Chlorophyll fluorescence response to herbicide stress in *Alopecurus myosuroides*

Chlorophyll-Fluoreszenz-Reaktion auf Herbizidstress bei Alopecurus myosuroides

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Abstract

Herbicide resistance has been a widely spread problem all over the world. The resistance detection is usually complicated and financial resources consuming. A sensor based monitoring of herbicide resistance would make the resistance detection much faster and spends less. In this research, greenhouse and field experiments with susceptive and resistant populations were conducted to study the chlorophyll fluorescence response of *Alopecurus myosuroides* after herbicide treatment. The fluorescence sensor WEED-PAM was used for measuring the maximum photosynthesis system II quantum yield (Fv/Fm) of *Alopecurus myosuroides* after the application for two weeks.

The Fv/Fm values of all untreated plants were around 0.7. In the greenhouse, Fv/Fm values of treated susceptive plants reduced significantly from 0.7 to 0.6-0.65 since 3 DAT (days after treatment), but Fv/Fm values of herbicide resistant populations show no significant reductions with ALS and ACCase treatments. With the treatment of PSII inhibitors the Fv/Fm values of susceptive and resistant plants decreased to 0.25 and 0.6 (3DAT) and further to 0.1 and 0.5 (14 DAT), respectively. The field experiments show corresponding results to the greenhouse test. With ALS and ACCase treatment, significant Fv/Fm reduction for the susceptive populations can be detected between 3-7 DAT. During the measurement of PS II inhibitor treatment, Fv/Fm values of susceptive plants decreased continuously from 0.5 to 0.2, while the value of resistant population kept stable in range 0.3-0.4.

The prototype WEED-PAM is suitable for the detection of herbicide effects based on Fv/Fm values and takes less time than visual assessment. This research shows the potential usage of fluorescence meter in herbicide resistance detection.

Keywords: Chlorophyll fluorescence, herbicide stress, resistance management, sensor detection, weed

Zusammenfassung

Herbizidresistenz ist zu einem weit verbreiteten Problem weltweit geworden. Die Detektion von Resistenzen ist in der Regel kompliziert und kostenintensiv. Eine sensorbasierte Überwachung von Herbizidresistenz würde deren Bestimmung viel schneller und günstiger machen. In dieser Arbeit wurden Feld- und Gewächshausexperimente mit sensitiven und resistenten Populationen von *Alopecurus myosuroides* durchgeführt. Hierbei wurde die Chlorophyllfluoreszenz der Pflanzen gemessen um die Wirkung von Herbizid-Behandlungen zu untersuchen. Die maximale Quantenausbeute des Photosystem II (Fv/Fm) von *Alopecurus myosuroides* wurde nach der Herbizid-Behandlung für zwei Wochen mit dem Chlorophyll-Fluoreszenz-Sensor WEEDPAM gemessen.

Die unbehandelten Kontrollpflanzen zeigten stabile Fv/Fm Werte von rund 0,7. In den Gewächshausversuchen mit ALS und ACCase Hemmern sanken die Fv/Fm Werte der behandelten sensitiven Pflanzen 3 TnA (Tage nach Applikation) signifikant von 0.7 auf 0.6-0.65 ab. Die resistenten Pflanzen hingegen zeigten keine signifikanten Abnahmen. Die Fv/Fm-Werte sanken in der Behandlung mit PS II-Inhibitoren innerhalb von 3-4 Tagen in der sensitiven Population auf 0.25 und in den resistenten Pflanzen auf 0.6, 14 TnA zeigten sich für sensitive Pflanzen noch niedrigere Werte von 0.1, wohingegen resistente Pflanzen Werte von 0.5 aufwiesen. Die Feldversuche zeigten ähnliche Ergebnisse wie die Gewächshausversuche. In den ALS- und ACCase Behandlungen wurden für die sensitiven Populationen signifikante Fv/Fm Reduktionen bereits nach 3-7 TnA festgestellt. In den Behandlungen mit PS II Inhibitoren fielen die Fv/Fm Werte der sensitiven Pflanzen kontinuerlich von 0.5 auf 0.2 ab (innerhalb von 14 Tagen), während die resistenten Pflanzen stabil auf einem Wert von 0.3-0.4 blieben. Der WEED-PAM Prototyp eignet sich für die sensorbasierte Stress-Detektion von Herbizideffekten mittels der Fv/Fm Werte. Weiterhin ist diese Methode sehr viel schneller als die visuelle Beurteilung der Herbizidsymptome. Diese Untersuchung zeigt den potentiellen Nutzen von Fluoreszenzmessgeräten für die Erfassung von Herbizidresistenzen.

Stichwörter: Chlorophyll-Fluoreszenz, Herbizid-Stress, Resistenzmanagement, Sensorerkennung, Unkraut

Introduction

Weeds are an important factor in agriculture. Weeds affects crop yield. Generally 13% yield losses due to the weed competition against the cereals are reported (OERKE et al., 1995**)**. What is worse, because continuous application of herbicides in same mode of actions, herbicide resistance has been discovered in 239 weed species (139 dicotyledons and 100 monocotyledons) during the past decades (HEAP, 2015). This made weed management strategies more complicated in recent years (MOSS ET AL., 2007).

In Europe, *Alopecurus myosuroides* (blackgrass) is one of the most widespread herbicide resistant grassweed. The occurrence has been reported in ten countries (MOSS, 2004). Both target site resistance (TSR) and non-target site resistance (NTSR) mechanisms have taken place to survive resistant *Alopecurus myosuroides* populations from herbicide treatments with different modes of action (DÉLYE et al., 2011). On worse aspect, cross-resistant and multiple resistant populations appeared after the over usage of herbicides mixture (DE PRADO et al., 2004). It results in greater economic losses for farmers, considering the additional input and lower yields (ORSON, 1999; GERHARDS, 2009).

In order to establish the control strategies for resistant weeds, diagnostic approaches of the specie characters should be constructed. Up to date, there are four methods, including greenhouse bioassays, biochemical assays, molecular assays and analytical assays, being generally used for the weed resistance diagnostic by weed researchers (BEFFA, 2012). However, the mentioned diagnostics are laboratory or greenhouse based. That request long period of time, high financial support, specific laboratory instruments and equipment, expertise knowledge and special experimental skills (KAISER et al., 2013; BURGOS et al., 2013).

In 1984, induction of chlorophyll fluorescence was observed on barley leaves when the carbon assimilation was affected (QUICK and HORTON, 1984). It starts an era for stress detection on photosynthesis with chlorophyll fluorescence measurement. In weed science, herbicide stress on PS II was used for the dose optimization by measuring photosynthetic efficiency (KEMPENAAR et al., 2010). Also in laboratory and greenhouse studies, the chlorophyll fluorescence was used to quantify the agar based Syngenta 'RISQ' test and detected the herbicide resistance in *Alopecurus myosuroides* depending on the dose response analysis (KAUNDUN et al., 2011; KAISER et al., 2013; WANG et al., 2015). There is few publications focus on the approaches for real-time herbicide resistance detection yet.

The objectives of this study is to develop a portable sensor which would be capable to use in the field for measuring chlorophyll fluorescence quantum yield of PSII (Fv/Fm); and secondly, work up a sensor based assessment to find out the herbicide stress shortly after application, which corresponds to the bioassay of the plants surviving herbicide treatments. The results would contribute to the strategy decision for farmers and researchers to control herbicide resistant weeds in the future.

The hypothesis of this paper is that the stress from inhibitors of ALS (branched chain amino acid synthesis) and ACCase (lipid synthesis) interfere the electron transport systems of plants including the steps in photosynthesis systems (RIETHMUELLER-HAAGE et al., 2006a, b). Furthermore, different responses of chlorophyll fluorescence intensity correspond to the herbicide mode of action and the duration after treatments (OORSCHOT and LEEUWEN, 1992).

Materials and Methods

Experiment design and seed origin for greenhouse tests

Seeds of one multiple resistant population and one susceptive population were sowed separately in the greenhouse. The multiple resistant seeds were collected from greenhouse cultivated

Alopecurus myosuroides that had been proved to be strongly resistant to herbicides with modes of action in ALS, ACCase and PS II due to the greenhouse bioassay (population from Heilbronn, Baden-Wuerttemberg, Germany) (GERHARDS, 2013). The seeds of susceptive population were bought from Herbiseed (Twyford, UK). The seeds were sown in 15 × 20 cm pots filled with vermiculite for germination. The plants were transplanted into 8 × 8cm paper pots (Jiffy, 4 plants per pot) when the first true leaf emerged from coleoptile (BBCH 10). The transferred plants were separated into eight groups. Three replicate of pots were setup for each subgroup. The plants were treated with herbicides at the growth stages of BBCH 22-23. The herbicide treatment was listed in Table 1. Herbicide spray was conducted with a spray chamber according to the recommended dose of each herbicide. The spray volume was calibrated to 200 L/ha. The pots were placed in greenhouse with completely randomized design.

Tab. 1 Treatment herbicides and dose rates for greenhouse test. MoA: Mode of Action.

Tab. 1 *Herbizidbehandlungen und Aufwandmengen im Gewächshausversuch. MoA: Wirkungsweise.*

No.	Treatments	MoA	Water [L/ha]	Herbicide [g/ha or L/ha]	Additive [L/ha]
1	control	-	200L	-	-
2	mesosulfuron-methyl + iodosulfuron-methyl-natrium	ALS	200L	500g	1L
3	propoxycarbazone-Na	ALS	200L	100g	-
4	pyroxsulam + florasulam	ALS	200L	220g	1L
5	pinoxaden	ACCase	200L	1.2L	-
6	pinoxaden + clodinafop-propargyl	ACCase	200L	1.2L	-
7	isoproturon	PS II	200L	3L	-

Experiment design and seed origin for field test

Four field experiments with randomized complete block design were conducted in three sites in south-western Germany, Wurmberg in April 2014 with resistant *Alopecurus myosuroides* populations, Renningen in April 2014 (Research station Ihinger Hof of the University of Hohenheim) and Hohenheim in December 2014 and March 2015 (Research station Goldener Acker of the University of Hohenheim) sown with susceptive *Alopecurus myosuroides* population. The herbicide resistant population at site Wurmberg was screened by GERHARDS (2014). It shew high tolerance to isoproturon. The susceptive population was ordered from the company HerbiSeed, Twyford, UK. Four blocks were set in each experiment. Each plot is set with the width of 2m and length of 5m. Three herbicides were applied at site Wurmberg, two herbicides were used at site Renningen, two herbicides were used at site Hohenheim in 2014 and five herbicides were used at site Hohenheim in 2015. Variant of control plot was reserved in each block. The herbicide treatments are listed in Table 2.

Herbicides in groups of ALS, ACCase and PS II inhibitors were involved in this research, including Attribut®, propoxycarbazone-Na, 700 g a.i. kg^{-1} (Bayer CropScience) (a.i., active ingredients); Atlantis® WG, mesosulfuron-methyl + iodosulfuron-methyl-natrium + mefenpyr-diethyl, 30 g a.i. kg^{-1} + 6 g a.i. kg^{-1} + 90 g a.i. kg^{-1} (safener) (Bayer CropScience); Broadway®, pyroxsulam + florasulam + cloquintocet - mexyl (safener), 68.3 g a.i. kg^{-1} + 22.8 g a.i. kg^{-1} + 68.3 g a.i. kg^{-1} (Dow AgroSciences); Topik® 100, Clodinafop, 89.1 g a.i. L^{-1} (Syngenta Agro); Arelon® Top, isoproturon, 500 g a.i. L^{-1} (Cheminova Deutschland GmbH); Axial® 50, pinoxaden + cloquintocet-mexyl (safener), 50 g a.i. L^{-1} + 12 g a.i. L^{-1} (Syngenta Agro); Atlantis adjunct, fatty alcohol ether sulphate, 27% (Bayer CropScience); Broadway adjunct, methyl esters in rapeseed oil (Dow AgroSciences)

Tab. 2 Treatment herbicides and dose rates for field test. MoA: Mode of Action.

***Tab. 2** Herbizidbehandlungen und Aufwandmengen in Feldversuchen. MoA: Wirkungsweise.*

Location	No.	Treatments	MoA	Water [L/ha]	Herbicide [g/ha or L/ha]	Additive [L/ha]
Wurmberg	1	control	-	200	-	-
	2	isoproturon	PS II	200	3 L	-
	3	mesosulfuron-methyl + iodosulfuron-methyl-natrium	ALS	200	500 g	1
	4	pyroxsulam + florasulam	ALS	200	220 g	1
Renningen	1	control	-	200	-	-
	2	clodinafop	ACCase	200	0.6 L	-
	3	pyroxsulam + florasulam	ALS	200	220 g	1
Hohenheim (Autumn 2014)	1	control	-	200	-	-
	2	pinoxaden	ACCase	200	1.2 L	-
	3	isoproturon	PS II	200	3 L	-
Hohenheim (Spring 2015)	1	control	-	200	-	-
	2	propoxycarbazone-Na	ALS	200	100 g	-
	3	mesosulfuron-methyl + iodosulfuron-methyl-natrium	ALS	200	500 g	1
	4	pyroxsulam + florasulam	ALS	200	220 g	1
	5	clodinafop	ACCase	200	0.6 L	-
	6	pinoxaden	ACCase	200	1.2 L	-

Chlorophyll fluorescence and WEED-PAM

Photosynthesis is driven by excited chlorophyll molecule and gains the energy when it dissipates the absorbed light. The rest energy is released either as heat in non-photochemical quenching or as fluorescence. When the chlorophyll molecules transition to non-excited state from excited state, energy is emitted as light, called chlorophyll fluorescence. It indicates the photosynthetic energy conversion.

The sensor WEED-PAM was developed from the MINI IMAGING-PAM® fluorescence sensor of the Heinz Walz GmbH, Effeltrich, Germany. The sensor is equipped with LEDs for generating blue light at 460 nm. These LEDs emit alternately measuring flashes with constant amplitude and strong pulses to light-saturate photosystem II photochemistry. Chlorophyll fluorescence is released from the plant after applying the measuring light. It can be detected by a camera that is mounted on the sensor head. The measured signal corresponds to the fluorescence yield variation during the light application. This fluorescence-meter is operated by the software "ImagingWin for WEED-PAM" (Heinz Walz GmbH). It controls the function of LED lights and generates pictures for the fluorescence response of the measured plants. By recording the value of Fo, minimum fluorescence in dark acclimated state, and Fm, maximum fluorescence in dark acclimated state in the presence of a saturation measuring pulse, the software calculates and the parameter Fv/Fm = (Fm - Fo) / Fm and generates the images describe the Fv/Fm value for plant leaf inside the measured area.

Measurement and data analysis

The Fv/Fm values of the weeds were measured after the herbicide treatment. The plants were dark acclimated with plastic covers for 30 ± 5 minutes before the measurement. The measurements were done 3, 7 and 14 DAT (days after treatment) on the plants in greenhouse and at sites Wurmberg and Renningen. For each treatment, measurements were done for three replicates in greenhouse and eight replicates in the two field sites. At site Hohenheim, measurements were done 1, 2, 3, 4, 5, 6, 7, 10 and 14 DAT and 40 replicates were done for each variant. Fv/Fm values were measured of the same plants on each measuring date. Visual assessment for the herbicide efficacy on *Alopecurus myosuroides* was taken on 21 DAT due to the method that was described by HESS et al. (2012).

Data analysis was done with statistic software R. Analysis of variance (ANOVA) and Tukey's HSD test were done to identify the herbicide effect on Fv/Fm. Package *multcomp* and ggplot2 were used (R DEVELOPMENT CORE TEAM, 2008).

Results

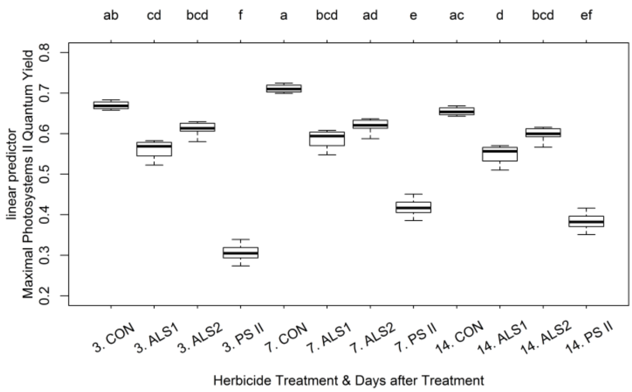
According to Table 3 - Treatment 1, values of control plants remain around 0.73 in low fluctuation during the 14 days after treatment. The analysis results show that Fv/Fm value of susceptive plants would reduce significantly since 3 DAT. Meanwhile, there is none significant decrease for the Fv/Fm value of resistant population except the treatment of isoproturon.

Tab. 3 Average value of Fv/Fm in greenhouse test and the Tukey's HSD test results of treated and untreated plants. Significant codes due to p values: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. The treatment numbers represent the herbicide treatments described in Table 1.

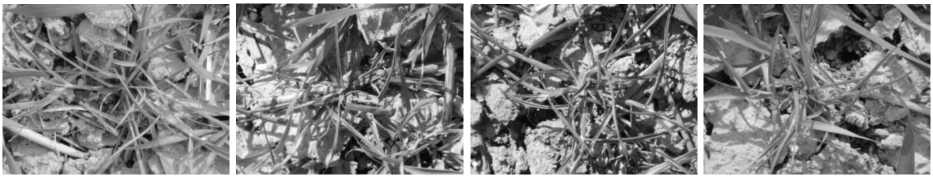
Tab. 3** Mittelwerte der Fv/Fm Werte im Gewächshausversuch sowie die Ergebnisse des paarweisen Tukey Tests für unbehandelte und behandelte Pflanzen. Kodierung der Signifikanzen anhand der p-Werte: 0 '' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. Die Behandlungszahlen beziehen sich auf die genannten Behandlungen in Tabelle 1.*

Population	DAT	Treatments						
		1	2	3	4	5	6	7
Susceptive	3	0.744	0.694*	0.712	0.703*	0.632**	0.699***	0.254***
	7	0.746	0.657***	0.662*	0.659***	0.532***	0.709***	0.207***
	14	0.720	0.693**	0.678***	0.672***	0.478***	0.645***	0.137***
Resistant	3	0.731	0.704	0.735	0.708	0.722	0.701	0.616***
	7	0.729	0.729	0.722	0.725	0.728	0.716	0.551***
	14	0.734	0.737	0.720	0.717	0.732	0.724	0.511***

Figure 1a presents the result of the experiment at site Wurmberg (Baden-Wuerttemberg, Germany). According to the figures and the data analysis of ANOVA, the Fv/Fm value of the herbicide treated plants reduced significantly since 3 DAT comparing with the control group. This phenomenon shows good efficacy of the ALS herbicide mesosulfuron-methyl plus iodosulfuron-methyl-natrium and the pyroxsulam plus florasulam. By treatment of PS II inhibitor isoproturon, the Fv/Fm value reduced significantly since 3 DAT and even lower than the other groups treated with ALS herbicides. Figure 1b shows the efficacy of each herbicide on *Alopecurus myosuroides* at 21 DAT. The plants by ALS treatments were dead and the plants with PSII treatment survived.



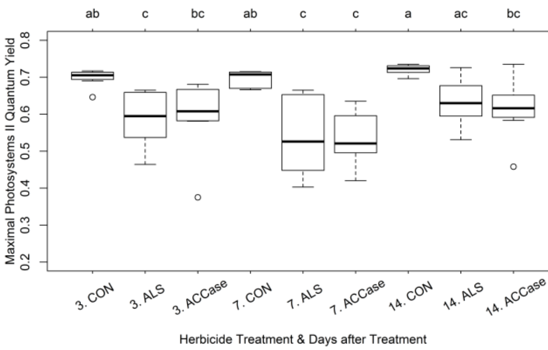
(a)



(b)

Fig. 1 Maximum PS II quantum yield for weeds after herbicide treatments at site Wurmberg. (a) Maximum PS II quantum yield; (b) Efficacy of herbicides (left to right: CON-ALS1-ALS2-PSII). Abbreviation: CON, control groups; ALS1, mesosulfuron-methyl + iodosulfuron-methyl-natrium; ALS2, pyroxsulam plus florasulam; PS II, isoproturon.

Abb. 1 *Maximale Quantenausbeute des PSII Systems für Ungräser nach den Herbizidbehandlungen am Standort Wurmberg. (a) Maximale PS II Quantenausbeute..(b) Wirksamkeit von Herbiziden (links nach rechts: CON-ALS1-ALS2-PSII). Abkürzung: CON, Kontrollgruppe; ALS1, Mesosulfuron-methyl + Iodosulfuron-methyl-natrium; ALS2, Pyroxsulam plus Florasulam; PS II, Isoproturon.*

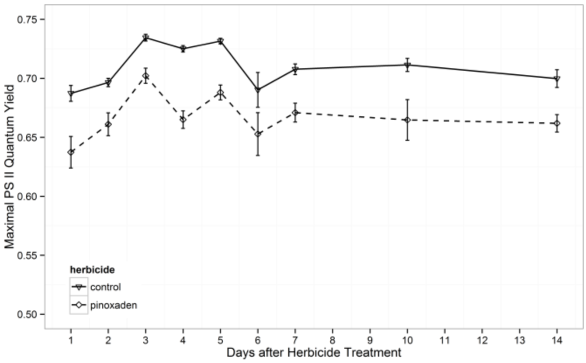


(a)

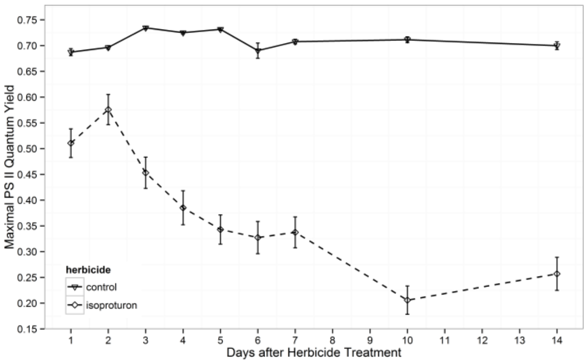
(b)

Fig. 2 Maximum PS II quantum yield for weeds after herbicide treatments at site Ihinger Hof. (a) Maximum PS II quantum yield; (b) Efficacy of herbicides (left to right: CON-ALS-ACCase). Abbreviation: CON, control; ALS, pyroxsulam plus florasulam; ACCase, clodinafop.

Abb. 2 *Maximale Quantenausbeute des PSII Systems für Ungräser nach den Herbizidbehandlungen am Standort Wurmberg. (a) Maximale PS II Quantenausbeute..(b) Wirksamkeit von Herbiziden (links nach rechts: CON-ALS-ACCase) …Abkürzung: CON, Kontrollgruppe; ALS, Pyroxsulam plus Florasulam; ACCase, Clodinafop.*



(a)



(b)

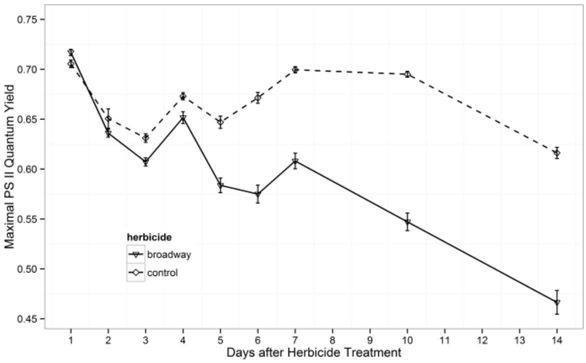
Fig. 3 Maximum PS II quantum yield for weeds after herbicide treatments at site Hohenheim of autumn application in December 2014. (a) Treatment of pinoxaden, (b) Treatment of isoproturon.

Abb. 3 *Maximale PS II Quantenausbeute für Ungräser nach der Herbstbehandlung mit Herbiziden im Dezember 2014 am Standort Hohenheim. (a) Behandlung mit Pinoxaden, (b) Behandlung mit Isoproturon.*

At site Ihinger Hof (Renningen, Baden-Wuerttemberg, Germany), Fv/Fm value for both of the ALS and ACCase herbicide treated plants decreased significantly since 3 to 7 DAT comparing with the control group. As is shown in Figure 2b the treated plants were proved to be dead as they were checked 21 DAT.

After the autumn treatment, significant reduction of Fv/Fm occurred since 1 DAT in the pinoxaden applied group. The value kept around 0.1 lower than the Fv/Fm value of the control group. The Fv/Fm value of isoproturon treated plants kept reducing until 10 DAT. All the plants with herbicide treatments were dead at 21 DAT.

After the spring application, the Fv/Fm value of herbicide treated plants got lower than the control group on 2 DAT. The significant difference occurred since 5 DAT for all the herbicides. By the visual assessment on 21 DAT, all the plants with herbicide treatments were proved to be dead.


0.75
0.70
0.65
0.60
0.55
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Maximal PS II Quantum Yield
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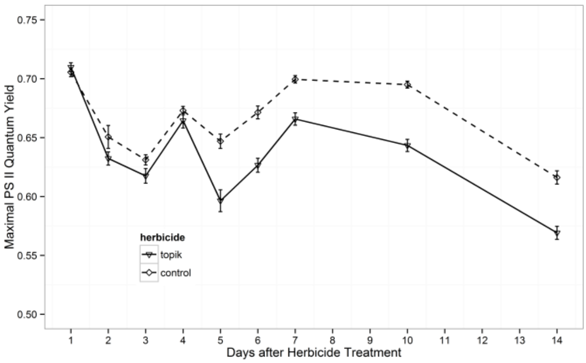
Days after Herbicide Treatment

(a)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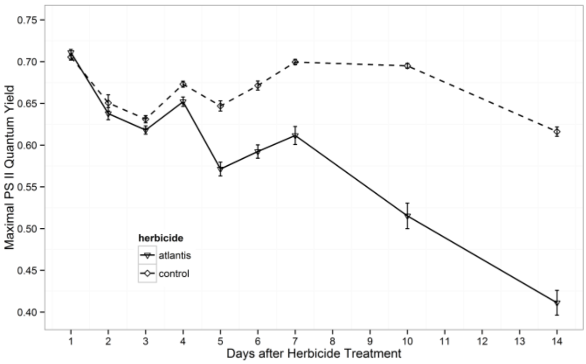
Days after Herbicide Treatment

(b)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Maximal PS II Quantum Yield
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control
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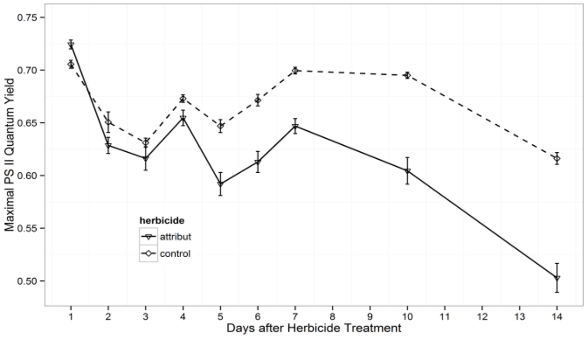
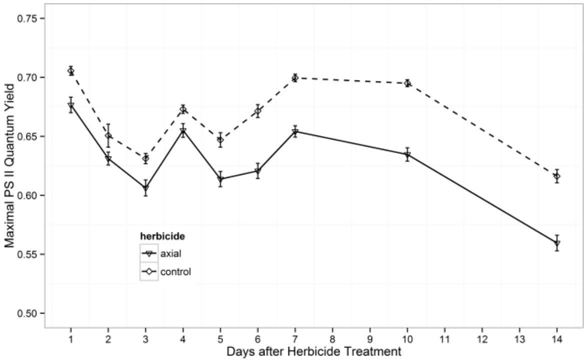
Days after Herbicide Treatment

(c)



(d)



(e)

Fig. 4 Maximum PS II quantum yield for weeds after herbicide treatments at site Hohenheim of spring application in March 2015. (a) Treatment of pyroxsulam plus florasulam, (b) Treatment of clodinafop, (c) Treatment of mesosulfuron-methyl plus iodosulfuron-methyl-natrium, (d) Treatment of propoxycarbazone-Na, (e) Treatment of pinoxaden.

Abb. 4 *Maximale PS II Quantenausbeute für Ungräser nach der Frühjahrsbehandlung mit Herbiziden im März 2015 am Standort Hohenheim (a) Behandlung mit Pyroxsulam plus Florasulam, (b) Behandlung mit Clodinafop, (c) Behandlung mit Mesosulfuron-methyl plus Iodosulfuron-methyl-natrium, (d) Behandlung mit Propoxycarbazone-Na, (e) Behandlung mit Pinoxaden.*

Discussion

All the herbicides involved in this research, as either PS II inhibitor or in other modes of action such as ALS and ACCase inhibitors, stressed the process of photosynthesis system II of susceptive population of *Alopecurus myosuroides* and decreased Fv/Fm value of the plants. The herbicide resistant plants exhibit none or less Fv/Fm reduction than the susceptive plants by the treatment respectively. Meanwhile, the study presented different performance of the herbicide modes on maximum PSII quantum yield.

According to the greenhouse test with ALS and ACCase inhibitors, the resistant plants show none significant lower Fv/Fm values than the control group. But the Fv/Fm values of susceptive plants reduced significantly on 3 DAT. In the field experiments at site Hohenheim, after the treatment of different ALS herbicides and ACCase herbicide clodinafop, the Fv/Fm values of the treated susceptive plants get lower than the untreated plants. But the difference of Fv/Fm values between control groups and treated groups is not significant during 1 to 2 DAT. Since 3 or 4 DAT, the Fv/Fm value of the treated susceptive plants decreased significantly, while the values of resistant and untreated plants still shew none significant difference. This means that the effect of ALS and ACCase inhibitors on PSII system of susceptive plants can be detected since 3 or 4 DAT.

Figure 2a presents the measurement results of site Renningen. This population was screened as susceptive to clodinafop and pyroxsulam plus florasulam (GERHARDS, 2014). The reduction of Fv/Fm values on 3 and 7 DAT corresponds to the other experiments on relative susceptive populations. In the Renningen experiment, increase of Fv/Fm values at 14th DAT in all groups can be found. That was influenced by the PS II fluorescence of moss. The *Alopecurus myosuroides* plants were too weak to process photosynthesis on 14 DAT. In that case, the measuring light on the plants was reflected and few photochemical quenching was released from the measured plant leaves. Then the chlorophyll fluorescence of the weed plants could no longer be detected. The sensor detected the PS II fluorescence emitted by moss instead of *Alopecurus myosuroides* plants.

According to Figure 3a of autumn treatment and Figure 4e of spring treatment, after the application of pinoxaden, an ACCase herbicide, the Fv/Fm values of *Alopecurus myosuroides* plants at site Hohenheim decreased and got significantly lower than the Fv/Fm values of control group since 1 DAT. The expanding of value difference between treated plants and the control group is not significant during the two weeks measuring duration. It means that the ingredient pinoxaden had affected PS II system of susceptive plants since 1 DAT (OETTMEIER, 2014).

The greenhouse experiment shows that Fv/Fm values of both susceptive and resistant plants reduced significantly after treatment of isoproturon. The average value of resistant group is higher than the value of susceptive group. Meanwhile, autumn experiment shows the same effect of isoproturon on the susceptive plants at site Hohenheim. The value difference between treated plants and control group keeps expanding during 1 to 14 DAT. For the PSII inhibitor resistant population at site Wurmberg, the Fv/Fm value difference between treated and untreated groups shrunk on 14 DAT. It indicates the plants recovered from PSII inhibitor stress (LEITSCH, 1994). This phenomenon corresponds the visual assessment result on 21 DAT at site Wurmberg that all the plants under PSII treatment survived. Therefore, the population with PS II inhibitor resistant can only be detect with the comparison with a standard Fv/Fm value of the susceptive plants.

In the greenhouse experiment, the Fv/Fm values of the control plants as both of susceptive and resistant populations are generally stable in the range 0.72 to 0.74. However in the field experiments, the Fv/Fm values of control groups exhibited a wider fluctuation range between 0.65 and 0.73. Furthermore, Fv/Fm values of herbicide treated plants present the fluctuation in similar trends with the value of relative control groups. This points out the research forward in the future, that environmental factors such as weather indexes or soil conditions should also be considered for influence on the activity of PSII unit (MAXWELL and JOHNSON, 2000). More statistical methods such as principal component analysis, support vector machine or neural networks may be applied for further model foundation of the resistance classification (WEIS and SOEKEFELD, 2009).

Conclusion

With the WEED-PAM sensor, it is able to detect herbicide resistance shortly after application. The duration takes less time than visual assessment, as visual test takes more than three weeks for *Alopecurus myosuroides* to display the typical herbicide stress symptoms. In the future, this sensor could be practically used in agricultural fields after the calibration of Fv/Fm values for different herbicides and environmental factors. By quick herbicide resistance test, this prototype sensor will save the precious time and financial input on herbicide resistance discovery and management for farmers and agricultural researchers.

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