EVALUATION OF HOST TOLERANCE, BIOLOGICAL, CHEMICAL, AND CULTURAL CONTROL OF *SCLEROTINIA SCLEROTIORUM* IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

BY

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A Thesis
Submitted to the Faculty of Graduate Studies
In Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

Department of Plant Science
University of Manitoba
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ABSTRACT

Duncan, Robert Wayne. M.Sc., The University of Manitoba, July, 2003. Evaluation of Host Tolerance, Biological, Chemical, and Cultural Control of *Sclerotinia sclerotiorum* in Sunflower (*Helianthus annuus* L.). Co-advisors; W.G.D. Fernando and K.Y. Rashid.

Sclerotinia sclerotiorum (Lib.) de Bary is an economically devastating pathogen in sunflower (*Helianthus annuus* L.) producing areas around the world. No single management practice provides consistent control of sclerotinia head rot (*Sclerotinia sclerotiorum*) in sunflower. An integrated approach utilizing host resistance, biological, chemical, and cultural control will minimize sclerotinia head rot losses.

Host resistance is the optimum method of controlling sclerotinia head rot in sunflower, however, a high level of resistance does not exist. Six oilseed and five confection sunflower hybrids were assessed for their tolerance levels to sclerotinia head rot. The oilseed hybrid SF125 was the most tolerant hybrid under natural ascospore, artificial ascospore, and ground millet inoculation. Pooled data showed greater tolerance to sclerotinia head rot in oilseed hybrids than in confection hybrids. The most susceptible growth stage was when 100% of disk flowers had completed flowering, while head rot susceptibility was strongly correlated with the percentage of disk florets present on the sunflower head.

Both introduced and natural bacterial biological control of *S. sclerotiorum* were investigated in this thesis. Two *Pseudomonas* spp., *P. chlororaphis* (strain PA-23), and *P. corrugata* (strain 41), along with a new fungicide, BAS 510 F (2-chloro-N-(4,-chloro-biphenyl-2-yl)nicotinamide), were assessed for their foliar protective ability against sclerotinia head rot. The biocontrol agents (BCA) produced effective results under natural infection, totally eliminating infection in 2001 at Carman, Manitoba. Control of

sclerotinia head rot was the most effective when treatments were applied at the 100% flowering stage. Strain PA-23 was the most effective BCA at reducing sclerotinia head rot infection, while the experimental fungicide BAS 510 F was more effective than both BCA.

Natural biological control was assessed by burying sclerotia in the soil at different depths and monitoring sclerotial viability and bacterial colonization of the sclerotia over time. A significant negative relationship between sclerotial viability and elapsed burial time (R^2 = -0.68, $P < 0.0001$), sclerotial viability and burial depth (R^2 = -0.58, $P < 0.0001$), and sclerotial viability and bacterial colonization (R^2 = -0.60, $P < 0.0001$) were found. A total of 268 bacteria isolates were isolated from the buried sclerotia, 29 of which were strongly inhibitory against *S. sclerotiorum* mycelial growth. *Bacillus amyloliquefaciens* strain 265 and *B. licheniformis* strain 223 were the most inhibitory bacteria, producing over 80% mycelial inhibition *in vitro*.

A high level of control of sclerotinia head rot in sunflower can only be achieved through an integrated approach incorporating all available management techniques. The knowledge accumulated over the course of this study, reiterates the fact that existing management strategies need further refinement to reach their full disease reduction potential.

FOREWARD

This thesis is written in manuscript style, with each manuscript having its own abstract, introduction, materials and methods, results and discussion sections. There is a general introduction and review of the literature prior to the manuscripts, followed by the general discussion and conclusions, and the literature cited section.

1.0 INTRODUCTION

Sunflower (*Helianthus annuus* L.) cultivation began in Arizona and New Mexico around 3000 B.C., (Semelczi-Kovacs 1975), and has progressed over time to become the world's fourth largest oilseed crop (Kleingartner 1997). Manitoba produces the majority of the Canadian sunflower crop, and in 2001, received $28.63 million in cash receipts from sunflower production (Statistics Canada 2001). Manitoba produces mostly confection sunflowers (Manitoba Agriculture and Food 2001), which are a larger seed type, mainly used for various forms of human consumption, and the remainder for bird and animal feed (Lofgren 1997). Oilseeds, a small seed with an elevated oil content are the main type of sunflower grown around the world. In 1995/96, worldwide sunflower production of oilseeds totaled 24.9 million tonnes, producing 9.0 million tonnes of vegetable oil (Kleingartner 1997). Oilseeds are produced mainly for the oil, in addition to limited production for the kernel, hull, and meal. Sunflower oil is processed into cooking oil, margarine, and occasionally industrial oil (Dorrell & Vick 1997).

Sunflower is the host of over thirty different diseases, including downy mildew (*Plasmopara halstedii* (Farl.) Berl. and de Toni), rust (*Puccinia helianthi* Schwein), and verticillium wilt (*Verticillium dahliae* Klebahnis) (Gulya *et al.* 1997). *Sclerotinia sclerotiorum* (Lib.) de Bary is also an economically devastating pathogen in sunflower production (Huang & Kozub 1990; Huang & Kozub 1993). *Sclerotinia sclerotiorum* was originally found in sunflower in 1861 (Gulya *et al.* 1997), and can infect sunflower roots, stems, leaves, and heads (Purdy 1979; Mestries *et al.* 1998). *Sclerotinia sclerotiorum* is a monocyclic pathogen (Agrios 1978), but disease can spread from infected to healthy plants by contact (Abawi & Grogan 1979). Ninety percent of the *S. sclerotiorum* life

cycle takes place in the form of over-wintering sclerotia, surviving as a compact mass of mycelia (Adams & Ayers 1979) until conditions are appropriate for germination. Germination can occur as two distinct mechanisms, either myceliogenic or carpogenic (Gulya *et al.* 1997). Myceliogenic germination occurs within the rhizosphere when exogenous conditions are appropriate (Bardin & Huang 2001). As the hyphae protrudes from the sclerotia it can infect healthy sunflower tap roots causing sclerotinia root rot (Willetts & Wong 1980). Carpogenic germination occurs when sclerotia are near the soil surface and germinate under water-saturated soil conditions to produce apothecia, which release air-borne ascospores, causing above-ground infection (Purdy 1979). Ascospores utilize an exogenous food source such as senescing disk florets (Willetts & Wong 1980), or sucrose reserves (Auger & Nome 1970) to initiate sclerotinia head rot infection. Sclerotinia head rot will appear as a water-soaked, light brown lesion, that can quickly spread in all directions, leaving only the vascular tissue intact and a broom-like appearance {Gulya, 1997 3 /id;Martens, 1988 267 /id}.

When inoculum is present and environmental conditions are appropriate, sclerotinia head rot can occur in all sunflower fields. Compiled Canadian Plant Disease Survey data suggests that the prevalence (presence of infected heads within a surveyed field) of sclerotinia head rot for the last eleven years is 62.4%, with a mean incidence (number of plants infected / total number of plants) of approximately 5.0%. Using average sunflower prices and yields (Rob Park, personal communication), over the same time period, this 5.0% incidence can result in approximate losses of $27/ha every year. Sayler (2003b) reports that United States sunflower producers loose $15 million each year to infection caused by *S. sclerotiorum*. With the broad host range of *S. sclerotiorum*

(Purdy 1979), these losses in sunflower are just a small view of the overall destruction caused by this destructive pathogen.

Control of *S. sclerotiorum* has rarely been achieved in any cropping system on a consistent basis (Grogan 1979). As the production of host crops such as beans and canola is increasing in Canada, the limited number of control practices are even further strained (Miller & Fick 1997). The main methods of *S. sclerotiorum* management are host resistance, biological, chemical, and cultural control. No satisfactory level of resistance to *S. sclerotiorum* exists in sunflower (Kohler & Friedt 1999). In Canada, no chemical or biological control products are registered to control sclerotinia head rot in sunflower, leaving cultural control practices as the main source of *S. sclerotiorum* management (Hoes & Huang 1985; Gracia-Garza *et al.* 2002).

Host resistance is the best possible method to control sclerotinia head rot in sunflowers (Gentzbittel *et al.* 1998; Hahn 2002), but the development of resistant hybrids is a long-term process. Studies have demonstrated that the incorporation of wild germplasm into commercial hybrids has developed a range of tolerance levels to *S. sclerotiorum* (Seiler & Rieseberg 1997). However, *S. sclerotiorum* resistance is difficult to develop because of the additive nature of resistance (Fuller *et al.* 1984). Further complicating *S. sclerotiorum* resistance, is the fact that the level of resistance within sunflower is not equal for every plant part (Degener *et al.* 1999).

Biological and chemical control of *S. sclerotiorum* have been effective in other host systems such as lettuce, bean, and rapeseed production (Budge *et al.* 1995; Boland 1997; Twengstrom *et al.* 1998), while studies in sunflower production have also shown success (McLaren *et al.* 1994; Expert & Digat 1995). Unfortunately, no chemical or

biological control products have been registered for control of sclerotinia head rot in sunflower. A chemical or biological product that is effective in combination with cultural practices, and host tolerance, is required.

Crop rotation is utilized for management of *S. sclerotiorum* (Adams & Ayers 1979), in addition to the agronomic advantages like proper soil-water and nutrient management that crop rotation provides (Campbell *et al.* 1994). To control sclerotinia head rot in sunflower, crop rotation is ineffective, as air-borne ascospores are the source of inoculum (Gulya *et al.* 1997). Field plans need to be developed in advance so that planting of sunflowers will occur at least 1 km from the previous production of any other host crops.

Tillage has also been reported to be effective in *S. sclerotiorum* management, distributing sclerotia throughout the soil profile (Mueller *et al.* 2002). The effects of this inoculum distribution on sclerotial viability and germination are not clearly understood, as results are conflicting (Merriman *et al.* 1979; Workneh & Yang 2000; Kurle *et al.* 2001; Gracia-Garza *et al.* 2002; Mueller *et al.* 2002). It is clear that tillage does affect *S. sclerotiorum* infection in sunflower; however, developing the proper tillage practices to minimize this infection will require further research.

It is evident from the economic loss caused by *S. sclerotiorum* that more effective sclerotinia head rot management in sunflower is essential. It was the objective of this research to elucidate information on several facets of sclerotinia head rot management, as only an integrated approached, utilizing host tolerance, biological, chemical and cultural control will manage *S. sclerotiorum* in sunflower. The research experiments within this objective were divided into three areas: host tolerance, as it may be the most effective

method of controlling *S. sclerotiorum* in sunflower; biological and chemical control, as chemical control of *S. sclerotiorum* is effective in other host-pathogen systems, but alternatives to chemical control are needed with today's environmental awareness; and cultural control, as tillage effects on the survival of *S. sclerotiorum* are unclear.

Host tolerance to sclerotinia head rot differs between hybrids and sunflower type, as does the susceptibility of sunflowers at each growth stage. The knowledge that select hybrids are more tolerant to *S. sclerotiorum* will direct producers to choose hybrids with the genetic background that will minimize losses to *S. sclerotiorum*. Confirming the general perception that oilseed hybrids are more tolerant than confections may aid breeders during the breeding process by incorporating genetic material from tolerant oilseeds into susceptible confection hybrids. The most susceptible growth stage to sclerotinia head rot infection has produced conflicting results in previous studies. The need to clarify this confliction is imperative as recognizing the most susceptible stage to sclerotinia head rot infection would directly aid producers during the application of biological or chemical control products.

The second research experiment was to assess two *Pseudomonas* species against *S. sclerotiorum*, along with an experimental fungicide (BAS 510 F), for their interaction with *S. sclerotiorum* and its disease-causing ability. *Pseudomonas chlororaphis* strain PA-23 and *P. corrugata* stain 41, have both been effective against *S. sclerotiorum* in canola production and *in vitro* studies. The intent of this experiment was to produce efficacy data from field trials to determine the bacteria's commercial applicability. BAS 510 F was added as a control to compare it's ability to the previously effective biocontrol

agents. BAS 510 F is a new experimental fungicide that has shown the ability to manage *S. sclerotiorum* in numerous host crops such as canola, tomato, beans and potatoes.

The third research experiment was to determine the effects of sclerotia placement at different depths within the soil over time, and isolate bacterial populations and assess their biological control potential. Tillage effects on sclerotial survival have been unclear in previous research, however it is clear that tillage effects sclerotial placement in the soil, impacting the microbial degradation of sclerotia. Determining the accurate time and soil depth for sclerotial degradation will direct known tillage practices to reduce losses to *S. sclerotiorum*. Screening for potential biocontrol agents is a continual process. Identifying effective biocontrol agents and their presence over time within the soil may benefit the management of *S. sclerotiorum* in numerous host-pathogen systems.

These advancements in host-tolerance, biological, chemical, and cultural control of *S. sclerotiorum* can then be pooled with existing management practices, moving one step closer to *S. sclerotiorum* control in sunflower.

2.0 LITERATURE REVIEW

2.1 *Helianthus annuus*

2.1.1 Sunflower History

Cultivation of sunflower (*Helianthus annuus* L.) is estimated to have begun in Arizona and New Mexico around 3000 B.C. (Semelczi-Kovacs 1975). Early use was by the North American Indians (Heiser 1951). The main use of sunflower was for food, usually ground; made into cakes, mush, or bread (Putt 1997). Hopi Indians of the Southwest produced a wafer-like bread (Whiting 1939), while North Dakota Indians used seeds in a mixture with bean (*Phaseolus* L.), squash (*Curcubita* spp.), and cornmeal (Wilson 1917). Sunflower was such an important food source that it was said to be "a staple from the Arctic Circle to the Tropics and from the Missouri River to the Pacific Ocean" (Harvard 1895). Additional minor uses were for medicinal purposes, anointing the hair and skin (Harvard 1895; Jenness 1958), in addition to uses in ceremonies (Heiser 1951). In the Southwest, the seed produced a purple dye for basketry and textiles, and the stems were utilized for ventilation structures (Whiting 1939). Not only were native spp. utilized, but the cultivated type was also grown, as there is records of sunflower heads up to 28 cm across (Wilson 1917).

Putt (1997) states that it is probable that early Spanish explorers first transported sunflower to Europe. The earliest record of this transportation from New Mexico to Madrid occurred in 1510 (Zukovsky 1950). From this point, the sunflower moved eastward and northward over the continent of Europe (Putt 1997). This movement across Europe is divided into two separate phases (Heiser *et al.* 1969). The first phase occurred for use of sunflower as an ornamental horticultural plant, followed by the second phase

where sunflowers were utilized as a food source. Sunflower dissemination from Spain first occurred through France and Italy (Putt 1997). By the late 16th century, sunflower was produced in gardens in Belgium, the Netherlands, Switzerland, Germany, and England (Putt 1997). Germany was the basis for eastward movement into countries like Hungary in 1664, but in eastern Europe sunflower was utilized only as a garden plant (Semelczi-Kovacs 1975). This European movement initiated the trend of human consumption of sunflower seeds (Putt 1997), where sunflower was regarded as a delicacy (Semelczi-Kovacs 1975). In 1716 an English patent was granted to Arthur Bunyan, describing the use of sunflower oil mostly as an industrial product, rather than for human consumption (Putt 1997).

The next step in sunflower distribution was the movement to Russia in the 18th century, originating from seed produced in the Netherlands (Semelczi-Kovacs 1975). The majority of use was as a garden ornamental, but cultivation for oil was also reported in 1769 (Semelczi-Kovacs 1975). During the early to mid-1800's, manufacturing of oil in Russia began on a commercial scale (Quesenberry *et al.* 1921). Interestingly, the Holy Orthodox Church of Russia played a significant role in increasing sunflower usage by excluding sunflower from a list of many oil foods that were prohibited (Putt 1997). By the year 1854, production had risen to approximately 150,000 ha in Russia (Semelczi-Kovacs 1975), and to around 21.5 million hectares in 1916 (Hensley 1924). It was around this time period that distinct sunflower seed types began to emerge; one smaller for extraction of edible oil, and the second larger, with a heavy hull for direct human consumption (Severin 1935).

As the specialization of sunflower types was taking place, so was the development of cultivar characteristics (Putt 1997). Using grower selections, local cultivars were developed for characteristics like maturity (Putt 1997), oil content (Zukovsky 1950), and resistance to the moth (*Homoeosoma nebulella* Hb.). Pustovoit made great advances in raising both the yield and oil content of sunflower (Putt 1997). "In 1940, average oil content in the main cultivar in the former USSR was 330g/kg, and by 1965, Pustovoit was testing cultivars with 550 g/kg oil" (Putt 1997).

It is reported that somewhere in the late 19th century, sunflower had come full circle, and sunflower types grown in North America were imported from Russia (Semelczi-Kovacs 1975). One of the main uses for sunflower after its reintroduction into North America was for silage to feed poultry (Wiley 1901). Production of sunflower in the early decades of the 19th century remained relatively low in North America, with the greatest production located in Missouri, Illinois, and California (Putt 1997). At that time, little sunflower production was intended for oil, as prices and the quality of other common oil crops were more suitable (Putt 1997).

In the mid 1930's the Canadian government realized the need for more domestic oilseed crops and began to research sunflower production and breeding at what is now known as the Saskatoon Research Station, Agriculture Agri-Food Canada (Putt 1997). Germplasm was collected from Mennonite gardens and multiplied at the research station, selecting for characteristics such as stem strength, maturity, vigor, yield, and oil content (Putt 1997). In 1943, oil supply was critical because of World War II and around 2000 ha were grown in Saskatchewan and Alberta (Putt 1997). Near the end of the war, the majority of production transferred to the Red River Valley of Manitoba, because of the

availability of germplasm resources imported by Mennonites, and the extended growing season in the area (Putt 1997). Yields and prices in the late 40's provided cash returns far greater than wheat, barley, oats and corn. However, soon after the first epidemic of sunflower rust in 1951, sunflower acreages declined to the lowest level since the reintroduction of sunflower (Putt 1997). This rust epidemic initiated research for disease resistance in sunflower. The discovery of rust resistant varieties (Putt 1949; Putt & Sackston 1955) increased planting in Manitoba to almost 11,000 ha for the remainder of the 1950s. Around this time "exploitation of the phenomenon of heterosis in sunflower" occurred, where hybrids were reported to out-yield the parental mean by almost 250% (Putt 1997). In the 1960s hybrids from the former USSR and the discovery of cytoplasmic male sterility and fertility-restoring genes to facilitate hybrid production had a dramatic effect on North American sunflower production (Putt 1997).

During the evolution of sunflower, the roles played by the North American origin and Russian influence are emphasized and other continents are often omitted from the literature. Asia, Australia, Africa, and South America all played roles in the evolution of sunflower cultivars in their respective regions. Following Europe and North America, South America quickly emerged as an important influence on sunflower development (Putt 1997).

2.1.2 Agronomics

Sunflower production agronomy has evolved to a highly specialized system since the days of ornamental production in European gardens. The two factors that are sought by sunflower producers are high seed quality and yield. Managing these two factors in an

economical manner is imperative, and is determined by sunflower genetics and the environmental conditions (Blamey *et al.* 1997).

Crop rotation in sunflower production is just as important as in any other production system for disease management (Gracia-Garza *et al.* 2002; Mueller *et al.* 2002). A crop sequence should be developed that will alternate between cereals and oilseeds, in addition to variation between crops that are susceptible to similar insects and diseases (Manitoba Agriculture and Food 2000). However, insects and diseases caused by air-borne spores are rarely controlled by crop rotation. Soil nutrient and moisture status are also important factors to consider when developing crop rotations including sunflower. Sunflower has high water use requirements, mainly from the deeper soil profiles due to its rooting system (Scheiner & Lavado 1999). It is suggested that a cereal crop such as wheat precede a sunflower crop (Dedio *et al.* 1980). This will provide a sequence of hosts that are susceptible to different pathogens and insects, in addition to distinct nutrient and moisture requirements.

Two systems of sunflower planting are used in North America, row crop planting is the most common method followed by solid seeding. Sunflowers have the ability to modify yield and quality characteristics depending on a range of physical characteristics and environmental conditions induced by plant populations (Blamey *et al.* 1997). Common row crop spacing is approximately 75 cm, and plant populations need to be adjusted according (Manitoba Agriculture and Food 2000). Oilseed types can have plant populations of 40,000-50,000 plants/ha, while confection sunflowers should not exceed plant populations of 44,000 plants/ha (Manitoba Agriculture and Food 2000). Plant populations will influence the head and seed size, and these consequences need to be

considered when planting, especially for confection sunflowers (Dedio *et al.* 1980). Seeds should ideally be placed in moisture, preferably 3-5 cm below the surface of the soil. Planting should occur any time during the month of May, depending on the weather conditions. Planting should not proceed past early June, as plant maturation will become an issue in the fall during harvest, especially for confection hybrids (Manitoba Agriculture and Food 2000).

Sunflowers are extremely susceptible to pathogens, either fungal, bacterial or viral, since the *Helianthus* genus is native to North America, these pathogens have evolved with the host (Gulya *et al.* 1997). Over thirty pathogenic organisms can infect sunflower, but approximately ten are economically important to sunflower production in general. The main pathogens to infect sunflower do so as seedling diseases, foliar diseases, wilts, and stalk and head rots (Gulya *et al.* 1997). Downy mildew (*Plasmopara halstedii* (Farl.) Berl. and de Toni) is the main seedling disease and can cause significant damage under the right environmental conditions. In recent years downy mildew infection has been minimized with resistant hybrids, and seed treatments (Gulya *et al.* 1997). The main foliar diseases include sunflower rust (*Puccinia helianthis* Schwein), Alternaria (*Alternaria helianthi* (Hansf.) Tubaki and Nishihara), septoria leaf spot (*Septoria helianthi* Ell and Kell.), and powdery mildew (*Erysiphe cichoracearum* DC. Ex Meret), along with a variety of bacterial and viral diseases (Gulya *et al.* 1997). The main wilts are sclerotinia wilt (*Sclerotinia sclerotiorum* (Lib.) de Bary) and verticillium wilt (*Verticillium dahliae* Klebahnis). *Sclerotinia sclerotiorum* is also the causal agent of stalk and head rot diseases which cause significant economic damage under the

appropriate environmental conditions (Gulya *et al.* 1997; Bailey *et al.* 2003). *Sclerotinia sclerotiorum* will be discussed in more detail in section 2.2.

Sunflower is usually the last crop to be harvested in the fall in Manitoba due to the late maturity of sunflower, in combination with the fact that frost is usually required to dry the foliage and reduce moisture content (Hofman & Hellevang 1997). A normal growing season is approximately 120 days in Manitoba, and a May planting will result in the crop maturing around late September or October, depending on the growing degree days during the summer. The longer the crop remains in the field, the lower the moisture percentage at the time of harvest. Delaying the date of harvest also increases the risk of seed loss due to birds, plant breakdown, seed shattering, and environmental conditions (Dedio *et al.* 1980). Producers may harvest sunflowers with seed moisture contents as high as 20 to 25%, so that losses are minimized (Hofman & Hellevang 1997). If the seed has a high moisture content at the time of harvest, it is imperative that a drying operation occur to reduce seed moisture content to approximately 10 to 12%, to reduce seed spoilage in the bin (Dedio *et al.* 1980; Hofman & Hellevang 1997).

2.1.3 Production

In 1947 and 1948, the mean annual gross return per hectare from sunflower was $102.92 in Manitoba (Putt 1997). In the 1960's, following the breeding advancements in sunflower yield, quality, and disease resistance, the overall market became even more economically attractive. Production reached its peak in 1979, when 150,000 ha were produced in Manitoba (Manitoba Agriculture and Food 2001). Soon after, the 15-year low was attained in 1986 when only 23,000 ha were produced, due to non-conducive weather, prices and disease epidemics (Manitoba Agriculture and Food 2001). In 2003 it

is estimated that 115,000 ha will be grown in Manitoba, with approximately 90,000 of those hectares as confection sunflowers. This is a significant increase from 1995 and 1996, where only 26,000 and 25,000 ha were in total production, respectively (Statistics Canada 2001).

Sunflower production contributions to the economy of Manitoba are relatively small in comparison to major prairie crops such as wheat and canola. However, Manitoba produced 88% of Canada's total sunflower crop in 2001, resulting in cash receipts of $28.63 million (Statistics Canada 2001). In 2001, Manitoba exported sunflower seed that totaled a value of $32.2 million, mainly to countries such as the United States, Mexico and Algeria (Manitoba Agriculture and Food 2001).

In the United states, one million total hectares were planted in 2002, with the majority of this production in the form of oilseed types (860,000 ha) (National Sunflower Association 2003). In 2003, the intended plantings are suspected to decrease to just under one million hectares, with the majority of the decline in confection production (National Sunflower Association 2003). Worldwide, sunflower is the fourth largest source of oil from plants, following soybean, cotton, and rapeseed. In 1995/96, worldwide sunflower production of oilseeds reached a value of 24.9 million tonnes, producing 9.0 million tonnes of vegetable oil (Kleingartner 1997).

2.1.4 Sunflower Usage

2.1.4.1 Oilseeds

Commercial crushing of oilseeds began in Manitoba in 1944 (Dorrell & Vick 1997). Manitoba crushing is currently discontinued, however, several United States processing plants crush approximately 0.6 to 1 million tons of seed annually (Dorrell &

Vick 1997). The industry utilizes oilseed sunflowers for their various components including the oil, kernel, hull, and meal.

Oilseed sunflowers can produce oils that are directly used in producing cooking oil, margarine, and occasionally industrial oils (Dorrell & Vick 1997). Cooking oil derived from sunflower, the main use of sunflower, is a high quality oil due to its light color and bland flavor (Dorrell & Vick 1997). Sunflower oil is regarded at a premium level in Europe. In the United States, sunflower oil is usually blended with canola and soybean oils (Dorrell & Vick 1997). Pure sunflower oil has a high level of polyunsaturated fatty acids (Table 2.1), and these characteristics are desired for certain cooking processes.

Oilseed kernels are directly utilized as food sources for animal feed, bird seed and as snack foods for humans (Park *et al.* 1997). Sunflower seeds are useful for feeding cows in early lactation because of the high oil/fat content and the compact, energy concentrated form of this feed (Park *et al.* 1997). Sunflower seeds contain 340 to 450g/kg crude fat, 170 to 210 g/kg crude protein, and 150 g/kg crude fiber (Park *et al.* 1997). Sunflower seed is high in unsaturated fatty acids (380-420 g/kg) but deficient in the protein lysine, hence sunflower kernels are combined with other food sources to compose an adequate diet for animals. Usually snack food for humans consists mostly of confection sunflower seeds, however, in some cases where oilseeds are of a large seed size, they can be utilized as human snack food (Park *et al.* 1997). Similarly, kernels for bird seed are usually composed of smaller confections, and oilseed screenings can be added to bird seed formulations with wheat, oat, corn, millet, and sorghum (Park *et al.* 1997).

Table 2.1. Fatty acid composition of selected oilseeds.

	Fatty acid (%)										
Oil	12:0	14:0	16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:1	Others
Sunflower			7	4	17	72					
Sunflower (high oleic)			3	4	88	3					2
Coconut	44	18	11	6	7	2					12
Corn			12	2	29	56	1				
Cottonseed		1	29	4	24	40					2
Olive			14	2	64	16	2				2
Palm		1	48	4	38	9					
Peanut			6	5	61	22		2			4
Rapeseed			4	2	17	13	9		15	41	
Safflower			7	2	13	78					
Soybean			11	4	25	51	9				

Adapted from Dorrell and Vick (1997).

Fatty acids are abbreviated with the first number indicating carbon number and the number after the colon indicating number of double bonds.

Sunflower meal is the fourth largest source of oilseed meal in the world, following, soybean, cottonseed, and rapeseed (Dorrell & Vick 1997). Hulls can be completely removed, partially removed, or not removed at all before oil processing (Dorrell & Vick 1997). The most efficient use of sunflower meal is in a mixture with soybean meal, for the purpose of rations in dairy, beef, sheep, swine, and poultry production. The reason that sunflower meal can be implemented in so many animal production systems is due to the high levels of oil, protein, and fiber (Park *et al.* 1997). Sunflower meal consists of 260 to 500g/kg crude protein, 120 to 350 g/kg crude fiber, and 10 to 90 g/kg crude fat (National Research Council 1989).

2.1.4.2 Confection Sunflowers

Confection sunflowers have larger seeds than oilseeds, and the hull is usually black and white striped. In the United States, the majority of sunflower production is oilseed and only about 18% is confection type, with the majority of overall production in North Dakota (National Sunflower Association 2003). In Manitoba, approximately 80% of sunflower production is of the confection type (Rob Park, Personal Communication). Worldwide, confection types are mainly grown in small quantities, mostly in gardens, for human consumption (Lofgren 1997). The largest confection seeds are used as "in-shell" product, to be salted and roasted. Medium sized seeds, or the "hulling size", are seeds that are dehulled and the kernels are roasted for snack food or used in a variety of baking products. The remaining confection seeds that are too small for human consumption are utilized as bird seed. This seed can be packaged purely as sunflower seed or incorporated with other grains and oilseeds to feed wild birds and pets (Lofgren 1997).

2.1.5 Growth Stages

The most common growth stage scale utilized for sunflowers was developed by Schneiter and Miller (1981) (Table 2.2). This growth stage scale does not measure all minute differences, but is adequate to make comparisons for field scientists and producers. The main use of this scale is for differentiating between growth stages for proper pesticide application, for breeding, and other agronomic practices (Blamey *et al.* 1997). This scale divides growth stages into two categories, vegetative, and reproductive. Vegetative stages are subdivided by the number of true leaves, while the reproductive stages are subdivided by the progression of anthesis and head development. For a description of sunflower physiology, a thorough review is suggested (Connor & Hall 1997).

2.2 *Sclerotinia sclerotiorum*

2.2.1 Introduction

Sclerotinia sclerotiorum is the causal agent for root rot, stem rot, and head rot in sunflower, and was originally identified on sunflower in 1861 (Gulya *et al.* 1997). *Sclerotinia sclerotiorum* was first described as *Peziza sclerotiorum* by Madame M. A. Libert in 1837 (Purdy 1979). In 1870, Funkel established the genus *Sclerotinia*, and renamed the pathogen *S. libertiana* Funkel. This binomial was accepted until it was demonstrated that this name was inconsistent with the International Rules of Botanical Nomenclature, renaming *S. libertiana* to *S. sclerotiorum* (Lib.) Massee (Wakefield 1924). However, it was later found that de Bary had first used this Latin name in an earlier publication, producing the proper name, *S. sclerotiorum* (Lib.) de Bary (Purdy 1979).

Table 2.2. Sunflower growth stages (Schneiter and Miller 1981).

Stage	Description
VE Vegetative emergence	Seedling has emerged and the first true leaf blade beyond the cotyledons is less than 4 cm long.
V(number) Vegetative stages (i.e.) V1 V2 V3 etc.	These are determined by counting the number of true leaves at least 4 cm in length beginning as V1, V2, V3, V4, etc. If senescence of the lower leaves has occurred, count leaf scars (excluding those where the cotyledons were attached) to determine the proper stage.
R1 Reproductive Stage	The terminal bud forms a miniature floral head rather than a cluster of leaves. When viewed from directly above, the immature bracts form a many-pointed star-like appearance.
R2	The immature bud elongates 0.5 to 2.0 cm above the nearest leaf attached to the stem. Disregard leaves attached directly to the back of the bud.
R3	The immature bud elongates to a distance more than 2.0 cm above the nearest leaf.
R4	The inflorescence begins to open. When viewed from directly above, immature ray flowers may be visible.
R5 (decimal) (i.e.) R5.1 R5.2 R5.3 etc.	This stage is the beginning of anthesis. The stage can be divided into substages dependent upon the percentage of the head area (disk flowers) that has completed or is in flowering. e.g. R5.3 (30%) R5.8 (80%), etc.
R6	Anthesis is complete and the ray flowers are wilting.
R7	The back of the head has started to turn a pale-yellow color.
R8	The back of the head is yellow but the bracts remain green.
R9	The bracts become yellow and brown. This stage is regarded as physiological maturity.

Sclerotinia sclerotiorum belongs to the Phylum Ascomycota of the Class Discomycete, of the Order Helotiales, and the Family Sclerotiniaceae (Ulloa & Hanlin 2000).

2.2.2 Taxonomy and Nomenclature

The differentiation between *S. sclerotiorum*, *S. trifoliorum,* and *S. minor* has been under question for many years (Grogan 1979; Willetts & Wong 1980). Separating the species has been based on morphological and physiological characteristics, such as sclerotial size, ascus and ascospore dimensions, and host isolation (Willetts & Wong 1980). Numerous reports have conferred regarding this taxonomy separation (Jagger 1920; Whetzel 1945), while others felt that this differentiation was inadequate and that all three fungi should be included into one species, *S. sclerotiorum* (Purdy 1955; Morrall *et al.* 1972). Purdy (1955) found that asci and ascospore size is variable within *S. sclerotiorum*, and concluded that previously distinct species were in actuality all from *S. sclerotiorum*. Grogan (1979) suspects that too much emphasis is placed on the host of origin, and that many isolates may just be variations of *S. sclerotiorum* and not different species. However, current studies now distinguish between the *Sclerotinia* species (Hubbard *et al.* 1997; Halmimi *et al.* 1998), and variations within certain species are differentiated by host isolation, physical and physiological characteristics, in addition to further genetic analysis (Errampalli & Kohn 1995; Kohli & Kohn 1996).

2.2.3 Host Range

Sclerotinia sclerotiorum has been described as one of the most nonspecific, omnivorous, successful plant pathogens (Purdy 1979). The success of *S. sclerotiorum* comes from it's non-host specific nature, contributing to the continuation of its life cycle even when a susceptible host is not present. Purdy (1979) reported that *S. sclerotiorum*

could infect 64 plant families, 225 genera, and a total of 383 plant species. This host index was updated in 1994 to include 75 plant families, 278 genera, 408 species, and 42 subspecies; over 100 of these species are present in Canada (Boland & Hall 1994). This list includes both Gymnospermae and Angiospermae, with the majority of hosts present in the Dicotyledonae subclass of Angiospermae (Boland & Hall 1994). The main host families are Solanaceae, Cruciferae, Umbelliferae, Compositae, Chenopodiaceae, and Leguminosae (Willetts & Wong 1980). The broad host range produces limitations on crop rotations (Boland & Hall 1994). In the past, flax (*Linum usitatissimum* L.) had been regarded as a non-susceptible host crop that could be utilized as a rotational crop. However, *S. sclerotiorum* has recently been reported to successfully infect flax in Manitoba and Saskachewan (Rashid 2000), placing further strains on prairie crop rotations. The destructive nature of *S. sclerotiorum* is better put into perspective when it is stated that 26% of all plant families contain hosts that are susceptible to *S. sclerotiorum* (Bailey & Bailey 1976).

2.2.4 Distribution, Prevalence, and Incidence

The broad host range of *S. sclerotiorum* exists in part due to the worldwide distribution of this pathogen (Twengstrom *et al.* 1998), and the expectation that *S. sclerotiorum* occurs in almost every country of the world (Purdy 1979). *Sclerotinia sclerotiorum* can occur in numerous climates around the world, ranging from hot and dry, to cool moist areas (Purdy 1979). Areas such as Florida have crops that are susceptible to *S. sclerotiorum*, but during the warm summer months in Florida, there is little prevalence. Cooler areas like Northern California, Nebraska, and New York are all known for their *S. sclerotiorum* presence (Purdy 1979). *Sclerotinia sclerotiorum* is the only species of the

genus *Sclerotinia* that has consistently been reported across Canada (Bardin & Huang 2001). In Southern Manitoba, environmental conditions are appropriate for *S. sclerotiorum* to infect a range of hosts in most growing seasons.

The prevalence and incidence of *S. sclerotiorum* are extremely variable in certain areas, and are dependant upon the environmental conditions and the frequency of host crops (Willetts & Wong 1980). In Canada, the main susceptible crops are dry beans, peas, lentils, soybeans, canola, and sunflower. Compiled Canadian Plant Disease Survey data suggests the mean prevalence (presence of infected heads within a surveyed field) of sclerotinia head rot for the last eleven years is 62.4%, with a mean head rot incidence (number of plants infected / total number of plants) of approximately 5.0%. In 2002, a sunflower crop survey across eight states produced a range of sclerotinia head rot incidences of 0% in Nebraska, Colorado, Kansas, Montana, and Texas, to 4.7% in North Dakota (Gulya 2003). These values are expected to only increase as the production of host crops increases, further limiting crop rotation as a management tool (Miller & Fick 1997; Gulya 2003).

2.2.5 Economic Importance

To examine the economic damage caused by *S. sclerotiorum*, numerous factors need to be taken into account. Within host crops of *S. sclerotiorum*, direct damage to yield and quality can occur, production losses due to the expenditures on control measures such as fungicides (Sayler 2003b), losses due to producing less lucrative non-host crops to avoid *S. sclerotiorum* infection (Purdy 1979), and finally losses during storage and transportation to the market place (Willetts & Wong 1980). Production losses caused by *S. sclerotiorum* were estimated to include $26 million annually to

United States dry bean production, $13 million/yr in United States snap bean losses, $24.5 million in 2000 to canola producers in North Dakota and Minnesota, 2% of the Midwest United States soybean crop is lost annually, and United States sunflower producers loose $15 million each year to infection caused by *S. sclerotiorum* (Sayler 2003b). The approximate 5% sclerotinia head rot incidence in Manitoba sunflowers translates into an economic loss of approximately $27/ha every year. Vegetable crops such as lettuce, celery, potato, tomato, and cabbage also exhibit drastic losses to *S. sclerotiorum* (Purdy 1979). These losses are extremely variable, and in some years *S. sclerotiorum* infection will not occur, whereas in optimal seasons, 100% yield losses can occur (Willetts & Wong 1980). As incidence increases (Willetts & Wong 1980), severe losses will become more common, contributing to the increased need for resources directed at solving *S. sclerotiorum* epidemics. This increased need was the reason for the United States Congress implementation of the Sclerotinia Initiative, which was allocated approximately $1 million in 2001 and another $496,750 in 2003 towards *S. sclerotiorum* research (Anonymous 2003; Sayler 2003b).

2.2.6 Disease Cycle, Infection, and Symptomology

The life cycle of *S. sclerotiorum* is monocyclic, with no secondary inoculum produced during the growing season (Agrios 1978). Nevertheless, the pathogen can spread during the growing season from infected to healthy plants by contact (Abawi & Grogan 1979). The life cycle of *S. sclerotiorum* (Figure 2.1) begins with sclerotia in the soil or on the soil surface. A high proportion of the life-cycle of *S. sclerotiorum* takes place in the form of this compact mycelial mass of sclerotia (Adams & Ayers 1979). The fungus uses the sclerotia to withstand conditions non-conducive to germination, and

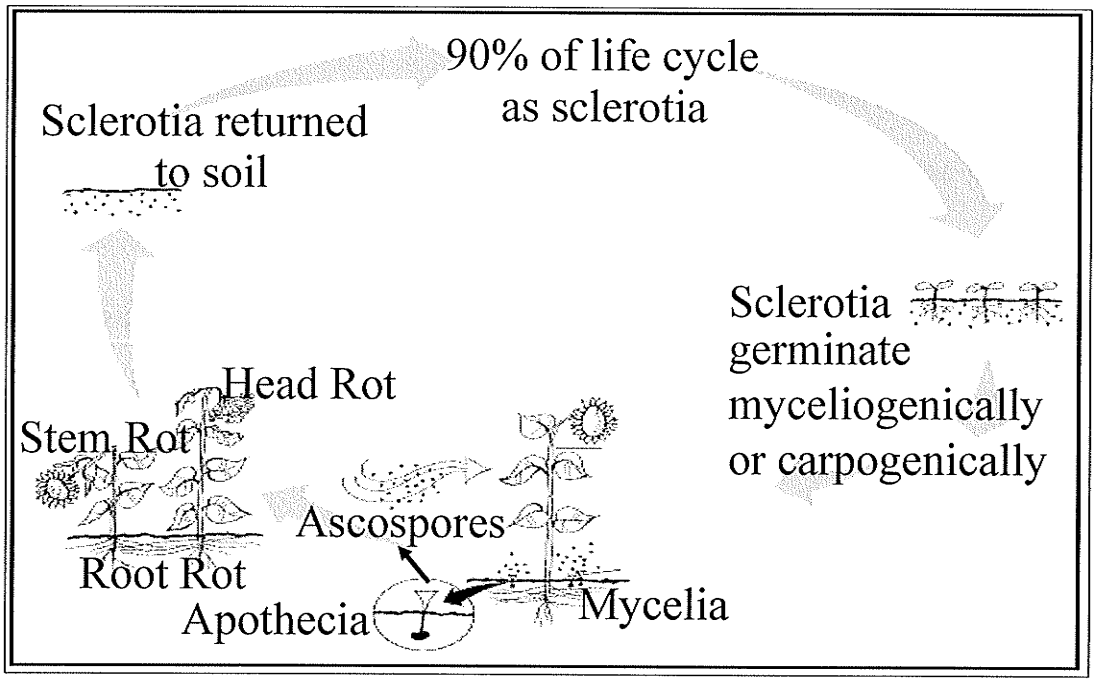


Figure 2.1. *Sclerotinia sclerotiorum* life cycle in the sunflower production system.

sclerotia have been reported to remain viable for seven years (Ben Yephet *et al.* 1993). However, sclerotial viability is highly dependant upon soil and environmental characteristics (Willetts & Wong 1980).

When conditions are appropriate, germination can occur through two distinct mechanisms (Adams & Tate 1976). Smaller sclerotia usually germinate myceliogenically (Purdy 1979), which is a type of asexual hyphal germination that protrudes from the sclerotia and infects plant tissue, usually below ground. This type of infection produces sclerotinia root rot in sunflowers, by penetrating root tissue and decomposing parenchymal tissue and the cortex (Gulya *et al.* 1997). Sclerotinia root rot will completely kill the plant if infection occurs at an early growth stage. Mycelia can continue to proliferate through the soil profile infecting nearby roots. Under row crop planting, it is common to see many plants in sequence die due to the progression of mycelia down the row (Bailey *et al.* 2003). Once roots are infected, watery-soft-rot symptoms can be observed above the soil surface producing white mycelia and subsequent sclerotia on the lower stem (Bailey *et al.* 2003). The lesion can progress up to 50 cm above the soil line. Stems become brittle and produce tan-colored fibers. Sclerotinia root rot can produce 50 to 100 sclerotia per plant (Enisz 1986), that may remain in the stem and root tissue or fall to the soil surface.

Carpogenic germination (sexual stage) will occur if environmental conditions are appropriate (Bardin & Huang 2001), and depends on the degree of melanization of the sclerotia (Willetts & Wong 1980). This type of germination produces apothecia, which are light brown, cup-shaped structures, borne on stipes that protrude from the sclerotia (Willetts & Wong 1980; Gulya *et al.* 1997). Apothecia forcefully release ascospores into

the air which are transported by air currents. Airborne ascospores will land on above-ground tissue and initiate infection through senesced tissue, such as disk or ray florets (Lamarque *et al.* 1985). In sunflower, ascospores can also initiate infection through living plant tissue (Purdy 1979), such as leaf axils, petioles, stems, and the receptacle of sunflower heads (Auger & Nome 1970). The majority of head rot infection occurs through senescing disk florets (Lamarque *et al.* 1985).

When leaf tissues are moist for a minimum of 16 hrs (Grogan & Abawi 1974), penetration of leaf tissue can proceed by mechanical pressure (Purdy 1979), enzyme degradation of the cuticle and epidermis, or by the growth of mycelia through stomatal openings (Prior & Owen 1964). It is also a requirement that an exogenous nutrient base be provided (Willetts & Wong 1980), however, in sunflower senesced tissue is not always required, and sucrose presence may aid in the infection of healthy tissue (Sedun & Brown 1987). After appressoria formation, penetration pegs force their way through the cuticle, and hyphal branches inflate between the cuticle and epidermal cells (Lumsden & Dow 1973). Lumsden and Dow (1973) found that intercellular growth in bean continues in the subcuticular area. Hyphal branches develop and progress through the host, inter and intracellularly with lesions developing within 48 to 72 hrs.

Symptoms of sclerotinia stem and head rot occur as a water-soaked, light brown lesion on the stem or receptacle of the head {Martens, 1988 267 /id}. The rot spreads quickly in both directions on the stem and can completely consume the head, including the seed, leaving only the vascular tissue intact. This produces sunflower heads with a broom-like appearance (Gulya *et al.* 1997). White mycelia is also evident, and sclerotia are produced in varying shapes and sizes. Rotted tissue, seeds and sclerotia fall to the

ground where the sclerotia over-winter in plant tissue or on the soil surface. Two to five times the amount of sclerotia can be produced in stem and head rot infections than compared to root rot (Gulya *et al.* 1997). Tillage operations incorporate sclerotia into the soil (Mueller *et al.* 2002), where they will degrade over time or germinate if conditions are appropriate, initiating the life cycle once again.

2.2.7 Environmental Requirements and Epidemiology

Proper environmental conditions are imperative to *S. sclerotiorum* infection (Willetts & Wong 1980). Every stage of the life cycle is dependant upon specific environmental factors including temperature, humidity, leaf wetness, crop canopy density, and soil moisture which differ for each stage of the life-cycle and type of germination (Abawi & Grogan 1979; Bardin & Huang 2001). The most common limiting factor for sclerotial germination is soil moisture (Coley-Smith & Cooke 1971; Grogan & Abawi 1974; Abawi & Grogan 1975). Continuous soil moisture for at least 10 days is required for apothecial development (Abawi & Grogan 1979). The reason that other factors like crop canopy, relative humidity, wind velocity, and temperature are important is mainly due to their direct effects on soil moisture, and not their direct effects on sclerotia themselves (Abawi & Grogan 1979). Temperature is most likely the next important factor affecting apothecial production, with 11 to 15 °C the optimum temperature range (Abawi & Grogan 1979). Nevertheless, temperature is rarely a limiting factor in temperate regions of the world, and it is the interaction between temperature and moisture that is the most significant parameter (Abawi & Grogan 1979). The optimum soil moisture and temperature for *S. sclerotiorum* germination also favors

host growth (Gulya *et al.* 1997), another reason why *S. sclerotiorum* is such a successful pathogen.

Under the appropriate conditions in Arizona, apothecia have been found as early as April 20, and will continue to germinate throughout the growing season as long as conditions remain conducive (Abawi & Grogan 1979). When a slight decrease in moisture tension occurs, "mature asci forcibly discharge the ascospores into the air to a distance of more than 1 cm" (Abawi & Grogan 1979). From this height, dispersal can take place in turbulent aboveground air layers, splashing rain, or by various insects (Abawi & Grogan 1979; McCartney & Lacey 1992). McCartney and Lacey (1992) report that ascospores can disperse up to 1 km, while other reports suggest several kilometers (Brown & Butler 1936). It is possible that a single sclerotiorum may produce 2.3×10^8 ascospores (Schwartz & Steadman 1978). Under low humidity and low temperatures, ascospores can remain viable for more than 45 days, or several months at 5 °C (Caesar & Pearson 1983).

Ascospores can cause infection within 2-3 days, however, at least 48-72 hours of continual leaf wetness is required (Abawi *et al.* 1975). Relative humidity alone is not appropriate, locations of infection need a thin layer of water present for infection (Abawi & Grogan 1979). If the moisture is no longer present after infection has been initiated, "lesion enlargement is stopped abruptly" (Abawi & Grogan 1979). It has also been determined that leaf wetness during inoculum availability is the most important factor, and that seasonal rainfall or irrigation amounts are of little consequence (Abawi & Grogan 1975). The optimum temperature range for ascospore germination and growth is

between 20 and 25 °C (Abawi & Grogan 1975). Sclerotia will develop within 7-10 days, and will fall to the base of the sunflower plant with the rotted tissue and kernels.

2.2.8 Sclerotinia Head Rot Assessment

Visual ratings are used for head rot assessment and progression. Single head ratings are recorded using a disease index of 0 to 5 (Rashid *et al.* 2002), 0 = no lesion, 1 = 1% to 5% head area infected (HAI), 2 = 5% to 20% HAI, 3 = 20% to 40% HAI, 4 = 40% to 60% HAI, 5 = greater than 60% HAI. This assessment method allows for comparison of disease incidence (number of plants infected / total number of plants) between treatments at harvest. The disease severity index (DSI) at harvest is calculated using the modified formula, DSI = (sum of individual plant ratings / 5 X number of plants rated) X 100) (Cober *et al.* 2003). This results in a DSI of 0 for treatments with all heads rated non-infected and a DSI of 100 for treatments with all heads rated 5 on the scale described above. The area under the disease progress curve (AUDPC) is also calculated (Shaner & Finney 1977) as a method of assessing disease development over time. Yield of seed and sclerotial production are also adequate measures of disease assessment, however, these are only effective in large scale plots. Analyzing yield on single head experimental units is extremely variable due to variation in head size.

2.3 Management of *Sclerotinia sclerotiorum*

2.3.1 Introduction

Control of *Sclerotinia* spp. has been inconsistent and uneconomical (Grogan 1979), most likely because of the wide host range and longevity of sclerotia (Gulya *et al.* 1997). Extensive research has progressed in *S. sclerotiorum* management, but only with "moderate success" (Gulya *et al.* 1997). Management of *S. sclerotiorum* in sunflowers

requires an integrated system of control, utilizing all available control methods (Gulya *et al.* 1997), including: 1) cultural practices, such as crop rotation, plant density management, and tillage, 2) the use of host resistance, 3) the use of chemical fungicides, and 4) and the use of natural and introduced biological control agents.

2.3.2 Cultural Management

Crop rotation is often the first cultural management method discussed when dealing with disease management. An optimal rotation to decrease *S. sclerotiorum* incidence in sunflower would be a 5-year rotation between any two *S. sclerotiorum* host crops (Gulya *et al.* 1997). This would include the control of susceptible weeds over this 5-year period. It was found that a 3 to 5 year rotation of non-host crops would reduce the number of sclerotia in the soil, reducing root rot incidence (Adams & Ayers 1979). It has also been reported that sclerotia samples collected from corn, sugar beet, and bean rotations were comparable despite different host crop history (Steadman 1983). Similarly, reports have shown that crop rotations were ineffective because of sclerotial longevity in the soil (Schwartz & Steadman 1978). Proper rotation will do little to reduce the influx of airborne ascospores (Gulya *et al.* 1997). Within the proper rotational schedule, field maps need to be developed that take into account the distance from other host crops, and this distance should be at least 1 km (Masirevic & Gulya 1992).

Plant densities affect both mycelial germination and apothecial germination of *S. sclerotiorum* (Gulya *et al.* 1997). Some studies have shown that plant densities have little effect on severity of sclerotinia root rot (Holley & Nelson 1986), while other studies have shown that increased plant populations increase the chances of plant-to-plant spread. (Huang & Hoes 1980; Hoes & Huang 1985). Due to the appropriate conditions for

carpogenic germination, a lower plant population will allow for greater air movement, producing a lower relative humidity and soil moisture content, decreasing ascospore production (Turkington & Morrall 1993). Planting date also affects *S. sclerotiorum* infection in sunflower (Gulya *et al.* 1989; Dedio 1992), especially if planting date can be manipulated to produce a host susceptibility period that does not coincide with prominent ascospore release periods.

The effects of tillage on sclerotial viability, root rot incidence, apothecial production, and stem and head rot incidence are not well understood, as many results are contrasting (Merriman *et al.* 1979; Workneh & Yang 2000; Kurle *et al.* 2001; Gracia-Garza *et al.* 2002; Mueller *et al.* 2002). The three main tillage practices studied are no-tillage, minimum tillage, and deep plowing which affect sclerotial placement in the soil. It is clear that tillage affects sclerotial placement (Subbarao *et al.* 1996), and that increased microbial colonization decreases sclerotia viability (Willetts & Wong 1980), however, the proper tillage practices to induce microbial colonization and sclerotial degradation are still unclear. Shallow cultivation or zero-tillage will keep sclerotia in the upper soil profile, which has been shown to increase the degradation of sclerotia (Cook *et al.* 1975). Carpogenic germination is more probable within the upper 5 cm profile (Kurle *et al.* 2001). Abawi and Grogan (1975) found that deep cultivation will bury sclerotia, increasing survival, and increasing mycelial germination for root rot, while decreasing the chance of carpogenic germination. Gulya et al. (1997) stated, that "theoretically, shallow tillage or no-tillage in dry areas, and deep tillage in areas with high precipitation may be effective in disrupting the two epidemiological systems in *S. sclerotiorum*". Deep plowing is often recommended for *S. sclerotiorum* control, but plowing to a depth

of 25 cm did not affect white mold severity in Nebraska (Steadman 1983). Continuous annual deep plowing may cause *S. sclerotiorum* infection every year, due to the theory that constant deep plowing will simply recover sclerotia that were previously buried (Subbarao *et al.* 1996). It is thus recommended, that deep plowing should take place after the growth of a host crop, burying the sclerotia; followed by minimum tillage in subsequent non-host seasons (Purdue University Department of Botany and Plant Pathology 2001). This practice will keep sclerotia in the deeper soil profiles where sclerotia degradation is accelerated (Merriman *et al.* 1979; Imolehin & Grogan 1980b), most likely by the degrading effects of microorganisms (Kurle *et al.* 2001; Gracia-Garza *et al.* 2002).

2.3.3. Host Resistance

Resistance is the most effective method to controlling disease in most host-pathogen systems (Gulya *et al.* 1997). Host resistance is also crucial to combating *S. sclerotiorum* in sunflowers, yet no high level of resistance to *S. sclerotiorum* in sunflower has been found (Gulya *et al.* 1997). Breeding programs have had little success due to *S. sclerotiorum* resistance being governed by additive gene action (Fuller *et al.* 1984).

Utilizing the genetic variability available from wild *Helianthus* spp. (Seiler & Rieseberg 1997), and incorporating this tolerance into commercial hybrids may be the answer to finding resistance. Resistance to head rot was discovered in *H. tuberosus* and *H. pauciflorus* (Pustovoit & Gubin 1974). Crosses between *Helianthus* species have produced a range of tolerance levels to *S. sclerotiorum* infection (Gulya *et al.* 1997; Kohler & Friedt 1999; Castano *et al.* 2001; Hahn 2002). Varying tolerance levels were found in North Dakota, where 26 different *Helianthus* plant introductions produced

significant levels of resistance to head rot (Gulya *et al.* 1986). More recently, Hahn (2002) found that sclerotinia head rot lesion size significantly varied between 45 sunflower hybrids. Similarly, 85 hybrids showed significant variation between sclerotinia stem rot lesions (Degener *et al.* 1999). These hybrids contained background germplasm incorporated from crosses with the wild species *H. tuberosus*, and *H. argophyllus*. Hybrids with this genetic background showed satisfactory stem rot resistance. Hybrids are regularly released that contain some level of tolerance to *S. sclerotiorum,* as eight sclerotinia-tolerant germplasm lines were released in 1999 (Miller & Gulya 1999). *Sclerotinia sclerotiorum* resistance is usually negatively correlated with height, oil concentration, days to bloom and days to maturity (Tourvieille & Vear 1990; Dedio 1992; Castano *et al.* 1993).

Sclerotinia sclerotiorum tolerance levels also fluctuate depending on the plant growth stages and infected tissues (Gentzbittel *et al.* 1998). Hybrids with tolerance to sclerotinia root rot did not necessarily express adequate levels of head rot tolerance (Rashid & Dedio 1992; Rashid & Dedio 1994). Even infections on the sunflower bud react differently than sclerotinia head rot infections (Auger & Nome 1970; Castano *et al.* 1993). Although, Castano et al. (1993) stated that some hybrids have similar infections in different plant parts. When this association between plant parts is better understood, along with the additive nature of *S. sclerotiorum* resistance genes (Gentzbittel *et al.* 1998), hybrid resistance may successfully be incorporated into *S. sclerotiorum* management strategies.

2.3.4 Chemical Control

The chemical fungicides, benomyl, vinclozolin, and iprodione have shown effectiveness against *S. sclerotiorum*, and are utilized effectively in other host-pathogen systems (Tu 1983; Morrall *et al.* 1985). In sunflower, benomyl has shown effectiveness (Auger & Nome 1970). Fungicide effectiveness against *S. sclerotiorum* in sunflower is hindered due to the morphology of the crop. Head rot is mainly caused by infection of the disk florets (Lamarque *et al.* 1985). To achieve control of sclerotinia head rot, the face of the sunflower would require adequate coverage (Gulya *et al.* 1997). Likewise, complete coverage of the stem, petioles, and leaves is needed to control any stem or leaf infection but is difficult to achieve because of the large, thick canopy of sunflower. Seed treatment has also been effective against *S. sclerotiorum* (Alabouvette & Louvet 1973; Rashid & Swanson 2002), but seed treatment only protects the seed from mycelial infection and not the root further into the growing season. Currently in Canada, no fungicide is registered for control of *S. sclerotiorum* in sunflower. Even if a fungicide is registered against *S. sclerotiorum* in sunflower, it may not be economical as a control procedure because of the difficulty in application due to the morphology of sunflowers (Mestries *et al.* 1998; Gentzbittel *et al.* 1998).

2.3.5 Biological Control

2.3.5.1 Introduction

Biological control is the "direct use of negative interactions – pathogenesis, competition, antibiosis, or antagonism – to regulate the population of a pathogen or pest" (Zadoks & Schein 1979). Biological control is a natural phenomenon in plant pathology, as microorganisms are among the most sustainable means of managing plant diseases

(Sutton & Peng 1993). Furthermore, emphasis has been placed on biocontrol to reduce the increasing use of pesticides (Fokkema 1993; Gerhardson 2002). Introduced biological control organisms have not been entirely successful, since their effectiveness depends on their suitability to the environment, and on how the species are introduced and maintained (Stack *et al.* 1988). Microclimatological conditions, along with the biochemical environment of the phylloshpere or rhizosphere directly impact the effectiveness of the biocontrol organisms (Blakeman 1973).

Optimal biocontrol agents (BCA) are only antagonists at the appropriate moment, which minimizes applications and the effects on other natural organisms in the cropping system (Sutton & Peng 1993; Cook 1993). To develop a successful BCA, thorough knowledge needs to be attained in regards to the economic feasibility, cropping system, disease epidemiology (biology, ecology, and population dynamics of the antagonists), and the interactions among these variables (Adams 1990; Deacon 1991; Sutton & Peng 1993). Temperatures on the leaves fluctuate along the phylloplane surface, making colonization and survival of BCA difficult; in contrast to the rhizosphere which is a much more stable environment for biological control (Blakeman & Fokkema 1982).

Potential BCA can be bacteria, filamentous fungi, and yeasts; applied as aqueous suspensions, wettable powders, or dusts (Sutton & Peng 1993). Important factors to consider are the type of BCA, the method in which the agent is applied, and the viability and longevity of the BCA (Sutton & Peng 1993). The longevity of the BCA will affect the biocontrol efficacy, frequency of applications, and production costs. The most common method of application is by spraying an aqueous BCA suspension at a high

concentration (Sutton & Peng 1993). This facilitates adequate coverage, and increases the possibility of successful colonization of the host or target.

2.3.5.2 Biological Control Mechanisms

The active ingredient in most chemical fungicides, and the manner in which this ingredient functions are usually well understood before chemicals are registered for disease control (Gerhardson 2002). For biological control, an array of conceptual theories and complex modes of action make it difficult to clearly define the mechanism of action for different BCA (Gerhardson 2002). It is rare that a particular BCA deploys only one type of biocontrol action (Cook 1993). Prominent mechanisms of biological control include parasitism, nutrient competition, and antibiosis (Blakeman & Fokkema 1982). Better understanding of the mechanisms and the organisms that employ these mechanisms will aid in optimum application timing, application in the appropriate form, and the appropriate concentration for application (Blakeman & Fokkema 1982).

Hyperparasites can affect plant pathogens by penetrating pathogen tissues and producing metabolic substances to destroy pathogen propagules, or by displacing pathogen tissues (Barnett 1963). *Trichoderma* spp. have restricted pathogen development simply by hyphal interactions, such as penetration of the pathogen by *Trichoderma* hyphae (Dennis & Webster 1971c). Mycoparasitism, is a form of hyperparasitism, involving the parasitism of a fungus by another fungus, and has been found in all major types of fungi (Lumsden 1981). Bacterial species such as *Bacillus* spp. and *Erwinia uredovora* Dye have also been reported to parasitize the fruiting structures of cereal rusts (Levine *et al.* 1936; Hevesi & Mashaal 1975).

Plant pathogen spores, such as *S. sclerotiorum* ascospores, often require exogenous nutrients for germination, mycelial growth, appressorium formation, and lesion development (Blakeman 1971; Clark & Lorbeer 1977; Fokkema 1981). If these nutrients or food sources were previously consumed by introduced organisms, infection may decrease. This mechanism of biological control is referred to as nutrient competition, defined as the "demand by two or more organisms for the same resource in excess of the immediate supply" (Singh & Faull 1988). Bacteria and yeasts are well suited towards nutrient competition because of their favorable surface-to-volume ratio, in comparison to fungal pathogens (Blakeman & Fokkema 1982). This type of biocontrol mechanism has been effective against such pathogens as *Botrytis cinerea* Pers.: Fr., and *Phoma betae* Frank (Blakeman & Fokkema 1982). Often this nutrient depletion is due to the limitation of amino acids, causing the inhibition of spore germination (Blakeman & Brodie 1977).

Antibiosis is the growth inhibition of a microorganism by another, through the production of antibiotics or toxic metabolites (Singh & Faull 1988). Filamentous fungi, yeasts, and bacteria have all been reported to produce antibiotics *in vitro* (Blakeman & Fokkema 1982). It is imperative that confusion between actual antibiosis and a simple delay in growth caused by the pathogen does not occur, as is often the case with *in vitro* inhibition zones (Fokkema 1973). To confirm that antibiotic production is the cause of pathogen inhibition, the antibiotic must be purified and tested directly, as opposed to introducing the living organism. This application of a cell-free culture filtrate to control the pathogen is advantageous when the producing organism may be poorly suited for biocontrol in certain environments (Blakeman & Fokkema 1982).

Both *Alternaria* and *Trichoderma* species have been reported to produce antibiotics. *Trichoderma* spp. were active against Gram-positive bacteria and fungi (Lindenfelser & Ciegler 1969; Blakeman & Fokkema 1982). *Trichoderma* spp. have also been identified to produce trichodermin, a sesquiterpene antibiotic effective against fungi, in addition to peptide antibiotics, active against fungi and bacteria (Dennis & Webster 1971a; Dennis & Webster 1971b). Antibiotic producing bacteria have also been active against both fungal and bacterial pathogens (Blakeman & Fokkema 1982). *Pseudomonas cepacia* Palleroni and Holmes, has been effective in inhibiting the germination and germ tube growth of *Bipolaris maydis* Shoemaker, as well as the formation of inhibition zones *in vitro* (Sleesman & Leben 1976). Likewise, *P. fluorescens* Migula is reported to have inhibited Phycomycetes, and the growth of *P. syringae* pv. *Phaseolicola* van Hall, by producing antibiotic compounds (Teliz-Ortiz & Burkholder 1960; Howell & Stipanovic 1980). Spore-forming bacteria such as *Bacillus subtilis* Cohn have been known to produce antifungal antibiotics, causing fungal germ tubes to swell and burst (Swinburne *et al.* 1975).

2.3.5.3 Biological Control of *Sclerotinia sclerotiorum*

Most biocontrol research of *S. sclerotiorum* has focused on the control of white mold of bean (Boland 1997), stem rot of canola (Savchuck 2002), and lettuce drop (Budge *et al.* 1995), along with soil applied treatments for sclerotinia root rot in sunflower (Bardin & Huang 2001). These BCA are most commonly in the form of mycoparasitic fungi, and occasionally, hypovirulent strains of *S. sclerotiorum*, bacteria, and insects (Bardin & Huang 2001). Steadman (1983) reported that at least 30 species of fungi, bacteria, and insects are parasites or antagonists of *Sclerotinia* spp. To be effective

these species need to be directed at the appropriate stage of the *S. sclerotiorum* life cycle that best suits the abilities of the specific BCA (Zhou & Boland 1998).

The first attempt at the biocontrol of sclerotinia root rot in the Canadian prairies evaluated *Coniothyrium minitans* Campbell, *Gliocladium catenulatum* Gilman and Abbott, and *Trichoderma viride* Pers. ex Fr., with *C. minitans* exhibiting the greatest BCA potential (Huang 1980). The majority of biocontrol research on S. *sclerotiorum* in sunflower has utilized *C. minitans* to manage sclerotinia root rot (Huang & Kozub 1991; McLaren *et al.* 1994). Numerous other studies demonstrate effective parasitism of *S. sclerotiorum* sclerotia using *C. minitans* (Ghaffar 1972; Turner & Tribe 1976; Budge & Whipps 1991; Budge *et al.* 1995; McQuilken *et al.* 1997). Foliar sprays of *C. minitans* have also been effective in reducing white mold of dry bean in the Canadian Prairies (Huang & Kokko 1993).

Biological control of *S. sclerotiorum* sclerotia using bacteria is less common than using fungal BCA (Willetts & Wong 1980; Bardin & Huang 2001), however, several genera have shown antagonistic effects towards *Sclerotinia* spp. (Wu 1988). *Bacillus cereus* Frankland and Frankland, reduced the incidence of pod rot in pea (Huang *et al.* 1993). *Bacillus subtilis* showed inconsistent results in the management of white mold of bean (Boland 1997). *Pseudomonas cepacia* and *B. subtilis* have reduced sclerotial germination and improved sunflower emergence (McLoughlin *et al.* 1992). Other *Pseudomonas* spp. such as *P. fluorescens* and *P. putida* Migula, have also been reported to be effective biocontrol agents of *S. sclerotiorum* (Expert & Digat 1995).

Previous research emphasizes the use of bacteria on sclerotial degradation and sclerotinia root rot (Expert & Digat 1995). The only report of bacteria evaluated against

S. sclerotiorum in the phyllosphere is in bean, using *B. polymyxa* Mace and *E. herbicola* Dye (Yuen *et al.* 1991). Further research is required into bacterial biocontrol on the phylloplane, as spore-forming bacteria in particular have the morphological ability (Emmert & Handelsman 1999) to receive close research attention as components in an integrated *S. sclerotiorum* management system.

2.3.5.4. Commercialization and the Future Outlook

The critical steps in the commercialization of biological control agents are: 1) discovering candidate agents, 2) performance testing, and 3) the scale-up for commercial use (Cook 1993). Within these three major categories are countless procedures and protocols, as "there are no short cuts to biological control" (Garrett 1965).

The discovery of BCA is an on-going process of screening masses of microorganisms so that no effective agent is overlooked (Cook & Baker 1983). This includes the actual isolation, and preliminary performance testing to identify any potential BCA. During the discovery process, the intended strategy of biological control needs to be determined. The main strategies include: 1) controlling the pathogen inoculum, 2) preventing the pathogen from infecting the plant, and 3) limiting disease development after infection (Cook & Baker 1983). As these strategies are being assessed for their effectiveness for each BCA, formulations should also be considered, but are often over-looked (Cook 1993). If a BCA exhibits potential during *in vitro* testing, but does not conform to formulation specifications for a specified use, the agent should be passed over (Schisler *et al.* 1992). Often this formulation assessment is neglected until the performance testing stage, causing extended time and monetary effort directed to a non-profitable identity.

Performance testing is an assessment of efficacy in replicated experiments under natural conditions with natural or artificial inoculation; only BCA with the greatest potential advance to this step (Cook 1993). The scale-up process is a major step, which requires an adequate amount of BCA biomass in the correct formulation to assess the biocontrol potential under field production systems (Cook 1993). This scale-up procedure is required to investigate the BCA effectiveness in combination with other agronomic practices and in the presence of other pathogens and microorganisms.

As the "biological systems mentality" is further being accepted (Sutton & Peng 1993), so is the idea that biological control is required in an integrated management system. Whether this biological control exists with the use of naturally occurring systems such as suppressive soils, or with the introduction of natural organisms such as *C. minitans*; it is imperative that researchers and producers take full advantage of these natural disease management organisms. Nevertheless, it is not a simple task to find, develop, and register an effective, economical BCA (Gerhardson 2002). In spite of many successful experimental results (Gerhardson 2002), the scale-up cost is limiting the production of BCA (Cook 1993). Advances in science, production of effective formulations, and further research into the integration of BCA with other disease management practices will demonstrate the requirement for biological control in the future.

3.0 Susceptibility of Confection and Oilseed Sunflowers to Sclerotinia Head Rot (*Sclerotinia sclerotiorum*)

3.1 Abstract

Sclerotinia sclerotiorum (Lib.) de Bary is the causal agent of sclerotinia head rot, an economically important disease of sunflower (*Helianthus annuus* L.). Host resistance is the most efficient method to control this pathogen, though no high level of resistance has been developed. The objectives of this study were to determine the differences in susceptibility between selected confection (P6946, MY9338, MY9490, IS8048, RH2073) and oilseed (CL 803, IS6111, P6230, 63A30, SF125, 8242NS) sunflower hybrids, compare artificial inoculation methods for infection and disease development, and investigate the relationship between sunflower growth stages and their susceptibility to infection. SF125 was the most tolerant commercial hybrid under all types of head rot inoculation. RH2073, IS8048, and P6230 were the most susceptible to head rot infection caused by natural, artificial ascospore, and ground millet infection, respectively. With natural infection, pooled incidence, disease severity index (DSI), and the area under the disease progress curve (AUDPC) for confection hybrids were significantly greater than the pooled data for all oilseed hybrids. Disease incidence, DSI, and the AUDPC for pooled confection hybrids were greater than for pooled oilseed hybrids in all cases for all inoculum sources, except for incidence when inoculated with ground millet. All three inoculation methods were significantly different from each other with infected ground millet causing the greatest infection, followed by artificial ascospore inoculation. The most susceptible flowering stage occurred when 100% of disk flowers had completed flowering (R6.0). Growth stage susceptibility seemed to correlate with the number of

disk florets. R7.0 was the second most susceptible stage followed by R5.9, R5.1-R5.5, R8.0-R9.0, and the lowest infection occurred at the R4.0-R5.0 growth stage. The information acquired from this study will benefit producers in current hybrid selection, and in determining the application timing of future fungicides or biocontrol products for the management of sclerotinia head rot.

3.2 Introduction

Sclerotinia sclerotiorum (Lib.) de Bary is an economically important disease that can infect sunflower (*Helianthus annuus* L.) roots, stems, and heads (Bisby 1921; Purdy 1979; Gulya *et al.* 1997). *Sclerotinia sclerotiorum* infects the roots through mycelial infection. If this infection occurs early in the life of the sunflower, 100% yield loss will occur. Infection via the stem and head occurs when sclerotia germinate carpogenically to produce apothecia which disperse airborne ascospores (Miller & Fick 1997). Yield and quality loss due to ascospore infection also depends on the stage of plant growth at the time of infection (Gulya *et al.* 1997). Canadian Plant Disease Survey data reveals that the mean head rot prevalence (presence of infected heads within a surveyed field) in Manitoba for the last eleven years was 62.4%, with a mean incidence (number of plants infected / total number of plants) of approximately 5.0%. The mean sunflower yield over the same period is 1,416 lbs/ac, with a mean price of 14.8 cents/lb (Rob Park, Personal Communication). With only a 5.0% yield loss (incidence), these values translate into an average loss of approximately $27/ha every year. These losses will significantly accumulate over time for large production systems, especially in seasons when prevalence can reach 100% (Rashid & Platford 1993).

Reductions in yield and quality of sunflower are not easily managed due to the limited number of management protocols in comparison to other sclerotinia host systems which utilize fungicides effectively (Twengstrom *et al.* 1998). The main control method utilized in sunflower production is the practice of managing infield inoculum (Hoes & Huang 1985). Crop rotation is an adequate method of reducing primary inoculum (Gracia-Garza *et al.* 2002), however, with today's large production area of host crops such as canola, beans, and peas, rotations are becoming less effective in reducing the overall inoculum (Miller & Fick 1997; Gulya 2003). Tillage is also an adequate method of reducing infield inoculum, resulting in decreased ascospore production (Merriman *et al.* 1979). Infield practices do little in regards to ascospore influx from neighboring fields which can be the main source of head rot infection (Gulya *et al.* 1989), justifying the recommendation to plant sunflower at least 1 km away from a previously *Sclerotinia*-infected field (Gulya *et al.* 1997). Other host production systems rely on fungicides as an effective source of protection to incoming ascospore infection (Gulya *et al.* 1997). Chemical control of *S. sclerotiorum* in sunflower is complex because roots, stems, leaves and the head are all susceptible to infection. Furthermore, chemical fungicides are difficult to apply in large scale sunflower production (Peres & Regnault 1985), and currently none are registered for control of sclerotinia head rot in Canada.

Studies have indicated that host resistance is appropriate to solving *S. sclerotiorum* infection in sunflower (Gentzbittel *et al.* 1998; Hahn 2002). A potential key to breeding for this resistance is utilizing the genetic variability available from wild *Helianthus* spp. (Seiler & Rieseberg 1997). Resistance to head rot was discovered in *H. tuberosus* Dumort and *H. pauciflorus* Dumort (Pustovoit & Gubin 1974). Crosses

between *Helianthus* species have produced a range of tolerance levels to *S. sclerotiorum* infection (Gulya *et al.* 1997; Kohler & Friedt 1999; Castano *et al.* 2001; Hahn 2002). Hahn (2002) showed the variation in head rot infection of 45 sunflower inbred lines. Cultivar HA850 demonstrated a low head rot rating of 3.0 on a scale of 1.0 to 8.0, while hybrids such as PLH2, HA300, and TUB-1789 all showed significantly higher infections of 6.2. Castano et al. (2001) demonstrated "continuous variation in resistance" to head rot between two inbred lines and their progenies. Disease incidence varied by four-fold in some cases when comparing parental lines to F_2 progeny. Similarly, resistance to sclerotinia head rot was significant in 26 sunflower lines in North Dakota (Gulya *et al.* 1986). Nonetheless, producers have no commercial hybrids available that provide a high level of resistance to sclerotinia head rot.

Susceptibility to sclerotinia head rot also varies between different sunflower growth stages (Auger & Nome 1970; Kondo *et al.* 1988). Limited data is available on the most susceptible growth stage in sunflower to sclerotinia head rot infection. Canadian Plant Disease Survey reports provide examples where incidence and severity of head rot drastically increase from ratings in August to ratings in late September within the same field (Rashid & Platford 2000; Rashid *et al.* 2001; Rashid *et al.* 2003). These increases in incidence and severity are most likely due to favorable weather conditions for disease development during the last month of the growing season. Additionally, heads that are infected in late July, August, and September, will all be visible before harvest, thus producing higher ratings towards the end of the growing season. Kondo et al. (1988) indicated "as the flowering time was delayed, incidence of head rot decreased". However, Auger and Nome (1970) showed the bud stage to be the most susceptible in

both field and greenhouse studies. Due to the previous contrasting research, the most susceptible growth stage to head rot is still unclear.

The objective of this study was to determine the differences in the level of tolerance between selected confection and oilseed sunflower hybrids. The general perception is that oilseed hybrids are more tolerant to head rot than confection hybrids, but no high level of resistance is available. Determining the most tolerant current hybrids and sunflower types would be beneficial to producers during hybrid selection and to seed companies during genetic evaluation and crossing. Comparing artificial inoculation methods for infection is equally important, as an economically efficient, high level of infection is required to effectively compare treatments. The information gained from determining the most appropriate artificial inoculation method will benefit future head rot research by inducing adequate levels of disease, for an appropriate cost. Identifying the most susceptible growth stage in sunflower to sclerotinia head rot is the next objective of this research. Knowledge into growth stage susceptibility in sunflower would benefit producers in managing head rot by directing proper chemical or biological control application timing. Knowing the most susceptible growth stage could also impact cultural practices such as seeding date, separating the most susceptible growth stage from prominent ascospore release periods.

3.3 Materials and Methods

3.3.1 Agronomics

3.3.1.1 Hybrid Comparison Experiment

The hybrid comparison experiments were located at the Agriculture and Agri-food Research Station in Morden, Manitoba, in 2001 and 2002, on a Hochfeld, fine sandy

loam, (well drained, orthic black, Chernozem). In 2001, only the confection type, P6946 (Pioneer®, Chatham, ON, Canada), and the oilseed type, CL 803 (Cloutier Agra Seeds Inc., Winnipeg, MB, Canada) were compared in a preliminary study of head rot susceptibility. In 2002, the experiment contained five oilseed hybrids, IS6111 (Interstate Seed Company, West Fargo ND, U.S.A.), P6230 and 63A30 (Pioneer®, Chatham, ON, Canada), SF125 and 8242NS (Mycogen Seeds, Indianapolis, IN, U.S.A.); and the following confection hybrids, P6946 (Pioneer®, Chatham, ON, Canada), MY9338 and MY9490 (Mycogen Seeds, Indianapolis, IN, U.S.A.), IS8048 (Interstate Seed Company, West Fargo, ND, U.S.A.), and RH2073 (Harvest States Sunflower, Grandin, ND, U.S.A.). A single treatment consisted of ten individually tagged plants within a 3 m row (17-22 plants), replicated in 4 blocks in a randomized complete block design. The spacing between treatments was 75 cm, with a border row separating each variety. The experiments were machine planted on May 15, 2001 and May 16, 2002.

3.3.1.2 Growth Stage Susceptibility Experiment

The growth stage susceptibility experiment was conducted using the hybrid Hysun 311 (Interstate Seed Company, West Fargo, ND, U.S.A.) in the greenhouse at the University of Manitoba, Department of Plant Science, Winnipeg, Manitoba, Canada, in 2002. Single sunflower seeds were planted in 210 mm x 210 mm pots (Listo Products Ltd., Vancouver, BC, Canada) containing a soil mixture consisting of two parts black top soil (Cheetham Soil Supplies Co Ltd., Winnipeg, MB, Canada), 2 parts washed concrete sand (Lafarge North America Inc., Winnipeg, MB, Canada) and 1 part sunshine peat moss (Sun Gro Horticulture Canada Ltd., Bellevue, WA, U.S.A.). Pots containing one sunflower comprised an experimental unit and ten pots were combined together randomly

to form a single treatment. The treatments in this experiment were the stage of flowering at the time of *S. sclerotiorum* ascospore inoculation. Ten sunflower seeds (one treatment) were stagger-planted every five days to produce this range of growth stages. Six different planting dates produced an acceptable range of growth stages to study the growth stage susceptibility. With limited greenhouse spacing, replications were produced on three consecutive occasions.

3.3.2 Artificial Inoculum Preparation

Ascospores were used as the primary source of inoculum in the 2002 hybrid comparison experiment, and in the growth stage susceptibility experiment. The ascospores were acquired from Dr. Mike Boosalis (University of Nebraska, Lincoln, NE). Ascospores were generated using a modification to the protocol described by (Lefol *et al.* 1998). Ascospores were recovered using the methods of (Hunter *et al.* 1982), by brushing and rinsing the ascospores off the filter paper with distilled H_20. Tween 20 (polyoxyethylene (20) sorbitan monlaurate, Mallinckrodt OR®, Paris, KY) was added as a surfactant to the spore suspension (10µl per liter) and vortexed at a medium-high rate for 45 seconds to disperse aggregated spores. Ascospores were then enumerated and their concentration adjusted with distilled water to a spore concentration of approximately 6.25×10^4 ascospores/ml using a hemacytometer. The appropriate volume of spore solution was transferred to a 1% sucrose solution contained in an E-Z Sprayer Vaporizer (Continental Industries, Brampton, ON). The E-Z Sprayer was set to a vapor pattern which produced a volume of 0.8 ml per single spray. This produced a spore concentration of 5.0×10^4 ascospores per single spray of the E-Z Sprayer.

The second source of inoculum used in the hybrid comparison trial was *S. sclerotiorum*-infected pearl millet seed (*Pennisetum glaucum* (L.) R. Br.). The pearl millet seed was autoclaved twice and amended with PDA plugs infected with *S. sclerotiorum* to cause mycelium infection of the pearl millet seed. This dried mycelial delivery mechanism could then be directly inserted into the receptacle of the head (utilized only in the 2001 preliminary study), or ground to a grainy dust using a Thomas-Wiley Laboratory Mill (Thomas Scientific, Swedesboro, NJ, U.S.A.), and lightly sprinkled on the face and receptacle of the sunflower heads.

3.3.3 Inoculation

3.3.3.1 Hybrid Comparison Experiment

Treatments composing the preliminary hybrid comparison experiment in 2001 were inoculated on August 16, 2001, by inserting infected millet directly into a wound induced with forceps, at the growth stage of R5.1-R5.5 (10% - 50% of all disk flowers have completed flowering) (Schneiter & Miller 1981). The hybrid comparison experiment in 2002 contained 10 different hybrids that were inoculated at the same growth stage, R5.1-R5.5. Hybrid variation in flowering resulted in multiple inoculation dates. On August 8th, 2002, IS6111, P6230, 8242NS, and P6946 were inoculated. The three inoculated treatments consisted of: 1) a non-inoculated control that was monitored for levels of natural ascospore infection, 2) an artificial inoculation with 5.0 x 10^4 ascospores on the face and receptacle of each experimental unit, and 3) an artificial inoculation of sprinkled ground infected pearl millet seed. Prior to infected millet sprinkling, each head was misted with 3 ml of double distilled H_20 on the face and receptacle to induce adhesion of the ground millet to the head. Following artificial

inoculation, heads were covered for 72 hrs with 10 lbs Poly plastic bags (Unisource, Winnipeg, MB, Canada), enclosed with 63 x 1.5 mm Premium Rubber Bands (Staples, Inc., Westborough, MA, U.S.A.). Prior to bag closure, the interior of each bag was misted with 4.0 ml of distilled water to induce infection. On August 12th, 2002, SF125, 63A30, and RH2073 were inoculated. The last inoculation date occurred on August 16th, 2002, when MY9338, IS8048, and MY9490 were inoculated.

3.3.3.2 Growth Stage Susceptibility Experiment

Treatments in the growth stage susceptibility experiment in the greenhouse were artificially inoculated only with ascospores. Ascospore inoculation was similar to the field inoculation, however, a spore concentration of only 1.5×10^4 ascospores on the face of each sunflower head was applied, followed by enclosing each head for 72 hrs with misted plastic bags. Inoculation for the first replication took place on July 30, 2002. The first planting date produced an inoculation stage of R8.0-R9.0 (back of the head is yellow) (Schneiter & Miller 1981), representing treatment one. R7.0 (back of the head has started to turn a pale yellow color), R6.0 (flowering is complete and the ray flowers are wilting), R5.9 (90% of disk flowers have completed flowering), R5.1-R5.5 (10-50% of disk flowers have completed flowering), and R4.0-R5.0 (inflorescence begins to open), represented treatments 2, 3, 4, 5, and 6, respectively. Ascospore inoculation for the second and third replications took place on January 17, 2003, and March 10, 2003, respectively, in the same manner as replication one.

3.3.4 Disease Assessment

Visual ratings were used for treatment comparison to assess the susceptibility of each hybrid and each inoculation stage. Visual rating began on the date of inoculation

and continued every seven days for the hybrid comparison experiment and the first replication of the growth stage susceptibility experiment. Visual ratings took place at five-day intervals for the second and third replications of the growth stage susceptibility experiment. Single head ratings were recorded using a disease index of 0 to 5 (Rashid *et al.* 2002), 0 = no lesion, 1 = 1% to 5% head area infected (HAI), 2 = 5% to 20% HAI, 3 = 20% to 40% HAI, 4 = 40% to 60% HAI, 5 = greater than 60% HAI. This assessment method allowed comparison of disease incidence (number of plants infected / total number of plants) at harvest. The disease severity index (DSI) at harvest was calculated for each treatment using the modified formula, DSI = (sum of individual plant ratings / 5 X number of plants rated) X 100) (Cober *et al.* 2003). This results in a DSI of 0 for treatments with all heads rated non-infected and a DSI of 100 for treatments with all heads rated 5 on the scale described above. The area under the disease progress curve (AUDPC) was also calculated (Shaner & Finney 1977) using the DSI values. Analyses of variance (ANOVA) and a mean separation test (Fisher's Least Significant Difference) were performed at $P = 0.05$, using the Analyst procedure of SAS (SAS Institute, Cary, NC).

3.4 Results

3.4.1 Hybrid Comparison

Disease incidence, DSI, and AUDPC, were higher for the confection hybrid, P6946 (Table 3.1) than for the oil hybrid CL803, yet the only significant difference between the two hybrids was in the AUDPC values for the 2001 preliminary study. In 2002, head rot incidence under natural infection ranged from 0 to 15.0% (Table 3.2). The confection hybrid, RH2073 had the highest incidence, DSI, and AUDPC in comparison

Table 3.1. Mean disease incidence, disease severity index (DSI), and area under the disease progress curve (AUDPC) for a hybrid comparison experiment at Morden, Manitoba, in 2001.

Hybrid	Disease Incidence (%)	DSI	AUDPC
Millet Mycelial Infection			
P6946 (Confection)	75.0a	75.0a	1515.1a
CL 803 (Oil)	55.0a	58.3a	904.1b
LSD *P* = 0.05	39.0	43.7	551.6

Note: No natural infection was observed in this experiment at Morden, in 2001.
Letters denote significance for Fisher's LSD value (P = 0.05)

Table 3.2. Mean disease incidence, disease severity index (DSI), and area under the disease progress curve (AUDPC) for 10 hybrids analyzed separately for the three inoculum sources at Morden, Manitoba, Canada, in 2002.

	Natural Ascospore Infection			Artificial Ascospore Infection			Ground Millet Mycelial Infection		
Hybrid	Incidence (%)	DSI	AUDPC	Incidence (%)	DSI	AUDPC	Incidence (%)	DSI	AUDPC
Oilseed									
IS6111	0.0b	0.0b	0.0b	40.0abc	38.0c	577.5bc	85.0ab	80.5abcd	1251.3ab
P6230	10.0ab	9.5ab	72.6ab	67.5a	66.5ab	1212.8a	97.5a	94.0a	1522.5a
SF125	0.0b	0.0b	0.0b	27.5c	27.0c	346.5c	47.5c	46.0e	532.0f
63A30	2.5b	2.5b	17.5b	52.5abc	46.5abc	544.3bc	75.0abc	67.5bcd	684.3ef
8242NS	2.5b	2.0b	14.0b	40.0abc	39.5bc	523.3bc	92.5a	85.5ab	1181.3bc
Oilseed Mean	3.0	2.8	20.8	45.5	43.5	640.9	79.5	74.7	1034.3
Confection									
P6946	2.5b	2.5b	17.5b	50.0abc	49.5abc	667.9bc	60.0bc	65.0d	814.6def
MY9338	7.5ab	7.5ab	61.3ab	37.5bc	39.2c	614.1bc	80.0ab	77.8abcd	1073.3bcd
IS8048	5.0ab	5.0ab	35.0ab	62.5ab	73.8a	1338.0a	87.5ab	84.1abc	1220.9abc
MY9490	10.0ab	9.5ab	74.4ab	42.5abc	40.6bc	759.1bc	80.0ab	87.0a	1351.0ab
RH2073	15.0a	15.0a	126.9a	42.5abc	42.5bc	779.5b	60.0bc	66.5cd	910.6cde
Confection Mean	8.0	7.9	63.0	47.0	49.1	831.7	73.5	76.1	1074.1
Hybrid Mean	5.5	5.4	41.9	46.3	46.3	736.3	76.5	75.4	1054.2
LSD P = 0.05	11.90	11.7	99.8	28.9	27.3	421.0	28.2	18.5	327.0

Letters denote significance for Fisher's LSD value (P = 0.05)

to all other hybrids under natural infection. The oilseed hybrids IS6111, SF125, 63A30, 8242NS, and the confection hybrid P6946 all had an incidence, DSI, and AUDPC, significantly lower than RH2073.

Artificial ascospore inoculation produced disease incidences ranging from 27.5% to 67.5%, a DSI range of 27.0 to 73.8, and an AUDPC range of 346.5 to 1338.0. P6230 produced the most infected heads while SF125 and MY9338 produced a significantly lower number of infected heads. The confectionery hybrid IS8048 had the highest DSI of 73.8 followed by P6230 with a DSI of 66.5. SF125 had the lowest DSI among the ten hybrids, significantly lower than IS8048 and P6230. When comparing the AUDPC, IS8048 had the highest AUDPC and all hybrids were significantly lower, except P6230.

Ground millet infection caused significantly higher head rot values under all three indexes, compared to natural and artificial ascospore infections; however, similar trends among the hybrids were produced with this mode of inoculation. Disease incidence ranged from 47.5% for SF125 to 97.5% for P6230. SF125 had significantly lower DSI than all other hybrids. Similarly, the AUDPC for SF125 was significantly lower than all other hybrids except 63A30 and P6946.

3.4.2 Susceptibility of Sunflower Types

Under all three inoculation treatments, pooled data for all confection hybrids produced greater indexes of head rot than the pooled data for all oilseed hybrids, except when comparing incidence under ground millet infection (Table 3.3). Under natural levels of infection, significant differences between oilseed and confection hybrids were produced for all three indexes. The same trend was expressed under artificial ascospore inoculation with no significant differences between confection and oilseed hybrids.

Table 3.3. Mean disease incidence, disease severity index (DSI), and area under the disease progress curve (AUDPC) for sunflower type with three inoculum sources, Morden, Manitoba, in 2002.

Sunflower Type	Incidence (%)	DSI	AUDPC
Natural Ascospore Infection			
Oilseed	3.0b	2.8b	20.8b
Confectionery	8.0a	7.9a	63.0a
LSD *P* = 0.05	4.4	4.3	35.6
Artificial Ascospore Infection			
Oilseed	45.5a	43.5a	640.9a
Confectionery	47.0a	49.1a	831.7a
LSD *P* = 0.05	15.8	16.2	298.1
Ground Millet Mycelial Infection			
Oilseed	79.5a	74.7a	1034.3a
Confectionery	73.5a	76.1a	1074.1a
LSD *P* = 0.05	16.9	12.9	285.3

Letters denote significance for Fisher's LSD value (P = 0.05)

Disease incidence under ground millet inoculation was actually greater in oilseeds, however not significantly different. The disease severity index and AUDPC were non-significantly greater for the pooled confection hybrids.

3.4.3 Inoculation Effectiveness

In 2002, the level of natural infection averaged across all hybrids was 5.5% (Table 3.4). Artificial ascospore infection was significantly greater than natural infection under all three indexes. Both natural and artificial ascospore infection were significantly lower than infection caused by ground millet. Disease incidence was 40.8, and 71.0 percentage points greater for the artificial ascospore and ground millet inoculation in comparison to natural infection. Similarly, DSI and AUDPC values were significantly different between all the three inoculation methods with ground millet producing the highest DSI and AUDPC values followed by artificial ascospore inoculation.

3.4.4 Growth Stage Susceptibility

Growth stages R6.0 and R7.0 had the highest head rot incidence of 73.3% (Table 3.5). The R5.1-R5.5 flowering stage was the next most susceptible growth stage with head rot incidence of 63.3%. The 90% flowering stage (R5.9) expressed a lower head rot incidence of 60.0%. Sunflowers that were nearly mature (R8.0-R9.0) by the time of ascospore application had an incidence of 46.7%, and the lowest incidence (16.7%) occurred when artificial inoculation with ascospores took place as the inflorescence began to open (R4.0-R5.0). Similar trends were produced when comparing the DSI data to the incidence data. Growth stage susceptibility followed a similar order with the R6.0 flowering stage having the greatest DSI followed by R7.0, R5.1-R5.5, R5.9, R8.0-R9.0, and the lowest DSI at R4.0-R5.0. Again, only the R4.0-R5.0 growth stage was

Table 3.4. Mean disease incidence, disease severity index (DSI), and area under the disease progress curve (AUDPC) for inoculum sources at Morden, Manitoba, in 2002.

Inoculum Source	Incidence (%)	DSI	AUDPC
Natural Ascospore Infection	5.5c	5.4c	41.9c
Artificial Ascospore Infection	46.3b	46.3b	736.3b
Ground Millet Mycelial Infection	76.5a	75.4a	1054.2a
LSD P = 0.05	7.6	6.6	114.0

Letters denote significance for Fisher's LSD value (P = 0.05)

Table 3.5. Mean disease incidence, disease severity index (DSI), and area under the disease progress curve (AUDPC) for growth stage susceptibility experiments, Winnipeg, Manitoba, 2002.

Inoculation Stage	Incidence (%)	DSI	AUDPC
R8.0 - R9.0 (Maturity)	46.7ab	53.8ab	1316.4ab
R7.0 (Back of the head yellow)	73.3a	73.3a	1706.7ab
R6.0 (100% Flowering)	73.3a	75.9a	1859.8a
R5.9 (90% Flowering)	60.0ab	61.9ab	1417.6ab
R5.1 - R5.5 (10-50% Flowering)	63.3ab	63.3ab	1355.0ab
R4.0 - R5.0 (Inflorescence begins to open)	16.7b	21.0b	416.7b
LSD *P* = 0.05	51.6	50.3	1362.2

Letters denote significance for Fisher's LSD value (P = 0.05)

significantly different from the R6.0 and R7.0, with all other growth stages non-significantly different from each other.

A difference in the growth stage susceptibility order was established when comparing the treatments by the AUDPC. The R5.9 stage had a higher AUDPC than the early-flowering stage (R5.1-R5.5). All other growth stages remained in the identical susceptibility order, and only the R4.0-R5.0 growth stage was significantly different from the 100% flowering stage (R6.0).

3.5 Discussion

This study was carried out with six oilseed and five confection hybrids available to sunflower producers in Manitoba. The results clearly suggest and confirm previous studies (Gulya 1985; Gulya *et al.* 1989; Rashid & Dedio 1992; Dedio 1992; Castano *et al.* 1993; Gulya *et al.* 1997; Degener *et al.* 1998; Kohler & Friedt 1999; Degener *et al.* 1999; Miller & Gulya 1999; Hahn 2002; Draper & Ruden 2002), that there is a significant range in tolerance to *S. sclerotiorum* infection in current sunflower hybrids. Continual assessment of current hybrids is required as varieties are selected primarily upon agronomic characteristics, while comparison of sclerotinia head rot tolerance levels needs exposure. Yields were not compared, as agronomic traits were not the objective of this study, but if a decrease in head rot susceptibility is an indicator of higher yield and enhanced quality (Gulya *et al.* 1989; Dedio 1992; Sadras *et al.* 2000; Mercau *et al.* 2001), current commercial hybrids with partial tolerance/resistance may prove beneficial to growers, and useful to breeders for further improvement in resistance to sclerotinia head rot.

One clear observation for all inoculum sources was that the oilseed hybrid SF125 was the most tolerant cultivar. When comparing the AUDPC, SF125 had significantly lower head rot levels than three hybrids under artificial ascospore infection, and seven hybrids under ground millet infection. Comparison under natural ascospore infection produced only one significant difference in incidence, however, no head rot occurred in SF125 plots, while P6230, MY9490, and RH2073 produced head rot incidences of 10, 10, and 15 %, respectively.

In a similar study, Castano et al. (1993) demonstrated that the genotype SD exhibited a high level of resistance to all inoculum sources and tests. The genotypes CD, SN, SD, SP, among others, also exhibited no symptoms under natural infection, while GH, CC40, and 2603 expressed significantly higher infections (Castano *et al.* 1993).

The ranking of the hybrids and severity of head rot differed depending on the inoculation method, a similar trend to what has been previously shown (Castano *et al.* 1993). In the present study, hybrids such as P6230 and MY9490 were consistently highly susceptible under all three inoculum sources. Artificial ascospore infection caused the confection hybrid IS8048 to have the highest AUDPC values, significantly higher than all other hybrids except the oilseed P6230, which also had an AUDPC significantly greater than all other hybrids. Ground millet infection caused P6230 to have the highest AUDPC values, significantly higher than all hybrids except for IS6111, MY9490 and IS8048. Under natural infection, hybrid rankings were similar with RH2073, MY9490, and P6230 exhibiting the highest susceptibility.

The purpose of the sunflower type comparison was to determine if any differences in tolerance exist between oilseed and confection hybrids. The general understanding is

that confection hybrids are less tolerant than oilseed hybrids to most diseases (Gulya 2003). Consistently, as a group confection hybrids had higher values for all three indexes, with all three-inoculation methods, except when comparing disease incidence under ground millet infection. Individually not all confection hybrids were more susceptible than all oilseed hybrids. In a related study, pooled data from oilseed hybrids produced a head rot incidence of 28.4% in comparison with an incidence of 41.4% in pooled data from confection hybrids (Van Becelaere & Miller 2003). Opposite results were reported from a field survey by Gulya (2003), where 394 oilseed fields had a higher incidence of head rot than the 78 confection fields surveyed, though these results were not produced under controlled experimental conditions. In the same survey, incidence of sclerotinia mid-stalk rot was higher in confection hybrids than in oilseed types (Gulya 2003), demonstrating how tolerance to sclerotinia can vary depending on the plant tissue and location of infection (Castano *et al.* 1993). Genetic differences between oilseed and confection hybrids and their impact on head rot susceptibility have not been documented. This comparison of phenotypic differences in oilseed and confection susceptibility will benefit producers in sunflower type selection, and breeders in directing future incorporation of oilseed tolerance into confection hybrids.

Numerous inoculation techniques have been utilized in studying sclerotinia head rot (Castano *et al.* 1993). Natural ascospore infection is the most realistic type of infection for researching head rot. Nevertheless, natural infection levels are often unreliable to allow effective comparison between hybrids or specific treatments. Castano et al. (1993) suggested artificial inoculation to counteract the unpredictable levels of natural infection. Artificial ascospore infection simulates natural infection well,

however, ascospore production is timely and a labor intensive process, while infected ground millet is as effective but less time consuming in preparing the inoculum. Ground millet inoculation does have its disadvantages; differences in head morphology among sunflower hybrids make it difficult to apply and retain equal amounts of ground millet to each head. The use of surfactants was suggested to help the adhesion of ground millet to the sunflower heads (Rashid & Seiler 2003). Covering the heads with plastic bags following inoculation was an effective method to induce infection. However, Rashid and Seiler (2003) demonstrated that plastic bags were the least effective in comparison to paper and pollination bags, and that paper bags provided the most favorable conditions for infection and disease development. The effectiveness of ground millet inoculation is sufficient to clearly compare head rot incidence, DSI, and the AUDPC between hybrids, which were all significantly greater than the index values under natural or artificial ascospore infections. These results are similar to those reported by Rashid and Seiler (2003), where ground millet infection was also the most effective followed by ascospore inoculation, with minimal infection under natural infection. A comprehensive breeding program should incorporate numerous inoculum sources and inoculation techniques in testing hybrids for tolerance (Castano *et al.* 1993).

Growth stage susceptibility information is a valuable tool to sunflower producers and researchers. Data explaining the relationship between sunflower growth stages and their susceptibility levels is conflicting. Auger and Nome (1970) reported that the most susceptible sunflower stage to ascospore attack was the bud stage (R4.0) followed by the fertilized flowering stage (R6.0). However, infection during early flowering (R5.1-R5.5) and after 100% flowering (R6.0) was minimal. Dedio (1992) showed significant

correlation between head rot incidence and sunflower bloom or maturity. Gulya (1989), demonstrated a significant correlation between head rot percentage and R5.5 bloom date, indicating that the later the 50% bloom date of a particular hybrid, the lower the percent head rot. Kondo et al. (1988) found similar results with greater infections occurring at earlier growth stages, while opposite results were reported by Castano et al. (1993). The effect of rainfall from the Gulya (1989) data, contributes to the possibility that decreased head rot could be due to disease escape and not resistance. Rashid and Seiler (2003) demonstrated that disease inoculations were more effective at mid-flowering and late flowering than at early flowering in wild sunflower species. The data from the present greenhouse study suggests that the most susceptible stage is when 100% of disk flowers have completed flowering (R6.0), since ascospores cause head rot through infection of senescing disk florets (Lamarque *et al.* 1985; Kondo *et al.* 1988). A higher number of senescing disk florets may increase the opportunity for ascospore infection, the reason why the R7.0 stage had a similar infection level to the R6.0 flowering stage. Following the R7.0 stage, disk florets tend to be removed by physiological processes and environmental conditions. This loss of senescing florets is the reason why head rot infection decreases as the plant matures, and perhaps the reason why the 90% flowering stage (R5.9) and the early flowering stage (R5.1-R5.5) have greater susceptibility levels than at maturity (R9.0). The only growth stage that is significantly lower than the R6.0 and R7.0 growth stage is the R4.0-R5.0 growth stage. This low susceptibility level is due to undeveloped floral tissue which is not yet conducive to head rot infection. This data emphasizes the destructive nature of this disease (Gulya *et al.* 1989), due to the fact that the sunflower heads are susceptible for a period of several weeks, giving the pathogen a

prolonged opportunity for infection. *S. sclerotiorum* infection in canola has an infection period with greater constraints, due to the fact that sclerotinia stem rot infection requires senescing petals (ephemeral existence) as an exogenous food source (Purdy 1958). If the 100% flowering stage in sunflower can be protected with potential fungicides or biocontrol products, or if bloom stage can be manipulated to avoid prominent ascospore release periods, either by adjusting cultivar selection or planting date, infection may decrease, maintaining seed yield and quality.

3.6 Conclusions

The data produced from this study can directly aid producers and researchers in head rot management and research. If appropriate hybrids are grown to decrease the incidence of head rot, this will not only impact the current sunflower crop, but also decrease inoculum for subsequent host crops, such as canola and a range of bean types. A connection between head rot tolerance and sunflower type was exhibited under natural infection, validating the present hypothesis that in general oilseed hybrids are more tolerant than confection hybrids. Inoculation data has confirmed previous techniques, proving that infected ground millet is an effective and inexpensive inoculation source.

Growth stage susceptibility data will be instrumental if a foliar fungicide or biological control agent is registered for control of sclerotinia head rot. The knowledge that head rot risk is high from early flowering to maturity further emphasizes the importance of multiple control applications that have been effective in the past (Kondo *et al.* 1988; Hagan *et al.* 1994; Jones 1995). Knowledge that the 100% flowering stage is the most susceptible flowering stage will aid in the timing of a single application of a control product during seasons not conducive to long-term ascospore release. Host

resistance may be the answer to controlling sclerotinia head rot in sunflowers, but until resistant hybrids are developed, knowledge of hybrid reactions and the epidemiology of *S. sclerotiorum* will lead to the improved management of sclerotinia head rot.

4.0 Management of Sclerotinia Head Rot (*Sclerotinia sclerotiorum*) in Sunflower

4.1 Abstract

Sclerotinia head rot (*Sclerotinia sclerotiorum* (Lib.) de Bary) in sunflower (*Helianthus annuus* L.) is a devastating pathogen affecting yield and seed quality. Present management practices to control head rot are limited. Research into biological control products, used in concert with existing chemical fungicides offers diversified control options for the sunflower industry. Two bacterial biological control agents (BCA), *Pseudomonas chlororaphis* (strain PA-23), and *P. corrugata* (strain 41), along with a new fungicide BAS 510 F (2-chloro-N-(4,-chloro-biphenyl-2-yl)nicotinamide), were assessed for their efficacy in foliar applications, and as *Sclerotinia*-inoculum coatings to control sunflower head rot under field conditions. In 2001 and 2002, field trials were conducted at Morden with two inoculation stages, and at Carman with one inoculation stage under a misting system. Products were tested against natural ascospore infection and artificial inoculation with *S. sclerotiorum* infected pearl millet seed (*Pennisetum glaucum* (L.) R. Br.). Natural ascospore infection was minimal, while artificial ascospore inoculation ensured adequate infection in the control plants, allowing for accurate treatment comparisons. BCA produced promising results under natural infection pressure, totally eliminating head rot incidence in 2001 at Carman. Artificial inoculation significantly increased sclerotinia head rot incidence over natural infection for both years and both locations. Coating the *S. sclerotinia* inoculum with strain PA-23 and strain 41 significantly reduced the area under the disease progress curve (AUDPC) in both trials at Morden in 2001 and 2002. All three products reduced the AUDPC in every

trial when applied to sunflower heads prior to artificial inoculation with *Sclerotinia*-infected millet. Control of sclerotinia head rot was more efficient when management products were applied at the R6.0 flowering stage. Strain PA-23 was more effective as a BCA, and the experimental fungicide, BAS 510 F, was more successful than both BCA.

4.2 Introduction

Sclerotinia head rot, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is a destructive disease that infects sunflower (*Helianthus annuus* L.) under favorable environmental conditions (Rashid 1993). Incidence and severity of head rot are variable in the Red River Valley depending on seasonal conditions (Gulya *et al.* 1997). An increase in *S. sclerotiorum* over the years can be attributed to the increased production of hosts, such as canola, dry beans, soybeans, peas, and sunflowers (Gulya 2003). From 1987 until 1991, no survey of sclerotinia head rot was recorded in Manitoba (Rashid & Platford 1992). Ten years later the prevalence of head rot in Manitoba was 60% of fields surveyed in 1997 (Rashid & Platford 1998), 15% in 1998 (Rashid & Platford 1999), 70% in 1999 (Rashid & Platford 2000), 65% in 2000 (Rashid *et al.* 2001), 33% in 2001 (Rashid *et al.* 2002), and 93% in 2002 (Rashid *et al.* 2003).

Sclerotinia sclerotiorum management in sunflower production is limited in comparison with other crops such as canola and dry beans that use chemical fungicides effectively to control *S. sclerotiorum*. No chemical fungicide is presently registered for control of head rot in sunflowers due to the lack of research on the effectiveness of fungicides, and for economic reasons (Mestries *et al.* 1998). Commercial sunflower hybrids lack a high level of resistance to *S. sclerotiorum* (Kohler & Friedt 1999). Nonetheless, research on resistance is progressing with the use of wild *Helianthus* species

as potential sources of resistance genes (Seiler 1992). Breeding for resistance is economical, but time-consuming because of the additive nature of sclerotinia resistance genes (Mestries *et al.* 1998). Immediate research targeted at developing effective alternative control measures for head rot management is required.

Bacterial biological control is the direct use of negative interactions caused by the bacteria to control a pathogen or pest population (Zadoks & Schein 1979). This mechanisms was seen as a possible alternative or an additional tool in managing sclerotinia head rot (Rashid & Dedio 1992). Numerous biocontrol agents (BCA) have been researched over the years to achieve a better understanding of their effects on *S. sclerotiorum* (McLaren *et al.* 1994; Budge *et al.* 1995; McQuilken *et al.* 1997; Boland 1997; Zhou & Boland 1998). *Pseudomonas* species have been used to manage different forms of *S. sclerotiorum* infection in sunflower (Expert & Digat 1995). The majority of biological control research within the sunflower production system has focused on soilborne pathogens, and the segment of a pathogen life-cycle within the rhizosphere (Inbar *et al.* 1996; Li *et al.* 2002). This method of management will decrease the initial inoculum in a particular field, but will not decrease the influx of *S. sclerotiorum* ascospores from neighboring fields. For this reason, it is required that a foliar treatment be applied to protect the sunflower heads from ascospore infection. Bacteria have been identified and demonstrated significant inhibitory effects on *S. sclerotiorum* mycelia *in vitro*. Two bacteria, *P. chlororaphis* (strain PA-23) and *P. corrugata* (strain 41), were previously isolated by serial dilution from soybean and canola fields, respectively, in Southern Manitoba. Both bacteria produced significant reduction in mycelial growth and exhibited biological control in a canola cropping system (Savchuck 2002). The success

of any potential control strategy requires efficacy data from commercial field trials with high disease severity (Perello *et al.* 2003).

The objective of this research was to compare the efficacy and assess two previously effective *Pseudomonas* species against *S. sclerotiorum*, *Pseudomonas chlororaphis* strain PA-23 and *P. corrugata* strain 41, along with a new experimental fungicide, BAS 510 F (2-chloro-N-(4,-chloro-biphenyl-2-yl)nicotinamide), BASF Corporation, Mount Olive, NJ, U.S.A.), for their reduction of sclerotinia head rot incidence, severity, and disease progression (AUDPC) under natural and artificial infections. Two treatment application techniques will be compared, including a foliar spray application and an *S. sclerotiorum* inoculum coating, along with two application timings, early (R5.1-R5.5) and late (R6.0). By incorporating two treatment application timings, the proper treatment application time will be developed. The use of a misting system to induce infection and increase BCA longevity will also be investigated. Overall, this information will provide incite into the BCA and chemical efficacy, proper application timing, and the effects of a misting system, that will aid in head rot management and future head rot research.

4.3 Materials and Methods

4.3.1 Agronomics

Two field experiments were conducted at the Agriculture and Agri-Food Canada Research Station in Morden, MB, and one experiment at the University of Manitoba, Carman Research Station, Carman, MB, in 2001 and 2002. The soil at the Morden site is a Hochfeld, fine sandy loam (well-drained, Orthic black Chernozem), while the soil at the Carman site is a Denham Loam, (Lacustrian loamy clay). The Morden site contained two

side-by-side trials representing two different inoculation stages (early-flowering stage, R5.1-R5.5, and late-flowering stage, R6.0). The Carman site differed from Morden in that it had an overhead misting system programmed to mist water five minutes every hour, beginning the day of product application and continuing for 14 days. Misting was carried out to create favorable conditions for a high incidence of disease and to increase the longevity of the BCA. All Morden trial locations were prepared with conventional tillage and a spring fertilizer application of 68 kg of 26-13-0 of N-P-K product. The Carman location was also prepared with conventional tillage, but with no fertilizer application (soil nutrient status was adequate). The sunflower hybrid Hysun 311 (Interstate Seed Company, West Fargo, ND, U.S.A.) was used in all trials to prevent any varietal variability. A single treatment consisted of ten randomly tagged (2.5 cm x 25 cm tags, C. Frensch Ltd., Beamsville, ON, Canada) plants (chosen from 17-22 plants) within a 3 m row, with four replications in a randomized complete block design. The spacing between treatments was 75 cm (standard sunflower production spacing). The trials were planted on May 15, 2001 and May 22, 2002 at Morden, and on June 4, 2001 and May 24, 2002 at Carman.

4.3.2 Biocontrol Agent Production

Pseudomonas chlororaphis (strain PA-23) and *P. corrugata* (strain 41) were retrieved from -80°C storage, and cultured onto Luria Bertani agar (LBA, 15.0 g agar, technical (Difco Laboratories, Detroit, Mich., U.S.A.), 10.0 g tryptone peptone (Becton/Dickinson, Sparks, MD, U.S.A.), 5.0 g yeast extract (SIGMA, St. Louis, MO., U.S.A.), and 5.0 g NaCl). After 48 hours of growth on LBA, a loop of bacteria was transferred to LB broth (same formulation as LBA, without 15.0 g of agar technical) and

cultured for 16 hours, at 28°C, shaking at 160 rpm. The bacteria culture concentrations were adjusted to log 8 cfu/ml by correlating with OD values from a standard curve generated for each bacterial strain. The bacterial solution was diluted in a potassium phosphate buffer solution (Fisher Scientific, Fair Lawn, NJ, U.S.A.) with Tween 20 (ICI Americas, Inc., SIGMA, St Louis, MO., U.S.A.) as a surfactant.

4.3.3 Pathogen Inoculum Production

A natural influx of ascospores was relied upon as a source of inoculum, though natural infection is highly variable and dependant on the environmental conditions. In 2001 and 2002, *Sclerotinia*-infected millet seed (*Pennisetum glaucum* (L.) R. Br.) was used to supplement natural inoculum. The millet seed was autoclaved twice (120°C for 25 min) in closed containers under aseptic conditions, then amended with potato dextrose agar (Becton/Dickinson, Sparks, MD, U.S.A.) plugs infected with *S. sclerotiorum* (14 days at room temperature) to cause mycelium infection of the millet seed. Infected millet was directly inserted into the receptacle of the sunflower head.

4.3.4 Biocontrol Agent and Fungicide Application

Two experiments were located at the Morden site in both 2001 and 2002. The purpose of the double timing was to obtain information on the appropriate application time to achieve the greatest head rot management. The Morden 1 experiment was carried out with the intent of applying the BCA and fungicide at an early flowering stage R5.1 – R5.5 (10% - 50% of disk flowers have completed flowering) (Schneiter & Miller 1981), while the Morden 2 experiment was intended to mimic a control application at the growth stage R6.0 (disk flowering is complete and ray flowers are wilting). Treatment application occurred July 30, 2001, and August 1st, 2002 for the Morden 1 experiment;

August 7, 2001 and August 13, 2002 for the Morden 2 trial; and August 14, 2001 and August 1st, 2002 for the Carman trial.

The two bacterial isolates and fungicide treatments were applied using an EZ-Sprayer Vaporizer (Continental Industries, Brampton, ON), set to a vapor pattern which produced a volume of 0.8 ml per single spray. A single spray of 0.8 ml was applied to both the face and the receptacle of each sunflower head that was naturally and artificially inoculated. This application ensured complete product coverage of each experimental unit.

Three additional treatments consisted of coating the *Sclerotinia*-infected millet with the bacteria or fungicide before insertion into the receptacle. This was accomplished by dipping the *Sclerotinia*-infected millet seed into the bacterial or fungicide solution, then inserting the coated inoculum in the sunflower head.

4.3.5 Pathogen Inoculation

Artificial inoculation with infected millet was carried out by puncturing the back of the sunflower head with sterile forceps, then placing the infected-millet seed in the wound. The wound was then closed and sealed with reinforced clear adhesive tape (3M, 3M Highland™ 897 Tape, London, Ontario) to provide favorable conditions for infection. Artificial inoculation for the coated inoculum treatments occurred at the time of coating the inoculum with the bacteria or fungicide. Artificial inoculation for the foliar treatments took place 24 hrs following bacteria or fungicide application. This provided time for the bacteria to colonize the sunflower head or inoculum prior to infection.

4.3.6 Disease Assessment

Visual rating took place to assess disease levels. Visual rating commenced the day of treatment application and continued every seven days until harvest. Single head ratings were recorded using a disease index of 0 to 5 (Rashid *et al.* 2002), 0 = no lesion, 1 = 1% to 5% head area infected (HAI), 2 = 5% to 20% HAI, 3 = 20% to 40% HAI, 4 = 40% to 60% HAI, 5 = greater than 60% HAI. This assessment method allowed comparison of disease incidence (number of plants infected / total number of plants) at harvest. Disease severity index (DSI) at harvest was calculated for each treatment using the modified formula, DSI = ((sum of individual plant ratings / 5 X number of plants rated) X 100) (Cober *et al.* 2003). This results in a DSI of 0 for plots in which all heads were rated non-infected and a DSI of 100 for plots in which all heads were rated 5 on the scale described above. The area under the disease progress curve (AUDPC) was also calculated (Shaner & Finney 1977) using the DSI values. Analyses of variance (ANOVA) and a mean separation test (Fisher's Least Significant Difference) were performed at $P = 0.05$, using the Analyst procedure of SAS (SAS Institute, Cary, NC).

4.4 Results

4.4.1 Natural Sclerotinia Head Rot Infection

In 2001, the incidences of natural sclerotinia head rot were 7.5%, 15.0%, and 3.3% at Morden 1, Morden 2, and Carman, respectively (Tables 4.1, 4.2, and 4.3). PA-23 application had little effect on incidence and DSI, but reduced the AUDPC from 404.1 in the non-inoculated control, to 127.1 at Morden 1 in 2001 (Table 4.1 and Figure 4.1A). The same trend was expressed for strain 41 at Morden 1 in 2001. Both bacteria reduced incidence, DSI, and AUDPC at Morden 2 in 2001 (Table 4.2 and Figure 4.2A). Strain 41

Table 4.1. Mean disease incidence, disease severity index (DSI), and area under the disease progress curve (AUDPC) for the early-flowering (R5.1-R5.5) inoculation experiment at Morden, Manitoba, in 2001 and 2002.

	MORDEN1					
	2001			2002		
Treatment	Incidence (%)	DSI	AUDPC	Incidence (%)	DSI	AUDPC
Natural Infection	7.5	17.5	404.1	12.5	12.5	120.8
Foliar PA-23	7.5	18.1	127.1	17.5	14.5	134.8
Foliar Strain 41	15.0	17.3	237.9	20.0	20.0	150.5
Foliar BAS 510 F	NA	NA	NA	20.0	19.0	196.0
Artificial Infection (Millet)	57.5	71.5	2114.3	97.5	97.5	3204.3
PA-23 Coated Millet	35.0*	41.9*	1237.9*	90.0	89.0	2604.0*
Strain 41 Coated Millet	47.5	56.9	1477.6*	90.0	90.0	2842.0*
BAS 510 F Coated Millet	NA	NA	NA	85.0	85.0	2308.8*
Foliar PA-23 + Millet	52.5	66.8	1855.2	95.0	95.0	2852.5*
Foliar Strain 41 + Millet	40.0	41.6*	1110.5*	97.5	97.0	2925.6
Foliar BAS 510 F + Millet	NA	NA	NA	87.5	100.0	3021.7
LSD $P = 0.05$	19.9	29.4	465.6	17.4	12.6	332.0

* Significant LSD value in comparison with appropriate control

Disease incidence and DSI ratings were recorded at sunflower maturity

Table 4.2. Mean disease incidence, disease severity index (DSI), and area under the disease progress curve (AUDPC) for the late-flowering (R6.0) inoculation experiment at Morden 2, Manitoba, in 2001 and 2002.

	MORDEN 2					
	2001			2002		
Treatment	Incidence (%)	DSI	AUDPC	Incidence (%)	DSI	AUDPC
Natural Infection	15.0	16.1	293.5	30.0	30.0	262.5
Foliar PA-23	7.5	5.8	35.0	22.5	22.5	211.8
Foliar Strain 41	2.5	2.5	8.8	27.5	27.5	250.3
Foliar BAS 510 F	NA	NA	NA	20.0	18.5	197.8
Artificial Infection (Millet)	90.0	92.2	2218.2	90.0	90.0	1711.5
PA-23 Coated Millet	75.0	74.5	1380.8*	67.5*	66.0*	1298.5*
Strain 41 Coated Millet	55.0*	58.9*	1547.4*	77.5	76.5	1359.8*
BAS 510 F Coated Millet	NA	NA	NA	52.2*	52.5*	698.3*
Foliar PA-23 + Millet	77.5	92.5	2211.2	95.0	93.5	1496.3
Foliar Strain 41 + Millet	85.0	89.4	2056.8	85.0	83.5	1478.8*
Foliar BAS 510 F + Millet	NA	NA	NA	72.5	72.5	1135.8*
LSD $P = 0.05$	26.0	26.9	475.9	18.1	18.1	229.8

* Significant LSD value in comparison with appropriate control

Disease incidence and DSI ratings were recorded at sunflower maturity

Table 4.3. Mean disease incidence, disease severity index (DSI), and area under the disease progress curve (AUDPC) for the early-flowering (R5.1-R5.5) inoculation experiment at Carman, Manitoba, in 2001 and 2002.

	CARMAN					
	2001			2002		
Treatment	Incidence (%)	DSI	AUDPC	Incidence (%)	DSI	AUDPC
Natural Infection	3.3	3.3	63.0	15.0	15.0	126.0
Foliar PA-23	0.0	0.0	0.0	12.5	12.5	110.3
Foliar Strain 41	0.0	0.0	0.0	5.0	5.3	134.0
Foliar BAS 510 F	NA	NA	NA	5.0	4.5*	54.3
Artificial Infection (Millet)	80.0	85.0	2257.5	100.0	100.0	3241.0
PA-23 Coated Millet	73.3	75.6	2000.7	95.0	95.0	3152.7
Strain 41 Coated Millet	80.0	80.0	1935.5	97.5	97.5	3081.8
BAS 510 F Coated Millet	NA	NA	NA	80.0*	79.5*	2108.8*
Foliar PA-23 + Millet	83.3	83.3	2102.3	100.0	100.0	3178.0
Foliar Strain 41 + Millet	80.0	78.7	2137.3	100.0	100.0	3237.5
Foliar BAS 510 F + Millet	NA	NA	NA	100.0	100.0	3024.0*
LSD $P = 0.05$	18.0	16.7	551.3	10.1	10.1	203.6

* Significant LSD value in comparison with appropriate control

Disease incidence and DSI ratings were recorded at sunflower maturity

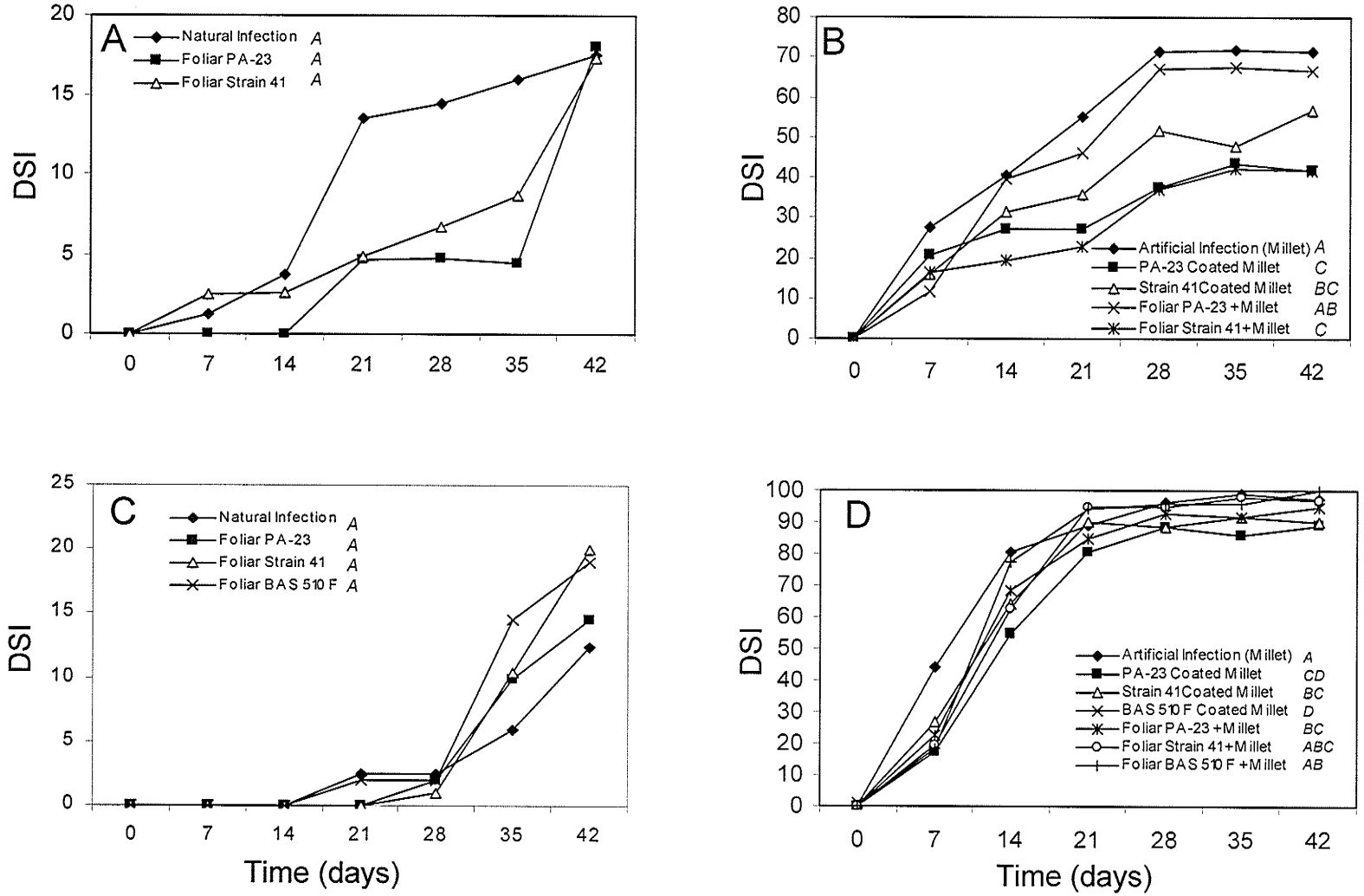


Figure 4.1A-D. Disease progress curves for the spread of *Sclerotinia sclerotiorum* head rot at Morden 1, Manitoba, Canada. Disease severity Index (DSI) values are means of four replications from the early-flowering stage (R5.1-R5.5). **A)** 2001, natural infection. **B)** 2001, artificial infection. **C)** 2002, natural infection. **D)** 2002, artificial infection. Italicized letters denote significance for the AUDPC.

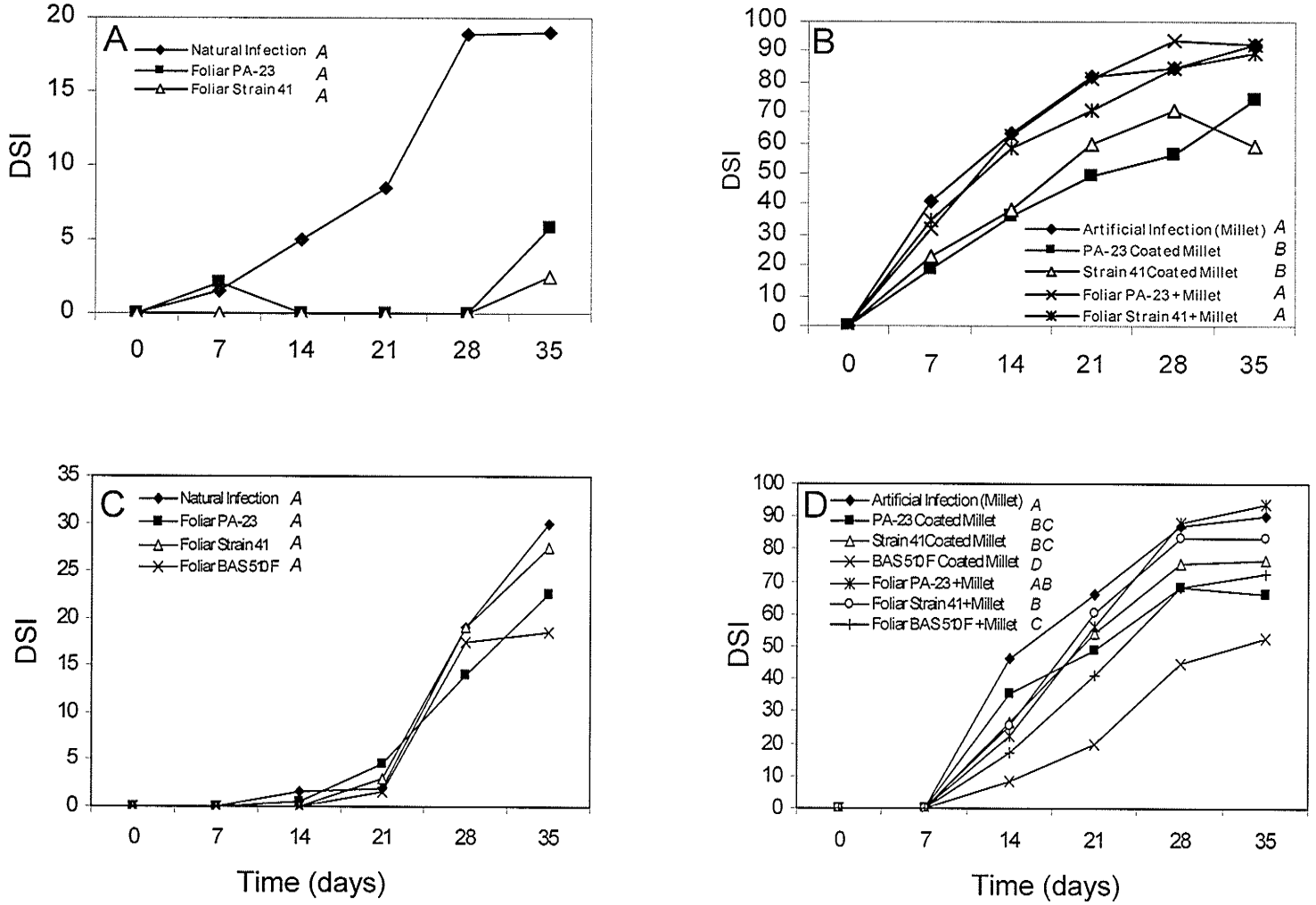


Figure 4.2A-D. Disease progress curves for the spread of *Sclerotinia sclerotiorum* head rot at Morden 2, Manitoba, Canada. Disease severity Index (DSI) values are means of four replications from the late-flowering stage (R6.0). **A)** 2001 natural infection. **B)** 2001 artificial infection. **C)** 2002, natural infection. **D)** 2002, artificial infection. Italicized letters denote significance for the AUDPC.

seemed to be more effective when applied at this late-flowering stage at Morden 2 in 2001. PA-23 and strain 41 provided complete control of natural sclerotinia head rot, reducing the incidence, DSI, and AUDPC to zero at Carman in 2001 (Table 4.3).

In 2002, natural sclerotinia head rot levels increased to 12.5%, 30.0%, and 15.0%, at Morden 1, Morden 2, and Carman, respectively. The treatments at Morden 1 increased head rot in all cases, with BAS 510 F producing the greatest increase (Table 4.1 and Figure 4.1C). Results from the Morden 2 trial showed a non-significant reduction in incidence, DSI, and AUDPC for all three products (Table 4.2 and Figure 4.2C). BAS 510 F provided the best control in this case, reducing incidence by 10%, DSI by 11.5 %, and the AUDPC by 64.7. In the Carman trial in 2002, treatments reduced incidence, DSI, and AUDPC, except for strain 41 which did not reduce the AUDPC in comparison to the control (Table 4.3). BAS 510 F provided the largest sclerotinia head rot reduction, significantly reducing DSI from 15.0 % to 4.5 %.

4.4.2 Artificial Sclerotinia Head Rot Infection

Inserting the *Sclerotinia*-infected millet into the receptacle of the sunflower head significantly increased incidence, DSI, and the AUDPC in comparison to natural infection in all six trials (Tables 4.1, 4.2, and 4.3). Artificial inoculation resulted in incidences of 57.5%, 90.0%, and 80.0% at Morden 1, Morden 2, and Carman, respectively in 2001. Levels were even greater in 2002, where Morden 1, Morden 2, and Carman artificial infection levels were 97.5%, 90.0%, and 100.0%, respectively (Tables 4.1, 4.2, and 4.3). These infection levels were significantly greater than the natural infection levels of 12.5%, 30%, and 15% at Morden 1, Morden 2, and Carman

respectively in 2002, and provided suitable conditions to study the effectiveness of strain PA-23, strain 41, and BAS 510 F.

4.4.3 Bacterial and Fungicide Coated *Sclerotinia sclerotiorum* Inoculum

In 2001, both bacterial coatings significantly reduced the AUDPC at Morden 1 (Table 4.1 and Figure 1B), but only PA-23 was effective in significantly reducing incidence by 22.5% and DSI by 29.6%. Strain 41 was effective in significantly reducing incidence by 35.0%, DSI by 33.3%, and the AUDPC by 670.8 units at the late-flowering period (Morden 2) in 2001 (Table 4.2 and Figure 4.2B). No significant differences were observed between the control and the coated inoculum with either bacterial strain in 2001 at Carman (Table 4.3).

In 2002, reductions in incidence, DSI, and the AUDPC were obtained with PA-23, strain 41, and BAS 510 F at Morden 1 (Table 4.1 and Figure 4.1D). All three treatments resulted in significant reductions in the AUDPC, with BAS 510 F producing the greatest reduction to 2308.8 units, from 3204.3 units in the inoculated control. Results from Morden 2 showed significant reductions in incidence, DSI, and the AUDPC for PA-23 and BAS 510 F (Table 4.2 and Figure 4.2D), but significant reductions only in the AUDPC for strain 41. Results at Carman in 2002 showed less effect than the treatments at the Morden trials, though all three treatments reduced sclerotinia head rot under all three indexes (Table 4.3). BAS 510 F was the only treatment that showed significant reductions in incidence, DSI, and the AUDPC in Carman in 2002.

4.4.4 Foliar Control of Artificially Induced Head rot

Application of the BCA or fungicide followed 24 hours later by insertion of the *Sclerotinia*-infected millet was not as effective in reducing head rot as coating the

inoculum with the control products. In 2001 at Morden 1, foliar applications of PA-23 and strain 41 decreased incidence, DSI, and the AUDPC (Table 4.1 and Figure 4.1B). Strain 41 was the most effective, significantly reducing DSI and the AUDPC by 29.8% and 1003.8 units, respectively. Seed and sclerotia yield results were analyzed for this treatment (data not shown), as it was the most effective. Seed yield did increase and sclerotia yield decreased in comparison to the control; however, results were not significant ($P = 0.05$). The head size variation in this single-head inoculation system was too large to efficiently compare seed and sclerotia yield, so no further yields were analyzed. In 2001, results from Morden 2 and Carman produced only minimal variations in sclerotinia head rot when PA-23 and strain 41 were foliar-applied compared to the control (Tables 4.2 and 4.3).

In 2002, PA-23 seemed to be the most effective at reducing head rot for the early application in Morden, significantly reducing the AUDPC down to 2852.5 units (Table 4.1 and Figure 4.1D). Strain 41 and BAS 510 F significantly reduced the AUDPC in the Morden 2 trial (R6.0 application) in 2002 (Table 4.2 and Figure 4.2D), but only BAS 510 F had a significant reduction in the AUDPC at Carman in 2002 (Table 4.3).

4.4.5 Application Timing

Under natural infection, both bacterial isolates resulted in greater reductions in head rot incidence, DSI, and AUDPC when applied at the late-flowering stage (Morden 2) in 2001, compared with application at the earlier-flowering stage (Morden 1) (Table 4.1A and 4.2A). Both bacteria worked well as an inoculum coating in 2001, but PA-23 seemed to provide more control at the early-flowering stage, and strain 41 at the late-

flowering stage. In terms of foliar control under artificial inoculation, head rot management was greatest when strain 41 was applied at the R5.1-R5.5 stage (Morden 1).

Under natural infection in 2002, control with all three products was more efficient when applied at the R6.0 stage (Morden 2). Both bacterial isolates were effective in reducing the AUDPC as an inoculum coating at early-flowering (R5.1-R5.5), but greater reductions in the AUDPC occurred at the late-flowering stage (R6.0). Similar results were obtained from BAS 510 F coated inoculum, where highly significant reductions in incidence, DSI, and the AUDPC were observed when applied at the R6.0 stage.

4.4.6 Effects of a Misting System

Biological control of sclerotinia head rot under the influence of a misting system was reduced in comparison to the biocontrol effectiveness where no misting system was present. The misting system provided optimum conditions for *S. sclerotiorum* development, reducing the biocontrol effectiveness. In 2001 and 2002, PA-23 and strain 41 were more effective at the Morden location without misting, compared to the Carman location that was misted. BAS 510 F performed well under the infection-inducing conditions provided by the misting system (Table 4.3). The misting system did not increase the incidence of natural infection in either season, but the misting system provided favorable conditions for severe artificial epidemics, especially in 2002. Under such severe epidemics, the BCA were not effective in controlling sclerotinia head rot.

4.5 Discussion

Biological control of sclerotinia head rot was demonstrated in 2001 and 2002, at Morden and Carman. Under natural infection, PA-23 slowed the progression of the disease in all trials except at Morden 1 in 2002. Results were similar for strain 41,

causing a reduction in the AUDPC in 2001 at Morden 1, Morden 2, and Carman, and in 2002 at Morden 2. As a foliar spray under natural infection conditions, PA-23 was equal or better than strain 41 in five of the six trials. As an inoculum coating, PA-23 was more effective than strain 41 in four of the six trials. This variation in effectiveness between PA-23 and strain 41 is normally expected, as the biocontrol activity of *Pseudomonas* species depends on the antifungal metabolites released by different species and strains (Pedras *et al.* 2003). In a former study, *in vitro* inhibition of *Sclerotinia homoeocarpa* (Bennet) with strains of *P. aeruginosa* (Schroeter) Migula varied by a factor of two, depending on the *Pseudomonas* isolate that was applied (Viji *et al.* 2003). Fluorescent pseudomonad strains had *in vitro* inhibition zones ranging from 14 mm to 41 mm when tested for their efficacy against *Fusarium oxysporum* f. sp. *ciceris* (Vidhyasekaran & Muthamilan 1995). It has been suggested that a combination application of more than one BCA can increase pathogen control and decrease the variability of effectiveness (Guetsky *et al.* 2001; Krauss & Soberanis 2002; de Boer *et al.* 2003). However, preliminary greenhouse data (data not shown) suggests that the combination of *P. chlororaphis* (strain PA-23) and *P. corrugata* (strain 41) was actually less effective in controlling sclerotinia head rot, most likely due to similar ecological requirements (Guetsky *et al.* 2001). It is likely that frequent applications of the BCA would further slow the progression of disease (Blakeman & Fokkema 1982; Krauss & Soberanis 2002), and should be the direction of further research. PA-23 slowed the progression of sclerotinia head rot more consistently, and expressed greater potential biocontrol ability than strain 41. Repeated applications of PA-23 may produce cumulative positive results

in an effective integrated management system, as demonstrated in controlling *S. sclerotiorum* in canola (Savchuk and Fernando Unpublished Data).

As a foliar application at Morden 2 and Carman, BAS 510 F provided better control than both BCA under both inoculation methods, and as an inoculum coating in 2002. As an inoculum coating, BAS 510 F was significantly better than the BCA in all experiments, except in the early-flowering stage at Morden in 2002 (Tables 4.1, 4.2, and 4.3). However, both foliar-applied biocontrol agents were more effective than BAS 510 F in reducing natural and artificial sclerotinia head rot DSI and the AUDPC at Morden 1 in 2002 (Figures 4.1C and 4.1D). *Pseudomonas* species have previously demonstrated equal or more effective control than registered fungicides in the control of cotton seedling damping-off (Zaki *et al.* 1998). Nonetheless, BAS 510 F exhibits the ability to combat sclerotinia head rot more effectively than strain PA-23 and strain 41 when disease levels were high. Other fungicides have also demonstrated this ability to consistently control disease when environmental conditions become increasingly conducive (Boland 1997). BAS 510 F may be effective against sclerotinia head rot due to its ability to inhibit spore germination, germ tube growth, and appressoria formation (BASF Corporation 2002).

Biocontrol effectiveness is often affected by the variable and complex environmental factors within the field (Kim & Misaghi 2003), the longevity of the specific bacteria (Vidhyasekaran & Muthamilan 1995), and the microclimatological conditions on the plant surface (Blakeman & Fokkema 1982). The intent of the misting system was to enhance the microclimate to induce bacterial colonization of the head. However, it has been stated that this alteration of the climate is not achievable or practical under field conditions (Blakeman & Fokkema 1982). The environmental variations

provided by the misting system altered the conditions for BCA effectiveness, and provided optimum conditions for sclerotinia head rot development. Neither BCA significantly reduced disease pressure under either inoculation or application technique under misting conditions. Boland (1997) has shown similar results of BCA efficiency decreasing when conditions for disease were conducive.

Time of fungicide and BCA application is crucial for effective disease control (Cooper 1989; Goulds & Fitt 1990). Under artificial inoculation, foliar application of PA-23 seemed to provide better control than strain 41 at three of the four trials when applications took place at the early-flowering stage (R5.1-R5.5). For the R6.0 application timing (late-flowering stage), strain 41 appeared to provide the greatest control in both years.

One of the most prominent research problems in plant pathology is the low occurrence of disease when higher levels are required to achieve consistent results when testing different treatments (Neya & Le Normand 1998; Carsten *et al.* 2000). Minimal natural head rot infection occurred in all experiments. The effectiveness of the BCA and BAS 510 F is still unclear under these inconsistent sclerotinia head rot levels. With low natural sclerotinia head rot levels likely to occur, it was the intent of this study to provide an inoculum source that would not fail under any environmental conditions. The infected millet insertion method is an effective strategy to ensure high incidence of head rot. The conditions provided by the insertion of mycelia directly into the receptacle of the head were extreme, and exemplified sufficient conditions for observing BCA and fungicide efficacy. Incidence levels in the inoculated controls increased in 2001 by 50, 75, and 76% at Morden 1, Morden 2, and Carman, respectively when compared with the non-

inoculated controls. In 2002, incidence levels increased by 85, 60, and 85 percentage points at Morden 1, Morden 2, and Carman, respectively when compared with the non-inoculated controls. These significant increases in sclerotinia head rot incidence produced disease levels where sclerotinia head rot management could adequately be studied, as elevated levels are vital (Neya & Le Normand 1998). Only in the Carman 2002 experiment were significant reductions produced under natural infections, likely due to the increased levels of head rot favored by the misting system.

The AUDPC proves to be the best index to compare PA-23, strain 41, and BAS 510 F because of the manner in which sclerotinia head rot symptoms occur and affect yield and quality. If sclerotinia head rot progression can be delayed as the seed develops, infected seed and yield losses may be minimized. Fewer differences were noted when comparing treatments in regard to their incidence and DSI, because these results were observed only once during the final rating prior to harvest. By the final rating, disease symptoms within BCA or fungicide treatments may have progressed to similar levels as the control. However, disease incidence and DSI do not express how sclerotinia head rot progressed over time (Gawande & Patil 2003). Since the AUDPC is a reliable parameter to rank host resistance and the effectiveness of fungicides (Wagonner 1986) it was the main comparison method utilized in this study.

4.6 Conclusions

Incidence, DSI, and the AUDPC were significantly reduced in experiments when strain PA-23, strain 41, and BAS 510 F were applied. Strain PA-23 was the most effective biocontrol agent, and treatment application at the R6.0 flowering stage was the most efficient at reducing sclerotinia head rot. These reductions will translate into

consistent levels of yield and seed quality, along with a decrease in the quantity of *S. sclerotiorum* sclerotial production, over large acreage production. Foliar management of sclerotinia head rot with either biological or chemical agents will have a positive impact on the yield and the quality of sunflowers, in an industry which faces higher quality restraints and an increasing prevalence of sclerotinia epidemics.

5.0 The Effects of Time and Burial Depth on Viability and Bacterial Colonization of Sclerotia of *Sclerotinia sclerotiorum*

5.1 Abstract

The effects of tillage on the primary inoculum of *Sclerotinia sclerotiorum* (Lib.) de Bary are not well understood. This research was conducted without the disturbance of tillage, by placing sclerotia at different depths within the soil. The purpose was to study sclerotial viability over time and between depths, to identify bacteria colonizing and degrading the sclerotia, and determine whether these bacteria may be utilized as biological control agents. Correlation analysis indicated that a significant negative relationship existed between sclerotial viability and elapsed burial time (R^2 = -0.68, $P <$ 0.0001), in addition to a significant negative relationship between sclerotial viability and depth of burial (R^2 = -0.58, $P < 0.0001$). After twelve months, sclerotia on the soil surface had the highest viability (57.5%), followed by the 5 cm depth (12.5%) and only 2.5% of sclerotia remained viable when placed at the 10 cm depth. A significant negative relationship between sclerotial viability and bacterial populations also existed (R^2 = -0.60, $P < 0.0001$). Bacterial populations were highest at the 10 cm depth, and decreased as soil depth decreased (R^2 = 0.49, $P < 0.0001$). The 12-month sampling date was the only analysis where bacterial populations were the lowest (4.9 log10 cfu/ml/sclerotia) at the 10 cm depth and increased as depth decreased. This trend was produced because sclerotial remnants were minimal after one year, leaving little sclerotial mass to be colonized. Bacterial colonization of sclerotia was also significantly correlated with elapsed burial time (R^2 = 0.56, $P < 0.0001$). Two hundred and sixty-eight bacteria were isolated from sclerotia, 29 of which were strongly antagonistic to *S. sclerotiorum* mycelial growth.

Bacillus spp., namely strains of *B. amyloliquefaciens* and *B. licheniformis* were effective inhibitory bacteria, producing over 80% *in vitro* mycelial inhibition. The biodiversity of the inhibitory bacteria was also analyzed for the 0, 5, and 10 cm depths over time. Inhibitory bacterial biodiversity was minimal within the 0 cm depths, and within all depths sampled at three months. All burial depths within the six and nine month sampling period produced bacterial diversities that were distinct from each other. Determining the effect of depth, time, and bacterial population levels on sclerotial degradation will direct tillage practices to disperse sclerotia to soil locations for the appropriate length of time, to achieve optimal sclerotial degradation.

5.2 Introduction

Sclerotinia sclerotiorum (Lib.) de Bary is a devastating pathogen affecting yield and product quality of a vast number of susceptible hosts. *S. sclerotiorum* is one of the most nonspecific and successful of plant pathogens (Purdy 1979) with a host range of over 408 species, 100 of which are present in Canada (Boland & Hall 1994). Purdy (1979) also reported that *S. sclerotiorum* is the causal agent of more than sixty different diseases, including, stem rot, stalk rot, head rot, pod rot, and wilt. This broad range of diseases covers the globe and occurs in almost every country of the world from the cool, moist regions to the hot, dry areas (Purdy 1979), but normally in temperate regions (Reichert 1958).

Sclerotinia sclerotiorum is the cause of drastic economic loss in numerous crops worldwide. Reported losses include, $26 million annually to United States dry bean production, $13 million annually in U.S. snap bean losses, $24.5 million in losses to North Dakota and Minnesota canola producers in 2000, 2% of the Midwest U.S. soybean

crop is lost every year, approximately $15 million in U.S. sunflower production is lost each year, in addition to the supplementary cost of fungicide applications to control this pathogen (Sayler 2003b). Yield and quality are not only affected in the field but also during transportation to market (Willetts & Wong 1980). Further economic losses can occur due to planting less lucrative non-host crops to avoid *S. sclerotiorum* infection (Purdy 1979). However, variation in crop loss is high, as incidence depends on environmental and crop canopy factors (Willetts & Wong 1980; Bardin & Huang 2001).

Sclerotinia sclerotiorum can cause disease through two distinct mechanisms, either germinating carpogenically to produce airborne ascospores or myceliogenically to infect roots of hosts such as sunflowers and carrots (Bardin & Huang 2001). These two modes of action cause infection of plants through a range of tissues including, sunflower heads (Huang 1983), canola stems and leaves (Gugel & Morrall 1986), pea pods (Huang & Kokko 1992), tubers of Jerusalem artichoke (Laberge & Sackston 1987), and alfalfa blossoms (Gossen & Platford 1999). In addition to the physiological mechanisms of disease spread, *S. sclerotiorum* can spread between diseased and healthy plants that come in contact (Huang & Hoes 1980), by transportation of infected pollen grains (Stelfox *et al.* 1978), and through infected seed lots (Mueller *et al.* 1999).

Biological control of *S. sclerotiorum* has received significant attention over the last few decades (Bardin & Huang 2001), because of the ineffectiveness of other management practices and the reliance on chemical fungicides. Registered biocontrol products such as Intercept (Prophyta Biologischer Planzenschutz, Malchow, Germany) seem effective in decreasing in-field inoculum (Sayler 2003a). The active ingredient in Intercept is *Coniothyrium minitans* Campbell, a naturally-occurring fungus that can

decrease the germination of sclerotia. Imperative to the effectiveness of Intercept is a light tillage operation following Intercept application. The purpose of the light tillage is to incorporate *C. minitans* into the soil to induce colonization. However, light tillage alone, such as mulch tillage, has actually increased the density of sclerotia in the soil surface and increased apothecial formation (Mueller *et al.* 2002). Conversely, Mueller et al. (2002) showed that deep plowing decreases sclerotinia infection, apothecial formation, and the density of sclerotia in the soil. This decrease in infection is mainly due to the burial of sclerotia deeper in the soil, which decreases sclerotia germination because of the inappropriate germination conditions deeper in the soil profile. However, previous studies have reported plowing is not effective in reducing disease caused by sclerotinia stem rot in soybean (Kurle *et al.* 2001). Similarly, no-till has been suggested as more effective than tillage because no-till soils have higher microbial activity causing sclerotial degradation (Workneh & Yang 2000). Keeping the sclerotia in the upper soil profile has been shown to increase sclerotial degradation (Cook *et al.* 1975).

Soil characteristics and microbial activity are instrumental in the degradation of *S. sclerotiorum* sclerotia. Positive correlation has been exhibited by the colonization of sclerotia (*Sclerotinia rolfsii* Saccardo) with *Gliocladium virens* Miller et al., and a decrease in sclerotial germination (Papavizas & Collins 1990). Sclerotia of *S. rolfsii* have also been antagonized by *Trichoderma harzianum* Rifai hyphae, which colonized the sclerotial surface and actually penetrated the rind (Benhamou & Chet 1996). Likewise, *Talaromyces flavus* (Kloecker) Stolk and Samson application decreased the germination of microsclerotia of *Verticillium dahliae* Klebahn from 84% to 17% in only 14 days, in comparison to the control where germination was only reduced to 74% (Fahima *et al.*

1992). Similarly, an isolate of *Trichoderma hamatum*, TMCS-3, also reduced the viability of *S. sclerotiorum* sclerotia (Gracia Garza *et al.* 1997). Limited research has progressed in bacterial effects on sclerotia of *S. sclerotiorum,* however, bacterial colonization has been reported to negatively correlate (R^2 = -0.84) with *Rhizoctonia solani* Kuhn sclerotial germination (Gupta *et al.* 1995). Further knowledge is required on the effects of bacterial colonization of sclerotia of *S. sclerotiorum*, as the majority of previous biocontrol research has concentrated on fungal antagonists (Oedjijono *et al.* 1993).

The objectives of this study were: 1) to determine the effects of sclerotia placement at different depths within the soil over time, isolating the effect of depth and time on sclerotial germination by omitting any soil disturbance caused by tillage; 2) to establish the relationship between sclerotial germination and bacterial colonization of sclerotia, and determine whether the bacterial populations interact with burial depth and time; 3) isolate bacterial populations and assess their *in vitro* inhibition of *S. sclerotiorum* mycelial growth; and 4) analyze the inhibitory bacterial population biodiversity. It is imperative that the effects of depth and time on sclerotial viability and bacterial colonization are better understood so that proper tillage practices can be implemented to increase sclerotia degradation. Previous natural and introduced biological control agents have shown effectiveness against sclerotia, however, greater efficacy is required. For this reason, new biocontrol agents need to be discovered and assessed for their ability to inhibit *S. sclerotiorum*. Understanding the bacterial biodiversity at different locations within the soil will aid in directing management practices to place sclerotia at locations that contain the greatest inhibitory populations.

5.3 Materials and Methods

5.3.1 Sclerotia Burial

On October 1, 2001 sclerotia were collected from a sunflower field just North of Sanford, Manitoba, Canada. All sclerotia were collected from a localized area (20 m x 20 m) within the field from sunflower basal stalk rot infections. Single uniform-shaped sclerotia that averaged 10 mm x 6 mm in size, weighing approximately 0.05 g each, were placed in ten separate compartments (5 cm x 5 cm) within mesh bags made from nylon window screening (Windsor Plywood, Winnipeg, MB, Canada). The trial was initiated on October 23, 2001, at the Department of Plant Science Field Station (Blacklake Silty Clay), on the University of Manitoba Fort Gary Campus, Winnipeg, Manitoba. The trial was designed as a repeated measures randomized complete block design, with burial depth representing the main plots and harvest date representing the sub-plots. The trial contained four replications. Sclerotia placed on the soil surface (0 cm), 5, and 10 cm, were the three burial depths. For the 0 cm depth, sclerotia packets were pinned down to the soil so that environmental conditions would not relocate the mesh bags. Sclerotia that were buried at the 5 and 10 cm depths were placed in level excavations and covered with the soil profile that was initially extracted. Within each main plot, four mesh bags were placed, each containing ten separately packaged sclerotia, representing the four sampling dates: three months (Time 1, January 23, 2002), six months (Time 2, April 23, 2002), nine months (Time 3, July 23, 2002), and twelve months (Time 4, October 23, 2002). An initial sampling analysis (Time 0) took place on a representative sample of collected sclerotia directly from the host. On the appropriate sampling date, the mesh bags containing ten separate sclerotia were recovered from the 0,

5, and 10 cm depths for 4 replications. The mesh bags collected on each sampling date were placed separately in 10 lbs poly plastic bags (Unisource, Winnipeg, MB, Canada) and placed at 4°C for approximately 2 weeks until analysis could take place.

5.3.2 Sclerotial Germination

Sclerotia from all sampling periods were analyzed for their percent germination. Percent germination was defined as the number of sclerotia that germinated myceliogenically divided by the total number of sclerotia sampled for each treatment (n = 40). Each sclerotium was cut in two with a scalpel so that one half of the sclerotium could be tested for viability and the other half was stored in 1.5 ml polypropylene micro centrifuge tubes (Fisher Scientific, Ottawa, ON, Canada) for isolation and identification of colonizing bacteria. The sclerotia halves analyzed for germination were surface sterilized with store brand bleach having an initial concentration of 4.0% NaOCl, diluted in distilled water to a concentration of 1.0% NaOCl for 3 minutes. Sclerotia were allowed to air dry and plated on potato dextrose agar until *S. sclerotiorum* mycelial growth was present (PDA, Beckton/Dickinson, Sparks, MD, U.S.A.). *Sclerotinia sclerotiorum* mycelial growth was the indicator of germination. Subsequent sclerotial and apothecial formation were not measured, as viable sclerotia did not consistently form new sclerotia or apothecia (Abawi & Grogan 1979).

5.3.3 Bacterial Colonization

The sclerotia halves for bacterial analysis were sonicated (Branson Ultrasonic cleaner™, Branson Cleaning Equipment Company, Shelton, Conn, U.S.A.) for 20 seconds in a sterile distilled water solution. Viable sclerotia were analyzed together, and sclerotia that did not germinate were analyzed separately. Serial dilutions were prepared

using standard dilution plating techniques, and bacteria were plated on half nutrient agar (11.5 g Nutrient Agar and 10.0 g Agar Technical (Becton/Dickinson, Sparks, MA, U.S.A.), amended with Nyastatin (Sigma Chemical CO., St. Louis, MO, U.S.A.). Bacterial colonies were enumerated after 72 hours, counting plates with 20-200 colonies and determining the colony forming units (cfu) per ml. The average colony count per sclerotia is reported (n = 40).

5.3.4 Biological Control Assessment

Visually distinct bacteria were isolated from the dilution plates onto Luria Bertani agar (LBA, 15.0 g agar, technical (Difco Laboratories, Detroit, Mich., U.S.A.), 10.0 g tryptone peptone (Becton/Dickinson, Sparks, MA, U.S.A.), 5.0 g yeast extract (Sigma Chemical CO., St. Louis, MO., U.S.A.), 5.0 g NaCl) and assessed for purity through morphological characteristics, then stored in LB broth amended with 20% glycerol at -80°C. All isolated bacterial were plated for percent inhibition of *S. sclerotiorum* mycelium growth on 30% Tryptic Soy Broth/70% Potato Dextrose Broth (TS/PD, 9 g Tryptic Soy Broth, 16.8 g Potato Dextrose Broth, and 17.0 g Agar Technical (Becton/Dickinson, Sparks, MA, U.S.A.) and PDA, in a similar manner as previously described (Wood 1951; Fernando & Pierson III 1999; Savchuck 2002). A loop of bacteria removed from a 24-hour sub-culture was placed in LB broth on an incubator shaker at 28°C for 16 hours at 160 rpm. Five micro liters of the bacterial suspension was pipetted onto both TSA/PDA and PDA in 15x100 mm petri plates at four equidistant points near the periphery of the plate. Bacteria were allowed to grow for 24 hours at room temperature. Mycelial plugs, 5 mm in diameter, were taken from the actively growing margin of *S. sclerotiorum* cultures and placed into the center of each bacterial

plate, and incubated for 14 days. *Sclerotinia sclerotiorum* cultures for *in vitro* biocontrol assessment trials were produced from the same sclerotia collection that was buried in the field. Measurements of radial mycelial growth were recorded at 48 hrs (mycelial growth had reached the circumference of the plate in the control, 80mm), and after 14 days. The percentage mycelial inhibition was calculated with the formula 100 x (R1 – R2)/R1 where R1 is the maximum radius of growth (80 mm) and R2 is the radius directly opposite the bacterial cultures (Fernando & Pierson III 1999). Only the 14-day assessment will be reported in this study, as it best represents the *in vitro* biological control activity of each bacterium. Each bacterium was replicated three times for the initial plate inhibition assays. Any bacterial isolate that produced greater than 40% mycelial inhibition in the initial screening was repeated in ten replications.

Bacterial isolates that produced over 40% mycelial inhibition were also assessed for volatile production (Fernando & Linderman 1994). Bacteria were streaked onto one half of a divided plate containing Tryptic Soy Agar (TSA, 30.0 g TSA and 10.0 g Agar Technical (Becton/Dickinson, Sparks, MA, U.S.A.), and then sealed with parafilm® (Fisher Scientific, Nepean, ON, Canada). After 72 hrs of incubation at room temperature, 5 mm plugs of *S. sclerotiorum* mycelial cultures were placed on the other half of the plate containing PDA, and the plates re-sealed. After 48 hours, mycelial growth had reached the furthest circumference of the plate (80 mm) in the control (no bacteria on the TSA half). Measurements of mycelial growth were recorded in a similar fashion as described in the previous mycelial inhibition study. Three replications were used for the initial screening of volatile production, and each bacterium producing volatiles that inhibited mycelial growth was replicated ten times.

5.3.5 Bacterial Identification

All inhibitory bacteria were identified using standard gram stain techniques followed by the use of the Biolog© Microstation, utilizing the Biolog Micrologᵀᴹ 3, Version 4.2 software (Microlog, Hayward, CA, U.S.A.). Each inhibitory bacterium isolated was identified once and repeated if confirmation was necessary (see appendix).

5.3.6 Data Analysis

Experiments were analyzed using analysis of variance (ANOVA) and a mean separation test (Fisher's Least Significant Difference) was performed at $P = 0.05$, using the Analyst procedure of SAS, Version 8.1 (SAS Institute, Cary, NC, U.S.A.). Correlation coefficients using Pearson's Rank Correlation were determined using the Descriptive procedure of SAS. Population biodiversity for the inhibitory bacteria using bacterial identities was compared for each sampling date and depth using the NTSYSpc, Numerical Taxonomy System, Version 2.1 software (Exeter Software, Setauket, NY, U.S.A.). The genetic distance between treatments was determined using the bacterial identity presence or absence for each sampling date and depth. This genetic distance between treatments was utilized to produce the dendogram using SAHN clustering and a UPGMA clustering method.

5.4 Results

5.4.1 Sclerotial Germination

Sclerotial germination analysis for time zero was accomplished using sclerotia collected directly from sunflower, the reason why there is no distinction between depths. The mean germination from the initial sclerotia collection is 80% (Figure 5.1). After three months in the field, viability of sclerotia increased for all three depths. Viability

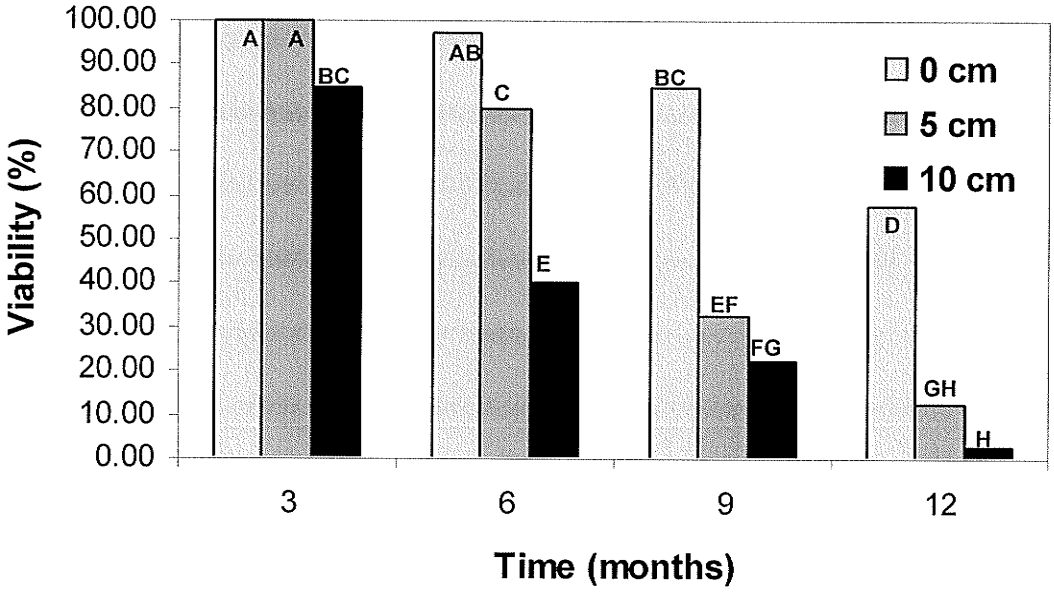


Figure 5.1. Viability of sclerotia buried at 0, 5, and 10 cm depths, sampled at 3, 6, 9, and 12 months, at Winnipeg, Manitoba, Canada, from 2001 to 2002. Initial viability for time 0 was 80.0%. Letters denote significance for Fisher's LSD value of 14.3 ($P = 0.05$).

for the 0 and 5 cm depths both significantly increased to 100%, while viability at the 10 cm depth non-significantly increased to 85%. At three months, a significant difference was expressed between the 0 and 5 cm depths compared to the 10 cm depth. After six months on the soil surface, viability did not show a significant change from the 3-month sampling date. After six months at 5 cm within the soil, viability significantly decreased, while viability significantly decreased to 40% at the 10 cm depth. All three burial depths were significantly different from each other after remaining in the field for six months. At nine months, sclerotial viability on the soil surface was still greater than the initial viability (80.0%), and did not decrease significantly from the six-month sampling period. From the six to nine month sampling period, significant decreases in viability for the 5 and 10 cm depths occurred to 32.5 and 22.5%, respectively. After nine months in the field, surface sclerotial viability was significantly different from the sclerotial viability at the 5 and 10 cm depths, however, the sclerotial viability at the 5 and 10 cm depths were not significantly different from each other. After 12 months in the field, sclerotial viability significantly decreased to 57.5, 12.5, and 2.5% for the 0, 5, and 10 cm depths respectively. All germination data was significantly different after 12 months, in comparison to any previous sampling period. A significant negative relationship existed between sclerotial viability and sampling time (R^2 = -0.68, $P < 0.0001$), and between sclerotial viability and burial depth (R^2 = -0.58, $P < 0.0001$) (Table 5.1).

5.4.2 Bacterial Colonization

No distinction between burial depths was made for time 0 when comparing bacterial populations. The initial bacterial population was 3.98 log10 (cfu/ml/sclerotia) (Figure 5.2). After three months in the field, the bacterial populations on sclerotia at the

Table 5.1. Correlation coefficients of Pearson's Rank Correlation for sclerotial germination and bacterial colonization levels from a sclerotial burial study at Winnipeg, Manitoba, Canada, from 2001 to 2002.

	Variable	
Variable	Sclerotial Germination	Bacteria Colonization Levels
Time	-0.68	0.56
Depth	-0.58	0.49
Bacteria Colonization Levels	-0.60	-

All correlation coefficients shown are significant at $P < 0.0001$.

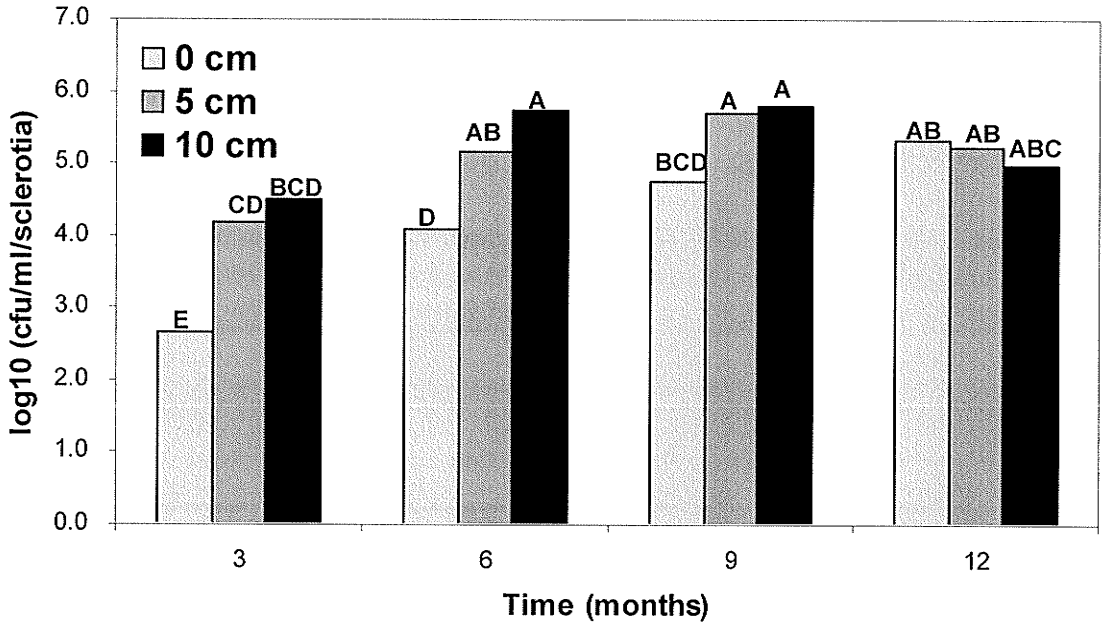


Figure 5.2. Bacterial populations colonizing sclerotia buried at 0, 5, and 10 cm, sampled at 3, 6, 9, and 12 months, from Winnipeg, Manitoba, Canada, from 2001 to 2002. Initial bacterial populations for time 0 were 3.98 log10 (cfu/ml/sclerotia) Letters denote significance for Fisher's LSD value of 0.8454 ($P = 0.05$).

soil surface significantly decreased to 2.7 log10 (cfu/ml/sclerotia), while the bacterial populations on sclerotia at the 5 and 10 cm depths were not significantly different from each other. After six months in the field, all bacterial populations significantly increased in comparison to samples from the same depth from the previous sampling period. Bacterial populations were 4.1, 5.2, and 5.7 log10 (cfu/ml/sclerotia) for the surface, 5, and 10 cm depths, respectively. Again, the surface sclerotia bacterial populations were significantly lower than the populations at the 5 and 10 cm depths. The 9-month sampling period produced bacterial populations within the same depth not significantly greater than the 6-month sampling period. Bacterial populations at 5 and 10 cm were not significantly different from each other but were significantly higher than the surface sclerotia bacterial populations. At the 12-month sampling, bacterial populations from the 5 and 10 cm depth decreased in comparison to the nine-month sampling period. Bacterial populations decreased to 5.2 and 5.0 log10 (cfu/ml/sclerotia) for the 5 and 10 cm depths, respectively, while the surface bacterial populations increased to 5.3 log10 (cfu/ml/sclerotia). A reverse trend in bacterial populations occurred at the 12-month sampling period. The surface sclerotia had the greatest bacterial populations, followed by the 5 and 10 cm depth, however, the bacterial populations were not significantly different among the three depths. Bacterial populations were significantly correlated with time ($R^2 = 0.56$, $P < 0.0001$) and depth ($R^2 = 0.49$, $P < 0.0001$) (Table 5.1).

5.4.3 Bacterial Isolation

Over the one-year sampling period, 268 morphologically different bacterial isolates were collected. Twenty-nine isolates were inhibitory *in vitro* to mycelial growth of *S. sclerotiorum* (Table 5.2). Of those 29 isolates, there were only 15 different bacterial

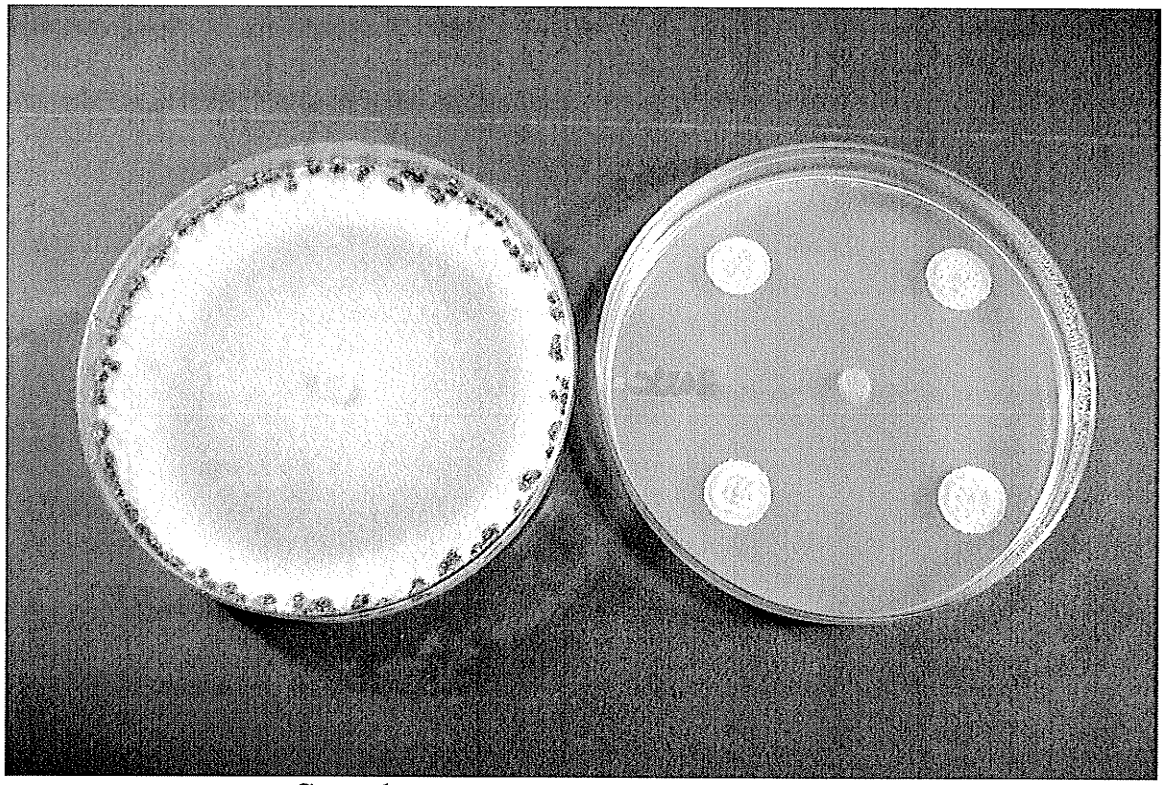
species. However, different strains of the same species often produced differing mycelial growth inhibition results. *Bacillus amyloliquefaciens* strain 2033, caused the greatest *in vitro* inhibition on TSA/PDA, reducing *S. sclerotiorum* mycelial growth by 77.3% (Figure 5.3). This was significantly more inhibition than all other bacterial isolates except *B. amyloliquefaciens* strain 268, which produced 73.8% mycelial inhibition on TSA/PDA. Four out of the top seven inhibitory bacteria on TSA/PDA were *B. amyloliquefaciens* isolates. Other bacteria that were effective on TSA/PDA were *Staphylococcus sciuri* strain 3055, *Bacillus licheniformis* strain 266, and *Mannheimia haemolytica* strain 230, which reduced mycelial growth by 72.6, 72.5, and 71.9 percentage points respectively. Sixteen isolates on TSA/PDA were more effective than the overall mean of all 29 bacteria.

Inhibition tests on PDA produced similar results, with isolates of *B. amyloliquefaciens* again producing four of the top seven *in vitro* inhibition results. The most effective bacteria on PDA was *B. licheniformis* strain 223, which caused 88.5% inhibition (Figure 5.4), significantly greater than all other isolates. However, on TSA/PDA, strain 223 only caused 53.3% mycelial inhibition. Sixteen isolates were more effective in reducing mycelial germination than the overall mean of all 29 isolates on PDA, however, some of the 16 effective isolates differed from the 16 isolates effective on TSA/PDA. *Kocuria rosea* strain 41, *B. cereus/thuringiensis* strain 54, and *B. amyloliquefaciens* strain 4078, had extremely low inhibition of *S. sclerotiorum* mycelial growth on PDA, but were effective on TSA/PDA media. Twenty-two of the 29 bacteria were more effective at reducing *S. sclerotiorum* mycelial growth on PDA than on TSA/PDA.

Table 5.2. Twenty-nine inhibitory bacteria to *Sclerotinia sclerotiorum* isolated from sunflower, 0, 5, and 10 cm depths at 0, 3, 6, 9, and 12 months in Winnipeg, Manitoba, Canada, from 2001 to 2002.

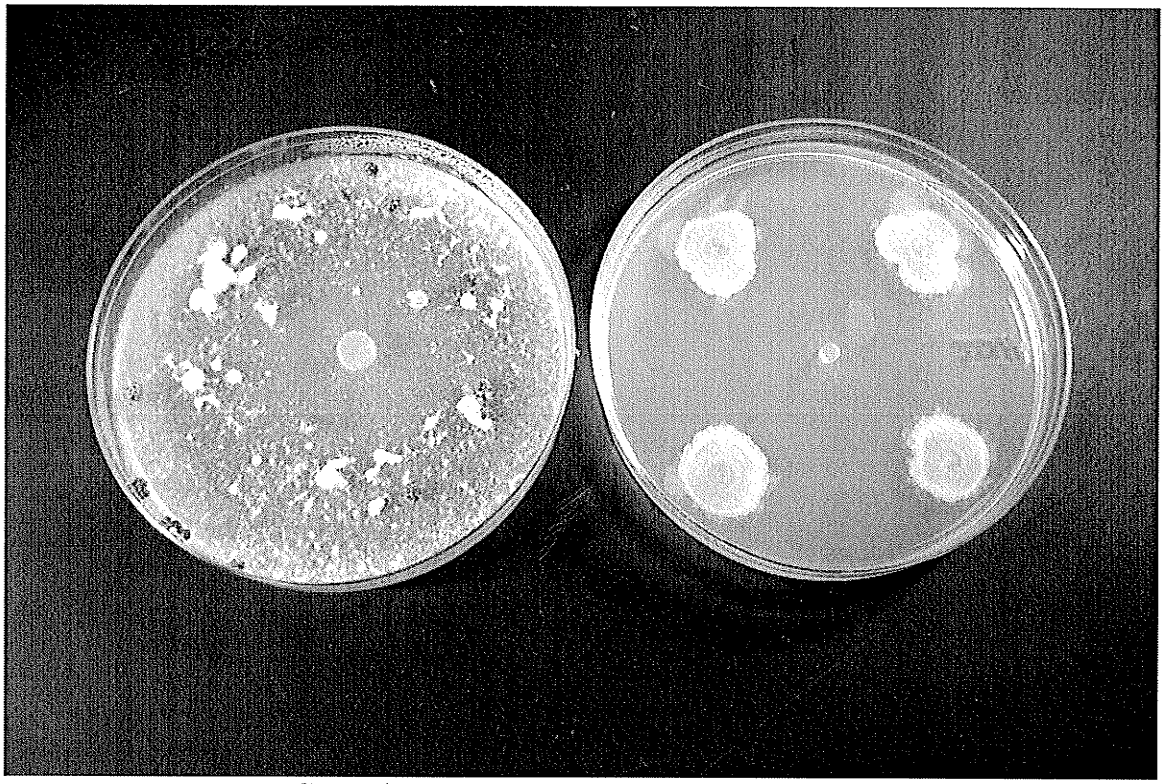
Strain	Location (cm)	Time (months)	Identity	Inhibition (%)		Volatile Inhibition (%)
				TS/PD	PDA	TSA/PDA
73	Host	0	*Hafnia alvei*	59.4	52.6	0.0
54	5	3	*Bacillus cereus/thuringiensis*	46.6	0.0	0.0
29	10	3	*Bacillus amyloliquefaciens*	62.1	54.9	0.0
41	10	3	*Kocuria rosea*	42.9	2.8	0.0
67	10	3	*Staphylococcus lentus*	62.5	72.0	0.0
207	0	6	*Brevibacterium otitidis*	52.5	71.4	0.0
240	0	6	*Bacillus subtilis*	68.4	78.1	0.0
2031	0	6	*Bacillus subtilis*	66.2	66.5	0.0
2033	0	6	*Bacillus amyloliquefaciens*	77.3	78.5	0.0
223	5	6	*Bacillus licheniformis*	53.3	88.5	0.0
248	5	6	*Bacillus amyloliquefaciens*	72.4	79.5	0.0
2056	5	6	*Pseudomonas corrugata*	50.0	56.9	28.6
226	10	6	*Bacillus amyloliquefaciens*	54.4	67.6	0.0
230	10	6	*Mannheimia haemolytica*	71.9	73.9	0.0
265	10	6	*Bacillus amyloliquefaciens*	71.7	82.6	0.0
266	10	6	*Bacillus licheniformis*	72.5	74.1	0.0
268	10	6	*Bacillus amyloliquefaciens*	73.8	76.4	0.0
2090	10	6	*Bacillus subtilis*	65.0	70.6	0.0
3055	10	9	*Staphylococcus sciuri*	72.6	76.3	0.0
3057	10	9	*Bacillus subtilis*	65.6	78.2	0.0
3060	10	9	*Staphylococcus sciuri*	50.4	63.6	0.0
3073	10	9	*Pseudomonas corrugata*	56.0	49.8	35.8
3008	10*	9	*Pseudomonas fluorescens*	31.9	47.3	17.8
3020	10*	9	*Macrococcus equipercicus*	56.9	55.9	0.0
3039	10*	9	*Bacillus licheniformis*	16.3	56.0	0.0
3045	10*	9	*Staphylococcus aureus ss aureus*	10.2	37.6	0.0
4076	10	12	*Bacillus licheniformis*	50.0	57.7	0.0
4078	10	12	*Bacillus amyloliquefaciens*	65.1	0.0	0.0
4079	10	12	*Bacillus mycoides*	47.6	55.5	43.2
			Mean	56.8	59.5	4.3
			LSD $P = 0.05$	4.5	3.3	3.7

* Similar morphological strains also found at 0 cm for strains 3008 and 3020, and at 5 cm for 3020, 3039, and 3045.



Control Strain 2033

Figure 5.3. *Sclerotinia sclerotiorum* mycelial inhibition of 77.3% by *Bacillus amyloliquefaciens* strain 2033 on TSA/PDA.



Control Strain 233

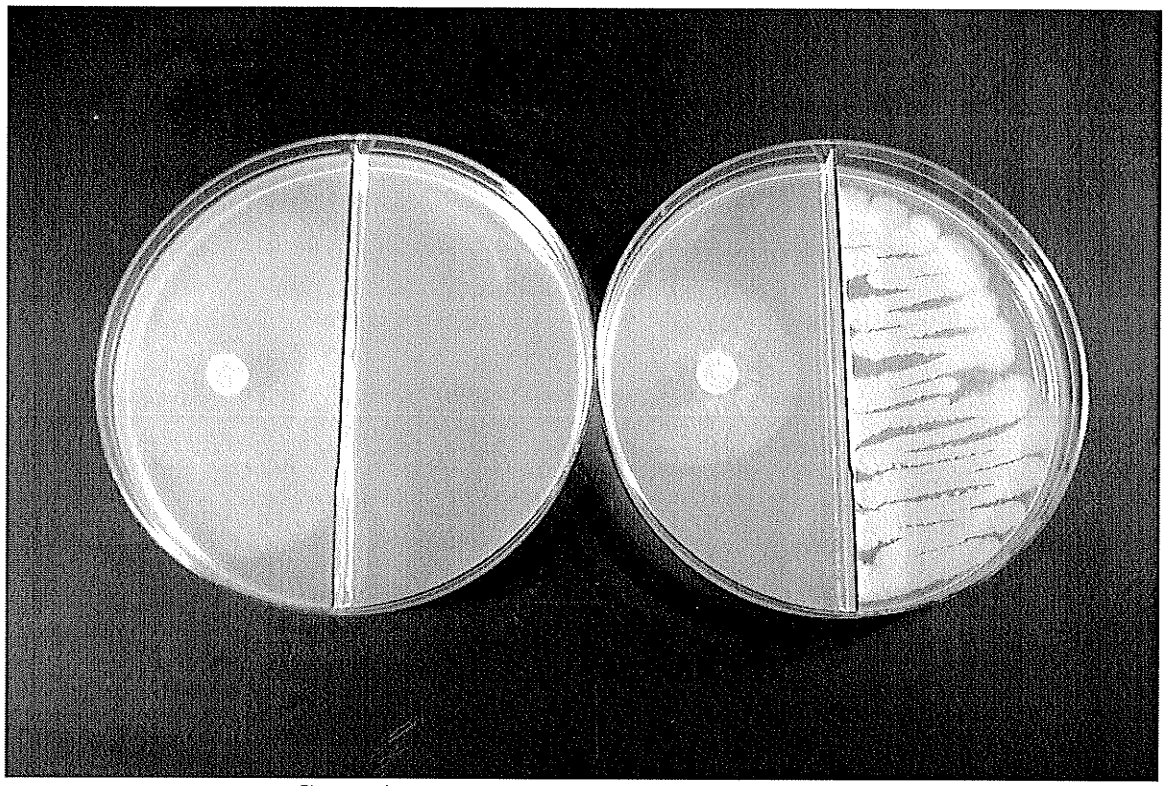
Figure 5.4. *Sclerotinia sclerotiorum* mycelial inhibition of 88.5% by *Bacillus licheniformis* strain 233 on PDA.

Only four of the 29 bacterial isolates caused reduced mycelial growth when tested for volatile production. All four bacteria produced *S. sclerotiorum* mycelial growth inhibition that was significantly different from each other isolate. *B. mycoides* strain 4079 produced the greatest volatile inhibition (43.2%) (Figure 5.5), followed by *Pseudomonas corrugata* strain 3073 (35.8%), *P. corrugata* strain 2056 (28.6%), and *P. fluorescens* strain 3008 (17.8%).

5.4.4. Inhibitory Bacterial Population Diversity

The sclerotia burial treatments were compared by analyzing the presence or absence of the inhibitory bacteria in each treatment. The inhibitory bacteria isolated from the initial analysis (0 months) were similar to inhibitory bacteria isolated from the 0, 5, and 10 cm depths at 3 months, the 0 cm depth at 9 months and the 0 and 5 cm depth at 12 months in the field (Figure 5.6). This similarity is due to the fact that few inhibitory bacteria were isolated from these treatments. The inhibitory bacterial populations isolated from 10 cm at nine months, 5 cm at six months, 10 cm at six months, and 10 cm at twelve months in the soil were all distinct from all other bacterial populations isolated from other sampling treatments. Inhibitory bacteria isolated from the surface at six months, and from 5 cm at nine months, were similar to each other, however, these two treatments produced inhibitory populations that were distinct from all other treatments.

All depths within the 3-month sampling period produced inhibitory populations similar to each other and the initial sampling period. All 0 cm inhibitory populations were similar to each other, except the 0 cm depth at six months. months. All inhibitory populations were distinct when sampled at six and nine months in the field (Figure 5.6).



Control Strain 4079

Figure 5.5. *Sclerotinia sclerotiorum* mycelial inhibition (43.2%) caused by volatile products from *Bacillus mycoides* strain 4079.

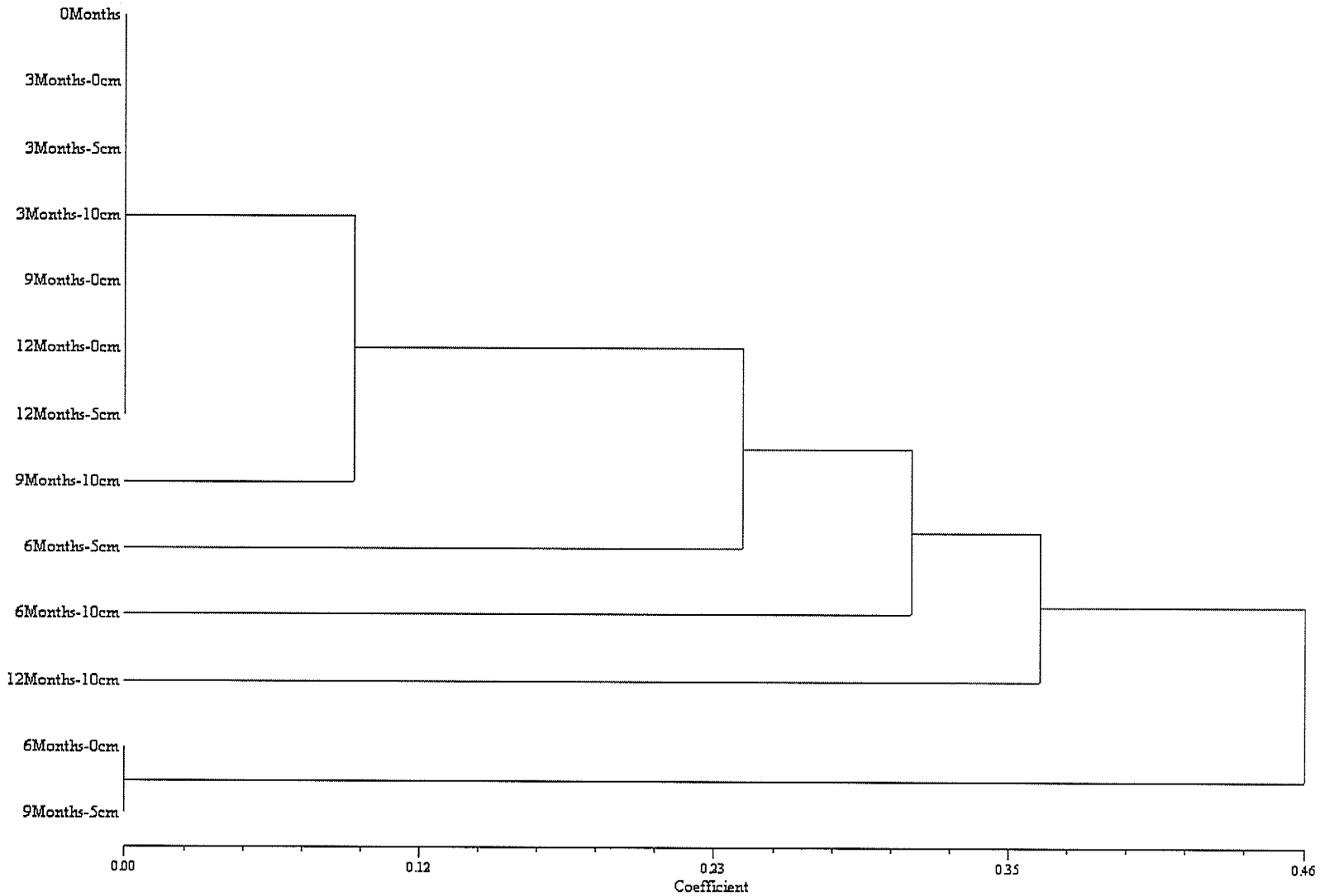


Figure 5.6. Inhibitory bacterial population diversity analysis for the 0, 5, and 10 cm depths, sampled at 0, 3, 6, 9, and 12 months, from Winnipeg, Manitoba, Canada, from 2001 to 2002. Each isolation depth and time composed a treatment and the 13 treatments were compared by the presence of the bacterial identities isolated from within each treatment.

5.5 Discussion

Sclerotial viability was the lowest for all sampling dates at the 10 cm depth, followed by the 5 cm depth, while the sclerotia that were placed on the soil surface had the highest viability for all sampling treatments. Sclerotial germination increased early in the winter when sclerotia are exposed to seasonal temperatures, similar to results found in Beltsville, MD (Adams 1975). As the temperatures begin to increase in the spring, along with the activity of soil microorganisms, sclerotial germination begins to decrease, just as mycelial viability decreases when temperatures increase (Huang & Kozub 1993). Merriman (1976) reported that sclerotia viability for sclerotia remaining on the soil surface is the least affected, perhaps due to the low bacterial colonization. Huang and Kozub (1993) reported similar results when examining the survival of *S. sclerotiorum* mycelium, and stated that buried mycelia have low viability, possibly because of the microorganisms in the soil. However, Cook et al. (1975) stated that sclerotia remaining in the upper soil profile degrade rapidly in comparison to sclerotia deeper in the soil profile. Imolehin and Grogan (1980) recovered *S. minor* sclerotia from 0, 5, 10, and 20 cm, finding similar results in regards to depth, but no viable sclerotia were found in the soil after 3 months. Kurle et al. (2001) also indicated that sclerotia viability decreased with increasing depth under chisel plow and no-tillage cultivation systems. Kurle et al. (2001) stated that sclerotia within the upper 5 cm soil profile will carpogenically germinate. Depths greater than 5 cm would not be conducive to carpogenic germination.

The sclerotial viability results were negatively correlated with the bacterial populations colonizing the sclerotia ($R^2 = -0.60$, $P < 0.0001$). The 10 cm depth produced the highest bacterial populations, while the lowest sclerotial viability was also found at

these sampling depths. Sclerotia placed on the soil surface consistently had the lowest bacterial colonization levels and greatest viability for each sampling date, probably due to periodic drying (Kurle *et al.* 2001). Only after 12 months did bacterial populations change, when sclerotia on the soil surface had the greatest bacterial colonization followed by the 5 and 10 cm depths. This inversion in bacterial colonization is due to partially or completely degraded sclerotia at the 5 and 10 cm depths. Sclerotial remnants were even difficult to locate in the mesh bag compartments at the depths of 5 and 10 cm. Kurle et al. (2001) indicated that germination was "sharply reduced" at 10-20 cm, but conceded that these results were produced by a low number of sclerotia found at this depth. Our results suggest that the reason a low number of sclerotia were found at this depth is not due to the fact that less sclerotia were located in this depth, but due to the high level of sclerotial degradation. Other factors affect the viability of sclerotia at different depths over time, such as, soil type and pH (Merriman 1976), tillage (Kurle *et al.* 2001), moisture (Moore 1949; Hao *et al.* 2003), humidity (Huang & Kozub 1993), temperature (Workneh & Yang 2000), gases (Imolehin & Grogan 1980a), fungal populations (Hoes & Huang 1975; Imolehin & Grogan 1980b), sclerotia size and shape (Hoes & Huang 1975; Hao *et al.* 2003), and sclerotia source (Merriman 1976). Nonetheless, it is important to recognize the impact that bacteria have contributed to sclerotial degradation in this experiment (R^2 = -0.60, P <0.0001). Kurle et al. (2001) also suggests that reduced sclerotia viability in chisel plow and moldboard plow tillage may be due to increased parasitism, in contrast to the findings of Gracia-Garza et al. (2002), who indicated no-till may increase microbial activity, thus increasing degradation of sclerotia. No-tillage has been shown to impact physical and chemical soil factors, affecting microorganisms that

decompose organic material (Lafond & Derksen 1996), further supporting the principle that tillage affecting sclerotial location, will in turn affect sclerotial viability.

Out of 268 bacteria, twenty-nine provided positive inhibition to *S. sclerotiorum* mycelial growth. Of the 29 inhibitory bacteria, 24 were gram-positive, 17 of which were spore-forming bacteria. The morphological ability of these spore-forming bacteria, and the previous industrial uses of these speices, supports their potential biocontrol success (Emmert & Handelsman 1999). Fifteen distinct bacterial species were present within the 29 isolates, with *Bacillus* spp. the most effective at inhibiting *S. sclerotiorum* mycelial growth. The *Bacillus* spp identified have potential for commercialization since *B. thuringiensis* (Bt) comprises 90% of the bio-insecticides market (Emmert & Handelsman 1999). Strains of *B. amyloliquefaciens, B. licheniformis,* and *B. subtilis* all provided over 70% inhibition on either TSA/PDA or PDA. *Bacillus amyloliquefaciens* has been reported to be effective on *Botrytis cinerea* Pers. Fr. in tomato (Mari *et al.* 1996), and against anthracnose (*Colletotrichum dematium* (Pers. Fr.) Grove) on mulberry leaves (Yoshida *et al.* 2001). Likewise, *B. licheniformis* has been effective against *Pyrenophora tritici-repentis* (Died.) Drechs. in wheat (Mehdizadegan & Gough 1987), and *Pyrenophora teres* Drechs. of barley (Scharen & Bryan 1981). *Bacillus subtilis* was reported to be effective against *Fusarium, Rhizoctonia,* and *Sclerotinia* pathogens, as well as stimulating plant growth (Turner & Backman 1991; Kondoh *et al.* 2000; Estevez de Jensen *et al.* 2002). *Bacillus mycoides* also provided positive inhibition of *S. sclerotiorum* mycelial growth, mostly due to volatile production in this study. Similarly, on strawberry leaves, *B. mycoides* was effective against *Botrytis cinerea*, and volatile production was also detected (Guetsky *et al.* 2002).

The bacteria with the greatest biocontrol potential were isolated from sclerotia that were in the soil or on the soil surface for six months. Effective biocontrol bacteria on PDA were also isolated from sclerotia sampled at nine months. Thirteen of the 29 inhibitory bacteria were isolated from the 6-month sampling date, followed by eight bacteria from the 9-month sampling date. Sclerotia in the field for three and twelve months had few inhibitory bacteria colonizing the sclerotia, with six of the seven bacteria from these two sampling dates isolated from the 10 cm depth. Twenty of the 29 inhibitory bacteria were isolated from the 10 cm depth, which had the highest bacterial population for all sampling dates, except, after twelve months when sclerotia were heavily degraded. Only seven inhibitory bacteria were isolated from the 5 cm depth, followed closely by six bacteria from the surface. Only one inhibitory bacterium was directly isolated from sclerotia retrieved from sunflower (time 0). Plants influence the biodiversity of bacteria in soils (Dunfield & Germida 2001), due to the release of amino acids, sugars and root exudates (Rovira 1956a), which impact the types of bacteria present at depths in the rhizosphere (Rovira 1956b). If root exudates can affect the bacterial biodiversity in this way, it may explain the bacterial biodiversity between sclerotial depths within the soil.

Inhibitory bacterial population biodiversity was evident between different treatments in this study. Bacterial content was similar for all 0 cm depths, except for the 6-month analysis. This exception at 0 cm was most likely due to the spring conditions of the sampling period, causing greater bacterial populations. Two 5 cm sampling dates that were similar were the 3 and 12-month sampling dates, due to no isolation of inhibitory bacteria from either of these dates. All 10 cm sampling dates were dissimilar from each

other, as over 66% of the bacteria were isolated from the 10 cm depth. Distinction in time was also clear when comparing the biodiversity of inhibitory bacteria. All depths within the 6-month sampling date as well as the 9-month sampling date contained inhibitory bacterial populations that were distinct.

5.6 Conclusions

The results in this study indicate that sclerotial longevity is negatively correlated with time of burial and depth within the field. Sclerotial longevity is often over-estimated, but a high percentage of sclerotia can remain viable if located on the soil surface. Decreasing sclerotial viability is one strategy of managing *S. sclerotiorum*, and it is evident that bacterial populations play a significant role in sclerotia degradation. The proper tillage practices to delay germination and increase the time for bacterial colonization will decrease *S. sclerotiorum* infection. Further research needs to determine exactly what these proper tillage practices will consist of.

This is the first study to compare inhibitory bacteria populations between depths, over time, and their effect on sclerotial germination. Further study into bacterial biological control will be beneficial in understanding the longevity of sclerotia in the soil, as the majority of previous research has concentrated on fungal antagonists. The isolated biocontrol agents can be investigated for their effectiveness against primary inoculum as soil applied treatments, along with analysis of their effectiveness as foliar treatments. Soil cover of sclerotia, promoting bacterial growth, will lead to increased sclerotial degradation. However, tillage needs to be manipulated so subsequent soil disruption does not simply recover previously buried sclerotia. With this information on sclerotial germination and bacterial colonization, known tillage effects can be properly

implemented and integrated with bacterial biocontrol agents, to limit the economic loss due to *S. sclerotiorum*.

6.0 GENERAL DISCUSSION AND CONCLUSIONS

Sclerotinia sclerotiorum (Lib.) de Bary infects over 400 plant species, and is economically devastating to numerous agricultural crops including sunflower. Research on *S. sclerotiorum* has occurred for well over a century, and although significant advances have taken place, a high level of control has not been achieved. It was the objective of this research to further investigate several facets of *S. sclerotiorum* management, with the intent of improving sclerotinia head rot management in sunflower. Host resistance, biological, chemical, and cultural control mechanisms were all investigated to elucidate further information that could be incorporated and combined with existing management practices. An integrated approach is required to reduce the economical loss caused by *S. sclerotiorum*, as this pathogen will most likely never be controlled by one mechanism alone.

Host resistance is the most economical method of disease control due to its simple deployment (Chapter 3). Unfortunately, no high level of resistance has been found. New sunflower hybrids are registered each year for their superior agronomic characteristics, such as yield, oil content, maturity, and height. However, these new hybrids also need to be assessed for their tolerance to *S. sclerotiorum*. The phenotypic reaction data verifies previous impressions, that in general oilseeds are more tolerant to sclerotinia head rot than confection sunflowers. With in depth phenotypic information, breeders may benefit by utilizing germplasm from tolerant oilseed hybrids and incorporating it with the agronomic and market traits of other sunflower hybrids.

The growth stage susceptibility to sclerotinia head rot in sunflowers was also investigated. Determining that the most susceptible stage to sclerotinia head rot is the

100% flowering stage will benefit researchers and producers in the future if a biological or chemical control product is registered. Discovering that sunflowers are highly susceptible to sclerotinia head rot from early flowering to maturity may lead to multiple applications of potential biological or chemical control treatments.

The emphasis of this thesis was on biological control, both natural (Chapter 5) and introduced (Chapter 4). Obviously, natural microorganism activity in the soil is not sufficient to degrade sclerotia at a rate in which germination will cease. Nonetheless, it is clear that natural sclerotial degradation does occur in the soil. By isolating beneficial organisms, and re-introducing them back into the rhizosphere, sclerotial degradation may increase. *Bacillus* spp. were isolated that produced over 80% inhibition of mycelial growth, and appear as excellent biocontrol candidates for reintroduction into the field. The reintroduction of isolated biocontrol agents from sclerotia was not investigated in this study, and should be the aim of future research on sclerotial degradation. This research should include direct assessment of bacteria effectiveness on reducing sclerotial germination, using *in vitro* studies that coat viable sclerotia with the appropriate concentrations of each bacterium. Formulations will need to be assessed along with the colonization potential and longevity of the bacteria. Any bacteria from the 29 inhibitory bacteria isolated that can be properly formulated, remain viable for an extended period of time, and most importantly reduce sclerotial germination, should progress to in-field performance testing. Clearly this research is a long-term project, but the initial biocontrol agent discovery has been accomplished.

Introduction of bacteria to the phylloplane to protect against *S. sclerotiorum* infection was the second biological control strategy utilized in this thesis (Chapter 4).

The purpose of this section was to assess *Pseudomonas chlororaphis* (strain PA-23), and *P. corrugata* (strain 41), for their ability to manage natural and induced *S. sclerotiorum* infection. Testing the performance *in vivo* is the second step in the process of biological control commercialization. Literature warns not to progress to performance testing too quickly, without fully assessing the biocontrol agents potential and all formulation combinations. In our study, bacterial formulations were not explored prior to performance testing. Results for the foliar biological control were variable; in the Morden 1 experiment in 2001, the area under the disease progress curve (AUDPC) was reduced by almost 50% with the foliar application of strain 41 under millet inoculation. In other experiments, effectiveness was minimal with the same strain. This variation in efficacy is because of numerous factors like, temperature, moisture, and humidity and their effects on bacterial longevity. With in depth formulation research prior to performance testing in the field, this variation in efficacy may be reduced. The results produced from this biocontrol performance experiment were not conclusive, indicating that neither bacterium should progress to the scale-up procedure. Future research should assess the potential of these bacteria under several different formulations and application procedures, determining the full extent of their applicability.

Chemical control is effective against *S. sclerotiorum* in other host crops such as canola and beans. Chemical control is utilized as the main method of control in these cropping systems, and is incorporated efficiently with other management strategies. The intent of incorporating a chemical fungicide into the head rot assessment experiment was to compare the effectiveness of an experimental chemical fungicide, BAS 510 F, to the effectiveness of the biological control bacteria. In all but one trial, BAS 510 F

outperformed both *Pseudomonas* spp., demonstrating the effectiveness of this fungicide. BAS 510 F was more effective when applied at the 100% flowering stage (R6.0), in comparison to application during early flowering (R5.1-R5.5). Whether this chemical control would be economical for the producer, or achieve similar results under natural production systems is a question that requires further research.

Cultural control is one of the most important protocols in an integrated disease management system, with tillage representing a major component of this system. The effects of tillage on the viability of sclerotia and incidence of *S. sclerotiorum* infection are not fully understood. In depth research has progressed under all types of tillage, but results are often contrasting within the same tillage system. The intent of this study was to omit this variation of tillage and isolate the effect of depth and time on the survival of sclerotia and bacterial colonization.

It is clear that the elapsed time in the soil (R^2 = -0.68, $P < 0.0001$) and depth of burial (R^2 = -0.58, $P < 0.0001$) were both negatively correlated with sclerotia viability. It is also evident that time and depth affect bacterial colonization which negatively correlates with sclerotial viability (R^2 = -0.60, $P < 0.0001$). Previous reports have stated that sclerotia near the soil surface degrade at a higher rate than sclerotia deeper in the soil profile. Under the soil and environmental conditions at this experimental site, burying sclerotia deeper in the soil profile will decrease sclerotial viability at a much greater rate than if sclerotia remain on the soil surface. Bacterial colonization of sclerotia plays a significant role in their degradation. Tillage effects on sclerotial placement should be studied over a simulation period of several years to better understand what tillage

practices will increase sclerotial degradation. With this information, producers could manage *S. sclerotiorum* inoculum effectively with current agronomic practices.

Four *S. sclerotiorum* management techniques were researched in this thesis. On their own, none provided complete control of *S. sclerotiorum.* However, if combined, it is possible that infection would decrease further, maintaining yield and seed quality. There is no doubt that an integration of management techniques will aid in *S. sclerotiorum* control until complete resistance is discovered. The research that has been conducted in this thesis will provide greater incite into the integration of these modified management techniques.

7.0 LITERATURE CITED

Abawi G. S. & Grogan R. G. (1975) Source of primary inoculum and effects of temperature and moisture on infection of beans by *Whetzelinia sclerotiorum*. *Phytopathology* 65: 300-309.

Abawi G. S. & Grogan R. G. (1979) Epidemiology of plant diseases caused by Sclerotinia species. *Phytopathology* 69: 899-904.

Abawi G. S., Polach F. J., & Molin W. T. (1975) Infection of bean by ascospores of *Whetzelinia sclerotiorum*. *Phytopathology* 65: 673-678.

Adams P. B. (1975) Factors affecting survival of *Sclerotinia sclerotiorum* in soil. *Plant Disease Reporter* 59: 599-603.

Adams P. B. (1990) The potential of mycoparasites for biological control of plant diseases. *Annual Review of Phytopathology* 28: 59-72.

Adams P. B. & Ayers W. A. (1979) Ecology of *Sclerotinia* species. *Phytopathology* 69: 896-899.

Adams P. B. & Tate C. J. (1976) Mycelial germination of sclerotia of *Sclerotinia sclerotiorum* on soil. *Plant Dis. Rep.* 60: 515-518.

Agrios G. (1978) Plant Pathology, 2nd edn. Academic Press, New York. 703 pp.

Alabouvette C. & Louvet J. (1973) *Sclerotinia sclerotiorum*. Information Bulletin. *CETIOM Inform. Tech.* 31: 7-26.

Anonymous (2003) Bill boosts sclerotinia initiative funding. *The Sunflower* 3.

Auger S. J. & Nome H. F. (1970) Effect of age on the susceptibility of sunflower (*Helianthus annuus* L.) to sclerotiniose (*Sclerotinia sclerotiorum* (Lib.) de Bary). *Agr. Tec.* 30: 161-165.

Bailey K. L., Gossen B. D., Gugel R. K., & Morrall R. A. A. (2003) Diseases of field crops in Canada, **3rd** edn. The Canadian Phytopathological Society, Canada. 304 pp.

Bailey L. H. & Bailey E. Z. (1976) Hortus Third. A Concise Dictionary of Plants Cultivated in the United States and Canada, 3rd edn. MacMillan Publishing Company Co., New York. 1290 pp.

Bardin S. D. & Huang H. C. (2001) Research on biology and control of *Sclerotinia* diseases in Canada. *Canadian Journal of Plant Pathology* 23: 88-98.

Barnett H. L. (1963) The nature of mycoparasitism by fungi. *Annual Review of Microbiology* 17: 1-14.

BASF Corporation (2002) Endura. *In Technical Information Bulletin.*

Ben Yephet Y., Genizi A., & Siti E. (1993) Sclerotial survival and apothecial production by *Sclerotinia sclerotiorum* following outbreaks of lettuce drop. *Phytopathology* 83: 509-513.

Benhamou N. & Chet I. (1996) Parasitism of sclerotia of *Sclerotium rolfsii* by *Trichoderma harzianum*: ultrastructural and cytochemical aspects of the interaction. *Phytopathology* 86: 405-416.

Bisby G. R. (1921) Stem-rot of sunflowers in Manitoba. *Sci. Agric.* 2: 58-61.

Blakeman J. P. (1971) The chemical environment of the leaf surface in relation to growth of pathogenic fungi. In: *Ecology of Leaf Surface Micro-organisms* pp. 255-268. Academic, London.

Blakeman J. P. (1973) The chemical environment of leaf surfaces with special reference to spore germination of pathogenic fungi. *Pestic. sci.* 4: 575-588.

Blakeman J. P. & Brodie I. D. S. (1977) Competition for nutrients between epiphytic micro-organisms and germination of spores of plant pathogens on beetroot leaves. *Physiol. Plant Pathol.* 10: 29-42.

Blakeman J. P. & Fokkema N. J. (1982) Potential for biological control of plant diseases on the phylloplane. *Annual Review of Phytopathology* 20: 167-192.

Blamey F. P. C., Zollinger R. K., & Schneiter A. A. (1997) Sunflower Production and Culture. In: *Sunflower Technology and Production* (ed A. A. Schneiter) pp. 595-670. American Society of Agronomy, Crop Science Society of American, Soil Science Society of America, Madison, Wisconsin. 834 pp.

Boland G. J. (1997) Stability analysis for evaluating the influence of environment on chemical and biological control of white mold (*Sclerotinia sclerotiorum*) of bean. *Biological Control* 9: 7-14.

Boland G. J. & Hall R. (1994) Index of plant hosts of *Sclerotinia sclerotiorum. Canadian Journal of Plant Pathology* 16: 93-108.

Brown J. G. & Butler K. D. (1936) Sclerotiniose of lettuce in Arizona. *Ariz. Agric. Exp. Stn. Tech. Bull.* 63: 475-506.

Budge S. P., McQuilken M. P., Fenlon J. S., & Whipps J. M. (1995) Use of *Coniothyrium minitans* and *Gliocladium virens* for biological control of *Sclerotinia sclerotiorum* in glasshouse lettuce. *Biological Control* 5: 513-522.

Budge S. P. & Whipps J. W. (1991) Glasshouse trials of *Coniothyrium minitans* and *Trichoderma* species for the biological control of *Sclerotinia sclerotiorum* in celery and lettuce. *Plant Pathology* 40: 59-66.

Caesar A. J. & Pearson R. C. (1983) Environmental factors affecting survival of ascospores of *Sclerotinia sclerotiorum*. *Phytopathology* 73: 1024-1030.

Campbell C. A., Lafond G. P., Zentner R. P., & Jame Y. W. (1994) Nitrate leaching in a udic haploboroll as influenced by fertilization and legumes. *Journ. Environ. Qual.* 23: 195-201.

Carsten L. D., Johnston M. R., Douglas L. I., & Sands D. C. (2000) A field trial of crown rust (*Puccinia coronata* f. sp. *avenae*) as a biocontrol agent of wild oats on San Clemente Island. *Biological Control* 19: 175-181.

Castano F., Vear F., & Tourvieille de Labrouhe D. (1993) Resistance of sunflower inbred lines to various forms of attack by *Sclerotinia sclerotiorum* and relations with some morphological characters. *Euphytica* 68: 98.

Castano F., Vear F., & Tourvieille de Labrouhe D. (2001) The genetics of resistance in sunflower capitula to *Sclerotinia sclerotiorum* mearsured by mycelium infections combined with ascospore tests. *Euphytica* 122: 373-380.

Clark C. A. & Lorbeer J. W. (1977) Comparative nutrient dependency of *Botrytis squamosa* and *B. cinerea* for germination of conidia and pathogenicity on onion leaves. *Phytopathology* 67: 212-218.

Cober E. R., Rioux S., Rajcan I., Donaldson P. A., & Simmonds D. H. (2003) Partial resistance to white mold in a transgenic soybean line. *Crop Science* 43: 92-95.

Coley-Smith J. R. & Cooke R. C. (1971) Survival and germination of fungal sclerotia. *Annual Review of Phytopathology* 9: 65-92.

Connor D. J. & Hall A. J. (1997) Sunflower Physiology. In: *Sunflower Technology and Production* pp. 113-182. American Society of Agronomy, Crop Science Society of American, Soil Science Society of America, Madison, Wisconsin.

Cook G. E., Steadman J. R., & Boosalis M. G. (1975) Survival of *Whetzelinia sclerotiorum* and initial infection of dry edible beans in Western Nebraska. *Phytopathology* 65: 250-255.

Cook R. J. (1993) Making greater use of introduced microorganisms for biological control of plant pathogens. *Annual Review of Phytopathology* 31: 53-80.

Cook R. J. & Baker K. R. (1983) The Nature and Practice of Biological Control of Plant Pathogens. Am. Phytopathol. Soc., St. Paul, MN.

Cooper R. L. (1989) Soybean yield response to benomyl fungicide application under maximum yield conditions. *Agron. J.* 81: 847-849.

de Boer M., Bom P., Kindt F., Keurentjes J. J. B., van der Sluis I., van Loon L. C., & Bakker P. A. H. M. (2003) Control of fusarium wilt of radish by combining *Pseudomonas putida* strains that have different disease-suppressive mechanisms. *Phytopathology* 93: 626-632.

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

Dedio W. (1992) Variability among cultivated sunflower genotypes to sclerotinia head rot. *Canadian Plant Disease Survey* 72: 13-16.

Dedio W., Hoes J. A., Campbell S. J., Ukrainetz H., & Arthur A. P. (1980) Sunflower Seed Crops. Agriculture and Agri-Food Canada, Ottawa. 31 pp.

Degener J., Melchinger A. E., Gumber R. K., & Hahn V. (1998) Breeding for *Sclerotinia* resistance in sunflower: A modified screening test and assessment of genetic variation in current germplasm. *Plant Breeding* 117: 367-372.

Degener J., Melchinger A. E., & Hahn V. (1999) Optimal allocation of resources in evaluating current sunflower inbred lines for resistance to *Sclerotinia*. *Plant Breeding* 118: 157-160.

Dennis C. & Webster J. (1971a) Antagonistic properties of species-groups of *Trichoderma*. I. Production of non-volatile antibiotics. *Trans. Br. Mycol. Soc.* 57: 25-39.

Dennis C. & Webster J. (1971b) Antagonistic properties of species-groups of *Trichoderma*. II. Production of volatile antibiotics. *Trans. Br. Mycol. Soc.* 57: 41-48.

Dennis C. & Webster J. (1971c) Antagonistic properties of species-groups of *Trichoderma*. III. Hyphal interactions. *Trans. Br. Mycol. Soc.* 57: 363-369.

Dorrell D. G. & Vick B. A. (1997) Properties and processing of oilseed sunflower. In: *Sunflower Technology and Production* (ed A. A. Schneiter) pp. 709-745. American Society of Agronomy, Crop Science Society of American, Soil Science Society of America, Madison, Wisconsin. 834 pp.

Draper M. A. & Ruden K. R. (2002) Variation in head rot reactions among sunflower hybrids. *In Proceedings: 24th Sunflower Research Workshop.* January 17-18, 2002, Fargo, ND: 162-164.

Dunfield K. E. & Germida J. J. (2001) Diversity of bacterial communities in the rhizosphere and root interior of field-grown genetically modified *Brassica napus*. *FEMS Microbiology Ecology* 38: 1-9.

Emmert E. A. B. & Handelsman J. (1999) Biocontrol of plant diseas: a (Gram-) positive perspective. *FEMS Microbiology Letters* 171: 1-9.

Enisz J. (1986) Sclerotium production of *Sclerotinia sclerotiorum* (Lib.) de Bary in sunflower. *Acta Phytopathol. Entom. Hung.* 21: 261-266.

Errampalli D. & Kohn L. (1995) Comparison of different pectic zymograms produced by different clones of *Sclerotinia sclerotiorum* in culture. *Phytopathology* 85: 292-298.

Estevez de Jensen C., Percich J. A., & Graham P. H. (2002) Integrated management strategies of bean root rot with *Bacillus subtilis* and *Rhizobium* in Minnesota. *Field Crops Research* 74: 107-115.

Expert J. M. & Digat B. (1995) Biocontrol of *Sclerotinia* wilt of sunflower by *Pseudomonas fluorescens* and *Pseudomonas putida* strains. *Canadian Journal of Microbiology* 41: 685-691.

Fahima T., Madi L., & Henis Y. (1992) Ultrastructure and germinability of *Verticillium dahliae* microsclerotia parasitized by *Talaromyces flavus* on agar medium and in treated soil. *Biocontrol Science and Technology* 2: 69-78.

Fernando W. G. D. & Linderman R. (1994) Inhibition of *Phytophthora vignae* and stem and root rot of cowpea by soil bacteria. *Biol. Agric. and Hort.* 12: 1-14.

Fernando W. G. D. & Pierson III L. S. (1999) The effect of increased phenazine antibiotic production on the inhibition of economically important soil-borne plant pathogens by *Pseudomonas aureofaciens* 30-84. *Arch. Phytopath. Pflanz.* 32: 491-502.

Fokkema N. J. (1973) The role of saprophytic fungi in antagonsim against *Dreschlera sorokiniana* (*Helminthosporium sativum*) on agar plates and on rye leaves with pollen. *Physiol. Plant Pathol.* 3: 195-205.

Fokkema N. J. (1981) Fungal leaf saprophytes, beneficial or detrimental? In: *Microbial Ecology of the Phylloplane* pp. 433-454. Academic, London.

Fokkema N. J. (1993) Opportunities and problems of control of foliar pathogens with micro-organisms. *Pestic. sci.* 37: 411-416.

Fuller P. A., Coyne D. P., & Steadman J. R. (1984) Inheritance of resistance to while mold disease in a diallel cross of dry beans. *Crop sci.* 24: 929-933.

Garrett S. D. (1965) Toward biological control of soil-borne plant pathogens. In: *Ecology of Soil-borne Plant Pathogens* (eds K. F. Baker and W. C. Snyder) pp. 4-17. Univ. Calif. Press, Berkeley, CA.

Gawande V. L. & Patil J. V. (2003) Genetics of powdery mildew (*Erysiphe polygoni* D.C.) resistance in Mungbean (*Vigna radiata* (L.) Wilczek). *Crop Protection* 22: 567-571.

Gentzbittel L., Mouzeyar S., Badaoui S., Mestries E., Vear F., Tourvieille de Labrouhe D., & Nicolas P. (1998) Cloning of molecular markers for disease resistance in sunflower, *Helanthus annuus* L. *Theory of Applied Genetics* 96: 519-525.

Gerhardson B. (2002) Biological substitutes for pesticides. *Trends in Biotechnology* 20: 338-343.

Ghaffar A. (1972) Some observations on the parasitism of *Coniothyrium minitans* on the sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary. *Pak. J. Bot.* 4: 85-87.

Gossen B. D. & Platford G. (1999) Blossom blight in alfalfa seed fields in Saskatchewan and Manitoba 1998. *Canadian Plant Disease Survey* 79: 95-96.

Goulds A. & Fitt B. D. L. (1990) Effects of fungicide timing on the severity of eyespot on winter wheat or winter barley inoculated with W-type or R-type isolates of *Pseudocercosporella herpotrichoides*. *Crop Protection* 9: 265-270.

Gracia Garza J. A., Reeleder R. D., & Paulitz T. C. (1997) Degradation of sclerotia of *Sclerotinia sclerotiorum* by fungus gnats (*Bradysia coprophila*) and the biocontrol fungi *Trichoderma* spp. *Soil Biology and Biochemistry* 29: 123-129.

Gracia-Garza J. A., Neumann S., Vyn T. J., & Boland G. J. (2002) Influence of crop rotation and tillage on production of apothecia by *Sclerotinia sclerotiorum*. *Canadian Journal of Plant Pathology* 24: 137-143.

Grogan R. G. (1979) *Sclerotinia* species: Summary and comments on needed research. *Phytopathology* 69: 908-910.

Grogan R. G. & Abawi G. S. (1974) The effect of water potential on the biology of *Whetzelinia sclerotiorum*. *Proc. Am. Phytopathol. Soc.* 1: 47.

Guetsky R., Shtienberg D., Elad Y., & Dinoor A. (2001) Combining biocontrol agents to reduce the variability of biological control. *Phytopathology* 91: 621-627.

Guetsky R., Shtienberg D., Elad Y., Fischer E., & Dinoor A. (2002) Improving biological control by combining biocontrol agents each with several mechanisms of disease suppression. *Phytopathology* 92: 976-985.

Gugel R. K. & Morrall R. A. A. (1986) Inoculum-disease relationships in Sclerotinia stem rot of rapeseed in Saskatchewan. *Canadian Journal of Plant Pathology* 8: 89-96.

Gulya T. (1985) Registration of five disease-resistant sunflower germplasms. *Crop Science* 25: 719-720.

Gulya T. (2003) Citing online sources: Sunflower crop survey - 2002: Disease assessment across eight states. *Available from* http://www.sunflowernsa.com/research_statistics/research_workshop/documents/102.PDF: Accessed April 5, 2003.

Gulya T., Rashid K. Y., & Masirevic S. M. (1997) Sunflower Diseases. In: *Sunflower Technology and Production* (ed A. A. Schneiter) pp. 263-379. American Society of Agronomy, Crop Science Society of American, Soil Science Society of America, Madison, Wisconsin. 834 pp.

Gulya T., Vick B. A., & Nelson B. D. (1986) Sclerotinia head rot of sunflower in North Dakota: 1986 incidence, effect on yield and oil components, and sources of resistance. *Plant Disease* 73: 504-507.

Gulya T. J., Vick B. A., & Nelson B. D. (1989) Sclerotinia head rot of sunflower in North Dakota: 1986 incidence, effect on yield and oil components, and sources of resistance. *Plant Disease* 73: 504-507.

Gupta S., Arora D. K., & Srivastava A. K. (1995) Growth promotion of tomato plants by rhizobacteria and imposition of energy stress on *Rhizoctonia solani*. *Soil Biol. Biochem.* 27: 1051-1053.

Hagan A. K., Bowen K. L., & Weeks J. R. (1994) Multiple applications of new fungicide needed to control white mold in peanuts. *Highlights. Agri. Res.* 41: 2-5.

Hahn V. (2002) Genetic variation for resistance to sclerotinia head rot in sunflower inbred lines. *Field Crops Research* 77: 153-159.

Halmimi E. S., Rowe D. E., & Pratt R. G. (1998) Responses of alfalfa to stem-tip inoculations with five isolates of *Sclerotinia trifoliorum*. *Crop Science* 38: 1179-1182.

Hao J. J., Subbarao K. V., & Duniway J. M. (2003) Germination of *Sclerotinia minor* and *S. sclerotiorum* Sclerotia under various soil moisture and temperature combinations. *Phytopathology* 93: 443-450.

Harvard (1895) Food plants of the North American Indians. *Bull. Torrey Bot. Club* 22: 98-123.

Heiser C. B. (1951) The sunflower among the North American Indians. *Proc. Am. Phil. Soc.* 95: 432-448.

Heiser C. B., Smith D. M., Clevenger S. B., & Martin W. C. (1969) The North American sunflowers (*Helianthus*). *Memoirs Torrey Bot. Club* 22: 1-218.

Hensley H. C. (1924) Production of sunflower seed in Missouri. *Missouri Univ. College Agric. Ext. Circ.* 140.

Hevesi M. & Mashaal S. F. (1975) Contributions to the mechanism of infection of *Erwinia uredovora*, a parasite of rust fungi. *Acta Phytopathol. Acad. Sci. Hung.* 10: 275-280.

Hoes J. A. & Huang H. C. (1975) *Sclerotinia sclerotiorum*: Viability and separation of sclerotia from soil. *Phytopathology* 65: 1431-1432.

Hoes J. A. & Huang H. C. (1985) Effect of between-row and within-row spacing on development of sclerotinia wilt and yield of sunflower. *Canadian Journal of Plant Pathology* 7: 98-102.

Hofman V. L. & Hellevang K. J. (1997) Harvesting, drying, and storage of sunflower. In: *Sunflower Technology and Production* (ed A. A. Schneiter) pp. 671-696. American Society of Agronomy, Crop Science Society of American, Soil Science Society of America, Madison, Wisconsin. 834 pp.

Holley R. C. & Nelson B. D. (1986) Effect of plant population and inoculum density on incidence of *Sclerotinia* wilt of sunflower. *Phytopathology* 86: 71-74.

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

Huang H. C. (1980) Control of *Sclerotinia* wilt of sunflower by hyperparasites. *Canadian Journal of Plant Pathology* 2: 26-32.

Huang H. C. (1983) Sclerotinia wilt and head rot of sunflower. *Agriculture Canada* Ont., Canadex 632.145.

Huang H. C. & Hoes J. A. (1980) Importance of plant spacing and sclerotial position to development of *Sclerotinia sclerotiorum* wilt of sunflower. *Plant Dis.* 64: 81-84.

Huang H. C. & Kokko E. G. (1992) Pod rot of dry peas due to infection by ascospores of Sclerotinia sclerotiorum. *Plant Dis.* 76: 597-600.

Huang H. C. & Kokko E. G. (1993) *Trichothecium roseum*, a mycoparasite of *Sclerotinia sclerotiorum*. *Can. J. Bot.* 71: 1631-1638.

Huang H. C., Kokko E. G., Yanke L. J., & Phillippe R. C. (1993) Bacterial suppression of basal pod rot and end rot of dry peas caused by *Sclerotinia sclerotiorum*. *Canadian Journal of Microbiology* 39: 227-233.

Huang H. C. & Kozub G. C. (1990) Cyclic occurrence of sclerotinia wilt of sunflower in Western Canada. *Plant Dis.* 74: 766-770.

Huang H. C. & Kozub G. C. (1991) Monocropping to sunflower and decline of *Sclerotinia* wilt. *Bot. Bull. Acad. Sin.* 32: 163-170.

Huang H. C. & Kozub G. C. (1993) Survival of mycelia of *Sclerotinia sclerotiorum* in infected stems of dry bean, sunflower, and canola. *Phytopathology* 83: 937-940.

Hubbard J. C., Subbarao K. V., & Koike S. T. (1997) Development and significance of dicarboximide resistance in Sclerotinia minor isolates from commercial lettuce fields in California. *Plant Dis.* 81: 148-153.

Hunter J., Steadman J. R., & Cigna J. (1982) Preservation of ascospore of *Sclerotinia sclerotiorum* on membrane filters. *Phytopathology* 72: 650-652.

Imolehin E. D. & Grogan R. G. (1980a) Effects of oxygen, carbon dioxide, and ethylene on growth, sclerotial production, germination, and infection by *Sclerotinia minor*. *Phytopathology* 70: 1158-1161.

Imolehin E. D. & Grogan R. G. (1980b) Factors affecting survival of sclerotia, and effects of inoculum density, relative position, and distance of sclerotia from the host on infection of lettuce by *Sclerotinia minor*. *Phytopathology* 70: 1162-1167.

Inbar J., Menendez A., & Chet I. (1996) Hyphal interaction between *Trichoderma harzianum* and *Sclerotinia sclerotiorum* and its role in biological control. *Biology and Biochemistry* 28: 757-763.

Jagger I. C. (1920) *Sclerotinia minor*, n. sp., the cause of a decay of lettuce, celery, and other crops. *J. Agric. Res.* 20: 331-334.

Jenness D. (1958) The Indians of Canada. *4th ed. Natl. Museum Can. Bull.* 65.

Jones D. R. (1995) Timing of fungicide application for control of eyespot disease of winter wheat. *Crop Protection* 14: 247-256.

Kim D. H. & Misaghi I. J. (2003) Biocontrol performance of two isolates of *Pseudomonas fluorescens* in modified soil atmospheres. *Phytopathology* 86: 1238-1241.

Kleingartner L. W. (1997) The World Oilseed Environment. In: *Sunflower Technology and Production* (ed A. A. Schneiter) pp. 809-824. American Society of Agronomy, Crop Science Society of American, Soil Science Society of America, Madison, Wisconsin. 834 pp.

Kohler H. & Friedt W. (1999) Genetic variability as identified by AP-PCR and reaction to mid-stem infection of *Sclerotinia sclerotiorum* among interspecific sunflower (*Helianthus annuus* L.) hybrid progenies. *Crop Science* 39: 1456-1463.

Kohli Y. & Kohn M. (1996) Mitochondrial haplotypes in populations of the plant-infecting fungus *Sclerotinia sclerotiorum*: wide distribution in agriculture, local distribution in the wild. *Molec. Ecol.* 5: 773-783.

Kondo N., Kodama M., Ozaki M., & Akai J. (1988) Occurrence and control of sclerotinia head rot of sunflower in Hokkaido. *Ann. Phytopathhol. Soc. Jpn.* 54: 198-203.

Kondoh M., Hirai M., & Shoda M. (2000) Co-utilization of *Bacillus subtilis* and Flutolanil in controlling damping -off of tomato caused by *Rhizoctonia solani*. *Biotechnology Letters* 22: 1693-1697.

Krauss U. & Soberanis W. (2002) Effect of fertilization and biocontrol application frequency on cocoa pod diseases. *Biological Control* 24: 82-89.

Kurle J. E., Grau C. R., Oplinger E. S., & Mengistu A. (2001) Tillage, crop sequence, and cultivar effects of Sclerotinia stem rot incidence and yield in soybean. *Agronomy Journal* 93: 973-982.

Laberge C. & Sackston W. E. (1987) Adaptability and diseases of jerusalem artichoke (*Helianthus tuberosus*) in Quebec. *Canadian Journal of Plant Science* 67: 349-352.

Lafond G. P. & Derksen D. A. (1996) Long-term potential of conservation tillage on the Canadian prairies. *Canadian Journal of Plant Pathology* 18: 151-158.

Lamarque C., Leconte M., Berrier J., & Jaunet A. M. (1985) Morphologie externe des differentes pieces florales du capitule de Tournesol et consequences sur les potentialites d'attaques par de sclerotinia. *CETIOM Inform. Tech.* 92: 5-35.

Lefol C., Nelson B., & Gulya T. (1998) Protocol to rapidly obtain apothecia of *Sclerotinia sclerotiorum*. *In Proceedings: 1998 International Sclerotinia Workshop: Methods for working with Sclerotinia.* 74.

Levine M. N., Bamberg R. H., & Atkinson R. E. (1936) Micro-organisms antibiotic or pathogenic to cereal rusts. *Phytopathology* 26: 99-100.

Li W., Roberts D. P., Dery P. D., Meyer S. L. F., Lohrke S., Lumsden R. D., & Hebbar K. P. (2002) Broad spectrum anti-biotic activity and disease suppression by the potential biocontrol agent *Burkholderia ambifaria* BC-F. *Crop Protection* 21: 129-135.

Lindenfelser L. A. & Ciegler A. (1969) Production of antibiotics by *Alternaria* species. *Dev. Ind. Microbiol.* 10: 271-278.

Lofgren J. R. (1997) Sunflower for confectionery food, bird food, and pet food. In: *Sunflower Technology and Production* (ed A. A. Schneiter) pp. 747-764. American Society of Agronomy, Crop Science Society of American, Soil Science Society of America, Madison, Wisconsin. 834 pp.

Lumsden R. D. (1981) Ecology of mycoparasitism. In: *The Fungal Community. Its Organization and Role in the Ecosystem* (eds D. Wicklow and G. Carroll) pp. 295-318. Marcel Dekker Inc., New York.

Lumsden R. D. & Dow R. L. (1973) Histopathology of *Sclerotinia sclerotiorum* infection of bean. *Phytopathology* 63: 708-715.

Manitoba Agriculture and Food (2000) Agronomy. In: *Sunflower Production Guide* pp. 4-9.

Manitoba Agriculture and Food (2001) Citing online sources: Manitoba grains and oilseed industry profiles 2001: Sunflower seed sector. *Available from* http://www.gov.mb.ca/agriculture/statistics/aac04s08.html: Accessed June 6, 2003.

Mari M., Guizzardi M., Brunelli M., & Folchi A. (1996) Postharvest biological control of grey mould (*Botrytis cinerea* Pers.: Fr.) on fresh-market tomatoes with *Bacillus amyloliquefaciens*. *Crop Protection* 15: 699-705.

Masirevic S. M. & Gulya T. (1992) *Sclerotinia* and *Phomposis* - two devastating sunflower pathogens. *Field Crops Research* 30: 271-300.

McCartney H. A. & Lacey M. E. (1992) Release and dispersal of *Sclerotinia* ascospores in relation to infection. *In Proc.* Brighton Crop Protection Conf. Brighton, England: 109-116.

McLaren D. L., Huang H. C., Kozub G. C., & Rimmer S. R. (1994) Biological control of Sclerotinia wilt of sunflower with *Talaromyces flavus* and *Coniothyrium minitans*. *Plant Dis.* 78: 231-235.

McLoughlin T. J., Quinn J. P., Bettermann A., & Bookland R. (1992) *Pseudomonas cepacia* suppression of sunflower wilt fungus and role of antifungal compounds in controlling the disease. *Applied and Environmental Microbiology* 58: 1760-1763.

McQuilken M. P., Budge S. P., & Whipps J. M. (1997) Biological control of *Sclerotinia sclerotiorum* by film-coating *Coniothyrium minitans* on to sunflower seed and sclerotia. *Plant Pathology* 46: 919-929.

Mehdizadegan F. & Gough F. J. (1987) Partial characterization of compounds produced by *Pseudomonas fluorescens* and *Bacillus licheniformis* antagonistic to *Pyrenopora tritici-repentis*, the cause of wheat tan spot. *Phytopathology* 77: 1720.

Mercau J. L., Sadras V. O., Satorre E. H., Messina C., Balbi C., Uribelarrea M., & Hall A. J. (2001) On-farm assessment of regional and seasonal variation in sunflower yield in Argentina. *Agricultural Systems* 67: 103.

Merriman P. R. (1976) Survival of sclerotia of *Sclerotinia sclerotiorum* in soil. *Soil Biology and Biochemistry* 8: 385-389.

Merriman P. R., Pywell M., Harrison G., & Nancarrow J. (1979) Survival of sclerotia of *Sclerotinia sclerotiorum* and effects of cultivation practices on disease. *Soil Biology and Biochemistry* 11: 567-570.

Mestries E., Gentzbittel L., Tourvieille de Labrouhe D., Nicolas P., & Vear F. (1998) Analyses of quantitative trait loci associated with resistance to *Sclerotinia sclerotiorum* in sunflowers (*Helianthus annuus* L.) using molecular markers. *Molecular Breeding* 4: 215-226.

Miller J. F. & Fick G. N. (1997) The Genetics of Sunflower. In: *Sunflower Technology and Production* (ed A. A. Schneiter) pp. 441-495. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, Wisconsin. 834 pp.

Miller J. F. & Gulya T. (1999) Registration of eight sclerotinia-tolerant sunflower germplasm lines. *Crop Science* 39: 301-302.

Moore W. D. (1949) Flooding as a means of destroying the sclerotia of *Sclerotinia sclerotiorum*. *Phytopathology* 39: 920-927.

Morrall R. A. A., Duczek L. J., & Sheard J. W. (1972) Variations and correlations within and between morphology, pathogenicity, and pectolytic enzyme activity in *Sclerotinia* from Saskatchewan. *Can. J. Bot.* 50: 767-786.

Morrall R. A. A., Verman P. R., & Dueck J. (1985) Recent progress in chemical control of *Sclerotinia* stem rot of rape in western Canada. *Meded. Fac. Landbouwwet. Rijksuniv. Gent.* 50: 1189-1194.

Mueller D. S., Hartman G. L., & Pedersen W. L. (1999) Development of sclerotia and apothecia of *Sclerotinia sclerotiorum* from infected soybean seed and its control by fungicide seed treatment. *Plant Dis.* 83: 1113-1115.

Mueller D. S., Hartman G. L., & Pedersen W. L. (2002) Effect of crop rotation and tillage system on sclerotinia stem rot on soybean. *Canadian Journal of Plant Pathology* 24: 450-456.

National Research Council (1989) Nutrient requirements of domestic animals, 6th edn. Natl. Acad. Sci., Washington, DC. 248 pp.

National Sunflower Association (2003) Citing online source: USDA: Prospective Plantings. *Available from* http://www.sunflowernsa.com/research_statistics/usda/prospective.asp: Accessed June 6, 2003.

Neya A. & Le Normand M. (1998) Responses of sorghum genotypes to leaf anthracnose (*Colletotrichum graminicola*) under field conditions in Burkina Faso. *Crop Protection* 17: 47-53.

Oedjijono, Line M. A., & Dragar C. (1993) Isolation of bacteria antagonistic to a range of plant pathogenic fungi. *Soil Biology and Biochemistry* 25: 247-250.

Papavizas G. C. & Collins D. J. (1990) Influence of *Gliocladium virens* on germination and infectivity of sclerotia of *Sclerotium rolfsii*. *Phytopathology* 80: 627-630.

Park C. S., Marx G. D., Moon Y. S., Wiesenborn D., Chang K. C., & Hofman V. L. (1997) Alternative uses of sunflower. In: *Sunflower Technology and Production* (ed A. A. Schneiter) pp. 765-807. American Society of Agronomy, Crop Science Society of American, Soil Science Society of America, Madison, Wisconsin. 834 pp.

Pedras M. S. C., Ismail N., Quail J. W., & Boyetchko S. M. (2003) Structure, chemistry, and biological activity of pseudophomins A and B, new cyclic lipodepsipeptides isolated from the biocontrol bacterium *Pseudomonas fluorescens*. *Phytochemistry* 62: 1105-1114.

Perello A., Moreno V., Simon M. R., & Sisterna M. (2003) Tan spot of wheat (*Triticum aestivum* L.) infection at different stages of crop development and inoculum type. *Crop Protection* 22: 157-169.

Peres J. & Regnault Y. (1985) *Sclerotinia sclerotiorum* (Lib.) de Bary: Recherche de moyens chimiques permettant de limiter la production d'inoculum par traitement du sol. *In: Proceedings, 11th International Sunflower Conference* 10-13/03/82, Mar del Planta, Argentina, II: 363-368.

Prior G. D. & Owen J. H. (1964) Pathological anatomy of *Sclerotinia trifoliorum* on clover and alfalfa. *Phytopathology* 54: 784-787.

Purdue University Department of Botany and Plant Pathology (2001) Crop diseases in corn, soybean, and wheat (online). *Available from http://www. btny. purdue. edu/Extension/Pathology/CropDiseases/Soybean/soybean. html#sclerotiniastemrot (updated 2 July 2001).*

Purdy L. H. (1955) A broader concept of the species *Sclerotinia sclerotiorum* based on variability. *Phytopathology* 45: 421-427.

Purdy L. H. (1958) Some factors affecting penetration and infection by *Sclerotinia sclerotiorum*. *Phytopathology* 48: 605-609.

Purdy L. H. (1979) *Sclerotinia sclerotiorum*: History, diseases and symptomology, host range, geographic distribution, and impact. *Phytopathology* 69: 875-880.

Pustovoit G. V. & Gubin I. A. (1974) Results and prospects in sunflower breeding for group immunity by using the interspecific hybridization method. *In Proceedings: 6th International Sunflower Conference* Bucharest, Romania: 373-381.

Putt E. D. (1949) Sunflower Seed Production. *Co-op Vegetable Oils Ltd.* Altona, Manitoba..

Putt E. D. & Sackston W. E. (1955) Rust resistance in sunflowers (*Helianthus annuus* L.). *Nature* 176: 77.

Putt E. D. (1997) Early History of Sunflower. In: *Sunflower Technology and Production* (ed A. A. Schneiter) pp. 1-19. American Society of Agronomy, Crop Science Society of American, Soil Science Society of America, Madison, Wisconsin. 834 pp.

Quesenberry G. R., Cunningham O. C., & Foster L. (1921) The culture and feeding of Russian sunflowers. *New Mexico Agric. Exp. Stn. Bull.* 126.

Rashid K. Y. (1993) *Sclerotinia* head-rot and mid-stem infection of sunflower in Manitoba. *In Proceedings: Sunflower Research Workshop* Fargo, ND, January 16, 1993: 10-11.

Rashid K. Y. (2000) *Sclerotinia* on flax in Western Canada - warning for a potential disease problem. *Canadian Journal of Plant Pathology* 21: 175.

Rashid K. Y. & Dedio W. (1992) Difference in the reaction of sunflower hybrids to sclerotinia wilt. *Canadian Journal of Plant Science* 72: 925-930.

Rashid K. Y. & Dedio W. (1994) Differences in the reaction of sunflower hybrids to *Sclerotinia* head-rot. *In Proceedings: 16th Sunflower Research Workshop* Fargo, ND, January 14-15: 88-91.

Rashid K. Y., Desjardins M., & Kaminski D. A. (2002) Diseases of sunflower in Manitoba in 2001. *Canadian Plant Disease Survey* 82: 109-111.

Rashid K. Y., Desjardins M., & Kaminski D. A. (2003) Diseases of sunflower in Manitoba in 2002. *Canadian Plant Disease Survey* 83: 133-134.

Rashid K. Y., Desjardins M., & Platford R. G. (2001) Diseases of sunflower in Manitoba in 2000. *Canadian Plant Disease Survey* 81: 138-140.

Rashid K. Y. & Platford R. G. (1992) Survey of sunflower diseases in Manitoba in 1991. *Canadian Plant Disease Survey* 72: 89.

Rashid K. Y. & Platford R. G. (1993) Diseases of sunflower in Manitoba in 1992. *Canadian Plant Disease Survey* 73: 101-102.

Rashid K. Y. & Platford R. G. (1998) Diseases of sunflower in Manitoba in 1997. *Candian Plant Disease Survey* 78: 107-108.

Rashid K. Y. & Platford R. G. (1999) Diseases of sunflower in Manitoba in 1998. *Canadian Plant Disease Survey* 79: 134-135.

Rashid K. Y. & Platford R. G. (2000) Diseases of Sunflower in Manitoba in 1999. *Canadian Plant Disease Survey* 80: 112-113.

Rashid K. Y. & Seiler G. J. (2003) Citing online sources: Epidemiology of sclerotinia head rot in wild sunflower species. *Available from* http://www.sunflowernsa.com/research_statistics/research_workshop/documents/104.PDF: Accessed April 7, 2003.

Rashid K. Y. & Swanson J. (2002) Effective seed treatments for the control of sclerotinia root infection in sunflower. *In Proceedings: 24th Sunflower Research Workshop* January 17-18, 2002, Fargo, ND: 44-47.

Reichert I. (1958) Fungi and plant diseases in relation to biogeography. *Trans. N. Y. Acad. Sci.* 20: 333-339.

Rovira A. D. (1956a) Plant root excretions in relation to the rhizosphere effect II. A study of the properties of root exudates and its effect on the growth of micro-organisms isolated from the rhizosphere and control soil. *Plant Soil* 7: 195-208.

Rovira A. D. (1956b) Plant root excretions in relation to the rhizosphere effect III. The effect of root exudates on the numbers and activity of micro-organisms in soil. *Plant Soil* 7: 209-217.

Sadras V. O., Trapani N., Pereyra V. R., Lopez Pereira M., Quiroz F., & Mortarini M. (2000) Intraspecific competition and fungal diseases as sources of variation in sunflower yield. *Field Crops Research* 67: 51-58.

Savchuck S. C. (2002) Evaluation of biological control of *Sclerotinia sclerotiorum* on canola (Brassica napus) in the laboratory, in the greenhouse, and in the field. *M. Sc. Thesis* 49-91.

Sayler T. (2003a) Controlling sclerotinia naturally: Growers, crop scientists gain additional information on biocontrol product 'Intercept'. *The Sunflower* 2: 20.

Sayler T. (2003b) Sclerotinia initiative underway. *The Sunflower* 2: 18.

Scharen A. L. & Bryan M. D. (1981) A possible biological control agent *Bacillus licheniformis* for net blotch *Pyrenophora teres* of barley. *Phytopathology* 71: 902-903.

Scheiner J. D. & Lavado R. S. (1999) Soil water content, absorption of nutrient elements, and responses to fertilization of sunflower: a case study. *J. plant nutr.* 22: 369-377.

Schisler D. A., Slininger P. J., & Hanneman R. E. (1992) Enrichment and selection of antagonists of *Fusarium sambucinum* based on efficacy and performance in liquid culture. *Phytopathology* 82: 1120.

Schneiter A. A. & Miller J. F. (1981) Description of sunflower growth stages. *Crop Science* 21: 901-903.

Schwartz H. F. & Steadman J. R. (1978) Factors affecting sclerotium populations of, and apothecium production by, *Sclerotinia sclerotiorum*. *Phytopathology* 68: 383-388.

Sedun F. S. & Brown J. F. (1987) Infection of sunflower leaves by ascospores of *Sclerotinia sclerotiorum*. *Annals of Applied Biology* 110: 275-285.

Seiler G. J. (1992) Utilization of wild sunflower species for the improvement of cultivated sunflower. *Field Crops Research* 30: 195-230.

Seiler G. J. & Rieseberg L. H. (1997) Systematics, origin, and germplasm resources of the wild and domesticated sunflower. In: *Sunflower Technology and Production* (ed A. A. Schneiter) pp. 21-65. American Society of Agronomy, Crop Science Society of American, Soil Science Society of America, Madison, Wisconsin. 834 pp.

Semelczi-Kovacs A. (1975) Acclimatization and dissemination of the sunflower in Europe. *Acta Ethnorg. Acad. Sci. Hung.* 24: 47-88.

Severin G. (1935) The trade in sunflower seed. *Int. Rev. Agric.* 26: 281S-284S.

Shaner G. & Finney R. E. (1977) The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology* 67: 1051-1056.

Singh J. & Faull J. (1988) Antagonism and biological control. In: *Biological Control of Plant Diseases (Vol II)* (eds K. G. Mukerji and K. Garg) pp. 167-177. CRC Press, Florida.

Sleesman J. P. & Leben C. (1976) Microbial antagonists of *Bipolaris maydis*. *Phytopathology* 66: 1214-1218.

Stack J. P., Kenerley C. M., & Pettit R. E. (1988) Application of biological control agents. In: *Biocontrol of Plant Disease* (eds K. G. Mukerji and K. L. Garg) pp. 43-54. CRC, Boca Raton, FL.

Statistics Canada (2001) Citing online source: Area of sunflowers seeded by province, 2001. *Available from* http://www.gov.mb.ca/agriculture/statistics/images/aac04s08e.gif: Accessed June 6, 2003.

Steadman J. R. (1983) White mold - A serious yield-limiting disease of bean. *Plant Dis.* 67: 346-350.

Stelfox D., Williams J. R., Soehngen U., & Topping R. C. (1978) Transport of Sclerotinia sclerotiorum ascospores by rapeseed pollen in Alberta. *Plant Dis. Rep.* 62: 576-579.

Subbarao K. V., Koike S. T., & Hubbard J. C. (1996) Effects of deep plowing on the distribution and density of *Sclerotinia minor* sclerotia and lettuce drop incidence. *Plant Dis.* 80: 28-33.

Sutton J. C. & Peng G. (1993) Manipulating and vectoring of biocontrol organsims to manage foliage and fruit diseases in cropping systems. *Annual Review of Phytopathology* 31: 473-493.

Swinburne T. R., Barr J., & Brown A. E. (1975) Production of anitbiotics by *Bacillus subtilis* and their effect on fungal colonists of apple leaf scars. *Trans. Br. Mycol. Soc.* 65: 211-217.

Teliz-Ortiz M. & Burkholder W. H. (1960) A strain of *Pseudomonas fluorescens* antagonistic to *Pseudomonas phaseolicola* and other bacterial plant pathogens. *Phytopathology* 50: 119-123.

Tourvieille D. L. & Vear F. (1990) Heredity of resistance to *Sclerotinia sclerotiorum* in sunflower. III. Study of reactions to artificial infections of roots and cotyledons. *Agronomie* 10: 323-330.

Tu J. C. (1983) Efficacy of iprodione against *Alternaria* black pod and white mold of white beans. *Canadian Journal of Plant Pathology* 5: 133-135.

Turkington T. K. & Morrall R. A. A. (1993) Use of petal infestation to forecast sclerotinia stem rot of canola: the influence of inoculum variation over the flowering period and canopy density. *Phytopathology* 83: 682-689.

Turner G. J. & Tribe H. T. (1976) On *Coniothyrium minitans* and its parasitism of *Sclerotinia* species. *Trans. Br. Mycol. Soc.* 66: 97-105.

Turner J. T. & Backman P. A. (1991) Factors relating to peanut yield increases after seed treatment with *Bacillus subtilis*. *Plant Dis.* 75: 347-353.

Twengstrom E., Sigvald R., Svensson C., & Yuen J. (1998) Forecasting sclerotinia stem rot in spring sown oilseed rape. *Crop Protection* 17: 405-411.

Ulloa M. & Hanlin R. T. (2000) Illustrated Dictionary of Mycology. APS Press, St. Paul, Minnesota. 448 pp.

Van Becelaere G. & Miller J. F. (2003) Citing online sources: Inheritance of resistance to sclerotinia head rot in sunflower. *Available from* http://www.sunflowernsa.com/research_statistics/research_workshop/documents/94.PDF: Accessed April 7, 2003.

Vidhyasekaran P. & Muthamilan M. (1995) Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. *Plant Dis.* 79: 782-786.

Viji G., Uddin W., & Romaine C. P. (2003) Suppression of gray leaf spot (blast) of perennial ryegrass turf by *Pseudomonas aeruginosa* from spent mushroom substrate. *Biological Control* 26: 233-243.

Wagonner P. E. (1986) Progress curves of foliar diseases: their interpretation and use. In: *Plant Disease Epidemiology, Vol. 1, Population Dynamics and Management* (eds K. J. Leonard and W. E. Fry) pp. 3-37. MacMillan, New York.

Wakefield I. N. (1924) On the names *Sclerotinia sclerotiorum* (Lib.) Massee, and *S. libertiana* Funkel. *Phytopathology* 14: 126-127.

Whetzel H. H. (1945) A synopsis of the genera and species of the Sclerotiniaceae, a family of stromatic inoperculate Discomycetes. *Mycologia* 37: 648-714.

Whiting A. E. (1939) Ethnobotany of the Hopi. *Mus. North. Ariz. Bull.* 15.

Wiley H. W. (1901) The funflower plant: Its cultivation, composition and uses. *USDA Div. Chem. Bull* 60. U.S. Gov. Print. Office, Washington, DC.

Willetts H. J. & Wong J. A. L. (1980) The biology of *Sclerotinia sclerotiorum, S. trifoliorum,* and *S. minor* with emphasis on specific nomenclature. *The Botanical Review* 46: 101-165.

Wilson G. L. (1917) Agriculture of the Hidsata Indians, an Indian interpretation. *Univ. Minnesota Stud. Soc. Sci.* 9.

Wood R. (1951) The control of diseases of lettuce by use of antagonistic organisms, I: The control of *Botrytis cinerea* Pers. *Annals of Applied Biology* 38: 203-216.

Workneh F. & Yang X. B. (2000) Prevalence of Sclerotinia stem rot of soybeans in the north-central United States in relation to tillage, climate, and latitudinal positions. *Phytopathology* 90: 1375-1382.

Wu H. L. (1988) Effects of bacteria on germination and degradation of sclerotia of *Sclerotinia sclerotiorum. M. Sc. Thesis* North Dakota State Univer., Fargo.

Yoshida S., Hiradate S., Tsukamoto T., Hatakeda K., & Shirata A. (2001) Antimicrobial activity of culture filtrate of *Bacillus amyloliquefaciens* RC -2 isolated from mulberry leaves. *Phytopathology* 91: 181-187.

Yuen G. Y., Godoy G., Steadman J. R., Kerr E. D., & Craig M. L. (1991) Epiphytic colonization of dry edible bean by bacteria antagonistic to *Sclerotinia sclerotiorum* and potential for biological control of white mold disease. *Biological Control* 1: 293-301.

Zadoks J. C. & Schein R. D. (1979) Epidemiology and Plant Disease Management. In: *Epidemiology and Plant Disease Management* pp. 364-365. Oxford University Press, Inc., United States. 427 pp.

Zaki K., Misaghi I. J., Heydari A., & Shatla M. N. (1998) Control of cotton seedling damping-off in the field by *Burkholderia* (*Pseudomonas*) *cepacia. Plant Dis.* 82: 291-293.

Zhou T. & Boland G. J. (1998) Biological control of strategies for *Sclerotinia* Diseases. In: *Plant-Microbe Interactions and Biological Control* (eds G. J. Boland and L. D. Kuykendall) pp. 127-156. Marcel Dekker, Inc., New York. 442 pp.

Zukovsky P. M. (1950) Cultivated plants and their wild relatives. *Commonw. Agric. Bur. Franham Royal, England.*

8.0 APPENDICES

Appendix 1. Biolog MicroLog3 Inhibitory Bacterial Identification Data

Program : Biolog MicroLog3 4.20
Save To File : C:\Biolog420\Rob.D4C
Unrestricted Access? : Yes
Read Time : May 06 2003 10:41
Parent File :
Plate Number : 12
Incubation Time : 16-24
Sample Number : 73 Plate Type: GN2
Strain Type : GN-ALL
Strain Number :
Strain Name : Hafnia alvei
Other :
Data Input Mode : Manual
Number +/b/- Reactions : 6 / 21 / 69
Database To Search : MicroLog
Data Base(s) Searched : C:\BIOLOG420\Databases\GN601.KID

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	-	{/}	-	-	-	-	-	-	-	-
B	-	{/}	{/}	{/}	-	{/}	-	-	-	-	-	-
C	-	-	-	-	-	-	-	-	-	-	-	-
D	-	{/}	-	-	-	-	{/}	-	-	{/}	{/}	{/}
E	-	-	{/}	{/}	-	-	-	-	-	-	-	{/}
F	-	-	-	-	-	-	-	{/}	{/}	<+-	-	-
G	-	-	-	-	-	{/}	<+-	{/}	<+>	{/}	-	-
H	-	<+>	<+>	<+>	-	{/}	-	{/}	{/}	-	-	-

=> Species ID: Hafnia alvei <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Hafnia alvei	92	0.79	2.24	GN-ENT
2)	Riemerella anatipestifer	4	0.03	3.32	GN-NENT OXI+
3)	Proteus myxofaciens	2	0.02	3.55	GN-ENT
4)	Salmonella gp 1 (choleraesuis)	1	0.01	3.87	GN-ENT
5)	Providencia rettgeri	0	0.00	4.18	GN-ENT
6)	Shewanella putrefaciens A	0	0.00	4.23	GN-NENT OXI+
7)	Xenorhabdus nematophila	0	0.00	4.50	GN-ENT
8)	Aeromonas veronii/sobria DNA group 8	0	0.00	4.54	GN-NENT OXI+
9)	Pseudomonas fluorescens biotype C	0	0.00	4.70	GN-NENT
10)	Empedobacter brevis	0	0.00	4.77	GN-NENT OXI+
Other)					

Print Time = May 06 2003 10:42 Page 1 of 1 pages

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 02 2003 10:14
Parent File	: Original Data Record
Plate Number	: 7
Incubation Time	: 16-24
Sample Number	: 54
Strain Type	: GP-ALL
Strain Number	:
Strain Name	: Bacillus cereus/thuringiensis
Other	:
Data Input Mode	: Reader
590/750 Filters Used	: 6 / 5
Threshold Mode	: Automatic: Color: 59/120
Number +/b/- Reactions	: 36 / 14 / 46
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GP601.KID

Plate Type: GP2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	0	18	{ 69}	< 525>	49+	52	< 135>	6+	{ 92}	< 384>	{ 64}	3
B	{ 87}	11	-23	< 140>	< 326>	-7	58	-3	11	44	< 593>	0
C	-7	22	< 468>	< 242>	18	{ 105}	26	-37	29	-42	< 432>	24
D	< 460-	11	< 193>	< 193>	18	10	{ 96}	0	-16	-11	-20	{ 76}
E	11	< 615>	{ 96}	-17	29	{ 80}	< 159>	24	56	39	32	< 502>
F	22	{ 108}	< 282>	12	< 313>	< 422>	52	58	< 641>	0	28	{ 75}
G	11+	< 310>	< 619>	< 701>	< 478>	< 349>	< 372>	55	< 866>	-8	{ 82}	< 455>
H	< 792>	< 515>	< 375>	< 672>	< 774>	< 561>	< 423>	< 513>	{ 85}	47	{ 73}	< 326>

=> Species ID: Bacillus cereus/thuringiensis <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Bacillus cereus/thuringiensis	94	0.69	4.15	GP-ROD SB
2)	Staphylococcus delphini	5	0.03	5.14	GP-COC CAT+
3)	Staphylococcus aureus ss aureus	1	0.00	5.83	GP-COC CAT+
4)	Staphylococcus xylosus	0	0.00	6.95	GP-COC CAT+
5)	Staphylococcus chromogenes	0	0.00	7.09	GP-COC CAT+
6)	Staphylococcus sciuri	0	0.00	7.89	GP-COC CAT+
7)	Bacillus mycoides	0	0.00	8.51	GP-ROD SB
8)	Staphylococcus muscae	0	0.00	8.94	GP-COC CAT+
9)	Staphylococcus intermedius	0	0.00	9.34	GP-COC CAT+
10)	Staphylococcus lutrae	0	0.00	9.84	GP-COC CAT+
Other)					

Print Time = May 02 2003 10:16

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 06 2003 10:52
Parent File	: Original Data Record
Plate Number	: 13
Incubation Time	: 16-24
Sample Number	: 29
Strain Type	: GP-ALL
Strain Number	:
Strain Name	: Bacillus amyloliquefaciens
Other	:
Data Input Mode	: Manual
Number +/b/- Reactions	: 35 / 23 / 38
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GP601.KID

Plate Type: GP2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	- +	<+>	<+>	-	{/}	{/}	-	{/}	-	{/}
B	-	-	<+>	<+>	<+>	-	<+-	-	<+>	{/}	<+>	-
C	-	-	<+>	<+>	<+>	<+>	{/}	-	-	-	<+>	<+>
D	<+>	-	<+>	<+>	<+-	-	{/}	{/}	-	<+>	-	<+>
E	-	<+>	<+>	-	-	{/}	-	{/}	-	-	-	{/}
F	{/}	-	<+>	-	{/}	<+>	{/}	{/}	<+>	{/}	{/}	{/}
G	-	{/}	<+>	- +	<+>	<+>	-	{/}	{/}	{/}	{/}	<+>
H	<+>	<+>	<+>	<+>	<+>	-	-	-	-	-	-	<+>

=> Species ID: Bacillus amyloliquefaciens <=

Species	PROB	SIM	DIST	TYPE
=>1) Bacillus amyloliquefaciens	91	0.63	4.62	GP-ROD SB
2) Bacillus licheniformis	6	0.04	5.53	GP-ROD SB
3) Microbacterium maritypicum	2	0.01	5.91	GP-ROD
4) Staphylococcus lentus	0	0.00	6.47	GP-COC CAT+
5) Bacillus megaterium	0	0.00	6.82	GP-ROD SB
6) Staphylococcus sciuri ss rodentium	0	0.00	6.86	GP-COC CAT+
7) Staphylococcus sciuri	0	0.00	7.48	GP-COC CAT+
8) Bacillus subtilis(ATCC 6633)	0	0.00	7.55	GP-ROD SB
9) Microbacterium testaceum	0	0.00	7.88	GP-ROD
10) Microbacterium spp. (CDC.A-4)	0	0.00	7.93	GP-ROD
Other)				

Print Time = May 06 2003 10:54

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 21 2003 16:39
Parent File	: Original Data Record
Plate Number	: 34
Incubation Time	: 4-6
Sample Number	: 41
Strain Type	: GP-ALL
Strain Number	:
Strain Name	: Kocuria rosea
Other	:
Data Input Mode	: Manual
Number +/b/- Reactions	: 42 / 23 / 31
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GP601.KID

Plate Type: GP2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	<+>	<+>	<+>	-	<+>	<+>	<+>	-	-	-
B	{/}	-	-	<+>	<+>	-	<+>	-	<+>	<+>	<+>	<+>
C	<+>	-	<+>	<+>	<+>	<+>	{/}	<+>	{/}	-	<+-	-
D	<+>	-	<+>	<+>	<+>	-	{/}	-	-	<+>	{/}	<+>
E	-	<+>	<+>	-	{/}	<+>	{/}	{/}	{/}	-	{/}	<+>
F	-	-	<+>	-	<+>	- +	<+>	<+>	<+>	<+>	{/}	-	-
G	{/}	-	{/}	{/}	<+>	<+>	{/}	<+>	<+>	-	{/}	<+-
H	<+>	{/}	{/}	{/}	<+>	{/}	{/}	-	-	{/}	{/}	-

=> Species ID: Kocuria rosea <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Kocuria rosea	100	0.75	3.81	GP-COC CAT+
2)	Cellulosimicrobium cellulans	0	0.00	10.06	GP-ROD CAT+
3)	Arthrobacter histidinolovorans	0	0.00	10.71	GP-ROD CAT+
4)	Microbacterium spp. (CDC A-4)	0	0.00	10.77	GP-ROD
5)	Arthrobacter ilicis	0	0.00	10.88	GP-ROD CAT+
6)	Bacillus megaterium	0	0.00	11.55	GP-ROD SB
7)	Staphylococcus arlettae	0	0.00	11.57	GP-COC CAT+
8)	Gordonia rubropertinctus	0	0.00	11.79	GP-ROD CAT+
9)	Rhodococcus rhodochrous	0	0.00	11.83	GP-ROD CAT+
10)	Dermacoccus nishinomiyaensis	0	0.00	12.30	GP-COC CAT+
Other)					

Print Time = May 21 2003 16:40

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 03 2003 10:31
Parent File	: Original Data Record
Plate Number	: 8
Incubation Time	: 16-24
Sample Number	: 67
Strain Type	: GP-ALL
Strain Number	:
Strain Name	: Staphylococcus lentus
Other	:
Data Input Mode	: Manual
Number +/b/- Reactions	: 46 / 20 / 30
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GP601.KID

Plate Type: GP2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	-	<+>	<+>	-	<+>	{/}	{/}	<+>	<+>	<+>
B	-	-	<+>	<+>	<+>	-	<+>	-	<+>	<+>	<+>	{/}
C	- +	-	<+>	<+>	<+>	<+>	{/}	- +	-	-	<+>	<+-
D	<+>	-	<+>	<+>	<+>	-	<+>	<+>	-	<+>	-	<+>
E	-	<+>	<+>	-	{/}	{/}	-	{/}	{/}	{/}	{/}	{/}
F	-	-	{/}	-	<+>	<+>	<+>	-	<+>	<+>	<+>	<+>
G	-	- +	{/}	{/}	<+-	<+>	-	{/}	{/}	{/}	<+-	<+>
H	<+>	<+>	<+>	<+>	<+>	{/}	{/}	{/}	-	-	-	<+>

=> Species ID: Staphylococcus lentus <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Staphylococcus lentus	99	0.56	6.76	GP-COC CAT+
2)	Staphylococcus sciuri	1	0.01	8.24	GP-COC CAT+
3)	Bacillus licheniformis	0	0.00	9.16	GP-ROD SB
4)	Bacillus amyloliquefaciens	0	0.00	9.45	GP-ROD SB
5)	Bacillus subtilis	0	0.00	10.15	GP-ROD SB
6)	Staphylococcus pulvereri/vitulinus	0	0.00	11.00	GP-COC CAT+
7)	Arthrobacter woluwensis	0	0.00	11.13	GP-ROD CAT+
8)	Rhodococcus rhodochrous	0	0.00	11.44	GP-ROD CAT+
9)	Bacillus psychrosaccharolyticus	0	0.00	11.94	GP-ROD SB
10)	Staphylococcus sciuri ss rodentium	0	0.00	12.29	GP-COC CAT+
Other)					

Print Time = May 03 2003 10:32

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 03 2003 10:44
Parent File	: Original Data Record
Plate Number	: 9
Incubation Time	: 16-24
Sample Number	: 207
Strain Type	: GP-ALL
Strain Number	:
Strain Name	: Brevibacterium otitidis
Other	:
Data Input Mode	: Manual
Number +/b/- Reactions	: 61 / 24 / 11
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GP601.KID

Plate Type: GP2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	<+>	<+-	<+>	-	<+>	<+>	<+>	<+>	<+>	<+>
B	{/}	{/}	<+-	<+>	<+>	-	-	-	<+>	--	<+>	<+>
C	-	-	<+>	<+>	<+>	<+>	<+>	<+>	<+>	<+>	<+>	<+>
D	<+>	{/}	<+>	<+>	<+>	{/}	<+>	{/}	{/}	{/}	{/}	<+>
E	-	<+>	<+>	-	<+>	{/}	{/}	<+>	{/}	{/}	{/}	<+>
F	{/}	{/}	<+>	{/}	{/}	<+>	<+>	{/}	<+>	<+>	<+>	<+>
G	{/}	<+>	<+>	<+>	<+>	<+>	{/}	<+>	<+>	<+-	<+>	<+>
H	<+>	<+>	<+>	<+>	<+>	<+>	{/}	<+>	<+>	{/}	{/}	{/}

=> Species ID: Brevibacterium otitidis <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Brevibacterium otitidis	100	0.72	4.26	GP-ROD CAT+
2)	Gordonia rubropertinctus	0	0.00	7.29	GP-ROD CAT+
3)	Corynebacterium nitrilophilus	0	0.00	8.48	GP-ROD CAT+
4)	Brevibacterium mcbrellneri	0	0.00	9.00	GP-ROD CAT+
5)	Bacillus amyloliquefaciens	0	0.00	9.51	GP-ROD SB
6)	Deinococcus grandis	0	0.00	9.93	GP-COC CAT+
7)	Tsukamurella inchonensis	0	0.00	10.04	GP-ROD CAT+
8)	Rhodococcus australis	0	0.00	10.25	GP-ROD CAT+
9)	Microbacterium terregens	0	0.00	10.48	GP-ROD
10)	Dermacoccus nishinomiyaensis	0	0.00	10.57	GP-COC CAT+
Other)					

Print Time = May 03 2003 10:45

Page 1 of 1 pages

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 06 2003 10:59
Parent File	: Original Data Record
Plate Number	: 14
Incubation Time	: 16-24
Sample Number	: 240
Strain Type	: GP-ALL
Strain Number	:
Strain Name	: Bacillus subtilis
Other	:
Data Input Mode	: Manual
Number +/b/- Reactions	: 28 / 10 / 58
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GP601.KID

Plate Type: GP2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	-	<+>	-	-	- +	-	-	<+>	-	-
B	-	-	{/}	<+>	<+>	-	{/}	-	<+>	-	<+>	-
C	-	-	<+>	- +	<+>	<+>	-	-	-	-	<+>	<+>
D	<+>	-	<+>	<+>	-	-	{/}	{/}	-	<+>	-	<+>
E	-	<+>	<+>	-	-	-	-	-	-	-	-	-
F	-	-	{/}	-	- +	<+>	{/}	-	<+>	-	{/}	-
G	-	{/}	<+>	-	<+>	<+>	-	-	<+>	-	{/}	<+>
H	{/}	-	<+>	<+>	<+>	-	-	-	-	-	-	-

=> Species ID: Bacillus subtilis <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Bacillus subtilis	96	0.75	3.31	GP-ROD SB
2)	Bacillus amyloliquefaciens	4	0.03	4.42	GP-ROD SB
3)	Staphylococcus lentus	0	0.00	6.52	GP-COC CAT+
4)	Staphylococcus sciuri ss rodentium	0	0.00	6.61	GP-COC CAT+
5)	Bacillus megaterium	0	0.00	6.78	GP-ROD SB
6)	Staphylococcus pulvereri/vitulinus	0	0.00	6.96	GP-COC CAT+
7)	Staphylococcus sciuri	0	0.00	7.37	GP-COC CAT+
8)	Bacillus subtilis(ATCC 6633)	0	0.00	7.67	GP-ROD SB
9)	Staphylococcus arlettae	0	0.00	7.68	GP-COC CAT+
10)	Bacillus licheniformis	0	0.00	7.70	GP-ROD SB
Other)					

Print Time = May 06 2003 10:59 Page 1 of 1 pages

Program : Biolog MicroLog3 4.20
Save To File : C:\Biolog420\Rob.D4C
Unrestricted Access? : Yes
Read Time : May 07 2003 10:15
Parent File : Original Data Record
Plate Number : 19
Incubation Time : 16-24
Sample Number : 2031 Plate Type: GP2
Strain Type : GP-ALL
Strain Number :
Strain Name : Bacillus subtilis
Other :
Data Input Mode : Manual
Number +/b/- Reactions : 28 / 8 / 60
Database To Search : MicroLog
Data Base(s) Searched : C:\BIOLOG420\Databases\GP601.KID

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	-	<+>	-	-	- +	-	-	<+>	-	-
B	-	-	<+>	<+>	<+>	-	{/}	-	<+>	-	<+>	-
C	-	-	<+>	- +	<+>	<+>	-	-	-	-	<+>	<+>
D	{/}	-	<+>	<+>	-	-	<+>	{/}	-	<+>	-	<+>
E	-	<+>	<+>	-	<+-	-	-	-	-	-	-	-
F	-	-	-	-	- +	<+>	-	-	<+>	-	-	-
G	-	{/}	<+>	-	{/}	<+>	-	-	{/}	-	-	<+>
H	{/}	-	<+>	<+>	<+>	-	-	-	-	-	-	{/}

=> Species ID: Bacillus subtilis <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Bacillus subtilis	100	0.72	4.23	GP-ROD SB
2)	Bacillus amyloliquefaciens	0	0.00	6.24	GP-ROD SB
3)	Bacillus megaterium	0	0.00	6.79	GP-ROD SB
4)	Staphylococcus sciuri ss rodentium	0	0.00	6.81	GP-COC CAT+
5)	Macrococcus equipercicus	0	0.00	7.16	GP-COC CAT+
6)	Microbacterium saperdae	0	0.00	7.17	GP-ROD
7)	Staphylococcus pulvereri/vitulinus	0	0.00	7.38	GP-COC CAT+
8)	Staphylococcus lentus	0	0.00	7.60	GP-COC CAT+
9)	Bacillus licheniformis	0	0.00	7.70	GP-ROD SB
10)	Macrococcus bovicus	0	0.00	8.04	GP-COC CAT+
Other)					

Print Time = May 07 2003 10:16 Page 1 of 1 pages

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 07 2003 10:21
Parent File	: Original Data Record
Plate Number	: 20
Incubation Time	: 16-24
Sample Number	: 2033
Strain Type	: GP-ALL
Strain Number	:
Strain Name	: Bacillus amyloliquefaciens
Other	:
Data Input Mode	: Reader
590/750 Filters Used	: 6 / 5
Threshold Mode	: Automatic: Color: 55/153
Number +/b/- Reactions	: 36 / 8 / 52
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GP601.KID

Plate Type: GP2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	0	5	-1+	< 493>	13	-6	4+	51+	18	< 315>	{ 79}	< 173>
B	17	-1	< 224>	< 332>	< 403>	-4	< 204-	-4	< 337>	{ 75}	< 399>	6
C	3	-2	< 331>	{ 149}	< 340>	< 319>	23	11	7	2	< 231>	< 317>
D	< 202>	-4	< 393>	< 335>	< 250-	-1	39	{ 142}	1	< 317>	4	< 450>
E	-1	< 443>	< 267>	11	8	1	-2	5	19	-7	7	29
F	4	9	{ 102}	-5	{ 82}	< 227>	{ 124}	-5	< 456>	1	{ 124}	0
G	3	< 302>	< 232>	5+	< 190>	< 305>	-1	12	< 282>	-3	18	< 343>
H	< 209>	< 279>	< 359>	< 277>	< 419>	-8	0	8	-1	-3	-1	< 420>

=> Species ID: Bacillus amyloliquefaciens <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Bacillus amyloliquefaciens	97	0.55	6.70	GP-ROD SB
2)	Staphylococcus lentus	2	0.01	7.97	GP-COC CAT+
3)	Staphylococcus sciuri	1	0.00	8.29	GP-COC CAT+
4)	Bacillus subtilis(ATCC 6633)	0	0.00	9.04	GP-ROD SB
5)	Bacillus licheniformis	0	0.00	9.37	GP-ROD SB
6)	Microbacterium testaceum	0	0.00	9.48	GP-ROD
7)	Staphylococcus sciuri ss rodentium	0	0.00	9.78	GP-COC CAT+
8)	Microbacterium maritypicum	0	0.00	10.70	GP-ROD
9)	Bacillus subtilis	0	0.00	10.70	GP-ROD SB
10)	Bacillus pumilus	0	0.00	11.00	GP-ROD SB
Other)					

Print Time = May 07 2003 10:22

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 03 2003 10:55
Parent File	: Original Data Record
Plate Number	: 10
Incubation Time	: 16-24
Sample Number	: 223
Strain Type	: GP-ALL
Strain Number	:
Strain Name	: Bacillus licheniformis
Other	:
Data Input Mode	: Manual
Number +/b/- Reactions	: 54 / 19 / 23
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GP601.KID

Plate Type: GP2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	{/}	{/}	<+>	<+>	-	<+>	<+-	{/}	<+>	<+>	<+>
B	-	-	<+>	<+>	<+>	-	{/}	-	<+>	<+>	<+>	<+>
C	{/}	-	<+>	{/}	<+>	<+>	{/}	-	-	-	<+>	<+>
D	<+>	-	<+>	<+>	<+>	-	<+>	{/}	-	<+>	{/}	<+>
E	{/}	<+>	<+>	-	{/}	-	-	{/}	{/}	<+-	<+>	<+>
F	<+-	{/}	{/}	-	<+>	<+>	{/}	-	<+>	<+>	<+>	<+-
G	-	<+>	<+>	<+>	<+>	<+>	-	<+>	<+>	<+-	<+>	<+>
H	<+>	<+>	<+>	<+>	<+>	-	-	{/}	{/}	-	{/}	<+>

=> Species ID: Bacillus licheniformis <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Bacillus licheniformis	100	0.58	6.47	GP-ROD SB
2)	Bacillus amyloliquefaciens	0	0.00	8.66	GP-ROD SB
3)	Staphylococcus lentus	0	0.00	12.63	GP-COC CAT+
4)	Bacillus subtilis	0	0.00	13.32	GP-ROD SB
5)	Bacillus megaterium	0	0.00	13.44	GP-ROD SB
6)	Staphylococcus pulvereri/vitulinus	0	0.00	13.56	GP-COC CAT+
7)	Arthrobacter woluwensis	0	0.00	14.21	GP-ROD CAT+
8)	Staphylococcus sciuri	0	0.00	14.26	GP-COC CAT+
9)	Brevibacterium otitidis	0	0.00	14.86	GP-ROD CAT+
10)	Gordonia rubropertinctus	0	0.00	14.87	GP-ROD CAT+
Other)					

Print Time = May 03 2003 10:56

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 06 2003 11:06
Parent File	: Original Data Record
Plate Number	: 15
Incubation Time	: 16-24
Sample Number	: 248
Strain Type	: GP-ALL
Strain Number	:
Strain Name	: Bacillus amyloliquefaciens
Other	:
Data Input Mode	: Manual
Number +/b/- Reactions	: 37 / 13 / 46
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GP601.KID

Plate Type: GP2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	- +	<+>	-	-	- +	<+>	<+>	<+>	-	- +
B	-	-	<+>	<+>	<+>	-	{/}	-	<+>	{/}	<+>	-
C	-	-	<+>	<+>	<+>	<+>	-	-	-	-	<+>	<+>
D	<+>	-	<+>	<+>	{/}	-	{/}	{/}	-	<+>	-	<+>
E	-	<+>	<+>	-	-	-	-	{/}	-	-	-	-
F	-	-	<+>	-	{/}	<+>	{/}	-	<+>	-	{/}	{/}
G	-	<+>	<+>	{/}	<+>	<+>	-	{/}	<+>	-	{/}	<+>
H	<+>	<+>	<+>	<+>	<+>	-	-	-	-	-	-	<+>

=> Species ID: Bacillus amyloliquefaciens <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Bacillus amyloliquefaciens	100	0.74	3.86	GP-ROD SB
2)	Bacillus licheniformis	0	0.00	6.66	GP-ROD SB
3)	Staphylococcus sciuri	0	0.00	7.40	GP-COC CAT+
4)	Staphylococcus lentus	0	0.00	7.85	GP-COC CAT+
5)	Bacillus subtilis	0	0.00	8.24	GP-ROD SB
6)	Microbacterium testaceum	0	0.00	8.30	GP-ROD
7)	Staphylococcus sciuri ss rodentium	0	0.00	8.51	GP-COC CAT+
8)	Bacillus subtilis(ATCC 6633)	0	0.00	8.97	GP-ROD SB
9)	Bacillus pumilus	0	0.00	10.00	GP-ROD SB
10)	Bacillus megaterium	0	0.00	10.32	GP-ROD SB
Other)					

Print Time = May 06 2003 11:08

Page 1 of 1 pages

Program	: Biolog MicroLog3 4.20
Worksheet File	: C:\Biolog420\ROB.W4C
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 01 2003 09:35
Parent File	: Original Data Record
Plate Number	: 3
Incubation Time	: 16-24
Sample Number	: 2056
Strain Type	: GN-ALL
Strain Number	:
Strain Name	: Pseudomonas corrugata
Other	:
Data Input Mode	: Reader
590/750 Filters Used	: 6 / 5
Threshold Mode	: Automatic: Color: 93/178
Number +/b/- Reactions	: 57 / 8 / 31
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GN601.KID

Plate Type: GN2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	0	17	89	{145}	<344>	<316>	5	<301>	26	<680>	<379>	7
B	43	<413>	67	<593>	42	<487>	<337>	7	15	52	<434>	<406>
C	49	{110}	<269>	14	74	<445-	<399>	<411>	21	19	<263>	<318>
D	<486>	<712>	<816>	<242>	<365>	26+	<478>	<354>	<274>	<197>	<450>	6
E	<414>	68	{158}	<542>	{151}	<481>	<515>	7+	{162}	<709>	6	<506>
F	<345>	<400>	31	<316>	<456>	<400>	<404>	<481>	<568>	<674>	32	<323>
G	<247>	{149}	<315>	{126}	48	<405>	<479>	<211-	<327>	<247>	<210>	<376>
H	<382>	<572>	<314>	8	-2	55	<223>	{167}	<299>	90	13	14

=> Species ID: Pseudomonas corrugata <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Pseudomonas corrugata	89	0.64	4.31	GN-NENT OXI+
2)	Pseudomonas marginalis	10	0.07	5.06	GN-NENT OXI+
3)	Pseudomonas synxantha	0	0.00	6.10	GN-NENT OXI+
4)	Pseudomonas fluorescens biotype F	0	0.00	6.28	GN-NENT
5)	Pseudomonas fluorescens biotype A	0	0.00	6.34	GN-NENT
6)	Pseudomonas fluorescens	0	0.00	7.44	GN-NENT
7)	Pseudomonas chlororaphis (fluor. biotype D)	0	0.00	8.00	GN-NENT OXI+
8)	Pseudomonas aurantiaca	0	0.00	8.17	GN-NENT OXI+
9)	Pseudomonas syringae pv papulans	0	0.00	10.52	GN-NENT
10)	Pseudomonas syringae pv primulae	0	0.00	10.58	GN-NENT OXI-
Other)					

Print Time = May 01 2003 09:35 Page 1 of 1 pages

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 03 2003 11:07
Parent File	: Original Data Record
Plate Number	: 11
Incubation Time	: 16-24
Sample Number	: 226
Strain Type	: GP-ALL
Strain Number	:
Strain Name	: Bacillus amyloliquefaciens
Other	:
Data Input Mode	: Manual
Number +/b/- Reactions	: 31 / 24 / 41
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GP601.KID

Plate Type: GP2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	- +	<+>	-	-	{/}	<+>	<+>	<+>	{/}	{/}
B	-	-	<+>	<+>	<+>	-	{/}	-	<+>	-	<+>	-
C	{/}	-	<+>	{/}	<+>	<+>	-	-	-	-	<+>	<+>
D	<+>	-	<+>	<+>	{/}	-	{/}	{/}	-	<+>	-	<+>
E	-	<+>	<+>	-	-	-	-	{/}	{/}	-	-	-
F	-	-	{/}	-	{/}	<+>	{/}	-	<+>	-	{/}	{/}
G	-	{/}	{/}	{/}	{/}	<+>	-	{/}	{/}	{/}	{/}	<+>
H	<+>	<+>	<+>	<+>	<+>	-	-	-	-	-	-	<+>

=> Species ID: Bacillus amyloliquefaciens <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Bacillus amyloliquefaciens	100	0.89	1.62	GP-ROD SB
2)	Staphylococcus sciuri ss rodentium	0	0.00	4.03	GP-COC CAT+
3)	Bacillus subtilis	0	0.00	4.16	GP-ROD SB
4)	Staphylococcus lentus	0	0.00	4.96	GP-COC CAT+
5)	Bacillus subtilis(ATCC 6633)	0	0.00	5.67	GP-ROD SB
6)	Bacillus licheniformis	0	0.00	6.20	GP-ROD SB
7)	Staphylococcus sciuri	0	0.00	6.26	GP-COC CAT+
8)	Microbacterium maritypicum	0	0.00	6.55	GP-ROD
9)	Staphylococcus arlettae	0	0.00	6.86	GP-COC CAT+
10)	Microbacterium saperdae	0	0.00	7.04	GP-ROD
Other)					

Print Time = May 03 2003 11:07

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 02 2003 10:05
Parent File	: Original Data Record
Plate Number	: 5
Incubation Time	: 16-24
Sample Number	: 230
Strain Type	: GN-ALL
Strain Number	:
Strain Name	: Mannheimia haemolytica
Other	:
Data Input Mode	: Manual
Number +/b/- Reactions	: 18 / 14 / 64
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GN601.KID

Plate Type: GN2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	<+>	-	-	-	-	- +	-	-	-	<+>
B	-	<+>	-	{/}	<+>	<+>	-	-	-	<+>	<+>	<+>
C	-	-	<+>	<+>	-	<+>	<+>	<+>	{/}	-	<+>	-
D	-	-	-	- +	-	-	<+-	-	-	-	-	-
E	-	-	- +	-	-	-	-	-	-	-	-	-
F	{/}	-	-	-	{/}	{/}	{/}	{/}	{/}	{/}	-	-
G	-	-	-	-	-	{/}	{/}	-	{/}	-	-	-
H	-	<+>	<+>	<+>	-	-	-	-	{/}	{/}	-	-

=> Species ID: Mannheimia haemolytica <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Mannheimia haemolytica	82	0.58	4.52	GN-NENT OXI+
2)	Pasteurella multocida ss multocida	7	0.05	5.35	GN-NENT OXI+
3)	Enterobacter cloacae	3	0.02	5.62	GN-ENT
4)	Pasteurella trehalosi	3	0.02	5.71	GN-NENT OXI+
5)	Serratia odorifera	1	0.01	6.02	GN-ENT
6)	Yersinia bercovieri	1	0.01	6.06	GN-ENT
7)	Rahnella aquatilis	1	0.01	6.11	GN-ENT
8)	Actinobacillus hominis	1	0.01	6.12	GN-FAS OXI+
9)	Vibrio diazotrophicus	1	0.00	6.21	GN-NENT OXI+
10)	Kluyvera ascorbata	1	0.00	6.24	GN-ENT
Other)					

Print Time = May 02 2003 10:06

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 06 2003 11:16
Parent File	: Original Data Record
Plate Number	: 16
Incubation Time	: 16-24
Sample Number	: 265
Strain Type	: GP-ALL
Strain Number	:
Strain Name	: Bacillus amyloliquefaciens
Other	:
Data Input Mode	: Manual
Number +/b/- Reactions	: 31 / 12 / 53
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GP601.KID

Plate Type: GP2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	- +	<+>	-	-	- +	<+>	{/}	<+>	-	{/}
B	-	-	<+>	<+>	<+>	-	{/}	-	<+>	-	<+>	-
C	-	-	<+>	{/}	<+>	<+>	-	-	-	-	<+>	<+>
D	<+>	-	<+>	<+>	-	-	{/}	{/}	-	<+>	-	<+>
E	-	<+>	<+>	-	{/}	-	-	-	-	-	-	-
F	-	-	{/}	-	{/}	<+>	{/}	-	<+>	{/}	-	-
G	-	- +	<+>	- +	{/}	<+>	-	-	-	-	-	<+>
H	<+>	<+>	<+>	<+>	<+>	-	-	-	-	-	-	<+>

=> Species ID: Bacillus amyloliquefaciens <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Bacillus amyloliquefaciens	98	0.71	4.09	GP-ROD SB
2)	Bacillus subtilis	1	0.01	5.58	GP-ROD SB
3)	Bacillus subtilis(ATCC 6633)	1	0.01	5.67	GP-ROD SB
4)	Microbacterium saperdae	0	0.00	7.13	GP-ROD
5)	Staphylococcus sciuri ss rodentium	0	0.00	7.37	GP-COC CAT+
6)	Bacillus licheniformis	0	0.00	8.49	GP-ROD SB
7)	Cellulomonas hominis (CDC.A-3)	0	0.00	8.67	GP-ROD CAT+
8)	Staphylococcus lentus	0	0.00	8.72	GP-COC CAT+
9)	Exiguobacterium acetylicum	0	0.00	8.84	GP-ROD CAT+
10)	Cellulosimicrobium cellulans	0	0.00	9.13	GP-ROD CAT+
Other)					

Print Time = May 06 2003 11:29

Program : Biolog MicroLog3 4.20
Save To File : C:\Biolog420\Rob.D4C
Unrestricted Access? : Yes
Read Time : May 07 2003 10:04
Parent File : Original Data Record
Plate Number : 17
Incubation Time : 16-24
Sample Number : 266 Plate Type: GP2
Strain Type : GP-ALL
Strain Number :
Strain Name : Bacillus licheniformis
Other :
Data Input Mode : Manual
Number +/b/- Reactions : 37 / 14 / 45
Database To Search : MicroLog
Data Base(s) Searched : C:\BIOLOG420\Databases\GP601.KID

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	- +	{/}	<+>	{/}	-	<+>	-	-	<+>	-	<+-
B	-	-	{/}	<+>	<+>	-	{/}	-	<+>	<+>	<+>	-
C	-	-	<+>	- +	<+>	<+>	-	-	-	-	<+>	<+>
D	{/}	-	<+>	<+>	{/}	-	{/}	{/}	-	<+-	-	<+>
E	- +	<+>	<+>	-	-	{/}	-	-	-	-	-	-
F	-	-	<+>	-	<+>	<+>	{/}	{/}	<+>	{/}	{/}	{/}
G	-	<+>	<+>	- +	<+>	<+>	-	-	<+>	-	<+>	<+>
H	<+>	<+>	<+>	<+>	<+>	-	-	-	-	-	-	<+>

=> Species ID: Bacillus licheniformis <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Bacillus licheniformis	96	0.66	4.72	GP-ROD SB
2)	Bacillus amyloliquefaciens	4	0.02	5.81	GP-ROD SB
3)	Bacillus subtilis(ATCC 6633)	0	0.00	6.97	GP-ROD SB
4)	Staphylococcus sciuri	0	0.00	7.85	GP-COC CAT+
5)	Bacillus subtilis	0	0.00	8.64	GP-ROD SB
6)	Staphylococcus sciuri ss rodentium	0	0.00	9.11	GP-COC CAT+
7)	Microbacterium spp. (CDC A-4)	0	0.00	9.84	GP-ROD
8)	Staphylococcus lentus	0	0.00	10.00	GP-COC CAT+
9)	Bacillus pumilus	0	0.00	10.00	GP-ROD SB
10)	Microbacterium testaceum	0	0.00	10.65	GP-ROD
Other)					

Print Time = May 07 2003 10:04 Page 1 of 1 pages

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 07 2003 10:09
Parent File	: Original Data Record
Plate Number	: 18
Incubation Time	: 16-24
Sample Number	: 268
Strain Type	: GP-ALL
Strain Number	:
Strain Name	: Bacillus amyloliquefaciens
Other	:
Data Input Mode	: Reader
590/750 Filters Used	: 6 / 5
Threshold Mode	: Automatic: Color: 53/145
Number +/b/- Reactions	: 37 / 24 / 35
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GP601.KID

Plate Type: GP2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	0	1	15+	< 364>	{ 74}	-16	{ 78}	{ 140}	{ 64}	< 269>	28	{ 95}
B	16	1	{ 89}	< 253>	< 331>	-6	< 209-	-12	< 299>	{ 107}	< 319>	28
C	0	10	< 359>	{ 133}	< 254>	< 373>	{ 65}	-3	4	13	< 331>	< 310>
D	< 349>	-8	< 388>	< 369>	{ 99}	36	{ 70}	{ 82}	29	< 313>	17	< 320>
E	27	< 378>	< 334>	22	{ 118}	{ 67}	-3	{ 97}	{ 56}	-6	{ 91}	{ 68}
F	{ 56}	38	< 181>	-2	{ 125}	< 323>	< 151>	-12	< 290>	{ 114}	< 159>	{ 133}
G	2	< 277>	< 239>	38+	< 197>	< 304>	-3	{ 74}	< 232>	{ 119}	< 213-	< 326>
H	< 300>	< 228>	< 339>	< 369>	< 398>	-4	15	39	26	29	44	< 340>

=> Species ID: Bacillus amyloliquefaciens <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Bacillus amyloliquefaciens	100	0.71	4.31	GP-ROD SB
2)	Bacillus licheniformis	0	0.00	6.33	GP-ROD SB
3)	Staphylococcus lentus	0	0.00	6.60	GP-COC CAT+
4)	Bacillus megaterium	0	0.00	7.32	GP-ROD SB
5)	Microbacterium testaceum	0	0.00	7.42	GP-ROD
6)	Staphylococcus sciuri	0	0.00	7.45	GP-COC CAT+
7)	Bacillus subtilis	0	0.00	8.20	GP-ROD SB
8)	Staphylococcus pulvereri/vitulinus	0	0.00	8.23	GP-COC CAT+
9)	Staphylococcus pasteuri	0	0.00	8.27	GP-COC CAT+
10)	Staphylococcus sciuri ss rodentium	0	0.00	8.91	GP-COC CAT+
Other)					

Print Time = May 07 2003 10:09

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 07 2003 10:25
Parent File	: Original Data Record
Plate Number	: 21
Incubation Time	: 16-24
Sample Number	: 2090
Strain Type	: GP-ALL
Strain Number	:
Strain Name	: Bacillus subtilis
Other	:
Data Input Mode	: Reader
590/750 Filters Used	: 6 / 5
Threshold Mode	: Automatic: Color: 31/65
Number +/b/- Reactions	: 31 / 10 / 55
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GP601.KID

Plate Type: GP2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	0	2	7	< 172>	23	-0	6+	{ 58}	{ 47}	< 126>	{ 51}	{ 54}
B	8	0	{ 57}	< 150>	< 151>	0	< 84-	0	< 200>	20	< 146>	-3
C	14	-3	< 168>	{ 37}	< 152>	< 162>	17	17	6	-0	< 180>	< 159>
D	< 135>	-1	< 114>	< 162>	< 77-	1	{ 40}	< 75>	2	< 152>	4	< 190>
E	3	< 181>	< 226>	7	16	9	1	18	18	-0	8	{ 35}
F	5	10	31	-1	3+	< 158>	22	2	< 135>	1	7	3
G	8	29	< 152>	4	< 91>	< 119>	2	8	{ 47}	-1	11	< 185>
H	{ 49}	29	< 180>	< 135>	< 166>	-4	3	-3	6	1	7	< 146-

=> Species ID: Bacillus subtilis <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Bacillus subtilis	99	0.65	5.16	GP-ROD SB
2)	Staphylococcus lentus	1	0.01	6.60	GP-COC CAT+
3)	Bacillus amyloliquefaciens	0	0.00	7.79	GP-ROD SB
4)	Bacillus megaterium	0	0.00	8.14	GP-ROD SB
5)	Staphylococcus sciuri ss rodentium	0	0.00	8.17	GP-COC CAT+
6)	Microbacterium maritypicum	0	0.00	8.62	GP-ROD
7)	Microbacterium saperdae	0	0.00	9.10	GP-ROD
8)	Bacillus licheniformis	0	0.00	9.67	GP-ROD SB
9)	Staphylococcus sciuri	0	0.00	9.85	GP-COC CAT+
10)	Enterococcus solitarius	0	0.00	10.00	GP-COC CAT-
Other)					

Print Time = May 07 2003 10:26

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 08 2003 11:04
Parent File	: Original Data Record
Plate Number	: 25
Incubation Time	: 16-24
Sample Number	: 3055
Strain Type	: GP-ALL
Strain Number	:
Strain Name	: Staphylococcus sciuri
Other	:
Data Input Mode	: Manual
Number +/b/- Reactions	: 47 / 25 / 24
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GP601.KID

Plate Type: GP2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	-	<+>	<+>	-	<+>	{/}	{/}	<+>	<+>	<+-
B	<+>	-	<+>	<+>	<+>	-	<+>	-	<+>	<+>	<+>	{/}
C	-	-	<+>	<+>	<+>	<+>	{/}	-	-	-	<+>	<+>
D	<+>	-	<+>	<+>	<+-	-	<+>	<+>	-	<+>	{/}	<+>
E	-	<+>	<+>	-	<+>	-	-	-	{/}	-	{/}	{/}
F	{/}	{/}	{/}	-	<+>	<+>	{/}	-	<+>	{/}	<+>	{/}
G	{/}	{/}	<+>	{/}	<+>	<+>	{/}	{/}	<+>	{/}	<+-	<+>
H	<+>	<+>	<+>	<+>	<+>	{/}	{/}	{/}	{/}	-	{/}	<+>

=> Species ID: Staphylococcus sciuri <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Staphylococcus sciuri	100	0.79	3.08	GP-COC CAT+
2)	Staphylococcus sciuri ss rodentium	0	0.00	5.62	GP-COC CAT+
3)	Staphylococcus lentus	0	0.00	6.17	GP-COC CAT+
4)	Staphylococcus pulvereri/vitulinus	0	0.00	6.31	GP-COC CAT+
5)	Bacillus amyloliquefaciens	0	0.00	7.95	GP-ROD SB
6)	Bacillus licheniformis	0	0.00	8.03	GP-ROD SB
7)	Microbacterium testaceum	0	0.00	8.46	GP-ROD
8)	Exiguobacterium acetylicum	0	0.00	9.02	GP-ROD CAT+
9)	Macrococcus equipercicus	0	0.00	9.30	GP-COC CAT+
10)	Bacillus subtilis	0	0.00	9.91	GP-ROD SB
Other)					

Print Time = May 08 2003 11:04

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 08 2003 11:10
Parent File	: Original Data Record
Plate Number	: 26
Incubation Time	: 16-24
Sample Number	: 3057
Strain Type	: GP-ALL
Strain Number	:
Strain Name	: Bacillus subtilis
Other	:
Data Input Mode	: Reader
590/750 Filters Used	: 6 / 5
Threshold Mode	: Automatic: Color: 31/73
Number +/b/- Reactions	: 28 / 11 / 57
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GP601.KID

Plate Type: GP2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	0	1	2	< 139>	24	1	-1+	{ 39}	{ 34}	< 107>	{ 41}	{ 70}
B	14	5	< 75>	< 179>	< 178>	2	{ 40}	-7	< 226>	< 89>	< 180>	-4
C	-1	2	< 146>	21+	< 116>	< 123>	18	0	-2	3	< 196>	< 121>
D	< 89>	0	< 110>	< 136>	< 84-	4	{ 52}	{ 65}	6	< 173>	27	< 283>
E	-1	< 238>	< 123>	10	16	-8	-1	22	7	-1	1	21
F	17	18	17	-6	11+	< 94>	30	-11	< 151>	13	22	12
G	13	9	{ 63}	13	{ 64}	< 101>	3	18	{ 32}	1	5	< 119>
H	24	15	{ 64}	< 107>	< 111>	-5	6	14	2	5	5	< 118-

=> Species ID: Bacillus subtilis <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Bacillus subtilis	98	0.64	5.29	GP-ROD SB
2)	Staphylococcus sciuri ss rodentium	2	0.01	6.61	GP-COC CAT+
3)	Bacillus amyloliquefaciens	0	0.00	7.30	GP-ROD SB
4)	Enterococcus solitarius	0	0.00	7.50	GP-COC CAT-
5)	Staphylococcus lentus	0	0.00	8.12	GP-COC CAT+
6)	Microbacterium arborescens	0	0.00	8.24	GP-ROD
7)	Bacillus licheniformis	0	0.00	8.43	GP-ROD SB
8)	Staphylococcus sciuri	0	0.00	8.46	GP-COC CAT+
9)	Bacillus subtilis(ATCC 6633)	0	0.00	8.67	GP-ROD SB
10)	Bacillus megaterium	0	0.00	9.47	GP-ROD SB
Other)					

Print Time = May 08 2003 11:10

Program : Biolog MicroLog3 4.20
Save To File : C:\Biolog420\Rob.D4C
Unrestricted Access? : Yes
Read Time : May 15 2003 22:49
Parent File : Original Data Record
Plate Number : 32
Incubation Time : 4-6
Sample Number : 3060 Plate Type: GP2
Strain Type : GP-ALL
Strain Number :
Strain Name : Staphylococcus sciuri
Other :
Data Input Mode : Manual
Number +/b/- Reactions : 37 / 18 / 41
Database To Search : MicroLog
Data Base(s) Searched : C:\BIOLOG420\Databases\GP601.KID

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	-	<+>	-	-	{/}	{/}	{/}	<+>	{/}	{/}
B	-	-	<+>	<+>	<+>	-	-	-	<+>	{/}	<+>	{/}
C	-	-	<+>	-	<+>	<+>	-	-	-	-	<+>	<+>
D	<+>	-	<+>	<+>	-	-	{/}	<+>	-	{/}	-	<+>
E	-	<+>	<+>	-	-	<+>	-	-	-	-	-	<+>
F	-	-	{/}	-	<+>	- +	-	-	<+>	-	{/}	{/}
G	{/}	<+>	<+>	<+>	<+>	<+>	<+>	{/}	<+>	-	{/}	<+>
H	<+>	<+>	<+>	<+>	<+>	-	{/}	{/}	-	-	{/}	<+>

=> Species ID: Staphylococcus sciuri <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Staphylococcus sciuri	100	0.84	2.44	GP-COC CAT+
2)	Staphylococcus sciuri ss rodentium	0	0.00	4.70	GP-COC CAT+
3)	Exiguobacterium acetylicum	0	0.00	6.25	GP-ROD CAT+
4)	Micrococcus equipercicus	0	0.00	6.52	GP-COC CAT+
5)	Bacillus amyloliquefaciens	0	0.00	6.70	GP-ROD SB
6)	Bacillus licheniformis	0	0.00	7.23	GP-ROD SB
7)	Staphylococcus pulvereri/vitulinus	0	0.00	7.72	GP-COC CAT+
8)	Bacillus maroccanus	0	0.00	8.94	GP-ROD SB
9)	Staphylococcus caprae	0	0.00	9.24	GP-COC CAT+
10)	Staphylococcus xylosus	0	0.00	9.25	GP-COC CAT+
Other)					

Print Time = May 15 2003 22:50 Page 1 of 1 pages

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob D4C
Unrestricted Access?	: Yes
Read Time	: May 02 2003 10:12
Parent File	: Original Data Record
Plate Number	: 6
Incubation Time	: 16-24
Sample Number	: 3073
Strain Type	: GN-ALL
Strain Number	:
Strain Name	: Pseudomonas corrugata
Other	:
Data Input Mode	: Reader
590/750 Filters Used	: 6 / 5
Threshold Mode	: Automatic: Color: 102/169
Number +/b/- Reactions	: 54 / 9 / 33
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GN601.KID

Plate Type: GN2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	0	13	91	{ 102}	< 315>	< 306>	5	{ 156}	12	< 648>	< 344>	-1
B	36	< 414>	24	< 504>	23	< 436>	< 309>	5	11	36	< 453>	< 348>
C	35	75	< 241>	34	45	< 354-	< 358>	< 392>	20	41	< 228>	< 178>
D	< 278>	< 464>	< 669>	< 236>	< 344>	23+	< 473>	< 350>	{ 139}	{ 139}	< 336>	7
E	< 476>	36	51	< 440>	99	< 361>	< 353>	< 278>	{ 132}	< 587>	-2	< 338>
F	< 419>	< 372>	18	< 203>	< 366>	< 302>	< 352>	< 377>	< 479>	< 417>	22	< 240>
G	< 200>	< 176>	< 231>	62	48	< 319>	< 331>	{ 152}	< 276>	{ 150}	< 193>	< 267>
H	< 352>	< 337>	< 229>	-2	8	35	{ 112}	{ 122}	< 302>	< 300-	4	1

=> Species ID: Pseudomonas corrugata <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Pseudomonas corrugata	98	0.79	3.00	GN-NENT OXI+
2)	Pseudomonas fluorescens biotype F	1	0.01	4.58	GN-NENT
3)	Pseudomonas chlororaphis (fluor. biotype D)	0	0.00	4.95	GN-NENT OXI+
4)	Pseudomonas synxantha	0	0.00	5.10	GN-NENT OXI+
5)	Pseudomonas fluorescens biotype A	0	0.00	5.69	GN-NENT
6)	Pseudomonas aurantiaca	0	0.00	6.38	GN-NENT OXI+
7)	Pseudomonas marginalis	0	0.00	6.81	GN-NENT OXI+
8)	Pseudomonas fluorescens	0	0.00	6.83	GN-NENT
9)	Pseudomonas asplenii	0	0.00	7.44	GN-NENT OXI+
10)	Pseudomonas syringae pv apii	0	0.00	8.01	GN-NENT
Other)					

Print Time = May 02 2003 10:12

Program : Biolog MicroLog3 4.20
Save To File : C:\Biolog420\Rob.D4C
Unrestricted Access? : Yes
Read Time : May 01 2003 09:42
Parent File : Original Data Record
Plate Number : 4
Incubation Time : 16-24
Sample Number : 3008 Plate Type: GN2
Strain Type : GN-ALL
Strain Number :
Strain Name : Pseudomonas fluorescens
Other :
Data Input Mode : Manual
Number +/b/- Reactions : 40 / 14 / 42
Database To Search : MicroLog
Data Base(s) Searched : C:\BIOLOG420\Databases\GN601.KID

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	-	-	{/}	{/}	-	-	<+>	<+>	<+>	-
B	-	{/}	-	{/}	-	<+>	<+>	-	-	-	<+>	<+>
C	-	-	{/}	-	-	<+>	<+>	- +	-	-	{/}	{/}
D	<+>	<+>	<+>	{/}	-	{/}	<+>	<+>	<+>	-	<+>	-
E	-	- +	-	<+>	-	<+>	<+>	<+>	<+>	<+>	-	<+>
F	<+>	<+>	{/}	{/}	<+>	<+>	<+>	<+>	<+>	<+>	-	-
G	- +	- +	-	-	-	<+>	<+>	-	<+>	{/}	{/}	<+>
H	<+>	<+>	<+>	-	-	-	<+>	-	<+>	{/}	-	-

=> Species ID: Pseudomonas fluorescens <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Pseudomonas fluorescens	85	0.55	5.43	GN-NENT
2)	Pseudomonas fluorescens biotype A	10	0.06	6.17	GN-NENT
3)	Pseudomonas syringae pv lachrymans	3	0.02	6.57	GN-NENT OXI-
4)	Pseudomonas syringae pv papulans	1	0.00	7.02	GN-NENT
5)	Pseudomonas syringae pv apii	1	0.00	7.12	GN-NENT
6)	Pseudomonas synxantha	0	0.00	7.50	GN-NENT OXI+
7)	Pseudomonas viridiflava (syringae)	0	0.00	7.60	GN-NENT OXI-
8)	Pseudomonas tolaasii	0	0.00	7.72	GN-NENT OXI+
9)	Pseudomonas syringae pv antirrhini	0	0.00	7.96	GN-NENT OXI-
10)	Pseudomonas fluorescens biotype F	0	0.00	8.30	GN-NENT
Other)					

Print Time = May 01 2003 09:42 Page 1 of 1 pages

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 08 2003 10:38
Parent File	: Original Data Record
Plate Number	: 22
Incubation Time	: 16-24
Sample Number	: 3020 Plate Type: GP2
Strain Type	: GP-ALL
Strain Number	:
Strain Name	: Micrococcus equipercicus
Other	:
Data Input Mode	: Manual
Number +/b/- Reactions	: 42 / 16 / 38
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GP601.KID

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	-	<+>	{/}	-	{/}	{/}	{/}	<+>	{/}	<+-
B	{/}	-	<+>	<+>	<+>	-	{/}	-	<+>	<+>	<+>	{/}
C	-	-	<+>	- +	<+>	<+>	{/}	-	-	-	<+>	<+-
D	<+>	-	<+>	<+>	{/}	-	<+>	<+>	-	<+>	-	<+>
E	-	<+>	<+>	-	{/}	<+>	-	-	-	-	-	<+>
F	{/}	{/}	{/}	-	<+>	<+>	-	{/}	<+>	-	-	-
G	- +	<+>	<+>	<+>	<+>	<+>	<+>	-	<+>	-	{/}	<+>
H	<+>	<+>	<+>	<+>	<+>	-	-	-	-	-	-	<+-

=> Species ID: Micrococcus equipercicus <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Micrococcus equipercicus	90	0.60	5.11	GP-COC CAT+
2)	Staphylococcus sciuri	9	0.05	5.91	GP-COC CAT+
3)	Bacillus licheniformis	1	0.01	6.70	GP-ROD SB
4)	Staphylococcus pulvereri/vitulinus	0	0.00	7.17	GP-COC CAT+
5)	Exiguobacterium acetylicum	0	0.00	7.29	GP-ROD CAT+
6)	Staphylococcus sciuri ss rodentium	0	0.00	7.48	GP-COC CAT+
7)	Bacillus amyloliquefaciens	0	0.00	8.17	GP-ROD SB
8)	Micrococcus bovicus	0	0.00	9.44	GP-COC CAT+
9)	Staphylococcus lentus	0	0.00	10.12	GP-COC CAT+
10)	Microbacterium testaceum	0	0.00	10.51	GP-ROD
Other)					

Print Time = May 08 2003 10:39 Page 1 of 1 pages

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 08 2003 10:42
Parent File	: Original Data Record
Plate Number	: 23
Incubation Time	: 16-24
Sample Number	: 3039
Strain Type	: GP-ALL
Strain Number	:
Strain Name	: Bacillus licheniformis
Other	:
Data Input Mode	: Reader
590/750 Filters Used	: 6 / 5
Threshold Mode	: Automatic: Color: 89/134
Number +/b/- Reactions	: 56 / 6 / 34
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GP601.KID

Plate Type: GP2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	0	25+	61+	< 688>	< 189>	6	< 301>	{ 94}	76	< 722>	< 430>	< 458>
B	{ 112}	15	< 540>	< 934>	< 808>	9	< 165>	19	< 619>	< 438>	< 975>	< 282>
C	5	30	< 603>	64+	< 734>	< 710>	< 157>	{ 122}	23	44	< 701>	< 501>
D	< 710>	12	< 717>	< 617>	< 140>	59	< 383>	< 663>	74	< 561>	23	< 931>
E	65+	< 801>	<1018>	62	{ 111}	< 585>	{ 102}	36	71	-11	68	< 532>
F	< 150>	< 360>	< 344>	4	< 524>	< 430>	< 159>	60	< 757>	64	< 190>	{ 111}
G	34	< 434>	< 438>	< 441>	< 740>	< 511>	< 543>	< 150>	< 537>	24	< 294>	< 572>
H	< 874>	< 591>	< 705>	< 735>	< 669>	39	45	22	82	59	65	< 410>

=> Species ID: Bacillus licheniformis <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Bacillus licheniformis	100	0.51	7.79	GP-ROD SB
2)	Staphylococcus pulvereri/vitulinus	0	0.00	11.63	GP-COC CAT+
3)	Bacillus amyloliquefaciens	0	0.00	12.32	GP-ROD SB
4)	Bacillus megaterium	0	0.00	13.12	GP-ROD SB
5)	Staphylococcus sciuri	0	0.00	13.78	GP-COC CAT+
6)	Staphylococcus pasteuri	0	0.00	14.47	GP-COC CAT+
7)	Macrococcus bovicus	0	0.00	14.69	GP-COC CAT+
8)	Macrococcus equipercicus	0	0.00	14.88	GP-COC CAT+
9)	Exiguobacterium acetylicum	0	0.00	15.13	GP-ROD CAT+
10)	Bacillus psychrosaccharolyticus	0	0.00	15.22	GP-ROD SB
Other)					

Print Time = May 08 2003 10:42

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 21 2003 16:47
Parent File	: Original Data Record
Plate Number	: 35
Incubation Time	: 4-6
Sample Number	: 3045
Strain Type	: GP-ALL
Strain Number	:
Strain Name	: Staphylococcus aureus ss aureus
Other	:
Data Input Mode	: Manual
Number +/b/- Reactions	: 34 / 15 / 47
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GP601.KID

Plate Type: GP2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	{/}	<+>	-	-	<+>	<+>	<+>	<+>	-	-
B	-	-	-	{/}	<+>	-	-	-	-	-	<+>	-
C	-	-	<+>	<+>	-	{/}	{/}	-	-	-	<+>	-
D	-	-	<+>	<+>	-	-	<+>	-	-	-	-	{/}
E	-	<+>	<+>	-	{/}	<+-	{/}	{/}	{/}	-	-	<+>
F	-	-	<+>	-	-	- +	-	{/}	<+>	-	-	-
G	-	-	<+>	<+>	<+-	<+>	<+>	{/}	<+>	-	-	<+>
H	<+>	<+>	<+>	<+>	<+>	<+>	<+>	<+>	{/}	{/}	{/}	{/}

=> Species ID: Staphylococcus aureus ss aureus <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Staphylococcus aureus ss aureus	100	0.80	3.01	GP-COC CAT+
2)	Staphylococcus delphini	0	0.00	6.00	GP-COC CAT+
3)	Bacillus cereus/thuringiensis	0	0.00	7.03	GP-ROD SB
4)	Staphylococcus xylosus	0	0.00	8.67	GP-COC CAT+
5)	Staphylococcus chromogenes	0	0.00	9.05	GP-COC CAT+
6)	Staphylococcus intermedius	0	0.00	9.50	GP-COC CAT+
7)	Bacillus mycoides	0	0.00	9.98	GP-ROD SB
8)	Staphylococcus lutrae	0	0.00	10.70	GP-COC CAT+
9)	Staphylococcus schleiferi	0	0.00	10.83	GP-COC CAT+
10)	Cellulosimicrobium cellulans	0	0.00	11.37	GP-ROD CAT+
Other)					

Print Time = May 21 2003 16:49

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 09 2003 11:16
Parent File	: Original Data Record
Plate Number	: 28
Incubation Time	: 16-24
Sample Number	: 4076
Strain Type	: GP-ALL
Strain Number	:
Strain Name	: Bacillus licheniformis
Other	:
Data Input Mode	: Manual
Number +/b/- Reactions	: 39 / 16 / 41
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GP601.KID

Plate Type: GP2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	- +	- +	<+>	{/}	-	{/}	-	-	<+>	{/}	{/}
B	{/}	-	<+>	<+>	<+>	-	<+-	-	<+>	<+>	<+>	{/}
C	-	-	<+>	- +	<+>	<+>	{/}	{/}	-	-	<+>	<+>
D	<+>	-	<+>	<+>	{/}	-	<+>	<+>	-	<+>	-	<+>
E	{/}	<+>	<+>	-	-	-	-	-	-	-	-	{/}
F	-	-	<+>	-	<+>	<+>	{/}	-	<+>	-	{/}	-
G	-	{/}	<+>	{/}	<+>	<+>	-	{/}	<+>	-	<+>	<+>
H	<+>	<+>	<+>	<+>	<+>	-	-	-	-	-	-	<+>

=> Species ID: Bacillus licheniformis <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Bacillus licheniformis	91	0.65	4.41	GP-ROD SB
2)	Staphylococcus sciuri	7	0.04	5.29	GP-COC CAT+
3)	Staphylococcus lentus	2	0.01	5.69	GP-COC CAT+
4)	Bacillus subtilis	0	0.00	6.76	GP-ROD SB
5)	Bacillus subtilis(ATCC 6633)	0	0.00	6.86	GP-ROD SB
6)	Staphylococcus sciuri ss rodentium	0	0.00	7.89	GP-COC CAT+
7)	Bacillus pumilus	0	0.00	8.00	GP-ROD SB
8)	Bacillus amyloliquefaciens	0	0.00	8.02	GP-ROD SB
9)	Staphylococcus pulvereri/vitulinus	0	0.00	9.07	GP-COC CAT+
10)	Microbacterium testaceum	0	0.00	9.45	GP-ROD
Other)					

Print Time = May 09 2003 11:17

Program : Biolog MicroLog3 4.20
Save To File : C:\Biolog420\Rob.D4C
Unrestricted Access? : Yes
Read Time : May 09 2003 11:29
Parent File : Original Data Record
Plate Number : 29
Incubation Time : 16-24
Sample Number : 4078 Plate Type: GP2
Strain Type : GP-ALL
Strain Number :
Strain Name : Bacillus amyloliquefaciens
Other :
Data Input Mode : Manual
Number +/b/- Reactions : 38 / 11 / 47
Database To Search : MicroLog
Data Base(s) Searched : C:\BIOLOG420\Databases\GP601.KID

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	- +	<+>	- +	-	- +	{/}	{/}	<+>	{/}	<+>
B	-	-	<+>	<+>	<+>	-	-	-	<+>	-	<+>	-
C	-	-	<+>	- +	<+>	<+>	-	-	-	-	<+>	<+>
D	<+>	-	<+>	<+>	-	-	{/}	<+>	-	<+>	-	<+>
E	-	<+>	<+>	-	-	{/}	-	-	-	-	- +	{/}
F	-	{/}	{/}	-	<+>	<+>	{/}	-	<+>	-	{/}	-
G	-	<+>	<+>	<+>	<+>	<+>	<+>	-	<+>	-	{/}	<+>
H	<+>	<+>	<+>	<+>	<+>	-	-	-	-	-	-	<+>

=> Species ID: Bacillus amyloliquefaciens <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Bacillus amyloliquefaciens	99	0.72	4.09	GP-ROD SB
2)	Staphylococcus sciuri	1	0.01	5.63	GP-COC CAT+
3)	Staphylococcus sciuri ss rodentium	0	0.00	7.61	GP-COC CAT+
4)	Bacillus licheniformis	0	0.00	8.07	GP-ROD SB
5)	Exiguobacterium acetylicum	0	0.00	8.23	GP-ROD CAT+
6)	Macrococcus equipercicus	0	0.00	8.27	GP-COC CAT+
7)	Staphylococcus lentus	0	0.00	8.77	GP-COC CAT+
8)	Bacillus pumilus	0	0.00	8.99	GP-ROD SB
9)	Deinococcus proteolyticus	0	0.00	9.73	GP-COC CAT+
10)	Staphylococcus pulvereri/vitulinus	0	0.00	9.79	GP-COC CAT+
Other)					

Print Time = May 09 2003 11:29 Page 1 of 1 pages

Program	: Biolog MicroLog3 4.20
Save To File	: C:\Biolog420\Rob.D4C
Unrestricted Access?	: Yes
Read Time	: May 15 2003 22:56
Parent File	: Original Data Record
Plate Number	: 33
Incubation Time	: 4-6
Sample Number	: 4079
Strain Type	: GP-ALL
Strain Number	:
Strain Name	: Bacillus mycoides
Other	:
Data Input Mode	: Manual
Number +/b/- Reactions	: 33 / 14 / 49
Database To Search	: MicroLog
Data Base(s) Searched	: C:\BIOLOG420\Databases\GP601.KID

Plate Type: GP2

Key : <X>: positive; <X-: mismatched positive; X: negative; X+: mismatched negative
{X}: borderline; -X: less than A1 well

Color	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	-	<+>	- +	-	{/}	<+>	<+>	{/}	{/}	-
B	-	-	-	{/}	{/}	-	-	-	-	-	<+>	-
C	-	-	<+>	<+>	-	<+>	-	-	-	-	<+>	-
D	<+>	{/}	{/}	{/}	-	-	{/}	-	-	-	-	{/}
E	-	<+>	{/}	-	-	{/}	<+>	<+>	-	-	-	<+>
F	-	-	<+>	-	{/}	<+>	-	-	<+>	-	-	-
G	<+>	<+>	<+>	<+>	<+>	<+>	<+>	- +	<+>	-	-	<+>
H	<+>	<+>	<+>	<+>	<+>	<+>	<+>	<+>	-	-	-	{/}

=> Species ID: Bacillus mycoides <=

	Species	PROB	SIM	DIST	TYPE
=>1)	Bacillus mycoides	99	0.85	2.18	GP-ROD SB
2)	Bacillus cereus/thuringiensis	1	0.01	3.73	GP-ROD SB
3)	Staphylococcus xylosus	0	0.00	6.67	GP-COC CAT+
4)	Staphylococcus aureus ss aureus	0	0.00	6.91	GP-COC CAT+
5)	Staphylococcus chromogenes	0	0.00	7.56	GP-COC CAT+
6)	Staphylococcus delphini	0	0.00	7.99	GP-COC CAT+
7)	Staphylococcus muscae	0	0.00	8.54	GP-COC CAT+
8)	Staphylococcus sciuri	0	0.00	9.46	GP-COC CAT+
9)	Staphylococcus hyicus	0	0.00	9.51	GP-COC CAT+
10)	Staphylococcus intermedius	0	0.00	9.70	GP-COC CAT+
Other)					

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