FACTORS AFFECTING THE GROWTH AND PATHOGENICITY OF *SCLEROTINIA SCLEROTIORUM* (Lib.) de Bary

by

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B.S.A., University of British Columbia, 1964

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN AGRICULTURE

in the Division

of

Plant Science

We accept this thesis as conforming to
the required standard.

THE UNIVERSITY OF BRITISH COLUMBIA
September, 1966.

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ABSTRACT

Effects of temperature and pentachloronitrobenzene on vegetative growth and development of *Sclerotinia sclerotiorum* (Lib.) de Bary were studied using Newton's synthetic liquid medium. Microconidial growth increased until the eighth day of incubation at 24.4° C., after which maximum dry weights were obtained. Growth and development of mycelia and sclerotia was most rapid at 24° C., although a similar, but slightly less response was obtained at 20° C. On a dry weight basis, inhibition of mycelial growth and development of *S. sclerotiorum* by PCNB was greater in shake culture than stationary culture at room temperature. PCNB was fungistatic at the concentrations used.

Pathogenicity of soil-borne inoculum of *S. sclerotiorum* on Penn Lake lettuce seeds was affected by temperature, inoculum density, PCNB and a *Trichoderma* sp. The number of emerged seedlings decreased with increasing concentration of soil inoculum and decreasing soil temperature. PCNB applied to U.C. Soil Mix C as a drench treatment not only failed to control disease caused by *S. sclerotiorum*, but also following use of the fungicide, disease increased appreciably at all concentrations used. *Trichoderma* sp. reduced disease only at 15° C. and was ineffective at lower soil temperatures. Decreases in height of lettuce seedlings were observed with increasing inoculum density of *S. sclerotiorum*.

ACKNOWLEDGEMENTS

The author would like to extend his appreciation to the following people for their help during the preparation of this thesis:

Dr. N. A. MacLean, Assistant Professor, Division of Plant Science, the University of British Columbia, under whose direction and supervision this study was carried out;

Dr. N. S. Wright, Head, Plant Pathology Section, Research Station, Canada Department of Agriculture, Vancouver, B.C., who kindly made facilities available for the incubation and centrifugation aspects of the study;

The members of the thesis committee, for their advice and help during the author's graduate study programme.

Thanks are also extended to those who offered assistance when timeliness of help was essential.

The author is especially grateful to Mr. Howard Severson, Research Station, Canada Department of Agriculture, Vancouver, B.C., for his help in the preparation of the photographs.

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INTRODUCTION

In the vegetable growing areas of the coastal region of the Lower Fraser Valley of British Columbia, losses due to Sclerotinia sclerotiorum (Lib.) de Bary emend. Purdy (42) have been serious in some years but not in others. In the summer of 1965 a vegetable disease survey was carried out (41) in order to establish a record of the occurrence, severity and regional distribution of vegetable diseases in the Musqueam (Southwest Marine Drive), Southeast Marine Drive, Richmond-Queensborough, Ladner-Boundary Bay and Cloverdale areas. Although all vegetable crops were included, special emphasis was placed on lettuce due to severe losses that occurred in May and June. S. sclerotiorum has an extremely wide host range as indicated by the extensive lists compiled by Brown and Butler (8) and Young (61). Since the above lists were formed, the pathogen has been found on the common bachelor's button (3), peas (21) and gloxinia (25). The fungus is also a parasite on several weed hosts (35), thus making it a very difficult pathogen to control.

In the field S. sclerotiorum was observed to attack all stages of growth from seedling transplants to mature fully headed lettuce. The severity of disease increased with the size of the plant as small-leaved seedlings were seldom infected while mature plants were often completely destroyed. Diseased lettuce plants in the two to five leaf stage wilted rapidly and either dried up or were

decomposed. It was difficult to determine whether S. sclerotiorum was responsible in all cases because of the rapidity of dehydration and degradation. The first symptom of parasitic attack on mature lettuce plants was a characteristic wilting of the large outer expanded leaves as observed by Brown and Butler (8). This wilting was invariably followed by a complete collapse of all plant leaves, which in very warm weather, resembled a light green folded cloth.

Sclerotiniose of lettuce was found in varying degrees of severity in all fields where lettuce was grown. Each field examined was of a different soil type although all had high organic matter contents characteristic of muck soils. Several fields were located on peat bogs. It was observed that very few plants, if any, were infected by S. sclerotiorum on newly planted peat soils. With the exception of the latter soil there appeared to be little difference among soil types as to disease severity. Much variation in the amount and distribution of disease was observed from field to field. These observations agree with most investigators such as Moore, et al. (35) who found that losses due to sclerotiniose ran from a trace in some fields to 100% in others.

Lettuce crops planted for the early market were often severely attacked by S. sclerotiorum and losses were high. As the growing season progressed, the incidence of the disease declined until very few diseased lettuce heads were noted. The development of the disease was favored by cool soils and high moisture conditions. With increasing soil temperatures,

drying out of soils took place, especially near the surface and sclerotiniose dropped off. In May and June the disease was most severe where early morning mists were heavy and where water was retained in low field areas. These observations are in accord with Partyka and Mai (40) who found that the percentage of potato hills infected by S. sclerotiorum was greater in moist soil and air than in dry soil and air. In a ten year study of rainfall-temperature relationships on the incidence of S. sclerotiorum in vegetables, Moore (34) reported that cool, wet soils induced heavy dews by chilling the warm moist air around growing plants.

In several instances, areas of soil were observed where no plants were growing. Although pre-emergence damping-off was suspected, post-emergence damping-off could not be disregarded because of the rapidity by which the wilted plants were decayed. Diseases of the pre-emergence type in lettuce are not common although Smieton and Brown (53) reported that low emergence of lettuce seed resulted even with conditions of good soil moisture and temperature.

In order to observe the method of infection by S. sclerotiorum several plants and the surrounding soil were examined carefully for sclerotia and apothecia. Apothecia were never found, even under conditions of heavy infection. Sclerotia were found in abundance on infected plant tissues. Infection appeared to take place near the collar of the plant, as noted by Williams and Western (60), progressing

upward along the large mid-rib of each lettuce leaf until the entire plant was colonized. Exposure of the infected area by removing the collapsed leaves revealed a white-cotton-like growth of the pathogen with often a narrow water-soaked area between uninfected and infected tissue. The root systems of several plants were removed from the soil and the external soil fragments were shaken off gently. In all cases the root systems were not colonized by S. sclerotiorum. The initial stages of infection leading to complete pathogenesis were not observed.

With the exception of late planting, no method of control was observed which was effective. Smieton and Brown (53) noted that late planting, however, was not always effective as unfavorable weather conditions could develop resulting in loss of the late crop. Row and drill spacing were observed which helped to provide for better air circulation around each lettuce plant and also permitted mechanized tillage and spraying operations. A form of crop rotation was observed in some areas but the disease did not appear to be reduced. Several fields were under water for part of the winter and early spring. In these fields, however, the disease rate was just as high if not higher than the water-free areas. The use of chemical sprays and dusts was not observed. The problem of sclerotiniose of lettuce was noted to be further accentuated by poor management practices in which diseased plant tissues, instead of being removed and destroyed, were returned to the soil. As a result, the field inoculum was increased.

Infection of lettuce by S. sclerotiorum appears to be

influenced greatly by the environment, especially temperature and moisture. Although not a serious problem, pre-emergence damping-off has not been investigated thoroughly enough. In general there is no efficient control for the disease despite several methods which have been recommended such as the use of pentachloronitrobenzene dusts and sprays (32, 51). A number of experiments, therefore, were outlined in order to determine the effects of various factors such as temperature, associated soil microflora and fungicides on the growth and pathogenicity of S. sclerotiorum. It was hoped that these studies would provide knowledge leading to a better understanding of the pathogen and the disease it causes on lettuce.

REVIEW OF THE LITERATURE

I. Physiology of Growth

Questionable use of agar media

Linear growth measurements of fungi on agar media do not correlate well with the total amount of fungus produced in liquid culture. Also, the interpretation of data obtained from linear growth of fungi on solid surfaces (agar) is not always clear. Purdy and Grogan (45) sharply criticized the work of Tanrikut and Vaughan (54) in which the latter concluded that the growth of S. sclerotiorum on agar media deficient in either of the following elements K, P, Fe or Mg, was as rapid as on a complete agar. Purdy and Grogan (45) compared the growth of S. sclerotiorum in a liquid medium and the same medium solidified with agar. They found that growth was significantly reduced in solutions lacking the essential elements P, Mg or the heavy metals and that little or no growth occurred if the solution lacked K. Barnett and Lilly (4) found that although Sclerotinia camelliae Hara covered a 0.2% malt agar surface faster than a 2% malt agar surface, the mycelial mat was much thicker on the 2% than on the 0.2% malt agar. In a comparison of the growth of two strains of S. trifoliorum Erikss., Held (24) observed a 45% greater amount of growth on agar media of the normal versus the degenerate strain. In liquid media, however, there was no significant difference in the dry weight of mycelium produced by the

two strains. Cochrane (13), while recommending the assay of growth gravimetrically in a liquid medium, criticized weaknesses in the agar-plate method which takes into account only radial growth and not mycelial density. In a study of fungal growth kinetics, Mandels (33) stated that dry weight measurement is the most basic and meaningful way of representing the amount of organism produced.

Effect of temperature on growth

Despite the above criticisms, studies of temperature effects on growth of Sclerotinia spp. demonstrate a lack of standardization of technique especially with regard to the type of substrate used.

Using measurements of linear growth of S. sclerotiorum on potato-dextrose agar, Tanrikut and Vaughan (54) noted that most growth occurred at 20° C. with the rate of growth decreasing above and below this figure. All cultures grew well at 30° C. Similar studies were made by Cappellini (11) who reported that the maximum growth of S. sclerotiorum took place at 20° and 25° C. Keay (28), measuring the radial growth of mycelia on malt-extract agar at various temperatures, found that S. sclerotiorum grew best at 20° to 25° C. Ramsey (46) studied the growth of various Sclerotinia spp. on potato-dextrose agar over a range of temperatures. He found that 20° C. was the optimum although there was much variation among isolates. Morgan (36) undertook a study of the growth rate of 23 Sclerotinia isolates

using the agar-plate method. He stated that optimum growth took place from 15.5° to 20° C. despite a wide variation between isolates. Hansen and Thomas (23) observed that mycelial growth and sclerotial production of S. camelliae on potato-dextrose agar was more rapid at 15° to 18° than at 24° C. Studies of the rate of growth of S. camelliae in liquid media were made by Barnett and Lilly (4) who mentioned that the growth rate at 22° C. was approximately the same as at 25° C., the optimum being between the two temperatures.

Effects of fungicides on growth

Very little laboratory work has been done on the effects of fungicides on the growth of S. sclerotiorum. Kendrick and Middleton (29) noted that 10 p.p.m. pentachloronitrobenzene (PCNB) suspended in potato-dextrose agar showed fungistatic effects against S. sclerotiorum. Grover (22) studied the effect of several fungicides suspended in Ashour's nutrient agar on mycelial growth and sporulation of S. sclerotiorum. He found that higher concentrations were required to inhibit mycelial growth than conidial formation.

PCNB affects the growth and development of several fungi other than S. sclerotiorum. Reavill (47) working with chlorinated nitrobenzenes, showed that growth of Trichoderma viride Pers. ex Fries on malt agar was retarded initially but was able to survive and colonize the plate.

Sporulation was severely depressed in the presence of both tetrachloro- and pentachloronitrobenzene. Gibson, et al. (20) found that PCNB in its vapor phase inhibited the growth of Penicillium paxilli Bain. Torgeson (55) showed that PCNB was ineffective in reducing oxygen uptake of Fusarium oxysporum f. cubense (E.F.Sm.) Snyd. and Hans., Sclerotium rolfsii Sacc. and Phytophthora parasitica (Dast.) var. nicotianae (B. de Haan) Tucker.

The biochemical mechanism of PCNB has not been clearly established in fungi. Burchfield (9) observed that PCNB participated in a true substitution reaction. Betts, et al. (7) reported that PCNB was metabolized in the rabbit by reduction of the nitro group yielding pentachloroaniline, hydroxylation yielding pentachlorophenol and loss of the nitro group giving N-acetyl-S-pentachlorophenyl L-cysteine. The majority of the dose administered to the rabbit was not absorbed. Horsfall (27) noted that chlorination of benzene derivatives and phenol gave improvement in fungicidal activity. This was thought to be due to an increase in fat solubility.

Vegetative growth in culture

Nutrition and temperature are the main factors affecting the formation of sclerotia in culture. Bedi (5) noted that formation of sclerotia was dependent on plentiful mycelium and a barrier such as the edge of two or more colonies coming together or the wall of the culture vessel. A further

stimulus to sclerotial formation is the accumulating products of metabolism. Demetriades (15) reported that a synthetic medium lacking Mg and S completely inhibited the formation of sclerotia but not the mycelium of S. sclerotiorum. The addition of either Mg or S alone did not favor sclerotial or mycelial development but when S and Mg were added together, growth equal to that of the control was obtained. Purdy and Grogan (45) found that sclerotia were formed on Houston's solution agar minus Mg after 12 days, whereas the control required 14 days. Bedi (6) examined the effects of temperature on sclerotial formation. He stated that the rate of formation increased between 5° and 25° C. Only a few large sclerotia formed at 5° C. while many small ones formed at 30° C.

The principal factors governing the formation of appressoria are the presence of a carbon source and the stimulus of a mechanical barrier. Purdy (43) noted that appressorial masses did not form at random but only in contact with a solid barrier such as a cover slip. He also found that appressoria did not form in solutions lacking a carbon source, regardless of the presence or absence of mineral elements essential for growth.

It appears that the age of the culture is the main factor in determining the formation of microconidia although temperature may also be important. Purdy (43) mentioned that microspores were produced at any locus along a hyphal filament in solutions that were lacking in a carbon source

as well as complete ones. Ramsey (46) observed that Sclerotinia can produce microconidia at all stages in its life history providing proper conditions are furnished. He found that most strains did not begin microconidial production until they were 20 to 30 days old. Hansen and Thomas (23) stated that S. camelliae produced microspores in abundance at 24° C. Barnett and Lilly (4) noted that the greatest microspore production took place near 25° C.

II. Pathogenicity

Growth rate and pathogenicity

Morgan (36) reported that the correlation of growth of Sclerotinia isolates in vitro with pathogenicity was poor due to the wide variation between isolates both in growth rate in vitro and in the amount of infection of the various hosts.

Infection and penetration

The mycelial growth of S. sclerotiorum in soil is important for parasitic colonization of host tissues. Williams and Western (60) found that mycelia grew to the surface of the soil and then infected the host through the collar. Vaughan and Dana (58) stated that most infections of beans resulted from mycelium growing saprophytically through the soil.

Non-living organic matter plays an important and underestimated role in the infection of mature plant tissues

by S. sclerotiorum. Chamberlain (12) observed that infection of soybean seedlings did not occur when mycelial suspensions or sclerotia alone were placed at the base of each seedling. Purdy and Grogan (44) showed that infection of lettuce from mycelia of germinating sclerotia took place only when non-living organic matter such as dead lettuce leaves was present. No infection resulted when germinating sclerotia alone were in contact with the stem. In a field study, Purdy (43) noted that lettuce plants remained free from infection until the lower leaves became senescent and died. Mycelium from germinating sclerotia colonized the leaves and infection of healthy tissues took place.

Pre-emergence damping-off of lettuce and other seeded crops by Sclerotinia spp. is less well known than post-emergence infection of mature plants. Smieton and Brown (53) found that the causal agents of pre-emergence damping-off were species of Pythium. Chamberlain (12) studied the effects of steamed versus non-steamed soil on the pathogenicity of Sclerotinia on soybean seeds. He reported a greater number of infected seeds in steamed soil than in the non-steamed field soil. Kerr (30) noted that seeds and seedlings of radish, lettuce, beet, and clover were severely reduced in size when grown in soil containing inocula of S. homeocarpa Bennett. A toxic substance was suspected as the organism was not recovered from dead seeds.

Pathogenicity of soil-borne inoculum

In general the quantitative measurement of the pathogenicity of soil-borne inoculum of Sclerotinia spp. has not been standardized. Seldom have seedling emergence counts been used to determine pathogenicity. Using a water suspension of macerated mycelium and sclerotia poured directly over soybean seeds planted in steamed soil, Chamberlain (12) noted 97% and 27% emergence in the control and inoculated flats respectively. Kerr (30) mixed 2 grams of sand-maize-meal inoculum with 100 grams of unsterilized soil and counted the emergence of seedlings from seeds planted in inoculated and uninoculated soil. He reported a large reduction in numbers of emerged seedlings of radish, lettuce, beet, clover, pea, bean and tomato in the inoculated soil as compared to the controls.

Control with pentachloronitrobenzene

PCNB has been recommended as a selective soil fungicide for the control of diseases caused by Rhizoctonia solani Kuhn, Plasmodiophora brassicae Wor., and Sclerotinia sclerotiorum (51). In general, however, the fungicide has not proven effective in the control of S. sclerotiorum (14, 40, 52).

The methods by which PCNB is applied in the field are numerous. Sharvelle (51) stated that PCNB may be applied either as a soil mix, surface applicant, transplant solution or as a seed treatment.

PCNB moves very little, if at all, in soil solution due to its insolubility in water (51). Kendrick and Middleton (29) noted that soil drenches of PCNB at 10, 100 and 1,000 p.p.m. failed to reach inoculum discs placed just beneath the soil surface.

The results concerning the effectiveness of PCNB in soil are contradictory. Munnecke, et al. (37) studied the fungicidal activity of air passed through a column of soil treated with PCNB. He found that PCNB did not produce toxic vapors in the effluent air even at concentrations of 2,000 p.p.m. for 305 hours. In these experiments the evaluation of fungicidal activity was made after exposure to the fungicide. Richardson and Munnecke (48) measured the toxicity of vapor escaping from soil to which had been applied PCNB. The degree of inhibition of radial growth of Pythium irregulare Buisman, Rhizoctonia solani and Trichoderma viride was recorded on potato-dextrose agar. In these experiments the assessment of fungicidal activity was made during the exposure of the test fungi to the toxic vapors of the fungicide-treated soil. They found that of the three organisms, T. viride was the most susceptible to the toxic vapors.

In the field PCNB has, in general, failed to give satisfactory control under a number of soil conditions. Smieton and Brown (53) found that PCNB dust was ineffective in controlling pre-emergence damping-off of lettuce seedlings. In a number of experiments designed to control S. sclerotiorum

on lettuce, Darby (14) found that drenching the soil before planting offered some protection but unless these treatments were followed by foliar applications, they were of little benefit. Skotland (52) failed to achieve control of S. sclerotiorum in lettuce using a number of sprays and dusts of PCNB. Partyka and Mai (40) observed that PCNB, when sprayed on the ground or placed in the soil, did not control S. sclerotiorum on potatoes.

Phytotoxicity due to PCNB sprays or dusts often is affected by environmental conditions. Smieton and Brown (53) found that PCNB checked the growth of lettuce seedlings and that after a frost the dusted plants showed more injury than the controls. Darby (14) mentioned that PCNB caused moderate injury at 15 pounds per 100 gallons per application under some environmental conditions, although details of the environmental conditions were not given. This injury was minimized by application in clear weather just after mid-day.

Highly undesirable results have followed from soil treatment using specific soil fungicides (31). Gibson, et al. (20) found that, after soil treatment with PCNB, increased losses due to a Pythium sp. occurred. This was due, at least in part, to the suppression of growth of Penicillium paxilli in the soil.

Effect of microflora on growth

Inoculation of unsterilized soil with a pathogen often

results in failure of the pathogen to cause infection of the test plants. In an experiment comparing infectivity of inoculum of S. sclerotiorum in sterilized and unsterilized soil, Chamberlain (12) noted a greater number of unemerged seedlings in the sterilized soil compared with non-sterilized soil. Sanford (49) observed that Trichoderma lignorum (Tode) Harz inhibited the formation of sclerotia of Rhizoctonia solani in sterilized soil but not in natural soil.

Sclerotia of soil-borne fungi such as S. sclerotiorum are subject to attack by parasitic fungi. Hino (26) reported that Trichoderma viride parasitized and often destroyed the sclerotia and mycelia of S. sclerotiorum. Campbell (10) found that sclerotia of S. sclerotiorum were readily parasitized by Coniothyrium minitans Campbell when pycnidiospores were brought into direct contact with the sclerotia or when they were poured on the surface of sand under which the sclerotia were buried. Tribe (56) showed that C. minitans actively killed sclerotia of S. trifoliorum within 11 weeks in soil. The soil inoculum mix, either a loamy sand or a heavy clay, remained infective for at least 14 months.

Despite the above reports of sclerotial parasitism of S. sclerotiorum by species of soil fungi, no studies of reduction in plant disease due to mycelial parasitism have been made, although fungi other than S. sclerotiorum have been tested. Allen and Haenseler (1) noted that with soil-inoculum mixtures of Trichoderma, Rhizoctonia and

Pythium, seedling emergence of peas and cucumbers were high in the series inoculated with *Trichoderma*. This indicated an interference, in some way, with the parasitic activity of *Pythium* and *Rhizoctonia*.

METHODS AND MATERIALS

I. Physiology of Growth

Organism

The organism used for physiological studies was a strain of *Sclerotinia sclerotiorum* recovered from infected bean plants in 1964. Stock cultures were grown in a liquid nutrient medium (see under media) and stored in a refrigerator at 4° C. for future use.

Media

Due to inconsistent results using agar media it was decided to select a simple nutrient solution which would support good growth of the organism used. Media tried were Houston's solution (44), Newton's solution (38) and Garrett's solution (19), the names referring to the author who first used these media. Chemicals were weighed to 0.1 mg. on an analytical balance, transferred to a 1 litre volumetric flask and brought to volume with distilled water. The solution was well shaken and filtered through #1 Whatman filter paper. Twenty-five mls. of media were pipetted to each of forty-five 250 ml. Erlenmeyer flasks and autoclaved at 15 lbs. pressure for 15 minutes. Inoculum plugs, cut with a 9.0 mm. cork borer from rapidly growing colonies on potato-dextrose agar plates were transferred to the cooled medium. Cultures were incubated under a variety of temperatures and growing conditions. Growth was determined after 4 days by the amount of visible mycelium produced, and rated from complete coverage of the

medium surface (++++) to no growth (-), (Table 1).

TABLE 1

GROWTH ON SEVERAL MEDIA

Condition of incubation	Media		
	Houston's	Newton's	Garrett's
20° C.	+++	+++	++
25° C.	+++	+++	++
30° C.	-	-	-
Shake culture (20-25° C.)	+	+	-
Still culture (20-25° C.)	++	++	+

Although both Houston's and Newton's media gave similar results, the latter medium was adopted for its ease of preparation. The formulation is as follows (38):

Dextrose	20.0 gm.
K_2HPO_4	0.2 "
KNO_3	0.2 "
$CaSO_4 \cdot 2H_2O$	0.1 "
$MgSO_4$	0.1 "

Distilled water to make 1 litre

Fungicide

The fungicide tested was pentachloronitrobenzene (PCNB) 75% wettable powder. Concentration of the fungicide was expressed as parts per million actual PCNB. The amount of

fungicide required to give the highest concentration in a series of concentrations was weighed to 0.1 mg., transferred to a volumetric flask and brought to volume with distilled water. The contents were shaken well, appropriate aliquots were removed and diluted to give a series of concentrations. A magnestir (Figure 1) was used to promote even suspension of the fungicide while pipetting 1 ml. aliquots of each concentration to 250 ml. Erlenmeyer flasks containing 25 mls. of nutrient solution. Flasks were stoppered with cotton wool and autoclaved at 15 lbs. pressure for 15 minutes.

Inoculum preparation

In order to obtain consistent results with replicated treatments, it was necessary to prepare and distribute inoculum in homogeneous condition. Several experiments were carried out toward the establishment of a technique which would satisfy both qualitatively and quantitatively the above requirements of inoculum preparation.

1) growth of inoculum

Several cultures of S. sclerotiorum were removed from refrigeration and allowed to reach room temperature. Using a nichrome needle, small pieces of peripheral mycelia were transferred to several (usually six to ten) 250 ml. culture vessels containing 25 mls. of freshly autoclaved liquid media. It was necessary to transfer mycelia from the growing edge of the colony due to the matted surface growth. Inoculated cultures remained at room temperature or were placed in

Plate 1.

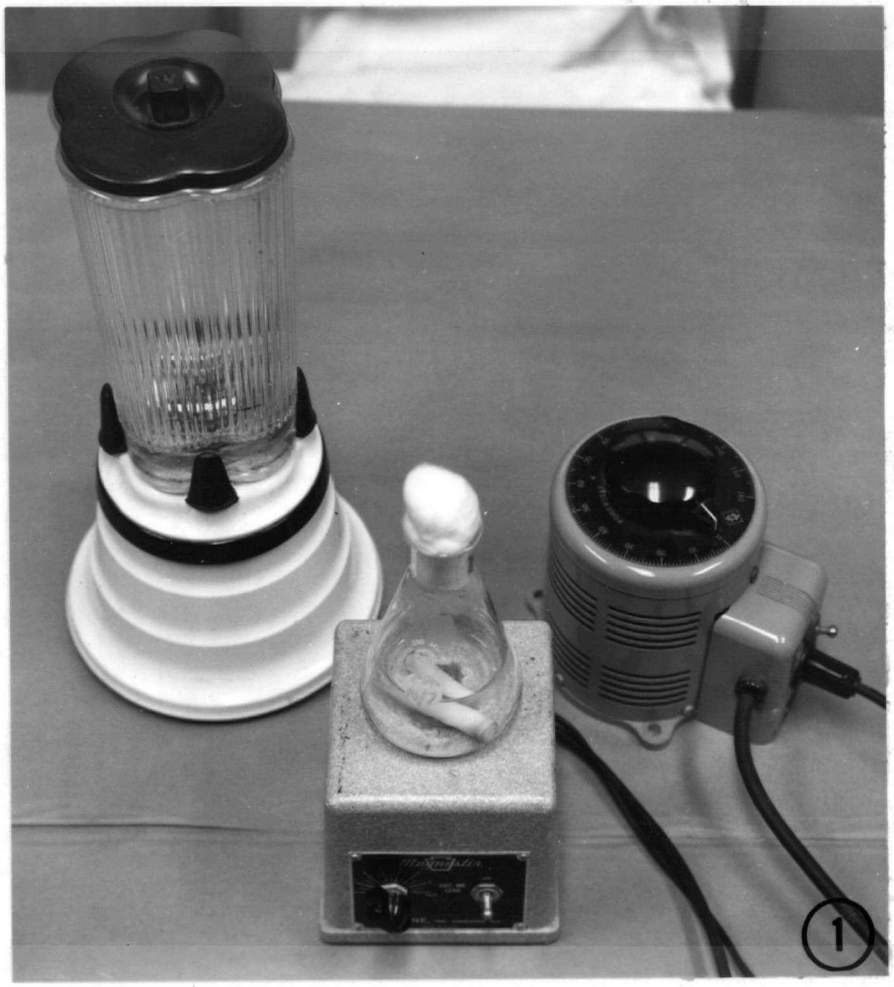


Figure 1. Apparatus used in fungicide and inoculum preparation. Left, Waring blender. Center, magnetic stirrer. Right, rheostat.

constant temperature cabinets at 24° C. Growth began almost immediately, forming a hyaline colony which grew submerged for several days. Depending on the temperature and amount of transferred mycelia, usually from 5 to 6 days were required for the submerged growth to occupy the full volume of the liquid medium. Upon contacting the surface of the medium, white aerial hyphae were formed which, within 24 hours, massed over several areas of the medium. These cultures were either used immediately or were refrigerated at 4° C. The latter was necessary due to the rapid massing action of the aerial hyphae which formed relatively compact masses of mycelia.

2) preparation of mycelial suspension

One to several cultures of S. sclerotiorum were added to an autoclaved and cooled Waring blender jar (Figure 1). A rheostat was used to adjust the momentum of the whirling blades in order to avoid the initial burst of speed and subsequent splattering of hyphae on the walls and lid of the jar (Figure 1). From 30-40 seconds were required to obtain a fine suspension of hyphal strands. It was felt that a longer period may have impaired the growth habit of the organism due to a physical shattering of the hyphae.

To determine the density of hyphal particles which would give uniform growth among replicates, a series of experiments were carried out in which the inoculum was diluted with distilled water. Typical weights in mgm. of several dilution experiments are given in Table 2.

TABLE 2

THE EFFECT OF INOCULUM DILUTION ON GROWTH

Replicate	Dilution of inoculum		
	1:0[a]	1:4[b]	1:6[c]
1	63.8	66.9	147.1
2	59.6	82.7	141.1
3	58.5	80.0	120.1
4	63.4	58.2	104.6

[a]25 mls. of inoculum culture:0 mls. distilled water

[b]25 mls. of inoculum culture:100 mls. distilled water

[c]25 mls. of inoculum culture:150 mls. distilled water

It was found that dilution of inoculum gave highly variable results among replicates of a treatment whereas direct pipetting of the homogenate gave more uniform results. These results are in agreement with Foster (16) who thoroughly reviewed the subject of inoculation technique. He postulated that dilution served to reduce the number of viable mycelial strands per unit volume, therefore the competition between growing hyphae was reduced and variation between replicates was more pronounced. Toxicity of distilled water to the homogenized hyphal strands cannot be ignored. The normal osmotic exchanges between the media and the hyphal wall cannot take place in an environment lacking essential ions.

3) distribution of inoculum

Immediately after homogenation, the hyphal suspension was transferred from the Waring blender jar to an autoclaved and cooled Erlenmeyer flask. The most efficient inoculum load was determined by measuring the growth obtained from aliquots of various sizes. The dry weights of growth in mg. of several volumes of mycelial suspensions are given in Table 3.

TABLE 3

THE EFFECT OF INOCULUM SIZE ON GROWTH

Replicate	Volume of inoculum		
	1 ml.	5 ml.	10 ml.
1	63.8	67.6	90.7
2	59.6	67.2	86.9
3	58.5	76.4	92.9
4	63.4	72.3	87.7
5	56.1	51.7	87.9
6	65.1	70.4	87.8

Growth was greater in those flasks inoculated with the larger inoculum suspensions. It was decided, however, to use the 1 ml. quantity because of the ease of handling smaller amounts of inoculum. Wide tipped pipettes (1/8" diameter) were used to ensure rapid delivery of inoculum in order to reduce the possibility of contamination while

transferring the inoculum. Sterilization of pipettes was secured by autoclaving at 15 lbs. pressure for 15 minutes. To prevent killing of the hyphal strands in suspension, the pipettes were not flamed between transfers. One pipette was used to inoculate all culture vessels in each experiment.

Inoculum was kept in suspension by swirling the contents of the flask vigorously before each transfer. It was found in previous experiments that even distribution of inoculum was critical for consistent results. Another method involving the use of a magnetic stirrer was tried but was abandoned due to the formation of long crystals in solution which interferred with the uptake of the hyphal suspension. Crystal formation did not take place when the inoculum was shaken by hand.

Culture vessel

Carefully washed conical shaped 250 ml. Erlenmeyer flasks were used in all growth experiments. A total of 25 mls. of medium was distributed to each flask using a 25 ml. volumetric pipette. The flasks were stoppered with cotton plugging and autoclaved at 15 lbs. pressure for 15 minutes. Cotton plugging was made as loose as possible so as not to interfere with gas exchange yet facilitate pure culture conditions.

The number of replicates used in all experiments was six. Preliminary work using three and four replicates was highly variable, however the cause of the variation was due

to a combination of factors and not the number of replicates alone. With six replicates the number of experimental units was increased and the use of a six-tube Servall-Refrigermatic centrifuge was facilitated.

Incubation

Depending on the experiment, culture vessels were incubated in three ways: 1) in small constant-temperature cabinets (capacity of sixty-250 ml. Erlenmeyer flasks), 2) at room temperature, or 3) on a mechanical shaker apparatus (120 cycles per minute). Temperature cabinets were placed in a large refrigerated room at 10° C. in order that temperatures higher than 16° C. could be obtained. Temperatures were measured at three levels in each incubator with thermometers placed in cotton-stoppered 125 ml. Erlenmeyer flasks containing 0.5" distilled water. Readings were taken three times daily. A tempscribe recorder was used to check for possible temperature fluctuations between the times of recording. The temperature of cultures on the rotary shaker (capacity 68 flasks) was measured as above.

Harvest of growth

At the desired time, two flasks were removed from each shelf of the incubator and either harvested immediately or stored at 4.5° C. until sufficient time could be had. Mycelial growth and sclerotia adhering to the walls of the

culture vessels were removed with a rubber-tipped stirring rod.

Filtration of the growth of S. sclerotiorum using #1 Whatman filter paper proved to be difficult and very slow due to the gelatinous and slimy growth in liquid culture. Attempts were made to weigh centrifuged mycelial growth on tared filter paper, but due to the rapid gain in weight through removal of moisture in the air by the hygroscopic filter paper, the method was abandoned.

Although time-consuming, the centrifugation method of washing mycelial growth was used because it proved to be the best method of washing the slimy and gelatinous growth of S. sclerotiorum. The contents of each culture were transferred to 200 ml. Nalgene centrifuge bottles with the aid of 150 mls. of distilled water. The mycelial mats and pieces of the colony were centrifuged at 8,000 x gravity for 5 minutes. The supernatant liquid was poured carefully into a beaker to check for loss of mycelial fragments. The centrifuged mycelial growth was re-floated with the addition of 150 mls. of distilled water, swirled well and centrifuged again. It was found that a total of 5 centrifugations were required to adequately wash the mycelial mats.

Weighing

The washed mycelial growth was transferred to tared weighing bottles (125 ml. Erlenmeyer flasks) with the aid of small streams of distilled water. The growth was oven-dried

at 70° C. to constant weight. After a period of five days at 70° C. the dried growth was placed in a desiccator for 10 hours. Weights were recorded on a Christian Becker Chainomatic swing balance to 0.1 mg.

II. Pathogenicity Studies

Organisms used

Studies on the growth of S. sclerotiorum in soil were made by determining the pathogenicity of various inoculum densities on the germination and emergence of lettuce seeds. The strain of Sclerotinia used was the same as that used in studies of the physiology of growth. To determine the effect of a soil-borne saprophyte on the growth and pathogenicity of S. sclerotiorum, a species of Trichoderma was isolated from soil and various inoculum mixtures used.

Inoculum

Large quantities of inoculum were prepared in one-litre flasks by soaking Terralite vermiculite with Newton's liquid media (unfiltered) at a ratio of 1:3 by volume as by the method of Varney (57). The flasks were stoppered with cotton plugs and autoclaved at 15 lbs. pressure for 15 minutes. When cool, the soaked vermiculite was inoculated with agar plugs cut from rapidly growing cultures. All inoculum was grown at room temperature (20° - 25° C.) for 1 month. After 2 weeks each flask was shaken to break up and distribute the inoculum more effectively. S. sclerotiorum grew abundantly throughout the medium producing large masses of white mycelium but few, if any, sclerotia were formed.

Sclerotia which were formed were very small and grew on the inside edge of the flask above the medium. This agrees with Purdy and Grogan (45) who found that media deficient in K have been shown to give small sclerotia in the above manner. The Trichoderma sp. grew very slowly and sporulated only at the edge of the inoculum adjacent to the glass container wall. Difficulty was experienced in obtaining abundant growth and several cultures failed to grow.

Studies with soil inoculum

1) soil used

The study of the pathogenicity of S. sclerotiorum in soil was carried out using the U.C. Soil Mix C (2). This soil, composed of 50% Evco dry mortar sand and 50% Canadian Acme peat moss, was selected because of its uniformity and reproducibility. The moisture holding capacity was determined to be 43.6%. The following chemicals were added to two cubic feet of sand and peat (1 cu. ft. sand plus 1 cu. ft. peat):

KNO_3	8.4 gm.
K_2SO_4	14.4 "
Single superphosphate	84.0 "
Calcium carbonate lime	84.0 "
Dolomite lime	252.0 "

Each chemical was ground in a large mortar in order to break up small lumps. The peat moss was screened through a #8 screen to remove large roots and plant fragments.

The sand was taken from bags directly and mixed with the peat moss in a wheelbarrow. The sand particle size was larger than recommended for the U.C. Soil Mix C but due to the convenience of using the sand directly from the bag, screening was not employed. The finely ground chemicals were added to the soil and mixed with a shovel.

The soil mix (moisture percentage of 2.56) was stored at room temperature (19° - 20° C.) in a polyethylene lined box. No attempt was made to sterilize the soil.

2) soil inoculum preparation

Appropriate weights of inoculum were added to the U.C. Mix so as to give a range of inoculum densities on a percentage by weight basis. Vermiculite, moistened with distilled water and autoclaved, was added as required to bring the vermiculite level of each inoculum density up to that of the highest inoculum level. The soil-inoculum mixture was blended in a two gallon plastic pail by mixing with a spoon for approximately 5 minutes.

3) seeding

Immediately after preparation, the soil inoculum mixtures were placed uniformly into 3 x 3" plastic pots. Twenty Penn Lake lettuce seeds were scattered evenly over the surface and covered to a depth of 0.5" with the appropriate soil-inoculum mixture. The soil in each pot was adjusted to 60% moisture holding capacity with distilled water. Due to the moistened vermiculite and inoculum added, the adjustment to 60% was only approximate.

4) fungicides

Soil fungicides used were PCNB (Terraclor) and Terrazole (3-trichloromethyl-5-ethoxy-1, 2, 4 thiadiazole) 60% W.P. Each fungicide was applied as a drench treatment to the soil surface. Formulations were made as parts per million on a solution basis. Five mls. were allowed to run slowly from a pipette over the soil surface. Adjustment to moisture holding capacity was then made with distilled water.

5) incubation

Immediately after moisture adjustment the pots were placed on racks and incubated at the required temperature in large refrigerated cabinets. The relative humidity inside each cabinet was raised to a maximum by pans of water placed near the top and bottom. Moist air was then circulated throughout each cabinet by a constantly running fan. The circulation of air throughout the cabinet was facilitated by proper spatial adjustment of the soil containers.

The soil temperature at a depth of 0.5" was obtained by inserting one thermometer horizontally into the soil through a hole drilled in the side of the pot.

Seedling counts were made for all treatments after the controls emerged and had reached a constant number.

OBSERVATIONS AND RESULTS

I. Physiology of Growth

Formation of microconidia

Although the factors of microconidial formation in culture by S. sclerotiorum have been elucidated (43, 46), a quantitative measurement of the growth rate has not been recorded in the literature. An experiment was devised so as to obtain information on the growth rate of the microconidial stage of S. sclerotiorum in liquid media. Inoculum was prepared using mycelium taken from the surface of 2-4 week old cultures. The contents of five (5) six day old cultures were used as homogenizing material. The pH of the medium was 6.50. The inoculated flasks were placed in a $24.4^{\circ} \pm 1^{\circ}$ C. temperature cabinet with two flasks removed from each shelf as required for weight determinations. Observations on growth were made daily.

After two days the first visible sign of growth was observed to be in the form of small, submerged colonies. Slight surface growth and the initiation of mycelial massing in the form of small tufts were observed on the third day. With the formation of aerial hyphae, growth appeared to be extremely rapid and on the fourth and fifth days, the surface of the medium was completely covered with a felt of light green mycelial growth. At this stage, the cultures resembled those of a contaminant, as noted by Ramsey (46). Dark colored appressoria were formed as a thick ring on the walls

of the culture vessel. Growth continued in this way, with the surface of the colony becoming densely covered with light green masses of mycelial tufts (Figure 2). In all cultures, sclerotia failed to form.

Dry weights of mycelium of S. sclerotiorum increased rapidly after the third day of growth, reaching a maximum average dry weight of 49 mg. on the eighth day (Table 4, Graph I).

TABLE 4

MICROCONIDIAL GROWTH OF S. sclerotiorum IN LIQUID MEDIA AT 24.4° C.

Replicate	Time in days						
	3	4	5	6	8	10	12
1	1.9*	11.7	12.8	29.4	37.9	40.0	45.4
2	3.3	6.7	20.7	35.4	39.0	44.7	42.7
3	2.7	13.3	19.1	35.0	51.9	41.0	46.7
4	3.6	14.1	11.7	46.6	36.5	41.1	45.9
5	2.7	12.2	22.2	30.7	58.2	38.5	55.6
6	1.9	5.5	21.7	28.7	70.1	39.9	54.2
Total	16.1	63.5	108.2	205.8	293.6	245.1	290.5
Average	2.7	10.6	18.0	34.3	48.9	40.8	48.4

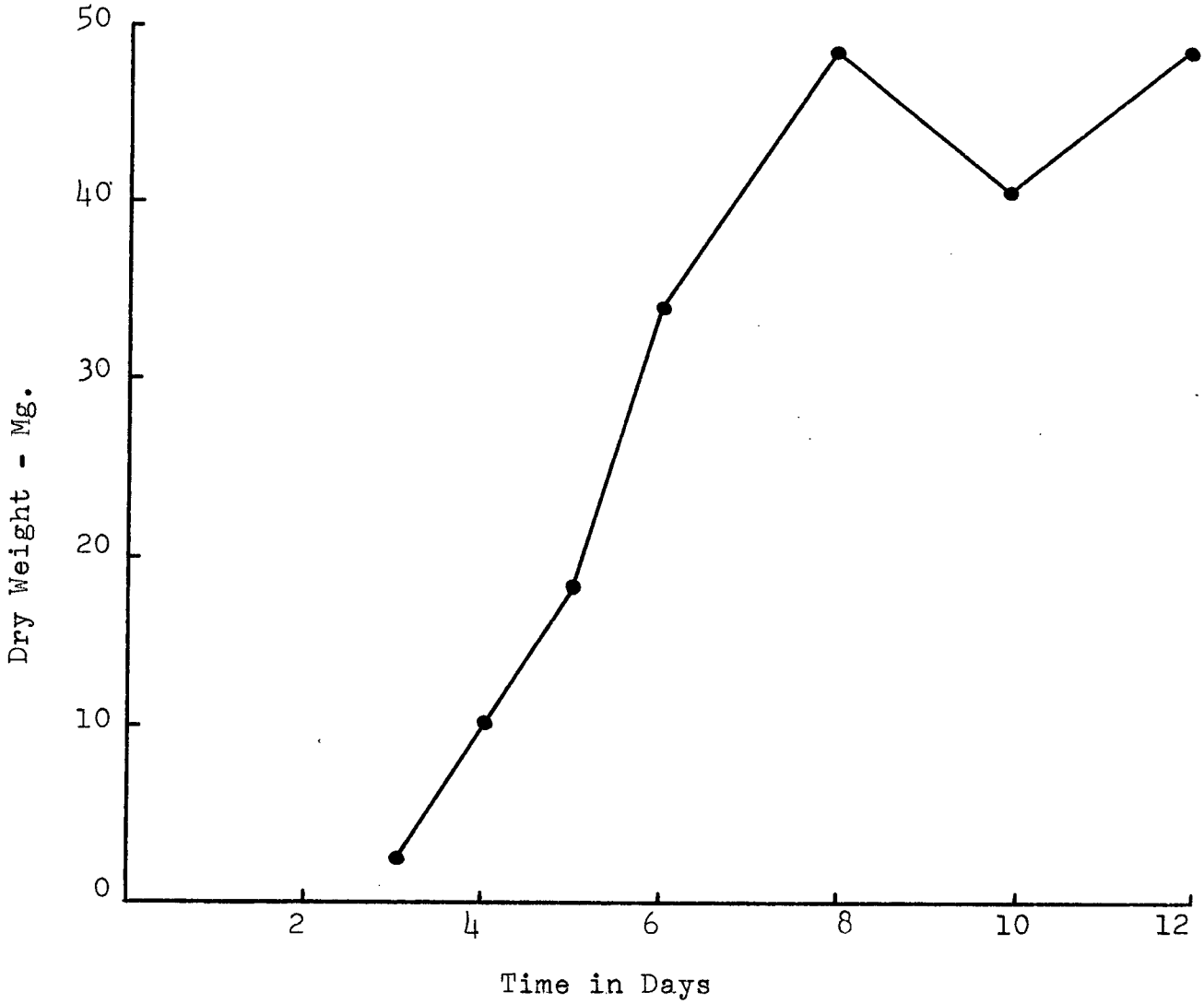
*milligrams dry weight.

The period of rapid growth between the third and eighth days was initiated when aerial mycelia were formed on the surface of the liquid medium. Formation of tuft-like masses of

Plate 2.

Figure 2. Microconidial growth of S. sclerotiorum in liquid culture at 20°C. Left, growth after 6 days. Center, growth after 10 days. Right, growth after 17 days.

GRAPH I



Microconidial Growth of
Sclerotinia sclerotiorum
in Liquid Culture at 24°C.

hyphae increased steadily also, after the third day of incubation. The effect of a temperature gradient in the incubator on growth was not reflected in the dry weights of the two cultures removed from each level in the incubator. Variation among replicates was slight except for the sixth replicate on the eighth day of growth. The average of the six replicates was, therefore, high and tended to indicate that the greatest amount of mycelial and microconidial production was on the eighth day.

The effect of temperature on growth

The growth of S. sclerotiorum at different temperatures in liquid media has not been recorded, although much work has been done using linear measurements on agar media (11, 28, 36, 46, 54). To compare the microconidial growth of S. sclerotiorum with mycelial growth and to gain an understanding of the effect of temperature on mycelial growth, further experiments were carried out using temperatures of 16°, 20° and 24° C. Inoculum was taken from eight cultures, grown for seven days at 24° C. One culture of inoculum was forming aerial tufts of mycelia on the surface of the liquid medium. After homogenation in a Waring blender, large pieces of massed mycelia were seen suspended in the homogenate. The pH of the medium inoculated was 6.51. Temperature gradients in each incubator were 24° ± 1.2° C. and 16° ± 0.6° C. Cultures were harvested at various intervals in the growth of the organism so as to obtain a

quantitative measurement of growth during the period of rapid formation of vegetative structures.

1) Growth habit

In general the formation and development of vegetative structures in stationary culture took place faster at 24° rather than at 20° or 16° C. (Tables 5, 6 and 7). The differences between 24 and 20° C. with regard to the time of formation of different vegetative stages were slight, being a matter of one day. Growth and development at 16° C. was decidedly slower, with formation of all individual vegetative stages approximately four days later than at 24° C.

The incubation period required at 24° C. for the formation of mature black sclerotia was eight days. The development of mature sclerotia followed a definite sequence of events, which was also observed in the 20° and 16° C. cultures.

Growth of mycelial fragments after inoculation was submerged since homogenized mycelial particles were either in suspension or had settled to the bottom of the culture vessel. Growth initially was in the form of small separate colonies, but after three days, all colonial growth became amalgamated into one large, coherent colony which continued to grow submerged. At this stage no appressoria were observed on the walls of the culture vessel. Aerial hyphae were produced on the fifth day. This marked the beginning

TABLE 5

GROWTH HABIT OF *S. sclerotiorum* IN STILL LIQUID CULTURE AT 24°C. (Solid line (-) indicates absence of structure, solid cross (+) indicates active formation of structure)

Growth characteristics	Time in days											
	1	2	3	4	5	6	7	8	9	10	11	12
Submerged growth	+											
Surface growth or aerial mycelia	-	-	-	-	+							
Mycelia massing on surface	-	-	-	-	-	+						
Formation of round bodies	-	-	-	-	-	+						
Extrusion of water from round bodies	-	-	-	-	-	-	+					
Darkening of round bodies	-	-	-	-	-	-	+					
Formation of black sclerotia	-	-	-	-	-	-	-	+				

TABLE 6

GROWTH HABIT OF *S. sclerotiorum* IN STILL LIQUID CULTURE AT 20°C.

Growth characteristics	Time in days											
	1	2	3	4	5	6	7	8	9	10	11	12
Submerged growth	+											
Surface growth or aerial mycelia	-	-	-	-	-	+						
Mycelia massing on surface	-	-	-	-	-	+						
Formation of round bodies	-	-	-	-	-	-	+					
Extrusion of water from round bodies	-	-	-	-	-	-	+					
Darkening of round bodies	-	-	-	-	-	-	-	+				
Formation of black sclerotia	-	-	-	-	-	-	-	+				

TABLE 7

GROWTH HABIT OF S. sclerotiorum
IN STILL LIQUID CULTURE AT 16°C.

Growth characteristics	Time in days											
	1	2	3	4	5	6	7	8	9	10	11	12
Submerged growth	+											
Surface growth or aerial mycelia	-	-	-	-	-	-	-	-	+[a]			
Mycelia massing on surface	-	-	-	-	-	-	-	-	+[a]			
Formation of round bodies	-	-	-	-	-	-	-	-	-	+[b]		
Extrusion of water from round bodies	-	-	-	-	-	-	-	-	-	+[b]		
Darkening of round bodies	-	-	-	-	-	-	-	-	-	-	+[c]	
Formation of black sclerotia	-	-	-	-	-	-	-	-	-	-	-	+[d]

[a] observed in cultures on bottom shelf.

b " " " " " "

c " " " " " "

d " " " " " "

of rapid growth which culminated in the formation of mature black sclerotia. The formation of aerial hyphae was followed immediately by mycelial massing on the surface of the liquid medium. As the white surface mycelium was rapidly massing over several areas of the culture medium, the transformation of these flat-appearing masses of mycelium into round immature sclerotia was taking place. On the seventh day, after massing action of the mycelium had ceased and definite round shaped white sclerotia were formed, extrusion of water droplets from several areas of the sclerotia began (Figure 3). This process of emanation continued for two or more days depending on the temperature and the individual culture. Color changes in the white immature sclerotia took place as soon as, or immediately after, the formation of water droplets was initiated. Each sclerotium, in the process of becoming darker, changed from pure white through several hues of brown until finally a jet black, carbonaceous structure was formed on the surface of the fungus colony. The time required for formation of black sclerotia after the inception of aerial growth was four days. The liquid medium, in which the fungus was growing, did not change color but remained clear throughout the growth of the organism.

The growth and development of S. sclerotiorum at 20° C. (Table 6) was slightly slower than at 24° C. although, in general, differences between the two temperatures were not large enough to indicate the favorability of one temperature to another. Surface growth, formation and darkening of

Plate 3.

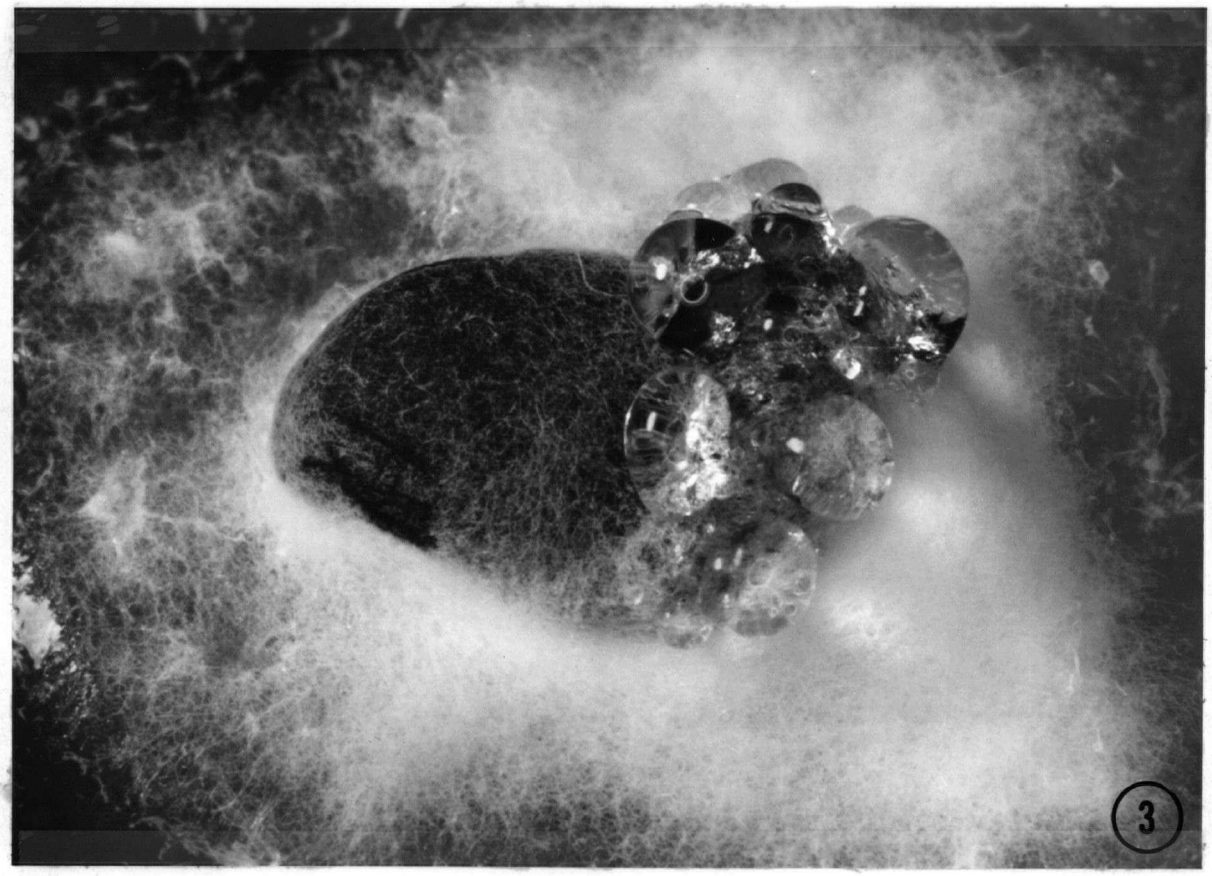


Figure 3. Extrusion of water droplets from an immature sclerotium developing immediately adjacent to a mature sclerotium (x10).

round bodies occurred one day later at 20° C. than at 24° C. In general the size, color and number of sclerotia formed at 20° C. was the same as at 24° C.

The pattern of development at 16° C. was similar to development at 20° and 24° C., with four days being required for the formation of black sclerotia after initiation of surface growth (Table 7). There were, however, variations among cultures situated at various levels in the incubator. In all cases the cultures on the lowest shelf showed signs of development before cultures on the middle or upper shelves because of the higher temperature at the bottom of the incubator where the heating element was located. Mature sclerotia were formed after twelve days on the lower shelf, while it was not until two days later that cultures on the upper and middle shelves were forming black sclerotia. This illustrates the sensitivity of S. sclerotiorum to slight changes in temperature at 16° C.

2) Rate of growth

Growth of S. sclerotiorum at 16°, 20° and 24° C. (Tables 8, 9 and 10), resulted in typical sigmoid curves (Graph II). In general cultures grown at 24° C. were harvested at one day intervals so as to obtain quantitative data on the growth of S. sclerotiorum during the period of maximum visible formation of vegetative structures in liquid culture. Cultures incubated at 20° C. were harvested every two days due to the slower rate of growth. Cultures incubated at 16° C. were harvested every three days after

TABLE 8

GROWTH OF *S. sclerotiorum* IN STILL CULTURE AT 24°C.

Replicate	Time in days						
	3	4	5	6	8	10	12
1	0.0*	0.8	5.7	17.9	72.6	82.5	69.0
2	0.2	1.0	6.1	29.5	73.5	61.2	80.7
3	0.2	1.6	8.9	25.7	84.5	85.6	90.6
4	0.2	2.1	10.0	31.2	94.6	85.9	86.2
5	0.3	0.5	9.4	38.6	67.1	93.3	99.1
6	0.5	1.5	14.9	58.5	85.4	88.7	89.5
Total	1.4	7.5	55.0	201.4	477.7	497.2	515.1
Average	0.2	1.3	9.2	33.6	79.6	82.9	85.9

*milligrams dry weight.

TABLE 9

GROWTH OF S. sclerotiorum
IN STILL CULTURE AT 20°C.

Replicate	Time in days						
	4	6	8	10	12	14	16
1	0.4*	11.9	19.5	69.6	90.1	69.6	76.0
2	0.2	4.9	57.6	75.2	54.2	76.4	69.8
3	0.1	12.1	59.6	67.0	77.7	73.5	77.5
4	0.7	11.4	46.4	52.9	76.2	73.2	74.5
5	0.6	13.3	83.2	87.3	90.8	101.3	87.0
6	1.9	31.8	59.8	76.2	86.2	78.6	87.5
Total	3.9	85.4	326.1	428.2	475.2	472.6	472.3
Average	0.7	14.2	54.4	71.4	79.2	78.8	78.7

*milligrams dry weight.

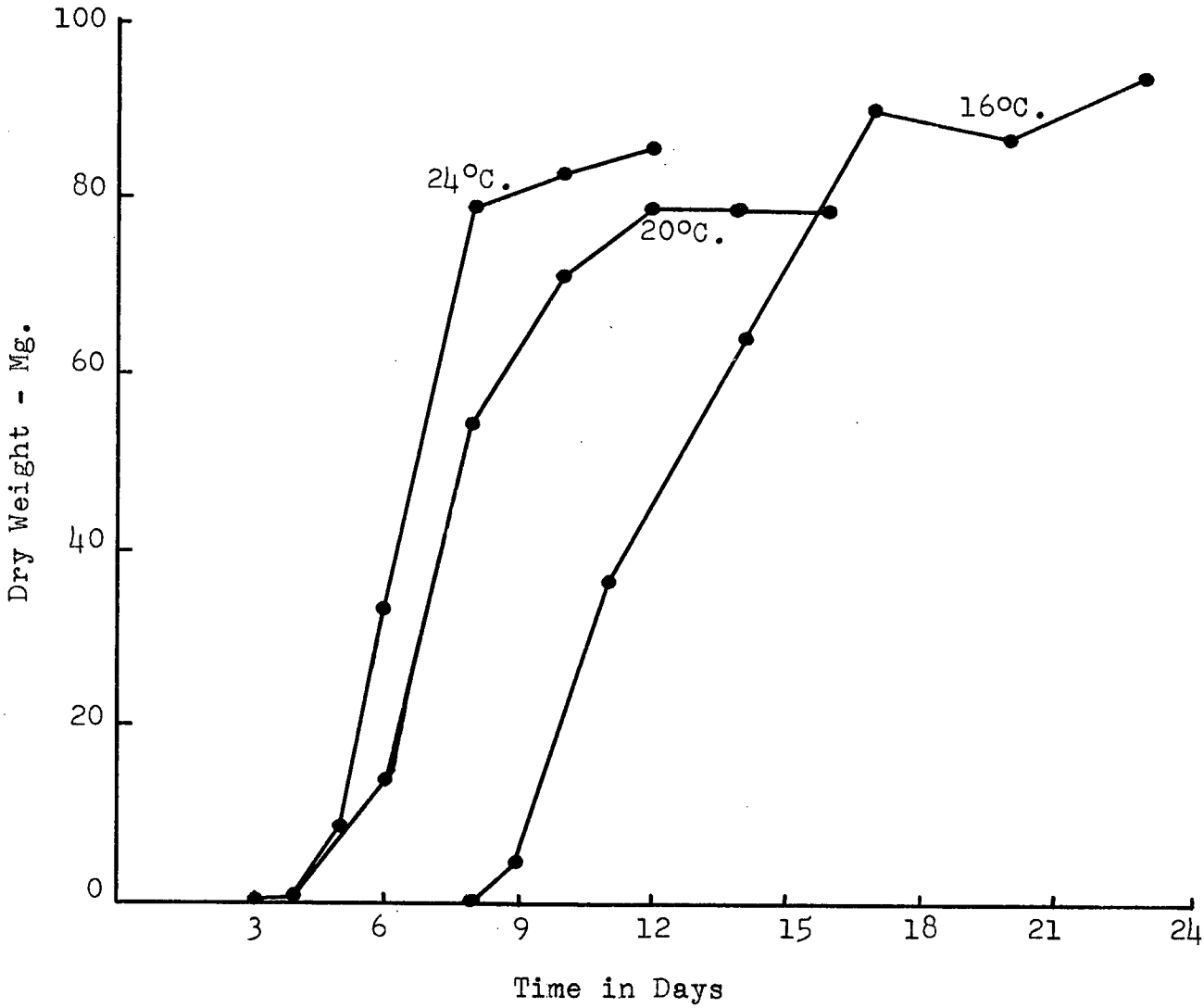
TABLE 10

GROWTH OF *S. sclerotiorum* IN STILL CULTURE AT 16°C.

Replicate	Time in days						
	8	9	11	14	17	20	23
1	0.5*	2.6	40.0	55.2	100.4	65.5	87.8
2	0.6	2.7	7.6	88.8	88.6	88.0	105.7
3	0.7	2.3	42.9	41.6	81.2	73.6	111.5
4	0.7	5.7	30.9	64.6	102.7	107.4	88.0
5	0.9	9.5	55.5	69.1	94.7	95.2	62.5
6	2.8	7.2	41.4	65.1	72.9	89.2	105.2
Total	6.2	30.0	218.3	384.4	540.5	518.9	560.7
Average	1.0	.5.0	36.4	64.1	90.1	86.5	93.5

*milligrams dry weight.

GRAPH II



The Effect of Temperature on the Growth of Sclerotinia sclerotiorum in Liquid Culture

the first three determinations were made at one and two days apart. The organism grew much faster at 24° than at 20° or 16° C. as indicated by the respective dry weight yields of 85.9, 79.2 and 45.0 mg. after twelve days. Rapid increases in dry weight at 24° C. from the fifth to the eighth day of incubation (Table 8) corresponded with the formation of vegetative structures for the same period (Table 5). Maximum growth of S. sclerotiorum at 24° C. was reached after twelve days. A further record of growth after the twelfth day was not obtained due to the lack of incubator space and the lengthy procedure for the harvest of mycelial growth.

Maximum yields of 79.2, 78.8 and 78.7 mg. were obtained from growth at 20° C. after 12, 14 and 16 days respectively. The maximum amount of growth, although less than that obtained at 24° C. was reached in the same period of incubation. The formation of vegetative structures took place between the sixth and eighth days of incubation as the dry weights increased from 14.2 to 54.4 mg. S. sclerotiorum continued to increase in dry weight after mature sclerotia were produced. Yields of 71.4 and 79.2 mg. were obtained on the tenth and twelfth days, an increase of 17.0 and 24.8 mg. respectively after the eighth day.

The rate of growth of S. sclerotiorum at 16° C. was considerably retarded. After eleven days of incubation only 36.4 mg. of mycelium was produced. As in the 20° C. cultures, growth continued to increase for five days after

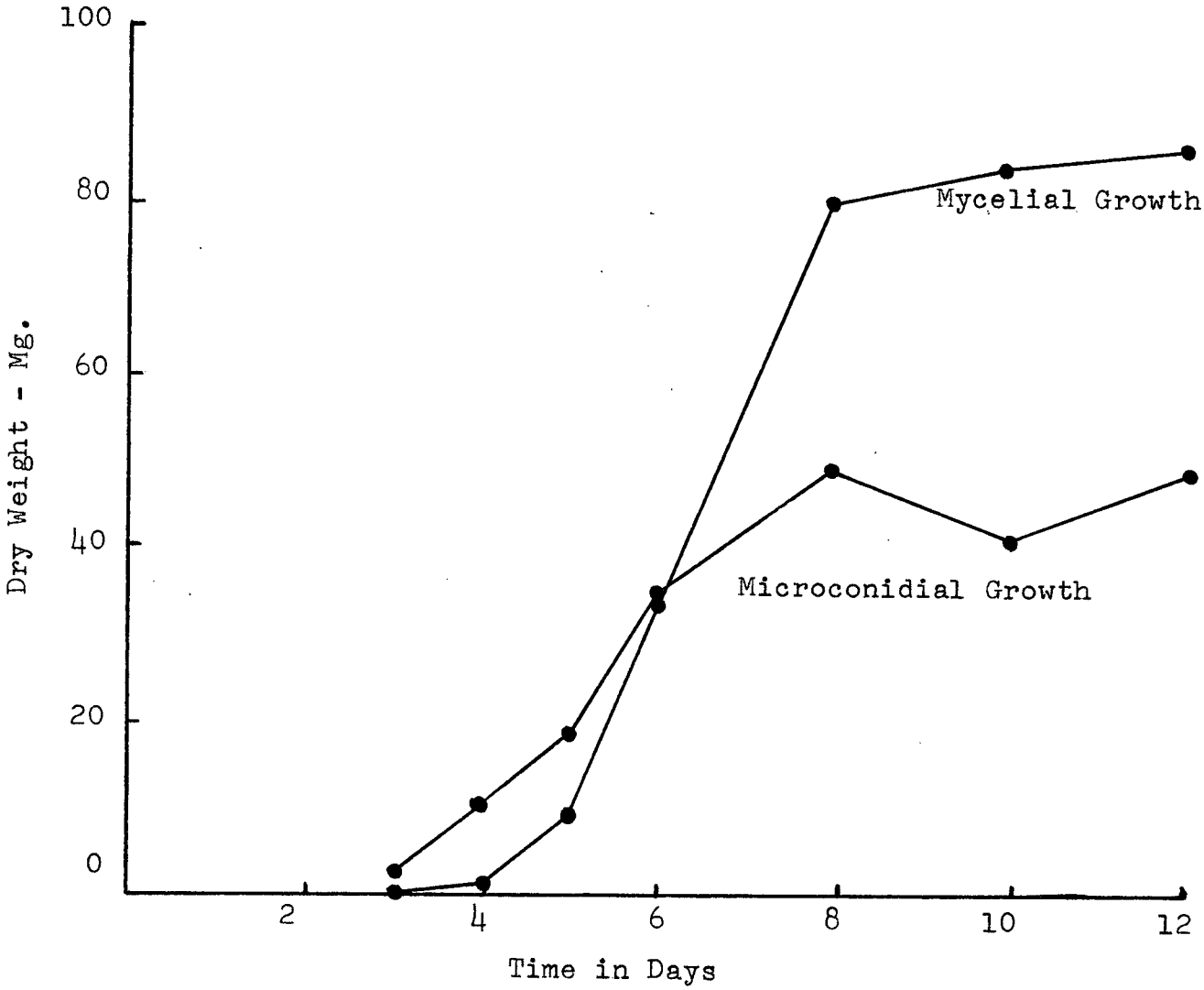
mature sclerotia were formed. Although fluctuations in dry weights were observed, maximum yields were obtained after the seventeenth day of growth.

The rate of growth of S. sclerotiorum while producing microconidia was faster than mycelial growth for the first six days of incubation (Graph III). At this point the rate of growth of the mycelial stage increased, reaching a maximum dry weight of 85.9 mg. as compared to 48.4 mg. for the microconidial growth. The final dry weight of the mycelial growth after twelve days was 77.4% greater than that of the microconidial growth.

The effect of pentachloronitrobenzene on growth

The effect of PCNB suspended in agar on the growth of S. sclerotiorum has been evaluated (29). The use of radial measurements for growth of fungi on agar media, however, has been criticized (4, 13, 45). It was decided, therefore, to evaluate the effects of PCNB on the growth of S. sclerotiorum by mixing the fungicide in liquid media and assaying the growth gravimetrically. Inoculum was taken from nine identical cultures grown for six days at 24° C. The contents were homogenized for 45 seconds in a Waring blender and 1 ml. was pipetted to 25 mls. of sterile liquid media containing the prepared fungicide. The pH of the medium inoculated was 6.05. Cultures were incubated at room temperature (20° - 24° C.) in still culture and on a rotary shaker for ten to twelve days. The mechanical shaking

GRAPH III



Microconidial Growth of
Sclerotinia sclerotiorum
Compared to Mycelial Growth
in Liquid Culture at 24°C.

device had a shaker velocity of 120 cycles per minute. Cultures were removed as desired for centrifugation and dry weight determination.

1) Growth habit

a) Still culture

The effect of PCNB at 250 and 500 p.p.m. acted principally to retard the formation and development of vegetative structures in culture (Tables 11, 12 and 13). The process of growth from the initiation of surface mycelia to the formation of mature black sclerotia took four days to complete in the control, six days in the 250 p.p.m. PCNB treatment and five days in the 500 p.p.m. PCNB treatment. The degree of inhibition of vegetative growth by PCNB depended mainly on the concentration of the fungicide. In treated cultures the growth of the homogenized inoculum was inhibited for several days. Mycelial fragments grew abnormally, forming very small separate colonies which grew slowly near the bottom of the culture vessel. The initial surface growth was very sparse as the mycelium formed one or two small surface colonies (Figure 4). In most cases the formation of sclerotia and other structures were delayed from three to five days by both concentrations of PCNB. The extrusion of water from sclerotia was more pronounced in fungicide treated cultures than in control cultures. The extruded water droplets were larger in size and remained on the sclerotium longer than in untreated cultures. As each sclerotium extruded water the color changed

Plate 4.

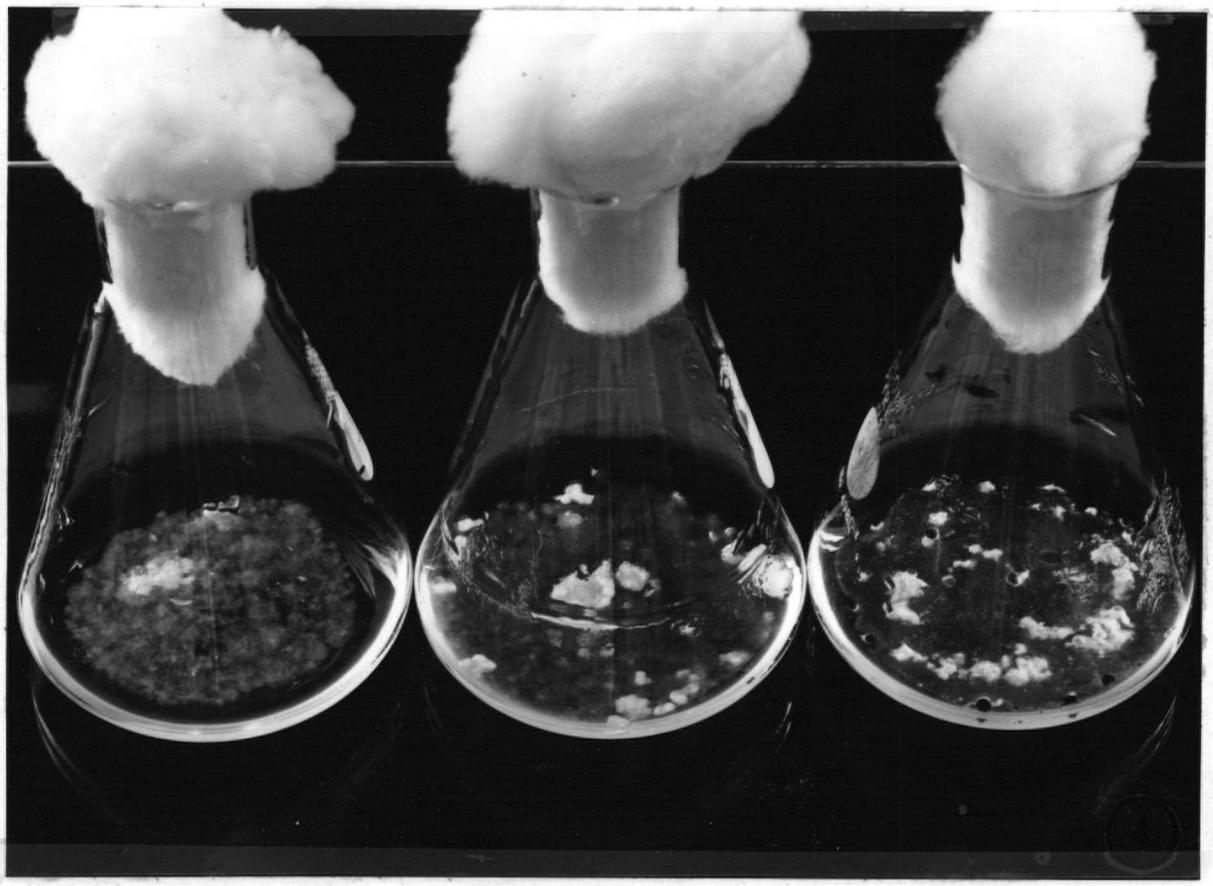


Figure 4. The effect of PCNB on S. sclerotiorum grown for 7 days in liquid culture at room temperature (20° - 24°C.). Left, 500 p.p.m. PCNB; growth mainly submerged. Centre, 250 p.p.m. PCNB; mycelial massing over several areas of colony, mature sclerotia absent. Right, 0 p.p.m. PCNB; several mature sclerotia formed on surface colony.

TABLE 11

GROWTH HABIT OF *S. sclerotiorum* IN STILL CULTURE AT ROOM TEMPERATURE (20° - 24°C.)
PCNB = 0 p.p.m.
(Solid line (-) indicates absence of structure, stippled cross (÷) indicates initial formation and solid cross (+) indicates active formation)

Growth characteristics	Time in days							
	1	4	5	6	7	8	9	10
Submerged growth	+							
Surface growth or aerial mycelia	-	+						
Mycelia massing on surface	-	-	+					
Formation of round bodies	-	-	+					
Extrusion of water from round bodies	-	-	÷	+				
Darkening of round bodies	-	-	-	+				
Formation of black sclerotia	-	-	-	-	+			

TABLE 12

GROWTH HABIT OF *S. sclerotiorum* IN STILL CULTURE AT ROOM TEMPERATURE (20° - 24°C.) PCNB = 250 p.p.m.

Growth characteristics	Time in days									
	1	4	5	6	7	8	9	10	11	12
Submerged growth	+									
Surface growth or aerial mycelia	-	-	-	-	+					
Mycelia massing on surface	-	-	-	-	-	+				
Formation of round bodies	-	-	-	-	-	-	+	+		
Extrusion of water from round bodies	-	-	-	-	-	-	+	+		
Darkening of round bodies (steel grey)	-	-	-	-	-	-	-	+	+	
Formation of black sclerotia	-	-	-	-	-	-	-	-	-	+

TABLE 13

GROWTH HABIT OF *S. sclerotiorum* IN STILL CULTURE AT ROOM TEMPERATURE (20° - 24°C.) PCNB = 500 p.p.m.

Growth characteristics	Time in days									
	1	4	5	6	7	8	9	10	11	12
Submerged growth	+									
Surface growth or aerial mycelia	-	-	-	-	-	±	+			
Mycelia massing on surface	-	-	-	-	-	-	-	+		
Formation of round bodies	-	-	-	-	-	-	-	±	+	
Extrusion of water from round bodies	-	-	-	-	-	-	-	±	+	
Darkening of round bodies	-	-	-	-	-	-	-	-	±	+
Formation of black sclerotia	-	-	-	-	-	-	-	-	-	+

very slowly from white to black. The characteristic golden-brown color of untreated sclerotia was not observed in sclerotia grown in the treated solutions. Instead, a grey color was observed which gradually became darker until the black color characteristic of normal sclerotia became evident. The number of sclerotia was visibly reduced in the treated versus the control cultures.

b) Shake culture

After forty-eight hours incubation on the rotary shaker, growth in each flask was observed to be in the form of a small (2 mm.) aggregated, submerged mycelial colony. Growth was slow and it was difficult to distinguish between the size of the treated and the untreated cultures. The form of growth in the shake cultures was of two types. One was a circular, flattened, disk-like object with feathery radiating flanges of mycelium. The other resembled a solid mass of mycelium with smooth edges from the repeated tumbling in solution while being shaken. The latter cultures often had a variety of shapes, none of which resembled the disk-like object with radiating mycelial growth described above. The color of each colony ranged from white to creamy white. In a few cultures, foaming was observed to take place toward the latter part of the incubation period.

2) Rate of growth

a) Still culture

PCNB inhibited the growth of S. sclerotiorum for the first six days of incubation (Table 14, Graph IV), but

after this period the organism was able to utilize the nutrients effectively and even appeared to be stimulated by the compound. Dry weights after four and six days were 8.0 and 48.4 mg. for the untreated cultures, whereas growth was not weighable after four days in both fungicide concentrations. After six days, dry weights of 5.9 and 4.2 mg. were obtained for the 250 and 500 p.p.m. PCNB treated cultures. On the eighth day of incubation, when the treated cultures were beginning to form aerial growth, the dry weights increased to 27.6 and 76.0 mg. for the 250 and 500 p.p.m. treatments. Cultures at both concentrations of PCNB continued to grow rapidly, surpassing the control after twelve days. Due to the trace amount of growth of the fungicide treated cultures after four days, weights were not taken. These cultures were harvested on the twelfth day of incubation. The data after twelve days indicate that S. sclerotiorum became adjusted to the initial toxicity of PCNB and grew linearly after a prolonged lag period.

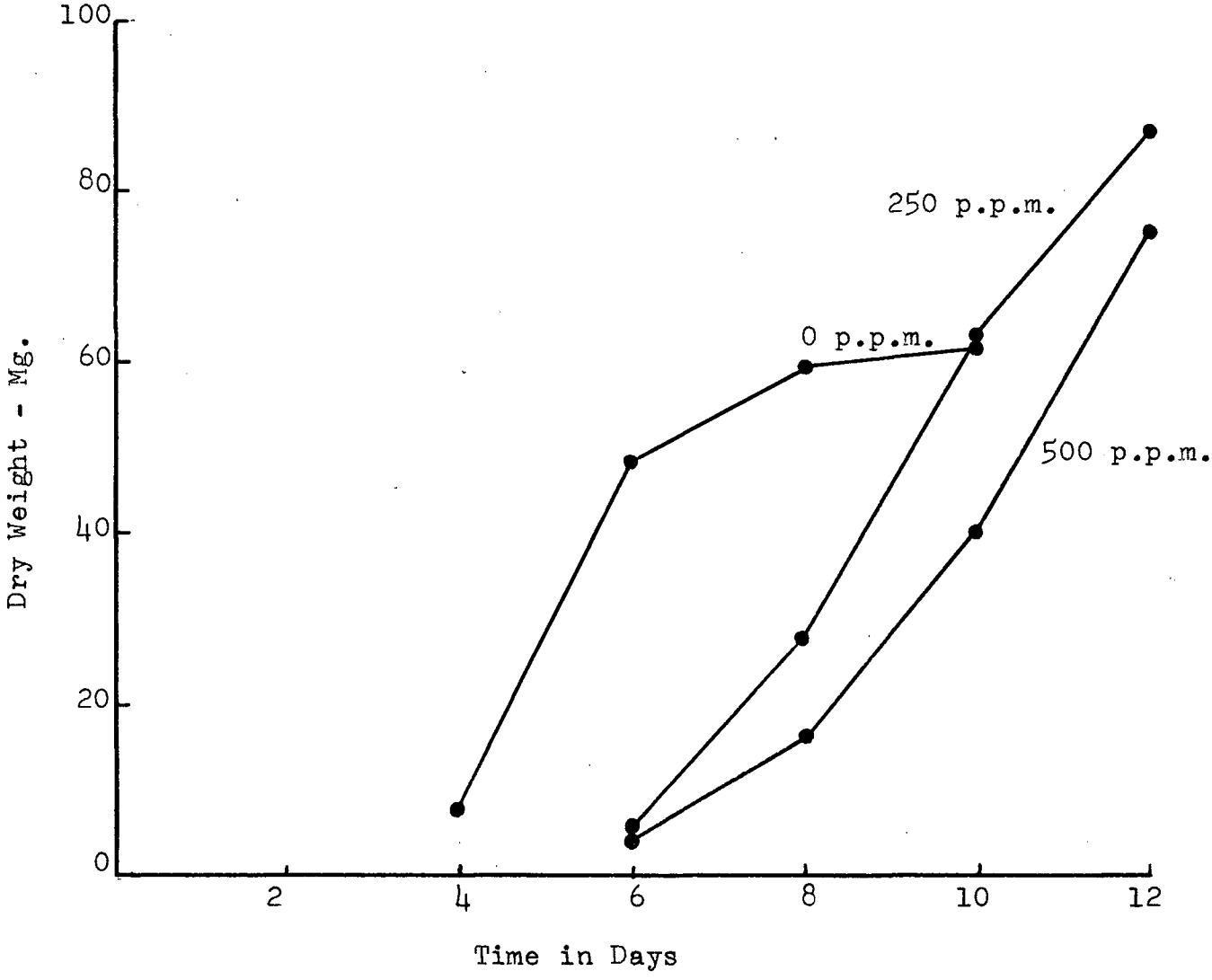
TABLE 14

THE EFFECT OF PCNB ON THE GROWTH OF S. sclerotiorum IN STILL CULTURE AT ROOM TEMPERATURE (20° - 24°C.)

Concentration of PCNB	Time in days				
	4	6	8	10	12
0 p.p.m.	8.0*	48.4	59.4	61.9	
250 "	trace	5.9	27.6	62.7	87.2
500 "	trace	4.2	16.0	40.5	75.0

*average of six replicates in milligrams.

GRAPH IV



The Effect of Pentachloronitrobenzene on the Growth of Sclerotinia sclerotiorum in Liquid Stationary Culture at Room Temperature (22° ± 2°C.)

b) Shake culture

The effect of PCNB on growth was more severe in shake culture (Graph V) than in the still culture experiments (Table 15). The control shake cultures continued to grow rapidly until the tenth day whereas growth had reached a peak in the still cultures. Yields were similar for the controls in both shake and still culture. Growth of the control was double that of the 250 p.p.m. treatment and four times the 500 p.p.m. treatment. Shaking of the culture media, therefore, appears to increase the efficiency of utilization of the fungicide. Yields of the 250 p.p.m. treatment after ten days for shake and non-shake incubation were 34.0 and 62.7 mg. respectively. Corresponding yields for the 500 p.p.m. PCNB treatment were 15.0 and 40.5 mg. The inhibitory action of PCNB, therefore, is appreciably increased by shaking the medium in which the organism grew.

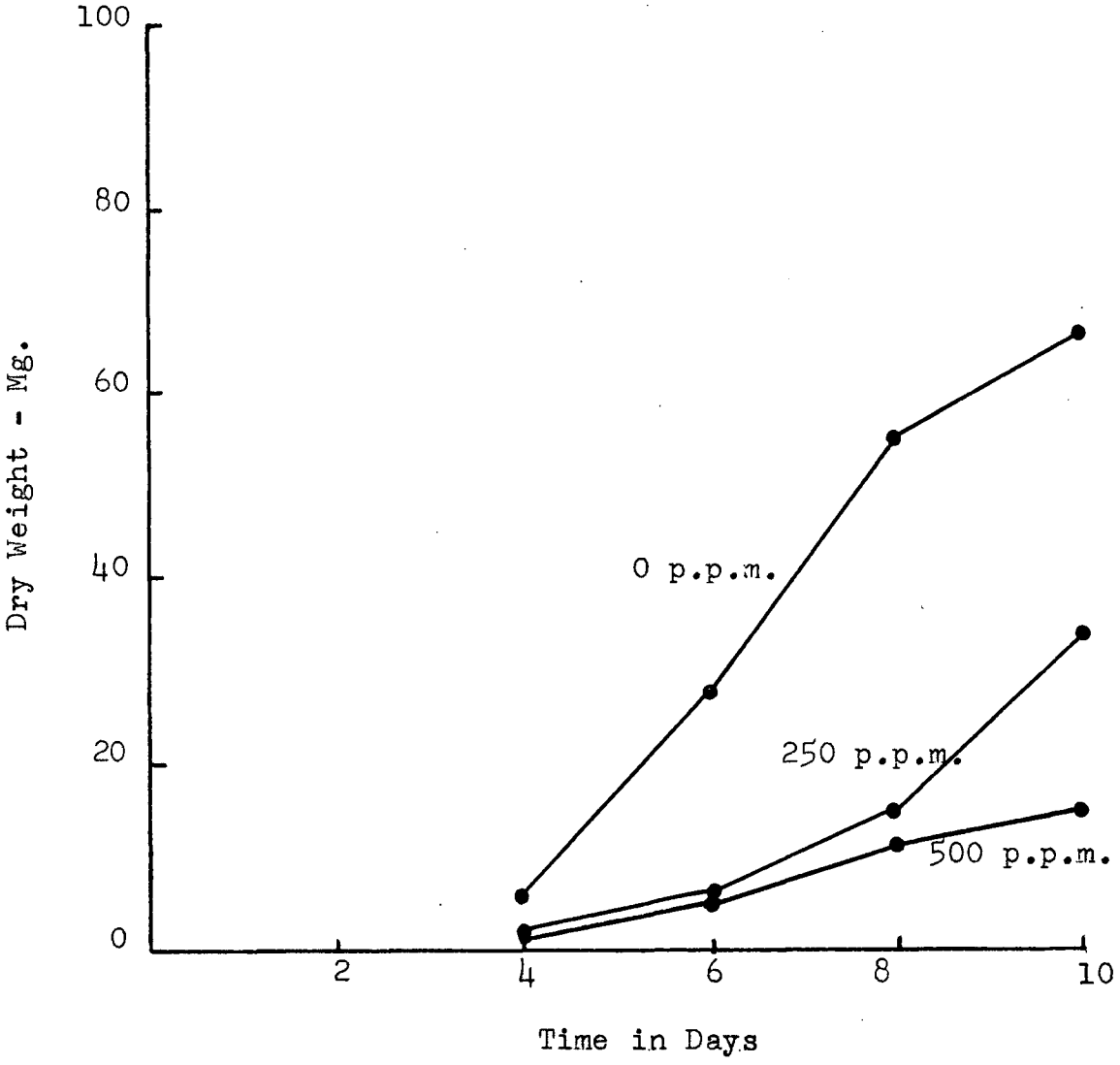
TABLE 15

THE EFFECT OF PCNB ON THE GROWTH OF S. sclerotiorum IN SHAKE CULTURE AT ROOM TEMPERATURE (20° - 24°C.)

Concentration of PCNB	Time in days			
	4	6	8	10
0 p.p.m.	6.0*	27.4	55.2	66.5
250 "	0.9	6.1	15.0	34.0
500 "	1.1	5.6	11.3	15.0

*average of six replicates in milligrams.

GRAPH V



The Effect of Pentachloronitrobenzene on the Growth of Sclerotinia sclerotiorum in Liquid Shake Culture at Room Temperature (22° ± 2°C.)

II. Pathogenicity Studies

Effect of temperature on pathogenicity

The effects of soil temperature on the pathogenicity of soil-borne inoculum of Sclerotinia spp. have been studied (12, 36), but in both instances the soil-inoculum ratio and the methods of inoculation were not given. Temperature often determines whether infection will take place (17). In the coastal area of the Lower Fraser Valley of British Columbia, S. sclerotiorum often causes 100% loss of beans and lettuce. The environment under these circumstances has been cool and moist. In order to investigate the temperature aspect of the problem, a number of experiments were conducted using large, temperature controlled, refrigerated incubators. Inoculum was mixed with unsterilized U.C. Mix soil to give a range of 2.5, 5.0, 7.5 and 10.0% inoculum densities on a weight/weight basis. Containers were placed in incubators at random. Seedling counts of the various treatments were made as soon as the number of emerged control seedlings became constant. Soil temperatures used were 6°, 9°, 12°, 15° and 18° C. To check for fungal attack, five to ten ungerminated seeds were removed from the treated soil, surface sterilized in 10% commercial chlorox solution for two minutes and plated on water agar. After two days, hyphal tips of fungal growth were transferred to tubes of potato-dextrose agar for identification.

The time required for the emergence of Penn Lake lettuce seedlings in uninoculated soil (Table 16) increased as the

soil temperature decreased. Several days were required after emergence until the number of emerged seedlings became constant. At all temperatures there was little variation among replicates.

TABLE 16

DAYS TO APPEARANCE OF PENN LAKE LETTUCE SEEDLINGS AT VARIOUS SOIL TEMPERATURES FROM SEED PLANTED AT A DEPTH OF 0.5 INCHES IN U.C. SOIL MIX C.

Temperature	Number of Days to emerge
18°C.	3
15 "	5
12 "	6
9 "	10
6 "	17

The effect of soil inoculum of S. sclerotiorum and soil temperature on the emergence of Penn Lake lettuce seedlings can be seen in Table 17 and Graph VI. From Table 17, several interesting points are clear.

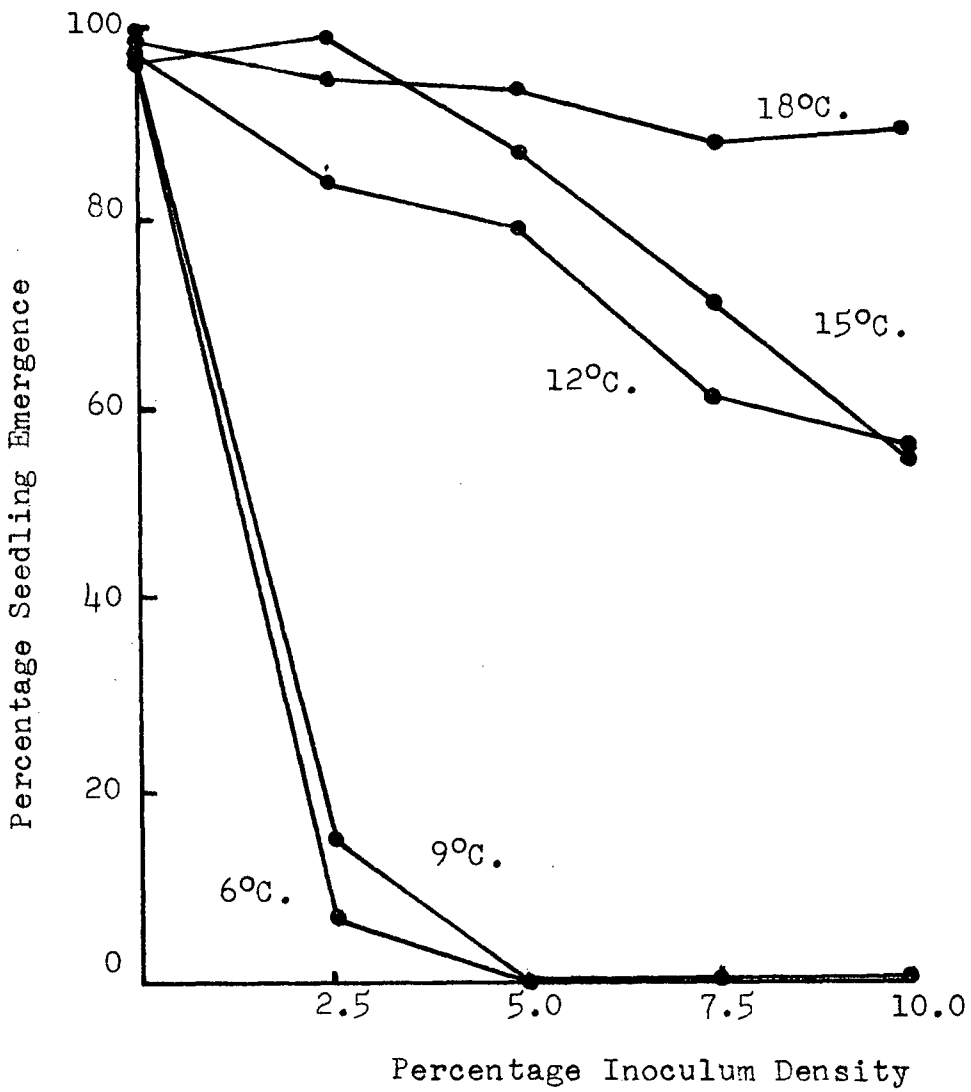
TABLE 17

EMERGENCE OF PENN LAKE LETTUCE SEEDLINGS AT DIFFERENT TEMPERATURES IN SOIL INFESTED WITH S. sclerotiorum

Inoculum density	Temperature				
	6°C.	9°C.	12°C.	15°C.	18°C.
0%	98*	98	98	97	99
2.5%	7	15	84	99	95
5.0%	0	0	79	87	94
7.5%	0	0	61	71	88
10.0%	0	0	56	55	89

*The total number of emerged seedlings from 100 seeds planted in five replicates.

GRAPH VI



The Emergence of Penn Lake Lettuce Seedlings at Different Temperatures in Soil Infested with Sclerotinia sclerotiorum

The germination and emergence of Penn Lake lettuce seed in the untreated soil (Figure 5), was 97 to 99% at all temperatures studied. This indicated that the seed was of high germination. As the soil inoculum increased the number of healthy emerged seedlings decreased. This was especially noticeable at temperatures of 6° and 9° C. However, as the temperature increased from 9° to 12° C., there was a large increase in the number of emerged seedlings even at the 10 per cent inoculum density. The pathogenicity of soil-borne inoculum of S. sclerotiorum was noted to be the highest at 6° C. although there were only slight differences between 6° and 9° C. Increase in soil temperature resulted in a decrease of pre-emergence damping-off, especially at 18° C. where pathogenicity was slight, even at the higher levels of inoculum density. Graph VI shows clearly, the above relationships.

In general there was little variation in the number of emerged seedlings among replicates of the various treatments. There were differences, however, among the various treatments with regard to seedling height. In high inoculum treatments seedlings of 0.5 to 1.0 cm. in height were observed to emerge much later than the majority of seedlings which were approximately 3.0 to 4.0 cm. in height. These stunted seedlings became more abundant with increasing soil-inoculum density. All emerged seedlings were etiolated due to growth in light-free incubators. Damping-off of emerged seedlings

(Figure 6) was rarely observed.

The growth of mycelia of S. sclerotiorum on the soil surface (Figure 7) was favored by high inoculum density and low soil temperatures. At temperatures of 6° and 9° C., the soil surface of each container was covered with white, fluffy, mycelial growth. With time the growth became dense and massing appeared to occur, however, sclerotia did not form. Mycelial growth on the surface of control soil was not observed.

Ungerminated seeds recovered from the soil generally appeared water soaked and necrotic (Figure 9). Examination of several seeds revealed the absence of tissues inside the seed coat. Fungi isolated most frequently were S. sclerotiorum and Penicillium spp. Seeds planted in and recovered from soils with high rates of inoculum generally gave S. sclerotiorum whereas Penicillium spp. and S. sclerotiorum were isolated equally at the lower inoculum densities.

Several seeds were found with small sclerotia attached to the seed (Figure 10, 11). These gave S. sclerotiorum when plated on agar.

Effect of PCNB and Terrazole on pathogenicity

Despite the recommended use of PCNB in the control of soil-borne diseases (32, 51), the fungicide has frequently failed to perform efficiently (14, 40, 52) and under certain circumstances highly unfavorable results were observed (20).

Plate 5.

Figure 5. Emergence of Penn Lake lettuce seedlings in uninoculated soil.

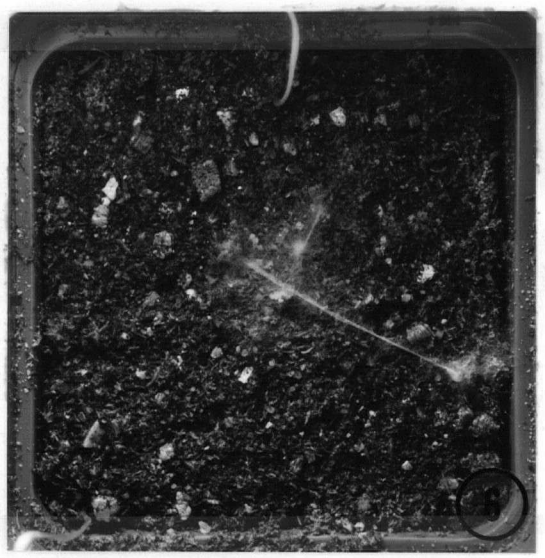


Figure 6. Damping-off of emerged Penn Lake lettuce seedlings.

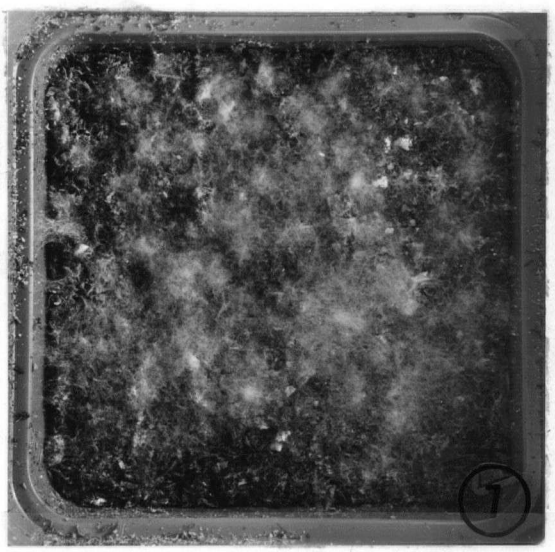


Figure 7. Mycelia of S. sclerotiorum on surface of soil incubated at 6°C.

Plate 6.

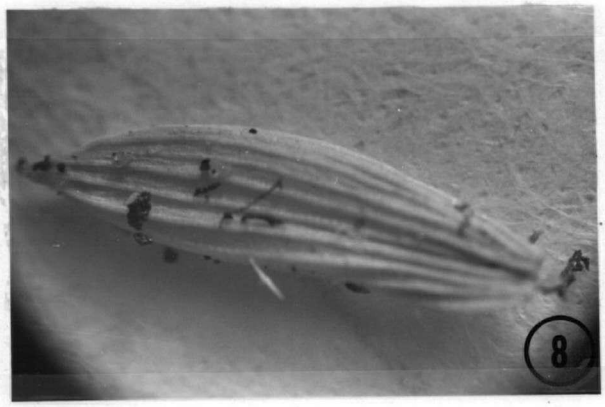


Figure 8. Healthy Penn Lake lettuce seed (x10).

Figure 9. Infected, necrotic lettuce seed with sclerotium at the right and mycelial tuft at the left (x10).

Figures 10 and 11. Infected lettuce seeds showing sclerotia being formed at various locations on the seed (x10).

Two experiments were devised to investigate the effects of PCNB and a newly introduced fungicide, Terrazole (39), on the pathogenicity of soil-borne inoculum of S. sclerotiorum. In the first experiment, PCNB at solution concentrations of 100, 200 and 400 p.p.m. was applied using a 5 ml. pipette to the surface of soil containing S. sclerotiorum and seeded with Penn Lake lettuce. The soil temperature was 12° C. Emergence counts were made when the number of emerged seedlings in the controls became constant.

In the second experiment, PCNB was applied as a drench treatment at a solution concentration of 800 and 1600 p.p.m. A third drench treatment, consisting of 800 p.p.m. PCNB and 1600 p.p.m. Terrazole, was applied in order to test the effect of PCNB and Terrazole in combination. All treatments were incubated at 12° and 15° C.

Experiment 1.

Table 18 and Graph VII show that the number of emerged seedlings decreases with increasing concentration of both PCNB and soil inoculum of S. sclerotiorum. The emergence of Penn Lake lettuce seedlings was not affected by any of the concentrations of PCNB used. Seedling emergence was decreased by 45.9 and 60.7% in soils containing inoculum densities of 7.5 and 10.0% and treated with 400 p.p.m. PCNB.

TABLE 18

EMERGENCE OF PENN LAKE LETTUCE SEEDLINGS IN SOIL INFESTED WITH *S. sclerotiorum* AND TREATED WITH PENTACHLORONITROBENZENE

Inoculum density	Concentration of PCNB in p.p.m.			
	0	100	200	400
0%	98*	100	97	99
2.5%	84	87	85	90
5.0%	79	72	72	66
7.5%	61	65	52	33
10.0%	56	48	44	22

*The total number of emerged seedlings from 100 seeds planted in five replicates.

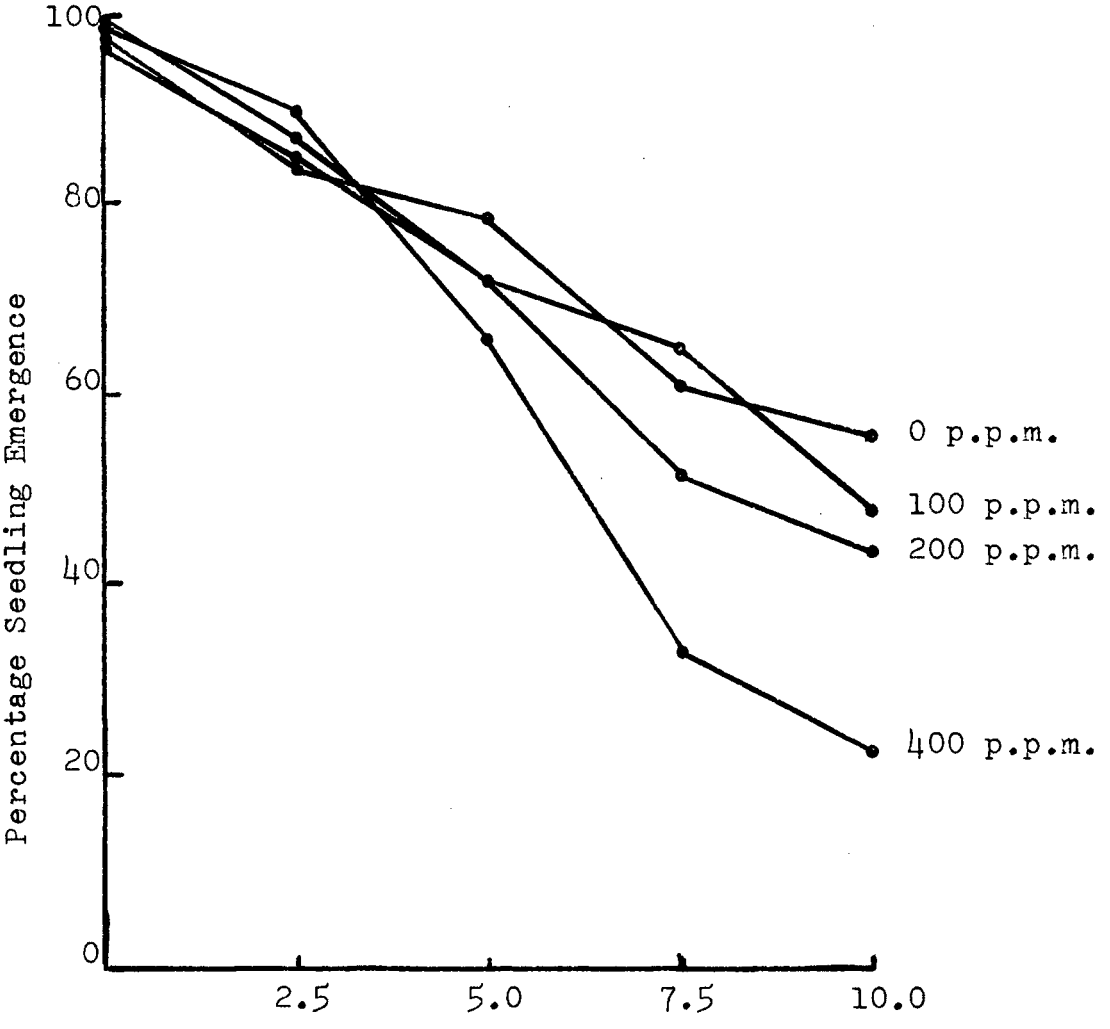
Experiment 2.

Drench treatments with the higher concentrations of PCNB and the PCNB-Terrazole combination gave erratic results. Several features of interest, however, were noted. The number of emerged seedlings at 15° C. was greater than at 12° C. Anomalous effects of the fungicides were apparent, but were not as severe as treatments in Experiment 1.

Phytotoxicity of PCNB and Terrazole on lettuce seedlings

Phytotoxic symptoms on lettuce plants have largely been due to application of PCNB dusts or sprays to the mature plants (14, 53). The effects of fungicides such as PCNB on the growth of lettuce plants has not been determined. The effect of drench applications of PCNB and PCNB-Terrazole on

GRAPH VII



The Emergence of Penn Lake Lettuce Seedlings in Soil at 12°C. Infested with *Sclerotinia sclerotiorum* and Treated with Pentachloronitrobenzene

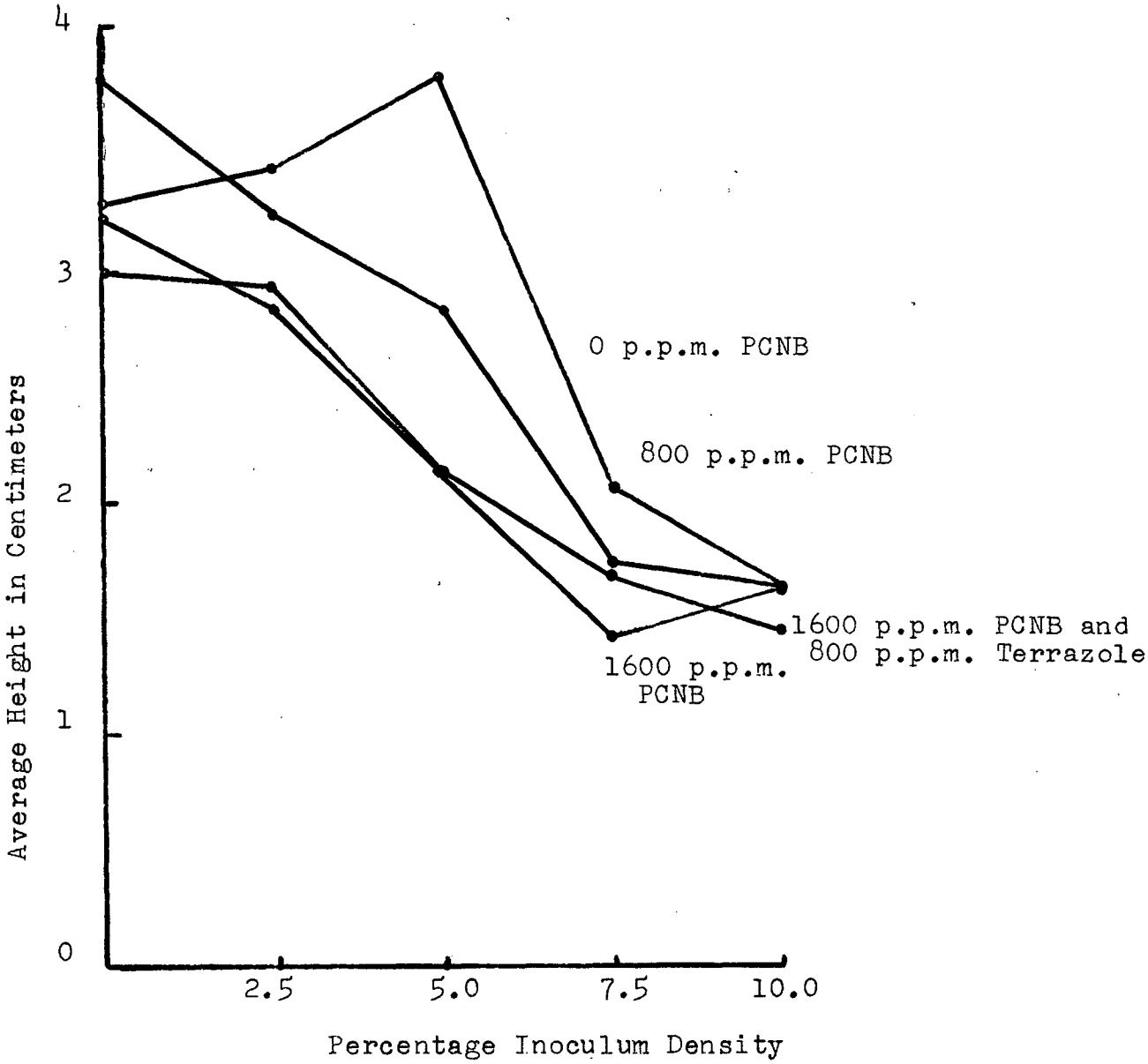
the seedling height of Penn Lake lettuce, grown in soil containing S. sclerotiorum, was determined. Containers were incubated at 15° and 12° C. for 6 and 8 days respectively. Heights were measured when the emergence counts of control treatments became constant.

Despite variation in the 800 p.p.m. PCNB treatment, the fungicides generally reduced the height of the lettuce seedlings grown in various inoculum densities of S. sclerotiorum (Graph VIII). The pathogen retarded the growth of the seedlings as indicated by heights of 3.27, 3.40, 2.80, 2.08 and 1.63 cm. for the 0, 2.5, 5.0, 7.5 and 10.0% soil inoculum densities. The data show that the seedling height was increased by 800 p.p.m. PCNB at both temperatures. This is probably not a realistic observation because all other treatments inhibited slightly the seedling height.

The effect of Trichoderma on pathogenicity

Biological control of soil-borne disease has revolved around pioneer studies involving the soil saprophyte Trichoderma viride (59). Since Weindling's work in 1932, much research has revealed that the success or failure of experiments involving one organism against another often depends on their respective inoculum potential (17, 18). Species of Trichoderma have been observed to attack sclerotia of S. sclerotiorum (26), however inhibition of mycelial growth in soil has not been demonstrated. An investigation into the effect of temperature on the emergence of Penn Lake

GRAPH VIII



The Average Height of Penn Lake Lettuce Seedlings Grown in Soil Infested with Sclerotinia sclerotiorum and Treated with PCNB and Terrazole

lettuce seedlings, in various combinations of soil inoculum densities of S. sclerotiorum and a Trichoderma sp., was made. Treatments were incubated at temperatures of 6°, 9°, 12° and 15° C. Emergence counts were made when the number of emerged seedlings in the control treatments became constant. In treatments where seedling emergence was low, ungerminated seeds were recovered from the soil and plated on water agar in order to determine the causal agent.

In general the effect of increasing inoculum density of the Trichoderma sp. on reducing the pre-emergence kill of lettuce seeds by S. sclerotiorum was obscured by variation among treatments (Tables 19, 20, 21, 22). Seedling emergence at 15° C. (Table 19) was notably increased at all inoculum densities of the Trichoderma sp. At 12° C., (Table 20) the addition of various amounts of inocula of Trichoderma resulted in a slight increase in seedling emergence, however results among treatments were erratic. At temperatures of 6° and 9° C., (Tables 21 and 22) no appreciable increase in emergence was observed with increasing rates of inoculum of the Trichoderma sp. Frequent isolation of the Trichoderma sp. from ungerminated lettuce seeds indicates that the organism was able to compete successfully for the dead substrate. Trichoderma alone did not infect or inhibit the Penn Lake lettuce seeds or seedlings.

TABLE 19

EMERGENCE OF PENN LAKE LETTUCE SEEDLINGS GROWN FOR 7 DAYS AT 15°C. IN VARIOUS SOIL INOCULUM DENSITIES OF *S. sclerotiorum* AND *Trichoderma* sp.

Percentage inoculum density of *S. sclerotiorum*	Percentage inoculum density of *Trichoderma* sp.				
	0	2.5	5.0	7.5	10.0
0	97*	98	99	98	97
2.5	99	99	93	96	97
5.0	87	93	82	93	95
7.5	71	89	76	93	82
10.0	55	82	79	78	90

*the total number of emerged seedlings from 100 seeds planted in five replicates.

TABLE 20

EMERGENCE OF PENN LAKE LETTUCE SEEDLINGS GROWN FOR 9 DAYS AT 12°C. IN VARIOUS SOIL INOCULUM DENSITIES OF *S. sclerotiorum* AND *Trichoderma* sp.

Percentage inoculum density of *S. sclerotiorum*	Percentage inoculum density of *Trichoderma* sp.				
	0	2.5	5.0	7.5	10.0
0	99*	98	100	100	99
2.5	91	88	91	96	88
5.0	88	80	82	93	83
7.5	72	77	62	68	79
10.0	58	59	56	66	63

*the total number of emerged seedlings from 100 seeds planted in five replicates.

TABLE 21

EMERGENCE OF PENN LAKE LETTUCE SEEDLINGS GROWN FOR 15 DAYS AT 9°C. IN VARIOUS SOIL INOCULUM DENSITIES OF *S. sclerotiorum* AND *Trichoderma* sp.

Percentage inoculum density of *S. sclerotiorum*	Percentage inoculum density of *Trichoderma* sp.				
	0	2.5	5.0	7.5	10.0
0	98*	95	95	97	98
2.5	15	10	5	5	23
5.0	0	1	0	0	1
7.5	0	0	0	0	1
10.0	0	0	0	0	0

*the total number of emerged seedlings from 100 seeds planted in five replicates.

TABLE 22

EMERGENCE OF PENN LAKE LETTUCE SEEDLINGS GROWN FOR 23 DAYS AT 6°C. IN VARIOUS SOIL INOCULUM DENSITIES OF *S. sclerotiorum* AND *Trichoderma* sp.

Percentage inoculum density of *S. sclerotiorum*	Percentage inoculum density of *Trichoderma* sp.				
	0	2.5	5.0	7.5	10.0
0	98*	98	98	95	96
2.5	7	5	0	0	5
5.0	0	0	1	0	0
7.5	0	0	0	0	0
10.0	0	0	0	0	0

*the total number of emerged seedlings from 100 seeds planted in five replicates.

DISCUSSION

The above experiments have shown the effects of some factors on the growth and pathogenicity of S. sclerotiorum. Growth and development of vegetative stages of S. sclerotiorum in culture were affected by temperature and pentachloronitrobenzene. Pathogenicity of soil-borne inoculum was affected not only by temperature, pentachloronitrobenzene and Terrazole but by certain interactions with inoculum of a Trichoderma sp.

The microconidial growth of S. sclerotiorum, measured in terms of dry weight per 25 mls. of media, increased steadily until the eighth day of incubation. Maximum growth, although fluctuating slightly, was reached after the eighth day. Determinations of microconidial growth in quantitative terms have not been made before, possibly because, as Ramsey (46) has mentioned, the microconidial stage of growth of S. sclerotiorum has the appearance of a contaminant in culture. It was found that cultures retained for inoculum and left at room temperature for long periods of time, gave rise to microconidial growth upon transfer to fresh media. Ramsey (46) found that the age of the fungus in culture was the chief determining factor governing microconidial formation. Observations made during the course of the present work tend to support his evidence.

Mycelial growth in liquid media, as measured by dry weights, was most rapid at 24° C. The development of mycelia

and sclerotia in culture was slightly faster at 24° than at 20° C. Barnett and Lilly (4) found that mycelial growth of S. camelliae at 22° C. was approximately the same as at 25° C. They found that sclerotial production was favored by a temperature of 18° C., whereas in the present studies sclerotial formation was favored by a temperature of 24° C. although development at 20° C. was very similar. Growth at 16° C. reached a maximum weight greater than that obtained at 24° or 20° C., although the incubation period was much longer at 16° C. Due to a lack of time and space, the extension of incubation periods at 24° and 20° C. was not possible.

For the most part, temperature studies involving linear measurements of growth on various agar media are not agreeable (11, 23, 28, 36, 46). It appears, therefore, that accurate measurements of the effects of temperature on fungal growth cannot be obtained using both liquid and solid media. Both systems are highly artificial; however dry weights of growth in liquid media include the total mycelium produced whereas radial measurements do not account for the three-dimensional growth of the organism.

Microconidial growth was more rapid than mycelial growth at 24° C. until the sixth day of growth. After this time the mycelial stage continued to increase steadily whereas the dry weights of the microconidial growth increased only slightly. At any given time after the eighth day of incuba-

tion, the average weight of the mycelial growth per culture was 40 to 50% greater than microconidial growth.

Pentachloronitrobenzene affected the mycelial dry weight and time of sclerotial formation in duplicate experiments, in shake and stationary cultures at room temperature (20° - 24° C.). In still culture, dry weights of the control increased steadily with time reaching a maximum yield of 61.9 mg. after ten days of incubation. In the 250 and 500 p.p.m. PCNB treatment, growth was inhibited until the eighth day, after which dry weights increased steadily giving yields of 63.0 and 40.0 mg. respectively after ten days.

The inhibition of growth by PCNB in liquid shake culture was increased throughout the time of the experiment. After ten days of incubation the dry weight yields were as follows: control, 66 mg.; 250 p.p.m. PCNB, 34 mg. and 500 p.p.m. PCNB, 15 mg. These results indicate that the inhibitory action of PCNB on growth of S. sclerotiorum grown for ten days in liquid media is increased in shake culture as compared to still culture. It should be noted, however, that in all concentrations of PCNB, S. sclerotiorum in still culture was able to form sclerotia and produce mycelial growth which steadily increased with time.

The above results differ radically from those of Kendrick and Middleton (29), who found that 10 p.p.m. of PCNB suspended in potato-dextrose agar was fungistatic to the growth of S. sclerotiorum. The present experiments

have shown that PCNB is fungistatic for a short period of time, after which growth increases steadily. The term "fungistatic" may, therefore, be interpretated differently under different conditions. Under the conditions of the present studies, PCNB was found to be fungistatic for a short time, after which (in still culture) it became ineffective. Whether this was due to diffusion, or biochemical conversion of the compound, is not known. Betts et al. (7) found that when a 2 gram portion of PCNB was administered into a rabbit, 12% was reduced to form nitroaniline, a trace was hydroxylated to form phenol, 14% was converted to N-acetyl-S-pentachlorophenyl-L-cysteine via loss of a nitro group and 62% was unabsorbed.

The emergence of Penn Lake lettuce seedlings in soil infested with S. sclerotiorum was reduced with decreasing soil temperature. Pathogenicity of soil-borne inoculum on lettuce seeds was favored by soil temperatures below 12° C. Chamberlain (12) reported variation in emergence of soybean seedlings in field soil infested with cornmeal-sand inoculum of Sclerotinia sp. He found that at 15° C., emergence was 53% in the uninoculated pots and 87% in the control. Little difference in emergence was observed at 10° or 20° C.

The recovery of S. sclerotiorum from ungerminated seeds planted in soil inoculum constitutes evidence that the pathogen can invade healthy seed tissues. In only a few cases was damping-off of seedlings observed, therefore it is

unlikely that S. sclerotiorum causes damping-off in the field. Kerr (30), working with S. homeocarpa found that penetration of seedlings of beets, raddish and lettuce did not take place.

In general the soil fungicides PCNB and Terrazole were ineffective in reducing the pathogenicity of S. sclerotiorum on lettuce seeds and seedlings. With the addition of PCNB at 100, 200 and 400 p.p.m. to soil surfaces, the number of emerged seedlings became reduced well below that of the control. Gibson, et al. (20) found that losses due to a Pythium sp. increased after soil treatment with PCNB. He assumed that this was due to suppression of Penicillium paxilli, a common saprophyte in the soil. In the present studies, soil dilution plates were used to observe the microflora in the untreated U.C. Mix. For the most part, species of Penicillium appeared on the plates. It may be assumed then, that upon application of PCNB not only is the inoculum of S. sclerotiorum killed or inhibited at the surface but also the Penicillium spp. Inoculum particles immediately below the surface, however, being unaffected by the fungicide, could utilize the dead microorganisms as organic food bases in order to parasitize the lettuce seeds. Purdy (43) and Purdy and Grogan (44) have demonstrated the requirement of organic matter for infection of lettuce plants. Organic matter may also be required for infection of seeds.

The above experiments have indicated that the use of so-called "specific" soil fungicides such as PCNB may result

in unexpected and unwanted results.

Reduction in the height of seedlings grown in soil infested with S. sclerotiorum and drench treated with fungicides is due mainly to the toxic effect of the pathogen. Secretion of a toxic substance by S. homeocarpa in sufficient concentration in the soil to depress the growth of seedlings of lettuce and other plant species was observed by Kerr (30). Other factors affecting seedling growth in the present studies were the phytotoxic effects of the fungicides and the combination of the fungicides with various inoculum densities of S. sclerotiorum. Seedling heights were visibly reduced with increasing inoculum density of the pathogen. Application of fungicides to non-infested soil did not result in inhibition of seedling growth to the same extent as in the inoculated soil. Combination of fungicides with soil inoculum caused a marked depression in seedling height.

Various inoculum densities of the Trichoderma sp. failed to improve seedling emergence in soil containing S. sclerotiorum at 6°, 9° and 12° C. A favorable response was observed at 15° C., where numbers of seedlings increased with increasing inoculum densities of the Trichoderma sp. The data indicate that the Trichoderma species used does not grow well in soils at low temperature. The variation observed may have been due to uneven growth of the inoculum in the vermiculite media and hence uneven distribution in the soil. Variation in the soil used and mixing techniques may have

been contributing factors to the overall variation from treatment to treatment.

LITERATURE CITED

1. Allen, M.C. and C.M. Haenseler. Antagonistic action of Trichoderma on Rhizoctonia and other soil fungi. Phytopath. 25:244-252. 1935.

2. Baker, K.F., ed. The U.C. system for producing healthy container-grown plants. California Agr. Expt. Sta. Manual 23. 1957.

3. Baker, K.F. and L.H.Davis. An unusual occurrence of sclerotia of Sclerotinia spp. with seed of Centaurea cyanus. Plant Dis. Reptr. 35:39-41. 1951.

4. Barnett, H.L. and V.G.Lilly. The interrelated effects of vitamins, temperature and pH upon vegetative growth of Sclerotinia camelliae. Am. J. Bot. 35: 297-302. 1948.

5. Bedi, K.S. Studies on Sclerotinia sclerotiorum (Lib.) de Bary. I. Some chemical factors affecting the formation of sclerotia. Proc. Nat'l. Acad. Sci. India. Sect. B. 26:112-130. 1956. (Biological Abstr. 31:No. 35623).

6. Bedi, K.S. Effect of temperature on the formation of sclerotia of Sclerotinia sclerotiorum (Lib.) de Bary. Indian Phytopath. 15:55-60. 1962. (Abstr. Rev. Appl. Mycol. 42:100. 1963).

7. Betts, J.J., S.P.James and W.V.Thorpe. The metabolism of PCNB and 2, 3, 4, 6-tetrachloronitrobenzene and the formation of mercapturic acids in the rabbit. Biochem. J. 61:611-617. 1955.

8. Brown, J.G. and K.D.Butler. Sclerotiniose of lettuce in Arizona. Bull. Ariz. Agric. Expt. Stn. 63:475. 1936.

9. Burchfield, H.P. Performance of fungicides on plants and in soil - physical, chemical and biological considerations. vol. 3, p.477-520. In J.G.Horsfall and A.E.Dimond (ed.), Plant pathology, an advanced treatise. Academic Press, Inc., New York. 1960.

10. Campbell, W.A. A new species of Coniothyrium parasitic on sclerotia. Mycologia 39:190-195. 1947.

11. Cappellini, R.A. Field inoculations of forage legumes and temperature studies with isolates of Sclerotinia trifoliorum and S. sclerotiorum. Plant Dis. Reptr. 44:862-864. 1960.

12. Chamberlain, D.W. Sclerotinia stem rot of soybeans. Plant Dis. Reptr. 35:490-491. 1951.

13. Cochrane, V.W. Physiology of Fungi. Wiley, New York. 1958.

14. Darby, J.F. Soil and foliar treatments for the control of sclerotiniose of lettuce. Plant Dis. Reptr. 45:552-556. 1961.

15. Demetriades, S.D. Studies on the biology of Sclerotinia sclerotiorum (Lib.) Massee. III. The action of magnesium and sulphur on the development of the mycelia and the formation of the sclerotia. Ann. Inst. Phytopath. Benaki. 7:15-20. 1953. (Abstr. Rev. Appl. Mycol. 34:740. 1955).

16. Foster, J.W. Chemical Activities of Fungi. New York, Academic Press. 1949.

17. Garrett, S.D. Biology of Root-Infecting Fungi. Cambridge University Press, London and New York. 1956.

18. Garrett, S.D. Inoculum potential as a factor limiting lethal action by Trichoderma viride Fr. on Armillaria mellea (.r.) Quel., Trans. Brit. Mycol. Soc. 41:157-164. 1958.

19. Garrett, S.D. Decomposition of cellulose in soil by Rhizoctonia solani Kuhn. Trans. Brit. Mycol. Soc. 45:115-120. 1962.

20. Gibson, I.A.S., M.Ledger and E.Boehm. An anomalous effect of pentachloronitrobenzene on the incidence of damping-off caused by a Pythium sp. Phytopath. 51:531-533. 1961.

21. Gray, E.G. and W.T.Findlater. Sclerotinia sclerotiorum on peas in Kincardineshire. Plant Pathol. 9:130-133. 1960.

22. Grover, R.K. Effect of some fungicides on pectolytic enzyme activity of Sclerotinia sclerotiorum and Botrytis allii. Phytopath. 54:130-133. 1964.

23. Hansen, H.N. and H.E.Thomas. Flower blight of camellias. Phytopath. 30:166-170. 1940.

24. Held, V.M. Physiological differences between a normal and degenerate strain of Sclerotinia trifoliorum. Phytopath. 45:39-42. 1955.

25. Hendrix, F.F. Jr., and R.D.Raabe. Sclerotinia blight of gloxinia. Phytopath. 50:638. 1960.

26. Hino, I. and S. Endo. Trichoderma parasitic on sclerotial fungi. Ann. Phytopath. Soc. Japan. 10:231-241. 1940. (Abstr. Rev. Appl. Mycol. 20:492. 1941).

27. Horsfall, J.G. Principles of Fungicidal Action. Chronica Botanica, Waltham, Massachusetts. 1956.

28. Keay, M.A. A study of certain species of the genus Sclerotinia. Ann. Appl. Biol. 26:227-246. 1939.

29. Kendrick, J.B. Jr., and J.T.Middleton. The efficacy of certain chemicals as fungicides for a variety of fruit, root and vascular pathogens. Plt. Dis. Reptr. 38:350-353. 1954.

30. Kerr, A. Some interactions between plant roots and pathogenic soil fungi. Aust. J. Biol. Sci. 9:45-52. 1956.

31. Kreutzer, W.A. Soil treatment. vol. 3, p.431-476. In J.G.Horsfall and A.E.Dimond (ed.), Plant pathology, an advanced treatise. Academic Press, Inc., New York. 1960.

32. MacSwan, I.C. and R.L.Powelson. Oregon plant disease control handbook. Oregon State University, Corvallis. 1966.

33. Mandels, G.R. Kinetics of fungal growth. vol. 1, p.599-612. In G.C.Ainsworth and A.S.Sussman (ed.), The fungi, an advanced treatise. Academic Press. 1965.

34. Moore, W.D. Relation of rainfall and temperature to the incidence of Sclerotinia sclerotiorum in vegetables in South Florida during 1944 to 1954. Plant Dis. Reptr. 39:470-472. 1955.

35. Moore, W.D., R.A.Conover and D.L.Stoddard. The sclerotiniose disease of vegetable crops in Florida. Florida Agric. Expt. Sta. Bull.457:1-20. 1949.

36. Morgan, O.D. Jr. Correlation of the growth rate of 23 Sclerotinia isolates in vitro with the rate of infectivity in five hosts at five different temperatures. Phytopath. 42:471. 1952.

37. Munnecke, D.E., K.H.Domsch and J.W.Eckert. Fungicidal activity of air passed through columns of soil treated with fungicides. Phytopath. 52:1298-1306. 1962.

38. Newton, W. The growth of Sclerotinia sclerotiorum and Alternaria solani in simple nutrient solutions. Scientific Agriculture. 26:303-304. 1946.

39. Olin Mathieson Chemical Corporation. Technical data on experimental fungicide No. 2424 (Terrazole). 1965.

40. Partyka, R.E. and W.F.Mai. Effects of environment and some chemicals on Sclerotinia sclerotiorum in laboratory and potato field. Phytopath. 52:766-770. 1962.

41. Porth, R.B. and H.N.W.Toms. Unpublished vegetable disease survey of the coastal region of the Lower Fraser Valley of British Columbia. 1965.

42. Purdy, L.H. A broader concept of the species Sclerotinia sclerotiorum based on variability. Phytopath. 45:421-427. 1955.

43. Purdy, L.H. Some factors affecting penetration and infection by Sclerotinia sclerotiorum. Phytopath. 48:605-509. 1958.

44. Purdy, L.H. and R.G.Grogan. Infection of lettuce by Sclerotinia sclerotiorum (Lib.) de Bary. Phytopath. 42:518. 1952.

45. Purdy, L.H. and R.G.Grogan. Physiological studies of Sclerotinia sclerotiorum in liquid and agar culture. Phytopath. 44:36-38. 1954.

46. Ramsey, G.B. Sclerotinia species causing decay of vegetables under transit and market conditions. J. Agr. Research. 31:597-632. 1925.

47. Reavill, M.J. Effect of certain chloronitrobenzenes on germination, growth and sporulation of some fungi. Ann. Appl. Biol. 41:448-460. 1954.

48. Richardson, L.T. and D.E.Munnecke. A bioassay for volatile toxicants from fungicides in soil. Phytopath. 54:836-839. 1964.

49. Sanford, G.B. Factors influencing formation of sclerotia by Rhizoctonia solani. Phytopath. 46: 281-284. 1956.

50. Savage, G.M. and M.J.Vanderbrook. The fragmentation of the mycelium of Penicillium notatum and Penicillium chrysogenum by a high speed blender and the evaluation of blended seed. Jour. Bact. 52: 385-391. 1946.

51. Sharvelle, E.G. The nature and uses of modern fungicides. Burges Publishing Company. 1961.

52. Skotland, C.B. Attempts to control Sclerotinia rot on irrigated lettuce. Plant Dis. Reptr. 45:51-53. 1961.

53. Smieton, M.J. and W.Brown. Botrytis disease of lettuce, its relation to damping-off and mildew, and its control by PCNB dust. Ann. Appl. Biol. 27:489-501. 1940.

54. Tanrikut, S. and E.K.Vaughan. Studies on the physiology of Sclerotinia sclerotiorum. Phytopath. 41: 1099-1103. 1951.

55. Torgeson, D.G. Effect of fungicides on the respiration of three species of soil fungi. Phytopath. 53:891. 1963.

56. Tribe, H.T. On the parasitism of Sclerotinia trifoliorum by Coniothyrium minitans. Trans. Brit. Mycol. Soc. 40:489-499. 1957.

57. Varney, E.H. Vermiculite media for growing fungi. Plant Dis. Reptr. 45:393. 1961.

58. Vaughan, E.K. and B.F.Dana. Experimental applications of dusts and sprays to beans for control of Sclerotinia sclerotiorum. Plant Dis. Reptr. 33: 12-15. 1949.

59. Weindling, R. Trichoderma lignorum as a parasite of other soil fungi. Phytopath. 22:837-845. 1932.

60. Williams, G.H. and J.H.Western. The biology of Sclerotinia trifoliorum Erikss. and other species of sclerotium-forming fungi. I. Apothecium formation from sclerotia. Ann. Appl. Biol. 56:253-260. 1965.

61. Young, P.A. Sclerotinia rot of squash and pumpkin. Phytopath. 26:184-190. 1936.