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GARDEN PEA VARIETAL SUSCEPTIBILITY TO *MYCOSPHAERELLA PINODES* AND ITS EFFECT ON YIELD COMPONENTS OF SINGLE PLANTS

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Summary

The susceptibility of 21 garden pea genotypes to *Mycosphaerella pinodes* was evaluated in a two years study with inoculation under controlled conditions and in field experiments. The tested genotypes differed significantly in their susceptibility to seedling foot rot and leaf and stem blight caused by *M. pinodes* under these tests. Average scores of leaf infection in laboratory testing were higher comparing to the degree of infection in field conditions with inoculation. The genotypes which were rated susceptible to foliar infection were also susceptible to stem infection, with the severity of stem infection being much lower. No significant correlation was obtained between foliar symptoms severity and seedling foot rot necrotic index for garden pea genotypes. Deleterious effects of the inoculation with *M. pinodes* in field experiments on all yield components of pea plants parameters were observed. The pathogen caused 14.6% reduction of number of pods per plant, 17.3% reduction of number of seed per plant, 23.5% reduction of seed weight per plant and 7.8% decrease of 1000 seed weight in comparison with protected controls. There were also quite large differences between the years of testing as well as among pea genotypes in reduction of yield components. Genotypes Mieszko and Jurand showed the lowest reduction of seed weight per plant and appeared to be more tolerant to *M. pinodes* among the tested. Overall, five of tested genotypes (Kadona, ZIF 2401, Juko Nike and Deko) were considered as moderately susceptible. The remaining were either susceptible or highly susceptible (Cud Kelwedonu, Pionier, Markus, Kosynier, Duet and Hetman). The obtained results will be useful for breeders in selecting the components for a crossing programme.

key words: *Pisum sativum*, *M. pinodes,* foot rot, stem and leaf blight, yield components

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INTRODUCTION

Pea (*Pisum sativum* L.) can be classified among the most important crop because of the wide diversity of its utilisation and high acreage (FAOSTAT 2007). The European catalogue of registered varieties has two different sections for *Pisum sativum*: field peas, including all peas used as dry seeds, which are part of 'forage plants' section, and 'garden peas' which include all peas used as immature seeds, whether fresh, frozen or canned.

Research and related works on pea crop are focused on the improvement of seed yield and its stability. Among other factors, resistance to pathogens influence on improvement of yield stability and the quality of seeds. This is particularly important in garden pea. Ascochyta blight is a serious pea disease worldwide. Its common occurrence also in Polish condition was demonstrated in earlier studies (Bajan 1968, Weber & Weber 1976, Bartłomiejczak & Bartłomiejczak 1978, Marcinkowska 1996, Marcinkowska 2002). Ascochyta blight is caused by three closely related pathogens with *Mycosphaerella pinodes* (Berk. Et Blox) Vestergren being the most frequent and destructive pea pathogen (Moussarat *et al*. 1998, Marcinkowska 2002, Warkentin *et al*. 1996, Xue 2003). Application of foliar fungicides can reduce blight severity and increase the seed yield (Hwang *et al*. 2006, Warkentin *et al*. 1996, Xue 2003). However, breeding resistant cultivars is a good alternative to the chemical controls. Variation in resistance to mycosphaerella blight has been observed among pea cultivars and germplasm (Xue *et al*.1996, Xue & Warkentin 2001, Kraft *et al.* 1998, Zhang *et al*. 2006). However promising sources of resistance have been found in exotic pea germplasm (Foundevilla *et al*. 2005). No study has specifically focused on assessing genotypic variation in the response of garden pea cultivars and lines to infection with *Mycosphaerella pinodes* under controlled and field conditions.

Studies were designed to evaluate genotypic reaction of garden pea genotypes to foot rot and seedling blight caused by *M. pinodes* under controlled and field conditions and to examine the effect of ascochyta blight on yield components of pea plant under field conditions.

MATERIALS AND METHODS

Twenty one garden pea cultivars were screened for their degree of resistance or susceptibility to foot rot and aerial parts of seedlings to ascochyta blight caused by *M. pinodes* in two separate tests under controlled conditions and in field conditions with inoculation.

***M. pinodes* inoculum preparation.** To prepare inoculum, cultures were grown on the Coon's medium for 14 days to produce pycnidiospores. Conidial suspensions were prepared by flooding the surface of cultures with sterile distilled water, gently scraping the colony and than filtering the suspension. The concentration was determined with a haemocytometer and adjusted to $5x10^5ml^{-1}$ for seed and seedling inoculation in controlled conditions and $2x10^6ml^{-1}$ for field

inoculation. One drop of Tween 20 was added as a wetting agent. A mixed inoculum of ten isolates made from equal volumes of each conidial suspension was prepared for cultivar susceptibility tests.

Seedling inoculation experiments were conducted in a growth room under controlled conditions with temperature of 20±2ºC, 70-80% RH and 16h of illumination. Surface sterilised seeds were planted in multi-pot plastic trays, thinned to one per pot 3x3-cm size. Three replicates of each genotype with eight plants per replication were used. Seedlings of 14-18 days old (4-leaf stage) were inoculated with a sprayer at a rate of 0.5 ml per plant. Trays were covered with plastic top for 48 h to maintain humid atmosphere around plants. Symptoms on leaves and stems were scored eight days after inoculation. Disease severity on leaves was assessed using a 0-5 scale, as defined by Roger & Tivoli (1996): 0 = no lesion; 1 = a few scattered flecks; 2 = numerous flecks; 3 = 10-15% of leaf area necrotic and appearance of coalesced necrosis; 4 = 50% of the leaf area dehydrated or covered by lesions; 5 = 75-100% of the leaf area dehydrated or necrotic. Disease severity on stems was scored with a 0-5 scale, as follows: 0 = no lesions; 1 = first strias; 2 = numerous strias along the stem; 3 = very numerous strias, some coalesced; 4 = necrosis (<3 mm) girdling the stem; 5 = necrosis (> 3 mm) girdling the stem (Roger & Tivoli, 1996).

Test with artificially infected seeds was done under controlled condition described above. Surface sterilized seeds, rinsed with sterile water were immersed in conidial suspension of *M. pinodes* ($5x10^5$ spores ml^{-1}) for 10 minutes and directly planted in multi-pot plastic tray along with the respective noninoculated controls. Three replicates of each genotype with ten plants per replication were used. Foot rot symptoms were scored two weeks after emergence on young plants carefully removed from the substrate and washed in water.

The seedling were examined for foot rot lesion using the following 0 to 5 scale adapted from Moussart *et al.* (1998) where: 0 = healthy plants; 1 = streaks on the hypocotyl or epicotyl; 2 = streaks on the hypocotyl and epicotyl; 3 = lesion girdling the hypocotyl and streaks on the epicotyl; 4 = lesion girdling the hypocotyl and the epicotyl; 5 = weak plants with lesion girdling the hypocotyl and the epicotyl. The necrotic index was calculated.

Field experiments were carried out in Radzików in a split-plot design with two treatments inoculated with *M. pinodes* (prior to the flowering stage of development) or sprayed with the chlorothalonil (Bravo) as the main plot and genotypes as the subplots. Pea were grown on one–row plots, 1.5 m long with 50 plants per plot and 50 cm row spacing with two in 2002 and three in 2003 replications. The inoculum was applied to pea plants with a compressed air sprayer to incipient run-off in the late evening. Disease intensity was assessed for leaves and stems using 0-5 scale where 0 = no lesion; 1 = a few scattered flecks; 2 = numerous flecks; 3 = 25-50% plant parts covered by small coalesced; 4 = 50-75% plant parts covered; 5 = 75-100% plant parts covered with extensive coalesced lesions (Tivoli *et al.* 1996). At the maturity stage 10 plants per plot were taken for yield components measurements including the following traits: plant height, number of pods, seeds and seed yield per plant, 1000 seed weight

(TSW). The percentage yield components reduction was calculated based on the differences between the chlorothalonil (Bravo) protected control and fungi inoculated combination using the formula: 100[(trait-control–trait fungi-inoc) /trait-control].

The data set was evaluate with the one way or multifactor analysis of variance (ANOVA). The t-test was used to evaluate the difference between factors for various attributes. Person correlation was used to evaluate the relationships among the results of test and between disease severity and yield component reductions.

RESULTS

Significant genotypic differences were found for foliar and stem infection scores (Table 1). Five of the tested genotypes (Kadona, ZIF 2401, Juko, Nike and Deko) showed the lowest infection of leaves and stems and were considered as moderately susceptible. The remaining sixteen genotypes were either susceptible or highly susceptible (Cud Kelwedonu, Duet, Markus, Pionier, Hetman and Kosynier). The average leaf infection scores in field experiments were lower than those in laboratory conditions. The Juko, Nike and Deko pea genotypes showed the lowest, while Cud Kelwedonu, Pionier and Jantar the highest infection rate in field experiments. Similarly to the results of test in controlled conditions, none of the tested genotypes displayed high level of resistance. Results of both tests allowed to identify Kadona, ZIF 2401, Juko Nike and Deko as moderately susceptible and Cud Kelwedonu, Pionier, Markus, Kosynier, Duet and Hetman as very susceptible. The pattern of response to *M. pinodes* infection was similar for leaves and stems in both laboratory and in field tests (r=0.90 and r=0.81 respectively).

Significant differences for foot rot symptoms among tested pea cultivars were found. The Hetman then ZIF 2401 and Duet cultivars revealed the lowest infection rate. The remaining genotypes were susceptible or as in case of Kujawski, Jurand and Cud Kelwedonu highly susceptible. No correlation was found between the foot rot necrotic index and the infection rate of leaves and stem caused by *M. pinodes* in laboratory and field test results.

The response of garden pea to infection with M. *pinodes* differed significantly in years of test (Table 2). However, the interaction between the genotypes and years was insignificant, indicating similarity in genotypes response. The Spearman correlation coefficients between years of the evaluation for final infection score of pea genotypes in seedling test (r=0.85 and r= 0.56, leaves and stems respectively), for foot rot test (r=0.79) and in field test (r=0.35 and r=0.25, leaves and stems respectively) indicated fair agreement of differences in the degree of infection among the cultivars throughout the study in controlled condition tests. The infection degrees of the least and most infected cultivars were generally quite consistent in both controlled conditions and field tests (r=0.58 and r = 0.60 for leaves and stems for both years).

Table 1. The mean infection scores for 21 garden pea genotypes in different tests calculated for two years of evaluation

Pea genotypes	Tests in controlled conditions			Field experiments	
	Leaves [a]	Stems [b]	Foot rot [c]	Leaves [d]	Stems [d]
Hetman	3.41	2.11	2.58	2.47	2.06
Duet	3.82	2.55	3.36	2.69	1.83
Cud Kelwedonu	3.89	2.68	4.34	3.17	2.50
Kaskada	3.09	2.00	3.69	2.52	1.77
Grand	3.18	1.74	3.76	2.83	2.00
Pionier	3.50	2.12	4.18	3.00	2.08
Maraton	3.16	1.71	4.21	2.48	1.81
Jantar	3.22	1.86	3.80	2.92	2.08
Helmo	3.15	2.06	3.86	2.24	1.73
Deko	2.86	1.79	4.04	2.08	1.68
Jurand	3.29	1.82	4.49	2.49	1.88
Kujawski	2.98	1.89	4.58	2.30	1.53
Nike	2.76	1.57	4.18	2.00	1.53
Markus	3.55	2.23	3.99	2.47	1.65
Kosynier	3.29	1.87	3.61	2.76	2.11
Mieszko	3.11	1.79	3.94	2.20	1.85
Juko	2.93	1.62	3.88	1.97	1.56
Walor	2.74	1.56	3.98	2.58	1.64
ZIF 2401	2.61	1.64	3.35	2.58	1.75
Kiler	3.13	1.59	3.83	2.50	1.63
Kadona	2.69	1.54	3.75	2.42	1.83
Overall mean	3.16	1.89	3.87	2.51	1.83
$LSD_{0.05}$	0.71	0.98	0.52	0.39	0.29

[a] 0-5 scale where 0=no lesion; 5= 5-100% of the leaf area dehydrated or necrotic (Roger & Tivoli 1996).

[b] 0-5 scale where 0=no lesions; 5=necrosis (>3 mm) girdling the stem (Roger & Tivoli 1996).

[c] 0 to 5 scale (Moussart *et al.* 1998) where: 0=healthy plants; 5=weak plants with lesion girdling the hypocotyl and the epicotyl.

[d] 0-5 scale where 0 = no lesions; 5 = 75-100% plant parts covered by extensive coalesced lesions (Tivoli *et al.* 1996)

Table 2. Mean disease severity of garden pea genotypes in different tests calculated for two years of evaluation

Year of tests	Mean disease severity in laboratory tests			Field experiments	
	leaves	stems	foot rot	leaves	stems
2002	3.30	2.12	3.38	2.79	2.10
2003	3.01	1.67	3.97	2.24	1.57
$LSD_{0.05}$	0.17	0.15	0.14	0.12	0.09

Note: rating scale see Table 1.

Table 3. The impact of plant inoculation with *M. pinodes* and foliar treatment with chlorothalonil (Bravo) on seed yield components of pea plant average for 21 genotypes in 2002-2003

Treatments	Pods per plant	Seeds per plant	Seed weight per plant (g)	Thousand seed weight (g)
Chlorothalonil	8.12	49.62	8.89	179.17
M. pinodes	6.90	41.19	6.88	165.25
$LSD_{0.05}$	0.27	1.96	0.36	2.55

Table 4. Genotype means and percentage reduction* of yield components of single plants tested in 2002-2003

Pea genotypes	Pods per plant		Seeds per plant		Seed weight per plant (g)		Thousand seed weight (g)	
	mean	% R**	mean	% R	mean	% R	mean	% R
Hetman	8.91	11.34	46.80	21.60	8.60	28.35	178.20	8.11
Duet	6.67	20.02	38.31	18.40	8.12	26.11	208.65	9.53
Cud Kelwedonu	5.51	17.82	33.22	23.85	6.83	31.30	200.13	9.27
Kaskada	9.88	21.95	54.11	28.22	8.75	30.48	163.79	8.68
Grand	5.86	9.40	40.90	10.70	7.55	18.02	175.05	6.94
Pionier	6.03	12.06	36.35	17.37	6.93	23.00	180.73	6.36
Maraton	8.24	12.16	45.76	13.96	7.89	21.89	167.41	8.88
Jantar	9.44	14.91	54.99	16.68	8.39	20.74	150.46	6.28
Helmo	8.28	23.38	52.69	25.71	7.09	30.06	139.81	7.55
Deko	5.82	11.23	39.24	21.23	6.46	28.33	157.32	8.97
Jurand	9.27	11.23	55.06	8.75	7.14	15.41	131.02	7.60
Kujawski	7.16	15.67	42.37	16.02	7.60	20.04	174.12	4.56
Nike	7.26	18.86	46.66	13.97	8.32	17.58	171.22	3.92
Markus	6.61	12.27	39.05	23.15	8.28	26.44	205.81	3.86
Kosynier	6.62	8.49	43.74	16.05	8.16	23.24	179.61	9.08
Mieszko	7.75	5.55	48.45	9.48	8.82	16.62	177.42	10.25
Juko	7.96	14.37	46.52	18.78	7.24	23.54	153.70	5.50
Walor	8.82	16.66	47.28	19.73	7.98	30.54	164.69	13.67
ZIF 2401	6.04	9.31	40.27	8.32	9.01	20.03	221.98	12.77
Kiler	7.56	14.51	51.24	16.30	8.31	21.65	158.83	6.35
Kadona	8.05	16.07	50.51	15.39	8.07	19.67	156.45	4.78
Overall mean	7.51	14.16	45.41	17.32	7.88	23.48	172.21	7.76
$LSD_{0.05}$	0.86	7.76	6.37	11.20	1.17	8.72	8.26	5.57

* calculation based on differences between the chlorothalonil (Bravo) protected control and *M. pinodes* inoculated combination for respective trait using the formula: 100[(trait-control – trait fungi-inoc)/trait-control].
** %R - % of reduction

Results from the field experiments indicate that the plant height and all seed yield components were significantly affected by mycosphaerella blight relative to fungicide protected treatment (Table 3). Likewise, significant differences among pea genotypes for yield components and percentage reduction due to infection with *M. pinodes* were observed (Table 4). The mean number of

pods per plant ranged from 5.51 to 9.88 and the percentage reduction ranged from 5.5 to 23.4% between genotypes. Subsequently, the number of seed per plant ranged from 33.2 to 55.1 and the reduction from 8.7 to 28.2%; the seed weight per plant ranged from 6.5 to 9.0 g and the reduction from 15,4 to 30.3%; the thousand seed weight ranged from 131 to 222 g and the reduction from 3.9 to 12.8%. Overall, the highest reduction cased by mycosphaerlla blight was found for the seed weight per plant and the lowest for the thousand seed weight. The genotypes Mieszko and Jurand showed the lowest reduction of the seed weight per plant and appeared to be more tolerant to *M. pinodes* among the tested.

All seed yield components and their reduction caused by infection with *M. pinodes* were significantly affected by the years of testing (Table 5). The plants of garden pea had significantly lower number of seeds and pods per plant, the seed weight per plant, and the thousand seed weight, as well as a significantly higher reduction of these parameters except for the thousand seed weight in the first year of test in comparison with second year.

Table 5. Means and percentage reduction of yield components of single plants of 21 garden pea genotypes in 2002 and 2003

Year	Pods per plant		Seeds per plant		Seed weight per plant (g)		Thousand seed weight (g)	
	mean	% R	mean	% R	mean	% R	mean	% R
Year 2002	6.08	16.40	33.15	21.71	5.09	28.22	154.21	8.8
Year 2003	8.94	11.91	57.66	12.92	10.67	18.74	190.21	7.33
$LSD_{0.05}$	0.27	2.39	1.96	3.46	0.36	2.69	2.55	n.s.

Note: see Table 4

DISCUSSION

In this study we assessed the response of selected garden pea genotypes to foot rot and seedling blight caused by *M. pinodes* under controlled and field conditions. Moreover, the effect of mycosphaerella blight on yield components of pea plants under field conditions was examined. In the seedling tests under controlled conditions, the disease symptoms developed on all genotypes and appeared earlier on leaflets and stipules than on stems. Significant genotypic differences were found for both foliar and stem disease severity. In the presented results, the pattern of response to *M. pinodes* infection and disease development for leaves and stems in both laboratory and in field tests was significantly correlated (r=0.90 and r=0.81 respectively) as previously reported by Wroth (1998), Onfroy *et al*. (1999) and Prioul *et al*. (2003).

The relatively high correlation values between the years of evaluation for the final infection score of pea genotypes in the seedling test indicated good agreement of differences in the infection degree among the cultivars throughout the study. The weak correlation between the years for field results was most probably caused by several factors that may influence disease development in field trials including daily temperature fluctuation, rainfall distribution, field

history and others. The infection degrees of the least and most infected cultivars were generally quite consistent in both controlled conditions and field tests (r=0.58 and r=0.60 for leaves and stems for both years) with genotypes rankings being more stable at the extremes (most resistant, most susceptible) than for the genotypes with intermediate reaction. Among the tested genotypes none were found to posses a high level of resistance to ascochyta blight. This result was similar to those reported earlier (Ali *et al*. 1978, Nasir & Hope 1997, Kraft *et al*. 1998, Xue & Warkentin 2001, Boros 2003, Foundevilla *et al*. 2005, Zhang *et al*. 2006, Boros & Wawer 2007).

The seedlings emerging from the artificially infected seeds almost always showed the foot rot symptoms. The lesions varied from small streaks around the point of cotyledonary attachment to lesions coalescing and completely girdling the epicotyl. Lesions caused by the fungus were always confined to the below soil surface level. Significant differences for the foot rot symptoms among the tested pea cultivars were found. The only three genotypes revealed a lower infection rate. The remaining genotypes were susceptible. On average, the tested garden pea genotypes showed higher susceptibility to foot rot compared to the field pea set of Polish origin screened in recent studies (Boros & Wiewióra 2004, Boros & Wawer 2007). The most resistant field pea genotypes identified in those studies possessed colored testa, suggesting that resistance to foot rot caused by *M. pinodes* is associated with the presence of phenolic compounds in the testa (Clauss 1963, Xue *et al.* 1996). The absence of a correlation between foot rot and blight phase of the disease implies that genetic factors for resistance to foot rot and leaf and stem infections are different. Pea lines may need to be screened separately for foot rot and leaf and stem infection to determine their overall resistance to *M. pinodes*.

Mycosphaerella blight had substantial impact on seed yield components. Mean losses of pods and seed per plant, seed weight and thousand seed weight of inoculated combination with *M. pinodes* in comparison with fungicide protected combination are consistent with the previous results found by Tivoli *et al*. (1996), Xue *et al*. (1997), Garry *et al*. (1998), Beasse *et al*. (1999) and the recent study with field pea (Boros & Wawer 2007). Genotypes Mieszko and Jurand showed the lowest reduction of seed weight per plant and appeared to be more tolerant to *M. pinodes* among the tested. This observation supports an earlier report that some cultivars yield reasonably well under severe disease pressure, while others yield poorly (Xue 2003, Boros & Wawer 2007). Since the high levels of resistance to mycosphaerella blight have not been found in *Pisum* identification of pea lines with higher tolerance to the disease is an important task.

Genotypes with the lowest leaf and stem infection scores and lowest foot rot necrotic index identified in this study may be useful for incorporating them into crosses programmes with high yielding and superior quality lines.

CONCLUSIONS

Among the evaluated pea accessions significant genotypic variation in susceptibility to *M. pinodes* have been found. However non of the genotypes displayed high level of resistance.

Both screening tests, carried out in controlled conditions, clearly discriminate between very susceptible and moderately susceptible classes.

The absence of a correlation between the foot rot and blight phase of the disease implies that genetic factors for resistance to foot rot and leaf and stem infections are different. Pea lines may need to be screened separately for foot rot as well as for leaf and stem infection to determine their overall resistance to *M. pinodes*.

Since a highly resistant source to *M. pinodes* is not available, breeding for resistance to this disease will most likely depend on recurrent selection to compile minor genes. Genotypes found to possess higher resistance could be intercrossed to compile minor genes for resistance in peas

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PODATNOŚĆ ODMIAN GROCHU SIEWNEGO OGRODOWEGO NA PORAŻENIE *MYCOSPHAERELLA PINODES* ORAZ WPŁYW PORAŻENIA NA KOMPONENTY PLONU ROŚLIN

Streszczenie

Oceniano podatność 21 odmian grochu siewnego ogrodowego na porażenie *Mycosphaerella pinodes* w dwuletnich badaniach z inokulacją, prowadzonych w warunkach kontrolowanych i polowych. Stwierdzono istotne genotypowe zróżnicowanie w podatności na zgorzel podstawy łodygi oraz na porażenie liści i łodyg. Porażenie liści i łodyg było istotnie dodatnio skorelowane, natomiast nie stwierdzono istotnego powiązania pomiędzy podatnością na porażenie liści z podatnością na zgorzel podstawy łodygi. Rośliny na poletkach inokulowanych *M. pinodes* posiadały o 14,6% strąków mniej, o 17,3% niższą liczbę nasion, o 23,5% niższą masę nasion z rośliny oraz 7,8% niższą masę tysiąca nasion w porównaniu do roślin z kombinacji chemicznie chronionej. Stwierdzono istotne różnice pomiędzy latami badań oraz genotypami dla redukcji komponentów plonu nasion. Genotypy Mieszko i Jurand wykazały najniższe wartości redukcji komponentów plonu świadczące o ich wyższej tolerancji w stosunku do pozostałych genotypów. W oparciu o przeprowadzone testy wyodrębniono pięć genotypów o średniej podatności na porażenie *M. pinodes,* są to Kadona, ZIF 2401, Juko Nike i Deko. Pozostałe to genotypy podatne lub bardzo podatne, takie jak Cud Kelwedonu, Pionier, Markus, Kosynier, Duet czy Hetman. Wyniki oceny podatności ułatwią hodowcom wybór komponentów do krzyżowań.