U. S. DEPARTMENT OF AGRICULTURE.
BUREAU OF PLANT INDUSTRY—BULLETIN NO. 171.
B. T. GALLOWAY, *Chief of Bureau.*

SOME FUNGOUS DISEASES OF ECONOMIC IMPORTANCE.

I.—MISCELLANEOUS DISEASES.

BY
FLORA W. PATTERSON, Mycologist,
AND
VERA K. CHARLES, Scientific Assistant.

II.—PINEAPPLE ROT CAUSED BY THIELAVIOPSIS PARADOXA.

BY
FLORA W. PATTERSON, Mycologist,
AND
VERA K. CHARLES AND FRANK J. VEIHMEYER,
Scientific Assistants.

Issued June 23, 1910.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1910.

BUREAU OF PLANT INDUSTRY.

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Assistant Chief of Bureau, G. Harold Powell.
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Pathological Collections and Inspection Work.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., March 8, 1910.

Sir: I have the honor to transmit herewith and to recommend for publication as Bulletin No. 171 of the Bureau series the accompanying manuscript entitled "Some Fungous Diseases of Economic Importance," consisting of two papers, "Miscellaneous Diseases" and "Pineapple Rot Caused by Thielaviopis Paradoxa," submitted with a view to publication by Mrs. Flora W. Patterson, Mycologist in Charge of Pathological Collections and Inspection Work.

The paper entitled "Miscellaneous Diseases" was prepared by Mrs. Flora W. Patterson, Mycologist, and Miss Vera K. Charles, Scientific Assistant. The observations reported in this manuscript relate to several fungous diseases of plants of economic and ornamental value.

The paper entitled "Pineapple Rot Caused by Thielaviopsis Paradoxa" was prepared under the supervision of Mrs. Flora W. Patterson, Mycologist. The fumigation experiments were originated and carried on by Miss Vera K. Charles and Mr. Frank J. Veihmeyer, Scientific Assistants. The purpose of the experiments was to control by fumigation with formaldehyde gas the growth of the fungus, the cause of a serious pineapple disease, so reducing the profits of shippers as to be in a sense prohibitive to the transportation of fruit from some localities. These experiments were undertaken at the request of the Director of the Hawaii Agricultural Experiment Station, and material assistance has been rendered by the Office of Experiment Stations of the United States Department of Agriculture.

Respectfully,

G. H. Powell,
Acting Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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SOME FUNGOUS DISEASES OF ECONOMIC IMPORTANCE.

I.—MISCELLANEOUS DISEASES.

By Flora W. Patterson, *Mycologist*, and Vera K. Charles, *Scientific Assistant*.

DISEASE OF SEDGE CAUSED BY KAWAKAMIA CYPERI.

INTRODUCTION.

For a number of years the Office of Seed and Plant Introduction of the Bureau of Plant Industry has been at work on the problem of the introduction into the Southern States of the Chinese and Japanese matting industries. One of the most important plants concerned in the industry is a species of sedge, *Cyperus tegetiformis* Roxb. It is a native of China, growing in the marshes along the coast. Large shipments of this sedge were secured by a representative of the United States Department of Agriculture who went to Japan for that purpose. These sedges were propagated at the Plant Introduction Garden at Chico, Cal., and were sent from there to Texas, Louisiana, and South Carolina, where they were planted in fields for the purpose of producing matting straw for manufacturers.

In the early spring of 1908 there appeared in the Texas plantation, located at Pierce, a very destructive disease which threatened to destroy the whole plantation. Specimens of this diseased sedge were submitted for examination, and it was found that the disease in question, which had been imported with the plants from Japan, was a new parasitic species recently described in Japanese literature. As soon as the serious character of this disease was known and determined as belonging to the destructive order of Peronosporaceæ, steps were immediately taken by the agent in the field, Mr. F. W. Clarke, to treat the disease according to the experience which has been gained by years of experiment with the grapevine disease *Plasmopara viticola* (B. & C.) Berl. and De Toni.

DESCRIPTION OF THE FUNGUS.

The fungus *Kawakamia cyperi* (Miyabe and Ideta) Miyabe was first tentatively described as *Peronospora* (*?*) *cyperi* M. and I. in Ideta's Lehrbuch der Pflanzenkrankheiten in Japan, page 122, figure 20. Later, Miyabe decided to establish a new genus, Kawakamia. This genus was fully described in the Botanical Magazine of Tokyo, Vol. XVII, No. 202, page 305. The name was given in honor of Mr. T. Kawakami, who succeeded in finding the conidial stage and its method of germination.

The material first received at the Bureau of Plant Industry showed no external evidence of the fungus, but there were slightly depressed brown areas which resembled more a condition caused by malnutrition than by a fungus. Numerous sections were made of the diseased portion of the stem and comparatively little mycelium was found to be present, but a few oöspores being observed led to the suspicion that the fungus might be the cause of the trouble. In more material received at a later date, a few conidia were found, while a third collection showed the fungus still more luxuriantly developed.

In the discussion of this fungus Doctor Miyabe remarks that the species is nearly allied to Phytophthora, but differs from it in the conidia possessing a prominent beak and a peculiar pedicel cell which persists as an appendage. The American specimens differ from Doctor Miyabe's species in the measurements of the conidia, but agree in all other essential respects. In the description by Doctor Miyabe the maximum dimensions of the conidia are given as 68 by 32 μ, while those studied by the writers of this paper are 130 by 30 μ with the beak included, the beak being 15 by 9 μ. It is difficult to get a conidium with the beak attached, as the beak is extremely fugacious, breaking off and leaving the spore with an abrupt, flattened apex. The progress of the disease was accompanied by a characteristic change in the color and turgidity of the diseased plant. In the very early stages the affected culms were bright yellow; as the disease developed they became reddish brown and wilted, changing in the most advanced stages to dull brown and showing a considerable collapse of the cell tissue. The conidia developed only in the later stages and appeared as a white, cobwebby mass on the diseased brown areas.

Kawakamia cyperi appears to be parasitic on *Cyperus tegetiformis* only; at least it has not been reported on any other species of Cyperus. Several sedges have been introduced by the Department of Agriculture, but no other parasite of importance has been observed.

METHOD OF CHECKING THE DISEASE.

This disease of sedge has been checked by careful cultural methods, as shown in the following extract from a letter of July 2, 1909, written by Mr. F. W. Clarke, Special Agent in Charge of Matting-Rush Investigations, United States Department of Agriculture:

I am in receipt of your letter of June 25, inquiring the present status of the sedge disease that appeared at Pierce, Tex., and Jacksonboro, S. C., last year. In answer would say that until June 24 I had the sedge patch under close observation and failed to find anything but a very slight trace of the disease that gave us so much trouble last season. What few diseased stalks I did find were confined to a very small space, and these I cut off and burned. Of course it is quite possible that it will appear in other parts of the field. However, it is my opinion that it will be impossible for it to do any material damage, as in two or three weeks the crop will be ready to harvest. My observations last year led me to the conclusion that the disease spreads most rapidly when the stalks are in a very succulent state. As this stage is passed, the stalks having attained the proper length and having nothing to do but fill out, I do not anticipate any danger from this source. At Jacksonboro, where the plants did not get the careful attention that I gave them in Texas, I find that the disease is very prevalent, at least 50 per cent of the stalks being affected. This, in my opinion, points conclusively to the fact that by burning off the stubble in the winter, thereby leaving the land perfectly bare, and by spraying with Bordeaux mixture just as soon as the disease appears, should it appear, the disease can be very readily controlled and possibly stamped out altogether.

WITCHES'-BROOM OF BAMBOO CAUSED BY A NEWLY DISCOVERED FUNGUS, LOCULISTROMA BAMBUSAE.

In the early autumn of 1908 a case of witches'-broom of a bamboo, *Phyllostachys* sp., was submitted for inspection to the Bureau of Plant Industry by Mr. Frank N. Meyer, one of the agricultural explorers of the United States Department of Agriculture. Mr. Meyer collected the material near Hankow, in central China, but he reported that it has a wide geographical distribution and causes loss in many sections.

GENERAL APPEARANCE OF THE DISEASE.

The general appearance of the disease is that of a witches'-broom formation, although there is no fasciation of the host as results from the attack of certain parasitic fungi, but the internodes are strikingly abbreviated and the diseased branches plumelike. The effect of the disease is more striking than the fungus itself, and is well shown by a comparison of healthy and infected material in Plate I.

HISTOLOGICAL CHANGES IN THE HOST.

The time and point of infection are unknown, but from the histological changes in the host the infection probably begins in the young tender tissue at the terminal node. In its mature stage the fungus

does not always bear a constant relation to its host, as sometimes the leaf enfolds the fungus without the leaf tissue being intermingled, or frequently isolated fibrovascular bundles are seen embedded in the stroma. Apparently this condition is produced by the fungus early attacking the parenchyma of the young leaf and finally appropriating all of the parenchymatous tissue, thus leaving the bundles in a stranded condition. This process was clearly observed in cross sections, which also showed secondary conidia of the fungus in early and successive stages of growth. Numerous sections were made in order to discover if the mycelium ran down in the stem tissue, but no evidence of such a fact could be found.

DESCRIPTION OF THE FUNGUS.

When fully developed the parasite somewhat resembles the sclerotia of *Claviceps purpurea*. These sclerotia-like bodies originate at the nodes, are generally sessile, and probably none exceed a centimeter in length. Their color when young could not be determined, as the specimens were mostly mature, and in that stage are dark green or black. A microscopic examination of the fungous body showed it to consist of a central, hyaline sclerotia-like tissue, in which are many large, round conidial chambers.

Perithecia are developed from the peripheral layer, mostly scattered, seldom more than two near together. The outer surface of the fungus has a more or less velvety appearance, due to the loose, dark-greenish peripheral hyphæ, bearing Cladosporium-like secondary conidia.

DISCUSSION OF RELATED FORMS.

Many difficulties were encountered in establishing the systematic position of this fungus on account of its unusual combination of characters. The writers have thought best to class it with Hypocreaceæ in the section Phragmosporæ.

An exhaustive search was made of the literature available to the United States Department of Agriculture for a fungus on any bamboo having the characters of the one now being described, but none could be found to which this could be referred, and but one species seems to have been reported as producing a comparable effect on its host. This other fungus causing witches'-broom of a bamboo is described by Dr. I. Miyake under the name *Aciculosporium take* in the Botanical Magazine of Tokyo, August, 1908. Doctor Miyake states that the disease occurs in Japan, possibly coming from China, Korea, or India, but that it probably does not occur in Europe, as there is no report concerning its presence there. The article is in Japanese, and when the name Aciculosporium, the only English word, was seen it was thought Doctor Miyake's fungus was located, as that name

was applicable to its primary conidia and the perfect stage had not yet been observed. However, a complete translation clearly demonstrated that there were more points of dissimilarity than resemblance between the two fungi. Aciculosporium differs from the Chinese species in general appearance, absence of secondary conidia, and in the shape, size, and color of the ascospores. Doctor Miyake considers Aciculosporium nearly related to Epichloë and Dusiella, but he states it possesses differences sufficient to establish a new genus. The Chinese bamboo fungus is among the stromatic Hypocreaceæ, most nearly related to Broomella and Peloronectria, but differs from these genera in morphological details and olivaceous spores; hence, the authors feel warranted in establishing a new genus to which the name Loculistroma is given. The generic and specific descriptions follow.

TECHNICAL DESCRIPTION OF THE NEW GENUS LOCULISTROMA.

Stroma upright, cylindrical, fleshy, soft, green or black, with conidial chambers. Perithecia scattered, partly immersed, ostiolate. Asci clavate, cylindrical, 8 spored, without paraphyses. Sporidia fusiform, 3-pluriseptate, olivaceous, biseriate.

TECHNICAL DESCRIPTION OF LOCULISTROMA BAMBUSAE.

Stroma erect, soft, fleshy, generally sessile, averaging 1 centimeter in length and 2 millimeters in diameter. Externally dark green or black. Perithecia two-thirds immersed in stroma, almost spherical, 125 by 100 μ. Asci 8 spored, clavate, cylindrical, 45 to 50 μ by 9 to 10 μ without paraphyses. Sporidia olivaceous, fusiform, 3 to 5 septate, 22 by 4.5 to 5 μ, biseriate. Primary conidia in large chambers, hyaline, filiform, 14 to 16 μ by $\frac{3}{4}$ to 1 μ; basidia 8 by $\frac{1}{2}$ μ. Secondary conidia dark olivaceous, Cladosporium-like, 1 to 3 celled, borne on external olivaceous hyphæ.

Habitat.—Phyllostachys sp.

DISEASES OF TWO ORNAMENTAL PLANTS CAUSED BY SPECIES OF BOTRYTIS.

Two species of Botrytis have been given considerable attention recently, one causing a serious disease of peonies and the other of chrysanthemums.

DISEASE OF PEONIES CAUSED BY BOTRYTIS.

The Botrytis on peonies was described by Oudemans[a] in 1897 as *Botrytis paeoniae*. Massee[b] later referred to it as *Sclerotinia paeoniae*, which appears to be a hypothetical stage and name. For several seasons diseased peonies have been received by the United States Department of Agriculture from various localities, including Canada,

[a] Oudemans, C. A. J. A. Sur une Maladie des Pivoines. Koninklijke Akademie van Wetenschappen te Amsterdam. April, 1897.

[b] Massee, G. The Gardeners' Chronicle. August 13, 1898.

Massachusetts, Rhode Island, Pennsylvania, and Maryland. From all of these localities a disease was reported as causing a serious loss to cultivators. The first collection of diseased plants made by one of the authors was in the autumn of 1907 in Massachusetts. Many plants were found to be in a very bad condition from an unknown cause that apparently could not be the result of improper cultivation. Upon pulling up the plants and cutting open the stems, in the lower portion of the latter were found numerous greenish-black, flattened, elliptical, hard bodies, sclerotia, averaging from 1 to 1½ centimeters in length. The Botrytis stage was not apparent, but in cultures made from a white mycelial layer lining the stems *Botrytis paeoniae* promptly developed, and later sclerotia in great abundance. In fact, a most remarkable phenomenon was the rapidity with which sclerotia were formed, as in four or five days they had attained the size of 1 centimeter.

While spraying with fungicides is to be recommended for the treatment of the disease in its early stages, when the Botrytis stage is apparent, it is not effective after the sclerotia by liberation from diseased plants have infected the soil. At this stage, soil treatment is the only efficient means of controlling the disease. Encouraging results have followed the use of lime when it was added to the soil in the proportion of 500 pounds to the acre. Some authorities do not consider this sufficient. In cases of marked acidity of the soil the quantity of lime could be increased even to 2,000 pounds to the acre.

DISEASE OF CHRYSANTHEMUMS CAUSED BY BOTRYTIS.

In the fall of 1908 some diseased chrysanthemums were received by the Office of Pathological Collections and Inspection Work from New York. The outer ray flowers were deformed and discolored and fungous mycelium was found to be present in the tissues. A second examination less than twenty-four hours after the specimens had been confined in a warm, moist temperature revealed the presence of *Botrytis cinerea* Pers. Cultures were made from the spores, and in this case, as with the peonies, sclerotia developed in from four to five days. Although this Botrytis species is recognized as being an omnivorous form, it was truly parasitic in this instance.

DISEASE OF CYCLAMEN CAUSED BY A VARIETY OF GLOMERELLA RUFOMACULANS.

APPEARANCE OF DISEASED MATERIAL.

Last season several collections of diseased cyclamen material from a greenhouse in Alexandria, Va., were studied. There was no diffused diseased area and the macroscopic appearance of certain spots

on the leaves suggested a physiological trouble, resulting perhaps from injudicious watering or some unfavorable cultural conditions rather than the effect of the presence of a fungus. The spots were circular, slightly water-logged in appearance, and with sharply defined outlines, adjacent cells being normal in color and seemingly rigid.

CULTURAL STUDIES OF THE PARASITE.

A microscopic examination of the diseased material revealed the presence of mycelium, and at the expiration of a week, the leaves meanwhile having been kept in a moist chamber, mature perithecia had developed. These perithecia proved to belong to the genus Glomerella, the ascogenous form of several anthracnoses.[a]

The material was regularly examined and it was found that the spots retained their definite form and a conidial stage did not precede the perfect one. Cultures from the ascospores were made and a Colletotrichum developed. A later collection of leaves showed the Colletotrichum in great abundance, and cultures made from these spores produced the Glomerella stage, thus unquestionably establishing the relationship of the conidial and ascogenous stages of the fungus. In every cultural experiment the perfect stage was developed in from eight to ten days. An anthracnose caused by Colletotrichum attacking cyclamen has been reported by Halsted,[b] but no technical description having been found a discussion of its probable identity with the conidial stage of the fungus here described can not be attempted.

DESCRIPTION OF THE NEW VARIETY.

Glomerella rufomaculans (Berk.) Spaulding and Von Schrenk *cyclaminis* nov. var.

Conidia.—Acervuli amphigenous, brownish, large; conidia oblong or linear obovate, straight or slightly curved, ends rounded, 12 to 15 μ by 4 to 5 μ; basidia long, slender; setæ few, short, rigid.

Perithecia.—Perithecia densely gregarious in definite light-colored round spots, brown, membranaceous, subglobose or distinctly rostrate, ostiolate; asci 8 spored, clavate-cylindric, apex pointed, short stipitate, 50 to 65 μ by 8.5 to 9 μ; spores subbiseriate, oblong to elliptic, 16 to 18 μ by 4 to 4.5 μ.

A NEW SPECIES OF STEMPHYLIUM ON ORANGES.

Specimens of oranges affected by what is locally known as "endrot" in Arizona were received from growers in that Territory in 1907 and again in 1908. The trouble was described as causing considerable loss from the falling of the fruit. Specimens in the early

[a] Shear, C. L., and Wood, Anna K. Ascogenous Forms of Gloeosporium and Colletotrichum. Botanical Gazette, vol. 43, April, 1907, pp. 259–266.

[b] Halsted, B. D. Report, Botanical Department, New Jersey Agricultural College Experiment Station, 1893, p. 399.

stages of the supposed disease were not secured, but in all the fruits examined fungous mycelium was found and this mycelium uniformly developed on the surface of the fruits as a blackish Stemphylium.

CULTURES OF THE FUNGUS.

The fruits after having been placed in 1 part of mercuric chlorid, by weight, to 500 parts of water for fifteen minutes and then thoroughly washed in distilled water were either placed entire in sterilized glassware, or sections of the fruits were removed by flamed scalpel and forceps and dropped in culture flasks. From these the same Stemphylium has always developed, even from sections of inner, apparently healthy tissue. Inoculations from pure cultures always developed satisfactorily on healthy experimental fruits when kept under favorable conditions of moisture and temperature, but it is not thereby claimed that the species to be described is of economic importance or a necessary factor in the so-called " end-rot," although it was always observed associated with this trouble.

DESCRIPTION OF THE FUNGUS STEMPHYLIUM CITRI.

Vegetative mycelium long, hyaline, later becoming dark, 4 μ in diameter, septate; conidiophores short; conidia dark brown, subglobose or oblong, apiculate, irregularly muriform, 20 to 30 μ by 12 to 15 μ, usually in chains of three with isthmus cells short, hyaline.

II.—PINEAPPLE ROT CAUSED BY THIELAVIOPSIS PARADOXA.

By Flora W. Patterson, *Mycologist*, and Vera K. Charles and Frank J. Veihmeyer, *Scientific Assistants*.

INTRODUCTION.

For some years *Thielaviopsis paradoxa* (De Seyn.) V. Höhn. has been recognized as the cause of a serious rot of pineapples. Since the species is not peculiar to this host or geographically restricted, the necessity of some method of controlling the fungus is very urgent. The present investigations of pineapple rot and the methods of checking the disease by using formaldehyde gas were undertaken by the Bureau of Plant Industry at the request of the director of the Hawaii Agricultural Experiment Station. The object of the study was to determine the death point of the fungus in the presence of the gas and from this information ascertain what the practical application might be to the pineapple industry.

SYNONYMY OF THE FUNGUS.

The synonymy of the fungus *Thielaviopsis paradoxa* is somewhat complex. It was described in 1886 by De Seynes[a] as occurring on pineapple and designated *Sporoschisma paradoxum*. In 1892 Saccardo[b] referred it to *Chalara paradoxa* (De Seyn.) Sacc. Went[c] in 1893 described a fungus causing a serious loss to the pineapple growers in Java and named it *Thielaviopsis ethaceticus*. Von Höhnel[d] later, in 1904, called attention to the identity of the two and included both under the name *Thielaviopsis paradoxa*, thus recognizing in the acceptance of the generic name the proper systematic position of the fungus and the rule of priority in the retention of the specific name.

[a] De Seynes. Recherches pour servir à l'histoire naturelle des végétaux inférieurs, III, 1886, pt. 1, p. 30.

[b] Saccardo. Sylloge Fungorum, vol. 10, 1892, p. 595.

[c] Went, F. A. F. C. De Ananasziekte van het Suikerriet. Mededeelingen van het Proefstation voor Suikerriet in West-Java te Kagok-Tegal. 1893.

[d] Von Höhnel. Hedwigia, vol. 43. 1904, p. 295.

DISCUSSION OF THE FUNGUS.

Both in cultures and in the diseased fruit the fungus *Thielaviopsis paradoxa*, studied in the Office of Pathological Collections and Inspection Work, adhered very closely to its characteristic morphology. In addition to the two kinds of spores, microspores and macrospores, a pycnidial stage was observed which appeared irregularly and without any unusual physical conditions, such as lack of moisture or reduction in the amount of media, being apparent.

When the fungus was found on sound fruit in association with insect remains or other foreign matter it exhibited morphological differences. In small particles of such extraneous substances as just mentioned, isolated fungous spores were found which resembled the macrospores of Thielaviopsis, but differed in possessing a more circular outline, denser cell contents, and a thicker wall. In these examinations no microspores were observed. In five days cultures made from the macrospores developed normal, remarkably pure cultures of Thielaviopsis. This phase is not only of interest in relation to the ecology of the fungus, but it has an important significance in the probable value of formaldehyde for pineapple disinfection.

HISTORICAL REVIEW OF FORMALDEHYDE AS A DISINFECTANT AND FUNGICIDE.

The important rôle of formaldehyde as a disinfectant and fungicide has been demonstrated within a comparatively recent date. Among the early workers who recognized its value were Guether,[a] Neger,[b] Tubeuf,[c] Kinzel,[d] and others. In 1898 Neger experimented with spores of *Ustilago hordei*, using formalin pastils to generate the gas. The treatment was found to be effective in its fungicidal action, and the degree to which it retarded germination was not serious. Kinzel's work was concerned with the effect of formalin on the germination of certain grains and legumes and its value in the treatment of smuts.

In 1906 McClintic,[e] of the United States Public Health and Marine-Hospital Service, published the results of his work on formaldehyde

[a] Guether, Th. Ueber die Einwirkung von Formaldehydlosungen auf Getreidebrand. Berichte der Pharmaceutischen Gesellschaft, vol. 5, no. 12, 1895. pp. 325–330.

[b] Neger, F. W. Ueber Desinfektion von Saatgut mittels Formaldehyd-Dämpfe. Praktische Blätter für Pflanzenbau und Pflanzenschutz, vol. 1, no. 11, November, 1898, pp. 84–85.

[c] Tubeuf, Carl von. Scherings Formalingas-Methode. Praktische Blätter für Pflanzenbau und Pflanzenschutz, vol. 1, no. 11, November, 1898, pp. 85–86.

[d] Kinzel, W. Ueber die Einwirkung des Formaldehyds auf die Keimkraft. Die Landwirthschaftlichen Versuchsstationen, vol. 49, 1898, pp. 461–466.

[e] McClintic, T. B. The Limitations of Formaldehyde Gas as a Disinfectant. Bulletin 27, Hygienic Laboratory. April 9, 1906.

gas as a disinfectant. The object of his investigation was to determine the effect of the gas on pathogenic bacteria. An exhaustive study was made of the subject and included delicate tests of the efficacy of the several means of generating the gas. The results of McClintic's work indicate the superiority of the formalin-permanganate method of generating the gas; therefore this method was used in the present experiments. In this method, formalin, a solution of formaldehyde in water, is poured on crystals of potassium permanganate; a vigorous reaction takes place, with sufficient heat to liberate a large quantity of formaldehyde gas, water vapor, carbon dioxid, and a small quantity of formic acid. The potassium permanganate is decomposed by a part of the formalin, and the heat of this reaction serves to liberate formaldehyde gas from the remaining formalin.

The first American pathologist to employ the gas in the control of fungous diseases was Jones,[a] of Vermont. His experiments were confined to the treatment for potato scab, and pastils were used to generate the gas. In subsequent work conducted by Jones and Morse,[b] the formalin-permanganate method was employed for generating the gas. Still later reports on the same line of work, the prevention of potato scab, were made by Morse[c] at the Maine Agricultural Experiment Station.

All of these experiments demonstrated the efficacy of formaldehyde gas as a disinfectant, but as potatoes and not cultures of the fungus were dealt with, the exact effect on the latter is still an unsolved problem.

J. E. Higgins,[d] of the Hawaii Agricultural Experiment Station, conducted preliminary experiments with formaldehyde gas as a means of checking pineapple rot. These were made in connection with investigations to determine the most efficient means of handling and shipping the Hawaiian fruit. Although the experiments were on a small scale they indicated the advantage of this means as a prophylactic measure.

APPARATUS USED IN THE EXPERIMENTS WITH THE FUNGUS.

In the experiments with the fungus *Thielaviopsis paradoxa*, conducted by this office, it was necessary to provide an especially con-

[a] Jones, L. R. Potato Diseases and Their Remedies. Thirteenth Annual Report, Vermont Agricultural Experiment Station, 1899–1900, pp. 268–281.

[b] Jones, L. R., and Morse, W. J. Potato Diseases and Their Remedies. Eighteenth Annual Report, Vermont Agricultural Experiment Station, 1904–5, pp. 272–291.

[c] Morse, W. J. Prevention of Potato Scab. Bulletins 141 and 149, Maine Agricultural Experiment Station. 1907.

[d] Higgins, J. E. Marketing Hawaiian Fruits. Bulletin 4, Hawaii Agricultural Experiment Station. 1907.

structed fumigating box which would be air-tight and provided with some means to allow of the ready removal of material from the chamber at different times during the process of fumigation, and at the same time to prevent the escape of an appreciable amount of the formaldehyde gas. To insure an absolutely air-tight compartment the box was constructed of an inner and outer casing with layers of building paper between the two. A large glass door swung on heavy hinges was fitted to the front of the box. The sectional view shows the construction of the door, which is similar to those of large refrigerators (see fig. 1). Double glass was provided and the edge of the door and the face of the casing were beveled, thus making larger bearing surfaces, which were covered with felt cloth. The salient feature of the box is the arrangement of the drawers for the removal of the fumigated material (see fig. 2). These were built in the side of the box and were supported in a large frame, the arrangement being such that when the drawers were closed and fastened, by means of the catches at the sides, the gas was prevented from escaping. When it was desired to remove the material in any particular drawer, that drawer was pulled out and the cultures removed, the back of the drawer coming in contact with the surface of the supporting frame closing the opening. The sides of the drawers were perforated and the bottom made up of cross strips. This arrangement permitted a free circulation of the gas through the drawers. The quantity of gas escaping when the drawers were pulled out during an experiment was negligible. An opening was arranged in the rear of the box and connected by a suitable casing to a laboratory hood, by means of which the gaseous contents of the box were exhausted after an experiment. This fumigating box had an air space of 50 cubic feet.

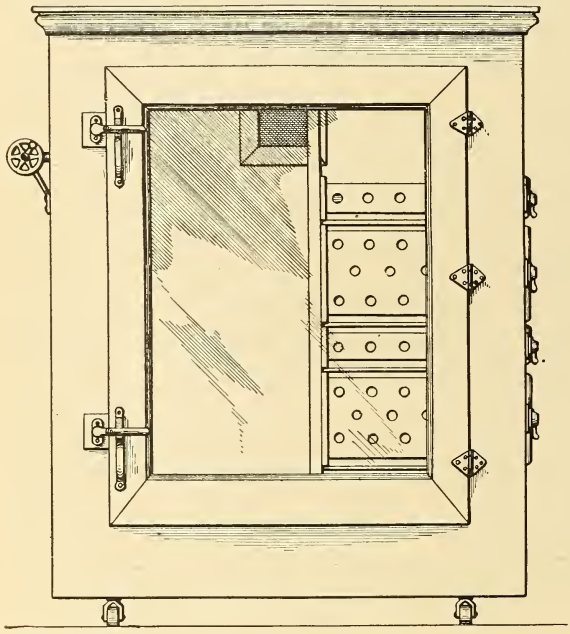


FIG. 1.—Sketch showing the front elevation of the fumigating box used in the experiments and the construction of the door.

To determine the increase in the quantity of moisture mixed with the air due to the generation of the different quantities of the gas, a psychrometer was fitted inside the box. This instrument was of the pattern used by the Weather Bureau. The handle was removed and the frame fastened to a hollow spindle which extended through the side of the box. The spindle was attached to the shaft of a centrifuge in order to whirl the psychrometer inside the box (see fig. 3). By means of the hollow spindle and a small rubber tubing leading to the wet bulb, just the sufficient quantity of moisture could be applied to it. The thermometers could be read through the glass door.

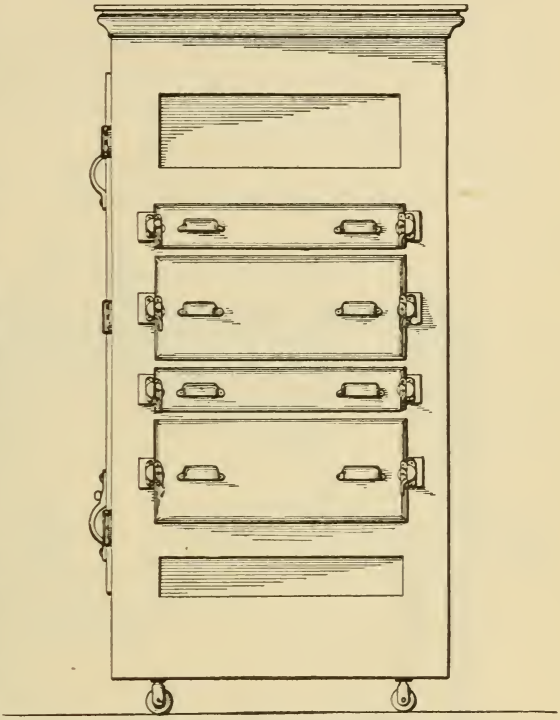


FIG. 2.—Sketch showing the side view of the fumigating box and the arrangement of the drawers.

The gas generator consisted of a beaker 8 inches deep and about 4 inches in diameter. This was placed in a large galvanized-iron pail in order to catch any overflow. In the experiments the generator was placed on the floor of the box, in the center, with the potassium permanganate crystals in it, the formalin poured upon them, and the door closed. There was sufficient time to employ this means of mixing the reagents as the reaction did not begin immediately.

CONTROL CONDITIONS AND EXPLANATION OF ARBITRARY UNITS.

It is thought that owing to the simplicity and efficacy of the formalin-permanganate method of evolving the formaldehyde gas this method will be the one which will be adopted in practical work.

No determination of the quantities of formaldehyde in the atmosphere of the box during the experiments was made. The data, given in terms of the cubic centimeters of formalin of known strength used per 1,000 cubic feet of air space, will therefore be as valuable as if given in terms of the quantity of formaldehyde per cubic foot.

This unit has been used throughout the experiments. McClintic[a] found that the percentage yield of formaldehyde is not much affected by variations in temperature when the experiments are performed at temperatures above 60° to 65° F. He also proved that successful disinfection varies directly with the humidity. The fumigating box was placed in a room which was provided with an automatic temperature and ventilating apparatus, thus permitting a perfect control of atmospheric conditions. The humidity in the room was found to be fairly constant during all the experiments, the average relative humidity being 38 per cent.

The formalin used was purchased in the open market and was supposed to be of 40 per cent strength, but on analysis it was found to

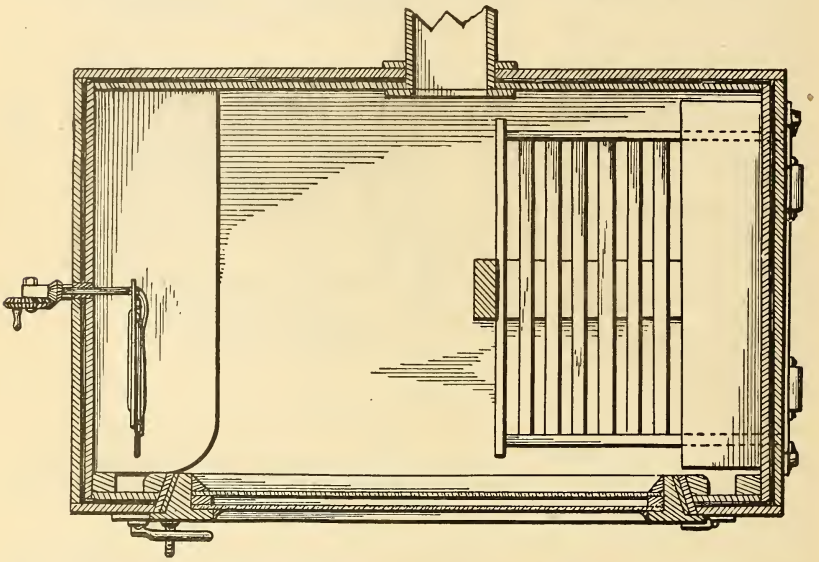


FIG. 3.—Sketch showing a sectional view of the interior of the fumigating box with the psychrometer attached.

contain 37.97 per cent of formaldehyde by weight, with a specific gravity of 1.0669 at 20° C. Chemically pure potassium permanganate in finely divided crystals was employed. In each of the experiments the formalin used was in the proportion of 100 c. c. to 50 grams of permanganate.

Preliminary experiments were made with cultures of the fungus in petri dishes, Van Tieghem cells, and test tubes, with quantities of formalin ranging from 300 to 1,200 c. c. per 1,000 cubic feet of space, to determine the approximate quantity of the gas fatal to Thielaviopsis. It was soon demonstrated that quantities of formalin less than 700 c. c. per 1,000 cubic feet were ineffectual in prohibiting the

[a] McClintic. Op. cit.

development of the fungus, therefore the critical and final work was concerned with quantities varying from 750 to 1,300 c. c. per 1,000 cubic feet of air space.

CULTURAL WORK ON THE FUNGUS.

For the cultural phase of the work the method was to isolate the fungus and grow pure cultures which were subjected to the direct action of the formaldehyde gas. Corn-meal agar proved a very satisfactory medium and was used throughout the experiments, during which over 1,500 cultures were made. Those intended for fumigation were smear cultures made in petri dishes. These dishes were well adapted to the purpose, as their shape permitted an equal distribution of the gas over their surface. Checks were made from the cultures immediately before they were subjected to the treatment, and transfers directly after.

Some authorities have contended that a dormant period precedes the germination of the macrospores, but in the present investigations no such condition was observed. Immediate germination was proved by transferring a definite number of macrospores from vigorously growing cultures to petri dishes containing 8 to 10 c. c. of media or by making drop cultures and noting the germination of the individual spores. Whether this is a cultural peculiarity due to favorable media or some other congenial condition can not be stated, as there was no opportunity for field observations on the behavior of the fungus or character of the infections under natural conditions.

EXPERIMENTS WITH PETRI-DISH CULTURES.

In each of the experiments in petri dishes many cultures were submitted to the action of the formaldehyde gas for different lengths of time, and a corresponding number of transfers were made after fumigation. In giving a record of the results of the effect of the gas, the initial or macroscopically visible and vigorous growths noted were in each case those of the first transfer showing any indication of growth. The data presented in the following tables show the minimum fungicidal effect of the gas, not the average period of retardation or the average number of cultures which grew. The period of retardation was estimated as the difference between the time of the initial growths of the checks and the fumigated material. The time of exposure is taken from the time the reaction commenced.

Experiment No. 1.

800 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 72° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	26	50
Fungus exposed 15 minutes	49	73
Fungus exposed 30 minutes	89	115
Fungus exposed 60 minutes	117	165

The relative retardation in the growth of the checks and the 15, 30, and 60 minute transfers one hundred and forty hours after fumigation is shown in Plate II.

Experiment No. 2.

850 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 72° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	25	50
Fungus exposed 15 minutes	65	113
Fungus exposed 30 minutes	92	143
Fungus exposed 60 minutes	144	61

Experiment No. 3.

900 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 72° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	24	49
Fungus exposed 15 minutes	96	134
Fungus exposed 30 minutes	124	168
Fungus exposed 60 minutes	168	189

Plate III shows the relative retardation in the growth of the cultures due to the use of the gas.

Experiment No. 4.

950 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 72° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	22	49
Fungus exposed 15 minutes	116	210
Fungus exposed 30 minutes	0	0
Fungus exposed 60 minutes	0	0

Experiment No. 5.

950 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 78° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	22	49
Fungus exposed 15 minutes	67	115
Fungus exposed 30 minutes	140	209
Fungus exposed 60 minutes	0	0

Experiment No. 6.

1,000 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 74° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	21	45
Fungus exposed 15 minutes	144	186
Fungus exposed 30 minutes	144	186
Fungus exposed 60 minutes	233	257

Experiment No. 7.

1,000 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 78° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	20	48
Fungus exposed 15 minutes	0	0
Fungus exposed 30 minutes	163	211
Fungus exposed 60 minutes	0	0

Experiment No. 8.

1,050 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 65° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	21	49
Fungus exposed 15 minutes	118	221
Fungus exposed 30 minutes	0	0
Fungus exposed 60 minutes	0	0

Experiment No. 9.

1,050 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 75° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	23	52
Fungus exposed 15 minutes	0	0
Fungus exposed 30 minutes	0	0
Fungus exposed 60 minutes	190	286

Experiment No. 10.

1,100 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 66° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	18	48
Fungus exposed 15 minutes	216	279
Fungus exposed 30 minutes	0	0
Fungus exposed 60 minutes	0	0

Experiment No. 11.

1,100 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 75° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	23	51
Fungus exposed 15 minutes	0	0
Fungus exposed 30 minutes	0	0
Fungus exposed 60 minutes	0	0

Experiment No. 12.

1,100 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 78° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	23	50
Fungus exposed 15 minutes	97	116
Fungus exposed 30 minutes	162	260
Fungus exposed 60 minutes	0	0

Experiment No. 13.

1,150 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 75° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	20	49
Fungus exposed 15 minutes	216	264
Fungus exposed 30 minutes	240	257
Fungus exposed 60 minutes	0	0

Experiment No. 14.

1,150 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 66° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	29	64
Fungus exposed 15 minutes	72	100
Fungus exposed 30 minutes	240	264
Fungus exposed 60 minutes	0	0

Experiment No. 15.

1,150 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 76° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	23	50
Fungus exposed 15 minutes	0	0
Fungus exposed 30 minutes	0	0
Fungus exposed 60 minutes	0	0

Experiment No. 16.

1,200 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 70° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	19	67
Fungus exposed 15 minutes	0	0
Fungus exposed 30 minutes	0	0
Fungus exposed 60 minutes	0	0

Experiment No. 17.

1,200 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 78° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	29	53
Fungus exposed 15 minutes	0	0
Fungus exposed 30 minutes	0	0
Fungus exposed 60 minutes	0	0

Experiment No. 18.

1,200 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 78° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	23	50
Fungus exposed 15 minutes	0	0
Fungus exposed 30 minutes	0	0
Fungus exposed 60 minutes	0	0

Several duplicate experiments were made with this quantity of gas and the growth was negative in every case.

Experiment No. 19.

1,250 c. c. formalin per 1,000 cubic feet or air space.
Temperature before mixing reagents, 76° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	23	50
Fungus exposed 15 minutes	0	0
Fungus exposed 30 minutes	0	0
Fungus exposed 60 minutes	0	0

Experiment No. 20.

1,250 c. c. formalin per 1,000 cubic feet or air space.
Temperature before mixing reagents, 80° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	24	50
Fungus exposed 15 minutes	0	0
Fungus exposed 30 minutes	0	0
Fungus exposed 60 minutes	0	0

Experiment No. 21.

1,300 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 76° F.

Thielaviopsis cultures in petri dishes.	Initial growth of fungus.	Vigorous growth of fungus.
	Hours.	*Hours.*
Checks; fungus not exposed to formaldehyde gas	22	46
Fungus exposed 15 minutes	0	0
Fungus exposed 30 minutes	0	0
Fungus exposed 60 minutes	0	0

TABLE I.—*Results of experiments with petri-dish cultures of Thielaviopsis.*

No. of experiment.	Formalin per 1,000 cubic feet.	Temperature before mixing reagents.	Retardation* of initial growth of the fungus exposed to formaldehyde gas for 15, 30, and 60 minutes, respectively.		
	c. c.	°F.	*Hours.*	*Hours.*	*Hours.*
1	800	72	23	63	91
2	850	72	40	67	119
3	900	72	72	100	144
4	950	72	94	0	0
5	950	78	45	118	0
6	1,000	74	123	123	212
7	1,000	78	0	143	0
8	1,050	65	97	0	0
9	1,050	75	0	0	167
10	1,100	66	198	0	0
11	1,100	75	0	0	0
12	1,100	78	74	139	0
13	1,150	75	196	220	0
14	1,150	66	43	211	0
15	1,150	76	0	0	0
16	1,200	70	0	0	0
17	1,200	78	9	0	0
18	1,200	78	0	0	0
19	1,250	76	0	0	0
20	1,250	80	3	0	0
21	1,300	76	0	3	0

* As previously stated, the retardation periods are reckoned from the first indication of growth in any single culture of any set of checks.

The results of the experiments with petri-dish cultures of Thielaviopsis show that formaldehyde gas is effective in retarding the growth of the fungus. The irregularities in the results obtained from fifteen-minute exposures suggest that exposures of less than thirty minutes are useless. There was no experimental evidence to show the effect of variation in temperature and humidity on the fungicidal property of the gas, but it is believed [a] that a high temperature and humidity increase its efficiency. Although the amount of moisture in the air of the room before starting any experiment was nearly uniform, the temperature was varied as shown in the tabulations, thus insuring comparable conditions with those encountered in actual practice, where temperature will necessarily be a variable factor.

[a] McClintic. Op. cit., p. 71.

VITALITY OF MICROSPORES AND MACROSPORES.

A series of experiments was made to determine the relative vitality of the microspores and macrospores of Thielaviopsis. The method was to simultaneously expose the two different kinds of spores suspended in distilled water to the action of the formaldehyde gas in various quantities and different time limits. At the conclusion of each experiment transfers were made to fresh culture media. Negative results as to growth were obtained in all the experiments with quantities of formalin exceeding 1,000 c. c. per 1,000 cubic feet of air space. Referring to the following tables it will be observed that the macrospores survived the fifteen and thirty minute treatment, while the microspores produced no growth.

Experiment No. 22.

750 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 68° F.

Thielaviopsis spores suspended in water.	Initial growth of fungus.	
	Microspores.	Macrospores.
	Hours.	*Hours.*
Check; fungus not exposed to formaldehyde gas	22	22
Fungus exposed 15 minutes	0	96
Fungus exposed 30 minutes	0	120
Fungus exposed 60 minutes	0	0

Experiment No. 23.

850 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 68° F.

Thielaviopsis spores suspended in water.	Initial growth of fungus.	
	Microspores.	Macrospores.
	Hours.	*Hours.*
Check; fungus not exposed to formaldehyde gas	22	22
Fungus exposed 15 minutes	0	190
Fungus exposed 30 minutes	0	200
Fungus exposed 60 minutes	0	0

Experiment No. 24.

950 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 78° F.

Thielaviopsis spores suspended in water.	Initial growth of fungus.	
	Microspores.	Macrospores.
	Hours.	*Hours.*
Check; fungus not exposed to formaldehyde gas	22	22
Fungus exposed 15 minutes	0	0
Fungus exposed 30 minutes	0	113
Fungus exposed 60 minutes	0	0

Experiment No. 25.

1,000 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 80° F.

Thielaviopsis spores suspended in water.	Initial growth of fungus.	
	Microspores.	Macrospores.
	Hours.	*Hours.*
Check; fungus not exposed to formaldehyde gas	23	23
Fungus exposed 15 minutes	0	0
Fungus exposed 30 minutes	0	88
Fungus exposed 60 minutes	0	0

Experiment No. 26.

1,050 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 80° F.

Thielaviopsis spores suspended in water.	Initial growth of fungus.	
	Microspores.	Macrospores.
	Hours.	*Hours.*
Check; fungus not exposed to formaldehyde gas	22	22
Fungus exposed 15 minutes	0	0
Fungus exposed 30 minutes	0	0
Fungus exposed 60 minutes	0	0

Experiment No. 27.

1,100 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 78° F.

Thielaviopsis spores suspended in water.	Initial growth of fungus.	
	Microspores.	Macrospores.
	Hours.	*Hours.*
Check; fungus not exposed to formaldehyde gas	23	23
Fungus exposed 15 minutes	0	0
Fungus exposed 30 minutes	0	0
Fungus exposed 60 minutes	0	0

Experiment No. 28.

1,150 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 66° F.

Thielaviopsis spores suspended in water.	Initial growth of fungus.	
	Microspores.	Macrospores.
	Hours.	*Hours.*
Check; fungus not exposed to formaldehyde gas	29	29
Fungus exposed 15 minutes	0	0
Fungus exposed 30 minutes	0	0
Fungus exposed 60 minutes	0	0

Experiment No. 29.

1,200 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 70° F.

Thielaviopsis spores suspended in water.	Initial growth of fungus.	
	Micro-spores.	Macro-spores.
	Hours.	*Hours.*
Check; fungus not exposed to formaldehyde gas	29	29
Fungus exposed 15 minutes	0	0
Fungus exposed 30 minutes	0	0
Fungus exposed 60 minutes	0	0

During the process of these experiments special attention was given to the microscopic appearance of the spores before and after fumigation. No remarkable change was observed in the color, size, or turgidity of the spores even when subjected to the gas generated from formalin in quantities as high as 2,600 c. c. per 1,000 cubic feet of air space.

INOCULATION EXPERIMENTS.

To determine what value fumigation may have in lessening or preventing the loss of pineapples by Thielaviopsis, it is necessary to consider the time and center of infection. Authorities differ upon this subject, some contending that there is an incipient infection before the fruit is pulled, others that infection takes place at the exposed cut portion of the stem or at points of mechanical injury. The last two possibilities presuppose the fact that the spores being abundant on the plant in many instances find a resting place on the fruit. Cultures of the fungus obtained from sound market fruit with superficially adhering spores indicate a strong probability of this condition. In order to determine what effect the formaldehyde gas might exert on spores superficially present on the fruit or in various stages of development, the following experiments were made. Pineapples were inoculated by pure cultures of the fungus being introduced into six separate incisions. The fruit was then kept in a warm, damp, and dark atmosphere for forty-eight hours, at the expiration of which it was subjected to fumigation. Just previous to the treatment a few spores were taken from the inoculated points and cultures started as checks for the transfers made from inoculated points after fumigation.

Experiment No. 30.

1.200 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 76° F.

Thielaviopsis on the fruit.	Initial growth of fungus.	Vigorous growth of fungus.	Per cent of fungus which grew.
	Hours.	*Hours.*	
Checks; transfers of the fungus before fumigation	26	42	100
Transfers of the fungus after 60 minutes of fumigation	40	131	60

After ninety-six hours the fungus was well developed from all centers of inoculation in nonfumigated fruit, while no growth of the fungus was apparent in the fumigated fruit. At the expiration of one hundred and twenty hours the nonfumigated material was badly decayed, although the fumigated fruit was still firm and in marketable condition.

Plate IV shows a typical set of fruit pertaining to the preceding experiment. *A*, *B*, and *C* represent the check, inoculated and fumigated, and inoculated and nonfumigated fruit, respectively, taken seventy-two hours after fumigation. *C* shows three separate centers of infection, while *B* similarly inoculated but fumigated has resisted the disease. The condition of *C* is still more strikingly represented in Plate VIII, figure 1.

Experiment No. 31.

1.250 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 76° F.

Thielaviopsis on the fruit.	Initial growth of fungus.	Vigorous growth of fungus.	Per cent of fungus which grew.
	Hours.	*Hours.*	
Checks; transfers of the fungus before fumigation	43	80	100.00
Transfers of the fungus after 60 minutes of fumigation	180	200	16.66

The nonfumigated fruit was badly decayed after one hundred and twenty hours. The fumigated fruit remained firm and in marketable condition.

Plate V is a reproduction of a photograph taken of cultures from the fruit before and after fumigation with 1,250 c. c. of formalin per 1,000 cubic feet of air space. The difference between the checks and transfers shows very strikingly the effect of the formaldehyde gas on the fungus.

Experiment No. 32.

1,250 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 80° F.

Thielaviopsis on the fruit.	Initial growth of fungus.	Vigorous growth of fungus.	Per cent of fungus which grew.
	Hours.	*Hours.*	
Checks; transfers of the fungus before fumigation	48	96	100
Transfers of the fungus after 60 minutes of fumigation	0	0	0

The nonfumigated fruit was badly decayed after one hundred and twenty hours. The fumigated fruit remained firm and in marketable condition.

Experiment No. 33.

1,300 c. c. formalin per 1,000 cubic feet of air space.
Temperature before mixing reagents, 78° F.

Thielaviopsis on the fruit.	Initial growth of fungus.	Vigorous growth of fungus.	Per cent of fungus which grew.
	Hours.	*Hours.*	
Checks; transfers of the fungus before fumigation	36	50	100
Transfers of the fungus after 60 minutes of fumigation	0	0	0

The nonfumigated fruit was badly decayed after one hundred and twenty hours. The fumigated fruit remained firm and unaffected.

Plate VI is a reproduction of a photograph of cultures from the fruit before and after fumigation, taken one hundred and twenty hours after fumigation.

Assuming that spores of Thielaviopsis may be present on the fruit after it is pulled, an attempt was made to reproduce such a condition. An abundant opportunity for infection was given by inserting or spraying the fungous spores directly on the fruit. After this treatment the pineapples were kept under conditions favorable to the development of the fungus and as nearly approximating natural conditions as possible. It will be observed from a comparison of the tables that on the fruit inoculated by the incision method and submitted to the gas generated from formalin in quantities less than 1,300 c. c. per 1,000 cubic feet there was some development of the fungus, while growth did not occur after subjection to the gas generated from 1,300 c. c. of formalin per 1,000 cubic feet of air space.

EXPERIMENTS WITH FRUIT SPRAYED WITH SPORES OF THIELAVIOPSIS.

Experiments corresponding with the inoculation work in number and in the quantities of formaldehyde gas used were made with fruit sprayed with spores of Thielaviopsis. After the fruit was sprayed it was kept in a warm, damp, and dark atmosphere for forty-eight hours previous to fumigation. The fungus did not develop on the fumigated fruit in a single case, though the nonfumigated fruit became diseased in every instance.

Plate VII shows what the effect of fumigation would be in preventing the infection of fruit by superficial spores. The photograph was taken one hundred and twenty hours after the fruit was sprayed with spores, or seventy-two hours after it was fumigated with 1,200 c. c. of formalin per 1,000 cubic feet of air space. The differences between check, fumigated, and nonfumigated fruits are very apparent. The development of the fungus in a sprayed and nonfumigated fruit is more strikingly shown in Plate VIII, figure 2.

EFFECT OF FUMIGATION ON THE FRUIT.

The fruit used in these experiments was obtained from different sources and comprised several varieties. Observations of the varietal peculiarities in the presence of the formaldehyde gas were not sufficiently extensive to be included in this paper.

An effort was made to determine the effect of fumigation on the fruit. Immature and mature pineapples were subjected in each experiment, after the completion of which they were kept indefinitely to observe any change which might take place in their appearance. It was found that the use of large quantities of formalin, as 2,600 c. c. per 1,000 cubic feet of air space, produced no immediate alteration. In quantities from 1,000 c. c. to 1,300 c. c. the fruit suffered a slight change in color, becoming somewhat brown. Besides this change in color there was a slight shrinkage and a loss of turgidity. None of these effects were remarkably conspicuous, and it seems probable that they may be controlled. There was no opportunity for observing the effect of fumigation on freshly pulled fruit.

In the experiments a small amount of moisture condensed on the fruit during the fumigating process. Quantitative determinations were not made of this amount, but it was so small that it could not be determined by means of delicate torsion balances by finding the difference in the weight of the fruit before fumigation and after subjection to formaldehyde gas. The moisture, together with the odor of formalin, which was very marked on the fruit immediately after it was taken from the fumigating box, quickly disappeared. The following table gives the increase in moisture in the atmosphere due to the liberation of the different quantities of the gas.

TABLE II.—*Results of experiments with different quantities of formalin per 1,000 cubic feet of air space for 15 and 30 minutes, respectively, showing the increase in the relative humidity of the atmosphere and the maximum percentage of increase in the grains of moisture per cubic foot.*

Formalin per 1,000 cubic feet.	Temperature before mixing reagents.	Relative humidity before experiment.	Relative humidity after 15 minutes.	Relative humidity after 30 minutes.	Increase in relative humidity.		Increase in grains of moisture per cubic foot.		Increase in grains of moisture per cubic foot.
					After 15 minutes.	After 30 minutes.	After 15 minutes.	After 30 minutes.	
c. c.	*° F.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*			*Per cent.*
900	76	38	51	50	13	12	1.412	1.236	38.50
1,000	78	38	53	50	15	12	1.714	1.395	43.88
1,100	76	39	53	51	15	12	1.857	1.559	49.30
1,200	76	39	56	54	17	15	1.961	1.615	52.88
1,300	76	37	54.5	51	17.5	14	2.117	1.762	57.02

CONCLUSIONS.

It is demonstrated by the experimental evidence presented in this paper that formaldehyde gas, even in small quantities, exerts a retarding action on the initial growth of *Thielaviopsis paradoxa*, while with certain quantities it is fatal in its fungicidal effect. It will be observed that in exposures to small quantities of the gas the results were not always identical in like experiments and did not exhibit a uniformity of gradation in periods of growth, although conditions, temperature excepted, were duplicated. From constant and uninterrupted observation of the fungus under different degrees of temperature in gaseous and nongaseous atmospheres it is not believed that these irregularities are due to differences in the temperature at which the experiments were performed, but may be attributed to a variation of the organism itself. Though the degree of retardation varies in like experiments, as just stated, it is constant in being proportional to the length of exposure in each set, 15, 30, and 60 minutes, respectively.

The quantity of gas fatal to *Thielaviopsis paradoxa* was obtained by the use of 1,200 c. c. or more of formalin per 1,000 cubic feet of air space. The proportions were used under varying degrees of temperature, ranging from 65° to 80° F., but with fairly constant humidity, the average being 38 per cent. The determinations were made by the use of an air-tight compartment in which all the gas was available and the fungus readily accessible to its fumes. Exposures of 30 minutes or more were found to be effective. The quantities of formalin ranging from 1,200 to 1,300 c. c. are the maxima which would be employed in the commercial use of the gas, since they were determined under most drastic conditions of temperature and humidity. The temperature in pineapple-growing countries would normally be above that under which some of the

experiments were performed, and the humidity would be considerably higher than 38 per cent.

The macrospores exhibited much greater resistance to the action of the gas than the microspores, and a dormant period did not precede their germination.

The degree of moisture present on the fruit at the conclusion of the fumigating process was not excessive, and although the odor of formaldehyde was quite apparent it was soon dissipated. The gas produced no immediate change in the consistency of the fruit, but a slight change was noted at the termination of four days. Whether this was an after effect of the gas is difficult to determine without a large quantity of fruit for comparison. The fruit did undergo an alteration in color, but it was not striking, and whether the gas would produce a similar effect in freshly picked fruit is yet to be determined.

The effect of the gas on cultures of the fungus and the fungus present on the fruit sprayed with spores or with spores introduced into cavities or the tissue was found to be constant. It is not claimed that the development of the fungus can be arrested if it has penetrated very far into the tissues of the fruit, but the fungus was killed when inserted in the fruit to a depth of one-half an inch. The development of the fungus on the fruit sprayed with spores and that inoculated by incisions was as great, if not greater, than it would be when the fruit is brought to the packing houses, since the danger is largely due to superficially adherent spores. Although unsubstantiated by field and packing-house observations, it is fair to assume that formaldehyde gas is an effective agent in controlling pineapple rot.

SUMMARY.

Formaldehyde gas is an efficient means of controlling *Thielaviopsis paradoxa* (De Seyn.) V. Höhn.

The macrospores exhibited a much greater resistance to the action of the gas than the microspores.

There was no marked anatomical change in the appearance of the fungus after fumigation, and it is believed that the effect is of a chemical rather than of a physical nature.

Even small quantities of the gas exert a retarding action upon the initial growth of the fungus.

The quantity of gas fatal to the fungus is generated from formalin in quantities ranging from 1,200 to 1,300 c. c. per 1,000 cubic feet of air space under physical conditions comparable to those used in the experiments described in this paper.

PLATES.

DESCRIPTION OF PLATES.

PLATE I. Normal and abnormal growths of bamboo: *A*, normal; *B*, abnormal. A striking example of a witches'-broom formation. These were not collected at the same time, but are presumably the same species.

PLATE II. The effect of formaldehyde gas on cultures of Thielaviopsis resulting from the use of 800 c. c. of formalin per 1,000 cubic feet of air space. *A*, Check; *B*, *C*, and *D*, transfers from cultures subjected for 15, 30, and 60 minutes, respectively, showing the gradation in the growth one hundred and ninety hours after fumigation.

PLATE III. The effect of formaldehyde gas on cultures of Thielaviopsis resulting from the use of 900 c. c. of formalin per 1,000 cubic feet of air space. *A*, Check; *B*, *C*, and *D*, transfers from cultures subjected for 15, 30, and 60 minutes, respectively, showing the gradation in the growth one hundred and ninety hours after fumigation.

PLATE IV. Fumigated and nonfumigated pineapples. *A*, Check; *B*, fruit inoculated and fumigated, and *C*, fruit inoculated and nonfumigated. The fruit was inoculated by deep incisions. Formalin was used in the proportion of 1,200 c. c. per 1,000 cubic feet of air space. Photograph was taken seventy-two hours after fumigation.

PLATE V. Cultures from inoculated pineapples before and after the fumigation of the fruit. *A* and *B*, Transfers before fumigation; *C* and *D*, transfers after fumigation. The photograph was taken one hundred and twenty-eight hours after fumigation with 1,250 c. c. of formalin per 1,000 cubic feet of air space, showing the negative growth in the transfers from fumigated fruit.

PLATE VI. Cultures from inoculated pineapples before and after the fumigation of the fruit. *A* and *B*, Transfers before fumigation; *C* and *D*, transfers after fumigation. Photograph was taken one hundred and twenty-eight hours after fumigation with 1,300 c. c. of formalin per 1,000 cubic feet of air space, showing the negative growth in the transfers from fumigated fruit.

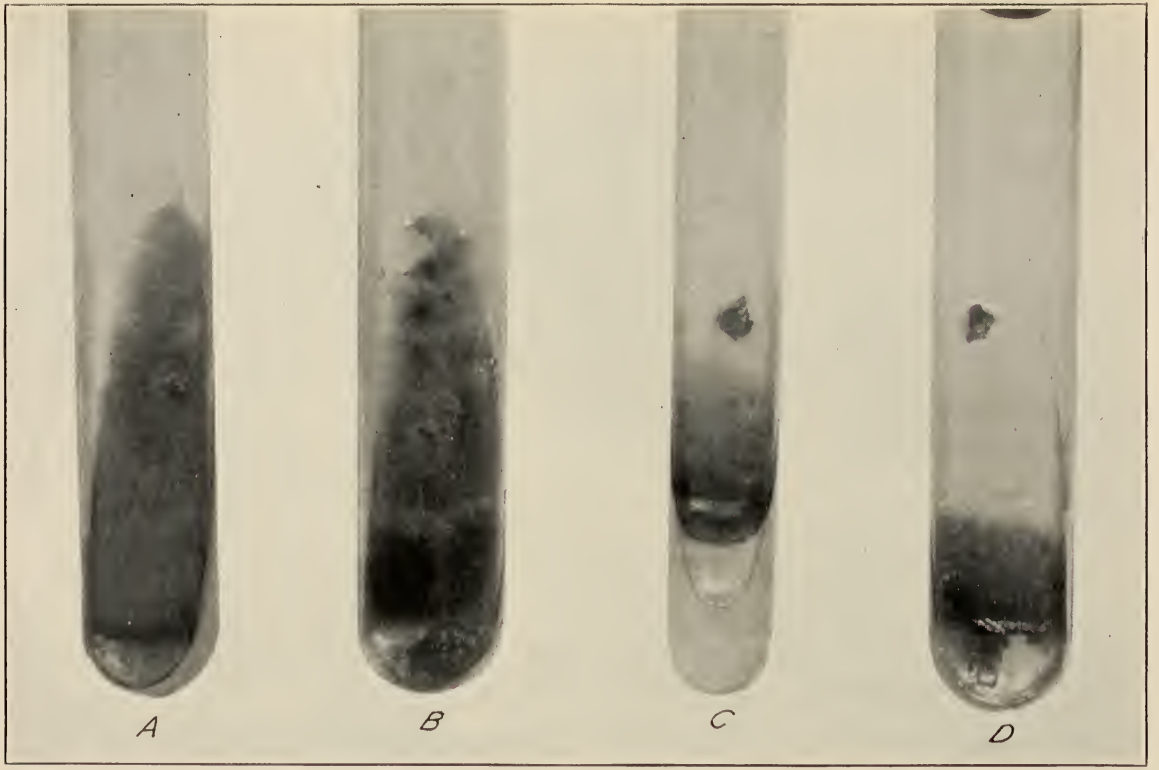
PLATE VII. Fumigated and nonfumigated pineapples. *A*, Check; *B*, sprayed with spores and fumigated, and *C*, sprayed with spores and nonfumigated. The photograph was taken seventy-two hours after fumigation with 1,200 c. c. of formalin per 1,000 cubic feet of air space. *C* shows the fungus developing from numerous centers of infection.

PLATE VIII. Artificially inoculated pineapples. Fig. 1.—Fruit inoculated by incision method. The fruit was not fumigated in this case and the fungus developed abundantly in forty-eight hours. Fig. 2.—Fruit sprayed with spores and not subjected to fumigation. Numerous centers of infection are visible.

NORMAL AND ABNORMAL GROWTHS OF BAMBOO.

A, normal; *B*, abnormal.

PLATE II.



EFFECT OF FORMALDEHYDE GAS ON CULTURES OF THIELAVIOPSIS. *A*, CHECK; *B*, *C*, AND *D*, TRANSFERS FROM CULTURES SUBJECTED FOR 15, 30, AND 60 MINUTES, RESPECTIVELY, TO GAS GENERATED FROM 800 C. C. OF FORMALIN PER 1,000 CUBIC FEET OF AIR SPACE.

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EFFECT OF FORMALDEHYDE GAS ON CULTURES OF THIELAVIOPSIS. *A*, CHECK; *B*, *C*, AND *D*, TRANSFERS FROM CULTURES SUBJECTED FOR 15, 30, AND 60 MINUTES, RESPECTIVELY, TO GAS GENERATED FROM 900 C. C. OF FORMALIN PER 1,000 CUBIC FEET OF AIR SPACE.

FUMIGATED AND NONFUMIGATED PINEAPPLES. *A*, CHECK; *B*, FRUIT INOCULATED AND FUMIGATED, AND *C*, FRUIT INOCULATED AND NONFUMIGATED.

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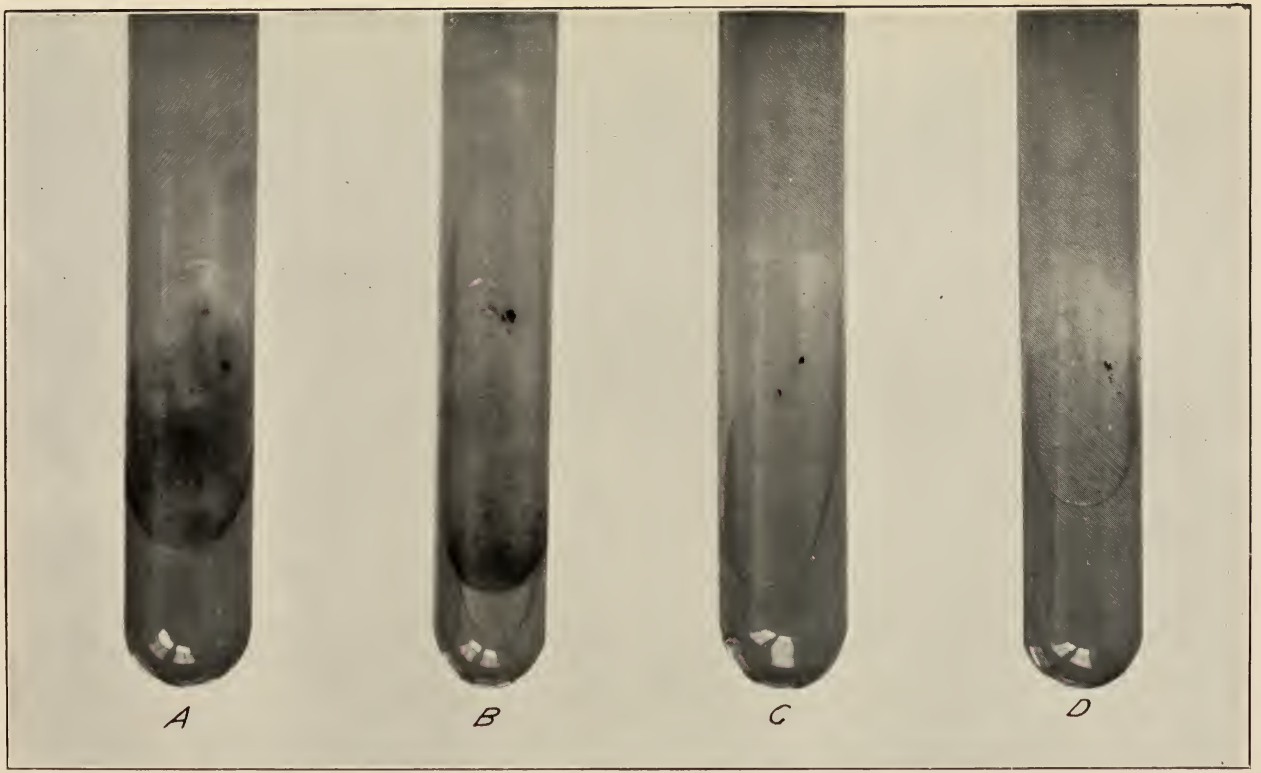
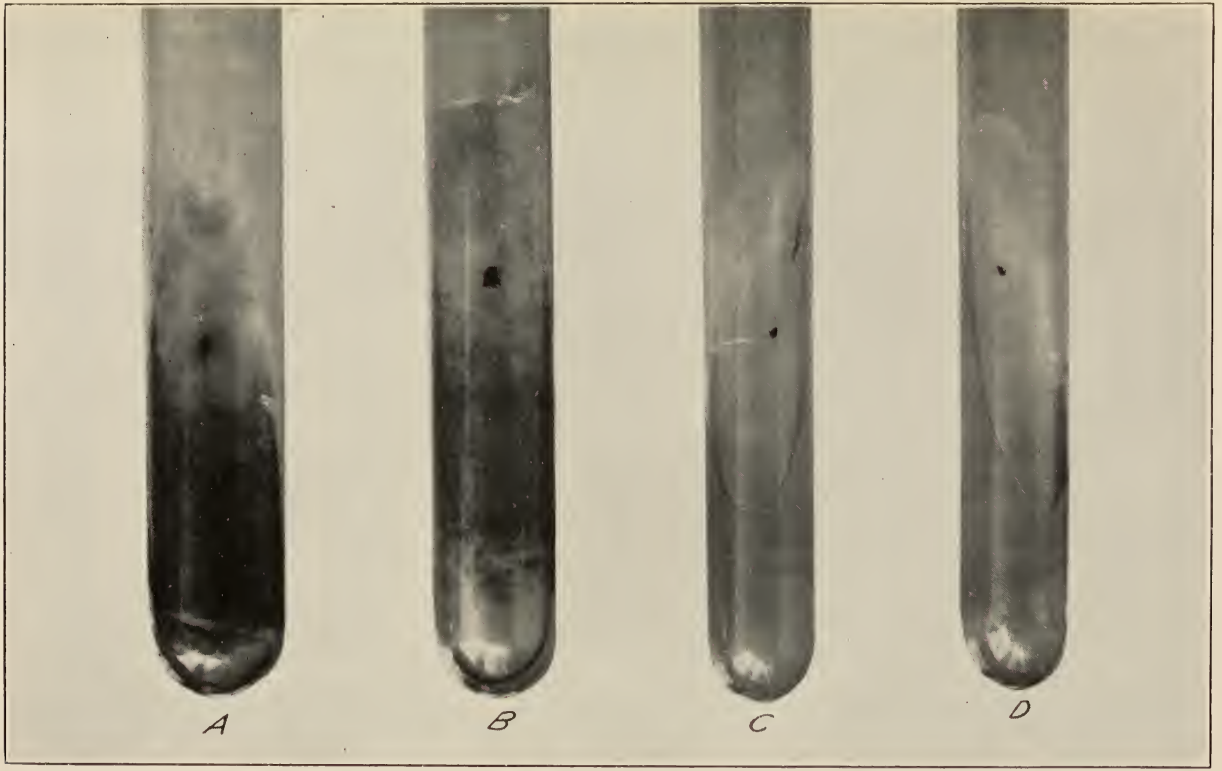


CULTURES FROM INOCULATED PINEAPPLES BEFORE AND AFTER THE FUMIGATION OF THE FRUIT. *A* AND *B*, TRANSFERS BEFORE FUMIGATION; *C* AND *D*, TRANSFERS AFTER FUMIGATION. THE FRUIT WAS SUBJECTED TO GAS GENERATED FROM 1,250 C. C. OF FORMALIN PER 1,000 CUBIC FEET OF AIR SPACE.



CULTURES FROM INOCULATED PINEAPPLES BEFORE AND AFTER THE FUMIGATION OF THE FRUIT. *A* AND *B*, TRANSFERS BEFORE FUMIGATION; *C* AND *D*, TRANSFERS AFTER FUMIGATION. THE FRUIT WAS SUBJECTED TO GAS GENERATED FROM 1,300 C. C. OF FORMALIN PER 1,000 CUBIC FEET OF AIR SPACE.

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FUMIGATED AND NONFUMIGATED PINEAPPLES. *A*, CHECK; *B*, SPRAYED WITH SPORES AND FUMIGATED, AND *C*, SPRAYED WITH SPORES AND NONFUMIGATED.

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D. G. Passmore

FIG. 1.—FRUIT INOCULATED BY INCISION METHOD.

A. A. Newton

FIG. 2.—FRUIT SPRAYED WITH SPORES.

ARTIFICIALLY INOCULATED PINEAPPLES.

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