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Effect of photosystem II, lipid biosynthesis and auxin inhibitor herbicides on fluorescence induction curve

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Abstract

Measuring chlorophyll fluorescence is a noninvasive, highly sensitive and fast technique for assaying photosynthetic apparatus status in plants. Chlorophyll fluorescence measurements were carried out to characterize how the fluorescence induction curve (Kautsky curve) and its parameters were affected by untreated control and seven doses of desmedipham + phenmedipham + ethofumesate (photosystem II (PSII) + lipid biosynthesis inhibitors), chloridazon (PSII inhibitor) and clopyralid (plant growth regulator (PGR) inhibitor) in common lambsquarters (*Chenopodium album* L.) and black nightshade (*Solanum nigrum* L.) at Ferdowsi University of Mashhad Greenhouse during 2013. Altogether, 42 treatments were used for both species with three replications per treatment and a completely randomized layout. Biomass effective dose (ED_{50} and/or ED_{90}), based on log-logistic dose–response curves, were more effective in black nightshade control than common lambsquarters, i.e. the minimum dose required for a satisfactory control with 90% reduction on black nightshade dry matter (ED_{90}) was 316.60, 1133.16 and 132.40 g ai ha^{-1} for desmedipham + phenmedipham + ethofumesate, chloridazon and clopyralid, respectively. The shape of the chlorophyll fluorescence induction curve were changed by desmedipham + phenmedipham + ethofumesate at doses higher than 308.25 mg ai ha^{-1} in common lambsquarters and in all doses of black nightshade at 4 hours after spraying (HAS). In contrast, chlorophyll fluorescence decay was obvious by chloridazon at 72 and 168 HAS in both species. Clopyralid had chlorophyll fluorescence inhibition only in black nightshade at doses higher than 60 mg ai ha^{-1}. The maximum quantum efficiency (F_V/F_m), the relative changes at the J step (F_{vj}) and area (the area between the Kautsky curve and maximum fluorescence (Fm)), were affected by desmedipham + phenmedipham + ethofumesate at rates of 51.38 mg ai ha^{-1} and up in both species 4 HAS, but F_V/F_m was stable for chloridazon and clopyralid in common lambsquarters. A good correlation (between 0.65 to 0.89) was existed between fluorescence parameters taken 24 hours after spraying and dry matter taken three weeks after spraying for both species. In conclusion, the chlorophyll fluorescence measurement may be used to shorten the bioassay screening period is a suitable and cost effective indicator for monitoring PSII and PGR inhibitors.

Keywords: Chloridazon, chlorophyll fluorescence, clopyralid, desmedipham + phenmedipham + ethofumesate, Kautsky curve.
Abbreviations: ABA_abscisic acid; Ai_active ingredient; CHEAL_*Chenopodium album* L. (Common lambsquarters); ED_effective dose; HAS_hours after spraying; Log (K_{ow})_Logarithm K Oil-Water partition coefficient; PGR_plant growth regulator; PSII_ photosystem 2; POST_ post-emergence; Q_A or Q_B_quinones A or B; ROS_ reactive oxygen species; SOLNI_*Solanum nigrum* L. (Black nightshade); WAS_week after spraying.

Introduction

Desmedipham + phenmedipham + ethofumesate, phenylcarbamates + benzofuranyl alkanesulfonate herbicides, is widely used for post-emergence broad-leaved weed control in sugar beet (Markovska et al., 2012). Chloridazon, a pyridazinone herbicide, is used as a pre- and post- emergence herbicide in sugar beet (Rouchaud et al., 1997). Desmedipham, phenmedipham and chloridazon, are photosystem II (PSII) inhibitors, their translocation via xylem are slow, mostly absorbed not only by roots, but also by foliage (Abbaspoor and Streibig, 2007). Their mode of action is through the blocking of electron transfer between the primary and secondary quinones (Q_A and Q_B) of PSII by binding to the Q_B-binding site and accepting electrons from Q_A in the chloroplasts (Govindjee et al., 1997; Hess, 2000; Ikeda et al., 2003). Consequently, the photosynthetic electron transport chain is interrupted, and leading to the concomitant inhibition of ATP production and carbon fixation (Van Rensen et al., 1999; Hess, 2000; Kohno et al., 2000). Eventually, plant death is caused by light induced oxidative stress, initiated via damaging caused by formation of reactive oxygen species (ROS) near the PSII reaction centers, leading to lipid peroxidation and proteolysis, as well as breaking off the protein–pigment complexes of PSII and plant death (Hess, 2000; Fufezan et al., 2002). Ethofumesate is readily absorbed by emerging shoots and roots, and is translocated readily to the foliage. Post-emergence applied ethofumesate is poorly absorbed by maturing leaves with a well-developed cuticle (Meyer et al., 2006). Clopyralid a growth regulator herbicide (PGR) is in the picolinic acid group and readily translocated apo-symplastic within the plant. It used for post-emergence broad-leaved weed control in sugar beet. Clopyralid has no direct impact on photosynthesis function, but it affects chlorosis and necrosis of the leaves indicating malfunctioning of the photosynthetic apparatus (Tu et al., 2001). Measures of changes to the chlorophyll fluorescence induction curve (Kautsky curve), is a rapid, non-invasive and simple method for monitoring the physiological status of the photosynthetic apparatus in plants (Matouskova et al., 1999; Strasser et al., 2000). It can be used for the study of the effect of PSII-inhibiting herbicides as well as herbicides with other modes of action (Percival and Baker 1991; Christensen et al. 2003). Analysis of the chlorophyll fluorescence induction

curve (Kautsky curve), means a series of changes in chlorophyll fluorescence yield are observed in plant leaves when light is abruptly turned on after a dark period (Maxwell and Johnson 2000; Baker and Rosenqvist, 2004). Illumination with 650 nm wavelength of healthy dark-adapted leaves provides a rise in amounts of chlorophyll fluorescence emission, with some trait phases. There are three phases found on the O, J, I and P steps. These phases primarily point out photochemical events relevant to PSII (Govindjee, 1995). The three phases are described as follows: at the O-J phase complete reduction of the primary electron acceptor Q_A of PSII takes place from 50 μs to 2 ms, the J-I phase corresponds to electron transfer from Q_A to $Q_B$happens between 2 to 30 ms and the I-P phase corresponds to the release of fluorescence quenching by the oxidized plastoquinone pool taking place within 30-500 ms (Appenroth et al., 2000; Strasser and Stirbet, 2001; Force et al., 2003) (Fig 1). From the numerous parameters which can be derived from the OJIP steps of the fluorescence induction curve, the relative changes at the J step e.g. [Fvj = (Fm – Fj)/Fm], prove to be a useful parameter to discern effects of herbicides with different mode of actions (Percival et al. 1992; Christensen et al. 2003; Abbaspoor and Streibig, 2005; 2007) (Fig 1). The shape of the Kautsky curve is affected by various stress factors, such as ambient illumination, nutrient supply, temperature and water stress, pathogens and herbicides (Habash et al., 1985; Bolhar-Nordenkampf and Oquist, 1993; Daley, 1995). In addition, fluorescence induction kinetics can be altered by many inhibitors of metabolic processes that are not directly involved in photosynthesis (Blowers, 1989; Percival and Baker, 1991; Crudace, 2000). The shape of the Kautsky curve and derived fluorescence parameters of *Arabidopsis thaliana* seedlings influenced by herbicides Asulam, Bifenox, 2,4-D, Diclofop-methyl, Glyphosate, and Imazapyr, which do not have a direct impact on photosynthesis (Barbagallo et al., 2003).
The objectives of this study were to determine how rapidly three herbicides with different mode of actions affect the shape of the Kautsky curve, defined by various fluorescence parameters in weed species, and whether possible herbicide variation can be observed in the shape of the Kautsky curve as a function of time after being exposed to herbicides. The final objective was to relate various fluorescence parameters, derived from the Kautsky curve, to the dry matter of these herbicides.

Results

Dose–response assays

A summary of the dose–response curve analysis for dry matter of common lambsquarters and black nightshade for the two experiments are shown in Table 1. In both experiments, desmedipham + phenmedipham + ethofumesate were more potent than chloridazon and clopyralid against common lambsquarters and black nightshade and their ED_{50} and ED_{90} values were lesser compared with chloridazon and clopyralid at cotyledon stage in either experiments. However, a lower dose was also required for 50% or 90% reduction in total dry matter for black nightshade by desmedipham + phenmedipham + ethofumesate, chloridazon and clopyralid application (Table 1). Consequently, we may recommend using desmedipham + phenmedipham + ethofumesate for adequate weed control in sugar beet instead of chloridazon and clopyralid.

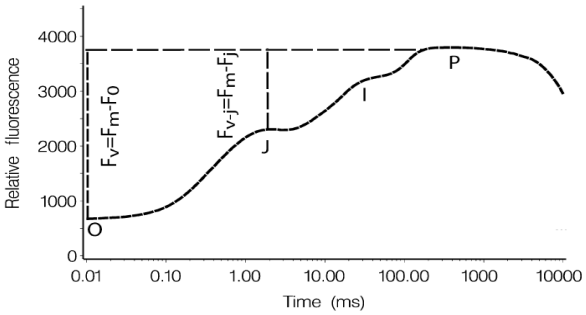

Fig 1. Kautsky curve recorded with Handy PEA instrument in a 30 minutes dark adapted leaf. The Kautsky curve rise from O to P levels is characterized by the OJIP steps reflecting PSII electron transport from water to PQ pool.

Chlorophyll fluorescence assays

The Kautsky curves

The effects of desmedipham + phenmedipham + ethofumesate, chloridazon and clopyralid on the shape of the Kautsky curves for common lambsquarters and black nightshade leaves are shown in Fig 2. In both experiments, 4 HAS, desmedipham + phenmedipham + ethofumesate changed the shape of the Kautsky curves, but chloridazon and clopyralid had no effect on it (Fig. 2).Kautsky curves were irreversibly affected and altered in an almost horizontal lines by desmedipham + phenmedipham + ethofumesate in both weeds species at 4 HAS at doses of 308.25 mg ai ha^{-1} and higher in common lambsquarters (first experiment), but all doses in black nightshade (second experiment) (Fig. 2 a,b), while chloridazon and clopyralid did not. Black nightshade was shown more sensitive to desmedipham + phenmedipham + ethofumesate application than common lambsquarters and excluding doses of 51.38 and 102.75 mg ai ha^{-1} was completely destroyed in all doses 168 HAS (Fig. 2). It seems that lag time of 168 HAS at the highest doses, was not suitable for desmedipham + phenmedipham + ethofumesate fluorescence measuring due to death of plants and the fluorescence virtually ceased in black nightshade (Fig. 2).
Chloridazon is a PSII inhibitor however, but had a little impact on the reduction of species florescence at 4, 24 and 48 HAS, but at 72 and 168 HAS the shape of the Kautsky curves was changed entirely. Lesser impact of chloridazon than that of desmedipham + phenmedipham + ethofumesate on chlorophyll fluorescence reduction of both species may be due to less solubility and more deposition of chloridazon in sprayer tank. Clopyralid did not well influence on Kautsky curves at all doses in common lambsquarters. While in black nightshade, the shape of Kautsky curves were influenced by clopyralid as a PGR inhibitor between 60-240 mg ai ha^{-1} (Fig. 2 f).

The fluorescence parameters

To describe the changes in the shape and form of the Kautsky curves in Fig. 2, plots of the selected fluorescence parameters e.g. (F_V/F_m, F_{Vj} and area) against doses are shown in Fig. 3. The value of the F_V/F_m parameter was close to 0.83 in all plants and consistent during the experimental period, while F_{Vj} and area somewhat were vacillated during the course of the experiment (Fig. 1) (Abbaspoor et al., 2006). The detailed changes of fluorescence parameters in treated plants are shown in Fig. 3 in both experiments. The herbicides effect on fluorescence parameters started 4 HAS, particularly for black nightshade in the second experiment.

Table 1. Summary of dose–response analysis of dry matter at 21 days after spray (DAS) (four to six true leaf stage) for both of experiments. In both experiments the test for lack of fit were not significant, indicating that the logistic model was able to describe the data better than an ordinary ANOVA

Weed species	Herbicide	Upper limit (SE)[a]	Slope (SE)	ED_{50} (SE)[b]	ED_{90} (SE)	Lack of fit test (5%)
First experiment						
Common lambsquarters (*Chenopodium album* L.) (CHEAL)	Desmedipham + phenmedipham + ethofumesate	4.13 (±0.71)	1.55 (±0.05)	93.21 (±11.12)	427.77 (±31.11)	0.69 (NS)[c]
	Chloridazon	4.01 (±0.65)	1.19 (±0.05)	387.79 (±60.88)	1361.83 (±134.13)	0.56 (NS)
	Clopyralid	3.98 (±0.58)	1.18 (±0.05)	17.09 (±2.55)	149.16 (±10.69)	0.27 (NS)
Second experiment						
Black nightshade (*Solanum nigrum L.*) (SOLNI)	Desmedipham + phenmedipham + ethofumesate	2.68 (±0.07)	1.02 (±0.04)	37.47 (±5.66)	316.60 (±33.81)	0.11 (NS)
	Chloridazon	2.52 (±0.06)	1.26 (±0.05)	198.35 (±24.77)	1133.16 (±140.53)	0.38 (NS)
	Clopyralid	2.48 (±0.06)	0.94 (±0.03)	12.66 (±2.55)	132.40 (±15.52)	0.97 (NS)

[a] SE = Standard Error, [b] ED_{50} or ED_{90} = Effective Dose at 50 or 90 percent, [c] NS: not significant at the 5% level, Abbreviations: CHEAL, *Chenopodium album* L. (common lambsquarters); SOLNI, *Solanum nigrum* L. (black nightshade).

Table 2. Assessment of relative potency between PSII + lipid biosynthesis inhibitor and plant growth regulators (PGR) herbicides

Weed species	Herbicide	Relative potency (RP)	
		ED_{50}[a] (SE)[b]	ED_{90} (SE)
First experiment			
Common lambsquarters	Desmedipham + phenmedipham + ethofumesate : Clopyralid	5.45 (±1.48)	2.87 (±0.85)
(*Chenopodium album* L.) (CHEAL)	Chloridazon : Clopyralid	22.69 (±6.49)	9.13 (±2.73)
Second experiment			
Black nightshade	Desmedipham + phenmedipham + ethofumesate : Clopyralid	2.96 (±0.71)	2.39 (±0.62)
(*Solanum nigrum* L.) (SOLNI)	Chloridazon : Clopyralid	15.67 (±2.43)	8.56 (±2.94)

[a] ED_{50} or ED_{90} = Effective Dose at 50 or 90 percent, [b] SE = Standard Error, Abbreviations: CHEAL, *Chenopodium album* L. (common lambsquarters); SOLNI, *Solanum nigrum* L. (black nightshade).

For desmedipham + phenmedipham + ethofumesate, significant decreases in parameters were evident at any time intervals measured after treatment in both species, while this was not the case with chloridazon and clopyralid (Fig. 3). Among fluorescence parameters, F_V/F_m was stable and not sensitive enough to trace minor herbicides effects in both species, but F_{vj} and area were decreased by desmedipham + phenmedipham + ethofumesate and chloridazon in all doses and time periods. In addition, F_{vj} and area were declined by clopyralid in black nightshade, whereas in common lambsquarters only F_{vj} were influenced by clopyralid, but F_V/F_m and area were consistent (Fig. 3).

The relationship between fluorescence parameter and dry weight

The relationship between dry matter, taken 21 DAS by desmedipham + phenmedipham + ethofumesate and chloridazon, and the selected fluorescence parameters, taken 24 HAS for common lambsquarters and black nightshade, respectively (Fig. 4). A linear regression of dry matter on fluorescence parameters was fitted to the data and the relationship between fluorescence parameters and dry matter was evident. The graphs show four HAS, F_V/F_m was more affected by the lipophilic desmediphamand + phenmedipham + ethofumesate [log (K_{ow}) = 9.68] and chloridazon [log (K_{ow}) = 1.19] than by the hydrophilic clopyralid [log (K_{ow}) = 2.34×10^{-2}]. It also shows that the plants die in the course of the experiment.

Discussion

The results of our study showed that desmedipham + phenmedipham + ethofumesate was more effective on common lambsquarters and black nightshade control compared to chloridazon and clopyralid. Starke and Renner (1996); Bosak et al. (2001); Abdollahi and Ghadiri (2004) reported that phenmedipham plus desmedipham showed much better weeds control than mere use of phenmedipham in sugar beet. Based on the results of the experiment, climatic conditions, phenological stage of plant and plant species could be effective on ED_x value discrepancy. Abbaspoor and Streibig (2007) reported that different ED_x such as ED_{50} or ED_{90} derived from log-logistic dose-response curves can be due to different plant growth, phenological stages at the time of herbicide treatment, weed species as well as climatic differences in the greenhouse conditions. They also, described that PSII and ACCase inhibitor herbicides such as

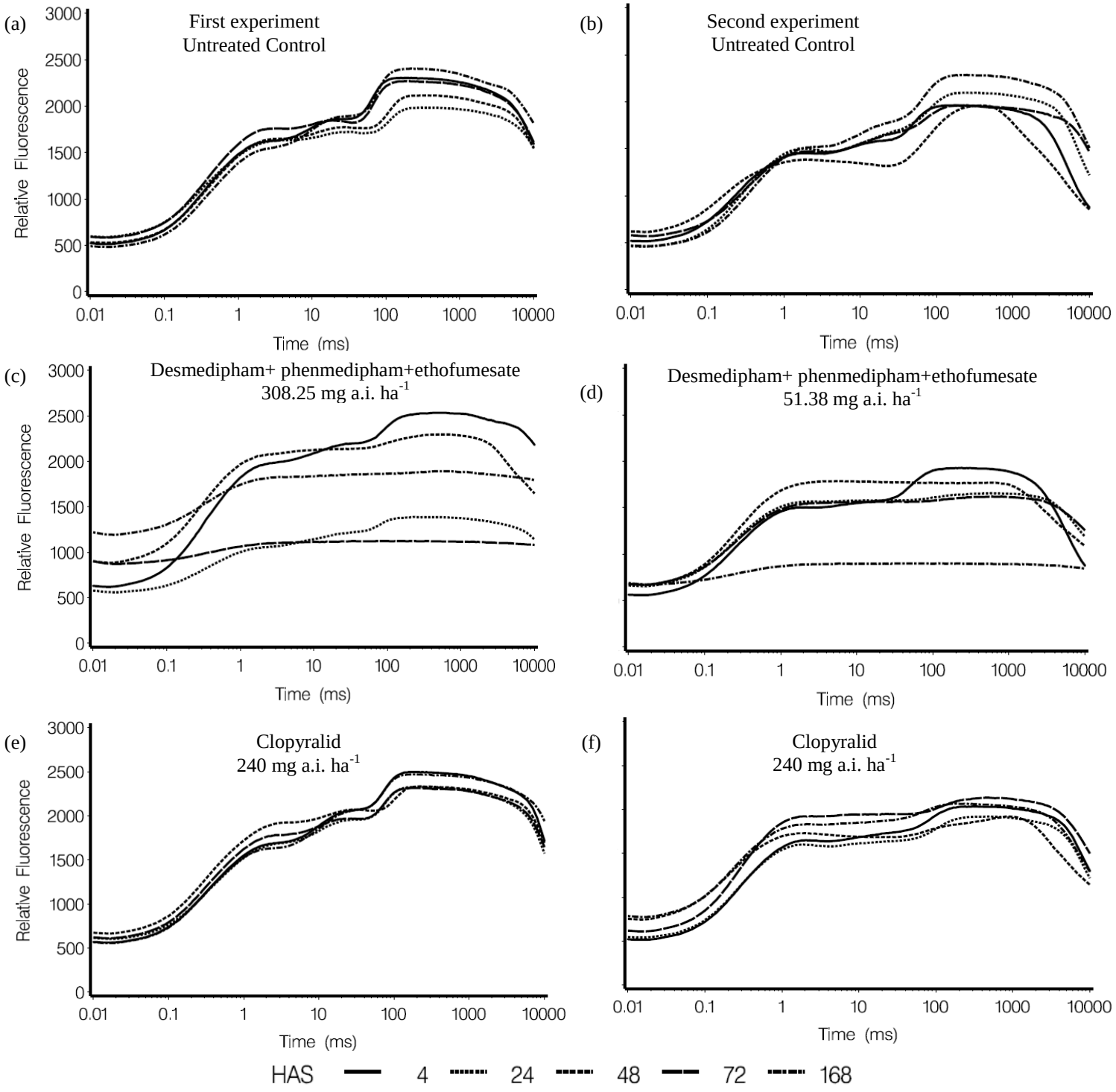

Fig 2. Effect of herbicides application on the shape of the Kautsky curve. Kautsky curve for untreated plants of common lambsquarters (a) and black nightshade (b), desmedipham + phenmedipham + ethofumesate effects on common lambsquarters (c) and black nightshade (d) at 308.25 and 51.38 mg a.i. ha^{-1} (c) respectively. Common lambsquarters unaffected (e) and affected on black nightshade (f) by clopyralid. Each curve is the mean of twelve replications for untreated controls and three replications for rest of the doses. HAS value is mean hours after spraying.

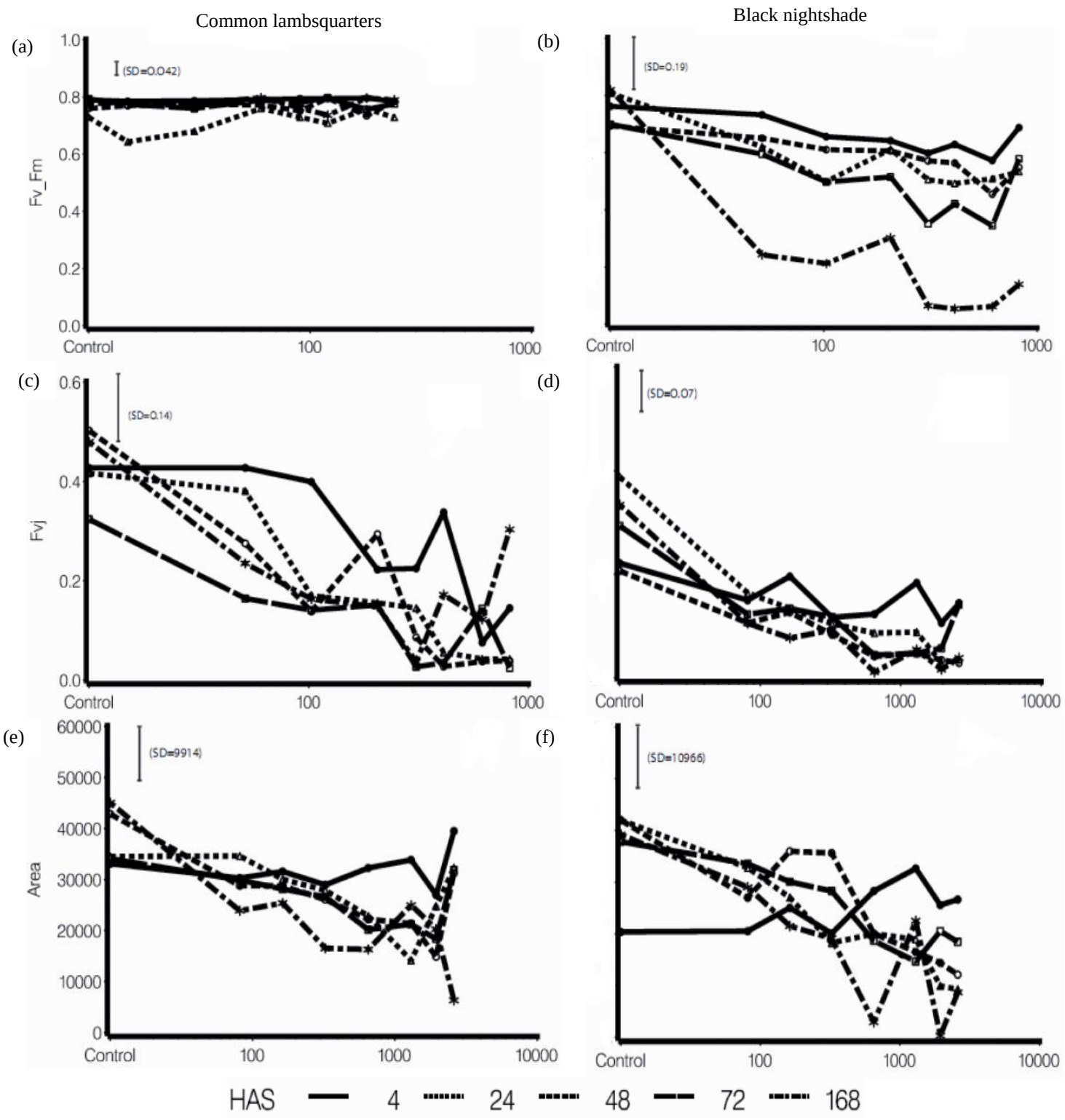

Fig 3. Effect of clopyralid on F_v/F_m in common lambsquarters (a), desmedipham + phenmedipham + ethofumesate on F_v/F_m and F_{vj} in black nightshade and common lambsquarters, respectively (b-c), chloridazon effect on F_{vj} in black nightshade (d), and chloridazon effect on area in common lambsquarters and black nightshade, respectively (e-f). Means values are shown. HAS: hours after spraying, SD: standard deviation.

desmedipham, phenmedipham and clodinafop respectively, affected variations in light and temperature conditions in greenhouse; moreover, the high post-spray light intensity resulted in greater inhibition of photosynthesis by desmedipham and phenmedipham than low light intensities (Abbaspoor and Streibig, 2005; 2007). Consequently, it seems that desmedipham + phenmedipham + ethofumesate, chloridazon and clopyralid usage resulted in different ED_{50} or ED_{90} values because of variation in light and temperature conditions of both species at the four- to six-true leaf stage. This is illustrated by the relative potency between the herbicides (Table 2). In addition, the results showed that the shape of the Kautsky curves changed by desmedipham + phenmedipham + ethofumesate at 4 HAS, but chloridazon and clopyralid did not. Abbaspoor et al. (2006) dipicted the rapidity of absorption and subsequent effect on fluorescence parameters is due to the contrasting properties of compounds. Desmedipham + phenmedipham + ethofumesate and chloridazon have a log (K_{ow}) of 9.68 and 1.19 respectively, and have direct impact on photosynthesis, whereas clopyralid with log (K_{ow}) 2.34×10^{-2}, due to solubility in aqueous solvents (polar solvent) did not show such effect on photosynthesis. Horgan and Zabkiewicz (2008) showed that log (K_{ow}) of herbicides could be a reflection of the performance of photosynthesis in plants exposed to herbicides. On the other hand, apparent discrepancy among the herbicides could be attributed to the different phenological stages of plant development at the time of treatment.

It seems that due to the high sensitivity of leaves and stems black nightshade is more affected by PSII inhibitors such as desmedipham + phenmedipham + ethofumesate, whereas Kautsky curve were less affected by common lambsquarters a few hours after the spray due to the existing powdery covering on the abaxial side of the leaves, resulted to lower penetration of herbicide into the plant tissue (Fig. 2 e-f) (Taylor et al., 1981). Abbaspoor et al. (2006); Abbaspoor and Streibig (2007) demonstrated that Kautsky curve was affected by different phenological stages of plant, environmental conditions, e.g. temperature, water stress, nutrient supply and weed species as well. Hence, the differences among response the phenological stages of plant should attributed to the differences weed species. This is again illustrated by the relative potency between the herbicides (Table 2). Despite the fact that clopyralid is a plant growth inhibitor, the shape of Kautsky curves were affected by it in black nightshade (Fig. 2f). Growth inhibition by auxins caused growth inhibition by ethylene evolution (Cobb and Reade 2010), cease the biosynthesis of abscisic acid (ABA) (Grossmann et al., 2001), stomata closure and restriction of CO_2 diffusion through stomata (Cronic, 2000), accumulation of reactive oxygen species (ROS) such as H_2O_2 and leakage to O_2 in the chloroplast (Dat et al., 2000) are the consequences of the clopyralid exposure to the susceptible weeds. H_2O_2 generated by hydroxyl radicals resulted in lipid peroxidation and oxidative damage (Dat et al., 2000). It seems that thylakoid membrane electron leakage and oxidative damage of thylakoid phospholipid membrane of the chloroplasts interrupts the electron transport chain (Z scheme) from PSII to PSI and this interruption of the Z scheme will change the shape of the Kautsky curves (Dayan and Zaccaro, 2012). Avarseji et al (2012) indicated that the usage of dicamba + 2, 4-D an auxin herbicide, changed the shape of the Kautsky curves two days after spraying (DAS). Abbaspoor and Streibig (2005) reported chlorophyll fluorescence halted after the P step in oat leaves by clodinafop application after seven DAS. Results showed that parameters derived from Kautsky curves had different variations as well. F_V/F_m parameter was stable, whereas F_{vj} and area reduced in both species. Abbaspoor and Streibig (2007) described the relative changes at the J step (F_{vj}) have also proved to useful parameters to discern effects of herbicides with other modes of action. Christensen et al. (2003); Abbaspoor and Streibig (2005; 2007) showed the F_v/F_m may not be the most sensitive parameter when early detection of fluorescence emission changes is required for herbicides with modes of action other than that of PSII inhibition. Abbaspoor and Streibig (2006) also reported that by spraying desmedipham, phenmedipham or a mixture of both, the F_{vj} and area parameters were much more sensitive than F_v/F_m measured in black nightshade and sugar beet. Furthermore based on the results, a good relationship was observed between dry matter and fluorescence parameters. Similar relation was previously reported in fluorescence parameters and dry matter for bentazone, desmedipham and phenmedipham (a PSII inhibitor) (Christensen et al., 2003; Abbaspoor and Streibig, 2007) and for glyphosate and clodinafop (non-PSII inhibitors) (Teicher et al., 2002; Abbaspoor and Streibig, 2005). Using these fluorescence parameters may suggest a rapid, noninvasive and cost-effective option to eliminate the need for whole plant bioassay screenings.

Materials and methods

Plant materials

Seeds of common lambsquarters (*Chenopodium album* L.) and black nightshade (*Solanum nigrum* L.) were collected from sugar beet fields in Mashhad, Iran, and grown in 2 litre pots filled with mixture of a sandy-loam soil, sand and peat (1:1:1wt/wt/wt), containing all necessary macro-and micro-nutrients. The experiments were conducted during a three-month period from April to June 2013 at the Research Greenhouse in Faculty of Agriculture, Ferdowsi University of Mashhad, Iran (Lat 36° 15' N, Long 59° 28' E; 985 m Altitude). The photoperiod was 16:8 h light:dark and temperature ranges were approximately 15 ± 1°C at night and 23 ± 2°C during the day. Four high-pressure sodium vapor lamps (Osram Sylvania, Lynn, MA, USA, 400 W, 55 000 lumens) were installed 2 m above the plants to accommodate the photoperiod as mentioned above. Plants were tinned to four plants per pot at cotyledon stage.

Chemical treatments and experimental design

Two independent experiments for common lambsquarters and black nightshade were conducted for three herbicides simultaneously, each of which consisted of a dose–response curves with seven doses plus an untreated check. The herbicides and doses were used for each, included of 1) desmedipham + phenmedipham + ethofumesate (Betanal Progress- OF®, 274 mg L^{-1}, Bayer Crop Science) at 0, 51.38, 102.75, 205.5, 308.25, 411, 616.5 and 822 mg ai ha^{-1}, 2) chloridazon (Pyramin®, 650 g L^{-1},BASF A/S) included of 0, 81.25, 162.5, 325, 650, 1300, 1950 and 2600 g ai ha^{-1} and 3) clopyralid doses were (Lontrel® 300 mg L^{-1}, Golsam Gorgan Chemicals Corporation, Gorgan, Iran) were: 0, 15, 30, 60, 90, 120, 180 and 240 mg ai ha^{-1}. Spraying was done using overhead trolley sprayer (Matabi 121030 Super Agro 20 litre sprayer; Agratech Services-Crop®, Spraying Equipment, Rossendale, UK), 8002 flat-fan nozzle at 300 kPa and a spray volume of 200 Lha^{-1}. The plants were treated 21 days (at the four- to six-true leaf stage) after planting. Altogether, 42 treatments were used for common lambsquarters and black

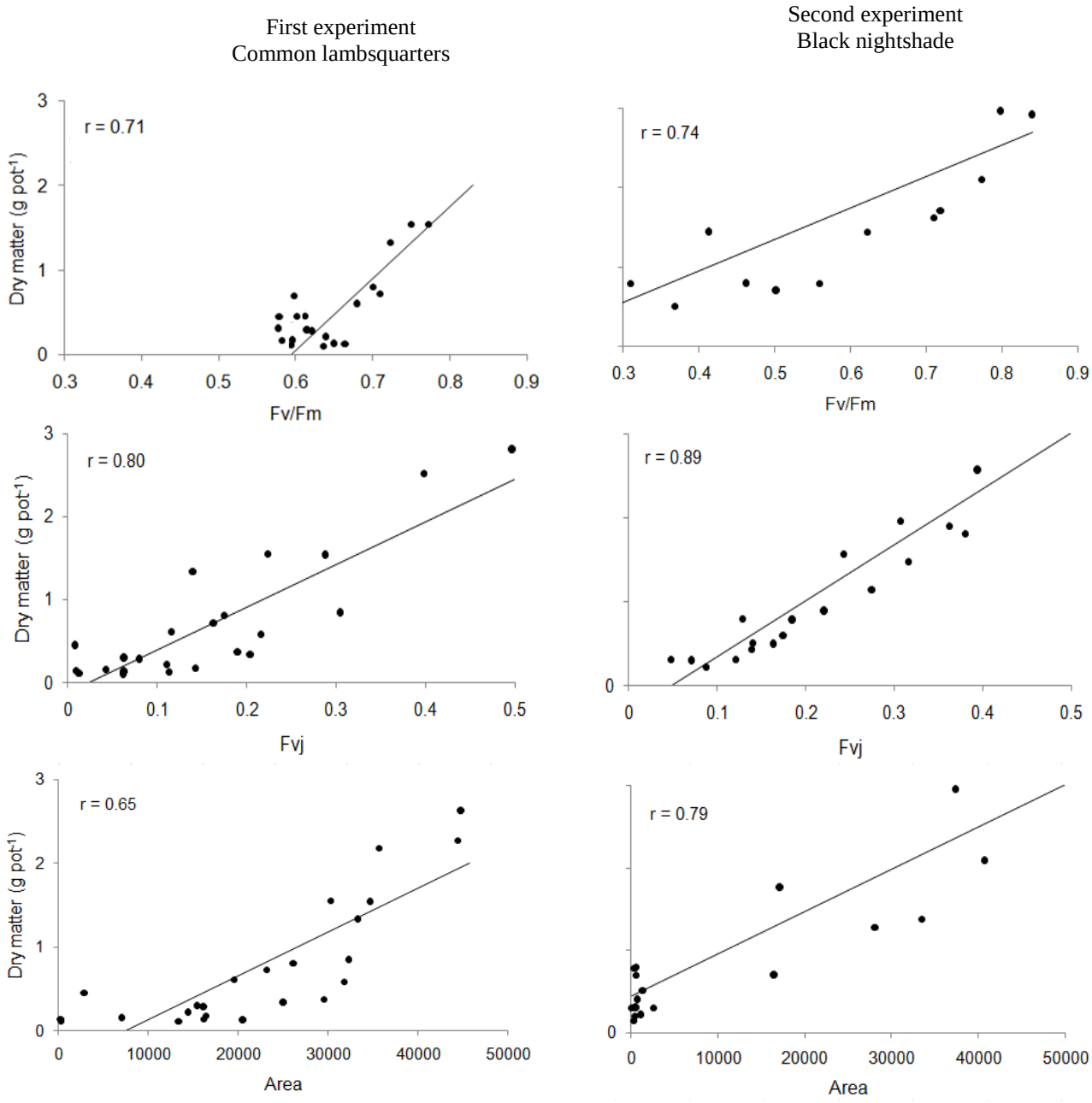

Fig 4. The relationship between dry matter and chlorophyll fluorescence parameters (F_V/F_m, F_{Vj} and area) at 24 h after spraying [HAS] in two experiments. (Left, common lambsquarters sprayed by desmedipham + phenmedipham + ethofumesate in the first experiment; right, black nightshade sprayed by chloridazon in the second experiment).

nightshade with three replications (i.e. totally 126 experimental units including check arranged) in completely randomized layout.

Chlorophyll fluorescence assays

Chlorophyll fluorescence measurements were carried out on dark-adapted leaves at the same stages of development among pots. Fluorescence emissions were measured using a portable chlorophyll fluorometer (Handy-PEA; Hansatech Instruments, King's Lynn, Norfolk, UK), which emits light of 650 nm wavelength with an intensity of 3000 µmol photons m^{-2} s^{-1} for 10s.

Leaves were dark adapted for a minimum of 30 min prior to measurement. The fluorescence measurements were taken 4 hours after spraying (HAS) for common lambsquarters and black nightshade and again at 24, 48, 72 and 168 HAS.

The Kautsky curves were visually examined for the effects of time and dose (Rodriguez and Strasser, 2002) by the BIOLYZER program with OJIP steps as fix points. The most important parameters derived from the Kautsky curve were: (i) F_V/F_m, maximum quantum efficiency of PS II (F_V/F_m = $(F_m - F_o)/F_m$), (ii) F_{Vj}, the relative changes at the J step (F_{vj} = $(F_m - F_j)/F_m$), where F_m was the maximum fluorescence, F_0 was ground state fluorescence and F_j was Fluorescence at J step and (iii) Area (area between Kautsky curve and Fm).

Three week after spraying (WAS), plants were harvested and their dry weights were measured.

Statistical analysis

If a significant dose effect was found, data were described by a log-logistic dose–response model against dose (Devilliers et al, 2001; Cedergreen et al., 2005):

$$U = \frac{d}{1+\exp\left[b\left(\log(z)-\log(ED50)\right)\right]} \qquad (1)$$

Where U is the dry matter production at dose z, d is the upper limit where the dose is zero, ED_{50} denotes the dose required for reducing dry matter by half and b is proportional to the slopes of the curves around ED_{50}. The ED_{50} parameter can be replaced by any ED level, so the selected model was used to estimate the dose of herbicides required to obtain 50% and 90% weed control (ED_{50} and ED_{90} values) when applied individually. The goodness-of-fit was assessed by graphical analyses of residuals and the tests for lack of fit of the models, and the biomass data were Box-Cox transformed to obtain variance homogeneity (Streibig et al. 1993). Data were analyzed by R statistical software (R Development Core Team, 2011) and the R extension package drc.

Assuming that Z_A and Z_B are the doses of herbicide A (PS II inhibitor herbicides) and B (Clopyralid) producing for example a 50% effect, i.e. the ED_{50} doses, the relative potency between the herbicides (function 2) was calculated as:

$$R = \frac{Z_A}{Z_B} \qquad (2)$$

The relative potency between herbicides A and B expresses the biological exchange rate between herbicides when applied separately (Streibig et al. 1993).

Conclusion

In conclusion, various fluorescence parameters can be used to describe the shape and change of Kautsky curves in different plant species. In this paper we focused on common fluorescence parameters for three tested herbicides. The parameter F_v/F_m seems to be less sensitive to detecting changes than are F_{vj} and area. Also, other or a combination of Kautsky curve parameters can be used for herbicides which do not directly affect the PSII system (Teicher et al., 2002). Thus, F_{vj} and area should be used as an early response parameter of herbicides action; it must be linked to the dry matter production used in classic assays, regardless of the herbicide ratio. In this method, the analysis of the Kautsky curve could enable weed scientist to increase their knowledge of the mechanisms of action primary differing inhibitory herbicides, and it also provides plant physiologists with a vital tool to widen their understanding of photosynthetic processes.

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