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Identification and characterization of *Fusarium* spp. associated with root rots of field pea in North Dakota

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Abstract Root rots are a major concern in field pea production in North Dakota. However, it is unclear which pathogens are involved in causing these diseases. This report brings together findings from surveys conducted over four years (2004, 2005, 2008, and 2009). The 2004 and 2005 surveys were mainly aimed at establishing the importance of pea root rot in North Dakota and providing an indication of the most prevalent root rot pathogens. The 2008 and 2009 surveys involved thorough evaluation of root rot incidence and severity, and included isolations and characterization of *Fusarium* species associated with the root rots. Greater mean root rot incidence and severity were observed in 2009 compared to three previous years. *Fusarium* species were the most frequently isolated fungal species from infected pea roots, of which *F. oxysporum* (66.7 and 94.7 % of the fields) and *F. avenaceum* (71.8 and 89.5 % of the fields) were most commonly isolated in 2008 and 2009, respectively. Pathogenicity tests showed that all nine *Fusarium* species isolated from symptomatic roots were capable of causing root rot of pea, and isolates of *F. avenaceum* were the most virulent at causing root rot. Significant differences in virulence were observed among *F. avenaceum* isolates. The prevalence of *F. avenaceum* on roots of field peas, and the ability of isolates of this species to cause severe root rot, emphasizes the possibility of this pathogen to emerge as a potential risk under the current cropping practices for pulse crops in North Dakota, and potentially in other regions with similar growing conditions.

Keywords *Fusarium* · Root rot · Field pea · *Fusarium avenaceum* · Virulence · Pathogenicity

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Introduction

Dry field pea (*Pisum sativum* L.) is an important cool season legume crop grown in the North Central plain states of the United States (McPhee et al. 2003). North Dakota produces nearly 38 % of the U.S. crop with 320,000 acres planted in 2013 (United States Department of Agriculture, National Agricultural Statistics Service [USDA-NASS]). Soilborne pea diseases, including wilts and root rots, are of major economic importance and can cause significant reduction in yield (Hwang and Chang 1989; Infantino et al. 2006). Root rots in field pea can be caused by many pathogens including *Aphanomyces euteiches, Fusarium oxysporum* f. sp. *pisi, F. solani* f. sp. *pisi, Mycosphaerella pinodes, Pythium* spp., and *Rhizoctonia solani.* The symptoms are often referred to as the pea root rot complex (Clarkson 1978; Mathew et al. 2012; Xue 2003). Among these, Fusarium

root rots are thought to cause the most serious disease (Kraft and Pfleger 2001). Fusarium root rot on pea was first reported in 1918 from Minnesota, and in 1923 from Wisconsin (Jones 1923). Yield losses to Fusarium root rots are significant. In eastern Washington State, soil infested with *F. solani* f. sp. *pisi,* reduced yields by 30 % (Kraft and Pfleger 2001). Mean percent yield losses of 35 to 57 % were reported for pea plants exhibiting moderate to severe symptoms of Fusarium root rot (Basu et al. 1976).

Fusarium solani f. sp. *pisi* has been thought to be the primary causal agent of the pea root rot complex in most areas of pea production (Kraft and Pfleger 2001). However, several other *Fusarium* species have been associated with root rots in field pea, including *F. avenaceum, F. culmorum, F. graminearum, F. sambucinum* var. *coeruleum, F. equiseti, F. poae, F. sporotrichioides,* and *F. tabacinum* (Clarkson 1978). Fernandez (2007) and Fernandez et al. (2008) reported that *F. avenaceum* was the most prevalent species of *Fusarium* isolated from the discolored roots of lentil and field pea plants grown in the eastern part of Saskatchewan, Canada. In addition to pea, *F. avenaceum* has also been isolated from crown and root tissue of other hosts such as clover, ryegrass, soybean, and potato.

In recent years, root rots appear to be developing as a major constraint to field pea production in North Dakota, and preliminary reports by Gregoire and Bradley (2005) suggested that *Fusarium* species were the predominant pathogens associated with root rot in this state. Therefore, the objectives of this study were: (i) to assess the incidence and severity of root rot in the major field pea production areas of North Dakota, (ii) to determine the major pathogens associated with pea root rots in North Dakota, and (iii) to characterize pathogenicity and aggressiveness of the root rot associated pathogens.

Materials and methods

Field surveys and disease rating

Two- year surveys were conducted in major field pea growing counties of North Dakota in 2004 to 2005, and 2008 to 2009, to assess the incidence and severity of pea root rots. Forty-seven and 41 fields in a total of 10 counties were surveyed in 2004 and 2005, respectively. In 2008, 77 fields were surveyed in 11 counties, of which 39 fields belonged to growers and the rest were research plots. An additional 38 grower fields in seven counties were surveyed in 2009. In both surveys, fields were selected arbitrarily and located at least three miles apart. Twenty plants were sampled from each field during 2004 and 2005, and 10 plants were collected from each field in 2008 and 2009. Plants were collected with a shovel, following a 'W' pattern across the field, placed in sealable plastic bags, and stored in coolers until brought to the laboratory. The roots were washed under running tap water and assessed for incidence and severity of root rot. Mean root rot incidence per field was calculated as the percentage of plants with symptomatic roots based on the total number of plants rated in a field. Root rot severity was measured as the percentage of root length with root rot lesions (length of lesions/total root length × 100) per plant, and then averaged for all plants sampled per field.

Pathogen isolation and identification

Root tissues with dark brown lesions on the tap root or hypocotyl were excised. In 2004 and 2005, excised lesions from 20 roots/field were surface-sterilized by soaking the roots in 0.5 % NaOCl followed by three rinses in sterilized water. In 2008 and 2009, excised lesions from five of the roots collected/field were surface-sterilized and the roots of five other plants were not surface-sterilized. In 2008, only roots from grower fields were used for isolation. Surface-sterilization involved soaking tissue in 0.5 % NaOCl and then in 70 % ethanol for 1 min each, followed by two rinses in sterilized distilled water. The samples were subsequently blotted dry on sterilized paper towels in a laminar flow hood. Dried root segments were then plated on half-strength potato dextrose agar (PDA 19.5 g/l; Difco Laboratories, Sparks, MD) containing streptomycin and penicillin (250 ppm each), and incubated for 3 to 5 days at 20 to 25 °C, under a 12 h photoperiod. Initial identification of the pure culture to genus was done based on colony characteristics including colony morphology, colony color on PDA, and spore morphology. Pure cultures of *Fusarium* species were obtained through single-spore transfers. For isolates for which sporulation could not be induced, pure cultures were established by hyphal tipping. *Fusarium* isolates were identified to species level using morphological keys as described in Booth (1971), and Leslie and Summerell (Leslie et al. 2006). In 2008 and 2009, morphological identification of representative isolates was confirmed by PCR assay and sequencing of

the translation elongation factor alpha 1 (TEF-1 α) region (Punja et al. 2007), followed by comparison of the sequences with TEF-1 α sequences of *Fusarium* available in GenBank and the Fusarium ID database (Geiser et al. 2004). Prevalence of each pathogenic fungal species was calculated as the percentage of the total number of fields from which each pathogen were isolated. Percent frequency distribution of each *Fusarium* spp. was calculated as the percentage of the total number of *Fusarium* spp. isolated in each year.

Plant material and inoculum preparation

Root rot susceptible pea cultivar DS Admiral was used in the pathogenicity and virulence assays conducted in this study. Seeds were surface-sterilized as mentioned above for root sterilizations, and pre-germinated on moist filter paper in petri dishes for 24 h. Inoculum for each *Fusarium* isolate was prepared by infesting pre-sterilized (at 121 °C, 1.05 kg/cm^2 (15 psi) for 45 min) sand-cornmeal mixture (45 g sand, 5 g cornmeal and 10 ml distilled water) in a 250 ml conical flask with three 5 mm plugs of 10 days old cultures grown on half-strength PDA incubated at 23 °C with a 12 h photoperiod/day. The flasks were incubated under the conditions mentioned above for 7 to 10 days to allow complete colonization of the sand-cornmeal mixture. Sand-cornmeal mixture infested with three pathogenic isolates of *F. solani* f. sp. *pisi* (Grünwald et al. 2003), and PDA plugs served as positive control and non-inoculated control treatments, respectively.

Pathogenicity tests

To determine the pathogenicity of the various *Fusarium* spp. recovered from symptomatic field pea roots, pathogenicity tests were conducted using a sand-cornmeal-inoculum-layer method (Bilgi et al. 2008) using two randomly selected isolates of each of nine *Fusarium* species isolated from field pea roots in this survey. Briefly, 266 ml plastic drinking cups, each with three small holes (1.5 mm ø) at the base to facilitate water drainage, were each filled with 15 g of sterilized premium grade coarse vermiculite (Sun Gro Horticulture, Bellevue, WA), followed by a 15 g layer of inoculum prepared as described above, and covered with 8 g of vermiculite. Three pre-germinated seeds of cv. DS Admiral were placed on this layer, and were then covered with another 8 g of vermiculite. Each cup was watered with 80 ml of sterilized tap water every other day and grown for 10 days in a greenhouse with a 14 h photoperiod/day and a 21/18 °C diurnal temperature regime. The experiment was laid out in a nested randomized complete block design with four replications, with one cup of three seedlings considered a replication, and the experiment was performed twice. Each plant was rated for root rot severity 10 days after planting. Disease severity/plant was calculated as the percentage of root length covered with lesions (length of lesions/total root length × 100 %) and averaged for the whole replication.

Virulence of F. avenaceum isolates

Variation in virulence of 17 randomly selected isolates of the most prevalent *Fusarium* species, *F. avenaceum,* was determined in a growth chamber experiment with alternating cycles of 14 h light, 10 h dark, with day and night temperatures of 21 and 18 °C, respectively. The experiment was a completely randomized design (CRD) with four replications per isolate tested using the sand-cornmeal-inoculum-layer method as previously described. Positive control, non-inoculated control treatments, and disease severity assessments were essentially the same as described previously. The experiment was performed twice. *Fusarium avenaceum* isolated were categorized into three severity classes according to Feng et al. (2010).

Statistical analyses

Data were analyzed statistically using SAS version 9.2 (SAS Institute Inc., Cary, NC). Homogeneity of variance between repeated experiments was tested using Levene's test. Experiments were combined and analyzed using GLM procedure, when the error variances between experiments were homogeneous and there was no significant experiment x treatment interaction. Means separation was performed using Fisher's protected least significant difference (LSD) test at P=0.05.

Results

Field surveys, isolation, and identification of root rot pathogens

The survey involved sampling more than 2900 roots from 203 fields, across 15 counties in North Dakota.

Table 1 Number of fields, mean root rot incidence (%), and mean root rot severity (%) observed during surveys of pea fields conducted in 2004, 2005, 2008, and 2009 in North Dakota

County	2004			2005			2008			2009		
	# of fields	Incidence[d]	Severity[e]	# of fields	Incidence	Severity	# of fields	Incidence	Severity	# of fields	Incidence	Severity
Benson	–[a]	–	–	1	10	0.7	–	–	–	–	–	–
Bottineau	4	28.8 (10–45)[b]	2.9 (0.6–5.8)[c]	4	10 (0–25)	1.3 (0.0–2.7)	7	38.6 (10–100)	10.9 (1.7–19.0)	1	6.0	25.2
Burke	5	18 (10–25)	2.7 (0.4–4.4)	3	6.7 (0–15)	0.5 (0.0–1.2)	–	–	–	–	–	–
Cass	–	–	–	–	–	–	3	0	0	–	–	–
Divide	12	12.5 (0–40)	0.8 (0.0–4.5)	10	11.5 (0–20)	0.8 (0.0–2.2)	–	–	–	3	66.7 (50–90)	22.9 (19.6–25.2)
Foster	–	–	–	–	–	–	1	40	4	1	50	16.64
Hettinger	3	26.7 (20–30)	2.5 (1.9–2.9)	3	13.3 (0–20)	1.1 (0.0–2.6)	2	55 (50–60)	16.5 (15.0–18.0)	–	–	–
McKenzie	1	50	5.44	5	7 (0–10)	0.38 (0.0–0.7)	–	–	–	–	–	–
McLean	6	22.5 (10–45)	2.1 (0.4–5.3)	–	–	–	15	7.3 (0–30)	4.7 (0.0–28.3)	11	61.8 (20–100)	29.8 (3.5–57.4)
Mountrail	4	23.7 (10–35)	2.1 (0.3–3.8)	–	–	–	2	40 (0.0–80)	7.7 (0.0–15.3)	4	42.5 (20–70)	13.3 (5.3–21.1)
Ramsey	–	–	–	1	25	3.4	–	–	–	–	–	–
Renville	3	13.3 (10–20)	0.7 (0.3–1.2)	4	2.5 (0–5)	0.1 (0.0–0.2)	7	17.1 (0–40)	6.9 (0.0–20.0)	–	–	–
Sheridan	–	–	–	–	–	–	2	15 (0–30)	2.5 (0.0–5.0)	–	–	–
Stutsman	–	–	–	–	–	–	1	60	11	–	–	–
Ward	3	26.7 (20–30)	0.8 (0.5–1.2)	4	6.3 (0.0–15)	0.6 (0.0–1.7)	25	26 (0.0–100)	9.7 (0.0–52.0)	10	52 (20–80)	18.8 (4.1–33.7)
Williams	6	12.5 (5–25)	1.2 (0.2–2.6)	6	14.2 (5–20)	1.4 (0.26–2.5)	12	29.2 (0.0–100)	9.1 (0.0–38.0)	8	57.5 (20–90)	20.6 (3.8–32.1)
Total	47	19.4	1.7	41	9.9	0.9	77	24	8.1	38	56.3	22.1

[a] Indicates pea fields were not surveyed or samples not collected from a county

[b] Numbers in parenthesis indicate the range of root rot incidence recorded in individual fields within a county

[c] Numbers in parenthesis indicate the range of root rot severity recorded in individual fields within a county

[d] Mean root rot incidence per field was calculated as the percentage of plants with symptomatic roots based on the total number of plants rated in a field. Twenty plants were collected from each field in 2004–2005 surveys, and 10 plants were collected from each field during 2008–2009 surveys

[e] Root rot severity/plant was measured as the percentage of root length with root rot lesions and then averaged per whole field

Plant samples were collected from 47 to 41 fields in a total of 10 counties in 2004 and 2005, respectively. In 2008 and 2009, plant samples were collected from a total of 77 fields in 11 counties and 38 fields in seven counties, respectively (Table 1). Infected roots were found in all the counties surveyed (Table 1). In 2004, mean root rot incidence and severity were 19.4 and 1.7 %, respectively, while in 2005, the mean root rot incidence and severity were 9.9 and 0.9 %, respectively. Mean root rot incidence in individual fields ranged from 0 to 50 % and 0 to 25 % in 2004 and 2005, respectively, while severity ranged from 0 to 5.8 % and 0 to 3.4 % in 2004 and 2005, respectively. In 2008, root rot incidence and severity ranged from 0 to 100 % and 4.0 to 52 %, respectively in individual fields with mean root rot incidence and severity of 24 and 8.1 %, respectively. Greater root rot incidence (56.3 %) and severity (22.1 %) were observed in 2009 compared to previous three years, with root rot incidence and severity in individual fields ranging from 20 to 100 % and 3.5 to 57.4 %, respectively (Table 1).

Fusarium was the most commonly isolated fungal genus in the 2004 to 2005 survey (Gregoire and Bradley 2005), when the preliminary study was initiated, and *F. avenaceum* was the predominant among the *Fusarium* spp. isolated (*data not shown*). In 2008, pathogen isolations from a subset of 39 of the total 77 fields surveyed revealed *F. avenaceum* to be the most prevalent species, found in 71.8 % of the fields (Fig. 1a). *Fusarium oxysporum*, the next most frequently isolated *Fusarium*

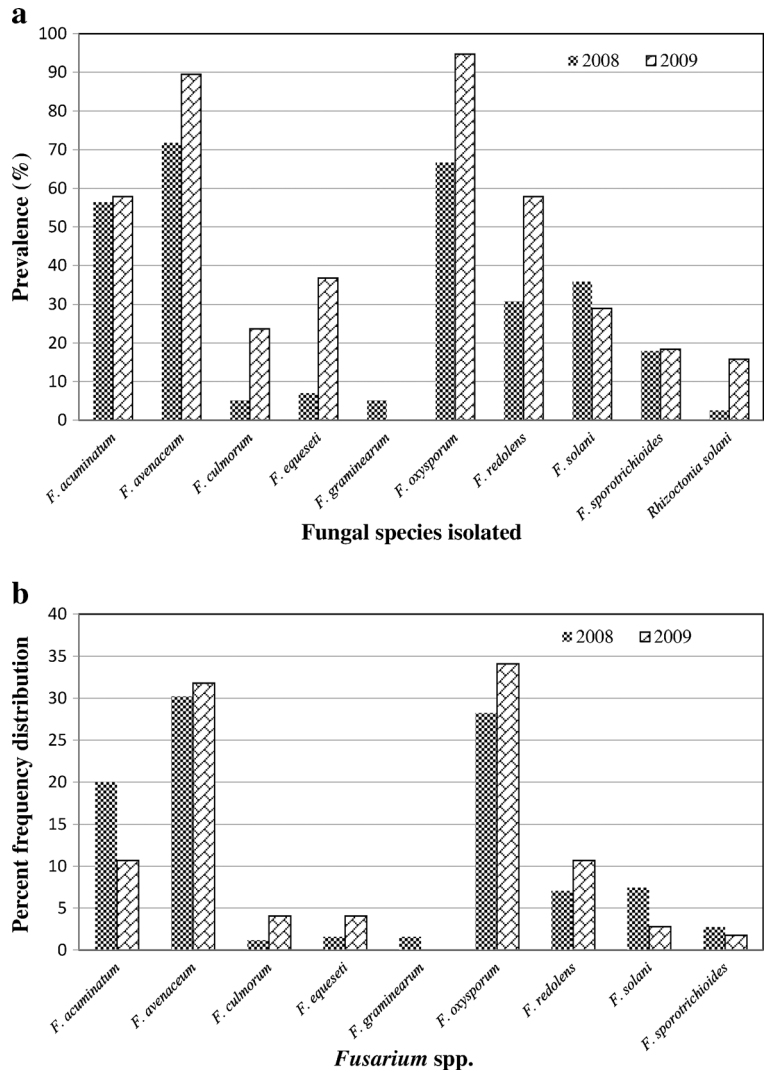

Fig. 1 Prevalence of pathogenic fungal species (**a**) and percentage frequency distribution of various *Fusarium* spp. (**b**) isolated from symptomatic field pea roots in 2008 and 2009 in North Dakota. Isolations were conducted from roots sampled from 39 fields in 2008, and from 38 fields in 2009. Prevalence was calculated as the percentage of the total number of fields from which each pathogen was isolated. '*F*'=*Fusarium* for the genus of the first nine fungal species listed. Percentage frequency distribution of each *Fusarium* spp. is calculated as the percentage of total *Fusarium* spp. isolated in 2008 (n=280) and 2009 (n=408)

species from symptomatic roots was isolated from 66.7 % of the fields. Additionally, *F. acuminatum* (56.4 %), *F. solani* (35.9 %), *F. redolens* (30.8 %), *F. sporotrichioides* (17.9 %), *F. equiseti* (7.0 %), *F. culmorum* (5.1 %), and *F. graminearum* (5.1 %) were isolated at varying frequencies (Fig. 1a). Of the total 280 *Fusarium* spp. isolated in 2008, *F. avenaceum* (30.2 %) and *F. oxysporum* (28.2 %) constituted nearly 60 % (Fig. 1b). Other *Fusarium* spp. *viz., F. acumatum* (20 %), *F. solani* (7.5 %), *F. redolens* (7.1 %), *F. sporotrichioides* (2.7 %), *F. equiseti* (1.6 %), *F. graminearum* (1.6 %), and *F. culmorum* (1.2 %) constituted the remaining *Fusarium* spp. isolated (Fig. 1b). Similar to 2008, *Fusarium* species were the most frequently isolated fungi from affected pea roots in fields surveyed in 2009, with *F. oxysporum* and *F. avenaceum* isolated from 94.4 to 89.5 % of the fields surveyed, respectively. *F. acuminatum* (57.9 %), *F. redolens* (57.9 %), *F. equiseti* (36.8 %), *F. solani* (28.9 %), *F. culmorum* (23.7 %), and *F. sporotrichioides* (18.4 %) were also isolated from fields surveyed in 2009 (Fig. 1a). *Rhizoctonia solani,* was also present in 2.6 % (2008) and 15.8 % of the fields surveyed in the state in 2008 and 2009, respectively (Mathew et al. 2012; Fig. 1a). A total of 408 *Fusarium* spp. were isolated in 2009, of which *F. oxysporumum* (34.1 %) and *F. avenaceum* (31.8 %) constituted approximately 65 % (Fig. 1b). Other *Fusarium* spp. *viz., F. acuminatum* (10.7 %), *F. redolens* (10.7 %), *F. equiseti* (4.1 %), *F. culmorum* (4.1 %), *F. solani* (2.8 %), and *F. sporotrichioides* (1.8 %) were isolated in varying frequency (Fig. 1b).

Pathogenicity tests

Tests for homogeneity of variance (P=0.8780), and experiment x treatment interaction (P=0.9999) were not significant, so data for the two experiments were combined for analysis. Pathogenicity tests showed that all the *Fusarium* species isolated from symptomatic roots were capable of causing root rot on the field pea cv. DS Admiral. The lesions on roots varied from dark brown to black, and rotted tissue was observed. Significant differences (P<0.0001) in severity of root rot symptoms were observed among isolates of each *Fusarium* species, and among *Fusarium* species (Fig. 2). Except for *F. oxysporum, F. redolens,* and *F. sporotrichioides*,

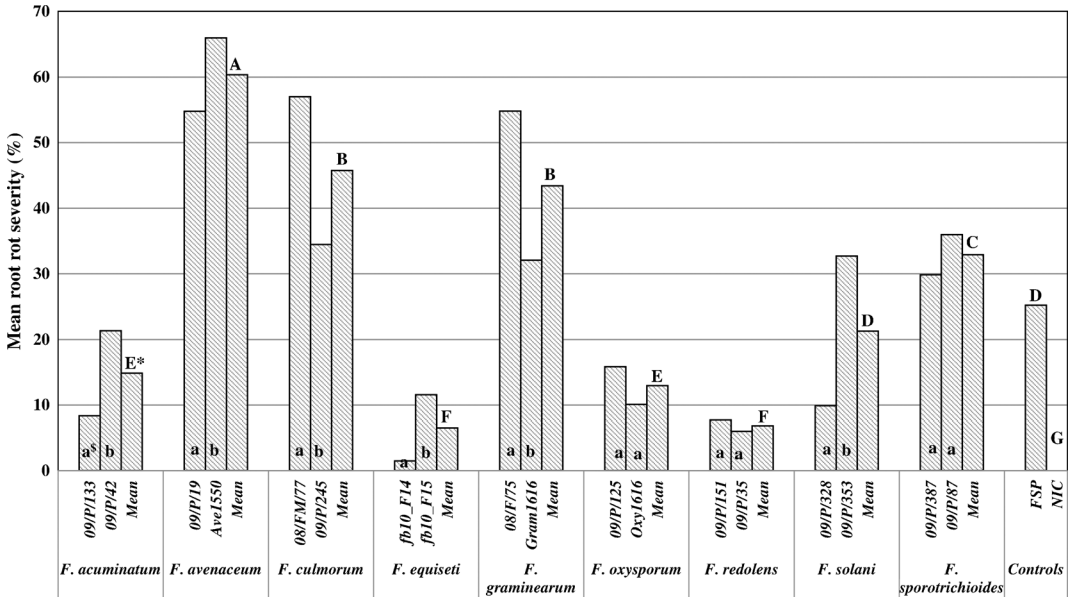

Fig. 2 Mean percent root rot severity of the field pea cv. DS Admiral caused by isolates of *Fusarium* species isolated from roots showing root rot symptoms collected during a survey of fields in North Dakota. Each bar represents mean percent disease severity measured 10 days after planting using a sand-corn meal-inoculum layer method. Disease severity was measured as percentage of root area covered by root rot lesions. Two isolates per each *Fusarium* species tested were used. Mixture of three isolates of *F. solani* f. sp. *pisi* were used as positive control (FSP) and sand-cornmeal mixture was used as non-inoculated control (NIC). *Same capital letters outside the bar indicate no significant difference (P=0.05) in mean root rot severity between different *Fusarium* species. $^{\$}$Same small letters inside the bar indicate no significant difference (P=0.05) in root rot severity between different isolates within the same *Fusarium* species

differences in root rot severity were significant between two isolates of each *Fusarium* species. Mean root rot severity caused by the nine *Fusarium* species varied from 6.5 to 60.3 %. Among the nine *Fusarium* species tested, *F. avenaceum* caused the most severe root rot (mean of 60.3 %), followed by *F. culmorum* (45.7 %), *F. graminearum* (43.42 %), and *F. sporotrichioides* (32.9 %). Isolates of these four *Fusarium* species were found to be more virulent than the *F. solani* f. sp. *pisi* (25.2 %) positive control. *Fusarium solani* (21.3 %) isolates recovered from pea roots in this survey were as virulent as the control isolate, while *F. acuminatum* (14.8 %) and *F. oxysporum* (12.9 %) were less virulent than the control isolate. *F. equiseti* (6.5 %), and *F. redolens* (6.8 %) were found to be weakly pathogenic on field pea roots.

Virulence of F. avenaceum isolates

Tests for homogeneity of variance (P=0.8061), and experiment x treatment interaction (P=0.9999) were not significant, so the two repeats of the experiments testing virulence of *F. avenaceum* isolates were combined for analysis. While the non-inoculated controls were free of any significant symptoms, all of the 17 *F. avenaceum* isolates were pathogenic on pea roots. Significant differences in virulence among the *F. avenaceum* isolates (P<0.0001) were observed (Table 2). Disease severity ranged from 6.3 to 88.7 %. Four *F. avenaceum* isolates (FA 0601, Pea 41, FPS M 60, and Ave 1614) were more virulent than the *F. solani* f. sp. *pisi* control isolate (56.6 %), resulting in 88.7, 87.9, 86.6 and 64.1 % of root rot, respectively. One isolate, PLE 1b SI (53.7 %) was as virulent as the *F. solani* f. sp. *pisi* control isolate, and 12 isolates were less virulent than the *F. solani* f. sp. *pisi* isolate.

Discussion

This study demonstrated that root rots of field pea were found in all counties surveyed in North Dakota over the four years of this survey. As many as 10 species of plant pathogens were isolated from infected roots, and *F. avenaceum* and *F. oxysporum* isolates were the most commonly detected. *F. avenaceum* isolates were also determined to be the most virulent, although variation in virulence among these isolates was observed.

This study not only illustrated the wide-spread nature of root rots in field pea crops in North Dakota, the largest producer of this crop in the USA, but also established, for the first time that *F. avenaceum* can be a major root rot pathogen in field pea in this area. This is in agreement with findings from Saskatchewan (Fernandez 2007) and Alberta (Feng et al. 2010) in Canada, and Sweden (Persson et al. 1997), where isolation of *F. avenaceum* ranged from 41 to 80 %. These findings are in contrast with earlier reports in which

Table 2 Variation in mean percent root rot severity among 17 isolates of *Fusarium avenaceum* obtained from field pea roots in North Dakota showing root rot symptoms during two-year surveys conducted in each of 2004–05 and 2008–09

F. avenaceum isolate	Mean disease severity (%)[a]	Severity Class[b]
FA 0601	88.7 a*	High
Pea 41	87.9 a	High
FPS M 60	86.6 a	High
Ave 1614	64.1 b	Moderate
PLE 1b SI	53.7 cd	Moderate
Ave 1550	51.1 de	Moderate
CTC 8G	49.1 ef	Moderate
CTC 6c	46.7 f	Moderate
FA 0602	46.6 f	Moderate
Pea 47 (e)	42.5 g	Moderate
CTC 6b	40.9 g	Moderate
Pea 9 (b)	29.8 h	Weak
Trt 6 NB (II)	26.7 hi	Weak
FA 0606	24.4 i	Weak
FA 0604	10.0 j	Weak
Pea 47	6.5 k	Weak
Pea 47 (a)	6.3 k	Weak
F.solani f. sp. *pisi*	56.6 c	

*Means with the same letter are not significantly different (α=0.05) according Fisher's least significant difference test (LSD=3.3)

[a] Mean root rot severity was calculated as the percentage of root length covered by lesions, measured 10 days after planting. Means are average of eight replications. Each replication consisted of three seedlings. Virulence assays were conducted following a sand-cornmeal-layer method in growth chamber conditions with 14 h light photoperiod/day, and 21/18 °C, day/night temperature regime. The experiment was completely randomized design (CRD) with four replications in each experiment and the whole experiment was performed twice

[b] Severity classes, 81 to 100 % highly virulent; 30 to 80 % moderately virulent; 0 to 30 % weakly virulent

F. solani f. sp. *pisi* was found to be the major root rot pathogen of field pea (Kraft and Pfleger 2001). *Fusarium avenaceum*, together with other *Fusarium* species can be associated with foot and root rot, crown rot, or Fusarium head blight (FHB) diseases of cereals (Fernandez 2007; Yli-Mattila et al. 2002), and seedling blight in canola (Calman et al. 1986), two crops commonly rotated with field pea. As suggested by Feng et al. (2010), lack of host specificity and rotation with other host crops could be attributed to the greater frequency of isolation of *F. avenaceum* from field pea than *F. solani* f. sp. *pisi*.

The pathogenicity tests in this study showed that *F. avenaceum* caused the most severe symptoms on the pea cv. DS Admiral among the *Fusarium* spp. isolated from symptomatic pea roots in North Dakota, which is similar to findings reported by Persson et al. (1997). This study also demonstrated significant variation in root rot severity among *F. avenaceum* isolates. Similar differences in severity of symptoms among 75 *F. avenaceum* isolates were observed by Feng et al. (2010), who grouped their isolates into three severity classes, *viz.,* weakly aggressive, moderately aggressive, and highly aggressive, which corresponded to 0–30 %, 31–80 %, and 80–100 % root discoloration, respectively. Using these severity classes, the 17 *F. avenaceum* isolates evaluated in this study could be classified into highly aggressive (three isolates), moderately aggressive (8 isolates), and weakly aggressive (six isolates) classes.

Fusarium oxysporum also was isolated frequently from infected pea roots in this North Dakota survey. This species is ubiquitous and is considered to be associated primarily with wilts of various crops (Gordon and Martyn 1997). However, reports from Europe and Canada have established *F. oxysporum* as a causal agent of pea root rot along with other *Fusarium* spp. (Hwang and Chang 1989; Persson et al. 1997; Skovgaard et al. 2002). In contrast, a study conducted in Southern Scandinavia (Persson et al. 1997) found *F. oxysporum* to cause severe root rot in pathogenicity tests. The *F. oxysporum* isolated in this study was found to be weak a pathogen of pea. This could be attributed to variation in aggressiveness among *F. oxysporum* isolates (Skovgaard et al. 2002). None of the field pea plants evaluated in this study showed symptoms of wilt and isolations were strictly conducted on roots. The pathogenicity test results suggest that although *F. oxysporum* was isolated commonly in both 2008 and 2009 in North Dakota pea fields, isolates of this species may not be as effective at causing significant root rot alone. These isolates could potentially be present as a part of a root rot complex however, and serve as a contributor to disease severity. Results from these surveys and those from different parts of the world demonstrated the prevalence of *Fusarium* species like *F. avenaceum* associated with pea root rots. These findings highlight the need for identifying sources of resistance to *F. avenaceum* and, possibly, re-evaluating existing sources of resistance identified based on reactions to *F. solani* f. sp. *pisi*, before incorporating the resistance into breeding programs aimed at developing varieties for the central plains region of the USA. This study highlights the prevalence of *F. avenaceum* on dry field pea, and the ability of these isolates to cause severe root rot, and the possibility of this pathogen emerging as a potential risk for pulse crops.

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