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Management of seed borne root and foot rot diseases of pea (*Pisum sativum* L.) with a fungicide seed treatment

Irena GAURILČIKIENĖ[1], Rūta ČESNULEVIČIENĖ[2], Dalia JANUŠAUSKAITĖ[1], Antanas RONIS[1]

[1]Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry
Instituto 1, Akademija, Kėdainiai distr., Lithuania
E-mail: irenag@lzi.lt

[2]Perloja Experimental Station, Lithuanian Research Centre for Agriculture and Forestry
Sodo 12, Perloja, Varėna distr., Lithuania

Abstract

Management of seed borne root and foot rot diseases of pea (*Pisum sativum* L.) through a seed treatment with Raxil extra (a.i. tebuconazole 15 g l^{-1} and thiram 500 g l^{-1}) at a dose of 2.0 l t^{-1} and Kinto (a.i. triticonazole 20 g l^{-1} and prochloraz 60 g l^{-1}) at a dose of 1.5 l t^{-1} was investigated during 2008–2010 under different soil and climatic conditions in two sites: 1) on a *Cambisol* (*CM*) in Central Lithuania, in Dotnuva, and 2) on a *Luvisol* (*LV*) in the south-east part of the country, in Perloja, and in a laboratory test. The commercial seed of semi-leafless cv. 'Pinochio' was used. High infestation of pea seeds by causal agents of seed borne foot and root rots, reduction of seed germination and considerable infection transmission from seed to seedling were determined. Fungicide seed treatment markedly decreased seed borne infection of seedlings' hypocotyls and rootlets and improved germination. In the field conditions, pea root and foot rot infestation varied between the sites mostly depending on soil moisture status, while the soil type is likely to have had little effect. Seed treatment gave a significant reduction in root and foot rot infection until flowering stage in both experimental sites. The area under disease progress curve (AUDPC) in the plots sown with fungicide-treated seed was significantly lower compared with the untreated in both sites. No consistent pea grain yield and thousand grain weight (TGW) increase resulting from chemical seed treatment was obtained.

Key words: *Pisum sativum*, root and foot rots, AUDPC, fungicide seed treatment.

Introduction

Pea (*Pisum sativum* L.) production is constrained by several soil borne and foliar diseases (McPhee, 2003). Pea is highly susceptible to pre-emergence damping off and to post-emergence root and foot rots caused by soil borne and seed borne fungal infection. In many areas, root and foot rots caused by a complex of soil borne pathogens are among the most destructive diseases of pea. More than 20 different fungi have been implicated as causal agents in different regions of the world. In Denmark and Sweden, the most frequently isolated pathogens from pea roots were *Phoma pinodella* and *Fusarium solani*, other pathogens involved in the root rot complex were *F. avenaceum*, *F. oxysporum*, *F. culmorum*, *Chalara elegans*, *Phytium irregulare* and *Mycosphaerella pinodes* (Persson et al., 1997). Three fungal species *Ascochyta pisi* Lib., *Mycosphaerella pinodes* (Berk.&A.Bloxam) Verstergr (anamorph *Ascochyta pinodes* L.K.Jones) and *Phoma pinodella* (L.K.Jones) Morgan-Jones & K.B.Burch (syn. *Phoma medicaginis* var. *pinodella* (L.K. Jones) Boerema) are responsible for *Ascochyta* blight disease of pea. All species can be seed borne and survive on plant residues in soil. They occur singly or in combination and are referred to as the *Ascochyta* complex (Wallen, 1965; Onfroy et al., 1999). Seed infestation can result in high levels of disease in subsequent crop. Seed borne infestation by *M. pinodes* and *P. pinodella* usually causes more widespread and severe seedling diseases than *A. pisi* infestation (Bowen, 1992). Infected seeds show varying degrees of shrivelling and discoloration, while other infected seeds remain symptomless. Planting of *Ascochyta*-infected seeds reduced number or vigour of emerging plants and similarly had deleterious effects on yield (Hwang et al., 1991; Tivoli et al., 1996; Marcinkowska et al., 2009; Setti et al., 2009; Boros, Marcinkowska, 2010). *P. pinodella* and *M. pinodes* resulted in black coloured epicotyls and slight plant weight reduction (Persson et al., 1997). These pathogens were prevalent in most pea crops in South Australia and were identified as probable major contributors to yield decline (Davidson, Ramsey, 2000). Pre-emergence damping off and post-emergence foot rot are characteristic symptoms of seed borne *M. pinodes* (Moussart et al., 1998). Infection caused by *M. pinodes* and *P. pinodella* produces indistinguishable symptoms that include stem base rot as well as necrotic spots on leaves, stems, and pods. Leaf and pod spots are usually caused by *A. pisi* (Wallen, 1965; 1974). Identification by cultural and morphological characteristics (size of pycnidia, shape and size of conidia, and presence or absence of chlamydospores, may be uncertain because it is based on small differences in morphological criteria that are often dependent on cultural conditions, leading to misidentification (Faris-Mokaiesh et al., 1996). *M. pinodes* was distinguished from *P. pinodella* on the basis of presence of pseudothecia, a higher number of larger, bicellular conidia, compared with the smaller, predominantly unicellular conidia of *P. pinodella* (Onfroy et al., 1999). The lesions caused by *A. pisi* are different from those caused by of *M. pinodes* and *P. pinodella*, both of which cause substantial losses in yield and seed quality (Allard et al., 1993). Apart from *Ascochyta* complex, seed borne are fungi of *Fusarium* genus, *Alternaria tenuissima*, *A. alternata*, *Penicillium* spp. and other fungi that can also cause root rots and deteriorate pea seedlings' germinating power, growth, and health (Prokinova, Markova, 1997; Marcinkowska, 2008).

Development of pea cultivars resistant to main causal agents of root rots would assist in controlling this disease and minimising its damage. Partial resistance to *M. pinodes* in pea is quantitatively inherited (Priol-Gervais et al., 2007). However, partial resistance was not expressed when pathogen inoculum concentration was high (Onfroy et al., 2007). Whereas, high levels of resistance to *Ascochyta* blight have not been found, the most effective practices could be an integrated disease management (Davidson, Kimber, 2007). Fungicide seed treatment prevents transmission of seed borne fungal pathogens and also controls soil borne fungi which induce seed rots and pre- and post-emergence root and stem disease of young seedlings, causing poor seedling establishment. Evidence is provided that crop rotation, destruction of infected pea trash and chemical seed treatments can significantly reduce the amount of primary inoculums (Bretag et al., 2006). Effective seed treatment managed to reduce seed borne inoculum and lessened introduction of the pathogens into the new areas (Hwang et al., 1991).

Earlier studies evidenced the importance of root and foot rots of pea crops in Lithuania (Gaurilčikienė, Janušauskaitė, 2007; Gaurilčikienė et al., 2008). The objective of the present study was to estimate the duration of chemical pea seed treatment effects on the progress of root and foot rot diseases in two different soils and sites of Lithuania.

Materials and methods

Field experiments. The field research was carried out during the period 2008–2010 at the Institute of Agriculture (Dotnuva in Central Lithuania) and Perloja Experimental Station (Perloja in South-east Lithuania) of the Lithuanian Research Centre for Agriculture and Forestry. The commercial seed cv. 'Pinochio' was used to assess the impact of fungicide seed treatments on the progress of pea root and foot rots. Kinto (a.i. triticonazole 20 g l^{-1} and prochloraz 60 g l^{-1}) at a dose of 1.5 l t^{-1} and Raxil extra (a.i. tebuconazole 15 g l^{-1} and thiram 500 g l^{-1}) at a dose of 2.0 l t^{-1} were used for pea seed treatment using a seed treating machine "Amazone" (Germany) with the water slurry of 10 l t^{1}. The seeds from the same lot were sown in both experimental sites. An untreated control was used. A split-plot randomized complete block design with four replicates was employed. The field trials were sown at a seed rate of 1 million viable seeds per ha with a drilling machine "Fiona" (Denmark). The plots were 3 m wide and 24 m long with a row spacing of 12.5 cm. The grain was harvested at complete maturity with a plot harvester "Wintersteiger Delta" (Germany).

The assessments of foot and root rots were made every 14 days from emergence to ripening. The disease severity assessments were done on a sample of 30 plants per plot: foot rots were assessed visually according to Garry et al. (1996) using a 0–5 point scale: 0 – no visible necrosis, 1 – necrosis represented by a few streaks, 2 – numerous streaks, 3 – coalesced streaks forming 3–5 mm lessons, 4 – up to 3 mm necrosis encircling the stem, 5 – necrotic zone wider than 3 mm encircling the stem. Root rots were assessed according to Grünwald et al. (2003) using a 0–5 point scale: 0 – no symptoms, 1 – slight lesions, 2 – lesions coalescing around main root, 3 – lesions starting to spread into the root system with root tips starting to be infected, 4 – root system almost completely infected and only slight amount of white, uninfected tissue left, 5 – completely infected root. A disease severity index (DSI %) of foot and root rots was calculated for each plot by the following formula: $\mathrm{DSI} = \sum (pn)100 / \mathrm{PN}$, were $\sum(pn)$ – sum of the point product with the number of plants affected at this point, P – the highest point value of the scale (5), N – number of plants assessed (Kim et al., 2000). Plant growth stages were identified according to the BBCH scale (Weber, Bleiholder, 1990). The area under disease progress curve (AUDPC) was used to summarize the progress of disease severity in untreated and treated seed assays. AUDPC was calculated by trapezoidal integration in accordance with 14 days' interval disease severity data over the season. $\mathrm{AUDPC} = \sum_{i=1}^{n-1}\left(\frac{y_i + y_{i-1}}{2}\right)(t_{i+1} - t_i)$, where y_i – disease severity %, t_i – interval of data records (days), n – number of assessments (Campbell, Madden, 1990).

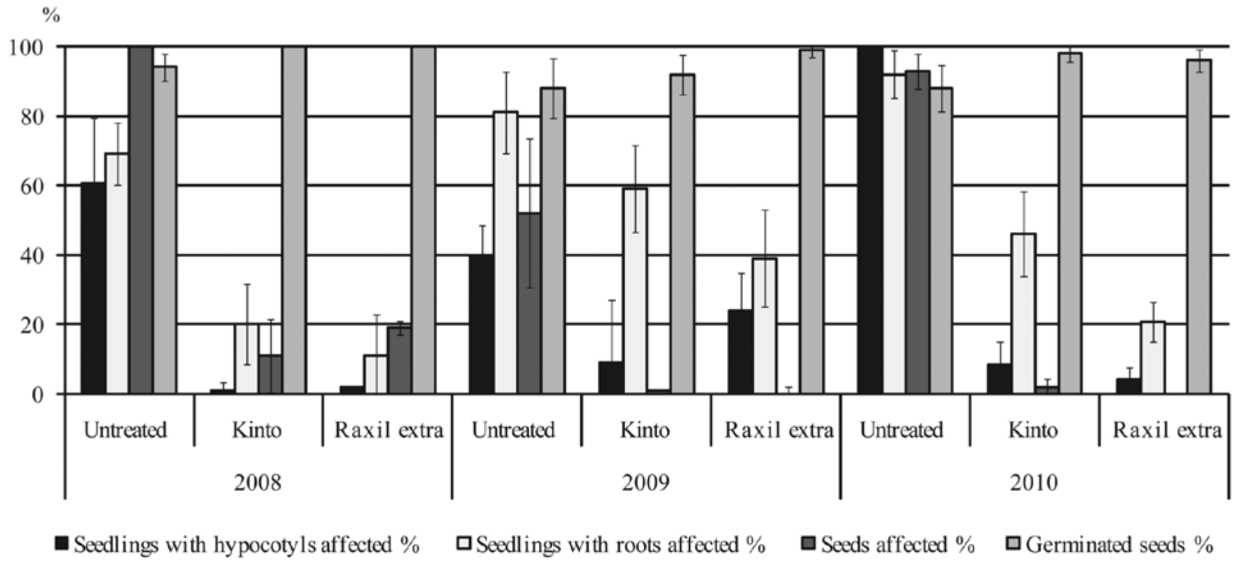
Pea seed and seedling infection test. Seed borne root rot infection of seedlings grown from untreated and treated seeds was estimated using the blotter rolls method (ISTA, 1996). One hundred (25 × 4) pea seeds were placed on 20 cm wide and 50 cm long blotter paper strips, covered by narrow (5 cm) blotter and oil-paper strips and rolled up. The rolls were stringed and soaked to 1/3 in water, and incubated for 21 days at 20°C and 12 h daylight photoperiod. Germinated seeds, seeds and seedlings showing discolorations (brownish or black lesions) on seeds, hypocotyls and rootlets were counted.

Meteorological data. During the period 2008–2010, metrological conditions varied between years and experimental sites – Dotnuva and Perloja. Over the entire period in Dotnuva (central zone), April-July monthly air temperature was slightly higher compared to Perloja (south-east zone). In May, over the whole period, substantially more precipitation was in Perloja, whereas in Dotnuva over the years 2008 and 2009, droughty conditions prevailed. June both in Dotnuva and Perloja in 2008 was droughty; however, excess humidity prevailed in 2009 and 2010. In July, lack of precipitation was observed only in 2008 in Dotnuva, whereas in 2009 and 2010, rainy weather prevailed both in Dotnuva and Perloja. Due to substantial higher wetness in Perloja, the conditions for root and stem base diseases of pea were more conductive compared with Dotnuva.

Statistical analyses. Statistical analyses were performed on the data of disease severity and AUDPC. The analysis of variance procedure (*ANOVA*) and Fisher test were used and standard deviations were calculated (Tarakanovas, Raudonius, 2003).

Results and discussion

Pea seedling infection by seed borne rots. This study was designed to assess the feasibility of reducing pea seedling rots' infestation with fungicide seed treatment. Kinto (triticonazole + prochloraz) and Raxil extra (tebuconazole and thiram) were chosen because of their non-phytotoxicity to pea seed germination (Gaurilčikienė et al., 2008). 'Pinochio' pea seeds used for seed treatment trials were tested in a wet blotter roll test. After three weeks' incubation, all untreated seeds in 2008 and 2010 showed brownish or black lesions resulting from seed borne infection, while in 2009 only half of the seeds were affected. The reduced germination of untreated seed was registered yearly (Fig. 1). Hypocotyls and rootlets of the seedlings grown from untreated seeds were severely affected by seed borne infection; especially high seedling infestation was observed in 2010. Lower seed infestation in 2009 resulted in a smaller number of seedlings with hypocotyl lesions. Fungicide treatment gave a very marked reduction of seedlings' hypocotyl and rootlet infection with seed borne causal agents. These results were obtained in perfect conditions for seed emergence and seed borne pathogens – sufficiently moist and warm. It has been documented that emerged seedlings from the seed lot with high rate of seed infestation was from 15% to 26% less than that of low seed infestation depending on soil moisture and temperature (Hwang et al., 1991). The main seed borne pea foot and root rots' pathogens were identified earlier as *Phoma pinodella* that prevailed among the fungus *Ascochyta* complex and *Fusarium* spp. (Gaurilčikienė, Janušauskaitė, 2007; Gaurilčikienė et al., 2008). *P. pinodella* and *M. pinodes* caused similar symptoms on seedling epicotyls – black coloured lesions (Persson et al., 1997), while *Fusarium* spp. was the most frequently isolated from root rot lesions of diseased pea plants (Hwang, Chang, 1989; Prokinova, Markova, 1997; Marcinkowska, 2008).

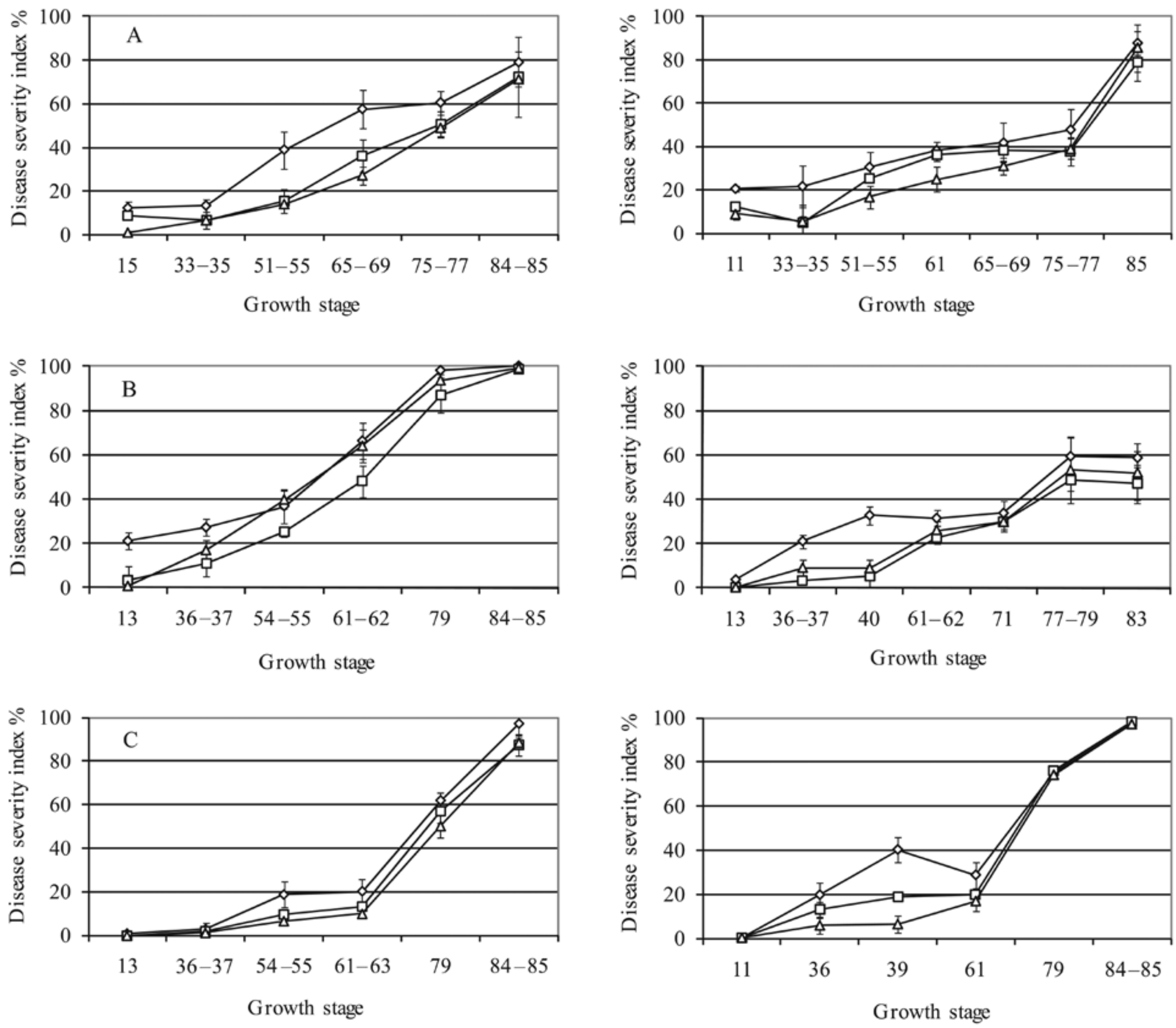


Note. Standard deviation bars are given (blotter roll test).

Figure 1. Disease incidence (%) on hypocotyls and roots of seedlings, seeds and percentage of germinated seeds in 2008, 2009 and 2010

Effects of fungicide seed treatment on foot and root rot infestation. Field experiments were done in two different sites: 1) on a *Cambisol* (CM) in Central Lithuania, in Dotnuva, and 2) on a *Luvisol* (LV) in the south-east part of the country, in Perloja. The weather conditions varied between the sites and growing seasons (2008–2010). There were no marked temperature differences between central and south-eastern parts of Lithuania; however, the amount of rainfall differed considerably.

During the period 2008–2009, higher pea root rot infestation was in Perloja compared with Dotnuva (Fig. 2). It is likely that wetter weather conditions in south-east part of the country were responsible for this. In 2008 and 2009, in both sites fungicide seed treatment with both Raxil extra and Kinto gave a very high efficacy against root rot diseases until stem elongation stage. Pea root rot infestation later in the season varied between the sites mostly depending on the soil moisture conditions. In 2008, the longest significant effect of root rots reduction lasted until the end of grain filling stage in both sites. During the period 2009–2010, the significant reduction effect of the disease lasted until flowering stage, except for Raxil extra treatment in Perloja (2009). Both in 2008 and 2009, more rapid disease severity increase in untreated plots was in Perloja, while the best seed treatment response to root rot reduction until the pea flowering stage was in 2008 in Perloja, and in 2009–2010 in Dotnuva. The least efficacy of fungicide seed treatment was in 2010 in Perloja, when rainy weather prevailed during the entire pea growing season. In the same year in Dotnuva, a good reduction of root rots was achieved until flowering stage due to dry period at pea stem elongation – bud formation stages, which inhibited infection.



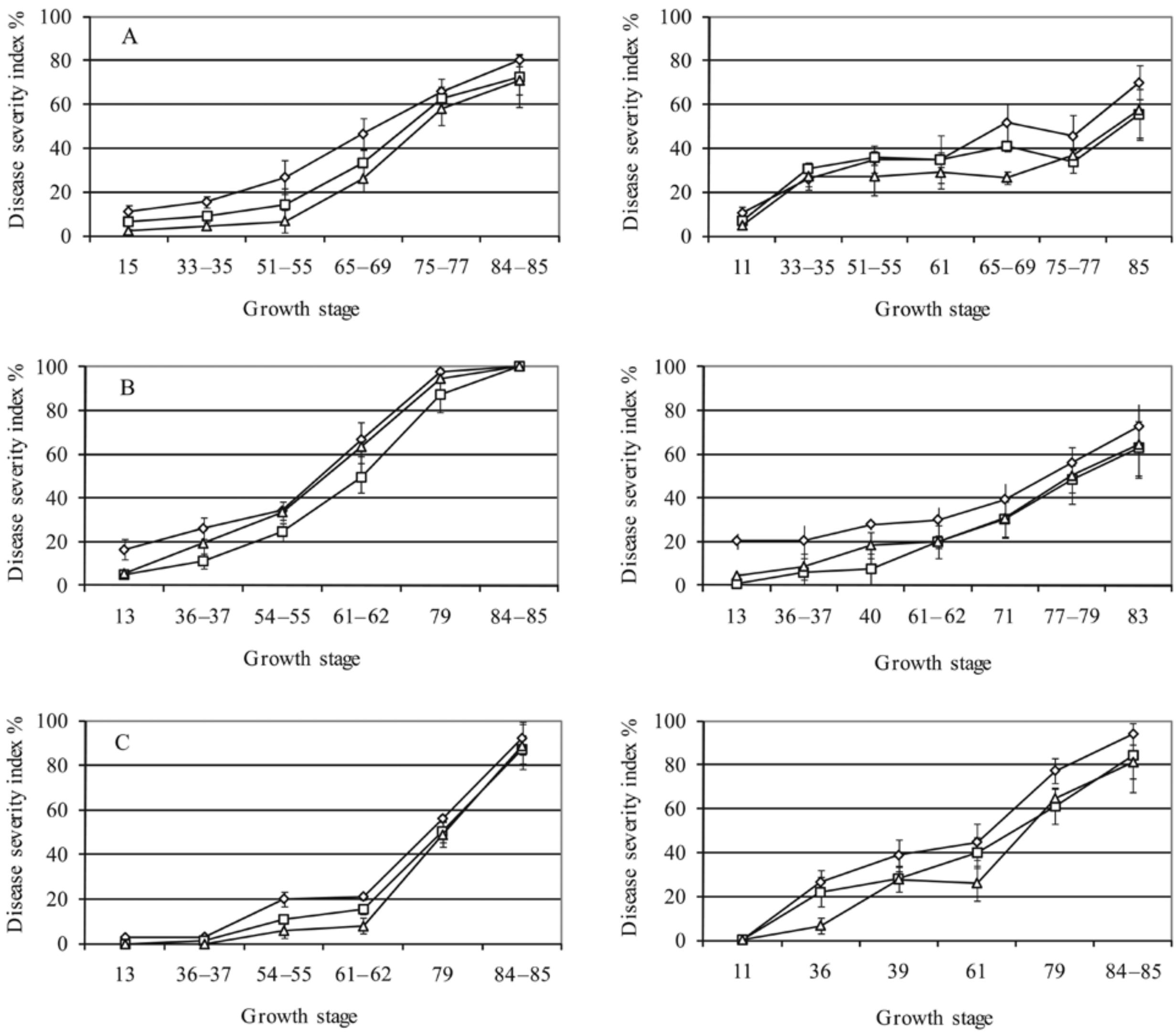
Note. Standard deviation bars are given.

Figure 2. Root disease development in 2008 (A), 2009 (B) and 2010 (C) in untreated (◊), treated by Kinto (□) and treated by Raxil extra (Δ) treatments in Perloja (left) and Dotnuva (right)

Pea foot rot infestation progress curves were comparable with those of root rot (Fig. 3). The highest level of foot rot severity at ripening stage was in 2009 in Perloja, and in 2010 both in Perloja and Dotnuva, while the least – in 2008 and 2009 in Dotnuva; however the infection rate curves differed between years and sites. Due to the seed treatment, the longest significant reduction of foot rot lasted until the ripening stage in 2008 in Dotnuva, while in most cases both in Perloja and Dotnuva the significant reduction of the disease lasted until flowering stage.

To summarize the progress of root and foot rots' severity in untreated and fungicide treated plots the area under disease progress curve (AUDPC) was calculated. AUDPC summarizes the rate of disease development over the time from seedling to maturity. Due to severe disease infection, the computed AUDPC values over the entire experimental period were very high (Fig. 4). In most cases, a significant reduction of AUDPC values in seed treated plots was determined. In 2009, a distinct difference in AUDPC of foot and root rots between the experimental sites was observed. Compared with the entire experimental period, that year the highest AUDPC of both root and foot rots was in Perloja, while the lowest in Dotnuva, where the development of rots was suppressed by droughty conditions that persisted from pea germination until flowering stage, and conversely, in Perloja unusually warm whether with heavy rains created favourable conditions for disease infection. However, in 2010 in Dotnuva, more intense root and foot rots at stem elongation determined higher AUDPC values compared with the Perloja site.

The severe root and foot rot infection in pea crop indicated a serious problem limiting pea production and increasing crop lodging. Using chemical seed treatment, a substantial reduction of infection could be achieved only until the flowering stage; however, later in the season in wet years pea plants became heavily infested by foot and root rots. The lack of significance among the fungicide seed treatments could be explained by the ability of pathogen populations to quickly increase and

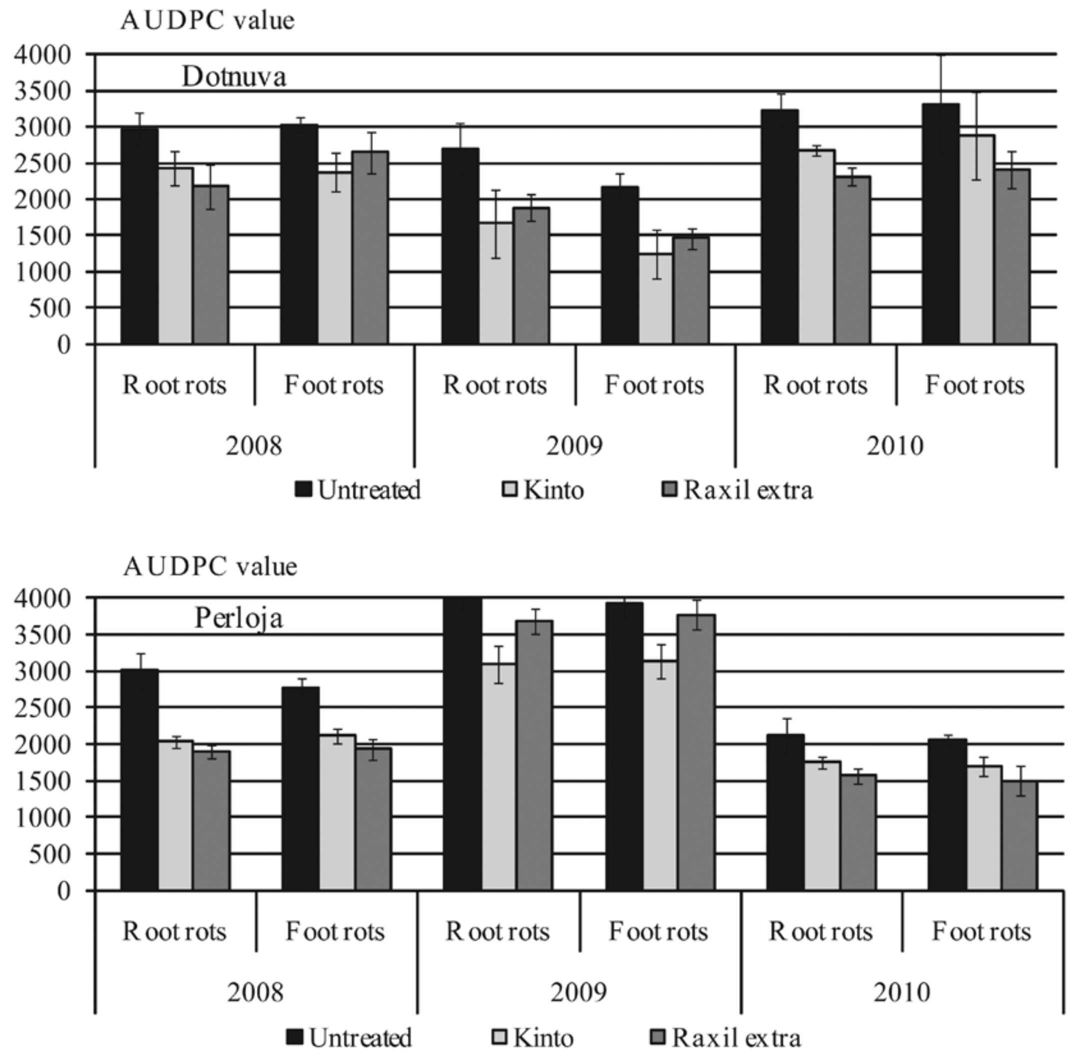


Note. Standard deviation bars are given.

Figure 3. Foot rot disease development in 2008 (A), 2009 (B) and 2010 (C) in untreated (◊), treated by Kinto (□) and treated by Raxil extra (Δ) treatments in Perloja (left) and Dotnuva (right)

rapidly spread when environmental factors favour their development, especially where peas have been intensively cropped (Wallen, 1965; 1974). Given the fact that *Ascochyta* complex and *Fusarium* spp. pathogens can be seed borne and survive on plant residues in soil, the sources of infection notably increased in pea intensive cropping areas. Davidson and Ramsey (2000) indicated that short rotation intervals (≤5 years) between pea crops were correlated with increased levels of *Phoma pinodella* infestation and lower grain yields. Many studies performed in different countries indicated that planting of *Ascochyta*-infected seeds led to the reduction of number or vigour of emerging plants and similarly had deleterious effects on yield (Tivoli et al., 1996; Marcinkowska et al., 2009; Setti et al., 2009; Boros, Marcinkowska, 2010). The assay of Moussart et al. (1998) identified that *Ascochyta* blight (*M. pinodes*)-infected seeds caused serious losses, as a result of poor germination and high transmission of the disease to underground plant parts, death of young seedlings. The authors of this research pointed out that seed borne *Ascochyta* blight remains near the basal parts of the plant as a foot rot symptom and seed borne inoculums of *M. pinodes* cannot be regarded as a source of contamination in the epidemiology of the disease.

Effects of fungicide seed treatment on pea grain yield and thousand grain weight (TGW). Although our findings showed a significant reduction of root and foot rots resulting from chemical seed treatment until flowering stage, the grain yield and TWG increase was not obtained, except for one case in 2010 in Perloja, when a significant pea grain yield increase was obtained (Table). It might have been determined by the fact that that year the infection process until flowering stage was slow and the significant foot and root rot reduction persisted until yellow ripening stage. Many studies suggest that the use of infected seeds induces a significant reduction in emergence, resulting in poor crop establishment (Wallen, 1965; 1974; Moussart et al., 1998). In our experiment, commercial seeds with good germination power were used and it might be the reason why seed treatment did not give any tangible



Note. Standard deviation bars are given.

Figure 4. The area under disease progress curve (AUDPC) of pea root and foot rots in the Perloja and Dotnuva sites in 2008, 2009 and 2010

Table. The influence of chemical seed treatment on pea grain yield and thousand grain weight (TGW)

Fungicide	Yield t ha^{-1}		TGW g	
	Perloja	Dotnuva	Perloja	Dotnuva
		2008		
Untreated	2.34 a	2.52 a	206.5 a	232.8 a
Kinto	2.32 a	2.41 a	206.0 a	239.2 a
Raxil extra	2.57 a	2.51 a	205.3 a	239.1 a
		2009		
Untreated	1.68 a	2.09 a	174.1 a	256.9 a
Kinto	1.51 a	2.11 a	168.2 a	259.5 a
Raxil extra	1.55 a	2.11 a	170.0 a	256.8 a
		2010		
Untreated	1.80 a	2.56 a	169.2 a	224.0 a
Kinto	1.74 a	2.41 a	162.9 b	225.9 a
Raxil extra	2.16 b	2.27 a	165.2 a	229.8 a

Note. The values of the year followed by the same letter in columns are not significantly different at $P \leq 0.05$.

yield increase. The lack of seed treatment efficiency for yield was reported by Hwang et al. (1991) who noted a significant grain yield increase only in Agrosol from 5 fungicide seed treatments tested. The number of emerged seedling and bushel weight of harvested seed were significantly reduced for seed with high *Ascochyta* infection and also for the pea after pea site; however seed yields were not affected by high or low levels of seed infestation. As Davidson and Kimber (2007) concluded, the most effective practice, established by decades of research, is the usage of combination of disease free seed, destruction or avoidance of inoculums sources, manipulation of sowing dates, seed and foliar fungicides, and cultivars with improved resistance.

Conclusions

1. High infestation of pea seeds by causal agents of seed borne foot and root rots, reduction of seed germination and considerable infection transmission from seed to seedling were determined. Fungicide seed treatment highly decreased seed borne infection of seedlings' hypocotyls and rootlets.

2. In the field conditions, pea seed treatment resulted in a significant reduction of root and foot rot infection until flowering stage in both experimental sites.

3. The level of pea root and foot rot infestation varied between the sites mostly depending on soil moisture conditions, while the soil type is likely to have had little effect. Disease AUDPC in the plots sown with fungicide-treated seeds was significantly lower than that in untreated in both Dotnuva and Perloja sites.

4. No consistent pea grain yield and thousand grain weight (TGW) increase resulting from chemical seed treatment was obtained.

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Sėjamojo žirnio (*Pisum sativum* L.) šaknų ir pašaknio puvinių žalos mažinimas, sėklą beicuojant cheminiais beicais

I. Gaurilčikienė[1], R. Česnulevičienė[2], D. Janušauskaitė[1], A. Ronis[1]

[1]Lietuvos agrarinių ir miškų mokslų centro Žemdirbystės institutas
[2]Lietuvos agrarinių ir miškų mokslų centro Perlojos bandymų stotis

Santrauka

2008–2010 m. lauko bandymuose skirtingų dirvožemių bei klimato zonose: 1) rudžemyje (RD) centrinėje Lietuvoje Dotnuvoje, 2) išplautžemyje (ID) Pietryčių Lietuvoje Perlojoje ir laboratorinėmis sąlygomis tirta žirnių šaknų bei pašaknio puvinių žalos mažinimo galimybė sėklą beicuojant cheminiais beicais Raxil ekstra (v. m. tebukonazolas 15 g l^{-1} bei tiramas 500 g l^{-1}) 2,0 l t^{-1} ir Kinto (v. m. tritikonazolas 20 g l^{-1} bei prochlorazas 60 g l^{-1}) 1,5 l t^{-1}. Tyrimų metu naudota veislės 'Pinochio' pusiau belapių žirnių sėkla. Nustatytas labai didelis sėklos užterštumas šaknų bei pašaknio ligų pradais, o dygimo metu – smarkus užkrato persidavimas nuo sėklų į daigus. Žirnių sėklą išbeicavus cheminiais beicais, labai sumažėjo daigų hipokotilio ir šaknelių pažeidimas, pagerėjo sėklos daigumas. Žirnių pažeidimas šaknų bei pašaknio puviniais lauko sąlygomis tarp Dotnuvos ir Perlojos vietovių įvairavo daugiausia priklausomai nuo dirvos drėgnio įvairiais augalų vegetacijos tarpsniais, o dirvos tipas, matyt, mažai lėmė ligos intensyvumą. Sėklą išbeicavus cheminiais beicais, žirnių šaknų ir pašaknio puvinių infekcijos esminis sumažėjimas buvo iki augalų žydėjimo tarpsnio. Šaknų ir pašaknio puvinių AUDPC (plotas po ligos vystymosi kreive per vegetaciją) buvo iš esmės mažesnis beicuota sėkla apsėtuose laukeliuose, lyginant su nebeicuota. Sėklos beicavimas cheminiais beicais žirnių derliui ir 1000-čio grūdų masei nuoseklios esminės įtakos neturėjo.

Reikšminiai žodžiai: *Pisum sativum*, šaknų ir pašaknio puviniai, AUDPC, sėklos beicavimas.