INTEGRATED MANAGEMENT AND CAUSES OF DAMPING OFF DISEASE OF CHICKPEA (*CICER ARIETINUM* L.) IN MONTANA

by

Rachel Sue Leisso

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Plant Pathology

MONTANA STATE UNIVERSITY
Bozeman, Montana

July 2008

APPROVAL

of a thesis submitted by

Rachel Sue Leisso

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

Dr. Mary Burrows

Approved for the Department Plant Sciences and Plant Pathology

Dr. John Sherwood

Approved for the Division of Graduate Education

Dr. Carl A. Fox

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library.

If I have indicated my intention to copyright this thesis by including a copyright notice page, copying is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for permission for extended quotation from or reproduction of this thesis in whole or in parts may be granted only by the copyright holder.

Rachel Sue Leisso

July 2008

ACKNOWLEDGEMENTS

I would like to thank the department of Plant Sciences and Plant Pathology as a whole for their technical, academic, and financial support. I am especially grateful for the advice, support, and editing of my graduate committee chairperson and major professor, Dr. Mary Burrows, as well as my committee members, Dr. Barry Jacobsen, and Dr. Perry Miller, who have been ever willing to provide assistance and advice with any facet of this project. I would also like to thank Mary Brennan Lollis and Ken Baker for field and lab assistance; Oliver Neher, Eva Grimme, Hope Talbert, John Ansley, and Ernesto Moya for lab support and invaluable advice; Jack Martin, for statistical advice; Tracy Dougher, for my teaching assistantship; Jeff Holmes, Mac Burgess, and Bernie Schaff for assistance at the Bozeman field site; Steve King, for help at the Huntley field site; Joyce Eckhoff, for support at the Sidney field site; and John Sherwood, for advice and support. I would like to extend special thanks to my friends and family, particularly Phil, Karel, Nathan, Sara, and Miriam Leisso.

TABLE OF CONTENTS

TABLE OF CONTENTS – CONTINUED

LIST OF TABLES

Table Page

LIST OF TABLES – CONTINUED

Table Page

LIST OF TABLES – CONTINUED

Table Page

LIST OF FIGURES

Figure Page

ABSTRACT

Chickpea is a minor crop in Montana with great potential for increase in both the conventional and organic sector. The semi-arid climate of much of Montana is well-suited to organic chickpea production, which commands a higher market price than conventional chickpea. Ranking third in the nation for certified organic cropland acres, many operations in Montana are already capable of organic chickpea production. There is a need for rotational crops such as chickpea that are compatible with organic wheat production. In addition to their profit potential, chickpea can perform valuable functions in wheat rotations such as fixing nitrogen and breaking insect pest and disease cycles. Damping-off of chickpea is one of the critical concerns of producers raising organic chickpea. Little was known about the pathogens causing damping off in Montana prior to this research. To determine the organisms responsible for damping off, pathogens were isolated from chickpeas affected by damping off at three field sites and identified to genera. *Pythium* spp. and *Fusarium* spp. are the predominate causes of damping off in Montana. *Fusarium* spp. have not been previously reported in association with damping off of chickpea. To determine if the *Fusarium* isolates were pathogenic or facultatively pathogenic, eight isolates of *Fusarium* were indentified to species and pathogenicity tests were performed under controlled conditions. All isolates caused damping off of chickpea. Damping off incidence and severity increased with increasing moisture levels for the majority of the *Fusarium* isolates. Seed treatments are the most common method of preventing damping off, and biological seed treatments are a control option for organic and conventional growers. The potential for control of chickpea damping off using biological and fungicide seed treatments was tested in greenhouse trials and at three field locations in Montana in 2007. Biological seed treatments *Bacillus pumilus* GB34 (Yield Shield), *B. subtilis* GB03 (Kodiak), and *Trichoderma harzianum* Rifai strain KRL-AG2 (T-22 Planter Box) were compared with conventional seed treatments fluidoxonil (Maxim) and mefenoxam (Apron XL LS) and combinations of biological and fungicide seed treatments in field trials. Treatments containing the chemical fungicide mefenoxam, which targets oomycete pathogens, were most effective for controlling damping off. Biological seed treatments were not effective at controlling damping off.

THE ROLE OF SOIL MOISTURE AND INOCULUM DENSITY IN *FUSARIUM* SPP. PATHOGENICITY IN CHICKPEA DAMPING OFF

Abstract

Damping off causes stand loss and reduces seedling vigor wherever chickpea is grown. Previous research cites *Pythium* spp., especially *Pythium ultimum*, as the cause of chickpea damping off in the Great Plains states and the Pacific Northwest. In the spring of 2007, both *Fusarium* spp. and *Pythium* spp. were isolated in similar proportions from chickpea affected by damping off in Montana in field trials. Previous research has reported that *Fusarium*-caused damping off and root and crown rot diseases are more severe in dry conditions, but the *Fusarium* spp. isolated in these trials were recovered from chickpeas growing in a range of soil moisture conditions, including wet soil. Eight of the *Fusarium* spp. isolated were identified to species using morphological characterization and sequence analysis of translation elongation factor gene (TEF) 1-α and the internal transcribed spacer (ITS) region. Eight isolates were tested for pathogenicity to kabuli chickpea in growth chamber experiments. All isolates were able to infect kabuli chickpea seeds. Incidence was the highest when soil water was near field capacity for five of the eight isolates. Disease incidence increased with inoculum density for all isolates. Most isolates caused more severe disease symptoms in cotyledons than roots. There were no relationships between the geographical location or soil conditions from which the isolates were obtained and disease incidence or severity.

Introduction

In North America, chickpea is grown in California, Idaho, Montana, North Dakota, Oregon, and South Dakota (USDA-ASB 2008) and in the Canadian provinces of Alberta and Saskatchewan (Saskatchewan Ministry of Agriculture 2007). Chickpea is grown in over 56 countries, with the highest production in India, Iran, and Pakistan (United Nations Food and Agriculture Organization [FAO] 2005). Damping off and seedling disease of chickpea can substantially reduce stand and decrease yield.

Damping off is characterized by seed decay and cotyledon degradation prior to above-ground growth, as well as discoloration and necrosis of emerging roots (Kaiser and Hannan 1983). In the Pacific Northwest of the USA, damping off of chickpea has been attributed to *Pythium* spp., in particular *Pythium ultimum* (Kaiser and Hannan 1983; Trapero-Casas 1990; Haware 1998).

Prior work regarding damping off of chickpea in the Great Plains has consisted primarily of applied agronomic studies, which have tested seed treatments in field settings (Brick et al. 1998; Chen et al. 2003; Leisso and Burrows 2007). Little published work exists regarding the pathogens causing damping off of chickpea in the Great Plains, particularly in Montana.

The objectives of this study were to determine the pathogenicity of eight *Fusarium* spp. isolates obtained from kabuli chickpeas affected by damping off disease at three field locations in Montana in the spring of 2007, and to determine how soil moisture and *Fusarium* spp. propagule concentration affect disease incidence and severity.

Materials and Methods

Collection and Identification of *Fusarium* spp. Isolates from Kabuli Chickpea

Decaying 'Sierra' kabuli chickpea seeds and seedlings were collected 3 to 4 wk after planting from control plots of a field experiment (Leisso and Burrows 2007). Diseased seeds and seedlings were collected 16 May 2007, 17 May 2007, and 23 May 2007, from Bozeman, Huntley, and Sidney, MT respectively. During these three weeks following planting and prior to seed collection, Bozeman soil temperatures averaged 14.1 ºC and plots received 42 mm of precipitation. At Huntley the average soil temperature during the three weeks following planting was 15.4 ºC and plots received 7 mm of precipitation. Mean Sidney soil temperature was 14.2 ºC, while plots received 49 mm of precipitation. Two rotted seeds were collected from inner rows from each of the of the control treatment plots for a total of 12 seeds per location. The site for seed collection was determined arbitrarily within row sections possessing low seedling emergence. Each seed was divided into eight pieces; four pieces were plated to potato dextrose agar (PDA) (EMD Chemicals Inc., Darmstadt, Germany) plus 50 ppm streptomycin (Sigma-Aldrich, Inc., St. Louis, MO) and to P_{10}ARP (17 g corn mean agar [Becton, Dickinson, and Company, Sparks, MD], 5 ppm pimaricin [Sigma-Aldrich, Inc., St. Louis, MO], 250 ppm ampicillin [Sigma-Aldrich, Inc., St. Louis, MO], 10 ppm rifampin [MP Biomedicals Inc., Solon, OH], and 100 ppm pentacloronitrobenzene [Chematura Corporation, Middlebury, CT, USA]). Each plate contained two pieces from each of the two cotyledons. Hyphal tip isolates of all morphologically distinct mycelial growth were transferred to PDA. The

isolates were initially identified visually utilizing microscopic characters described in a number of laboratory identification manuals (Barnett 1972; Van der Plaats-Niterink 1981; Leslie and Summerell 2006).

Eight *Fusarium* isolates were selected from each location representing the most commonly isolated *Fusarium spp.* based on initial identification as described above. Single spore isolates of the eight *Fusarium* spp. tested were obtained by performing serial dilution plating of spores and transferring a single germinating spore to new media.

DNA was extracted from fungal mycelia of the eight *Fusarium* spp. isolates using E.Z.N.A. Fungal DNA kit (Omega Bio-tek, Doraville, GA) according to manufacturer's instructions. Approximately 250 mg of mycelia were sterilely removed from a 1-wk-old Petri dish culture of the isolate on PDA (EMD Chemicals Inc., Darmstadt, Germany). Mycelia were placed in a sterile mortar, frozen (with liquid nitrogen), and ground into fine powder. Approximately 100 mg of mycelia were used for DNA extraction.

Primers ITS 1F and ITS 4 (see Appendix A, Table A-1), were used to amplify the rDNA ITS region, specifically, a portion of the18S rRNA gene, internal transcribed spacer region one, 5.8S rRNA gene, and internal transcribed spacer region II and a portion of the 28S rRNA gene. These primers amplify the ITS region as for a wide array of fungal taxa (Gardes and Bruns 1993). The 20 µl reaction mixture consisted of 1X GoTaq Master mix (Promega Corporation, Madison, WI, USA), 1.25 µM primer ITS 1F, 1.25 µM primer ITS 4, and 250 ng of extracted DNA. PCR conditions were: denaturation at 95ºC for 5 min, followed by 35 cycles of denaturation at 95 ºC for 30 s, annealing at 55 ºC for 30 s, and extension at 72 ºC for 1 min with a final extension step at 72 ºC for 7

min. The amplification was performed using an iCycler Thermal Cycler (Bio-Rad Laboratories Ltd, Hemel Hempsted, United Kingdom). Amplified DNA was purified using QIAquick PCR purification kit (Quiagen Sciences Inc., Germantown, Maryland) and sequenced using ITS1F by Functional BioSciences of Madison, WI, USA.

The translation elongation factor 1-α (TEF) gene, which has the highest phylogenetic utility for identification of *Fusarium* spp. (Geiser et al. 2004) was also amplified and sequenced. Primers ef1 and ef2 (O'Donnell et al. 1998; see Appendix A, Table A-1) were used to amplify the translation elongation factor gene as described by Geiser et al. (2004). The 20 µl reaction mixture consisted of 1X GoTaq Master mix (Promega Corpporation, Madison, WI, USA), 1.25 µM primer ef1, 1.25 µM primer ef2, and 250 ng of extracted DNA. PCR conditions were: denaturation at 95ºC for 5 min, followed by 35 cycles of denaturation at 95 ºC for 30 s, annealing at 53 ºC for 30 s, and extension at 72 ºC for 1 min with a final extension step at 72 ºC for 7 min.

ITS region and TEF 1-α sequences were compared to sequence collections on GenBank (National Center for Biotechnology Information [NCBI] 2008b) using BLAST (NCBI 2008a) algorithm 'blastn.' Translation elongation factor sequences were queried to the FUSARIUM-ID server at http://fusarium.cbio.psu.edu (Gieser et al. 2004) which contains a BLAST search tool. Phylogenetic relationships between the isolates were determined using neighbor-joining bootstrap analysis with CLC Combined Workbench 3 (CLC bio USA, Cambridge, MA, USA).

Effects of Soil Moisture and Incoculum Density
on Germination, Disease Incidence,
and Severity in Growth Chamber Experiments

The eight *Fusarium* spp. isolates identified as described above were tested for pathogenicity to chickpea seeds in relation to soil moisture and inoculum levels. A factorial treatment design was used with 4 moisture levels (10, 20, 30 and 40% v/v [volume water to volume sand]) and 5 inoculum levels (0, 10, 100, 1000, and 10,000 propagules g^{-1} of soil) for a total of 20 treatments. The lower limit of the moisture gradient was 10% v/v; permanent wilting point in sand is 6% v/v (Ley et al. 1994). Chickpea seeds were reported not to germinate in soil containing less than 20% moisture v/v (ICRISAT 1981). The highest moisture level was 40% v/v; the field saturation point for sand is 42.5% v/v, as calculated using the Soil Water Characteristics Hydraulic Properties Calculator (version 6.02.74, K.E. Saxton, United States Department of Agriculture-Agricultural Research Service, and Department of Biological Systems Engineering, Pullman Washington, USA [Saxton 2006; Saxton and Rawls 2006]). Inoculum levels ranged from 0 to 10,000 propagules (mainly conidia or clamydospores, occasional hyphal fragments) g^{-1} sand, with 0 serving as the control, and 10,000 as the upper bound based on preliminary experiments and previous studies in legumes (Kraft 1975; Kaiser and Hannan 1983). Propagule determination is as described below.

Sand (Silica sand 20/30, Lane Mountain Co., Valley, WA) was chosen as a media due to its pH and nutrition neutrality (Dhingra and Sinclair 1985). Seeds of kabuli chickpea cultivar Sierra, which has a pale to un-pigmented seed coat, were used in field and growth chamber trials; desi chickpea seeds with darkly-pigmented seed coats are less

susceptible to seed decay and damping off (Kaiser and Hannan 1983; Leisso and Burrows 2007) and were not used in these trials.

Fusarium spp. were grown in 25% potato dextrose broth (EMD Chemicals Inc., Darmstadt, Germany) for 15-20 d by inoculating the culture with three 7-mm diam. circular agar plugs cut from the edges of actively growing 3 to 5 d-old-cultures. Spores were extracted from cultures by straining through four layers of sterile cheesecloth. Conidia, chlamydospores, and hyphal fragments in solution were quantified using a phase haemacytometer (Hausser Scientific, Horsham, PA, USA). Prior to sand inoculation, 250 µl of propagule solution was plated onto PDA and the percentage of germinating and actively growing conidia, clamydospores, or growing hyphal fragments was quantified after 12 h incubation at 25 ºC in the dark. Quantities of spore solutions added to media were adjusted so that the levels of propagules inoculated into plates reflected the number of viable propagules.

Chickpea seeds were surface-sterilized by placing them in 0.25% NaOCl for 3 min, rinsing for 30 s in 70% ethanol, and then rinsed three times with sterilized de-ionized water and drying overnight (at least 10 h) on autoclaved paper towels in a laminar flow hood. Chickpea seeds were planted in 70 g of sand in sterile Petri dishes and the appropriate treatment applied. Sand moisture levels and conidia concentration were obtained by aseptically adding autoclaved sand (autoclaved twice, 24 h between each autoclaving) to individual Petri dishes with the required quantity of sterile distilled water containing the appropriate quantity of conidia and mixing with a flame-sterilized glass rod. Ten seeds were aseptically buried in sand in each inoculated plate and plates were

sealed with parafilm to prevent moisture loss. Plates were placed in a growth chamber set at 15 ºC d/ 10 ºC n and a 14 h photoperiod. Ideal temperature for chickpea germination is 10-15 ºC (Ellis et al. 1986). The 14 h photoperiod approximates Montana day length in spring (United States Naval Observatory [USNO] 2008). Three replications of the 20 treatments were arranged in a completely randomized block design with one replication per block. The experiment was performed twice for each isolate.

Ten days after planting, Petri plates were removed from incubation. Number of germinated seeds was recorded, and cotyledon and root disease assessed visually on a 1-5 scale. The rating scale is a modification of seedling and root rot rating scales described previously for legume seed and root diseases (Kraft 1975; Peters and Grau 2002). Five diseased seeds from each trial were plated to PDA and *Fusarium* spp. identity confirmed microscopically.

Statistical Analyses

Data analysis was performed using Statistical Analysis System (SAS, v. 9.1, Cary, NC, USA 2003). Residuals for each parameter were examined for normality and homogeneity of variances. Assumptions of normality were examined using the Shapiro-Wilk's test (Shapiro and Wilk 1965) performed by PROC UNIVARIATE. Homogeneity of variance between isolates and between experiments was examined using Hartley's F-max test (Hartley 1950). Data was log transformed for analysis as necessary, although untransformed data has been presented in figures and tables. Orthogonal polynomial statements were constructed in PROC GLM to compare germination and disease

incidence to soil moisture levels and concentration of *Fusarium* sp. propagules per g of soil, as well as to determine linear and quadratic trends in disease incidence as affected by soil moisture and propagules per gram of soil. Multilinear regression using PROC REG was performed to determine the relationship between the two independent variables, soil moisture and soil propagule concentration and the dependant variable, disease incidence. Cotyledon and root disease severity of affected seeds were analyzed using analysis of variance in PROC GLM. For cotyledon and root severity means comparisons, as data sets were unbalanced due to differing numbers of affected seeds, letter groupings were generated by comparing results of the *lsmeans pdiff* option, which performs pair-wise comparisons of least squares means. Pearson's correlation coefficients were generated by comparing least square means of affected isolates of disease incidence, cotyledon disease severity and root disease severity. For all analyses $P < 0.05$ was considered significant.

Results

Pathogen Isolation and *Fusarium* spp. Identification

Pathogens isolated from kabuli chickpea seeds affected by damping off in field trials at three locations in Montana in 2007 were identified to genus using morphological characters (Nelson et al. 1983; Leslie and Summerell 2006). In most cases multiple genera of fungi were isolated from one seed (Table 1-1).

Table 1-1. Pathogens isolated from kabuli chickpea affected by damping off in field trials in at three locations in Montana in 2007.

	Bozeman	Huntley	Sidney
Fusarium spp.	8	10	8
Pythium spp.	7	0	7
Rhizopus spp.	4	4	7
Other	*Alternari*a spp. (2 isolates)	*Alternaria* spp. (2 isolates)	*Acremonium* sp. (1 isolate)
	Cladosporium sp. (1 isolate)	*Rhizoctonia* sp. (2 isolates)	*Lecancillum* sp. (1 isolate)

Eight *Fusarium* sp. were selected, two from Bozeman, and three each from Huntley and Sidney for identification to species, based on the most commonly isolated genera from initial identification. Morphological identifications to genus for the eight *Fusarium* spp. are listed in Table 1-2. Identification of isolates according to sequence comparison of the internal transcribed spacer region (ITS) rDNA to GenBank using BLAST is listed in Table 1-3. Identification of isolates by comparison of translation elongation factor 1-α gene sequence to GenBank using BLAST is listed in Table 1-4. Identification and accession number of isolates deposited in GenBank are listed in Table 1-5.

The phylogenetic relationship between isolates using rDNA ITS region sequence is diagrammed in Figure 1-1; the phylogenetic relationships between isolates using the ITS region sequence is shown in Figure 1-2.

Table 1-2. Morphological identification of *Fusarium* spp. isolated from kabuli chickpea affected by damping off in Montana field trials in 2007.

Isolate number	Location of origin	Morphological identification
1.3	Bozeman, MT	*Fusarium sp.**
3.5	Bozeman, MT	*F. oxysporum*
DI	Huntley, MT	*F. sambucinum*
3.2	Huntley, MT	*F. trincintum*
4.6	Huntley, MT	*F. culmorum*
1.7	Sidney, MT	*F. solani*
2.2	Sidney, MT	*F. solani*
2.6	Sidney, MT	*F. rendolens*

*in *F. semitectum, nelsonii, camptoceras* group; isolate does not completely fit morphological characters for *Fusarium* sp. germane to temperate zones.

.

Table 1-3. Identification of *Fusarium* isolates by sequence comparison of internal transcribed spacer region (ITS) sequence to GenBank.*

Isolate number	Location of origin	ITS sequence length (bp†)	GenBank hit for rDNA ITS region sequence	ITS score (bits)	Query coverage	E-value	GenBank hit accession number
1.3	Bozeman, MT	543	*Gibberella avenacea* FA12	1000	100%	0.0	EU255802.1
3.5	Bozeman, MT	537	*Fusarium oxysporum* isolate XSD-78	966	99%	0.0	EU326216.1
DI	Huntley, MT	516	*Fusarium camptoceras* isolate NW643c	953	100%	0.0	EU520082.1
3.2	Huntley, MT	545	*Fusarium trincintum*	996	100%	0.0	AY188923.1
4.6	Huntley, MT	520	Uncultured endophytic fungus clone 51-01-16	936	100%	0.0	EF505484.1
1.7	Sidney, MT	542	*Fusarium solani*	996	100%	0.0	AB369907.1
2.2	Sidney, MT	521	Uncultured *Fusarium* clone 7g	953	99%	0.0	EU002984.1
2.6	Sidney, MT	534	*Fusarium redolens*	987	100%	0.0	X94169.1

*Sequence amplified using primer pair ITS1F and ITS4, and sequenced using forward primer ITS1F. See Appendix A, Table A-1 for primer sequences.
†Base-pairs.

Figure 1-1. Neighboring joining phylogentic tree of amplified internal transcribed spacer (ITS) rDNA regions of selected *Fusarium* spp. generated by bootstrap analysis.

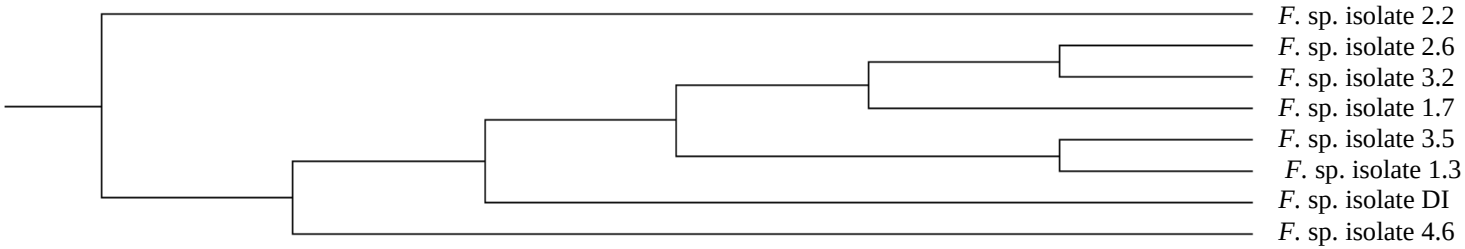
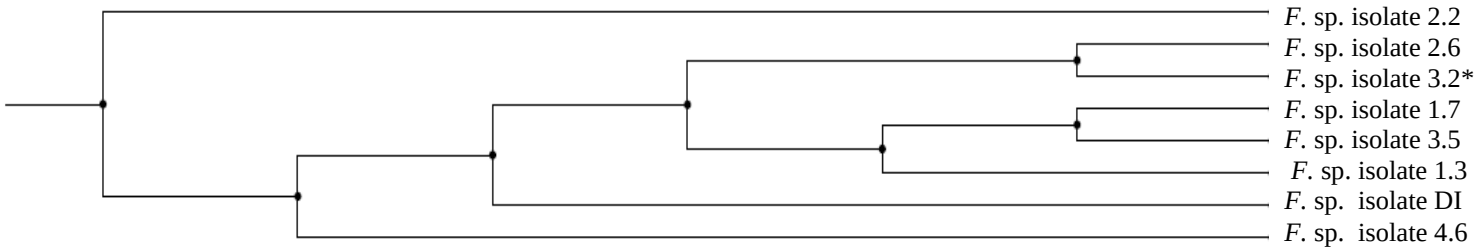


Table 1-4. Identification of *Fusarium* isolates by sequence comparison of transcription elongation factor (TEF) 1-α to GenBank.*

Isolate number	Location of origin	TEF sequence length (bp)	GenBank hit for TEF gene sequence	TEF score (bits)	Query coverage	E-value	GenBank hit accession number
1.3	Bozeman	590	*Fusarium reticulatum* var. *negundinis* isolate 15/2.3.1	1171	100%	0.0	DQ854864.1
3.5	Bozeman	653	*Fusarium oxysporum* isolate F84	1179	99%	0.0	EF531699.1
DI	Huntley	576	*Gibberella zeae* PH-1 hypothetical protein	117	28%	6^{e-23}	XM_383563.1
4.6	Huntley	568	*Gibberella zeae* PH-1 hypothetical protein	117	28%	6^{e-23}	XM_383563.1
1.7	Sidney	682	*Fusarium sp.* NRRL 43704 haplotype FSSC	1221	99%	0.0	EF453029.1
2.2	Sidney	528	*Fusarium sp.* 43407	1223	99%	0.0	DQ986181.1
2.6	Sidney	674	*Fusarium redolens* isolate 13/3.5.1	1219	100%	0.0	DQ854915.1

*Sequence amplified using primer pair ef1 and ef2, and sequenced using forward primer ef1. See Appendix A, Table A-1 for primer sequences. DNA extraction, amplification, and sequencing performed three times for isolate 3.2; sequence traces were of poor quality compared to other isolates and were not submitted for BLAST analysis.

Figure 1-2. Neighboring joining phylogentic tree of amplified translation elongation factor (1-α) genes of selected *Fusarium* spp. generated by bootstrap analysis.



*Sequence trace was low quality compared to other sequence traces.

Table 1-5. Sequence accession numbers in GenBank of Fusarium spp. isolated from kabuli chickpea affected by damping off in Montana field trials 2007.*

Lab number	Location of origin	ITS isolate identification submitted to GenBank	ITS isolate GenBank accession number	TEF isolate identification submitted to GenBank	TEF isolate GenBank accession number
1.3	Bozeman	*Fusarium* sp.	EU910075	*Fusarium* sp.	EU910069
3.5	Bozeman	*Fusarium* sp.	EU910080	*Fusarium* sp.	EU910072
DI	Huntley	*Fusarium* sp.	EU910082	*Fusarium* sp.	EU910074
3.2	Huntley	*Fusarium* sp.	EU910079	*Fusarium* sp.	NA*
4.6	Huntley	*Fusarium* sp.	EU910081	*Fusarium* sp.	EU910073
1.7	Sidney	*Fusarium* sp.	EU910077	*Fusarium* sp.	EU910068
2.2	Sidney	*Fusarium* sp.	EU910076	*Fusarium* sp.	EU910070
2.6	Sidney	*Fusarium* sp.	EU910078	*Fusarium* sp.	EU910071

*DNA extraction, amplification, and sequencing performed three times for isolate 3.2; sequence traces were of poor quality compared to other isolates and were not submitted to GenBank.

Effects of Moisture and Propagule Concentration on Disease Incidence

All *Fusarium* spp. isolates caused damping off in kabuli chickpea seedlings under controlled conditions. Moisture and propagule inoculum concentration had highly significant effects on disease incidence for most isolates (Table 1-8). The interaction effect of moisture and inoculum concentration on disease incidence was not significant at $P < 0.05$ for most isolates (Table 1-8).

Disease occurred at lowest moisture level (10% v/v) and at the highest moisture (40% v/v), as well as at the lowest concentration of propagules (10 propagules g^{-1}) (Table 1-9 and Figure 1-3). All of the isolates except *F.* sp. isolate DI and *F.* sp. isolate 2.6 exhibited a linear relationship between soil moisture and disease incidence (Table 1-11).

Low levels of propagules (10 propagules g^{-1} of soil) caused disease for all of the isolates, except for *F.* sp. isolate 3.5 whose disease incidence was not different from zero

at 10 propagules g^{-1} of soil (Table 1-10). All of the isolates had linear relationships between log propagules g^{-1} of soil and disease incidence; for many isolates, this relationship also had quadratic trend (Table 1-11). For complete table of orthogonal contrasts delineating linear and quadratic trends see Appendix A, Table A-2.

Disease Severity

Seedlings were rated for disease severity on roots and cotyledons using the rating scale described below in Table 1-6.

Table 1-6. Cotyledon and root disease severity rating scale used in evaluating disease of kabuli chickpea seeds and seedlings caused by *Fusarium* spp. in growth chamber pathogenicity and virulence trials.

Cotyledon rating scale	Description	Root rating scale	Description
1	seed germinated, no disease apparent on cotyledons	1	seed germinated, no disease apparent on roots
2	seed germinated, <25% of cotyledons necrotic or covered by mycelia	2	seed germinated, <25% of roots necrotic
3	seed germinated, 25-50% of cotyledons necrotic or covered by mycelia	3	seed germinated, 25-50% of roots necrotic
4	seed germinated, 25-50% of cotyledons necrotic or covered by mycelia	4	seed germinated, 50-75% of roots necrotic
5	seed failed to germinate (due to disease) or 75-100% of cotyledons necrotic	5	seed failed to germinate (due to disease) or 75-100% of roots necrotic

Cotyledons exhibited more severe disease symptoms than roots for most isolates (Table 1-7). *F.* sp. isolate 2.2 had more severe disease symptoms on roots than cotyledons; *F.* sp. isolate 3.2 had equally severe symptoms on roots and cotyledons. *F.*

sp. isolate 3.2 caused the most severe disease on cotyledons, followed by *F.* sp. isolate DI; *F.* sp. isolate 3.2 also caused the most severe disease on roots, followed by *F.* sp. isolate 1.7 (Table 1-7).

Table 1-7. Disease severity of cotyledons and roots infected by *Fusarium* spp. isolates.

Isolate	Location	Cotyledon	Root
F. sp. isolate 1.3	Bozeman, MT	3.6 c	2.1 de
F. sp. isolate 3.5	Bozeman, MT	2.8 e	1.6 f
F. sp. isolate 3.2	Huntley, MT	4.3 a	4.3 a
F. sp. isolate 4.6	Huntley, MT	3.3 d	2.2 d
F. sp. isolate D.I.	Huntley, MT	4.0 b	2.6 c
F. sp. isolate 1.7	Sidney, MT	3.2 d	3.0 b
F. sp. isolate 2.2	Sidney, MT	2.3 f	2.4 cd
F. sp. isolate 2.6	Sidney, MT	2.8 e	1.7 ef

Note: Numbers in a column for each isolate followed by the same letter are not significantly different at $P < 0.05$ (Fischer's LSD). Severity was rated using a 1 to 5 scale described in Table 1-6.

Relationship between Disease Incidence and Severity

Pearson's correlation coefficients found a positive relationship between disease incidence and severity for both cotyledons and roots for most isolates (Table 1-12). These results indicated higher levels of disease incidence corresponded with higher levels of disease severity.

Table 1-8. Analysis of variance of *in vitro* disease incidence of *Fusarium* spp. isolates as affected by moisture and inoculum density.

Isolate	Location of origin	Source of variation	df	*P*
F. sp. isolate 1.3	Bozeman, MT	soil moisture	3	<0.01
		inoculum density	4	<0.01
		soil moist. x inoc. density	12	<0.01
F. sp. isolate 3.5	Bozeman MT	soil moisture	3	<0.01
		inoculum density	4	<0.01
		soil moist. x inoc. density	12	0.71
F. sp. isolate3.2	Huntley, MT	soil moisture	3	<0.01
		inoculum density	4	<0.01
		soil moist. x inoc. density sity	12	0.01
F. sp. isolate 4.6	Huntley, MT	soil moisture	3	<0.01
		inoculum density	4	<0.01
		soil moist. x inoc. density	12	0.09
F. sp. isolate DI	Huntley, MT	soil moisture	3	0.17
		inoculum density	4	<0.01
		soil moist. x inoc. density	12	0.77
F. sp. isolate 1.7	Sidney, MT	soil moisture	3	<0.01
		inoculum density	4	<0.01
		soil moist. x inoc. density	12	0.08
F. sp. isolate 2.2	Sidney, MT	soil moisture	3	<0.01
		inoculum density	4	<0.01
		soil moist. x inoc. density	12	0.44
F. sp. isolate 2.6	Sidney, MT	soil moisture	3	0.04
		inoculum density	4	<0.01
		soil moist. x inoc. density	12	0.10

Figure 1-3. Interaction between sand moisture, propagule density and damping off incidence caused by selected *Fusarium* spp. isolates under controlled conditions in growth chamber experiments.

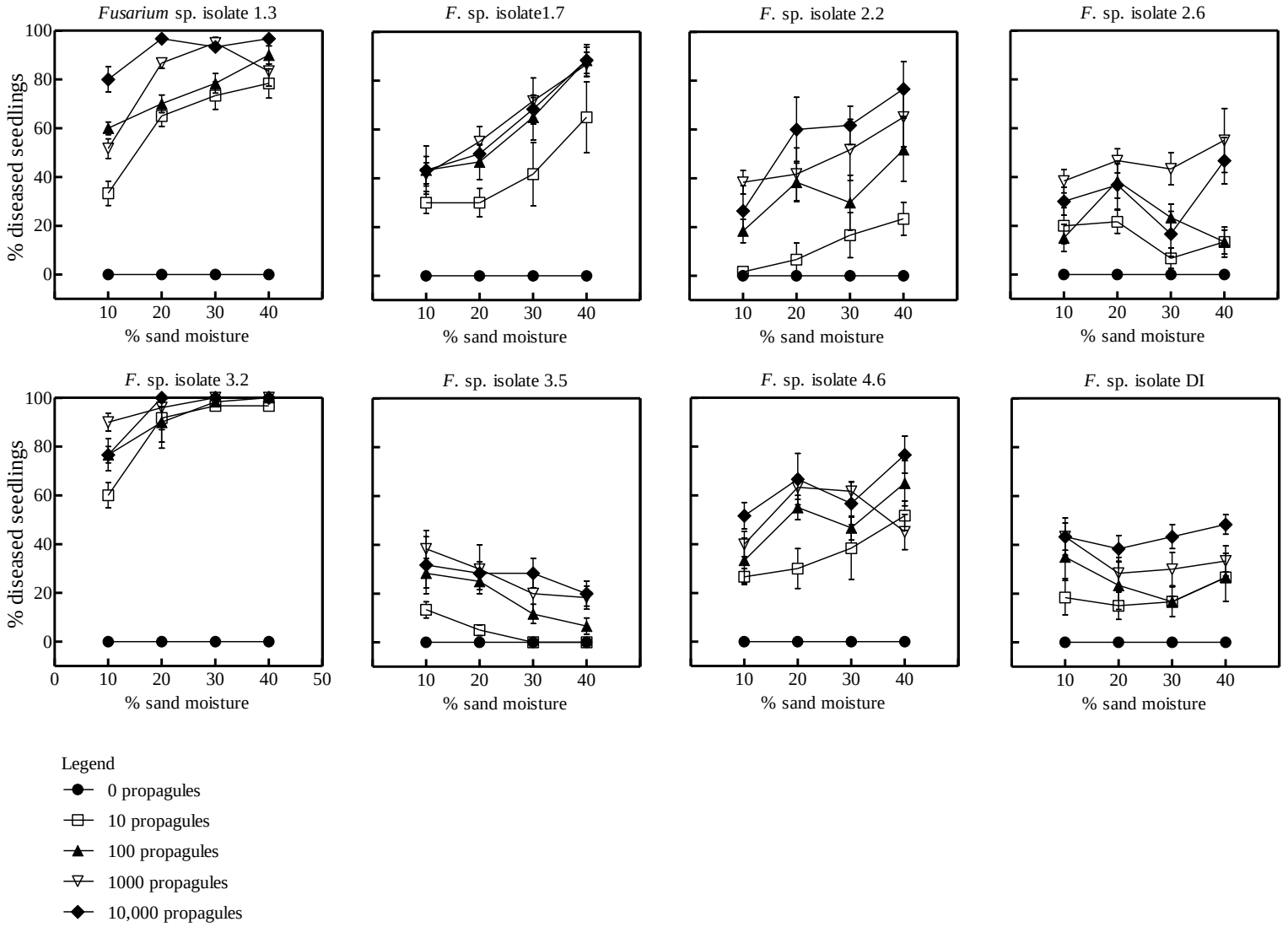


Table 1-9. Percent damping off incidence of *Fusarium spp.* isolates as affected by moisture in growth chamber experiments.

	Percent diseased seeds							
Percent soil moisture	*F*. sp. isolate 1.3 Bozeman, MT	*F*. sp. isolate 3.5 Bozeman, MT	*F*. sp. isolate 3.2 Huntley, Mt	*F*. sp. isolate 4.6 Huntley, MT	*F*. sp. isolate DI Huntley, MT	*F*. sp. isolate 1.7 Sidney, MT	*F*. sp. isolate 2.2 Sidney, MT	*F*. sp. isolate 2.6 Sidney, MT
10	45 a	21 a	59 a	30 a	28 a	32 a	17 a	20 ab
20	64 b	18 ab	77 b	43 b	21 a	36 a	29 b	28 b
30	68 b	16 bc	77 b	41 b	22 a	49 b	32 b	18 a
40	70 b	12 c	77 b	48 b	27 a	66 b	43 c	26 ab

Note: Numbers in a column for each isolate followed by the same letter are not significantly different at $P < 0.05$ (orthogonal polynomial contrasts).

Table 1-10. Percent damping off incidence of *Fusarium spp.* isolates as influenced by inoculum density in growth chamber experiments.

	Percent diseased seeds							
Inoculum density (propagules g^{-1} soil)	*F*. sp. isolate 1.3 Bozeman, MT	*F*. sp. isolate 3.5 Bozeman, MT	*F*. sp. isolate 3.2 Huntley, Mt	*F*. sp. isolate 4.6 Huntley, MT	*F*. sp. isolate DI Huntley, MT	*F*. sp. isolate 1.7 Sidney, MT	*F*. sp. isolate 2.2 Sidney, MT	*F*. sp. isolate 2.6 Sidney, MT
0	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
10	63 b	5 a	86 b	37 b	19 b	42 b	12 b	15 b
100	75 c	14 b	91 b	50 c	25 b	61 c	35 c	23 b
1000	79 c	20 c	100 c	53 c	34 c	64 c	49 d	46 d
10,000	92 d	18 c	94 bc	63 d	43 d	63 c	56 d	33 c

Note: Numbers in a column for each isolate followed by the same letter are not significantly different at $P < 0.05$ (orthogonal polynomial contrasts).

Table 1-11. Significance of contrasts for linear and quadratic trends of damping off incidence of kabuli chickpea cause by *Fusarium* spp. as affected by moisture and inoculum density in growth chamber trials.

		Soil moisture		Log propagules g^{-1} soil	
Isolate	Location	Linear trend	Quadratic trend	Linear trend	Quadratic trend
F. sp. isolate 1.3	Bozeman, MT	***	**	***	***
F. sp. isolate 3.5	Bozeman, MT	*	0.1873	***	0.9471
F. sp. isolate 3.2	Huntley, MT	***	0.0045	***	***
F. sp. isolate 4.6	Huntley, MT	**	0.2851	***	**
F. sp. isolate D.I.	Huntley, MT	0.8474	0.2014	***	**
F. sp. isolate 1.7	Sidney, MT	***	0.7391	***	***
F. sp. isolate 2.2	Sidney, MT	***	0.1586	***	*
F. sp. isolate 2.6	Sidney, MT	0.8022	0.0881	***	0.2013

Note: All linear and quadratic trend contrasts have 1 df. *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.0001$.

Table 1-12. Correlation between disease incidence and severity of damping off of kabuli chickpea caused by *Fusarium* spp. in growth chamber trials.

Isolates	Location of origin	Incidence x cotyledon disease severity	Incidence x root disease severity
F. sp. isolate 1.3	Bozeman, MT	0.7549	0.5447
F. sp. isolate 3.5	Bozeman, MT	0.7290	NS
F. sp. isolate 3.2	Huntley, MT	0.9767	0.9827
F. sp. isolate 4.6	Huntley, MT	0.5243	NS
F. sp. isolate D.I.	Huntley, MT	0.7324	0.5009
F. sp. isolate 1.7	Sidney, MT	0.6451	0.8794
F. sp. isolate 2.2	Sidney, MT	0.5983	0.5625
F. sp. isolate 2.6	Sidney, MT	NS	NS

Note: Pearson's correlation coefficient (r). NS = not significant at $P < 0.05$.

Discussion

The *Fusarium* spp. tested for pathogenicity in chickpea damping off in this study were identified as *F. culmorum*, *F. oxysporum*, *F. rendolens*, *F. sambucinum*, *F. solani*, and *F. trincintum* using a combination of morphological and molecular techniques as suggested by Summerell et al. (2006). However, isolates are referred to as *Fusarium* spp. in text and sequences were submitted to GenBank as *Fusarium* spp., due to the lack of synchrony between morphological and molecular identification. Morphological characters of *F.* sp. isolate 1.3 did not fit any description referenced in most recent source for morphological identification of *Fusarium*, The Fusarium Laboratory Manual (Leslie and Summerell 2006). The morphological characters are most similar to *F. semitectum*, *F. nelsonii,* or *F. camptoceras*; it may be that this isolate has not been previously described or that aberrations in morphology complicated the identification. The TEF 1-α gene of isolate 3.2 was poorly amplified by the primers used; the sequence obtained was not compared to existing sequences in GenBank using BLAST analysis nor was the sequence submitted to GenBank. In this application, no attempt was made to design primers better suited for amplification of this gene for *F.* sp. isolate 3.2. The phylogenetic trees generated based upon the two regions of DNA sequenced also did not suggest a clear picture of the evolutionary history and relatedness of the *Fusarium* spp. utilized in this study. Isolates were often located at differing branch points in each tree, which indicates either differing evolutionary histories for each gene region or that the methods were not ideal for delineating this relationship. The lack of synchrony between

morphological and sequence identification is not entirely unexpected; molecular identification of *Fusarium* is an imperfect science (Windels 1991) and not all *Fusarium* spp. have good representation in GenBank (Leslie and Summerell 2006). The purpose of this paper is not to discuss the complexities of *Fusarium* identification and taxonomy, but to report on the ability of *Fusarium* to cause damping off disease in chickpea.

This is the first report of *Fusarium* spp. causing damping off disease in chickpea. This research found that kabuli chickpea is vulnerable to infection by the *Fusarium* spp. tested in damping off disease under controlled conditions in growth chamber experiments. Levels of disease incidence and severity varied depending upon the isolate, but all isolates were able to infect kabuli chickpea. Moisture and propagule density increases caused an increase in disease incidence for five of the eight isolates examined. Cotyledon disease severity symptoms were generally more severe than root disease severity symptoms. Disease severity on cotyledons correlated positively with moisture increase. No clear trends can be observed between disease incidence or severity in relation to the source location of the isolations, nor does phylogenetic analysis suggest that a particular group causes higher disease incidence or severity in infection of kabuli chickpea. Aside from the lack of such trends, this study was performed on a small scale and to make inferences in regards to disease incidence and severity as influenced by source location and phylogenetic relationships would be unwarranted.

Fusarium spp. have been documented to cause a number of chickpea diseases in addition to damping off. *F. oxysporum* f sp. *ciceris*, *F. avenaceaum*, *F. equiseti*, and *F. solani* are all listed as pathogens involved in Fusarium root rot of chickpeas (Kraft et al.

1981; Nene and Sheila 1996). The strain *F. oxysporum* f. sp. *ciceris* causes vascular wilt disease in chickpea (Burgess 1981).

Past research has indicated *Fusarium* spp. thrive in dry soils, and are frequently reported as important disease agents in dry conditions (Stover 1953; Cook and Papendick 1972; Cook 1981). In contrast with these findings, disease incidence increased with soil moisture for five of the eight *Fusarium* isolates tested. The remaining isolates had either a neutral or inverse relationship between disease incidence and soil moisture. This unique relationship between soil moisture and disease incidence could be due to moisture dynamics in the chickpea germination process. Chickpeas exude a higher quantity of compounds such as carbohydrates and amino acids as seed moisture increases, similar to peas (Cook and Flentje 1967). Seed exudates may stimulate pathogen propagule germination (Schroth and Snyder 1961) and serve as a food sources for both *Fusarium* spp. and other organisms (Singh and Merhota 1980; Nelson 1990). From utilizing exudates as a food source, *Fusarium* spp. may opportunistically infect the susceptible juvenile tissue. To describe the chickpea and *Fusarium* spp. disease dynamic in terms of Lockwood's concepts of soilborne plant pathogens (1988), kabuli chickpeas are predisposed to infection during the seedling stage due to a lack of innate resistance to pathogens and the nature of their seed exudates, which, as a viable pathogen food source, increases *F.* spp. inoculum potential.

Results of chickpea seed treatment studies in Montana in 2007 found that the seed treatments containing the chemical mefenoxam (marketed as Apron XL LS), which targets oomycetes, was effective for controlling damping off, while seed treatments

containing fluidoxonil (Maxim), targeting fungi, were not (Leisso and Burrows 2007). This indicated that oomycetes, such as *Pythium* spp., are more important in damping off in Montana than fungal pathogens such as *Fusarium* sp. However, the results of this research found that *Fusarium* spp. can cause seed rot and damping off in chickpea. The dynamics of *Fusarium* spp. and *Pythium* spp. in damping off disease were not explored in this research, but previous work has indicated that simultaneous infections by oomycetes and fungi are additive in disease severity; the combination of these organisms in disease infection causes higher levels of disease severity than either organism alone (Peters and Grau 2002). Therefore, in order to ensure protection against damping off, we recommend that producers utilize seed treatments which include metalaxyl and a fungicide which target both oomycetes such as *Pythium* spp. and other fungi such as *Fusarium* spp.

References

Agricultural Statistics Board (ASB). 2008. U.S. & All States Data - Chickpeas (Garbanzo) [online] ASB, United States Department of Agriculture (USDA)-National Agricultural Statistics Service (NASS). Available from http://www.nass.usda.gov/QuickStats/PullData_US.jsp [accessed 2 June 2008].

Brick, M. A., Berrada, A., Schwartz, H.F., and Krall, J. 1998. Technical Bulletin TB 98-2: Garbanzo Bean Production Trials in Colorado and Wyoming [online]. Colorado Agricultural Experiment Station and Wyoming Agricultural Experiment Station, Colorado Department of Education, Denver, CO. Available from http://www.cde.state.co.us/artemis/ucsu20/UCSU20210TB982INTERNET.pdf [accessed 27 May 2008].

Burgess, L. W. 1981. General Ecology of Fusaria. *In* Fusarium: diseases, biology, and taxonomy. *Edited by*: P. E. Nelson, T. A. Toussoun and R. J. Cook. The Pennsylvania State University Press, University Park, PA. pp. 225-235.

Chen, W., Paulitz, T.C., McPhee, K.E., and Muehlbauer, F.J. 2004. Field evaluation of seed treatment fungicides for control of root rot and damping-off of chickpea, 2003. Fungicide and Nematicide Tests [online] 59: ST017. The American Phytopathological Society, St. Paul, MN. doi:10.1094/FN59. Available from http://www.plantmanagementnetwork.org/pub/trial/fntests/vol59/abstracts/st017.htm (subscription required).

CLC bio. 2007. CLC combined workbench user manual. Version 3.5 (3.6.2) [computer program]. CLC bio USA, Cambridge, MA.

Cook, R. J. 1981. Water relations in the biology of Fusarium. *In* Fusarium: diseases, biology, and taxonomy. *Edited by* P. E. Nelson, T. A. Toussoun and R. J. Cook. The Pennsylvania State University Press, University Park, PA. pp. 236-244.

Cook, R. J. and Flentje, N.T. 1967). Clamydospore germination and germling survival of *Fusarium solani* f. sp. *pisi* in soil as affected by soil water and pea seed exudation. Phytopathology 57: 178-182.

Cook, R. J. and Papendick, R. I. 1972. Influence of water potential of soils and plants on root disease. Annual Review of Phytopathology: 349-374.

Dhingra, O. D. and Sinclair, J. B. 1985. Basic plant pathology methods. Boca Raton, FL, CRC Press, Inc.

Food and Agriculture Organization (FAO). 2005. Table 13-- Chickpeas: World Harvested acreage, 1970-2005 (05/206) [online]. *In* Chickpea World Acreage. USDA-ERS, Washington, DC. Available from http://usda.mannlib.cornell.edu/usda/ers/02001/CkpWAcres.xls [accessed 16 June 2008].

Gardes, M. and Bruns, TD. 1993. ITS primers with enhanced specificity for basidiomycetes: application to the identificatioin of mycorrhizae and rusts. Molecular Ecology 2: 113-118.

Geiser, D.M., del Mar Jiménez-Gasco, M., Kang, S., Makalowska, I., Veeraraghaven, N., Ward, T.J., Zhang, N., Kuldau, G.A., and O'Donnell, K. 2004. FUSARIUM-ID v. 1.0: A DNA sequence database for identifying *Fusarium*. European Journal of Plant Pathology 110: 273-479. doi: 10.1023/B:EJPP.0000032386.75915.a0.

Hadas, A. 1977. Water Uptake and Germination of Leguminous Seeds in Soils of Changing Matric and Osmotic Water Potential. Journal of Experimental Botany 28(4): 977-985.

Hartley, H. O. 1950. The maximum *F*-ratio as a shortcut for heterogeneity of variance. Biometrika 37: 308-312. doi:10.1093/biomet/37.3-4.308.

Harware, M. P. 1998. Diseases of chickpea. *In* The Pathology of Food and Pasture Legumes. *Edited by* D. J. Allen and J. M. Lenne. CAB International, New York NY.

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). 1981. Annual Report 1979/80. ICRISAT, Patancheru A.P., India.

Kaiser, W. J. and R. M. Hannan. 1983. Etiology and control of seed decay and preemergence damping-off of chickpea by *Pythium ultimum*. Plant Disease 67(1): 77-81. doi: 10.1094/PD-67-77.

Kraft, J. M. 1975. A rapid technique for evaluating pea lines for resistance to Fusarium root rot. Plant Disease Reporter 59(12): 1007-1011.

Kraft, J. M., Burke, D.W., and Haglund, W.A. 1981. Fusarium diseases of beans, peas, and lentils. *In* Fusarium: diseases, biology, and taxonomy. *Edited by* P. E. Nelson, T. A. Toussoun and R. J. Cook. The Pennsylvania State University Press, University Park, PA and London, UK. pp. 141-156.

Leisso, R. L. and M. E. Burrows. 2007. Field evaluation of biological control and fungicide seed treatments for pre-emergence damping off of chickpeas. Pisum Genetics 39: 28-31.

Leslie, J. F. and Summerell, B.A. 2006. The Fusarium Laboratory Manual. Blackwell Publishing, Ames, IA.

Ley, T.W., Stevens, R.G., Topielec, R.R., and Niebling, W.H. Soil water monitoring and measurement [online]. Pacific Northwest Extension Publications: PNW0475. Available from http://cru.cahe.wsu.edu/CEPublications/pnw0475/pnw0475.html [accessed 9 July 2008].

Lockwood, J.L. 1988. Evolution of concepts associated with soilborne plant pathogens. Ann. Rev. Phytopathol. 26:93-121. doi:10.1146/annurev.py.26.090188.000521.

Manter, D. K. and Vivanco, J. M. 2007. Use of the ITS primers, ITS1F and ITS4, to characterize fungal abundance and diversity in mixed template samples by qPCR and length heterogeneity analysis. Journal of Microbiological Methods 71(1): 7-14.

National Center for Biotechnology Information. 2008a. Basic Local Alignment Search Tool (BLAST) [online]. NCBI, National Library of Medicine. Available from http://www.ncbi.nlm.nih.gov/blast/Blast.cgi [accessed 28 May 2008].

National Center for Biotechnology Information. 2008b. GenBank [online]. NCBI, National Library of Medicine. Available from http://www.ncbi.nlm.nih.gov/Genbank/GenbankSearch.html [accessed 28 May 2008].

Nelson, E. N. 1990. Exudate molecules initiating fungal responses to seeds and roots. Plant and Soil 129: 61-73.

Nelson, P.E., Toussoun, T.A. and Marasas, W.F.0. 1983. Fusarium Species: An Illustrated Manual for Identification. The Pennyslvania University Press, University Park, PA.

Nene, Y. L. and Sheila, V. K. 1996. Common names of plant diseases: Diseases of chickpea (*Cicer arietinum* L.)[online]. The American Phytopathological Society, Minneapolis, MN. Available from http://www.apsnet.org/online/common/names/chickpea.asp [accessed 29 May 2008].

O'Donnell, K.; Kistler, H.C., Cigeruk, E., and Ploetz, R.C. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National Academy of Sciences of the United States of America. 95: 2044-2049.

Peters, R. D. and Grau, C. R. 2002. Inoculation with nonpathogenic *Fusarium solani* increases severity of pea root rot caused by *Aphanomyces euteiches*." Plant Disease 86: 411-414.

SAS Institute Inc. 2002-2003. SAS/STAT user's guide. Version 9.1 (TS1M3) [computer program]. SAS Institute Inc., Cary, N.C.

Saxton, K. E. 2006. Soil Water Characteristics Hydraulic Properties Calculator (SPAW) [public download available online]. United States Department of Agriculture (USDA)- Agricultural Research Service (ERS) and Department of Biological Systems Engineering, Washington State University, Pullman, WA. Available from http://hydrolab.arsusda.gov/SPAW/newregistration.html [accessed 29 May 2008].

Saxton, K. E. and Rawls W. J. 2006. Soil water characteristic estimates by texture and organic matter for hydrologic solutions. Soil Sci. Soc. Am. J. 70: 1569-1578. doi: 10.2136/sssaj2005.0117.

Schroth, M. N. and Snyder, W.C. 1961. Effect of host exudates on chlamydospore germination of the bean root rot fungus, *Fusarium solani* f. *phaseoli*. Phytopathology 51: 389-393.

Shapiro, S. S. and M. B. Wilk (1965). An analysis of variance test for normality (complete samples). Biometrika 52(3 and 4): 591-611.

Singh, P. J. and Mehrotra, R. S. 1980. Relation between seed exudate and host susceptibility in gram (*Cicer arietinum* L.) to *Rhizoctonia bataticola*. Plant and Soil 56(2): 265-271.

Stover, R. H. 1953. The effect of soil moisture on Fusarium species. Can. J. Bot. 31: 693-697.

Summerell, B. A., B. Salleh, et al. 2003. A utilitarian approach to Fusarium identification. Plant Disease 87(2): 117-128.

Trapero-Casas, A., Kaiser, W.J., and Ingram, D.M. 1990. Control of Pythium seed rot and preemergence damping-off of chickpea in the U.S. Pacific Northwest and Spain. Plant Disease 74: 563-569. doi: 10.1094/PD-74-0563.

United States Naval Obervatory (USNO). 2008. Sun or moon rise/set table for one year [computer applet][online]. UNSO, Washington, DC, USA. Available fromhttp://aa.usno.navy.mil/data/docs/RS_OneYear.php [accessed 13 June 2008].

Urbianik, G. C. and Plous, S. 1997. Research Randomizer [computer applet, online]. Available from www.randomizer.org [Accessed 29 May 2008].

Van der Plaats-Niterink, A. J. 1981. Monograph of genus Pythium. Centraalbureau Voor Schimmelcultures, Baarn, The Netherlands.

Windels, C. E. 1991. Current status of Fusarium taxonomy. Phytopathology 81(9): 1048-10

THE USE OF BIOLOGICAL AND CONVENTIONAL SEED TREATMENTS TO CONTROL CHICKPEA (*CICER ARIETINUM* L.) DAMPING OFF

Abstract

Damping off of chickpea (*Cicer arietinum* L.), caused by *Pythium* spp., *Fusarium* spp., *Rhizoctonia solani*, and other soil-borne plant pathogens, can lead to stand loss and yield reduction. The objective of this study was to determine the effects of commercially available biological and conventional fungicide seed treatments on damping off of kabuli and desi chickpea. Biological seed treatments including *Bacillus pumilus* GB34 (Yield Shield), *Bacillus subtilis* GB03 (Kodiak), *Bacillus subtilis* MBI 600 (Subtilex), *Streptomyces lydicus* WYEC 108 (Actinovate), *Streptomyces griseoviridis* K61 (Mycostop), *Trichoderma harzianum* Rifai strain KRL-AG2 (T-22 Planter Box), the fungicide treatments fluidoxonil (Maxim) and mefenoxam (Apron XL LS), and combined biological and fungicide seed treatments were tested in greenhouse and field experiments. Two chickpea cultivars, a desi type (CDC-Anna), and a kabuli type (Sierra), were used. The desi cultivar exhibited lower incidence of damping off in greenhouse and field trials than the kabuli cultivar. Several biological seed treatments inhibited germination and growth of kabuli chickpea in the absence of pathogens in greenhouse experiments, but not in field trials. In greenhouse experiments where soil was artificially infested with the damping off pathogen *Pythium ultimum* var. *ultimum*, kabuli chickpea emergence was increased by the application of mefenoxam but not by biological seed treatments. Mefenoxam was the most effective seed treatment in field trials at three locations in

Montana in the spring of 2007 for the control of damping off for both the kabuli and the desi cultivar. Biological seed treatments were generally ineffective for reducing damping off and increasing plant growth measures above untreated controls, even in combination with fungicides. These results indication the use of mefenoxam is critical to control Pythium spp. in Montana and biological controls are not effective control measures for damping off.

Introduction

Chickpea (*Cicer arietinum* L.) is a drought tolerant, cool season legume crop grown for human consumption. In the United States, chickpea is divided into two market classes: kabuli and desi. Desi chickpea cultivar seeds are small (6-7 mm diameter), angular, and have a thick, pigmented seed coat while kabuli chickpea cultivars have large (>8 mm diameter), rounded seeds and a thin cream-colored seed coat (Singh and Saxena 1999). In North America, chickpea is grown throughout the semiarid regions of the Great Plains and Pacific Northwest. The United States reported 70,470 tonnes of chickpea harvested in 2007 (USDA-NASS 2007), while the Canadian province Saskatchewan alone harvested 192,000 tonnes in 2007 (Saskatchewan Ministry of Agriculture 2007). In Montana, 3,266 tonnes of kabuli and 635 tonnes of desi chickpea were harvested in 2007 (USDA-NASS 2008b). Cutforth et al. (2007) reported an increasing interest in chickpea and other pulse crops production throughout the northern Great Plains.

Montana is well-suited for chickpea production (Miller et al. 2002). Montana has potential for development of a high-quality organic chickpea market for two reasons: the

semiarid climate is unfavorable to foliar diseases including ascochyta blight, and more than 50,000 ha of land is certified for organic production (USDA-ERS 2005) which could be managed for premium organic chickpea. Plant diseases pose the most significant challenges to organic chickpea production, in particular the management of seedling damping off and ascochyta blight.

Cool, wet springs cause stand loss due to damping off and seed rot (Kaiser and Hannon 1983; Miller et al. 2002).The oomycetes *Pythium ultimum* var. *ultimum*, *Pythium ultimum* var. *sporangiiferum*, *Pythium irregulare*, and the fungi *Fusarium solani*, *Rhizoctonia solani*, as well as other soil-borne organisms cause damping off of chickpea (Kaiser and Hannan 1983; Trapero-Casas 1990; Harware 1998; Schwartz et al. 2007). Damping off, as described by Kaiser and Hannon (1983), is characterized by seed rot prior to emergence, and seedling stunting due to necrosis and decay of roots. Cultivars possessing darkly pigmented seed coats, primarily desi cultivars, have much higher resistance to damping off than kabuli cultivars (Kaiser and Hannan 1983). With the exception of certain specialty markets, desi chickpea does not command as high a market value as kabuli chickpea (USDA-NASS 2008a).

Damping off is controlled using fungicide seed treatments in conventional farming systems, but non-chemical methods must be used for organic production. Previous research has indicated potential for biological seed treatments to control damping off in chickpea (Kaiser and Hannan 1984; Trapero-Casas 1990; Hammon and Berrada 2001; Stack et al. 2006). Other field trials found biological seed treatments were not effective against damping off (Brick et al. 1998; Smiley et al. 2001; Chen et al. 2004;

Lauver and Guy 2005). At the initiation of this study, nothing was known about the ability of biological seed treatments to control damping off of chickpea in Montana.

Past research has found the combination of biological and chemical seed treatment beneficial for control of seedling disease due to additive benefits of the combined treatments (Taylor and Harman 1990; Estevez de Jensen et al. 2001; Yang and del Rio 2002). This study tested both chemical and biological seed treatments alone and in combination with one another. With this strategy, the results of this research are applicable to both organic and conventional growers seeking the best seed treatments for chickpea.

The objectives of this study were to better understand damping off disease of chickpea, and the effects of biological and fungicide options for control of damping off for both organic and conventional producers. Greenhouse trials compared (i) desi and kabuli chickpea varieties susceptibility to damping off in field soil, (ii) the phytotoxicity of six biological seed treatments to kabuli chickpea in the absence of pathogens (iii) biological seed treatment control of kabuli chickpea damping off caused by *Pythium ultimum*. Field trials tested the effectiveness of (i) conventional fungicide treatments alone, (ii) biological seed treatments alone, and (iii) the combination of biological with fungicide seed treatments for management of chickpea damping off and yield in both kabuli and desi cultivars at three sites in Montana in 2007. Two biological control organisms, *Bacillus subtilis* GB03 and *Bacillus pumilus* GB34, were also tested for their ability to grow in desi and kabuli chickpea seed exudates.

Materials and Methods

Resistance of Chickpea Varieties to Damping Off in Field Soil

The emergence of three chickpea cultivars in field soil and autoclaved field soil was compared under greenhouse conditions to determine if cultivars varied in their susceptibility to damping off. The chickpea cultivars tested were: Dylan, a large kabuli cultivar; Amit (B-90), a small kabuli cultivar; and CDC-Desiray, a desi cultivar. Soil samples were obtained from a field near Big Sandy, MT from which chickpea was harvested approximately two months prior to the sampling date on 17 Oct 2006. Soil was stored at 6 ºC and desiccation was prevented with additions of sterile distilled water until use three weeks after the soil was obtained. Soil was mixed 3 parts field soil to 2 parts peat-based media Sunshine Mix #2 Basic (Sun Gro Horticulture, Vancouver, Canada) by hand in order to improve aeration of soil. Planting media was left unsterilized or autoclaved twice at 121 ºC for 40 min, with 24 h between each sterilization. Chickpea seeds of each cultivar were surface sterilized by immersion in a solution of 0.25% NaOCl for 3 min, 95% ethanol for 30 s, and three rinses in sterile distilled water before allowing the seeds to dry on sterile paper towels in a laminar flow hood. Discolored seeds and those with a cracked seed coats were discarded. Three repetitions of 100 seeds each were sown in 2.5 cm deep in soil-filled metal trays (40 x 32 x 6 cm) in a 5 x 20 array for each treatment. Trays were placed in a greenhouse with a 16 h photoperiod and temperatures of 24±4 ºC d / 18±4 ºC n. Trays were watered daily to field capacity. Emergence was measured 2 wk after planting. The experiment was performed twice.

Seed Treatment Impacts on
Emergence and Seedling Growth

A series of greenhouse experiments were performed to determine if seed treatments altered emergence or seedling growth in the absence of pathogens. Preliminary assays indicated that some biological seed treatments negatively impacted kabuli chickpea, but not desi chickpea seedling health; therefore, these experiments used a kabuli variety, Sierra. Seeds were surface sterilized as previously described. Manufacturer's formulations of biological seed control treatments were applied to seeds using 1% carboxymethylcellulose (Sigma-Aldrich Corp, St. Louis, MO) as a mixing and sticking agent (0.1 ml g^{-1} seed) to ensure even distribution of the treatment. The seed treatments tested were *Bacillus subtilis* GB03 (Kodiak; Bayer CropScience LP, Research Triangle Park, NC, USA), *B. subtilis* MBI 600 (Subtilex; Becker Underwood, Ames, IA, USA), *B. pumilus* GB34 (Yield Shield; Bayer CropScience LP, Research Triangle Park, NC, USA), *Streptomyces lydicus* WYEC 108 (Actinovate; Natural Industries, Inc., Hudson, TX, USA), *S. griseoviridis* strain K61 (Mycostop; Kemira Agra Oy, Helsinki, Finland), and *Trichoderma harzianum* KRL-AG2 (RootShield; Bioworks, Geneva, NY, USA). Treatments were applied in manufacturer's formulation at highest recommended rate; rate and active ingredients are listed in Table 2-1.

Table 2-1. Seed treatments and application rates for control of chickpea damping off in greenhouse and field trials.

Active ingredient	Trade name	Rate (g treatment kg^{-1} seed)
Bacillus pumilus GB34	Yield Shield Concentrate Biological Fungicide	0.08
Bacillus subtilis GB03	Kodiak Concentrate Biological Fungicide	0.08
Bacillus subtilis MBI 600	Subtilex	0.20
Streptomyces griseoviridis K61	Mycostop	5.00
Streptomyces lydicus WYEC 108	Actinovate	5.30
Trichoderma harzianum Rifai strain KRL-AG2	T-22 Planter Box® Granules Biological Fungicide	5.00
Fludioxonil	Maxim 4 FS	0.05†
Mefenoxam	Apron XL LS	0.15†

*Target organisms listed in Appendix B, Table B-1
†g active ingredient per kg seed

The presence of viable biological control organisms on seeds was confirmed by performing three repetitions of washing of two seeds in 4.5 ml phosphate buffered saline (PBS) (6800 ppm K_2PO_4, 1600 ppm NaOH, 8500 ppm NaCl, pH 7.4) and performing serial dilution plating to media favorable for growth of the organism 12 h after seed inoculation. Tryptic soy agar (TSA)(EMD Chemicals Inc., Darmstadt, Germany) was used for the three *Bacillus* spp. Yeast malt extract agar amended with glucose (4 g d(+)-glucose [Sigma Chemical Co., St. Louis, MO], 4 g yeast extract [Becton, Dickson and Company, Sparks, MD], 10 g malt extract [Becton, Dickson and Company, Sparks, MD], 2 g calcium carbonate [Sigma Chemical Co., St. Louis, MO], and 12 g agar (Agar-Agar, EMD Chemicals Inc., Darmstadt, Germany) per liter distilled water; pH adjusted to7.2 with KOH [J.T. Baker, Phillipsburg, NJ]) was used for the *Streptomyces* spp. Water

agar (WA) (Agar-Agar, EMD Chemicals Inc., Darmstadt, Germany) was used for *Trichoderma harzianum* Rifai strain KRL-AG2.

Twelve seeds were planted per pot (16 x 12 x 5 cm), with eight pots in each treatment. The soil mixture used was a 1:1 ratio of Montana State University (MSU) mix (a 1:1:1 blend of mineral soil, Canadian Sphagnum peat moss, Aquagro 2000 G [a wetting agent; Aquatrols, Paulsboro, NJ] and washed concrete sand steam pasteurized at 80 ºC for 45 min) and Sunshine Mix # 2 Basic. Pots were placed on a greenhouse bench with a 16 h photoperiod and temperatures of 24± 4 ºC d / 18±4 ºC n. Eighteen days after planting, the height of all emerged seedlings was measured from the soil line to the highest node and the plants were removed from the pots. Shoots and roots were separated and the roots washed. Fresh weight of plant tissue was measured at harvest. Shoot and root weight was measured after four days of oven drying at 46±3 ºC. The experiment was performed twice.

Efficacy of Biological Seed Treatments in Soil Infested with *Pythium ultimum*

An isolate of *Pythium ultimum* was isolated from a germinated but diseased kabuli chickpea seed grown in field soil obtained from Big Sandy, MT where chickpea had been grown the previous year. Disease symptoms included severe necrosis and softening in the cotyledons. This isolate, B6, was identified visually according to Van der Plaats-Niterink (1981) as *Pythium ultimum* and also identified by sequencing of the rDNA ITS region and comparison to the GenBank database (NCBI 2008b). DNA was extracted using E.Z.N.A. Fungal DNA kit (Omega Bio-tek, Doraville, GA, USA). Approximately 250

mg of mycelia were sampled from a 1-wk-old Petri dish culture of the isolate on potato dextrose agar (PDA, EMD Chemicals Inc., Darmstadt, Germany). Mycelia were placed in a sterile mortar and frozen with liquid nitrogen, then ground into fine powder. Less than 100 mg of mycelia were used for DNA extraction according to manufacturer's instructions. Universal primers ITS5 and ITS4 were used to amplify the rDNA ITS region as described by White et al. (1990); primers are recommended for oomyetes by Ristiano et al. (1998). The reaction mixture consisted of 10 µl GoTaq Master mix (Promega Corporation, Madison, WI, USA), 2 µl (1.25 µM) primer ITS 5, 2 µl (1.25 µM) primer ITS 4, and 6 µl of extracted DNA (250 ng) in a final volume of 20 µl. PCR conditions were: denaturation at 95 ºC for 5 min, followed by 35 cycles of denaturation at 95 ºC for 30 s, annealing at 55 ºC for 30 s, and extension at 72 ºC for 60 s with a final extension step at 72 ºC for 7 min (White et al. 1990). The amplification was performed using an iCycler Thermal Cycler (Bio-Rad Laboratories Ltd, Hemel Hempsted, United Kingdom). Amplified DNA was purified using QIAquick PCR purification kit (Qiagen Sciences Inc., Germantown, MD, USA) and sequenced using ITS5 (Functional BioSciences, Madison, WI, USA). The resulting 880 base pair (bp) sequence was compared to the online sequence database GenBank (NCBI 2008) using the basic local search alignment tool (BLAST) (NCBI 2008a). Both the sequence database GenBank and the search tool BLAST are available online through the National Center for Biotechnology Information of the United States National Library of Medicine (NLM). The BLAST algorithm 'blastn' was used to search the nucleotide collection (nr/nt); the search returned accession number AB355596.1, *Pythium ultimum* as the closest match, differing by 4 bp with a

score of 1605 bits and achieving 100% coverage of queried sequence. The sequence of *P. ultimum* isolate B6 was deposited in GenBank, accession number EU792322. See Appendix B, Table B-2 for complete submission and sequence.

To produce inoculum for pathogenicity studies, *P. ultimum* isolate B6 was grown for 3 wk on 2% water agar (Difco Laboratories, Detroit, MI, USA) to allow for the formation of oospores, and 0.7 cm agar plugs (containing 37 ± 11 oospores) were cut using a circular agar borer from the actively growing edge of the culture. Seeds of kabuli chickpea cultivar 'Sierra' were surface sterilized as described above. Seed treatments *B. pumilus* GB34, *B. subtilis* GB03, *B. subtilis* MBI 600, *S. lydicus* WYEC 108, *S. griseoviridis* strain K61, *T. harzianum* KLR-AG2, and mefenoxam were applied in manufacturer's formulation at the highest recommended rate. The presence of viable biological organisms on seeds was determined as described above. Application rates are listed Table 2-1.

Seeds were planted in 10 x 10 x 10 cm pots. The potting mixture used was the same as in the greenhouse study described above. Two hundred cm^3 of steam pasteurized potting mixture was placed in the bottom of each pot, followed by an agar plug of *Pythium ultimum* var. *ultimum* in each of four quadrants, then covered with 50 cm^3 of soil. Ten chickpea seeds were placed in each pot and covered by an additional 200 $cm^3$of soil.

Four replications of each treatment were arranged in a completely randomized block design. Seedlings were grown at temperatures of 18±2 ºC day/13±2 ºC in a growth chamber with a 16 h photoperiod and maintained near field capacity by daily watering.

The plants were removed from the pots 18 d after planting. Roots were washed and separated from the shoots; both were weighed at the time of plant harvest. Dry weights were measured after 4 d of oven drying at 46±3 ºC. The experiment was performed three times.

Field trials of Biological and Chemical Seed Treatments at Three Locations in 2007

Twelve seed treatments comprised of combinations of the biological control fungicide treatments *B. pumilus* GB34, *B. subtilis* GB03, and *T. harzianum* KRL-AG2, and individually or combined with the chemical fungicides mefenoxam and fluidoxonil were tested on the kabuli chickpea variety Sierra and the desi chickpea variety CDC-Anna at three locations in Montana in the summer of 2007. Application rates are listed in Table 2-1. Carboxymethylcellulose (CMC, 1%, 45 ml/kg seed) was used as a sticking agent and to help disburse the seed treatments. The twelve seed treatments were: 1) untreated control (1% CMC), 2) *B. pumilus* GB34, 3) *B. subtilis* GB03, 4) *T. harzianum* KRL-AG2, 5) mefenoxam 6) fluidoxonil 7) *B. pumilus* GB34 and mefenoxam, 8) *B. subtilis* GB03 and mefenoxam, 9) *T. harzianum* KRL-AG2 and mefenoxam, 10) *B. pumilus* GB34 and fluidoxonil, 11) *B. subtilis* GB03 and fluidoxonil 12) *T. harzianum* KRL-AG2 and fluidoxonil.

Field plots were located at Montana Agricultural Experiment Stations (MAES) in Bozeman (Arthur H. Post Agronomy Research Farm), Huntley (Southern Agricultural Research Center), and Sidney (Eastern Agricultural Research Center), MT. Winter wheat was grown in the winter of 2005-2006 at the Bozeman site prior to the spring seeding of

chickpea in 2007. Bozeman field soil is an Amsterdam-Quagle silt loam, pH 7.5. The Huntley site had been fallow for 2 yr and the soil is a Fort Collins/Thurlow clay loam with a pH of 7.8. Plots at Sidney were seeded following safflower in 2005 and fallow in 2006. The soil at Sidney is Williams loam with a pH of 7.7. At Bozeman, plots were 1.8 m by 4.6 m. Huntley plots were 1.8 m by 7.6 m. Field plots at Sidney were 1.2 m by 3.1 m. At Bozeman, rows were spaced 26 cm apart; at Huntley and Sidney, 30 cm. The experiment was arranged as a randomized complete block design with six replications of each treatment. Prior to planting, pre-emergence herbicides were applied to field plots. At Bozeman, trifluran (Treflan HFP, Dow AgroSciences LLC, Indianapolis, IN, USA) was applied around 15 April at the rate of 3.4 kg ai ha^{-1} using a 30 cm Valmar applicator; 0.34 kg a.i. ha^{-1} of sulfentrazone (Spartan 4F, FMC Corporation, Philedelphia, PA, USA) mixed with 0.7 kg ammonium sulfate, as well as 1.8 kg a.i. ha^{-1} glyphosate in 90 L ha^{-1} water on 27 April. At Huntley, ethafluranlin (Sonalan, Dow AgroSciences LLC, Indianapolis, IN, USA) at the rate of 1.7 kg a.i. ha^{-1} and incorporated immediately; date of application is unknown. Ethafluranlin (Sonalan) was also applied at Sidney on17 April, at the rate of 0.20 kg a.i. ha^{-1} and incorporated immediately. Seeds were planted 4 to 5 cm deep with plot seeders. Seeds were planted at the rate of sixty kabuli cultivar Sierra seeds per m^2, and seventy desi cultivar CDC-Anna seeds per m^2. Plots were seeded at Bozeman 1 May, Huntley 1 May, and Sidney 27 April, 2007. A peat-based rhizobial inoculant containing *Mesorhizobium ciceri* was included in each packet (18.5 kg/ha, Soil Implant+, Nitragin, Milwaukee, WI, USA). During the 3 wk following planting, Bozeman soil temperatures averaged 14.1 ºC and plots received 42 mm of precipitation.

At Huntley the average soil temperature during the three weeks following planting was 15 ºC and 7 mm of precipitation accumulated. Mean Sidney soil temperature was 14 ºC; precipitation reached 50 mm. Stand counts were taken three weeks after planting, on 20 May at Bozeman, 21 May at Huntley, and 18 May at Sidney.

Ascochyta blight was identified at the Huntley field site on 5 June, the Bozeman field site on 7 June and at Sidney on 11 June, 2007. The disease was controlled by foliar fungicide applications until dry conditions commenced in early July or the beginning of plant senescence. Fungicides applied were azoxystrobin+chlorothalonil (Quadris Opti SC; Syngenta Crop Protection Inc., Greensboro, NC, USA) and prothioconazole (Proline 480; Bayer CropScience, Research Triangle Park, NC, USA) at the rates of 1.7 kg a.i. and 0.16 kg a.i./ha. See Table 2-2.

Table 2-2. Application of foliar fungicides to control ascochyta blight in chickpea field trials in Montana in 2007

Location	Date	Treament
Bozeman	8 June	prothioconazole
	15 June	azoxystrobin+chlorothalonil
	22 June	prothioconazole
	11 July	azoxystrobin+chlorothalonil
Huntley	9 June	prothioconazole
	14 June	azoxystrobin+chlorothalonil
	27 June	prothioconazole
	9 July	azoxystrobin+chlorothalonil
Sidney	14 June	prothioconazole
	20 June	azoxystrobin+chlorothalonil
	11 July	prothioconazole

At Bozeman, treatments were applied using a handheld CO_2-powered sprayer with four, 8002 VS nozzles spaced at 30 cm with pressure of 2.07 GPa. Huntley field

plots were treated with using a 6 m wide sprayer with Teejet XR80015 nozzles spaced 50 cm apart at and delivered at10 GPa. At Sidney treatments were applied using a custom-built sprayer mounted on a Kobota tractor with nozzle spray pressure of 15 GPa. Ascochyta blight was rated every 7 to 10 d using the 1-9 (1 = no disease, 9 = plant death) rating scale described by Singh et al. (1981). For complete description of rating scale see Appendix B, Table B-3.

Plant heights were taken just prior to flowering for kabuli varieties and at early flowering for desi varieties on 21 June at Bozeman, 21 June at Huntley and 19 June at Sidney. Heights were measured from the soil line to the highest fully developed node. In cases where a single plant had multiple branches originating at the base, the longest branch was selected. Four plant heights were randomly collected per plot from the inner rows at least 0.6 m into the plot from the ends of the rows.

Plant canopy closure was estimated using a LAI-2000 Plant Canopy Analyzer (LI-COR Biosciences, Lincoln, NE, USA) at the phenological stages of green pod for the kabuli chickpeas, and at expanded pod for the desi chickpeas as described by Verghis et al. (1999). Measurements were taken with a 45 º optical blocker between 5 A.M. and 9 A.M. on 16 July in Huntley, 18 July in Sidney, and 20 July in Bozeman. Five measurements (one above and four below canopy) were taken in each plot. Below canopy measurements were taken 6±2 cm from the ground. LAI measurements were collected systematically in a diagonal from the lower left of the plot to the upper right of the plot.

Plant weights were obtained at expanded pod for kabuli variety and near mature-pod stage for the desi variety (described by Verghis et al. 1999) on 25 July at Bozeman

and Huntley, and 26 July in Sidney. Four plants were randomly selected per plot from the inner rows, at least 0.6 m from the either end of the rows. Plants were placed in paper bags and oven-dried for 7 d at 46±3 ºC. For complete data of chickpea phenology, see Appendix B, Table B-3.

Plots were harvested using plot combines at Bozeman and Huntley. Bozeman plots were harvested 27 August; Huntley plots 31 August. At Sidney, desi plots were harvested 9 August; plants were cut by hand and fed into plot combine for threshing. No yield was obtained from the kabuli cultivar 'Sierra' plots at Sidney due to low initial stand and severe ascochyta blight.

Seed quality of kabuli chickpea was measured by sieving seeds through sieves with hole sizes of 8, 9, and 10 mm, and by obtaining seed weights. Seed quality of desi treatments was determined by obtaining seed weights.

Multiplication of Biological Control Organisms in Chickpea Seed Exudates

To determine if chickpea seed exudates are able to support the growth of biological control organisms, exudates were collected from desi and kabuli chickpea seeds and inoculated with the bacterial biological control organisms tested in the field trials, *Bacillus pumilus* GB34 (Yield Shield) and *B. subtilis* GB03 (Kodiak); colony forming units (cfu) of the biological control organisms in seed exudates was quantified. The growth of the fungal biological control organism used in field trials, *Trichoderma harzianum* (T-22 Planter Box), in exudates was not tested since preliminary experiments indicated that much of the fungal growth in exudates broth culture within 36 h is

vegetative mycelia, rendering serial dilution plating less meaningful. To obtain chickpea seed exudates, 25 g each of desi cultivar CDC-Anna and kabuli cultivar Sierra were surface sterilized as previously described. Seeds were placed in 50 ml sterile distilled water in the dark on a rotary shaker at 130 rpm for 24 h. Seed exudates were extracted by vacuum filtration through a sterile 0.2 µm filter disc 82 mm in diameter (Gelman Sciences, Ann Arbor, MI, USA). Fifteen ml of water was used to rinse seeds and pooled with primary exudates for a total of approximately 50 ml seed exudate suspension from each variety. Ten µl of a 24 hr culture of *B. subtilis* GB34 (Kodiak) and *B. pumilus* GB34 (Yield Shield) were added to 10 ml of seed exudate suspension in 125 ml Erlenmeyer flask. Ten ml of 25% tryptic soy broth (TSB, EMD Chemicals Inc., Darmstadt, Germany) was used as a control, representing bacterial growth under optimal conditions. Two repetitions of each bacterium in each broth culture media were performed. At 12, 24, and 36 h, 1.5 ml of the bacterial suspension were collected from each flask. The suspension was centrifuged for 5 min at 8000 x *g*, and the supernatant was decanted. The pellet was resuspended in 1.5 ml phosphate buffere saline (PBS, 6800 ppm K_2PO_4, 1600 ppm, NaOH, 8500 ppm NaCl, pH 7.4) and centrifuged for 5 m at 8000 x *g*, again the supernatant was decanted and the pellet was resuspended in 1.5 ml PBS. Serial dilutions of the suspension (10^{-1} to 10^{-8}) were performed by adding 0.5 ml of suspension to 4.5 ml of sterile PBS in 50 ml sterile test tubes. Enumeration of bacteria was performed using the drop plate method (Herigstad 2001). The experiment was performed twice.

Statistical Analyses

Data analysis was performed using Statistical Analysis System (SAS) (v. 9.1, Cary, NC, USA 2003). Residuals for experimental data both within an individual experiment and between repetitions of the same experiment were examined for normality and homogeneity of variances. Homogeneity of variance was examined using Hartley's F-max test (Hartley 1950), while the assumption of normality was examined using the Shapiro-Wilk's test (Shapiro and Wilk 1965) performed by the univariate procedure. Data from greenhouse and growth chamber experiments, both as individual repetitions of experiments, and as combined data from multiple repeats of the same experiment when variance was homogenous, were subjected to analysis of variance (ANOVA) for parameters of interest. Treatment means separation for germination data in greenhouse experiments were determined by using Fischer's Least Significant Difference (LSD) at a probability level of $P < 0.05$. For root and shoot weight data in greenhouse and growth chamber experiments, least squares treatment means were generated and all possible pairwise t-tests were performed due to unbalanced data sets. Letter groupings were delineated based on results of pairwise t-tests as generated by the *pdiff* option, and pairwise treatment comparisons where $P < 0.05$ were considered significantly different. ANOVA was utilized for field data. Since chickpea cultivar was consistently a significant source of variation based on type III sums of squares in Proc GLM ($P < 0.05$), results from kabuli and desi chickpea field plots were analyzed separately. A logarithmic transformation was used in the analysis of variance ANOVA for field emergence data of the kabuli chickpea at the Huntley and Sidney locations, kabuli and desi chickpea plant

weights from the Bozeman and Huntley locations, and desi chickpea plant weights from the Sidney location. Emergence data for both kabuli and desi chickpea plots at Bozeman and Huntley, emergence data for desi chickpea plots at Sidney and plant heights, LAI measurements, yield and all seed quality data for all field locations and both seed types were subjected to ANOVA without transformation. Treatment means of untransformed data have been used in all tables and figures in the text. For field germination data, t-tests of least squares means were used to compare treatment means, due to missing or omitted data. Due to clogging in the seeder while planting kabuli chickpea seeds at the Huntley and Sidney, individual plot stand counts were low. Outliers which met Chauvenet's criterion (Taylor 1997) were omitted. Simple linear regression analysis was performed on an individual site basis and separately by cultivar to determine if the number of seeds germinated consistently predicted the yield harvested. Bacterial multiplication over time in chickpea seed exudate was analyzed using repeated measures analysis in PROC MIXED; means were estimated and compared as per Littlell et al. (1997). Results from *Bacillus subtilis* GB03 and *B. pumilus* GB34 were analyzed separately.

Results

Resistance of Chickpea Varieties to Damping Off in Field Soil

The emergence of kabuli and desi chickpea cultivars in field soil and autoclaved field soil was tested to determine if the cultivars differed in their resistance to damping off disease. In autoclaved soil, the desi chickpea cultivar had higher emergence than the

large kabuli cultivar (98% compared to 84%) (Table 2-3). The desi cultivar had higher emergence in field soil (78%) than either the small kabuli (3%) or kabuli chickpea cultivar (3%) (Table 2-3).

Table 2-3. Percent emergence of three chickpea cultivars in field soil and autoclaved field soil in greenhouse experiments

		Autoclaved field soil		Field soil	
Cultivar	Seed type	% emergence		% emergence	
Desiray	desi	98	a	78	c
Amit B-90	small kabuli	89	ab	3	d
Dylan	large kabuli	84	bc	3	d

Note: Values followed by the same letter in a column are not significantly different at $p < 0.05$ (Fischer's LSD).

Seed Treatment Impacts on Emergence and Seedling Growth

Chickpea seeds treated with biological organisms were planted in steam-sterilized soil under controlled conditions to determine if the effects of treatments on emergence and plant health measurements in the absence of pathogens. *B. subtilis* GB03, *B. subtilis* MB1600, *S. griseoviridis* K61, and *T. harzanium* KRL-AG2 to kabuli chickpea reduced emergence as compared to the untreated control, while *Bacillus pumilus* GB34 and *S. lydicus* WYEC 108 had no effect on emergence in pasteurized soil (Table 2-4). Seedlings treated with *B. pumilus* GB34 emerged earlier than the untreated control as measured by the number of emerged seedlings 6 d after planting, although overall emergence did not differ from the control (data not shown). Dry weights of roots and shoots reflected trends seen in the emergence data. All of the treatments except *S. lydicus* WYEC 108 had lower root dry weights than the untreated control. Shoot dry weights of seedlings from

treatments *B. subtilis* GB03 and *Trichoderma harzianum* Rifai strain KRL-AG2 were lower than the control, while the rest did not differ from the control (Table 2-4).

Table 2-4. Effects of biological control seed treatments on kabuli chickpea emergence and seedling dry weight in steam sterilized soil in greenhouse experiments.

Treatment*	% emergence	Root dry weight (mg)	Shoot dry weight (mg)
Control	81 a	64 a	107 ab
Bacilllus pumilus GB34	78 ab	52 b	107 b
B. subtilis GB03	68 bc	34 c	83 c
B. subtilis MBI 600	65 c	51 b	95 bc
Streptomyces griseoviridis K61	67 bc	36 c	92 bc
S. lydicus WYEC 108	75 a	56 ab	127 a
Trichoderma harzianum Rifai strain KRL-AG2	63 c	38 c	87 c

Note: Values followed by the same letter in a column are not different at $P < 0.05$ (least significant difference for percent emergence; pair-wise Student's t-test for weights). Application rates: *B. pumilus* GB34, 0.08 g kg^{-1}; *B. subtilis* GB03, 0.08 g kg^{-1}; *B. subtilis* MBI600; *S. griseoviridis* K61, 5.0 g kg^{-1}; *S. lydicus* WYEC 108, 5.0 g kg^{-1}; *T. harzianum* Rifai strain KRL-AG2, 5.0 g kg^{-1}.
* All treatments applied with 1% carboxymethylcellulose as a mixing and sticking agent (0.1 ml g^{-1} seed).

Efficacy of Seed Treatments in Soil
Infested with *Pythium ultimum*

The ability of biological seed treatments and mefenoxam seed treatment to control damping off caused by *Pythium ultimum* was tested. Growth chamber experiments indicated that the fungicide mefenoxam was more effective than any of the biological seed treatments for reducing stand loss on kabuli chickpea caused by *Pythium ultimum* (Table 2-5). Mefenoxam increased stand emergence by at least 30% over untreated seeds in pathogen infested soil, and did not differ in emergence from seeds grown in

uninoculated soil in all repetitions of the experiment. Biological control seed treatments did not increase seedling emergence compared to the control (Table 2-5).

Table 2-5. Emergence of kabuli chickpea treated with six biological control seed treatments and mefenoxam in soil treated with *Pythium ultimum*

Treatment*	%Emergence	
Healthy control†	66.6	a
Inoculated control‡	30.0	bcd
Mefenoxam	60.8	a
Bacillus pumilus GB34	39.2	bc
B. subtilis GB03	42.5	b
B. subtilis MBI 600	31.6	bcd
Streptomyces griseoviridis K61	18.3	d
S. lydicus WYEC 108	22.5	cd
Trichoderma harzianum Rifai strain KLR-AG2	40.8	b

Note: Values followed by the same letter in a column are not different at $P < 0.05$ (Fischer's least significant difference).
Application rates: mefenoxam, 0.05 g a.i. kg^{-1}; *B. pumilus* GB34, 0.08 g kg^{-1}; *B. subtilis* GB03, 0.08 g kg^{-1}; *B. subtilis* MBI600; *S. griseoviridis* K61, 5.0 g kg^{-1}; *S. lydicus* WYEC 108, 5.0 g kg^{-1}; *T. harzianum* Rifai strain KRL-AG2, 5.0 g kg^{-1}.
*All treatments applied with 1% carboxymethylcellulose as a mixing and sticking agent (0.1 ml g-1 seed).
†Untreated seeds, un-inoculated soil.
‡Untreated seeds, *Pythium ultimum*-inoculated soil.

Seed Treatment Effects on Emergence and Yield
of a Kabuli and a Desi Chickpea Cultivar in Field Experiments

Biological and chemical treatments were applied to kabuli and desi chickpea planted at three sites in Montana in 2007 to determine which seed treatments were most effective for reducing stand loss due to damping off. Treatments and results are listed in Table 2-6. Emergence of the desi chickpea cultivar was higher than the kabuli chickpea cultivar at all locations at $P < 0.05$. Results for each cultivar were analyzed separately.

Seed treatments containing mefenoxam alone or in combination with biological treatments were the most effective for reducing damping off for the kabuli cultivar at all three field locations (Table 2-6). For the kabuli cultivar, biological treatments applied alone did not increase seedling emergence compared to the untreated control. Fluidoxonil was also ineffective for increasing emergence as compared to the untreated control for the kabuli cultivar, although fluidoxonil combined with *B. subtilis* GB03 did increase emergence at two of the three locations. Within the desi chickpea plots, no seed treatment consistently increased emergence. At Bozeman, *T. harzianum* Rifai strain KRL-AG2, mefenoxam, and *T. hazianum* Rifai strain KRL-AG2 combined with fluidoxonil increased desi chickpea emergence compared to the untreated control. At Huntley, there were no differences in emergence due to seed treatment. At Sidney, desi chickpea seeds treated with *B. subtilis* GB03 alone had increased emergence compared to the untreated control (Table 2-6).

Results for kabuli yield at Bozeman and Huntley indicated modest differences in yield due to seed treatments (Table 2-6). Kabuli plots at Sidney were not harvested due to low initial stands and ascochyta blight damage. No differences in yield due to seed treatment were observed for the desi cultivar at either Bozeman or Huntley. For the desi variety at Sidney, treatments *T. hazanium* KRL-AG2and *B. pumilus* GB34+mefenoxam yielded more than the untreated control (Table 2-6). Simple linear regression indicated that the number of emerged seedlings could not reliably predict yield for either of the cultivars at any of the locations: higher emergence rates did not lead to higher yield.

Table 2-6. Effects of seed treatments on emergence and yield of a kabuli and desi chickpea cultivar at three field locations in Montana in the spring of 2007

	Bozeman, MT				Huntley, MT				Sidney, MT			
Treatment	%Emergence		Yield(kg/ha)		%Emergence		Yield(kg/ha)		%Emergence		Yield (kg/ha)*	
Sierra (kabuli cultivar)												
Control	58	cd	1234	ab	65	cd	1920	NS	7	bc	--	--
fluidoxonil	59	bc	1280	ab	60	d	1827	NS	2	c	--	--
mefenoxam	96	a	1335	a	94	a	1815	NS	64	a	--	--
Bacillus pumilus GB34	55	cd	1136	ab	64	d	1978	NS	5	c	--	--
B. subtilis GB03	65	bc	1248	ab	71	bc	1918	NS	17	b	--	--
Trichoderma harzianum Rifai strain KRL-AG2	64	bc	1349	a	71	bc	1854	NS	18	b	--	--
mefenoxam + *B. pumilus* GB34	90	a	1304	a	90	ab	1905	NS	74	a	--	--
mefenoxam + *B. subtilis* GB03	89	a	1316	a	93	a	1942	NS	66	a	--	--
mefenoxam + *T. harzianum* Rifai strain KRL-AG2	89	a	968	b	83	a	2024	NS	54	a	--	--
fluidoxonil + *B. pumilus* GB34	56	cd	1313	a	63	cd	1698	NS	3	c	--	--
fluidoxonil + *B. subtilis* GB03	67	b	1398	a	89	a	1928	NS	9	b	--	--
fluidoxonil + *T. harzianum* Rifai strain KRL-AG2	56	cd	1184	ab	71	bcd	1857	NS	3	c	--	--
CDC-Anna (desi cultivar)												
Control	80	b	1799	NS	85	ab	2704	NS	69	bcd	1598	bc
fluidoxonil	88	ab	1990	NS	97	a	2507	NS	63	cd	1997	ab
mefenoxam	92	ab	1916	NS	91	a	2560	NS	54	d	1553	c
Bacillus pumilus GB34	89	ab	1893	NS	93	a	2769	NS	73	abc	1708	abc
B. subtilis GB03	79	b	1554	NS	90	ab	2779	NS	89	a	1547	c
Trichoderma harzianum Rifai strain KRL-AG2	97	a	1901	NS	89	ab	2769	NS	76	abc	2087	a
mefenoxam + *B. pumilus* GB34	97	a	1873	NS	89	ab	2945	NS	85	ab	2081	a
mefenoxam + *B. subtilis* GB03	86	ab	1998	NS	93	a	2741	NS	71	abcd	1939	abc
mefenoxam + *T. harzianum* Rifai strain KRL-AG2	87	ab	1902	NS	76	b	2335	NS	80	abc	1842	abc
fluidoxonil + *B. pumilus* GB34	91	ab	1849	NS	91	a	2682	NS	68	bcd	1529	c
fluidoxonil + *B. subtilis* GB03	81	b	1826	NS	95	a	2784	NS	84	ab	1878	abc
fluidoxonil + *T. harzianum* Rifai strain KRL-AG2	96	a	1905	NS	95	a	2540	NS	74	abc	1729	abc

Note: Values followed by the same letter in a column not different at $P < 0.05$ (least significant difference). Values followed by NS indicate that *F*-ratio from ANOVA was not significant. Application rates, both in solo and combined treatments: mefenoxam, 0.05 g a.i. kg^{-1}; fluidoxonil 0.05 g a.i. kg^{-1}; *B. pumilus* GB34, 0.08 g kg^{-1}; *B. subtilis* GB03, 0.08 g kg^{-1}; *B. subtilis* MBI600; *S. griseoviridis* K61, 5.0 g kg^{-1}; *S. lydicus* WYEC 108, 5.0 g kg^{-1}; *T. harzianum* Rifai strain KRL-AG2, 5.0 g kg^{-1}.
*No harvest for kabuli cultivar Sierra at Sidney.

Seed Treatments Effects on Plant Health
in Field trials

Plant growth as measured by plant weight, leaf area index (LAI), plant height, and ascochyta blight severity were not consistently affected by seed treatments at three field locations. Mean values for these variables are presented in Table 2-7. Only measurements of plant height and LAI in the kabuli cultivar at Sidney had differences due to seed treatment (data not shown). At Sidney, most treatments, except those containing mefenoxam, had poor kabuli stand establishment due to heavy spring rains and cool soil temperatures. Seedlings that did establish in these plots appeared unthrifty compared to seeds treated with mefenoxam (personal observation). This observation was reflected in taller plants (36 cm) compared to the control (30 cm) at $P < 0.05$ for kabuli seeds treated with mefenoxam at Sidney. Differences in LAI existed for kabuli plots at Sidney due to the differing seedling emergence in the plots. Emergence correlated significantly with LAI ($P < 0.05$, Pearson's correlation coefficient r= 0.60, n= 72). Seed weights and size did not differ among treatments for either the kabuli or desi chickpea trial locations (data not shown).

Table 2-7. Plant health measurements from treatment trials in Montana, 2007.

	Plant height (cm)		Plant weight (g)		Leaf area index (LAI)		Ascochyta severity rating*		1000 seed weights (g)		Seed size (kabuli)			
Location	kabuli	desi	kabuli†	desi	kabuli	desi	kabuli	desi	kabuli†	desi	<8 mm	8-9 mm	9-10 mm	>10 mm
Bozeman	39	33	42	26	1.8	2.1	3.2	3.0	434	178	7%	15%	60%	18%
Huntley	42	34	54	27	1.5	1.7	5.2	3.1	405	182	8%	20%	56%	16%
Sidney†	28	28	--	24	0.7	1.5	7.1	7.3	--	191	--	--	--	--

Note: Mean values for each variable presented. Few differences existed in measurements of plant health, with the exception of kabuli height and LAI at Sidney, where treatments containing mefexonam increased height and LAI.
*Final rating; 1=no disease, 9 = plant death. A total of 3 to 4 fungicide applications of Quadris Opti SC (active ingredients azoxystrobin and chlorothanolil) alternated with Proline (active ingredient prothioconazole) to control ascochyta blight were made at each location.
†No kabuli plant weights, seed weights, or seed size obtained from Sidney for kabuli cultivar.

Multiplication of Biological Control Organisms in Chickpea Seed Exudates

Twenty-four hr old desi and kabuli chickpea seed exudates, and 25% tryptic soy broth (TSB) were inoculated with the bacterial biological control organisms *B. pumilus* GB34 and *B. subtilis* GB03 to determine if the organisms could grow and multiply in chickpea seed exudates. Both bacteria were able to grow in both desi and kabuli chickpea seed exudates. Both *B. pumilus* GB34 and *B. subtilis* GB03 multiplied less quickly and had overall lower colony forming units (cfu) in desi chickpea seed exudate over a 36 h period than either kabuli chickpea seed exudate or 25% TSB. Exudates of kabuli chickpea supported a similar population as 25% TSB, a nutrient rich media broth (Table 2-8).

Table 2-8. Quantification of biological control organisms *Bacillus pumilus* GB34 and *Bacillus subtilis* GB03 grown in desi chickpea seed exudate, kabuli seed exudate, and 25% tryptic soy broth and quantified at 12, 24, and 36 h by serial dilution plating

	Log cfu/ml		
Biological control organism	12 h	24 h	36 h
B.pumilus GB34			
Desi chickpea seed exudate	0.0 a	5.2 a	5.4 a
Kabuli chickpea seed exudate	7.2 b	8.8 b	8.6 b
25% tryptic soy broth	8.7 c	9.3 c	9.0 c
B. subtilis GB03			
Desi chickpea seed exudate	0.0 a	7.0 a	6.9 a
Kabuli chickpea seed exudate	7.0 b	8.2 b	8.4 b
25% tryptic soy broth	7.0 b	8.4 b	8.8 c

Note: cfu = colony forming units. *B. pumilus* GB34 and *B.subtilis* GB03 analyzed separately. Within each biological control organism, numbers in columns followed by the same letter are not significantly different at $p < 0.05$ (orthogonal polynomial contrasts).

Discussion

Biological control fungicide seed treatments are a promising tool for managing seedling damping off of many crops in organic and conventional systems (Weller 1998; Taylor and Harman 1990; Mao et al. 1997; Estevez de Jensen 2001). However, our tests indicated that biological seed treatments were not consistently effective for control of damping off of chickpea in Montana.

Mefenoxam (Apron XL LS) was the most effective seed treatment for controlling damping off for both kabuli and desi chickpea cultivars, in both greenhouse and field trials. In field trials, the chemical mefenoxam, which targets oomycetes that cause damping off, was effective for reducing stand loss. Conversely, the other chemical treatment tested, fluidoxonil (Maxim), which targets a variety of fungal pathogens, was not as effective as mefenoxam for reducing stand loss, indicating that oomycetes are a more important cause of damping off disease of chickpea in Montana than fungal pathogens. In field trials, treatments containing mefenoxam increased emergence over the untreated control, but no consistent yield increase was observed due to the success of this seed treatment. At Sidney, seedling emergence was extremely low in kabuli chickpea plots where seeds were not treated by mefenoxam, but kabuli chickpea was not harvested at Sidney due to the severity of ascochyta blight. At this site, had plants not been decimated by ascochyta blight, yield differences due to mefenoxam seed application in the kabuli chickpea plots would have been likely. Additionally, plant biomass compensation in response to reduction of plant density coupled with the lack of weed

pressure to weed-free plot maintenance may have contributed to the lack of yield difference. A study in Saskatchewan found that more than doubling plant density resulted in a 27% increase in yield for a desi cultivar, and only a 17% increase in yield for a kabuli cultivar (Liu et al. 2003). The relationship between increased plant density and yield was not directly proportional, implying that plants at lower densities can increase biomass and yield to compensate for the lower initial stand density.

Bacillus subtilis GB03 (Kodiak) exhibited the most potential for control of damping off, although results were inconsistent. Percent emergence of seeds treated with Kodiak did not differ from the healthy control in two of three experiments under controlled conditions, indicating *B. subtilis* GB03 was effective for reducing damping off. However, *B. subtilis* GB03 reduced emergence in the absence of a pathogen in greenhouse trials. It may be that in the absence of other microbes, this organism overwhelms the chickpea spermosphere and rhizosphere to a degree where it inhibits seed germination and growth. For the desi chickpea cultivar in field trials at Sidney, only seeds treated with *B. subtilis* GB03 had increased emergence (89%) compared to the untreated control (69%). Notably, other treatments containing Kodiak also had relatively high emergence: *B. subtilis* GB03+mefenoxam (71%) and *B. subtilis* GB03 +fluidoxonil (81%). This was the only field location where *B.subtilis* GB03-treated seeds had greater seedling emergence compared to the control. Potentially, the cool wet conditions at Sidney, which were conducive to higher disease pressure than at the other two locations, contributed to better resolution of seed treatment efficacy.

Previous research has indicated the combination of biological control treatments with low levels of chemical treatments can increase the reliability of disease control (Hwang and Chakravarty 1992; Zablotowicz et al. 1992). For example, chemicals may reduce competition of other soil microbes with biological control organisms, and the two treatments may target different pathogens or have synergistic effects against pathogens (Taylor and Harman 1990). This study found combinations of biological seed treatments with fungicides differed little from solo fungicide application in their effect on damping off incidence or other measures of plant health, including yield.

No beneficial effects in plant growth or resistance to ascochyta blight were noted despite previously documented plant health benefits related to biological control treatments in legumes and other field crops (Backman et al 1994; Nemec et al. 1996; Raupach and Kloepper 1998; Tokala et al. 2002; Kloepper et al. 2004). The application of treatment *B. pumilus* GB34 to seeds has been documented to reduce the severity ascochyta blight of chickpea, although this work has not been published (personal communication, M.S. Reddy, 2008). Apparent detrimental effects on emergence in greenhouse trials were not observed in field trials.

Direct application of the biological control organisms to the seed coat, which is also the infection site for damping off diseases, would imply that biological control for damping off disease should be among the most effective applications of biological control (Mathre et al. 1999). However, the inability to find a reliable biological control seed treatment for chickpea from among commercially available treatments may be a reflection of the inconsistencies typical to biological control as discussed by Ojiambo and

Sherm (2006). A plethora of factors such as soil pH, soil nutrient status, soil moisture and ecology of other competitive soil microbes can affect the ability of biological control organisms to survive in the rhizosphere (Weller 1988; Taylor and Harman 1990; Marschner et al. 2004; Ghini et al. 2006). In this case soil pH may be an important contributing factor: a substantial portion of soils in Montana tend to be alkaline (Montange et al. 1982), and high pH has been shown to inhibit biological control seed treatment organisms (Marshall 1982; Huber et al. 1989).

The two bacterial biological control organisms used in the field trials, *B. pumilus* GB34 and *B. subtilis* GB03, were able to multiply in both desi and kabuli chickpea seed exudates. The ability of the organisms to multiply in seed exudates, while proving that there is no fundamental incompatibility between chickpea seed exudates and these two organisms' ability to use exudates for a food source, does not explain the inability of the seed treatments to provide protection against damping off.

Desi chickpea grown in field soil in a greenhouse experiment had markedly greater emergence than a small kabuli chickpea and a kabuli chickpea cultivar grown in the same soil, while the same cultivars grown in autoclaved field soils had similar emergence. Desi chickpea planted in field trials had also significantly higher emergence rates than the kabuli variety. These findings echo those of Kaiser and Hannan (1983) in which desi chickpea were highly resistance to increasing inoculum levels of *Pythium ultimum*, while emergence of kabuli dropped to only 2% in soil with high inoculum levels. The pigmented seed coat of desi chickpea cultivars, containing polyphenolic compounds (Singh and Jambunathan 1981) is thought to confer this innate resistance

(Smiley 2001). This suggests that desi chickpea may be a viable option for organic growers who have difficulty achieving reasonable kabuli chickpea emergence.

Based on growth chamber, greenhouse, and field experiments, the biological control seed treatments used in this study were not effective for reducing seed and seedling loss due to damping off. Biological seed treatments did not confer advantages in terms of plant health, such as resistance to ascochyta blight or other measured parameters. Fungicide treatments and cultural control methods, such as delayed planting date, remain the most viable management practices for reducing stand loss due to damping off of chickpea. Based on the results of this research, organic growers will likely obtain reasonable stands of desi cultivars with no seed treatments for desi cultivars where healthy seed and best management practices are employed.

References

Backman, P. A., Brannen, P. M. et al. 1994. Plant response and disease control following seed inoculation with *Bacillus subtilis*. *In* Improving plant productivity with rhizosphere bacteria. *Edited by* M.H. Ryder, P. M. Stephens, G. D. Bowen. Commonwealth Scientific and Industrial Research Organization (CSIRO) Division of Soils, Adelaide, South Australia. pp. 3-8.

Brick, M. A., Berrada, A., Schwartz, H.F., and Krall, J. 1998. Technical Bulletin TB 98-2: Garbanzo Bean Production Trials in Colorado and Wyoming [online]. Colorado Agricultural Experiment Station and Wyoming Agricultural Experiment Station, Colorado Department of Education, Denver, CO. Available from http://www.cde.state.co.us/artemis/ucsu20/UCSU20210TB982INTERNET.pdf [accessed 27 May 2008].

Chen, W., Coyne, C.J., Peever, T.L., and Muehlbauer, F.J. 2004. Characterization of chickpea differentials for pathogenicity assay of ascochyta blight and identification of chickpea accessions resistant to *Didymella rabiei*. Plant Pathology 53: 759-769. doi:10.1111/j.1365-3059.2004.01103.x.

Cutforth, H. W., McGinn, S. M., McPhee, K.E., and Miller, P.R. 2007. Adaptation of pulse crops to the changing climate of the northern Great Plains [online]. Agronomy Journal 99: 1684-1699. Available from http://agron.scijournals.org/cgi/content/full/94/2/261 [accessed 27 May 2008].

Estevez de Jensen, C., Percich, J.A., and Graham, P.H. 2001. Integrated management strategies of bean root rot with *Bacillus subtilis* and *Rhizobium* in Minnesota. Field Crops Research 74: 107-115. doi:10.1016/S0378-4290(01)00200-3.

Ghini, R. and Morandi, M. 2006. Biotic and abiotic factors associated with soil suppressiveness to *Rhizoctonia solani*. Sci. Agric. 63(2): 153-160.

Hammon, R. and A. Berrada. 2001. Evaluation of Kodiak® biological seed treatment to control seedling diseases of chickpea (summary). *In* Western Colorado Research Center 2001 Research Report. Tech. Rep. TR01-13. *Edited by* Caspari, H., R. Godin, R.W. Hammon, H.J. Larsen, C.H. Calvin, and R. J. Zimmerman. Agric. Exp. Sta., Coop. Ext., Western Colo. Res. Ctr., Colorado State Univ., Ft. Collins, CO. p. 16.

Hartley, H. O. 1950. The maximum *F*-ratio as a shortcut for heterogeneity of variance. Biometrika 37: 308-312. doi:10.1093/biomet/37.3-4.308.

Harware, M. P. 1998. Diseases of chickpea. *In* The Pathology of Food and Pasture Legumes. *Edited by* D. J. Allen and J. M. Lenne. CAB International, New York NY.

Herigstad, B., Hamilton, M., and Heersink, J. 2001. How to optimize the drop plate method for enumerating bacteria. J Microbial Meth 44(2): 121-129. doi: 10.1016/S0167-7012(00)00241-4.

Huber, D. M., El-Nasshar, H., Moore, L.W., Mathre, D.E., and Wagner, J.E. 1989. Interaction between a peat carrier and bacterial seed treatments evaluated for biological control of take-all diseases of wheat (*Triticum aestivum* L.). Biology and Fertility of Soils 8(2): 166-171. doi: 10.1007/BF00257761.

Hwang, S. F. and P. Chakravarty. 1992. Potential for the integrated control of Rhizoctonia root-rot of *Pisum sativum* using *Bacillus subtilis* and a fungicide. Journal of Plant Disease Protection 99: 626-636.

Kaiser, W. J. and R. M. Hannan. 1983. Etiology and control of seed decay and preemergence damping-off of chickpea by *Pythium ultimum*. Plant Disease 67(1): 77-81. doi: 10.1094/PD-67-77.

Kaiser, W. J. and R. M. Hannan. 1984. Biological control of seed rot and pre-emergence damping-off of chickpea with *Penicillium oxalicum*. Plant Disease 68: 806-811. doi: 10.1094/PD-68-806.

Kloepper, J. W., Ryu, C., and Zhang, S. 2004. Induced systemic resistance and promotion of plant growth by *Bacillus spp*. Phytopathology 94: 1259-1266. doi: 10.1094/PHYTO.2004.94.11.1259.

Lauver, M. and Guy, S. 2006. Field evaluation of seed treatments for control of soil-borne pathogens and Ascochyta blight of chickpea, 2005. Fungicide and Nematicide Reports. Report 61: ST003. The American Phytopathological Society, St. Paul, MN. doi: OI:10.1094/FN60.

Liu, P., Gan, Y., Warkentin, T., and McDonald, C. (2003). Morphological plasticity of chickpea in a semiarid environment [online]. Crop Science 43: 426-429. Available from http://crop.scijournals.org/cgi/content/full/43/1/426 [accessed 17 May 2008].

Mao, W., Lewis, J.S., Hebber, P.K., and Lumsden, R.D. 1997. Seed treatment with a fungal or a bacterial antagonist for reducing corn damping-off by species of *Pythium* and *Fusarium*. Plant Disease 81: 450-454. doi: 10.1094/PDIS.1997.81.5.450.

Marshall, D. 1982. Effect of *Trichoderma harzianum* seed treatment and *Rhizoctonia solani* inoculum concentration on damping-off of snap bean in acidic soils. Plant Disease 66(9): 788-789.

Marschner, P., Crowley, D. and Hong Yang, C. 2004. Development of specific rhizosphere bacterial communities in relation to plant species, nutrition, and soil type. Plant and Soil 261: 199-208. doi: 10.1023/B:PLSO.0000035569.80747.c5.

Mathre, D. E., Cook, R.J., and Callan, N.W. 1999. From discovery to use: *Transversing the world of commercializing biocontrol agents for plant disease control.* Plant Disease 83(11): 972-983. doi: 10.1094/PDIS.1999.83.11.972.

Miller, P. R., McConkey, B.G., Clayton, G.W., Brandt, S.A., Staricka, J.A., Johnston, A.M., Lafond, G.P, Schatz, B.G., Baltensperger, D.D., and Niel, K.E. Pulse crop adaptation in the northern Great Plains [online]. Agronomy Journal 94: 261-272. Available from http://agron.scijournals.org/cgi/content/full/94/2/261 [accessed 27 May 2008].

Montange, C., Munn, L.C, Nielson, G.A., Rogers, J.W., and Hunter, H.E. 1982. Bulletin 744: Soils of Montana. Montana Agricultural Experiment Station (MAES), Bozeman, MT.

National Center for Biotechnology Information. 2008. Basic Local Alignment Search Tool (BLAST) [online]. NCBI, National Library of Medicine. Available from http://www.ncbi.nlm.nih.gov/blast/Blast.cgi [accessed 28 May 2008].

National Center for Biotechnology Information. 2008. GenBank [online]. NCBI, National Library of Medicine. Available from http://www.ncbi.nlm.nih.gov/Genbank/GenbankSearch.html [accessed 28 May 2008].

Nemec, S., Dantoff, L.E., and Strandberg, J. 1996. Efficacy of biocontrol agents in planting mixes to colonize plant roots and control root diseases of vegetables and citrus. Crop Protection 15(8): 735-742. doi: 10.1016/S0261-2194(96)00048-8.

Ojiambo, P. S. and H. Scherm. 2006. Biological and Application-Oriented Factors Influencing Plant disease suppression by biological control: A meta-analytical review. Phytopathology 96(11): 1168-1174. doi: 10.1094/PHYTO-96-1168.

Raupach, G. S. and J. W. Kloepper. 1998. Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Biological Control 88: 1158-1164. doi: 10.1094/PHYTO.1998.88.11.1158.

Reddy, M. S. (2008). The effect of Yield Shield seed treatment on Ascochyta blight of chickpeas. Personal communication to R. Leisso. Auburn University, AL.

Ristaino, J. B., Madritch, M., Trout, C.L., and Papa, G. 1998. PCR amplification of ribosomal DNA for species identification in the plant pathogen genus *Phytophthora* [online]. Applied and Environmental Microbiology 64(3): 948-954. Available from http://aem.asm.org/cgi/content/full/64/3/948 [accessed 27 May 2008].

SAS Institute Inc. 2002-2003. SAS/STAT user's guide. Version 9.1 (TS1M3) [computer program]. SAS Institute Inc., Cary, N.C.

Saskatchewan Agriculture and Food. 2007. Crops – StatFact, November Estimate of 2007 Crop Production [online]. Saskatchewan Agriculture, Saskatchewan Ministry of Agriculture, Regina, Saskatchewan. Available from http://www.agriculture.gov.sk.ca/Default.aspx?DN=247628e2-0f10-4a30-a3c6-4c8ee516b624 [accessed 27 May 2008].

Schmidt, C. S., Agostini, F., Simon, A., Whyte, J., Townend, J., Leifert, C., Kilham, K., and Mullins, C. 2004. Influence of soil type and pH on the colonization of sugar beet seedlings by antagonist *Pseudomonas* and *Bacillus* strains, and other their control of Pythium damping-off." European Journal of Plant Pathology 110(10): 1025-1046.

Schwartz, H.F., Gent, D.H., Mikkelson, M. and Riesselman, J. 2007. Pulse Crops:Root Rot and Seedling Blight of Chickpeas [online]. *In* High Plains Integrated Management Guide. Available from http://highplainsipm.org/HpIPMSearch/Docs/RootRotSeedlingBlight-Chickpea.htm [accessed 27 May 2008].

Shapiro, S. S. and Wilk, M.B. 1965. An analysis of variance test for normality (complete samples). Biometrika 52(3 and 4): 591-611. doi:10.1093/biomet/52.3-4.591.

Singh, K. B., Hawtin, G.C., Nene, Y.L., and Reddy, M.V. 1981. Resistance in chickpeas to *Ascochyta rabiei*. Plant Disease 65(7): 586-587.

Singh, K. B. and Saxena, M.C. 1999. Chickpeas. Macmillan Education, Ede, Netherlands.

Singh, U. and Jambunathan, R. 1981. Studies on desi and kabuli chickpea (*Cicer arietinum* L.) cultivars: levels of protease inhibitors, levels of polyphenolic compounds and in vitro protein digestability. Journal of Food Science 46(5): 1364-1367. doi:10.1111/j.1365-2621.1981.tb04176.x.

Smiley, R.J., Gourlie, R., Whittake, S., Easley, K., Rhinhard, E., Burnett, J., Kellogg, D., and Zeckman, T. 2001. Seed treatments for chickpea (garbanzo bean). *In* Oregon Agricultural Experiment Station Special Report 1054: pp. 120-127.

Stack, M., Berrada, A., Hammon, R., and Hooten, T. 2006. Technical Report TR06-03. Research Report 2004. Colorado State University, Southwestern Colorado Research Center, Yellow Jacket, CO.

Taylor, A. G. and Harman, G.E. 1990. Concepts and technologies of selected seed treatments. Annual Review of Phytopathology 28: 321-339. doi:10.1146/annurev.py.28.090190.001541.

Taylor, J. R. 1997. An introduction to error analysis. University Science Books, Sausolito, CA.

Tokala, R. K., Strap, J.L., Jung, C.M., Crawford, D.L., Saylor, M.H., Deobald, L.A., Bailey, J.F., and Morra, M.J. 2002. Novel plant-microbe rhizosphere interaction involving *Streptomyces lydicus* WYE108 and the pea plant (*Pisum sativum*). Applied and Environmental Microbiology 68(5): 2161-2171.

Trapero-Casas, A., Kaiser, W.J., and Ingram, D.M. 1990. Control of Pythium seed rot and preemergence damping-off of chickpea in the U.S. Pacific Northwest and Spain. Plant Disease 74: 563-569. doi: 10.1094/PD-74-0563.

United States Department of Agriculture (USDA)-Economic Reporting Service (ERS). 2005. Table 4—Certified organic pasture and cropland, 2005, by State. *In* Data sets, Organic production, State-level tables [online]. USDA-ERS, Washington, DC. Available from http://www.ers.usda.gov/Data/organic/Data/PastrCropbyState.xls [accessed 27 May 2008].

USDA-National Agriculture Statistics Service (NASS). 2007. Chickpeas (Garbanzo) report [online]. USDA-NASS, Washington, DC. Available from http://www.nass.usda.gov/QuickStats/indexbysubject.jsp?Pass_group=Crops+%26+Plants, *select and click search:* Chickpeas (Garbanzo) [accessed 27 May 2008].

USDA-NASS. 2008. Chickpeas (Gabanzo Beans): Price per Cwt and Value of Production, by United States, 2005-2007 (table). *In* Crop Values 2007 Summary [online]. USDA-NASS, Washington, DC. Available from http://usda.mannlib.cornell.edu/usda/current/CropValuSu/CropValuSu-02-14-2008.pdf [accessed 27 May 2008]. p.33.

USDA-NASS. 2008. News Release: 2007 Pulse Crop Summary [online]. USDA-NASS, Washington, DC. Available from http://www.nass.usda.gov/Statistics_by_State/Montana/Publications/Press_Releases_Crops/forecast/pulsesum.pdf [accessed 27 May 2008].

Van der Plaats-Niterink, A. J. 1981. Monograph of genus Pythium. Centraalbureau Voor Schimmelcultures, Baarn, The Netherlands.

Verghis, T. I., McKenzie, B.A., and Hill, G.D. 1999. Phenological development of chickpeas (*Cicer arietinum*) in Canterbury, New Zealand [online]. New Zealand Journal of Crop and Horticultural Science 27: 249-256. Available from www.rsnz.org/publish/nzjchs/1999/30.pdf [accessed 27 May 2008].

Weller, D. M. 1988. Biological control of soilborne pathogens in the rhizosphere with bacteria. Annual Review Phytopathologia 26: 379-407. doi:10.1146/annurev.py.26.090188.002115.

White, T.J., Bruns, T.J., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* PCR protocols: a guide to methods and applications. *Edited by* M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White. Academic Press, Inc., New York, NY.

Yang, X. B. and del Rio, L. 2002. Implementation of biological control of plant diseases in integrated pest-management systems. *In* Biological Control of Crop Diseases. *Edited by* S. S. Gnanamanickam, CRC Press, Boca Raton, FL. pp. 339-354.

Zablotowicz, R. M., C. M. Press, Lyng, N., Brown, G.L., and Kloepper, J.W. 1992. Compatibility of plant growth promoting rhizobacterial strains with agrichemicals applied to seed. Can. J. Microbiol. 38: 45-

EPILOGUE

Chickpea Seedling Disease

Environmental conditions, in particular temperature and water relations, are the most important factors contributing to the development of chickpea damping off. Only a narrow range of environmental conditions are ideal for chickpea germination, which predisposes the seed to be susceptible to damping off. Chickpea damping off is non-specific in terms of causal pathogens; as illustrated by the first chapter, pathogens vary in their ability to cause damping off, even fungi within the same species. It also appears that the pathogens involved in damping off of chickpea need not have innately high virulence to cause disease. Cool wet conditions, which are common in Montana in the spring, tend to favor the inoculum potential of *Pythium* spp., which has caused damping off disease of chickpea to be attributed these pathogens, but dry weather may also compromise seedling health and favor the establishment of pathogens.

Large seeded chickpea cultivars require a great quantity of water for germination; if this quantity is not reached, the seed may initiate germination but not possess sufficient water resources to emerge. The initiation of germination causes the production of nutrient-rich seed exudates, feeding growth of common soil inhabitants such as the *Fusarium* spp. capable of causing damping off to the compromised host. In this case, nutrient-rich chickpea seed exudates favor pathogen germination and infection, while environmental conditions compromise the chickpea seedling.

Qualities of the seed and seed coat also are important in chickpea damping off disease. This research found that chickpea cultivars possessing a pigmented seed coat are far less susceptible to damping off disease than cultivars with an un-pigmented seed coat. Although this research did not explore the qualities of pigmented seed coats that might contribute to damping off resistance, it is clear that the seed coat has an important role in damping off disease. Seed size may also have an important role: larger seeds require more water to germinate, predisposing them to water stress and opportunistic pathogen infection. Larger seeds likely also exude a higher quantity of seed exudates which may stimulate a larger number of pathogen propagules to initiate infection.

Biological versus Chemical Seed Treatments

Biological control has a tendency to be viewed as the organic counterpart of chemical treatments for plant disease. This perception of biological control as an analog to chemical treatments, that is, a "silver-bullet" treatment, contributes to the well-known inconsistency of results in biological control. While chemical seed treatments target a critical biological function with a relatively stable molecule or molecules, biological control treatments are applied as living organisms, and enact disease control through complex mechanisms which are still not fully understood. These disease control mechanisms are affected by all manner of parameters that affect biota, and thus their functions are susceptible to environmental compromise. Unfortunately, in many cases, little is known about ideal environmental conditions for the biological control organisms in a field setting, and therefore no efforts are or can be made to achieve these parameters.

Much research is needed to achieve a better understanding of biological control in an agricultural system before the success of biological control can be better guaranteed. Molecular mechanisms of disease control, ecology of biological control organisms in field soil with other microbes, and the effects of abiotic factors on biological control organisms' growth and survival are just several of many topics that need further attention in the study of biological control for plant disease.

Research in Biological Control

Biological control is a large and complex topic. Most research studies performed do not have the resources to answer all of the questions that might be addressed. For example, consider a portion of the research detailed in this thesis: commercialized biological control organisms were applied to chickpea seeds as seed treatments, and tested in greenhouse and field trials. This approach towards biological control is commendable in that if the treatments were successful, they could be quickly available to growers, since they are already produced and sold on commercial scale. However, the end result of this research is that the treatments tested were not effective for controlling damping off disease of chickpea, and there is no clear answer as to why this result occurred. It is not a failure for the seed treatments to be ineffective; at least chickpea producers may know to save their money for other needs. But no understanding of molecular mechanisms in biological control has been gained, no knowledge of biological control organisms' population dynamics in soil, and perhaps most importantly, no new methods for such research have been achieved. In order to achieve the understanding of

biological control that is necessary for consistent field success, it would be ideal for studies in biological control to encompass a broad range of topics in biological control. This would involve the inclusion of researchers from a variety of disciplines in plant pathogy and agronomy, comprehensive study designs, as well as long-term study periods to observe results of multi-faceted questions and approaches. Since funding for such an endeavor is rare, scientists involved in biological control and plant pathology must seek intradiciplinary collaboration, and strive to encompass both these broad and detailed questions in the biological control of plant pathogens.

Recommended Revisions to Methods

If this research is continued or repeated, several changes to the methods involved are suggested.

Regarding *Fusarium* spp. pathogenicity to chickpea, the *Fusarium* spp. tested in growth chamber trials should be tested using propagules grown in broth originating from either chickpea or other field susbstrate, as opposed to the tryptic soy broth that was used. Subsequent literature review indicates that pathogens grown in media of varying nutrient composition have differing levels of energy to devote to infection of plant material. In order to better mimic natural states, propagules should be grown in a broth more similar to a substrate that might be available to it in the field. A more extensive survey of the organisms associated with chickpea damping off disease should also be performed.

In testing seed treatments, the application of higher quantities of biological controls could be tested; treatments were applied only at maximum recommended rates as

per manufacturer's instructions. Previous researchers have found increased success with higher application rates of biological control treatments. Another method of seed preparation prior to planting, bio-priming, which is the soaking of seeds in liquid formulations of biological control, has been highly successful for reducing damping off of chickpea at test scales. However, priming softens the tissues of the seeds, and makes the radical prone to breakage during mechanized planting. Labor and materials involved would likely make priming prohibitively expensive for growers in the United States, unless chickpea prices rise dramatically. This is an area of applied research to which effort might be devoted should economic incentive allow. Coating seeds in an inert substance prior to planting may also provide a physical barrier to pathogen infection that could be worthy of further attention.

In order for chickpea to be a viable crop for organic growers, and a less expensive crop for conventional growers, the problem of ascochyta blight must also be addressed. Biological control foliar sprays for other crops do exist, and it would be worthwhile to perform simple greenhouse tests to determine the potential of these treatments in commercial application. Breeding for resistance to ascochyta blight is continually progressing, but even with these advances, cultural strategies such as the use of pure seed lots and the isolation of chickpea fields must still be employed to prevent or delay initial infection, and the development of inexpensive and organic control strategies would be ideal.

A better understanding of microbial population dynamics and soil physical state as they affect biological control is also needed. The parameters most suitable for the

establishment of any biological control organism should be determined and included in labeling of such treatments, so that producers can seek to achieve field conditions most suitable for the success of biological control treatments.

Biological control was not found successful for the control of damping off of chickpea, but this should not imply that it could not be. Application rates, the organisms used, and environmental parameters may still be modified to achieve the success of these treatments. In addition, other organic strategies, such as seed priming, seed coating, or new methods in cultural control may prove successful in managing damping off disease of chickpea without the use of chemical control.

APPENDICES

APPENDIX A

ADDITIONAL INFORMATION ON METHODS AND RESULTS

FOR

THE ROLE OF SOIL MOISTURE AND INOCULUM DENSITY IN *FUSARIUM* SPP. PATHOGENICITY IN CHICKPEA DAMPING OFF

Table A-1: Primers used in rDNA ITS region and TEF 1-α sequence amplification of *Fusarium* spp. for identification purposes.

Target sequence	Forward primer name and sequence	Reverse primer name and sequence
rDNA ITS region*	ITS1F 5′- CTTGGTCATTTAGAGGAAGTA	ITS4 5′- TCCTCCGCTTATTGATATGC
TEF 1-α †	ef1 5′-ATGGGTAAGGA(A/G)GACAAGAC	ef2 5′-GGA(G/A)GTACCAGT(G/C)ATCAGTT

*Ribosomal DNA internal transcribed spacer region. Includes partial 18S, internal transcribed spacer region I, 5.8 S, internal transcribed spacer region II, and partical 28S. ITS1F used for sequencing. Use of primer ITS1-F for amplification of ITS region for wide array of fungal taxa described by Gardes and Bruns (1993).

†Translation elongation factor 1-α gene. Primer ef1 used for sequencing. Primer sequences created by O'Donnell et al. (1998). Additional information can be found in Geiser et al. (2004).

Table A-2. Significance of contrasts for linear and quadratic trends of seed and seedling disease incidence of kabuli chickpea caused by *Fusarium* spp. under controlled conditions as influenced by moisture and propagule density.

			Soil moisture		Propagules g^{-1} soil	
Isolate	Location of origin	ANOVA term	Linear trend	Quadratic trend	Linear trend	Quadratic trend
F. sp.	Bozeman,	df	1	1	1	1
isolate	MT	*F*	38.5	9.8	442.5	94.3
1.3		*P*	0.0001	0.0033	0.0001	0.0001
F. sp.	Bozeman,	df	1	1	1	1
isolate	MT	*F*	6.3	1.8	43.1	0.0
3.5		*P*	0.0166	0.1873	0.0001	0.9471
F. sp.	Huntley,	df	1	1	1	1
isolate	MT	*F*	27.8	9.3	1275.8	495.4
3.2		*P*	0.0001	0.0045	0.0001	0.0001
F. sp.	Huntley,	df	1	1	1	1
isolate	MT	*F*	14.2	1.2	102.7	17.5
4.6		*P*	0.0005	0.2851	0.00001	0.0002
F. sp.	Huntley,	df	1	1	1	1
isolate	MT	*F*	0.0	1.7	189.8	11.3
DI		*P*	0.8474	0.2014	0.0001	0.0017
F. sp.	Sidney,	df	1	1	1	1
isolate	MT	*F*	54.0	0.1	240.1	44.6
1.7		*P*	0.0001	0.7391	0.0001	0.0001
F. sp.	Sidney,	df	1	1	1	1
isolate	MT	*F*	50.9	2.1	161.2	6.4
2.2		*P*	0.0001	0.1586	0.0001	0.0155
F. sp.	Sidney,	df	1	1	1	1
isolate	MT	*F*	0.06	2.97	96.71	1.65
2.6		*P*	0.8022	0.0881	<0.0001	0.2013

Table A-3. Regression term coefficients and coefficients of determination for multiple linear regression equations describing the effects of *Fusarium* spp. moisture and propagule density on seed and seedling disease incidence of kabuli chickpea under in a growth chamber.

Isolate	Location of origin	F-stat[a]	P-value	R^2	Constant coefficient	Independent variables coefficients: Percent soil moisture (v/v)[b]	Independent variables coefficients: Log propagules g^{-1} soil[c]
F. sp. isolate 1.3	Bozeman, MT	137.39	<0.0001	0.70	-18.00	0.78	20.00
F. sp. isolate 3.5	Bozeman, MT	51.22	<0.0001	0.46	3.79	-0.46	7.63
F. sp. isolate 3.2	Huntley, MT	66.35	<0.0001	0.56	-5.96	0.54	23.41
F. sp. isolate 4.6	Huntley, MT	70.85	<0.0001	0.55	-14.08	0.49	14.08
F. sp. isolate D.I.	Huntley, MT	56.19	<0.0001	0.49	-4.62	NS	9.88
F. sp. isolate 1.7	Sidney, MT	76.08	<0.0001	0.56	-27.13	1.15	14.70
F. sp. isolate 2.2	Sidney, MT	72.82	<0.0001	0.55	-34.87	0.82	14.95
F. sp. isolate 2.6	Sidney, MT	32.86	<0.0001	0.36	-6.46	NS	9.54

[a]119 df per test.

[b,c] Significant at $P < 0.05$, unless noted by NS (not significant).

Table A-4. *Fusarium* sp. isolate 1.3 ITS region GenBank submission.

```
LOCUS                       537 bp    DNA     linear   PLN 18-JUL-2008
DEFINITION  Fusarium sp. isolate 1.3 partial 18S ribosomal RNA gene, internal
            transcribed spacer 1, 5.8S ribosomal RNA gene, and internal
            transcribed spacer 2, and partial 28S ribosomal RNA gene.
ACCESSION   EU910075
VERSION
KEYWORDS    .
SOURCE      Fusarium sp.
  ORGANISM  Fusarium sp.
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; mitosporic
            Hypocreales; Fusarium.
REFERENCE   1  (bases 1 to 537)
  AUTHORS   Leisso,R.
  TITLE     FUSARIUM SPP. CAUSING DAMPING OFF OF KABULI CHICKPEAS:
            PATHOGENICITY OF ISOLATES IN RELATION TO SOIL MOISTURE AND
            PROPAGULE CONCENTRATION
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 537)
  AUTHORS   Leisso,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-JUL-2008) Plant Sciences and Plant Pathology, Montana
            State University, 119 Plant Biosciences Building, Bozeman, MT
            59715, United States
COMMENT     Bankit Comment: rachel.leisso@gmail.com.
FEATURES             Location/Qualifiers
     source          1..537
                     /organism="Fusarium sp."
                     /mol_type="genomic DNA"
                     /isolation_source="Cicer arietinum"
                     /db_xref="taxon:29916"
                     /note="PCR primers: forward_name = ITS1F,
                     reverse_name=ITS4"
     misc_RNA        <1..>537
                     /note="contains partial 18S ribosomal RNA , internal
                     transcribed spacer 1, 5.8S ribosomal RNA, internal
                     transcribed spacer 2, and partial 28S ribosomal RNA"
BASE COUNT      138 a    150 c    127 g    122 t
ORIGIN
        1 gaccagcgga gggatcatta ccgagtttac aactcccaaa cccctgtgaa cataccttaa
       61 tgttgcctcg gcggatcagc ccgcgccccg taaaacggga cggcccgcca gaggacccaa
      121 actctaatgt ttcttattgt aacttctgag taaaacaaac aaataaatca aaactttcaa
      181 caacggatct cttggttctg gcatcgatga agaacgcagc aaaatgcgat aagtaatgtg
      241 aattgcagaa ttcagtgaat catcgaatct ttgaacgcac attgcgcccg ctggtattcc
      301 ggcgggcatg cctgttcgag cgtcatttca accctcaagc ccccgggttt ggtgttgggg
      361 atcggctctg ccttctggcg gtgccgcccc cgaaatacat tggcggtctc gctgcagcct
      421 ccattgcgta gtagctaaca cctcgcaact ggaacgcggc gcggccatgc cgtaaaaccc
      481 caacttctga atgttgacct cggatcaggt aggaataccc gctgaactta agcatat
```

Table A-5. *Fusarium* sp. isolate 1.7 ITS region GenBank submission.

```
LOCUS                       542 bp    DNA     linear   PLN 18-JUL-2008
DEFINITION  Fusarium sp. isolate 1.7 partial 18S ribosomal RNA gene, internal
            transcribed spacer 1, 5.8S ribosomal RNA gene, and internal
            transcribed spacer 2, and partial 28S ribosomal RNA gene.
ACCESSION   EU910077
VERSION
KEYWORDS    .
SOURCE      Fusarium sp.
  ORGANISM  Fusarium sp.
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; mitosporic
            Hypocreales; Fusarium.
REFERENCE   1  (bases 1 to 542)
  AUTHORS   Leisso,R.
  TITLE     FUSARIUM SPP. CAUSING DAMPING OFF OF KABULI CHICKPEAS:
            PATHOGENICITY OF ISOLATES IN RELATION TO SOIL MOISTURE AND
            PROPAGULE CONCENTRATION
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 542)
  AUTHORS   Leisso,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-JUL-2008) Plant Sciences and Plant Pathology, Montana
            State University, 119 Plant Biosciences Building, Bozeman, MT
            59715, United States
COMMENT     Bankit Comment: rachel.leisso@gmail.com.
FEATURES             Location/Qualifiers
     source          1..542
                     /organism="Fusarium sp."
                     /mol_type="genomic DNA"
                     /isolation_source="Cicer arietinum"
                     /db_xref="taxon:29916"
                     /note="PCR primers: forward_name=ITS1F, reverse_name=ITS4"
     misc_RNA        <1..>542
                     /note="contains partial 18S ribosomal RNA, internal
                     transcribed spacer 1, 5.8S ribosomal RNA, internal
                     transcribed spacer 2, and partial 28S ribosomal RNA"
BASE COUNT      145 a    151 c    129 g    117 t
ORIGIN
        1 agcggaggga tcattaccga gttatacaac tcatcaaccc tgtgaacata cctaaaacgt
       61 tgcttcggcg ggaacagacg gccctgtaac aacgggccgc ccccgccaga ggacccctaa
      121 ctctgttttt ataatgtttt tctgagtaaa caagcaaata aattaaaact ttcaacaacg
      181 gatctcttgg ctctggcatc gatgaagaac gcagcgaaat gcgataagta atgtgaattg
      241 cagaattcag tgaatcatcg aatctttgaa cgcacattgc gcccgccagt attctggcgg
      301 gcatgcctgt tcgagcgtca ttacaaccct caggcccccg ggcctggcgt tggggatcgg
      361 cagaagcccc ctgtgggcac acgccgtccc tcaaatacag tggcggtccc gccgcagctt
      421 ccattgcgta gtagctaaca cctcgcaact ggagagcggc gcggccatgc cgtaaaacac
      481 ccaacttctg aatgttgacc tcgaatcagg taggaatacc cgctgaactt aagcatatca
      541 at
```

Table A-6. *Fusarium* sp. isolate 2.2 ITS region GenBank submission.

```
LOCUS                        521 bp    DNA     linear   PLN 18-JUL-2008
DEFINITION  Fusarium sp. isolate 2.2 partial 18S ribosomal RNA gene, internal
            transcribed spacer 1, 5.8S ribosomal RNA gene, and internal
            transcribed spacer 2, and partial 28S ribosomal RNA gene.
ACCESSION   EU910076
VERSION
KEYWORDS    .
SOURCE      Fusarium sp.
  ORGANISM  Fusarium sp.
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; mitosporic
            Hypocreales; Fusarium.
REFERENCE   1  (bases 1 to 521)
  AUTHORS   Leisso,R.
  TITLE     FUSARIUM SPP. CAUSING DAMPING OFF OF KABULI CHICKPEAS:
            PATHOGENICITY OF ISOLATES IN RELATION TO SOIL MOISTURE AND
            PROPAGULE CONCENTRATION
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 521)
  AUTHORS   Leisso,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-JUL-2008) 119 Plant Biosciences Building, Bozeman, MT
            59715, United States
COMMENT     Bankit Comment: rachel.leisso@gmail.com.
FEATURES             Location/Qualifiers
     source          1..521
                     /organism="Fusarium sp."
                     /mol_type="genomic DNA"
                     /isolation_source="Cicer arietinum"
                     /db_xref="taxon:29916"
                     /note="PCR primers: forward_name=ITS1F, reverse_name=ITS4"
     misc_RNA        <1..>521
                     /note="contains partial 18S ribosomal RNA, internal
                     transcribed spacer 1, 5.8S ribosomal RNA, internal
                     transcribed spacer 2, and partial 28S ribosomal RNA"
BASE COUNT      141 a    137 c    114 g    128 t      1 others
ORIGIN
        1 acggagggat cattaccgag tttacaactc ccaaacccct gtgaacatac cacttgnttg
       61 cctcggcgga tcagcccgct cccggtaaaa cgggacggcc cgccagagga cccctaaact
      121 ctgtttctat atgtaacttc tgagtaaaac cataaataaa tcaaaacttt caacaacgga
      181 tctcttggtt ctggcatcga tgaagaacgc agcaaaatgc gataagtaat gtgaattgca
      241 gaattcagtg aatcatcgaa tctttgaacg cacattgcgc ccgccagtat tctggcgggc
      301 atgcctgttc gagcgtcatt tcaaccctca agcacagctt ggtgttggga ctcgcgttaa
      361 ttcgcgttcc tcaaattgat tggcggtcac gtcgagcttc catagcgtag tagtaaaacc
      421 ctcgttactg gtaatcgtcg cggccacgcc gttaaacccc aacttctgaa tgttgacctc
      481 ggatcaggta ggaatacccg ctgaacttaa gcatatcaat a
```

Table A-7. *Fusarium* sp. isolate 2.6 ITS region GenBank submission.

```
LOCUS                       534 bp    DNA     linear   PLN 18-JUL-2008
DEFINITION  Fusarium sp. isolate 2.6 partial 18S ribosomal RNA gene, internal
            transcribed spacer 1, 5.8S ribosomal RNA gene, and internal
            transcribed spacer 2, and partial 28S ribosomal RNA gene.
ACCESSION   EU910078
VERSION
KEYWORDS    .
SOURCE      Fusarium sp.
  ORGANISM  Fusarium sp.
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; mitosporic
            Hypocreales; Fusarium.
REFERENCE   1  (bases 1 to 534)
  AUTHORS   Leisso,R.
  TITLE     FUSARIUM SPP. CAUSING DAMPING OFF OF KABULI CHICKPEAS:
            PATHOGENICITY OF ISOLATES IN RELATION TO SOIL MOISTURE AND
            PROPAGULE CONCENTRATION
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 534)
  AUTHORS   Leisso,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-JUL-2008) Plant Sciences and Plant Pathology, Montana
            State University, 119 Plant Biosciences Building, Bozeman, MT
            59715, United States
COMMENT     Bankit Comment: rachel.leisso@gmail.com.
FEATURES             Location/Qualifiers
     source          1..534
                     /organism="Fusarium sp."
                     /mol_type="genomic DNA"
                     /isolation_source="Cicer arietinum"
                     /db_xref="taxon:29916"
                     /note="PCR primers: forward_name=ITS1F, reverse_name=ITS4"
     misc_RNA        <1..>534
                     /note="contains partial 18S ribosomal RNA, internal
                     transcribed spacer 1, 5.8S ribosomal RNA, internal
                     transcribed spacer 2, and partial 28S ribosomal RNA"
BASE COUNT      138 a    148 c    126 g    122 t
ORIGIN
        1 accagcggag ggatcattac cgagtttaca actcccaaac ccctgtgaac ataccttact
       61 gttgcctcgg cggatcagcc cgctcccggt aaaacgggac ggcccgccag aggaccccta
      121 aactctgttt ctatatgtaa cttctgagta aaaccataaa taaatcaaaa ctttcaacaa
      181 cggatctctt ggttctggca tcgatgaaga acgcagcaaa atgcgataag taatgtgaat
      241 tgcagaattc agtgaatcat cgaatctttg aacgcacatt gcgcccgcca gtattctggc
      301 gggcatgcct gttcgagcgt catttcaacc ctcaagccct cgggtttggt gttggggatc
      361 ggcgagcctt tctggcaagc cggccccgaa atctagtggc ggtctcgctg cagcctccat
      421 tgcgtagtag taaaaccctc gcaactggaa cgcggcgcgg ccaagccgtt aaaccccaa
      481 cttctgaatg ttgacctcgg atcaggtagg aatacccgct gaacttaagc atat
```

Table A-8. *Fusarium* sp. isolate 3.2 ITS region GenBank submission.

```
LOCUS                        545 bp    DNA     linear   PLN 18-JUL-2008
DEFINITION  Fusarium sp. isolate 3.2 partial 18S ribosomal RNA gene, internal
            transcribed spacer 1, 5.8S ribosomal RNA gene, and internal
            transcribed spacer 2, and partial 28S ribosomal RNA gene.
ACCESSION   EU910079
VERSION
KEYWORDS    .
SOURCE      Fusarium sp.
  ORGANISM  Fusarium sp.
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; mitosporic
            Hypocreales; Fusarium.
REFERENCE   1  (bases 1 to 545)
  AUTHORS   Leisso,R.
  TITLE     FUSARIUM SPP. CAUSING DAMPING OFF OF KABULI CHICKPEAS:
            PATHOGENICITY OF ISOLATES IN RELATION TO SOIL MOISTURE AND
            PROPAGULE CONCENTRATION
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 545)
  AUTHORS   Leisso,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-JUL-2008) Plant Sciences and Plant Pathology, Montana
            State University, 119 Plant Biosciences Building, Bozeman, MT
            59715, United States
COMMENT     Bankit Comment: rachel.leisso@gmail.com.
FEATURES             Location/Qualifiers
     source          1..545
                     /organism="Fusarium sp."
                     /mol_type="genomic DNA"
                     /isolation_source="Cicer arietinum"
                     /db_xref="taxon:29916"
                     /note="PCR primers: forward_name=ITS1F, reverse_name=ITS4"
     misc_RNA        <1..>545
                     /note="contains partial 18S ribosomal RNA, internal
                     transcribed spacer 1, 5.8S ribosomal RNA, internal
                     transcribed spacer 2, and partial 28S ribosomal RNA"
BASE COUNT      141 a    151 c    129 g    124 t
ORIGIN
        1 gtgacagcgg agggatcatt accgagttta caactcccaa acccctgtga acatacctta
       61 atgttgcctc ggcggatcag cccgcgcccc gtaaaacggg acggcccgcc agaggaccca
      121 aactctaatg tttcttattg taacttctga gtaaaacaaa caaataaatc aaaactttca
      181 acaacggatc tcttggttct ggcatcgatg aagaacgcag caaaatgcga taagtaatgt
      241 gaattgcaga attcagtgaa tcatcgaatc tttgaacgca cattgcgccc gctggtattc
      301 cggcgggcat gcctgttcga gcgtcatttc aaccctcaag cccccgggtt tggtgttggg
      361 gatcggctct gcccttctgg gcggtgccgc ccccgaaata cattggcggt ctcgctgcag
      421 cctccattgc gtagtagcta acacctcgca actggaacgc ggcgcggcca tgccgtaaaa
      481 ccccaacttc tgaatgttga cctcggatca ggtaggaata cccgctgaac ttaagcatat
      541 caata
```

Table A-9. *Fusarium* sp. isolate 3.5 ITS region GenBank submission.

```
LOCUS                       530 bp    DNA     linear   PLN 18-JUL-2008
DEFINITION  Fusarium sp. isolate 3.5 partial 18S ribosomal RNA gene, internal
            transcribed spacer 1, 5.8S ribosomal RNA gene, and internal
            transcribed spacer 2, and partial 28S ribosomal RNA gene.
ACCESSION   EU910080
VERSION
KEYWORDS    .
SOURCE      Fusarium sp.
  ORGANISM  Fusarium sp.
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; mitosporic
            Hypocreales; Fusarium.
REFERENCE   1  (bases 1 to 530)
  AUTHORS   Leisso,R.
  TITLE     FUSARIUM SPP. CAUSING DAMPING OFF OF KABULI CHICKPEAS:
            PATHOGENICITY OF ISOLATES IN RELATION TO SOIL MOISTURE AND
            PROPAGULE CONCENTRATION
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 530)
  AUTHORS   Leisso,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-JUL-2008) Plant Sciences and Plant Pathology, Montana
            State University, 119 Plant Biosciences Building, Bozeman, MT
            59715, United States
COMMENT     Bankit Comment: rachel.leisso@gmail.com.
FEATURES             Location/Qualifiers
     source          1..530
                     /organism="Fusarium sp."
                     /mol_type="genomic DNA"
                     /isolation_source="Cicer arietinum"
                     /db_xref="taxon:29916"
                     /note="PCR primers: forward_name=ITS1F, reverse_name=ITS4"
     misc_feature    <1..>530
                     /note="contains partial 18S ribosomal RNA, internal
                     transcribed spacer 1, 5.8S ribosomal RNA, internal
                     transcribed spacer 2, and partial 28S ribosomal RNA"
BASE COUNT      142 a    138 c    120 g    130 t
ORIGIN
        1 ggtggtgaac agcggaggga tcattaccga gtttacaact cccaaacccc tgtgaacata
       61 ccacttgttg cctcggcgga tcagcccgct cccggtaaaa cgggacggcc cgccagagga
      121 cccctaaact ctgtttctat atgtaacttc tgagtaaaac cataaataaa tcaaaacttt
      181 caacaacgga tctcttggtt ctggcatcga tgaagaacgc agcaaaatgc gataagtaat
      241 gtgaattgca gaattcagtg aatcatcgaa tctttgaacg cacattgcgc ccgccagtat
      301 tctggcgggc atgcctgttc gagcgtcatt tcaaccctca agcacagctt ggtgttggga
      361 ctcgcgttaa ttcgcgttcc tcaaattgat tggcggtcac gtcgagcttc catagcgtag
      421 tagtaaaacc ctcgttactg gtaatcgtcg cggccacgcc gttaaacccc aacttctgaa
      481 tgttgacctc ggatcaggta ggaatacccg ctgaacttaa gcatatcata
```

Table A-10. *Fusarium* sp. isolate 4.6 ITS region GenBank submission.

```
LOCUS                       520 bp    DNA     linear   PLN 18-JUL-2008
DEFINITION  Fusarium sp. isolate 4.6 partial 18S ribosomal RNA gene, internal
            transcribed spacer 1, 5.8S ribosomal RNA gene, and internal
            transcribed spacer 2, and partial 28S ribosomal RNA gene.
ACCESSION   EU910081
VERSION
KEYWORDS    .
SOURCE      Fusarium sp.
  ORGANISM  Fusarium sp.
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; mitosporic
            Hypocreales; Fusarium.
REFERENCE   1  (bases 1 to 520)
  AUTHORS   Leisso,R.
  TITLE     FUSARIUM SPP. CAUSING DAMPING OFF OF KABULI CHICKPEAS:
            PATHOGENICITY OF ISOLATES IN RELATION TO SOIL MOISTURE AND
            PROPAGULE CONCENTRATION
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 520)
  AUTHORS   Leisso,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-JUL-2008) Plant Sciences and Plant Pathology, Montana
            State University, 119 Plant Biosciences Building, Bozeman, MT
            59715, United States
COMMENT     Bankit Comment: rachel.leisso@gmail.com.
FEATURES             Location/Qualifiers
     source          1..520
                     /organism="Fusarium sp."
                     /mol_type="genomic DNA"
                     /isolation_source="Cicer arietinum"
                     /db_xref="taxon:29916"
                     /note="PCR primers: forward_name=ITS1F, reverse_name=ITS4"
     misc_RNA        <1..>520
                     /note="contains partial 18S ribosomal RNA, internal
                     transcribed spacer 1, 5.8S ribosomal RNA, internal
                     transcribed spacer 2, and partial 28S ribosomal RNA"
BASE COUNT      141 a    141 c    117 g    121 t
ORIGIN
        1 accagcggag ggatcattac cgagtttaca actcccaaac ccctgtgaac atacctatac
       61 gttgcctcgg cggatcagcc cgcgccctgt aaaaagggac ggcccgcccg aggaccctaa
      121 actctgtttt tagtggaact tctgagtaaa acaaacaaat aaatcaaaac tttcaacaac
      181 ggatctcttg gttctggcat cgatgaagaa cgcagcaaaa tgcgataagt aatgtgaatt
      241 gcagaattca gtgaatcatc gaatctttga acgcacattg cgcccgccag tattctggcg
      301 ggcatgcctg ttcgagcgtc atttcaaccc tcaagctcag cttggtgttg ggactcgcgg
      361 taacccgcgt tccccaaatc gattggcggt cacgtcgagc ttccatagcg tagtaatcat
      421 acacctcgtt actggtaatc gtcgcggcca cgccgtaaaa ccccaacttc tgaatgttga
      481 cctcggatca ggtaggaata cccgctgaac ttaagcatat
```

Table A-11. *Fusarium* sp. isolate DI ITS region GenBank submission.

```
LOCUS                       516 bp    DNA     linear   PLN 18-JUL-2008
DEFINITION  Fusarium sp. isolate DI partial 18S ribosomal RNA gene, internal
            transcribed spacer 1, 5.8S ribosomal RNA gene, and internal
            transcribed spacer 2, and partial 28S ribosomal RNA gene.
ACCESSION   EU910082
VERSION
KEYWORDS    .
SOURCE      Fusarium sp.
  ORGANISM  Fusarium sp.
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; mitosporic
            Hypocreales; Fusarium.
REFERENCE   1  (bases 1 to 516)
  AUTHORS   Leisso,R.
  TITLE     FUSARIUM SPP. CAUSING DAMPING OFF OF KABULI CHICKPEAS:
            PATHOGENICITY OF ISOLATES IN RELATION TO SOIL MOISTURE AND
            PROPAGULE CONCENTRATION
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 516)
  AUTHORS   Leisso,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-JUL-2008) Plant Sciences and Plant Pathology, Montana
            State University, 119 Plant Biosciences Building, Bozeman, MT
            59715, United States
COMMENT     Bankit Comment: rachel.leisso@gmail.com.
FEATURES             Location/Qualifiers
     source          1..516
                     /organism="Fusarium sp."
                     /mol_type="genomic DNA"
                     /isolation_source="Cicer arietinum"
                     /db_xref="taxon:29916"
                     /note="PCR primers: forward_name=ITS1F, reverse_name=ITS4"
     misc_feature    <1..>516
                     /note="contains partial 18S ribosomal RNA, internal
                     transcribed spacer 1, 5.8S ribosomal RNA, internal
                     transcribed spacer 2, and partial 28S ribosomal RNA"
BASE COUNT      141 a    141 c    114 g    120 t
ORIGIN
        1 agggatcatt accgagttta caactcccaa acccctgtga acatacctat acgttgcctc
       61 ggcggatcag cccgcgcccc gtaaaaaggg acggcccgcc cgaggacccc taaactctgt
      121 ttttagtgga acttctgagt aaaacaaaca aataaatcaa aactttcaac aacggatctc
      181 ttggttctgg catcgatgaa gaacgcagca aaatgcgata agtaatgtga attgcagaat
      241 tcagtgaatc atcgaatctt tgaacgcaca ttgcgcccgc cagtattctg gcgggcatgc
      301 ctgttcgagc gtcatttcaa ccctcaagct cagcttggtg ttgggactcg cggtaacccg
      361 cgttccccaa atcgattggc ggtcacgtcg agcttccata gcgtagtaat catacacctc
      421 gttactggta atcgtcgcgg ccacgccgta aaaccccaac ttctgaatgt tgacctcgga
      481 tcaggtagga atacccgctg aacttaagca tatcaa
```

Table A-12. *Fusarium* sp. isolate 1.3 TEF 1-α gene GenBank submission.

```
LOCUS                        652 bp    DNA     linear   PLN 17-JUL-2008
DEFINITION  Fusarium sp. isolate 1.3 partial tef-1alpha gene for translation
            elongation factor 1-alpha.
ACCESSION   EU10069
VERSION
KEYWORDS    .
SOURCE      Fusarium sp.
  ORGANISM  Fusarium sp.
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; mitosporic
            Hypocreales; Fusarium.
REFERENCE   1  (bases 1 to 652)
  AUTHORS   Leisso,R.
  TITLE     THE ROLE OF SOIL MOISTURE AND INOCULUM DENSITY IN FUSARIUM SPP.
            PATHOGENICITY IN CHICKPEA DAMPING OFF
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 652)
  AUTHORS   Leisso,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-JUL-2008) Plant Sciences and Plant Pathology, Montana
            State University, 119 Plant Biosciences Building, Bozeman, MT
            59715, USA
COMMENT     Bankit Comment: rachel.leisso@gmail.com.
FEATURES             Location/Qualifiers
     source          1..652
                     /organism="Fusarium sp."
                     /mol_type="genomic DNA"
                     /isolation_source="Cicer arietinum"
                     /db_xref="taxon:29916"
  gene       <1..>652
             /gene="EF1-alpha"
  CDS              join(<1..20,113..175,415..553,610..>652)
                     /gene="EF1-alpha"
                     /codon_start=1
                     /product="(partial) translation elongation factor 1-alpha"
                     /translation="DSGRSTTTGHLIYQCGGIDKRTIEKFEKEAAELGKGSFKYAWVL
                     DKLKAERERGITIDIALWKFETPRYYVTVIDAPGHRDFIKNMIT"
BASE COUNT      150 a    194 c    142 g    166 t
ORIGIN
        1 gactctggca gatcgaccac tgtaagtaca accatcagcg agtcgcttat ctgcactcag
       61 agcctgccaa acctggcggg gtattaccac aacagtttgc taacttttga tagaccggtc
      121 acttgatcta ccagtgcggt ggtatcgaca agcgaaccat cgagaagttc gagaaggtta
      181 gtcaatatcc cttcgattgc gcgctcctat cgatccccac gactcgctcc ttcactcgaa
      241 acgcatccat taccccgctc gagcccgaaa attttgcggt gcgaccgtga ttttttggt
      301 ggggtatctt accccgccac tcgagtgacg gatgcgcttg ccctgttccc acaaaacctt
      361 actaccctgc cgcgcactat catatgtctt gcagtcacta accactggac aataggaagc
      421 cgccgagctc ggaaagggtt ccttcaagta cgcctgggtt cttgacaagc tcaaagccga
      481 gcgtgagcgt ggtatcacca ttgatatcgc tctctggaag ttcgagactc ctcgctacta
      541 tgtcaccgtc attggtatgt tgtcactgtc tcataccacc atgctttcat catgctaaca
      601 tccctctcag atgcccccgg tcatcgtgat ttcatcaaga acatgatcac tg
```

Table A-13. *Fusarium* sp. isolate 1.7 TEF 1-α gene GenBank submission.

```
LOCUS                        680 bp    DNA     linear   PLN 17-JUL-2008
DEFINITION  Fusarium sp. isolate 1.7 partial tef-1alpha gene for translation
            elongation factor 1-alpha.
ACCESSION   EU10073
VERSION
KEYWORDS    .
SOURCE      Fusarium sp.
  ORGANISM  Fusarium sp.
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; mitosporic
            Hypocreales; Fusarium.
REFERENCE   1  (bases 1 to 680)
  AUTHORS   Leisso,R.
  TITLE     THE ROLE OF SOIL MOISTURE AND INOCULUM DENSITY IN FUSARIUM SPP.
            PATHOGENICITY IN CHICKPEA DAMPING OFF
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 680)
  AUTHORS   Leisso,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-JUL-2008) Plant Sciences and Plant Pathology, Montana
            State University, 119 Plant Biosciences Building, Bozeman, MT
            59715, United States
COMMENT     Bankit Comment: rachel.leisso@gmail.com.
FEATURES             Location/Qualifiers
     source          1..680
                     /organism="Fusarium sp."
                     /mol_type="genomic DNA"
                     /isolation_source="Cicer arietinum"
                     /db_xref="taxon:29916"
     mRNA            join(<1..20,127..187,453..591,643..>680)
                     /note="EF1-alpha"
     CDS             join(<1..20,127..187,453..591,643..>680)
                     /codon_start=1
                     /product="translation elongation factor 1-alpha"
                     /translation="DSGKSTTTGHLIYQCGGIDKRTIEKFEEAAELGKGSFKYAWVLD
                     KLKAERERGITIDIALWKFETPRYYVTVIDAPGHRDFIKNMI"
BASE COUNT      153 a    216 c    152 g    159 t
ORIGIN
        1 gactctggca agtcgaccac cgtaagtcaa accctcatcg cgatctgctt atctcgggtc
       61 gtggaacccc gcctggcatc tcgggcgggg tattcatcat tcacttcatg ctgacaatca
      121 tctacagacc ggtcacttga tctaccagtg cggtggtatc gacaagcgaa ccatcgagaa
      181 gttcgagaag gttggtgaca tctcccccga tcgcgccttg atattccaca tcgaattccc
      241 tgtcgaattc cctccatcgc gatacgctct gcgcccgctt ctcccgagtc ccaaaatttt
      301 tgcggtccga ccgtaatttt tttggtgggg catttacccc gccactcggg cgacgttgga
      361 caaagccctg atccctgcac acaaaaacac caaatcctct tggcgcgcat catcacgtgg
      421 ttcacgacag acgctaactg gtccaacaat aggaagccgc tgagctcggt aagggttcct
      481 tcaagtacgc ctgggtcctt gacaagctca aggccgagcg tgagcgtggt atcaccatcg
      541 acattgccct ctggaagttc gagactcccc gctactatgt caccgtcatt ggtatgttgc
      601 tgtcacctct ctcacacatg tctcaccact aacaatcaac agacgccccc ggccaccgtg
      661 acttcatcaa gaacatgatc
```

Table A-14. *Fusarium* sp. isolate 2.2 TEF 1-α gene GenBank submission.

```
LOCUS                        685 bp    DNA     linear   PLN 18-JUL-2008
DEFINITION  Fusarium sp. isolate 2.2 partial ef1-alpha gene for translation
            elongation factor 1-alpha.
ACCESSION   EU910070
VERSION
KEYWORDS    .
SOURCE      Fusarium sp.
  ORGANISM  Fusarium sp.
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; mitosporic
            Hypocreales; Fusarium.
REFERENCE   1  (bases 1 to 685)
  AUTHORS   Leisso,R.
  TITLE     THE ROLE OF SOIL MOISTURE AND INOCULUM DENSITY IN FUSARIUM SPP.
            PATHOGENICITY IN CHICKPEA DAMPING OFF
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 685)
  AUTHORS   Leisso,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-JUL-2008) Plant Sciences and Plant Pathology, Montana
            State University, 119 Plant Biosciences Building, Bozeman, MT
            59715, USA
COMMENT     Bankit Comment: rachel.leisso@gmail.com.
FEATURES             Location/Qualifiers
     source          1..685
                     /organism="Fusarium sp."
                     /mol_type="genomic DNA"
                     /isolation_source="Cicer arietinum"
                     /db_xref="taxon:29916"
     misc_feature    <1..>685
                     /note="partial gene for product translation elongation
                     factor 1-alpha; coding region not determined"
BASE COUNT      154 a    218 c    153 g    160 t
ORIGIN
        1 cgatcgactc tggcaagtcg accaccgtaa gtcaaaccct catcgcgatc tgcttatctc
       61 gggtcgtgga accccgcctg gcatctcggg cggggtattc atcattcact tcatgctgac
      121 aatcatctac agaccggtca cttgatctac cagtgcggtg gtatcgacaa gcgaaccatc
      181 gagaagttcg agaaggttgg tgacatctcc cccgatcgcg ccttgatatt ccacatcgaa
      241 ttccctgtcg aattccctcc atcgcgatac gctctgcgcc cgcttctccc gagtcccaaa
      301 atttttgcgg tccgaccgta attttttttgg tggggcattt accccgccac tcgggcgacg
      361 ttggacaaag ccctgatccc tgcacacaaa aacaccaaat cctcttggcg cgcatcatca
      421 cgtggttcac gacagacgct aactggtcca acaataggaa gccgctgagc tcggtaaggg
      481 ttccttcaag tacgcctggg tccttgacaa gctcaaggcc gagcgtgagc gtggtatcac
      541 catcgacatt gccctctgga agttcgagac tccccgctac tatgtcaccg tcattggtat
      601 gttgctgtca cctctctcac acatgtctca ccactaacaa tcaacagacg cccccggcca
      661 ccgtgacttc atcaagaaca tgatc
```

Table A-15. *Fusarium* sp. isolate 2.6 TEF 1-α gene GenBank submission.

```
LOCUS                        650 bp    DNA     linear   PLN 18-JUL-2008
DEFINITION  Fusarium sp. isolate 2.6 partial ef-1alpha gene for translation
            elongation factor 1-alpha.
ACCESSION   EU910071
VERSION
KEYWORDS    .
SOURCE      Fusarium sp.
  ORGANISM  Fusarium sp.
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; mitosporic
            Hypocreales; Fusarium.
REFERENCE   1  (bases 1 to 650)
  AUTHORS   Leisso,R.
  TITLE     FUSARIUM SPP. CAUSING DAMPING OFF OF KABULI CHICKPEAS:
            PATHOGENICITY OF ISOLATES IN RELATION TO SOIL MOISTURE AND
            PROPAGULE CONCENTRATION
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 650)
  AUTHORS   Leisso,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-JUL-2008) Plant Sciences and Plant Pathology, Montana
            State University, 119 Plant Biosciences Building, Bozeman, MT
            59715, United States
COMMENT     Bankit Comment: rachel.leisso@gmail.com.
FEATURES             Location/Qualifiers
     source          1..650
                     /organism="Fusarium sp."
                     /mol_type="genomic DNA"
                     /isolation_source="Cicer arietinum"
                     /db_xref="taxon:29916"
     gene            <1..>650
                     /gene="(partial) EF1-alpha"
     CDS             join(<1..21,122..184,433..571,627..>650)
                     /gene="(partial) EF1-alpha"
                     /codon_start=1
                     /product="(partial) translation elongation factor 1-alpha"
                     /translation="DSGSRPLTGHLIYQCGGIDKRTIEKFEKEAAELGKGSFKYAWVL
                     DKLKAERERGITIDIALWKFETPRYYVTVIDAPGHRDF"
BASE COUNT      149 a    194 c    142 g    165 t
ORIGIN
        1 gactctggca gtcgaccact gtaagtacaa ccctcgacga tatgcttatc tgctttcgtc
       61 aatcccgacc aagacctggt ggggtatgct tcaatcgcaa catgctgaca gtatttcaca
      121 gaccggtcac ttgatctacc agtgcggtgg tatcgacaag cgaaccatcg agaagttcga
      181 gaaggttagt cactttccct tcgatcgcgc gccctttgc ccatcgattt ccccttcgac
      241 tcgccgctcc catacaactc gaaacctgcc cgctaccccg ctcgaggcca aaaattttgc
      301 ggtgcgaccg taatattttt tggtggggca tttaccccgc cactcgagac gggcgcgctt
      361 gccctcttcc cacacaatca caatgagcgc ggatcatcac gtgtcaaaca gtaactaacc
      421 actcaataat aggaagccgc tgagctcggt aagggttcct tcaagtacgc ctgggttctt
      481 gacaagctca aagccgagcg tgagcgtggt atcaccatcg atattgctct ctggaagttc
      541 gagactcctc gctactatgt caccgtcatt ggtatgttgt cgctaatgtc tcatcacaat
      601 catcattgta ctaacgtttc atccagacgc tcccggtcac cgtgatttca
```

Table A-16. *Fusarium* sp. isolate 3.5 TEF 1-α gene GenBank submission.

```
LOCUS                       650 bp    DNA     linear   PLN 18-JUL-2008
DEFINITION  Fusarium sp. isolate 3.5 partial ef1-alpha gene for translation
            elongation factor 1-alpha.
ACCESSION   EU910072
VERSION
KEYWORDS    .
SOURCE      Fusarium sp.
  ORGANISM  Fusarium sp.
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; mitosporic
            Hypocreales; Fusarium.
REFERENCE   1  (bases 1 to 650)
  AUTHORS   Leisso,R.
  TITLE     FUSARIUM SPP. CAUSING DAMPING OFF OF KABULI CHICKPEAS:
            PATHOGENICITY OF ISOLATES IN RELATION TO SOIL MOISTURE AND
            PROPAGULE CONCENTRATION
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 650)
  AUTHORS   Leisso,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-JUL-2008) Plant Sciences and Plant Pathology, Montana
            State University, 119 Plant Biosciences Building, Bozeman, m 59715,
            United States
COMMENT     Bankit Comment: rachel.leisso@gmail.com.
FEATURES             Location/Qualifiers
     source          1..650
                     /organism="Fusarium sp."
                     /mol_type="genomic DNA"
                     /isolation_source="Cicer arietinum"
                     /db_xref="taxon:29916"
     gene            <1..>650
                     /gene="(partial) EF1-alpha"
     CDS             join(<1..21,123..185,420..558,614..>650)
                     /gene="(partial) EF1-alpha"
                     /codon_start=1
                     /product="(partial) translation elongation factor 1-alpha"
                     /translation="DSGKSTTTGHLIYQCGGIDKRTIEKFEKEAAELGKGSFKYAWVL
                     DKLKAERERGITIDIALWKFETPRYYVTVIDAPGHRDFIKNM"
BASE COUNT      150 a    187 c    140 g    173 t
ORIGIN
        1 gactctggca agtcgaccac tgtgagtact ctcctcgaca atgagcttat ctgccatcgt
       61 caatcccgac caagacctgg tggggtattt ctcaaagtca acatactgac atcgtttcac
      121 agaccggtca cttgatctac cagtgcggtg gtatcgacaa gcgaaccatc gagaagttcg
      181 agaaggttag tcactttccc ttcgatcgcg cgtcctttgc ccatcgattt cccctacgac
      241 tcgaaatgtg cccgctaccc cgctcgagac caaaaatttt gcaatatgac cgtaattttt
      301 tttggtgggg cacttacccc gccacttgag cgacgggagc gtttgccctc ttaaccattc
      361 tcacaacctc aatgagtgcg tcgtcacgtg tcaagcagtc actaaccatc caacaatagg
      421 aagccgctga gctcggtaag ggttccttca agtacgcctg ggttcttgac aagctcaagg
      481 ccgagcgtga gcgtggtatc accatcgata ttgctctctg gaagttcgag actcctcgct
      541 actatgtcac cgtcattggt atgttgtcgc tcatgcttca ttctacttct cttcgtacta
      601 acatatcact cagacgctcc cggtcaccgt gatttcatca agaacatgat
```

Table A-17. *Fusarium* sp. isolate 4.6 TEF 1-α gene GenBank submission.

```
LOCUS                        568 bp    DNA     linear   PLN 18-JUL-2008
DEFINITION  Fusarium sp. isolate 4.6.
ACCESSION   EU910073
VERSION
KEYWORDS    .
SOURCE      Fusarium sp.
  ORGANISM  Fusarium sp.
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; mitosporic
            Hypocreales; Fusarium.
REFERENCE   1  (bases 1 to 568)
  AUTHORS   Leisso,R.
  TITLE     FUSARIUM SPP. CAUSING DAMPING OFF OF KABULI CHICKPEAS:
            PATHOGENICITY OF ISOLATES IN RELATION TO SOIL MOISTURE AND
            PROPAGULE CONCENTRATION
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 568)
  AUTHORS   Leisso,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-JUL-2008) Plant Sciences and Plant Pathology, Montana
            State University, 119 Plant Biosciences Building, Bozeman, MT
            59715, United States
COMMENT     Bankit Comment: rachel.leisso@gmail.com.
FEATURES             Location/Qualifiers
     source          1..568
                     /organism="Fusarium sp."
                     /mol_type="genomic DNA"
                     /isolation_source="Cicer arietinum"
                     /db_xref="taxon:29916"
     misc_feature    <1..>568
                     /note="coding region not determined"
BASE COUNT      122 a    143 c    155 g    148 t
ORIGIN
        1 tcttggtcgt gtagttgagg gtggtaggaa ttgggagggc ttttctaatg atccttatct
       61 tgcgggtcag cttggacggg aggctgttgt tggtattcag gatgctggtg ttattgcttg
      121 tggaaagcat tggcttgcgc aagagcagga gacacatcga cttaatgctg ccatcacaga
      181 agcccaaccc gtgtcttcca acgttgacga caagactctt catgagcttt acatgtggta
      241 agtacccaaa gtccaactgt attacgaact cactgactga cttcgacagg cccttcgccg
      301 acgccgtcca tgccggactc ggtagtatca tgtgcagtta caaccgcgcc aacaactcgc
      361 aagcctgcca gaactccaag ctcctcaacg gtcttctcaa gggcgagcta ggcttccagg
      421 gctttgttgt ttctgactgg ggtgctcagc actctggtat ggcgtctgct ttagctggta
      481 tggatatggc catgcctacc aacaagctgt ggggtggaaa ccttactgct ggtgttaaca
      541 acggcaccat tcccgaggct cagctcga
```

Table A-18. *Fusarium* sp. isolate DI TEF 1-α gene GenBank submission.

```
LOCUS                       576 bp    DNA     linear   PLN 11-JUL-2008
DEFINITION  Fusarium sp. isolate DI partial tef-1alpha gene for translation
            elongation factor 1 alpha, exons 1-4.
ACCESSION   EU910074
VERSION
KEYWORDS    .
SOURCE      Fusarium sp.
  ORGANISM  Fusarium sp.
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; mitosporic
            Hypocreales; Fusarium.
REFERENCE   1  (bases 1 to 576)
  AUTHORS   Leisso,R.
  TITLE     FUSARIUM SPP. CAUSING DAMPING OFF OF KABULI CHICKPEAS:
            PATHOGENICITY OF ISOLATES IN RELATION TO SOIL MOISTURE AND
            PROPAGULE CONCENTRATION
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 576)
  AUTHORS   Leisso,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (11-JUL-2008) Plant Sciences and Plant Pathology, Montana
            State University, 119 Plant Biosciences Building, Bozeman, MT
            59715, United States
COMMENT     Bankit Comment: rachel.leisso@gmail.com.
FEATURES             Location/Qualifiers
     source          1..576
                     /organism="Fusarium sp."
                     /mol_type="genomic DNA"
                     /isolation_source="Cicer arietinum"
                     /db_xref="taxon:29916"
BASE COUNT      127 a    146 c    154 g    149 t
ORIGIN
        1 tcttggtcgt gtagttgagg gtggtaggaa ttgggagggc ttttctaatg atccttatct
       61 tgcgggtcag cttggacggg aggctgttgt tggtattcag gatgctggtg ttattgcttg
      121 tggaaagcat tggcttgcgc aagagcagga gacacatcga cttaatgctg ccatcacaga
      181 agcccaaccc gtgtcttcca acgttgacga caagactctt catgagcttt acatgtggta
      241 agtacccaaa gtccaactgt attacgaact cactgactga cttcgacagg cccttcgccg
      301 acgccgtcca tgccggactc ggtagtatca tgtgcagtta caaccgcgcc aacaactcgc
      361 aagcctgcca gaactccaag ctcctcaacg gtcttctcaa gggcgagcta ggcttccagg
      421 gctttgtggt ttccgattgg ggtgctcaac actctggtat ggcttctgcc cttgccggta
      481 tggatatggc catgcctacc aacaaactgt ggggtggaaa ccttactgct ggtgtcaaca
      541 atggtactat tcccgaggct cagctcgaca acatga
```

APPENDIX B

ADDITIONAL INFORMATION ON METHODS AND RESULTS

FOR

THE USE OF CONVENTIONAL AND BIOLOGICAL CONTROL SEED TREATMENTS TO CONTROL CHICKPEA (*CICER ARIENTINUM* L.) DAMPING OFF

Table B-1. Biological control seed treatments control of selected damping off pathogens, according to manufacturer's label.

Active ingredient	Trade name	Pathogens labeled to control			Other pathogens
		Pythium spp.	*Fusarium* spp.	*Rhizoctonia* spp.	
Bacillus pumilus GB34	Yield Shield	no	yes	yes	none listed
B. subtilis GB03	Kodiak	yes	yes	yes	*Aspergillus* sp.
B. subtilis MBI 600	Subtilex	yes	yes	yes	*Aspergillus* sp.
Streptomyces lydicus WYEC108	Actinovate SP	yes	yes	yes	*Phytophthora* sp., *Verticillium* sp.
S. griseoviridis K61	Mycostop	yes	yes	yes	*Phytophthora* sp., *Alternaria* sp.
Trichoderma harzanium KLR AG-13	T-22 Planter Box	yes	yes	yes	*Cylindrocarpon* sp., *Thielaviopsis* sp.

Table B-2. Chickpea damping off pathogen *Pythium ultimum* isolate B6 GenBank submission.

```
LOCUS       bankit1100701            880 bp    DNA     linear   PLN 04-JUN-2008
DEFINITION  Pythium ultimum partial 18S ribosomal RNA gene, internal
            transcribed spacer region I, 5.8S ribosomal RNA gene, internal
            transcribed spacer region II, partial 28S ribosomal RNA gene.
ACCESSION   1100701 (EU792322)
VERSION
KEYWORDS    .
SOURCE      Pythium ultimum
  ORGANISM  Pythium ultimum
            Eukaryota; stramenopiles; Oomycetes; Pythiales; Pythiaceae;
            Pythium.
REFERENCE   1  (bases 1 to 880)
  AUTHORS   Leisso,R.S., Miller,P.R. and Burrows,M.E.
  TITLE     Integrated management of chickpea (Cicer arietinum L.) damping off
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 880)
  AUTHORS   Leisso,R.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-JUN-2008) 119 Plant Biosciences Building, Bozeman, MT
            59715, United States
COMMENT     Bankit Comment: rachel.leisso@gmail.com.
FEATURES             Location/Qualifiers
     source          1..880
                     /organism="Pythium ultimum"
                     /mol_type="other DNA"
                     /isolate="B6"
                     /isolation_source="chickpea (Cicer arietinum L.)"
                     /db_xref="taxon:65071"
                     /tissue_type="mycelium"
                     /dev_stage="vegetative"
     rRNA            1..13
                     /note="partial 18S ribosomal RNA"
     misc_feature    14..243
                     /note="internal transcribed spacer region I"
     rRNA            244..403
                     /product="5.8S ribosomal RNA"
     misc_feature    404..838
                     /note="internal transcribed spacer region II"
     rRNA            839..880
                     /note="partial 28S ribosomal RNA"
BASE COUNT      211 a    147 c    230 g    292 t
ORIGIN
        1 cggaggacat taccacactt taaaaaactg tccacgctga actgtaagca agtctagcgc
       61 tgtgactgag ctggtgtttt catttttgga cactggaacg ggagtcagca ggacgaaggt
      121 tggtctgttg taatgcaagt tatgatggac tagctgatga acttttgttt ttaaaccctt
      181 acctaaatac tgatttatac tgtggggacg aaagtccttg cttttactag ataacaactt
      241 tcagcagtgg atgtctaggc tcgcacatcg atgaagaacg ctgcgaactg cgatacgtaa
      301 tgcgaattgc agaattcagt gagtcatcga aattttgaac gcatattgca ctttcgggtt
      361 atgcctggaa gtatgtctgt atcagtgtcc gtaaatcaaa cttgcctttc tttttctgtg
      421 tagtcaggga tggaatgtgc agatgtgaag tgtctcgcat ggttgcgttc gttttttcga
      481 tcgagaatct gtcgagtcct tttaaatgga cacggtcttt tctatggttt ctatgaagtg
      541 taatggttgg aaggcagtga ttttcggatt gctggcggct tttggcgact tcggtatgaa
      601 cgtatggaga ctagctcaat tcgtggtatg ttaggcttcg gctcgacaat gttgcgtaat
      661 tgtgtgtggt ctttgtttgt gccttgaggt gtactagagg ttgtcggttt gaaccgtaag
      721 tgattgttta gtagagcatt ttcacgatgt atggagacgc tgcatttagt tgcgtagaga
      781 gattgatttg ggaaattttg tatcattgtc aattgcaaga ttgtgtatgg tatctcaatt
      841 ggacctgata tcagacaaga ctacccgctg aacttagcat
```

Table B-3. Rating scale for foliar disease ascochyta blight caused by the fungal pathogen Ascochtya rabiei on chickpea*

Rating	Symptoms
1	no visible lesions on any plants
3	lesions visible on less than 10% of plants, no stem girdling (resistant)
5	lesions visible on up to 25% of the plants, stem girdling on less than 10% of plant but little damage (tolerant)
7	lesions on most plants, stem girdling on less than 50% of the plants resulting in the death of a few plants (susceptible)
9	lesions profuse on all plants, stem girdling on more than 50% of plants, and death of most plants (highly susceptible)

*Singh, K.B., Hawtin, G.C., Nene, Y.L., and Reddy, M.V. 1981. Resistance in chickpeas to *Ascochyta rabiei*. Plant Disease 65(7):586-587.

Table B-4**.** Kabuli and desi chickpea phenology at field trials in Bozeman, Huntley and Sidney, MT 2007.

Location	Seed type	Planting date	First emergence	Ascohyta blight	Flowering	Green pod	Expanded pod	Mature pod	Harvest maturity
Bozeman	kabuli	1 May	13 May	7 June	25 June	17 July	27 July	3 Aug	21 Aug
	desi	1 May	11 May	15 June	21 June	11 July	18 July	27 July	3 Aug
Huntley	kabuli	1 May	13 May	5 June	25 June	9 July	17 July	25 July	20 Aug
	desi	1 May	11 May	18 June	20 June	3 July	9 July	17 July	25 July
Sidney	kabuli	27 Apr	13 May	11 June*	19 June	15 July	25 July	NA‡	NA‡
	desi	27 Apr	11 May	11 June	15 June†	11 July	18 July	25 July	9 Aug

Note: Bozeman location was Montana Agricultural Experiment Station (MAES) Arthur H. Post Agronomy Research Farm; Huntley, MAES-Southern Agricultural Research Center; Sidney, MAES-Eastern Agricultural Research Center. Due to the inability to observe plots at Huntley and Sidney on a daily basis, dates may be estimated. Kabuli chickpea cultivar is Sierria; desi, CDC-Anna.

*Due to location of plots, and to the severity of disease upon first notice, ascochyta blight was likely present sooner than noted.

†Date of first flowering approximated.

‡Kabuli chickpea not harvested at Sidney location