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OCCURRENCE AND INTENSITY OF DOWNY MILDEW ON SOYBEAN SEED IN RELATION TO PLANTING DATE

T. DUVNJAK[1], Marija VRATARIĆ[1], Aleksandra SUDARIĆ[1], Jasenka ČOSIĆ[2], Karolina VRANDEČIĆ[2], M. KRIZMANIĆ[1] and A. MIJIĆ[1]

[1]The Agricultural Institute Osijek
Poljoprivredni institut Osijek

[2]The Faculty of Agriculture in Osijek
Poljoprivredni fakultet u Osijeku

SUMMARY

The main objective of this research was to determinate the impact of downy mildew on soybean seed regarding to planting dates, as well as evaluated differences in occurrence and intensity of disease on seed among tested genotype (cultivar) and years, respectively. Infection of downy mildew in this trial was natural under field conditions. Four-year investigation (1998-2001) was undertaken on 10 domestic soybean cultivars with different level of resistance on downy mildew. The investigation was set up in two planting date (optimal and delayed) on experimental fields of the Agricultural Institute Osijek, Croatia. The obtained results indicate on significant differences in the occurrence and intensity of downy mildew on soybean seed among tested planting dates, cultivars and years. Variability of expression grain yield and percentage of diseased seed were caused by genotypic variability (cultivar) and environmental variability (year and planting date).

Keywords: soybean seed, cultivar, planting date, downy mildew, climatic conditions

INTRODUCTION

Health conditions are very important for the production of every crop, including soybeans, because occurrence and intensity of diseases affect grain yield and grain quality. They could even jeopardize crop production. According to H a r t m a n *et al*., (1999) more then 100 pathogens are known to affect soybean but only 35 of them are economically important. Among them is downy mildew of soybean (*Glycine max* (L.) Merrill), caused by fungus *Peronospora manshurica* (Naum.) Syd. ex Gaum. This is one of the most common foliar diseases of this crop worldwide (L i m *et al*., 1984; D u n l e a v y, 1987; L i m,

1989; P h i l l i p s, 1999). The fungus is an obligate parasite that infects leaves, grows within the plant by establishing mycelium with haustoria, invades pods, and covers seeds with a crust of oospores. Attacked seed often have reduced quality, may be smaller or lighter in weight than normal seeds, and can produce systemically infected seedlings (P h i l l i p s, 1999). These seedlings can produce sporangiosphore and become source of secondary inoculum in the field. Estimates of soybean damage caused by *P. manshurica* can vary from none to severe, (A t h o w, 1973; D u n l e a v y, 1971). Pod infections may occur without external symptoms. The interior of the pods and the seed coat may be encrusted with a whitish mass of mycelium and oospores. Seeds partly or completely encrusted with oospores often appear dull white and have craks in the seed coat (P h i l l i p s, 1999). Temperatures of 20-22°C and high humidity favor downy mildew. No sporulation occurs below 10°C and above 30°C; sporulation occurs at 10-25°C.

In large-scale soybean production in Croatia downy mildew regularly occurs, along with other several diseases, but serious losses are not recorded yet. Occurrence and intensity of disease vary depending on climatic conditions during the growing period, the aggressiveness of pathogens and the susceptibility of cultivars (J u r k o v i c and V r a t a r i c, 1986; V r a t a r i c *et al*., 2002; D u v n j a k *et al*., 2002).

The main objective of this report was to evaluate the occurrence and intensity of downy mildew on soybean seed regarding to planting dates, as well as evaluated differences in occurrence and intensity of the disease on seed among tested genotypes (cultivars) and years, respectively. Also, we want to establish correlation between incidence of the disease on seed and grain yield.

MATERIAL AND METHODS

The experimental material involved 10 soybean cultivars (1-10) originate from different hybridization within the soybean program at the Department of Breeding and Genetics for Industrial Plants of The Agricultural Institute Osijek. These cultivars have different level of resistance on downy mildew and according to length of vegetation period; they belong to maturity group 0 and I. The investigation was conducted over four years (1998-2001) on experimental field of The Agricultural Institute Osijek (Eastern Croatia). The trial was designed as a randomized complete block (RCBD) in four replications on basic plot of 10 m^2. Plots were sown by precise planting machine in last decade of April (optimal planting date - planting date I) and two weeks later (delayed planting date – planting date II). Seed was health before sowing. Currently accepted levels of management and cultural practices for soybean were applied each year in trial.

Plots were harvested with small combine when cultivars reached the full harvest maturity in R8 soybean stage of development (F e h r and C a v i n e s s,

1977). After harvest grain yield from each was measured and converted to kg/ha on standard of 13% seed moisture content. In order to determine incidence of downy mildew on seed, 2x100 seed were used from each seed lot and checked on incidence of fungus crusts under stereo binocular in laboratory. Number of infected seeds was expressed as percentage.

The summarized experimental data for disease intensity and grain yield were subjected with analysis of variance. The significance was tested at 5% level with Fisher's Least Significance Test. Coefficient of correlation were calculated due to evaluation of direction and power of interrelationship of analyzed traits. Data were statistically processed using SAS System 8.2 (2001).

Meteorological data (air temperature and rainfall) for investigated years (1998-2001) as well as 90-years average were shown in Table 1.

Table 1 Mean monthly air temperature (T-˚C) and monthly total precipitation (P-mm) per years, 1998-2001 and 90-year average for Osijek

Tablica 1. Srednje mjesečne temperature zraka (T-˚C) i ukupne mjesečne oborine (P-mm) po godinama, 1998.-2001. i 90-godišnji prosjek za Osijek

	1998		1999		2000		2001		1901-1990	
Month *Mjesec*	T (˚C)	P (mm)	T (˚C)	P (mm)	T (˚C)	P (mm)	T (˚C)	P (mm)	T (˚C)	P (mm)
I	2.7	90.7	0.4	35.7	-1.7	17.5	17.5	72.9	- 6.0	43.9
II	5.0	0.7	1.1	59.9	4.2	14.8	4.2	21.5	1.5	44.2
III	4.8	21.2	8.2	28.7	7.0	41.0	9.9	82.5	6.3	42.0
IV	12.6	53.5	12.6	44.9	14.9	27.4	10.8	71.5	11.2	55.7
V	16.2	48.6	17.3	88.8	18.4	26.1	18.4	59.5	16.4	62.7
VI	21.4	26.1	20.3	149.6	22.5	9.6	18.1	238.9	19.4	87.6
VII	22.2	83.7	21.9	95.3	21.7	62.3	21.8	77.1	21.1	66.3
VIII	21.8	99.4	21.3	73.5	23.7	5.3	22.7	7.1	20.4	61.2
IX	15.9	64.4	18.8	50.8	16.1	15.0	14.9	195.2	16.8	46.4
X	12.3	96.5	11.7	22.1	14.1	10.2	13.9	5.1	10.8	56.2
XI	3.9	68.7	4.0	122.2	10.0	42.4	3.5	74.0	5.4	58.8
XII	-3.3	29.7	0.7	95.6	3.0	36.6	-3.8	33.9	1.2	54.7
Total *Ukupno*		683.2		867.1		308.2		939.2		679.7
Mean *Prosjek*	11.3		11.5		12.8		11.4		10.4	

* Data from Meteorological station Osijek; Podaci sa meteorološke stanice Osijek

RESULTS

Grain yield (G) values for all cultivars in this investigation are ranged from 2185.0 kg/ha to 5080.0 kg/ha for all four investigated years and both planting date. The results showed in Table 4 were separated by years and planting date of investigation. Average grain yield per year was ranged from 2996.3 in 1998 to 4214.0 kg/ha in 1999, with significant differences among them. In planting date I, the highest average grain yield per year was obtained in 2001 (4006.2 t/ha) and the lowest average grain yield was 2926.0 obtained in 1999. The highest grain yield in planting date II was obtained in 1999 (4454.0 t/ha), while in 1998 was again obtained the lowest grain yield (3046.5 t/ha). Average grain yield for all investigated years and cultivars was significantly higher in II planting date (3866.9 t/ha) in comparison to 3612.1 t/ha in planting date I. In each year of investigation was obtained significant difference among investigated cultivars. Beside statistically significant differences among years, planting dates and cultivars statistically significant differences was also established for interaction cultivar x year.

Table 2 Soybean grain yield (kg/ha) by years and planting dates, 1998-2001, Osijek

Tablica 2. Urod zrna soje (kg/ha) po godinama i rokovima sjetve, 1998.-2001., Osijek

Cultivar *Kultivar*	1998		1999		2000		2001		Mean *Prosjek*	
Planting date I - *Rok sjetve I*										
1-10	2185.0-3767.5		3092.5-4757.5		3163.9-4069.4		3457.8-4693.6		3083.5-4149.5	
Mean *Prosjek*	2926.0	*a*	3974.0	*b*	3542.2	*b*	4006.2	*b*	3612.1	*B*
Planting date II - *Rok sjetve II*										
1-10	2687.5-3612.5		3880.0-5080.0		3419.5-4398.3		3770.1-4825.1		3545.6-4231.7	
Mean *Prosjek*	3046.5	*a*	4454.0	*a*	3828.4	*a*	4138.5	*a*	3866.9	*A*
Year mean *Prosjek godine*	2996.3	*C*	4214.0	*A*	3685.3	*B*	4072.4	*A*	3736.1	

* Means within a column followed by the same letter do not differ significantly according to Fisher's Least Significance Difference Test (*P*=0.05); small letters shows differences between planting dates and capital letters show differences between tested years

** Srednje vrijednosti u stupcima koje su označne istim slovom ne razlikuju se značajno prema Fišerovom LSD testu (P=0.05); mala slova pokazuju razliku između rokova sjetve a velika slova između testiranih godina*

Table 3 Statistical data analysis for grain yield and diseased seed

Tablica 3. Statistička analiza za urod zrna i oboljelo zrno

		Grain yield *Urod zrna*			Seed disease *Oboljelo zrno*		
Source/*Izvor*	DF	F Value	Pr > F		F Value	Pr > F	
Cultivar/*Kultivar*	9	10.05	<.0001	**	54.07	<.0001	**
Year/*Godina*	3	109.98	<.0001	**	47.15	<.0001	**
Planting date *Rok sjetve*	1	22.21	<.0001	**	52.01	<.0001	**
Cultivar x Year *Kultivar x Godina*	27	2.75	<.0001	**	10.50	<.0001	**
Cultivar x Pl.date *Kultivar x Rok sjetve*	9	1.37	0.2012	n.s.	3.28	0.0009	n.s.
Year x Pl.date *Godina x Rok sjetve*	3	1.51	0.2124	n.s.	20.03	<.0001	**
Cult. x Year x Pl. date *Kult. x God. x Rok sj.*	27	0.58	0.9551	n.s.	1.98	0.0037	n.s.

** - highly significant, n.s. – non significant

** - *visoko značajno, n.s. – nema značajnosti*

Values of seed disease intensity (D) for all cultivars in this investigation was measured in percentage (%) and ranged from 0.3 % to 22.3 % for all four investigated years and two planting date. The results were separated by years and planting date of investigation was showed in Table 2. Average percentage of disease intensity per year was ranged from 4.5 in 1998 to 8.8 % in 1999, with significant differences among them. In planting date I, the highest average disease incidence per year was obtained in 2001 (10.9 %) and the lowest average disease incidence was 4.0 obtained in 1998. The highest disease incidence in planting date II was obtained in 1999 (8.0 %), while in 1998 was again obtained the lowest value of disease incidence (4.9 %). Average disease incidence for all investigated years and cultivars was higher in planting date I (8.7 %) in comparison to 6.1 % in planting date II with significant differences among these two planting dates. Statistical data analysis for grain yield and seed disease was obtained in Table 3. In each year of investigation was obtained significant difference among investigated cultivar, as well. Beside statistically significant differences among years, planting dates and cultivars, statistically significant differences were also established for interactions cultivar x year and year x planting date.

Table 4 Percentage (%) of diseased seed by years and planting dates, 1998-2001, Osijek

Tablica 4. Postotak (%) oboljelog zrna po godinama i rokovima sjetve, 1998.-2001., Osijek

Cultivar *Kultivar*	1998		1999		2000		2001		Mean *Prosjek*	
Planting date I - *Rok sjetve I*										
1-10	0.3-0.9		0.8-22.3		2.5-21.0		2.5-20.5		1.5-12.2	
Mean - *Prosjek*	0.4	*a*	9.7	*a*	10.1	*a*	10.9	*a*	8.7	*a*
Planting date II - *Rok sjetve II*										
1-10	1.0-10.3		0.8-19.0		0.3-8.5		2.0-10.0		1.1-9.6	
Mean - *Prosjek*	4.9	*a*	8.0	*b*	5.4	*b*	6.2	*b*	6.1	*b*
Year mean *Prosjek godine*	4.5	*C*	8.8	*A*	7.7	*B*	8.6	*A*	7.4	

* Means within a column followed by the same letter do not differ significantly according to Fisher's Least Significance Difference Test (*P*=0.05); small letters shows differences between planting dates and capital letters show differences between tested years

** Srednje vrijednosti u stupcima koje su označne istim slovom ne razlikuju se značajno prema Fišerovom LSD testu (P=0.05); mala slova pokazuju razliku između rokova sjetve a velika slova između testiranih godina*

The estimations of direction and power of correlation between grain yield and disease intensity for all investigated years obtained in this investigation are listed in Table 5 in order to determine the degree of association existing among the values analyzed. Correlation coefficients for both investigated planting dates, all investigated years, grain yield and diseased seed were significant

Table 5 Spearman correlation coefficients

Tablica 5. Spearmanovi koeficijenti korelacije

	P	Y	G	D
Planting date (P) - *Rok sjetve*	-	1.00000	0.15196	-0.19962
Year (Y) - *Godina*	1.00000	-	0.43033**	0.28200**
Grain yield (G) - *Urod zrna*	0.15196	0.43033**	-	-0.07020
Diseased seed (D) - *Oboljela zrna*	-0.19962	0.28200**	-0.07020	-

	Planting date I - *Rok sjetve I*			Planting date II - *Rok sjetve II*		
	D	G	Y	D	G	Y
Year (Y) - *Godina*	0.44621**	0.43276**	-	0.09900	0.43343**	-
Grain yield (G) - *Urod zrna*	0.21059	-		0.02657	-	
Diseased seed (D) - *Oboljela zrna*	-			-		

between grain yield (G) and year (Y)(r = 0.43033) as well as between diseased seed (D) and year (Y)(r = 0.28200). We also divide results for correlation coefficients on two separate planting dates. Obtained Spearman correlation coefficient in planting date I were highly significant between year and diseased seed (r = 0.44621) and year and grain yield (r = 0.43276). Correlation between grain yield and diseases seed in I planting date (r = 0.21059) was not significant. In planting date II, only correlation between year and grain yield (r = 0.43343) was highly significant, while correlations between year and diseased seed (r = 0.099) and grain yield and diseased seed (r = 0.02657) were not significant.

DISCUSSION

The obtained differences in grain yield among investigated years, planting dates and cultivars during the present investigation is necessary to look over climatic conditions shown in Table 1. Rainfall and temperatures during vegetation period as well as during whole year have great impact to soybean grain yield and disease incidence. All four investigated years were warmer than 90-year average. Among them, year 2000 was the warmest and in combination with total precipitation (only 308.2 mm and considerably lower than 90-year average) significantly impacted on the lowest soybean grain yield obtained in investigation. Among all investigated years, only total rainfall in 1998 was on same level of 90-year average. Total rainfall in 1999 and 2001 were significantly higher than 90-year average. In these years grain yield was significantly higher than in other investigated years, and that were years with significantly higher soybean grain yield.

Estimates of soybean damage caused by downy mildew varied from none to severe (D u n l e a v y, 1971; A t h o w, 1973). D u n l e a v y (1987) claims that one possibility which impacts on measured damages are weather conditions, host susceptibility, and physiologic race of pathogen. According to same author these are factors that interact to favor downy mildew and determine seed infection. Physiologic race of pathogen was not object of this investigation and in all years of investigation we use data collected on basis of natural infection caused by pathogen races present in field. Significant difference in percentage of diseased seed among investigated cultivars was obtained in each year of investigation. Range between disease incidences among cultivars was wide and the widest was in 1999. Difference obtained in all years in investigations was caused by different cultivar susceptibility as well as climatic conditions during investigation. The biggest difference between planting dates were obtained in last two years of investigation. Probably main factor which impacts on this difference were weather conditions. In both these years were measured enough rainfalls in April and May, while in June only 2001 had enough rainfall for infection. On the other hand, air temperatures in June in most years in Eastern Croatia are too high (often above 30°C) to allow disease

development. That is probably the main reason why in I planting date was measured higher percentage of seed infection. Only in first year of investigation (1998) I planting date obtained lower seed infection than II planting date but this year was significantly lower disease incidence than other years in investigation. Also, lower disease incidence in 1998 could be explained by lowest percentage of initial inoculum in previous year. In 1999, which is one of the best years for soybean production in last 30 years, climatic conditions were very suitable for soybean production as well as for downy mildew development. P h i l l i p s (1999) cite that *P. manshurica* as an obligate parasite is adapted to conditions which favor soybean development that could explain high disease seed incidence in this year. One proof of this we get if compares differences in grain yield and diseased seed among years it is possible to notice similarity between them. In both case, years with the highest yield is years with the highest percentage of diseases seed. On the other hand, in 1998 was obtained the lowest grain yield and the lowest percentage of diseased seed as well.

Regarding to results from Table 5, only correlation coefficients calculated between year and grain yield as well as between year and diseased seed were highly significant. This shows that only year significantly impact on grain yield and percentage of diseased seed. Other calculated correlation coefficients were not significant.

Furthermore, the correlation coefficients were determined in terms of planting date. Obtained positive correlations in I planting date were highly significant between year and diseased seed as well as between year and grain yield shows that year has a great impact on these two traits. In II planting date, only correlation between year and grain yield was significant and correlation coefficient was almost the same as in I planting date. Year has not such great impact on percentage of diseased seed as in I planting date. In both planting dates, obtained correlation coefficients between grain yield and diseased seed was low and not significant what shows that disease incidence during growing season have not significant impact on grain yield decrease which correspond with researches of B e r n a r d *et al*., (1971), D u n l e a v y, (1987), H i l d e b r a n d and K o c h, (1951), L i m, (1985) and L i m (1989). Also, obtained correlation coefficient in planting date I was highly significant what could be explained with better condition for pathogen growing in early soybean planting dates. On the other hand, in planting date II there was not correlation.

Variability of expression grain yield and percentage of diseased seed were caused by genotypic variability (cultivar) and environmental variability (year and planting date).

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POJAVA I INTENZITET PLAMENJAČE NA SJEMENU SOJE U ODNOSU NA ROKOVE SJETVE

SAŽETAK

Glavni cilj ovog istraživanja bio je odrediti pojavu i intenzitet plamenjače na sjemenu soje u odnosu na rok sjetve kao i procijeniti razlike u pojavi i intenzitetu bolesti na sjemenu između testiranih genotipova (kultivara) i godina. Provedeno je četverogodišnje istraživanje (1998.-2001.) na 10 domaćih kultivara soje sa različitim nivoom otpornosti na plamenjaču. Istraživanje je postavljeno u dva roka sjetve (optimalni i odgođeni) na eksperimentalnom polju Poljoprivrednog instituta Osijek, Hrvatska. Dobiveni rezultati ukazuju na značajne razlike u pojavi i intenzitetu plamenjače na sjemenu soje između testiranih rokova sjetve, kultivara i godina. Varijabilnost u ekspresiji uroda zrna i postotku oboljelog zrna uzrokovana je genetskom varijabilnošću (kultivar) i varijabilnošću okoline (godina i rok sjetve).

Ključne riječi: sjeme soje, plamenjača, rok sjetve, kultivar, klimatski uvjeti

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Adresa autora – Authors' address:
Tomislav Duvnjak, Ph.D.
Marija Vratarić, Ph.D.
Aleksandra Sudarić, Ph.D.
Miroslav Krizmanić, Ph.D.
Anto Mijić, Ph.D.
Agricultural Institute Osijek
Department for Breeding and Genetics of Industrial Plants
Južno predgrađe 17. HR-31000 Osijek
e-mail: tomislav.duvnjak@poljinos.hr

Prof. Jasenka Ćosić, Ph.D.
Karolina Vrandečić, M.Sc.
The Faculty of Agriculture in Osijek
Trg. Sv. Trojstva 3, HR-31 000 Osijek

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