

THE POSSIBILITIES OF REDUCTION OF WINTER BARLEY CHEMICAL PROTECTION BY GROWING VARIETY MIXTURES. PART I. EFFECT ON POWDERY MILDEW LEVEL

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Abstract: In the four-year experiment the impact of four different winter barley varieties and selected two- and three-component mixtures were tested. Reduced dosages of fungicides on disease reduction in the mixtures compared with pure stands were evaluated. The studies were carried out at two sites: Experimental Station for Variety Testing Słupia Wlk. (Wielkopolska region) and Plant Breeding Station Bąków (Opole District). Observations on powdery mildew occurrence during the vegetation season were done every 7–10 days. In order to compare the disease occurrence levels on different cultivars in pure stands and on their mixtures combined with different fungicide treatments the Area Under Disease Progress Curve (AUDPC) was evaluated. On the base of the AUDPC values the reduction of powdery mildew occurring in the mixtures due to epidemiological and ecological factors functioning in mixed stands were also evaluated. On the base of obtained results it can be stated that winter barley variety mixtures combined with different fungicide treatments do reduce the powdery mildew incidence comparing to pure stands and winter barley variety mixtures can constitute an alternative way of growing winter barley, especially at low-input and ecological agriculture.

Key words: pro-ecological agriculture, variety mixtures, powdery mildew, winter barley, low-input husbandry

INTRODUCTION

Monocultures of modern cereal crops are popular due to technical and organizational reasons. In particular they are easier to manage in terms of crop husbandry and marketing (consistent quality and product use). However, in this monoculture chemical protection of crops is the norm, to reduce yield loss due to diseases, pests and sometimes weed infestation. In order to keep high and stable grain yields with appropriate grain quality characteristics in the monoculture, relatively high inputs may be needed. Experimentally and practically it has been demonstrated that culti-

var mixtures and also species mixtures can constitute an alternative to the traditional monoculture. It has been found that crops sown and managed as mixtures operate different epidemiological and ecological factors, which lead to considerable disease reduction, better pest and weed control, which translate into higher and more stable grain yields when compared to the mixture components grown in pure stands. (Finckh et al. 2000; Gacek et al. 1994; Wolfe et al. 1997).

Appropriate mixtures of winter barley cultivar can considerably restrict the development of powdery mildew (*Blumeria graminis* f. sp. *hordei*) and to some extent other airborne diseases (Gacek et al. 1996). Cultivar mixtures can provide functional diversity that limits pathogen and pest expansion by making use of knowledge known about interactions between hosts and their pests and pathogens to direct pathogen evolution. Indeed, one of the most powerful ways to reduce risk of resistance breakdown and to still make use of defeated resistance genes is to use cereal variety and species mixtures (Finckh et al. 1999, 2000).

The results of four years field experiments designed to evaluate epidemiological and economical effects of winter barley cultivar mixtures are presented. The aim of the studies was to evaluate the possibility of reduction of powdery mildew (*B. graminis* f. sp. *hordei*) through growing variety mixtures in combination with fungicide reduced use.

MATERIALS AND METHODS

In the growing seasons 2001/2002–2004/2005, experiments with winter barley variety mixtures combined with different treatments of fungicides were carried out at two sites, namely the Experimental Station for Variety Testing Słupia Wlk. (Wielkopolska District) and the Plant Breeding Station Bąków (Opole District).

During the growing season 2002/2003 the studies were carried out in one site, the Experimental Station for Variety Testing Słupia Wielka. The experiment at the Plant Breeding Station Bąków was completely destroyed by late frost in the spring (March). In the experiment at Słupia Wlk., because of late frost, 25% of plots were destroyed.

In the experiments, four different winter barley cultivars and three, two- and three-component mixtures, composed of these varieties, were tested on 5m² plots in four replicates. The winter barley cultivars: Bombay (BO), Gil (GI), Gregor (GR) and Bażant (BA), and the following mixtures: Bombay/Gil (BOGI), Bombay/Gregor (BOGR), Gil/Gregor/Bażant (GIGRBA) were used.

On the experimental plots seven different treatments with fungicides were applied:

- untreated plots (control),
- single treatment application with ¼, ½ and full dosage of fungicides (at the beginning of shooting),
- treatments with ¼, ½ and full dosages of fungicides but applied twice over the growing season (at the beginning of shooting and at the full/end of shooting).

At the beginning of shooting mixture of two fungicides was used – Amistar 250 SC + Tilt Plus 400 EC. At the full/end of shooting Tilt Plus 400 EC was used.

During the vegetation season powdery mildew infection was observed 3–5 times using 1–9 scale (where 9 – fully resistant, 1 – fully susceptible).

In order to compare the disease occurrence levels on different cultivars in pure stands and on their mixtures combined with different fungicide treatments the Area Under Disease Progress Curve (AUDPC) (Finckh et al.1997) was evaluated. This is defined as follows:

$$\text{AUDPC}_i = \sum_{j=1}^{n-1} (x_j \cdot y_{ij} + x_j \frac{y_{i(j+1)} - y_{ij}}{2})$$

where:

AUDPC_i – (Area Under Disease Progress Curve for i-variety (mixture),

X_j – number of days between j and j+1 observations,

y_{ij} – % of infected plant area by powdery mildew of i-variety (mixture) at the time of j-observation.

The results were statistically evaluated.

RESULTS AND DISCUSSION

The development of powdery mildew in different treatments was analysed on the basis of the Area Under Disease Progress Curve (AUDPC). On the base of the AUDPC value the reduction of powdery mildew in the mixtures occurring due to epidemiological and ecological factors functioning in mixed stands (Wolfe et al. 1975) were also evaluated.

The factors denoted for the analysis were Barley Variety, Treatment, Site and Year, where 2002/2003 was excluded from the analysis since it was at one site only and the data was not considered sufficiently reliable.

An Analysis of Variance (ANOVA) was then carried out on all data. It was found that all main factors were significant at 0.1% level. However looking at the F-Ratio's it showed Year > Site > Year >> Variety > Treatment. Both year and site was highly dominant and further analyses were carried out using separate sites e.g. Bąków and Słupia Wlk.

Table 1. Analysis of variance of Area Under Disease Progress Curve (AUDPC) from Bąków
Variate: Area Under Disease Progress Curve

Source of variation	Degree of freedom	Mean square	F statistic	P-value
Variety	6	1445617	55.30	< 0.001
Treatment	6	793803	30.37	< 0.001
Year	2	24209787	926.14	< 0.001
Variety x Treatment	36	31830	1.22	0.186
Variety x Year	12	258107	9.87	< 0.001
Treatment x Year	12	278880	10.67	< 0.001*
Variety x Treatment x Year	72	29916	1.14	0.210
Residual	441	26140		
Total	587			

*see C and 2TF

Table 2. Analysis of variance of Area Under Disease Progress Curve (AUDPC) from Słupia Wlk. Variate: Area Under Disease Progress Curve

Source of variation	Degree of freedom	Mean square	F statistic	P-value
Variety	6	89603	12.95	< 0.001
Treatment	6	136157	19.67	< 0.001
Year	2	670424.	96.87	< 0.001
Variety x Treatment	36	4720	0.68	0.920
Variety x Year	12	13435	1.94	0.028
Treatment x Year	12	44078	6.37	< 0.001*
Variety x Treatment x Year	72	3609	0.52	1.000
Residual	441	6921		
Total	587			

* see C and 2TF

Separating the sites for analysis gives a fairer comparison of what is being looked at, e.g. "do variety mixtures give better disease protection than separate ones and what are the optimal levels of fungicides to apply?" Tables 1 and 2 show the ANOVA's from individual sites.

The year effect is quite amazing with more AUDPC occurring after each year. However only in the first year was actually more disease at Słupia Wlk. than at Bąków and the rate of increase at Bąków was far greater than at Słupia Wlk. The next most noticeable effect is the treatment and year interaction where in the first year results appear as expected at both sites (i.e. control having the most disease and 2TF having the least). It is interesting to note, that the 2nd highest levels of disease (at both sites) were actually for fungicide combination 1TF (explanations are in Tables 3–5).

For the next two years of the study, the 2TF combination had the lowest levels of disease at Bąków as would be expected, but the control treatment ranked better than other 1-treatment combinations at both sites.

Out of the seven treatments, 1TF and 2TH plus 1TH and 2TQ each give the same level of overall applied fungicide. These were looked at more closely. Table 6 gives the observed differences, in disease levels (AUDPC), between the fungicide combinations at both sites for the three individual years.

Both sites give very much the same overall outcome and all main factors are significant at 0.1% level and only the three way interaction and the variety by treatment interaction are not significant. As year is very much a dominant factor the analysis was repeated using the individual years to see if variety and treatment interaction could be seen if the year effect was removed. In Bąków it was found in the first year that the variety and treatment interaction was significant ($p = 0.003$) (e.g. the average 'control' score was the highest as expected, however, for variety Bazant it was the second lowest). Both Log_{10} and square root transformations of the data were carried out at this stage to 'pull in' any outliers and to help reduce the overall variability of the scores. The analyses were then repeated and in these cases there were no significant variety and treatment interactions. Tables 3, 4 and 5 contain Variety by Treatment results split between the 2 sites for each year.

Table 3. Powdery mildew incidence on winter barley varieties and their mixtures in the vegetation season 2001/2002

Site	Chemical treatment	Area Under Disease Progress Curve							
		Bombay	Gil	Gregor	Bazant	Bombay/ Gil	Bombay/ Gregor	Gil/Gregor/ Bazant	average
Bańków	control	64.8	288.3	43.4	33.0	127.4	48.1	90.8	99.5
	1 treatment ¼ dose (1TQ)	47.5	139.0	29.5	38.5	81.2	61.3	57.2	64.9
	1 treatment ½ dose (1TH)	75.7	124.4	29.5	47.5	84.7	38.5	89.5	69.9
	1 treatment full dose (1TF)	60.5	129.2	42.0	38.5	114.1	54.5	79.8	74.1
	2 treatments ¼ dose (2TQ)	53.0	121.0	29.5	38.5	58.6	40.5	53.0	56.3
	2 treatments ½ dose (2TH)	49.5	84.5	37.0	38.5	63.3	35.0	46.1	50.6
	2 treatments full dose (2TF)	29.5	76.2	29.5	29.5	48.1	29.5	29.5	38.8
	average	54.4	137.5	34.3	37.7	82.5	44.1	63.7	64.9
	control	144.4	188.4	108.2	164.3	106.7	116.3	93.8	131.7
	1 treatment ¼ dose (1TQ)	124.6	116.3	59.7	99.9	74.0	72.0	65.8	87.5
Stupia Wlk.	1 treatment ½ dose (1TH)	121.1	147.9	78.1	100.6	65.8	102.1	74.0	98.5
	1 treatment full dose (1TF)	125.9	134.2	82.1	101.3	110.2	81.6	51.6	100.3
	2 treatments ¼ dose (2TQ)	115.7	113.7	53.6	67.9	93.8	61.8	65.8	81.8
	2 treatments ½ dose (2TH)	119.8	123.9	47.5	67.9	89.7	61.8	65.8	81.0
	2 treatments full dose (2TF)	110.2	93.8	49.6	55.7	61.8	59.7	51.6	68.9
	average	123.1	132.2	68.4	95.0	86.0	77.9	66.9	92.8

Table 4. Powdery mildew incidence on winter barley varieties and their mixtures in the vegetation season 2003/2004

Site	Chemical treatment	Area Under Disease Progress Curve							
		Bombay	Gil	Gregor	Bażant	Bombay/Gil	Bombay/Gregor	Gil/Gregor/Bażant	average
Bałków	control	349.0	481.7	82.1	101.5	409.7	123.0	147.7	242.1
	1 treatment ¼ dose (1TQ)	348.5	677.5	126.1	94.0	370.8	271.0	222.5	301.5
	1 treatment ½ dose (1TH)	476.7	708.1	80.4	80.4	319.3	184.7	261.7	301.5
	1 treatment full dose (1TF)	305.2	537.8	81.3	74.3	285.4	179.4	181.5	235.0
	2 treatments ¼ dose (2TQ)	373.6	711.6	89.1	89.1	308.4	210.8	119.8	271.8
	2 treatments ½ dose (2TH)	216.4	181.7	64.7	55.1	178.3	95.8	79.5	124.5
	2 treatments full dose (2TF)	155.4	178.5	56.8	62.9	97.8	69.0	87.1	101.1
	average	317.7	496.7	82.9	79.6	281.4	162.0	157.1	225.4
Stupia Wlk.	control	236.4	133.3	134.1	143.7	117.0	151.9	110.8	146.7
	1 treatment ¼ dose (1TQ)	338.6	218.9	145.4	192.2	228.3	161.8	154.9	205.7
	1 treatment ½ dose (1TH)	161.4	182.0	125.2	139.0	141.0	94.5	104.8	135.5
	1 treatment full dose (1TF)	231.9	284.1	106.4	167.7	163.2	165.3	173.1	184.5
	2 treatments ¼ dose (2TQ)	194.4	161.8	84.2	113.6	137.8	134.2	59.7	126.5
	2 treatments ½ dose (2TH)	170.4	106.0	69.9	147.9	76.0	88.4	100.6	108.5
	2 treatments full dose (2TF)	201.2	140.9	101.9	182.3	94.5	84.2	110.2	130.7
	average	219.2	175.3	109.5	155.2	136.8	125.7	116.4	148.3

Table 6. Observed differences in disease levels

1TF – 2TH	2001/2002	2003/2004	2004/2005
Bąków	23.5	110.5	367.4
Słupia Wlk.	19.3	76.0	143.4
1TH – 2TQ	2001/2002	2003/2004	2004/2005
Bąków	13.6	29.7	31.6
Słupia Wlk.	16.7	9.0	4.6

1TF – one treatment with full dose

2TH – two treatments with half dose

2TQ – two treatments with quarter dose

Bąków LSD for Treatment.Year is 84.92

Słupia Wlk. LSD for Treatment.Year is 43.70

There appears to be no difference between fungicide levels 1TH and 2TQ across all years and sites or between any varieties. This suggests whichever fungicide application is the most cost effective would be the right one to choose and no more disease would expect to occur irrespective of season. In this case, it would be considered cheaper to apply 1-application of half dosage rather than 2-applications of quarter dosage. However, it was interesting from the yield study that there was a significant difference at Bąków for this combination of treatments.

Looking at 1TF V 2TH all values are lower for the 2TH fungicide level and it does appear to be significantly better at both sites in the years 2003/2004 and 2004/2005 which suggests applying half rate dosage twice rather than full dosage once would reduce disease levels. Looking more closely, taking variety into account, only Gil is significantly better for this treatment (2TH) in 2003/2004 (at both sites). This suggests the level of disease in the variety Gil is dominating, the high disease values seen for 1TF and when the analyses is rerun excluding Gil the variety and treatment interaction, at both sites, becomes non-significant for 1TF and 2TH. In 2004/2005 many varieties are also significantly lower for 2TH compared with 1TF: 2 in 7 varieties (BO and BOGI) at Słupia Wlk, 5 out of 7 at Bąków. Bażant and BOGR are not significant at either site. Knowing whether there were some very different growing conditions in these years (especially 2004/2005) compared with 2001/2002 would be useful in helping decide when to go for the more frequent application of fungicide.

As for variety selection, there is evidence at both sites that this is significant (see Tables 1 and 2). As it is the variety mixtures that we want to compare with the respective pure stands further work was carried out looking at the variety mixtures compared to the mean of the respective pure stands. The following ANOVA's were looked at for both sites and are shown in Tables 7 and 8.

There are no available papers aimed at the influence of winter barley variety mixtures combined with different fungicide treatments on disease reduction. Other authors (Newton et al. 2002) in the experiment with spring barley variety mixtures combined with standard fungicide treatments showed 30–60% of powdery mildew reduction in mixtures (AUDPC reductions in mixtures compared to pure stands).

In the experiment with spring barley variety mixtures (without fungicide control) other authors (Gacek 1986; Gacek and Nadziak 2000) revealed 30–70% of powdery mildew reductions.

Table 7. Analysis of variance of Area Under Disease Progress Curve at Bąków over three growing seasons.

Variate: Area Under Disease Progress Curve

Source of variation	Degree of freedom	Mean square	F statistic	P-value
Variety	6	1445617	55.91	< 0.001
BO and GI x BOGI	1	286758	11.09	< 0.001
BO and GR x BOGR	1	222191	8.59	0.004
GI,GR and BA x GIGRBA	1	93746	3.63	0.058
Variety x Treatment	36	31830	1.23	0.173
BO and GI x BOGI x Treatment	6	33665	1.30	0.255
BO and GR x BOGR x Treatment	6	10832	0.42	0.866
GI,GR and BA x GIGRBA x Treatment	6	36460	1.41	0.209
Variety x Year	12	258107	9.98	< 0.001
BO and GI V BOGI x Year	2	59131	2.29	0.103
BO and GR x BOGR x Year	2	113466	4.39	0.013
GI,GR and BA x GIGRBA x Year	2	17841	0.69	0.502

Table 8. Analysis of variance of Area Under Disease Progress Curve at Słupia Wlk. over three growing seasons.

Variate: Area Under Disease Progress Curve

Source of variation	Degree of freedom	Mean square	F statistic	P-value
Variety	6	89603	13.97	< .001
BO and GI x BOGI	1	183907	28.66	< .001
BO and GR x BOGR	1	64277	10.02	0.002
GI,GR and BA x GIGRBA	1	97378	15.18	< .001
Variety x Treatment	36	4720	0.74	0.870
BO and GI x BOGI x Treatment	6	2255	0.35	0.909
BO and GR x BOGR x Treatment	6	5481	0.85	0.529
GI,GR and BA x GIGRBA x Treatment	6	1853	0.29	0.942
Variety x Year	12	13435	2.09	0.016
BO and GI V BOGI x Year	2	3834	0.60	0.551
BO and GR x BOGR x Year	2	3795	0.59	0.554
GI,GR and BA x GIGRBA x Year	2	4410	0.69	0.503

BO – Bombay, GI – Gil, GR – Gregor, BA – Bażant, BOGI – Bombay/Gil,
BOGR – Bombay/Gregor, GIGRBA – Gil/Gregor/Bażant

Experiments with winter wheat variety mixtures (Gacek et al. 1997) showed that thanks to growing two-component variety mixtures 6–34% reduction of powdery mildew were observed while thanks to growing three-component mixtures – 5–41% of disease reductions were achieved.

Summary of variety performance of AUDPC

Looking at tables 7 and 8 there does appear to be strong evidence that variety mixtures do reduce the AUDPC's. Using mixture of Bombay and Gil instead of the individual component stands does significantly reduce AUDPC at the 0.1% level; Bombay and Gregor is significant at the 1% level, for both sites. Although the 3-way mixture of Gil, Gregory and Bažant is significant at the 0.1% level at Słupia Wlk, it just fails to reach a significant level at Bąków. Table 9 shows the variety means in each year at Bąków:

Table 9. Variety by year means for AUDPC at Bąków

Variety	2001/2002	2003/2004	2004/2005	Average
Bombay	54.4	317.7	761.6	377.9
Bažant	37.7	79.6	552.1	223.1
Bombay/Gil	82.5	281.4	873.9	412.6
Bombay/Gregor	44.0	162.0	528.7	244.9
Gil/Gregor/Bažant	63.7	157.1	714.8	311.9
Gil	137.5	496.7	1136.9	590.4
Gregor	34.4	82.9	596.4	237.9

Given below are the observed difference between the mean of the pure stands and the respective variety mixture (mean of pure stands – mixture)

Bombay/Gil	13.5	125.8	75.4	71.5
Bombay/Gregor	0.4	38.3	150.3	63.0
Gil/Gregor/Bažant	6.2	62.6	47.0	38.6
LSD	84.5			
Variety x Year				
LSD Variety	48.8			

All figures are positive showing that the levels of powdery mildew in the variety mixtures are actually lower than the mean of the pure stands in all cases but they are not necessarily lower than the actual individual pure stands. The bold figures are the relevant significant ones. The 150.3 shows that in the year 2004/2005 (very high AUDPC year) the reduction of AUDPC for mixture Bombay and Gregor compared with the mean of the respective pure stands is significantly more than the other two years. However, the variety mixture does not significantly reduce disease levels compared with the pure stand Gregor. There appears to be no significant interaction with regard to treatment. Also of interest is the reduction of AUDPC for BOGI compared with both BO and GI in 2003/2004 (125.8) but not significantly compared with the overall effect.

Results from Słupia Wlk are similar but in this case all variety mixture combinations do appear to reduce AUDPC levels significantly including the three way mixture of Gil, Gregory and Bażant at the 0.1% level. The patterns over the years is similar i.e. no significant interaction as well as for treatments. Table 10 shows the variety means in each year at Słupia Wlk.

Table 10. Variety by year means for AUDPC at Słupia Wlk.

Variety	2001/2002	2003/2004	2004/2005	Average
Bombay	123.1	219.2	231.9	191.4
Bażant	95.0	155.2	175.0	141.7
Bombay/Gil	86.0	136.8	195.1	139.3*
Bombay/Gregor	77.9	125.8	178.8	127.5
Gil/Gregor/Bażant	66.9	116.4	173.6	119.0
Gil	132.2	175.3	297.8	201.8
Gregor	68.4	109.5	216.0	131.3

Observed difference between mean of the pure stands and the respective variety mixture (mean of pure stands – mixture)

Bombay/Gil	41.7	60.5	69.8	57.3
Bombay/Gregor	17.9	38.6	45.2	33.9
Gil/Gregor/Bażant	31.6	30.3	56.0	39.3
LSD Variety x Year	42.1			
LSD Variety	24.3			

All bold values are above 24.3 showing that using variety mixtures should indeed reduce the levels of AUDPC compared with the mean of the respective pure stands. In addition, the use of variety mixture Bombay and Gil will significantly reduce the levels of AUDPC compared with both the individual pure stands over the three growing seasons. Taking individual years 2003/2004 and 2004/2005 it was found that this mixture does not significantly reduce disease levels compared with GI in 2003/2004 and BO in 2004/2005.

CONCLUSIONS

1. Meteorological conditions in growing seasons had essential influence on experiment results.
2. Winter barley variety mixtures do reduce the powdery mildew (*B. graminis* f. sp. *hordei*) occurrence comparing to pure stands.
3. Highest powdery mildew reductions were observed in mixtures with variety Gil as the one of the component.
4. The results of the study show that the combination of variety mixtures with reduced use of fungicides can be regarded as a low – input and environment – friendly method of winter barley growing.

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POLISH SUMMARY**MOŻLIWOŚĆ OGRANICZENIA CHEMICZNEJ OCHRONY JĘCZMIENIA OZIMEGO POPRZEZ UPRAWĘ MIESZANEK ODMIAN. CZĘŚĆ I. WPŁYW NA NASILENIE WYSTĘPOWANIA MĄCZNIAKA PRAWDZIWEGO**

W czteroletnim doświadczeniu polowym (2001/2002–2004/2005), w dwóch miejscowościach (Stacja Doświadczalna Oceny Odmian Słupia Wlk. – woj. wielkopolskie i Hodowla Roślin Smolice Oddział Bąków – woj. opolskie) badano wpływ uprawy czterech odmian jęczmienia ozimego w siewie czystym i w mieszankach w połączeniu ze stosowaniem fungicydów w różnych dawkach i ilościach zabiegów na ograniczenie występowania mączniaka prawdziwego (*Blumeria graminis* f. sp. *hordei*).

W sezonie wegetacyjnym nasilenie występowania choroby na roślinach przedstawiano wartością AUDPC (Area Under Disease Progress Curve) tj. wielkością powierzchni pod krzywą postępu choroby. Na podstawie uzyskanych wartości AUDPC wyliczono poziom redukcji występowania mączniaka prawdziwego na mieszankach odmian jęczmienia ozimego w różnych kombinacjach ochrony w porównaniu do siewów czystych. Na podstawie uzyskanych wyników można stwierdzić, że uprawa mieszanek odmianowych może być alternatywną formą uprawy jęczmienia ozimego, zwłaszcza w rolnictwie niskonakładowym i ekologicznym, gdyż dzięki ich uprawie notowano ograniczenie nasilenia występowania mączniaka prawdziwego.