Eur J Plant Pathol (2018) 152:469–477
https://doi.org/10.1007/s10658-018-1494-8

The combined effects of multiple diseases and climatic conditions on thousand kernel weight losses in winter wheat

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Accepted: 27 April 2018 / Published online: 5 May 2018

Abstract Thousand kernel weight (TKW) is a yield component associated with grain quality. It is reported in the literature that TKW is significantly influenced by varieties, agro-ecological conditions and disease indices, but the influence of their interactions on TKW loss has rarely been taken into consideration. The main objective of this study was to examine the combined effects of multiple diseases and climatic conditions on TKW losses in winter wheat. Leaf rust, powdery mildew, and Septoria tritici blotch were considered biotic predictor variables in regression models explaining TKW losses. Monthly averages of temperature, relative humidity and total rainfall in May and June in the 2006–2013 growing seasons were used as abiotic predictor variables. The results of this study indicated a significant low positive correlation between yield loss and TKW loss in the two varieties. TKW losses were less influenced by leaf rust, powdery mildew, and Septoria tritici blotch than yield losses. The significant influence of the interaction between variety and the environmental conditions on TKW loss was confirmed from the general linear model function. The results of this study indicated that factors influencing yield and yield component losses are part of the complex environment, and the relationship between them should be investigated with respect to their interactions.

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Keywords Thousand kernel weight · Winter wheat · Regression models · Leaf rust · Powdery mildew · Septoria tritici blotch

Introduction

The negative impact of climate change on agricultural production and environmental degradation are some of the challenges for wheat production (FAO 2011; Alexandratos and Bruinsma 2012). The improvement in quality and productivity of cereal crops is a major goal of breeders, considering the growing global population. Breeding high yielding wheat varieties with good quality is not an easy task as these traits can be negatively correlated (Laidig et al. 2017) or not correlated at all (Mladenov et al. 2011).

The factors influencing yield and yield components have been studied for decades, but the influence of major factors was analysed separately without considering their interactions (Juroszek and von Tiedemann 2013; White et al. 2011). Mohammadi et al. (2012) reported that analysing simple correlations between yield and yield components without taking into account their interactions may mislead breeders in reaching their goals. Jevtić et al. (2017) noted that climate changes have an impact on wheat yield not only directly but also through interactions with biotic factors. However, their combined effects on yield components have yet to be investigated.

Thousand kernel weight (TKW) is one of the yield components associated with grain quality. Higher TKW indicates better milling quality and better germination of wheat grain (Protic et al. 2007). Although the significant effect of varieties, agro-ecological conditions and diseases on TKW has been reported in several studies (Draz et al. 2015; Cao et al. 2014), the impact of interactions between disease indices and abiotic factors has rarely been taken into consideration.

Wheat breeding for high TKW is one of the goals of breeding programmes, but yield quality traits need to be combined with good disease resistance to generate varieties suitable for a reduced fungicide integrated pest management (IPM) regime. On the basis of observations in the earlier studies, it was hypothesized in this study that different environmental factors impact TKW and yield loss. As a result, the main objective of this study was to evaluate the combined effects of biotic and abiotic factors on TKW losses. The influence of multiple disease systems on TKW losses was analysed using *Puccinia triticina* (leaf rust)*, Blumeria graminis* f. sp. *tritici* (powdery mildew), and *Zymoseptoria tritici* (Septoria tritici blotch) as biotic predictor variables. Abiotic predictor variables included monthly averages of temperature, total rainfall and relative humidity in May and June for the period 2006 to 2013. Climatic elements in May and June were considered the most influential on TKW since they were related to anthesis, fruit development and ripening (BBCH 61–89) of wheat in agro-ecological conditions of Serbia.

Materials and methods

Plant material

Data originated from fungicide efficacy trials conducted in the locality Rimski Šančevi (Vojvodina, the northern province of Serbia) over the period 2006 to 2013. The influence of the changing climate elements and disease indices on yield and TKW losses was examined on two model varieties: club wheat Barbee (*Triticum aestivum spp. compactum*) and durum wheat Durumko (*Triticum turgidum subsp. durum*). Barbee is known for increased susceptibility to obligate parasites (*Blumeria* and *Puccinia*), while Durumko is usually used as a susceptible check for leaf blotch diseases such as *Zymoseptoria tritici*.

Field trials

Field trials were set up under naturally occurring inoculum conditions in a randomized block design in four replicates for each non-sprayed check and fungicide-sprayed treatment. The plot size was 10 m^2. A trial usually included 10 fungicide-sprayed treatments applied at growth stage BBCH 36–37 (flag leaf just visible, rolled) and BBCH 51–59 (inflorescence emergence, heading**)**. Different types of active ingredients, such as amides, azoles, aromatics, benzimidazoles, oxazoles, morpholines, pyridines, pyrazoles and strobilurins, were applied at the recommended dosage rates using calibrated field crop sprayers with fan nozzles at 300 kPa pressure and 200 L of water per hectare. The mean sowing date for winter wheat in Serbian agro-ecological conditions is 20 October (optimal time of sowing).

Disease assessment

Assessments of leaf rust and powdery mildew severity were made at the growth stage 71–73 BBCH (kernel watery; early milk) using a modified Cobb's scale (Peterson et al. 1948). Disease severity of Septoria tritici blotch was assessed using the disease rating keys devised by James (1971) at the growth stage 71–73 BBCH. The disease indices (%) were calculated by taking into consideration disease incidence and average disease severity (Cao et al. 2014). The upper three leaves were assessed for symptoms.

Yield and TKW losses

Yield and TKW were measured for each plot at 15% water content. The losses of yield and TKW (%) were determined as yield and TKW reductions in untreated plots compared with those in treated plots showing the best control of wheat diseases (Eq. 1). The efficacy of fungicides showing the best control of wheat diseases exceeded 80%.

$$Y(\%) = ((Y_1 - Y_2)/Y_1) \times 100 \quad (1)$$

Y_1 grain yield or TKW of fungicide treatment for the best wheat disease control

Y_2 grain yield or TKW of the non-sprayed check treatment.

Statistical methods

The effects of year, variety and their interactions on yield and TKW were analysed using the General Linear

Model function in Minitab 17 Statistical Software (trial version). The correlations between yield and TKW and between yield and TKW losses were estimated using Spearman's coefficient of correlation. Further, multivariate regression models were used to estimate the relationship between disease indices, abiotic factors and TKW losses. Disease indices were considered as biotic predictive variables, while monthly averages of temperatures, relative humidity and total rainfall taken from May to June in the period 2006–2013 (http://www.hidmet.gov.rs/) were considered as abiotic predictor variables. In 2014, yellow rust became predominant over leaf rust; thus, the period from 2014 was excluded from regression models. Since biotic and abiotic factors are correlated (multicollinearity), stepwise regression and best subset regression were applied to make predictions of TKW losses in Barbee and Durumko. Interactions between predictor variables were also considered for regression model building. Regression models were as follows: coefficient of determination (R^2) expressing the percentage of response variation explained by the model; coefficient of prediction (R^2_{pred}) determining possibilities of response predictions with new observations; and Mallow's Cp comparing the precision and bias of the full model to models with a subset of predictors. Multivariate regression analysis was performed using Minitab 17 Statistical Software (trial version).

Results and discussion

Average yield (3.6 t/ha) and average TKW (23.7 g) of Barbee in untreated plots were significantly lower ($P < 0.001$) than average yield (6.5 t/ha) and average TKW (43.7 g) of Durumko. A significant influence of year ($P < 0.001$) and variety ($P < 0.001$) on yield and TKW was observed. The average yield loss of variety Barbee (30%) was much higher than the average yield loss of Durumko (10%), but the average TKW losses of Barbee and Durumko were the same (12.6%). TKW losses of Barbee and Durumko followed a similar trend up to 2011 and 2012 when environmental conditions favoured the TKW of Durumko, which exceeded the ten-year average (Fig. 1a). As a result, Durumko did not suffer TKW losses in 2011 and 2012, in contrast to Barbee, and a significant influence ($P < 0.001$) of interactions between variety and year on TKW loss was confirmed (Fig. 1b).

The potential of prediction of TKW and TKW losses from yield and yield losses is shown to be limited (11% $< R^2 <$ 22%) (Fig. 2). Spearman's coefficients of correlation also showed a significant low positive correlation between yield and TKW, as well as between yield and TKW losses (Table 1). These results indicated the possibility that different factors impact TKW and yield loss, which is examined in more detail further.

To investigate the most influencing factors on TKW loss in the Barbee and Durumko varieties, disease indices and climatic factors were subjected to stepwise and best subset regression. Average disease indices of powdery mildew, leaf rust and Septoria tritici blotch in the 2006–2013 period are shown in Table 2.

In stepwise regression, relative humidity in May, total rainfall in June and leaf rust index proved to be the strongest predictors for TKW losses of the Barbee variety with $P < 0.001$. The effect of interactions between analysed factors on TKW loss was also examined, and it was shown that interaction between powdery mildew and

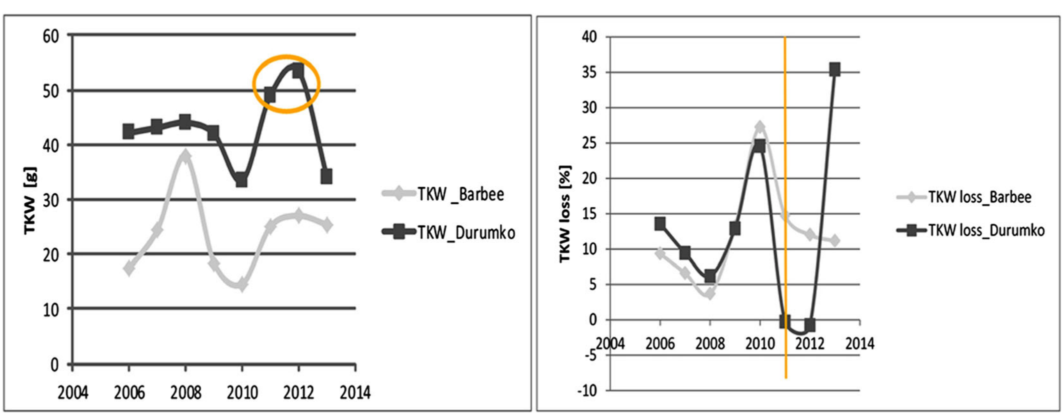
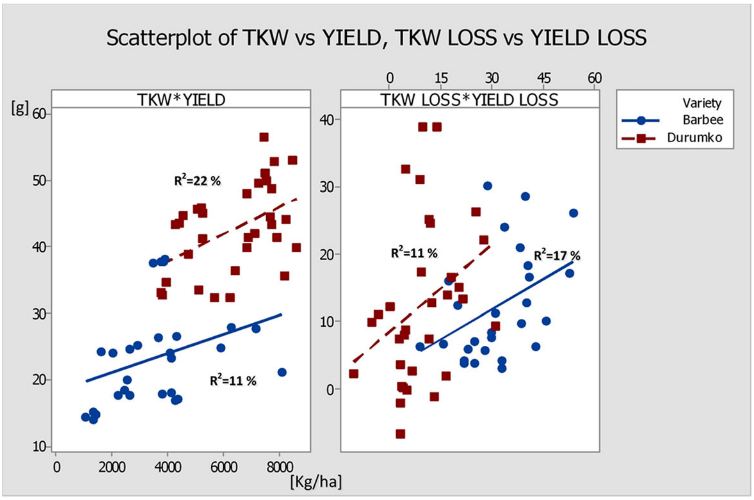


Fig. 1 TKW and TKW loss of the Barbee and Durumko varieties in the 2006–2013 period

Fig. 2 Dispersion of TKW, yield and losses of the Barbee and Durumko varieties



leaf rust had a significant influence on TKW loss, but the significance level was higher than 0.05. Alpha to enter the predictor into the stepwise model and Alpha to remove the predictor from the stepwise model was set by default to be 0.15, so the effect of the interaction between powdery mildew and leaf rust on TKW loss was considered significant at $P = 0.106$. Powdery mildew was not a significant predictor since $P = 0.978$, but it was introduced in the model as a part of the higher-order term (interaction with leaf rust) The percentage of variation in the TKW loss obtained by the model was R^2 of 80.4% with an R^2_{pred} of 68.0% (Eq. 2).

$$Y = -116.7 + 1.656\, x_1 + 0.0795\, x_2 + 0.324\, x_3 + 0.1398\, x_4 - 0.00610\, x_3 \times x_4 \quad (2)$$

Y TKW loss of the Barbee variety
x_1 Relative humidity in May
x_2 Total rainfall in June
x_3 Leaf rust index
x_4 Powdery mildew index

When best subset regression was conducted, all possible models were displayed, and the climatic elements in May and relative humidity in June composed the model with the somewhat lower R^2 (79.7%) and somewhat higher R^2_{pred} (69.4%) than the model selected by stepwise regression (Table 3). In addition, the model revealed by the best subsets regression had the lowest Mallow's Cp (1.3) compared to Mallow's Cp of the model chosen by stepwise regression (Cp = 2.6). Low Mallow's Cp indicates relatively unbiased estimates of true regression coefficients. Multiple regression analysis with climatic elements in May and relative humidity in June gave the following equation:

$$Y = -576.4 + 9.40\, x_1 - 0.3480\, x_2 + 4.774\, x_3 + 1.677\, x_4 \quad (3)$$

Y TKW loss of the Barbee variety
x_1 T in May
x_2 Total rainfall in May

Table 1 The correlation between TKW, yield and their losses of Barbee and Durumko varieties

	Barbee		Durumko	
	Yield	Yield loss	Yield	Yield loss
TKW	0.449 (*P* = 0.016)		0.406 (*P* = 0.021)	
TKW loss		0.427 (P = 0.023)		0.437 (P = 0.012)

Table 2 Disease indices of powdery mildew, leaf rust and Septoria tritici blotch in the 2006–2013 period

Barbee				Durumko		
Average disease index [%]				Average disease index [%]		
	Leaf rust	Powdery mildew	Septoria tritici blotch	Leaf rust	Powdery mildew	Septoria tritici blotch
2013	0.2	5.9	5.1	tR	0.3	6.6
2012	tR	11.3	21.3	tR	tR	25.0
2011	47.1	31.6	3.3	tR	tR	25.9
2010	20.0	35.0	18.8	tR	tR	30.0
2009	67.5	30.0	11.3	tR	0.1	tR
2008	12.3	17.8	0.5	tR	tR	tR
2007	15.8	10.8	9.5	tR	tR	2.6
2006	tR	17.4	tR	17.8	tR	47.3

tR-Trace

x_3 Relative humidity in May
x_4 Relative humidity in June

Although analysis of variance showed that both models (Eqs. 2 and 3) were significant ($P < 0.001$) at an α-level of 0.05, the normal probability plot of best subsets regression model evinced a more linear pattern of residuals, which was an advantage over the model built by stepwise regression (Fig. 3a). To evaluate the potential of the best subset regression model to predict the TKW loss of Barbee variety, the actual and predicted TKW losses were regressed, and an R^2 of 79.7% was obtained. (Fig. 3b).

The most influencing predictor variables on TKW losses of the Durumko variety were all climatic elements in May and June, and these were selected by both stepwise and best subsets regression. Neither the Septoria tritici blotch index nor the interactions between predictor variables were recognized as significant effects on TKW loss of the Durumko variety. Multiple regression analysis was performed with selected predictor variables, and the following equation was produced:

$$Y = 912 + 0.6444\ x_1 \text{-} 5.85\ x_2 \text{-} 2.976\ x_3 \text{-} 11.09\ x_4 + 0.3688\ x_5 \text{-} 6.165\ x_6 \quad (4)$$

Y TKW loss of the Durumko variety
x_1 Total rainfall in May
x_2 Temperature in May
x_3 Relative humidity in May
x_4 T in June
x_5 Precipitation in June
x_6 Relative humidity in June

The P value for the regression model was estimated as significant ($P < 0.001$) at an α-level of 0.05. Regression coefficients of all climatic factors in June, total rainfall in May and relative humidity in May were significantly related to TKW loss, with $P < 0.001$. T in May was significantly related to TKW loss, with $P =$ 0.006. The percentage of variation in TKW loss explained by the model was $R^2 = 94\%$, and the possibility of prediction of new TKW losses was 90%. The normal probability plot evinced a linear pattern of residuals that was consistent with a normal distribution (Fig. 4a). Predicted TKW losses were regressed with actual TKW losses, and a high R^2 of 94% value was obtained, indicating good potential of selected variables to predict TKW losses of the Durumko variety (Fig. 4b).

Discussion

The results of our study indicated that the TKW loss of the Barbee variety was mostly influenced by climatic elements and the variety itself. The significant influence of leaf rust on TKW loss was shown by stepwise regression, but with best subset regression, it was evident that the leaf rust index was not as significant as climatic elements for building prediction models. According to Jevtić et al. (2017), leaf rust together with temperature in April was the most influential factor on yield loss of the Barbee variety. As a consequence, it could be expected that the impact of leaf rust on TKW loss was also

Table 3 Best subsets regression for TKW loss prediction of Barbee variety

Number of predictors	R^2	R^2 pred	Mallow's Cp	T May [°C]	Total rainfall in May [mm]	Rel. hum. in May	T June [°C]	Total rainfall in June [mm]	Rel. hum. in June	Leaf rust index [%]	Powdery mildew index [%]	Septoria tritici blotch index [%]	Interaction leaf rust x powdery mildew
1	41.1	30.0	32.1			X							
1	37.1	28.1	35.9									X	
2	61.7	54.0	14.5	X								X	
2	59.5	48.2	16.6			X				X			
3	77.2	67.7	1.7			X		X		X			
3	73.8	62.3	5.0			X			X	X			
4	**79.7**	**69.4**	**1.3**	**X**	**X**	**X**			**X**				
4	78.3	67.1	2.7		X	X		X		X			
5	80.6	67.9	2.5	X	X	X	X		X				
5	**80.4**	**68.0**	**2.6**			**X**		**X**		**X**	**X**		**X**
6	81.6	67.8	3.5	X	X	X	X		X				X
6	81.4	67.1	3.7	X	X	X	X					X	X
7	82.0	64.2	5.1	X	X	X	X		X			X	X
7	81.8	65.7	5.3	X	X	X	X	X	X				X
8	82.1	58.7	7.0	X	X	X	X	X	X			X	X
8	82.1	61.1	7.1	X	X	X	X		X		X	X	X
9	82.1	45.3	9.0	X	X	X	X		X	X	X	X	X
9	82.1	51.6	9.0	X	X	X	X	X	X		X	X	X
10	82.2	39.9	11.0	X	X	X	X	X	X	X	X	X	X

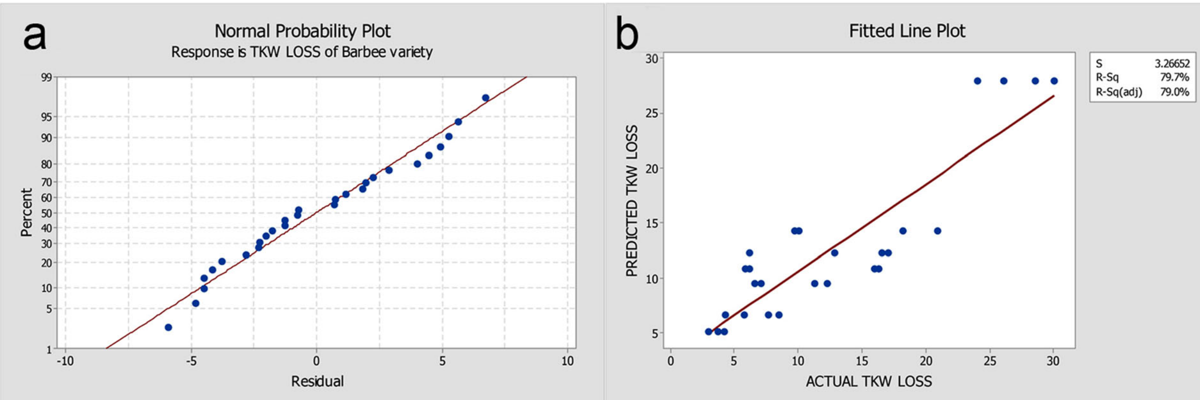


Fig. 3 **a** Residual analysis in the multiple regression model; **b** Regression of actual and predicted yield losses of the Barbee variety

prominent, but the results of our study indicated that leaf rust had more influence on yield loss than on TKW loss. According to Herrera-Foessel et al. (2006), if leaf rust appears at earlier plant development stages, both kernel number per spike and kernel size would be seriously affected, resulting in yield loss rather than in TKW loss. In addition, in the study of Herrera-Foessel et al. (2006), TKW loss was more associated with the date of sowing than with the leaf rust pressure. The significant influence of sowing date on TKW was also confirmed by other authors (Fayed et al. 2015; Protic et al. 2007).

The results of this study showed that the influence of wheat diseases on TKW losses of the Durumko variety was not significant and that the influence of climatic elements and variety itself predominated. According to Jevtić et al. (2017), yield loss of the Durumko variety was significantly determined by Septoria tritici blotch and T in June in agro-ecological conditions of Serbia, but in this study, a significant influence of Septoria tritici blotch on TKW loss was not determined. El Wazziki et al. (2015) reported that the two upper leaves of a durum variety contributed differently to TKW (9%) and grain yield (25%). As a result, it can be assumed that factors influencing the photosynthetic activity of two upper leaves, including the impact of wheat diseases, have a greater influence on yield than on the TKW losses of the Durumko variety in field conditions.

The results of this study indicated limited potential for the prediction of TKW or TKW losses from yield or yield losses ($11\% < R^2 < 22\%$) (Fig. 2). Spearman's coefficients of correlation also showed a significant low positive correlation between yield and TKW, as well as between yield and TKW losses. According to Herrera-Foessel et al. (2006), environmental factors in the period before and just after anthesis are more critical for yield achievements than factors occurring in the grain-filling

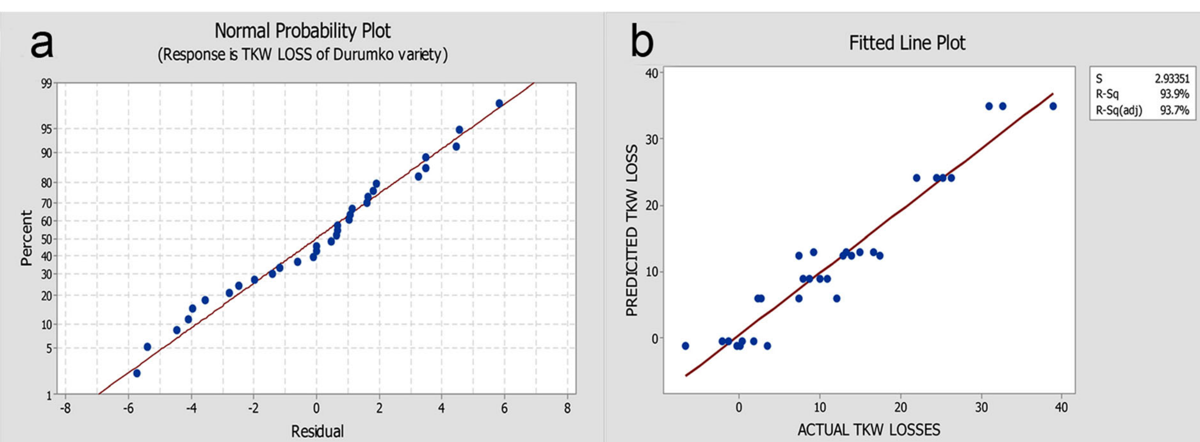


Fig. 4 **a** Residual analysis in the multiple regression model; **b** regression of actual and predicted yield losses of the Durumko variety

period. Knowing that kernel weight is defined in the grain filling stage (Herrera-Foessel et al. 2006), the low correlation between yield and TKW losses in this study can be attributed to non-correlated changes in factors affecting wheat development at different growth stages. In this study, factors affecting yield and TKW loss were not the same, and this was evidenced not only by regression models but also by Spearman's coefficients of correlation. Spearman's coefficients of correlation showed a significant low positive correlation between yield and TKW loss of Barbee ($r = 0.427$, $P = 0.023$) and Durumko ($r = 0.437$, $P = 0.012$) varieties. In the study of Harasim et al. (2016), TKW contributed to grain yield from −0.4% to 13.3% depending on the growing season, which indicated that the effect of yield components on yield quantity cannot unambiguously be determined. Moreover, it was emphasized that deterioration in the value of one yield component can be compensated by a high value of another component, reducing a decrease in grain yield. El Wazziki et al. (2015) also reported that defoliation of flag leaves could improve the photosynthetic activity of the other leaves and that disease severity is not equivalent to the loss of the same percentage of green photosynthetic leaf area. The results of our study showed that the influence of wheat diseases on TKW losses is not straightforward and that more attention should be paid to the interactions between biotic and abiotic factors if losses of yield components have to be analysed and predicted.

Conclusions

1. We found only a small positive correlation between yield and TKW loss in the winter wheat varieties studied herein, although this correlation was significant.
2. TKW loss was less influenced by leaf rust and Septoria tritici blotch than yield loss.
3. TKW loss was significantly influenced by both variety and different environmental conditions. The influence of the climatic elements on TKW loss in the period from anthesis to ripening of wheat grain was more prominent than the influence of leaf rust and Septoria tritici blotch.
4. Yield, yield components, environmental factors (e.g., air humidity), and management factors (e.g., sowing date), including their interactions, are important to better understand thousand kernel weight development patterns, including TKW loss in winter wheat.
5. The best subset regression was a useful tool for comparing different regression models related to the impact of biotic and abiotic factors on TKW losses of winter wheat.

Acknowledgements This study was realized as part of the project TR 31066 - Contemporary breeding of small grains for current and future needs financed by the Ministry of Education and Science of the Republic of Serbia.

Funding This study was realized as part of the project TR 31066 - Contemporary breeding of small grains for current and future needs financed by the Ministry of Education, Science and Technological Development of Republic of Serbia.

Compliance with ethical standards All authors are aware of the content of the manuscript and have agreed upon its submission to European Journal of Plant Pathology.

Conflict of interest 1) The authors declare no conflicts of interest;
2) The manuscript has not been published in whole or in part elsewhere;
3) The manuscript is not currently being considered for publication in another journal;
4) The manuscript is not split up into several parts to increase the number of submissions.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Not applicable.

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