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M. F. Kernkamp
D. J. de Zeeuw, S. M. Chen, B. C. Ortega, C. T. Tsiang, and A. M. Khan
Division of Plant Pathology and Botany

University of Minnesota
Agricultural Experiment Station

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M. F. Kernkamp[1]

D. J. de Zeeuw, S. M. Chen, B. C. Ortega, C. T. Tsiang, and A. M. Khan[2]

Introduction

THE PURPOSE of this bulletin is to bring up to date the knowledge on physiologic specialization and parasitism of *Rhizoctonia solani* Kühn. More specifically, it is the intent of the author to review and put into the published records the results of several investigations on this subject made in the Division of Plant Pathology, University of Minnesota, during the past 15 years. A complete understanding of the problems involved cannot be attained without a review of literature from other sources. The latter are therefore incorporated where pertinent.

The literature on this subject prior to 1934 was reviewed adequately by LeClerg *(35)*[3], and several other writers have presented comprehensive summaries of various phases of studies on Rhizoctonia *(8, 52)*. The host range of the organism will not be discussed since the literature on that subject is voluminous. One only needs to peruse the *Review of Applied Mycology* to gain some comprehension of the host range of *R. solani*. It was reported recently that a species of Rhizoctonia is even parasitic on other fungi *(11)*. The taxonomy of the organism, although extremely important, will not receive major emphasis. It has been treated recently by Rogers *(59)*.

Since LeClerg's review *(35)*, considerable work has been done by various investigators on the cultural and pathogenic variation of *Rhizoctonia solani*, on the origin and stability of races, on control of *R. solani* in relation to the above, on the antagonism of other organisms to *R. solani*, on the toxins produced by *R. solani*, and on certain phases of pathological histology relative to infection by the organism. The author and his associates believe that a comprehensive review and evaluation of these recent contributions is appropriate at this time and may be of special benefit to others who are investigating problems on *R. solani*.

Cultural Variation

The literature concerning cultural variation of *Rhizoctonia solani* prior to

[1] Associate professor of plant pathology and botany.

[2] This bulletin is based on investigations made by the six authors at different times. The results are unpublished except in fragmentary form, but the manuscripts or theses on which they are based are on file in the Division of Plant Pathology and Botany. The senior author assumed responsibility for selecting those data that illustrate certain principles and assumes responsibility for all conclusions and interpretations. For this reason the expression "the author" is used frequently in the bulletin.

[3] Italic figures in parentheses refer to Literature Cited, page 32.

1934 was reviewed by LeClerg *(35)* and will not be repeated here. Since that time investigations on cultural variation of *R. Solani* have included the following: (1) studies of the types and range of cultural variations (figure 1); (2) attempts to determine if there is a correlation between cultural characters and pathogenicity; and (3) attempts to determine if certain cultural, morphological, or physiologic characters are of sufficient magnitude to be used for distinguishing species of Rhizoctonia.

During these studies many investigators have studied isolates from different crop plants, and studies have been made with tissue-culture, sclerotial, single basidiospore, and hyphal-tip isolates.

The various investigations showed that there are cultural races of *Rhizoctonia solani* that differ in color, zonation, growth rate, type of sclerotia *(35)*, structure of sclerotia, width of hyphae *(12)*, and enzyme production *(22)*. Other investigations demonstrated that races of *R. solani* respond differently to temperature *(5, 12, 32, 35, 41, 45, 73)*, nutrients *(7, 12, 21, 32, 35, 51, 73)*, hydrogen-ion concentration *(13, 21, 22, 32, 35, 43, 73)*, and to toxic chemicals *(12, 32)*.

Several examples will serve to illustrate the above conclusions. LeClerg *(35)* studied 78 cultures, 51 from sugar beets and 27 from potatoes. He showed that the optimum temperature for growth in artificial culture of some isolates is 25° C. while for others it is 30° C. LeClerg, Person, and Meadows *(41)* compared 63 sclerotial isolates from potatoes on potato-dextrose agar at 20°, 25°, and 30° C. with two crown-rot and two dry-rot isolates from sugar beets. The isolates from potatoes grew best at 25° C., and the isolates from sugar beets grew best at 30° C. Chen *(12)* studied 47 cultures selected from more than 200 isolates from 10 crops, including potatoes, sugar beets, barley, oats, cotton, flax, sweet clover, wheat, *Agropyron cristatum*, and *Delphinium*.

Others *(5, 32, 45, 73)* worked with smaller numbers of cultures, but all concluded that different races require different temperatures for growth. Some races have different optima than others, and some tolerate wider temperature ranges than others. Furthermore, the effects of temperature and media are interrelated since the effects of temperature are expressed differently on different media. For example, a race may have one optimum temperature on one medium and a different optimum on another.

The fact that different races respond differently to different nutrients has been demonstrated by LeClerg *(35)*, Chen *(12)*, and de Zeeuw *(21)* who studied 78, 47, and 39 isolates, respectively, from different crops.

LeClerg *(35)* summarized the conclusions aptly when he stated, "There is a marked difference in the growth of isolates of *Rhizoctonia solani* on the same and different media."

It is noteworthy that all of Chen's *(12)* cultures were started from hyphal-tip isolates that were cut off with a microrazor. He made his cultural studies on five different media and came to the same conclusions as LeClerg (figure 1). Only two of the 47 isolates were alike; the others differed in one or more characters, and sometimes two races were alike on one medium, but entirely different on another. Similar conclusions were reached by de Zeeuw *(21)*, Ortega *(51)*, Tsiang *(73)*, Boosalis *(7)*, and Khan *(32)*.

It likewise has been shown that different races react differently to hydrogen-ion concentration. This was first established by Matsumoto *(43)* and later confirmed by LeClerg *(35)*, Cherewick *(13)*, de Zeeuw *(21)*, and Tsiang *(73)*.

Since Tsiang *(73)* investigated this phase quite extensively, his results illustrate the facts well, and his work will be discussed in some detail. He studied 13 races in culture at *pH* values of 2.8, 4.2, 5.2, 6.0, 7.2, and 9.2. The

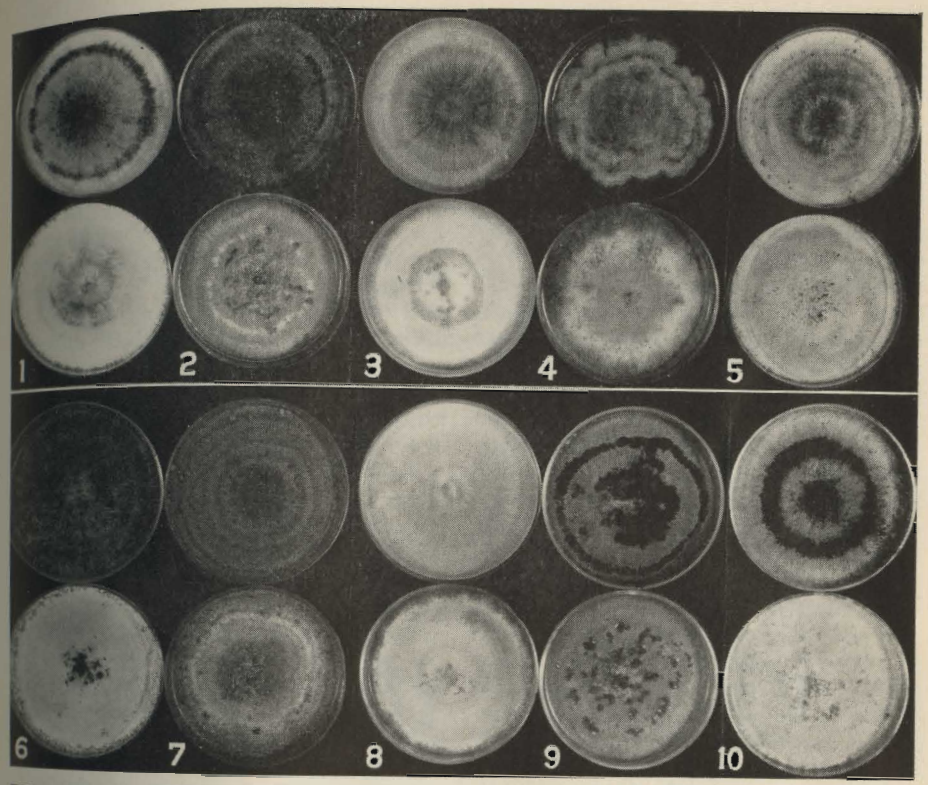

FIG. 1. Rows 1-5, cultural differences between races of *Rhizoctonia solani* growing on potato-dextrose agar. Rows 6-10, the same 10 races in the same relative positions on nitrate-dextrose-agar. Photograph from Ph.D. thesis by Shan-Ming Chen *(12)*.

Table 1. **The Relative Growth of 12 Isolates of *Rhizoctonia solani* after Seven Days in Richard's Solution Adjusted to Different Hydrogen-ion Concentrations at the Beginning of the Experiment***

Isolate	Relative growth at different *pH* values†					
	2.8	4.2	5.2	6.0	7.2	9.2
1	0	2	3	3	3	1
2	0	1	3	3	3	T
3	0	1	3	3	2	T
4	0	1	2	3	1	T
5	0	T	2	3	1	T
6	0	1	1	3	1	T
7	0	T	1	3	1	T
8	0	1	1	3	T	T
9	0	2	3	3	T	T
10	0	3	2	1	T	0

* Table from Ph.D. thesis of Chen-Tong Tsiang (73).
† Growth ratings: 0 = none; T = trace; 1 = fair; 2 = good; 3 = vigorous.

results are presented in table 1. They show that all of the races except one tolerated a wide range of hydrogen-ion concentration. Except for one race the maximum growth was at *pH* 6 and growths of different races varied at higher and lower *pH* values. The exceptional race grew best at *pH* 4.2 and did not grow at all at *pH* 9.2.

Khan's results *(32)* agreed with these, the three races he studied having an optimum *pH* of between 6 and 7, with differences in tolerance appearing at the extremities of the *pH* scale.

From the results of experiments on types and ranges of cultural variants in *Rhizoctonia solani*, it is obvious that many races exist and that the races respond very differently to individual en-

vironmental factors or combinations of them. Thus, there are an infinite number of races that parasitize many hosts. In view of this, various experimenters were interested in determining whether there is a correlation between certain cultural characters and parasitic capabilities. For example, would cultures isolated from flax have any cultural characters that would distinguish them from cultures isolated from potatoes? Some reports indicate that there is a correlation between cultural characters and pathogenicity, and other reports do not support this view.

Houston *(29)* studied 52 cultures from a collection of 260 isolates from 15 California crop plants. He divided them into three cultural types and indicated some correlation between pathogenicity and cultural types.

Growth rate and cultural characters of 39 races isolated from potato tubers and stems, flax, peanuts, and sweet clover were compared by de Zeeuw *(21)*. Growth rate was so variable that it was not a valid character for separating races. He also studied the 39 races on cornmeal, malt, prune, and potato-dextrose agars. On these media there were great differences in cultural characters. He stated that the greatest cultural differences were between isolates from different host species. This would indicate some degree of correlation between cultural type and pathogenicity, but he went on to state that differences between isolates from a single host were also great.

Other workers found no indications of such correlation. Chen *(12)* studied 47 cultures isolated from 10 different hosts and attempted to place races in distinct cultural groups on the basis of hosts from which they were isolated. This failed because no characters were consistent enough, nor were they correlated with hosts from which they were isolated.

Person *(54)* showed that 12 isolates from eight hosts varied considerably in culture at seven different temperatures. He separated them into four fairly distinct groups by pathogenicity tests, but did not mention a correlation between pathogenicity and cultural type.

Tsiang *(73)* compared 13 isolates in artificial culture to determine if flax isolates resembled each other more closely than they resembled isolates from other hosts. He compared them on four media, potato-dextrose, malt, cornmeal, and water agars. The cultures differed from each other in one or more characters, and the flax isolates differed as much among themselves as did the entire group. Thus, the flax isolates could not be distinguished as a group from the other isolates.

Boosalis *(7)* reported that six isolates from five crops differed on four different media, but found no correlation between cultural characters and pathogenicity.

The taxonomy of the genus Rhizoctonia is complex and probably will not be elucidated until the perfect stage of the organism is found or produced with regularity. An approach to the problem, however, has been made through studies of the physiological and morphological characters of the cultures of Rhizoctonia. Matz *(48)* described several new species, chiefly on the basis of location, size, shape, and color of sclerotia. Chen *(12)* found that sclerotia might be borne in aerial mycelium on one medium but submerged in the surface of another. The shape, size, and color of sclerotia of a single race differed on two different media. To illustrate, one race was an albino on potato-dextrose agar, but on media containing gallic acid it produced typical brown sclerotia.

Detailed studies of the structure of sclerotia of 10 different cultural races proved that there are considerable differences in size of core and thickness of rind of sclerotia from different races (figure 2). In some cases sclerotia are really aggregates of several sclerotia, having several cores and several rinds all in one aggregation. Some sclerotia

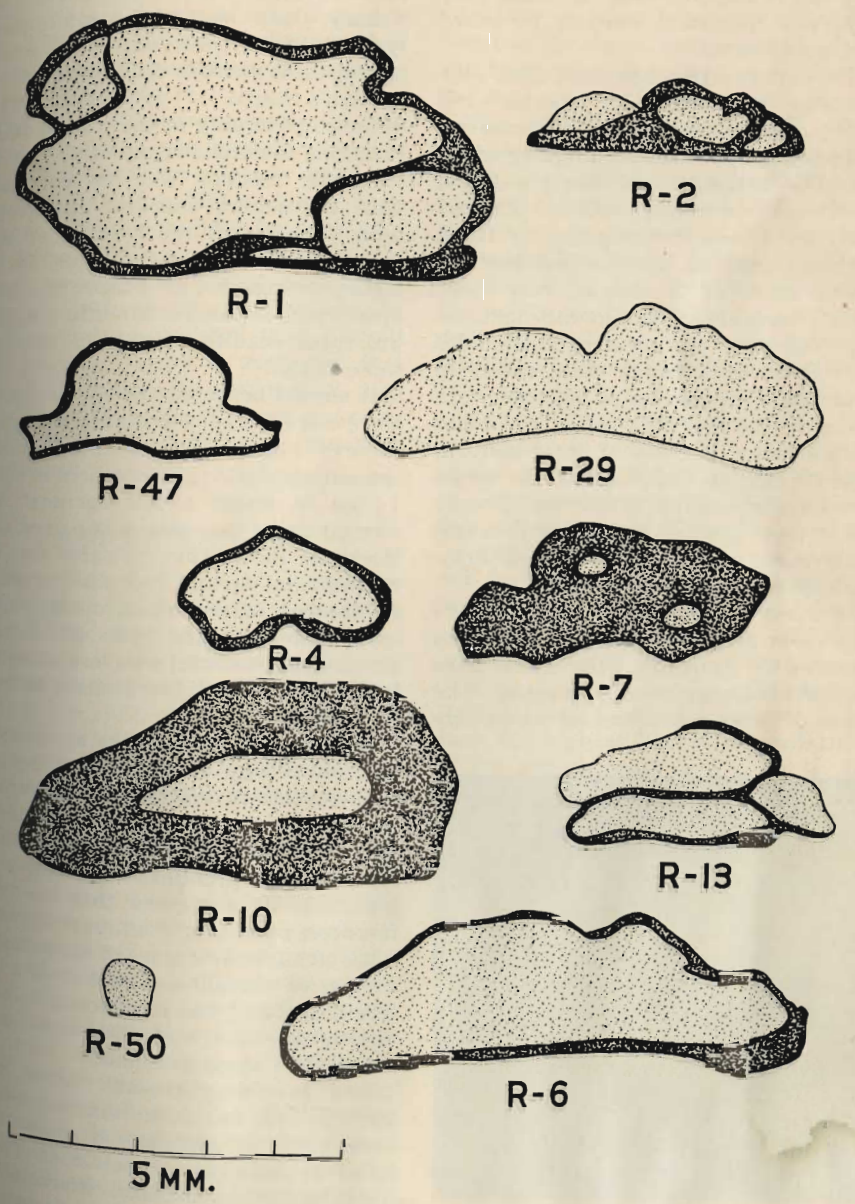

FIG. 2. The sclerotial structure of ten races of *Rhizoctonia solani*. Figure from Ph.D. thesis by Shan-Ming Chen *(12)*.

appear to be made up of sclerotia within sclerotia, and races differ in these respects *(12)*. Thus, it appears that the characters used by Matz *(46)* were not consistent enough to be of taxonomic value.

Cultural aversion has been used successfully to distinguish species and races, and the extensive literature on this phenomenon was reviewed recently *(9)*. The feasibility of using cultural aversion to determine races of *Rhizoctonia solani* was investigated by Chen *(12)*. In order to test the reaction between races of *R. solani*, two races were transferred to potato-dextrose agar and were placed at opposite sides of a Petri dish. Notes were taken 10 days later. Five series of pairings were made. In the first two series, the races were selected because of their cultural resemblance or dissimilarity. In series 3 and 4, the races were selected according to their original hosts; and in series 5, the races were chosen for their similar hyphal diameters.

In many cases, a thin line or "barrage" was produced when the race was paired with itself, but after a few days the two colonies usually merged. The types of reactions obtained when two races were paired were very different. Thus, one colony might grow completely over another colony, or a "barrage" might be formed and persist. Obviously, there were many intergrading types between these extremes, but no attempt was made to differentiate these.

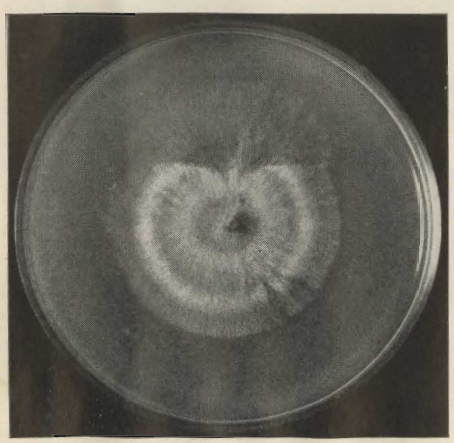

FIG. 3. A colony of a sugar-beet isolate of *Rhizoctonia solani* showing a large variant. Photo courtesy of E. L. LeClerg.

Chen's results *(12)* indicate that races differ considerably in their antagonistic reactions toward one another. The antagonistic reaction was not correlated with the general cultural appearance, with diameter of the hyphae, or with the original hosts of the races. In two instances, a race was antagonistic to all other races isolated from different hosts but fused readily with races from the same host.

It should be noted that the reactions were not always consistent throughout the experiments. For instance, one race was antagonistic to four others in series 1; but in series 4, no "barrage" was formed when that race was paired with the same races. This indicates that environmental factors and possibly slight differences in the medium may influence materially the type of reaction. Apparently, cultural aversion alone has limited value in differentiating races in *R. solani*.

LeClerg *(39)* studied the morphology and physiology of sugar beet dry rot canker isolates and sugar beet crown rot isolates. He found that they differed in size of hyphae, rates of growth in culture, and optimum temperatures for infection. He suggested that the "differences, particularly as regards symptoms on sugar beets, are of such magnitude as to warrant designation of a different species," but considered it inadvisable to make such distinction until the perfect stage is found.

The preceding reports prove that there are an indefinite number of cultural races of *Rhizoctonia solani* that differ in pathogenic capabilities. There seems to be no cultural character or group of characters that serve to distinguish isolates from one host or another, nor is there any character that distinguishes one pathogenic race from another on a given host variety or species. The problems of pathogenic variation will be discussed in a later section.

Likewise, it does not seem that species of Rhizoctonia can be distinguished by cultural, physiological, or morphological characters.

The origin of races is a matter of conjecture, but it is pertinent to note that similar studies have been made by Exner and Chilton *(24)* on single basidiospore isolates and by LeClerg *(37)* and Person *(53)* on sector variants that arose in cultures of *Rhizoctonia solani* (figure 3). These cultures behaved the same as cultures isolated from various host plants. Thus, it is suggested that the origin of races, at least in part, may be through reduction division occurring at the time of basidiospore formation or through mutation.

Induced Variation

Since *Rhizoctonia solani* comprises numerous cultural and parasitic races, the question arises as to how these races originated. It has been suggested by Sanford *(60)*, Exner and Chilton *(24)*, and Kotila *(33)* that one source of variation is segregation in the perfect stage, resulting in different biotypes that can be identified by isolating single basidiospores.

Origin of races from sectors in cultures is relatively rare. This writer is aware of only two such variants that have been studied *(37, 53)*. Most investigators state categorically or imply that cultural races of *R. solani* are very stable in culture. If these cultural variants can be considered mutants, it is apparent that mutation is relatively rare in this organism under ordinary cultural conditions. For this reason Chen *(12)* determined to what extent mutation could be induced and the range of variation of induced mutants.

In the writer's opinion Chen's *(12)* contribution to the knowledge of variation of *R. solani* is highly significant because he succeeded in inducing mutants or variants whose cultural characters differed in the same magnitude as any of the races that have been isolated from nature.

The interpretation of induced mutation may be open to question since there is no clear-cut demonstration that the variants Chen perceived and isolated were actually induced or whether they were selected from a mixed population by the techniques he used. Biologists are aware of the phenomenon wherein different media have different selective effects on mutants that arise in colonies. It is possible that this phenomenon occurred in Chen's work. The writer does not intend to enter into a controversy on this question, but to present the facts as Chen recorded them. Therefore, for the purpose of this discussion the term "induced mutation," as Chen used it, will be used throughout this section of the bulletin.

Chen *(12)* worked with the same cultures referred to in the section on cultural variation. All of his cultures were started from hyphal-tip isolates to insure the highest degree of uniformity or the purest genic base possible. Potato-dextrose or nitrate-dextrose agars[4] were used throughout most of the experiments. Uniform quantities of inoculum were used in each test and each treatment was replicated three times. Radial growth was measured and color was determined with Ridgeway's color standards *(58)*. Variants were isolated by cutting off hyphal tips from mycelia of sectors or patches in the original colonies. Agents used to induce variation were nutrients, chemicals, hydrogen-ion concentration, visible light, temperature, and metabolic by-products of microorganisms, and they will be discussed in that order.

[4] Nitrate-dextrose agar was composed of the following: dextrose, 30.0 grams; NH_4NO_3, 2.0 grams; K_2HPO_4, 0.4 grams; $MgSO_4 \cdot 7\ H_2O$, 0.25 grams; agar, 15.0 grams; distilled water to make 1,000 cubic centimeters.

In the first experiment attempts were made to induce variation in 16 races of *R. solani* on potato-dextrose, Czapeck's, 2.5 per cent malt, sepedonica[5], and Leonian's nutrient[6] agars. Only three races sectored, producing one sector each on Leonian's nutrient, potato-dextrose, and sepedonica agars.

Another experiment was made to determine if different concentrations of sucrose in the medium would stimulate sectoring. Richard's nutrient agar was used as the basic medium and sucrose contents of 0, 1, 10, 50, and 100 grams per 1,000 cubic centimeters of agar were used. None of the same races used in the preceding experiment sectored on media having different concentrations of sucrose.

In another experiment a certain race and a variant from it were grown on potato-dextrose agar and malt agar. The race gave rise to two sectors, and its variant gave rise to another sector on potato-dextrose agar. Neither sectored on malt agar. Chen observed this particular race for five years and concluded that it was a rare race of *Rhizoctonia solani* because it sectored more frequently than others, and potato-dextrose agar seemed to be more conducive to sectoring in this particular race than any other common medium.

Twelve chemicals were tested for their ability to induce mutation in 14 races of *Rhizoctonia solani*. The chemicals, their concentrations, and the results are presented in table 2. The results show that mutation can be induced in *R. solani* with certain chemicals. Sodium arsenite was most effective, inducing sectoring in all races tested except one and being most effective at concentrations of 1:6000 and 1:8000. Lithium chloride was next in effectiveness, while the remaining chemicals had little or no influence on sectoring in these tests.

Chen *(12)* was also interested in whether treatment of sclerotia would give similar results. He picked sclerotia of nearly the same size from 10 different races, treated them with various chemicals for 15 minutes, and then planted them on potato-dextrose agar. Eight sectors were observed from subsequent growth from the sclerotia. One race produced six sectors, one each from sclerotia treated with sodium arsenite 1:500 and 1:1000, and one each from sclerotia treated with copper sulfate 1:500, potassium dichromate 1:500, mercuric chloride 1:2000, and formalin 1:500. Two other races produced one sector each from sclerotia treated with copper sulfate 1:250 and sodium arsenite 1:500, respectively. Lithium chloride 1:500 and 1:250, mercury bichloride 1:1000, formalin 1:200, potassium dichromate 1:250, and 5 per cent ethyl mercury phosphate (New Improved Ceresan) 1:2000 did not stimulate sectoring.

Chen *(12)* also compared the effectiveness of sodium nitrite and sodium nitrate in the stimulation of sectoring. He added 2 and 4 grams of these chemicals per liter of Steinberg and Thom's medium *(70, 71)* and grew 16 races of *Rhizoctonia solani* on them. Chen *(12)* stated that the concentrations of the chemicals he used were too high to support the growth of most of the races, but a total of 14 mutants were produced on nitrite media while none were produced with the nitrate. Hydrogen-ion concentration had no effect on mutation nor did various amounts of visible radiations. A limited test with 10 races grown at 2, 4, 15, 25, 30, and 40° C. indicated that higher temperatures tended to stimulate sectoring.

Since it is known that metabolic by-products of certain microorganisms stimulate mutation of certain species of fungi in culture *(15)*, Chen *(12)* studied

[5] Sepedonica agar was composed of the following: potato, 300 grams; peptone, 5 grams; Na_2HPO_4, 2 grams; NaCl, 2 grams; sodium citrate, 1 gram; asparagine, 1 gram; dextrose, 6 grams; agar, 12 grams; water to make 1,000 cubic centimeters.

[6] Leonian's nutrient agar was composed of the following: dextrose, 5 grams; potassium nitrate, 1 gram; H_2NaPO_4, 0.5 grams; $MgSO_4$, 0.25 grams; agar, 20 grams; water to make 1,000 cubic centimeters.

Table 2. Number of Sectors that Developed in Duplicate Plates of 14 Races of *Rhizoctonia solani* When 12 Chemicals in Different Concentrations Were Added to Potato-dextrose Agar*

Chemicals and concentrations	Races														Totals
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
	number of sectors														
Na_2HAsO_3															
1:2000†	... ‡	.....	2	6	.....	.....	0	.....	.....	.....	1	.....	.....	.....	9
1:4000	.....	8	3	4	0	2	2	2	3	0	0	.....	0	0	24
1:6000	26	7	16	3	4	2	5	2	0	1	0	2	1	0	69
1:8000	20	14	7	4	5	5	2	1	2	6	0	1	0	0	67
LiCl															
1:500	.....	.....	.....	0	1	1	1	0	2	1	0	1	1	1	8
1:1000	.....	.....	.....	0	0	0	0	0	1	0	5	0	0	0	6
1:10,000	0	0	0	2	0	0	0	1	0	0	0	0	1	0	4
$CuSO_4$															
1:1000	0	0	0	0	1	1	0	1	0	0	0	0	0	0	3
1:2000	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
$K_2Cr_2O_7$															
1:1000	0	2	0	0	0	0	0	0	0	0	0	0	0	0	2
1:2000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
$HgCl_2$															
1:2000	0	2	0	0	0	0	0	0	0	0	0	0	0	0	2
$ZnSO_4$															
1:1000	0	1	0	0	0	0	0	0	0	0	1	0	0	0	2
1:2000	0	1	0	0	0	0	0	½	0	0	0	0	0	0	2
Cu_2O															
1:1000	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
1:2000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Formalin															
1:2000	.....	.....	0	0	.....	.....	.....	.....	.....	0	0	.....	.....	0	0
Gentian violet															
1:1,000,000	0	0	0	1	2	0	0	0	0	0	1	0	.....	0	4
Methyl blue															
1:100,000	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Colchicine															
1:1000	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Eosin															
1:100,000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Check	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total number of sectors	46	36	28	20	13	11	11	9	8	8	8	4	3	1	206

* Table from Ph.D. thesis by Shao-Ming Chen, 1943 *(12)*.

† The 1:4000, 1:6000, and 1:8000 concentrations of sodium arsenite were added to 50 cubic centimeters of nutrient agar in 250 cubic centimeter Erlenmyer flasks, in which the colonies were grown.

‡ Indicates no growth.

the effects of certain toxins from microorganisms on *Rhizoctonia solani*. He grew *Helminthosporium sativum*, *Rhizoctonia solani*, an undetermined species of Penicillium, and an unknown bacterium in potato-dextrose broth. Filtrates from these cultures were added in two concentrations to potato-dextrose agar, and 14 races of *R. solani* were grown on this agar. Only five sectors appeared in 252 Petri plate cultures. These results indicate that metabolic by-products of these organisms are not particularly active in inducing mutation.

From Chen's experiments *(12)* on induced variation, several generalizations are apparent. The frequency of mutation in *R. solani* is not very high (table 3), but certain races mutate more frequently than others. Magnitude of differences between the induced variants is comparable to the differences between races isolated from their natural habitats. To illustrate, one race produced 46 sectors in 128 colonies in different environmental conditions, another race produced only seven sectors, and other races did not produce any sectors in the same number of colonies under the same conditions. The fact that the variants differ in the same magnitude as races isolated from nature is illustrated in paragraphs that follow.

Table 3. Number of Sectors Developed in Nine Races of *Rhizoctonia solani* on 64 Media in Duplicate Petri Dishes or Flasks*

Race	Number of sectors	Per cent of colonies in which sectors appeared
71	46	35.9
4	16	12.5
36	14	10.9
37	11	8.5
34	10	7.8
5	9	7.0
18	8	6.3
19	8	6.3
2	7	5.5

* Table from Ph.D. thesis by Shan-Ming Chen, 1943 *(12)*.

TYPES OF SECTORS AND CHARACTERISTICS OF VARIANTS

Variants appeared in colonies as wedge-shaped sectors (figure 3), patches, or tufts. In most cases there was only one sector per colony, but colonies of two races on sodium arsenite, 1:6000 and 1:8000, usually gave rise to five or more sectors. In one case an unusually large sclerotium was produced. A hyphal tip was isolated from it when it germinated and the resulting culture was distinctly different from the parent culture, demonstrating that variants occasionally arise from sclerotia.

Most sectors were distinctly different from parental colonies. Some grew faster, others more slowly than their parents. The type of growth also differed from that of parent cultures. Some sectors, in contrast to parental cultures, developed only a relatively thin mycelial growth, and others produced an abundance of sclerotia.

Color of sectors often differed from that of parental growth, and white sectors from colored colonies were not uncommon. Sectors often differed from their parents in degree and frequency of zonation. The margin of sectors also differed from the margin of parental colonies, so that parents with smooth margins gave rise to sectors with rough margins or vice versa.

CULTURAL AND PHYSIOLOGICAL STUDIES OF VARIANTS

Variants in some fungi have been studied extensively, but since cultural variants in *Rhizoctonia solani* are rare, only two had been studied, one by LeClerg *(37)* and one by Person *(53)*, until Chen *(12)* succeeded in inducing a relatively large number of variants.

Chen *(12)* studied differences in cultural characters of 29 induced variants and their respective parents on potato-dextrose agar and nitrate-dextrose agar (see page 9). It is impossible to record all of the details here, but there were differences in color, zonation, topography, general appearance, color of substrates, amount of aerial mycelium, and other characters of variants and parents of those investigated. Some differences were slight but others were great. Based on repeated comparisons on various media, none of the 29 variants except two resembled other variants or their parents in cultural characters. It was further evident that two variants might look more or less alike on one medium but on a second medium, they were completely different.

Sclerotia

Chen *(12)* stated that variations in sclerotial formation were perhaps the most striking differences between many variants and their parents. A study of sclerotia of more than 30 variants and their respective parents proved that they differed in distribution, abundance, color, shape, size, and type of aggregation. For instance, one race developed abundant sclerotia on potato-dextrose agar in test tubes, but under identical conditions its variant seldom produced sclerotia. Another race ordinarily formed a crust-like mycelial growth on the surface of agar followed by numerous small sclerotia in chains. As the culture grew older, larger sclerotia developed. One of its variants produced definite sclerotia, even when the culture was relatively young, while a sister variant produced sclerotia in approximately the same manner as the parent, although the variants differed in some other characters. Sclerotial formation of a variant could be different on two kinds of media, and two variants that had similar sclerotial development on one medium could differ on another.

Hyphal Diameter

As stated previously *(12)*, the diameter of hyphae of different races varies from 5.17 to 10.23 microns. In order to determine if such differences occurred among hyphae of induced variants, Chen measured the diameters of hyphae of 27 variants and their four parents. Two hundred hyphae of each parent and the variants were measured with a micrometer.

The hyphae of one parent measured 9.48 microns, while those of nine of its variants ranged from 9.19 to 10.15 microns. Another's hyphae measured 8.97 microns, while those of two of its variants measured 9.00 and 9.48 microns, respectively. Hyphae of another parent measured 6.08 microns, and those of 10 of its variants ranged from 5.66 to 6.22 microns. The hyphae of still another parent measured 6.90 microns, and those of six of its variants were from 6.39 to 7.23 microns. A seventh variant, however, had hyphae only 3.47 microns in diameter. Chen cites another example where hyphae of a race were 9.47 microns in diameter, and hyphae of one of its variants were 5.58 microns.

The differences between the hyphae of 30 of the variants and their parents are so slight that they are probably of little significance, but in the latter two cases, where hyphae of variants are less than half the diameter of their parents the difference is highly significant. These results prove that diameters of hyphae of induced variants have as great a range as diameters of hyphae of races isolated from their natural habitats.

Growth Rate

Seventeen variants and their parents were grown on potato-dextrose agar at room temperature, and measurements of their daily growth were made after 48 hours. There were marked differences in daily growth rates between variants and their parents. For example, one race grew 5.4 millimeters per day, while growth rate for seven of its variants ranged from 5.0 to 10.1 millimeters per day. In another experiment 19 vari-

Table 4. Average Daily Growth of Variants and Parents of *Rhizoctonia solani* on Potato-Dextrose Agar and Nitrate-Dextrose Agar Growing in Duplicate Flasks*

Race	Average daily growth on: Potato-dextrose agar	Nitrate-dextrose agar†	Race	Average daily growth on: Potato-dextrose agar	Nitrate-dextrose agar
	millimeters			millimeters	
15	18.0	10.1	15-6	2.5	0.5
15-14	19.3	1.0	15-3	2.4	1.3
15-15	19.3	1.4	15-10	1.2	0.5
15-5	16.5	1.3			
15-13	16.1	5.9	36	8.3	0.1
15-7	15.0	5.7	36-3	10.1	0.1
15-9	14.0	5.3	36-5	8.7	2.7
15-11	10.4	3.9	36-4	8.6	1.1
15-1	9.9	0.8	36-8	7.5	0.4
15-12	9.3	0.2	36-6	2.7	0.3
15-4	4.3	0.1	36-7	1.5	1.1

* Table from Ph.D. thesis by Shan-Ming Chen, 1943 *(12)*.
† For nitrate-dextrose agar see page 9.

ants from two races were grown on potato-dextrose and nitrate-dextrose agars. The results of this experiment are given in table 4. It is obvious that the differences between variants and parents were striking in many cases, and that growth rate of a race and its variants on one medium was not a criterion of its growth rate on another medium.

Tolerance to Toxic Substances

LeClerg *(37)* and Khan *(32)* presented data showing that races of *Rhizoctonia solani* have different degrees of tolerance to certain chemicals. Chen *(12)* showed that variants from cultures of *R. solani* responded similarly to chemicals. He studied many variants from several races on media containing a wide range of chemicals. All of the details cannot be given here, but in table 5 are listed the chemicals and concentrations on which a race and one of its variants were grown. These results are typical of many others, showing that variants respond differently to chemicals than do their parents, and other results show that variants themselves respond differently to the same or different chemicals. These

Table 5. Tolerance of Race 8 and Its Variant, 8-2, to Different Toxic Chemicals Added to Potato-Dextrose Agar*

Chemical added	Concentration	Average daily growth: Race 8	Race 8-2†
		millimeters	
Gentian violet	1:1,000,000	trace	trace
New Improved Ceresan	1:50,000	2.5	9.5
	1:10,000	3.5	10.0
$ZnSO_4$	1:1000	3.5	5.0
LiCl	1:2000	4.5	3.5
$CuSO_4$	1:1000	5.0	8.0
$K_2Cr_2O_7$	1:1000	5.5	10.5
Colchicine	1:1000	6.5	10.0
$HgCl_2$	1:1000	8.0	9.5
$ZnSO_4$	1:2000	10.0	13.0
Check		11.5	12.0

* Table from Ph.D. thesis by Shan-Ming Chen, 1943 *(12)*.
† Race 8-2 is a variant from race 8.

results may be of practical significance relative to the chemical control of *R. solani*.

Metabolic By-Products

Chen *(12)* determined the hydrogen-ion concentration of substrates after races and variants had grown on liquid media for four months. He found differences in the hydrogen-ion concentrations of the media on which different variants were grown and attributed this to different physiological capabilities of the races and their variants.

Enzyme Production

Edwards and Newton *(22)* distinguished three strains of *Rhizoctonia solani* on the basis of their enzyme activity. Chen *(12)*, using the technique of Davidson, Campbell, and Blaisdell *(19)*, compared oxidase production of induced variants with that of strains isolated from nature. Some variants grew faster and some more slowly than their parents, but no radical difference in the production of oxidase was detected, with the exception of three variants, which failed to change the color of gallic acid medium while their respective parents gave a positive reaction.

Pathogenicity

Preliminary tests on the pathogenicity of variants and their parents were made on potatoes according to the technique described by LeClerg *(35)*. Potato tubers were washed and their surfaces disinfected with formalin, after which they were cut into slices about 5 millimeters thick and 40 millimeters in diameter. Two slices each were inoculated with a variant and its parent and then placed in Petri dishes. After 10 days, readings were made on the amount of rot in the tuber slices. The results showed differences between the destructiveness of parents and variants. In some cases the variants decayed tubers more rapidly than their parents, and in other cases the reverse was true. It was unfortunate that Chen could not test the pathogenicity of induced variants in more detail, but a field trial failed because of adverse weather conditions. The preliminary results, however, indicate that differences in pathogenicity of induced variants exist as much as differences in other characters.

Cultural Aversion

Cultural aversion had been used successfully to distinguish species and races of fungi and the literature on that subject was reviewed by Brown *(9)*. As was pointed out earlier (see page 8), Chen *(12)* attempted to distinguish races of *Rhizoctonia solani* on the basis of cultural aversion. He was not successful because the environment changed the reactions more than the cultures themselves.

Similar tests were made on aversion between variants and their parents, variants and sister variants from the same parent, and variants from different parents. As in the tests with isolates, there was no consistent reaction between any of the cultures tested. Environment seemed to play a more important role than the cultures themselves. It is significant to note, however, that in one case a variant was antagonistic to its parent and to all of the other variants from that race except one. Results of this kind tend to invalidate the use of cultural aversion to distinguish species, at least of *Rhizoctonia*.

Hyphal Fusion

In investigating the difference between *Rhizoctonia solani* and the so-called "*Hypochnus sasakii* Shirai," Matsumoto, Yamamoto, and Hirane *(45)* observed that hyphal fusions occurred between strains within each group but not between the two. They believed that this is one basic criterion for differentiating species.

Chen *(12)* tested whether all the variants obtained in his work would fuse with each other by placing small pieces of the colonies for pairing on opposite sides of a drop of non-nutrient agar on a cover slip. After incubation for about 12-24 hours in a van Tieghem cell, they were observed under a microscope.

At least one variant failed to fuse with all other variants of the same parent. It apparently formed a kind of false fusion with the parent, but whether or not this was true hyphal fusion could not be determined. Four other doubtful cases of fusion were observed in the group; all other races fused freely within the group. It was also observed that there is no correlation between hyphal fusion and the cultural aversion of races.

Stability of Induced Variants

Chen *(12)* kept large numbers of variants under constant observation for more than four years. Many of them were transferred as many as twenty times. Cultural comparisons on various media also were made from time to time. In general, the variants appeared to be very stable. None of the forty variants which were studied extensively underwent any noticeable change. This also was true of all the original races.

Cultural reversions were observed by Chen *(12)*, although they occurred in a very small percentage of the variants. He believes that the original sectors of the reverted variants did not arise by true genetical change; thus, this is not a reversion in the real genetical sense. The belief is based on the observation that when this kind of reversion occurred, it was usually during the first few transfers. The exact nature of this temporary change was not determined. The constancy of the variants, in general, was rather significant and was in accord with the apparent stability of isolates of *R. solani*.

Chen proved the stability of the variants in another experiment. A few variants were inoculated into potato tubers or seedlings of several plants and then reisolated. The passage through hosts did not change their cultural characteristics.

Cytological Study of Induced Variants

It is important to know the origin of nuclei in hyphal branches and tips, in order to account for possible origin of variants. Therefore, Chen *(12)* attempted to ascertain the nuclear condition in the hyphal tips and side branches in a number of races of *Rhizoctonia solani*.

The cytological preparations were made by placing a drop of fresh egg albumen on a glass slide along with a piece of mycelium. The slide was dried sufficiently to coagulate the egg albumen. Then another thin film of egg albumen was smeared on it and a drop of sterile water or Leonian's nutrient solution was poured on it. The slide was placed on glass rods in a Petri dish lined with moist filter paper. When the fungus reached the required stage of development, the slide was drained, dried, and then fixed with Carnoy's acetic alcohol fluid. Feulgen's stain was used; potassium metabisulfate was substituted for SO_2 solution as described by de Tomasi *(20)* and basic-fuchsin was prepared by the method of Coleman *(16)*. Five- to ten-minute hydrolysis proved to be satisfactory. Four races and a variant from one of them were studied.

Müller *(48)* found that the cells of *Rhizoctonia solani* were multinucleate, usually containing about 16 nuclei. Chen's *(12)* findings agree. Terminal cells of young hyphal branches and the main hyphal cells were usually multinucleate and contained an average of six nuclei. Hyphal branches with a length of about two or three times their diameter contained up to ten or more nuclei. In a few cases Chen *(12)* found only a single nucleus in a very short branch, but he stated that that was perhaps before the complete formation of the septum of the cell in the hyphal branch, and more nuclei might migrate into it later. It is uncertain whether these arose through the division of a single nucleus or migrated from the main branch. In some cases he noticed that two nuclei were located at the base of a young branch which was just beginning to form. This indicated to him that more than one nucleus might enter a hyphal branch. Conjugated dicaryotic condition was also observed, although usually it occurred in only one portion of the mycelial mat. The exact reason for this distribution is unknown. Furthermore, it was not certain whether this was truly dicaryotic or simply due to the fact that the nuclei of this portion of the mycelial mat underwent mitosis simultaneously.

NATURE OF THE INDUCED VARIATION

In the study of the nature of variation it is essential that isolates be genetically pure. Single spore or single hyphal-tip cultures are often considered genetically pure cultures. Recent studies indicate, however, that a single spore or hyphal tip does not necessarily guarantee the genetic purity of an isolate. The variation of such a culture may be simply the result of nuclear dissociation of genetically different nuclei present in the original multinucleate culture (heterocaryosis). If a culture originated from a single nucleus, however, variation could be interpreted more readily as being due to mutation.

Single hyphal segments of *Rhizoctonia solani* containing a single nucleus cannot be isolated and propagated in culture, so one cannot prove absolutely the purity of a given isolate. But understanding of the nature of variation of such a fungus can be approached reasonably well through indirect evidence. The following assumption, based on the knowledge of other fungi, is probably essentially correct: the more stable the isolate is in culture the greater is the possibility that the fungus is not in a heterocaryotic condition.

Most of these races were kept in culture for more than six years, and during this time many transfers and subcultures were made on various media *(12)*. Except in a relatively few cases no variations were noticed. In fact, most of the races were so stable that they rarely sectored, even on media with heavy doses of toxic chemicals which are known to induce variation in other fungi. This consistent infrequency of variation could be explained by assuming that the nuclei were all alike, and the isolates, therefore, were genetically pure, or by assuming that the nuclei divide at the same rate and are evenly distributed in the hyphae. Since there is no mechanism of this kind known in fungi, except in the dicaryotic phase in basidiomycetes, it seemed to Chen *(12)* that the first assumption is the more probable.

If nuclei were present in a heterocaryotic condition, then one might expect hyphal-tip isolates from the same race to be different, at least in some cases. This would be particularly true if no conjugate division of nuclei took place and thus the nuclei of different kinds were unevenly distributed throughout all the hyphae. Chen *(12)* cut off about 120 hyphal tips from two races. One hundred of them were plated on potato-dextrose agar, and their cultural aspects were compared. If any of the cultures appeared to be different, comparisons were repeated. Without exception, these cultures were identical to races from which they were isolated.

On the evidence presented above, Chen *(12)* believed that the original hyphal-tip isolates were relatively pure genetically; therefore, the variations induced in his experiments were believed to be true mutations and not

the results of the dissociation of several kinds of nuclei.

Pathogenic Variation in Relation to Host Species and Varieties

The literature on physiologic specialization of *Rhizoctonia solani* prior to 1934 was reviewed by LeClerg *(35)*. Since 1934 approximately 20 papers have been published regarding various phases of variation in the parasitism of cultures of *R. solani* and several theses have been devoted to studies on this subject at the University of Minnesota. A review of these papers follows.

LeClerg *(35)* published results of studies on the parasitism of 78 cultures, of which 51 were isolated from sugar beets and 27 from potatoes. Sugar beets were susceptible to *Rhizoctonia solani* at all stages of development, but there were wide differences in the ability of different isolates to rot sugar-beet slices. LeClerg *(35)* found that sugar-beet isolates were pathogenic to large sugar-beet roots, while the potato isolates were not. He concluded that "rhizoctonia root rot of sugar beets is probably caused by strains of *Rhizoctonia solani* distinct pathogenically from those on potato." He also demonstrated that sugar-beet isolates caused a high percentage of damping off of sugar-beet and table-beet seedlings, while potato isolates caused considerably less. Both groups were equally destructive to alfalfa seedlings. Throughout all of the experiments there were differences in pathogenicity of different isolates. The type of variation in pathogenicity of different isolates is illustrated in figure 4.

After further studies on the parasitism of *Rhizoctonia solani*, LeClerg *(36)* found sugar-beet isolates that were pathogenic, as a group, to potatoes; he also isolated strains of *R. solani* from potato sprouts that were virulent on sugar-beet roots. Three isolates were strongly pathogenic to sugar beets, and 25 others caused less decay. One hundred sixteen additional isolates from older potato plants and from sclerotia on tubers were nonpathogenic to sugar beets. Further studies by LeClerg *(40)* showed that none of 89 isolates from underground stems of older potato plants or sclerotia on tubers from widely separated areas in the United States were pathogenic on sugar-beet roots. Beet isolates were more injurious to potatoes than isolates from potato tubers. Twenty-five per cent of 97 isolates from potato stolons were pathogenic on beets.

Sanford's results *(60)* were in general agreement with LeClerg's. He isolated parasitic races from sclerotia, stem lesions, and basidiospores on potatoes and reported a wide range in pathogenicity of the isolates. From 20 to 25 per cent of the sclerotial isolates were weakly pathogenic to nonpathogenic on potato stems, whereas the stem isolates were pathogenic. Basidiospore isolates gave rise to a large proportion of virulent cultures.

In 1941, Sanford *(63)* reported the results of three years' tests on the pathogenicity of 148 isolates of *Rhizoctonia solani* from sclerotia on potato tubers, lesions on potato stems, and basidiospores on potato stems. All of them failed to infect sugar beets, but in the same investigations, sugar-beet isolates killed sugar-beet seedlings.

In 1939 LeClerg *(38)* attempted to distinguish races of *Rhizoctonia* on the basis of parasitism on several crop plants. He studied 29 isolates from sugar beets and potatoes, the possible differential hosts being a variety of sugar beet,[7] Detroit Dark Red table beets, and Grimm alfalfa. There was so much variation among results of repeated tests that races could not be accurately distinguished by this method.

[7] K. W. Normal brand, produced by Zuckerfabrik Kleinwanzleben.

FIG. 4. Virulence of eight races of *Rhizoctonia solani* on the soybean variety Habaro. Ten seeds were planted in each pot. Plants in pots 1-8 were inoculated with eight different races; 9 was control. Photo courtesy of M. G. Boosalis *(8)*.

Attempts were made to identify races by direct inoculation of six varieties of beans. Again, the degree of variability was too great to warrant using this method for identification of races. Other tests indicated parasitic differences between isolates. For example, potato isolates were nonpathogenic to cabbage, and carrots more resistant to potato than to sugar-beet isolates.

LeClerg *(39)* also studied the parasitism of dry-rot canker isolates and crown-rot isolates from sugar beets and potato isolates on peas, sugar beets, and cabbage. In general, the crown-rot strains caused the greatest reduction in stand of the three crops tested. Inoculation with the dry-rot strain caused deep localized lesions on beets, while the crown-rot strains caused generalized decay. LeClerg suggested that these differences, along with differences in size of hyphae, rate of growth in culture, and different optimum temperatures for infection, warrant specific distinction but considered it inadvisable until the perfect stage of the dry-rot strain is found.

Person (54) studied parasitism of *Rhizoctonia solani* on beans. He reported that 12 isolates from eight hosts varied considerably in cultural characters and was able to distinguish four fairly clear-cut groups in pathogenicity tests. One group from sugar cane and sclerotia of potato tubers was weakly pathogenic on beans; a second group from peas was slightly infectious on beans; group three, a rice strain, prevented emergence of beans; and a fourth group from beans, tomatoes, egg plant, and sugar beets caused loss in stand and severe damage to stems of beans. In addition, he found that sugarcane and potato strains were harmless to soybeans, cowpeas, peas, and broad beans; but a rice strain was destructive to these crops.

Other investigators (3, 7, 13, 14, 25, 29, 42, 67, 69, 72) have studied the parasitism of *Rhizoctonia solani* on a wide range of crop hosts. Their results are as varied as the fungus itself. Houston (29), for example, described three cultural types and claimed indications that there is a correlation between cultural types and pathogenicity. Storey (72) stated that some strains have a restricted host range while the host range for other strains is not so specific. For example, one strain each from tomato and potato isolates infected only solonaceous plants. Another isolate from tomatoes infected lettuce. Isolates from crucifers infected only crucifers, and isolates from lettuce and zinnia infected only those crops. An endive strain attacked swede but not lettuce or other hosts tested, and a strain from beets did not infect other hosts. On the other hand, isolates from cotton and grass attacked both cruciferous and solonaceous plants. Similar results were obtained by Marchionatto (42) with isolates from carnation, potato, egg plant, cicer, chilli, and *Pinus spp.*

A report from the Argentina Ministry of Agriculture (3) described six races isolated from flax, and Sprague (69) differentiated five physiologic races by inoculation of Kubanka wheat, Marion oats, *Bouteloua oligostachya*, *Agropyron cristatum*, Turghai proso millet, Black Amber sorghum, and Grimm alfalfa. Cherewick (14) reported a Rhizoctonia that was parasitic on *Melilotus alba*, *M. officinalis*, *M. suaveolens*, *Trifolium pratense*, *T. hybridum*, *Medicago sativa*, *Glycine max*, and *Vicia angustifolia*, but it was not pathogenic on any of the grasses.

The most recently published data on parasitism of *R. solani* appears to be that of Boosalis (7). He studied 16 races of the pathogen on five varieties of soybeans and concluded that the pathogenicity of this fungus on soybeans is generalized rather than specific.

A series of studies was made in the Division of Plant Pathology, University of Minnesota, on the physiologic specialization and parasitism of *Rhizoctonia solani* on various crops. Its relation to various vegetables was studied by de Zeeuw (21) and Ortega (51); Chen (12) studied the pathogenicity of the organism on cereals and grasses; Tsiang (73) studied it in relation to flax; and Khan (32) continued studies of *R. solani* on soybeans.

In his studies, de Zeeuw (21) worked with 65 isolates from potato tubers, potato stems, flax, peanuts, sweet clover, Delphinium, muskmelon, and sugar beets. He studied the parasitism of these isolates on 16 varieties of potatoes, pumpkin, watermelon, muskmelon, citron, and several varieties of squash. He demonstrated that there were many differences among the isolates, the most important of which was the differential parasitism of isolates on potato varieties. As far as the writer is aware, this was the first positive demonstration of physiologic specialization of *R. solani* for potato varieties. These results agree with those of Le-Clerg (36) that certain sugar-beet isolates are pathogenic to potatoes.

Furthermore, de Zeeuw (21) found that four isolates, one from flax, one from sweet clover, and two from peanuts, were markedly pathogenic on eight cucurbits while 15 others were not, in field and greenhouse tests. Little or no difference was found in varietal susceptibility of cucurbits to *Rhizoctonia solani* in the greenhouse. Two isolates from flax and sweet clover were very pathogenic to seven cucurbits in the field and three isolates from sugar beets were not.

Ortega (51) studied six isolates of *Rhizoctonia solani* that differed culturally. They were isolated from sugar beets, tomatoes, peas, sweet clover, and flax. He studied their pathogenicity on tomatoes, cucumbers, beans, peas, and peppers. The cultures had different degrees of pathogenicity on the different hosts, again indicating physiologic specialization for the strains that he studied.

Chen (12) made a rather extensive study of the parasitism of *Rhizoctonia solani* on cereals and grasses. He investigated 24 isolates from potatoes, sugar beets, cotton, flax, and sweet clover, and studied their parasitism on Thatcher wheat, Wisconsin 38 barley, Anthony oats, Dakold rye, *Agropyron cristatum*, *Bromus inermis*, *Dactylis glomerata*, *Festuca elatior*, *Phleum pratense*, and *Poa pratensis*. As with other crops, there was pre-emergence and post-emergence damping off of the various Gramineae. The behavior of the isolates on these hosts was generally the same as other workers had found. Some isolates were strongly pathogenic, others weakly pathogenic, and others intermediate on the same or different hosts. Some isolates had a wider host range than others. Thus, one isolate may attack only two or three hosts, while another one may attack all of the hosts.

Tsiang (73) made a similar study with 13 isolates of *Rhizoctonia solani* on approximately 60 varieties, hybrids, and selections of flax. He concluded that isolates of *R. solani* from flax and other hosts vary greatly in their virulence on different flax varieties. One isolate was so virulent that it killed almost entire stands of all varieties tested. One isolate from sugar beets was extremely virulent on flax, much more so than some isolates from flax. He concluded that pathogenicity of strains of *R. solani* for flax varieties is not specialized, because one strain highly virulent on one variety of flax was usually highly virulent on all varieties of flax. These conclusions agree with those of Boosalis (7).

The Effects of Environment on Parasitism

It is obvious from the preceding studies that variation in pathogenicity of *Rhizoctonia solani* is extreme. Different isolates usually have different degrees of pathogenicity on the crop from which they were isolated or on different crops regardless of the host from which they were isolated. Some isolates have a wider host range than others. Pathogenicity, however, usually differs only in degree of virulence on a crop rather than being very specific for varieties of crops. Attempts to classify or separate physiologic races by using certain crop species as differential hosts have failed. About all that can be said then is that the species, *R. solani*, is comprised of an infinite number of pathogenic and cultural races. These races behave differently in different organic and meteorological environments. Some of the studies on the latter are reviewed in the following paragraphs.

Many observations and experiments have been made on the effect of soil temperature on the incidence and virulence of *Rhizoctonia solani*. Richards (56, 57) was probably one of the first to call attention to the effect of soil temperature on Rhizoctonia infection of potatoes, peas, beans, and cotton. He pointed out that the optimum temperature for infection of these crops was 18° C. and that this temperature corre-

lated closely with the optimum required for saprophytic activity of the fungus.

Vasudeva and Ashraf *(77)* concluded that cotton infected with *Rhizoctonia solani* suffered the highest degree of mortality at 35° or 39° C., and the severity of the disease was reduced as the temperature was reduced. Sanford *(62)* claimed that 16° to 20° C. was favorable for vegetative growth of the organism and depressed its virulence. Elmer *(23)* studied the incidence of *R. solani* for 13 years and concluded that incidence is always highest during the coolest seasons. Smith *(68)* reported that the optimum temperature for growth in culture and for infection of alfalfa with *R. solani* was between 24° and 30° C. He found few cankers in the field when temperatures were below 20° C. or above 35° C. and found few cankers either in the spring or fall. Leach *(34)* concluded that spinach, watermelon, and sugar beets suffered mostly from Rhizoctonia when the temperatures were less favorable for the host than for the pathogen. Tsiang *(73)* reported that temperatures between 15° and 20° C. were most conducive to the parasitism of Rhizoctonia isolates to flax, but soil temperature for infection was not correlated with optimum temperature for growth in culture. Boosalis *(7)* found that 25° to 29° C. was the optimum temperature for infection of soybeans with two races of *R. solani*.

These reports would lead one to believe that different crops require different optimum temperatures for infection with *Rhizoctonia solani*. This is partly true, but not entirely, since the optimum temperature for infection is also dependent on the particular parasitic strain with which one is dealing. Sanford *(61)* reported that virulence and rate of growth of different isolates varies with different temperatures. This was reaffirmed by Ortega *(51)*, working with different isolates of the parasite on several vegetable hosts.

Khan *(32)* probably has some of the best evidence to support those conclusions. He studied the relationship of soil temperature to the pathogenicity of three races of *Rhizoctonia solani* on several legume crops. The pathogenicity of two of the races differed markedly in response to temperature in temperature tank and field tests. One race caused greatest damage to peas at temperatures between 14° and 17° C. and those between 28° and 32° C., while it caused greatest damage to soybeans only at temperatures between 14° and 17° C. The latter is considerably lower than the optimum reported by Boosalis *(7)*, who found 25° to 29° C. optimum for infection of soybeans. A second race of Khan's, however, caused most damage to both soybeans and peas at temperatures between 21° and 28° C., and a third race also was most pathogenic to legumes at these temperatures in field tests.

These results serve to emphasize the interrelationship of host, strain of the pathogen, and temperature as they affect virulence or susceptibility, and point out that one cannot generalize too broadly regarding the influence of temperature on the pathogenicity of a fungus such as *Rhizoctonia solani*.

Generally, soil moisture does not appear to have as much influence on pathogenicity of *Rhizoctonia solani* as soil temperature. It appears, however, that soil that is neither too wet nor too dry is most conducive to infection. Sanford *(62)* reported that with potatoes sclerotial development seems to be stimulated when soil moisture is slightly above optimum for the host.

Soil nutrients greatly affect parasitism of *Rhizoctonia solani*. Much has been written about this with particular emphasis on the relation of soil nutrients to crop rotation and control. That phase is presented in the section on control, but at this point direct experimental results on soil nutrients in relation to parasitism will be discussed.

Van Beekom *(75)* reported that application of potash reduced the incidence of Rhizoctonia on potatoes on reclaimed land in south Holland. Elmer *(23)* stated that when vetch and cowpeas were grown as fall fertility crops to supply nutrients for potatoes, the incidence of *R. solani* was greater the following year. Sanford *(65)* claimed that nitrogenous salts, especially nitrate, and maize meal tended to reduce the disease and the persistence of the pathogen. Addition of sucrose, calcium hydroxide, magnesium sulfate, and sulfur to soil tended to favor both the disease and persistence of the pathogen on potatoes. He tentatively attributed the reduction of disease and decline of the pathogen to antibiotic effects of soil fungi and bacteria as modified by the various soil supplements.

Khan *(32)*, on the other hand, found direct evidence that absence of calcium and magnesium predisposes soybeans and peas to infection by *Rhizoctonia solani*. He grew these crops in washed quartz sand to which were added various nutrient solutions and the pathogen. With calcium- and magnesium-deficient nutrients the number of infected plants was almost double the number of those with normal nutrients.

Soil *p*H apparently has little effect on parasitism of *Rhizoctonia solani* except where *p*H is at such a level that it interferes with the availability of certain nutrients. Khan's results *(32)* indicated that *p*H itself had no great effect on parasitism, but that calcium- and magnesium-deficient soils had a low *p*H and the absence of these elements predisposed the plants to attack by *R. solani*.

Soil aeration is among the factors involved in parasitism of a soil-borne fungus such as *Rhizoctonia solani*. Blair *(6)* demonstrated that improved aeration of soil stimulated the growth of this fungus. Tsiang *(73)* made further tests and showed that root rot of flax was increased in proportion to the amount of oxygen supplied to a culture for a period up to 40 minutes. When O_2 was supplied for a longer time, the amount of root rot decreased.

Parasitism in Relation to Control

All of the preceding factors are important in the control of root rot and damping off caused by *Rhizoctonia solani*, since they are inseparable from crop rotation, soil amendments, and host range. Since the pathogen is soil-borne, much thought has been given to the use of crop rotation as a means of control. The pathogen has such a wide host range and is composed of many parasitic strains, however, and rotation would appear to be an inadequate means of control.

Braun *(8)* and Peltier *(52)* probably have two of the most extensive summaries of *R. solani* to date. Let us, however, examine some of the more recent literature regarding control of diseases caused by this fungus.

Buchholtz *(10)* reported a severe epidemic of root rot in sugar beets following potatoes, but a portion of the same field that had been in barley the previous year had scarcely any root rot. Goss and Afanasiev *(27)* reported the least infection of potatoes with a four- and six-year rotation when lucerne preceded the potato crop. A maize-potato rotation or continuous potatoes was least satisfactory.

Later Goss and Livingston *(28)* reported that *Rhizoctonia solani* increased rapidly when potatoes were planted consecutively for two years. Less than half as much *R. solani* was noted in plots not planted to potatoes consecutively. Schleusener *(66)* claimed that one of the best ways to combat *R. solani* on potatoes is to apply green manure by growing lupin in the rotation immediately preceding the potato crop. Elmer *(23)* claimed that the incidence of *R. solani* on potatoes was higher following a fall crop of potatoes, vetch, or cowpeas, when the latter two were sown as fertility crops. Afanasiev and Morris *(1)* reported that Rhizoctonia builds up rapidly in soil after succes-

sive potato crops, requiring two favorable years for maximum infection.

Maxon *(47)*, reporting on incidence of *Rhizoctonia solani* on beets, stated that there was an increase of the disease in fields with beets planted continuously, and likewise in fields where potatoes preceded beets. He claimed that maize and small grains minimized the losses, with no rot in beets following four years of grain. Coons, Stewart, and Kotila *(18)* agreed that beets following maize are freer from root rot than when they follow alfalfa, sweet clover, red clover, or crimson clover. Later Coons, Kotila, and Bockstahler *(17)* reported that "Black root" of sugar beet, a complex disease caused by *Pythium spp., Phoma betae, R. solani,* and *Aphanomyces cochliodes,* is increased following a sweet clover crop.

How do these observations fit in with host range and pathogenic variation in relation to hosts and varieties? The results of LeClerg, Person, Houston, Sanford, Sprague, Smith, Boosalis, Marchionatto, Storey, de Zeeuw, Ortega, Chen, Tsiang, and Khan show that many pathogenic races of the pathogen exist, that they are widespread, and that they have no particular specialization for individual host species.

There seems to be some hope, however. In general, legume and flax isolates seem to be more pathogenic to flax and legumes than other isolates. Isolates from sclerotia of potatoes seem to be less pathogenic to potatoes and sugar beets than isolates from sugar beets or potato stems. Isolates from cereals and grasses appear to be less pathogenic to legume and vegetable crops than isolates from those crops themselves. These results and observations have been confirmed, at least in part, by Gibler *(26)*, who reported that races from flax and legumes were most pathogenic to soybeans, while those from potatoes, sugar beets, and cereals were not pathogenic to soybeans. Thus, it appears that there is some scientific basis for rotations that have been recommended and that the method has virtue in controlling diseases caused by *Rhizoctonia solani.*

The writer wishes to emphasize the importance of proper soil amendments in farm practice to assist in the control of *Rhizoctonia solani.*

Antagonisms to *Rhizoctonia Solani* in Relation to Control

No soil-borne pathogen can be considered fully without consideration of antagonisms of other soil microflora. Since some of the investigators at the University of Minnesota have studied this phase of *Rhizoctonia solani,* with a view toward using this phenomenon for control, a review of their work is included.

In some of the experiments it was apparent that there was more root rot in sterilized soil than in nonsterilized soil that had been inoculated with *Rhizoctonia solani (62, 73).* This probably is due to the presence of an inhibitory organism in the nonsterilized soil. Various workers have demonstrated that several fungi and bacteria are antibiotic to *R. solani (2, 4, 30, 31, 64).* Sanford *(65)* proposed that the antibiotic effects of soil fungi are modified by soil amendments. Vasudeva *(76)* obtained evidence that the virulence of *R. solani* was enhanced by the presence of other fungi such as *Fusarium solani.*

Weindling *(79, 80, 81)* showed that *Trichoderma lignorum* inhibits the growth of *Rhizoctonia solani* and parasitizes it. Tsiang *(73)* made further experiments to determine what limited infection by *R. solani* in nonsterilized soil. His results agreed with those of Weindling that *Trichoderma lignorum* is not only antibiotic to *R. solani* but actually parasitizes it. In culture *R. solani* stopped growing when it came in contact with *T. lignorum* in the same culture. Hyphae of *T. lignorum* coiled tightly around the aerial mycelium of *R. solani,* and the hyphae of the parasite frequently were found inside the host hyphae. The parasite seemed to penetrate through the septa, although Tsiang observed internal hyphae linked with the hyphae coiling outside at the point of contact indicating direct penetration through hyphal walls. Tsiang commonly observed lysis of the host hyphae at a distance from the parasite. He grew *R. solani* in liquid media for three weeks and then added *T. lignorum.* After one week the mycelial mat of *R. solani* was completely disintegrated, leaving a turbid brown solution.

Tsiang *(73)* also showed that *Bacillus subtilis* was very toxic to *Rhizoctonia solani.* Autoclaving reduced the toxicity of the filtrate from cultures of *Trichoderma lignorum,* but did not significantly affect that from *B. subtilis.* In studying the effect of pH on antibiosis by *T. lignorum,* Tsiang concluded that the pH most favorable for the lethal effect on *R. solani* was most favorable for the growth of *T. lignorum.*

Weindling and Emerson *(82)* successfully isolated the toxic principle produced by *Trichoderma lignorum,* and Tsiang *(73)* was successful in duplicating their work. Tsiang also found that the extract in crystalline form was very lethal to *R. solani,* although it is significant that some of his races responded differently to the extract. One race failed to grow even at a dilution of 1:1,000,000, whereas two other races grew moderately well at the same dilution.

Tsiang *(73)* made experiments in the greenhouse to determine if *Trichoderma lignorum* and *Bacillus subtilis* would control damping off of flax. Soil was mixed with the two organisms separately. *Rhizoctonia solani* was added and flaxseeds were planted in the mixture. There was a general increase in stand in the soils treated with *T. lignorum* and *B. subtilis,* but it is significant that again there were different responses from different races of *R. solani.* Plants inoculated with one race were protected more by *T. lignorum* and *B. subtilis* than plants inoculated with another race, and stands of plants inoculated with a third race of *R. solani* were intermediate between the first two. Tsiang pointed out the possibilities of controlling soil-borne diseases by the use of antibiotic microflora.

Survival of Sclerotia in Relation to Control

Since *Rhizoctonia solani* does not form spores commonly, sclerotia are important in the survival and dissemination of the pathogen. It is apparent then that any factors that influence the survival of sclerotia may be directly or indirectly implicated in the control of diseases caused by this organism. Tsiang *(73)*, therefore, investigated some of the factors that influence the survival of sclerotia.

He studied the effects of temperature, moisture, chemical treatments, and radiation on the longevity of sclerotia from 47 isolates of *Rhizoctonia solani.* Sclerotia were subjected to temperatures ranging from 15° to 70° C. for 35 days. Then they were removed and placed on potato-dextrose agar in Petri dishes. In this test low temperature had no appreciable effect on their viability, but high temperature did. Generally, growth was retarded when they were stored at 50° C., or above. After storage at 70° C. for 35 days, none of them grew except four which produced only a trace of mycelium. After storage at 65° C. there were marked differences in the growth of different races. Tsiang *(73)* found that the critical temperature for growth of sclerotia from all races but four of the 47 isolates was 68° C. The remaining four grew only slightly at that temperature but were killed at 71° C.

From studies of the effect of moisture on the survival of sclerotia, Tsiang *(73)* also found differences among sclerotia from different races. At 60° C. and 95 per cent moisture all sclerotia were

killed in 14 days, but at the same temperature and 50 or 75 per cent moisture four isolates survived. At 60° C. for seven days at 50, 75, and 95 per cent moisture, three of the isolates were killed, but seven others survived. At 40° C. and 50, 75, and 95 per cent moisture all of the isolates were alive at 21 days, the duration of test. In general, survival was better at lower temperature and moisture levels than at higher temperature and moisture levels.

There is much literature on chemical treatment of potato seed pieces for the control of *Rhizoctonia solani*. Presumably, the chemicals kill the sclerotia on the seed pieces, but more knowledge of the effect of different chemicals on longevity of sclerotia is still needed.

Tsiang *(73)* tested the effects of nine chemicals on sclerotia from 40 races of *R. solani*. The treatments and the data relative to the sclerotia of 16 of the cultures are given in table 6. In the writer's opinion these results are very significant. It will be noted that there were great differences in the ability of sclerotia of different isolates to survive the treatments. Sclerotia of some isolates were very sensitive to all treatments while some of them survived all of the treatments. There was a great variation in the response of the remaining sclerotia, some surviving certain treatments and being very sensitive to other treatments.

What seems even more significant is that Tsiang *(73)* pointed out that the characters of cultures from treated sclerotia were frequently different from those of the parent culture, and that mutants frequently arose in cultures resulting from chemically treated sclerotia as reported by Chen *(12)*.

These results serve notice that chemical treatment to kill sclerotia cannot be taken for granted. There is the warning that improper chemical treatment may be worse than none, because cultures resulting from improperly treated sclerotia might mutate to produce more virulent races than the original.

Tsiang *(73)* also attempted to determine the effect of radiation on longevity of sclerotia. He found that ultraviolet light had little effect even after 144-hour exposure. After exposure to X-ray for 240 minutes, however, all of the sclerotia took twice as long to germinate, and after 270 minutes irradiation, practically all of them failed to germinate. There were no apparent differences among the 40 isolates tested.

Pathological Histology

Matsumoto *(44)* stated, "The penetration of cuticle, as well as cell wall by Rhizoctonia might not be brought about by mechanical pressure alone, but at the same time be assisted by the enzymes or other related substances liberated from the invading hyphae." The basic principles of this have not changed since that time, but more recent literature on penetration and the toxins associated with penetration will be reviewed here.

Ullstrup *(74)* reported that an aster strain and a sugar-beet strain formed "infection cushions" on the surface of leaves of asters, beets, and begonia and then penetrated directly. Other strains could penetrate only through stomata. Nakayama *(49)* stated that hyphae proceed along the depressions of the epidermis of roots, hypocotyl, and cotyledons. The root tip is very susceptible and Rhizoctonia will penetrate the epidermis, then branch and grow intercellularly and intracellularly. There is also penetration through injuries and natural wounds. The formation of "infection cushions" is the principal means for Rhizoctonia to penetrate the hypocotyl, whereas cuticle apertures and stomata are the principal avenues of entrance into cotyledons.

Cherewick *(13)*, studying Rhizoctonia root rot of sweet clover, did not observe direct penetration. He claims that the most common procedure is as follows: There is an aggregation of my-

Table 6. The Growth of Colonies, in Millimeters, of *Rhizoctonia solani* from Sclerotia Treated with Different Chemicals*

Isolates of Rhizoctonia	Chemicals, concentrations, and duration of treatment										Time for check to reach full growth
	70 per cent alcohol 1:2	Copper sulfate 1:250	Formalin 1:250	Potassium dichromate 1:260	Mercuric chloride 1:2000	New Improved Ceresan 1:1000	Sodium hypochlorite 1:100	Sodium arsenate 1:1000	Gentian violet 1:100,000	
	30 minutes	120 minutes	120 minutes	120 minutes	120 minutes	120 minutes	120 minutes	120 minutes	120 minutes	Days
1	0	22	0	0	0	0	0	0	0	6
2	0	58	41	34	0	0	32	0	20	7
3	0	34	49	45	5	0	35	0	18	6
4	0	15	54	59	48	0	T	0	T	6
5	0	3	0	T†	T	0	0	0	0	5
6	0	0	0	T	0	0	0	0	0	5
7	0	20	0	T	0	0	0	0	0	5
8	0	0	T	T	0	0	0	0	0	5
9	0	32	34	48	32	T	T	0	T	6
10	0	0	0	0	0	0	0	0	0	5
11	0	18	18	28	19	0	T	0	0	5
12	0	50	63	62	47	T	T	0	T	7
13	0	43	T	50	T	0	T	0	T	6
14	0	42	60	52	T	0	8	0	T	6
15	0	0	T	T	0	0	0	0	T	5
16	20	60	41	52	17	10	15	T	T	7

* Table from Ph.D. thesis by Chen-Ling Tsiang *(73)*.
† T = trace.

celium (pseudo-sclerenchymatous sclerotium) appressed against the epidermis of the root. This kills underlying cells. Wedge-like projections invade the dead tissue by schizogenous action, and from these wedges normal hyphae arise and ramify throughout the cortical tissue both intercellulary and intracellularly. Tsiang *(73)* reported essentially the same thing with flax. Chen *(12)* reported direct penetration of epidermal cells and root hairs of cereals and grasses by *R. solani.*

Boosalis *(7)* studied the penetration of soybean roots by *Rhizoctonia solani.* He reported that there appeared to be a definite dark brown discoloration of cell walls and often of cell protoplasm before hyphae penetrated (figure 5). The discolored area extended 2 to 10 cell layers beyond the hyphae and was frequently observed in the sections of necrotic lesions on the cracked side of a taproot. Boosalis concluded that the natural cracks in soybean roots can be an excellent avenue of entrance, and that discoloration occurred before the host actually was penetrated. In other words certain isolates of *R. solani* can cause necrosis of host tissues without actually contacting them.

Boosalis *(7)* studied the effects of filtrates from cultures of *Rhizoctonia solani* and found that they prevented root formation of seedlings and actually caused discoloration of root tissue in the absence of mycelium of the pathogen. He also showed that the activity of filtrates from different races was different. For example, seed germination and root development were inhibited when treated with the filtrates of a strongly pathogenic race, but filtrates from a weakly pathogenic race had no effect on seedlings of Minsoy soybeans.

These results, however, are not completely new. Ramsey *(55)* reported that in infected potato tubers host cells die, lose their contents, and cell walls suberize and break down several cells in advance of the fungus filaments.

Newton and Mayers *(50)* stated that a heat-stable toxin is liberated during growth and is present in the mycelium of *Rhizoctonia solani.* The heat-sterilized filtrates of old cultures were more toxic to carrots and turnips than to wheat seedlings. Hot-water extracts of washed, dried, and ground mycelium were toxic to turnips but not to wheat.

Vasudeva and Sikka *(78)* found that filtrates of *R. solani* caused wilting of cotton plants in 60 minutes. The writer observed a similar phenomenon when alfalfa cuttings were inoculated with a heavy dosage of *R. solani.* In this case cuttings wilted completely in 24 hours, too short a time for complete infection by the pathogen. Ortega *(51)* found one culture whose toxic by-products wilted tomatoes and retarded germination of tomato, cucumber, pea, and bean seed. Ortega found that the toxic properties of the culture filtrates were partially adsorbed by charcoal and were reduced by dilution with water. Tsiang *(73)* reported that two of his cultures produced a toxic substance that wilted flax seedlings, the toxin from one being approximately twice as potent as the toxin from the other.

A recent contribution to the parasitism, penetration, and infection of plants by *Rhizoctonia solani* is that of Khan *(32).* He studied the penetration of *R. solani* into peas and soybeans in the hope of obtaining some indications of the nature of resistance, and he made microchemical tests and histological studies of host tissue from plants grown with calcium and magnesium deficiencies to determine relationships of these with infection.

In normal plants Khan *(32)* found that entrance through the epidermis was accomplished by a peglike structure which penetrated directly. He observed both intercellular and intracellular mycelium even in the innermost cortical tissue and noted that root hairs were destroyed by the pathogen.

Khan *(32)* made histological studies of healthy and diseased tissue and found that the middle lamella of diseased tissue was absent. This indicated that the pathogen has the ability to digest pectin. He proved in subsequent laboratory tests with the fungus grown on media containing pectin that this was true. His tests also proved that *R. solani* digested cellulose when it was grown on media containing cellulose as the only source of carbon.

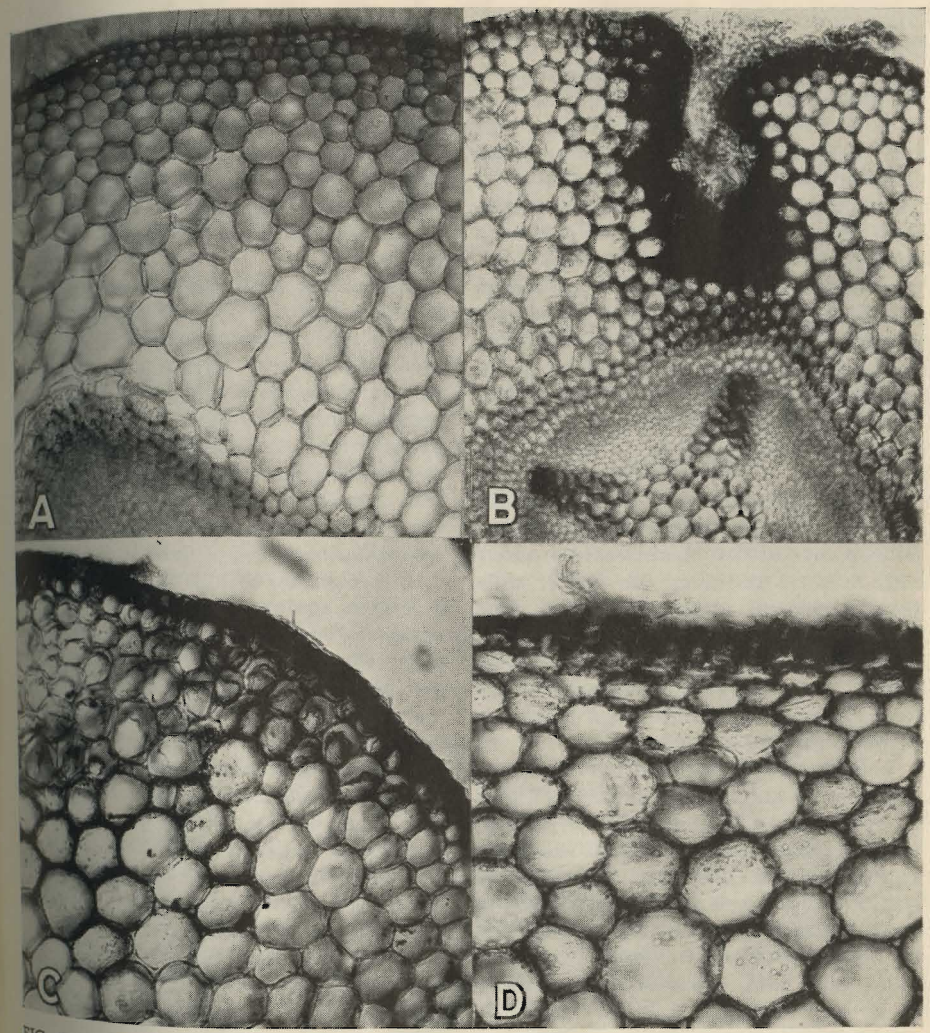

FIG. 5. Freehand, unstained cross sections of soybean tap roots: A. healthy tap root; B. cracked tap root infected; C. tap root infected; D. tap root naturally infected with *Rhizoctonia solani* in the field. Photo courtesy of M. G. Boosalis *(8).*

Khan *(32)* had previously shown that deficiencies of calcium and magnesium predisposed soybeans to attack by *Rhizoctonia solani.* Deficiencies of calcium in nutrient media had no appreciable effect on the pathogen so Khan hypothesized that the effect must be mostly on the host. Histological studies of calcium- and magnesium-deficient plants demonstrated that they have

large intercellular spaces with poor delimitation of cell walls in the cortex.

Thus, if the organism can digest pectin and cellulose and if cortical tissue is abnormally developed in calcium- and magnesium-deficient plants, one can readily see the relationship between host, parasite, nutrition of both, and the interrelationships between all of the factors. Practically, these results suggest a reason for patches of infected plants in fields where areas lack calcium or magnesium or these nutrients are tied up, and also suggest that these nutrients be applied to plants either through soil amendments or spraying.

Discussion

From the results of the various workers it is obvious that the species, *Rhizoctonia solani*, comprises an indefinite number of races that differ culturally as well as pathogenically. The organism has one of the widest host ranges of any plant pathogen known. Some races have an extremely wide host range, while others appear to have rather limited host range.

In culture the races differ in color; zonation of colony; type of growth, that is, suppressed or aerial mycelium; number, size, and position of sclerotia; aggregation of sclerotia; diameter of hyphae; and rate of growth. All of these characters appear to be influenced by environment. For example, two cultures may look alike on one medium or at one temperature, but if either the medium or temperature is changed, the two cultures may appear to be entirely different from each other.

Certain investigations *(29)* indicate that cultural characters have a tendency to be correlated with pathogenicity. Some workers studied many isolates from different hosts to determine if races pathogenic on a given host have cultural characters in common *(7, 12, 54, 73)*, but they report that there is as much difference between cultures from the same host as between cultures from different hosts.

Attempts to identify physiologic races by using different crop plants as differential hosts showed some promise *(38)*, but it is the consensus among most investigators that little can be done to identify races until the sexual stage of the organism can be reproduced readily and the genetic nature of races of the organisms is thoroughly understood.

The origin and stability of races of *Rhizoctonia solani* is an important practical as well as scientific question. Studies by Exner and Chilton *(24)*, Sanford *(60)*, and Kotila *(33)* indicate that segregation in the formation of the basidiospores may account for some of the variation. Chen *(12)* showed that variation can be induced in cultures of isolates from natural habitats by proper manipulation of the environment.

Although sectoring is rare in this organism under ordinary cultural conditions, Chen was able to stimulate considerable sectoring in certain races. After subjecting the artificially induced variants to many different environmental conditions, it was obvious that the induced variants differed in the same magnitude, in every respect, as did races isolated from nature. Chen concluded that the variants arose from mutation in a manner similar to that in other fungi. It has been suggested that aversion between cultures of Rhizoctonia could be used as a means of identifying species *(45)*. It is interesting to note that Chen *(12)* found as much aversion between a variant and its parent as occurs between cultures isolated from nature.

The diameter of hyphae of Rhizoctonia is another character used for separating species. Chen *(12)* reported that diameter of hyphae of races varied from 5.17 microns to 10.23 microns. He found a similar range of hyphal diameter of induced variants, and in two instances races gave rise to mutants whose hyphae were half the diameter of the hyphae of their respective parents. These results appear to invalidate the use of cultural aversion as a means of separating species and to throw doubt on the value of hyphal diameter as a character for separating species.

As stated previously, there are innumerable pathogenic races of *Rhizoctonia solani* with extremely wide host ranges. This is significant from the standpoint of control, for presumably it would be useless to practice any type of rotation or sanitation program when all crops are hosts of the pathogen. There are, however, indications that certain races appear to be more specific for certain crops than others. Some results indicate that isolates from flax and legumes are more pathogenic to these crops than isolates from cereals and grasses. Isolates from sclerotia of potato tubers seem to be less pathogenic to potatoes and sugar beets than isolates from sugar beets or potato stems. If these observations are sound, more work may lead to the practical use of crop rotations as one means of controlling diseases caused by *R. solani*.

From the standpoint of control, other factors have been considered. One of the most important means of survival of the pathogen is by sclerotia. Potato seed treatment is practiced to control *Rhizoctonia solani*, presumably by killing the sclerotia on the tubers. Chen *(12)* found a wide variation in size, structure, and aggregation of sclerotia, and Tsiang *(73)* investigated some of the factors that influence the survival of sclerotia. Many factors influence the latter, but the effects of chemicals on sclerotia are particularly significant. Sclerotia from different races respond quite differently to the chemicals, some being killed by all, some surviving all, and others either being killed or surviving different treatments. But of greater significance is the fact that cultures arising from some chemically treated sclerotia gave rise to a considerable number of variants. These results show that improper chemical treatment not only may fail to control *R. solani*, but also may induce new strains that might conceivably be more pathogenic than the original.

The antagonisms of other organisms to *R. solani* is another factor in the control of diseases caused by *R. solani*. Although considerable work has been done on this, nothing fundamentally new has been added since the work of Weindling *(79, 80, 81)* and Weindling and Emerson *(82)*. It is apparent that antagonism of different organisms to *R. solani* is influenced by environment such as cropping sequence, soil amendments, or use of green manure. More information may lead to an intelligent use of the antagonism phenomenon in the control of diseases caused by this pathogen.

Several investigations have been made on the methods by which *Rhizoctonia solani* gains entrance to its hosts and ramifies through the host tissue. Most workers agree that the invading hyphae penetrate by pressure and are assisted by the action of enzymes or other chemicals the hyphae liberate. Some minor variations of this process are reported, such as the formation of an "infection cushion" or an aggregation of mycelium appressed against the epidermis from which peglike invasion hyphae proceed. Generally, however, the workers agree on the method of penetration. Once the hyphae enter they grow both intercellularly and intracellularly throughout the invaded tissue.

Significantly, however, there is considerable deterioration of host tissue well ahead of the parasitic hyphae *(7, 55)*. The tissue may have all the characteristics of parasitized tissue even in the absence of hyphae when filtrates from some cultures are applied to tissues of certain plants *(7)*. The parasitized tissue is characterized by dark discoloration of cell walls and cell protoplasm as much as 8 to 10 cell layers ahead of the invading hyphae.

The toxic principle involved is produced by the hyphae, since filtrates of

certain cultures prevent the germination of seed, prevent root formation, and cause wilting of certain plants *(7, 50, 51, 73, 78)*. The toxic principle is thermostable and is partially adsorbed by charcoal. It is significant that the toxicity of filtrates of different races of the organisms are different in degree of activity.

Apparently, host resistance, host nutrition, fungus toxins, and possibly fungus nutrition are all interrelated. Khan *(32)* demonstrated that soybeans grown in calcium- and magnesium-deficient soils were predisposed to infection by *Rhizoctonia solani*. He also showed that cortical tissues of these plants were deficient in calcium and magnesium, and that the cells of that tissue were improperly laid down with very thin cell walls and large intercellular spaces. He also reported that the organism digests cellulose and pectin, important complements of cell walls. Invaded tissue was characterized by dissolution of the middle lamella. Thus, there is the phenomenon of deficiencies of calcium and magnesium with resultant improper formation of cortical tissue. The fungus, being able to dissolve cellulose and pectin, could penetrate cell walls directly. These results suggest the possibility of aiding the control of diseases caused by *R. solani* by proper amendment of soil with calcium or magnesium.

The things that are known about *R. solani* from work at Minnesota and at other places have been discussed. The following are some of the things that are not known about the organism or diseases caused by it:

(1). How can the perfect stage be produced at will and what part does it play in the genetic variation of the fungus?

(2). What is the nuclear life cycle of the fungus?

(3). What are the host range and specificity of races for host species and varieties?

(4). How do chemicals affect the survival of sclerotia in soil?

(5). What factors are involved in utilizing antibiosis as a means of control?

(6). What is the nature of the toxins produced by *R. solani*?

(7). What is the place of soil amendments in the control of diseases caused by *R. solani* and how do they influence host tissue formation?

(8). What levels of calcium and magnesium are necessary for normal growth of hosts and for prevention of disease?

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