



Cultivar mixtures as part of integrated protection of spring barley

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Abstract

Barley powdery mildew is caused by the fungus *Blumeria graminis* f. sp. *Hordei*. Monoculture and the use of disease-resistant varieties on large-scale usually lead to the selection of new pathogen races able to overcome the resistance. The use of variety mixtures can significantly improve the control of the disease and provides stable yield among different environments. To assess the effects of the variety mixtures of spring barley on the intensity of powdery mildew infection, we conducted field trials during four growing seasons at two locations contrasting in soil and environmental conditions. The experiments were based on five varieties of spring barley (Basza, Blask, Skarb, Rubinek, Antek) sown in pure stands as well as in their mixtures. No fungicide and insecticides were used, only herbicide protection was implemented. Our studies have shown that in both locations the intensity of the disease was reduced in varieties grown in mixtures when compared to pure stands. This confirms that cultivation of genetically diversified varieties of spring barley in mixtures provides economically and environmentally sustainable tool which allows to control powdery mildew disease.

Keywords Spring barley · Variety mixtures · Powdery mildew · Disease reduction

Introduction

Powdery mildew is the most destructive disease of barley and is caused by the biotrophic fungus (*Blumeria graminis* f. sp. *hordei*) (Tratwal and Bocianowski 2014, Wolfe et al. 1997) that is of great economic importance in many barley production regions. In order to achieve sustainable use of pesticides, rules and guidelines for integrated pest control in Annex III “General principles of integrated pest management” to the Directive of the European Parliament and of the Council 2009/128/CE of 21 October 2009 established a framework for Community action which emphasize the use of all possible methods which reduce population of pests to the level of harmlessness. Section 4 of the annex points that “Sustainable biological, physical and other non-chemical

methods must be preferred to chemical methods if they provide satisfactory pest control.” Practical application of integrated pest control comprises of using alternative methods to chemical pest control. The general principle is to apply most effective and least harmful to the natural environment methods in a particular period of development of the cultivated plant. One of the relatively cheap and easy methods which increases the durability of genetic resistance of contemporary cultivars is their cultivation in different types of mixtures and in complex interbred populations according to the concept of evolutionary plant cultivation (Frison et al. 2001; Gacek 2000; Matyjaszczyk 2015; Newton et al. 2009, 2010). Mixed stands are less susceptible to unfavorable environmental conditions, such as weather fluctuations and other abiotic factors as well as more resistant to biotic stress (diseases, pests, weeds) (Finckh et al. 1998, 2000; Philips and Wolfe 2005; Wolfe 1990; Wolfe et al. 1997).

The aim of the research was to determine the influence of genetically diversified cultivar mixtures of spring barley on the development of epidemics of barley powdery mildew (*B. graminis* f. sp. *hordei*). For this we examined the level of infection of powdery mildew in variety mixtures of spring barley in field trials at two different locations in four growing seasons (2010–2013).

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Table 1 Characteristic features of spring barley cultivars used in the experiment (www.coboru.pl)

Variety	Cultivar type	Resistance to <i>B. graminis</i>	
		Level ¹	Source of resistance
Basza	Two-row, malting	9.0	<i>mlo</i>
Blask	Two-row, malting	7.2	<i>Ly + un</i>
Antek	Two-row, fodder	6.1	<i>Ly + un</i>
Skarb	Two-row, fodder	7.2	<i>Mla + IM9</i>
Rubinek	Two-row, fodder	8.3	<i>Mlo</i>

¹Resistance is expressed in a 9-grade scale, where 9 indicated full resistance, while 1—full susceptibility

Materials and methods

Field trials

Field experiments with spring barley were carried out in four growing seasons: 2010, 2011, 2012 and 2013 at two locations: “Plant Breeding Smolice—IHAR Group Bakow Division, Opolskie (50°42’N, 18°28’E) and Experimental

Station for Variety Station, Koscielna Wies, Wielkopolskie Province (51°47’N, 18°00’E).” The two sites differed in terms of soil and weather conditions. Five cultivars of spring barley, Basza, Blask, Antek, Skarb and Rubinek (Table 1), varying in resistance to barley powdery mildew were cultivated on plots of 10 m² (Bakow) and 16.5 m² (Koscielna Wies) in the split-plot system. The varieties were sown in pure stands and in all possible 10 two-way mixtures and 10 three-way mixtures combinations. The ratio of cultivar components in mixtures was 1:1 or 1:1:1. Rows spacing in plots is 12.5 cm, seed drill type—Oyord (Bakow), SPZ 1,5/2 (Koscielna Wies). Fertilization—N: 60–60 kg/ha, P: 30–40 kg/ha, K: 50–60 kg/ha. Soil types: podzolic soil and leached brown soil (Bakow); leached brown soil; and acid brown soil (Koscielna Wies). No fungicide treatments were applied in the experiments.

The assessment of resistance of barley to *Blumeria graminis*, powdery mildew

Natural powdery mildew infections and pests on spring barley plants were assessed every 7–10 days during the

Table 2 Level of infection of powdery mildew (*B. graminis* f. sp. *hordei*) (standard deviation) in varieties of spring barley in pure stands and in mixtures in growing season 2010

Combination	AUDPC		RAUDPC	
	Bakow	Koscielna Wies	Bakow	Koscielna Wies
Basza	70.6 (34.93)	270.2 (323.79)		
Blask	185.7 (48.04)	277.4 (248.24)		
Antek	620.3 (199.89)	488.7 (374.28)		
Skarb	262.2 (53.36)	272.3 (268.1)		
Rubinek	84.6 (17.93)	207.3 (143.66)		
Basza/Blask	74.1 (17.47)	154.9 (86.86)	41.20	31.19
Antek/Basza	198.2 (35.65)	192.5 (105.86)	40.97	9.06
Basza/Skarb	74.1 (17.47)	115.5 (34.83)	55.07	35.15
Basza/Rubinek	77.5 (22.11)	135.3 (92.53)	No reduction	16.94
Antek/Blask	244.4 (79.46)	246.8 (87.63)	36.19	6.91
Antek/Skarb	346 (75.09)	290.7 (319.97)	16.48	22.66
Antek/Rubinek	220.8 (67.73)	218.6 (78.45)	36.13	25.07
Blask/Skarb	141.8 (5.82)	85.3 (0)	34.43	59.38
Blask/Rubinek	117.5 (25.82)	154.7 (98.69)	11.06	9.10
Rubinek/Skarb	142.9 (52.99)	110 (49.36)	17.93	43.01
Antek/Basza/Blask	159.7 (37.99)	211.5 (87.15)	43.35	10.01
Antek/Blask/Skarb	200.6 (46.08)	130.6 (30.17)	41.62	62.28
Antek/Basza/Rubinek	138.9 (6.72)	356.8 (296.1)	44.76	No reduction
Antek/Blask/Rubinek	156.7 (39.91)	447.8 (386)	45.14	No reduction
Antek/Basza/Skarb	114.1 (29.08)	207.6 (69.81)	64.04	18.78
Blask/Rubinek/Skarb	103.6 (36.24)	369.5 (212.42)	42.83	No reduction
Basza/Blask/Rubinek	85.1 (35.06)	288.2 (101.11)	26.88	No reduction
Antek/Rubinek/Skarb	174 (81.86)	121 (29.87)	43.86	60.65
Basza/Blask/Skarb	98.4 (35.55)	343.1 (438.76)	38.80	No reduction
Basza/Rubinek/Skarb	95.5 (35.77)	177.4 (112.06)	28.17	5.74

Table 3 Level of infection of powdery mildew (*B. graminis* f. sp. *hordei*) (standard deviation) in varieties of spring barley in pure stands and in mixtures in growing season 2011

Combination	AUDPC		RAUDPC	
	Bakow	Koscielna Wies	Bakow	Koscielna Wies
Basza	38.8 (0)	37.2 (0)		
Blask	190.2 (43.59)	184 (127.41)		
Antek	514.5 (90.6)	367.3 (84.08)		
Skarb	224.9 (76.87)	172.6 (60.17)		
Rubinek	50.5 (23.44)	37.2 (0)		
Basza/Blask	94.8 (34.47)	85 (9.41)	14.51	4.42
Antek/Basza	109.7 (24.17)	128.8 (66.46)	60.49	27.45
Basza/Skarb	83.1 (32.45)	86 (34.97)	28.24	10.88
Basza/Rubinek	42.3 (6.98)	68.1 (29.27)	3.81	No reduction
Antek/Blask	233.6 (28.3)	205.5 (22.17)	33.58	20.28
Antek/Skarb	286.5 (89.9)	323.3 (214.73)	20.62	No reduction
Antek/Rubinek	182.2 (81.68)	163.8 (90.54)	34.41	12.94
Blask/Skarb	181.3 (34.28)	95.9 (18.13)	6.19	42.12
Blask/Rubinek	71.4 (30.1)	108 (81.3)	35.54	7.38
Rubinek/Skarb	130.6 (47.83)	95.9 (18.13)	3.45	No reduction
Antek/Basza/Blask	138.8 (42.68)	168.5 (66.78)	43.32	6.45
Antek/Blask/Skarb	212.5 (25.7)	205.5 (22.17)	31.05	12.33
Antek/Basza/Rubinek	123 (55.15)	152.8 (89.61)	40.83	No reduction
Antek/Blask/Rubinek	190.2 (43.59)	109.6 (21.86)	23.42	42.05
Antek/Basza/Skarb	169.5 (79.05)	109.6 (13.41)	36.12	42.85
Blask/Rubinek/Skarb	117.9 (24.02)	95.9 (18.13)	22.19	17.68
Basza/Blask/Rubinek	89.4 (8.73)	94.6 (25.8)	1.85	No reduction
Antek/Rubinek/Skarb	173.8 (70.95)	146.6 (47.26)	31.17	23.03
Basza/Blask/Skarb	143.9 (63.25)	173.4 (95.63)	2.51	No reduction
Basza/Rubinek/Skarb	83.1 (37.52)	88.9 (23.99)	16.71	No reduction

growing season (on mature plants?). The intensity of the disease infection was assessed using the following 9-grade scale where “1” indicated full susceptibility, while “9” full resistance (no symptoms of the disease) (Kaczynski et al. 1998). The obtained results were expressed as a percentage value of damage caused by the disease to particular plants of pure cultivars and mixtures. Analyses were carried out on individually converted percentage value.

The area under disease progress curve

The area under disease progress curve (AUDPC) value was calculated according to the following formula (Tratwal and Bocianowski 2014)

$$\text{AUDPC} = \sum_{i=1}^n \left\{ \left(\frac{y_i + y_{i-1}}{2} \right) (x_i - x_{i-1}) \right\},$$

where AUDPC is the area under disease progress curve, y_i is the percentage of visible infected area ($y_i/100$) at the i -th observation, x_i is the day of the i -th observation, and n indicates the total number of observations (modified from Shaner and Finney 1977).

The relative area under disease progress curve (RAUDPC) for mixtures was calculated by comparing the area under disease for mixtures with the area under disease for cultivars in pure stands (mixture components).

The assessment of yield

Mature grain was harvested from each plot using combine. Weight of grain from each plot was measured and used to calculate yield in dt/ha. Yield from mixtures was calculated as an expected yield. The expected yield is a comparison between average yield from pure stands (mixture components) and yield from mixture.

Statistical analysis

The normality of distribution of the studied traits was tested using the Shapiro–Wilk’s normality test (Shapiro and Wilk 1965). Three-way analysis of variance (ANOVA) was performed to verify the hypotheses of lack of effects of locations, years and cultivars, cultivars/mixtures and the hypotheses about a lack of all interactions on the variability

Table 4 Level of infection of powdery mildew (*B. graminis* f. sp. *hordei*) (standard deviation) on cultivars of spring barley in pure stands and in mixtures in the 2012 season

Combination	AUDPC		RAUDPC	
	Bakow	Koscielna Wies	Bakow	Koscielna Wies
Basza	57.7 (19.2)	31.9 (15.42)		
Blask	123.5 (44.81)	94.5 (97.41)		
Antek	781.3 (13.71)	162.4 (81.55)		
Skarb	168.8 (16.45)	56.5 (38.69)		
Rubinek	99.5 (54.73)	23.8 (4.07)		
Basza/Blask	86.5 (0)	38 (14.87)	1.15	10.38
Antek/Basza	195.8 (78.47)	89.4 (52.63)	53.75	23.36
Basza/Skarb	109.1 (45.25)	38 (18.81)	3.98	12.18
Basza/Rubinek	57.7 (19.2)	57.1 (11.61)	24.01	No reduction
Antek/Blask	319.1 (126.89)	55.8 (24.43)	29.68	47.39
Antek/Skarb	365.5 (70.41)	46.9 (21.15)	23.04	63.19
Antek/Rubinek	221.3 (66.86)	44.9 (23.59)	49.37	59.04
Blask/Skarb	114.1 (42.98)	29.9 (16.29)	19.17	25.11
Blask/Rubinek	86.5 (0)	33.9 (14.11)	12.98	16.46
Rubinek/Skarb	123.5 (44.81)	42.1 (14.11)	0.81	9.29
Antek/Basza/Blask	154.5 (38.04)	59.1 (9.6)	51.64	44.67
Antek/Blask/Skarb	224.8 (83.65)	33.9 (8.14)	37.42	59.22
Antek/Basza/Rubinek	174.5 (74.48)	53 (17.88)	43.94	41.30
Antek/Blask/Rubinek	149 (47.39)	46.9 (11.61)	54.64	48.69
Antek/Basza/Skarb	179.8 (26)	33.9 (14.11)	46.50	64.87
Blask/Rubinek/Skarb	120 (49)	33.9 (14.11)	No reduction	17.56
Basza/Blask/Rubinek	86.5 (0)	44.9 (15.61)	1.10	No reduction
Antek/Rubinek/Skarb	271.4 (50.02)	52.3 (4.07)	21.75	43.01
Basza/Blask/Skarb	86.5 (0)	33.8 (15.53)	25.01	27.48
Basza/Rubinek/Skarb	52.8 (0)	66.3 (25.53)	50.50	No reduction

of level of infection of powdery mildew and grain yield. Mean values, standard deviations and least significant differences (LSDs) were calculated which permitted creation of homogeneous groups for level of infection of powdery mildew and grain yield. Contrast analysis was performed to test the difference in powdery mildew resistance between cultivars and their mixtures, between mixtures and single cultivars, as well as between three-way and two-way mixtures. Data analysis was performed using GenStat 17th edition.

Results

In order to test the influence of genetically diversified cultivar mixtures of spring barley on the development of epidemics of barley powdery mildew, five cultivars of spring barley varying in resistance to barley powdery mildew were cultivated (Table 1) at two locations. The studies were carried out in four consecutive years from 2010 to 2013. The most prevalent among other observed

disease was powdery mildew; other diseases such as leaf rust (*Puccinia hordei*) or scald were observed at much smaller intensity, at the trace level (data not shown).

The analysis of variance indicated that AUDPC was not influenced by the location ($F_{1600} = 1.06$, $p = 0.573$) or the interaction of location \times cultivar/mixture ($F_{24,600} = 1.07$, $p = 0.501$). There was statistically significant influence of years ($F_{3600} = 47.43$, $p < 0.001$), cultivars/mixtures ($F_{24,600} = 31.01$, $p < 0.001$), the interaction of years \times cultivars/mixtures ($F_{72,600} = 2.50$, $p < 0.001$), as well as interaction of location \times years \times cultivars/mixtures ($F_{72,600} = 2.12$, $p < 0.001$).

Average AUDPC for varieties in pure stands also varied in all four growing seasons. In Bakow average AUDPC in pure stands ranged from 203.79 (2011) to 283.23 in growing season 2013. The biggest differences in AUDPC were observed for varieties in growing season 2011 and ranged from 57.71 (Basza) to 781.34 (Antek) (Table 2).

In Koscielna Wies AUDPC in pure stands ranged from 73.83 (2012) to 412.54 in growing season 2013. The biggest differences in AUDPC were recorded for the year

Table 5 Level of infection of powdery mildew (*B. graminis* f. sp. *hordei*) (standard deviation) on cultivars of spring barley in pure stands and in mixtures in the 2013 season

Kombinacja	AUDPC		RAUDPC	
	Bakow	Koscielna Wies	Bakow	Koscielna Wies
Basza	66.3 (0)	71 (15.94)		
Blask	251.4 (39)	361.5 (233.57)		
Antek	770.8 (197.56)	1209.2 (428.7)		
Skarb	272.3 (49.1)	313.6 (264.56)		
Rubinek	55.3 (17.45)	107.4 (69.61)		
Basza/Blask	132.3 (28.57)	78.4 (20.64)	15.52	51.99
Antek/Basza	238.2 (115.51)	500.9 (392.25)	45.66	11.82
Basza/Skarb	89.3 (26.8)	194.9 (257.01)	44.27	No reduction
Basza/Rubinek	56.4 (11.42)	107.8 (68.87)	6.94	No reduction
Antek/Blask	310.4 (162.82)	446.5 (272.28)	32.10	34.92
Antek/Skarb	334.9 (134.93)	747.8 (267.64)	31.46	No reduction
Antek/Rubinek	229.8 (57.17)	292.6 (274.44)	44.49	47.23
Blask/Skarb	144.8 (38.84)	155.2 (37.33)	43.84	38.31
Blask/Rubinek	103.9 (16.55)	244.1 (140.84)	29.91	No reduction
Rubinek/Skarb	76.5 (9.89)	198.8 (170.77)	51.51	No reduction
Antek/Basza/Blask	177 (79.02)	246.2 (148.21)	51.93	47.64
Antek/Blask/Skarb	160.4 (74.91)	294.9 (87.6)	63.39	46.84
Antek/Basza/Rubinek	160.3 (60.79)	216.3 (118.34)	39.86	52.83
Antek/Blask/Rubinek	227.1 (38.84)	321.3 (172.83)	34.53	37.31
Antek/Basza/Skarb	188.9 (86.37)	233.7 (118.34)	47.54	57.14
Blask/Rubinek/Skarb	137.3 (62.33)	144.1 (97.27)	29.00	15.20
Basza/Blask/Rubinek	111.9 (59.55)	107.8 (70.94)	10.75	7.16
Antek/Rubinek/Skarb	165.4 (81.38)	452.6 (246.72)	55.90	3.16
Basza/Blask/Skarb	98.9 (20.15)	95.1 (28.71)	49.54	46.19
Basza/Rubinek/Skarb	82.6 (14.31)	117.8 (75.45)	35.97	25.14

Table 6 Assessment of differences between cultivars and their mixtures for the AUDPC value

Contrast	Bakow 2010	Bakow 2011	Bakow 2012	Bakow 2013	Koscielna Wies 2010	Koscielna Wies 2011	Koscielna Wies 2012	Koscielna Wies 2013
Basza—Basza in mixtures	– 41	– 69*	– 61*	– 67	52	– 78*	– 19	– 119
Blask—Blask in mixtures	47	43	– 19	91*	34	50	54**	148
Antek—Antek in mixtures	425***	333***	556***	552***	246*	196***	111***	834***
Skarb—Skarb in mixture	113***	67*	4	124**	77	31	15	50
Rubinek—Rubinek in mixtures	– 47	– 70*	– 35	– 80	– 31	– 75*	– 24	– 113
Varieties—varieties in all mixtures	96***	61***	87***	122***	85	24	27.1**	153**
Varieties—varieties in 2 components	81***	62***	78***	112***	133*	24	26.2**	116*
Varieties—varieties in 3 components	112***	60***	96***	132***	38	25	28**	190***
Two-row mixtures—three-row mixtures	31*	– 3	18	21	– 95*	1	1.8	74

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 7 Results of three-factorial variance analysis

Source of variation	<i>df</i>	Sum of squares	Mean square	<i>F</i> statistic	<i>F</i> pr.
Object	24	4569.49	190.4	8.99	<.001
Place	1	15,566.61	15,566.61	735.27	<.001
Year	3	28,267.27	9422.42	445.06	<.001
Object × place	24	233.34	9.72	0.46	0.988
Object × year	72	2979.45	41.38	1.95	<.001
Place × year	3	6844.29	2281.43	107.76	<.001
Object × place × year	72	2500.94	34.74	1.64	0.001
Residual	600	12,702.81	21.17		
Total	799	73,664.2			

2013, including cultivars Basza and Antek. They ranged from 71.05 to 1209.19 (Tables 2, 3, 4, 5).

In all three growing seasons, at both sites Antek was the most susceptible to powdery mildew in pure stands when compared to other tested cultivars (Tables 2, 3, 4, 5). In growing season 2010 similar level of powdery mildew infection was observed at both locations. Basza was more susceptible to powdery mildew in Koscielna Wies than in Bakow. The intensity of infections was reduced in variety mixtures to the level of 11.06–64.04% for Bakow (excluding Basza/Rubinek combination) and 5.74–62.28% for Koscielna Wies (excluding Antek/Basza/Rubinek, Antek/Blask/Rubinek, Blask/Rubinek/Skarb, Basza/Blask/Rubinek, Basza/Blask/Skarb combinations) when compared to pure stands (Table 2).

In the 2011, bigger differences in the level of disease infection were recorded only for the Antek and the intensity of disease symptoms was much higher in Bakow than in Koscielna Wies (Table 3).

At both sites, the level of powdery mildew infection in mixtures was reduced to the levels of 1.85–60.49% for Bakow and 4.42–42.85% for Koscielna Wies (excluding combinations: Basza/Rubinek, Antek/Skarb, Rubinek/Skarb, Antek/Basza/Rubinek, Basza/Blask/Rubinek, Basza/Blask/Skarb, Basza/Rubinek/Skarb), when compared to pure stands (Table 3).

In growing season 2012 the intensity of powdery mildew infection in Koscielna Wies was much higher on all tested cultivars in pure stands when compared to the previous 2011 season. At the same growing season in Bakow higher level of infection in pure stands was only observed on Basza and Antek (Table 4).

For both locations, the disease infection was reduced in mixture to the levels of 0.81% to 54.64% for Bakow (excluding Blask/Rubinek/Skarb combination) and 9.29–64.87% (for Koscielna Wies, excluding combinations: Basza/Rubinek, Basza/Blask/Rubinek and Basza/Rubinek/Skarb) when compared with pure stands (Table 4).

In Koscielna Wies, higher level of infection of powdery mildew was observed on the varieties in pure stands in growing season 2013 when compared to the season 2012. In Bakow, higher level of infection was observed on four cultivars excluding Rubinek in 2013 when compared to growing season 2012 (Table 5).

For both locations, the disease infection was much lower and reduced in mixtures when compared to pure stands to the level of 6.94% (Basza/Rubinek) to 63.39% (Antek/Blask/Skarb) in Bakow and 3.16–57.14% in Koscielna Wies (except combinations of Basza/Skarb, Basza/Rubinek, Antek/Skarb, Blask/Rubinek i Rubinek/Skarb) (Table 5).

The difference between the cultivars and their mixtures in terms of the AUDPC value in particular locations and years was expressed as relevant contrast values (Table 6). The AUDPC values were significantly higher in pure stands when compared with mixtures and were observed in Antek at both sites and all three growing seasons. Mixtures with the Basza showed significantly bigger AUDPC values than the pure stand in 2011 growing season in both locations and in 2012 in Bakow. On average, single cultivars showed bigger AUDPC values than the two-way, three-way and all mixtures together in Bakow in all three growing seasons. In Koscielna Wies, such correlation was observed in 2012 and 2013 (Table 6).

The results of analysis of variance indicated that grain yield was influenced by the cultivar/mixture ($F_{24,600} = 8.99$, $p < 0.001$), location ($F_{1600} = 735.27$, $p < 0.001$), year ($F_{3600} = 445.06$, $p < 0.001$), the interaction of cultivar/mixture × year ($F_{72,600} = 1.95$, $p < 0.001$), location × year interaction ($F_{3600} = 107.76$, $p < 0.001$) and cultivar/mixture × location × year interaction ($F_{72,600} = 1.64$, $p = 0.001$) (Table 7).

In Bakow, grain yield (dt/ha) of varieties in pure stands ranged from 45.78 (Antek) to 63.68 (Skarb) in season 2010; 35.38 (Blask) to 40.88 (Rubinek) in 2011; 53.55 (Antek) to 65.98 (Blask) in 2012; and 31.73 (Antek) to 51.55 (Basza) in 2013.

Table 8 Grain yield (dt/ha) of spring barley varieties in pure stands and mixtures in Bakow

Object	2010			2011			2012			2013		
	Yield	Average-pure stands	Yield increase	Yield	Average-pure stands	Yield increase	Yield	Average-pure stands	Yield increase	Yield	Average-pure stands	Yield increase
Basza	54.60			36.73			63.65			48.78		
Blask	53.92			35.38			65.98			45.22		
Antek	45.78			39.55			53.55			31.73		
Skarb	63.68			39.38			59.42			51.55		
Rubinek	62.17			40.88			55.85			49.42		
Basza/Blask	54.20	54.26	–*	35.67	36.06	–	63.43	64.82	–	48.62	47.00	1.62
Antek/Basza	52.60	50.19	2.41	39.97	38.14	1.83	60.25	58.60	1.65	39.83	40.26	–
Basza/Skarb	63.00	59.14	3.86	37.90	38.06	–	61.77	61.54	0.24	51.65	50.17	1.49
Basza/Rubinek	59.55	58.39	1.16	41.38	38.81	2.58	60.10	59.75	0.35	51.77	49.10	2.67
Antek/Blask	52.27	49.85	2.42	37.63	37.47	0.16	57.75	59.77	–	39.88	38.48	1.41
Antek/Skarb	55.58	54.73	0.85	39.93	39.47	0.46	58.52	56.49	2.04	44.17	41.64	2.53
Antek/Rubinek	54.83	53.98	0.85	41.92	40.22	1.71	56.73	54.70	2.03	43.32	40.58	2.75
Blask/Skarb	58.20	58.80	–	38.58	37.38	1.20	61.28	62.70	–	49.28	48.39	0.90
Blask/Rubinek	60.02	58.05	1.98	38.02	38.13	–	60.65	60.92	–	49.88	47.32	2.56
Rubinek/Skarb	65.32	62.93	2.40	40.38	40.13	0.25	56.60	57.64	–	51.93	50.49	1.45
Antek/Basza/Blask	54.10	51.43	2.67	37.25	37.22	0.03	59.58	61.06	–	41.15	41.91	–
Antek/Blask/Skarb	56.15	54.46	1.69	38.40	38.10	0.30	59.45	59.65	–	41.70	42.83	–
Antek/Basza/Rubinek	57.33	54.18	3.15	40.67	39.05	1.62	58.63	57.68	0.95	44.10	43.31	0.79
Antek/Blask/Rubinek	56.88	53.96	2.92	39.02	38.60	0.42	57.92	58.46	–	42.55	42.12	0.43
Antek/Basza/Skarb	57.15	54.69	2.46	39.00	38.55	0.45	58.52	58.87	–	44.33	44.02	0.31
Blask/Rubinek/Skarb	62.30	59.92	2.38	38.60	38.55	0.05	60.35	60.42	–	48.80	48.73	0.07
Basza/Blask/Rubinek	57.45	56.90	0.55	39.60	37.66	1.94	61.55	61.83	–	47.75	47.81	–
Antek/Rubinek/Skarb	60.42	57.21	3.21	40.80	39.94	0.86	58.65	56.27	2.38	45.73	44.23	1.50
Basza/Blask/Skarb	59.08	57.40	1.68	36.35	37.16	–	63.42	63.02	0.40	47.93	48.52	–
Basza/Rubinek/Skarb	63.45	60.15	3.30	37.92	39.00	–	58.80	59.64	–	50.92	49.92	1.00
LSD _{0.05}	5.80			4.71			3.70			5.02		

* No yield increase

Table 9 Grain yield (dt/ha) of spring barley varieties in pure stands and mixtures in Koscielna Wies

Object	2010			2011			2012			2013		
	Yield	Average—pure stands	Yield increase	Yield	Average—pure stands	Yield increase	Yield	Average—pure stands	Yield increase	Yield	Average—pure stands	Yield increase
Basza	53.71			53.23			75.26			59.68		
Blask	62.36			60.97			68.14			53.77		
Antek	59.53			51.67			55.56			45.74		
Skarb	58.11			56.74			73.86			58.02		
Rubinek	58.68			61.74			64.20			53.77		
Basza/Blask	58.61	58.04	0.58	53.45	57.10	–	66.02	71.70	–	54.79	56.73	–
Antek/Basza	60.52	56.62	3.90	51.80	52.45	–	64.77	65.41	–	52.33	52.71	–
Basza/Skarb	62.39	55.91	6.48	54.32	54.99	–	68.71	74.56	–	61.71	58.85	2.86
Basza/Rubinek	59.88	56.20	3.69	59.14	57.49	1.66	69.97	69.73	0.24	60.35	56.73	3.63
Antek/Blask	59.08	60.95	–	55.21	56.32	–	57.55	61.85	–	49.09	49.76	–
Antek/Skarb	59.71	58.82	0.89	51.68	54.21	–	63.79	64.71	–	50.14	51.88	–
Antek/Rubinek	59.83	59.11	0.72	53.89	56.71	–	62.76	59.88	2.88	52.12	49.76	2.36
Blask/Skarb	62.98	60.24	2.75	59.83	58.86	0.97	68.53	71.00	–	57.68	55.90	1.79
Blask/Rubinek	62.97	60.52	2.45	58.79	61.36	–	67.47	66.17	1.30	57.09	53.77	3.32
Rubinek/Skarb	59.14	58.40	0.75	57.26	59.24	–	71.20	69.03	2.17	60.58	55.90	4.69
Antek/Basza/Blask	59.58	58.53	1.05	56.45	55.29	1.16	61.32	66.32	–	51.94	53.06	–
Antek/Blask/Skarb	61.52	60.00	1.52	55.52	56.46	–	62.83	65.85	–	54.45	52.51	1.94
Antek/Basza/Rubinek	59.36	57.31	2.05	58.71	55.55	3.16	59.62	65.01	–	56.20	53.06	3.14
Antek/Blask/Rubinek	57.77	60.19	–	56.50	58.13	–	62.70	62.63	0.07	52.11	51.09	1.02
Antek/Basza/Skarb	59.55	57.12	2.43	53.71	53.88	–	62.12	68.23	–	55.36	54.48	0.88
Blask/Rubinek/Skarb	57.29	59.72	–	60.27	59.82	0.45	68.23	68.73	–	59.02	55.19	3.83
Basza/Blask/Rubinek	57.76	58.25	–	59.77	58.65	1.12	66.56	69.20	–	57.52	55.74	1.78
Antek/Rubinek/Skarb	57.06	58.77	–	59.08	56.72	2.36	64.79	64.54	0.25	57.42	52.51	4.91
Basza/Blask/Skarb	59.73	58.06	1.67	60.97	56.98	3.99	68.48	72.42	–	61.48	57.16	4.32
Basza/Rubinek/Skarb	61.91	56.83	5.08	55.65	57.24	–	66.52	71.11	–	58.85	57.16	1.69
LSD _{0.05}	5.84			10.24			5.56			8.46		

– No yield increase

Variety mixtures yielded (dt/ha) 52.27 (Antek/Blask) to 65.32 (Rubinek/Skarb) in season 2010; 35.67 (Basza/Blask) to 41.92 (Antek/Rubinek) in 2011; 56.60 (Rubinek/Skarb) to 63.43 (Basza/Blask) in 2012; 39.83 (Antek/Basza) to 51.93 (Rubinek/Skarb) in 2013.

The growth of yield in mixtures when compared to pure stands (dt/ha) ranged from 0.55 (Basza/Blask/Rubinek) to 3.86 (Basza/Skarb) in 2010; 0.03 (Antek/Basza/Blask) to 2.58 (Basza/Rubinek) in 2011; 0.24 (Basza/Skarb) to 2.38 (Antek/Rubinek/Skarb) in 2012; and 0.07 (Blask/Rubinek/Skarb) to 2.75 (Antek/Rubinek) in 2013 (Table 8).

In Koscielna Wies grain yield (dt/ha) of varieties in pure stands ranged from 5371 (Basza) to 62.36 (Blask) in 2010; 51.57 (Antek) to 61.47 (Rubinek) in 2011; 55.56 (Antek) to 75.26 (Basza) in 2012; 45.74 (Antek) to 59.68 (Basza) in 2013.

Variety mixtures yielded (dt/ha) 57.06 (Antek/Rubinek/Skarb) to 60.52 (Blask/Rubinek) in 2010; 51.68 (Antek/Skarb) to 60.97 (Basza/Blask/Skarb) in 2011; 57.55 (Antek/Blask) to 71.20 (Rubinek/Skarb) in 2012; 49.09 (Antek/Blask) to 61.71 (Basza/Skarb) in 2013.

Yield growth in mixtures when compared to pure stands (dt/ha) ranged from 0.58 (Basza/Blask) to 6.48 (Basza/Skarb) in 2010; 0.45 (Blask/Rubinek/Skarb) to 3.99 (Basza/Blask/Skarb) in 2011; 0.07 (Antek/Blask/Rubinek) to 2.88 (Antek/Rubinek) in 2012; 0.88 (Antek/Basza/Skarb) to 4.69 (Rubinek/Skarb) in 2013 (Table 9).

Discussion

It has been shown that the intensification of the disease caused by monoculture could be avoided by retaining diversity within the cereal crops (Finckh et al. 2000; Newton et al. 2009). Our study has shown that cultivation of varieties of spring barley in mixtures reduces the intensity of powdery mildew disease when compared to pure stands.

Modern plant protection methods have to be compliant with the ideas of sustainable agriculture, whose premise is plant protection that is economically profitable, environmentally friendly and accepted in the community. In light of that, contemporary systems of cultivated plant protection should incorporate any available methods of pest control. Those include both interspecies mixtures and intercultural mixtures within the same species. In recent years, in Poland about 17% of general growing area has been sown with mixtures (cereal and cereal–leguminous).

The biggest advantage of cultivating crops in mixtures is introducing biodiversity and individual features of particular plants which allows a better use of the environmental resources without disturbing its biological balance. Genetic diversity in mixed stands is increased when compared to

pure stands. Main advantages of cultivating crops in variety mixtures involve an increased genetic distance between plants with the same genetic resistance to diseases, restriction of pathogen spread by resistant plants which form a natural barrier, competitive interactions among host plants affecting their disease susceptibility (Finckh et al. 2000).

Relationship between disease severity and mixtures composition was found in the experiments involving different sets of mixtures of four near-isogenic barley lines. The powdery mildew levels in the mixtures were reduced most when the mean resistance of pure stands was intermediate (Kolster et al. 1989). Other researchers (Gacek 2000; Tratwal et al. 2007; Tratwal and Walczak 2010) also observed a reduction in the level of powdery mildew infection in mixtures of spring cereal when compared to pure stands of up to 75%. The Directive of the European Parliament and of the Council 2009/128/CE of 21 October 2009 established a framework for Community action which emphasize the use of all possible sustainable methods which reduce the use of pesticides. Together, our study confirms that cultivation of spring barley varieties in mixtures might be used in both industrialized and organic agriculture which is of great economic importance as integrated plant protection requires the use of non-chemical methods (biological, physical) against pathogens.

Conclusions

1. Powdery mildew (*B. graminis* f. sp. *hordei*) was the most prevalent among other observed diseases, and Antek cultivar was the most susceptible to powdery mildew in each trial (year and location).
2. Our study has shown that there was a positive effect of cultivating mixtures of spring barley varieties in terms of their resistance to powdery mildew (*B. graminis* f. sp. *hordei*) and yield increase.
3. Cultivation of spring barley as a variety mixture is an alternative way of growing this crop instead of monoculture (single variety) in low cost and ecological farming.
4. Further work is required to develop mixtures of spring barley on a wider scale, especially selecting new varieties for mixtures development.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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