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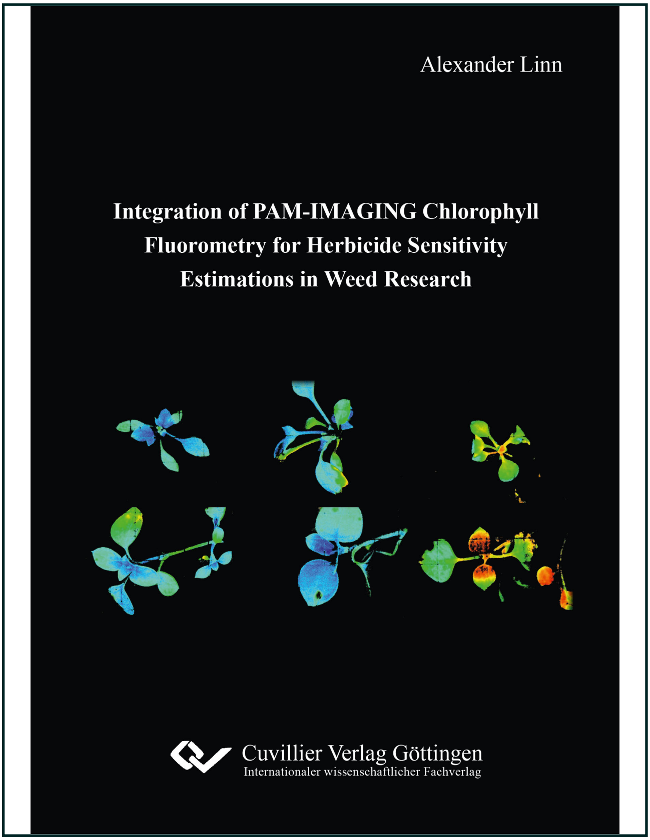
# Integration of PAM-IMAGING Chlorophyll Fluorometry for Herbicide Sensitivity Estimations in Weed Research

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# Summary

Since the introduction of synthetic herbicides, almost all mechanical weed control measures have been replaced by chemical control practices. Inevitably, the repeated weed control with herbicides, especially with the same mode of action, combined with the reduction of alternative indirect and direct weed control methods boosted the development of herbicide-resistant weeds. Fast in-field herbicide resistance detection is a key requirement for future herbicide resistance management, yet secure and rapid herbicide resistance tests are still missing. Chlorophyll fluorescence and chlorophyll fluorescence ratios can be very sensitive to changes in plant health status. Recent studies have demonstrated the potential of chlorophyll fluorescence measurements to evaluate the herbicide impact on crops and weeds. The fluorescence ratio $F_v/F_m$ quantifies the maximum quantum efficiency of photosystem II ($F_v/F_m$) and indicates reliably plant health status. Hitherto, research on herbicide resistance detection via the $F_v/F_m$ value have concentrated on *Alopecurus myosuroides* Huds. Investigations concerning herbicide resistance detection in other important weed species, both monocotyledonous as well as dicotyledonous, have not been carried out to a large extent. Therefore, more research is needed to investigate whether chlorophyll fluorescence, in particular $F_v/F_m$ measurements, can be similarly useful in other agricultural crops and weeds. This work addresses the apparent gap in knowledge by four lines of research:

- Setup of a laboratory chlorophyll fluorescence agar test for the detection of herbicide resistance in *Apera spica-venti* L. Beauv;
- Development of a field-portable pulse amplitude modulated (PAM)-imaging chlorophyll fluorometer to determine plant stress in weeds and crops *in situ*;
- Field studies analyzing the $F_v/F_m$ value for herbicide resistance detection in *Stellaria media* L. Vill and *Papaver rhoeas* L.;
- Design and testing of classifiers, in order to automatically assign individual herbicide-treated plants to the classes “resistant” or “susceptible” on the basis of their determined $F_v/F_m$ value.

The outcome of the research has been published in three scientific papers:

1st paper: The development, application possibilities and limitations of a portable PAM-imaging chlorophyll fluorometer were reviewed. By measuring the fluorescence ratio $F_v/F_m$ the device is capable to detect plant stress by herbicides, not only interacting directly with the photosystem II but also induced by herbicides acting on distant biological pathways. This stress can be determined within 1-5 days after treatment (DAT) before any damage becomes visible. Due to the compact design, the device is optimised to estimate herbicide sensitivity in crops and weeds in greenhouse and field studies. The device can be used for the detection of herbicide-resistant weed populations and single resistant specimens in combination with decision protocols. Furthermore, the herbicide sensitivity of crops can be estimated unbiased prior to visual assessments. It is concluded that the PAM-imaging sensor is suitable as an expert tool for decision support in integrated weed management.

2nd paper: In order to validate the possibility of accelerating screenings by a $F_v/F_m$ survey, an agar test on herbicide resistance in *A. spica-venti* was investigated. Sensitive and resistant *A. spica-venti* plants were cultivated in herbicide treated agar. The plant stress was quantified by the $F_v/F_m$ value with the PAM-imaging chlorophyll fluorometer. Though plant stress increased with increasing herbicide concentrations in all experiments, the results of the agar test on herbicide sensitivity were highly variable and did not represent results from standard greenhouse pot trials. It is assumed that the variability in the results is due to the increased herbicide concentrations used for inducing the rapid plant response.

3rd paper: The sensor capabilities for herbicide resistance detection in dicotyledonous weeds have been investigated in the field. Herbicide-resistant and sensitive *S. media* and *P. rhoeas* plants were treated with acetolactate-synthase inhibitors. The plant stress was quantified by the determination of the $F_v/F_m$ value with the portable PAM-imaging chlorophyll fluorometer. Discriminant maximum likelihood classifiers were created and tested with independent data sets, assigning single plants based on the measured $F_v/F_m$ values to the classes "resistant" or "susceptible". The $F_v/F_m$ values of sensitive *P. rhoeas* and *S. media* plants decreased within 3 DAT by 28–43%. The $F_v/F_m$ values of the resistant plants were 20% higher than those of the sensitive plants in all herbicide treatments. The classifiers separated sensitive and resistant plants within 3 DAT with accuracies of 62% to 100%. Therefore, an estimation of herbicide resistance can be performed within 3 days after herbicide treatment.

In summary, these papers demonstrate that a mobile PAM-imaging chlorophyll fluorometer can perform fast estimations of herbicide stress in laboratory and field conditions. By employing this PAM device, it is shown that the detection of herbicide action by $F_v/F_m$ also works for dicotyledonous species. Moreover, it was possible to identify individual herbicide-resistant plants based on its value of $F_v/F_m$. Hence, PAM fluorometry can play a significant role in a decision support system in integrated weed management.

# Zusammenfassung

Synthetische Herbizide haben seit deren Einführung fast alle mechanischen Unkrautbekämpfungsmaßnahmen verdrängt. Jedoch fördert der wiederholte Einsatz von Herbiziden - oftmals mit gleicher Wirkungsweise -, kombiniert mit einer Reduzierung indirekter und anderer direkter Bekämpfungsmaßnahmen, zwangsläufig die Selektion herbizidresistenter Unkrautpopulationen. Eine frühe Erkennung dieser resistenten Unkrautpopulationen ist ein zentraler Baustein des Resistenzmanagements. Eine Möglichkeit Veränderungen im Gesundheitszustand von Pflanzen schnell zu erkennen, bieten Chlorophyllfluoreszenzmessungen, da die Chlorophyllfluoreszenz sowie Chlorophyllfluoreszenzquotienten sehr empfindlich auf diese Veränderungen reagieren können. Jüngere Studien zeigten bereits das Potential von Chlorophyllfluoreszenzmessungen zur Bewertung, der durch Herbizide induzierten Veränderungen in Kulturpflanzen und Unkräutern. Der Fluoreszenzquotient $F_v/F_m$ quantifiziert die maximale Quantenausbeute des Photosystems II ($F_v/F_m$) und ermittelt somit zuverlässig Pflanzenstress. Bisher konzentrierte sich die Forschung hauptsächlich auf *Alopecurus myosuroides* Huds., um herbizidresistente Unkräuter anhand des $F_v/F_m$-Wertes nachzuweisen. Umfangreiche Studien zur Detektion weiterer monokotyler und dikotyler herbizidresistenter Unkrautpopulationen unter Verwendung des $F_v/F_m$-Wertes sind bislang rar. Um zu evaluieren, ob sich der $F_v/F_m$ Wert eignet Herbizidresistenz in weiteren Kulturpflanzen und Unkräutern zu erkennen, wurden in dieser Arbeit folgende Forschungsschwerpunkte bearbeitet:

- Entwicklung eines chlorophyllfluoreszenzbasierenden Agartests im Labor zum Nachweis der Herbizidresistenz in *Apera spica-venti* L. Beauv.;
- Entwicklung eines tragbaren, bildgebenden Puls-Amplituden-Modulations (PAM)-Chlorophyllfluorometers zur Erkennung des Pflanzenstresses in Unkräutern und Kulturpflanzen *in situ*;
- Feldstudien zur Bewertung des $F_v/F_m$-Wertes zum Nachweis der Herbizidresistenz in *Stellaria media* L. Vill. und *Papaver rhoeas* L.;
- Erstellung und Prüfung von Klassifikatoren, um einzelne herbizidbehandelte Pflanzen anhand ihres $F_v/F_m$-Wertes automatisch den Klassen „resistent“ oder „sensitiv“ zuzuordnen.

Die Ergebnisse wurden in drei wissenschaftlichen Veröffentlichungen zusammengefasst und diskutiert:

1. Artikel: Es wurde die Eignung eines tragbaren PAM-Chlorophyllfluorometers zur Ermittlung von herbizidinduziertem Pflanzenstress, dessen Anwendungsbereiche und systembedingte Limitierungen untersucht. Aufgrund der kompakten Bauweise des PAM-Fluorometers eignet es sich hervorragend für Studien zur Quantifizierung der Herbizidempfindlichkeit von Pflanzen im Gewächshaus und Feld. Durch die Messung des Chlorophyllfluoreszenzquotienten $F_v/F_m$ ist das tragbare, bildgebende PAM-Fluorometer in der Lage herbizidinduzierten Pflanzenstress zu identifizieren. Dies gilt nicht nur für Herbizide, die direkt mit dem Photosystem II interagieren, sondern auch für Herbizide, deren Wirkort außerhalb des Photosystems II liegt. Dieser herbizidinduzierte Pflanzenstress kann innerhalb von 1-5 Tagen nach der Herbizidbehandlung (DAT) ermittelt werden. Zu diesem Zeitpunkt sind visuell noch keine Schäden an der Pflanze erkennbar. In Kombination mit Entscheidungsprotokollen kann dieses PAM-Fluorometer sowohl zum Nachweis herbizidresistenter Unkrautpopulationen als auch einzelner resistenter Pflanzen eingesetzt werden. Darüber hinaus ist eine objektive Einschätzung der Herbizidsensitivität in Kulturpflanzen noch vor einer visuellen Bonitur möglich. Zusammenfassend ist das bildgebende PAM-Chlorophyllfluorometer als Werkzeug zur Einschätzung der Herbizidsensitivität von Kulturpflanzen sowie zur Detektion von Herbizidresitenz in Unkräutern geeignet.

2. Artikel: Um die Erkennung herbizideresistenter Unkräuter mit Hilfe von $F_v/F_m$-Messungen zu beschleunigen, wurde ein Agartest zur Herbizidresistenzdetektion in *A. spica-venti* untersucht. Herbizidsensitive und -resistente *A. spica-venti* Pflanzen wurden in herbizidhaltigem Agar etabliert. Der Pflanzenstress wurde mit einem PAM-Chlorophyllfluorometer anhand des $F_v/F_m$-Wertes quantifiziert. Obwohl in allen Experimenten der Pflanzenstress mit zunehmender Herbizidkonzentration zunahm, waren die Ergebnisse des Agartests sehr variabel und spiegelten die erhobenen Ergebnisse aus herkömmlichen Resistenztests im Gewächshaus nicht wieder. Es wird davon ausgegangen, dass die Variabilität der Ergebnisse auf die im Agartest verwendeten, erhöhten Herbizidkonzentrationen zurückzuführen ist.

3. Artikel: In Feldversuchen wurde die Möglichkeit für den Nachweis herbizidresistenter dikotyler Unkräuter unter Zuhilfenahme des tragbaren PAM-Chlorophyllfluorometers untersucht. Herbizidresistente und -sensitive *S. media* und *P. rhoeas* Pflanzen wurden mit Acetolactat-Synthase-Hemmern behandelt. Der Pflanzenstress wurde anhand des $F_v/F_m$-Wertes mit dem tragbaren PAM-Chlorophyllfluorometer quantifiziert. Es wurden, auf den gemessensen $F_v/F_m$-Werten basierende, diskriminierende Maximalwahrscheinlichkeits-Klassifikatoren erstellt und mit unabhängigen Datensätzen getestet. Die Klassifikatoren

ordneten einzelne Pflanzen den Klassen „resistent" oder „sensitiv" zu. Die $F_v/F_m$-Werte von sensitiven *P. rhoeas* und *S. media* Pflanzen sanken innerhalb von 3 DAT um 28-43%. Die $F_v/F_m$-Werte der resistenten Pflanzen waren bei allen Herbizidbehandlungen 20% höher als die der sensitiven Pflanzen. Die Klassifikatoren ordneten sensitive und resistente Pflanzen innerhalb von 3 DAT mit Genauigkeiten von 62% bis 100% ein. Dies ermöglicht eine Einschätzung zur Herbizidresistenz in *S. media* und *P. rhoeas* binnen 3 DAT.

Zusammenfassend zeigen diese Arbeiten, dass die PAM-Chlorophyllfluorometrie zur Bestimmung des Pflanzengesundheitszustandes innerhalb weniger Tage nach der Herbizidapplikation im Labor sowie im Feld geeignet ist. Unter Zuhilfenahme des mobilen PAM-Fluorometers konnten herbizidresistente dikotyle Unkräuter durch Bestimmung des $F_v/F_m$-Wertes identifiziert werden. Darüber hinaus war eine automatisierte Erkennung einzelner herbizidresistenter Pflanzen anhand des $F_v/F_m$-Wertes möglich. Damit kann die PAM-Chlorophyllfluoreszenzmessung einen wertvollen Beitrag zum Resitenzmanagement leisten.

# Chapter I

# General Introduction

# 1 General Introduction

Herbicide-resistant weeds constitute one of the most serious problems in agriculture. In the current agricultural practice, almost all mechanical weed control measures have been replaced by herbicides (Heap, 2014). The strong and repeated selection pressure by herbicides forced the development of herbicide-resistant weed populations. Since the first documentation of herbicide-resistant weed plants in 1957 (Hilton, 1957; Switzer, 1957), the number of herbicide-resistant weed species and populations, and the affected herbicide modes of action increased steadily (Heap, 2019). With the rise of herbicide-resistant weed populations, herbicide resistance management became a fundamental part of crop management. An integral part of herbicide resistance management is the safe and rapid testing for herbicide resistance.

The pulse amplitude modulated (PAM)-imaging chlorophyll fluorometry is an analytical tool to study photosynthesis. Chlorophyll fluorescence parameters and ratios can be very sensitive indicators for changes in the plant health status. The maximum quantum efficiency of photosystem II ($F_v/F_m$) is a chlorophyll fluorescence ratio that represents the performance of photosystem II photochemistry (Baker, 2008). After the determination of the ground fluorescence ($F_o$) and the maximum fluorescence ($F_m$) of dark acclimated plants, the $F_v/F_m$ value can be derived ($F_v/F_m = (F_m-F_o)/F_m$). In most higher plants, the $F_v/F_m$ value ranges from 0.78 and 0.84 in healthy conditions, while lower $F_v/F_m$ values indicate plant stress (Stirbet & Govindjee, 2011). The $F_v/F_m$ value quantifies plant stress, independent of the stress origin (Rosenqvist & van Kooten, 2003). Therefore, the $F_v/F_m$ has proven to be a reliable parameter for plant stress identification.

The potential of PAM-imaging chlorophyll fluorometry for herbicide sensitivity assessments using the $F_v/F_m$ value in weeds and crops has already been demonstrated (Kaiser *et al.*, 2013; Wang *et al.*, 2016; Wang *et al.*, 2018; Weber *et al.*, 2017; Li *et al.*, 2017). By employing the PAM-imaging sensor, herbicide-induced plant stress in crops can be quantified by determining the $F_v/F_m$ value and thereby deriving statements on the crop sensitivity to the herbicides as well as variety-related herbicide sensitivities (Weber *et al.*, 2017). Likewise, the $F_v/F_m$ value can provide information on the herbicide resistance status of weed populations after herbicide application even before visible symptoms of herbicide damage occur (Kaiser *et al.*, 2013; Wang *et al.*, 2018; Wang *et al.*, 2016). Here, the research focus was on *Alopecurus myosuroides* Huds. Since the potential for the detection of herbicide-resistant *A. myosuroides* has been demonstrated, it should be investigated whether conclusions about the herbicide-resistance status can also be drawn in other weed species, monocotyledonous as well as dicotyledonous. This would significantly expand the range of applications for the PAM-imaging chlorophyll fluorometry and enhance the system's versatility. A mobile PAM-imaging chlorophyll fluorometer for field use was introduced for the use of the PAM-imaging chlorophyll fluorometry outside laboratories in field trials (Wang *et al.*, 2016). Since that the fluorometer was continuously advanced. Due to the many applications of PAM-imaging chlorophyll fluorometry with respect to the detection of herbicide-induced plant stress, a detailed description of the system, the discussion of possible areas of application, and limitations for its use in arable farming are necessary.

## 1.1 Objectives

The aims of this research work were: First, the adjustment of a mobile PAM-imaging chlorophyll fluorometer to identify the induced stress in agricultural plants by measuring the $F_v/F_m$ value. Second, to develop a modification of a pre-existing laboratory agar test for the detection of herbicide resistance in *Apera spica-venti* (L.) Beauv by the determination of the $F_v/F_m$ value after the herbicide treatment. Third, to investigate the employment of $F_v/F_m$ value for the identification of herbicide-resistant *Papaver rhoeas* L. and *Stellaria media* L. Vill populations in-field, and, further, to create an autonomous assignment of single herbicide treated *P. rhoeas* and *S. media* plants based on their $F_v/F_m$ value to the classes "susceptible" or "resistant".

## 1.2 Structure of the Dissertation

The dissertation is presented in a cumulative thesis of three articles. At the current state, one article is submitted and under review in a peer-reviewed journal. The other two articles are published in peer-reviewed journals. The publications are presented in a unified format and reference style.

The first article entitled "Development and applications of a field imaging chlorophyll fluorometer to measure stress in agricultural plants" is submitted to the Journal "Precision Agriculture". This review describes a mobile PAM-imaging chlorophyll fluorometer, including the special physical properties of the sensor and gives examples of applications.

The second article entitled "Detecting herbicide-resistant *Apera spica-venti* with a chlorophyll fluorescence agar test" is published in the Journal "Plant Soil and Environment". It describes the approach of adopting a chlorophyll fluorescence agar test for herbicide resistance detection in *A. spica-venti*.

The third article entitled "In-field classification of herbicide-resistant *Papaver rhoeas* and *Stellaria media* using an imaging sensor of the maximum quantum efficiency of photosystem II" is published in the Journal "Weed Research". It describes the introduction of PAM-imaging chlorophyll fluorometry for the detection of herbicide-resistant *S. media* and *P. rhoeas* in the field. In addition, a classifier is presented that assigns single plants to the classes "susceptible" and "resistant" based on their $F_v/F_m$ value after herbicide treatment.

Besides the presented publications in this thesis, three contributions to international conferences were accepted:

- A. Menegat, B. Sievernich, A. Linn, R. Mink & R. Gerhards (2016): Development of a standardised test system for detection of resistance against per-emergence herbicides. *Proceedings 7th International Weed Science Congress*. "Weed science and management to feed the planet", Prague, Czech Republic. ISBN 978-80-213-2648-4
- A. Linn, R. Mink, G. Peteinatos & R. Gerhards (2018): Herbicide efficacy estimation of ALS-inhibitors in *Stellaria media* L. and *Papaver rhoeas* L. *Proceedings 18th European Weed Science Society Symposium*. "New approaches for smarter weed management", Ljubljana, Slovenia. ISBN 978-961-6998-21-5
- P. Kosnarova, K. Hamouzova, A. Linn, P. Hamouz & J. Soukup (2018): *Apera spica-venti* biotype from the Czech Republic resistant to three herbicide modes of action. *Proceedings 18th European Weed Science Society Symposium*. "New approaches for smarter weed management", Ljubljana, Slovenia. ISBN 978-961-6998-21-5

# Chapter II

# Publications