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Soil Water Pressure Affects Population Dynamics of Biocontrol Agents of *Verticillium dahliae*, the Cause of Potato Early Dying

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Abstract: Studies were conducted to determine the effect of different osmotic potentials on time required for 95% spore germination, mycelial growth and sporulation of *Verticillium dahliae* and its four known biocontrol agents. Time for 95% spore germination increased as osmotic potential of the medium decreased from -0.12 to -8.13 MPa and ranged from 8-29, 16-72, 16-46 and 13-60 h for *Fusarium equiseti, Gliocladium virens, Trichoderma viride* and *Verticillium dahliae*. Ascospores of *Talaromyces flavus* did not obtain 95% germination even after heat treatment at 70°C for one hour. Mycelial growth decreased as osmotic potential of the medium decreased. In a greenhouse study *F. equiseti* reduced colonization of roots by *V. dahliae* and increased foliar dry weight. *Gliocladium virens*, on the other hand, did not have an effect on colonization of roots by *V. dahliae*, it did increase both foliar dry weight and log root/shoot ratio. Fresh root weight, root/shoot ratio and colonization of roots by *G. virens* was greater at -0.15 than at -0.03 MPa whereas infection of roots by *V. dahliae* was reduced at -0.03 MPa. Population density of *F. equiseti* on roots was not affected by soil water pressure.

Key words: Potato soil water pressure, *Verticillium dahliae*, biocontrol, osmotic potential

INTRODUCTION

Potato early dying, caused by *Verticillium dahliae* kleb, is an important disease, limiting potato production in many parts of the world[1-4]. The most common symptoms of the disease are chlorosis, which often is manifested unilaterally in leaves[5] and necrosis, which results in retarded growth[6] followed by premature defoliation. Discoloration of vascular tissues[5] and root deterioration[7] are also commonly associated with the disease. The net result is the reduction in tuber yield and quality as plant senesce after tuber initiation[8].

Crop rotation, resistant cultivars and soil fumigation are the primary practices used for disease management. Because of the broad host range of the pathogen and its persistence in soil for many years[9], crop rotation has not been very effective. Cultivars with resistance to *V. dahliae* have been grown with varying degree of success. Due to long term effects on the environment, including ground water quality and high cost of soil fumigation, concerns have been raised about the use of chemicals[10]. Because of the limitations of the above mentioned strategies, alternative methods for managing this disease were investigated.

Management through biological agents, which can be added directly to the soil to suppress the disease is one such possibility. Further, intelligent manipulation of cultural practices can enhance the effectiveness of biocontrol agents. Of interest in these studies was soil moisture. It has been reported that *T. harzianum* produces a higher hyphal mass under dry conditions[11]. Manipulation of soil water pressure to enhance the efficacy of biocontrol agents, therefore has a potential as a practical disease management strategy. In view of this, present studies were designed to determine the effect of osmotic potential on growth and reproduction of both *V. dahliae* and its biocontrol agents *in vitro* and the influence of soil water pressure alone or in combination with *G. virens* and *F. equiseti* on potato growth, root and vascular colonization by the pathogen and root colonization by biocontrol agents over time.

MATERIALS AND METHODS

Fungal cultures, biocontrol agents and soil: Cultures of *Glicoladium virens* Miller, Giddens and Fester, *Talaromyces flavus* (klocker) Stolk and Samson and *Trichoderma viride* Pres. ex Gray were provided by

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D. Fravel and a culture of *F. equiseti* (corda) sacc. Sense Gordon was provided by O. Huisman. *Verticillium dahliae* kleb. was a mixture of isolates from the Agriculture Research and Extension center Hermiston, Hermiston. The soil was a quincy fine, sandy loam; mixed, mesic, xeric. torripsamment. The soil was steam pasteurized for 1 h at 65°C, air-dried on greenhouse benches for 1 month and then sieved to remove stones and debris.

Mycelial and spore germination studies: For the mycelial growth study, treatments consisted of five fungi (*F. equiseti*, *G. virens*, *T. flavus, T. viridae* and *V. dahliae*) and 10 osmotic potentials (-0.12, -0.18, -0.59, -1.10, -2.15, -3.06, -5.12, -6.17, -7.16 and -8.13 MPa) for a total of 50 treatments. *Talaromyces flavus* was not included in the spore germination study. The factorial combination of treatments was replicated five or four times for the mycelial growth study and spore germination study, respectively.

Malt-Yeast-extract (MYP) medium[12] was adjusted to selected molal concentrations by amending with potassium chloride (KCl). Plates lacking KCl served as the control. The actual osmotic potential of the treatments was determined with an osmometer.

For the mycelial growth experiment, a 5 mm agar plug, cut from the periphery of an actively growing colony of the test fungus, was transferred to the center of each MYP plate. Plates were incubated at 24±2°C for eight days. Growth was determined by measuring the colony diameter along the two perpendicular lines.

For the spore germination experiments, the colony surface of two week old cultures of each fungus grown on Potato Dextrose Agar (PDA), was flooded with 5 mL of Sterile Distilled Water (SDW) and scraped with a rubber spatula. The spore suspension was filtered through cheese cloth and adjusted to a concentration of 10^4 propagules mL^{-1}. Aliquots (200 μL) of the resulting suspension were spread across the surface of MYP agar with a glass rod. Plates were incubated at 25°C. Number of germinated propagules in 15-20 microscopic fields at 10X magnification were determined every 2 h until 95% of the propagules had germinated. A minimum of 200 propagules per plate was counted.

Data were subjected to regression analysis using SAS version 6.04 (Statistical Analysis Systems, SAS institute, Inc. cary, NC). Data values that corresponded to maximum colony diameter were removed from the data set before analysis to avoid censored effects. Linear and curvilinear regression analyses were used to determine the relationship between osmotic potential and mycelial growth or incubation time for each fungus.

Greenhouse study: Single spore cultures of *V. dahliae* were grown on plates containing a modified minimal agar[13] overlain with cellophane. Cultures were incubated for 3-4 week at 22±2°C. The cellophane covered with microclerotia, was scraped from each plate and processed in SDW in a Waring blender for 4-5 min. The slurry was filtered through nested 140 and 175 μm mesh sieves. Microsclerotia collected on the 140 μm sieve were washed with tap water and allowed to dry at room temperature for 48-72 h.

The air–dried inoculum was gathered and mixed with soil in a twin shell blender to obtain the inoculum concentrate. Treatments consisted of two soil water pressures (-0.15 and –0.03 MPa corresponding to 7.8 and 15.4% water per dry weight of soil) combined with no pathogen (control), *V. dahliae* alone or in combination with one of the two biocontrol agents (*G. virens* and *F. equiseti*) for a total of eight treatments. The experiment was designed as a randomized complete block with a factorial combination of treatments replicated eight times for each of the four harvest weeks. The pathogen was added to soil such that the final inoculum density of 25 colony forming units, cfu/g of soil was obtained.

Fusarium equiseti and *G. virens* were grown on wheat grains in glass jars (13 cm diameter), which were incubated at room temperature for two weeks until the grains were covered with the fungus. The grains were then ground in a Wiley mill for approximately two minutes at high speed. The resulting concentration of each biocontrol was added at the rate of 4 g /7100 g of soil to obtain 25 cfu/g of soil.

Inoculum of each fungus was mixed with soil in a cement mixer, which was disinfected with 95% alcohol between treatments. About 7100 g of soil were placed in each 22 cm diameter plastic pot. Based on 7.8 and 15.4% water per weight of soil, enough water was added to each pot to achieve the desired water pressure.

Pre-germinated potato seed pieces were planted into the center of each pot at a depth of 6 cm. The surface of the soil was covered with a 3 cm deep layer of perlite to minimize evaporation. Each pot was weighed daily and water was added to maintain the desired water pressure. Plants were maintained on greenhouse benches under natural daylight at a temperature of 17-30°C.

Plants were harvested weekly for 4 weeks. At each harvest plants were cut near the soil line and then fresh and dry foliar weight was recorded. The below ground portion of each plant was removed from the soil, washed in running tap water, blotted dry, weighed and placed in plastic bags containing moist filter papers. Samples were refrigerated until processed. Fresh root shoot ratio was calculated from fresh weight of roots and foliage.

Both roots and stems were assayed for *V. dahliae* on Sorenson's NP10 medium[14] and roots were assayed for *F. equiseti* and *G. virens* on Nash Snyder[15] or Elad medium[16], respectively. Roots from each treatment were cut into 1 cm long pieces and placed onto their respective medium to determine the number of cfu/cm of roots. A total of 100 cm of roots/plant was plated.

To determine vascular populations of *V. dahliae*, a garlic press was used to express the sap from the stem of each plant. Aliquots of stem sap were spread onto NP10 plates, which were incubated at room temperature for seven days. At that time, colonies of *V. dahliae* were enumerated.

For statistical analysis, root/shoot ratio was transformed to natural logarithms (ln) and data on colonies of *V. dahliae* from stem sap were transferred to square root values in order to stabilize variances as determined by residual plots. Procedures on analysis of variance for balanced data and general linear models for unbalanced data were used. Two-way analysis of variance was performed on the data. Means were separated by least significance difference (LSD) procedure.

RESULTS

Spore germination: In general, spore germination time increased as osmotic potential decreased from -0.12 to -8.13 MPa and ranged from 8-29, 16-72, 16-46 and 13-60 h for *F. equiseti, G. virens, T. viride* and *V. dahliae*, respectively (Table 1). Regression analysis of time for 95% spore germination versus osmotic potential revealed significantly different slopes of regression lines ($p = 0.05$). Regression equations were $Y=7.57 + 0.21\ X$ ($R^2 = 0.90$) for *F. equiseti*; $Y= 13.74 + 0.38\ X$ ($R^2 = 0.93$) for *G. virens*; $Y= 11.80 + 0.36\ X$ ($R^2 = 0.92$) for *T. viride* and $Y= 13.61-0.25\ X + 0.01\ X^2$ ($R^2 = 0.99$) for *V. dahliae*, where, Y and X represent the time to 95% spore germination and osmotic potential of the medium, respectively. Ascospores of *T. flavus* did not attain 95% germination even after heat treatment at 70°C for 1 h.

Mycelial growth and sporalation: *Fusarium equiseti*, *T. viride* and *V. dahliae* grew at all osmotic potentials tested. In contrast, *G. virens* and *T. flavus* did not grow at -8.13 MPa and below -6.17 MPa, respectively (Table 2). Mycelial growth of the four-biocontrol agents decreased as osmotic potential decreased. *Verticillium dahliae*, on the other hand showed a slight increase in mycelial growth below -0.59 MPa with maximum growth occurring between -1.10 and -3.06 MPa and a linear decrease in growth below -3.06 MPa. When mycelial growth was regressed on osmotic potential for each fungus separately, slopes of regression lines for mycelial growth were significantly different at different osmotic potentials for all the fungi except *F. equiseti* ($p = 0.05$). Regression equations were $Y= 8.17 -0.11\ X$ ($R^2 = 0.95$) for *G. virens*;

Table 1: Effect of different osmotic potentials on time to 95% germination of *V. dahliae* and its biocontrol agents

	95% spore germination (h)				
Osmotic potential (MPa)	*T. flavus*	*T. viride*	*G. virens*	*V. dahliae*	*F. equiseti*
-0.12	0	15.5	15.5	13.0	9.00
-0.18	0	14.5	16.0	12.0	9.50
-0.59	0	14.5	16.0	12.5	8.00
-1.10	0	14.5	17.5	14.0	9.50
-2.15	0	16.0	18.0	14.0	11.00
-3.06	0	17.0	21.0	16.0	14.00
-5.12	0	26.0	34.5	25.0	15.50
-6.17	0	31.5	38.0	34.0	17.54
-7.16	0	37.5	72.0	46.5	22.00
-8.13	0	46.0	72.0	59.5	29.00

Table 2: Effect of different osmotic potentials on colony diameter of *V. dahliae* and its biocontrol agents

	Colony diameter (cm)				
Osmotic potential (MPa)	*T. flavus*	*T. viride*	*G. virens*	*V. dahliae*	*F. equiseti*
-0.12	5.00	9.00	9.00	1.65	8.36
-0.18	5.06	9.00	9.00	1.55	9.00
-0.59	5.27	9.00	9.00	1.63	9.00
-1.10	4.08	9.00	9.00	2.84	9.00
-2.15	3.00	9.00	9.00	2.41	9.00
-3.06	2.20	7.43	5.19	2.53	9.00
-5.12	0.93	4.52	2.28	1.77	8.41
-6.17	0.00	3.11	1.29	1.06	8.15
-7.16	0.00	2.43	0.98	1.04	7.75
-8.13	0.00	1.31	0.00	0.62	6.34

Table 3: Effect of soil water pressure on fresh root weight (g) of potato grown in soil non-infested and infested with *Verticillium dahliae* alone or in combination with either *Gliocladium virens* or *Fusarium equiseti*. Within weeks, columns with different letters are significantly different ($p \leq 0.05$) from each other according to LSD test

Soil water pressure (MPa)	Week 1	Week 2	Week 3	Week 4
-0.03	3.48	6.75B	13.57B	15.26
-0.15	5.65	11.01A	16.17A	14.91

$Y = 10.82 - 0.12\ X$ ($R^2 = 0.99$) for *T. viride*; $Y = 5.13 - 0.09\ X$ ($R^2 = 0.96$) for *T. flavus*; $Y = 1.53 + 0.11\ X - 0.003\ X^2 + 0.00002\ X^3$ ($R^2 = 0.88$) for *V. dahliae*, where, Y and X represent the colony diameter and osmotic potential of the medium, respectively.

Glicladium virens, *T. viride* and *T. flavus* did not sporulate below -1.10 MPa where as *V. dahliae* did not produce microsclerotia below -0.59 MPa. *Fusarium equiseti* produced macroconidia at all osmotic potentials although the number of macroconidia was reduced below -5.12 MPa. Chlamydospores were not produced by this fungus at any osmotic potential.

Fresh root weight: Soil water pressure had a significant ($p \leq 0.05$) effect on fresh root weight. Main effect of soil water pressure on fresh root weight was significant ($p \leq 0.05$) at weeks 2 and 3 (Table 3). Fresh root weight was significantly greater at low compared to high soil water pressure; i.e. 36 and 17% greater for plants grown at -0.15 MPa than those grown at -0.03 MPa.

None of the fungal treatments had an effect on fresh root weight. In addition, interactions between soil water pressure and fungal treatment were not significant.

Ln root/shoot ratio: A significant ($p \leq 0.05$) interactive effect of soil water pressure and fungal treatments on ln root/shoot ratio was observed at week 3 (Table 4). The ln root/ shoot ratio was inversely proportional to soil water pressure for all fungal treatments except *V. dahliae* alone for which it was directly proportional to soil water pressure. The ratio was 26% larger for plants grown in soil infested with *V. dahliae* plus *F. equiseti* at -0.15 compared to -0.03 MPa.

Significant ($p \leq 0.05$) differences among fungal treatments were observed at week 4. The ratio was significantly larger in the control (no fungus) compared to the other treatments. Among biocontrol agents, the ln root/ shoot ratio was larger for plants grown in soil infested with *G. virens* plus *V. dahliae* compared to those grown in soil infested with *F. equiseti* plus *V. dahliae*.

Soil water pressure effect on ln root/shoot ratio was significant ($p \leq 0.05$) at week 2. The ratio was 10% larger in plants grown at -0.15 MPa compared to those at -0.03 MPa.

Foliar dry weight: Interactive effects of soil water pressure and fungi were significant ($p \leq 0.05$) at week 2 (Table 5). In plants grown in soil infested with *G. virens*

Table 4: Effect of *Verticillium dahliae* alone or in combination with *Gliocladium virens* or *Fusarium equiseti* and soil water pressure (MPa) on ln root/shoot ratio of potatoes

	Soil water pressure										
	Week 1			Week 2			Week 3		Week 4		
Fungal treatments	-0.15	-0.03	Mean	-0.15	-0.03	Mean	-0.15	-0.03	-0.15	-0.03	Mean
control	-1.62	-0.98	-1.30	-1.46	-1.63	-1.54	-1.68	-2.06	-1.44	-1.61	-1.52a
V. dahliae	-0.99	-0.56	-0.77	-1.39	-1.55	-1.47	-1.60	-1.50	-1.81	-1.88	-1.84c
G. virens + *V. dahliae*	-1.29	-1.05	-1.17	-1.47	-1.53	-1.50	-1.72	-1.80	-1.66	-1.70	-1.68b
F. equiseti + *V. dahliae*	-1.16	-0.97	-1.06	-1.51	-1.80	-1.65	-1.49	-2.02	-1.84	-1.93	-1.88c
Mean	-1.26	-0.89		-1.45A	-1.62B	*			-1.68	-1.78	

Within weeks fungal main effects are shown across rows and soil water pressure main effects within columns, Means followed by different letters are significantly different at $p \leq 0.05$, *indicates the significance of interaction as determined by analysis of variance ($p = 0.003$)

Table 5: Effect of *Verticillium dahliae* alone or in combination with *Gliocladium virens* or *Fusarium equiseti* and soil water pressure (MPa) on foliar dry weight of potatoes

	Soil water pressure										
	Week 1			Week 2		Week 3			Week 4		
Fungal treatments	-0.15	-0.03	Mean	-0.15	-0.03	-0.15	-0.03	Mean	-0.15	-0.03	Mean
control	1.09	0.87	0.98	3.36	2.78	8.48	9.63	9.05a	9.02	11.35	10.18
V. dahliae	1.09	0.50	0.79	3.34	2.07	8.16	6.58	7.37b	9.66	9.97	9.81
G. virens + *V. dahliae*	1.27	0.61	0.94	5.81	2.37	10.31	8.90	9.60a	9.28	11.01	10.14
F. equiseti + *V. dahliae*	1.11	0.83	0.97	3.42	3.96	9.92	11.39	10.65a	11.79	14.71	13.25
Mean	1.14A	0.70B		*		9.21	9.12		9.93B	11.76A	

Within weeks fungal main effects are shown across rows and soil water pressure main effects within columns, Means followed by different letters are significantly different at $p \leq 0.05$, *indicates the significance of interaction as determined by analysis of variance ($p = 0.003$)

Table 6: Effect of fungal treatments on potato root colonization by *Verticillium dahliae* at –0.03 and –0.15 MPa. Mean colony density is presented as colony forming units (CFU) per centimeter of root. Within weeks, columns with different letters are significantly different ($p \leq 0.05$) from each other according to LSD test

Treatments	Week 1	Week 2	Week 3	Week 4
Verticillium	22.47A	14.0A	14.98C	15.78
Vert + *Gliocladium*	26.11A	14.16A	24.03A	21.71
Vert + *Fusarium*	15.16B	4.75B	18.08B	19.06

Table 7: Effect of soil water pressure on potato root colonization (cfu/cm) by *Verticillium dahliae* grown in soil infested with *Verticillium dahliae* alone or in combination with either *Gliocladium virens* or *Fusarium equiseti*. Within weeks, columns with different letters are significantly different ($p \leq 0.05$) from each other according to LSD test

Soil water pressure (MPa)	Week 1	Week 2	Week 3	Week 4
-0.03	16.92B	9.83	16.08B	19.41
-0.15	25.58A	12.12	21.98A	18.28

Table 8: Effect of soil water pressure on potato root colonization (cfu/cm) by *Gliocladium virens* grown in soil infested with *Verticillium dahliae* in combination with either *Gliocladium virens*. Within weeks, columns with different letters are significantly different ($p \leq 0.05$) from each other according to LSD test

Soil water pressure (MPa)	Week 1	Week 2	Week 3	Week 4
-0.03	15.69	13.90	14.43B	5.83
-0.15	20.38	10.3	24.7A	1.29

plus *V. dahliae*, foliar dry weight was 59% greater at -0.15 than at -0.03 MPa. Moreover foliar dry weight was inversely proportional to soil water pressure for all the treatments except *F. equiseti* plus *V. dahliae*.

Significant differences among fungal treatments with respect to foliar dry weight occurred at week 3. Foliar dry weight of plants grown in soil noninfested or infested with *V. dahliae* and either *G. virens* or *F. equiseti* was significantly greater than that of plants grown in *V. dahliae* infested soil. Differences between the biocontrol agents were not significant.

Differences between soil water pressures were significant at week 1 and week 4. Foliar dry weight was 38% greater in plants maintained at -0.15 MPa compared to those at -0.03 MPa at week 1. The effect, however, was reversed at week 4. Foliar dry weight of plants maintained at -0.03 MPa was 15% greater than those at -0.15 MPa (Table 5).

Recovery of *Verticillium dahliae* from roots: Recovery of *V. dahliae* differed significantly ($p \leq 0.05$) among fungal treatments during the first three weeks of the experiment (Table 6). *Fusarium equiseti* suppressed the number of root infections by *V. dahliae* at weeks 1 and 2. Plants grown in soil infested with *F. equiseti* plus *V. dahliae* had 32% and 66% fewer root infections by *V. dahliae* compared to those grown in soils infested with *V. dahliae* alone at week 1 and week 2, respectively, At week 3, however, number of *V. dahliae* infections were greater with *F. equiseti* compared to *V. dahliae* alone. Plants grown in soil infested with *F. equiseti* plus *V. dahliae* resulted in 17% more colony forming units of *V. dahliae* per centimeter of root than those grown in soil infested with *V. dahliae* alone. *Gliocladium virens* had no effect on frequency of root colonization by *V. dahliae*.

Effect of soil water pressure on root colonization of *V. dahliae* was significant ($p \leq 0.05$) at weeks 1 and 3 (Table 7). For these two respective sampling dates, plants grown at -0.15 MPa had 34 and 27% more root infections by *V. dahliae* than those grown at -0.03 MPa. No interactive effect of main effect treatments was observed.

Recovery of the biocontrol agents from roots: Effect of soil water pressure on root colonization by *G. virens* was significant ($p \leq 0.05$) at week 3 (Table 8). Number of root infections by *G. virens* was 42% greater in plants grown at -0.15 MPa than those at -0.03 MPa. Frequency of root colonization by *F. equiseti* was not affected by soil water pressure.

Recovery of *Verticillium dahliae* from stem sap: Interactive effect of soil water pressure and fungal treatments on the square root transformed data was significant ($p \leq 0.05$) at week 2 of the experiment (Table 9). Number of vascular infections was inversely proportional to soil water pressure for plants grown in soil infested with *V. dahliae* alone or in combination with *G. virens*. The relationship, however, was reversed for plants grown in soil infested with *V. dahliae* plus *F. equiseti*. Plants

Table 9: Effect of *Verticillium dahliae* alone or in combination with *Gliocladium virens* or *Fusarium equiseti* and soil water pressure (MPa) on square root of mean vascular population of *V. dahliae* in potatoes

	Soil water pressure										
	Week 1			Week 2		Week 3			Week 4		
Fungal treatments	-0.15	-0.03	Mean	-0.15	-0.03	-0.15	-0.03	Mean	-0.15	-0.03	Mean
V. dahliae	3.57	0.93	2.25	4.57	1.84	2.21	1.80	2.01	3.84	3.06	3.45
G. virens + *V. dahliae*	1.37	1.00	1.19	2.40	1.00	4.19	1.62	2.91	3.04	1.27	2.98
F. equiseti + *V. dahliae*	0.08	1.50	1.19	0.62	2.21	1.06	1.66	1.36	1.27	2.94	2.11
Mean	1.94	1.14		*		2.49	1.69		1.58	2.42	

Within weeks fungal main effects are shown across rows and soil water pressure main effects within columns, *indicates the significance of interaction as determined by analysis of variance ($p = 0.009$)

grown in soil infested with *V. dahliae* plus *F. equiseti* at -0.15 MPa had 72% fewer colony forming units of *V. dahliae* per mL of sap than those at -0.03 MPa. No significant effect of the main effect treatments was observed at any of the sampling dates.

DISCUSSION

Time required for 95% spore germination, mycelial growth and production of reproductive structures by *F. equiseti*, *G. virens*, *T. flavus*, *T. viride* and *V. dahliae* were qualitatively similar but quantitatively different over the range of osmotic potentials tested. In general, time for 95% spore germination increased whereas mycelial growth decreased as osmotic potential of the agar medium decreased from -0.12 to -8.13 MPa. *Fusarium equiseti* took the least amount of time to complete 95% germination whereas *G. virens* took the longest. Germination of *F. equiseti* spores was two-fold faster than microsclerotia of *V. dahliae* below -3.16 MPa. The ability of *F. equiseti* spores to germinate quickly may prove to be a factor in its success as a biocontrol agent of *V. dahliae*. Spores of *G. virens* did not obtain 95% germination below -7.16 MPa. The spores were either dead, dormant or the osmotic potential of the medium was too low for the spores to germinate. Above -5.13 MPa, spores of *T. viride* completed 95% germination comparatively slowly compared to microsclerotia of *V. dahliae*. *Trichoderma viride*, therefore, could be at a relative disadvantage compared to *V. dahliae* in wet soils. The decreasing rate of germination of microsclerotia of *V. dahliae* with a decrease in osmotic potential of the medium is in agreement with the results of Mozumder *et al.*[17] who reported a similar trend in germination of conidia of the fungus. Microsclerotia of *V. dahliae* germinate, penetrate and infect the root cortex. Some of the cortical infections successively invade the stele where the fungus produces conidia, which are disseminated in the xylem. The water potential of xylem fluid, therefore, may affect systemic infection by influencing both production of conidia and their movement in the vascular tissue. *Talaromyces flavus* has been reported as a potential antagonist of many soilborne plant pathogens including *V. dahliae*[18-20]. In this study, however, none of the spores germinated at and below -2.15 MPa and despite a heat treatment, only 45% germinated at osmotic potentials above -2.15 Mpa after 60 h. Katan[19] reported that if ascospores of the fungus are heated to a temperature of 70°C for 1 h, only about 5-10% germinate. The lower percent spore germination in our study might be due to the use of MYP instead of PDA, the medium used by Katan.

The reduction in mycelial growth with a decrease in osmotic potential of the medium and/ or soil water pressure is in agreement with previous studies[12,21,22]. Results obtained with KCl are largely attributed to osmotic stress rather than toxicity of the salt[23-26]. *F. equiseti, T. flavus* and *V. dahliae* showed a trend of slight increase in mycelial growth as the osmotic potential of the medium was lowered up to a certain point, after which each showed a reduction in growth with further lowering of osmotic potential. Cook and Duniway[27] reported that growth of many fungi is stimulated by reduction of the osmotic potential of the medium by -0.5 to -2. MPa. The optimal osmotic potential, however, depends upon the fungus. Moreover, several other factors such as solute[28] and temperature [29]also affect the osmotic potential at which optimal growth of a fungus occurs. Maximal radial growth was observed at -0.12 to -3.16 MPa for both *G. virens* and *T. viride*. However, unlike *T. viride*, which grew at all osmotic potentials tested, *G. virens* did not grow at -8.13 MPa suggesting that the latter was less tolerant to low osmotic potentials than the former.

Formation of reproductive structures in all the fungi except *F. equiseti* ceased well above the osmotic potential at which mycelial growth continued to occur. Cook and Duniway[27] reported that water potential requirements for sporulation of fungi were more strict than those for initiation and maintenance of mycelial growth. *Gliocladium virens*, *T. flavus* and *T. viride* did not produce spores below -1.10 MPa, which is in agreement with Cook and Duniway[27] who reported a range of 0 to -1.5 MPa for the production of sporangia, apothecia or other reproductive structures. *V. dahliae* did not produce microsclerotia below -0.59 MPa. Ioannou *et al.*[22] reported that production of microsclerotia in *V. dahliae* was more sensitive to decreases in osmotic potential than was mycelial growth. Results of the present study, however, differ from those of Ioannou *et al.*[22] who reported formation of microsclerotia at osmotic potentials of -7.0 and -8.0 MPa. Water requirements of isolates of a species or subspecies may be different[30]. Maximal chalmydospore production among isolates of *Fusarium roseum graminearum, culmorum and avenaceum* collected from dry areas of Washington State occurs at -1.5 MPa compared with Pennsylvania isolates of *F. roseum graminearum,* which occurs at -0.14 Mpa. In addition to different isolates, use of MYP instead of PDA may explain, in part, differences in results between the studies. Production of macroconidia by *F. equiseti* at all osmotic potentials tested is in agreement with Sung and Cook[30] who reported that *F. roseum* produced macroconidia at osmotic potentials as low as -6 to -8 MPa.

In the greenhouse study, treatments with biocontrol agents were variable in their effects on the population dynamics of *V. dahliae* in roots. Recovery of *V. dahliae* from roots was significantly reduced only during the first two weeks of the experiment with the *F. equiseti* plus *V. dahliae* treatment. *Fusarium equiseti* has been reported as a weak root pathogen[31]. Early colonization of infection courts by *F. equiseti* may have resulted in the lower frequency of root infection by *V. dahliae* during the first two weeks. The lack of an effect at the later sampling dates could have resulted from a loss in viability of propagules of *F. equiseti*. In contrast, *G. virens* had no effect on root colonization by *V. dahliae*. *Gliocladium virens* is a soil saprophyte and is not regarded as effective as *F. equiseti* in colonizing roots. Dix[32] reported *G. virens* as a root surface colonizer of older roots in beans. By the time roots were old enough to be colonized by *G. virens*, they were already colonized by *V. dahliae*. Thus *G. virens* was not successful in preventing root colonization by *V. dahliae*.

Plant growth responses including foliar dry weight and ln root/shoot ratio were significantly affected by both *G. virens* and *F. equiseti*. In the presence of *F. equiseti*, there was an increase in foliar dry weight at week 3. A similar effect on both foliar dry weight and ln root/shoot ratio was observed with *G. virens* at the last two sampling dates. Biocontrol agents are known to enhance plant growth responses. Baker *et al.*[33] reported an increase in height, weight, branch and flower production in *T. harzianum* treated chrysanthemum and peanut plants. Similarly, Elad and Chet[34] demonstrated that *Trichoderma* porpagules were present in plants that showed increased growth responses. Similar effects have been documented with Plant Growth Promoting Rhizobacteria (PGPR) on potato[35,36]. Improved plant growth by PGPR has been attributed to the ability of PGPR to suppress the pathogen by out competing it for nutrients[37,35]. Moreover, alteration of host physiology resulting in subsequent production of plant growth hormones has also been implicated as one of the mechanisms[36]. Thus the increased growth responses seen in our study could be due to suppression of pathogen, increase in plant vigor or induced host resistance. These hypotheses, however, warrant further investigation.

Plant growth responses were affected differentially by soil water pressure. Fresh root weight at weeks 2 and 3 and ln root/shoot ratio at week 2 were significantly lower for plants maintained at -0.03 compared to -0.15 MPa. Poor aeration of the soil due to excessive moisture may have contributed to reduced fresh root weight at this soil water pressure. Our results, however, differ from those of Gaudreault *et al.*[38] who reported a higher fresh root weight at -0.03 than at -0.15 MPa. A possible reason is that the soil in our experiment was mixed before planting which could have resulted in different bulk densities. A higher fresh root weight at -0.15 than at -0.03 MPa resulted in larger ln root/shoot ratio at this soil water pressure.

Soil water pressure effect on foliar dry weight varied across sampling dates. Plants maintained at -0.15 compared to -0.03 MPa had greater foliar dry weight at week 1. The effect was reversed at week 4. In another study Gaudreault *et al.*[38] concluded that these two soil water pressures had no differential effect on foliar dry weight. A relatively higher greenhouse temperature in our study may have promoted plant growth. Reasons for inconsistent effect of soil water pressure at different harvest dates are, however, unclear.

Colonization of roots by both *V. dahliae* and *G. virens* was affected by soil water pressure. Number of root infections by *V. dahliae* was lower at high compared to low soil water pressure, which could be due to anaerobic conditions. Pullman and Devay[39] reported that flooding resulted in a reduction in population of microsclerotia of *V. dahliae* in soil. Factors contributing to this reduction included antagonism by anaerobic soil microorganisms. Soils at -0.03 MPa could, therefore, be suppressive to *V. dahliae*. A higher rate of root colonization by *G. virens* a -0.15 MPa is in agreement with a previous study in which Keinath *et al.*[40] reported significant reduction in viability of microsclerotia of *V. dahliae* by *G. roseum* in soil at -0.01 and -0.1 MPa. Colonization of roots by *F. equiseti*, however, was not affected by soil water pressure.

Fungal treatments and soil water pressure interacted to affect foliar dry weight, ln root/shoot ratio and vascular colonization by *V. dahliae*. *Gliocladium virens* and *F. equiseti* at -0.15 MPa increased foliar dry weight and ln root/shoot ratio, respectively. *Fusarium equiseti* at -0.15 MPa probably prevented *V. dahliae* from colonizing the roots and hence its transport to the vascular system.

This study showed that the potential for biocontrol of *V. dahliae* exists. However, a better understanding of the effects of moisture on growth and survival of potential biocontrol agents of *V. dahliae* are needed before they can be effectively integrated into normal production systems for suppression of diseases and/or pathogens.

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