THE EPIDEMIOLOGY AND CONTROL OF *PYTHIUM* ROOT DIEBACK OF MUCK-GROWN CARROTS

by

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ABSTRACT

Olpidium brassicae was observed in both brown and healthy carrot roots from Pythium root dieback (PRD) problem and non-problem fields. The incidence of Olpidium was correlated with the frequency of precipitation greater than one half inch but was not correlated with root temperature, CO_2 or O_2 concentration, saturated hydraulic conductivity, the height of the carrot beds, marketable yield or cull rate. Olpidium isolates with and without TNV did not produce lesions on carrot roots under greenhouse conditions. TNV was detected in both brown and white roots but only from problem fields. Carrot rootlets rub-inoculated with TNV failed to produce necrotic symptoms. Olpidium and TNV were found in onion, lettuce, celery and some weed species common to PRD problem fields. However, no root tip browning was observed in any of these hosts.

Fast growing Pythium species were recovered equally frequently in brown and symptomless rootlets and from problem and non-problem soils. Most weeds, celery, onion and lettuce also had a high incidence of fast growing Pythium.

The highly pathogenic, slow growing Pythium sulcatum was recovered only from problem soil. The recovery rate from symptomless roots was very low compared to brown roots. P. sulcatum was not isolated from celery or any of the weed species common in problem soil. Lettuce and onion were found to support low levels of infection. Evidence suggests that P. sulcatum is a primary incitant.

PRD losses can be kept to a minimum and marketable yields increased by using tolerant varieties, such as HiPak; raised beds, if there is a readily available supply of irrigation water; precision seeding at 1 1/4 inches; and a crop rotation of onions preceding carrots.

Matric potential was controlled in small containers separated from osmotic solutions of polyethylene glycol (PEG) 6000 by Pellicon ultrafiltration membranes (nominal molecular weight cutoff:500, Millipore Corp.). Matric potentials could be maintained for periods of 3-5 weeks before microbial breakdown of membranes occurred. Flow rate for the membranes was 1.0 $cm^3 cm^{-2} day^{-1}$ for a water potential difference across the membrane of 0.2 bar. Water potential measured with tensiometers or thermocouple psychrometers in a cylindrical container (4.3 cm diam. x 10 cm) with a membrane acrosss the bottom, remained relatively constant under conditions of soil surface evaporation but decreased rapidly when young plants were grown in the system. Soil cells (5.5 x 2.0 x 10 cm with one 43 mm diameter membrane in each side), containing two young carrots, and emersed in a -0.2 and -2.0 bar PEG solution had an average matric potential of -0.4 and -2.5 bars respectively over a three week period. The carrots transpired 7.8 and 3.9 ml/day at osmotic potentials of -0.2 and -2.0 bars respectively which suggests that sufficient water was passing through the membrane to meet the needs of a growing carrot.

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INTRODUCTION

A root disorder of muck-grown carrots, Daucus carota L., observed in several locations of North America has received several descriptive names. In Ontario, Fushtey and Filman (1968) described the disease as "rusty root." Workers in British Columbia called a similar disease "lateral root dieback" of carrot (Ormrod, 1969). Pythium debaryanum Hesse. has been shown to be associated with the disease in British Columbia and McElroy et al. (1971) felt that the name "Pythium Root Dieback" of carrot was more descriptive. Researchers in Wisconsin, who have also associated several Pythium species with the disorder, named the problem "brown root" (Mildenhall et al., 1971). Since there is now evidence that: 1) the diseases are the same; and 2) Pythium is the primary organism responsible for the disease, Pythium Root Dieback (PRD), the name first associating Pythium with the problem, will be used throughout this thesis.

In British Columbia, three organisms are associated with the disorder. High levels of Olpidium spores are observed in diseased tissue, and tobacco necrosis virus can also be detected in this tissue. Pythium species highly pathogenic to carrot have been recovered from necrotic root tissue and from soil. The etiology and epidemiology of these three organisms in PRD incidence and development will be considered in Chapter I.

Since chemical control of PRD has been shown to be either ineffective or uneconomical, other methods of control had to be devised. An integrated method of control using four cultural practices is discussed in Chapter I. Because soil moisture appears to be the most important parameter in the epidemiology of PRD, Chapter II is devoted to the role of soil water in disease development.

CHAPTER I

ETIOLOGY, EPIDEMIOLOGY AND CONTROL OF *PYTHIUM* ROOT DIEBACK

INTRODUCTION

History and Importance

Pythium Root Dieback (PRD) of carrot was first observed on two muck soil farms in the Bradford Marsh area of Ontario in 1962, and the problem reoccurred in 1965 and 1968 on several more farms in that area (Fushtey and Filman, 1968). A similar problem had been observed for several years in muck soils in the Fraser Valley of British Columbia, but it received little attention until severe losses in 1968. Surveys of carrot fields around Cloverdale and Marine Drive, suggested that it was present in many fields and caused average crop losses of 35% in 1969 (McElroy *et al.*, 1971) and 10-15% in 1970 (D.J. Ormrod *et al.*, *unpublished data*). One small field was disked under as a result of PRD. In the Fraser Valley, there were moderate losses to PRD in 1971 and 1972 and little loss in 1973. Presently PRD is considered to be the most important disease problem of carrot in organic muck soils of Florida (Pratt and Mitchell, 1973), Wisconsin (Mildenhall *et al.*, 1971), Ontario (Fushtey and Filman, 1968), and British Columbia (McElroy *et al.*, 1971).

Symptoms

Above ground symptoms were first observed as a wilt when the plants were 4 to 6 inches tall. The tops wilted during the day and recovered at night. After several days of wilting, lower leaves began to show necrosis of the margins and affected areas became quite conspicuous because of yellowing foliage and reduced growth. Below ground symptoms appeared as a root tip necrosis. Rootlets became a distinctive rusty red colour in the vicinity of the necrotic area (Fushtey and Filman, 1968). The decay of the taproot of seedlings to within several centimeters of the soil surface was another phase of the disease (Mildenhall et al., 1971). Extensive proliferation and branching just above the point of necrosis then occurred (McElroy et al., 1971). Depending upon the weather, the plants were either killed or they continued producing new rootlets to replace those lost. At harvest, foliage gave the appearance of a healthy stand, but below ground the roots were short, stubby and forked. Many carrots were rough and had an excessive number of feeder roots which held the soil at harvest.

Etiology

Abiotic. A number of attempts were initiated to determine if abiotic agents might be responsible for PRD. Salinity was measured in carrot growing muck-soils of British Columbia, but there was no correlation between soil salt content and PRD (M. Driehuyzen, unpublished data). Leaching of PRD soils in a pot experiment had no effect on reducing root symptoms (Filman and Fushtey, 1972). It was hypothesized that pesticide residues might have some effect on PRD but Oloffs et al. (1971) found

lower levels of chlorinated hydrocarbons in muck-grown carrots than mineral-grown carrots. Linuron came into use at about the time PRD was first recognized, but applications of Linuron at rates 2 to 4 times the recommended rate had no effect on root dieback symptoms (Filman, 1972a). Applications of lime (Filman, 1972d) and different rates and forms of nitrogen (Filman, 1972c) also had no effect on disease incidence. New seed reduced disease severity compared with two-year-old seed in some varieties but this observation has been inconsistent between experiments (Filman and Andersen, 1972; Filman and Fushtey, 1970).

Plant density had an effect on disease incidence. In a trial with different numbers of carrots per foot of row, a seeding rate of 12 carrots per foot of row (recommended rate) had such a high PRD loss, that it would have been uneconomical to harvest. Seeding at half the rate reduced disease incidence by 10% but PRD was still too severe for economical harvesting (Filman, 1972b). Less forking and fewer culls were observed with precision seeding than Planet Junior seeding (A. R. Maurer, personal communication).

Biotic. A number of fungi have been associated with PRD. McElroy et al. (1971) consistently isolated P. debaryanum from brown rootlets and soil from disease problem areas. Since their publication, this isolate has been reidentified as a closely related species, P. sylvaticum Campbell and Hendrix. Therefore, throughout the rest of this thesis McElroy's isolate will be referred to as P. sylvaticum. In pathogenicity trials, P. sylvaticum was capable of causing brown roots (McElroy et al. 1971) and carrots infected with P. sylvaticum had only one third the amount of root growth as non-inoculated plants (Blok, 1970).

A large number of Pythium species have been isolated from muck soil in carrots from British Columbia by Wisconsin researchers. These include P. irregulare Buisman, P. paroecandrum "classical form," P. paroecandrum "P. ultimum form," P. debaryanum, P. coloratum Vaartaja, P. sylvaticum, P. sulcatum Pratt and Mitchell and several unclassified Pythium species (R. G. Pratt and R. J. Howard, personal communication).

Four species of Pythium have been isolated by baiting PRD problem soils from Wisconsin (Mildenhall et al., 1971). P. irregulare, P. paroecandrum Drechsler, P. sylvaticum, and P. sulcatum all induced root browning, but only P. irregulare, P. paroecandrum and P. sulcatum reduced germination. Root tip necrosis was most severe with P. sulcatum and P. irregulare. P. sulcatum was easily distinguished from the other three pathogens by its much slower growth habit (Pratt and Mitchell, 1973).

A wide range of genera of fungi has been isolated from carrots in Ontario but none was pathogenic (Sutton, 1973). The fungi most frequently recovered from PRD roots were Alternaria, Cylindrocarpon, Fusarium, Gliocladium, Mucor, and Penicillum. Similar fungi were often found in roots from non-problem areas, but they were usually fewer in number. Sutton concluded that filamentous fungi do not initiate PRD but were probably important as secondary organisms in disease development. He also concluded that Pythium was unimportant in the initiation and development of PRD because he isolated very low levels from roots. However, he did not use a selective medium which is necessary when attempting to recover Pythium from plant tissue.

Numerous chytrid spores have been observed in roots of Ontario carrots (Sutton, 1973). Olpidium brassicae (Woron.) Dang. was found

in 18 of 30 fields in Ontario (Anon., 1972). Tobacco necrosis virus (TNV), carried by zoospores of O. brassicae, was detected in 3 of 30 fields by indexing carrot roots (Anon., 1972). The chytrid and virus have been detected in carrot roots just after plant emergence (Kemp and Filman, 1972). O. brassicae zoospores, liberated from a chytrid-virus-infected carrot, and added to carrots grown in sand under sterile conditions, transmitted TNV. Some brown rootlets developed but they were not as discoloured, as those associated with PRD under field conditions (Anon., 1973a). O. brassicae and TNV have been detected in PRD soils from British Columbia (W. G. Kemp, personal communication).

Carrot rootlets in two fields of Ontario were 50-100% mycorrhizal but most fields were 1-12% mycorrhizal (Sutton, 1973).

A nematode survey of PRD problem and non-problem fields in British Columbia indicated the presence of several species of stylet bearing nematodes, but there was no correlation between a particular species of nematode and the dieback problem (McElroy et al., 1971).

Epidemiology

Carrots, replanted in fields disked under because of severe PRD losses, grew well, suggesting that soil temperature or moisture may be important (Fushtey and Filman, 1968). In British Columbia, P. sylvaticum was active throughout the year, but it was less active during the dry summer months (McElroy et al., 1971). A number of watering regimes, in a pot experiment, had no effect on PRD if P. sylvaticum was already present. If the soil was free of the incitant, carrots responded to the watering regimes (Maurer et al., 1971).

Inoculation of carrots with P. ultimum at cool temperatures (7-18 C), led to a greater PRD infection than high temperatures (18-30 C) (A. R. Maurer et al., unpublished data).

Cranston and Copeman (1972) attempted to separate the effects of temperature and water in a greenhouse experiment. They found that infection by P. sylvaticum was greatest at 18.5 C and was higher in pots maintained at a high soil moisture level.

Soil temperature and moisture were measured under field conditions in an attempt to relate PRD to critical conditions in the microenvironment (Copeman and Black, 1972). A greater disease incidence was found in plots maintained at a high soil moisture level by irrigation than drier plots that received only rainfall. Raising the moisture level later in the season to the spring level by irrigation increased disease incidence, as measured by the number of spores in brown rootlets, while incidence decreased in the non-irrigated plot. Moisture was considered more critical than soil temperature.

The effect of soil moisture content on PRD was also studied by growing carrots under different moisture regimes in infested field soil in plastic lined bins (Filman and Andersen, 1972). Water treatments had little effect on the incidence of PRD as measured by carrot cullage, but they significantly affected total carrot yields. Flooding the soil for 7 days before planting reduced symptoms from 73% to 57% but this reduction was not enough to be of practical value.

Control of Pythium Root Dieback

A number of fungicide trials have been attempted but no effective control has been found. McElroy et al. (1971) found that a Dexon drench under greenhouse conditions reduced culls from 76.5 to 5.5%. However, this chemical was ineffective under field conditions (Ormrod et al., 1970). After four years of trials in British Columbia, Ormrod and Castley (1973) concluded that fumigants such as Brom-O-gas were uneconomical; Terrachlor, Dexon and Demosan were not likely to be registered; and Terrazole, though promising, needed more study. Field trials applying Dexon against Pythiaceous fungi, Benlate against ascomycetes and some basidiomycetes, Terrachlor against basidiomycetes and Nemagon against nematodes, had no effect in Ontario (Anon., 1973b).

The most successful results in reducing losses to PRD have been obtained by selection of more resistant varieties. Hybrids from Michigan State with 5988 or Spartan parentage are more tolerant or resistant to PRD than any other varieties or hybrids on the market today (Baker et al., 1972). Spartan Sweet, Spartan Fancy, HiPak and Grenadier in British Columbia had significantly less forking and cullage due to PRD than other varieties tested (Copeman and Black, 1973). HiPak's acceptance by British Columbia growers has been one important factor in reducing losses in the past few years.

To date Olpidium spp., Pythium spp., and TNV have been associated with carrot roots exhibiting PRD symptoms in British Columbia. Since P. sulcatum, a very pathogenic species capable of causing rootlet dieback in Wisconsin and Florida (Pratt and Mitchell, 1973), has been found in

British Columbia soils, it was considered important to determine its role in PRD. Olpidium is often regarded as a non-pathogenic parasite causing no macroscopic symptoms (Temmink and Campbell, 1968). However, it is hard to imagine that the large number of spores observed in rootlets from British Columbia grown carrots are not reducing yields. If soil moisture is the most important parameter of the microenvironment affecting the incidence of PRD (Copeman and Black, 1972), can PRD severity be modified by cultural techniques which alter soil moisture? The unknown role of alternate weed or crop hosts on pathogen populations deserves attention. Therefore, the objectives of this study were:

1) to determine the importance of P. sulcatum in PRD soils of British Columbia;

2) to duplicate field infection of Olpidium in the laboratory and determine if Olpidium was capable of causing PRD symptoms;

3) to determine if PRD losses and pathogen populations were reduced on raised beds where the microenvironment had been modified;

4) to determine if precision seeding and seed spacing affect PRD severity, and;

5) to observe the effects of weed hosts and alternate crops on the populations of the organisms associated with PRD.

METHODS AND MATERIALS

Field Studies

Microenvironment plot. In 1972, a field experiment was conducted by Drs. R. J. Copeman and T. A. Black to determined if "raised" carrot

beds would reduce PRD losses by modifying the microenvironment. Mr. Hedi Trabelsi maintained the plot and collected soil and microenvironmental data, while Mr. Frank Schneider collected and prepared carrot root samples for later examination. The author observed the carrot rootlets and analyzed the soil, yield, and disease rating data.

The field plot was situated on a Lumbrum Muck near Cloverdale, B. C. on the farm of Cloverdale Produce Ltd. Carrots had been grown on this land for the two preceding years, and the preceding year's crop had suffered heavy losses from PRD. The cooperating grower cultivated and fertilized the land as he would have done for a commercial carrot crop, and then prepared beds of maximum height with his equipment. The top 2 or 3 inches of soil were removed from half of the beds to form "conventional" height beds, and placed on the adjacent beds to form "raised" beds. This resulted in beds having a height difference of 4 to 5 inches. The removal of soil from "conventional" beds may have reduced inoculum potential and plant nutrients, but it was considered more desirable than introducing top soil from the adjacent field, had this been possible. A side dressing of nitrogen was applied after a heavy rain in early July. Sprinkler irrigation was applied to both plots as was necessary to maintain a high soil moisture in the "conventional" bed.

The plot design was a 4 x 4 Latin square split plot, with planting dates the major factor, being split into "raised" and "conventional" bed heights. Replicate beds were 10 feet long on 72 inch centers. Carrot cultivar 'GoldPak' was planted May 11 and at three successive 10 day intervals, with a Planet Junior belt seeder with a 4 inch scatter shoe.

Only three rows per bed could be fitted on the "raised" beds due to a reduced planting surface.

The following soil and microenvironmental measurements were made every other day.

1) Soil moisture was determined gravimetrically, by oven drying at 105 C. Two soil samples were taken at 0-3, 3-8, 8-15 cm.

2) Temperature was measured with a germanium diode-bridge circuit (Sargent, 1965) at 5, 10, and 15 cm.

3) O_2 and CO_2 were measured at 5, 10, and 15 cm (Black et al., 1965).

4) Precipitation was measured with a standard rain gauge.

5) Height of the water table in a "raised" and "conventional" bed, and level of water in the adjacent drainage ditch was determined.

The saturated hydraulic conductivity, bulk density, and partial retention curve of a "raised" and "conventional" bed at 0-3, and 4-7 cm were determined at the end of the growing season (Black et al., 1965).

Root samples from the outside rows in two locations of each replicate were taken at successive 10 day intervals from 30 to 70 days after planting. Twenty discoloured rootlets were selected, fixed in formalin glacial actic acid (FAA) (Phillips and Hayman, 1970), cleared and stained with trypan blue (Phillips and Hayman, 1970) and microscopically examined under phase contrast (560 μ field diameter). Counts of Pythium and Olpidium spores were made at five locations having the highest concentration of spores per root. The remaining root system was indexed for TNV by rub inoculating carborundum-dusted Chenopodium

quinoa Willd. (Teakle, 1962a). The incidence of Olpidium in carrots at four planting dates on "raised" and "conventional" beds, and at five sampling times was analyzed by a latin square split-split plot method of analysis (Winer, 1971).

Approximately three months after planting, the center row of carrots was harvested, graded into A's, B's, culls, and smalls, and the number and weight of each class recorded.

Grower's raised bed trial 1973. Two cooperating Cloverdale growers, one with a PRD problem soil and the other with no history of PRD, cultivated and fertilized plot areas as they would have done for a commercial carrot crop. Then they prepared three conventional, 300 foot long beds, and four raised, 300 foot long beds, made as high as possible with their existing equipment. Raised beds were constructed by throwing soil up into beds with a wide flanged cultivator shoe, and then shaping with a bed shaper to give a firm planting surface and firm sides. Conventional beds were constructed only with the bed shaper and were 2-3 inches lower than the raised beds. Carrot cultivar 'GoldPak' was precision seeded using a Stan Hay seeder with a belt pattern of 90:48:90. The three lines were 1 1/2 inches apart and the distance between seeds of the outside lines was 1 1/2 inches. The following row spacings were used: 1) three rows 14 inch centers, raised beds, 2) four rows 12 inch centers, raised beds, 3) four rows 12 inch centers, conventional beds, and 4) four rows 14 inch centers, conventional beds. An adjacent conventional bed, seeded by the grower using a Planet Junior Seeder with a 4 inch scatter shoe, was also used in the comparison. After seeding, the grower managed the plot according to current recommended practices.

Five, 20 foot plots evenly spaced in the 300 foot row served as the sample areas. Carrot emergence in a 3 foot section of row was determined for each replicate. Root samples were taken from two locations within a replicate at 50 and 80 days after planting and 50 discoloured and 50 white rootlets were selected from each sample. Twenty roots were fixed in FAA, stained with Phloxine and KOH (Tuite, 1969), and microscopically examined with phase contrast at five locations of highest spore concentration per root for Pythium and Olpidium spores. Twenty-one rootlets were washed for 24 hours in cold tap water and a section of each was plated on a medium selective for Pythium. The selective medium was a modification of the medium of Tsao and Ocana (1969). Benlate (10 ppm active ingredient, 50% WP, E.I. Dupont de Nemours and Co., Wilmington) was used in place of Pimaricin. Ingredients for the media were 10 ppm Benlate, 100 ppm PCNB (75% WP, Olin Mathieson Chem. Corp., N.Y.), 10 ppm Vancomycin HCl (Sigma Chem. Co., St. Louis), 17 g Corn meal agar (CMA, pH 5.6, BBL), and 1 liter distilled water. The medium was autoclaved for 15 minutes at 15 psi. Plates were incubated in the dark at 25 $\pm$ 1 C and were examined every 12 hours for Pythium growth. The remaining rootlets and the whole root system were separately indexed to carborundum-dusted C. quinoa.

Carrots were harvested 100 to 110 days after planting, graded into A's, B's, culls, and smalls, and the number and weight of each class recorded.

Precision seeding trial. The plot was situated on the land previously used for this project in 1971 and 1972. The land and raised

beds were prepared by the grower in the same fashion as 1972. A replicated 3 x 3 latin square split plot design was used, with plant spacing the major factor being split by two carrot cultivars. GoldPak, a PRD susceptible variety, and HiPak, a PRD tolerant variety, were precision seeded with a Stan Hay Seeder at seed spacings of 1 1/4, 1 1/2, and 2 inches with four rows per bed. Plots were 20 feet in length. Irrigation was applied to maintain a high soil water content to favor disease incidence. Weeds were controlled with three applications of Stoddard Solvent at 60-80 gallons per acre.

Carrot emergence and sampling for disease incidence were performed in the same manner as previously discussed. Samples for the disease survey were taken at 40 and 55 days after seeding. Weed samples were collected from the plot in the second sampling and roots were processed in the same manner as the carrot roots.

Harvesting was done as in the grower's raised bed trial except that the culls were broken into two categories: 1) "culls" which included split, bent and green shouldered carrots, and 2) "forks" which included forked, rough, and hairy carrots.

Laboratory Studies

Olpidium. Cultures of *Olpidium* were obtained by baiting soil samples with carrot cultivar 'GoldPak Elite' in the greenhouse. Field soil held at greenhouse temperatures for one year, and fresh field soil that had TNV-infected carrots were used. Seedling carrots were indexed on *C. quinoa* to confirm presence or absence of TNV.

Attempts were made to culture Olpidium from the seedling carrots. Fifteen ml plastic beakers with no drainage, 2 x 2 inch and 4 x 4 inch plastic pots with drainage, and 3 inch clay pots with drainage were filled with washed Fraser River sand, and 14-21 day old carrot or lettuce (Lactuca sativa L. 'Grande Rapids') seedlings were transplanted in the pots. Incoulation of the seedlings was done by: 1) pouring actively swimming zoospore suspensions over the roots of seedlings (Campbell and Grogan, 1964), 2) soaking seedlings in zoospore suspensions for 15-60 minutes before transplanting (Teakle, 1962a), 3) transplanting an infected plant in with the seedlings, or 4) mixing infected roots in the sand before transplanting (D.J.S. Barr, personal communication). Zoospore suspensions were obtained by removing infected plants from soil or sand, quickly washing roots in cool tap water, and soaking roots in either distilled water, tap water, 1:3 or 1:20 Hoagland's nutrient solution, or pond water (Kassanis and Macfarlane, 1964; Teakle, 1962a). The presence of Olpidium was confirmed by examining several rootlets under the microscope for actively swimming zoospores or zoosporangia. Cultures were maintained in a growth cabinet at the optimum temperature of 18 $\pm$ 2 C (Fry and Campbell, 1966) with a photoperiod of 12 hours and a light intensity of 4300 lux. Cultures were watered daily with either tap water supplemented with nutrient solution once a week, or full strength nutrient solution. Transfer of Olpidium from inoculated to healthy plants was attempted at 1 to 8 weeks after inoculation.

Tobacco necrosis virus. 'GoldPak Elite' carrot seed was germinated in the dark at 25 $\pm$ 1 C on moist filter paper until the roots were

approximately 3 cm in length at which time they were inoculated with TNV. The virus source was obtained by single lesion isolation from C. quinoa that had been inoculated with PRD symptomed roots. Inoculated C. quinoa leaves having almost confluent lesions, were ground in .05 M phosphate buffer, pH 8.5, carborundum was added and 50 carrot roots were either dipped into the virus solution, or brushed 10 times with a paint brush dipped in the virus solution. Control seedlings were treated similarly except that only carborundum and phosphate buffer were used. After four days, roots were examined for brown lesions, dipped in 10% Concentrate R.B.S. 25* (Fisher Scientific Co. Ltd.) for 30 seconds to inactivate surface virus contamination and indexed on C. quinoa.

Greenhouse Crop Rotation

A greenhouse crop rotation experiment was conducted from October 1973 to June 1974. Lumbrum muck, from two farms with a known PRD problem, was thoroughly mixed, potted in 19.5 x 25 cm plastic pots and arranged on the greenhouse bench in a randomized block design. Five crop rotations (Table I) with six replicates per sequence were used. A known number of seeds of carrot, cultivar 'GoldPak Elite,' onion (Allium cepa L. 'Autumn Spice'), or lettuce cultivar 'Pennlake' were planted. Emergence was determined after 2 to 3 weeks. The percent germination for each cropping was estimated by incubating six lots of 30 or 50 seed on moist filter paper in petri dishes in the dark at 25 $\pm$ 1 C. The first cropping was grown at 16-20 C and subsequent croppings were at 10-16 C. Plants received supplement lighting of 16 hours per day at an intensity of

3600 lux. Pots were frequently sprinkle watered so as to maintain a high soil moisture content. At 3 weeks, pots were thinned to either 12 carrot, 20 onion or 20 lettuce plants. Four weeks after planting, 20-20-20 fertilizer (500 lb/acre) was applied as a drench.

Table I. Sequence of the crops utilized in the rotation experiment

	Cropping sequence			
Rotation	1	2	3	4
A	C[1]	C	C	C
B	C	L	O	C
C	C	O	L	C
D	C	L	L	C
E	C	O	O	C

[1] C = carrot; L = lettuce; O = onion.

Sixty days after planting, plants were removed from the soil, roots and tops were weighed and 41 discoloured roots were removed and treated as previously described. Within a day of harvesting, the next crop was planted.

A composite soil sample, made up of small quantities of soil from pots within a treatment, was analyzed for Pythium propagule number. The soil was thoroughly mixed and soil dilutions of 1:25, 1:50, 1:100, 1:200 were made with 100 ml of 0.25% water agar (Difco) plus 200 ppm Vancomycin. While the soil agar mixture was being stirred, 1 ml of solution was removed and pipetted onto each of five petri plates of

Pythium selective medium. The plates were incubated in the dark at 25 ± 1 C for 18-24 hours. The soil water agar suspension was washed off, plates were stained with KOH and Phloxine, and colonies counted. Propagule number was calculated relative to oven dry soil (Ocana and Tsao, 1966).

RESULTS

Etiology

Indirect determination of organism responsible for PRD. The incidence of Pythium, Olpidium and TNV were compared between a non-problem and two PRD problem fields and between brown and white roots (Table II). Fast and slow growing Pythium spp. grew out of roots plated on the selective medium.

The root systems of carrots in PRD soil showed typical root tip browning, whereas carrot roots from non-problem soil were only greyish brown in colour. These greyish brown roots were analyzed as brown roots. Microscopic examination of typical diseased rootlets revealed that the epidermal cells were often sloughed off. Epidermal cells and root hairs were intact on rootlets from non-problem [redacted].

Olpidium, TNV and both slow and fast growing Pythium spp. were found in roots from PRD soil, but only Olpidium and fast growing Pythium spp. were found in roots from non-problem areas (Table II). Olpidium spores were observed much more frequently in problem than non-problem fields. In PRD fields, all four organisms were found in brown and white

Table II. Incidence of *Olpidium*, fast and slow growing *Pythium* spp. and TNV in brown and white roots of a non-problem and two PRD problem fields at two sampling times in 1973

	Olpidium Av. spores/field		Fast *Pythium* % roots infected		Slow *Pythium* % roots infected		TNV % recovery	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
Brown Roots								
Precision seeded problem soil	12.9	7.7	11.0	9.5	15.0	14.5	55	53
Grower's trial problem soil	21.5	16.4	12.5	2.7	8.6	8.8	80	76
Grower's trial non-problem soil	2.2	-	22.1	-	0.0	-	0	-
White Roots								
Precision seeded problem soil	2.1	7.3	3.0	12.5	0.5	2.5	19	42
Grower's trial problem soil	5.0	9.0	6.5	1.9	1.3	2.5	85	60
Grower's trial non-problem soil	0.2	-	7.2	-	0.0	-	0	-

roots, but only the slow growing Pythium isolate was consistently recovered at much lower levels in white roots (Table II). Olpidium spore levels decreased in brown roots and increased in white roots in the second sampling. The incidence of slow growing Pythium remained constant in brown roots and increased in white roots, but the recovery of slow Pythium in the second sampling was still 6 times less in white roots than brown roots. The selection of white roots at the second sampling was much more difficult as the roots were not a true white but rather an off-white or grey colour.

PRD symptom production by Olpidium and TNV. Cultures of Olpidium, free of TNV, were successfully obtained by baiting one-year-old stored field soil with carrots, while Olpidium-TNV cultures were obtained by baiting freshly collected field soil.

Carrot and lettuce roots inoculated with Olpidium were white or grey-white in colour. TNV in the presence of Olpidium had no effect on root tip browning. No orange-brown lateral roots were observed throughout the study even if high levels of spores were present in the roots. Microscopic examination of the grey coloured roots sometimes revealed Olpidium zoosporangia and resting spores. Mycelium was sometimes observed in the grey rootlets and was most common in roots from plants in poorly drained pots.

If zoosporangia were observed, zoospores could usually be seen swimming around the root within 5 minutes of soaking the roots in water. Zoospores were most active in tap water and 1:20 nutrient solution, and remained active for about 20 minutes. Zoospores in distilled water

and nutrient solution more concentrated than 1:20 were sluggish and became immobile within 5 minutes of release. Several sources of pond and stream water were used to soak roots, but none was superior to tap water in maintaining zoospore activity.

Olpidium cultures transferred on carrot were very difficult to maintain from one transfer to the next. Olpidium infection of carrots could rarely be detected by microscopic examination after 2 or 3 transfers. However, cultures of Olpidium on lettuce, obtained from the same source as the carrot isolates, produced abundant zoospores at each transfer and were maintained for at least six transfers. Olpidium cultures maintained on lettuce and transferred to carrot also died out after several transfers, but if zoospores from carrots were transferred to lettuce, Olpidium reproduced well on the lettuce. The different types of pots and inoculation techniques had little effect on the ability to culture Olpidium on lettuce, but the same procedures with carrot, resulted in inconsistent survival of Olpidium.

When care was taken to keep TNV-infected and TNV-free Olpidium cultures separate, TNV was never detected in cultures originating from stored, field soil. Both lettuce and carrot became infected with TNV when incoulated with Olpidium-TNV cultures. Sometimes carrots inoculated with Olpidium-TNV cultures, while not having Olpidium visible in the roots, were infected with the virus.

Necrotic spots developed on carrot roots rub-inoculated with TNV (Table III). However, control rub-inoculated and dip-inoculated roots also developed lesions. Lesions developed at points where the

roots were damaged and were more prevalent on long than short roots. The long roots were more easily damaged in handling. TNV was detected only from carrots that had been rub or dip-inoculated in the TNV solution. Symptomless rub-inoculated and dip-inoculated roots in TNV extracts were positive for TNV (Table III).

Table III. Number of carrot root systems developing necrotic symptoms and indexing TNV positive after rub or dip-inoculation in carborundum and .05 M phosphate buffer with and without TNV.

Treatment	Roots with necrotic symptoms		Symptomless roots	
	No.	No. TNV	No.	No. TNV
TNV rub	27	14	23	6
TNV dip	6	2	44	8
Control rub	13	0	37	0
Control dip	10	0	40	0

Epidemiology

Olpidium population and the microenvironment. The field experiment in 1972 was designed to study Olpidium incidence of carrot on "raised" and "conventional" beds at different planting dates. Olpidium incidence was significantly influenced by planting date. The May 23 planting had a significantly greater ($P = .05$) average number of spores per

microscope field than the late planting of June 13 (Figure 1). Carrot age at sampling was also a significant factor in the incidence of Olpidium (Figure 2). There was a significant interaction between planting date and sampling time as can be seen in Figure 3. Late-planted carrots always had a low Olpidium population throughout the growing season. By contrast, carrots that were planted early, had a low infection when the carrots were young, but a high infection level as they grew older. Bed height and any interactions of planting date or age of carrots at sampling with bed height had no significant effect on Olpidium population (Appendix I). Carrot rootlets in the earliest planted "raised" beds had 34.8% of the microscope fields with Olpidium. This was significantly fewer ($P = .05$) than "conventional" beds which had 50.3% of the microscope fields with Olpidium. However, in later plantings there was no significant difference of Olpidium incidence in "raised" and "conventional" beds. In the fourth planting, "conventional" beds had a slightly lower average number of spores per microscope field than "raised" beds; 1.8 and 2.2 spores/field respectively.

The data on the number of Olpidium spores per microscope field was transformed into the number of microscope fields with: one and greater than one, five and greater than five, and 10 and greater than 10 spores. An analysis of variance of the transformed data revealed no changes in significant factors except that carrot age at sampling was no longer significant ($P = .05$).

There was a significant increase in the marketable yield on "raised" beds over "conventional" beds (Table IV). Both bed types had

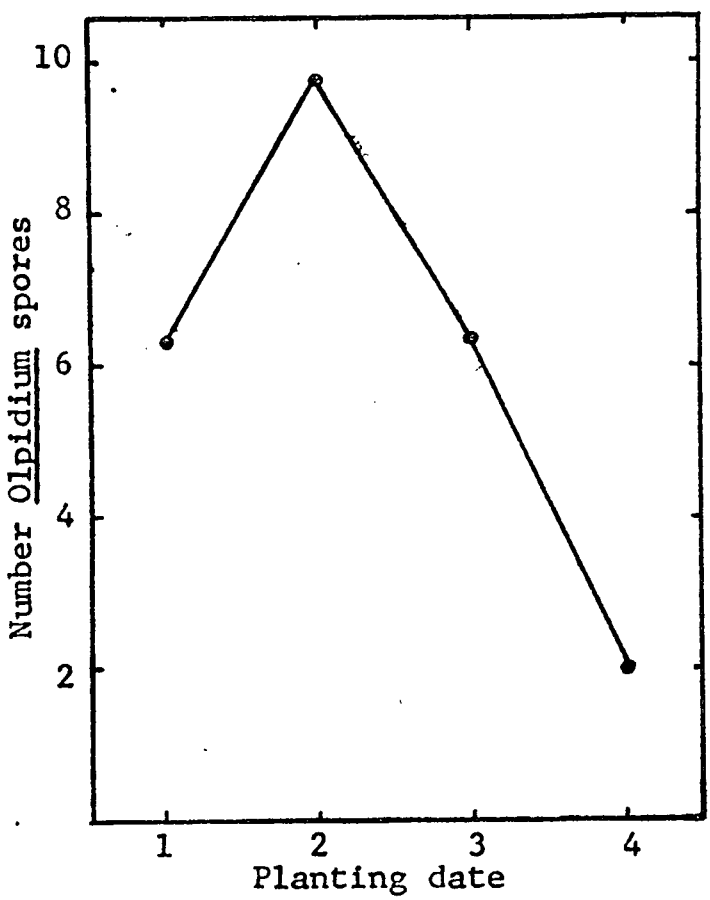

Figure 1. Average number of Olpidium spores per microscope field (560 μ) at four planting dates. 1 = May 11, 2 = May 23, 3 = May 30, 4 = June 13. Each point is the mean of 4000 counts.

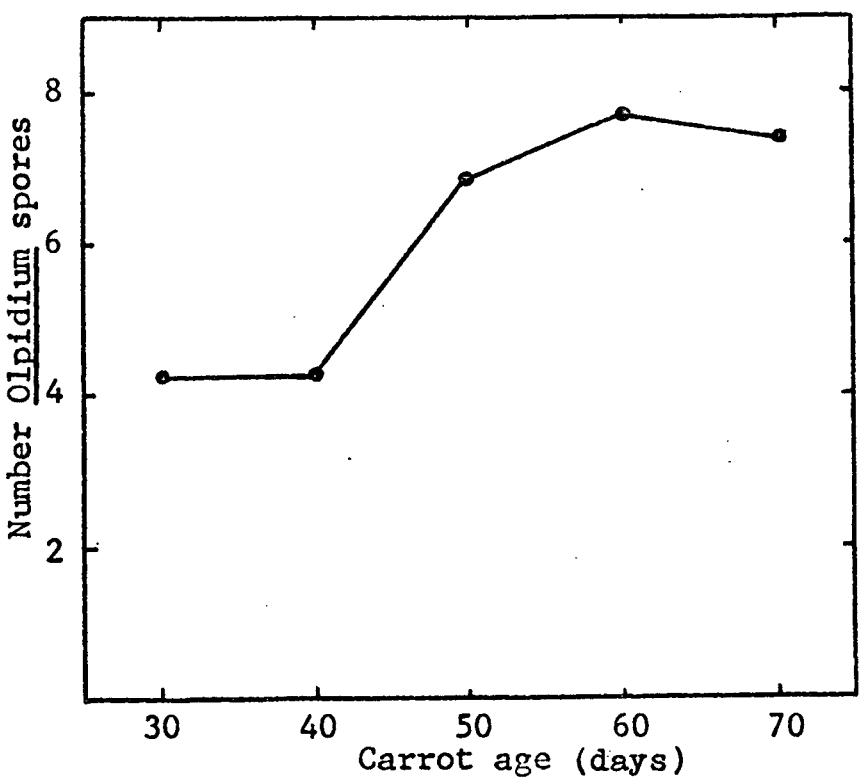

Figure 2. Average number of Olpidium spores per microscope field (560 μ) at five carrot ages. Each point represents the mean of 3200 counts.

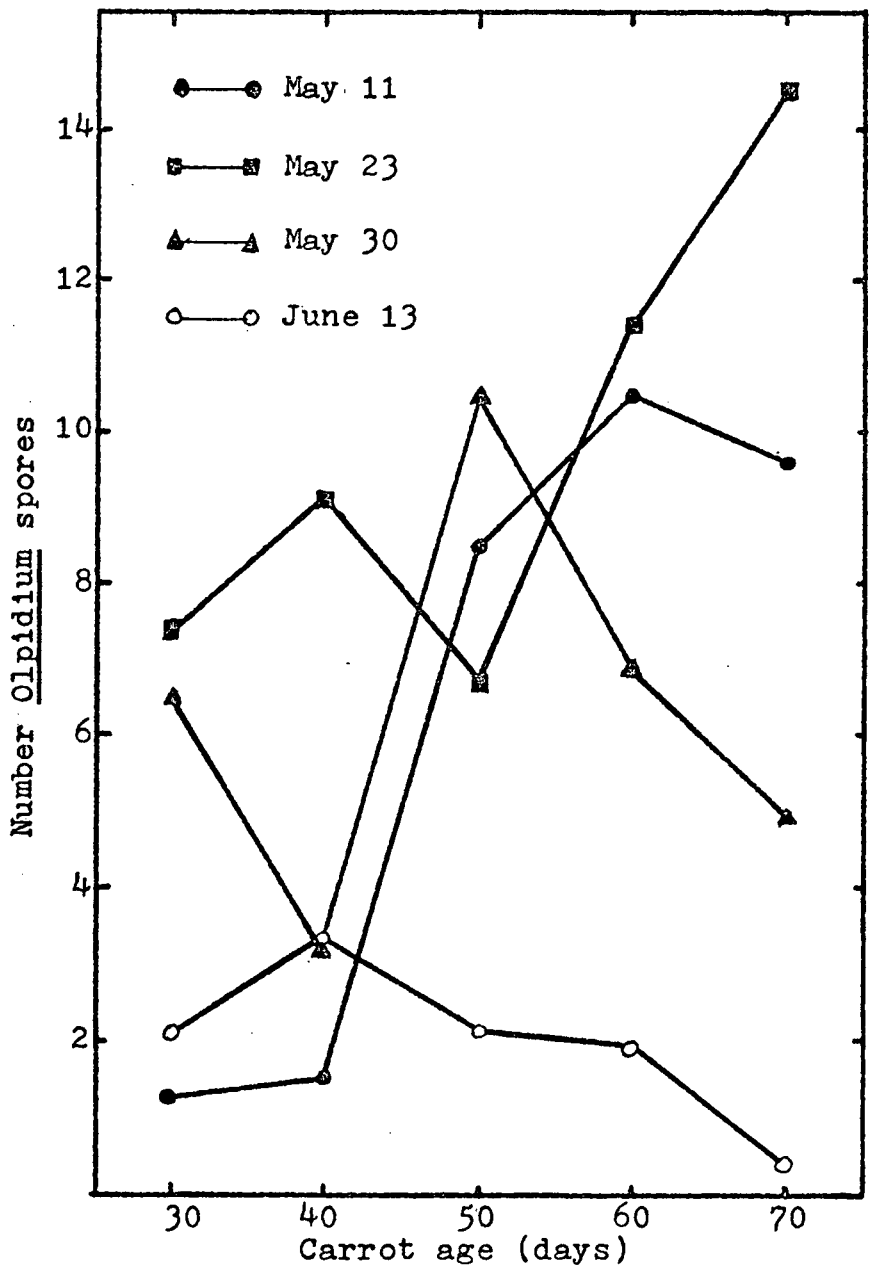

Figure 3. Average number of Olpidium spores at five sampling ages and four planting dates. Each point represents the mean of 800 counts.

Table IV. Carrot yield in "raised" and "conventional" beds and at four planting dates in 1972

Planting Date	A		B		Marketable		Culls		Smalls	
	No.	wt	No.	wt	No.	wt	No.	wt	No.	wt
May 11	49.8 a	7.1 a	0.0 b	0.0 b	49.8 a	7.1 a	72.0 a	5.1 a	82.4 a	2.6 b
May 23	41.5 a	4.9 a	2.9 a	1.1 a	44.4 a	6.1 a	36.0 b	3.6 a	39.5 b	1.8 ab
May 30	43.1 a	6.3 a	0.1 b	0.1 b	43.4 a	6.4 a	35.0 b	3.3 a	39.4 b	1.5 a
June 11	38.8 a	6.0 a	0.0 b	0.0 b	38.8 a	6.1 a	37.0 b	4.0 a	25.6 b	1.1 a
Bed Height										
Raised	55.3 a	7.9 a	1.2 a	0.5 a	56.5 a	8.4 a	42.6 a	4.5 a	34.8 a	1.3 a
Conventional	31.3 b	4.3 b	0.3 a	0.1 a	31.6 b	4.4 b	47.4 a	3.5 b	58.6 b	2.1 b

[1]Means within a column with the same letter do not differ significantly (P = .05).

the same total number of carrots but there was a shift from a large number of small carrots in "conventional" beds to a greater number of A carrots in "raised" beds (Table IV). The carrots in "raised" beds were larger in size (.105 lb/carrot versus .071 lb/carrot), and as a consequence there was a significantly greater weight of culls from "raised" beds than "conventional" beds even though "conventional" beds had a greater number of culls. However, on a percent basis (wt culls/total wt), "raised" beds had a lower, but not significantly lower, percent weight culls than "conventional" beds (31.8% versus 36.4%).

Marketable yield and weight of culls were not affected by planting dates. The number of small and cull carrots in the earliest planting was significantly greater ($P = .05$) than the later plantings. The early planting had a density of 20 carrots per foot of row compared to an average of 11.3 carrots per foot of row in later plantings. There was a significant interaction of bed height and planting date with respect to the number of culls. "Raised" beds at the earliest planting date had significantly fewer culls than "conventional" beds at the same date, but in the third planting, "conventional" beds had significantly fewer number of culls than raised beds (Table V).

Attempts were made to correlate changes in Olpidium population with the soil microenvironmental measurements collected by Hedi Trabelsi. Temperature did not differ greatly in "raised" and "conventional" beds. At the 15 cm depth, soil temperature slowly increased from 12 C to 16-18 C, the latter level being optimal for Olpidium reproduction. There was generally higher Olpidium population later in the growing season, but

the late planting of carrots had very low levels of Olpidium during the period of most favorable temperature.

Table V. Number of culls from raised and conventional beds at four planting dates

Planting date	Number of culls Raised	Conventional
May 11	58.3 b[1]	85.8 a
May 23	36.0 cd	36.0 cd
May 30	45.3 bc	24.8 d
June 13	30.8 cd	43.3 bcd

[1]Means with the same letter do not differ significantly.

A three times greater saturated hydraulic conductivity in "raised" beds than in "conventional" beds had no significant effect on Olpidium incidence.

Measurements of O_2 and CO_2 in the soil atmosphere showed slight changes. The concentration of CO_2 slightly increased after a period of rain and was at a higher concentration as the depth increased. After a rain, the oxygen concentration in the soil decreased but the lowest level measured was 19%. This small change in concentration was considered unlikely to have had any affect on Olpidium.

Soil water content, measured gravimetrically every other day at three depths was higher in "conventional" beds than "raised" beds throughout the growing season (Figure 4). The water content at the 8-15 cm

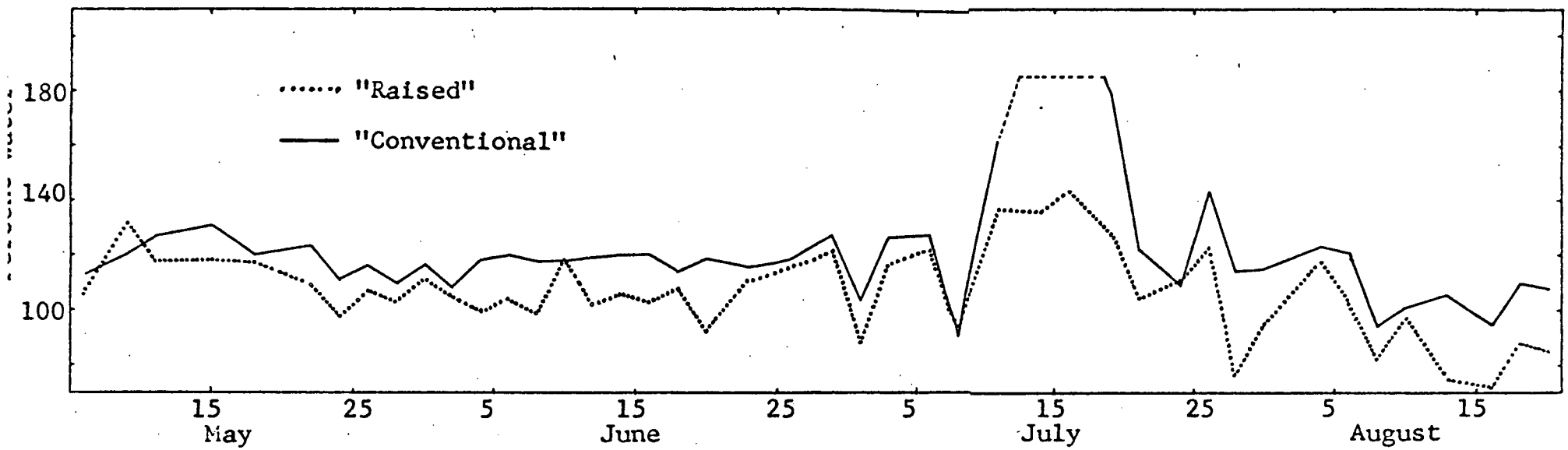

Figure 4. Soil water content of raised and conventional beds at 8-15 cm depth in 1972.

depth remained relatively constant between 100 and 120% water content until July 11, when a heavy rainfall increased soil water content for 10 days (Figure 4). There was no significant difference in Olpidium population in "raised" and "conventional" beds, even though "conventional" beds had about a 10% greater water content.

The water table was measured in one "raised" and one "conventional" bed at opposite edges of the plot. The piezometric level of the "raised" bed was about 20 cm higher than the "conventional" bed.

The frequency and quantity of rainfall was correlated with Olpidium population changes. Rainfall had been very low during May and the earliest planted carrots had low levels of Olpidium (Table VI). The second planting of carrots had high level of Olpidium when first sampled on June 21. This planting had received three good waterings at nearly weekly intervals from planting (Table VI). The third planting also had high levels of infection at the first sampling on July 1 (Table VI). It had received two good rainfalls 14 days apart which would also favor Olpidium spread. The mid June planting of carrots had a very low level of infection even though it had received four heavy periods of water (Table VI). On July 20, high levels of Olpidium spores were found in roots of the first three plantings. The second and third plantings had higher infections of Olpidium on July 20 than four days earlier (Table VI). The heavy rainfall of July 10th to 13th favored spread and infection resulting in the Olpidium build up evident in the later sample. After the deluge of mid-July, there was only one potential infection period as the rest of the summer was dry. Olpidium infection in the

Table VI. Correlation between the average number of Olpidium spores per microscope field at various sampling times and the frequence of infection periods of precipitation greater than 1/2 inch in 1972

Sampling date	June 13	June 21	July 1	July 16	July 20	July 28	Aug. 9	Aug. 19
Planting date								
May 11	1.3[1]	1.6	8.5	10.5	9.6			
May 23		7.4	9.1	6.7	11.4	14.5		
May 30			6.5	3.2	10.5	6.9	4.9	
June 13				2.1	3.4	2.2	1.9	0.4
No. infection periods 7-21 days prior to sampling	1	3	2	4	4	3	1	1

[1]Number spores per microscope field.

third planting decreased in later samplings but was still moderately high, while in the fourth planting, Olpidium incidence decreased after the heavy rainfall and was at a very low level as the summer progressed (Table VI).

In the June and early July samplings, a greater percentage of the Olpidium spores were zoosporangia. However, later in the summer, a greater proportion of the spores were resting spores. Many of the roots after July 28 had vesicular arbuscular mycorrhizae.

Grower's raised bed trial. During 1973, the performance of raised beds was evaluated under different commercial cultural management regimes. One farm, using 68 inch wheel centers, made conventional beds with a 51 inch planting surface and raised beds with a 43 inch surface. The second farm used 72 inch centers and formed beds with planting surface widths of approximately 46 and 50 inches for raised and conventional beds respectively.

The emergence in the problem and non-problem soil was 57% and 46% respectively. The number of carrots per foot of row varied for precision and scatter shoe seeded plots (Table VII). The carrots were growing in the same location an average of 10.3% of the time in the precision seeded plot.

A comparison of the disease organisms found in problem and non-problem fields, and brown and white roots has been discussed in the section of "Indirect determination of organism responsible for PRD." Only the disease rating of the various treatments will be discussed for each field separately.

Table VII. Average carrot emergence and percentage of doubles in precision-seeded and scatter shoe-seeded beds

	No. carrots/ft row		% doubles	
	PRD field	Non-problem field	PRD field	Non-problem field
Precision seeded	9.2	11.4	8.0	12.5
Scatter shoe seeded	8.7	7.0	-	-

On the non-problem soil, there was no significant difference ($P = .05$) in the incidence of Olpidium and fast-growing Pythium in conventional and raised beds (Table VIII). The first sampling of carrot rootlets from conventional beds on problem soil had a higher number of Olpidium spores than those from raised beds (28 versus 16 spores/microscope field), but in the later sample there was little difference in spore levels (19.4 versus 18.1 spores/field) (Table VIII). The grower's scatter shoe-seeded bed had fewer spores than the precision-seeded beds (7.1 versus 19.7 spores/field) in the second sample. The percentage of roots infected with slow-growing Pythium was nearly the same in raised and conventional beds and between the first and second samples. There was no significant difference ($P = .05$) in fast-growing Pythium and TNV recovery in raised and conventional beds (Table VIII). Fast growing Pythium was recovered less frequently from carrots in the second sample than the first (Table VIII). 12 inch and 14 inch row centers appeared to have little effect on disease incidence.

Table VIII. Organism survey in raised and conventional beds on PRD problem and non-problem soil at two sample times

Treatment	*Olpidium* Av. spores/field Sample 1	*Olpidium* Av. spores/field Sample 2	Fast *Pythium* % roots infected Sample 1	Fast *Pythium* % roots infected Sample 2	Slow *Pythium* % roots infected Sample 1	Slow *Pythium* % roots infected Sample 2	TNV % recovery Sample 1	TNV % recovery Sample 2
Problem Soil								
Scatter shoe	19.9 a[1]	7.1 a	12.4 a	0.9 a	9.5 a	12.4 a	100 a	20 a
3 row 14" raised	15.8 a	15.3 ab	10.5 a	2.9 a	6.7 a	4.8 a	100 a	40 a
4 row 12" raised	16.3 a	23.5 b	15.2 a	1.9 a	9.5 a	11.0 a	100 a	20 a
4 row 12" conventional	25.1 a	15.0 ab	12.4 a	3.8 a	9.5 a	2.9 a	100 a	0 a
4 row 14" conventional	30.6 a	21.1 b	12.4 a	0.9 a	7.6 a	13.3 a	100 a	40 a
Non-problem Soil								
Scatter shoe	1.4 a	-	13.4 a	-	0.0 a	-	0 a	-
3 row 14" raised	3.0 a	-	17.1 a	-	0.0 a	-	0 a	-
4 row 12" raised	2.5 a	-	24.8 a	-	0.0 a	-	0 a	-
4 row 12" conventional	1.7 a	-	27.6 a	-	0.0 a	-	0 a	-
4 row 14" conventional	2.6 a	-	27.6 a	-	0.0 a	-	0 a	-

[1]Means within the same column with the same letter do not differ significantly (P = .05).

Marketable yield of carrots, from raised and conventional beds with four rows per bed on non-problem soil were not significantly different (P = .05). The raised bed with four rows on 12 inch centers yielded equally well as four row 12 inch spaced conventional beds (Table IX). More culls were harvested from the scatter shoe-seeded bed than the precision seeded beds (Table IX). There was no advantage of using 14 inch centers over 12 inch spaced conventional beds.

On the problem soil, carrot yield on conventional beds was consistently better than on raised beds (Table IX). The scatter shoe seeded bed and the four row, 12 inch center conventional bed produced a significantly greater (P = .05) weight of marketable carrots than the raised beds. There was a greater weight of marketable carrots from the grower-planted scatter shoe-seeded bed because they were planted earlier and were more mature. In addition, the plant density was lower (Table VII), resulting in more carrots being in the B class (Table IX). Fewer culls (P = .05) were found in the four row, 14 inch conventional bed than in the scatter shoe-seeded and the four row 12 inch center raised bed. The three row 14 inch center raised bed had nearly the same marketable yield, and significantly fewer small carrots (P = .05) than the four row 12 inch center, raised bed.

The plot on the problem soil contained two distinct soil types: an organic, and an inorganic clay. Marketable yield was better in conventional beds compared to the raised beds on both soil types. However, the cull rate was twice as great on raised compared to conventional beds in organic-clay soil, but there was no difference between bed types

Table IX. Carrot yield in PRD problem and non-problem soil

Treatment	A lb.	B lb.	Culls lb.	Smalls lb.
Problem soil				
Scatter shoe	96.5 b[1]	45.0 a	24.4 a	0.5 c
3 row 14 inch raised	95.8 b	7.0 b	20.2 ab	5.5 b
4 row 12 inch raised	97.6 b	8.6 b	26.6 a	8.5 a
4 row 12 inch conventional	114.9 a	14.3 b	21.0 ab	4.1 b
4 row 14 inch	112.2 ab	9.6 b	17.6 b	6.9 ab
Non-problem soil				
Scatter shoe	89.0 bc	15.6 a	21.4 a	4.8 a
3 row 14 inch raised	78.6 c	5.5 b	9.9 b	3.0 a
4 row 12 inch raised	96.4 ab	5.7 b	13.6 b	5.7 a
4 row 12 inch conventional	103.3 a	8.7 b	11.6 b	3.8 a
4 row 14 inch conventional	88.0 c	13.9 a	13.7 b	3.1 a

[1]Means within a column with the same letter do not differ significantly (P = .05).

on the organic soil. Due to distribution of the clay area in the plot, only two replicates were used to make these comparisons.

Carrots in the problem soil suffered a water stress. Irrigation could not be applied as the water in the drainage ditches was too saline. The drought was so severe that some of the carrots in the raised beds felt rubbery at harvest.

Precision seeding trial. A field trial was conducted in 1973 to determine if precision seed spacing had any effect on the incidence of PRD in susceptible and tolerant carrot cultivars. HiPak (HP), a PRD tolerant variety, had 59% emergence which was significantly greater ($P = .05$) than the 52% emergence recorded for GoldPak (GP), a susceptible variety. The number of carrots per foot of row at the three seed spacings is given in Table X. There was a higher percentage of two carrots growing at the same location for HP than GP, and for the 1 1/4 inch seed spacing than other spacings (Table X). HP was much more vigorous than GP. Early in the season the differences in growth were especially noticable. The foliage growth of GP eventually caught up to that of HP.

Table X. Carrot emergence and the percent doubles (two carrots growing in the same location) of two varieties precision seeded at a spacing of 1 1/4, 1 1/2, and 2 inches

Variety	Emergence No. carrots/ ft of row 1 1/4"	1 1/2"	2"	Doubles % 1 1/4"	1 1/2"	2"
GoldPak	11.7	10.9	8.1	10.3	9.2	6.2
HiPak	14.5	11.0	9.2	15.2	12.7	8.7
Theoretical	24	20	15	0	0	0

The three spacings had no significant effect on the incidence of Olpidium, TNV, and fast and slow growing Pythium spp. HP usually had greater levels of the four organisms than GP (Table XI). A significant (P = .05) variety x spacing interaction occurred with Olpidium and fast growing Pythium. In the first sample the number of Olpidium spores decreased in GP but increased in HP with increasing plant spacing (Figure 5). The incidence of fast growing Pythium in the second sample at 1 1/4, 1 1/2, and 2 inch was 10.3, 11.9, 4.8, and 7.1, 9.5, 11.9% for GP and HP respectively. Fast growing Pythium incidence at the 2 inch spacing compared to other spacings significantly decreased (P = .05) in GP but significantly increased in HP.

At harvest, the culls were subdivided into two classes, "forks" and culls. Since PRD is capable of causing forked, hairy, rough, and stubby carrots, the "fork" class provided an estimate of the loss in yield due to PRD. As the spacing distance increased from 1 1/4 to 2 inches, the weight of the classes of A's, marketables (A + B), and forks, significantly increased (P = .05) and the weight of the classes of B's, culls, and smalls significantly decreased (P = .05) (Table XII). If yield of the classes is analyzed on a percentage basis (weight of class/ total weight x 100), there was no significant difference in the percentage weight of A's, B's, marketables (A + B), culls and smalls at the three plant spacings. However, the percentage weight of forks significantly decreased (P = .05) as the planting distance increased.

Table XI. Organism survey of brown roots at two sampling times of HiPak and GoldPak and precision seed spacings of 1 1/4, 1 1/2, 2 inches

	Olpidium Av. spores/field		Fast *Pythium* % roots infected		Slow *Pythium* % roots infected		TNV % recovery	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
Spacing								
1 1/4"	12.7 a[1]	8.4 a	11.1 a	8.7 a	10.3 a	11.1 a	59 a	50 a
1 1/2"	12.2 a	6.9 a	9.1 a	10.7 a	16.7 a	12.3 a	75 a	59 a
2"	13.8 a	7.7 a	10.7 a	8.3 a	16.2 a	17.9 a	33 a	50 a
Variety								
GoldPak	11.7 a	6.7 a	10.8 a	9.0 a	10.8 a	13.0 a	61 a	45 a
HiPak	14.1 b	8.6 a	9.7 a	9.5 a	17.9 b	14.5 a	50 a	61 a

[1]Means within a column with the same letter do not different significantly (P = .05).

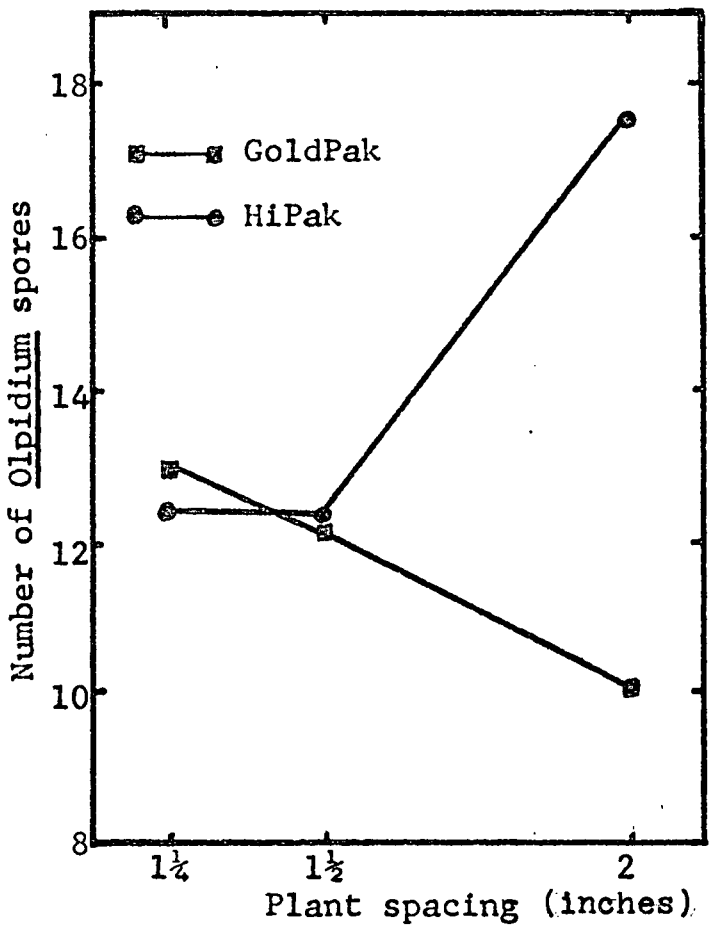

Figure 5. Average number of Olpidium spores per microscope field in rootlets of HiPak and GoldPak at three precision seed spacings. Each point represents the mean of 600 observations.

Table XII. Carrot yield of GoldPak and HiPak at precision seed spacing of 1 1/4, 1 1/2, and 2 inches

	A lb.	B lb.	Culls lb.	Forks lb.	Smalls lb.
Spacing					
1 1/4"	129.5 a[1]	3.5 a	11.4 a	18.5 a	5.0 a
1 1/2"	119.9 b	5.1 b	10.9 a	21.2 b	3.7 b
2"	107.3 c	5.4 b	8.5 b	20.7 b	2.2 c
Variety					
GoldPak	103.0 a	2.5 a	8.3 a	19.8 a	3.7 a
HiPak	134.8 b	6.8 b	12.2 b	20.5 a	3.6 a

[1]Means within a column with the same letter do not differ significantly (P = .05).

HP had a significantly greater (P = .05) weight of A's, B's, marketables, and culls than GP (Table XII). The marketable yield at spacings of 1 1/4, 1 1/2, and 2 inches was 28.1, 25.5, and 23.5 tons/acre, and 20.1, 19.9, and 17.4 tons/acre for HP and GP respectively. GP had a greater number (P = .05) of forked carrots, and a significantly greater (P = .05) percentage weight of smalls than HP.

There was no significant space x variety interaction in the weight of the classes of A's, B's, marketables, and smalls. HP had a significant decrease in the weight of culls at three spacings compared to closer spacings while GP had no significant change in the weight of culls at the three spacings. Similarly, GP had little change in the

weight of forks at the three spacings whereas HP had a significant increase (P = .05) in the weight of forks at spacings greater than 1 1/4 inches.

Weeds as alternate hosts for PRD incitants. A number of weeds growing in the precision-seeded plot were indexed for fast and slow growing Pythium spp., TNV, and Olpidium (Table XIII). Yellowed, stunted celery plants suffering from an unknown root disorder, and lettuce plants suffering from hollow root were also surveyed.

None of the weed species exhibited root tip necrosis. The epidermis and root hairs showed no signs of breakdown and decay as is commonly observed in carrot. Celery roots from both stunted and healthy plants contained high levels of Olpidium. Mycelium was frequently observed in the cortex of roots from only the stunted celery plants.

Crop rotation. The effects of a carrot, lettuce and onion rotation on the populations of PRD organisms was studied in the greenhouse. After the first crop of carrots, high levels of Olpidium, fast and slow growing Pythium spp. and TNV were recovered from all pots (Tables XIV-XVII). However, in the second and third croppings, low levels of Olpidium and slow growing Pythium were recovered only from rotations containing lettuce and onion. In carrots by contrast, the incidence of these organisms remained at the original recovery rate. In the second cropping, there was no significant difference (P = .05) in the level of fast growing Pythium but carrot roots had the highest level (Table XVI). After the third cropping, there was significantly (P = .05) more fast growing Pythium recovered from carrot than from lettuce or onion roots.

Table XIII. Organism incidence in some common weeds growing in soils with a history of severe PRD

Host	Fast *Pythium*	Slow *Pythium*	*Olpidium*	TNV
Lamb's quarters *Chenopodium album* L.	+++[1]	-	-	+[2]
Common chickweed *Stellaria media* (L.) Vill.	+	-	+	-
Sheperd's purse *Capsella bursa-pastoris* (L.) Medic.	+	-	-	+
Barnyard grass *Echinochloa crusgalli* (L.) Beauv.	++++	-	+	+
Redroot pigweed *Amaranthus retroflexus* L.	++++	-	-	-
Pineapple weed *Matricaria matricarioides* (Less.) Porter	+	-	++	+
Hop clover (?) *Trifolium* sp.	-	-	-	-
Celery *Apium graveolens* L.	++++	-	++++	-
Lettuce *Lactuca sativa* L.	++	+	++	-

[1] - = 0; + = 1-3; ++ = 4-7; +++ = 8-12; ++++ = 12; roots infected with fast and slow growing *Pythium* spp. or *Olpidium*.

[2] + = positive; - = negative TNV on *Chenopodium quinoa*.

Table XIV. Average number of Olpidium spores per microscope field in roots of crops from five rotations

Rotation	Croppings 1	2	3	4
A (C-C-C-C)	10.3 a[1]	7.4 a	7.0 a	3.1 a
B (C-L-O-C)	11.0 a	1.1 b	0.1 b	2.5 a
C (C-O-L-C)	12.6 a	0.4 b	0.8 b	2.3 a
D (C-L-L-C)	7.8 a	0.5 b	0.4 b	2.7 c
E (C-O-O-C),	8.0 a	0.4 b	0.2 b	3.8 a

[1] Means with the same letter within a column do not differ significantly (P = .05).

C = carrot; L = lettuce; O = onion.

Table XV. Percent slow growing Pythium recovered from roots of crops from five rotations

Rotation	Croppings 1	2	3	4
A (C-C-C-C)	17.5 abc[1]	33.5 a	41.5 a	34.0 a
B (C-L-O-C)	15.0 bc	0.0 b	0.9 b	36.5 a
C (C-O-L-C)	26.5 a	0.9 b	1.8 b	24.0 a
D (C-L-L-C)	22.5 ab	0.9 b	0.9 b	39.0 a
E (C-O-O-C)	11.0 c	0.9 b	0.9 b	21.5 a

[1] Means with the same letter within a column do not differ significantly (P = .05).

Table XVI. Percent fast growing Pythium recovered from roots of crops from five rotations

Rotation	Croppings 1	2	3	4
A (C-C-C-C)	18.5 a[1]	14.0 a	17.5 a	12.5 a
B (C-L-O-C)	21.0 a	2.0 a	0.9 b	16.0 a
C (C-O-L-C)	20.0 a	0.9 a	4.2 b	10.0 a
D (C-L-L-C)	11.5 a	9.0 a	7.5 b	11.0 a
E (C-O-O-C)	29.0 a	6.5 a	5.0 b	13.5 a

[1]Means within a column with the same letter do not differ significantly (P = .05).

Table XVII. Percent detection of TNV in roots of crops from five rotations

Rotation	Croppings 1	2	3	4
A (C-C-C-C)	100 a[1]	67 a	100 a	33 a
B (C-L-O-C)	100 a	17 b	33 b	0 a
C (C-O-L-C)	100 a	0 b	100 a	33 a
D (C-L-L-C)	100 a	33 ab	100 a	66 a
E (C-O-O-C)	100 a	0 b	66 ab	33 a

[1]Means within a column with the same letter do not differ significantly (P = .05).

TNV was not recovered from onion in the second cropping and was recovered less frequently from onion than from carrot and lettuce in the third cropping (Table XVII).

In the fourth cropping, when all pots were planted back to carrot, no significant difference (P = .05) was detected in the incidence of all four organisms (Tables XIV-XVII). The change in recovery rate of fast growing Pythium between the first and fourth croppings was significant only for crop rotation B. The number of roots infected with both fast and slow growing Pythium spp. were significantly higher in the last crop of carrots than the first crop.

In the first harvest, there were some brown carrot rootlets, but very few carrots exhibited forking. In the second and third croppings, only carrots had brown rootlets. The lettuce rootlets were an off-white colour and the onion roots were pearly white. No breakdown of the epidermis and cortex and little mycelium in the rootlets of lettuce and onion were observed by microscopic examination. By contrast carrot rootlets showed breakdown of the epidermis and had mycelium permeating the cortex. In the final harvest, the crop sequence of four successive carrot crops contained more typical PRD symptoms than carrot grown after other crop sequences. Between 10-20% of the carrots from the "only carrots" rotation displayed forking and stubby root symptoms. Carrots from the other crop sequences, had few red brown rootlets and only a few were forked. Generally, their rootlets were grey or grey-brown in colour.

Throughout the experiment, the carrots had an emergence of 55 to 62%; onions, 84 to 93%; and lettuce, 2.3 to 4.5% of maximum germination. Germination was determined by the petri dish germination test, and these results were used to correct the percent emergence. Lettuce required reseeding to get a sufficiently good stand, so that the soil would be well permeated with roots.

Supplementary illumination was insufficient to compensate for changes in day light between croppings, which prevented meaningful statistical analysis of yields between rotations. The first three croppings were conducted through the winter months and had much shorter periods of daylight than the final cropping which was conducted during the late spring. The effect of daylight was quite evident in a comparison of the root yields in crop sequence A (Table XVIII). In the first three harvests, carrot root weight was one third to one quarter the top weight but in the final harvest, root weight was 60% greater than top weight. Carrots grown in soil that had previously been in onions had a significantly greater ($P = .05$) root weight than carrots grown in soil that had previously been in lettuce or carrots (Table XVIII). Carrots grown after two crops of onions had a significantly greater root weight than after all other rotations.

The population of Pythium spp. in the soil as measured by dilution plating appeared to follow a cyclic pattern. No crop sequence significantly changed the level of Pythium in the soil (Table XIX). A decrease from 3600 to 1600 propagules of Pythium per gram oven dry soil was found after the first cropping of carrots. After the second

Tablve XVIII. Root weight of crops from five rotations

Rotation	Harvest 1	2	3	4
A (C-C-C-C)	10.6 a[1]	14.1	15.1	45.3 c
B (C-L-O-C)	9.9 a	8.4	16.6	61.5 b
C (C-O-L-C)	8.5 a	2.3	14.6	45.6 c
D (C-L-L-C)	7.5 a	6.4	17.8	40.2 c
E (C-O-O-C)	8.8 a	1.3	19.9	80.0 a

[1]Means within a column with the same letter do not significantly differ (P = .05).

Table XIX. *Pythium* propagule number x 10^2 per gram of over dry soil from five rotations

Rotation	Pre-plant	Croppings 1	2	3	4
A (C-C-C-C)	36	13 a[1]	22 a	11 bc	21 a
B (C-L-O-C)	36	17 a	25 a	11 bc	28 a
C (C-O-L-C)	36	14 a	13 b	8 c	26 a
D (C-L-L-C)	36	17 a	13 b	18 a	31 a
E (C-O-O-C)	36	21 a	8 b	16 ab	25 a

[1]Means within a column with the same letter do not significantly differ (P = .05).

harvest, Pythium propagule number in soil that had been in onions was significantly less (P = .05) than soil in carrots. The propagule level in soil in which lettuce had grown, showed an inconsistent change. In rotation B, the level of Pythium in the soil increased and in rotation D, the level decreased, even though both sequences had been in the same crops up to this stage of the experiment. There were often large variations in the number of Pythium propagules recovered at the four soil dilutions. Usually the 1:200 dilution had only one half to one third the propagule number per gram oven dry soil, as the 1:100 and 1:50 dilutions.

DISCUSSION

Etiology and Epidemiology of PRD

Koch's first postulate requires the pathogen always to be associated with the disease. An indirect method of proving that a given incitant is the cause of a disease when there is more than one organism present is to show that the suspected incitant is not present while the others are present in symptomless tissue. To this end, potential pathogens in brown carrot roots were compared with those in symptomless roots from both PRD problem and non-problem soils. The number of Olpidium spores, the presence of TNV, and the recovery rate of fast growing Pythium were similar in brown and white roots from PRD problem soils. Very low levels of slow growing Pythium were recovered from symptomless roots, while high levels were recovered from brown roots. Slow growing Pythium

spp. could be recovered from rootlets of plants grown only in problem fields and were not recovered from carrots grown in non-problem soils. In a second sampling of the same plots, there was an increase in the incidence in the rate of recovery of slow growing Pythium from white roots. Since it was very difficult to find white rootlets in the second sampling some of the off-white or grey rootlets selected may have been in the early stages of developing PRD. By contrast none of the weed species investigated was found to harbor the slow growing Pythium. Based on field isolations and greenhouse rotations in PRD problem soil, both lettuce and onion supported only very low levels of this organism. However, the incidence in greenhouse grown carrots following either lettuce or onion crops was not significantly reduced, even though the two preceding crops supported very low levels. This slow growing Pythium has been identified on the basis of growth rate and diagnostic antheridal morphology as the very pathogenic Pythium sulcatum. These results certainly implicate P. sulcatum as a causal agent of PRD in British Columbia soils and suggest that the organism has a rather narrow host range.

A causal role for the fast growing Pythium species in PRD has not been ruled out, but there was a high recovery rate from both white and brown carrot roots. Lettuce, onion, celery, and a number of weed species (Table XIII) were good hosts for the fast growing Pythium species. P. irregular, P. paroecandrum "classical form," P. paroecandrum "P. ultimum" form, P. debaryanum, P. sylvaticum, and P. coloratum and several unidentified fast growing Pythium sp. were isolated from British Columbia soils (R. J. Howard and R. G. Pratt, personal communication). Probably

not all of these species are pathogenic to carrot, but at least two species, P. debaryanum and P. irregulare have also been isolated from lettuce and onion (MacFarlane, 1968).

In Florida and Wisconsin, Pythium spp. are responsible for 50% pre-emergence damping off (R. J. Howard and J. O. Strandburg, personal communication). Similarly, 40-50% seed failure has been observed in field trials and greenhouse experiments using PRD infested soil. Preliminary experiments suggest that some isolates of P. sylvaticum and P. sulcatum caused seed failure. In pathogenicity trials, F. D. McElroy (unpublished results) found that it was necessary to germinate seeds on filter paper before inoculating with P. sylvaticum, because if seeding and inoculating were done simultaneously, a higher rate of seed failure occurred. Pythium species were probably responsible for the poor emergence rate of lettuce in PRD soil. Farmers on muck soil in the Fraser Valley sow lettuce at very heavy rates to compensate for poor emergence. The importance and role of Pythium in pre-emergence damping off of carrots and lettuce requires further study.

The presence of 80-100 Olpidium zoosporangia and resting spores per 560 μ section of rootlet and the sporadic recovery of TNV from symptomed roots were early observations. As a consequence, considerable time was spent in determining what role TNV, Olpidium brassicae, or both, were playing in the disease complex.

TNV was recovered only from carrot roots in PRD problem soil which suggests a causal role. Smith (1937) concluded that TNV occurred in symptomless roots, but Teakle (1962b) has shown that sterile cowpea

roots produce necrotic lesions when rub-inoculated with TNV. Attempts to determine if TNV alone could reproduce field symptoms, were inconclusive. Lesions were only sporadically observed on TNV rub-inoculated carrot roots, and these were similar to lesions which developed on phosphate buffer inoculated control plants. Because carrot roots are much more fragile and more easily injured than cowpea or mung bean roots used by Teakle (1962b), the lesions observed were probably the result of injury during inoculation rather than TNV infection. Roots dipped in TNV extracts also developed symptoms and indexed positive for virus. Handling the seedlings during dip-inoculation, must have sufficiently injured the roots to enable virus penetration and infection to occur. TNV could also have been recovered from symptomless and dip-inoculated roots if infective TNV particles on the root surface were not inactivated by surface disinfecting the roots in Concentrate R.B.S. *.

Attempts to establish a role for Olpidium alone as the causative agent of PRD were unsuccessful. The incidence of spores was not highly correlated with disease severity as measured by marketable yield and cull rate. Typical necrotic root symptoms were not observed with Olpidium alone or in mixed infections with TNV. No symptoms were produced on lettuce even when infection levels were greater than 50 sporangia per 560 μ diameter field. Furthermore, the presence of Olpidium was observed in both PRD problem and non-problem fields but TNV, transmitted by Olpidium was only observed in PRD problem fields. The correlation of the virus but not the vector to PRD problem fields may possibly be explained by Teakle's (1962b) observation that root necrosis of lettuce

occurred only under conditions of high inoculum potentials of both Olpidium and TNV. Perhaps the virus titer in the non-problem soil was too low to initiate symptom development.

Olpidium was also observed in roots of a number of weeds, celery, lettuce and onion and TNV was detected in all but a few weed species. In the crop rotation experiments, onion was the poorest host, and a significantly lower level of Olpidium was also observed in lettuce than in carrot. This later observation is contradictory to culture studies of Olpidium where on carrot, Olpidium isolates died after several transfers but on lettuce, the isolates reproduced well. Different lettuce varieties were used in two experiments. Host specificity may also be involved (D.J.S. Barr, personal communication). Garnet and Tomlinson (1967) found that lettuce isolates failed to infect brussel sprout, cabbage and turnip and conversely cabbage isolates infected these crops but not lettuce. However, it is strange that the Olpidium isolates recovered from carrot were not more specific to carrot considering the problem soil used for baiting had been planted to carrots the three preceding years. Another complicating factor in this issue, and the field surveys as well, is that Pythium species are capable of reducing Olpidium infection (D.J.S. Barr, personal communication). The high levels of Pythium in the rotation experiment may have inhibited Olpidium infection of lettuce. This mechanism could also be important in preventing Olpidium infections from becoming so numerous as to cause a measurable yield loss. Experimentally determining a role for Olpidium at high populations must await the development of suitable techniques for the maintenance of these apparently strain specific isolates.

Previous disease rating had been based on observations of unstained infected roots which did not permit distinguishing Pythium and Olpidium sporangia. With the introduction of the staining technique in 1972 field studies, it was apparent that only a few of the sporangia were Pythiaceous while the majority were Olpidiaceous. This resulted in the switch to root isolations on selective media in subsequent attempts to monitor Pythium. Attempts to evaluate microenvironmental factors with Olpidium incidence were continued, since conclusive proof of the non-involvement of Olpidium had not been shown.

The only environmental factor that appears to be related to Olpidium population fluctuations in 1972 field studies was rainfall. Soil temperature, soil CO_2 and O_2, saturated hydraulic conductivity, and soil moisture difference between "raised" and "conventional" beds, apparently had no direct effects on Olpidium. Rainfall or irrigations in sufficient quantity to saturate the soil for a short period appeared to influence Olpidium spread and build up in carrot roots. This was not unexpected because Olpidium requires free water for zoospore release and spread. Heavy rainfalls or irrigation may increase the free water in the root zone for a short period and thus allow for zoospore release and infection of roots. Under optimum conditions of temperature and moisture, Olpidium's life cycle requires 5 to 7 days but under less ideal conditions it is 7-14 days (Fry and Campbell, 1966). Therefore, the amount and frequency of rainfall and irrigation 7 to 21 days prior to sampling are important in determining the spread and build up of Olpidium in young carrot rootlets. One half inch of precipitation is generally considered to be the minimum beneficial quantity of water

required to wet a soil, and amounts less than this only dampen the soil surface. Rainfall of less than 1/2 inch was thought not to influence Olpidium.

A very dry May resulted in a low initial infection of early planted carrots. Carrots planted in late May were subjected to 2 or 3 periods of soil saturation and thus had high levels of Olpidium infection. The mid-June planting of carrots appeared to be more resistant to Olpidium despite being subjected to at least four potential infection periods. It appears that later plantings of carrots escaped infection even though conditions were favorable for spread of Olpidium. Olpidium levels decreased during August, a month with only one infection period. It appeared that once there was a high inoculum level in the roots, the population of Olpidium was high in new rootlets even if infection periods were less favorable.

Even though there was no precipitation after the July cloudburst, soil moisture was maintained at an optimum level for carrot growth by adjusting the level of water in the drainage ditches to maintain a constant water table. Soil moisture during this period was less favorable for zoospore spread but was optimum for carrot growth.

Zoospore movement is dependent upon the water filled pore size. Flagella propel zoospores relatively short distances in water filled pores. The maximum movement of zoospores becomes possible when the matric potential allows for the greatest frequency of water filled pores large enough to accommodate the zoospore (Cook and Papendick, 1972). The maximum pore diameter that remains filled with water as a soil drains can be determined from the capillary rise equation $\psi_m d = 2.94$, where

ψ_m = matric potential in bars, and d = pore diameter in μ. Stolzy et al. (1965) suggested (without any supporting data) that a water filled pore diameter of 40 to 60 μ is necessary for Phytophthora zoospore movement. Phytophthora zoospores are slightly larger than Olpidium zoospores, but if relatively the same pore diameter is necessary for movement of Olpidium zoospores, this would correspond to a matric potential of -.05 to -.07 bar. These potentials are equal to a soil water content of 110 to 115% (calculated from the retention curve Figure 3, Russel, 1972). At the 15 cm depth, the soil moisture in "conventional" beds was greater than 110% until August. Soil moisture in the "raised" bed was below 110% during late May and mid-June, it then increased and did not become less than 110% until late July (Figure 4). The increase in water content above 110% in "raised" beds corresponded to the period of highest Olpidium infection.

The type of spore formed by Olpidium will influence population build up. Under favorable conditions, zoosporangia are formed in roots and there is a short period between zoospore infection and release. Under less favorable conditions, resting spores develop. The resting spores are resistant to adverse conditions and require the presence of certain factors to trigger the breaking of dormancy and production of zoospores to initiate new infections. Therefore, roots with a high proportion of zoosporangia have a higher infection capability than roots with a high proportion of resting spores. As the season progressed, a higher proportion of the spores were resting spores, so that these roots were less capable of spreading Olpidium even if weather conditions had been favorable for spread.

Modification of the microenvironment with "raised" beds, in 1972, influenced Olpidium incidence only in the earliest planted carrots. In the first sampling of 1973, Olpidium spore levels were less in raised beds made on a commercial scale than conventional beds. However it was concluded that raised beds had little overall effect in reducing Olpidium infection. Olpidium appears to have no direct effect on yield, as yield was significantly affected by bed height, whereas the incidence of Olpidium was not. The use of precision seeding to modify the microenvironment of individual plants also had no effect on Olpidium incidence.

Evaluation of Cultural Practices to Control PRD

Raised beds . Yield of marketable carrots from "raised" beds was greater than that from "conventional" beds in 1972 but not 1973. In 1972, "raised" beds were made higher by removing soil from adjacent beds, whereas in 1973, raised beds were made only as high as was possible with the grower's equipment. Therefore, the comparison between bed heights of raised and conventional beds was much more extreme in 1972 than in 1973. In 1972 there was a shift in the population from a large number of small carrots in "conventional" beds to fewer small carrots and more marketable carrots in "raised" beds. If the lateral root dieback phase of the disease is important this would prevent many carrots from reaching a larger size. It would seem that one of the major benefits of "raised" beds was to increase marketable yield by decreasing the time from planting to maturity. In California, narrow beds, 20 inches from

center to center with an 8 inch wide top are being used because narrow beds have a greater surface area exposed to heat units so crop maturity is earlier (Anon., 1973c). "Raised" beds also increase the exposed surface area, and this may explain why carrots in "raised" beds appear to mature earlier.

On non-problem soil, where there was no visible water stress, bed height had no effect on marketable yield. The average marketable yield from beds with four rows was 18.5 T/acre for raised beds and 19.4 T/acre for conventional beds. On the problem soil, where there was a serious water stress, four row conventional beds out-yielded the four row raised beds, 22.8 to 19.3 T/acre respectively. Under conditions where moisture was limiting and irrigation was unavailable, carrots on raised beds suffered a greater water stress than carrots on conventional beds. The carrots from the raised beds were smaller than those from conventional beds: .156 lb/carrot and .174 lb/carrot respectively. Under severe water stress, the marketable yield from raised beds with three rows per bed nearly equalled that from the four rows raised bed. Plants on the four row raised beds did not have enough water to "size up" as was indicated at harvest by greater percent weight of small carrots from the four row raised beds than from the three row raised bed. Raised beds with four rows per bed will yield as well as conventional four row beds if soil moisture is adequate, and therefore should only be used if irrigation water is available.

Bed height had no effect on the cull rate of carrots on non-problem soil. The 1973 growing season was very dry and as a result

there was only a minor incidence of PRD. There was no opportunity to evaluate the raised beds' ability to reduce disease incidence and cull rate due to PRD.

The grower with the problem soil used much higher conventional beds than employed by most other growers. As a consequence, the width of the planting surface of his conventional beds was less. Problems were encountered in keeping the seeder on the bed when seeding four rows with 14 inch centers. This may have been one cause for the poor performance of the four row 14 inch center conventional beds.

Several factors other than the quality and availability of irrigation water should be considered when using raised beds. Equipment must be capable of throwing the soil up into a bed, rather than just making furrows. A bed shaper is necessary to firm the soil on the sides of the bed and make a smooth packed surface for precision seeding. Raised beds should be 45 inches wide if four row 12 inch spacing is used, otherwise it is difficult to keep the seeder on the bed, and the soil may fall away from the edges of the bed later in the season and expose the roots. Mechanical weeders must be adjusted for the narrower between row spacing. The between row spacing of 14 inch center to center precision seeded carrots seemed slightly less than the between row spacing of the 14 inch center to center scatter shoe seeded carrots and therefore, the precision seeded carrots were damaged by the mechanical weeder set for the scatter shoe between row spacing.

Yield was increased by 10-25% with four rows of carrots per bed over 3 rows per bed. The yield increase was most noticeable in

carrots not suffering from drought. If moisture is available, five rows per bed may further increase yield. The between row space does not have to be as great as in the past because more effective herbicides are now used so less cultivation is required. Filman (1972e) has found that yields were significantly increased, the nearer the plants were evenly spaced at about 12 cm^2 per plant (4 x 3 inch grid) compared to the standard 16 inch row spacing. However, British Columbia growers have expressed doubt that present harvesting combines could handle the increased carrot density of a solidly sown bed or five row bed.

Precision seeding and tolerant varieties. HiPak (HP), a PRD tolerant variety, had a greater marketable yield and fewer number of forked carrots than GoldPak (GP), a PRD susceptible variety despite having a higher incidence of Olpidium and slow growing Pythium. Forking of carrots was considered one measure of PRD incidence. On this basis GP was more adversely affected by PRD than HP. The loss of yield due to PRD was 13.5-15.8% and 9.2-13.3% for GP and HP respectively at the different spacings. HP's ability to tolerate PRD has been previously observed (McElroy et al., 1971).

In the grower's raised bed trial, precision seeding significantly reduced cull rate over scatter shoe-seeding, confirming A. R. Maurer's unpublished results. However there was no significant difference in the incidence of the PRD associated organisms.

Marketable yield was greater as the precision plant spacing decreased. At a spacing of 1 1/4 inches, marketable yield was the greatest but on a percentage weight basis, plant spacing had no

significant effect on yield. Yield increased at the closer spacing only because plant population was greater. The incidence of Olpidium, fast and slow growing Pythium spp., and TNV were not affected by plant spacing, but the percent weight of forks significantly decreased as plant spacing increased. The increase in marketable yield far exceeded the small increase in culls at the closer spacings. Filman (1972b) found that by reducing the seeding rate by half from the recommended rate of 15 to 20 seed per foot, PRD was reduced by 10%, but the disease incidence was still too high for economic harvesting. In the present trial, PRD, as measured by forking, was least at the closest spacing. A high disease incidence would have been predicted because the plot had been in carrots the 3 previous years, the area had a history of severe PRD incidence, and the soil was maintained at a high soil water content throughout the growing season. Precision seeding and raised beds appear to have sufficiently modified the soil microenvironment to limit build up and spread of the PRD associated organisms and as a consequence, PRD losses were kept to a minimum. Nonnecke (1973) has also noted the PRD was kept to a minimum by precision seeding, because "stress" between individual plants was removed.

The high frequency of two carrots growing in the same location in precision seeded beds could be caused by a number of factors. The seed may have been poorly coated, and crumbled off in the planter, so that two seeds could then fall through the same hole in the belt. This explanation seems unlikely as the two varieties had been coated by different companies. The stop and start planting of short plots may have caused some seeding irregularities, but nearly the same percentage of

doubles were observed in the grower's trial. Two carrots growing in the same location may become culls by twining around each other, but in this trial, they helped to fill in the row because of the poor emergence. The cause of two carrots growing in the same location requires further study.

Crop rotation. Lettuce and onion, two crops commonly grown in rotation with carrots on muck soil in the Fraser Valley, were evaluated as to their effectiveness in reducing PRD. There was no simple correlation between crop yield and the incidence of the four PRD organisms. Carrots, grown after onions had a significantly greater yield, but the same level of infection of Olpidium, fast and slow growing Pythium and TNV as carrots grown after lettuce or carrots. A higher percentage of the carrots were forked and exhibited PRD symptoms in soil that was continuously cropped in carrots than soil that had been in onions or lettuce. This was in conflict with Sutton's (1973) questionable observation correlating PRD incidence and previous crops of onions.

Olpidium and slow growing Pythium were at low levels in lettuce and onion for two crop periods, but were at high levels when the soil was returned to carrots. These organisms may maintain their population in the soil by a resting spore, which can survive adverse conditions such as poor hosts, or by infecting the poor host just enough to maintain its population, but not enough to cause damage. A greenhouse crop rotation experiment is unable to duplicate the effects of winter. Periods of soil freezing and flooding, and the long period the soil is idle without a crop would probably reduce the hold-over of an organism from

one crop to the next. In the greenhouse experiment another crop was planted within a day after harvesting the previous crop. Filman and Andersen (1972) have shown that PRD is reduced 16% by 10 days of soil flooding before planting. Many of the muck souls around Cloverdale are flooded for a considerable part of the winter but PRD is still a problem. Flooding during the winter may reduce the PRD associated organisms enough to permit economic growing of carrots in British Columbia. Under field conditions, lettuce and onions in a rotation with carrots, would probably have greater effects on Pythium and Olpidium than was observed in the greenhouse.

Losses due to PRD can be kept to a minimum by following an integrated method of control. Firstly, Copeman and Black (1973) showed that marketable yield could be increased and cull rate decreased by using PRD tolerant varieties. Raised beds significantly increased marketable yield over low conventional height beds if there is a sufficient soil moisture throughout the season. Precision seeding reduces the cull rate over scatter shoe-seeding. The carrots are more evenly spaced so there is probably less spread of the disease from one carrot to the next. And finally, PRD is reduced if other crops such as lettuce and onion are included in the rotation. Onions appear to be the best crop to precede carrots. PRD losses can probably be kept to a minimum by using crop rotation, tolerant varieties, raised beds, and precision seeding.

CONCLUSIONS

1. Negative evidence from pathogenicity tests and the lack of correlation between the incidence of Olpidium and carrot marketable yield or forking suggest that Olpidium and TNV play a minor role in PRD.

2. Indirect field evidence suggests that Pythium sulcatum is a primary causal agent. Other fast growing Pythium spp. may also be important.

3. Olpidium incidence appears to be directly correlated with frequency of precipitation greater than 1/2 inch.

4. PRD losses can be kept to a minimum and marketable yields increased by following an integrated method of control using a) tolerant varieties, b) raised beds, c) precision seeding, d) crop rotation.

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APPENDIX I

Analysis of Variance of Olpidium spores per microscope field from the 1972 microenvironment plot

Line No.	Source	df	MS	EMS	F
1	Row	3	4680.6	Error A	2.5
2	Column	3	845.1	Error A	0.5
3	Date	3	41355.0	I	3.8559*
4	Error A	6	1854.4	Error B	0.9
5	Water	1	2488.5	II	1.067
6	W x D	3	2518.0	III	1.0485
7	Error B	12	1965.4	Error C	1.0
8	Age	4	8973.4	IV	3.1104*
9	A x W	4	1779.8	AxWxD	0.9
10	A x W	12	7406.7	AxWxD	3.53*
11	A x W x D	12	2098.5	Error C	1.11
12	Error C	96	1889.2	Root	3.47**
13	Roots	3040	543.8	L/Rt	6.95**
14	L/Rt	12800	78.2		

* Significance at the 5% level (P = .05).

** Significance at the 1% level (P = .01).

Quasi F" test and degrees of freedom for numerator/denominator.

I MS_{3+7+11}/MS_{4+6+10} df 3.6/19.0

II MS_{5+11}/MS_{6+9} df 3.2/19.0

III MS_{6+12}/MS_{7+11} df 9.0/24.0

IV MS_{8+11}/MS_{9+10} df 6.0/15.7

CHAPTER II

CONTROLLED WATER RELATIONS AND *PYTHIUM* ROOT DIEBACK DEVELOPMENT

INTRODUCTION

Field work conducted by Copeman and Black (1972) had suggested that soil moisture was the most important soil parameter in the incidence of *Pythium* root dieback (PRD) of carrot. Roots from plots maintained at a high soil water content contained a greater number of *Pythium* and *Olpidium* spores than roots from drier plots. Greater marketable yields were also harvested from these drier plots, suggesting that soil moisture, the presence of spores in the roots and yields were correlated.

Control of Soil Water

Soil water is best described in terms of potential energy. Water (like heat) flows from areas of high to low potential energy, in accordance with the Second Law of Thermodynamics, until the two areas are in equilibrium. Soil water potential is the sum of pressure, matric, osmotic, gravitational, and several negligible potentials. The pressure potential results when hydrostatic pressures occur in the soil, for example when the soil surface is flooded. The matric potential results from the physical affinity of water to the soil particle surfaces and

capillary pores. Osmotic potential is influenced by the presence of solutes in the soil water. The gravitational potential is the result of gravitational forces and is important only between saturation capacity (SC) and field capacity (FC). Under most field conditions, except those of high water and high salt content, matric potential is the most important component of the total soil water potential.

Osmotic potential may dominate in the rhizosphere and root since roots are known to exude salts, amino acids and sugars. Earlier work on the effects of osmotic potential on fungal germination and growth have been reviewed by Scott (1957) and Griffin (1963a). Sommers et al (1970) have recently shown that growth of Phytophthora species is stimulated as potential energy decreases from -1.2 bars to -8.0 bars on agar media osmotically amended with salts or sucrose. Phytophthora parasitica was inactivated at a potential of -40 to -50 bars (Sommers et al., 1970) and Fusarium oxysporum f. sp. vasinfectum at -120 bars (Manandhar and Bruehl, 1973).

Experimental methods for controlling soil moisture of growing plants within narrow limit are subject to a great deal of error. High soil water potentials can be maintained by the Haines apparatus; a sintered glass funnel connected to a hanging water column (Haines, 1930). The Haines technique is generally limited to potentials of greater than -0.15 bars because the flow rate of water through the funnel is too slow at lower potentials. This technique is ideal for spore germination studies (Bainbridge, 1970) and seed exudate studies (Kerr, 1964).

Weighing pots and adding enough water to maintain the plants at the desired level of soil moisture works reasonably well in the range

between SC and FC but is not satisfactory in the range of FC to permanent wilting point (PWP) (Couch et al., 1967). The whole soil mass cannot be uniformly wetted at stresses below FC because when water is added to the top of a pot, it tends to move down along the sides. As a result some areas of soil are saturated and others are near the PWP (Hendickson and Veihmeyer, 1941). To avoid the problem of only wetting part of the soil, the recommended procedure is to allow the pots to dry out to a predetermined moisture level and then add enough water to bring the soil back up to FC (Couch et al., 1967). Essentially, this technique only provides information on the effects of adding different amounts of water to pots. For example, pea root rot, caused by Pythium ultimum was most severe when water fluctuated between -1/3 bar and -1 bar, and greater drying prior to rewatering to FC, reduced disease severity (Kraft and Roberts, 1967).

Roth and Riker (1943) studied the effects of moisture on damping off of red pine seedlings by Pythium and Rhizoctonia by using different heights of soil cylinders above a constant water table. Bateman (1961) improved the technique by restricting the root zone in small clay pots buried in varying depths of sand above the water table. The height of sand column necessary for a certain water potential is obtained by trial and error and is difficult to duplicate. Capillary movement of water through the sand, the walls of the pot, and the soil within the pot probably will not keep up with the demands of a growing plant, and as a result, water potential decreases. Double-walled porous pots have a similar short coming--the flow rate of water through them is very slow at water potentials less than FC.

An osmotic system has been developed to control soil water matric potential (Zur, 1966). A narrow slice of soil is separated from an osmotic solution by a semi-permeable membrane. Water moves from the osmotic solution to the soil when the potential of the soil is less than that of the solution. By maintaining the volume of the osmotic solution constant, water lost by soil surface evaporation and transpiration is continuously replaced. A membrane with a high water conductivity and resistance to microbial breakdown is necessary if this technique is to be used to control soil moisture for several weeks.

Effects of Soil Water on *Pythium*

Pythium diseases are usually associated with soils of high soil water content. A number of roles for soil water in disease development and *Pythium* population dynamics have been suggested. Oogonia of *P. ultimum* were more prevalent at high soil potentials than sporangia (Bainbridge, 1970). Oogonia production took place in water filled pores, whereas sporangial production only took place in air filled pores. Griffin (1963b) found that *P. ultimum* produced oospores in wet soils only when soil pores were of sufficient size to accommodate the oogonia. Sexual reproduction did not occur in artificial soils with a pore space less than 15 μ diameter, though mycelial growth was unaffected.

The size of water-filled pores or pore necks and pore size distribution may limit the passage of certain Phycomycetes such as *Olpidium* which rely on zoospores for spread. As the matric potential decreases, the diameter of water-filled pores decreases. Stolzy *et al.* (1965) suggested that zoospores of *Phytophthora* species required water-filled

pores at least 40-60 μ in diameter. At saturation with distilled water, no zoospores were produced by oospores or sporangia of P. aphanidermatum but at three times soil saturation, 30% of the oospores and 90% of the sporangia formed zoospores. If nutrients were in the water, the propagules germinated directly (Stanghellini and Burr, 1973). They concluded that zoospores are only produced in surface water of saturated soils and are not an important form of inoculum spread.

Kerr (1964)concluded that soil moisture did not affect P. ultimum per se but influenced the amount of sugar exuded from pea seeds. Under high soil moisture, bean exudates permeated further from the seed so that a greater number of Pythium spores were stimulated to germinate and infect the seedlings (Stanghellini and Burr, 1973). Germination and growth was restricted at higher water potentials because of a reduced availability of nutrients.

When a soil becomes saturated, the concentration of O_2 decreases and CO_2 increases. Griffin (1963b) has suggested that the ability of Pythium to withstand saturated conditions is related to the ability of the fungus to withstand either low concentrations of O_2 or high concentrations of CO_2. P. ultimum growth was reduced at 1.3% O_2 but not 4% O_2 and P. irregulare and P. vexans were relatively unaffected by high CO_2 and low O_2 concentrations (Gardner and Hendrix, 1973).

Periodic or continuous saturation may have different effects on Pythium species. P. irregulare was unaffected by periodic or continuous saturation because it infected holly roots only by direct germination of the sporangia, whereas P. vexans was most severe under continuous saturation because it produced zoospores (Biesbrock and Hendrix, 1970a).

Since soil moisture appeared to be the critical factor in PRD, its role in disease development was studied. The objectives of the study were 1) to determine the effects of periodic soil saturation on Pythium and Olpidium populations, 2) to observe the effect of osmotic potential on Pythium growth, and 3) to develop a technique for controlling soil moisture of a growing carrot.

METHODS AND MATERIALS

Periodic Saturation Experiment

A greenhouse experiment was done to determine the effects of periodic soil saturation on PRD incidence. The soil for the experiment was a Lumbrum Muck from the farm of Cloverdale Produce Farms Ltd., which had produced a carrot crop with symptoms of PRD the past summer. A portion of the soil was autoclaved at 15 psi for 90 minutes. One week after autoclaving, the autoclaved and unautoclaved soil was potted in square 4 inch plastic pots.

Carrot, Daucas carota L. 'GoldPak Elite', was seeded and lightly covered with soil. After seedlings had emerged, they were thinned to two uniform seedlings per pot. The carrots were grown at 16-20 C, under a 14 hour photoperiod (3600 lux) provided by "Cool White" fluorescent lamps and incadescent bulbs.

Pythium sylvaticum Campbell and Hendrix was grown on Difco Corn meal agar (CMA, 17 g/l) plates at 25 $\pm$ 1 C for eight days. The cultures were examined for sporangia and then the agar and the organism were

homogenized with an Osterizer blender at low speed for 10 seconds in 250 ml of distilled water. Subsequent to thinning, four 1 ml aliquants of solution were injected with a syringe into half of the pots containing autoclaved soil. Naturally-infested, field soil served as a third soil treatment. Pots were placed in plastic lined cedar flats (12 x 18 inches) with a drain hole at one end.

Four watering regimes were used: 1) sprinkling the pots from above when they appeared to be nearing the point of wilting; 2) saturating pots by filling the flats with water for 10 minutes daily; 3) saturating pots 10 minutes every fourth day; and, 4) saturating the pots 8 hours every fourth day. Pots of different inoculum treatments were kept in separate flats.

Every two weeks, for 8 weeks, three pots in each water and soil treatment were harvested. The total fresh weight of the carrots was measured and 20 brown or discoloured rootlets from plants in each pot were picked off and preserved in formalin acetic acid (FAA) (Phillips and Hayman, 1970). Roots were stained with Phloxine and KOH (Tuite, 1969), and examined under phase contrast. The number of spores at five locations of estimated highest density were recorded for each root. The remainder of the root system was macerated in a small amount of 0.05 M phosphate buffer, pH 8.5 (Teakle, 1962), and rub-inoculated on carborundum-dusted leaves of Chenopodium quinoa Willd. The data was analyzed by a 3 x 4 factorial analysis.

Effects of Osmotic Potential on Pythium

To determine the ability of Pythium to infect and colonize a root, it was necessary to determine Pythium's ability to tolerate various osmotic potentials.

The osmotic potentials of agar media were adjusted to various levels by adding solutes of the appropriate molal concentrations. For a single solute, the molal concentrations were determined by interpolation from the water activity (a_w) tables of Robinson and Stokes (1955). A salt mixture consisting of $5NaCl:3KCl:2Na_2SO_4$ was also used to adjust the osmotic potentials. The a_w values of Na_2SO_4 were calculated from the formula:

$$\log a_w = -0.007824vm\emptyset$$

where v = no. of ions per molecule (3), m = molality and $\emptyset$ = osmotic coefficient. Robinson and Stokes (1955) list the osmotic coefficients. The a_w values of the salt mixture is the sum of the 3 salt a_w values at the 5:3:2 molal ratio. Water potential (Ψ) is derived from the a_w values by the formula:

$$\Psi = \frac{RT}{V} \log_{10} a_w = 10.6\ T \log a_w$$

where R = ideal gas constant, T = absolute temperature and V = volume of a mole of water (Manandhar and Bruehl, 1973).

The general procedure used in all experiments was to dissolve the salts in distilled water before the agar medium was added. Calcium chloride was dissolved in distilled water and autoclaved, before adding the agar to prevent precipitation. The potentials of the solute amended

media were calculated as the sum of the medium plus solute osmotic potentials. Media were autoclaved at 15 psi for 15 minutes. After the media had cooled to about 46 C, 20-25 mls were poured into 8.5 cm ID plastic petri dishes. Dishes were left ajar in a laminar air flow bench to prevent condensation on the lids. A mycelial agar plug taken with a #1 cork bore (4 mm diameter) from the leading edge of an actively growing Pythium culture (grown on the same base medium) served as inoculum. Each treatment was replicated four times. Petri dishes were sealed in plastic bags and incubated at 25 $\pm$ 1 C. Colony diameter was measured at regular intervals until the fastest growing treatment had covered the plate.

The effect of the solutes NaCl, KCl, $CaCl_2$, 5NaCl:3KCl:2$NaSO_4$ and sucrose on growth of P. sylvaticum on CMA (Difco 17 g/l) and P. sulcatum Pratt and Mitchell on basal medium (Sommers et al., 1970) were tested. To determine if nutrition might have an effect on osmotic potential tolerance, CMA, potato dextrose agar (PDA, Difco 39 g/l) and basal medium were adjusted osmotically with KCl and inoculated with P. sulcatum. The osmotic potential of nonamended basal medium, CMA and PDA were -1.2, -1.2 and -3.2 bars respectively (Sommers et al., 1970). P. ultimum Trow, and two isolates each of P. sylvaticum and P. sulcatum were inoculated on KCl osmotically adjusted basal medium to determine if different species and isolates of Pythium act similarly to osmotic potentials.

Development of a Technique to Control Matric Water Potential

After a comparison of the techniques used to control soil moisture, I decided that Zur's osmotic system had the greatest potential in controlling soil moisture within narrow limits.

Cellulose dialysis membrane.

Flow rate. The flow rate of water through the membrane was determined by measuring the quantity of water passing through the membrane from a reservoir of distilled water to a reservoir of Polyethylene glycol (PEG) solution. A glass millipore filter holder was attached to a funnel by means of rubber tubing (Figure 1). A single thickness of a 1 5/16 inch flat diameter, 0.001" wall thickness cellulose dialysis membrane (Arthur H. Thomas Co., Philadelphia) was placed on the screen of the millipore filter holder. The funnel was filled with distilled water and all the air bubbles were removed from between the screen and dialysis membrane. A 2.5 bar PEG 6000 solution (J. T. Baker Chemical Co., New Jersey) was placed in the filter holder. The concentration of PEG at various osmotic potentials was determined by interpolation from graphs of Zur (1966) and Williams and Shaykewich (1970) (Table I). The levels of the two solutions were adjusted to the same initial height. The change in volume of the PEG solution was measured and removed daily.

Moisture controlling prototypes using dialysis membrane. Soil cells were constructed by fitting a cellulose dialysis membrane over a Polyvinylchloride (PVC) frame. Three sixteenth inch PVC was used to make a frame 12.5 x 1.0 x 30.0 cm. Dialysis tubing, 5 3/4 inch flat

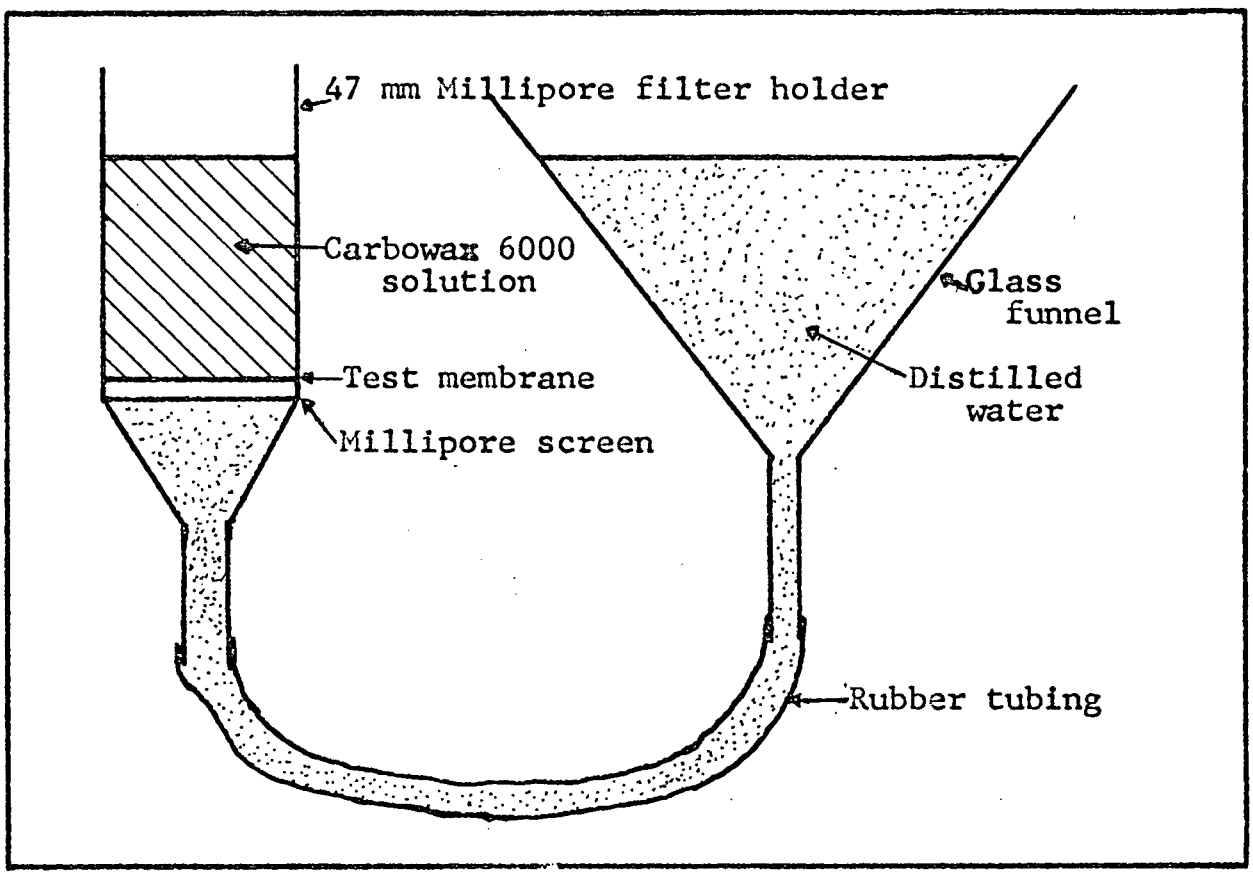


Figure 1. Apparatus used to measure the flow rate of water through test membranes.

Table I. The concentration of Polyethylene glycol 6000 at various osmotic potentials

Osmotic potential (-bars)	Concentration PEG 6000 (g/l)
0.0	0
0.1	20
0.2	30
0.4	42
0.5	55
0.6	60
1.0	88
1.2	90
2.0	125
2.5	132
5.0	175

diameter, 0.0043 inch wall thickness (Arthur H. Thomas Co., Philadelphia) was soaked in water, for 15 minutes, pulled over the frame, and sealed at the base by clamping the end of the tubing between a plastic book binder. The cell was placed between two sheets of plywood and then carefully filled and packed with freshly autoclaved greenhouse soil. The cells were then placed in -0.4 bar and -2.5 bar PEG solutions and the plywood sides were removed.

Soil tubes were constructed by knotting one end of a 15 cm, 1 5/16 inch flat diameter, 0.001 inch wall thickness dialysis tube and fitting the other end over a single holed No. 3 1/2 cork. The tubing was held on the cork with an elastic band. Tubes were filled with freshly autoclaved soil and placed in 600 ml glass beakers of PEG solutions of 0, -0.2, -0.4, -0.6, -1.0, -1.5, or -2.0 bar (Table I).

Antibiotics for the PEG solutions. Bacteria and fungi growing in the PEG solution, soil, and on the membrane surface were isolated by plating small quantities of soil and dialysis membrane or by making streaks of PEG solution on PDA and Nutrient Agar. All cultures were incubated in the dark at 25 $\pm$ 1 C.

The isolated organisms capable of degrading the cellulose membrane were determined by the following technique. Eight cm long soil tubes were placed in 250 ml Erlenmeyer flasks and held upright by a string attached to the cork and passed to the outside of the flask. Approximately 100 ml of a Glucose Yeast Extract broth (1% Glucose, 0.2% Yeast Extract, 1 liter water) was poured in the flask and in the tube so as to half fill the soil tube. The flask was stoppered with a foam plug and

the whole assembly was autoclaved for 20 minutes at 15 psi. A 1 cm agar disk of inoculum from each isolate was placed inside a soil tube, taking care not to contaminate the solution around the soil tube. Flasks were gently shaken at a temperature of 20 ± 3 C. After 9 days, the soil tube was removed and the strength of the dialysis tubing was tested by gently pulling on the membrane.

Benlate (50% WP, E.I. DuPont de Nemours and Co., Wilmington), PCNB (75% WP, Olin Mathieson Chem. Corp., New York), and Streptomycin sulfate (Sigma Chem. Co., St. Louis) were tested for their effectiveness against the organisms capable of degrading the cellulose membrane, by incorporating 1, 10, 100, and 1000 ppm active ingredient of each chemical separately into autoclaved CMA at 45 to 50 C. Mycelial disks from the leading edge of actively growing fungal cultures and streaks of bacterial isolates were used as inocula. Plates were incubated at 25 ± 1 C and colony diameter or growth was determined daily.

Benlate, PCNB, and Streptomycin were added separately in concentrations of 1000 ppm, 1000 ppm, and 125 ppm respectively to a -0.4 bar PEG solution in 600 ml beakers. Soil tubes containing either freshly autoclaved or aged autoclaved soil were placed in the PEG solutions. Carrot seeds were planted in the soil of some of the tubes. The soil water controlling apparatus was set up on a laboratory bench at 20 ± 3 C. After two weeks, the water content of the soil was determined gravimetrically by oven drying the soil at 105 C. Throughout the thesis, water content will be expressed on a weight basis.

Pellicon membrane

In preliminary experiments, the Pellicon ultrafiltration membranes (Millipore Corp., Bedford) appeared capable of restricting large molecules such as PEG 6000, and were durable for at least three weeks.

Flow rate. The flow rate of water through a Pellicon PSAC, 500 nominal molecular weight limit (NMWL) and a PSAL 1000 NMWL ultrafiltration membrane were determined by the procedure previously outlined. The concentration of PEG was monitored throughout the experiment by drying and weighing the PEG solution removed from the Millipore filter holder. To determine if any PEG passed through the membrane, 100 ml of solution from the distilled water side of the apparatus was dried and weighed for PEG residue.

Moisture controlling prototypes. A preliminary experiment was conducted to test the membranes' ability to control the moisture of small quantities of soil. Acrylonitrile Butadiene Stryrene (ABS) plumbing adapters (4 cm inside diameter, ID) were used to hold 4.3 cm diameter 500 NMWL Pellicon membranes. Membranes were placed between the nylon rings and caps, which had been lightly coated with stop cock grease. Chambers were then placed in a distilled water bath for one hour to check for leaks before filling with soil. Pasteurized greenhouse mix soil, screened through a 10 mesh sieve was used in this and all other soil moisture controlling experiments. The soil was packed several times during the filling of the chambers to a height of 3 cm. Extreme care had to be exercised not to rupture the membrane. Chambers were

placed in solutions of PEG of 0, -0.2, -0.4, -1.0 or -2.5 bars. After 6 weeks, the water content of the bottom 2 cm of soil was determined gravimetrically.

Tensiometers and thermocouple psychrometers were used to measure soil water potentials over time in the ranges of 0 to -0.8 bars and less than -1.0 bars respectively. Soil chambers were made larger by fitting a 4 cm ID ABS pipe in the plumbing adapter to give a total height of 9.5 cm (Figure 2a). A 2 cm x 0.6 cm outside diameter (OD) ceramic tensiometer (Soil Moisture Equip. Corp., Santa Barbara) was glued with epoxy to a 0.65 cm OD acrylic tube. Tensiometers were inserted through the wall of the soil chambers and cemented in place with epoxy at 2 cm and 7.5 cm from the membrane surface. Tensiometers were connected to mercury manometers by means of tygon tubing. Three terminal double loop thermocouple psychrometers were built according to the specifications of Chow and De Vries (1973) and were calibrated with KCl solutions (Campbell et al., 1966). Thermocouple output was measured with a Keithley Model 155 Microvoltmeter and recorded on a strip chart recorder. A ceramic bulb made from a 1.0 cm outside diameter (OD) tensiometer and ground to 0.8 cm OD was used to protect the thermocouple psychrometer. The thermocouple psychrometer was held in the bulb by means of a tapered PVC plug (Figure 3). The ceramic bulbs were inserted through the wall of soil chambers at 2 cm and 7.5 cm from the membrane surface. Rubber cement (Black Plastic Rubber, Duro-Plastic, Woodhill Chem Sales Corp., Cleveland) was applied to prevent water from leaking into the thermocouple psychrometer chamber.

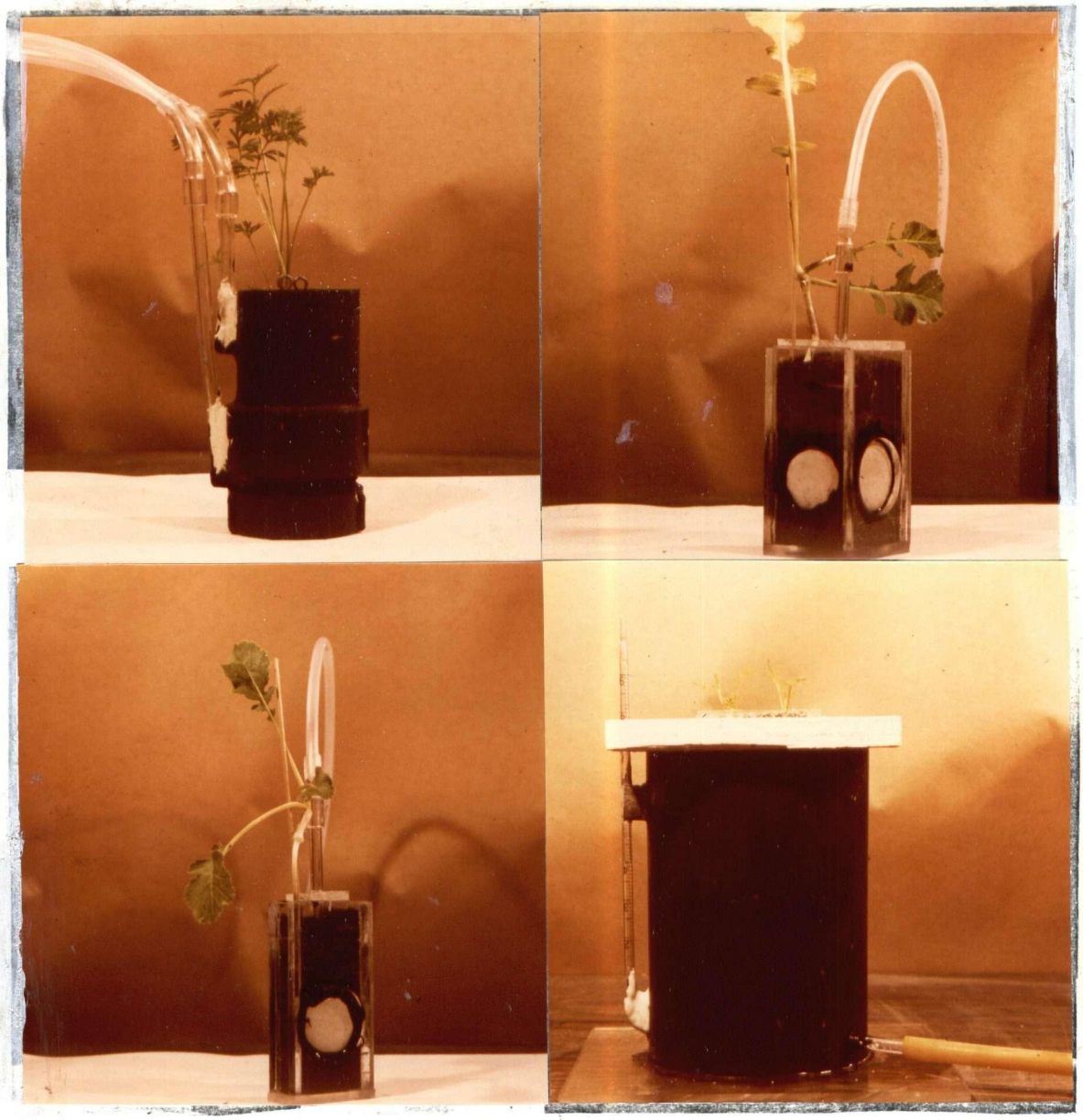


Figure 2. Prototypes using Pellicon membranes for controlling soil matric potential. (A) plumbing adaptor chamber (B) cubical chamber (C) narrow soil cell (D) osmotic solution chamber.

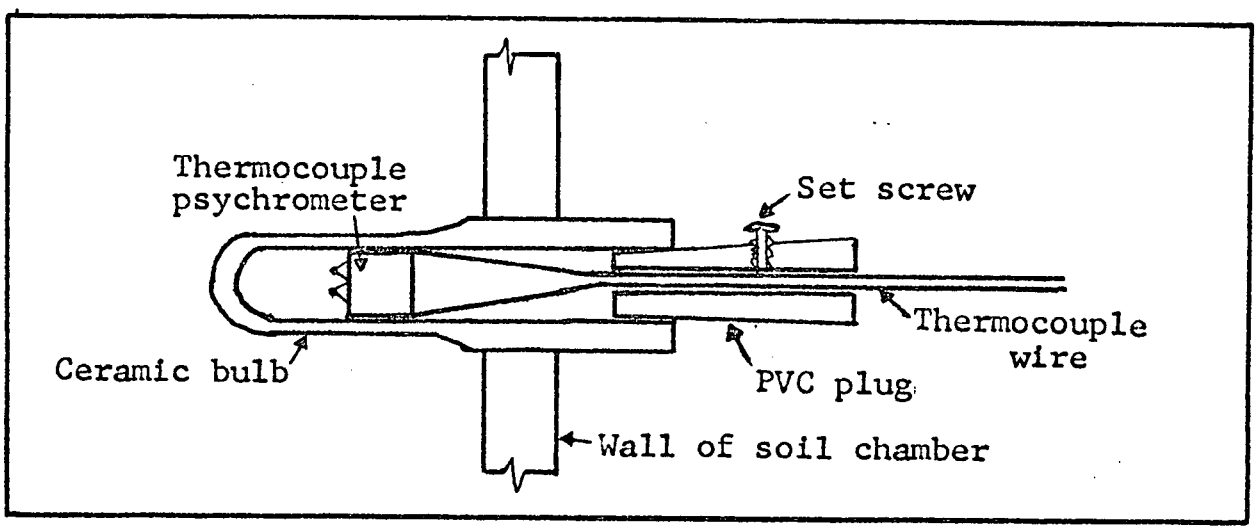


Figure 3. Cross section of ceramic bulb with PVC plug holding thermocouple psychorometer in place.

PEG solution containers were made of 15 x 10 cm ID ABS sewer pipe glued to a 0.65 cm clear plexiglass bottom (Figure 2d). A 0.65 cm acrylic tube near the base of the container was connected to an air manifold system. A small stream of air agitated the PEG solution. The solution containers were covered with plywood lids having holes only large enough to accommodate the soil chambers.

Soil chambers were filled with soil and placed in PEG solutions of -0.1, -0.2, -0.4, -0.6, -1.2, -2.5 or -5.0 bars. Saran wrap was used to cover the soil chambers for several days while the system came to equilibrium. Soil surface evaporation was then allowed to take place and the water potential of the chambers was measured 3 or 4 times daily. In some cases, surface evaporation was hastened with the aid of a fan. The volume of the PEG solution was kept constant throughout the experiment by adding water every second day. PEG solutions were renewed weekly.

To determine whether the system could keep up with the water demands of a young plant, radish, Raphanus sativus L., was planted in soil chambers which were then placed in PEG solutions of -0.1 and -0.2 bars. The water potential was measured with tensiometers. The plants were grown at 20 $\pm$ 3 C under a 14 hour photoperiod (2800 lux) provided by "cool white" fluorescent lamps and incandescent bulbs.

Two soil cell designs were evaluated in an attempt to maintain the water potential within a narrower range than previously done. A cubical soil chamber, 5.6 x 5.6 x 10 cm of 1/4 inch clear plexiglass, had a 3.8 cm diameter hole in each of the four sides and bottom. A 4.3 cm diameter 500 NMWL Pellicon membrane was cemented with rubber cement

(Black Plastic Rubber) over the inside of each hole (Figure 2b). The second design was 5.6 x 2.5 x 10 cm of 1/4 inch clear plexiglass with a membrane in each side (Figure 2c). A tensiometer, entering the chambers from the soil surface, was placed at a point equal distance from the membranes. The soil cells were placed in a -0.2 bar PEG solution and water potentials were monitored during the growth of a radish seedling. Transpiration was approximated from the amount of water added to maintain a constant level of the PEG solution.

Constant water potential as determined gravimetrically. The measurement of water potential by instrumentation for extended periods of time in the -0.4 to -2.0 bar range is subject to many problems. Gravimetric measurement of soil water content is the most reliable indirect method of determining the water potential and therefore, it was used as a check to see if water potentials were constant for several weeks.

Soil cells, 5.6 x 2.0 x 10.5 cm were constructed of 1/8 inch plexiglass (Figure 2c). One Pellicon (4.3 cm diameter, 500 NMWL) membrane was glued with rubber cement (Devcon Rubber, Devcon Canada Ltd., Scarborough) on the inside of each side. Soil cells were filled with soil and placed in osmotic solutions of -0.2, -0.5, -1.0, -2.0 bars. Two chambers were placed in each bath. The change in volume of the PEG solution resulting from transpiration was determined daily by measuring the change in height of the solution in a 1 ml disposable pipette that was bent and fitted in the base of the PEG container to function as a level gauge.

Two uniform 15 day old carrot seedlings were transplanted into each cell and were grown at 20 ± 3 C under a 14 hour photoperiod (2800 lux) provided by "cool white" fluorescent lamps and incandescent bulbs.

Soil water content was determined for twelve locations in each pot by measuring the loss of water at 105 C. Two cells at each of four osmotic potentials were analyzed each week for 3 weeks. Leaf area was measured with a planimeter at the end of the third week.

The water retention characteristics of the greenhouse soil was determined by a hanging column apparatus and a pressure plate extractor (Richards, 1947). Soil water contents could then be converted into potentials.

RESULTS

Periodic Soil Saturation

Carrots grown in naturally infested soil, under all four watering regimes, had at each harvest some necrotic root tips. Post emergence damping off was observed and at harvest, some of the carrots exhibited typical field symptoms of forking. By contrast, carrots from autoclaved soil were not forked, but some of the rootlets from *Pythium* inoculated plants were light grey in colour. Any roots that had grown through the drainage holes in the bottom of the pots were brown, but these were easily distinguished because of their thicker diameter and bright brown or orange colour.

A visual comparison of carrots at each harvest suggested that growth in autoclaved soil was much greater than in non-autoclaved soil.

Fresh weight of carrots in autoclaved soil was significantly greater ($P = .05$) at each harvest than in non-autoclaved soil (Figure 4). Weight of carrots was not significantly different in Pythium inoculated and control treatments. Periodic soil saturation increased yields over sprinkle watering in autoclaved soil, whereas in naturally-infested soil, water regimes had no significant effect on yield.

Olpidium brassicae (Woron.) Dang. was the only organism consistently observed in roots from naturally-infested soil. While there was mycelium in the roots, rarely were Pythium spores observed. Olpidium spore counts per microscope field were significantly greater ($P = .05$) in rootlets exposed to daily 10 minute saturation than all other water treatments (Figure 5). Rootlets saturated for 10 minutes every fourth day had a significantly greater ($P = .05$) level of spores than those saturated for 8 hours every fourth day or sprinkle watered. If data were analyzed on the basis of positive incidence of Olpidium, counts greater than or equal to five spores, or counts greater than or equal to 10 spores, the same basic results were obtained. Carrots at six weeks of age, had a significantly greater ($P = .05$) number of spores than all other ages. No Olpidium spores were observed in rootlets from autoclaved soil.

Tobacco necrosis virus (TNV) was recovered only from carrots grown in naturally-infested soil that was subject to periodic saturation. Carrots from naturally-infested soil that was sprinkle watered, while having relatively high levels of Olpidium, did not contain the virus at levels detectable by the assay used.

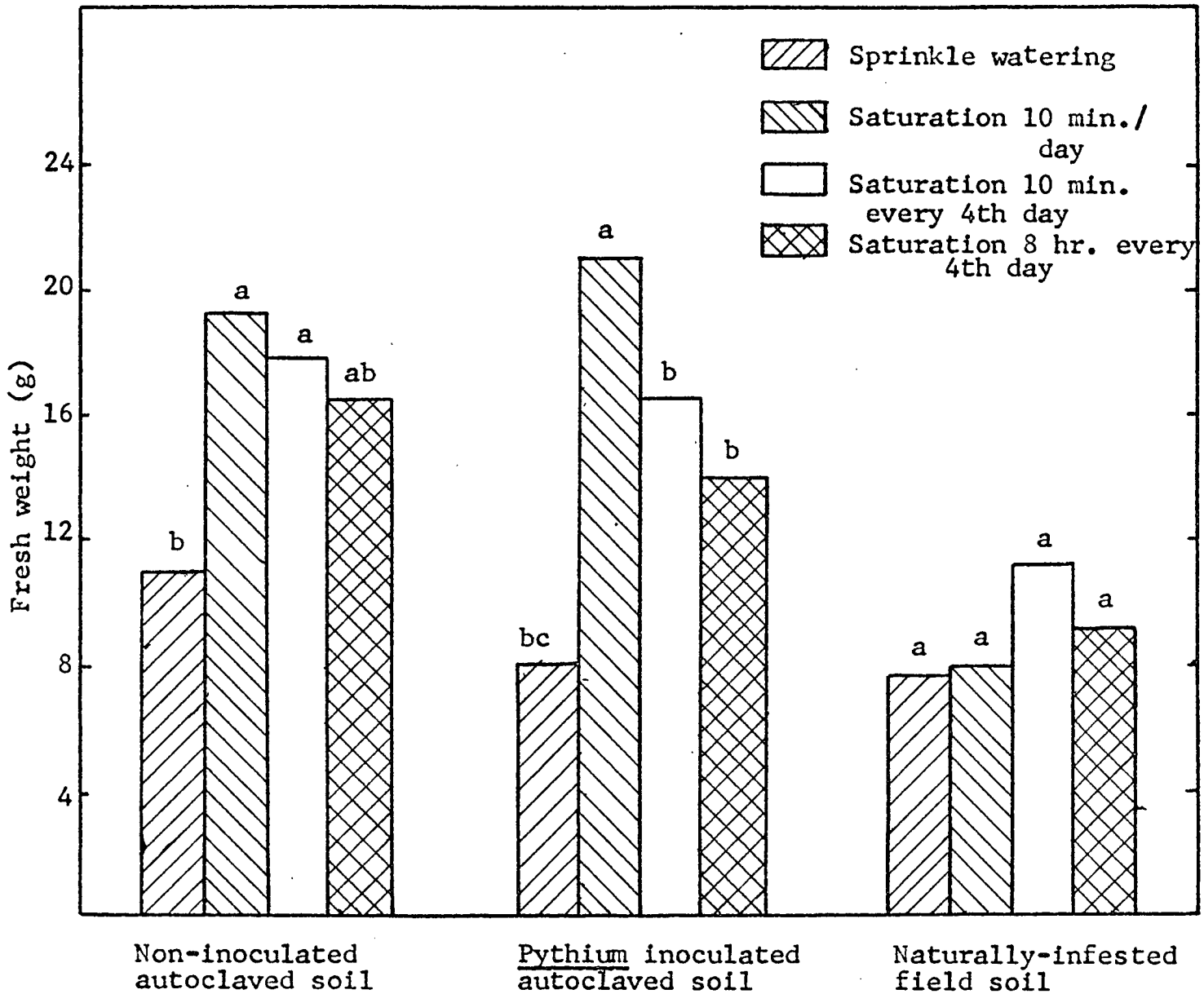


Figure 4. Mean total fresh weight yield of carrots at 8 weeks of age in naturally-infested soil, Pythium sylvaticum-inoculated autoclaved soil, and non-inoculated autoclaved soil. Bars within a soil treatment with the same letter do not significantly (P = .05) differ.

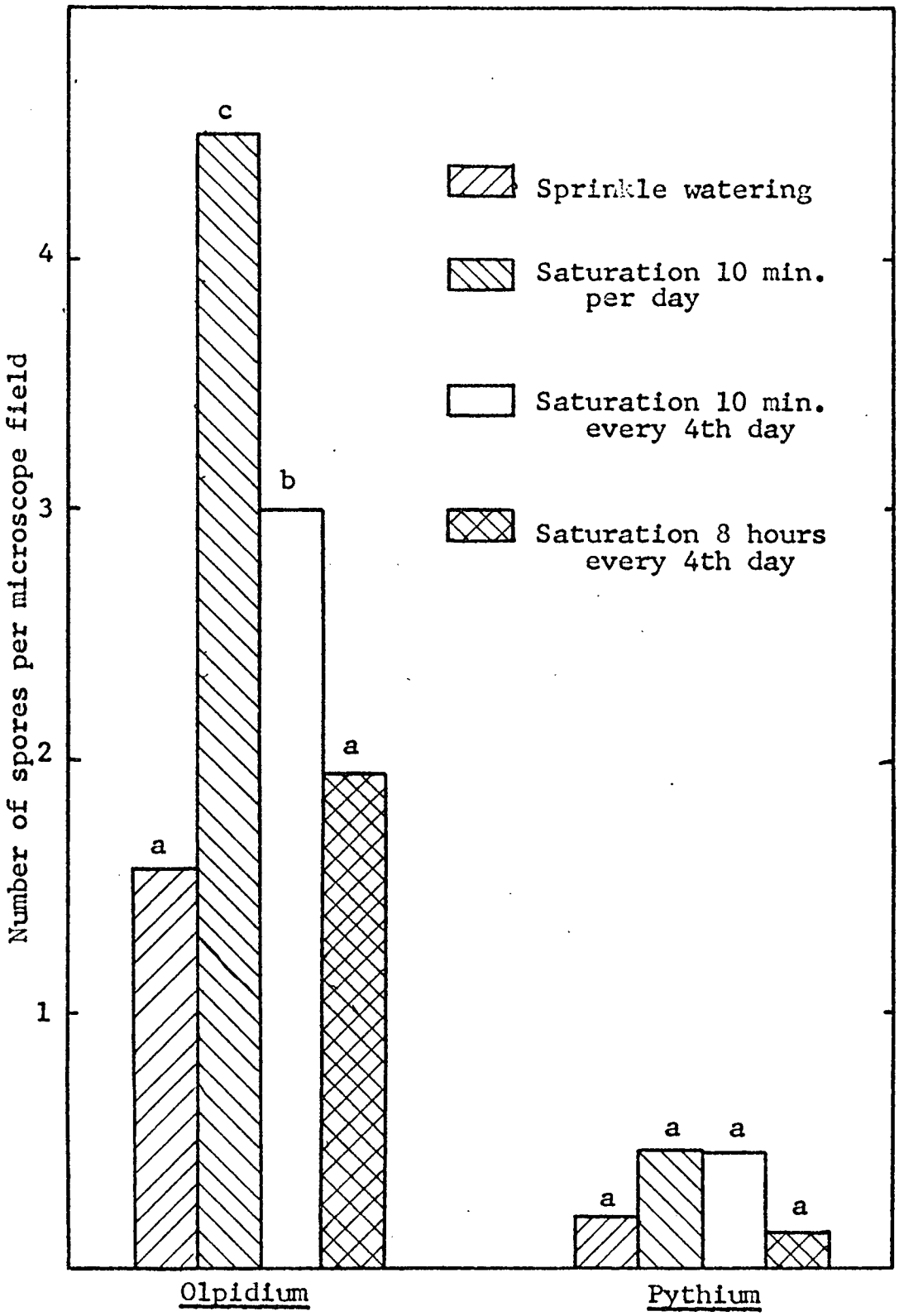


Figure 5. Average number of Olpidium and Pythium spores per microscope field, in carrot rootlets under four watering regimes (4 carrot ages combined). Bars with the same letter do not differ significantly (P = .05).

Low levels of Pythium infection were observed in roots from artificially infested soil, and no spores were observed in non-inoculated autoclaved soil. Mycelium was observed in the cortex of the rootlets, and in some cases, mycelia and sporangia were observed externally around the rootlets. No oospores were observed in any of the rootlets. Watering regimes had no significant effect on the number of Pythium spores observed per microscope field (Figure 5). Carrots at 10 weeks of age had a significantly greater ($P = .05$) Pythium infection than four week old carrots. There was a significant ($P = .05$) sample date X water regime interaction, but this was caused by one replicate having an exceptionally high infection level, and therefore, the interaction was not considered important.

Effects of Osmotic Potential on Pythium

For all solutes tested, growth of P. sulcatum at decreasing osmotic potentials was nearly identical (Figure 6). The growth rate of Pythium was expressed in mm of radial growth per day, to facilitate direct comparisons between experiments. Radial growth decreased as the water potential decreased from -1.2 bars. P. sylvaticum exhibited a greater variability in growth to different solutes than P. sulcatum (Figure 7). Radial growth was stimulated, approximately 25%, over non-amended medium at a potential of -3.2 bars created by KCl or NaCl and -4.2 bars created by sucrose. The growth rate of P. sulcatum on CMA and basal medium was nearly identical; however, on PDA, growth ceased at -15 bars as compared to -30 bars on the other media (Figure 8).

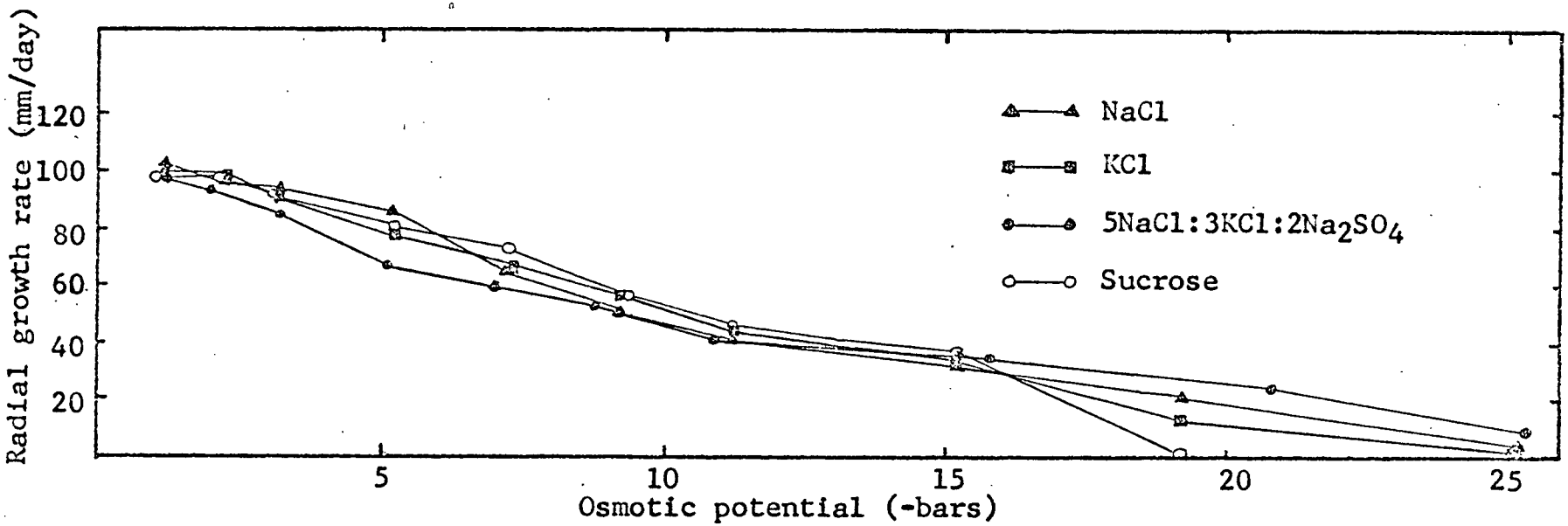


Figure 6. Radial growth rate of Pythium sulcatum on basal medium amended osmotically with NaCl, KCl, 5NaCl:3KCl:$2Na_2SO_4$, sucrose. Each point represents the mean of four observations.

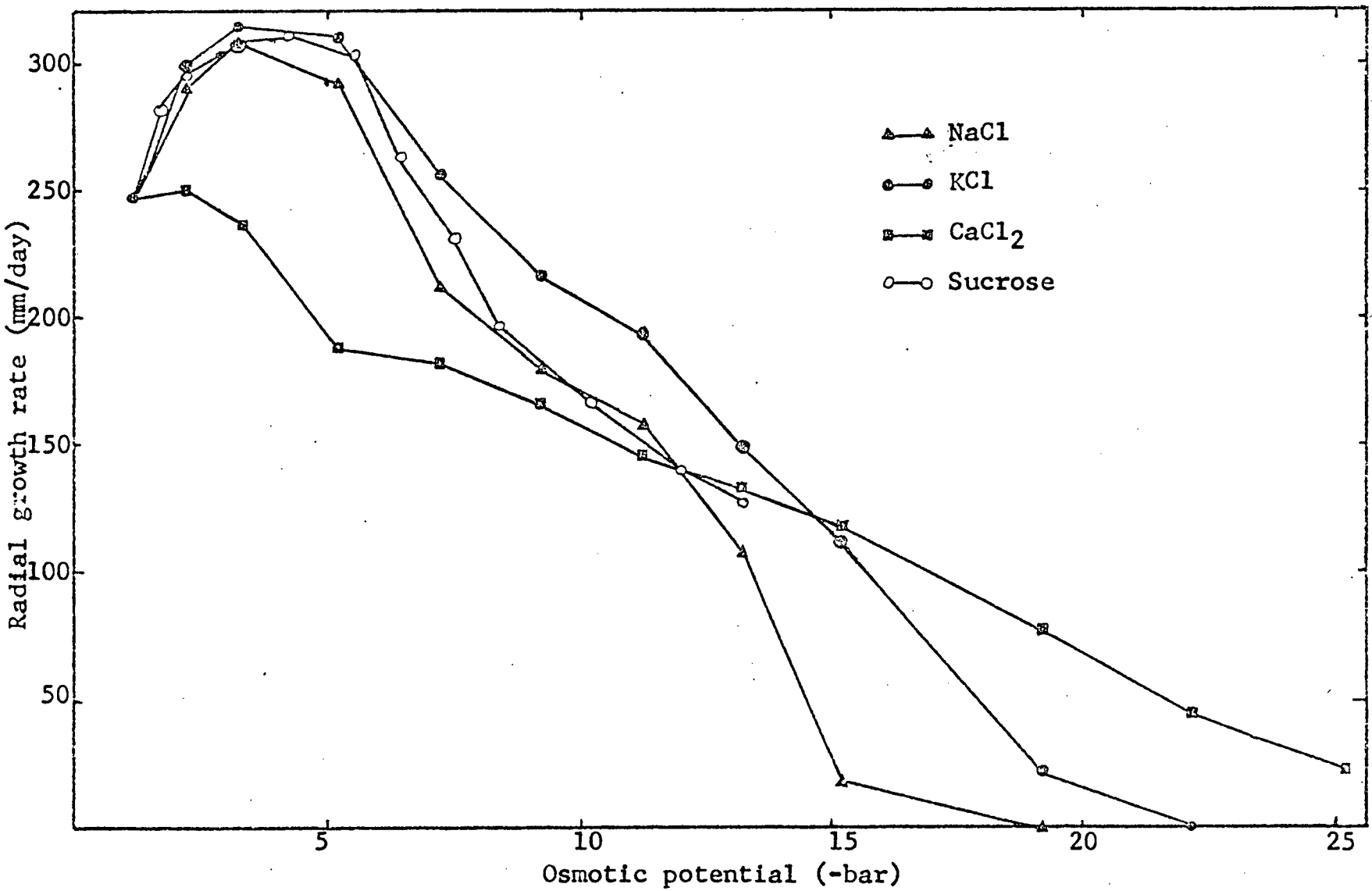


Figure 7. Radial growth rate of Pythium sylvaticum on CMA amended osmotically with NaCl, KCl, $CaCl_2$, sucrose. Each point represents a mean of four observations.

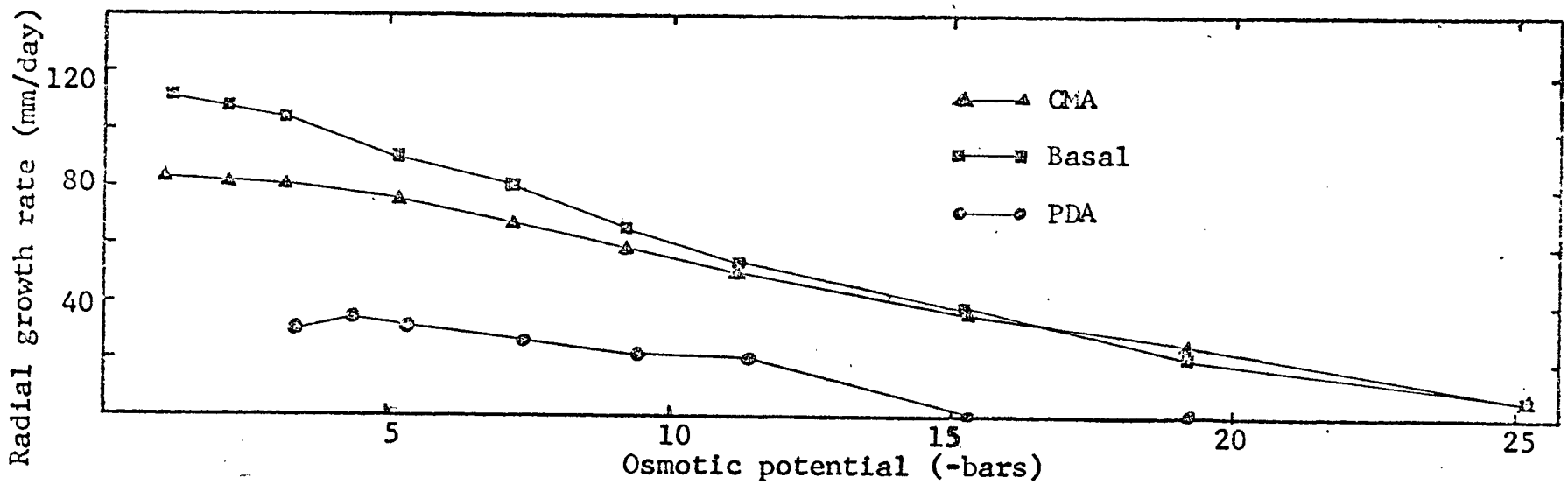


Figure 8. Radial growth rate of Pythium sulcatum on CMA, PDA, and basal medium amended with KCl. Each point represents a mean of four observations.

Colony diameter of P. sulcatum was measured at three times intervals to determine if there was a lag phase at lower potentials. Growth did show a slight lag at potentials less than -15 bars but there was no growth at -30 bars even after 2 to 3 weeks (Table II).

Table II. Radial growth rate of Pythium sulcatum on KCl osmotically amended basal media at 34, 57 and 83 hours after inoculation. Each measurement is the mean of four observations

Osmotic potential (-bars)	Radial growth rate (mm/day) 34 hours	57 hours	83 hours
1.2	1.01	.97	1.01
2.2	.99	.97	.98
3.2	.89	.90	.94
5.2	.86	.81	.78
7.2	.69	.72	.67
9.2	.63	.60	.57
11.2	.47	.45	.44
15.2	.27	.31	.34
19.2	.09	.10	.13
25.2	Trace	.02	.02
30.2	.00	.00	.00

P. ultimum, two isolates of P. sylvaticum and an isolate of P. sulcatum responded similarly to osmotic potentials. Each exhibited stimulated growth at osmotic potentials in the range of -1.2 to -3.2

bars, over non-amended media (Figure 9). Only the Wisconsin P. sulcatum (65) isolate had decreasing growth rate with decreasing osmotic potential in the range of -1.2 to -3.2 bars (Figure 9). The growth rate was 50% less than on non-amended media at osmotic potentials of approximately -11.5, -19.0 and -20.8 bars for P. sulcatum, P. sylvaticum, and P. ultimum respectively. However, mycelial density at low potentials appeared less than at high water potentials. The growth rate as measured by colony diameter does not reflect changes in density.

Control of Soil Moisture using Cellulose Dialysis Membranes

The flow rate of water across a membrane is directly related to the potential energy gradient and the thickness of the membrane. Preliminary experiments indicated that the 5 3/4 inch flat diameter (FD) membrane had a much slower flow rate than the 1 5/16 inch FD membrane at the same pressure gradient, because of its greater thickness. The flow rates of water across the 1 5/16 inch FD membrane determined at various pressure gradients are shown in Figure 10. For a pressure differential of 1.0 bar, the flow rate of water across the cellulose membrane was 0.65 $ml/day^{-1}cm^{-2}$.

Usually within 5 to 10 days, the 1 5/16 inch FD membrane had deteriorated and was allowing uncontrolled flow of water into the soil. A white and blue-green coloured fungus was observed growing in the soil tubes and in a bag of week old autoclaved soil. This fungus was identified as Trichoderma sp., a common soil fungus that is active in freshly autoclaved soil. Several mucor like fingi and bacteria were isolated from the membranes, soil, and PEG solution.

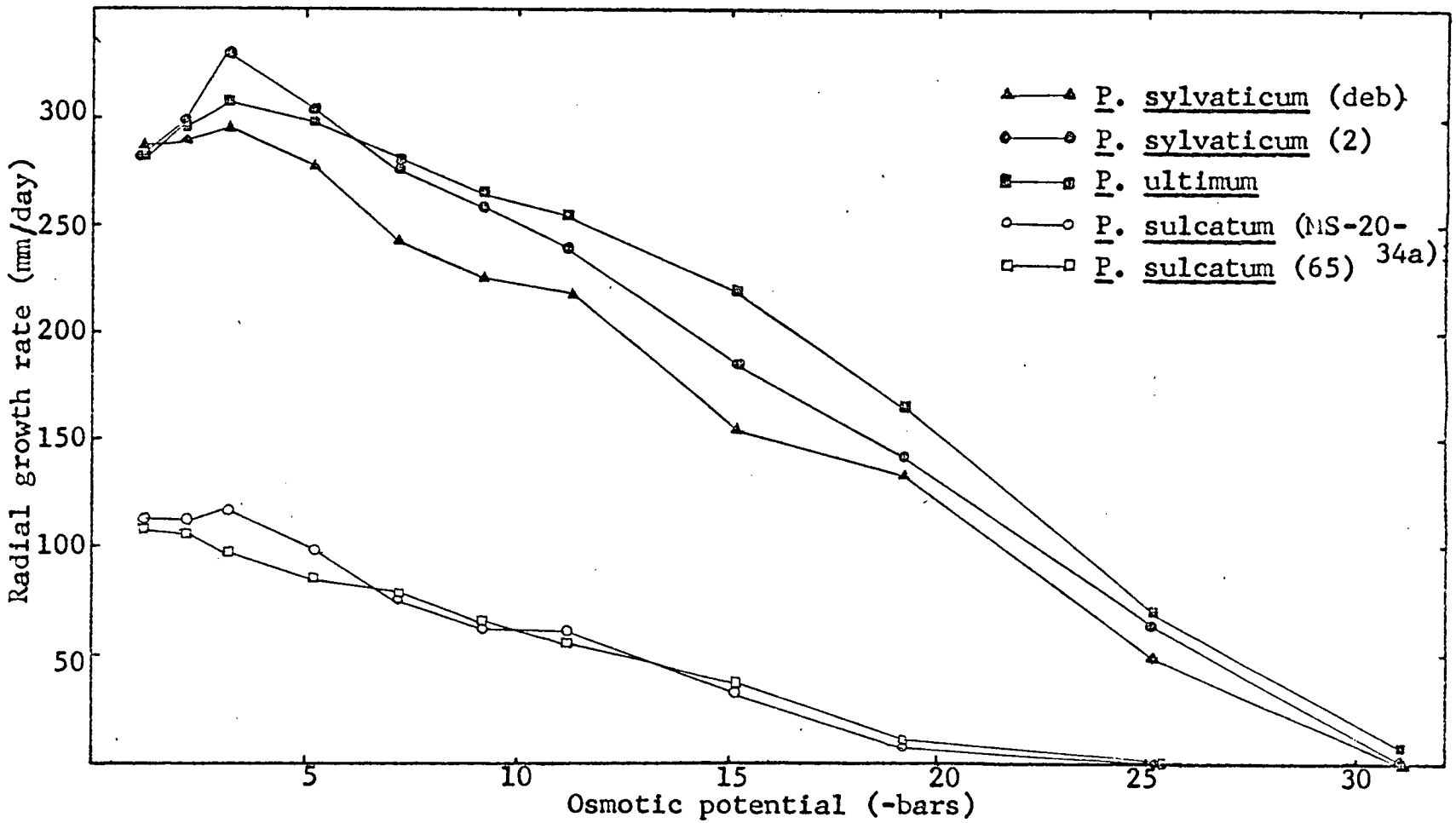


Figure 9. Radial growth rate of P. ultimum, two isolates of P. sylvaticum and two isolates of P. sulcatum on basal medium amended with KCl. Each point is the mean of four observations.

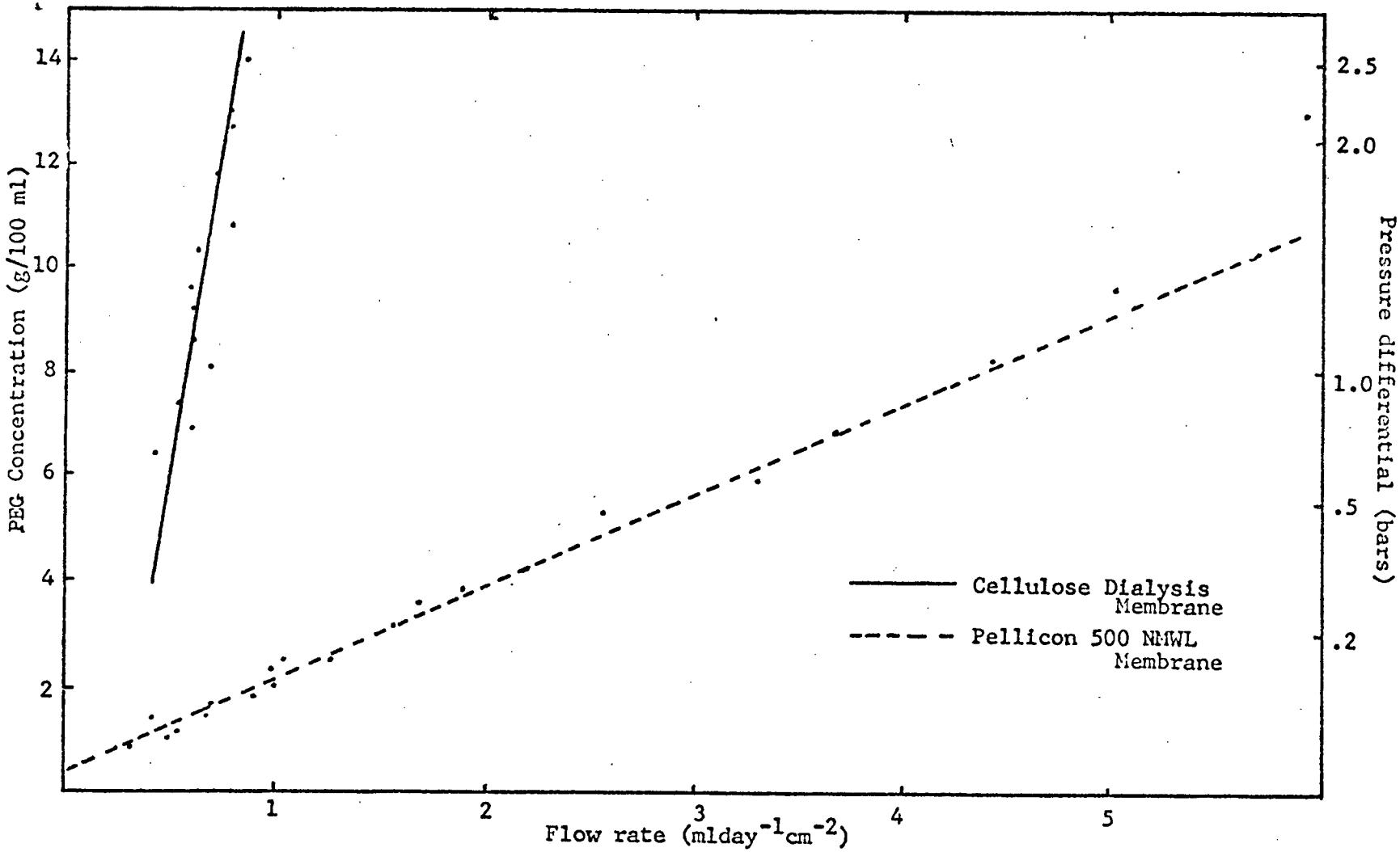


Figure 10. Flow rate of water across a 0.001 inch thick cellulose dialysis membrane and a 500 NMWL Pellicon ultrafiltration membrane at various potential differences.

Soil tubes, in the glucose yeast broth, inoculated with Pythium, Trichoderma and bacteria were still intact after nine days. The tubes inoculated with the mucors, were brittle and tore easily. In some cases, the fungi had pierced and grown through the wall of the membrane.

The feasibility of using fungicides and antibiotics of slight toxicity to Pythium species to protect the membrane from cellulose degrading organisms was tested. Trichoderma was completely inhibited by 10 ppm Benlate and partially by 1000 ppm PCNB. The growth of one mucor-like isolate was 70% inhibited by 1 to 1000 ppm Benlate, and that of a second mucor isolate was completely inhibited by 1 ppm PCNB and 80% inhibited by 1000 ppm Benlate. The bacteria were only slightly inhibited by 100 ppm Streptomycin. Pythium growth was slightly inhibited by 100 ppm Streptomycin, 80% inhibited by 1000 ppm Benlate and not inhibited by 100 ppm PCNB. 10 ppm Benlate had no observable effect on Pythium growth. This finding was later used in the modification of the medium of Tsao and Ocana (1969). 10 ppm Benlate replaced Pimaricin when it became unavailable.

The addition of Benlate, PCNB and Streptomycin increased membrane longevity to 14-17 days. However, any handling of the tubes after this time caused them to fall apart as they were very brittle. The use of aged autoclaved soil in which Trichoderma had been growing presented no advantage over freshly autoclaved soil by increasing the life of the membranes. Carrots germinated and grew to a height of about 2 inches in soil tubes that had been placed in fungicide-PEG solutions. Carrots in the soil tubes immersed in PEG solutions containing

Streptomycin died shortly after emergence. Some of the soil tubes were so fragile that the carrot root grew through the membrane.

Control of Soil Moisture using Pellicon Membranes

The flow rates of water through the Pellicon 500 NMWL membrane at various pressure gradients exceeded that of the cellulose membranes (Figure 10). At a pressure differential of 1 bar, the flow of water across the membrane was 4.35 $ml day^{-1} cm^{-2}$ and even at a low pressure differential of 0.2 bar, the flow rate was nearly 1 $ml day^{-1} cm^{-2}$. No PEG residue was detected in 100 ml of solution from the distilled water reservoir after 7 days of operation. The 1000 NMWL Pellicon membrane had slightly greater flow rates but 0.081 g of PEG per 100 ml water was detected as having passed through the membrane after 7 days of operation. Therefore, the 500 NMWL membrane was used in all further experiments.

Soil moisture controlling prototypes. Preliminary experiments using the Pellicon membrane in the plumbing adaptor apparatus indicated that soil moisture could be controlled for 4 to 6 weeks. The water content of small quantities of soil in the apparatus decreased as the osmotic potential of the solution decreased (Table III).

When a larger volume of soil was used, and there was only surface evaporation, the soil in the top of the chamber was drier than soil near the membrane (Figure 11). Water was not able to pass through the membrane and the 9.5 cm column of soil fast enough to keep up with evaporative demand, so the potential of the top soil decreased. However, the potential of the lower layer of soil remained constant (Figure 11).

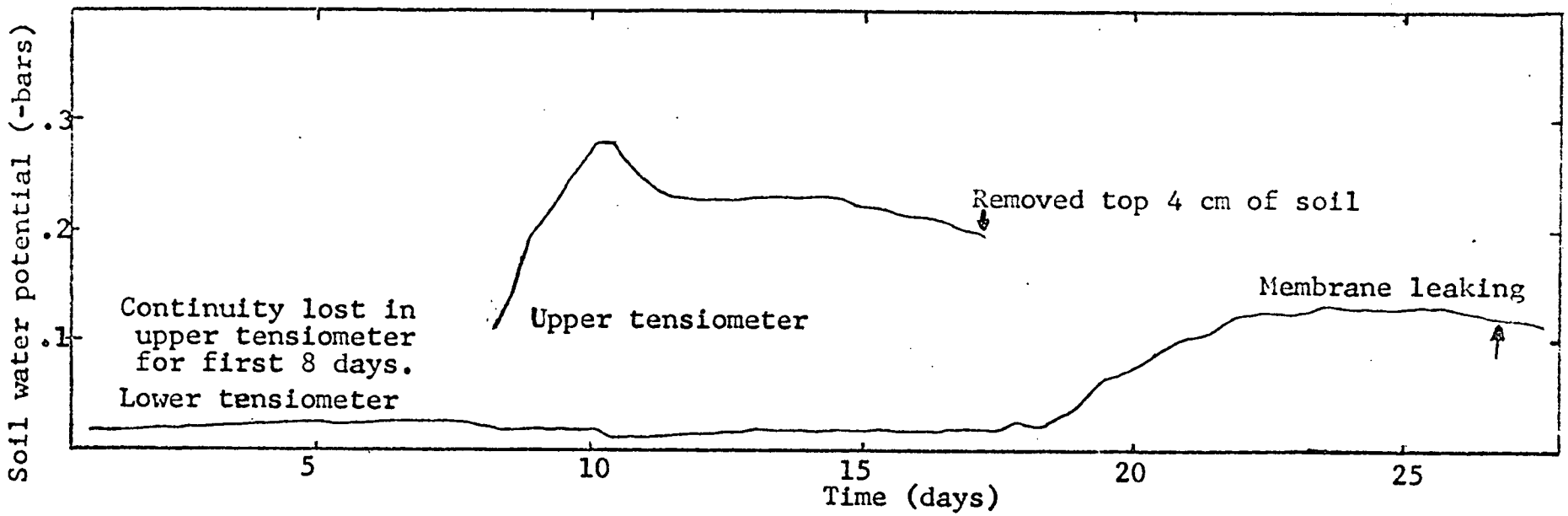


Figure 11. Soil water potential as measured with two tensiometers in the plumbing adaptor apparatus emersed in a -0.1 bar PEG solution.

The top 4 cm of soil was removed to permit drying out of the lower soil regions because the capillary conductivity of the soil was so low in the upper region. After removing the soil, the water potential in the lower region of the chamber decreased from -.02 to -.125 bars in 5 days (a change of .02 bars per day) and remained constant for the next 6 days (Figure 11).

Table III. The percent water content of soil in the plumbing adaptor chamber emersed in PEG solutions of various osmotic potential

Osmotic potential (-bars)	Water content (%)
0.0	63.3
0.2	51.9
0.4	34.1
1.0	25.1
2.5	15.3

The water potential in the lower region of the plumbing adaptor chambers remained relatively constant for seven days after planting a radish seed. Thereafter, as the plant grew, the potential decreased slowly over an 18 day period at .023 and .025 bars per day for a -0.1 and -0.2 bar osmotic solution respectively (Figure 12). The rate of potential decrease appeared to be slowly accelerating as the plants became older.

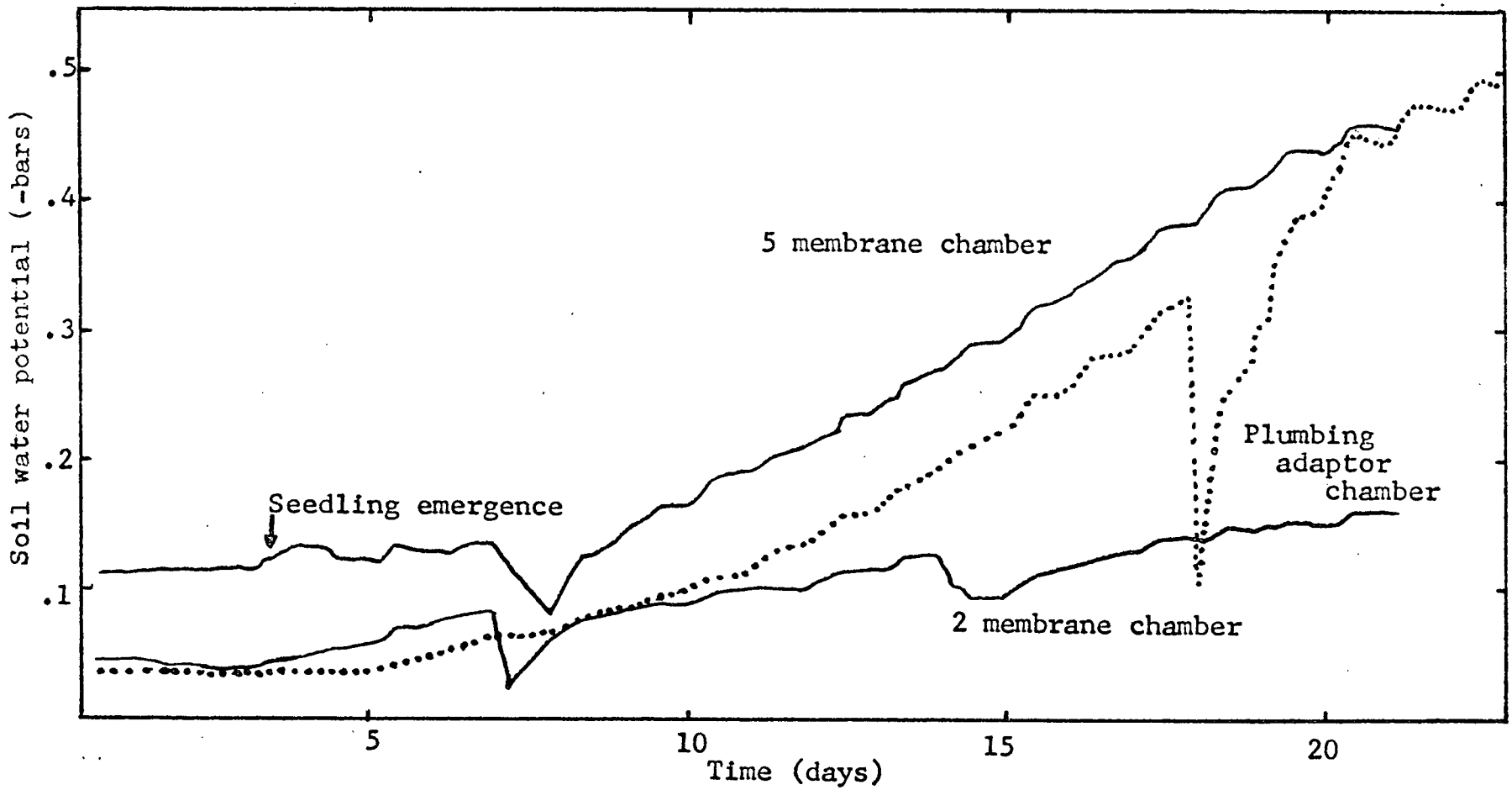


Figure 12. Soil water potential of plumbing adaptor chamber, 5 membrane cubical chamber, and 2 membrane soil cell with a radish seedling. Chambers were emersed in -0.2 bar PEG.

On dismantling the chamber, a dense mat of fine roots was found in the first 0.5 cm of soil from the membrane. The water content of the soil at different heights above the membrane are given in Table IV.

Table IV. Percent water content of soil at various heights above the membrane in the plumbing adaptor chambers

Height above membrane (cm)	Soil water content (%) of chambers in osmotic solutions	
	-0.1 bar	-0.2 bar
0.0 to 0.5	62.3	74.2
0.5 to 1.5	44.3	55.4
1.5 to 3.0	46.6	52.3
3.0 to 4.5	44.3	49.3
4.5 to 6.0	36.1	39.1
6.0 to 9.5	-	-
Leaf area (cm^2)	42	62

The soil chamber with five membrane surfaces hada slow and steady decrease in potential of 0.02 bars per day over 17 days after seedling emergence (Figure 12). However, the two membrane chamber had only a decrease of 0.007 bar/day over the same 17 day period (Figure 12). The radish seedlings were approximately transpiring 8 ml/day towards the end of the 17 day period.

The water potential of the five membrane chamber decreased more rapidly during the late hours of the light period than during the night period (Figure 13). In some cases the potential even increased during the night period.

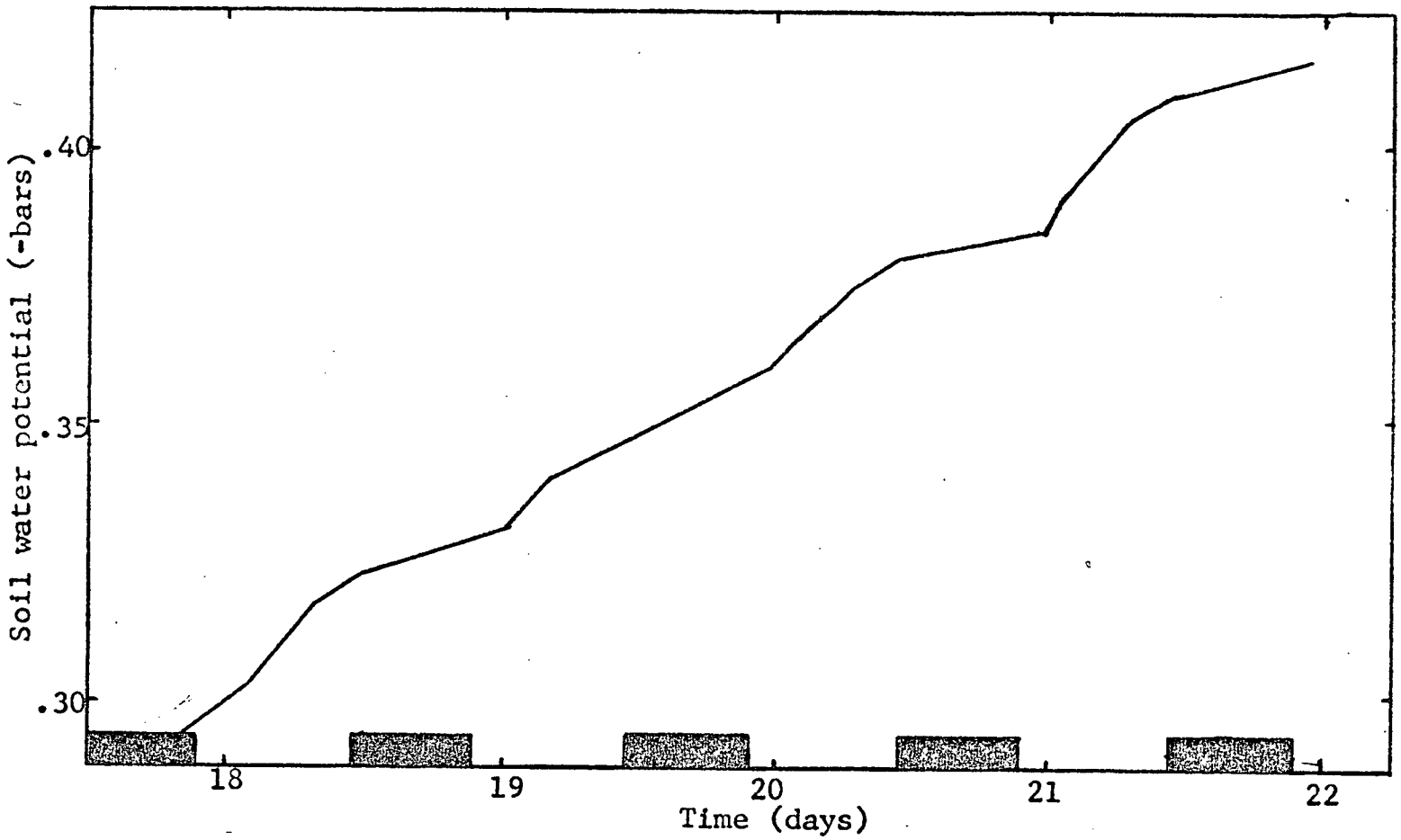


Figure 13. Soil water potential of the five membrane chamber over a four day period.

Soil moisture control as measured gravimetrically. Due to innumerable problems of measuring water potential with tensiometers (Figure 11) and thermocouple psychrometers (Figure 14), an experiment was designed whereby moisture could be determined gravimetrically for several weeks. However, it was first necessary to determine the soil water retention curve for the greenhouse soil (Figure 15) to enable conversion of soil water contents into potentials. The greenhouse soil was packed in the soil cells to give an average bulk density of 0.7 $gmcm^{-3}$.

The water content of the soils decreased as the osmotic potential of the solution decreased (Table V). The soil water content of soil cells in the -0.2 bar solution remained constant at 46% over the 3 week period. After one week, soil cells in the -2 bar solution had a potential of -1.0 bar. The system had probably not reached equilibrium as the carrots had been "watered in" at transplanting.

The water distribution within a soil cell at 2 weeks shows that the top layer of soil furthest from the membrane was the driest (Table VI). This was more noticable at lower potentials. The soil region directly between the two membranes had the highest water content and its potential was the closest to that of the osmotic solution.

Transpiration, estimated by measuring the change in level of the PEG solution and calculating the change in volume, is given in Table VII. After 3 weeks, transpiration was greater for carrots in the -0.2 bar than the -2.0 bar PEG solutions. At the end of the 3 week period, plants at -0.2 bar had three true leaves and a leaf area of 8.5 cm^2 while plants at 2 bar had one to two true leaves and a leaf area of 3 cm^2. In this experiment 75% of the cells were controlling soil moisture after two weeks but only 37.5% were operating properly after 3 weeks.

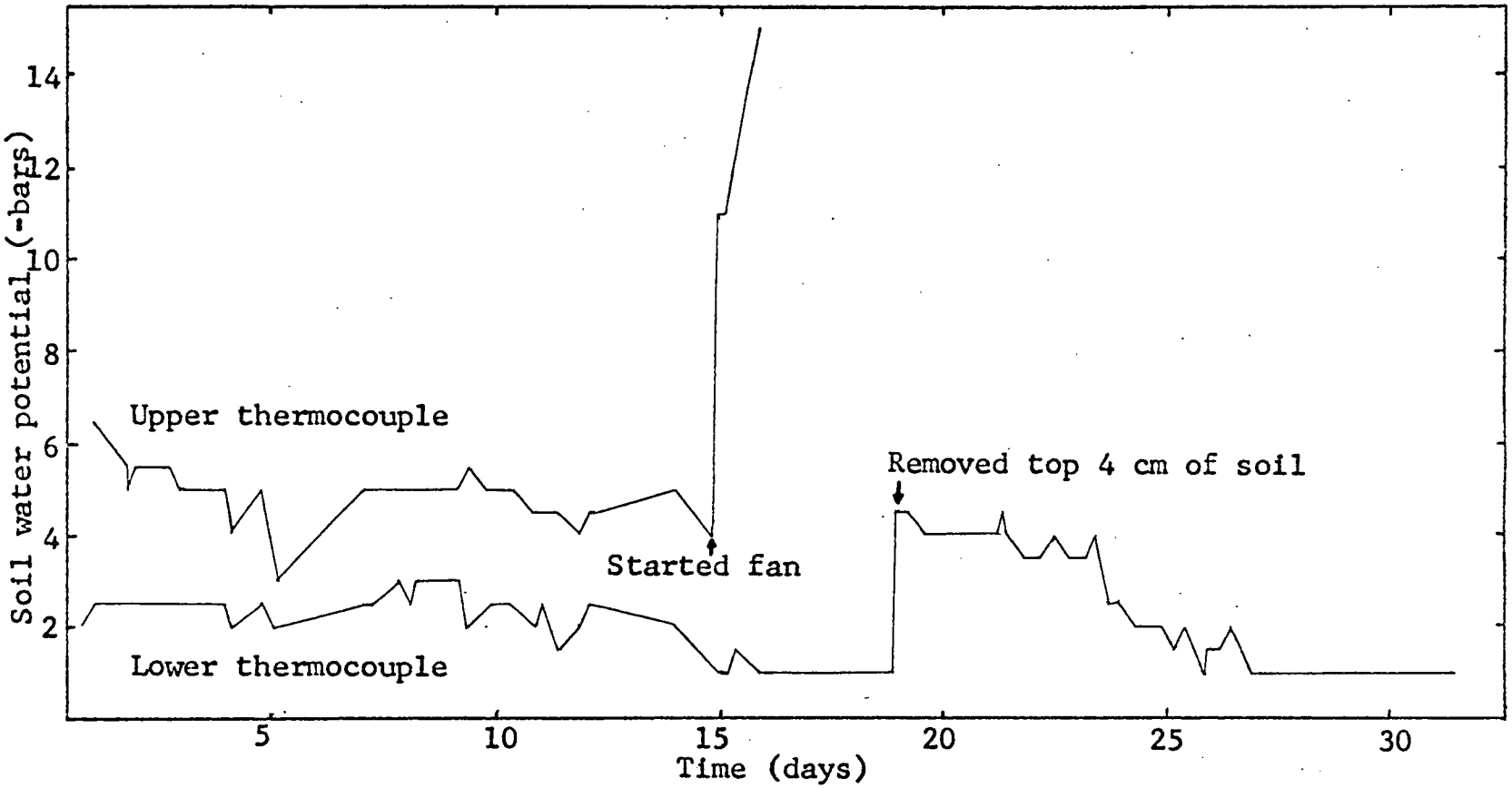


Figure 14. Soil water potential measured with two triple junction two loop thermocouple psychrometers in the plumbing adaptor apparatus emersed in -1.2 bar PEG.

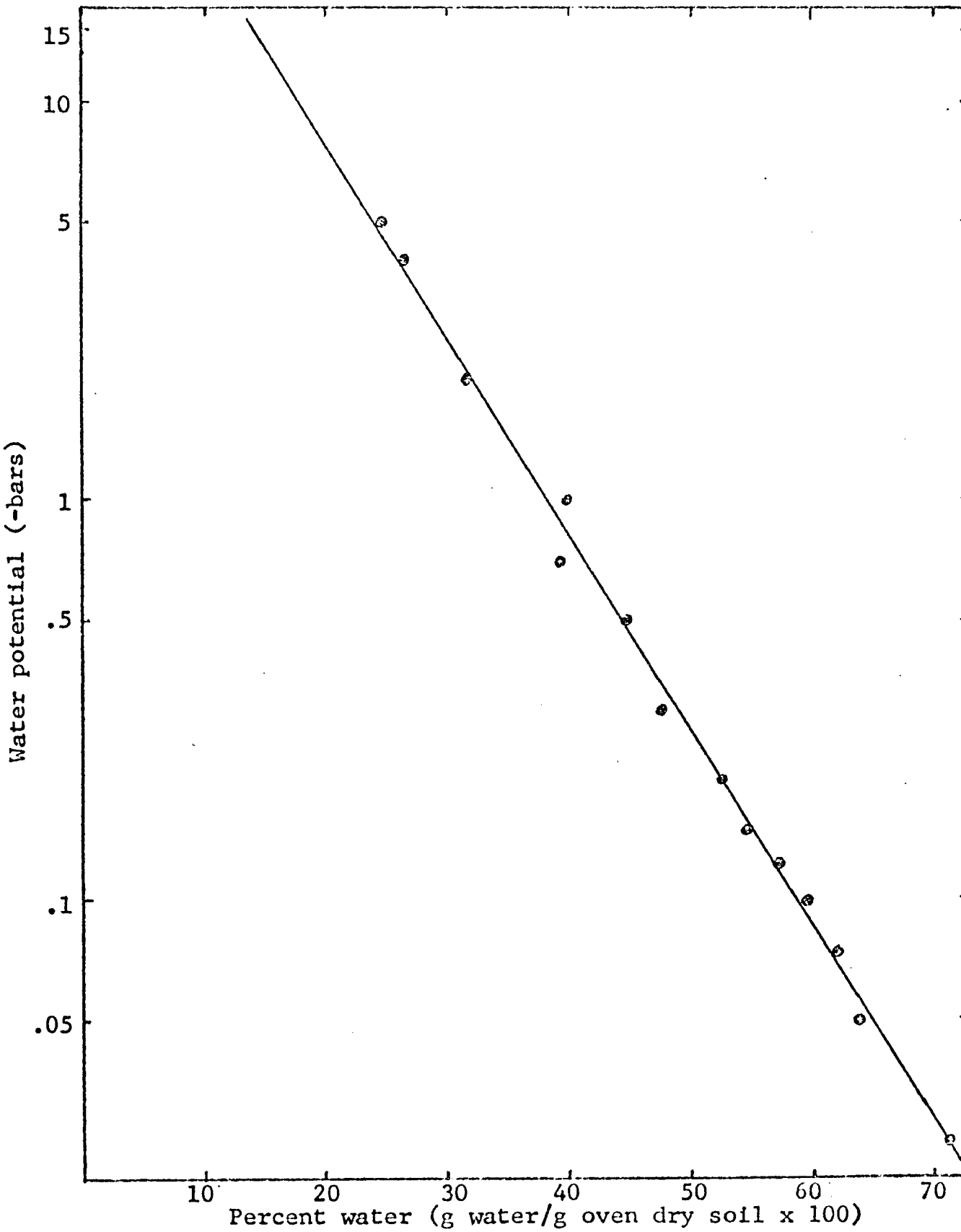


Figure 15. Soil water retention curve for UBC greenhouse soil.

Table V. Soil water content and potential of soil cells containing carrots over a three week interval maintained at four osmotic potentials

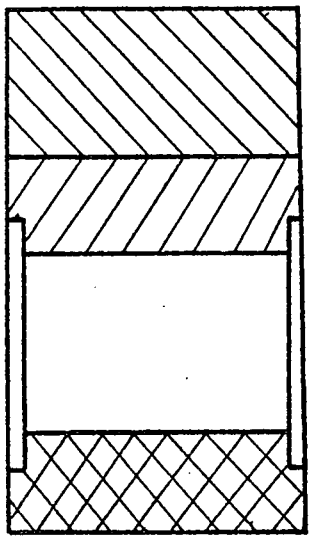
Weeks	Soil water content (%) of cells in osmotic solutions							
	-0.2 bar		-0.5 bar		1.0 bar		2.0 bars	
1	46.3[1]	(.40)[2]	42.6	(.60)	38.3	(.98)	37.8	(1.0)
	46.3	(.40)	43.6	(.54)	39.0	(.88)	37.8	(1.0)
2	48.4	(.33)	-[3]	-	35.3	(1.35)	26.8	(3.6)
	46.3	(.40)	-	-	39.5	(.85)	33.5	(1.7)
3	46.2	(.40)	-	-	-	-	33.0	(1.8)
	-	-	-	-	-	-	25.3	(4.2)

[1]Each measurement is an average of 3 soil samples taken from the region between the two membranes.

[2]Soil water potential (-bars).

[3]Chambers developed leaks.

Table VI. Soil water content at four locations within soil cells after two weeks of operation

Location	Soil water content (%) of cells in osmotic solutions -0.2 bar	-1.0 bar	-2.0 bars
	16.7[1]	9.4	10.4
	19.8	12.7	9.5
	37.3	30.3	22.8
	39.0	34.0	24.6
	48.4	35.4	26.6
	46.0	39.5	33.5
	51.3	35.4	23.8
	47.1	39.5	31.6

[1]Average of 3 determinations per location.

Table VII. Average transpiration of two carrots grown in soil cells at four osmotic potentials

Time	Transpiration (ml/day) in osmotic solutions -0.2 bar	-0.5 bar	-1.0 bar	-2.0 bars
3 to 7	1.6	0.8	1.2	1.2
8 to 10	3.2	1.9	1.9	3.8
11 to 15	11.3	-	3.3	5.6
16 to 18	13.6	-	8.0	4.8
19 to 21	7.8	-	-	3.9

DISCUSSION

Periodic Soil Saturation

Olpidium population was affected by different watering regimes. Daily saturation and saturation for short periods of time, induced greater infection levels than sprinkle watering or saturation for a prolonged period of time. These findings confirm field results of 1972 which suggested that *Olpidium* infection was related to the number of rainfalls or irrigations that saturated the soil (Wisbey, 1974). Early planted carrots which received only 1 or 2 heavy waterings had lower levels of *Olpidium* than later planted carrots that had received 3 or 4 soil saturations. *Olpidium*'s only form of spread is by swimming zoospores and under experimental conditions zoospore release is stimulated by saturating roots of nearly wilted plants. Frequent periodic soil saturation provided the best growing conditions for carrots while stimulating zoospore release. Carrots grown under "the 4 days between saturation" watering regimes were nearly wilted before resaturating the soil. Under such stress growing conditions, *Olpidium* may have been induced to form a greater percentage of resting spores than zoosporangia. Therefore, inoculum potential was reduced even though saturation of nearly wilted plants was ideal for zoospore release.

This experiment has also confirmed conclusions that *Olpidium* has no direct effect on yield (Wisbey, 1974). Watering regimes affected *Olpidium* incidence but not yield, while in the field, raised and conventional beds had no significant effect on *Olpidium* incidence, but marketable yield from raised beds was significantly greater.

The different soil saturation periods of 10 minutes or 8 hours, were devised to simulate conditions of high and low saturated hydraulic conductivity (HC) respectively. A soil with a high HC drains quickly, so the root system is saturated for a short length of time, whereas, a soil with a low HC remains saturated a longer period of time. Olpidium infection was significantly greater ($P = .05$) under conditions of 10 minutes saturation (high HC) than under 8 hours saturation (low HC, Table V), but there was no significant difference in carrot yield at these two simulated HC's in naturally-infested soil.

In the 1972 field study, the HC was determined to be three times greater in raised than conventional beds, but Olpidium incidence was not significantly different. These field results are in direct contradiction to greenhouse results. A soil with a high HC would be expected to have less Olpidium infection because zoospores would have less chance to infect roots before soil pore diameter restricted their movement. As a soil drains, the large pores are the first to become air filled. Prolonged saturation for 8 hours may not simulate a soil with a low HC, as the saturation period may have been too long. Concentrations of O_2 and CO_2 in the soil may change considerably under prolonged saturation, and these concentration changes may have had unknown effects on zoospore infection. No provision was made in this simulation to slowly drain the soil as would happen under natural conditions of low HC.

Olpidium was present in all water treatments but TNV was recovered only in the periodic saturation treatments. TNV is closely associated with the zoospore so it is difficult to explain the reason for the apparent lack of virus in carrots under the sprinkle water regime.

Carrots in autoclaved soil responded to the various water regimes, whereas carrots in non-autoclaved soil showed no response to water. Maurer et al. (1971) found that carrots grown in soil which was allowed to dry to various moisture levels before resaturating, showed a yield response to water only when infection with Pythium debaryanum (P. sylvaticum ?) was not a factor. In both studies, carrot yields were greater under frequent watering regimes.

P. sylvaticum was a poor choice of pathogen in this experiment for two reasons. Firstly, this species does not produce zoospores (Campbell and Hendrix, 1967) which are dependent upon water for spread. In pure culture, terminal and intercelary sporangia are the only infection structures. Infection takes place by germination of the sporangia to form a germ tube which infects roots. In similar studies, Biesbrock and Hendrix (1970b) found that root damage to peach by P. irregulare was not affected by excess water because it infected peach roots only by direct germination of the sporangia. However, damage induced by P. vexans was most severe at conditions of excess water and was related to the capacity of P. vexans to produce zoospores. Secondly, a low incidence of P. sylvaticum was observed because it may not be the primary pathogenic Pythium species infecting muck grown carrots. P. sulcatum is now considered the most pathogenic species on carrot, and it produces some zoospores (Pratt and Mitchell, 1973). Had the latter been used in the autoclaved soil, the results may have been quite different.

Effect of Osmotic Potential on Pythium

Roots are known to exude a number of compounds that have an effect on the osmotic potential of the rhizosphere. Therefore, the capability of Pythium spp. to infect and colonize carrot roots may be related to its ability to tolerate osmotic potentials. Pythium growth at decreasing osmotic potentials was related more to water stress than specific ion toxicity. Radial growth rates of P. sylvaticum and P. sulcatum responded similarly to different solutes. Sommers et al. (1970) obtained similar results with Phytophthora spp.

P. sulcatum, P. sylvaticum, and P. ultimum ceased growth at -25, -31 and less than -31 bars respectively. According to the classification of Walter (Griffin, 1963a), Pythium would be considered a hygrophile; minimum relative humidity for growth of 95% or higher, and maximum growth at about 100% R.H. On PDA, P. sulcatum was inactivated at a much higher potential than on other media. This relatively rich media and basal medium amended with sucrose did not increase growth rates as Sommers et al. (1970) found with Phytophthora parasitica. The effect of nutrition on the growth response curve at various potentials with different media suggest that the effect of water potential should not be interpreted solely on a water potential basis but that the nutrient status of the soil or plant may be important (Sommers et al., 1970). Side dressings of fertilizer may affect the osmotic potential and nutrient status of the root zone, and thus affect Pythium infection and colonization of rootlets growing into the fertilizer zone.

Pythium diseases are associated with soils of high water content, but the three Pythium species were growing well on osmotically amended media at -15 bars, a potential which is considered to be the PWP of most plants. Gardner (1968) has hypothesized that the water potential in the rhizosphere is considerably less than in the soil mass a distance from roots. In a saturated soil there is probably only a small potential gradient near the root. If the soil is at a potential of -1 bar, there is probably a potential gradient of 1 bar near the root so the potential at the soil-root interface would be -2.0 bar. But at the PWP (-15 bars), the potential gradient between the soil and the root surface will probably be 100 bars (Gardner, 1968). A potential of -115 bars at the root surface is prohibitive to Pythium growth. In order to colonize a root, Pythium spp. must be capable of growing at lower water potentials than are usually associated with soils because the root surface-soil interface is at a lower potential. Average soil water potentials determined in field plots in 1972 in the 0-3 cm, 4-8 cm and 9-15 cm of soil of raised and conventional beds were -0.4 to -2.0, -0.02 to -0.8, and -0.02 to -0.2 bars respectively (calculated from soil water retention curve, Figure 3, Russell, 1972). Mycelial growth and infection of carrot rootlets was probably not restricted at these potentials.

The stimulation effect of osmotic potentials in the range of -2 to -5 bars, was observed with all Pythium isolates tested, except one isolate of P. sulcatum. This increased growth rate may be due to an increased energy requirement by cells to retain solutes in very dilute media (Scott, 1957). Another possibility is that an ion deficiency in

dilute media may restrict the function of some enzymes, resulting in a slower growth rate (Manandhar and Bruehl, 1973). Manandhar and Bruehl (1973) have suggested that the stimulation effect of lower water potentials is not due to decreased water per se.

Adebayo and Harris (1971) compared the effects of matric and osmotic potential on fungal growth. They found that growth was not stimulated as the matric potential decreased from -1 bar, and that growth was not nearly as great at low matric potentials. The matric potential at which growth extinction occured was one half to two thirds of the corresponding osmotic potential. Fungal response to decreasing water cannot be explained solely on an energy basis but includes other changes in water soil properties such as solute transport. Their findings emphasize a need for both matric and osmotic potential studies on Pythium growth.

Controlled Matric Potential

In designing a soil moisture controlling apparatus for a growing plant an important consideration is the ratio of the volume of soil to the membrane surface area. For a constant membrane surface area, a design with a large volume of soil (e.g. tube) will be subject to greater water potential gradients than a design with a smaller volume of soil (e.g. narrow soil cell). The 1 5/16 inch FD dialysis tube works well as a tube because it has a large surface area to soil volume. The 5 3/4 inch FD diameter dialysis tube would not work well as a tube because the ratio of soil volume to membrane surface area is very large. Soil chambers

had to be constructed by fitting the membrane over a frame so as to limit soil volume and decrease the ratio of soil volume to surface area.

However, the chambers of this design were subject to several problems. It was impossible to get a permanent, water tight seal with rubber or silicone cement when a thermocouple psychrometer was fitted through the membrane and the wall of the frame. Pressure had to be maintained against the frame and membrane surface at all times, otherwise the membrane would bulge and the soil would move. This was not a problem for Babalola et al. (1968) because they put the membrane around a developed root system that held the soil in place. The narrow diameter soil tubes avoided the above problems, were easy to construct, had a relatively large surface area to volume and were made of a thinner membrane that had a greater flow rate.

One of the problems with the thinner membrane soil tubes was that the membranes were degraded by microorganisms within two weeks. Mucor-like fungi were capable of degrading the cellulose membrane in liquid culture after only nine days. Tribe (1966) studying the interaction of soil fingi on cellulose film in the soil, found that Trichoderma was cellulolytic and that Pythium was not. The availability of nutrients may be an important factor in the cellulolytic ability shown by fungi. Under conditions of high nutrition such as Glucose Yeast broth, fungi may not produce cellulose degrading enzymes.

Membrane longevity was not increased by fungicides or aged autoclaved soil in which Trichoderma had been growing. Trichoderma is one of the first organisms to recolonize autoclaved soil; it builds up rapidly,

and produces an antibiotic, inhibitory to many other soil microorganisms (Agrios, 1969).

Preliminary experiments indicated that the Pellicon 500 NMWL membrane had the following desirable characteristics: 1) it resisted microbial breakdown for 4 to 6 weeks, 2) it had a flow rate sufficient to meet the water requirements of a carrot for the first four weeks after emergence, 3) the design of the chamber therefore did not require as much conducting surface as previously needed in cellulose models (Cox and Boersma, 1966).

The working hypothesis was that a plant in such a system would send its roots down to the membrane and extract most of its water at the membrane surface. As a result, the water potential of the whole soil system would be relatively constant throughout. However, when a plant was grown in the chamber, the water potential slowly decreased after germination of the plant until it could no longer be monitored with the tensiometers. There was a high concentration of roots next to the membrane and the water content of the soil in the 0.5 to 4.5 cm region varied slightly. As the distance from the membrane increased, the soil became drier. This fact strongly suggested that the flow rate of water through the membrane was not the limiting factor, but rather that the capillary conductivity of water through the soil was not great enough to maintain a constant water potential of the whole soil mass.

A change in soil cell design confirmed that the capillary conductivity of the soil was the limiting factor, as a narrow soil cell, with two membrane conducting surfaces maintained a constant water potential,

while a cubical chamber with five membrane surfaces had a decreasing potential as seedlings grew. The narrow soil cell design decreased the volume of soil, while increasing the membrane surface area and the cubical chamber, only increased the membrane surface area, while the soil volume remained nearly the same as with the plumbing adaptor chamber. The centre of the narrow soil cell was only 0.7 cm from the membrane compared to 2.2 cm to the centre of the cubical chamber. Since water moves from one point to another along an energy gradient, a larger energy gradient would be required for water to move this greater distance. The greater variability of water potential observed in the cubical chamber compared to the narrow soil cell was not unexpected from theoretical considerations.

The measurement of water potential by instrumentation for extended periods of time in the 0-.5 bar to -2 bar range is subject to many problems. Tensiometers draw air relatively rapidly at tensions greater than -0.4 bars. When air is removed from the system, the pressure is released and water passes from the tensiometer into the soil because the soil is at a lower potential. It takes several days for the system to return to its original potential, by which time the tensiometers again require bleeding. On several occasions great difficulty was experienced in getting the tensiometers to operate after bleeding. If an air bubble was trapped in the tensiometer cup instead of rising to the highest point in the tube, the tensiometer dried out. It was difficult to saturate the porous cup and reestablish continuity so tensions could be again measured. In one case, where continuity was lost and

reestablished (Figure 11), soil potential readings were still questionable. The tension readings indicated a soil potential of -0.225 bars but a gravimetric determination indicated a tension of -0.6 bars.

The triple junction two loop thermocouple psychrometer, used to measure potentials less than -1 bar, also posed problems. Measurements were probably only accurate within 0.5 bars as the thermocouple output fluctuated quite widely from one reading to the next (Figure 14). Immediately after starting the fan to increase soil surface evaporation, measurements of water potential in the top region of the pots decreased rapidly and much more than was expected in such a short time interval. Gravimetric determination of soil water content indicated that psychrometer readings were in fact much too low. The psychrometer reading was less than -15 bars but gravimetric determination suggested a potential of -2 bars. While dismantling the soil chambers, a white salt deposit was found around some of the porous bulbs. A few of the psychrometers had visible corrosion at the copper-constantan, copper-chromel junctions. Calibration curves after the six week experiment were much lower than curves before the experiment. Interpretation of results using the thermocouple psychrometer to measure water potentials of soil maintained at osmotic potentials of less than -1 bar were therefore impossible because of these unforseen problems.

Gravimetric determination of the soil water potential eventually had to be used because of the problems in measurement with the available instruments. This indirect method of determining water potential has the advantage of high accuracy, but the disadvantage that it is a

destructive method. The water potentials of soil cells in -0.2 bar osmotic solution were constant at -0.4 bars at each of the three weeks. The water potential of the soil cells in -2.0 bars was more variable and potentials ranged from -1.7 to -4.2 bars in the second and third weeks of the experiment. This technique of using osmotic solutions to control the soil matric potential, permitted control at much lower water potentials than are possible using other techniques.

To show that this system could keep up with the water demands of young plants, the following calculations were done. The soil cells had a soil volume of 57.5 cm^3, and at a bulk density of 0.7 g/cm^3, they would contain 40 g of oven dry soil. If no water was entering the system, and a plant was transpiring 7.8 ml/day, it would take only one day for the soil water potential to decrease from -0.2 bar to -2.0 bars and less than a further half day for the soil to have reached a potential of the permanent wilting point. Since the carrots were growing well and the water content of the soil remained constant, water must have been flowing from the PEG solution into the soil. Therefore, this system is able to maintain the water requirement of a young actively transpiring plant.

Transpiration decreased as the osmotic potential of the PEG solution decreased. Plants at -2.0 bars transpired about half as much water as plants at -0.2 bars. Plant growth after 3 weeks was less at -2.0 bars than -0.2 bars. It appeared that the lower water potential was placing a stress on the plants which resulted in decreased growth. Rawlins et al. (1968) found that transpiration was unaffected by soil

water content until soil potentials were less than -6 to -8 bars. A short period of low soil potential before irrigating the soil to saturation had little effect on transpiration (Rawlins et al., 1968).

The limiting factor in this technique for controlling soil moisture is the capillary conductivity of the soil. This was particularly noticable inthe upper layers of the narrow soil cells at low water potentials. In cells at -2 bars, the soil directly between the membranes was closer to the potential of the solution than the soil above or below the membranes. As the potential of a soil decreased, the capillary conductivity rapidly decreased (e.g. the conductivity of a saturated, -1 bar and -15 bar soil is in the order of 1, 10^{-3} and 10^{-5} cm/day respectively (Gardner, 1968)), so that water movement at lower potentials is very limited. If this technique is to be successfully used at low potentials, the distance between membranes should not be any greater than 1.4 cm as used in this experiment. At high potentials such as -0.2 bars, the cell width could be slightly increased without causing a decrease in soil potential.

A high percentage of soil cells, after 3 weeks, had developed leaks. A different lot of membranes was used in this experiment than previous experiments. Since these membranes were still in the experimental stages of development at the time of use, the quality may not have been reproducible from one lot to the next. In addition, the membranes were cemented to the plexiglass with Devcon rubber cement, a different brand of rubber cement than used previously. This change was necessitated by a temporary removal of Dexon rubber cement from the

market, pending relicensing. Soil cells that were neglected controlled soil moisture longer than cells that were weekly washed and placed in fresh PEG solutions. Chambers were washed with a squirt of water from a plastic wash bottle to remove slime that built up on the chamber surface. The force of the spray did not seem excessive enough to damage the membrane.

If the problems observed with the 500 NMWL Pellicon membrane can be overcome or if 1000 NMWL membranes and PEG 20,000 are used in place of the 500 NMWL membranes and PEG 6000, this technique will be ideal for controlling soil moisture within a narrow range for a growing plant. The technique is now at a stage where the organisms associated with PRD can be added to the system, and disease development at various water potentials can be observed. This technique is also ideal for studying the effect of soil moisture on spore germination and behavior at potentials much lower than is possible with other techniques.

CONCLUSIONS

1. A greenhouse periodic, soil-saturation experiment confirmed field observations that the frequency of soil saturation is directly correlated with Olpidium incidence.

2. Pythium species ceased growth on osmotically amended media at about -30 bars, while at -15 bars they generally had a growth rate of greater than 50% that on non-amended media. Therefore soil water content per se may not be the limiting factor in Pythium infection.

3. A method of controlling soil water matric potential of a growing plant for within narrow limits for at least three weeks was developed.

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