Sclerotinia spp., a potential biological control agent of Canada thistle (Cirsium arvense [L.] Scop.)
by Brenda Simmonds Brosten

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Pathology
Montana State University

Abstract:
In a search for a biological control agent of Canada thistle (Cirsium arvense [L.] Scop.), a survey of endemic pathogens of Canada thistle was conducted in Montana during the summer of 1981. Pathogens from six genera, Alternaria, Fusarium, Septoria, Puccinia, Pseudomonas, and Sclerotinia were obtained. Of these pathogens, Sclerotinia was selected for further study due to its lethality on Canada thistle.

Sclerotinia inoculum for greenhouse or field studies was prepared by culturing isolates of Sclerotinia spp. on autoclaved oat or wheat kernels. The infested kernels were applied fresh or after being air-dried. In greenhouse studies using a kernel inoculation technique, the isolates of Sclerotinia differed in virulence on Canada thistle and also on eleven crops tested. A S. sclerotiorum (Lib.) de Bary isolate was used in all of the field studies.

In field trials conducted on cultivated land infested with Canada thistle, up to 73% of the thistle shoots died within nine weeks of treatment. In addition to attacking the thistle crown and causing wilting and death of the shoots, Sclerotinia infected the roots, extending more than 35 cm into the root system. The damage to the root system reduced the thistle shoot density by 8 to 51% the following year, while the density increased over 40% in the control plots. Soil incorporation of the inoculum reduced the thistle density to lower levels than surface application.

In statewide field trials to test S. sclerotiorum under varied environmental and habitat conditions, 19 to 80% kill of the thistle shoots died within 4 to 8 weeks. The trial site with the highest level of thistle control was in a pasture and, contrary to most of the sites, had not been disturbed by cultivation. At this site, the thistle density in the treated plots was reduced 95% the following year.

Additional research into the host range and genetics of Sclerotinia would be necessary in the development of this fungus as a biological control agent of Canada thistle.

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APPROVAL

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Brenda Simmonds Brosten

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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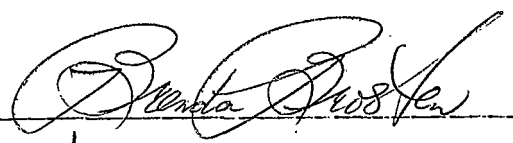
Signature 

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ABSTRACT

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INTRODUCTION

Canada thistle is a serious problem weed infesting range and cropland in Montana and the surrounding states. This noxious perennial weed is estimated to infest over 600,000 hectares in Montana, 680,000 hectares of wheat in North Dakota and 35% of all cultivated fields in Alberta (34,54,73). In addition to rangeland, pasture, dryland crops, and irrigated crops, Canada thistle can also be found in home gardens, along roadways and ditches. Its windblown seeds allow the weed to spread and its extensive perennial root system makes it difficult to control.

The best current control practices include the use of herbicides, intensive summer cultivation, and competitive crops such as alfalfa or perennial forage grasses. However, herbicides recommended for thistle control are often too expensive to be used on rangeland and application of most herbicides is severely restricted or forbidden near waterways because of water quality considerations. Consequently, effective control in cropland areas is usually only temporary due to reinfestation from surrounding rangeland, roadways or waterways. In addition, farmers have recently been

forced to examine the economic tradeoffs caused by higher fuel, equipment, and herbicide costs. As they shift to more sophisticated management systems involving reduced tillage, flexible cropping, and integrated pest management, Canada thistle control could become an even worse economic problem.

With the recent successes in using plant pathogens as biological agents to control weeds, it was desirable to investigate the possibility of using a plant pathogen to control Canada thistle. It was the objective of this study to conduct a survey of endemic Canada thistle diseases in Montana and study the feasibility of using any of the associated pathogens as biological control agents of Canada thistle.

LITERATURE REVIEW

Biological Control of Weeds with Plant Pathogens

Much of the early research in biological weed control was with insects, with several well documented successes (15,120). In reference to thistles, Trumble and Kok (104) have recently reported on the exotic insects that have been introduced into North America to control *Carduus* and *Cirsium*. Interest in using plant pathogens to control weeds dates back to the late 1800's (121). In the last twenty years, this interest has increased dramatically due to economic incentives and improved understanding of the organisms involved. Today, diseases are being actively researched by several scientists (83).

Biological control can involve the use of an exotic or a native pathogen to control a weed. The classical approach involves the importation of an exotic pathogen to control an introduced or native weed. Often a weed introduced into a country is brought in without its associated diseases that help to limit the weed's population in its native habitat. This classical type of control has been successful for rush skeletonweed in Australia and pamakani weed in Hawaii (37). In the

United States, use of an exotic pathogen is reviewed by the U.S. Department of Agriculture Working Group on Biological Control of Weeds and U.S. Department of the Interior Weed Committee, and tested in quarantine facilities before being released in the United States (126).

A second tactic is the microbial or bio-herbicide approach that can apply to either a native or an approved exotic pathogen. It involves mass production and application of the pathogen to the target weed. This overcomes the natural constraints that limit the pathogen's dissemination, survival, and inoculum increase in nature. Approval of the Environmental Protection Agency (EPA) must be obtained before a pathogen can be marketed as a bio-herbicide in the United States. Within the past two years, the first two microbial herbicides, both native pathogens, have been registered by the EPA and commercialized in the United States. Phytophthora palmivora (Butler) Butler (DeVine^{TM}) is registered to control strangler vine in Florida citrus groves, and Colletotrichum gloeosporioides (Penz.) Sacc. f.sp. aeschynomene (Collego^{TM}) to control northern jointvetch in rice and soybean fields in Arkansas (56,102).

Canada Thistle

Cirsium arvense (L.) Scop. has several common names, including Canada thistle, creeping thistle, Californian thistle, and perennial thistle. This plant is a serious introduced weed of pastures, rangeland, and croplands in the northern United States and Canada. Once indigenous to southeastern Europe and the eastern Mediterranean area, it has spread throughout Europe, North Africa, and Asia, and is found in South Africa, New Zealand, and parts of Australia (67). It is now a serious weed in five countries and a principal weed in over 17 countries (48).

Canada thistle reduces crop or forage yields by competing with the crop or rangeland plants for space, light, moisture, and nutrients. It can also harbour crop insects and diseases. Canada thistle is a highly competitive weed and one of the major weeds for small grains in Montana (55,77). A thistle infestation of 2.4 shoots/m^2 may reduce the yield of spring wheat or barley by 15%, and 30 shoots/m^2 can reduce the yields of spring wheat by 60% and barley by 40% (47,77).

Cirsium arvense is in the subtribe Cardueae in the family Compositae. This perennial plant has extensive horizontal roots that overwinter and give rise to numerous aerial shoots. The shoots first form a rosette

upon emergence. Seven to eight weeks later at maturity, these shoots will have attained a height of 0.3 to 1 meter. The leaf margins can vary from deeply ruffled and lobed with numerous spines to entire margins without spines. The plants are dioecious, although the male plants can sometimes produce a few viable seeds (46). Numerous small purple flower heads form at the ends of branches. These flowers are cross-pollinated by insects, and one female plant may produce up to 1530 seeds (42).

Canada thistle seed is dispersed by wind, water, and animals, and is frequently introduced into an area by hay, contaminated crop seed or pasture seed (12,43). Recently disturbed areas such as bare land, croplands, and poorly managed pastures that are overgrazed or underfertilized are the most susceptible to colonization by seeds (13).

Once introduced into an area, C. arvense also spreads by vegetative propagation of its root system. The invasive capability of this weed is demonstrated in the ability of the root system of a single thistle to spread outwards 3 to 4 m in one season (47). This annual rate of vegetative spread is dependent on seasonal conditions, vigor of associated vegetation, soil type, and land use. In pasture lands, the annual rate of vegetative spread may vary from 4 cm to 3.4 m (13). Moreover, cultivation enhances the spread of Canada

thistle in croplands as root fragments as small as one cm long and one mm in diameter are able to produce shoots (41). This regenerative capacity of the root system, that may extend to a soil depth of over a meter, makes extermination of the weed very difficult. The best current control practices involve an integrated approach of cultural and chemical methods.

There is considerable genetic diversity in morphological and physiological characters within the species C. arvense (46,67). Based on leaf morphology alone, Wimmer and Grabowski in 1829 (122) separated C. arvense into four varieties: vestitum, integrifolium, mite (arvense), and horridum. These varieties have the same chromosome number (2n = 34) both in Europe and in Canada, and hybridize freely, so that true varietal forms are rarely found (33,38,67). Within these varieties, many clones or ecotypes have been reported that exhibit differences in photoperiodism, growth habit, vigor, seed dormancy and germination, herbicide reponse, and disease susceptiblity (38,46,67,109).

Canada Thistle Diseases

There are over forty genera of microorganisms, fungi, bacteria, nematodes, and viruses found to occur on Canada thistle in the United States and Canada, according to host indexes and a list compiled by Libscomb in 1974

(29,61,94,112). Many of these microorganisms cause only minor damage to the weed.

Puccinia punctiformis (Str.) Rohl. (= P. obtegens [Link.] Tul. = P. suaveolens [Pers.] Rost.) is the best documented of these pathogens. Systemically infected thistles are pale green, have long internodes, small leaves with incurled leaf margins, and usually produce no flowers or viable seeds (18). Interest in using this fungus as a biocontrol agent dates back to 1900 (16,28). Watson (115) and Turner et al. (108,109) have recently investigated the potential of P. punctiformis as a biological control agent for Canada thistle.

A root rot caused by Fusarium roseum (Link.) Snyder and Hans. was studied in Wyoming (62,63,86). Under natural conditions, the fungus caused wilting, yellowing, and reduced vigor in thistles. The pathogen infected a prickly denticulate-leaved thistle ecotype, but not a smooth-leaved spinulose ecotype in greenhouse studies (62).

Verticillium albo-atrum Rke. and Berth. caused a wilt of Canada thistle in Oregon and Germany (49,95). The fungus infects the roots, as well as the stem.

Detmers (33) observed bacteria that caused stunting and death of Canada thistle in Ohio, but gave no further information. Mycoplasmalike organisms have been found in Denmark and South Dakota in C. arvense plants exhibiting

chlorotic and wrinkled leaves, dwarfism, shoot proliferation, and abnormal flowers (19,89).

The disease caused by Septoria cirsii Niessl. has been reported by Detmers (33), and results in large necrotic spots on the lower leaves in mid-summer. The infected leaves turn brown and dry, and the disease slowly spreads to the upper leaves.

Another fungus, Sclerotinia, was reported to cause a wilt and crown rot of Canada thistle resulting in the death of the plant shoot (20,21,127). More information on this disease is presented in tne following section. In addition to Canada thistle, other weeds including wild mustard (Brassica kaber [Dc.] Wheeler), field pennycress (Thlaspi arvense L.), redroot pigweed (Amaranthus retroflexus L.), lambsquarters (Chenopodium album L.), sowthistle (Sonchus spp.), diffuse knapweed (Centaurea diffusa Lam.), and toothed spurge (Euphorbia dentata Michx.) have been reported to be killed by Sclerotinia spp. (20,23,70,114,127).

Sclerotinia

Taxonomy

Kohn in 1979 (58) limited the fungal genus Sclerotinia to three species, S. sclerotiorum (Lib.) de Bary, S. trifoliorum Erikss. and S. minor Jagger. The

other species previously placed in this genus were assigned to other genera.

Prior to 1980, controversy had prevailed for two decades concerning the delineation of these three species. Since 1955 when Purdy first suggested that the three species be combined into a single species, extensive studies have indicated three distinct species (81,82,117). Morphologically, special distinguishing characteristics are the small sized sclerotia of S. minor and the ascospore dimorphism of S. trifoliorum (58,110). Dissimilarities have been proven in ontogenetic, electrophoretic, mycelial interaction and cytological research (32,117,118,123,124,125). In cytological studies, Wong and Willetts (125) found for S. minor, S. trifoliorum and S. sclerotiorum, the number of nuclei per ascospore and the haploid chromosome count to be 4,4; 4,8; and 2,8, respectively. In 1983, Uhm and Fujii (111) observed 9 bivalents during S. trifoliorum ascosporogenesis. Additionally, Petersen et al. (78) have recently reported that the major sclerotia proteins vary in the three species. Serologically, the three species have common antigens (92). Differences in host range and epidemiology are discussed in subsequent parts of this section. In reviewing the host range, epidemiology and other aspects of these species, it should be remembered that identification of

S. trifoliorum in past literature may be incorrect as it was often based only on the host of origin.

Host Range

Sclerotinia occurs throughout the world, including Europe, Africa, Asia, South Pacific and North and South America, and is most common in the temperate regions. These climatic areas also enclose the geographical range of Canada thistle. Although the genus has a wide host range, corn, wheat, barley, oats, and other cereal grain crops are not affected by any of the three species (65,82).

The only host range research done to compare the three pathogens was limited to testing a small number of isolates in greenhouse studies. Keay (57), and Held and Haenseler (44) both found *S. trifoliorum* to be restricted to legumes, tomato, lettuce and beans under optimal greenhouse conditions. *Sclerotinia minor* and *S. sclerotiorum* in these tests had broad host ranges including legumes.

General field observations show *S. sclerotiorum* is most damaging to vegetables and oilseed crops, *S. minor* to peanuts and lettuce, and *S. trifoliorum* to forage legumes (98). Some field studies have shown alfalfa and red clover are not susceptible to isolates of *S. sclerotiorum* (25). In addition, Burke et al. (23)

found little damage from S. sclerotiorum in bean fields in which alfalfa had been grown for three or more years between bean crops. One explanation for these conflicting reports on host susceptibility is the diversity found among isolates within each of these species, S. sclerotiorum, S. minor and S. trifoliorum. Considerable variability in virulence is exhibited by these isolates when tested on different hosts (25,57,71,80).

Incompatible mycelial interactions have been observed between some S. sclerotiorum isolates as well as between S. trifoliorum isolates (79,91). Price and Colhoun (79,80) suggest that the differences in virulence reflect small genetic differences in the isolates.

Genetics and Pathogenesis

Sclerotinia sclerotiorum and S. trifoliorum were considered to be homothallic. Recent studies by Uhm and Fujii (110,111) have demonstrated that S. trifoliorum is partly heterothallic. This species produces dimorphic ascospores. The small ascospore strains are heterothallic and self-sterile, while the large ascospore strains are self-fertile. They also established that the microcondia produced by this fungus are functional spermatia through mating crosses between sclerotia from

the small ascospore strains and microcondia from the large ascospore strains.

A second means of genetic exchange in fungi is the transfer of genetic material between the mycelium of two strains through heterokaryosis. While the extent to which this occurs between strains of a Sclerotinia species is unknown, incompatible mycelial interactions have been observed among S. sclerotiorum isolates as well as among S. trifoliorum isolates (79,91).

The genome can also be changed through mutation. As Sclerotinia is haploid for most of its life cycle, any mutation in the genes in the genome that are expressed phenotypically would be immediately revealed.

There are many factors involved in Sclerotinia pathogenesis, the ability of Sclerotinia spp. to successfully infect a host plant and cause disease. The first step is the formation of an infection cushion leading to mechanical penetration of the host cuticle. Ascospores and S. sclerotiorum sclerotia usually require an external nutrient source to form the infection cushion. Once inside the host, the fungus produces three pectolytic enzymes and oxalic acid that work in combination to break the pectin layer between the host cells. Oxalic acid chelates cation inhibitors of pectinase activity and lowers the pH making conditions more favorable for enzymatic action. In addition,

degradative enzymes, including cellulases, hemicellulases, proteases and phosphatidase are produced by the fungus to supply nutrients for the rapid growth and development of infection hyphae (65). The differences in virulence observed between Sclerotinia spp. isolates may be a result of variations in these pathogenesis factors.

Host Resistance

Within the past 15 years, resistance to Sclerotinia has been reported in some crops. Field tolerance to S. trifoliorum has been shown in cultivars and experimental lines of alfalfa, and in cultivars of white clover (11,116). Similarly, field tolerance to S. sclerotiorum has been observed in cultivars and breeding lines of dry beans (Phaseolus vulgaris L.), and in cultivars of soybeans (14,31,39,106). Some types of tolerance are due to plant canopy structure that influences the microclimatal conditions and the development of the disease. Another type of resistance is found in the highly resistant runner bean Phaseolus coccineus L. Abawi et al. (4) transferred this resistance into P. vulgaris through backcrossing and suggested a single completely dominant gene was involved. Recently, Hunter et al. (53) screened over 400 lines of P. vulgaris for resistance using a limited-term

inoculation method under growth chamber conditions. They detected levels of resistance in lines from Central and South America, Turkey and Europe.

Resistance has been found in closely related wild plant species. Three wild Lactuca species were found to be highly resistant to S. minor (5). One of these wild species can be crossed with lettuce. Resistance to S. sclerotiorum also exists in the North America native perennial Helianthus spp., as well as in Jerusalem artichoke (H. tuberosus L.). Hybrid crosses of Jerusalem artichoke and the annual sunflower (H. annuus L.) were completely resistant in greenhouse studies (76).

Four mechanisms of host resistance to Sclerotinia have been theorized by Lumsden (65). Little research has been done in this area. Physical barriers may slow the penetration and development of the fungus, and nutritional factors within the host may be inadequate for rapid infection hyphae formation. The host may also have performed inhibitors of Sclerotinia or the host may be induced by the fungus to form phytoalexins.

Infection

Sclerotinia spp. can survive and infect over wide temperature and pH ranges. Depending on the species involved, disease can result from airborne ascospore

infection or soilborne mycelial infection. No asexual spores are produced.

Ascospores are the prime source of infection in diseases caused by both S. sclerotiorum and S. trifoliorum, but of limited importance in the epidemiology of S. minor caused diseases. In the latter case, soilborne mycelial infection is important (2).

Ascospores are released from apothecia produced by sclerotia. Much of the following information on ascospore infection is based on S. sclerotiorum. Less is known about S. trifoliorum ascospore infection, that usually occurs in the autumn. Apothecia initiation is closely tied to soil depth and soil moisture. Only preconditioned sclerotia within the top 3 to 5 cm of the soil surface are capable of forming apothecia, as stipes over this length are rarely produced (2,30). Furthermore, continuous soil moisture for 10 to 14 days is required for apothecia to form. Sclerotinia sclerotiorum isolates from New York State required a soil matric potential of -80 to -160 millibars (mb), while isolates from Nebraska required -250 mb for optimal apothecia production (2,98). In California and other southern areas, S. sclerotiorum apothecia are produced in late winter through early spring. At more northerly locations, ascospores are formed throughout the growing season provided the top 3 cm of the soil is near

saturation. In the northern semi-arid regions, apothecia formation does not occur until early summer when the plant canopy has covered the soil surface decreasing water evaporation (2,72,98).

Sclerotinia sclerotiorum ascospores require 48-72 hours of continuous free water and an exogenous food source such as pollen, dying flower petals, or senescing or injured plant tissue to infect a host (1,30). The lack of this exogenous energy source prevents infection. Ascospores have been found to survive 12 days in the field in New York state (2,24). In vitro studies have shown survival is enhanced by temperatures below 20° C and relative humidities below 35% (24). Free water is also required for lesion expansion. If the surface of infected tissues, except for bulky stem tissue, becomes dry, lesion enlargement is abruptly stopped until free water is again available (2).

Soilborne mycelial infection results from mycelial germination of sclerotia. This is the principal means of infection in S. minor and no outside food source is required (2). While the optimal soil water potential for mycelial germination of S. minor sclerotia is the same as for carpogenic (apothecial) germination of S. sclerotiorum sclerotia at -250 mb, germination can occur as low as -2 bars (3). In addition, prior drying of S. minor sclerotia at the soil surface, stimulates

mycelial germination. With S. sclerotiorum, the importance of mycelial sclerotia germination depends upon the crop involved. In beans, this type of infection is of minor importance and occurs only when an exogenous energy source is in direct contact between the sclerotium and the host tissue (1). It is a different case in sunflowers, where the basal stem rot phase of Sclerotinia wilt is due entirely to mycelial infection from germinating sclerotia (51,52). With sunflowers, no exogenous food source is needed. Recently, host root exudates have been implicated in influencing the type of sclerotia germination. Rimmer and Menzies (87) found rape and corn seedling exudates could affect mycelial and carpogenic germination of single spore isolates of S. sclerotiorum. Sclerotia placed with sunflower plants had increased mycelial but reduced carpogenic germination, in comparison to sclerotia placed with rape plants.

Sclerotinia sclerotiorum and S. minor have been described as simple interest pathogens as defined by Van der Plank (2,113). Nonetheless, some secondary spread may occur from contact with mycelium from infected plants, including spread through root contact or interconnecting roots. With S. sclerotiorum, actively growing mycelium on moist infected plant tissue requires only 16 - 24 hours of leaf wetness to infect a new host

plant, whereas dry infected plant tissue requires over 72 hours of continuous moisture (1).

Dissemination

In the Sclerotinia diseases where ascospore infection is important, windblown ascospores may spread the fungus from field to field. The ascospores are discharged in a mucilaginous material that cements them to any object encountered. Most of the ascospores are trapped by the plant canopy, but some will escape to above the crop canopy and be carried in air currents. Spores have been trapped at distances of 150 m to several hundred meters from the nearest source (111,119). Hims (45) observed an outbreak of *S. sclerotiorum* restricted to one corner of a 23 ha field of oilseed rape and suggested that the source of inoculum was ascospores from weeds in an adjacent woodland. Ascospores can also be transmitted with pollen by honeybees, and in irrigation runoff water (97,100). Spores of *S. sclerotiorum* have been shown to survive up to 5 days in deionized water (99).

Sclerotia dissemination is limited. In *S. minor* where the sclerotium is the infective propagule, dispersal is generally restricted to secondary plant to plant spread. Sclerotia can, however, be moved from field to field with machinery and in irrigation runoff

(90). It is possible for S. sclerotiorum sclerotia to remain viable up to 21 days in flowing ditch water but longer periods may destroy the sclerotia (69,99). Animals eating Sclerotinia infested crop refuse containing masses of sclerotia may transmit the fungus at very low levels. Brown (22) found only 2% of the sclerotia still intact and viable after passage through sheep. Sclerotinia can also be spread in seed lots contaminated with sclerotia or with mycelium infected seed. Steadman (96) found S. sclerotiorum mycelium in 12% of abnormal chalky, discolored bean seed and 0.5% of normal bean seed.

Survival

Sclerotinia is a facultative saprophyte and grows rapidly in pure culture. Yet, this fungus is not a vigorous competitor in the soil, and aside from invading necrotic or senescent plant parts, colonizes only plant tissue previously killed by the pathogen. Cook et al. (30) found S. sclerotiorum was unable to survive as mycelium on soil plant residues but could overwinter as mycelium in 2% of the bean seeds left on the soil surface after harvesting. Sclerotia are the principal means Sclerotinia survives environmental extremes.

Many studies have been done on Sclerotinia sclerotia survival in the soil. Due to non-comparable soil and

environmental conditions, secondary sclerotia formation as well as unrelated soil microflora and methodology, the results have varied considerably, with survival times from a few weeks to 8 years. Based on growers' experience with crop rotation, sclerotia of S. trifoliorum survive in nature for about 8 years, and sclerotia of S. sclerotiorum and S. minor for 4 to 5 years (6). The use of crop rotation to nonsusceptible hosts as a control, may be of limited value unless all inoculum sources and susceptible weed hosts are eliminated.

Various factors may be reducing sclerotia viability in nature. Troutman and Matejka (103) noted no build-up of S. sclerotiorum sclerotia over a 12 year period in irrigated lettuce plots in Arizona, despite being inoculated each year with the fungus. Destruction by soil microorganisms is one of these factors and appears to be greatly affecting sclerotia survival. A natural decline of S. sclerotiorum was observed in potato fields in Oregon, recently put into production with the installation of center-pivot irrigation. Some of these fields had moderate to severe sclerotinia stem rot and only 0 to 16% sclerotia infection from the hyperparasite Sporidesmium sclerotivorum Uecker, Ayers and Adams. Another field had little stem rot but over 92% sclerotia infection by S. sclerotivorum (7).

Sporidesmium sclerotivorum, as well as other microorganisms, are being studied as possible Sclerotinia biological control agents. Much of this work has been done within the past five years. In field testing, Sporidesmium sclerotivorum provided 40 to 83% disease control of sclerotinia lettuce drop caused by Sclerotinia minor over a three year period, and reduced the sclerotia populations by 75 to 95% (9). This mycoparasite grew intercellularly through the sclerotium, did not invade sclerotial cells, and produced large quantities of macroconidia on the sclerotium surface (10). Methods to cultivate this fungus have now been patented (8). Another fungus, Talaromyces flavus (Klocker) Stolk and Samson reduced the percentage of Sclerotinia infected sunflowers to 27 and 4% in field trials, verus 81 and 47% disease in the controls (66). Coniothyrium minitans Camp., Trichoderma koningii Oud. and Teratosperma oligocladum Uecker, Ayers and Adams also greatly reduce the number of viable sclerotia and show potential as biological control agents of Sclerotinia spp. (17,36,50,105,107).

MATERIALS AND METHODS

Disease Survey

A disease survey was conducted in Montana to find diseased Canada thistle plants in the fall of 1980 and spring and summer of 1981. Isolations were done in the laboratory using water agar, potato dextrose agar and BCBRVB media (69,88). Some of these isolates were tested on young Canada thistle plants in the greenhouse. The thistle plants had previously been started from thistle root sections placed in pots containing a sand-steamed soil mix. Three inoculation techniques were attempted to obtain successful infection of Canada thistle with the fungal isolates.

1. The thistle plant was put in a mist chamber for 24 hours, sprayed with a suspension of mycelial fragments and possibly spores, then either covered with a plastic bag or placed back in the mist chamber for 2 days.

2. Mycelial fragments were applied to the soil around the crown of the thistle plant and then covered with a 2.5 cm layer of sand.

3. A mycelial agar plug was placed next to the uninjured thistle stem and held in place with a piece of parafilm.

If the organism was a bacterium, a bacterial suspension was injected into the stem of the Canada thistle plant.