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Growth, population dynamics, and diversity of *Fusarium equiseti* in ginseng fields

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Abstract *Fusarium equiseti* is prevalent in ginseng soil, straw mulch and in ginseng root tissues and is the cause of a root surface discolouration on ginseng grown in British Columbia. Population levels of the fungus in ginseng fields ranged from 3.8×10^3 cfu g^{-1} soil to 1.4×10^4 cfu g^{-1} soil and were highest at 0–5 cm soil depths compared to 10–15 cm. Soil population levels were negatively correlated with S content in soil and positively correlated with Zn levels. Barley or wheat straw added to soil significantly increased population levels under laboratory conditions. Mycelial growth in culture was highest at 26–30°C and at pH 7.2–7.8. Samples of flowers and berries, and harvested seed, contained DNA of *F. equiseti* detected using a *Fusarium*-specific DNA array and the fungus was isolated from these tissues on agar medium. A high degree of genetic variation in the EF-1 alpha gene sequence was present among 52 isolates of *F. equiseti* which originated from ginseng fields. At least seven clades were identified. Inoculum dispersal from straw mulch used in ginseng gardens can result in seed contamination by the fungus. In addition, fungal growth near the soil surface under warm summer conditions can result in infection and crown discolouration of ginseng roots.

Keywords Epidemiology · Floral infection · Soilborne pathogen · Straw mulch

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Introduction

North American ginseng (*Panax quinquefolius*) is a perennial herbaceous plant in the family Araliaceae which is grown in British Columbia and Ontario, Canada and exported to markets in Asia for use as a medicinal herb. The plants are initiated from seed, which are produced within fleshy berries on large inflorescences, and require an 8-month period of cold-warm temperature stratification under controlled conditions after harvest for the embryo to mature (Schluter and Punja 2000). The seeds are planted in the fall season on raised beds and covered with cereal straw mulch and the seedlings emerge the following spring. The crop requires an additional 3–4 years of growth prior to harvest of the tap roots.

Several *Fusarium* species, including *F. solani*, *F. oxysporum* and *F. equiseti*, are reported to cause

seed decay and damping-off on ginseng seedlings (Brammall 1994; Punja 1997). In addition, *F. equiseti* and *F. sporotrichioides* were reported to cause a superficial root surface discolouration on ginseng roots (Punja et al. 2008) that significantly reduces marketability. *Fusarium equiseti* was the most frequently recovered species of *Fusarium* from straw mulch used during ginseng production and from ginseng soil, and was the most pathogenic species to ginseng roots (Punja 1997). As such, it represents the most important species in the *Fusarium* complex affecting ginseng.

There is little published information on the factors which affect growth of *F. equiseti*. This species produces several mycotoxins (Bottalico 1998; Hestbjerg et al. 2002; Kosiak et al. 2005; Logrieco et al. 2003) and while it has been recovered from cereal tissues, it is not considered to be a component of the *Fusarium* head blight disease complex (Bottalico 1998; Gale 2003; Logrieco et al. 2003; Parry et al. 1995; Shaner 2003). Isolates of *F. equiseti* are reported to cause diseases on a number of other plant species, including muskmelon (Adams et al. 1987; Sumbali and Mehrotra 1982), cotton (Chimbekujwo 2000), cumin (Reuveni 1982), potato (Rai 1979) and cowpea (Aigbe and Fawole 1999). The fungus infects the crown and root, and can cause fruit, tuber and seed decay. It has also been isolated from roots of diseased asparagus and wheat plants (Demirci and Dane 2003; Nash and Snyder 1965; Strausbaugh et al. 2005; Vujanovic et al. 2006).

In the light of the prevalence of *F. equiseti* in ginseng fields in British Columbia and its role in causing a root surface discolouration symptom (Punja et al. 2008), and the lack of information on factors influencing growth of *F. equiseti*, a better understanding of the epidemiology of this pathogen was needed. The objectives of this research were to: (1) study factors affecting growth of *F. equiseti*, specifically temperature, pH, fungicides and plant residues; and (2) determine the population dynamics and extent of genetic diversity among isolates originating from ginseng fields.

Materials and methods

Growth studies

Two isolates of *F. equiseti* recovered from straw mulch in 4 year-old ginseng gardens located in Kamloops, British Columbia (HE farm) were used for growth studies. To determine the effect of temperature on colony growth, mycelial plugs (5 mm diam) were transferred to the centre of 9 cm diam Petri dishes containing potato dextrose agar (Difco) which were then placed in incubators ranging from 5 to 37°C. There were three replicate dishes per isolate and measurements were taken in two perpendicular directions for each colony after 14 days of growth. The experiment was conducted twice. The data were averaged and standard errors determined from all replicates.

To determine the effect of pH, potato dextrose broth (Difco) was made up in buffered solutions containing appropriate mixtures of 0.1 M KH_2PO_4 and K_2HPO_4 to achieve pH values ranging from 4.5 to 7.8. The broth was autoclaved and the pH was recorded after cooling and just prior to inoculation with mycelial plugs of *F. equiseti.* Two replicate flasks containing the broth solutions per isolate were placed on a shaker (125 rpm) at ambient temperature (21–23°C) for 21 days. The contents were filtered through a Buchner funnel and the mycelial mats were dried at 40°C for 3 days and weighed. The final pH of the filtrate was recorded. The experiment was conducted twice.

To determine the effects of fungicides on colony growth, 1 to 25 μg ml^{-1} active ingredients of four fungicides were added to potato dextrose agar following autoclaving. The fungicides tested were propiconazole, benomyl, fludioxonil and thiophanate-methyl. Colony diameter was measured after 14 days of growth from two replicate dishes per isolate and compared to the control without fungicide.

Population dynamics of *F. equiseti* in soil

Inoculum densities of *F. equiseti* in soil in ginseng fields were determined by plating soil suspensions after serial dilution onto Komada's medium (Komada 1975). A 1 cm^3 soil sample was diluted to 10^{-2} and 0.5 ml was plated onto each of four replicate dishes and incubated at 21–23°C for 7–10 days. Colonies of *F. equiseti* appeared whitish-pink on this medium (Punja et al. 2008). Colony counts were converted to cfu g^{-1} of soil after determining the moisture content in each sample. Soil samples for these studies were obtained from a number of sources: (1) taken at depths of 0, 5, 10 and 15 cm from two ginseng fields; (2) from the soil surface under the straw mulch from

five different ginseng fields; and (3) from the rhizosphere of a number of weed species and forage crops growing in areas adjacent to ginseng fields. These soil samples were collected during July, 2005 and the sampling was repeated from the same fields during July, 2006.

To determine the effect of straw mulch residues on populations of *F. equiseti*, field soil containing naturally occurring predetermined cfu g^{-1} was amended with autoclaved wheat or barley straw mulch segments at a level of 1% (dry weight basis). The soil was characterized as a silt-loam, pH 7.1, organic matter content of 1.6%, with the following nutrient levels: NO_3 (16 ppm), P (44 ppm), K (330 ppm), Ca (3660 ppm), Mg (419 ppm) and S (20 ppm). The straw segments were mixed with the soil, which was moistened to 65% moisture -holding capacity (close to field capacity based on a moisture-release curve) and placed in Styrofoam containers. The containers were covered with parafilm and incubated at 21–23°C. At weekly intervals for up to 7 weeks, two 0.5 cm^3 soil samples were taken from two replicate containers and diluted as described above to determine cfu g^{-1}. Soil moisture content was determined and moisture was added to the containers if required. Changes in population density of *F. equiseti* were compared to that in soil receiving no amendment.

Relationship of soil factors to population levels

Soil samples from 10 ginseng fields were collected from the soil surface during July, 2005 and analyzed for organic matter content and pH, as well as a range of macronutrients (N, P, K, Ca, Mg, S) and micronutrients (Fe, Cu, Zn, B, Mn) using standard analytical procedures in a commercial laboratory (Norwest Labs., Langley, B.C.). The population density of *F. equiseti* in each sample was determined by dilution plating as described above. To determine if there were any correlations between soil parameters and population levels, principal components analysis (PCA) was conducted as described by other authors (Martinez et al. 2002; Termorshuizen et al. 2006). Principal components and correlation matrix was analyzed using SAS (version 9.2, SAS Institute Inc, Cary, NC) under multivariate analysis. This analysis reduces the dimensionality of the original space by creating component axes and provides patterns of correlations among the measured variables. Each component axis was given a descriptive label representing the common soil nutrients.

Occurrence of *F. equiseti* on ginseng seed and floral parts

To establish whether *F. equiseti* could colonize ginseng seed, samples of stratified seed (exposed to an 8 month cool–warm temperature cycle under controlled conditions after harvest) as well as seed

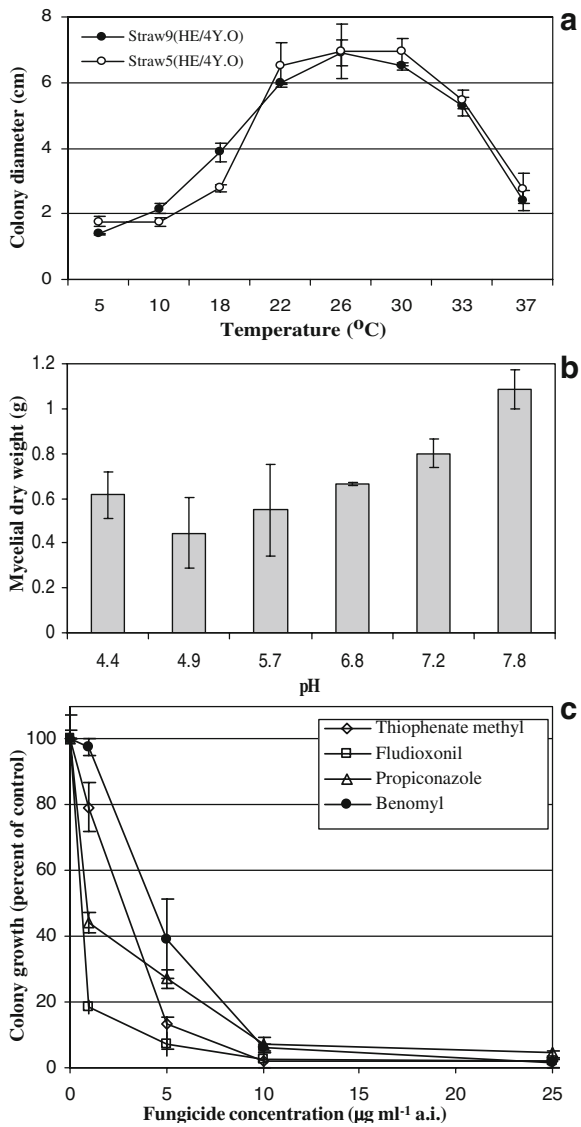
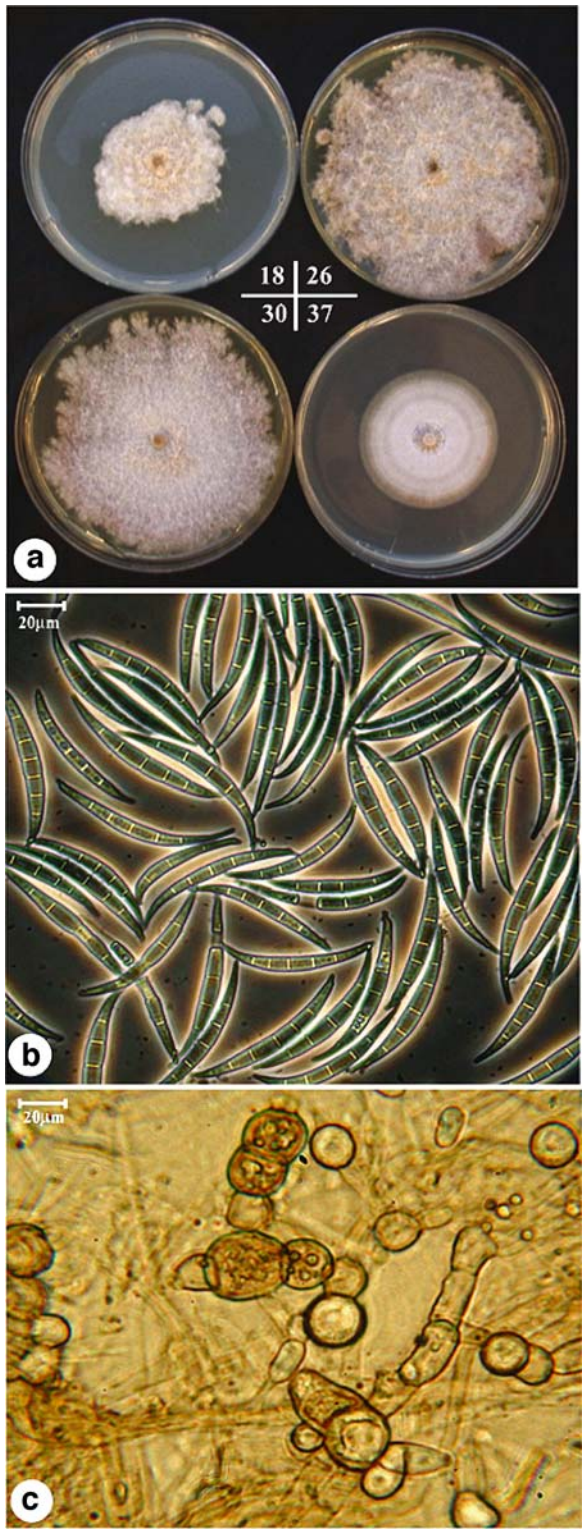


Fig. 1 Factors affecting growth of *Fusarium equiseti* in culture. **a** Effect of temperature. **b** Effect of pH. **c** Effect of fungicides. *Error bars* denote standard error of the mean



◀**Fig. 2** Growth and spore production by *Fusarium equiseti* in culture. **a** Growth on potato dextrose agar at four different temperatures. **b** Spore production. *Bar*=20 µm. **c** Chlamydospore production. *Bar*=20 µm

that had been planted in field soil were collected during March, 2006 from four ginseng fields. The seeds were surface-disinfested after washing in water by immersion in 70% EtOH for 30 s followed by 10% bleach (containing 5.25% NaOCl) for 1 min and then plated onto Komada's medium and incubated for 14 days. In addition, samples of ginseng flowers, small green berries, mature red berries, and freshly harvested nonstratified seed were obtained during July–September, 2006 and surface-disinfested as described above and plated onto Komada's medium. Samples consisted of at least 25–30 flowers or berries from each of three ginseng fields. The pathogenicity of a subset of isolates recovered from ginseng seeds and floral parts (around 50 isolates) was determined using an in vitro inoculation method utilizing ginseng roots as described previously (Punja et al. 2008).

To confirm the presence of *F. equiseti* on seed and floral parts, a DNA array based on oligonucleotides designed from sequences of a portion of the EF-1 alpha gene of 26 *Fusarium* species was used (Barasubiye et al. 2005; Punja et al. 2008). The DNeasy Plant Mini Kit (Qiagen, Valencia, CA) or the Ultraclean ™ Soil DNA Kit (MOBIO, Carlsbad, CA) was used to extract DNA from surface-disinfested tissues. Digoxigenin-labelled PCR-derived EF-1 amplicons were generated as described previously (Barasubiye et al. 2005) and hybridized onto the DNA array panel.

Genetic diversity in *F. equiseti*

A total of 52 isolates of *F. equiseti* originating from ginseng root tissues, straw mulch and soil from eight ginseng fields was analyzed for the extent of genetic diversity based on an alignment of their EF-1 alpha gene sequences. Genomic DNA was extracted from single-spore derived cultures. A portion of the EF-1 alpha gene was amplified by PCR as described previously (Barasubiye et al. 2005) and sequenced. Sequences were aligned using Clustal W as implemented in MegAlign 5.06 software for windows (DNASTAR INC., Madison, WI), with minor adjustments made by hand to correct obvious alignment

errors. Sequences of five reference strains of *Fusarium acuminatum* from the National Mycological Herbarium, Department of Agriculture, Ottawa, Mycology (DAOM) were used as the outgroup. In addition, a collection of 27 isolates of *F. equiseti* from a wide range of sources, deposited in the DAOM culture collection (Ottawa, Canada), were included in the analysis. Phylogenetic analysis was undertaken with PAUP version 4.0b10 (Swofford 2001) using heuristic searches and default options. For heuristic searches, 'MaxTrees' was set to 5,000. Consensus trees (70%) were calculated from equally parsimonious trees and bootstrap values were calculated using 1,000 replicates with 'fast' stepwise addition analysis.

Results

Growth studies

Colonies of *F. equiseti* grew best at 22–30°C and growth was reduced at 5–10 and 37°C (Fig. 1a). The appearance of colonies at four different temperatures is shown in Fig. 2a. Growth at different pHs was highest

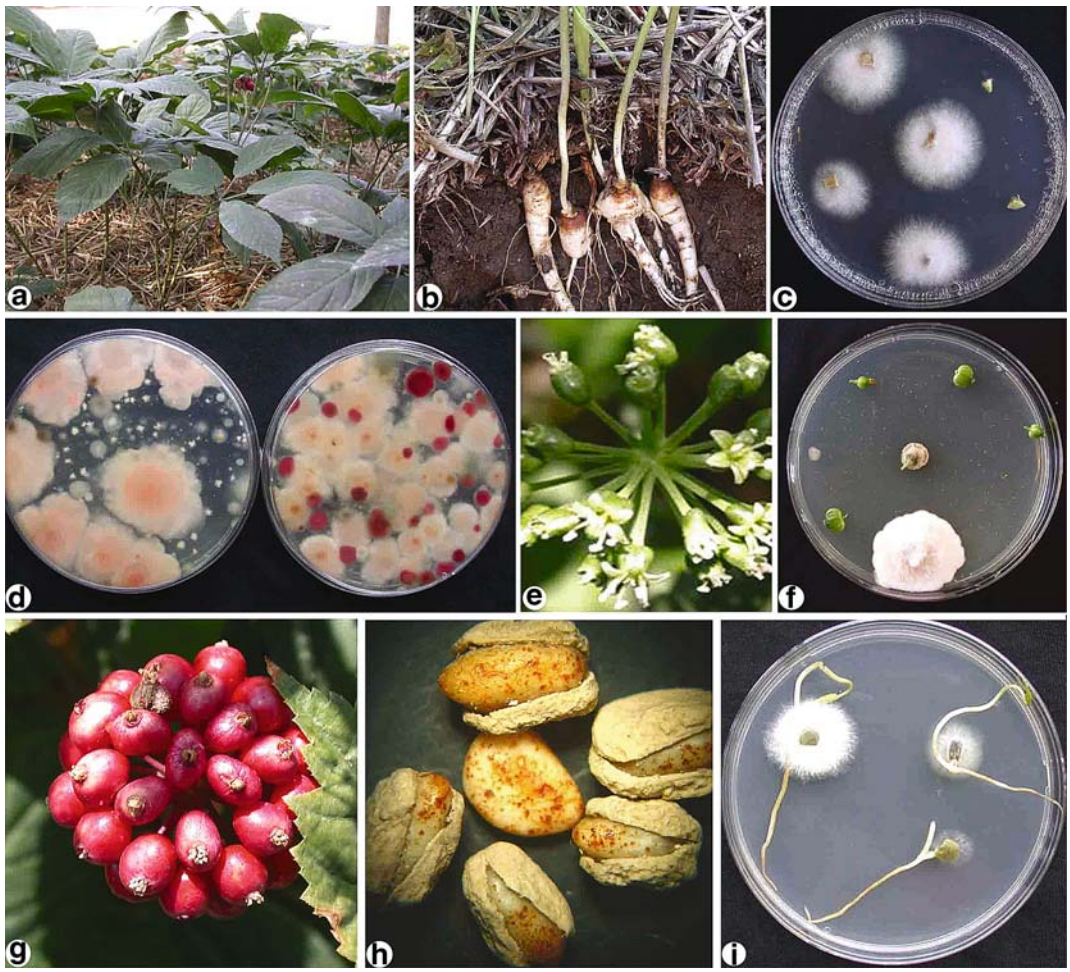


Fig. 3 Recovery of *Fusarium equiseti* from ginseng fields. **a** Ginseng canopy showing placement of straw mulch on the soil surface. **b** View of ginseng roots at the straw–soil interface. **c** Colonies of *F. equiseti* growing from straw segments placed on Komada's medium. **d** Colonies of *F. equiseti* following dilution-plating of soil taken from 15 cm depth (*left*) and soil surface (*right*) on Komada's medium. **e** Ginseng flowers and green berries sampled to detect *Fusarium*. **f** A colony of *F. equiseti* emerging from a green berry. **g** Red berries sampled to detect *Fusarium*. **h** Stratified seed showing splitting of seed coat to reveal the endosperm. **i** Recovery of *F. equiseti* from germinating seeds

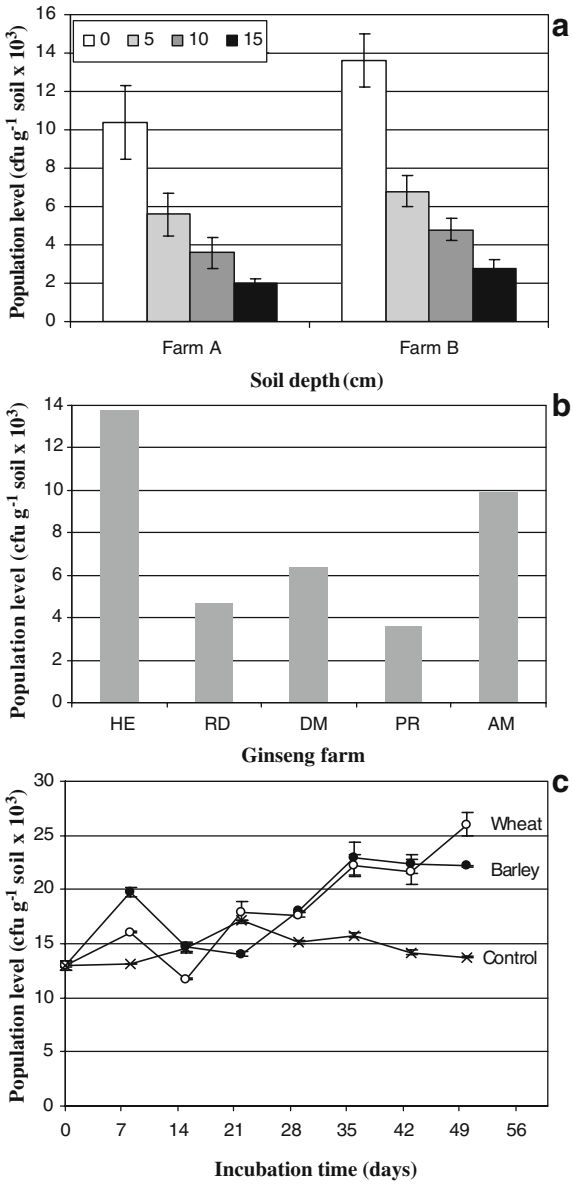


Fig. 4 Factors affecting population levels of *Fusarium equiseti* in soil. **a** Effect of depth in soil. **b** Effect of ginseng farm. **c** Effect of amendment with wheat or barley straw

at pH 7.2–7.8 and the fungus grew well at pH 4.4–6.8 (Fig. 1b). The final pH of the medium at the end of the experiment was changed by about ±0.2. Macroconidia and microconidia of *F. equiseti* were produced in abundance in culture (Fig. 2b) and were accompanied by the appearance of one- to-three-celled thick-walled chlamydospores produced in chains (Fig. 2c).

The effect of four fungicides on growth of *F. equiseti* is shown in Fig. 1c. All fungicides reduced growth at 1 μg ml^{-1} a.i., with fludioxonil being the most effective. At 10 μg ml^{-1}, growth was reduced by 90–95% of that in the control by all fungicides. No growth occurred at 25 μg ml^{-1}.

Population dynamics of *F. equiseti* in soil

Isolates of *F. equiseti* recovered from ginseng fields (Fig. 3a) from samples of soil under the straw mulch layer (Fig. 3b) and from colonized straw segments grew well on Komada's medium (Fig. 3c,d). With increasing depths in soil, the population levels declined from the highest level at 0 cm to the lowest level at 15 cm (Figs. 3d and 4a). The population levels in soil under the straw mulch were significantly different among the five ginseng fields sampled and ranged from 3.8×10^{3} to 1.4×10^{4} cfu g^{-1} of soil (Fig. 4b). In contrast, the population levels in soil in adjacent areas not planted to ginseng but containing weed species or forage crops (alfalfa, clover) were considerably lower, ranging from 1.2×10^{1} to 2.9×10^{2} cfu g^{-1} of soil. These same trends were also found for soil samples collected in July, 2006 from the same fields (data not shown). The addition of wheat or barley straw mulch to soil enhanced the population levels of *F. equiseti*, and the levels after 50 days of

Table 1 Correlation coefficients derived from principal components analysis following regression of soil nutrient levels against population levels of *Fusarium equiseti* (cfu g^{-1} soil)

	CFU	$P\leq 0.05$
CFU	1.00	
OM	0.3702	0.292
pH	0.3676	0.296
P	0.4880	0.153
K	0.4696	0.171
Ca	0.4067	0.243
Mg	0.5086	0.133
S	−0.7871	0.006*
N	0.5702	0.085
Fe	0.0875	0.810
Cu	0.4902	0.150
Zn	0.6214	0.054*
	0.4315	0.213
Mn	0.2450	0.495

Data from 10 ginseng fields in British Columbia were used in the analysis. Probability values ($P\leq0.05$) marked with an asterisk were significant.

incubation had doubled when compared to the unamended control (Fig. 4c).

An analysis of the soil factors that were associated with population levels (cfu g^{-1}) of *F. equiseti* in ginseng fields using PCA revealed that the measured parameters varied widely in their ability to explain the variation in cfu. An analysis of correlation matrix indicated a significant correlation with only two soil nutrients (S and Zn; Table 1) while PC1 and PC2 explained 39.4 and 20.1% of the variation associated with soil nutrient levels (data not shown).

Occurrence of *F. equiseti* on ginseng seed and floral parts

Colonies of *F. equiseti* were recovered from ginseng flowers and green berries originating from three ginseng fields at a frequency of 1–5% on Komada's medium (Fig. 3e,f). From red berries (Fig. 3g) and unstratified seed, the frequency of recovery was 5–10%, which increased to 10–30% from stratified seed, depending on the ginseng field (Fig. 3h). Seed that had been planted in soil and initiated germination

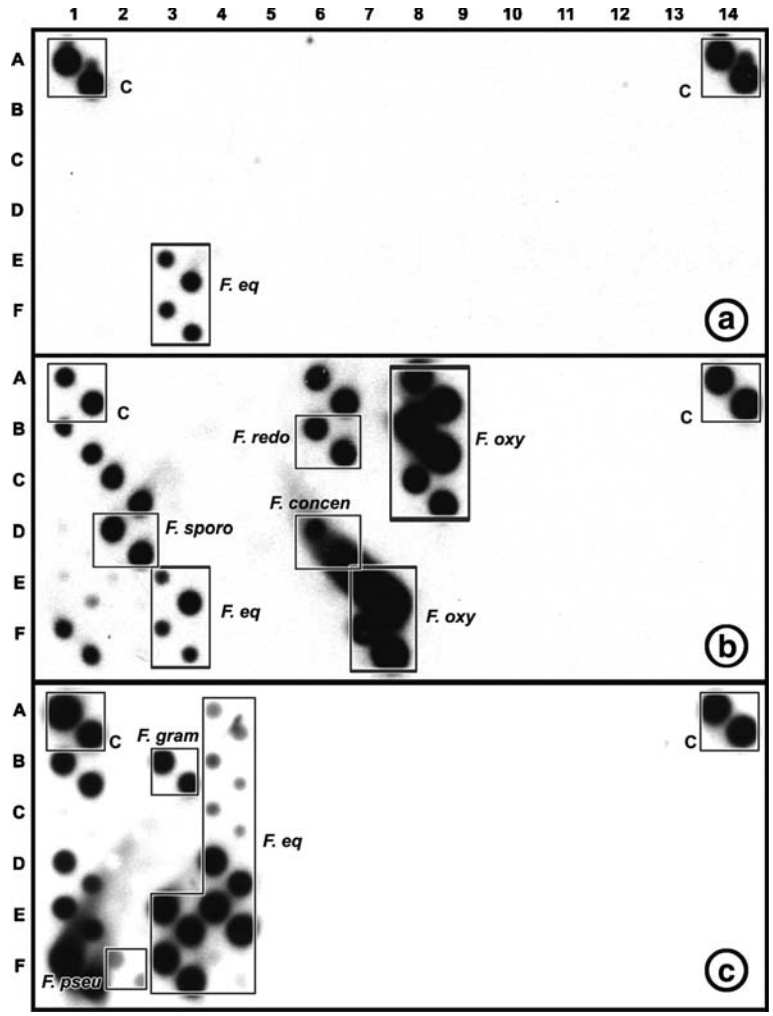
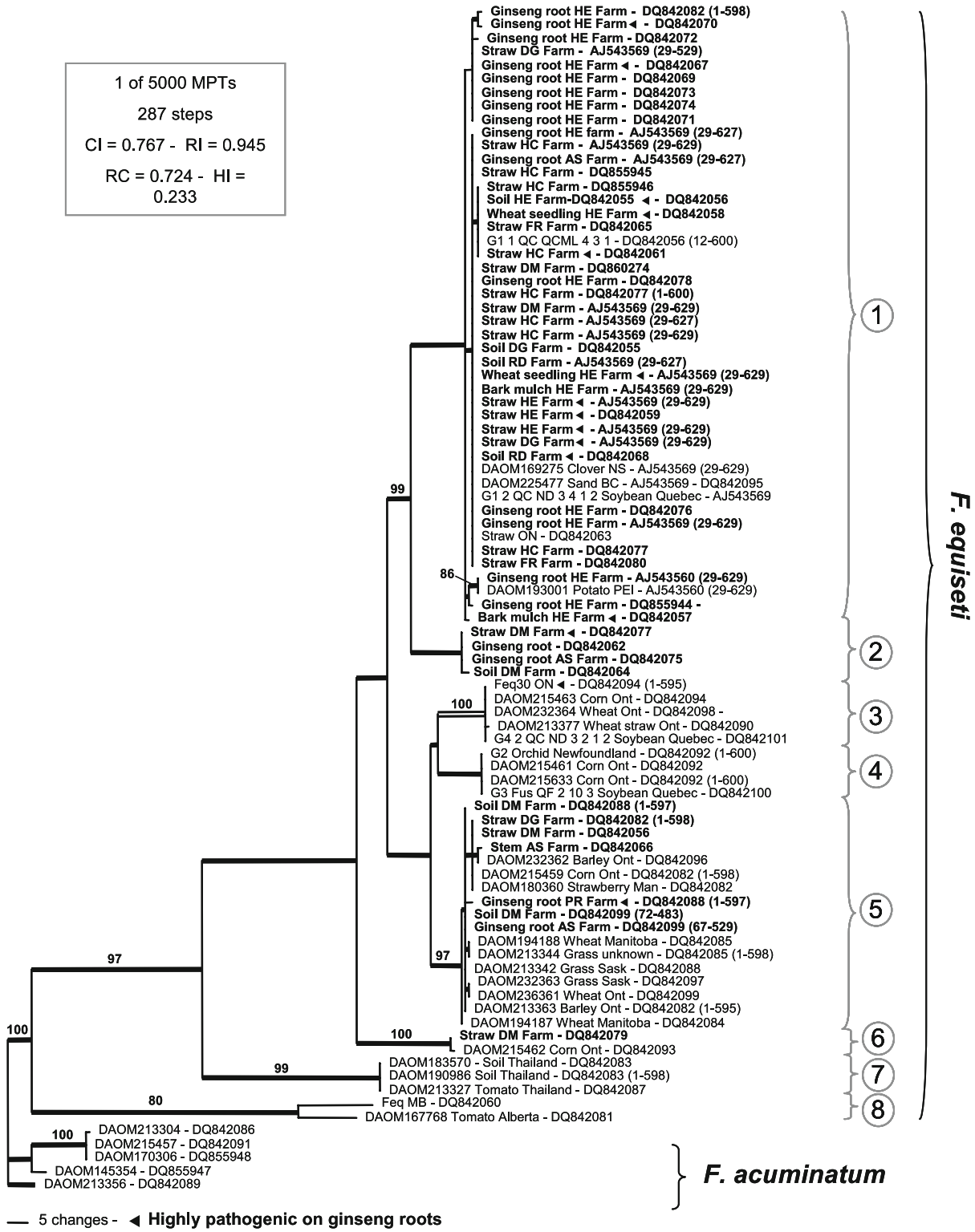


Fig. 5 DNA array hybridization assay to a panel of 82 specific oligonucleotides representing 26 different *Fusarium* species and several clades above or below the species level. The specific oligonucleotides designed from EF-1 alpha gene and intron sequences were amino-modified and spotted in duplicate on nylon membranes. They were arranged in 6 rows × 14 columns in two replicates. Positive hybridizations can be seen as chemiluminescent labelled spots at specific locations along the array representing each species. DNA samples originated from ginseng plants from British Columbia. **a** Green berries. **b** Red berries. **c** Seedling root. *C* Control, reverse complement oligo of forward PCR primer. F. eq., *F. equiseti*; F. spo, *F. sporotrichioides*; F. oxy, *F. oxysporum*; F. redo, *F. redolens*; F. concen, *F. concentricum*; F. pseu, *F. pseudograminearum*; F. gram, *F. graminearum*


1 of 5000 MPTs
287 steps
CI = 0.767 - RI = 0.945
RC = 0.724 - HI = 0.233
Ginseng root HE Farm - DQ842082 (1-598)
Ginseng root HE Farm◄ - DQ842070
Ginseng root HE Farm - DQ842072
Straw DG Farm - AJ543569 (29-529)
Ginseng root HE Farm◄ - DQ842067
Ginseng root HE Farm - DQ842069
Ginseng root HE Farm - DQ842073
Ginseng root HE Farm - DQ842074
Ginseng root HE Farm - DQ842071
Ginseng root HE farm - AJ543569 (29-627)
Straw HC Farm - AJ543569 (29-629)
Ginseng root AS Farm - AJ543569 (29-627)
Straw HC Farm - DQ855945
Straw HC Farm - DQ855946
Soil HE Farm-DQ842055 ◄ - DQ842056
Wheat seedling HE Farm ◄ - DQ842058
Straw FR Farm - DQ842065
G1 1 QC QCML 4 3 1 - DQ842056 (12-600)
Straw HC Farm ◄ - DQ842061
Straw DM Farm - DQ860274
Ginseng root HE Farm - DQ842078
Straw HC Farm - DQ842077 (1-600)
Straw DM Farm - AJ543569 (29-629)
Straw HC Farm - AJ543569 (29-627)
Straw HC Farm - AJ543569 (29-629)
Soil DG Farm - DQ842055
Soil RD Farm - AJ543569 (29-627)
Wheat seedling HE Farm ◄ - AJ543569 (29-629)
Bark mulch HE Farm - AJ543569 (29-629)
Straw HE Farm◄ - AJ543569 (29-629)
Straw HE Farm◄ - DQ842059
Straw HE Farm◄ - AJ543569 (29-629)
Straw DG Farm◄ - AJ543569 (29-629)
Soil RD Farm◄ - DQ842068
DAOM169275 Clover NS - AJ543569 (29-629)
DAOM225477 Sand BC - AJ543569 - DQ842095
G1 2 QC ND 3 4 1 2 Soybean Quebec - AJ543569
Ginseng root HE Farm - DQ842076
Ginseng root HE Farm - AJ543569 (29-629)
Straw ON - DQ842063
Straw HC Farm - DQ842077
Straw FR Farm - DQ842080
Ginseng root HE Farm - AJ543560 (29-629)
DAOM193001 Potato PEI - AJ543560 (29-629)
Ginseng root HE Farm - DQ855944 -
Bark mulch HE Farm◄ - DQ842057
Straw DM Farm◄ - DQ842077
Ginseng root - DQ842062
Ginseng root AS Farm - DQ842075
Soil DM Farm - DQ842064
Feq30 ON◄ - DQ842094 (1-595)
DAOM215463 Corn Ont - DQ842094
DAOM232364 Wheat Ont - DQ842098 -
DAOM213377 Wheat straw Ont - DQ842090
G4 2 QC ND 3 2 1 2 Soybean Quebec - DQ842101
G2 Orchid Newfoundland - DQ842092 (1-600)
DAOM215461 Corn Ont - DQ842092
DAOM215633 Corn Ont - DQ842092 (1-600)
G3 Fus QF 2 10 3 Soybean Quebec - DQ842100
Soil DM Farm - DQ842088 (1-597)
Straw DG Farm - DQ842082 (1-598)
Straw DM Farm - DQ842056
Stem AS Farm - DQ842066
DAOM232362 Barley Ont - DQ842096
DAOM215459 Corn Ont - DQ842082 (1-598)
DAOM180360 Strawberry Man - DQ842082
Ginseng root PR Farm◄ - DQ842088 (1-597)
Soil DM Farm - DQ842099 (72-483)
Ginseng root AS Farm - DQ842099 (67-529)
DAOM194188 Wheat Manitoba - DQ842085
DAOM213344 Grass unknown - DQ842085 (1-598)
DAOM213342 Grass Sask - DQ842088
DAOM232363 Grass Sask - DQ842097
DAOM236361 Wheat Ont - DQ842099
DAOM213363 Barley Ont - DQ842082 (1-595)
DAOM194187 Wheat Manitoba - DQ842084
Straw DM Farm - DQ842079
DAOM215462 Corn Ont - DQ842093
DAOM183570 - Soil Thailand - DQ842083
DAOM190986 Soil Thailand - DQ842083 (1-598)
DAOM213327 Tomato Thailand - DQ842087
Feq MB - DQ842060
DAOM167768 Tomato Alberta - DQ842081
DAOM213304 - DQ842086
DAOM215457 - DQ842091
DAOM170306 - DQ855948
DAOM145354 - DQ855947
DAOM213356 - DQ842089
99
86
100
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97
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99
80
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1
2
3
4
5
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7
8
F. equiseti
F. acuminatum
5 changes - ◄ Highly pathogenic on ginseng roots

◀ **Fig. 6** Phylogenetic analysis of elongation factor 1-alpha sequences from 52 isolates of *F. equiseti* originating from ginseng roots, straw or bark mulch or soil from eight farms (HE, HC, AS, PR, FR, DM, DG, RD) in British Columbia (shown in *bold*), and 27 reference strains from DAOM, with five strains of *F. acuminatum* used as an outgroup. Strains marked with an arrowhead are highly aggressive on ginseng roots (Punja et al. 2008). Sequences from the EF-1 alpha gene were amplified, sequenced, and aligned using MegAlign 5.06 software. Phylogenetic and bootstrap analyses were conducted using PAUP version 4.0b.10. The GenBank Accession numbers for the sequences are included in the phylogenetic tree. Sequences that were identical to existing entries in GenBank have the region of identity mentioned in parentheses following the accession numbers. One of more than 5000 most parsimonious tree (MPTs) 287 steps, (C1=0.767, RI=0.945, RC=0.724, HI=0.233). Bootstrap values above 70% are given as *numbers along branches*. Thick branches occurred in 100% of the MPTs, and the branches represented by double lines in 90–99% of the MPTs. The *bracketed groups* indicated by *circled numbers* represent eight major sequence groups discussed in the text

yielded *F. equiseti* at a frequency of 35–50% (Fig. 3i). The pathogenicity of a subset of the isolates recovered from ginseng seed (50 isolates) was tested on ginseng roots. Twenty-six isolates (52%) produced reddish-brown lesions on the surface of inoculated roots after 7–10 days.

The presence of *F. equiseti* was confirmed using a DNA array which detected this species on small green berries (Fig. 5a), red berries (Fig. 5b) and on seedling roots (Fig. 5c). A number of other species, including *F. sporotrichioides*, *F. oxysporum* and *F. concentricum*, were also detected on red berries.

Genetic diversity in *Fusarium equiseti*

A phylogenetic analysis of 52 isolates of *F. equiseti* originating from ginseng fields, along with numerous reference strains of this species from different substrates and geographical origins, is shown in Fig. 6. The sequence alignment included 653 characters, of which 169 were phylogenetically informative. The topology of the tree was well-supported by 90% or greater consensus bootstrap values, but only the basal parts of the tree, and some terminal and subterminal nodes, were well-supported by bootstrap values. These sequences have been submitted to GenBank and the corresponding accession numbers are included in Fig. 6. There was considerable genetic diversity among the isolates, with the ginseng isolates occurring in seven of the eight sequence groups identified. Most of the isolates from this study were in clade 1. Clade 2 was comprised only of isolates obtained from ginseng. The remaining isolates originating from ginseng farms were scattered in four of the six remaining clades. Many highly aggressive strains on ginseng roots determined in a previous study (Punja et al. 2008) were in clade 1, with other ginseng-pathogenic strains found in clades 2, 3 and 5. Some isolates recovered from ginseng roots on the HE farm belonged to the same sequence group (clade 1) as the isolates from straw mulch from the same farm.

Discussion

The results from this study revealed that population levels of *F. equiseti* were consistently higher in soil taken from beneath the straw mulch in ginseng fields compared to adjacent areas which contained weeds or forage crops. The ability of *F. equiseti* to colonize wheat and barley mulch was demonstrated in this study, and *F. equiseti* is considered to be a highly competitive colonizer of cereal straw residues (Pereyra et al. 2004) and demonstrates prolonged survival in soil (Sangalang et al. 1995a). As a result, population levels were highest near the soil surface in ginseng fields compared to depths of 10–15 cm. In addition, soil population levels were negatively correlated with S content in soil and positively correlated with Zn levels, as revealed by principal components analysis. Martinez et al. (2002) showed correlations between soil nutrient factors and population levels of *Helminthosporium solani*, while Termorshuizen et al. (2006) demonstrated that PCA analysis was an effective tool to minimize the dimensionality of variables to find components which explained the variability related to the suppressive effects of composts against different pathosystems.

Temperatures of 22–30°C were optimal for growth of *F. equiseti*, which correlates with the warmer climatic regions from which this species has been frequently recovered (Aigbe and Fawole 1999; Chimbekujwo 2000; Demirci and Dane 2003; Fedel-Moen and Harris 1987; Gale 2003; Reuveni 1982; Sangalang et al. 1995b). The fungus grew well over a wide range of pHs, although growth was best at pH 7.2–7.8, suggesting a preference for alkaline conditions. Many of the soils in British Columbia ginseng fields from

which *F. equiseti* was recovered are in the range of pH 6.5–8.0 (authors, unpublished observations), providing suitable soil conditions for fungal growth. One soil factor which was negatively correlated with soil population levels of *F. equiseti* was sulphur, which is associated with low soil pH. While Zn was positively correlated with population levels, the ecological significance of this observation is not known.

While *F. equiseti* has been previously isolated from cereal tissues, it is not considered to be a component of the *Fusarium* head blight disease complex on cereals (Bottalico 1998; Gale 2003; Logrieco et al. 2003; Parry et al. 1995; Shaner 2003). However, this fungus can cause root and crown rot, in addition to seed, fruit and tuber decay, on a number of plant species, including cereals (Adams et al. 1987; Aigbe and Fawole 1999; Chimbekujwo 2000; Demirci and Dane 2003; Fedel-Moen and Harris 1987; Nash and Snyder 1965; Rai 1979; Reuveni 1982; Strausbaugh et al. 2005; Sumbali and Mehrotra 1982). On ginseng, *F. equiseti* causes seed decay and damping-off on seedlings (Punja 1997). The practice of using straw mulch during ginseng production encourages the proliferation of *F. equiseti* and other straw-inhabiting *Fusarium* species, especially under warm and humid summer conditions (Punja et al. 2008). At the present time, there are no alternatives to the use of straw mulch by ginseng growers, as it is readily available and cost-effective. However, new straw mulch first introduced into ginseng fields may contain *Fusarium* species at frequencies of 10–40%, depending on the source (authors, unpublished data). A number of these species, especially *F. equiseti*, were shown to cause a surface discolouration upon contact of the mycelium with the ginseng root below the straw mulch, resulting in reduced quality of the roots (Punja et al. 2008).

The ability of *F. equiseti* to also colonize ginseng floral parts and berries, as well as seeds, was demonstrated in this study. Inflorescences of wild grasses have been reported to contain *F. equiseti* (Inch and Gilbert, 2003) and this species has been recovered from *Fusarium* head blight affected seed at low levels (Bottalico 1998; Logrieco et al. 2003; Parry et al. 1995). The source of inoculum for floral and berry infection in ginseng fields is most likely the straw mulch, and colony-forming-units of *F. equiseti* have been recovered from air samples collected within the ginseng canopy during mid-summer (authors, unpublished data). Therefore, seed contamination by *F. equiseti* can play a role in dispersal of the pathogen as well as provide a source of inoculum for seedling infection. Colonization of straw mulch and crop residues on the soil surface was also shown to significantly increase the inoculum potential of *Fusarium* species and the severity of head blight (Dill-Macky and Jones 2000; Parry et al. 1995; Pereyra et al. 2004; Shaner 2003). A number of other *Fusarium* species, including *F. sporotrichioides* and *F. oxysporum*, which are frequently recovered from cereal straw residues (Gale 2003; Parry et al. 1995; Pereyra et al. 2004; Shaner 2003) were also present on ripening ginseng berries. Other fungi, such as *Alternaria alternata* and *Botrytis cinerea*, were also recovered from these berries when plated onto agar medium. The time period from flowering to berry ripening is about 6–8 weeks (Schluter and Punja 2000), providing sufficient opportunity for the ginseng inflorescences to be colonized by air-borne inoculum of different fungi.

Stratified seed yielded a higher recovery of *F. equiseti* compared to freshly-harvested seed, suggesting that during the 8-month stratification period, in which seed is incubated in moist sand at 8–10°C, further colonization of the seed had occurred. Many of these seed-derived isolates of *F. equiseti* were pathogenic to ginseng roots. Seed colonization by *F. equiseti* has also been reported in other plant species, such as cowpea (Aigbe and Fawole 1999), resulting in seed decay and seedling infection.

Several fungicides were identified that inhibited growth of *F. equiseti* at 10 μg ml^{-1} and could potentially be used as seed treatments or applied to the developing inflorescences in the summer to reduce berry and seed contamination by air-borne inoculum. The fungicides benomyl, propiconazole and thiophanate-methyl reduced *Fusarium* head blight development on wheat when applied to coincide with spike emergence and anthesis, or in some cases when used as a seed treatment (Ioos et al. 2005; Mesterhazy 2003; Parry et al. 1995).

The extensive genetic diversity among isolates of *F. equiseti* in this study based on a sequence comparison of a portion of the EF-1 alpha gene indicates that this cosmopolitan species may be comprised of at least eight sub-groups. Further molecular characterization using additional molecular markers is needed to resolve this species complex. There was no pattern of grouping of isolates of *F. equiseti* from ginseng fields to suggest an intra-specific differentiation based on host or

substrate of origin. This is consistent with the widespread occurrence and lack of host specialization within *F. equiseti*.

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