1922

Damping-off of onion seedlings due to Rhizoctonia

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DAMPING-OFF OF ONION SEEDLINGS DUE TO RHIZOCTONIA

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Thesis Submitted for the Degree

of

Master of Science

Massachusetts Agricultural College

Amherst, Mass.

June 1922

OUTLINE

ACKNOWLEDGEMENTS.

The author wishes to express her heartiest thanks to Doctor P. J. Anderson and Doctor W. H. Davis for direction and assistance in this work, and her sincere appreciation of the never failing kindness and encouragement of all those connected with the Department of Botany.

INTRODUCTION

During an investigation of onion smut (*Urocystis cepulae*) by the Department of Botany of the Massachusetts Agricultural College, it was frequently observed that damping-off caused considerable injury in the onion fields of the Connecticut valley. When later work was undertaken in the Department greenhouse with soil from a Sunderland onion field, damping-off occured to such a degree that it was difficult to determine what percentage of loss was due to smut and what to the seedling rot. From these observations it was concluded that the disease was of sufficient importance to warrant further investigation to determine, primarily, what fungi were responsible and how they might be controled.

Damping-off in general.

Definition: Damping-off is a general term for a group of diseases which cause rapid wilting and decay of young plants by rotting them off at the surface of the ground. While the term is most commonly used in reference to rots of seedlings or soft cuttings, cases of damping-off of fern prothallia (2) and of young shoots from parasitized root-stocks (29) have been included under the same general name. Damping-off may occur wherever plants are

grown: in the field, forest, nursery, garden, seed-bed, or greenhouse. It is most prevalent, however, where plants are crowded together under conditions of high temperatures, and poor soil drainage, and takes its name from the fact that a state of excessive moisture is most favorable to its spread and development.

Symptoms of damping-off of seedlings: Damping-off occurs typically as a rot of seedlings and originates at or near the surface of the ground, or it may attack the hypocotyl upon germination, killing the plant even before it reaches the surface. The tissues of the affected part at first lose their turgidity and appear water-soaked; later, as the decay progresses, they collapse, causing the plant to topple over or "damp-off". The mycelium then quickly invades the tissue of the entire plant, and often forms a white felt over the surface of the soil surrounding the fallen seedling, thus spreading to adjacent hosts.

Hosts: Since some of the soil inhabiting fungi seem almost unlimited as to host, it may eventually be found that any seedling is susceptible to attack by one or more of these disease producing organisms. At present there is a wide phylogenetic range of hosts,

from the prothallia of certain Fillicales to the highest angiosperms. Damping-off has been reported much less frequently among monocotyledonous plants than among dicotyledonous. Certain monocotyledons, however, are known to be affected--Asparagus sprengeri (15), grass, and Allium sps. (41), and it is probable that further investigation will show that a large number are susceptible. In forest nurseries the damage is so serious that Hartley (25) says; "With the exception of cedars, damping-off of seedlings is a serious hindrance to the raising of coniferous seedlings." Broad-leaved trees, both fruit and shade trees, are also often subject to injury, while among flowering plants, ornamentals, truck and field crops, new hosts are constantly being discovered.

Causal Organisms: With such a wide range of hosts it might easily be suspected that there would also be a large number of organisms involved, and such is the case. Stevens (64), in "Diseases of Economic Plants," lists as general damping-off fungi the following: Pythium, Thielavia, Corticium, Fusarium, Botyrtis, Sclerotinia, Sclerotium, Sclerotium, Phoma, Volutella, Phytophthora, Colletotrichum, and Gloeosporium. A more complete account is given by Hartley (28), who separates damping-off fungi into two types on the

basis of parasitism and host. In the first type he includes soil inhabiting and primarily saprophytic fungi, indifferent as to host. Among these may be noted Phytophthora fagi, Aphanomyces levis, Rheosporangium aphanidermatus, Botrytis cinera, Fusarium, and the two most common of damping-off fungi, Pythium de Baryanum Hesse and Corticium vagum B. and C. The second type, on the other hand, includes the more strongly parasitic fungi and bacteria with a limited host range. Into this group Hartley puts the following: Phoma betae on beed seedlings; Phomopsis vexans, causing damping-off of egg-plants; Fusarium Lini, parasitic on flax; Phoma lingam and Peronospora parasitica, destructive to crucifers; Completonia complens on fern prothallia; Gloeosporium and Volutella sps. attacking respectively greenhouse roses and carnations; as well as Bacillus malvacearum on cotton, and bacteria reported by Halstead as causing damping-off of cucumber seedlings.

Literature relating to Damping-off of Onion Seedlings.

Very few records are to be found in the literature of plant pathology relative to damping-off of onion seedlings. Thaxter (66), reporting fungous diseases of onions from Connecticut in 1889, writes: "Another fungus which did great injury to onion seedlings in the

hothouse may also be mentioned. Plants attacked by this disease become white and flaccid near the base, soon falling over and withering. The cause in this case was a fungus related to the molds (Saprolegnia) often destructive to fishes; and belonged to the genus Pythium. Whether the species is identical with Pythium de Baryanum, also said to produce disease among various seedlings, was not determined". In 1914, according to Robson (49) of the Imperial Department of Agriculture, Montserrat, considerable loss occured to the onion crop of the West Indies due to damping-off in the seed-bed. The causal organism was not determined, but was said to be neither Pythium nor apparently Rhizoctonia. Several precautionary measures were recommended for control, roasting of the surface soil being considered the most practical for local purposes. The use of formalin, one part to fifty, and surface treatment with a layer of sand were also advised.

A culture of Rhizoctonia isolated from damped-off onion seedlings was obtained from Dr. I. C. Jagger of Cornell in 1911 by Mr. H. W. Anderson of the University of Illinois. The investigation of this strain is reported by Peltier, (41) who, on the basis of culture comparison with R. Solani Kühn, would rank the onion

fungus as a different species.

Rogers (50), writing on onion growing in California in 1918, includes damping-off among the most common crop troubles. He suggests methods of treatment, but does not ascribe the injury to any definite organisms, merely stating that it may be caused by "several fungi which live in the soil". In view of the apparent prevalence of this disease in the onion seed-beds of California, it is strange that there is no mention of it in the reports of the other important onion-growing states of the southern section, Texas and Louisiana, since in this district planting is done in warm weather most favorable to the growth of the causal organisms.

In the northern section, where spring planting is most extensively practised, Massachusetts (31) is the only state reporting damping-off of this crop. Rhizoctonia on onion was reported from Washington(30) in 1920, but whether it was from seedlings or from mature bulbs was not stated. No reference to damping-off in this connection is to be found in the latest United States Department of Agriculture Bulletin (69) on "Onion Diseases and their Control."

Scope of the present investigation.

From the above history it will be noted that

although the cause of damping-off of onion seedlings has not been fully determined, it has been definitely ascribed to three fungi, Pythium, Rhizoctonia, and Fusarium, with the possibility of others being involved. Species of these three and of Botyrtis were isolated by Doctor P. J. Anderson[#] from damped-off seedlings from fields near Amherst, Massachusetts. Work started by the writer in late September, 1921, resulted in isolations of Pythium, Rhizoctonia, and Fusarium. The present paper deals with the pathological relations of Rhizoctonia to the disease from the following standpoints: Identity of the fungus, based on morphological and cultural characters; Pathogenicity; Infection; and Control.

THE DISEASE

Economic Importance.

The amount of damage caused by this fungus to onion seedlings under field conditions has not been fully determined, as limited field inspection in May, 1922, resulted in the finding of but two areas in which damping-off was serious and in both of these cases the trouble was due to Pythium.

(# Unpublished data from private notes of Doctor P. J. Anderson, Department of Botany, Massachusetts Agricultural Experiment Station.)

In other localities and during other years Rhizoctonia might be found to be of great importance. In the greenhouse, where infection increases with each planting, practically 100% damping-off may occur, especially where there are other contributing factors to weaken the young plants. In a series of observations, based on plantings made on an average of every two weeks from October to June, infection was invariable, ranging from 10% to as high as 96% in one instance. There was also a consistent variation in the amount of damage per row, those rows on the outside of the bed, which dried out most rapidly, having the highest percentage of injury. This seems to be a contradiction to the name "damping-off," but under the uncontrolled conditions of a greenhouse bed there are so many factors involved, that it is not possible to ascribe the cause definitely to any one. It might rather be presumed that the cause of the increased activity of the fungus at such points was due not so much to lack of moisture, as to an increased soil temperature which produced the more rapid evaporation, since this part of the bed was in closest proximity to the glass and steam pipes, and also received the heat reflected from the cement walks and bed foundations. Hartley (28) found fluctuations in damping-

off to be in direct proportion to temperature and evaporation, thus proving the soil heat of greater factorage in producing the disease than the moisture content.

Symptoms.

The first symptom of damping-off is the presence of breaks in the row, as the seedlings come up. About the third or fourth day after the knees come through the soil, those seedlings nearest the barren portions of the row will begin to fall over (Plate I, Fig. 1.). If damping-off is in the early stages, examination will reveal a hygrophorous area just at the surface of the ground, or if has developed a little more, a decided lesion or contraction may be found at the collar. As the fungus penetrates more deeply, the tissues collapse at this point, and only a feeble white thread ties the still green upper part of the cotyledon to the root. The fungus then spreads into the root and cotyledon, and with secondary agents completes the destruction of the seedling. (Plate I, Fig. 2)

This is the most common outward appearance of the disease, it is found in those plants where infection occurs in the neighborhood of the root joint. Cases occur frequently, however, where multiple infection of the cotyledon causes it to grow shriveled and fibrous while still

green, and this dessicated area may extend over the whole cotyledon. If such seedlings are examined, the tissue will be found tough, and hard to tear apart, while microscopic examination will show the cells so packed with mycelium that only the chloroplasts can be distinguished.

CAUSAL ORGANISM

Taxonomy

The form genus Rhizoctonia was established in 1815 by DeCandolle (9), who recognized two species, R. Crocorum DC., primarily inhabiting the crocus, and R. Medicaginis DC., on alfalfa and other hosts. He also considered a third doubtful species, reported on apple, as R. Mali. Nearly a century before this attempt to systematize the fungus, Duhamel (17), also working in Southern France on Crocus sativus, had described a sclerotial disease of the saffron. Although he regarded this fungus as allied to the Truffles, and considered the sclerotium, "tubercle," a special form of fruiting body of which the hyphae were the roots, his discussion of the macroscopic features of the mycelium and sclerotia identifies them with the violet hyphae and knot-like swellings of R. Crocorum of De Candolle.

In 1785, Fougeroux de Bondaroy (8) in a further

study of this disease of the saffron recorded its occurence on asparagus, grown in soil previously occupied by the infected crocus bulbs. In 1791, Bulliard (3), following Duhamel, included the fungus among the Truffles, and named it Tuber parasiticum. Shortly afterwards, however, Persoon (42), distinguishing between the sclerotium and a true sporocarp, changed the name to Sclerotium Crocorum, and placed it in the genus Sclerotium. Persoon's specific name was retained by DeCandolle, when he established the genus Rhizoctonia, and later (1823) by Fries (22), thus establishing the priority of R. Crocorum over the more descriptive names of R. Medicaginis or R. violacea.

Nees(40) in 1817 placed the crocus fungus in Thanatophytum under the name T. Crocorum, although it is evident from his description and figures that he was dealing with Rhizoctonia. Besides R. Crocorum DC., Fries (22) recognized R. Medicaginia DC., R. Batatas Fr. on Ipomoea Batatas, and R. muscorum Fr., with R. Mali DC. among species ignota. Link (36:1824) excluded the last two forms as doubtful, and added a species R. strobilina. Fries had assigned Rhizoctonia to the Sclerotiaceae following the genus Sclerotium, and this arrangment was adopted by Duby (11:1830), with the addition of a species R. Allii on Allium Ascalonicum. In 1843, Leveille (35) gave as

additional hosts, Rubia tinctorum, Solanum tuberosum, Phaseolus, and Tuplipa, without any attempt to classify the species of the parasite.

The first systematic work of real importance was done in 1851 by L. and C. Tulasne (67) in France, and by Kühn (34:1858) in Germany. The former reduced R. Crocorum DC. and R. Medicaginis DC. to one form, which they named from the characteristically colored mycelium R. violacea. This classification has been favored by most later investigators. Previous to 1858 the reports of Rhizoctonia were confined to France. Kühn not only extended the range of the genus, but also established its importance as a pathological organism, since in addition to the fungus on crocus, he reported root-rots of the carrot and beet due to R. Medicaginis DC. Considerable confusion has resulted at this point due to the use by later writers of the terms R. Dauci Kühn and R. Betae Kühn, although Kühn himself employed no such nomenclature, but referred the diseases directly to the violet fungus.. Kühn's most important contribution to the history of the fungus, however, was the establishment by him of the new species, R. Solani, causing "Schorf oder grind" of potatoes. This form he distinguished carefully from R. Medicaginis.

Due to the fact that the life history of this form and the symptoms of disease induced by it were very imperfectly known, those who subsequently discussed the genus have sometimes recognized R. Solani Kühn, while others have referred the organism to R. Crocorum DC. As both species are found not only on the potato but also on alfalfa, this fact has lead to considerable confusion among writers, especially in Europe where R. Crocorum is more prevalent than it is here. Thus Saccardo[A] (54:1899) included both R. Solani Kühn and R. Medicaginis DC. under R. Violacea Tul. (See synonymy for R. Crocorum.), and this same classification is followed in Rabenhorst's "Kryptogamen-Flora" (45:1910). Shaw (58) in India has apparently correctly referred to Corticium Vagum B. and C. a fungus from the groundnut and cowpea, but his description of a species isolated from damped-off jute seedlings hardly concurs in formation and type of sclerotia with R. Solani Kühn to which he refers it. In Shaw's first article (58:1912) he suggests that the name R. Violacea be retained for the macrosclerotial form of the fungus of which Corticium vagum B. and C. is the fertile stage. Later (59:1915), he classifies as synonymous R. Solani Kühn and R. Medicaginis D. C. As will be seen from the

classification given by Duggar and from the results of those who have worked out the perfect stage of the fungus, Shaw's classification is contrary to generally accepted taxonomy.

Recently (16:1915) Duggar, following the classifications of Tulasne and Kühn, has presented a comparative study of the two main species (or groups of species) which should serve as a standard for differentiation between them: (1) to facilitate the interpretation of past literature; and (2) to prevent future confusion. Duggar gives the following provisional synonymies. Under R. Crocorum (Pers.) DC. he lists:

Tuber parasiticum Bull. (1791)

Sclerotium Crocorum Pers. (1801)

Rhizoctonia Crocorum DC. (1815)

Rhizoctonia Medicaginis DC (1815)

Thantophytum Crocorum Nees (1816)

Tuber Croci Duby (1830)

Rhizoctonia Rubiae Dcne. (1837)

Rhizoctonia Dauci Rabenh. (1859)

Rhizoctonia violacea Tul. (1862)

Rhizoctonia Asparagi Fckl. (non Fr.) (1869)

Hypochnus violaceus Eriks. (1913)

Under R. Solani Kühn (Corticium vagum B. & C.)

Rhizoctonia Solani Kühn (1858)

Rhizoctonia Betae Eidam (non Kühn) (1887)

Rhizoctonia Napaeae West. (1846)

Rhizoctonia Rapae West. (1852)

Hypochnus Solani Prill. & Del. (1891)

The first account of a true fruiting stage of either of these species was the description of Hypochnus Solani in 1891, by Prillieux and Delacroix (44) from potato stems in Germany, This was not associated with R. Solani, however, until 1903, when Rolfs (51) found the same fungus on potato in Colorado. Professor E. A. Burt, the American authority on Thelephoraceae, identified Rolf's material as Corticium vagum B. & C., synonymous with Hypochnus Solani Prill & Del.# Later (1904) a typical culture of R. Solani was obtained by Rolfs (52) from germinating basidiospores. The work of Riehm (48:1911) in Germany and Pethybridge (43:1915) in Ireland substantiates that of Rolfs. The perfect stage of R. violacea Tul.,

(# Burt, E. A. The Thelephoracea of North America I. Ann. Mo. Bot. Gard., 1, 193. 1914.
"To include in Corticium species always resupinate, which have colorless spores and lack cystidia, excepting those species which for other reasons are placed in Exobasidium. Include in Corticium hypochnoid as well as compact species."

moreover, is probably to be referred to Hypochnus violacens (Tul.) Eriks. following Eriksson (21:1913).

In regard to other species described previous to 1915, Duggar after careful examination concluded that their affinities with Rhizoctonia were insufficient to include them in the genus (16,p451). Peltier (41), however, recognized the validity of Corticium orchraleucum (Noack) Burt (63,64) on the leaves of pomaceous trees, and the species isolated from damped-off onion seedlings (41,p376), in addition to the two major species. Rosenbaum and Shapovalow (53), as a result of their study of Rhizoctonia diseases of the potato, proposed a new strain of Rhizoctonia Solani Kühn, differentiated from the common form in the following characters: (1) more pronounced lesions produced when inoculated into injured stems or tubers; (2) reaction, growth, and character of sclerotia on definite media; (3) morphological characters; (4) difference in diameter of germ tubes. Ramsey (46) would also divide the species on the basis of two types of symptoms produced on the potato. Later, Matsumoto (37) studied the physiological specialization of so called strains of Rhizoctonia Solani Kühn, and isolated one which he believed to be identical with Rosenbaum's "new" strain. As no perfect stage

has been found for this form, it cannot be considered as a different species. Matz (38:1917) described a new species on the fig. (Ficus Carica), which he named R. Microsclerotia. A Corticium found in association with the sclerotia, was identified by Burt as C. Salmonicolor Br. & Brs. Recently a species of Rhizoctonia[#] found attacking grass and clover at Sunderland, Massachusetts has been under investigation at Massachusetts Agricultural Experiment Station.

Of the above species, R. Solani Kuhn, the species from onion seedlings described by Peltier, Shaw's Rhizoctonia from jute, and the grass fungus, are all known to cause damping-off. Because of the total lack of similarity between the last two species mentioned and the material studied by the writer, they may be omitted from further discussion in this connection. In general, cases of damping-off have been referred to R. Solani Kühn.

Atkinson (1:1892) first observed this fungus on cotton seedlings; damping-off, or causing the production of stem ulcers at the ground level. This disease, known

(# Unpublished data of the Department of Botany, Massachusetts Agricultural Experiment Station.)

among planters as "sore shin," was thought by them to be wounds due to contact with cultivating tools. In 1895, Atkinson (2) again found the "sterile fungus" as a common cause of injury to seedling beets, radishes, lettuce, egg-plants, and cabbages. To B. M. Duggar (12:1899) is due the determination of this organism as Rhizoctonia, and the demonstration of its etiological relationships. The association of the fungus with the damping-off of other seedling hosts, among them Asparagus Sprengeri, bean celery, cucumber, and white pine, was further determined by Duggar and Stewart (13-15).

These preliminary observations brought forth a mass of literature on the subject, much of it of a popular character dealing with the economic importance, hosts, and control. Of those who made a special study of Rhizoctonia in connection with the damping-off of some particular host the following may be mentioned: Van Hook (68), and Whetzel and Rosenbaum (70) on ginseng; Selby (55-57) and Johnson (33) on Tobacco; Edgerton and Moreland (18) on tomato; Edson (19) on sugar beet; and Hartley (24-28) on conifers. In Connecticut, Clinton (4-7) has made numerous observations on seedling troubles due to Rhizoctonia, and Sherbakoff (60-62) found this fungus the most im-

portant damping-off parasite of truck crops in Florida. From culture comparisons of many strains of Rhizoctonia isolated from different hosts, Peltier reported Rhizoctonia Solani Kühn as the cause of damping-off of seedlings of over fifty species of plants. In the opinion of Duggar (16) it is in general the worse enemy of seedling plants, but he adds that as far as he has been able to ascertain there has been no report of the damping-off of monocotyledonous plants under normal seed bed conditions. The question under consideration is, therefore, whether to include the fungus which causes damping-off of onion seedlings as a strain of R. Solani Kuhn, or whether to consider it a separate species, following Peltier (41).

Rhizoctonia Solani Kühn commonly lives as a saprophyte, maintaining its existence on the dead organic matter in the soil. It is also a facultative parasite, i.e., it may enter living tissue and feed upon it; and it is in connection with this stage of its life cycle that the fruiting form, Corticium vagum B. and C. has generally been found.

The strain from onions is primarily saprophytic, developing extensively in culture or in the soil. That it is also parasitic on the living tissues of the young seedlings, is indicated by the data presented below. The lack

of a perfect stage, however, prevents certain identification of this strain, but its relationship to R. Solani may reasonably be hypothecated from a comparison of the morphological features and from cultural experiments and cross inoculations of the two.

Morphological Features.

The Rhizoctonia attacking onion seedlings has the same general characters as R. Solani Kühn. The young hyphae are hyaline, septate, densely vacuolate, and branch at an acute angle from the parent hypha, subsequently extending parallel to it. As the hyphae mature, the branching is less constricted, and at right angles to the main hyphae; the contents of the mycelial threads become granular and are finally lost, the walls becoming stained a deep brown. Hyphal fusion may occur.

In order to compare the morphological features of the form from onion seedlings with those of a typical strain of R. Solani Kühn, cultures of both were obtained in the following manner: Damped-off seedlings were removed from the bed, care being taken to remove the whole seedling. They were washed in a 1:1000 solution of mercuric bichloride for 30 seconds, and transferred through several washings of sterile water. Two millimeter sections were cut from

them with a sterile scalpel, and transferred to tubes of potato agar by a flamed platinum needle. Pure cultures of Rhizoctonia resulted, in every case. Hereafter, for the sake of convenience, the symbol RA (Rhizoctonia from Allium cepa) may be used to designate this strain. Typical cultures of R. Solani Kühn were made from sclerotia from a diseased tuber. The tuber was first thoroughly scrubbed, and the same method of isolation followed as for the onion seedlings, except that the sclerotia were picked from the potato and no tissue was transferred. This strain may be designated as RS (Rhizoctonia Solani Kühn).

Hyphae of each strain, selected at random from the edge of twenty four hour old plate cultures on potato agar, were measured as to the length and width of their individual cells. The following table is significant:

Measurements of Mycelial Cells of Rhizoctonia

Strain	Length			Width		
	Least	greatest	average for 100 cells	Least	greatest	average for 100 cells
RS	42 u	245 u	114.7 u	8 u	12 u	10.5 u
RA	35 u	259 u	114.4 u	7 u	12 u	9.2 u

Another type of mycelium also appears on the affected plants and in cultures, forming a tufted growth, has much more closely septate, ovoid cells, sometimes regular and moniliforme, sometimes very irregular, lobed, elbowed, or branching dicotomously. These give rise to sclerotia, which appear as knots in the mycelial threads. Appearing at first as fluffy white masses of tangled hyphae, they turn brown centrifugally, becoming hard and dark with age. The strains RS and RA compare in size and shape of the individual cells, but they differ in size and type of the total sclerotial body. In culture the sclerotia of the onion fungus are globose to ovoid, .25 mm. to 1 mm. in diameter, smooth, and coalesce rarely, while those from the potato Rhizoctonia are flat, irregular and tufted, with great variance in size. Although when separate, the sclerotia of RS may be as minute as those of RA, yet because of their extreme propensity for coalescence, they often form a rough irregular lump as much as 5 cm. in diameter. The sclerotia of both strains, however, are homogeneous in structure without any distinction into medulla and cortex.

Any differences found in either the mycelial or sclerotial cells of RS and RA are so slight as to be less

than might occur in cells of two cultures from the same strain. They cannot, therefore, be considered as different species morphologically. Both fall well within the limits of Rhizoctonia Solani Kühn.

Cultural Characters.

Methods: Rhizoctonia is no more selective as to cultural media than it is in its host, generally making a vigorous growth on any common laboratory media. For the purposes of comparison, parallel cultures were made of the Rhizoctonia from onion, and R. Solani Kühn. The former was obtained in the following manner: From the original cultures of RA and RS transfers of sclerotia were made to cold plates of the following media, prepared in accordance with the directions given in the Appendix: Corn-Meal Agar, Green-Bean Agar, Green-Bean Agar, Oat Agar, Potato Agar, Potato-Glucose Agar, Synthetic Agar (Cook). These were of the same composition as those used by Peltier (41) in his experiments. Eight plates of each agar were poured at a time and the experiment was repeated four times at intervals of about three weeks, or long enough for the cultures to show all their characteristic features and to develop sclerotia. In this way it was possible to compare not only the original cultures with each other, but every subsequent set with the original and with each other. Trans-

fers were made from plates of each set to the next, instead of from the original cultures, in order to observe whether repeated culturing had any noticeable effect on the fungus. In each case, the inoculum was taken from green -bean agar and consisted of a small piece of agar about two mm. square bearing both sclerotia and mycelium. The Petri dishes after inoculation were kept under a bell jar to prevent excessive evaporation and conditions were maintained as uniform as possible throughout.

Growth on Media: A brief statement of the peculiarities of each organism, as shown in the different culture media used, is given below:

Potato Agar

RS. Growth of RS rapid, vigorous, but not profuse, covering plate in 4-5 days: margin definite and entire. Zonation of six, rarely eight, rings of alternately prostrate or submerged fine hyphae and coarser, erect, aerial hyphae (Plate 2, Fig. A and B.). Mycelium at first fluffy white, turning a dirty brown centrifugally with slight discoloration of the substrate and of the guttation drops which are formed freely. Sclerotia develop sparsely in from 7-18 days, and may form either beneath or on the surface of the agar, where they show a tendency to coalesce and

lump up in the center, or scattered over the lid of the Petri dish (Plate 6, Fig. Z.) Sclerotia formed on the lid are white at first, turning brown in the centre with a scarious white border; later, becoming chestnut brown, throughout[#] These are small, .25 to 2mm. in diameter and irregular. The sclerotia formed beneath the agar are the same size and color as those on the upper lid while those formed upon the agar are more difficult to distinguish because of the web of aerial mycelium covering them, as well as a more or less adherent investiture of hyphae which causes them to appear drab or mouse gray. In old cultures, however, where the investing web has dried off these sclerotia appear chestnut brown, as they do when viewed from the under side of the plate. Because of their tendency to coalesce, sclerotia may vary greatly in size and shape.

RA. Growth parallel to RS in rapidity, amount of mycelium and zonation, but certain differences are so constant as to enable one familiar with the strains to tell them apart at a glance. RA makes a denser, more compact, felt-like growth, dry and flat with much shorter aerial mycelium. No guttation drops are formed, and there is rather less discoloration of the agar. (Plate 2, Fig. C and D.) Sclerotia are formed much more abundantly both against

(# All colors according to Ridgway's Color Standards)

the lid and on the agar, the minute white balls of mycelium appearing in 5 or 6 days. (The beginning of sclerotia formation may be seen by close examination of Plate 2, Fig. C and D.) These are small, .25 to 1 mm., distinct, and separate, with slight coalescence, even though they show a tendency to aggregate. They have no investing hyphae; and when they turn dark have a glistening metallic appearance due to exudation. Although scattered freely over the plate, they form more densely at the zones. With age they became chestnut brown to blackish brown. (Plate 6, Fig. S.)

Potato-Glucose Agar.

RS. Much more luxuriant development of mycelium and sclerotia with deeper staining of the agar are the only features which differentiate this culture of RS from that on potato agar; in other respects growth is closely similar. (Plate 3, Fig. E and F, and Plate 6, Fig. R.).

RA. Growth similar to culture on potato agar, but much more profuse development of close, dry, woolly, white mycelium, and more extensive formation of sclerotia. The distinct feature of this culture is the formation of a definite coalescent ring of partly submerged sclerotia. (Plate 3, Fig. G and H, and Plate 6, Fig. T.).

Green-Bean Agar.

RS. Growth of RS on this medium is intermediate be-

tween that on potato agar and on potato-glucose agar. (Plate 4, Fig. I and J, and Plate 7, Fig. U).

RS. The same may be said of this strain. (Plate 4, Fig. K and L and Plate 7, Fig. W.)

Oat Agar.

RS. While the growth on this medium is not sufficiently different from that on the above agars to have any diagnostic value, by far the heaviest growth is produced. The mycelium shows dense and white against the agar, and zonation is very marked. (Plate 5, Fig. M and N.) Sclerotia formation is abundant and takes place in 7-15 days, being slightly earlier on this agar than on the others. The small sclerotia formed against the lid often coalesce and are so plentiful as to form with the connecting aerial hyphae a sort of upper crust while those sclerotia formed on the surface of the agar produce an encrusted cake in the centre which is often 3-5 cm. in diameter. Smaller, irregular lumps of coalescent sclerotia are also found scattered about on the remaining surface or formed against the edge of the plate. (Plate 7, Fig. V., and Plate 8, Fig. Y). Some discoloration of the agar is noticeable.

RA. As seen for strain RS the growth of this strain is similar to that on other vegetable extract agars, the individual features being more intensified because of the

more luxuriant development. The very dry, woolly, dense, snow-white mycelium is 2-3 days slower in covering the plate and sclerotia formation begins correspondingly earlier. (Plate 5, Fig. O and P.) There is some tendency for the sclerotia to coalesce, especially in the outer zones, and the lump formed in the center is encrusted at the base, but on this agar, as in others used, sclerotia of this strain remain distinct, and piled up like grains of sand. (Plate 8, Fig.2)

Corn-Meal Agar.

RS. Very scanty, indistinct growth, no marked features. Aerial hyphae becoming rugose with age while/but a few irregular flat auburn sclerotia formed.

RA. Similar to RS but sclerotia smaller and darker, between chestnut and blackish brown.

Agar XII. (Cook's)

RS. Cobwebby, rather scanty growth, more compact in central ring, no zonation and plumose border. Sclerotia Vandyke brown, formation slight as on corn meal.

RA. Growth similar to RS except that the minute sclerotia are formed separately and are darker brown.

Discussion of Habits of Growth: The habit and characters of both strains of Rhizoctoni varied so slightly

in repeated culturing that the variation can be considered negligible. As great variations would appear on the same agar in any one set of plates. It is interesting to note that R. Solani Kühn from potato and the strain from the onion seedlings had the same cultural affinities. Both make a progressively vigorous growth, on potato, potato-glucose, bean, and oat agars. Both produced poor mycelical development and a scanty formation of sclerotia on the corn-meal agar, and a rather intermediate growth on the synthetic medium. Similar parallel tendencies were seen in the habits of growth of each such as zonation, and their physiological effect on the media. Zonation and an even margin were evident in a greater or lesser degree on all but Cook's Agar. On this medium, however, both maintained an even growth at first, but after the primary central zone was formed a difference in the habit was noted, with a tendency to radial development, instead of concentric, zonation, which resulted in a plumose margin. Both strains produced a deep discoloration of the potato-glucose and string-bean agar, slight discoloration of the potato and oat agars, and no discoloration when grown on cornmeal or synthetic agar.

There are certain features, however, in which RS and RA differ. The mycelial growth of RS exhibits a ten-

dency to be more rapid than that of RA, especially on oat agar, but this variation in rate is too slight to be a marked difference. There is a greater dissimilarity in the type of mycelium, that of RS making a more abundant aerial growth. RA tends to form a denser, dry, woolly mat closely appressed to the substratum, its rather scanty aerial mycelium being of the same nature. The more rapid, more luxuriant formation of sclerotia in RA, as well as their distinctive size, position, surface characters, and color are even more striking. A graphic comparison of these features of development will be had by referring to Plate 8, Fig. Y and Z.).

Evidence of biologic specialization among the fungi is common. Magnus was one of the first to suggest that a particular biologic form might, by constant association with one host, change its physiological capabilities to such an extent as to develop a new race. Since then much notable work has been done along this line, especially with the rusts and powdery mildews. Eriksson, (20) working with Rhizoctonia violacea on carrots and beets, found not only a variation in the virulence of the strains, but also a pathogenic specialization on certain varieties of root crops. Those who have dealt with R. Solani Kühn preeminently from this angle are: Peltier(41), Duggar (16),

and Matsumoto (37). From the detailed and careful observations and experiments of these men, it is evident that great variations in virulence, morphological and physiological characteristics may be expected within the species. From fifteen isolations of Rhizoctonia, Matsumoto obtained six which differed widely in such features as dimensions and color of sclerotia, enzymatic activities, temperature requirement, cultural characters, and pathogenicity, yet which might not be considered separate species since these properties did not remain constant, but were subject to modifications by environment and change of host. In regard to raising to specific rank forms now put in R. Solani Duggar (16) says. "In the different strains which have been studied originating from different hosts, certain minor modifications of the general habit of the fungus in culture have been observed. But these have not seemed to be sufficient to be of specific importance except in the case of the form on rhubarb. In general the differences referred to consist in a variable amount of the mealy or tufted growth, or of the amount of aerial growth. Differences in the color of the colony are also observable; while the rapidity with which sclerotia are formed is also a minor distinguishing feature. The subject needs further investigation, but in general it is felt that these differences

are such as might be due either to permanent alterations in the pathological strains on the one hand, or to temporary differences induced by recent environment on the other. It may be pointed out that the appearance of the mycelium of the beet fungus from the damping-off seedlings is not exactly comparable with that of the mycelium derived from the beet rot. When the organisms from both sources are grown in culture they are found to be identical. Strains do occur, however, evidence of which may persist for some time in the general appearance of the cultures." Peltier furnishes further evidence of biologic specialization through morphological and cultural comparisons of nearly fifty strains obtained from different hosts of wide geographical distribution.

The differences which exist in culture between the strains RS and RA, while striking to the eye, resolve themselves on close analysis into just such modifications as may be produced by environmental factors. The characters of growth, which the two forms have in common, predominate by their constancy, making it appear, that in spite of outward differences, the fundamental characteristics of the two strains are the same. From these experiments, therefore, it may be concluded that such minor differences as occur in the cultural characters of RS and RA are due to biologic

specialization, and are insufficient to justify raising RA to specific rank.

The Rhizoctonia isolated from onion seedlings, for which Peltier proposed specific, rank, does not exactly compare with RA, as far as can be determined from his description. More definite information is needed, and cultural comparisons of the two, in order to establish their relationships. It is probable, however, that both are strains of the same species, R. Solani, Kühn.

Pathogenesis.

Proof of Pathogenicity: By microscopic examination of onion seedlings from the greenhouse, it was apparent that Rhizoctonia entered the epidermal cells and permeated the tissues in all directions, but due to the presence of other fungi, Pythium and a large percentage of onion smut (Urocystis Cepulae), the question arose whether Rhizoctonia was a primary parasite or whether it was merely secondary, entering thru wounds or avenues opened by other organisms. The opinion that this fungus might be only secondary was strengthened by the facts that such soil fungi are notably weak parasites, and that Peltier considered the strain from onion seedlings, with which he worked, to be doubtfully parasitic (41, pp. 376). Accordingly, inoculation experiments were carried on to determine the pathogenicity of

the strain RA on onion seedlings, and at the same time cross inoculations with RS were made to further check the morphological and cultural comparisons of these two strains.

To prevent the introduction of seed-borne microorganisms, seed sterilization was practised in the following manner: Seeds of known germination were soaked 15 minutes in 1:1000 solution mercuric bichloride, then transferred through three washings of sterile water. A section lifter dipt in 95% alcohol and flamed was found a most convenient and efficient instrument with which to handle the seeds. In every case a number of seeds were grown on potato agar in test tubes to test their sterility, and in no instance did any contamination develop.

The following experiments were preformed:

(1) Erlmeyer flasks were half filled with moist soil, plugged, and sterilized in the autoclave at fifteen pounds pressure for two hours on each of three successive days. They were then inoculated under sterile conditions with about five square millimeters of green bean agar bearing sclerotia and mycelium of the fungus. In each experiment, six flasks were inoculated with RS, six with RA, while two were retained as checks. In every case the fungus made a vigorous growth, forming in four to five days

a dense mass of fluffy white mycelium on the surface of the soil. After one week the flasks were opened, the inoculum stirred into the soil with a sterile glass rod, and the flasks were planted with fifteen seeds each. This experiment was repeated twice with no results. Germination was very poor, not more than one third to one half of the seeds came up even in the controls, and although both strains of the fungus made a luxuriant growth, both on the soil and over the seedlings, no damping-off occured.

(2) Flasks were prepared as in experiment (1), but in order to obtain a greater percentage of plants the seed was first sown on moist filter paper in sterile Petri dishes, and transfered to the flasks as soon as the radicle appeared. In this way a better stand was secured, but as before no infection took place.

(3) With the idea of altering somewhat the atmospheric conditions and providing better aeration, the next planting was made in three-inch earthen ware pots. These were sterilized in a 1-1000 solution of mercuric bicloride, dried, and filled with soil, after which they were autoclaved at seventeen to twenty pounds pressure for two hours on each of three successive days. After considerable difficulty in keeping these free from contamination, the author

found that by covering them with a bell jar, which had been treated with formaldehyde (1-100 solution), as soon as they were removed from the autoclave, air-borne spores could be excluded. Eight pots were inoculated with strain RS, eight with RA, and two were retained as checks. The fungus was given a week to thoroughly penetrate the soil, at the end of which time it was stirred in, and fresh inoculum was introduced. At the same time each pot was planted with twenty five seeds. A large percentage of the seeds germinated, but again no visible signs of damping off of the seedlings. Since even with the increased space provided by this method, the moisture content of the air and soil was still very high, half the pots of each strain were removed from the bell-jars, and fresh inoculum added. As this was not done until two weeks after planting, the seedlings were well up and had pulled the seeds out of the ground. In the eight pots removed from the bell jars, no damping-off appeared in those inoculated with RS, and only 7 seedlings out of the 53 (total number developed in four pots inoculated with RA) were killed by the fungus. It was hardly expected that infection would occur so late in the development of the seedlings, and it is therefore not surprising that only a few damped off. The diseased

plants were removed from the pots, washed in mercuric bichloride solution, well rinsed in sterile water, and transfered to potato agar slants. From these strains RA was reisolated.

(4) The previous experiments were performed under laboratory conditions and unregulated room temperature, ranging from 18° to 30° C. In order to simulate field conditions as near as possible and yet grow the seedlings in inoculated plats without contamination the following method was devised: Small flats (10x12 inches) were disinfected by soaking in a strong formalin solution, filled with soil, and sterilized in the autoclave as in (3). To prevent contamination on removal each flat was covered with a sheet of glass. The soil was inoculated as before from cultures, one flat with RS, one with RA, and one retained as a control. After the fungus had spread over the soil, it was stirred in, and in each flat three rows of seeds were planted, 150 seeds to the row. As soon as the knees appeared above ground, the glass was lifted three inches above the top of the flat by means of props set in the corners of the flat, and two thicknesses of cheese cloth was used to cover the glass and the sides, partly to prevent burning and partly to increase aeration. These flats were set in a sunny spot out-of-doors.

In this experiment no damping-off occured in either the flat inoculated with RS or the control, and in each 90% germination was produced. In the flat inoculated with RA, however, the stand was reduced very markedly by infection below the surface of the soil. Only 107 seedlings from approximately 450 seeds planted appeared. Of these 66 survived, and 41 damped-off before the first leaf developed. Since seed from the same lot was used in all the plats, it is seemed reasonable to expect an equal germination in all. Basing percentages on a 90% germination, therefore, 74% damped off below the ground, and of the remaining 26% over one third ultimately became infected, making a total of 84% damping-off. To verify the belief that the poor stand was caused by Rhizoctonia in this instance, the flat was dug up, and seeds found in all stages of germination, but dead and shriveled. Transfers made from these and from the seedlings which damped off above the ground resulted in the reisolation of strain RA.

(5) To check the above experiments sterile seeds were germinated on agar slants and as soon as growth started, mycelium of RA was transfered to the agar, resulting in a few days in the infection and death of all the seedlings. Transfers of tissue of the infected seedlings to fresh agar slants gave pure cultures of RA.

Through isolation of the fungus associated with damping off of onions in the greenhouse, and by inoculation and reisolation of it in these experiments, the requirements of Koch's four rules of the proof of pathogenicity have been complied with, and the parasitism of strain RA definitely established. By the same methods RS is proven to have no parasitic activity in regard to this host, indicating the pathological specialization of the two strains.

Factors influencing pathogenicity: In interpeting the results of these experiments it should be borne in mind that no satisfactory work has yet been done relative to the optimum conditions for growth and that many external factors are correlated with its maximum parasitic action. If we consider soil temperature, generally conceded to be the most important factor in damping-off, we find that Peltier (41; pp. 375) sets the optimum for infection by Rhizoctonia at 88° F. (31° C) Johnson (33; pp. 56) at 25° C, while Matsumoto (37; pp.12-13) found temperature variable for different strains. It is evident, therefore, that external factors may largely determine the virulence of any given strain. The auther feels that thorough investigation of these physiological features would solve many of the

problems of both the taxonomist and the pathologist.

Penetration.

In studying the parasitism of the Rhizoctonia from damped off onion seedlings, special attention was paid to the infection of the host, to penetration, and to the course of the hyphae in the tissues, since in reviewing the literature of R. Solani Kühn, the author found very little work had been done on these important phases of the life history of the fungus. From Atkinson's early observations of "sore-shin" of cotton, the following account of penetration, probably based on microscopic examination, was obtained (2; p. 265). "The trouble is caused by the fungus growing first in the superficial tissues of the stem near the ground and disintegrating them before it passes to the deeper tissues, in other words the fungus never seems to penetrate far in the living tissues but 'kills as it goes' and the tissues become brown, depressed, and present the appearance of the plant having a deep and ugly ulcer at the surface of the ground. The fungus does not spread into the tissues either above or below the ulcer to any extent, but literally eats away at that point until it has severed the stem at the affected place or the plant has recovered from its effects." Drayton (10) undertook to prove the stem parasitism of the fungus by microscopical

examinations of transverse and longitudinal sections of lesions from diseased potato stems. He showed that cells of the cortex, vascular bundles and pith were all invaded by mycelium, and that the course of the hyphae might be either inter or intracellular. Gussow (23) remarked the parasitism of the fungus on the tips of the young rootlets with a consequent inhibiting effect on the formation of soil tubers, and considered that most infection took place in this way. More recently, Matsumoto (37) experimented with strains of Rhizoctonia on young pea plants, and determined that infection occured most readily through the root, and that penetration was chiefly a mechanical process. No research has been conducted, however, on the nature of the infection by Rhizoctonia of seedlings, resulting in damping-off.

Period of Susceptibility: In order to determine the period of susceptibility of the seedling, point of infection, method of entrance, and course of the fungus in the plant tissues, onion seeds were planted in soil known to produce nearly 100% infection. Beginning with the fourth day after planting, at which time the projecting radicle was about 4mm. long, twenty five plants were taken from the bed each day, washed in sterile water, and fixed in Flemming's weaker solution. After dehydration they were run into paraffin, sectioned longitudinally, mounted seriall

and stained with the triple stain, safranin, gentian violet, and orange G. Mycroscopic examination of the four day seedlings at the time they were dug revealed the presence of the mycelium of Rhizoctonia, but no lesions were found in the stained sections of the plants earlier than the sixth day. Because of the marked failure of the onions to produce a good stand in soil infected with Rhizoctonia, it is probable that considerable infection takes place at this early date, and that the seedlings are killed before reaching the surface. In the hundreds of seedlings examined by the author only two seedlings were found which damped off after the appearance of the first leaf, and since the cotyledons of these were almost entirely rotted away, it is safe to assume that the infection occured previous to the emergence of the leaf bud, although the symptoms were not apparent or observed earlier.

It can be concluded, therefore, that infection may take place at any time from the emergence of the hypocotyle until the emergence of the first leaf (a period of about fifteen days.)

Point of Entrance and Multiple Infections: By examination of the stained slides of the onion seedlings it was possible to determine the points of entrance of

the fungus. The majority of the infections were found taking place at the base of the cotyledon in the neighborhood of the root joint, but they were by no means confined to this region, as any part of the cotyledon between the root joint and the knee was susceptible to attack. Since seedlings were often found with feeble root development or with some of the roots developing and others apparently cut off, lesions were sought and found on these, as well. Traces of mycelium were frequently found in the cotyledonary cavity, and in one instance where a sclerotium was formed this became the infection court, penetration taking place thru the cavity walls. Infection of the roots and of the cotyledon in the neighborhood of the root-joint obviously occurs below the soil, but whether in the case of infections near the knee joint the mycelium gains entrance before the plant leaves the soil and develops slowly, or whether it enters later cannot be judged with any certainty. Mycelium is often found climbing over the cotyledon, the threads tieing the ascending and descending legs, but apparently doing no damage. Numerous infections, of the same plant may occur, in fact single infections were more rare than multiple infections, a plant attacked in one part possibly being so weakened that it becomes more susceptible.

In damping-off of onion seedlings by Rhizoctonia, therefore, infection may take place not only at the root joint or growing zone, but also through the cotyledon at any point between this zone and the knee. Less frequently penetration occurs from within the cotyledonary cavity and through the roots. All such infections may be localized, or multiple, resulting in a concerted attack on the plant.

Method of Entrance and Character of Inoculum: The facts that Rhizoctonia is so universally present in the soil and that it grows indefinitely without producing spores lead one to expect infection to be produced directly by hyphae of the saprophytic mycelium. The literature of the subject is rather hazy on this point, but the general implication is that such is the case. In no instance, however, did the author find mycelium approaching and penetrating the seedlings directly. Considerable loose mycelium was found associated with the sections, but wherever infection occured the hyphae had gathered together into a mass, forming a sclerotium. Beneath these sclerotia a depression developed, so that they appeared sunken in the tissue. (Plate 8 Fig. 1).

The contents of the cells of this depressed area and of several rows beneath it were apparently acted upon by an enzym or toxin secreted from the sclerotium.

Those cells nearest the lesion, in which the contents were destroyed, contained a gummy substance, while back of these the cells were badly plasmolized. This disruption of the cell contents invariably preceded penetration, but no dissolution of the cell walls occured, although cytase is known to be present in the mycelium.

In many instances the outer cells were so crushed by the germinating sclerotium that it was impossible to distinguish the method of infection, but from other sections where the sclerotia were not so deeply sunken in the tissue, it became apparent that the epidermal walls were pushed in by the invading hyphae. It is evident, therefore, that the primary infection takes place by mechanical pressure from hyphae of a germinating sclerotium after it has first destroyed the cell contents chemically.

Development of Interior Mycelium: The hyphae from the sclerotia penetrate the walls of the inner cells in the same manner as they entered the epidermis--the contents are destroyed before penetration and the walls are punctured by the growing tips. (Plate 8 Fig. 2) Generally the infecting hypha bores directly into the cell, its passage through the tissues being intracellular, but it is sometimes found in the intercellular spaces, following along the longitudinal walls and gaining entrance where two cells come together. Considerable distortion of the

cell wall may be caused by the pressure of the hypha against it before entrance is affected. The invading mycelium quickly fills the collapsed cells with short, constricted, closely packed hyphae of the sclerotia-forming type, and not only "kills as it goes", but apparently sends out a toxin to poison the cells in its path. In this manner the thin walled tissues of the cotyledon are quickly invaded and the seedling is destroyed.

CONTROL

Many measures for the control of damping-off by Rhizoctonia have been recommended, special favor being given to soil treatment by formaldehyde. Accordingly it was at first thought that the application used to control onion smut would at the same time effectively combat Rhizoctonia. Various solutions of formaldehyde, applied by the drip method, were tested to prove its efficacy. Several tests were made with each of the following strengths:

pints of formaldehyde	pints of water	feet of row
1	50	3000
1	50	4000
1	50	5000
1	50	8000
1	100	3000
1	100	4000
1	100	5000
1	100	8000
1	32	3000
1	32	4000
1	64	3000
1	128	3000

No one of these was found to affect any control of damping-off, which was as prevalent in the treated rows as in those left as controls. Not only was there no cessation of the disease, but much poorer stands were obtained than in the check rows, indicating a decided impairment of the viability of the seeds, due to the formaldehyde.

Because of the very resistant sclerotia formed by Rhizoctonia, the only thoroughly satisfactory method of ridding the soil of this organism seems to be steam sterilization. While this is very efficient and available for the greenhouse or seed-bed, it is not generally practical in the field. Where transplanting is practised, however, and damping-off becomes troublesome in the seed-bed, any method by which the soil can be thoroughly sterilized with steam may be employed with satisfactory results. This procedure not only eliminates damping-off, but has the additional advantages of freeing the soil of other microorganisms, insect pests, and weeds, as well as stimulating plant growth.

SUMMARY

1. The species of Rhizoctonia commonly found damping off seedlings is Rhizoctonia Solani Kuhn.

2. Cultural differences between the damping-off fungus from onion seedlings and strains from other hosts lead Peltier to conclude that the onion fungus was a species distinct from R. Solani.

3. Microscopic examinations of damped-off onion seedlings in the greenhouse beds at Massachusetts Agricultural College revealed the presence of Rhizoctonia in over 95% of the diseased plants.

4. Although the fungus is exceedingly virulent in the seed-bed, its importance as a field parasite has not been determined except thru limited field observations made in May 1922 in the Sunderland onion-growing district. Investigations should be extended further before the economic status can be decided.

5. The presence of the disease is first indicated by failure of seedlings to develop at various places in the rows. After the cotyledons have pushed through the soil the most typical outward symptom is the appearance of a contracted, water-soaked area at the collar, followed by the subsequent collapse of the seedling.

6. Rhizoctonia Solani Kühn is primarily a soil saprophyte, but may become a facultative parasite on immature plant tissues. This is also true of the strain isolated from damped-off onions.

7. Two strains of Rhizoctonia were used in all experiments: (1) RS obtained from sclerotia of Rhizoctonia Solani Kühn on potato tubers. (2) RA isolated from diseased seedlings of Allium cepa.

8. Comparisons and measurements of the individual cells of the vegetative hyphae and sclerotia showed such slight variations that the two strains may be considered morphologically identical. Both fall well within the species limits of R. Solani Kühn.

9. That RS and RA are biologic strains of the same species, and not separate species, is indicated by their cultural characteristics.

10. RA does not agree in all particulars with the Rhizoctonia from onion seedlings described by Peltier. It seems probable, however, that both are strains of R. Solani Kühn.

11. Inoculation experiments prove that RA is parasitic on onion seedlings, causing damping-off. RS did not manifest any pathogenic capacity for this host, indicating either lack of virulence or pathological speci-

alization.

12. Infection by RA occurs during the period between germination and the appearance of the first leaf.

13. Infection may take place through any part of the cotyledon between the root-joint and the knee, through the cotyledonary cavity, or through the roots, but the majority of the infections occur through the cotyledon in the neighborhood of the root joint.

14. Infections may be single and localized or multiple and general.

15. Saprophytic hyphae from the soil form sclerotia at any of the above foci, and the fungus gains entrance to the plant tissues through the epidermis by means of hyphae from the germinating sclerotial cells.

16. The fungus apparently secretes a toxin which kills the cell contents, but penetration of the cell walls is chiefly a mechanical process.

17. The course of the hyphae within the tissues may be intra- or intercellular.

18. The invading mycelium fills the cells with hyphae of the sclerotia-forming type.

19. Formaldehyde was not only ineffective in preventing injury by Rhizoctonia, but considerably reduced the percentage of germination of the seeds.

20. Thorough control of damping-off may be effected by steam sterilization of the infected soil.

CONCLUSIONS

1. From comparisons of the morphological and cultural features of the Rhizoctonia isolated from damped-off onion seedlings with those of a typical strain of R. Solani Kühn, it is evident that the onion fungus is a specialized strain of R. Solani and not a distinct species.

2. Constant association of the isolated strain with the disease, and inoculation experiments resulting in the reisolation of this strain prove its pathogenicity.

3. Saprophytic mycelium from the soil forms sclerotia on the cotyledon or roots of the seedling, and infection occurs by the penetration of hyphae from the germinating sclerotial cells through the epidermis. Although the contents of the cells are first destroyed chemically, penetration of the cell walls is due to mechanical pressure. The course of the hyphae in the plant tissues may be either intra- or intercellular.

4. Formaldehyde will not prevent the rhizoctonia damping-off, but satisfactory control may be obtained by steam sterilization of the infected soil.

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APPENDIX

Composition of Media Used in Culture Experiments.#

Corn-Meal Agar (Shear) -- To 4 teaspoonfuls of corn meal add 1 liter of distilled water. Keep in water bath for one hour at a temperature below 60° C. Strain thru gauze, and to the filtrate add 15 grams agar. Steam three-quarters of an hour. Filter thru paper tube and place in autoclave for 15 minutes at 115 degrees C. Tube and sterilize 20 minutes.

Green-Bean Agar. -- 300 grams young string beans cooked in 500 cc water for one hour and strained thru cloth. 15 grams agar melted in 500 cc water. Mix the two, add enough water to make 1000 cc., and boil in autoclave. Filter thru cotton. Tube and sterilize 20 minutes.

Oat Agar (Clinton) -- 200 grams oats ground fine thru a coffee mill and soaked in 500 cc. water for one hour. 15 grams agar melted in 500 cc water and strained thru cheesecloth. Mix the two but do not filter, since the most nutrient part of the medium would be lost. Tube and sterilize 20 minutes.

Potato-Agar -- 300 grams peeled potatoes, sliced as thin as possible and cooked in 500 cc. water for one hour. Strain thru cloth. 15 grams agar melted in 500 cc. water. Mix the two and add enough water to make 1000 cc. and

boil in autoclave for a short time. Filter thru cotton. Tube and sterilize 20 minutes.

Potato-Glucose Agar. -- 300 grams peeled potatoes, sliced as thin as possible and cooked in 500 cc. water for one hour. Strain thru cloth and add 20 grams of glucose. 15 grams agar melted in 500 cc. water. Mix the two, add enough water to make 1000 cc., and boil in autoclave for short time. Filter thru cotton. Tube and sterilize for 20 minutes.

Agar (Cook)

Water	1000.00	cc.
Agar	15.00	grams
Glucose	20.00	"
Ammonium nitrate	1.00	"
Potassium nitrate	1.00	"
Ammonium sulfate	1.00	"
Magnesium sulfate	.25	"
Dipotassium phosphate	.25	"
Calcium chlorid	.01	"

Dissolve in autoclave, tube and sterilize.

(# Peltier, G. L. Parasitic Rhizoctonias in America. Ill. Agr. Exp. Sta. Bul. 189:283 - 388. 1916.)

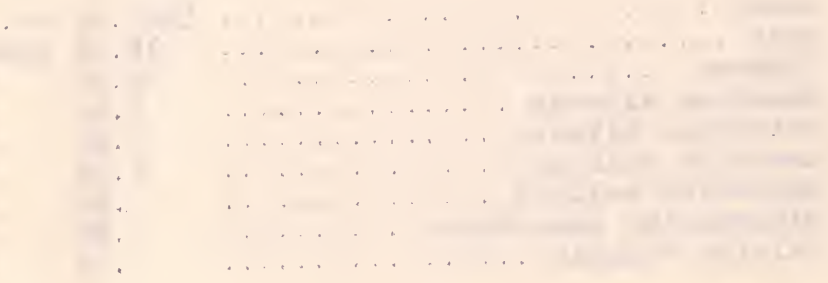

PLATE I.

Fig. 1. Onion seedlings, damped-off by Rhizoctonia in greenhouse bed, photographed 10 days after planting.

Fig. 2. Enlargement of group of diseased seedlings from seed-bed, shown in Fig. 1.

A. Healthy seedlings
B. Damped-off seedlings
C. Section of drill in which seedlings failed to appear above ground.

PLATE I.

Fig. 1

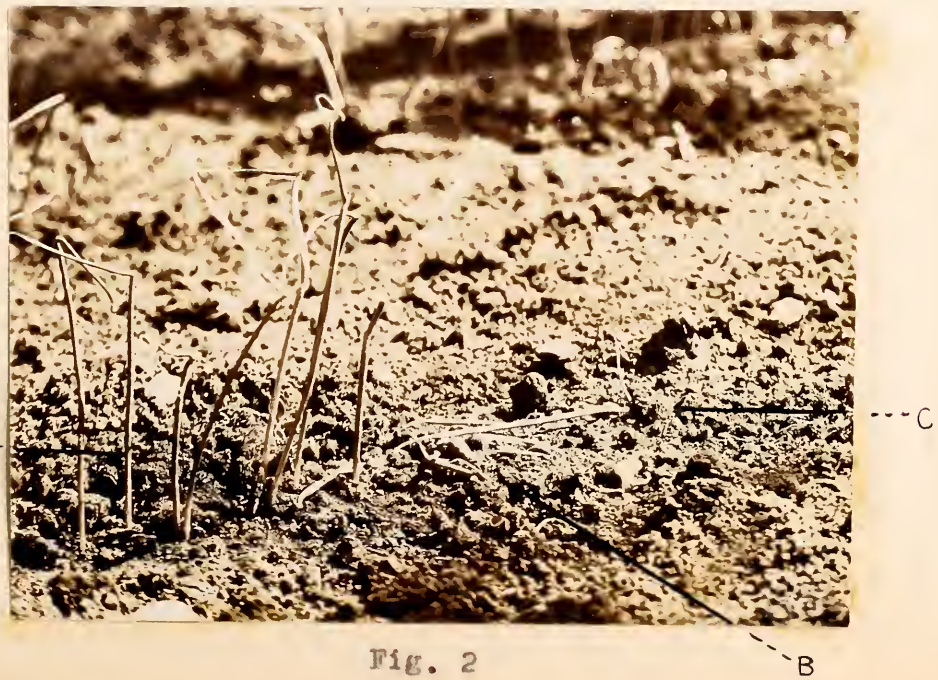

Fig. 2

PLATE II.

Cultures of Rhizoctonia on potato agar after 5 day's growth.

Figs. A and B. Mycelial growth of strain RS.

Figs.C and D. Mycelial growth of strain RA.

PLATE II.

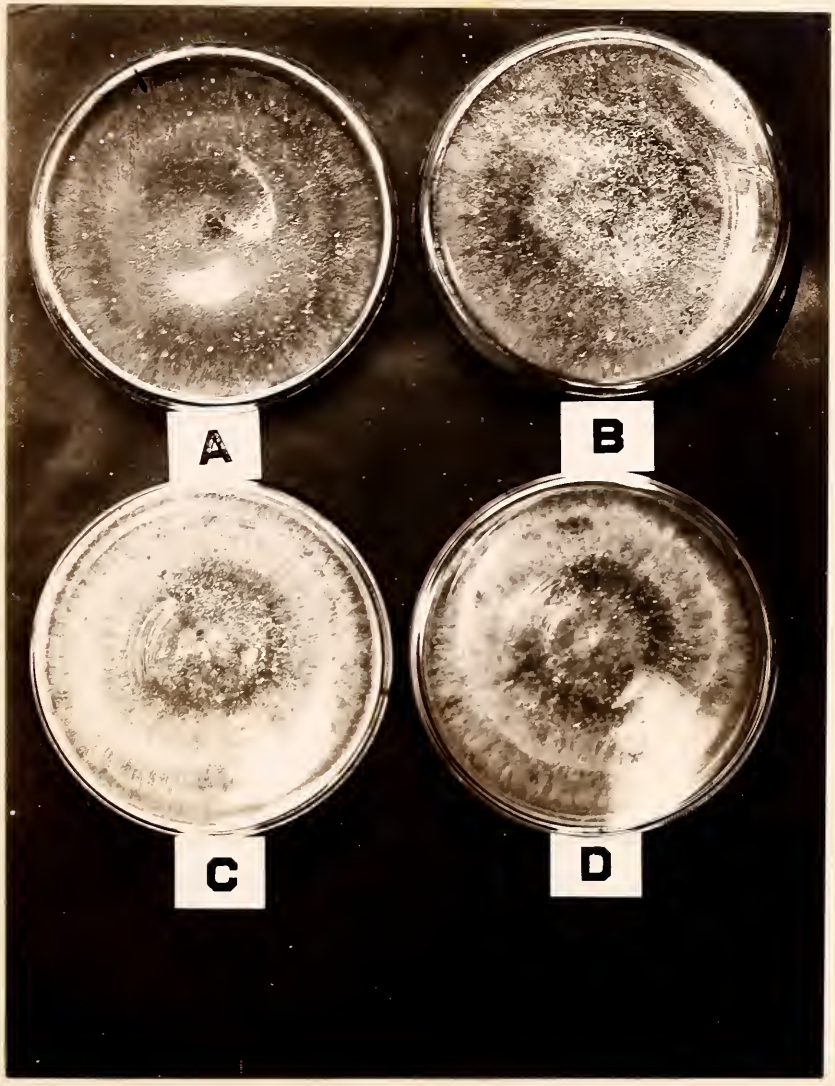

PLATE III.

Cultures of Rhizoctonia on potato glucose agar after 5 day's growth.

Figs. E and F. Mycelial growth of strain RS.

Figs. G and H. Mycelial growth of strain RA.

PLATE III.

PLATE IV.

Cultures of Rhizoctonia on green-bean agar after 5 day's growth.

Figs. I and J. Mycelial growth of strain RS.

Figs. K and L. Mycelial growth of strain RA.

PLATE IV.

PLATE V.

Cultures of Rhizoctonia on potato agar after 5 day's growth.

Figs. M and N. Mycelial growth of strain RS.

Figs. O and P. Mycelial growth of strain RA.

PLATE V.

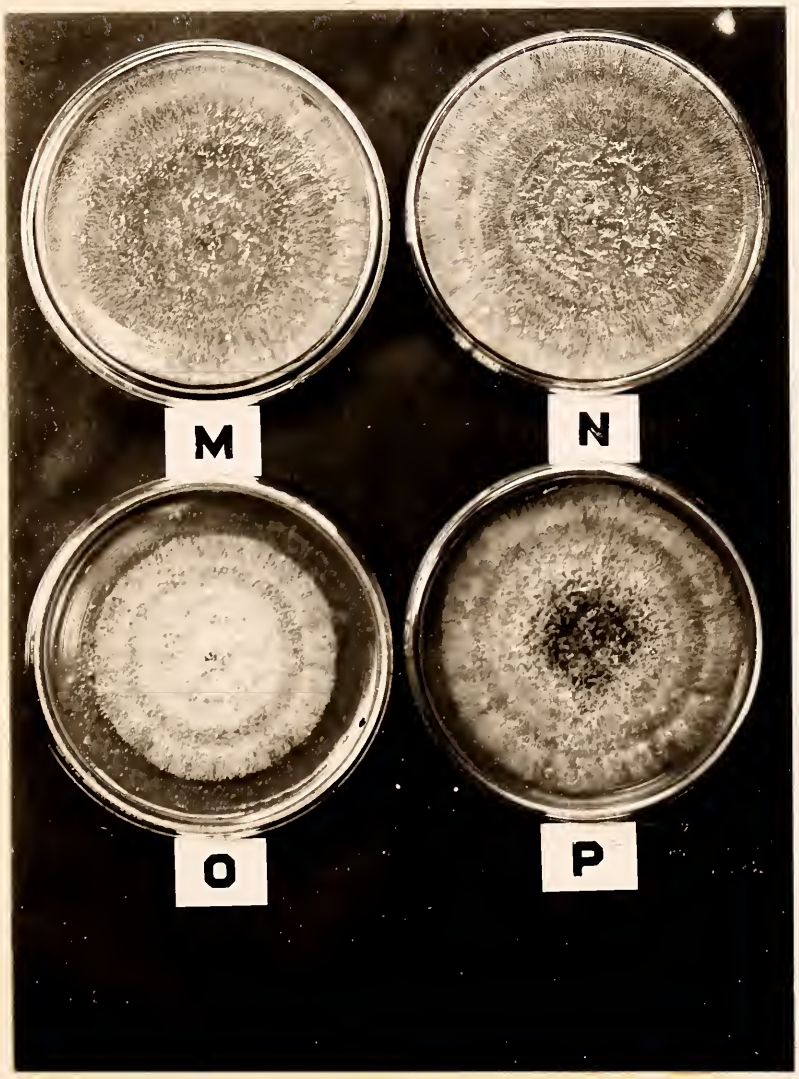

PLATE VI.

Cultures of Rhizoctonia after 3 week's growth.

Fig. Q. Sclerotial growth of strain RS on potato agar.

Fig. R. Sclerotial growth of strain RS on potato glucose agar.

Fig. S. Sclerotial growth of strain RA on potato agar.

Fig. T. Sclerotial growth of strain RA on potato glucose agar.

PLATE VI.

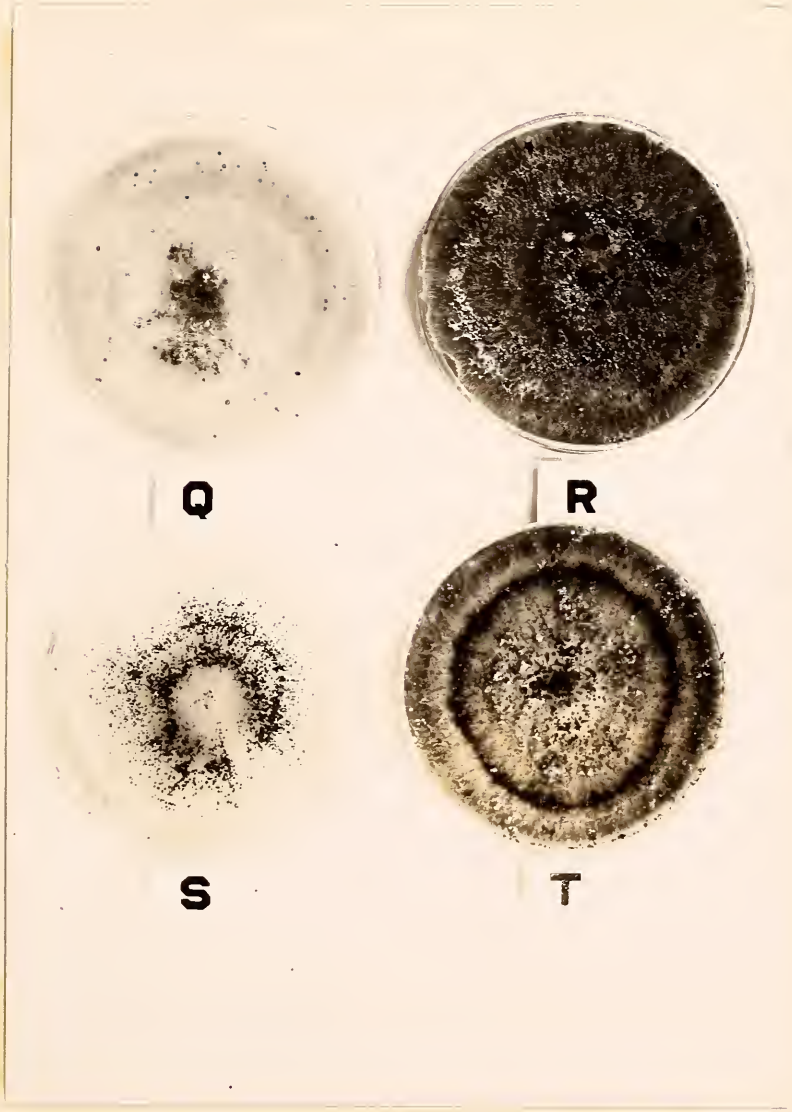

PLATE VII.

Cultures of Rhizoctonia after 3 week's growth.

Fig. U. Sclerotial growth of strain RS on green-bean agar.

Fig. V. Sclerotial growth of strain RS on oat agar.

Fig. W. Sclerotial growth of strain RA on green-bean agar.

Fig. X. Sclerotial growth of strain RA on oat agar.

PLATE VII.

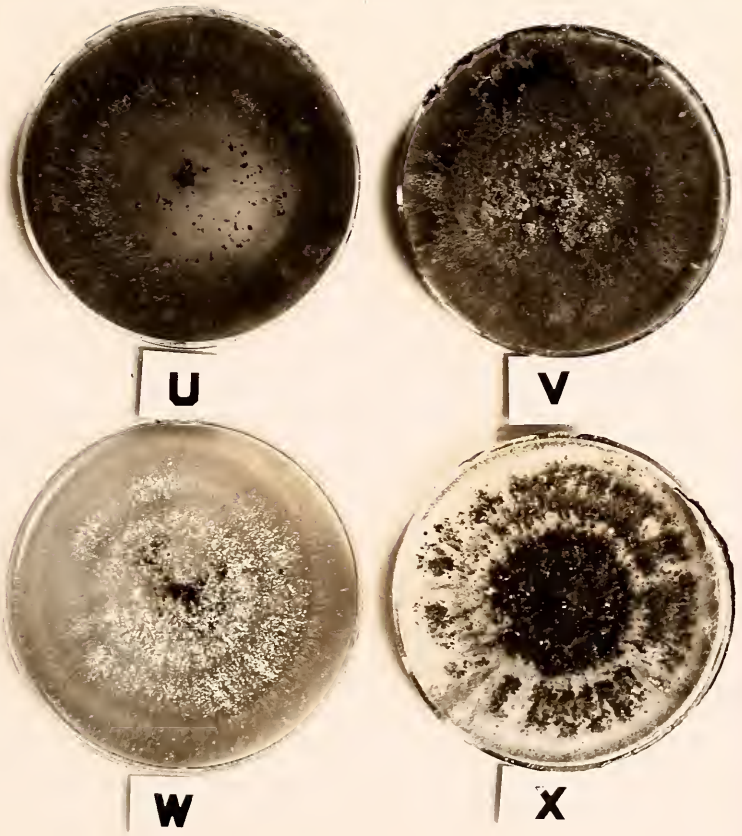

PLATE VIII.

Fig. Y. Sclerotial growth of strain RS on oat agar.

Fig. Z. Sclerotial growth of strain RA on oat agar.

PLATE VIII.

PLATE IX.

Fig. 1. Photomicrograph of section of onion seedling just above root joint, showing formation of sclerotia of Rhizoctonia and penetration of the onion tissues by hyphae from the germinating sclerotial cells.

Fig. 2. Photomicrograph of one sclerotia of section shown in Fig. 1. m ny times enlarged.

A. Mechanical pressure of growing tip of hypha causing bending of cell wall.

PLATE IX.

Fig. 1

Fig. 2