AN ABSTRACT OF THE THESIS OF

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(Name) (Degree) (Major)

Date Thesis presented May 15, 1940

Title PHYSIOLOGY OF RHIZOCTONIA AND ITS RELATION TO BOTRYOBASIDIUM

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(1) The first description of the disease caused by Rhizoctonia was made by Duhamel in 1728; but the genus itself was established by De Candolle in 1815. This Rhizoctonia was the violet-colored species R. Crocorum (Pers.) DC., growing on saffron, asparagus, alfalfa, and some other hosts. In 1858 Kühn first described the commoner species R. Solani, from potato in Germany.

(2) R. Solani is a very destructive cosmopolitan species, having as hosts, reported from time to time, more than 200 species, in more than fifty families. The very destructive disease on potato caused by this species has been studied by hundreds of investigators.

(3) The perfect stage of R. Solani was first carefully observed and accurately described by Rolfs in Colorado in 1903; he then called it Corticium vagum Berk. and Curt. var. Solani. Several other species of Basidiomycetes and Ascomycetes had been suspected of being related to this Rhizoctonia. Hypochnus Solani Prill. and Del. had been known in Europe since 1891, but no definite claim had been made before Rolfs of its association with the Rhizoctonia.

(4) The perfect form of R. Crocorum has also been found, in England, by Buddin and Wakefield in 1927, and shown to be Helicobasidium purpureum (Tul.) Pat.

(5) A third perfect form of a Rhizoctonia was found on alfalfa in Michigan by Kotila in 1924 and named Corticium praticola Kotila; it is easily distinguishable by its white mycelium and other morphological characters from the other two species.

(6) Recent mycological studies have shown the heterogeneity of the old genus Corticium and have resulted in the segregation of a number of distinct small genera from it. In 1931 Donk thus separated the genus Botryobasidium, and assigned to it the species orginally known as Hypochnus Solani.

In 1935 Rogers described differences between that parasitic form and the true C. vagum, and added the latter to the genus Botryobasidium. Kotila's C. praticola, according to this concept of the genus Corticium, would belong either in Botryobasidium or in the closely related genus Ceratobasidium.

(7) Fries, whose work is the starting point for the mycological nomenclature of this group, in 1822 defined the genus Rhizoctonia first of all by the presence of sclerotia--irregular in form, of uniform texture inside and out, and possessing a thin inseparable cortex; and further by the uniting of many sclerotia by visible mycelial strands, by subterranean growth, and by attachment to the roots of living plants. It is now known that Rhizoctonia also attacks the aerial parts of plants --for example, R. microsclerotia Matz, on living leaves, branches and fruits of the cultivated fig. The sclerotia are not always associated with the mycelia in nature; for example, the endophytic mycelia in orchid mycorrhizae belonging to several species of Rhizoctonia never form sclerotia in the natural condition, but do so in culture. In pathological literature many fungi with mycelium similar to that of typical species, but without sclerotia, are assigned to the genus Rhizoctonia.

(8) The only generally recognized diagnostic character for Rhizoctonia is the right angled branching, usually accompanied by sclerotium formation either on the host or in culture. This genus is easily confused with Sclerotium, which is differentiated by more solid, black-colored, smoother, shiny, unconnected sclerotia with differentiated, light-colored medulla and no aerial mycelia.

(9) Seven parasitic strains of R. Solani were used in this study, isolated from potato, carnation, tomato, strawberry, barley, oats, velvet grass. Another strain, isolated from strawberry in the course of this study, possessed different morphological characters. Five single-basidiospore cultures isolated from the saprophytic Botryobasidium vagum of rotten wood were also used in this study, since they show the same morphological characters.

The results of the physiological experiments are summarized in the following:

(10) For nutrition, organic compounds, peptone and sugars, are best utilized; inorganic carbon compounds, such as $CaCO_3$, cannot be utilized at all; inorganic nitrogenous salts can be fairly well utilized; ammonium salts are less readily used than nitrate salts and seem toxic to the growth. The brownish pigment formed in the mycelia

results from sufficient available nitrogen in the media. The formation of sclerotia occurred only when abundant available carbon and nitrogen compounds were both present. The best medium tested is Cook's medium.

(11) In nutrient sugar media, dextrose is the sugar best utilized, sucrose next and lactose last. In synthetic sugar media, sucrose is first, dextrose next, and lactose last. In all cases sugar utilization is much faster in synthetic media than in nutrient ones.

(12) Starch is hydrolyzed by all strains, the enzyme amylase, being always secreted ahead of the growth of the mycelium.

(13) Gelatine can be liquefied by all strains but the rapidity varies a great deal with the strain. A dark brownish pigment is associated with potato, tomato, and carnation strains in the liquid and to some depth also in the solid layer of gelatine.

(14) Nitrate can be reduced to nitrite and nitrite in turn to ammonia but with different speeds by different strains.

(15) Eight kinds of metal salts ($ZnSO_4$, $NaNo_3$, $HgCl_2$, $CuSo_4$, $MnSo_4$, $FeSo_4$, $AgNo_3$and NaCl) were used for studying the stimulating and intoxicating effects of different dilutions. All salts in very great dilutions (5:100,000 to 5:1,000,000,000) have definite stimulating effects, especially when several salts are used together. The greater the atomic weight the greater the dilution needed to produce stimulating effects.

(16) The plant hormone indole-butyric acid shows the same effect as metal salts. The stimulating effect is apparent at the dilution of 5:100,000 to 5:10,000,000. Higher concentrations than 1:10,000 inibit growth.

(17) The optimum temperature for growth is around 20° C., but the strawberry and carnation strains prefer a somewhat higher temperature. The growth rate is very sensitive to temperature variation.

(18) All strains grow best at the high atmospheric humidities of 80--100%; at a humidity lower than 50% growth ceases.

(19) Growth occurs through a wide range of pH, (3-10) but at lower pH, 5-6, the growth is more luxuriant.

(20) All strains show best growth if kept most of the time in the dark, with only 2-3 hours of light per day. Continued exposure of the culture to light inhibits growth to a great extent. Mycelial growth shows a much lighter color in the dark than in the light. The brownish pigments formed probably serve as a screen in protecting the protoplasm in the cell. Concentric rings are formed in the culture chiefly as a result of intermittent illumination.

(21) Ultra-violet radiation of 15 minutes' duration checks the growth of all strains, but the carnation and potato strains, with their thicker mycelium shielding deeper layers of cells, can remain viable even after 1 hour's radiation.

(22) A rapid and accurate inoculation method was devised, the seeds of plants to be tested being germinated in Knop's solution and the seedlings then being aseptically transferred to culture tubes.

(23) The cross-inoculation tests show that different strains have a different virulence on different hosts. The virulence seems generally to coincide with the morphological and physiological characters of the strain: the more vigorous the growth and the darker the mycelium, the more abundant the enzymes secreted and the more virulent the strain. The saprophytic strain isolated from single basidiospores of Botryobasidium vagum also attacked certain hosts.

(24) An improved isolation method was devised, to be used with infected plant parts too delicate to be sterilized for the removal of contaminated organisms. The infected part was placed in a petri dish on a layer of small sterilized discs of filter paper; on this substratum the mycelium grew more rapidly than did contaminants; discs taken from the periphery of the Rhizoctonia mycelium gave pure cultures on agar plates.

(25) Contaminated mycelia were somewhat similarly purified of bacterial contaminants by being placed on the glass bottom of a petri dish only one side of which was covered with a layer of agar. The mycelium grew out across the glass and finally reached the agar, leaving contaminants behind.

(26) Septum formation and the streaming of protoplasm through the central pores of the septa were observed in growing hyphae.

(27) In cultures of the saprophytic strain chains of short barrel-shaped cells with 1 or 2 nuclei were observed, growing along the cover of the petri dish. The same structure was observed in parasitic strains, especially the strawberry strain, just before the formation of sclerotia.

They are not conidia, because they never break off, but seem to be structures preparatory for sclerotium formation.

(28) The statement that vagum and Solani are distinct species is confirmed by the morphological characters of the perfect forms; biologically the two are not completely different, since, according to the inoculation test, either can be parasitic or saprophytic.

(29) A standard method for classifying different strains of R. Solani and related species, based on physiological and inoculation tests, is formulated and suggested for future work with Rhizoctonia.

(30) The suspicion that the Rhizoctonia of strawberry is closely related to the common saprophyte Botryobasidium vagum was confirmed by the numerous similarities between the two fungi in culture. However, the one fruiting-body of the strawberry fungus, formed on strawberry leaves in the greenhouse, was not B. vagum, but B. Solani; and the Rhizoctonia must be considered to be ~~even an~~ an imperfect stage ~~of~~ of the latter.

PHYSIOLOGY OF RHIZOCTONIA AND ITS RELATION TO BOTRYOBASIDIUM

by

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A THESIS

submitted to the

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

May 1940

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ACKNOWLEDGMENT

I am indebted to a number of professors and other friends from whom I have received suggestions and encouragement in this work. For specimens and cultures I must thank Dr. C. E. Owens, Dr. D. P. Rogers, Dr. S. M. Zeller, Dr. R. Sprague and Mr. Alvin Braun. For suggestions concerning various techniques, Dr. W. B. Bollen, Dr. C. P. Hegarty, Dr. W. M. Atwood, Dr. F. H. Smith, and Dr. Elizabeth C. Smith are to be especially thanked. For equipment and apparatus for carrying on the experiments special credit should be given to the Departments of Botany, Bacteriology, and Food Industries. Dr. H. M. Gilkey, Mrs. D. P. Rogers, Dr. E. I. Sanborn, Dr. J. A. Milbrath, and many others give me help in many different ways. Above all I acknowledge indebtedness to the instructors more immediately concerned with this research, Dr. Owens, Dr. Hegarty, and Dr. Rogers, for their guidance and encouragement. For the detailed correction of my manuscript Dr. Rogers is largely responsible. Thanks for the typing are due to Miss Edith Boehm and Miss Rachel Elderl

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PHYSIOLOGY OF RHIZOCTONIA AND ITS RELATION TO BOTRYOBASIDIUM

STATEMENT OF PROBLEM

The work described in the following pages was performed to obtain a more satisfactory knowledge of the physiology, host relations, and taxonomy of the genus Rhizoctonia of the Fungi Imperfecti. The physiological studies were planned to yield, besides such general light as they can throw on the physiology of fungi, some knowledge of use in developing control methods, and also a further means of classifying isolates assigned to Rhizoctonia -- that is, to form the basis of a standard method of classification. The history and comparative structure of Rhizoctonia and similar genera were traced through the available literature to furnish the basis for an acceptable definition of the genus. An attempt to indicate the relation of this fungus imperfectus with certain perfect forms was made, through the use of single basidiospore isolates of the perfect phase. Host relations were investigated by means of cross inoculations. The physiological studies were carried out on the general nutrition of the fungus, sugar fermentation, starch hydrolysis, gelatine liquefaction, ammonium and nitrate-salt metabolism, stimulating and toxic action of metal salts and plant hormones, influence of pH, of humidity, of temperature, of light and dark, of ultraviolet radiation, the oxygen relation, and

so on. The results of some of the experiments are less satisfactory than was expected; but care and patience were exercised throughout.

HISTORICAL REVIEW

The disease caused by a species of Rhizoctonia was described long before that generic name was used, by the French botanist Duhamel, who in 1728 published an account of sclerotia associated with a disease of saffron (Crocus sativus). The sclerotia he believed to be individual plants, and the associated hyphal strands, their roots; he called the organism Tuberoides. In 1791 the great mycologist Bulliard placed the fungus among the truffles, as Tuber parasiticum. Persoon, in 1801, assigned it to the genus Sclerotium, as S. Crocorum. In 1815 A. P. De Candolle placed it in a genus of its own, Rhizoctonia (meaning root-destroyer) within which he distinguished three species -- R. Crocorum, R. Medicaginis, and R. Mali. In 1822 Fries incorporated De Candolle's genus in his Systema mycologicum.

Various additional hosts and species were described by other early authors; the disease was reported by Fougeroux de Bondaroy on asparagus growing in a field formerly planted to saffron; by Duby on Allium escalonicum (R. Allii); by Léveillé on Rubia tinctorum, Solanum tuberosum, Phaseolus, and Tulipa. The Tulasnes, in their great work on hypogeous fungi, included all species known to them, including all of De Candolle's, under the single

name R. violaceum. In 1858 Kühn reported it from Germany on Crocus and sugar beet, and at the same time described the species R. Solani on potato. In spite of the clear distinction which Kühn made between the violet and the potato rhizoctonias, Saccardo in 1899 included them both under R. violacea. In this he has had little following. Kühn gave an excellent account of the development of sclerotia, contrasting the smooth ones of the potato fungus with the wooly sclerotia of R. Medicaginis (R. Crocorum) on beet, lucerne, and carrot. His figures show also the characteristic right-angled branching of the hyphae, and their anastomosis. The fungus, at first found only in France, after Kühn's work was reported from many other parts of Europe, and on roots of beech, pine, and larch as well as from field crops. Eriksson in 1903 showed that considerable variability exists in the susceptibility to the disease of different varieties of carrot. Güssow, who reported Rhizoctonia from England in 1905, considered Solani and violacea the same.

Rhizoctonia was first reported from the United States by Webber in 1890. He assigned the fungus, which attacked alfalfa, to R. Medicaginis. Pammel presently reported a Rhizoctonia on beets, and Atkinson, one causing damping-off of cotton seedlings and parasitic on other plants also. In 1901 Duggar and Stewart added a long list to those hosts

already known to be subject to attack by Rhizoctonia; their descriptions refer to the hyphae only, omitting mention of of sclerotia; and so it is not possible to determine whether one or several strains or species were included.

A number of reports, early and recent, have been made of possible perfect stages. In 1869 Fuckel asserted that Byssothecium circinans Fckl. (Leptosphaeria circinans Sacc.) was that perfect stage. No connection was made beyond the occurrence of both on decaying stems of alfalfa. Prunet in 1893 similarly reported an ascomycete as the perfect form, and similarly neglected to offer details or evidence. Hartig, as long ago as 1888, found a fungus resembling Rhizoctonia on the roots of oak, together with a Rosellinia; and Massee in 1910 suggested that the two were segments of the same life cycle; this conjecture was based on association and on similarities in habit and mycelium. In 1897 Frank named a Thelephora, found along with Rhizoctonia violacea on grape vines, Th. Rhizoctoniae. In 1913 Cook found, on potatoes parasitized by Rhizoctonia a sclerotium-like body containing asci and ascospores; the connection could not be proved. Prillieux and Delacroix in 1891 had described a basidiomycete on potato stems as Hypochnus Solani; Apparently they did not suspect its connection with the potato Rhizoctonia. But this connection was finally announced, and their name is the

one commonly employed in Europe for the perfect fungus. Rolfs, who demonstrated the connection, in 1904, chose rather to call the fungus Corticium vagum Berk, and Curt., following Burt in using the name of a basidiomycete found in or before 1873 on pine bark. Burt later treated the potato fungus as a var. Solani, and still later (1926), returned it to complete synonymy with C. vagum. Since Rolfs's proof of the identity of the perfect and imperfect fungi, both by tracing mycelium from one to the other and by culture experiments, the perfect stage has been generally known in this country as C. vagum, although the potato Rhizoctonia and all similar fungi have still been treated as R. Solani.

In addition to C. vagum, Burt describes in his last work (1926) four other species of Corticium causing plant diseases, C. Koleroga on coffee branches and leaves, C. Stevensii on leaves of apple, pear, and quince in Brazil, C. salmonicolor on various cultivated trees and shrubs in the tropics, Florida, and Louisiana, and C. centrifugum.

In 1924 and 1927 Buddin and Wakefield published morphological, cultural, and pathological evidence that the perfect stage of R. Crocorum is the auriculariaceous Helicobasidium purpureum. Thus the two best-known species of Rhizoctonia have been definitely connected with basidial fructifications. The status of numerous other fungi

is less satisfactorily determined. The eastern Asiatic Corticium Sasakii is a case in point. Wei in China and Matsumoto and Yamamoto in Japan have recently collected specimens of this fungus; the latter authors compared it with C. Stevensii and C. Koleroga, and decided that they are three closely related species, but their decision is not universally accepted. The "propagation-fungus" (Vermehrungspilze), a serious pathogen in seedbeds in Germany and France, is another such doubtful form. It was at first referred to various well known imperfect genera and presently segregated in its own genus as Moniliopsis Aderholdii. Duggar stated that it was no more than a form of R. Solani; and this may well be the case, since subsequent attempts at separation have had nothing more definite as a basis than extremely variable pathogenicity criteria and minor differences in the dimensions of organs. Both saprophytic and parasitic fungi of this group show great variability; and except perhaps for the perfect stages of the saprophytes, their taxonomy is uncertain.

SCLEROTIA AND SCLEROTIUM-BEARING FUNGI

A sclerotium is a compact pseudoparenchymatous resting body, made up of thick-walled mycelium. It is usually dark-colored, at least externally, with oil or an oil-

like substance in the cells and much less water than ordinary vegetative mycelium. It serves to carry the fungus over periods unfavorable for active growth. Various sclerotia vary in size from those hardly visible to some as large as a canteloupe. Many sclerotia or sclerotium-forming fungi have been associated with perfect -- ascus or basidial -- fruiting stages, and are properly classified according to those stages: Sclerotinia, Claviceps, Cordyceps, Rosellinia of the Ascomycetes, and Poria, Coprinus, Collybia, and Typhula of the Basidiomycetes; no phycomycetous sclerotia are known. Since Sclerotium-forming fungi when considered apart from their perfect stages are classified among the Fungi imperfecti, there is no obligation upon the botanist to arrange them according to their natural relationships, and one genus of sclerotial fungi may have perfect stages in more than one division of the natural -- that is, phyletic -- classification. Such a genus is Rhizoctonia, containing forms related to both Hetero -- and Homobasidiomycetes, and possibly to ascomycetous groups also.

In the Systema mycologicum of Fries (1822), with which the nomenclature of sclerotium-forming imperfects commences, there is described a family Sclerotiaceae, now no longer recognized as distinct. It is there divided into two sub-groups, the first earth-inhabiting -- that is,

formed apart from any living plant -- and the second epiphytic -- that is, definitely connected with a living plant. In the epiphytic section are five genera. The first is Acrospermum, characterized, as the name implies, by the production of conidia on the apex of the sclerotium: it is now recognized as Claviceps, the ergot sclerotium. The second is Periola, one of the Tuberculariaceae, with setulose sporodochia and hyaline conidia borne on the conidiophores. The remaining three are still recognized as members of the mycelia sterilia, having no conidia. Of these Acinula is readily distinguishable by having a discrete and separable cortex. Sclerotium and Rhizoctonia have no cortex separable from the interior of the fungus; the first, according to Fries, has sclerotia unconnected by fibrils, and the second shows such connections. To this distinction, often difficult to apply, may be added the further points of difference that the sclerotia of Sclerotium are (or are said by Fries to be) regular in shape and those of Rhizoctonia irregular; that those of Sclerotium usually have an interior lighter in color than the rind, while those of Rhizoctonia are uniform; that the resting bodies of Sclerotium are often formed within host tissue, but those of Rhizoctonia are always external. Fries's complete description reads as follows: "Of various forms, concrescent, cartilaginous-fleshy, inseparable,

persistent; fibers root-like, cottony, springing in fascicles from the angles of the sclerotia, joining many individuals together. No fructification evident. Smooth, but rooted.....Subterranean fungi, formed free, growing on the roots of living plants and killing them."

It will be noted that this classification and description furnish a satisfactorily detailed set of criteria for the genus, and that these are nearly all derived from the sclerotia. However, in recent years sterile mycelia have repeatedly been studied which showed the characteristic right-angled branching of the typical species-- a hyphal character unknown to Fries -- but that produced no sclerotia in nature, and sometimes few or none on the host. These have been often added to the genus, especially by students of pathology. A whole group of fungi thus lacking sclerotia has been isolated from orchid mycorrhizae and assigned to the genus Rhizoctonia. Other fungi show the characteristic mycelium and sclerotia, but grow over stems and leaves instead of underground; and these also have been added. Rhizoctonia is thus more heterogeneous than at first, and the emphasis has been shifted in defining it; but probably the fungi it embraces are as similar as those in many other genera of Fungi imperfecti, and no inconvenience has resulted from the enlargement of the generic limits.

THE PERFECT FORM OF RHIZOCTONIA SOLANI

As has already been stated, Rolfs in 1904 found on the stem of potato what he believed to be the perfect stage of Rhizoctonia Solani; a mycelium produced in culture from a single basidiospore of this fungus gave rise to typical sclerotia of the Rhizoctonia, and so the connection was established; on Burt's advice, he assigned the basidial fungus to Corticium vagum. But Corticium vagum was based on a tufted, yellowish basidial layer growing on pine bark, and thus differed in structure, color, and biology from the potato parasite. Because of the great authority possessed by Burt's name and his work, there has rarely been serious doubt expressed, in spite of the obvious superficial differences between Rolfs's fungus and that on pine, of the correctness of the identification. In 1935, however, Rogers examined portions of the original collection of C. vagum and of fructifications on potato and separated Solani, the parasite, from vagum. The differences as he gave them are as follows: Solani is a parasite and vagum a saprophyte; the hymenium of Solani is continuous and of vagum, tufted and interrupted; the basidia of Solani are often ovoid and always with four sterigmata, and those of vagum are cylindric and usually with six; the spores of Solani are oblong-ovoid to

short-reniform and those of vagum fusiform-cylindric, flattened on one side, and obliquely depressed next to the apiculus. Further, he recognized the segregation from Corticium of a number of small and homogeneous genera, of which one, Botryobasidium Donk, includes both Solani and vagum; this genus is characterized by peculiar short-cylindric basidia resembling hyphal segments, short-celled, stout hyphae in the basidiocarp which branch at right angles; candelabrum-like clusters of basidia; and loose texture; it appears to be a very natural and homogeneous systematic unit.

The youngest mycelia of Botryobasidium Solani do not show right-angled branching; instead, branches diverge from the parent hypha at an angle of 20 - 40°. A constriction is generally present at the base of the branch, and near it, a septum. These young threads are hyaline and slender. In older sterile mycelium, as in the fruiting-body, the constriction is less conspicuous, the color brownish, and the angle of divergence of branches approaches and equals 90°. Anastomoses of mycelia are abundant at all stages, but most so in young transfers; they can occur between two branches of the same hypha or between different transfers of the same strain.

Sclerotia appear in culture as soft white knots of

mycelium, which gradually increase in size and hardness and take on a brown color. The number of sclerotia depends on the age of the culture, the medium, and other factors. Sometimes, especially when the temperature is high and the agar fairly fresh, watery drops of exudate appear on the surface of the sclerotia; tests have shown that these drops contain little or nothing but water. Presumably these drops are formed as the result of secretion and as a part of the maturing -- that is, becoming dormant -- of the sclerotia. The cells of sclerotia are dark brown, barrel-shaped, thick-walled, and heavily loaded with oil globules. The cells can germinate at any time by the protrusion of a hypha from the wall; some germ-hyphae of interior cells pass directly through cells of outer layers. Apparently the cortical cells are dead. By some strains sclerotia are produced very abundantly in nature; this is especially true of Rhizoctonia on potato.

The perfect stage, Botryobasidium Solani (Prill. & Del.) Donk (Hypochnus Solani Prill. & Del.; Corticium vagum in Burt's sense), is rarely seen; its development is apparently dependent on peculiar weather conditions. It occurs on the host stem, in a collar just above the surface of the soil. It consists of a loosely attached web of grayish hyphae, supporting a layer of club-shaped basidia. The spores are ovate, colorless, somewhat

tapered toward the point of attachment, smooth, 9 - 14 x 6 - 8 μ. This perfect stage seems to be produced during a cool season with abundant moisture; it lasts only a short time, even when conditions chance to favor its development.

HOSTS AND DISTRIBUTION

Rhizoctonia Solani is a thoroughly cosmopolitan species. It is also omnivorous. The appended list of hosts numbers more than 200; and as long as the fungus or the disease it produces are studied, the list will continue to grow. The families Amaranthaceae, Caryophyllaceae, Cruciferae, Leguminosae, Solanaceae, and Compositae appear particularly susceptible. Under the proper conditions the fungus can attack susceptible plants at any stage of their growth, from germination to harvesting, as damping-off, foot-rot, root-rot, stem-rot, fruit-rot, pod spot, crown rot, wilt, blight, rosette, or tuber scurf. It is one of the commonest and destructive of plant pathogens. Hosts reported to the present are as follows:

A List of Hosts of Botryobasidium Solani, Known to 1939

Abroma augusta . . . . . Steyaert

Abutilon hybridum, var. Savitzii . . . . Peltier

Acacia decurrens . . . . . Gadd & Bertus

Acalypha Wilkesiana, var. bicolor . . . . Peltier

" " " tricolor . . . Peltier

" " " marginata . . . Peltier

Achillea Millefolium. Milfoil . . . . . Peltier

Actinidia polygama . . . . . Stewart

Adenanthera povonia . . . . . Deighton

Agave rigida . . . . . Shaw

Ageratum mexicanum vars. . . . . Peltier

Agrostis canina . . . . . Piper & Coe

" palustris . . . . . " " "

stolonifera . . . . . " " "

tenuis . . . . . " " "

Albizzia moluccana . . . . . Gadd

Allium sp. Onion . . . . . Rolfs

Alyssum odoratum. Sweet alyssum . . . . Peltier

Amaranthus albus . . . . . Duggar & Stewart

" caudatus . . . . . Peltier

" retroflexus. Pigweed . . . . Duggar & Stewart

" salicifolius . . . . . Peltier

" spinosus . . . . . Rolfs

Ambrosia sp. Ragweed . . . . . Burkholder

Ambrosia psilostachya. Ragweed . . . . . Hartley & Bruner

Antirrhinum sp. Snapdragon . . . . . Duggar & Stewart

" majus. Snapdragon . . . . . Peltier

Apium graveolens. Celery . . . . . Duggar & Stewart

Aquilegia sp. . . . . Peltier

Arachis hypogaea. Peanut . . . . . Shaw

Arancaria sp. . . . . Marchal

Arundinaria sp. Bamboo . . . . . Ogilvie

Asclepias sp. Milkweed . . . . . Stakman

Asparagus Sprengeri . . . . . Duggar & Stewart

Aucuba sp. . . . . Stevens & Wilson

Avena sp. Side Oats . . . . . Temple

Bartonia aurea . . . . . Peltier

Begonia sp. . . . . Duggar & Stewart

Berberis sp. . . . . Stevens & Wilson

Beta vulgaris var. Garden beet . . . . . Johnson

" " " Sugar beet . . . . . Pammel

Bidens pilosa . . . . . Steyeart

Bouvardia sp. . . . . Van Poeteren

Brassica arvensis. Charlock . . . . . Burkholder

Brassica chinensis . . . . . Bunschoten

Brassica oleracea var. Cabbage . . . . . Atkinson

" " " Cauliflower . . . Duggar & Stewart

" rapa. Turnip . . . . . Rolfs

" sp. Mustard . . . . . Briton-Jones

Bromus carinatum Velvet grass . . . . . Sprague

Cactus sp. . . . . Van Poeteren

Calendula Pongei. (Demorphotheca) . . . . Peltier

Callisthephus hortensis. China Aster . . Duggar & Stewart

Calopogonium muncunoides . . . . . Palm

Campanula sp. . . . . Peltier

" 8 sp. . . . . Peltier

" persica . . . . . Jensen

Canavalia ensiformis . . . . . Steyeart

Capsicum sp. Pepper . . . . . Edgerton

Cassia hersuta . . . . . Gadd

Cedrus Deodara . . . . . Auchinleck

Celosia Huttonia "var. Thompsonii magnifica" . Peltier

Centaurea gymnocarpa . . . . . Peltier

Centrosema Peluemieri . . . . . Steyeart

" pubescens . . . . . Ultee

Cerastrum vulgatum . . . . . Piper & Coe

Chenopodium album. Lamb's quarters . . . Duggar & Stewart

" leptophyllum . . . . . Hartley & Bruner

Chrysanthemum hortorum . . . . . Peltier

Cicer arietinum . . . . . McRae

Cicharium Intybus. Chicory . . . . . Weber

Cichorium endiva. Endive . . . . . Thomas

Cineraria sp. . . . . Peltier

Cirsium sp. Thistle . . . . . Peltier

Citrullus vulgaris. Watermelon . . . . . Rolfs

Citrus sp. Orange . . . . . Smith

" Vulgaris. Bitter orange . . . . . Petri

Clitoria cajanifolia . . . . . Gadd

Coffea arabica. Coffee . . . . . MacDonald

Coleus sp. . . . . Duggar & Stewart

Colocasia antiquorum . . . . . Auchinleck

Colcasia sp. . . . . Briton-Jones

Convolvulus arvensis. Bindweed . . . . . Stakman

Corchorus capsularis. Jute . . . . . Shaw

Coreopsis lanceolata . . . . . Duggar & Stewart

Crataegus sp. Hawthorn . . . . . Stevens & Wilson

Crotalaria sp. Rattle-box . . . . . Rolfs

" incana . . . . . Gadd

" Juncea. Sannhemp . . . . . Metri

" retusa . . . . . Steyeart

" spectabilis var. speciosa. . . McKee & Enlon

" striata . . . . . McKee & Enlon

" verrucosa . . . . . Gadd

Cucumis melo. Muskmelon . . . . . Selby

" sativus. Cucumber. . . . . Duggar & Stewart

Cucurbita maxima. Squash . . . . . Duggar & Stewart

" pepo. Pumpkin . . . . . Rolfs

Cuphea platycenta. Cigar Plant . . . . . Clinton

Cyclamen persicum . . . . . Van Poeteren

Cynodon Dactylon. Bermuda Grass . . . . . Thompson

Cyperus rotundus. Nut grass . . . . . Rolfs

Dahlia sp. . . . . Matsumotao

Daucus carota. Carrot . . . . . Duggar & Stewart

Dianthus barbatus. Sweet William . . . . Duggar & Stewart

" caryophyllus . . . . . Duggar

" Chinensis . . . . . Peltier

" deltoides . . . . . Peltier

" Hedewigii . . . . . Peltier

" latifolius . . . . . Peltier

" plumarius . . . . . Peltier

" Sequeri . . . . . Peltier

Digitaria sanguinalis . . . . . Wei

Dioscorea sp. Yam . . . . . Martyn

Dolichos Junghunianus . . . . . Ultee

" Hosei . . . . . Ultee

Dunbaria Heynei . . . . . Park

Elletaria Cardamomum . . . . . Park

Equisetum sp. . . . . Stakman

Eriobotrya japonica. Loquat . . . . . Stevens & Wilson

Erysimum pulchellum . . . . . Peltier

Euphorbia pulcherrima. Poinsettia . . . Peltier

Fagopyrum esculentum. Buckwheat . . . . Stevens & Wilson

Festuca rubra . . . . . Tilford

Fragaria californica . . . . . Zeller

Fragaria cuneifolia. Field strawberry . Zeller

Gliricidia maculata . . . . . Gadd

Glycine Max. Soybean . . . . . Nacion

Gypsophila paniculata . . . . . Malencon

Glycine soja. Soybean . . . . . Shaw

Goetia sp. . . . . Peltier

Gossypium herbaceum. Cotton . . . . . Atkinson

Gypsophila muralis . . . . . Peltier

" repens . . . . . Peltier

Habenaria sp. . . . . Matsumoto

Helianthus annuus. Sunflower . . . . . Temple

" sp. . . . . Johnson

Heterotheca Lamarkii . . . . . Rolfs

" subaxillaris . . . . . Rolfs

Hevea brasiliensis. Rubber . . . . . Schweizer

Hibiscus cannabiscus . . . . . Balls

" esculentus. Okra . . . . . Heald & Wolf

" Sabdariffa. Roselle . . . . . Matz

" sp. . . . . Stevens & Wilson

Holcus Sorghum. Sorghum . . . . . Wei

Hordeum vulgare. Barley . . . . . Samuel & Garrett

Impatiens sp. . . . . Stewart

Indigofera arrecta . . . . . Gadd

" endecaphylla . . . . . Schweizer

" hirsuta . . . . . Gadd

Indigofera sumatrana . . . . . Gadd

Ipomea batatas. Sweet Potato . . . . . Rolfs

Iresine sp. (Achyranthus) . . . . . Peltier

Iris sp. . . . . Dodge

Kochia trichophylla . . . . . Peltier

Lactuca sativa. Lettuce . . . . . Atkinson

" scariola. Prickly Lettuce . . . Stewart

Larix sp. Larch . . . . . Delevoy

Lathyrus odoratus. Sweet Pea . . . . . Clinton

Lavatera arborea "variegata" . . . . . Peltier

Lepidium sativum. Cress . . . . . Johnson

Leucaena glauca . . . . . s'Jacob

Ligustrum sp. Privet . . . . . Stevens & Wilson

Lilium sp. . . . . Ogilvie

Linaria Cymbalaria . . . . . Peltier

" Maroccana . . . . . Peltier

Linum grandiflorum "rubrum" . . . . . Peltier

" usitatissimum. Flax . . . . . Thomas

Lobelia cardinalis . . . . . Teng

" erinus. Single Blue . . . . . Peltier

Lolium perenne. Perennial rye grass . . Newton & Mayers

Lupinus digitatus . . . . . Balls

" Luteus . . . . . Muller

Lycopersicum esculentum. Tomato . . . . Selby

Lythrum sp. . . . . Peltier

Matthiola incana. Stocks . . . . . Peltier

Medicago sativa. Alfalfa . . . . . Rolfs

Melilotus alba var. annua. Hubam clover Gadd

Mentha nana . . . . . Malencon

Morus alba . . . . . Shaw

Musa paradisiaca . . . . . Gadd & Bertus

" vulgaris. Banana . . . . . Matz

Naias flexilis . . . . . Bourn

Nicotiana sp. Tobacco . . . . . Clinton

Olea laurifolia . . . . . Phillips

Oncidium grande . . . . . Schenk

Ornithopus sativus . . . . . Johnson

Oryza sativa. Rice . . . . . Gadd

Paeonia sp. Peony . . . . . Stakman

Panax sp. Ginseng . . . . . Van Hook

Panicum sanguinale . . . . . Wei

Pastinaca sativa. Parsnip . . . . . Heald & Wolf

Pelargonium zonale. Geranium . . . . . Johnson

Pentus carnea . . . . . Bertus

Petunia sp. . . . . Higgins

Phaseolus lunatus. Lima beans . . . . . Nacion

" mungo . . . . . Nacion

" vulgaris. Bean . . . . . Atkinson

Phleum pratense. Timothy . . . . . Newton & Mayers

Phlox sp. . . . . Duggar & Stewart

Physalis Franchetii. Chinese Lantern Plant . Tolaas

Phytolacca decandra. Pokeweed . . . . . Rolfs

Picea excelsa. Norway Spruce . . . . . Wiant

Picea pungens. Colorado Spruce . . . . . Wiant

Picea sp. . . . . Selby

Pinus ponderosa . . . . . Hartley & Bruner

" nigra var. austriaca . . . . . Wiant

" resinosa. Red pine . . . . . Wiant

" sylvestris. Scotch pine . . . . . Wiant

" sp. . . . . Hartley

" Strobus . . . . . Duggar & Stewart

Piper Betle . . . . . McRae

" nigrum . . . . . Deighton

Piqueria trinervia (Stevia) . . . . . Peltier

Pisum sativum. Pea . . . . . Paddock

Plantago sp. Plantain . . . . . Burkholder

" aristata . . . . . Peltier

Platycodon sp. . . . . Clinton

Poa pratensis . . . . . Piper & Coe

" trivialis . . . . . Piper & Coe

Portulaca sp. Purslane . . . . . Rolfs

" oleracea . . . . . Peltier

Potamogeton pectinus . . . . . Bourn

" perfoliatus . . . . . Bourn

Primula malacoides . . . . . Peltier

Potentilla sp. Five-finger . . . . . Stakman

Primula obconica "grandiflora" . . . . . Peltier

Prunus sp. Cherry . . . . . Duggar & Stewart

" " Laurel Cherry . . . . . Stevens & Wilson

Pseudotsuga taxifolia . . . . . Eliason

Pteridium aquilinum var. pubescens . . . Zeller

Punica granatum. Pomegrante . . . . . Stevens & Wilson

Pyrethrum sp. . . . . Duggar & Stewart

Radicula armoracia. Horse-radish . . . . Burkholder

Raphanus sativus. Radish . . . . . Atkinson

Ruppia maritima . . . . . Bourn

Reseda odorata. Mignonette . . . . . Clinton

Rheum rhaponticum. Rhubarb . . . . . Duggar & Stewart

Rhododendron carolineanum . . . . . White

" ponticum . . . . . White

Richardia scabra . . . . . Rolfs

Ricinus sp. Castor Bean . . . . . Melchers

Rosa. Rose . . . . . Briton-Jones

Rubus sp. Raspberry . . . . . Duggar & Stewart

" " Blackberry . . . . . Paddock

Rumex acetosella. Sorrel . . . . . Peltier

" sp. Dock . . . . . Buckholder

Saccharum officinarum. Sugar Cane . . . Edgerton

Salvia officinalis. Mammoth Sage . . . . Duggar & Stewart

" sp. . . . . Selby

Salvia splendens var. . . . . Peltier

Sambus Ebulus . . . . . DuHamel

Santolina chamaecyparissus . . . . . Peltier

Schizanthus sp. . . . . Peltier

Secale cereale. Rye . . . . . Stakman

Sedum anglicum . . . . . Peltier

" apectabile . . . . . Peltier

seradella sp. . . . . Johnson

Sesamum indicum . . . . . Balls

Sesbania aculeata . . . . . Gadd

Setaria glauca. Foxtail Grass . . . . . Peltier

" italica. Italian millet . . . . Wei

Silene Schafta . . . . . Peltier

Solanum melongena. Eggplant . . . . . Atkinson

" Tuberosum. Potato . . . . . Duggar & Stewart

" verbascifolium . . . . . Rolfs

Spinacea oleracea. Spinach . . . . . Wei

Stachys lantata . . . . . Peltier

Stellaria graminea . . . . . Piper & Coe

" sp. Chickweed . . . . . Ferdinandsen

Stizolobium atterimum . . . . . Briant & Martyn

Sweitenia macrophylla. Mahogany . . . . Kalshoven

Tagetes sp. . . . . Steyeart

Taxus canadensis. Yew . . . . . Clinton

" cuspidata . . . . . Clinton

In the course of these studies the literature of the last two decades was combed for references to Rhizoctonia, and an extensive digest of control methods prepared. Since, however, no field investigations of control were contemplated, no summary of this digest is here made; from it were taken such data as are of significance in the aspects of the Rhizoctonia problem which are under discussion.

Cultures of seven parasitic strains of Rhizoctonia were employed in the experiments later described. Their hosts, and the symptoms they produce, are briefly listed: 1) Potato. The typical sign of Rhizoctonia infection of potato is the presence of black sclerotia on the tubers; symptoms are russeting, cracking, and pitting of tubers, girdling or cankering of stems, aerial tubers, little potatoes, decay of "seedlings." 2) Carnation. Rhizoctonia here is extremely destructive, causing stem rot of mature plants and damping-off of cuttings. 3) Tomato. Symptoms here are damping-off, stem and root canker, leaf rosetting, and fruit rot. 4) Strawberry. Rhizoctonia has caused such serious disease of strawberries in the Willamette and Hood River valleys that whole regions have largely been replanted to other crops. The most obvious symptoms are dark lesions on new roots and fall yellowing of leaflets and purpling of veins. Weakening and

winter-killing of the plants follow. 5, 6, 7) Barley, oats, velvet grass. The symptoms on these grasses are eyespot, or occurrence of elliptical lesions on the culms, and a severe root-rot.

In addition to these strains, two basidiospore isolates of the saprophytic C. vagum, already described, were used in some of the tests.

COMPARISON OF STRAINS OF RHIZOCTONIA

Microscopic differentiation of different strains of Rhizoctonia is a difficult and unsatisfactory procedure. The variation among different parts of a single mycelium is so great that only by carefully choosing a single type of cell for measurement can even remotely comparable results be obtained. The dimensions given here are those of old mycelia of the superficial layer, and those of young sclerotial cells. Measurements are in microns, made under 10X ocular and 43X dry objective with an ocular micrometer.

	Old mycelium cells	Young sclerotial cells
Potato	6.8 x 144 - 180	18 x 33
Carnation	7.2 x 120 - 300	15 x 33
Tomato	8 x 162 - 330	18 x 46
Barley	5 x 150 - 210	10 x 21
Oats	7 x 144 - 200	9 x 18
Velvet gr.	5 x 108 - 150	10 x 28
Strawberry	7 x 150 - 240	10 x 36
Strawberry B	8 x 160 - 300	14 x 25
Botryob. I	6 x 108 - 180	10 x 24
Botryob. II	6 x 70 - 140	10 x 23

The variation in length of old superficial cells is, according to the table, 70 - 33 μ, and in width, 5 - 8 μ; that of the young sclerotial cells lies between 18 - 46 μ and 9 - 18 μ. The stouter mycelium and larger sclerotial cells belong to the more vigorous and more virulent strains; these mycelia show also a darker color and the formation of larger or more numerous sclerotia. Short-barrel-shaped cells seemed at first characteristic of the Botryobasidium strains alone; but examination of mycelium of all stages showed that they were found in all strains, with the possible exception of that from potato, just before the formation of sclerotia. The sclerotia of the Botryobasidium strains are quite small, but the sclerotial cells are like those of the parasitic strains; and so no clear difference exists in that character.

One accustomed to working with a series of strains of Rhizoctonia can distinguish them, largely by macroscopic characters; but the differences are not readily described. As concise a description as is possible follows for each strain. Since the young cultures resemble each other more than the old, reference is to the latter.

1) Potato strain. Easily recognized on petri dishes by the abundant sclerotia and the presence on the large sclerotia of exuded drops of brown watery liquid, which when evaporated leave black scars on the surface of the

sclerotia. Formation of sclerotia commenced at the inoculum in the center of the dish and spread out to a diameter of 3-7 cm. In the space of two weeks many of the sclerotia germinated in place, by short white mycelia. Mycelium was in contact with the substratum, and never lifted as free aerial mycelium. Viewed from the underside the culture was brownish, with the part bearing the sclerotia dark brown.

2) Tomato strain. More like the potato strain than were any others; but with smaller, almost mealy, sclerotia, spread clear to the edge of the dish. The young sclerotia looked like mealy bugs or aphids on the mycelium. Sclerotia more pinkish than those of the potato strain.

3) Carnation strain. The most aggressive and rapid growing of all. Sclerotia so numerous and so minute as to form a powdery layer. Luxuriant aerial mycelium formed, reaching the cover of the petri dish. Brownish sclerotia distributed through the mycelial mat. The culture viewed from below is darker than any other.

4) Barley strain. Characterized by the formation of a white ring of mycelium, up to 5 cm. in diameter, in the middle of the dish, with a few scattered sclerotia inside the ring. Sclerotia dark brown, more prominent than in other strains, since they stand out as isolated bodies against the white background. Crystal-clear drops of

exudate always associated with the sclerotia. The culture is light brownish from below.

5) Oats strain. Characterized by formation of sclerotia in a ring, usually connected together and so forming a solid ring, along the periphery of the dish. Young sclerotia cream-colored, changing to pink, pinkish-brown, and finally dirty dark gray. Very minute scars of exudate drops visible on large old sclerotia.

6) Velvet-grass strain. A slow-growing form. Old culture powdery, with sclerotia formed usually in two concentric rings. The inoculum here, as in the oat strain, is usually also covered by sclerotia. Sclerotia in these strains less abundant than in the first three.

7) Strawberry strain. Characterized by the rarity of sclerotia; sometimes not one is present on a petri dish even in very old cultures. But when this mycelium is being grown in test-tubes along with susceptible seedlings sclerotia are formed along the edges of the culture and also on the host; consequently the lack of sclerotia in culture may be attributed to some nutritional deficiency. Mycelium more whitish than that of the other strains. Medium colored brownish, with dark spots which ordinarily would indicate the position of sclerotia. Clear concentric rings in the mycelium. Three cultures have been in stock at one time or another; one died out, and the second near-

ly did so, but had sufficient vigor that it could be kept alive. This and the third, isolated at different times and from different sources, are the same in appearance, growth rate, and other characters.

8) Saprophytic strain, derived by single-basidiospore isolation from Botryobasidium growing on rotten wood. Characterized by luxuriant mycelium and regular formation of short-barrel-shaped cells about the periphery of the dish. Sclerotia not ordinarily observed on the petri dish; present in tube cultures when the mycelium was associated with living seedlings; sclerotia from 0.2 to 1 cm. in diameter formed when the mycelium was grown on a medium containing only inorganic (nitrate) nitrogen, but then less brownish than those of parasitic strains. Medium colored light brown, as seen through the bottom of the dish. Five isolates, all the same in appearance.

The foregoing descriptions probably would not serve to identify an additional isolate with any one of the eight, in the absence of cultures of these strains; but they will serve to suggest general groupings, and to indicate the types of growth dealt with in these studies.

TECHNIQUE OF ISOLATION AND PURIFICATION

In these studies several isolation methods were used. The strawberry strain was derived from disinfected host tissue, the commonest method. Roots of strawberry showing lesions possibly caused by Rhizoctonia were washed thoroughly, disinfected in 0.1% $HgCl_2$ for 0.5 - 2 min., depending on the size of the root, and then rinsed in 3 - 4 changes of sterile water. The section of root bearing the lesion was cut out with a flamed scalpel and placed on agar on a petri dish. Mycelia which grew out of the cut ends of the root could then be transferred to stock culture. The second method was isolation from sclerotia; in this way were derived the potato mycelia. The sclerotia were treated as were roots under the first procedure. A third method was used to isolate the Rhizoctonia mycelium from diseased leaves of oats, barley, and velvet grass. Since the leaves are quite thin and delicate, neither $HgCl_2$ nor alcohol can be used to remove contaminants from the surface without killing the Rhizoctonia mycelium also. An improved filter-paper isolation method was here employed. Two layers of filter- or towel-paper, cut to fit, were placed in a petri dish. On them were scattered 20 - 40 8 - mm. paper discs cut from filter-paper with a punch. Enough water was added

to moisten the paper, and the whole then autoclaved. The diseased leaf was then placed in the center. In a few days, mycelia had grown out from the leaf and spread over the paper. Paper disks carrying mycelium were then transferred to agar plates. The advantage of the method is that mycelia can grow rapidly over the filter-paper, but bacteria scarcely at all. Care needs to be exercised in adding water; too much would serve to distribute bacteria, and too little would discourage the growth of mycelium. The fourth method of isolation is that involving transfer of single basidiospores, to be described later.

A simple purification method devised for these studies proved very successful. Melted agar was poured into a tilted petri dish, so as to leave at one side 3 cm. of the bottom uncovered. A 6 - mm. inoculum from a contaminated culture was placed 5 - 20 mm. distant from the margin of the agar. In a day or two the mycelium had reached the agar. It had been shown by microscopic examination that bacteria followed growing mycelium for only a short distance; the new growth made on the slanted agar was entirely free from bacterial contamination. The filter-paper isolation method and this method of purification are shown by photographs at the end of this account.

BOTRYOBASIDIUM AND ITS RELATION TO RHIZOCTONIA

Over a period of two years repeated attempts were made to obtain isolations from friuting-bodies of species of Botryobasidium, collected on decaying wood at various places near Corvallis. The mycelial growth which supports the basidia is so loose and thin that it is impossible to obtain from it tissue cultures which can with certainty be assigned to the Botryobasidium. Because of this looseness of the mycelium, it proved equally impossible to obtain basidiospores uncontaminated by bacteria or mold spores. The dilution method of isolation, spore-print method, micro-cutter method, and dry-needle method, all repeatedly employed on the best material available, gave no pure basidiospore cultures. Finally, when a micromanipulator became available, cultures were started from single basidiospores lifted off their sterigmata by a microneedle, under observation with the microscope. The material could not be attached directly to the cover-class of the micromanipulator moist-chamber, and so a short piece of glass rod was cemented to the cover with balsam, and a fragment of rotten wood bearing the hymenium stuck to the rod, with the upper margin in contact with the cover and the hymenial surface forming an angle of 100° with the cover, so that the lower part did not

interfere with the illumination. Before the cover slip bearing the fungus was placed on the microscope, a glass needle carried by the micromanipulator was adjusted so that its tip lay within one edge of the microscope field. When the material was placed on the stage the basidia and spores and the needle could all be seen at once. The needle was then brought in contact with a basidiospore; if the spore was mature it came off the sterigma readily, held by a tiny drop of agar on the needle's tip. The needle tip was then moved across a drop of solidified sterile agar on another cover-slip, leaving the spore to grow; the spore was incubated over a van Tieghem cell. For each operation a new needle was drawn and bent over a micro-flame; a satisfactory diameter for the tip is 10 μ. Dipping the tip in a tube of melted agar served to coat it with a thin film, to which the basidiospore would adhere. Of 200 such cultures, five were successful -- uncontaminated, and clearly the development of Botryobasidium spores. Many failed completely to germinate.

The mycelium produced by these five isolates compares well with that of the fungi from which they were derived. The characteristic right-angled and cruciform branching, the thick brownish walls of the older hyphae and the thin and hyaline younger strands are as in the Botryobasidium. The cells of the aerial mycelium are 6 - 7 μ thick and

50 - 200 μ long. The growth in the petri dishes was very vigorous. When the culture grew old peculiar short cells were produced, mostly 7 - 10 together, barrel-shaped, and 25 - 32 x 14 μ. These cells are not conidia or oidia, since they never separate. In contact with the glass of petri dish or test-tube they are interwoven much as are the elements of a sclerotium; but the groups never attain the size of the sclerotia of other strains. They did, however, approach closely those of the strawberry strain.

The fruiting body from which the Botryobasidium mycelia were derived was largely composed of brownish hyphae whose cells measured about 50 x 9 μ; the basidia were evenly cylindric, about 15 x 7 μ; the spores, borne 4 - 6 to a basidium, measured about 10 x 4 μ. In all points the fungus agreed with Botryobasidium vagum (Berk. & Curt.) as described by Rogers. That fungus is known only as a saprophyte; but in the inoculation experiments later reported the Botryobasidium isolates attacked radish, sunflower, and bean seedlings, although not very vigorously. From these experiments it then follows that B. vagum on rotten wood has as its imperfect stage a Rhizoctonia very near R. Solani or even included within that form species, and capable of parasitism.

INOCULATION EXPERIMENT

In the summer of 1939, 10 pots each of some 50 kinds of plants were started and grown in the greenhouse as a cross-inoculation test. But the temperatures were too high; many of the strains lost their virulence; and because of the difficulty of washing out large root systems intact and the impossibility of preventing contamination of the cultures, the results were both difficult to observe and of doubtful significance. The need for a better method for inoculation tests was apparent. After many trials and modifications, the method here summarized was determined upon. Seeds were soaked for 30 min. in water and then placed in a four-inch section of one-inch glass tubing closed at one end with a piece of cheese-cloth. The seeds in this tube were dipped in 95% alcohol for 0.5 - 2 min., depending on size and the character of the seed coat, washed in running water for 1 min., dipped in 0.1% $HgCl_2$ for 0.5 - 2 min., and rinsed with four or five changes of sterile distilled water. The seeds were then aseptically transferred to a sterile germination chamber consisting of a petri dish with two layers of filter paper in the bottom. To the petri dish were then added 4 - 20 cc. of a nutrient solution, made up as follows:

$Ca(NO_3)_2$	0.8 g.
KNO_3	0.2 g.
KH_2PO_4	0.2 g.
$MgSO_4$	0.2 g.
$FePO_4$	0.005 g.
Water	1 liter

The seeds were left for 3 - 7 da., until they had germinated. Meanwhile tube cultures of the Rhizoctonia strains had been started, so as to have 2 - 7 days' growth before seedlings were ready. One to three seedlings, depending on their size, were transferred aseptically to each fungus culture, a few cc. of the culture solution pipetted into the tube, and the double culture set aside for observation. Checks -- tubes with seedlings but no fungus -- were grown for comparison, in addition to four tubes for each combination of plant and Rhizoctonia. Of 400 tubes only two or three became contaminated. If the fungus was able to attack the plant, symptoms appeared in 2 - 14 da. These were readily visible since even the finest roots could be observed without injury, and even very small discolored areas or cankers were easily visible. An additional advantage of the method is the complete elimination of the activity of organisms other than the one tested, and a still further advantage, the brevity of the time within which accurate date can be obtained.

The only difficulty is that connected with the necessity of performing every step aseptically. The data are summarized in the following table, where 0 indicates absence of infection and 1 - 4 increasing virulence. Nine strains of Rhizoctonia and 11 hosts are represented in this test.

host	car.	bar.	oat.	pot.	str.	tom.	vel.	bot.1	bot.2	check
radish	4	1	1	3	1	3	2	2	2	0
red clover	4	1	1	3	1	3	1	2	2	0
barley	2	3	3	0	0	1	2	0	0	0
oats	2	3	3	0	1	1	2	0	0	0
corn	4	2	2	0	1	1	4	1	1	0
field bean	3	2	1	1	2	1	1	0	1	0
garden bean	2	1	1	2	1	2	2	2	2	0
squash	3	1	0	1	0	2	0	2	3	0
sun flower	4	2	2	3	1	3	1	2	2	0
tomato	4	1	0	3	2	4	2	1	1	0
lettuce	4	2	1	3	0	1	3	3	3	0

Of the strains tested, the isolate from carnation is by far the most aggressive. Every seedling in the tubes was killed; and in a preliminary test in which seeds instead of seedlings were placed on the mycelium, 90% of the seeds failed to germinate. The tomato and potato strains came next in order of virulence, and following them barley, oats, and velvet grass. The strawberry strain was very weak. The two strains derived from basidiospores of B. vagum were able to attack the seedlings, but were less virulent than any except the strawberry isolate. It is also clear from the table that the three strains placed in contact with their original hosts -- oats, barley, and tomato -- produced more severe injury than on any other hosts. In this connection other experiments performed in the investigation of susceptibility and virulence should be mentioned. One was a greenhouse experiment in which three flower-pots of potatoes were inoculated with each strain. Even though other strains attacked somewhat the roots and stems, none produced sclerotia on the tubers except the potato isolate, which formed them abundantly, both on the seed pieces and on the young tubers. The second was an experiment in which strawberry plants were inoculated. None of the strains produced root lesions or rot except the ordinarily only moderately vigorous strawberry strain, which even produced a basidial

hymenium on one leaf, and basidiospores with nearly the same measurements as some examined on potato stems. The third experiment was performed with the tops of tomato plants, cut away from the roots while held under water, and placed upright in test-tubes of nutrient solution, with a plug of cotton to hold the stem in place, the cut end in the solution, and most of the top extending into the air. A 6-mm. disk of the mycelium was placed in contact with the uninjured stem just above the cotton plug and held in place with more cotton; a little sterile water was dropped on the cotton to keep the inoculum moist. After one week lesions were observed on several stems, the most severe being produced by the carnation, tomato, and potato strains, which were about equal; less severe infection resulted from inoculation with velvet-grass and strawberry isolates; the others showed no symptoms. The results here reported are regarded as reliable indications of relative virulence; the method of seedling inoculation seems particularly useful for testing the activity of Rhizoctonia, which is a principal pathogen of seedlings.

MEDIUM EXPERIMENT I

The purpose of the first experiment was to determine whether organic or inorganic sources of nitrogen and carbon are more favorable for the growth of Rhizoctonia. $CaCO_3$ was used as the inorganic carbon source and dextrose as the organic; KNO_3 was the inorganic nitrogen source, and peptone the organic. Seven kinds of agar (1.8%) were prepared, with the nutrients added in the amounts per liter given below. Petri dishes of each medium were inoculated with the tomato strain of Rhizoctonia. Media numbers 3 and 4, containing $CaCO_3$, were not transparent, and consequently measurements of early growth could not be made; growth on other media was measured daily for four days. The results follow; each figure below is the sum of the growth on five petri dishes, measured in millimeters.

Medium No.	Nutrients	Character of mycelia	Sclerotia	Diameter of mycelium			
				1 da.	2 da.	3 da.	4 da.
1	dextrose 20g.	aerial mycelium colorless	no sclerotia in 1 wk.; a few minute ones in 1 mo.	104	222	335	442
2	dextrose 20g. peptone 10g.	strong growth; brown	plate covered with well-developed sclerotia in 1 wk.	105	235	353	475

Medium No.	Nutrients	Character of mycelia	Sclerotia	Diameter of mycelium 1 da.	2 da.	3 da.	4 da.
3	$CaCO_3$ 20g.	growth as feeble as on No. 7	none	---	---	---	409
4	$CaCO_3$ 20g. peptone 10g.	weak; brown	none	---	---	---	400
5	peptone 10g.	weak; brown	none	103	194	252	294
6	dextrose 20g. KNO_3 10g.	weaker than on No. 2; brown	sclerotia, but fewer and less developed than on No. 2	75	187	281	384
7	none	scant, poor, almost invisible; slightly brown at point of inoculation	2 or 3 after 1 wk.	122	247	355	475

It is apparent that $CaCO_3$ cannot be utilized as a source of carbon, or is even toxic; that dextrose is a good source of carbon; and that KNO_3 can be utilized, but not so well as peptone. The brownish color of the mycelium depends on abundant available nitrogen compounds; dextrose alone never gave this color. For the formation of sclerotia both carbon and nitrogen must be abundantly available. Rapid

growth can be made for the period of the experiment on glucose medium alone; possibly a minute amount of nitrogen was present in the agar used to solidify the media; or it may have been absorbed as ammonia from the air. Certainly a trace was carried with the inoculum from the stock culture.

MEDIUM EXPERIMENT II

It has been stated that application of $(NH_4)_2SO_4$ to soil infected with Rhizoctonia will improve the crop and reduce the amount of infection by the fungus; the present experiment was carried out to test the effect of this salt on the fungus in pure culture. To a basic medium containing 10g. of glucose, 1g. of K_2HPO_4, and 20g. of agar to the liter, $(NH_4)_2SO_4$was added at rates of 2.4g. and 5g. to the liter. Petri dishes of the basic medium (used as a check) and of each of the two modifications were inoculated with the various strains of Rhizoctonia; growth was measured at the end of two and of four days. The results are summarized in the following table and graph; each figure represents the sum of the diameters of the growth on two petri dishes, measured in millimeters.

		Car.	Bar.	Oat.	Pot.	Str.	Vel.	Total
Check	2 day	11.2	46	51	65	44	44	362
	4 day	18.7	96	96	123	84	77	662
0.25% $(NH_4)_2SO_4$	2 day	10.5	39	44	62	35	41	326
	4 day	18.0	60	85	118	68	74	585
0.5% $(NH_4)_2SO_4$	2 day	8.7	37	46	52	36	33	291
	4 day	15.2	55	80	100	60	66	513

It is clear that in the concentrations employed $(NH_4)_2SO_4$ inhibits the growth of Rhizoctonia. On the medium containing 0.25%, growth was an average of 14.4% less than in the check cultures; on the 0.5% medium, 26.4% less.

MEDIUM EXPERIMENT III

The purpose of this experiment was to determine the influence of different organic and inorganic nitrogen compounds on growth of Rhizoctonia. The basic medium used contained 3.4 g. (M/40) of KH_2PO_4, 0.6 g. (M/200) of $MgSO_4$, 23 g. (M/8) of dextrose, 0.01 g. of $FeCl_2$, and 20 g. of agar to the liter. To five lots of this medium were added, singly, to each liter of basic media, the following nitrogen compounds, in the amount indicated: 1) KNO_3, 10.11 g.; 2) $NaNO_3$, 8.501 g.; 3) $(NH_4)_2SO_4$, 6.607 g.; 4) NH_4HO_3, 8.501 g.; 5) peptone, 8.89 g. The media numbers 1 to 4

contained N/10 solutions of the various salts; the amount of peptone was such as to supply the same amount of nitrogen as a N/10 solution of $NaNO_3$. The basic solution was used as a check. Petri plates of these six media were inoculated with the various strains of Rhizoctonia, and growth measured at the end of two days. Each figure in the table is the sum of the diameters of growth on two petri dishes, measured in millimeters.

Medium No.	1	2	3	4	5	6
Nitrogen Compound	KNO_3	$NaNO_3$	$(NH_4)_2SO_4$	NH_4NO_3	peptone	check
pH	4.1	3.6	3.75	3.85	4.7	5.0
Carnation	133	128	98	103	152	147
Barley	50	49	40	40	74	52
Oats	46	47	44	37	61	64
Potato	52	45	37	33	74	59
Strawberry	56	54	76	34	78	71
Velvet Gr.	38	38	44	34	56	38
Ave.	62.5	60.1	55.6	47.1	81.8	74.8

As is shown by the table, the best growth appeared on medium number 5, made up with peptone; not only was growth most extensive on this medium, but the mycelium appeared most vigorous, and sclerotia developed in abundance. The next most favorable media were numbers 2 ($NaNO_3$) and 1 (KNO_3); of these, number 1 supported a slightly more rapid

growth, but number 2 a more vigorous-appearing mycelium. Growth on numbers 3 ($(NH_4)_2SO_4$) and 4 (NH_4NO_3) was poor, being irregular rather than uniform in all directions, and, especially on number 3, with weak, scattered mycelia, often erect rather than appressed to the substratum. Even number 6 (control) was more favorable than 3 and 4.

From this experiment it is clear that the organic nitrogen source peptone is more favorable for the growth of Rhizoctonia than the inorganic salts employed, and that of the latter the nitrates are more favorable than the ammonium salts. $NaNO_3$ seemed slightly better than KNO_3, and NH_4NO_3 distinctly better than $(NH_4)_2SO_4$. Ammonium salts seem actually toxic to the fungus; since this is the contrary of their effect on higher plants, the use of ammonium salts seems a promising method of control.

MEDIUM EXPERIMENT IV

The purpose of this experiment was to determine the most favorable medium (of a number used in routine culturing of fungi) for the cultivation of various strains of Rhizoctonia. Seven were tested, made up as follows:

1) Corn meal agar

Corn meal, 4 teaspoonsful -- cooked in 1 l. distilled water at 60° C. for 1 hour, and the

solid material then strained off.

Agar, 10g. -- added to the above liquid, and the whole then steamed 45 min., filtered, and tubed.

2) Green bean agar

Green string beans, 300g. -- cut, steamed in 0.5 l. water for 1 hour, and strained.

Agar, 15g. -- dissolved in 0.5 l. water, added to the first liquid, and the whole stirred with whites of 2 eggs, autoclaved at 15 lb. for 10 minutes, filtered, and tubed.

3) Oat meal agar

Rolled oats, 200g. -- steamed in 1 l. water for one hour, and strained.

Agar, 15g. -- added to the above liquid, and the whole then steamed for 30 min., strained, and tubed.

4) Potato agar

Potato, peeled and sliced, 300g. -- steamed in 1 l. water for 1 hour, and strained.

Agar, 15g. -- added to the liquid, steamed 30 min., stirred with the whites of 2 eggs, and the whole then autoclaved for 10 min., filtered and tubed.

5) Potato glucose agar

Glucose, 20g. -- added to 1 l. of medium No. 4.

6) Cook's agar

Agar, 15g. -- steamed in 1 l. distilled water for 45 min.

Glucose, 20g. --

NH_4NO_3, 1g. --

KNO_3, 1g. --

$(NH_4)_2SO_4$, 1g. --

$MgSO_4$, 0.25g. --

K_2HPO_4, 0.25g. -- added to liquid, and the whole then steamed for 30 min., filtered, and tubed.

7) Cook's II agar

Agar, 18g. -- steamed in 1 l. distilled water for 45 min.

Glucose, 20g. --

Peptone, 10g. --

$MgSO_4$, 0.25g. --

KH_2PO_4, 0.25g. -- added to the liquid, and the filtered and tubed.

All of these media were autoclaved in the tubes for 20 min. at 15 lb. pressure. Ten cc. of each medium were used for each petri dish. Different plates were inoculated with a disk of inoculum 6 mm. in diameter of each of five strains of Rhizoctonia, and the growth measured once a day

until the mycelium had reached the edge of the dish (9.5 cm. in diameter). In the table below are listed the numbers of days and hours required for thy mycelium to reach the edge of the petri dish; such figures could not be obtained directly except with hourly measurements; they are read from a curve constructed from the diameters and times of the daily readings.

Medium No. Medium name pH	1 CMA 6.35	2 GBA 6.30	3 OA 5.94	4 PA 6.11	5 PDA 6.11	6 OCA 5.81	7 CA 5.90
Strain							
Potato	5:1	3:13	3:04	3:09	3:08	7:20	3:08
Oats	5:3	5:	4:18	5:01	5:05	11:14	4:22
Velvet	5:7	5:03	5:	5:	5:06	15:20	5:04
Strawberry	9:	8:15	9:03	8:18	7:16	8:14	7:20
Barley	5:6	5:02	5:01	5:	5:03	11:18	5:05
Average for the medium	5:23	5:12	5:10	5:11	5:08	11:03	5:07

It is clear that from rapidity of growth alone little difference can be shown among the media tested, except for number 6. In this medium the nitrogen sources are $(NH_4)_2SO_4$ and NH_4NO_3; it has already been shown that ammonium is toxic to the strains of Rhizoctonia being studied; presumably the greater acidity of this medium is also unfavorable. The mycelium produced on this medium was very poor, and

sclerotia appeared only near the transfer disk, while they occurred abundantly on all other media. The value of the other media was judged chiefly by the color and vigor of the mycelium and the abundance of sclerotia. Medium number 7 (Cook's II) was easily the best; the order of desirability of the others was 3, 5, 4, 2, 1. For the remainder of the experiments, except where a special medium was required, Cook's II was used. It not only gives the best growth, but also is easily prepared, and so long as a single brand of peptone is used, can be kept uniform in composition.

SUGAR FERMENTATION

The purpose of this experiment was to determine what sugars can be utilized by Rhizoctonia, whether there is any difference in the fermenting power of the different strains, and, if possible, the relation of such differences to differences in virulence. Two basic types of liquid media were used:

1) Nutrient sugar media (containing nutrients of unknown chemical composition)

Meat extract, 3 g.

Peptone, 5 g.

Sugar, 10 g.

2) Synthetic sugar media (containing, in addition to sugar, only salts)

$(NH_4)_2SO_4$, 1 g.

KCl, 0.2 g.

$MgSO_4$, 0.2 g.

Sugar, 10 g.

With each of these the sugars dextrose, sucrose, and lactose were used. The ingredients were steamed in 1 l. of water for 30 min. and adjusted to a pH of 7; 10cc. of 0.25% aqueous solution of the indicator bromcresol purple were then added, to give an indicator concentration of about 0.0025%. The media so prepared were tubed, about 10 cc. being used in each tube; a small fermentation tube was then placed in each tube, mouth down; the tubes were then plugged and autoclaved for 20 min. at 15 lb. After the autoclaving the medium rose inside the small fermentation tubes and filled them. Inocula of the various strains of Rhizoctonia were added. The cultures were placed where they would not be shaken, and the amount of time required for the color of the medium to change from purple to yellow was determined. This color change commenced near the inoculum and gradually extended to the whole body of medium; it occurred first outside, and later inside, the small tube. In no case was gas collected in the small tube; however, bubbles sometimes appeared outside the small tube, in

very old cultures, under a dense felt of mycelium. To make certain whether these bubbles were a product of the metabolism of the fungus, the experiment was repeated with duplicate sets of tubes, in one of which the inoculum was inserted in the small tube, and in the other, left outside. Again no gas was collected in the fermentation tubes; and consequently it is judged that none is released by Rhizoctonia. The observations on the time of color-change are tabulated below. The numbers indicate the order in which complete change from purple to yellow occured in the various cultures; thus the lower the number the more rapid the fermentation in that culture.

	Carnation	Barley	Oats	Potato	Strawberry	Velvet	Total
ND	17	20	22	23	13	24	119
NS	19	25	26	27	35	29	161
NL	18	21	30	28	34	33	164
SD	6	15	9	4	14	12	66
SS	1	10	8	2	36	11	68
SL	5	7	32	3	31	16	94
Total	66	98	127	87	163	125	

From these results it is clear, first of all, that sugar fermentation is more rapid in the synthetic than in the nutrient media. This is probably attributable to the greater abundance of available food materials in the media

containing peptone and meat extract in addition to the sugar; probably the fungus utilized the sugars much more slowly when a considerable part of its food came from the other nutrients. But it is possible also that the difference was brought about by the fact that the nutrient media were much more highly buffered, and that in consequence the fermentation of a given amount of sugar would produce a slighter color-change. That the former--decreased utilization of sugar in media containing other organic nutrients--is the true explanation of the differences between the two sets of cultures was demonstrated by sugar titration, by the very delicate method of Shaffer and Hartman. This test showed in all cases that more sugar remained in the nutrient than in the synthetic media.

The experiment also shows that lactose is the sugar least easily fermented, in either nutrient of synthetic medium, and by all strains of Rhizoctonia. The strawberry and oat strains, in particular, scarcely used it at all. This is what would be expected from the origin and chemical structure of lactose. This sugar is present in quantities only in milk, and never found in plant tissues. Like the great majority of fungi, Rhizoctonia derives its food from living or dead plant tissues, and has no occasion for digesting lactose. The fermenting power, such as it is, of Rhizoctonia in lactose media is probably not the power of

acting directly on lactose. In the autoclave lactose is partly broken down into glucose and galactose. It has been shown that only with difficulty can lactose be hydrolized by such microorganisms as bacteria, and that when it is hydrolized only the glucose residue can be utilized; lactose itself is even toxic to bacteria. Another factor is that the favorable point for the action of lactase is pH7, a point too high for active growth of Rhizoctonia, even if this fungus could produce lactase.

Of the other sugars dextrose is the more rapidly attacked in the nutrient medium, and sucrose in the synthetic. Two factors enter here, acidity of the medium, and complexity of the sugars. The most favorable range for the action of sucrase, or invertase, is pH 4.2-5.2; in the poorly-buffered synthetic medium this degree of acidity will be more quickly attained than in the well-buffered nutrient medium. On the other hand, the most favorable range for the assimilation of dextrose is around the neutral point, and so the dextrose will be more readily utilized in the nutrient medium. As for the availability of other nutrients: Since no other nutrients are present in the synthetic medium, sucrase would presumably be there secreted abundantly and the sucrose vigorously broken down and absorbed. In the nutrient medium, pepsin, trypsin, and other proteases would be secreted, and a smaller amount of sucrase

than in the synthetic medium; but dextrose, more readily available than the other substances, would be rapidly absorbed.

Of the various strains of the fungus, the carnation strain is shown in the table to be most active in the fermentation of sugar, and after it, in order, the potato, barley, velvet-grass, oats, and strawberry strains. This order is nearly the same as the order of destructiveness of these strains on various hosts, as will be shown later. A supplementary test which included strains of mycelium arising from single-spore isolations of saprobic species of Botryobasidium showed the latter to be much weaker in fermentive power than any of the parasitic strains of Rhizoctonia; this serves as confirmation of the relation stated between fermentive power and pathogenicity.

HYDROLYSIS OF STARCH

The purpose of this experiment was to determine whether differences exist among the various strains of Rhizoctonia in their ability to hydrolyze starch. Starch is of course not a single chemical substance; the intermediate products in the hydrolysis of starches from different plants are sometimes different, although all finally yield dextrose as the main product; some starches yield small

amounts of phosphoric acid. Two kinds of starch were used in the experiment: commercial corn starch, of the "Red and White" brand, and Merck's soluble starch. The commercial corn starch consists of amylose and amylopectin in about the proportion of 2:1. The amylose is found in the interior of the granules; it is soluble in water and colored blue by iodine solution; the structure formula is essentially like the disaccharides, except for being about twelve times as large, and thus containing 24 linked glucoside residues. The amylopectin occurs in the outer layers of the granules; it is insoluble in water and gives no color reaction with iodine; it is more resistant to hydrolysis than the inner portion; in it is contained most of the phosphorus, presumable as a phosphate ester. The soluble starch is prepared by treating starch granules with dilute HCl or H_2SO_4, which acts on the amylopectin so as to cause it to disintegrate rapidly when heated with water. Starch can be hydrolyzed in the laboratory by mineral acids or by diastase (amylase). When starch is hydrolyzed by diastase there is first formed soluble starch, which gives a blue color reaction with iodine, next erythrodextrin, pink or red with iodine, again achroodextrin, colorless with iodine, and finally maltose. In acid hydrolysis of starch the same reactions take place, and a further one: the maltose is hydrolyzed to form glucose.

In enzyme hydrolysis a second enzyme, maltase, is needed to complete the breaking down of starch.

Two kinds of media were used for the experiment, starch broth and starch agar, each prepared with both kinds of starch. Corn starch was mixed into a paste with hot water and then stirred into the required quantity of boiling water; soluble starch was added directly. The broth contained

Meat extract, 3 g.

Starch, 10 g.

Water, boiling, 1 liter, the whole adjusted to pH 6.5, filtered, tubed, and sterilized at 15 lb. for 30 min. Starch agar contained the same ingredients as starch broth, solidified with 20 g. of agar to the liter, and poured into petri dishes after sterilization. Tubes and plates were inoculated with the various strains of Rhizoctonia and tested over a period of five days with iodine solution, to determine the extent of hydrolysis. The broth was tested by withdrawing 1 cc. and mixing it with 1 drop of iodine solution (Lugol's: 5% I, 10% KI in water) on a drop plate, observing the color reaction; the agar was tested by placing a small amount of iodine solution on the margin of the growth. No appreciable differences appeared in the reaction of the corn starch and soluble starch media; presumably the corn starch was hydrolyzed to the soluble

starch stage in autoclaving. The reactions of all strains in the petri dishes were about the same: a clear area (unstained by iodine) underlying the mycelium and extending beyond its margin; hydrolysis must then occur as the result of the action of extracellular diastase and maltase. The remainder of the agar was blue; no intermediate colors appeared. The results of observations on the starch broth of the test-tube cultures follow:

	Carna.	Potato	Tomato	Strawb.	Barley	Oats	Velvet Gr.
1 day	3	4	4	4	4	4	4
2 days	1	2	2	2.5	3	3	3
3 days	0	1	1	1	1	1	1
5 days	0	0	0	0	0	0	0

4 = blue black 3 = blue 2 = bluish pink

1 = pink 0 = colorless

The hydrolyzing power of the different strains is therefore different. It is highest in the carnation strain, and decreases for other strains in the following order: potato, tomato, strawberry, barley, oats, and velvet grass.

GELATINE LIQUEFACTION

The purpose of this experiment is to determine

differences in the extent of gelatine liquefaction brought about by the various strains of Rhizoctonia. Gelatine is a protein prepared by acid hydrolysis of the insoluble protein collagen contained in cartilage and connective tissue. Gelatine cannot furnish all of the amino acids necessary for growth, but can support life for a time. The gel which it forms after being dissolved in warm water and cooled can be attacked and destroyed by gelatinase secreted by microorganisms. The medium used contained the following:

Meat extract, 3 g.
Peptone, 5 g.
Gelatine, 150 g.
Distilled water, 1 liter, the whole heated to boiling, adjusted to pH 7.00, filtered, tubed, and sterilized for 3 successive days at 100° C. for 20 min. each.

The Rhizoctonia mycelia were transferred to the surface of the medium in tubes. The depth of the layer of liquefied gelatine in centimeters was measured in 1 wk., 6 wk., and 16 wk. The results follow:

	Potato	Tomato	Carna.	Barley	Oats	Strawb.	Velvet G.
1 wk.	2.5	2.4	2.0	2.0	2.0	2.0	1.3
6 wks.	3.9	3.6	3.4	3.0	2.8	2.6	2.6
16 wks.	5.6	5.6	5.5	4.6	4.5	4.5	4.5

The potato and tomato strains of Rhizoctonia gave the greatest liquefaction, and after them, in order, carnation, oats, barley, strawberry, and velvet grass. The liquefied part in cultures of the first three had a strong brownish color, the strawberry strain a faint brownish color, and the others none at all. At the last reading (16 wk.) the carnation, potato, and tomato strains had produced a dark, smoky brown color which penetrated into the solid gelatine to a depth of 0.5 -- 1 cm.; a layer of mycelium was only visible at the surface. None of the others showed any mycelium at this time.

Sugar was omitted from the medium because the acid resulting from carbohydrate hydrolysis would retard the digestion of gelatin, and the presence of available carbohydrate food would also reduce the amount of gelatinase elaborated. Since there was no sugar, no great amount of mycelium was produced in any of the cultures; and finally, much of the mycelium was destroyed by the action of its own enzymes. The amount of gelatinase produced after the first few days was very slight; the curve which follows,

constructed from the data already given, shows by the slowing down of liquefaction the low rate at which the enzyme is produced.

REDUCTION OF NITRATE TO NITRITE

This experiment was performed to determine whether Rhizoctonia can reduce nitrate to nitrite, and whether differences in this respect exist among the various strains. The medium employed consisted of:

Meat extract, 3 g.
Peptone, 5 g.
KNO_3, 1 g.
Water 1 liter, the solution adjusted to pH 6.5, filtered, tubed, and sterilized for 30 min. at 15 lb.

Each strain was introduced into three tubes of this medium. The tests for reduction of the nitrate were made by adding 3 drops of Tromsdorf's reagent and 2 drops of 33% H_2SO_4 to the culture and observing the resulting color. A blue color indicates the presence of nitrite -- and hence, of reduction of nitrate; a colorless solution indicates the absence of nitrite -- and thus either the absence of reduction or reduction which has been carried to the point of yielding ammonia. The results follow:

	Car.	Pot.	Tom.	Bar.	Oats	Straw.	Vel.	Check
2 days	1	2	2	3	3	3	3	0
7 days	0	1	1	3	3	3	3	0
21 days	0	0	0	1	1	0	1	0

3 = dark blue 2 = blue 1 = reddish blue 0 = colorless

The data show that all strains reduce nitrate, and that the rapidity of reduction varies considerably with the strain. The carnation strain reduced all nitrates through the nitrite stage to ammonia in 7 da.; the tomato and potato strains showed only a slight nitrite reaction in 7 da. and complete reduction in 21 da. The other four showed a strong nitrite reaction the 7th day; in 21 da., the strawberry strain showed no nitrite and the barley, oats, and velvet-grass strains had still failed to reduce the nitrite completely. Tests of the media in which no nitrite was shown, made with Nessler's reagent, confirmed the presence of ammonia. Early in the experiment the media became acid, a condition favoring reduction of NO_2 to NH_3. The experiment could probably have been hastened by sealing the tubes, the lack of atmospheric oxygen then acting to increase the rate of nitrate reduction.

LIGHT AND GROWTH

The purpose of this experiment was to determine the relation between illumination and the growth of Rhizoctonia mycelium. The light was furnished by a 120 V., 75 W. lamp placed two feet from the petri-dish cultures. The cultures were treated in five different ways:

Group A. The cultures were placed in a paper carton, wrapped in three layers of heavy black paper, and placed in a dark locker. They received light only briefly, when measurements were being taken.

Group B. Exposed to light 1 hr. a day; the rest of the time treated as were the cultures in group A.

Group C. Exposed to light 3 hr. a day.

Group D. Exposed to light 24 hr. a day.

Group E. Placed on the laboratory shelf, and exposed to the ordinary day-and night-alternation; used as a check.

Measurements were made once a day. The figures given are for the fourth day, and represent the sums of the diameters of mycelium in two cultures.

Group	A	B	C	D	E
Time in light (Hours)	0	1	3	24	check
Potato	125	128	129	113	135
Oats	98	103	103	70	97
Barley	102	100	101	43	94
Velvet grass	66	84	109	33	70
Strawberry	110	95	93	32	78
Total mm.	501	510	535	291	479

It is clear from the table that the best growth was made by the cultures of group C, exposed to 3 hours of light each day. There follow in order B, A, E, and D. The growth of group D, exposed to continuous illumination, was only 3/5 of that of the check, grown under the usual laboratory conditions; continuous illumination is then highly unfavorable to the development of Rhizoctonia. Continuous darkness is unfavorable also, but not equally so. The retarding effect of prolonged illumination in the laboratory is consistent with the habit of the mycelium in the field, where it grows in partial or complete darkness.

The cultures grown in this experiment showed also the relation between light and the formation of concentric rings in the mycelium. Rings were formed in the cultures exposed to the light only long enough for measurements to be made, and occurred along the line reached by the myce-

lium when the measuring was done; the same is true of those exposed 1 hr. and 3 hr. to light. The rings were less clear in the cultures kept on the shelf, and absent from those exposed continuously to light. The clearness of the rings varied with the strain and also (as observed in a later experiment) with variation in acidity of the medium.

TEMPERATURE AND GROWTH

The object of this experiment was to determine for each of the strains of Rhizoctonia on hand the optimum temperature for growth. Seven strains were tested at six temperatures each -- 5°, 10°, 20°, 30°, 37°, and 45° C. Fresh petri-dish cultures were placed in incubators whose temperatures varied only within 1° C. Growth was measured five times in a period of one week. The complete data follow:

	Day	Car.	Bar.	Oats	Bot. I	Str.	Pot.	Tom.
5°	0.0	.6	.6	.6	.6	.6	.6	.6
	2.0	.6	1.3	.7	1.6	.7	.7	.7
	3.5	.6	1.7	1.1	2.2	.7	1.0	1.0
	5.0	.6	2.1	1.5	2.8	.8	1.3	1.3
	6.5	.6	2.4	1.9	3.3	.9	1.6	1.6
	8.0	.6	2.8	2.3	3.9	1.0	1.9	1.9

	Day	Car.	Bar.	Oats	Bot. I	Str.	Pot.	Tom.
10°	0.0	.6	.6	.6	.6	.6	.6	.6
	2.0	2.4	2.6	2.0	3.0	1.2	1.6	3.7
	3.5	3.0	3.6	2.4	3.5	1.5	1.9	4.4
	5.0	3.2	4.8	3.4	4.8	2.4	2.5	6.0
	6.5	3.3	6.0	4.4	6.4	3.0	3.6	7.6
	8.0	4.0	7.2	5.6	8.0	3.6	4.6	9.5
20°	0.0	.6	.6	.6	.6	.6	.6	.6
	2.0	9.0	4.0	3.5	6.3	4.3	4.6	4.9
	3.5	9.5	6.6	5.8	9.5	7.0	7.5	8.0
	6.0	9.5	9.2	8.1	9.5	9.5	9.5	9.5
	6.5	9.5	9.2	9.5	9.5	9.5	9.5	9.5
	8.0	9.5	9.3	9.5	9.5	9.5	9.5	9.5
30°	0.0	.6	.6	.6	.6	.6	.6	.6
	2.0	6.3	1.3	1.2	1.7	2.9	1.5	2.1
	3.5	9.5	1.3	1.2	1.7	5.4	1.5	2.2
	5.0	9.5	1.3	1.2	1.7	7.2	1.6	2.2
	6.5	9.5	1.4	1.2	1.8	9.5	1.6	2.3
	8.0	9.5	1.4	1.2	1.8	9.5	1.6	2.4
37°	0.0	.6	.6	.6	.6	.6	.6	.6
	2.0	2.5	1.2	1.1	1.5	3.3	1.1	2.0
	3.5	2.6	1.2	1.2	1.6	4.8	1.2	2.1
	5.0	3.1	1.2	1.2	1.6	5.7	1.2	2.1
	6.5	3.4	1.2	1.2	1.7	5.7	1.2	2.1
	8.0	3.7	1.2	1.2	1.7	5.8	1.3	2.1
45°	0.0	.6	.6	.6	.6	.6	.6	.6
	2.0	1.7	1.1	1.1	1.5	1.0	1.1	1.9
	3.5	1.7	1.1	1.1	1.5	1.0	1.1	1.9
	5.0	1.8	1.1	1.1	1.6	1.0	1.2	1.9
	6.5	1.8	1.2	1.1	1.6	1.0	1.2	1.9
	8.0	1.9	1.2	1.1	1.6	1.0	1.2	1.9

From this table it is clear that 45° and 37° C. were too high for all strains. At 30° C. five of the seven strains, after making a small initial growth, ceased development long before the end of the period; and only two, those isolated from carnation and strawberry, were able to

continue. Of the temperatures tested, 20° C. proved to be the optimum for all strains. Growth was much lessened at 10°, and was very slow at 5°, for all except carnation and strawberry strains. To furnish a basis for comparison of the different isolates, the following table is presented, showing the number of days required at 20° for the mycelia to reach the margin of a 95 mm. petri dish.

	Car.	Bot. I	Tom.	Pot.	Str.	Bar.	Oats
For 95 mm. growth	2.04 *	3.04	4.05	4.16	4.20	5.05	5.22

* It reads as 2 days and 4 hours.

These figures are derived from the curves of growth also here presented.

It is probable that the exact optima departed two or three degrees from 20° C.; apparatus was not available to determine these figures more precisely. The results compare well with the experiment of Richards, who found that the greatest damage from Rhizoctonia to potato crops occurred between 15° and 21° C., that damage was inconsiderable above 24°, and that none occurred below 5°. Richards found also that in a year with average soil temperature of 20.1° a loss of 50% in the potato crop occurred but in a year with the average 23°, only 15.45. The optimum may then be considered about 20° C., both for labora-

tory culture and for field development of Rhizoctonia.

HYDROGEN ION CONCENTRATION AND GROWTH

In this experiment, to determine the response of Rhizoctonia to variation in pH, six strains of the fungus were tested. Oatmeal agar, in petri dishes, was the medium employed; H_2SO_4 and NaOH were used for adjusting the reaction: the pH was determined by the glass-electrode method. In the beginning, samples of the agar were kept melted at 45° C; to one was added (in six small amounts of N/20 solution) enough NaOH to raise the pH to about 10; to another (in eight small amounts of N/20 solution) enough H_2SO_4 to lower the pH to 3. From a curve drawn from the quantities of NaOH and H_2SO_4 added and the resulting pH were determined the amounts of acid or base needed to produce reactions intermediate between pH 3 and pH 10. Wherever more than 10cc. of N/20 solution was needed for the adjustment of 100cc. of the medium, N/1 solution was used, to avoid over-dilution. To melted, sterile agar were added the proper quantities of NaOH and H_2SO_4 to provide media of eight different reactions, pH 3,4,5,6,7,8,9,10. The extent of growth was measured daily for five days beginning three days after the inoculation of the plates. The table which follows presents measurements made on the third and

sixth days; each figure is the average diameter in millimeters of the growth on four petri dishes.

Strain	Days	pH 3	4	5	6	7	8	9	10
Car.	3 days	35	73	80	87	67	60	40	35
	6 days	52	90	90	90	90	90	74	63
Pot.	3 days	0	14	41	40	48	47	42	40
	6 days	0	30	85	90	90	90	78	75
Bar.	3 days	0	6	43	48	49	41	29	27
	6 days	0	10	90	90	90	80	68	57
Vel.	3 days	0	20	23	24	23	20	19	19
	6 days	0	44	50	52	51	46	43	42
Oat	3 days	0	11	30	31	31	29	23	12
	6 days	0	38	75	89	85	70	55	25
Str.	3 days	10	27	27	30	27	24	22	10
	6 days	27	65	70	72	66	57	52	30

In general, the optimum reaction was a slightly acid one; for five of the strains, greatest growth occurred on a medium of pH 6, and for the sixth, the barley strain, about equal growth at pH 6 and pH 7. Only the carnation and strawberry strains showed any growth at pH 3. The growth on the most alkaline medium probably was dependent on reduction of the original pH by absorption of oxygen from the air. These results -- optimum, about pH 6; toleration, pH 3 to pH 10 -- agree well with the findings of other investigators. Matsumoto reported no growth at pH 2.5

and fairly luxuriant growth at pH 9.8. Melin's experiments showed Rhizoctonia to grow equally well in neutral or acid soil. For a strain isolated from cabbage, Gratz found the range to be 2 -- 10.4, and the optimum 6.2. Sammel and Garrett, and LeClerg, found the optimum for a sugar beet Rhizoctonia to be 5.6 - 6.2, for one on cereals 6-6.5, and for potato Rhizoctonia 6.5 - 7.

ATMOSPHERIC HUMIDITY AND GROWTH

The relation of atmospheric humidity to growth of Rhizoctonia is one of significance in field control of the diseases produced by this group of fungi. The present experiment was undertaken to determine the limits of tolerance of the mycelium. The humidity of the air surrounding the cultures was regulated by the use 1) of different concentrations of H_2SO_4, and 2) of saturated solutions of various salts. Agar cultures of the strains of Rhizoctonia were started in the usual manner; the petri dishes were then inverted and 10 cc. of the proper solution of H_2SO_4 or a salt was poured in the cover. Growth was measured in 2, 4, and 6 days. The results follow:

Humidity H_2SO_4	60% 38%			70% 33%			80% 27%		
Car.	35	37	37	40	45	47	49	59	64
Bar.	13	13	13	21	24	25	24	35	35
Oat	11	12	12	18	19	19	16	16	16
Pot.	25	28	29	27	28	28	31	37	37
Str.	6	6	6	20	30	35	25	35	35
Vel.	14	14	15	16	18	19	18	18	19
Total	104	110	112	142	164	173	163	250	206

Humidity H_2SO_4	90% 20%			100% 0%			Check		
Car.	44	59	61	56	81	81	58	85	95
Bar.	25	37	47	25	45	49	27	50	70
Oat	20	28	28	23	37	38	23	40	68
Pot.	33	49	49	38	68	73	40	70	85
Str.	20	38	48	22	47	70	23	50	75
Vel.	19	25	25	24	35	42	24	40	69
Total	161	236	258	188	313	353	195	333	462

Moisture	15%			32%			66%		
Salts (Sat. sol.)	LiCl			$CaCl_2$			$NaNo_2$		
Car.	13	13	14	29	30	30	37	45	45
Bar.	10	10	10	14	15	15	21	22	23
Oats	9	9	9	14	15	15	18	20	20
Pot.	14	14	15	17	18	20	16	16	16
Str.	7	7	7	15	16	16	16	20	22
Vel.	9	10	10	9	10	10	18	19	20
Total	62	63	65	98	104	106	126	142	146

Moisture	81%			91%			100%		
Salts (Sat. sol.)	$(NH_4)_2SO_4$			Na_2CO_3					
Car.	55	77	84	60	85	86	56	81	87
Bar.	22	40	70	25	45	80	25	45	80
Oats	21	30	50	20	34	55	23	37	60
Pot.	28	38	40	28	40	42	30	42	43
Str.	13	30	43	18	35	45	22	47	50
Vel.	18	25	27	21	33	52	24	35	53
Total	157	240	314	172	272	360	180	287	373

From these tables it is clear that mycelial growth increases with humidity. Below a humidity of 60% no growth was shown after two days; this is to be explained by the reduction in water-content of the agar medium. Such

reduction was apparent from examination of the plates; in those incubated in an atmosphere with less than 60% humidity the agar shrank down to a thin, obviously dry layer, in contrast to the thicker, softer agar of the other plates. In no case was growth so rapid as in the control cultures, where no sealing layer of liquid prevented free exchange of gases. At a given humidity, growth was more rapid on the media over salt solutions than over H_2SO_4; presumably a minute amount of toxic vapor was released by the latter. Whatever incidental effects were present, it can safely be concluded that on any substratum whose water-retaining power is no greater than that of ager, an atmospheric humidity of less than 60% is highly detrimental to the growth of Rhizoctonia. Since growth increased with humidity even in the upper range, it can also be concluded that high atmospheric humidity directly favors growth.

ULTRAVIOLET RADIATION AND GROWTH

This experiment was performed to determine the effect of ultaviolet radiation on Rhizoctonia, and the necessary dosage. Petri-dish cultures of four strains of the fungus were used. At the end of one day's growth the outline of the mycelium was marked with a wax pencil on the bottom of

the petri dish; and the culture was then irradiated. Measurement from this line gave the amount of growth following irradiation. The source of ultra-violet light was a 30-inch "Sterilamp", operated at approximately 475 volts on a current of 41-49 milliamperes, and delivering 90% of its energy at the wave length of 2537 Ångstrom units. The temperature of the radiation chamber was 24° C.; the distance from the lamp to the petri dish, 8 in. The whole apparatus was housed in a wooden cabinet; and it was found possible to irradiate the mycelium in open petri dishes without contamination. For the first experiment, the duration of exposure was varied between 30 sec. and 60 min. Measurements of growth were made 1 da. and 3 da. after irradiation. The results follow:

	Check		30"		1'		2'		4'		8'		15'		30'		60'	
Car.	9	31	8	30	7	24	4	22	1	12	0	3	0	0	0	0	0	0
Bar.	9	25	7	25	6	25	5	25	4	22	3	20	0	0	0	0	0	0
Oat.	8	24	5	21	5	20	4	18	4	16	0	12	0	0	0	0	0	0
Pot.	9	27	8	25	7	25	5	23	5	21	4	19	0	0	0	0	0	0
Total	35	107	28	101	25	94	18	88	14	71	7	54	0	0	0	0	0	0

The minimum exposure capable of stopping growth of the mycelium appeared to be around 15 min.; and so a second set of cultures was exposed, for periods from 8 min. to 16 min.

After 3 days and 6 hours the results follow:

	Check	8'	10'	12'	14'	16'
Carnation	35	4	4	1	0	0
Barley	28	22	15	8	1	0
Oats	25	14	9	7	0	0
Potato	29	24	20	15	2	0
Total	117	64	48	31	3	0

From these experiments it is clear that ultraviolet radiation under the stated conditions for 15 min. was sufficient to stop the growth of all strains. The later history of the cultures showed, however, that inhibition of growth is not the same as the killing of all cells; for after two weeks further growth appeared even on some of the 60-minute plates. Transfers were consequently made to fresh petri dishes. Of these transfers, those from cultures of the carnation and potato strains irradiated for 60 min. were viable; for the isolates from barley and oats, 30 min. was the longest exposure permitting subsequent growth. Microscopic examination of mycelia immediately after 15-minute irradiation showed that the cells along the margin of growth and also in the upper layers of the older, central portion had the protoplasm concentrated at one corner of

the cell; but in deeper layers of the central portion the protoplasm was distributed within the cells as in unirradiated material. The two more resistant strains had produced before exposure a mycelium thicker than the less resistant strains, and brownish in color; the upper layers consequently formed a better screen than in the thinner, colorless cultures. Presumably in nature dark color affords protection to cell contents from sunlight.

OXYGEN AND GROWTH

The purpose of these experiments was to determine whether Rhizoctonia is aerobic, anaerobic, or microaerobic. Three different procedures were followed. Under the first, alkaline pyrogallol solution was used (2 parts pyrogallol, 5 parts NaOH, 30 parts water) to absorb oxygen from the air. Petri-dish cultures were placed in a large glass desiccator with a beaker, the alkaline pyrogallol solution was poured into the beaker through a funnel, and the desiccator tightly closed. The total volume of the solution was about 1/20 the volume of air in the desiccator -- a safe margin, since the solution can absorb 10 volums of oxygen. Another set of preparations, lacking only the alkaline pyrogallol solution, served as a check. Measurement of growth at the end of three days gave the following results:

	Car.	Bar.	Oat.	Pot.	Str.	Vel.	Total growth
O_2 absorbed	9.5	7.2	4.8	6.8	6.7	4.5	39.5
Check	9.5	8.0	6.5	9.0	8.5	6.0	47.5
Decrease of growth	0%	11%	35%	32%	27%	33%	20%

From these data it is clear that growth was reduced by removal of oxygen, but that all strains were still able to make considerable growth. Stunting was most marked in cultures of the less vigorous strains; the fastest growing, that isolated from carnation, showed no reduction in diameter.

To determine whether atmospheric oxygen was actually absorbed in the growth of the fungus, cultures were started on 20 cc. of agar in 125- cc. Erlenmeyer flasks. These flasks, and also several checks containing no mycelium, were stoppered with one-hole rubber stoppers; in each was inserted a 10-cc. graduated pipette; the flasks were reversed and the open ends of the pipettes immersed in a concentrated solution of NaOH in a glass cylinder. At the end of ten days a considerable amount of the NaOH solution had risen into the culture flasks, while the checks remained as before. The amount of solution that had risen in each culture was measured, and in every case had a volume about 20% of the original air content of the flask and pipette. Clearly, then, the oxygen originally

present had been absorbed during the growth of the fungi; since the NaOH solution absorbed whatever CO_2 was released, it had risen in the pipettes to replace the volume of gas lost.

In the third experiment, mycelia of nine strains of Rhizoctonia were grown on 4 cc. of agar in test-tubes. After good growth had occurred, melted agar at 40° C. was poured over the mycelium, to depths of 1 to 5 cm. After two weeks the cultures were examined to determine through how great a depth of agar the mycelia were able to grow up to the surface. The results follow, X indicating penetration, and O, failure to penetrate:

Agar Depth	Car.	Bot. I	Bot. II	Pot.	Tom.	Str.	Bar.	Oat.	Vel.
1 cm.	X	X	X	X	X	X	X	X	X
2 cm.	X	X	X	X	X	X	X	0	0
3 cm.	X	X	X	X	X	0	0	0	0
4 cm.	X	0	0	0	0	0	0	0	0
5 cm.	0	0	0	0	0	0	0	0	0

It is clear that the strain isolated from carnation, whose growth was least affected by anaerobic conditions produced by alkaline pyrogallol, was able to grow to the surface from under the greatest depth of agar; that those strains most affected in the first experiment -- velvet-grass and oats -- were prevented by the least amount of

agar from reaching the surface; and that one strain, potato, changed its relative position, being less limited by the layer of agar than was the strawberry strain. Since incubation for several additional weeks failed to change the results, it is clear also that the differences indicated are not merely differences in rapidity of growth. Rhizoctonia then cannot grow indefinitely under anaerobic conditions.

PLANT HORMONES AND THE GROWTH OF RHIZOCTONIA

The purpose of this experiment was to determine the effect of plant hormones on the growth of Rhizoctonia. There are many contradictory data, and equally contradictory theories in explanation of them, published on the subject of fungus response to hormones. Perhaps all of them are correct; certainly different fungi react differently, and the concentration used is as important a factor as the organism. For preliminary tests two chemical hormones, indole-3-acetic acid (hetero-auxin) and -indole-3n-butyric acid (auxilin), were used, dissolved in methanol. But it was learned from these tests that methanol in the concentrations employed is toxic to the fungus; subsequently only indole-butyric acid was used, in water solution. Preliminary tests also showed that the fungus must

be grown on a medium containing only inorganic nitrogen; on a peptone medium the same growth was produced either with or without the hormone. For a stock solution, 4 mg. of the hormone were dissolved in 10 cc. of water; this was added to the medium in such quantities as to produce dilutions of 4: 10^4 -- 4: 10^9. The carnation strain was used; cultures were grown in quadruplicate; the figures given are the sums of measurements on four plates.

Real Conc. 4:	10^4	10^5	10^6	10^7	10^8	10^9	Check
24 hr. growth	64	112	106	108	93	80	99
36 hr. growth	84	186	190	192	181	160	197
60 hr. growth	117	308	333	347	306	308	338

The greatest growth was produced with a hormone concentration of 4: 10^7, except for the first day when the growth was greater with a hundred times that amount. Concentrations of 4: 10^5 -- 10^7 all gave better growth on the first day than the check, and 4: 10^6, 10^7 at the end of 60 hr. also. The peculiarity in the results lies in the behavior of cultures with the two highest dilutions which, like those in which the hormone was most concentrated, gave poorer growth than the check. Presumably too great a concentration (4: 10^4) is toxic; too great a dilution might be expected to be simply ineffective. The experiment was repeated, using 5 mg. to 10 cc. for the stock solution

instead of 4 mg., and discarding the final dilution; thus each dilution contained 25% more of the hormone than in the earlier experiment. The results were as follows:

Real conc. 5:	10^4	10^5	10^6	10^7	10^8	Check
26 hr. growth	123	136	132	127	127	127
38 hr. growth	190	225	222	226	222	220
50 hr. growth	233	273	276	279	273	268
58 hr. growth	276	331	321	331	321	314

The data correspond very well to the earlier data. Again the 10^7 dilution produced the greatest stimulation; again the first day's growth was best at 10^5. The higher dilutions showed less stimulation than that produced by the 10^7, but all were better than the check. Evidently the range of most favorable reaction is here 4 -- 5: 10^5 -- 10^7; in higher concentrations the hormone is toxic. The stimulating effect shown in this experiment is at best only about 7%. The most effective concentration is here 4 - 5 parts in 10 million, about 40 - 400 times more dilute than the solution used on cuttings of ornamental plants.

STIMULATING AND TOXIC EFFECTS OF METAL SALTS ON THE GROWTH OF RHIZOCTONIA

It is the toxic effect of metal salts on fungus mycelia which is of chief importance in developing control methods; but the stimulating effects are of greater physiological interest, and less well known. Of a long series of delicate experiments carried out through many weeks only two which serve as summaries, and such details as are necessary for an understanding of the work, are here given.

The basic medium used consisted of

KNO_3, 5 g.
Dextrose, 10 g.
$MgSO_4$, 0.25 g.
KH_2PO_4, 0.25 g.
Agar, 18 g.
Water, 1 liter

With this medium were used eight salts: $ZnSO_4$, $CuSO_4$, $MnSO_4$, $NiNO_3$, $FeSO_4$, $HgCl_2$, $AgNO_3$, and NaCl. Stock solutions of these salts were prepared and sterilized separately; the proper solution was added by means of a sterilized 1-cc. graduated pipette to 10 cc. of melted agar in the petri dish, and the whole mixed evenly by tilting the dish. Tomato Rhizoctonia was used in all experiments.

In one experiment, three concentrations of each salt were used. The diameter of growth was measured six times in three days. The last measurements only are given in the table, each figure representing the total growth on four petri dishes, measured in millimeters.

Salt	$AgNO_3$			$CuSO_4$		
Concentration	$5:10^6$	$:10^7$	$:10^8$	$5:10^6$	$:10^7$	$:10^8$
No.	A	B	C	A	B	C
Growth mm.	65	235	269	235	258	296
Difference	-202	-32	+2	-14	-9	+29

$MnSO_4$			Check
$1:10^4$	$:10^5$	$:10^6$	
A	B	C	
249	251	283	267
-18	-16	+16	

Salt	$NiNO_3$			$ZnSO_4$		
Concentration	$1:10^6$	$:10^7$	$:10^8$	$1:10^5$	$:10^6$	$:10^7$
No.	A	B	C	A	B	C
Growth mm.	273	270	278	281	255	283
Difference	-3	-10	-2	+1	-25	+3

$HgCl_2$			Check
$1:10^6$	$:10^7$	$:10^8$	
A	B	C	
159	261	267	280
-121	-19	-13	

Salt	$FeSO_4$			NaCl			
Concentration	$1:10^5$	$:10^6$	$:10^7$	$5:10^5$	$:10^6$	$:10^7$	Check
No.	A	B	C	A	B	C	
Growth mm.	279	275	244	263	251	250	248
Difference	+23	+27	-4	+15	+3	+2	

As the data show, some salts in very great dilution cause increase of growth up to 10%, as $FeSO_4$ at 1:1,000,000 and $CuSO_4$ at 5:100,000,000. On the other hand, some salts in considerable dilution were extremely toxic, as $AgNO_3$, which at 5:1,000,000 caused a decrease in growth of 75%, and $HgCl_2$, which at 1:1,000,000, caused a decrease of 60%; these completely prevented growth at, respectively, 5:100,000 and 1:100,000.

A second experiment showed the stimulating effect of salts present in combination. The concentrations used were as follows:

$AgNO_3$, $5:10^9$	$ZnSO_4$, $1:10^7$
$CuSO_4$, $5:10^8$	$HgCl_2$, $1:10^8$
$MnSO_4$, $1:10^6$	$FeSO_4$, $1:10^6$
$NiNO_3$, $1:10^8$	NaCl, $5:10^5$

These dilutions were believed, from preliminary tests, to be well suited to the production of stimulation; from later work it appeared that 1/10 the amount of salts of Cu, Fe, Ag, and Hg would have given better results. Time could not be spared for a repetition to test the further dilutions. The results follow:

No.	Metals in	Growth mm.	Growth difference	Increase %
check		270		
1	Mn, Na,	290	20	7%
2	Zn, Ag,	300	30	11%
3	Ni, Hg,	299	29	11%
4	Cu, Mn, Zn,	303	33	12%
5	Cu, Ni, Hg,	309	39	15%
6	Cu, Fe, Mn,	314	44	17%
7	Zn, Na, Ag,	310	40	15%
8	Cu, Fe, Mn, Na,	317	47	18%
9	An, Ag, Ni, Hg	312	42	16%
10	Mn, Na, Zn, Ag	314	44	17%
11	Cu, Fe, Mn, Na Zn, Ag, Ni, Hg	311	41	16%

Traces of inorganic salts, added singly or in combination to a culture medium, have here produced increases in growth of 10 - 18%. The need for the so-called "minor elements" in the growth of higher plants has been extensively studied; only a few investigations have been attempted of their effect on fungi. Such marked stimulation as is here shown has not before been reported.

PROTOPLASMIC STREAMING AND SEPTATION OF THE HYPHAE

The mycelium of Rhizoctonia provides a favorable material for an attempt to confirm the observations of Buller on the translocation of protoplasm, and such an attempt was accordingly made. Hanging drops of cleared Cook's agar were prepared and inoculated with mycelium from a stock culture of Rhizoctonia Solani. On the following day the hyphae had grown out to the periphery of the drop. The material was illuminated with daylight and studied under the high power of the microscope.

The young vegetative hyphae are about 8 μ in diameter and 100 - 200 μ long. The walls are colorless; the protoplasm is not quite hyaline, but faintly clouded, as though very minutely granular. What vacuoles are present arise about the periphery of the cell and remain there. In each septum is a pore, 1 μ or less in diameter, through which passes a bridge of cytoplasm. In several hyphae the streaming of protoplasm was observed, as a faint gray cloud moving continuously in one direction. As the protoplasm came to a septum it passed without evident obstruction through the pore; the stream widened out abruptly on the other side of the septum. Such streaming was observed to be continuous through a dozen long cells. The hyphae in which it was most active appeared less vacuolate than most;

and no large vacuoles came in contact with the septa.

The straming is caused by increase in volume of the vacuoles in certain cells, and presumably by increase in the mass of protoplasm. In an old hypha, the end cell of a side branch became emptied of protoplasm first, then the penultimate cell, and so on. When a cell has lost nearly all of its protoplasm it comes to contain only one large vacuole; then suddenly its turgidity is lost, and the remaining protoplasm becomes contracted in death. The adjacent cell exerts sufficient pressure to bow the common septum into the cavity of the dead cell; and it plugs the connecting pore. Where a living and a dead cell lie end to end this plug in the septal pore can be clearly seen when the light is properly regulated by manipulation of the diaphragm, condenser, and mirror. The body formed in the pore has the shape of a flattened ovoid. Not only can it be seen, but its formation can be induced by using a glass needle (about 20 μ in diameter) attached to a micro- micromanipulator to cut and kill a living cell; the uninjured adjacent cell then forms the plug and so prevents continued bleeding of protoplasm into the broken cell. As the mycelium comes to contain numbers of such dead and emptied cells, the streaming of protoplasm is interrupted, and continues in new directions, toward the tips of side branches in which active growth is occurring.

From observation under similar conditions of a number of strains of Rhizoctonia it appears that speed of flow is greater in mycelium of the more vigorous strains, such as carnation, and less in less vigorous strains, such as velvet grass. Streaming of protoplasm is not in every case from an older cell into a younger; the direction may be the reverse of this when such direction brings the protoplasm to a rapidly developing region.

At a growing point the protoplasm is seen to move slowly forward through the last-formed septum and into the enlarging apical cell. The last few septa back of the apical cell are bulged forward, as though under pressure; this would seem to indicate that the movement into these cells was more rapid than the possible rate of exit through the successive pores, or perhaps that the protoplasm of the youngest cells is enough more viscous, enough less hydrated, than that in the older portions that its movement is slowed up, and pressure is exerted from behind against the whole terminal group. A new septum is formed in the terminal cell by annular growth from the lateral wall. This new septum is also bulged forward, apparently by the movement of protoplasm past the wall as it forms; possibly the bulge of the cells just back of the tip indicates nothing more than the persistence for the time of an original convexity. Cellular elongation occurs chiefly at

the tip, and not at all is apparent back of the few newest cells; this is in contrast to the uniform alongation, throughout its extent, of a bacterial filament, and to the growth of those filamentous algae in which it is both apical and intercalary. Branching may be induced by removing the end of a filament with a needle attached to the micromanipulator. The uninjured cell next to the one destroyed puts out a peg-like lateral protrusion which continues the growth of the hypha. Injury to the growing tip may also cause reversal of the direction of streaming; such injury may be produced by a hot needle, or by crystals of iodine, copper sulphate, or sodium choride. For a time after injury the protoplasm in the terminal cells circulates within the cell, and then, in 2 - 3 min., flows in the direction opposite to that taken before injury.

DISCUSSION

Since the form genus Rhizoctonia was first established in 1815 by DeCandole, there have been about thirty species described for it. Undoubtedly many of them are not to be regarded as species, but rather to be ranked as strains, forms, or varieties; and still some others are not true species of Rhizoctonia. Rhizoctonia belongs to the group Mycelia sterilia of the form class Fungi imperfecti; if any spore-forms are found for any species formerly put in this genus, the species in question must be removed to the position to which its spores would assign it. For instance Macrophomina Phaseoli (Mankl.) Ashby is known to be the right name for Rhizoctonia Bataticola (Taub.) Butl., since 1927, when Ashby found the relation of the Rhizoctonia and Macrophomina stages.

More significant taxonomically, but perhaps less useful, are the identifications of several species of Rhizoctonia with Basidiomycetes. The first, with which every one is familiar, is the identification of Rhizoctonia Solani Kühn as the imperfect stage of Corticium vagum B & C., the result of the work of Rolfs in 1903. Later work has shown that the group of fungi of which this perfect stage is a member is better segregated from Corticium, as Botryobasidium Donk (1931). Further more, the parasitic perfect stage typically has spores which are oblong-ovoid to

short-reniform, and usually four sterigmata to the basidium; whereas the type collection of C. vagum has laterally flattened, slender, fusiform spores and six sterigmata to the basidium. This difference was at first regarded by Burt as worthy of varietal recognition (C. vagum var. Solani) and later as of no significance at all. Rogers has recently revived the original specific separation, treating the parasite as Botryobasidium Solani (Prill. & Del.) Donk, and the saprophyte as B. vagum (B. & C.) Now it has been shown in these studies that the fungus isolated from rotting wood, B. vagum in the sense of Rogers, can under favorable circumstances become a parasite, and that in culture it closely resembles the strawberry and velvet-grass strains of Rhizoctonia Solani. The biology of the fungi, then, is not a significant basis for separation of vagum and Solani. As to the number of sterigmata on the basidia of the potato fungus; Matsumoto in 1930 saw 4 - 6 sterigmata per basidium on a specimen from Germany also growing on potato. As to spore dimensions: Rolfs reported 10 - 12 x 6 - 8 μ, Matsumoto 8 - 14 x 4 - 6 μ, Peltier 9 - 14 x 6 - 8 μ; for the saprophyte, Rogers gives 6.5 - 10.5 x 3 - 5.5 μ. Specimens on potato examined during the present studies showed spores 10 x 7 μ (average of 10); specimens of the saprophyte showed 10 x 3.5 μ (average of 20. Apparently only this single difference exists between

the two forms, that the spores of the parasite are oval, the length being 1 - 2 times the width, while those of the saprophyte are cylindric, and 2 - 3 times as long as wide. It is a question then whether the fungi are to be regarded as two species, two varieties, or two ecological variants. Apparently Matsumoto's German specimens resembled more closely the saprophyte.

Rhizoctonia Crocorum DC. ex Fr. was connected with Helicobasidium purpureum (Tul.) Pat., a member of the Auriculariaceae, by Buddin and Wakefield in 1927. They isolated mycelia from the perfect stage and compared it with the imperfect form, Rhizoctonia Crocorum, with respect to both growth and characters and pathogenicity. They felt well satisfied with their prediction, in 1924, that the Helicobasidium would prove to be the perfect stage of the Rhizoctonia, a prediction based on work with the disease on black currant. Helicobasidium purpureum was first described by Tulasne in 1865, as Hypochnus purpureus, from specimens found on the base of small trees or herbaceous plants. In 1885 Patouillard, who apparently had overlooked Tulasne's work, called the fungus Helicobasidium purpureum; in 1900 he set down Hypochnus purpureus as a synonym. The peculiar curved, septate basidia bear 1 - 4 basidiospores, usually 2 or 3, one on each cell. The perfect stage is closely set on the base of herbaceous or

young woody plants as an effused, thick, dense felt, in growth like Sebacina incrustans Tul., purple or violet in color, and not gelatinous or waxy in texture as are most the other Auriculariaceae. A curious aspect of the work of Buddin and Wakefield is that a **Tuberculina** which usually is found only as the parasite of the summer sori of rusts was always associated with the cultures both of the Helicobasidium and of R. Crocorum.

Corticium praticola, a third perfect form of a Rhizoctonia, was isolated from alfalfa in 1924 by Kotila while studying the violet Rhizoctonia of alfalfa. The vegetative hyphae are hyaline, and so it can not be either R. Crocorum or R. Solani. The sclerotia in culture were smaller and less abundant than those of R. Solani. The basidia were 15.6 x 6.5 μ, with 1 - 4, usually 3, sterigmata, measuring 13 - 18.8 - 26.5 x 2.6 μ, and ovate, apiculate, smooth-walled basidiospores, 5.2 - 7.7 - 7.8 x 4.9 - 5.2 - 5.5 μ. Clearly it is different from Helicobasidium purpureum, and apparently also from the perfect stage of R. Solani, differing in the fewer and much longer sterigmata. According to Rogers's classification, this should probably be put in the genus Ceratobasidium.

Some investigators have tried to prove a connection of Rhizoctonia with ascomycetes, but no one has been able

to give strong proof. The form genus Rhizoctonia is still very heterogeneous, some species probably forming part of the life cycle of pycnidial fungi and others representing the vegetative stage of Basidiomycetes. The former should be separated off, and the genus then divided into two subgenera; 1) Hetero-Rhizoctonia to include R. Crocorum (Helicobasidium purpureum) and any similar species which, presumably, are part of the life cycle of Heterobasidiomycetes, with divided basidia, and 2) Homo- Rhizoctonia to include R. Solani (Botryobasidium Solani) and analogous species such as Ceratobasidium praticola (Kotila) com. nov., all of which appear to have their perfect form with aseptate basidia of the type of Corticium in the wider sense.

The genus Rhizoctonia is easily confused with the genus Sclerotium, the only difference between the two being that the sclerotia of Rhizoctonia are not differentiated clearly into cortex and medulla, are scattered in a mycelial mat and connected with each other by mycelia, are not so hard, and are less regular, smooth, dark-colored and globular than those of Sclerotium. The characteristic Rhizoctonia-type branching may be more or less similar to that of certain species of Sclerotinia, Morchella, Pleospora, Rosellinia and many others; but those can be easily recognized by their spores and many other

characters. Because of the similarity of the mycelia, some have predicted that the perfect stages of Rhizoctonia will prove to be certain species of Ascomycetes, such as Rosellinia Quercina; but this has never been proved. So far, according to our present knowledge, the only possible perfect forms are lower Basidiomycetes, and not Phycomycetes, Ascomycetes or higher Basidiomycetes.

The common impression that sclerotia of the Rhizoctonia disease are produced only on the underground parts of plants is not right. In tropical and subtropical countries there are several species of Rhizoctonia whose sclerotia are produced on the aerial parts of the plants, such as R. microsclerotia on leaves and stems of fig, cowpea, carrot, and all kinds of beans. Thus from their ecology there are really two distinct groups of Rhizoctonia, one comprising those species which inhabit and infest the aerial parts, especially the foliage, of higher plants, and the other containing root-infesting species. This distinction, though primarily an ecological one, also entails morphological and physiological features. The sclerotia and mycelia in some and sclerotia alone in others are more distinct, and of a harder consistency, in the aerial group; this is probably an adaptation for adverse conditions, especially the drying effect of air currents.

The two most important species of the genus are R. Solani and R. Crocorum, the latter being the type species. It was first found in France and later in other continental European countries, attacking many kinds of plants; It seems rare in United States. R. Solani is very common in United States and attacks over 200 species of plants, especially potatoes, carnations, other vegetables, ornamentals, and crops. Thetwo species are easily distinguished by many characters, the most important ones being 1) color of the mycelial felt: pink, red, or violet, (to violet brown with age) in R. Crocorum; in R. Solani the color of the mycelial web, if evident, is dirty yellow or yellow brown; 2) the presence in R. Crocorum of an external felt or mantle of investing hyphae, confined to the underground parts; while in R. Solani the external mycelium, if noticeable, occurs only as an open web, or sometimes with flaky tufts, the formation of a continuous collar of mycellium occuring only at the time of fruiting; 3) the sclerotia, in R. Crocorum densely wooly, with investing mycelia and filaments of short ovoidal cells, in internal structure not truly plectenchymatic; while those of R. Solani are normally free from investing mycelia and abnormal filaments, and the internal structure is homogeneous; 4) infection cushions, present on the root-infesting mycelia of R. Crocorum on most hosts and

absent from R. Solani; 5) a biological difference: R. Crocorum is very hard to isolate and grow on culture media -- probably an indication of a high degree of parasitism --; while R. Solani is very easily isolated and grown on any kind of culture medium.

Reference to Rhizoctonia, always implies sclerotium-formation, but in nature in some species sclerotia are normally not produced. This is especially true of endotrophic-mycorrhizal species, which never form sclerotia in root tissue, even when they produce sclerotia in culture. Even the parasitic strains of R. Solani do not always produce sclerotia under natural conditions; for example, the strawberry strain, which also rarely forms sclerotia on agar. Sclerotia, then, are not always associated with the diseases or formed in cultures where typical Rhizoctonia mycelia occur.

For the classification of Rhizoctonia into species and strains, several methods have been proposed, but none seems quite sound. Most investigators depend upon the host and the characters of mycelia and sclerotia for classification. As to the host; one species may attack many hosts and one host may be attacked by more than one species or strain, and so classification according to host is not dependable. The width, length, color, size, and other characters of the mycelia and sclerotia are so

variable in different environments and on different media, that they likewise are not dependable. But in addition to these, no other morphological characters can be found, since Rhizoctonia has only sterile mycelia and sclerotia, without any kind of spores. Thus the only method that can be used there is to compare growth characters under standard conditions and make cross inoculation tests on a certain number of selected hosts by standard methods. At least, this is the conclusion to which the experiments here described surely lead. From these studies there are to be suggested the following conditions as standard for comparing growth rate and other growth characters; Cook's medium, prepared as described earlier, to be the standard medium. The pH, if adjusted, to be 6; but it is quite near that in this medium even without adjustment. Fifteen cc. of the medium to be used for a 95 mm. petri dish. The inoculum to be uniformly 6 mm. in diameter, and cut from the margin of a young culture. The petri dish to be kept in a 20° C. incubator, variation in temperature to be within the limit of 1° C. The inoculum to be placed on the medium with the upper side down and in the center of the Petri dish. The time of the transfer to be marked clearly in hours and minutes. The standard times for measurement to be 48 and 96 hours, counting from the minute of inoculation. The measures to be taken from 5

similar cultures, each measured twice, once along the largest and once the shortest diameter, in millimeters. No light to be used in the incubator, or Petri dishes to be put in paper boxes to avoid the variation arising from light. Two days' growth to be the standard for classifying into 4 growth divisions, each with a range of 25 mm., between 10 and 100 mm. of total diameters, these divisions to be subdivided by sclerotium formation and related characters. Classification from sclerotia to be into groups which form sclerotia in one week, in three weeks, and not at all. Size of the sclerotium to be stated in terms of the average; color to be judged from observation at the end of two weeks' sclerotial growth. Other obvious and striking characters to be used as further classifying characters. The cross-inoculation test to use the method already described in this paper, growth conditions to be the same as for the growth rate test in respect to temperature, light and other factors, and also the medium used. The fungus to be grown one week ahead of the transfer of seedlings, which are to grow in Knop's nutrient solution until the seedlings are ready for transfer to the tube cultures. The degree of infection to be stated in terms of 4 classes: 1, completely killed: 2, the roots or leaves heavily attacked; 3, lightly attacked; and 4, no symptom of infection. Standard data to be taken one

week from the transfering day. Ten standard hosts to be corn, sunflower, field bean, tomato, cucumber, mustard, beet, buckwheat, turnip, and castor bean. From the growth rate, growth characters, sclerotium size, other sclerotial characters, and inoculation test, the species and strains could be classified easily. The advantage of this method is that everybody can duplicate the standard conditions, that the data required are easily taken and more dependable than those formerly considered by different workers, and that all data can be gathered in a space of one or two months. This suggestion is fully practicable except for some of the more specialized parasites, not ordinarily encountered, which cannot be isolated and grown on this medium; for such fungi, modifications should be developed after experience with them.

The physiological tests on different strains of Rhizoctonia isolated from different hosts show some variations, but can be fairly well summarized as follows: Generally these fungi prefer a pH on the acid side; 5-6 is ideal for the growth of the fungi studied; but the tolerated range is very wide, pH 3-9. The optimum temperature is around 20° C., as would be expected, since under field conditions the early-planted and late-harvested varieties of potato are most severely affected by the disease. High moisture is needed for good growth of the mycelium;

this also coincides with the field observations. The enzymes produced by the mycelia are numerous and very active. The parasitic strains have much more destructive power on different sugars than the saprophytic ones. This can be explained by the fact that saprophytic strains can use the foods already partially broken down by other organisms, such as bacteria or other fungi, but parasitic ones must attack healthy living tissue and work their own way in. Rhizoctonias rather prefer dark to bright light, which inhibits the growth a great deal; the brownish color of the mycelia is darker on dishes exposed to the light than those kept in the dark, the pigment evidently serving to protect the protoplasm from radiation. Ultraviolet radiation, except of long duration, can kill only the superfial mycelia, but not the lower layer; since aerial hyphae in nature are always in the shade, they probably avoid most solar ultraviolet light. For the nutrition of Rhizoctonia, organic nitrogen is much better than inorganic, and ammonium salts worse than nitrate salts, ammonium sulphate definitely inhibiting the growth of the fungus; nitrogen from air can not be utilized by Rhizoctonia. For carbon, inorganic salts cannot be utilized; of the sugars, sucrose and dextrose can be utilized most rapidly in the absence of other food materials; lactose is utilized more slowly. The brownish pigment produced either in the

mycelia or in the sclerotia is dependent on the presence of abundant nitrogenous material. The formation of sclerotia is dependent upon abundant available carbon and nitrogenous foods; the lact of either prevents it. The formation of sclerotia always takes place after the whole Petri dish is covered by the mycelium.

In the inoculation test, a strain attacks the host from which it was isolated, or a related genus, more severely than other hosts; the mycelia isolated from saprophytes also attacked living hosts; thus if the environment is favorable, the saprophytic can behave the same as the parasitic strains. The saprophyte isolated from single basidiospores failed to form basidia when grown on agar and even on living hosts, and remained as sterile as the original sterile parasitic strains. The short barrel-shaped cells formed by the saprophytic strain are also found on the parasitic strains. Small sclerotia are also produced on the saprophytic forms, less rarely than by the strawberry strain. The darkening of the medium after the mycelia have grown over it is a universal character; the shade of the color seems related to the virulence of the strains, the carnation with the darkest color being the most destructive one, even killing seeds before germination and the strawberry strain, the weakest one, having the lighest color; the others were about in order.

The perfect stage was never found in the culture, except that in the inoculation test several strains formed basidia but not [illegible] basidiospores, even though several recommended methods of inducing fruiting were tried; however, only once, one piece of strawberry leaf showed the basidial stage perfectly. The formation of the perfect stage seems to depend upon two main factors, one inherited, and the other environmental. Kotila showed clearly that a sterile strain was always sterile, and a fertile strain always could form spores. This inherited character has also been shown by other workers, like Buddin and Wakefield, Matsumoto, Shaw and several others. Environmental factors are also important, even strains which produce basidia quite easily being prevented from doing so by unsuitable conditions. But the hereditary factor seems to be the more effective.

SUMMARY

(1) The first description of the disease caused by Rhizoctonia was made by Duhamel in 1728; but the genus itself was established by De Candolle in 1815. This Rhizoctonia was the violet-colored species R. Crocorum (Pers.) DC., growing on saffron, asparagus, alfalfa, and some other hosts. In 1858 Kühn first described the commoner species R. Solani, from potato in Germany.

(2) R. Solani is a very destructive cosmopolitan species, having as hosts, reported from time to time, more than 200 species, in more than fifty families. The very destructive disease on potato caused by this species has been studied by hundreds of investigators.

(3) The perfect stage of R. Solani was first carefully observed and accurately described by Rolfs in Colorado in 1903; he then called it Corticium vagum Berk. and Curt. var. Solani. Several other species of Basidiomycetes and Ascomycetes had been suspected of being related to this Rhizoctonia. Hypochnus Solani Prill. and Del. had been known in Europe since 1891, but no definite claim had been made before Rolfs of its association with the Rhizoctonia.

(4) The perfect form of R. Crocorum has also been found, in England, by Buddin and Wakefield in 1927, and

shown to be Helicobasidium purpureum (Tul.) Pat.

(5) A third perfect form of a Rhizoctonia was found on alfalfa in Michigan by Kotila in 1924 and named Corticium praticola Kotila; it is easily distinguishable by its white mycelium and other morphological characters from the other two species.

(6) Recent mycological studies have shown the heterogeneity of the old genus Corticium and have resulted in the segregation of a number of distinct small genera from it. In 1931 Donk thus separated the genus Botryobasidium, and assigned to it the species originally known as Hypochnus Solani. In 1935 Rogers described differences between that parasitic form and the true C. vagum, and added the latter to the genus Botryobasidium. Kotila's C. praticola, according to this concept of the genus Corticium, would belong either in Botryobasidium or in the closely related genus Ceratobasidium.

(7) Fries, whose work is the starting point for the mycological nomenclature of this group, in 1822 defined the genus Rhizoctonia first of all by the presence of sclerotia -- irregular in form, of uniform texture inside and out, and possessing a thin inseparable cortex; and further by the uniting of many sclerotia by visible mycelial strands, by subterranean growth, and by attachment to the roots of living plants. It is now known that

Rhizoctonia also attacks the aerial parts of plants -- for example, R. Microsclerotia Matz, on living leaves, branches and fruits of the cultivated fig. The sclerotia are not always associated with the mycelia in nature; for example, the endophytic mycelia in orchid mycorrhizae belonging to several species of Rhizoctonia never form sclerotia in the natural condition, but do so in culture. In pathological literature many fungi with mycelium similar to that of typical species, but without sclerotia, are assigned to the genus Rhizoctonia.

(8) The only generally recognized diagnostic character for Rhizoctonia is the right-angled branching, usually accompanied by sclerotium formation either on the host or in culture. This genus is easily confused with Sclerotium, which is differentiated by more solid, black-colored, smoother, shiny, unconnected sclerotia with differentiated, light-colored medulla and no aerial mycelia.

(9) Seven parasitic strains of R. Solani were used in this study, isolated from potato, carnation, tomato, strawberry, barley, oats, velvet grass. Another strain, isolated from strawberry in the course of this study, possessed different morphological characters. Five single-basidiospore cultures isolated from the saprophytic Botryobasidium vagum of rotten wood were also used in this study, since they show the same morphological characters.

The results of the physiological experiments are summarized in the following:

(10) For nutrition, organic compounds, peptone and sugars, are best utilized; inorganic carbon compounds, such as $CaCO_3$, cannot be utilized at all; inorganic nitrogenous salts can be fairly well utilized; ammonium salts are less readily used than nitrate salts and seem toxic to the growth. The brownish pigment formed in the mycelia results from sufficient available nitrogen in the media. The formation of sclerotia occurred only when abundant available carbon and nitrogen compounds were both present. The best medium tested is Cook's medium.

(11) In nutrient sugar media, dextrose is the sugar best utilized, sucrose next, and lactose last. In synthetic sugar media, sucrose is first, dextrose next, and lactose last. In all cases sugar utilization is much faster in synthetic media than in nutrient ones.

(12) Starch is hydrolyzed by all strains, the enzyme amylase being always secreted ahead of the growth of the mycelium.

(13) Gelatine can be liquefied by all strains but the rapidity varies a great deal with the strain. A dark brownish pigment is associated with potato, tomato, and carnation strains in the liquid and to some depth also in the solid layer of gelatine.

(14) Nitrate can be reduced to nitrite and nitrite in turn to ammonia but with different speeds by different strains.

(15) Eight kinds of metal salts ($ZnSO_4$, $NaNo_3$, $HgCl_2$, $CuSo_4$, $MnSo_4$, $FeSo_4$, $AgNo_3$ and NaCl) were used for studying the stimulating and intoxicating effects of different dilutions. All salts in very great dilutions (5:100,000 to 5:1,000,000,000) have definite stimulating effects, especially when several salts are used together. The greater the atomic weight the greater the dilution needed to produce stimulating effects.

(16) The plant hormone indole-butyric acid shows the same effect as metal salts. The stimulating effect is apparent at the dilution of 5:100,000 to 5:10,000,000. Higher concentrations than 1:10,000 inhibit growth.

(17) The optimum temperature for growth is around 20^{o} C., but the strawberry and carnation strains prefer a somewhat higher temperature. The growth rate is very sensitive to temperature variation.

(18) All strains grow best at the high atmospheric humidities of 80--100%; at a humidity lower than 50% growth ceases.

(19) Growth occurs through a wide range of pH, (3-10) but at lower pH, 5-6, the growth is more luxuriant.

(20) All strains show best growth if kept most of the time in the dark, with only 2-3 hours of light per day. Continued exposure of the culture to light inhibits growth to a great extent. Mycelial growth shows a much lighter color in the dark than in the light. The brownish pigments formed probably serve as a screen in protecting the protoplasm in the cell. Concentric rings are formed in the culture chiefly as a result of intermittent illumination.

(21) Ultra-violet radiation of 15 minutes' duration checks the growth of all strains, but the carnation and potato strains, with their thicker mycelium shielding deeper layers of cells, can remain viable even after 1 hour's radiation.

(22) A rapid and accurate inoculation method was devised, the seeds of plants to be tested being germinated in Knop's solution and the seedlings then being aseptically transferred to culture tubes.

(23) The cross-inoculation tests show that different strains have a different virulence on different hosts. The virulence seems generally to coincide with the morphological and physiological characters of the strain: the more vigorous the growth and the darker the mycelium, the more abundant the enzymes secreted and the more virulent the strain. The saprophytic strain isolated from single basidiospores of Botryobasidium vagum also attacked

certain hosts.

(24) An improved isolation method was devised, to be used with infected plant parts too delicate to be sterilized for the removal of contaminated organisms. The infected part was placed in a petri dish on a layer of small sterilized discs of filter paper; on this substratum the mycelium grew more rapidly than did contaminants; discs taken from the periphery of the Rhizoctonia mycelium gave pure cultures on agar plates.

(25) Contaminated mycelia were somewhat similarly purified of bacterial contaminants by being placed on the glass bottom of a petri dish only one side of which was covered with a layer of agar. The mycelium grew out across the glass and finally reached the agar, leaving contaminants behind.

(26) Septum formation and the streaming of protoplasm through the central pores of the septa were observed in growing hyphae.

(27) In cultures of the saprophytic strain chains of short barrel-shaped cells with 1 or 2 nuclei were observed, growing along the cover of the petri dish. The same structure was observed in parasitic strains, especially the strawberry strain, just before the formation of sclerotia. They are not conidia, because they never break off, but seem to be structures preparatory for sclerotium formation.

(28) The statement that vagum and Solani are distinct species is confirmed by the morphological characters of the perfect forms; biologically the two are not completely different, since, according to the inoculation test, either can be parasitic or saprophytic.

(29) A standard method for classifying different strains of R. Solani and related species, based on physiological and inoculation tests, is formulated and suggested for future work with Rhizoctonia.

(30) The suspicion that the Rhizoctonia of strawberry is closely related to the common saprophyte Botryobasidium vagum was confirmed by the numerous similarities between the two fungi in culture. However, the one fruiting-body of the strawberry fungus, formed on strawberry leaves in the greenhouse, was not B. vagum, but B. Solani; and the Rhizoctonia must be considered to be ~~even~~ an imperfect stage ~~of~~ the latter.

EXPLANATION OF GRAPHS

1. The toxic effect of ammonium sulphate on the growth of Rhizoctonia mycelia.
2. Gelatine liquefaction caused by the mycelia of Rhizoctonia Solani.
3. Growth rate of different strains of Rhizoctonia Solani at 20° C.
4. The effect of different pH on the mycelial growth rate of Rhizoctonia Solani (Carnation strain).
5. The effect of different humidities (obtained by using various concentrations of sulphuric acid) on the mycelium growth rate of Rhizoctonia Solani.
6. The effect of different humidities (obtained by using various saturated salt solutions) on the mycelium growth rate of Rhizoctonia Solani.
7. The effect of ultraviolet radiation for various lengths of time on the growth of Rhizoctonia on mycelia.

EXPLANATION OF PLATES

The numbers 1--11 used in the pictures indicate the different strains of Rhizoctonia Solani isolated from various hosts and rotten wood (except No. 10, the check material in the inoculation test).

1. carnation
2. barley
3. oats
4. potato
5. tomato
6. strawberry
7. velvet grass
8. Botryobasidium No. 1
9. Botryobasidium No. 2
10. strawberry

Plate I. One month old cultures in test tubes.

Plate II. Cultures of different ages of strains 1 - 4, in petri dishes; upper row, 40 hours, middle row, 10 days; lower row, 1 month. From the one month old culture of barley strain, 6 inoculum discs have been removed leaving 6 circular black spots.

Plate III. Cultures of strains 5--8.

Plate IV, left half. Cultures of strains 9 and 11.

right half. No. 12, the improved isolation or reisolation method. A piece of diseased corn leaf is in the dish. No. 17, dish with paper disc covered with mycelia transferred from No. 12. No. 22, the new technique for making slides. Four sterilized round cover glasses were put in the dish with the

inoculum; after they are covered with growing mycelia the cover glass can be taken off and put in a wire basket, and treated and stained as usual in Coplin jars. For microchemical tests also this is a very successful method because no media interfere with the test. For observations of tip-growing, septum formation, protoplasmic streaming, and method of branching, and for many other delicate physiological studies, this method is much better than the hanging drop method. No. 28, the new purification method; pure transfers can be made from the marginal growth. No. 26, same as No. 28, 1 week old; the dried original inoculum still can be seen as a white spot in the dish, far away from the agar. No. 19, germinating corn seedlings, grown aseptically in Knop's solution in a petri dish, at about the stage for transfer to culture tubes for inoculation test.

Plate V. Inoculation test of wheat seedlings, in test tubes.

Plate VI. Test of sunflower seedlings inoculated with the tomato strain of Rhizoctonia. No. 20 is the check.

Plate VII. Inoculation test of corn seedlings.

Plate VIII. Inoculation test of field-bean seedlings.

Plate IX. Inoculation test of squash seedlings.

Plate X. Inoculation test of lettuce seedlings (upper row) and radish seedlings (lower row).

Plate XI. Inoculation test of tomato stems.

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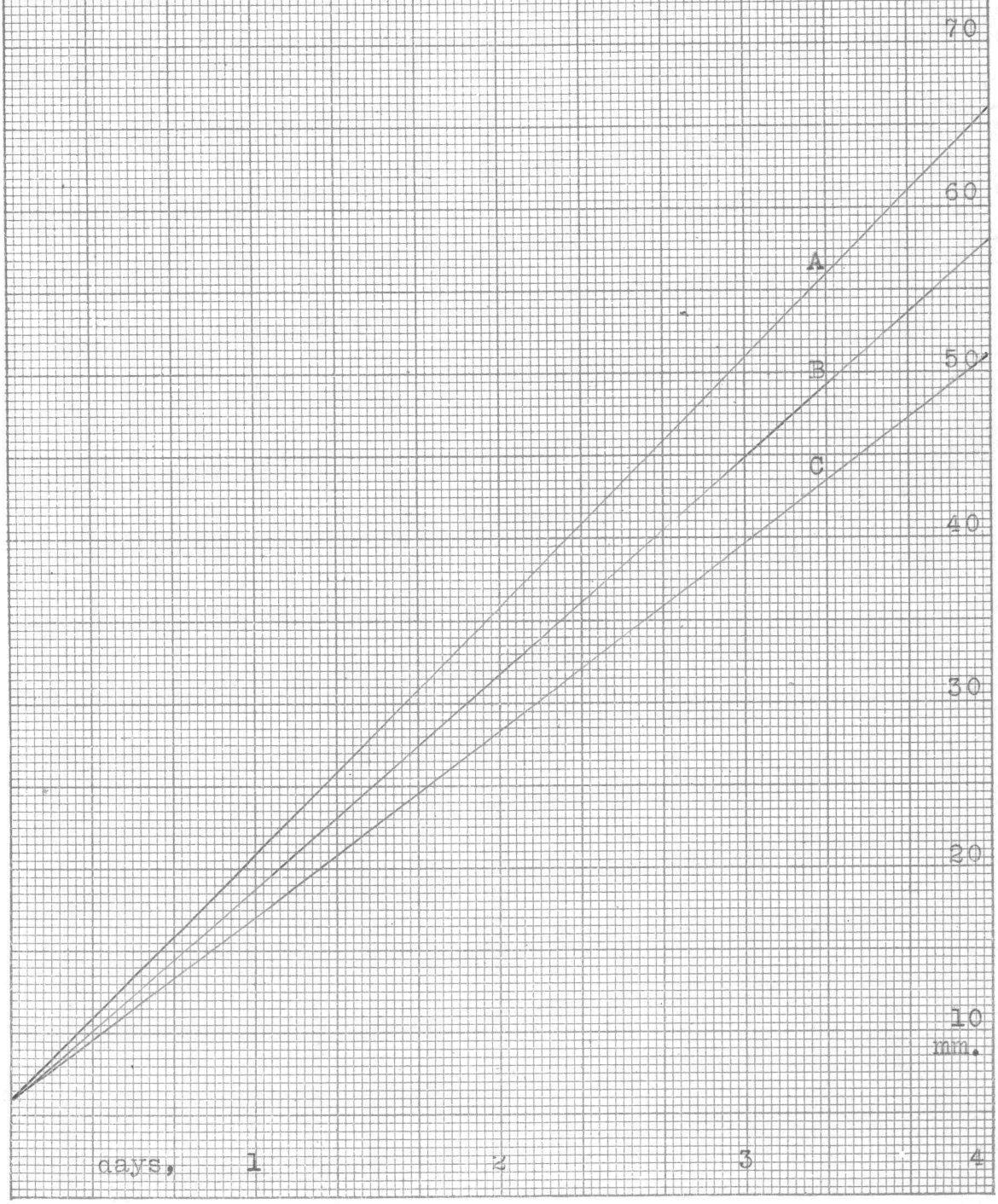
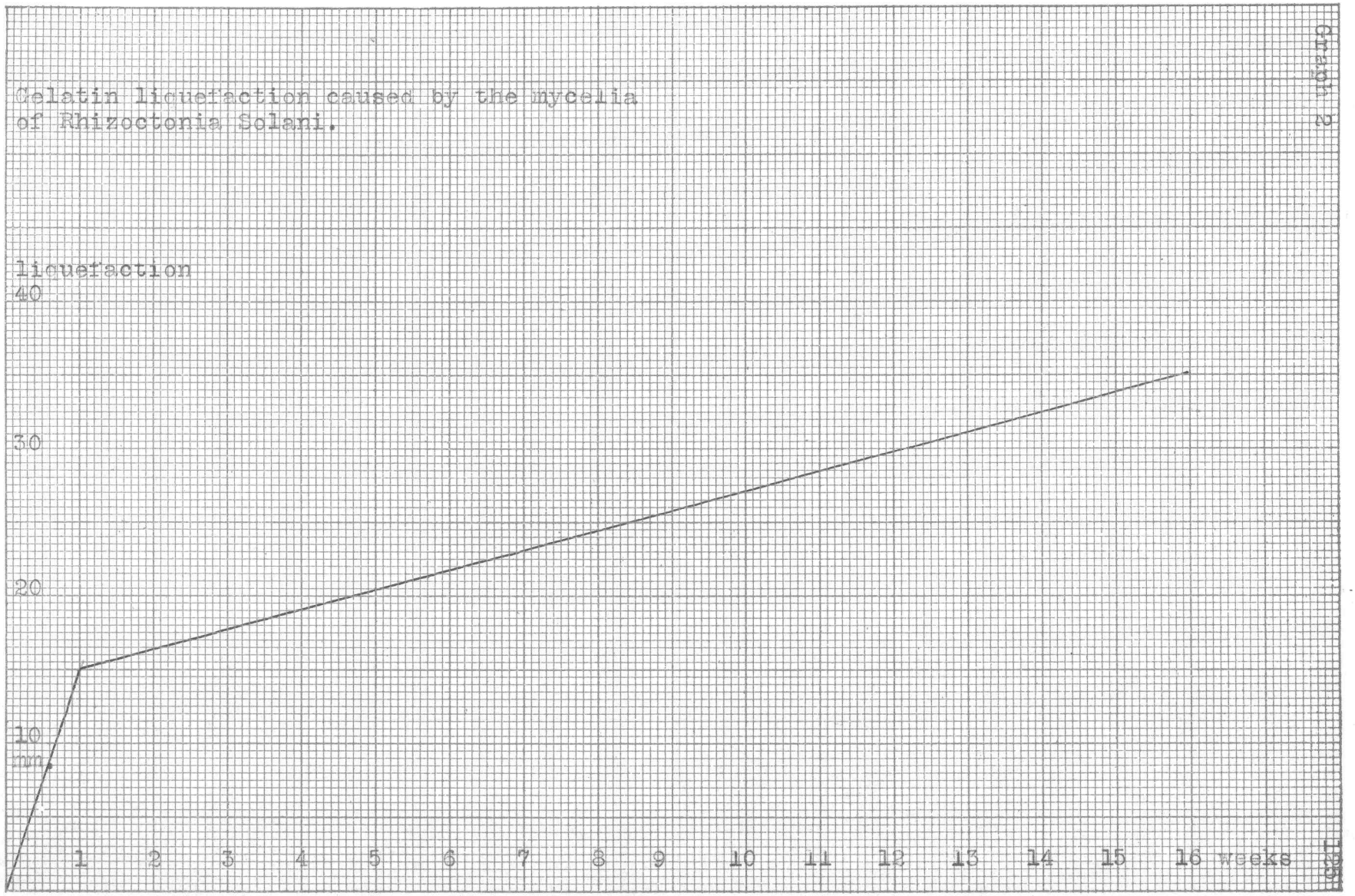
Graph 1

The toxic effect of ammonium sulphate to the growth of Rhizoctonia mycelia.
A, check
B, 0.25% ammonium sulphate
C, 0.50% ammonium sulphate



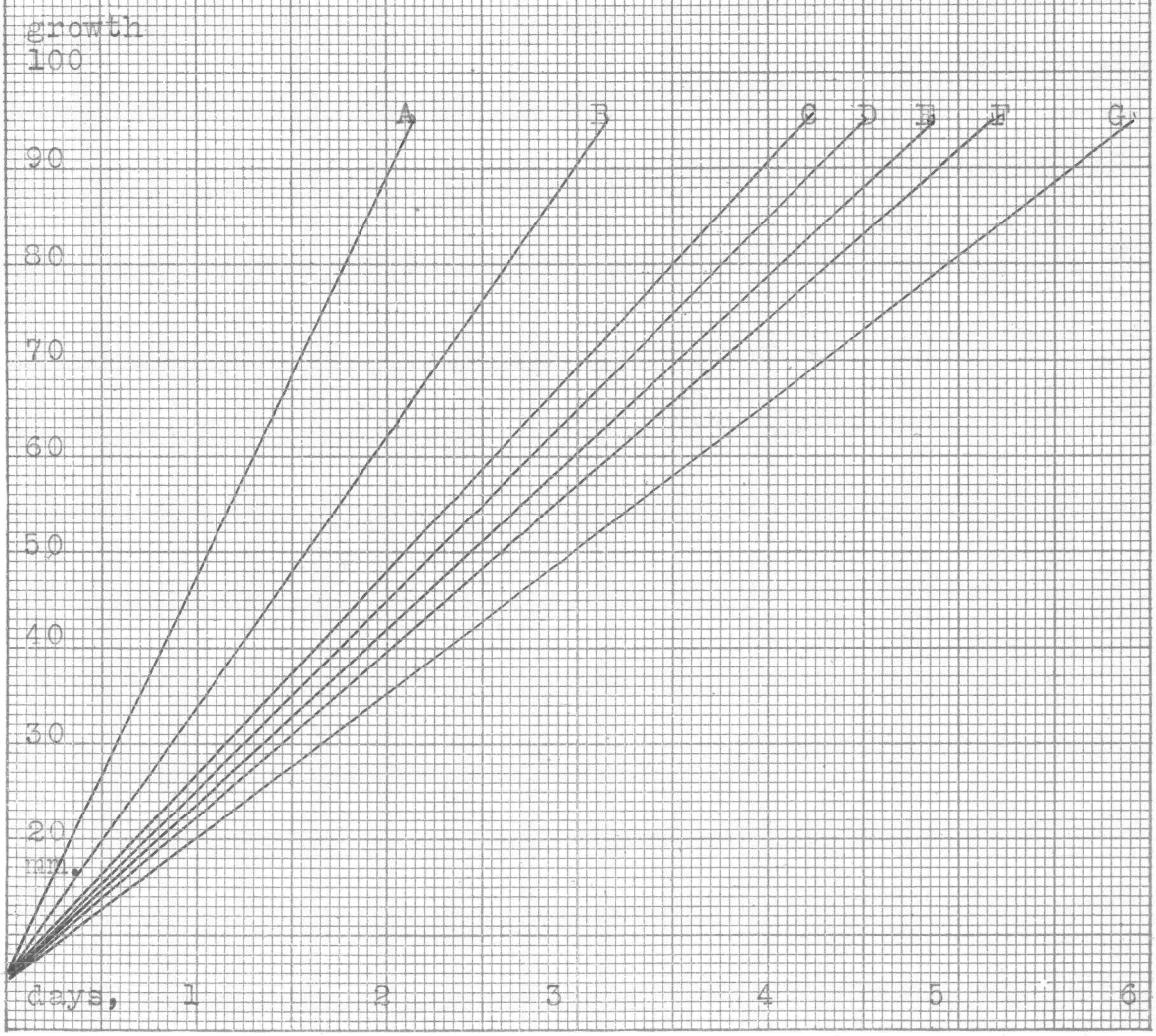
Graph 2

Gelatin liquefaction caused by the mycelia of Rhizoctonia Solani.



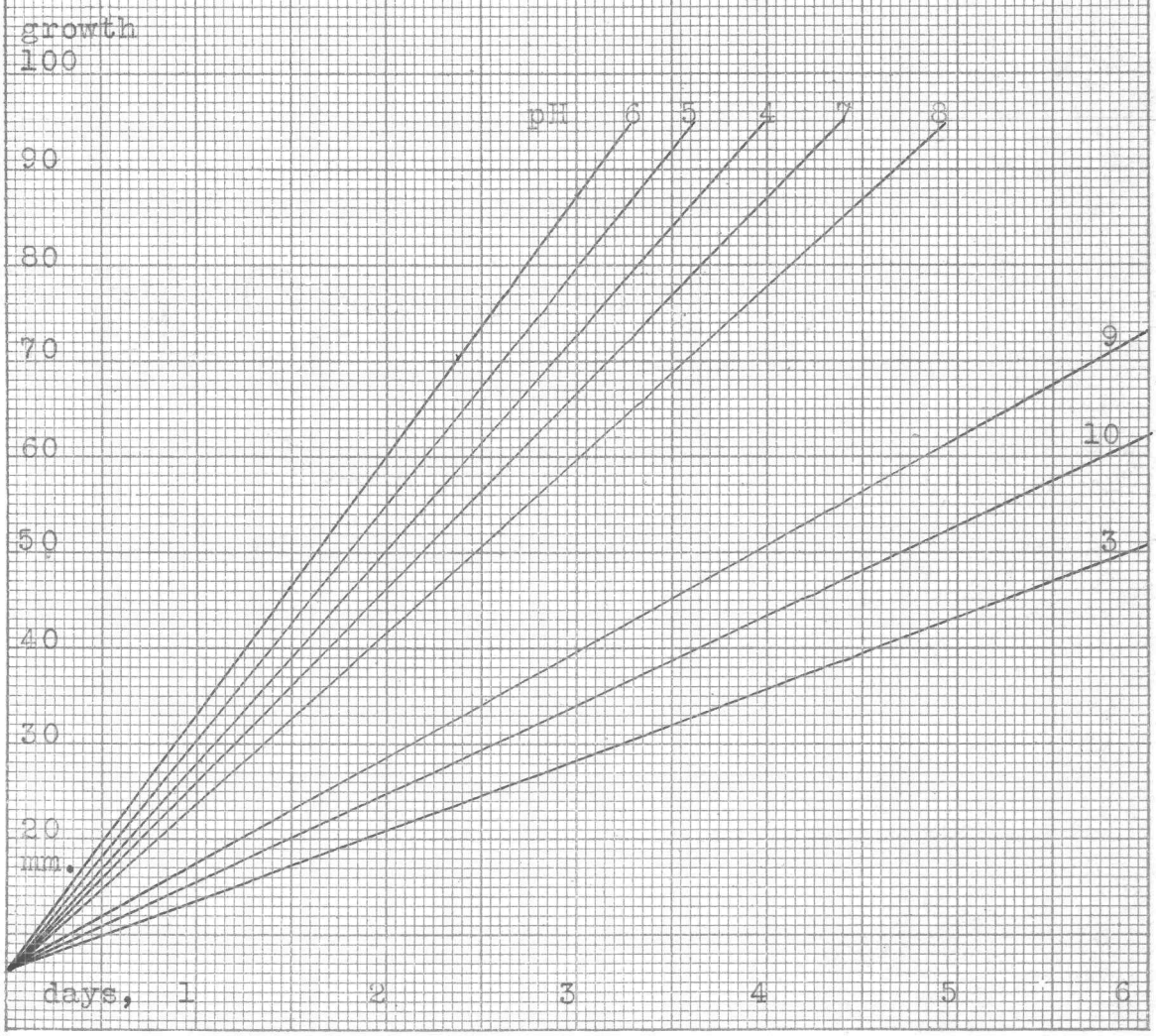
Graph 3

Growth rate of different strains of Rhizoctonia Solani at 20 degree C.
A, carnation strain
B, Botryobasidium no. 1
C, Tomato strain
D, potato strain
E, strawberry strain
F, barley strain
G, oats strain



Graph 4

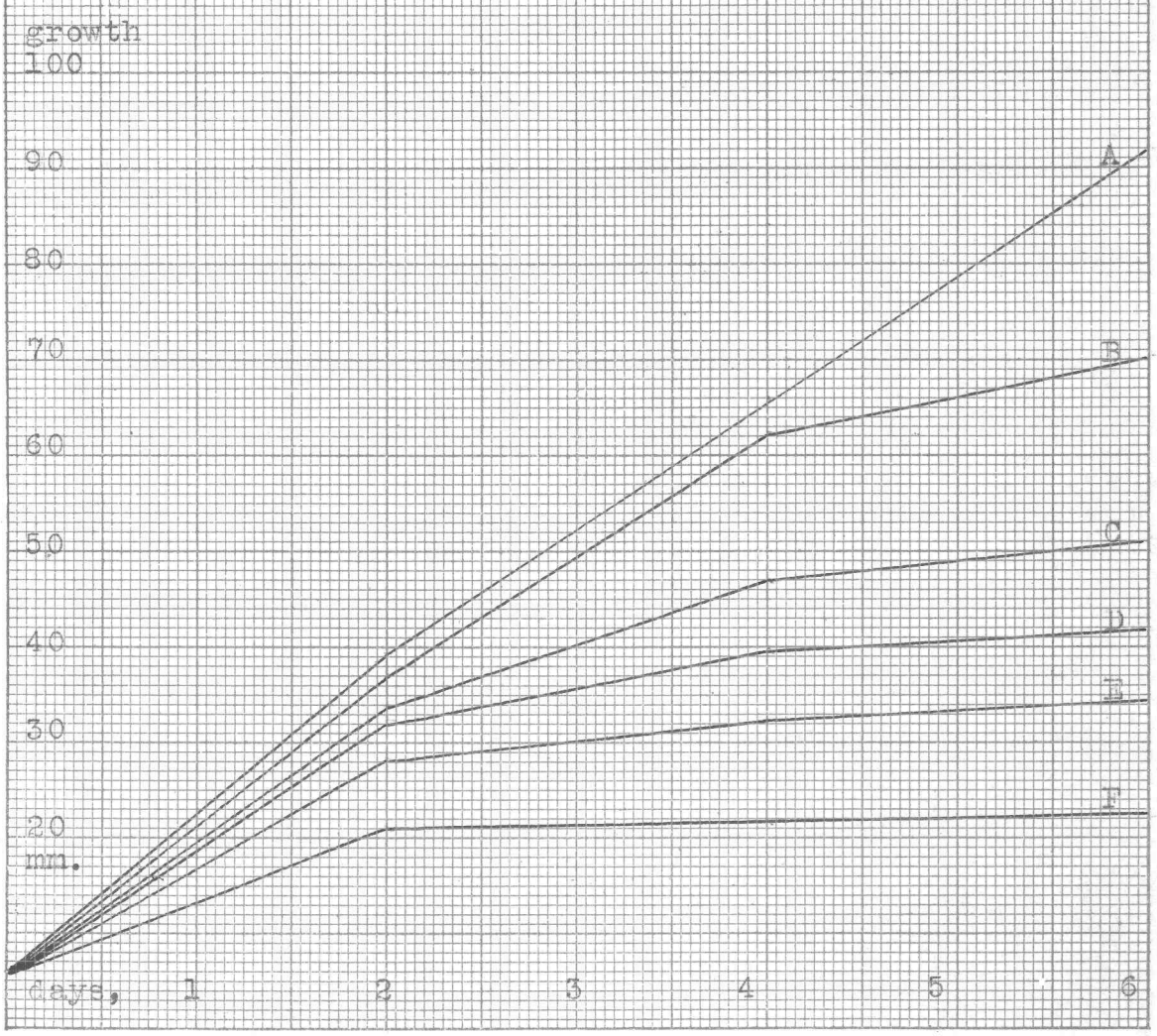
The effect of different pH to the mycelium growth rate of Rhizoctonia Solani (carnation strain).



Graph 5

The effect of different humidity (by using various concentrations of sulphuric acid) to the mycelium growth rate of Rhizoctonia Solani.

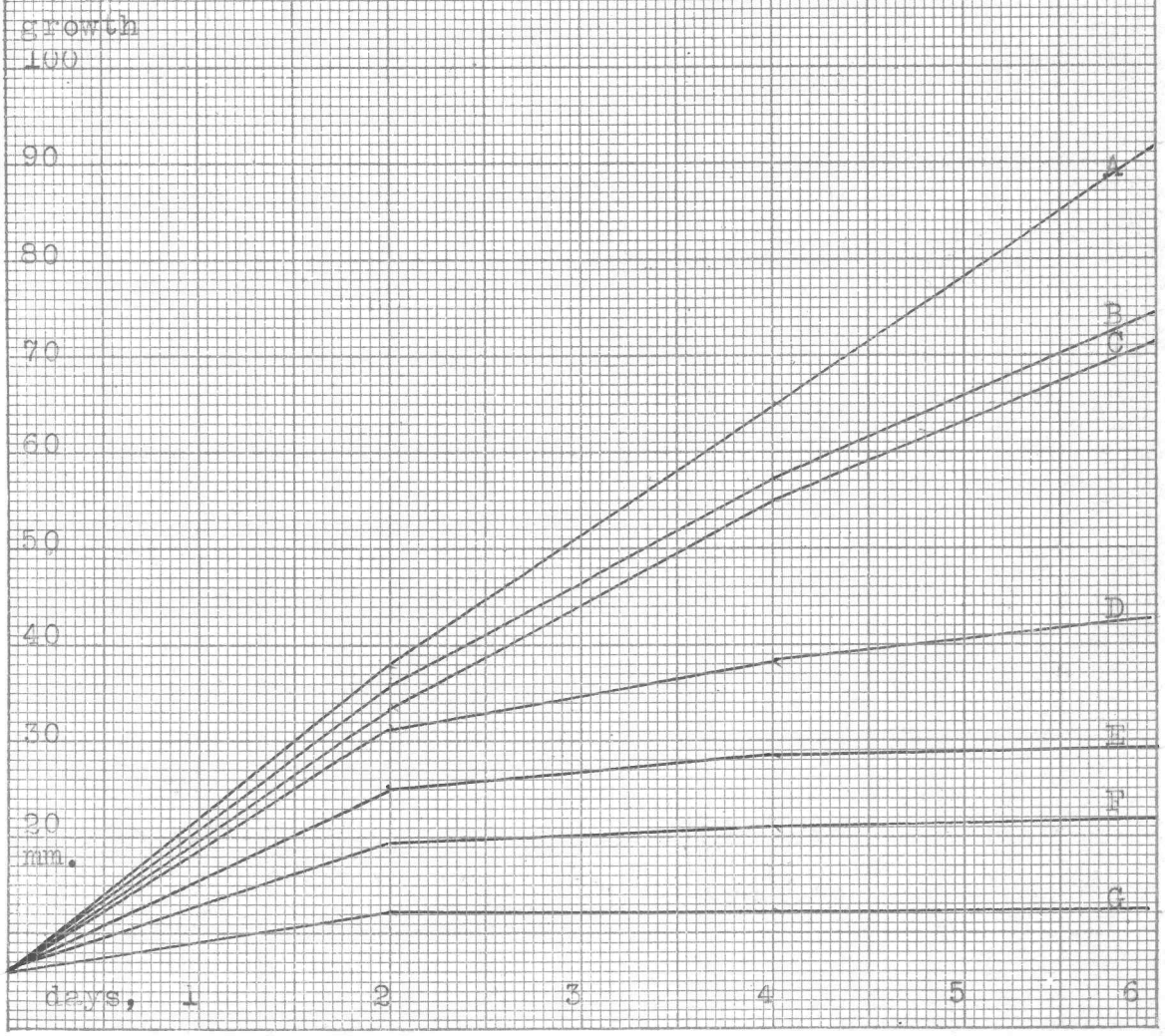
A, check
B, 100% humidity
C, 90% "
D, 80% "
E, 70% "
F, 60% "



Graph 6

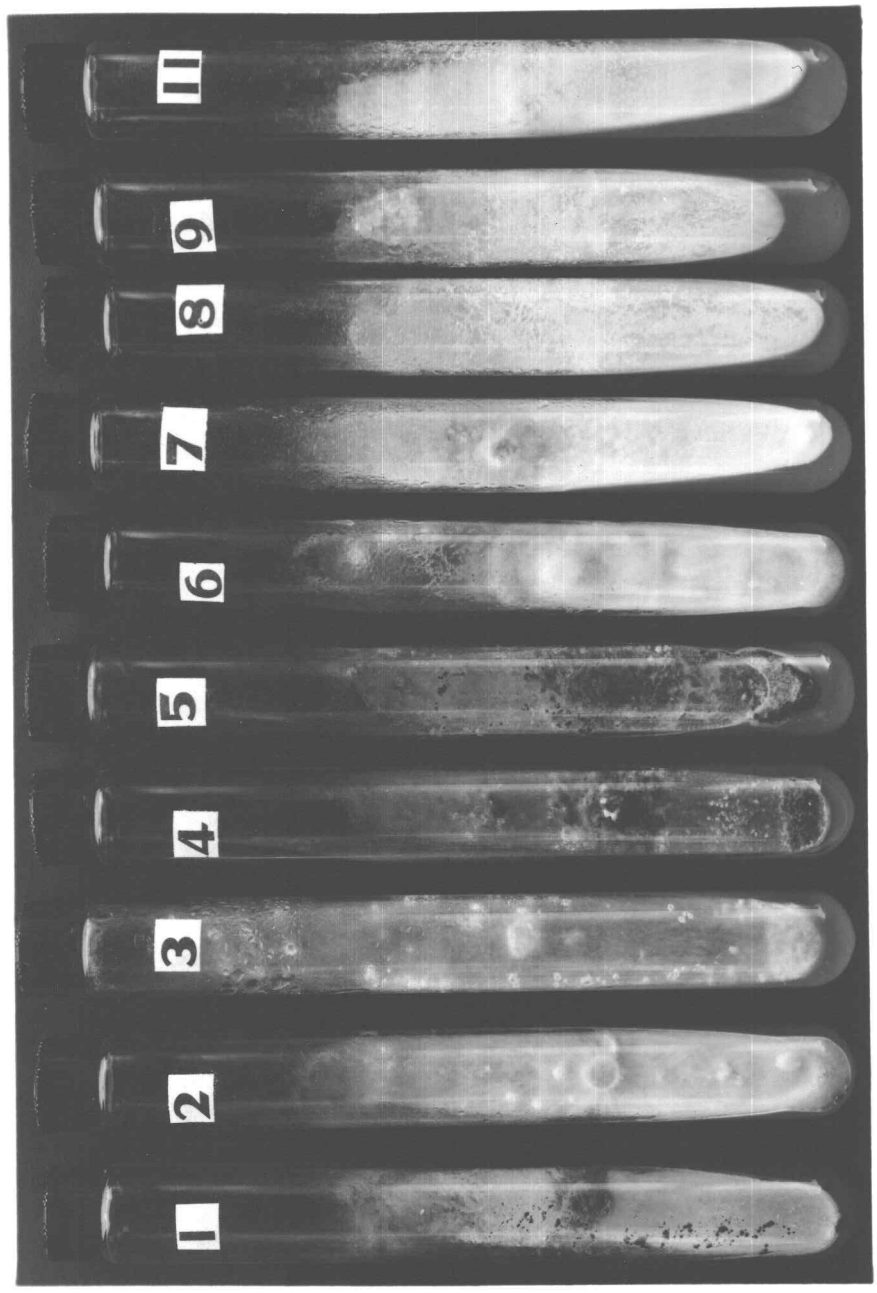
The effect of dif erent humidity (by using various saturated salt solution) to the mycelium growth rate of Rhizoctonia Solani.

A, check
B, 100% humidity
C, 91% "
D, 81% "
E, 66% "
F, 33% "
G, 15% "



The effect of ultraviolet light radiation of various length of time to the growth of Rhizoctonia mycelia.
A, 1 day growth after radiation
B, 3 days growth after radiation
C, 3 days and 6 hours growth after radiation

Green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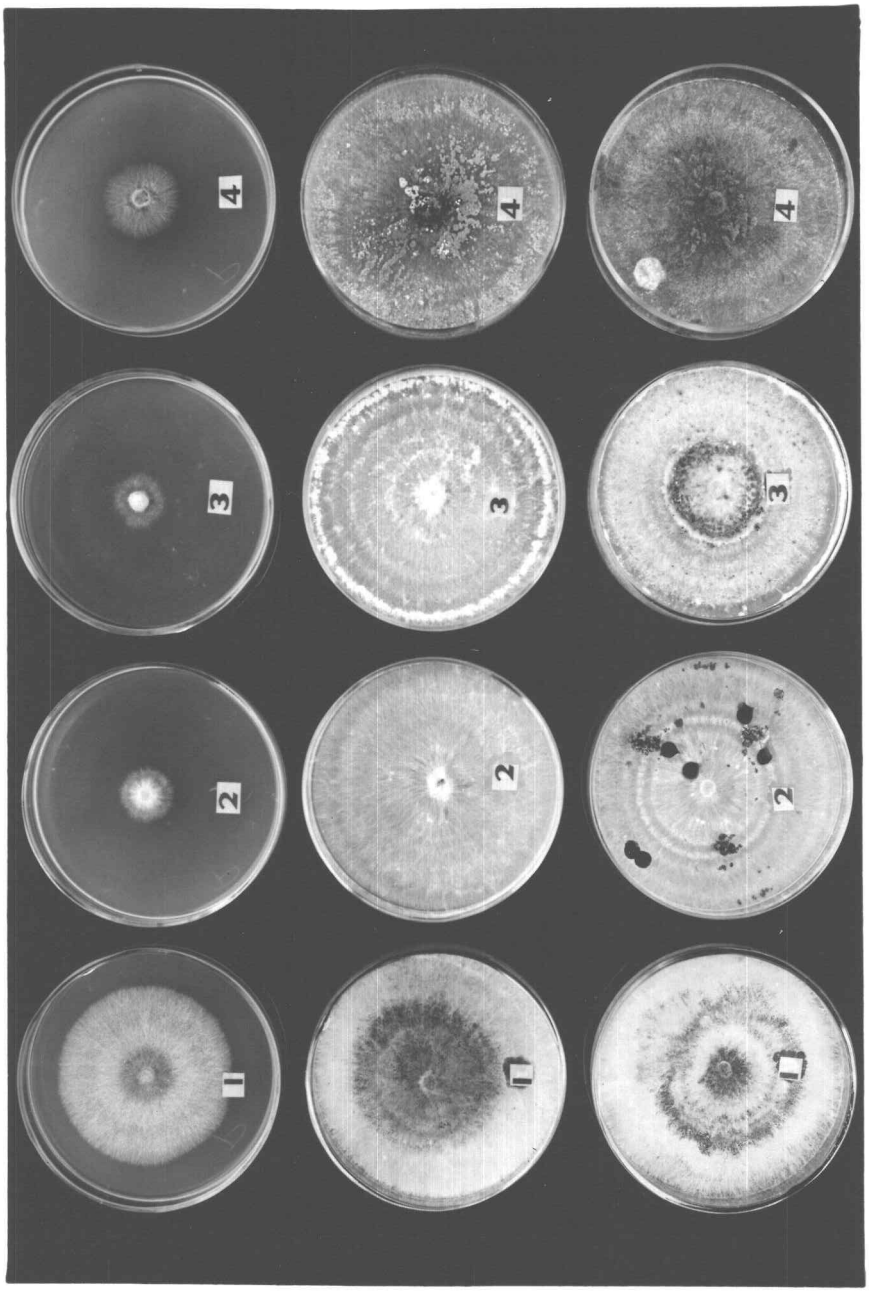
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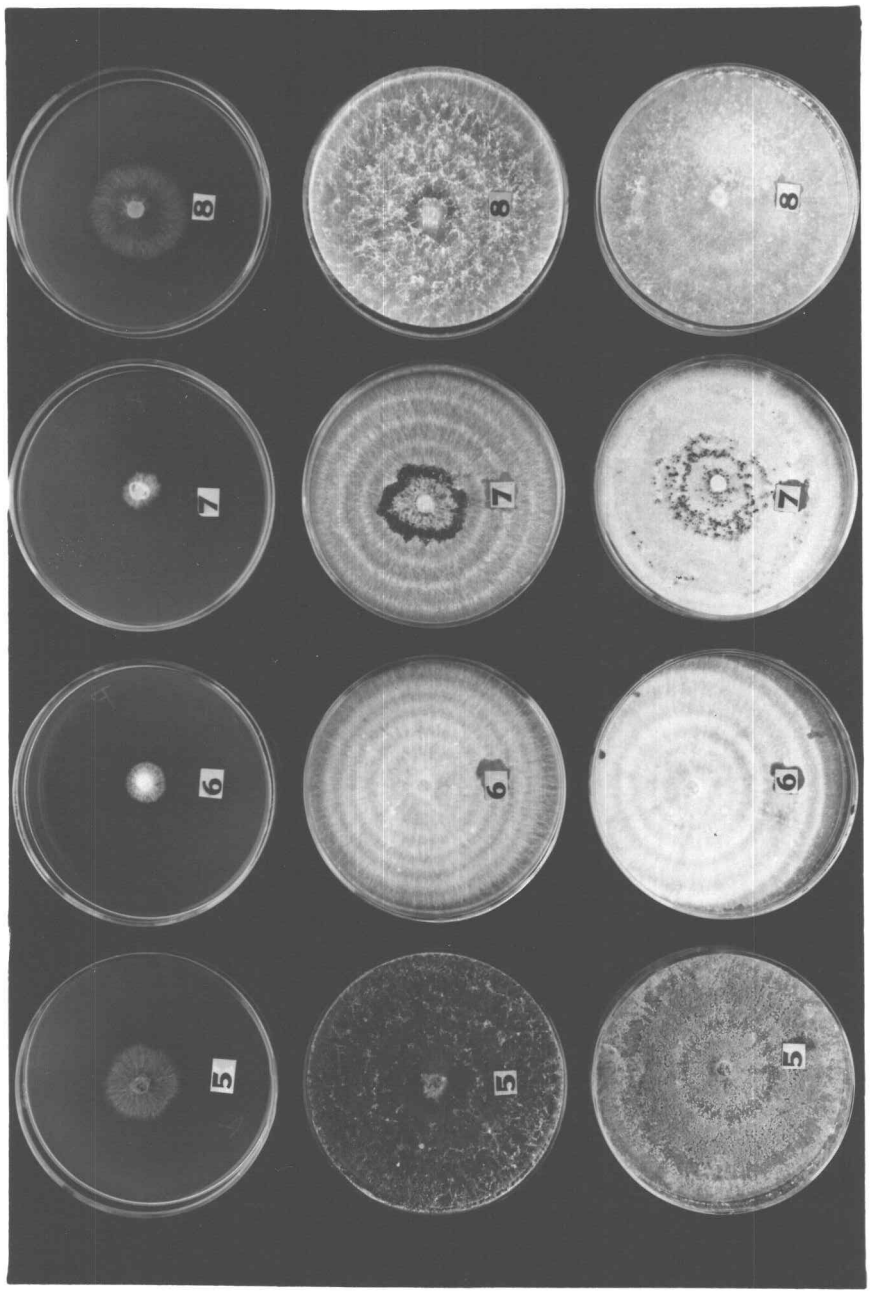
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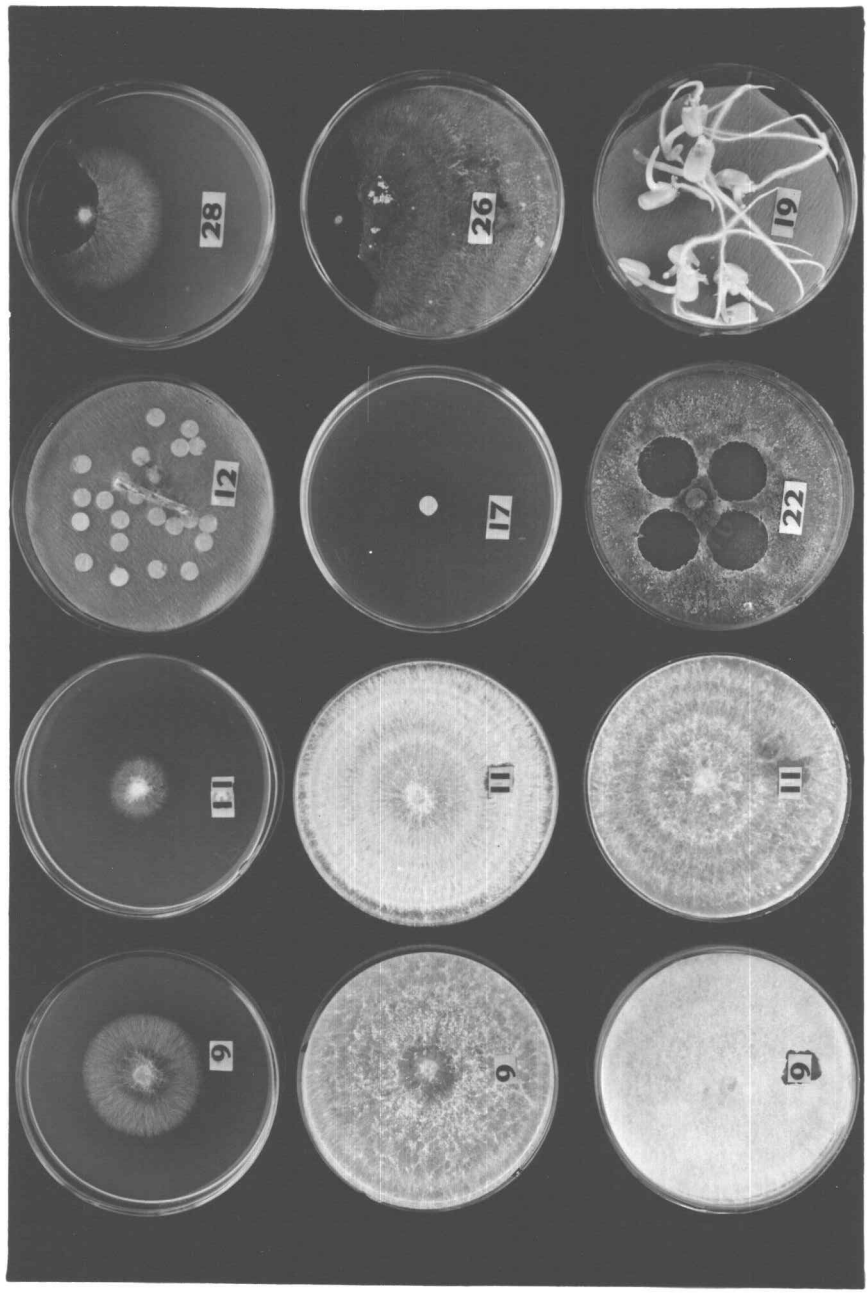
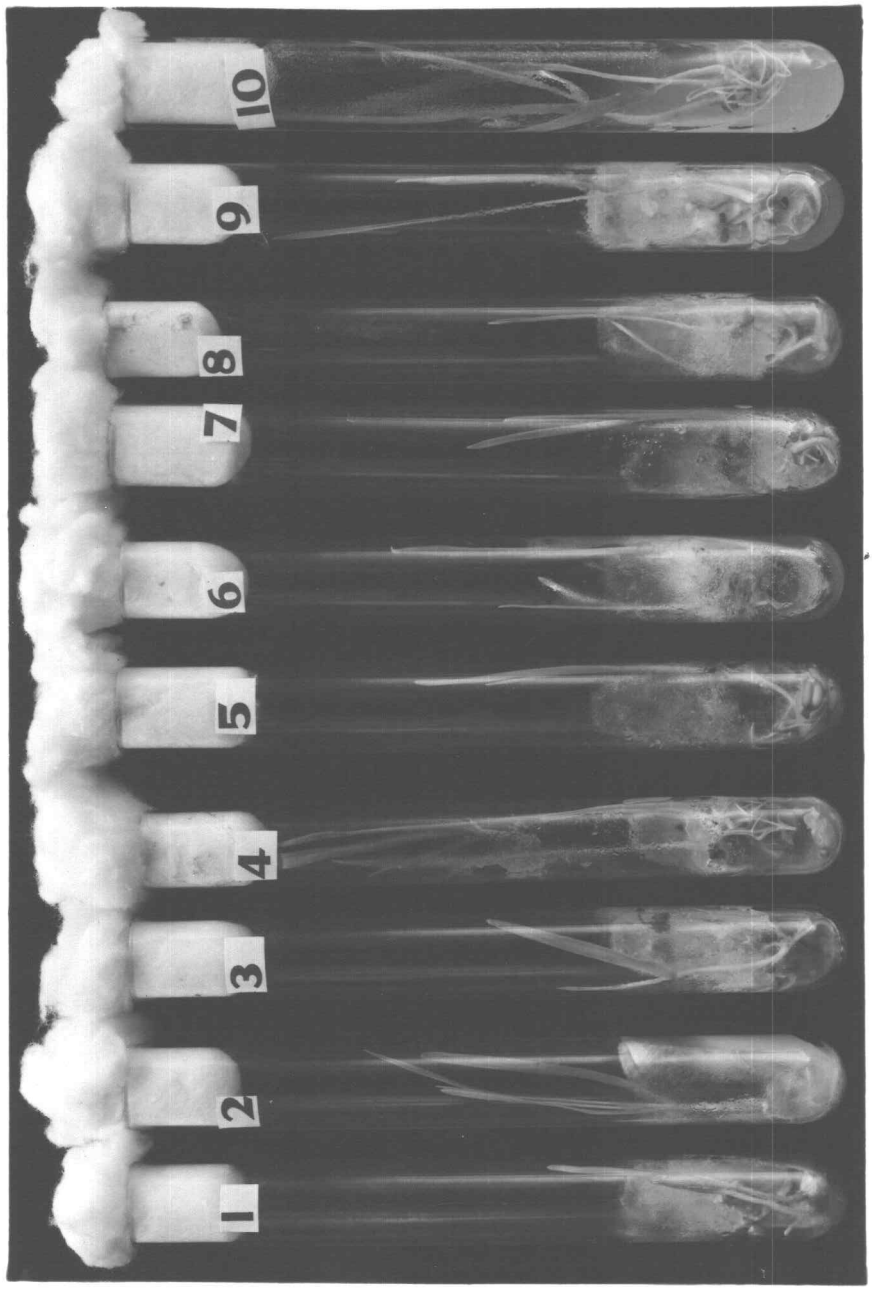
Plate IV

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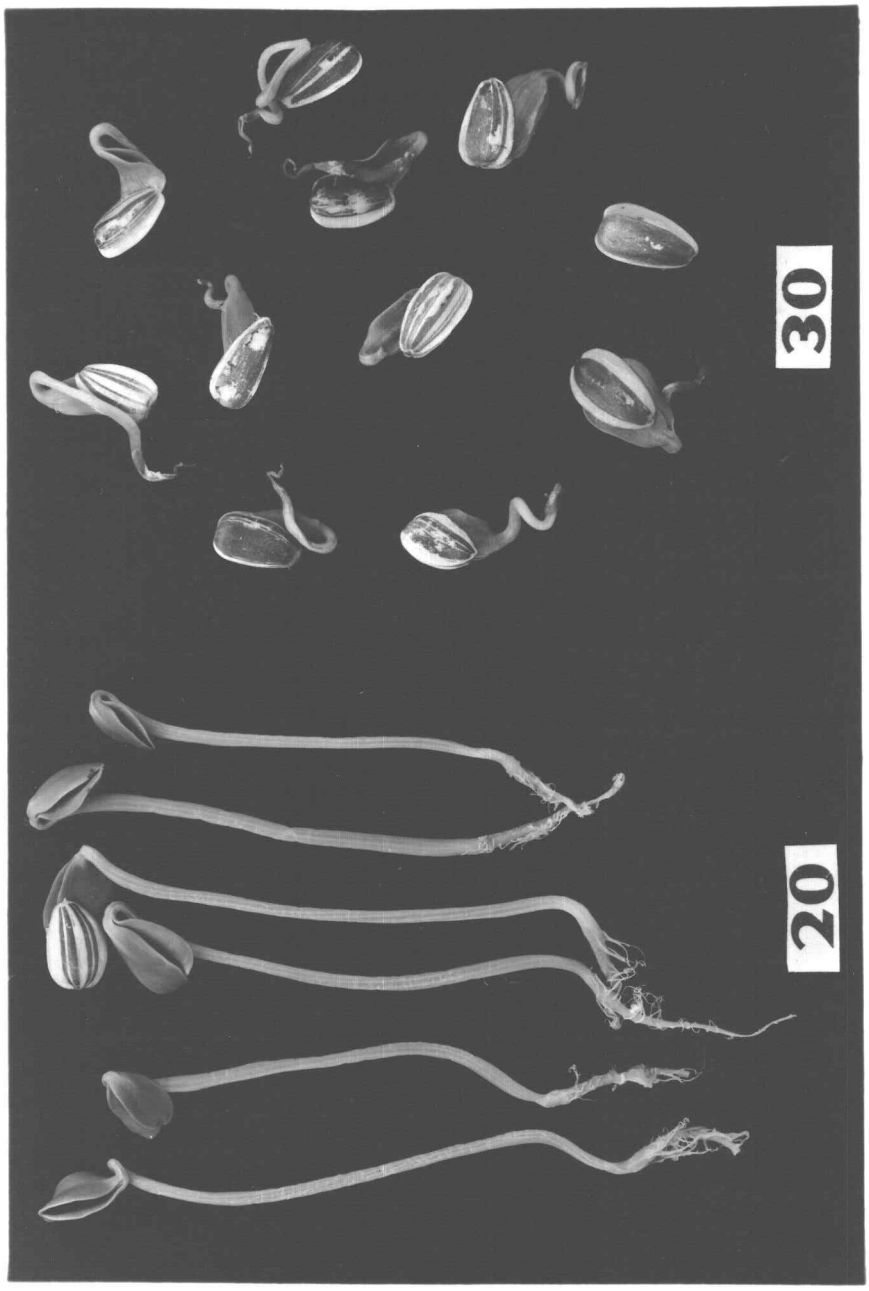
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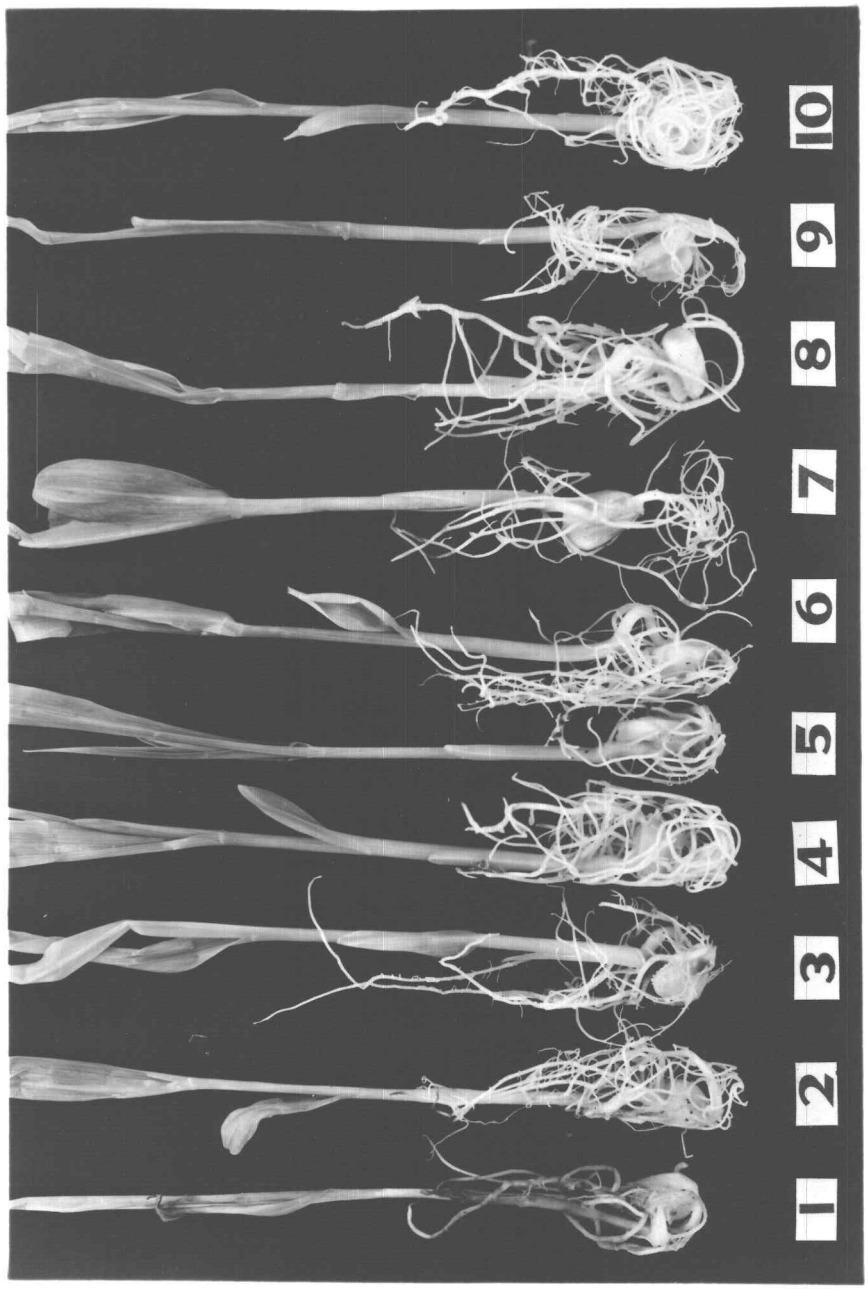
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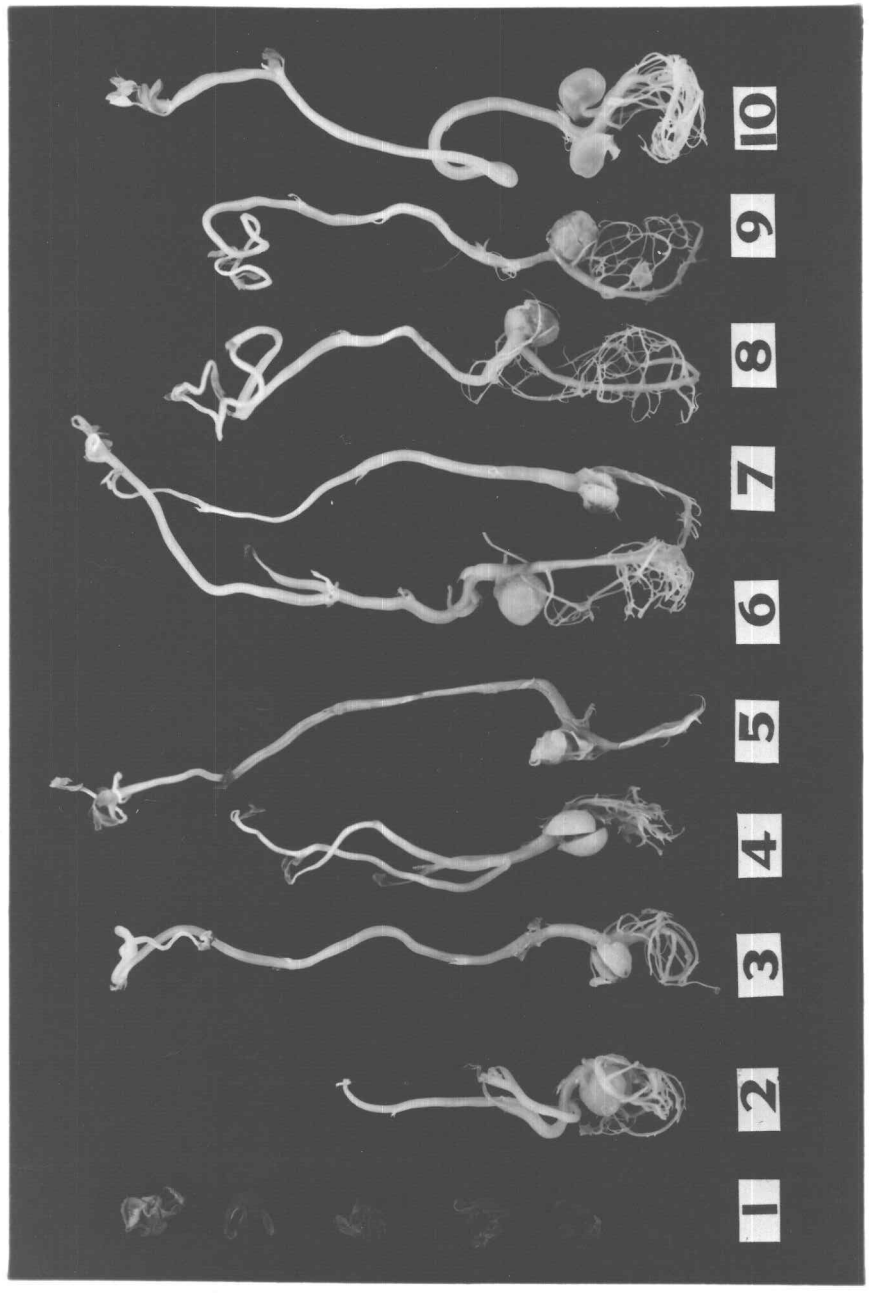
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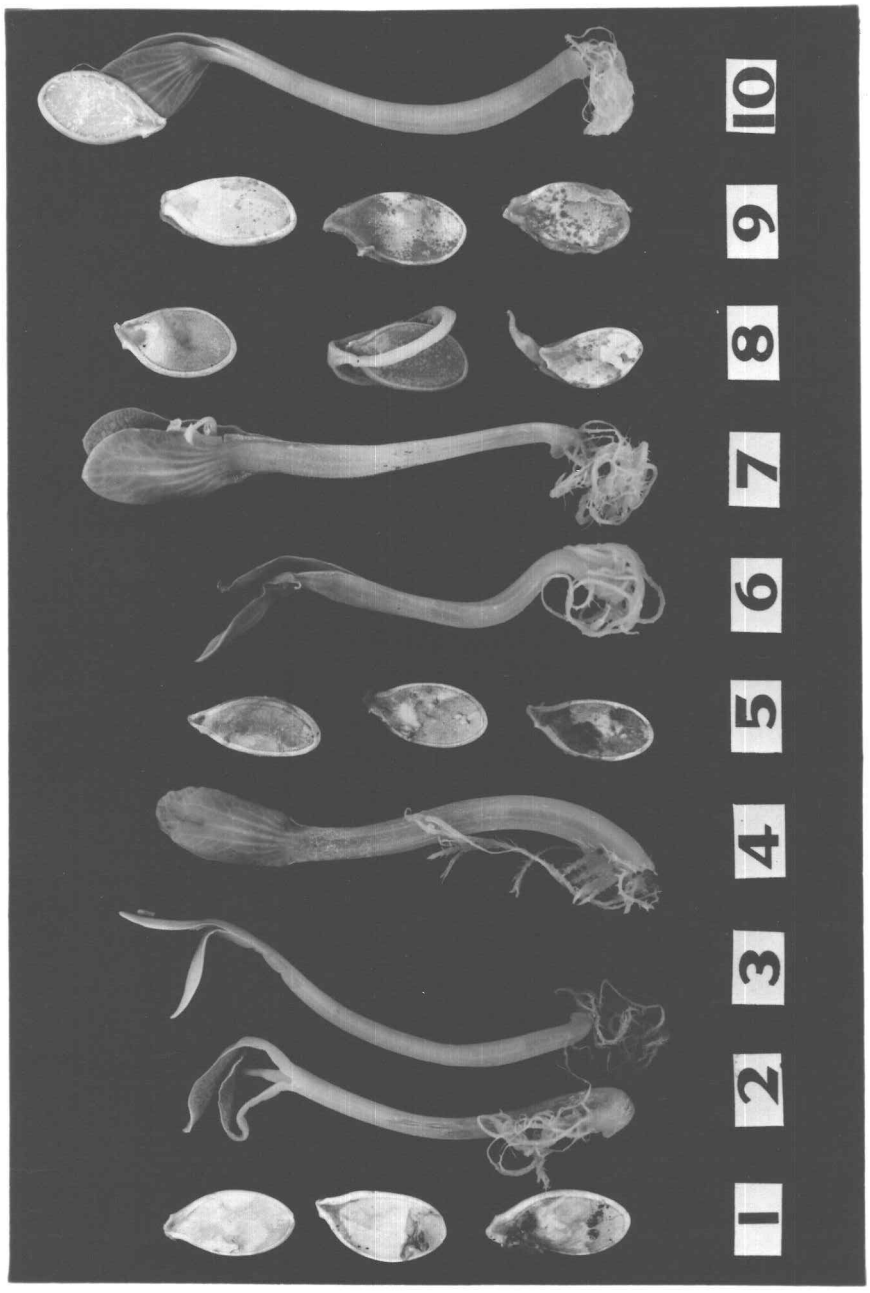
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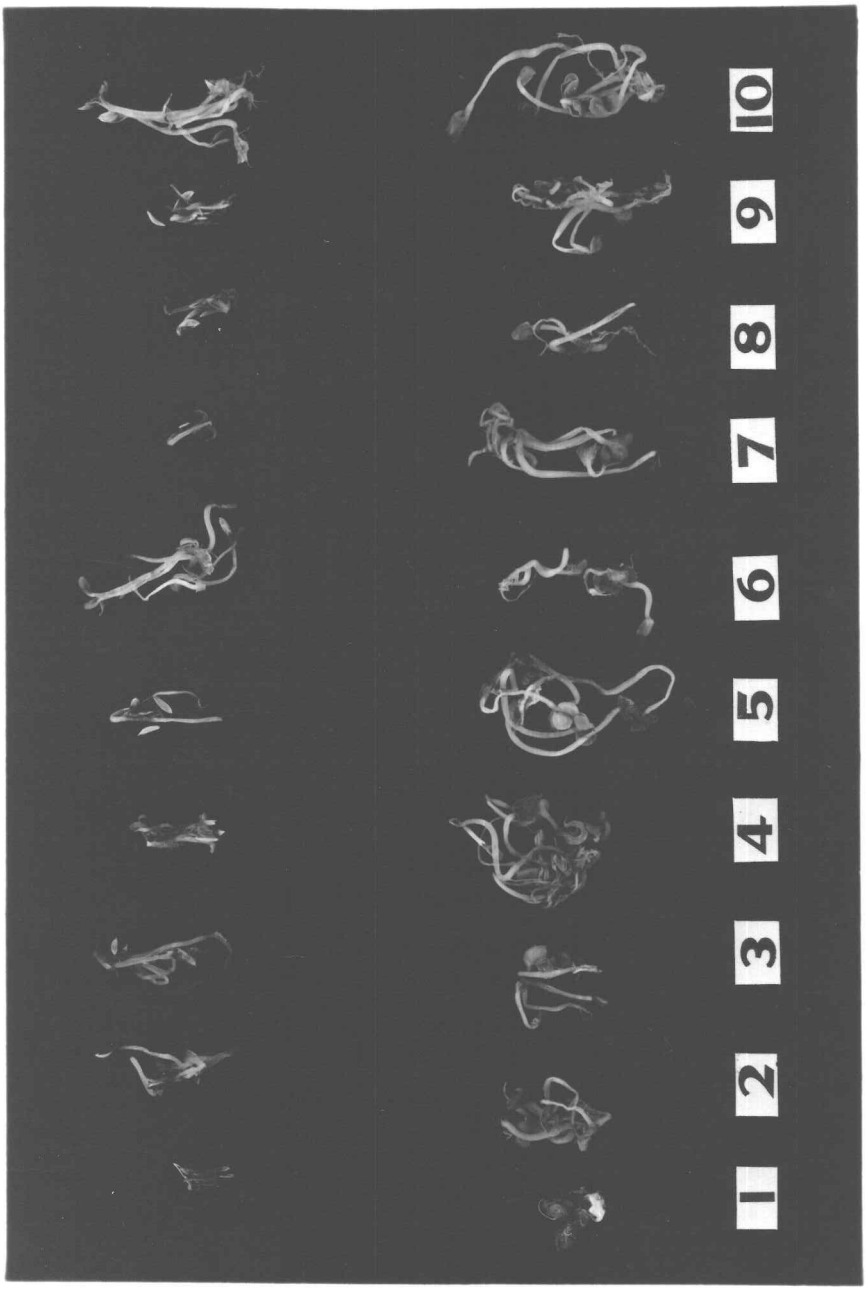
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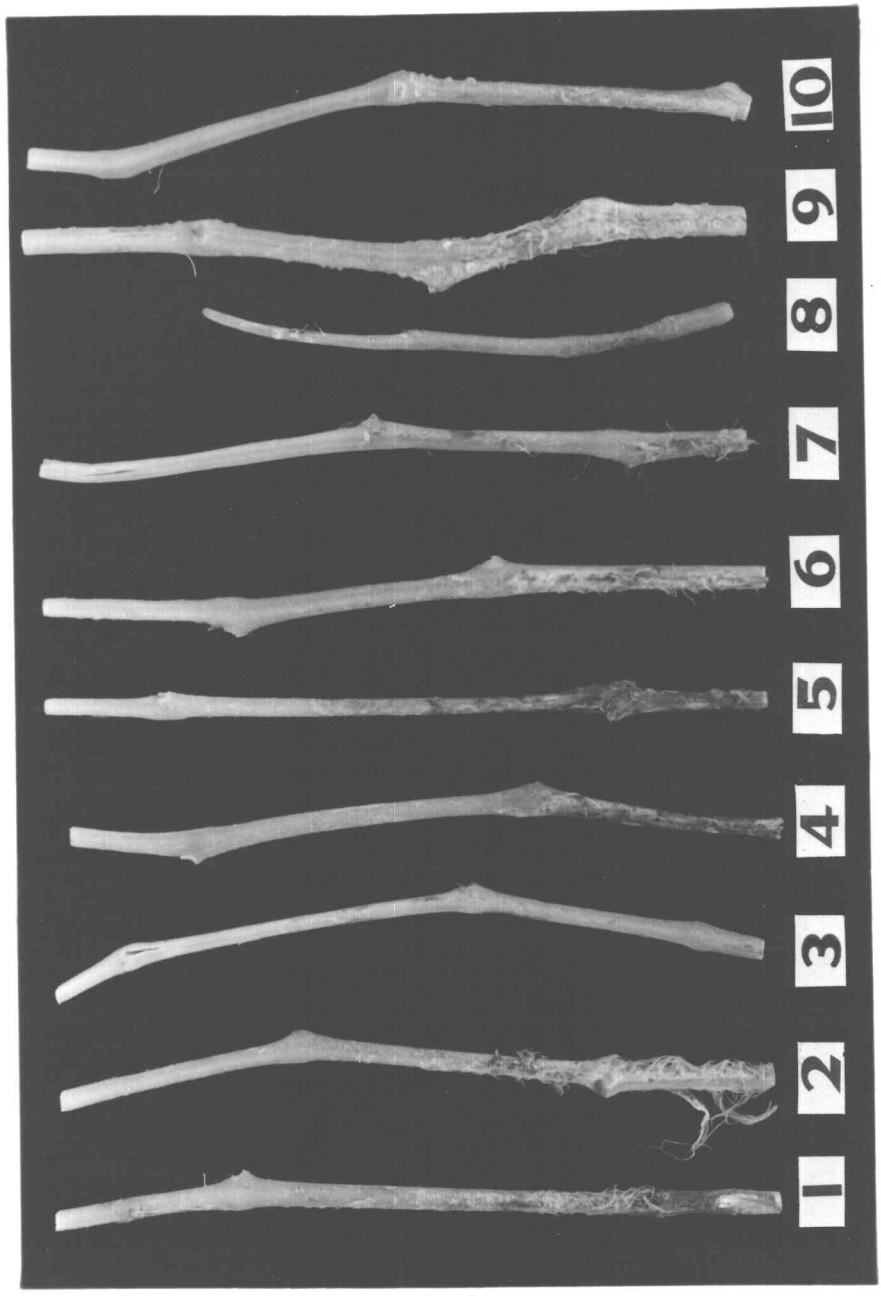
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