Vol. 62, 2016, No. 3: 114–120 Plant Soil Environ.

doi: 10.17221/778/2015-PSE

Effect of application of growth regulators on the physiological and yield parameters of winter wheat under water deficit

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ABSTRACT

The objective of this study was to evaluate the effect of growth regulator applications on yield and physiological parameters of winter wheat (*Triticum aestivum* L.) under water deficit. Water deficit was induced artificially by using rain-out shelters in period between booting and late milk ripeness. The effect of growth regulators was evaluated in two contrast years in terms of yield formation (2014–2015). In both years the water deficit caused a significant reduction of grain yield. The negative effect of water deficit was partly alleviated by application of growth regulators. However, the effect of individual growth regulators varied depending on year. In favourable conditions for grain filling (2014) the alleviating effect was more pronounced in application of azoxystrobin which slows down the leaf senescence. On the contrary, in the year with suitable conditions for tillering (2015), the alleviating effect was most apparent in application of chlormequate-chloride. The applications of trinexapac-ethyl and ethephon showed small reduction of negative effect of water deficit consistently in both years. The CO_2 assimilation rate and stomatal conductance were recovered particularly by applications of chlormequate-chloride and azoxystrobin. Chlorophyll content and chlorophyll fluorescence parameters were more affected by water deficit in 2014, and the alleviating effect of growth regulators corresponded with yield response.

Keywords: PSII photochemistry; number of spikes; photosynthesis; spike productivity; water limitation

Water shortage is one of the most limiting factors for wheat production not only in the Central Europe (Hlavinka et al. 2009) but also worldwide (Lobell et al. 2011). Moreover, climate modelling showed increased probability of drought events (Trnka et al. 2014) and their increasing length (Burke et al. 2006) for Europe in the coming decades.

The critical period for effect of water deficit on wheat grain yield and quality is defined particularly by the stages between stem elongation and early ripening (Entz and Fowler 1988). Water deficit decreases the growth of plants, influences various physiological and biochemical processes such as photosynthesis, respiration, metabolism of nutrients etc. Severe water stress may result in the cessation of photosynthesis, disturbance of metabolism and finally the death of plants (Jaleel et al. 2008).

To be able to meet the challenge of increased frequency and severity of drought events, the agriculture is forced to implement a range of adaptation measures that would be able to alleviate the negative impacts of water limitation during critical growth stages. Such measures can be divided into three main categories: (i) the selection and breeding of genotypes with improved drought tolerance; (ii) an increase in the soil retention capacity and reduction of evaporation from soil surface, and (iii) cultivation measures improving the efficient use of water by plants, which include, for example, the use of growth regulators. In general, the growth regulators can contribute to improving effective

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Supported by the Internal Grant Agency of the Faculty of Agronomy, Mendel University in Brno, No. IP 23/2015, and by the Ministry of Agriculture of the Czech Republic, Project No. QJ1530373.

doi: 10.17221/778/2015-PSE

use of water in particular by increasing the rooting depth or root water extraction from soil (Marcum and Jiang 1997), increasing the root:shoot ratio (Rajala and Peltonen-Sainio 2001), reducing leaf area (Beasley et al. 2007) and accumulation of osmoregulatory substances (Akram et al. 2012), increasing the accumulation of antioxidants (Wu and von Tiedemann 2001), supporting stomatal regulation (Bingham and McCabe 2006), reducing rate of leaf senescence (Cromey et al. 2004) etc. However, very often contradictory effects of plant regulators on plant physiology and yield formation under water deficit are reported (e.g. Kasele et al. 1994).

According to available information, comparison of the alleviating effects of different growth regulators which belong to the group of gibberellin biosynthesis inhibitors (chlormequate-chloride [CCC], trinexapac-ethyl), liberation of ethylene (ethephon) and promoting cytokinin biosynthesis (strobilurins) under water limiting conditions has not been performed in wheat so far.

MATERIAL AND METHODS

The field trials were carried out at the field experimental station in Žabčice, Czech Republic (49°01'22"N, 16°37'04"E) located in a warm area with transitional climate (average annual rainfall 482 mm and temperature 9.3°C). The soil type is fluvi-eutric gley soil, and soil texture is clay-loam. The soil reaction in the arable horizon of the soil is neutral, pH is 6.9 and the content of humus is 2.28%. The winter wheat cv. Matylda was sown on October 15th, 2013 and October 8th, 2014 in sowing density of 400 germinating seeds/m^2. The standard crop protection measures were carried out to ensure weed and disease control throughout the vegetation season. The total nitrogen dose was 190 kg N/ha. Growth regulators were sprayed (spray volume 300 L/ha) using wheeled small plot sprayer. The growth regulator treatments were randomized in blocks (3 replications). Within the experiment following growth regulators were applied: 1354 g/ha chlormequat-chloride (Retacel extra R68, Lučební závody Draslovka a.s., Kolín, Czech Republic) at growth stage 31 BBCH, 100 g/ha trinexapac-ethyl (trinexapac; Moddus, Syngenta Crop Protection AG, Basel, Switzerland) at 32–35 BBCH, 288 g/ha ethephon (Cerone 480 SL, Bayer S.A.S., Lyon, France) at 39–49 BBCH and 200 g/ha azoxystrobin (Amistar, Syngenta Supply AG, Basel, Switzerland) at 49–55 BBCH.

At the growth stage 39–45 BBCH (April 24th, 2014 and May 12th, 2015), the rain-out shelters covering the half of the experimental plots were built from clear PVC material Ondex Ecolux of thickness 0.8 mm (Zenit, Prague, Czech Republic) mounted on light wooden construction. The rain-out shelters divided each plot on two subplots of area 4 m^2 each, one with induced water deficit and the other with ambient precipitation. The sheltering was terminated at the growth stage 77–81 BBCH (June 20th, 2014 and July 1st, 2015). During the induction of water deficit 67 and 51 mm of precipitation was excluded in 2014 and 2015, respectively.

The measurements of physiological parameters were done at the end of water deficit period (June 14th, 2014 and June 26th, 2015). An open gas-exchange system Li-6400 XT (Li-Cor, Lincoln, USA) was used to estimate the light-saturated (1200 μmol photons/m^2/s) CO_2 assimilation rate (A_{max}) and stomatal conductance (g_s). Simultaneously, measurements of maximum quantum yield of chlorophyll fluorescence (F_v/F_m) and a parameter 1-Vj in dark-adapted leaves were made by FluorPen FP 100 (PSI, Brno, Czech Republic). Chlorophyll content was measured by transmittance method using the instrument Dualex 4 Flav (Force-A, F, Orsay). All physiological measurements were performed on flag leaves of 3 plants per each replication.

After wheat ripening evaluation of yield and yield structure has been done. The number of spikes was calculated on the area of 1 m^2; then the area of 1 m^2 from the central part of each plot was manually harvested and the grain was threshed using the plot harvester Sampo 2010 (Sampo Rosenlew, Pori, Finland) on July 14th, 2014 and July 17th 2015.

The two-way ANOVA followed by the Fisher's *LSD* post-hoc test was completed using the Statistica 12 software (Statsoft, Tulsa, USA).

RESULTS AND DISCUSSION

Grain yield and yield parameters. Both grain yield and yield parameters studied (number of spikes and spike productivity) were affected significantly by year ($P < 0.01$; Table 1). The year 2014 was characterized by generally lower yield levels and spike numbers per unit area (Figure 1).

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Table 1. The average daily temperature and sum of precipitation per month in the main vegetation period of the year 2014 and 2015 and comparison with long-term average (1991–2010)

Month	Temperature (°C)					Precipitation (mm)				
	long-term average (1991–2010)	2014	difference	2015	difference	long-term average (1991–2010)	2014	difference	2015	difference
III.	4.8	8.5	3.7	5.5	0.7	33.4	5.6	–27.8	28	–5.4
IV.	10.9	11.8	0.9	10.1	–0.8	30.5	11.2	–19.3	9.4	–21.1
V.	15.8	14.5	–1.3	14.7	–1.1	51.2	62.8	11.6	33.8	–17.4
VI.	18.9	18.8	–0.1	19.1	0.2	59.6	43.4	–16.2	22.4	–37.2
VII.	20.7	21.5	0.8	22.9	2.2	73.1	85	11.9	22.4	–50.7

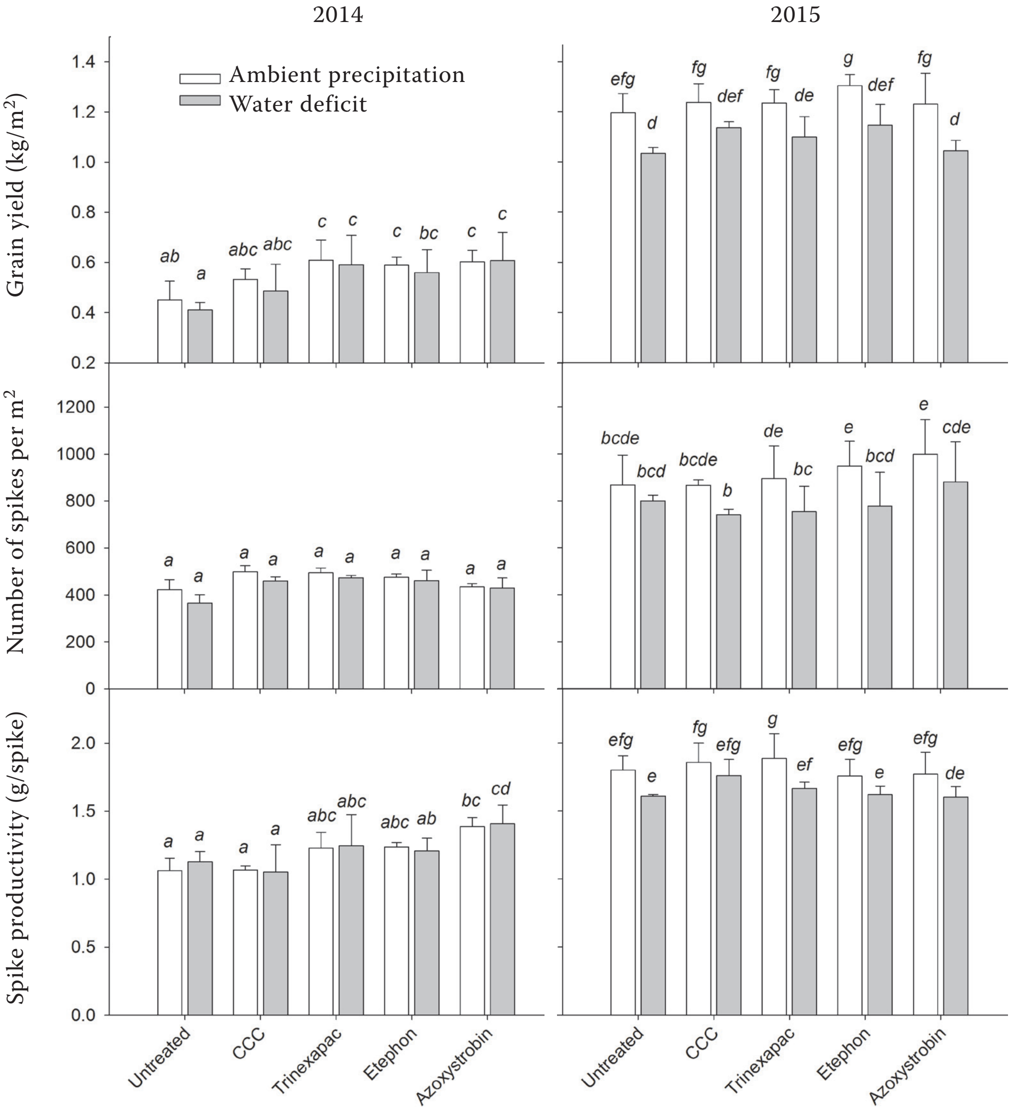

Figure 1. Effects of water deficit in period between booting and late milk ripeness and application of growth regulators on yield parameters (grain yield, number of spikes and spike productivity) in years 2014 and 2015. Means (columns) and standard deviations (error bars) are presented ($n = 3$). Different letters denote statistically significant differences ($P < 0.05$) between water deficit and ambient precipitation treatment, growth regulator applications and the year using the Fisher's *LSD* (least significant difference) ANOVA post-hoc test

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The highest differences in grain yield between the years were recorded in the untreated control and CCC, whereas the lowest inter-annual differences were found for growth regulators azoxystrobin and trinexapac-ethyl. These results suggest that the growth regulators, particularly azoxystrobin and trinexapac-ethyl, were able to partially eliminate the adverse effect of the year 2014, which was characterized by lower formation of productive tillers. Relatively dry weather and high temperatures during tillering in 2014 (Table 1) were probably the cause of accelerated plant development and reduced number of strong tillers able to form productive stems (Křen et al. 2014).

Similarly to grain yield the inter-annual differences in spike productivity were mostly reduced by the application of azoxystrobin. This implies the ability of azoxystrobin to markedly compensate the negative effect of the year 2014 on spike number by spike productivity increase, particularly due to delayed leaf senescence (Grossmann et al. 1999). Higher effect of azoxystrobin in 2014 can be also attributed to more favourable conditions for grain filling after drought induction given by sufficient rainfall and lower temperatures. Under these conditions the beneficial effect of azoxystrobin on slowing down leaf senescence is probably more pronounced as the grain filling period is extended.

Also the water deficit effect caused statistically significant changes in grain yield and yield parameters (Table 2). The greatest impact on reduction of yield losses caused by water deficit in 2014 was observed with late application of azoxystrobin and in 2015 with early applied CCC (Figure 1); however, this effect was significant only for azoxystrobin. Higher effect of CCC on yield parameters in 2015 was probably promoted by colder weather during tillering and stem elongation, which resulted in tiller equalization and increased root:shoot ratio strengthening the drought resistance (e.g. Emam and Cartwright 1990).

These results confirm the hypothesis, that the alleviation of water deficit effect by growth regulators is modulated by year. The positive effect on reduction of water deficit impacts was observed in both years also in applications of trinexapac-ethyl and ethephon, however, this effect was relatively low and statistically insignificant. Trinexapac-ethyl exhibits both an indirect effect on drought tolerance through reduction of aboveground biomass and increasing the root length and area (Beasley

Table 2. Results of three-way ANOVA for effects of water deficit, growth regulators and year on yield characteristics, gas exchange parameters (A_{max}, g_s), chlorophyll content and chlorophyll fluorescence (1-Vj, F_v/F_m) parameters. *F*-ratio and *P*-value are presented for each combination of factor (interaction) and parameter measured. Significant effects and interactions ($P < 0.05$) are highlighted in bold

	Degrees of freedom	Grain yield		Number of spikes		Spike productivity		A_{max}		g_s		Chlorophyll content		1-Vj		F_v/F_m	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Year (Y)	1	1037.5	**< 0.001**	344.1	**< 0.001**	297.2	**< 0.001**	374.9	**< 0.001**	17.6	**< 0.001**	60.7	**< 0.001**	1659.1	**< 0.001**	537.1	**< 0.001**
Growth regulators (GR)	4	5.3	**0.002**	1.2	0.309	2.7	**0.044**	6.1	**< 0.001**	8.9	**< 0.001**	4.1	**0.007**	3.5	**0.015**	16.5	**< 0.001**
Water deficit (WD)	1	20.4	**< 0.001**	12.3	**0.001**	6.0	**0.018**	201.4	**< 0.001**	110.0	**< 0.001**	68.3	**< 0.001**	29.3	**< 0.001**	3.2	0.079
Y × GR	4	2.5	0.055	2.4	0.065	6.1	**< 0.001**	3.9	**0.009**	2.3	0.080	0.4	0.783	3.0	**0.029**	10.6	**< 0.001**
Y × WD	1	10.1	**0.003**	5.1	**0.030**	8.1	**0.007**	0.0	0.988	0.9	0.362	29.5	**< 0.001**	27.8	**< 0.001**	1.6	0.210
GR × WD	4	0.1	0.991	0.1	0.988	0.1	0.992	3.4	**0.016**	0.3	0.849	0.5	0.701	5.5	**0.001**	1.8	0.146
Y × GR × WD	4	0.3	0.865	0.3	0.864	0.3	0.868	1.2	0.339	1.0	0.428	0.9	0.490	2.1	0.093	0.6	0.688

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et al. 2005) and the direct effect increasing the efficient use of water, reducing transpiration and greater osmotic adjustment (Bian et al. 2009). Very variable results of ethephon effects under water deficit ranging from deteriorating to alleviating effects were reported, whereby the positive effect of ethephon on drought tolerance is probably associated with reduction of leaf area and effective use of water (Kasele et al. 1994).

Physiological parameters. The gas exchange parameters A_{max} and g_s were statistically significantly affected by all three factors, year, water deficit and growth regulators at $P < 0.01$ (Table 2). Similarly to the grain yield, the gas exchange parameters were statistically significantly lower in 2014 (Figure 2). In contrast to yield parameters the effect of water deficit was in A_{max} and g_s significant also for comparison within individual growth regulator treatments ($P < 0.05$; Figure 2). The A_{max} decline under water deficit was mostly alleviated by application of azoxystrobin and CCC. Several studies have shown increases of CO_2 assimilation rate after application of strobilurin fungicides (e.g. Grossmann et al. 1999), which is often associated with delayed senescence and an increase of antioxidant activity (Wu and von Tiedemann 2001). All the growth regulators acted positively to g_s decrease under water deficit but the differences between distinct types of growth regulators were relatively low. The chlorophyll content and chlorophyll fluorescence parameter 1-Vj were both affected significantly at $P < 0.01$ by year and water deficit and at $P < 0.05$ by growth regulators (Table 2). In contrast, F_v/F_m was not

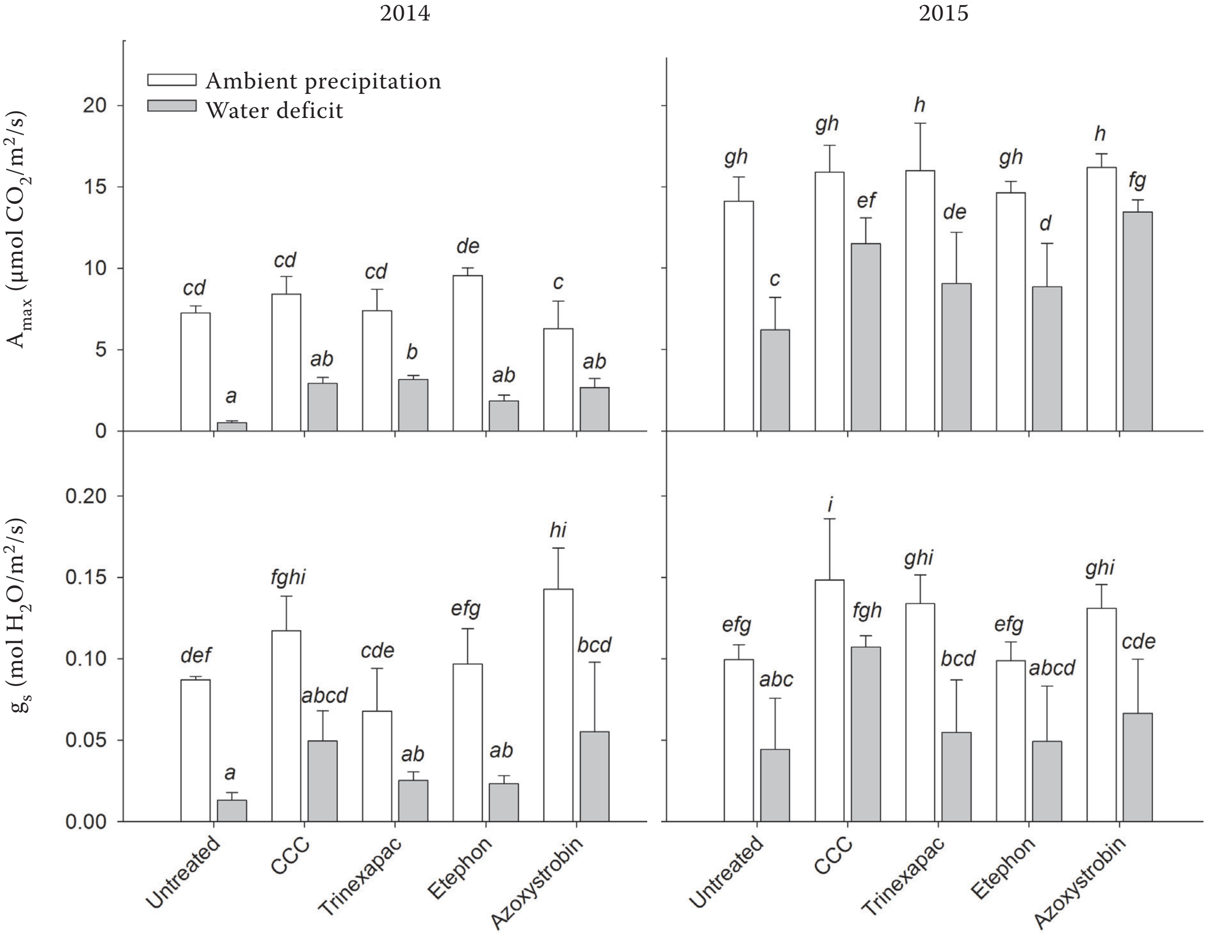

Figure 2. Effects of water deficit in period between booting and late milk ripeness and application of growth regulators on gas exchange parameters light saturated CO_2 assimilation rate (A_{max}) and stomatal conductance (g_s) measured at the end of water deficit period on the flag leaf. Means (columns) and standard deviations (error bars) are presented (n = 3). Different letters denote statistically significant differences ($P < 0.05$) between water deficit and ambient precipitation treatment, growth regulator applications and the year using Fisher's *LSD* (least significant difference) ANOVA post-hoc test

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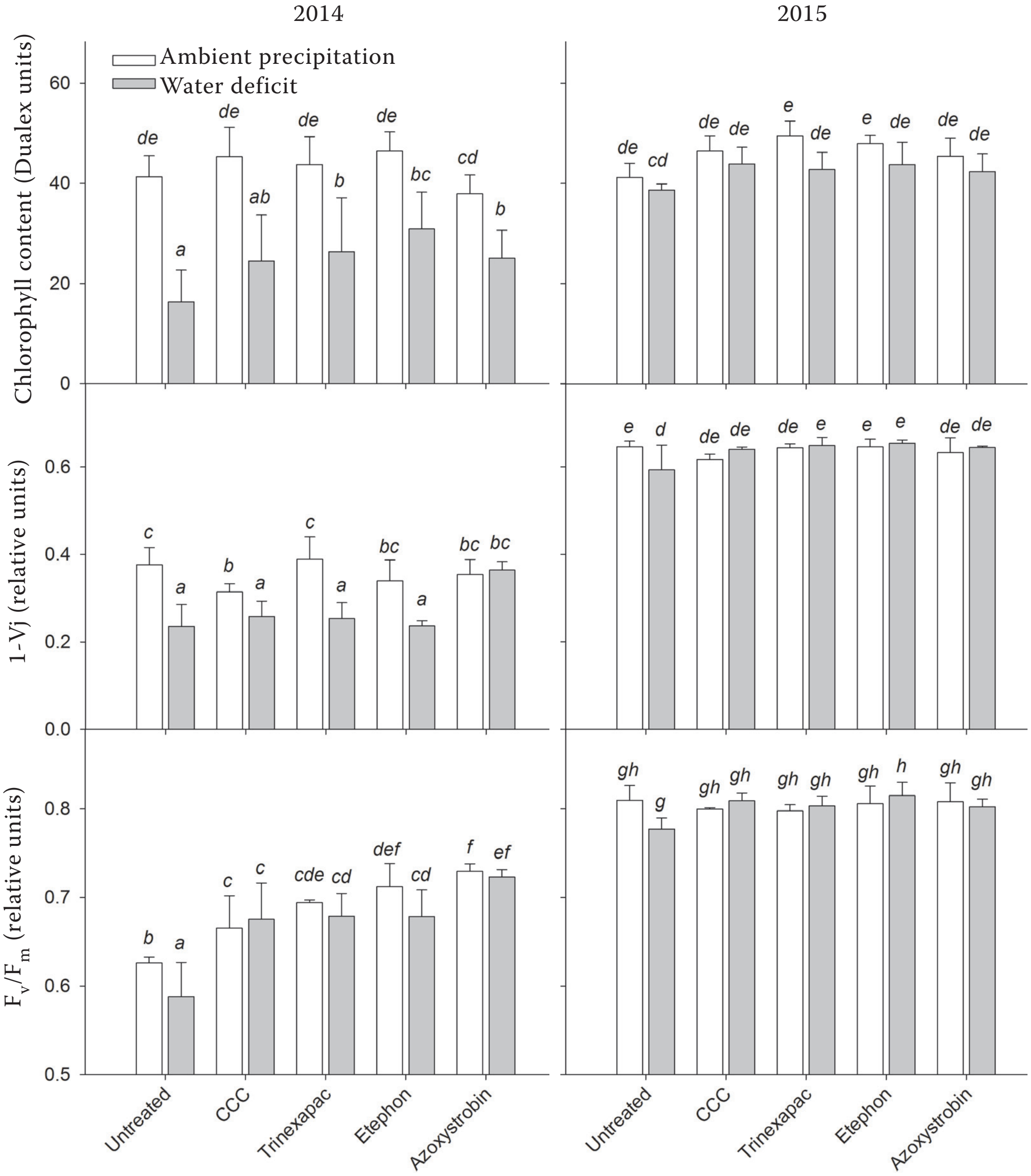

Figure 3. Effects of water deficit in period between booting and late milk ripeness and application of growth regulators on *in vivo* chlorophyll content and chlorophyll fluorescence parameters 1-Vj (variable chlorophyll fluorescence at wave J) and F_v/F_m (maximum quantum yield of photosystem II photochemistry) measured at the end of water deficit period on the flag leaf. Means (columns) and standard deviations (error bars) are presented ($n = 3$). Different letters denote statistically significant differences ($P < 0.05$) between water deficit and ambient precipitation treatment, growth regulator applications and the year using the Fisher's *LSD* (least significant difference) ANOVA post-hoc test

significantly affected by water deficit, but it was significantly influenced at $P < 0.01$ by year and growth regulators (Table 2).

The comparison of differences between individual growth regulators and water deficit treatments (Figure 3) shows that the content of chlorophyll changed in 2015 only little, whereas in 2014 it declined significantly under water deficit. The decrease in chlorophyll content caused by water deficit was alleviated particularly by applications of etephon and azoxystrobin.

Similarly, the chlorophyll fluorescence parameters were affected more by water deficit in 2014 (Figure 3). In 2014, both 1-Vj and F_v/F_m reductions under water deficit were mostly alleviated by application of azoxystrobin and CCC. Accordingly,

doi: 10.17221/778/2015-PSE

Beck et al. (2001) reported improvement in electron transport determined by chlorophyll fluorescence after application of strobilurins and the differences increased with growth stage, which indicated a delay in senescence.

It can be concluded that growth regulators can alleviate the negative effect of water deficit both on plant physiology and grain yield. This effect, however, varies according to the conditions for yield formation. In the year with lower tillering and favourable conditions for grain filling the best effect was achieved with late applied azoxystrobin. On the other hand, in the year with favourable conditions for tillering a positive effect was observed in early applied CCC.

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Received on December 14, 2015
Accepted on February 24, 2016

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