ISSN 1392-3196
Zemdirbyste-Agriculture, vol. 95, No. 3 (2008), p. 50–57
UDK 633.358:632.4:[631.531.01:632.95]

THE SUPPRESSION OF STEM BASE AND ROOT ROT DISEASES OF PEA AS AFFECTED BY FUNGICIDAL SEED TREATMENT

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Abstract

Field trials were conducted in Dotnuva during 2005–2006 with the aim of determining the impact of fungicidal seed treatment on the severity of stem base and root rot diseases of pea. Seed treaters reduced the severity of stem base and root rot diseases in pea. The severity of stem base diseases of the plants that emerged from treated seed was lower until stem elongation stage, whereas that of root rot until the end of flowering. With plant development, the severity of both root and stem base rots increased and at ripening stage pea plants were heavily affected by rots. The content of propagules of the pathogenic fungus *Phoma pinodella* (L. K. Jones) Morgan-Jones & K. B. Burch, one of the main causal agents of plant root and stem base rot in pea rhizosphere increased with advancing maturity of plants. When chemical seed treaters had been used, the content of *P. pinodella* in pea rhizosphere declined significantly. Pea grain yield data indicate no increase through seed treatment.

Key words: pea, root and stem base rots, seed treatment

Introduction

In many areas, root and stem base rots caused by a complex of soil-borne and seed-borne pathogens are among the most destructive diseases of pea (*Pisum sativum* L.). In the countries similar to Lithuania in climate – Southern Sweden and Denmark pea root rots are one of the most devastating diseases. The most important pathogens mentioned in those countries are *Aphanomyces euteiches, Phoma pinodella, Fusarium solani, Fusarium* spp., *Mycosphaerella pinodes* and others /Perssson et al., 1997/. The prevalent species in the Netherlands are *Thielaviopsis basicola, A. euteiches, F. solani* f. sp. *pisi*. /Oyarzun et al., 1997/. The main soil-borne causal agents affect not only plant roots but also overground part. The most devastating seed-borne pathogens the causal agents of Ascochyta blight are three related 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). They occur singly or in combination and are referred to as the *Ascochyta* complex /Wallen, 1965; Onfroy et al.,

1999/. 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/. The three above mentioned pathogens are distributed in pea-growing regions worldwide. Complete resistance to infection by either pathogen has not been observed in pea. Breeding of pea varieties resistant to the diseases caused by *Aschohyta* complex, is difficult due to the availability of only partial resistance /Timmerman-Vaughan, et al., 2002; Prioul et al., 2004; Fondevilla et al., 2007; Zhang et al., 2007/. Integrated disease management is essential to take advantage of cultivars with partial resistance to this disease. The most effective practices, established by decades of researches, use a combination of disease–free seed, destruction or avoidance of inoculums sources, seed and foliar fungicides and cultivars with improved resistance /Davidson, Kimber, 2007/. It is already half a century since seed treatment aimed to protect peas from root rots was first employed. Many literature sources indicate that chemical seed treaters protect well emerging plants against root rots but cannot control the disease at more advanced plant growth stages and seed treatment not always results in a seed yield increase /Harper, 1964; Hwang et al., 1991; Xue et al., 2003/. In Lithuania, more comprehensive research on the diseases of the *Fabaceae* plant family has been done in beans and lupine /Strukčinskas, 1974/. The phytosanitary state of semi-leafless pea, whose cultivation has been started in Lithuania only recently, has not been investigated before. Fungal diseases in semi-leafless peas have not been researched, although in most pea crops up to 100% of plants are severely affected by root rots (data not published). Such severe occurrence of root rots can result in very low yields of pea in the country.

The objective of the present study was to identify the species composition of the causal agents of seed and soil borne foot and root diseases in semi-leafless pea crops and to estimate the possibility to control fungal seed-borne diseases by chemical seed treatment.

Materials and Methods

The field and laboratory research was carried out at the Lithuanian Institute of Agriculture in 2006–2007. The semi-leafless pea cv. 'Pinochio' was used to assess the impact of fungicide seed treatments on seed health, germination, seed-borne root rot diseases and grain yield. Since there are no chemical seed treaters registered for pea seed treatment in Lithuania, the six seed treaters registered for cereal seed treatment were chosen for the experiments. In 2006, we used Bariton (fluaxistrobin + prothioconazole 37.5 + 37.5 g l^{-1}) 1.5 l t^{-1}, Kinto (triticonazole + prochloraz 20 + 60 g l^{-1}) 2 l t^{-1}, Maxim extra (fludioxonil + difeconazole 25 + 25 g l^{-1}) 1.5 l t^{-1}, Maxim star (fludioxonil + ciproconazole 18,75 + 6,25 g l^{-1}) 1.5 l t^{-1}, Raxil extra (tebuconazole + thiram 15 + 500 g l^{-1}) 2 l t^{-1}, and Kemikar T (karboxin + thiram 200 + 200 g l^{-1}) 2 l t^{-1}. In 2007, we used Bariton 1.5 l t^{-1} and Raxil extra 2 l t^{-1} as the model seed treaters from different chemical groups (strobilurin and triazole + dimetilditiocarbamat) that showed good results in 2006. The method of blotter rolls was used for estimating of seed-borne root rot infection of seedlings. The seed was incubated for 21 days. The phytotoxicity of the seed treaters for seed germinating power and germination was estimated. The tests were done on wet sand, in wet blotter rolls and in field conditions by sowing 100 seeds in 4

replicates. The field trial was sown at a seed rate of 1 million viable seeds per ha with a drill "Fiona" and replicated four times. The plots were 3 m wide, and in 2006 – 10 m and 2007 – 24 m long with row spacing of 12.5 cm. The grain was harvested at complete maturity with a plot harvester Wintersteiger Delta.

The assessments of root rots were made in 2006 at seedling (BBCH 13–17) growth stage, bud formation – flowering (BBCH 51–65) and ripening (BBCH 87) stages; in 2007 – every 14 days from emergence to ripening. The disease incidence and severity assessments were done on a sample of 30 plants per plot: root rot was estimated in points (0 – healthy, 1 – weakly, 2 – moderately, 3 – heavily affected), and the disease severity index (DSI) % was calculated: $DSI = \sum (pn) / PN$, 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 (3); N – number of plants assessed. Plant growth stages were identified according to the BBCH scale /Weber, Bleiholder, 1990/.

For identification of pathogens, plant samples were taken from the plots every 14 days from emergence to ripening. Roots from the randomly selected plants were washed and placed under running tap water for at least 1 h. For *Pythium* spp. and *Rhizoctonia solani* determination, firm tissue near the margin of the lesion (hypocotyls or roots of seedlings) was selected and immersed in 0.5% NaOCl for 15 sec, rinsed twice in sterile distilled water, dried between pieces of sterile filter paper and placed on corn meal agar media. For identification of other fungi, the diseased parts of pea plant were surface disinfected in 1.0% sodium hypochlorite for 2 min. The small transverse sections of diseased tissue were cut and transferred to plates with potato dextrose agar (PDA) and incubated in a thermostat for 7–21 days at 21° C. *Ascochyta* spp. was identified through microscopical observations of the characteristics of the pycnidiospores. *Fusarium* spp. and other fungi were identified through colour, cultural characteristics on PDA, and through macro- and microconidia, conidiaphores, and chlamidospores. The soil infestation with pathogenic fungi was estimated by dilution plate spread method. Fungal species were determined according to LugauskaS et al. (2001), Leslie, Summerell (2006), Satton et al. (2001), Malone, Muskett (1997).

Statistical analyses were performed on the data of disease incidence and severity. The data were arcsine-transformed to stabilize error variance prior to the analysis of variance (ANOVA). The analysis of variance procedure and Fisher test were used /Tarakanovas, Raudonius, 2003/.

Results and Discussion

In 2006, the phytotoxic effect of some seed treaters on seed germination in blotter rolls test was revealed, although in the field conditions the seed treated with all seed treaters germinated well (Table 1). In the spring of 2007, during pea emergence period the soil was cold and wet, which might have caused very distinct phytotoxicity of the seed treater Bariton for seed germination in the field conditions. With the aim of determining the seed-borne root rot infection pea seeds were grown for three weeks in wet blotter rolls, i.e. without any contact with soil. One third of the plants emerged from the treated seeds had damaged hypocotyl and 30–50% had rot-affected rootlets (Table 2). Most of the seed treaters used gave a good protection of seedlings against root

Table 1. The impact of seed treatment on pea seed germination

Chemical product	Dose l t^{-1}	Germination test on wet sand		Field germination		Wet blotter rolls test (after 21 days)	
		Germinating power % (after 4 days)	Germination % (after 8 days)	Initial (after 14–16 days)	Final (after 30 days)	Germination %	Abnormal sprouts %
2006							
Untreated	–	96.7	100	85.2	95.5	100	0.7
Bariton	1.5	97.6	99.7	86.5	91.8	100	7.3
Kinto Pro	2.0	98.0	100	90.0	96.5	99.3	10.1
Maxim extra	1.5	98.2	99.7	78.2	90.2	100	13.3
Maxim star	1.5	97.7	99.3	65.8	77.0	100	12.0
Raxil extra	2.0	96.7	99.7	73.0	86.0	100	2.0
Kemikar T	2.0	98.3	99.3	79.2	87.5	99.3	6.0
$LSD_{0.05}$		1.68	0.91	15.28	8.13		
2007							
Untreated	–	88.0	98.0	76.8	86.0	95.0	3.2
Bariton	1.5	90.5	95.5	0	41.8	95.0	66.8
Raxil extra	2.0	97.0	99.5	70.8	90.8	97.5	8.4
$LSD_{0.05}$		7.04	3.0		10.58	3.20	5.8

Table 2. The impact of seed treatment on the incidence of root rot diseases of pea at the seedling stage and on the grain yield

Chemical product	Dose l t^{-1}	Incidence of seed-borne root rot % (wet blotter rolls test)		Incidence of root rot (in field conditions) %		Grain yield t ha^{-1}
		Hypocotil	Roots	Hypocotil	Roots	
2006						
Untreated	–	33.3	50.0	98.4	96.6	2.41
Bariton	1.5	4.0	4.0	56.7	87.5	2.45
Kinto Pro	2.0	20.1	8.7	80.8	73.4	2.47
Maxim extra	1.5	6.0	5.3	64.2	76.6	2.38
Maxim star	1.5	4.0	17.3	75.8	80.0	2.51
Raxil extra	2.0	2.7	0.7	49.2	41.7	2.45
Kemikar T	2.0	2.0	0	71.7	65.8	2.64
$LSD_{0.05}$				17.94	19.0	2.490
2007						
Untreated	–	36.0	30.7	60.0	100	3.87
Bariton	1.5	14.9	51.9	34.6	72.7	–
Raxil extra	2.0	3.4	11.1	13.8	76.2	3.80
$LSD_{0.05}$				20.99	17.0	

rots and only separate plants had disease-affected main root or hypocotyls, except Bariton in 2007. The seed treaters provided a good protection of pea seedlings against root rots also in the field conditions. The plots sown with treated seed, except for Kinto,

contained significantly fewer seedlings affected by root rot compared with the plots sown with untreated seed. The meteorological conditions in 2006 and 2007 were different. In the 2006 growing season, cool and dry weather prevailed. The lack of rainfall was appreciable during the whole growing season. In June cool and in July hot and extremely dry weather prevailed. Rainy weather at the end of July and August delayed harvesting. Conversely, in 2007 rainy and moderately warm weather conditions prevailed during the growing season. Irrespective of different conditions of the seasons both 2006 and 2007 during the pea bud formation – flowering stage root rots had already spread on all plants in the treatments sown both with treated and untreated seed. However, the disease severity was significantly lower in the plots sown with treated seed (Figure). Later, when the plants had matured, the severity of root rots either did not differ between the plots sown with treated and untreated seed or the differences were not significant in both 2006 and 2007. Pea grain yield data show no yield increase resulting from seed treatment (Table 2). Results of this study agreed with those of Harper (1964), Hwang et al. (1991), Xue et al., (2003), Oyarzun et al. (1990) and many others suggesting that seed treatment protect well emerging plants against root rots but cannot control root rots at more advanced plant growth stages and seed treatment not always results in a seed yield increase. The seed treatment is only one tool in integrated disease management system. As Davidson and Kimber (2007) reported the use of disease free seeds or seed treatments, is crucial as seed-borne infection is highly effective as primary inocolum and epidemic develops rapidly from foci in favourable conditions.

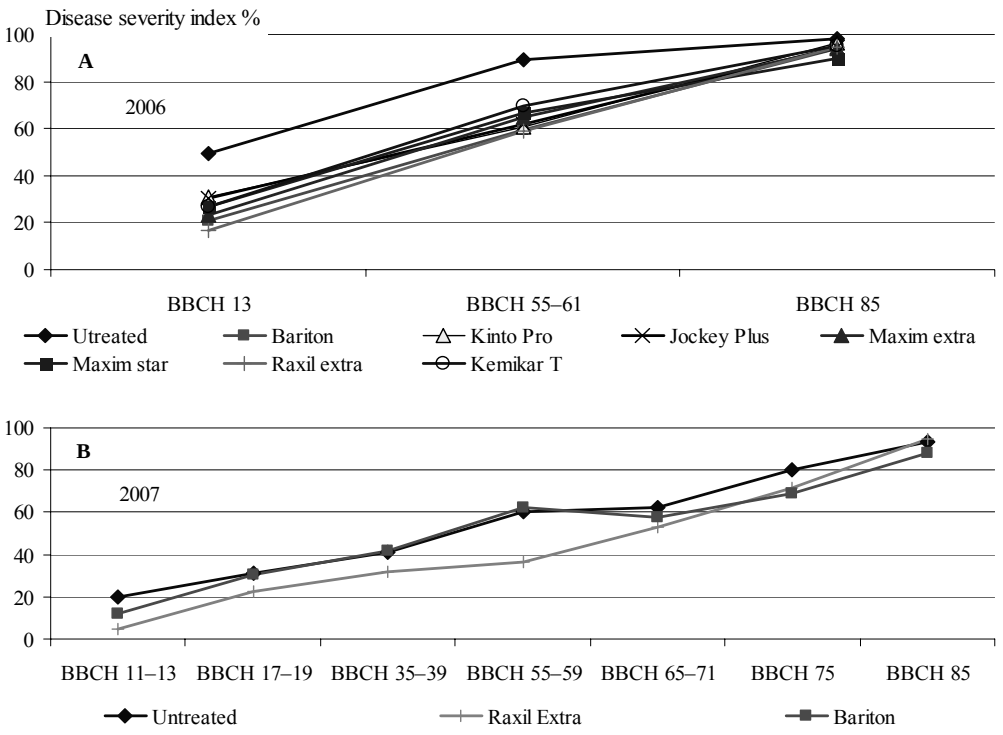

Figure. The severity index of stem base rots on peas during the growing seasons of 2006 (A) and 2007 (B) as affected by the fungicide seed treatment

Our experimental data suggest that the main pathogens causing pea stem base and root rots in the plots sown with untreated and treated seed were found to be *Phoma pinodella* – one of *Ascochyta* blight causal agents and *Fusarium* genus fungi, among which the most prevalent were *F. oxysporum*, *F. moniliforme* and *F. avenaceum*. These fungi were isolated from semi-leafless pea stem base from the very beginning of emergence to hard maturity. Our experimental findings indicate that the range of prevalent pea root and foot pathogenic fungi practically does not differ from that occurring in south Scandinavia. Like in Lithuania, both in Denmark and Sweden the most frequent pathogenic fungi isolated from pea roots were *Phoma pinodella* and *Fusarium spp.* /Perssson et al., 1997/. Comparison of the pathogens isolated from the plants grown from treated and untreated seed revealed that on the plants grown from untreated seed *Phoma pinodella* was isolated already at seedling stage, whereas from plants grown from treated seed – only at stem elongation stage (Table 3). The plants grown from Raxil extra treated seed were protected from this one of *Ascochyta* complex fungi for more than a month. Already at seedling stage pea plants were affected by a complex of seed and soil-borne pathogens. During the ripening stage, the plants of semi-leafless pea were affected by all the three *Aschohyta* complex pathogens: *Aschochyta pisi, Mycosphaerella pinodes* and *Phoma pinodella.* On the upper plant part and pods *Aschochyta pisi* prevailed.

Table 3. The variation of fungi composition on semi-leafless pea plants as affected by seed treatment

Date; growth stage BBCH	Most frequent fungi	
	Untreated	Raxil extra 2 l t^{-1}
27 April; BBCH 11–13	*Fusarium* spp. (*F. oxysporum, F. moniliforme*), *Phoma pinodella*	*Fusarium* spp. (*F. oxysporum, F. moniliforme)*
10 May; BBCH 17–19	*Fusarium* spp. (*F. oxysporum, F. moniliforme*), *Phoma pinodella, Mucor* spp.	*Fusarium* spp. (*F. oxysporum, F. moniliforme*)
24 May; BBCH 35–39	*Phoma pinodella, Fusarium* spp. (*F. oxysporum, F. moniliforme, F. equisetum*), *Trichoderma* spp., *Botrytis* spp.	*F. oxysporum, F. equisetum, Phoma pinodella Penicilium* spp., *Alternaria* spp.
8 June; BBCH 55–59	*F. oxysporum, F. moniliforme, F. poae*, *Phoma pinodella, Botrytis* spp., *Rizoctonia* spp.	*P. pinodella , F. sporotrichoides*, *F. oxysporum, F. moniliforme, F. avenaceaum*
22 June; BBCH 65–71	*F. oxysporum, F. moniliforme, F. graminearum*, *Phoma pinodella, Trichoderma* spp., *Alternaria* spp.	*F. oxysporum, F. moniliforme, F. avenaceum*), *Phoma pinodella Rizoctonia* spp.

In 2006 the amount of fungi was estimated on rhizosphere of plants sown with treated and untreated seed and in the soil 5 cm away from both treated and untreated pea roots. A significantly lower amount of fungi was detected on treated pea rhizosphere compared with the untreated (Table 4). There was a significantly lower amount of fungi 5 cm away from pea roots one month after sowing. In summer, the number of fungi was growing due to the fact that more developed roots secrete more exudates and create

favourable conditions for the growth of microorganisms. The repeated analysis in July showed that in the rhizosphere of treated seeds the plants had significantly lower amount of fungi compared with the untreated. However, 5 cm away from plants the soil contained almost the same amount of fungi in all the treatments. The accumulation of fungi propagules in the rhizosphere might be a source of infection for forthcoming plants. Johnston et al. (2005) reported that pea yields were reduced when seeded on pea stuble in all years of their study. Panicker and Ramraj's (2008) experimental evidence on pathogen survival in the soil showed that in the fields with peas as a preceding crop, the population of *Ascochyta* increased from 12.6×10^3 cfu g^{-1} of soil at sowing to 25.9×10^3 cfu g^{-1} of soil at harvesting and the pathogen survived in the soil even up to one year after harvest.

Table 4. The number of fungi (colony forming units) in the rhizosphere and soil sown with treated and untreated seeds

Treatment	Number of fungi in the soil (cfu g^{-1})			
	BBCH 37–39		BBCH 75–77	
	Rhizosphere	Soil in 5 cm range of plant	Rhizosphere	Soil in 5 cm range of plant
Untreated	56×10^3	38.5×10^3	80.8×10^3	15.7×10^3
Bariton	30.3×10^3	34.3×10^3	59.3×10^3	19.3×10^3
Maxim star	26.7×10^3	34×10^3	58.8×10^3	20.0×10^3
LSD_{05}	23.1×10^3	3.8×10^3	19.6×10^3	8.9×10^3

Conclusions

Pea seed treatment gave a good protection of seedlings against seed-borne root rot infection and significantly reduced disease severity until the bud formation-flowering stage. The pea grain yield data showed no increase resulting from the seed treatment. *Phoma pinodella* – one of the *Ascochyta* blight causal agents and fungi of *Fusarium* genus prevailed on stem base and roots of diseased pea plants.

Acknowledgements

The study was supported by the Lithuanian State Science and Studies Foundation (agreements G-34/06 and G-18/07).

Received 2008-07-11
Accepted 2008-08-22

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