

CHARACTERISATION OF
RHIZOCTONIA SOLANI
(THANATEPHORUS CUCUMERIS) ISOLATES
IN MANITOBA
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Colin Ntilimuna Mwiindilila

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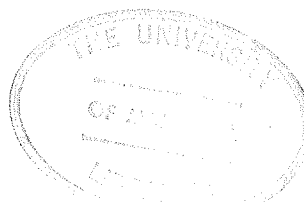


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GENERAL ABSTRACT

Mwiindilila, C.N. M.Sc., the University of Manitoba, February, 1984.
Characterisation of Rhizoctonia solani Isolates from Manitoba Fields.
Major Professor: C.C. Bernier

Seventy four isolates of R. solani were recovered from diseased seedlings of rapeseed, faba bean, peas, lentils, sugarbeet, potato and other special crops in Manitoba, in 1981 and 1982. The isolates were grouped on the basis of the color and texture of mycelium, and the amount and color of sclerotia produced. Mycelial zonation, the size and distribution of sclerotia were not consistent features of any single group and were thus not used in the assignment of isolates into cultural morphology groups.

Isolates were also grouped according to their anastomosis groups (AG) determined by pairing with known-AG tester cultures. All AG-4 cultures were correctly assigned to their anastomosis group on the basis of their morphological characteristics. Other isolates, especially slow growing cultures and those with inconsistent pigmentation, showed overlapping cultural features and could not be correctly assigned to their AGs on the basis of cultural morphology alone. Isolates were distributed among anastomosis groups as follows: six in 'AG-2' composed of isolates that anastomosed with both AG-2T1 and AG-2T2 testers; seven in AG-2T1, one in AG-2T2; four in AG-3; forty

six in AG-4; and ten in AG-5. Two of the isolates in 'AG-2' also anastomosed with the AG-3 tester, with each other and with other 'AG-2' isolates. These isolates may belong to AG-B1, a group that has not been reported in North America before. No AG-1 isolates were recovered in Manitoba during 1981 and 1982.

Isolates in each AG varied in pathogenicity from non-pathogenic to highly pathogenic on a given host. Some isolates were pathogenic on faba bean, peas, lentil and rapeseed, while some isolates were pathogenic on three, two or only one of the greenhouse test hosts. With a few exceptions, AG-4 isolates were largely pathogenic on legume hosts, while 'AG-2' and AG-2T1 were largely pathogens of rapeseed. AG-5 isolates were mostly non-pathogenic with the exception of a few isolates that elicited superficial lesions on some hosts. However, there were some AG-4 isolates that were pathogenic on legumes as well as rapeseed, and some AG-2T1 isolates that were pathogenic on rapeseed as well as legumes. This indicates that a certain minimum number of isolates is required to characterise an AG's cultural features, pathogenicity, or any other physiological feature under study.

GENERAL INTRODUCTION

R. solani Kuehn is a composite species of isolates with different morphological, physiological, and pathological attributes. An important concept in studies of R. solani is that of anastomosis groups (AGs), which are determined by pairing isolates with testers of known AG (Parmeter et al., 1969). The relations between AGs and morphological and physiological norms, serological relations, DNA base composition, or pathological activity have been established (Sherwood, 1969; Ogoshi and Ui, 1979; Adams and Butler, 1979; Kuninaga and Yokosawa, 1979; Anderson, 1982).

Diseases incited by Rhizoctonia solani have been reported in individual Manitoba fields and in eastern and western Canada on potato, rapeseed, faba bean, lentils and peas. In Manitoba, disease on potato is endemic every year and R. solani has been cited as being capable of causing serious losses elsewhere in Canada every year. (Frank and Leach, 1980; Zimmer and Russell, 1981; Lamari, 1982; Acharya et al., 1983). A severe root and foot rot in a rapeseed field at the University of Manitoba arboretum has also been observed since 1980. The nature and types of R. solani causing diseases in these crops have not been elaborated yet.

This study was initiated to determine the nature of populations of R. solani associated with diseased seedlings in Manitoba fields. Seventy four isolates were obtained from diseased seedlings of rapeseed, faba bean, peas, lentils, sugarbeet, potato and other special crops. The isolates were grouped on the basis of mycelial color and texture, sclerotial color and amount produced, and also

according to their anastomosis groups. The relations established between the source hosts, cultural groups AGs, or pathogenicity are reported.

Chapter I
REVIEW OF LITERATURE

RHIZOCTONIA SOLANI

1.1 HISTORY

The form genus *Rhizoctonia* was erected by De Candolle in 1815 to accommodate the non-sporulating root pathogen *Rhizoctonia crococom* DC. ex Fr., (Tu and Kimbrough, 1975). The genus has since then become a heterogeneous assemblage of mycelia of basidiomycetes, ascomycetes and Imperfect Fungi (Parmeter et al., 1967). Only a few criteria are used to delimit this genus. In general, these fungi are characterised by the possession of relatively wide, colored basal hyphae that branch at an acute angle when young, but later almost at right angles from the main axis. A septum is formed in each branch close to the point of branching, and the branches are slightly constricted at the junction, or at the septum. Such mycelia may also produce sclerotia (Tu and Kimbrough, 1975).

Rhizoctonia solani Kuehn, the mycelial state of *Thanatephorus cucumeris* (Frank) Donk, was first described by Julius Kuehn in 1858 (Menzies, 1970). Early work on the taxonomy and nomenclature of *R. solani* was controversial due to the lack of comparative studies and the wide variation in morphology, pathology and physiology of the pathogen. The first attempt to critically review the taxonomy and

nomenclature was by Duggar in 1915 (Menzies, 1970). Several workers since then, attempted to group isolates of R. solani according to various cultural, physiological or pathological criteria (Exner, 1953; Houston, 1945; Luttrell, 1962). Such groups though considered artificial, contributed towards understanding the species (Adams and Butler, 1979).

In a taxonomic review by Parmeter and Whitney (1970), the criteria for distinguishing R. solani from other fungi closely resembling it, were set to include the presence of a prominent, doliform, septal pore apparatus, typical of basidiomycetes, and a multinucleate condition in vegetative hyphal cells, which aids in distinguishing R. solani from binucleate, Rhizoctonia - like fungi with similar cultural appearance, morphology and habitat (Ruppell, 1972). Usually but not invariably, field isolates possess monilioid cells, undifferentiated sclerotia, hyphae greater than 5 μ in diameter, a rapid growth rate, and pathogenic characteristics (Parmeter and Whitney, 1970).

1.2 ANASTOMOSIS GROUPS

Not all isolates of R. solani Kuehn form, or have been observed to form, the perfect state. With a complete description and a precise species concept yet unavailable, the relationship of these isolates is determined by anastomosis tests.

In 1921, Matsumoto observed that hyphal fusion might take place only between hyphae from strains that were rather closely related or which had recently originated from the same ancestral type. The first natural subdivision of R. solani was made by Schultz in 1936 who

divided the species into five groups on the basis of hyphal anastomosis. Richter and Schneider in 1953, differentiated 176 strains of R. solani from Asia, Europe, North and South America, and Australia, into six anastomosis groups. They indicated that, but for a few exceptions, the groups corresponded to six morphological groups, (Parmeter et al. 1969). From studies of more than 300 isolates by Parmeter et al. (1969), and of 188 isolates by Sherwood (1969), four subdivisions of anastomosis groups (AG), were recognised. In studies involving 234 isolates, Ogoshi (1972) recognised five AGs, with two subdivisions in each of AG-1 and AG-2, based on cultural, pathological and ecological characteristics. AG-1 is subdivided on the basis of distinct morphology and pathology (Anderson, 1983). AG-2 is subdivided on the basis of the infrequency of hyphal anastomosis and of cultural characteristics. Isolates anastomosing within the subgroups designated AG-2 Type 1 (AG-2T1) have been established to be thiamine autotrophic, whereas AG-2T2 isolates are thiamine auxotrophic (Ogoshi, 1979). This physiological evidence of the subdivision of AG-2 confirmed earlier observations by Sherwood (1969), that there were both thiamine-autotrophic and thiamine-auxotrophic isolates in AG-2. Serological studies of anastomosis groups of R. solani by Adams and Butler (1979), demonstrated that the antisera of AG-2 types 1 and 2 were not identical. However, antigens from the subgroups of AG-2 formed identical precipitin bands with six test antisera. This was taken as an indication of a basic homogeneity within AG-2.

Anastomosis group AG-5 was first reported by Ogoshi (1972) in Japan using isolates from soil. Bandy et al. (1983) reported the first

occurrence of AG-5 in North America from potato fields in Maine. Two more groups have been reported in Japan by Kuninaga et al. (1979), AG-6 and AG-B1. AG-B1 is of particular interest because anastomosis has been reported between field isolates of this group and several isolates of the other groups.

The infrequency of hyphal fusion between two isolates has often caused difficulties in tests for anastomosis, especially within AG-2. Few isolates readily anastomose with AG-2 reference cultures. This prompted the suggestion by Herr and Roberts (1980), that more than one tester isolate should be used, especially in AG-2, to facilitate the assignment of an isolate to its proper group. In addition, there are isolates that will not anastomose within any of the established groups (Parmeter et al., 1969; Sherwood, 1969; Ogoshi, 1972). These problems and the now proven suspicion that isolates that "bridge" the groups may be found, cast some doubt on the validity of the anastomosis groups (Adams and Butler, 1980).

There, however, exists good evidence that the groups represent biological species. They have been independently discovered four times since 1936, by researchers from three continents. Sherwood (1969), reported that each AG was characterised by distinctive morphological, pathological and physiological norms, with a few overlapping isolates. Kuninaga and Yokosawa (1980), compared DNA base composition among anastomosis groups of R. solani. They observed that the Guanine plus Cytosine (GC) content of DNA of isolates within the same AG, was almost identical and concluded that each AG should be considered as a genetically distinct unit. These results are in

agreement with those of Mutsuyama (1978), who reported that non-specific zymograms of isolates of the same AG gave identical patterns, and those of Adams and Butler (1980) who found a correspondence between serological groups and AGs. In genetic studies of R. solani, Anderson (1983), reported that death of anastomosed cells on either side of fusion, was a somatic, or vegetative incompatibility reaction. Somatic incompatibility restricts outbreeding and can make a race the evolutionary unit instead of the species. According to current concepts then, each AG is regarded as an independent and genetically isolated, non-interbreeding population (Anderson, 1982).

1.3 ECOLOGY AND EPIDEMIOLOGY

Survival in Soil

R. solani Kuehn, described as a soil inhabiting fungus (Garrett, 1956), is able to carry on an active saprophytic existence in soil as vegetative mycelia in plant debris colonized during parasitism, or by colonizing dead plant tissue (Boosalis and Scharen, 1959). R. solani can remain, active or dormant, in this state for varying periods of time (Papavizas and Davey, 1962), or as viable sclerotia on the surface of plant debris particles (Boosalis and Scharen, 1959).

R. solani generally exhibits physiological characteristics that are associated with a high competitive saprophytic ability (Garrett, 1956). The position occupied by a fungus in the succession of substrate colonization, possession and utilization is influenced by its intrinsic growth rate (Garrett, 1956, Wastie, 1961), tolerance of competition from other soil microorganisms and their activities, the

nature of the substrate and other soil micro-ecological factors (Wastie, 1961). Davey and Papavizas (1963), determined that a substrate's nitrogen to carbon ratio influenced its suitability as an energy source, but that the relative availability of carbon and nitrogen, other organic and inorganic nutrients, as well as the nature of the isolate in question were also important. Papavizas and Davey (1962), showed that R. solani survived in soil as morphologically, saprophytically and parasitically distinct clones. Single basidiospore isolates that showed high saprophytic survival in soil also showed higher tolerance to carbon dioxide and anti-microbial agents, and had superior cellulose decomposing ability and thus slower rates of decolonization of precolonized substrates. They also established that mycelia of R. solani within precolonized substrates was less sensitive to adverse micro-environmental conditions created by decomposing organic amendments, than mycelia of the active saprophytic phase (Papavizas et al., 1962; Papavizas and Davey, 1965).

The use of saprophytic activity as an estimate of parasitic activity rests on the premise that the saprophytism of primitive parasites such as R. solani is closely related to parasitism (Garrett, 1956). Sanford (1941), however noted that conditions favouring saprophytism may not be optimal for parasitism. The observation that R. solani was more virulent in natural soils than in sterilized soil was attributed to possible presence of autotoxic staling products resulting from abundant mycelial growth in sterilized soil. The accumulation of carbon dioxide as a result of increased mycelial growth in sterilized soil could also suppress parasitic activity more

than saprophytic activity. Changes in nutrient availability, for instance, an increase in available nitrogen in autoclaved soils, may reduce disease, depending on the relative availability of other nutrients. In unsterilized soils, more organisms including weak pathogens and saprophytes, may have a synergistic effect on disease development (Papavizas and Davey, 1962).

Sanford (1952), considered parasitism as being more important to the survival of R. solani in soil. Papavizas and Davey (1961), however disputed this on the basis that a greater inoculum potential is required for infection than for saprophytic colonization. The rapid decline of parasitic activity relative to saprophytic activity, explained as a result of the depletion of certain nutrients in the ephemeral food bases in the soil, appears to support the view that saprophytic activity is more important than parasitic activity for survival.

Evidence of the correspondence of saprophytism and parasitism, has been presented by Papavizas and Ayres (1965) who found that single basidiospore isolates of T. cucumeris that had strong saprophytic growth in natural soil were also among the most virulent. Martinson (1963), showed a significant linear relationship between saprophytic activity measured by soil microbiological sampling tubes (SMST), and the frequency of emergence of radish seedlings. Sneh et al. (1968), obtained high correlations between the saprophytic activity of R. solani estimated by plant segment colonization (Papavizas and Davey, 1962), by SMST (Martinson, 1963) and the plant debris isolation method (Boosalis and Scharen, 1959), and the level of soil infestation

estimated by the disease severity index (DSI), of the seedling infection method using bean plants. Papavizas and Davey (1959), obtained similar results but they used the percentage of infected plants in relation to inoculum concentration. DSI seems to be a better parameter since it incorporates virulence characteristics. The estimation of soil infestation by the percentage of diseased plants is limited by multiple infections that may occur especially at high inoculum concentrations and thereby underestimating the intensity of soil infestation. The plant segment colonization method was found to be statistically the most accurate and sensitive method, for estimating R. solani saprophytically existing in soil (Papavizas, 1970). Ko and Hora (1971), noted that R. solani propagule concentrations were low in soil and pointed out that estimates of populations using baits were inadequate since a single propagule could colonize more than one bait.

To avoid problems associated with soil mixing, incorporation of baits and the screening required to recover baits, Herr (1973), developed a disc plate method for selective isolation of R. solani existing saprophytically in soil. He reported that the technique was more sensitive in assessing inoculum potential, especially at low inoculum concentration, than seedling disease assays. Weinhold (1977), developed a wet screening procedure for the quantitative determination of R. solani propagules in soil. He obtained a good agreement between this method and cotton plant segment colonization. He also reported that R. solani populations in natural soil were relatively low.

The low inoculum densities observed have an epidemiological significance. These soils induce disease when cropped to plants. It has been established that the pathogen can assume a parasitic existence from its saprophytic condition (Papavizas and Davey, 1961). What is yet unclear is whether or not the low inoculum density in absence of a host is concomitant with the elimination of propagules from the soil, due to various factors, or just a temporal transformation to a resistant, or inert survival state (Papavizas and Davey, 1962).

Host range

Based on the current definition of R. solani Kuehn, the pathogen has perhaps, the broadest host range of any plant pathogenic species. Strains have been reported causing root rot of wheat under semiarid conditions, and others causing disease of submerged plants, and yet others causing web blights of aerial parts in rice, soybean and sorghum in the humid tropics and the subtropics (Baker, 1970; Scott et al, 1979). R. solani has also been reported as a seed borne pathogen in 53 plant species (Crosier et al., 1970).

The concept of a host range of R. solani is somewhat confused. The adaptability of the pathogen is more apparent than real and is largely a result of the extremely wide variation of the numerous strains that constitute this species (Baker, 1970). In addition, an isolate's host range may be altered by time, the nature of the host plant used, varietal resistance or susceptibility, and by other soil microecological factors (Papavizas and Ayres, 1965). It therefore becomes necessary to establish a working definition of the host range in R. solani studies.

In many studies, numerous R. solani isolates have been found to exhibit little or no host specificity (Boosalis, 1950; Tolbat and Moubasher, 1955). Other workers have established some general trends relating the parasitic activities of isolates and either, their cultural appearance, anastomosis behaviour, organ on the plant from which they were isolated, or the source host. Flentje and Saskena (1957), found many host specific isolates and some that were not. Sanford (1952) reported that isolates from sclerotia on potatoes have little effect on R. solani potential in soil. Others reported that isolates from potatoes, especially those from sclerotia, varied from being non-pathogenic to strongly pathogenic and that the soil type and other environmental factors were important in the expression of disease on potato (Person, 1955; James and Mckenzie, 1972; Bolkan and Wenham, 1973; Frank and Leach, 1980). Herr and Roberts (1980), characterised R. solani populations from sugarbeet fields with different soil textures. Isolates in anastomosis series AG-2, predominated in fine textured soils and were more virulent on 6 - 8 week old sugarbeet plants. Isolates in AG-4 predominated in coarser textured soils and were significantly more virulent on sugarbeet seedlings. Ruppel (1972), used a number of different media to characterise R. solani populations from sugarbeet. The isolates were distinguished by cultural characteristics. Foliar isolates had higher growth rates than crown isolates. Root isolates had the lowest rates of growth. Crown and foliar isolates were predominantly AG-4 types, while root isolates were AG-2 types. Though the relative behaviour of isolates was similar in susceptible and resistant lines within isolate

groupings, it was established that greater root rot severity was inflicted on susceptible cultivars by root rot isolates than by crown or foliar isolates.

In an evaluation of the *Rhizoctonia* complex in relation to flax seedling blight, Anderson (1977), reported that anastomosis series AG-2, AG-3 and AG-4, all caused seed decay. AG-2 and AG-3 were limited to root rots, whereas AG-4 also caused hypocotyl infection. He also reported that AG-1 types had a wide host range causing aerial blights in warm humid climates. Aerial blights of soybean caused by AG-1 type isolates have been reported in Louisiana on soybean and also rice, sorghum and wheat in greenhouse tests (O'Neill et al, 1977). The AG-1 types were also shown to infect the weeds Echinochloa crus-galli (barnyard grass), E. colonum (jungle rice) and Cynodon dactylon (bermuda grass) in diseased soybean fields. Lee and Rush (1983), also reported AG-1 isolates of R. solani as the causal agents of a severe rice sheath blight disease in the southern rice-producing areas of the United States. Yield losses of up to 50% were reported. In the United States, AG-2 isolates are mainly pathogens of cruciferous hosts, whereas AG-3 isolates are predominantly potato pathogens. The most important group is AG-4 whose isolates have been reported to cause damping-off and crown rot of legumes, sugar beet, alfalfa and cotton. A thorough treatment of the pathological patterns of R. solani on the basis of anastomosis grouping was reviewed recently by Anderson (1982).

Isolation and Identification

Most methods used to study saprophytic survival and inoculum density in soil, have been used to recover R. solani from soil and colonized plant debris particles.

Isolation of R. solani from infected plant tissue is relatively simple due to its ability to grow on routine laboratory media. The best growth is obtained on potato dextrose agar (PDA), and Richards solution (Parmeter, 1970). To check bacterial contamination, the medium may be acidified or antibiotics may be added. Ko and Hora (1971), developed a media selective for R. solani isolation (RSM), fortified with antibiotics and fungistatics, and also containing sources of minerals and basic nutrients.

The identification of R. solani on isolation media is primarily based on its mycelial growth habit as described by Parmeter (1970). The dark colour of RSM does not facilitate easy identification. Hyphal tip initiated cultures are thus established on either water agar (WA) or PDA. The identity of cultures in the absence of the T. cucumeris perfect state, is confirmed by nuclear staining of young, vegetative hyphae to establish the presence of multinucleate cells. Various staining techniques have been described by Parmeter (1970), Tu and Kimbrough (1973), Burpee et al. (1978), Herr (1979), and Yamamoto and Uchida (1981). Isolates are then assigned to their respective anastomosis groups on the basis of their reaction with known-AG tester isolates.

Chapter II

THE HOSTS

2.1 FABA BEAN

Faba bean (Vicia faba L.), is an annual legume grown in Manitoba for export and for on-farm use as a livestock protein feed. In the semiarid areas of North Africa and the Middle east, and in China, faba beans have been used for hundreds of years as a low cost dietary protein source (Hanounik, 1978). Faba bean plays an important role in maintaining soil productivity in rotations by accumulating plant nutrients such as nitrogen, and improving the soil physical condition (Djerbi and Bouslama, 1978).

Root rots and wilts limit yields of faba bean in many parts of the world, the severity of losses depending on the stage at which the plants are attacked. Young plants are generally more susceptible than older ones (Hanounik, 1978; Salt, 1982). The two disease complexes are not easily distinguished because the most obvious symptoms are similar including, general wilting and plant collapse, pale dark leaves turning black from the margin inwards, reduced growth and yields, or premature death. The characteristic root rot is a black tissue disintegration of the cortex of the tap root and the laterals which results in damping-off of seedlings. In older plants, wilting may occur when roots have been extensively damaged. Usually, plants reduce their water demand by shedding of leaves and flowers, and allowing pods to dry prematurely (Salt, 1982).

Root rots can however be distinguished from wilts by the infection of the vascular tissues and a brown discoloration of the vascular elements extending up the stem. In root rots the discoloration of vascular elements would either be absent or confined to the roots and basal parts of the stem (Salt, 1982).

Abdallah (1969) reported R. solani as being the most important faba bean root rot pathogen in Egypt, followed by Fusarium solani f. sp. fabae, F. oxysporum and Sclerotium rolfsii Sacc. The most damaging soil-borne disease of faba bean, especially in the dry areas, is a complex involving several fungal species. Abol-wafa (1964) reported that Aspergillus, Penicillium and Alternaria spp., are the most prevalent species associated with leguminous seed. In addition, Fusarium roseum and Fusarium tricinctum were found to be pathogenic on seedlings of faba bean. A severe damping-off of faba bean in a naturally infested soil in Iran was associated with a complex involving R. solani, Fusarium solani (Mart.) Sacc. and Fusarium oxysporum (Eisa and Barakat, 1978). In Egypt, R. solani was identified as the main predisposing factor to Fusarium sp. infection causing wilts and damping-off in faba bean (Mansour et al., 1976). Other fungi that may be involved and have a wide host range include Fusarium avenaceum, Fusarium graminearum, and species of Pythium, Phoma and Cylindrocarpon. R. solani has been reported in other root rot and wilt complexes with Phialophora sp. and Pythium spp., by Hanounik (1978), and with F. oxysporum, Sclerotium rolfsii and Rhizoctonia bataticola (Mengistu, 1978). Lamari (1982), reported R. solani and a Fusarium sp. as a part of a root rot complex primarily caused by Aphanomyces euteiches in field plots at the University of Manitoba.

2.2 PEAS

Peas (*Pisum sativum* L.), are grown primarily as a dry pulse but can also be harvested immature as a green vegetable, processed and either frozen or canned for off-season consumption. In the dry regions of North Africa and West Asia, they are an important source of dietary protein. In world legume production, peas rank second overall (Hamawi, 1978).

In Ontario root rot was reported to be the single most important factor limiting the quantity of peas available for canning and freezing. The fungi most implicated in the complex were *Fusarium* spp., *Pythium* spp., and *R. solani*. *R. solani* was reported to cause a stem canker of peas (Benedict, 1969). Severe root rot caused by *R. solani* in emerging canning peas has also been reported in Wisconsin associated with *Fusarium* spp. in fields with a history of severe root rot (Flentje and Hagedorn, 1964; Burke and Hagedorn, 1968). In Saskatchewan, pea root rot caused by *R. solani* and *Fusarium* spp. was listed as the second most important disease of peas after *Sclerotinia sclerotiorum* (Morrall et al., 1972). Root rot of processing peas has also been reported in Alberta associated with *R. solani*, *Fusarium* spp. and *Pythium* spp. (Flores and Howard, 1982). Other complexes reported with *R. solani* include, *F. solani* f.sp. pisi and *Pythium* spp. in Ontario (Reyes, 1980), with *F. solani* f. sp. pisi, *Pythium* spp. and *A. euteiches* (Hampton and Ford, 1965), and with *Pythium* spp., *F. oxysporum* and *F. solani* in Iran (Kaiser et al., 1971). Crosier et al. (1970), reported *R. solani*, as a companion to *Pythium* spp. or as the predominant pathogen, in seed decay and damping-off of peas. *R.*

solani has also been reported to cause a serious disease syndrome including seed rot, stem rot, tip blight and damping-off (Jhooty and Behar, 1970; Shehata et al., 1981).

2.3 LENTILS

Lentils (Lens culinaris Medikus) are well adapted to a temperate climate and have been grown on an increasing basis in western Canada since 1970. A gradually increasing domestic market in North America may sustain production of lentils. They are used as a protein source in soups and other lentil dishes. Crops grown after lentils yield higher than if grown after cereals or flax. Lentils may thus be used to extend the rotation (Slinkard and Drew, 1980). Lentils are one of the oldest crops cultivated crops in the dry tropics as a source of dietary protein. Most production takes place at high altitudes. The leading producing countries include India, Ethiopia, Syria, Turkey, USSR, South Africa and Poland (Robinson, 1975).

Soil borne root rots caused by R. solani and a Fusarium sp., and a Sclerotinia wilt have been recorded in Western Canadian fields at various growth stages of the crop. Without an effective method of control, except rotation, they represent a major threat to future production of lentils in Western Canada (Morrall and McKenzie, 1972; Slinkard and Drew, 1980). When attacked by R. solani, lentil roots show a reddish brown, dry necrosis. All tissues except the xylem elements, are infected and disintegrate completely. Overall root development is poor and wilting is prevalent. In addition to seedling damage in the pre- and post-emergence stages, losses may result from

seed rot (Khare, 1981). Heavy losses occur when R. solani and other fungi are associated with the root disease at the same time. A root disease complex including F. oxysporum, S. rolfsii, R. bataticola, with R. solani was reported in Ethiopia (Mengistu, 1978). In Syria, the most important lentil disease was a complex involving R. solani, Phialophora spp. and Fusarium spp. (Hanounik, 1978). A root rot and wilt complex of R. solani and Fusarium spp. was reported as the most serious lentil disease in Egypt (Ibrahim et al., 1978). Other complexes that have been associated with R. solani in lentil diseases include Pythium ultimum and F. solani (Shatla et al. 1975), S. sclerotiorum, Verticillium alboatrum, and Fusarium spp. (Wilson and Bandsbury 1965), and Macrophomina phaseolina, Pythium aphanidermatum, Pythium ultimum, (Kaiser and Homer, 1980).

2.4 CANOLA - RAPESEED

Canada is the world's leading exporter of rapeseed (Brassica napus L. and B. campestris L.). The crop is used locally for oil extraction and the seed cake as livestock feed.

Beside seed decay and pre- and post-emergence damping-off in spring, R. solani causes two distinct diseases in rapeseed and other Brassica spp. The pathogen may attack plants beyond the seedling stage and if the weather becomes dry after infection, the cortex decays in sharply defined areas encircling the stem. The cortex collapses but since the toughened stele provides support, the plant may remain erect. However, such plants are unthrifty, non-productive and usually succumb completely to the disease (Baker, 1970).

Infection in older plants is evidenced by prematurely ripened plants appearing in patches in the field during the summer. Stem lesions or cankers develop at the stem base. The hard, brown, rough-surfaced lesions, have a clearly defined border, sometimes marked by a black margin. Severe infections may girdle the stem completely and eventually kill the plant. White mycelial growth may be present on discoloured root tissue.

None of the presently cultivated B. napus and B. campestris genotypes have complete resistance to R. solani (Acharya, et al., 1983).

Chapter III

FACTORS THAT AFFECT DISEASE DEVELOPMENT

3.1 INOCULUM POTENTIAL

The most pertinent component of inoculum potential as defined by Garrett (1960), is inoculum density. Inoculum of R. solani Kuehn may be present in soil as hyphae, sclerotia or basidiospores at varying depths extending to 10-15cm. The persistence of R. solani in soil will depend on the inherent survivability of the fungus, the sum of abilities of other antagonistic soil microorganisms and the influence of the soil environment (Baker et al., 1967).

The inoculum density of R. solani is difficult to enumerate since the propagative unit is not easy to define. Inoculum density is thus, usually stated in relative terms. This makes comparison of various studies difficult. In addition, various reports have indicated that R. solani inoculum density in soil is relatively low (Ko and Hora, 1971; Herr, 1973; Weinhold, 1977). It however, has been demonstrated that R. solani inoculum is aggressive and can increase rapidly in presence of a host and cause considerable disease (Papavizas, 1964).

Most workers studying the epidemiology of R. solani, have used the damping - off phase of the pathogen's disease syndrome. This is due to the fact that it is the most prevalent phase. It also is an attempt to reduce the number of interacting variables (Baker and Martinson, 1970). Since the period when infection courts for

damping-off and many canker diseases are available is fairly brief, the probability of a propagule being in the infection court at the appropriate time to cause disease is increased with a large amount of inoculum.

3.2 DISEASE POTENTIAL

Disease potential is a function of the resistance or susceptibility of a host at the different stages of growth, and the environmental factors that may affect its vigour (Baker and Martinson, 1970). Most hosts are particularly susceptible to penetration during seedling and juvenile stages of growth. Tissues may become more resistant with age. This may result from a thickening of host cell walls (McClure and Robbins, 1942), wound periderm formation (Thatcher, 1942), increase in calcium content of the tissue (Bateman and Lumsden, 1965), and extrusion of phenols (Stockwell and Hanchey, 1982).

Different strains of R. solani may attack a crop at its different ages. Ui et al. (1963), reported one strain of R. solani that attacked flax seedlings in spring, and another strain within the same field, that was weakly pathogenic on seedlings but severely attacked mature plants in summer.

3.3 NUTRITION

Weinhold (1969), indicated that a superior food base increased the virulence of R. solani. Three sources of nutriment are available in the infection court to satisfy energy requirements for processes prior to and during penetration and establishment of the fungus in the host.

These are propagules of the fungus, the soil solution and organic matter in the soil, and exudates from the host plant (Toussoun et al., 1960). Exudates have been shown to play a role in the formation of infection structures and provide nutrients for growth prior to penetration (Flentje et al., 1963; Schroth and Cook, 1964; Martinson, 1965). Weinhold et al. (1969), pointed out that a lowered nutritional status of the pathogen may be the operative effect in cultural practises such as rotation, rather than a reduction in the pathogen population.

Mineral imbalances in soil may affect either inoculum potential or disease potential. The apparent suppression of R. solani activity in soil by decomposing organic amendments, may be a result of an increase in the carbon to nitrogen ratio. This would increase the total microbial population and result in immobilization of nutrients such as nitrogen (Papavizas and Davey, 1960). Bateman (1964), observed that plants grown in calcium deficient soils had poor root systems with very soft cortical tissues, an indistinct middle lamella, and large intercellular spaces. He speculated that the role of multivalent ions such as calcium, magnesium and phosphorus, could be the formation of insoluble pectate complexes in cell walls, which are able to resist hydrolysis by polygalacturonases produced by R. solani.

3.4 TEMPERATURE

Most evidence suggests that R. solani is parasitically active over a wide range of temperatures, certainly within the range of temperatures that most agricultural crops show optimal growth (Baker and Martinson, 1970). Of Richter and Schneider's natural groupings of R. solani, Parmeter and Whitney, 1970), two groups, mostly strains attacking potatoes and crucifers, had an optimum growth temperature between 21 C and 25 C. Another four groups showed their best growth at temperatures between 25 C and 29 C

Some workers have established that the effect of temperature on disease development may express itself through host vigour. Bolkan et al. (1974), reported that at low temperatures, relatively low levels of inoculum caused disease in potatoes because of delayed emergence. This extended the duration of the period susceptible stem tissue was exposed to inoculum in soil. Tissues become more resistant after emergence. However, he also reported that at high inoculum densities, a rise in temperature did not suppress disease. Benedict (1968), reported that pea (P. sativum) cultivars grew better at cooler temperatures in absence of the pathogen, and that the incidence of pre-emergence damping off showed a greater variation in relation to cultivar than soil temperature. The incidence of post-emergence wilting was however more closely correlated with soil temperature. Lewis and Papavizas (1977) reported that the appropriate environmental conditions for soybean disease included high levels of temperature, moisture and pH.

Leach (1947), found that disease incidence didn't correspond closely with either the growth rate of the host or that of the pathogen at different temperatures. He established that it was inversely related to the ratio of the coefficient of the velocity of seedling emergence (CVE), and the growth rate of the fungus (GR). The CVE was used as an indication of host vigour. The isolate used had a temperature growth range of 8 - 40 C, and exhibited maximum growth between 25-30 C. Thus in spring, low temperature crops like sugarbeet and spinach would give a high CVE:GR ratio and therefore show low pre-emergence damping-off. Warm temperature crops would give a low CVE:GR ratio in spring and thus show high disease incidences. Martinson (1963), observed similar results but indicated that seedling emergence in the presence of R. solani correlated more closely with CVE to inoculum potential ratio, than CVE to growth rate ratio. Such findings would explain why some workers find close relation between optimal culture growth temperature and temperature favouring disease development (Smith, 1946), while others do not find any relation at all (Richards, 1923).

3.5 MOISTURE

The expression of the condition of soil water has also suffered from the multiplicity of terminologies used by workers which make comparison or even comprehension difficult. Baker and Martinson (1970), suggested soil moisture be expressed as the amount of water in a given mass or volume of soil, or as the energy of retention. The latter is preferred because it can be compared to a moisture tension

curve and related to physical soil characteristics such as field capacity, permanent wilting point and saturation point. Thus if aeration is a problem, porosity, bulk density and pore space can be determined. This would be the case for moisture levels above field capacity, while low soil moisture stress occurs below field capacity.

Most literature on moisture is complicated by the various interactions and assumptions made. Pitt (1964) reported that sharp eyespot of cereals was favoured by low soil moisture. However, conditions of low temperatures existed in this instance too and the expression could have been due to an increase in disease potential. Bloom and Couch (1960) could not show any difference in severity of brown patch disease of seaside bent grass under varying moisture but implied that concentrations approaching saturation resulted in higher disease severity. Lewis and Papavizas (1977) reported that high temperatures and moisture increase R. solani disease of soybean. This is in agreement with the observation that soil moisture stress and low temperature reduce R. solani growth (Baker and Martinson, 1970).

Diseases of aerial plant parts require conditions of free water or relative humidity approaching 99% for growth and infection. Increases in the use of irrigation seem to accentuate the severity of rice and soybean diseases (O'Neill et al., 1977; Lee and Rush, 1983). Fruit rots of tomato or cucumbers are favoured by high moisture and occur through infection of plant organs in contact with moist soil (Jones and Carter, 1974).

Low soil moisture stress may increase the disease potential more than the reduction in inoculum potential. This would explain apparent

contradictions, for instance increases in severity of R. solani disease of potato in sandy soil (Baker and Martinson, 1970)

3.6 AERATION

The role of aeration in disease development is primarily related to a soil's texture, structure and therefore, the pore size distribution and air capacity, for a given level of soil moisture (Baker and Martinson, 1970). Papavizas and Davey (1961) demonstrated that poor aeration due to high soil moisture content reduced the competitive saprophytic ability (CSA) of R. solani. In vitro, CSA was reduced by 20-25% carbon dioxide (CO₂). They also showed that oxygen supply only became limiting when atmospheric levels were reduced to 1% or less. The effect of poor aeration was therefore ascribed to CO₂ toxicity rather than oxygen deficiency. Baker and Martinson (1970), indicated that there was less pronounced CO₂ toxicity in alkaline soils, which act as CO₂ receptors, than in neutral to acidic soils. Increases in disease severity under poor aeration conditions were explained to be a result of increased disease potential in a host sensitive to high soil moisture.

R. solani appears to exhibit distinct ecological specialization with regard to aeration. Strains primarily causing root rot are more tolerant to higher concentrations of CO₂ than those that cause foliar, or stem diseases (Durbin, 1959, Davey and Papavizas, 1960).

3.7 HYDROGEN ION CONCENTRATION

R. solani isolates are capable of growth over a broad pH range while plants have a more restricted range of optimal growth, and are particularly sensitive at the pH extremes (Sherwood, 1970; Papavizas and Lewis, 1977). The effect of pH may be an increase in the disease potential due to, for instance, an upset in the mineral nutrient balance. The inoculum potential of the pathogen may also be affected by pH. Baker and Martinson (1970), pointed out that, if penetration was primarily enzymatic, pH may affect the enzyme production and therefore the parasitic activity of the pathogen. They also noted that the components of the associated microfloras may vary with pH thereby producing a different set of interactions.

3.8 MICROBIAL INTERACTIONS

Various antagonistic or synergistic interactions may occur in the rhizosphere. Favourable conditions created by exudates from roots may benefit a wide range of soil microflora. The microbial population may thus show an overall increase (Mitchell, 1970).

The build-up of antagonistic microflora takes place within a certain time frame. Chances of suppressing a pathogen by antagonists are improved if the initial population of antagonists is sufficiently high and conditions are favourable for their persistence during the crop's growth (Jager et al., 1967). In addition, antagonists must be able to resist or tolerate activities of other microorganisms and their metabolic products, and conditions that may be created by their mutual presence including nutrient competition or mineral imbalances,

changes in pH, and mechanical obstruction (Baker et al. 1967; Arora et al., 1977).

Organisms that have been reported to have antagonistic effects on the activity of R. solani, with varying degrees of disease suppression include Trichoderma spp., (Weindling, 1932

Boosalis, 1956; Rich and Miller, 1962; Hussain and Mckeen, 1962; Mall, 1973; Hadar et al., 1979; Elad et al., 1980; and Marshall, 1982). Mall (1973), reported antagonism in vivo by Pseudomonas spp., Streptomyces spp. and Aspergillus spp., which were frequently isolated from potato rhizospheres, but failed to reproduce the effect in soil. Talaromyces flavus has been reported as a R. solani antagonist by Boosalis (1956), and Husain and Mckeen (1962). Gliocladium roseum was shown to infect and kill both hyphae and sclerotia of R. solani under certain conditions in potato fields (Pugh and Van Emden, 1969; Jager et al., 1979). G. virens (Aluko, 1970), Pseudomonas fluorescens (Howell and Stipanovic, 1979) and Pythium oligandrum (Dreschler, 1946; Deacon, 1976, Jager et al., 1974), have also been reported to have strong antagonistic properties against R. solani. A Corticium spp., recently included in the newly described species of Laetisaria, was reported by Odvody et al. (1980), as another antagonistic rhizosphere - fungus. The above interactions have the effect of lowering the inoculum potential of the pathogen.

On another level, microbial associations may increase the disease potential of the host. Either R. solani would be the predisposing organism as in Verticillium disease of olive rootlets (Wilhem et al., 1962), or other organisms may predispose the host to R. solani

infection. Greater disease incidences have been reported in associations of R. solani with Fusarium solani, as in wheat eyespot disease (Price and Stubbs, 1963), with F. roseum in crown-bud rot of alfalfa (Hawn and Cormack, 1952) and with Thielaviopsis basicola in beans (Macer, 1961).

Synergism with virus diseases has been demonstrated in Fragaria vesca infected with chlorosis virus which showed greater susceptibility to R. solani root rot than virus free plants (Skiles, 1953). Bateman (1961) also showed that cucumber seedlings infected with mosaic virus were more susceptible to R. solani damping-off than healthy seedlings. Baker and Martinson (1970) have reviewed the subject in considerable detail.

3.9 HERBICIDES

As with most pesticides, the biological effects of herbicides may extend to non-target organisms. Depending on the nature of the herbicide, the pathogen, and the host involved, effects may extend from inhibition to stimulation of the pathogen, or an increase or a decrease in disease potential (Katan and Eshel, 1974).

The herbicide Trifluralin (common name - Treflan), has been reported to increase the incidence of diseases caused by R. solani in snap beans and cotton (Altman and Campbell, 1977), post-emergence damping-off of beans (Katan and Eshel, 1974), and in cotton and safflower (Wrona et al., 1981). The latter established that normal mitosis, and subsequently hypocotyl maturation, was disrupted in red kidney bean. The plants did not exhibit the enhanced resistance to R.

solani with age. Increased pectolytic activity in trifluralin-treated bean and pea plants, over the non-treated plants, was also shown.

Katan and Eshel (1974), reported that damping-off of pepper caused by R.solani was increased by diphenamid (N, N-dimethyl-2, 2-dipheynlacetamide) although the herbicide was shown to be slightly toxic to R. solani in vitro. The herbicide enhanced the initial increase in R. solani colonization and also decreased the decolonization process. Decolonization has been attributed to the activities of antagonists of R. solani (Papavizas and Davey, 1960). Katan and Eshel therefore concluded that the increase in disease incidence was due to a relative suppression of associated soil microorganisms.

Grinstein et al., (1976), reported that there was increased resistance to R. solani by solanaceous crops when trifluralin was used. Increased resistance to R. solani was also reported in tomato plants in diphenamid treated soil (Katan and Eshel, 1974). They suggested that altered host metabolism by diphenamid or its by-products, may have led to stimulation of host defence mechanisms.

Characterisation of Rhizoctonia solani Isolates from Manitoba Fields

ABSTRACT

Seventy four pure cultures of R. solani were established from hyphal tips of colonies on isolation media in 1981 and 1982. In order of decreasing number of isolates obtained from diseased seedlings, the major host sources were rapeseed, faba bean, sugarbeet, lentil, peas, and potato.

Isolates were classed into ten groups on basis of the color and texture of mycelium, and the amount and color of sclerotia produced. The zonation of colonies in culture was not a consistent feature of any single group. The size of sclerotia tended to be extremely variable and was of minor importance in grouping the isolates. With a few exceptions, isolates in each of the ten cultural groups corresponded to one of the five anastomosis groups determined by pairings with AG-tester isolates.

Five anastomosis groups were identified among isolates from Manitoba. Isolates in AG-4 were the most prevalent and exhibited the most uniform characteristics in culture even though they were isolated from a variety of hosts. Forty six isolates were assigned to AG-4, ten to AG-5, seven to AG-2T1, six to 'AG-2', four to AG-3, and one to AG-2T2. 'AG-2' was composed of isolates that anastomosed with AG-2T1 and AG-2T2 testers. Two of the 'AG-2' isolates also anastomosed with the AG-3 tester and may belong to AG-B1. AG-B1 has been reported by Kuninaga in Japan and consists of field isolates that anastomose with several isolates of other AGs. No AG-1 isolates were recovered from diseased seedlings in Manitoba.

In greenhouse pathogenicity tests, eleven AG-4 isolates, and two isolates of each of AG-2T1 and AG-2T2 were pathogenic on faba bean, peas, lentils, and rapeseed. Thirty two AG-4 isolates and one AG-2T1 isolate were pathogenic on all the legume hosts. Five 'AG-2' isolates four AG-2T1 isolates, three AG-5 isolates, and one isolate from each of AG-1 and AG-3, were pathogenic only on rapeseed. Six AG-5 isolates and one AG-3 isolate were not pathogenic on any of the four hosts used. The remaining eight isolates were pathogenic on at least two of the four hosts in various combinations.

INTRODUCTION

Rhizoctonia solani Kuehn (Thanatephorus cucumeris (Frank) Donk, has been isolated from diseased seedlings of faba bean, peas, lentils, rapeseed, sugarbeet and potato from individual fields in Manitoba (Ali-Khan and McVetty, 1980; Rimmer and Platford, 1980; Zimmer and Russell, 1981; Rimmer, 1982).

R. solani is currently considered as a composite species of isolates that show different morphological, physiological and pathological characteristics. Previous workers have attempted to group R. solani on the basis of culture types, anastomosis groups (AG) and pathogenicity (Houston, 1945; Exner, 1953; Luttrell, 1962; Parmeter, et al., 1969). Sherwood (1969), established that each AG was characterised by distinct morphological and physiological norms, although some isolates that lacked sclerotia, distinct pigmentation or those with slow growth rates could not be easily assigned to their proper AG on basis of cultural characteristics alone. Ruppell (1972), noted a constant association of anastomosis behaviour and pathological activity, and was able to distinguish sugarbeet foliar isolates from root isolates by either cultural characteristics or anastomosis behaviour. A similar observation was made by O'Neill (1977), in aerial blights of soybean and foliar blights of rice and sorghum. Anderson (1982) acknowledged the importance of AG's in relation to pathological activity and indicated that there was a tendency of each AG to have a restricted host range.

Isolates within each group may however, vary from non-pathogenic to highly pathogenic on a given host. The population of R. solani,

even in a small field may be composed of a number of distinct groups of isolates, each with different saprophytic and parasitic potentialities (Papavizas, et al., 1975

Weinhold, 1977; Herr and Roberts, 1980).

No study has thus far been done on the cultural types, anastomosis groups or the pathogenicity of R. solani from Manitoba. This paper reports on the isolation, identification, cultural characterisation, and pathogenicity of R. solani isolates associated with diseased seedlings of selected legumes, rapeseed, sugarbeet and potato in Manitoba. The cultural characteristics and pathogenicity of isolates from a single field were also compared.

Chapter IV
MATERIALS AND METHODS

4.1 SOURCE OF ISOLATES

In 1982, twenty eight cultivars of legumes and special crops were sown in a field naturally infested with R. solani at the U of M arboretum. Each cultivar was planted in 5m row plots repeated six times in a randomized complete block design. Disease incidence was assessed 33, 43, 53, and 64 days after planting. The susceptibility of some of the cultivars was verified in greenhouse tests using naturally infested soil.

Isolations of R. solani and other fungi associated with diseased plants were made during the season. Isolations were also made from disease samples of legumes, rapeseed and sugarbeet seedlings obtained from field surveys, the Manitoba Department of Agriculture, and the Manitoba Sugar Company. Other isolates were kindly provided by Lamari ((University of Manitoba, Dept. Plant of Science)) and Dr N.A. Anderson (University of Minnesota, St. Paul).

The cultures used in this study were obtained by hyphal tip isolation from representative colonies. Small pieces of root or stem were sectioned from areas adjacent to lesions and washed in a seive nested in a beaker under running tap water. The pieces were then surface disinfected in 1% sodium hypochlorite solution for thirty to sixty seconds, rinsed in sterile distilled water, and blotted dry on

filter paper before being plated. Water agar (WA), Difco potato dextrose agar (PDA), and a Rhizoctonia-selective media (RSM) developed by Ko and Hora (1971), were used as plating media (See Appendix 1 for description of RSM). When RSM was used, the pieces were not surface disinfected. The plates were incubated at room temperature (ca 22 C) for 2 to 3 days. Hyphal tips transferred to PDA, incubated at 25 C for 2 to 4 days and then examined for mycelial characteristics. All fungi recovered were recorded. Colonies that showed morphological features characteristic of R. solani were propagated for further characterisation.

4.2 MICROSCOPIC OBSERVATIONS

Cultures characteristic of R. solani were grown on slides coated with 2% water agar (WA), and potato dextrose agar (PDA), resting on 2% WA in 9cm diameter petri dishes, and incubated at 25 C for 2 to 3 days. The slides were stained with 0.05% aniline blue in dilute lactophenol or, with 1% aniline blue in 50% glycerine slightly acidified with HCl directly on the slide (Tu and Kimbrough, 1973). A cover slip was added and microscopic examination of the branching pattern, septation and septal pore character was made at X100 and X400 magnifications. The multinucleate condition of actively growing vegetative cells was established to confirm the culture's identity as that of R. solani as outlined by Parmeter and Whitney (1970).

The culture was designated by a code with the first two letters indicating the source host (See Appendix 2). Stock cultures were maintained on PDA slants stored at 4C, and transferred every six months (Butler, 1980).

4.3 CULTURAL CHARACTERISTICS

Cultural characteristics of isolates were determined on two week old cultures grown on 20ml PDA in 9cm diameter petri dishes incubated at 25 C. All cultures were initiated from 8mm diameter inoculum plugs from the margins of 3-4 day old cultures also grown on PDA at 25 C. The characteristics recorded were colour, texture, growth rate and zonation of the mycelia, and where sclerotia were present, the colour, distribution, size, amount, and shape of the sclerotia. The descriptions were based on those given by Sherwood (1969).

Colony diameter of each isolate was measured every 24 hours, or until the whole petri dish was covered with mycelia. Three replications were allowed for each isolate. The cultures were grouped according to their mycelial and sclerotial characteristics.

4.4 ANASTOMOSIS GROUPS

Anastomosis reactions were determined by the method of Parmeter et al. (1970). Each isolate was opposed with a representative of each of the six known-AGs tester isolates on 30ml of PDA in a 15cm diameter petri dish. The unknown-AG isolate was initiated from a 10mm diameter plug off a 2-3 day old culture grown at 25 C, and placed in the centre of the dish. Tester isolates were started with 8mm inoculum plugs from cultures grown under the same conditions as above, and placed equidistantly from, and around the unknown-AG isolate. The plates were examined for a distinct, clear and depressed zone at the junction of the cultures. Pairs showing a positive interaction were paired in PDA plates to confirm the presence of the junction and then opposed on

sterile, agar-coated slides, incubated in water agar moisture chambers at 25 C for 2-5 days depending on the growth rates of the cultures. After mingling and a slight overlap of hyphae, the slides were stained and examined for hyphal anastomosis (Tu and Kimbrough, 1973). Hyphal anastomosis was examined at x500 magnification to confirm the dissolution of cell walls (Sherwood, 1969). Anastomosing hyphae were retraced to their respective sources to ascertain that no cases of self anastomosis were mistaken for anastomosis between isolates.

4.5 PATHOGENICITY TESTS

Inoculum production and inoculation

Potato dextrose agar inoculum was incubated for 5-7 days at 25 C. Jars of infested corn-kernel inoculum or cornmeal-sand inoculum (cornmeal: sand:perlite 1:10:10) were incubated for 14-21 days at room temperature. The jars were shaken every three days to prevent the inoculum from caking and to evenly distribute mycelial growth.

Plants were inoculated with either a 10mm diameter mycelial disc or a corn kernel placed next to the hypocotyl region and held securely in place by covering with soil. Alternately, one tablespoonful of cornmeal sand inoculum (CMS), was applied at planting above a layer of sand covering the seed, and covered with another layer of sand or soil. This was done to allow the seed to germinate before coming into contact with the layer of inoculum thus preventing pre-emergence seed rot.

Hosts

Seedlings were grown in a steam pasteurized mixture of greenhouse soil, sand and peat moss (3:3:1 v:v:v), passed through a 2cm mesh screen to remove clods and large plant pieces.

Seven to ten seeds were planted 3-5cm apart, 2cm deep, in rows across the width of 55 X 37 X 9cm styrofoam flats and covered with soil. The flats were watered daily or as necessary. The seedlings were inoculated 7 to 9 days after planting.

Each isolate was initially tested for pathogenicity on the source host in a controlled environment chamber (25 C, 16 hours day; 20 C night). Subsequent pathogenicity tests were done in the greenhouse. All tests were repeated two or more times and corresponding controls were maintained consisting of uninoculated seedling rows in each flat. Disease reactions were recorded 10-14 days after inoculation.

The accession PI222125 was used as the susceptible faba bean host. It consistently gave rapid germination rates and thus even and uniform seedlings at inoculation. PI222125 was not different in susceptibility from the registered cultivars Diana, Ackerperle, Aladin and Herz freya (Appendix 2). Typical faba bean disease symptoms range from pin-point superficial hypocotyl lesions that sometime merge into larger lesions, to a deep dry, black rot girdling the stem. Some isolates produce a wet, black stem rot. Severely infected plants show extensive leaf chlorosis, collapse at the stem base and die. Disease on faba bean was assessed by rating individual plants on a scale of 0 (healthy), to 5 (plant dead). A mean disease severity index (MDSI), was calculated as follows (Sneh et al., 1975):

$$\text{MDSI} = \text{SUM } Y_i X_i / N$$

where: X_i = Class value (i=0 to 5)

Y_i = Number of plants in class i

and N = Total number of plants

An isolate was considered pathogenic on fababean if it gave a MDSI of 2.5 or greater.

The selected pea cultivar Trapper was as susceptible as Century, Tara, and Triumph. Seeds of Trapper were however more easily available. Infected plants show chlorosis of lower leaves followed by a relatively dry stem rot at the base of the plant. Severely infected plants wilt, collapse at the base and die.

The Lentil cultivar Laird was preferred over the equally susceptible cultivar Eston because the larger seed give more vigorous seedlings at inoculation. Characteristic R. solani symptoms on lentils include a reddish brown wet necrosis of the stem, a marked leaf chlorosis and necrosis followed by plant death.

The Brassica campestris cultivar, Candle, is more susceptible than either Tobin or the B. napus cultivars and was thus used as the rapeseed test host. Wire stem constriction of the stem bases, stunting and a purplish red leaf discoloration are some of the symptoms characteristic of R. solani infection on rapeseed.

An isolate was considered pathogenic on peas, lentils and rapeseed if it elicited characteristic symptoms on more than half the number of plants inoculated.

Chapter V

RESULTS

5.1 ISOLATION AND IDENTIFICATION

There were significant differences among crops in the incidence of disease in the naturally infested field, but not among cultivars of a crop at a given stage of growth. The incidence of disease on faba bean, peas, lentils and rapeseed on the first date of sampling was significantly less than that on later dates (see Appendix 3). Pre-emergence damping-off was prevalent in the legume crops while root rot and stem infection were observed in both legumes and rapeseed (fig. 1). Faba bean cultivars Diana, Ackerperle, Herz Freya, and Aladin all showed extensive stem cankers and root rot (fig.2). Pea cultivars Trapper, Tara, Century and Triumph all showed general chlorosis, basal stem rot, and plants that were severely infected usually succumbed to disease completely and died (fig.4). Lentil cultivars Laird and Eston were both susceptible and showed extensive stem decay. Dead leaves remained attached to the plant. The Brassica campestris cultivars Candle and Tobin generally showed a higher level of disease than B. napus cultivars, Tower, Andor, and Altex. Infected plants showed basal stem cankers and root rot later in the season.

By the end of the second month after planting, some apparently healthy surviving field bean plants of the cultivar Seafarer, and soybean plants of the cultivars Maple Amber, Maple Presto and McCall,

FIGURE 1-5. Field symptoms of Rhizoctonia solani diseases.

1. seed rot of a legume showing excision of the epicotyl (e),
and the hypocotyl (h).
2. faba bean a) damping-off.
b) stem lesions.
c) root rot.
3. stem and stolon pruning on the potato cultivar Norland.
4. basal stem rot of peas.
5. soybean root rot.



exhibited some reddish, distinctly delimited dry lesions on the main root. Although pre-emergence damage was not assessed, seed rots and epicotyl excision were observed and may have caused the reduced plant stands of field bean and soybean in the field (fig.1 and 5).

Cultivars of flax, Culbert, Linott, McGregor and Norlin did not show any signs of infection. However, R. solani was isolated from one dead seedling of the cultivar Dufferin.

Lesions characteristic of R. solani infection on potato were observed on Norland but not on Russet Burbank (fig.3). Only a binucleate Rhizoctonia-like fungus was isolated from the lesions. The sugarbeet cultivar Mono Hybrid-1 did not show any disease symptoms in the field.

R. solani was isolated from diseased seedlings of faba bean, peas, lentils, sugarbeet, potato and other special crops and was recovered without contamination on the selective media of Ko and Hora (1971). Contamination by bacteria and species of Fusarium was common when water agar (WA), or potato dextrose agar (PDA) were used. However, the faster growing rate of R. solani on either medium facilitated the isolation of the fungus free of the contaminants after 2-3 days of incubation.

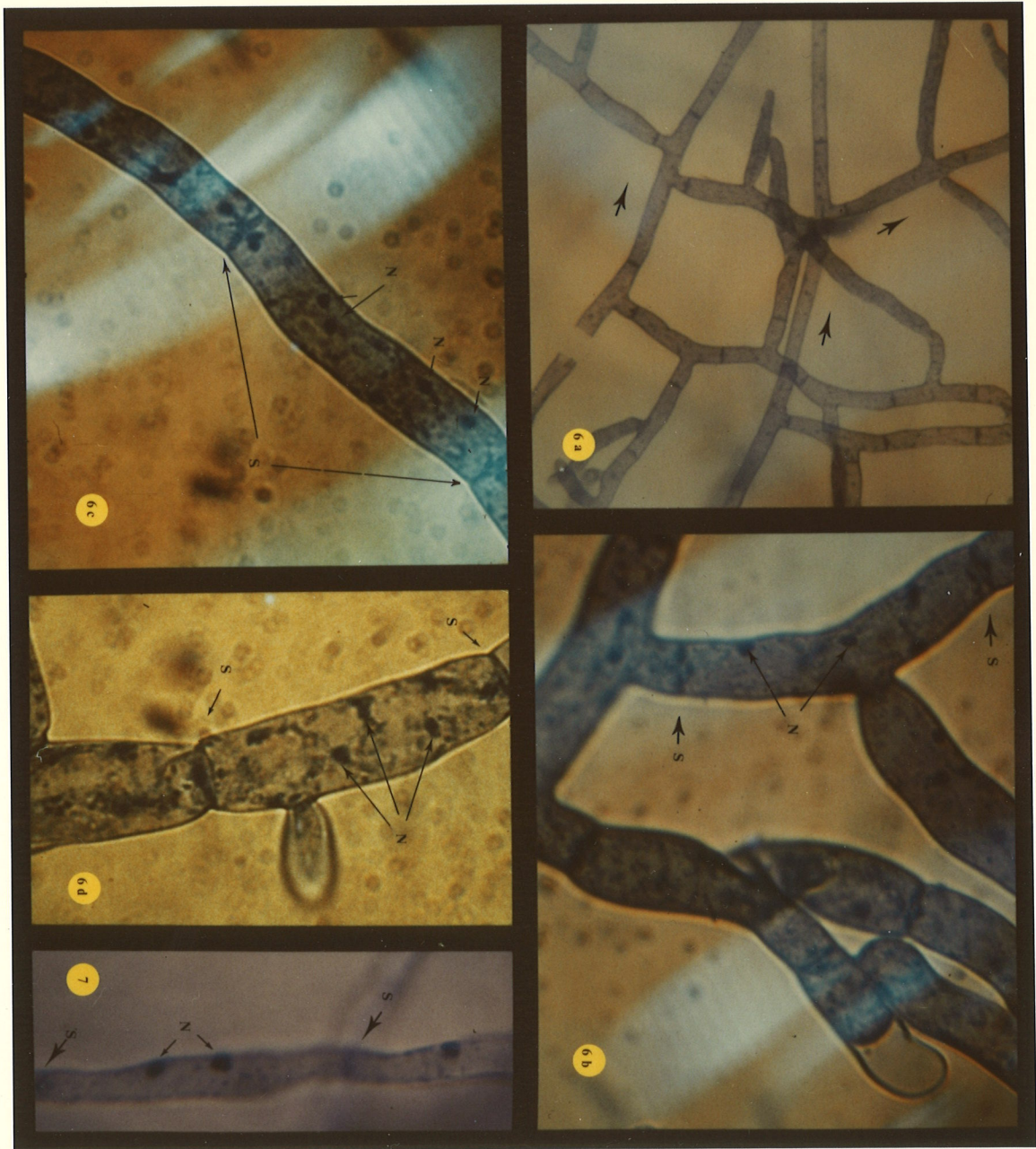
The identification of R. solani was based solely on the asexual state, as outlined by Parmeter (1970). Typical characteristics used included the branching pattern and septation (fig.6a), the presence of multinucleate cells in young vegetative hyphae, and the doliform septal pore structure (fig. 6b,c,d).

FIGURE 6. Mycelial characteristics used in the identification of the asexual state of Rhizoctonia solani.

a) septation and branching pattern, x100.

b-d) doliform septa (S), and multinucleate cells (N): x400, x400, x500 respectively.

FIGURE 7. Binucleate cell in hypha of a Rhizoctonia-like fungus x400.



Fusarium spp. were the most prevalent group of fungi isolated with R. solani from diseased seedlings. Other fungi isolated included Alternaria spp., Rhizopus nigricans Ehr., Aphanomyces euteiches Drechs. and a few other unidentified fungi. Binucleate Rhizoctonia-like fungi were also isolated from faba bean, pea and potato plants. Rhizoctonia-like fungi could only be distinguished from R. solani with certainty, on the basis of their binucleate condition in vegetative cells and their generally smaller hyphal widths (fig.7).

The frequencies with which the various fungal species were isolated, given in Table 1, indicate that there was a greater incidence of root rot in 1981 than in 1982 in Manitoba.

5.2 GROUPING OF ISOLATES BASED ON GROSS CULTURAL CHARACTERISTICS

The cultures of Rhizoctonia were separated and grouped into ten broad groups based on the color and texture of mycelia, and the amount, size and distribution of sclerotia. The groups were designated 'A' through 'J' and are shown in fig.8, Table 2 (Appendix 4). Only 'G' and 'H' did not form sclerotia after two weeks of incubation. Cultures in groups 'I' and 'J' also did not form sclerotia but were later shown to be binucleate Rhizoctonia-like fungi. Binucleate Rhizoctonia-like cultures could not be distinguished from R. solani on the basis of gross morphology, with the exception of isolate 87 which grew very slowly on PDA and had an uneven or serrated colony margin.

The cultural characteristics of all isolates were consistently stable over the two years the cultures were observed.

TABLE 1. Frequency of isolation of fungal species from diseased seedlings of legumes, oilseed and special crops.

Fungal species	% isolations *	
	1981	1982
<u>Rhizoctonia solani</u>	26	23
<u>Fusarium spp.</u>	49	60
Others **	25	17

* Percentage of isolation based on the number of colonies established from diseased samples.

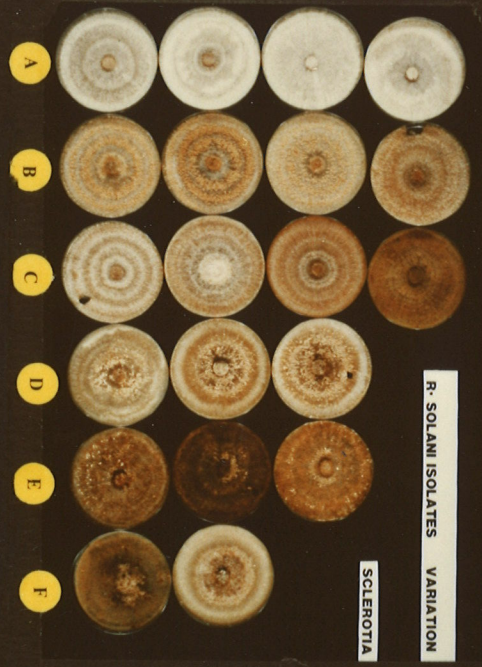
** Includes Rhizopus spp., Aphanomyces euteiches, Trichoderma spp., Alternaria spp., Pythium spp., and a few unidentified fungi.

FIGURE 8. Morphological groups and anastomosis group testers on potato dextrose agar.

'A' to 'H'- Rhizoctonia solani.

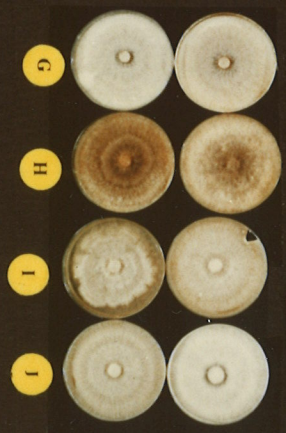
'I' and 'J'- binucleate Rhizoctonia-like cultures.

Insert shows brown exudates from sclerotia.



R. SOLANI ISOLATES VARIATION

SCLEROTIA



NO SCLEROTIA



ANASTOMOSIS GROUP TESTERS

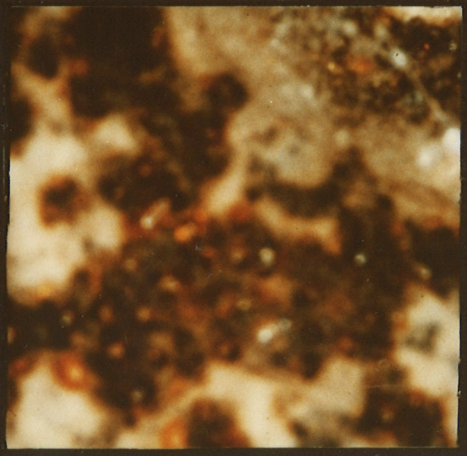


TABLE 2 Criteria used for grouping *R. solani* cultures on basis of gross morphology.

	A	B	C	D	E	F	G	H	I & J
mycelial color	grayish white to light brown, coarsely radiate	light brown to brown	grayish white to brown	light brown to brown	brown to chocolate brown	light brown to brown	grayish white to light brown coarsely radiate	light brown to brown	white to light brown
mycelial texture	appressed compact mealy	zonate, appressed & moderately aerial	zonate as in 'B'	aerial to cottony	moderately aerial to cottony	appressed	appressed compact smooth	aerial to cottony	I-moderately aerial J-appressed radiate
sclerotia color	light brown, embedded	light brown to brown	light brown	white to brown	white to dark brown	light to dark brown	absent	absent	-
sclerotia production	moderate	high	moderate	high	high	high confluent	absent	absent	-
AG's	4	2, 2T1	2, 2T1	5	3	5, 2T2	4	5	Binucleates

*Recorded on 14 day old cultures on potato dextrose agar , 25 C.

5.3 GROUPING OF ISOLATES ON THE BASIS OF THEIR ANASTOMOSIS REACTIONS

Any one, or a combination of six anastomosis reactions, was observed between paired isolates. A 'neutral', or no reaction, was observed when opposing hyphae intermingled and mycelia blended without fusion. 'Contact' fusion involved flattening of the tips of hyphae in contact. After contact, a wall fusion reaction would occur, and could be followed by complete cytoplasmic fusion. The above reactions could occur within the same isolate. The anastomosis group of an isolate was however, only identified when cell death, cytoplasmic granulation or degeneration of anastomosing cells occurred with a known-AG tester isolate. The death of cells adjacent to anastomosing cells was confirmed. This reaction is what constitutes the clear depressed zone in pairings of isolates in PDA plates, (fig.9). (Parmeter and Whitney, 1970). Cytoplasmic contact with cell death was more frequent in fast growing cultures such as AG-4. Neutral reactions, contact or wall fusions were more frequent in slow growing cultures which usually had to be repeatedly paired with testers and also other morphologically similar isolates to facilitate assignment to their proper group. Due to the consistent branching and septation patterns of R. solani, and the presence of distinctly stained 'runner' hyphae, the sources of anastomosing hyphae were easily established (fig.10). The distribution of the collection of isolates among the anastomosis groups is given in fig.11. No isolates in AG-1 were recovered in Manitoba. AG-4 was the most prevalent group, followed by AG-5, AG-2T1, 'AG-2' and AG-3 in that order. There was only one isolate belonging to AG-2T2 recovered from Manitoba.

FIGURE 9. Anastomosis tests on potato dextrose agar plates.

a) against all testers- from top (arrow) clockwise, AG-1,
AG-2T1, AG-2T2, AG-3, AG-4 and AG-5.

left to right: anastomoses AG-4 x VF58

AG-4 x VF72

negative AGs x VF8

AGs x VF14

b) in pairs - top row: anastomoses AG-4 x VF13, VF6 and VF12.

bottom row, left to right: no anastomosis

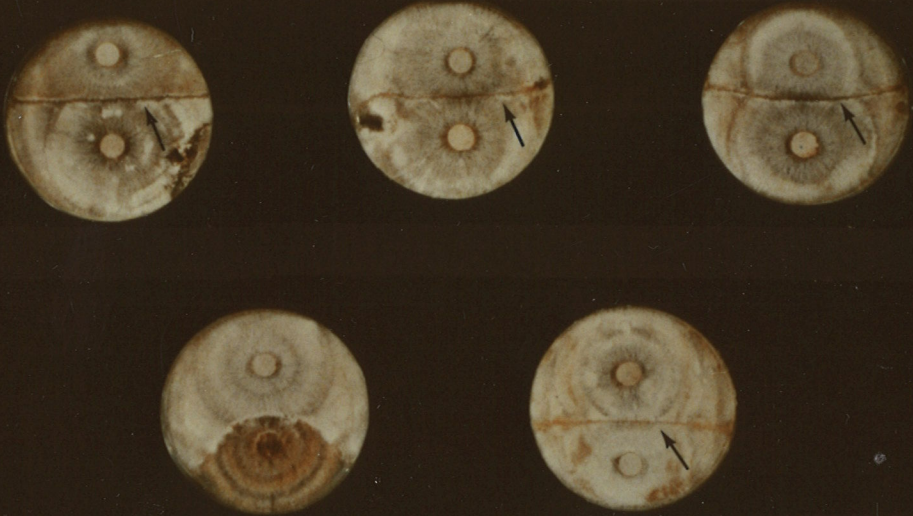
AG-2T2 x VF2; anastomosis AG-4 x VF2.



AG REACTIONS

NO AG REACTION

9 a



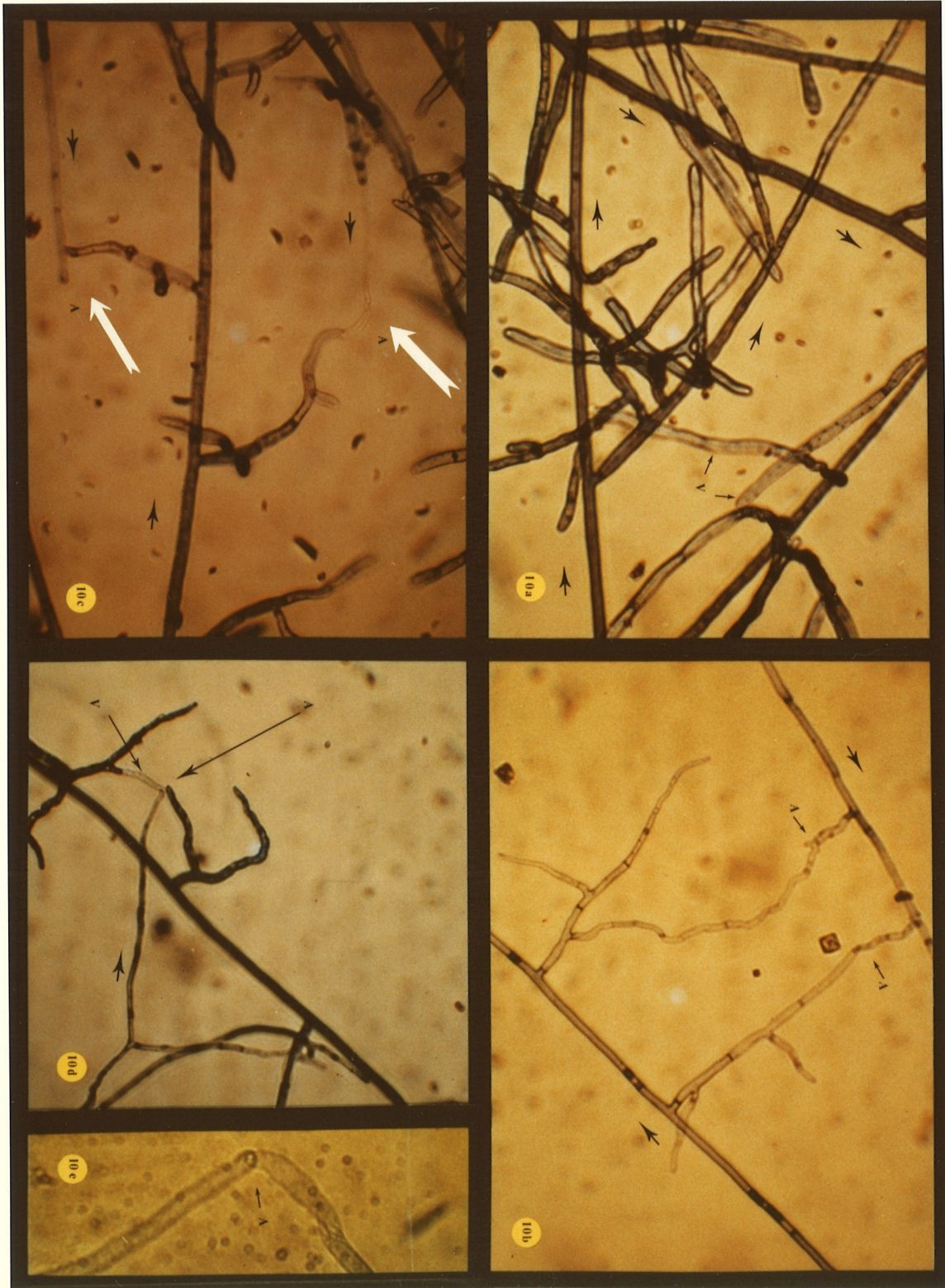
AG REACTIONS

9 b

FIGURE 10. Anastomosis tests on potato dextrose and water agar coated slides.

Unmarked arrows indicate the direction of growth of hyphae.

- a) vacuolated cells in the region of anastomosis (V). x100
- b-d) complete anastomosis (A), with cell death. x100
- e) anastomosing hyphae x500.



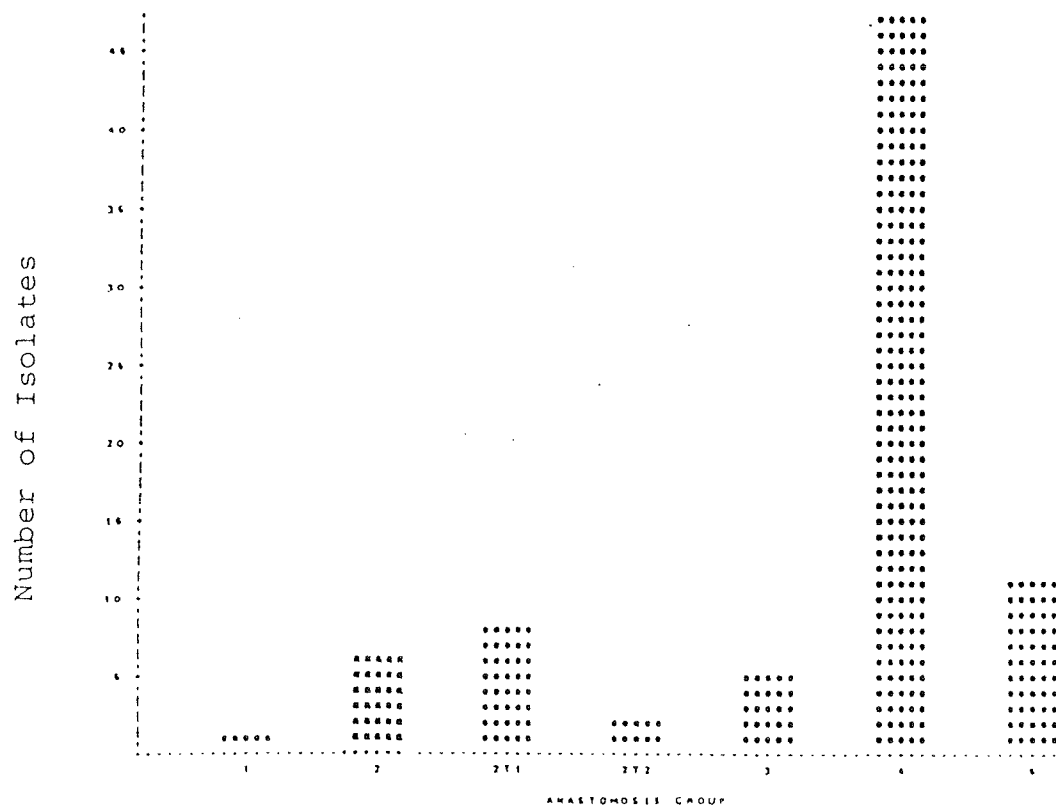


Figure 11. Distribution of *R. solani* isolates among the five anastomosis groups.

Each anastomosis group had characteristic cultural features and with a few exceptions, it was possible to correctly assign an isolate to its proper AG on the basis of gross morphology.

5.4 DESCRIPTION OF CULTURAL CHARACTERISTICS OF ANASTOMOSIS GROUP

The growth rates of each anastomosis group at 25 C are given in Table 3. The AG-1 tester isolate and isolates in AG-4 had the highest mycelial growth rates with averages greater than 30mm per day. Isolates in 'AG-2' and AGs 2T1, 2T2, 3 and 5 had growth rates averaging 17mm or less per day. These results agree with those of Sherwood (1969) and Herr and Roberts (1980).

AG-1

Only the AG-1 tester isolate, RS100, was present in this group. It had appressed, coarsely radiate whitish mycelium which turned to a light brown with age. Cultures formed numerous tiny black, or brown immature sclerotia, which later turned a very dark brown, forming initially at the edge of the colony but later scattered singly over the culture giving it a 'salt and pepper' appearance. Pits sometimes formed on confluent sclerotia where dark exudates occurred. The isolate had the highest average growth rate of 37mm per day at 25 C.

'AG-2', AG-2T1, and AG-2T2

Isolates grouped under AG-2T1 anastomosed only with the respective tester or with isolates that anastomosed with the AG-2T1 tester. Isolate VF1 in AG-2T2 anastomosed only with the AG-2T2 tester. There was another group of isolates that anastomosed with both AG-2T1 and

TABLE 3 Growth Rates of *R. solani* AG's on PDA

AG	Number of Isolates	Growth Rate (mm/day) [*]		
		Minimum	Maximum	Average ^{**}
1	1	-	-	37
2	6	8	23	17
2T1	8	4	34	17
2T2	2	11	18	15
3	5	14	22	18
4	47	27	38	34
5	11	10	31	17

*
Growth rate based on observations between the second and third day of incubation on PDA, at 25 C.

**
Average of two diameters measured at right angles, three replications for each isolate, all isolates in each AG.

AG-2T2 isolates. These isolates were tentatively grouped simply as 'AG-2'. Two of these isolates BR66 and BR67, also anastomosed with the AG-3 tester isolate and may belong to AG-B1 described by Kuninaga et al. (1979). Known AG-B1 isolates were not available to confirm the assignment of isolates BR66 and BR67 to AG-B1.

The majority of isolates in the three groups had light brown to brown mycelia. Cultures showed concentric zones of appressed and aerial mycelia of alternating coloration with varying sizes of sclerotia distributed over the surface of the colonies in the zones that had aerial mycelia. Other isolates had whitish to light brown aerial mycelia and wooly sclerotia distributed over the culture and light brown to brown sclerotia embedded in the agar. The AG-2T2 isolate VF1, had sparse brown appressed mycelia and distinct brown 'runner' hyphae. It also had large confluent sclerotia concentrated mostly around the inoculum disc. sclerotia ranged in color from light brown to dark chocolate brown and were sometimes pitted with a dark brown to almost black viscous exudate. Isolates in the three groups had low growth rates with an average of 16mm per day at 25 C. Isolates in the morphological groups 'B' and 'C' belong to one of these three AGs, with the exception of VF1 which was incorrectly grouped under morphological group 'F'.

AG-3

The isolates in this group all corresponded to the morphological group 'E' and were characterised by coarse, brown to dark chocolate brown, aerial or cottony mycelium. Varying sizes of white sclerotia, changing to a dark brown with age, formed profusely both on the

surface and embedded in the agar. Confluent masses of sclerotia sometimes formed crusts on the surface. The agar was turned to deep dark brown color. The average growth rate of isolates in this group was 22mm per day at 25 C.

AG-4

Isolates in AG-4 were in morphological groups 'A' and 'G' and had almost white, to light brown mycelial color of a leathery, appressed or mealy texture and a high average mycelial growth rate of 35mm per day at 25 C. Isolates in group 'A' formed tiny, light brown sclerotia embedded in the agar. Isolates in morphological group 'G' did not form sclerotia after two weeks of incubation .

AG-5

Two morphological groups, 'H' and 'D' contained isolates that belonged to AG-5. All isolates had characteristic light brown to brown aerial or wooly mycelia. Sclerotia were absent in morphological group 'H'. Isolates in group 'D' formed abundant, white to brown sclerotia concentrated mostly around the inoculum disc. The average mycelial growth rate of this group was 17mm per day at 25 C.

5.5 PATHOGENICITY TESTS

The infested corn kernel method (CK), gave greater disease severity indices for pathogenic isolates than the PDA mycelial disc technique. The corn meal-sand (CMS) technique gave results comparable to the CK inoculation method, however, the CMS induced greater mortalities than the CK method because plants were usually infected as they emerged from the soil (refer to Appendix 2).

The CK and the CMS inoculation methods were limited by the labourious nature of inoculum preparation. At least three weeks of growth in glass jars was required before the inoculum was used. In addition, AG-4 isolates did not show vigorous growth in the enclosed environment of glass jars. A 10mm diameter PDA mycelial disc, or a 2cm wide PDA mycelial strip, was found to be a sufficient amount of inoculum allowing consistent separation of pathogenic and non-pathogenic isolates on susceptible hosts. Disease reactions comparable to those produced by the corn-kernel technique were produced with the PDA disc 7 to 10 days after inoculation. A PDA mycelial disc was easily placed in proximity to juvenile and potentially susceptible hypocotyl tissue eliminating chances of the infection courts escaping the inoculum. The PDA disc was therefore adopted as the basic inoculum unit in subsequent pathogenicity tests.

Disease symptoms in greenhouse tests were comparable to field symptoms. Deep, black stem lesions, and sometimes wet stem rots were observed on faba bean. Severe infections in seedlings resulted in damping-off (fig.12). Symptoms on pea seedlings started with a chlorosis of older leaves followed by a distinct stem rot at the base of the plant (fig. 13). Typical leaf chlorosis and disintegration of hypocotyl tissues in lentil seedlings were observed in the greenhouse. The plants however, rapidly shed their leaves within two days of restricting water supply. The characteristic symptoms on rapeseed seedlings included leaf discoloration, stunting and 'wirestem' constrictions of the stem base (fig.14). All cultivars that were susceptible in the field, were susceptible in the greenhouse.

FIGURES 12-14. Greenhouse symptoms of Rhizoctonia solani infection:

12 a) faba bean wet stem rot.

b) faba bean disease rating scale. 0=healthy; 5=dead.

13. basal stem rot of peas

14. stunting, leaf discoloration and 'wirestem' in rapeseed.



Ten pathogenicity patterns emerged among the isolates in tests on faba bean, peas, lentils and rapeseed, (Table 4). Eleven of the fifteen isolates pathogenic on all hosts were AG-4 isolates. Three of these were isolated from rapeseed, four from faba bean, and one of each from lathyrus, potato, soybean and sugarbeet. Two isolates in AG-2T1 from rapeseed, and two AG-2T2 isolates, one from carrot (DC300), and one from faba bean (VF1), were also pathogenic on all four hosts.

Thirty two isolates in AG-4 and one AG-2T1 isolate from field bean were pathogenic only on the three legume hosts. Of the AG-4 isolates there were 7 isolates from each of faba bean and sugarbeet, 6 isolates from each of peas, lentils, and rapeseed, and 1 isolate from alfalfa.

Ten rapeseed isolates, five in 'AG-2', four in AG-2T1 and one in AG-3, were pathogenic only on rapeseed. The AG-1 tester isolate RS100, and three AG-5 isolates, two from faba bean and one from rapeseed, were considered pathogenic on rapeseed but produced only superficial lesions.

Two AG-4 isolates, one from lentils, the other from flax, and an AG-5 isolate from petunia leaves, were pathogenic on peas, lentils and rapeseed but not on faba bean. Two AG-4 isolates, one from lentils, and the other from rapeseed, and an AG-5 isolate from field bean, were pathogenic only on lentil and peas. Two rapeseed isolates one in 'AG-2', and the other in AG-2T1, infected only lentil and rapeseed. The AG-3 tester isolate ST400, was pathogenic only on peas and rapeseed. Only one isolate, ST75, in AG-3 infected faba bean alone. Another AG-3 isolate, ST56, and an AG-4 isolate from rapeseed infected

TABLE 4. Pathogenicity of Rhizoctonia solani isolates.

Isolates	Pathogenicity				
	AG	Faba bean	Pea	Lentil	Rapeseed
BR60; BR68	2T1	+	+	+	+
VF1; DC300	2T2				
VF13;VF20;VF59;LT31;BR71;BR84 BR85;ST49;BV53;GM96.					
PV43	2T1	+	+	+	-
VF2;VF6;VF12;VF19;VF58;VF59;VF73	4				
PS3;PS30;PS72;PS81;PS82;LC25;LC48 LC87;LC88;LC89;LC92;BR11;BR23;BR26 BR63;BR64;BR68;BV51;BV52;BV76;BV77 BV78;BV79;BV80;MS500.					
LC47; LU94	4	-	+	+	+
PE16	5				
ST56	3	+	-	-	+
BR27	4				
ST400	3	-	+	-	+
BR9	2	-	-	+	+
BR46	2T1				
LC50; BR74	4	-	+	+	-
PV21	5				
ST75	3	+	-	-	-
RS100	1	-	-	-	+
BR24;BR65;BR66;BR67;BR69.	2				
BR28;BR61;BR62;BR200	2T1				
BR29	3				
VF4; VF7; VF70.	5				
ST95	3	-	-	-	-
VF5;VF8;VF14;VF15;VF57;RS600	5				

* Source hosts: BR= Brassica spp.; BV=Beta vulgaris; GM=Glycine max
LC=Lens culinaris; LT=Lathyrus spp.; LU=Linum usitatissimum
MS=Medicago sativum; PE=Petunia spp.; PS=Pisum sativum; PV=Pisum
vulgaris; ST=Solanum tuberosum; VF=Vicia faba.

** (+)=Pathogenic on host indicated; (-)=Not pathogenic.
Test cultivars: Faba bean (PI222125); Pea (Trapper); Lentil (Laird);
Rapeseed (Candle).
Note: The AG-tester isolates RS100, BR200, DC300, ST400, MS500, and
RS600 for AG-1, AG-2T1, AG-3, AG-4, and AG-5 respectively, are
included in this table.

only faba bean and rapeseed. One AG-3 isolate, ST95, five AG-5 isolates from faba bean and the AG-5 tester isolate from soil were not pathogenic on any test host.

The population of R. solani in the naturally infested field at the arboretum was composed of isolates in four anastomosis groups: 'AG-2', AG-2T1, AG-3 and AG-4. Sixteen R. solani isolates were recovered from rapeseed, faba bean, lentil, peas, and flax. Of the eleven isolates from rapeseed, BR86 in AG-2T1, and BR84 and BR85 in AG-4, were pathogenic on faba bean, peas, lentils and rapeseed. BR11 and BR26 in AG-4, were pathogenic on the legume hosts but not on rapeseed. An AG-4 isolate, BR27 was pathogenic only on faba bean and rapeseed. BR9 in 'AG-2', and BR46 in AG-2T1, were pathogenic on only lentils and rapeseed. BR24 in AG-2, BR28 in AG-2T1, and BR29 in AG-3 were pathogenic only on rapeseed. The AG-4 isolates, LU92 from flax, LC94 from lentil, PS81 and PS82 from peas were pathogenic only on the legume hosts. VF90, an AG-4 isolate from faba bean was pathogenic both on legumes and rapeseed hosts.

A considerable amount of specificity was observed with some isolates pathogenic on all the four hosts whereas others were pathogenic on three, two, or even only one host. The data show that there was a general tendency for more AG-4 isolates to be pathogenic on the legume hosts than on rapeseed, and for more of the 'AG-2' and AG-2T1 isolates to be pathogenic on rapeseed than on legumes (Appendix 5). However the source an isolate did not seem to correlate with its AG. There also did not appear to be any correlation between the AG of an isolate and its pathogenicity. For example, although 34 AG-4

isolates from various hosts were pathogenic on all the legumes but not on rapeseed, another 11 AG-4 isolates were pathogenic on all the legumes as well as rapeseed. Furthermore, two isolates from each of 'AG-2' and AG-2T1, were pathogenic on all the four test hosts. In addition, isolates within each AG varied in virulence.. Some isolates were not pathogenic on their source host but were pathogenic on other hosts. Their association with the diseased seedlings could not be determined from this study.

Chapter VI

DISCUSSION

Isolates of Rhizoctonia solani from diseased seedlings in Manitoba fields exhibited definite morphological differences. Each isolate consistently maintained those characteristics in culture over the two years they were observed. No sectoring or variants were observed in any culture indicating their stability. The close association of the morphological classes and the anastomosis groups (AG) classification of isolates agrees with observations made by other workers that each AG has distinctive morphological characteristics, although there are isolates that overlap among groups (Sherwood, 1969; Parmeter et al., 1969).

The number of morphological classes in this study is however conservative since only one medium was used for culturing. Boosalis and Scharen (1969), were able to distinguish three distinct clones from transfers off morphologically similar cultures on PDA by using other media. The use of groups or classes based on cultural features is appropriate for initial screening of large numbers of isolates preparatory to more precise characterisations, which are more time consuming. Castro et al. (1983), reported a Steward's medium on which AG-3 isolates could be distinguished by their brown mycelial color whereas isolates from other groups produced white mycelia. However, AG-3 isolates regularly have darker brown coloration on PDA than other

isolates. The use of Steward's medium would be important in work involving a large number of isolates especially in studies of R. solani in potato fields. Because of the presence of overlapping characteristics of some isolates, actual anastomosis pairing tests are still the only reliable method of determining the proper AG of an isolate. Color of mycelia in culture depends on the medium, the age of the culture, and also the temperature of incubation. Most R. solani cultures tend to a brown coloration with age. Some isolates tend to have a more aerial mycelial texture when the incubation temperature is above 25 C and aerial mycelia are generally lighter colored than mycelia appressed on the culture media.

The inability to distinguish between binucleate Rhizoctonia-like isolates and multinucleate isolates by cultural appearance is a serious shortcoming of the cultural typing method. This could lead to misidentification of the anastomosis group of an isolate and may also affect inoculum density determinations based on recognition of R. solani colonies in isolation plates. It would then be important to ascertain the relative proportions of binucleate and multinucleate propagules in the soil being assayed. This further demonstrates the need for determinations of the number of nuclei and anastomosis pairing.

With the exception of isolates in AG-4, isolates in other groups did not all anastomose with tester isolates. Such isolates were paired with a number of morphologically similar cultures of known AG to confirm their assignment to their proper group. Isolates that do not anastomose with testers of the presently recognised AGs have been

reported before (Sherwood, 1969; Parmeter, 1969; Ogoshi, 1979). The mechanism controlling the failure of one isolate to anastomose with a given tester, and yet anastomose with another isolate capable of anastomosis with the same tester, is unknown. Parmeter et al. (1969), pointed out that anastomosis is an indication of a relation between isolates but that the failure to anastomose was not necessarily an indication that the isolates were unrelated.

A group of field isolates capable of anastomosis with isolates from several other groups has been reported so far only in Japan, and designated AG-B1 (Kuninaga et al., 1979). Isolates that anastomosed with testers of both AG-2T1 and AG-2T2 were observed in this study and designated simply as 'AG-2'. Two of these isolates, BR66 and BR67, also anastomosed with the AG-3 tester, with each other and with other isolates in 'AG-2', for instance BR9. There is a possibility that these isolates belong to Kuninaga's AG-B1. This appears to be the first report of isolates with such anastomosing behaviour in North America.

The faster growing cultures in AG-4 and AG-5, appeared to anastomose much more readily than those in AGs 2, 2T1, 2T2 and 3. The latter groups have been reported to have an optimum growth temperature in the 21-25 C range, while the former groups have their optimum growth temperature in the 25-29 C range (Parmeter and Whitney 1970). Preliminary temperature studies in our laboratory indicated a similar trend. The incubation temperature may have affected the relative ability of isolates to anastomose. These observations confirm those of Herr and Roberts (1980), that more than one tester isolate should be used for each anastomosis group. Anastomosis tests should also be

done at a number of predetermined temperatures corresponding to the isolates growth optima.

No isolates in AG-1 were recovered from diseased plants in Manitoba fields. This group has been associated with soybean aerial blight and rice sheath blight in the warm humid tropics (O'Neill et al., 1977). The tester isolate had the highest mycelial growth rate on PDA at 25 C. This may be an indication of an ecological adaptation to the rapidly fluctuating aerial environment, and therefore the short duration of the presence of favourable infection conditions, and potential infection courts that may be available. The profuse production of numerous, tiny, black sclerotia and the prevalence of the sexual state may be of survival value also (Rush and Lee, 1983).

With the exception of BR9, isolates in 'AG-2' were pathogenic only on rapeseed. BR9 was also pathogenic on lentil as well as rapeseed. All AG-2T1 isolates were pathogenic on rapeseed but five out of eight were pathogenic on at least one legume host. AG-2T2 isolates, VF1 and DC300, were highly pathogenic on all hosts tested. The isolates in 'AG-2', AG-2T1 and AG-2T2 had the lowest mycelial growth rates at 25 C on PDA. As a grouping, these isolates produced abundant sclerotia in culture. Isolates in AG-3 had similar features including sclerotia production, distinctive brown coloration and low mycelial growth rates.

The low growth rates of AG-2 types and AG-3 isolates may have been related to the incubation temperature. These isolates show optimum growth in the 21-25 C range. Isolates in AGs 2, 2T1, 2T2 and 3 have a more restricted host range and cause primarily root rots and stem or

stolon diseases. The ecological adaptation to a subterranean environment may explain the low optimum growth temperature. The abundant sclerotia production would also be of survival value given the more restricted host range. Isolates in AG-3 are particularly noteworthy in this regard being the group that is regularly present on tubers in the black scurf disease of potato.

There is a possibility that 'AG-2', AG-2T1 and AG-3 isolates present in the naturally infested field may become parasitically active earlier in spring than isolates in AG-4. This may explain why most isolates from stem cankers of mature rapeseed plants were in AG-4. Initial disease levels were higher on Brassica napus than B. campestris. This trend was reversed on subsequent dates of sampling, with B. campestris cultivars giving higher disease incidence levels. B. napus may be more susceptible than B. campestris early in the season. Although none of the presently cultivated rapeseed cultivars have complete resistance to R. solani, Acharya *et al.* (1983), showed increased resistance in progeny of B. napus and B. campestris crosses, to R. solani seedling damping-off. On the other hand, the essentially different disease syndromes on rapeseed seedlings and mature plants may be caused by different components of the pathogen's soil population. This could however, not be determined from this study.

Isolates in AG-4 had a wide host range and being the most prevalent group in Manitoba, they are of etiological importance in any control efforts that may be envisaged. In culture, this group had a very consistent morphology and exhibited vigorous mycelial growth ranking second only to the AG-1 isolate on PDA. However, sclerotia production

in AG-4 was generally poor. Pathologically, this group has been reported to cause a number of disease syndromes including seedling damping-off, stem cankers, and foot rots (Anderson, 1982). Given the poor sclerotial production, high mycelial growth rates would contribute to saprophytic colonization of organic matter and therefore enhance the survival and persistence of the pathogen in the soil. The wide host range may also allow colonization of non-hosts in absence of a susceptible host.

AG-5 is of recent discovery and its pathology has not been well elaborated yet. Isolates in this group were either non-pathogenic or weakly pathogenic on rapeseed with the exception of PE16 and PE21 which were pathogenic on peas and lentil. Sclerotia production was higher than that of AG-4 isolates but lower than that of AG-2 type isolates and that of AG-3. The low mycelial growth rates of AG-5 isolates suggests that these isolates may subsist in soil as weak pathogens whose aggressiveness may increase when the host is stressed by other factors. It would be interesting to determine the nature of R. solani cultures from soil that are not associated with diseased seedlings. This group has been reported in potato fields in Maine but no indication of the pathogenicity of those isolates was given (Bandy, et al., 1983). Grisham and Anderson (1983) isolated AG-5 isolates from carrots but failed to reproduce the disease in greenhouse tests. This report of the occurrence of non-pathogenic AG-5 isolates from petunia leaves, field bean, rapeseed and fababean, supports the suggestion that AG-5 isolates are weak pathogens.

Some isolates were not pathogenic on their source host, and yet some isolates not pathogenic on their source host were pathogenic on other hosts. This may be attributed to the fact that in a field soil, many organisms including weak pathogens and saprophytes, as well as cultural practises, may have a predisposing, or synergistic effect on disease development. It also indicates that a certain minimum number of isolates should be used in pathogenicity tests, or for any cultural characterisation within each anastomosis group.

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APPENDIX 1 Medium for the selective isolation of *R.solani*

Ko and Hora (1971).

K_2HPO_4	1.0g
$MgSO_4 \cdot 7H_2O$	0.5g
KCl	0.5g
$FeSO_4 \cdot 7H_2O$	10.0mg
$NaNO_2$	0.2g
Gallic acid	0.4g
Dexon	90.0mg
Chloramphenicol	50.0mg
Streptomycin	50.0mg
Agar	20.0g
Water (distilled)	1.0 l

APPENDIX 2: Comparison of inoculation methods on faba bean .

Isolate	AG	MDSI *			
		Control	PDA disc	Corn kernal	Corn meal-sand
VF1	2T2	0	5	5	5
VF20	4	0	2.9	3.5	nt
VF59	4	0	3.0	4.9	nt
VF5	5	0	1.8	1.8	nt

* MDSI = Mean Disease Severity Index observed on five cultivars;

Ten plants per cultivar inoculated. Observations made seven days after inoculation,

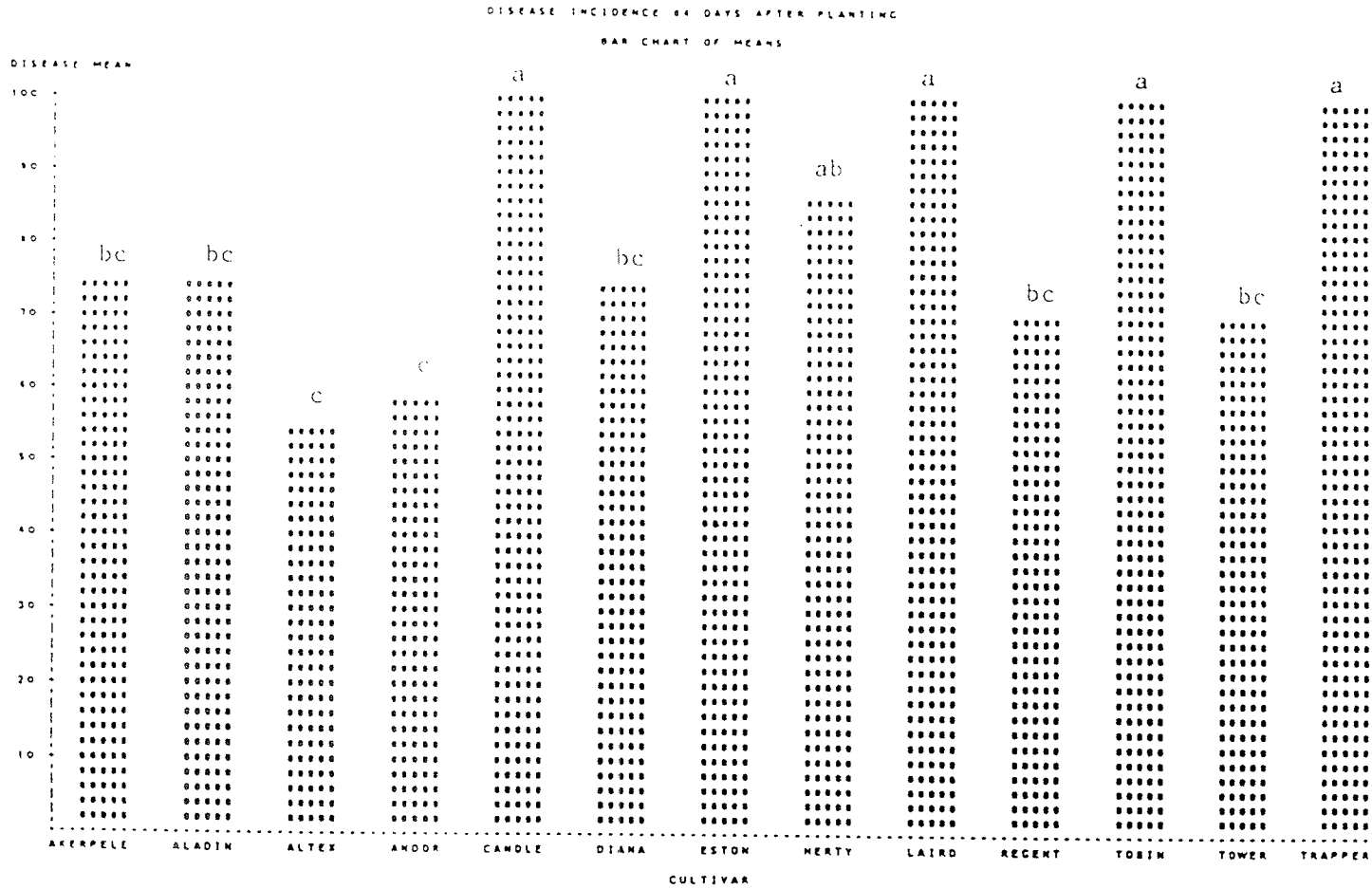
MDSI = $\frac{\sum (Y_i X_i)}{N}$ where Y_i = number of plants in disease severity

class X_i , and

N = total number of plants,

nt = not tested

APPENDIX 3.



Means with the same letter are not significantly different.

Tukey's (HSD) $p = 0.05$

cv. = 16.89 (Arcsine transformation)

APPENDIX 3. (continued) Anova - Disease incidence in naturally infested field.

33 DAYS AFTER PLANTING

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE DISEASE					
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F
MODEL	17	5.44881000	0.37934176	3.84	0.0001
ERROR	80	5.77598124	0.08426602		ROOT MSE
CORRECTED TOTAL	77	12.22479124			0.31026786
SOURCE		ANOVA SS	F VALUE	PR > F	
REP	5	0.45547500	0.95	0.4590	
CULTIVAR	12	5.99333500	5.19	0.0001	

43 DAYS AFTER PLANTING

ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE DISEASE					
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F
MODEL	17	6.44737743	0.37928750	8.10	0.0001
ERROR	80	2.80889713	0.04681162		ROOT MSE
CORRECTED TOTAL	77	9.25627456			0.21635883
SOURCE		ANOVA SS	F VALUE	PR > F	
REP	5	0.91804754	3.91	0.0040	
CULTIVAR	12	5.53132989	8.85	0.0001	

53 DAYS AFTER PLANTING

ANALYSIS OF VARIANCE PROCEDURE

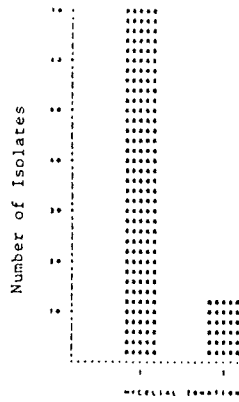
DEPENDENT VARIABLE DISEASE					
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F
MODEL	17	3.25730711	0.54454748	7.61	0.0001
ERROR	80	4.35127080	0.07252118		ROOT MSE
CORRECTED TOTAL	77	13.40857791			0.28928757
SOURCE		ANOVA SS	F VALUE	PR > F	
REP	8	0.20853018	0.52	0.7210	
CULTIVAR	12	8.04677893	10.40	0.0001	

64 DAYS AFTER PLANTING

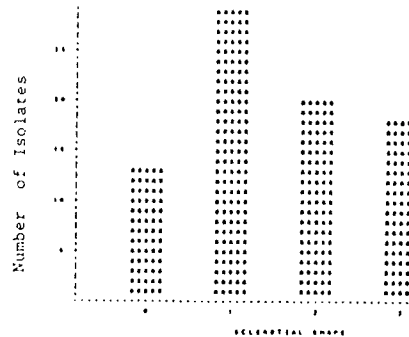
ANALYSIS OF VARIANCE PROCEDURE

DEPENDENT VARIABLE DISEASE					
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F
MODEL	17	8.46036847	0.38002151	8.88	0.0001
ERROR	60	2.62678053	0.04377984		ROOT MSE
CORRECTED TOTAL	77	11.08715700			0.20923833
SOURCE		ANOVA SS	F VALUE	PR > F	
REP	5	0.21822388	1.00	0.4255	
CULTIVAR	12	8.24114259	11.88	0.0001	

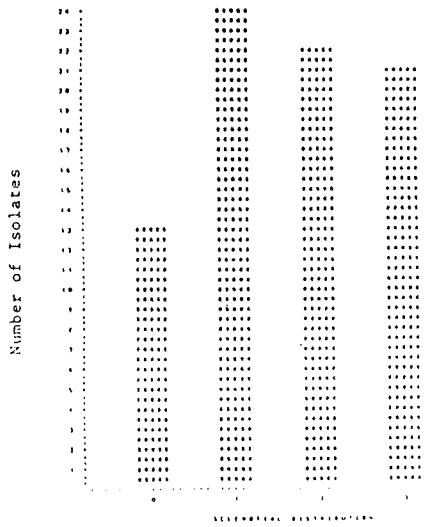
APPENDIX 4. Distribution of isolates into classes of mycelial and sclerotial characteristics.



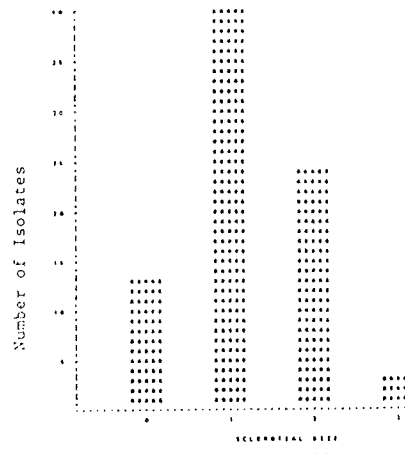
1= uniform
2= concentric



0= no sclerotia; 1= regular;
2= Irregular; 3= mixed.

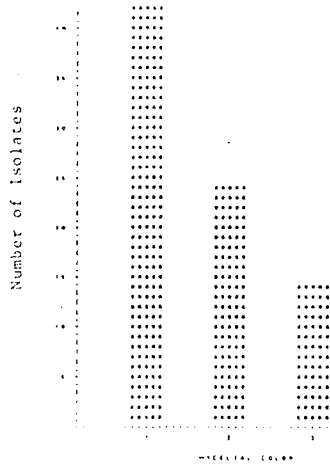


0= no sclerotia; 1= surface; 2= embedded
3= surface and embedded

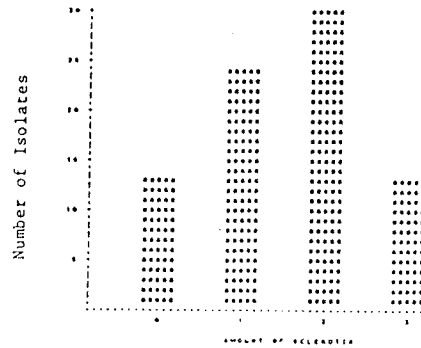


0= no sclerotia; 1= small;
2= medium; 3= large/confluent.

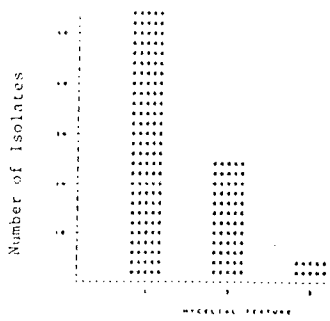
APPENDIX 4 (continued).



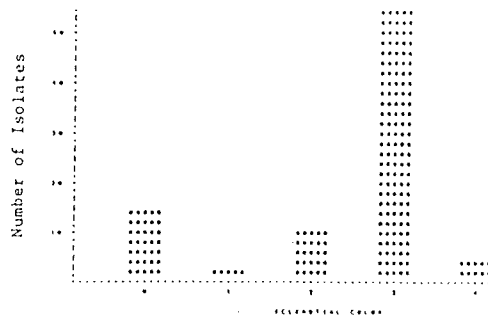
1= white; 2= light brown;
3= brown.



0= no sclerotia; 1= few; 2=moderate;
3= abundant.



1= appressed; 2= moderately aerial
3= aerial/cottony



0= no sclerotia; 1= white; 2= light brown;
3= brown; 4= black

APPENDIX 5 Source hosts, anastomosis group and pathogenicity of *R. solani* isolates.

ORS	ISOLATE	HOST	AG	faba			
				bean	peas	lentil	rapeseed
1	VF1	FABABEAN	2T7	1	1	1	1
2	VF2	FABABEAN	4	1	1	1	0
3	VF4	FABABEAN	5	0	0	0	1
4	VF5	FABABEAN	5	0	0	0	0
5	VF6	FABABEAN	4	1	1	1	1
6	VF7	FABABEAN	5	0	0	0	1
7	VF8	FABABEAN	5	0	0	0	0
8	VF12	FABABEAN	4	1	1	1	0
9	VF13	FABABEAN	4	1	1	1	0
10	VF14	FABABEAN	5	0	0	0	0
11	VF15	FABABEAN	5	0	0	0	0
12	VF19	FABABEAN	4	1	1	1	0
13	VF20	FABABEAN	4	1	1	1	1
14	VFS7	FABABEAN	5	0	0	0	0
15	VFS4	FABABEAN	4	1	1	1	0
16	VFS9	FABABEAN	4	1	1	1	1
17	VF73	FABABEAN	4	1	1	1	0
18	VF90	FABABEAN	4	1	1	1	1
19	PS3	PEAS	4	1	1	1	0
20	PS14	PEAS	4	1	1	1	0
21	PS30	PEAS	4	1	1	1	0
22	PS72	PEAS	4	1	1	1	0
23	PS81	PEAS	4	1	1	1	0
24	PS82	PEAS	4	1	1	1	0
25	GM6	SOYBEAN	4	1	1	1	1
26	LC25	LENTIL	4	1	1	1	0
27	LC47	LENTIL	4	0	1	1	1
28	LC48	LENTIL	4	1	1	1	0
29	LC50	LENTIL	4	0	1	1	0
30	LC88	LENTIL	4	1	1	1	0
31	LC89	LENTIL	4	1	1	1	0
32	LC92	LENTIL	4	1	1	1	0
33	PV21	BEANS	5	0	1	1	0
34	PV43	BEANS	2T1	1	1	1	0
35	PE16	PETUNIA	5	0	1	1	1
36	LT31	LATHYRUS	4	1	1	1	1
37	BR26	MUSTARD	4	1	1	1	0
38	LUR4	FLAX	4	0	1	1	1
39	RR9	CANOLA	2	0	0	1	1
40	BR11	CANOLA	4	1	1	1	0
41	BR23	CANOLA	4	1	1	1	0
42	BR24	CANOLA	2	0	0	0	1
43	BR27	CANOLA	4	1	0	0	1
44	BR28	CANOLA	2T1	0	0	0	1
45	BR29	CANOLA	3	0	0	0	1
46	BR46	CANOLA	2T1	0	0	1	1
47	BR40	CANOLA	2T1	1	1	1	1
48	BR61	CANOLA	2T1	0	0	0	1
49	BR62	CANOLA	2T1	0	0	0	1
50	BR63	CANOLA	4	1	1	1	0
51	BR64	CANOLA	4	1	1	1	0
52	BR65	CANOLA	2	0	0	0	1
53	BR66	CANOLA	2	0	0	0	1
54	BR67	CANOLA	2	0	0	0	1
55	BR68	CANOLA	4	1	1	1	0
56	BR69	CANOLA	2	0	0	0	1
57	BR70	CANOLA	5	0	0	0	1
58	BR71	CANOLA	4	1	1	1	1
59	BR74	CANOLA	4	0	1	1	0
60	BR84	CANOLA	4	1	1	1	1
61	BR85	CANOLA	4	1	1	1	1
62	BR86	CANOLA	2T1	1	1	1	1
63	ST49	POTATO	4	1	1	1	1
64	ST56	POTATO	3	1	0	0	1
65	ST75	POTATO	3	1	0	0	0
66	ST95	POTATO	3	0	0	0	0
67	BY51	SBEET	4	1	1	1	0
68	BY52	SBEET	4	1	1	1	0
69	BY53	SBEET	4	1	1	1	1
70	BY74	SBEET	4	1	1	1	0
71	BY77	SBEET	4	1	1	1	0
72	BY78	SBEET	4	1	1	1	0
73	BY79	SBEET	4	1	1	1	0
74	BY80	SBEET	4	1	1	1	0
75	RS100	PIHF	1	0	0	0	1
76	RR200	CRUCIFER	2T1	0	0	0	1
77	JC300	CARROT	2T1	1	1	1	1
78	ST400	POTATO	3	0	1	1	0
79	MS500	LEPILFA	4	1	1	1	1
80	MS400	SILE	1	1	1	1	1

1= pathogenic on host indicated

0= non-pathogenic.

APPENDIX 5 (continued); number of isolates pathogenic on faba bean, peas, lentil and rapeseed.

AG	number * of isolates tested	number of pathogenic isolates			
		faba bean (PI222125)	peas (Trapper)	lentil (Laird)	rapeseed (Candle)
1	1	0	0	0	0
2	6	0	0	0	6
2T1	8	3	3	4	7
2T2	2	2	2	2	2
3	5	2	1	0	3
4	47	43	46	46	14
5	11	0	2	2	4
Totals 80		50	54	54	37

* Number of isolates includes AG testers.