EVALUATION OF NATIVE RHIZOSPHERE BACTERIA FOR USE AS BIOLOGICAL CONTROL AGENTS AGAINST *PYTHIUM APHANIDERMATUM* ROOT ROT OF EUROPEAN GREENHOUSE CUCUMBERS

by

© Lynda Rankin

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science.

Department of Plant Science
MacDonald Campus
McGill University
Montreal

Date: July, 1992

SHORT TITLE

BIOLOGICAL CONTROL OF *P. APHANIDERMATUM* IN GREENHOUSE CUCUMBER

SHORT TITLE

BIOLOGICAL CONTROL OF *P. APHANIDERMATUM* IN GREENHOUSE CUCUMBER

PREFACE

This thesis consists of five sections . The first gives a perspective on the greenhouse cucumber industry and provides a scope of the problem. Parts two and three detail preliminary in vitro screening of the bacteria and further testing of the bacteria in small scale growth bench experiments. The fourth part consists of the full scale greenhouse testing of the bacteria and is presented as a complete manuscript. Part five is an overall discussion and conclusion.

This format has the approval of the Faculty of Graduate Studies and Research of the University of McGill ; as outlined in Guidelines Concerning Thesis Preparation (Revised 1991) , section 2 , Manuscripts and Authorship. The aforementioned section reads as follows :

" The candidate has the option, subject to the approval of the department, of including as part of the thesis the text, or duplicated published text, of an original paper or papers.

Manuscript-style theses must still conform to all other requirements explained in the Guidelines Concerning Thesis Preparation. Additional material (procedural and design data as well as descriptions of equipment) must be provided in sufficient detail (eg. in appendices) to allow clear and precise judgement to be made of the importance and originality of the research reported. The thesis should be more than a mere collection of manuscripts published or to be published. It must include a general abstract, a full introduction and literature review and a final overall conclusion. connecting texts which provide logical bridges between different manuscripts are usually desirable in the interest of cohesion.

It is acceptable for theses to include , as chapters, authentic copies of papers already published, provided these are duplicated clearly and bound as an integral part of the thesis. In such instances, connecting texts are mandatory and supplementary material is always necessary. Photographs or other materials which do not duplicate well must be included in their original form.

While the inclusion of manuscripts co-authored by the candidate and others is acceptable, the candidate is required to make an explicit statement in the thesis of who contributed to the work and to what extent, and supervisors must attest to the accuracy of the claims at the PhD. Oral defense. Since the task of the Examiners is made more difficult in these cases, it is in the candidate's interest to make the responsibilities of authors perfectly clear.

With the exception of the bacteria identification done by Dr. Zhou Ting , all the work reported here was done by the candidate between Sept. 1990 and June 1992. These studies were done under the supervision of Dr. Timothy C. Paulitz, Assistant professor, Department of Plant Science, MacDonald Campus, McGill University. Data presented in section two have been submitted for publication under the authorship of Paulitz, T.C. Zhou, T. and Rankin L. 1992 as part of the paper entitled Selection of rhizosphere bacteria for biological control of Pythium aphanidermatum on hydroponically-grown cucumber. The manuscript which comprises section four of this thesis will be submitted in appropriate format to the journal Plant Disease.

ABSTRACT

Thirty-two isolates of rhizosphere bacteria , selected for their ability to inhibit zoospore germination and/or mycelial growth of Pythium aphanidermatum (Pa) in vitro, were evaluated in a test tube bioassay using cucumber c.v. 'Straight 8'. From the bioassay the five most effective isolates were chosen for further characterization in long term growth bench studies and full scale greenhouse testing. These isolates were identified as Pseudomonas corrugata (Pc13 or 35) and P. fluorescens (Pf15, 16 or 27). All but one of the five isolates effectively colonised the roots of cucumber plants in short term studies. In a 7-week study to assess the rhizosphere competence of the bacterial isolates, isolates 15 and 35 were found to maintain high population densities throughout the time period and both were recovered from the endo-rhizosphere at the end of the 7 weeks. Plant growth promotion characteristics were associated with Pf15 in the bioassay and in both growth bench and greenhouse studies. Growth bench studies however, were not found to be useful to assess the effects of the bacterial isolates and the Pythium in planta. In 2 out of 3 greenhouse crops, two isolates were effective at suppressing the effects of Pythium root rot. In the spring 1991 crop , Pa-inoculated plants treated with the Pc13 or Pf15 produced fruit yields equal to 92 and 74% respectively of the control (no Pa , no bacteria). Pa-inoculated plants without bacteria yielded only

46% of the control. In the fall crop, Pa-inoculated plants treated with Pc13 or Pf15 yielded 52 and 47% of the control compared to Pa-only treatment, which yielded 12.5% of the control. In both crops, treatment with any of the bacterial isolates resulted in significantly reduced cull rates compared to the Pa-only treatment. Conclusions are drawn as to the predictive value of the various screening processes used here and the potential of the bacterial isolates as biocontrol agents for use in commercial greenhouses to control root rot caused by Pa.

Trente-deux isolate de bactérie rhizosphère, selectioné pour leur nabilité a pronibité la germinaison de zoosphère et/ou la croissance de mycelial Pythium aphanidermatum (Pa) en vitro ont étés évalué à l'eprouvette en employant le concombre c.v. 'Straight 8'. De ces études, cinq isolate, choisi parmi les plus effetif, ont été soumis a des études de characterization a court term et a des tests de serre. Ces isolates sont identifié comme Pseudomonas corrugata (Pc13 ou 35) et P. fluorescens (Pf15, 16, ou 27). Pendant ces études a court term, tous les cinq isolate, saut un, ont colonizé éffectivement les racine des plantes de concombres. Durant une étude de 7 semaine pour asseser la competance des rhizosphere d'isolate bacterial, l'isolate Pf15 et Pc35 ont maintenu des population a haute densite, et tous deux ont été récupéret du endo-rhizosphere. Des characteristic de croissance ont été associe avec le Pf15 dans cette assesement (biologic) dans les étube et aussi a court term et dans les serre. Cependant études à court terms ne se s'ont pas trouve util pour asseser les effets de isolute bacterial et du Pythium in planta. Dans le 2 et 3 récoltes de serre, deux isolates ont été effectif a supprimer les effet de la pouriture de racine Pythium . Pendant la récolte de printemp 1991, des plant inocule avec Pa traité avec du Pc13 ou de Pf15 on produit des fruit egálent 92 et 74%, respectivement, du group de control (pas de Pa, pas de

bactérie). Des plants inocule avec Pa sans bacterie ont produit seleument 46% de group de control. Pendant la récolte d'autaumne des plant inoculé avec Pa traite avec du Pc13 et du Pf15 ont produit 52 et 47% du group de control, compare a 12.5% pour le group traite avec du Pa seulement. Dans les deux récoltes tout traitement avec de l'isolate bactérial ont donne des cueillets beaucoup moins significant compare au group traité avec du Pa seulement. Des conclusion sont issues tant qu'a la valeur predictive des process de selection utilizer ici et du potentiel de l'isolate bacterial comme agent de control biologic pour l'utilization dans des serre comercial comme controlleur de pourritures des racine causé par le Pythium aphanidermatum

ACKNOWLEDGEMENTS

I wish to express my appreciation to all those who were involved with this project and without whom this work would not have been possible:

Firstly, Dr. Timothy C. Paulitz, primary academic supervisor , whose knowledge of this field and guidance was invaluable. Also for going 'above and beyond' as a supervisor. Thanks Tim for the extra time spent on discussions of 'New Directions in Biological Control' which contributed greatly to my understanding of the field of biocontrol as a whole. Dr. Katrine Stewart for advice concerning plant physiology and for providing space in her greenhouses for the trials which were conducted, and the late Dr. M. Fanous for guidance with the statistical analysis.

Thanks also to my colleagues from the lab: Dr. Ting Zhou for invaluable advice, assistance with the lab work and for helpful discussions throughout. Dr. Dilantha Fernando for guidance with the statistical analysis and informative discussions about microbiology. Dr. Osama Anas for advice on a wide variety of topics and for handing out jokes like vitamins - one a day. Also Javier Gracia and Taufik Fauzi for helpful discussions. I would also like to acknowledge the two under-grads who worked in the lab during the summers of 1992 and 1991 respectively. Cyrla Hoffert, whose assistance with the final greenhouse crop was much appreciated and Louis Sergent, whose unabashed enjoyment of pathology affected us

all. Thank you all for making it an enjoyable working environment. A major component of this work was done in the greenhouse. The assistance of Mike Bleho and J.P. Leblanc, horticultural technicians, with crop establishment , post cropping cleanup and maintenance of the greenhouse equipment was greatly appreciated.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. GENERAL INTRODUCTION

A PERSPECTIVE OF THE GREENHOUSE CUCUMBER INDUSTRY IN CANADA

European (seedless) cucumber , Cucumis sativus L. , is the second most prevalent greenhouse vegetable crop grown in Canada , its production volume exceeded only by that of tomatoes. The production of greenhouse cucumbers has been increasing in recent years; up from 3.8 million dozen produced in the 1987-88 growing season to 5.6 million dozen in 1990-91 (Statistics Canada. 1989 ,1990a ,1991) The most rapid growth in the industry has occurred in British Columbia where the volume produced has essentially doubled each year since 1988.

The total market value of the crop for the 1990 - 91 growing season was $29 million and on average growers received a wholesale price of $5.15 per dozen although this varied considerably by province (Statistics Canada 1991). High production costs, especially when energy costs are high such as during the early 80's and in 1990 , have resulted in a situation of marginal profitability for the greenhouse vegetable producer in most years (Ingratta et al.1985 , Statistics Canada 1991).

The majority of the greenhouse cucumbers produced in Canada are grown in Ontario ,British Columbia and Alberta .These provinces produce 61, 17, and 15% of the total production

respectively. Quebec , New Brunswick and Nova Scotia combined produce 7% (Statistics Canada 1991). Greenhouse cucumbers are not produced on any significant commercial scale in any of the other provinces. In Quebec the annual production of cucumbers currently satisfies 30% of local consumption needs (Andre Carrier , personal communication). In 1990 -91 Quebec produced 232,000 dozen European cucumbers. (Statistics Canada 1991).

Soilless culture is used widely throughout the country, while classic hydroponic culture is used in less than 5% of the area in production. Classic hydroponic culture is used somewhat more widely in Alberta than in the rest of Canada. Sawdust , peat , rockwool , oasis (expanded phenolic formaldehyde foam) NFT (nutrient film technique), sand , and gravel are the production systems used ; listed here roughly in their order of popularity (Ingratta et al. 1985). Sawdust tops this list only by virtue of the fact that the majority of greenhouse vegetable production in British Columbia at that time used this substrate (Resh, 1985). Sawdust was initially introduced as a growth media to avoid nematode infestations which plagued the industry when a soil based culture system was used (Ingratta et al. 1985). It was the substrate of choice in B.C. because of its availability and low cost (Resh , 1985) .

More recently however, rockwool has become more popular in B.C. than sawdust (R.J. Copeman , personal communication).

Peat is a readily available , extensively used horticultural

substrate in the rest of Canada. There are over 250,000 ha of peat bogs throughout the country , although not all have good horticultural characteristics or are accessible by equipment. The use of peat in greenhouse vegetable production is expected to continue to increase in the future. (Ingratta et al. 1985). Rockwool , a third popular substrate , is produced by melting a mixture of 60% diabase and 20% each of limestone and coke then spinning the melted material out into fibres. The addition of a binder and various additives to the fibres allows the cooling rockwool to be compressed into water-absorbing mats which are finally cut into blocks. Rockwool can be reused after steam sterilization, however it may loose its shape with repeated cropping. There is also the danger of increasing the incidence of soilborne disease such as Pythium root rot.

Ingratta et al. (1985) note that soilless or hydroponic production systems are not a panacea for the greenhouse vegetable industry in Canada. The growers decision to use these production methods is often governed by a lack of feasibility for soil based production on his/her site. Once soilless production is chosen there are a variety of considerations governing the choice of media . One aspect that is especially noteworthy in these times of increased concern over waste management is post-cropping disposal of the substrate. Both sawdust and peat are organic materials and therefore will degrade over time . Discarded peat media may in

fact be used as a soil amendment. Rockwool, on the other hand, has no post-cropping use nor does it breakdown - dumping is the only disposal option at present (Larsson, 1980).

Effective biological control strategies exist for most of the pest problems encountered during the production of European cucumbers and the majority of the diseases never reach economically damaging levels in the well managed greenhouse. For those diseases that do, recommended cultural (environmental manipulation, choice of resistant cultivars) or chemical control measures exist. The notable exception to this are diseases caused by the root rotting fungi , Pythium spp., Phytophthora spp. , Olpidium sp. and Phomopsis sp.. Of these fungi , Pythium spp. are the most prevalent and devastating pathogens. There is no registered chemical control for Pythium spp. in mature cucumber plants. Continual exclusion of the pathogen , which is difficult to accomplish , is the only recommended control measure at present.

To address this problem, a biological control strategy using native rhizobacteria has been investigated and is the subject of this thesis. Prior to the research reported here , six hundred bacteria isolates were cultured from the roots of European cucumber c.v.'Corona', which were grown in 34 different agricultural and forest soils. During 1990 these bacteria were screened to determine their in vitro effect on

Pythium aphanidermatum (Edson) Fitz. strain 186 (Paulitz et al., 1992). Following this screening process, 32 isolates were selected for further testing in vivo. These isolates were inhibitory to zoospore germination and/or mycelial growth of the fungus.

The research presented in this thesis is divided into 3 sections. Chapter 2 deals with the initial screening of the 32 bacterial isolates using cucumber seedlings in a test tube bioassay system. Chapters 3 and 4 describe the growth bench studies and greenhouse testing of the bacterial isolates found to be most effective in the bioassay.

1.2 LITERATURE REVIEW

1.2.1 AN OVERVIEW OF HYDROPONIC CROP PRODUCTION

Water culture systems have been used in plant physiology studies since the late 16th century. (Jones 1983). Hydroponics, the name derived from 'hydro' (water) and 'ponos' (labour) (Jones 1983) , have been used in commercial production since the 1930's (Zinnen 1988). Because of the variety of systems now in use, Jones (1983) and others have proposed that the term hydroponics be used only to refer to culture systems in which bare plant roots are bathed in the nutrient solution. All other water culture systems which use a solid medium to support the plants should be referred to as soilless culture. In current literature this is not strictly adhered to, and terms such as classic hydroponics are used to differentiate systems which use no media from those that do. Due to the ambiguity associated with this term in some of the literature referenced in this review , hydroponic production as used here, will refer to both classic hydroponic production and soilless culture unless otherwise specified.

Classic hydroponic systems and many soilless systems can be either closed (recirculating the nutrient solution) or open (non-recirculating). These two types of systems present different considerations for fertility control and disease management. Non recirculating systems are appropriate for tomato and cucumber production but inappropriate for the

production of leafy vegetables due to the wasting of nutrients (Zinnen 1988). Closed systems are necessary in areas where water supplies are limited or where drainage and discharge ordinances exist.

Hydroponic production affords the grower a stable, highly controllable production system. Plant nutrition and the physical environment, both in the root zone and above ground, are fully controllable by the grower. Due to this, increased fruit quality and greater control of crop scheduling can be achieved (Resh 1985). Hydroponic production reduces plant nutrition to its simplest form where all elements which are applied are available to the plant without the buffering action of the soil. As a result, plant competition is reduced and a greater planting density is possible, thus increasing yields . Resh (1985) suggests yields of 30480 kg/ha are possible for greenhouse cucumbers grown in a soilless system, compared to yields of 7842 kg/ha in a soil-based system. Yield increases of a similar magnitude are possible for other crops.

1.2.2 DISEASES IN THE HYDROPONIC SYSTEM

The absence of soil from hydroponic and soilless production systems theoretically precludes soil borne diseases (Jenkins and Averre 1983, Mohyuddin 1985a, Zinnen 1988). However until recently, many plant pathologists have had reservations about hydroponic production . Efficient dissemination of infective

propagules by the nutrient solution (Davies 1980, Jenkins and Averre 1983, Price and Dickenson 1980, Stanghellini et al. 1984, Tomlinson and Faithfull 1979, Vanachter et al. 1983) coupled with low populations of competing microbes and a uniform crop (Price and Dickenson 1980, Stanghellini et al. 1984, Zinnen 1988) were thought to increase the potential for rapid and catastrophic disease development.

In studies to assess this risk and control disease development, some researchers have failed to produce high levels of disease after infesting the nutrient solution of NFT systems with Colletotrichum (Daughtrey and Schippers 1980), Fusarium oxysporum f.sp. lycopersici, Didymella lycopersici , or Phytophthora nicotianae (Staunton and Cormican 1978). Others report rapid disease development under similar conditions using Pythium spp. (Evans 1979, Jenkins and Averre 1983) Colletotrichum , Fusarium oxysporum f.sp. cucumerinum (Jenkins and Averre 1983) and Didymella lycopersici (Evans 1979).

In soilless production systems using individual growing modules, plants are more isolated and were thought to be less prone to the rapid spread of root pathogens. (Zinnen 1988) . This has not proven to be the case (Carrier 1990, Favrin et al. 1988).

Reports dealing with disease incidence in commercial production facilities indicate that the number of root diseases is less than in the field, but the severity is greater due to favourable conditions (Zinnen 1988). On the

whole , severe infestations of root infecting pathogens have not been reported often but high losses have occurred in some areas (Price and Dickenson 1980) occasionally causing culture of the crop to be abandoned. (Stanghellini et al. 1984)

Several root infecting pathogens that have been reported in hydroponic vegetables are; Pythium spp. (Favrin et al. 1988, Gold and Stanghellini 1985, Jenkins and Averre 1983, Price and Dickenson 1980, Stanghellini and Tomlinson 1987, Stanghellini et al 1984, Zinnen 1988) Phytophthora (Price and Dickenson 1980) Colletotrichum , Fusarium (Jenkins and Averre 1983) Spongospora and Verticillium (Davies 1980).

1.2.3 THE PREVALENCE OF PYTHIUM SPP. IN HYDROPONIC PRODUCTION SYSTEMS

The zoosporic fungi Pythium and Phytophthora are particularly well adapted to the hydroponic system which affords the zoospores a method of rapid unobstructed dissemination (Gold and Stanghellini 1985). Pythium spp. are the most commonly reported pathogens encountered during hydroponic production and are often considered the most serious (Favrin et al.1988, Goldberg and Stanghellini 1990, Jenkins and Averre 1983, Stanghellini and Kronland 1986, Stanghellini et al. 1984).

Pythium species have been reported as the causal agents of

root disease in hydroponically grown vegetables which resulted in crop damage ranging from slight yield loss to complete crop failure (Gold and Stanghellini 1985, Jenkins and Averre 1983, Mohyuddin 1985b, Price and Dickenson 1980, Stanghellini et al. 1984, Zinnen 1988). Pythium root and basal stem rot (foot rot) is a significant limiting factor in the production of cucumbers in Quebec (Carrier 1990)and has been reported in commercial greenhouse facilities in British Columbia (Favrin et al. 1988).

Numerous Pythium species have been reported as the causal agents of cucumber root rot. P. aphanidermatum has been widely reported (Favrin et al. 1988, Jenkins and Averre 1983, Stanghellini and Phillips 1975, Zinnen 1988). It is the species most commonly associated with root rot of hydroponic cucumbers and has been reported as the predominant and most virulent species present in several commercial greenhouses (Favrin et al. 1988, Jenkins and Averre 1983) P.debaryanum (Jenkins and Averre 1983, Zinnen 1988) P. dissoticum (Stanghellini and Tomlinson 1987) P.myriotylum (Jenkins and Averre 1983, Zinnen 1988) and P.ultimum (Jenkins and Averre 1983) have also been widely reported. P.irregulare (Favrin et al. 1988) was reported in B.C. greenhouses and P. intermedium (Stanghellini et al. 1988) has been reported in England .

Both P. ultimum and P. aphanidermatum have been isolated from the roots of cucumber growing in a hydroponic system near Montreal (Paulitz unpub.). P.ultimum has also been recovered

from cucumbers grown in a hydroponic system in which the temperature was lowered to avoid a previous problem with P. aphanidermatum (Jenkins and Averre 1983). P. aphanidermatum and P. dissoticum often exist simultaneously in the hydroponic system . Differences in pathogenicity at different temperatures temporarily impart a competitive advantage to one species (Gold and Stanghellini 1985). The temperature optimum of P. aphanidermatum in situ is 23-27C and for P. dissoticum is 17-23C. Both species grow between 10-25C but P. aphanidermatum is the more virulent above 25C and causes more damage than P. dissoticum at 21-2[illegible]. P. dissoticum is more aggressive at 17C. Hydroponic solutions generally range in temperature from 17-27C (Ayers and Lumsden 1975, Gold and Stanghellini 1985).

1.2.4 GEOGRAPHIC DISTRIBUTION , HOST RANGE AND CONDITIONS FAVOURING DISEASE DEVELOPMENT

Pythium species are widely distributed throughout the world in agricultural and undisturbed soils (Kommedahl and Windels 1979, Waterhouse and Waterston 1964). They have an extensive host range covering most plant families (Hendrix and Campbell 1973, Waterhouse and Waterston 1964). Few varieties of crop plants are resistant to Pythium (Whipps and Lumsden 1991) Pythium spp. are best known as the causal organism of both pre and post-emergence damping-off which results in considerable

yield losses in many crops (Elad and Chet 1987). Pythium spp. also cause cottony blight of turf and cottony leak of cucurbit fruit (Waterhouse and Waterston 1964). There have also been reports of involvement of Pythium spp. in the pathogen complexes causing peach tree decline (Hendrix et al. 1966, Miller et al. 1966, Mircetech 1971) and more recently in apple replant disease in Nova Scotia (Braun 1991). Csnios and Hendrix (1978) also note that Pythium can affect plants as a minor pathogen or in ways that fall short of pathogenesis. Pythium spp. tend to be more efficient pathogens of young root and soft fruit tissue than of older root tissue. Various reasons have been postulated for this: Pythium has a poor competitive ability relative to other root colonising organisms and usually acts as a primary coloniser (Kommedahl and Windels 1979). Mellano et al. (1970a) suggest that differences in the cell wall and the composition of plant exudates makes mature roots less attractive to Pythium spp.. They note that these differences are physiological rather than anatomical and are not related to the extent of tissue lignification. With maturation of the root system comes tolerance to the pathogen , although the plants are not fully resistant. Older plants can be infected. Gotlieb and Butler (1939) reported yield losses of 20 -50 % in field grown melons and squash and similar losses were reported in muskmelon in Canada (McKeen and Thorpe 1968). Severe Pythium root rot occurring in mature hydroponically grown plants has

been frequently reported (Favrin et al. 1988 , Goldberg and Stanghellini 1990, Jenkins and Averre 1983, Sonoda 1983, Stanghellini and Phillips 1975, Stanghellini et al. 1984). In the hydroponic system, older plants remain in a more succulent state. In addition the entire root system is exposed to the inoculum . Rapid pathogenesis leading to severe root rot and plant death can occur in a matter of days (Jenkins and Averre 1983). Conversely Pythium may exist in the root system of hydroponically grown plants as a minor pathogen without causing recognisable symptoms until plant stress , brought about by fruiting (Favrin et al. 1988) or a check in growth (Davis 1980).

1.2.5 PATHOGENESIS AND SYMPTOM EXPRESSION

Pythium species are capable of direct attack on roots (Miller et al. 1966) and there is evidence to suggest that toxins (Csnios and Hendrix 1978, Woltz 1973) and pectic enzymes (Mellano et al. 1970b, Turner and Bateman 1968) are also produced.

Following mycelial or zoospore contact with the host tissue, small appressoria may be produced, usually at epidermal cell junctions. An infection peg then penetrates the tissue between the cells , or penetrates the cell directly. Hyphal growth then occurs in the plant tissue (Miller et al. 1966). Pathogenesis can be rapid with extensive root rotting

occurring in 3-9 days after introduction of the Pythium (Gold and Stanghellini 1985, Jenkins and Averre 1983). Lag periods of 8-10 weeks between introduction and symptom expression have also been reported (Evans 1977).

Symptoms range from subclinical infections (Stanghellini and Kronland 1986) to distinctive stem and root rots with wilting and chlorosis and stunting of the plant (Evans 1979 , Jenkins and Averre , Mellano et al. 1970a). Severe root infection does not produce obvious above ground symptoms in all cases (Mellano et al. 1970a) The exact mechanism of yield loss due to subclinical infections is unknown , however Stanghellini (unpublished) has suggested that rootlet maturation and senescence may be accelerated . Root formation and function may also be impaired. Stem rot is characterised by a dry, whitish-yellow rot of the basal stem . A brownish-orange discolouration of the roots also occurs. Severely affected plants are poorly anchored and daytime wilting often occurs.

1.2.6 BIOLOGY AND LIFE CYCLE OF PYTHIUM SPP.

Pythium spp. produce pale coloured, branched , rapidly growing mycelium. Arising from the mycelium are short hyphae which terminate in spherical oogonia or club shaped antheridium. The antheridium attachment may be monoclinous or diclinous, depending on the species. When the antheridia contacts the oogonium, fertilization occurs through the production of a

fertilization tube, and the expulsion of a male nucleus from the antheridium. This nucleus fuses with a female nucleus in the oogonium. Following this, the oogonial wall thickens and the resulting structure is the oospore (Agrios 1978). Exposure to cholesterol (Ayers and Lunmsden 1975) and other sterols (Mellano et al. 1970b) stimulates the production and maturation of the oospores. Mellano et al. (1970b) speculate that substances such as B-sitosterol present in plant tissue may cause the fungus to switch from a vegetative mode to a reproductive one. McKeen and Thorpe (1968) found oospores forming only in roots which were at advanced stages of degradation . The oospores of Pythium are resting structures which permit long term survival under unfavourable conditions (Ayers and Lumsden 1975, Stanghellini and Burr 1973). Temperature optima for oospore production and pre-germination dormancy period vary with species (Ayers and Lumsden 1975). The presence of root exudates induces the germination of oospores (Elad and Chet 1987, Stanghellini and Burr 1973). Bimodal germination has been observed in most species of Pythium , however the mode of germination is partially controlled by the presence of nutrients, free water (Stanghellini and Burr 1973) and favourable temperature (Agrios 1978). Under conditions where nutrients are present (ie: aspargine , glucose) oospores germinate directly via a germ tube, following which appresorium formation and/or host penetration occurs (Agrios 1978). If conditions revert to an

unfavourable state, some species can re-form the resting structure. This has been observed in P. ultimum but not in P. aphanidermatum , indicating it is at best a variable phenomenon (Stanghellini and Burr 1973).

Under low nutrient and/or high moisture conditions (flooding), oospores germinate to form a short hypha which terminates in a vesicle (Agrios 1978, Stanghellini and Burr 1973). The protoplasm passes from the oospore to the vesicle where the zoospores are formed. Upon maturation, the unicellular 3-10 um diameter zoospores are released and become free swimming (Stanghellini unpub). Zoospores remain motile for 4-24 hours, (Stanghellini unpub) during which time they are attracted to sugars and amino acids present in plant root exudates (Jones et al.1991). Zoospores accumulate around the root and bind to it in the region of elongation or near the root tip (Longman and Callow 1987, Mellano et al. 1970a). Binding is facilitated by the interaction of protein receptors on the zoospore surface interacting with root surface mucilage (Longman and Callow 1987). There is a retraction or loss of the flagella followed by encystment on the host (Agrios 1978, Gold and Stanghellini 1985). Sugars and amino acids also stimulate the germination of the zoospore (Jones et al. 1991, Kraft and Erwin 1967) which is followed by appresorium formation in some species such as P.ultimum (Miller et al. 1966) or direct penetration of the host as is the case with P.aphanidermatum (Gold and Stanghellini 1985). Jones et al. (1991), in studies

with P. aphanidermatum, found that while a variety of sugars and amino acids stimulated one or more steps in the sequence of infection by the zoospore only glutamic and aspartic acid incited all the steps. Zoospores function as the primary dispersal and infective propagules in liquid media ; however in soil their effectiveness is limited to flood conditions (Agrios 1978, Gold and Stanghellini 1985, Stanghellini and Burr 1973). In the soil system the main infective propagule of Pythium is the oospore. (Stanghellini and Burr 1973).
Sporangia , which can be spherical, filamentous or variously shaped, are also produced on the mycelium of Pythium. The sporangia exhibit the same type of bimodal germination and infection processes as the oospores (Agrios 1978). Volatile exudates, including low concentrations (1-7 nmol/ ml) of ethanol, also stimulate sporangia germination (Nelson 1987).

1.2.7 BIOLOGY OF PYTHIUM APHANIDERMATUM (EDSON) FITZ.

Pythium aphanidermatum (Edson) Fitzpatrick is characterised by luxuriant white mycelial growth and abundant oogonial production in culture (Gotlieb and Butler 1939). Waterhouse and Waterston (1964) give a temperature range for this species as 8-46 C, with the optimum range falling between 34-36 C. Its optimum pH range is 5.5 to 7.0 and mycelial growth is sparse below 4.5 (Bolton 1980a) Oospores are readily produced in culture in the 15-35 C range and do not exhibit the strong

dormancy associated with other species such as P ultimum. (Ayers and Lumsden 1975). P. aphanidermatum readily produces zoospores at temperatures between 25-30 C (Van der Plaats-Niterink 1981) when placed in a liquid medium (Stanghellini and Burr 1973). Temperatures of 30-35 C most favour infection by P. aphanidermatum (Van der Plaats-Niterink 1981).

1.2.8 INTRODUCTION OF THE PATHOGEN

Pythium can be easily introduced to the soilless system by infested surface and well water (Mohyuddin 1985b) or contaminated soil (Sonoda 1983, Stanghellini and Phillips 1975). Favrin et al. (1988) found that the majority of the pre- mixed (commercial) peat-based propagation media and peat-based custom mixes used by greenhouse growers in the Fraser Valley of B.C. harboured populations of Pythium spp.. It has also been suggested by some authors that fungus gnats (Bradysia impatiens) (Gardiner et al.1990) and shore flies (Scatella stagnalis) (Goldberg and Stanghellini 1990, Goldberg et al.1991) may be partially responsible for the introduction and spread of Pythium in commercial greenhouse facilities . Both of these insects are abundant in areas where plants are grown in rockwool (Lindquist 1985) and both have been shown to be capable of excreting viable oospores after feeding on the fungus or Pythium infected cucumber roots.

1.2.9 CHEMICAL PHYSICAL AND CULTURAL CONTROL

Currently , few effective control measures exist for root rot caused by Pythium spp. in hydroponically grown vegetables. At present there are no fungicides registered for use against Pythium on greenhouse cucumbers in Canada (Boivin 1988) Initially it was hoped that crop protection in hydroponic production systems would be a matter of adding a chemical to the nutrient solution reservoir and allowing it to circulate to the crop plants (Staunton and Cormican 1980). As this method of application was investigated, it became evident that it would not succeed. The concentrations of fungicides which are used in soil to compensate for adsorption and dispersal are generally above the LD_{50} for the plants in the hydroponic system (Price and Dickenson 1980). Roots lack the protective cuticle of leaves, so fungicides suitable for aerial application can be phytotoxic when the roots are directly exposed to them. Staunton and Cormican (1980) tested a variety of fungicides on hydroponically grown tomatoes ; the majority were found to be phytotoxic at rates of 10 - 50 ppm a.i.. This list included benomyl (Benlate™) which was slightly phytotoxic at 50 ppm and severely phytotoxic at 100 ppm. Price and Fox (1986) found that levels of benomyl low enough to avoid phytotoxicity are only fungistatic to Pythium and Phytophthora. Metalaxyl (Ridomil) a fungicide specific for pythiaceous fungi ,has been tested for its potential to

control Pythium in the hydroponic system. The current Canadian registration is for use in controlling Pythium in potato and tobacco only, although its registration in other countries is more extensive. Metalaxyl concentrations as low as 5-10 ug/ml prevented zoospore formation. (Gold and Stanghellini 1985, Price and Dickenson 1980). Furalaxyl (Fonganil, Fongarid), a related compound, also prevented zoospore formation at 10 ug/ml in all species tested except P. nicotianae (Price and Dickenson 1980). Species of the pythiaceous fungi differ widely in their tolerance to these chemicals (Price 1980 ,Price and Dickenson 1980). Metalaxyl is fungistatic (Favrin et al.1988, Gold and Stanghellini 1985, Price and Dickenson 1980 ,) and viable Pythium has been recovered from treated plants. (Favrin et al. 1988). Price and Maxwell (1980) found Fongarid produced a fungistatic effect in Pythium following 48 hour exposures to 50ppm.

Metalaxyl resistance has also been found in closely related fungi. Pseudoperonospora cubensis (cucumber downy mildew) with stable resistance to metalaxyl concentrations 2 - 10.5 times the recommended rates was recovered from greenhouses in Greece (Georgopoulos and Grigoriu 1981). Bruin and Edgington (1980) induced Ridomil resistance in Phytophthora capsici and Pythium spp. by repeated culturing of the fungi on media containing sublethal doses of the fungicide or by exposure of the mycelium to UV light.

In addition, metalayxl is stable in the nutrient solution .

Vanachter et.al. (1983) found 1/3 to 1/2 of the original dose remained three weeks after application. Furalaxyl is also stable in the nutrient solution . Price and Fox (1986) found 50% of the original dose remained in the solution seven days after application. In the same trial furalaxyl treatment of the nutrient solution resulted in decreased fruit yield but no decrease in dry weight of leaves or stems. Staunton and Cormican (1980) also noted phytotoxicity with 20 ppm a.i. metalaxyl in tomatoes .

Low potential sales and high potential liabilities make agrochemical manufacturers reluctant to pursue the development of new chemicals or pursue minor use registration of old chemicals for use in hydroponic production (Zinnen 1988). Another problem associated with fungicide use is the preharvest interval (14 days for Ridomil) which may not be suitable for a daily harvest system. Residue in the fruit is also of concern. Three weeks after the second of two applications of metalaxyl at a rate of 10 ppm a.i., tomato fruit residues up to 0.42 ppm were detected. (Vanachter et al. 1983)

The addition of surfactants to the nutrient solution presents a more promising approach . A number of surfactants representing non-ionic, anionic, and cationic classes were shown to be toxic to the zoospores of Olpidium sp., a related phycomycete (Tomlinson and Faithfull 1979). Twenty to twenty-five ug/ml of Agral™ ,a non-ionic surfactant , caused zoospore

lysis and inhibited zoospore formation in Pythium spp.. However the surfactant did not affect mycelial growth or direct germination of zoospore cysts and sporangia (Stanghellini and Tomlinson 1987). Agral™ is biodegradable , non-systemic and non-phytotoxic to cucumber at 20 ug/ml (Stanghellini and Tomlinson 1987).

UV irradiation is a commonly used water purification treatment in food processing and manufacturing industries. Irradiation of the nutrient solution has been shown to reduce populations of Pythium (Moyhuddin 1985b, Stanghellini et al.1984). However , irradiation causes soluble iron to be precipitated out as ferric oxide (Moyhuddin 1985b, Stanghellini et al.1984). In a recent trial, the decrease in iron concentration was 96.1 % over a 24 hour period. The resulting precipitate may coat equipment and nutrient delivery lines. (Moyhuddin 1985b). Solution concentrations of boron, manganese and phosphorous are also reduced to a lesser extent (Moyhuddin 1985b). However, this system has been used by several Alberta growers for 3 years, with no serious disease problems encountered (Moyhuddin 1985b). Irradiation appears promising but effectiveness of the treatment is limited in systems with high flow rates (Stanghellini et al. 1984)

Filtration (Goldberg et al.1991) and the addition of silicates to the nutrient solution (R. Belanger, personal communication) also have shown promise.

Manipulation of light, temperature, or nutrient composition

may also reduce disease severity (Funck-Jenson and Hockenhull 1983, Gold and Stanghellini 1985). Zinc (10 ug/ml) and copper (4 ug/ml) were found to be toxic to the zoospores of Olpidium brassicae in under one hour in laboratory assays. However ,these concentrations may be too high for practical use and phycomycetes differ in tolerance to zinc (Tomlinson and Faithful 1979).

Resistance is not available in commercial European cucumber cultivars, nor is it likely to be available in the foreseeable future (Whipps and Lumsden 1991). As with the development of chemical controls, the development of resistance is limited by the small potential market. Due to the ubiquitous nature of Pythium and its multiple modes of entry into the greenhouse, attempts at exclusion are unlikely to provide adequate control.

1.2.10 THE POTENTIAL FOR BIOLOGICAL CONTROL

Rhizobacteria and many plant pathogens including Pythium are involved in an equilibrium in the rhizosphere which is partially controlled by nutrient availability (Elad and Chet 1987).

Rhizosphere bacteria utilize carbon and nitrogen sources present in the root exudates. This reduces the total available carbon and nitrogen and affects the processes within the disease cycle of Pythium which depend on the presence of those

nutrients (Elad and Chet 1987). The lack of a normal complement of rhizosphere flora and fauna (Van Peer and Schipppers 1989) in the hydroponic system precludes the relationship from establishing and potential disease control effects are lost. Yet this drawback of hydroponic production is turned to an advantage when biological control is considered.

The idea of establishing antagonist populations in the microenvironment of the pathogen is not a new one in biocontrol research. Many attempts have been made to introduce antagonists to the soil system in an effort to control plant pathogens , but few have succeeded. (Deacon 1991, Fry 1982) The most common cause of failure is that the antagonist , while effective under controlled laboratory conditions, is rendered ineffective or suppressed by indigenous microbes in the soil (Fry 1982). The hydroponic system, due to the stability of the environment and reduced diversity of competing organisms, would seem ideally suited to the introduction of an antagonist population. In addition, this type of system could provide a very effective dispersal mechanism for the introduced antagonist (Jenkins and Averre 1983).

Seed rot damping-off and post-emergent root rot caused by Pythium and other soil borne fungi have been the focus of a great deal of research attention in recent years (Callan et al. 1990, Hadar et al. 1983, Lifshitz et al. 1984, Osburn and

Schroth 1988, Weller and Cook 1983). Disease control by antagonists and competitors in this area appears promising because establishment of the control agent on the seed coat (biopriming) prior to encounters with the pathogen is possible.(Harman and Taylor 1988). Low amounts of inoculum can also be used (Howell 1991). Various soil fungi (Bolton 1980b, Bolton 1978, Hadar et al. 1984, Howell 1991) including non pathogenic species in the pathogen genera (Cubeta and Echandi 1991, Foley and Deacon 1986, Martin and Hancock 1987, Paulitz and Baker 1987a, 1987b) and rhizobacteria (Chang and Kommedahl 1968, Cubeta and Echandi 1991, Hadar et al. 1983) most notably Pseudomonads (Howell and Stipanovic 1979, Howell and Stipanovic 1980, Kaiser and Hannan 1988, Lifshitz et al. 1986, Osburn et al. 1989, Parke 1990, Trapero-Casas et al. 1990) have been exploited for this use. In 1989 a Pseudomonas fluorescens product, Dagger ^{R}, was commercially available for use against cotton damping-off caused by Pythium. This product has since been removed from the market (Whipps and Lumsden 1991). A Streptomyces product with the proposed name Mycostop is currently being developed and projected to be available in the early 1990's (Whipps and Lumsden 1991) .

Soil solarization (Gamliel and Katan 1991, Greenberger et al. 1987, Stapleton and DeVay 1984) and partial sterilization have been used in an attempt to increase population densities of beneficial bacteria while decreasing pathogen populations in field and pot crops.

The introduction of antagonists to the hydroponic system has received less attention (Goldberg and Stanghellini 1991, Okamoto and Isaka 1989) but partial successes have been realized (Okamoto and Isaka 1989).

Studies into the mode of action of these biocontrol measures have been less revealing. Currently a considerable amount of contradictory literature exists regarding the role of nutrient competition (Becker and Cook 1988, Elad and Chet 1987, Loper 1988, Osburn and Schroth 1988,Paulitz and Loper 1991). antibiotic and antifungal metabolite production (Howell and Stipanovic 1980, Howell et al. 1988, Thompson and Burns 1989) parasitism (Nelson et al. 1986, Sneh et al. 1977) and induced resistance (van Peer et al. 1991, Wei et al. 1991) in biocontrol by rhizobacteria.

The beneficial effects of biopriming and osmopriming have been attributed to increased nutrient competition from populations of indigenous seed coat bacteria rather than a reduction in seed exudate production (Osburn and Schroth 1988).

Recently it has been reported that bacteria isolated from the soil rhizosphere (Pseudomonas spp. and Alcaligens sp.) have been effective in suppressing diseases caused by Pythium species in greenhouse crops (Elad and Chet 1987, Parke and King personal communication). The suppressive effect resulted from the bacteria establishing along the roots and preventing the establishment of Pythium by competition for nutrients (Elad and Chet 1987).

1.2.11 PLANT GROWTH PROMOTION AND BIOCONTROL AGENTS

Some rhizobacteria which have been associated with biological control activity also have been reported to induce plant growth promotion in their host plants, and are called plant growth promoting rhizobacteria (PGPR) (de Frietas and Germida 1990, Gamliel and Katan 1991, Kloepper 1983, Kloepper et al. 1980, Weller and Cook 1986). Recent studies have attributed this response to ; displacement of indigenous root colonisers (Becker and Cook 1988 , Kloepper and Schroth 1981, van Peer and Schippers 1989) production of gibberellin-like substances (Katznelson and Cole 1965) and increased phosphate uptake (Lifshitz et al. 1987).

LITERATURE CITED

Agrios, G.N. 1978. Plant Pathology . Academic Press Inc., New York. 703 pp.

Anonymous 1989. Seasonal Fruit and Vegetable Production. Statistics Canada Publication 22-203

Anonymous 1990a. Seasonal Fruit and Vegetable Production. Statistics Canada Publication 22-203

Anonymous 1991. Seasonal Fruit and Vegetable Production. Statistics Canada Publication 22-203

Anonymous 1990b. Greenhouse Industry 1989 and 1990. Statistics Canada Publication 22-202.

Ayers, W.A. and Lumsden, R.D., 1975. Factors affecting production and germination of oospores of three Pythium species. Phytopathology. 65: 1094-1100

Becker, J.O. and Cook, R.J. 1988. Role of siderophores in suppression of Pythium species and production of increased growth response of wheat by fluorescent pseudomonads. Phytopathology. 78: 778-782.

Boivin, C. 1988. Les maladies racinaires en culture hydroponique prevention et control. L'Horticuleur 3: 12-13.

Bolton, A.T. 1980a. Effects of temperature and pH of soilless media on root rot of poinsettia caused by Pythium aphanidermatum. Can. J. Plant Pathol. 2: 83-85

Bolton, A.T. 1980b. Control of Pythium aphanidermatum in poinsettia in a soilless culture by Trichoderma viride and a Streptomyces sp. Can. J. Plant Pathol. 2: 93-95.

Bolton, A.T. 1978.Effects of amending soilless growing mixtures with soil containing antagonistic organisms on root rot and blackleg of geranium (Pelargonium hortorum) caused by Pythium splendens. Can. J. Plant Science. 58: 379-383.

Braun, P.G. 1991. The combination of Cylindrocarpon lucidum and Pythium irregulare as a possible cause of apple replant disease in Nova Scotia. Can. J. Plant Pathol. 13: 291-297.

Bruin, G.C. and Edgington, L.V. 1980. Induced resistance to ridomil of some oomycetes. Phytopathology 70: 459-460 (abst.)

Callan, N.W., Mathre,D.E. and Miller, J.B. 1990. Bio-priming seed treatment for biological control of Pythium ultimum preemergence damping-off in sh2 sweet corn. Plant Disease 74:368-372.

Carrier, A. 1990. Concombres de Serre: nos plants vieillissent mal! Le Producteur Horticole (5)3:11-12

Chang, I. and Kommedahl, T. 1968. Biological control of seedling blight of corn by coating kernals with antagonistic microorganisms. Phytopathology 58: 1395-1401

Csnios, A., and Hendrix, J.W. 1978. Parasitic and non parasitic pathogenesis of tomato plants by Pythium myriotylum. Can. J. Botany 56: 2334-2339.

Cubeta, M.A. and Echandi, E. 1991. Biological control of Rhizoctonia and Pythium damping off of cucumber: an integrated approach. Biological Control 1:227-236.

Daughtrey, M.L. and Schippers, P.A., 1980. Root death and associated problems. Acta Hortic. 98:283-291

Davies, J.M.L. 1980. Disease problems encountered in commercially grown tomato crops. Acta Hortic. 98:299-305

Deacon, J.W. 1991. Significance of ecology in the development of biocontrol agents against soil-borne plant pathogens. Biocontrol Science and Tech. 1:5-20.

de Freitas, J.R. and Germida, J.J. 1990. Plant growth promoting rhizobacteria for winter wheat. Can. J. Microbiol. 36: 265- 272.

Elad, Y. and Chet H., 1987. Possible role of competition for nutrients in biocontrol of Pythium damping-off by bacteria. Phytopathology 77:190-195

Evans, S.G. 1977. Disease risks affect the entire crop in NFT systems. The Grower 15: 1233-1239

Evans, S.G. 1979. Suceptibility of plants to fungal pathogens when grown by the nutrient film technique (NFT) Plant Pathology. 28: 45-58

Favrin, R.J., Rahe, J.E. and Mauza, B., 1988. Pythium spp. associated with crown rot of cucumbers in British Columbia greenhouses. Plant Disease 72:683-687

Foley, M.F. and Deacon J.W. 1986. Suceptibility of Pythium spp. and other fungi to antagonism by the mycoparasite Pythium oligandrum. Soil Biol. Bichem. 18:1 91-95

Fry W.F. 1982. Principles of Plant Disease Management. Academic Press Inc., New York . 378 pp.

Funck-Jenson, D. and Hockenhull, J. 1983. The influence of some factors on the severity of Pythium root rot of lettuce in soiless (hydroponic) growing systems. Acta Hortic. 133: 129-136

Gamliel, A. and Katan, J. 1991. Involvement of fluorescent pseudomonads and other soil microorganisms in increased growth response of plants in solarized soils. Phytopathology 81:494-502.

Gardiner, R.B., Jarvis, W.R. and Shipp, L. 1990 . Ingestion of Pythium spp. by larvae of the fungus gnat Bradysia impatiens (Diptera: Sciaridae) Ann. Appl. Biol. 116: 1-8

Georgopoulos, S.G. and Grigoriu, A.C. 1981. Metalaxyl resistant strains of Pseudoperonospora cubensis in cucumber greenhouses in southern Greece. Plant Disease 65:729-731

Gold, S.E. and Stanghellini, M.E., 1985. Effects of temperature on Pythium root rot of spinach grown under hydroponic conditions. Phytopathology 75:333-337

Goldberg, N.P. and Stanghellini, M.E. 1990. Ingestion - egestion and aerial transmission of Pythium aphanidermatum by shore flies (Ephydrinae: Scatella stagnalis) Phytopathology 80: 1244-1246.

Goldberg, N.P. and Stanghellini, M.E. 1991. The potential use of bacteria as biological control agents for Pythium in hydroponics. Phytopathology 81:1345 (abst)

Goldberg, N.P., Stanghellini, M.E. and Rasmussen, S.L. 1991. Filtration as a strategy for the control of Pythium in recirculating hydroponics. Phytopathology 81:1345 (abst)

Gottlieb, M. and Butler, K.D. 1939 A Pythium root rot of cucurbits. Phytopathology 29: 624-628

Greenberger, A. Yogev, A. and Katan, J. 1987. Induced suppressiveness in solarized soils. Phtyopathology 77: 1663-1667.

Hadar, Y. Harman, G.E. and Taylor, A.G.1984. Evaluation of Trichoderma koningii and T. harzianum from New York soils for the biological control of seed rot caused by Pythium spp. Phytopathology 74: 106-110

Hadar, Y. Harman, G.E. Taylor, A.G.and Norton J.M. 1983. Effects of pregermination of pea and cucumber seeds and of seed treatment with Enterobacter cloacae on rots caused by Pythium spp. Phytopathology 73:1322-1325.

Harman, G.E. and Taylor , A.G. 1988. Improved seedling performance by intergration of biological control agents at favorable pH levels with solid matrix priming. Phytopathology 78: 520-525

Hendrix, F.F. and Campbell, W.A. 1973. Pythiums as Plant Pathogens. Ann. Rev. of Phytopathology 11:77-99

Hendrix,F.F. , Powell, W.M. and Owen, J.H. 1966. Relation of root necrosis caused by Pythium species to Peach tree decline. Phytopathology 56: 1229-1232

Howell, C.R. 1991. Biological control of Pythium damping-off of cotton with seed coating preparations of Gliocladium virens. Phytopathology 81:738-741

Howell, C.R., Beier, R.C., and Stipanovic, R.D. 1988. Production of ammonia by Enterobacter cloacae and its possible role in the biological control of Pythium preemergence damping-off by the bacterium. Phytopathology 78: 1075- 1078.

Howell, C.R. and Stipanovic, R.D. 1979. Control of Rhizoctonia solani on cotton seedlings with Pseudomonas fluorescens and with an antibiotic produced by the bacterium. Phtyopathology 69:480-482.

Howell, C.R. and Stipanovic, R.D. 1980. Supression of Pythium ultimum - induced damping-off of cotton seedlings by Pseudomonas fluorescens and its antibiotic, pyoluteorin. Phytopathology 70:712-715.

Ingratta , F.J., Bloom, T.J., Straver, W.A., 1985. p. 95-102 in Hydroponics Worldwide: State of the art in Soilless Crop Production.A.J. Savage ed. International Center for Special Studies , Honalulu,Hawaii. 194 pp.

Jenkins, S.F. and Averre, C.W., 1983. Root diseases of vegetables in hydroponic culture systems in North Carolina greenhouses. Plant Disease 67:968-970

Jones, J.B. Jr. 1983. A guide for the hydroponic and soiless culture grower. Timber press Portland Oregon. 124 pp

Jones, S.W. Donaldson, S.P. and Deacon, J.W. 1991. Behavior of zoospores and zoospore cysts in relation to root infection by Pythium aphanidermatum. New Phytol. 117: 89-301.

Kaiser, W.J. and Hannan, R.M. 1989. Biological control of seed rot and preemergence damping-off of chickpea with fluorescent pseudomonads. Soil Biol. Biochem. 21:2 269-273.

Katznelson, H. and Cole, S.E. 1965. Production of gibberellin-like substances by bacteria and actinomycetes. Can. J. Microbiol. 11: 733-741.

Kloepper, J.W. and Schroth, M.N. 1981. Relationship of in vitro antibiosis of plant growth promoting rhizobacteria to plant growth and the displacement of root microflora. Phtyopathology 71:1020-1024

Kloepper, J.W. , Schroth, M.N. and Miller, T.D. 1980. Effects of rhizosphere colonization by plant growth promoting rhizobacteria on potato plant development and yield. Phytopathology 70:1078-1082

Kommedahl T. and Windels C.E. 1979. Pathogen host dominance in disease. p 1-103 in Ecology of root pathogens Elsevier Scientific Publishing C. New York. 281pp. Krupa,S.V. and Dommergues Y.R. eds.

Kraft, J.M. and Erwin, D.C. 1967.Stimulation of Pythium aphanidermatum by exudates from mung bean seeds. Phytopathology 57:866-868

Larsson, G. 1980. Experiences in growing cucumbers and tomatoes in peat in Sweden during the last 10 years. Acta Hortic 99:39-46

Lifshitz, R., Kloepper,J.W., Kozlowski, M., Simonson C., Carlson, J., Tipping, E.M. and Zaleska I. 1987. Growth promotion of canola (rapeseed) seedlings by a strain of Pseudomonas putida under gnotobiotic conditions. Can. J. Microbiol.33: 390-395.

Lifshitz, R., Simonson, C., Scher, F.M., Kloepper,J.W., Roderick-Semple, C., and Zaleska, I. 1986. Effect of rhizobacteria on the severity of phytophthora root rot of soybean. Can. J. Plant Pathol. 8: 102-106.

Lifshitz, R. Sneh, B. and Baker, R. 1984. Soil suppressivness to a plant pathogenic Pythium species. Phytopathology 74:1054-1061

Lindquist, R.K. 1985 Insect and mite pest control for crops grown under protected cultivation p 51-57 in Hydroponics Worldwide: State of the art in Soilless Crop Production. Savage, A.T.(ed) International Center for Special Studies Honolulu, Hawaii 194 pp.

Longman, D. and Callow, J.A. 1987. Specific saccharide residues are involved in the recognition of plant root surfaces by zoospores of Pythium aphanidermatum. Physiological and Molecular Plant Pathol. 30: 139-150

Loper, J.E. 1988. Role of fluorescent siderophore production in biological contol of Pythium ultimum by a Pseudomonas fluorescens strain. Phytopathology 78:166-172

Martin, F.N. and Hancock, J.G. 1987. The use of Pythium oligandrum for the biological control of preemergence damping-off caused by P. ultimum. Phytopathology 77: 1013-1020.

Mellano, H.M. , Munnecke, D.E. and Endo, R.M. 1970a. Relationship of seedling age to development of Pythium ultimum on roots of Antirrhinum majus. Phytopathology 60: 935-942

Mellano, H.M. Munnecke, D.E. and Sims, J.J. 1970b. Relationship of pectic enzyme activity and presence of sterols to pathogenesis of Pythium ultimum on roots of Antirrhinum majus. Phytopathology 60: 943-950

McKeen, C.D. and Thorpe, H.J. 1968. A Pythium root rot of muskmelon. Can J. Bot. 46: 1165- 1171

Mohyuddin, M. 1985a. Greenhouse Cucumber Production Guide For Commercial Growers 1985-86. Alberta Agriculture 42 pp.

Mohyuddin, M. 1985b .Crop Cultivars and Disease Control. p 42-50 in Hydroponics Worldwide: State of the Art in Soilless Crop Production. Savage, A.T.(ed) International Center for Special Studies Honolulu, Hawaii 194 pp.

Miller, C.R., Dowler, W.M., Peterson, D.H., and Ashworth, R.P. 1966. Observations on mode of infection of Pythium ultimum and Phytophthora cactorum on young roots of peach. Phytopathology 56: 46-49

Mircetich, S.M. 1971. The role of Pythium in feeder roots of diseased and symptomless peach trees and in orchard soils in peach tree decline. Phytopathology 61:357-360

Nelson, E.B. 1987. Rapid germination of sporangia of Pythium species in response to volatiles from germinating seeds. Phytopathology 77: 1108-1112.

Nelson, E.B., Chao, W.L., Norton, J.M., Nash, G.T. and Harman, G.E. 1986. Attachment of Enterobacter cloacae to hyphae of Pythium ultimum: Possible role in the biological control of Pythium preemergence damping-off. Phytopathology 76:327-335.

Okamoto H. and Isaka M. 1989. Studies on biocontrol of plant diseases - control of cucumber disease caused by Phytophthora capsici by applied antagonistic bacteria. Bulletin of the Fukui Agricultural Experiment Station 1989. No. 14:33-44

Osburn, R. M. and Schroth, M.N. 1988. Effect of osmopriming sugarbeet on exudation and subsequent damping-off caused by Pythium ultimum. Phytopathology 78: 1246-1250

Osburn, R.M., Schroth,M.N., Hancock, J.G. and Hendson, M. 1989. Dynamics of sugar beet seed colonization by Pythium ultimum and Pseudomonas species: Effects on seed rot and damping-off. Phytopathology 79: 709-716.

Parke, J.L. 1990. Population dynamics of Pseudomonas cepacia in the pea spermosphere in relation to biological control of Pythium. Phytopathology 80:1307-1311

Parke J.L. and King E.B. unpub. Effects of Pseudomonas cepacia on zoospores of Aphanomyces and Pythium in relation to biocontrol.

Paulitz, T.C. and Loper, J.E. 1991. Lack of a role of fluorescent siderophore production in the biological control of Pythium damping-off of cucumber by a strain of Pseudomonas putida. Phytopathology 81:930-935

Paulitz, T.C., Zhou, T., and Rankin, L. 1992. Selection of rhizosphere bacteria for biological control of Pythium aphanidermatum on hydroponically-grown cucumbers. Biological Control: in press

Paulitz, T.C. and Baker, R. 1987a. Biological control of Pythium damping-off of cucumbers with Pythium nunn:Population dynamics and disease supression. Phytopathology 77: 335-340.

Paulitz, T.C. and Baker, R. 1987b. Biological control of Pythium damping-off of cucumbers with Pythium nunn:influence of soil environment and organic amendments. Phytopathology 77: 341-346.

Price, D. 1980. Fungal flora of tomato roots in nutrient film technique. Acta Hortic. 98: 269-275.

Price,D. and Dickenson, A., 1980. Fungicides and the nutrient film technique. Acta. Hortic. 98:277-283

Price, T.V. and Fox, P. 1986. Studies on the behavior of furalaxyl on pythiaceous fungi and cucumbers in recirculating hydroponic systems. Aust. J. Agric. Res. 37: 65-77

Price, T.V. and Maxwell, M.K. 1980. Studies of disease problems and their control in hydroponics in Australia. Acta Hortic. 98: 307-316

Resh, H.M. 1985. Hydroponic food production: A definitive guidebook for the advanced home gardener and the commercial hydroponic grower. 3rd ed. Woodbridge Press Pub. Company, Santa Barbara, California 384 pp.

Rush C.M. 1991. Comparison of seed priming techniques with regard to seedling emergence and Pythium damping-off in sugar beet. Phytopathol. 81:878-882

Sneh,B. Humble,S.J. and Lockwood,J.L. 1977. Parasitism of oospores of Phytophthora megasperma var. sojae, P. cactorum, Pythium sp. and Aphanomyces euteiches in soil by oomycetes, chytridiomycetes, hyphomycetes, actinomycetes and bacteria. Phytopathology 67:662-668.

Sonoda, R.M. 1983. On the lookout for Greenhouse Cucumber Diseases. Am. Veg. Grower 31:32-36.

Stanghellini, M.E. unpub. Root Disease in Hydroponically Grown Plants.

Stanghellini ,M.E. and Burr, T.J., 1973. Germination in vivo of Pythium aphanidermatum oospores and sporangia. Phytopathology 63:1493-1496

Stanghellini, M.E. and Kronland, W.C. 1986. Yield loss in hydroponically grown lettuce attributed to subclinical infection of feeder rootlets by Pythium dissoticum. Plant Disease 70: 1053-1056.

Stanghellini, M.E., and Phillips, J.M. 1975. Pythium aphanidermatum: Its occurance and control with pyroxychlor in the Arabian desert at Abu Dhabi. Plant Dis. Rep. 59:559-563.

Stanghellini, M.E., Stowell, L.J. and Bates, M.L., 1984. Control of root rot of spinach caused by Pythium aphanidermatum in a recirculating hydroponic system by ultraviolet Light. Plant Diseasse 68: 1075-1076

Stanghellini, M.E. and Tomlinson, J.A. 1987. Inhibitory and lytic effects of a non ionic surfactant on various asexual stages in the lifecycle of Pythium and Phytophthora species. Phytopathology 77: 112-114

Stanghellini, M.E., White, J.G., Tomlinson, J.A. and Clay, C., 1988. Root rot of hydroponically grown cucumbers caused by zoospore-producing isolates of Pythium intermedium. Plant Disease 72: 358-359.

Stapleton, J.J. and DeVay, J.E. 1984. Thermal components of soil solarization as related to changes in soil and root microflora and increased plant growth response. Phytopathology 74: 255-259

Staunton, W.P. and Cormican,T.P. 1978. The behavior of tomato pathogens in a hydroponic system. Acta Hortic. 82: 133-135

Staunton, W.P. and Cormican,,T.P. 1980. The effects of pathogens and fungicides on tomatoes in a hydroponic system. Acta Hortic. 98 293-297

Thompson, R.J. and Burns, R.G. 1989. Control of Pythium ultimum with antagonistic fungal metabolites incorporated into sugarbeet seed pellets. Soil Biol. Biochem. 21: 745-748

Tomlinson, J.A. and Faithfull, E.M., 1979. Effects of fungicides and surfactants on the zoospores of Olpidium brassicae. Ann. Appl. Biol. 93: 13-19.

Trapero-Casas, A. ,Kaiser, W.J. and Ingram, D.M. 1990. Control of Pythium seed rot and preemergence damping-off of chickpea in the U.S. Pacific Northwest and Spain. Plant Disease 74: 563-569

Turner, M.T. and Bateman, D.F. 1968. Maceration of plant tissues suceptible and resistant to soft rot pathogens by enzymes from compatible host pathogen combinations. Phytopathology 58: 1509- 1515

Vanachter, A. Van Wambeke, E. and Van Assche, C. 1983. Potential danger for infection and spread of root diseases of tomatoes in hydroponics. Acta Hortic. 133: 119-145

van der Plaats-Niterink 1981. Monograph of the genus Pythium. Institute of the Royal Netherlands Academy of Science and Letters. 242 pp.

van Peer, R., Niemann, G.J., Schippers, B. 1991. Induced resistance and phytoalexin accumulation in biological control of fusarium wilt of carnation by Pseudomonas sp. Strain WCS417r. Phytopathology 81:728-734

van Peer, R. and Schippers, B. 1989. Plant growth reponses to bacterization with selected Pseudomonas spp. strains and rhizosphere microbial development in hydroponic cultures. Can. J. Microbiol. 35: 456-463.

Waterhouse, G.M. and Waterson, J.M. 1964. Pythium aphanidermatum. # 36 in C.M.I. Descriptions of Pathogenic Fungi and Bacteria. Commonwealth Mycological Institute Surrey, England.

Wei, G., Kloepper, J.W., and Tuzan, S. 1991. Induction of systemic resistance of cucumber to Colletotrichum orbiculare by select strains of plant growth promoting rhizobacteria. Phytopathology 81:1508-1512

Weller, D.M. and Cook, R.J. 1983. Suppression of take-all fo wheat by seed treatments with fluorescent pseudomonads. Phytopathology 73:463-469

Weller, D.M. and Cook, R.J. 1986. Increased growth of wheat by seed treatments with fluorescent pseudomonads, and implications of Pythium control. Can. J. Plant Pathol. 8:328-334.

Whipps, J.M. and Lumsden, R.D. 1991. Biocontrol of Pythium species. Biocontrol Science and Technology 1: 75-90.

Woltz. S.S. 1978. Non parasitic plant pathogens. Annu. Rev. Phytopathol. 16:403-430.

Zinnen, T.M. 1988. Assesssment of plant disease in hydroponic culture. Pl. Dis. 72:2 96-99

2 SCREENING OF POTENTIAL BIOCONTROL AGENTS USING A TEST TUBE BIOASSAY SYSTEM

2.1 ABSTRACT

Thirty-two isolates of rhizosphere bacteria , selected for their ability to inhibit zoospore germination and/or mycelial growth of Pythium aphanidermatum in vitro, were evaluated in a test tube bioassay using cucumber c.v. 'Straight 8'. Isolates were tested in planta both with and without Pythium. Eleven of the isolates, when inoculated with Pythium, produced root lengths > 80% of a non-inoculated control. However, treatment with isolate 15 plus Pythium resulted in the only significant increase in root length over that of the Pythium control. In non-inoculated plants treatment with three of the bacterial isolates (13,14,15) increased plant root length over that of an non-inoculated control. Eight of the eleven isolates which reduced the root damage by Pythium were not significantly different (P=0.05) from Pseudomonas putida N1R in colonization ability in at least one trial.

The 32 isolates were identified and the majority were found to be species of Pseudomonas ; P.fluorescens (subgroup C or E), P.corrugata ,P.cichorii or Pseudomonas sp. One Escherichia vulneris was also identified.

Conclusions are drawn about the usefulness of this type of screening system for research into the biocontrol of Pythium.

2.2 INTRODUCTION

The Pythium species most commonly associated with root rot of hydroponically grown cucumbers in Quebec are P. aphanidermatum (Pa) and P. ultimum (Paulitz unpub.). While Pa. and P. ultimum may exist simultaneously in hydroponic systems, the temperature optimum of Pa (23-27 C) corresponds more closely to the temperatures found in Canadian greenhouses during the late spring and early fall production cycles. This may give Pa a competitive advantage in most years. Jenkins and Averre (1983) working in North Carolina recovered P.ultimum from cucumbers grown in a hydroponic system where the temperature had been lowered to avoid a previous problem with Pa. P.aphanidermatum unlike P. ultimum is a prolific producer of zoospores ,the main infective propagule responsible for the spread of Pythium in the greenhouse. This capacity might also give Pa. a competitive advantage over P.ultimum. Zoospores are the infective propagule used in the inoculum throughout these studies therefore, zoospore production capacity was also factored into the choice of Pa. as the pathogen. The Pa. strain 186 used in these experiments was originally isolated from diseased greenhouse cucumbers by W. Jarvis, Agriculture Canada , Harrow Ontario. In preliminary work, strain 186 proved to be a most prolific producer of zoospores compared to three other isolates from Ontario and three from Quebec. As detailed in Section 1.1 of this manuscript, the 32 bacterial

isolates being evaluated in this section are the successful candidates resulting from a mass collection and in vitro screening in 1990. The isolates were evaluated in vitro for their ability to inhibit mycelial growth or zoospore germination , motility and chemotaxis of P.a. (Paulitz et al.1992). Previous workers have found that in vitro inhibition of mycelial growth was a poor indicator of biological control ability (Broadbent et al. 1971), however the correlation between in vitro effects on the zoospores and biocontrol ability was good. Others (Okamoto and Isaka 1989) have found that the effects on zoospores in vitro were a useful predictor of biocontrol ability in the soil system but some isolates failed to have an effect in the hydroponic system. This would suggest that in vitro zoospore assays are a useful but incomplete prescreening process. To further screen the thirty-two isolates which performed well in the in vitro assays, a test tube plant-bioassay system was developed and the isolates were screened for their ability to control Pythium root rot in cucumber seedlings. This would facilitate the selection of a few effective isolates for full scale greenhouse testing. The work reported here details the preliminary testing of the plant bioassay system and the screening of the 32 isolates. The results of this screening have been previously reported (Paulitz et al. 1992).

2.3 MATERIALS AND METHODS

2.3.1 PRODUCTION OF ZOOSPORES

Pythium aphanidermatum (strain 186) was grown on clarified V8 juice (CV8J) agar for 48 hours at 35 C in an incubator with constant fluorescent light. The agar in each plate was then cut into 1-cm strips, half of which were placed in a second sterile petri dish. All plates were flooded with 20 ml sterile distilled water (SDW) and left standing for 30 minutes to leach the nutrients from the agar. The water was replaced with fresh SDW and the plates were returned to the incubator for 20 hours. The plates were then moved to a 20 C incubator to initiate zoospore formation. After 4 hours the liquid in the plates was filtered through paper tissue (Kimwipes) to remove coarse debris (mycelium and agar) and zoospore concentration in the filtrate was estimated with a haemocytometer.

2.3.2 PRODUCTION OF CUCUMBER PLANTS

Cucumber (Cucumis sativus L. c.v. 'Straight 8') seeds were placed in petri plates containing filter paper (Whatman #1) saturated with SDW. The seeds were germinated by incubating the plates for 48 hours in a dark, 26 C incubator , followed by 24 hours on a growth bench under a combination fluorescent-incandescent light source. Each seedling was then suspended at

the mouth of a 150 mm X 18 mm diameter test tube filled with 28 ml nutrient solution (97 g Peter's Hydrogro and 64 g $CaNO_3$ per L SDW). The seeds were secured in place with parafilm (American Can Co. ,Greenwich, Conn.) so the roots were immersed in the nutrient solution. The test tubes were agitated on a rotary shaker at 80 RPM to provide aeration of the solution.

2.3.3 DETERMINATION OF OPTIMUM INOCULUM DENSITY

The purpose of this experiment was to determine the concentration of P.aphanidermatum zoopores that would induce a disease severity level of approximately 50%. This experiment used the bioassay system described in 2.3.2 . After 48 hours zoospores of P.a. were added to the test tubes to give final concentrations of 0, 10, 30, 100, 300, 1000 or 3000 cfu/ml in the nutrient solution. The plants were grown for 8 days and during this period , nutrient solution depleted by the plants was replaced daily with SDW. After 8 days the plants were harvested and the roots were removed and dried for two days at 70C. Regressions of disease severity corrected for multiple infection, according to the methods of Baker 1978, plotted against log inoculum density were used to determine the zoospore concentration which would induce a disease severity level of approximately 50%. Five replicates of each concentration were tested. This experiment was repeated twice.

2.3.4 PRODUCTION OF BACTERIA

Each of the 32 bacterial isolates were grown on nutrient agar amended with 100 ug/ml rifampicin to produce spontaneous rifampicin - resistant strains. These resistant strains were used for all further testing. Bacterial suspensions for the bioassay were produced in nutrient broth culture incubated for 24 hours at 25 C on a rotating shaker. The optical density of bacterial cells was quantified with a spectrophotometer by measuring the absorbance at 640 nm and comparing to a standard curve determined by dilution plating.

2.3.5 IDENTIFICATION OF THE BACTERIA

A gram stain was performed on all isolates. The isolates were then further identified using the Biolog GN Log microplates and the microcomputer database. (Biolog Inc. Hayward CA, USA 94545)

2.3.6 PRELIMINARY TEST OF TEST TUBE BIOASSAY SYSTEM

The three bacterial isolates were chosen from the group of thirty - two to run a preliminary test of the bioassay system. These isolates , numbered 1, 2,and 3, were produced and quantified as described in 2.3.4. The cucumber plants were produced and the test tube system described in 2.3.2 was set

up. Immediately after the plants were set in the test tubes , the bacterial isolates were added to give a final concentration in the tubes of 10^6 cells/ml. Twenty four hours after the introduction of the bacteria , P.aphanidermatum was introduced to the test tubes to give a final concentration in the nutrient solution of 100 zoospores per ml. The plants were grown for 8 days following the inoculation with P.a. and then harvested. The roots were then dried for two days at 70C and the dry weight was determined. Five replicate plants were treated with each bacterial isolate alone and five replicate plants were treated with each bacterial isolate plus Pa.. Five non-inoculated plants and five plants inoculated with Pa. only were included as controls.

2.3.7 DETERMINATION OF OPTIMAL BACTERIAL CONCENTRATION FOR THE BIOASSAY

Bacterial isolate number 2 , the best performing isolate in the preliminary test, was used to determine the optimum bacterial concentration. The bioassay system described above was set up and bacterial isolate 2 was immediately added to the test tubes to give final concentrations of 10^2, 10^4, 10^6,or 10^8 cells/ml. Treatments receiving P.aphanidermatum were inoculated as in 2.3.5. Five replicate tubes of each bacterial concentration and five replicate tubes of each bacterial concentration with 100 zoospores /ml of P.a. were tested. Five

non-inoculated plants and five plants inoculated with P.a. only were included as controls. The plants were grown for 8 days and then harvested and root dry weights were determined.

2.3.8 PLANT BIOASSAY - SCREENING OF POTENTIAL BIOCONTROL BACTERIA

The bioassay system previously described was set up. Bacterial suspensions were added to the test tubes to give a final concentration of 10^6 cells /ml. Treatments receiving P.a. were inoculated to give a final concentration in the test tubes of 100 zoospores /ml. Each of the thirty-two bacterial isolates were evaluated in five replicate test tubes with P.a. and in 5 replicate tubes without the P.a. Due to the large number of isolates tested , the screening process was conducted in a number of runs . Controls consisting of a blank treatment (no bacteria, no Pythium) and a Pythium treatment (no bacteria) were included in each run. The plants were grown for 8 days following the inoculation with P.a. and then harvested. Root lengths were determined by the line intersect method of Newman (1966). All isolates were evaluated twice.

2.3.9 BACTERIAL COLONIZATION OF CUCUMBER ROOTS

Bacteria that resulted in root lengths of inoculated plants that were equal or greater to 80% of the non-inoculated

control (no Pythium, no bacteria) were tested for their ability to colonize the roots of young cucumber plants.

Cucumber seeds (c.v. Corona) were set up in the test tube system described in 2.3.2. After the seeds were secured in place , bacteria were added to the test tubes to give a final concentration of 10^6 cells/ml. The plants were grown for 4 days on a rotary shaker at 80 RPM and depleted nutrient solution was replaced with SDW.

The plants were harvested and the shoots were discarded. The roots were placed in 125 mm X 16 mm diameter test tubes containing 9 ml SDW and were vortexed for 20 seconds. The roots were then blotted on sterile paper towel to remove excess water and placed on nutrient agar amended with rifampicin (100 ug/ml). The plates were incubated for 48 hours at 26 C. The total root length was determined by the line intersect method and the extent of bacterial colonization was determined by counting those intersections which contained bacterial colonies. A colonization index was derived by dividing the colonised root length by total root length. Each bacterium was tested in five replicate plants . A control treatment with no bacteria and a treatment with Pseudomonas putida N1R, a well documented colonizer (Dupler and Baker 1984) were also included. The experiment was repeated once.

2.3.10 PLANT BIOASSAY - EVALUATING THE BEST FIVE BACTERIAL ISOLATES WITH CUCUMBER C.V. CORONA

The top five performing bacterial isolates form the original plant bioassay using cucumber c.v. 'Straight 8' were retested using cucumber c.v. 'Corona' as the test plant. The bioassay system described in 2.3.8 was used and each isolate was tested in four replicated test tubes. Isolates were tested with and without Pythium as described in 2.3.8. Control treatments consisting of a blank (no bacteria, no Pythium) and a Pythium treatment (no bacteria) were also included. Upon termination of the experiment, bacterial concentrations in the nutrient solution were determined by dilution plating and the production of new zoospores was assayed using the millet baiting method described in 3.3.1.

2.3.11 STATISTICAL ANALYSIS

Data from the optimum inoculum density experiment were analyzed using a linear regression analysis. An F-test indicated that the data from the second and third trials of this experiment could be pooled , based on the homogeneity of variances . Data from the first trial were analyzed separately . Data from all other experiments were analyzed with ANOVA

protected Duncan's Multiple Range tests. Because of the large number of isolates tested in the plant bioassay, isolates were grouped into runs. F - tests showed no significant difference between the variances of the runs so the data were pooled. Data from the first and second (repeat) trials of the isolates were also pooled.

2.4 RESULTS

2.4.1 OPTIMUM INOCULUM DENSITY

Regression analysis and subsequent F- test of non-transformed data from all trials indicated that trial 1 data was significantly different from the other two trials and could not be pooled with them. Salt damage resulting in retarded growth of the some of the plants, most notably the non - inoculated blanks, and was believed to be a source of variance in this trial. As a result, the data from this trial was discarded and the pooled results of trial two and three were used to determine the 50% disease severity level. Disease severity was determined by calculating the average root dry weight from each inoculated treatment as a percentage of the average root dry weight of the control (no Pythium) treatment. Data was then corrected for multiple infections by the Van der Plank multiple infection correction :

$$\text{Disease Severity} = \text{Log n } (1/\ 1-y)$$

(Baker , 1978)

This resulted in a regression with the equation $Y = 0.04757\ X + 0.0763$ $R^2 = 71.90\%$ $(P=0.015)$. From the regression (Figure 2.1) an inoculum density of 100 zoospores/ml was selected for the bioassay.

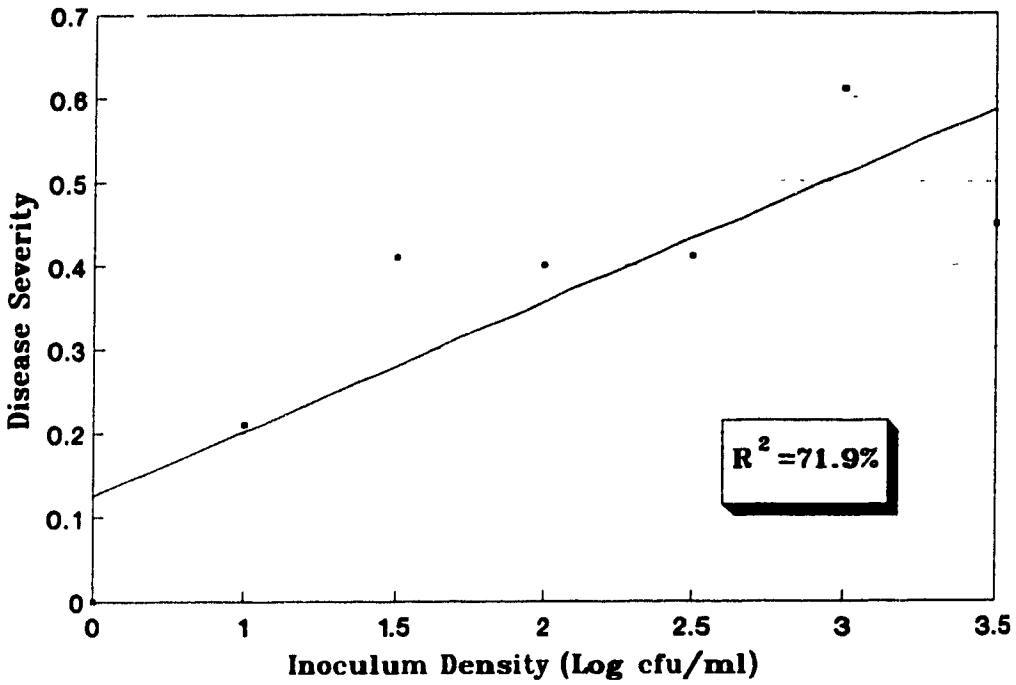

Figure 2.1

Regression of disease severity (corrected) against inoculum density of Pythium aphanidermatum

2.4.2 IDENTIFICATION OF BACTERIA

Twenty four isolates were positively identified (Table 2.4). With one exception, these were all species of Pseudomonas , predominantly P. corrugata or P. fluorescens subgroup B,C, or E. Some isolates could not be identified to the species level.

Table 2.1 Identification of bacteria isolated from the rhizosphere of Cucumis sativus var. Corona grown in Quebec soils [a]

ISOLATE	IDENTIFICATION
1	NI
2	Pseudomonas corrugata
3	NI
4	Pseudomonas corrugata
5	Pseudomonas sp.
6	Pseudomonas sp.
7	Pseudomonas corrugata
8	Pseudomonas corrugata
9	NI
10	Escherichia vulneris
11	Pseudomonas cichorii
13	Pseudomonas corrugata
14	Pseudomonas corrugata
15	Pseudomonas fluorescens subgroup C
16	Pseudomonas fluorescens subgroup C
17	NI
18	NI
19	Pseudomonas sp.
20	Pseudomonas sp.
21	Pseudomonas fluorescens subgroup C
22	Pseudomonas sp.
23	Pseudomonas corrugata
24	NI
25	Pseudomonas corrugata
26	NI
27	Pseudomonas fluorescens subgroup E
28	Pseudomonas corrugata
31	Pseudomonas corrugata
32	Pseudomonas corrugata
33	Pseudomonas corrugata
34	NI
35	Pseudomonas corrugata

[a] - Data previously reported in Paulitz et al. 1992.
NI - not identified

2.4.3 DEVELOPMENT OF TEST TUBE BIOASSAY SYSTEM

The most effective isolate, #2, of the three evaluated in a preliminary test (Table 2.2) was used to determine the optimum bacteria concentration for use in the bioassay. There was no significant difference (Table 2.3) in root dry weight of Pythium inoculated plants treated with 10^2, 10^4 or 10^6 cells/ml of the bacterial isolate. However, nutrient solution containing 10^8 cells/ml significantly reduced root dry weight over that of the blank . The greatest non-inhibitory bacterial suspension density 10^6 was chosen for the assay.

Table 2.2 Effect of Three Bacterial Isolates on the Root Dry Weight of Pythium Inoculated and Non-inoculated Plants

TREATMENT	ROOT DRY WEIGHT (g)
1	213.00 a*
1P	82.07 b
2	209.40 a
2P	142.80 ab
3	209.62 a
3P	72.00 b
BLANK	220.99 a
PYTHIUM	99.30 b

* Treatments followed by the same letter are not significantly different (P = 0.05)

The treatment identified as Pythium and numbered treatments followed by 'P' were inoculated with Pythium aphanidermatum

Table 2.3 Effect of Bacterial Concentration on Root Dry Weight of Pythium Inoculated and Non-inoculated Plants

TREATMENT	ROOT DRY WEIGHT (g)
10^2	321.80 a*
10^2 P	159.69 bc
10^4	304.60 a
10^4 P	212.00 b
10^6	335.60 a
10^6 P	161.20 bc
10^8	114.40 c
10^8 P	125.80 c
BLANK	306.40 a
PYTHIUM	106.60 c

* Treatments followed by the same letter are not significantly different (P = 0.05)

The treatment identified as Pythium and numbered treatments followed by 'P' were inoculated with Pythium aphanidermatum

2.4.4 PLANT BIOASSAY - SCREENING OF POTENTIAL BIOCONTROL BACTERIA

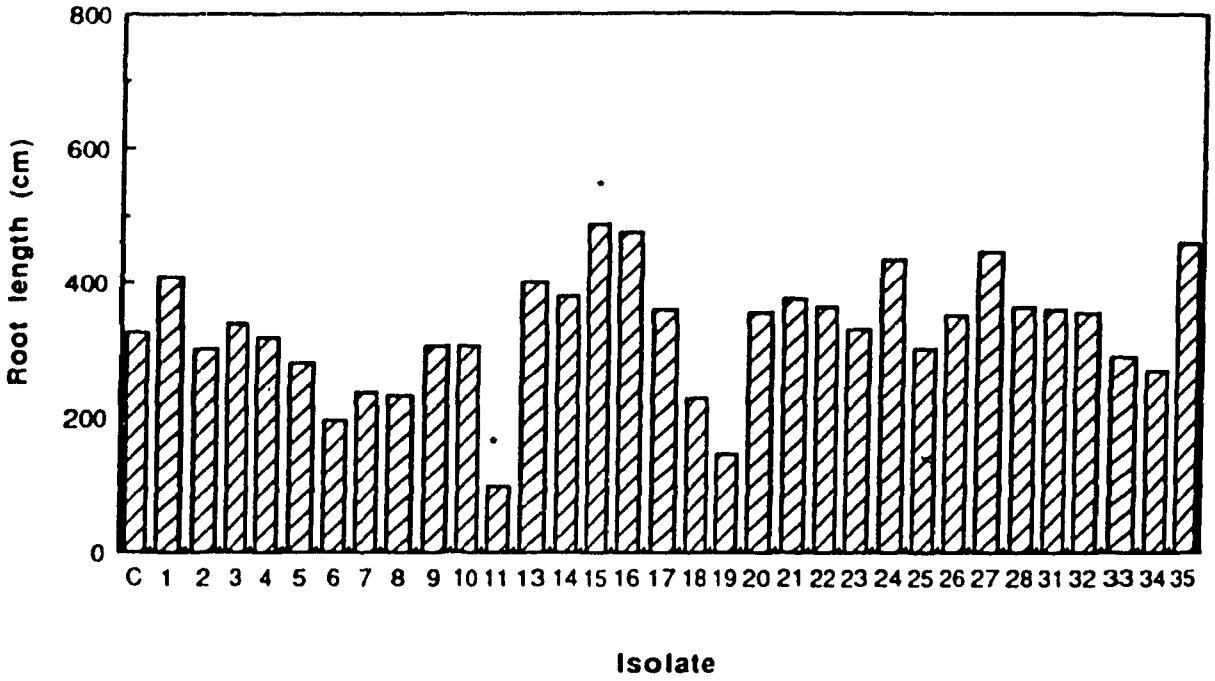

Figure 2.2

Effect of 32 bacterial isolates on the root length of cucumber grown in a nutrient solution infested with Pa.. Isolates marked * are significantly different from the Pa. inoculated control in a DMR test (P=0.05)

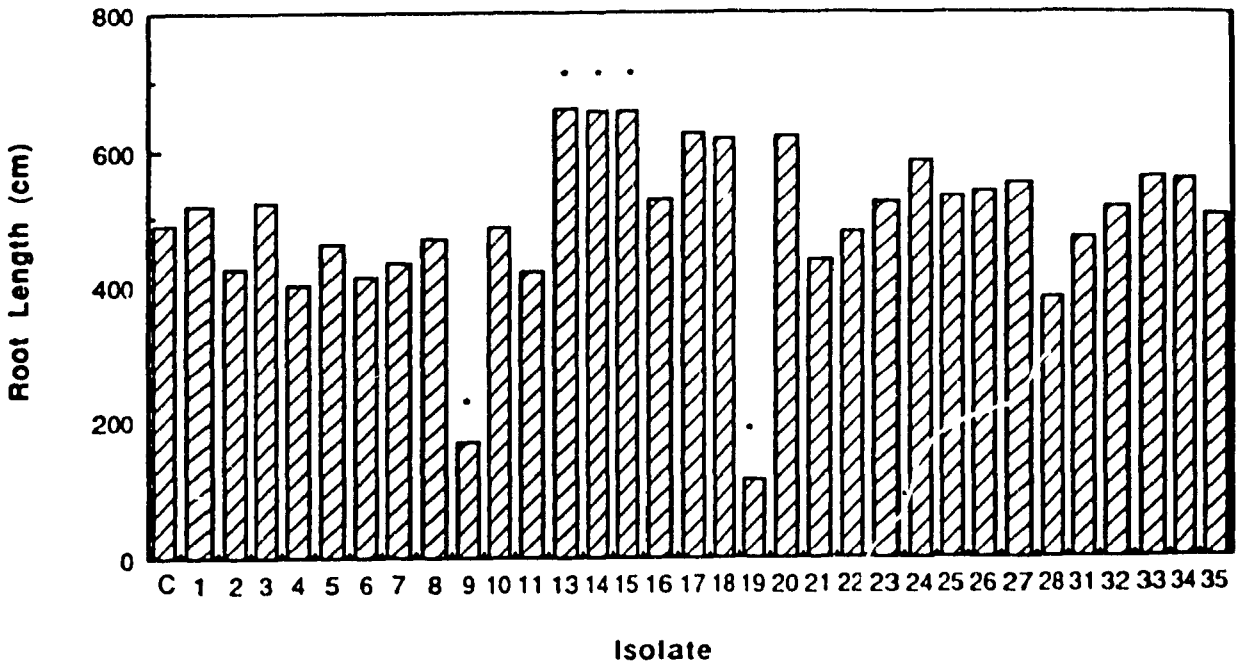

Figure 2.3

Effect of 32 bacterial isolates on the root length of cucumber grown in a nutrient solution . Isolates marked * are significantly different from the non-inoculated control in a DMR test (P=0.05)

2.4.5 PLANT BIOASSAY - EVALUATING THE BEST FIVE BACTERIAL ISOLATES WITH CUCUMBER C.V. CORONA

Table 2.4 Effect of Bacterial Isolates on Root Length of Pythium Inoculated and Non-inoculated Cucumber Seedlings c.v. Corona

TREATMENT	ROOT LENGTH (cm)
BLANK	951.30 a*
PYTHIUM	325.15 de
13	711.58 abcd
13 + P	282.13 e
15	794.63 ab
15 + P	368.75 cde
16	450.60 bcde
16 + P	328.08 de
27	699.80 abcd
27 + P	283.93 e
35	739.25 abc
35 + P	193.23 e

* Treatments followed by the same letter are not significantly different (P = 0.05)

The treatment identified as Pythium and numbered treatments followed by 'P' were inoculated with Pythium aphanidermatum

The bacteria concentration in the nutrient solution ranged from 10^4 to 10^5 cfu/ml in tubes which were inoculated with isolate 13 , 15, or 35 , both with and without Pythium. Isolates 16 and 27 were below detection level in some cases . However, bacterial concentrations ranging from 10^2 to 10^3 cfu/ml were recovered from some tubes. Zoopores were recovered in low levels from all treatments inoculated with Pythium except 15 + P.

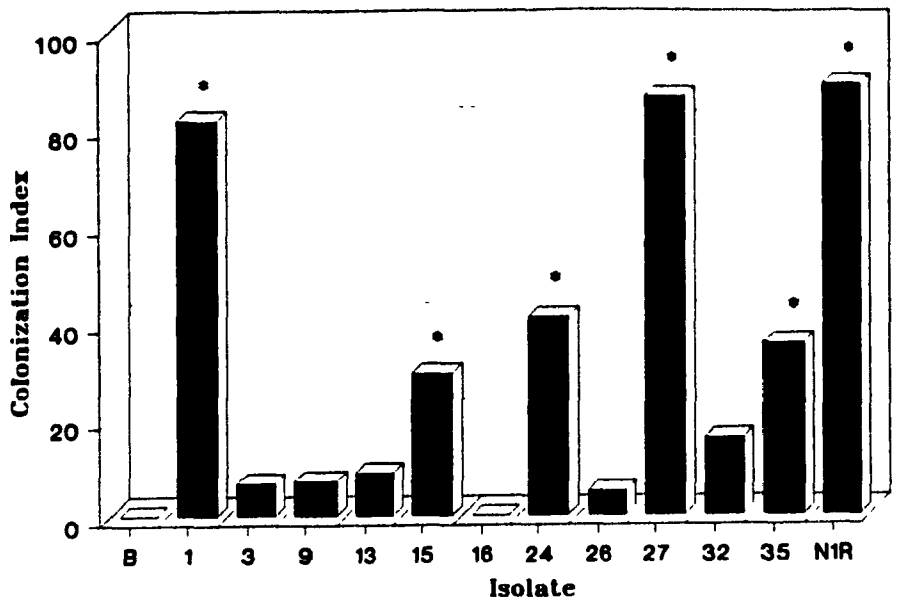

Figure 2.4 - Root colonization index values for bacterial isolates in colonization run 1. Isolates marked * are significantly different from a non-inoculated control (B)

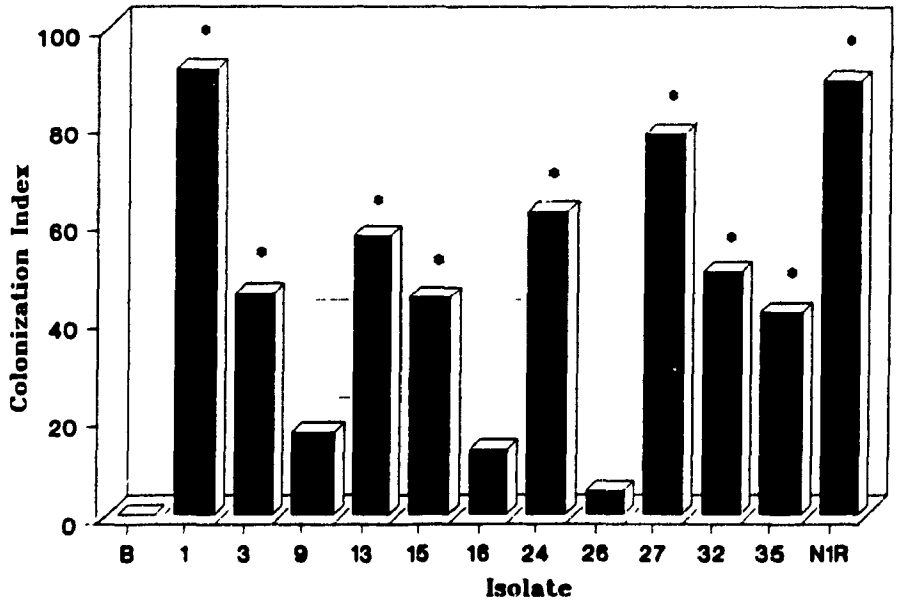

Figure 2.5 - Root colonization index values for bacterial isolates in colonization run 2. Isolates marked * are significantly different from a non-inoculated control (B)

2.5 DISCUSSION AND CONCLUSIONS

In preliminary tests to determine optimum inoculum density, non-transformed data yielded a weak regression. However when data was transformed using the multiple infection correction an R^2 of 71.9% was obtained. This would indicate that increasing the inoculum density increased the number of infections but not the overall severity of the symptoms. In other words, once the root is saturated with infections , additional inoculum does not increase the overall disease severity. The pathogenesis of Pythium in the young root is rapid under most conditions, yet in these studies 'misses' were frequently encountered both in the preliminary tests and in the bioassay itself. This may have resulted from an uneven distribution of zoospores in the inoculating solution , or contaminating microbes from the seed coat interfering with pathogenesis. It is possible that pregermination of the cucumber seeds in sterile distilled water allowed the populations of microorganisms indigenous to the seed coat to increase. Surface sterilization of the seeds was attempted in preliminary work but abandoned due to the harsh effects of the process on the emergent seedling.

In the bioassay, the root length of cucumber seedlings grown in nutrient solution infested with the Pythium was reduced to 67% of the root length of seedlings grown in the non infested control. Addition of eleven of the bacterial isolates ,

one day before addition of the Pythium, resulted in root lengths of 80-99% of the non-inoculated control. However only treatment with isolate 15 resulted in a significant increase in root length. Eight of these eleven isolates were effective root colonizers , having colonization indices which were not significantly different from Pseudomonas putida N1R.

The remaining 21 bacterial isolates produced marginal to no increase in root length. The lack of activity associated with the non-performing isolates is not surprising. Previous workers have demonstrated that while zoospore assays could be useful to select strains of bacteria with protectant properties against soil born pathogens (Lifshitz et al. 1986) , mycelial growth inhibition is not a useful predictor (Fravel 1988).

When non-inoculated cucumber plants were treated with the bacteria , three isolates (#13, 14 and 15) resulted in significantly greater plant root length (approx. 134%) than in the untreated control. Two other isolates (# 9, and 19) resulted in significantly decreased root growth compared to the untreated control.

The cucumber c.v. 'Straight 8' was used as the test plant in the bioassay. Straight 8 , a field cucumber cultivar , is in many respects more robust than the newer, gyonecious, greenhouse cultivars. Seed for this variety could be obtained for < $0.01 each while seed for the gynoecious hybrids used in commercial greenhouse production cost $0.50 each. A final test

of the five most effective bacterial isolates was conducted using seedling of the greenhouse cultivar Corona. Results presented in Table 2.4 show that under the assay conditions the severity of root damage by the Pythium was much greater relative to the control than in assays using Straight 8. It should also be noted that the top five performing bacteria failed to have any significant effect on the severity of the root rot in the Pythium inoculated treatments. While Straight 8 was chosen for purely economic reasons, it is likely that greenhouse house cultivars would be too susceptible to root rot under the assay conditions.

LITERATURE CITED

Baker 1978 The multiple infection correction ref ????

Broadbent, P., Baker K.F., and Waterworth, Y. 1971. Bacteria and actinomycetes antagonistic to fungal root pathogens in Australian soil. Aust. J. Biol. Sci. 24: 925-944.

Callan, N.W., Mathre D.E. and Miller J.B. 1990. Bio-priming seed treatment for biological control of Pythium ultimum preemergence damping-off in sh2 sweet corn. Plant Dis. 74:368-372.

Dupler,M. and Baker R. 1984. Survival of Pseudomonas putida , a biological control agent, in soil. Phytopatholgy 74: 195-200.

Fravel,D.R.,1988. Role of Antibiosis in the biocontrol of plant diseases. Ann. Rev. Phytopathol. 26: 75-91

Hadar, Y. Harman, G.E. Taylor, A.G.and Norton J.M. 1983. Effects of pregermination of Pea and cucumber seeds and of seed treatment with enterobacter cloacae on rots caused by Pythium spp. Phytopathol. 73:1322-1325.

Jenkins S.F. and Averre C.W., 1983. Root Diseases of Vegetables in Hydroponic Culture Systems in North Carolina Greenhouses. Pl. Dis. 67:968-970

Lifshitz, R., Simonson C., Scher F.M., Kloepper,J.W., Roderick-Semple C., and Zaleska I. 1986. Effect of rhizobacteria on the severity of phytopthora root rot of soybean. Can. J. Plant Pathol. 8: 102-106.

Newman E.I. 1966. A method of estimating the total length of root in a sample. J. Appl. Ecol. 3:139

Okamoto H. and Isaka M. 1989. Studies on biocontrol of plant diseases - control of cucumber disease caused by Phytophthora capsici by applied antagonistic bacteria. Bulletin of the Fukui Agricultural Experiment Station 1989. No. 14 33-44

Parke J.L. 1990. Population dynamics of Pseudiomonas cepacia in the pea spermosphere in relation to biological control of Pythium. Phytopathol. 80:1307-1311

Paulitz, T.C., Zhou, T., and Rankin, L. 1992. Biocontrol of Pythium on greenhouse cucumbers.(in press)

3 FURTHER CHARACTERIZATION OF EFFECTIVE BACTERIAL ISOLATES IN SHORT-TERM GROWTH BENCH STUDIES USING ROCKWOOL CUBES

3.1 ABSTRACT

The five best performing bacterial isolates from the cucumber bioassay described in chapter 2 were further evaluated in long-term studies using cucumber c.v. 'Corona' growing in rockwool cubes. The effect of the bacterial isolates on a variety of plant growth parameters was studied in both Pythium inoculated and non-inoculated plants. Significant effects of the Pythium were not seen in most of the growth parameters that were measured. Water uptake also showed little response to Pythium inoculation. A growth promotion effect was seen in some plant growth parameters when plants were treated with isolate 15 alone. In studies to assess the rhizosphere competence of the bacterial isolates, the two isolates 15 and 35 were found to maintain high population densities throughout a 7-week study and both were recovered from the rhizoplane and inside the root at the end of the 7 weeks.

3.2 INTRODUCTION

The three experiments reported in this section were designed to study in detail the effect of the five most6 effective bacterial isolates on plant growth parameters and root function of Pythium inoculated and non-inoculated plants. In the third experiment rhizosphere competence of the bacteria was also evaluated .

In the rockwool production system used in the greenhouse, the plant root system is too large and tangled to facilitate sampling and study without using a destructive harvest method. Because of this, a smaller more manageable system was designed using rockwool cubes which could easily be sampled to provide thorough and rapid evaluation of the root system and its function throughout the growing period.

In the first experiment , which was a preliminary test of the system, shoot and root dry weight were evaluated and populations of the pathogen and the control agent were also monitored .

The second experiment was a study to determine the effect of the Pythium and the potential biocontrol bacteria on root functional parameters. Pythium has been reported to cause an asymptomatic infection (Stanghellini and Kronland 1986) especially in the case of young plants where the infection may be a latent one until the plant undergoes some physiological stress , at which time the typical root rot symptoms appear

(Hendrix and Campbell 1973).

In the third experiment, the rhizosphere competence of the bacteria was evaluated. Rhizosphere competence has often been cited as one of the main requirements for a successful biocontrol agent for use against root infecting pathogens (Baker and Scher 1987). Natural inhabitants of the rhizosphere or introduced organisms which can establish and maintain high populations stand the best chance of disrupting pathogenesis. Control of this nature has been attributed to competition for infection sites (Mandeel and Baker 1991) , and nutrients (Elad and Chet 1987) or to antibiosis (Fravel 1988). Plant growth promotion through the production of phytohormones or nutrients has also been attributed to rhizosphere microbes. Growth promotion may give the plant an extra boost by allowing it extra reserves for defense against the infection or by allowing it to withstand physiological stresses better and thereby be less susceptible (Kloepper et al. 1991a) . Failures of biocontrol in field situations have often been attributed to low competence of the control agent . As reported in Chapter two, the top performing bacterial isolates were found to have at least a partial ability to colonise the root . The experiment reported here is an attempt to further characterise this ability.

3.3 MATERIALS AND METHODS

3.3.1 DEVELOPMENT OF MILLET BAITING METHOD TO DETERMINE ZOOSPORE POPULATION DENSITIES IN INOCULATED TREATMENTS

The purpose of this experiment was to develop a rapid method of detecting low zoospore numbers in inoculated treatments. Zoospores of P. aphanidermatum were produced according to the methods described in section 2.3.1 The zoospores were added to 20 ml test tubes containing 9 ml SDW and 20 pearl millet seeds. The test tubes were incubated for 24 hours in a dark, 26 C incubator. The millet seeds were then placed on Pythium selective media (Mircetich and Kraft 1973) and returned to the incubator for a further 24 hours. Following the second incubation, the number of infected seeds was determined. Zoospore concentrations of 0, 1, 3, 10, 30, 100, and 300 zoospores/ml were tested. There were four replicate tubes per concentration and the experiment was repeated three times. Zoospore concentration was plotted against number of seeds infected to establish a standard curve for later use in monitoring zoospore levels in the growth bench and greenhouse experiments.

3.3.2 PRODUCTION OF CUCUMBER PLANTS

Cucumber c.v. 'Corona' was seeded into plug trays containing granular rockwool media (Pargro) and grown for 21 days on a 26 C growth bench with a 16 h of light (combined incandescent-fluorescent) per day. The plants were then set into 10 X 10 X 6.5 cm rockwool cubes (Pargro) which had been previously saturated with Peter's 10-52-10 (2 g/L). During the course of the experiment all plants were watered daily to saturation with either tap water or 10-52-10 (2 g/L) on alternate days.

3.3.3 GROWTH BENCH EXPERIMENT 1 - EFFECT OF PYTHIUM AND BACTERIA ON PLANT GROWTH PARAMETERS

The rockwool cubes were dried at 70 C for 4 days and dry weighs were determined prior to setting the plants into the cubes. The cubes were then saturated with 10-52-10 fertilizer solution and cucumber plants produced as described in 3.3.2 were transplanted into the cubes. Forty-eight hours after transplanting, the bacteria were applied by watering the cubes with 100 ml of a 10^6 cells/ml bacterial suspension. Forty eight hours later a solution containing 500 zoospores/ml of P.aphanidermatum was applied at the rate of 100 ml/cube. Plants were watered with 10-52-10 fertilizer solution (2 g/L) at every second watering . Pythium and bacteria populations were sampled during the growing period using the millet baiting method described in section 3.3.1 and dilution plating respectively. At the end of the 4 weeks Pythium populations

were sampled a second time. The number of leaves per plant were counted and the shoots were harvested and dried for 2 days at 70 C . The cubes containing the roots were dried for six days at 70 C.

Experimental design was a randomized complete block with five replicates and 12 treatments. Treatments were as follows; bacteria isolates 13,15,16,27 or 35 alone or in combination with P.a. , and two control treatments: P.a. alone (no bacteria) and a blank (no Pythium, no bacteria).

3.3.4 GROWTH BENCH EXPERIMENT 2 - EFFECT OF PYTHIUM AND BACTERIA ON ROOT FUNCTION

This experiment was designed to study the effect of the potential biocontrol bacteria on Pythium root rot as determined by measuring plant functional parameters.

Cucumber plants were produced and transplanted to the cubes as described in 3.3.1. The cubes were placed in 250 ml weighing boats and returned to the growth bench. Starting at transplanting the plants were fertilized at every second watering with 10-52-10 fertilizer solution (2 g/L). Five days after transplanting the bacteria were applied to the cubes at the rate of 200 ml/cube (10^6 cfu/ml) and 48 hours later a solution containing 500 zoospores/ml of P. aphanidermatum was applied at the rate of 100 ml/cube. Twenty-four hours later the cubes were flooded to run-off and the run-off was

discarded. The first water uptake measurement was taken 48 hours later. Water deficit was determined by applying a known volume of water or fertilizer solution and measuring the run-off. This was done every 48 hours to provide a total of 9 measurements. Upon termination of the experiment the shoots were harvested and dried for 4 days at 70 C and the weights were recorded.

Experimental design was a randomized complete block with 4 replicates and 12 treatments. Treatments were as follows; bacteria isolates 13,15,16,27 or 35 alone ,or in combination with Pythium, and two control treatments; Pythium alone (no bacteria) and a blank (no Pythium no bacteria). The experiment was repeated once. Isolate 16 was not included in the repeat of this experiment due to failure of the culture to grow at that time.

3.3.5 EVALUATION OF RHIZOSPHERE COMPETENCE OF BACTERIAL ISOLATES AND FURTHER STUDIES ON THE EFFECT OF PYTHIUM AND BACTERIAL ISOLATES ON PLANT GROWTH PARAMETERS

Cucumber plants were produced and set into the rockwool cubes as described in section 3.3.2. The plants were treated with the bacteria and inoculated with the Pythium as described in 3.3.3. Two weeks after the application of the bacteria to the cubes, bacterial populations on the newest roots were

monitored by sampling 3 cm root tips as they emerged from the base of the cube. Sampling was done such that each root tip was composed of a section which was growing on the cube surface and a section which was growing inside the cube. Five root tips were taken per cube . The root tips were then placed in 9 ml SDW and sonicated for 30 seconds. The suspension was then vortexed and the resulting dilution was plated on nutrient agar amended with 100 ug/ml rifampicin and 200 ug cyclohexamide. The plates were incubated at 26 C for 48 hours and the colonies were counted. The rockwool cubes were sampled at 2, 3, 5, and 7 weeks after the application of the bacteria. The experiment was terminated at 7 weeks after the application of the bacteria. At that time a sample of old roots was also taken. These samples were treated as previously described . The basal stems of the plants in trial 1 were also evaluated for the presence of the bacteria. This was done by surface sterilizing a 5-cm stem sample in 10% sodium hypochlorite solution for 1 minute. The stem sections were rinsed three times in SDW , aseptically split lengthwise and printed on the culture media previously described. Following removal of the stem sections the following plant growth parameters were evaluated: plant height, number of leaves, leaf area (determined with Leco Image Analyzer , Leco Systems, Longueil Que.) , plant dry weight , number of cucumbers and weight of cucumbers. A root length index was determined for each plant by placing a transparent grid over the four sides

of the cube and counting the number of root intersects. Water usage was recorded for the last two watering periods in each trial. Pythium populations were also determined . Experimental design was a randomized complete block and the experiment was repeated twice. There were three replicate cubes of each treatment in trial 1 and four replicates in trial 2.

3.3.6 STATISTICAL ANALYSIS

Data from all experiments were analyzed ANOVA protected Duncan's Multiple Range tests. The non parametric Cochran Q test was used to analyze differences in mortality between the Pythium inoculated bacteria treatments and the pythium blank.

3.4 RESULTS

3.4.1 MILLET BAITING TO DETERMINE ZOOSPORE POPULATION DENSITIES IN INOCULATED TREATMENTS

The number of seeds colonized at each inoculum density in trials 1, 2, and 3, was similar (Figure 3.1). The results of these trials were used in subsequent studies to determine zoospore population densities.

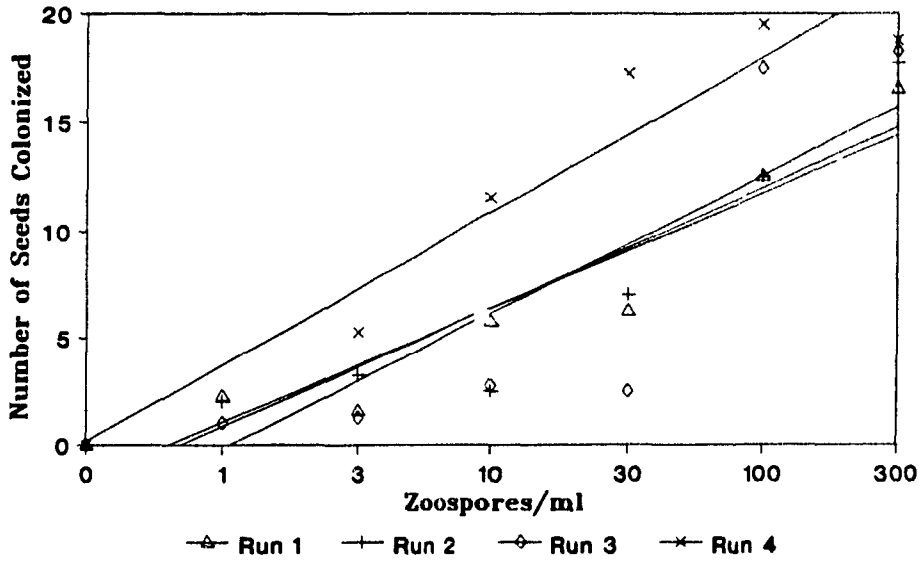

Figure 3.1 The number of millet seeds colonized in 24 hours by P.aphanidermatum at various inoculum densities

3.4.2 EFFECTS OF PYTHIUM AND BACTERIA ON PLANT GROWTH PARAMETERS

No significant difference (P=0.05) was found between any of

the treatments for either root or shoot dry weight. The coefficients of variation for these two parameters were 46.2% and 37.8% respectively. All Pythium inoculated bacterial treatments resulted in leaf numbers (Table 3.2) which were intermediate between the Pythium (alone) control and the non inoculated control. Plant survival in the Pythium inoculated bacteria treatments was not significantly different (P=0.05) from the Pythium blank (Table 3.1). Pythium was recovered on at least one sampling date from all plants treated with isolate 16 + Pythium , from 4/5 plants treated with either isolate 35, 13 or 27 + Pythium and from 3/5 plants treated with isolate 15 + Pythium. Pythium was also recovered from 4/5 plants treated with Pythium alone but not recovered from the blank or any of the treatments with the bacteria alone.

Table 3.1 Plant Survival in Pythium Inoculated Treatments Four Weeks After Inoculation with P. aphanidermatum at 100 ml/cube (500 cfu/ml)

Treatment	Plant Survival
Pythium	2/5
35 + Pythium	4/5
13 + Pythium	2/5
15 + Pythium	3/5
16 + Pythium	3/5
27 + Pythium	3/5

Table 3.2 Effect of Rhizobacteria and P. aphanidermatum on the Number of Leaves per Plant at Seven Weeks after Emergence

Treatment	Number of Leaves
Blank	6.2 b*
Pythium	4.2 c
35	5.8 abc
35 + Pythium	5.0 abc
13	5.6 abc
13 + Pythium	4.4 bc
15	6.4 a
15 + Pythium	5.2 abc
16	6.0 abc
16 + Pythium	5.2 abc
27	6.6 a
27 + Pythium	5.8 abc

* Treatments followed by the same letter are not significantly different (P=0.05)

Table 3.3 Population Densities of Bacteria In Rockwool Cubes Three Weeks After Application

Treatment	Blocks [a]	Bacteria X 10^b
Blank	0	-
Pythium	0	-
35	5	40.4
35 + P	4	5.1
13	4	2.2
13 + P	3	2.8
15	5	3.2
15 + P	4	3.2
16	4	2.4
16 + P	4	1.3
27	3	1.8
27 + P	2	4.1

a - number of blocks from which greater than 10^5 bacteria per g dry weight of rockwool was recovered
b - cfu/g dry weight of sample containing rockwool and roots

3.4.3 GROWTH BENCH EXPERIMENT 2 - EFFECT OF PYTHIUM ON ROOT FUNCTION

There was no significant difference (P=0.05) in shoot dry weight between any of the treatments in either trial. The total water usage and the volume of water used during the final measurement period also were not significant in trial 1. In trial 2 the difference in total water usage (Table 3.4) between the non-inoculated blank and the Pythium treatments was not significant. Treatments with bacteria plus Pythium were intermediate between the Pythium and the blank. When the volume of water used on the last measurement date was compared , the Pythium treatment used significantly less water than the blank control. Treatments with bacterial isolates 35, 27 or 15 plus Pythium resulted in intermediate water usage on this date. However treatment with isolate 13 plus Pythium was not statistically different from the Pythium control.

Table 3.4 Trial 2 - Average[z] Total Water Usage (ml) over Four Weeks and Average[z] Volume Used During Final Measurement Period per Plant

Treatment	Total Volume Used Over 4 Weeks	Volume Used At Last Measurement
Blank	1802.25 abc*	218.25 abc
Pythium	1631.00 c	172.75 d
35	1838.75 ab	218.25 abc
35 + P	1704.50 bc	185.25 cd
15	184/.00 ab	237.50 a
15 + P	1786.62 abc	194.20 bcd
27	1846.75 ab	223.75 abc
27 + P	1790.25 abc	199.00 abcd
13	1901.75 a	227.25 ab
13 + P	1654.50 c	175.25 d

* Treatments followed by the same letter are not significantly different (P=0.05)
z- average of four replicate plants

3.4.4 GROWTH BENCH EXPERIMENT 3 - FURTHER STUDIES ON THE EFFECT OF PYTHIUM AND BACTERIAL ISOLATES ON PLANT GROWTH PARAMETERS

Water usage and the number and weight of immature cucumbers did not significantly differ amoung treatments in either trial . Little difference was also noted in the majority of parameters presented in Table 3.5. In trial 1 the plants treated with 15+P were significantly taller than those treated with 13+P but in trial 2 this trend was reversed. In trial 1, 16+P produced significantly more leaves than 13+P although neither were significantly different from either the blank or the Pythium control. The relative performance of 16+P and 13+P was reversed in trial 2. Isolate 35+P produced significantly

more leaves than the Pythium control in trial 2. Leaf area and plant dry weight were not significantly different in trial 1. However in trial 2, the blank control produced significantly more leaves than the Pythium treatment. Treatments with bacteria 35, 15 16 or 27 plus P were intermediate while 15+P was not significantly different from the Pythium control. 15+P significantly more roots than the Pythium control in trial 1 intermediate between Pythium and non-inoculated controls in trial 2. In trial 2, 15 produced the greatest number of roots while in trial 1 it was 15+P.

Table 3.5 Comparrison of Plant Growth Parameters Measured at 7 Weeks After the Application of Rhizobacteria in Trial 1 and Trial 2 of Experiment 3

	Plant Height (cm)		Leaf Number		Leaf Area (cm^2)		Dry Weight (g)		Root Index [a]	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Blank	27.7 ab	38.5 abc	9.3 ab	9.5 ab	710.7 a	799.7 a	2.91 a	3.81 ab	291 ab	442 ab
Pythium	28.0 ab	31.3 bc	9.3 ab	8.5 b	627.0 a	531.4 b	3.01 a	3.02 ab	162 b	210 b
35	29.7 ab	34.3 abc	9.0 ab	9.7 ab	693.5 a	719.5 ab	3.25 a	3.27 ab	280 ab	429 ab
35 + P	27.7 ab	34.8 abc	8.3 ab	10.5 a	690.7 a	647.7 ab	3.49 a	3.47 ab	304 ab	275 ab
15	31.0 ab	38.5 abc	9.0 ab	10.5 a	674.4 a	736.3 ab	3.44 a	4.17 ab	433 ab	551 a
15 + P	36.0 a	32.0 bc	9.5 ab	9.5 ab	685.0 a	537.1 b	3.62 a	2.95 b	487 a	283 ab
16	35.0 ab	39.3 ab	9.7 a	10.5 a	685.5 a	712.0 ab	3.53 a	3.80 ab	513 a	399 ab
16 + P	34.3 ab	39.0 ab	10.0 a	9.3 ab	815.3 a	622.4 ab	3.60 a	3.61 ab	316 ab	458 ab
27	29.7 ab	43.7 a	9.3 ab	10.5 a	728.2 a	804.3 a	3.30 a	4.38 a	373 ab	485 ab
27 + P	29.7 ab	32.7 bc	9.0 ab	9.8 ab	531.2 a	700.5 ab	3.12 a	3.71 ab	258 ab	266 ab
13	31.0 ab	29.3 c	9.0 ab	8.8 ab	722.7 a	540.1 b	3.48 a	2.85 b	378 ab	261 ab
13 + P	24.0 b	37.5 abc	7.3 b	9.5 ab	530.1 a	707.2 ab	2.70 a	4.26 ab	255 ab	476 ab

* Treatments followed by the same letter are not significantly different (P=0.05)

a - Root index was determined for each plant by placing a transparent grid over the four sides of the cube and counting the number of root intersects.

3.4.5 GROWTH BENCH EXPERIMENT 3 - EVALUATION OF RHIZOSPHERE COMPETENCE OF BACTERIAL ISOLATES

Isolates 35 and 15 were consistently recovered from the new roots of both Pythium inoculated and non-inoculated plants in both trials at each sampling period. Population densities exceeding 1 X 10^7 cfu/g (dry weight) were present on the new roots in more than half the samples taken three weeks after the application of the bacteria. Equivalent population densities were still recovered from new roots in some samples at five weeks although the population had dropped in many samples. At seven weeks (Table 3.6), population densities on the new roots were below the detection threshold in many cases for both isolates. Population densities of isolate 35 exceeded 10^5 cfu/g (dry weight) root material in the older roots. Recovery of isolate 15 was more sporadic but the population densities in the positive samples also exceeded 1 X 10^5 cfu/g. Isolate 13 and 16 were recovered at the three-week sampling period from the new roots of plants not inoculated with Pythium. Population densities of isolate 16 exceeding 1 X 10^7 cfu/g were recovered from one plant in each trial while isolate 13 was recovered from one plant in trial 1 and two in trial 2. By the five-week sampling period, isolate 16 was below the detection threshold and isolate 13 was only recovered from one plant in trial 2. Isolate 27 was only recovered once (Table 3.6).

Table 3.6 Recovery of the Five Best Performing Bacterial Isolates Seven Weeks After Application to the Rockwool Cubes

Treatment	Trial 1			Trial 2	
	Basal Stem	Roots		Roots	
		New	Old	New	Old
Blank	0/3[a] (-)[b]	-[c]	-	-	-
Pythium	0/3 (-)	-	-	-	-
35	2/3 (2)	1/3[d]	2/3	1/4	3/4
35 + P	3/3 (3)	1/3	1/3	1/4	4/4
15	1/3 (3)	3/3	-	-	2/4
15 + P	0/3 (-)	-	-	-	-
16	2/3 (1)	-	-	-	3/4
16 + P	0/3 (-)	-	-	-	-
27	1/3 (3)	-	-	-	1/4
27 + P	1/3 (3)	-	-	-	-
13	1/2 (1)	-	-	-	-
13 + P	3/3 (2)	-	-	1/4	-

a - number of replicates from which a positive print was obtained
b - colony density in print area (1)- 1-5 colonies ,(2)- >5 colonies but colonies still distinct, (3)- solid smear of bacteria in print area.
c - bacteria not recovered
d - bacteria recovered in population densities greater than 1×10^5

3.5 DISCUSSION

Experiments 3.4.2, 3.4.3 and the plant growth parameter studies in 3.4.4 were designed to study the effects of Pythium and the bacteria in a manageable system. It was hoped that some parameter would consistently show a measurable reaction to Pythium so that an assay could be developed for future use in efficacy screening of potential biocontrol agents. It was evident from the variety of parameters measured in these trials that Pythium has no consistent, measurable effect on the shoots of plants of that age growing in rockwool, although differences in leaf number were noted in some trials. Measurable effects were also seen on the final water uptake and root indices, but these too were inconsistent. The total volume of water used and water usage on the last date of the trials in experiments 3.4.3 and the last date of the trials in experiment 3.4.4 would be expected to be a parameter indicative of root function. Although some root necrosis was evident in Pythium inoculated plants in both experiments the water uptake ability of the root was apparently unaffected.

Possibly the disease didn't have time to develop. Evans (1977) reports an 8-10 week lag time between inoculation and disease development. It is also feasible that the infections occurred but no recognisable symptoms were produced , similar to the effect reported by (Stanghellini and Kronland 1986).

PAGINATION ERROR.

TEXT COMPLETE.

ERREUR DE PAGINATION.

LE TEXTE EST COMPLET.

However the most likely explanation is that plants growing in such a uniform environment received very little stress. Davies (1980) reports stress is often a mitigating factor governing symptom expression in Pythium infected plants. Plants in all the experiments were grown on a growth bench which was under automatic temperature and photoperiod regulation, diurnal variation in temperature was virtually nonexistent. This provides an ideal environment for plant growth.

Many reports cite Pythium as causing damping off both, pre-emergence and in the 1-2 weeks following emergence. Pythium diseases occurring at or near the time of fruit production, a time of high plant stress, have also been reported both in the field and hydroponic production. But there are few if any reports dealing with Pythium alone (not as part of a complex) causing symptoms or an "early death" in unstressed herbaceous plants during the active vegetative growth phase . Mellano et al.(1970a, 1970b) reported that snapdragons (Antirrhinum majus) were susceptible to Pythium until they were about 15 days old , at which time a tolerance to the pathogen developed. It is likely that cucumber plants at this stage also have a reduced susceptibility to Pythium and that, coupled with the very stable environmental conditions, resulted in a lack of disease development in these experiments. Failure of the pathogen to establish was ruled out, due to consistent recovery during the experiments.

More promising results were obtained in the final experiments

on rhizosphere competence. The two bacterial isolates 15 and 35, which were found in section 2.4.5 to have good short term colonisation ability, also proved to have good long-term colonisation ability. According to Weller (1988) this would make them excellent candidates for biocontrol agent. He lists colonisation ability among the most important factors. Isolate 16 which had a poor colonisation ability in 2.4.5 was also a poor colonizer in the long-term tests. Isolate 13 was a variable colonizer in the short term and was found to be poor in the long term. Of note is isolate 27, which was among the top colonizers relative to N1R in the short term tests. It exhibited a lack of tenacity on the new roots in the long term tests. However this isolate was recovered in high numbers from internal stem prints in 2/6 stems. It is possible that isolate 27 does not move well on the root surface but rather is an endorhizosphere dweller. The movement of Pseudomonas spp. into the endorhizosphere was reported by Kloepper et al. (1991b) Isolate 35 was recovered from 5/6 stems and also had high population densities on new roots throughout the study period and on both old and new roots at 7 weeks. This is indicative of a good distribution in the root system, considered critical by (Weller, 1988), and also a high degree of movement in the rhizosphere .

Isolate 15 was also recovered from 1/6 stems and had high, although inconsistent, populations on old roots at 7-weeks and new roots throughout the experiment. Isolate 15 again

exhibited some growth promotion effects when plant growth parameters were measured. Leaf number and root index exceeded that of the blank treatment (although not significantly) in trials 1 and 2 and trial 2 respectively. A significantly greater number of leaves were produced by treatments receiving isolate 15 alone in trial 3.4.2 than in the blank of the same trial.

LITERATURE CITED

Becker, R. and Scher, F. 1987. Enhancing the activity of biological control agents. In " Innovative Approaches to Plant Disease Control". Chet, I. (ed) John Wiley and Sons N.Y.

Davies J.M.L. 1980. Disease Problems Encountered in Commercially Grown Tomato Crops. Acta. Hortic. 98:299-305

Elad, Y. and Chet H., 1987. Possible Role of Competition for Nutrients in Biocontrol of Pythium Damping-Off by Bacteria. Phytopathology 77:190-195

Evans, S.G. 1977. Disease risks affect the entire crop in NFT systems. The Grower 15: 1233-1239

Fravel, D.R., 1988. Role of antibiosis in the biocontrol of plant diseases. Ann. Rev. Phytopath. 26:75-91

Hendrix F.F. and Campbell W.A. 1973. Pythiums as Plant Pathogens. Ann. Rev. of Phytopathology 11:77-99

Kloepper, J.W., Zablotowivz, R.M., Tipping, E.M. and Lifshitz, R. 1991a. Plant growth promotion mediated by bacterial rhizosphere colonisers. p 315-326 in " The Rhizosphere and Plant Growth" Keister,D. L. and Cregan P.B. (eds), Kluwer Academic Publishers, Dordecht

Kloepper, J.W., Wei, G., and Tuzan, S. 1991b. Rhizosphere population dynamics and internal colonization by plant-growth promoting rhizobacteria which induce systemic resistance to Colletotrichum orbiculare. pg.24 in: "Biological Control of Plant Diseases: Progress and Challenges for the Future", proceedings of a NATO Advanced Research Workshop, Cape Sounion, Greece, May 19-24, 1991.

Mandeel,Q. and Baker, R. 1991. Mechanisms involved in biological control of Fusarium wilt of cucumber with strains of nonpathogenic Fusarium oxysporium. Phytopathology 81: 462-469.

Mellano, H.M. , Munnecke, D.E. and Endo, R.M. 1970a. Relationship of seedling age to development of Pythium ultimum on roots of Antirrhinum majus. Phytopathology 60: 935-942

Mellano, H.M. Munnecke, D.E. and Sims, J.J. 1970b. Relationship of pectic enzyme activity and presence of sterols to pathogenesis of Pythium ultimum on roots of Antirrhinum majus. Phytopathology 60: 943-950

Mircetich S.M. and Kraft O.M. 1973. Efficiency of various selective media in determining Pythium populations in soil. Mycopathol. Mycol. Appl. 50:151-161

Stanghellini M.E. and Kronland W.C. 1986. Yield loss in hydroponically grown lettuce attributed to subclinical infection of feeder rootlets by Pythium dissoticum. Plant Disease 70: 1053-1056.

Weller, D.M. 1988. Biological control of soil born plant pathogens in the rhizosphere with bacteria. Ann. Rev. Phytopahtol. 26: 379-407

4 GREENHOUSE EXPERIMENTS

4.1 ABSTRACT

Two isolates of Pseudomonas corrugata (Pc13 or 35) and three isolates of P. fluorescens (Pf.15, 16 or 27) were evaluated for their ability to control pythium root rot of European cucumbers (Cucumis sativus L. cv. 'Corona') grown in a rockwool hydroponic system . All five isolates were evaluated in both a spring and early fall crop in 1991. Three of the isolates Pc13, Pc35 and Pf15 were also evaluated in a spring crop in 1992. In all crops seven week old plants were set onto rockwool slabs and treated with water or 200 (10^6 cells/ml) of one of the bacterial isolates. Half the plants were inoculated with 10,000 zoospores of Pythium aphanidermatum (Pa) strain 186; 6 days after the bacterial introduction in the 1991 crops and at three inoculation times in the 1992 crop. In the spring 1991 crop , Pa-inoculated plants treated with the Pc13 or Pf15 produced fruit yields equal to 92 and 74% respectively of the control (no Pa , no bacteria). Pa-inoculated plants without bacteria yielded only 46% of the control. In the fall crop, Pa-inoculated plants treated with Pc13 or Pf15 yielded 52 and 47% of the control compared to Pa-only treatment, which yielded 12.5% of the control. In both crops treatment with any of the bacterial isolates resulted in significantly reduced cull rates compared to the Pa-only treatment. In the fall

crop, treatment with Pf15 alone (no Pa) increased shoot dry weight compared to the non-treated control. Disease development in the 1992 crop was much less than in the previous two crops resulting in a much less noticeable effect of the bacterial treatments in Pa-inoculated plants. Treatment with Pf15 (alone) resulted in significantly greater fruit production than the control.

4.2 INTRODUCTION

European cucumber (Cucumis sativus L.), is the second most prevalent greenhouse vegetable crop grown in Canada and much of the production is hydroponic. The market value of the crop for 1990-91 was $29 million. Root rot caused by Pythium spp. is a significant limiting factor in the production of cucumbers in Quebec (Carrier 1990) and has been reported in commercial greenhouses in British Columbia (Favrin et al. 1988). Currently there are no chemicals registered for the control of Pythium spp. in mature cucumber plants. Nor are there likely to be in the future due to phytotoxicity problems associated with the use of chemicals in the hydroponic system (Price and Dickenson 1980, Price and Fox 1986) and the lack of financial incentive to pursue their development (Zinnen 1988). Continual exclusion of the pathogen, which is difficult to accomplish, is the only recommended control measure at present. Cultural controls have been investigated (Moyhuddin 1985, Goldberg et al. 1991) and results show promise. Biological control is another valid alternative which has been investigated by other researchers (Goldberg and Stanghellini 1991) although success to date has not been great. The experiments presented in this chapter represent the final greenhouse testing of five bacterial isolates selected by in vitro screening (Paulitz et al. 1992) and plant bioassays (Chapter 2 and 3). Conclusions are drawn as to their potential value for use in commercial cucumber production.

4.3 MATERIALS AND METHODS

4.3.1 PRODUCTION OF CUCUMBER SEEDLINGS

Cucumber c.v. 'Corona' was seeded into plug trays containing granular rockwool medium (Pargro) and grown for 21 days on a 26 C growth bench with 16 h of light (combined incandescent-fluorescent) per day. The plants were then set into 10 X 10 X 6.5 cm rockwool cubes (Pargro) which had been previously saturated with Peter's 10-52-10 (2 g/L). The plants were grown in the cubes for four weeks and were watered daily to saturation with either tap water or 10-52-10 (2 g/L) on alternate days.

This method was used to produce seedlings for all greenhouse experiments.

4.3.2 GREENHOUSE EXPERIMENT 1 : APRIL - JUNE 1991

The greenhouse used for all the experiments was a 30 X 5.5 m quonset house ,covered with clear plastic. Random fibre rockwool slabs (Pargro) measuring 100 X 6.5 X 20 cm were laid out in a double row configuration with the slabs butted together in the row. The slabs were soaked with nutrient solution (1.26 g/L Hydrosol + 0.83 g/L $CaNO_3$) for three days to adjust the pH to 6.5 before setting out the plants. On April 15, two rockwool cubes were placed on eacn slab. The

centre of each cube was set 25 cm from the centre of the slab to provide a spacing of 50 cm between plants in the row and a final spacing of 0.6 m^2 per plant.

During the cropping period the slabs were fertigated with Peter's Hydrosol and $CaNO_3$ at stock concentrations of 126 g/L and 83 g/L respectively. Stock solutions were diluted 1:100 at the point of delivery to the slabs with Dosmatic Plus liquid dispensers (J.F. Equipment Co., Dosmatic International, Lewisville Tx., 75067). Stock solution pH was adjusted periodically with phosphoric acid to produce an output pH of 6.5. The output cycle was 2 minutes per hour between 8 a.m and 4 p.m. , 11 p.m. to 12 p.m. and 4 a.m. to 5 a.m.. Once the fruiting stage was initiated the cycle was increased to 3 minutes per hour and the daytime cycle was extended to 7 a.m. to 6 p.m.. This produced a slab E.C. of 2.3 millimhos. Nighttime supplemental heating was provided during April.

The experimental design was a randomized complete block design, blocked against the light intensity. There were 12 treatments and three replicates, each replicate consisted of one slab . Treatments were ; bacterial isolates, 13, 15, 16, 27, or 35 alone , each bacterium with Pythium aphanidermatum , P. aphanidermatum with no bacteria and a blank treatment (no Pythium or bacteria. Bacterial suspensions of 10^6 cfu/ml were added to the cubes at a rate of 200 ml/ cube , two and four days after setting the plants on the slabs. Six days after the second bacteria introduction , zoospores of Pythium

were introduced at the rate of 200 ml/cube (500 zoopores/ml). The fertigation pumps were shut off for 12 hours following the introductions of the Pythium and the bacteria.

Pythium and bacteria population levels were monitored twice during the cropping period. Bacteria populations were determined by dilution plating and Pythium zoospore levels were determined by baiting with pearl millet seeds (Section 4.3.8). During the cropping period, fruit were harvested daily and plant wilting was recorded when it occurred. Plants were examined periodically for fruit abortion and the number of aborted fruit was recorded. Leaf production was determined twice and leaf diameter was measured once. The experiment was terminated after seven weeks of harvest. The plant shoots were removed from the greenhouse , dried for four days at 70 C and dry weights were determined. Plant roots were also examined visually for signs of necrosis.

4.3.3 GREENHOUSE EXPERIMENT 2 : AUGUST - SEPTEMBER 1991

Cucumber transplants were produced , set out in the greenhouse and inoculated as outlined in 4.3.2. The plants were set onto slabs on August 9,1991.

The experimental design was a randomized complete block design with 16 treatments and four replicates, each replicate consisted of one slab . Treatments were bacterial isolates, 13, 15, 16, 27, or 35 alone and each bacterium treatment with

Pythium aphanidermatum . Two mixed bacterium treatments were also included ; isolate 13+15 , the two most promising isolates from the first crop and isolate 15+27 which were found in previous work to be a good coloniser (Section 2.4.6) and an antibiotic producer (Paulitz et al. 1992) respectively. These mixed treatments were tested alone and with the Pythium. The two control treatments were P. aphanidermatum with no bacteria and a blank treatment (no Pythium or bacteria). Bacteria and Pythium were introduced as outlined in 4.3.2 . Pythium and bacteria population levels were monitored once during the cropping period. Data on yield , plant wilting and fruit abortion were recorded. Due to excessive levels of fruit abortion caused by high daytime temperatures and recurrent exhaust fan problems this experiment was terminated after only four weeks of harvest. At that time the plant shoots were removed from the greenhouse , dried for four days at 70 C and dry weights were determined. Plant roots were also examined visually for signs of necrosis.

4.3.4 GREENHOUSE EXPERIMENT 3: APRIL - JUNE 1992

Cucumber transplants were produced , set out in the greenhouse and inoculated as outlined in 4.3.2 , however vertical fibre rockwool slabs were used in this experiment. The plants were set onto slabs on April 7, 1992 and nighttime supplemental heating was provided for the remainder of April. The

experimental design was a randomized complete block design with 16 treatments and four replicates, each replicate consisted of one slab . Only the three most effective bacterial isolates , numbers 13, 15, and 35 , from the two previous crops were tested . The treatments consisted of one of each of the three bacterial isolates alone and each isolate tested with Pythium inoculated at either ; time 1 (P1)- Pythium applied before the bacteria, time 2 (P2)- Pythium and bacteria applied simultaneously or time 3 (P3) - Pythium applied after the bacteria. Three Pythium controls , one for each introduction time and a blank treatment (no bacteria, no Pythium) were also included. Bacterial inoculum was applied to the cubes at a rate of 200 ml/cube (10^6 cfu/ml) on April 17 and again on April 20. Pythium inoculum was applied as follows ; P1 - April 10 , P2 - April 17 , P3 - April 22. Bacteria and Pythium populations in the slabs were monitored at the end of the cropping period as previously described. The presence of the bacteria in the basal stem area of plants in the first and second replicate was determined by the method outlined in section 3.3.5

4.3.5 HARVEST

In all greenhouse crops , the fruit were harvested when they had attained a diameter of 4.5 cm at any point along the length. Each fruit was individually weighed and graded

according to the grades set out in table 4 .1 (Anonymous, 1989). Fruit which were soft at harvest or had severe shape deformities including multiple bulges and under developed blossom ends were considered unmarketable (cull grade). These fruit were included in the total harvest data but were also considered separately to determine the non-marketable percentage of harvest which is presented in Figures 4.3 , 4.6 and 4.7 .

Table 4 .1 Commercial Grade Standards For Greenhouse Cucumbers

Grade 1	
Grade	Length (cm)
small	28 - 32
medium	32 - 37
large	37 - 42
x - large	42 +
Grade 2	
Any cucumber < 28 cm in length or which has a bend , crook or bulge greater than the diameter of the fruit	

4.3.6 WILTING INDEX

Plant wilting was determined by daily examination of the plants between the hours of 3 p.m. and 5 p.m. The extent of wilting was rated as follows:

0 - No wilting.
1 - Very slight wilting , leaf blades have lost hard turgor but do not droop.
2 - Leaves droop slightly at the margin only , the petiole is

unaffected. Plant recovers overnight.

3 - The entire leaf blades droop and the petioles have lost some of thier turgor. Plant recovers only if the following day is overcast or cool.

4 - The leaf blades have entirely collapsed and the petiole droops. Plant will not recover.

4.3.7 BACTERIA POPULATION MONITORING

All bacterial isolates used in the greenhouse experiments had been previously marked for rifampicin resistance. Bacteria populations in the greenhouse were sampled by inserting a forceps into the slab and removing a small sample of rockwool and root material from the root zone in the area just below the cube/slab interface. The sample was placed in a 125 X 16 mm test tube containing 9 ml of sterile distilled water and sonicated for 30 seconds and then vortexed. One or two serial 1:10 dilutions were made from the solution and 0.01 ml of the final solution was plated on nutrient agar amended with 100 ug/L rifampicin and incubated at 26 C for 48 hours. Colony counts were made for each plate. The original rockwool sample was drained and then dried for 4 days at 70 C and dry weight was determined. On all sampling dates one sample was taken from each replicate of each treatment.

4.3.8 PYTHIUM POPULATION MONITORING

A sample of rockwool and root material was removed as described above. The sample was then placed in a 125 X 16 mm

test tube containing 9 ml SDW and 20 pearl millet seeds. The sample was placed in the tube so it was in contact with the water but not fully immersed. This allowed the zoospores access to the millet seeds but did not allow direct contact between the sample and the millet seeds. The test tubes were incubated for 24 hours at 26 C and then the seeds were removed and plated on Pythium selective media. The plates were incubated for a further 48 hours and then the number of infected seeds was determined and compared to the curve developed in Section 3.3.1.

4.3.9 STATISTICAL ANALYSIS

The results of all greenhouse experiments were analyzed using a Two - way Anova followed by a Duncans Multiple Range test . In greenhouse experiment three , bacterial isolate and Pythium inoculation time were also analyzed to determine if either factor was significant and if interactions between the factors occurred. The non - parametric Cochran Q test was used to analyze the plant mortality rates of the Pythium inoculated treatments in greenhouse crop 2.

4.4 RESULTS

4.4.1 GREENHOUSE CROP 1

Late afternoon wilting occurred almost two weeks earlier in Pythium inoculated treatments (Figure 4.1) than in treatments not inoculated with Pythium (Figure 4.2). Of the Pythium inoculated treatments , the most severe wilting was observed in the Pythium control (no bacteria) , while treatments with bacterial isolate 13 or 15 and the Pythium showed markedly less wilting. Premature plant death from non- recoverable wilt was not very prevalent in this crop, as only seven plants died prior to the termination of the experiment after seven weeks of harvest. Two of the seven were treated with Pythium alone , two were treated with bacterial isolate 13 alone (no Pythium) and the three remaining plants came from treatments 35P, 16, and 16P.

All treatments were sampled to determine zoospore levels two weeks after the Pythium was applied. Greater than 300 zoospores per ml were recovered from all replicates of all Pythium - inoculated treatments. Pythium was not recovered from either the blank treatment nor any of the treatments which received the bacterial isolates alone. Inoculation with Pythium caused discolouration of the roots of all treated plants however poor anchorage to the slab was not noted. The roots of plants receiving treatments with the bacterial

isolates alone and the roots of plants in the blank treatment were not discoloured.

Bacteria populations were sampled at three weeks and again at seven weeks after application of the bacterial inoculum. At the three week sampling time population levels ranged from 5×10^5 to 1×10^7 cfu / g dry weight of sample (Table 4.2). Bacteria were not recovered from the blank or Pythium control treatments. At the seven week sampling period the presence of contaminating organisms rendered the plate counts unreliable, however at dilutions of 1 : 10,000 , bacteria were recovered from treatments which had received the bacterial inoculum.

Table 4.2 Bacterial Populations in Rockwool Slabs Three Weeks After Application

Treatment	Bacteria/gram dry weight of sample
Blank	0
Pythium	0
35	5.3×10^5
35 + P	1.1×10^7
15	7.7×10^5
15 + P	2.4×10^6
16	9.1×10^5
16 + P	9.7×10^6
27	7.4×10^6
27 + P	1.7×10^6
13	1.2×10^6
13 + P	4.5×10^6

The treatment identified as Pythium and the numbered treatments followed by 'P' were inoculated with Pythium aphanidermatum

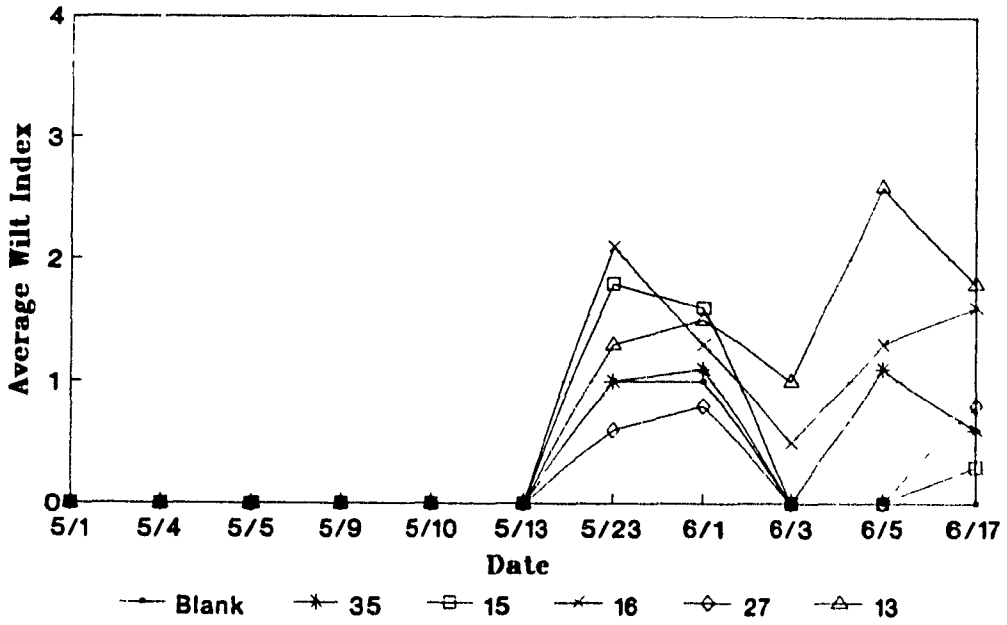

Figure 4.1 Severity of Wilting in Non Inoculated Treatments in Greenhouse Crop 1

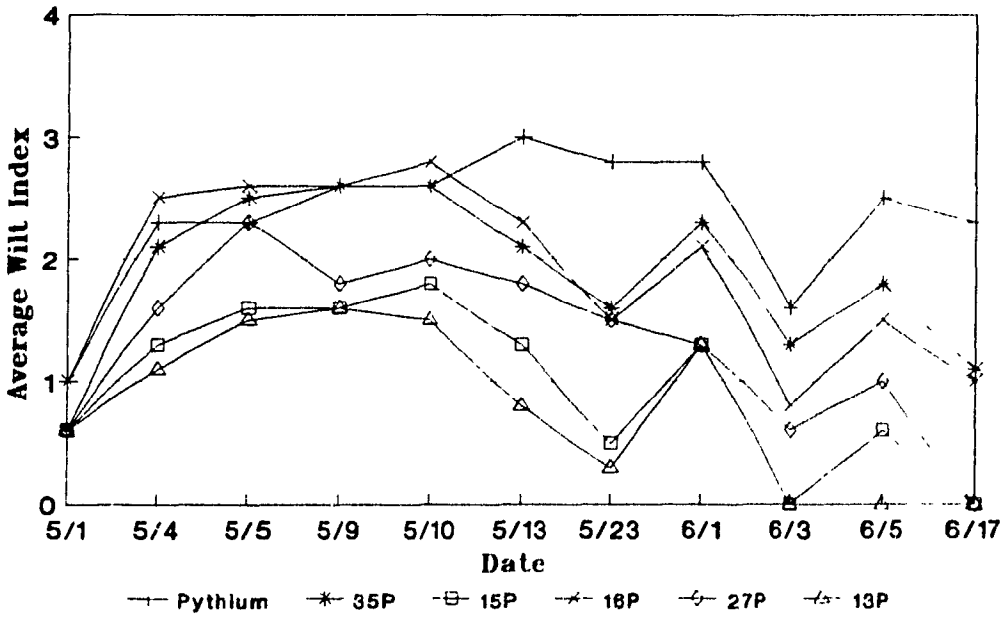

Figure 4.2 Severity of Wilting in Pythium Inoculated Treatments in Greenhouse Crop 1

Note - Plant wilting was rated as outlined in Section 4.3.6. The average wilt index for a treatment includes plants which have died and been removed from the greenhouse. Dead plants were given a wilt index value of 4 , the same value used for non reversible wilt.

Table 4.3 Leaf Production and Final Shoot Dry Weights in Greenhouse Crop 1

Treatment	# of Leaves produced by May 9[x]	# of Leaves produced by May 23[x]	Shoot Dry Weight
Blank	3.83 ab*	11.33 a	97.28 abc
Pythium	1.83 cd	8.53 abc	50.89 c
35	4.17 a	10.83 ab	98.47 abc
35 + P	1.17 d	6.17 c	55.58 bc
15	4.50 a	11.33 a	98.63 abc
15 + P	2.00 cd	7.83 bc	70.44 bc
16	4.33 a	10.67 ab	77.20 abc
16 + P	2.33 cd	9.17 abc	54.27 bc
27	4.83 a	11.67 a	127.17 a
27 + P	1.67 cd	6.67 c	53.41 bc
13	4.17 a	10.83 ab	107.06 ab
13 + P	2.83 bc	9.33 abc	75.07 abc

x - the most recently emerged leaf was flagged on May 2 and the number of leaves produced was determined by counting from this leaf.

* - Treatments followed by the same letter are not significantly different at (P= 0.05)

The treatment identified as Pythium and the numbered treatments followed by 'P' were inoculated with Pythium aphanidermatum

Table 4.4 Greatest Diameter (cm) of Basal Leaves on May 23/1991

Treatment	7th leaf from base of plant	10th leaf from base of plant
Blank	26.17 abc*	25.83 a
Pythium	23.62 c	19.72 d
35	25.83 abc	25.83 a
35 + P	22.50 c	19.17 d
15	27.50 ab	25.83 a
15 + P	24.00 bc	21.00 d
16	25.50 abc	24.67 abc
16 + P	23.83 bc	21.33 cd
27	28.33 a	25.33 ab
27 + P	22.67 c	19.83 d
13	27.33 ab	27.17 a
13 + P	25.00 abc	21.83 bcd

* - Treatments followed by the same letter are not significantly different at (P=0.05)

The treatment identified as Pythium and the numbered treatments followed by 'P' were inoculated with Pythium aphanidermatum

Table 4.5 Fruit Production From Greenhouse Crop 1

Treatment	Percent fruit abortion May 9	Total # of Fruit produced
Blank	3.33 b*	24.67 abc
Pythium	40.00 ab	11.33 d
35	5.00 b	25.00 ab
35 + P	68.50 a	13.00 cd
15	0.00 b	26.33 a
15 + P	43.17 ab	18.33 abcd
16	0.00 b	21.67 abcd
16 + P	64.50 a	13.00 bcd
27	0.00 b	24.47 abc
27 + P	68.67 a	11.56 d
13	3.33 b	22.33 abcd
13 + P	34.50 ab	22.67 abcd

* - Treatments followed by the same letter are not significantly different at (P=0.05)

Note - data for fruit yield in kg was not significantly different at P=0.05 therefore was not included in table

The treatment identified as Pythium and the numbered treatments followed by 'P' were inoculated with Pythium aphanidermatum

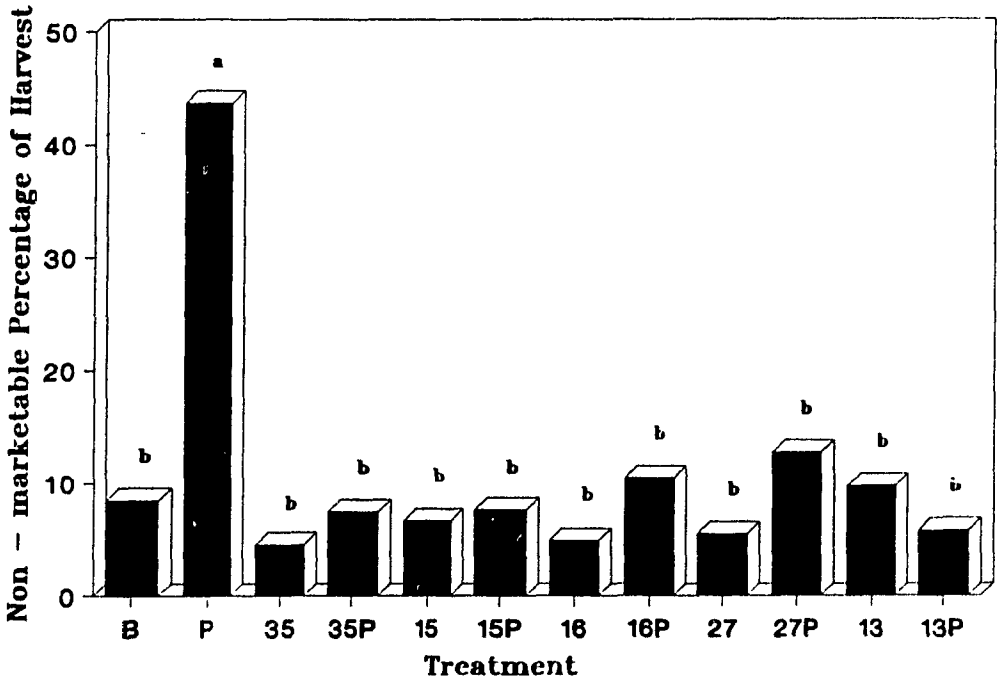

Figure 4.3 Non-marketable Percentage of Harvest From Greenhouse Crop 1

Pythium significantly reduced the number of leaves at May 9, but not at May 23 (Table 4.3). Treatment with bacteria had no effect on the number of leaves. Neither Pythium nor the bacterial isolates significantly affected shoot dry weight. Pythium did significantly reduce the diameter of the 10th leaf from the base of the plant (Table 4.4) but treatment of inoculated plants with bacteria did not result in increased leaf diameter. Inoculation with Pythium alone significantly reduced the number of fruit produced (Table 4.5). When plants treated with the bacterial isolates were inoculated with Pythium , the number of fruit was increased to a level intermediate between the inoculated and noninoculated control, not significantly different from either. Treatment with the bacterial isolates prior to Pythium inoculation significantly reduced the percentage of non-marketable fruit (Fig 4.3).

4.4.2 GREENHOUSE CROP 2

Higher daytime temperatures during this cropping period resulted in increased water demands by the plants causing a more rapid onset of wilting in both Pythium inoculated and Pythium non - inoculated treatments. Wilting in treatments which were not inoculated with the Pythium was very slight however, with wilt indexes rarely exceeding 1 (Figure 4.4). Plant wilting severity in Pythium inoculated treatments rose rapidly following its onset. The wilting was most severe in

the Pythium control and least severe in treatments with bacterial isolate 13 + Pythium or 27 + Pythium. Overall wilting in this crop was more severe than in the first crop and premature plant death also reached higher levels. However , comparison of the Pythium control to all treatments receiving a bacterial isolate and the Pythium showed no significant difference in mortality (P=0.05) by a Cochran Q test. Treatment with some bacterial isolates delayed severe plant wilting and plant death. On Sept. 1, 5/8 plants were dead in the inoculated control treatment without the bacteria. Three out of eight, 1/8 and 3/8 plants were dead in inoculated treatments with Isolate 13 , 15, and 27 respectively. On Sept. 9, 7/8 plants were dead in the inoculated control, compared to 4/8, 5/8, and 4/8 of the inoculated plants treated with isolate 13, 15, and 27 respectively. Inoculation with Pythium caused discolouration of the root system of all treated plants. In addition most which died prematurely were poorly anchored to the rockwool slabs. Many of the surviving plants in the Pythium control treatment were also poorly anchored. Neither of these symptoms were seen on the roots of plants from the blank treatment or treatments with the bacterial isolates alone. Plant dry weight of Pythium inoculated plants receiving bacterial treatments was not significantly higher than in the Pythium control (Table 4.7) However treatment of plants with isolate 15 in the absence of Pythium produced an increased growth response. Pythium inoculation significantly

decreased both total fruit weight and number. Treatments with the bacterial isolates plus the Pythium resulted in total fruit weight and number which were intermediate between the Pythium control and the blank control. However, treatment with isolate 16+P and isolates 13+15+P resulted in fruit weight and numbers which were not significantly different from the Pythium control (Table 4.6). Treatment of Pythium inoculated plants with all bacteria isolates reduced the percentage of non - marketable fruit (Figure 4.6).

Prior to termination of the experiment, samples were removed from all treatments in order to determine Pythium and bacteria population levels. Between 100 - 300 zoospores per ml were present in all replicates of all Pythium inoculated treatments. Pythium was also recovered at low levels from one replicate of the blank treatment but was not recovered from any of the treatments which received the bacterial isolates alone. At the time of sampling ,on Sept. 18 , bacteria population levels ranged from 10^5 to 10^7 cfu / g dry weight of sample. Bacteria were also recovered from one replicate of the Pythium control and three replicates of the blank control. Various disease and insect problems were encountered during the cropping period , but chemical control measures were not used . Powdery mildew became apparent on the lower leaves of the plants during the second week the crop was in the greenhouse and it progressed unabated throughout the cropping period.

Cucumber beetles moved into the greenhouse from adjacent field crops during the initial two weeks of the cropping period. Plants were examined daily and beetles were removed . By the end of the second week they were no longer present in the greenhouse and plant damage as a result of their feeding was very slight.

Fungus gnats became a problem during the cropping period. High temperatures combined with abundant algal growth in gutters and under slabs provided a favourable breeding environment for the gnats and a large population ensued. Algal growth was partially controlled with household bleach (5% Sodium hypochlorite) applied at full strength to the gutter areas. Algal growth on exposed rockwool surfaces and fungus gnat feeding both in this area and in the root zone could not be controlled.

Failure of the high speed fan setting on Sept 9, 10 , 1991 caused a major episode of fruit abortion and some high temperature injury to the plants as temperatures in the greenhouse reached 46 C by mid afternoon on Sept 9. Due to this the trial was terminated approximately one week later on Sept. 18 , after allowing maturation of the remaining fruit which were beyond the stage that would abort.

Throughout the cropping period late afternoon temperatures consistently exceeded 30 C and often reached 35-37 C. Slab temperatures ranged from 25 - 30 C. These temperatures resulted in hard and rapid plant growth. Low levels of male

flower production and slight leaf scorching also resulted .

Table 4.6 Plant Dry Weights

Treatment	Plant dry weight(g)
Blank	41.52 bcde*
Pythium	29.46 e
35	52.36 ab
35 + P	30.16 e
15	57.22 a
15 + P	30.95 e
16	51.09 ab
16 + P	35.42 de
27	50.59 abc
27 + P	30.74 e
13	52.03 ab
13 + P	38.00 cde
13 + 15	48.23 abcd
13 + 15 + P	29.83 e
15 + 27	47.39 abcd
15 + 27 + P	30.80 e

* - Treatments followed by the same letter are not significantly different at P=0.01

The treatment identified as Pythium and the numbered treatments followed by 'P' were inoculated with Pythium aphanidermatum

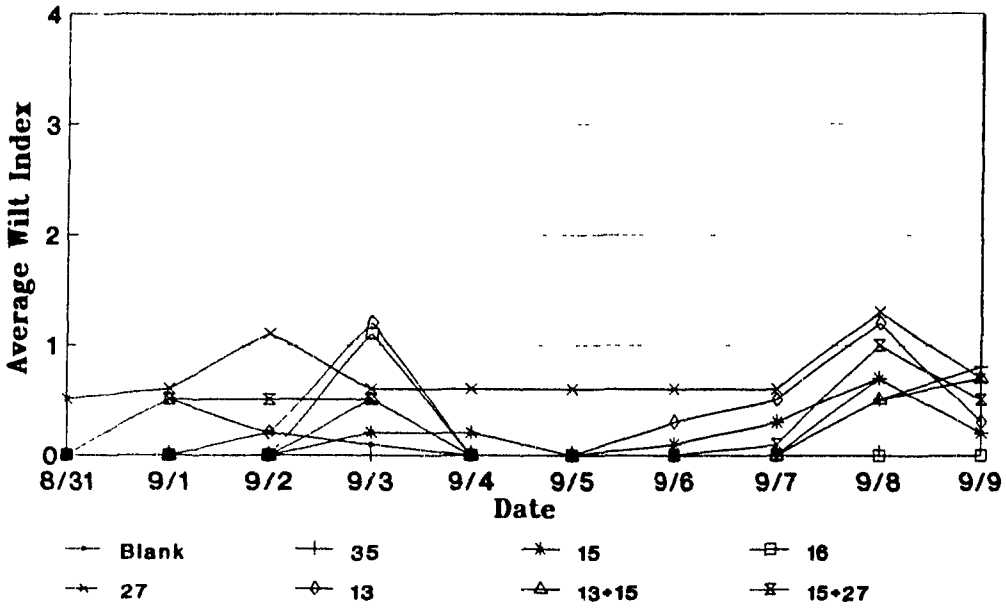

Figure 4.4 The Severity of Wilting in Non Inoculated Treatments in Greenhouse Crop 2

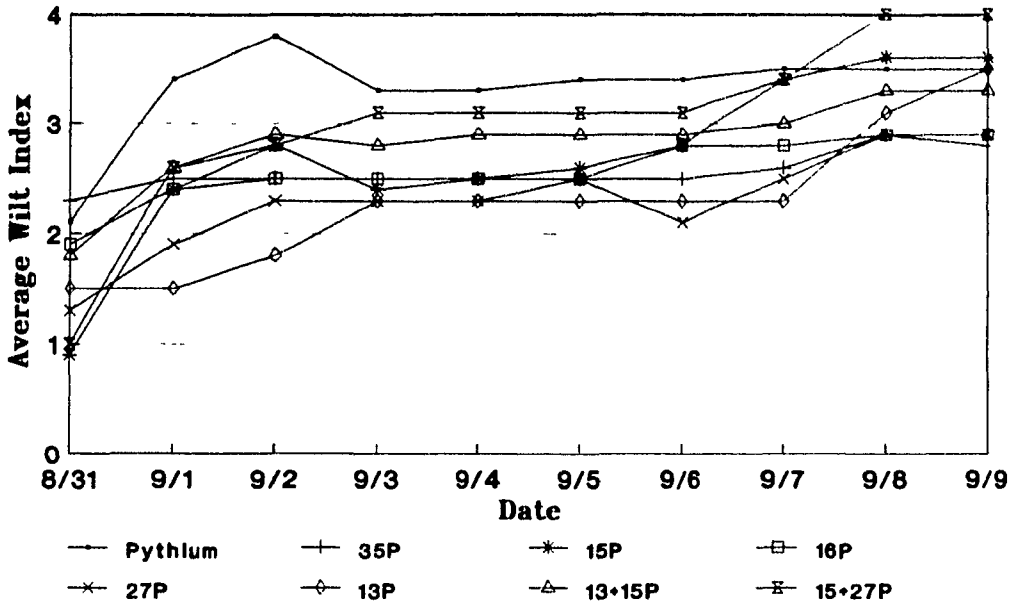

Figure 4.5 Severity of Wilting in Pythium Inoculate Treatments in Greenhouse Crop 2

Note - Plant wilting was rated as outlined in Section 4.3.6. The average wilt index for a treatment includes plants which have died and been removed from the greenhouse. Dead plants were given a wilt index value of 4 , the same value used for non reversible wilt.

Table 4.7 Total Fruit Production in Greenhouse Crop 2

Treatment	Total fruit yield in Kg	Total fruit number
Blank	3.92 ab*	9.00 abcd
Pythium	0.49 d	1.25 f
35	4.61 a	10.25 ab
35 + P	1.43 bcd	3.25 def
15	4.68 a	11.00 a
15 + P	1.86 bcd	5.00 abcdef
16	4.61 a	9.75 abc
16 + P	1.22 cd	2.75 ef
27	3.51 abc	8.25 abcde
27 + P	1.91 bcd	4.75 bcdef
13	3.58 abc	8.50 abcde
13 + P	2.04 bcd	5.25 abcdef
13 + 15	5.03 a	10.75 ab
13 + 15 + P	0.90 d	2.50 ef
15 + 27	4.85 a	10.75 ab
15 + 27 + P	1.29 cd	3.75 cdef

* - Treatments followed by the same letter are not significantly different at P=0.01
Note - total fruit yield represents the average yield for a two plant replicate slab of each treatment

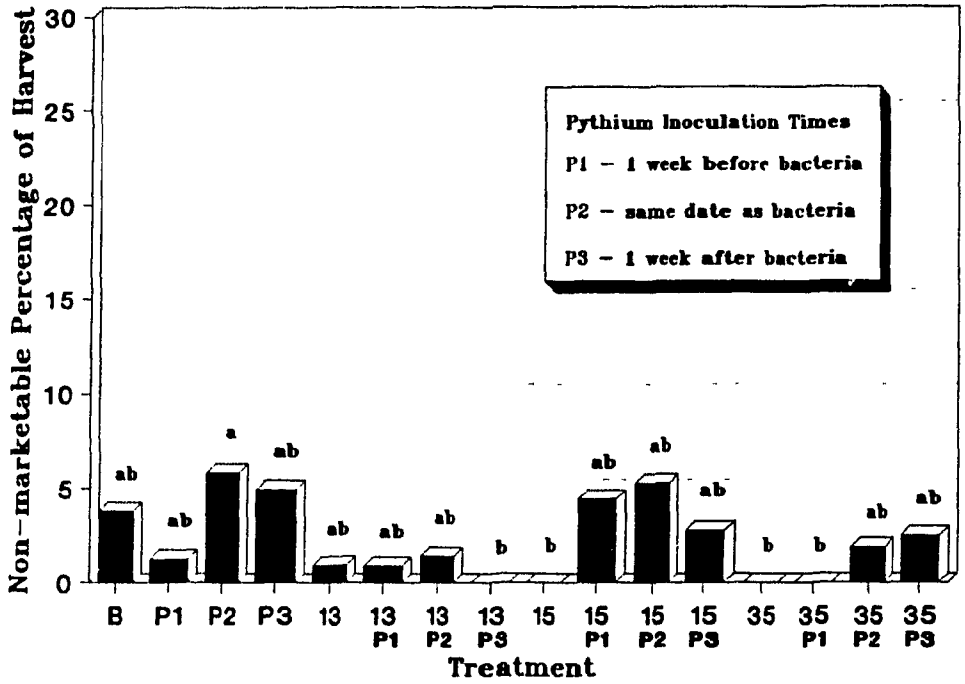

Figure 4.6 Non-marketable Percentage of Harvest From Greenhouse Crop 2

4.4.3 GREENHOUSE CROP 3

The data for all plant growth parameters and harvest parameters was analyzed using bacterial isolate and Pythium inoculation date as main effects. Neither of these factors were significant nor was there any significant (P=0.05) interaction between the two. Due to this the data was analyzed using a simple ANOVA analysis. The results for these analysis are presented below in tables 4.7 and 4.8.

Data for plant dry weight and number of leaves produced by 2 weeks after inoculation are not presented as no significant difference was found between the treatments for these parameters. Disease development in this crop was apparently slower than in the spring 1991 crop although slab temperatures were similar (20-25 C). Plant wilting was very slight and only occurred during 2 days in May. Plant collapse was not preceded by periods of wilting as had occurred in previous crops. Pythium recovery from all treated slabs was low. Pythium levels were below the detection threshold of the millet baiting method however Pythium was recovered from all treated slabs by direct plating of root samples on Pythium selective media. Bacteria populations in the slabs were low at the end of the harvest period , ranging from 5 X 10^3 cfu/g to levels that were below the detection threshold at the dilutions used. Isolates 15 and 35 were recovered from both Pythium inoculated and non-inoculated treatments while isolate 13 was only recovered from 1/2 reps of the Pythium non- inoculated

treatment. Isolate 13 was recovered from the basal stem of 1/8 plants tested while isolates 15 and 35 were each recovered from the stems of 5/8 plants.

Treatments with the Pythium alone at all three introduction times resulted in discolouration of the root system although poor anchorage was not noted. Plants treated with bacterial isolate 13 or 15 and 35P3 and the Pythium were similarly discoloured , however plants treated with isolate 35 and P1 or P2 were not discoloured. Roots from treatments with the bacteria alone or the blank control were not discoloured.

Fruit number and total fruit weight and non-marketable percentage of harvest were not significantly decreased by inoculation with Pythium in this crop. However treatment with isolate 15 alone (no Pythium) resulted in fruit number and total fruit weight which were significantly greater than the blank treatment (no Pythium, no bacteria)

Table 4.8 Total Fruit Production in Greenhouse Crop 3

Treatment	Total fruit yield in Kg	Total fruit number
Blank	12.45 bcd*	26.25 bcd
Pythium 1	12.30 bcd	26.70 abcd
Pythium 2	9.10 d	21.25 d
Pythium 3	10.00 cd	22.75 cd
13	14.10 ab	30.00 ab
13 + P1	12.36 bcd	27.30 abcd
13 + P2	11.18 bcd	24.25 bcd
13 + P3	11.68 bcd	26.25 bcd
15	15.90 a	33.00 a
15 + P1	12.27 bcd	29.00 abc
15 + P2	11.33 bcd	24.00 bcd
15 + P3	11.53 bcd	25.25 bcd
35	13.30 abc	29.50 abc
35 + P1	12.13 bcd	26.30 bcd
35 + P2	11.20 bcd	25.00 bcd
35 + P3	12.45 bcd	26.00 bcd

* - Treatments followed by the same letter are not significantly different at P=0.05
Note - total fruit yield represents the average yield for a two plant replicate slab of each treatment
P1,P2,P3 - Pythium applied at time 1, time 2 and time 3 respectivly

Table 4.9 Leaf Production and Final Plant Height

Treatment	# of Leaves produced by week 1	# of Leaves produced by week 3	Plant Height
Blank	4.50 abc*	13.80 ab	165.40 b
Pythium 1	4.30 abc	13.50 ab	163.30 b
Pythium 2	4.50 abc	12.12 b	155.40 b
Pythium 3	4.60 abc	14.25 a	172.20 ab
13	4.60 abc	14.00 ab	170.40 ab
13 + P1	4.60 abc	12.60 ab	171.20 ab
13 + P2	4.00 c	12.50 ab	154.40 b
13 + P3	4.37 abc	12.50 ab	163.10 b
15	4.80 ab	14.16 a	180.00 ab
15 + P1	5.00 a	13.60 ab	168.30 b
15 + P2	4.25 abc	14.12 ab	169.25 ab
15 + P3	4.37 abc	13.60 ab	161.25 b
35	4.50 abc	13.80 ab	169.63 ab
35 + P1	4.16 bc	13.16 ab	146.25 b
35 + P2	4.13 bc	13.00 ab	155.67 b
35 + P3	4.00 c	13.80 ab	202.50 a

Note - Plant dry weight was not significantly different at P=0.05 therefor was not included
P1,P2,P3 - Pythium applied at time 1, time 2 and time 3 respectivly

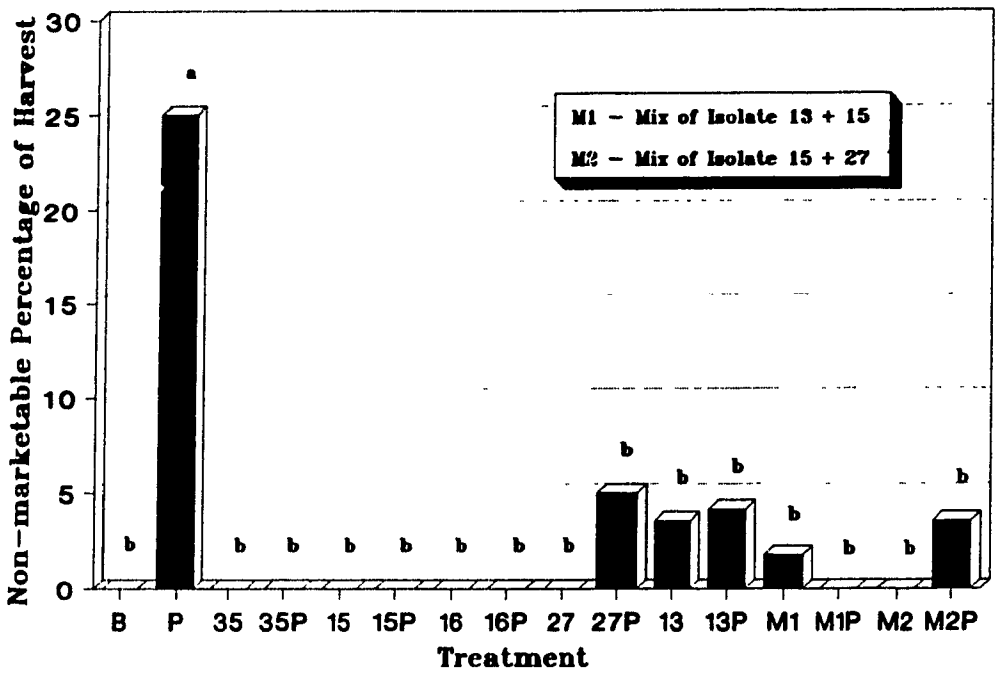

Figure 4.7 Non-marketable Percentage of Harvest From Greenhouse Crop 3

4.5 DISCUSSION AND CONCLUSIONS

In all greenhouse crops it was apparent that plant growth parameters such as leaf diameter, plant height, leaf production and dry weight were poor indicators of the presence of Pythium. The exception to this was plant wilting. In both greenhouse crops 1 and 2 , the highest level of wilting was always seen in the Pythium control treatment (no bacteria). Lower levels of wilting were present in those treatments where bacterial isolates 13 , 15 or 27 had been applied prior to the Pythium inoculation.

The presence of Pythium in the root system had a more obvious effect on the plant yield. Greenhouse cucumbers are always sold by the unit therefore, fruit number is a more useful measure of production than the weight of the total yield. In the first two crops, treatment with the bacterial isolates prior to inoculation with the Pythium produced yields that were intermediate between those of the plants treated with the Pythium alone (no bacteria) and the plants in the blank treatment which received neither the Pythium nor the bacteria. However when the fruit was graded it became evident that the cull rate in the Pythium treatment was significantly higher ($P=0.05$) than the blank. Pythium plus bacteria treatments had cull rates which were not significantly different from the blank. Cucumbers tend to respond to stress initially by sacrificing fruit production. This can be seen in increased

abortion rates at times of high stress to the plant. It is plausible that at times when plant stress is not sufficient to cause abortion , both fruit development rate (indicated by fruit number) and quality are compromised. Average fruit production levels coupled with high rates of cullage are more economically damaging to the grower than are low yields alone. Labour must be expended to harvest and grade poor quality fruit. This would suggest that applications of the bacteria would be beneficial to growers in terms of a greater marketable yield.

Although the precise mode of action of the potential biocontrol bacteria used here is outside the scope of this thesis, sufficient data exist for speculation that a plant growth promotion response is at least partially responsible for the activity of isolate 15. This has been seen in test tube studies in the initial screening stages and in the greenhouse crops . Treatments with isolate 15 in the absence of Pythium resulted in higher yields while treatments of isolate 15 plus the Pythium reduced the effects of disease. It can be suggested that mitigation of the disease losses could result from one of two scenarios. Isolate 15 is increasing plant vigour which would tend to bolster natural plant defenses and increase its resistance to stress . This would in turn reduce damage from Pythium as it is most damaging to plants under stress conditions (Davies 1980). The other scenario is that isolate 15 is enhancing plant vigour even in

the presence of a Pythium infection, thereby masking the effects of the disease. From the Pythium sampling it is evident that the Pythium was still present in the slabs. Isolate 13 has also shown an ability to lessen the effects of the Pythium . However no PGPR response has been associated with this isolate in the absence of the Pythium except in the initial bioassay. Speculation on its mode of action would be without basis.

Disease development was greater in the summer greenhouse crop than in either spring crop. Increased disease pressure resulted from higher slab temperatures which were closer to the optimum of the Pythium, but daytime air temperatures which were stressful to the cucumbers . Stress responses such as abortion , male flower production and slight leaf scorching were evident in all treatments and controls. Also other pest problems during this cropping period contributed to the plant stress possibly causing greater disease development (indicated by the wilting). As the impaired root function resulting from the presence of Pythium interfered with water relations, plant mortality increased.

In greenhouse crop 3 all treatments having Pythium applied at time 1 (before the bacteria) fared well. However plants in the Pythium control treatment for this inoculation time were only slightly affected by the pathogen. If PGPR was the mode of action a marginal effect would be expected in this inoculation situation, whereas if the bacteria were simply

interacting with the Pythium to prevent pathogenesis no effect would be expected. Pythium causes rapid pathogenesis in situations where it can act as a primary coloniser (Kommedahl and Windels 1979), therefore it is assumed it would have been well established before the bacteria were applied. However, as a result of the lack of disease development in the Pythium control for this inoculation time ,no conclusions can be drawn from treatments receiving P1. It is possible that the Pythium inoculum was of low viability on this date. Also with the short lag time between P1 and P2 inoculation dates, any appreciable difference in plant susceptibility would not be expected to account for the difference in disease development. It is also possible that this lack of disease development in any P1 treatments could have skewed the data set, resulting in the lack of significant effects of the bacterial isolates, inoculation times and interactions between the two. Inoculations P2 and P3 fared as expected ,however greater disease development was noted with inoculation of the Pythium at time P2. It is possible that P3 was too late to cause devastating levels of disease development. Disease pressure was lower overall in this crop than in the spring 1991 crop. It is possible that this could be attributed to slightly earlier Pythium inoculation times and lower slab temperature which were not as favourable to the Pythium. It is also possible that the use of rockwool slabs with a vertical fibre orientation (as opposed to random fibre in the 1991 crops) had

an effect on disease incidence. Vertical fibre orientation would improve drainage possibly causing the Pythium inoculum to be flushed from the slab more rapidly. Pythium recovery from the slabs was lower in this crop than in either 1991 crop. Bacteria populations were also low , however this could have been affected by the time lag between application and sampling which was approximately 3 weeks longer than in previous crops.

Overall it was apparent that treatment with the bacterial isolates prior to the inoculation of Pythium results in increased fruit production. Fruit numbers which did not differ significantly from those of non-inoculated plants were obtained. Possibly of more importance is the reduction in cull grade fruit. In 2/3 crops treatments which received the bacterial isolates plus the Pythium had a significantly lower cull rate than treatments receiving the Pythium alone . The greatest disease development occurred in the summer crop where slab temperatures remained continuously near the optimum of Pythium aphanidermatum . Results from the third crop did not follow any of the trends present in the first two , this leaves open the question of the effect of rockwool fibre orientation on disease development. It is possible that this fibre orientation resulted in poor establishment of both the pathogen and the bacteria in Crop 3.

LITERATURE CITED

Anonymous 1989. Pargro rockwool reference guide. Plant Products Co. Ltd.

Carrier, A. 1990. Concombres de Serre: nos plants vieillissent mal! Le Producteur Horticole (5)3:11-12

Davies, J.M.L. 1980. Disease problems encountered in commercially grown tomato crops. Acta Hortic. 98:299-305

Favrin, R.J., Rahe, J.E. and Mauza, B., 1988. Pythium spp. associated with crown rot of cucumbers in British Columbia Greenhouses. Plant Disease 72:683-687

Goldberg, N.P. and Stanghellini, M.E. 1991. The potential use of bacteria as biological control agents for Pythium in hydroponics. Phytopathology 81:1345 (abst)

Goldberg, N.P., Stanghellini, M.E. and Rasmussen, S.L. 1991. Filtration as a strategy for the control of Pythium in recirculating hydroponics. Phytopathology 81:1345 (abst)

Kommedahl T. and Windels C.E. 1979. Pathogen host dominanace in disease. p 1-103 in Ecology of root pathogens Elsevier Scientific Publishing C. New York. 281pp. Krupa,S.V. and Dommergues Y.R. eds.

Mohyuddin, M. 1985b .Crop cultivars and disease control. p 42-50 in: Hydroponics Worldwide: State of the Art in Soilless Crop Production. Savage, A.T.(ed) International Center for Special Studies Honolulu, Hawaii 194 pp.

Paulitz, T.C., Zhou, T., and Rankin, L. 1992. Selection of rhizosphere bacteria for biological control of Pythium aphanidermatum on hydroponically-grown cucumber. Biological Control: in press.

Price,D. and Dickinson, A., 1980. Fungicides and the nutrient film technique. Acta. Hortic. 98:277-283

Price, T.V. and Fox, P. 1986. Studies on the behaviour of furalaxyl on pythiaceous fungi and cucumbers in recirculating hydroponic systems. Aust. J. Agric. Res. 37: 65-77

Zinnen, T.M. 1988. Assessment of plant disease in hydroponic culture. Pl. Dis. 72:2 96-99

5 DISCUSSION AND OVERALL CONCLUSIONS

In the test tube bioassay, eleven out 32 bacteria, previously selected for their ability to inhibit zoospore germination and/or mycelial growth of Pythium aphanidermatum (Pa) in vitro, resulted in root lengths of Pa-inoculated plants > 80% of that of a non-inoculated control. Inoculation with Pa alone reduced root lengths to 67% of the non-inoculated control. These results agree with the conclusions of Broadbent et al. (1971), who state that in vitro testing is not an adequate predictor of biological control activity in planta. However, in vitro testing is an effective prescreening to rapidly assess a large number of bacterial isolates whereas use of the bioassay for this purpose would be very labour intensive. Also of note are the two isolates which performed favourably in vitro tests but produced a deleterious effect when tested alone on cucumber plants. This type of effect, by its very nature, would not be apparent in an in vitro screening. Therefore the bioassay serves another function , that of the further weeding out deleterious organisms. The assay was also found to be a useful indicator of the plant growth promotion ability of the bacterial isolates.

Problems with high variances were encountered in the bioassay ,these probably resulted from the lack of disease development in the Pa-inoculated controls. This variance may have been due to non-uniformity of seedlings, uneven distribution of zoospores in the inoculating solution, or contaminating microbes from the seed coat. Complete decontamination of the seed coat without affecting the

viability of the seed is difficult, and previous attempts in our lab to accomplish this were unsuccessful. In addition assay conditions as similar to a commercial situation as possible were desirable. Surface disinfestation of the seed coat would have introduced another possible artifact.

The best five of the eleven best performing isolates were selected for further study in a rockwool cube system on the growth bench. This system, while useful in the number of plant growth parameters which can be readily measured , is apparently inappropriate for use in studying a pathogen with the etiology of Pythium spp. Possibly it would have an application in the study of other pathogens in the hydroponic system such as Phytophthora spp. which tend to be more aggressive in young plants and have a more rapid symptom development.

Studies to determine the colonization abilities of the bacteria , both in the rockwool cube system and the test tube system, proved useful. Short term colonization of the cucumber root system was achieved by eight of the eleven top performing bacteria from the bioassay in at least one colonization trial. However these results were not entirely reproducible in the cube system. Results from both of these trials indicate a number of things. Firstly, that colonization ability is an important component of disease suppression ability in this pathogen system. The relationship between colonization ability and the efficacy of biological control agents against soil-born pathogens has been documented (Baker and Scher 1987, Kloepper et al. 1991a, 1991b, Weller 1988) Secondly,

that short- term studies of colonization ability such as those conducted in the test tube system do not have a high predictive value for the long-term colonization ability of the bacteria. This is especially evident with isolate 27 which built up high population densities in the rhizosphere in the short-term studies but showed low external colonization in the longer term studies. Short term studies serve only as a very rough screen and tend only to eliminate the least effective colonizers as was seen with isolate 16.

It is interesting to note thac the isolates 15 and 35, with the best long term colonization ability, were among the top three isolates producing disease suppression effects in greenhouse tests. In the greenhouse studies isolate 13 performed well in all tests , equalling or exceeding the performance of isolate 15. Since the colonization ability of isolate 13 is variable and its PGPR response was weak, its mode of action remains to be elucidated.

Isolate 15, which is considered the top performing isolate overall, exhibited a good degree of colonization ability and a consistent PGPR response in both the bioassay and in growth bench and greenhouse testing. The involvement of the PGPR response in the in the disease suppression activity of this isolate is likely.

Isolate 35 had excellent colonization abilities but no PGPR activity. Its performance in the greenhouse crops was moderate and consistent.

A solid argument for the use of isolate 15 in commercial production can be made. Considering the ease of application of a biocontrol

agent through the nutrient solution, application of the bacterium would not be labour intensive for the grower. Also isolate 15 has been shown to cause a growth promotion in the absence of Pythium and a disease suppression effect when Pythium is present. Use of this isolate as a prophylactic treatment could have economic benefits far in excess of the potential application costs. However a great deal of further research is required before this becomes a valid consideration. Efficacy studies to determine the activity of this isolate against other strains and species of Pythium and related pathogens such as Phytophthora spp. must be conducted. Also, application methods and optimum timing of application requires study. Finally, with the prevalence of open hydroponic systems in this country, the fate of the organism in the environment must be assessed. Although it is a naturally occurring rhizosphere microbe, artificially increased populations in the environment resulting from drainage from hydroponic systems may have deleterious effects on other plant species.

Isolate 35 may have less value in hydroponic production systems than either isolate 13 or 15. However, given its disease suppression ability in young plants (in the bioassay) coupled with its superior colonization ability, this isolate may have greater value as a seed treatment for damping-off. Colonization and competitive exclusion of the pathogen seem to be the primary factors in the suppression of both pre and post-emergent damping off . Evaluating isolate 35 for this use could prove valuable. Testing the bacteria isolate in bioassay using Pythium ultimum ,

the pathogen more commonly associated with damping-off, would be an important first step.

From the studies presented in this thesis, it would seem that both isolates 15 and 35 warrant further study. It is believed that isolate 15 could have commercial benefit as a prophylactic treatment for Pythium aphanidermatum in hydroponically produced greenhouse cucumbers.

LITERATURE CITED

Broadbent, P., Baker K.F., and Waterworth, Y. 1971. Bacteria and actinomycetes antagonistic to fungal root pathogens in Australian soil. Aust. J. Biol. Sci. 24: 925-944.

Baker, R. and Scher, F. 1987. Enhancing the activity of biological control agents. In " Innovative Approaches to Plant Disease Control". Chet, I. (ed) John Wiley and Sons N.Y.

Kloepper, J.W., Zablotowivz, R.M., Tipping, E.M. and Lifshitz,R. 1991a. Plant growth promotion mediated by bacterial rhizosphere colonizers. p 315-326 in " The Rhizosphere and Plant Growth" Keister,D. L. and Cregan P.B. (eds), Kluwer Academic Publishers, Dordecht.

Kloepper, J.W., Wei, G., and Tuzan, S. 1991b. Rhizosphere population dynamics and internal colonization by plant-growth promoting rhizobacteria which induce systemic resistance to Colletotrichum orbiculare. pg.24 in: "Biological Control of Plant Diseases: Progress and Challenges for the Future", proceedings of a NATO Advanced Research Workshop, Cape Sounion, Greece, May 19-24, 1991.

Weller, D.M. 1988. Biological control of soil borne plant pathogens in the rhizosphere with bacteria. Ann. Rev. Phytopahtol. 26: 379-407

APPENDIX 1

STATEMENT OF ORIGINAL CONTRIBUTIONS TO KNOWLEDGE

The research reported in this thesis contains the following original contributions to knowledge:

1. Development of a simple test tube- bioassay using cucumber c.v. 'Straight 8', to select bacterial isolates effective in the biocontrol of Pythium aphanidermatum.

2. First demonstration of bacteria effective in suppressing Pythium aphanidermatum on cucumber grown in rockwool under simulated commercial conditions.