Piriformospora indica reduces Fusarium head blight disease severity and mycotoxin DON contamination in wheat under UK weather conditions

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Short title: *Piriformospora indica* reduces Fusarium

Piriformospora indica **reduces Fusarium head blight disease severity and mycotoxin DON contamination in wheat under UK weather conditions**

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Key words: *Piriformospora indica*, root endophytic fungus, Fusarium head blight, Fusarium crown rot, *Triticum aestivum*, mycotoxin DON

Summary

Piriformospora indica (Sebacinaceae) is a cultivable root endophytic fungus. It colonises the roots of a wide range of host plants. In many settings colonisation promotes host growth, increases yield and protects the host from fungal diseases. We evaluated the effect of *P. indica* on Fusarium head blight (FHB) disease of winter (cv. Battalion) and spring (cv. Paragon, Mulika, Zircon, Granary, KWS Willow and KWS Kilburn) wheat and consequent contamination by the mycotoxin deoxynivalenol (DON) under UK weather conditions. Interactions of *P. indica* with an arbuscular mycorrhizal fungus (*Funneliformis mosseae*), fungicide application (Aviator Xpro) and low and high fertiliser levels were considered. *P. indica* application reduced FHB disease severity and incidence by 70%. It decreased mycotoxin DON concentration of winter and spring wheat samples by 70% and 80% respectively. *P. indica* also increased above ground biomass, 1000 grain weight and total grain weight. *P. indica* reduced disease severity and increased yield in both high and low fertiliser levels. The effect of *P. indica* was compatible with *F. mosseae* and foliar fungicide application. *P. indica* did not have any effects on plant tissue nutrients. These results suggest that *P. indica* might be useful in biological control of Fusarium diseases of wheat.

Introduction

Fusarium crown rot (FCR) and head blight (FHB) are two of the most important diseases of wheat globally. The two most prevalent causal organisms are *Fusarium culmorum* and *F. graminearum* (Fernandez & Chen, 2005). *Fusarium* spp. produce a range of mycotoxins that can accumulate in the grain and, if they enter the food chain, can cause a risk to human and animal health (Xu et al., 2008). The mycotoxin deoxynivalenol (DON), which is produced during head infection, has been identified as the most frequent contaminant associated with FHB in wheat (Bai & Shaner, 2004). European Union legislation has set a legal limit for DON

of 1250 µg kg^{-1} for cereals intended for human consumption (Anon, 2006), but even low level contamination of grain can reduce market prices or cause the grain to be rejected entirely (Bai & Shaner, 2004). *Fusarium* species overwinter in soil and crop residues for several seasons. They survive as saprophytes on dead host tissues, especially if susceptible crops are planted in successive years. The most important sources of inoculum are ascospores from the sexual stage and macroconidia from the anamorph stage but chlamydospores and hyphal fragments can also act as sources of inoculum (Leplat et al., 2013). During warm, moist and windy environmental conditions the ascospores or macroconidia are dispersed by water-splash or air currents onto wheat heads and initiate infection of wheat spikes. Infections can occur as early as spike emergence, but the flowering stage or shortly after is considered the most vulnerable stage for *Fusarium* infection (Madgwick et al., 2011). No highly resistant commercial cultivars are yet available. Agronomic practices intended to reduce these diseases are only partially effective, because the necessary actions depend on the causal species and the environmental conditions, and the results are often unpredictable (Paulitz et al., 2002). Currently, control of Fusarium diseases relies on high inputs of fungicide in FHB-endemic regions (Mesterházy, 2003). Two factors are currently increasing the *Fusarium* problem in the UK. First, the UK is predicted to experience more often weather (UKCIP; www.ukcip.org.uk/) which will increase the risks of infection, colonisation, reproduction and dispersal of Fusarium diseases (West et al., 2012) leading to increased severity and incidence. Second, maize cultivation is increasing, leading to increased populations of *F. graminearum*; as maize debris is a potent source of inoculum of *Fusarium* (West et al., 2012).

Plant roots are associated with beneficial fungi in the majority of soils. For example, arbuscular mycorrhizal fungi (AMF), such as *Funneliformis mosseae* (=*Glomus mosseae)*, are important soil microorganisms forming beneficial symbiotic associations with most land plants. AMF are

obligate biotrophs which provide mineral nutrients, specifically phosphate and nitrogen, to their host plant in exchange for carbohydrates and therefore stimulate plant growth (Bucher, 2007, Schalamuk et al., 2011).

Piriformospora indica is a root endophyte with a wide host range belonging to the Sebacinaceae (Sebacinales, Basidiomycota). It was originally found in the Thar desert of Rajasthan, an arid region in India (Verma et al., 1998), which experiences extreme day-time heat and diurnal temperature fluctuations as well as extended drought. *P. indica* promotes plant growth, increases root and above ground biomass and final yield of a broad range of host plants, including many plants of economic importance (Shrivastava & Varma, 2014) and helps plants to grow under temperature, water and physical stresses (Alikhani et al., 2013, Ghabooli et al., 2013). Evidence suggests that *P. indica* protects plants against pathogens of roots (caused by *Fusarium culmorum, F. graminearum, Gaeumannomyces graminis* var. *tritici*), stems (caused by *Oculimacula* Spp.) and leaves (caused by *Blumeria graminis* f.sp. *tritici* and *B. graminis* f.sp. *hordei*) under glasshouse and field conditions (Deshmukh & Kogel, 2007, Ghahfarokhy et al., 2011, Harrach et al., 2013, Waller et al., 2005). Our previous work shows that *P. indica* association protected wheat seedlings from FCR damage in simulated UK autumn conditions (Rabiey et al., 2015).

The effect of some root associated fungi is to improve plant nutrient uptake (Miransari, 2010, Wu et al., 2011). For instance, AMF obtain fixed carbon compounds from host plants, while plants benefit from increased nutrient supply (Finlay, 2008). Research so far suggests that *P. indica* association improves plant mineral nutrient acquisition from the soil. It can mobilise and transport phosphorus, nitrogen and micronutrients from soil to the infected host plant via plant-fungal interfaces (Sherameti et al., 2005, Yadav et al., 2010). However, it is not yet clear if *P. indica* can increase nutrient uptake in all of its hosts.

The present study investigated the effect of *P. indica* on *Fusarium* infection of parts of the host not directly colonised by *P. indica*. We tested the following hypotheses: *P. indica* would reduce damage to wheat grains caused by FHB and mycotoxin contamination; any effect of *P. indica* on FHB would be greater at low soil fertility levels as in AMF, such as *F. mosseae* (Nouri et al., 2015); *P. indica* application would be as effective as fungicide application; and *P. indica* would increase plant tissue nutrients. We scored FHB disease severity and incidence, analysed mycotoxin DON, yield parameters and nutrients level in wheat grown in pots with factorial combinations of inoculation with *F. culmorum*, *F. graminearum*, *P. indica*, or *F. mosseae*, foliar fungicide and low and high fertiliser application rates. Plants were grown outdoors.

Materials and Methods

Fungal inoculation

Piriformospora indica

P. indica was obtained from Dr. Patrick Schafer, Warwick University, UK and was grown on agar containing complex modified *Aspergillus* medium (CM medium). Inoculum prepared by the methods described by Rabiey et al. (2015). *P. indica* liquid culture containing an unquantified mixture of chlamydospores and mycelium was used for inoculation. The inoculum was mixed with soil at sowing time.

Fusarium isolates

Isolates of *F. culmorum* and *F. graminearum* (576 and 602.1) of UK origin were obtained from the School of Biological Sciences, University of Reading and Rothamsted Research Centre, UK, respectively. Inocula of *F. culmorum* were prepared by the methods described by Ghahfarokhy et al. (2011).

Conidia of *F. graminearum* 576 and *F. graminearum* 602.1were harvested from the surface of sporulating PDA cultures in sterile distilled water so that the resulting suspension contained 1×10^6 spores mL^{-1}.

Funneliformis mosseae

F. mosseae was obtained from Prof. Alan Gange, Royal Holloway/University of London. The fungus was propagated on maize plants grown in a 3:1 mixture of steam sterilised compost (John Innes Composts, BHGS Ltd, UK) and sand. After 3 months, the contents of each pot (including compost and roots) were chopped on a sterilised surface and transferred into a zip-lock bag and stored at 4 °C until required.

Plant materials and pot experiments

The effect of *Piriformospora indica* and *Funneliformis mosseae* on Fusarium crown rot and Fusarium head blight of winter wheat under low and high fertiliser regimes

Winter wheat seeds, cv. Battalion, were surface disinfected by rinsing for 2 mins in 20mL L^{-1} (2%) sodium hypochlorite (Fisher scientific, UK), followed by three rinses in sterilized distilled water, and germinated on damp filter paper in a Petri dish at room temperature under natural indoor light for 48 hours. No micro-organisms grew from a sample of seeds so treated and placed on PDA plates for one week. Eight germinated seeds per pot were planted in 12L pots at a depth of 2 cm in a mixture of 2 parts non-sterilised compost (No 2, John Innes Compost, BHGS Ltd, UK) and one part sand, mixed with 11 grams (1 g L^{-1}) or 44 grams (4 gL^{-1}) of slow release fertiliser (8-9 months, Osmocote® Pro, the Scott Company, UK, contains 16% nitrogen, 11% phosphorus, 10% potassium, 2% magnesium oxide, 0.01% boron, 0.042% copper, 0.3% iron, 0.04% manganese, 0.015% molybdenum and 0.01% zinc) to provide wheat macro- and micro-nutrients during the experiment. Seeds were planted in 2 rows 11 cm apart with 2 cm

between each seed to simulate field spacing. Non-sterilised compost and sand were used to simulated field soil conditions.

In all experiments, pots were watered as necessary to maintain the compost moist, and the experimental area was surrounded by pots filled with sand to reduce edge effects on microclimate.

The experiment was carried out in the 2013-14 growing season at the University of Reading (grid ref: SU733719), under natural conditions. The experiment had 32 treatments with two replicates (giving 32 df for error), distributed in two randomised blocks, with the following factorial combinations of treatments = ±*P.indica*, ±*F. mosseae*, ±*F.culmorum* (FCR), ±*F. graminearum* (FHB) and ±fertiliser (1 g L^{-1} or 4 g L^{-1}). Inoculations with *P. indica* (6 g liquid culture mixed with soil) and *F. mosseae* (50 g, 20 spores per g, mixed with soil) and *F. culmorum* (6 g of prepared inocula mixed with soil) were performed at sowing and *F. graminearum* was applied at flowering. All disease symptoms, whether from inoculations or natural infections were recorded when appropriate, including Septoria leaf blotch and yellow rust.

In this experiment, extra nitrogen and sulphur fertiliser were applied in two split applications, with the first dose applied in late March and the second in late April, including 1.4 g N pot^{-1} (over 2 splits) and 28 mg S pot^{-1} (in one application). The first dose was made up of ammonium nitrate (34.5%N) and ammonium sulphate nitrate (27%N, 30% SO_4). The second dose was ammonium nitrate (34.5%N).

The effect of *Piriformospora indica, Funneliformis mosseae* and fungicide application on Fusarium head blight of spring wheat

Spring wheat seeds, cv. Paragon, were surface disinfected and pre-germinated as described above. Eight germinated seeds per pot were planted in 12L pots at a depth of 2 cm in non-

sterilised compost and sand (2:1), mixed with 44 grams (4 g L^{-1}) of slow release fertiliser as for winter wheat.

The experiment was carried out in the 2014 growing season. It had 16 treatments with three replicates, distributed in three randomised blocks, with the following factorial combinations of treatments: ±*P.indica*, ±*F. mosseae*, ±*F. graminearum* (FHB) and ±fungicide. Inoculations with *P. indica* (6g liquid culture mixed with soil) and *F. mosseae* (50 g, 20 spores per g mixed with soil) were performed at sowing. The fungicide, Aviator Xpro (Bayer CropScience, UK) with active ingredients of prothioconazole (15.84%) and bixafen (7.43%), was applied at the concentration of 2 ml L^{-1}, diluted with water, when flag leaf was fully emerged (Zadoks Growth Stage (GS) 39; T2; Zadoks et al. (1974)) and also 72 hours after plants were artificially sprayed with spore suspension of *F. graminearum* (GS 65; T3) at both stages for the selected treatments only. The fungicide Aviator Xpro exhibits both translaminar (within and across the leaf) and systemic movement (around the plant).

The effect of *Piriformospora indica* on *Fusarium* head blight of different cultivars of spring wheat

It is possible that some wheat cultivars benefit more than others from association with *P. indica*. In another experiment, the effect of *P. indica* on Fusarium head blight of spring wheat was assessed on 6 different spring wheat cultivars: Paragon, Mulika, Zircon (group 1), Granary, KWS Willow (group 2) and KWS Kilburn (group 4), chosen from HGCA recommended list for spring sowing and were supplied by KWS UK Ltd. Eight germinated seeds per pot were planted in 12L pots at a depth of 2 cm in a mixture of 2 parts non-sterilised compost and one part sand, mixed with 44 grams (4 g L^{-1}) of slow release fertiliser (3-4 months, Osmocote® Pro).

The experiment was done in the 2015 growing season. The experiment had 24 treatments with three replicates, distributed in three randomised blocks, with the following factorial combinations of treatments: ±*P indica*, ±*F. graminearum* (FHB), and 6 cultivars of spring wheat. Inoculations with *P. indica* (6 g liquid culture mixed with soil) was performed at sowing and *F. graminearum* was applied at flowering. All disease symptoms, whether from inoculations or natural infections, were recorded when appropriate including yellow rust and powdery mildew.

Fusarium ear inoculation

In all experiments, when most tillers of each pot were at mid-anthesis stage (GS 65), all tillers in the pot were sprayed with 1 mL of a 50:50 mixed conidia suspension of *F. graminearum* 576 and *F. graminearum* 602.1. In all experiments inoculation was done in a cloudy evening with rain afterward.

Fusarium head blight visual disease assessment and yield determination

Visual disease assessment, based on the percentage of infected spikelets per ear, was made two weeks after artificial inoculation on each of the treated ears from each pot. *F.graminearum* disease symptoms were recognized by pink fungal growth, brown-coloured lesions and premature bleaching of spikelets.

Plants were hand harvested at GS 92. The total above ground dry weight, total grain weight at 15% moisture content, 1000 grain weight (TGW), harvest index (total grain weight/total above grain weight), number of ears and root dry weight were measured.

Mycotoxin Analysis

Determination of mycotoxin DON in all samples from the winter and spring experiments was performed using ELISA testing by RomerLabs (Romer Labs Ltd, UK).

206 **The effect of *P. indica* on plant tissue nutrients under low and high fertiliser regimes**

207 Winter wheat seeds, cv. Battalion, were surface disinfected and pre-germinated. Eight seeds
208 per pot were planted in 12L pots at a depth of 2 cm in 2 parts non-sterilised and one part sand,
209 mixed with 11 grams (1 g L^{-1}) or 44 grams (4 g L^{-1}) of slow release fertiliser (8-9 months,
210 Osmocote® Pro). The experiment was carried out in the 2014/15 growing season. The
211 experiment had 8 treatments with three replicates, distributed in three randomised blocks, with
212 the following factorial combinations of treatments: ±*P indica*, ±*F. mosseae*, and ±fertiliser (1
213 g L^{-1} or 4 g L^{-1}). Inoculations with *P. indica* (6 g liquid cultures mixed with soil) and *F. mosseae*
214 (50 g, 20 spores per g mixed with soil) were done at the time of sowing. Around 200g leaf
215 materials of each treatment at GS 27-29 (tillering, main shoot with 7-9 or more tillers) were
216 sent for analysis in the first week of April 2015. The plant tissue analysis included total nitrogen
217 (N) and sulphur (S) with N:S ratio, total phosphorus (P), potassium (K), magnesium (Mg),
218 calcium (Ca), copper (Cu), zinc (Zn), Iron (Fe) and Boron (B).

219 **Weather conditions during 2013-15**

220 Winter 2013/14 was an exceptionally stormy season, with at least 12 major winter storms
221 affecting the UK. Mean temperatures and total rainfall over Reading were well above the long-
222 term average (Nov-Mar 2013-14 average 7.1°C). Following this, the weather of spring and
223 summer 2014 was warm (April-June 2014 average 13.5°C) with rainfall above the average.
224 The weather of Sep-Nov 2014 was exceptionally warm (average 12.7°C) with all months
225 warmer than average and the number of air frosts well below average. Rainfall was slightly
226 above average. Dec-Mar 2014/15 was sunny with mean temperature (5.6°C) near average.
227 Rainfall totals were slightly below average. April-Jun 2015 mean temperature (12.5°C) was,
228 close to average, with rainfall well below the average. (www.met.reading.ac.uk/weatherdata).

229 **Statistical analysis of experiments**

230 ANOVA was used to analyse all data using GenStat 17th ed, (VSN, UK) with appropriate
231 blocking. Where judged necessary from residual plots, data were $\log_{10}$ or square root
232 transformed to stabilize the residual variance and aid interpretation.

233 **Results**

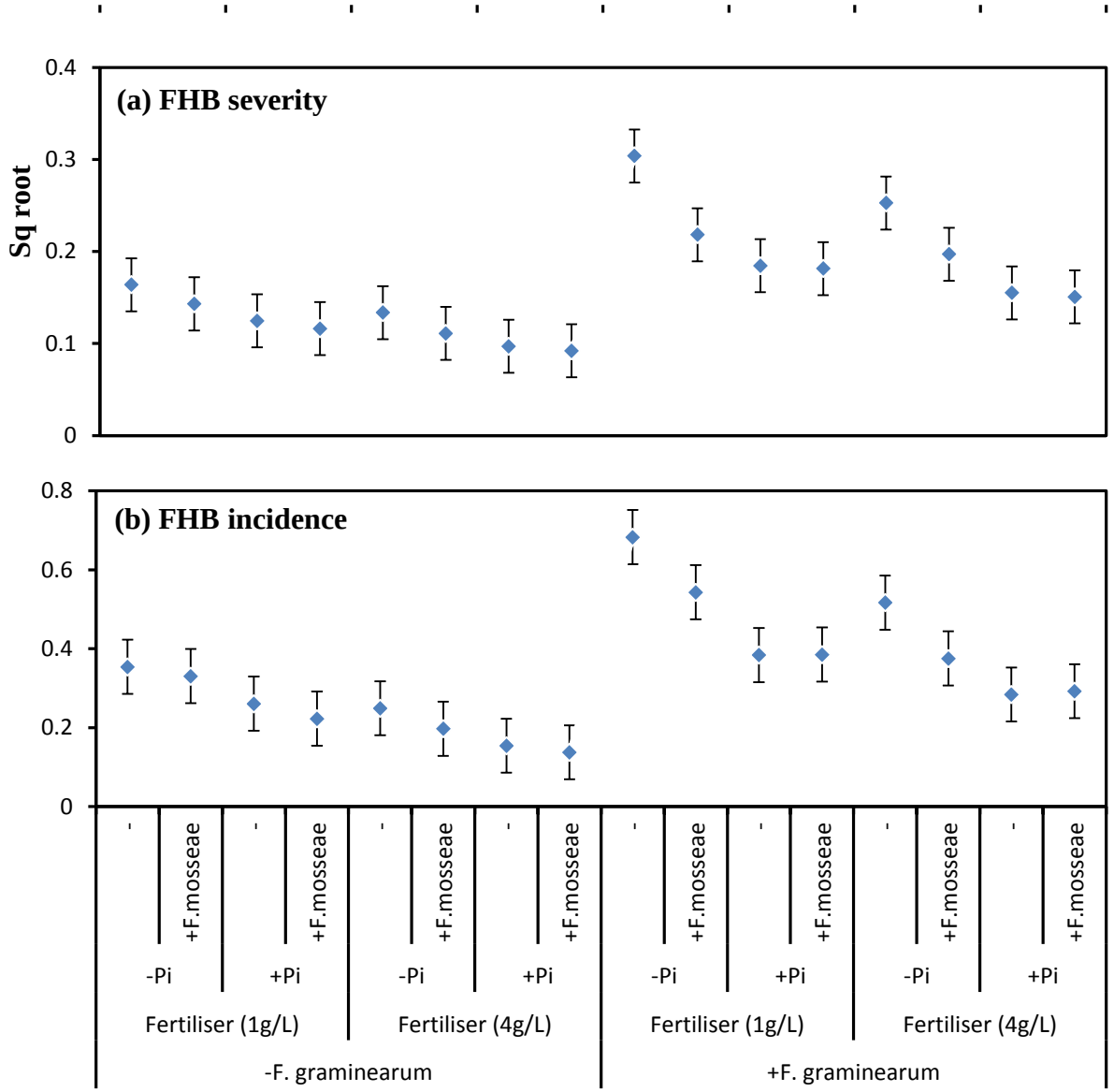
234 **Emergence rate**

235 The emergence rate of cv. Battalion (winter 2013), cv. Paragon (spring 2014) and the average
236 of six cultivars of spring wheat seedlings (spring 2015) from control treatments 14 days after
237 sowing was 90%, 98% and 95% respectively. *F. culmorum* application at sowing time reduced
238 the emergence rate by 10% (P=0.04). There were no other significant differences between
239 treatments.

240 **Fusarium head blight disease severity and incidence**

241 FHB disease severity of winter wheat cv. Battalion was assessed two weeks after artificial
242 inoculation at GS65. The main effects of fungicide and inoculation were large and significant,
243 but interactions between them and with *P. indica* were also important. Third and fourth order
244 interactions were not significant. Inoculation of ears with *Fusarium* increased the disease
245 severity and incidence significantly (P<0.001) compared to non-inoculated samples, but there
246 was also some natural background infection of *Fusarium* spp. present. *F. culmorum* application
247 at the time of sowing did not have a significant effect on FHB disease severity or incidence.
248 FHB severity and incidence in pots inoculated with *P. indica* (at sowing) and *F. graminearum*
249 (at flowering) was reduced by 70% (severity interaction P=0.004; incidence interaction
250 P=0.005), compared to *F. graminearum* inoculated pots. Disease severity and incidence were
251 higher in the low fertilisation level than the high level (main effect P<0.001). *F. mosseae*
252 reduced severity and incidence of FHB, but this effect was not additive to that of *P. indica*, so

253 *F. mosseae* in co-inoculation with *P. indica* gave no extra advantage (Fig. 1 a,b, supporting
254 information 1).



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256

257 **Fig 1.** The effect of *Piriformospora indica* (Pi) and *Funneliformis mosseae* under low (1g L^{-1})
258 and high (4g L^{-1}) fertiliser levels (Osmocote® Pro slow release fertiliser) on Fusarium head
259 blight (FHB) disease severity and incidence of winter wheat (cv. Battalion), recorded at two
260 weeks after artificial inoculation with *Fusarium graminearum*. (a) FHB disease severity, s.e.d
261 = 0.02; d.f = 31 (data were square root transformed); (b) FHB disease incidence, s.e.d = 0.05;
262 d.f = 31; Each point represents mean ± 2SEM.

263

264

In spring wheat cv. Paragon, inoculation of ears with Fusarium spores significantly increased the disease severity and incidence of FHB (main effect of inoculation $P<0.001$), but there was also some natural background infection of *Fusarium* spp. (Fig. 2 a,b). The application of fungicide following *F. graminearum* inoculation reduced FHB severity by 80% (fungicide.FHB interaction $P=0.04$). *P. indica* soil inoculation resulted in a reduction in FHB severity, but the effect was only marginally significant (*P. indica*. FHB interaction $P=0.08$; Fig. 2 a,b, supporting information 2).

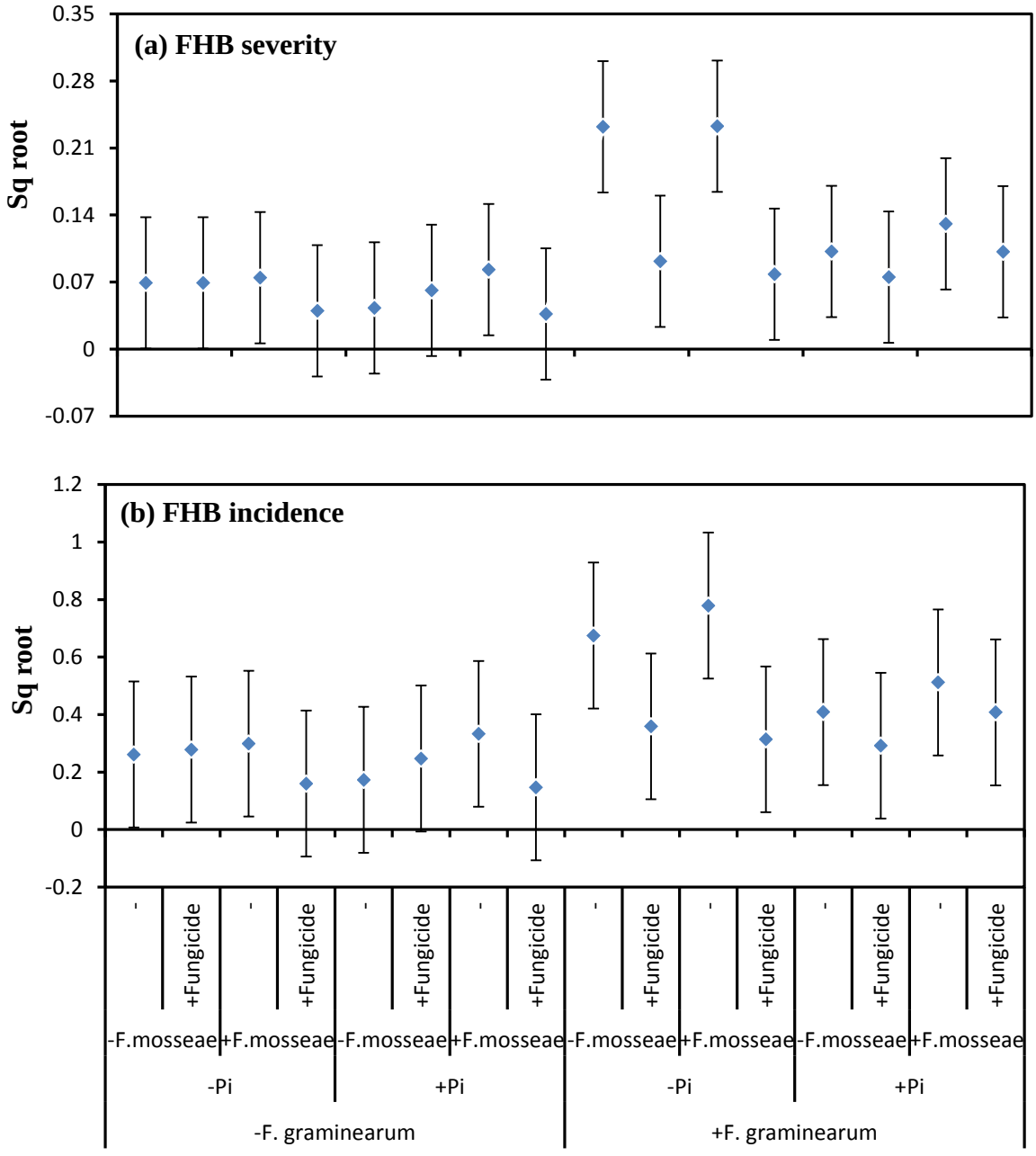


Fig 2. The effect of *Piriformospora indica* (Pi), *Funneliformis mosseae* and fungicide Aviator Xpro on *Fusarium* head blight (FHB) disease severity and incidence of spring wheat (cv. Paragon), recorded at two weeks after artificial inoculation with *Fusarium graminearum*. (a) FHB disease severity, s.e.d = 0.05, d.f = 30 (data were square root transformed); (b) FHB disease incidence, s.e.d = 0.18, d.f = 30, (data were square root transformed); Each point represents mean ± 2SEM.

Ears inoculation of six cultivars of spring wheat with *F. graminearum* spores significantly increased the disease severity and incidence of FHB (main effect of inoculation P<0.001), but there was also some natural background infection of *Fusarium* spp. (Fig 3 a,b). FHB severity and incidence in pots inoculated with *P. indica* (at sowing) and *F. graminearum* (at flowering) was reduced by around 80% (severity *P. indica*. FHB interaction P<0.001; incidence interaction P=0.02), compared to *F. graminearum* inoculated pots (Fig 3 a,b, supporting information 3).

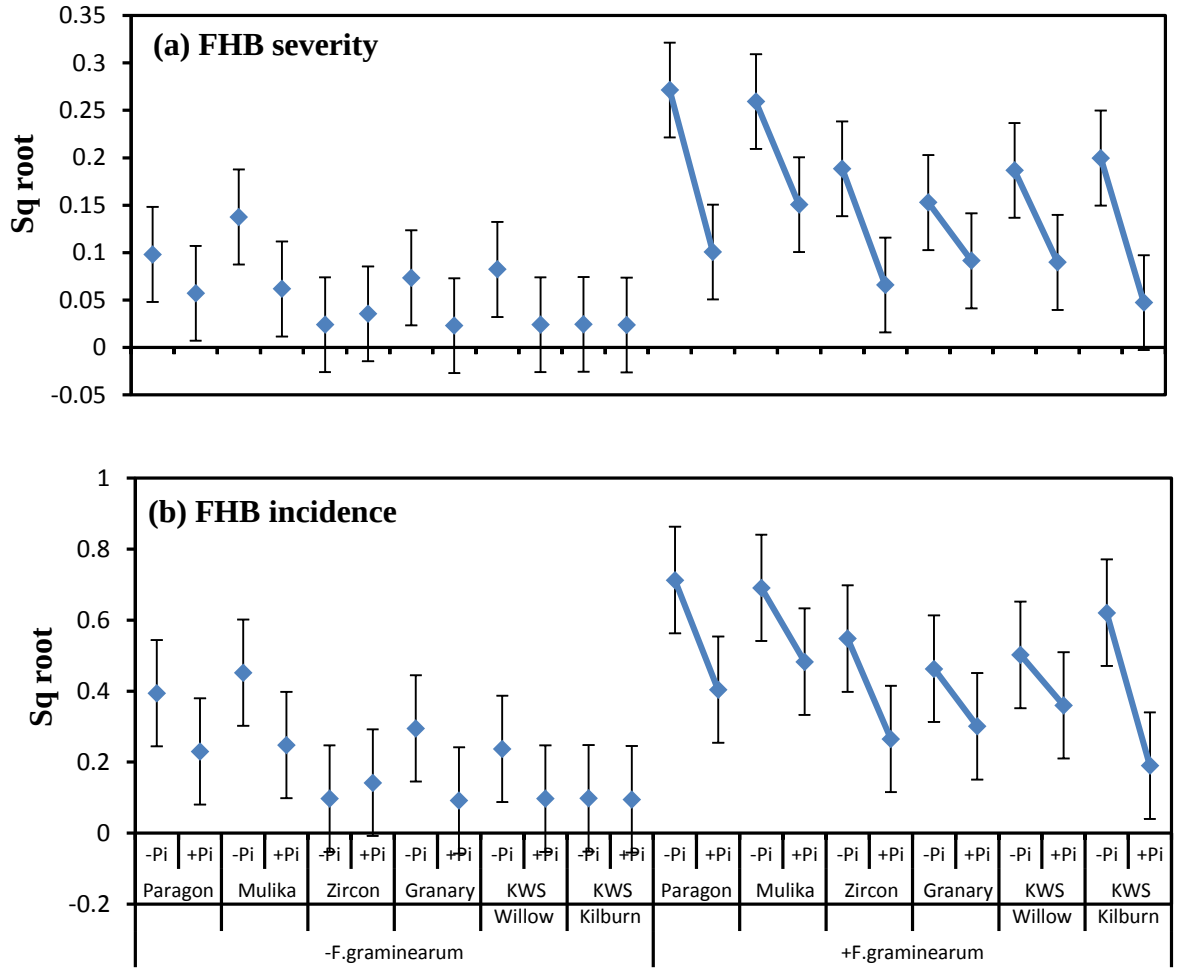


Fig 3. The effect of *Piriformospora indica* (Pi) on Fusarium head blight (FHB) disease severity and incidence of six cultivars of spring wheat (cv. Paragon, Mulika, Zircon, Granary, KWS Willow and KWS Kilburn), recorded at two weeks after artificial inoculation with *Fusarium*

311 *graminearum*. (a) FHB disease severity, s.e.d = 0.04; d.f = 46; (b) FHB disease incidence s.e.d
312 = 0.1; d.f = 46; (data were square root transformed). Each point represents mean ± 2 SEM.
313

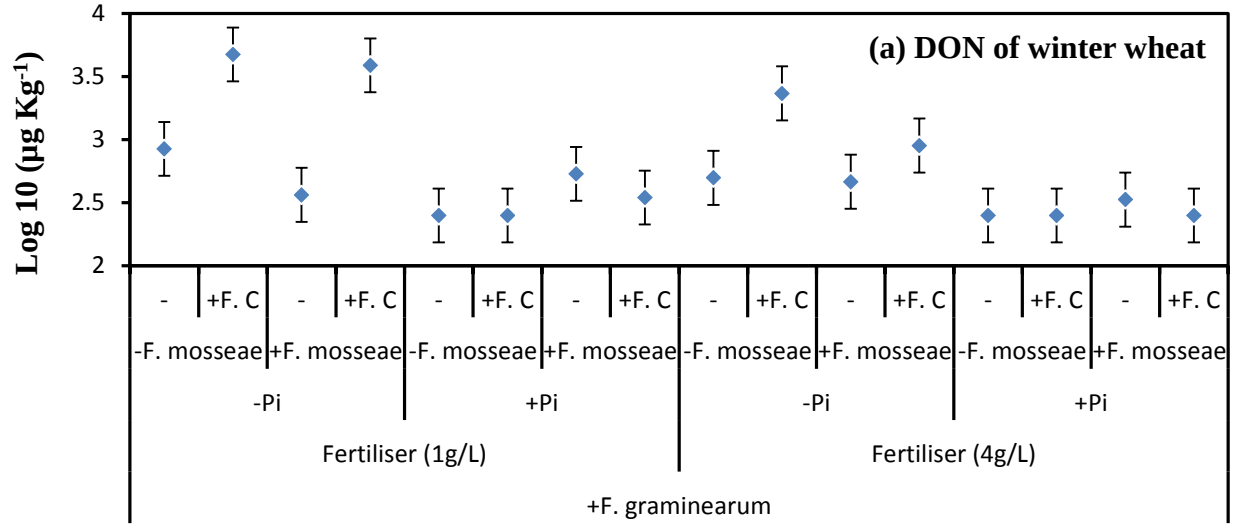
314 **Mycotoxin DON analysis**

315 ELISA testing could only detect DON level of above the limit of detection. For both winter
316 and spring wheat samples with no Fusarium head inoculation, DON concentrations were below
317 the limit of detection (<250 µg kg^{-1}). We therefore restricted the analysis to those samples from
318 plants which were artificially inoculated with *F. graminearum* and considered those lower than
319 the limit of detection as 250µg kg^{-1}. The following results concern *F. graminearum*-inoculated
320 samples only, in the cv. Battalion in 2014: *P. indica* application reduced DON concentrations
321 by 70% at low fertilisation and 50% at high fertilisation (Fig 4a; *P. indica*. fertiliser interaction
322 P<0.001), to levels close to the limit of detection. In the absence of *P. indica*, DON
323 concentrations were 70% higher at low fertilisation (fertiliser main effect P=0.005) than high
324 fertilisation. DON concentrations were higher in the samples inoculated at sowing with *F.*
325 *culmorum* (P<0.001); however, *P. indica* reduced DON concentrations in these samples to
326 below the limit of detection (P<0.001). *F. mosseae* had no main effect (P=0.5) and no
327 significant interactions (Fig. 4a, supporting information 4).
328 In the cv. Paragon spring wheat samples in 2014 inoculation with *F. graminearum* significantly
329 increased DON concentrations (main effect P<0.001). The following results concern *F.*
330 *graminearum*-inoculated samples only: *P. indica* application (main effect P=0.01) reduced
331 DON concentrations by 80% (Fig 4b). Fungicide application (main effect P=0.001) also
332 reduced the mycotoxin concentrations by 70%, but the effect was not additional to that of *P.*
333 *indica* (interaction P=0.03). *F. mosseae* had no effect on average (main effect, P=0.5) but had
334 a significant interaction with *P. indica* (P=0.009): without *P. indica*, *F. mosseae* reduced DON

by roughly 50%, but in the presence of *P. indica*, *F. mosseae* increased DON by about 50% (Fig. 4b, supporting information 5).

In 2015, inoculation of six cultivars of spring wheat samples with *F. graminearum* significantly increased DON concentrations (main effect $P<0.001$); very few positive samples were found in the uninoculated pots, and with low levels of contamination. The following results concern *F. graminearum*-inoculated samples only: The cultivars differed in mycotoxin DON concentration ($P<0.001$). *P. indica* application reduced DON concentration by around 90% (main effect $P<0.001$). *P. indica* reduced DON concentration in all cultivars, with an interaction arising because cv. KWS Willow and cv. Granary had low concentrations of DON even in non-*P. indica* treated pots (interaction $P=0.002$, Fig 4c, supporting information 6).

FHB severity was well correlated to DON ($r=0.7$). Both FHB severity and DON were weakly related to yield, but not to root-shoot ratio, above ground biomass or root biomass.


(a) DON of winter wheat
Log 10 (µg Kg-1)
4
3.5
3
2.5
2
- +F. C - +F. C - +F. C - +F. C - +F. C - +F. C - +F. C - +F. C
-F. mosseae +F. mosseae -F. mosseae +F. mosseae -F. mosseae +F. mosseae -F. mosseae +F. mosseae
-Pi +Pi -Pi +Pi
Fertiliser (1g/L) Fertiliser (4g/L)
+F. graminearum

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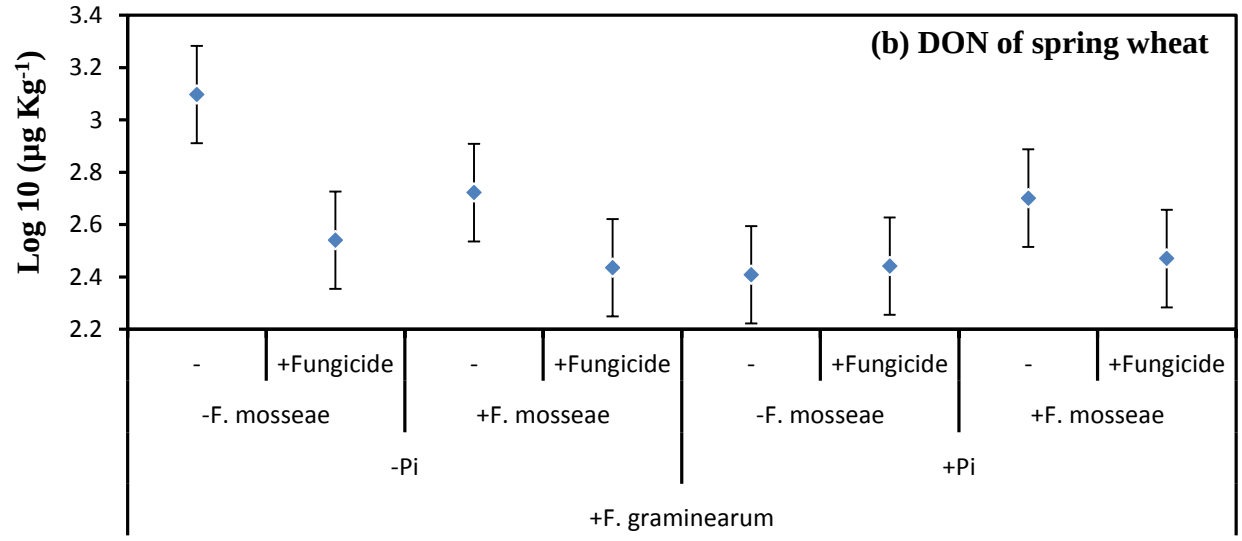
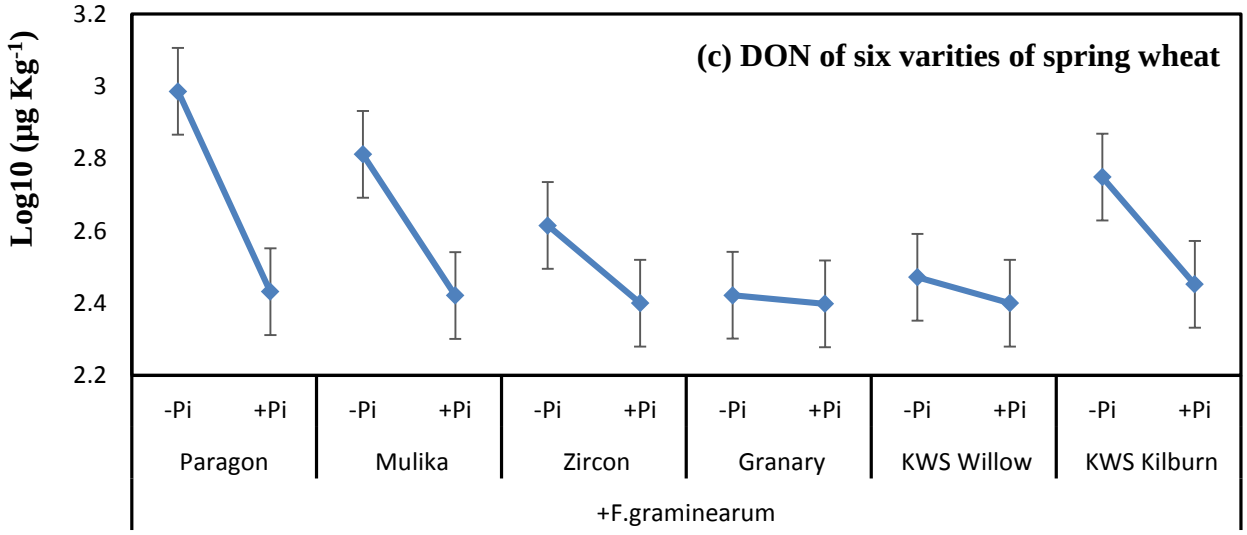

(b) DON of spring wheat
Log 10 (µg Kg-1)
3.4
3.2
3
2.8
2.6
2.4
2.2
- +Fungicide - +Fungicide - +Fungicide - +Fungicide
-F. mosseae +F. mosseae -F. mosseae +F. mosseae
-Pi +Pi
+F. graminearum

359


(c) DON of six varities of spring wheat
Log10 (µg Kg-1)
3.2
3
2.8
2.6
2.4
2.2
-Pi +Pi -Pi +Pi -Pi +Pi -Pi +Pi -Pi +Pi -Pi +Pi
Paragon Mulika Zircon Granary KWS Willow KWS Kilburn
+F.graminearum

360

Fig 4. The effect of *Piriformospora indica* (Pi), *Funneliformis mosseae* and fungicide Aviator Xpro, under low (1g L^{-1}) and high (4g L^{-1}) fertiliser levels (Osmocote® Pro slow release fertiliser) on *Fusarium* mycotoxin deoxynivalenol (DON) of winter and spring wheat grain samples. (a) DON of winter wheat samples (cv. Battalion), s.e.d = 0.15, d.f = 15; (b) DON of spring wheat samples (cv. Paragon), s.e.d = 0.13, d.f =14; (c) DON of six cultivars of spring wheat samples (cv. Paragon, Mulika, Zircon, Granary, KWS Willow and KWS Kilburn), s.e.d = 0.08, d.f. = 22; (data were Log_{10} transformed); Each point represents mean ± 2SEM; (F.c: *F. culmorum*).

Final harvest results

Winter wheat cv. Battalion, 2014

Above ground biomass*: F. mosseae* increased the above ground biomass in the presence of *F. culmorum* by 17% at high fertilisation and by 10% at low fertilisation, compared to *F. culmorum*-inoculated samples (*F. mosseae. F. culmorum* interaction P<0.001). *P. indica* inoculation increased biomass on average (main effect P=0.06). Its combination with *F. mosseae* increased the above ground biomass in the presence of *F. graminearum* by 25% at low fertilisation (*P. indica. F. mosseae. F. graminearum* interaction *P* =0.008), compared to samples inoculated with *F. graminearum* alone. The co-inoculation increased biomass also in plants inoculated with *F. culmorum*, by 15% at low fertilisation and 34% at high fertilisation (*P. indica. F. mosseae. F. culmorum* interaction P=0.07). At low fertilisation, in the presence of *F. graminearum*, *F. mosseae* increased the above ground biomass by 30% (*F. mosseae, fertiliser. F. graminearum* interaction P=0.001), compared to *F. graminearum*-inoculated samples at low fertilisation. *F. culmorum* application at sowing time reduced the above ground weight by 7%, but the effect could have been chance (P=0.09).

Root biomass: Roots were heavier at high fertilisation than low fertilisation (main effect P<0.001). *P. indica* application increased the root weight by 55% at both low and high fertilisation (main effect P<0.001), compared to non-*P. indica* inoculated samples. The co-

389 inoculation of *F. mosseae* with *P. indica* also increased the root weight by 52% at low
390 fertilisation and 37% at high fertilisation (*P. indica*. *F. mosseae* P<0.001). *F. culmorum*
391 reduced the root weight by 40% at both low and high fertilisation (interaction P<0.001). This
392 reduction was smaller when *P. indica* (P=0.01) or *F. mosseae* (P=0.01) were also applied.
393 Yield: *F. mosseae* at low fertilisation increased the total grain weight by 5%, but at high
394 fertilisation it decreased the weight by 20% (*F. mosseae*. fertiliser interaction P=0.03),
395 compared to non-*F. mosseae*-inoculated samples. The combination of *P. indica* and *F. mosseae*
396 increased the total grain weight by 60% in the presence of *F. graminearum* (*P. indica. F.mosseae. F. graminearum* interaction P=0.09) at low fertilisation level, compared to *F.*
398 *graminearum*-inoculated samples. The combination of *P. indica* and *F. mosseae* increased the
399 total grain weight in the presence of *F. culmorum* at both low and high fertilisation (*P. indica.*
400 *F.mosseae. F. culmorum* interaction P=0.05).
401 TGW*: P. indica* application increased 1000 grain weight (TGW) by 8% at low fertility (main
402 effect P=0.02). The application of *F. graminearum* reduced TGW by 10% (P=0.06) at both low
403 and high fertilisation. However, *P. indica* maintained TGW in the presence of *F. graminearum*
404 at low fertilisation (*P. indica. F. graminearum* interaction P=0.04). The combination of *P.*
405 *indica* and *F. mosseae* increased TGW at high fertilisation, but not at low fertilisation (*P.*
406 *indica*. *F. mosseae.* fertiliser interaction P=0.008).
407 Harvest index
408 : There were no significant differences among treatments for harvest index.
409 Ears: Fertilisation increased the number of ears per pot (main effect P=<0.001). The
410 combination of *P. indica* and *F. mosseae* increased the number of ears at both low and high
411 fertilisation (*P. indica*. *F. mosseae*. fertiliser interaction P=0.02), compared to non-*P. indica*-
412 inoculated samples (table 1 & supporting information 1).

Table 1. Final harvest results of winter wheat samples (cv. Battalion) inoculated with *Piriformospora indica* (Pi), *Funneliformis mosseae*, *Fusarium culmorum* (F. c; at sowing time) and *F. graminearum* (F. g; at flowering time) under low (1g L^{-1}) and high (4g L^{-1}) fertiliser levels (Osmocote® Pro slow release fertiliser; d.f. = 31).

Fertiliser	P.indica	F.g	F.mosseae	F.c	Total above ground weight (g)	Root weight	Total grain weight per pot (g)	1000 grain weight (g)	Harvest index	no of ears per pot (Log10)
1 g/L	-	-	-	-	243	23	78	68	0.3	1.4
				+	227	16	77	66	0.3	1.4
			+	-	264	21	82	71	0.3	1.4
				+	251	27	84	70	0.3	1.4
		+	-	-	204	21	57	60	0.3	1.4
				+	195	17	62	63	0.3	1.4
			+	-	266	27	83	69	0.3	1.4
				+	274	33	79	67	0.3	1.4
				mean	**241**	**23**	**75**	**67**	**0.3**	**1.4**
	+	-	-	-	272	34	85	73	0.3	1.4
				+	217	38	63	68	0.3	1.3
			+	-	257	35	83	67	0.3	1.4
				+	261	34	90	68	0.3	1.4
		+	-	-	247	28	77	66	0.3	1.3
				+	221	35	65	73	0.3	1
			+	-	257	32	92	68	0.4	1.4
				+	276	32	88	69	0.3	1.4
				mean	**251**	**34**	**80**	**69**	**0.3**	**1.3**
4 g/L	-	-	-	-	336	27	120	69	0.4	2
				+	276	19	95	67	0.3	1.6
			+	-	303	38	96	71	0.3	1.7
				+	326	34	94	68	0.3	2
		+	-	-	307	31	89	64	0.3	1.6
				+	277	18	93	69	0.3	2
			+	-	305	38	110	65	0.4	1.7
				+	298	32	92	67	0.3	1.7
				mean	**304**	**30**	**99**	**68**	**0.3**	**1.8**
	+	-	-	-	317	42	125	68	0.4	1.7
				+	281	37	94	69	0.3	1.6
			+	-	301	37	102	71	0.3	1.6
				+	372	37	129	71	0.3	1.7
		+	-	-	380	41	122	65	0.3	1.6
				+	316	38	97	69	0.3	2
			+	-	266	37	81	70	0.3	1.6
				+	297	39	92	68	0.3	2
				mean	**316**	**39**	**105**	**69**	**0.3**	**1.7**
				s.e.d	24	3.09	17.3	3.07	0.05	0.05

Spring wheat cv. Paragon, 2014

The application of *P. indica* increased total above ground weight by 16% (main effect P=0.05), root weight by 20% (main effect P=0.02), total grain weight by 23% (main effect P=0.02), TGW by 23% (main effect P=0.08), harvest index by 8% (main effect P=0.07), and number of ears by 12% (main effect P=0.003), compared to samples without *P. indica*. The interaction of *P. indica* with *F. graminearum* increased total grain weight of *F. graminearum*-inoculated samples by 54% (P=0.08) and harvest index by 13% (P=0.07). Also, the combination of *P. indica*, *F. mosseae* and fungicide increased total above ground weight (P=0.03), total grain weight (P=0.003), TGW (P=0.01), harvest index (P=0.009) and number of ears (P=0.003) (Table 2 & supporting information 2).

Table 2. Final harvest results of spring wheat samples (cv. Paragon) inoculated with *Piriformospora indica* (Pi), *Funneliformis mosseae* (at sowing time), *Fusarium graminearum* (F. g; at flowering time) and fungicide Aviator Xpro (at growth stage 39 and 72 hours after artificial inoculation at flowering time) (d.f. = 26).

P. indica	F.g	F.mosseae	Fungicide	Total above ground weight (g)	Root weight (g)	Total grain weight per pot (g)	1000 grain weight (g)	Harvest index	no of ears per pot (Log10)
-	-	-	-	193	23	73	43	0.4	39
			+	229	28	103	52	0.5	41
		+	-	212	24	98	50	0.5	39
			+	201	24	79	46	0.4	35
	+	-	-	183	21	62	38	0.3	36
			+	199	22	83	45	0.4	38
		+	-	213	29	86	50	0.4	38
			+	214	30	90	45	0.4	35
			mean	**206**	**25**	**84**	**46**	**0.4**	**38**
+	-	-	-	225	28	89	53	0.4	44
			+	205	28	91	47	0.5	40
		+	-	205	29	82	46	0.4	39
			+	232	28	102	47	0.4	41
	+	-	-	217	28	96	51	0.4	40
			+	204	28	91	47	0.4	37
		+	-	236	28	95	51	0.4	40
			+	226	25	108	48	0.5	39
			mean	**219**	**28**	**94**	**49**	**0.4**	**40**
			s.e.d	18.5	2.8	10.9	4.1	0.04	2.01

Six cultivars of spring wheat, 2015

Averaged over other treatments, the cultivars of spring wheat differed in above ground biomass (P=0.02), root weight (P=0.09), total grain weight (P=0.001), and the number of ears per pot (P<0.001). Averaged over cultivars *P. indica* inoculation increased the above ground biomass (P<0.002), root weight (P= 0.002), total grain weight (P<0.001), TGW (P<0.001), harvest index (P<0.001) and the number of ears per pot (P=0.002). *F. graminearum* application at flowering reduced the above ground biomass (P=0.06), total grain weight (P<0.001), and harvest index (P=0.03) of all cultivars. In the presence of *F. gramineraum*, *P. indica* inoculation

455 increased the above ground biomass and TGW (*P. indica*.*F. graminearum* interaction P=0.04
456 and P=0.03, respectively). There was no interaction between *P. indica* or *F. graminearum* with
457 cultivars (Table 3 & supporting information 3).
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Table 3. Final harvest results of six **cultivars** of spring wheat samples (cv. Paragon, Mulika, Zircon, Granary, KWS Willow and KWS Kilburn) inoculated with *Piriformospora indica* (at sowing time), and *Fusarium graminearum* (F. g; at flowering time, d.f. = 46).

P.indica	F.g	Spring wheat cultivars	Total above ground weight (g)	Root weight (g)	Total grain weight per pot (g)	1000 grain weight (g)	Harvest index	No of ears
		Paragon	267	18.6	82	45	0.3	51
		Mulika	267	15.3	94	47	0.4	52
		Zircon	289	17.9	103	48	0.4	66
	-	Granary	250	16.2	87	46	0.4	60
		KWS Willow	283	14.8	105	45	0.4	59
		KWS Kilburn	257	16.1	93	44	0.4	62
-		**mean**	269	16.5	94	46	0.4	58
		Paragon	201	17.2	61	39	0.3	54
		Mulika	228	16.8	72	43	0.3	53
		Zircon	245	17.4	88	45	0.4	61
	+	Granary	219	15.7	74	44	0.3	60
		KWS Willow	257	17.4	71	41	0.3	65
		KWS Kilburn	251	17.1	74	41	0.3	58
		mean	234	16.9	73	42	0.3	59
		Paragon	223	27.4	102	65	0.5	56
		Mulika	284	20.1	127	65	0.4	57
		Zircon	338	22.8	154	62	0.5	74
	-	Granary	257	20.8	111	61	0.4	68
		KWS Willow	302	22.4	97	61	0.3	70
		KWS Kilburn	269	21.3	97	55	0.4	61
+		**mean**	279	22.5	115	62	0.4	64
		Paragon	280	21.7	89	60	0.3	61
		Mulika	273	23.01	108	65	0.4	58
		Zircon	269	24.6	115	60	0.4	69
	+	Granary	269	22.7	105	59	0.4	65
		KWS Willow	325	22.9	102	64	0.3	62
		KWS Kilburn	268	21.1	103	66	0.4	61
		mean	281	22.7	104	62	0.4	63
		s.e.d	30.9	2.1	13.4	3.6	0.05	5.3

Leaf tissue nutrients analysis

The concentrations of leaf total N, P, K, Ca, Mg, S, Mn, Cu, Zn and B were all higher at high fertilisation (main effect P<0.001). However, the concentration of Fe was higher at low fertilisation (main effect P=0.002). The concentration of B in the leaves was lower in the presence of *P. indica* (main effect P=0.01). The combination of *P. indica* and *F. mosseae*, at high fertilisation, increased the total concentration of N in the leaves (*P. indica*, *F. mosseae* and fertiliser interaction P=0.04), but on their own, each decreased the level (table 4 & supporting information 7).

Table 4. Leaf tissue nutrient analysis results of winter wheat samples (cv. Battalion) with *Piriformospora indica* and *Funneliformis mosseae* at sowing time (fertiliser: Table 4.3. Leaf nutrient analysis results of winter wheat samples inoculated with *Piriformospora indica* and *Funneliformis mosseae* at sowing time (fertiliser: Osmocote® Pro slow release fertiliser, N: Nitrogen, P: phosphorus, K: potassium, Ca: calcium, Mg: magnesium, S: sulphur, Mn: manganese, Cu: copper, Zn: zinc, Fe: Iron, B: boron ; d.f. = 14).

Fertiliser	P.indica	F.mosseae	Total N	Total P	Total K	Ttal Ca	Total Mg	Total S	Total Mn	Total Cu	Total Zn	Total Fe	Total B
1 g/L	-	-	3	4471	35830	2849	908	2649	123	4	29	517	3
		+	3	5163	40524	2803	1020	3564	139	4	32	192	3
		Mean	3	4817	38177	2826	964	3107	131	4	31	355	3
	+	-	3	4989	39836	2771	1029	3443	152	5	31	214	3
		+	3	4906	38003	2689	1003	3125	142	4	31	173	3
		Mean	3	4948	38920	2730	1016	3284	147	5	31	194	3
4 g/L	-	-	5	7803	52583	4120	1540	7440	216	8	60	157	4
		+	4	7465	51042	3638	1382	6479	209	6	53	121	4
		Mean	5	7634	51813	3879	1461	6960	213	7	57	139	4
	+	-	4	7995	52829	3668	1406	6790	204	7	56	135	3
		+	5	7106	52588	4065	1553	6018	177	6	54	121	3
		Mean	5	7551	52709	3867	1480	6404	191	7	55	128	3
		s.e.d	0.3	632	3500	450	132	715	15	0.6	5	76	0.3

Discussion

P. indica effectively reduced FHB disease severity and incidence, and also grain DON contamination. It was as effective as fungicide applied 72 hours after *F. graminearum* inoculation, and the effect was consistent across years and cultivars. *P. indica* also increased yield in both high and low fertilisation, suggesting *P. indica* application is compatible with low-input systems. However, unlike mycorrhizal fungi, its effect was greater at the high fertilisation level. *P. indica* application was compatible with *F. mosseae* and fungicide, but effects of these were not additive. Collectively, these results suggest that *P. indica* application could be useful in the long-term. *P. indica* reduced FCR at sowing, FHB at flowering and grain DON contamination, suggesting there would be fewer spores, hyphae and macroconidia overwintering in soil and crop residues; as a result there would be less inoculum available for the disease to occur in the next season.

Fungicide application during wheat growing stages can reduce the risk of FHB and mycotoxin contamination (Edwards & Godley, 2010, Paul et al., 2008). However, inconsistent control of FHB disease with fungicide has been found in several reports (Horsley et al., 2006, McMullen, 1994). Yoshida et al. (2012) indicated that the timing of fungicide application differentially affected FHB disease and mycotoxin concentration, considering anthesis as the crucial stage for fungicide application. The application of fungicide, in our spring wheat experiment, at GS 39 (when flag leaf was fully emerged), and then at anthesis GS 65 (72 hours after *Fusarium* inoculation), reduced both FHB and DON concentration. In all spring and winter wheat experiments, *P. indica* application at sowing also reduced FHB severity and incidence as effectively as fungicide. The application of *P. indica* not only might reduce the use of fungicide and any environmental damage from fungicide use, but also increase plant resistance against other pathogens (Franken, 2012).

The DON concentration in samples inoculated at sowing with *F. culmorum* and then at heading with *F. graminearum* was much higher than in samples inoculated only with *F. graminearum*. This suggests that when *Fusarium* is already present in the plant, there is an increased risk of mycotoxin production in the grains by FHB. *F. culmorum* might have produced DON that moved from lower parts of the plants to the heads, consistent with the results of Moretti et al. (2014) and Covarelli et al. (2012) who demonstrated that although *F. graminearum* and *F. culmorum* could not be detected beyond the third internode, a low concentration of DON was found in the kernels beyond those tissues colonized by the fungus; suggesting that DON can be moved from lower parts of the plants to the heads. This is probably due to its water solubility, which can cause a reduction in concentration at late harvest, but in this case led to transfer upwards. Alternatively, Mudge et al. (2006) isolated *F. graminearum* and DON from wheat heads and flag leaf nodes following inoculation of the stem base. Xu et al. (2007) indicated that the mycotoxin productivity of *F. graminearum* in the co-inoculation with *F. culmorum* and *F. poae* was higher of that in the single-isolate inoculations. However, in the present case DON levels in the ear were not detectably increased by root infection with *F. culmorum* in the absence of *F. graminearum* inoculation. The increased production of DON is therefore presumably connected to increased plant resistance.

In the winter wheat experiment, *P. indica* increased the above ground weight, total grain weight and 1000 grain weight by similar amounts under both low and high fertilisation, suggesting that the *P. indica* effect on grain yield was independent of fertiliser levels. Similarly Achatz et al. (2010) found that increased grain yield in *P. indica* inoculated barley was independent of the fertilisation level. Murphy et al. (2014) found that *P. indica*-inoculated barley had greater grain weight in higher nutrient input. These indicates that *P. indica*-induced yield increase does not result from relief of low phosphorus or nitrogen supply. By contrast, both our results

549 and those of Achatz et al. suggest that the increase in the above ground weight caused by *F.*
550 *mosseae* only occurred under low fertility. The difference in response to high fertility shows
551 that the beneficial effects of *P. indica* are based on different mechanisms from mycorrhizal
552 fungi. The effect of *P. indica* under low and high fertilisation levels on final yield of winter
553 wheat was confirmed on a small scale experiment (data not shown, see supportive information
554 8 &9).
555 Consistently with our results, Shahabivand et al. (2012) and Yaghoubian et al. (2014) reported
556 that *P. indica* increased wheat growth more than *F. mosseae* and that their co-inoculation
557 improved the defence mechanisms, drought resistance, and growth of wheat plants, suggesting
558 *P. indica* application was compatible with *F. mosseae* application.
559 During the experiments we scored the severity of any air borne diseases which occurred
560 naturally. *P. indica* reduced disease severity and incidence of Septoria leaf blotch at GS 22
561 (tillering stage) and yellow rust at GS 35-37 (stem elongation, 5th node detectable to flag leaf
562 just visible) for the winter wheat cv. Battalion, and yellow rust and powdery mildew at GS 70
563 (milk development) for six different cultivars of spring wheat (data not shown). In a small-
564 scale experiment the effect of *P. indica* on Septoria leaf blotch was confirmed at seedling stage;
565 this is consistent with *P. indica* producing a generalised increase in resistance to a wide class
566 of fungi.
567 These results show that *P. indica* colonised and increased shoot and final yield of the winter
568 wheat (cv. Battalion) and 6 cultivars of spring wheat. *P. indica* reduced disease severity and
569 incidence of FHB, and other foliar diseases and DON concentration of all cultivars. It is
570 consistent with Deshmukh et al. (2006), Deshmukh and Kogel (2007)'s study. They inoculated
571 different barley cultivar seedlings with *P. indica* and different isolates of *Sebacina vermifera*
572 (member of Sebacinaceae, genetically close to *P. indica*). Despite considerable variation of the

fungal activity of the different isolates, they found an increase in shoot and root biomass with consistent resistance-inducing activity of all strains of the *S. vermifera* against powdery mildew (caused by *Blumeria graminis* f.sp. *hordei*) as with *P. indica.* In contrast, Gravouil (2012) showed that different barley cultivars had different rates of colonisation by *P. indica*. Some barley cultivars had the highest rate of *P. indica* colonisation and the best increase in shoot biomass and protection against pathogens such as *Rhynchosporium commune.*

The leaf tissue nutrient analysis showed that *P. indica* did not have any effect on leaf nutrients, suggesting that at least in the case of this experiment, *P. indica* effects on growth and yield were not due to better nutrient uptake. These results are inconsistent with others that suggest *P. indica* increasing the uptake of micro- and macro-nutrients leads to growth promotion (Shrivastava & Varma, 2014). Gosal et al. (2010) reported that *P. indica* increased the amount of Cu, Zn and Mn in *Chlorophytum sp*. and promoted plant growth and biomass. *P. indica* increased the amount of Zn in Turmeric (*Curcuma longa* L.) which enhanced the growth, yield and active ingredients (Bajaj et al., 2014). The inconsistency with their results might have various causes. It might be due to the host differences, the methods of plant cultivations and inoculations, environmental effects or differences in the fertilisers and their concentrations. However, *F. mosseae* also did not have any effects on leaf nutrients, suggesting no effect of *P. indica* and/or *F. mosseae* might be because of the experimental conditions. So more experiments are needed to confirm that.

These results suggest that *P. indica* could be useful in control of Fusarium crown rot and head blight, mycotoxin contamination and other air borne diseases. However, *P. indica* is probably an alien species in many parts of the world including the UK, so its releases into the open environments in these regions, to confirm its beneficial effects, requires consideration also of physiological trade-offs and ecological and agronomical side-effects. The wider effects of *P.*

indica and similar organisms also need to be better understood before agricultural deployment.

A search for native organisms with similar characteristics might be a safer direction to go in.

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767 **Supporting information**

768 **Table 1.** ANOVA P-value for variable measured in pots of winter wheat (cv. Battalion) and
769 treated in a full factorial design with the factors shown. The experiment carried out in the
770 2013/14 growing season.

	P value

	FHB severity	FHB incidence	Total above ground weight	Root weight	Total grain weight	TGW	Harvest index	No of ears
Main effect								
P.indica	**<.001**	**<.001**	**0.06**	**<.001**	0.2	**0.02**	0.6	0.2
F.mosseae	**0.001**	**0.006**	**0.01**	**<.001**	0.3	**0.05**	0.9	**0.02**
Fertiliser	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	0.6	0.3	**<.001**
F.graminearum	**<.001**	**<.001**	0.2	0.9	0.09	**0.06**	0.2	0.8
F.culmorum	0.09	0.1	0.09	**0.05**	0.2	0.8	0.6	0.9
2nd order interaction								
P.indica.F.mosseae	**0.008**	**0.03**	**0.06**	**<.001**	0.7	0.2	0.6	0.3
P.indica.Fertiliser	0.7	0.2	0.9	0.3	0.8	0.5	0.9	0.7
F.mosseae.Fertiliser	0.6	0.9	**0.004**	0.4	**0.03**	0.8	0.2	0.7
P.indica.F.g	**0.004**	**0.005**	0.4	**0.03**	0.9	**0.04**	0.8	0.1
F.mosseae.F.g	0.1	0.3	0.4	0.2	0.6	0.7	0.4	0.7
Fertiliser.F.g	0.7	0.5	0.9	0.8	0.6	0.5	0.7	0.3
P.indica.F.c	0.2	0.1	0.6	**0.01**	0.9	0.8	0.5	0.7
F.mosseae.F.c	**0.03**	**0.01**	**<.001**	**0.01**	**0.07**	0.6	0.7	0.5
Fertiliser.F.c	0.7	0.9	0.8	**<.001**	0.7	0.7	0.6	0.3
F.graminearum.F.c	0.4	0.5	0.9	0.6	0.9	**0.02**	0.8	0.9
3rd order interaction								
P.indica.F.mosseae.Fertiliser	0.6	0.7	0.9	**0.05**	0.5	**0.008**	0.4	**0.02**
P.indica.F.mosseae.F.g	0.08	**0.05**	**0.008**	0.7	0.09	0.7	0.5	0.9
P.indica.Fertiliser.F.g	0.9	0.5	0.9	0.04	0.2	0.8	0.1	0.6
F.mosseae.Fertiliser.F.g	0.6	0.9	**0.001**	0.1	0.4	0.09	0.5	0.7
P.indica.F.mosseae.F.c	0.4	0.7	**0.07**	**0.008**	**0.05**	0.4	0.2	0.3
P.indica.Fertiliser.F.c	0.6	0.7	0.3	0.2	0.4	0.3	0.5	0.8
F.mosseae.Fertiliser.F.c	0.6	0.4	0.06	0.4	0.3	0.7	0.9	0.4
P.indica.F.g.F.c	0.8	0.2	0.6	0.3	0.7	0.9	0.9	0.3
F.mosseae.F.g.F.c	0.6	0.3	0.4	0.9	0.1	**0.04**	0.2	0.7
Fertiliser.F.g.F.c	0.07	**0.04**	0.1	0.5	0.9	0.8	0.4	0.5
4th order interaction								
P.indica.F.mosseae.Fertiliser.F.g	0.5	0.7	0.1	0.2	0.2	0.7	0.6	0.9
P.indica.F.mosseae.Fertiliser.F.c	0.2	0.03	0.9	**0.008**	0.4	0.7	0.6	0.8
P.indica.F.mosseae.F.g.F.c	**0.05**	**0.01**	0.8	0.8	0.8	0.4	0.7	0.09
P.indica.Fertiliser.F.g.F.c	0.06	0.04	0.4	0.4	0.7	0.1	0.8	0.9
F.mosseae.Fertiliser.F.g.F.c	0.4	0.3	0.4	0.5	0.6	0.3	0.9	0.6
5th order interaction								
P.indica.F.mosseae.Fertiliser.F.g.F.c	0.7	0.9	0.4	0.8	0.6	0.8	0.8	0.5

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772 **Table 2.** ANOVA P-value for variable measured in pots of spring wheat (cv. Paragon) and
773 treated in a full factorial design with the factors shown. The experiment carried out in the 2014
774 growing season.

	P value							
Main effect	FHB severity	FHB incidence	Total above ground weight	Root weight	Total grain weight	1000 grain weight	Harvest index	No of ears
P.indica	0.07	0.2	**0.05**	**0.02**	**0.02**	0.08	0.07	**0.003**
F.mosseae	0.8	0.6	0.1	0.2	0.1	0.5	0.5	0.1
F.graminearum	**<.001**	**<.001**	0.8	0.8	0.8	0.4	0.7	**0.03**
Fungicide	**0.005**	**0.02**	0.6	0.7	0.05	0.7	**0.03**	0.12
2nd order interaction								
P.indica.F.mosseae	0.4	0.5	0.8	**0.03**	0.7	0.1	0.3	0.4
P.indica.F.g	0.2	0.4	0.4	0.6	0.08	0.1	0.07	0.9
F.mosseae.F.g	0.7	0.6	0.09	**0.05**	0.2	0.1	0.7	**0.06**
P.indica. Fungicide	0.08	0.3	0.3	0.2	0.8	0.1	0.4	0.6
F.mosseae. Fungicide	0.4	0.3	0.8	0.3	0.3	0.2	0.1	0.8
Fusarium. Fungicide	0.04	0.1	0.5	0.4	0.9	0.8	0.4	0.9
3rd order interaction								
P.indica.F.mosseae.F.g	0.8	0.9	0.7	**0.01**	0.6	0.6	0.7	0.7
P.indica.F.mosseae. Fungicide	0.9	0.9	**0.03**	0.9	**0.003**	**0.01**	**0.009**	**0.003**
P.indica.F.g. Fungicide	0.1	0.3	0.7	0.8	0.4	0.9	0.3	0.7
F.mosseae.F.g.Fungicide	0.5	0.6	0.8	0.6	0.4	0.8	0.1	0.4
4th order interaction								
P.indica.F.mosseae.F.g. Fungicide	0.7	0.6	0.2	0.3	0.3	0.5	0.9	0.5

Table 3. ANOVA P-value for variable measured in pots of six cultivars of spring wheat (cv. Paragon, Mulika, Zircon, Granary, KWS Willow and KWS Kilburn) and treated in a full factorial design with the factors shown. The experiment carried out in the 2015 growing season.

	P value							
Main effect	FHB severity	FHB incidence	Total above ground weight (g)	Root weight (g)	Total grain weight per pot (g)	1000 grain weight (g)	Harvest index	No of ears
P.indica	**<.001**	**<.001**	**0.002**	**<.001**	**<.001**	**<.001**	**<.001**	**0.002**
F.graminearum	**<.001**	**<.001**	**0.06**	0.6	**<.001**	0.2	**0.034**	0.6
Wheat cultivars	**<.001**	**<.001**	**0.02**	0.09	**0.001**	0.1	0.1	**<.001**
2nd order interaction								
P.indica.F.g	**<.001**	**0.02**	**0.04**	0.8	0.2	0.03	0.6	0.6
P.indica.wheat cultivars	0.7	0.8	0.9	0.9	0.3	0.4	0.6	0.8
FHB.wheat cultivars	0.9	0.9	0.5	0.1	0.7	0.8	0.9	0.7
3rd order interaction								
P.indica.F.g.wheat cultivars	0.2	0.2	0.3	0.5	0.3	0.5	0.2	0.6

Table 4. for Fig 4a. ANOVA table of mycotoxin DON of winter wheat (cv. Battalion).

	P value
main effect	mycotoxin DON
P. indica	**<.001**
F. culmorum	**<.001**
Fertiliser	**0.005**
F. mosseae	0.5
2rd order interaction	
P.indica.F. culmorum	**<.001**
P_indica.Fertiliser	**0.001**
Fertiliser.F.culmorum	0.09
P.indica.F.mosseae	**0.003**
F.mosseae.F. culmorum	0.3
F.mosseae.Fertiliser	0.4
3rd order interaction	
P.indica.Fertiliser.F.c	**0.05**
P.indica.F.mosseae.F.c	0.6
P.indica.F.mosseae.Fertiliser	0.4
F.mosseae.Fertiliser.F.c	0.2
4th order interaction	
P.indica.F.mosseae.Fertiliser.F.c	0.1

Table 5. for Fig 4b. ANOVA table of mycotoxin DON of spring wheat (cv. Paragon).

	P value
main effect	Mycotoxin DON
P.indica	**0.01**
F.mosseae	0.5
Fungicide	**0.001**
2nd way interaction	
P.indica.F.mosseae	**0.009**
P.indica.Fungicide	**0.03**
F.mosseae.Fungicide	0.9
3rd way interaction	
P.indica.F.mosseae.Fungicide	0.06

Table 6. for Fig 4c. ANOVA table of mycotoxin DON of six cultivars of spring wheat (cv. Paragon, Mulika, Zircon, Granary, KWS Willow and KWS Kilburn).

	P value
Main effect	Mycotoxin DON
P.indica	**<.001**
Wheat cultivars	**<.001**
2nd order interaction	
P.indica.Wheat cultivars	**0.002**

Table 7. ANOVA P-value for variable leaf tissue nutrients measured in pots of winter wheat (cv. Battalion) and treated in a full factorial design with the factors shown. The experiment carried out in the 2014/15 growing season.

Main effect	Total N	Total P	Total K	Ttal Ca	Total Mg	Total S	Total Mn	Total Cu	Total Zn	Total Fe	Total B
P.indica	0.6	0.9	0.6	0.8	0.6	0.6	0.6	0.7	0.9	**0.04**	**0.01**
F.mosseae	0.7	0.6	0.9	0.8	0.7	0.4	0.4	0.3	0.5	**0.01**	0.2
Fertliser	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**0.002**	**<.001**
2nd order interaction											
P.indica.F.mosseae	0.4	0.3	0.5	0.4	0.5	0.5	0.1	0.8	0.9	0.06	1
P.indica.Fertliser	0.6	0.7	0.9	0.8	0.8	0.3	**0.03**	0.5	0.6	0.07	**0.02**
F.mosseae.Fertliser	0.8	0.2	0.5	0.9	0.7	0.1	0.2	0.3	0.3	0.05	0.7
3rd order interaction											
P.indica.F.mosseae.Fertliser	0.04	0.9	0.3	0.3	0.1	0.3	0.8	0.3	0.4	0.1	0.3

Table 8. The effect of *P. indica* under low and high fertilisation levels on final yield of winter wheat (cv. Battalion) was confirmed on a small scale experiment in 2014/15 growing season.

Fertiliser	P.indica	Total above ground weight (g)	Root weight (g)	Total grain weight per pot (g)	1000 grain weight (g)	Harvest index	No of ears
1 g/L	-	184	8.9	69	48	0.4	30
	+	232	19.4	92	60	0.4	33
4 g/L	-	273	18.9	88	54	0.3	47
	+	296	22.05	122	61	0.4	52
	s.e.d	15.2	2.3	8.7	3.5	0.05	2.3

Table 9. ANOVA P-value for variable measured in pots of winter wheat (cv. Battalion) and treated in a full factorial design with the factors shown.

	P value					
Main effect	Total above ground weight (g)	Root weight (g)	Total grain weight per pot (g)	1000 grain weight (g)	Harvest index	No of ears
P.inidca	**0.007**	**0.001**	**<.001**	**0.003**	0.2	**0.05**
Fertliser	**<.001**	**0.002**	**0.002**	0.2	0.6	**<.001**
2nd order interaction						
P.inidca.Fertliser	0.3	**0.04**	0.5	0.3	0.4	0.7