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Effectiveness of seed treatment against *Fusarium* spp. and *Cochliobolus sativus* of spring barley in different conditions

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Abstract. Effect of fungicide seed treatments on initial growth of spring barley (*Hordeum vulgare* L.) was evaluated in greenhouse trials. The soil collected from minimum tillage fields where spring barley, spring wheat (*Triticum* L.) and oilseed rape (*Brassica napus* L.) have been cultivated in previous growing season were used in trials. Eight fungicide seed treatments and untreated seed as the control were evaluated. Root rot severity and seedling emergence rate were assessed at growth stages 20–22. In addition the incidence of seed-borne *Fusarium* spp. and *Cochliobolus sativus* and germination were assessed in treated and untreated spring barley seeds in laboratory condition. Fungicides prothioconazole and tebuconazole significantly reduced incidence of seed-borne *Fusarium* spp. Seed treated with fludioxonil and tebuconazole more effectively decreased root rot infection in soil from minimum tilled barley field, fludioxonil + difenoconazole in soil from minimum tilled spring wheat field and prothioconazole mixes with tebuconazole or fluoxastrobin in soil from minimum tilled oilseed rape field. This study brings out the pre-crop and seed treatment interaction effect on control of root rot in spring barley.

Key words: barley; fungicide; root rot disease; germination; emergence rate.

INTRODUCTION

Common root rot and seedling blight are early season diseases that occur shortly after germination and have been attributed to infection of cereal seedlings by fungi including *Fusarium* spp. and *Cochliobolus sativus*. These pathogens live in the soil and are a serious threat to spring barley causing yield losses (Kumar et al., 2002). Seed-borne pathogen *C. sativus* contributes to the development of brownish roots and coleoptiles resulting in seedling blight. A numbers of conidia of *C. sativus* varies greatly in soils from the various rotations, the highest number being in barley stubble (Piening & Orr, 1988). Sources of inoculum for *Fusarium* spp. include seed, crop residue and soil (McGee, 1995). *Fusarium* spp. that causes crown rot can infect any tissues but often invade the coleoptile as it emerges (Smiley et al., 2013). Increased levels of crop debris influence the incidence and severity of plant diseases (Bailey & Lazarovits, 2003; Paulitz, 2006; Matusinsky et al., 2009). There are larger microbial populations in the upper layers of soil in reduced tillage conditions (Krupinsky et al., 2002).

Fungicide seed treatment is widely used in grain crops for disease management because positive impact on early plant growth (Pike et al., 1993; Bradley et al., 2001; Cook et al., 2002). Fungicides form a protective zone around germinating seeds and reduce diseases caused by soil-borne pathogens (Galperin et al., 2003). Seed treatment is an advanced and economic delivery system to protect the seed from the moment of sowing. The specific action of the fungicide seed treatment on the interaction between *Fusarium* spp. and seed treatment fungicides is limited. The studies done with barley, wheat (Jones, 2000) and maize (Munkvold & O'Mara, 2002) have determined that most seed treatments are effective against soil-borne infections of *Fusarium* spp.

The objective of the study was to investigate the efficacy of different fungicides on *Fusarium* spp. and *C. sativus* on barley and evaluate germination and seedling emergence ability in different conditions.

MATERIALS AND METHODS

Studies were carried out in autumn 2012 in Estonian Crop Research Institute. Three greenhouse experiments were done with spring barley variety 'Barke'. The soil collected from minimum tillage fields where spring barley, spring wheat and oilseed rape have been cultivated in previous growing season were used in trials. The soil from minimum tilled barley field further in the text will be named as barley pre-crop soil, from spring wheat field as wheat pre-crop soil and from oilseed rape field as oilseed rape pre-crop soil. Eight seed treatment fungicides and untreated control were compared for control of root rot in spring barley. The trials were conducted in boxes for plant cultivation containing different pre crop soils using four replicats. (Table 1). The soils used in trials were collected after the harvest and soil cultivation from top 10 cm layer of minimum tillage spring barley, spring wheat and oilseed rape fields. The soils contain also stubble and straw from the crop. A mix containing field soil and sand in ratio 90:10 (%) was prepared for trials. Complex fertilizer Kemira $N_{10}P_{23}K_{14}$ was used at the rate of 20 g per box (60 x 40 cm², 8 cm deep). The boxes were kept 7 days in warm and moist conditions to propitiate pathogen mycelium growth. Boxes were maintained under cool and moist conditions for 10 days in temperatures 4–8 °C to predispose the infection and delay the seed germination. The plants were then grown in a greenhouse at 23 ± 2 °C with supplemental lighting for 14 h day^{-1} during the test period from leaf development to tillering (GS 10–22 according Zadoks et al., 1974). Root rot disease was measured using the sub-crop internode index (Ledingham et al., 1973): 1– no symptoms of disease; 2–25%; 3–50%; 4–75% of tissue with disease symptoms. 100 plants from each variant were individually scored at shooting stage (GS 20–22). These scores were transformed to root rot index (RRI) and were expressed as a percentage of the root rot by using the formula:

$$RRI = \frac{\sum 100nv}{NV} \quad (1)$$

where *n* – number of plants with the respective infection level; *v* – infection level 1–4 as described above; *N* – the number of scored plants; *V* – a maximal level of the infection.

Incidence of seed-borne *Fusarium* spp. and *Cochliobolus sativus* was assessed in laboratory conditions using one sample of 100 seeds from each treatment. All 100 fungicide treated seeds were plated onto potato-dextrose agar medium supplemented with streptomycin solution in Petri dishes and incubated at 20–22 °C, 12 hr day^{-1} 12 hr night for seven days. All seeds checked under the microscope. Incidence of both seed-borne fungal genera was calculated as the percentage of seeds infected with that genus.

Seed germination was assessed in laboratory conditions. Treated and untreated seeds were placed in Petri dishes on moist filter paper in four replicates per treatment, 25 seeds per replicate in randomized design. The tests were performed under laboratory conditions at 20 °C and natural room light and monitored daily. Seeds were considered germinated upon radicle emergence. Germination was counted at 4, 7, 11 and 14 days after the start of the trials and germination expressed as a percentage by number of normal seedlings.

Plant vigour was estimated by counting seedlings of each treatment from each pre-crop soil. The emergence percentage is calculated by dividing the number of normal seedlings per 100 seeds obtained at each treatment in the greenhouse. The emergence rate is calculated by dividing the number of days for emergence in the greenhouse (Maguire, 1962).

The Agrobase (release 20; Winnipeg, Canada) software package was used for statistical analysis. Factorial analysis of variance (ANOVA) and one-way ANOVA were applied to test the results also coefficient of determination was founded. The level of statistical significance was set at $P < 0.05$.

Table 1. Product names, active ingredients and doses used in greenhouse trials

	Product	Active ingredients per litre	Dose l t^{-1}
1	Premis® 25 FS	25 g triticonazole	2.0
2	Kinto®	20 g triticonazole + 60 g prochloraz	2.0
3	Bariton® 075 FS	37.5 g fluoxastrobin + 37.5 g prothioconazole	1.25
4	Lamardor® 400 FS	250 g prothioconazole + 150 g tebuconazole	0.2
5	Raxil® 060 FS	60 g tebuconazole	0.5
6	Maxim® 025 FS	25 g fludioxonil	2.0
7	Maxim Extra® 050 FS	25 g fludioxonil + 25 g difenoconazole	2.0
8	Maxim Star® 025 FS	18.8 g fludioxonil + 6.3 g cyproconazole	1.5
9	Untreated control	–	–

RESULTS AND DISCUSSION

Effect of fungicide seed treatment on plant emergence

Fungicide seed treatments had a significant effect on emergence of spring barley in different pre-crop soil (Table 2). Fludioxonil + difenoconazole treated seeds had significantly faster emergence rate in barley pre-crop soil and triticonazole treated seeds slower emergence rate when compared with untreated seeds. The proportion of emerged plants increased by using active substances tebuconazole, triticonazole and fludioxonil, when compared with untreated seeds.

In wheat pre-crop soil emergence rate of tebuconazole and fludioxonil treated seeds was faster when compared with untreated seeds. The emergence was most delayed in seeds treated with triticonazole + prochloraz. Significantly highest emergence rate was observed in barley seeds treated with triticonazole + prochloraz and fludioxonil + difenoconazole. Compared with untreated seeds lower emergence rate values had seed treated with tebuconazole, fludioxonil and prothioconazole + tebuconazole.

In oilseed rape pre-crop soil the emergence rate differences between treated and untreated seeds were very small. Emergence rate was significantly higher in treatments with fluoxastrobin + prothioconazole, fludioxonil + difenoconazole and fludioxonil, when compared with untreated seeds. Treatment with fluoxastrobin + prothioconazole had significantly higher emergence % when compared with untreated seeds. Other fungicides had lower values compared with those of fluoxastrobin + prothioconazole treated seed.

The effectiveness of seed treatment fungicides are usually evaluated by emergence used as an indication of plant vigor (Munkvold & O'Mara, 2002). Speed of seed emergence and seedling growth has been widely accepted as main parameter to monitor growth responses (Briggs & Dunn, 2000). In our trials it was observed at all pre-crop soils that fungicidal treatment affects seed germination and seedling vigor. Seed treatment fungicidal compounds had different decelerate effects of barley emergence. It depends on the plant protection products used. According to Platz et al. (2001) most triazole fungicides have indicated possible effect on emergence. Our results demonstrated decelerate effect on seedlings grown from seeds treated with triticonazole and mixes with prochloraz. Seedlings grown from seeds treated with tebuconazole, mixes with prothioconazole and fludioxonil indicated faster emergence rates in all pre-crop soils. In oilseed rape pre-crop soil, only slight differences were observed in emergence rates between treated and untreated seeds.

Table 2. Seed treated spring barley seedlings emergence from different pre-crop soil in greenhouse trials

Fungicide	Barley pre-crop soil		Wheat pre-crop soil		Oilseed rape pre-crop soil	
	emergence (%)	emergence rate (days)	emergence (%)	emergence rate (days)	emergence (%)	emergence rate (days)
Triticonazole	94	15.7	86	14.3	75	10.7
Triticonazole, prochloraz	84	14.0	92	15.3	80	11.4
Fluoxastrobin, prothioconazole	86	14.3	85	14.2	92	11.5
Prothioconazole, tebuconazole	86	12.3	83	11.9	83	10.3
Tebuconazole	95	11.9	81	11.6	78	9.8
Fludioxonil	93	13.3	82	11.7	83	11.9
Fludioxonil, difenoconazole	82	11.7	92	13.1	81	11.6
Fludioxonil, cyproconazole	90	12.9	91	13.0	80	10.0
Untreated	89	14.8	86	14.3	88	11.0
Average	89	13.4	86	13.3	82	10.9
$LSD_{0.05}$	1.02	0.35	1.19	0.50	1.26	0.43

Effect of fungicide seed treatment on root rot infection

Root rot infection caused by *Fusarium* spp. and *C. sativus* was observed in all pre-crop soils (Fig. 1). In untreated spring barley seedlings 10–11% of roots and crowns were colonized with root rot. All treatments, except tebuconazole in wheat and oilseed rape pre-crop soils and triticonazole in oilseed rape pre-crop soil reduced mean root rot index. Least root rot symptoms was observed in seedlings grown in wheat pre-crop soil from seeds treated with fludioxonil + difenoconazole and in barley pre-crop soil from seeds treated with fludioxonil. However no differences between pre-crop soils were observed in case of seeds treated with prothioconazole + tebuconazole. Seed treated with fluoxastrobine and prothioconazole or prothioconazole and tebuconazole worked more effectively in oilseed rape pre-crop soil. Obtained results demonstrate that minimum tillage pre-crop soils have influence to soil-borne infection of *Fusarium* spp. when no above ground symptoms are evident and the management of early season barley diseases with fungicide seed treatments can result in reduction of root rot. Fungicide active ingredients form a protective barrier around the seed against soil-borne pathogens and have a limited period of activity (Charnay et al., 2000). Systemic fungicides are able stop the progress of *Fusarium* infections (Boyacioglu, 1992). Triticonazole has been tested as seed treatment fungicide in wheat, barley and other small grains protecting against *Fusarium* spp. and other soil-borne fungi (Biradar et al., 1994). In previous studies tebuconazole selectively (Simpson et al., 2001) and prothioconazole (Suty-Heinze & Dutzmann, 2004) highly controlled *Fusarium* spp. Fungicides include fludioxonil reduce damage caused by common root rot.

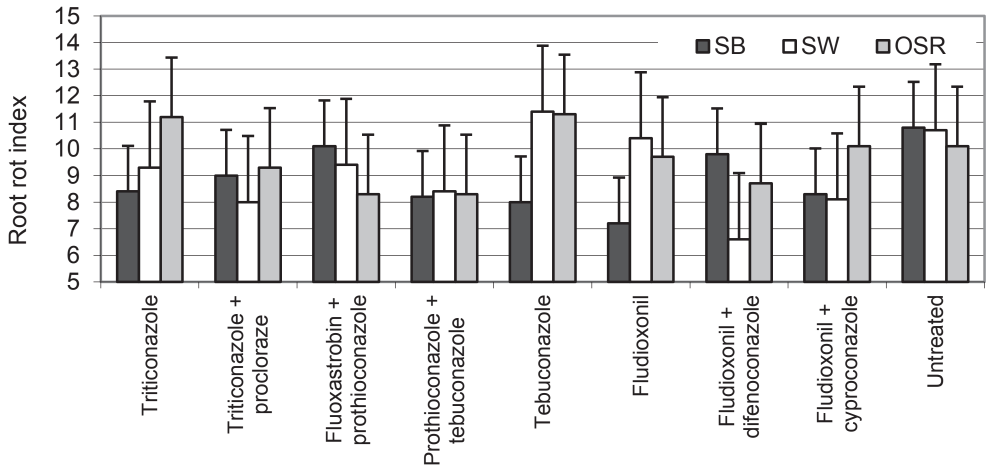

Figure 1. A percentage root rot index in seed treated spring barley seedlings grown in different pre-crop soil in greenhouse trials: SB – barley pre-crop soil; SW – wheat pre-crop soil; OSR – oilseed rape pre-crop soil. LSD0.05 = 1.72 (SB), 2.49 (SW), 2.24 (OSR).

Presence of seed-borne *Fusarium* spp. and *C. sativus* in fungicide treated barley seed

The choice of seed treatment fungicide had significant impact on the incidence and proportion of pathogenic fungi on grains. The evaluation of barley grain showed, that more prevalent fungus in treated seeds was *C. sativus* (range 33–69%) (Fig. 2). Although *Fusarium* spp. was not found so frequently as *C. sativus*, the incidence of infections by *Fusarium* spp. ranged 11–37%. *Fusarium* species was found in rate of 37% in tebuconazole treated seeds, followed by triticonazole + prochloraz (36%), fludioxonil + cyproconazole (35%) and fludioxonil + difenoconazole (34%). These treatments did not show any control of this species, when compared to 32% infections of untreated seeds. It is on contrary with results obtained by Galperin et al. (2003) where prochloraz completely suppressed seed-borne *Fusarium* in maize. According to Rodriguez-Brljevich et al. (2008) triazole active ingredients were more effective against seed-borne *Fusarium* than strobilurins. In our experiment most effective were triazoles containing active ingredients prothioconazole and tebuconazole. In untreated control 84% of seeds were contaminated with *C. sativus*. This pathogen had the high incidence of infection in seeds treated with triticonazole + prochloraz (69%) and with triticonazole (66%).

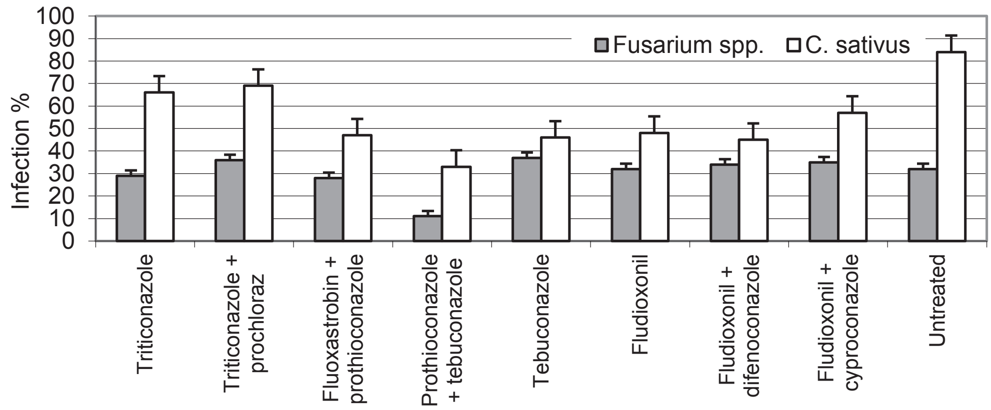

Figure 2. Incidence (%) of *Fusarium* spp. and *Cochliobolus sativus* in seed treated spring barley grains under laboratory conditions: LSD0.05 = 4.99 (*Fusarium* spp.), 15.57 (*C. sativus*).

Effect of fungicide seed treatment on germination rate

The germination of untreated spring barley seeds under laboratory conditions was 80% (Fig. 3). Nearly all seed treatment fungicides increased initial germination in days 4 compared with untreated seeds. Only treatment with fludioxonil delayed germination in all assessed days. In addition, initial germination was somewhat delayed in seeds treated with triticonazole + prochloraz and this effect was slightly observed after 14 days. Fungicide seed treatments increased the germination of barley seeds when compared with untreated seeds in use of fludioxonil + difenoconazole, fludioxonil + cyproconazole and prothioconazole + tebuconazole. A few days difference in time of germination may significantly affect seedling development. The evaluation of barley grain showed, that the germination was more uniform in seeds treated with prothioconazole + tebuconazole and fludioxonil + cyproconazole. This is in agreement with the findings of Querou et al. (1997) where the germination of wheat seeds was positively influenced by the treatment with triazoles. On the contrary to our results, the findings of Rufino et al. (2013) have shown in wheat germination test that the seed treatment with fungicide fludioxonil presented better results than without treatment.

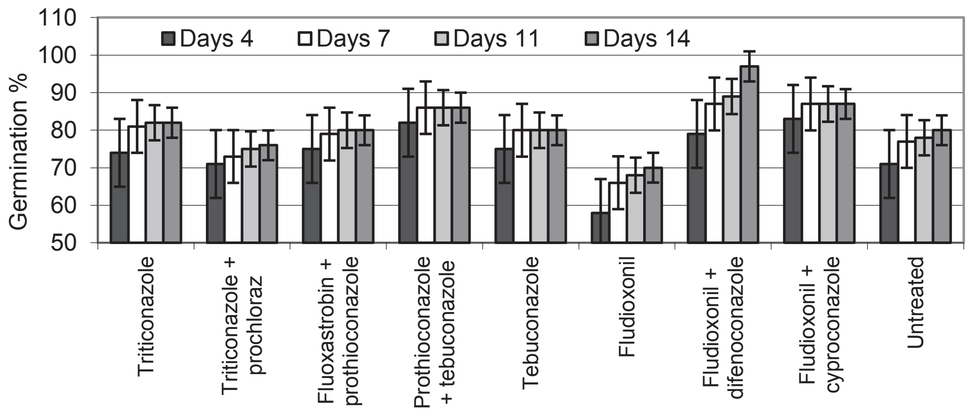

Figure 3. Effect of seed treatments on percentage of germination of spring barley 4, 7, 11 and 14 days after starting: LSD0.05 = 9.01 (days 4), 7.02 (days 7), 4.71 (days 11), 3.98 (days 14).

Results of ANOVA verified that the impact of the pre-crop soil had the biggest influence on the emergence rate and germination (Table 3). The fungicide seed treatment was major factor determining the infection level with root rot disease. The coefficients of determination indicate that environmental and fungicidal factor's contribution to the emergence rate was 70% ($R^2 = 0.70$). The occurrence of root rot was less dependent on environmental and fungicidal factors ($R^2 = 0.65$). The rest is related to some other factors.

Table 3. Mean squares of ANOVA of infection data of root rot disease, germination and emergence rate

	Root rot			Germination			Emergence rate		
	MS	*F*-ratio	*P*	*MS*	*F*-ratio	*P*	*MS*	*F*-ratio	*P*
Treatment (T)	49.4	14.07	0.000	2.8	0.37	0.019	14.4	8.52	0.000
Pre-crop soil (P)	44.0	12.5	0.000	51.3	6.82	0.932	73.0	43.23	0.000
T x P	3.1	0.9	0.587	15.6	2.08	0.018	1.6	0.96	0.512
R^2	0.6520			0.4302			0.7049		

MS - mean square, R^2 - correlation between trial factors.

CONCLUSIONS

This experiment brings out the pre-crop and seed treatment efficacy interaction and their compatibility with reduced tillage soil. The results of the study show that the benefit of fungicide seed treatment in reduction infection and colonization of spring barley by *Fusarium* spp. and *Cochliobolus sativus* is evident at seedling growth stage. Prothioconazole and tebuconazole are capable to significantly reduce and suppress seed-borne inoculum of *Fusarium* spp. The seed treatments with fludioxonil, tebuconazole or prothioconazole showed best results in increasing plant stand and vigour and reducing seedling root rot in minimum tillage soil where spring barley was used as preceding crop. This is an important contribution to the epidemiology of soil-borne *Fusarium* spp. and root rot infection.

REFERENCES

Bailey, K.L. & Lazarovits, G. 2003. Suppressing soil-borne diseases with residue management and organic amendment. *Soil Tillage Res*. **72**, 169–180.

Biradar, D.P., Pedersen, W.L. & Rayburn, A.L. 1994. Nuclear DNA analysis of maize seedlings treated with the triazole fungicide, triticonazole. *Pestic. Sci.* **41**, 291–295.

Boyacioglu, D., Hettiarachchy, N.S. &Stack, R.W. 1992. Effect of three systemic fungicides on deoxynivalenol (vomitoxin) production by Fusarium graminearum in wheat. *Can. J. Plant Sci.* **72**, 93–101.

Bradley, C.A., Wax, L.M., Ebelhar, S.A., Bollero, G.A. & Pedersen, W.L. 2001. The effects of fungicide seed protectants, seeding rates, and reduced rated of herbicides on no-till soybean. *Crop Prot*. **20**, 615–622.

Briggs, K.G. & Dunn, G.J. 2000. Variation amongst Canadian six-row spring barley cultivars for germination and emergence characteristics in controlled environments and in the field. *Can. J. Plant Sci*. **80**, 247–253.

Charnay, M.P., Vergé, C. & Barriuso, E. 2000. Influence of soil type and water content on release of triticonazole from coated maize seed. *Pest Management Sci.* **56**, 249–256.

Cook, J.R., Weller, D.M., El-Banna, A.Y., Vakoch, D. & Zhang, H. 2002. Yield responses of direct-seeded wheat to rhizobacteria and fungicide seed treatments. *Plant Dis*. **86**, 780–784.

Galperin, M., Graf, S. & Kenigsbuch, D. 2003. Seed treatment prevents vertical transmission of Fusarium moniliforme, making a significant contribution to disease control. *Phytoparasitica* **31**, 344–352.

Jones, R.K. 2000. Assessments of Fusarium head blight of wheat and barley in response to fungicide treatment. *Plant Dis*. **84**, 1021–1031.

Krupinsky, J.M., Bailey, K.L., McMullen, M.P., Gossen, B.D. & Turkington, T.K. 2002. Managing plant disease risk in diversified cropping systems. *Agron. J.* **94**, 198–209.

Kumar, J., Schafer, P., Hückelhoven, R., Langen, G., Baltruschat, H., Stein, E., Nagarajan, S. & Kogel, K. 2002. *Bipolaris sorokiniana* a cereal pathogen of global concern: cytological and molecular approaches towards better control. *Mol. Plant Pathol.* **3**(4), 185–195.

Ledingham, R.J., Atkinson, T.G., Horrocks, J.S., Piening, L.J., Mills, J.T. & Tinline, R.D. 1973. Wheat losses due to common root rot in the prairie provinces of Canada. *Can. J. Plant Dis. Surv*. **53**, 113–122.

Maguire, J.D. 1962. Speed of germination aid in selection and evolution for seedling emergence and vigour. *Crop Sci.* **2**, 176–177.

Matusinsky, P., Mikolasova, R., Klem, K. & Spitzer, T. 2009. Eyespot infection risks on wheat with respect to climatic conditions and soil management. *J. Plant Pathol.* **91**, 93–101.

McGee, D. 1995. Epidemiological approach to disease management through seed technology. *Annu. Rev. Phytopathol*. **33**, 445–466.

Munkvold, G.P. & O'Mara, J.K. 2002. Laboratory and growth chamber evaluation of fungicidal seed treatments for maize seedling blight caused by Fusarium species. *Plant Dis*. **86**, 143–150.

Paulitz, T.Z. 2006. Low input no-till cereal production in the Pacific Northwest of the U.S.: the challenges of root diseases. *Eur. J. Plant Pathol.* **115**, 271–281.

Piening, L.J. & Orr, D. 1988. Effects of crop rotation on common root rot of barley. *Can. J. Plant Pathol.* **10**, 61–65.

Pike, K.S., Reed, G.L., Graft, G.T. & Allison, D. 1993. Compatibility of imidacloprid with fungicides as a seed-treatment control of Russian wheat aphid 22 (Homoptera: Aphididae) and effect on germination, growth, and yield of wheat and barley. *J. Econ. Entom.* **86**, 586–593.

Platz, G.J., Meldrum, S.I. & Webb, N.A. 2001. Chemical control of seed borne diseases of barley. *Proceeding of the 10th Australian Barley Technical Symposium*. Australia: Canberra; p.16–20.

Querou, R., Euvrard, M. & Gauvrit, C. 1997. Uptake of triticonazole, during imbibition, by wheat caryopses after seed treatment. *Pestic. Sci*. **49**, 284–290.

Rodriguez-Brljevich, C., Robertson, A.E. & Nordman, D.J. 2008. Effectiveness of fungicide seed treatments against seed-borne *Fusarium verticillioides* in maize (*Zea mays* L.). *2008 Annual Meeting Abstract. Phytopath* **98**, S134.

Rufino, C.A., Tavares, L. C., Brunes, A.P., Lemes, E.S. & Villela, F.A. 2013. Treatment of wheat seed with zinc, fungicide, and polymer: seed quality and yield. *J. Seed Sci.* **35**(1), 106–112.

Simpson, D.R., Weston, G.E., Turner, J.A., Jennings, P. & Nicholson, P. 2001. Differential control of head blight pathogens of wheat by fungicides and consequences for mycotoxin contamination of grain. *Eur. J. Plant Pathol.* **107**, 421–431.

Smiley, R.W., Machado, S., Gourlie, J.A., Pritchett, L.C., Yen, G.P. & Jacobsen, E.E. 2013. Influence of semiarid cropping systems on root diseases and inoculum density of soil-borne pathogens. *Plant Dis.* **97**, 547–555.

Suty-Heinze, A. & Dutzmann, S. 2004. *Fusarium* head blight: an additional strength of Prothioconazole. *Pflanzenschutz-Nachrichten Bayer* **57**, 265–282.

Zadoks, J.C., Chang, T.T. & Konzak, C.F. 1974. A decimal codes for the growth stages of cereals. *Weed Res.* **14**, 415–421.