown; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, concentric, white; vegetative mycelium dull dark brown; soluble pigment absent.

4. Citrate-Glycerin Agar: Aerial mycelium present, white; vegetative growth dull medium brown, soluble pigment absent.

5. Gelatin: Vegetative growth turned from whitish to tawny yellow; no soluble pigment; liquefaction of 1" in 30 days.

6. Milk, 25° C.: Vegetative growth white; after 20 days peptonizing to amount of 1 inch of heavy white coagulum; whey colorless.

7. Milk, 37° C.: Heavy coagulum after 15 days, with peptonization proceeding to include 1 inch of milk, colorless.

8. Potato Plug: Aerial mycelium present, at first (7 days) white; later white to light violaceous gray; vegetative mycelium potato-flesh colored; plug turned slightly flesh pink-orange.

9. Starch Agar: Aerial mycelium present, white to light gray; vegetative mycelium buff to pale orange buff, later pallid white to light-brown; colony pulvinate; soluble pigment absent.

10. Nutrient-Peptone Agar: Aerial mycelium absent; vegetative growth dull pale yellow, soluble pigment absent.

11. Tyrosin Agar: Aerial mycelium present, ranging from white through pale orange-brown to light gray; vegetative mycelium deep brown; soluble pigment present, dark orange-red brown.

Conclusions regarding identity: The above reactions have some of the characteristics of both A. fradii and A. alboflavus, but the divergencies are sufficiently great to regard the organism as a different species - A. K678.

10.

ISOLATE NO. 680

SOURCE OF ISOLATE: Same as that for Isolate No. 1264, save from a different tuber.

1. Czapek's Agar: Aerial mycelium present, pale green; vegetative mycelium buff yellow-green; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, pale green, vegetative mycelium buff yellow-green; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, whitish; vegetative growth dull gray green, soluble pigment present, lightly olivaceous.

4. Citrate-Glycerin Agar: Aerial mycelium present, pale green; vegetative mycelium buff yellow-green; soluble pigment absent.

5. Gelatin: Aerial mycelium present, gray; vegeative growth pallid, complete liquefaction in 14 days.

6. Milk, 25° C.: Aerial mycelium present, white; vegetative growth whitish forming a pellicle; in 14 days complete hydrolysis (no curd formation); all whey turned bright orange in 30 days.

7. Potato Plug: Aerial mycelium present, light gray green; plug turned slightly gray green.

8. Starch Agar: Aerial mycelium present, whitish; vegetative growth pallid; soluble pigment absent.

9. Nutrient Peptone Agar: Aerial mycelium present, white; vegetative growth ranging from pallid to dull light green; soluble pigment absent.

10. Tyrosin Agar: Aerial mycelium present, greenish; vegetative growth light brown; soluble pigment absent.

Conclusions regarding identity: This isolate shows very close agreement with Isolate No. 640, and like the latter, is considered to be A. flavovirens Waksman.

11.

ISOLATE NO. 686

SOURCE OF ISOLATE: Same as for Isolate No. 638 (soil) save from Series II, No. 2, which had a pH of 5.3.

1. Czapek's Agar: Aerial mycelium present, gray; growth concentric; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, gray; vegetative growth greenish-yellow; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, gray; vegetative growth greenish-yellow; soluble pigment absent.

4. Citrate-Glycerin Agar: Aerial mycelium present, gray; vegetative growth greenish-yellow; soluble pigment absent.

5. Gelatin: Aerial mycelium present, white; vegetative growth yellow; complete liquefaction in 15 days; soluble pigment present, faint oily yellow.

6. Milk, 25° C.: Digestion of curd beginning in 10 days, proceeding to peptonization of all curd except a white cap at bottom of tube; whey turned yellow.

7. Milk, 37° C.: Curd formation in 7 days; peptonization beginning in 10 days, to 1½ inches (3/4 of tube) in 30 days; soluble pigment pastel yellow.

8. Potato Plug: Heavy growth; water below plug yellow.

9. Starch Agar: Colonies white, circular; soluble pigment absent.

10. Nutrient Peptone Agar: Colonies white; circular; soluble pigment absent.

Conclusions regarding identity: Agreement of the above with what Waksman describes as being A. olivaceus is good, and this binomial is being applied to the above organism.

12.

ISOLATE NO. 748

SOURCE OF ISOLATE: Same as for Isolate No. 638 (soil) but from Series III, #5, having a pH value of 5.0.

1. Czapek's Agar: Vegetative growth disc-and-halo effect, pinkish; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, gray-white; vegetative mycelium brilliant orange red, turning brown orange with age; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium absent; vegetative growth orange and yellow, later becoming brown orange; soluble pigment absent.

4. Citrate-Glycerin Agar: Aerial mycelium present, gray; vegetative growth brilliant blood red.

5. Gelatin: Aerial mycelium present, gray; vegetative growth red; no liquefaction or soluble pigment.

6. Milk, 25° C.: Vegetative growth orange red; heavy curd with slight amount of colorless whey after 25 days.

7. Milk, 37° C.: Vegetative growth red; heavy white coagulum with slight amount of colorless whey after 20 days.

8. Potato Plug: Aerial mycelium light pink outlined with brick red; plug and water below not changed in color.

9. Starch Agar: Aerial mycelium present, gray; vegetative growth at first (7 days) blood red, later (15 days) deep pink and red; soluble pigment absent.

10. Nutrient Peptone Agar: Vegetative growth orange; soluble pigment absent.

Conclusions regarding identity: There is a degree of agreement between some of the reactions above and those given by Waksman for A. albosporeus, but the exceptions (e.g. the non-liquefaction of gelatin, the coagulation of milk, and the brilliant colors of the vegetative mycelium) are so marked that a separate category must be established, which is called A. K748.

13.

ISOLATE NO. 1057

SOURCE OF ISOLATE: Same as for Isolate No. 638 (soil) but from Series I, No. 7, having a pH value of 5.0.

1. Czapek's Agar: Vegetative mycelium pallid to faint orange; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present scant, white; vegetative mycelium ranging in color from pale pink to reddish bright orange; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, small tufts of moldy white; vegetative growth from buff to dark reddish brown; older colonies appear as quartered discs; soluble pigment absent.

4. Citrate-Glycerin Agar: Aerial mycelium present, heavy; light gray; vegetative growth from pink to blood red; soluble pigment absent.

5. Gelatin: Aerial mycelium present, white to slight gray; vegetative growth dark orange red; soluble pigment present, dark brown.

6. Milk, 25° C.: After 25 days milk turned light lavender gray; vegetative mycelium (ring on glass) blood-red inner ring, middle ring dull salmon red, topmost ring dark brown.

7. Milk, 37° C.: After 20 days milk light coffee-and-cream colored.

8. Potato Plug: Vegetative growth moist, dull pink red; plug turned blackish; water below plug dark brown.

9. Starch Agar: At first (7 days) light orange; vegetative growth orange pink; later aerial mycelium and vegetative growth "strawberry-frosting" pink; soluble pigment absent.

10. Nutrient Peptone Agar: Vegetative growth pale pink; soluble pigment present, dark brown.

11. Tyrosin Agar: Aerial mycelium present, white; vegetative growth pink orange; soluble pigment present, faint brown.

Conclusions regarding identity: The above reactions, vivid as they are, have no approximation in the descriptions of Waksman. In certain respects they are similar to those of A. reticulus-ruber and A. albosporeus. A separate category is established for this organism - A. K1057.

14.

ISOLATE NO. 1059

SOURCE OF ISOLATE: Same as for Isolate No. 1264 (tuber).

1. Czapek's Agar: Aerial mycelium present, dark gray; vegetative growth light to dark olive drab; soluble pigment absent.

2. Glucose Agar: At first (7 days) aerial mycelium gray; vegetative growth buff gray. Later (30 days) aerial mycelium dark gray; vegetative growth greenish brown. Questionable soluble pigment formation.

3. Malate-Glycerin Agar: At first (7 days) aerial mycelium gray; vegetative growth grayish green. Later (30 days) aerial mycelium gray; vegetative mycelium light and dark greenish brown; soluble pigment present, yellowish green.

4. Citrate-Glycerin Agar: At first (7 days) aerial mycelium gray; vegetative growth greenish gray; no soluble pigment. Later (30 days) aerial mycelium gray; vegetative mycelium light and dark greenish brown; soluble pigment present, yellowish green.

5. Gelatin: Vegetative growth grayish green; after 25 days 1/4th inch liquefaction; soluble pigment present, light yellow.

6. Milk, 25° C.: Vegetative growth whitish; heavy coagulum after 15 days with few drops of light orange buff whey; complete peptonization in 30 days and whey entirely orange.

7. Milk, 37° C.: Vegetative growth brown; heavy coagulum after 10 days; complete peptonization in 30 days with orange soluble pigment.

8. Potato Plug: Aerial mycelium present, gray; vegetative growth pulvinate, moist, buff; plug and water beneath uncolored. Later (30 days) aerial mycelium dark gray speckled with lighter gray; potato plug colored dark green; water below plug greenish brown.

9. Starch Agar: Aerial mycelium present, scant, covering older colonies, white to gray; vegetative growth buff, glassy.

10. Nutrient Peptone Agar: Aerial mycelium present, scant, white, appearing around periphery of some colonies; vegetative

growth buff; with age appearing glassy; soluble pigment present, faint yellow.

Conclusions regarding identity: The above description finds no counterpart in Waksman's species, but is congruent with the writer's previously described organism, A. K676, and it is therefore considered as another isolate of that species.

15.

ISOLATE NO. 1177

SOURCE OF ISOLATE: Same as for Isolate No. 638 (soil) save from Series I, #6, having a pH value of 3.4.

1. Czapek's Agar: Aerial mycelium present, gray; vegetative growth light gray, some colonies pinkish; soluble pigment absent.

2. Glucose Agar: At first (7 days) aerial mycelium gray; sometimes tinged with pink; vegetative growth ranging from white to pink to violaceous red. Later (10 days) some colonies diffusing a soluble violaceous pigment into the medium. Still later (15 days) aerial mycelium light brown; vegetative growth ranging from pale brown to ultramarine.

3. Malate-Glycerin Agar: At first (7 days) aerial mycelium white; vegetative growth ranging from gray through vinaceous to deep indigo; soluble pigment indigo. Later (15 days) vegetative growth deep purple; soluble pigment purple.

4. Citrate-Glycerin Agar: At first (7 days) aerial mycelium whitish; vegetative growth deep vinaceous; soluble pigment purplish. Later (15 days) aerial mycelium hoary, light gray; vegetative growth in part dark blue, emitting blue soluble pigment, and in part purple, emitting violet soluble pigment.

5. Gelatin: At first (15 days) aerial mycelium pink; vegetative growth dull light violaceous red. Later (25 days) a trace of brown soluble pigment.

6. Milk, 25° C.: At first (5 days) ring of vegetative growth top of milk, interspersed with pink, lavender, and red. Later (15 days) vegetative growth all deep blue; soluble pigment deep brown for 1½ inch down into

milk, aerial mycelium present, white; still later (25 days) complete hydrolysis (no prior coagulation), with top 1½ inch deep violet, and bottom ½ inch dark brown.

7. Milk, 37° C.: At first (14 days) vegetative growth ranging from blackish red to pink; soluble pigment pinkish buff. Later (25 days) soluble pigment inky black; consistency of water (whey?).

8. Potato Plug: At first (7 days) aerial mycelium found as a small patch, grayish; vegetative growth moist, potato-colored; no color change affecting potato and water below plug. Later (15 days) vegetative growth, moist, dull orange. Still later (30 days) all of vegetative growth covered with pinkish white aerial mycelium.

9. Starch Agar: Aerial mycelium present, grayish white; vegetative growth pink orange; soluble pigment absent.

10. Nutrient Peptone Agar: Aerial mycelium present, white to light gray; vegetative growth from pallid to orange; soluble pigment absent.

11. Tyrosin Agar: Aerial mycelium present, gray with patches of white; vegetative growth mostly wine red with areas of gray, pink, blue, etc.; soluble pigment absent.

Conclusions regarding identity: In comparing the several tests made of this isolate, there is revealed some variation in the color of vegetative growth and soluble pigment. When the nature of successive color change, as it is found here, is realized then a more orderly pattern becomes evident. According to Waksman (70) this successive color change can be readily explained by the change in the reaction of the medium. The organism produces a soluble red pigment at a pH of 7.0, then as the medium becomes more alkaline through the metabolic activity of the organism, the red changes to a blue color, acting as an indicator. Since the reaction of media (particularly the organic substrata) at the time of inoculation may vary, some of the reactions may not duplicate those of another test. Also the rapidity of the change of pigment differs with the strain of the organism, depending probably on the rapidity of alkali production. Furthermore, this pigment is only one of two or even three pigments present in a mixture, and it may therefore often be obscured.

The differences in the reactions found in the above

organism when compared with those of the organism described by Waksman as A. violaceus-ruber are found for the most part to be attributable to the reaction of the medium.

In view of this, the organism is being regarded as a variation of A. violaceus-ruber.

16.

ISOLATE NO. 1239

SOURCE OF ISOLATE: Same as Isolate No. 638 (soil) save from Series I, #7, which had a pH value of 5.0.

1. Czapek's Agar: Aerial mycelium absent; vegetative growth thin, dirty brown; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, dirty white gray; vegetative growth dark brownish yellow; soluble pigment present, surrounding colonies with golden brown halo.

3. Malate-Glycerin Agar: Aerial mycelium present, gray; vegetative growth brown-black; soluble pigment surrounding colonies dark yellow-brown.

4. Citrate-Glycerin Agar: Aerial mycelium present, white ringed with buff; vegetative growth dark brownish-yellow; soluble pigment present, golden brown, surrounding colonies.

5. Gelatin: Soluble pigment dark brown diffusing through solid gelatin; slight liquefaction.

6. Milk, 25° C.: Vegetative growth buff; soluble pigment green-gray after 7 days turning to gray-black in 20 days; no coagulation observed.

7. Potato Plug: Aerial mycelium present, grayish; vegetative growth punctiform; potato plug blackened.

8. Starch Agar: Aerial mycelium present, velvety gray centered surrounded by buff; soluble pigment absent.

9. Nutrient Peptone Agar: Aerial mycelium present, velvety gray

gray centered surrounded by buff; vegetative growth buff; presence of brown soluble pigment questionable.

10. Tyrosin Agar: Aerial mycelium present, white; vegetative growth dark brown; soluble pigment present, dark brown.

Conclusions regarding identity: The approximation of the above reactions to those of A. pheochromogenus Conn is sufficiently close to impel the writer to an adoption of that name for the above organism. Conn originally named this organism, having isolated it from New York soil, and it was subsequently found by Waksman and Curtis in New Jersey orchard soil.

17.

ISOLATE NO. 1242

SOURCE OF ISOLATE: Same as for Isolate No. 638 (soil) save from Series II, #9, which had a pH value of 5.5.

1. Czapek's Agar: Aerial mycelium present, concentrically white and buff; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, velvety white; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, velvety white; soluble pigment absent.

4. Citrate-Glycerin Agar: Aerial mycelium present, velvety white; soluble pigment absent.

5. Gelatin: Liquefaction, with soluble pigment dark brown.

6. Milk, 25° C.: Vegetative growth from white to brown with age; milk turned gray on 20th day, on 30th day dark gray with cap of light blue sediment.

7. Potato Plug: Aerial mycelium absent; vegetative growth black, wet; potato plug turned black in 5 days; water below plug dark brown.

8. Starch Agar: Aerial mycelium present, dull gray; concentric; soluble pigment absent.

9. Nutrient Peptone Agar: Aerial mycelium present, dull gray, concentric; soluble pigment absent.

10. Tyrosin Agar: Aerial mycelium present, white, pitted and fleecy, vegetative mycelium dark olive-brown soluble pigment present, brown.

11. Creatinin Solution: Growth but soluble pigment absent.

Conclusions regarding identity: White there are minor variations from the reactions described by Waksman, on the whole the similarity with those of A. scabies is sufficient to regard the organism as a strain of that species.

18.

ISOLATE NO. 1244

SOURCE OF ISOLATE: Same as for Isolate No. 638 (soil) save from Series I, #7, with a pH of 5.0.

1. Czapek's Agar: Aerial mycelium present, scant; dirty white; vegetative growth orange yellow; soluble pigment present, faint orange yellow.

2. Glucose Agar: Aerial mycelium present, whitish yellow; vegetative mycelium yellow; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, whitish yellow; vegetative mycelium yellow; soluble pigment absent.

4. Citrate-Glycerin Agar: Aerial mycelium present, whitish yellow; vegetative mycelium yellow; soluble pigment absent.

5. Gelatin: Soluble pigment present, dark brown.

6. Milk, 25° C.: Soluble pigment after 15 days light brown buff; after 30 days dark brown.

7. Milk, 37° C.: Milk colored dark cream after 15 days; after 25 days all milk colored khaki buff.

8. Potato Plug: Aerial mycelium absent; vegetative growth wet,

dark brownish gray; plug turned black; water below plug dark brown.

9. Starch Agar: Aerial mycelium present, white and gray; vegetative growth buff; soluble pigment absent.

10. Nutrient Peptone Agar: Aerial mycelium absent; vegetative growth moist, dirty buff; soluble pigment present, brown.

11. Tyrosin Agar: Aerial mycelium present, scant, gray; vegetative growth yellow; soluble pigment present, oily yellow.

12. Creatinin Solution: Soluble pigment absent.

Conclusions regarding identiy: The above reactions are somewhat like those obtained by Waksman for the species he called A. flavus; however, since agreement is not good in all points, it is thought best to regard this as a species of another category, as A. K1244.

19.

ISOLATE NO. 1246

SOURCE OF ISOLATE: Same as for Isolate No. 638 (soil), but from Series III, No. 4, having a pH value of 5.4.

1. Czapek's Agar: Vegetative mycelium dull, dark brown; soluble pigment present, light brown.

2. Glucose Agar: Aerial mycelium present, velvety white bordered by buff; vegetative mycelium deep yellow brown; soluble pigment present, light brown.

3. Malate-Glycerin Agar: Aerial mycelium present, velvety white bordered by buff; vegetative growth deep yellow brown; soluble pigment present, dull brown.

4. Citrate-Glycerin Agar: Aerial mycelium present, velvety white bordered by buff; vegetative mycelium deep yellow brown; soluble pigment present, light brown.

5. Gelatin: After 15 days 1/16th inch liquefied and that much containing dark soluble pigment; after

30 days liquefaction increased to 1 inch and soluble pigment to ½ inch.

6. Milk, 25° C.: In 12 days 1 inch of milk light brown; in 24 days brown soluble pigment becomes khaki; no coagulation or peptonization observed.

7. Milk, 37° C.: After 6 days top 1/4th inch consists of dark brown whey, with rest of 1-3/4th inch consisting of white curd. In 15 days all of tube whey and brown soluble pigment including 1½ inch of tube. In 30 days milk all whey and all dark brown.

8. Potato Plug: Vegetative growth dark buff, slimy; potato plug turned black along line of growth; water below plug dark brown.

9. Starch Agar: Aerial mycelium present, velvety gray; vegetative mycelium yellow buff; soluble pigment absent.

10. Nutrient Peptone Agar: Vegetative growth dull white; soluble pigment present, brown.

11. Tyrosin Agar: Aerial mycelium scant; soluble pigment present, deep brown.

Conclusions regarding identity: Were it not for the positive presumptive test of pigment formation on tyrosin agar, the above organism would fit quite satisfactorily the description of A. purpeochromogenus Waksman and Curtis. Conversely, because of the definite soluble pigment formation on the synthetic agars, this organism cannot be considered as of the species A. scabies. Consequently, the writer is placing it in a separate category designated A. K1246.

20.

ISOLATE NO. 1248

SOURCE OF ISOLATE: Same as Isolate No. 1242 (soil), but from Series II, #11, having a pH of 5.3.

1. Czapek's Agar: Aerial mycelium present, velvety white; vegetative growth buff; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, velvety gray; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, velvety gray; soluble pigment absent.

4. Citrate-Glycerin Agar: Aerial mycelium present, velvety gray; soluble pigment absent.

5. Gelatin: Complete liquefaction in 15 days; soluble pigment dark brown for top 1/4th inch from 15th to 30th day.

6. Milk, 25° C.: At 15 days whey (1 inch) dark brown; loose curd light brown; at 30 days entire tube black brown.

7. Milk, 37° C.: Milk slightly coagulated in 6 days and somewhat browned; in 30 days all whey, medium brown.

8. Potato Plug: Vegetative growth grayish black, becoming greenish and slimy with age; plug turned purple black after 3 days; water below plug golden brown.

9. Starch Agar: Aerial mycelium present, velvety white; vegetative growth buff; soluble pigment absent.

10. Nutrient Peptone Agar: Vegetative growth dull buff; soluble pigment production questionable.

11. Tyrosin Agar: Soluble pigment production questionable.

Conclusions regarding identity: The growth of the above organism on potato, milk, gelatin and the synthetic agars is characteristic of A. scabies, but not so are the growths on nutrient peptone and on tyrosin agar. Waksman (68) points out that only some cultures of A. scabies are able to produce a soluble dark pigment on tyrosin; furthermore as stated by Henrici (27) the ability to produce soluble pigments is often lost with continued cultivation. In the light of these observations, less weight is therefore being placed on the two above mentioned incongruities, and the organism is being considered as a strain of A. scabies.

21.

ISOLATE NO. 1254

SOURCE OF ISOLATE: Same as for Isolate No. 1262 (tuber).

1. Czapek's Agar: Aerial mycelium present, pulvinate, white; vegetative growth light buff. Later (30 days) aerial mycelium white to faint gray; vegetative growth lemon yellow. Soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, feathery, gray; vegetative growth yellowish; later olivaceous; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, whitish and yellowish; vegetative growth yellowish; soluble pigment absent.

4. Citrate-Glycerin Agar: At first (15 days) aerial mycelium gray, feathery; vegetative growth tinged yellow. Later (30 days) vegetative growth olive gray to olive. Soluble pigment absent.

5. Gelatin: At first (7 days) aerial mycelium white; vegetative growth yellowish. Later (30 days) aerial mycelium gray; vegetative growth greenish gray; 1/4th inch of gelatin liquefied; soluble pigment present, brown.

6. Milk, 25° C.: At first (7 days) ring of dark brown vegetative growth top of milk; soluble pigment greenish gray. Later (20 days) ring transformed to a deep reddish pellicle; soluble pigment dark reddish brown.

7. Milk, 37° C.: At first (5 days) vegetative growth top of milk brown; milk somewhat thickened and buff. Later (20 days) ring of growth dark brown; soluble pigment gray brown.

8. Potato Plug: Vegetative growth moist, dull brownish black; plug blackened; water below plug turned black; later aerial mycelium present, gray.

9. Starch Agar: Aerial mycelium present, scant, white; vegetative growth gray buff, soluble pigment absent.

10. Nutrient Peptone Agar: Aerial mycelium present, scant, white; vegetative growth water white; soluble pigment brown. Later (30 days) vegetative growth yellow brown; soluble pigment dark brown.

11. Tyrosin Agar: Aerial mycelium present, white and gray; surface poroid; vegetative mycelium dull olive; soluble pigment slightly present, faint brown.

12. Creatinin Solution: Growth present on meniscus, but no soluble pigment.

Conclusions regarding identity: The reactions are very much like those of Isolate No. 1262, and consequently this organism is considered to be A. scabies. The same reasoning as under Isolate No. 1262 applies here.

22.

ISOLATE NO. 1256

SOURCE OF ISOLATE: Same as for Isolate No. 638 (soil) save from Series III, #9 which had a pH of 4.8.

1. Czapek's Agar: Soluble pigment absent.

2. Glucose Agar: Aerial mycelium white; vegetative growth buff; soluble pigment absent.

3. Malate-Glycerin Agar: Vegetative mycelium dull, dirty buff; questionable browning of the medium.

4. Citrate-Glycerin Agar: Aerial mycelium gray-brown; vegetative growth yellow buff; soluble pigment absent.

5. Gelatin: Peptonization slow, liquefying 1/2 inch ($\frac{1}{4}$ of tube) in 30 days; soluble pigment present, dark brown.

6. Milk, 25° C.: Vegetative growth yellowish; coagulation and diffusing brown soluble pigment as early as 4th day, peptonization producing to 1 inch ($\frac{1}{2}$ of tube) within 8 days; later whey increasing and turning black brown.

7. Milk, 37° C.: Slight liquefaction by 20th day; soluble pigment present, at first deep brown, later becoming black brown.

8. Potato Plug: Aerial mycelium covering upper drier portion of plug, white, vegetative growth slimy dirty

gray brown; water below plug lemon yellow.

9. Starch Agar: Aerial mycelium present, white; vegetative growth buff; soluble pigment absent.

10. Nutrient Peptone Agar: Vegetative growth buff; soluble pigment present, brown.

11. Tyrosin Agar: Aerial mycelium present, white and grayish, fleecy, surface pitted; vegetative growth dark olivaceous brown; soluble pigment formation faint brown.

12. Creatinin Solution: Soluble pigment absent.

Conclusions regarding identity: This organism is designated A. scabies in consequence of its satisfactory agreement with reactions for this species given by Waksman.

23.

ISOLATE NO. 1258

SOURCE OF ISOLATE: Scab lesion on a potato tuber labelled "L-1a" lifted October 1940 from Lincoln Farm, Greenville, Michigan by Dr. J. H. Muncie. The pH value of the soil was 4.9.

1. Czapek's Agar: Aerial mycelium present, cottony white; vegetative mycelium yellowish below; soluble pigment absent.

2. Glucose Agar: At first (7 days) aerial mycelium present, matted, white; vegetative growth buff. Later (15 days) aerial mycelium stringy, dirty gray; vegetative growth yellow buff; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, white; vegetative growth at first (7 days) buff with centers turning yellow, later (15 days) sand brown; soluble pigment absent.

4. Citrate-Glycerin Agar: Aerial mycelium present, scant, white; vegetative growth sand brown; soluble pigment absent.

5. Gelatin: Aerial mycelium present, white; vegetative growth yellowish brown; soluble pigment present, brown.

6. Milk, 25° C.: After 14 days milk turned dark gray green capped by a dark brown pellicle; in 30 days milk deep brown with a deep reddish pellicle; no change in consistency.

7. Milk, 37° C.: After 7 days milk turned light gray buff; in 15 days dark brown soluble pigment coloring milk with dark brown ring of growth at top; after 30 days color is violaceous gray.

8. Potato Plug: At first (7 days) vegetative growth punctiform, moist, gray; plug turned black; water below plug black. Later, colonies whitish aerially.

9. Starch Agar: Aerial mycelium present, scant at first, white; vegetative growth yellowish; soluble pigment absent.

10. Nutrient Peptone Agar: Aerial mycelium present, scant; vegetative growth brown; soluble pigment brown.

11. Tyrosin Agar: Aerial mycelium present, white to light gray; vegetative growth from greenish gray to darkly olivaceous; soluble pigment present, dark brown.

Conclusions regarding identity: The above description coincides closely with A. scabies, and is considered to be that species.

24.

ISOLATE NO. 1260

SOURCE OF ISOLATE: Same as for Isolate No. 638 (soil) save from Series III, #2, which had a pH of 5.0.

1. Czapek's Agar: Aerial mycelium white, appearing gradually from a buff vegetative mycelium, reverse side of growth pallid through buff brown to brown; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, white; vegetative growth ranging from white to chrome yellow; soluble pigment present, light chrome yellow.

3. Malate-Glycerin Agar: Aerial mycelium present ranging in color from white to gray; vegetative growth from pale brown to dark brown; colonies small, restricted.

4. Citrate-Glycerin Agar: Aerial mycelium present, light gray; vegetative mycelium ranging from white through dark chrome-yellow to dark orange brown; soluble pigment absent.

5. Gelatin: Aerial mycelium present, gray bordered by white; vegetative growth dark gray; soluble pigment present, dark brown.

6. Milk, 25° C.: Aerial mycelium present, white; vegetative growth yellowish brown; becoming black-brown with age; slight coagulation in 7 days; in 15 days milk contains khaki brown soluble pigment.

7. Milk, 37° C.: Slight coagulation occurring in 10 days, assuming a slight buff color, turning khaki brown to dark brown throughout.

8. Potato Plug: Aerial mycelium absent; vegetative growth moist and black; potato plug turned black; soluble pigment in water under plug faint brown; grayish colonies growing on surface of water.

9. Starch Agar: Aerial mycelium present, whitish; vegetative growth whitish to buff orange brown, soluble pigment absent.

10. Nutrient Peptone Agar: Aerial mycelium absent; vegetative growth at first dull gray, later dull yellow brown; soluble pigment present, dark yellow brown.

11. Tyrosin Agar: Aerial mycelium present, light brownish gray; vegetative growth dark brown; soluble pigment present, dark brown.

12. Creatinin Solution: Growth present, soluble pigment pale yellow.

Conclusions regarding identity: Waksman cites but one organism of the chromogenus group which is capable of producing a soluble yellow pigment in Creatinin Solution, viz., A. olivochromogenus. This same reaction was recorded for the above organism, and the other reactions fit reasonably well Waksman's description. This isolate is therefore being referred to the species A. olivochromogenus.

25.

ISOLATE NO. 1262

SOURCE OF ISOLATE: Same as for 1258, save from a deep scab on a tuber marked "L4" coming from soil with a pH value of 4.9.

1. Czapek's Agar: Aerial mycelium present, concentric, white to gray; vegetative growth yellowish, later (30 days) becoming lemon yellow; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, cottony white to gray; vegetative growth yellowish; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, grayish white; vegetative mycelium dirty brown; soluble pigment absent.

4. Citrate-Glycerin Agar: Aerial mycelium present, concentric, white to gray; vegetative growth yellowish, later (30 days) becoming lemon yellow; soluble pigment absent.

5. Gelatin: Aerial mycelium present, gray centered, bordered by white; vegetative mycelium yellowish; soluble pigment dark brown, extending into medium 1/4th inch.

6. Milk, 25° C.: Vegetative growth a brown ring top of milk; grayish soluble pigment; later (30 days) aerial mycelium present, white, vegetative mycelium black brown; coloration in banded zones, top 1 inch black-brown, middle 1/2 inch lighter brown; bottom 1/2 inch whitish.

7. Milk, 37°C.: After 15 days brown pellicle top of milk and soluble pigment light brown buff; later (30 days) top 1/4th inch of milk black brown, middle 1/2 inch zone buff orange, and bottom 1-1/4th inch white.

8. Potato Plug: Aerial mycelium absent at first; vegetative growth moist, grayish; water below plug brownish. Later (30 days) slight amount of white aerial mycelium, vegetative growth slimy, convoluted, dirty gray, black near top; water below dark brown.

9. Starch Agar: Aerial mycelium present, feathery, white to light gray; vegetative growth white to yellowish; soluble pigment absent.

10. Nutrient Peptone Agar: Vegetative growth moist and dirty gray above, brown on obverse; soluble pigment dark brown.

11. Tyrosin Agar: Aerial mycelium present, white, vegetative growth poroid, lightly olivaceous; soluble pigment present, faint brown.

12. Creatinin Solution: Vegetative growth, but no soluble pigment present.

Conclusions regarding identity: The characteristic reaction of A. scabies is the production of tyrosinase from tyrosin, though Waksman has found that only some strains of A. scabies are capable of producing this reaction. The above described organism showed only a faint brown soluble pigment, forming presumptive evidence that the organism might be A. olivochromogenus. However, upon culture on Creatinin Solution the characteristic yellow soluble pigment for A. olivochromogenus was not produced. Since all other reactions of the above organism are in good accord with A. scabies, it is being regarded as a strain of this species.

26.

ISOLATE NO. 1264

SOURCE OF ISOLATE: From a scab lesion on a potato tuber originating from the Potato Experimental Farm, Lake City, Michigan. Lifted fall of 1939. Isolation made August 3, 1940.

1. Czapek's Agar: Vegetative growth restricted, white; questionable soluble pigment formation.

2. Glucose Agar: Vegetative growth restricted, white; soluble pigment absent.

3. Malate-Glycerin Agar: Vegetative growth buff, questionable browning of medium.

4. Citrate-Glycerin Agar: Vegetative growth restricted, white; soluble pigment absent.

5. Gelatin: Aerial mycelium present, white; vegetative growth yellow; complete liquefaction in 7 days; soluble pigment present after 14 days, dark brown.

6. Milk, 25° C.: Slight coagulation after 5 days, in 7 days light brown soluble pigment; after 15 days soluble pigment dark orange brown.

7. Milk, 37° C.: Light brown soluble pigment in 3 days; turning to dark grayish brown in 15 days.

8. Potato Plug: Vegetative growth black; plug turned black; water below plug turned dark brown.

9. Starch Agar: Vegetative growth small, white; soluble pigment absent.

10. Nutrient Peptone Agar: Vegetative growth buff; soluble pigment present, yellow brown.

11. Tyrosin Agar: Aerial mycelium present, white, poroid, fleecy, vegetative growth dark olive-brown; soluble pigment present, brown.

Conclusions regarding identity: The above description bears close congruity with that for A. scabies, and is considered to be this species by the writer.

27.

ISOLATE NO. 1285

SOURCE OF ISOLATE: Subculture of isolation made by KenKnight (33) labelled "KenKnight's Act. No. 37, Warba Tuber, Lake City, Michigan, 11-2-39". Subcultured November 7, 1940.

1. Czapek's Agar: Aerial mycelium present, dark gray; vegetative growth darkly olivaceous; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, concentrically ringed, from light to dark gray; vegetative growth at first (7 days) yellow to dark green brown, later (15 days) more darkly olivaceous; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, light gray, papillate; vegetative growth yellowish to dark green brown, later becoming black brown and surrounded by a thin brown halo which appears to be attenuation of colony growth.

4. Citrate-Glycerin Agar: At first (7 days) aerial mycelium present, papillately gray with white aerola; vegetative growth light yellow to dark green brown. Later (30 days) aerial mycelium dark gray with concentric bands of vegetative brown; vegetative growth (seen from underneath) concentrically ringed and deep brown; soluble pigment questionably present, faint olive.

5. Gelatin: In 7 days slight, liquefaction with colorless vegetative growth. Later (30 days) liquefaction of 1 inch (half of tube); blob of pallid vegetative growth at bottom of tube; soluble pigment present, faint yellow.

6. Milk, 25° C.: In 14 days complete coagulation, faint pink in color; vegetative growth a ring top of milk, faintly yellow. In 30 days curd transformed into a jelly-like pinkish orange mass.

7. Milk, 37° C.: In 14 days complete coagulation, faint pink in color; vegetative growth a ring top of milk, faintly yellow. In 30 days curd transformed into a jelly-like pinkish orange mass.

8. Potato Plug: Aerial mycelium absent; vegetative growth yellowish, moist, maggot-like rugosity. Potato plug and water below uncolored.

9. Starch Agar: Aerial mycelium present, restricted to center, dark gray fringed by white, rest of top surface brownish vegetable mycelium; below vegetative growth dark brown; soluble pigment absent.

10. Nutrient Peptone Agar: Aerial mycelium absent; vegetative growth buff colored; soluble pigment absent. Colonies 4 mm. in diameter.

11. Tyrosin Agar: Aerial mycelium present, dark gray; vegetative growth grayish; soluble pigment present, amber brown.

Conclusions regarding identity: There is some degree of variation in the above description when compared with that of Waksman's A. olivaceus, particularly the absence

of aerial mycelium on potato plug and the distinctive growth on starch agar. However, for the most part there is sufficient similarity of reactions to regard the above organism as a strain of A. olivaceus. This species has heretofore been isolated from Oregon, California adobe, and Maine Aroostook soils.

28.

ISOLATE NO. 1434

SOURCE OF ISOLATE: Scab lesion on a potato tuber lifted from Series I, #1, Potato Experimental Farm, Lake City, Michigan, soil of which showed a pH of 4.8.

1. Czapek's Agar: Aerial mycelium present, ranging in color from white to gray; vegetative growth pastel yellow; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, concentric, white to light gray; vegetative growth pale yellow centered; large colonies; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, white; vegetative growth from pallid white to yellow brown; soluble pigment absent.

4. Citrate-Glycerin Agar: Aerial mycelium present, concentric, light gray; vegetative growth yellow centered; clearing of calcium particles around colonies; soluble pigment absent.

5. Gelatin: Aerial mycelium present, white, partly covering yellowish vegetative mycelium; soluble pigment present, dark brown.

6. Milk, 25° C.: At first (7 days) ring of brown vegetative mycelium top of milk; soluble pigment faintly lavender. Later (20 days) top 1 inch of milk darkly violaceous brown, grading off by zones through lavender into white at bottom of tube.

7. Milk, 37° C.: Vegetative growth on glass orange; soluble pigment pinkish light brown.

8. Potato Plug: Aerial mycelium present, scant, white covering dry vegetative growth; later moist, dirty gray;

potato plug turned black brown; water below plug black brown.

9. Starch Agar: Aerial mycelium present, white; vegetative growth pale yellow buff; halo around colonies cleared of starch; later (12 days) a few colonies turning from white to gray aerially.

10. Nutrient Peptone Agar: Aerial mycelium absent; top of vegetative growth dirty light gray, obverse pale dull buff yellow; soluble pigment present, dark brown.

11. Tyrosin Agar: Aerial mycelium present, white; vegetative growth darkly olivaceous; soluble pigment present, dark brown.

12. Creatinin Solution: Good colony development but no production of soluble pigment.

Conclusions regarding identity: The above organism is being considered as a strain of A. scabies. A characterizing reaction of this particular strain is the unique process by which soluble pigment is produced in skimmed milk at 25° C., giving the resultant pigmentation the appearance of a Liesegang phenomenon.

29.

ISOLATE NO. 1436

SOURCE OF ISOLATE: Same as for Isolate No. 1258 save from a surface-scabbed tuber originating from plot "L-5", the pH value of which was 4.9.

1. Czapek's Agar: Aerial mycelium present, concentric, white; vegetative growth yellowish; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, gray; vegetative mycelium pastel yellow; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, concentric, white; vegetative growth yellowish; soluble pigment absent.

4. Citrate-Glycerin Agar: Aerial mycelium present, concentric, white; vegetative growth yellowish; soluble pigment absent.

5. Gelatin: Aerial mycelium present, gray ringed with white;

vegetative growth dark gray surrounded by white; soluble pigment present, dark brown.

6. Milk, 25° C.: After 15 days milk slightly coagulated, vegetative growth as a ring around top of milk deep red; soluble pigment present, yellowish-greenish brown. After 24th day milk partly peptonized; soluble pigment dark cloudy gray-green.

7. Milk, 37° C.: After 15 days deep reddish pellicle capping milk; soluble pigment mouse gray, later dark brownish gray.

8. Potato Plug: Vegetative growth pulvinate, black, emarginated by whitish colonies, plug turned black; water under plug not colored.

9. Starch Agar: Vegetative growth gray bordered with white; soluble pigment absent.

10. Nutrient Peptone Agar: Vegetative growth at first (7 days) dull brown, later becoming yellowish brown and ringed by a circle of white aerial mycelium; soluble pigment present, brown.

11. Tyrosin Agar: Aerial mycelium present, white; vegetative growth darkly olivaceous; soluble pigment present, faint brown.

12. Creatinin Solution: Vegetative growth active but no production of soluble pigment.

Conclusions regarding identity: The above organism is regarded as a strain of A. scabies. The non-characteristic reaction mentioned under tyrosin agar is explained under Isolate No. 1262.

30.

ISOLATE NO. 1339

SOURCE OF ISOLATE: This is a subculture of an isolate made by Ken-Knight (33) and labelled: "KenKnight's Actinomyces No. 10 - air, apparently pathogenic, 10-20-39." Subcultured November 7, 1940.

1. Czapek's Agar: Aerial mycelium present, powdery to poroid, white; vegetative growth pale yellow centered, rest white except pale watery lavender around periphery; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, white; vegetative growth lemon yellow; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, white to pink; vegetative growth brownish yellow; soluble pigment present, lavender, bordering colonies.

4. Citrate-Glycerin Agar: Aerial mycelium present, white to buff; vegetative growth bay; soluble pigment absent.

5. Gelatin: Aerial mycelium present, white; vegetative growth yellow brown, soluble pigment present, tokay red; liquefaction to 1 inch of gelatin in 30 days.

6. Milk, 25° C.: In 12 days slight coagulation; ring of vegetative growth top of milk buff yellow; in 15 days curd almost all peptonized, colored cloudy lemon; in 20 days whey colored cloudy orange; aerial mycelium white; vegetative growth yellow.

7. Milk, 37° C.: In 12 days aerial mycelium present, white; vegetative growth dark brown centered surrounded by lighter yellowish-brown margins; some colonies emarginated by a narrow rim of purple soluble pigment; in 30 days entire milk turned orange colored whey.

8. Potato Plug: Aerial mycelium present, white, cracking on 12th day; underneath crack amber brown vegetative mycelium; plug and water beneath uncolored.

9. Starch Agar: Aerial mycelium present, white; vegetative growth lemon yellow; soluble pigment absent.

10. Nutrient Peptone Agar: Aerial mycelium present, white to pinkish; vegetative mycelium brownish yellow bordered in 10 days with margins of lavender soluble pigment; later (15 days) also general browning of the medium.

Conclusions regarding identity: The above description tallies closely with that given by Waksman for A. erythreus except for the brown pigment on nutrient agar and the tokay red pigment in gelatin. Waksman (70) in his

introductory paragraph to the species A. erythreus says "This species resembles in many respects Krainsky's A. erythrochromogenus, but it does not produce the brown pigment characteristic of the chromogenus species on nutrient agar and gelatin." Syllogistically then, the above species would be A. erythrochromogenus. However, Krainsky utilized media in his characterization which are of other compositions than the writer's; therefore working merely from his description (36) it is impossible to ascertain whether or not these two species are actually the same. For this reason, it has been decided to place the above described organism into a separate category (A. K1339) until such time as the two cultures can be run side by side.

31.

ISOLATE NO. 1341

SOURCE OF ISOLATE: Subculture of an isolate obtained by KenKnight (33) from radish grown on Michigan soil. Tube labelled "KenKnight's Act. 43, 11-5-39".

1. Czapek's Agar: Aerial mycelium present, poroid, faint greenish white; vegetative mycelium faint yellow green buff. Later (10 days) colonies merged to form an entire sheet over surface of plate; aerial mycelium yellowish white; vegetative growth pastel yellow. Soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, scant, late, white, vegetative growth pallid to buff brown; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, concentrically ringed with gray and white; vegetative growth dark brown green; soluble pigment present, dark brown.

4. Citrate-Glycerin Agar: Aerial mycelium present, pale light green; vegetative mycelium light yellow brown; soluble pigment absent.

5. Gelatin: Aerial mycelium present, white; vegetative growth dark brown bordered by pallid white. Liequfaction in 10 days, and soluble pigment golden brown in 30 days.

6. Milk, 25° C.: In 7 days hydrolysis (without prior coagulation) of 1/8th inch top of milk; vegetative growth white; in 20 days all whey turned orange.

7. Milk, 37° C.: Solid curd in 10 days; in 13 days half of curd (1 inch) peptonized, clear; in 20 days whey turned watery cloudy orange.

8. Potato Plug: In 3 days at lower end colonies punctiform moist, dirty gray; colonies at upper end covered with powdery white aerial mycelium; in 30 days entire potato covered with pebbly growth overlaid with dingy white aerial mycelium; potato plug colored deep brown.

9. Starch Agar: Aerial mycelium present, sometimes absent, concentrically ringed, white; vegetative growth pallid; soluble pigment none.

10. Nutrient Peptone Agar: Aerial mycelium present, white, disc-and-halo type of growth; vegetative mycelium pallid to yellowish; soluble pigment present, brown.

11. Tyrosin Agar: Aerial mycelium present, white; vegetative mycelium slightly yellow; soluble pigment questionable.

Conclusions regarding identity: There is good congruity of this description and the one given by Waksman for A. griseolus; therefore the above organism is considered to be of that species. According to Waksman, this is a very common soil organism.

32.

ISOLATE NO. 1343

SOURCE OF ISOLATE: A subculture of an isolate made by KenKnight (33) and labelled: "KenKnight's Act. No. 13, from Solanum nigrum." Subculture Nov. 11, 1940.

1. Czapek's Agar: At first (15 days) aerial mycelium present, white; vegetative growth yellowish. Later (30 days) aerial mycelium pinkish and a variegated gray; vegetative growth mahogany red, soluble pigment present, brown.

2. Glucose Agar: At first (15 days) aerial mycelium white; vegetative growth yellowish. Later (30 days) aerial mycelium grayish white; vegetative growth yellowish in concentric circles; soluble pigment absent.

3. Malate-Glycerin Agar: At first (15 days) aerial mycelium white; vegetative growth pale to dark yellow; soluble pigment light yellow. Later (30 days) aerial mycelium fleecy, grayish-white; vegetative growth almost hemispherical, mahogany colored; soluble pigment cloudy pinkish light brown.

4. Citrate-Glycerin Agar: At first (15 days) aerial mycelium white; vegetative growth yellow brown to bay; soluble pigment dark brown. Later (30 days) aerial mycelium light gray; vegetative growth ranging in color from yellow brown through mahogany red to purple; soluble pigment brownish pink with some purple soluble pigment around colonies.

5. Gelatin: Aerial mycelium present, white; vegetative growth yellowish; soluble pigment brown; liquefaction 1/2 inch from 14 to 30 days.

6. Milk, 25° C.: At first (15 days) vegetative growth on glass bay brown at top, below pale yellow. Later vegetative growth light mustard brown; soluble pigment present, dark brown.

7. Milk, 37° C.: At first (15 days) vegetative growth amber yellow; soluble pigment light pinkish brown. Later (30 days) milk turned light "cream-and-cocoa" color.

8. Potato Plug: At first (7 days) white colony surrounded by blackening of the plug. Later (30 days) aerial mycelium velvety white; potato plug black; water below plug black brown.

9. Starch Agar: Aerial mycelium present, white to gray; vegetative growth pallid to yellowish to gray. Later (30 days) aerial mycelium powdery gray; vegetative growth dirty yellow brown; presence of brown soluble pigment questionable.

10. Nutrient Peptone Agar: At first (15 days) aerial mycelium white;

vegetative growth yellow brown to bay; soluble pigment dark brown. Later (30 days) aerial mycelium scanty, white, disc-and-halo type; vegetative growth pale yellow brown; soluble pigment dark yellow brown.

11. Tyrosin Agar: Aerial mycelium present, white, filiform growth; vegetative mycelium yellow; soluble pigment present, faint yellow.

Conclusions regarding identity: No description by Waksman approximates the above reactions, though it is a member of the chromogenus group due to its soluble pigment formation in proteinaceous media. An outstanding characteristic of this organism is its rapid alteration of form and color over a period of 30 days. This organism is being placed in a new category - A. K1343.

33.

ISOLATE NO. 1345

SOURCE OF ISOLATE: Subculture of an isolation made by KenKnight (33) labelled "KenKnight's Act. No. 5, beet scab, 11-7-38". Subcultured November 7, 1940.

1. Czapek's Agar: Aerial mycelium present, greenish; vegetative growth pale yellow; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, cottony greenish white; vegetative growth greenish surrounded by white; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, white at first (7 days), later (15 days) velvety light green; vegetative growth yellowish brown surrounded by white, later (15 days) orange-brown centered surrounded by buff; soluble pigment absent.

4. Citrate-Glycerin Agar: Aerial mycelium present, cottony greenish white; vegetative mycelium at first (7 days) greenish surrounded by white, later (15 days) orange brown centered, ringed by buff, soluble pigment absent.

5. Gelatin: Liquefaction 1/2 inch (1/4th of tube) in 5 days, 1 inch from 20 to 30 days. Aerial mycelium

present, powdery, white; vegetative growth pallid; soluble pigment dark reddish brown, becoming lighter with age.

6. Milk, 25° C.: Coagulation in 10 days, peptonized 1/2 inch on 18th day, and complete digestion in 25 days, with whey orange colored. Vegetative growth as a ring at top of milk, yellowish.

7. Milk, 37° C.: Coagulation in 10 days, peptonization 1/2 inch by 15th day; practically complete digestion by 30th day; whey orange colored.

8. Potato Plug: Growth covering plug faint green; water below plug turned brown.

9. Starch Agar: Growth whitish above and below; soluble pigment absent.

10. Nutrient Peptone Agar: Aerial mycelium present, white; vegetative growth white to greenish pallid; soluble pigment present, brown.

11. Tyrosin Agar: Aerial mycelium present, dingy white; vegetative growth faint yellow; soluble pigment absent.

Conclusions regarding identity: There is good agreement between the above described organism and Waksman's A. 218, the latter being described by him as "closely related to A. griseus, but producing a brown pigment on protein-containing media and not so strongly proteolytic". His isolation was made from sewage of a trickling filter at Plainfield, N.J. The above organism is considered to be the same A. W218.

34.

ISOLATE NO. 1347

SOURCE OF ISOLATE: Subculture of an isolation made by KenKnight (33) and labelled "KenKnight's Act. No. 7, turnip scab, apparently pathogenic on potato". Subculture made November 7, 1940.

1. Czapek's Agar: Aerial mycelium present, scant, white; vegetative growth dull pallid white; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, scant, white; vegetative growth pallid above, obverse brown buff centered, surrounded by ring of pallid white; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, at first white, later gray; vegetative growth at first yellowish brown, later dark brown; soluble pigment present, brown.

4. Citrate-Glycerin Agar: Aerial mycelium present, white; vegetative growth at first greenish yellow, later (15 days) becoming different shades of brown; soluble pigment present, at first faint yellow, later becoming brown.

5. Gelatin: Aerial mycelium present, white; vegetative growth yellowish; soluble pigment present, dark tokay red; liquefaction 1 inch gelatin from 15 to 30 days.

6. Milk, 25° C.: Aerial mycelium present, scant, white, appearing in 15 days; vegetative growth a ring of faint yellow; coagulation in 15 days with beginning of peptonization; in 30 days peptonization complete, whey cloudy orange colored.

7. Milk, 37° C.: Coagulation in 10 days; peptonization taking in 1/2 inch of milk in 15 days and 2 inches (entire tube) in 30 days; whey dark cloudy orange colored.

8. Potato Plug: Colonies slightly greenish at top of potato, descending white; in 20 days plug abundantly covered with white tumulous aerial mycelium; plug turned dark reddish brown; water below plug orange.

9. Starch Agar: Aerial mycelium present, concentric, white; vegetative growth pallid to yellowish, later (15 days) brown centered, surrounded by ring of yellow and that bordered by ring of pallid white.

10. Nutrient Peptone Agar: Aerial mycelium present, white; disc-and-halo type of growth; vegetative growth pallid to light yellow; soluble pigment present, at first faint yellow, later (15 days) faint brown.

11. Tyrosin Agar: Aerial mycelium present, white; vegetative growth faint yellow; soluble pigment absent.

Conclusions regarding identity: The above reactions concide closely with those given by Waksman for A. griseolus, also with other species isolated by the writer that have been determined to be A. griseolus. The soluble pigment on gelatin, however, varies from the described "faint yellowish" of Waksman, and this organism is therefore considered as perhaps a strain of A. griseolus. This species is a very common soil inhabitant.

35.

ISOLATE NO. 1386

SOURCE OF ISOLATE: Subculture of isolate made by KenKnight (33) labelled "KenKnight's Act. No. 38, isolated from Warba tuber, Lake City, Michigan, apparently pathogenic." Subcultured November 7, 1940.

1. Czapek's Agar: Vegetative growth thin, pallid; soluble pigment absent.

2. Glucose Agar: Aerial mycelium absent; vegetative growth on top watery yellow centered, obverse colored pallid yellow; soluble pigment absent.

3. Malate-Glycerin Agar: At first (7 days) aerial mycelium white; vegetative mycelium deep blue centered margined with white. Later (15 days) vegetative growth purple centered, some colonies fringed with a lighter purple, some with a purplish red. Soluble pigment absent.

4. Citrate-Glycerin Agar: Aerial mycelium present, white and pink; vegetative growth pink orange with pallid borders; soluble pigment absent.

5. Gelatin: At first (7 days) aerial mycelium white and pinkish; vegetative growth yellow and red margined; no soluble pigment or liquefaction. Later (15 days) vegetative growth pinkish; liquefaction; no soluble pigment.

6. Milk, 25° C.: At first (10 days) vegetative growth ochroleucous with a narrow band of faint pink. Later (30 days) milk is pinkish orange and whey-like with a loose jelly-like consistency.

7. Milk, 37° C.: After 15 days milk slightly pink and incipiently curdled. Later (after 20 days) milk solidly coagulated with drop of orange-colored whey.

8. Potato Plug: Aerial mycelium present, rugose, velvety, white; plug and water beneath unchanged. Later reflexed edge of vegetative growth brown yellow.

9. Starch Agar: Aerial mycelium present, scant, white; vegetative growth flat and thin, dull pallid; one colony sectored with pink.

10. Nutrient Peptone Agar: Aerial mycelium absent; vegetative growth paraffin-colored on top, on obverse from yellow to light green to greenish blue centered; soluble pigment absent.

11. Tyrosin Agar: Aerial mycelium present, scant, white; vegetative growth yellowish; soluble pigment absent.

Conclusions regarding identity: Many of the above reactions conform to those of Waksman and Curtis' A. alboflavus. Some, however, do not, and these reactions are so striking and characteristic (e.g., the purple vegetative growth on Malate-Glycerin Agar) that there can be no question but what the above organism is a different species. It is being designated A. K1386.

36.

ISOLATE NO. 1390

SOURCE OF ISOLATE: Subculture of isolation made by KenKnight (33) labelled "KenKnight's Act. No. 4, soil, East Lansing, 10-20-39." Subcultured November 10,1940.

1. Czapek's Agar: Aerial mycelium present, white; vegetative growth white; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, white; vegetative growth yellow buff; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium absent; vegetative growth yellowish; soluble pigment present,yellow.

4. Citrate-Glycerin Agar: Aerial mycelium white, scant, or lacking; vegetative mycelium light mustard brown; soluble pigment absent.

5. Gelatin: Aerial mycelium present, white; vegetative growth yellowish; slight liquefaction in 7 days with 1 inch liquefied to 30 days; soluble pigment present, dark brown.

6. Milk, 25° C.: Aerial mycelium present, white; vegetative growth yellowish; milk sand buff colored; after 18 days all of milk dark brown whey.

7. Milk, 37° C.: In 7 days complete coagulation; in 18 days complete peptonization with whey colored dark brown.

8. Potato Plug: Aerial mycelium present, white; potato plug turned pinkish, later (30 days) plug turned deep brown; water below plug light brown.

9. Starch Agar: Aerial mycelium present, white; vegetative growth faint orange yellow; soluble pigment absent.

10. Nutrient Peptone Agar: Aerial mycelium present, disc-and-halo effect, white; vegetative growth yellow to brown pallid; soluble pigment brown.

11. Tyrosin Agar: Aerial mycelium present, white; vegetative mycelium yellowish; soluble pigment present, faint brown.

Conclusions regarding identity: The above description has no approximation in Waksman's species; it is therefore designated A. K1390.

37.

ISOLATE NO. 1396

SOURCE OF ISOLATE: Subculture of isolation made by KenKnight (33) and labelled "KenKnight's Act. No. 9, soil apparently pathogenic, 11-22-1938". Subcultured November 7,1940.

1. Czapek's Agar: Aerial mycelium present, white, becoming tinged with green; vegetative growth slightly yellow; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, white; vegetative growth grayish centered surrounded by ring of white; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium at first brownish centered, later pale yellow; soluble pigment present, faint yellow.

4. Citrate-Glycerin Agar: Aerial mycelium present, white; vegetative growth brown centered; questionable yellowing of medium.

5. Gelatin: In 7 days aerial mycelium present, white; vegetative growth brownish; liquefaction to 1 inch; soluble pigment present, light brown. Later (12 to 30 days) liquefaction to 1-1/2 inches; soluble pigment reddish brown.

6. Milk, 25° C.: Aerial mycelium present, scant, white; 7 days loose coagulation of milk with 1/2 inch peptonized to a water-white whey. In 30 days peptonization completed, colorless.

7. Milk, 37° C.: Milk coagulated, with colorless peptonization proceeding to 1 inch (half of tube) in 30 days.

8. Potato Plug: At first (4 days) vegetative growth, water, yellow; later (progressively after 7th day) becoming overrun with abundant chalky white to slightly pink aerial mycelium; plug changed to deep red; water under plug brownish.

9. Starch Agar: Vegetative growth pallid; soluble pigment absent.

10. Nutrient Peptone Agar: Aerial mycelium present, white; vegetative growth pallid centered surrounded by white; soluble pigment present, faint brown.

11. Tyrosin Agar: Aerial mycelium present, cottony, white; vegetative growth yellow; soluble pigment absent.

Conclusions regarding identity: No description presented by Waksman accommodates the above organism. It is being designated A. K1396.

38.

ISOLATE NO. 1392

SOURCE OF ISOLATE: Subculture of isolation made by KenKngith (33) labelled "KenKnight's Act. No. 16, Solanum nigrum, 10-20-39." Subcultured November 7, 1940.

1. Czapek's Agar: Aerial mycelium present, at first (7 days) white; bordered by gray, later (12 days) gray. Vegetative growth yellow green; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, gray; vegetative growth at first light greenish yellow, later green gray; questionable greenish soluble pigment.

3. Malate-Glycerin Agar: Aerial mycelium present, at first white, later gray; vegetative growth yellow brown; soluble pigment present, faint yellow.

4. Citrate-Glycerin Agar: Aerial mycelium present, brownish gray; vegetative mycelium yellow brown; soluble pigment present, faint brown.

5. Gelatin: Aerial mycelium present, at first white, later gray; vegetative grwoth at first yellow, later yellow brown; soluble pigment dark brown; liquefaction of 1/2 inch in 20 days to 30 days.

6. Milk, 25° C.: Aerial mycelium present, developing after 12 days, white, later becoming greenish yellow; vegetative growth yellowish green brown. Hydrolysis (without prior coagulation) beginning after 12th day, and coming to completion (2 inches) on the 18th day. Soluble pigment present, orange brown.

7. Milk, 37° C.: Aerial mycelium present, white; vegetative growth white; coagulation and peptonization proceeding to 1-1/2 inches of tube in 7 to 30 days; whey water-white.

8. Potato Plug: At first (7 days) aerial mycelium near top of slant, grayish, lower greenish white; vegetative growth watery yellow. Later (25 days) aerial mycelium rugose, dark gray; bordered by white; exuding droplets of amber-colored fluid; plug darkened; water under plug yellowish.

9. Starch Agar: Aerial mycelium present, scant, white; vegetative growth pallid; soluble pigment absent.

10. Nutrient Peptone Agar: Aerial mycelium present, scant, at first (7 days) white, later (15 days) turning grayish; vegetative growth pallid orange yellow; soluble pigment present, pale orange brown.

11. Tyrosin Agar: Aerial mycelium present, gray; vegetative growth darkly olivaceous; soluble pigment present, pale brown.

Conclsuions regarding identity: With the notable exception of the soluble pigment formation on gelatin (which in

the above case is dark brown) this organism bears a close resemblance to Waksman's A. griseolus. The latter is described as forming a faint yellowish soluble pigment, but this difference in degree of color intensity is not considered sufficient to offset the agreement found in most other reactions. The above organism is therefore considered to be a strain of A. griseolus. This is a very common soil organism, and was isolated by Waksman from California adobe soils.

39.

ISOLATE NO. 1424

SOURCE OF ISOLATE: Subculture of isolate made by KenKnight (33) labelled "KenKnight's Act. No. 53, turnip". Subcultured November 7, 1940.

1. Czapek's Agar: Aerial mycelium present, white; vegetative growth dull whitish brown; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, white; vegetative growth dull whitish brown; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, scant, white; vegetative mycelium yellow; soluble pigment present, yellow.

4. Citrate-Glycerin Agar: Aerial mycelium present, scant, white; vegetative growth yellowish; soluble pigment present; faint yellow.

5. Gelatin: Aerial mycelium present, white; vegetative growth greenish bordered by yellow; soluble pigment present, dark brown. After 7 days peptonization including 1-1/2 inches of gelatin. After 30 days complete liquefaction; aerial mycelium powdery, dirty white; vegetative growth brownish; soluble pigment pale yellowish brown.

6. Milk, 25° C.: Milk loosely coagulated with slight colorless whey in 7 days; peptonization proceeding to completion (2 inches) in 15 days, resulting in a colorless whey. Aerial mycelium present, scant, white.

7. Milk, 37° C.: Milk coagulated with peptonization to 1 inch (half of tube) in 7 days; whey water-white.

8. Potato Plug: Aerial mycelium present, white, rugose; vegetative growth watery yellow; plug unchanged.

9. Starch Agar: Aerial mycelium present, scant, white, vegetative growth dull yellow brown; soluble pigment absent.

10. Nutrient Peptone Agar: Aerial mycelium present, s cant, white; vegetative growth yellowish; soluble pigment present; faint yellow.

11. Tyrosin Agar: Aerial mycelium present, white; vegetative growth dark yellow; soluble pigment present, light brown.

Conclusions regarding identity: While there are certain reactions which agree with those of A. alboflavus and A. griseolus, it is thought better, on account of certain distinct reactions (e.g. the vegetative coloration and pigment formation on gelatin, and the growth on potato) to place this organism in a new category - A. K1424.

40.

ISOLATE NO. 1426

SOURCE OF ISOLATE: Same as for Isolate No. 638 save from a scabby tuber grown in Series I, #1, having a pH value of 4.8.

1. Czapek's Agar: Vegetative mycelium buff yellow; soluble pigment absent.

2. Glucose Agar: Aerial mycelium gray; vegetative growth yellow buff; soluble pigment none.

3. Malate-Glycerin Agar: Aerial mycelium gray; vegetative growth yellow buff; soluble pigment none.

4. Citrate-Glycerin Agar: Aerial mycelium gray; vegetative growth yellow buff; soluble pigment none.

5. Gelatin: Liquefaction 1/4th inch in 14 days, increasing in 25 days to 3/4th inch; soluble brown pigment present, dark brown.

6. Milk, 25° C.: In 15 days milk is unaffected save for soluble gray green pigment; in 30 days still no proteolysis, but color is olive drab.

7. Milk, 37° C.: After 10th day, color of milk is buff khaki; no proteolysis.

8. Potato Plug: Aerial mycelium absent till 15th day when a scant ring of white covers radiately wrinkled border surrounding cokey to moist vegetative growth; plug slightly browned; water underneath faint brown.

9. Starch Agar: Aerial mycelium occurring late, white, umbonate; vegetative growth buff yellow; soluble pigment absent.

10. Nutrient Peptone Agar: Aerial mycelium occurring late, white; vegetative growth dirty brown; soluble pigment present, dark brown.

11. Tyrosin Agar: Aerial mycelium present, white; vegetative growth white; restricted filiform growth; soluble pigment absent.

Conclusions regarding identity: The above description is in fair enough agreement with the description given for A. aureus Waksman and Curtis to be considered that species. This is a member of the chromogenus group and is one of the most common soil organisms, having been isolated from New Jersey garden, orchard, meadow and forest soils, Iowa, Louisiana, North Dakota, Hawaii, Texas, Alaska and Colorado soils. The species has been found to vary in some details, a number of strains having been isolated.

41.

ISOLATE NO. 1430

SOURCE OF ISOLATE: Same as for Isolate No. 1242 save from a scabby tuber grown in Series I, #11, having a pH of 5.1.

1. Czapek's Agar: At first aerial mycelium velvety white; vegetative growth faint buff; later aerial mycelium gray; vegetative growth lemon buff; soluble pigment absent.

2. Glucose Agar: At first aerial mycelium velvety white; vegetative growth faint buff; later aerial mycelium gray; vegetative growth lemon buff; soluble pigment absent.

3. Citrate-Glycerin Agar: At first aerial mycelium velvety white; vegetative growth faint buff; later aerial mycelium gray; vegetative growth lemon buff; soluble pigment absent.

4. Gelatin: Liquefaction 1/4th inch (1/8th of tube) in 15 days, with no further peptonization to 30 days; soluble pigment present, dark brown.

5. Milk, 25° C.: Milk at first yellowish brown, changing to black in 30 days.

6. Milk, 37° C.: No apparent effect on milk soluble pigment grayish chocolate.

7. Potato Plug: Aerial mycelium absent; vegetative growth at first moist and buff, later (15 days) dry granular and gray; plug turned dark brown to black; water below plug dark brown.

8. Starch Agar: Aerial mycelium present, velvety white; vegetative growth faintly buff; soluble pigment absent.

9. Nutrient Peptone Agar: Vegetative growth dirty buff; soluble pigment present, dark brown.

10. Tyrosin Agar: Aerial mycelium present, white to gray; vegetative growth dark olive; soluble pigment present, dark brown.

Conclusions regarding identity: The above account is in very good agreement with Waksman's physiological description of A. scabies and the above organism being regarded as that species.

42.

ISOLATE NO. 1432

SOURCE OF ISOLATE: Same as for Isolate No. 1264 but from a different tuber.

1. Czapek's Agar: Aerial mycelium present, whitish; vegetative growth from white to pale bronze brown; soluble

pigment absent.

2. Glucose Agar: Aerial mycelium present, slightly green; vegetative mycelium white to pallid; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, white; vegetative mycelium pallid to brown; soluble pigment present, brown.

4. Citrate-Glycerin Agar: Aerial mycelium present, whitish to faint green; vegetative mycelium ranging from pallid through light gray to buff brown; soluble pigment absent.

5. Gelatin: Aerial mycelium present, white; complete liquefaction in 14 days; soluble pigment dark red brown, enveloping 1 inch in 15 days.

6. Milk, 25° C.: Aerial mycelium present, white; vegetative mycelium yellowish; milk hydrolized (no prior curd) in 15 days; soluble pigment light orange.

7. Potato Plug: Aerial mycelium scant, white, on 10th day, exuding orange colored droplets; vegetative mycelium pimpled and convoluted; potato at first turned brown, later dark red violet.

8. Starch Agar: Aerial mycelium absent; vegetative growth pallid; soluble pigment absent.

9. Nutrient Peptone Agar: Aerial mycelium present, white; vegetative growth pallid to yellow buff; soluble pigment present, slightly brown.

10. Tyrosin Agar: Aerial mycelium present, whitish; vegetative mycelium brown; soluble pigment slightly brown.

11. Carrot Agar: Heavy white growth.

Conclusions regarding identity: The description given above does not coincide with the accound of any species given by Waksman; it is accordingly placed in a new category, viz., A. K1432.

43.

ISOLATE NO. 1478

SOURCE OF ISOLATE: Deep lesion from a scabby tuber grown on Plot

Series I, #6, Potato Experimental Farm, Lake City, Michigan. Soil had a pH of 3.4. Tuber lifted October 21, 1940, and brought in by Dr. J. H. Muncie. Cultured November 30, 1940.

1. Czapek's Agar: Aerial mycelium present, from white to gray; vegetative growth from white to pastel yellow; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, white to gray, exuding droplets of liquid; vegetative growth pallid to pale yellow to light gray; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, white; vegetative growth light brown; soluble pigment absent.

4. Citrate-Glycerin Agar: Aerial mycelium present, ranging from white to hoary light gray; vegetative growth faint yellow buff; soluble pigment absent.

5. Gelatin: Aerial mycelium present, ranging from white to grayish; vegetative growth yellowish; soluble pigment present light brown, extending into medium 1/4th inch.

6. Milk, 25° C.: Vegetative growth as a ring top of milk dark brown; milk turning black brown. Coagulation and peptonization not observed.

7. Milk, 37° C.: Coagulation in 15 days, with no observable peptonization to 30 days.

8. Potato Plug: Aerial mycelium absent; vegetative growth moist, dirty gray, becoming dry, cokey black; potato plug and water below turned black brown.

9. Starch Agar: Aerial mycelium present, scant, occurring as white points; vegetative growth faint brown buff; soluble pigment absent.

10. Nutrient Peptone Agar: Aerial mycelium absent; vegetative mycelium dirty gray and moist on top, on obverse dull brown yellow; soluble pigment present, dark yellow brown.

11. Tyrosin Agar: Aerial mycelium present, white, becoming gray, poroid; vegetative growth darkly olivaceous; soluble pigment present, dark brown.

Conclusions regarding identity: The reactions of this organism are in good agreement with those described by Waksman

for A. scabies, and this designation is being applied to it. The pH value (3.4) of the soil from which this tuber was obtained leads to an interesting situation for it has long been held that A. scabies does not tolerate a hydrogen-ion concentration of much below 5.0.

44.

ISOLATE NO. 1480

SOURCE OF ISOLATE: Deep lesion from a scabby tuber grown on Plot Series II, #11, Potato Experiment Farm, Lake City, Michigan. Soil had a pH value of 5.3. Tuber lifted October 21, 1940, and was brought in by Dr. J. H. Muncie. Cultured November 30, 1940.

1. Czapek's Agar: Aerial mycelium present, white to gray; vegetative growth yellow; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, dark gray fringed with a translucent halo; vegetative growth yellow-olive centered; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium occurring late (after 20 days) and then scant, white; vegetative growth above papilliform, cream yellow surrounded by whitish translucent halo, below yellow orange; soluble pigment absent.

4. Citrate-Glycerin Agar: Aerial mycelium present, scant, white, concentric; vegetative growth ranging from pallid white to yellow to brown yellow; soluble pigment absent.

5. Gelatin: Aerial mycelium present, white; vegetative growth yellow buff below; soluble pigment present, dark brown; no liquefaction observed.

6. Milk, 25° C.: Ring of light brown vegetative growth at top of milk; soluble pigment present, dark brown.

7. Milk, 37° C.: Complete coagulation in 15 days, slightly pink colored; peptonization of 1 inch in 20 days.

8. Starch Agar: Aerial mycelium present, scant, white; vegetative growth pallid white to buff; soluble pigment absent.

9. Nutrient Peptone Agar: Aerial mycelium present, scant, white; vegetative growth yellow brown; soluble pigment present, dark brown.

10. Tyrosin Agar: Vegetative growth white; soluble pigment dark brown.

Conclusions regarding identity: There is good agreement between this description and that given by Waksman for A. scabies, and the organism is considered to be this species. The isolation was made from the same soil as in the case of Isolate No. 1478 with the exception of a different pH value. It is a possibility that the difference in the two descriptions have their origin in the nature of the isolates' former environments.

45.

ISOLATE NO. 1548

SOURCE OF ISOLATE: Subculture of an isolation made by KenKnight (33) labelled "KenKnight's Act. No. 38, Warba Tuber, Lake City, Michigan, apparently pathogenic". However, this is not the same organism represented by Isolate No. 1285, since when isolations were made from the original stock culture, several colony types were observed. This is a subculture of a pink colony appearing on KenKnight and Muncie's Agar, the only one of its kind among the other white and buff ones.

1. Czapek's Agar: Aerial mycelium present, scant, thin, white; vegetative growth papilliform, pallid with some development of pink; soluble pigment absent.

2. Glucose Agar: Aerial mycelium present, pulvinate, white, feathery; vegetative growth yellowish brown centered encircled with margin of white; soluble pigment absent.

3. Malate-Glycerin Agar: Aerial mycelium present, white to light gray; vegetative mycelium with centers ranging from dull pink to dark red to (the majority) violet centered, outlined by pallid white. Subsurface colonies blood red to dull purple on top. Soluble pigment absent.

4. Citrate-Glycerin Agar: Aerial mycelium present, whitish to pink, feathery, forming a ring around a center of deeper pink vegetative growth; vegetative growth on underside blood-red centered. Soluble pigment present, faint pink.

5. Gelatin: At first (7 days) vegetative growth as a ring at top of gelatin dull brown buff. Later (30 days) aerial mycelium dull white; vegetative growth brownish; liquefaction 1/2 inch; soluble pigment present, dull dark brown.

6. Milk, 25° C.: Aerial mycelium present, white; vegetative growth as a ring top of milk zonally variegated white, yellow, orange-spotted. Later colonies of blue and red occurring in zones; milk turned cloudy buff yellow whey, finally to a cloudy orange whey.

7. Milk, 37° C.: Coagulation in 4 days with ring of vegetative growth at top of milk, white, and on milk-ward side, blood red. In 7 days half of milk (1 inch) colorless whey; later (30 days) milk completely peptonized, whey colored orange.

8. Potato Plug: At first (7 days) at upper end of plug grayish white punctiform colonies; at lower end water-soaked dirty yellow buff colonies. After 30 days colonies on upper portion of plug covered with gray aerial mycelium, lower on plug with pinkish white aerial mycelium. Potato plug uncolored.

9. Starch Agar: Aerial mycelium present, white to pink, surrounded by pink to red vegetative border; vegetative growth underneath ranging from whitish to buff to pink orange to pink to blood red. Soluble pigment absent.

10. Nutrient Peptone Agar: Aerial mycelium present, scant, white; vegetative growth pallid white to yellow; soluble pigment absent.

11. Tyrosin Agar: Aerial mycelium present, filiform white; vegetative growth yellow buff; soluble pigment absent.

Conclusions regarding identity: This organism might well be a strain of Krainsky's A. albosporeus as amended by Waksman and Curtis were it not for certain rather definite reactions not included in Waksman's descriptions.

> On certain points there is better agreement with Krainsky's original account, for which variation in the composition of the media may be held responsible. It is thought best because of certain distinctive reastions (soluble pigment formation in Gelatin, characteristic pigmentation of colonies in Starch Agar, etc.) to place this organism in a new category, viz., A. K1548.

The following key is given as a brief summarization of the descriptions that have been presented. The scheme is, of course, artificial, but will serve to bring out the more important diagnostic characters of the different species. The key is vulnerable because the major and minor divisions have been based on speed of proteolysis and pigment formation; and as Waksman (70) has pointed out, these characters are subject to considerable variation, their reliability depending on a number of factors including nature of the organism, its prior substrate, and its present one. The key is similar to the one designed by Waksman (67).

A PARTIAL KEY TO THE ACTINOMYCETAL FLORA OF MICHIGAN SOILS

A. Production of soluble pigment on proteinaceous media:

I. Soluble pigment dark brown:

1. Brown soluble pigment produced on tyrosin agar:

a. Vegetative growth on potato dark gray to black; on citrate-glycerin agar becoming yellow buff:

(1) Soluble pigment on tyrosin agar usually dark brown; sulfur-yellow pigment absent on creatinin solution; quinone reaction absent.....A. scabies

(2) Soluble pigment on tyrosin agar usually light brown; sulfur-yellow pigment in creatinin solution; quinone reaction present.....A. olivochromogenus

(3) Soluble pigment on tyrosin agar dark brown; soluble pigment brown on synthetic and glycerin agars.....A. 1246 Knorr

b. Vegetative growth on potato red; on citrate-glycerin agar becoming vinaceous red.....A. 1057 Knorr

2. Soluble brown pigment produced on glucose and malate glycerin agar.....A. pheochromogenus

3. Vegetative growth on tyrosin agar white, filiform, no soluble pigment.....A. aureus

4. Aerial mycelium on inorganic media greenish.....A. 218 Waksman

5. Vegetative growth on Czapek and glycerin agars mahogany red.....A. 1343 Knorr

6. Soluble yellow pigment on malate-glycerin agar, potato turned pink in 7 days.....A. 1390 Knorr

7. Vegetative growth and soluble pigment on Czapek's agar orange yellow.....A. 1244 Knorr

II. Soluble pigment faint brown, golden, yellow or blue:

1. Soluble pigment on synthetic agar red, turning blue.....A. violaceus-ruber

2. Aerial mycelium on potato greenish; potato plug becoming deep brown.....A. griseolus

3. Orange or lemon soluble pigment dispersed in the whey of peptonized milk:

a. Vegetative growth on starch agar pink to blood red.....A. 1548 Knorr

b. Soluble lavender pigment bordering colonies on malate-glycerin agar.....A. 1339 Knorr

c. Growth on potato exuding orange colored droplets.....A. 1432 Knorr

4. Vegetative growth on potato yellow in 4 days, turning potato deep red.....A. 1396 Knorr

B. Soluble pigment not produced on proteinaceous media:

I. Species strongly proteolytic; gelatin liquefied rapidly and/or milk coagulated and peptonized rapidly:

1. Vegetative growth greenish yellow and aerial mycelium grayish on synthetic agar; growth on potato exuding black droplets.....A. flavovirens

2. Aerial mycelium white on most media, gray on tyrosin agar with vegetative growth reddish brown.....A. poolensis

3. Grayish aerial mycelium on synthetic agar; vegetative growth on citrate-glycerin agar greenish yellow.....A. olivaceus

4. Orange or lemon soluble pigment dispersed in the whey of peptonized milk:

a. Vegetative growth on potato concolorous with flesh of tuber.....A. 638 Knorr

b. Vegetative growth dull green on synthetic and glycerin agars.....A. 676 Knorr

c. Vegetative growth on citrate-glycerin agar dull brown, with halos of calcium-cleared agar surrounding colonies....A. 484 Knorr

5. Yellow soluble pigment present in glycerin agars .....A. 1424 Knorr

II. Proteolytic action on gelatin and milk weak:

1. No soluble pigment present on synthetic agar:

a. Vegetative growth on malate-glycerin agar deep blue centered, bordered by red..... .....A. 1386 Knorr

b. Vegetative growth on malate-glycerin agar dark brown.....A. 678 Knorr

c. Vegetative growth on malate-glycerin and glucose agar brilliant orange..... .....A. 748 Knorr

The susceptibility of A. scabies to an acid soil reaction has constituted the basis for the major control measure against soil-borne Actinomyces - viz., the sulfuring of the soil. Most workers are of the opinion that the check on the scab organism is brought about by the oxidation of the sulfur to produce an acid reaction, though certain investigators, particularly Duff and Welch (16) and Graham (24) have demonstrated an inhibition of scab without any appreciable alteration in the hydrogen-ion concentration of the soil.

Gillespie (21, 22) made an extended study of A. scabies and its reaction to acid pH values, and found that a hydrogen-ion concentration of 5.2 was approximately the lower limit of growth for this organism though slight development occurred in a few instances at pH 4.8. Sanford (56) found for the strain of A. scabies with which he worked that a pH of 5.36 was the limiting acidity for germination, but that severe scab could occur in soils of pH 5 because of the higher pH value of the tuber and the tendency of the organism to make alkaline the scab pustule.

In the course of these investigations strains of A. scabies were isolated from soils of various pH values. Table 1 brings together some of these isolates and the pH values which accompanied them.

Table 1 - Isolates of A. scabies from Michigan Soil, and Their Accompanying pH Values

Isolate No.	Page No.	Description of locale, substrate, etc.	Soil pH Value
1242	38	Soil from Lake City Sulfur Plot, Series I, #7, 1940.....	5.0
1248	41	Soil from Lake City Sulfur Plot, Series II, #11, 1940.....	5.3
1254	43	Tuber from Lincoln Farm, Greenville, Michigan, Plot L-4, 1940.....	4.9
1256	44	Soil from Lake City Sulfur Plot, Series III, #9, 1940.....	4.8
1258	45	Tuber from Lincoln Farm, Greenville, Michigan, Plot L-1a, 1940.....	4.9
1262	48	Tuber from Lincoln Farm, Greenville, Michigan, Plot L-4, 1940.....	4.9
1430	69	Tuber from Lake City Sulfur Plot, Series I, #11, 1940.....	5.1
1434	52	Tuber from Lake City Sulfur Plot, Series I, #1, 1940.....	4.8
1436	53	Tuber from Lincoln Farm, Greenville, Michigan, Plot L-5, 1940.....	4.9
1478	71	Tuber from Lake City Sulfur Plot, Series I, #6, 1940.....	3.4
1480	73	Tuber from Lake City Sulfur Plot, Series II, #11, 1940.....	5.3

The pH value of 3.4 given above for Isolate No. 1478 is of particular interest when compared with findings cited in the foregoing history of Actinomycetal susceptibility to high hydrogen-ion concentrations.

No isolations of A. scabies are listed in the above table that were made from soils of pH values in which scab would normally be expected to exist as shown by growing on artificial media. Most of the information here given concerns the state of the A. scabies strain flora of the Lake City Sulfur Plots. Other Actinomycetes found in these plots are listed in Table 2.

Table 2 - Isolates of Actinomyces, other than A. scabies, from the Lake City Sulfur Plots, and Their pH Values

Isolate No.	Page No.	Location	Name of Organism	Soil pH Value
636	21	Series III, #7	A.poolensis	4.8
638	22	Series III, #7	A.638 Knorr	4.8
640	23	Series I, #7	A.flavovirens	5.0
686	31	Series II, #2	A.olivaceus	5.3
678	28	Series I, #5	A.678 Knorr	3.7
748	31	Series III, #5	A.748 Knorr	5.0
1057	32	Series I, #7	A.1057 Knorr	5.0
1177	35	Series I, #6	A.violaceus-ruber	3.4
1239	37	Series I, #7	A.pheochromogenus	5.0
1244	39	Series I, #7	A.1244 Knorr	5.0
1246	40	Series III, #4	A.1246 Knorr	5.4
1260	46	Series III, #2	A.olivochromogenus	5.0
1426	68	Series I, #1	A. aureus	4.8

PART II

PATHOGENICITY TESTS

Historical: Prior to 1890, theories regarding the etiology of potato scab fell mainly into four divisions: 1) insect and other animal parasites; 2) chemical erosion; 3) mechanical irritation; and 4) excessive moisture. Thaxter (65) first attributed the malady to a fungus parasite, and named the organism Oospora scabies. Many workers after him have confirmed his conclusions, but little information exists in the literature for the 25 years following his announcement regarding methods of inoculation and tests for pathogenicity. As late as 1915 Pethybridge (52) wrote: "Although some writers have gone so far as definitely to ascribe some of our scab to this (Thaxter's) organism, no scientific evidence appears as yet to have been published proving that the said organism is really responsible for our ordinary potato scab."

The indefinite state of the literature made it possible for Roze (55) in 1896 to attribute scab to a primary organism, Micrococcus pellucidus, which he thought to be the active agent which prepared the entry into the tuber for the "saprophytic" A. scabies. Later, in 1905 Güssow (25) postulated that deep scab was but a stage in the rhizoctoniose of potatoes, and in the same year Paddock (51) also considered "Eastern" potato scab to be due to Rhizoctonia solani and that A. scabies was merely parasitic upon this primary invader.

Pethybridge (52) mentioned the absence of details regarding inoculation and pathogenicity tests prior to 1915. Subsequent to that date, data have not been very much more complete, for information

concerning procedures followed in these matters seem to have been taken much for granted. In the following paragraphs are presented some of the more detailed methods for testing pathogenicity that have appeared in the literature.

Thaxter's original method of reinoculating potato tubers with a pure culture of the etiologic agent was carried out under no certain terms of sterility. The soil was removed from stolons to be inoculated, and they were washed with sterile water. Pieces of broken pots were laid around the stolon and a pot with its drainage hole firmly plugged was inverted over the rim of broken crockery. Inoculation was accomplished by transferring an emulsion of spores on to the tuber. The pot was firmly replaced, and the whole heaped over with soil.

Millard and Burr (48) again used Thaxter's technique in 1926, and while recognizing the objections which could be raised on account of septic conditions they nevertheless felt that weight could be given to the results of such a procedure.

Millard (49) also employed a soil inoculation procedure in his infectivity tests. Pots were filled with soil and autoclaved at 130° C. for one hour. Seed pieces were disinfected by immersion in one-sixth per cent formaldehyde solution for two hours. These were planted in the sterilized pots, and at the same time three sterilized glass tubes were inserted obliquely into each pot for the purpose of subsequent inoculation. These pots were then placed in a greenhouse, and further attempts at aseptic culture were restricted to a periodic washing of the floor and benches with a

disinfectant. In another paper (48) Millard and Burr reported that with this technique ".....we have found that on no occasion did infection spread to the control pots, and we feel justified in assuming that the scab produced in any individual pot was due solely to the infection material added to it. Further proof, if necessary, was afforded by the different types of scab produced in the different pots."

Goss (23) used seven-inch clay pots filled with soil. These were autoclaved for two and one-half to three hours at a pressure of 25 pounds. Scab-free tubers, treated with hot formaldehyde (1 to 120) for three minutes at 52° C. were used for seed pieces. They were first planted in sterilized sand until rooted and sprouted, when they were then transplanted to the clay pots. Inoculation of the soil in the clay pots was made at the time of transplantation. Water was applied at the surface or by means of a glass tube to the center of the container. The pots were then placed in a greenhouse, and attempts to eliminate contamination were limited to the cleaning of floors and benches with water.

The method employed by Afanasiev (2) in his study of pathogenicity is much the same as that used by Goss. Sterile water was used in watering the plants, and the cement floors were never swept with a broom but were kept clean by washing with water and a strong disinfectant.

KenKnight (33) utilized several methods in his tests for pathogenicity. In one he planted sprouts in six inch pots filled

with steam sterilized soil. These pots he then placed out of doors on a lawn and kept them supplied with tap water. In the other method he prepared similar pots, and placed these in a greenhouse which was kept as clean as possible by keeping the floors wet. In the first method only three of the thirty sterile uninoculated control pots produced scabby tubers; in the second, all controls were heavily scabby. A subsequent modification in the design of pathogenicity testing was made the following year. In order to keep down contamination asphalt roofing paper was laid over the greenhouse floor. The soil was steamed in six inch pots for 15 hours, and the pots were placed four each in galvanized pans specially constructed for the purpose and watered from below. Over the open surface of the pots was laid rock wool in an attempt to exclude air borne Actinomycetal contamination. The results with this method were also unsatisfactory since tubers in five out of the sixteen uninoculated control pots were scab-infected.

KenKnight came to the conclusion that planting detached potato sprouts rather than tubers and covering the pots with rock wool were not instrumental in reducing contamination, though covering the greenhouse floor with asphalt-coated roofing paper did appear to reduce contamination somewhat.

Techniques: In the light of difficulties experienced here with pathogenicity trials in the past, the writer has attempted to devise and test some new procedures for proving the pathogenic nature of isolates. The ultimate goal in these designs has been to perfect a method of

testing pathogenicity of a particular isolate toward any particular variety of potato by means of a short-cut, laboratory method. It is only by means of the precise techniques of the laboratory that the multifarious conditions which obtain in the field can be held in check.

However, before such a technique can be developed, be it chemical, physical or biological, there must first be some stardard against which to check results, and for these results to be significant it is necessary that the standard be a true representation of conditions as they occur in the field. As a consequence, various gross attempts at proof of pathogenicity have been made under conditions that are as nearly typical of the field as possible and yet which will give some protection against contamination.

As one of these gross methods, a repetition of KenKnight's greenhouse procedure has been attempted, with the introduction of a few modifications. Soil, instead of having been steam sterilized, was placed in eight inch clay pots, and autoclaved for two and one-half to three hours at 15 pounds pressure. Waksman (70) stated that a soil temperature of 100° C. was sufficient to kill all Actinomycetes with the possible exception of A. invulnerabilis, and tests by the writer have shown that not even bacterial growth will follow plating out of soil sterilized in these containers for two hours at 15 pounds. After cooling, the soil in these pots was mixed thoroughly with inoculum. It has been shown that the depth of the Actinomycetal flora zones is of considerable importance in producing infection; therefore the inoculum was mixed throughout the soil and

not merely applied as a water suspension on the surface of pots. Rubber gloves were worn during the mixing operations, and these were sterilized in a strong solution of mercuric chloride before mixing each new culture. In addition to this initial application of inoculum, repeated dosages were administered to the soil at four different intervals during growth of the plants. The inoculum was poured into a hole driven into the soil of each pot by a sterile test tube. After the addition the hole was tamped shut.

Inoculum was grown on sterile diced potatoes in a 1000 cc. Erlenmyer flask. This method of producing inoculum was much superior to the oat culture in that it could be produced abundantly in five to seven days. When ready to use, the flasks were filled with water, shaken, and the entire contents mixed with the soil.

A total of 120 such pots were prepared in the testing of ten Actinomycetal isolates. Each isolate was introduced into ten pots, and twenty pots were left uninoculated as checks. Of the 120 pots half the number were placed out of doors in an uncovered cold frame adjoining the Botany Department greenhouses. The other 60 pots were located in a greenhouse that had previously been thoroughly cleaned and wetted down. Tar paper was used to cover the central earthen floor space, and on this surface the pots were placed, resting them on inverted saucers to prevent contamination through capillary attraction of water via the drain holes. The floor of the greenhouse was kept flushed with water in an attempt to accomplish a dual purpose: 1) a reduction in the amount of airborne contamination, and 2) a reduction of the high summer tempera-

tures. The greenhouse was periodically fumigated with nicotine to keep out aphids and the plants were sprayed with a proprietary compound to control thrips and white flies.

A more serious attempt to approximate aseptic conditions of culture led to the second method. Theoretically Koch's postulates have never been entirely satisfied with the case of potato scab. The literature of scab deals with no attempt to exclude all organisms save the suspected pathogen. The original method by which Thaxter demonstrated A. scabies to be the etiologic agent of potato scab, while empirically correct, nevertheless did not preclude the association of other microorganisms. Since Thaxter's announcement, there have been several claims that other organisms are more basically involved than A. scabies. Primarily to dissociate all other microorganisms from the one to be studied, and secondarily, more in the spirit of incidental curiosity, to ascertain the effect on tubers of a pure culture of Actinomyces, an attempt was made to grow potato tubers and roots under sterile conditions.

A previous attempt to culture potatoes aseptically is described by MacMillan (41), who sought to produce sterile plants in connection with research on potato Fusaria. Elaborate precautions were taken to insure sterility. One hundred ash cans were employed which were filled with soil, sealed with metal covers, hoisted into high pressure processing kettles, autoclaved for nine hours at 65 pounds pressure, cooled, planted in a sterile culture house of special construction, and each can then supplied with a sterile glass lamp chimney. The completed assembly was then placed

in a sunken cement trough almost as deep as the cans, and the trough filled with water to equalize the temperature. A watering machine was constructed which supplied the cans with sterile water, and each can was weighed daily to maintain a uniform state of moisture. Despite the vigilant attention given these plants, MacMillan made the following concluding observation:

> The growth of plants was feeble, even under what appeared to be the optimum conditions. At no time was there anything approaching a normal rate of growth, the plants growing usually only a very few inches during the entire season. On some plants the leaves were yellow and small and, although showing no signs of contamination or disease, were obviously devitalized. The lack of vitality appeared to be caused in part by the soil, the humus of which had been destroyed by the steam.....
>
> Also, the plants were definitely hurt by the process of paring, which took away the best part of the eye. From this loss they seemed never to recover. Add to these destructive influences the exposure to unfavorable temperatures, and it is evident that the plant growth must have been weak indeed.....No plants were obtained which could be used for inoculation with Fusaria.

In view of the above, which resulted after much care and diligence on the part of MacMillan, the writer should not have been disposed to pursue the same matter further. But on the other hand, as pointed out by MacMillan there were certain shortcomings in his procedure and it was in the hope that with the rectification of these shortcomings a successful conclusion could be attained that the writer attempted the following described procedure for growing potatoes aseptically.

Number ten size cans, such as are used in packaging fruits

and vegetables were used as the culture receptacle. These cans had an approximate diameter of six inches and a height of seven inches. The tops were removed and the cans painted inside and out with asphaltum. Near the base of each can, a hole was punched barely large enough to accommodate a galvanized iron nipple (Figure 1, A) one inch in diameter and approximately five inches long. A rubber gasket (B) was fitted on the end protruding into the can and this was forced against the wall by a nut (I). On the outside the junction of the can and the nipple was made water-tight by a durable coating of solder. Into the exterior end of the nipple was driven a tightly-fitting rubber stopper (C) that accommodated an ell of glass tubing (D) 7/16th inch in diameter. The can was filled with a layer of clinkers (F) to a height slightly above the top of the nipple (A), and then more clinkers were slanted as a truncated cylinder with its base on the bottom layer and its uppermost end at the top of the can opposite the opening of the nipple.

Soil (G) - consisting of two parts composted earth, one part washed sand and one part small gravel, all well mixed - was then used to fill the rest of the can to within one inch of the top. A glass tube (E) 7/16th inch in diameter was then sunk perpendicularly into the soil at such a position that its lower end became embedded in the upper portion of the cinders. The soil was watered with 500 cc. of tap water, and a hole (H) two inches deep and 3/4th inch in diameter was made into the soil at the center. The hole was loosely stuffed with a wad of cotton, and a layer of cotton was also placed over the top of the can so that its edges

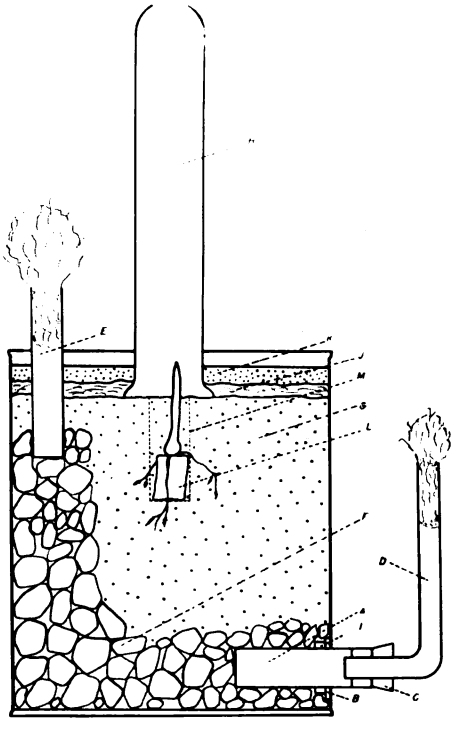


FIGURE 1: STERILE CAN ASSEMBLY

Figure 1 - Sterile Can Assembly

fitted on the inside wall of the can and encircled tube (E). Both tubes (D) and (E) were loosely plugged with wads of cotton. Another layer of cotton was placed over the first layer and secured to the outside of the can with rubber bands. In order to prevent drops of condensing steam from falling onto the top of the cans and to keep out dust after removal from the autoclave paper hoods consisting of inverted paper bags, #25's, cut in halves, with the open ends discarded, were placed over the entire assembly. The pots were then ready for autoclaving.

Prior experiments were conducted to ascertain the length of time necessary to secure complete sterilization in these cans. Three cans filled and covered in a manner similar to the above were autoclaved for two hours at 15 pounds, and another set of three for four hours. At the end of these periods, the pots were removed from the autoclave, the cotton coverings taken off, the surface flame-sterilized, a sterile auger sunk to the bottom of the can, and the core removed to a 500 cc. Erlenmeyer flask containing sterile water. Dilution plates were then run from this flask and incubated for seven days. The pots were then flamed and the cotton and paper bag coverings replaced, and held at room temperature for 24 hours when they were again autoclaved for two and four hours per set, respectively. Core samples were taken daily after each autoclaving for four days. No cans were tested at a time several days after an autoclaving for germinating spore formers, since it was to be expected that any resistant spores present in the can would be revealed sooner or later on the dilution plates. The record of this experiment showed that

it was unnecessary to autoclave such cans longer than two hours at 15 pounds pressure to insure soil sterility.

Concern was felt over the matter of too long a period of soil heating, else the two hour period could have been prolonged for safety's sake. As MacMillan pointed out, soil sterilization was in all probability one of the chief factors resulting in the abnormal development of his potato plants. There existed the likelihood that the humic content of the soil may have been destroyed or that an increase in soluble salts may have resulted or that poisons may have been liberated due to the heat of sterilization. Tests were carried out to ascertain the effect of varying periods of autoclaving on the soil. Cans the same as described above were filled with various soil and sand mixtures, autoclaved for different periods, and then planted to two different indicator plants: tomatoes and barley, which were chosen for their extra sensitivity to abnormal soil conditions and for their ability to make a rapid growth during the winter season. A summary of the experiment and its results are indicated in Table 3.

Table 3 - Effects of Different Soil Mixtures and Periods of Sterilization upon the Subsequent Growth of Tomatoes and Barley. Readings at 40 Days' Growth.

Soil Mixture	Period and Pressure of Sterilization	Tomatoes		Barley	
		Average Length of All Leaves	Values of Extremes	Average Length of All Leaves	Values of Extremes
		cm.	cm.	cm.	cm.
50% Composted Soil + 50% Gravel	3 hrs. at 15 lbs.	2.1	1.5-3.5	9.8	7-14
	Unsterilized	2.6	1.0-3.5	11.4	10-13
100% Gravel	3 hrs. at 15 lbs.	0.9	0.5-1.3	9.1	8-11
	Unsterilized	1.1	0.4-2.1	9.9	8-13
Washed* 50% Composted Soil + 50% Gravel	4 hrs. at 15 lbs.	2.3	0.7-3.0	12.0	8-14
Gravel	Unsterilized	3.2	1.7-4.2	14.2	11-17
Washed* 100% Gravel	4 hrs. at 15 lbs.	1.3	0.5-2.1	9.3	8-11
	Unsterilized	1.6	1.0-2.2	9.6	8-11

*The soils in these pots were washed with tap water after they had been autoclaved two hours with the expectation of removing any poisonous substances or excess soluble salts that may have formed as the result of autoclaving. The cans were then reautoclaved for another two hours.

In all cases the best growth was made on an unsterilized 50% composted soil + 50% gravel mixture, showing that autoclaving did have a deleterious effect on subsequent plant culture. However, the unfavorable action by no means precluded reasonable growth even in the case of the unwashed, three-hour treatment. The value of the washing procedure appears at first to hold some merit when compared to the unwashed autoclaved soil, but when growth of plants in the unsterilized, unwashed soil is compared with that in the sterilized, unwashed soil this difference becomes of doubtful significance. In conclusion, it was deemed that sterilization of soil at 15 points for two hours would produce no marked ill effects on subsequent plant culture, neither as the result of an increase in soluble salts, the formation of toxins from the heated soil or asphaltum lining of the cans,nor from destruction of humic matter. As pointed out above, this two-hour sterilizing period was also sufficient to insure an aseptic condition of the soil.

It was felt that in the gentler procedure of autoclaving (two hours at 15 pounds) as compared to the necessarily greater increase in autoclaving (nine hours at 65 pounds) for the larger bulk of soil used by MacMillan, there might lie some basis for a more successful growth of potatoes under sterile conditions. Furthermore, in this procedure there was no necessity of confining the vines within a glass lamp chimney which would act as a greenhouse within a greenhouse, since sterility of only the rhizosphere was required.

At the end of the two-hour autoclaving period, the cans were allowed to cool, the paper hood and the upper layer of cotton

functioning to trap any air-borne contamination. When sufficiently cool, the cans were placed one at a time in a steamed inoculating chamber, the paper hood and topmost layer of cotton and the cotton plug removed, a sterile potato seed piece (Fig. 1 (L)) inserted in the hole, soil pressed over the seed piece with sterile tampers, a sterile glass chimney (H), consisting of an inverted 300 cc. test tube) centered over the place of ultimate sprout emergence, and over the top of the can, onto the bottom batting of cotton still in place (J), was poured a layer of melted paraffin approximately 1/4th inch thick (K). The function of the embedded layer of cotton was to keep the melted paraffin from sinking into the soil, and possibly producing a deleterious effect on sprout development.

The cans were then taken to the greenhouse in the condition shown in Figure 2. Watering when necessary was accomplished through tube E, and enough sterile water was admitted until its presence became manifest in tube D. The clinker passage-way from tube E and D also served as a runway for soil ventilation, though its efficiency in this respect was somewhat doubted.

When a sprout emerged through the soil and had attained a degree of growth the can was again placed in a steamed inoculating chamber, the glass chimney removed, sterile cotton plugged around the stem, melted paraffin applied to the new cotton, and then the can was replaced in the greenhouse. Care had to be exercised to prevent the stem from coming in direct contact with paraffin for when this happened, the plant in its delicate immature condition was no match for the toxic property inherent in paraffin, and promptly

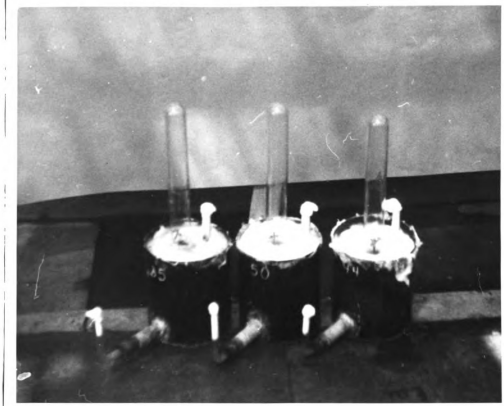


Figure 2 - Sterile Can Assembly at Time of Sprout Emergence

died. When once the plant had made its growth beyond the first few trying stages, development proceeded with a fair degree of success, though the plants never recovered from the small size of the seed piece and the stems at their crowns remained thin and wiry despite the rather good development of leaves and stem diameters higher up the plant. The amount and type of grwoth after approximately one month is shown in Figure 3.

Inoculum in the form of a water suspension of spores was introduced on several occasions through the watering tube E. The inoculum was grown on diced potatoes as mentioned under the discussion dealing with the clay pot procedure.

The processing of the sterile seed pieces which were used in planting the above cans constituted an extended problem of its own. The task would have been simple indeed if true potato seed could have been used, for their sterilization is an elementary procedure. The use of true seed, however, was not feasible since such wide variation in their development ensues as the result of genetic recombination. For example, the genetic combination in a Katahdin potato is such that resistance to potato scab is low. Upon recombination, however, the seed may possess a high degree of resistance, and were a particular strain of Actinomycetal inoculum to be tested on this seedling there would be introduced the new factor of tuber resistance.

Other devices to obviate the use of seed pieces were considered, such as the development of sterile "blind tubers", and the use of sprout cuttings, but resort in the last analysis had to be

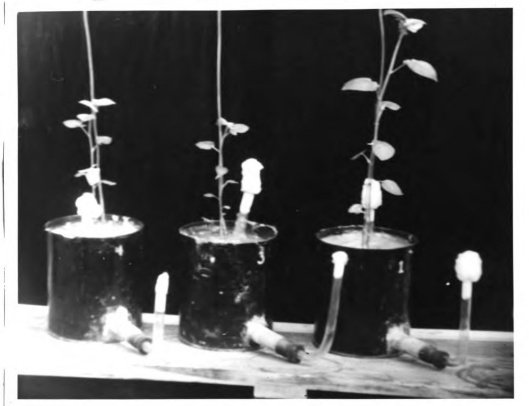


Figure 3 - Growth one month after the planting of seed pieces

taken of the unwieldy seed piece. The eye of the potato is surrounded by folds and crevices, and the destruction of all organisms which are here concealed is a task that cannot be accomplished by chemical means alone and still leave the germ in a viable condition. The method employed by MacMillan was to scrub a tuber, treat it for an hour in 1-1000 mercuric chloride solution, concentrate on one eye, and, with sterile knives cut away all superfluous tissue, leaving a square block of potato with an eye in the center of the upper side. Then the top side was cut across in the hope of leaving the germ of the eye, and the piece was then dropped into the agar which was to constitute the test for its sterility. Following this procedure, MacMillan was able to obtain after a very considerable amount of time, a yield of five per cent usable seed pieces.

The writer employed a procedure for accomplishing this same purpose which was a combination of that used by MacMillan (41) and that by Young (74). Chippewa potatoes, a year removed from certification, were washed under a spray of tap water for at least two hours. Eyes on the tubers were then removed by withdrawing them with a cork borer approximately 1/2 inch in diameter. The diameter was chosen only after trials had shown that a smaller diameter reduced the initial food supply of the seedling sprout too considerably and that a larger diameter increased the contamination load. The plugs were then cut to a length of approximately 3/4ths inch, as much of the epidermis removed as was possible, washed again briefly under a spray of tap water, dipped momentarily in 70 per cent ethyl alcohol, transferred immediately to a one per cent aqueous solution of hydrogen peroxide

for ten minutes, and then placed in a four per cent aqueous solution of formaldehyde for 25 to 30 minutes. During the course of this phase of the work many different percentages of formaldehyde and lengths of soaks were tried, but it was found that a stronger formaldehyde solution for a shorter period produced more usable seed pieces than did a gentler solution for a longer period.

At the end of the 25-30 minute formaldehyde treatment, the seed pieces were dropped into cotton plugged quart fruit jars filled with sterile water to allow the formaldehyde to diffuse out of the plugs. After this half-hour wash, each seed piece was lifted out by means of sterile tongs and dropped into a half-pint milk bottle containing a layer of sterile agar 1/2 inch deep. The eyes were placed upright, out of the agar, since when eyes were submerged, germination was precluded. The bottle was then whirled around so that the whole plug became coated with a layer of agar. The cotton plug was replaced, and the bottle was incubated until, in the absence of contamination, sprout growth was initiated. This required at times a period of from 7 to 25 days.

The task of obtaining satisfactory seed pieces was beset with considerable difficulty. On the one hand, a procedure designed to obtain viable seed pieces by gentle disinfection led to a flourishing growth of molds and bacteria. On the other hand a procedure calculated to wipe out contamination left a plug of potato devoid of any germinative tissue. Out of over 1500 platings only six or seven per cent combined sterility with viability.

Some of the contaminating colonies were so hyaline as at times to escape notice. A procedure was therefore adopted whereby each seed piece before being planted in a can was dipped momentarily into sterile water. An agar plate was then poured using this water as a source of inoculum, and the resulting growth, if any, was then readily observable after a period of incubation.

During the course of this phase of the work it became evident that growth under absolutely sterile conditions was within the realm of possibility, but that a considerably greater amount of time would have to be spent in the attempt than was at the writer's disposal. It is believed that with certain modifications the above procedure would provide a satisfactory technique for this as well as other problems affecting the potato.

The several factors making sterility within the cans a doubtful matter may have their foundations in one, several, or all of the following possibilities. The medium used in testing the seed pieces was the standard potato-dextrose agar formula. While this would have revealed the presence of contamination in most cases, there were still those bacteria and fungi which could not grow on such a substrate but which remained on the seed piece as a latent source of contamination. Under a soil environment they may again have resumed growth. This difficulty is always present in bacteriological work as pointed out by Brierley (5), who said: "All more recent critical experience demonstrates the frequency and tenacity with which infinitesimal numbers of one organism may persist, sometimes

apparently for years, in "pure cultures" of another organism and are only revealed by special or selective technique. Several transfers from single colonies on plates or hosts may not eliminate this initial "contamination" which may only become apparent under some particular circumstance."

A second source of possible contamination resided in an infiltratation of microorganisms through the cotton plugs of the water and aeration tubes and through the collar of cotton surrounding the stem. Within the four or five months in which the plugs were exposed to the dust of a greenhouse it is entirely possible that a spore might have worked itself into the soil and begun proliferation.

Another possibility of contamination lay in the tissues of the seed piece. While these potatoes were but one year removed from certification, there remained the danger of their being infested with tuber-inhabiting fungi or bacteria. These, if present, may have been killed in the outer layers of the plug thus giving a sterile reading on agar, but when it was planted, and the piece underwent disintegration, these concealed inhabitants may have become liberated in the cans. Actual instances were noticed of plugs that appeared sterile in a week's time but later developed a flow of bacterial ooze from around the ends of the cut vascular system.

In the light of these possibilities of contamination the writer feels that this attempt should be not described as a sterile technique but may be regarded as an "Actinomycete-free culture of potatoes".

Another attack on the problem of testing pathogenicity has been made by means of growing potatoes under chemiculture. Various preliminary attempts with aggregate and hydroponic methods were undertaken before the modifications outlined below were decided upon. Cans, the same as those used in the preceding experiments, were painted with asphaltum and each was provided with a hole 1/2 inch in diameter close to the bottom. This hole was plugged with a tight-fitting rubber stopper. A riser four inches deep with lips holding it in place on top of the can was made using 1/2 inch galvanized wire cloth, painted with asphaltum. The riser was then lowered into the can, and a layer of excelsior 1/2 inch deep was placed on the bottom of the riser, and on this was placed a previously disinfected two-ounce potato seed piece. The can was then filled with wood shavings, watered and placed in the greenhouse to await germination of the seed. Gericke (20) has shown the importance of covering the tubers with an adequate thickness of litter, since a deep bed is coolest and a shallow one warmest at the level of the tubers. Especially was such a precaution necessary with the cultures located in a greenhouse. The cans on the first few days became so hot, that they were distinctly uncomfortable to the touch. Subsequently they were all kept in a shallow water bath to equalize the "soil" temperature.

When the sprout appeared above the surface of the shavings, the water in the can was drained through the hole near the bottom, the stopper replaced, and a nutrient solution added to a point just

below the riser. Care had always to be exercised to keep the contents of the riser above water else tuber formation would have been interfered with.

The hydroponic solution was one recommended especially for the growing of potatoes by Shive and Robbins (59) and consisted of the following formula:

Monopotassium phosphate.....	4.7 grams
Sodium nitrate.....	7.6 grams
Magnesium sulfate.....	12.3 grams
Calcium chloride.....	3.8 grams
Water.....	5 gallons

To the above were added various trace elements in the form of two stock solutions:

Stock Solution A:

Boric acid.....	3.2 grams
Manganese sulfate.....	3.2 grams
Zinc sulfate.....	3.2 grams
Water.....	64 ounces

Stock Solution B:

Iron ammonium citrate.....	0.8 grams
Water.....	16 ounces

Ten cc. of Stock Solution A was added to each five gallons of nutrient solution, and just before use 20 cc. of Stock Solution B was mixed with each gallon. An addition of 0.7 grams of copper sulfate to Stock Solution A was made to keep down algae.

The solution was drained from each pot every seven days and new solution added. This was necessary in order to keep the concentration of salts constant and to prevent adverse changes in pH values.

Since light inhibits the formation of tubers on the stolons the pots were covered with heavy denim cloth, leaving only a hole through which the potato vine could grow and through which nutrient solution could be added. The cloth covering also helped to reduce the amount of contamination entering the can.

At the time of tuber formation the risers were removed from the cans and several tuberiferous stolons in each pot separated from the shavings. These were enclosed in a small sterile flask containing sterilized soil inoculated with the Actinomycetal isolate whose pathogenicity was to be tested, sterile cotton plugged into the space left in the opening of the flask, and the flask-enclosed stolon replaced in the litter and sunk into the can with the rest of the plant. The rest of the tubers that remained uncovered constituted the checks.

Results: The foregoing techniques for testing pathogenicities were run using 10 different isolates. These 10 were selected from among 45 isolates for various reasons in themselves. Isolates 1262, 1434, and 1478 keyed out as A. scabies in physiological tests; the ability of these isolates to reproduce scabs would confirm these tests. Several isolates, such as 1341 and 1432 were selected because of their allegedly saprophytic nature. Others, whose pathogenic nature was not known, were selected for elucidation on this point.

The open-top pots which had been placed in the greenhouse as mentioned above were harvested on September 13. The following tabulation (Table 4) sets forth the results obtained. The presence or absence of scab took into account all tubers in a pot. If one out of five tubers in a pot was infected so that 20% of the surface was scabbed then the final reading for the pot was "+", 20%. The percentage stood for the tuber most heavily scabbed. A pot was designated "-" if none of the tubers were scabbed.

Table 4 - Scabbing of Potato Tubers Harvested September 13, 1941 from Open Top Pots in Greenhouse

Pot Number	Isolate Number	Presence (+) or Absence (-) of Scab	Percentage of Surface Scabbed	Type of Scab
2429	1057 (A.1057K)	+	10	Surface
2430	"	+	20	Deep,whitish
2431	"	-	-	-
2432	"	-	-	-
2448	"	+	Trace	Russet
2394	1177 (A.violaceous-ruber)	+	50	Deep
2402	"	+	50	Deep,whitish
2403	"	-	-	-
2404	"	+	20	Surface
2405	"	+	10	Deep,whitish
2424	1260 (A.olivochromogenus)	+	-10	Russet
2425	"	-	-	-
2426	"	-	-	-
2427	"	-	-	-
2428	"	-	-	-
2409	1262 (A. scabies)	+	50	Surface
2410	"	+	80	Surface
2411	"	+	70	Deep
2412	"	+	50	Surface
2413	"	+	50	Surface
2397	1341 (A. griseolus)	-	-	-
2398	"	+	-10	Surface
2399	"	-	-	-
2400	"	+	-10	Deep
2401	"	-	-	-
2384	1394 (A. viridochromogenus)*	-	-	-
2385	"	+	30	Deep,whitish
2386	"	-	-	-
2387	"	-	-	-
2388	"	+	50	Surface
2389	1432 (A. 1432K)	-	-	-
2390	"	-	-	-
2391	"	-	-	-
2392	"	-	-	-
2393	"	-	-	-
2414	1434 (A. scabies)	+	50	Surface
2415	"	+	50	Surface,whitish

*This is a culture obtained from Wollemweber and declared by him to be A. viridochromogenus and pathogenic in Germany.

Table 4 continued

Pot Number	Isolate Number	Presence (+) or Absence (-) of Scab	Percentage of Surface Scabbed	Type of Scab
2416	1434 (A. scabies)	+	50	Deep, whitish
2417	"	No tubers	-	-
2418	"	+	10	Surface,whitish
2419	1478 (A. scabies)	+	80	Surface
2420	"	+	20	Surface
2421	"	+	90	Surface
2422	"	+	80	Surface,whitish
2423	"	+	80	Surface,whitish
2395	1548 (A. 1548K)	-	-	-
2396	"	-	-	-
2400	"	+	10	Surface
2407	"	+	-10	Deep
2408	"	-	-	-
2433	Sterilized soil, uninoculated check	+	20	Deep
2434	"	+	20	Deep,whitish
2435	"	-	-	-
2436	"	-	-	-
2437	"	+	-10	Surface,whitish
2438	"	+	50	Deep
2439	"	+	-10	Pinpoint lenticel infection
2440	"	-	-	-
2441	"	-	-	-
2442	"	-	-	-
2443	Unsterilized soil, uninoculated check	+	10	Surface
2444	"	-	-	-
2445	"	+	10	Deep
2446	"	-	-	-
2447	"	-	-	-

As mentioned above, the same series of pots, inoculated with the same isolates were also placed in an open cold frame outside the northern end of the Botany Building greenhouses. The tubers were harvested on the same day (September 13) and the results are indicated in Table 5.

Table 5 - Scabbing of Potato Tubers Harvested September 13, 1941 from Open Top Pots in Open Cold Frame, outside Northern End of Botany Building Greenhouse

Pot Number	Isolate Number	Presence (+) or Absence (-) of Scab	Percentage of Surface Scabbed	Type of of Scab
2369	1057 (A. 1057K)	+	10	Deep
2370	"	-	-	-
2371	"	+	10	Surface
2372	"	+	10	Deep
2373	"	+	10	Surface
2344	1177 (A. violaceus-ruber)	-	-	-
2345	"	-	-	-
2346	"	-	-	-
2347	"	-	-	-
2348	"	-	-	-
2359	1260 (A. olivochromogenus)	-	-	-
2360	"	+	10	Deep
2361	"	+	10	Surface
2362	"	+	20	Surface
2363	"	+	-10	Surface
2374	1262 (A. scabies)	+	80	Deep
2375	"	+	90	Surface
2376	"	+	80	Surface
2377	"	+	80	Deep
2378	"	+	80	Deep
2349	1341 (A. griseolus)	-	-	-
2350	"	-	-	-
2351	"	-	-	-
2352	"	+	-10	Russet
2353	"	-	-	-
2336	1394 (A. viridochromogenus)	-	-	-
2337	"	-	-	-
2338	"	-	-	-
2339	"	+	10	Deep
2340	"	+	10	Deep
2239	1432 (A. 1432K)	-	-	-
2240	"	-	-	-
2241	"	-	-	-
2242	"	-	-	-
2243	"	-	-	-

Table 5 - Continued

Pot Number	Isolate Number	Presence (+) or Absence (-) of Scab	Percentage of Surface Scabbed	Type of of Scab
2379	1434 (*A. scabies*)	+	80	Surface
2380	"	+	50	Surface
2381	"	+	70	Surface
2382	"	+	20	Surface
2383	"	+	70	Surface
2354	1478 (*A. scabies*)	+	90	Surface
2355	"	+	90	Surface
2356	"	+	80	Surface
2357	"	+	50	Surface
2358	"	+	70	Deep
2364	1548 (*A.* 1548K)	-	-	-
2365	"	+	10	Surface
2366	"	+	10	Deep
2367	"	+	-10	Surface
2368	"	+	10	Surface

A comparison of both the indoor and outdoor trials, together with those based on the sealed pot and the chemiculture techniques is found in Table 7. A discussion of the above figures is deferred to the end of Table 7.

Much time was spent in attempting to culture potato tubers aseptically or at least *Actinomycete*-free, as described under "Techniques". In many cases the sterile seed piece, once laboriously obtained and planted, failed to emerge above ground. These unsuccessful pots were then dismantled, resterilized, and replanted with new sterile seed pieces. In the end only 17 pots developed vines. The aerial parts grew slowly and spindly but ultimately reached a height of about three feet. The tuber growth, however, was disappointing. The tubers were limited in number, ranging from one to six, with an average of two per pot; and their diameters ranged from 7/8ths inch to 1-3/4ths inches

with the mean at 1-3/10ths inches.

More important is the complete failure of scab development in these pots. Not one tuber developed Actinomycetal lesions. Most tubers were without blemish except a few which were corroded and covered with a horny growth, not, however, Actinomycetal in nature.

It is now evident that the biological requirements of the inoculated Actinomyces were not given adequate attention in the construction of these pots. Probable deficiencies may have ben suffered in their air and water relations. That one or the other or possibly both were at fault seems probable because many tubers removed from these pots had an extraordinary degree of lenticel development.

While the shortcomings of this technique could be overcome, it is felt that the lengths to which it has been necessary to go are not justified in the light of the simpler and more successful technique attempted, viz., that of testing pathogenicity under chemicultural methods.

The results of pathogenicity tests using the chemicultural technique are given in Table 6. As has been mentioned under "Techniques", tuberiferous stolons were removed from the wood shavings, enclosed in inoculated flasks, and replaced in their beds, along with uninoculated and unenclosed tubers. Several flasks were often placed in one can, thus the several readings under different isolate numbers for the same can.

Table 6 - Scabbing of Potato Tubers Harvested September 13, 1941 from Hydroponic Culture Cans in Greenhouse

Can Number	Flask Containing Isolate Number	Presence (+) or or Absence of Scab (-)
63	1260	+
"	1434	+
"	Unenclosed check	-
67	1177	-
"	Unenclosed check	-
72	Unenclosed check	-
75	1432	-
"	Unenclosed check	-
76	1394	-
77	1341	-
"	Unenclosed check	-
79	1478	+
"	1432	-
"	Unenclosed check	-
80	1478	+
"	1260	-
"	Unenclosed check	-
81	1549	-
"	Unenclosed check	-

A summary of the results obtained from open-top pots in the greenhouse, and those out of doors, and from the hydroponic cans is presented in comparative form in Table 7.

Table 7- Comparative Tabulation of Results Obtained under Various Techniques of Testing Pathogenicity

Isolate Number	Greenhouse Open Pots No. of Pots Showing Scab	Greenhouse Open Pots No. of Pots Showing No Scab	Outdoor Open Pots No. of Pots Showing Scab	Outdoor Open Pots No. of Pots Showing No Scab	Hydroponic Cans No. of Flasks Showing Scab	Hydroponic Cans No. of Flasks Showing No Scab
1057 (A.1057K)	3	2	4	1	-	-
1177 (A.violaceous-ruber	4	1	0	5	0	1
1260 (A.olivochromogenus	1	4	4	1	1	1
1262 (A. scabies)	5	0	5	0	-	-
1341 (A.griseolus)	2	3	1	4	0	1
1394 (A.viridochromogenus)	2	3	2	3	0	1
1432 (A. 1432K)	0	5	0	5	0	2
1434 (A. scabies)	4	0	5	0	2	0
1478 (A. scabies)	5	0	5	0	2	0
1548 (A. 1548K)	2	3	4	1	1	1
Uninoculated checks	5	5	5	2	0	8

The data contained in Table 7 and subsidiary ones point by no means in one direction. First of all it is evident that the open-pot culture method has failed to an extent because of contamination from the air. This fact is revealed by the almost equal amounts of scabbed and non-scabbed tubers obtained from the sterilized uninoculated check pots. That more contamination took place within the greenhouse than outdoors seems probable from the fact that there are four times as many "two to three" combinations in the former than in the latter; also that under greenhouse conditions five check pots became contaminated whereas only two became contaminated out of doors. With the possible exception, that of Isolate 1260, no contamination occurred in the hydroponic series.

While the unreliability which has been introduced by the existence of contamination might invalidate any further conclusions, it is interesting to note that where a pathogen has been especially virulent, as in the cases of Isolates Nos. 1262, 1434, and 1478, the reactions have been exclusively in one direction throughout the two or three series. For example, Inoculum 1434 brought about scabbing in every pot of the outdoor, indoor, and hydroponic series.

That such complete results would be secured with a virulent pathogen regardless of contamination is not surprising. However, the situation is quite extraordinary with Isolate 1432 where complete agreement in all series is found involving a saprophyte. In view of the limited number of pots, however, this situation cannot be attributed to more than coincidence.

The maintenance of disease-free controls has been a serious problem among those investigating the pathogenicity of Actinomyces. Leach et al (37) and Goss (23) among others have experiences contamination of check pots, and DeBruyn (6) reports that it is impossible to grow disease-free checks in the greenhouse. KenKnight (33), using the same greenhouse as the writer - but a technique somewhat different in the manner of watering - also obtained check tubers that were scabbed.

On the other hand Decker (13) reports that in his work "tubers from 300 control pots placed at random among the pots containing the scab inoculum were absolutely scab-free". From personal conversation with Decker and an inspection of his greenhouse layout, the one difference from the writer's technique which appeared of greatest consequences was that Decker's greenhouse was situated in a location well removed from a dust-laden atmosphere, whereas the writer's greenhouse was directly adjacent to a frequently travelled earth road.

The belief that failure to secure disease-free checks was mainly the result of location may be further borne out by the fact that KenKnight obtained much cleaner checks when he placed his pots in the middle of a field somewhat sheltered by trees and grass than when they were placed in the roadside greenhouse.

Results obtained through the hydroponic culture technique were considerably more gratifying from the standpoint of the development of scab-free checks. However, since these hydroponic cultures were conducted primarily to test the efficacy of the technique, and since too few replicates were run, no significance can be attached

to the readings as measures of the pathogenicity of isolates used.

From the standpoint of technique there are a number of favorable aspects to this method. Considerably less pains need be taken to insure disease-free checks than with the open-pot technique. Since each tuberiferous stolon of a plant is enclosed and made to act as one trial unit, the number of plants necessary to test a collection of Actinomycetal isolates is appreciably reduced. Furthermore, sequential studies on stages of infection are possible since the tubers can be "unearthed" in situ at any time during growth. Inasmuch as three or four or more tubers on the same plant can be made to test that many different isolates, this method provides a means for negating the differences that might exist from plant to plant such as variations in water, temperature, or light relations. In the light of recent studies on accelerator and inhibitor microorganisms as they affect pathogenicity of the primary pathogen, this technique is of especial value in that these accessory organisms are excluded along with other contamination, whereas in the open pot method such contaminants, while not Actinomycetes themselves might, however, be one of such accessory organisms.

In some measure this technique provides the controlled environment for the developing tubers required by Goss (23) when he wrote:

> The development of an experimental technique which will permit the study of the pathogene in the soil immediately surrounding the susceptible plant tissues, as contrasted with the present gross methods, is essential for the determination of the effects of many of these soil factors upon the disease.

SUMMARY

Conflicting reports have attended the use of mercurials as a soil disinfectant for the control of potato scab. A history of these opposing results is given.

In the light of prior investigations under Michigan conditions, which suggest that tolerance or susceptibility to mercurials is a quality of the particular Actinomyces population, a study of the local Actinomycetal flora and its biology has been undertaken. Isolations of Actinomycetes were made from soils and tubers collected from various locations in Michigan. Physiological and morphological studies were made of each isolate by culturing it on some 12 different media. A summarization of the reactions covering the 45 isolates is given in a key to the isolated species. Soil acidity values for those organisms cultured from soil are given together with the names of these organisms.

The problem of which of the isolates were involved in the scabbing of potatoes necessitated tests for the pathogenicity of these isolates. The shortcomings of various techniques to accomplish this purpose have been noted, and three new methods were devised. One was a modification of the open-pot culture as heretofore used in the Department. The second was an attempt to grow tubers under aseptic, or at least Actinomycete-free, conditions. The third method consisted of an assembly whereby potatoes were grown under hydroponic culture, the tuberiferous stolons meantime being enclosed in flasks containing the isolate to be tested.

The open-pot method produced scabby checks and consequently results were difficult or impossible to interpret, although isolates of undoubted pathogens produced consistent results throughout the members of all series. Such was the case with Actinomyces scabies.

The Actinomycete-free culture method produced a fair amount of aerial growth, but tubers remained quite small. None were attacked by scab, since apparently air and moisture relations were unsuited to the growth or infectiousness of Actinomyces.

The results secured from the hydroponic culture method, though in themselves not significant because of limited replicates, indicate that this procedure may be of value in combatting the problems of contaminated checks, contaminating accelerator and inhibitor organisms, and variations from plant to plant, as well as affording a means of studying pathogens in the soil immediately surrounding the susceptible plant tissues.

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