Plant Pathology Bulletin 11:207-214, 2002

Organic Soil Amendments for Control of Apothecial Production of *Sclerotinia sclerotiorum*

Hung-Chang Huang[1,3], R. Scott Erickson[1], Chi Chang[1], Jim R. Moyer[1], Francis J. Larney[1], and Jenn-Wen Huang[2]

1 Agriculture and Agri-Food Canada, Lethbridge Research Centre, PO Box 3000, Lethbridge, Alberta, T1J 4B1 Canada
2 Department of Plant Pathology, National Chung-Hsing University, Taichung, Taiwan
3 Corresponding author, E-mail: huangh@agr.gc.ca; Fax: (403) 382-3156
Accepted for publication: Nov, 5 , 2002.

ABSTRACT

Huang, H. C., Erickson, R. S., Chang, C., Moyer, J. R., Larney, F. J. and Huang, J. W. 2002. Organic soil amendments for control of apothecial production of *Sclerotinia sclerotiorum*. Plant Pathol. Bull. 11:207-214.

An indoor study was conducted to assess the potential of organic residues, with or without biocontrol agents, as soil amendments for control of carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* and production of apothecia. Of the 87 organic residues tested including crop straws, animal manures and processing by-products, 46 inhibited carpogenic germination of *S. sclerotiorum* when applied to soil at the rate of 3% (w/w), 21 were effective at 2%, 4 were effective at 1%, and 3 were effective at 0.5%. Ammonia released from the decomposition of organic residues appeared to be the key factor in suppression of carpogenic germination. Organic soil amendments combined with biocontrol agents such as *Coniothyrium minitans* and *Trichoderma virens*, enhanced the control of apothecia of *S. sclerotiorum* by killing of sclerotia through mycoparasitism. The soil amendment with combination of organic materials and biocontrol agents represents an environmentally sustainable strategy for management of crop diseases caused by *S. sclerotiorum*.

Key words : *Sclerotinia sclerotiorum*, sclerotia, carpogenic germination, apothecia, soil amendment, biological control, *Coniothyrium minitans*, *Trichoderma virens*, *Penicillium griseofulvum*

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is a pathogen with worldwide distribution and a wide range of hosts including many broadleaf crops [20]. Sclerotia of *S. sclerotiorum*, when properly conditioned, can produce apothecia [14], which release airborne ascospores serving as the primary source of inoculum for diseases such as white mold of bean (*Phaseolus vulgaris* L.) [13,23], pod rot of pea (*Pisum sativum* L.) [12], stem rot of canola/rapeseed (*Brassica napus* L. and *B. rapa* L.) [4], and head rots of sunflower (*Helianthus annuus* L.) [5], and safflower (*Carthamus tinctorius* L.) [19].

Disposal of organic debris such as animal manures and crop straws has become a problem in modern day agriculture. There is considerable interest in the use of residue-based organic amendment products for control of soilborne plant pathogens [9]. For example, studies on suppression of Rhizoctonia damping-off of cabbage in the greenhouse have shown that SSC-06, a container medium using spent forest mushroom compost as a major ingredient [16] and the commercial product FBN-5A, at rates of 0.1 and 0.2%, were effective in suppressing the disease [22]. Another study demonstrated that the commercial product CF-5, which contains liquid extracts of fermented agricultural wastes, is effective in suppressing the production of apothecia of *S. sclerotiorum* when applied to soil at rates of 150 to 400 ppm, and enhances the colonization of sclerotia by *Trichoderma* spp. [10]. Another commercial product from Taiwan, S-H mixture, is based on bagasse and rice husks, and is also highly inhibitory to *S. sclerotiorum* at rates of 1 to 2% [15].

Coniothyrium minitans Campbell [3] is a mycoparasite which is effective in reducing viability of sclerotia of *S. sclerotiorum* in sunflower fields [6,7]. Soil treated with *C. minitans* can reduce the incidence of wilt of sunflower [1,7,17] and drop of lettuce (*Lactuca sativa* L.) [2] caused by *S. sclerotiorum*. Further field studies in western Canada demonstrated that soil treatment with *C. minitans* is effective in reducing carpogenic germination, and thereby reduces production of apothecia in *S. sclerotiorum* under the canopies of bean, pea [8], canola and safflower (Huang and Erickson, unpublished), as well as the non-host crops, wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) [18].

This study was undertaken to determine the effectiveness of control of carpogenic germination and apothecial production of *S. sclerotiorum* under controlled environments, by soil amendment with organic matter alone, or in combination with fungal biocontrol agents.

MATERIALS AND METHODS

Soil samples (sandy clay loam, pH 7.5) collected from the Fairfield Farm at the Agriculture and Agri-Food Canada Research Centre (LRC) near Lethbridge, Alberta, Canada, were air dried, sieved through a 2 mm mesh screen, and stored in cans for use in the experiments. *Sclerotinia sclerotiorum* isolate LRC 2148 (syn. sun-87), was grown on potato dextrose agar (PDA) at 10℃ for 8 weeks, to produce sclerotia conditioned for carpogenic germination [14]. Sclerotia from 4-7 mm in diameter were selected for use in the experiments.

Organic matters used for the amendment tests were crop straws, manures, processing by- products, or commercial products. Crop straws were collected from research and demonstration plots at LRC. The above-ground plant material was harvested at anthesis, except for cilantro (*Coriandrum sativum* L.), which was collected at the late vegetative stage. Straws were dried for 14 days in an oven at 55℃, ground using a Thomas-Wiley® laboratory mill, model #4 (Thomas Scientific, Philadelphia, PA, USA), and sieved through a 2 mm meshed screen. The crop straws used included alfalfa (*Medicago sativa* L. cv. Barrier), amaranth (*Amaranthus hybridus* L.), two cultivars of barley (*Hordeum vulgare* L. cvs. Galt and Harrington), black bean (*Phaseolus vulgaris* L. cv. UI 906), small red bean (*Phaseolus vulgaris* L. cv. NW 63), borage (*Borago officinalis* L.), buckwheat (*Fagopyrum esculentum* Moench.), canary grass (*Phalaris canariensis* L. cv. Keet), argentine type of canola (*Brassica napus* L. cv. Westar), polish type of canola (*Brassica rapa* L. cv. Tobin), kabuli chickpea (*Cicer arietinum* L. cv. Sanford), cilantro, coriander (*Coriandrum sativum* L.), dwarf corn (*Zea mays* L.), silage corn (*Zea mays* L.), dill (*Anethum graveolens* L.), fababean (*Vicia faba* L.), fenugreek (*Trigonella foenum-graecum* L.), flax (*Linum usitatissimum* L.), lathyrus (*Lathyrus sativus* L.), four cultivars of lentil (*Lens culinaris* Medik. cvs. CDC Richlea, Eston, Indianhead and Laird), lupine (*Lupinus angustifolius* L.), brown mustard (*Brassica nigra* (L.) Koch cv. Common Brown), oriental mustard (*Brassica juncea* L. cv. Forge), yellow mustard (*Brassica hirta* L. cv. Tilney), two cultivars of oats (*Avena sativa* L. cvs. Cascade and Mustang), three cultivars of pea (*Pisum sativum* L. cvs. Carneval, Radley and Trapper), quinoa (*Chenopodium quinoa* L.), rye (*Secale cereale* L.), two cultivars of safflower (*Carthamus tinctorius* L. cvs. AC Stirling and Saffire), soybean (*Glycine max* (L.) Merrill), sunola (*Helianthus annuus* L.), sunwheat (*Helianthus annuus* L.), sweet clover (*Melilotus officinalis* L. cv. Polara), and wheat (*Triticum aestivum* L. cv. Fielder).

The manures tested included cattle manures collected from the feedlot and composting complex at LRC, chicken manures obtained from Coaldale Egg Farms Ltd., Coaldale, Alberta, Canada, and hog manure collected from Veurink Hog Farm, Lethbridge, Alberta, Canada. Manures were oven-dried, ground and sieved by the methods described for crop straws. Cattle manures were composed of either pure manure, manure with wheat straw bedding, manure with wood chip bedding, or manure with lamb carcasses, and were used either raw, passively composted (never turned during the composting process) for 29, 50 or 84 days, or actively composted (turned at day 14, 21, 29, 50, 70 and 84 of the composting process) for 29, 50 or 84 days. Chicken manures were either pure manure or chicken pen scrapings, and were used either raw or passively composted for 50 days. Hog manures were used only in raw form.

Processing by-products included blood meal and fish meal from Unifeed, Lethbridge, Alberta, Canada; bone meal from Green Valley Fertilizer, Abbotsford, British Columbia, Canada; canola meal, canola screenings, and spent clay from Canbra Foods Ltd., Lethbridge, Alberta, Canada; distiller's dry grain from Black Velvet Distilling Co., Lethbridge, Alberta, Canada; spent lime and sugar beet pulp from Rogers Sugar Ltd., Taber, Alberta, Canada; mushroom media from Tiger Mushroom Farm Ltd., Nanton, Alberta, Canada; potato mash and potato peel from Hostess Frito-Lay Co., Taber, Alberta, Canada; cedar (*Thuja occidentalis* L.) sawdust and fir (*Pseudotsuga* spp.) sawdust from Revelstoke Lumber, Lethbridge, Alberta, Canada; and whey from Parmalat Canada, Lethbridge, Alberta, Canada. The mushroom media were either unused, used, or used and passively composted. Blood meal, bone meal, canola meal, spent clay, distiller's dry grain, fish meal, spent lime, cedar sawdust and fir sawdust were obtained in ground form, so no drying or grinding was necessary. Canola screenings, mushroom media, potato mash, potato peel, and sugar beet pulp were dried, ground and sieved as described above for crop straws. Whey was obtained as a liquid and was stored at 0.5℃ until used for the experiments.

The commercial preparations used were: CF-5 liquid [9], provided by Dr. J.W. Huang, Department of Plant Pathology, National Chung-Hsing University, Taichung, Taiwan, Republic of China; Perlka™, a slow-release product supplied by SKW Trostberg Aktiengesellschaft, Trostberg, Germany; and S-H mixture [24] provided by Professor S.K. Sun, Department of Plant Pathology, National Chung-Hsing University, Taichung, Taiwan, Republic of China.

Field soil was placed in plastic Petri dishes (9 cm diameter by 2.5 cm deep), 50 g per dish. Each organic matter was added to the soil at rates of 0.5, 1, 2, and 3% (w/w for solids; w/v for liquids) and thoroughly mixed with the soil. Ten sclerotia of *S. sclerotiorum* were buried in the soil in each dish, and soil moisture was adjusted to near field capacity by adding 20 ml distilled water to each dish. The dishes were

sealed individually with parafilm™ and incubated at 20℃ under continuous fluorescent light (15.2 μE s^{-1} m^{-2}). After 3 wk, each dish was rated for number of carpogenically germinated sclerotia and number of apothecia produced. Ungerminated sclerotia were removed, surface sterilized in 70% ethanol for 90 sec, air-dried on paper towel, and placed onto PDA containing 200 ppm streptomycin sulfate in petri dishes. After 10 days of incubation at 20℃, they were examined for viability. The control was set up by burying sclerotia in moistened, unamended soil. There were three replicates (dishes) for each treatment, arranged in a complete randomization design. The experiment was repeated 3 times.

Eight organic matters with varying effects on carpogenic germination of sclerotia of *S. sclerotiorum* were selected from the above experiment for a second study. They were bone meal, canola (cv. Westar) straw, cattle manure (raw), cattle manure (actively composted for 84 days), cattle manure with wood chips (actively composted for 84 days), distiller's dry grain, fish meal, and sweet clover (cv. Norgold) straw. The experiment was set up by the same Petri dish techniques described above, using each organic matter at rates of 1 and 3% (w/w). Unamended soil was used as control. After collection of carpogenic germination data, the sclerotia were removed from each dish, a 10 g subsample of moist soil was removed, and was combined with 1 mol of 2M HCl. The subsample was acidified to pH 2.3 (determined with litmus paper), air-dried, finely ground, and used to determine total C, total N and available N. Total C and total N were determined using an automated combustion analyser (Carlo Erba, Milan, Italy). Available N was determined as KCl extractable NO_3^- and NH_4^+. This was done by preparing a suspension from the subsample using a 5:1 ratio of 2M KCl:soil, shaking for 1 h at 180 rpm, and filtering through filter paper that had been previously wetted with 2M KCl. Samples were then analysed for NO_3^- and NH_4^+ using a Bran+Luebbe autoanalyser 3 (Norderstedt, Germany). The remaining soil from each sample was air-dried and coarsely ground (2 mm), an aqueous suspension was prepared by mixing 7.5 g of the soil with 37.5 ml of distilled water, and the pH and electrical conductivity (EC) of the suspension was determined using a Model 50 pH/ion/conductivity meter (Denver Instrument Co., Arvada, Colorado, USA). The experiment was conducted twice.

A third study was conducted to determine the effects of soil amendment using six different composite formulae. Mixtures 1 and 2 were composed of wheat straw, raw cattle manure, distiller's dry grain, and fish meal in a 3:4:2:1 (weight basis) ratio. Mixtures 3 and 4 were composed of canola straw (cv. Westar), canola screenings, and raw cattle manure in a 2:1:4 ratio. Mixtures 5 and 6 were composed of canola straw (cv. Westar), canola screenings, and chicken pen scrapings in a 2:1:4 ratio. Mixtures 1, 3, and 5 were not composted, whereas mixtures 2, 4, and 6 were actively composted (turned every 4 days) for 60 days. Each of the composites was mixed with soil at concentrations of 1 and 3% (w/w) and tested for effects on production of apothecia of *S. sclerotiorum* and soil properties using the same methods described in the second study above.

A fourth study was conducted to assess the effectiveness of amendments with an added microbial (biocontrol agent) component. Two mycoparasites, *Coniothyrium minitans* isolate LRC 2137 and *Trichoderma virens* isolate LRC 2425, and one antagonist, *Penicillium griseofulvum* isolate LRC 2461, were used as biological control agents. All of the biocontrol agents originated from the western Canadian prairie region [8]. Inoculum of each fungus was prepared by solid state fermentation on a mixture of wheat bran and corn meal. Aluminum pans (23 x 29 x 6.5 cm, W x L x H) were filled with a mixture of 150 g each of wheat bran and corn meal, and 300 ml of sterile distilled water. The pans were covered with aluminum foil and autoclaved twice with 48 hrs between sterilization cycles. Biocontrol agents were grown on PDA at 20±2℃ under continuous fluorescent light for three wks. Agar plugs of 8 mm diameter were cut from the edge of the colony using a cork borer, and 20 agar plugs were placed, evenly spread over the surface of the wheat bran - corn meal mixture. The pans were incubated at 20℃ for three wks, and stirred weekly using a sterilized spatula, to ensure thorough colonization of the wheat bran - corn meal medium by the organism. The inoculum was air dried at room temperature, and ground to 1 mm particle size using a Thomas-Wiley® laboratory mill (see above). Microbial amendments were then created by mixing each of the biocontrol agents with 84-day-old composted cattle manure with wood chips, and 84-day-old composted cattle manure with wheat straw. The ratio of biocontrol agent and compost was 2:3 (w/w). The resulting microbial infested composts were amended with soil at concentrations of 1 and 3% (w/w) and tested for production of apothecia of *S. sclerotiorum*, using the same methods described in the second and third studies.

Within each study, differences between treatments in percent carpogenic germination, number of apothecia produced, total C, total N, available N as NO_3^-, available N as NH_4^+, C:N ratio, pH and EC were analyzed for statistical significance using analysis of variance (ANOVA) for a complete randomization design. Means were separated using Duncan's multiple range test (DMRT). All statistical analyses were conducted using the GLM procedure of SAS/STAT® software [21].

RESULTS

Sclerotia of *S. sclerotiorum* in the unamended controls germinated carpogenically to produce small, light brown colored apothecia capable of releasing ascospores. The frequency of germinated sclerotia in the untreated controls from the four studies ranged from 76 to 87%. In the first study, carpogenic germination was significantly (P<0.05) reduced for 46 of the 87 organic residues amended with soil at

the rate of 3%, whereas 21 were effective at 2%, 4 were effective at 1%, and 3 were effective at 0.5% (Table 1). The frequency of germinated sclerotia in the effective treatments ranged from 0 to 49%, compared to 87% for the untreated control. The organic amendments effective at the rate of 1% were fish meal, CF-5, Perlka™, and S-H mixture. Fish meal, CF-5, and Perlka™ also suppressed carpogenic germination of sclerotia at the lowest rate of 0.5%. A concentration effect was observed for some, but not all of the treatments. For example, canola straw was effective in reducing carpogenic germination of sclerotia at concentrations of 3 or 2%, but not at 1 or 0.5%, and the frequency of germinated sclerotia was inversely proportional to the concentration of amendment applied to the soil (Table 1). In contrast, fish meal was effective at all concentrations from 0.5 to 3%, whereas cattle manures were ineffective for all the concentrations tested.

In the second study, sweet clover straw, canola straw, fish meal, and distiller's dry grain significantly (P<0.05) reduced carpogenic germination of sclerotia of *S. sclerotiorum* at amendment rates of 1 and 3%, whereas bone meal and raw cattle manure did so only at the rate of 3% (Table 2). The frequency of germinated sclerotia for the effective amendments ranged from 0 to 42%, compared to 76% for the untreated control. Significant reductions in the number of apothecia per germinated sclerotium were observed for the treatments of bone meal (3%), distiller's dry grain (1 or 3%), canola straw (3%), raw cattle manure (3%), fish meal (1 or 3%), and sweet clover straw (3%). The number of apothecia per germinated sclerotium for the effective treatments ranged from 0 to 1.8, compared to 3.6 for the untreated control. Fish meal (1 or 3%) and distiller's dry grain (3%) completely prevented carpogenic germination and apothecial production of *S. sclerotiorum*.

Testing of different composted and non-composted mixtures of organic residues in the third study revealed that all of the mixtures were effective (P<0.05) in suppressing carpogenic germination of *S. sclerotiorum* when applied at the rate of 3%, and all except mixture 2 were effective when applied at the rate of 1% (Table 3). The frequency of germinated sclerotia for the effective mixtures ranged from 0 to 46%, compared to 79% for the untreated control. Significant reductions in the number of apothecia per germinated sclerotium were observed for all of the mixtures except mixture 6 at the rate of 3%, and for none of the mixtures at the rate of 1%. The number of apothecia per germinated sclerotium for the effective mixtures ranged from 0 to 1.2, compared to 3.3 for the untreated control. Mixture 1 (wheat straw, raw cattle manure, distiller's dry grain and fish meal mixed at 3:4:2:1 ratio, non composted) and mixture 3 (canola straw, canola screenings and raw cattle manure mixed at 2:1:4 ratio, non composted) completely prevented carpogenic germination and production of apothecia of *S. sclerotiorum* when amended with soil at the rate of 3%.

Results of testing microbial infested organic matters for soil amendments in the fourth study showed that all of the treatments significantly (P<0.05) reduced carpogenic germination of sclerotia of *S. sclerotiorum* at both rates of 1 and 3% (Table 4). The frequency of germinated sclerotia for the effective treatments ranged from 0 to 60%, compared to 86% for the untreated control. Significant reductions in the number of apothecia per germinated sclerotium were observed for all of the treatments at both amendment rates. The number of apothecia per germinated sclerotium for the treatments of soil amended with microbial infested organic matters ranged from 0 to 3.0, compared to 5.3 for the untreated control. Soil treatment with mixtures of *T. virens*/cattle manure/wood chips or *T. virens*/cattle manure/wheat straw completely prevented carpogenic germination and production of apothecia of *S. sclerotiorum*.

In the studies where NO_3^- and NH_4^+ levels and C:N ratios were determined, reductions in carpogenic germination of sclerotia of *S. sclerotiorum* were often accompanied by elevated levels of nitrogen compounds, resulting in reduced C:N ratios (Tables 2, 3, and 4). For example, amendment of soil with fish meal at the rate of 3% resulted in a C:N ratio of 7.3, compared to 16.7 in the untreated control (Table 2). No significant effect of amendment with any of the agricultural residues was observed on pH, EC, or total C. Also, all of the ungerminated sclerotia retrieved at the end of each of the first three experiments were nonviable. In the fourth study with soil amended with organic matters infested with *C. minitans* or *T. virens*, the sclerotia were nonviable and frequently colonized by the mycoparasites.

DISCUSSION

This study demonstrates that organic soil amendment with agricultural residues can be an effective method for controlling carpogenic germination of sclerotia of *S. sclerotiorum* and therefore reducing the production of ascospores, the primary source of inoculum for sclerotinia diseases on numerous crops. Moreover, the study further demonstrates that some agricultural wastes such as crop straws or animal manures can be made into valuable commodities for pulse crop production because of their suppressive effects to this important pathogen, and can thereby reduce the effort required for disposal of such agricultural residues.

The elevated levels of N compounds and corresponding reductions of C:N ratio in the effective treatments such as fish meal suggest that the mode of action for suppression of carpogenic germination of sclerotia of *S. sclerotiorum* in these amendments may be related to the production of ammonia and ammonia-related compounds. Huang and Janzen [11] found that application of urea to soil was effective in preventing carpogenic germination of sclerotia of *S. sclerotiorum* that were not in direct contact with the soil, and they suggested that ammonia was the key toxic component for the

Table 1. Effect of organic matters on carpogenic germination of sclerotia of *Sclerotinia sclerotiorum*. (growth cabinet experiments).

Category	Treatment	Amendment Concentration (%) [1]				
		3	2	1	0.5	0
		Germinated sclerotia (%)				
Control		-	-	-	-	87
Crop straws	Alfalfa	35*	75	70	93	-
	Amaranth	0*	90	97	100	-
	Barley (cv. Harrington)	41*	68	83	85	-
	Bean, Black	17*	51	61	68	-
	Bean, Small Red	22*	48*	70	71	-
	Borage	38*	65	67	62	-
	Buckwheat	0*	78	87	93	-
	Canary Grass	49*	83	78	90	-
	Canola, Argentine	7*	23*	57	90	-
	Canola, Polish	10*	22*	67	92	-
	Chickpea	13*	27*	53	60	-
	Cilantro	20*	50	50	50	-
	Coriander	23*	63	63	80	-
	Corn, Dwarf	38*	67	78	83	-
	Dill	13*	53	53	73	-
	Fababean	30*	36*	59	77	-
	Fenugreek	10*	83	93	97	-
	Flax	37*	83	85	78	-
	Lathyrus	0*	12*	87	97	-
	Lentil (cv. Eston)	17*	93	97	100	-
	Lentil (cv. Laird)	0*	17*	51	57	-
	Lentil (cv. CDC Richlea)	6*	29*	57	64	-
	Lupine	37*	43*	80	87	-
	Mustard, Brown	23*	72	95	95	-
	Mustard, Oriental	49*	72	81	75	-
	Mustard, Yellow	10*	80	95	93	-
	Oats (cv. Mustang)	17*	72	78	73	-
	Pea (cv. Carneval)	2*	74	78	80	-
	Pea (cv. Trapper)	10*	75	91	82	-
	Quinoa	0*	5*	68	63	-
	Rye	0*	80	75	78	-
	Safflower	35*	37*	70	90	-
	Soybean	20*	43*	60	70	-
	Sunwheat	17*	45*	58	62	-
	Sweet Clover	27*	45*	60	63	-
	Wheat	32*	68	73	72	-
Animal manures	Hog manure, raw	17*	88	77	92	-
Processing by-products	Blood meal	8*	50	67	72	-
	Bone meal	15*	32*	62	62	-
	Canola meal	0*	17*	90	100	-
	Canola screenings	31*	50	97	100	-
	Distiller's Dry Grain	0*	10*	97	100	-
	Fish meal	0*	0*	0*	47*	-
Commercial preparations	CF-5 liquid	0*	0*	0*	0*	-
	Perlka™ solid	2*	0*	3*	0*	-
	SH solid mixture	0*	0*	2*	70	-

[1.] Data presented are the results of three runs.

* Significant reduction of carpogenic germination (Duncan's multiple range test, $P<0.05$).

Table 2. Effect of soil amendment with agricultural residues on carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* and soil properties (growth cabinet experiments [1]).

Treatment	Germinated sclerotia (%) [2]	No. apothecia per sclerotium [2]	NO_3^- (ppm) [2]	NH_4^+ (ppm) [2]	C:N ratio [2]
Control	76 ab	3.6 ab	15 gh	12 d	16.7 bcd
Sweet clover straw, 1%	42 cd	2.3 a-e	21 fgh	32 d	17.2 bcd
Sweet clover straw, 3%	2 e	0.8 ef	5 gh	175 c	13.4 de
Canola straw, 1%	39 cd	2.0 b-e	54 c-f	7 d	16.8 bcd
Canola straw, 3%	4 e	0.2 f	103 b	77 d	14.4 cde
Bone meal, 1%	86 a	3.6 ab	7 gh	13 d	18.9 bc
Bone meal, 3%	41 cd	1.8 cde	13 gh	11 d	14.7 cde
Fish meal, 1%	0 e	0 f	191 a	54 d	13.1 de
Fish meal, 3%	0 e	0 f	2 h	1023 a	7.3 f
Distiller's dry grain, 1%	27 de	1.2 def	80 bc	10 d	15.0 cde
Distiller's dry grain, 3%	0 e	0 f	4 gh	441 b	11.7 e
Manure [3], 1%	57 bc	4 a	6 gh	7 d	17.6 bcd
Manure [3], 3%	20 de	1.4 def	14 gh	50 d	17.1 bcd
Manure [3] + straw [4], 1%	73 ab	3.5 abc	34 e-h	6 d	23.3 a
Manure [3] + straw [4], 3%	48 bcd	2.8 a-d	42 d-g	7 d	17.1 bcd
Manure [3] + wood chips, 1%	64 abc	2.7 a-d	75 bcd	23 d	20.1 ab
Manure [3] + wood chips, 3%	48 bcd	2.5 a-e	61 cde	15 d	18.1 bc
Standard Error (8 df)	8.7	0.5	6.4	12.3	0.8

[1.] Data presented are the results of two runs.
[2.] Means within a column followed by the same letter are not significantly different at $P<0.05$ level (Duncan's multiple range test).
[3.] Raw cattle manure.
[4.] Wheat straw.

Table 3. Effect of formulated organic soil amendments on carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* and soil properties (growth cabinet experiments [1]).

Treatment [2]	Germinated sclerotia (%) [3]	No. apothecia per sclerotium [3]	NO_3^- (ppm) [3]	NH_4^+ (ppm) [3]	C:N ratio [3]
Control	79 a	3.3 ab	67 e	14 b	17.4 a
Mixture 1, 1%	40 bcd	3.4 ab	128 cd	16 b	14.9 c
Mixture 1, 3%	0 e	0 e	139 c	25 b	12.9 d
Mixture 2, 1%	63 ab	3.6 a	19 f	16 b	16.3 ab
Mixture 2, 3%	16 de	0.8 de	83 de	20 b	15.8 bc
Mixture 3, 1%	39 bcd	2.8 a-d	81 e	16 b	14.7 c
Mixture 3, 3%	0 e	0 e	345 a	22 b	12.4 d
Mixture 4, 1%	36 bcd	2.6 abc	7 f	15 b	16.7 ab
Mixture 4, 3%	19 cde	1.2 cde	1 f	41 a	16.9 ab
Mixture 5, 1%	40 bcd	2.9 abc	95 cde	13 b	14.8 c
Mixture 5, 3%	3 e	0.3 e	267 b	23 b	13.1 d
Mixture 6, 1%	46 bc	3.1 ab	11 f	16 b	17.1 ab
Mixture 6, 3%	19 cde	1.7 b-e	3 f	28 b	17.3 a
Standard Error (8 df)	8.9	0.6	7.7	2.2	0.3

[1.] Data presented are the results of two runs.
[2.] Composition of mixtures:
Mixture 1 = wheat straw, raw cattle manure, distiller's dry grain, fish meal (3:4:2:1); not composted.
Mixture 2 = wheat straw, raw cattle manure, distiller's dry grain, fish meal (3:4:2:1); composted 60 d.
Mixture 3 = canola straw, canola screenings, raw cattle manure (2:1:4); not composted.
Mixture 4 = canola straw, canola screenings, raw cattle manure (2:1:4); composted 60 d.
Mixture 5 = canola straw, canola screenings, chicken pen scrapings (2:1:4); not composted.
Mixture 6 = canola straw, canola screenings, chicken pen scrapings (2:1:4); composted 60 d.
[3.] Means within a column followed by the same letter are not significantly different at $P<0.05$ level (Duncan's multiple range test).

Table 4. Effect of combined application of biocontrol agents and organic matters on carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* and soil properties (growth cabinet experiments [1]).

Treatment [2]	Germinated sclerotia (%) [3]	No. apothecia per sclerotium [3]	NO_3^- (ppm) [3]	NH_4^+ (ppm) [3]	C:N ratio [3]
Control	86 a	5.3 a	34 d	9 b	19.9 abc
Penicillium + wheat, 1%	60 b	3.0 b	85 b	5 b	18.0 b-e
Penicillium + wheat, 3%	42 bc	2.7 b	144 b	11 ab	16.6 e
Penicillium + wood, 1%	52 bc	2.8 b	72 bc	17 ab	20.4 ab
Penicillium + wood, 3%	40 bc	2.7 b	139 a	12 ab	17.7 cde
Trichoderma + wheat, 1%	0 e	0 e	22 d	13 ab	17.4 de
Trichoderma + wheat, 3%	0 e	0 e	26 d	17 ab	16.9 e
Trichoderma + wood, 1%	0 e	0 e	23 d	16 ab	18.9 a-e
Trichoderma + wood, 3%	0 e	0 e	30 d	17 ab	18.2 b-e
Coniothyrium + wheat, 1%	39 bc	2.4 bc	26 d	5 b	17.2 e
Coniothyrium + wheat, 3%	13 de	0.9 cde	19 d	12 ab	17.2 e
Coniothyrium + wood, 1%	32 cd	1.7 bcd	27 d	14 ab	19.7 a-d
Coniothyrium + wood, 3%	3 e	0.2 de	49 cd	28 a	20.9 a
Standard Error (8 df)	8.1	0.5	5.3	2.9	0.4

1. Data presented are the results of two runs.
2. *Coniothyrium* = *C. minitans* isolate LRC 2137; *Trichoderma* = *T. virens* isolate LRC 2425; *Penicillium* = *P. griseofulvum* isolate LRC 2461.
3. Means within a column followed by the same letter are not significantly different at $P<0.05$ level (Duncan's multiple range test).

suppression of this pathogen. Although soil amendment with some organic debris alone, composted or non-composted, was suppressive to *S. sclerotiorum*, the present study reveals that such a pathogen-suppression effect can be further enhanced by soil amendment with organic debris infested with *C. minitans* or *T. virens* (Table 4), which are biocontrol agents of *S. sclerotiorum* [8,18]. This suggests that toxic substances from organic matter and mycoparasitism by the biocontrol agents may have contributed to the enhancement of control of *S. sclerotiorum*.

The loss of viability of ungerminated sclerotia in the soil amended with certain organic matters indicates that the effect of such treatments is fungicidal and not fungistatic. This suggests that, in addition to suppressing *S. sclerotiorum*, amendment of soil with agricultural residues might have additional long-term benefits of reducing populations of sclerotia, which are overwintering bodies of *S. sclerotiorum*. The implications of this for agricultural producers are great, since it may represent an effective means of reducing disease pressure and rehabilitating *S. sclerotiorum*- infested fields.

The lack of effects of organic soil amendment on pH, EC, and total C implies that the organic materials used in this study may have no significant negative effects on soil health and plant growth. Thus, the organic soil amendments identified as effective in this study offer a means for environmentally sustainable management of sclerotinia diseases. New studies have been initiated at LRC to determine if the superior organic soil amendment methods identified in this study are effective for controlling apothecial production of *S. sclerotiorum* in the field and thereby, reducing incidence of white mold of bean.

ACKNOWLEDGEMENTS

This study was funded by Farming for the Future Research Project No. 19990073, Alberta Agricultural Research Institute, Edmonton, Alberta, Canada. The authors thank K. Hironaka and G. Travis for technical assistance. This is LRC Contribution No. 387-02086.

LITERATURE CITED

1. Bogdanova, V.N., Karadzhova, L.V. and Klimenko, T.F. 1986. Use of *Coniothyrium minitans* Campbell as a hyperparasite in controlling the pathogen of white rot of sunflower. Sel'skokhozyaistvennaya Biologiya (Agr. Biol.) 5:80-84.
2. Budge, S.P. and Whipps, J.M. 1991. Glasshouse trials of *Coniothyrium minitans* and *Trichoderma* species for the biological control of *Sclerotinia sclerotiorum* in celery and lettuce. Plant Pathol. 40:59-66.
3. Campbell, W.A. 1947. A new species of *Coniothyrium* parasitic on sclerotia. Mycologia 39:190-195.
4. Gugel, R.K. and Morrall, R.A.A. 1986. Inoculum-disease relationships in Sclerotinia stem rot of rapeseed in Saskatchewan. Can. J. Plant Pathol. 8:89-96.
5. Gulya, T.J. and Vick, B.A. 1986. Sclerotinia head rot of sunflower in North Dakota: 1986 incidence, effect on yield and oil components, and sources of resistance. Plant Dis. 73:504-507.
6. Huang, H.C. 1977. Importance of *Coniothyrium minitans* in survival of sclerotia of *Sclerotinia sclerotiorum* in wilted sunflower. Can. J. Bot. 55:289-295.

7. Huang, H.C. 1980. Control of sclerotinia wilt of sunflower by hyperparasites. Can. J. Plant Pathol. 2:26-32.
8. Huang, H.C. and Erickson, R.S. 2000. Soil treatment with fungal agents for control of apothecia of *Sclerotinia sclerotiorum* in bean and pea crops. Plant Pathol. Bull. 9:53-58.
9. Huang, H.C. and Huang, J.W. 1993. Prospects for control of soilborne plant pathogens by soil amendment. Current Topics in Bot. Research 1:223-235.
10. Huang, H.C., Huang, J.W., Saindon, G. and Erickson, R.S. 1997. Effect of allyl alcohol and fermented agricultural wastes on carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* and colonization by *Trichoderma* spp. Can J. Plant Pathol. 19:43-46.
11. Huang, H.C. and Janzen, H.H. 1991. Control of carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* by volatile substances from urea. Plant Prot. Bull. 33:283-289.
12. Huang, H.C. and Kokko, E.G. 1992. Pod rot of dry peas due to infection by ascospores of *Sclerotinia sclerotiorum*. Plant Dis. 76:597-600.
13. Huang, H.C., Kokko, M.J. and Phillippe, L.M. 1988. White mold of dry bean (*Phaseolus vulgaris* L.) in southern Alberta, 1983-87. Can. Plant Dis. Surv. 68:11-13.
14. Huang, H.C. and Kozub, G.C. 1989. A simple method for production of apothecia from sclerotia of *Sclerotinia sclerotiorum*. Plant Prot. Bull. 31:333-345.
15. Huang, H.C. and Sun, S.K. 1991. Effects of S-H mixture or Perlka™ on carpogenic germination and survival of sclerotia of *Sclerotinia sclerotiorum*. Soil Biol. Biochem. 23:809- 813.
16. Huang, J.W. and Huang, H.C. 2000. A formulated container medium suppressive to Rhizoctonia damping-off of cabbage. Bot. Bull. Acad. Sin. 41:49-56.
17. McLaren, D.L., Huang, H.C., Kozub, G.C. and Rimmer, S.R. 1994. Biological control of Sclerotinia wilt of sunflower with *Talaromyces flavus* and *Coniothyrium minitans*. Plant Dis. 78:231-235.
18. McLaren, D.L., Huang, H.C. and Rimmer, S.R. 1996. Control of apothecial production of *Sclerotinia sclerotiorum* by *Coniothyrium minitans* and *Talaromyces flavus*. Plant Dis. 80:1373-1378.
19. Muendel, H.H., Huang, H.C. and Kozub, G.C. 1985. Sclerotinia head rot in safflower: Assessment of resistance and effects on yield and oil content. Can. J. Plant Sci. 65:259-265.
20. Purdy, L.H. 1979. *Sclerotinia sclerotiorum*: History, diseases and symptomatology, host range, geographic distribution and impact. Phytopathology 69:875-880.
21. SAS Insitute Inc. 1989. SAS/STAT® User's Guide, Version 6, 4th Ed. SAS Institute Inc., Cary, North Carolina. 1686 pp.
22. Shiau, F.L., Chung, W.C., Huang, J.W. and Huang, H.C. 1999. Organic amendment of commercial culture media for improving control of *Rhizoctonia* damping-off of cabbage. Can. J. Plant Pathol. 21:368-374.
23. Steadman, J.R. 1983. White mold - A serious yield-limiting disease of bean. Plant Dis. 67:346-350.
24. Sun, S.K. and Huang, J.W. 1985. Formulated soil amendment for controlling *Fusarium* wilt and other soil-borne diseases. Plant Dis. 69: 917-920.

摘 要

黃鴻章[1,3]、R. S. Erickson [1]、張治[1]、J. R. Moyer [1]、F. J. Larney [1]、黃振文[2]. 2002. 應用土壤有機添加物防治菌核病菌 (*Sclerotinia sclerotiorum*) 產生子囊盤. 植病會刊 11:207-214. ([1.] 加拿大農部 Lethbridge 研究中心；[2.] 台灣國立中興大學植病系；[3.] 聯絡作者：電子郵件：Huangh@em.agr.ca；傳眞：+0021-403-382-3156)

本研究旨在應用一系列室內實驗來探討土壤有機添加物防治菌核病菌 (*Sclerotinia sclerotiorum*) 產生子囊盤 (apothecia) 的效果。將 87 種農業有機殘留物，包括作物莖葉，動物糞便及加工副產品等進行測試篩選，結果顯示不同有機物抑制菌核產生子囊盤的效果與它們施用濃度間呈顯著相關性。其中在添加濃度 3%，2%，1% 及0.5% (w/w) 下，具有抑制功效的有機物種類，分別爲46 種，21 種，4 種及 3 種。進一步，將供試土壤進行分析，結果顯示有機物分解後，釋放出氨氣可抑制子囊盤的產生。將有機添加物與 *Coniothyrium minitans* 或 *Trichoderma virens* 等生物防治菌混合使用，則更具有抑制菌核產生子囊盤的效果。本研究顯示混合使用有機物及重寄生菌防治作物菌核病，是一種頗具環保的防病策略。

關鍵詞：菌核病菌、菌核、子囊盤、土壤添加物、生物防治、*Coniothyrium minitans*、*Trichoderma virens*、*Penicillium griseofulvum*