ISOLATION AND SCREENING OF PSEUDOMONAD ISOLATES FOR ANTAGONISM TOWARDS *GAEUMANNOMYCES GRAMINIS* VAR. *TRITICI*, CAUSAL AGENT OF THE TAKE-ALL DISEASE OF WINTER WHEAT

BY

JO-ANN STEBBING

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TABLEOFCONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Stebbing, J., M.Sc., The University of Manitoba. Isolation and screening of pseudomonad isolates for antagonism towards Gaeumannomyces graminis var. tritici, causal agent of the take-all disease of winter wheat. Major Professor: Dr. C.C. Bernier.

Fluorescent pseudomonad isolates recovered from two field soils continuously cropped to wheat for several years were compared for inhibitory activity towards the take-all disease of winter wheat. The occurrence of take-all was confirmed at the Minto, Manitoba location, but remained undetermined at the Indian Head, Saskatchewan location. Fluorescent pseudomonads were isolated from each of the soils using the selective King's Medium B. A total of 64 isolates was recovered from the two sources of soil. Each pseudomonad isolate was tested for its ability to inhibit Gaeumannomyces graminis var. tritici (Ggt) in vitro based on the production of zones of inhibition. The inhibitory actions of the pseudomonads were classified as non-inhibitory, moderately inhibitory and highly inhibitory. The isolates from the Minto location were more inhibitory than those from the Indian Head location.

In vivo antagonism of take-all by the fluorescent pseudomonads was assessed by applying isolates to Norstar winter wheat seeds using methylcellulose, and sowing the seeds into soil artificially inoculated with Ggt. The roots of the seedlings were washed after 4 weeks and rated for plant growth and disease parameters. There was a poor correlation between in vitro and in vivo inhibition of take-all by the fluorescent pseudomonads. Twenty-five

isolates demonstrating a range of antagonism, expressed as percent root infection and root dry weight were used in further assessment studies.

Inhibitory activity of the fluorescent pseudomonad isolates towards Ggt in a sterile and non-sterile soil was compared. The fluorescent pseudomonads were more effective in the sterile soil than the non-sterile soil due to the absence of competition in the sterile soil. Further evaluation of the pseudomonads in a non-sterile soil at low and normal temperature regimes indicated the isolates were more effective at the lower temperature, likely due to reduced microbial competition. In addition to assesssment of the pseudomonad isolates as individual treatments, Norstar seeds were treated with combinations of isolates to determine the effects of interactions between isolates on inhibitory action. Positive and negative synergistic reactions were observed between the isolates. Some combinations induced disease severity that exceeded either of the individual treatments, while other combinations reduced disease severity below the level observed for the individual treatments.

Chapter I

General Introduction

The take-all disease caused by the fungal pathogen Gaeumannomyces graminis Sacc. von Arx and Olivier var. tritici Walker (Ggt) has become a significant problem in areas continuously cropped to winter wheat. Growing concern has been expressed for the control of this disease since conventional methods do not provide adequate control or may be economically unfeasible (Lester, 1981; Weller, 1983; Weller and Cook, 1983; Capper and Campbell, 1986; Chao et al. 1986).

The practice of growing wheat in monoculture has led to the development of suppressive soils responsible for take-all decline (Slope and Cox, 1964; Weller and Cook, 1983; Wong and Baker, 1984; Weller et al. 1985; Wilkinson et al. 1985). This phenomenon is characterized by an increase in the disease severity during the initial years of monoculture, reaching a maximum of intensity, followed by a decline as the soils become suppressive (Weller et al. 1985).

The mechanisms governing suppressive soils are not fully understood, but antagonistic bacteria have been implicated in this role (Cook and Rovira, 1976; Smiley, 1978a; Weller and Cook, 1983; Wong and Baker, 1984; Weller et al., 1985). Cook and Rovira (1976) concluded the presence of fluorescent pseudomonad bacteria was necessary for the suppression of take-all either in natural field conditions or in pot bioassays. Smiley (1978a) demonstrated that populations of antagonistic fluorescent pseudomonads were higher on roots grown in take-all suppressive soils compared to take-all conducive soils. Weller and Cook (1983) were able to duplicate the effects of suppressive soils by

isolating and reintroducing antagonistic strains of fluorescent pseudomonads to the conducive system. They achieved yield increases ranging from 5 - 27 % as a result of the suppression of take-all rather than the inhibition of other pathogens or plant growth promotion. They suggested the most likely source of recovery of these antagonistic bacteria was the roots of wheat grown in soils where take-all decline had occurred or the wheat roots and the rhizosphere. Weller and Cook (1983) theorized that the probability of recovering antagonistic bacteria would be enhanced by isolating from the system where the bacteria were expected to function. Bacterial strains were isolated from soils where take-all decline had occurred, referred to as suppressive soils, and from non-decline or conducive soils. For the purpose of rapidly assessing the antagonistic abilities of these strains, Weller et al. (1985) developed a rapid screening test by applying bacteria in methylcellulose to wheat seeds, and growing these in the presence of take-all. They concluded that a greater proportion of the strains isolated from roots grown in take-all decline soil suppressed take-all compared with strains isolated from the take-all conducive soils.

This study was initiated to determine whether similar pseudomonad bacteria exist in soils originating from Minto, Manitoba and Indian Head, Saskatchewan, and to assess their inhibitory action towards the take-all pathogen. The first step in this study was the isolation of bacteria from sources most likely to contain the pseudomonad bacteria. The clay loam soil originating in Minto, Mb. was collected from a field continuously cropped to winter wheat for several years. The take-all disease was reported to occur in this field, but there was no indication of take-all decline. The soil from Indian Head, Sask. was collected from a number of plots located at the Agriculture Canada Research

Station in Indian Head, Sask. These plots had been continuously cropped to spring wheat, but there were no reports on the occurrence of take-all.

The soil from Minto was selected because, as suggested by Rovira and Wildermuth (1981) cereal roots infected with Ggt, in a non-suppressive system, may harbour a variety of fluorescent pseudomonads including those responsible for take-all decline. The Indian Head soil was selected for its cropping history of continuous spring wheat which may contribute to a rich bacterial microflora. According to Cook (1982), soils suppressive to take-all are the most likely source of recovery of antagonistic fluorescent pseudomonads, but they can also be recovered from soils cropped to wheat (Cook and Weller, 1983).

Once the bacteria had been isolated from their respective sources they were assessed for their ability to inhibit Ggt in vitro measured by the production of zones of inhibition. Isolates were then applied with methylcellulose to Norstar winter wheat seeds to assess their ability to inhibit take-all in vivo. Comparison of inhibitory activity in sterile and non-sterile soils and under different temperature regimes provided an indication of antagonistic activity when microbial competition is altered. Norstar seeds were treated with bacterial isolates individually and in combinations to observe the interactions between isolates and how this affects their ability to antagonize take-all.

Chapter II

Review of Literature

2.1 The Host

2.1.1 Origin and History

Currently 70% of the annual world wheat production is winter wheat (Fowler, 1983). In western Canada the area sown to winter wheat has increased considerably since 1980 (Austenson, 1986). This is attributable to herbicide development and the use of zero-tillage practices, permitting direct drilling of winter wheat into standing stubble. The stubble provides better moisture retention and snow cover, both of which are necessary for winter survival. Winter wheat production is preferable to spring wheat, where appropriate, due to its efficacy in terms of early moisture use and higher yields in response to nitrogen fertilizers.

Winter wheat is not a new crop to the prairie provinces. The earliest attempts at production were prior to 1790 in Manitoba and Saskatchewan. Winter wheat is believed to have been introduced to the prairie provinces by early settlers from areas familiar with winter wheat production such as eastern Canada, the U.S.A. and Europe (Austenson, 1986). Records dating back to 1912 indicate winter wheat production exceeded that of the spring wheats initially, but as the spring wheats became more common they soon replaced winter wheat.

Prior to the development of an export market, western Canadian winter wheat remained on the domestic market or in reserves for foreign aid. Recent increases in winter wheat quotas have provided an incentive for producers to expand their acreage and the Canadian Wheatboard will likely continue to

develop this market (Fowler, 1983).

2.2 The Pathogen

2.2.1 History and Distribution

The take-all disease is one of the classic plant diseases. The name derives from the extensive damage the pathogen causes to cereal roots (Nilsson, 1969). Among the root rot pathogens, it has one of the longest historical records dating back to 1852 in South Australia. In 1890 Prillieux and Delacroix identified a fungus as the causal organism of take-all and the name Ophiobolus graminis Sacc. was assigned to the pathogen (Garrett, 1981). The fungus has since been renamed Gaeumannomyces graminis Sacc. von Arx and Olivier var. tritici Walker (Ggt) (Walker, 1981). Gaeumannomyces graminis var. tritici is a member of the subdivision Ascomycotina in the order Diaporthales. In addition to the variety tritici, G. graminis var. avenae (Gga) and G. graminis var. graminis (Ggg) have also been identified. The former is capable of infecting oats as well as wheat and barley. The host range for the latter variety does not include wheat, barley and oats (Garrett, 1981) and is somewhat less defined. It is however associated with several grasses and a crown sheath rot in rice. The take-all disease is currently worldwide in distribution and is of particular prominence in areas continuously cropped to wheat. Initially Australia was considered to be the source of introduction of the take-all disease into North America, but a more likely explanation for its presence is a latent infection in native grasses which surfaced when the land was broken and sown to wheat (Russell, 1930).

The take-all disease did not pose a threat to wheat production in Canada until 1923, although reports of its existence in Saskatchewan date back to 1916.

Presently this pathogen can be found in all of the provinces (Martens et al. 1984).

2.2.2 Host Range

The take-all disease is mainly restricted to the family Gramineae. The genera Triticum, Hordeum and Agropyron are regarded as the most susceptible to take-all (Hollins et al. 1986). Phleum, Poa, Oryza sativa (rice), Setaria (millet) and Sorghum species are the least susceptible. Nilsson(1969) summarized the literature and presented an extensive list of more than 350 gramineous species demonstrating some degree of susceptibility to G. graminis including wheat, barley and rye. There have been reports of G. graminis infecting plants from other families, but these infections were largely the result of heavy inoculation under laboratory conditions. The resultant infections were never severe enough to penetrate the underlying vascular tissue.

The majority of the grasses infected by G. graminis belong to the Festucoideae subfamily. The order of increasing resistance of cereals to all isolates is generally wheat, barley, rye and oats with triticale somewhere intermediate between wheat and rye. Variability in resistance of these species may be due to differences in the root systems and root placement (Scott, 1981). The degree of susceptibility may also be related to the rate of natural cortical cell death where the ranking is wheat > barley > oats (Yeates and Parker, 1986). Take-all infection has the tendency to stimulate adventitious root production and rye has a greater capacity for root replacement than barley which in turn has a greater capacity than wheat (Scott, 1981). Nilsson (1969) reported six row barleys were less infected when compared to the two row varieties and susceptibility in spring cultivars was greater than winter cultivars

within the same species.

Barley appears to tolerate the disease better than wheat when artificially inoculated with Ggt at comparable rates. Garrett (1948) attributed this tolerance to a disease escape mechanism as opposed to a physiological phenomenon. Both wheat and barley produced more crown roots in response to infection by Ggt compared to uninoculated plants (Asher, 1972). Barley plants compensate for the loss of root material, as a result of infection, by producing more crown roots for a prolonged period of time.

Oats are resistant to infection by G. graminis var. tritici due to the presence of the inhibitory compound, avenacin. Gaeumannomyces graminis var. avenae has the ability to infect oats in addition to a broad range of cereals because it contains the enzyme avenacinase, which is able to degrade avenacin (Asher, 1981).

2.2.3 Epidemiology

The source of inoculum in a cereal crop is infected stubble residues or other plant debris (Martens et al. 1984; Skou, 1981). Infection may also arise from germinating ascospores, but their contribution is minimal (Garrett, 1939; Nilsson, 1969; Prew, 1980). The hyphae arising from the infected residues are thick-walled macrohyphae referred to as runner hyphae. These runner hyphae may grow ectotrophically along the surface of the wheat roots or between cortical cell layers (Gilligan, 1985). Runner hyphae characteristically have few organelles and limited endoplasmic reticulum and vesicles.

Branching of the runner hyphae gives rise to thin walled , hyaline microhyphae or infection hyphae (Skou, 1981). They are smaller in diameter with highly convoluted plasma membranes and more numerous endoplasmic

reticulum and vesicles. Gaeumannomyces graminis var. tritici is described as a highly invasive pathogen. The infection hyphae are capable of penetrating all six of the cell layers comprising the root cortex. This is accomplished through the process of enzymically eroding the cell walls. Growth of infection hyphae is radial from cell to cell once within the cortex. The hyphae pass through the endodermis and the stele to eventually come into contact with the vascular system. The combination of these events produces the early symptoms of take-all including the brown discolouration of the central vascular tissue and the appearance of the coarse, darkly pigmented hyphae on the root surface (Penrose, 1987).

At the point of penetration of the cell wall, a protuberance occurs, elongating at right angles to the cell wall. This protuberance is referred to as a lignituber and primarily occurs in those cell walls that reach maturity prior to fungal infection. The infection hyphae either outgrow the lignituber to enter the lumen or further advancement is restricted. Microhyphae failing to penetrate the epidermis turn brown and their walls become thickened resembling the macrohyphae. Once the hyphae have penetrated the stele, movement is in a lengthwise direction along the root.

Within the vascular system, the phloem tissue is colonized more rapidly than the xylem. The phloem is destroyed near the point of penetration. The roots cease to function below the point of penetration and the supply of assimilates is prevented (Gilligan, 1980a). Although the xylem tissue is colonized at a slower pace and is not rapidly destroyed, it eventually develops a brown discolouration and becomes blocked by gum-like deposits (Deacon, 1981). Extensive root damage during the course of the growing season can lead to the eventual death of the plant as a result of water and nutrient stress.

Severely infected plants are stunted and produce few tillers, particularly when the nodal roots are infected (Gilligan, 1980b). Infected tiller bases and roots appear dark brown or shiny black and the plants are prone to lodging. Plants in the heading stage may be bleached, an occurrence referred to as whiteheads, and often die prematurely, due to water restriction, or are exposed to a form of secondary infection (Sivasithamparam and Parker, 1978). The whiteheads contain shrivelled kernels or no kernels at all and are the result of a reduction in the supply of assimilates coinciding with the grain filling stage. Invasion by saprophytic moulds can cause the heads to become darkened. Winter wheat varieties are susceptible to freezing-thawing injury as a result of take-all infection. These plants dry out in the late winter or spring due to restricted water supply (Nilsson, 1969).

2.2.4 The Disease Cycle

The fungus overwinters in infected wheat and grass or other infected plant debris. An important source of infection in wheat is hyphae arising from fragments of host debris. The presence of take-all in a wheat field may be confirmed by examining the roots for vascular lesions. Early infections in which the fungus moves from the roots into the crowns are most damaging. Both old and young plants are susceptible to infection although the latter more readily (Deacon and Henry, 1980). During the seedling stage infection is primarily restricted to the roots although runner hyphae have been observed on the coleoptile. Sexual fruiting bodies, referred to as perithecia, may be observed on the stem base and leaf sheaths of heavily infected plants. These structures are bottle-shaped, occurring on the roots or inside the sheath of the outer leaf. The ascospores are exuded during moist conditions as a

yellowish-red mucoid mass. The ascospores germinate to produce a germ tube which may directly penetrate the host plant (Nilsson, 1969). As mentioned previously, this form of infection is of minor importance. This is attributed to a form of natural biological control imparted by the soil microflora. Brooks (1965) reported the inhibition of ascospore germination by the natural soil microflora, however ascospores were able to infect those portions of the seminal roots on the surface of the soil.

2.2.5 Saprophytic Survival

The duration of saprophytic survival is questionable. Saprophytic growth may continue for a few months to several years (Butler, 1953; Nilsson, 1969; Kollmorgen et al. 1985a; Wong, 1984; Wong, 1985) and its longevity may be influenced by soil type, texture, nutrients, pH, temperature, humidity, cropping history and soil microflora (Nilsson, 1969).

In a survey conducted by Sturz and Bernier (1985), the occurrence of take-all in winter wheat was greater in crop rotations of wheat, barley and rape compared to rotations with oats and flax. During the initial years of wheat monoculture selection pressure is for parasitic features and thus an increase in virulence. Once the disease reaches a peak in intensity the virulent portion of the fungal population is unable to survive between successive cereal crops. With the aid of other inhibitory factors such as the soil microflora, there is a shift in the population towards saprophytism (Shipton, 1981).

Shipton (1981) regarded nitrogen as the single most important factor governing survival, enhancing it when in surplus through facilitating assimilation of carbohydrate reserves. Garrett (1939) observed soluble nitrogen requirements were associated with available food material and the assimilation

of undecomposed carbohydrate reserves. Nitrogen uptake related to the soil pH influences the rate of hyphal growth. The rate of advancement of Ggt along the roots has been reported to increase as the soil pH rises above 5.0 (Garrett, 1936), the optimum for hyphal extension in culture being pH 6.0 -7.0. Reis et al. (1983) studied the relationship between soil liming, to increase the pH, and the availability of trace nutrients. They observed decreases in the tissue concentrations of the trace nutrients copper, zinc, manganese and iron in response to a pH rise from 6.5 to 7.5. Smiley and Cook (1973) and Smiley (1974) demonstrated ammonium-nitrogen uptake reduced the soil pH in close proximity to the root surface, but did not do so to the same extent in the surrounding bulk soil. The reverse effect was produced by the uptake of nitrate-nitrogen. This helps to explain the observation of rapid hyphal growth along the roots when nitrate is the source of nitrogen. Smiley (1978b) continued to work with this relationship and its influence on the soil microflora.

The disease is favoured by cool temperatures, the optimum being 12-20^{o} C, and poorly drained, compacted soils to the extent that oxygen does not become a limiting factor (Cook et al. 1972; Cook, 1981a; Glenn et al. 1985; Wong, 1984). In contrast, the saprophytic phase of Ggt is favoured by cool, dry conditions (15^{o} C, < -10 MPa). Wong (1984) reported cool and moist, warm and dry or warm and moist soil conditions did not promote saprophytic survival. In fact, the latter regime was responsible for virtually eliminating the pathogen. Soil moisture is important to the rate of growth of Ggt on the root surface. Cook et al. (1972) observed hyphal growth to be greatest in wet soils and decreased as the matric potential was lowered.

2.3 Control Practices

2.3.1 Crop Rotation

Crop rotation is considered to be a valid means of controlling take-all due to the pathogen's shortlived saprophytic ability. Numerous studies have been conducted on the use of break crops or non-host crops to reduce the amount of inoculum in the soil (Angell and Mills, 1951; Slope and Etheridge, 1971; Cook, 1981b; Kollmorgen et al. 1983; Kollmorgen et al. 1985b). Sturz and Bernier (1987) observed a general increase in frequency of both field occurrence and relative proportion of Ggt in winter wheat with each successive year of continuous cropping to cereals. Kollmorgen et al. (1983) reported higher grain yields in wheat following non-cereals or fallow. Some of the reasons provided for this success are a stimulation of the soil microflora unfavourable to the pathogen, exudation of antifungal compounds by non-host crops and depletion of the soil nitrate.

Nilsson (1969) recommended a break in cultivation, using the least susceptible crops, for a period of 3-4 years to provide adequate control. Sturz and Bernier (in press) concluded a 1 year rotation using either flax or canola was insufficient to completely eradicate the take-all pathogen in Manitoba. Winter wheat performed better when grown in rotation with non-cereals and barley. Improved agronomic practices were likely responsible for the reduction in inoculum potential of the pathogen. Although crop rotation can provide adequate control of take-all, it has not become a widely accepted practice. This is largely the result of economic conditions and the restrictions of rotations to a few profitable crops.

2.3.2 Fertilizers

The use of fertilizers in itself is not a method of control, but may be effective when used in an integrated control program (Huber, 1981). The most significant losses to take-all can be attributed to the combined effects of the pathogen and poor nutrition of the host plants. An inverse relationship exists between the severity of take-all and the nutritional status of the host plant. Glynne (1953) reported losses from take-all were more severe in inadequately fertilized crops simply because well nourished plants suffer less yield reduction than poorly nourished plants. The well nourished plants are able to replace the roots damaged by take-all. Response to fertilizer application is not an isolated event, but may be affected by other conditions such as pH, moisture, soil texture and competition (Trolldenier, 1985).

A reduction in take-all severity was observed in response to the application of the mineral nutrients nitrogen, phosphorous, potassium, sulphur, magnesium and chlorine, calcium, copper, iron, manganese and zinc (Nilsson, 1969; Huber, 1981; Christensen and Brett, 1985; Christensen et al. 1981; Powelson and Christensen, 1985; Huber, 1989). Deficiencies in these nutrients pre-dispose the plant to severe take-all infection. Six of these elements, nitrogen, phosphorous, calcium, potassium, magnesium and molybdenum have also been reported to increase take-all severity. The impact a nutrient has on take-all depends on the form of the nutrient and the rate of application (Huber, 1989).

A number of researchers have looked at the form of nitrogen and the time of application (Huber et al. 1968; Smiley, 1978b; Smiley, 1979; Huber, 1981; MacNish and Speijers, 1982; Gutteridge et al. 1987). Smiley and Cook (1973) offer two explanations for the effect of nitrogen on take-all severity.

Ammonium (NH_4^+) as a nitrogen source lowers the soil pH and by doing so directly affects the vigor of the take-all pathogen. Ammonium is also more readily available to the soil microflora due to a reduction in the rate of nitrification. Indirectly, ammonium enhances the soil microflora while at the same time rendering the take-all pathogen more susceptible to biological control. The nutrient status of a crop is influenced by a number of factors including moisture, temperature, pH, soil type, oxygen, compaction, weed and plant density competition, tillage, cropping sequence and seeding date. In order to minimize the severity of take-all using mineral nutrients it is necessary to adopt sound agricultural practices maintaining a balance of N, P and K and avoiding alkaline soils (Yarham, 1981). The advantage of supplying mineral fertilizers is two-fold, first it may offset the degree of damage from reduced plant efficiency and second aid in disease escape mechanisms through the production of new root growth (Huber, 1989).

2.3.3 Chemical Control

Fungicides have been proposed for the control of take-all, but little success has been achieved for various reasons (Jenkyn and Prew, 1973; Prew and McIntosh, 1975). One of the main problems encountered with fungicidal control of take-all is that the pathogen remains infective throughout the growing season and early applications of fungicides are often rendered ineffective later in the season (Bateman, 1980). The pathogen may be susceptible to the fungicide, but conventional applications as seed treatments or soil drenches often do not affect much of the inoculum. There is also some concern regarding the effects of fungicides on the soil microflora (Bockus,1983; Rovira and Whitehead, 1985). Fungicides have the potential to drastically alter the soil

microflora. Natural competitors of Ggt may be eliminated by fungicide application favouring re-colonization by the take-all fungus. And finally there is the problem of economics and the high cost associated with the application of fungicides. Fungicides are limited in their range of effectiveness and presently there is no commercial fungicide available that is registered for the specific control of take-all.

Bateman (1985) found Ggt to be susceptible to many fungicides when treated in vitro and Mathre and Johnston (1986) worked with a fungicide that proved to be successful in delaying infection. Garcia and Mathre (1987) obtained some promising results with the fungicide triadimenol. This treatment functions as a protectant of the crown tissue, restricting invasion by the pathogen and permitting the plants to continue adventitious root production even after the crown area has become damaged. Bateman and Nicholls (1982) concluded fungicides prolong the production of replacement roots and contribute to higher yields, but the rates of application make them uneconomical.

Crombie et al. (1986) worked with the antifungal agents, avenacins, found in oats. These compounds were found to inhibit take-all thus explaining the resistance of oats to Ggt. Gaeumannomyces graminis var. avenae, unlike Ggt, has the ability to infect oats due to enzymic deglucosylation.

Nilsson (1969) found that herbicides could influence the degree of infection by take-all. He observed an increase in take-all associated with herbicide use and attributed this to phytotoxicity which in turn predisposed the plant to take-all infection.

Soil fumigation with methyl bromide has been proposed as an alternative to fungicide application. Fumigation of the soil was demonstrated to enhance mineralization and inhibit the process of nitrification. The effects may however

be nullified by the addition of nitrate nitrogen and enhanced by the addition of ammonium (Bockus, 1983). As with the fungicides, there is some concern about the effects of fumigants on the soil microflora (Bateman, 1980). Ridge (1976) reported fumigation enhanced populations of fluorescent pseudomonads by providing them with a source of organic matter. The pseudomonads have the capacity to rapidly multiply and populate an area where other organisms are limited.

2.3.4 Breeding for Resistance

This is an attractive alternative in view of the lack of other effective control measures. Several attempts have been made to screen cultivars demonstrating sufficient resistance to take-all to warrant incorporation into a breeding program (Nilsson, 1969; Halloran, 1974; Scott and Hollins, 1985; Scott et al. 1985). Plant breeders frequently observed greater success based on seed source as opposed to cultivar. The selection pressure for take-all resistance has probably been slight for two reasons. First of all intensive cereal production, as opposed to conventional crop rotation programs, resulted in an increase in take-all and secondly, the existing microflora likely functioned in a manner similar to what is now referred to as biological control.

Rye possesses moderate resistance to take-all. Scott et al. (1981; 1985) worked with triticale in the hope that resistance from the rye parent could be incorporated into the hybrid, but its performance was closer to that of the wheat parent. Triticale seedlings were found to be similar to wheat seedlings in susceptibility, while plants in the adult stages demonstrated greater resistance. Scott et al. (1985) recommended triticale as a substitute for wheat in areas where take-all is severe.

Consideration has also been given to the transfer of the resistance factor found in oats, but there still remains the problem of Gga (Scott, 1981). In view of these problems, it would appear that wild species of Triticum and Hordeum might prove to be genetically convenient as sources of genetic resistance to take-all. To date, no species have been identified with greater variation than the existing wheat and barley cultivars. Nilsson (1969) reported a greater degree of resistance at a higher frequency in diploids compared to polyploids, but not to the extent observed in rye species. Phleum and Poa species demonstrated a greater degree of resistance to take-all, but they do not form fertile hybrids when crossed with wheat. Halloran (1974) working with Agropyron found two species, A. caninum and A. donianum, with resistance approaching that of rye, but again hybridization with wheat was unsuccessful. Transfer of the resistant material by means of chromosome substitution, translocation or homologous recombination may be too complicated and the wheat cultivar may not respond well to the introduction of large amounts of chromatin.

Induced mutations were considered as a means of incorporating resistance. A mutation could provide a new source of genetic variation since attempts at selection over several years have resulted in minimal success (Scott, 1981). Several aspects must be considered in a plant breeding program. The most obvious objective is to develop a high degree of resistance, but this may be an unrealistic goal. Perhaps a more realistic approach would be the identification and use of species exhibiting a tolerance to take-all infection or the ability to recover once infection has occurred (Nilsson, 1969).

2.3.5 Cross Protection

Cross protection is a form of biological control involving the suppression of a pathogen with the aid of a hypovirulent strain of the pathogen or an unrelated pathogen (Cook and Baker, 1983). Some of the mechanisms proposed to explain the concept of cross protection include antagonism and induced resistance. The former mechanism, antagonism, is a direct relationship involving antibiosis, competition or parasitism. The latter, induced resistance, is indirect and involves the induction of the host plant to activate its defense mechanisms in response to invasion by a non-pathogenic isolate.

Several reports of substantial take-all damage in the first wheat crop following grass appear in the literature. Contrary to this occurrence, Deacon (1973) reported reduced levels of take-all in the first year of a wheat-grass rotation and proposed the incorporation of short-term grass leys to reduce the severity of take-all under intensive cropping situations. He observed high populations of Phialophora radicicola to restrict the take-all fungus from actively colonizing wheat roots. This relationship persisted in subsequent wheat crops and the population of P. radicicola could be rapidly re-established with a variety of grasses and grass mixtures.

Wong (1975) described a similar occurrence where a nonpathogenic isolate of G. graminis var. graminis (Ggg) promoted cross protection in wheat infected with Ggt. Significantly greater grain yields were produced by the cross protected plants when compared to unprotected plants. Wong suggests this form of cross protection was achieved through a host induced response in the presence of Ggg. Gaeumannomyces graminis var. graminis did not penetrate the cortical tissues of the host plant, yet Ggt was restricted from the underlying vascular tissues. Asher (1978) suggested that a secondary thickening

of the host plant tissue, by means of suberization and lignification, may prevent a virulent isolate of Ggt from penetrating the endodermis. Wong and Southwell (1980) concluded that Ggt could be significantly reduced by the early colonization of seminal roots with hypovirulent strains of the fungus, particularly if the population of antagonists were larger than that of the pathogen.

2.3.6 The Take-all Decline Phenomenon

Slope and Cox (1964) were the first to coin the term "take-all decline" based on their research at the Rothamsted Experimental Station. With each successive crop of wheat, they observed less infection than in the previous crop. Take-all decline has since been described by Rovira and Wildermuth (1981) as "the spontaneous reduction in take-all and the subsequent increase in yield associated with the practice of continuous cropping to wheat". Disease incidence usually reaches a peak within 5 years after which time the disease declines (Shipton, 1972). According to Shipton, prior to the onset of decline, the disease must reach a maximum intensity in conjunction with a maximum inoculum concentration.

Take-all decline is not a new concept. The first report of this phenomenon dates back to 1935 based on work by Glynne (1935) at Rothamsted. She observed a reduction in take-all when wheat was continuously cropped. Outbreaks of take-all may occur from time to time during the decline phase, possibly in response to environmental conditions (Hornby and Henden, 1986). A break in monoculture with a nonhost crop may disrupt take-all decline while a prolonged break in cereal cropping reduces the inoculum level. Upon a return to continuous cereal production the disease cycle must develop all over again

leading to the decline phase (Cook, 1981b). Although a nonhost crop may help to reduce the amount of inoculum, such a break in cereals may result in more severe take-all outbreaks in a second or third crop when compared with cereal monoculture (Slope and Etheridge, 1971).

Soils from a field where take-all decline has occurred are referred to as decline or suppressive soils while those obtained from fields lacking take-all decline are referred to as non-decline or conducive soils. Huber and Schneider (1982) defined suppressive soils as "those soils in which disease development is suppressed even though the pathogen is introduced in the presence of a susceptible host". The term suppressive soils is used to describe the disease and pathogen interactions, including fungistasis and competitive saprophytic ability, where the disease is reduced in the presence of a susceptible host and the pathogen. The suppressive factor, in soils with low levels of disease, may be transferred to a soil conducive to take-all, rendering it suppressive (Shipton et al. 1972; Rovira and Wildermuth, 1981; Rovira, 1983).

Suppressiveness can be induced by a number of treatments including organic matter amendments, increasing the soil temperature to the range of 25-28^{o} C, minimum tillage practices and ammonium fertilization (Cook and Rovira, 1976). Baker and Cook (1983) offered three explanations for the occurrence of suppressive soils. One of the most obvious situations is failure of the pathogen to become established. In the event the pathogen succeeds in colonizing the host plant, damage as a result of infection, is minimal. In the third situation the pathogen becomes established causing disease for a period of time then becomes insignificant in terms of disease severity.

Wildermuth and Rovira (1977) demonstrated the ability of suppressive soils to lessen the hyphal growth of Ggt. With a reduction in root infection, top

growth was enhanced providing a greater photosynthetic area which in turn facilitated root replacement. They correlated hyphal density with the degree of disease suppression. Wildermuth et al. (1979) later reported a reduction in the number of emerging hyphae growing towards, and colonizing, the cereal host. The emerging hyaline hyphae were lysed, resulting in a subsequent reduction in both the number of lesions, and of hyphae colonizing the roots. Cook (1981b) confirmed suppressive soils prevented the progression of the disease rather than inhibiting the initial infection process. Simon et al. (1987) demonstrated suppressive soils have the capacity to inhibit both the parasitic and saprophytic phases of G. graminis var. tritici. Wildermuth (1982) found take-all suppressive soils reduced infection caused by other pathogens of wheat in addition to suppressing Ggt. Rhizoctonia solani, Gibberella zeae, Pythium irregulare, Cochliobolus sativus and Fusarium culmorum were reduced by soils suppressive to Ggt.

Simon et al. (1987) suggested the suppressive factor is not restricted to the surface of the root, but that its zone of effectiveness includes the rhizosphere and non-rhizosphere soil. Cook and Rovira (1976) described two kinds of suppression, specific and general suppression. The former type is thought to be responsible for the take-all decline phenomenon. Specific suppression is transferable and eliminated by moist heat of 60^{o} C, methyl bromide fumigation, soil pH and some forms of nitrogen (Hornby, 1979). The latter is considered a property of the soil and is associated with the overall soil microflora (Cook and Rovira, 1976). It is a non-transferable component, surviving moist heat treatment of 70^{o} C and methyl bromide soil fumigation (Cook and Rovira, 1976). The modes of action of general and specific suppression differ. Cook et al. (1986) attributed general suppression to a reduction in nutrient availability

enhancing competition within the soil microflora. Specific suppression on the other hand is thought to occur only after initiation of infection as opposed to pre-penetration of the host tissue.

Wildermuth (1980) was able to suppress Ggt in the absence of a cereal host implying the fungal hyphae may be the component upon which the suppressive factor is acting. Cook and Naiki (1982) considered the determinant responsible for take-all decline to be external to the take-all fungus and not an internal factor within the pathogen. Pope and Hornby (1975) concluded a biological factor, eliminated by moist heat treatments of 70^{o} C for 30 minutes, was responsible for the take-all decline phenomenon. Baker and Chet (1982) were further convinced the suppressive factor was biological when they confirmed its ability to multiply. The suppressive factor is present in the smaller particles of decline soils as well as the water extracts associated with particles in the range 0.2-2.0 μm.

Several hypotheses have been proposed to explain the take-all decline phenomenon including hypovirulence and mycophagous amoebae (Baker and Cook, 1983). Recently attention has been focused on the association of take-all decline with Pseudomonas fluorescens sp. Examination of soil samples where take-all decline is known to occur revealed higher populations of non-spore forming bacteria that are destroyed by moist heat treatments (Linderman et al. 1983).

2.4. Fluorescent Pseudomonads

2.4.1 Taxonomy

The genus Pseudomonas contains many plant pathogenic bacteria including those causing wilts, cankers, blights and soft rots, as well as non-pathogenic

difficult when they are not the dominant species (Sands and Rovira, 1970). The majority of the fluorescent pseudomonad species isolated for the purpose of biological control agents have belonged to the species Pseudomonas fluorescens and P. putida. Generally identification beyond the species level is difficult and no further attempts at classification are made; suffice it to say they are fluorescent pseudomonads.

2.4.2 Biological Control Agents

Weller (1985) regarded the pseudomonads as suitable biological control agents for numerous reasons. Fluorescent pseudomonads occur naturally in the root rhizosphere and are classified as nutritionally diverse. They have a rapid growth rate when compared to other rhizosphere inhabiting bacteria. Antibiotic- and siderophore-producing Pseudomonas spp. have been identified in relation to take-all decline. The bacteria, when introduced into a crop as a seed treatment, multiply and colonize the root system.

The fluorescent pseudomonads have received a great deal of attention as plant growth promoting rhizobacteria (PGPR) (Kloepper et al. 1980) and biological control agents (Weller and Cook, 1983; Weller et al. 1985). Several hypotheses have been proposed for the activity of fluorescent pseudomonads including siderophore and antibiotic production. Siderophores are iron chelators that function to tie up the available iron causing it to become limiting to the microflora, including fungal pathogens. The siderophores, pseudobactin and pyroverdine, have been identified in some strains of pseudomonads (Burr and Caesar, 1984).

Nielands and Leong (1986) reported the inhibition of G. graminis by Pseudomonas spp. producing siderophores and the antibiotic, phenazine. The

Pseudomonas spp. producing siderophores and the antibiotic, phenazine. The take-all fungus requires iron for growth along the root surface. According to Nielands and Leong, the siderophore production by fluorescent pseudomonads restricts this phase of growth along the root, while antibiotic production prevents the infection hyphae from penetrating the host. Rovira (1983) found that a soil conducive to take-all could be rendered suppressive by the addition of siderophore-producing pseudomonads, while the addition of ferric iron to a soil suppressive to take-all could render it conducive. Smiley (1978) suggests antibiotics are responsible for the reduction in take-all. Kloepper and Schroth (1981) support the suggestion that antibiotics are functional in the rhizosphere and are responsible for antagonism along the root surface.

2.4.3 Antagonism

The environment provided by roots infected with take-all promotes the activity of fluorescent pseudomonad species (Cook, 1982). Their frequency of recovery is enhanced by directly isolating from roots grown in soil exhibiting take-all decline. The higher frequency of recovery of fluorescent Pseudomonas bacteria associated with infected roots as opposed to healthy roots is well documented (Smiley, 1978a; Charigkapakorn and Sivasithamparam, 1987). Weller (1983) sampled populations of fluorescent pseudomonads from suppressive and conducive soils and concluded they were similar, but was able to demonstrate the population of inhibitory pseudomonads was 5-10 times greater on roots growing in suppressive soils. Brown (1981) monitored the populations of fluorescent pseudomonads in a wheat monoculture system. During the early years of monoculture, bacterial populations decreased, but subsequently increased. As the roots of a crop aged, there was an increase in the number

surrounding rhizosphere decreased (Sivasithamparam et al. 1979).

It was previously mentioned that the older roots are less susceptible to take-all infection possibly because of the activity of the bacteria interfering with hyphal growth and penetration of the root surface (Sivasithamparam et al. 1979). Cook (1985) proposed that since fluorescent Pseudomonas spp. normally colonize the roots they immediately respond to infected lesions on the wheat roots and utilize them as a nutrient source. With the aid of electron microscopy the bacteria were observed to proliferate in the lesions and provide protection against the secondary spread of take-all. Secondary colonization of the roots by these organisms is evident in very high populations thereby creating competition between the bacterial strains.

Electron microscopy revealed the bacteria colonize the healthy hyphae by directly attaching to them. They attack the outer layer of the bilayer cell wall, producing zones of lysis. In the more advanced stages of colonization, lysis pits, which coalesce to form small holes, may be observed in the cell wall. Eventually the hyphae lose their rigidity and collapse. The holes formed in the hyphae may be the result of the production of lytic enzymes by the bacteria (Foster et al. 1983). The role of bacteria in suppressing take-all is two-fold. They reduce the number of hyphae emerging from the inoculum source and cause lysis of the existing hyphae (Rovira and Wildermuth, 1981).

2.4.4 Bacterization

Seed bacterization is the process of applying beneficial bacteria to seeds. Biological control is now becoming a widely accepted means of controlling plant diseases. Weller and Cook (1983) and Weller et al. (1985) have researched the use of seed bacterization to reduce take-all in wheat. Strains of

fluorescent pseudomonads were isolated from wheat roots grown in soils suppressive to the take-all disease to increase the probability of recovering antagonistic bacteria. They worked with single isolates and various combinations thereof. Bacteria did not promote plant growth (Cook, 1982). They obtained variable results and degrees of success. Cook (1985) reported some degree of success, but disease suppression levels did not approach those achieved with take-all decline in monoculture wheat. The level of suppression attained was closer to the non-bacterized, infected check than the non-bacterized, non-infected control while there have been other reports of up to 27% yield increases with bacterization (Weller and Cook, 1983). This method of take-all suppression may be an alternative to wheat monoculture where severe losses are experienced during the first 3-4 years before take-all decline occurs (Weller and Cook, 1983).

The fluorescent pseudomonads, when applied as a seed treatment, move down the root surface from the site of seed inoculation with the aid of the vertical movement of water (Parke et al. 1986). Howie et al. (1987) reported the flagella were not important to the motility down the root surface, but the more likely explanation was a passive movement of the bacteria. The number of colonies decreased as the distance from the site of seed treatment increased and under optimal matric potential detection of colony forming units indicated an increase in the pseudomonad population.

Weller et al. (1985) developed a rapid screening technique for the selection of bacteria with the ability to antagonize the take-all pathogen. This technique enabled them to screen large quantities of bacteria in a limited space. Once potentially antagonistic strains were identified their pergormance could be evaluated in field tests. Capper and Campbell (1986) investigated the timing of

application of the suppressive bacteria and concluded pre-treatment of the plant roots prior to take-all infection was a more effective means of reducing infection than applying bacteria to a pre-existing infection.

Cook et al. (1988) have more recently researched the effects of bacterial treatment of wheat seeds in conjunction with fungicide treatments. They have also looked at the possibility of using Bacillus isolates to suppress take-all. In addition to this work, some researchers are starting to look at some of the deleterious aspects of certain pseudomonad isolates (Stroo et al. 1988).

Chapter III

Methods and Materials

3.1 Isolation and Storage of Bacteria

Three soil types were selected as potential sources of fluorescent pseudomonad bacteria; a clay loam from Minto, Manitoba, a sandy loam from Indian Head, Saskatchewan and a 2:1:1 greenhouse mixture comprised of black soil, sand and peat respectively. The third soil, the greenhouse mixture, was included as a form of control. Fluorescent pseudomonads antagonistic to the take-all disease were not expected to be recovered from this source, or at least not in high numbers.

Norstar winter wheat and HY320 seeds were surface-sterilized for 10 minutes in 0.1 % silver nitrate with a drop of Tween 80 wetting agent. The seeds were rinsed with three washings of sterile distilled water (SDW). Seven seeds of each cultivar were sown into each of the soils in 15.5 cm clay pots. The plants were thinned to four per pot after emergence. The plants were grown in the greenhouse for 4 weeks, after which the roots of three plants were removed from each pot and washed under running tap water to remove adhering soil.

The soils were retained in their respective pots after the seedlings had been removed. The pots were again sown to Norstar or HY320 seeds for two more cycles. Bacterial isolations were made from the roots and crowns of the plants from each of the soil types in each of the three cycles. Soil was also collected from each of the pots for use in serial dilutions. The purpose of recycling the soil and growing wheat repeatedly was to attempt to build up bacterial populations and enhance the probability of isolating potentially antagonistic pseudomonads.

Three methods of recovering bacteria from plant roots were used. In each, plants were selected whether they showed symptoms of take-all or not. In the first method, one plant from each of the samples was selected and the roots were surface-sterilized in a 2.5 % solution of sodium hypochlorite for 2 minutes then rinsed in sterile distilled water (SDW). The roots and crowns, including a 1 cm length of the stem above the crown, were sectioned and plated on two different media, modified King's Medium B (KMB), a selective medium for fluorescent pseudomonads, (Sands and Rovira, 1970, Appendix 1) and PDA, a non-selective medium. The PDA was included to provide an indication of the microflora present in addition to the fluorescent pseudomonads. The plates were incubated for 3 days at room temperature. The modified KMB plates were viewed under ultra violet light for production of fluorescent pigments.

In a second technique isolations were made from a non-sterilized root. A second plant was selected from each of the samples and the roots were crushed with a mortar and pestle in 100 ml phosphate buffer (pH 7.2). Serial dilutions with SDW ranging from 10^4 - 10^7 were plated on modified KMB. The plates were incubated and viewed as above.

In a third method, the non-sterile root surface of a sampled plant was pressed directly on to the surface of a modified KMB plate, leaving a slight imprint, then removed. The plates were incubated and observed as above.

In addition to isolation of bacteria from the roots, isolations were made from the soil. Soil samples previously collected from each pot were weighed into 1 g portions and placed in 99 ml SDW. The samples were shaken vigorously and dilutions of 10^4 - 10^8 were plated on modified KMB and nutrient agar. The nutrient agar was included to determine the composition of the soil microflora. After incubation as above, the modified KMB plates were

examined for fluorescence.

Bacterial isolates were retained on the basis of differences in cultural characteristics and source of isolation. The isolates were stored using the methodology of Weller and Cook (1983). Single colonies of each of the isolates were subcultured on Nutrient Broth Yeast Extract (NBY) for 24 h. A 3 ml sample of the bacterial suspension was then transferred to 3 ml of an 80% glycerol solution in a vial and shaken vigorously. The vials were sealed and stored at 3-4^{o} C. These cultures served as the source of bacteria for future experiments.

3.2 Preparation of Ggt Inoculum

Isolate WH.1 of Ggt used throughout these studies was originally isolated from roots of a winter wheat plant sown into winter wheat stubble in Minto, Manitoba in 1985 (Sturz, A.V., pers. comm.). The isolate, derived from a single ascospore, was identified as Gaeumannomyces graminis var. tritici and shown to be pathogenic on winter wheat. Gaeumannomyces graminis var. tritici cultures were established on PDA and incubated for future use.

One liter mason jars were filled 1/3 with gamma irradiated millet seed (24 h at 33 rad/sec) and tap water and allowed to soak for 20 h. The water was decanted and the jars sealed. A styrofoam plug was placed in the lid of each jar to facilitate gas exchange. The jars were autoclaved on 3 consecutive days for 1 h at 121^{o} C and 15 lbs pressure. The combination of irradiated millet seed and autoclaving was essential to destroy all possible contaminants. Agar plates colonized by Ggt were cut up and the pieces from one plate added to each jar of sterilized millet. The jars were incubated at room temperature for 4 weeks then stored at 3-4^{o} C and used for inoculum as required.

3.3 In vitro Testing for Antagonism

Of the 132 bacteria isolated from the three soils, using the various techniques, 64 were selected for further in vitro and in vivo assessment based on visual differences in colony appearance and source of isolation.

An 11 mm diameter plug was taken from the margin of an actively growing Ggt colony on PDA and transferred to the center of PDA plates and incubated at room temperature for 3 days. Each of the bacterial isolates stored in glycerol were re-suspended in nutrient broth and incubated until the suspension was turbid. A loop-full of the bacterial suspension was spotted on the periphery of a plate at four points equidistant from the fungal colony. The plates were again incubated at room temperature for 1 week. The degree of antagonism was measured by the width of the zone of inhibition produced between the bacterial isolate and the fungal colony. This test was repeated three times.

3.4 Bacterial Treatment of Wheat Seeds

Wheat seeds were coated with individual bacterial isolates as described by Weller et al. (1985). Norstar winter wheat seed was soaked in a steady flow of tap water for 20 h, then surface-sterilized as described previously. The seeds were dried under a flow of sterile air and 5 g added to each test tube. Two ml of a turbid nutrient broth suspension of bacteria were placed on a modified KMB plate and incubated at room temperature for 48 h. Four ml of a 1 % solution of methylcellulose (Methocel A15 C, Dow Chemical, Midland, MI) was mixed with the modified KMB plate and scraped into the test tube containing the wheat seeds. The test tubes were shaken vigorously and the seeds were dried under a steady flow of sterile air. Prior to assessing the bacteria coated seeds for activity towards take-all, ten seeds were selected from each treatment

and sampled to assess the number of colony forming units (cfu) present on each seed. The seeds were crushed with a mortar and pestle in 100 ml phosphate buffer (pH 7.2). Dilutions ranging from 10^4 - 10^9 were plated on modified KMB and nutrient agar and the numbers of colonies per treatment were determined.

3.5 Assessment of Antagonistic Activity

The methods used to assess the bacterial activity were as described by Weller et al. (1985) with some modifications. The experiments were conducted in plastic tubes (Ray Leach Conetainer, Canby, Or) suspended in a rack. The tubes were sterilized in a sodium hypochlorite solution and a sterile cotton plug was placed in the bottom of each tube. A 7 cm column of soil, inoculated with Ggt colonized millet kernels at 10 g per kg of soil, was added to the tube. The soil consisted of an Almasippi clay loam, gamma irradiated for 24 h at 33 rad/sec. Two bacteria-treated wheat seeds were placed directly on the inoculated soil and covered with a 2.5 cm layer of uninoculated Almasippi soil followed by a 2.5 cm layer of autoclaved vermiculite. Tubes using non-inoculated Ggt soil served as controls. The experiment was a completely randomized design with 10 replications.

The tubes were placed in a growth room with a diurnal cycle of 20^{o} C day and 15^{o} C night, 16 h/8 h respectively. The average light intensity recorded was 360 $\mu E\ m^{-2}\ s^{-1}$ and the humidity was set at 40 %. Initially, each tube received 10 ml of a 1/3 strength (v/v) Hoagland's solution. Thereafter the plants were watered twice a week with 10 ml SDW in addition to 10 ml of 1/3 strength (v/v) Hoagland's solution once a week. After emergence the seedlings were thinned to one per tube. The plants were removed after 4 weeks and the roots were washed free of soil. A total of 64 bacterial isolates were assessed

using this methodology. Two additional isolates, B-15132 (2-79) and B-15134 (13-79) (Weller et al. 1985), received from the United States Department of Agriculture, Pullman, Washington were included in the assays as standards to which the other isolates were compared.

The initial assessment was repeated three times to evaluate the repeatability of the experiment. The methods were modified slightly after each assessment. To facilitate tube drainage and root washing, the gamma irradiated Almasippi soil was mixed with autoclaved, washed sand at 3:1 (v/v) and 100 ml SDW. The amount of inoculum was reduced to five Ggt-colonized millet kernels per tube. The infected millet was placed on top of the Almasippi/sand mixture creating a zone of inoculum through which the wheat roots must pass followed by a 0.5 cm layer of uninoculated soil. The bacteria-treated seeds were placed on top of the soil and covered with autoclaved vermiculite. The nutrient solution was increased to 10 ml of full strength Hoagland's solution.

Two final repetitions of the bacterial seed treatment assessment were conducted simultaneously in separate controlled environment facilities, one in a growth room with the same growing conditions as described previously, and the second in a growth cabinet. The growth cabinet was programmed for the same temperature and daylength regime, but had a higher light intensity of 660 μE $m^{-}2\ s^{-1}$. Twenty-five representative isolates were selected for further evaluation in different soil and temperature systems.

3.6 Assessment of Bacterial Activity in a Non-sterile verses Sterile Soil

The inhibitory activity of 25 fluorescent pseudomonad isolates, representing a range of activity against take-all, was compared in sterile (gamma irradiated) and non-sterile Almasippi soil. Of the 25 isolates, 13 were from Minto and 12

from Indian Head. The isolates were selected on the basis of the plant growth and disease parameters of the previous assessments. The two U.S.D.A isolates were also included for comparison. The experiment was conducted in a growth cabinet and the procedures and experimental design were as described for the final assessment.

3.7 Temperature Effects on Bacterial Treatment of Seeds

The experiment was designed to compare the effects of two temperature regimes on the antagonistic activity of fluorescent pseudomonads towards the take-all pathogen in a non-sterile soil. The temperature regimes were established in separate growth cabinets. A diurnal cycle of 15^{o} C day and 10^{o} C night (16h/8h) respectively represented the lower temperature. The temperature and daylength conditions used in the previous experiment served as a comparison. In this test as in the previous, sterile versus non-sterile test, the root dry weights (RDW) and disease severity indices (DSI) were compared for each of the plants from bacteria-treated seed in the two temperature regimes.

Several of the isolates initially selected from the screening tests were eliminated prior to this experiment. The most effective isolates, namely 1, 6, 11, 36, 49 and 59 were retained. In addition to these, four new isolates, 10, 29, 41 and 58 were introduced to this trial. Isolates 10 and 29 from the greenhouse soil were included as a comparison of bacteria derived from a soil where Ggt is absent. Isolates 41 and 58 were included as they had demonstrated some potential in the initial assessments. The U.S.D.A. isolates 2-79 and 13-79 were again included in addition to the test isolates and ten replicates were set up for each treatment.

3.8 Mixtures of Bacterial Isolates

Isolates demonstrating the greatest advantage as seed treatments, based on root dry weights and the disease severity index in the previous temperature experiment, were selected for use as mixtures of bacterial isolates. Among the selected isolates were 36, 41, 49 and the U.S.D.A. isolates 2-79 and 13-79 which served as checks. The isolates were tested individually as well as in pairs. To mix the isolates for seed treatment, 1 ml samples were taken from turbid suspensions in nutrient broth and cultured together on modified KMB for 48 h.

The experiment was set up in two stages; one to assess the effects of seed treatment with mixtures of isolates after 4 weeks and a second to assess the effects after 8 weeks. Initially all the treated seeds were sown into inoculated, non-sterile Almasippi soil and placed in a growth cabinet. After 2 weeks, 10 plants from each of the treatments were transplanted into 500 ml cardboard containers. The remaining plants were returned to the growth cabinet for the duration of the 4 week period. The plants in the cardboard containers were also placed in the growth cabinet for the remaining 2 weeks and an additional 4 weeks (8 weeks total). The watering regime for the transplanted treatments was changed to 20:20:20 NPK fertilizer three times a week. The procedures and experimental design were as previously described. Upon completion of these two trials, the plants were removed and the roots were washed free of soil under running tap water. Plant growth and root disease severity observations were made on the two sets of plants.

3.9 Disease Assessment

When assessing the plants for the extent of disease, both the plant growth parameters and the disease severity were evaluated. Plant growth was measured

by plant height (cm), root vigor, shoot dry weight (g) and root dry weight (g). Root vigor was determined on a scale of 1-4 where 1=vigorous, 2=moderate, 3=poor and 4=dead. Disease was assessed on a scale based on the the percentage of roots infected by the take-all pathogen and ranged from 0-4 (Sturz, A.V., pers. comm.) where 0=no disease, 1=1-25%, 2=26-50%, 3=51-75% and 4=76-100%. The crowns of the plants were visually rated on a scale from 1-3 based on the extent of discolouration where 1=no discolouration, 2=moderate discolouration and 3=severe discolouration. Potentially suppressive bacteria were selected on the basis of a disease severity index (DSI), derived from the root infection scale.

Disease severity index (DSI) was computed as follows (Nilsson, 1969):

$$DSI\ (\%) = \frac{100 \times \Sigma (x_i \times f_i)}{\text{Total number of plants examined} \times \text{Maximum rating}}$$

where

x = disease number
f = number of plants in class

classes 0-4 of the root infection scale

Chapter IV

Results

4.1 Isolation of Bacteria

A total of 132 bacteria were isolated from the three soils, using the various techniques. The isolates were segregated according to source of isolation. Sixty-four isolates could be distinguished based on visual differences. The frequency of recovery of pseudomonad bacteria was greatest from the Minto and Indian Head soils and least from the greenhouse mixture (Table 1). More bacteria were recovered by plating the roots and crowns than by the other two methods.

The bacteria isolated were referred to as fluorescent pseudomonads if they produced a fluorescent pigment on modified KMB under ultra violet light, and no further attempts at identification were made.

4.2 In vitro Antagonism

A variety of reactions were observed between Ggt and the potentially antagonistic bacteria during the in vitro testing for antagonism (Table 2). Although a definite zone of inhibition was not evident for all of the bacterial isolates, other reactions were observed. Figure 1 represents four stages of in vitro antagonism. Generally, when there was no inhibition, the Ggt colony overgrew the bacterial isolate. Some isolates initiated variations of this reaction causing the aerial hyphae to be flattened while the fungal colony continued to overgrow the bacteria.

Another common reaction was the prevention of advancement of the leading edge of the Ggt colony in the presence of the bacterial isolate. Initially a

Table 1. Recovery of fluorescent pseudomonad bacteria from three soils using three isolation techniques

Isolation Technique	Origin of Soil		
	Minto [a]	Indian Head [b]	Greenhouse [c]
Root/Crown Section (KMB and PDA)	28	29	7
Crushed/Pressed Root (KMB)	22	17	0
Soil Serial Dilution (KMB and NA)	21	6	2
Total	71	52	9

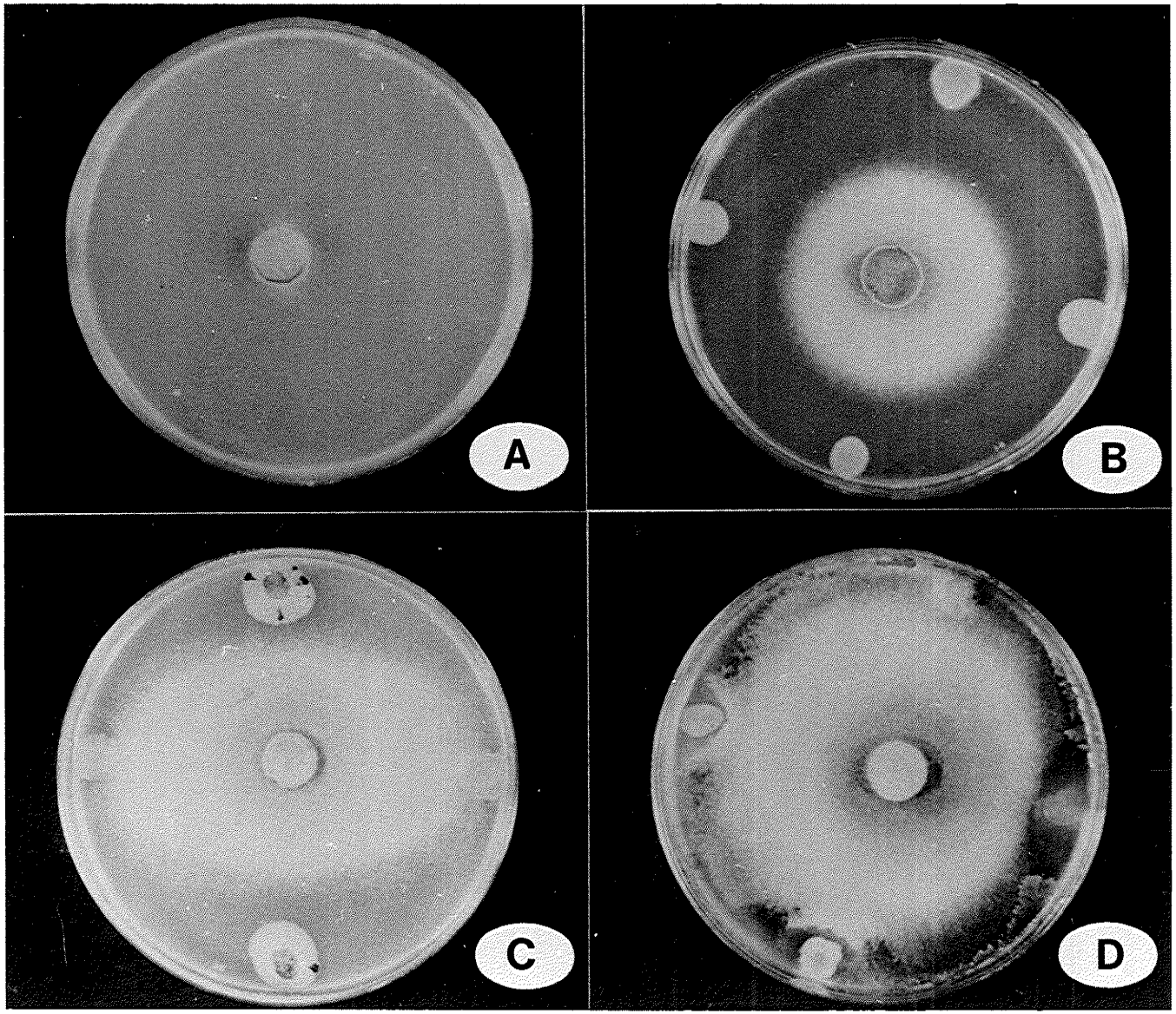
[a]Take-all present
[b]Take-all absent, cropping history of continuous wheat
[c]No take-all or cropping history of continuous wheat

Table 2. Summary of in vitro interactions between fluorescent pseudomonad bacteria recovered from three soils and Gaeumannomyces graminis var. tritici on PDA after 8 days

Interaction	Origin of Soil		
	Minto	Indian Head	Greenhouse
1*	10	13	1
1a	1	5	1
2	8	7	0
2a	3	1	0
3	7	5	0
3a	1	1	0
Total	30	32	2

*Explanation of interactions:
1 - Ggt uninhibited by bacterial isolate
1a - Ggt overgrows bacterial colony, but aerial hyphae are flattened
2 - Ggt inhibited, but continues to grow around the bacterial colony
2a - the aerial hyphae of the Ggt colony are flattened on the leading edge growing towards the bacterial colony
3 - advancement of Ggt colony margin inhibited forming a defined zone of inhibition
3a - zone of inhibition and flattening of aerial hyphae

Figure 1. In vitro antagonism of Gaeumannomyces graminis var. tritici when challenged with four isolates of pseudomonads. a)unchallenged Ggt culture. b)three days after challenging c)seven days after challenging, inhibition by two isolates. d)twelve days after challenging, the fungal colony has overgrown three of the four isolates


A
B
C
D

clearly defined zone was evident. In some instances superficial mycelium overgrew the bacterial isolate, but in other reactions the zone persisted. In the latter case the Ggt continued to grow around the bacteria making minimal contact with the periphery of the colony. The most pronounced of the reactions was the formation of a measurable zone of inhibition. The width of the zone ranged from 1.0-9.0 mm after 8 days of incubation.

The reactions consisted of three classes representing no inhibition, moderate inhibition and severe inhibition of the fungal pathogen. Of the 30 fluorescent pseudomonad isolates recovered from Minto, 19 isolates induced some degree of inhibitory action towards the pathogen in vitro. Eight of these isolates were considered highly inhibitory with a 3 or a 3a interaction causing the formation of a defined zone of inhibition. The other 11 isolates were classified as moderately inhibitory, with a 2 or a 2a interaction causing the Ggt colony to grow around the bacterial colony. The 11 isolates classified as non-inhibitory, with a 1 or a 1a interaction were overgrown by the Ggt colony and no zone of inhibition was induced. In comparison, 14 of the 32 Indian Head isolates tested expressed inhibitory activity. Six isolates were highly inhibitory and eight were moderately inhibitory. The remaining 18 isolates were regarded as non-inhibitory. The two isolates from the greenhouse soil mixture failed to demonstrate any degree of antagonism in vitro.

4.3 Assessment of Antagonistic Activity

The results of the colony forming units for the bacterial seed treatments are presented in Appendix 2. The bacterial isolates were classified based on their ability to antagonize the take-all pathogen in vivo. Table 3 summarizes the level of activity of the bacteria-treated seeds towards Ggt compared to the

Table 3. Summary of effects based on disease severity index of isolates of fluorescent pseudomonads from Minto and Indian Head when applied as a seed treatment to Norstar winter wheat for the control of Gaeumannomyces graminis var. tritici

Repetition	Class I[a]		Class II[b]		Class III[c]	
	Minto	Indian Head	Minto	Indian Head	Minto	Indian Head
1	25	27	0	1	4	3
2	14	12	0	0	16	20
3	22	20	0	1	8	9
4	3	1	0	0	27	31
5	15	11	0	0	15	21

[a]Class I - Bacterial seed treatment producing plants with a DSI lower than the Ggt check

[b]Class II - Bacterial seed treatment producing plants with a DSI equal to the Ggt check

[c]Class III - Bacterial seed treatment associated with a DSI greater than the Ggt check

Ggt infected check. The isolates were segregated into one of three classes depending on whether the disease rating was greater, less than or equal to the Ggt check.

The disease severity values for the first three repetitions ranged from 25 - 67.5 %. In the fourth and fifth repetitions the ratings were higher ranging from 37.5 - 80 % (Appendices 3 - 7). From this information, 25 bacterial isolates were selected representing a range of activity towards Ggt. Each of the classes I and III were represented by the selected isolates. Of the 25 isolates, 13 were derived from the soil originating from Minto and 12 from the soil originating from the Indian Head source. None of the isolates recovered from the greenhouse soil demonstrated any degree of antagonism either in vitro or in vivo.

4.4 Activity of Bacteria in a Sterile vs Non-sterile Soil

Plants grown from bacteria treated seeds in the sterile soil did not demonstrate significant increases in root weight compared to the Ggt infected check (Appendix 8). Isolates 27, 6 and 14 produced plants with greater dry weights than the Ggt check (Figure 2a and 2b), but these differences were not significant. Seeds treated with these isolates were associated with root weights exceeding all other treatments including the two isolates, 2-79 and 13-79 from the U.S.D.A. Although treatments with these isolates resulted in plants with higher root dry weights, the other growth parameters (Appendix 10) didn't always reflect this advantage.

In terms of disease severity in the sterile soil, 88 % of the bacteria-treated seeds produced plants rated lower than the Ggt check for disease severity (Appendix 10). Plants grown from seeds treated with isolates 5, 16, 35, 49 and

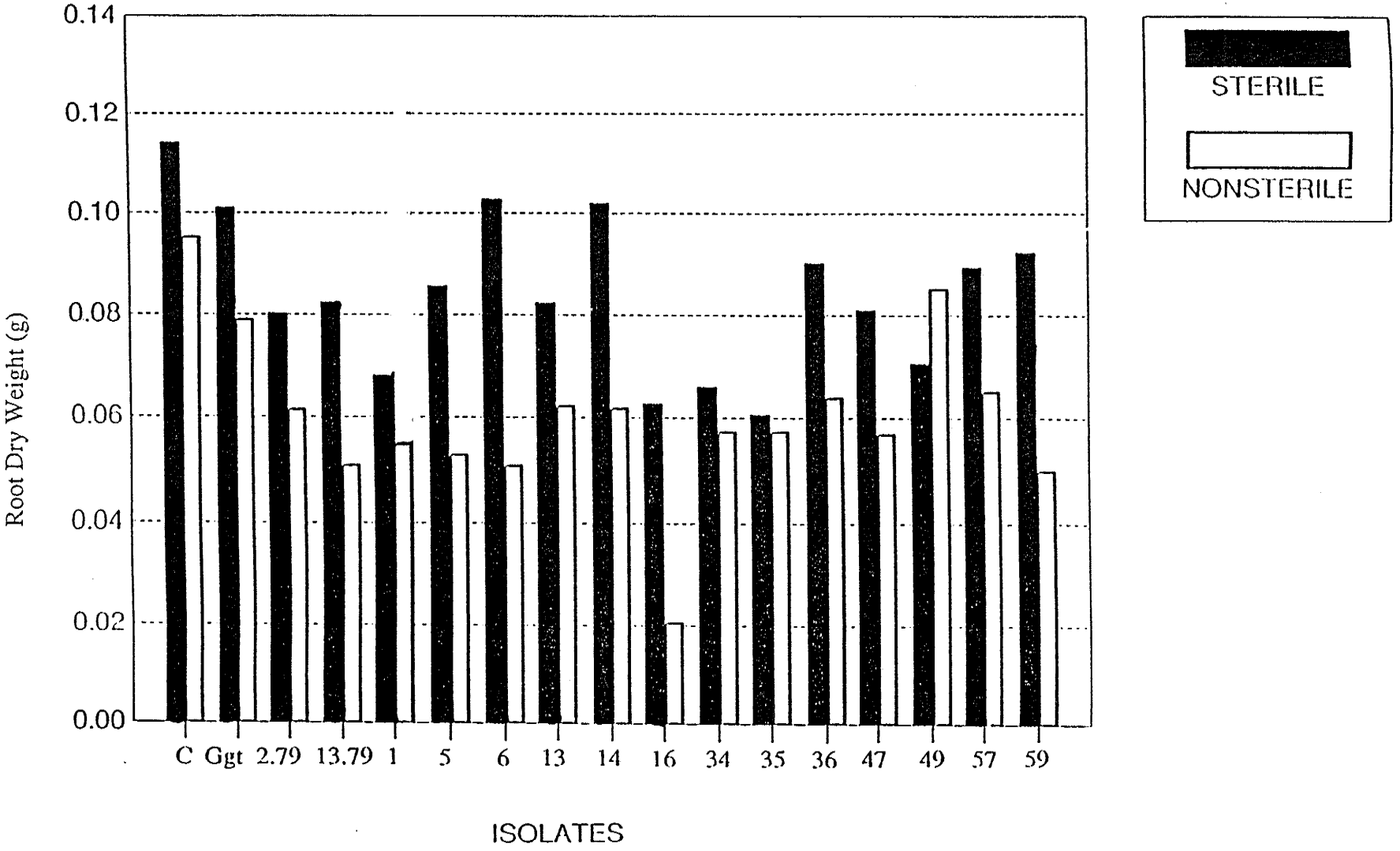


Figure 2a. Root dry weight of Norstar seedlings treated with isolates of pseudomonads from Minto for the control of Gaeumannomyces graminis var. tritici in sterile and non-sterile systems.

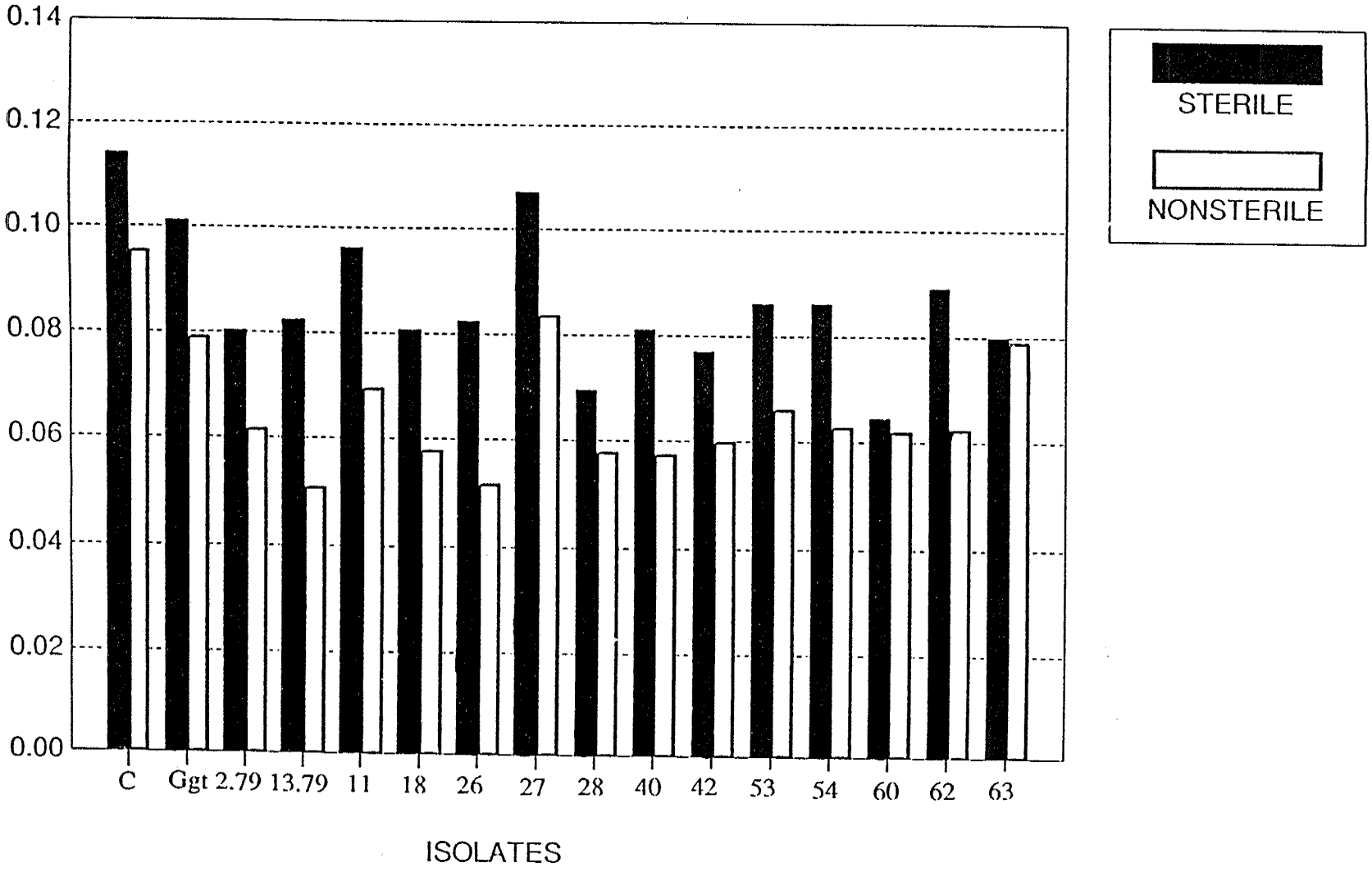


Figure 2b. Root dry weight of Norstar seedlings treated with isolates of pseudomonads from Indian Head for the control of Gaeumannomyces graminis var. tritici in sterile and non-sterile systems.

60 had disease severity values considerably lower than the Ggt infected check.

In the non-sterile soil, isolates 49, 27 and 63 were associated with plants with root dry weights higher than the Ggt check (Appendix 9), but with regards to the other plant growth parameters there was poor expression (Appendix 10). The disease severity index in the non-sterile soil indicated treatments with isolates 5, 26 and 36, were effective in reducing the amount of infection.

A comparison of the two soil regimes indicated 92 % of the isolates produced greater root mass in the sterile soil, compared to the corresponding isolates in the non-sterile soil. Only 12 % and 16 % of the isolates in the sterile and non-sterile soil, respectively, produced greater root dry weights than the infected check. Generally there was no significant increase in the root dry weights of the bacteria treated seeds, including isolates 2-79 and 13-79, compared to the Ggt inoculated check.

With regards to disease severity, 60 % of the isolates had a lower index rating in the sterile soil compared to the non-sterile soil. The advantage of seed treatment was not very significant when compared to the Ggt inoculated check. The disease severity ranges were 41.7 % -97.5 % in the sterile and 60.0 -100 % in the non-sterile. In the sterile soil 88 % of the isolates had lower ratings than the infected check whereas only 80 % were rated lower in the non-sterile system.

Differences in the levels of effectiveness of bacterial seed treatment were not as evident in the plant height and shoot dry weight parameters. Only 44 % of the isolates demonstrated an advantage in terms of plant height, while 59 % produced greater shoot dry weights. These latter two parameters are not very useful measurements of the effects of bacterial seed treatments on take-all.

The U.S.D.A. isolate, 2-79, did not perform well in either of the soil regimes. In both systems, 2-79 had a higher disease rating than the infected check. Isolate 13-79, also from U.S.D.A., was more effective in the sterile soil, but again the disease severity associated with this treatment was higher than the check. Figure 3 provides a visual comparison of seedlings in the sterile and non-sterile regimes.

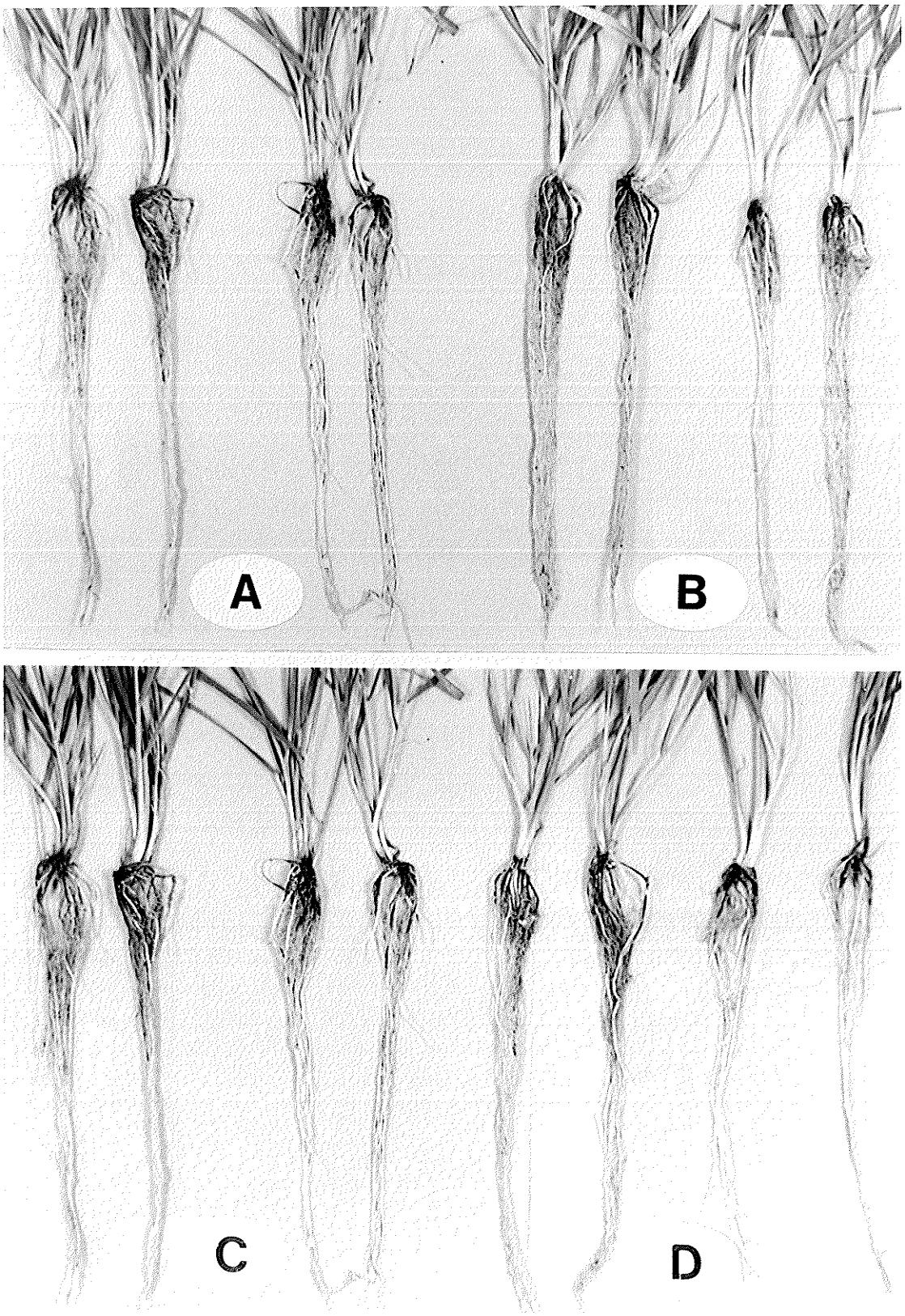
4.5 Temperature Effect on Seed Treatment

At the $20^{o}/15^{o}$ C temperature none of the bacteria-treated seeds produced plant root dry weights significantly greater than the Ggt check (Appendix 11). The root dry weights of plants from seeds treated with bacterial isolates 6 and 29 were significantly lower than that of the Ggt check suggesting that these treatments enhanced the severity of take-all.

In the low temperature regime ($15^{o}/10^{o}$ C) there were no significant differences in root dry weight with the exception of isolates 29, 36 and 49 which had very low root mass (Appendix 12). A comparison of the two temperature regimes and the root dry weights for each of the treatments are presented in Figure 4.

Isolates 29 and 49 produced plants with a lower disease severity index at the $20^{o}/15^{o}$ C temperature (Appendix 13). There were no differences between the remaining isolates. With the exception of isolates 1, 11 and 59, all other isolates generated a lower disease severity index than the Ggt infected check at the lower temperature. Although the differences were not significant, isolates 6, 29, 36, 41, 49 and 13-79 were fairly effective reducing the percentage of root infection. Isolates 29 and 49 were exceptionally good treatments based strictly on disease severity, but there was a poor correlation between disease severity

Figure 3. Level of infection induced by Gaeumannomyces graminis var. tritici on seedlings treated with isolates of pseudomonads in a sterile and non-sterile system. a) and c) Ggt inoculated check in sterile (left) and non-sterile soil (right) b)isolate # 36 in a sterile (left)and non-sterile soil (right) d)isolate 13-79 in sterile (left) and non-sterile (right).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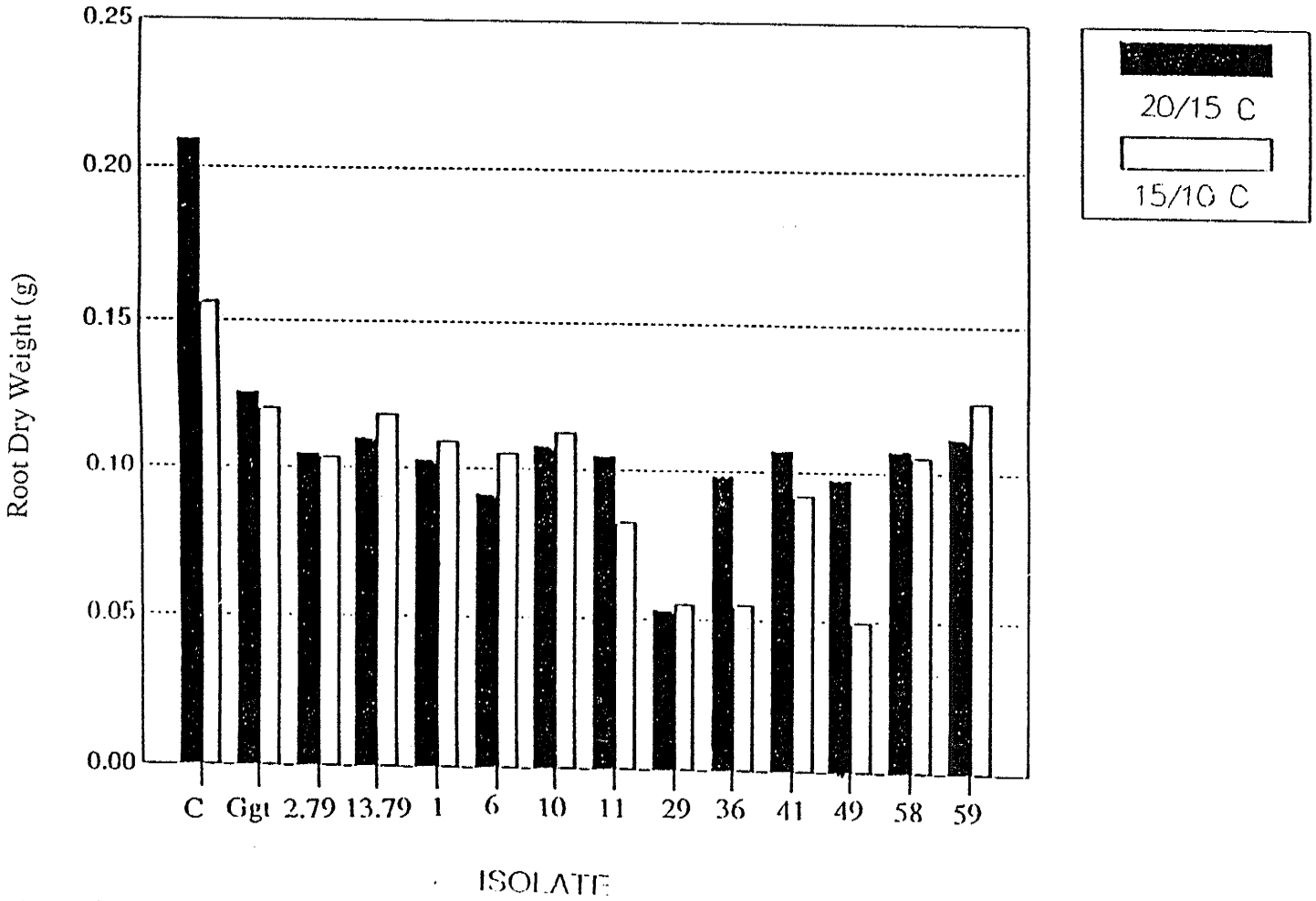


Figure 4. Root dry weights of Norstar winter wheat seedlings treated with isolates of pseudomonads and inoculated with Gaeumannomyces graminis var. tritici in normal and low temperature regimes.

and root dry weight. Methylcellulose seed treatment with isolate 29 severely impeded germination and only three of the ten treated seeds germinated.

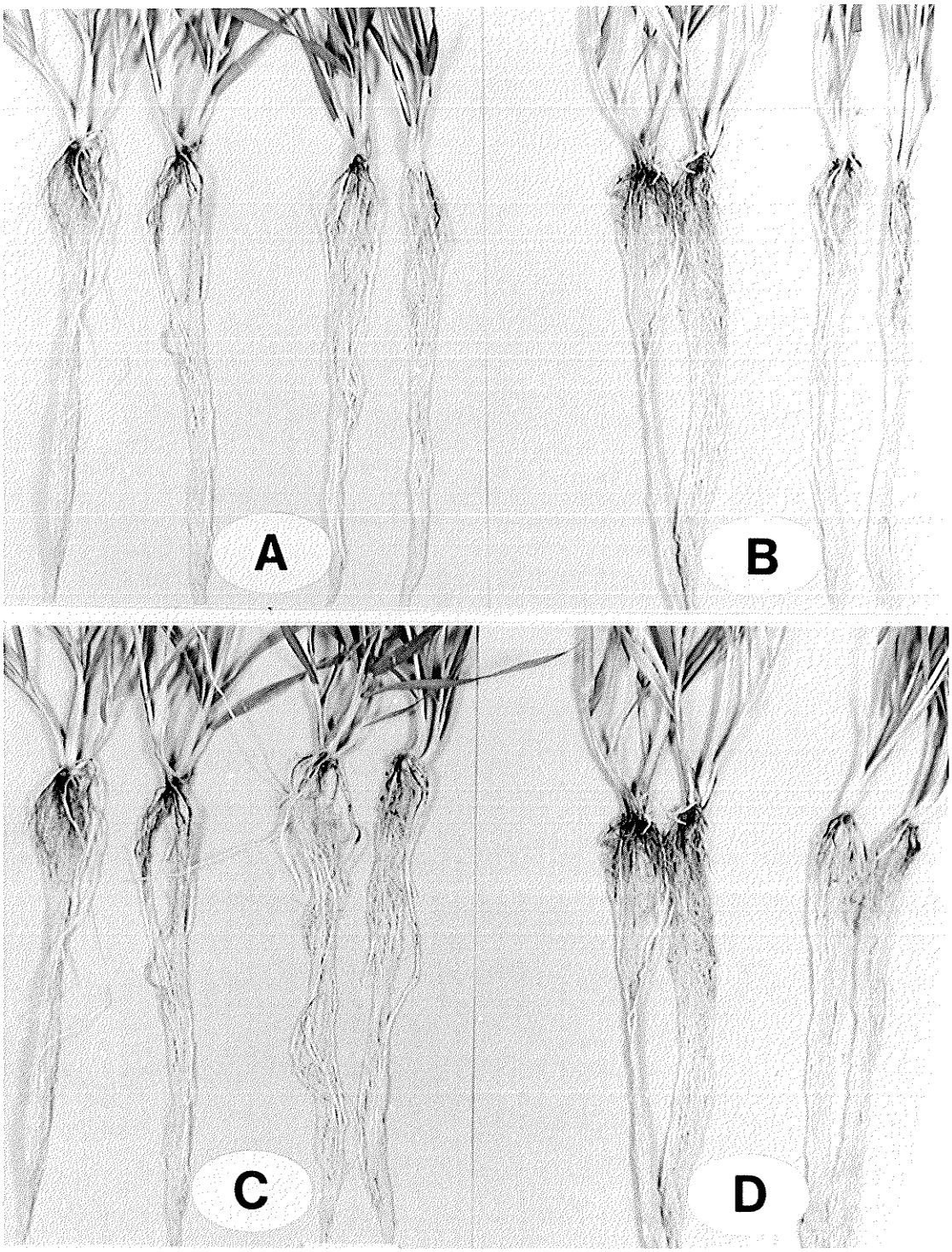
More isolates were beneficial at the lower temperature contributing to reduced disease severity ratings, however this may not be an indication of the bacteria being effective antagonists, but rather that the lower temperature reduced the competition of the indigenous microflora. Figure 5 illustrates the root systems of plants grown from seeds treated with fluorescent pseudomonad isolates 36 and 41 in each of the two temperature regimes.

A linear regression analysis was performed on the data to determine the relationship between root dry weight and the disease severity index in the two temperature regimes (Appendix 14 and 15). The lower temperature indicated a non-significant relationship between the extent of root infection and the root dry weight. At the higher temperature, however, there was a significant relationship at the 1 % level of significance for disease severity and the 5 % level for root infection. This implies that at the higher temperature, a linear relationship exists between the disease severity and the root dry weight as well as the root infection and the root dry weight.

4.6 Bacterial Treatment of Seeds Using Mixtures of Bacterial Isolates

A comparison of the root dry weights indicated no significant differences between the treatments using individual and combined isolates and the Ggt infected check (Appendix 16). Combinations of isolates were however more effective than others in reducing the level of infection on the roots (Figure 6), but again these differences were not significant. The most effective treatment was evident in plants treated with the combination of isolates 36 and 41 which received a disease index value lower than all the other treatments involving

Figure 5. Comparison of the root systems and degree of infection at two temperature regimes. a)Ggt infected check (left) and isolate 36 (right) at $15^{o}/10^{o}$ C. b)Ggt infected check (left) and isolate 36 (right) at $20^{o}/15^{o}$ C. c)Ggt infected check (left) and isolate 41 (right) at $15^{o}/10^{o}$ C. d)Ggt infected check (left) and isolate 41 (right) at $20^{o}/15^{o}$ C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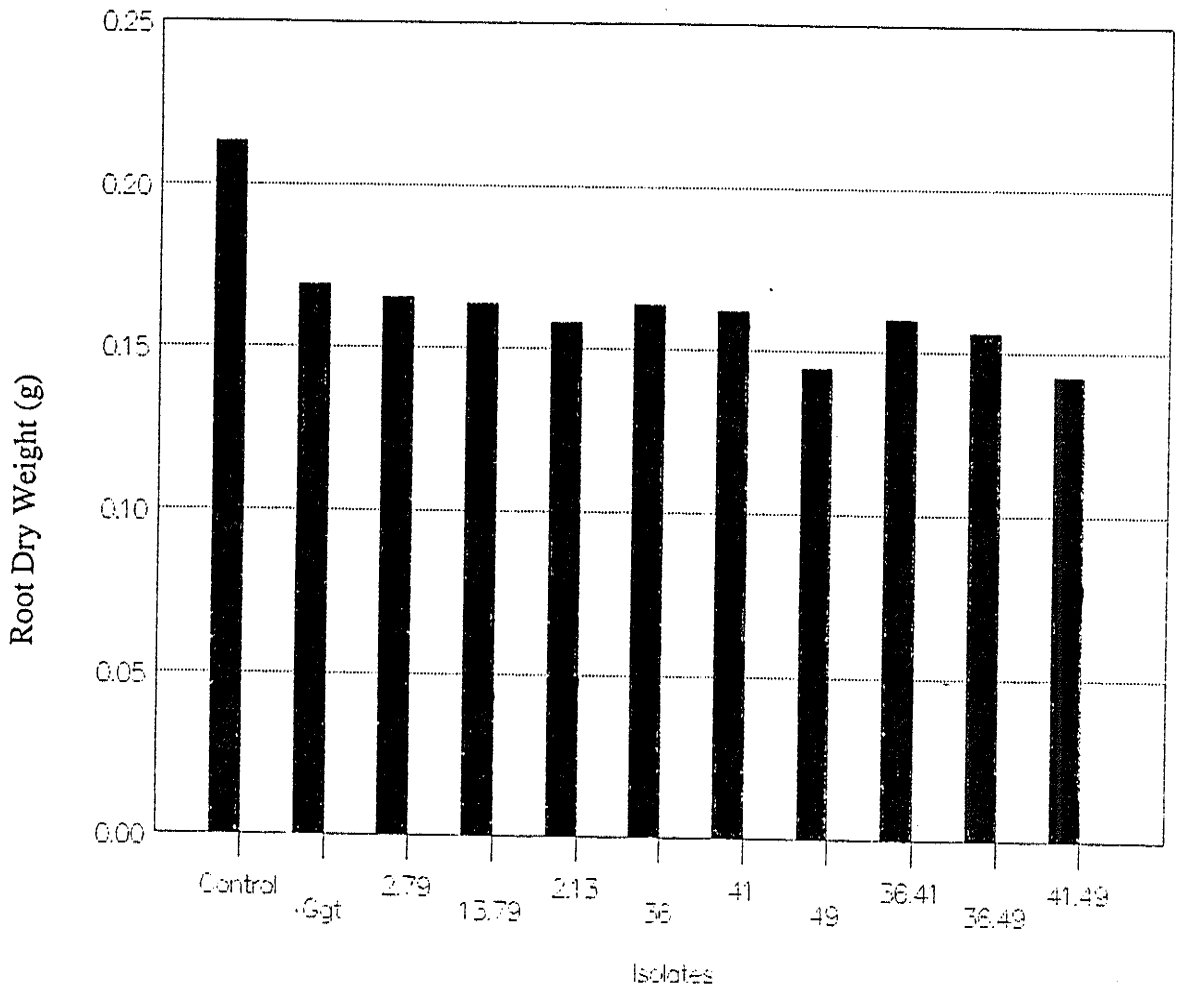

Figure 6. Comparison of individual and combinations of pseudomonad isolates for effects on root dry weight of 4 week old Norstar winter wheat when inoculated with Gaeumannomyces graminis var. tritici.

combined or individual isolates. Plants grown from seeds treated with this combination of isolates had the highest root dry weight after 8 weeks and the lowest disease severity index (Appendix 17) compared to other combination treatments. Root dry weight production for this treatment exceeded the uninoculated check after eight weeks (Figure 7). Another effective combination was comprised of isolates 41 and 49. Although the root dry weight for this treatment was lower than either of the individual treatments, the roots appeared to be less infected than the Ggt check. All other treatments had significantly lower root dry weights and higher disease severity ratings than the inoculated check.

The poorest treatment exhibiting 75 - 100 % infection of the roots was the mixture comprised of the two isolates, 2-79 and 13-79, from the United States. The disease severity of the combined treatment exceeded the percent root infection of the individual isolates. Isolate 13-79 received a disease index of 82.5% while 2-79 received 92.5%. As a combined treatment these isolates were more detrimental than beneficial to the plant based on root infection. Figure 8 illustrates the degree of infection on the root systems of plants treated with isolates 36 and 41 individually and in combination. Figure 9 provides a visual comparison of plants grown from seeds treated with the individual isolates 36 and 41 and the combined treatment using the same isolates after 8 weeks.

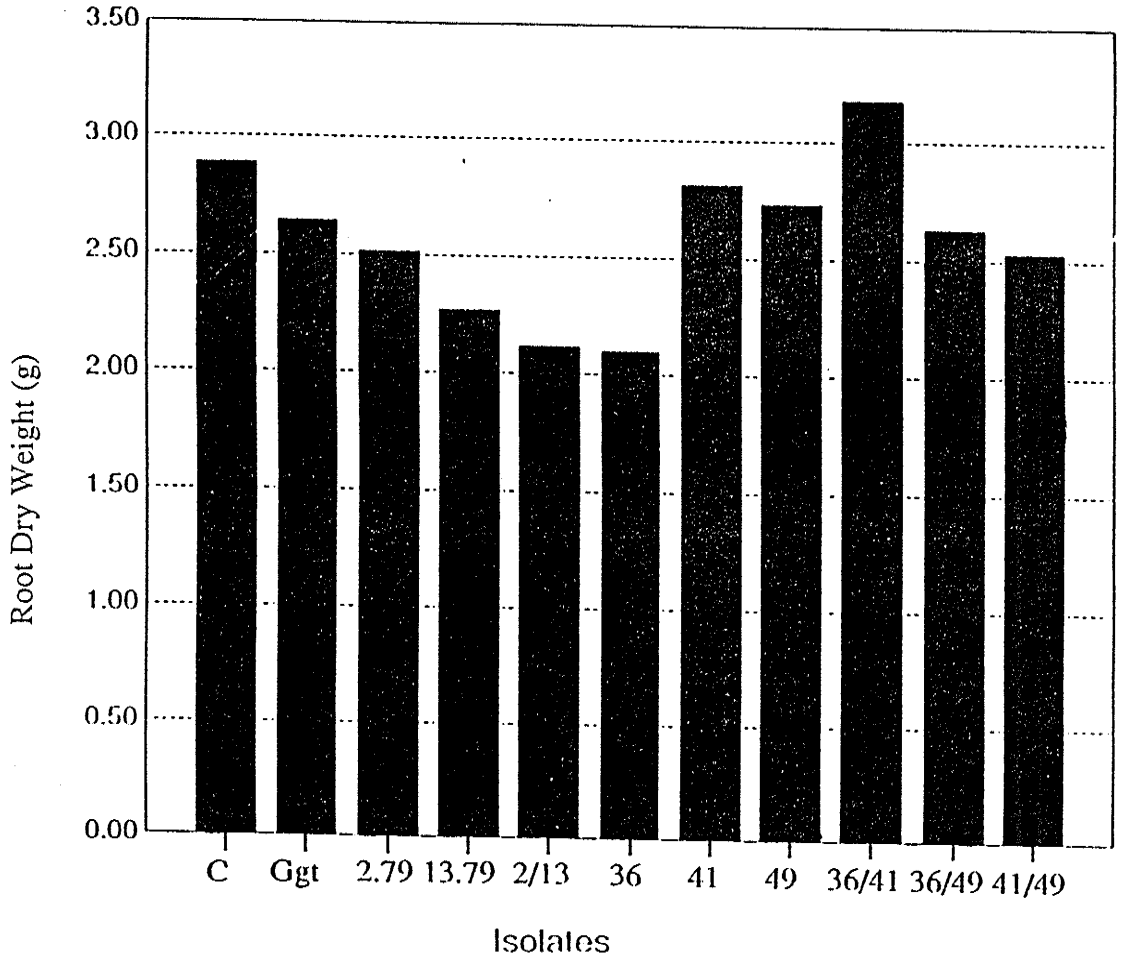

Figure 7. Comparison of individual and combinations of pseudomonad isolates for effects on root dry weight of 8 week old Norstar winter wheat when inoculated with Gaeumannomyces graminis var. tritici.

Figure 8. Four week old Norstar winter wheat seedlins treated with mixtures and single isolates of pseudomonads. a)Ggt inoculated check. b)combined treatment of isolates 36 and 41. c)isolate 36. d)isolate 41.

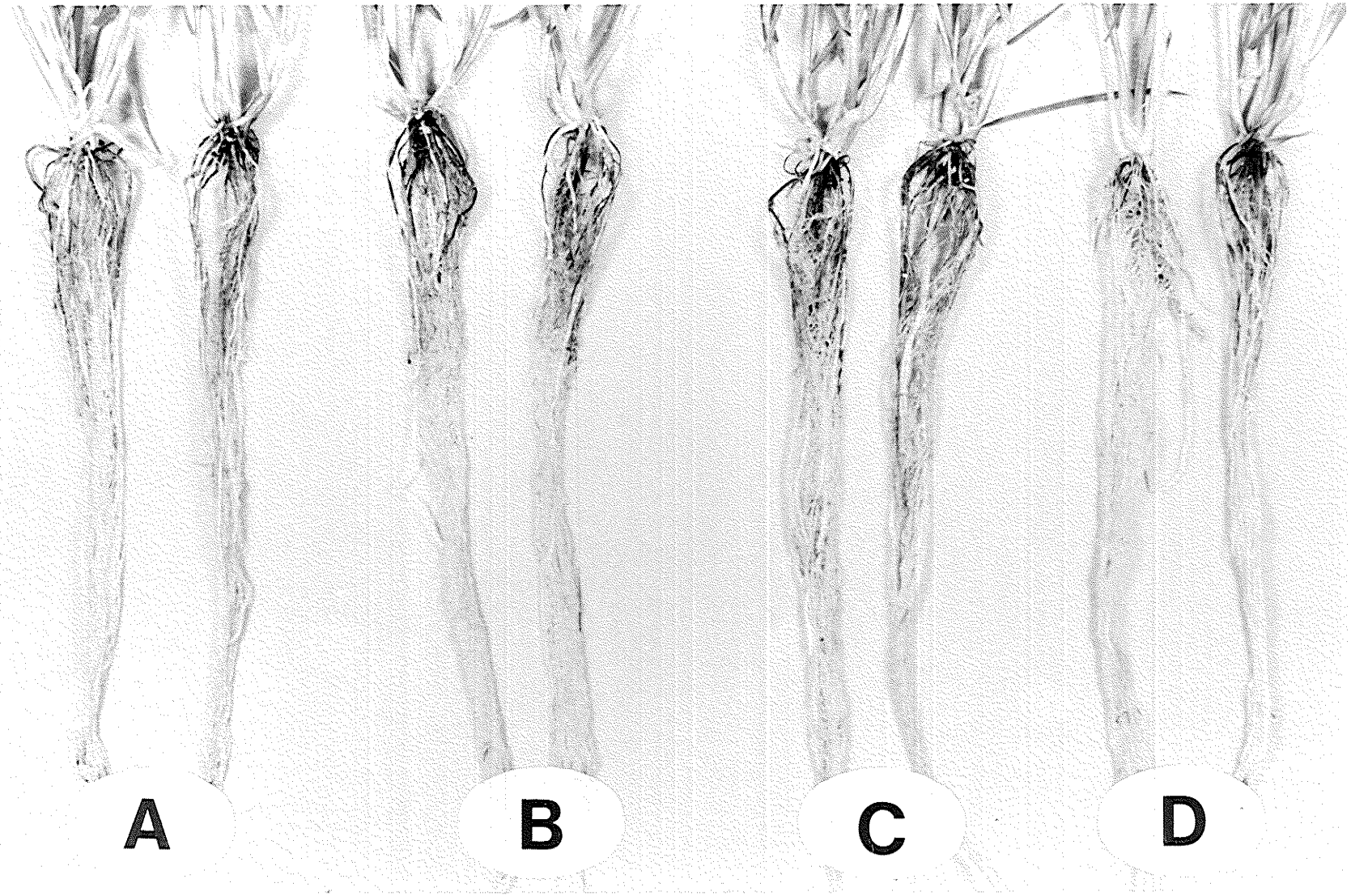
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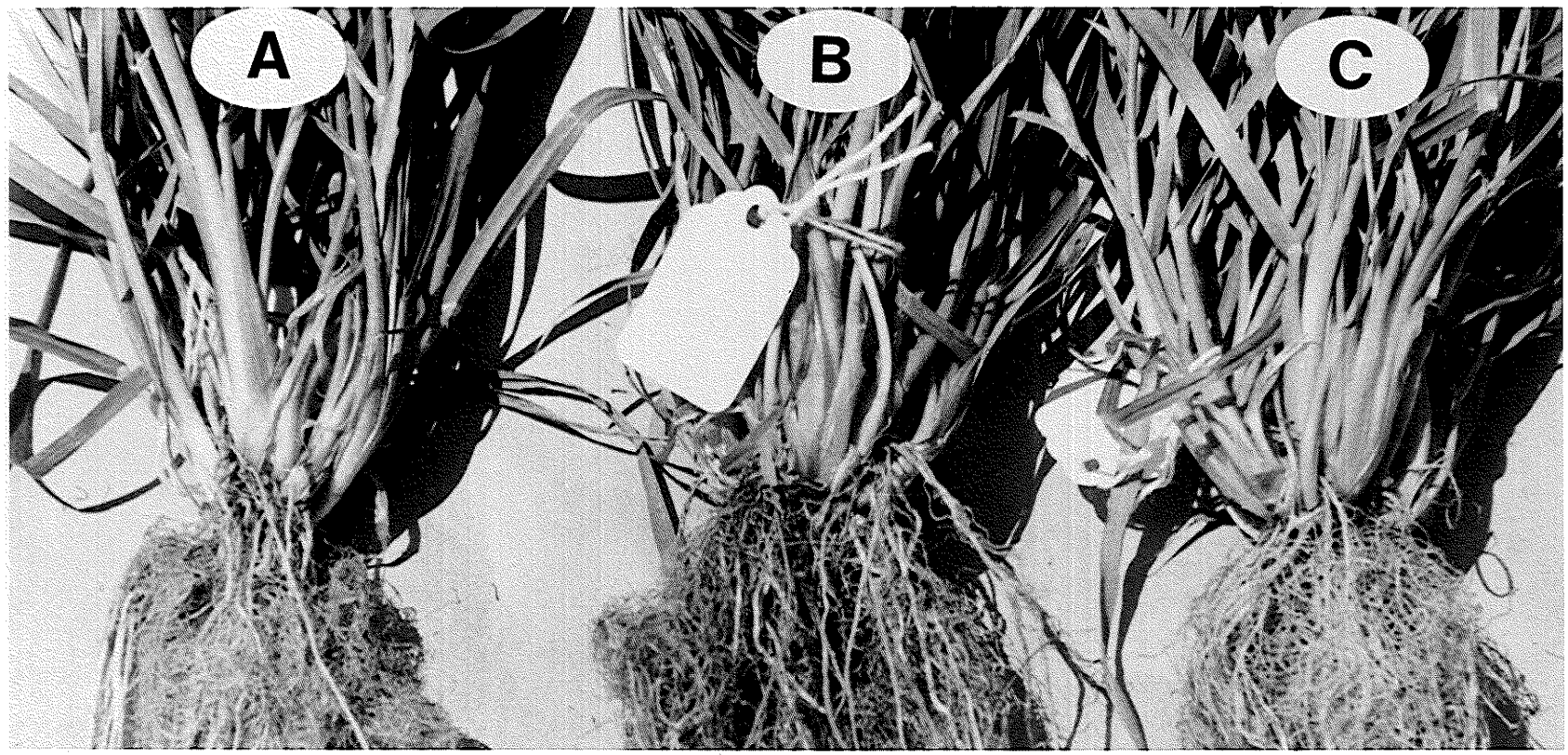
Figure 9. Eight week old Norstar winter wheat plants treated with pseudomonad isolates individually and as a mixture and inoculated with Ggt. a)individual treatment with isolate 36. b)combined treatment using isolates 36 and 41. c)individual treatment with isolate 41.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Chapter V

Discussion

The accurate assessment of bacterial isolates as potential biological control agents is a multi-faceted task. The origin and cropping history of the soils from which bacteria are isolated have considerable impact on the frequency of recovery of antagonistic bacteria (Rovira and Wildermuth, 1981; Cook and Weller, 1983). The soils selected from Minto, Mb. and Indian Head, Sask. had cropping histories of continuous cereals for several years. The frequency of isolation of pseudomonads that fluoresce was greater from these two soils than from the greenhouse soil mixture.

The occurrence of Ggt in the Minto soil was confirmed and the higher frequency of recovery of pseudomonad bacteria from this soil supports Cook's (1982) observations. He proposed the isolation of bacteria from wheat roots grown in the presence of take-all as the most likely source of recovery. Weller et al. (1985) isolated specifically from roots of plants grown in take-all decline soils. The occurrence of take-all decline has not been confirmed in Manitoba soils.

The number of bacterial isolates recovered from the Indian Head soil was fairly comparable to that from Minto. Take-all had not been reported in Indian Head unlike Minto, but a long cropping history of cereals is of some importance in recovering pseudomonad isolates. The greenhouse soil mixture only provided two isolates of pseudomonad bacteria.

The position of bacteria at the time of isolation is also of some significance. According to Elliott and Lynch (1985) the root surface is the site

most likely to harbour antagonistic bacteria. They observed that fewer inhibitory bacteria were recovered from the macerated root tissue. In this study, the root surface provided the most antagonistic isolates, supporting the conclusions of Elliott and Lynch.

With in vitro assessment, the most antagonistic isolates, both in terms of number and of degree of inhibition, were recovered from the Minto soil. Weller et al. (1985) recommended as seed treatments, bacterial strains that demonstrate antibiosis in vitro. They indicated antibiotic and siderophore production to be important properties of some fluorescent Pseudomonas spp. inhibitory to take-all. This method of in vitro screening may be a valid prerequisite for in vivo assessment if there is a good correlation between antibiotic and/or siderophore production and the ability to antagonize Ggt. However, screening for a particularly high degree of inhibition may inadvertently lead to overlooking some strains which may otherwise be effective treatments. In a comparison between in vitro and in vivo antagonism, Wong and Baker (1984) concluded there was no correlation between in vitro antagonism and the ability to suppress take-all.

The results in this study support the conclusions of Wong and Baker (1984) since isolates demonstrating reduced disease severity and greater root dry weights in the in vivo tests did not produce the largest zones of inhibition in the in vitro tests. Isolate 36 effectively reduced the level of disease in the non-sterile soil, while placement of this isolate next to the Ggt colony in vitro caused a flattening of the aerial hyphae, but there was no defined zone of inhibition. On the basis of the in vitro test this isolate might have been overlooked. The in vitro results indicated isolates 9, 35 and 59, recovered from Minto, and 17 and 42 from Indian Head induced the largest zones of inhibition

and should have been selected for further assessment. When these isolates were applied as seed treatments in the in vivo analysis they were associated with a high incidence of disease and/or reduced root mass.

Considering the nutritional status of the rhizosphere in comparison to an artificial medium, Rovira and Wildermuth (1981) pointed out that it is not surprising that a poor correlation exists between in vitro and in vivo antagonism of take-all. Other factors play a role in the relationship between the pathogen and antagonist in addition to the production of antibiotics and siderophores. Activities within the soil such as competition for space and nutrients can greatly influence the behaviour of the bacterial isolate. In the laboratory situation, conditions governing the storing and handling of bacteria may be of significance when testing for in vitro inhibition of Ggt. Aspects such as the timing of inoculation of the test isolate, duration of incubation, type of medium and pH may all play a role in the degree of expression of antagonism. Multiple subcultures and transfers and even mutations during short term storage can alter the stability of the isolate and its antagonistic capabilities. Stability of the isolate population is an important consideration when screening for potential antagonists. Weller and Cook (1983) reported fairly stable populations for 3 weeks at 5^{o} and 15^{o} C. By the fifth week stability was significantly lower and at higher temperatures the populations declined steadily.

In the in vivo assessment of the bacteria, the methodology developed by Weller et al. (1985) for the rapid screening and selection of potentially antagonistic bacteria was used. Many of the isolates did not perform consistently over the five repetitions and variability was observed in the levels of antagonistic activity between initial tests. This is not uncommon and

Weller (1985) suggested inconsistencies could be expected between repetitions due to a lack of understanding of the ecology and to the interaction of the bacteria with the root. Selection of consistently antagonistic bacterial isolates from the five repetitions of the initial in vivo assessment was difficult for a number of reasoms. Variability in this study was observed in the levels of antagonistic activity and might have been the result of uneven distribution of inoculum, nutrient deficiencies, poor seed germination and the lack of an effective delivery system for the introduction of the bacteria to the treatment.

The inconsistencies of inoculum placement were evident in the distribution of the infected millet kernels in the tubes. This was remedied by creating a zone of inoculum comprised of five Ggt infected millet kernels placed on top of the column of soil and covered with a layer of Almasippi soil. The roots of the germinating seeds ultimately grow through the zone coming into contact with the inoculum. In the previous inoculation technique the entire root surface was exposed to the inoculum.

Chlorosis of the lower leaves was evident in all the treatments, including the uninoculated checks. This was partially attributed to a nutrient deficiency and for this reason the nutrient solution was increased to 10 ml full strength Hoaglands solution three times a week. The poor germination was due to the quality of the seed lot and the bacterization process. Germination and seedling emergence were often severely impeded or retarded when seeds were treated with bacteria and methylcellulose. As a result, seedlings were readily infected by Ggt. There was no evidence of delayed germination when Norstar seeds were treated with methylcellulose in the absence of the bacteria. The combination of the bacteria and methylcellulose together was likely responsible for the delay of emergence. Cook et al. (1988) and Basset et al. (1987) also

reported problems with a reduced percentage of emergence and a delay in the date of emergence.

Inhibitory and stimulatory effects of bacteria during transfer from culture medium to wheat seeds may be responsible for some of the inconsistencies observed in the assessments of the bacteria as seed treatments. Changes due to mutations during storage or transfer from one medium to another may influence the activity of the pseudomonad isolates from one screening to the next (Elliott and Lynch, 1985). Capper and Campbell (1986) commented on the possibility of mutations occurring in bacterial isolates in storage or during inoculum preparation rendering initially good antagonists ineffective. Suggestions have been made for the use of genetically more stable bacteria than the pseudomonads, which would be tolerant of environmental stress and easily stored.

Variability was also evident in the number of colony forming units observed with the bacterial seed treatments. In the treatment combining isolates 36 and 49, the number of cfu were considered non-detectable at 2×10^2 cfu/10 seeds. Most of the treatments ranged from 1×10^8 - 3×10^9 cfu per 10 seeds or in some instances were too numerous to count. To better assess the antagonistic activity of combinations of pseudomonad isolates the cultures should be incubated separately prior to introduction to the seed. This would prevent one culture becoming predominant in the seed treatment and allow equal expression of both the isolates.

Another source of variability is the subjectivity of the visual rating system used to measure the degree of infection. The development of a more precise rating scale taking into account root and crown infection would reduce some of the subjectivity of the rating process. The other plant growth characteristics

including plant height, shoot dry weight, vigor and the disease assessment variables were also taken into consideration, but the root dry weight and root infection were the variables most indicative of the disease severity.

In several treatments in this study, plants grown from bacteria-treated seed and inoculated with Ggt more closely resembled the Ggt infected checks than the uninoculated control. Generally the take-all infected plants were stunted and more chlorotic than the uninfected checks. The roots from bacteria-treated seeds had higher disease severity values and reduced shoot and root growth. Cook and Weller (1983) reported that the condition of the plants produced from bacteria treated seed was generally closer to that of the Ggt infected check than to that of the non-infected control. The level of antagonism reported by Weller and Cook (1983) was never achieved with any of the selected bacterial isolates, although, in terms of overall plant health, they were comparable to the infected checks as observed by Weller and Cook (1983).

This type of interaction between the bacteria seed treatment and Ggt has been attributed to deleterious bacteria. Recently attention has been focused on the deleterious aspects of some bacterial isolates. Evidence suggests some pseudomonads produce a toxin that is inhibitory to the host plant (Cherrington and Elliott, 1987; Stroo et al. 1988). The pseudomonads reportedly exhibited a certain degree of specificity. The toxin produced by these inhibitory pseudomonads caused such reactions as chlorosis, necrosis, darkening of the roots, and facilitated infection by other pathogens. Some of these symptoms were evident in plants grown from bacteria treated seeds in this study, but whether they were attributable to deleterious effects of the bacteria, to the pathogen or to some other circumstances remains unanswered.

Another consideration that might have an impact on the bacterial population

is the cultivar from which the bacteria are isolated. Weller (1988) commented on the influence of plant genotype on the quantity and composition of the rhizosphere microflora in response to root exudates. He suggested the possibility of manipulating the host genotype to improve the efficiency and root colonization by the introduced bacteria.

The aggressiveness of the pathogen is also of some significance in a biological control system. A highly virulent organism may be difficult to overcome and rapid infection of the host plant may occur before the antagonist has a chance to become established. The soil pH is another aspect of this system that may have influenced the antagonism of take-all by the pseudomonad isolates. Cook and Weller were using soils in the pH range of 5.5 - 6.0. Under such conditions Ggt is reported to be less aggressive. The Almasippi soil used in this study was approximately pH 7.0. Such a pH may have increased the aggressiveness of the Ggt isolate allowing it to resist the bacterial antagonist and overcome the host plant defenses. Reis et al. (1983) reported that a deficiency in any one of the nutrients essential to the host as a result of higher soil pH may pre-dispose the plant to take-all infection. They concluded that elevated pH levels above 6.5 could induce deficiencies of trace nutrients thereby favouring the development of take-all. With regards to pH and the antagonist, a neutral pH is suitable for the in vitro growth of pseudomonads, but there is evidence of greater colonization of wheat roots when the rhizosphere pH is 6.0 - 6.5 (Weller, 1988).

Initially the bacterial isolates were evaluated as seed treatments in a sterile soil. The purpose of this system was to eliminate any microbial competition that may be naturally present and thus interfere with the activity of the test isolate. Testing of bacterial isolates in a sterile soil may only

provide information on their ability to interact with the pathogen. A better indication of an isolate's ability to antagonize Ggt is in a non-sterile soil where microbial competition is present. The non-sterile Almasippi soil was introduced to the system to determine if the bacterial isolates demonstrated the same level of activity in the presence of microbial competition. Competition exists for a variety of components including space and nutrients in a non-sterile soil. An introduced biological control agent must become rapidly established and be able to compete with the existing microflora as well as the target pathogen.

The bacterial seed treatments were generally more effective in the sterile soil than in the non-sterile soil in terms of higher root dry weights and reduced disease severity. This supports some of the work Weller et al. (1985) conducted comparing bacterial seed treatments in fumigated and non-fumigated soils. They reported that more strains of pseudomonads were effective against take-all in a fumigated system than in a non-fumigated system. They attributed their results to reduced competition between the pre-existing microflora and the newly introduced pseudomonad isolates. In a sterile or fumigated system the introduced bacteria can rapidly colonize the root systems without having first to overcome the existing microflora.

Lack of successful disease control in the field while success is experienced in the greenhouse and in culture is attributed to the inability of organisms to spread through root systems and soil. Chao et al. (1986) studied the movement of bacteria in sterile and non-sterile soils. In a sterile system bacteria were observed moving from the site of initial seed treatment down along the entire length of the root surface. In contrast, in the non-sterile system, the bacteria were only evident in the upper 25 % of the root. They attributed this latter observation to competition and concluded that the success of a newly introduced

biological control agent is dependent upon its ability to compete with the soil microflora. This is essential if the bacteria are to be of any benefit to the host crop.

Microbial competition is an important aspect of a non-sterile system and is subject to changes in response to temperature. Assessment of antagonism at a lower temperature is more representative of field temperatures in the early spring and for this reason the two temperature regimes were selected. The increase in the disease severity index in the $20^{o}/15^{o}$ C temperature test can be attributed to the responses of both the pathogen and the bacterial antagonist to the temperature. Gaeumannomyces graminis var. tritici is reportedly favoured by cool, moist conditions during the parasitic phase (Wong, 1984). The optimum temperature range lies between 12^{o} and 20^{o} C. The conditions of the normal temperature regime, 20^{o} day and 15^{o} C night were within this range and may have been somewhat more favourable to the pathogen than to the bacterial isolate. The optimum temperature for P. fluorescens is 25^{o} - 30^{o} C in culture, while its root colonizing capacity is greatest at temperatures below 20^{o} C (Weller, 1988). The low temperature regime in which reduced disease levels were observed falls below the optimum range for Ggt, but not for the P. fluorescens. Weller (1988) reported the bacteria to be more effective as root colonizers at the lower temperatures due to a reduction in competition from the indigenous microflora allowing the pseudomonads to become better established. The low temperature regime may therefore indirectly favour the antagonist over the pathogen.

Henry (1932) demonstrated the activity of the resident antagonists to Ggt was influenced by soil temperature. In a sterile soil, take-all severity increased as soil temperature increased from 18^{o} to 23 - 27^{o} C. In comparison, in a non-

sterile soil in the same temperature regime, take-all severity decreased in response to an increase in the activity of the resident antagonists. Higher temperatures in a non-sterile soil are associated with higher respiratory activity resulting in greater demand for nutrients. Competition for carbon and energy required for microbial growth leaves less for penetration growth by Ggt (Cook and Baker, 1983).

Weller and Cook (1983) looked at the effect of storage temperature on the survival of bacteria-treated seeds. They observed a steady decline in the number of colony forming units at 25^{o} C over 5 weeks, but the cultures were stable at lower temperatures of 5^{o} and 15^{o} C. Although the activity of the bacteria and the soil microflora in general may be manipulated by changing temperatures, a suitable biological control agent must be expected to function at the temperatures that will be encountered in field situations regardless of whether they are more favourable to the pathogen.

Competition is a significant concern in the selection of competent bacterial isolates. Under natural field conditions isolates can not be expected to function the same as they would in sterile conditions. They are constantly interacting with the microbial population. Pseudomonad species are estimated to account for 1 % of the total bacterial populations in rhizospheres, while higher percentages are associated with the rhizoplane (Miller et al. 1989).

The initial tube assays were conducted with single bacterial isolates applied to the seeds. In natural soil environments, the biological activity is the result of a few distinct populations of a species interacting with a diverse population of microorganisms. There are several types of relationships that exist between microorganisms and their environment. Positive synergism was apparent in the treatment combining isolates 36 and 41. Disease severity index results from the

combined treatment were lower than that observed for the individual isolates. In contrast, the combination of isolates 36 and 49 contributed to a higher disease severity than either of the individual isolate treatments and thus represented a negative synergism. The same results were observed with the combination of the U.S.D.A. isolates 2-79 and 13-79. This finding contradicts previous work by Weller and Cook (1983). They used the same combination of 2-79 and 13-79 and found this treatment to be superior to the individual isolates. They suggested this combination enhanced root colonization or increased the complexity of the protective barrier to the take-all pathogen. This contradiction could be attributed to a number of aspects of the assessment methodology. The modifications made to the application and screening methodology could affect their ability to antagonize take-all. The wheat cultivar selected for this study may not have been conducive to colonization by the bacteria. Another factor that might affect the performance of the test isolate is the degree of aggressiveness of the Ggt isolate. Bacterial isolates may not be able to overcome a highly aggressive strain of the pathogen. The texture and pH of the Almasippi soil might also be of significance as well as the nutritional status of the soil. In many of the experiments, only a few of the pseudomonad isolates were superior to the U.S.D.A. isolates. The results achieved by Weller and Cook were not attained. Perhaps many of the isolates recovered from Minto and Indian Head were not of the beneficial, but rather of the deleterious type of pseudomonad.

Developing a system for assessing the ability of bacteria to antagonize Ggt while not favouring the host plant over the pathogen is difficult. Stress to either the pathogen, antagonist or host must be eliminated in order to effectively measure the antagonist's ability to suppress the pathogen. The

results of this study suggest that the ability of selected bacterial isolates to antagonize the take-all pathogen is dependent upon the environmental conditions to some extent. Manipulation of the rhizosphere conditions to favour the antagonist over the pathogen may contribute to reduced losses from take-all although an effective antagonist must be able to function under those conditions most suitable for the pathogen.

Conclusions

1) Cropping history has an impact on the frequency of recovery of fluorescent pseudomonad bacteria. More fluorescent pseudomonads were recovered from soils cropped to cereals for several years than from non-cropped soil.

2) Site of bacterial isolation from the plant roots is significant to the recovery of antagonistic bacteria. More antagonistic isolates were recovered from the root surface than from the macerated root tissue.

3) In vitro antagonism of fluorescent pseudomonad bacteria to Ggt is not well correlated with in vivo antagonism. Fluorescent pseudomonad isolates producing defined zones of inhibition in vitro did not effectively antagonize Ggt when applied to the seed with methylcellulose.

4) Bacterial isolates are likely to be favoured over the pathogen in sterile soil compared to non-sterile soil due to a reduction in the amount of competition.

5) Lower temperatures in a non-sterile soil favour the antagonistic bacteria over the fungal pathogen as a result of reduced competition by the indigenous microflora.

6) Combinations of fluorescent pseudomonad isolates that were effective as individual isolate treatments interacted synergistically. Some combinations of isolates induced disease severity in excess of the individual isolate treatments, other combinations reduced disease severity.

7) The ability of an antagonist to suppress take-all is dependent upon environmental conditions and the effect they have on the soil microflora.

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Appendix 1. Modified King's Medium B
(Sands and Rovira, 1970)

Difco proteose peptone no. 3	20 g
Difco agar purified	12 g
Glycerol	8 ml
K_2SO_4	1.5 g
$MgSO_4 - 7H_20$	1.5 g
Distilled water	940 ml
Penicillin G	45.4 mg
Novobiocin	45.0 mg
Cycloheximide	75.0 mg

Appendix 2. Number of colony forming units of fluorescent pseudomonads on bacteria treated wheat seeds.

Strain	**cfu/10 seeds**
2-79	1×10^9
13-79	1 x 109
36	1×10^8
41	3×10^9
49	TNTC[a]
2-79/13-79	1×10^8
36/41	TNTC
36/49	ND[b]
41/49	1×10^9

[a]TNTC - too numerous to count

[b]ND - not detectable (2×10^2 cfu/10 seeds)

Appendix 3. Effectiveness of selected isolates of pseudomonads as seed treatments on Norstar winter wheat for the control of Gaeumannomyces graminis var. tritici in the first screening.

Seed Trt	**Plant Height**(cm)	**Shoot Dry Weight**(g)	**Root Dry Weight**(g)	**DSI** (%)
Check	20.29	.1054	.0850	0
Ggt	17.25	.1015	.0623	35.0
2-79	17.33	.0935	.0512	27.8
13-79	16.43	.0805	.0453	27.5
1	18.79	.0998	.0562	25.0
5	16.25	.0840	.0373	37.5
6	20.03	.1318	.0695	25.0
11	10.00	.0375	.0260	25.0
13	17.95	.1095	.0753	25.0
14	17.20	.0910	.0600	25.0
16	15.90	.1005	.0625	37.5
18	18.15	.0995	.0627	32.5
26	16.10	.0794	.0454	30.6
27	18.56	.1247	.0730	37.5
28	17.56	.0890	.0520	30.0
34	14.80	.0763	.0527	33.3
35	16.12	.1055	.0587	32.5
36	15.81	.1160	.0622	27.8
40	15.55	.1074	.0520	34.4
42	16.60	.1088	.0472	25.0
47	20.70	.1360	.0910	25.0
49	12.25	.0450	.0325	37.5
53	19.27	.0914	.0640	30.0
54	18.61	.0800	.0530	27.8
57	18.99	.0940	.0560	25.0
59	20.73	.0765	.0353	25.0
60	17.96	.0678	.0456	35.0
62	21.47	.0920	.0530	25.0
63	18.80	.0840	.0320	25.0

Appendix 4. Effectiveness of selected isolates of fluorescent pseudomonads as seed treatments on Norstar winter wheat for the control of Gaeumannomyces graminis var. tritici in the second screening.

Seed Trt	**Plant Height** (cm)	**Shoot Dry Weight** (gm)	**Root Dry Weight** (gm)	**DSI** (%)
Check	24.27	.0886	.0626	0
Ggt	20.39	.0854	.0573	33.9
2-79	21.44	.0720	.0481	31.3
13-79	18.65	.0585	.0392	34.4
1	0 *	0	0	0
5	18.91	.0879	.0693	34.4
6	19.77	.0833	.0612	50.0
11	22.28	.0920	.0652	33.3
13	22.50	.0965	.0735	27.5
14	22.59	.0886	.0650	27.5
16	23.06	.0778	.0645	34.5
18	21.33	.0548	.0290	50.0
26	21.99	.0808	.0533	45.0
27	18.55	.0593	.0429	67.5
28	21.80	.0648	.0538	30.0
34	22.10	.0775	.0517	29.2
35	17.90	.0398	.0243	31.3
36	22.10	.0896	.0583	27.5
40	22.22	.0686	.0420	30.0
42	18.67	.0805	.0547	32.5
47	17.50	.0712	.0474	40.0
49	16.94	.0740	.0483	41.7
53	15.43	.0503	.0286	46.4
54	15.81	.0662	.0377	41.7
57	16.06	.0630	.0336	42.9
59	14.70	.0542	.0375	25.0
60	22.23	.0830	.0558	25.0
62	16.40	.0583	.0332	41.7
63	20.93	.0850	.0593	25.0

* no seedlings germinated in this treatment

Appendix 5. Effectiveness of selected isolates of fluorescent pseudomonads as seed treatments on Norstar winter wheat for the control of Gaeumannomyces graminis var. tritici in the third screening.

Seed Trt	**Plant Height** (cm)	**Shoot Dry Weight** (gm)	**Root Dry Weight** (gm)	**DSI** (%)
Check	22.28	.1260	.1022	0
Ggt	16.91	.0532	.0341	42.5
2-79	18.48	.0714	.0403	36.3
13-79	19.10	.0806	.0486	40.6
1	20.04	.0540	.0350	40.0
5	18.10	.0668	.0430	25.0
6	15.47	.0453	.0304	27.5
11	17.04	.0634	.0462	50.0
13	15.38	.0376	.0266	25.0
14	16.47	.0655	.0363	37.5
16	17.43	.0620	.0378	37.5
18	17.38	.0592	.0385	32.5
26	17.56	.0682	.0424	30.0
27	16.60	.0474	.0272	50.0
28	17.40	.0530	.0357	33.3
34	22.18	.0818	.0520	31.3
35	17.97	.0637	.0417	45.8
36	21.33	.0930	.0610	25.0
40	22.13	.1043	.0565	25.0
42	19.70	.0950	.0513	37.5
47	16.41	.0535	.0364	50.0
49	17.26	.0720	.0494	40.6
53	20.63	.0788	.0383	31.3
54	19.89	.0874	.0527	25.0
57	17.23	.0488	.0253	43.8
59	18.05	.0643	.0395	37.5
60	17.83	.0677	.0473	41.7
62	18.75	.0610	.0397	45.8
63	17.96	.0660	.0450	50.0

Appendix 6. Effectiveness of selected isolates of pseudomonads that fluoresce as seed treatments on Norstar winter wheat for the control of Gaeumannomyces graminis var. tritici in the fourth screening.

Seed Treatment	**Plant Height** (cm)	**DSI** (%)
Check	35.07	0
Ggt	29.65	51.7
2-79	28.32	42.5
13-79	30.52	42.5
1	28.95	59.4
5	27.93	57.5
6	29.45	37.5
11	25.93	57.5
13	30.17	60.0
14	28.82	47.5
16	26.28	52.5
18	26.22	57.5
26	30.16	57.5
27	29.86	57.5
28	26.70	65.0
34	29.86	62.5
35	28.59	65.0
36	30.63	60.0
40	32.06	60.0
42	28.99	60.0
47	29.35	80.0
49	28.34	77.5
53	33.32	62.5
54	32.60	52.5
57	30.46	70.0
59	31.39	67.5
60	31.72	75.0
62	31.52	70.0
63	28.50	65.6

Appendix 7. Effectiveness of selected isolates of pseudomonads as seed treatments on Norstar winter wheat for the control of Gaeumannomyces graminis var. tritici in the fifth screening.

Seed Trt	**Plant Height** (cm)	**Shoot Dry Weight** (gm)	**Root Dry Weight** (gm)	**DSI** (%)
Check	34.09	.2896	.1320	0
Ggt	28.99	.2599	.1164	49.1
2-79	29.23	.2117	.0980	25.0
13-79	28.15	.2046	.0969	27.5
1	26.31	.2433	.1209	40.0
5	27.49	.2252	.1108	42.5
6	28.43	.2155	.1007	29.2
11	27.47	.1497	.0700	42.5
13	25.68	.1935	.0976	57.5
14	27.73	.2030	.0930	50.0
16	28.43	.2242	.0853	45.0
18	29.12	.2289	.0906	52.5
26	29.69	.2590	.1134	57.5
27	30.79	.2552	.0992	52.5
28	29.07	.2392	.1019	55.0
34	31.49	.2278	.0892	52.8
35	31.61	.2346	.0861	47.5
36	34.41	.2217	.0869	37.5
40	29.82	.2710	.1115	50.0
42	26.90	.2798	.1134	52.5
47	31.51	.2448	.0952	55.0
49	31.75	.2268	.0874	55.0
53	32.61	.2101	.0834	52.5
54	33.58	.2227	.0824	47.5
57	32.90	.2627	.0966	47.5
59	31.32	.2450	.1045	50.0
60	32.73	.2408	.0976	40.6
62	25.52	.3205	.1441	50.0
63	30.12	.2948	.1230	50.0

Appendix 8. Comparison of plant root dry weights from bacteria-treated seed in sterile soil inoculated with Gaeumannomyces graminis var. tritici

Treatment	Mean Dry Weight (g)	Grouping
Control	0.1144	a*
27	0.1072	ab
6,14,Ggt	0.1030 - 0.1014	abc
11	0.0965	abcd
59	0.0925	bcde
36,40	0.0904 - 0.0903	bcdef
57,62	0.0899 - 0.0895	bcdefg
53,54	0.0864 - 0.0863	cdefgh
5	0.0858	defgh
26,13,13-79	0.0827 - 0.0801	defghi
47,18,2-79,63		
42,49	0.0772 - 0.0712	efghi
28	0.0697	fghi
1	0.0687	ghij
34	0.0666	hij
60,16	0.0648 - 0.0603	ij
35	0.0610	j

*means with the same letter are not significantly different $p > 0.05$ LSD Test

Appendix 9. Comparison of plant root dry weights of bacteria-treated seeds in a non-sterile soil inoculated with Gaeumannomyces graminis var. tritici

Treatment	Mean Dry Weight (g)	Grouping
Control	0.0957	a*
49	0.0855	ab
27	0.0839	bc
63	0.0794	bcd
Ggt Check	0.0791	bcde
11	0.0698	bcdef
53	0.0665	cdefg
57	0.0656	defgh
36	0.0642	efgh
54,13,62,60 14,2-79	0.0633 - 0.0618	fgh
42,28,18,34 35,40,47,1 5,26,13-79,6	0.0602 - 0.0510	gh
59	0.0503	h
16	0.0205	i

*means with the same letter are not significantly different $p > 0.05$ LSD Test

Appendix 10. Effect of norstar winter wheat seed treatment with selected isolates of pseudomonads in sterile and non-sterile soil for the control of Gaeumannomyces graminis var. tritici based on plant growth and disease parameters.

Seed Trt	Plant Height		Shoot Dry Weight		Root Dry Weight		DSI (%)	
	S	N	S	N	S	N	S	N
Check	30.76	35.20	.2606	.2296	.1144	.0957	0	0
Ggt	28.99	27.98	.2464	.2225	.1014	.0791	87.7	87.9
2-79	30.17	27.49	.2057	.1686	.0804	.0618	92.5	100.0
13-79	31.60	26.81	.1987	.1401	.0825	.0510	82.5	90.0
1	25.47	24.80	.1583	.1559	.0687	.1312	75.0	83.3
5	27.43	29.40	.2318	.1776	.0858	.0533	58.3	67.9
6	25.73	23.55	.2843	.1525	.1030	.0510	83.3	100.0
11	27.58	28.41	.2406	.2314	.0965	.0698	84.4	75.0
13	29.17	29.43	.1856	.2435	.0826	.0626	87.5	90.0
14	29.51	28.92	.2627	.2124	.1024	.0622	87.5	87.5
16	19.50	24.60	.1420	.0590	.0630	.0205	41.7	75.0
18	26.19	29.31	.1705	.2403	.0809	.0580	97.5	75.0
26	28.42	28.67	.1941	.1956	.0827	.0520	82.5	70.0
27	28.04	29.29	.2153	.2132	.1072	.0839	87.5	82.5
28	23.01	29.28	.1429	.1698	.0697	.0581	67.5	77.8
34	28.21	29.12	.2063	.1726	.0666	.0577	67.9	80.6
35	28.70	27.97	.1453	.1903	.0610	.0577	58.3	75.0
36	29.20	28.44	.2410	.1793	.0904	.0642	80.0	60.0
40	27.41	27.66	.1768	.2228	.0816	.0576	72.2	80.0
42	27.36	28.09	.1584	.1471	.0772	.0602	75.0	80.6
47	28.21	27.87	.1914	.2064	.0813	.0572	80.0	90.0
49	20.56	26.55	.1632	.2438	.0712	.0855	60.0	79.2
53	29.41	29.08	.1974	.2554	.0864	.0665	92.5	92.5
54	28.44	30.32	.1699	.1841	.0863	.0633	90.0	77.8
57	29.10	29.84	.2407	.2238	.0899	.0656	75.0	86.1
59	27.32	27.07	.2049	.1391	.0925	.0503	67.5	80.0
60	29.81	27.46	.1572	.1383	.0648	.0624	65.0	85.7
62	29.47	27.92	.1921	.1572	.0895	.0625	85.0	85.0
63	29.06	29.76	.1704	.2347	.0801	.0794	75.0	89.3

S = sterile
N = non-sterile

Appendix 11. Comparison of root dry weights of plants grown from bacteria-treated seed in $20^{o}/15^{o}$ C temperature regime when inoculated with Gaeumannomyces graminis var. tritici

Treatment	Mean Dry Weight (g)	Grouping
Control	0.2098	a*
Ggt Check 1	0.1348	b
Ggt Check 2	0.1158	bc
59,13-79,10 41,58,11,2-79 1,36,49	0.1122 - 0.0980	cd
6	0.0918	d
29	0.05367	e

*means with the same letter are not significantly different $p > 0.05$ LSD Test

Appendix 12. Comparison of root dry weights of plants grown from bacteria treated seed at $15^{o}/10^{o}$ C temperature and inoculated with Gaeumannomyces graminis var. tritici

Treatment	Mean Dry Weight (g)	Grouping
Control	0.1563	a*
59,Ggt Check 1	0.1240 - 0.1225	ab
Ggt Check 2,13-79	0.1189 - 0.1188	b
10,1,6,58 2-79,41	0.1130 - 0.0922	bc
11	0.0829	cd
36,29,49	0.0556 - 0.0500	d

*means with the same letter are not significantly different $p > 0.05$ LSD Test

Appendix 13. Effectiveness of Norstar winter wheat seed treatment with isolates of pseudomonads for the control of Geaumannomyces graminis var. tritici in two temperature regimes.

Seed Trt	Plant Height H	Plant Height L	Shoot Dry Weight H	Shoot Dry Weight L	Root Dry weight H	Root Dry weight L	DSI (%) H	DSI (%) L
Check	22.77	16.99	.3453	.2595	.2098	.1563	0	0
Ggt	22.97	16.99	.3013	.2399	.1254	.1207	90.0	81.3
2-79	25.18	16.75	.2550	.1889	.1052	.1037	80.0	77.5
13-79	24.80	17.67	.2587	.1929	.1104	.1188	85.0	67.5
1	23.02	15.64	.2654	.1979	.1031	.1096	95.0	87.5
6	25.86	16.81	.2258	.1812	.0918	.1059	87.5	69.4
10	25.07	16.46	.2757	.2019	.1082	.1130	82.5	80.0
11	24.98	14.24	.2378	.1495	.1053	.0829	86.1	87.5
29	27.93	14.13	.1270	.1080	.0537	.0553	58.3	56.3
36	26.28	13.71	.2438	.0991	.0988	.0556	80.0	66.7
41	27.02	15.34	.2557	.1709	.1079	.0922	85.0	65.0
49	26.98	10.82	.2447	.0874	.0980	.0500	62.5	50.0
58	24.73	16.28	.2689	.1926	.1074	.1058	97.5	77.5
59	25.02	16.91	.2736	.2242	.1122	.1240	85.0	85.0

H = $20^{o}/15^{o}$ C
L = $15^{o}/10^{o}$ C

Appendix 14. Linear regression analysis for root dry weight and disease severity in the 15/10^{O} C temperature regime.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RDW ROOT DRY WEIGHT

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	2	0.13955818	0.06977909	76.94
ERROR	12	0.01088335	0.00090695	
UNCORRECTED TOTAL	14	0.15044153		

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF
INTERCEPT	1	0.13874283	152.98	0.0001	1
DSI	1	0.00081535	0.90	0.3618	1

PARAMETER	ESTIMATE	T FOR H0: PARAMETER=0	PR > |T|	STD ERROR OF ESTIMATE
INTERCEPT	0.12477545	4.49	0.0007	0.02779552
DSI	-0.00034572	-0.95	0.3618	0.00036463

PR > F	R-SQUARE	C.V.
0.0001	0.069696	30.2517

ROOT MSE	RDW MEAN
0.03011553	0.09955000

TYPE III SS	F VALUE	PR > F
0.01827637	20.15	0.0007
0.00081535	0.90	0.3618

Appendix 15. Linear regression for root dry weight and disease severity index in the 20/15^{O} C temperature regime.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RDW ROOT DRY WEIGHT

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	2	0.17573459	0.08786729	147.84
ERROR	12	0.00713223	0.00059435	
UNCORRECTED TOTAL	14	0.18286682		

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF
INTERCEPT	1	0.16878456	283.98	0.0001	1
DSI	1	0.00695003	11.69	0.0051	1

PARAMETER	ESTIMATE	T FOR H0: PARAMETER=0	PR > |T|	STD ERROR OF ESTIMATE
INTERCEPT	0.18509525	8.06	0.0001	0.02296274
DSI	-0.00094153	-3.42	0.0051	0.00027534

PR > F	R-SQUARE	C.V.
0.0001	0.493531	22.2034

ROOT MSE	RDW MEAN
0.02437935	0.10980000

TYPE III SS	F VALUE	PR > F
0.03861777	64.97	0.0001
0.00695003	11.69	0.0051

Appendix 16. Comparison of root dry weights of plants grown from bacteria treated seed as individual and combinations of isolates and inoculated with Gauemannomyces graminis var. tritici

Treatment	Mean Dry Weight (g)	Grouping
Control	0.2135	a*
Ggt Check	0.1692	b
2-79	0.1658	b
13-79	0.1640	b
36	0.1640	b
41	0.1623	b
36/41	0.1603	b
2-79/13-79	0.1584	b
36/49	0.1562	b
49	0.1448	b
41/49	0.1430	b

*means with the same letter are not significantly different $p > 0.05$ LSD Test

Appendix 17. Effectiveness of fluorescent pseudomonads against Gaeumannomyces graminis var. tritici when applied to Norstar winter wheat seeds as mixtures of bacterial isolates.

Seed	Plant Height	Shoot Dry Weight		Root Dry Weight		DSI(%)
		4wks	8wks	4wks	8wks	
Check	22.75	.3752	3.9651	.2135	2.8853	0
Ggt	19.47	.4573	4.3131	.1692	2.6388	80.0
2-79	20.81	.4132	4.2972	.1658	2.5154	92.5
13-79	20.21	.3810	3.3929	.1640	2.2663	82.5
2-79/13-79	19.34	.4238	3.6842	.1584	2.1150	100.0
36	18.60	.3703	3.5136	.1640	2.0975	92.5
41	20.68	.3900	3.7367	.1623	2.8167	85.0
49	18.95	.3438	3.3894	.1448	2.7281	87.5
36/41	20.27	.3874	3.8113	.1603	3.1902	72.5
36/49	19.33	.3930	3.3458	.1562	2.6334	92.5
41/49	20.83	.3457	3.8113	.1430	2.5353	80.0