FUNGAL BIODIVERSITY ON RUNNER BEAN GROWING IN TWO SYSTEMS OF PLANT PROTECTION

Marek KOPACKI¹, Stanisław PARAFINIUK², Barbara SKWARYŁO-BEDNARZ¹

¹Department of Plant Protection and Quarantine, University of Life Sciences in Lublin, POLAND

²Department of Machinery Exploitation and Management of Production Processes, University of Life Sciences in Lublin, POLAND

e-mail of corresponding author: marek.kopacki@up.lublin.pl

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ABSTRACT

The aim of the investigation was to evaluate three fungicides (Dithane NeoTec 75 WG, Sumilex 500 SC, Amistar 250SC) for their antifungal activity against fungi occurring on bean seeds using two types of nozzles: standard RS-MM 110 03 and injector type ID 120 03 C. Studies of three years (2007-2009) were conducted at Czesławice in the south-east of Poland. The objects of research were the plants and seeds of cultivar Blanka. The following agents were used for protection. Observations were carried out in October in each year. Diseased plants were collected for mycological analysis. Seeds with symptoms were noticed on the investigated plantations. The results of mycological analysis showed that bean plants were colonized by *Fusarium* spp., *Sclerotinia* spp. and *Alternaria alternata*. The best health status and the lowest number of colonies were noticed on plants protected by azoxystrobin with both types of nozzles.

INTRODUCTION

Runner bean (*Phaseolus coccineus* L.) is an important legume with high nutritional value commercially grown in different parts of the world. The health of bean is spoiled by many pathogens. Seedborne diseases have been found to affect the growth and productivity of the legume. The greatest yield losses owing to pathogens occur when seeds used for planting are infected. Seedborne pathogenic fungi can prevent germination, kill seedlings, or reduce plant growth by damaging the roots and the vascular system, which prevents the transport of water and nutrients (Mancini et al. 2016). Seedborne pathogenic fungi that cause losses of yield and quality of bean worldwide include *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *F. solani*, and *Rhizoctonia solani* (Schwartz et al. 2005; Naseri et al. 2008). Recommended fungicides are often effective but windy weather weakens their effectiveness. In Poland the pesticides should be applied with wind lighter than 4 m/sec and that is why attempts are being made to improve the effectiveness of spraying and looking for modern methods of application (Doruchowski 2013; Subr et al. 2015). The main goal in pest-management research is to improve pesticide application technology for its effective action and rapid dissipation from crop tissues (Gamliel 2010).

Effectiveness of spraying depends largely on the technique which was used for doing it. In practice of plant protection different types of nozzles are used. In field spraying flat-stream standard nozzles are used most frequently. With higher work pressure they give small and very small droplets which perfectly cover the spraying surface but are prone to drifting. The second kind of tested nozzles were air-ejector nozzles. These nozzles generate a lot of aerated droplets which are less prone to drifting and to a lesser extent cover the spraying surface. Nowadays on the labels of pesticides there is information on parameters of doing spraying and recommended spectrum of droplets (Nuyttens 2009).

The aim of investigation was to determine the influence of the fungicides sprayed by means of standard and thick drop nozzles on the healthiness of plants of the cv. Blanka and to estimate the fungal communities colonising seeds of bean grown in the field.

MATERIAL AND METHODS

The experiment was conducted at the Agricultural Research Station in Czesławice, Lublin region, Poland (51°18'23''N 22°16'02''E). The experiment was conducted in a randomized complete blocks design with four replicates where block was the random effect. The seeds were planted in the field in May. The experimental combination consisted of 40 plants of each plot (in 4 replicates). Bean cv. Blanka received three levels of fungicide treatments: (1) – non- treated control and (2)- three fungicide applications with the use of standard RS-MM 110 03 (Marian Mikołajczyk) sprayers and (3) - three fungicide applications with the use of thick drop nozzles ID 120 03 (Lechler). Fungicide was applied starting 6 weeks after planting and repeated every 14 days thereafter. Three products were used: Dithane NeoTec 75 WG (a.i. mancozebe), Sumilex 500 SC (a.i. procymidone) and Amistar 250 SC (a.i. azoxystrobin). Pressure of spray amounted to 3 bars and working speed was 4 km/h. To define the degree of covering plants with fungicide when doing plant spraying with two kinds of nozzles, water sensitive papers were placed on plants on the upper part. The healthiness of plants and seeds was estimated after harvest in October.

Seeds of common bean cv. Blanka (200 from each combination) produced in the crop seasons were subjected to asepsis with a sodium hypochlorite solution and immediately rinsed in sterile distilled water. After such procedure, the seeds were placed on the mineral medium on Petri dishes. The obtained fungal colonies were transferred to potato dextrose medium (PDA, Difco) and identified to the species with the available monographs (Kopacki and Wagner 2006).

CONCLUSIONS

Black or brown spots were observed on diseased seeds. Weather conditions in 2007–2009 were very favourable for the development of pathogenic fungi.

Percentage of seed infection determined by visual observation and fungal isolation ranged from 19,2-63,0% in all as shown in Table 1.

Table 1. Percentage number of seeds infected with fungi

Combination	Seeds with diseases symptoms (%) in year		
	2007	2008	2009
Control	60.5	53.2	63.0
Dithane NeoTec 75WG	47.2	57.2	28.2
Dithane NeoTec 75 WG ID	38.1	39.0	33.1
Sumilex 500 SC	53.2	36.4	45.1
Sumilex 500 SC ID	48.2	39.0	47.2
Amistar 250 SC	33.2	20.9	28.3
Amistar 250 SC ID	29.0	21.4	19.2

The results of three-year investigation showed that the type of atomizer had no effect on the populations of bean seeds pathogens. We saw the differences between monitored fields where we did not carry out the security plants and fields where this operation was. On the monitored fields all plants had diseases symptoms. The chemical preparation which was used has some influence on this illness. The best was Amistar 250 SC chemical preparation especially in 2008. Amistar 250 SC and Sumilex 500 SC

preparations, used in thick drops nozzles ID" with broad-spectrum of drops, were effective. Where we used Dithane NeoTec 75 WG preparation and standard RS-MM nozzles all plants were infected with lots of symptoms. Because we didn't have enough parameters we must carry out more investigation about effectiveness protection at use of different kinds of nozzles.

To define the degree of covering plants with fungicide when doing plant spraying with two kinds of nozzles, water sensitive papers were placed on plants on the upper part. To measure the degree of covering water sensitive papers were used. The papers were attached to leaves of sprayed plants. On each paper the area of 1000 mm² surface was analysed. After spraying the papers were scanned and the obtained image was analysed. The image analysis was done with the use of Image-Pro Premier 3D. The degree of covering the water sensitive papers was defined in percentage. The diameter of droplets which fell on the plants was measured and the number of droplets which were on the analysed surface was defined. Percentage share in particular fractions of droplets in eight size intervals was defined. Average results from eight measurements for each kind of nozzles were presented in Table 2.

Table 2. The degree of covering the sprayed surface with the use of two kinds of spraying nozzles.

	Average sample from RS- MM 110 03	Average sample from Lechler ID 120 03
Sample surface area [mm ²]	1000.00	1000.00
Droplets surface area [um ²]	4968647.25	1168369.53
Degree of covering [%]	49.19	22.81
Number of droplets [pcs.]	360	199

In 2007–2009 from seeds, as the result of mycological analysis, 1456 fungal colonies belonging to 17 species were isolated. The predominating species were *Alternaria alternata*, *Fusarium* spp., and *Sclerotinia sclerotiorum* (Tab. 3). More colonies were obtained in autumn than in summer.

A. alternata, *Fusarium* spp. and *S. sclerotiorum* predominated among fungi which are regarded as pathogenic. The occurrence of individual species of *Fusarium* depended on the used fungicides. *F. oxysporum* was isolated frequently from seeds, especially from plants treated by azoksystrobine with both types of nozzles. Numerous colonies of *F. avenaceum* were obtained every year especially from seeds treated with mancozeb, procymidone and azoksystrobine.

A. alternata occurred on all combinations but more frequently on seeds from plants treated with mancozeb and procymidone. *S. sclerotiorum* colonised frequently seeds especially in 2007 and 2008. *Thanatephorus cucumeris*, *B. cinerea* and *C. gloeosporioides* colonised the seeds very rarely.

Table 3. Fungi colonizing seeds of runner bean

Year	Fungus species								Total (%)
		Control	Dithane		Sumilex		Amistar		
			S	ID	S	ID	S	ID	
	<i>Acremonium scirctum</i> W.Gams	27							27(5)
	<i>Alternaria alternata</i> (Fr.) Keiss.	58	56	41	21	12	12	16	216 (38)
	<i>Epicoccum nigrum</i> Link	14	8	11	1				34(6)
	<i>Fusarium avenaceum</i> (Corda ex	13	14	2	6	4	2	2	43(7)

Year	Fungus species								Total (%)
	Fries)Sacc.								
07	<i>Fusarium equiseti</i> (Corda) Sacc.	21				12			33(6)
	<i>Fusarium oxysporum</i> Schlecht.	6	4	8	4	2	2	12	38(7)
	<i>Gliocladium catenulatum</i> Gill. et Abb..	16		4		1			21(4)
	<i>Penicillium</i> spp.	22					1	2	25(4)
	<i>Thanatophorus cucumeris</i> Kühn	21	11	1		1			34(6)
	<i>Sclerotinia sclerotiorum</i> (Lib.) de By	21	11						32(6)
	<i>Trichoderma harzianum</i> Rifai	2	14				1	1	18(3)
	<i>Trichotecium roseum</i> (Pers.) Link	14	1	2		1	14	18	50(8)
	total	235	119	69	32	33	32	51	571
	<i>Alternaria alternata</i> (Fr.) Keiss.	13	31	29	12	1	22	40	148 (24)
	<i>Aspergillus niger</i> van Tieghem		34	11			4		49(8)
	<i>Botrytis cinerea</i> Pers. ex Fries.		8		5	23			36(6)
	<i>Epicoccum nigrum</i> Link	3	21	23					47(8)
	<i>Fusarium avenaceum</i> (Corda ex Fries) Sacc.	3	22	2	4	12	8		51(8)
	<i>Fusarium culmorum</i> (Smith) Sacc.	3		14					17(3)
08	<i>Fusarium equiseti</i> (Corda) Sacc.				2	2			4 (1)
	<i>Fusarium oxysporum</i> Schlecht.	6	31	6	2	14	6		65 (11)
	<i>Mucor mucedo</i> Mich. Ex St.-Am.				7	12			19 (3)
	<i>Penicillium</i> spp.	6	12	3			4		25 (4)
	<i>Sclerotinia sclerotiorum</i> (Lib.) de By		32			12	2		46 (8)
	<i>Trichoderma harzianum</i> Rifai		21	2	11	5	6		45 (7)
	<i>Trichoderma koningii</i> Oud.	3	14				4		21 (3)
	<i>Trichotecium roseum</i> (Pers.) Link		22				14		36 (6)
	total	37	248	90	43	111	70	40	609
	<i>Alternaria alternata</i> (Fr.) Keiss.	11	7	6			2	2	28(10)
	<i>Botrytis cinerea</i> Pers. ex Fries.	2							2(1)
	<i>Colletotrichum gloesporioides</i> (Penz.)	4	7		11		4	2	28(10)
	<i>Epicoccum nigrum</i> Link						6		6(2)
	<i>Fusarium avenaceum</i> (Corda ex Fries) Sacc.	4		6		4	4	4	22(8)
	<i>Fusarium equiseti</i> (Corda) Sacc.	2							2(1)
09	<i>Fusarium oxysporum</i> Schlecht.	13	13	26	7	18	2	18	97(35)
	<i>Penicillium</i> spp.	1		6		8		6	21(7)
	<i>Stemhyllium botryosum</i> Wallr.	4			2				6(2)
	<i>Thanatophorus cucumeris</i> Kühn	4	10		8	8	17	8	55(20)
	<i>Trichoderma harzianum</i> Rifai	2							2(1)
	<i>Trichoderma koningii</i> Oud.			2			5		7(3)
	total	47	37	46	28	38	49	40	276

The results of investigations showed the effect of tested fungicides on the health status of seeds of bean. Probably with stronger infection pressure it would be possible to observe the greater differences in the effectiveness of tested fungicides with the application of various drop nozzles (Foqu'e and Nuytens 2011). The results proved that *A. alternata*, *Fusarium* spp., *S. sclerotiorum* were most probably the cause of diseases of bean seeds from plants protected with fungicides. These known fungi cause bean seed diseases worldwide (Marcenaro and Valkonen 2016). The best results are connected with the use of azoxystrobin in all combinations both types of nozzles.

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