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Identification and characterization of pathogens associated with root rot of winter peas grown under organic management in Germany

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Abstract Root rots are limiting factor for pea production worldwide. This disease is caused by a pathogen complex and the role of single pathogens is unclear. This study aimed at identifying pathogens involved in a root rot of organically grown field pea in Germany, and establishing their importance in the disease complex. The potential of yard waste compost to suppress the diseased was also studied. Average disease severity index was similar in 2010 and 2011 (DI of 4.56 to 4.59, respectively) but it increased in 2012 to DI 5.8. *Peyronellaea pinodella* was most frequently isolated pathogen, with isolation frequency from 86%, 73% and 86% in 2010, 2011 and 2012, respectively. In addition, *Didymella pinodes*, *Fusarium solani* f. sp. *pisi*, *F. oxysporum* f. sp. *pisi* and *F. avenaceum* were the main fungi recovered from pea roots. In pathogenicity test all of the tested pathogens caused weak symptoms on the pigmented winter variety EFB33 and moderate to severe symptoms on the white flowering summer variety Santana. *F. avenaceum* was the most aggressive pathogen on Santana with DI of 7.4 followed by *P. pinodella* with DI of 5.7. The high aggressiveness combined with the wide host range highlights the possibility of *F. avenaceum* emerging as potential risk for organic crop rotation. High levels of resistance of EFB33 against all pathogens shows the potential of this variety to serve as a resource in further research for identification and development of new sources of resistance against root rot diseases of pea.

Keywords Root rot · Pea · *Fusarium* · *Peyronellaea pinodella* · Pathogenicity · Organic agriculture

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Introduction

Field pea (*Pisum sativum* L.) is after soybeans the most produced grain legume in Europe, with a production area of about 3.7 million ha in 2014 (FAOSTAT data, 2014; http://faostat.fao.org/). Pea is one of the most common grain legumes grown in Germany. However, despite its importance, especially in organic agriculture, production is declining (Sass 2009; Urbatzka et al. 2011). Soil-borne pathogens causing root and foot rot disease are considered to be the most important biotic limiting factor worldwide (Ali et al. 1993; Bretag and Ramsey 2001; Gaurilckiene and Cesnuleviciene 2013; Oyarzun 1994). Root rot affected plants exhibit poor growth and root symptoms, including brown to black lesions, especially on the upper part of the main root. A complex of more than 20 different species of soil-borne fungal pathogens have been associated with foot and root rot of pea and many of them often infect a wide range of other legumes such as chickpea, lentil, soybean, faba bean and lupin (Arias Diaz et al. 2013a, b; Hwang et al.

1994; Kraft 1994; Persson et al. 1997). The most important species of the complex are *Didymella pinodes* (syn *Mycospharella pinodes*), *Peyronellaea pinodella* (syn *Phoma pinodella), Fusarium solani* f. sp. *pisi*, *F. oxysporum* f. sp. *pisi*, *F. avenaceum*, *Pythium spp.* and *Aphanomyces euteiches* (Feng et al. 2010; Nasir et al. 1992; Oyarzun 1993; Xue et al. 1997). Usually, several of these pathogens are occurring simultaneously on diseased plants and the role of single pathogens in disease development and severity is not clear. Their prevalence, dominance and importance in the complex vary greatly depending on location, climate, and agricultural practices (Gaurilckiene and Cesnuleviciene 2013; Jensen et al. 2004; Persson et al. 1997) and some shifts have occurred over time. Recent reports indicate declining importance of *F. solani* and increasing presence and significance of *F. avenaceum* (Chittem et al. 2015; Esmaeili Taheri et al. 2016; Feng et al. 2010; Fernandez et al. 2008; Fernandez 2007; Pflughöft et al. 2012). *Fusarium avenaceum* is a pathogen with a wide host range and, in addition to pea, it have been associated with root diseases of faba bean, soybean, lentil, canola, potatoes and a range of different cereals (Arias Diaz et al. 2013a, b; Chang et al. 2014; Chen et al. 2014; Hwang et al. 1994).

One of the potential means to control soil-borne diseases in organic agriculture is application of compost. Compost provides plant nutrients, and improves physical properties of soil and introduces or enhances communities of beneficial microorganisms which contribute to soil health and disease suppression (Boutler et al. 2000; Dick and Gregorich 2004; Hoitink et al. 1997). Its success in disease suppression depends strongly on the type of raw material, composting procedure and maturity (Hoitink and Fahy 1986) and inconsistency in effectiveness have often been reported (Hoitink et al. 1997; Litterick et al. 2004; Noble and Coventry 2005).

In order to increase knowledge on diversity, frequency and pathogenicity of pathogens associated with pea root rots as well as the potential of compost to suppress foot and root rot of pea, field and greenhouse experiments were conducted at the organically managed farm at the Kassel University. The objectives of the study were to: (i) identify pathogens involved in foot and root rot of pea, (ii) assess the effect of compost application in the field on disease severity and incidence of *Didymella pinodes*, *Peyronellaea pinodella, Fusarium solani* f. sp. *pisi*, *F. oxysporum* f. sp. *pisi* and *F. avenaceum*, and (iii) determine the aggressiveness of the pathogens isolated from symptomatic roots of field grown pea plants in the greenhouse trial.

Material and methods

Field experiment

Field experiments were carried out from October 2009 to May 2012 at the experimental farm of the University of Kassel in Neu Eichenberg (51°22' N.L. and 9°54′ E.L, and 247 m ASL). The soil type is classified as a deep Haplic Luvisol, long-term annual mean temperature is 7.9 °C and mean annual precipitation is 619 mm. The experimental farm has been managed organically since 1984.

Experiments took place within a crop rotation of six years consisting of two years grass-clover followed by winter wheat, a cover crop of winter pea (cv. EFB33), maize, winter wheat, and spring cereal. Each pea main plot was divided into four subplots (7.5 × 20 m). One day prior to sowing, a total of 5 t of dry matter ha^{-1} yard waste composts (Table 1) was applied by hand to two of the subplots. To ensure disease development two of the subplots were inoculated with laboratory prepared inoculum of *Peyronellaea pinodella,* resulting in a factorial set-up with four treatments: Control **(0),** Inoculated with *P. pinodella* **(I),** Compost **(C)** and Inoculated with *P. pinodella* + Compost **(I + C),** with four replications. Seeding density of 80 seeds m^{-2} in 2010 was increased to 120 seeds m^{-2} in 2011 and 2012.

Inoculum of *P. pinodella* was prepared on broken oat grains. Approximately 40 kg of grains were soaked in water for 24 h, autoclaved at 121 °C for 15 min, and 24 h later a second time. Agar plugs, 1 cm in diameter, of fungal cultures from two fully covered Petri dishes were added and incubated in a covered plastic box for two weeks at room temperature. During this time, the material was regularly stirred by hand to ensure homogenous fungal growth and prevent anaerobic conditions. Inoculation in the field was done by hand broadcasting approximately 4 kg of inoculum per plot at the time of compost application.

Sampling, pathogen isolation and identification

The first sampling of pea plants was at BBCH stage 15/17, i.e. with five to seven fully developed leaves (middle to end March) and the second at the beginning of

Table 1 Chemical characteristic of composts used in the field from 2009 to 2012

Compost	Dry matter content (%)	Bulk density (g/L)	pH	EC (μS/cm)	K (mg/kg)	P (mg/kg)	Total N (%)	Total C (%)	C/N ratio	N_{min}
YWC 1	67	408	6.9	533	3397	477	1.18	26.9	22.9	0
YWC 2	95	375	6.6	758	5347	541	1.09	12.5	11.4	0
YWC 3	63	339	6.6	617	4598	637	1.31	35.5	27.1	0

flowering, BBCH 59–61 (middle to end of May). Twenty pea plants together with roots were taken following a "W" sampling route throughout each plot and taken to the laboratory for assessment and isolation of pathogens. Root and foot rot on pea was assessed using the scale 1 to 9, where score 1 = healthy plant and 9 = dead plant (Pflughöft et al. 2012) (Table 2). A Disease index (DI) was calculated as the mean of external and internal tissue score.

For pathogen isolations plants were surface disinfected by placing them in 3% NaClO for 10 s. Afterwards they were thoroughly rinsed under running distilled water and placed on filter paper under a laminar flow hood for 1 h to dry. From the stem base and root of each plant three 1 cm pieces were cut, placed on Coon's agar media (Maltose 4 g, KNO_3 2 g, $MgSO_4$ 1.20 g, KH_2PO_4 2.68 g, Agar 20 g, H_2O 1 L) and incubated for a week under black-light blue fluorescent light (F40 BLB; range 315–400 nm with the peak at 365 nm) at 23 ± 3 °C. Isolation frequency of the most commonly isolated fungi associated with roots of pea from all experimental treatments in each experimental year was defined as the percentage of all roots that yielded a given species.

After isolation, pathogenic fungi belonging to *Fusarium* spp. and the Ascochyta complex, i.e. *Ascochyta pisi, Peyronellaea pinodella, Didymella pinodes*, were identified to the species level. For that purpose the one-week old mycelium from the original Coon's plate was subcultured and incubated again under the same conditions. Subculturing was done on half strength Potato Dextrose Agar (PDA), for colony formation, and on Synthetic Nutrient Agar (SNA) medium for the sporulation of *Fusarium* spp. (KH_2PO_4 1 g, KNO_3 1 g, $MgSO_4$•$7H_2O$ 0.5 g, KCl 0.5 g, Glucose 0.2 g, Sucrose 0.2 g, Agar 20 g, H_2O 1 L), whereas for the Ascochyta complex pathogens Coon's agar was used again. After 10 to 14 days pathogens were identified microscopically based on colony and spore characteristics (Leslie and Summerell 2006; Nasir et al. 1992).

The identity of selected representative isolates of *Fusarium* spp. was molecularly confirmed in the

Table 2 Scoring scheme for assessment of root and foot rot of peas (Pflughöft, 2008)

Score	State of plant external tissues at root and stem	State of plant internal tissues at root and stem by cutting through the lesion
1	No visible symptoms	No visible symptoms
2	Streaks at the transition zone or at epicotyl or hypocotyl (not the discoloration of the seed and seed remaining attached)	Epidermis/rhizodermis is brownish to black
3	Brownish lesion covers <50% perimeter	Brownish discoloration on cortical tissues
4	Brownish-black lesion covers >50% perimeter	Cortical tissues partially black, but center and endodermis are still brownish or healthy
5	Black lesion covers 100% of stem	Cortex tissue is completely black
6	Black color and intensive spread of lesion	Cortex tissue begins to rot (bursting of epicotyl or rhizodermis on the root)
7	Lesions spread up to the first lower leaf or <3 cm on the tap root	Cortex tissue is completely rotten
8	Lesions spread above the first lower leaf and/or >3 cm on the tap root	Shedding of the cortex tissue of endodermis
9	Dead plant	Dead plant

Karlovsky laboratory of the Georg-August University in Göttingen, following methods previously described by Dastjerdi (2014). The identity of *Peyronellaea pinodella* and related spp. was confirmed by amplifying and sequencing a portion of the β-tubulin (tub2) gene region using primer pairs TUB2Fd and TUB2Fd (Woudenberg et al. 2009) and method as described in Chen et al. (2015).

Aggressiveness test

To compare the aggressiveness of the most common species isolated from pea roots, five isolates of each *Fusarium solani* (Fs), *Fusarium avenaceum* (Fa), *Peyronellaea pinodella* (Pp) and *Didymella pinodes* (Dp) were tested on two pea varieties - the white flowering summer variety Santana (KWS Lochow, Gmbh) and the winter variety EFB33 with pigmented flowers.

Inoculum preparation and inoculation

All of the tested isolates originated from pea plants with exception of *F. avenaceum* isolates Fa1 and Fa2 that were isolated from wheat, and Fa5 isolated from subterranean clover seeds. *Didymella pinodes* and *P. pinodella* isolates were grown in Petri dishes containing Coon's agar while *F. solani* and *F. avenaceum* cultures were grown on half strength PDA. Petri dishes were incubated at 20 °C and subjected to 12 h cycles of BLB and 12 h darkness for 15 to 20 days. Inoculation was done with spore suspensions at the time of sowing. Spores were prepared by flooding the surface of cultures with distilled water and gently scraping them with a disposable cell spreader. The concentrations of spores were determined with a Fuchs Rosenthal haemocytometer and adjusted to the concentration of 10^5 spores g^{-1} of growing substrate by adding sterilized distilled water.

Experimental set-up

Pea seeds were surface disinfected with 70% ethanol for five minutes, rinsed with running distilled water and air dried under a laminar flow cabinet. Plants were grown in 6 cm diameter and 8 cm deep plastic pots filled with 150 mL (~200 g) of sterilized yellow sand or non-sterilized soil taken from the field experiment. Four seeds were sown in each pot at 2 cm depth. Directly after sowing, prepared spore suspensions were added. Treatments with sand substrate were fertilized with a total of 100 mg of N L^{-1} substrate of complex N:P:K fertilizer Wuxal® Super (8:8:6 + microelements), divided in two equal applications 10 and 15 days after sowing. No fertilizer was used for plants grown in soil. The experiment was arranged in a completely randomized design with five replications. Plants were grown for 21 days with a day/night air temperature regime of 19/16 °C and watered daily with approx. 15 mL of tap water.

Harvest and disease assessment

Plants were harvested 21 days after sowing. Roots were washed with running tap water and disease severity was assessed according to the key of Pflughöft et al. (2012) (Table 2). Plant shoots were weighted directly after harvest and dry weights were measured after oven drying at 105 °C until constant weight. In order to follow Koch's postulates each pathogen was re-isolated from two inoculated, symptomatic plants per replication following the same isolation procedure as described for the field experiment.

Data analysis

Statistical analyses were conducted using R statistical software (version 3.2.2, R Core Team 2013).

In the field experiment, differences in disease index (DI) among plants sampled in March and May were tested by using Mann-Whitney's U test. The differences in mean disease index among experimental years for samplings done in May, using Dunn's multiple comparison test.

Effects of experimental factors on the isolation frequency of the most commonly isolated fungi were tested using ANOVA. Prior to ANOVA, data were checked for normality using Shapiro-Wilks-W-Test and Levene's test was applied to test the homogeneity of variance. Mean separations were made by Tukey HSD test and treatments were considered significantly different if $P \leq 0.05$.

Results

Field experiment

Weather conditions

Overall, temperatures measured from October 2009 until September 2013 followed the thirty years trend

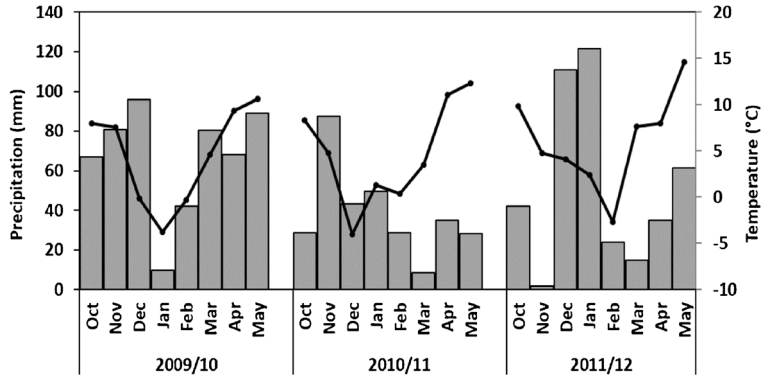

Fig. 1 Monthly mean temperatures (°C) and precipitation (mm) in the period between 2009 and 2012

(1970–2000) with a few extremes mainly in the winter months (Fig. 1). A sudden drop of the temperature in the first two weeks of February 2012 after a relatively warm December and January followed by warm and dry March resulted in an almost complete failure of the pea crop in the season 2011/12. There were thirteen icing days (daily maximum temperature below 0 °C) in February 2012, and in this period, average maximal temperature was −5.7 °C. The temperatures increased sharply starting from 15th of February and this continued in March. The mean monthly temperature of 7.5 °C in March was 3 °C higher than the long-term average and at the same time precipitation was 14.7 mm, only 26% of the thirty-year average.

Root rot severity in the field trial and relative abundance of pea root rot pathogens

The results obtained indicated high levels of the inoculum of *Peyronellaea pinodella* naturally present in the experimental field, and application of laboratory prepared inoculum did not increase the frequency of isolation of *P. pinodella* (data not shown). However, in order to follow consistent methodology, we continued to apply laboratory prepared inoculum in the second and third experimental year. Because there was no significant difference in the relative abundance of pathogens in *P. pinodella* inoculated and non-inoculated treatments, the abundance data were analyzed across all treatments.

Root rot was present in all three experimental years. No significant interactions between experimental factors were observed. Neither inoculation with *P. pinodella* nor compost application had an effect on the disease severity (Fig. 2a). Mean disease severity was similar in May 2010 and 2011 (DI 4.56 and 4.59, respectively), and slightly higher in 2012 with DI of 5.8 (Fig. 2b). As well as in 2010 as in 2011, disease was more severe at the second sampling in May compared with the sampling in March (Fig. 2b).

Peyronellaea pinodella was the most frequently isolated pathogen in all three years, isolated from 86%, 73% and 86% of the sampled plants in 2010, 2011 and 2012, respectively (Fig. 3). In addition to *P. pinodella, D. pinodes, F. solani, F. oxysporum* and *F. avenaceum* were the main fungi isolated from symptomatic pea roots. Fungi like *Ascochyta pisi*, *Alternaria* spp., *Rhizopus* spp., *F. culmorum*, *F. equiseti*, *and F. redolens* were rarely isolated from pea roots (< 2% of roots, data not shown). With the exception of *P. pinodella,* the relative abundance of fungi varied significantly among years (Fig. 3). Overall, the lowest incidence of pea root rot pathogens was in 2011. *F. avenaceum* was not found in 2010 and rarely isolated in 2011 (< 8% of roots). In 2012, following the extreme cold period, *F. avenaceum* was isolated from 40% of sampled roots.

Aggressiveness test

All of the tested pathogens were capable to cause root rot symptoms on both pea varieties in both growing substrates. On EFB33, all pathogens caused weak symptoms in both sand and soil. Disease was more severe on the susceptible variety Santana, where all pathogens caused moderate symptoms when grown in soil and moderate to severe disease symptoms in sand (Table 3). All of the experimental factors significantly affected disease severity in pea plants and interactions among all main factor combinations were significant ($P \leq 0.01$). Dry weight of plants was significantly affected by pathogen inoculation and growing substrate, and significant interactions were observed ($P \leq 0.01$)

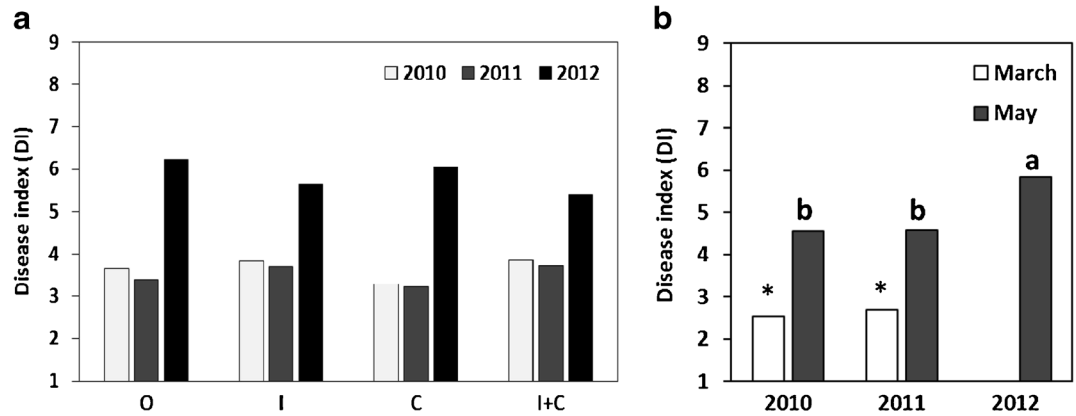

Fig. 2 Mean root rot disease severity of pea plants sampled in May 2010, 2011 and 2012 (a), where O = control, I = Inoculated with *P. pinodella*, C = compost, I + C = Inoculated with *P. pinodella* and compost applied. (b) Mean disease severity across treatments in March (2010–2011) and May (2010–2012). Different letters above bars indicate significant differences in mean disease index among experimental years for samplings done in May, using Dunn's multiple comparison test ($P \leq 0.05$). * Indicate significant differences in mean disease index between plants sampled in March and May within experimental year at $P \leq 0.05$ level using Mann-Whitney's U test (sampling in March 2012 was not possible due to winter kill)

among pathogen and variety, pathogen and substrate, as well as among all three experimental factors.

In all of the pathogen treatments and in both substrates disease indices on the variety Santana were higher than on EFB33 (Table 3). Significant differences in dry weights between varieties were observed only in treatments inoculated with *F. avenaceum* and *F. solani*. EFB33 plants inoculated with *F. avenaceum* had higher dry weights compared to Santana ($P \leq 0.01$). In contrast, in the *F. solani* treatment dry weights of Santana plants were somewhat higher than those of EFB33. Plants grown in soil had generally lower disease index compared to plants grown in sand and higher dry weights. Exception was the treatment inoculated with *D. pinodes* where no significant differences among two substrates were measured.

Fusarium avenaceum was the most aggressive pathogen on Santana grown in sand (DI 7.4), followed by *P. pinodella* (DI 5.7), *D. pinodes* (DI 4.8) and *F. solani* (DI 4.0). These effects mirrored in the effects of pathogens on the dry weights of peas. The highest reduction of dry weight was caused by *F. avenaceum*, followed by *P. pinodella* and *D. pinodes* (Table 3). Inoculation with *F. solani* did not significantly affect dry weights of Santana plants grown in sand. In soil, mean disease severities on Santana did not differ significantly among pathogens and were only significantly higher than in control plants for *F. avenaceum* and *D. pinodes*. Dry weights of plants inoculated with these two pathogens were also lowest but did not differ significantly from the control (Table 3).

Although all pathogens caused disease symptoms on variety EFB33, disease severity was low to moderate. In both substrates, the highest DI was measured in the treatment inoculated with *P. pinodella* (DI 3.6 and 2.3 in sand and soil, respectively). *F. avenaceum* was the second most aggressive pathogen in sand (DI 2.7) and *D. pinodes* (DI 2.2) in soil. *F. solani* was the least aggressive pathogen in all of the variety-substrate combinations, and only in sand, it differed significantly in root rot severity from the non-inoculated control. None of pathogens affected dry weight of EFB33 in sand except for *P. pinodella* isolate Pp1 (Table 3). In contrast, in soil *F. avenaceum* and *D. pinodes* significantly reduced dry weight compared to the control.

Among individual isolates, significant variation in aggressiveness was measured for *D. pinodes* isolates on EFB33 in both substrates and on Santana in soil (Table 3). In addition, isolates of *F. avenaceum* differed in aggressiveness on Santana grown in sand. Some variability in the effect of isolates of *P. pinodella*, *F. solani* and *F. avenaceum* on the dry weights was also observed (Table 3).

Discussion

Overall severity of root and foot rots of pea was moderate in 2010 and 2011 and severe in 2012 when pea crop failed. Among fungi isolated from the roots, *Peyronellaea pinodella, Didymella pinodes, Fusarium*

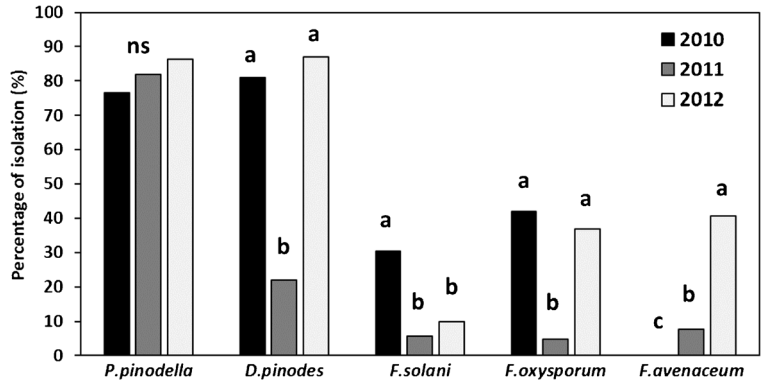

Fig. 3 Frequency of isolation (%) of fungi from pea roots in May 2010 to 2012. Different letters above bars indicate significant differences between sampling years in mean percentage of isolation for each pathogen, according to Tukey's HSD ($P \leq 0.05$)

oxysporum, F. avenaceum and *F. solani* were most commonly detected. *Peyronellaea pinodella* dominated this complex and was isolated from 82% of the sampled plants, and was together with *F. avenaceum* the most aggressive pathogen on pea in the pathogenicity test.

The dominance *of P. pinodella* and *D. pinodes* in three experimental years, together with *F. avenaceum* in 2012 are in contrast to previous findings of Oyarzun (1994) and Kraft and Pfleger (2001) where *F. solani* f. sp. *pisi* was identified as the most important pathogen of the foot rot complex. *Peyronellaea pinodella* was considered more damaging as causal agent of foliar blights rather than root rots. However, our data are in agreement with the results of Persson et al. (1997), who found that *P. pinodella* was dominating the pathogen complex in Sweden, whereas *F. solani* f. sp. *pisi* dominated in Denmark. *Peyronellaea pinodella* together with *F. redolens* and *F. avenaceum* was in the period 2005 to 2007 the most frequently isolated pathogen from roots of pea in Germany (Pflughöft et al. 2012). In Australia Tran et al. (2016) reported widespread presence of *P. pinodella* and *D. pinodes* in soils where pea had been grown up to ten years prior to tests, indicating that there may be a risk of high inoculum potential when susceptible crops like pea are grown.

Our results are in line with findings of recent studies in the midwestern region of USA and Canada where *F. avenaceum* was the most abundant pathogen associated with the roots of field pea (Chittem et al. 2015; Esmaeili Taheri et al. 2016; Feng et al. 2010). This increased abundance in the disease complex has been mainly attributed to the generalist nature and lack of host specificity of the fungus (Feng et al. 2010). Moreover, due to its genetic and ecological diversity *F. avenaceum* can occupy several ecological niches allowing selection for different fitness traits for both saprophytic and pathogenic phases of its life cycle (Holtz et al. 2011). In our study, unusually high precipitation in December 2011 and January 2012 accompanied with extremely low temperatures in the second two weeks of February has been conductive for the infection with *F. aveanceum*. The fungus was isolated in 2012 from 40% of sampled roots, compared to less than 8% in 2011. *Fusarium avenaceum* is capable of continuous growth in wet and cool soils which gives it an advantage over other soil microorganisms and other *Fusarium* spp. (Brennan et al. 2003). It can also thrive at relatively high partial pressures of carbon dioxide and reduced oxygen leveIs in wet soils (Forbes and Dickinson 1977) which we experienced in March 2012. Esmaeili Taheri et al. (2016) also observed an increase in the isolation frequency of *F. avenaceum* in a year with higher than normal precipitation.

Application of yard waste compost overall did not have an effect on the severity of foot rot of peas in our study. Litterick et al. (2004) suggested that besides compost composition, application rates in the field are usually inadequate to achieve the desired level of suppression. Bruns et al. (2013) also reports variable effects on the root rot severity of the highly susceptible pea variety Santana after the application of compost into the rows at sowing.

Regardless of the growing substrate used, EFB33 with pigmented flowers and seeds were more resistant to inoculation with all tested pathogens compared to white flowering Santana. All pathogens tested caused only weak disease symptoms on EFB33, and plant weights were only affected negatively by *F. avenaceum* and *D. pinodes* when plants were grown in the soil. This is consistent with work by Bodah et al. (2016) who reported that among 33 pea genotypes tested those with pigmented flowers were the most resistant to *Fusarium solani* f. sp. *pisi*. Weeden and Porter, (2007) suggest that

Table 3 Effects of *Fusarium avenaceum* (Fa), *F. solani* (Fs), *Peyronellaea pinodella* (Pp) and *Didymella pinodes* (Dp) on dry weights (g) and disease index (DI) of pea plants under greenhouse conditions

Pathogen/ Isolate	Santana				EFB33			
	Sand		Soil		Sand		Soil	
	Dry weight (g)	Disease index	Dry weight (g)	Disease index	Dry weight (g)	Disease index	Dry weight (g)	Disease index
Control	0.25 A[1]	1.5 E	0.27 AB	2.8 B	0.20 A	1.2 C	0.26 BC	1.6 B
Fa1	0.06 bc	7.3 a	0.23	3.9	0.17	3.4	0.22	2.2
Fa2	0.04 c	9.0 a	0.21	4.9	0.18	3.7	0.23	2.0
Fa3	0.15 a	6.7 b	0.22	2.9	0.17	3.1	0.22	1.7
Fa4	0.07 bc	7.8 ab	0.27	3.5	0.18	2.7	0.25	2.2
Fa5	0.12 ab	6.2 b	0.20	4.3	0.22	2.4	0.20	2.0
Mean Fa	0.09 C	7.4 A	0.23 B	3.9 A	0.18 A	2.7 AB	0.22 CD	2.0 AB
Fs1	0.19	3.9	0.29 ab	3.4	0.21	2.8	0.29	2.0
Fs2	0.22	3.9	0.34 a	3.6	0.19	2.4	0.28	2.3
Fs3	0.28	4.3	0.36 a	3.1	0.19	2.5	0.28	1.7
Fs4	0.23	3.6	0.33 ab	3.6	0.20	2.2	0.26	1.8
Fs5	0.23	4.3	0.21 b	4.7	0.15	2.7	0.30	1.8
Mean Fs	0.23 A	4.0 D	0.31 A	3.7 AB	0.19 A	2.5 B	0.28 AB	1.9 AB
Pp1	0.16	6.1	0.13 b	4.7	0.10 b	3.8	0.24	2.6
Pp2	0.13	6.0	0.28 ab	3.9	0.17 ab	3.9	0.30	1.9
Pp3	0.19	5.4	0.32 a	3.4	0.21 a	3.0	0.33	2.1
Pp4	0.18	5.0	0.29 a	3.6	0.22 a	3.2	0.33	2.5
Pp5	0.19	6.1	0.29 a	3.1	0.23 a	3.8	0.31	2.5
Mean Pp	0.17 B	5.7 B	0.26 AB	3.7 AB	0.19 A	3.6 A	0.30 A	2.3 A
Dp1	0.21	4.8	0.22	3.4 b	0.17	2.0 b	0.20	1.4 b
Dp2	0.17	5.0	0.19	5.6 a	0.18	2.7 ab	0.22	2.4 ab
Dp3	0.12	4.9	0.22	3.7 b	0.17	3.6 a	0.17	3.2 a
Dp4	0.15	4.9	0.24	4.4 ab	0.20	2.1 b	0.21	2.2 ab
Dp5	0.14	4.2	0.21	4.0 ab	0.16	2.2 b	0.22	1.7 ab
Mean Dp	0.16 B	4.7 C	0.21 B	4.2 A	0.17 A	2.5 B	0.21 D	2.2 AB

[1] Capital letters indicate significant difference in dry weights and DI between tested pathogens within pea variety and substrate, whereas lower case letters indicate significant difference among isolates within pathogen, variety and substrate combination according to Tukey's HSD $P \leq 0.05$

the gene A that codes for pigmented flowers might be linked to a gene or genes responsible for higher partial resistance to *F. solani* f. sp. *pisi*. In our study, all tested pathogens caused only weak disease symptoms, suggesting that resistance is not only restricted to *F. solani*, but also to the other pathogens of the root rot complex. Thus, pigmented pea varieties may be interesting plant material for further breeding studies.

Disease symptoms and weight reductions on Santana grown in sand were moderate to severe, whereas only weak effects of all tested pathogens were observed in soil substrate. Overall, the most severe symptoms where caused by *F. avenaceum* followed by *P. pinodella*. These results are in agreement with findings of Chittem et al. (2015), Feng et al. (2010), and Persson et al. (1997) who found that *F. avenaceum* isolates where highly aggressive to pea. However, these studies observed significant variation in aggressiveness among tested isolates and classified them into weakly, moderately and highly aggressive groups, whereas, in our study variations among isolates within one pea variety and one substrate was only moderate. The differences in aggressiveness of

F. avenaceum on Santana when grown in sterilized sand and non-sterilized field soil can be explained by its low competitive ability and inability to compete with fast growing soil saprophytes (Fletcher et al. 1991; Šišić et al. 2016). This resulted in lower disease severity in the soil compared to sand substrate.

Moderate disease symptoms and significant reduction of plant weights of Santana variety in sand caused by *P. pinodella* are in contrast with the findings of Persson et al. (1997) who reports that although *P. pinodella* caused disease symptoms on pea in their study, the reduction of fresh weight was only slight. Similarly to our results, Tran et al. (2016) showed that different isolates of *P. pinodella, D. pinodes,* and *Phoma koolunga*, previously described as aggressive foliar pathogens, are able to cause severe disease symptoms on pea roots. These recent findings highlight that the role of Ascochyta complex pathogens in root rot is more important than previously considered, and their contribution to the disease complex is likely to be as important as those of *Fusarium* spp.

Fusarium solani was the least aggressive pathogen in our study and did not affect adversely biomass production of inoculated plants compared with non-inoculated control. The possible reasons for these weak effects of *F. solani* could be lack of external stress and the short time for colonization of the root system by this pathogen. It is very likely that three weeks for the experiments were not long enough for the slow root colonizing fungi like *F. solani* (Huisman 1982) to infect and colonize roots of pea and cause more severe damage.

In conclusion, this study demonstrates that *P. pinodella* and *D. pinodes* are important pathogens of root rot disease complex in Germany as they were the most frequently isolated from the roots of field-grown peas, and both of them were pathogenic to pea in the greenhouse trial. The high aggressiveness combined with its wide host range highlights the possibility of *F. avenaceum* emerging as a potential risk for organic crop rotation. Lastly, high level of resistance of the pigmented variety EFB33 against all tested pathogens observed in this study, points to the potential of this variety to serve as an important resource in further research and breeding programs for identification and development of new sources of resistance against root rot disease of pea.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This work does not contain any studies with human participants or animals performed by any of the authors.

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