Die Versuchsanstellung war genau die gleiche wie oben beschrieben.

Ein Zusammenhang der „*Urocystis italica* Speg." (= *Stephanoma italica* Sacc.) mit einer *Melanospora*-Art konnte als um so wahrscheinlicher angesehen werden, da auf dem gleichen Substrat (Edelkastanien) in Italien zwei *Melanospora*-Arten beobachtet worden sind, nämlich *M. Gibelliana* Matt. und *M. styranophora* Matt.[1]).

Leider erwies sich die Hoffnung, die beiden fraglichen *Stephanoma*-Arten auf die angegebene Weise zur Perithecienbildung zu zwingen, als trügerisch.

Trotzdem, daß die Bedingungen mannigfach variiert wurden und andauernd ein lebhafter, feuchtgehaltener Luftstrom durch die Culturen geleitet wurde, entstand in keiner derselben auch nur eine Spur von Perithecien.

Die Zugehörigkeit der beiden genannten *Stephanoma*-Arten zu *Hypocreaceen* könnte also höchstens ex analogia geschlossen werden.

Egg plant rots.

By

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(With 4 textfigures.)

Introduction.

Several more or less serious diseases of Egg plants (*Solanum Melongena* L.) occur within the United States and have been subjected at various times to investigation. Among the organisms causing these diseases may be mentioned *Bacillus solanacearum* Smith, *Fusarium* sp., *Pythium de Baryanum* Hesse, *Sclerotium Rolfsii* Sacc., *Ascochyta hortorum* (Speg.) C. O. Smith and *Corticium vagum* B. et C. var. *Solani* Burt. The first of these has been reported by Sackett (1, 2) as causing a fruit rot in Michigan and Colorado. Clinton (3) mentions the occurence of a root rot in Connecticut caused by a species of *Fusarium*. *Pythium de Baryanum* causes a damping off of seedlings (4). *Sclerotium Rolfsii* (5) has been recorded as occurring on the hypocotyl of Egg plants producing a „Fußkrankheit". A disease, appearing upon all of the above ground parts of the plant and caused by *Ascochyta hortorum* seems to occasion the most serious losses and to be the most widely distributed malady of Egg plants. The malady is not confined to this country alone but has been recorded in Cuba, France, Italy and other foreign parts. *Corticium vagum* appears to have been previously reported only from Florida as attacking fruits and

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1) Herr Prof. Saccardo hatte die Güte, mir mitzuteilen, daß das im Museum zu Padua befindliche Material der beiden Pilze so spärlich ist, daß eine Untersuchung nichts Neues zutage fördern kann. Man könnte sonst erwarten, daß neben den *Melanospora*-Perithecien etwa *Urocystis*-ähnliche Conidien auftreten.

stems and it is productive of no serious injury. The two organisms last named have been under investigation during the past two years and will receive further consideration.

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1. *Ascochyta hortorum* (Speg.) C. O. Smith.

This organism together with the nature of the disease which it produces and methods of control have several times (4, 7, 8, 9, 10, 11, 12) been subjected to careful study and consequently are quite well known. The present intention is, therefore, only to confirm certain of these conclusions and to contribute additional obervations.

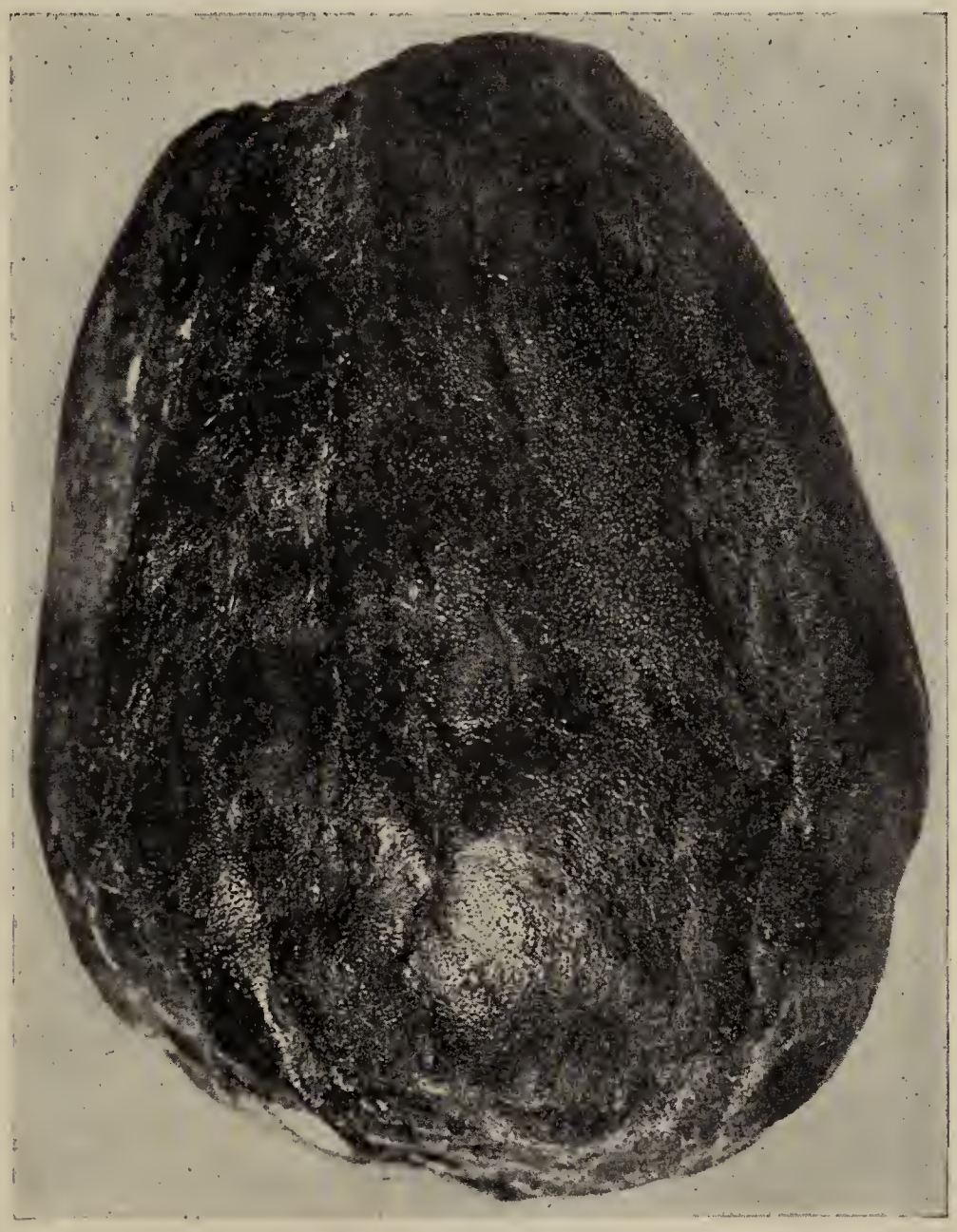

Fig. 1. Egg plant fruit attacked by *Ascochyta hortorum*. $\times$ 1/2.

Appearance of the disease: The disease may be present on leaves, stems and fruits but by far the greatest amount of damage results from the decay of the fruits. Egg plants of all ages are subject to attack. The disease may even evidence itself as a damping off of seedlings before they have been transplanted to the field. Lesions, beginning as water soaked areas, appear upon the stems near the surface of the ground. These become sunken and the plant topples over, the stem bending at the point of constriction as is characteristic of an attack by a damping off fungus. Infected seedlings, for the most part, perish. The minute, dark fruit bodies of the fungus may appear on these sunken areas. The leaves, too, may be affected before the plants are removed from the seed bed and the disease continues throughout the summer and autumn until the plants succumb to the effects of frosts. A discolored spot, less green than the surrounding tissue, first appears at the point of infection. This diseased area increases in size, becoming dead and brown at the center, and adjacent tissues are chlorotic. These spots vary in size from 2 to 10 mm and are in the main circular to ellipitical in outline. Large dead patches several centimeters across and more or less irregular may eventually be formed or by confluence a large portion of the leaf may

become involved. Frequently entire leaves are destroyed. Under favorable conditions, pycnidia, the fruit bodies of the fungus, appear as small black dots on the dead leaf tissues.

Later in the season the stems, fruit pedicels and fruit become diseased. Grayish areas dotted with numerous black pycnidia appear on the stems often completely girdling them and the fruit pedicels are generally entirely involved.

The decay of the fruit first manifests itself by the formation of a circular, brown area. This rapidly enlarges, depending upon atmospheric conditions, so that within a few days the entire fruit may be attacked and will fall from the plant. The fruit may maintain its conformation with but little shrinkage until the numerous pycnidia have been formed and the epidermis has been ruptured (s. fig. 1). This affords an avenue of entrance for various organisms which soon reduce the tissues to a wet pulp, so thoroughly disintegrated that the fruits readily fall to pieces. If in this rotted condition they remain undisturbed, they may dry up becoming black, shriveled mummies. During the season of 1912 this disease was unusually common and destructive in Alabama even in fields in which but little leaf spot was in evidence.

The fungus: The pycnidia of the fungus producing this malady vary in size from 100 to 150 μ. The conidia are extruded from the pore or ostiolum in rope-like coils, being held together by a mucilaginous substance which readily dissolves in water. Water, by splashing upon healthy plant parts or trickling down upon them, serves, perhaps, as the chief agent in the dissemination of the disease. Because of the fact that this fungus was first observed on leaves and the conidia were hyaline and continuous, it was regarded as *Phyllosticta*, receiving the name *P. hortorum* Speg. (13). Later Halsted observed it in New Jersey as the cause of a damping off disease of Egg plants and named it *Phoma Solani* Hals. (14). In the autumn of 1903, Smith found, in Deleware, an Egg plant fungus which he regarded at first as *P. hortorum*. The uniseptate conidia later lead him to believe it was an *Ascochyta*, and because of its close agreement with *Ascochyta Lycopersici*, of which there were no available authentic specimens for comparison, he decided it should properly be referred to *A. Lycopersici*. In order to verify the presence of a septum in the conidia he subsequently sent material to Halsted, Atkinson, Kellerman and others, all of whom regarded it as an *Ascochyta*. By inoculation experiments this *Ascochyta* proved to be parasitic on the Tomato also, establishing the fact that *A. Lycopersici* Brun. is identical with *P. hortorum* Speg. as reported in New Jersey. Since the latter name had priority, the name properly became *A. hortorum* (Speg.) C. O. Smith.

Quite frequently, it is to be noted, the conidia remain continuous or septation occurs only as they become old. Never have any septate conidia been found, however, by the writer, during the course of this work. Mummified fruits a year old and old agar cultures have been repeatedly examined. In addition to the typical conidia which are 6—10 $\times$ 2,5—4 μ, there occurs, morever, a second type of spore, which is hyaline, continuous, frequently curved or hooked at one end and 14—17 $\times$ 2—2,5 μ. These spores may occur in the pycnidium together with the typical pycnospores or alone in other pycnidia upon fruit pedicels, fruits and in cultures.

Morphologically, at least, they are identical with the stylospores of NITCHKE (15) in *Diaporthe*, the "B" spores of DIEDICKE (16) in *Phomopsis*, the scolecospores of SHEAR (17) and the paraphyses of REDDICK (18) in *Fusicoccum* the pycnidial form of *Cytosporella viticola*. Only failure has thus far met the various attempts to germinate these bodies.

No particular difficulty is experienced in isolating this organism in pure culture by the dilution plate method. The conidia germinate within 24 hours by the formation of one or more germ tubes. Septations are formed in these germ tubes at irregular intervals. Within a week, on bean agar, a loose whitish or grayish growth of mycelium has been formed. This subsequently becomes darker in color and pycnidia are sparsely developed. These pycnidia bear typical conidia but they themselves differ from those formed in the host tissue in that many of them are beaked, the neck often being as long or longer than the diameter of the pycnidial cavity.

Unsuccessful attempts have thus far been made to induce the fungus to develop an ascigerous stage both in culture and upon decaying fruits and fruit pedicels, which were permitted to be exposed to open air conditions during the winter of 1912—13. Cultures were made from the overwintered pycnidia on March 25th. Within eight days typical fungus fruits had appeared in these cultures. This observation is in confirmation of SMITH's observations. Egg plant fruits, exposed to the rigorous winter weather of Delaware in 1904, possessed viable pycnospores in March. These facts, besides showing that the organism can survive the winter in it pycnidial form, increase the probability that no ascigerous stage ever occurs in its cycle of development.

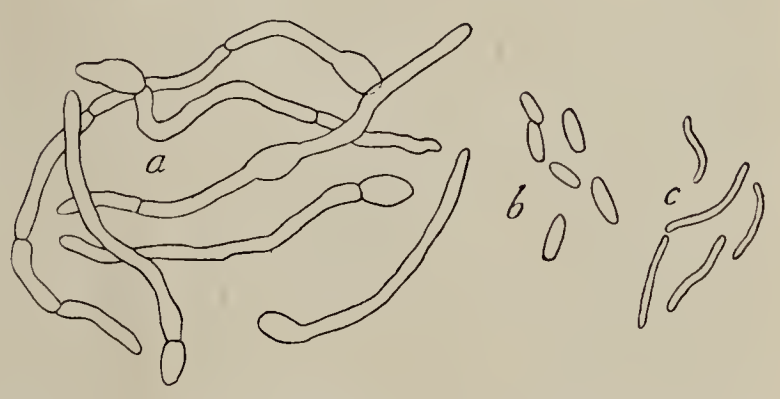

Fig. 2. *a* Germination of conidia of *Ascochyta hortorum*; — *b* Typical conidia; — *c* Thread like bodies borne in pycnidia either alone or with typical conidia (enlarg. 300×).

Host plants: Through the infection experiments of SMITH (10), *Ascochyta hortorum* has been shown to be parasitic upon *Solanum Melongena, S. Lycopersicum, S. carolinense*, and *Datura patula*. VOGLINO (11) entended this list to include *Solanum nigrum, S. Dulcamara, Physalis Alkekengi, Datura Metel,* and *Atropa Belladonna*. He suggests too that this fungus is probably the cause of a destructive disease of Peppers in Piemont (12) and has also observed it upon green or maturing Tomato fruits. Other solanaceous hosts are very probably susceptible to the attacks of this fungus. Under *Ascochyta hortorum* are to be included *Phoma Solani* HALSTED, *Ascochyta Lycopersici* BRUNARD, *A. solanicola* OUDEMANNS, *A. Atropae* BRESADOLA. *A. Alkekengi* MASSALONGO, *A. physalicola* OUDEMANNS, and *A. pyrzolensis* BUBAK and KAB. Other forms, among which are species of *Phoma* on *Solanaceae*, are beyond doubt to be referred to this same form.

Control measures: As the result of several seasons work in New Jersey, HALSTED reports that Bordeaux mixture gives very satisfactory results in the control of both leaf spot and fruits rot of Egg plants. In addition to Bordeaux mixture he used several modifications,

substituting soda and potash for lime with equal effectiveness. Eau celeste and potassium sulphide were entirely unsatisfactory. Bordeaux mixture has been successfully used, during the past summer, under conditions such as obtain in Alabama. A plot which bore a badly diseased crop in 1912 was selected upon which to grow the plants. All of the old plants and decaying fruits had been gathered up from this plot and burned during the preceeding autumn. Three applications of Bordeaux mixture, 4—4—50, were given on the following dates, Aug .27th, Sept. 10th and Sept. 22nd. The plot comprised 60 plants, 48 of which were sprayed and 12 left as checks or controls. These 48 plants produced 14 diseased fruits and the 12 checks bore 25. No record of sound fruits was made yet it may be noted that the plants bore heavily.

The work both of Smith previously mentioned, and myself, establishes the fact that the organism survives the winter upon diseased plant parts more frequently perhaps on fruit pedicels and stems because they resist the decay better than fruits and leaves. Halsted has shown too that the disease is more destructive when the old plants have not been removed frem the field. From these facts it is to be expected that a judicious rotation would be a sufficient safe guard. Such is not the case, however, in that the disease appeared during the past season in a garden which had never before been cultivated and was removed by several hundred yards from the nearest cultivated soil. It also appeared in a green house in which Egg plants had never been grown previously and in a bench, the soil of which had been hauled in from the woods with consequently no chance of previous infection. Halsted reports a similar condition concerning which he states that 16 percent of the fruits on new ground decayed as compared with 61 percent on old ground.

From the fact that the trouble appeared on soil not previously cultivated, it might be conjectured that the disease is transmitted through the seed. Thus far no evidence has been secured to substantiate this probability. Seeds, taken from the same packages as those which produced diseased plants in new ground, were centrifuged and the washings plated on agar. Several samples were thus tested with only negative results.

Varietal resistance seems not to be a promising field for investigation in the control of this disease. Among the varieties grown in these experiments were „New York Improved“, „Florida High Bush“, „Early Large Purple“, „Perfection Purple“, „Black Pekin“ and „Black Beauty“, all of which seem equally susceptible to attack. Halsted reports a similar experience with four different varieties.

Satisfactory control of this disease must depend, therefore, upon the observance of proper sanitary measures, relative to the destruction of diseased plants and rubbish, coupled with a judicious system of rotation and the employment of Bordeaux mixture as a fungicide.

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2. *Corticium vagum* B. et C. var. *Solani* Burt.

The *Rhizoctonia* stage of this fungus is the cause of serious root diseases of many of the field and truck crops, and also of many ornamental and wild plants. It is not to be regarded, however, as the cause of serious injury to Egg plants. Rolfs (6) does not so regard it from his observations in Florida and it cannot be said to be of economic

importance in Alabama. Plants in the seed bed have been observed to damp off in a manner characteristic of this disease. The writer, moreover, has never observed the fungus on more mature stems, and it has been noted in one locality only as productive of a fruit rot. Fruits of all ages, when in contact with the soil seem to be subject to attack. However it is unknown whether or not a wound or some break in the epidermis is necessary to penetration.

Fig. 3. *Rhizoctonia* on fruit of egg plant. $\times$ 1/2.

While the rot does not evidence itself in a strikingly characteristic manner yet there is little or no chance of its confusion with the few other Egg plant rots. The decay extends equally in all directions from the point of infection making a circular brown area. It also extends deeply into the fruit, reaching even to the center or beyond. Affected tissues are soft and collapsed, leaving a deep depression in the fruit. The fungus may appear on the surface of affected tissues as a cottony growth especially if the atmosphere is humid making easy a diagnosis. Affected fruits, when placed in a moist chamber, develop a condition as shown in fig. 3 within forty-eight hours. Again it may be necessary to make a microscopical examination before it can be known that a fungus is involved in the decay. It is to be noted that the tissues on the margin of the decay are often free from fungous filaments. Entire fruits are sometimes destroyed within a week or ten days.

I. The fungus.

Thus far only the mycelial stage has been found in Alabama although Rolfs observed a fruiting stage which he identified as *Corticium vagum* B. et C. There is no doubt, however, that the form under observation is identical with this species. A. *Rhizoctonia* isolated from Potato stems when used for inoculation in Egg plants, produced the characteristic rot. In cultural characters too it was indistinguishable from the form

originally isolated from Egg plants. A fungus, to all appearances identical with the Potato- and Egg plant-*Rhizictonia*, has also been isolated from green Tomato fruits. Rolfs (6) has previously reported, on Tomatoes, an organism which agrees quite well, in size and shape of the spores and the structure of the hyphae with the *Corticium* of the Potato. An unidentified *Rhizoctonia* has also been observed on Tomato fruits in Nebraska (19). More recently Wollenweber (5) has noted a form, *Rhizoctonia potomacensis*, Wr. which is productive of a fruit rot of Tomatoes. This species differs from *R. Solani* in the character of its attack in that concentric, subepidermal mycelial zones are formed within the Tomatoes [1]). The successful inoculation of Egg plants, by the writer, with the form isolated from Tomatoes furnishes conclusive proof that it is also physiologically identical with the form originally isolated from Egg plants and from Potatoes.

II. Occurrence of an enzyme and its isolation.

Because of the fact that tissues were observed to be decayed, as has been noted before, in advance of the fungus, the idea was suggested that the decay must be due to enzymotic activity and that the enzyme is either activated by the presence of the fungus within the tissues of the fruit or is itself a secretory product of the fungus. The problem was then to determine the presence of an enzyme, its origin, and the manner in which it affects living tissues. In order to do this attempts were simultaneously made to extract an enzyme from ripe, healthy fruits, from decaying fruits, and from the mycelium itself. Since the fungus can be made to produce a luxuriant superficial growth of mycelium in a humid atmosphere, no difficulty was experienced in securing twenty grams of this mycelium. This was macerated in a mortar, sixty cubic centimeters of distilled water were added, and the whole was allowed to stand for six hours. By means of a press forty-six cubic centimeters of liquid were recovered from the twenty grams of mycelium together with the sixty cubic centimeters of water. An extract from ripe fruits and decaying fruits was obtained in much the same way. 362 grams of ripe Egg plants were first ground through a meat chopper, were then further macerated

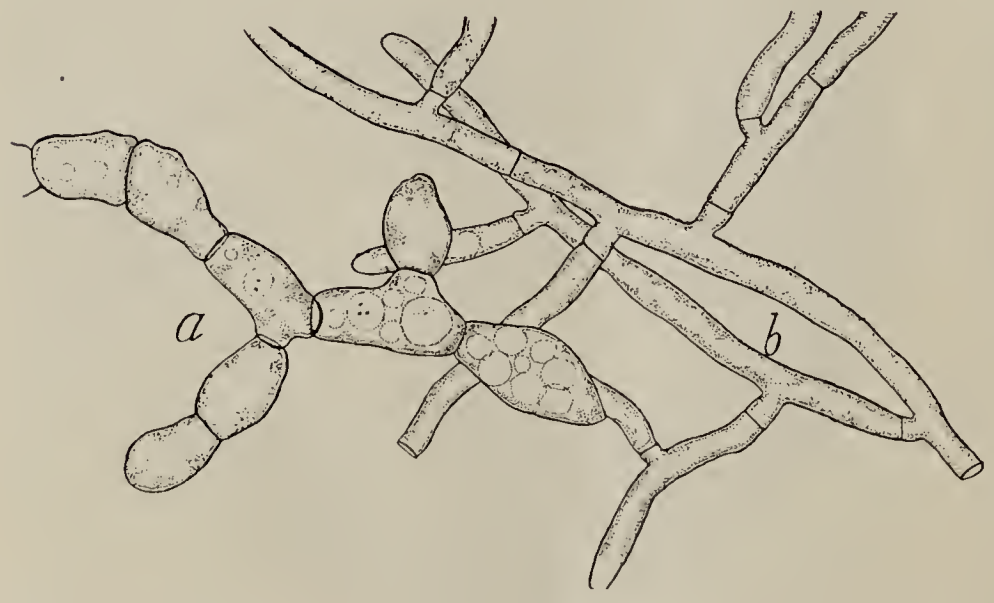

Fig. 4. *a* Chlamydospores of *Rhizoctonia* on Egg plants; — *b* Mycelium showing characteristic method of branching and septation (enlarge 300×).

1) „Von *Rhizoctonia potomacensis* unterscheidet sich diese (*R. Solani*) durch das Krankheitsbild an Tomate, das auffällige subepidermale concentrische Mycelzonen zeigt, die *R. Solani* fehlen"; l. c. p. 30.

in a mortar, 125 cc of water were added and after having been allowed to stand for 6 hours, 312 cc of liquid were expressed. 800 g of rotted fruits were used, to which 50 centimeters were added, and from this 576 cc of liquid were extracted.

Since precipitation with strong alcohol is one of the commonest methods employed [1]) in securing enzymes it has been used in these experiments. To each of the liquid extracts was added 4 volumes of 95 per cent alcohol. This caused the formation of a gummous precipitate, probably carbohydrate in nature, but as yet unknown, which included or carried down with it the enzyme present. The precipitates from the Egg plant extracts were brownish black and from the *Rhizoctonia* mycelium grayish. The color imparted to the fruit extract precipitates was due, beyond doubt, to the presence of an oxidizing enzyme, laccase, which KASTLE (20) [see p. 63] finds to be readily obtained in aqueous solutions from Egg plant fruits. BACH and CHODAT (20) [see p. 118 to 120] have showed that this enzyme is composed of two distinct substances, an oxygenase — that is a substance which forms a peroxyde by taking up oxygen and which is replacable by hydrogen peroxide — and a peroxydase, which activates this peroxide or the hydrogen peroxide added. After 24 hours the material was filtered through filter paper and the precipitate was washed with 95 per cent alcohol and permitted to air dry. Meanwhile, test tubes containing a small amount of sterile distilled water had been prepared. Cylinders of tissue from Egg plant fruits, made with a six millimeter cork borer, were placed in these test tubes, using every care to preserve aseptic conditions. Cylinders of Carrots, Irish Potatoes, and Pears were prepared in the same way. An attempt was then made to redissolve the dried precipitate in a small amount of water. Water was found to have no appreciable effect upon the coagulated gummous material in this precipitate. Nevertheless quantities of the partially dissolved precipitate, varying from a few drops to several cubic centimeters, from normal and rotted Egg plants and from the mycelium were added to the tubes containing the cylinders. Within 13 hours the Egg plant cylinders were completely disinteregrated by the enzyme isolated from the decaying fruits and from the mycelium. The enzyme from both sources seemed equally active. Since it has not been possible to isolate the enzyme free from gums, no quantative results were obtained, nothing is known of the time relations and the effects of temperature, acids, alkalies etc. on the action of the enzyme.

No evidence of softening occurred in the cylinders treated with the extract from healthy fruits. The cylinders, used as checks, remained firm and sterile. Carrots, Pears and Irish Potatoes showed after several days no evidence of enzymotic activity. A second set of cylinders was prepared with the same result.

Tissues which had been acted upon by the enzyme was examined microscopically and it was found that the cells were intact, the middle lamella having been dissolved thus permitting the cells to become dissociated. The enzyme must therefore be one of the cytolytic enzymes, pectinase. Cytolytic enzymes are not at all uncommon in various

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1) Consult EULER, H., General Chemistry of the Enzymes, I—IX, p. 319, 1912.

fungi (21) [1]) and so far as it is known no one has previously determined the presence of pectinase in *Rhizoctonia*. No evidence of this enzyme could be found in fruits decayed through the attack of *Ascochyta hortorum*.

The work thus far conducted clearly indicates that *Rhizoctonia* secretes a cytolytic enzyme, since none was found in normal Egg plants, and that it is in a measure specific since only Egg plant tissues were softened. It is to be understood, however, that the investigation thus far conducted is entirely preliminary.

Summary: A serious disease of Egg plants appearing upon leaves, stems, fruit pedicels and fruits is caused by *Ascochyta hortorum* (Speg.) C. O. Smith.

The fungus overwinters in its conidial condition on fruit pedicels and fruits. There are two kinds of pycnospores, one of which has thus far failed to germinate in cultures. Septate conidia have not been observed during this investigation.

Indirect evidence that seed serve in the dissemination of the disease comes from the fact that the disease appeared in fresh soil in the green house and in a new garden.

The disease can be controlled by sanitation, coupled with judicious rotation and the use of Bordeaux mixture. — *Corticium vagum* B. et C. causes both a damping off disease and a fruit rot.

An enzyme, pectinase, secreted by the *Rhizoctonia* stage, causes a softening of the tissues in advance of the fungus.

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Versuche über die Bedingungen der Holz-Ansteckung und -Zersetzung durch *Merulius* [Hausschwammstudien V].

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(Schluß.)

(Mit 2 Tafeln.)

6. Unterschied von Splint und Reifholz (Taf. I, Fig. 1—4).

Nach obigen Resultaten über die Wirkung der Holztränkung muß man bei Culturversuchen im kleinen sich offenbar die Beschaffenheit des benutzten Holzes vorher etwas genauer ansehen, nährstoffreiches Holz gibt zweifellos ganz andere Ergebnisse als nährstoffarmes, der Begriff „Holz“ und selbst Fichtenholz ist chemisch wie ernährungsphysiologisch recht unbestimmt, Art und Mengenverhältnis der mancherlei Bestandteile des in ihm vorliegenden schwankenden Gemenges verschiedener Substanzen wechseln nach Jahreszeit, Alter, Behandlung usw. innerhalb weiter Grenzen. Daß junges und altes Holz sich nicht gleich verhalten, ist vorauszusehen, es hat dann auch der besondere Verfolg dieses Punktes das ohne weiteres gezeigt [1]).

Mit dem früher von mir benutzten reifen Bretterholz wurde also das Holz einer jungen ca. 7jährigen Fichte, dünnes Zweigholz derselben und frischer Splint älterer Bäume (bei Fällung in Gestalt der abfallenden Späne gesammelt) in übrigens ganz gleich angeordneten Kolbenversuchen verglichen. Es kam da in der Tat derselbe Unterschied heraus wie bei natürlichem und mit Nährlösung getränktem Reifholz (p. 247); nur das junge Holz lieferte üppige hohe Pilzrasen, nach deren schließlichem Verfall die Stücke verfärbt, morsch und mit Schwindrissen bedeckt (Würfel-

1) Ähnliche Angaben über Einfluß der Holzbeschaffenheit sind früher schon wiederholt gemacht, genaue Nachweise fehlen bislang.