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# Causal Agents of Root Rot and the Effect of Vesicular-Arbuscular Mycorrhizal Fungi in Seedlings of *Rhodiola rosea* in Alberta, Canada

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Abstract: Rhodiola (*Rhodiola rosea*) is a plant with adaptogenic properties and is suitable for cultivation in Alberta, Canada. Disease surveys indicated the occurrence of root rots in rhodiola plantations in the Province. A total of 74 fungal isolates were associated with discoloration and rotting in the crown and root regions of the plants. Among these, 15 isolates were identified as *Fusarium* sp., three as *Pythium* sp. and eight as *Rhizoctonia* sp. This is the first report of root rot in rhodiola in Alberta, Canada. These soil-borne pathogens are the potential threat to the quality and quantity of rhodiola production. Experiments were conducted to determine the effect of vesicular-arbuscular mycorrhizal fungi and these soil pathogens on rhodiola growth and development under greenhouse conditions. Overall results indicated that *Fusarium*, *Pythium* and *Rhizoctonia* sp. are all capable of reducing rhodiola biomass. However, biomass was significantly higher when vesicular-arbuscular mycorrhizal fungi were applied in conjunction with these pathogens or in non-inoculated controls. This suggests that vesicular-arbuscular mycorrhizal fungi could be used as a management tool for the control of seedling root rot diseases of rhodiola.

Key words: Rhodiola rosea, root rot, pathogenicity, biological control, vesicular-arbuscular mycorhizal fungi

## INTRODUCTION

Rhodiola rosea L. (roseroot, golden root or arctic root) is a yellow-flowered herbaceous perennial plant of the Crassulaceae family. The plant is a circumpolar species of cool temperate and sub-arctic areas of the Northern hemisphere, including North America, Greenland, Iceland and the Altai, Tien-Shan and Himalaya mountains in Asia. The European distribution includes Scandinavia and most of the mountains of Central Europe.

Rhodiola is a multipurpose medicinal herb with adaptogenic properties that increase the body's nonspecific resistance and normalize body functions (Brown et al., 2002; Galambosi, 2005).

Based on the documented beneficial pharmacological effects and product safety, commercial interest in roseroot-based products has increased worldwide. Commercial development, however, has been hindered by insufficient quantities of rhodiola to meet the growing industrial demand. Rhodiola cultivation is the only

solution to this lack of raw materials for the production of roseroot-based products. Rhodiola cultivation experiments are being conducted at various locations in Alberta, since the province has an environment that is ideal for the growth of this crop (Ampong-Nyarko, 2004). To cultivate rhodiola, the seedlings need to be established in a greenhouse and transplanted to the field. Therefore, the crop requires a 4-5 year commitment of time, land and labour before it reaches harvestable maturity.

As with other plant species, *R. rosea* is susceptible to attack by various pathogenic microorganisms. However, there have been few plant pathological studies on rhodiola (Hwang *et al.*, 2007) because, until recently, rhodiola has not been extensively grown as a crop. Losses due to disease may be a critical factor in adoption of the crop by Alberta producers. Root rot has caused particular concern because the crop derives its economic value from its roots. Since, rhodiola needs a long time from planting to harvesting, soil-borne pathogens are of

prime importance and monitoring their prevalence and identification of the pathogens are necessary for successful disease management.

Vesicular-Arbuscular Mycorrhizal (VAM) fungi are reported to be effective biological control agents for the control of many soil-borne fungal root diseases, including those caused by *Fusarium*, *Pythium* and *Rhizoctonia* sp. (Chang *et al.*, 2006; Hwang, 1988; Hwang *et al.*, 1992; Yao *et al.*, 2002). Since, no research results are available on the interaction of VAM, root rot pathogens and rhodiola, the objectives of this study were to: (1) isolate and identify mycoflora associated with discoloured and diseased rhodiola roots, (2) evaluate the effect of root-associated pathogens on rhodiola seedlings and (3) understand the influence of VAM and root pathogens on rhodiola seedlings.

#### MATERIALS AND METHODS

### Collection of diseased plant samples and fungal isolation:

Plantations of rhodiola were surveyed for the presence of diseased plants in the greenhouse and experimental plots at Crop Diversification Center (CDC) North, Edmonton, Alberta and in farmers' fields in the spring of 2005, 2006 and 2007. The plots at each site were checked for the presence of diseased or dead plants. A total of 50 rhodiola seedlings showing disease lesions on the roots were collected from several locations in central Alberta. Roots and stem bases bearing distinct lesions were washed thoroughly in running tap water and cut into 2×2 mm pieces. Ten pieces of discoloured root tissue from each of the 50 plant samples were surface-disinfested in 0.6% sodium hypochlorite (v/v) for 1 min, rinsed in sterile distilled water and plated on pentachloronitrobenzene (PCNB) medium (Nash and Snyder, 1962) and potato dextrose agar acidified with lactic acid to pH 5.0 (APDA). After seven to 10 days, hyphal tips of representative isolates (based on colony characteristics) were transferred onto fresh Potato Dextrose Agar (PDA) tubes. Fusarium isolates were transferred from the PDA slants onto Carnation Leaf Agar (CLA) (Leslie and Summerell, 2006) and PDA (BD/Difco, Sparks, MD) for identification. The isolates were incubated at room temperature under fluorescent light with a 16/8 h photoperiod. Observations of the cultural and microscopic characteristics of the isolates were recorded 10 to 14 days after plating. Isolates were identified using the taxonomic key of Nelson et al. (1983) and the additional species descriptions of Leslie and Summerell (2006). Pythium isolates were identified to the genus level, by colony morphology on APDA and Rhizoctonia isolates were identified to the species level based on colony morphology on PDA and anastomosis grouping as described below.

Anastomosis grouping of Rhizoctonia isolates: Anastomosis grouping of Rhizoctonia isolates was conducted using tester isolates representing AG-1, AG-2-1, AG-2-2, AG-3, AG-4, AG-5, AG-6, AG-7, AG-8 and AG-9 of R. solani. Tests were conducted on a clean slide in Petri dishes, which served as moisture chambers. Slides were dipped into 70% ethanol, flamed and placed on top of two pieces of water agar. Agar plugs of the tester strains and the isolate to be tested were placed on the slide, 2 cm apart. Five milliliters of sterilized distilled water were added to each dish to provide high humidity. At least two slides were prepared for each isolate. Petri dishes were incubated at 25°C and growth was monitored every 24 h. When hyphae of the two isolates touched, slides were removed from the Petri dishes and examined under a microscope to determine whether anastomosis had occurred.

# Effect of Fusarium, Pythium and Rhizoctonia inoculum on rhodiola

Rhodiola seedlings and growth conditions: Rhodiola seedlings were grown in a greenhouse on Metro-Mix® (Sun-Gro Horticulture, Vancouver, BC) in 96-cell (3×3 cm) plastic trays (ITML Horticultural Products, Brantford, ON). Surface-sterilized rhodiola seeds were sown and covered with a thin layer of the soil mixture. The seedlings were grown for 30 days and then transplanted into 450 mL plastic cups (Georgia-Pacific Canada Ltd., Edmonton, AB) filled with pasteurized Metro-mix, at a rate of three seedlings per cup during inoculation. The experiment was conducted under greenhouse conditions at 20±5°C temperature and a 16/8 h photoperiod.

**Inoculum preparation:** Mycelial inoculum of 15 Fusarium isolates, three Pythium isolates and eight Rhizoctonia isolates was prepared in 250 mL Erlenmeyer flasks containing 100 mL of sterile Potato Dextrose (PD) broth. The PD broth was inoculated with a 9 mm diameter mycelial agar disc from a 7 day old culture and shaken continuously at 200 rpm for seven days at room temperature (20°C) under ambient light. The culture in each flask was comminuted for 15 sec in a waring blender. For each isolate, 20 mL of the inoculum was poured into pots containing a steam-pasteurized soil mixture during seedling transplanting. Non-inoculated PD broth was used as a control. Three separate experiments were conducted for the three pathogens. For each experiment ten replicate pots were used for each treatment (isolate) in every repetition of the experiments. The experiments were

repeated once. All pots in an experiment were randomly arranged on a greenhouse bench in a Complete Randomized Design (CRD). Two months after inoculation data on plant height, shoot vigour and shoot dry mass were recorded. Shoot vigour was assessed subjectively on a scale of 1-4 where, 1 = poor, 2 = fair, 3 = fairly good and <math>4 = good. Re-isolations were made from the roots of diseased plants to confirm the pathogenicity of the isolates.

**Data analysis:** Prior to analysis of variance, data were tested for homogeneity using a normal probability plot. The data distributions for the three pathogens were normal. Analysis of Variance (ANOVA) of data was performed following the General Linear Model procedure using SAS Statistical software (SAS Institute Inc., 2005). The trial×treatment (isolate) interaction was not significant for any of the pathogens at  $p \le 0.05$ . Therefore, the data were pooled over the trials before analysis. The means separation was performed using Duncan's new multiple range test ( $p \le 0.05$ ).

Influence of VAM and root pathogens on rhodiola **seedlings:** To test the interaction of *Fusarium avenaceum* (isolate RD18), Pythium (isolate RD50) and Rhizoctonia solani (RD12) (one isolate of each pathogen) with VAM (Glomus intraradices) (Myke Pro®; Premier Tech Biotechnologies, Rivière-du-Loup QC), three separate experiments (one for each pathogen) were conducted under greenhouse conditions. This VAM strain has been found to provide effective control of Fusarium, Pythium and Rhizoctonia root disease in other crops (Chang et al., 2006; Hwang, 1988; Hwang et al., 1992). The rhodiola plants used for these experiments were grown and transplanted as described above. The treatments in each experiment included: (1) pathogen+VAM, (2) no pathogen +VAM, (3) pathogen+no VAM and (4) no pathogen+no VAM. The treatment that included pathogen and VAM was inoculated with 5 mL cup<sup>-1</sup> of each. The pathogen was grown on sterilized wheat grains for 15 days and then dried at 29°C and ground to powder. The VAM or pathogen inoculum was applied in a 5 mL volume to the top of the soil when the seedlings were growing in the 450 mL plastic cups, as described above. Two months after inoculation, the plants were harvested, the roots washed under tap water and data on shoot dry mass, root dry mass and shoot length and shoot dry mass were recorded and analyzed. Shoot vigour was assessed following the same scale (1-4) as described above.

Data analysis: Exploratory analysis using a normal probability plot indicated heterogeneity in the original distribution of the three sets of data obtained with the three pathogens to determine their effect on rhodiola. The data were square root transformed to provide the best fit for a normal distribution. Analysis of variance (ANOVA) was performed following the General Linear Model (SAS Institute Inc., 2005) procedure. The class variables included trial, treatment and trial × treatment interactions; the response variables included plant number, shoot length, shoot dry mass and root dry mass. Since, there were significant trial×treatment interactions (although not for all response variables) in the data sets of the three pathogens, the data were analyzed separately by trial. The means separation was performed using Fisher's Protected Least Significant Difference (LSD) at p≤0.05.

#### RESULTS

### Isolation and identification of root-associated mycoflora:

A total of 74 fungal isolates were isolated from the roots and crowns of plant samples exhibiting necrosis or discoloration. Among the isolates, 15 were identified as *Fusarium* sp., three were *Pythium* sp. and eight isolates were *Rhizoctonia solani*. The remaining isolates were not identified and not included in the study. Among the *Fusarium* isolates, three were classified as *F. avenaceum*, two as *F. culmorum* and two as *F. equiseti* (Fig. 1a-c), whilst the other *Fusarium* isolates could not be identified to the species level because of an absence of identifying characteristics. Anastomosis testing indicated that all eight *R. solani* isolates belonged to AG-4. The *Pythium* isolates were not identified at the species level.

Effect of Fusarium, Rhizoctonia and Pythium on rhodiola: Analysis of variance indicated that plant height, shoot vigour and shoot dry mass were significantly reduced in inoculated plants relative to the noninoculated control. There also were differences among individual isolates of Fusarium and Rhizoctonia (Table 1, 2), but no significant differences were observed among Pythium isolates (Table 3). In the case of seedlings inoculated with Fusarium sp., plant heights ranged from 1.8-3.3 cm, shoot vigour from 1.5-2.8 and shoot dry mass from 0.19 -0.57 g, two months after inoculation (Table 1). In the case of R. solani-inoculated seedlings, the plant heights ranged from 1.9-3.3 cm, shoot vigour from 1.5-2.5 and shoot dry mass from 0.15-0.42 g (Table 2). In the case of seedlings inoculated with Pythium, plant height ranged from 2.0-2.4 cm, shoot vigour from 1.5-1.8 and shoot dry mass from 0.21-0.33 g (Table 3).

Response of VAM and root pathogens on rhodiola seedlings: With few exceptions, there were significant differences among the variables for all pathogens in the

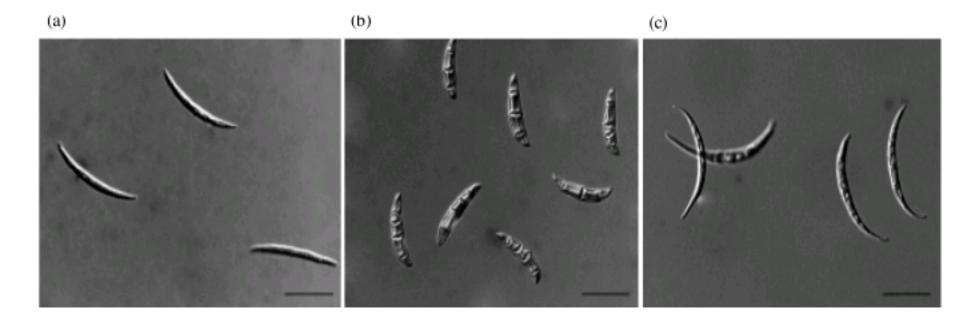


Fig. 1: Fusarium sp. associated with Rhodiola rosea root rot in Alberta. (a), Fusarium avenaceum, (b) Fusarium culmorum and (c) Fusarium equiseti). Scale bar = 25 μm

Table 1: Effect of Fusarium sp. on rhodiola height, shoot vigour and shoot dry mass when grown under greenhouse conditions

Fusarium	Plant height	Shoot vigour	Shoot dry
isolate code	(cm)	(1-4 scale)	mass (g)
RD17	2.4cde	2.3cd	0.38bcde
RD18	1.8ef	1.8de	0.30cdef
RD20	2.3cdef	2.4cd	0.31cdef
RD24	1.8f	1.5e	0.19f
RD25	1.8f	1.8de	0.23ef
RD26	2.6cd	2.5bc	0.42abcd
RD27	2.1def	2.0cd	0.24ef
RD28	2.6cd	2.4bc	0.38bcde
RD31	2.1def	1.8de	0.23ef
RD49	2.6cd	2.2cd	0.42abcd
RD65	2.5cd	2.2cd	0.27def
RD68	2.0cdef	2.4bc	0.45abc
RD86	2.8bc	2.8b	0.49ab
RD95(1)	3.3b	2.8b	0.57a
RD95(2)	2.2def	2.0cd	0.30cdef
Control	4.3a	3.2a	0.56a

Data are the means of 10 replications. Means followed by the same letter(s) are not significantly different according to Duncan's new multiple range test  $(p \le 0.05)$ . Vigour was assessed using 1-4 scale, where, 1 = Poor; 2 = Fair; 3 = Fairly good and <math>4 = Good growth of shoots and roots

Table 2: Effect of Rhizoctonia solani on rhodiola height, shoot vigour and shoot dry mass when grown under greenhouse conditions

Rhizoctonia isolate code	Plant height (cm)	Shoot vigour (1-4 scale) <sup>1</sup>	Shoot dry mass (g)
RD1	2.3cd	2.2cde	0.32bc
RD12	3.2b	2.5bc	0.42b
RD14	2.3cd	1.8ef	0.22cd
RD4	3.0b	2.0de	0.27cd
RD46	3.3b	2.3cd	0.32bc
RD5	2.7b	2.2cde	0.22cd
RD6	2.8b	2.0de	0.25cd
RD9	1.9d	1.5f	0.15d
Control	4.3a	3.3a	0.56a

Data are the means of 10 replications. Means followed by the same letter(s) are not significantly different according to Duncan's new multiple range test ( $p \le 0.05$ ). <sup>1</sup>Vigour was assessed using 1-4 scale, where, 1 = Poor; 2 = Fair; 3 = Fairly good and <math>4 = Good growth of shoots and roots

trial ( $p \le 0.05$ ). In the case of seedlings inoculated with F. avenaceum, the shoot dry mass was significantly higher in the control (no pathogen; no VAM) followed

Table 3: Effect of Pythium sp. on rhodiola height, shoot vigour and shoot dry mass when grown under greenhouse conditions

Pythium	Plant height	Shoot vigour	Shoot dry
isolate code	(cm)	(1-4 scale)	mass (g)
RD42	2.0cd	1.8b	0.26b
RD50	2.4b	1.5b	0.33b
RD52	2.3cd	1.8b	0.21b
Control	4.2a	3.3a	0.53a

Data are the means of 10 replications. Means followed by the same letter(s) are not significantly different according to Fisher's protected least significant difference test (p≤0.05). ¹Vigour was assessed using 1-4 scale, where, 1 = poor; 2 = fair; 3 = fairly good and 4 = good growth of shoots and roots

Table 4: Effects of the interaction of Fusarium avenaceum (isolate RD18) and vesicular-arbuscular mycorrhizal fungi on growth of rhodiola under greenhouse conditions

	Shoot dry	Root dry	Stem length
Treatments	mass (g)	mass (g)	(cm)
Trial 1			
Fusarium + VAM	0.31	0.32b	4.04b
No Fusarium; VAM	0.39	0.40a	5.04a
Fusarium; no VAM	0.29	0.23c	3.90b
No Fusarium; no VAM	0.38	0.39ab	4.52ab
Trial 2			
Fusarium + VAM	0.32c	0.14b	4.92b
No Fusarium; VAM	0.53a	0.13bc	5.60a
Fusarium; no VAM	0.24d	0.08c	3.94c
No Fusarium; no VAM	0.41b	0.25a	5.23ab

Data are the means of 10 replications. Means followed by the same letter(s) are not significantly different according to Fisher's protected least significant difference test ( $p \le 0.05$ )

VAM (Table 4). The lowest shoot dry mass was recorded when the pathogen was inoculated alone in trial 2. The trend was similar in trial 1, although the treatment effects were not statistically significant. In both trials, the root dry mass was highest when VAM was added alone followed by treatments with no VAM or pathogen. This was followed by the treatments with both pathogen and VAM and was the lowest when the pathogen was added alone. A similar trend was observed for stem length.

Table 5: Effects of the interaction of Pythium sp. (isolate RD50) and vesicular-arbuscular mycorrhizal fungi on the growth of rhodiola under greenhouse conditions

under greenhouse conditions				
Treatments	Shoot dry mass (g)	Root dry mass (g)	Stem length (cm)	
Trial 1				
Pythium + VAM	0.40	0.39b	4.63	
No Pythium; VAM	0.41	0.50a	4.96	
Pythium; no VAM	0.30	0.25c	3.75	
No Pythium; no VAM	0.31	0.39b	4.33	
Trial 2				
Pythium + VAM	0.29a	0.12	3.75	
No Pythium; VAM	0.35a	0.14	4.46	
Pythium; no VAM	0.21b	0.12	3.76	
No Pythium; no VAM	0.31a	0.16	4.13	

Data are the means of 10 replications. Means followed by the same letter(s) are not significantly different according to Fisher's protected least significant difference test ( $p \le 0.05$ )

Table 6: Effects of the interaction of Rhizoctonia solani (isolate RD12) and vesicular-arbuscular mycorrhizal fungi on the growth of Rhodiola under greenhouse conditions.

_	Shoot dry	Root dry	Stem length
Treatments	mass (g)	mass (g)	(cm)
Trial 1			
Rhizoctonia + VAM	0.33	0.30a	4.83
No Rhizoctonia; VAM	0.36	0.34a	4.38
Rhizoctonia; no VAM	0.19	0.10b	4.24
No Rhizoctonia; no VAM	0.30	0.35a	4.32
Trial 2			
Rhizoctonia + VAM	0.32b	0.14	4.78a
No Rhizoctonia; VAM	0.45a	0.19	5.32a
Rhizoctonia; no VAM	0.34b	0.16	2.70b
No Rhizoctonia; no VAM	0.42a	0.21	5.08a

Data are the means of 10 replications. Means followed by the same letter(s) are not significantly different according to Fisher's protected least significant difference test ( $p \le 0.05$ )

In the case of seedlings inoculated with *R. solani* in trial 1, the shoot dry mass, the root dry mass and stem length were the lowest when VAM was not added with the pathogen, although shoot dry mass and stem length were not significantly different (Table 5). In trial 2, the shoot dry mass was the same in inoculated treatments whether VAM was applied or not. Similarly, for non-inoculated treatments, the shoot dry mass was similar in both VAM and non-VAM treatments. Similar trend was evident for root dry mass. However, stem length was significantly shorter when VAM was not added to *Rhizoctonia*-inoculated soil.

Shoot dry mass was significantly higher when VAM was added to *Pythium*-inoculated soil compared to pathogen inoculation alone in trial 2 (Table 6). However, the difference in shoot dry mass in trial 1 was not statistically significant. In trial 1, the root dry mass was highest in the treatment with VAM alone. Soil inoculated with *Pythium* sp. alone yielded the lowest root dry mass. In trial 2 the plant survival was reduced by *Pythium* sp. when VAM was not added. No significant differences in stem length were observed.

#### DISCUSSION

Because of the many beneficial pharmacological effects of rhodiola the commercial cultivation of this crop is increasing in Alberta. In this study, we identified *Fusarium* and *Pythium* sp. and *R. solani* as the principal root rot causing pathogens. These soil-borne pathogens could be of particular significance, since it takes 4-5 years after seeding to produce a rhodiola crop and the roots are the economically important organ. All three are common soil-borne pathogens and have wide host ranges (Adam, 1988; Chang *et al.*, 2006; Leslie and Summerell, 2006). They may also remain viable in the soil for extended periods of time and can reduce seedling establishment in other crops (Hwang, 1992; Hwang *et al.*, 2001).

In this study, we identified three Fusarium species, F. avenaceum, F. culmorum and F. equiseti in diseased rhodiola roots. These pathogens may cross-infect other crops like canola (Brassica napus L.), pea (Pisum sativum L.) and wheat (Triticum aestivum L.) and may cause severe stand and yield loss. A study by Zhou et al. (2009) indicated that the predominant anastomosis solani affecting lupin group of Rhizoctonia (Lupinus angustifolius L.) crops was AG-4, followed by AG-2-1 and AG-2-2. In this study, we showed that all of the Rhizoctonia isolates affecting rhodiola belonged to AG-4. Pythium sp. becomes most active moist soils. They are important earlyin cool, season pathogens of canola, pulse crops and forage legumes.

Fusarium and Pythium sp. and R. solani all significantly reduced plant height, shoot vigour and shoot dry mass in rhodiola. Therefore, it is essential to develop a suitable disease management strategy to minimize the impact of these pathogens on rhodiola productivity. Among disease management strategies, seed treatments with fungicides may be one option to control damping off or seedling blight, thereby allowing for better stand establishment (Hwang et al., 2001). Considering the long cultivation period for rhodiola and the need to transplant the seedlings from greenhouse to field, it may be difficult to apply any chemical control measures other than chemical drenching, which has a negative impact on the environment and on the organic nature of the end product.

Vesicular-Arbuscular Mycorrhizal (VAM) fungi, used as biological control agents, have proven to be effective in controlling fungal pathogens (Pal and Gardener, 2006). In this study, while we demonstrated that *Fusarium* and *Pythium* sp. and *R. solani* all are capable of reducing

rhodiola biomass, application of VAM not only ameliorated the effects of these pathogens, improved biomass production in non-infested soils. Shoot dry mass was significantly lower in our study in Pythium-inoculated soil compared with non-inoculated soil. VAM fungi increase efficiency of phosphorus uptake (Chaudhary et al., 2008) which may contribute to the increase in shoot biomass found in this study. Several mechanisms are described how VAM interacts with the root systems to prevent pathogen infections. Pal and Gardener (2006) mentioned that during colonization VAM reduce the infection sites, stimulate host defenses and form an intricate network of fungal hyphae around the roots. This may block pathogen access to infection sites and may have resulted in the increase in biomass found in this study. However, this study suggests that VAM could be useful and appropriate as an organic disease management tool in the cultivation of rhodiola in Alberta. In addition, the VAM fungi are critically important for all crop plants, except for species of the Brassica family. A number of researchers have shown that an absence of VAM inoculum, or the insufficient concentrations of inoculum, can result in poor plant growth, in weak competition with other plants and/or in the inability to reproduce or survive under extreme (Abbott and Robson, 1982; Haas and Krikun, 1985; Fernando and Liderman, 1997). Future studies should be directed towards optimizing the application of VAM fungi, in conjunction with the manipulation of environmental factors such as soil type, soil moisture, soil chemistry and temperature to improve the productivity of rhodiola cultivation in Alberta.

A total of 74 isolates were found to be associated with the root rot symptoms of rhodiola and in this study we included only the Fusarium, Pythium and Rhizoctonia isolates since these pathogens in most cases are the causal organisms of damping off, seedling blight and root rot in Alberta. However, the role of rest of the root rot associated mycoflora warrants investigation to explore their presence with root rot as they may be either beneficial as biocontrol agents or pathogenic to rhodiola. Furthermore, this study was conducted under controlled environmental conditions; field experiments should be conducted to validate the benefit of the use of VAM in the rhodiola cultivation system. We concluded that Fusarium, Pythium and Rhizoctonia are associated with root rot and reduced growth parameters of rhodiola and VAM was found to be effective to combat the negative impact of these pathogens on rhodiola.

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