The effects of soil temperature and moisture and certain biological factors on the pathogenicity of Rhizoctonia Solani Kuhn to sugar beet seedlings
by Harry S Fenwick

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science in Botany
Montana State University

Abstract:
The effect of temperature and moisture and of certain biological factors on the pathogenicity of several isolates of Rhizoctonia solani to sugar beet seedlings tbs studied.

Pathogenicity of six isolates of Rhizoctonia solani obtained from seedling and matured sugar beets was established on sugar beet seedlings in the greenhouse. Four of the six isolates were selected for further study on the basis of their virulence and total per cent disease produced on the beets. Two of the isolates were obtained from beet seedlings, the other two were obtained from matured beets.

The minimum temperature for growth of these isolates in culture was, found to be near 10° C. The optimum growth for these cultures was at 29° C, and the maximum was near 40° C.

Rhizoctonia disease was produced at both temperatures used (15° and 25° C) and at all moisture levels (55, 70, and 85 per cent of the total moisture holding capacity). More disease occurred at 25° C than at 15° C. Moisture level of the soil appeared to have less effect on the incidence of the disease than did temperature. The total per cent disease was greater with isolates obtained from seedling beets, also the average weight per plant and the average length of tops were smaller with these isolates than with isolates from matured beets.

A test of the effect of inoculum of varying ages on beet seedlings In different stages of growth revealed that beets are subject to attack by Rhizoctonia at any stage of growth and that the age of the beet is more important than the age of the inoculum in total incidence of disease.

Antagonistic action was exhibited by Trichoderma lignorum toward Rhizoctonia in soil and culture tests.

Penicillium notatum did not appear to have any effect on Rhizoctonia in soil or culture tests.

Streptomyces griseus appeared to increase the pathogenicity of Rhizoctonia in soil tests, but no conclusive results could be obtained in culture tests.

THE EFFECTS OF SOIL TEMPERATURE AND MOISTURE AND CERTAIN BIOLOGICAL FACTORS ON THE PATHOGENICITY OF RHIZOCTONIA SOLANI KÜHN TO SUGAR BEET SEEDLINGS

by

HARRY S. FENWICK

A THESIS

Submitted to the Graduate Faculty

in

partial fulfillment of the requirements

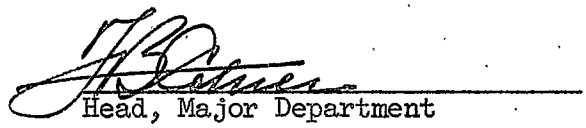
for the degree of

Master of Science in Botany

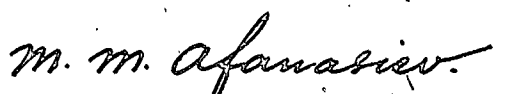
at

Montana State College

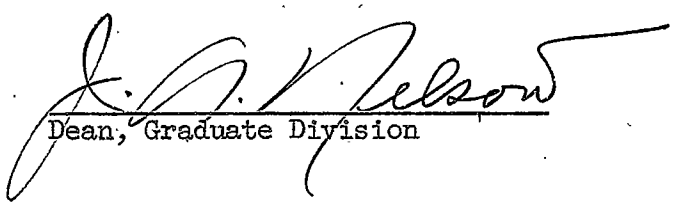
Approved:



Head, Major Department



Chairman, Examining Committee



Dean, Graduate Division

Bozeman, Montana
August, 1952

ACKNOWLEDGMENT

Grateful acknowledgment is made to Dr. M. M. Afanasiev, who directed this study and was most helpful in all of its phases. The assistance of Professor H. E. Morris and other staff members of the Department of Botany and Bacteriology is also greatly appreciated.

TABLE OF CONTENTS

ABSTRACT

The effect of temperature and moisture and of certain biological factors on the pathogenicity of several isolates of Rhizoctonia solani to sugar beet seedlings was studied.

Pathogenicity of six isolates of Rhizoctonia solani obtained from seedling and matured sugar beets was established on sugar beet seedlings in the greenhouse. Four of the six isolates were selected for further study on the basis of their virulence and total per cent disease produced on the beets. Two of the isolates were obtained from beet seedlings, the other two were obtained from matured beets.

The minimum temperature for growth of these isolates in culture was found to be near 10° C. The optimum growth for these cultures was at 29° C, and the maximum was near 40° C.

Rhizoctonia disease was produced at both temperatures used (15° and 25° C) and at all moisture levels (55, 70, and 85 per cent of the total moisture holding capacity). More disease occurred at 25° C than at 15° C. Moisture level of the soil appeared to have less effect on the incidence of the disease than did temperature. The total per cent disease was greater with isolates obtained from seedling beets, also the average weight per plant and the average length of tops were smaller with these isolates than with isolates from matured beets.

A test of the effect of inoculum of varying ages on beet seedlings in different stages of growth revealed that beets are subject to attack by Rhizoctonia at any stage of growth and that the age of the beet is more important than the age of the inoculum in total incidence of disease.

Antagonistic action was exhibited by Trichoderma lignorum toward Rhizoctonia in soil and culture tests.

Penicillium notatum did not appear to have any effect on Rhizoctonia in soil or culture tests.

Streptomyces griseus appeared to increase the pathogenicity of Rhizoctonia in soil tests, but no conclusive results could be obtained in culture tests.

THE EFFECTS OF SOIL TEMPERATURE AND MOISTURE AND CERTAIN BIOLOGICAL FACTORS ON THE PATHOGENICITY OF RHIZOCTONIA SOLANI KÜHN TO SUGAR BEET SEEDLINGS

INTRODUCTION

One of the most serious and troublesome diseases of sugar beets (Beta vulgaris L.) is the so-called root rot due to the fungus Rhizoctonia solani Kühn (Pellicularia filamentosa (Pat.) Rogers). This fungus is responsible for damping off of seedings as well as decay and death of older sugar beets. In the heavy soils typical of many of the sugar beet growing areas of Montana, the occurrence of Rhizoctonia disease varies from a trace to severe, resulting in reduced stands and many beets of poor market value.

Coons and Stewart (5) showed that the color of the young sugar beet leaves gives the first indication of Rhizoctonia disease on seedlings. The leaves may be merely deeper green in color, but occasionally they are blue green. Associated with this sign, one often finds a lemon-yellow color of the stem. Later, the point of attack appears to be at the ground level or just below the soil surface. Other points of attack are at the base of the leaf petioles or on the main taproot. On the stem it produces dry local lesions that extend upward and downward along it and often completely girdle the stem, killing the beet. Often infected seedlings appear stunted and, in general, show evidence of malnutrition. On removal of such a seedling from the soil, the taproot is found decayed at the lower end, but the rootlets have developed above the decayed region giving the appearance that the primary root is being replaced by them.

Sometimes the plants may appear normal and healthy up to the time of thinning, but when these beets are dug and examined, small black lesions may be present near the end of the main taproot. These lesions may give rise to the symptoms found on beets half grown or older.

The first visible evidence of the disease on older beets is a slight wilting of the plant, which appears to recover temporarily in the cool part of the day. This sign is closely followed by a darkening of the bases of the petioles and by the rotting of the crown. The leaves may retain their color for some time, or until the leaf petioles rot off completely. With the rotting of the crown, the leaves turn yellow, begin to wilt, and the plant dies. Upon closer examination, the root may be found to be rotted off at the basal end and large cracks may extend half way through the root. Often the entire root may appear to be rotted, but when cut radially, there may be a small portion of firm, healthy-appearing tissue within. A beet may be badly infected and show little or no symptoms above ground, and when conditions become unfavorable to the fungus, the rotting and cracking slow down and the plant usually survives until the harvest.

A considerable amount of root rot of sugar beets occurred in 1948 and 1949 in several one-quarter acre plots in Field O-II at the Huntley Branch Station, Huntley, Montana. In 1949 more than 90 per cent of all the sugar beets in these plots were dead or diseased at harvest time. Successive crops of sugar beets were grown in these plots during the years 1950, 1951, and 1952. The amount of disease has subsequently decreased and varied each year. Epidemics of Rhizoctonia disease such as the one

in 1949 are apparently the result of a combination of factors, such as the presence of a virulent strain of the fungus, a susceptible variety of plant, and optimum temperature, moisture, and other conditions favorable for infection and development of the disease.

The present study was undertaken to investigate the effect of several environmental and biological factors on the pathogenicity of Rhizoctonia cultures to sugar beets, and to attempt to explain the occurrence and variation in this disease at the Huntley Station.

REVIEW OF LITERATURE

Seedling disease of sugar beets may be caused by several fungi. Those fungi most prevalent as seedling pathogens are of the genera Rhizoctonia, Pythium, Aphanomyces, and Phoma. Afanasiev (1) isolated these four genera as well as Fusarium and Macrosporium from diseased seedlings. Leach (14) reported that the three most common fungi to cause damping off of sugar beets in California were Rhizoctonia solani, Pythium ultimum Trow, and Phoma betae Fr. The first two are common in field soils, while Phoma appears to originate only from imported seed. Tilford and Young (31) stated that Aphanomyces cochlioides Drechs is responsible for the major part of losses in Ohio from seedling diseases, but that R. solani, although less common, may cause severe damage. Edson (8) reported the distribution of the fungus Rhizoctonia to be very general, but under field conditions, damping off of sugar beets due to Rhizoctonia was far more general in the soils of the semi-arid West. He stated that the fungus was a cause of a very destructive crown rot in the

West, where it frequently became epidemic. It was not uncommon to see entire fields of 50 or 100 acres practically destroyed in August by root rot of which there was no evidence earlier in the season. This form of rot is seen only occasionally in the more eastern beet growing districts where it appears to be of less economic importance.

The actual control of Rhizoctonia disease is a problem of great difficulty, since the sugar beet is susceptible to the ravages of Rhizoctonia at any stage of growth and because of the high degree of virulence of the fungus under various environmental conditions.

Sugar beets, as a rule, are grown in the West in irrigated areas where a crop rotation is practiced. The extremely wide host range of Rhizoctonia enables the fungus to live from year to year in these fields on many of the crops used in the rotation system. Peltier (22) in 1916 reported about 165 species of plants were subject to attacks by Rhizoctonia. Included in these species were all the more important families of dicotyledons as well as a number of monocotyledons, and several gymnosperms. He reported that most of the floricultural plants, vegetable and field crops, herbaceous plants, and many weeds were susceptible to attacks of Rhizoctonia. In addition to its occurrence on many different hosts, Rhizoctonia also forms specialized races or strains, some of which differ morphologically as studied by Matsumoto (19) and LeClerg (15). They have shown that there may be a slight or occasionally somewhat more pronounced morphologic difference between isolates. More recently, Exner and Chilton (9) found as many as 29 distinct cultural types differing in

rate of growth, color, size, and position of sclerotia occurring among isolates from a single basidial mat.

The criteria most often employed in strain or racial differentiation have been differences in pathogenicity or growth form in artificial culture. Investigations on racial specialization have been undertaken by several workers including Duggar (7), LeClerg (16, 17, 18), Sanford (27, 28), Storey (30), and more recently, Houston (12). LeClerg (15) tested 116 isolates obtained chiefly from sclerotia formed on potato tubers and from the lesions on stems of older plants. Among these isolates he did not find any that were pathogenic to half-grown or mature sugar beets. On the other hand, Rhizoctonia isolates obtained from sugar beets were pathogenic on potatoes. However, he later found that some potato isolates were pathogenic to sugar beets. Storey (30) reported that some strains had a wide host range, whereas others exhibited a more selective parasitism. Buchholtz (4) reported a severe case of Rhizoctonia root rot of sugar beets on land previously planted to potatoes. Adjoining land that had been previously planted to barley showed very little root rot. On the potato ground, 50 per cent of the stand of beets was rotted; only 1.6 per cent was rotted on the barley ground. Houston (12) made additional studies on 260 isolates of Rhizoctonia from 15 different crop plants. He classified the isolates into cultural types. Differences in growth habit on nutrient media such as presence or absence of stroma, nature, size, and abundance of sclerotia and absence or presence of substances that darken the medium were criteria for differentiation. Although intergrades

occurred, he stated that most isolates could readily be assigned to their appropriate types. Type A was obtained from many hosts and was a polyphagus type, whereas type B and C were highly specific as to hosts. Type C was essentially non-pathogenic to sugar beet roots, and Type B was, from a practical standpoint, non-pathogenic on sugar beet seedlings but was highly pathogenic on older beet roots. Kotila (13) reported finding a strain of Rhizoctonia that attacked only the foliage of sugar beets and did not cause any damage to the older roots but was capable of causing both pre-emergence and post-emergence damping off of sugar beet seedlings, often killing 100 per cent. In addition, the strain was pathogenic to beans, alfalfa, bromegrass, and potato.

The observation that Rhizoctonia disease develops best in wet soil and under relatively wet conditions has been recorded by several investigators. Harter (11) reported Rhizoctonia disease of sweet potatoes to be more prevalent in beds that had been too frequently watered. Roth and Riker (27), while working with damping off of red pine seedlings in the greenhouse, found soil moistures somewhat less than 70 per cent of the soil moisture holding capacity were favorable to Rhizoctonia and those above 70 per cent were favorable to Pythium. Alexander et al. (2) reported dry soil was found to be unfavorable to the Rhizoctonia damping off disease of tomato seedlings. Morris and Afanasiev (21) reported the disease to be most prevalent in Montana in heavy, wet soils rich in organic matter.

Numerous investigations show that the development of certain soil-

borne diseases of plants also depends on a specific range of soil temperatures, which in combination with the other soil factors, creates favorable conditions for the development of disease. Richards (24) found that Rhizoctonia was most virulent to potatoes at soil temperatures of 15° to 21° C. He also concluded that the greatest damage to peas was between 12° and 27° C with the maximum injury near 18° C, and 15° to 18° C was the optimum temperature for maximum injury to beans. On the other hand, Peltier (22) found that high temperature (30°) together with too little or too much moisture determined to a large degree the virulence of various strains of Rhizoctonia. LeClerg (15) reported soil temperatures of 25° to 33° to be the most favorable for development of Rhizoctonia root rot of sugar beets.

Definite experimental evidence on the combined effects of soil temperature and soil moisture on parasitism of Rhizoctonia is apparently very meagre. Sanford (29) studied the effects of soil temperatures between 16° C and 25° C and of soil moisture content between 19 and 40 per cent of the moisture holding capacity on the virulence and type of attack of Rhizoctonia on young potato sprouts. He found the pathogen to be equally virulent throughout the range of soil moisture between the temperatures of 16° and 23° C, but he was unable to obtain any conclusive results that the virulence of Rhizoctonia was greater at 16° C than at 23° C, or that a dry soil was more or less favorable for the development of Rhizoctonia disease than a wet soil. Separately conducted temperature and moisture studies were undertaken on the pathogenicity of Rhizoctonia

on beans by Person (23). He tested the pathogenicity of Rhizoctonia at temperatures of 15° to 28° C, and at moisture levels of 40, 60, and 80 per cent, he studied the effect of soil moisture on the emergence of bean seedlings. His results showed that Rhizoctonia was pathogenic on beans from 15° to 28° C, and that it was most pathogenic at the lower temperature. In the moisture studies, the stands were reduced about equally at all three soil moisture levels, but the average degree of infection was more severe at 60 and 80 per cent soil moisture. Unfortunately, neither of the above named authors stated the total moisture holding capacity of the soil with which they worked. As far as it is known, no study has been made on the combined effects of temperature and moisture on the pathogenicity of Rhizoctonia isolates on sugar beet seedlings.

In recent years, considerable evidence has been accumulated to bring out the importance of antagonism among microorganisms. Weindling (34) in 1932 observed Trichoderma lignorum (Tode) Harz. coiling around the hyphae of Rhizoctonia, destroying the colonies, and suggested that T. lignorum might be used for the biological control of Rhizoctonia and other fungus diseases. Later he reported various fungi grown on culture media with Rhizoctonia attacked the latter in a manner resembling the parasitic action of Trichoderma. Gliocladium fimbriatum G and A was also reported (34) capable of attacking and destroying the hyphae of Rhizoctonia and other fungi when grown in conjunction on nutrient media. A Streptomycete antagonistic to a Pythium root parasite of sugar cane was reported by Tims (32). His tests showed the Streptomycete

produced a toxic principle lethal to the Pythium rather than exhaustion of the nutrients in the medium which would starve the Pythium. Diachun (6) reported Penicillium notatum Westl. offered some protection against infection by Penicillium oxalicum Currie and Thom.

MATERIALS AND METHODS

A mixture of sugar beet seed of U.S. 22 and 268 of the Great Western Sugar Company was used throughout the course of the experiments. This seed was treated with New Improved Ceresan on a basis of one ounce per 20 pounds of seed, or 0.31 grams per 100 grams of seed.

The soil used in all the experiments was obtained from Field O-II, plots 1 to 3, at the Huntley Branch Station, Huntley, Montana, and was naturally infested with Rhizoctonia. This soil is classified as a heavy clay soil, characteristic of much of the irrigated land planted to sugar beets in this area. Prior to any experiment, the soil was sifted through a No. 16 mesh screen and air dried. All soil disinfecting was done in an autoclave (15 pounds pressure for 3 hours).

In experiments carried out under ordinary environmental conditions in the greenhouse, 6-inch clay pots filled with the above mentioned soil were employed. In the main tests in which the effect of soil moisture and temperature was studied on the development of Rhizoctonia disease of sugar beets, the plants were grown in soil under controlled conditions in the control cage, in metal flats 9 inches by seven and three-eighths inches by four and one-half inches, and stone jars 5 inches in diameter and three and three-eighths inches high.

The control cage was a simply constructed chamber enclosed on three sides and bottom, and with a swinging door on the fourth side. Double glass constituted the roof of the chamber. Above the glass, 12 fluorescent tubes were installed. Within the chamber, 14 coils were fastened to the three enclosed walls for uniform refrigeration. A thermostat was installed within the chamber for constant temperature control. A refrigeration unit was installed on the floor beneath the entire chamber.

For the determination of the total moisture holding capacity (TMHC) of Huntley soil, a method described by Goss (10) was employed. Three metal tubes 25 cm long and 5 cm wide were filled with the air-dried soil and tapped 10 times from a height of 3 inches. The tubes of soil were placed in water for 6 hours until the soil was thoroughly saturated, then drained for 3 hours and weighed. The tubes of soil were then placed in a drying oven for three days at a constant temperature of 105° C. Simultaneously, 2 small soil cans containing air-dried soil, both of which had been weighed, were also placed in the drying oven to determine the hydroscopic moisture in air-dried Huntley soil. After the three-day period of oven drying, the tubes and cans of soil were weighed, re-heated, and re-weighed until a constant weight was maintained. The TMHC of this soil was then computed and found to be equal to 38.3 per cent by weight. The hydroscopic moisture was found to be equal to 2.12 per cent.

The medium used in isolations, laboratory tests, and for growing

cultures was potato dextrose agar prepared by adding 17 grams of dextrose, 20 grams of agar agar, and 200 grams of sliced potatoes to 1000 cc of tap water.

Several cultures of Rhizoctonia were isolated from matured, infected sugar beet roots collected at the Huntley Station during the fall of 1950. The beets were washed in tap water and broken by hand. Small pieces of firm, infected tissue taken as near as possible to the healthy tissue were imbedded in the media in sterile Petri dishes. Rhizoctonia cultures were also isolated from infected beet seedlings grown in the Huntley soil in the greenhouse during the fall of 1950. In making isolations from these seedlings, the following procedure was used: the seedlings were placed in a beaker; cheese cloth was placed over the mouth of the beaker and held in place by a rubber band. The seedlings were washed with running tap water for four hours, and then washed several times with sterile distilled water. Small pieces of the diseased roots were imbedded in the media. All cultures were grown at room temperature.

Several contaminating organisms were encountered in each isolation method. Those most prevalent were Fusarium spp, Phoma spp, Rhizopus spp, Penicillium spp, and bacterial contaminates. Rhizoctonia cultures were separated from contaminating fungi by the dilution method and from the contaminating bacteria by the following method, which was described by Ark and Dickey (3). Three small pellets of clay were placed on one edge of Van Tiegham rings. The rings were placed in Petri dishes with the clay touching the glass. The Petri dish covers were replaced and the entire

apparatus sterilized in an autoclave (15 pounds pressure for 30 minutes). Melted potato dextrose agar of pH 3.8 was poured into the plates. After the agar was solidified, the contaminated material was placed in the ring. The fungus hypha tend to grow into the agar and come out from under the ring to the surface of the agar outside the ring, free from any bacteria. The acidified agar was prepared by adding 10 drops of 25 per cent lactic acid to 1000 cc of potato dextrose agar.

As a result of these isolations, six cultures of Rhizoctonia were obtained. They were numbered 1, 2, 3, 595, 596, and 599. Cultures 1, 2, and 3 were isolated from mature sugar beets. Cultures 595, 596, and 599 were isolated from seedlings.

EXPERIMENTAL PROCEDURE AND RESULTS

In an attempt to explain the reasons for the sudden increase and subsequent variation in intensity of Rhizoctonia disease of sugar beets at the Huntley Station, a series of experiments were conducted in which various environmental and biological factors were investigated. These studies were as follows:

(1) A test of pathogenicity of the isolates of Rhizoctonia.

(2) The effects of temperature on the growth of the isolates in culture.

(3) The effects of temperature and moisture on the amount of disease caused by the isolates of Rhizoctonia.

(4) The effects of inoculum of varying ages on beet seedlings at the different stages of growth.

(5) Antagonistic effects of other fungi on Rhizoctonia.

Tests of Pathogenicity of the Isolates of Rhizoctonia

These tests were conducted in the greenhouse during the winter of 1950. In these experiments, the pathogenicity of the three cultures (1, 2, 3) of Rhizoctonia isolated from matured beets, and of the three cultures (595, 596, 599) isolated from seedlings, were tested. Twelve 6-inch clay pots containing sterile soil were inoculated with the six isolates. Two pots were used for each isolate. The inoculum was grown on the media mentioned above. Two plates of inoculum were thoroughly mixed into the top layer of the soil in each pot. Twenty segmented beet seeds were planted at a depth of one-half inch in each pot. The beets planted in the inoculated soil emerged in about five days. The first reading for healthy and diseased beets was made one day later, and at three- to four-day intervals thereafter until harvest. The beets were grown 38 days after emergence and harvested. Final readings for healthy, diseased, and the total number of dead beets were recorded at the time of harvest. The percentages of diseased beets recorded during the 38-day growing period are compared in figure 1 and table 1. The cultures (595, 596, 599) isolated from seedling beets developed the greatest amount of disease (100 per cent, 95 per cent, and 95 per cent respectively, with an average of 96.7 per cent). The cultures isolated from mature beets (1, 2, 3), although causing slightly less infection, also produced a considerable amount of disease (94 per cent, 88 per cent, and 91 per cent, respectively, with an average of 91 per cent).

Limitations of equipment and time for the temperature and moisture studies planned made it necessary to select only 4 of the 6 isolates for use in the temperature and moisture studies and subsequent experiments. The isolates (1, 2, 595, 599) were selected on the basis of (1) early virulence and (2) total number of beets killed. Isolate 1 killed 38 beets, or 65 per cent of the total number of emerged plants. Isolate 2 killed 36, or 62 per cent of the plants, and also showed a relatively high degree of early virulence. Isolate 595 killed 41, or 89 per cent of the plants, and isolate 599, although causing the least killing percentage (48 per cent), was selected on the basis of early virulence. No pre-emergence damping off was observed, but it is suspected that some did occur, especially with isolate 599, since the number of plants that emerged in the pots inoculated with this culture was considerably less than with the other isolates. Although the total per cent of disease as produced by the 6 isolates was practically the same, these results show that isolates from the same host differed in virulence. The existence of different strains of Rhizoctonia which vary in their pathogenicity on the same or different hosts is a well-known fact, as reported by LeClerg (17), Kotila (13), and Houston (12).

Table 1. Pathogenicity test in the greenhouse, showing the mortality and total disease per cent produced by each isolate of Rhizoctonia on sugar beet seedlings.

Isolate Number	Plants Emerged Number	Plants Harvested Number	Mortality at Harvest Per cent	Total Diseased Per cent
1	58	20	65	94
2	58	22	62	88
3	61	30	51	91
595	46	5	89	100
596	40	19	52	95
599	31	16	48	95
Check	57	57	-	-

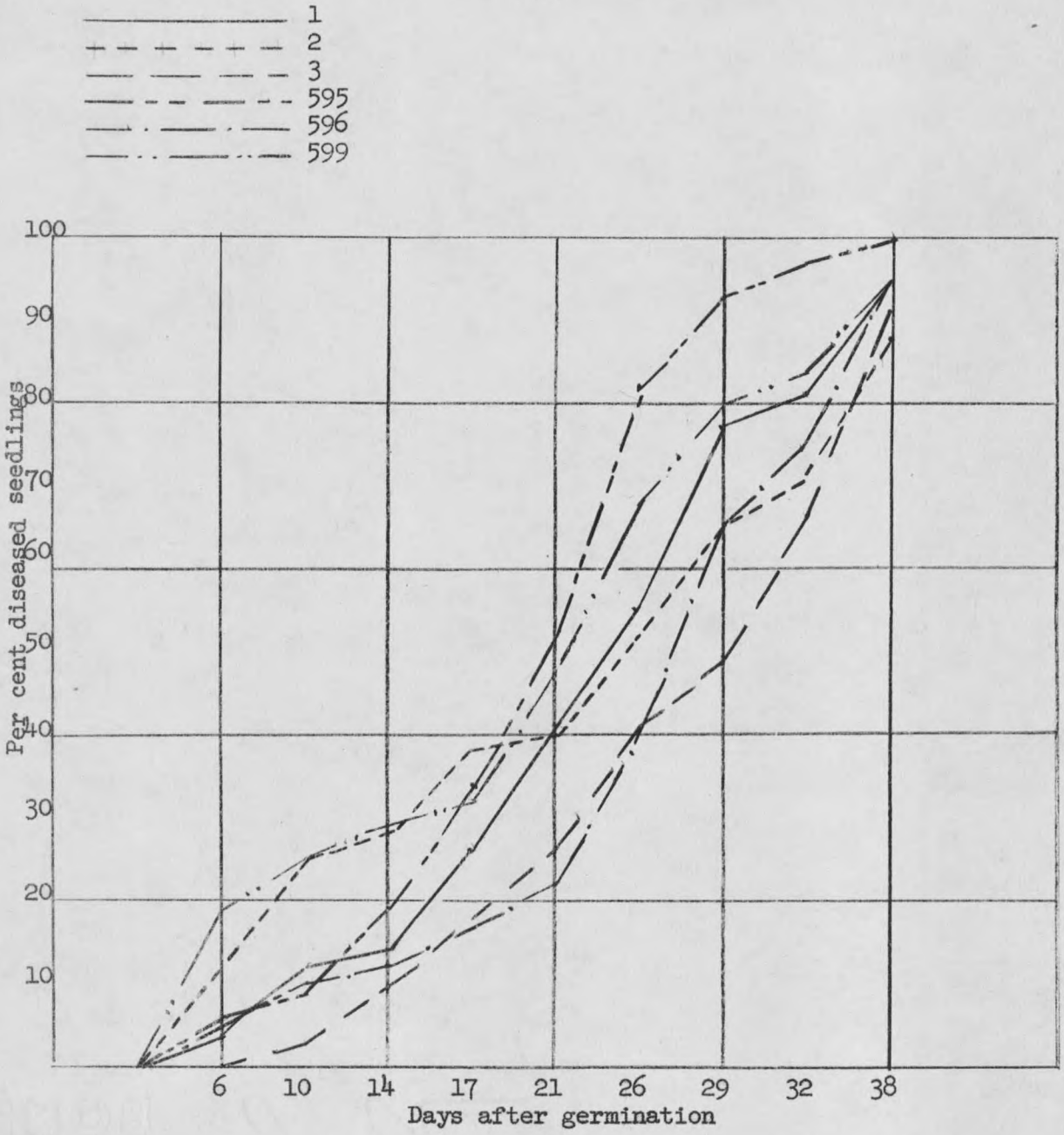


Figure 1. The progress of Rhizoctonia disease of sugar beet seedlings as caused by each isolate in the greenhouse.

The Effect of Temperature on Radial Growth in Culture

Differences in growth habit on media is often used as a criterion for strain differentiation of Rhizoctonia isolates. The present test of the effect of temperature on the radial growth of the 4 isolates was undertaken to determine if any of the isolates could be placed in cultural types as suggested by Houston (12).

Pure cultures of each isolate of Rhizoctonia were grown on potato dextrose agar at room temperature. When the mycelium filled the plates, discs 1.5 mm in diameter were cut from the margins of the colonies and placed upside down in freshly poured agar in Petri dishes. Four dishes were used for each isolate. These were then immediately placed in control chambers at temperatures of 10°, 15°, 20°, 25°, 29°, 34°, and 39° C. Measurements of the greatest and smallest diameters were made each 24 hours. At the end of 72 hours, the average diameter of the isolates was determined and recorded. The results of the experiment are summarized in figure 2. The optimum temperature for the 4 isolates was found to lie between 25° and 29° C. The growth rate of all the isolates was practically the same throughout except at temperatures of 10° and 15°C. At 10°C, isolate 1 made some growth, whereas isolates 2, 595, and 599 showed only a trace of growth or none at all. It is therefore assumed that the minimum temperature required for growth on this culture medium for isolate 1 lies between 5° and 10° C, and the minimum temperature for the other isolates lies between 8° and 10° C. At 15° C, isolates 1 and 599 showed a slightly greater rate of growth than isolates 2 and 595.

The maximum temperature for all isolates was found to be near 40° C.

These results generally agree with previous work that has been done on different isolates of Rhizoctonia. LeClerg (18) reported the optimum temperature for sugar beet isolates as 25° to 30° C, and for potato isolates, 20° to 25° C. Walker (33), while working with an isolate of Rhizoctonia from cotton, found that the optimum temperature for its growth was between 27° and 29° C, with no growth above 38° C, and a minimum temperature between 7° and 11° C. Montieth and Dahl (20), working with grass and potato strains of Rhizoctonia, found wide variations in growth rates between the potato isolates with the optimum growth between 25° and 30° C, and in most cases nearer to 25° than 30° . Houston (12) reported a strain of type B obtained from sugar beets to achieve maximum growth at 28°—29° C.

On the basis of these results, it is believed that the cultural type of all the isolates used in this test was similar.

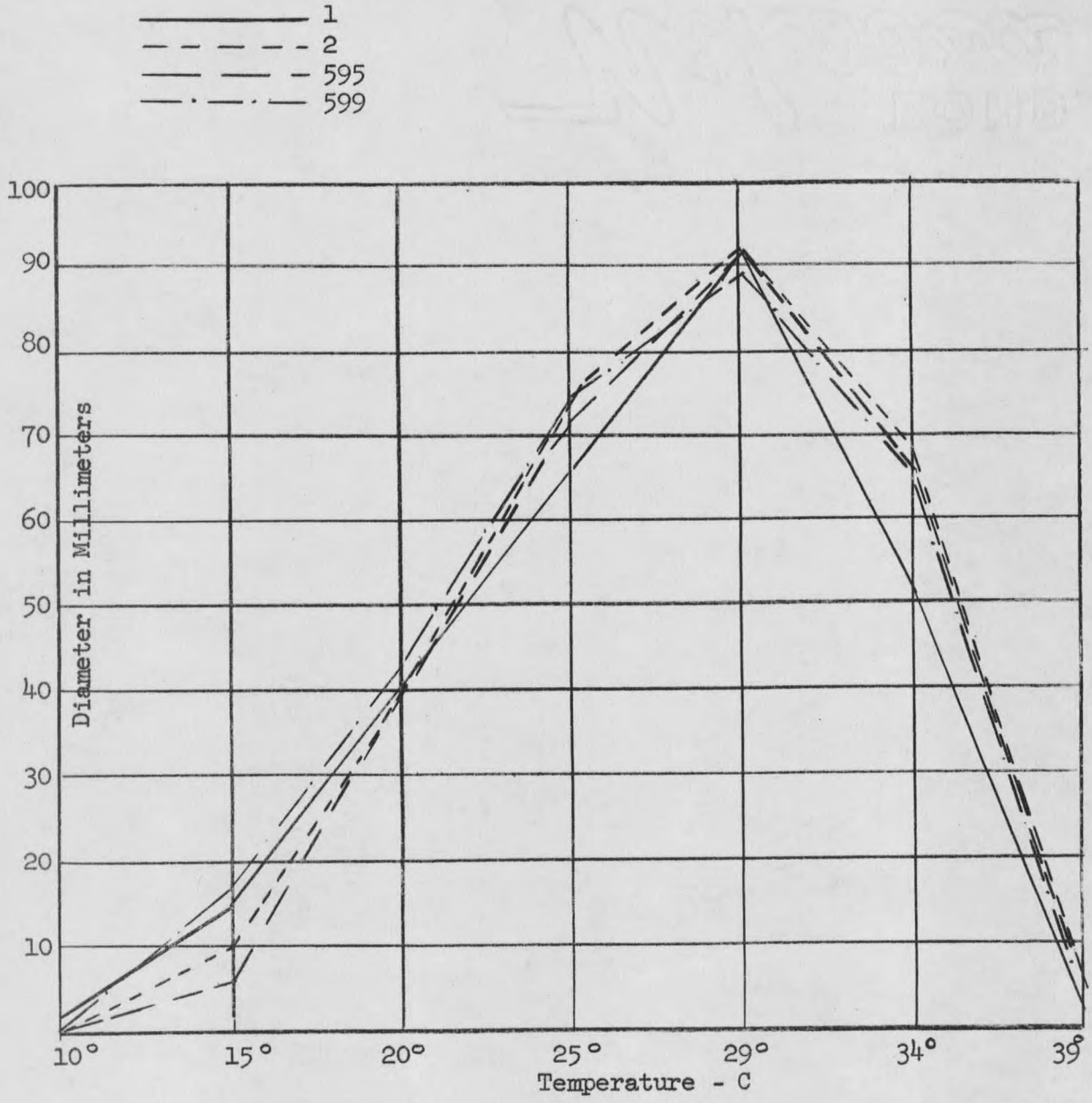


Figure 2. The average growth on potato dextrose agar of four isolates of Rhizoctonia solani after 72 hours at seven different temperatures.

The Effect of Temperature and Moisture on the Amount of Rhizoctonia Disease

The effect of temperature and moisture on the pathogenicity of Rhizoctonia to beet seedlings was studied in a parallel series. Temperatures of 15° and 25° C and moisture levels of 55, 70, and 80 per cent of the total moisture holding capacity were selected. The moisture level of 70 per cent was considered as optimum, the level of 55 per cent minimum, and the 85 per cent level was considered as maximum moisture. Two flats and one jar were used for each moisture level. All flats and jars were weighed separately, filled with air-dried soil, and re-weighed. Tap water was added to each; then they were sterilized in the autoclave as mentioned above. All the flats were inoculated with a 7-day-old culture of Rhizoctonia grown on potato dextrose agar in Petri dishes. Four Petri dishes of inoculum were added to each flat, and the inoculum was thoroughly mixed with the top three inches of soil. The jars were retained as controls. In each flat two rows of sugar beet seeds with twenty seeds per row were planted at a depth of one-half inch. Twenty seeds were planted in each jar. The amount of water to be added to each flat and jar to bring the soil up to its respective moisture level was computed. Flats 1 and 2 and jar 1 were kept at 55 per cent TMHC, flats 3 and 4 and jar 2 were kept at 70 per cent TMHC, and flats 5 and 6 and jar 3 were kept at 85 per cent TMHC. All flats and jars were placed in the control cage. The seedlings were subjected to a fifteen-hour light period by turning on the lights at 5:00 p.m. and turning them off at 8:00 a.m. Throughout