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EFFECT OF DIFFERENT ADJUVANTS ON PHYTOTOXICITY OF FLUMIOXAZIN TO SUNFLOWER IN DIFFERENT GROWTH STAGES

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ABSTRACT

The aim of this work was to determine the effect of different adjuvants on phytotoxicity of flumioxazin on sunflower. Highest selectivity to sunflower was found in flumioxazin without adjuvant (phytotoxicity 8-25%). Symptoms of flumioxazin phytotoxicity were growth inhibition and leaf necroses. All tested adjuvants increased phytotoxicity of flumioxazin. Highest phytotoxicity (72%), reduction of net photosynthesis (by 80%) and stomata conductance of sunflower was recorded when flumioxazin was mixed with methylester of rape seed oil. Phytotoxicity of flumioxazin in combination with heptamethyltrisiloxan and isodecylalkohol-ethoxylate was 32, and 26% respectively. The results of experiments showed that all measured physiological parameters (maximum quantum yield of PSII, photosynthetic rate, transpiration rate and stomata conductance) are sensitive and give information on the toxicity of flumioxazin on sunflower. The response of plants when exposed to herbicides was faster than visible symptoms of damage. Therefore, the methods of gas exchange and chlorophyll fluorescence may be recommended to be used to determine effect of flumioxazin on target plants.

Key words: sunflower, flumioxazin, herbicide phytotoxicity, adjuvants.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is very sensitive to post-emergence herbicides; thus, weed control is not easily achieved using these kinds of herbicides (Pannacci et al., 2007; Jursík et al., 2011a).

Pendimethalin, prosulfocarb, bifenox and aclonifen are used for early post-emergent control of annual dicotyledonous weeds, but these herbicides only affect weeds in early growth stages. In addition to these, only flumioxazin can be used for control of dicotyledonous weeds in later growth stages (Jursík et al., 2011a). For post-emergent control of grass weeds, the ACCase inhibitor herbicides may be used, as sunflower showed good selectivity for these herbicides (Bedmar, 1997). In herbicide tolerant sunflower hybrids (ClearField or Express technology), some acetolactate synthase inhibitors are used for post-emergent weed control (Baumgartner et al., 1999; Pfenning et al., 2008).

Flumioxazin is inhibitor of protoporphyrinogen oxidase (PPO), the last common enzyme to both heme and chlorophyll biosynthesis. Flumioxazin is taken up by both the leaves and roots. When it is applied after the emergence of weeds, the protoplast membranes of sensitive weeds are disrupted during the few hours after application, and the affected tissues turn brown and display necrosis. A high intensity of solar radiation accelerates the efficacy of PPO inhibitors (Dayan and Duke, 1997). Annual weeds are only sensitive to flumioxazin at early growth stages (maximum of 4 to 8 true leaves). Perennial weeds cannot be completely controlled, because their underground system is not affected, and new stems are promptly formed (Jursík et al., 2010). Flumioxazin has a lower selectivity to sunflower and to get the maximum performance it needs to be applied exactly under low sunshine and temperature (Jursík et al., 2011a). Different adjuvants have been used to increase efficacy of this herbicide on less sensitive weeds, but may also cause crop/sunflower injury (Torma et al., 2006). Also, TM combination of ACCase-inhibiting herbicides with flumioxazin resulted in

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sunflower phytotoxicity, because ACCase-inhibiting herbicides are formulated with oil adjuvants. In addition, reduction of control of grass weeds, as the result of applying mixtures of ACCase inhibitors with herbicide controlling dicotyledonous weeds, was detected as a herbicide antagonism (Culpepper et al., 1999; Brommer et al., 2000).

Adjuvants are additives used to make herbicide treatment more effective by lowering herbicide rate together with maintaining or enhancing biological efficacy and crop selectivity. Spray solution and herbicide activity can be influenced by adjuvants in many ways (Jursík et al., 2011b). The most important group of employed adjuvants is represented by surfactants. These agents enhance biological activity of herbicides by increasing and accelerating the penetration of active ingredient into leaf tissues. Surfactant molecules are amphipatic – composed of two parts, each of them attracted by different phase. One end of a molecule (head group) is hydrophilic, while other one (tail) hydrophobic creates „bridge“ between two phases, decreasing surface tension of spray drops (Nikolov and Wasan, 2011). Most common adjuvants are mineral and plant seed (mostly esterified) oils, or synthetic compounds like dissociable salts or esters, nonionic surfactants based on alcohols and fatty acids and organosilicates. Each group of adjuvants has specific effect that can be attributed to their chemical and physical properties. In this regard, the efficacy and selectivity of different herbicides may be affected (Jursík et al., 2011b).

As referred in Ferrel and Vencill (2003), the herbicide treatment effect on leaf area changes the biomass accumulation, the most commonly used parameter in weed/crop competitive studies. This parameter can be highly correlated with changes in net CO_2 assimilation rates, the latter having the advantage of early results. Flumioxazin was found to affect a number of parameters related to photosynthesis (Saladin et al., 2003; Bigot et al., 2007; Geoffroy et al., 2004). Bigot et al. (2007) confirmed the strong inhibition of net photosynthesis and parallel decrease of stomata conductance and transpiration, as well as the photosystem II activity decline in *Vitis vinifera*. Moreover, this active ingredient alters the plastid structure, inhibits O_2 production and induces decline in chlorophyll content. Additionally, the amount of carotenoids in the leaves is reduced. The former studies confirmed a strong oxidative stress due to an overproduction of excited chlorophyll molecules and oxygen (Hess, 1993). Nevertheless, Saladin et al. (2003) observed recovery of some physiological parameters under low flumioxazin concentration.

The aim of this work was to determine the effect of different adjuvants on sunflower phytotoxicity of flumioxazin.

MATERIAL AND METHODS

Two identical pot trials were carried out with sunflower in vegetation house in May 2009 and 2010. Each experiment had three replicates. During the experiments, the temperature fluctuated between 16 and 24°C and relative air humidity between 60 and 80%, respectively. Plastic pots were filled with loamy soil. The soil type was chernozem, clay content 46% (loamy soil), soil pH (KCl) 7.5, sorption capacity of soil: 209 $mmol^{(+)}$ kg^{-1}. Nutrient content was 87 μg g^{-1} P, 203 μg g^{-1} K, 197 μg g^{-1} Mg, 8.073 μg g^{-1} Ca. Three sunflower achenes (hybrid Alexandra) were sown to plastic pots (0.10 x 0.10 x 0.12 m) to depth 0.02 m. After sunflower emergence, the plants in pots were thinned to one per pot to prevent intra-specific competition. Delaying of the sunflower sowing by consecutive three weeks at one week interval resulted in optimal growth stage at the time of herbicide application.

Herbicide application

At herbicide application time the sunflower was at three different growth stages: BBCH 10 (cotyledonous leaves), BBCH 12 (first two true leaves) and BBCH 16 (six true leaves). Each growth stage was treated by same herbicide treatments (Table 1). A laboratory-pot sprayer was used to apply the herbicides. The water volume applied was 250 l ha^{-1}, the nozzles were Lurmark 015 F 80, and

the application pressure was 0.2 MPa. The herbicide Pledge 50 WP (500 g kg^{-1} flumioxazin) was used at two application rate (30 and 60 g ha^{-1} a.i). Nonionic adjuvant Trend 90 (900 g L^{-1} isodecyl alcohol ethoxylate), organosilicate adjuvant Silwet L-77 (840 g L^{-1} heptamethyltrisiloxan) and esterified seed oil Mero (730 g L^{-1} methylester of rape seed oil) were used at recommended application rates (Table 1).

Table 1. Description of herbicide treatments

No.	Herbicide		Adjuvant	
	Active ingredient	Rate (g ha^{-1})	Active ingredient	Rate (g ha^{-1} a.i.)
Untreated	-	-	-	-
1N FLM	flumioxazin	30	-	-
2N FLM	flumioxazin	60	-	-
1N FLM + OSS	flumioxazin	30	heptamethyltrisiloxan	84
1N FLM + NIS	flumioxazin	30	isodecylalkohol-ethoxylate	90
1N FLM + MSO	flumioxazin	30	methylester of rape seed oil	730

Visual phytotoxicity assessments

Sunflower phytotoxicity was assessed visually and by measuring the weight of the sunflower aboveground biomass. Visually phytotoxicity symptoms were assessed by estimation method using a percentage scale from 0 to 100% (0% = untreated, 100% = full control) according to the European and Mediterranean Plant Protection Organisation (EPPO) 1/153 (3) guidelines. The first assessment was carried out one week after application (WAT), and the second one two WAT, respectively. The weight of sunflower aboveground biomass (g) grown in pots was recorded two WAT, related to untreated check and expressed as relative weight of aboveground biomass [RWAB (%)].

Chlorophyll fluorescence and gas-exchange measurement

Measurements of chlorophyll fluorescence and gas exchange characteristics were carried out one day after the treatment. The same representative leaves were chosen for chlorophyll fluorescence monitoring and gas exchange measurements. Measured leaf area was determined by measuring chamber and was 0.7 cm^2 for chlorophyll fluorescence and 1.7 cm^2 for gas-exchange, respectively.

Chlorophyll fluorescence measurement was performed using a portable FMS-2 pulse modulated fluorometer (Hansatech, UK). The leaves were dark adapted with leaf clips (Hansatech, UK) for 30 min. Then the measuring radiation was switched on to determine the dark-adapted minimum fluorescence (F_0) with all PSII reaction centres open. It was measured by the measuring modulated light sufficiently low (0.1 μmol $m^{-2}s^{-1}$) not to induce any significant variable fluorescence. The maximal fluorescence level (F_M) with all PSII reaction centres closed were determined by a 2.5 s saturating pulse at 10,000 μmol $m^{-2}s^{-1}$. Next, the actinic radiation (100 μmol $m^{-2}s^{-1}$) was turned on after the fluorescence signal levelled off, the steady-state fluorescence (F_S) and the light-adapted maximum fluorescence (F_M') were determined, then the actinic radiation was removed and the minimal fluorescence in the light-adapted state (F_0') was determined by irradiating the leaf segment for 2 s with infrared radiation. Maximum quantum yield of PSII (F_V/F_M) was calculated according to Genty et al. (1989), $F_V/F_M = (F_M - F_0)/F_M$.

The photosynthetic gas-exchange parameters including net photosynthetic rate (P_N, μmol m^2s^{-1}), transpiration rate (E, mmol m^2s^{-1}) and stomata conductance (g_s, mmol m^2s^{-1}) were analysed. The measurements were carried out on fully expanded youngest leaves of sunflower using a portable infrared gas analyser CIRAS-2 (PP Systems, UK) assessed with the leaf chamber model PLC6 (U) Rice (PP Systems, UK). Based on the measured variables and pre-set values, all parameters mentioned above were calculated according to the manufacturers' settings.

For recording, the timed recording measurement method was used with automatically pre-set time intervals at 120 s. System setup box was adjusted as follows: light type LED, leaf temperature measured with IR sensor, leaf area was 1.7 cm^2, boundary layer 0.35, PAR: energy factor equals 0.17, zero/diff bal mode set to automatic values and analysis flow was 100 ml min^{-1}, respectively. The CO_2 concentration, air humidity and leaf temperature were maintained at 370+/-2 ppm and 980 Pa, respectively. The LED radiation source was used and set to 500 µmol m^2s^{-1} as recommended by Hay and Porter (2006) for C3 plant carbon fixation metabolism.

Leaves were adapted to radiation for 10 min under given photon flux density. The measurements on these photosynthetic parameters lasted approximately 10 min, during which no significant recovery was observed. All the measurements were repeated five times.

Percentage of stomata on upper/lower leaf surfaces (n=30) was calculated. A rather thick layer of clear nail polish was painted on respective part of the leaf. After the nail polish has dried, the layer was peeled off and the leaf imprints were viewed under the microscope (Orthoplan, Leitz, Germany) and digital images of stomata were recorded at 630x magnification.

Statistic methods

The experimental data were evaluated using the software package Statgraphics Plus. Multiple and one-way analysis of variance was used. The contrasts between treatments were verified by the LSD test ($P<0.05$).

RESULTS AND DISCUSSION

Visual phytotoxicity and weight of sunflower aboveground biomass

Highest selectivity to sunflower was showed by flumioxazin without adjuvant (Table 2). Phytotoxicity was lowest (below 10%) when application was used at BBCH 16 and highest (20-25%) at BBCH 10 and BBCH 12, but differences among growth stages were not significant (Figure 1). Symptoms of flumioxazin phytotoxicity were growth inhibition and leaf necroses. RWBA of sunflower was not affected when application was carried out at BBCH 16 (Figure 2), while at other growth stages, RWBA were between 67 and 77% compared to untreated sunflower. Between tested application rates there were not significant difference in phytotoxicity and RWBA at each tested growth stage. Effect of adjuvants on sunflower phytotoxicity was significant.

All tested adjuvants significantly increased phytotoxicity of flumioxazin. Main symptoms of phytotoxicity were identical with symptoms phytotoxicity of flumioxazin alone (growth inhibition and leaf necroses), with different intensity.

Only, heptamethyltrisiloxan + flumioxazin applied at BBCH 12 and 16 caused growing point injury in addition. Highest phytotoxicity (72 %) was recorded when flumioxazin was mixed with methylester of rape seed oil. Phytotoxicity of flumioxazin at combination with heptamethyltrisiloxan and isodecylalkohol-ethoxylate was 32, resp. 26% (Table 2). Effect of adjuvant on RWBA of sunflower was significantly proved only for methylester of rape seed oil (RWBA 39.8%), because sunflower plant quickly regenerated on another treatments. For individual adjuvants, difference in phytotoxicity among tested growth stages was not significant one week after application (Figure 1).

Subsequently, the sunflower plants treated at BBCH 16 quickly regenerated, while plants treated at BBCH 10 regenerated very slowly.

Therefore, RWBA of plant treated at BBCH 16 was significantly higher compared to plants treated at BBCH 10, only with heptamethyltrisiloxan; the RWBA differences among growth stages were not significant (Figure 2). Similar results were found by Price et al. (2004), who studied phytotoxicity, absorption, translocation, and metabolism of flumioxazin after post-emergence-directed spray in cotton.

Table 2. Sunflower phytotoxicity and relative weight of sunflower aboveground biomass (in relation to untreated check) after application of flumioxazin in relationship to growth stage, used adjuvant and rate of herbicide [Data were pooled for both experiments, while showing no statistically significant difference (LSD 0.05)]

	Effect of growth stage at application		
	Phytotoxicity (%)		Relative weight of sunflower aboveground biomass (%)
	1 WAT	2 WAT	
BBCH 10	38 [a]	33 [a]	50.4 [a]
BBCH 12	39 [a]	30 [a]	59.2 [a]
BBCH16	33 [a]	31 [a]	82.7 [b]
LSD (0.05)	*6*	*7*	*11.6*
F-Ratio	*2.19*	*0.51*	*16.34*
P-Value	*0.1184*	*0.6052*	*0.0000*
Effect of adjuvant			
Without adjuvant	18 [a]	17 [a]	78.9 [b]
Heptamethyltrisiloxan	32 [b]	27 [b]	69.0 [b]
Isodecylalkohol-ethoxylate	26 [b]	27 [b]	68.7 [b]
Methylester of seed rape oil	72 [c]	55 [c]	39.8 [a]
LSD (0.05)	*6*	*8*	*13.0*
F-Ratio	*80.36*	*25.08*	*9.98*
P-Value	*0.0000*	*0.0000*	*0.0000*
Effect of application rate of flumioxazin			
30 g ha^{-1} a.i.	19 [a]	16 [a]	77.8 [a]
60 g ha^{-1} a.i.	16 [a]	18 [a]	79.9 [a]
LSD (0.05)	*7*	*6*	*11.9*
F-Ratio	*0.35*	*0.11*	*0.08*
P-Value	*0.5532*	*0.7452*	*0.7812*

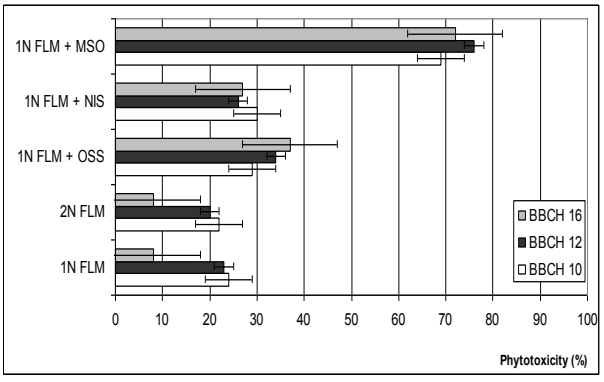

Figure 1. Phytotoxicity of tested herbicide on sunflower in three growth stages, one week after application [Different letters indicate significant difference (LSD 0.05).]

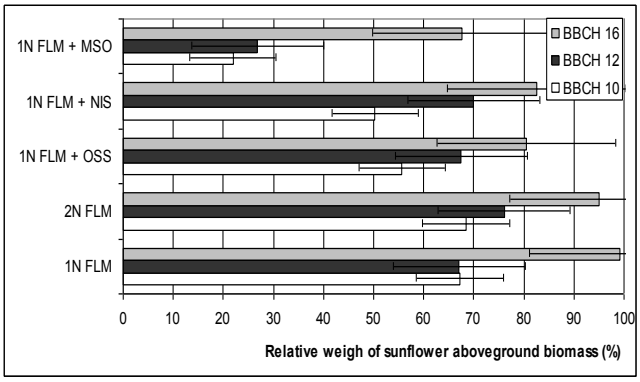

Figure 2. Relative weight of sunflower aboveground biomass after herbicide treatments in tested growth stages. [Different letters indicate significant difference (LSD 0.05).]

Chlorophyll fluorescence and gas-exchange measurements

Flumioxazin application significantly induced a strong net photosynthesis inhibition and a parallel decrease of stomata conductance and transpiration. The flumioxazin treatment leads to the closure of stomata in sunflower after 3 days in all treated variants (data not shown). In these paragraphs, we only present the data measured 1 day after treatment, while all the parameters are related to the measured area. The necrosis significantly affected the leaves area; therefore the observed changes were often caused by the decrease of the photosynthetically active leaf surface. The differences observed between the two experimental years can be partly attributed to the weather condition at the time of measurement. In 2009, the ambient temperature was about 15°C, day global radiation reached 20,200 kJ $m^{-2}day^{-1}$ and photosynthetically active radiation was about

750 mmol $m^{-2}s^{-1}$. In 2010, the ambient temperature reached 9.5°C, global radiation 15,000 kJ $m^{-2}day^{-1}$ and PAR 755 mmol m^{-2} s^{-1}, respectively.

In the present work, at the 2 leaves growth stage, the photosynthetic characteristics were significantly lower 24 hours after the herbicide treatment (Figure 3). The highest reduction of net photosynthesis, by 80%, was shown in flumioxazin + methylester of rape seed oil, followed by other adjuvants. No differences between recommended and double dose of flumioxazin were detected when treated at later growth stages ($\alpha = 0.05$). Furthermore, there appeared to be a consistent relationship between P_N and *gs* across all treatments. As P_N declined in response to each herbicide treatment, g_s declined proportionally (data not shown). The flumioxazin + methylester of rape seed oil treatment decreased g_s most readily, followed by flumioxazin + heptamethyltrisiloxan and double dose of flumioxazin.

Net photosynthesis and transpiration of sunflower following flumioxazin treatment was also reduced significantly. The flumioxazin alone caused a reduction in these parameters retaining up to 65% of initial P_N values in 2009.

Probably due to the lower temperature, in 2010 the same reduction was observed the second day after the treatment.

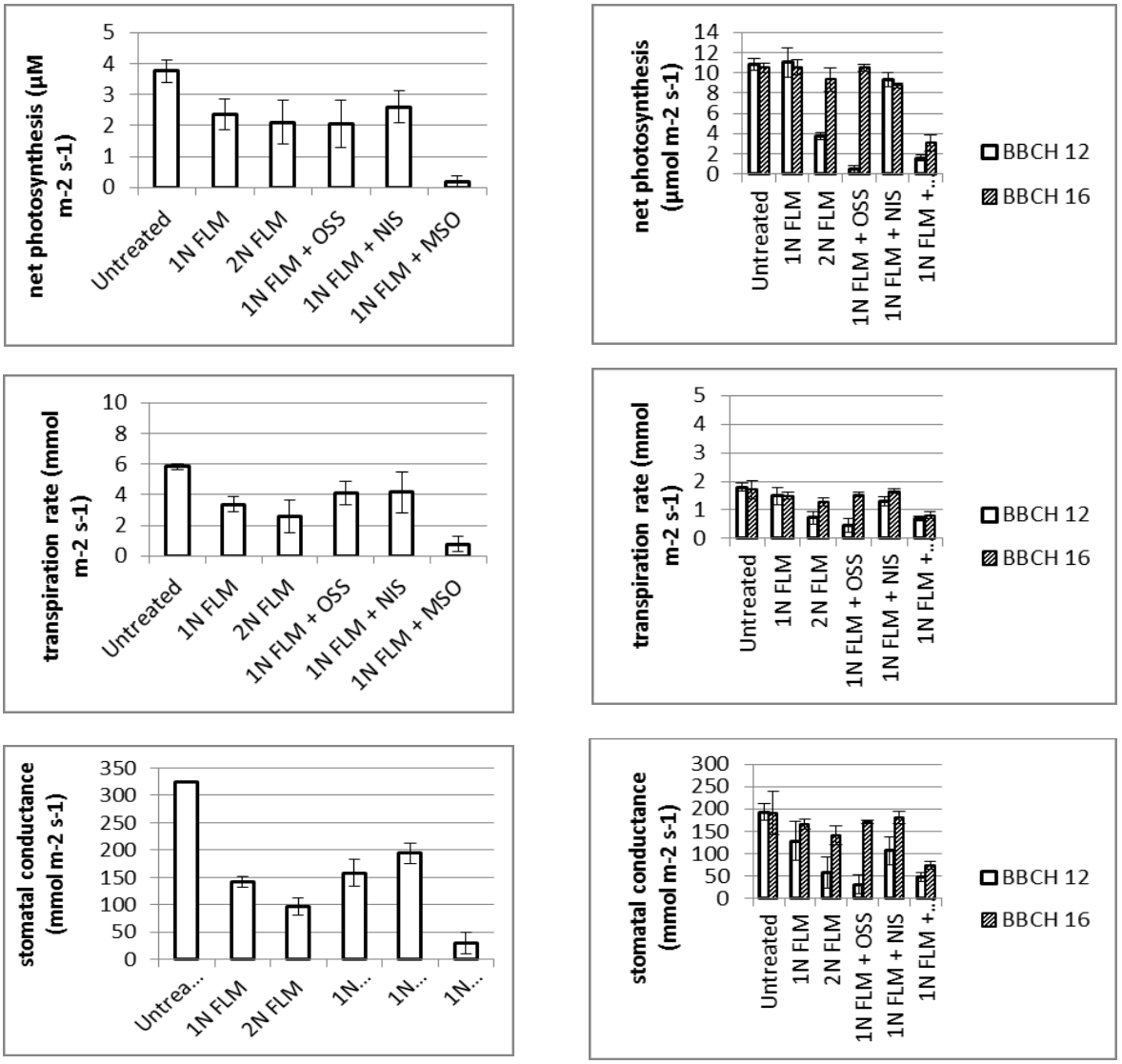

Figure 3. Net photosynthesis (P_N), transpiration rate (*T*) and stomata conductance (g_s) in leaves of sunflower after the spraying of flumioxazin alone or with adjuvants one day after treatment
(The left column represents data of sunflower treated at the growth stage BBCH 16 from 2009. Right column shows data from 2010. The vertical bars represent standard deviation.)

To characterise the functioning of photosynthetic apparatus, the chlorophyll fluorescence parameters were calculated. As in many studies, the dark adapted leaf reflects the potential quantum efficiency of PSII was used as a sensitive indicator of plant photosynthetic performance with optimal values around 0.83 in most plants. Plants exposed to stress showed lower value, indicating the phenomenon of photoinhibition. The F_V/F_M ratio, used as a parameter of maximal photochemical efficiency of PSII, dropped down in all treated variants few hours after herbicide treatment in 2009. Statistically significant differences were observed between flumioxazin + methylester of rape seed oil and all other variants. Surprisingly, one day after treatment no statistical differences were observed when double dose of flumioxazin was used. Clearly higher damage was observed in younger plants than in plants treated at the growth stage BBCH 16. One day after herbicide application, no statistical differences were observed in 2010; there was only slight decline of F_V/F_M value (Figure 4).

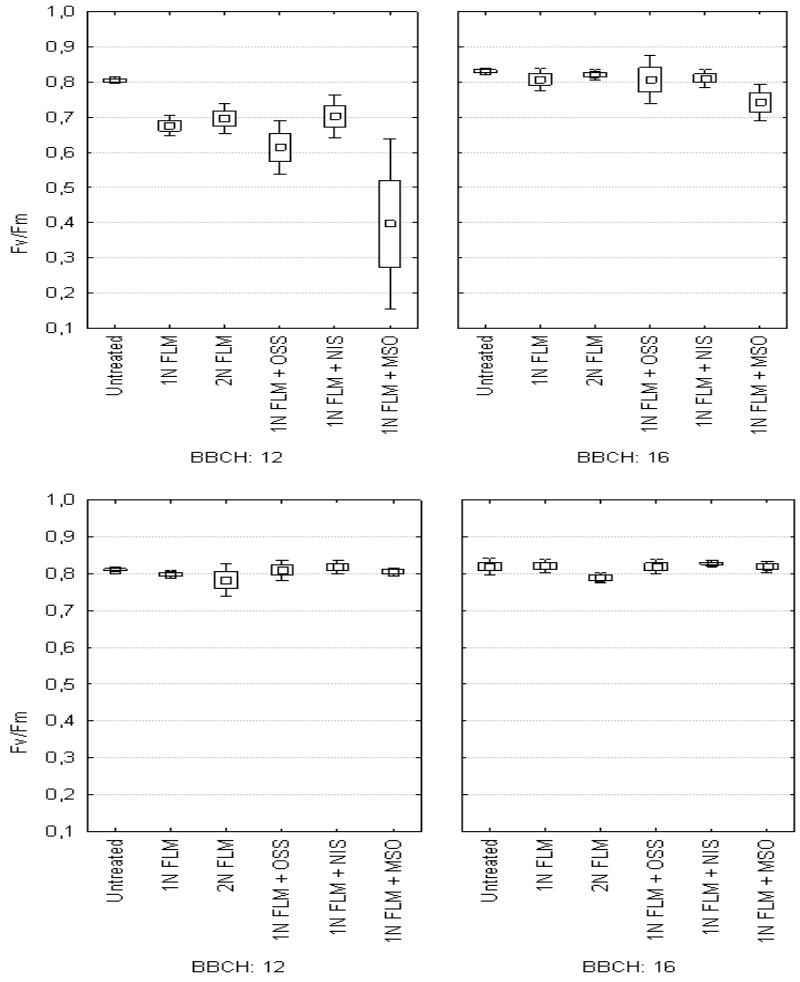

Figure 4. Maximum quantum yield of PSII (F_V/F_M) in sunflower induced by flumioxazin alone or with adjuvants at different growth stages in both experimental years (2009 upper row, 2010 lower row) one day after treatment
(The small box represents mean values, the larger box standard errors and vertical bars intervals of confidence, 95%.)

Simultaneous stomata closure and decrease in the quantum yield of CO_2 assimilation indicate a chase in energy metabolism following flumioxazin stress (Bigot et al., 2007). That decline confirms that the photochemistry of PSII and its ability to reduce the primary acceptor Q_A are affected by herbicide.

The results show that all measured physiological parameters are sensitive and

give information on the toxicity of flumioxazin on sunflower. The response of plants when exposed to herbicides was faster than visible symptoms of damage. Therefore, the methods of gas exchange and chlorophyll fluorescence may be recommended to be use to determine effect of flumioxazin on target plants. Further detailed investigation can contribute to better understanding of herbicide mode of action.

CONCLUSIONS

Flumioxazin may be used as post-emergent herbicide in sunflower without high level of crop injury, but sunflower must have at least two true leaves. For control of some problematic weeds, using a suitable adjuvant added to flumioxazin has been recommended (Jursík et al., 2011a), but all tested adjuvant in this work decreased selectivity of flumioxazin to sunflower, especially at early growth stage. Most selective adjuvant to sunflower was isodecylalkohol-ethoxylate.

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