INTERRELATIONSHIPS BETWEEN SOIL-BORNE PATHOGENS ON *TRITICUM AESTIVUM*

by

Bharati K. Patel B.Sc. (Bombay) M.Sc. (Exon)

Department of Plant Pathology
Waite Agricultural Research Institute
The University of Adelaide
South Australia

Thesis submitted to The University of Adelaide
in fulfilment of the requirements for the degree of
Doctor of Philosophy

October 1983

-----xx-----

TABLE OF CONTENTS

SUMMARY

The aim was to study interrelationships between the complex of pathogens found on wheat roots mainly *Heterodera avenae (Ha)*, *Gaeumannomyces graminis* var. *tritici (Ggt)*, *Rhizoctonia solani (Rs)* and *Pratylenchus minyus (Pm)*.

Patches of poor growth in the field in the study area were not caused by pathogens alone. All plants whether from patches or non-patches had one or more pathogens on their roots. The most consistent correlation was a positive one between *Ggt* and *Ha*, at all sampling times for both years of the study. *Ggt* and *Rs* were negatively correlated in the second year. *Rs* and *Ha* were positively correlated at the second sampling time in the first year when *Rs* was the dominant fungal pathogen found on the roots and negatively correlated in the second year when *Rs* was not the dominant fungal pathogen. All pathogens except *Pm* were negatively associated with growth in the field and pathogens on seminal roots had a greater effect on growth than those on coronal roots.

The relationship between *Ggt* and *Ha* measured at maturity in laboratory studies was negative, the opposite of that found in the field measured during earlier stages of growth. The strain of *Ggt* also influenced the relationship.

Rs and *Ha* were also found to be negatively related experimentally. *Rs* suppressed *Ha* populations but also had an effect on penetration and establishment of *Ha* as early as 10 days after sowing. Greater reduction in growth occurred with the combinations of both pathogens.

Antagonism did occur in *'in vitro'* testing between *Ggt* and *Rs*; not between hyphae but in the establishment of lesions. Studies in soil partially confirmed the antagonism between the fungal pathogens. *Ggt* reduced *Rs* lesion length and *Rs* also reduced *Ggt* infection.

Both *Ggt* and *Rs* individually suppressed *Ha* populations. In the presence of *Ha*, the ability of *Ggt* to suppress *Rs* was reduced. *Ha* survival was greater due to the antagonism between the fungi, when all three pathogens were studied in combination.

In the multifactorial studies of the interrelationships between the pathogens under laboratory conditions, first order interactions accounted for more variation and were more frequent than second order interactions.

The sheer size of multifactorially designed experiments puts constraints on the number of factors, the levels at which they can be tested and the number of replicates to overcome the many sources of variation inherent to large experiments eg. seed size, inoculum preparation and incorporation, containers etc. Interpretation of second order interactions are difficult and though F values are useful, a difference may be trivial.

STATEMENT

This thesis has not been previously submitted for a degree at this or any other University, and is the original work of the writer except where due reference is made in the text.

_____

BHARATI PATEL

ACKNOWLEDGEMENTS

I wish to thank Dr. J.M. Fisher and Professor H.R. Wallace for encouragement, discussion and criticism during their supervision of this work. I also thank other members of staff and students for their help and interest.

I am also grateful to Ms. Sally Wayte, Mr. R. Cook and Mr. T. Hancock for statistical help; Mrs. L. Wichman who drew the figures; Mrs. M. Brock for typing this thesis and Mr. B. Palk for photography.

I acknowledge the assistance of the Australian Government (Commonwealth Scholarship and Fellowship Plan) for financial support throughout these studies and the Government of the Republic of Zambia for granting study leave.

CHAPTER I

INTRODUCTION

The list of soil-borne pathogens which attack roots of wheat is extensive in Australia. Among the fungi, *Gaeumannomyces graminis* (Sacc) van Arx. & Olivier var. tritici (*Ggt*) which occurs in all states is generally considered to be the most important (Garrett, 1942; McKnight, 1960; Butler, 1961). *Rhizoctonia solani* Kühn *(Rs)* - the imperfect state of *Thanatephorus cucumeris* (Frank) is of local importance in South Australia (McKnight, 1960) and parts of New South Wales (Butler, 1961). The nematode, *Heterodera avenae*, Wollenweber 1924 (Filipjev, 1934) (*Ha*), is the main nematode parasite of cereals in South Australia, although *Pratylenchus minyus*, Sher and Allen, 1953 *(Pm)* is also commonly found in roots of wheat (de Beer, 1965; Kimpinski, 1972; Stynes, 1975).

The constant association of (*Pm*) with (*Rs*) in wheat was first recognized by de Beer (1965). Stynes (1975) found that *Ggt*, *Rs*, *Ha* and *Pm*, commonly occurred together on wheat.

Ggt, commonly referred to as "take-all" or "hay-die" was first recorded in South Australia in 1852 and McAlpine (1904) concluded that *Ggt* was the primary cause of take-all. Historically *Ggt* is very much an Australian disease, associated with wheat since cereal cultivation began. The natural host range of *Ggt* is confined to Poaceae, especially wheat, barley, rye and oats. Several genera of grasses are susceptible to *Ggt* and it has been found to infect maize (Robinson and Lucas, 1967) and sorghum (Tarr, 1962). Root infection and perithecium formation on plants in other families including dicotyledons have been reported under sterile conditions (Nilsson, 1969; Zogg, 1969).

Take-all is widespread in the temperate cereal growing areas of the world but has also been found in Brazil and Kenya (CMI Map 334, ed.3, 1972). Extensive variation in pathogenicity within isolates was noted in the early studies of Davis (1925) and Padwick (1936) and the problem of attenuation in culture resulting in loss of virulence has often been encountered (Russel, 1939; Chambers, 1970). Differences within naturally-occurring populations are now well documented. The interplay between the effect of climatic conditions, soil factors, host susceptibility and change in virulence in the pathogen which ultimately results in disease in the field, are difficult to reproduce under laboratory conditions.

Under natural conditions infection *via* air-borne spores is not considered of much consequence due to microbial competition (Brooks, 1965), but spread through ascospores in cereals on the recently reclaimed polders of Netherlands, where the low number and activity of other micro-organisms provided minimum competition, has been documented by Gerlagh (1968). Soil-borne inoculum is the most important biological factor in the survival and spread of the disease.

Ggt survives between crops on cereal and grass residues. Most survival studies have been made with artificially colonized straws and these did not give reliable estimates of survival ability in naturally infected material (Macnish and Dodman, 1973). Macnish and Dodman (1973) found that there was viable *Ggt* on stubble after 52 weeks in 82% of sites sampled. Shipton (1972) confirmed this survival capacity under nonsusceptible break crops for up to 66 months and stated that survival seemed to have been in stubble rather than on weeds and self-sown cereals. These observations indicate that field survival may be much longer than initially realized with laboratory studies using artificially colonized straws. Survival of *Ggt* in wheat straw is greater when nitrogen is not limiting (Garrett, 1938; Chambers, 1971).

In Australia, take-all is generally more severe in light soils (Butler, 1961) and increases in severity with rising soil pH and decline in fertility (Garrett, 1942). There is general agreement that wet soil conditions favour take-all development in temperate cereal growing areas. Garrett (1934a) established a definite relationship between high spring rainfall incidence and occurrence of take-all, based on 33 years of South Australian records.

The effect of nitrogen - NH_4^+N and NO_3^-N on *Ggt* has been studied by Smiley and Cook (1973) and Huber and Watson (1972). One effect of NH_4^+N is to increase host resistance, as indicated by infected tissue, while NO_3^-N seems to increase susceptibility to *Ggt* (Huber and Watson, 1972). The final effect would be the result of many factors influenced by fertilizers.

Root damage can occur on plants at any growth stage; seedlings and young plants may be killed in patches (Walker, 1975). General stunting, reduction in tillering and appearance of dead bleached inflorescences (whiteheads) are the symptoms associated with severe take-all infection. "Whiteheads" or "hay-die" are seen under hot dry condtiions when nodal root development is restricted and water stress resulting from earlier root damage is severe (White, 1947). Infected root systems exhibit characteristic blackening of the stele. When infection is severe, the root system may be reduced. Discrete small lesions with slight darkening of stele may be seen in slightly infected roots.

Different measurements of infection have been used for *Ggt*: percentage of plants infected, number and percentage of infected roots per plant, disease indices and growth of runner hyphae on roots. Nillson (1969) suggested that the effects of the disease on seminal roots, nodal roots, subcrown internode and on above ground parts should be

recorded separately. Greater reduction in yield has been associated with nodal root damage (Garrett, 1942; Rothamsted Reports 1963-1966). Severe seminal root infection also reduces dry weight, leaf area, water content of shoots and tillering (Asher, 1972). Plants with infected seminal roots produced more crown roots than healthy ones (Asher, 1972).

Nilsson (1969) obtained a correlation between discoloration due to *Ggt* used as a disease rating and grain yield reduction. Significant regression coefficients for grain yield and percentage plants infected were obtained by Slope (1967) and Rosser and Chadburn (1968). Slope later (1973) pointed out that on some take-all soils, weather and soil conditions sometimes reduced grain yield more effectively than the pathogen.

Take-all development is greatly affected by previous cropping history (Slope & Etherbridge, 1971). Take-all has become most destructive in areas where intensive cereal cultivation has taken place with little reference to rotations (Hynes, 1935; Garrett, 1942). However, it was due to continuous susceptible cereal cultivation that a decline in the severity of take-all after a peak disease year was first noted (Slope and Cox, 1964; Cox, 1965). Take-all decline (T.A.D.) was confirmed by Gerlagh (1968) on cereals on the reclaimed polders in Holland and has now been observed in many parts of the World. In Australia it is of a far less stable kind than in Europe.

Successful infection by *Ggt* under laboratory conditions has been achieved using inocula developed on agar media and those developed on a base such as maize-meal, wheat or barley straw or cereal grains (Nilsson, 1969). The cereal based inocula, following colonization, are incorporated into soil as a mixture or layer (Skou, 1968) whereas the agar inocula have usually been introduced as a disc, on which seed is sown

(Garrett, 1934; Chambers and Flentje, 1967). The inoculum base used e.g. wheat straw, oat or barley kernals and soil, has been shown to affect the virulence of isolates differentially (White and McIntyre, 1943). Differential behaviour in terms of virulence by the same isolate in steam sterilized and natural soil was reported by Henry and McKenzie (1959). Blanche (1977) found significant interactions between isolates and John Innes composts that had been stored for different lengths of time when testing the composts for an effect on pathogenicity. The sensitivity of *Ggt* to microbial antagonism is well established (Slagg and Fellows, 1947).

Microbial activity is often higher where artificial inoculum is introduced into soil and where soil is contaminated following partial or complete sterilization. This problem might be further exacerbated if the inoculum substrate is not fully colonized or the nutrient status of the soils is too high. Thus it is not surprising that results vary greatly with type of inoculum, soil medium and the test conditions (Nilsson, 1969).

The occurrence and interrelationship of *Ggt* with the cereal cyst nematode, *H. avenae (Ha)* were first noticed on barley in England (Cook, 1969). Low levels of *Ha* were associated with high levels of *Ggt*. Decrease in natural and artificial nematode populations by high levels of take-all damage was shown in pot experiemnts (Cook, 1969; 1975).

Rhizoctonia solani was first reported as a cereal pathogen in Australia (Samuel, 1928) and is the cause of "purple or bare patches" (Samuel and Garrett, 1932; Hynes, 1933; Kerr, 1955; de Beer, 1965). *Rs* is a cosmopolitan species reported as a field pathogen of wheat in England (Dillon Weston and Garrett, 1943), Canada (Benedict and Mountain, 1956), and U.S.A. (Sprague, 1950; Bruehl, 1951). In Australia it is of

major concern in wheat in South Australia (McKnight, 1960) and parts of New South Wales.

The host range is extensive and includes wheat, barley and oats among the cereals. It has been recorded on a wide variety of plants such as *Hordeum leporinum* (barley grass); *Bromus* spp; *Cryptosterra calendula* (Cape weed); *Medicago sativa* (lucerne); *Pisum sativum* (garden pea) and *Trifolium* spp. (Ludbrook, Brockwell and Riceman, 1953).

Two basic general strains are associated with cereals in South Australia; for convenience referred to as the 'stem strain' and the 'root strain' (Kerr, 1955; de Beer, 1965; Chambers, 1966). Variation between the isolates of root-attacking strains has been recognized (de Beer, 1965). Most local studies have been confined to the root attacking strain. *Rs* is capable of extensive saprophytic growth in soil (Blair, 1943) and is widely distributed in natural soils. Its ability to survive competition in the saprophytic soil phase has long been recognized (Sanford, 1959). Suppression of its activity by addition of easily decomposable organic matter to natural soil, was also recognized and contributed to the development of antagonistic microflora (Blair, 1943; Sanford, 1952; Davey and Papavizas, 1960; Papavizas and Davey, 1960). Nitrate and ammonium nitrogen sources added with the organic amendments reduced the depressing effect of the organic material on *Rs* growth. The role of organic amendments in disease control seems to be quite complex.

Infections in soil are confined to immature root tissues and usually result in death of the root tips (Samuel and Garrett, 1932). Extensive rotting can proceed from the root tips leaving characteristic short root stumps. Discrete lesions are seen in the early stages of infection in which only necrosis or concaving of the cortex has occurred. Growth of

the plant can be greatly affected by *Rs*. General symptons given include stunting and yellowing of leaves; a spindly appearance of the plant and extensive rotting of the roots. The description of field and plant symptoms in South Australia (Samuel and Garrett, 1932); New South Wales (Hynes, 1933); England (Dillon Weston and Garrett, 1943) and Canada (Benedict and Mountain, 1956), are similar. Both in Canada and South Australia, the nematode, *Pratylenchus minyus (Pm)* is found in association with *Rs* in patches of unthrifty plants (Benedict and Mountain, 1956; de Beer, 1965).

Infection by *Rs* was favoured by low soil temperatures in the range of 12-18°C (Samuel and Garrett, 1932), and at low temperatures soil moisture was not a limiting factor to infection; but at high temperatures, infection was favoured by low levels of soil moisture (Hynes, 1937). *Rs* is more prevalent on the light alkaline sandy, mallee-type soils of pH 8.0-9.0 in South Australia and the lime deficient soils of pH 6.0-6.3 in New South Wales (Samuel and Garrett, 1932; Hynes, 1937).

Kerr (1955) examined the population of the root-attacking strain in a cereal field with patches and found a higher population inside the patches. De Beer (1965) found similar differences, although he noted that the higher populations occurred on the periphery of the patches rather than in the centre.

Survival of the root attacking strain was studied by Kerr (1955), and Flentje and Saksena (1957). They introduced the fungus into cereal fields at Waite Institute, but it failed to survive from one season to the next. De Beer (1965) also studied its mode of survival and concluded that it persists in soil as hyphae or resistant cells in organic matter e.g. stubble and crowns of cereals and grasses. De Beer (1965) found no evidence that sclerotia were formed in nature.

Rs is often found in association with other pathogens. Its association with *Pm* has been mentioned previously. Synergism to some degree has been demonstrated with *Heterodera schachtii* on radishes (Polychronopoulos *et al.*, 1969), and *G. rostochiensis* on potatoes (Dunn and Hughes, 1967), with *Fusarium oxysporum* f. sp. *vasinfectum* in cotton (Sabet and Khan, 1969), and with *F. solani* in tomato (Goujon, 1967), and with *Oospora pustulans* in potatoes (Dunn and Hughes, 1967).

On wheat, *Rs* and *H. avenae* in combination caused greater reduction in tillering, plant height, fresh weight, and root number and length, than when acting alone under glasshouse conditions (Meagher and Chambers, 1970; Meagher *et al.*, 1978).

H. avenae (Ha) commonly known as cereal cyst nematode was first reported in South Australia by Davidson and Hickinbotham in 1930. It is a serious pathogen of cereals in Europe, India and Australia and has been found in U.S.A., Canada and Japan.

Several pathotypes have been recognized in Europe and India. In Australia only one pathotype has been found (McLeod and Khair, 1977). The U.S.A. and Canada, where the occurrence of *H. avenae* is limited, have only one pathotype.

The cereal hosts include wheat, barley, oats and rye. Maize can be attacked but it is not a good host (Johnson and Fushtey, 1967; Gill and Swarup, 1971; Behringer *et al.*, 1975). Also a wide range of grasses have been reported as hosts.

In southern Australia the mobile second stage larvae emerge from the dormant cysts from May to June and enter the roots just behind the growir tips (Banyer and Fisher, 1971). Larvae are capable of surviving in soils at low temperatures (5°C) with little loss in infectivity up to seven weeks but at higher temperatures they are short lived (Davies and Fisher,

1976). Not all eggs in a cyst hatch, about 15% remain dormant. Giant cells develop in the host in response to larval infection and the characteristic root swellings or knots are used in diagnosis. In August and September the swollen white sedentary, egg-bearing females are found protruding from the roots. Much branching of the root system occurs under a heavy attack of *Ha* and this together with the swollen knots indicate the presence of *Ha*. Usually the seminal roots are more affected than nodal roots. The temperature and moisture requirements for larval hatch are rather complex (Banyer and Fisher, 1971). Only one generation per year is completed. Although *Ha* is more often a serious pest in lighter soils, it can be found in heavier soils (Meagher, 1968).

Fertilization is necessary for reproduction. The ratio of males to females produced in the roots is variable and is affected by the host, population density and moisture. Under laboratory conditions the ratio of males to females produced on susceptible wheats was 1.33 males to 1 female but on resistant wheats the ratio was 20 males to 1 female (Brown, 1974). Jakobsen (1972) noted that under excess moisture conditions the ratio was 1 : 0.63 (2 x normal water supply) and under low moisture conditions it was 1 : 3.26 (half normal water supply). With increasing population density more males are also produced (Lindhardt, 1961). The sex ratio can also be altered by the presence of fungi in roots. Cook (1975) observed changes in sex ratio of *Ha* due to the take-all fungus. Alteration in the sex ratio of *H. rostochiensis* in the presence of *R. solani*, *Verticillium alboatrum*, or a grey sterile fungus is reported by Ketudat (1969).

It is necessary for the female to continue feeding, after protruding from the surface of the roots, for both egg survival and cyst formation, (Banyer and Fisher, 1976). Disruption of food supply or

changes in the host may affect the size and survival of *Ha*.

Under an initial heavy attack, aboveground symptoms such as yellowing of leaves and a general reduction in growth may be seen under field conditions. There are no specific symptoms that can be attributed to a low or mild attack. Intensity of attack on the seminal roots can be compensated for by the coronal or nodal roots, thus the relationship between damage and yield is not easily correlated (Williams and Salt, 1970). Reports of population levels at which plant growth is affected are variable due to effect of climatic conditions, nutrition, soil type, extraction methods, cultivars tested and other pathogens. Degree of invasion is not necessarily related directly to populations (Stone, 1968).

Duggan (1961) was among the first to quantify the relationship between *Ha* numbers (eggs/g of soil) and cereal growth and yield. At the level of 4 eggs/g of soil no symptoms were seen and yield was not affected in wheat. Hesling (1957) showed that height was affected in wheat with increasing density of larvae/g of soil.

Seinhorst (1965) suggested that the yield of plants was unaffected when nematode density at planting or sowing was below a certain threshold. Above this threshold, yield decreased exponentially with density. This hypothesis with *Ha* on wheat needs to be tested experimentally over a range of densities.

The association of *Ha* and *Ggt* in barley has been mentioned previously. The presence of *Ggt* not only affects the male-female sex ratio of *Ha* but also the multiplication rate (Williams and Hornby, 1970; Cook, 1975). The earliest record of *Rs* and *Ha* occurring in patches was by Davidson (1930). Kimpinski found *Ha* in patches of poor growth of wheat with *Rs*, *Ggt* and *Pm*. Stynes (1975) emphasized that these four pathogens frequently

occurred together. Interrelationship between *Ha*, *Fusarium moniliforme* and *Helminthosporium gramineum* on barley has also been studied (Gill and Swarup, 1977). Each of the fungi suppressed multiplication of *Ha* but in the presence of both fungi, cyst recovery was greater. The effect of *H. schachtii* in combination with *Rs* on young sugar beets (Polychronopoules *et al.*, 1969) resulted in more serious damage; the nematode provided avenues for entry of fungi and an improved substrate for colonization deep into the cortex.

Pratylenchus minyus (Sher and Allen, 1953) (*Pm*) is regarded as being conspecific and a synonym of *Pratylenchus neglectus* (Rensch, 1924) Filipjev and Shuurmans Stekhoven, 1941 (Hopper, 1971). Both names occur in current literature, *P. minyus* consistently in North America, England, South Africa and Australia, and *P. neglectus* in continental Europe. It is mainly found in the temperate regions of the world. Its distribution in Canada is well documented in tobacco, cereal and forage growing areas (Olthof and Hopper, 1973; Potter and Townshend, 1973; Willis *et al.*, 1976).

Pm is primarily a parasite of grasses including cereals, forage, turf and wild grasses (Loof, 1960; Wetzel, 1962; Townshend *et al.*, 1973). It is also found in other crops such as crucifers, flowers and legumes (Goodey *et al.*, 1965); it is pathogenic to tobacco (Mountain, 1954), and destructive to peppermint (Falkner and Bolander, 1969).

Distribution of this migratory, endoparasitic nematode has been recently studied in Denmark (Andersen, 1979a,b,c), West Germany (Dern, 1977) and Italy (Inserra *et al.*, 1978), in cereal growing areas.

Pm exhibits a considerable capacity to survive under varied soil conditions and temperature (Meagher, 1970; Townshend, 1973; Fuchs, 1975). It multiplies readily on many crops (Mountain, 1954; Townshend and Potter,

1976). It is an obligate parasite feeding first externally then becoming a migratory endoparasite. Its life history may be completed in 28 days (Mountain, 1954). The attack on roots of maize, tobacco (Mountain, 1954) and red kidney bean (Varo Alcala *et al.*, 1970), has been extensively studied. In maize *Pm* prefers to penetrate the feeder roots rather than the coarser lateral or main roots.

In peppermint low populations (7462 nematodes/plant) caused an increase in dry weight of 17-25% over that of uninfected plants. Only when nematodes exceeded 14,000 in number, was a reduction in dry weight recorded (Faulkner and Skotland, 1965). Among the cereal species in glasshouse tests, the tolerance limit in barley appears to be less than 4500 nematodes per 200 gms of soil (Andersen, 1979c). Yield loss in cereals in parts of Germany was associated with more than 500 *Pm* in 250 gms of soil (Dern, 1978). Occurrence of *Ha* with *Pm* together in cereals was found in Italy (Inserra *et al.*, 1978). *Pm* reduced growth of *Triticum durum* in glasshouse tests.

In wheat, inoculations with 1000 nematodes per plant did not effect growth (Kimpinski, 1972). Stynes (1975) reported an increase in dry weight of wheat plants, similar to that found in peppermint, at low population levels (500-1000 nematodes).

Benedict and Mountain (1956) studied the root rot complex of *Rs* and *Pm* on wheat in the field. Their combined effect was found to be additive. In glasshouse studies on wheat, these two pathogens together reduced yield and size of grains (Kimpinski, 1972). Only in peppermint is the role of *Pm* in the wilt disease complex clear. Even when the two pathogens, *Pm* and *Verticillium dahliae* f *menthae* were kept separate on different parts of the root system, the incidence and severity of wilt increased when *Pm* was present. It also reduced the incubation period for the fungus and reproduction of *Pm* increased in infected plants

(Faulkner and Skotland, 1965; Faulkner *et al.*, 1970). Kimpinski (1976) found a positive correlation between *Ggt* and *Pm* in patches in South Australia. The association of *Pm* with *Rs* in wheat plants (de Beer, 1965), was confirmed by Kimpinski (1972).

The distribution of *Pm* in seminal and nodal roots of wheat was recorded by both Kimpinski (1972) and Stynes (1975). Higher numbers were recorded in the seminal roots and there was a marked variation in numbers. *Pm* has been cultured monoxenically on lucerne callus (Andersen, 1972a) and on excised roots of maize, tobacco and clover (Mountain, 1954).

Ggt, *Rs* and *Ha* have been individually associated with 'patches' in the classical descriptions of diseases caused by them in Australia : *Ggt* with "more or less circular patches" (Butler, 1961); *Rs* with "purple or bare-patch" (Samuel and Garrett, 1932; Hynes, 1933), and *Ha* with "no-growth patches" (Hickinbotham, 1930).

Davidson (1930) reported that some plants from patches had eelworms on them and others a fungal disease due to a species of *Rhizoctonia*. De Beer (1965) referred to the constant association with *Pm* and *Rs* in plants from patches. Kimpinski (1972) established the presence of *Ggt*, *Rs*, *Ha* and *Pm* in patches in South Australia. Stynes (1975) found that these four pathogens did occur together on wheat plants generally and were not confined to patches. These are the predominant pathogens on wheat. Certain species of *Fusarium* and *Helminthosporium* are also found but occur to a lesser extent.

The aim of this project was to study the interrelationships between nematodes and fungi. The complex of pathogens found on wheat provided the basis for the study and a field survey was undertaken to examine any association between them. To maximize the information on pathogen

distribution, it was decided that the survey should include 'patches' and adjacent areas of good growth. Higher pathogen populations have been recorded in patches. The reason for choosing patches and adjacent areas of good growth was to determine whether pathogens were responsible for causing patches and whether stronger or more associations among pathogens occurred at higher or lower densities. Interrelationships that emerged from statistical analysis of the field data as being important were then studied under laboratory conditions.

Most pathogen association or relationships studies have been conducted at one density of each pathogen. The literature abounds with experiments of the following format: Nematode alone; fungus alone; nematode + fungus; uninfected control. Conclusions are drawn on the basis of the effects measured at one density or level. As suggested by Wallace (1983) interaction studies should be conducted at several levels of each pathogen in a factorial experiment with adequate replication and the effects on plants should be measured at intervals.

Considerable time and effort was spent on selection of sites. They needed to be representative of the area. Patches of ~~good~~ poor growth associated with patches of good growth was the main consideration but the size of the patch needed to be such that sampling could be carried out over the whole growing season without affecting later samples.

CHAPTER II

FIELD DISTRIBUTION

INTRODUCTION

A cohesive area in which sampling sites showed similar environmental and soil conditions and which was situated close to Adelaide and had a history of 'patches' in fields was chosen for study. The first object was to investigate interactions between pathogens in the field and secondly to examine the part that pathogens played in the occurrence of patches.

2.1 Methods and Procedures

A. Site Selection and Sampling Procedures

Field data were collected in both 1979 and 1980 in the immediate cereal growing area north of Murray Bridge (Hundred of Finiss and Mobilong), which is situated 70 kilometres east of Adelaide (Fig.1). The eleven paddocks sampled per year contained 'patches' of poor growth of wheat. Each 'patch' was marked and numbered as was the adjacent area of good or normal growth from which samples were taken. A typical patch is shown in Plate 1. The survey area contained two principal soil types - red brown earths and calcareous mallee soils. (SEE PARAGRAPH ON FACING PAGE.)

A total of twenty plants was collected from each paddock; ten from a 'patch' and ten from the adjacent normal growth area. Samples were collected at intervals of four weeks from sowing over three months during the early part of the growing season. The first sampling was carried out in late July in both years when 'patches' were clearly visible. Plants were randomly selected from within patches and healthy areas. Plants were carefully dug up to include most of the root system

Fig.1: Map of study area for 1979 and 1980 showing sampling sites.

1

● 1979
▲ 1980

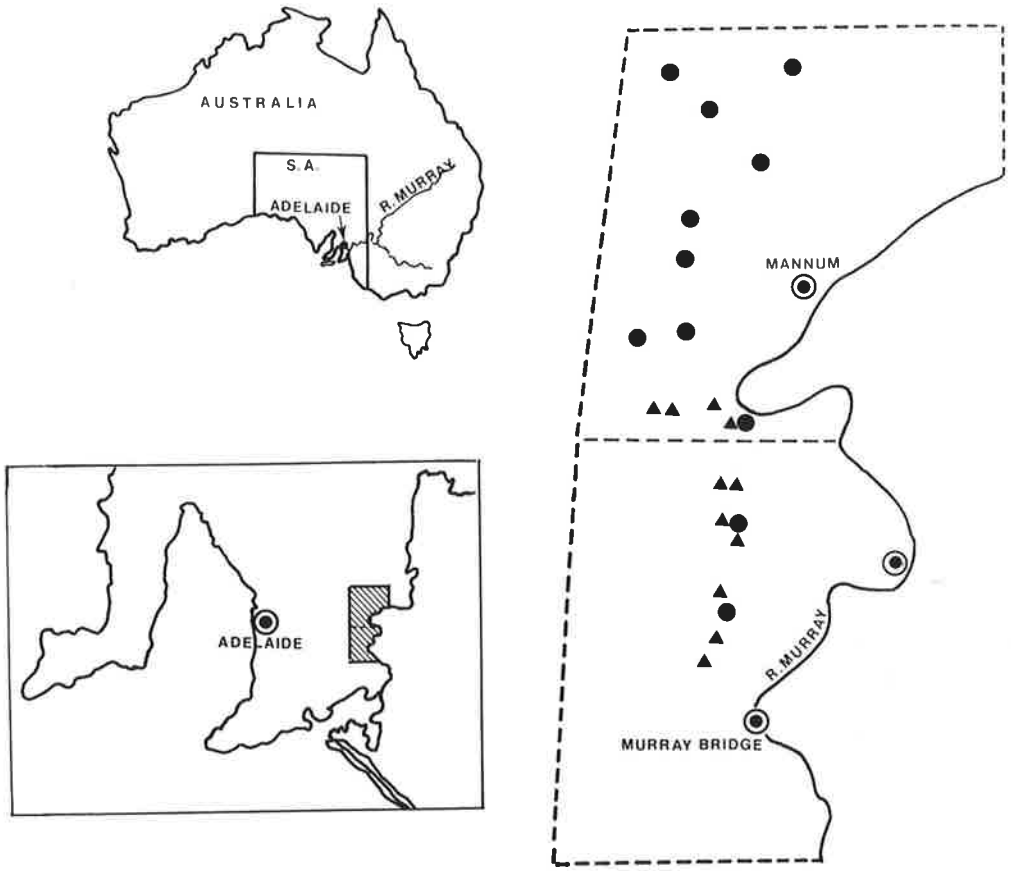

AUSTRALIA
S.A.
ADELAIDE
R. MURRAY
ADELAIDE
MANNUM
R. MURRAY
MURRAY BRIDGE

Plate 1: Paddock with a 'patch' of poor growth.

and the surrounding soil and placed in plastic bags for transport back to the laboratory.

B. *Measurements of Plant and Pathogen Variables*

Plants were carefully washed and excess moisture removed by blotting before the fresh weight of the whole plant and of the root system was recorded. After drying at 110°C for 72 hours, the dry weight of the shoot was recorded. The number and length of both the seminal and coronal roots were noted separately. Only the length of the main root was measured, not the laterals.

Roots were placed lengthwise in a rectangular perspex dish, 20 cms long and 10 cms wide which had 1 cm^2 grid markings on its base. The fungal pathogens, *Ggt* and *Rs* were assessed by measuring the length of the root lesions caused by each of them on the seminal and coronal root systems. The number of intervals containing whole or a portion of a lesion were counted. For *Ggt*, a measured lesion referred to the extent of blackened vascular tissue. *Rs* was recognized by cortical breakdown, necrosis and in advanced cases by the snapped and rotted end, that is characteristic of *Rs* damage. The identification of both these fungi was supported by isolations from characteristic lesions.

Direct counts of all *Ha* and *Pm* were carried out by staining the nematodes within the root systems with lactophenol cotton-blue (Southey, 1970). The swollen larvae of *Ha* were conspicuous and easily separated from *Pm*. Males of *Ha* were not counted. Numbers of *Pm* included larvae.

C. *Statistical Procedures*

Discriminant Analysis was performed initially on the data to test

the adequacy of the variables to measure the visual differences between 'patchy' areas and adjacent areas of good growth. It was also used to examine the extent to which it was possible to distinguish patches from non-patches on the basis of the recorded measurements of pathogen density (Kendall and Stuart, 1968; Blakith and Reyment, 1971.)

Correlation Coefficients were obtained to demonstrate the presence or absence of a relationship between the variables, and also to provide an exploratory indication of the closeness of relationship between variables, especially among the pathogen variables. Correlations also indicated the type of relationship between the variables.

Principal Component Analysis was used mainly to examine the interrelationships between all the variables measured i.e. plant parameters and pathogen level variables; and to see if a clearer pattern of relationships emerged. This method of analysis is general for it makes no assumptions about the original variables, neither does it test any hypothesis. It is simply a different way of expressing the same set of results (Clifford and Stephenson, 1975; Daultrey, 1976.)

Principal component analysis may reduce dimensionality of a space by forming linear combinations of the original variates which attempt to account for most of the variation in the data set. The first principal component is the line or axis on which the projections of the data points have maximum spread. Subsequent components are projected orthogonally and describe successively smaller amounts of variation in the data. This redescription of the data thus produces a set of uncorrelated principal components. Usually the first few components account for a large proportion of the variation in the system. The contribution of the original variables to this variation is determined by the magnitude and sign of their coefficients in each component.

Thus the interrelationships between the variates can be studied by comparing the principal component coefficients.

As the predetermined variables measured were separated out initially into two separate groups (growth and pathogen parameters), principal component analysis was applied separately for indications of possible relationships between growth variables and pathogen variables that occurred in the field and which were not consistently identified by correlations. Inconsistent variable relationships may also be better explained by this analysis.

Multiple Regression Analysis was used so that all pathogen variables which substantially contributed to the variance, could be identified and also to find out to what extent pathogens were responsible for variation in growth. All four pathogens measured were the independent variables in the equation against the dependent variable of dry shoot weight. The analysis was also carried out with interaction terms of pathogens (*Rs*, *Ha* and *Ggt*) for the 2nd and 3rd sampling times to see if any interactions contributed to the variance.

2.2 RESULTS

A. Discriminant Analysis

When all parameters were analysed, the differences between areas of good and poor growth at each sampling in each year were distinct (Figs. 2 to 7). When only the plant parameters, plant weight and length and number of roots were analysed, separation of the two types of growth was still distinct. When the analysis was applied only to pathogen variables, the separation was not as distinct with considerable overlapping between the two areas. Greater overlapping in pathogen measurements occurred at the first and third sampling in 1979.

In the second year, all samples with all variables included, clearly illustrated that patches and non-patches were different. Discriminant analysis with only growth parameters also clearly separated the two areas. With pathogen variables only, the trend to separation of the two areas persisted but considerable overlap remained although less than in 1979.

Although patches in the study area were not entirely due to pathogens, higher numbers of organisms (*Ha*, *Rs* and *Ggt*) were found in the roots of plants from patches, but plants from adjacent areas of normal growth also had these pathogens on them. There were no plants without any pathogen damage in either year.

Discussion

This analysis confirmed that the measurements used were adequate, that the chosen areas of patches and non-patches were statistically different and clearly indicated that pathogens alone were not responsible for the occurrence of patches. Some other factor or factors, not measured, had an important influence in the manifestation of patches. The added stress on the wheat plant by pathogens in high salinity areas was offered as a plausible explanation for the occurrence of patches by Kimpinski (1972) who observed that patchy areas could be distinguished in paddocks within two weeks after crop emergence. Because of the lack of association between pathogens and patches, all data were pooled and further analysis carried out on all samples from the eleven paddocks, ignoring the separation of patches and non-patches.

Figure 2: Histogram of results of Discriminant Analysis at first sampling time, July 1979.

A: All variables (growth + pathogens)

B: Growth variables only

C: Pathogen variables only

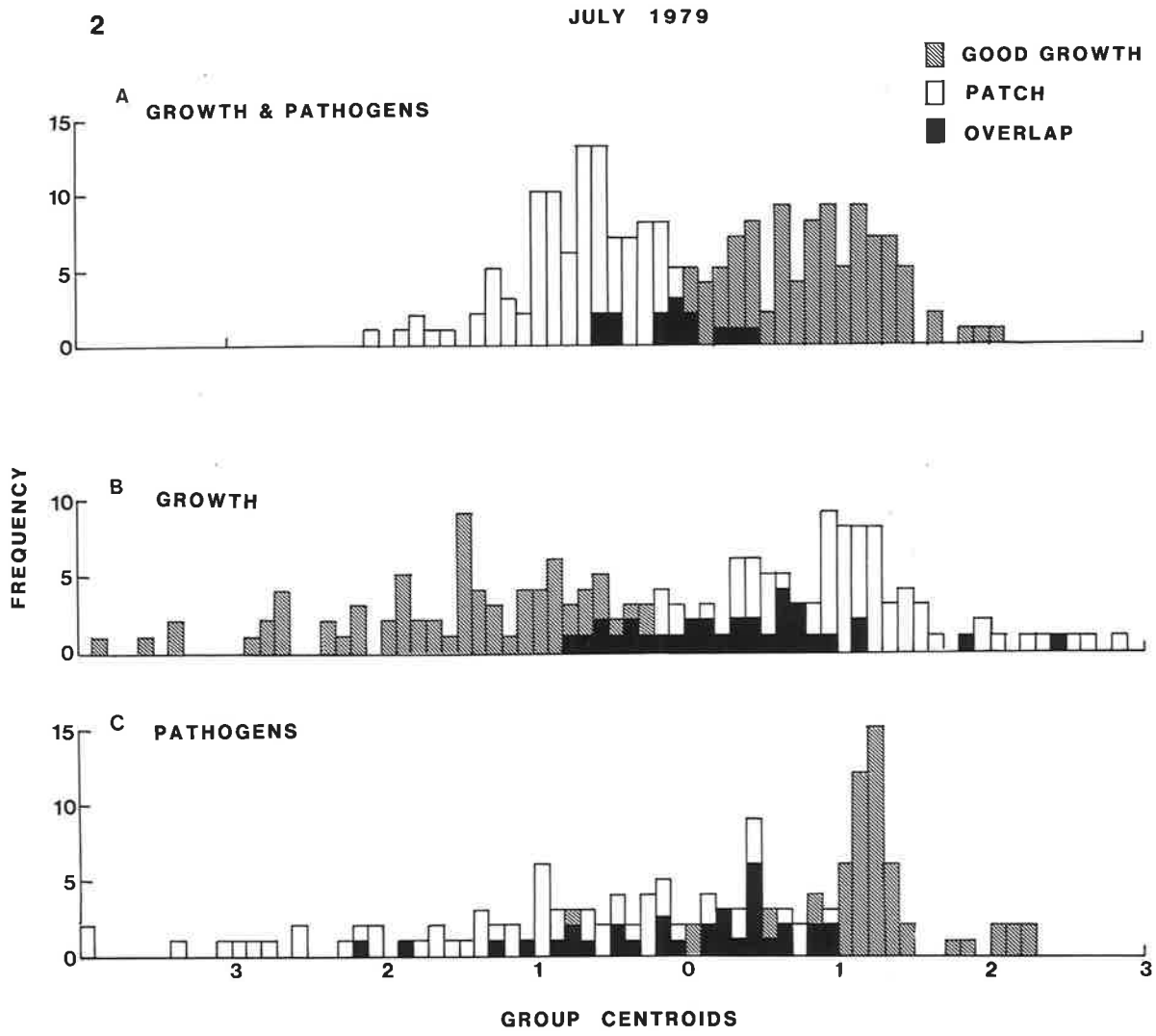

2
JULY 1979
GOOD GROWTH
PATCH
OVERLAP
A
GROWTH & PATHOGENS
15
10
5
0
B
GROWTH
10
5
0
FREQUENCY
C
PATHOGENS
15
10
5
0
3
2
1
0
1
2
3
GROUP CENTROIDS

Figure 3: Histogram of results of Discriminant Analysis at second sampling time, August, 1979.

A: All variables (growth + pathogens)

B: Growth variables only

C: Pathogen variables only

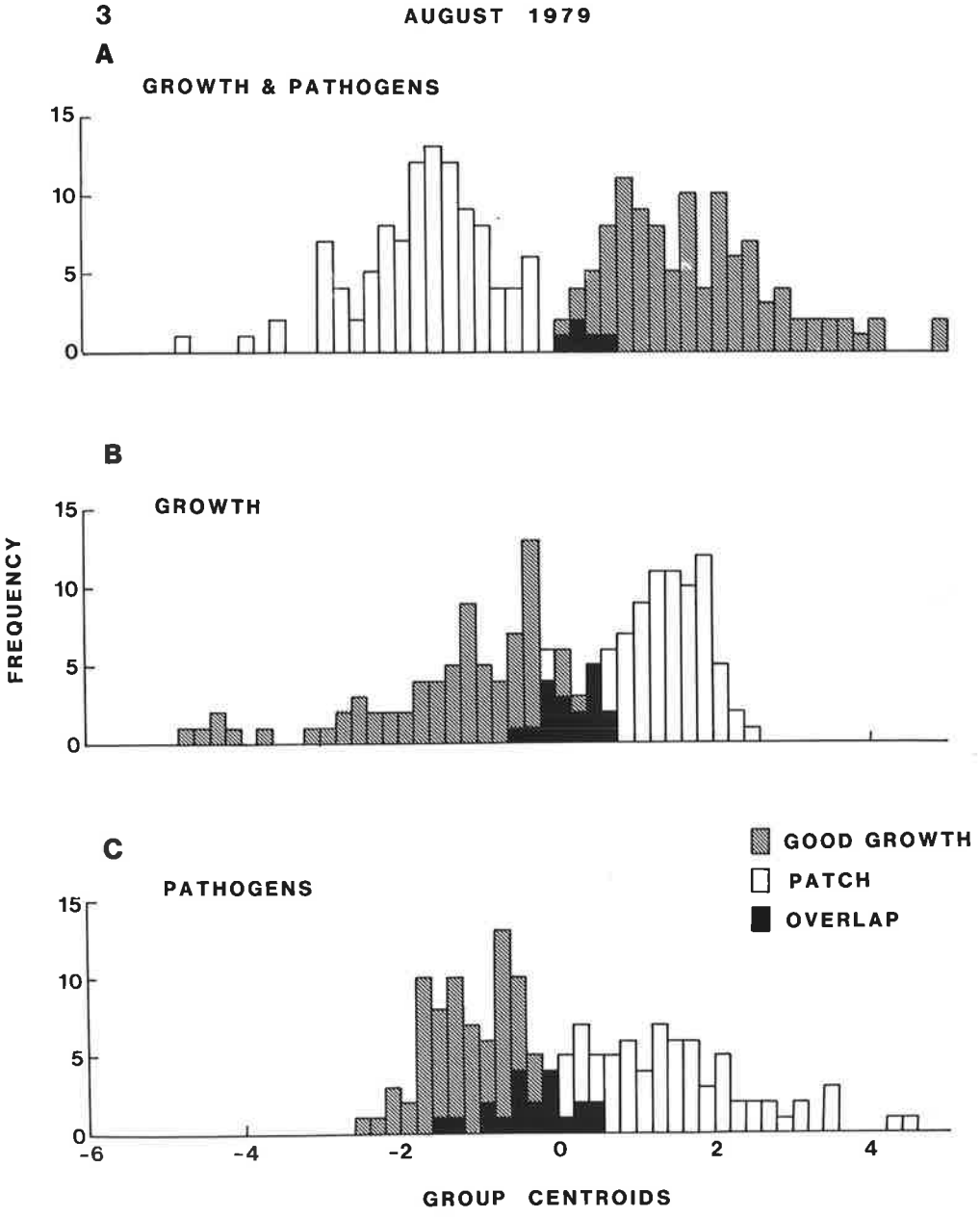

3
AUGUST 1979
A
GROWTH & PATHOGENS
15
10
5
0
B
GROWTH
FREQUENCY
C
PATHOGENS
GOOD GROWTH
PATCH
OVERLAP
-6
-4
-2
0
2
4
GROUP CENTROIDS

Figure 4: Histogram of results of Discriminant Analysis at third sampling time, September, 1979.

A: All variables (growth + pathogens)

B: Growth variables only

C: Pathogen variables only

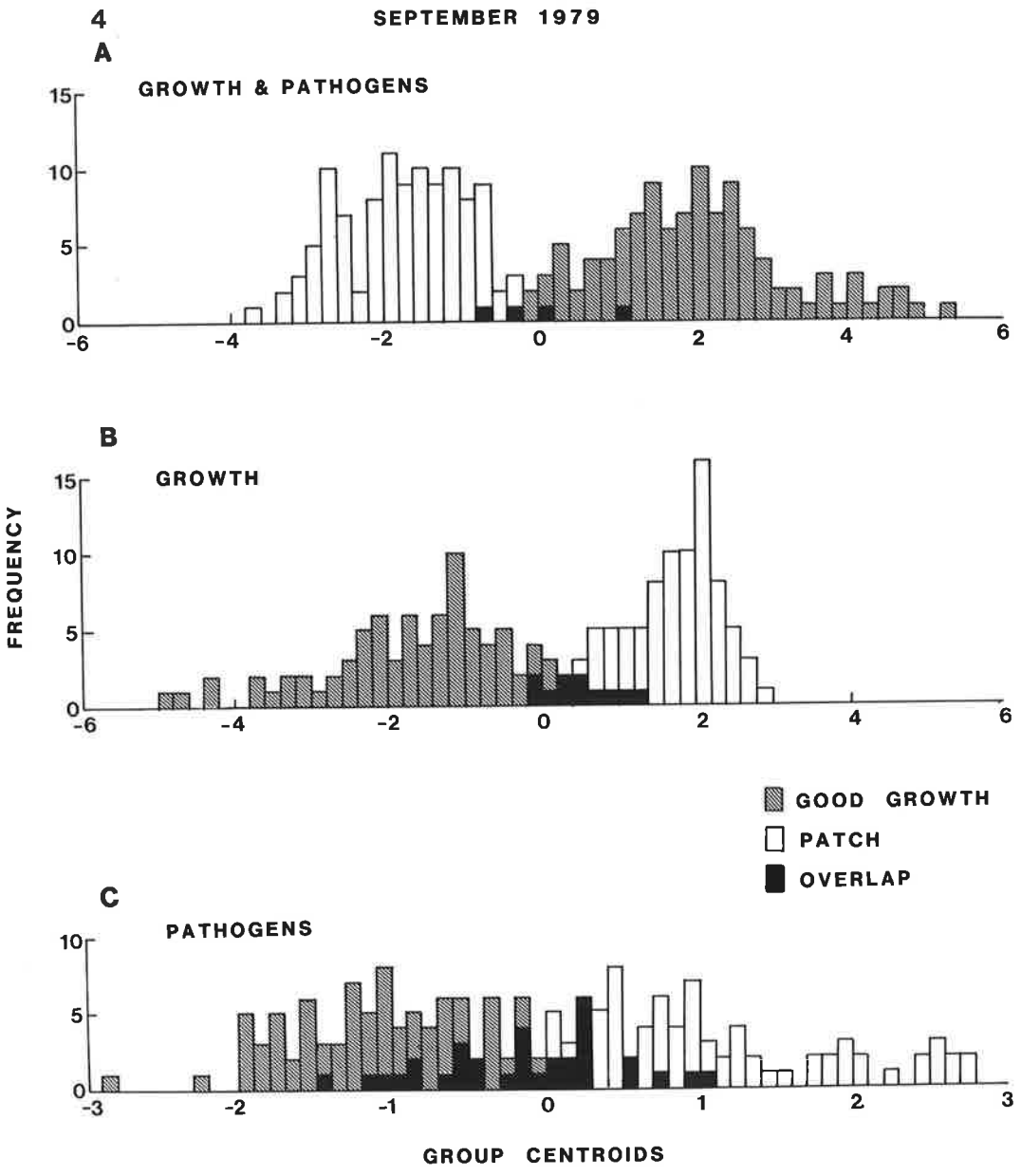

4
SEPTEMBER 1979
A
GROWTH & PATHOGENS
15
10
5
0
-6
-4
-2
0
2
4
6
B
GROWTH
FREQUENCY
15
10
5
0
-6
-4
-2
0
2
4
6
GOOD GROWTH
PATCH
OVERLAP
C
PATHOGENS
10
5
0
-3
-2
-1
0
1
2
3
GROUP CENTROIDS

Figure 5: Histogram of results of Discriminant Analysis at first sampling time, July, 1980.

A: All variables (growth + pathogens)

B: Growth variables only

C: Pathogen variables only

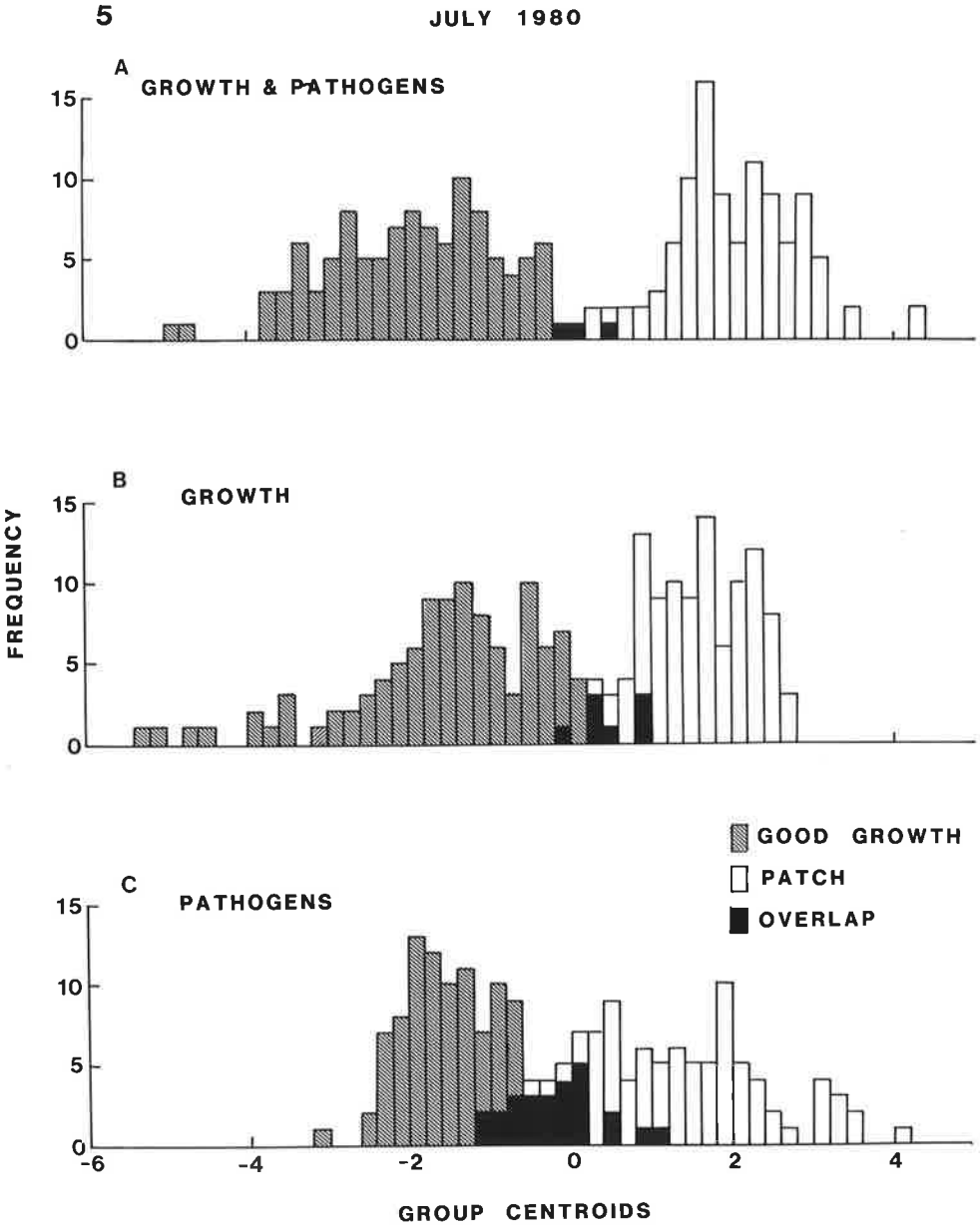

5
JULY 1980
A
GROWTH & PATHOGENS
15
10
5
0
B
GROWTH
FREQUENCY
C
PATHOGENS
GOOD GROWTH
PATCH
OVERLAP
-6
-4
-2
0
2
4
GROUP CENTROIDS

Figure 6: Histogram of results of Discriminant Analysis at second sampling time, August, 1980.

A: All variables (growth + pathogens)

B: Growth variables only

C: Pathogen variables only

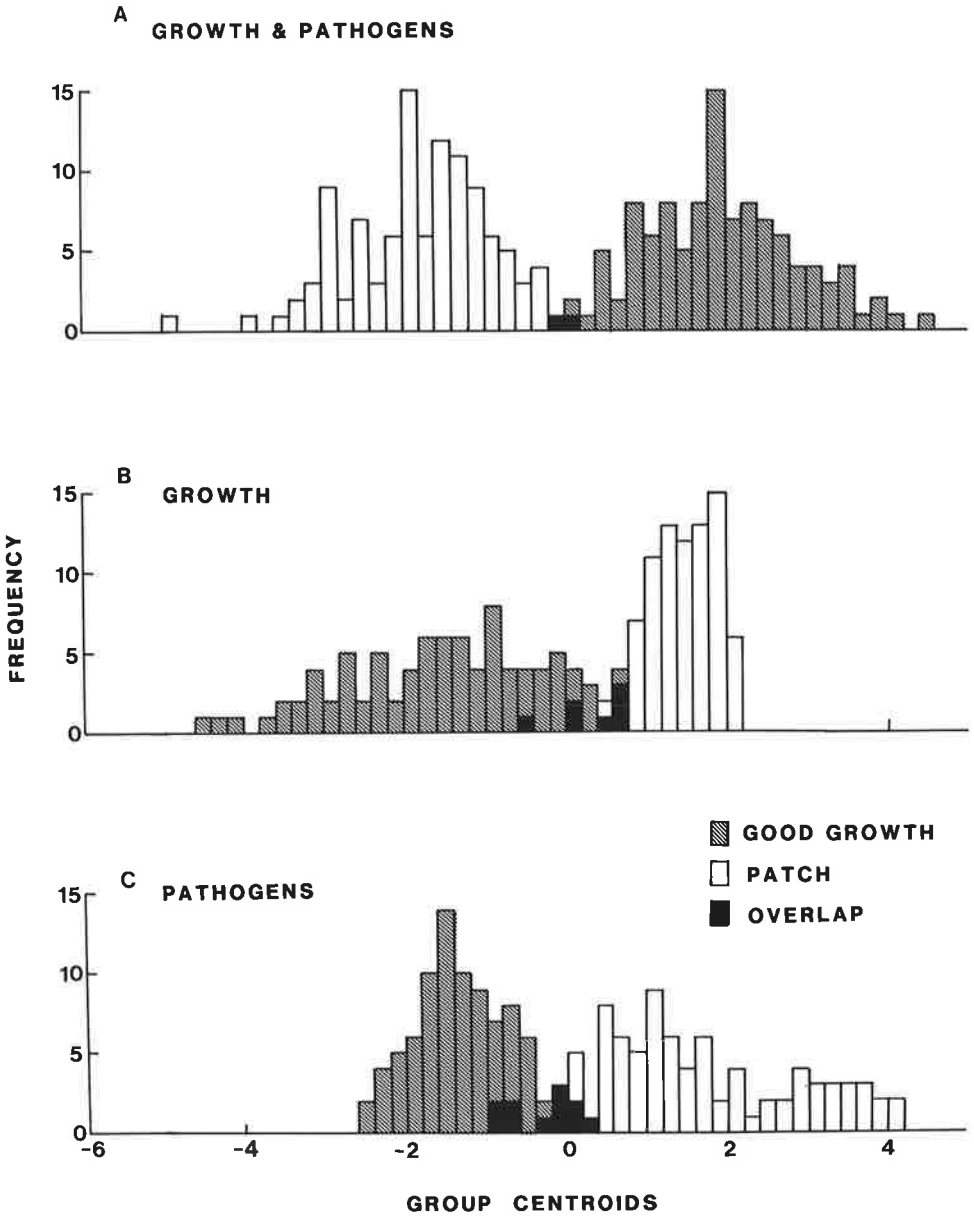
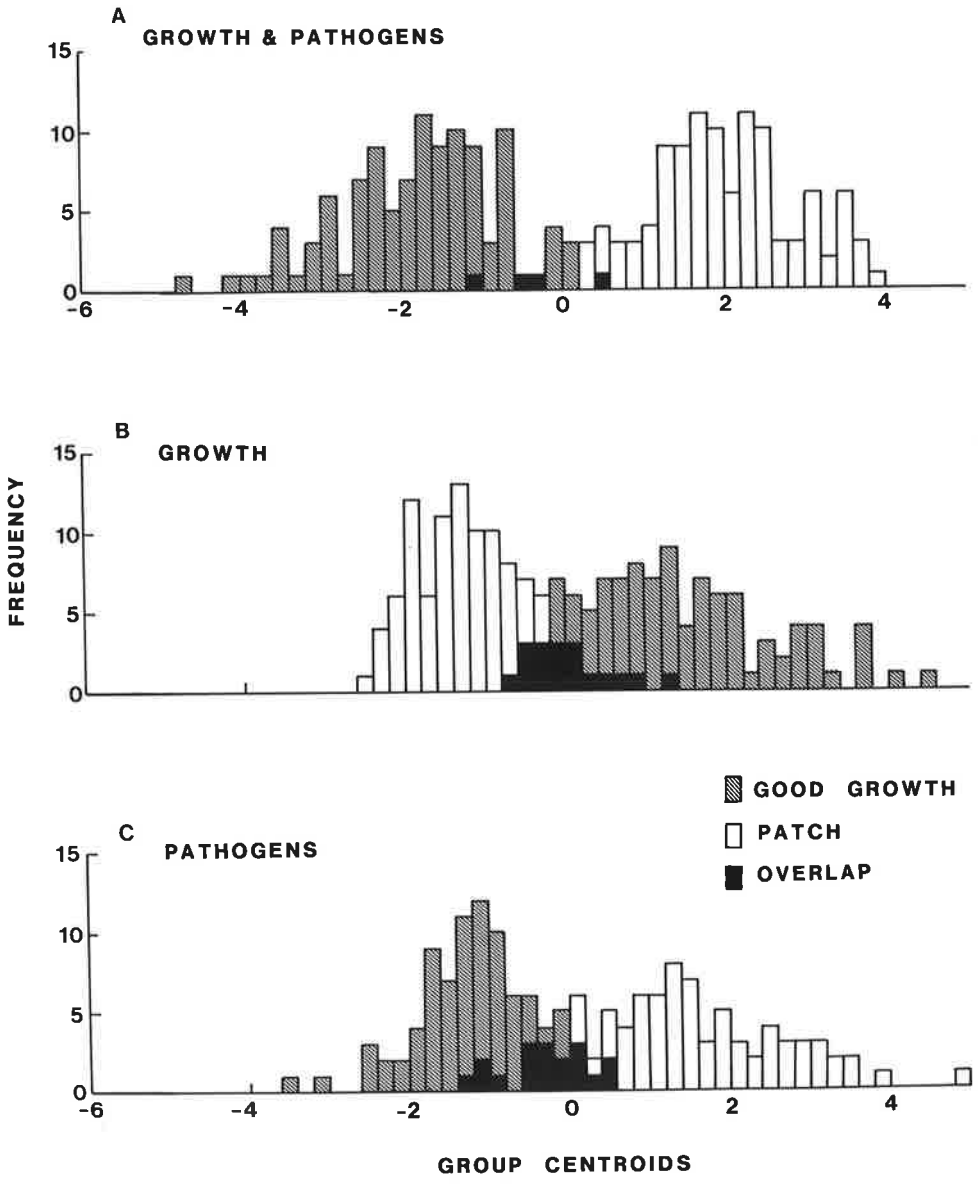

A
GROWTH & PATHOGENS
15
10
5
0
B
GROWTH
15
10
5
0
FREQUENCY
C
PATHOGENS
15
10
5
0
-6
-4
-2
0
2
4
GROUP CENTROIDS
GOOD GROWTH
PATCH
OVERLAP

Figure 7: Histogram of results of Discriminant Analysis at third sampling time, September, 1980.

A: All variables (growth + pathogens)

B: Growth variables only

C: Pathogen variables only


A
GROWTH & PATHOGENS
B
GROWTH
C
PATHOGENS
FREQUENCY
15
10
5
0
-6
-4
-2
0
2
4
GOOD GROWTH
PATCH
OVERLAP
GROUP CENTROIDS

B. *Means of Variables*

Year 1, 1979 - Rainfall in April and May was adequate to give a reasonable opening to the season but June and July were relatively dry months with little rainfall. August, September and October had adequate rainfall (Table 1). The variables recorded on the plants in July, August and September are listed in Table 2 and the means are given in Table 3.

At the first sampling time at the end of July only seminal roots were present. Variation in the plant variables between paddocks was not extreme but the pathogens did vary. The mean length of *Ggt* lesions was small and only 21 of the 220 plants sampled were infected with *Ggt* (Table 4); *Rs* and *Pm* were found on all plants and this is reflected in the length of *Rs* lesions and the numbers of *Pm* in the root systems. Numbers of *Ha* in the root systems were variable but were mainly small with more nematodes in plants from patches.

At the second and third sampling times, in August and September, coronal roots were present. At the second sampling time 60 plants out of 220 plants (Table 4) had *Ggt* lesions on the seminal roots. There was a corresponding increase in the lesion length as well (Table 3). *Rs* was still the dominant fungal pathogen and like *Pm* was found in all plants. Swollen sedentary females of *Ha* were seen in the seminal roots. Males were also observed in the roots but were not counted.

Plants from patchy areas had fewer coronal roots. *Rs* was the main pathogen found on these roots and only 34 plants out of 220 plants showed *Rs* root lesions (Table 4).

At third sampling time in 1979 there was almost a ten-fold increase in *Ggt* infection with most plants having at least one lesion of *Ggt*. *Rs* was found in all plants sampled, the incidence of this fungus

TABLE 1: Average monthly rainfall (mm) during 1979 and 1980

	MONTH											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1979												
Murray Bridge	22.2	36.2	15.0	20.8	36.6	4.8	12.2	54.6	78.4	66.0	37.4	3.8
Mannum	29.4	29.2	16.2	20.4	38.2	6.6	6.6	58.8	88.3	48.8	39.0	6.2
1980												
Murray Bridge	1.8	2.6	2.2	62.6	49.0	50.0	12.6	15.0	17.8	83.7	9.6	18.0
Mannum	1.8	1.4	6.2	107.6	33.6	36.4	15.0	11.2	11.0	82.4	5.2	7.0

The survey areas lay between Murray Bridge and Mannum.

TABLE 2: Variables recorded on wheat plants collected in survey area over 2 years

Variable number	Variable		
1st Sampling time:			
1	Fresh total weight of plant	(g)	
2	Fresh root weight	(g)	
3	Dry shoot weight	(g)	
4	Number of roots		
5	Length of primary roots	(cm)	(S)
6	Length of *Ggt* lesions	(cm)	(S)
7	Length of *Rs* lesions	(cm)	(S)
8	Number of *Pm*		(S)
9	Number of *Ha*		(S)
2nd and 3rd Sampling time:			
1	Fresh total weight of plant	(g)	
2	Fresh root weight	(g)	
3	Dry shoot weight	(g)	
4	Number of seminal roots		
5	Length of seminal roots	(cm)	
6	Length of *Ggt* lesions	(cm)	(S)
7	Length of *Rs* lesions	(cm)	(S)
8	Number of *Pm*		(S)
9	Number of *Ha*		(S)
10	Number of coronal roots		
11	Length of coronal roots	(cm)	
12	Length of *Ggt* lesions	(cm)	(C)
13	Length of *Rs* lesions	(cm	(C)
14	Number of *Pm*		(C)
15	Number of *Ha*		(C)

(S) - On seminal roots
(C) - On coronal roots

TABLE 3: Means and range of variables sampled 3 times per year for 2 years

1979	1	2	3	4	5	6	7
July	.449 (0.323- 0.574)	.168 (0.127-0.209)	0.0348 (0.0252-0.0445)	4.62 (4.26-4.98)	24.66 (20.83-28.49)	0.105 (0.036-0.173)	1.636 (1.21-2.05)
August	1.83 (0.84-2.81)	0.401 (0.267-0.536)	0.2611 (0.1158-0.4064)	4.68 (4.48-4.88)	28.26 (24.87-31.64)	0.400 (0.264-0.536)	2.40 (1.92-2.87)
September	6.82 (3.03-10.61)	1.267 (0.650-1.882)	1.195 (0.491-1.890)	4.95 (4.59-5.32)	27.88 (24.79-30.98)	2.50 (1.75-3.26)	2.08 (1.89-2.27)
1980							
July	2.35 (1.00-3.69)	0.561 (0.465-0.657)	0.2317 (0.0798-0.3836)	5.79 (5.74-5.83)	26.54 (19.54-33.53)	3.81 (2.29-5.33)	1.34 (0.99-1.69)
August	4.169 (1.67-6.66)	0.867 (0.533-1.202)	0.631 (0.211-1.052)	5.80 (5.64-5.97)	21.54 (19.56-23.52)	3.56 (1.12-5.01)	1.37 (1.09-1.66)
September	6.04 (3.15-8.93)	0.986 (0.635-1.337)	1.555 (0.774-2.365)	5.70 (5.58-5.83)	23.50 (19.60-27.40)	2.75 (1.29-4.22)	2.58 (2.40-2.76)

continued/..

8	9	10	11	12	13	14	15
45.5 (38.6-52.3)	6.47 (2.8-10.0)						
78.7 (68.3-89.0)	8.89 (3.93-13.85)	3.07 (1.63-4.52)	11.9 (5.1-18.7)	0.068 (0.0-0.136)	0.30 (0.3-0.318)	.61 (0.47-0.75)	0.10 (0.08-0.13)
58.8 (46.2-71.5)	7.01 (2.89-11.13)	10.22 (6.15-14.20)	59.9 (32.5-87.3)	2.50 (1.75-3.26)	2.60 (2.43-2.77)	21.26 (18.34-24.18)	1.88 (1.55-2.20)
62.7 (45.5-79.7)	5.52 (2.04-9.01)						
58.8 (51.8-65.8)	4.10 (0.45-7.75)	7.37 (4.44-10.30)	25.77 (11.86-39.67)	1.15 (0.85-1.46)	2.98 (1.93-4.04)	6.52 (3.95-9.09)	0.273 (0.173-0.373)
81.2 (66.8-95.7)	3.63 (0.80-6.46)	9.87 (6.80-12.94)	36.54 (20.24-52.84)	0.750 (0.6-0.9)	5.60 (4.75-6.45)	17.04 (9.63-24.45)	0.664 (0.345-0.982)

TABLE 4: Number of plants from a total of 220 at each sampling time infected with *Ggt*, *Rs*, *Pm* and *Ha* over 2 years

	Seminal roots				Coronal roots			
	Ggt	*Rs*	*Pm*	*Ha*	*Ggt*	*Rs*	*Pm*	*Ha*
1979								
July	21	208	206	162				
August	60	220	220	206	3	34	21	10
September	196	220	220	187	172	212	203	146
1980								
July	207	151	202	163				
August	202	158	220	136	110	180	92	18
September	170	202	220	146	57	214	184	65

being similar to that found at the two earlier samling dates. There were no significant differences in the level of *Pm* and *Ha* (Table 3).

All four pathogens were found on the coronal roots and the incidence of both fungi was similar. Low numbers of *Ha* were recorded on the coronal roots (Table 3) which were fewer on plants from patchy areas.

Year 2, 1980 - Rainfall in April, May and June was adequate for early growth but July, August and September were relatively dry months with low rainfall. The variables recorded on the plants in 1980 are also given in Table 2 and the means in Table 3.

At the first sampling in July, only seminal roots were found. In contrast to the previous year's results, *Ggt* was the dominant fungal pathogen and was found in most plants (Table 4). There were however more lesions on plants from patchy areas. Although mean length of *Rs* lesions did not differ substantially from the previous year, numbers of plants with *Rs* did; about 50% of plants from patches were infected with *Rs* whereas nearly all plants from areas of normal growth had *Rs* on the roots. Plants that had more *Ggt* on them, had less *Rs*. All plants from patches were infected with *Ha* but half the plants from non-patches were free from *Ha*. Only 163 of the 220 plants sampled were infected with *Ha* (Table 4).

At the second and third sampling times, coronal roots were present and plants from patches had fewer coronal roots. This delay in production and number of coronal roots was similar to that in the previous year. Length of *Ggt* lesions and number of plants with *Ggt* were the same at the first and second sampling times; 62 plants out of 220 plants

did not exhibit any *Rs* lesions. With *Pm* there was no discernable pattern. All plants had *Pm* in the seminal roots. The pattern of the infection was similar to that found at the first sampling: outside patches, more plants were without *Ha* on them whereas all plants within patches were infested with *Ha*.

Both fungal pathogens were found on the coronal roots although *Ggt* to only a minor extent. *Rs* was equally abundant on both seminal and coronal roots. Nematode counts were very low on the coronal roots (Table 3).

At the third sampling time the length of *Rs* lesions increased and *Ggt* levels were lower than those at the second sampling time (Table 3). *Pm* was found in all roots whereas *Ha* occurred in only 172 plants out of 220 plants. Swollen, white, protruding females of *Ha* were present at this sampling time.

On the coronal roots, only 57 plants out of 220 (Table 4), had *Ggt* lesions on them while nearly all plants were infected by *Rs*. More *Rs* lesions were found on plants from outside patches. The coronal roots only contained low numbers of *Pm* and *Ha*.

Discussion

Patches could be easily identified because plants from them were smaller and unthrifty. The only pathogen consistently found at high density in plants from patches was *Ha*. The numbers of *Ha* in patches in the survey area was high although there were variations in incidence between patches in different paddocks. Both *Ha* and *Pm* were found in the roots at the first sampling probably because sufficient water was available for penetration. With fungal pathogens, the pattern changed between the two years and this may have been related to rainfall. In

1979, in April and May there was barely sufficient rain for sowing and this was followed by two dry months. In 1980, rainfall in April, May and June was plentiful, but July, August and September, were dry. In 1979, in early and mid samples, *Rs* was dominant but *Ggt* increased in the last wet sample. In 1980, *Ggt* was dominant early in the wetter conditions whereas infection of coronal roots by the fungi probably depended on water availability at their time of emergence.

C. *Correlations*

Correlations between plant variables

All plant variables at all times in both years were significantly correlated with each other (Tables 5 and 6). The weakest relationship was between number of seminal roots with fresh weight of plant at all sampling times in 1979. In 1980, number of seminal roots and their length also were not strongly correlated with fresh plant weight.

Correlations between plant growth and pathogens

In the first sample in the first year, there were few significant relations between the organisms and plant variables (Table 7). Length of *Ggt* and *Rs* lesions were negatively related to length of seminal roots while *Ha* was the only factor negatively related to shoot dry weight. The heavier the root system, the more *Ha* were present. At the second sampling, all organisms were negatively correlated with fresh weight of the plants and dry weight of shoots; all organisms except *Rs* were significantly correlated with fresh weight of roots. Only *Ha* and *Ggt* were negatively correlated to seminal root length and only *Ha* to the number of seminal roots.

TABLE 5: Correlation coefficients among growth measurements in Year 1, 1979

		1	2	3	4	5
1st Sampling						
Total fresh wt	1	1.000				
Root fresh wt	2	0.831***	1.000			
Dry shoot wt	3	0.900***	0.660***	1.000		
No. of S. roots	4	0.520***	0.549***	0.418***	1.000	
Length of S. roots	5	0.667***	0.734***	0.552***	0.588***	1.000

2nd Sampling		1	2	3	4	5	6	7
Total fresh wt	1	1.000						
Root fresh wt	2	0.759***	1.0000					
Dry whoot wt	3	0.912***	0.567***	1.0000				
No. of S. roots	4	0.432***	0.308***	0.441***	1.000			
Length of S. roots	5	0.758***	0.559***	0.754***	0.656***	1.000		
No. of C. roots	6	0.692***	0.520***	0.660***	0.269***	0.479***	1.000	
Length of C. roots	7	0.388***	0.314***	0.369***	0.174*	0.226**	0.319***	1.000

3rd Sampling		1	2	3	4	5	6	7
Total fresh wt	1	1.000						
Root fresh wt	2	0.866***	1.000					
Dry shoot wt	3	0.941***	0.769***	1.000				
No. of S. roots	4	0.321***	0.261***	0.401***	1.000			
Length of S. roots	5	0.498***	0.431***	0.514***	0.582***	1.000		
No. of C. Roots	6	0.822***	0.702***	0.741***	0.235***	0.336***	1.000	
Length of C. roots	7	0.850***	0.758***	0.772***	0.236***	0.403***	0.943***	1.000

S. - Seminal; C. - Coronal.

TABLE 6: Correlation coefficients among growth measurements in Year 2, 1980

		1	2	3	4	5
1st Sampling						
Total fresh wt	1	1.000				
Root fresh wt	2	0.653***	1.000			
Dry shoot wt	3	0.984***	0.571***	1.000		
No. of S. roots	4	0.078	0.069	0.084	1.000	
Length of S. roots	5	0.555***	0.367***	0.519***	0.249***	1.000

2nd Sampling		1	2	3	4	5	6	7
Total fresh wt	1	1.000						
Root fresh wt	2	0.848***	1.000					
Dry shoot wt	3	0.972***	0.749***	1.000				
No. of S. roots	4	0.252***	0.326***	0.222*	1.000			
Length of S. roots	5	0.340***	0.344***	0.313***	0.158*	1.000		
No. of C. roots	6	0.871***	0.689***	0.852***	0.164*	0.298***	1.000	
Length of C. roots	7	0.831***	0.695***	0.817***	0.077	0.403***	0.869***	1.000

3rd Sampling		1	2	3	4	5	6	7
Total fresh wt	1	1.000						
Root fresh wt	2	0.846***	1.000					
Dry shoot wt	3	0.975***	0.790***	1.000				
No. of S. roots	4	0.116	0.137	0.102	1.000			
Length of S. roots	5	0.500***	0.469***	0.491***	0.338***	1.000		
No. of C. roots	6	0.870***	0.785***	0.863***	0.098	0.488***	1.000	
Length of C. roots	7	0.715***	0.755***	0.700***	6.122	0.486***	0.824***	1.000

S. - Seminal; C. - Coronal.

TABLE 7: Correlations between growth parameters and pathogens on seminal roots in 1979

Pathogen Variables	Fresh weight of plants	Fresh root weight	Dry shoot weight	Number of S. roots	Length of S. roots
1st Sampling					
Length of *Ggt* lesions	-0.083	-0.049	-0.098	-0.004	-0.140*
Length of *Rs* lesions	-0.085	-0.101	-0.079	-0.060	-0.175*
Number of *Pm*	0.063	0.179*	0.056	0.099	0.097
Number of *Ha*	-0.084	0.161*	-0.195**	0.086	-0.046
2nd Sampling					
Length of *Ggt* lesions	-0.310***	-0.318***	-0.232***	-0.039	-0.140*
Length of *Rs* lesions	-0.266***	-0.118	-0.369***	0.088	-0.057
Number of *Pm*	-0.226**	-0.195**	-0.190**	0.018	-0.086
Number of *Ha*	-0.443***	-0.247***	-0.512***	-0.183**	-0.411***
3rd Sampling					
Length of *Ggt* lesions	-0.362***	-0.208**	-0.461***	-0.115	-0.148*
Length of *Rs* lesions	-0.215**	-0.179*	-0.127	0.040	0.054
Number of *Pm*	-0.085	0.021	-0.156*	-0.178*	-0.083
Number of *Ha*	-0.433***	-0.260***	-0.483***	-0.410***	-0.340***

TABLE 8: Correlations between growth parameters and pathogens on seminal roots in 1980

Pathogen Variables	Fresh weight of plants	Fresh root weight	Dry shoot weight	Number of S. roots	Length of S. roots
1st Sampling					
Length of *Ggt* lesions	-0.343***	-0.059	-0.322***	0.196**	-0.482***
Length of *Rs* lesions	0.245***	0.092	0.238***	0.010	0.408***
Number of *Pm*	0.088	-0.110	0.089	0.096	0.278***
Number of *Ha*	-0.510***	0.009	-0.544***	0.034	-0.411***
2nd Sampling					
Length of *Ggt* lesions	-0.477***	-0.302***	-0.450***	-0.108	-0.122
Length of *Rs* lesions	0.181**	0.171*	0.168*	0.210**	0.026
Number of *Pm*	-0.167*	0.085	-0.169*	0.013	0.103
Number of *Ha*	-0.610***	-0.415***	-0.601***	-0.025	-0.188**
3rd Sampling					
Length of *Ggt* lesions	-0.394***	-0.279***	-0.391***	-0.038	-0.279***
Length of *Rs* lesions	0.121	0.040	0.160*	0.131	0.207**
Number of *Pm*	0.323***	0.334***	0.319***	0.178**	0.394***
Number of *Ha*	-0.506***	-0.368***	-0.506***	-0.053	-0.352***

At the third sample, *Ha* again was negatively correlated with the majority of plant variables as were *Ggt* and *Rs*. *Pm* had only low correlations.

In the second year (Table 8) the pattern was similar at the second and third sampling. *Rs* was positively correlated with the plant variables whereas the relation of *Pm* to plant variables was again inconsistent.

Correlations between Pathogens

At the first sampling time in the first year, only two positive correlations were recorded (Table 9) with *Ha* - *Ggt* and *Ha* - *Pm*. At the second sampling time this changed to relationships of *Ha* with *Ggt* and *Rs* with *Ggt*. The coronal roots showed a similar relationship of *Ha* with *Ggt* and *Rs* but *Pm* was also related to *Rs* and *Ha*. Essentially similar situations occurred in the third sample. The most consistent relationship occurred between *Ggt* and *Ha*.

In the second year (Table 10) the correlation between *Ggt* and *Ha* was again consistent but inverse relationships were now evident between *Ggt* and *Rs* and *Rs* and *Ha* . Although significant correlations between *Pm* and other organisms were recorded, they were sometimes positive and sometimes negative and therefore difficult to interpret. Relationships between organisms were generally more consistent on the seminal than coronal roots.

Discussion

The strong correlations among plant parameters measured at all sampling times in both years, illustrated that the growth of plants was closely related to morphological parameters except those which

TABLE 9: Correlation coefficients among pathogens in Year 1, 1979

	Ggt	*Rs*	*Pm*	*Ha*
1st Sampling				
Ggt (S)	1.000			
Rs (S)	0.086	1.000		
Pm (S)	0.065	0.065		
Ha (S)	0.235***	0.130	0.498***	1.000
2nd Sampling				
Ggt (S)	1.000			
Rs (S)	0.159*	1.000		
Pm (S)	0.092	0.056	1.000	
Ha (S)	0.183**	0.347***	0.057	1.000
Ggt (C)	1.000			
Rs (C)	-0.045	1.000		
Pm (C)	0.060	0.246**	1.000	
Ha (C)	0.335***	0.161*	0.534***	1.000
3rd Sampling				
Ggt (S)	1.000			
Rs (S)	0.002	1.000		
Pm (S)	0.398***	-0.011	1.000	
Ha (S)	0.422***	0.130	0.572***	1.000
Ggt (C)	1.000			
Rs (C)	0.293***	1.000		
Pm (C)	0.260***	0.265***	1.000	
Ha (C)	0.261***	0.155*	0.280***	1.000

(S): Seminal roots; (C): Coronal roots.

TABLE 10: Correlation coefficients among pathogens in Year 2, 1980

	Ggt	*Rs*	*Pm*	*Ha*
1st Sampling				
Ggt (S)	1.000			
Rs (S)	-0.578***	1.000		
Pm (S)	-0.199**	0.071	1.000	
Ha (S)	0.500***	-0.370***	-0.197**	1.000
2nd Sampling				
Ggt (S)	1.000			
Rs (S)	-0.497***	1.000		
Pm (S)	0.192**	-0.005	1.000	
Ha (S)	0.339***	-0.196**	0.325***	1.000
Ggt (C)	1.000			
Rs (C)	-0.126	1.000		
Pm (C)	0.105	0.505***	1.000	
Ha (C)	-0.003	-0.024	0.077	1.000
3rd Sampling				
Ggt (S)	1.000			
Rs (S)	-0.464***	1.000		
Pm (S)	-0.114	0.103	1.000	
Ha (S)	0.458***	-0.187**	-0.218**	1.000
Ggt (C)	1.000			
Rs (C)	-0.018	1.000		
Pm (C)	0.248***	0.111	1.000	
Ha (C)	0.139*	0.079	0.081	1.000

(S): Seminal roots; (C): Coronal roots.

describe roots. Inability to measure the complete length of roots may explain this difference.

The association of pathogens on seminal roots with growth variables was predominantly negative, indicating that damage to seminal roots reduced growth. *Ggt*, *Rs* and *Ha* decreased growth in the first year of survey whereas only *Ggt* and *Ha* were responsible in the second year. *Rs* in the second year did not have much effect when *Ggt* was the dominant fungal pathogen on the roots, and *Pm* appeared to have little effect.

The only consistent relationship among the plant pathogens in both years, was the positive correlation between *Ggt* and *Ha* on seminal roots. It is possible that *Ha*, at the time of penetration may facilitate infection by *Ggt*. In many samples *Ggt* lesions were found in the vicinity of syncytia radiating into the lateral roots especially in the second year when *Ggt* was present in greater amounts early in the season.

The association between *Ggt* and *Rs* was negative in all three samples in the second year. This inverse relationship was strong, suggesting that they might be antagonistic to each other.

Rs was also consistently negatively correlated with *Ha* in the second year, suggesting that an increase in *Rs* might reduce the numbers of *Ha* in the roots. Reduction of other kinds of cyst populations by fungi has been reported (James, 1966, 1968; Jorgenson, 1970). Suppression of *H. glycines* populations by *R. solani* was found by Dave (1975) in soybeans.

The association between *Ggt* and *Pm* was not consistent and no strong association was found between *Rs* and *Pm*. The change in relationships between *Ha* and *Pm* and *Ggt* and *Pm*, at different sampling times may have been due to the great variation in numbers of *Pm*, found in the

samples in both seminal and nodal roots. In any event, inconsistent and weak relationships do not provide useful hypotheses for further study. The correlation coefficients, even in the highly significant consistent relationships, tend to be low, reflecting the multifactorial nature of disease causation. Nevertheless, such associations do give a picture of what might be happening in the field.

D. Principal Component Analysis

Tables 11 to 16 show the results for both 1979 and 1980 at each sampling time for both growth and pathogen variables separately. With both sets of variables the first three or four components accounted for most of the variation in plant growth and pathogen measurements. That the first component often has the character of a 'size' vector (Blackith and Reyment, 1971) is clearly demonstrated by the analysis of the plant parameters. The first principal component indicated that the growth of the plants at all sampling times was proportional to the measurements recorded as seen by the similar magnitude and sign of coefficient values in its structure. The exception was the parameter of number of seminal roots. The variables describing one or both root systems were identified by the second and third components. Thus components 2 and 3 in this study can be considered as root factors. Overall the number and length of seminal roots dominated the second component, but the association between number and length of roots was not the same at all sampling times indicating that size of the root system was very variable in the samples.

At the first sampling date in 1979, the second principal component identified that among the plants assayed, there was much variation in numbers of seminal roots irrespective of their length. In the second sample, many plants with more and longer seminal roots sometimes had

TABLE 11: Principal component analyses of growth and pathogen variables on seminal roots at 1st sampling date in Year 1, 1979

Variable	Principal component		
	1	2	3
Total fresh wt of plant	.937	-.278	.085
Fresh root wt	.900	-.018	-.221
Dry shoot wt	.845	-.427	.236
No. of seminal roots	.703	.609	.363
Length of seminal roots	.834	.250	-.403
Eigen value	3.596	.695	.406
% of variation	71.9	13.9	8.1
Cumulative %	71.9	85.8	94.0

Organism	Seminal roots			
	1	2	3	4
Ggt	.449	.516	-.711	.159
Rs	.323	.718	.614	.041
Pm	.766	-.430	.177	.442
Ha	.853	-.157	-.018	-.497
Eigen value	1.620	.993	.915	.470
% of variation	40.5	24.8	22.9	11.8
Cumulative %	40.5	65.4	88.2	100.0

TABLE 12: Principal component analyses of growth and pathogen variables in seminal and coronal roots at 2nd sampling date in Year 1, 1979

Variable	Principal components			
	1	2	3	4
Total fresh wt	.950	.079	-.151	.043
Fresh root wt	.767	.155	-.211	.545
Dry shoot wt	.906	.018	-.092	-.179
No. of S. roots	.595	-.621	.410	-.037
Length of S. roots	.846	-.369	.039	.026
No. of N. roots	.749	.265	-.263	-.408
Length of N. roots	.466	.584	.661	.017
Eigen value	4.168	.965	.753	.500
% of variation	59.6	13.8	10.8	7.1
Cumulative %	59.6	73.3	84.1	91.3

	Seminal roots				Coronal roots		
	PRINCIPAL COMPONENTS						
	1	2	3	4	1	2	3
Ggt	.563	.217	-.795	.054	.428	.754	.450
Rs	.735	-.257	.287	.556	.426	.662	.615
Pm	.274	.905	.324	-.009	.797	-.241	-.397
Ha	.751	-.240	.195	-.581	.860	.176	-.160
Eigen value	1.499	.990	.859	.650	1.741	1.098	.765
% of variation	37.5	24.8	21.5	16.3	43.5	27.5	19.1
Cumulative %	37.5	62.2	83.7	100.0	43.5	71.0	90.1

TABLE 13: Principal component analysis of growth and pathogen variables on seminal and coronal roots at 3rd sampling date in Year 1, 1979

Variable	Principal components			
	1	2	3	4
Total fresh wt	.962	-.121	-.086	-.136
Fresh root wt	.870	-.150	0.200	-.259
Dry shoot wt	.923	-.007	-.051	-.202
No. of S. roots	.453	.785	.387	-.150
Length of S. roots	.608	.632	-.371	.302
No. of N. roots	.877	-.285	.253	.247
Length of N. roots	.909	-.258	.146	.231
Eigen value	4.709	1.202	.424	.356
% of variation	67.3	17.2	6.1	5.1
Cumulation %	67.3	84.5	90.5	95.6

Organism	Seminal roots				Coronal roots			
	PRINCIPAL COMPONENTS							
	1	2	3	4	1	2	3	4
Ggt	.730	-.130	.668	.048	.697	-.143	.569	-.411
Rs	.109	.985	.068	.110	.637	-.622	-.102	.442
Pm	.824	-.124	-.358	.420	.691	.129	-.614	-.356
Ha	.845	.106	-.237	-.466	.625	.651	.149	.402
Eigen value	1.939	1.015	.636	.408	1.76	.849	.734	.655
% of variation	48.5	25.4	15.9	10.2	44.0	21.2	18.4	16.4
Cumulative %	48.5	73.9	89.8	100.0	44.0	65.3	83.6	100.0

TABLE 14: Principal component analysis of growth and pathogen variables on seminal roots at 1st sampling date in Year 2, 1980

Growth variables	Principal components		
	1	2	3
Total fresh wt	.958	-.153	.005
Fresh root wt	.755	-.161	.467
Dry shoot wt	.928	-.145	-.023
No. of S. roots	.201	.937	.256
Length of S. roots	.708	.304	-.549
Eigen value	2.895	1.043	.586
% of variation	57.9	20.9	11.7
Cumulative %	57.9	78.8	90.5

Organisms	Seminal roots		
	1	2	3
Ggt	.865	.077	-.116
Rs	-.778	-.309	.419
Pm	-.305	.934	.169
Ha	.756	-.031	.633
Eigen value	2.020	.976	.619
% of variation	50.5	24.4	15.5
Cumulative %	50.5	74.9	90.4

TABLE 15: Principal component analysis of growth and pathogen variables on seminal and coronal roots at 2nd sampling date in Year 2, 1980

Growth variables	Principal components		
	1	2	3
Total fresh wt	.972	-.047	-.116
Fresh root wt	.863	.128	-.087
Shoot dry wt	.942	-.090	-.123
No. of S. roots	.292	.912	-.241
Length of S. roots	.456	.254	.850
No. of N. roots	.912	-.172	-.091
Length of N. roots	.902	-.226	.078
Eigen value	4.521	1.004	.832
% of variation	64.6	14.3	11.9
Cumulative %	64.6	78.9	90.8

Organisms	Seminal roots			Coronal roots		
	1	2	3	1	2	3
Ggt	.813	-.262	-.131	-.041	.870	-.457
Rs	-.669	.586	.135	.865	-.217	-.043
Pm	.472	.732	-.474	.865	.212	-.068
Ha	.686	.379	.615	.0884	.457	.881
Eigen value	1.802	1.093	.639	1.508	1.058	.992
% of variation	45.1	27.3	16.0	37.7	26.5	24.8
Cumulative %	45.1	72.4	88.4	37.7	64.2	89.0

TABLE 16: Principal component analysis of growth and pathogen variables on seminal and coronal roots at 3rd sampling date in Year 2, 1980

Growth variables	Principal components		
	1	2	3
Total fresh wt	.954	-.132	-.151
Fresh root wt	.890	-.088	-.070
Dry shoot wt	.939	-.142	-.135
No. of S. roots	.218	.905	.087
Length of S. roots	.634	.466	-.154
No. of N. roots	.923	-.138	.003
Length of N. roots	.468	-.059	.874
Eigen value	4.108	1.106	.842
% of variation	58.7	15.8	12.0
Cumulative %	58.7	74.5	86.5

Organisms	Seminal roots			Coronal roots		
	1	2	3	1	2	3
Ggt	.834	.247	.108	.695	-.452	-.045
Rs	-.679	-.401	.534	.307	.885	-.198
Pm	-.389	.839	.371	.712	-.054	-.460
Ha	.719	.212	.581	.513	.158	.820
Eigen value	1.027	.972	.773	1.349	1.016	.926
% of variation	45.7	24.3	19.3	33.7	25.4	23.2
Cumulative %	45.7	70.0	89.3	33.7	59.2	82.3

fewer and shorter coronal roots which accounted for 13.8% of the total variation. In the third sample there was also a lot of variation in the length and number of seminal roots on the plants. Similar variation of the root system was also found at all the three sampling dates in 1980 in the second component.

The third component in 1979, at the second sampling date, was dominated by the number of seminal roots and length of coronal roots. In 1980, at the first sampling date, the third component identified the plants that had long but not heavy roots. Length of seminal roots and coronal roots contributed to the variation in measurements at these times.

The pathogens on the two root systems were analysed separately because of the possibility of different pathogen colonization pattern occurring on the later developing coronal roots. In both years of the field study, analysis of pathogens on the seminal roots, identified that conditions were similar for colonization by all four pathogens. In 1979, *Ha* and *Pm* were the most variable pathogens in the principal components during the first and last sampling times, but *Rs* and *Ha* dominated at the second sampling time. *Rs* and *Pm* were the most variable pathogens on many plants identified by the second component. *Rs*, *Pm* and *Ggt* were the main pathogens contributing to the variability on the coronal roots.

In 1980, at all the sampling times, the first component identified the plants which had more variable *Ggt* and *Ha* on the seminal roots. The second component indicated that there was much variation in numbers of *Pm* found in the seminal roots.

Discussion

Principal components describe variability. Each principal component contains a contribution from each of the variables. For example, the fact that a contrast between two parameters dominates a component does not necessarily mean that a negative association exists between these two variables in general, but rather that when a negative association does exist, it contributes a lot to the variability of measurements. If there was an overall consistent negative association it would not contribute to the variability, so would not feature in a principal component.

Among the growth parameters, the first component was consistently a 'size' vector. The second and third components showed that there was a lot of variability in number and length of root systems, especially seminal roots.

The results with the pathogens were not as clear cut as with growth parameters. There were no consistent trends and the first component did not account for a very high proportion of variation (40-50% as opposed to 60-70% for growth variables). There was a lot more variability inherent in the pathogen parameters that was not consistently described over sampling dates or years. Each of the pathogens in turn accounted for a large proportion of the variation without dominating a component. Relationships between pathogens changed over time. As there was no consistent pattern, attempting to interpret coefficients would add little to our understanding of the interrelationships among the pathogens. In general terms, the results corresponded to the correlations found among the pathogens, but added little new information.

E. Regression Analysis (Table 17)

Ha was the important pathogen at first sampling time in both 1979 and 1980 and was responsible for approximately 38% and 29% of the variation in dry shoot weight in Year 1 and Year 2, respectively. All four organisms on the seminal roots had an effect on growth. *Ggt*, *Rs* and *Ha* were consistently negatively related to dry shoot weight in both years. *Pm* in Year 1 at second sampling was negatively related with dry shoot weight and positively at the third sampling time. The interaction terms involving *Pm* were dropped from the equations because their association with other pathogens and growth was inconsistent. Pathogens on the coronal roots were positively correlated with dry shoot weight in both years at the second and third sampling times. All combinations between *Ggt*, *Rs* and *Ha*, contributed to the variation in growth with inclusion of interaction terms.

Discussion

This method revealed and confirmed that the *Ha*, *Ggt* and *Rs* infections had an effect on growth (dry shoot weight) mainly through damage to the seminal roots. Effect of early infection by *Ha* persisted till the third sampling time. Fungal damage later in the growing season was also important. *Rs* and *Pm* on the coronal roots had very little effect on growth with which they were positively associated indicating that coronal roots can tolerate a population of pathogens to certain thresholds.

2.3 GENERAL DISCUSSION

The two years during which this study were undertaken, had different weather patterns, particularly with respect to rainfall, and

TABLE 17: Results of regression analysis : pathogens causing variation on dry shoot weight

Time	Year 1		Year 2	
1st Sampling				
	Ha on S. roots	-.486	*Ha* on S. roots	-.211
	Constant	.380	Constant	.348
	R^2	0.376	R^2	.295
	F	8.5	F	91.6
	RSS	.085	RSS	6.94
	CV	56.9	CV	77.0
2nd Sampling				
	Ha on S. roots	-.207	*Ha* on S. roots	-.372
	Rs on S. roots	-.924	*Rs* on C. roots	.635
	Pm on S. roots	-.567	*Ggt* on S. roots	-.766
	Pm on C. roots	.178	*Rs* on S. roots	-.106
	Rs, *Ha* on S. roots	.375	*Pm* on C. roots	.799
	Ggt on S. roots	-.137	Constant	.963
	Ggt, *Rs* on S. roots	.403		
	Constant	.625		
	R^2	.414	R^2	.551
	F	21.31	F	65.9
	RSS	4.90	RSS	27.79
	CV	58.5	CV	56.0
3rd Sampling				
	Ha on S. roots	-.826	*Ha* on S. roots	-.982
	Ggt on S. roots	-.276	*Rs* on C. roots	.124
	Rs on C. roots	.184	*Pm* on C. roots	.229
	Pm on S. roots	.402	*Ggt* on S. roots	-.115
	Ggt, *Ha* on S. roots	.920	*Ggt*, *Ha* on S. roots	.126
	Constant	1.547	Constant	.035
	R^2	.414	R^2	.515
	F	30.27	F	45.41
	RSS	103.53	RSS	128.04
	CV	58.2	CV	50.3

weather influenced the variables, but in both years patches were present hence weather presumably had little influence on the incidence of patches. Patches were present at the first sampling time, about four weeks after sowing. Discriminant analysis confirmed that patches and non-patches were different but pathogens alone were not responsible for the occurence of patches. Some other factors not measured in this study were mainly responsible for patches.

The strong correlations among plant parameters measured at all sampling times in both years, illustrated that the growth of plants was closely related to morphological parameters except those which described roots. Inability to measure the complete length of roots or loss of parts of the root system due to rotting may explain this difference. This was confirmed by principal component analysis.

Most plants whether healthy or not had one or more organisms (Stynes, 1975) but *Ha* most consistently reduced growth; this was most noticeable on the seminal roots. *Ggt* was abundant when rainfall was more than adequate and *Rs* was largely the converse. *Pm* appeared to have little effect.

The most consistent relationship among the plant pathogens was the positive correlation between *Ggt* and *Ha* on the seminal roots at all sampling times though Cook (1969) reported a negative relationship on barley. It is possible that *Ha*, at the time of penetration or establishment, may aid infection by *Ggt*. In many samples, *Ggt* lesions were found in the vicinity of nematodes syncytia radiating into lateral roots.

One surprising association was the strong negative correlation between *Ggt* and *Rs* in the second year. Usually the lesions of each occured on separate roots or if they were on the same roots the lesions were well separated. Although Lal (1939) recorded this antagonism, it has not been mentioned since but it could be important in the field behaviour of the two fungi.

The association between *Ha* and *Rs* was positive once in 1979, but consistently negative in 1980. This may be a reflection of the timing of sampling. Infestation

by *Ha* may encourage the infection by *Rs* but the ability of *Rs* to rapidly damage the root tissue including the nematode syncytia would lead to rapid death of the nematode. Thus a positive correlation could quickly change to a negative one. James, (1966; 1968) and Jorgensen (1970) both reported reduction of *Globodera rostochiensis* and *H. schactii* respectively.

Regression analysis showed that these interactions between pathogens accounted for approximately 5% of the variation in growth; not a dominant proportion but they do contribute to reduction in growth.

Varietal differences, age of crop, soil type, rotations (Slope and Etherbrige 1971), fertilization and strain difference of fungi (de Beer, 1965) indirectly affected the field data and results.

Sampling techniques probably influenced the results to some extent. *Rs* when severe, damages the roots to such an extent that despite the utmost care, some parts of the root system could not be extracted from soil. Those parts of the root system that were lost could have contained other organisms (particularly nematodes) which would not be counted. The natural loss of cortical tissue with age, from the roots also probably influenced the results, particularly those of *Pm* which inhabits the cortex.

The relative importance of fungi and nematodes was also influenced by the methods used to assess them. While direct counts of nematodes were used, fungi were assessed by symptoms. This difference in assessment tends to give a closer correlation between fungi and damage than that of nematodes to damage. The difference may be small or large. There is no statistical method other than subjective weighting for equating differences.

Despite the deficiencies in the procedure used here, the associations that were found in the field between *Ggt* and *Ha*, *Ggt* and *Rs* and *Ha* and *Rs* provide useful hypotheses for further study. Meagher *et al* (1978) have claimed that the association of *Ha* and *Rs* is the most important cause of damage to wheat in southern Australia. The field study described here did not support that hypothesis.

CHAPTER III

LABORATORY EXPERIMENTS ON THE INTERRELATIONSHIPS BETWEEN ORGANISMS

INTRODUCTION

The constraint of time (1 or 2 years) required for a thesis and the bias of chosen variables limit the amount of information that can be obtained from a field study. Whatever the limitations, such a study should nevertheless form a basis on which hypotheses, trends or associations can be based. This field study established that there were strong and consistent associations between the root pathogens of wheat, and which of these were important. It also established the range of pathogen damage that occurred under natural conditions and provided a guide to the levels of pathogens that should be used in experiments.

Most studies on pathogen interaction in the literature have been conducted under laboratory conditions at one level of both pathogens. The format for studying the interrelationship of two pathogens, e.g. nematode and fungus so far has been: nematode alone; fungus alone; nematode + fungus; and control. The information obtained from such a study at one level is extremely limited and does not provide any indication of what could occur at lower or higher densities. Wallace (1983) suggested that interaction studies should be ideally conducted at several levels of each pathogen so that greater understanding of the relationship between pathogens occupying the same niche might be gleaned.

Synergism occurs when the combined effect (as measured by plant parameters) is greater than the sum of each factor alone and antagonism, when the combined effect is less than the sum of each factor alone. Either of these reactions may show as a significant interaction in an analysis of variance of a factorial design.

Diomande and Beute (1981) studied the effect of *Meloidogyne hapla* and *Macroposthonia ornata* on Cylindrocarpon black rot of peanuts in all possible combinations in a factorial experiment. The relationship between *Fusarium graminearum* and *Bipolaris sorokiniana* on barley was evaluated in an inoculum time sequence study by Scardaci and Webster (1981). In both of these studies the value of factorial experiments at several rates of pathogen combinations was realised. All experiments in this study had a factorial design.

3.1 Materials and Methods

A. Soil

John Innes nutrients (half strength) in soil were used in all the experiments. After being prepared and steam sterilized the soil was transferred to a covered bin for at least six weeks before use.

B. The source of pathogens and inoculation methods

G. graminis var. *tritici* was isolated from a wheat root from the field survey area in 1979. It was maintained on Czapex-Dox plus yeast extract ($^1/6$ dilution) medium (NDY/6). *Ggt* inoculum was prepared by the following procedure: Oat grains were soaked for 16 hours in rain water; sterilized for one hour each day on three consecutive days and then inoculated with *Ggt* growing on NDY/6 on agar plates. The flasks of inoculated grains were maintained at 25°C and shaken every three days for a month. The infected grains were then thoroughly dried in laminar flow cabinets, ground up in a coffee blender and sieved through a 0.5 mm sieve. This sieved material was used as *Ggt* inoculum in the John Innes soil. The inoculum suspension was serially diluted to give a range of % inoculum.

Ggt attenuates readily on artificial media and is known to lose pathogenicity in culture. Partial restoration of virulence can be achieved by infecting a susceptible host and re-isolating (Chambers, 1966). Virulence is not fully restored by inoculation of a susceptible host and re-isolation from roots (Asher, 1981). One lot of inoculum of *Ggt* was prepared from the culture of *Ggt* maintained on agar medium for two years and this inoculum was used in one experiment. All other inocula of *Ggt* were prepared from *Ggt* re-isolated after passage through barley grass (*Hordeum leporinum*).

R. solani (Thanatephorus cucumeris) root strain was originally isolated from wheat in 1979 by S.N. Neate and given the number *Rs 96*. It was maintained on NDY/6 and 5 mm diameter plugs were used to inoculate 100 ml of de Beer's liquid medium (de Beer, 1965).

The fungus was allowed to grow for one month at 25°C before being used. Mats of *Rs* were removed, washed in sterile water and excess moisture removed in a Buchner funnel (on sterile filter paper) attached to a vacuum pump. The mats were then weighed and the required quantity of mycelium was placed in a sterile blender with a measured quantity of sterile water and blended to break up the mat into small hyphal pieces. This blended soup of *Rs* was used for inoculating the experimental soil.

H. avenae larvae were hatched from cysts collected from soil and maintained at 10°C. Larvae were collected daily (Davies and Fisher, 1976). None of the larvae used for inoculating was more than 7 days old. Appropriate numbers of larvae were added to the surface of the tubes in 2 ml aliquots after pregerminated seeds had been planted.

C. *Growth Conditions*

Lengths of plastic conduit tubes, 12 cms long, with a diameter of

27 mm were used as growth containers (Plate 2). Each tube was sealed at its base with parafilm and filled with 100 gms of experimental soil. No extra nutrients were given to the plants to avoid any added effect on the pathogens and to aid the restriction of the root system to the tube. The tubes were stood in trays (Plate 2).

All experiments were conducted in a controlled environment growth room, at a constant temperature of 15°C (day and night) with a 14 hour day-length and light of 16355.2 lux provided by fluorescent lights.

The cultivar of wheat used in all the experiments was 'Condor'. Seeds were surface sterilized by soaking in a 5% sodium-hypochlorite solution for 5 minutes, thoroughly washed with sterile water and placed on moist filter paper in petri dishes at 15°C for 2 to 3 days to germinate. Seedlings were selected for uniformity.

D. Statistical Analysis

Two-way or three-way analysis of variance was performed on non-transformed or transformed data (log or $\sqrt{\ }$). In the analysis of variance of three-way factorial trials, the means obtained for the highest order of interaction are the means over the replicates of each combination of pathogens. For two-factor interactions means are calculated for the two pathogens or variables over all levels of the third pathogen or variable. For main effect of individual pathogen or any parameter, the means are calculated over all levels of other pathogens. Thus the means of the effect of individual pathogen or parameter differ from the means for first order and second order interactions.

Non-parametric or distribution free method of analysis was used on some data (e.g. number of cysts or number of roots) where there were only a few possible values within the observed range. Such data did

PLATE 2: Tubes (containers) used for growing wheat plants under experimental conditions in growth chambers.


R1
R1

not meet the assumptions required for analysis of variance (such as normality of errors).

E. Use of Statistics

In discussing the results of the experiments on the interrelationships between pathogens, certain clear-cut conclusions can be drawn about the major treatment effects and their interactions. Conclusions are justified when differences are statistically significant and are not trivial. However, in discussing the reasons for major treatment effects and in looking for biological explanations of interaction terms, it is necessary to examine the results of the numerous individual treatments. Here, L.S.D's are used to draw tentative conclusions which are little more than further hypotheses. Even differences which are statistically significant at levels greater than P = 0.05 are worth mentioning when the next step in an investigation is being considered. In multifactorial studies, significant F values are useful but even if significant, a difference may be trivial. Treatment means for pathogens and pathogen combinations are given for all variables (including those for which interaction terms are not significant) to illustrate trends and facilitate explanations. The term interaction refers to a statistical interaction. L.S.D's given in both tables and graphs are for interactions.

F. Results

In the many sections that follow, significant results were obtained for the effect of individual organisms on the growth of 'Condor' wheat. This is not remarkable as each is a recognised pathogen on wheat. In some instances, the lower densities of *Ha* had no effect or had a stimulatory effect. Neither of these responses are remarkable. Therefore in the presentation of the results, the effects of the individual organisms

will not be commented on unless they are considered pertinent. Mostly *Ggt*, with its oatmeal inoculum base, caused a stimulation to growth. This aspect will be covered in Section 3.6. Where an L.S.D. is indicated on a figure, it is at the 5% level.

3.2 Interrelationships between *Gaeumannomyces graminis* var *tritici (Ggt)* and *Heterodera avenae (Ha)*

Field studies indicated that *Ggt* and *Ha* were often positively correlated. The data did not reveal the reasons for such an association although it is possible that both organisms had similar environmental requirements and co-existed with little interference between them.

To test the hypothesis of positive correlation two experiments were done. Both experiments used four levels of *Ggt* (0, 0.5, 1.0 and 1.5%), four rates of *Ha* (0, 25, 50 and 125 larvae/100g soil) with eight-fold replication.

Two 'strains' of *Ggt*, a 'mild strain' that had been maintained on the agar for 2 years, and a 're-isolated strain', which was the 'mild strain' inoculated to barley grass *(Hordeum leporinum)* and re-isolated, were used.

Lengths of leaves were taken at weekly intervals from 10 days after emergence. All other measurements were taken at maturity.

A. Results - 'Mild Strain'

Growth

The influence of *Ggt* and *Ha* on six parameters of growth of wheat plants is shown in Table 18. A statistically significant interaction between *Ggt* and *Ha* was recorded for shoot dry weight, root length and yield (Table 18). This suggests that the effect of the organisms on these measurements of growth were not consistent but changed with density.

TABLE 18: The effects of *Ggt* (mild strain) and *Ha* on various plant characters at maturity

Pathogen and density	Shoot dry weight (g) (log)	Root dry weight (g) (log)	Number of roots ($\sqrt{\ }$)	Root length (cm)	Number of grains/plant ($\sqrt{\ }$)	Yield (g) (log)
0% *Ggt*	.832(2.29)	-1.266(0.281)	4.67	265.2	5.37	-0.075(0.927)
0.5% *Ggt*	1.111(3.03)	-1.145(0.318)	5.07	287.4	6.00	0.145(1.15)
1.0% *Ggt*	0.938(2.55)	-1.308(0.270)	4.59	210.1	5.69	-0.017(0.983)
1.5% *Ggt*	1.091(2.97)	-1.445(0.235)	4.48	185.0	5.84	0.101(1.10)
25 *Ha*	-.080(0.923)	-2.050(0.128)	3.74	148.3	3.69	-1.024(0.359)
50 *Ha*	-.298(0.742)	-1.832(0.160)	3.68	154.0	3.34	-1.188(0.304)
125 *Ha*	-.438(0.645)	-2.305(0.099)	3.33	121.8	2.86	-1.419(0.241)
0.5% *Ggt* + 25 *Ha*	1.036(2.81)	-1.254(0.285)	5.08	254.0	6.03	0.135(1.144)
" + 50 *Ha*	1.253(3.50)	-1.225(0.293)	5.09	279.0	6.90	0.341(1.406)
" + 125 *Ha*	0.739(2.09)	-1.514(0.220)	4.30	200.0	5.19	-0.274(0.760)
1.0% *Ggt* + 25 *Ha*	1.156(3.17)	-1.295(0.273)	4.68	239.0	6.29	0.255(1.29)
" + 50 *Ha*	0.912(2.48)	-1.273(0.279)	4.41	207.0	5.89	-0.094(0.91)
" + 125 *Ha*	0.675(1.96)	-1.611(0.199)	4.38	183.2	5.02	-0.279(0.75)
1.5% *Ggt* + 25 *Ha*	1.076(2.93)	-1.432(0.238)	4.70	235.7	6.12	0.140(1.15)
" + 50 *Ha*	0.916(2.49)	-1.397(0.247)	4.44	209.0	5.72	0.021(1.02)
" + 125 *Ha*	0.139(1.14)	-1.440(0.236)	4.07	169.9	3.85	-0.829(0.436)
L.S.D; P = .05	0.425	0.453	0.61	62.15	1.26	0.467
F values						
Ggt	37.679**	10.081**	14.819**	9.666**	18.907**	30.137**
Ha	15.645**	4.810**	6.992**	7.083**	9.018**	14.465**
Ggt, *Ha*	3.633**	1.528	1.541	2.325*	1.911	3.277**

Values in parenthesis are antilogs.

With shoot weight, these variable effects can be seen. With combination of the two organisms, generally an increase in shoot weight occurred but at the two higher densities of *Ggt*, as *Ha* density increased reduction in shoot weight was greater. At the lowest density of *Ggt*, the effects of the combination were variable. Similar results but varying in detail were obtained with length of roots and yield (Table 18). Inoculation with *Ha* prevented tiller formation and this was responsible for the large reductions in the parameters.

When *Ggt* was added alone, it increased shoot weight at all densities, but more at the lowest and highest densities than at the intermediate density. *Ha* alone decreased shoot weight greatly and the amount of reduction increased with density.

With root dry weight, number of roots and number of grains, no significant interaction was obtained suggesting consistent trends throughout. *Ggt* had little effect alone, *Ha* tended to decrease root weight and the combinations tended to have intermediate effects, though the results were not always consistent (Table 18).

Leaf length

No significant interactions between *Ggt* and *Ha* on leaf lengths could be measured, but the main effects of both *Ggt* and *Ha* individually were mostly significant (Table 19).

Ggt had two effects on the length of leaves. The lower concentrations of 0.5% and 1.0% increased the lengths of leaves 3 to 8 inclusive (Table 19). The highest concentration (1.5%) increased leaf length over that of the controls, but decreased it compared to that of the two lower concentrations of *Ggt*, but from leaf 6 to 8, this effect largely disappeared. *Ha* at the highest density reduced the length of all leaves

TABLE 19: Main effects of *Ggt* and *Ha* inoculation on means of final leaf lengths of 'Condor' plants

G. graminis var. *tritici*

Leaf No.	0%	0.5%	1%	1.5%	LSD; P=.05
1	6.44	6.66	6.05	5.92	N.S.
2	10.01	10.71	10.54	9.28	1.13
3	16.48	18.37	18.28	15.52	1.40
4	17.65	21.73	21.99	18.52	1.30
5	19.07	23.52	24.05	21.13	1.39
6	18.02	22.57	22.93	21.14	1.43
7	14.47	20.71	19.26	19.78	1.76
8	11.82	17.09	15.15	15.89	1.80

H. avenae

Leaf No.	0	25	50	125	LSD; P=.05
1	6.43	6.93	5.69	6.02	0.72
2	10.77	10.78	9.70	9.29	1.13
3	17.73	17.97	17.00	15.87	1.40
4	20.95	20.52	20.11	18.31	1.30
5	22.72	22.46	22.27	20.32	1.39
6	21.72	21.05	22.24	19.65	1.43
7	19.28	18.51	19.54	16.88	1.76
8	15.75	14.48	15.73	13.98	N.S.

except leaves 1 and 8.

Effect of *Ggt* and *Ha* on each other

The influence of pathogens on each other was assessed by measuring the length of *Ggt* lesions and the number of cysts and eggs at maturity as shown in Table 20. There was no significant interaction between the pathogens. *Ggt* alone had no significant effect at any concentration on the length of lesions. *Ha* had no effect on the length of lesions formed by *Ggt*. Number of cysts increased only at the highest initial density of *Ha* but there was no effect on egg numbers. Cyst and egg numbers were affected by *Ggt* but the results did not give consistent trends with densities of *Ggt* (Table 20). The significant results are probably not meaningful.

B. *Results - 'Re-isolated strain'*

Growth

The effect of *Ggt* (re-isolated strain) and *Ha* on growth of wheat was assessed by measuring seven plant parameters as shown in Table 21. Most results showed consistent trends but a significant interaction occurred only with root dry weight. When added together, the combination of *Ggt* and *Ha* at all densities reduced root weight roughly in the same pattern as *Ggt* but with minor variations. At the two higher concentrations of *Ggt*, density of nematodes had no effect but at the lowest concentration of *Ggt*, nematodes caused significant variation.

Ggt increased some growth parameters (shoot dry weight, number of grains and yield) and reduced others (number and length of roots and height of plants). In most instances effects were obtained only at the two higher concentrations. *Ha*, either alone or in combination had no effect on any growth parameter.

TABLE 20: Effects of *Ggt* (mild strain) and *Ha* on each other at maturity

Pathogen and density	Length of *Ggt* lesions	Number of cysts	*Ha* eggs
0% *Ggt*	-	-	-
0.5% *Ggt*	128.1	-	-
1.0% *Ggt*	116.9	-	-
1.5% *Ggt*	86.9	-	-
25 *Ha*	-	3.12	1798
50 *Ha*	-	3.00	1532
125 *Ha*	-	4.12	1633
0.5% *Ggt* + 25 *Ha*	108.5	2.12	895
" + 50 *Ha*	110.6	3.00	1455
" + 125 *Ha*	90.9	3.25	1473
1.0% *Ggt* + 25 *Ha*	106.3	2.50	1154
" + 50 *Ha*	94.1	2.00	1195
" + 125 *Ha*	104.2	3.00	1457
1.5% *Ggt* + 25 *Ha*	104.1	2.87	1307
" + 50 *Ha*	114.8	1.87	873
" + 125 *Ha*	93.7	4.20	1609
L.S.D; P = .05	29.1	Non-parametric analysis	523.9

	F value	χ^2 statistic	F value
Ggt	0.862	7.487	3.188*
Ha	1.035	15.309**	2.726*
Ggt, *Ha*	1.643	9.453	1.850

TABLE 21: Effect of *Ggt* (re-isolated strain) and *Ha* on various plant growth characters at maturity

Pathogen and density	Shoot dry weight (g)	Root dry weight (g)	No. of roots	Length of roots (cm)	Height (cm)	No. of grains	Yield (g)
0% *Ggt*	1.630	0.335	18.75	230.6	54.35	15.38	0.616
0.5% *Ggt*	1.535	0.300	17.50	197.8	52.31	10.63	0.490
1.0% *Ggt*	1.770	0.248	17.63	202.6	47.81	16.63	0.621
1.5% *Ggt*	1.834	0.205	15.25	147.9	49.83	22.38	0.899
25 *Ha*	1.735	0.426	19.38	222.8	52.69	17.88	0.670
50 *Ha*	1.614	0.440	19.38	224.4	53.38	16.13	0.571
125 *Ha*	1.508	0.335	17.25	205.7	53.66	15.75	0.539
0.5% *Ggt* + 25 *Ha*	1.578	0.263	17.75	188.6	53.86	14.63	0.594
" + 50 *Ha*	1.668	0.332	17.25	189.9	58.85	14.13	0.595
" + 125 *Ha*	1.518	0.268	16.25	183.8	51.89	15.25	0.640
1.0% *Ggt* + 25 *Ha*	1.865	0.270	17.63	198.6	51.83	16.13	0.806
" + 50 *Ha*	1.959	0.285	17.38	196.1	51.03	21.38	0.893
" + 125 *Ha*	1.785	0.267	17.25	187.4	49.31	15.88	0.650
1.5% *Ggt* + 25 *Ha*	1.904	0.198	15.63	170.9	51.83	22.75	0.909
" + 50 *Ha*	1.689	0.176	14.63	155.6	50.00	20.13	0.836
" + 125 *Ha*	1.865	0.223	14.88	153.9	47.39	22.50	0.846
L.S.D; P = .05	0.199	0.061	1.98	29.16	3.90	5.35	0.175
F values							
Ggt	14.573**	48.988**	17.692**	25.427**	7.164**	13.066**	19.146**
Ha	1.538	1.983	2.012	1.236	1.154	0.656	1.844
Ggt, *Ha*	1.374	2.230*	0.430	0.463	0.912	1.036	1.533

Leaf length

The main effects were consistent on all leaves except leaf 8 where a significant interaction occurred (Table 22). Many plants had leaf 7 as the flag leaf and so, the results for leaf 8 must be doubtful because they are based on a reduced number of leaves. Generally *Ggt* affected the lengths of all leaves whereas *Ha* had no effect except on leaf 4 (Table 22). *Ggt* affected the whole pattern of development of the leaves, particularly at the highest concentration (Fig.8) such that the reduction in lengths of the earlier leaves was changed to an increase in the later leaves. The effects of the combination of organisms largely followed that of *Ggt*.

Effect of *Ggt* and *Ha* on each other

The effect of pathogens on each other are shown in Table 23. *Ggt* at the lower level of 0.5% increased the number of cysts and eggs but at the two higher concentrations tended to reduce numbers particularly at the two higher densities of nematodes. *Ha* had little effect on the length of lesions of *Ggt*.

C. *Discussion*

This is a complex situation that these experiments by and large failed to resolve. Nevertheless some conclusions can be drawn. The 'mild strain' of *Ggt* caused little reaction in the plants, even though it initiated lesions, and therefore no synergistic effect in causing damage. *Ha* was not able to influence the virulence sufficiently to affect damage.

The oat-meal of the *Ggt* inoculum stimulated growth (Chapter III, 3.6). With the re-isolated strain, the effects measured were the result

TABLE 22: Effect of *Ggt* (re-isolated strain) and *Ha* on final leaf lengths

Pathogen status	Leaf 1	2	3	4	5	6	7	8
0% *Ggt*	9.25	16.01	23.00	25.29	22.61	15.61	13.12	12.14
0.5% *Ggt*	9.27	15.50	21.84	12.05	21.34	17.04	14.74	12.16
1.0% *Ggt*	7.70	13.15	17.88	18.00	18.73	20.76	17.72	16.47
1.5% *Ggt*	8.15	11.20	13.56	14.15	18.28	23.56	21.81	20.04
25 *Ha*	9.74	17.32	24.63	26.30	23.49	16.94	13.37	11.73
50 *Ha*	9.07	16.04	23.09	25.35	23.24	16.40	13.64	11.73
125 *Ha*	9.01	15.74	23.10	24.92	22.07	15.92	12.66	11.25
0.5% *Ggt* + 25 *Ha*	8.51	15.14	22.31	24.39	21.16	16.31	14.93	12.26
" + 50 *Ha*	9.19	16.00	22.79	23.99	22.14	16.75	14.43	12.49
" + 125 *Ha*	8.37	14.25	21.23	22.12	21.64	17.00	14.16	12.94
1.0% *Ggt* + 25 *Ha*	8.77	14.64	20.13	20.55	21.55	20.27	17.44	13.75
" + 50 *Ha*	8.21	13.85	18.41	18.70	22.14	20.71	18.74	14.72
" + 125 *Ha*	7.75	13.24	17.90	18.70	18.96	18.92	19.26	13.92
1.5% *Ggt* + 25 *Ha*	8.22	11.72	13.30	14.91	18.59	24.15	21.96	14.54
" + 50 *Ha*	8.14	11.69	13.60	15.27	17.21	21.72	20.07	15.71
" + 125 *Ha*	8.09	12.15	13.89	14.86	19.05	25.58	23.39	16.32
L.S.D; P = .05	1.20	1.74	2.44	2.25	2.91	2.89	3.02	1.88
F values								
Ggt	6.857**	40.840**	101.194**	130.109**	13.990**	44.871**	50.534**	43.365**
Ha	0.971	1.624	1.275	2.745*	0.923	0.212	0.247	7.365**
Ggt, *Ha*	0.713	0.896	0.555	0.505	1.005	1.041	0.695	3.331**

Figure 8: Effect of *Ggt* (re-isolated strain) on growth pattern of leaves (means of leaf lengths).

8

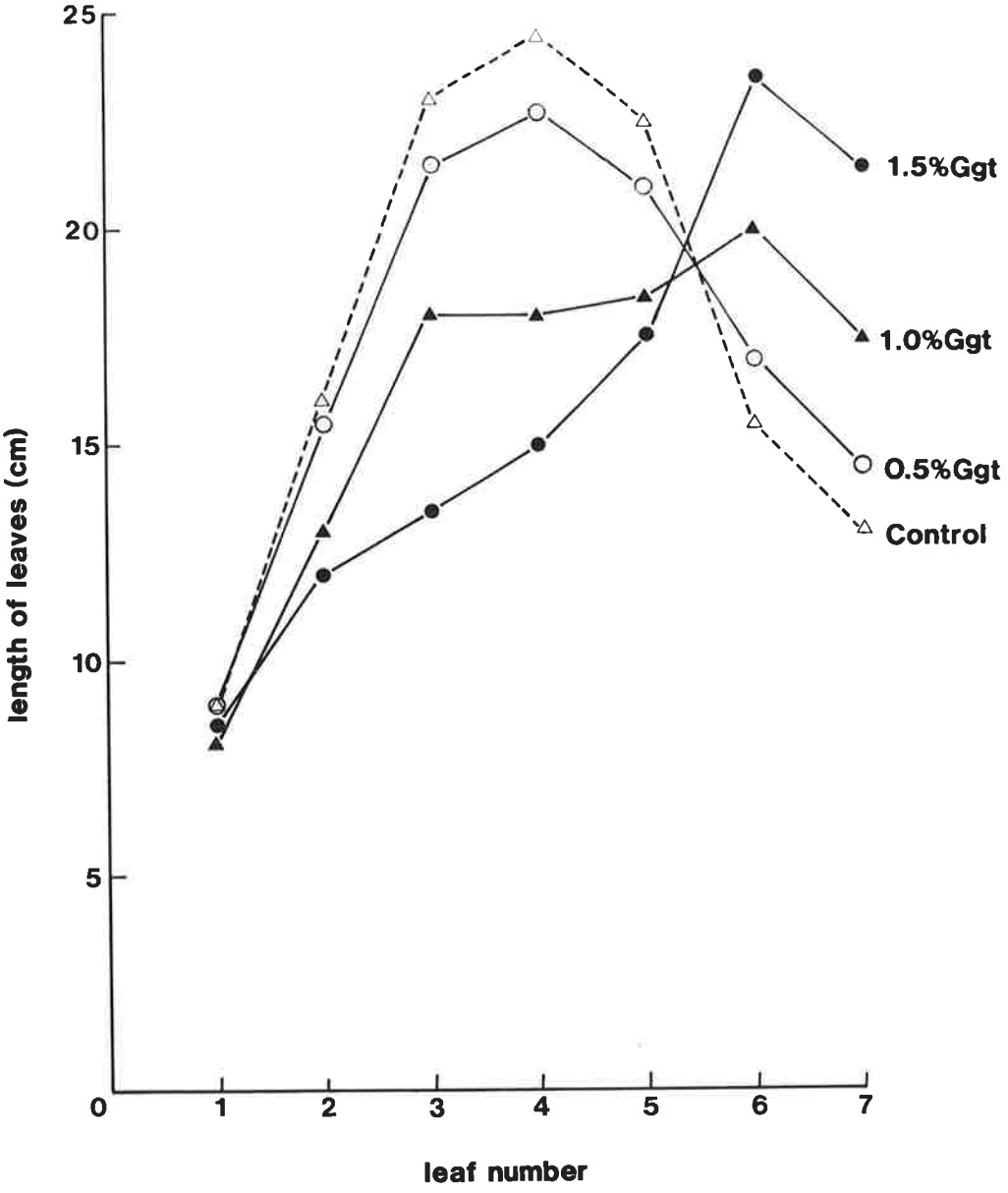

25
20
15
10
5
0
1
2
3
4
5
6
7
length of leaves (cm)
leaf number
1.5%Ggt
1.0%Ggt
0.5%Ggt
Control

TABLE 23: Effect of *Ggt* (re-isolated strain) and *Ha* on each other at maturity

Pathogen and density	Length of *Ggt* lesions (cm)	Number of cysts	Eggs of *Ha*
0% *Ggt*	-	-	-
0.5% *Ggt*	90.1	-	-
1.0% *Ggt*	102.2	-	-
1.5% *Ggt*	113.6	-	-
25 *Ha*	-	1.88	708
50 *Ha*	-	1.75	766
125 *Ha*	-	3.88	1623
0.5% *Ggt* + 25 *Ha*	100.9	3.25	1383
" + 50 *Ha*	77.8	2.38	1003
" + 125 *Ha*	82.4	4.38	1718
1.0% *Ggt* + 25 *Ha*	116.7	0.88	392
" + 50 *Ha*	103.1	1.00	372
" + 125 *Ha*	85.4	1.00	427
1.5 *Ggt* + 25 *Ha*	129.4	1.00	422
" + 50 *Ha*	120.4	0.75	244
" + 125 *Ha*	111.3	0.87	342
L.S.D; P = .05	26.6	Non-parametric analysis	461

	F value	χ^2 statistic	F value
Ggt	10.547**	36.342**	27.647**
Ha	2.961*	1.114	7.196*
Ggt, *Ha*	0.360	1.971	2.491*

of this stimulation minus the reductions due to *Ggt*. To add oatmeal to further controls would have made the experiments too large to assess, so the effect of the oatmeal will be examined later.

The effect of the re-isolated *Ggt* on the length of leaves show how complicated the relationship is. The normal pattern of development is shown by the control plants with the longest leaves being 4,5 or 6. *Ggt* at all densities but particularly at the highest, affected this pattern such that the flag leaf was the longest leaf on some plants and longer than on the controls. This would affect yield in the same way so that measurements taken at maturity may not reflect what is happening. Initial density influenced the final result and higher initial densities may change the results further. The results here suggest that conditions for *Ggt* were favourable early but perhaps were not favourable during later development. There was a suggestion from the field data that water is important so that further work may resolve this.

Ha, at the same densities, caused quite different effects in the two experiments. Normally in tubes of this size, 60% penetration of the larvae is obtained (Fisher, J.M., private communication). The difference between the two experiments was that in the first experiment, all plants inoculated with *Ha* failed to produce tillers while in the second experiment, tillers were produced. This suggests that an unknown variable was inadequately controlled. Long day conditions in the growth room would have influenced this.

Container size may have imposed constraints which were more severe at harvest than earlier and which may have affected undamaged plants more than the damaged ones. Choice of the initial densities also limits comparison to the field situation as well as results.

Although no synergism was recorded between *Ggt* and *Ha* in the effects on the host, *Ggt* at higher concentrations did reduce the ability of *Ha* to reproduce so that occurrence of both pathogens in the one plant might eventually lead to reduction in the numbers of *Ha*. At low concentrations of *Ggt* there was no effect on *Ha* and *Ha* did not affect growth of *Ggt*.

3.3 Interrelationships between *Rhizoctonia solani (Rs)* and *Heterodera avenae (Ha)*

Both *Rs* and *Ha* were found on wheat plants in the field in each year. These two pathogens were negatively correlated with the growth parameter of dry shoot weight. The consistent negative correlation at all sampling times in the second year of the field study between the two pathogens suggested that a relationship existed between these two pathogens but did not reveal the effect of *Rs* on *Ha* or *vice versa*. Meagher (1981) reported that an interaction between *Rs* and *Ha* is probably the most frequent cause of severe disease and yield reduction in wheat in south-eastern Australia.

The test the hypothesis of negative correlation two experiments were done. The first experiment used four levels of *Rs* (0, 0.5, 1.0 and 1.5%), four rates of *Ha* (0, 25, 50 and 125 larvae/100g of soil) with a seven-fold replication. Leaf length measurements were taken weekly and all other parameters (plant and organism) were measured at maturity.

The second experiment also used four levels of *Rs*, but the concentrations used were reduced (0, 0.05, 0.1 and 0.5%); *Ha* concentrations were unchanged and replication was eight-fold. Plants were assayed at 10, 20 and 30 days to reveal the relationship between the pathogens during early growth.

A. *Results - At maturity*

Growth

No significant interaction occurred (Table 24) and only *Rs* affected plant growth. *Rs* reduced dry shoot weight, dry root weight, number of roots, length of roots, height and yield. At the highest concentration growth was most severely affected. Although not significant, combination of *Rs* with *Ha* tended to reduce all parameters of growth more than *Rs* or *Ha* independently.

Leaf length

Interaction between *Rs* and *Ha* was significant for leaves 8 and 9. There was an increase in the length of leaf 8 with the combination of 0.5% *Rs* with 125 larvae and 1.5% *Rs* with 50 larvae. This increase may have caused the interaction (Fig.9). Since there were many plants in which leaf 8 was the flag leaf, the interaction on leaf 9 is not considered to be meaningful. *Rs* had a significant effect on the lengths of all leaves (Table 25). The lowest concentration of 0.5% reduced the lengths of leaves 6 to 9. The intermediate concentration of 1.0% reduced the lengths of leaves 4 to 9, and the highest concentration of 1.5% reduced lengths of leaves 3 to 9. As the concentration increased, the number of leaves affected also increased.

Effect of *Rs* and *Ha* on each other

Combinations of *Rs* and *Ha* had no effect on lesion length. Lesion length was lower at the highest initial concentration (Table 26) because the root length was also greatly reduced at this rate of *Rs*. With each initial increasing concentration of *Ha*, more cysts were recovered at maturity. Combinations of *Rs* and *Ha* at all densities, reduced the

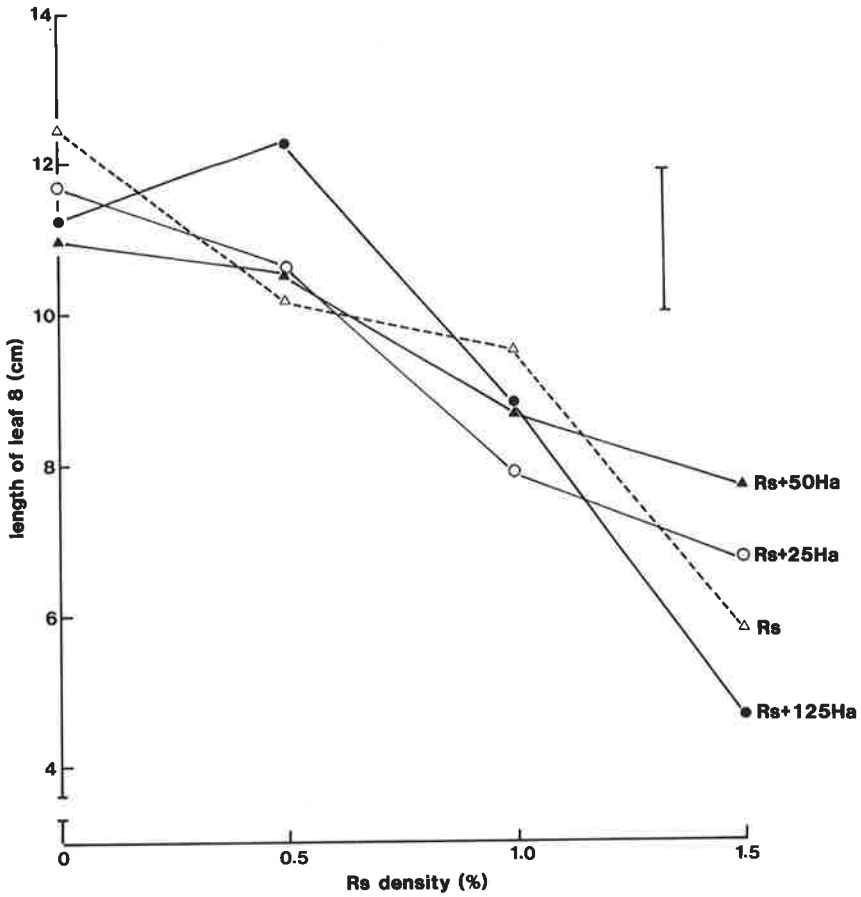
TABLE 24: The effects of *Rs* and *Ha* on various plant growth characters at maturity

Pathogen and density	Dry shoot weight (g)	Dry root weight (g)	Number of roots	Length of roots (cm)	Height (cm)	Yield (g)
0% *Rs*	1.143	0.237	13.71	160.2	53.4	0.433
0.5% *Rs*	0.766	0.138	12.43	54.4	47.0	0.281
1.0% *Rs*	0.569	0.101	11.41	42.6	41.6	0.210
1.5% *Rs*	0.159	0.054	7.41	20.9	22.6	0.043
25 *Ha*	1.013	0.248	12.71	149.5	49.4	0.360
50 *Ha*	1.110	0.238	14.43	169.4	51.9	0.427
125 *Ha*	1.006	0.215	13.57	120.8	49.0	0.371
0.5% *Rs* + 25 *Ha*	0.763	0.138	11.43	60.7	51.2	0.283
" + 50 *Ha*	0.647	0.132	10.00	47.0	46.9	0.247
" + 125 *Ha*	0.621	0.104	11.04	55.3	44.5	0.225
1.0% *Rs* + 25 *Ha*	0.434	0.091	10.43	43.7	37.3	0.126
" + 50 *Ha*	0.559	0.090	11.29	42.4	42.4	0.197
" + 125 *Ha*	0.462	0.088	9.32	30.8	43.7	0.176
1.5% *Rs* + 25 *Ha*	0.211	0.057	8.43	24.4	31.3	0.069
" + 50 *Ha*	0.234	0.058	10.71	34.2	29.6	0.056
" + 125 *Ha*	0.109	0.040	8.17	19.2	24.1	0.032
L.S.D; P = .05	0.291	0.055	2.92	27.44	11.36	0.124
F values						
Rs	51.984**	62.807**	15.837**	135.425**	27.776**	44.445**
Ha	0.854	1.034	0.854	2.290	0.297	0.754
Rs, *Ha*	0.288	0.129	1.138	1.210	0.670	0.412

Figure 9: Effect of *Rs* and *Ha* combination on length of leaf 8.

Figure 10: Effect of *Rs* on *Ha* survival.

9


14
12
10
8
6
4
length of leaf 8 (cm)
Rs+50Ha
Rs+25Ha
Rs
Rs+125Ha
0
0.5
1.0
1.5
Rs density (%)

10

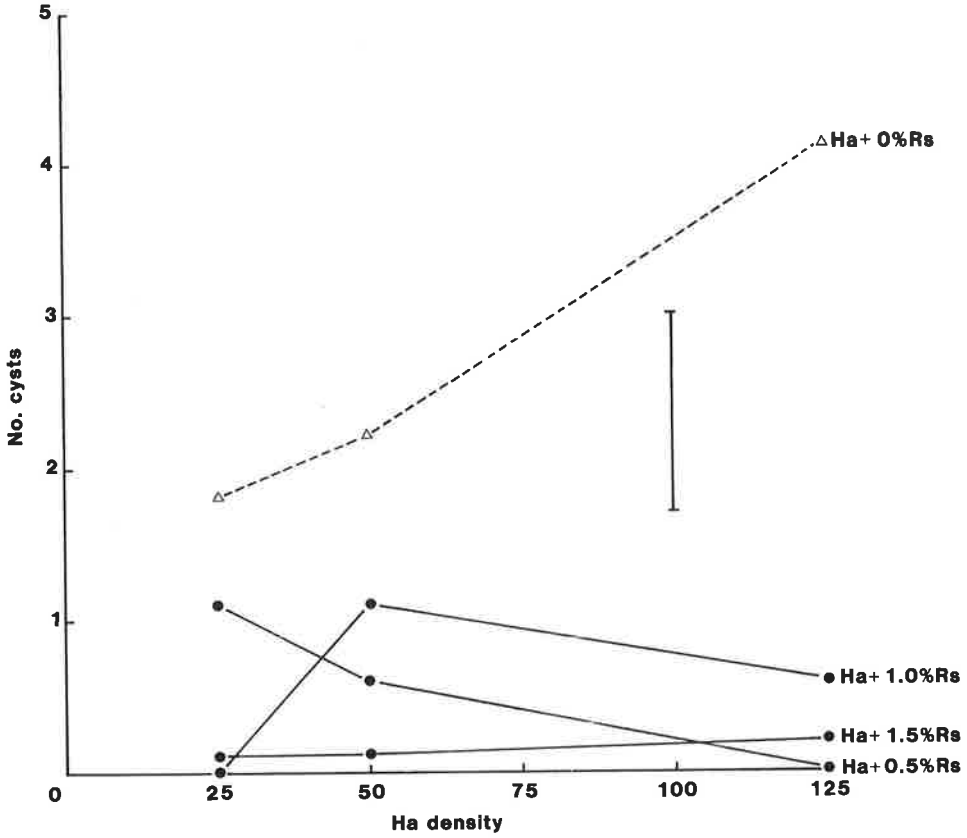

5
4
3
2
1
No. cysts
Ha+ 0%Rs
Ha+ 1.0%Rs
Ha+ 1.5%Rs
Ha+ 0.5%Rs
0
25
50
75
100
125
Ha density

TABLE 25: The effects of *Rs* and *Ha* on leaf lengths (cm)

Pathogen and density	Leaf 1	Leaf 2	Leaf 3	Leaf 4	Leaf 5	Leaf 6	Leaf 7	Leaf 8	Leaf 9
0% *Rs*	4.19	8.13	14.74	21.29	22.44	20.46	16.27	12.47	9.57
0.5% *Rs*	5.40	10.74	16.76	18.80	18.89	16.63	13.00	10.17	8.19
1.0% *Rs*	4.71	8.58	12.82	15.84	16.46	14.94	12.45	9.51	8.42
1.5% *Rs*	3.06	5.27	7.80	11.51	10.67	8.29	6.31	5.78	5.07
25 *Ha*	3.41	9.39	16.34	20.80	22.27	19.13	13.74	11.73	9.85
50 *Ha*	3.54	7.83	14.33	21.63	22.24	18.96	14.09	10.99	9.49
125 *Ha*	3.77	8.37	15.51	18.59	20.53	18.73	14.00	11.21	8.65
0.5% *Rs* + 25 *Ha*	4.64	10.29	15.40	17.57	19.71	18.06	15.27	10.59	9.13
" + 50 *Ha*	5.34	10.31	14.29	16.80	17.04	15.53	12.11	10.56	9.66
" + 125 *Ha*	3.43	6.36	11.40	14.77	16.27	17.60	15.83	12.28	10.11
1.0% *Rs* + 25 *Ha*	5.87	9.23	12.59	14.27	15.83	12.40	10.43	7.89	7.22
" + 50 *Ha*	6.93	11.71	16.24	17.41	16.39	14.09	12.11	8.70	7.78
" + 125 *Ha*	5.55	11.01	14.15	14.04	13.89	11.41	10.10	8.80	8.26
1.5% *Rs* + 25 *Ha*	3.56	5.64	8.81	10.36	10.96	9.00	8.48	6.76	5.89
" + 50 *Ha*	3.54	7.16	10.16	10.70	10.96	9.33	8.11	7.69	7.99
" + 125 *Ha*	3.52	5.32	8.57	9.94	9.24	7.47	6.31	4.68	5.99
L.S.D; P = .05	2.10	4.00	4.90	4.38	3.90	3.68	3.08	1.94	1.31
F values									
Rs	8.089**	6.990**	11.229**	28.355**	47.909**	52.145**	36.401**	49.599**	41.040**
Ha	0.741	0.808	0.419	2.187	2.234	0.660	0.166	0.169	2.940*
Rs, *Ha*	0.867	0.996	0.941	0.331	0.215	0.777	1.943	2.362*	3.400**

TABLE 26: The effects of *Rs* and *Ha* on each other at maturity

Pathogen and density	Length of *Rs* lesions (cm)	Cysts
0% *Rs*	-	-
0.5% *Rs*	19.73	-
1.0% *Rs*	20.15	-
1.5% *Rs*	11.57	-
25 *Ha*	-	1.86
50 *Ha*	-	2.29
125 *Ha*	-	4.14
0.5% *Rs* + 25 *Ha*	13.64	1.14
" + 50 *Ha*	15.36	1.00
" + 125 *Ha*	15.60	1.33
1.0% *Rs* + 25 *Ha*	17.00	0.00
" + 50 *Ha*	19.07	1.14
" + 125 *Ha*	12.53	0.59
1.5% *Rs* + 25 *Ha*	11.07	0.14
" + 50 *Ha*	13.50	0.14
" + 125 *Ha*	9.30	0.21
L.S.D; P = .05	5.96	1.32
F values		
Rs	55.275**	13.411**
Ha	2.196	8.214**
Rs, *Ha*	0.834	2.688*

number of cysts. The highest rate of *Rs* with all concentrations of *Ha*, reduced the cyst population to the same level (Table 26, Fig.10).

B. *Results at 10, 20 and 30 days*

The influence of *Rs* and *Ha* on early growth of plants and on each other was assessed by measuring the following variables: fresh shoot and root weight; dry shoot weight, length of roots, lengths of leaves, length and total number of *Rs* lesions, number of main root tips damaged by *Rs*, number of other lesions (besides root tips), number of whole roots destroyed by *Rs* and percentage of *Rs* on roots (length of *Rs* lesions ÷ length of roots x 100). The roots were then stained in lactophenol-cotton-blue and cleared in lactophenol for *Ha* larval counts.

Growth at 10 days

The interaction between *Rs* and *Ha* significantly reduced both fresh root weight and the length of roots. The interaction was not significant for other growth parameters at this early stage of growth (Table 27). The combination of 0.05% *Rs* with 25 and 50 larvae reduced the fresh weight of root more than 0.05% with the highest density of *Ha*. At the intermediate level of *Rs* significant reduction with 125 larvae occurred compared with the combination with 50 larvae. The increase in root weight due to galls and lateral roots and this may have caused the interaction (Fig.11). There was a general reduction of the root length with all combinations of both pathogens. Not all combinations caused a significant reduction (Fig.12).

Rs significantly reduced all growth variables (Table 27) including the length of the first leaf. *Ha* only at the highest density reduced root length. *Ha* also increased the length of leaf 1.

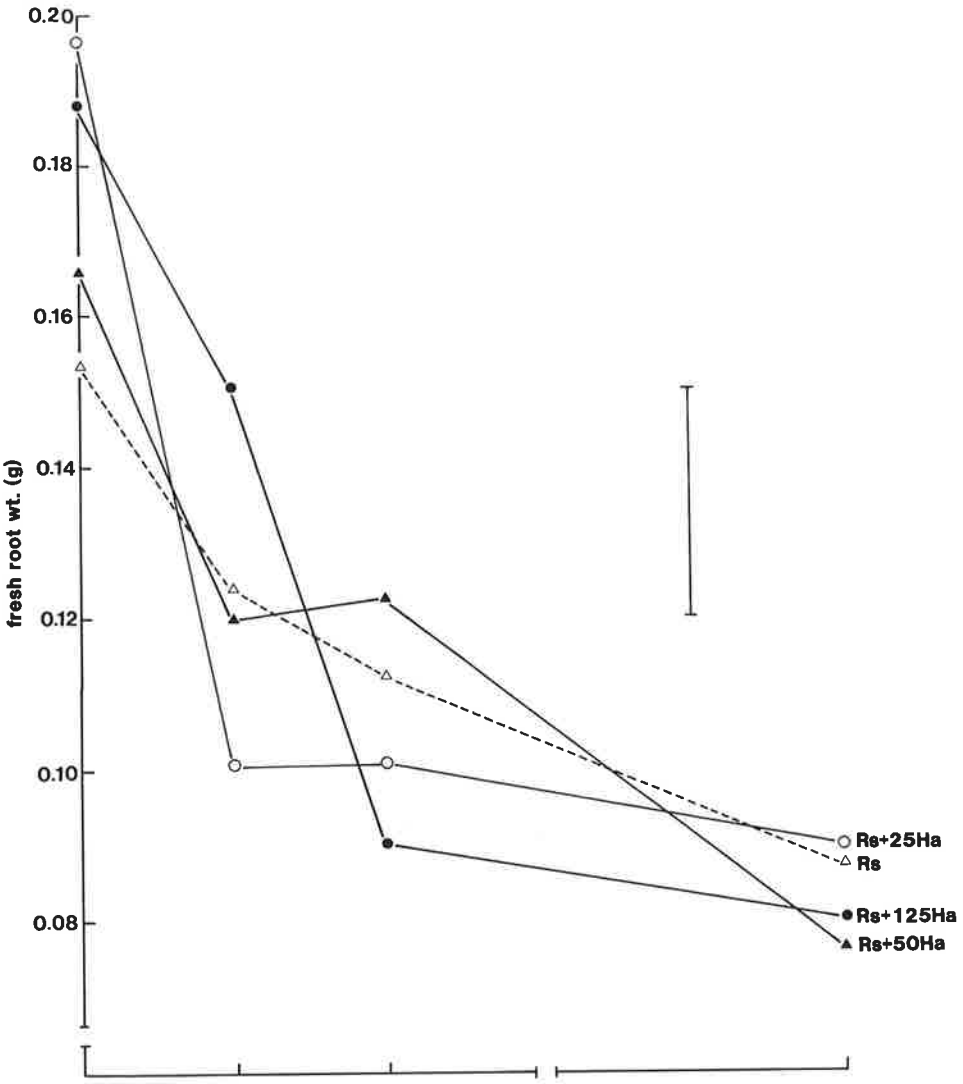
TABLE 27: The effects of *Rs* and *Ha* on plant growth characters at 10 days

Pathogen and density	Fresh shoot weight (g)	Fresh root weight (g)	Dry shoot weight (g)	Root length (cm)	Length of Leaf 1 (cm)
0 % *Rs*	0.2050	0.1538	0.01963	49.63	9.89
0.05% *Rs*	0.1212	0.1238	0.01263	32.00	7.84
0.1 % *Rs*	0.0900	0.1125	0.01013	31.75	7.54
0.5 % *Rs*	0.0725	0.0875	0.00880	23.88	7.59
25 *Ha*	0.2112	0.1963	0.02025	56.25	10.63
50 *Ha*	0.1850	0.1663	0.01788	45.81	9.71
125 *Ha*	0.1712	0.1888	0.01700	29.56	10.11
0.05% *Rs* + 25 *Ha*	0.0975	0.1050	0.01100	23.75	8.63
" + 50 *Ha*	0.0987	0.1200	0.01138	26.69	9.00
" + 125 *Ha*	0.1100	0.1513	0.01275	28.63	9.24
0.1% *Rs* + 25 *Ha*	0.0900	0.1013	0.01063	22.50	8.51
" + 50 *Ha*	0.1025	0.1225	0.01313	24.00	9.88
" + 125 *Ha*	0.0800	0.0900	0.01063	18.69	8.91
0.5% *Rs* + 25 *Ha*	0.0900	0.0900	0.01138	25.94	8.80
" + 50 *Ha*	0.0725	0.0763	0.00975	17.44	8.38
" + 125 *Ha*	0.0662	0.0800	0.00950	17.38	8.16
L.S.D; P = .05	0.028	0.031	0.00296	7.116	1.54
F values					
Rs	103.632**	49.605**	56.200**	72.332**	8.074**
Ha	1.998	0.385	0.458	13.668**	2.866*
Rs, *Ha*	1.358	2.518*	1.658	5.272**	0.843

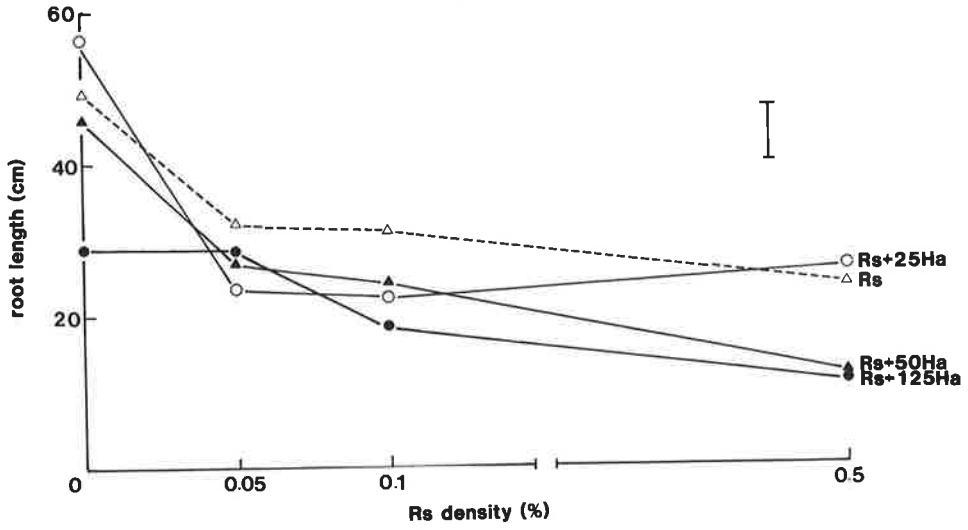
Figure 11: Effect of *Rs* and *Ha* combination on fresh root weight (g) at 10 days.

Figure 12: Effect of *Rs* and *Ha* combination on length of roots at 10 days.

11


0.20
0.18
0.16
0.14
0.12
0.10
0.08
fresh root wt. (g)
Rs+25Ha
Rs
Rs+125Ha
Rs+50Ha

12


60
40
20
0
root length (cm)
0.05
0.1
0.5
Rs density (%)
Rs+25Ha
Rs
Rs+50Ha
Rs+125Ha

Effect of *Rs* and *Ha* on each other

The interaction between the pathogens was significant for the length of *Rs* lesions, number of lesions other than those on root tips, % *Rs* on roots and number of *Ha* larvae in the roots (Table 28). When combinations of pathogens were inoculated there was a general increase in lesion length as *Rs* concentration increased and this contrasted with no change in lesion length with concentration when *Rs* was inoculated alone (Fig.13). At the highest concentration of *Rs*, lesion length decreased as *Ha* density increased. When lesion length was expressed as a percentage (Fig.14), the pattern was similar except that the relation at the highest concentration changed. This was probably because part of the root system had been severely rotted by *Rs* and this affected both length of roots and lesions. This is exemplified by the change in the points for the *Rs* with 125 *Ha* for the length of lesions relative to *Rs* alone (Fig.13) compared to the same data plotted as percentage of *Rs* on roots (Fig.14). It is probably involved in the data for 0.5% *Rs* with 50 *Ha* (Figs. 13 and 14) as well.

The number of other lesions produced at 0.5% *Rs* with 25 *Ha* was markedly increased and this probably caused the interaction. Generally more whole roots were destroyed as concentration of *Rs* increased and *Ha* influenced this (Table 28). Numbers of *Ha* larvae in the roots were severely reduced as concentration of *Rs* increased such that at the highest concentration of *Rs*, different initial densities of *Ha* had no effect on numbers of larvae in the roots (Fig.15).

Growth at 20 days

Significant interactions were obtained with fresh shoot weight, dry shoot weight, root length and length of leaf 2 (Table 29). When plotted (Figs.16 and 17) it is difficult to see consistent changes related to a significant interaction; they may be trivial.

TABLE 28: The effects of *Rs* and *Ha* on each other at 10 days

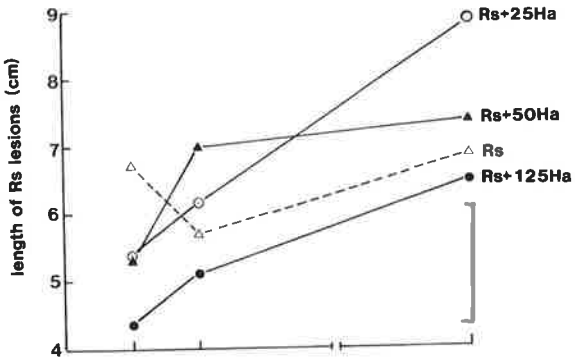
Pathogen and density	Length of *Rs* lesions (cm)	Number of *Rs* lesions (Total)	Number of root-tips attacked by *Rs*	Number of other lesions (*Rs*)	Number of whole roots destroyed by *Rs*	% *Rs* on roots	Number of *Ha*
0 % *Rs*	–	–	–	–	–	–	–
0.05% *Rs*	6.75	6.63	3.13	3.50	0.000	21.97	–
0.1 % *Rs*	5.75	5.75	3.25	2.50	0.000	19.95	–
0.5 % *Rs*	6.87	7.00	3.63	3.38	0.250	29.15	–
25 *Ha*	–	–	–	–	–	–	5.38
50 *Ha*	–	–	–	–	–	–	6.63
125 *Ha*	–	–	–	–	–	–	7.63
0.05% *Rs* + 25 *Ha*	5.38	5.75	3.25	2.38	0.125	23.33	4.25
" + 50 *Ha*	5.37	5.38	3.00	2.37	0.000	20.83	4.38
" + 125 *Ha*	4.37	4.63	2.38	2.25	0.125	16.38	6.88
0.1 % *Rs* + 25 *Ha*	6.13	6.13	3.50	2.50	0.375	28.00	2.88
" + 50 *Ha*	7.00	7.00	3.13	3.88	0.500	29.34	4.75
" + 125 *Ha*	5.13*	5.62	3.38	2.25	0.500	28.84	3.88
0.5 % *Rs* + 25 *Ha*	8.87*	9.00	2.88	6.13	1.000	36.21	2.63
" + 50 *Ha*	7.37	7.50	3.75	3.75	0.750	44.02	1.88
" + 125 *Ha*	6.50	6.50	3.88	2.63	1.000	38.65	3.25
L.S.D; P = .05	1.57	1.65	0.98	1.77	0.473	6.01	1.58
F values							
Rs	12.686**	11.084**	2.921	5.411**	16.692**	59.630**	27.052**
Ha	4.054*	2.957*	0.095	2.253	4.533**	6.822**	8.331**
Rs, *Ha*	2.171*	1.734	1.487	2.850*	0.985	3.810**	2.201*

Figure 13: Effect of *Rs* and *Ha* combination on *Rs* lesion length at 10 days.

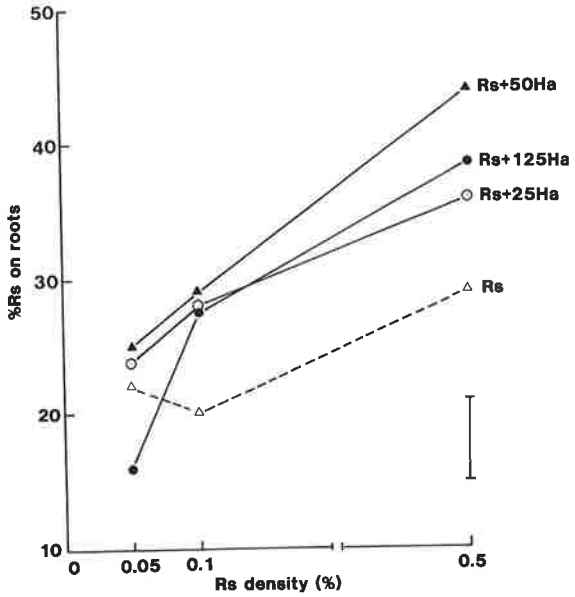
Figure 14: Effect of *Rs* and *Ha* combination on % *Rs* on roots at 10 days.

Figure 15: Effect of *Rs* and *Ha* combination on number of *Ha* larvae in roots at 10 days.

13


length of Rs lesions (cm)
9
8
7
6
5
4
Rs+25Ha
Rs+50Ha
Rs
Rs+125Ha

14


%Rs on roots
50
40
30
20
10
0
0.05
0.1
0.5
Rs density (%)
Rs+50Ha
Rs+125Ha
Rs+25Ha
Rs

15

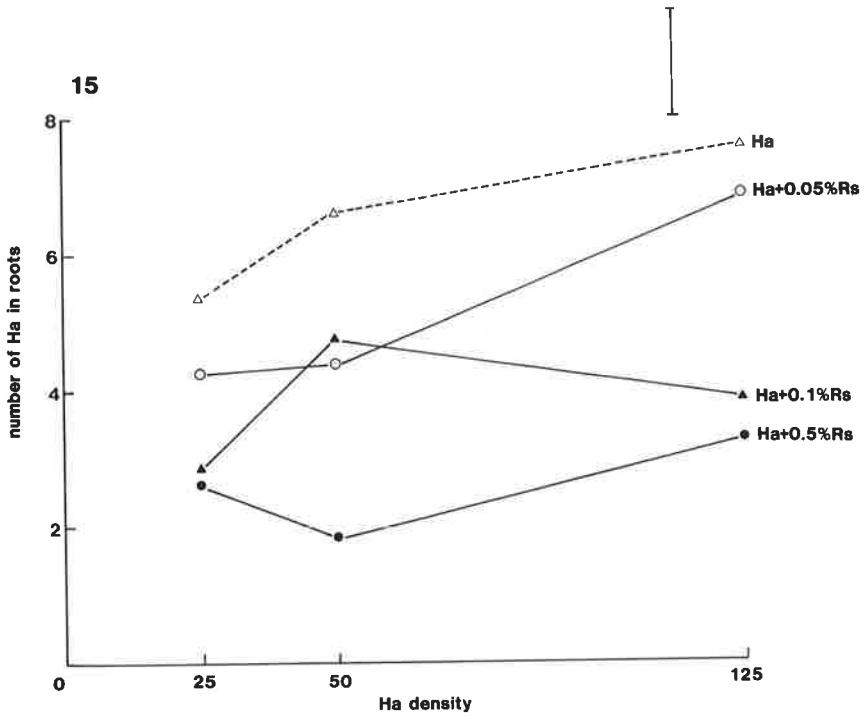

number of Ha in roots
8
6
4
2
0
25
50
125
Ha density
Ha
Ha+0.05%Rs
Ha+0.1%Rs
Ha+0.5%Rs

TABLE 29: The effects of *Rs* and *Ha* on various growth characters at 20 days

Pathogen and density	Fresh shoot weight (g)	Fresh root weight (g)	Dry shoot weight (g)	Root length (cm)	Length of Leaf 1 (cm)	Length of Leaf 2 (cm)	Length of Leaf 3 (cm)
0 % *Rs*	0.7850	0.914	0.0862	90.94	9.23	18.29	27.31
0.05% *Rs*	0.4850	0.355	0.0571	53.57	8.94	17.76	23.71
0.1 % *Rs*	0.2738	0.228	0.0330	32.12	7.31	13.80	16.38
0.5 % *Rs*	0.2313	0.171	0.0292	26.62	7.68	13.46	14.76
25 *Ha*	0.7363	0.816	0.0788	92.25	9.73	19.40	26.16
50 *Ha*	0.7000	0.740	0.0755	87.81	9.75	19.79	26.84
125 *Ha*	0.6450	0.824	0.0693	70.88	8.95	17.00	25.77
0.05% *Rs* + 25 *Ha*	0.2800	0.295	0.0362	30.56	8.19	14.07	17.38
" + 50 *Ha*	0.3600	0.356	0.0431	35.62	9.01	16.51	20.47
" + 125 *Ha*	0.2550	0.253	0.0323	23.94	7.98	13.96	16.56
0.1 % *Rs* + 25 *Ha*	0.3000	0.270	0.0375	31.56	8.98	15.34	18.05
" + 50 *Ha*	0.2638	0.268	0.0346	29.50	8.97	14.79	17.95
" + 125 *Ha*	0.1825	0.159	0.0241	21.19	8.11	11.37	11.55
0.5 % *Rs* + 25 *Ha*	0.2213	0.164	0.0298	33.75	8.58	12.12	13.27
" + 50 *Ha*	0.1813	0.125	0.0267	22.69	8.89	12.06	11.30
" + 125 *Ha*	0.2000	0.150	0.0266	23.62	8.14	12.55	11.14
L.S.D; P = .05	0.0870	0.126	0.0097	10.19	1.47	2.29	4.03
F values							
Rs	220.004**	175.346**	165.172**	232.989**	3.828*	41.091**	67.731**
Ha	10.502**	1.657	9.514**	13.869**	2.686*	5.773**	6.088**
Rs, *Ha*	2.454*	1.154	2.248*	3.158**	0.714	2.319*	1.698

TABLE 30: The effect of *Rs* and *Ha* on each other at 20 days

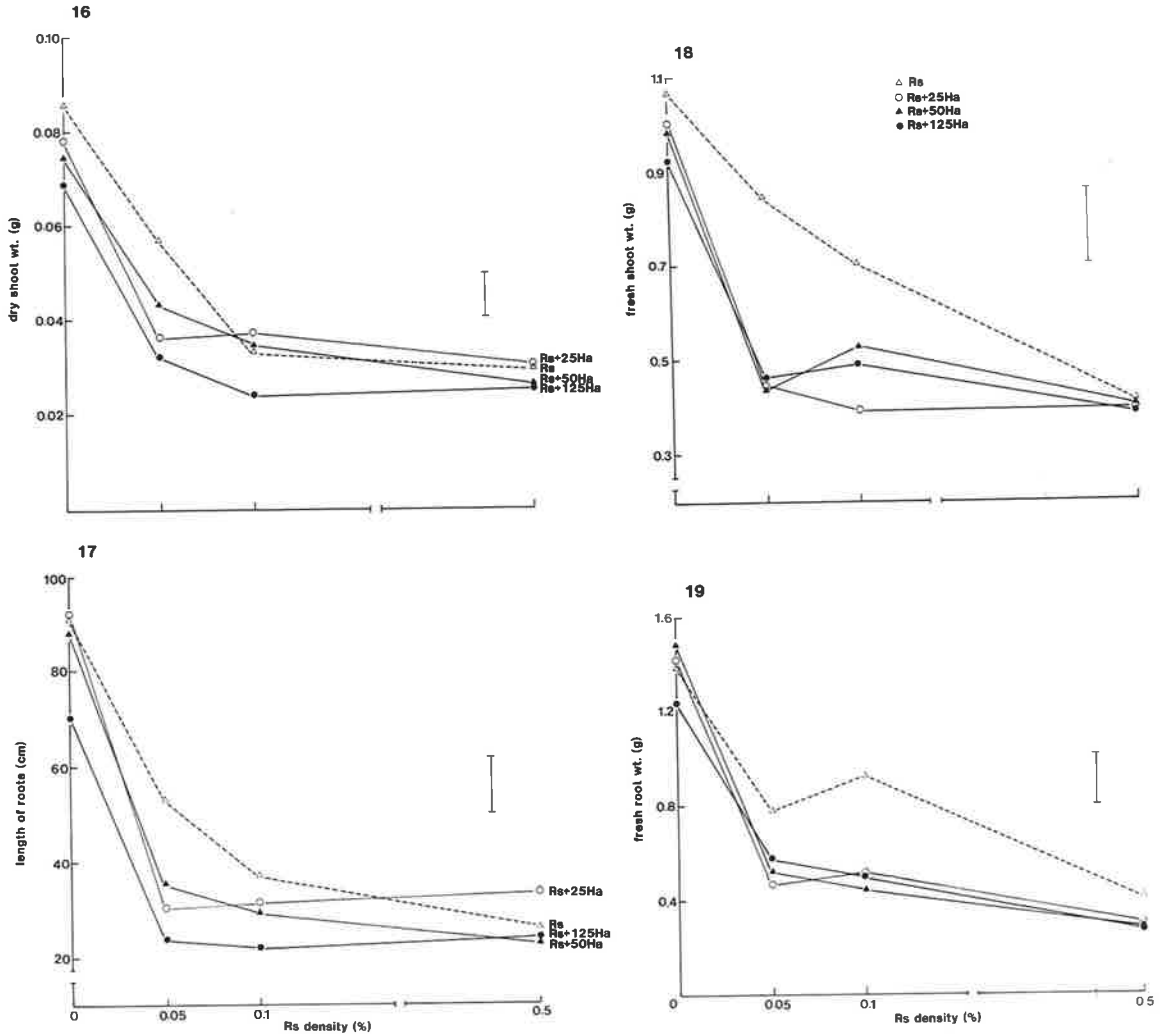
Pathogen and density	Length of *Rs* lesions (cm)	Number of *Rs* lesions (Total)	Number of root-tips attacked by *Rs*	Number of other lesions	Number of whole roots destroyed by *Rs*	% *Rs* on roots	Number of *Ha*
0 % *Rs*	-	-	-	-	-	-	-
0.05% *Rs*	12.75	14.13	5.63	8.50	1.25	23.88	-
0.1 % *Rs*	10.06	10.58	5.25	5.25	1.25	33.60	-
0.5 % *Rs*	10.25	9.63	5.63	4.00	1.50	37.54	-
25 *Ha*	-	-	-	-	-	-	9.38
50 *Ha*	-	-	-	-	-	-	10.25
125 *Ha*	-	-	-	-	-	-	11.00
0.05% *Rs* + 25 *Ha*	10.44	10.50	5.25	5.25	1.12	35.25	3.13
" + 50 *Ha*	11.81	12.50	5.50	7.00	1.12	33.32	3.75
" + 125 *Ha*	8.63	8.75	4.75	4.00	1.25	36.68	6.88
0.1 % *Rs* + 25 *Ha*	10.69	10.75	5.00	5.75	1.25	36.33	3.00
" + 50 *Ha*	11.75	10.88	5.63	5.25	1.12	39.81	4.13
" + 125 *Ha*	9.06	8.88	4.63	4.25	2.25	44.11	4.13
0.5 % *Rs* + 25 *Ha*	16.75	14.63	5.63	9.00	2.12	47.70	2.12
" + 50 *Ha*	11.44	10.75	4.88	5.88	2.37	50.99	2.25
" + 125 *Ha*	12.25	11.63	5.63	6.00	2.25	52.81	3.37
L.S.D; P = .05	3.16	3.26	N.S.	3.24	0.90	7.68	1.81
F values							
Rs	4.439**	1.707	0.749	1.141	7.568**	30.041**	81.953**
Ha	2.877*	2.000	0.996	1.411	1.724	11.936**	9.198**
Rs, *Ha*	3.427**	3.001*	1.295	2.444*	1.181	0.684	1.378

Figure 16: Effect of *Rs* and *Ha* combinaton on dry shoot weight at 20 days.

Figure 17: Effect of *Rs* and *Ha* combinaton on length of roots at 20 days.

Figure 18: Effect of *Rs* and *Ha* combination on fresh shoot weight at 30 days.

Figure 19: Effect of *Rs* and *Ha* combination on fresh root weight at 30 days.


16
0.10
0.08
0.06
0.04
0.02
dry shoot wt. (g)
Rs+25Ha
Rs
Rs+50Ha
Rs+125Ha
18
1.1
0.9
0.7
0.5
0.3
fresh shoot wt. (g)
Rs
Rs+25Ha
Rs+50Ha
Rs+125Ha
17
100
80
60
40
20
length of roots (cm)
Rs+25Ha
Rs
Rs+125Ha
Rs+50Ha
0
0.05
0.1
0.5
Rs density (%)
19
1.6
1.2
0.8
0.4
fresh root wt. (g)
0
0.05
0.1
0.5
Rs density (%)

Combinations of *Rs* with *Ha*, at all concentrations reduced the above mentioned parameters. The effect of 0.05% *Rs* with all *Ha* densities on fresh shoot weight and length of roots was severe (Fig.17). Severe reduction also occurred with dry shoot weight at the 25 and 50 larval densities in combination with the low rate of 0.05% *Rs* (Fig.16). All concentrations of *Rs* significantly reduced all growth parameters except the length of the first three leaves at the lowest rate of *Rs*. *Ha* at the highest density of 125 larvae significantly reduced fresh and dry whoot weight, and the length of roots. *Ha* had little effect on the length of leaves though its effect was significant for all three leaves.

Effect of *Rs* and *Ha* on each other

Combination of the low rate of *Rs* with all densities of *Ha* reduced the length of *Rs* lesions, total number of lesions and number of lesions other than that on root tips; parameters for which the interaction terms were significant (Table 30). Only % *Rs* on roots increased with increasing concentration of *Rs*. Numbers of *Ha* in the roots were significantly reduced in all combinations of *Rs* and *Ha* densities.

Growth at 30 days

Significant interactions were obtained with fresh and dry shoot weights and with fresh root weight (Table 31). This was probably because the combination of the two lower concentrations of *Rs* with all nematode densities severely reduced shoot weights (Fig.18). This reduction did not occur at the highest concentration of *Rs* (Fig.18). Fresh root weight was reduced by all combinations of *Rs* and *Ha* densities except the combination of 0.5% *Rs* with 125 larvae (Fig.19). A similar reduction in the length of leaf 4 would explain the significant interaction (Table 31).

Rs at all concentrations reduced the length of roots and the length of the first three leaves significantly (Table 31). The reduction was also significant between the lowest and the highest concentrations of *Rs*. *Ha* at all concentrations reduced the length of roots.

Effect of *Rs* and *Ha* on each other

Significant interactions between the pathogens resulted because more whole roots were destroyed at the 25 and 125 larval densities in combination with the highest concentration of *Rs* (Table 32). Number of root tips and percentage of *Rs* on roots increased significantly at the intermediate and highest concentrations of *Rs*. *Rs* greatly reduced the number of larvae in the roots and the reduction was greater with increasing concentration of *Rs*.

C. *Discussion*

Both *Rs* and *Ha* under field conditions were negatively related to growth (dry shoot weight). Under laboratory conditions *Rs* was extremely damaging, although its effect on growth, yield and root damage was probably exaggerated at the concentrations used, by the method of inoculation and the type and size of containers. Under field conditions roots can grow away from the inoculum but under these experimental conditions all roots were closely associated with the inoculum throughout the life of the plant. *H. avenae* in these experiments did not affect growth to the same extent found in the field. Only with the highest concentration of 125 larvae did a significant reduction in shoot growth occur. During early growth of the plant *Ha* damage was limited to the seminal roots because these were the only roots present, whereas *Rs* caused damage to both seminal and coronal roots.

TABLE 31: The effects of *Rs* and *Ha* on various growth characters at 30 days

Pathogen and density	Fresh shoot weight (g)	Fresh root weight (g)	Dry shoot weight (g)	Root length (cm)	Length of Leaf 1 (cm)	Leaf 2 (cm)	Leaf 3 (cm)	Leaf 4 (cm)
0 % *Rs*	1.070	1.399	0.1680	112.3	11.11	21.36	28.52	24.15
0.05% *Rs*	0.845	0.788	0.1223	62.8	8.65	16.85	23.69	23.09
0.1 % *Rs*	0.701	0.934	0.1000	69.9	9.09	16.57	23.46	22.02
0.5 % *Rs*	0.415	0.411	0.0600	38.0	7.40	13.86	18.91	18.76
25 *Ha*	0.986	1.415	0.1446	90.4	10.01	20.32	28.21	23.49
50 *Ha*	1.001	1.486	0.1483	89.6	10.26	19.90	27.69	24.89
125 *Ha*	0.924	1.251	0.1515	77.9	10.34	18.76	27.26	23.97
0.05% *Rs* + 25 *Ha*	0.435	0.471	0.0636	35.9	8.29	13.52	18.29	17.81
" + 50 *Ha*	0.446	0.522	0.0739	42.3	8.49	14.84	19.40	18.31
" + 125 *Ha*	0.460	0.580	0.0638	40.1	6.75	15.13	19.93	18.72
0.1 % *Rs* + 25 *Ha*	0.525	0.522	0.0750	39.5	8.25	14.86	20.25	19.70
" + 50 *Ha*	0.394	0.447	0.0561	39.0	9.31	15.46	18.64	17.60
" + 125 *Ha*	0.496	0.519	0.0690	46.2	8.23	13.57	18.70	19.11
0.5 % *Rs* + 25 *Ha*	0.409	0.294	0.0593	34.4	8.10	12.99	16.31	15.92
" + 50 *Ha*	0.416	0.280	0.0623	32.7	8.80	13.49	16.47	16.27
" + 125 *Ha*	0.382	0.276	0.0575	29.8	7.94	13.36	15.91	16.12
L.S.D; P = .05	0.157	0.213	0.0204	13.38	1.38	2.03	2.49	2.05
F values								
Rs	84.396**	147.245**	129.912**	116.513**	20.501**	62.610**	109.166**	68.516**
Ha	10.950**	7.616**	13.874**	19.292**	2.680	5.774	11.821**	13.343**
Rs, *Ha*	2.653**	2.131*	3.103**	1.894	1.325	1.042	1.398	2.573*

TABLE 32: The effects of *Rs* and *Ha* on each other at 30 days

Pathogen and density	Length of *Rs* lesions (cm)	Number of *Rs* lesions (Total) (cm)	Number of root-tips attacked by *Rs*	Number of other lesions	Number of whole roots destroyed by *Rs*	% *Rs* on roots	Number of *Ha*
0 % *Rs*	-	-	-	-	-	-	-
0.05% *Rs*	19.00	18.63	8.00	10.63	1.12	25.96	-
0.1 % *Rs*	22.81	21.50	10.13	11.38	4.00	37.59	-
0.5 % *Rs*	15.63	15.13	10.00	5.25	3.50	43.34	-
25 *Ha*	-	-	-	-	-	-	7.25
50 *Ha*	-	-	-	-	-	-	9.38
125 *Ha*	-	-	-	-	-	-	9.75
0.05% *Rs* + 25 *Ha*	14.13	14.25	9.00	5.25	1.87	41.32	2.38
" + 50 *Ha*	19.63	17.75	9.75	8.00	2.50	48.97	3.50
" + 125 *Ha*	16.75	14.50	7.75	6.75	1.75	39.40	5.63
0.1 % *Rs* + 25 *Ha*	18.88	19.13	9.00	10.12	3.00	49.09	2.25
" + 50 *Ha*	18.25	16.50	9.12	7.37	2.13	47.45	3.08
" + 125 *Ha*	19.63	18.93	8.84	10.09	2.45	43.33	3.11
0.5 % *Rs* + 25 *Ha*	20.00	18.13	11.62	6.50	5.50	59.10	1.00
" + 50 *Ha*	19.88	17.75	11.13	6.62	3.75	60.90	1.12
" + 125 *Ha*	16.75	17.00	10.50	6.50	4.88	56.38	2.50
L.S.D; P = .05	5.17	4.33	1.63	4.37	1.38	7.48	1.70
F values							
Rs	1.981	3.347	14.590**	5.112	27.941**	37.155**	82.142**
Ha	0.668	0.599	1.751	0.857	1.087	22.918**	11.145**
Rs, *Ha*	1.893	1.981	1.573	1.293	3.325**	1.205	1.216

Measurements of both *Rs* and *Ha* on the roots at any sampling time reflect the damage found on the remaining roots at that time. Damage to the already destroyed roots or parts of roots cannot be accounted for, resulting in inconsistent anomalies in measurements. Both early and frequent assessments in this study illustrated the effect of *Rs* on penetration and establishment of *Ha*. When nematodes were present the percentage of *Rs* on the roots increased and more whole roots were destroyed by *Rs*.

With the initial densities of larvae, penetration and establishment in the roots continued up to 20 days. At 30 days no increase was found. These results are in agreement with that of Davies and Fisher (1976). In the presence of *Rs*, number of *Ha* larvae in the roots were reduced by 10 days after inoculation and little further reduction in numbers occurred up to 30 days. This suggests that larvae either did not penetrate or failed to establish and left the roots. Both *Rs* and *Ha* can penetrate at the same site but *Rs* quickly destroys the root tips. If such destruction occurred rapidly enough then *Ha* larvae would leave the roots before establishing. Thus time of inoculation of each organism may be important in determining the type of relation between them.

The major effect on early growth of combined simultaneous nematode and fungus infection at all three concentrations were either additive or synergistic. Only additive effects were recorded at 10 days; some synergistic effects appeared at 20 days but these dominated at 30 days. This suggests that whatever is responsible for synergism, develops after the stage of penetration (although fewer larvae penetrate) and the development of syncytia in response to the nematode may be involved. Polychronopoulos (1969) reported that *H. schactii* provided avenues for entry of *Rs* into roots of sugar beets. (~~They also found that~~) The syncytia

as well as adjacent areas provided suitable substrates for growth of *Rs*. This process may also occur in wheat but it was not clear from observations how *Rs* damage increased in the presence of *H. avenae*. The increase in fresh weight at 10 days at all densities of *Ha* and at the two lower densities at 30 days of growth, agrees with the findings of Madamba *et al*. (1965). They reported an increase in root weight at low densities of nematodes due to the reduction of lateral roots.

Meagher and Chambers (1970) in their study of these two pathogens at only one high initial level of both pathogens reported a greater reduction in growth due to the combination of both than to either alone, on 8 week old plants. They also reported that growth differences that were apparent soon after emergence, were not so apparent after 4 weeks of growth. In this study synergistic effects occurred initially with the low and intermediate concentrations of *Rs* and became more general with time. The effect of the high concentration of *Rs* on growth alone was drastic so that the little effect of *Ha* densities was masked. It would be unwise to expect a consistent synergisitic effect under a different set of conditions and with other densities. If *Ha* was the first pathogen in the roots followed by *Rs* later or *vice versa*, a different effect might be found. The effect of *Rs* on growth was evident at maturity. The significant early effect on growth due to *Ha* largely disappeared at maturity. This could have been due to recovery of plants during later stages of growth. Damage may have increased or been sustained with higher populations of nematodes. Meagher *et al*. (1978) reported recovery of plants at 23 weeks, with a high density of nema. Combination of *Rs* and *Ha* reduced the number of cysts at maturity in this study. Meagher *et al*. (1978) reported a reduced egg count due to the effect of *Rs*. The reduction in their study could have been due to the excessive high initial density used leading to competition which

would also result in smaller egg output, or due to the effect of *Rs*.

Other variables, such as moisture availability, virulence of fungus, density and sequence of infection may also affect the relationship between the pathogens. The rapidity of *Rs* damage at even lower densities needs further study with inoculations at different times.

Until we fully understand the complex of relationships possible between the pathogens in terms of all relevant variables and the effect of such relationships on the host, it would be unwise to draw conclusions or offer suggestions as to what might occur. The effect and relationship in one year will differ from that in the next year.

3.4 Interrelationships between *G. graminis* var. *tritici* and *R. solani*

The strong negative correlation between *Ggt* and *Rs* in the second year of the field distribution study and the observation that the damage due to each fungus, often occurred on separate roots, indicated that perhaps the relationship between *Ggt* and *Rs* was an antagonistic one.

To test this observation experiments were conducted in liquid media under sterile conditions and in soil.

A. Study of relationships in vitro

Method

Surface sterilized seeds were germinated at 25°C and 4 day old seedlings were used in all experiments. Each seedling was placed on a sterile glass slide (215 mm in length and 42 mm in width) and held in place with a sterile rubber band. The three roots were carefully laid out on the slide. An 8 mm disc of *Ggt* or *Rs*, both grown on

Czapex-dox + yeast agar, was placed equidistant from the middle seminal root or on it or both sides of it. The glass slide with the seedling plus inoculum disc or discs was placed in a sterile glass jar (diameter of 85 mm and 245 mm in length) at an angle, upright, so that the root tips touched the Hoaglands nutrient solution ($^1/10$ strength) which covered the base of the jar to a depth of 1 cm. Figure 20 shows the system used in these experiments, similar to that used by Flentje *et al.* 1963, 1967), in the study of *Rs* on radish seedlings. The jars were placed in incubators at 10°, 15° or 20°C in the dark and assayed after 2 weeks. Figure 21 shows the positions of inoculum of discs for the three experiments. There were four treatments each with 3 replicates in the first experiment which was designed to determine if lesion formation would occur on wheat roots with the *in vitro* experimental method. The second experiment had three treatments replicated four times, to determine the effect of the presence of each fungus on the other, in lesion formation when inocula were placed horizontally equidistant from the root (0.5 cm from root), and vertically adjacent to each other (Fig.21).

The third experiment with three treatments replicated four times, was to examine the effect on lesion formation when the inocula were placed (i) 1 cm apart in a horizontal plane or (ii) 2 cms apart vertically (Fig.20). In all experiments the 8 mm discs were placed 2 cms below the junction of root and seed, at a distance of 0.5 cm or 1 cm away from the middle seminal root. The discs were placed with the plain agar bottom on the glass slide. Discs were taken from the edge of a week-old culture. Plants of similar size with similar lengths of roots were used.

Figure 20: System used to study the interrelationship between *Ggt* and *Rs 'in vitro'*.

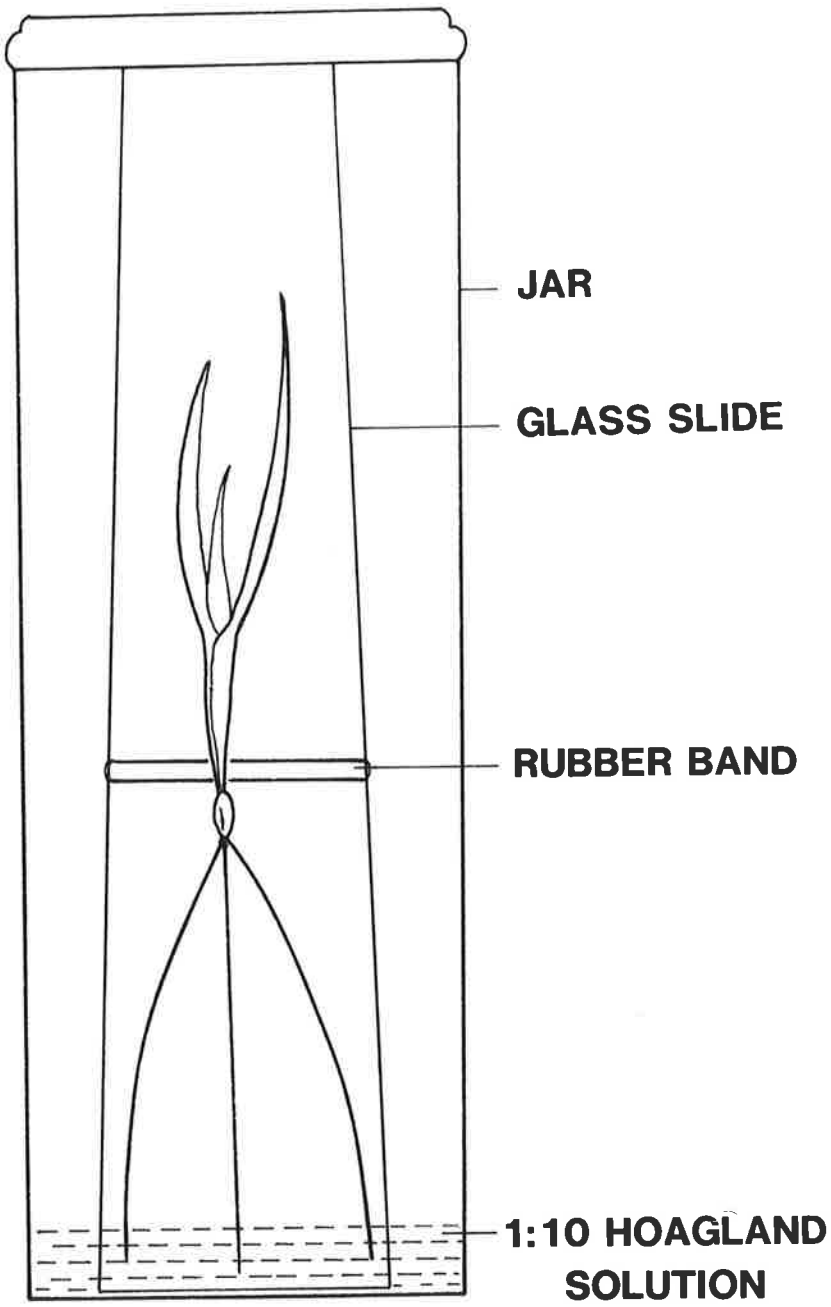
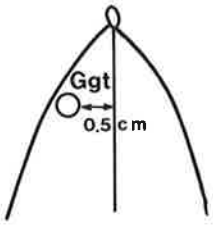
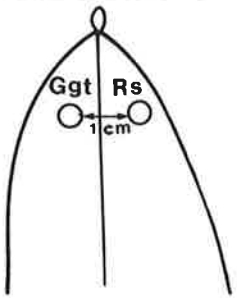
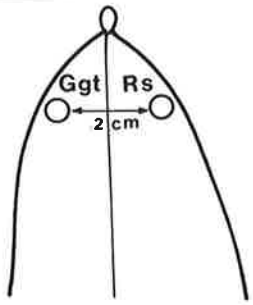
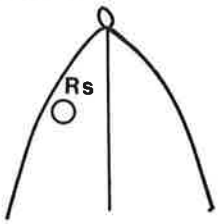
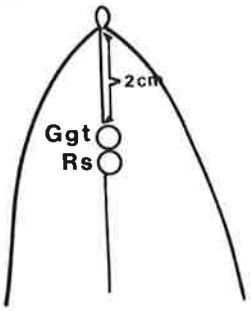
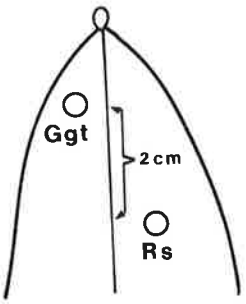
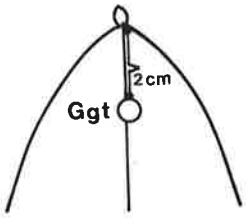
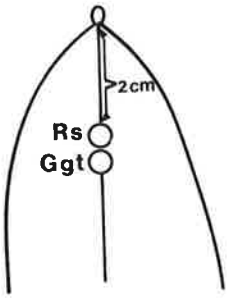
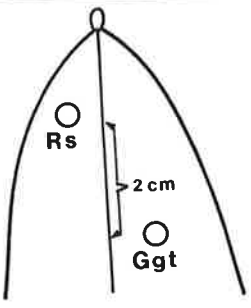
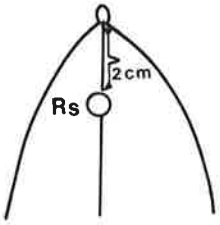

JAR
GLASS SLIDE
RUBBER BAND
1:10 HOAGLAND SOLUTION

Figure 21: *Ggt* and *Rs* inoculum agar disc positions used in the *'in vitro'* experiments.

To test the ability of Ggt and Rs to form lesions (Expt. 1)	To test the ability of Ggt and Rs together to form lesions (Expt. 2)	To test the ability of Ggt and Rs to form lesions when separated (Expt. 3)
		
		
		
		

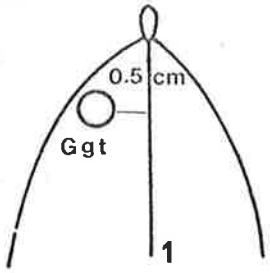
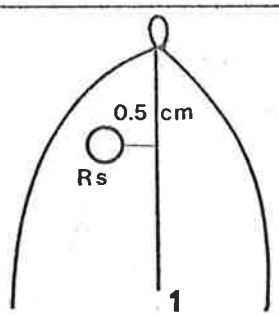
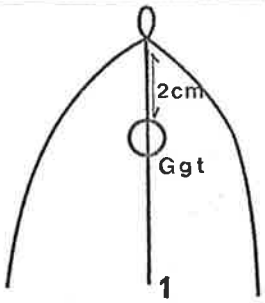
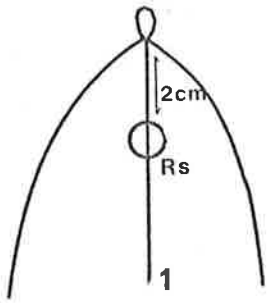
Results

In the first experiment, lesions were visible (Table 33) on the middle seminal root (numbered as 1), the only root considered in this experiment. With *Ggt* the distinct blackening or darkening of the stele was seen and with *Rs*, a little concaving and softening of the cortex occurred. Hyphae grew from the disc of inoculum on to the glass slide and then on to the root. Growth of *Ggt* was slower than *Rs* which affected a large area of the root. Temperature did not influence lesion formation. The results showed that it was possible to test the relationship between the fungi by this simple method.

In the second experiment only the middle (or first) seminal root was used in evaluating the response because of the difficulty in positioning the second and third seminal roots. No lesions were formed on this root at 14 or 21 days at any temperature when the inocula were placed 1 cm apart in the same horizontal plane. Neither of the fungi was able to establish itself in the first seminal root. However, lesions were formed by each fungus on the seminal root that was distal to the piece of inoculum of the other fungus. *Rs* grew prolifically on the glass slide, even down to the nutrient solution and there penetrated the tips of all roots (Table 34).

When *Ggt* disc was placed directly adjacent to the *Rs* disc but closer to the seed, *Ggt* formed lesions underneath the disc and closer to the seed, but *Rs* did not form lesions directly underneath the disc but only a short distance distal to the *Ggt* inoculum. On

TABLE 33: Ability of Ggt and Rs to form lesions on wheat roots at varying temperatures

Temperature	10°C			15°C			20°c		
Replicate	1	2	3	1	2	3	1	2	3
	+	+	-	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+

+ = lesion formed; - = no lesion formed

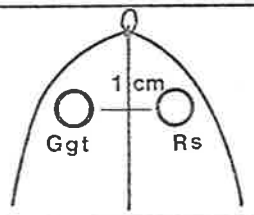
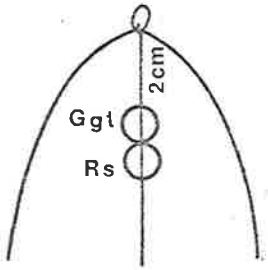
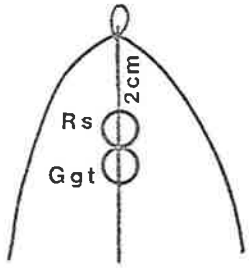
Root labelled as 1 was the only root assayed.

the area immediately under the *Rs* inoculum only hyphae were seen. The immediate area of the root adjacent to *Rs* was also intact, with no softening of the cortex. Only at 20°C did the *Ggt* lesion extend proximally to the junction between root and seed and onto the roots on either side. At 20°C *Rs* formed lesions beneath the inoculum disc. *Rs* grew to the nutrient medium and attacked all three root tips.

When the positions of the fungal inocula were reversed lesions were found between the *Rs* disc and the seed at all temperatures. *Ggt* lesions were found only at 10°C and 15°C distal to the inoculum. At 20°C no *Ggt* lesion was formed on the first seminal root. The area immediately underneath the two fungal inoculum discs was intact and hyphae were seen on the root surface. Only at 15°C did *Rs* attack roots 2 and 3 by extending *via* the seed from the middle root.

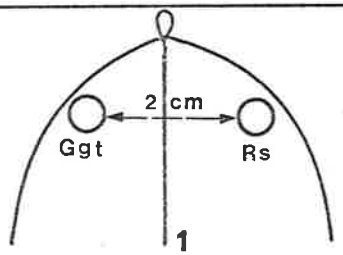
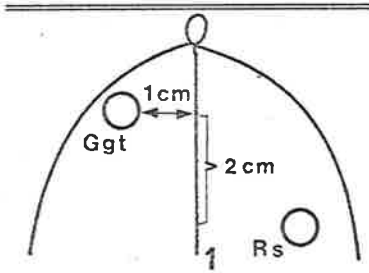
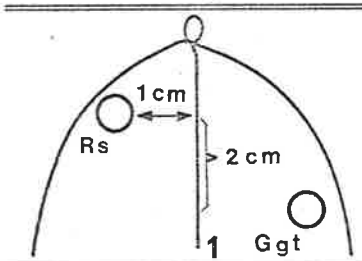
In the third experiment no lesions of either *Ggt* and *Rs* were found on the middle root. When the inocula were separated 2 cm horizontally at both 15°C and 20°C, slight browning of the root tissue was noticed along with hyphae of both fungi. When separated 2 cms vertically, hyphae were found girdling the root. Hyphae of *Ggt* and *Rs* intermingled and grew over each other (Table 35), but no lesions were formed.

TABLE 34: Ability of Ggt and Rs to form lesions in the presence of each other when horizontally separate or placed together

Temperature	10°C				15°C				20°C			
Replicate	1	2	3	4	1	2	3	4	1	2	3	4
	-X	-X	-X	B	-X	-X	-X	-X	-X	-X	-X	-X
	Formation of Ggt lesion*											
	+	+	+	B	+	+	+	+	+	+	+	+
	Formation of Rs lesion†											
	+	+	+	+	+	+	+	+	+	+	+	+
	Formation of Rs lesion*											
	+	+	+	X	+	+	+	+	+	+	+	+
	Formation of Ggt lesion†											
	+	+	+	-	+	+	+	+	-	-	-	-

- = No visible lesion. + = Lesion formed. B = Slight darkening or browning of root.
X = Hyphae seen on root. * = Root area between disc and seed. † = Root area between disc and root tip.

TABLE 35: Ability of Ggt and Rs to form lesions *in vitro* when separated vertically and horizontally

Treatment	Rep. No.	15°C				20°C			
		Ggt lesion	Rs lesion	Browning of root	Hyphae only	Ggt lesion	Rs lesion	Browning of root	Hyphae only
	1	-	-		X	-	-	B	X
	2	-	-	B	X	-	-	B	X
	3	-	-	B	X	-	-		X
	4	+	-		X	+	-		X
	1	-	-	B	X	-	-		X
	2	-	-		X	-	-		X
	3	-	-		X	-	-		X
	4	-	-		X	-	-		X
	1	-	-		X	-	-		X
	2	-	-		X	-	-		X
	3	-	-		X	-	-		X
	4	-	-		X	-	-		X

- = No lesion; + = Lesion formed; B = Browning; X = Hyphae on the root.

B. *Study of relationship in soil*

Method

Interrelationships between *Ggt* and *Rs* were studied in soil in a factorial experiment, at 4 rates of *Ggt* (0%, 1.5%, 2.0% and 2.5%), 4 rates of *Rs* (0%, 0.05%, 0.1% and 0.5%), assayed at 35 days and at maturity and the whole was replicated 8 times. Up to 35 days, all treatments received the same amount of water (5 ml/100 gms of soil every 3 days). After 35 days half the remaining treatments continued to receive the same amount of water (5 ml/100 gms of soil every 3 days) whereas remaining treatments received double the amount of water (10 ml/100 gms of soil every 3 days).

Leaf length measurements were taken weekly; all other measurements were taken at 35 days and at maturity. Soil was inoculated with pathogen or pathogens four days before sowing pregerminated seeds.

B.1. *Results at 35 days*

Growth

There was no significant interaction between *Ggt* and *Rs* on growth (Table 36). There was a significant increase in the dry weight of the plants at the highest concentration of *Ggt* over the two lower concentrations of *Ggt*. The reduction in dry weight with the highest concentration of *Rs* was not significant. Plants infected with *Rs* had more roots, and the highest concentration of *Rs* reduced root length significantly

Leaf length

No significant interaction occurred (Table 37) on the leaves. *Ggt* at 1.5% concentration reduced the length of leaves 2 and 3, but the

TABLE 36: Effect of *Ggt* and *Rs* on growth on 35 day old plants (means)

Pathogen and density	Dry weight of plant (g)	Number of roots	Length of roots (cm)
0% *Ggt*	.159	8.00	90.6
1.5% *Ggt*	.136	7.00	73.5
2.0% *Ggt*	.149	7.75	75.8
2.5% *Ggt*	.201	7.63	81.6
0.05% *Rs*	.150	9.50	80.5
0.1 % *Rs*	.177	9.75	84.5
0.5 % *Rs*	.116	8.88	62.5
0.5% *Ggt* + 0.05% *Rs*	.137	8.38	54.6
" + 0.1 % *Rs*	.125	8.25	50.6
" + 0.5 % *Rs*	.151	8.88	56.5
2.0% *Ggt* + 0.05% *Rs*	.162	9.25	61.3
" + 0.1 % *Rs*	.158	8.38	55.0
" + 0.5 % *Rs*	.154	11.25	57.6
2.5% *Ggt* + 0.05% *Rs*	.172	10.00	62.6
" + 0.1 % *Rs*	.165	9.75	77.3
" + 0.5 % *Rs*	.174	9.75	60.4
L.S.D; P = .05	0.050	Non-parametric analysis	18.1

	F value	χ^2 statistic	F value
Ggt	3.620*	7.095	8.037**
Rs	0.271	24.099**	7.583**
Ggt, *Rs*	0.961	8.283	0.964

TABLE 37: Effect of *Ggt* and *Rs* on lengths of leaves on 35 day old plants (means)

Pathogen and density	Leaf 1 (log)	Leaf 2 (log)	Leaf 3	Leaf 4	Leaf 5
0% *Ggt*	1.18	2.07	12.69	19.06	20.29
1.5% *Ggt*	0.98	1.73	9.86	18.57	18.30
2.0% *Ggt*	0.94	1.69	10.96	17.45	17.81
2.5% *Ggt*	1.13	2.05	12.64	19.50	20.35
0.05% *Rs*	1.21	2.13	14.20	19.61	18.29
0.1 % *Rs*	1.25	2.18	15.98	20.41	16.86
0.5 % *Rs*	1.17	2.03	12.96	18.07	15.95
0.5% *Ggt* + 0.05% *Rs*	0.64	1.59	11.04	19.99	17.70
" + 0.1 % *Rs*	1.20	2.08	13.79	18.37	17.16
" + 0.5 % *Rs*	1.26	1.98	13.21	19.80	20.33
2.0% *Ggt* + 0.05% *Rs*	1.25	2.08	13.69	18.72	18.79
" + 0.1 % *Rs*	1.15	2.01	13.50	17.96	18.58
" + 0.5 % *Rs*	1.19	2.03	13.34	19.57	20.06
2.5% *Ggt* + 0.05% *Rs*	1.20	1.96	13.64	19.96	20.21
" + 0.1 % *Rs*	1.10	2.01	14.49	20.59	21.11
" + 0.5 % *Rs*	1.12	2.08	14.23	19.96	21.24
L.S.D; P = .05	0.344	0.332	2.67	2.71	3.44
F values					
Ggt	1.440	3.394*	3.560*	1.771	4.144**
Rs	1.178	2.013	6.268**	0.686	0.497
Ggt, *Rs*	1.818	1.429	0.780	0.732	1.285

2.0% concentration only reduced the length of leaf 2. *Rs* had little effect on the lengths of leaves.

Effect of *Ggt* and *Rs* on each other

No significant interaction was obtained for the effect of the pathogens on each other at 35 days (Table 38). More whole roots were destroyed by *Rs* in the presence of *Ggt*. Length of *Ggt* lesions increased with increasing concentrations of *Ggt* and length of *Rs* lesions with increasing concentrations of *Rs*.

B.2. *Results - At maturity*

Dry shoot weight

This was a large and complex experiment with 180 degrees of freedom which reduced the error term in the analysis to small proportions. The result was that many factors appeared significantly different even when the differences were small. Some of the differences were trivial. To make assessment consistent, differences were considered important when trends were consistent over a range of concentrations of the variables.

A significant second order interaction was obtained (Fig.22, Table 39) between water, *Ggt* and *Rs* on dry shoot weight and this is difficult to interpret. Significant first order interactions were obtained between water and *Ggt*, between *Ggt* and *Rs* but not between water and *Rs*. Generally additional water increased shoot weight and so did *Ggt* (Fig.22) but *Rs* decreased shoot weight. The overall effect was probably that additional water increased the stimulation from *Ggt* and this overcame some of the reduction due to *Rs*. Additional water had no direct effect on *Rs*.

TABLE 38: Effect of *Ggt* and *Rs* on each other on 35 day old plants (means)

Pathogen and density	Length of *Ggt* lesions (cm)	Length of *Rs* lesions (cm)	Number of root tips damaged by *Rs*	Number of whole roots destroyed by *Rs*
0% *Ggt*	-	-	-	-
1.5% *Ggt*	3.88	-	-	-
2.0% *Ggt*	6.63	-	-	-
2.5% *Ggt*	7.78	-	-	-
0.05% *Rs*	-	9.50	2.75	1.12
0.1 % *Rs*	-	12.00	1.75	0.37
0.5 % *Rs*	-	15.75	1.25	0.62
1.5% *Ggt* + 0.05% *Rs*	3.62	6.63	3.13	2.25
" + 0.1 % *Rs*	3.63	8.13	2.25	2.62
" + 0.5 % *Rs*	4.50	14.00	3.38	3.13
2.0% *Ggt* + 0.05% *Rs*	5.75	12.06	2.63	2.00
" + 0.1 % *Rs*	5.50	9.63	2.13	1.12
" + 0.5 % *Rs*	6.63	14.75	5.00	4.00
2.5% *Ggt* + 0.05% *Rs*	6.38	11.13	3.38	3.25
" + 0.1 % *Rs*	6.67	11.00	2.50	1.00
" + 0.5 % *Rs*	6.75	12.75	3.25	2.62
L.S.D; P = .05	Non-parametric analysis	4.17	Non-parametric analysis	Non-parametric analysis

	χ^2 statistic	F value	χ^2 statistic	χ^2 statistic
Ggt	31.137**	1.245	4.333	7.961*
Rs	2.838	9.799**	3.250	5.704
Ggt, *Rs*	2.921	0.907	6.416	2.128

TABLE 39: F values obtained by ANOVA for the effect of Water, *Ggt* and *Rs* on various growth characters at maturity

Water and Pathogens	Dry shoot weight	Dry root weight	Height	Number of roots	Length of roots	Yield
Water	106.030**	14.055**	128.882**	3.259**	1.168	49.750**
Ggt	81.035**	67.868**	26.156**	10.652**	1.796	3.076**
Rs	8.750**	12.396**	4.237**	4.477**	55.364**	1.468
Water, *Ggt*	18.600**	5.493**	6.320**	1.128	0.663	6.074**
Water, *Rs*	0.652	0.933	0.479	0.128	1.744	3.063**
Ggt, *Rs*	13.292*	2.216*	6.828**	1.605	2.670**	14.244**
Water, *Ggt*, *Rs*	3.379**	3.281**	1.695	1.595	0.915	1.049

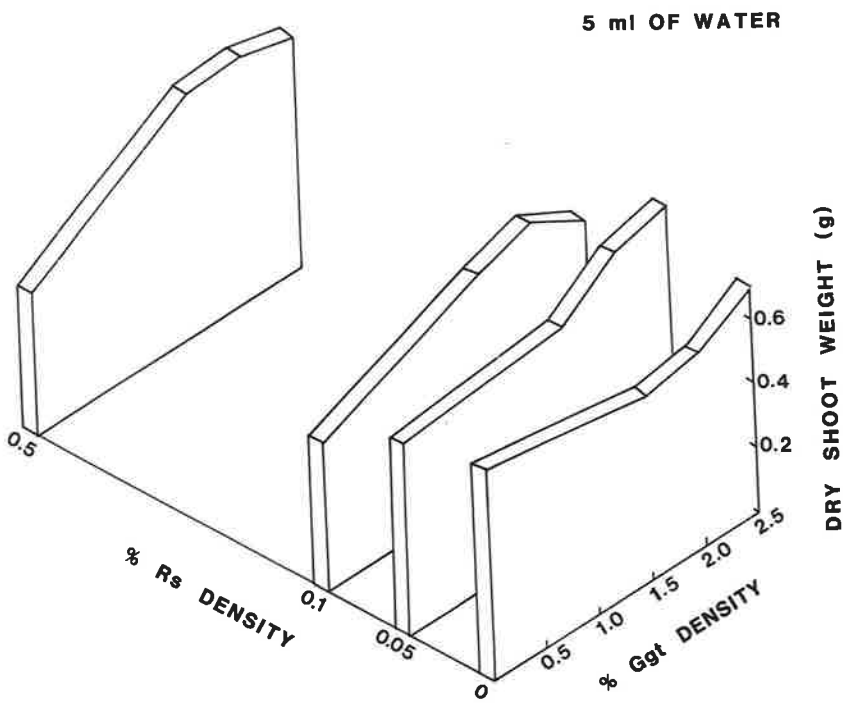
Figure 22: Effect of Water, *Ggt* and *Rs* on dry shoot weight at maturity.

A: Dry shoot weight at half rate of water (5 ml/3 days)

B: Dry shoot weight at full rate of water (10 ml/3 days)

22

A


5 ml OF WATER
DRY SHOOT WEIGHT (g)
0.6
0.4
0.2
% Rs DENSITY
0.5
0.1
0.05
0
% Ggt DENSITY
0.5
1.0
1.5
2.0
2.5

B

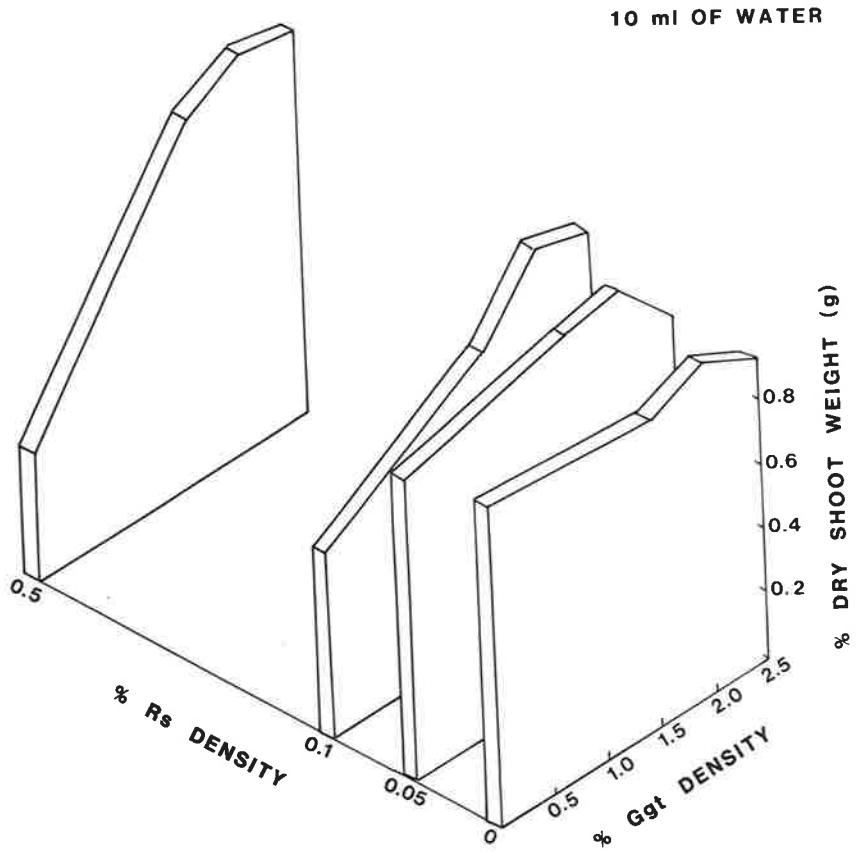

10 ml OF WATER
% DRY SHOOT WEIGHT (g)
0.8
0.6
0.4
0.2
% Rs DENSITY
0.5
0.1
0.05
0
% Ggt DENSITY
0.5
1.0
1.5
2.0
2.5

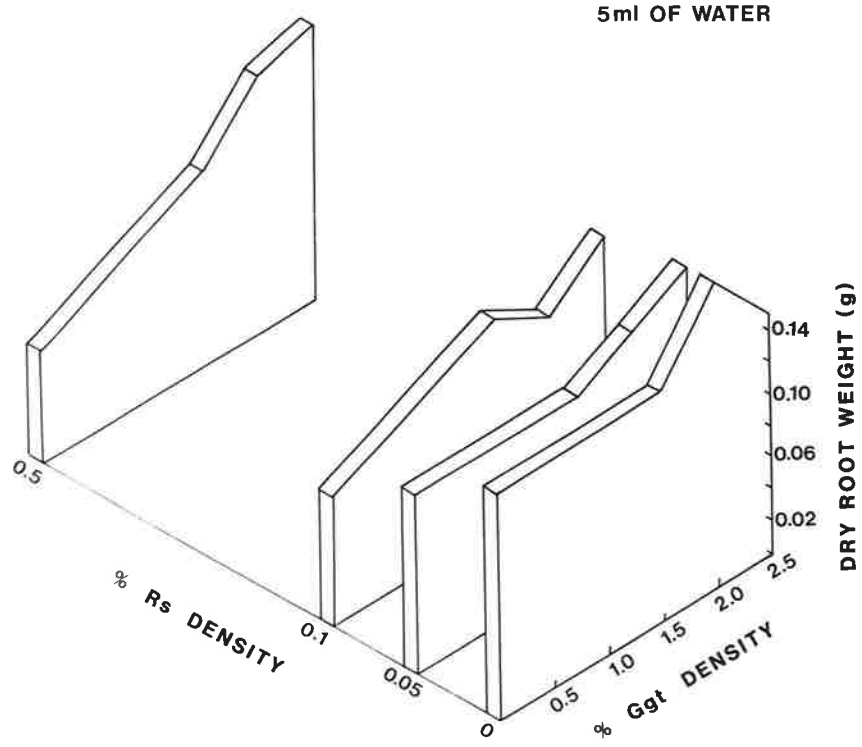
Figure 23: Effect of Water, *Ggt* and *Rs* on dry root weight at maturity.

A: Dry root weight at half rate of water (5 ml/3 days)

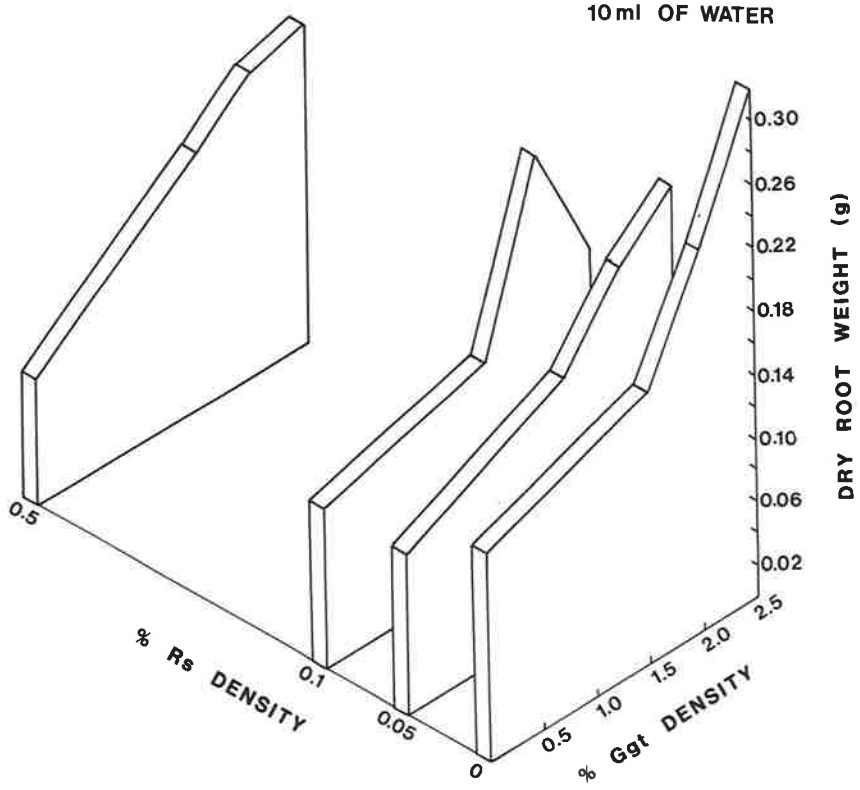
B: Dry root weight at full rate of water (10 ml/3 days)

23

A


5ml OF WATER
DRY ROOT WEIGHT (g)
0.14
0.10
0.06
0.02
% Rs DENSITY
0.5
0.1
0.05
0
% Ggt DENSITY
0.5
1.0
1.5
2.0
2.5

B


10ml OF WATER
DRY ROOT WEIGHT (g)
0.30
0.26
0.22
0.18
0.14
0.10
0.06
0.02
% Rs DENSITY
0.5
0.1
0.05
0
% Ggt DENSITY
0.5
1.0
1.5
2.0
2.5

Dry root weight

Essentially similar results were obtained for the effects of water, *Ggt* and *Rs* on dry root weight, though the effects were not as clear (Fig.23, Table 39).

Height

A significant interaction between water and *Ggt* occurred with respect to height. At the lower water rate, *Ggt* had little effect on height whereas at the higher level, all concentrations of *Ggt* increased height (Fig.24, Table 39). The significant interaction between *Ggt* and *Rs* occurred, because although *Rs* alone tended to reduce height, in the presence of *Ggt* at all concentrations, height either remained the same or was increased (Fig.25, Table 39).

Number and length of roots

There was no significant interaction of *Ggt* and *Rs* on the number of roots (Table 39). Water and all levels of *Ggt* increased the number of roots but only the highest level of *Rs* increased the number of roots (Table 40). However, there was a significant interaction of *Ggt* and *Rs* on the length of roots (Table 39). *Ggt* at all levels reduced the effect of the highest level of *Rs* (Fig.26).

Yield

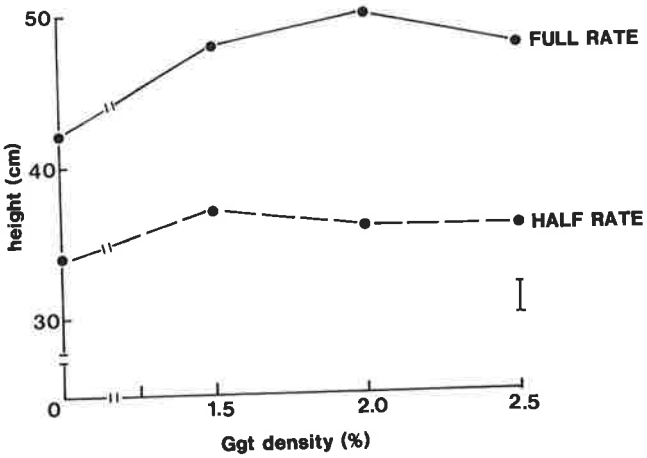
All three first order interactions (water and *Ggt*, water and *Rs*, *Ggt* and *Rs*) were significant (Table 39). In the absence of *Ggt*, water level did not affect yield but at the highest level, *Ggt* increased yield (Fig.27). Similar results were obtained for *Rs* and water (Fig.28).

Figure 24: Effect of Water and *Ggt* on height of plants at maturity.

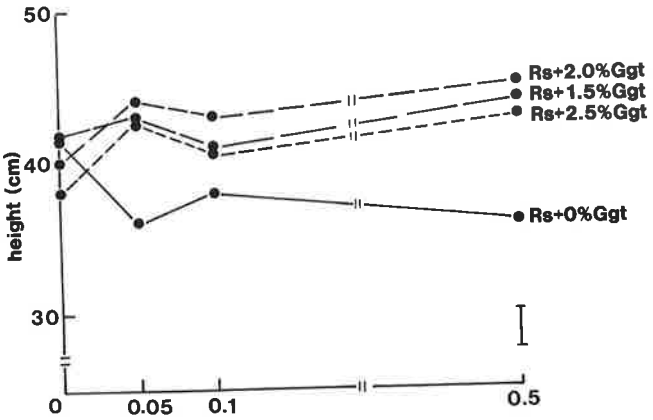
Figure 25: Effect of *Ggt* and *Rs* combination on height of plants at maturity.

Figure 26: Effect of *Ggt* and *Rs* combination on length of roots at maturity.

24


50
40
30
height (cm)
FULL RATE
HALF RATE
0
1.5
2.0
2.5
Ggt density (%)

25


50
40
30
height (cm)
Rs+2.0%Ggt
Rs+1.5%Ggt
Rs+2.5%Ggt
Rs+0%Ggt
0
0.05
0.1
0.5

26

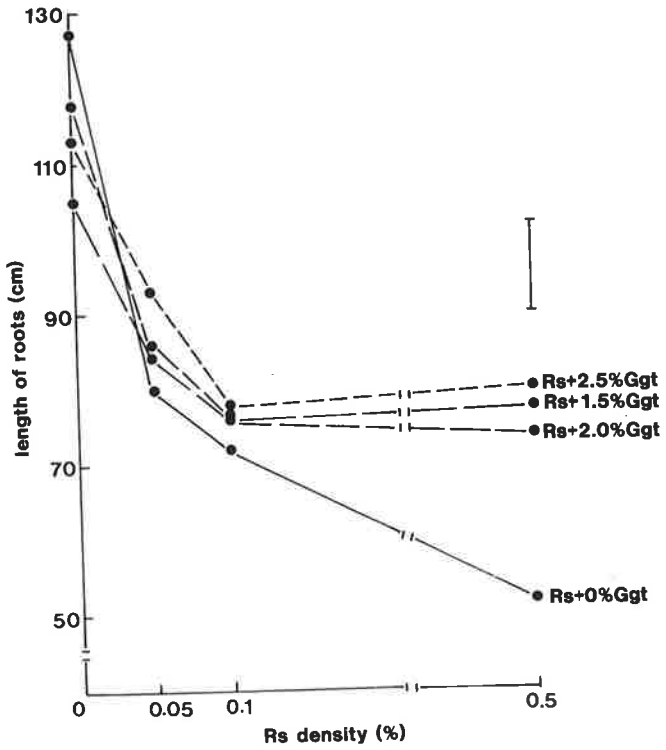

130
110
90
70
50
length of roots (cm)
Rs+2.5%Ggt
Rs+1.5%Ggt
Rs+2.0%Ggt
Rs+0%Ggt
0
0.05
0.1
0.5
Rs density (%)

TABLE 40: Effect of water, *Ggt* and *Rs* on number and length of roots, height and yield at maturity

Number of roots:	Effect of individual variables				
Water	5 ml 12.53		10 ml 12.93		LSD; P = .05 NS*
Ggt	0% 11.61	1.5% 12.82	2% 13.11	2.5% 13.38	.64
Rs	0% 12.38	0.05% 12.77	0.1% 12.34	0.5% 13.43	.64

Length of roots:	Effect of individual variables				
Water	5 ml 86.1		10 ml 88.8		LSD; P = .05 NS
Ggt	0% 83.2	1.5% 86.2	2.0% 88.9	2.5% 91.6	NS
Rs	0% 116.1	0.05% 86.4	0.1% 75.8	0.5% 71.5	7.48

Height of plants:	Effect of individual variables				
Water	5 ml 36.20		10 ml 47.33		LSD; P = .05 1.92
Ggt	0% 38.24	1.5% 43.16	2.0% 43.36	2.5% 42.30	1.29
Rs	0% 40.87	42.08	41.13	42.98	1.29

Yield:	Effect of individual variables				
Water	5 ml .1785		10 ml .2889		LSD; P = .05 .0306
Ggt	0% 0.2013	1.5% 0.2461	2.0% 0.2691	2.5% 0.2181	.0474
Rs	0% 0.2081	0.05% 0.2471	0.1% 0.2261	0.5% 0.2535	.0474

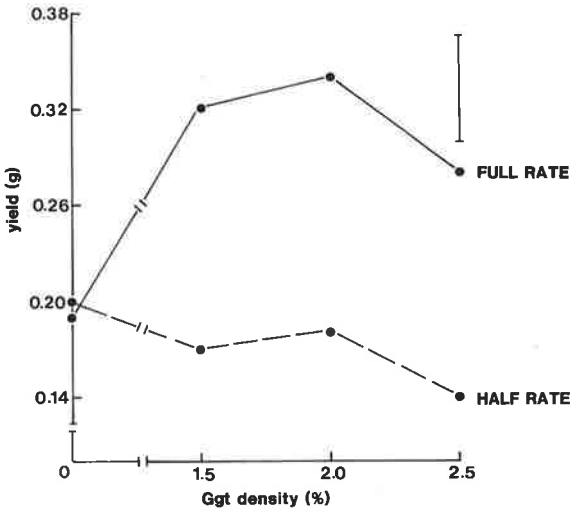
*NS = Not significant.

Figure 27: Effect of Water and *Ggt* on yield.

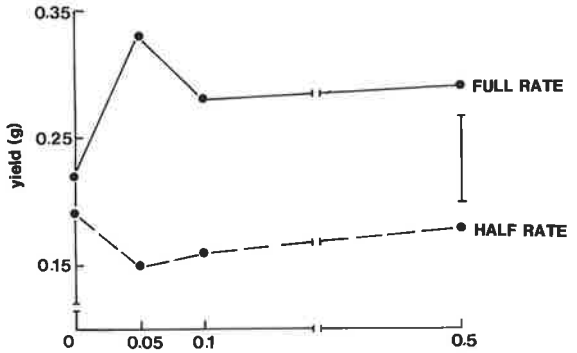
Figure 28: Effect of Water and *Rs* on yield.

Figure 29: Effect of *Ggt* and *Rs* combination on yield (log)

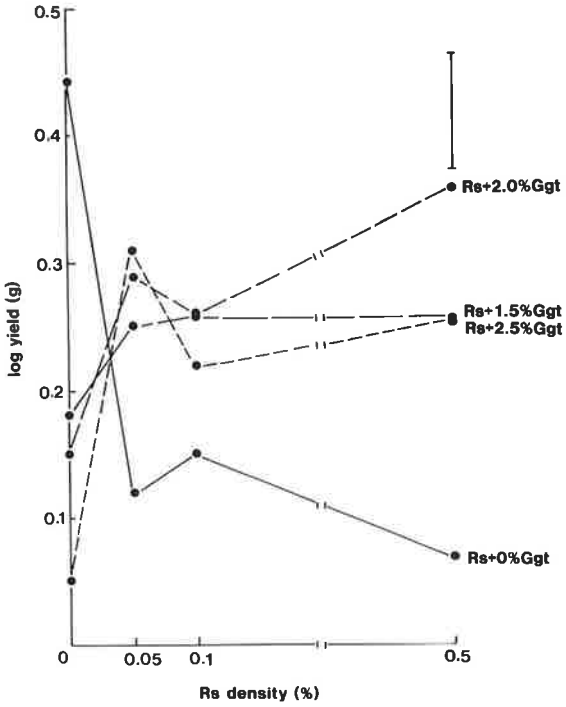
27


0.38
0.32
0.26
0.20
0.14
yield (g)
FULL RATE
HALF RATE
0
1.5
2.0
2.5
Ggt density (%)

28


0.35
0.25
0.15
yield (g)
FULL RATE
HALF RATE
0
0.05
0.1
0.5

29


0.5
0.4
0.3
0.2
0.1
log yield (g)
Rs+2.0%Ggt
Rs+1.5%Ggt
Rs+2.5%Ggt
Rs+0%Ggt
0
0.05
0.1
0.5
Rs density (%)

Although *Rs* alone reduced yield, in the presence of *Ggt* yield increased (Fig.29).

Length of leaves

The first five leaves were fully developed when the two levels of water were introduced so water level could have no influence. The significant interaction between *Ggt* and *Rs* on the length of leaf 5, occurred because the reduction in the length of leaf 5 caused by the two higher concentrations of *Rs* was removed by the presence of *Ggt* (Table 42).

Leaf 6 behaved in much the same way as leaf 5 due to the *Ggt*, *Rs* interaction (Fig.31), but with an additional significant interaction between water and *Ggt* which was not very important (Fig.30). Leaves 7 and 8, both showed the effects of the two water levels (Table 41) but the significant interaction between water and *Ggt* was replaced by one between water and *Rs* (Table 41); however this appeared trivial (Fig.32 and 34). The significant interactions between *Ggt* and *Rs* continued (Fig. 33 and 35), though in both these leaves *Rs* failed to reduce length (Table 41).

Effect of *Ggt* and *Rs* on each other

Few significant results were obtained (Table 43 and 44). Only one significant interaction between *Ggt* and *Rs* occurred in which *Ggt* suppressed the number of root tips destroyed by *Rs* at high initial concentration. Water had no effect on any of the parameters; *Ggt* reduced the length of *Rs* lesions and *Rs* reduced the length of *Ggt* lesions and incresed the number of root tips and whole roots destroyed (Table 43 and 44).

TABLE 41: F values obtained by ANOVA for the effect of Water, *Ggt* and *Rs* on lengths of leaves

Water and Pathogens	Leaf 6	Leaf 7	Leaf 8
Water	0.073	10.539**	25.884**
Ggt	21.934**	37.432**	45.374**
Rs	23.778**	1.834	2.289
Water, *Ggt*	4.707**	0.970	1.715
Water, *Rs*	2.301	3.014*	2.624*
Ggt, *Rs*	2.175*	4.368**	4.091**
Water, *Ggt*, *Rs*	0.503	0.691	0.536

TABLE 42: Effect of *Ggt* and *Rs* on leaf lengths of first 5 leaves

	Leaf 1 (log)	Leaf 2 (log	Leaf 3	Leaf 4	Leaf 5
0% *Ggt*	1.199	2.065	13.84	18.36	21.06
1.5% *Ggt*	1.250	2.089	13.46	17.26	20.43
2.0% *Ggt*	1.192	2.080	12.89	17.38	21.00
2.5% *Ggt*	1.136	2.006	12.91	18.09	21.50
0.05% *Rs*	1.066	2.009	13.46	18.59	20.51
0.1 % *Rs*	1.247	2.060	13.45	18.10	19.63
0.5 % *Rs*	1.085	1.962	11.72	16.35	16.64
1.5% *Ggt* + 0.05% *Rs*	1.120	2.071	13.76	18.77	21.05
" + 0.1 % *Rs*	1.202	2.092	13.89	18.11	20.40
" + 0.5 % *Rs*	1.238	2.093	14.19	18.46	20.43
2.0% *Ggt* + 0.05% *Rs*	1.127	2.112	14.15	18.91	20.93
" + 0.1 % *Rs*	1.166	2.033	13.10	17.93	20.32
" + 0.5 % *Rs*	1.230	2.081	14.33	19.15	21.45
2.5% *Ggt* + 0.05% *Rs*	1.134	2.113	14.66	19.79	22.04
" + 0.1 % *Rs*	1.190	2.113	13.97	17.71	20.06
" + 0.5 % *Rs*	1.212	2.107	13.83	19.07	20.63
L.S.D; P = .05	0.150	0.151	1.68	1.70	1.25
F values					
Ggt	0.666	1.166	1.229	1.243	10.352**
Rs	2.486	0.100	0.989	3.165*	8.566**
Ggt, *Rs*	0.845	0.666	1.499	1.634	5.268**

Figure 30: Effect of water and *Ggt* on length of leaf 6.

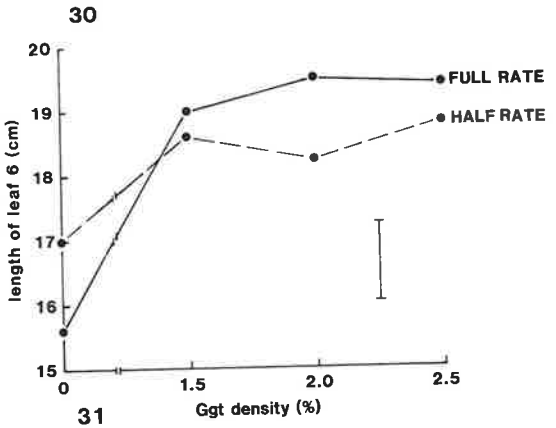
Figure 31: Effect of *Ggt* and *Rs* combination on length of leaf 6.

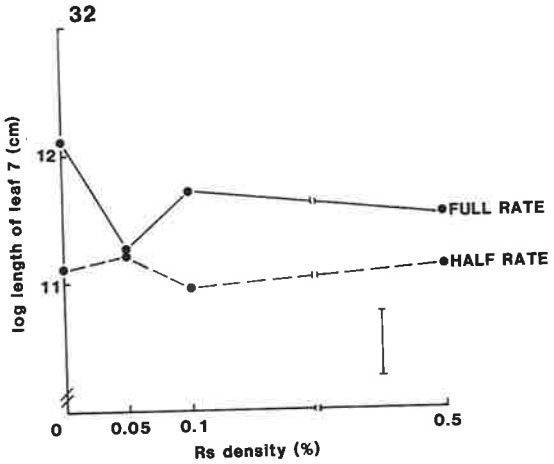
Figure 32: Effect of water and *Rs* on length of leaf 7.

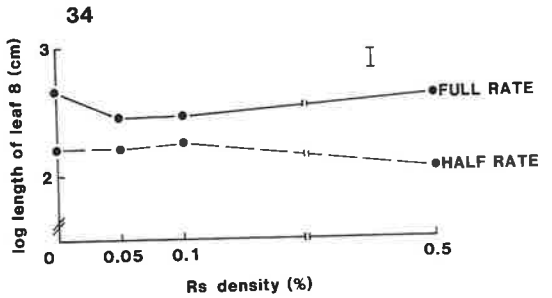
Figure 33: Effect of *Ggt* and *Rs* combination on length of leaf 7.

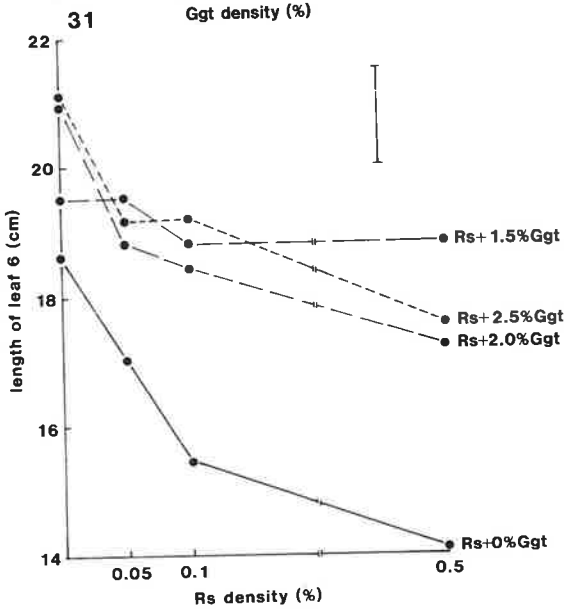
Figure 34: Effect of water and *Rs* on length of leaf 8 (log).

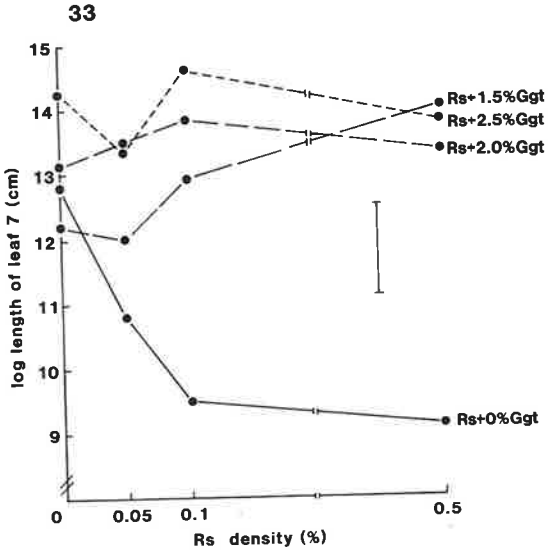
Figure 35: Effect of *Ggt* and *Rs* combination on length of leaf 8.


30
length of leaf 6 (cm)
20
19
18
17
16
15
FULL RATE
HALF RATE
0
1.5
2.0
2.5
Ggt density (%)


32
log length of leaf 7 (cm)
12
11
FULL RATE
HALF RATE
0
0.05
0.1
0.5
Rs density (%)


34
log length of leaf 8 (cm)
3
2
FULL RATE
HALF RATE
0
0.05
0.1
0.5
Rs density (%)


31
length of leaf 6 (cm)
22
20
18
16
14
Rs+1.5%Ggt
Rs+2.5%Ggt
Rs+2.0%Ggt
Rs+0%Ggt
0.05
0.1
0.5
Rs density (%)


33
log length of leaf 7 (cm)
15
14
13
12
11
10
9
Rs+1.5%Ggt
Rs+2.5%Ggt
Rs+2.0%Ggt
Rs+0%Ggt
0
0.05
0.1
0.5
Rs density (%)

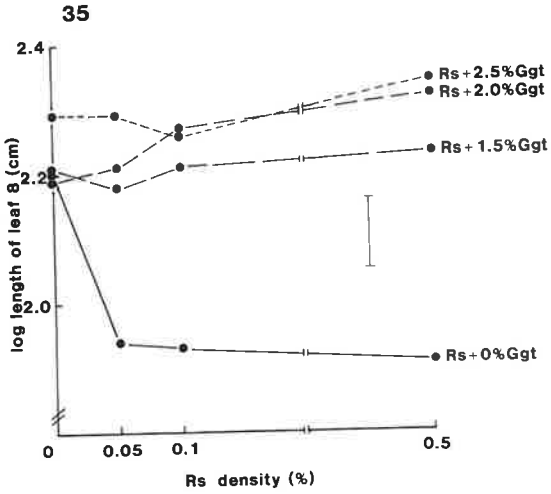

35
log length of leaf 8 (cm)
2.4
2.2
2.0
Rs+2.5%Ggt
Rs+2.0%Ggt
Rs+1.5%Ggt
Rs+0%Ggt
0
0.05
0.1
0.5
Rs density (%)

TABLE 43: F values obtained by ANOVA for the effect of Water, *Ggt* and *Rs* on each other

Water and Pathogens	Length of *Ggt* lesions	Length of *Rs* lesions	No. of whole roots destroyed by *Rs*	No. of root tips destroyed by *Rs*
Water	0.197	0.014	0.348	0.281
Ggt	0.789	7.014**	1.409	1.932
Rs	32.782**	2.179	34.075**	15.588**
Water, *Ggt*	1.560	2.120	1.149	0.565
Water, *Rs*	0.283	1.088	1.327	1.531
Ggt, *Rs*	0.862	1.852	1.008	2.561*
Water, *Ggt*, *Rs*	1.015	1.072	1.286	0.840

TABLE 44: Effect of Water, *Ggt* and *Rs* on length of *Ggt* and *Rs* lesions, number of whole roots and root-tips destroyed by *Rs*.

Water

Rate (ml/3 days)	5 ml	10 ml	LSD; P = .05
Length of *Ggt* lesions	2.70	2.66	0.19
Length of *Rs* lesions	14.99	15.12	2.05
No. of whole roots destroyed by *Rs*	2.47	2.66	0.63
No. of root-tips destroyed by *Rs*	6.82	7.04	0.79

Ggt

Density	%	1.5%	2.0%	2.5%	LSD: P = .05
Length of *Ggt* lesions	-	2.66	2.65	2.73	0.14
Length of *Rs* lesions	17.26	13.69	15.25	14.02	1.69
No. of whole roots destroyed by *Rs*	2.93	2.40	2.57	2.35	0.60
No. of root-tips destroyed by *Rs*	6.86	6.83	7.52	6.50	0.85

Rs

Density	%	1.5%	2.0%	2.5%	LSD: P = .05
Length of *Ggt* lesions	3.17	2.62	2.48	2.45	0.16
Length of *Rs* lesions	-	14.73	14.49	15.95	1.46
No. of whole roots destroyed by *Rs*	-	1.51	2.46	3.72	0.52
No. of root-tips destroyed by *Rs*	-	6.41	6.23	8.14	0.74

C. Discussion

The results obtained with these studies of the relationship between *Ggt* and *Rs*, confirmed the negative correlations that were obtained in the field study. The initial study of the relationship *in vitro* in jars under sterile conditions clearly illustrated the antagonism between *G. graminis* var. *tritici* and *R. solani* on wheat cultivar 'Condor'. The study in soil further substantiated the inverse relationship between these two important pathogens on wheat.

The *in vitro* study, also indicated that neither fungus had an effect on hyphal growth, for the hyphae of both *Ggt* and *Rs* grew, intermingled and girdled the root without causing lesions. Whether penetration of the root was prevented by each of the fungi, was not checked. Both *Ggt* and *Rs*, penetrated and formed lesions on the first seminal root in the absence of the other fungus. Perhaps *Ggt* prevents direct penetration, or affects cushion and appresoria formation, thus preventing infection peg penetration by *Rs*. *Rs* may, in turn, prevent infection peg formation and penetration from *Ggt* runner hyphae. Inhibition may occur externally or internally. It might be due to antibiotic production, lysis of hyphal tips of the fungi, competition for site or due to host tissue reaction. When the inoculum source was separated up to a distance of 2 cm vertically and horizontally, antagonism was effective in preventing lesion formation, but when inocula were placed adjacent to each other directly on the root, both fungi were able to form lesions distally and proximally to the inoculum. This suggests that establishment of the lesion is dependent on the position, size and density of inoculum propagule. Thus the distribution and size under field conditions would be important. The first fungus to reach a root would have the advantage. *Rs* was able to attack all root tips because of its greater growth rate under these experimental conditions. The mode of antagonism needs further investigation.

The pattern of extension by *Ggt* and *Rs* into other seminal roots from the main seminal root and seed junction, suggested that the area of antagonism is limited.

The extension with *Rs* occurred at 15°C and with *Ggt* at 20°C. *Rs* infection even in soil is favoured by low temperatures in the range of 12-18°C (Samuel and Garrett, 1932) and *Ggt* infection under natural conditions is favoured by soil temperatures of 12-20°C (Butler, 1961).

In soil at 35 days, the combination of *Ggt* and *Rs* had little effect on any growth or disease variable. At maturity the full effects of the antagonism between *Ggt* and *Rs* resulted in lower levels of root rot as measured by length of lesions caused by both fungi. The reduced damage to the roots was correlated with better growth of the plant. Combination of *Ggt* and *Rs* increased dry shoot weight, dry root weight, length of roots, yield and length of leaves. Damage from *Rs* alone reduced growth. The growth enhancement effect of the oatmeal base of *Ggt* increased growth but it had little effect on yield. Many infertile heads were found at the highest concentration of *Ggt*.

Water, had the greatest effect and dominated all variables. Plant growth was greater at the full rate of water than at the lower rate. Plants that received more water had more roots and had higher yields despite damage by the fungi. The relationship between the two fungi was not affected by water. Stynes (1975) in his synoptic study of wheat in South Australia found that the amount of water in the soil during the year had the greatest effect on growth and yield although pathogens did affect the efficient functioning of roots.

Ledingham (1942) first demonstrated antagonism between *Helminthosporium sativum* and *Fusarium culmorum* in affecting germination of wheat.

He also showed that in the presence of *F. culmorum* conidia, germination of *H. sativum* conidia was inhibited. Scardaci and Webster (1981) have also demonstrated antagonism between the cereal root pathogens, *F. graminearum* and *Bipolaris sorokiniana* when both were inoculated simultaneously in barley, resulting in lower levels of seedling blight and root rot.

Rhizoctonia sp. was one of the many soil organisms whose relationship with *Ophiobolus graminis* (= *G. graminis*) was studied by Lal (1939). He isolated *Rhizoctonia* sp. with *O. graminis* from roots of wheat and reported a strong antagonism between the two fungi on agar plates as well as restricted growth of *O. graminis* on soil in the presence of *Rhizoctonia* sp. Lal also reported that pathogenicity of *O. graminis* was affected by metabolites of *Rhizoctonia* grown in liquid medium. Height of plants grown in the presence of both fungi was greater than with *O. graminis* and less than with *Rhizoctonia* sp. The results of this study are in general agreement with the findings of Lal (1939) except that *Rs* was more damaging in this study than *Ggt*.

The effect of water in pots on disease development differed from that found in the field study where greater *Rs* damage was associated with dry moisture conditions and *Ggt* with higher moisture levels. Such differences suggest that the watering regime in the pot experiments inadequately simulated soil water conditions in the field.

3.5 Interrelationships between *G. graminis* var. *tritici*, *R. solani* and *H. avenae*

Ggt, *Rs* and *Ha* were found together on the roots of wheat in the field study. Since relationships between pairs of these pathogens has already been studied and the effects of the pathogens under experimental conditions is known, an experiment using all three pathogens was conducted. The aim was to find out whether high order interactions occurred at the densities chosen and, if these did not occur, which first order interactions were important when all three pathogens were together. Also to examine whether behaviour of pathogens or the effects of pathogens differed when all three pathogens were together and to rigorously test earlier results.

Method

Simultaneous inoculation of *Ggt* (0, 1.5, 2.0 and 2.5%), *Rs* (0, 0.5, 0.1 and 0.5%) and *Ha* (0, 25, 50 and 125 larvae/100 gm of soil) was used in a multifactorial experiment with an eight-fold replication to examine the effects on growth of the host and on the organisms.

A. Results at maturity

Growth

No second order interaction between all pathogens was significant for any of the growth variables measured (Table 45). First order interaction between *Ggt* and *Rs*, had a significant effect on all growth characters except on height of plants (Table 45). The *Rs*,*Ha* interaction had an effect on dry shoot weight, length of roots and on yield. The *Ggt*,*Ha* interaction was not significant for any character.

Means of the 64 treatments for all growth variables are given in the appendices (Appendix A). These results give an overall view of the effect

TABLE 45: F values and χ^2 statistic obtained by ANOVA for the effect of *Ggt*, *Rs* and *Ha* on growth variables at maturity

Pathogens	Dry shoot weight	Dry root weight	Number of † roots	Length of roots	Height	Number of grains	Yield (log)
Ggt	303.401**	27.622**	7.7736	6.793**	10.578**	116.956**	194.543**
Rs	79.252**	27.363**	26.7113**	225.257**	11.200**	8.559**	20.399**
Ha	2.264	2.563	4.0235	3.296**	1.699	3.131**	1.418
Ggt, *Rs*	21.058**	10.085**	33.3833**	21.234**	1.579	6.241**	13.463**
Ggt, *Ha*	0.795	1.895	5.5704	1.646	1.799	1.226	1.452
Rs, *Ha*	2.010*	0.850	14.8830	3.974**	0.924	1.264	2.147*
Ggt, *Rs*, *Ha*	0.916	1.057	28.1489	0.927	0.916	0.939	0.787

† - χ^2 statistic

of pathogens independently and in all combinations.

The reduced growth associated with *Rs* did not occur when both fungi at all concentrations were inoculated together (Figs. 36 to 40). The interaction effect was to increase growth. This was because *Ggt* suppressed the reduction in the growth parameters due to *Rs*. There was clear suppression of the reduction in all parameters except number of roots (Fig.38) where limited variation was possible and length of roots (Fig.39) where suppression increased with increasing density of *Ggt*.

The effects of *Ggt*,*Rs* interaction on number of grains and yield are seen in Fig.40 and 41. *Ggt* alone and in combination with *Rs* produced more grains and increased yield with all combinations.

Rs and *Ha* combination reduced dry weight of shoot more than *Rs* alone (Fig.42). Reduction in length of root was more apparent with the combination of both pathogens (Fig.43). The two higher concentrations of *Rs* with 25 and 50 *Ha* larval densities reduced yield significantly as did 0.5% *Rs* with 125 *Ha* (Fig.44).

Height measurements at maturity only reflected the effect of *Ggt* and *Rs*. Plants were taller in the *Ggt* treatments and shorter in the *Rs* treatments (Table 45).

Length of leaves and width of leaf 5

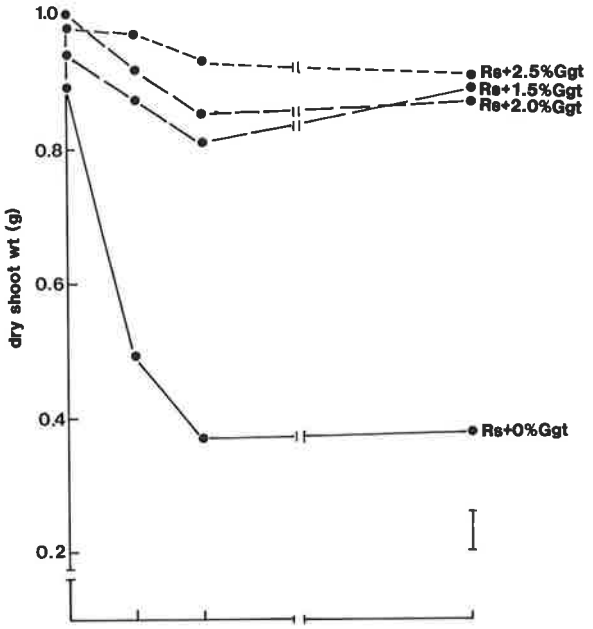
The effect of all three pathogens on lengths of leaves can be separated into 3 groups of similar reactions (Table 46). For the first three leaves, only the first order interaction between *Ggt* and *Rs* was significant. Neither of the other first order interactions (*Ggt* and *Ha* or *Rs* and *Ha*) nor the second order interaction between *Ggt*, *Rs* and *Ha* was significant. Leaf 4 was the only leaf for which the second order interaction between all three pathogens was significant at the 1% level; all the

Figure 36: Effect of *Ggt* and *Rs* combination on dry shoot weight at maturity.

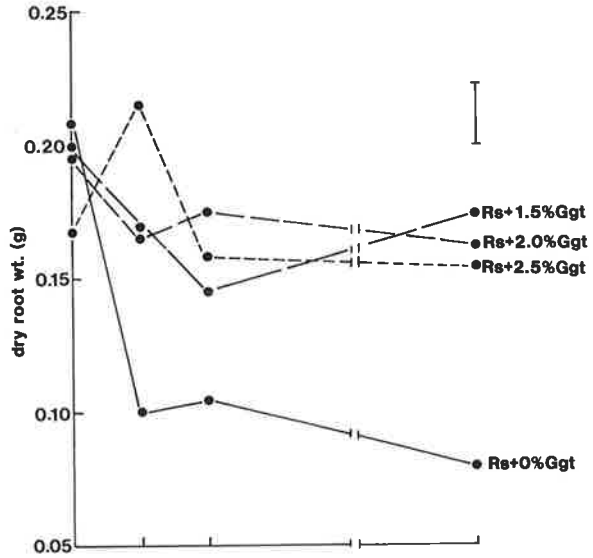
Figure 37: Effect of *Ggt* and *Rs* combination on dry root weight at maturity.

Figure 38: Effect of *Ggt* and *Rs* combination on number of roots at maturity.

36


1.0
0.8
0.6
0.4
0.2
dry shoot wt (g)
Rs+2.5%Ggt
Rs+1.5%Ggt
Rs+2.0%Ggt
Rs+0%Ggt

37


0.25
0.20
0.15
0.10
0.05
dry root wt. (g)
Rs+1.5%Ggt
Rs+2.0%Ggt
Rs+2.5%Ggt
Rs+0%Ggt

38

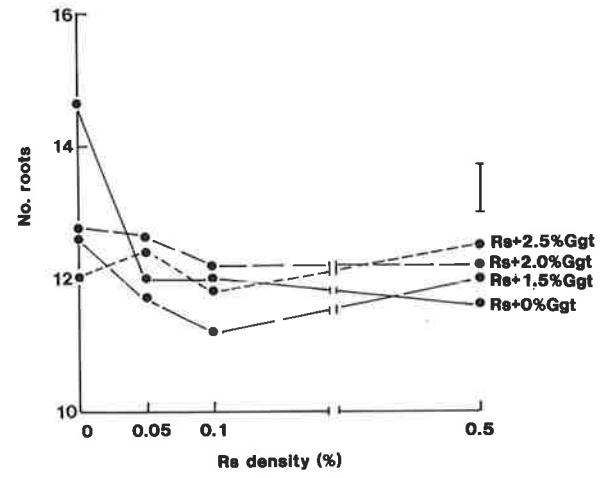
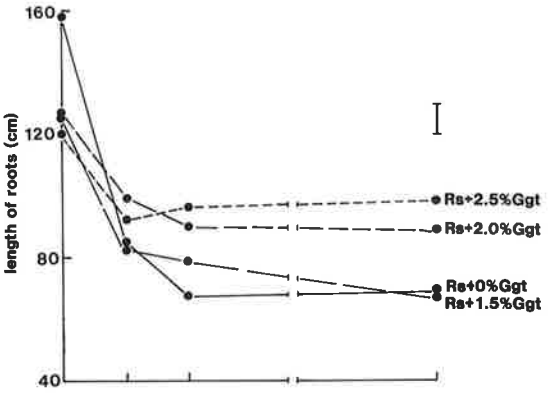

16
14
12
10
No. roots
0
0.05
0.1
0.5
Rs density (%)
Rs+2.5%Ggt
Rs+2.0%Ggt
Rs+1.5%Ggt
Rs+0%Ggt

Figure 39: Effect of *Ggt* and *Rs* combination on length of roots at maturity.

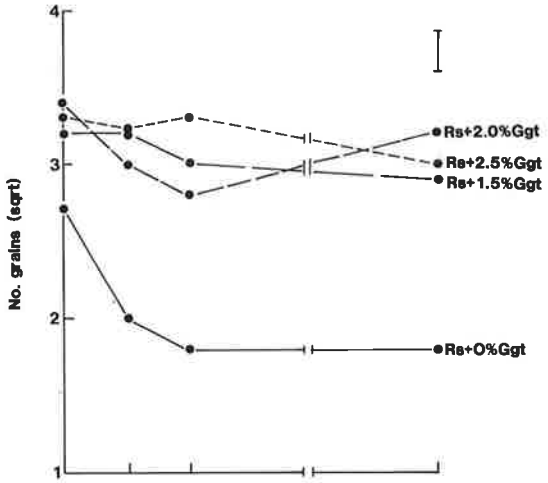
Figure 40: Effect of *Ggt* and *Rs* combination on number of grains (sq.rt.).

Figure 41: Effect of *Ggt* and *Rs* combination on yield (log).

39


160
120
80
40
length of roots (cm)
Rs+2.5%Ggt
Rs+2.0%Ggt
Rs+0%Ggt
Rs+1.5%Ggt

40


4
3
2
1
No. grains (sqrt)
Rs+2.0%Ggt
Rs+2.5%Ggt
Rs+1.5%Ggt
Rs+0%Ggt

41

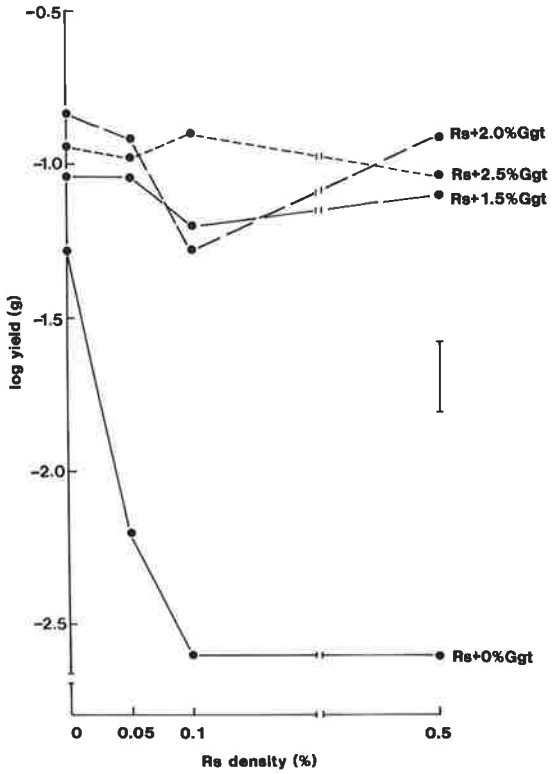

-0.5
-1.0
-1.5
-2.0
-2.5
log yield (g)
Rs+2.0%Ggt
Rs+2.5%Ggt
Rs+1.5%Ggt
Rs+0%Ggt
0
0.05
0.1
0.5
Rs density (%)

Figure 42: Effect of *Rs* and *Ha* combination on dry shoot weight at maturity.

Figure 43: Effect of *Rs* and *Ha* combination on length of roots at maturity.

Figure 44: Effect of *Rs* and *Ha* combination on yield.

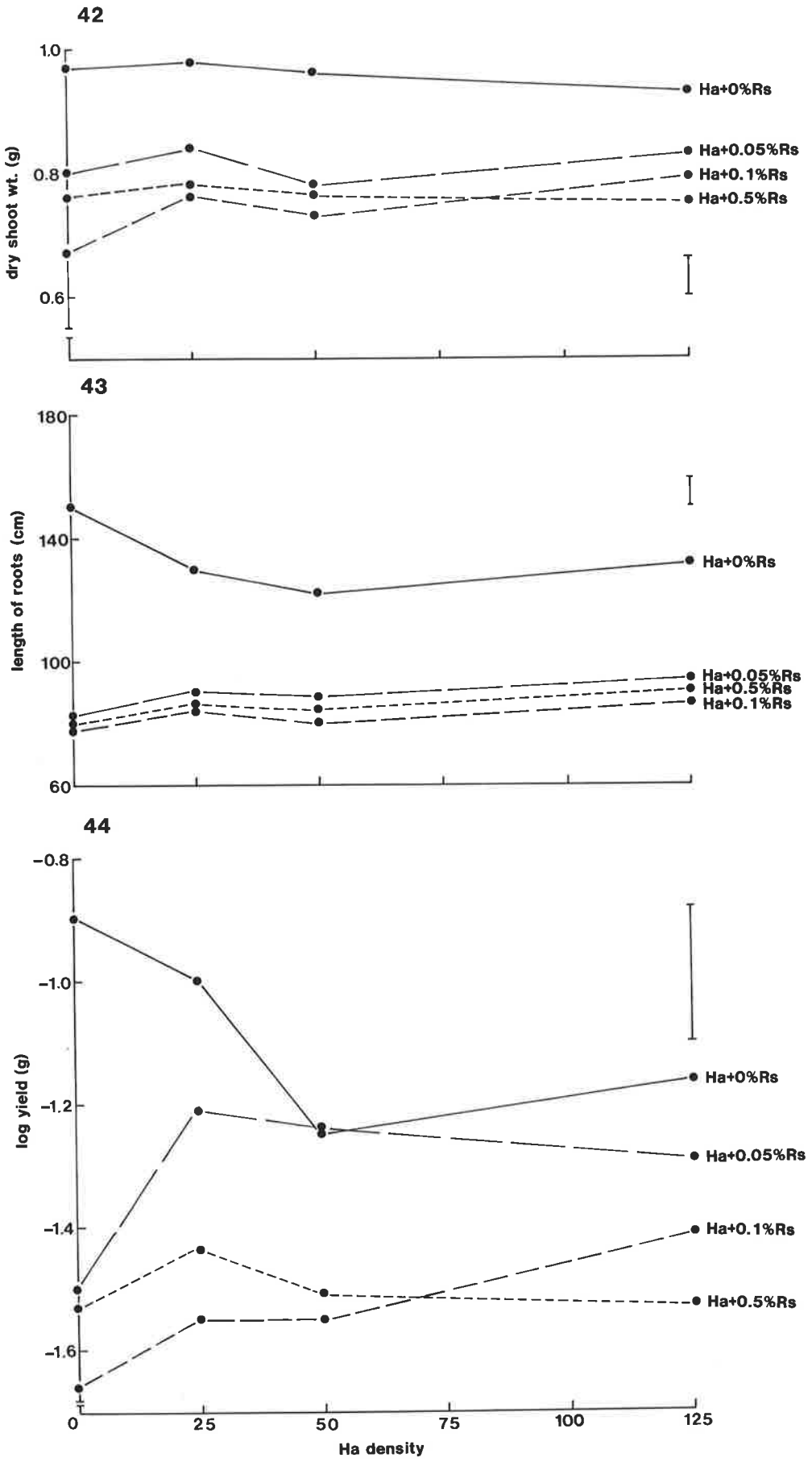

42
1.0
0.8
0.6
dry shoot wt. (g)
Ha+0%Rs
Ha+0.05%Rs
Ha+0.1%Rs
Ha+0.5%Rs
43
180
140
100
60
length of roots (cm)
Ha+0%Rs
Ha+0.05%Rs
Ha+0.5%Rs
Ha+0.1%Rs
44
-0.8
-1.0
-1.2
-1.4
-1.6
log yield (g)
Ha+0%Rs
Ha+0.05%Rs
Ha+0.1%Rs
Ha+0.5%Rs
0
25
50
75
100
125
Ha density

TABLE 46: F values obtained by ANOVA for the effect of *Ggt*, *Rs* and *Ha* on length of leaves and width of leaf 5

Pathogens	Leaf 1	2	3	4	5	6	7	Width of Leaf 5
Ggt	6.68 **	29.70**	60.65**	29.57**	12.15**	90.98 **	50.59**	11.87**
Rs	3.134*	28.87**	39.77**	53.45**	6.91**	12.149**	6.56**	88.90**
Ha	3.66 *	6.17**	14.89**	13.20**	1.07	16.90 **	1.00	4.18**
Ggt, *Rs*	6.94 **	25.67**	24.77**	8.39**	6.35**	12.00 **	7.37**	8.23**
Ggt, *Ha*	1.54	1.13	1.07	4.88**	1.97*	1.53	1.00	1.04
Rs, *Ha*	1.39	1.02	1.39	3.54**	1.03	2.02 **	1.20	1.26
Ggt, *Rs*, *Ha*	1.24	0.89	0.73	2.95**	1.58*	1.46	1.74*	1.13

first order interactions were significant at the 1% level. The effect on leaves 5,6 and 7 were more variable. Second order interactions were significant for leaves 6 and 7 at the 5% level. The *Ggt*,*Rs* interaction was significant for leaves 5,6 and 7; *Ggt*,*Ha* interaction for leaf 5 and the *Rs*,*Ha* interaction for leaf 6.

The effect of the *Ggt*,*Rs* interaction was the same as that found earlier in Section 3.4 on leaves 1,2 and 3 (Table 47, Fig.45 and 46). Due to the presence of *Ggt*, the effect of *Rs* was suppressed.

The means for the length of leaf 4 are given in Table 48. It is difficult to interpret second order interactions. Therefore only consistent trends are noted. The second order interaction was responsible for 2.7% variation in length. Combination of *Ggt* with *Rs* at all concentrations gave the normal increase (Fig.47). The decrease in length of leaf 4 due to *Rs* alone was relatively unaffected by the presence of 125 *Ha* larvae. Combination of all concentrations of *Ggt* and *Rs* with the 125 larval density reduced the ability of *Ggt* to suppress *Rs* damage (Fig.48). Similar trends were found with the two lower densities.

Similar but less obvious trends were observed with leaf 5 (Fig.48). The combination of the three pathogens increased the length of leaf 6, more than the *Ggt* with *Rs* combinations particularly at the two higher levels of *Ggt* (Fig.49). The negative relation between the three pathogens and length of leaf 4 (Fig.47), changed to no relation with leaf 5 (Fig.48) and to a positive one for leaves 6 and 7 (Fig. 49 and 50). Means for lengths of leaves 5,6 and 7 are given in Appendix B.

The means for all treatments for the width of leaf 5 are given in Appendix B. The effect of the significant interaction between *Ggt* and *Rs* are shown in Fig.51. The interaction is probably significant because of the change in slope at the lower densities of both pathogens (Fig.51).

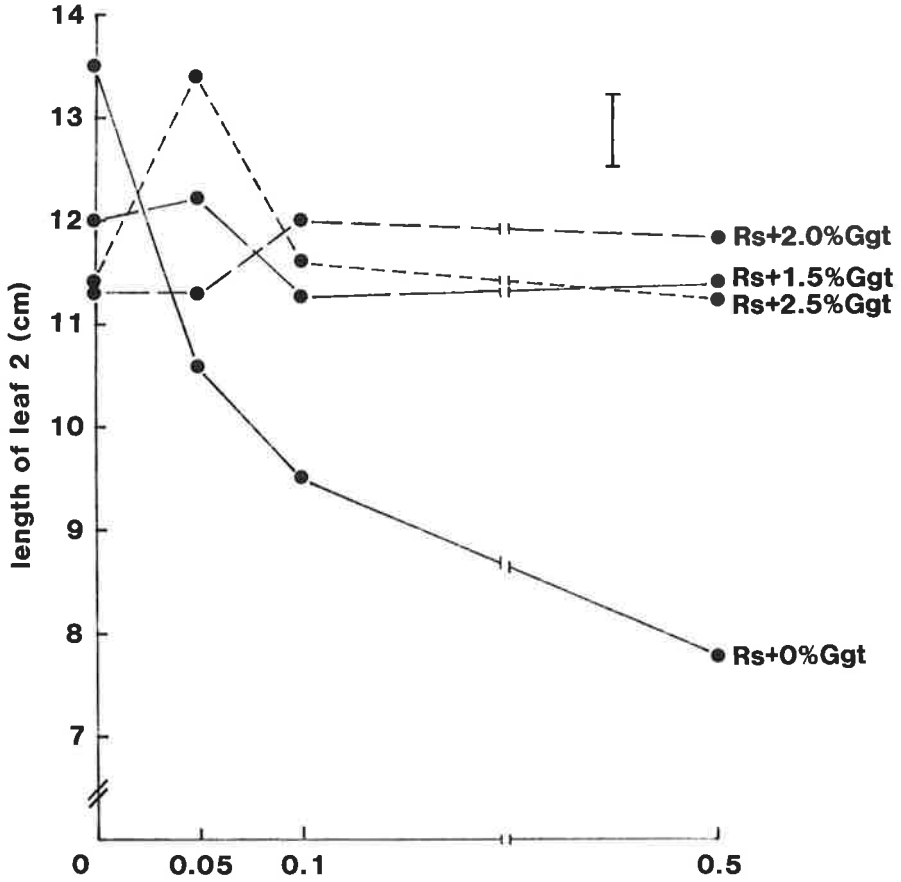
TABLE 47: The effect of *Ggt*,*Rs* combination on length of leaves 1, 2 and 3

Pathogen and density	Leaf 1	Leaf 2	Leaf 3
0% *Ggt*	6.71	13.59	19.10
1.5% *Ggt*	7.17	11.94	18.58
2.0% *Ggt*	7.04	11.39	17.73
2.5% *Ggt*	6.46	11.48	17.01
0.05% *Rs*	7.36	10.58	13.05
0.1 % *Rs*	6.56	9.55	12.16
0.5 % *Rs*	5.72	7.89	11.70
1.5% *Ggt* + 0.05% *Rs*	7.45	12.27	18.56
" 0.1 % *Rs*	7.47	11.33	15.41
" 0.5 % *Rs*	6.68	11.44	16.50
2.0 *Ggt* 0.05% *Rs*	6.42	11.36	15.22
" 0.1 % *Rs*	7.15	12.00	17.66
" 0.5 % *Rs*	7.64	11.83	16.62
2.5% *Ggt* 0.05% *Rs*	7.25	13.43	21.00
" 0.1 % *Rs*	7.18	11.58	16.32
" 0.5% *Rs*	7.01	11.32	16.37
L.S.D; P = .05	0.56	0.72	1.20

Figure 45: Effect of *Ggt* and *Rs* combination on length of leaf 2.

Figure 46: Effect of *Ggt* and *Rs* combination on length of leaf 3.

45


14
13
12
11
10
9
8
7
length of leaf 2 (cm)
Rs+2.0%Ggt
Rs+1.5%Ggt
Rs+2.5%Ggt
Rs+0%Ggt
0
0.05
0.1
0.5

46

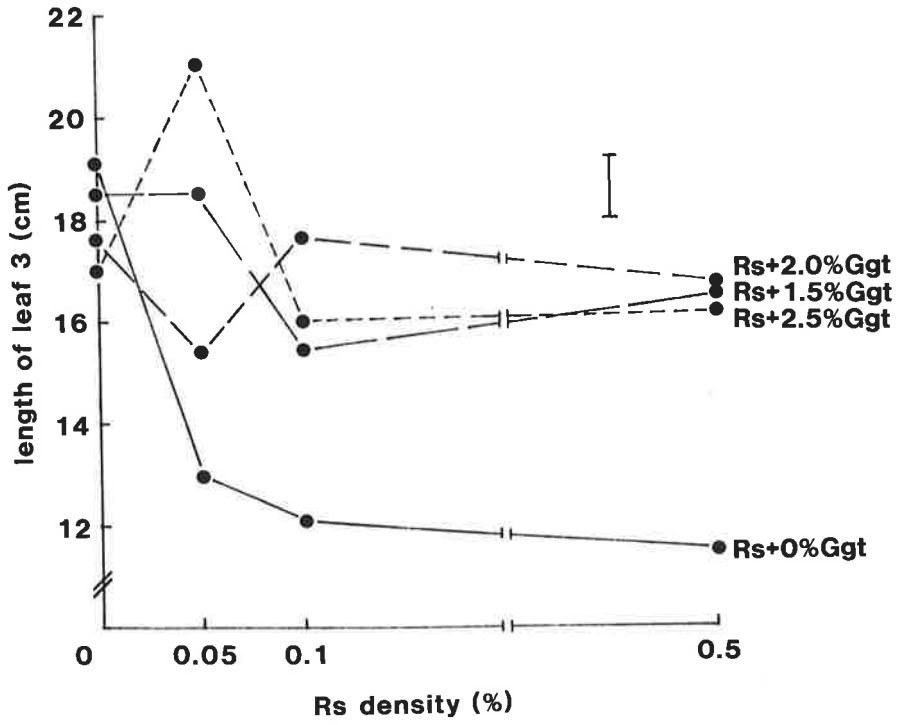

22
20
18
16
14
12
length of leaf 3 (cm)
Rs+2.0%Ggt
Rs+1.5%Ggt
Rs+2.5%Ggt
Rs+0%Ggt
0
0.05
0.1
0.5
Rs density (%)

Figure 47: Effect of *Rs*, *Ggt* + *Rs* and *Ggt* + *Rs* + 125 *Ha* larvae on length of leaf 4.

Figure 48: Effect of *Rs*, *Ggt* + *Rs* and *Ggt* + *Rs* + 125 *Ha* larvae on length of leaf 5.

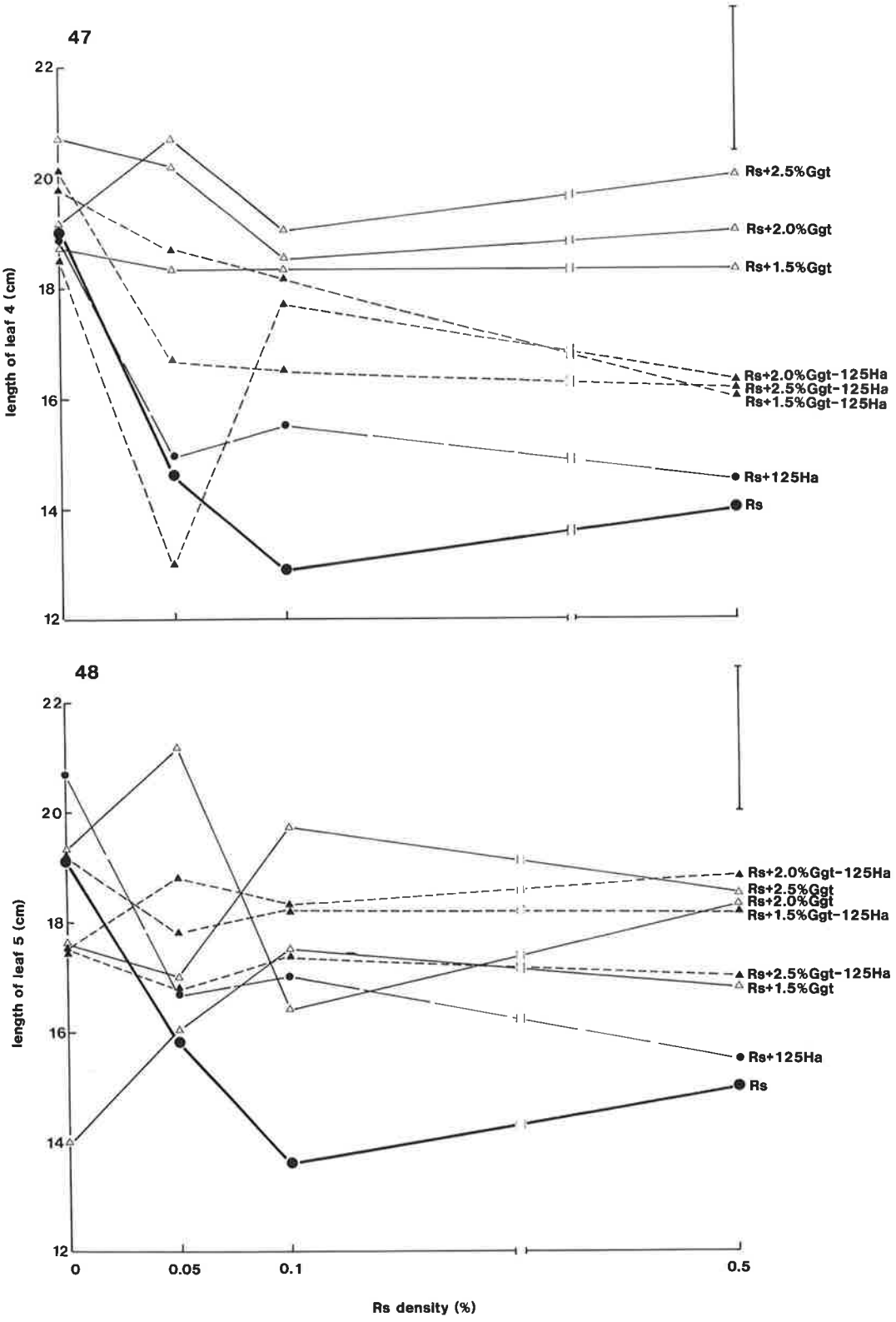

47
22
20
18
16
14
12
length of leaf 4 (cm)
Rs+2.5%Ggt
Rs+2.0%Ggt
Rs+1.5%Ggt
Rs+2.0%Ggt-125Ha
Rs+2.5%Ggt-125Ha
Rs+1.5%Ggt-125Ha
Rs+125Ha
Rs
48
22
20
18
16
14
12
length of leaf 5 (cm)
Rs+2.0%Ggt-125Ha
Rs+2.5%Ggt
Rs+2.0%Ggt
Rs+1.5%Ggt-125Ha
Rs+2.5%Ggt-125Ha
Rs+1.5%Ggt
Rs+125Ha
Rs
0
0.05
0.1
0.5
Rs density (%)

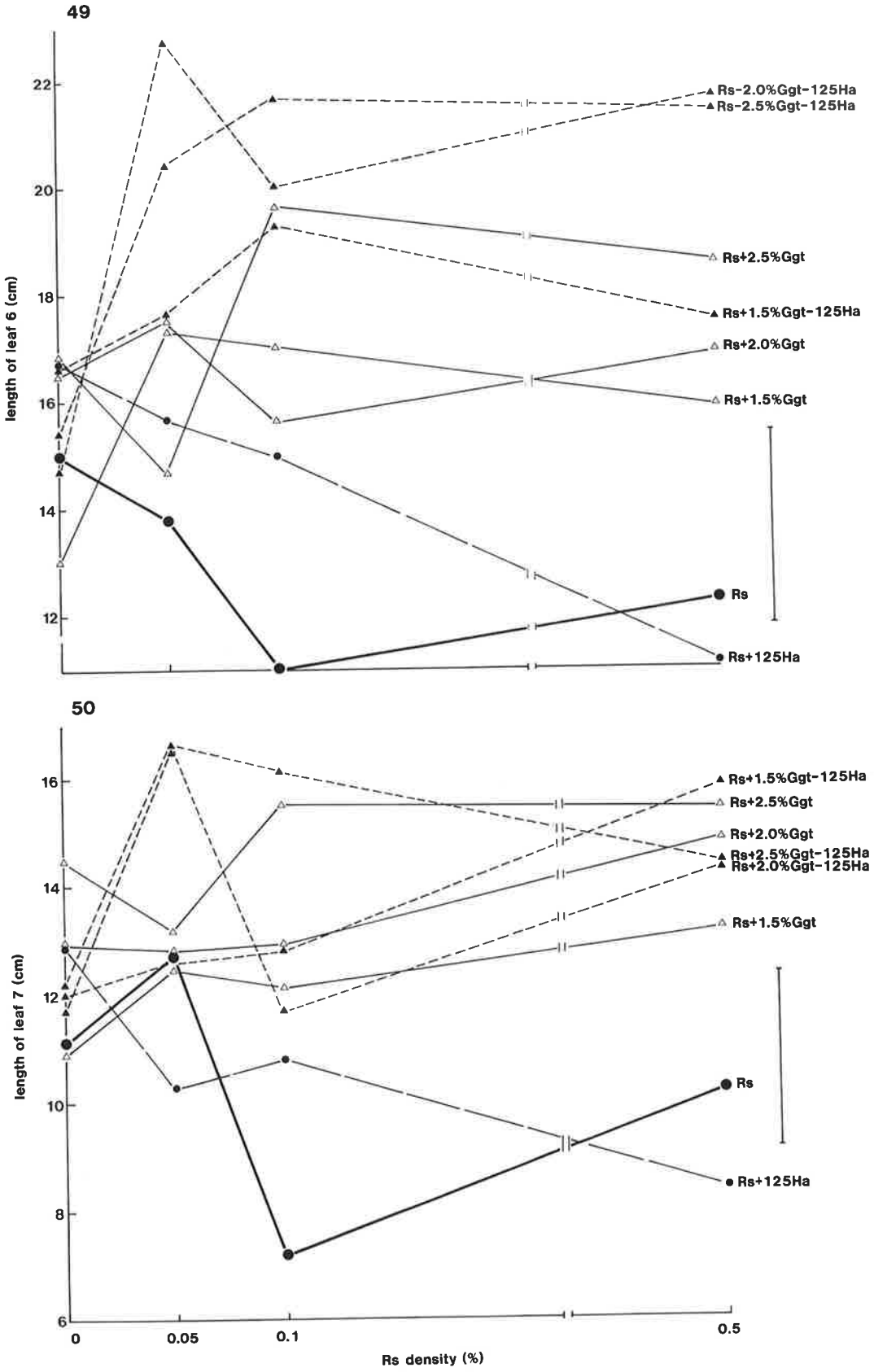
TABLE 48: Effect of *Ggt*, *Rs* and *Ha* on length of leaf 4

		Initial density of *Ha*			
Ggt	*Rs*	0	25	50	125
0%	0	19.06	19.18	19.10	18.84
	0.05%	14.68	15.69	15.26	14.99
	0.1 %	12.89	13.21	13.01	15.55
	0.5 %	14.09	14.68	14.05	14.51
1.5%	0	18.83	18.01	20.31	19.86
	0.05%	18.29	16.48	17.13	18.78
	0.1 %	18.34	17.08	16.58	18.29
	0.5 %	18.36	16.96	15.99	16.05
2.0%	0	20.71	21.22	21.04	18.58
	0.05%	21.72	13.40	10.90	13.04
	0.1 %	18.50	17.69	19.53	17.73
	0.5 %	19.06	16.64	17.73	16.34
2.5%	0	19.19	20.48	17.40	20.10
	0.05%	20.78	16.78	17.74	16.69
	0.1 %	19.08	17.05	16.35	16.49
	0.5 %	19.99	16.00	14.93	16.19

L.S.D; P = 0.05 = 2.34.

Figure 49: Effect of *Rs*, *Ggt* + *Rs* and *Ggt* + *Rs* + 125 *Ha* larvae on length of leaf 6.

Figure 50: Effect of *Rs*, *Ggt* + *Rs* and *Ggt* + *Rs* + 125 *Ha* larvae on length of leaf 7.


49
length of leaf 6 (cm)
22
20
18
16
14
12
Rs-2.0%Ggt-125Ha
Rs-2.5%Ggt-125Ha
Rs+2.5%Ggt
Rs+1.5%Ggt-125Ha
Rs+2.0%Ggt
Rs+1.5%Ggt
Rs
Rs+125Ha
50
length of leaf 7 (cm)
16
14
12
10
8
6
Rs+1.5%Ggt-125Ha
Rs+2.5%Ggt
Rs+2.0%Ggt
Rs+2.5%Ggt-125Ha
Rs+2.0%Ggt-125Ha
Rs+1.5%Ggt
Rs
Rs+125Ha
0
0.05
0.1
0.5
Rs density (%)

Combinations of these fungi increased the width of leaf 5 to a greater extent than *Rs* alone but decreased it compared to *Ggt* concentrations alone.

Effect of *Ggt*, *Rs* and *Ha* on each other

No second order interaction was significant (Table 49) but first order interactions between *Ggt* and *Rs* had significant effects on length of *Rs* lesions and number of cysts. A significant interaction was obtained between *Ggt* and *Ha* on length of *Ggt* lesions.

The significant *Ggt*,*Rs* interaction on length of *Rs* lesions probably resulted from the removal of the reduction in length of *Rs* lesions between the two lower concentrations of *Rs* (Fig.52) by the addition of *Ggt* - a small change. Not only did *Ggt* influence this change, it also suppressed length of *Rs* lesions at all densities. *Ggt* by suppressing *Rs*, also increased cyst production by *Ha* and restricted the damaging effects of *Rs* on cysts (Fig.53).

The effect of *Ha* with *Ggt* on the length of *Ggt* lesions was variable (Fig.54). The combination of 2.5% *Ggt* with 50 larvae gave a remarkably different result and this was probably responsible for the significant interaction (Table 49). Whether this was a true or spurious result can only be decided from further experiments.

Ggt reduced the length of *Rs* lesions (Fig.52, Table 49) and the number of whole roots destroyed by *Rs* (Appendix C). It also reduced the number of cysts produced (Appendix C). *Rs* decreased the length of *Ggt* lesions and severely reduced the number of *Ha* cysts. *Ha* had no effect on length of *Ggt* lesions, reduced the length of *Rs* lesions at particular densities and had variable effects on number of whole roots destroyed (Appendix C).

TABLE 49: F values obtained by ANOVA for the effect of *Ggt*, *Rs* and *Ha* on each other

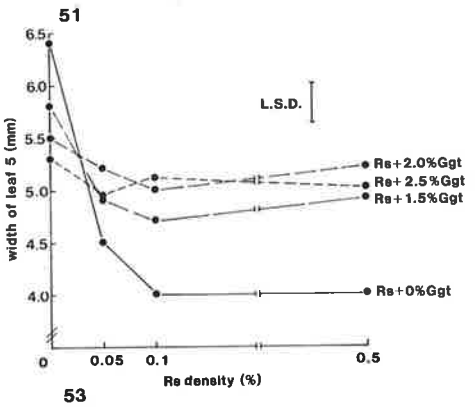
Pathogens	*Ggt* lesion length (F values)	*Rs* lesion length (F values)	Number of roots destroyed by *Rs* (χ^2 statistic)	*Ha* Cysts (χ^2 statistic)
Ggt	8.71 **	243.23**	70.04**	13.36**
Rs	31.35 **	1.88	1.32	20.53**
Ha	0.825	10.14**	11.68**	22.64**
Ggt, *Rs*	0.844	4.45**	6.39	51.59**
Ggt, *Ha*	1.88*	1.84	7.30	11.85
Rs, *Ha*	1.04	1.09	2.87	2.68
Ggt, *Rs*, *Ha*	0.79	0.83	19.26	15.81

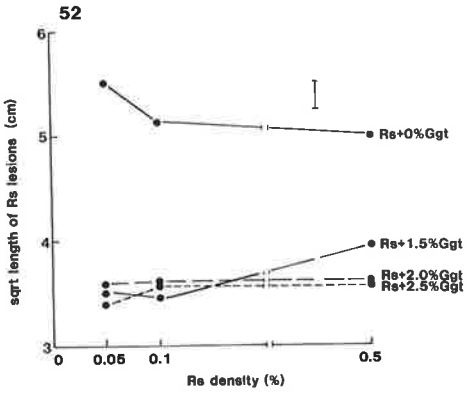
Figure 51: Effect of *Ggt* and *Rs* combination on width of leaf 5.

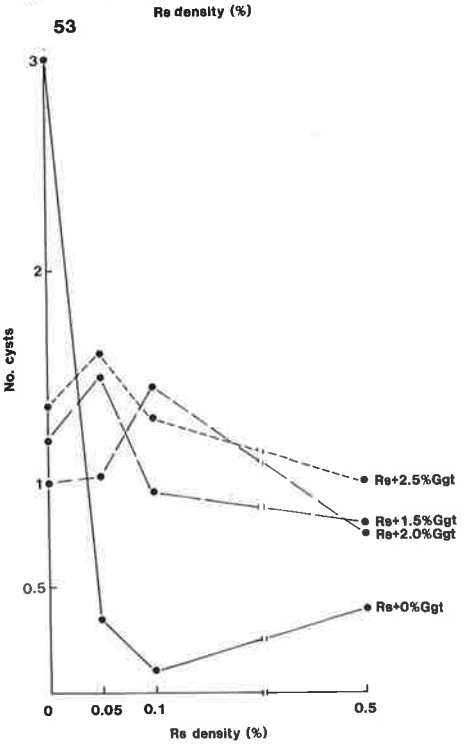
Figure 52: Effect of *Ggt* and *Rs* combination on length of *Rs* lesions at maturity (sq.rt.).

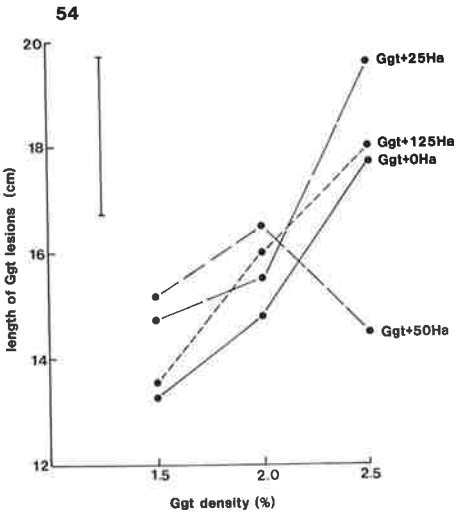
Figure 53: Effect of *Ggt* and *Rs* combination on *Ha* survival (cysts) at maturity.

Figure 54: Effect of *Ggt* and *Ha* combination on length of *Ggt* lesions at maturity.


51
6.5
6.0
5.5
5.0
4.5
4.0
width of leaf 5 (mm)
L.S.D.
Rs+2.0%Ggt
Rs+2.5%Ggt
Rs+1.5%Ggt
Rs+0%Ggt
0
0.05
0.1
0.5
Rs density (%)


52
6
5
4
3
sqrt length of Rs lesions (cm)
Rs+0%Ggt
Rs+1.5%Ggt
Rs+2.0%Ggt
Rs+2.5%Ggt
0
0.05
0.1
0.5
Rs density (%)


53
3
2
1
0.5
No. cysts
Rs+2.5%Ggt
Rs+1.5%Ggt
Rs+2.0%Ggt
Rs+0%Ggt
0
0.05
0.1
0.5
Rs density (%)


54
20
18
16
14
12
length of Ggt lesions (cm)
Ggt+25Ha
Ggt+125Ha
Ggt+0Ha
Ggt+50Ha
1.5
2.0
2.5
Ggt density (%)

B. Discussion

This experiment, to examine the effects of three pathogenic variables at one time, was ambitious but it does represent the situation that was found in the field. One important point has emerged; that few significant second order interactions on growth were recorded. This suggests, though it needs confirmation that second or higher order interactions may be relatively unimportant in the relationship of a number of organisms to the growth of plants. Although the experiment had a large number of degrees of freedom and one could expect small differences to be significant, this did not help to demonstrate significant second order interactions.

Conversely significant first order interactions were common between *Ggt* and *Rs*. These were obtained chiefly because the reduction in the growth parameters due to *Rs*, was removed by the *Ggt* inoculum so changing the shape of the curve. Whether this was wholly due to the antagonism between the two fungi or partly due to the addition of oatmeal still remains to be demonstrated.

Few significant first order interactions between *Ggt* and *Ha*, and *Rs* and *Ha* were found. This may have been due to the relatively low initial densities of *Ha* and higher densities may give different results.

The numerous fluctuations in the means possibly reflect the many sources of variations that can influence such a large experiment, eg. seed size, germination, inoculum preparation, maceration and incorporation of pathogens into soil. Three different methods of inoculation were used here, one for each pathogen, and this probably caused variation. Although broad trends were apparent, there were no clear cut and consistent effects in all the variables. It is difficult to interpret individual significant means. One of the important facets in these

experiments is to try to remove this source of variation.

Ggt (+ oatmeal) stimulated growth producing an increase in dry shoot weight, height, number of grains and yield; the only reduction due to *Ggt* was that in root length. *Rs* reduced all growth variables measured. Its effect on growth was immediate and could be measured at maturity. There was no effect of *Ha* at the low densities used.

The effect of oatmeal on *Rs* has been reported before. Davey and Papavizas (1960) observed that soybean, corn and oat amendments reduced disease due to *Rhizoctonia solani* and that sawdust had no effect. The reduction in disease development seemed to be greatest, in their experiments, when the amendment was undergoing rapid decomposition. The *in vitro* experiments in my studies of the relationship between *Ggt* and *Rs*, comprehensively illustrated the antagonism between these two fungi. The decrease in length of *Rs* lesions and the number of whole roots destroyed by *Rs*, support the antagonism hypothesis. *Rs* was unable to cause damage in the presence of *Ggt* and *vice versa*. Over 1000 roots were measured and scored for *Ggt* and *Rs* damage in this study and lesions of both *Ggt* and *Rs* were found together on one root in less than 1% of the roots. In many instances there was damage to the laterals by both fungi but both fungi rarely occurred on the same main root. *Rs* damage was greater on the coronals and *Ggt* on the seminal roots.

Rs,*Ha* combinations caused greater damage than the sum of both pathogens alone at all concentrations. These results are in agreement with those of Meagher and Chambers (1970). Dunn and Hughes (1967), and Polychronopoulos *et al.* (1969), had similar results with *Globodera rostochiensis* and *Rs*; *H. schachtii* and *Rs*, respectively.

The most important limiting factor affecting the length of leaves was *Rs*. Its effect on leaves was immediate and was apparent in leaves 2 to 7. Significant second order interactions were derived for leaves

4,5 and 7. Leaf 4, the first leaf initiated outside the embryo (Williams, 1960), was the first leaf to show the effect of all three pathogens. All the first order interactions were also significant for this leaf. *Ha* affected the ability of *Ggt* to suppress *Rs* ie. in the presence of *Ha*, the increase due to the *Ggt*,*Rs* interaction in the length of leaf 4 was reduced. Leaf 4 showed the complexity of the second order interaction. The complex effect carried through to the following leaves but gradually disappeared due to the recovery of the plant. The difficulty in interpretation of second order interactions, envisaged by Wallace (1983) was encountered. Further experimentation, it is felt, would be required to confirm that interaction effects are clearly shown on leaf lengths in wheat at other densities of pathogens. Effect of *Ha* damage to cereals is reflected in the leaves but there are no examples of the influence of soil fungi on the length of leaves in cereals.

The reduction of *Ha* population by both *Ggt* and *Rs* in this study confirmed the results of the experiment described earlier in this thesis. These findings are in agreement with those reported by Cook (1975) and Meagher *et al*. (1978).

3.6 The effect of *Ggt* oatmeal inoculum on growth

In the previous experiments, the oatmeal in the *Ggt* inoculum caused significant nutritional effects which confused the assessment of damage due to *Ggt*. This could not be done in the experiments because the addition of another variable would have made the experiments unmanageable. It was decided to examine this by growing inoculum, killing the *Ggt* in a proportion of it and examining the effects of live and dead inoculum on growth.

Method

Ggt oatmeal inoculum was sterilized for one hour and used as dead inoculum for control treatments. A two factor experiment was conducted using five rates of dead (sterilized) and live (unsterilized) *Ggt* inoculum (0.5, 1.0, 1.5, 2.0 and 2.5% / 100g of soil) with six replicates. All measurements of plant growth were taken 42 days after sowing.

Effect on growth

Greater increase in both fresh and dry shoot and root weight was found with the dead inoculum than with the live *Ggt* inoculum (Figs. 55 to 58). The increase was significant between the two media but not between all densities. The 4th, 5th and 6th leaves were longer in the dead control treatments (Table 50).

Discussion

Ggt had an inhibitory effect on growth, but due to the inoculum base used, the true effect on growth i.e. reduction of growth parameters was masked. The increase in growth due to the oatmeal base of the sterilized dead inoculum was obvious. The second sterilization of the inoculum probably would not have as large an effect on the release of nutrients as the first sterilization of oat grains prior to infection by *Ggt*. It is not possible to predict, however, the complexity of effects of the increased nutrition which is known to have varying effects on the amount of disease and the growth of organisms (Baker and Martinson, 1970; Huber, 1981). Increased nutrition may

Figure 55: Effect of live and dead *Ggt* inoculum on fresh shoot weight at 42 days.

Figure 56: Effect of live and dead *Ggt* inoculum on fresh root weight at 42 days.

Figure 57: Effect of live and dead *Ggt* inoculum on dry shoot weight at 42 days.

Figure 58: Effect of live and dead *Ggt* inoculum on dry root weight at 42 days.

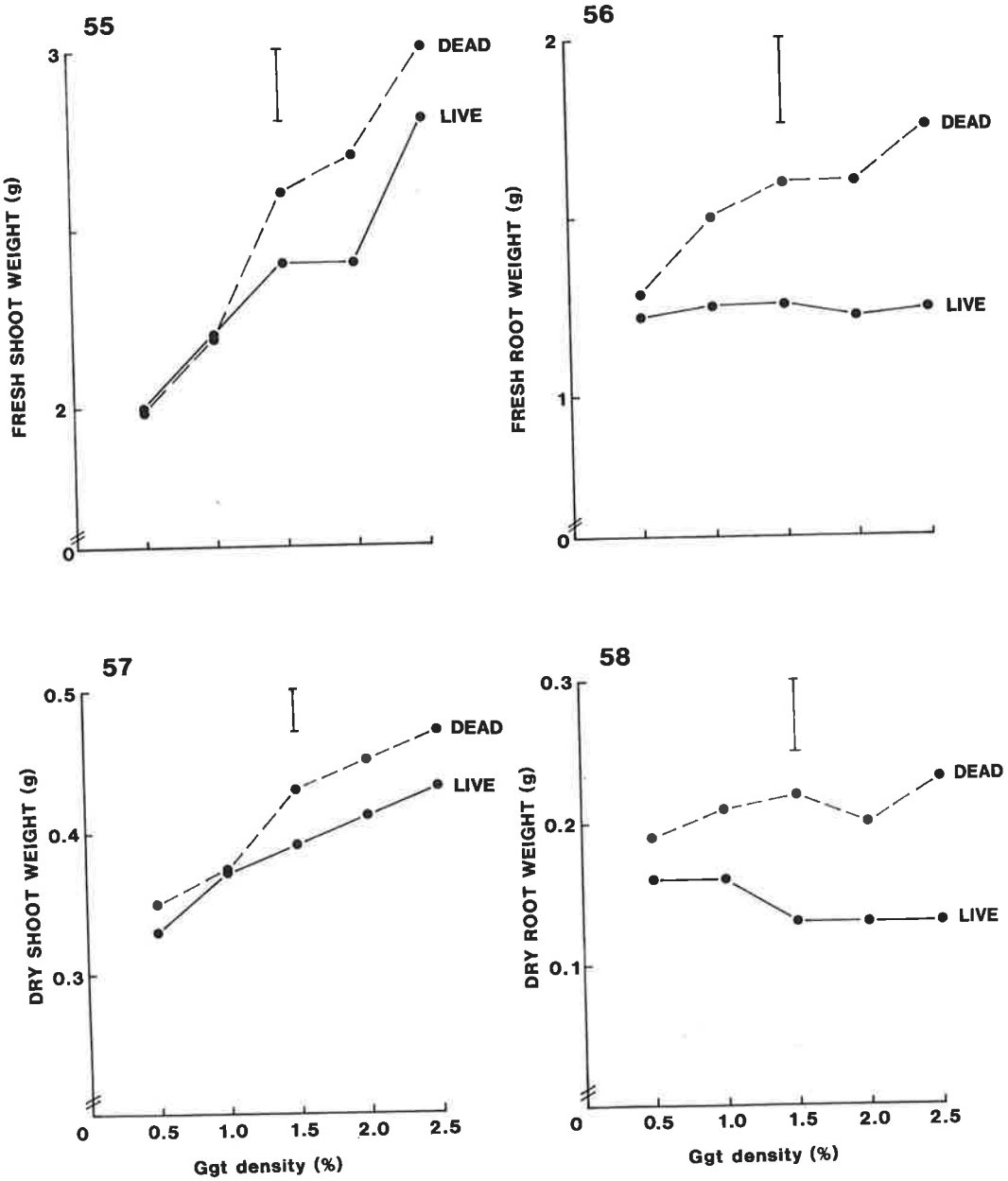

55
FRESH SHOOT WEIGHT (g)
3
2
0
DEAD
LIVE
56
FRESH ROOT WEIGHT (g)
2
1
0
DEAD
LIVE
57
DRY SHOOT WEIGHT (g)
0.5
0.4
0.3
0
0.5
1.0
1.5
2.0
2.5
Ggt density (%)
DEAD
LIVE
58
DRY ROOT WEIGHT (g)
0.3
0.2
0.1
0
0.5
1.0
1.5
2.0
2.5
Ggt density (%)
DEAD
LIVE

TABLE 50: Effect of dead and live *Ggt* inoculum on final leaf length

Oatmeal and pathogen density		Length of leaves					
		Leaf 1	Leaf 2	Leaf 3	Leaf 4	Leaf 5	Leaf 6
Oatmeal	0.5%	9.53	16.27	23.13	24.92	24.77	20.48
	1.0%	9.63	16.57	23.75	25.83	24.53	22.45
	1.5%	10.10	17.45	25.45	28.07	25.62	22.72
	2.0%	9.65	17.13	25.12	28.10	27.18	22.00
	2.5%	10.03	18.00	26.17	29.00	27.02	23.73
Ggt oatmeal inoculum	0.5%	10.20	17.32	24.20	25.07	24.17	20.48
	1.0%	9.10	16.27	23.28	25.65	24.72	22.45
	1.5%	9.23	14.82	22.42	25.80	25.60	22.72
	2.0%	10.32	16.87	24.42	25.92	23.98	22.00
	2.5%	9.83	17.25	24.97	26.32	25.53	23.73
L.S.D; P = .05		1.09	1.61	1.93	1.44	1.52	1.65

increase damage or it may decrease damage, so that without experimental evidence it is not possible to predict what would happen to *Rs* or *Ha* in the presence of *Ggt*. It may not have any effect or it may change the effects.

Thus it is important to choose an inoculum medium that will have few such effects. The oatmeal inoculum, which is the one normally employed with *Ggt*, is unsatisfactory and a suitable means of growing *Ggt* needs to be investigated. Until such a medium is available it is unlikely that simulation with field conditions will be approached and this should be borne in mind in interpreting results.

CHAPTER IV

GENERAL DISCUSSION

Studies of plant disease usually consider the influence of single pathogen species on the host plant. Such studies have clearly increased our knowledge of disease in spite of the fact that, in nature, disease is the result of numerous factors, environmental as well as pathogenic.

The relationships between the root pathogens of wheat studied here show that it is just as important to study communities of organisms and their relation to damage and each other. The conventional terms antagonism and synergism have largely been replaced by the positive and negative relation between the organisms.

Four organisms (*Heterdoera avenae*, *Gaeumannomyces graminis var. tritici*, *Rhizoctonia solani* and *Pratylenchus minyus*) were present in the area and all plants had at least one or more organisms in their roots but discriminant analysis showed that the organisms were not strongly related, though they contributed, to the damage that occurred in the patches. Some other factor or factors were involved in the causation of patches.

Established statistical procedures such as correlations coefficients clearly demonstrated the presence of relationship between the pathogens and also indicated the type of relationships.

Principal Component analysis successfully used by Stynes (1975) in his synoptic study of wheat did not clarify nor add any new information about inter-relationships between pathogens in this study.

Regression analysis using interation terms demonstrated that all the pathogen combinations studied, were responsible for about 5% of the variation in growth in the field. In the laboratory tests with the desities chosen, interactions were

responsible for between 5% (*Ggt, Ha* combination) and 12% (*Ggt, Rs* combination). The *Rs, Ha* combination averaged about 9%. Thus the overall contribution of interactions to the variance under experimental conditions was similar to the field situation. The F value (variance ratio) reflected the order of importance of each factor in ANOVA but it was the size of the mean squares that indicated the contribution of the interaction to the total variation of the factors in the study.

Pathogens that attacked the seminal roots had a greater effect on growth than those which attacked coronal roots (in some cases the same organisms) suggesting that early infection was more damaging. Among the organisms, *Ha* was responsible for the greatest variation in growth and was most consistently associated with damage while the relation of *Pm* to damage was variable. Although both *Ggt* and *Rs* were negatively correlated with growth, the relationship varied between the two years and the effects of the two fungi seemed to be affected more by environment (particularly water), as *Ggt* damage occurred when rainfall was high.

The most consistent relation in the field was the positive relation between *Ggt* and *Ha* even under different environmental conditions. This relationship was found when sampling occurred earlier than maturity. When sampled in the laboratory at maturity, a negative relation was found. In England on barley (Cook, 1969) low numbers of cysts were associated with high levels of *Ggt* at maturity. Cook (1975) examined the effect of adding the inocula at different times; later inoculation with *Ggt* did not affect the numbers of larvae in the root system but earlier inoculation did. A similar situation occurred with *Rs* and *Ha*. The association, which occurred in the field mainly in the second year, varied from positive to negative but in pots was mainly negative whether sampled early or late. What may happen is that if fungal penetration precedes nematode penetration, the root is made unsuitable for *Ha* and numbers are reduced ie. an effect on penetration. If nematodes precede fungi by some time, then the fungus may

find suitable sites for entry or for growth (syncytia) and a positive relation may be found. But both damage to the plant and damage to the syncytia would both reduce numbers of cysts at maturity. Initial relative densities of organisms could effect the relationship.

Antagonism did occur in *'in vitro'* testing between *Ggt* and *Rs*, not between the hyphae but perhaps at penetration and establishment in the root as lesions did not form in the immediate vicinity of the inoculum of the other fungus. This suggested a limited (in area) antagonism that is important in the field but that requires further examination. But antagonism is not the only factor involved as there was an association between water and *Ggt* infection in the field. This was not confirmed in the laboratory when the amount of water was varied but this just demonstrates the difficulties of working in pots of soil. The water regimes in tubs may not have simulated the water regimes in the field. The association of the two fungal pathogens (*Ggt* and *Rs*) with wet and dry conditions has been reported (Garrett, 1944; Butler, 1961; Hynes, 1937; Blair, 1943; Das and Western, 1959; Cook *et al.*, 1972). Whether the relation of the water to the fungi is to the antagonistic interaction or merely to direct effect on fungal growth needs further investigation.

Such a complicated relationship has implications for the relation between yield and organisms at maturity and for the expected amount of damage in the following year in the field. Thus, a positive rather than a negative relation between yield and cyst number might be expected. On the other hand, in the presence of both fungi in the lab tests, reduction in the *Ha* population was not as great as that due to *Rs* alone. The antagonism between the two fungi allowed more females of *Ha* to survive. In the laboratory test, *Rs* was dominant because of the high initial densities. These relationships might influence yields of crops the following year but it is probably too early to speculate on what might occur as insufficient data is available. Such relationships between fungi and nematodes indicate the care that is needed when interpreting the effects of chemical control

or the use of resistant varieties where reduction in one pathogen might enable another to be more damaging.

The sheer size of factorial designed glasshouse experiments puts constraints on the number of factors, the levels at which they are used, the number of replicates and the size of pots.

The laboratory experiments high-lighted some of the problems associated with demonstrating the effects of soil-borne fungi. Firstly there was the loss of virulence associated with lengthy culture of the fungus *(Ggt)* and the partial restoration by passage through a host. Secondly there was the problem of using realisitic densities. This was a problem with both fungi but particularly with *Rs* where all the densities were relatively high (in terms of damage caused) and tended to dominate because of this. Many authors (eg. Meagher, 1970) do not even record the exact concentration of fungus used. Thirdly, the problem of the medium used for growing the fungus, especially *Ggt* . This caused problems in trying to distinguish the effects of increased nutrition and the damage due to the fungus. It probably also affected the interrelationships with other organisms.

Microplot technique or the use of field soil, in a dilution series would reduce the number of variables in such complex studies. The survival of pathogens and their ability to infect after handling and mixing would need testing.

This study emphasises that formulation of hypotheses and testing of them should be based on many years of field data

In effect, pot experiments are useful for testing specific hypotheses generated in the field and in revealing complex relationships between pathogens.

Discretion has to be used in drawing conclusions, however, due to marked differences between the environment of the pot and that of the field; for example, no strong interaction was detected between *Rs* and *Ha* in one year when *Rs* was the predominant fungal pathogen. However, synergism occurred under experimental conditions. Such a result demands further investigation in the field to see if synergism does in fact occur with densities different from those previously recorded in the field study.

The interrelationship between *Ggt*, *Rs* and *Ha*, measured, established and studied are only a part of the whole complex of interactions and interrelationships that occur at all levels, between the microbial population, physical nature and chemical composition of the soil, environment and the plant. Nevertheless studies of parts of systems are necessary and useful as they produce hypotheses for further testing. This project has attempted to study relationships of four pathogens which form a part of the complex ecosystem.

A P P E N D I C E S

A P P E N D I X A.

TABLE 1: Means of dry shoot weight

Ggt	Rs	Initial density of Ha 0	25	50	125
0	0	0.9575	0.8550	0.8775	0.8887
	0.05%	0.4625	0.5462	0.4600	0.5212
	0.1 %	0.3475	0.3762	0.3173	0.4625
	0.5 %	0.4107	0.3850	0.3675	0.3687
1.5%	0	0.9287	0.9662	0.9825	0.9025
	0.05%	0.8575	0.8637	0.8650	0.9112
	0.1 %	0.7637	0.8150	0.8187	0.8462
	0.5 %	0.8562	0.9712	0.9075	0.8350
2.0%	0	1.0325	1.0819	1.0200	0.9500
	0.05%	0.9799	0.9850	0.8562	0.8750
	0.1 %	0.7450	0.8675	0.9162	0.8987
	0.5 %	0.8637	0.8637	0.8562	0.9087
2.5%	0	0.9825	1.8200	0.9625	0.9912
	0.05%	0.9312	0.9925	0.9475	1.0375
	0.1 %	0.8337	0.9862	0.9475	0.9550
	0.5 %	0.9225	0.9200	0.9350	0.8937

L.S.D; P = .05 = 0.120

TABLE 2: Means of dry root weight

		Initial density of Ha			
Ggt	Rs	0	25	50	125
0	0	0.1897	0.2096	0.2421	0.1879
	0.05%	0.0943	0.1122	0.1004	0.1115
	0.1 %	0.0807	0.0869	0.1539	0.1017
	0.5 %	0.0937	0.0912	0.0824	0.0869
1.5%	0	0.1632	0.1755	0.1986	0.2532
	0.05%	0.1476	0.1607	0.1630	0.2068
	0.1 %	0.1251	0.1385	0.1559	0.1625
	0.5 %	0.1567	0.2020	0.1737	0.1666
2.0%	0	0.2021	0.1807	0.2252	0.1974
	0.05%	0.1861	0.1593	0.1475	0.1678
	0.1 %	0.1616	0.1587	0.1600	0.1831
	0.5 %	0.1730	0.1624	0.1672	0.1501
2.5%	0	0.1767	0.1752	0.1620	0.1587
	0.05%	0.2116	0.1883	0.2465	0.2136
	0.1 %	0.1492	0.1550	0.1617	0.1626
	0.5 %	0.1636	0.1417	0.1501	0.1646

L.S.D; P = 0.05 = .0492

TABLE 3: Means of number of roots

		Initial density of Ha			
Ggt	Rs	0	25	50	125
0	0	15.250	13.875	14.125	13.875
	0.05%	12.375	12.750	11.750	11.500
	0.1 %	12.000	11 250	11.797	11.750
	0.5 %	12.375	11.250	11.250	11.375
1.5%	0	12.250	12.375	13.000	12.875
	0.05%	11.750	11.375	11.375	12.125
	0.1 %	11.750	11.250	11.125	10.750
	0.5 %	11.875	12.250	11.750	12.125
2.0%	0	12.875	12.625	12.750	12.625
	0.05%	12.699	12.750	12.125	12.750
	0.1 %	12.750	11.625	11.875	12.125
	0.5 %	12.875	11.500	12.000	11.500
2.5%	0	13.250	11.875	11.375	11.875
	0.05%	12.625	12.500	11.875	12.875
	0.1 %	12.125	11.750	11.625	12.125
	0.5 %	12.625	12.000	12.625	12.875

Non-parametric analysis.

TABLE 4: Means of length of roots

Ggt	Rs	Initial density of Ha 0	25	50	125
0	0	174.05	151.12	155.25	153.31
	0.05%	79.75	88.06	86.25	88.50
	0.1 %	68.25	70.56	61.36	72.81
	0.5 %	69.81	79.81	68.44	61.56
1.5%	0	129.25	126.50	122.38	123.75
	0.05%	73.69	88.00	84.81	89.38
	0.1 %	76.81	77.38	84.00	80.81
	0.5 %	75.38	87.75	92.75	99.44
2.0%	0	142.75	121.44	114.19	127.75
	0.05%	92.36	100.63	98.00	105.38
	0.1 %	88.96	92.75	80.69	102.00
	0.5 %	94.50	85.94	81.94	93.88
2.5%	0	135.31	121.86	102.44	123.25
	0.05%	89.12	90.81	89.75	99.31
	0.1 %	85.81	103.69	99.69	98.37
	0.5 %	86.62	95.75	103.81	112.94

L.S.D; P = .05 = 16.99

TABLE 5: Means of heights of plants

		Initial density of Ha			
Ggt	Rs	0	25	50	125
0	0	40.64	42.39	40.17	41.95
	0.05%	37.29	38.37	39.29	39.54
	0.1 %	34.94	40.74	38.72	40.82
	0.5 %	40.07	39.24	38.94	39.97
1.5%	0	45.34	42.24	42.52	42.22
	0.05%	41.60	41.89	41.45	42.94
	0.1 %	44.45	43.06	41.60	41.14
	0.5 %	45.21	43.60	41.35	39.25
2.0%	0	44.76	41.94	42.29	43.84
	0.05%	42.91	39.61	37.72	35.60
	0.1 %	38.91	40.14	39.55	39.01
	0.5 %	41.01	39.86	40.45	39.71
2.5%	0	42.12	45.67	43.11	43.05
	0.05%	42.75	40.16	39.94	42.36
	0.1 %	39.22	40.77	39.20	41.54
	0.5%	40.69	38.91	38.04	38.19

L.S.D; P = 0.05 = 4.17.

TABLE 6: Means of number of grains ($\sqrt{\ }$)

		Initial density of Ha			
Ggt	Rs	0	25	50	125
0	0	3.093	2.793	2.305	2.776
	0.05%	1.969	2.086	2.023	2.268
	0.1 %	1.783	1.812	1.737	2.163
	0.5 %	1.863	1.867	1.793	1.897
1.5%	0	3.378	3.174	2.815	2.752
	0.05%	3.171	2.996	3.129	2.978
	0.1 %	3.225	3.281	3.047	3.131
	0.5 %	3.397	3.369	3.216	2.999
2.0%	0	3.327	3.884	3.217	3.206
	0.05%	3.193	3.315	2.964	2.863
	0.1 %	3.163	2.704	3.025	2.671
	0.5 %	3.230	3.286	3.184	3.418
2.5%	0	3.592	3.312	3.189	3.160
	0.05%	3.142	3.229	3.358	3.324
	0.1 %	3.236	3.563	3.176	3.291
	0.5 %	3.059	3.177	3.317	2.567

L.S.D; P = 0.05 = .5329.

TABLE 7: Means of yield (log)

		Initial density of Ha			
Ggt	Rs	0	25	50	125
0	0	-1.139	-1.381	-1.689	-1.346
	0.05%	-2.405	-2.158	-2.270	-2.016
	0.1 %	-2.708	-2.726	-2.869	-2.235
	0.5 %	-2.553	-2.798	-2.803	-2.615
1.5%	0	-0.893	-1.041	-1.302	-1.445
	0.05%	-1.146	-1.196	-1.063	-1.245
	0.1 %	-1.326	-1.159	-1.179	-1.142
	0.5%	-1.161	-1.808	-1.129	-1.144
2.0%	0	-0.987	-0.723	-1.016	-1.149
	0.05%	-1.080	-0.902	-1.155	-1.180
	0.1 %	-1.465	-1.465	-1.156	-1.269
	0.5 %	-1.202	-1.060	-1.169	-0.943
2.5%	0	-0.894	-0.957	-1.324	-1.039
	0.05%	-1.415	-0.920	-0.959	-0.822
	0.1 %	-1.149	-0.871	-1.026	-0.950
	0.5 %	-1.201	-1.035	-0.958	-1.447

L.S.D; P = .05 = 0.4455.

A P P E N D I X B.

TABLE 1: Means of final lengths of leaf 5

Ggt	Rs	Initial density of Ha 0	25	50	125
0	0	19.06	18.50	19.94	20.73
	0.05%	15.80	17.16	16.79	16.71
	0.1 %	13.63	13.68	13.34	17.00
	0.5 %	15.09	15.33	16.66	15.50
1.5%	0	14.04	16.99	18.05	19.16
	0.05%	15.98	15.95	15.50	17.85
	0.1 %	17.60	17.76	16.90	18.21
	0.5 %	16.78	17.63	17.45	18.20
2.0%	0	19.25	20.66	20.24	17.56
	0.05%	21.16	17.45	15.68	18.84
	0.1 %	16.45	17.54	19.10	18.28
	0.5 %	18.29	19.41	17.78	18.91
2.5%	0	17.65	18.96	16.45	17.54
	0.05%	17.10	16.95	16.93	16.66
	0.1 %	19.75	18.18	19.14	17.58
	0.5 %	18.48	17.51	18.20	16.85

L.S.D; P = 0.05 = 2.68.

TABLE 2: Mean leaf lengths for leaf 6

		Initial density of Ha			
Ggt	Rs	0	25	50	125
0	0	15.05	14.23	16.26	16.64
	0.05%	13.83	16.36	14.86	15.73
	0.1 %	10.96	12.20	11.11	14.99
	0.5 %	12.38	11.41	13.48	11.10
1.5%	0	12.99	16.14	16.25	16.49
	0.05%	17.25	17.64	17.03	17.56
	0.1 %	17.00	16.80	18.51	19.35
	0.5 %	15.91	18.88	19.98	17.49
2.0%	0	16.45	18.36	16.83	14.65
	0.05%	17.54	22.65	22.69	23.59
	0.1 %	15.63	20.20	19.11	20.04
	0.5%	16.93	20.78	20.59	21.71
2.5%	0	16.80	18.50	15.55	15.41
	0.05%	14.71	21.03	18.26	20.43
	0.1 %	19.58	20.75	22.33	21.73
	0.5%	18.56	22.68	22.78	21.40

L.S.D; P = 0.05 = 3.01.

TABLE 3: Mean of final lengths of leaf 7

Ggt	Rs	Initial density of Ha			
		0	25	50	125
0	0	11.03	11.26	12.40	12.95
	0.05%	12.75	10.38	11.08	10.15
	0.1 %	7.23	8.26	9.44	10.82
	0.5 %	10.24	7.17	6.26	8.40
1.5%	0	10.98	13.21	13.19	11.98
	0.05%	12.56	13.23	13.66	12.54
	0.1 %	12.08	11.17	9.74	12.88
	0.5 %	13.21	14.01	13.85	15.81
2.0%	0	11.68	14.05	11.73	12.23
	0.05%	12.87	17.22	17.36	16.68
	0.1 %	12.99	15.05	11.63	11.72
	0.5%	14.88	12.79	11.49	14.33
2.5%	0	14.55	12.38	12.11	11.74
	0.05%	13.18	16.06	13.83	16.44
	0.1 %	15.56	14.91	14.81	16.19
	0.5 %	15.43	14.20	16.70	14.46

L.S.D; P = 0.05 = 3.12.

TABLE 4: Means of width of leaf 5

		Initial density of Ha			
GGt	Rs	0	25	50	125
0	0	6.600	6.438	6.225	6.175
	0.05%	4.187	4.688	4.513	4.537
	0.1 %	3.862	4.462	3.608	4.262
	0.5 %	4.125	3.919	3.825	4.130
1.5%	0	5.762	5.625	6.025	5.800
	0.05%	5.125	4.825	4.838	4.938
	0.1 %	5.225	4.525	4.662	4.650
	0.5 %	5.512	4.817	4.530	4.750
2.0%	0	5.975	6.805	5.838	5.912
	0.05%	5.894	4.963	4.625	5.700
	0.1 %	4.812	5.037	5.062	4.900
	0.5 %	5.287	4.787	4.800	4.300
2.5%	0	5.350	5.675	5.737	5.575
	0.05%	5.775	5.050	5.600	5.462
	0.1 %	5.050	4.337	4.462	4.675
	0.5 %	5.300	4.712	4.600	4.575

L.S.D; P = 0105 = .7253.

A P P E N D I X C.

TABLE 1: Means of length of Ggt lesions

		Initial density of Ha			
Ggt	Rs	0	25	50	125
1.5%	0	18.87	21.00	20.25	18.13
	0.05%	10.51	12.44	12.56	13.25
	0.1 %	11.75	11.31	13.75	10.13
	0.5 %	12.19	14.13	13.94	12.25
2.0%	0	21.38	21.88	23.69	21.44
	0.05%	15.15	13.44	15.06	15.44
	0.1 %	10.69	12.31	13.38	14.25
	0.5 %	12.31	14.31	13.81	12.56
2.5%	0	29.25	23.25	17.00	18.50
	0.05%	13.31	16.12	12.94	18.06
	0.1 %	15.25	19.94	13.00	18.06
	0.5 %	13.50	19.25	15.19	16.94

L.S.D; P = 0.05 = 6.238.

TABLE 2: Means of length of Rs lesions ($\sqrt{\ }$)

Ggt	Rs	Initial density of Ha 0	25	50	125
0	0.05%	5.510	4.457	5.777	5.176
	0.1 %	5.599	5.084	4.962	4.867
	0.5 %	5.525	4.878	5.163	4.740
1.5%	0.05%	3.668	3.372	3.435	3.658
	0.1 %	3.897	3.417	3.355	3.173
	0.5 %	4.111	3.882	3.891	3.703
2.0%	0.05%	3.952	3.346	3.475	3.446
	0.1 %	3.823	3.709	3.607	3.351
	0.5 %	4.132	3.238	3.635	3.716
2.5%	0.05%	3.371	3.452	3.253	3.639
	0.1 %	3.734	3.522	3.604	3.665
	0.5 %	3.769	3.553	3.509	3.823

L.S.D; P = 0.05 = .499.

TABLE 3: Means of number of whole roots destroyed by Rs

Ggt	Rs	Initial density of Ha 0	25	50	125
0	0.05%	2.500	2.875	1.875	2.125
	0.1 %	2.375	2.750	3.044	3.250
	0.5 %	3.375	3.000	3.250	3.125
1.5%	0.05%	1.750	1.875	1.500	0.875
	0.1 %	1.000	0.875	1.125	1.000
	0.5 %	2.375	1.625	1.125	0.875
2.0%	0.05%	1.761	1.000	1.125	0.625
	0.1 %	1.250	0.875	1.250	0.625
	0.5 %	0.625	1.375	1.500	1.000
2.5%	0.05%	1.750	0.875	1.125	0.750
	0.1 %	1.750	0.625	0.875	0.750
	0.5 %	1.500	1.125	0.875	0.875

Non-parametric analysis.

TABLE 4: Means of number of cysts recovered

		Initial density of Ha			
Ggt	Rs	0	25	50	125
0	0		2.375	2.750	4.250
	0.05%		0.375	0.375	0.375
	0.1 %		0.125	0.003	0.375
	0.5 %		0.125	0.000	0.000
1.5%	0		1.125	1.375	1.125
	0.05%		0.750	1.625	2.125
	0.1 %		0.750	0.750	1.375
	0.5 %		0.250	0.500	1.375
2.0%	0		0.375	0.500	2.125
	0.05%		0.625	0.625	1.875
	0.1 %		1.250	1.750	1.375
	0.5 %		0.250	0.375	1.625
2.5%	0		0.250	1.375	2.500
	0.05%		0.375	2.125	2.375
	0.1 %		1.000	1.375	1.625
	0.5 %		0.375	0.750	1.875

L.S.D; P = 0.05 = 1.4.

ANDERSEN, H.J. (1979). Migratory nematodes in Danish barley fields. I. The qualitative and quantitative composition of the fauna. *Tidsskr. Planteavl.* 83,1: 1-8.

ANDERSEN, H.J. (1979a). Migratory nematodes in Danish barley fields. II. Population dynamics in relation to continuous barley growing. *Tidsskr. Planteavl.* 83,1: 9-27.

ANDERSEN, H.J. (1979b). Migratory nematodes in Danish barley fields. III. *Pratylenchus neglectus*. A potential pathogen of barley. *Tidsskr. Planteavl.* 83,1: 28-31.

ASHER, M.J.C. (1972). Effect of *Ophiobolus graminis* infection on growth of wheat and barley. *Ann. Appl. Biol.* 70: 215-223.

ASHER, M.J.C. (1981). In: *'Biology and Control of Take-all'*. Eds. Asher, M.J.C and Shipton, P.J. 538 pp. Academic Press, London, New York.

BAKER, R. & MARTINSON, C.A. (1970). In: *'Rhizoctonia solani : Biology and Pathology'*. Ed. Parmeter, J.R. Jnr., University of California Press, Berkeley, Los Angeles and London.

BANYER, R.J. and FISHER, J.M. (1971). Seasonal variation in hatching of eggs of *Heterodera avenae*. *Nematologica* 17: 225-236.

BANYER, R.J. and FISHER, J.M. (1976). Effect of host and environment on some aspects of the biology of *Heterodera avenae*. *Nematologica* 22: 298-305.

BEHRINGER, P., FURST, L. and GRAMATTE, K. (1975). Full cysts of *Heterodera avenae* in maize established for the first time in the German Federal Republic. *Gesunde Pflanz.* 27: 234-236.

BENEDICT, W.E. and MOUNTAIN, W.B. (1956). Studies on the etiology of a root rot of winter wheat in South-western Ontario. *Can. J. Bot.* 34: 159-174.

BLACKITH, R.E. and REYMENT, R.A. (1971). *'Multivariate Morphometrics'*, 412 pp. Academic Press, London and New York.

BLAIR, I.D. (1943). Behaviour of the fungus *Rhizoctonia solani* Kühn in the soil. *Ann. Appl. Biol.* 30: 118-127.

BLANCHE, P.A. (1977). Pathogenic variation in *Gaeumannomyces graminis*. Ph.D. thesis, Univ. of Oxford.

BROOKS, D.H. (1965). Wild and cultivated grasses as carriers of the take-all fungus *(Ophiobolus graminis)*. *Ann. Appl. Biol.* 55: 307-316.

BROWN, J.A.M. (1974). Test tube reproduction of *Heterodera avenae* on resistant and susceptible wheats. *Nematologica* 20: 192-203.

BRUEHL, A.W. (1951). *Rhizoctonia solani* in relation to cereal crown and root rots. *Phytopathology* 41: 375.

BUTLER, F.C. (1961). Root and foot rot diseases of wheat. Dept. Agric., N.S.W. Sci. Bull. No. 77.

DAULTREY, S. (1976). *"Principal Components Analysis"* Concepts and Techniques in Modern Geography No. 8; 51pp. Geo Abstracts, Univ. of East Anglia, NORWICH.

CHAMBERS, S.C. (1966). Studies on *Ophiobolus graminis* and a root-attacking strain of *Rhizoctonia solani*. Ph.D. Thesis, Univ. of Adelaide.

CHAMBERS, S.C. (1970). Pathogenic variation in *Ophiobolus graminis*. *Aust. J. Biol. Sci.* 23: 1099-1103.

CHAMBERS, S.C. (1971). Some factors affecting the survival of the cereal root pathogen *Ophiobolus graminis* in wheat straw. *Aust. J. Exp. Agric. Anim. Husb.* 11: 90-93.

CHAMBERS, S.C. and FLENTJE, N.T. (1967). Studies on variation with *Ophiobolus graminis*. *Aust. J. Biol. Sci.* 20: 941-951.

C.M.I. DIST. MAP OF PLANT DISEASES No.334 (ed.3) (1972).

COOK, R. (1969). Studies on the interrelationship of the cereal cyst nematode *Heterodera avenae* and the take-all fungus *Ophiobolus graminis* on barley. Proceedings of the 5th British Insecticide and Fungicide Conference 3: 626-634.

COOK, R. (1975). Invasion and development of the cereal cyst-nematode *Heterodera avenae* in roots of barley infected by the take-all fungus *Gaeumannomyces graminis*. *Ann. Appl. Biol.* 81: 349-357.

COOK, R.J., PAPENDICK, R.I. and GRIFFIN, D.M. (1972). Growth of two root-rot fungi as affected by osmotic and matric water potentials. *Soil Sci. Soc. Am. Proc.* 36: 78-82.

COX, J. (1965). Continuous wheat growing and the decline of take-all. Rep. Rothamsted Exp. Stn. 1964. Pt.1, 133-134.

DAS, A.C. and WESTERN, J.H. (1959). The effect of inorganic manures, moisture and inoculum on the incidence of root disease caused by *Rhizoctonia solani* Kühn in cultivated soil. *Ann. Appl. Biol.* 47: 37-48.

DAVE, G.B. (1975). Interrelationships of *Rhizoctonia solani* with *Heterodera glycines*, *Pratylenchus scribneri* and *Tylenchorhynchus martini* on "Clark 63" soybeans. Ph.D. Thesis, Univ. of Illinois at Urbana-Champaign.

DAVEY, C.B. and PAPAVIZAS, G.C. (1960). Effect of dry mature plant materials and nitrogen on *Rhizoctonia solani* in soil. *Phytopathology* 50: 522-525.

DAVIDSON, J. (1930). Eelworms (*Heterodera schachtii* Schm.) affecting cereals in South Australia. *J. Dep. Agric. S.Aust.* 34: 378-85.

DAVIES, K.A. and FISHER, J.M. (1976). Duration of infectivity of second stage larvae of *Heterodera avenae*. *Nematologica* 22: 163-168.

DAVIS, R.J. (1925). Studies on *Ophiobolus graminis* Sacc. and the take-all disease of wheat. *J. Agric. Res.* 31: 801-825.

de BEER, J.F. (1965). Studies on the ecology of *Rhizoctonia solani* Kühn. Ph.D. Thesis, Univ. of Adelaide.

DERN, R. (1977). Freeliving root-nematodes as pests of agricultural plants in Hessen-Nassau. *Gesunde Pflanzen*, 29: 287-289.

DILLON WESTON, W.A.R. and GARRETT, S.D. (1943). *Rhizoctonia solani* associated with a root rot of cereals in Norfolk. *Ann. Appl. Biol.* 30: 79.

DIOMANDE, M. and BEUTE, M.K. (1981). Effects of *Meloidogyne hapla* and *Macropostonia ornata* on *Cylindrocarpon* Black rot of peanuts. *Phytopathology* 71: 491-495.

DONEY, D.L., WHITNEY, E.D. and STEELE, A.E. (1971). Effects of *Heterodera schachtii* infection on sugarbeet leaf growth. *Phytopathology* 61:40-41

DUGGAN, J.J. (1961). The effect of cereal root eelworm (*Heterodera major* O. Schmidt) on its hosts. *Ir. J. Agric. Res.* 1(1): 7-16.

DUNN, E. and HUGHES, W.H. (1967). Interaction of *Oospora pustulans*, *Rhizoctonia solani* and *Heterodera rostochiensis* on potato. *Eur. Potato J.* 10(4): 327-328.

FAULKNER, L.R. and SKOTLAND, C.B. (1965). Interactions of *Verticillium dahliae* and *Pratylenchus minyus* in Verticillium wilt of peppermint. *Phytopathology* 55: 583-586.

FAULKNER, L.R. and BOLANDER, W.J. (1969). Interaction of *Verticillium dahliae* and *Pratylenchus minyus* in Verticillium wilt of peppermint: Effect of soil temperature. *Phytopathology* 59: 868-870.

FAULKNER, L.R., BOLANDER, W.J. and SKOTLAND, C.B. (1970). Interaction of *Verticillium dahliae* and *Pratylenchus minyus* in Verticillium wilt of peppermint : Influence of the nematode as determined by the double root technique. *Phytopathology* 60: 100-103.

FLENTJE, N.T. and SAKSENA, H.K. (1957). Studies on *Pellicularia filamentosa* (Pat.) Rogers II. Occurence and distribution of pathogenic strains. *Trans. Br. Mycol. Soc.* 40: 95-108.

FLENTJE, N.T., DODMAN, R.L. and KERR, A. (1963). The mechanism of host penetration by *Thanatephorus cucumeris*. *Aust. J. Biol. Sci.* 16: 784-99.

FLENTJE, N.T., STRETTON HELENA, M. and McKENZIE, A.R. (1967). Mutation in *Thanatephorus cucumeris*. *Aust. J. Biol. Sci.* 20: 1173-80.

FUCHS, E. (1975). The effect of intensity factors on the population dynamics of migrating root nematodes on cereals, with particular reference to *Pratylenchus* Filipjev. I. The effect of sprinkler irrigation. II. The effect of nitrogen fertilizers. *Zentralbl. Bakteriol. Parasitenk. Infektionskr. Hyg.* 130: 7. 130(7):654-672; 673-688.

GARRETT, S.D. (1934). Factors affecting the severity of take-all. II. Soil temperature. *J. Dep. Agric. S.Aust.* 37: 799-805.

GARRETT, S.D. (1934a). Factors affecting severity of take-all. III. The climatic factor. *J. Dep. Agric. S.Aust.* 37: 976-983.

GARRETT, S.D. (1938). Soil conditions and take-all disease of wheat. III. Decomposition of the resting mycelium of *Ophiobolus graminis* in infected wheat stubble buried in the soil. *Ann. Appl. Biol.* 25: 742-766.

GARRETT, S.D. (1942). The take-all disease of cereals. Tech. Commun. Bur. Soil Sci., Harpenden 41.

GERLAGH, M. (1968). Introduction of *Ophiobolus graminis* into new polders and its decline. *Neth. J. Plant Pathol.* 74 (Suppl.2): 1-97.

GILL, J.S. and SWARUP, G. (1971). On the host range of the cereal cyst nematode *Heterodera avenae* Woll., 1924, the causal organism of Molya disease of wheat and barley in Rajasthan, India. *Indian J. Nematol.* 1: 63-67.

GILL, J.S. and SWARUP, G. (1977). Effect of interaction between *Heterodera avenae*, *Fusarium moniliforme* and *Helminthosporium gramineum* on barley plants and nematode reproduction. *Indian J. Nematol.* 7(1): 42-45.

GOODEY, J.B., FRANKLIN, M.T. and HOPPER, D.E. (1965). The nematode parasites of plants catalogued under their hosts. Commonwealth Agric. Bur. (3rd ed.) pp. 214.

GOUJON, M. (1967). Étude de Pa synergie parasitaire *Rhizoctonia solani* Kühn-*Fusarium solani* Snyder et Hansen chez la tomate. *Can. Orstom. Ser. Biol.* 4: 43-51.

HENRY, A.W. and McKENZIE, A.R. (1959). Note on the comparative activity of monosporous and mycelial isolates of *Ophiobolus graminis* Sacc. from the same source as wheat pathogens in natural and sterilized soil. *Can. J. Plant Sci.* 39: 405-407.

HESLING, J.J. (1957). *Heterodera major* O. Schmidt. 1930 on cereals - a population study. *Nematologica* 2: 285-99.

HICKINBOTHAM, A.R. (1930). Eelworm and "no-growth" patches. J. Dept. Agric. S.Aust. 34: 386-392.

HOPPER, B.C. (1971). The taxonomic status of *Pratylenchus neglectus* (Rensch) *J. Nematol.* 3: 313-314.

HUBER, D.M. (1981). In: *'Biology and Control of Take-all'*. (Eds. Asher, M.J.C. and Shipton, P.J.) Academic Press, London, New York.

HUBER, D.M. and WATSON, R.W. (1974). Nitrogen form and plant disease. *Annu. Rev. Phytopathol.* 12: 139-165.

HYNES, H.J. (1933). 'Purple Patch' of wheat and oats. A disease caused by the fungus *Rhizoctonia solani*. *Agric. Gaz. N.S.W.* 44: 879-883.

HYNES, H.J. (1937). Studies on "take-all" of wheat. 2. *Aust. Inst. Agric. Sci.* 3: 102-106.

INSERRA, R.N., VOVLAS, N. and BRANDINISIO, A. (1978). A survey of parasitic nematodes on cereals in Puglia Basilicata and Calibria, Italy. *Nematologia Mediterranea* 6(2): 163-174.

JAKOBSEN, J. (1972). The effect of population density, water and fertilizer supply on the sex ratio of *Heterodera avenae*. *Tidssk. Planteavl.* 76: 244-253.

KENDALL, M.G. and Stuart A. (1968). *"The Advanced Theory of Statistics"* Vol 3, 557pp. Charles Griffin & Co. Ltd. London.

JAMES, G.L. (1966). Effect of the causal fungus of brown root rot of tomatoes on the hatch of the potato root eelworm *Heterodera rostochiensis* Woll. *Nature (Lond.)* 212: 1466.

JAMES, G.L. (1968). The interrelationships of the causal fungus of brown root rot of tomatoes and ptato root eelworm *Heterodera rostochiensis* Woll. *Ann. Appl. Biol.* 61: 503-510.

JOHNSON, P.W. and FUSHTEY, S.G. (1967). The biology of the oat cyst nematode, *Heterodera avenae*, in Canada. II. Nematode development and related anatomical changes in roots of oats and corn. *Nematologica* 12: 630-636.

JORGENSON, E.C. (1970). Antagonistic interaction of *Heterodera schachtii* Schmidt and *Fusarium oxysporum* (Woll.) on sugarbeets. *J. Nematol.* 2: 393-398.

KERR, A. (1955). Studies on the parasitic and saprophytic activities of *Pellicularia filamentosa* (Pat.) Rogers and *Sclerotinia homeocarpa* Bennett. Ph.D. Thesis, Univ. of Adelaide.

KIMPINSKI, J. (1972). The ecology of *Pratylenchus minyus*. Ph.D. Thesis, Univ. of Adelaide.

KIMPINSKI, J., WALLACE, H.R. and CUNNINGHAM, R.B. (1976). Influence of some environmental factors on populations of *Pratylenchus minyus* in wheat. *J. Nematol.* 8: 310-314.

KETUDAT, U. (1969). The effects of some soil-borne fungi on the sex ratio of *Heterodera rostochiensis* on tomato. *Nematologica* 15: 229-233.

LAL, A. (1939). Interaction of soil micro-organisms with *Ophiobolus graminis* Sacc; the fungus causing take-all disease of wheat. *Ann. Appl. Biol.* 26: 247-61.

LEDINGHAM, R.J. (1942). Observations on antagonism in inoculation tests of wheat with *Helminthosporium sativum* P, K, and B; and *Fusarium culmorum* (W. Cr. Sm.) Sacc. *Sci. Agric.* 22: 688-697.

LINDHARDT, K. (1961). Some observation on sex determination influenced by degree of infection by the cereal root eelworm, *Heterodera major* O. Schmidt, 1939. *Tidssk. Planteavl.* 46: 889-896.

LOOF, P.A.A. (1960). Taxonomic studies on the genus *Pratylenchus (Nematoda)*. *Tijschr. Plantenziekten* 66: 29-90.

LUDBROOK, W.V., BROCKWELL, J. and RICEMAN, D.S. (1953). Bare-patch disease and associated problems in subterranean clover pastures in South Australia. *Aust. J. Agric. Res.* 4: 403-414.

MacNISH, G.C. and DODMAN, R.L. (1973). Survival of *Gaeumannomyces graminis* var. *tritici* in the field. *Aust. J. Biol. Sci.* 26: 1309-1317.

MADAMBA, C.P., SASSER, J.N. and NELSON, L.A. (1965). Some characteristics of the effects of *Meloidogyne* spp. on unstable host crops. North Carolina Ag. Exp. Stn. Tech. Bull. 169.

MEAGHER, J.W. (1968). The distribution of cereal cyst nematode (*Heterodera avenae*) in Victoria and its relation to soil type. *Aust. J. Exp. Agric. Anim. Husb.* 8: 637-640.

MEAGHER, J.W. (1970). Seasonal fluctuations in number of larvae of the cereal cyst nematode (*Heterodera avenae*) and of *Pratylenchus minyus* and *Tylenchorhynchus brevidens* in soil. *Nematologica* 16: 333-347.

MEAGHER, J.W. (1981). Yield losses caused by *Heterodera avenae* in cereal crops grown in a Mediterranean climate. EPPO Bull. 12(4): 325-331 (1982).

MEAGHER, J.W. and CHAMBERS, S.C. (1971). Pathogenic effects of *Heterodera avenae* and *Rhizoctonia solani* and their interaction on wheat. *Aust. J. Agric. Res.* 22: 189-194.

MEAGHER, J.W., BROWN, R.H. and ROVIRA, A.D. (1978). The effects of cereal cyst nematode (*Heterodera avenae*) and *Rhizoctonia solani* on the growth and yield of wheat. *Aust. J. Agric. Res.* 29: 1127-1137.

MOUNTAIN, W.B. (1954). Studies of nematodes in relation to brown root rot of tobacco in Ontario. *Can. J. Bot.* 32: 737-759.

McALPINE, D. (1904). Take-all and whiteheads in wheat (*Ophiobolus graminis* Sacc.). *J. Dep. Agric. Vic.* 2: 410-426.

McKNIGHT, T. (1960). Survey of present incidence, importance and cause of cereal root rots in Australia. Proc. Conf. on root rots of wheat, Wagga Wagga, N.S.W., Paper 1, 12 pp.

McLEOD, R.W. and KHAIR, G.T. (1977). Observations on the morphology of *Heterodera avenae* in Australia. *Nematologica* 23(3): 382-389.

NILSSON, H.E. (1969). Studies of root and foot rot diseases of cereals and grasses. I. On resistance to *Ophiobolus graminis* Sacc. *Lantbrukshogsk Ann.* 35: 275-807.

OLTHOF, T.H.A. and HOPPER, B.E. (1973). Distribution of *Pratylenchus*- spp and other stylet bearing nematode genera in soils in the flue cured tobacco area of Southern Ontario. *Can. Plant. Dis. Surv.* 53(1): 31-34.

PADWICK, G.W. (1936). Biological strains of *Ophiobolus graminis* Sacc. *Ann. Appl. Biol.* 23: 45-56.

PAPAVIZAS, G.C. and DAVEY, C.B. (1960). *Rhizoctonia* disease of bean as affected by decomposing green plant materials and associated microfloras. *Phytopathology* 50: 516-522.

POLYCHRONOPOULOS, A. (1967). Effect on young sugarbeet (*Beta vulgaris* L.) plants of *Heterodera schachtii*, Schmidt 1871, alone and in combination with *Rhizoctonia*. Ph.D. Thesis, Univ. of California, Davis.

POLYCHRONOPOULOS, A.G., HOUSTON, B.R. and LOWNSBERRY, B.F. (1969). Penetration and development of *Rhizoctonia solani* in sugarbeet seedlings infected with *H. schactii*. *Phytopathology* 59: 482-485.

POTTER, J.W. and TOWNSHEND, J.L. (1973). Distribution of plant-parasitic nematodes in field crop soils of southwestern and central Ontario. *Can. Plant. Dis. Surv.* 53,(1): 39-48.

ROBINSON, R.K. and LUCAS, R.L. (1967). Observation on the infection of *Zea mays* by *Ophiobolus graminis*. *Plant Pathol.* 16: 75-77.

ROSSER, W.R. and CHADBURN, B.L. (1968). Cereal diseases and their effects on intensive wheat cropping in the East Midland Region, 1963-65. *Plant Pathol.* 17: 51-60.

ROTHAMSTED EXP. STN. REPS. 1963-1966.

RUSSEL, R.C. (1939). Pathogenecity tests with cultures of *Ophiobolus graminis* Sacc. *Scient. Agric.* 19: 662-669.

SABET, K.A. and KHAN, I.D. (1969). Interaction of Cotton root-infecting fungi. *Cott. Grow. Rev.* 46(3): 210-222.

SAMUEL, G. (1928). Two "stunting" diseases of wheat and oats. J. Dep. Agric., S.Aust. 32: 40-43.

SAMUEL, G. and GARRETT, S.D. (1932). *Rhizoctonia solani* on cereals in South Australia. *Phytopathology* 22: 827-836.

SANFORD, G.B. (1952). Persistance of *Rhizoctonia solani* in soil. *Can. J. Bot.* 30: 362-664.

SCARDACI, S.C. and WEBSTER, R.K. (1981). Antagonism between the cereal root rot pathogens *Fusarium graminearum* and *Bipolaris sorokiniana*. *Plant Dis.* 65(12):965-967.

SEINHORST, J.W. (1965). The relation between nematode density and damage to plants. *Nematologica* 11: 137-154.

SHIPTON, P.J. (1972). Influence of stubble treatment and autumn application of nitrogen to stubble on subsequent incidence of take-all and eye-spot. *Pl. Pathol.* 21: 147-155.

SKOU, J.P. (1968). Studies on take-all fungus, *Gaeumannomyces graminis*. II. The virulence as measured in infection experiments with cereal seedlings. *K. Vet. Landbohojsk. Arsskr.* 1968: 117-133.

SLAGG, C.M. and FELLOWS, H. (1947). Effects of certain soil fungi and their by-products on *Ophiobolus graminis*. *J. agric. Res.* 57: 279-293.

SLOPE, D.B. (1967). Disease problems of intensive cereal growing. *Ann. Appl. Biol.* 59: 317-319.

SLOPE, D.B. and COX, J. (1964). Continuous wheat growing and the decline of take-all. Rep. Rothamstead Exp. Stn., 1963. Pt.1, 108.

SLOPE, D.B. and ETHERIDGE, J. (1971). Grain yield and incidence of take-all (*Ophiobolus graminis* Sacc.) in wheat grown in different crop sequences. *Ann. Appl. Biol.* 67: 13-22.

SMILEY, R.W. and COOK, R.J. (1973). Relationship between take-all of wheat and rhizosphere pH in soils fertilized with ammonium *vs.* nitrate nitrogen. *Phytopathology* 63: 882-890.

SOUTHEY, J.F. (Ed.) (1970). Laboratory methods for work with Plant and Soil nematodes. Tech. Bull. Minist. Agric. Fish. Fd. 2 (5th edn.) 148 pp.

SPRAGUE, R. (1950). *"Diseases of cereals and grasses in North America".* New York : The Ronald Press Co.

STONE, L.E.W. (1968). Cereal cyst nematode in spring barley damage assessment experiments 1963-1964. *Plant Pathol.* 17: 145-150.

STYNES, B.A. (1975). A synoptic Study of Wheat. Ph.D. Thesis, Univ. of Ade.

TARR, S.A.J. (1962). Diseases of sorghum, sudan grass and broom corn. Commonwealth Mycological Institute, Kew, 380 pp.

TOWNSHEND, J.L. (1973). Survival of *Pratylenchus penetrans* and *P. minyus* in two Ontario soils. *Nematologica* 19: 35-42.

TOWNSHEND, J.L., EGGENS, J.L. and McCOLLUM, N.K. (1973). Turf grass hosts of three species of nematodes associated with forage crops. *Can. Plant Dis. Surv.* 53,3: 137-141.

TOWNSHEND, J.L. and POTTER, J.W. (1976). Evaluation of forage legumes grasses and cereals as hosts of forage nematodes. *Nematologica* 22(2): 196-201.

VARO ALCALA, J., TOBAR JIMENEZ, A. and MUNOZ MEDINA, J.M. (1970). Lesiones causadas y reacciones provocadas por algunos nematodes en las raíces de ciertas plantas (Abstract). H.A. (B) 40: No. 701.

WALKER, J.C. (1975). Take-all diseases of *Graminaea* - A review of recent work. *Rev. Plant Pathol.* 54: 113-144.

WALLACE, H.R. (1983). Interactions between nematodes and other factors on plants. *J. Nematol.* 15,2: 221-227.

WETZEL, Th. (1962). Zum auftreten und zur Schadwirfung des freilebenden Nematoden Pratylenchus neglectus (Rensch) an futtergrasern. Schriftenr. Karl-Marx. Univ. Leipzig zu Fragen Sozialist. Landw. H.8: 79-84.

WHITE, N.H. (1947). The etiology of take-all disease of wheat. 4. The effect of agronomic practices on the incidence and severity of take-all. *J. Coun. scient. ind. Res. Aust.* 20: 82-86.

WHITE, N.H. and McINTYRE, G.A. (1943). The pathogenicity of single spare isolates of *Ophiobolus graminis* under field conditions. *J. Coun. scient. ind. Res. Aust.* 16: 93-94.

WILLIAMS, R.F. (1960). The physiology of growth in the wheat plant. I. Seedling growth and the pattern of growth at the shoot apex. *Aust. J. Biol. Sci.* 13: 401-428.

WILLIAMS, T.D. and HORNBY, D. (1970). The interaction between cereal cyst nematode and the take-all fungus. Rothamsted Exp. Stn. Rep. Part I: 196-198.

WILLIAMS, T.D. and SALT, G.A. (1970). The effects of soil sterilants on the cereal cyst-nematode (*Heterodera avenae* Woll.), take-all (*Ophiobolus graminis* Sacc.) and the yields of spring wheat and barley. *Ann. Appl. Biol.* 66: 329-338.

WILLIS, C.B., TOWNSHEND, J.L., ANDERSON, R.V., KIMPINSKI, J., MULVEY, R.H., POTTER, J.W., SANTERRE, J. and WU, L.Y. (1976). Species of Plant-Parasitic nematodes associated with forage crops in Eastern Canada. *Plant Dis. Rep.* 60: 207-210.

ZOGG, H. (1969). Crop rotation and biological soil disinfection. *Qualitas Plant Mater. Veg.* 18: 256-273.