Determination of the influence of herbicides on dicotyledons plant transpiration using the sap flow method

V. Brant, J. Pivec, K. Hamouzová, P. Zábranský, J. Satrapová, M. Škeříková

Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic

ABSTRACT

Physiological parameters are sensitive and provide information on the toxicity of herbicides in plants. The impact of herbicide application on plant transpiration was evaluated by the sap flow method during 2009–2011. The aim of this work was to verify the sap flow method for determining the effect of herbicides on the basis of continuous measurements of the transpiration flow. *Helianthus annuus* was used as a model plant species. The two different herbicides tested in this study differed by the effect of active ingredients bromoxynil and clopyralid. The water flow was measured using sap flow meter T4.2. The impact of herbicides was assessed by comparing measured transpiration rate (Q) after herbicide application with an extrapolation of transpiration rate of plants before herbicide treatment (Q_{calc}). After treatment with bromoxynil the Q values decreased significantly compared to Q_{calc}. For plants treated by clopyralid, the decline of actual transpiration (Q) compared with the modelled one (Q_{calc}) was less substantial and the plants continued to transpire after the treatment. The effect of herbicides was also verified using infrared gas analyser and chlorophyll fluorescence meter.

Keywords: stomatal conductance; photosynthesis; weed

Competition for water is one of the most crucial processes that cause water stress, resulting in reductions in both weed growth and crop yield (Acciaresi and Guiamet 2010). The presence of weeds in crops is associated with a decrease in water content in the soil and leads to the reduction of its availability (Dalley et al. 2006, Sadeghi et al. 2007). The effect of herbicidal treatments on transpiration processes is important in terms of reducing the weed's competition for water. Van Oorschot (1970) states that specific inhibitors of the photosynthetic process had a more pronounced effect on the photosynthetic activity than on the transpiration rate. Ferrell et al. (2004) comment, that halosulfuron, imazapic, and glyphosate treatments decrease the leaf net carbon assimilation and stomatal conductance (g_s) in *Cyperus esculentus*. This simultaneous decline of carbon assimilation and g_s with time was also observed for *Sorghum halepense* treated with various herbicides (Ferrell et al. 2003). Application of MSMA (monosodium acid methanearsonate) and halosulfuron strongly suppressed water use by *C. esculentus*, but the mesotrione treatment had no effect on water use at all (Earl et al. 2004). Determination of the transpiration of plants is possible with the gasometric method (Flexas et al. 1999, Lopes et al. 2004) or by direct determination of water flow in plants (Gordon et al. 1999, Angadi et al. 2003, Pivec et al. 2011). Kjelgaard et al. (1997) and Jara et al. (1998) reported that sap flow measurements in the same plant were practicable up to one week in duration, depending on weather conditions and stem thickening. Cohen and Li (1996) reported a positive correlation between the flow of water in the plant and leaf area. Application of measurements of gas exchange to determine the effect of herbicides on plant physiological manifestations can be considered as a standard method (Ferrell et al. 2003, Kaňa et al. 2004). Literature data about the sap flow method usage for the determination of the effect of herbicides on plant transpiration demands are not available.

The aim of this work was to verify the sap flow method for determining the effect of herbicides on the basis of continuous measurements of the transpiration flow, and demonstrate different effects of active substances on the plant transpiration with respect to the weed competition for water after application of herbicide.

MATERIAL AND METHODS

The impact of herbicide application on plant transpiration was evaluated by the sap flow method in the field and under laboratory conditions in 2009–2011. The evaluation of the effect of herbicide on the plant was also verified by using gas exchange and chlorophyll fluorescence measurements.

Helianthus annuus L. (cv. Pikasol) was used as a model plant species because of their high sensitivity to wide spectrum of herbicides (Jursík et al. 2011). In terms of morphology, they are appropriate for installation of a sap flow meter. Herbicides with a different mode of action were used – inhibitor of photosystem II (bromoxynil) and a synthetic growth-blocking auxin (clopyralid), respectively. Herbicides Pardner 22,5EC (225 g/L bromoxynil, Bayer CropScience, Leverkusen, Germany) at the dose of the active ingredient 337.5 g/ha and Lontrel 300 (300 g/L clopyralid, Dow AgroSciences, Indianapolis, USA) at 120 g/ha, were evaluated within the framework of the experiment. The dose of water was 30 mL/m². Table 1 documents the number of evaluated plants, the measurement period, the application term of herbicides and BBCH stages (Meier 2001) of plants at the beginning of measurements in 2009–2011. The later growth phases of plants were chosen because of the possibility of installing sap flow meter in the plant's stem.

The sap flow meter T4.2 (EMS Brno, Czech Republic) is designed for stems with a diameter of 6–20 mm. The measuring principle is based on the stem heat balance method (SHB) with external heating and internal temperature sensing (Lindroth et al. 1995, Čermák et al. 2004). The sensor consists of the two similar cylindrical parts. One part contains linear heating elements which are gently pressed to the stem by soft foam. A needle thermocouple is inserted to the stem in radial direction at the level of upper edge of heating elements (in direction of water movement). Second cylinder has no heaters and it just covers the reference needle thermocouple located with respect to the thermal symmetry. The water passing along the sensor is warmed and the increase of temperature is measured with thermocouples as the water leaves the heated space. The input power is directly proportional to the amount of water passing the sensor in terms of kg/hr. Plants grown under field conditions were evaluated in 2009 (location Prague-Suchdol, GPS 50°7'40.583''N, 14°22'22.755''E). The main soil unit is Haplic Luvisols. The plants were not artificially irrigated. The average daily value of volumetric water content in the soil (VWC, %) was 30.1% in 2009 (the average value for the level 0–200 mm of the depth), and was measured by ML2 sensor in combination with the datalogger HH2 Moisture Meter (Delta-T Devices, Cambridge, UK). The average daily values of temperature/relative humidity/global solar radiation (t_a/RH_a/Rg) 18.2°C/70.5%/17 084.7 kJ/m²/day were observed in the mentioned period (Table 1) of 2009. Laboratory experiments were carried out in 2010–2011 (Prague-Suchdol). Plants were grown in plastic containers (size 0.15 × 0.15 × 0.15 m). The bulk density of the soil in pots was 1.2 t/m³ (Haplic Luvisols). The average daily value of volumetric water content in the soil in containers was 34.3% in 2010, and 35.5% in 2011 at the time of measurement. The humidity in the containers was measured by ML2 with the datalogger HH2. The average daily values of temperature/relative humidity/global solar radiation (t_a/RH_a/R_g)

Table 1. Number of evaluated plants (n_p), the measuring period, the date of application of herbicides and plant BBCH stage at the beginning of the measurements period 2009–2011

| Year | Active ingredient | | Control | Measured interval | Herbicide application | BBCH |
	bromoxynil	clopyralid				
2009[f]	$n_p = 3$	$n_p = 3$	$n_p = 3$	8.7.–21.7.	13.7.	56
2010[g]	$n_p = 4$	$n_p = 4$	$n_p = 4$	13.4.–3.5.	20.4.	35
2011[g]	$n_p = 3$	$n_p = 3$	$n_p = 3$	8.5.–31.5.	16.5.	36

f – field conditions; g – greenhouse conditions

21.1°C/54.6%/17 312.6 kJ/m²/day were observed in the mentioned period of 2010. The same variables $t_a/RH_a/R_g$ equal to 26.9°C/46.0%/22 150.0 kJ/m²/day were observed in the mentioned period of 2011. The effect of herbicides on water regime of plants was measured by the sap flow method, gasometrical system and the method of chlorophyll fluorescence. The measuring terms of water flow in plants and the number of measured plants are listed in Table 1.

The so-called heat balance method is based on the relationship between the input amount of heat and the increase in temperature within a defined space (Tatarinov et al. 2005). The measured values were recorded at 10 min intervals during the entire period of individual measurements. To assess the impact of herbicide effect on the transpiration, the daily amounts of the measured sap flow (transpiration) Q (kg/day) and daily values of calculated sap flow Q_{calc} (kg/day) were used. An approximation of the measured sap flow by the calculated one was made using the special Mini32 software, ver. 4.2.31.0 (EMS Brno, Czech Republic), based upon the following algorithm (Kučera, EMS Brno, pers. comm.; Pivec et al. 2011):

$$Q_{calc} = par1 \frac{R_g}{(R_g + par2)} \frac{VPD}{(VPD + par3)} \qquad (1)$$

Where: R_g – global solar radiation (W/m²); VPD – vapour pressure deficit (hPa). The parameters (par) 1–3 for the Q_{calc} (kg/h) calculation were estimated for measurement period before the herbicide application. Tetens's (1930) algorithm for the saturation vapour pressure calculation was used. Q_{calc} values were calculated from the set of data measured at 10 min intervals for the entire measured period, R_g by pyranometer CM11 (K&Z, Delft, Netherlands), air temperature and humidity by Minikin TH (EMS Brno, Czech Republic).

A portable infra-red gas analyser CIRAS 2 (PP Systems, Amesbury, USA) was used to estimate the transpiration rate (T_r, H_2O mmol/m²/s), photosynthesis rate (P_n, CO_2 μmol/m²/s) and stomatal conductance (g_s, H_2O mmol/m²/s) assessed by the leaf chamber model PLC6 (U) Rice (PP Systems, Amesbury, USA) head plates 25 mm × 7 mm. Based on the measured variables and pre-set values, all parameters mentioned above were calculated according to the manufacturers' settings (equations).

Measurement of parameters was carried out on one of the treated plants within the individual variations on the same leaf. The order of the measured leaf from the base of plant is always shown in the tables' results. In 2009 (field condition), the values of

T_r, P_n and g_s were measured under natural light. A LED light unit was used in 2010 and 2011 (photosynthetically active radiation (PAR) = 750 μmol/m²/s, greenhouse conditions).

Chlorophyll fluorescence measurements were performed using an IMAGING-PAM chlorophyll fluorometer and Imaging Win software application (Walz, Germany). The plants were adapted to dark for 1 h before the measurement. Dark-adapted plants were subjected to an initial saturating pulse (1800 μmol photons/m²/s), followed by a 40 s delay in darkness and subsequently 10 s of actinic illumination with saturating flashes at 20 s intervals. Induction and recovery kinetics were measured. Maximum quantum yield of PSII (F_v/F_m) was calculated according to Genty et al. (1989):

$$F_v/F_m = (F_m − F_0)/F_m$$

Where: F_0 – minimal fluorescence yield of dark-adapted sample with all PS II centres open; F_m – maximal fluorescence yield of dark-adapted sample with all PS II centres closed; F_v – variable fluorescence, calculated as $F_m − F_0$.

In 2010, dry weight of aboveground biomass (g), dry weight of roots (g) and the size of the active leaf area (m²) of plants were estimated in the monitored plants. The root system of plants was rinsed in a sieve with the mesh size of 0.25 mm. The biomass was oven-dried at 105°C for 48 h.

Photosynthetically active leaf area of plants was determined. The Nikon Coolpix 995 digital camera (Tokyo, Japan) was modified by removing the NIR-blocking filter and assessing with Infrared R72 (Hoya, Japan) filter mounted in front of the lens. The images were processed with the analytical tool in the Adobe Photoshop CS5 (Adobe Systems Software, Dublin, Ireland). Data were processed using the Mini32 (v. 4.2.31.0) program and Statgraphics®Plus, v. 4.0 (Warrenton, USA). Simple analysis of variance (ANOVA) and simple linear regression were used.

RESULTS AND DISCUSSION

Based on the experiments, the influence of herbicide application on the transpiration of *H. annuus* plants has been established. Figure 1 illustrate the dependency between the average values of Q_{calc} (kg/day) and Q (kg/day) for the control plants and plants treated with herbicides. Table 2 illustrate the relation between measured and calculated transpiration. The Q values in the control plants were higher or equal to Q_{calc} in the second half
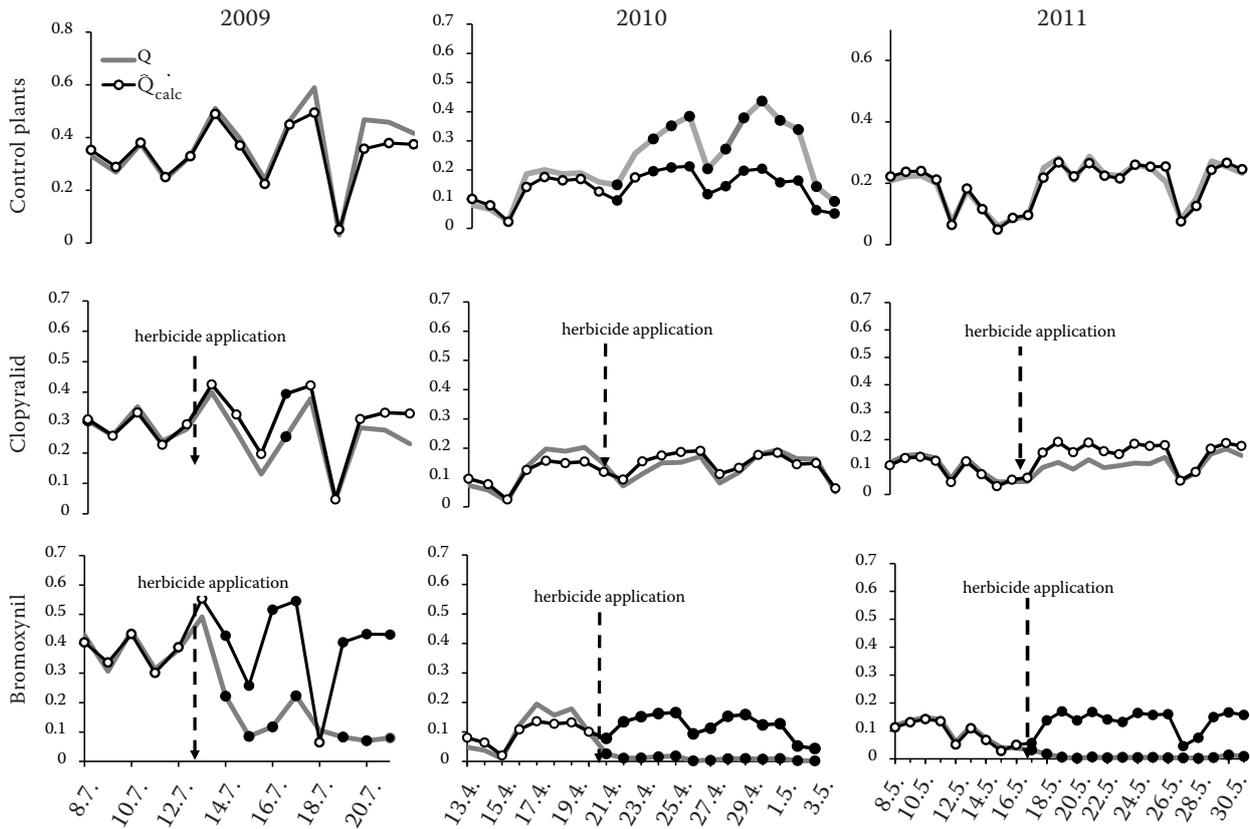
Figure 1. Average daily values of transpiration (Q, kg/day) and calculated sap flow (Q_{calc}, kg/day) in the control plants and plants of the *Helianthus annuus* after clopyralid and bromoxynil treatment (2009, 2010 and 2011). The couple of black filled circles indicates statistically significant differences (ANOVA, α = 0.05) between daily average values of Q and Q_{calc}

of the measurement. A significant decrease in the Q values compared with Q_{calc} after the treatment with the herbicide bromoxynil was observed. When bromoxynil was applied, the Q decreased by the

second day after its application (Figure 1). The decrease of the actual transpiration (Q) in plants treated with clopyralid was less pronounced in comparison with the modelled transpiration (Q_{calc})

Table 2. The relationship between calculated sap flow (Q_{calc}, kg/day) and transpiration (Q, kg/day) in the year 2009, 2010 and 2011

Treatment	Period before application	*r*	cl	Period after application	*r*	cl
2009	8.7.–13.7.			14.7.–21.7.		
Control plants	$Q_{calc} = 0.05064 + 0.86762 \times Q$	0.994	*	$Q_{calc} = 0.03433 + 0.79244 \times Q$	0.980	*
Clopyralid	$Q_{calc} = -0.02932 + 1.10393 \times Q$	0.974	*	$Q_{calc} = 0.0374 + 1.10409 \times Q$	0.939	*
Moxynil	$Q_{calc} = -0.03169 + 1.10163 \times Q$	0.934	*	$Q_{calc} = 0.27617 + 0.88264 \times Q$	0.359	**
2010	13.4.–20.4.			21.4.–4.5.		
Control plants	$Q_{calc} = 0.02185 + 0.73669 \times Q$	0.964	*	$Q_{calc} = 0.01375 + 0.48752 \times Q$	0.931	*
Clopyralid	$Q_{calc} = 0.03282 + 0.62601 \times Q$	0.974	*	$Q_{calc} = 0.03206 + 0.84879 \times Q$	0.939	*
Bromoxynil	$Q_{calc} = 0.03793 + 0.55004 \times Q$	0.934	*	$Q_{calc} = 0.08735 + 2.90541 \times Q$	0.359	**
2011	12.5.–16.5.			17.5.–31.5.		
Control plants	$Q_{cal} = -0.01315 + 1.12162 \times Q$	0.995	*	$Q_{cal} = 0.00760 + 0.94999 \times Q$	0.945	*
Clopyralid	$Q_{cal} = -0.00091 + 0.91737 \times Q$	0.969	**	$Q_{cal} = 0.01858 + 1.21365 \times Q$	0.865	*
Bromoxynil	$Q_{cal} = 0.00443 + 0.88225 \times Q$	0.957	**	$Q_{cal} = 0.14727 - 1.60577 \times Q$	−0.302	***

Regression analysis – linear model. *r* – correlation coefficient; cl – confidence level; *99%; **95%; ***90%

Table 3. The average (4 plants) total dry weight of aboveground biomass (g), of roots (g) and size of the active leaf area (m^2) of plants *Helianthus annuus*, depending on treatment (5.5. 2010). Different indices between average values in columns illustrate statistically significant differences (α = 0.05)

Treatment	Dry weight of aboveground biomass	Dry weight of roots	Size of the active leaf area
Control plants	38.3[b]	9.5[b]	0.271[c]
Clopyralid	19.5[a]	4.4[a]	0.193[b]
Bromoxynil	9.0[a]	3.0[a]	0.017[a]

and the plants continue to transpire after the treatment. The Table 2 illustrate the tightness of dependency between the average values of Q_{calc} (kg/day) and Q (kg/day) after the treatment of plants by herbicides. Slightly higher values of Q (kg/day) than Q_{calc} (kg/day) were noticed in the control plants without herbicide treatment (Figure 1 upper part). This is because the period selected for computation of regression analysis for Q_{calc} estimation preceded the herbicides application period and the plants were growing continuously without any disruption. The biomass increased in control plants (Table 3), where a difference in the assessed physiological parameters is noticeable. Comparison of the results obtained with similar work cannot be carried out, as the literature data concerning the use of sap flow methods to determine the effect of herbicides on transpiration of plants are not available.

The effectiveness of herbicides, expressed in terms of net photosynthesis rate, transpiration rate and stomatal conductance, is presented in Table 4. In each case, the parameters were significantly reduced after the bromoxynil treatment shortly after the application. Changes in photosynthetic and transpiration rates showed a similar pattern as stomatal conductance. Moreover, the results show that the decrease in net photosynthesis is accompanied by a decrease in internal CO_2 concentration (data not shown) indicating that the lower photosynthetic capacity was due to stomatal limitation.

Results of fluorescence measurements in sunflower treated with herbicides with a different mode of action are summarized in Figures 2 and 3. No statistical differences were observed in F_v/F_m shortly after the herbicide application, even though the decrease could be observed after bromoxynil treatment (Figure 2). Bromoxynil significantly reduced the photosystem II efficacy (F_v/F_m < 0.1) of sunflower, whereas it was unaffected by clopyralid (F_v/F_m = 0.83) two days after the treatment. Comparison of the induction and recovery curve of untreated plants with that of the bromoxynil treated plants revealed that the F_0 levels were increased (Figure 3). At the same time, a decrease of about 25% of F_m value was observed and the fluorescence kinetics was largely modified compared to untreated plants. The maximum quantum efficacy of photosystem II seems to be a less appropriate indicator for herbicides with different mode of action than that of inhibiting photosystem II.
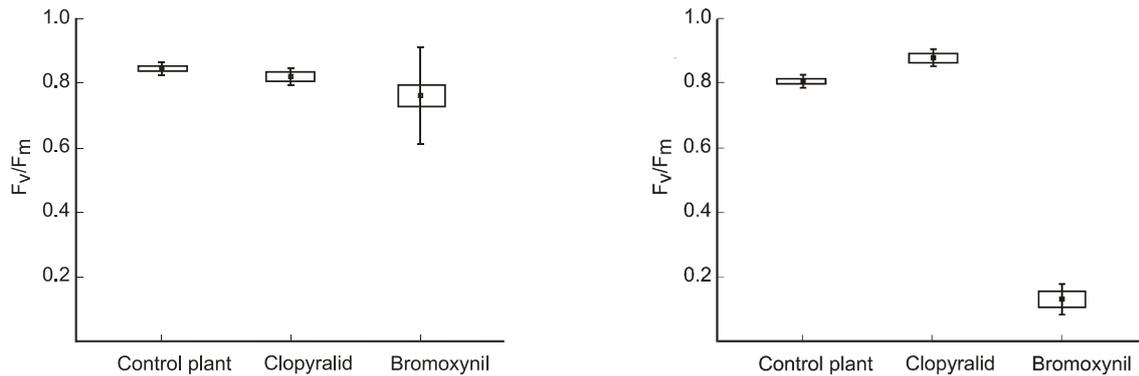
Table 4. Photosynthesis rate (P_n, CO_2 $\mu mol/m^2/s$), photosynthetically active radiation (PAR, $\mu mol/m^2/s$), transpiration rate (T_r, H_2O $mmol/m^2/s$) and stomatal conductance (g_s, H_2O $mmol/m^2/s$) of plant after application of herbicides in 2009 and 2011. Different indices between averages within a column document statistically significant difference (α = 0.05)

Day	Treatment	P_n	PAR	T_r	g_s	Measurement
14.7. 2009	control plants	16.2[c]	1077.3[a]	9.0[c]	929.7[c]	natural light, on the 14th fully developed leaf from the base of the measured plant
	clopyralid	14.1[b]	1076.7[a]	5.1[b]	261.3[b]	
	bromoxynil	–2.0[a]	1067.4[a]	2.0[a]	69.7[a]	
16.7. 2009	control plants	29.4[b]	3131.8[a]	11.7[c]	12760[c]	
	clopyralid	30.4[b]	2989.0[a]	10.1[b]	782.5[b]	
	bromoxynil	–1.5[a]	3186.8[a]	1.7[a]	45.5[a]	
16.5. 2011	control plants	17.5[c]	737.8[a]	6.2[b]	643.4[b]	on the 11th fully developed leaf from the base of the measured plant
	clopyralid	17.0[c]	745.0[a]	5.3[ab]	540.6[b]	
	bromoxynil	2.6[a]	746.8[a]	4.6[a]	367.9[a]	
29.5. 2011	control plants	11.3[a]	595.9[a]	10.4[b]	758.3[b]	
	clopyralid	10.9[a]	596.0[a]	6.1[a]	552.4[a]	

Figure 2. Mean values (4 plants) of maximal quantum yield of photosystem II photochemistry (F_v/F_m) 4 h (left) and 2 days (right) after the treatment of herbicides under laboratory conditions as measured in 2010. The boxes show mean ± standard errors. Vertical bars represent confidence intervals (95%)

The application of clopyralid caused restriction of the transpiration in comparison with the control. However, the plants continued to transpire after the application of the herbicide. The transpiration rate fits well with the values obtained before the application. Based on this fact, it can be assumed that even after clopyralid application on the weed plants they can significantly compete with the crop plants. The applied herbicides affected photosynthesis via different ways by inhibiting electron transport from PS II, blocking auxin and protein biosynthesis, thus we suspect a different response in chlorophyll fluorescence curves and photosynthetic parameters. Bromoxynil affects photosynthesis by binding to the second electron acceptor and strongly inhibiting the electron transport shortly after PS II, therefore it is appropriate to use photosynthetically-based method to measure the influence of the herbicide. Similarly, the effect of clopyralid is detectable as it disrupts the photosynthesis and influences the nucleic acid metabolism. After the exposure to bromoxynil and clopyralid the decrease of F_v/F_m was detected. The reduction of F_v/F_m was mainly due to the increased F_0 value. In plants F_0 reflects

the state of antenna chlorophyll and it is a measure for the initial distribution of energy to PS II and the efficacy of excitation capture in P680 (Rintamaki et al. 1994). It is clear that induction and recovery curves and parameters derived from these curves could provide more information on the photochemical state of plants, but this was not a subject of this study.

On the basis of the evaluation of the herbicides impact on the physiological characteristics of plants, a statistically significant effect of the herbicides bromoxynil and clopyralid to reduce aboveground and root dry matter in comparison with control plants was demonstrated (Table 3).

Results of this work demonstrated the applicability of the sap flow method for continuous evaluation of the impact of herbicides on plant transpiration. The advantage of the method, based on a comparison of modelled transpiration of plant after herbicide application and actual transpiration, is the ability to verify the effect of used herbicides on an individual plant of *H. annuus*. This prevents the variability of plants from influencing the results. The advantage may be even longer measurement on the plant, which is particularly important for
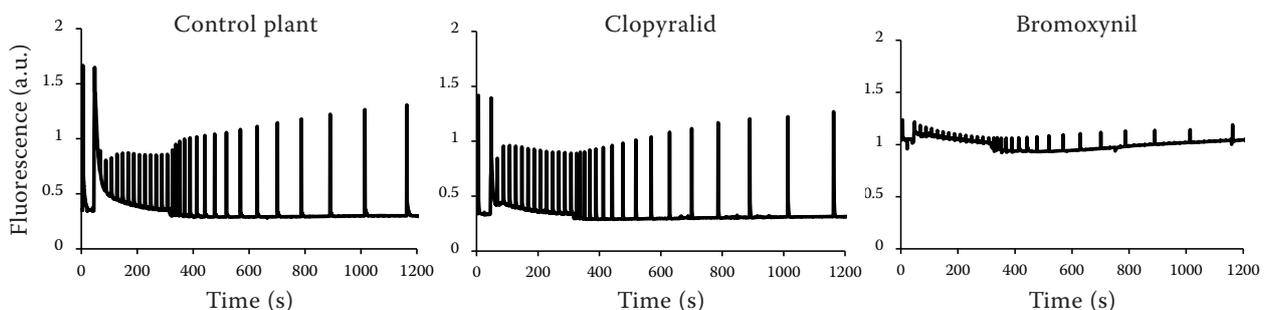

Figure 3. Courses of chlorophyll fluorescence induction and recovery kinetics for control and herbicide-treated sunflower 4 h after the herbicide treatment under laboratory conditions in 2011

herbicides with indirect effect on photosynthesis and transpiration. The limiting factor is that the sensors for measuring the flow of sap cannot be applied to plants in the early growth phases, or in a small diameter stem. The bromoxynil and clopyralid treated sunflower provided information concerning phytochemical responses, detectable before the visual symptoms appeared and thus these changes could provide the basis for early detection of lethal effects. In comparing the methods, the fluorescence bioassay was more/less sensitive than the sap flow method for all three herbicide tested (or for some of them).

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Corresponding author:

Ing. Václav Brant, Ph.D., Česká zemědělská univerzita v Praze, Fakulta agrobiologie, potravinových a přírodních zdrojů, Katedra agroekologie a biometeorologie, Kamýcká 129, 165 21 Praha, Česká republika
e-mail: brant@af.czu.cz