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Studies on the efficacy, composition and mode of action of an ethoxylated soybean oil adjuvant for herbicides

Thesis submitted in fulfilment of the requirements of the degree
Doctor sc. agr. of the Faculty of Agricultural Sciences, University of
Hohenheim, Stuttgart, Germany

by

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Stuttgart 2012

Date of oral examination: 15.11.2012

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I declare that I wrote this thesis independently according to the doctoral regulations of the University of Hohenheim (§ 8.2.2). I clearly marked any language or ideas borrowed from other sources as not my own and documented their sources.

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Abstract

The potential of an ethoxylated soybean oil adjuvant – Agnique® SBO 10 – to increase the efficacy of different herbicides was investigated in the present thesis. Furthermore, Agnique® SBO 10 was fractioned by preparative High-Performance Liquid Chromatography (prep. HPLC) to elucidate the composition of the ethoxylated soybean oil (ESBO). In addition, experiments with fluorescein-labelled Agnique® SBO 10 were conducted to evaluate the fate of modified seed oil adjuvants on the leaf surface.

The efficacy of many herbicides can be increased by adding adjuvants to the spray solution. Adjuvants, in particular surfactants, are able to increase the foliar uptake of active ingredients for example, by enhancing the retention of spray droplets on cuticles, penetration and absorption into leaf tissue. Agnique® SBO 10 belongs to a group of environmental friendly surfactant containing ten ethylene oxide units. Modified seed or vegetable oils are biodegradable and are proposed to be as effective as petroleum oils. These facts make them very interesting for the usage as adjuvants for agrochemicals. To evaluate the potency of Agnique® SBO 10 and further ethoxylated vegetable oil adjuvants based on linseed, safflower and high oleic sunflower oil, dose-response studies were accomplished. Therefore, the ethoxylated vegetable oils were mixed with the herbicides sulfosulfuron, topramezone, carfentrazone-ethyl and foramsulfuron & iodosulfuron and applied to the test species velvetleaf (*Abutilon theophrasti* Medik.). Commercial adjuvants (polyethylated fatty alcohol, polyether siloxane, rapeseed oil methyl-ester and a mixture of fatty acid methyl-ester, fatty alcohol alkoxylate and oleic acid) served as standards. Results showed that sulfosulfuron, topramezone, and foramsulfuron & iodosulfuron did not control velvetleaf sufficiently when they were applied without adjuvant. The ethoxylated linseed oil demonstrated the best efficacy in combination with the sulfonylureas, whereas the ethoxylated safflower oil was most effective with carfentrazone-ethyl. The least pronounced effect was demonstrated for the ethoxylated high-oleic sunflower oil, which did not show a good potential compared with the other ethoxylated vegetable oils. Agnique® SBO 10 acted 2-fold better compared to the recommended adjuvants. Thus, Agnique® SBO 10 could present an alternative adjuvant for a widespread use. Furthermore, none of the herbicides developed its best efficacy in combination with the respective recommended commercial adjuvant in this study. These results show, that a certain adjuvant has the potential to increase the efficacy of a herbicide to its maximum. However, for the user it is not easy to choose a proper adjuvant of the broad range of available products. Thus, integrating additives into pesticide formulations is desirable. Due to the fact, that Agnique® SBO 10 is a huge and complex product it cannot be included into a formulation. For this reason, an experiment was accomplished dividing Agnique® SBO 10 into four fractions by using preparative HPLC. The earlier eluting fractions could be termed as rather polar and the later eluting as non-polar substances. These four fractions were isolated of Agnique® SBO 10 and tested in combination with commercially formulated herbicides (foramsulfuron & iodosulfuron and bromoxynil octanoate) and a technical grade bromoxynil product. The aim was to find a fraction with a higher potential compared to Agnique® SBO 10 to be used as low-molecular adjuvant. The herbicide-fraction mixtures were applied as droplets with a pipette on velvetleaf leaves. Dry weight and leaf area measurements of test plants demonstrated that the efficacy of foramsulfuron & iodosulfuron and the technical bromoxynil was increased with decreasing polarity of isolated fractions of Agnique® SBO 10, whereas the efficiency of bromoxynil octanoate was tendentially enhanced adding more polar fractions to the herbicide solution. Again, it was demonstrated that the herbicide’s efficacy is strongly dependent of the adjuvant/fractions.

Though a lot of research was conducted to confirm the positive effect of adjuvants on herbicides, their mode of action is still not completely clear. Because modified vegetable oils are complex compounds, a radioactive labelling is not easy. Therefore, in this study Agnique® SBO 10 was labelled with fluorescein to investigate, whether Agnique® SBO 10 remains on the leaf surface, accumulates in the cuticle or even penetrates into the underlying plant tissue. Fluorescein-labelled Agnique® SBO 10 (AF) was applied to one leaf of the test species velvetleaf, wild mustard and sugar beet. At different time intervals, treated leaves were harvested and rinsed with different washing solutions (deionised water, methanol/water and chloroform). The washed leaves and the respective washing solutions were processed separately and analyzed with HPLC coupled with an UV detector. Results lead to the assumption that AF might have been passed through the cuticle. However, AF is not expected to be very mobile because it is lipophilic and electrically neutral. If there was a penetration through the cuticle, an enzymatic metabolisation of Agnique® SBO 10 into fatty acids and ethoxylated glycerol is likely to occur.

With this thesis the herbicide-enhancing effect of Agnique® SBO 10 was confirmed. Furthermore, a new approach for the design of optimized adjuvants for precise herbicide-adjuvant mixtures was presented. Though a lot of research has to be conducted to elucidate the mode of action of adjuvants, this study gives an approach to investigate the behaviour of foliar applied adjuvants.

Kurzfassung

In der vorliegenden Arbeit wurde das Potential eines ethoxylierten Sojabohnenöl Adjuvants – Agnique® SBO 10 – zur Wirkungssteigerung verschiedener Herbizide untersucht. Des Weiteren wurde Agnique® SBO 10 mittels präparativer Hochleistungs-Flüssigkeits-Chromatographie (präp. HPLC) fraktioniert, um Aufschluss über die Zusammensetzung des ethoxylierten Sojabohnenöls (ESBO) zu erlangen. Zusätzliche Experimente mit Fluorescein-markiertem Agnique® SBO 10 wurden durchgeführt, um den Wirkmechanismus von modifizierten Saatöl-Adjuvantien zu untersuchen.

Die Wirksamkeit oder die Anwendung vieler Herbizide kann durch den Einsatz von Adjuvantien, speziell durch so genannte Surfactants, erhöht werden, indem diese die Retention der Spritzlösung auf Kutikeln erhöhen oder die Penetration und Aufnahme in das Blattgewebe fördern. Agnique® SBO 10 gehört zu einer Gruppe von umweltfreundlichen Adjuvantien und weist zehn Ethylenoxid-Gruppen pro Molekül auf. Modifizierte Saatöle oder Pflanzenöle sind biologisch abbaubar und zeigen eine vergleichbare Wirkung wie Petroleum-Öle. Diese Fakten machen sie sehr beliebt für den Einsatz als Adjuvantien für Pflanzenschutzmittel. Um die Wirkung von Agnique® SBO 10 und weiteren ethoxylierten Pflanzenölen, basierend auf Lein-, Färberdistel- und „High-Oleic“-Sonnenblumenöl, zu beurteilen, wurden Dosis-Wirkungsversuche durchgeführt. Dazu wurden die ethoxylierten Pflanzenöle den Herbiziden Sulfosulfuron, Topramezone, Foramsulfuron & Iodosulfuron und Carfentrazon-ethyl zugegeben und auf die Testpflanze Schönmalve (*Abutilon theophrasti* MEDIK.) appliziert. Als Standard dienten kommerziell erhältliche Adjuvantien (polyethylierter Fettsäurealkohol, Polyether Siloxan und Rapsöl Methylester). Die Ergebnisse zeigten eine unzureichende Kontrolle der Schönmalve nach einer Behandlung mit Sulfosulfuron, Topramezone und Foramsulfuron & Iodosulfuron ohne die Zugabe von Adjuvantien. Das ethoxylierte Leinöl wies die beste Wirkung in Kombination mit den Sulfonylharnstoffen auf, das ethoxylierte Färberdistelöl hingegen war am Effektivsten in Kombination mit Carfentrazone-ethyl. Der schwächste Effekt wurde für das ethoxylierte „High-Oleic“-Sonnenblumenöl nachgewiesen. Es zeigte keine gute Wirkung im Vergleich zu den anderen ethoxylierten Pflanzenölen. Agnique® SBO 10 war doppelt so effektiv im Vergleich zu den kommerziellen Adjuvantien. Agnique® SBO 10 könnte deshalb ein alternatives Adjvuant für einen breit gefächerten Einsatz sein. Des Weiteren entfaltete in dieser Studie keines der Herbizide seine beste Wirksamkeit in Kombination mit den empfohlenen kommerziellen Adjuvantien. Diese Ergebnisse zeigen, dass bestimmte Adjuvantien das Potenzial besitzen, die Wirksamkeit eines Herbizides maximal zu steigern.

Für den Anwender ist es jedoch nicht einfach ein passendes Adjuvant aus dem umfassenden Angebot auszuwählen. Aus diesem Grunde ist eine Einbindung des Additives in eine Pflanzenschutzmittelformulierung wünschenswert. Da Agnique® SBO 10 ein sehr großes, komplexes Produkt darstellt, kann es nicht ohne weiteres in eine Formulierung integriert werden.

Deshalb wurde ein Versuch durchgeführt, bei dem Agnique® SBO 10 mittels präparativer HPLC in vier Fraktionen unterteilt wurde. Die früher eluierenden Fraktionen können als eher polar bezeichnet werden, die späteren als eher unpolar. Diese Fraktionen wurden aus Agnique® SBO 10 isoliert und in Kombination mit kommerziell erhältlichen Herbiziden (Foramsulfuron & Iodosulfuron und Bromoxynil Octanoate) und einem technischen Bromoxynil Produkt gemischt. Das Ziel war es, herauszufinden, ob eine der Fraktionen ein höheres Potential als Agnique® SBO 10 besitzt um als niedermolekulares Adjuvant genutzt zu werden. Die Herbizid-Fraktion Mischungen wurden in Form von Tröpfchen auf jeweils ein Blatt der Schönmalve pipettiert. Messungen des Trockengewichtes und der Blattfläche ergaben, dass die Wirksamkeit von Foramsulfuron & Iodosulfuron und des technischen Bromoxynils mit der Zumischung von Fraktionen mit abnehmender Polarität erhöht wurden. Die Effektivität von Bromoxynil Octanoat wurde jedoch eher durch die Zugabe von polaren Fraktionen gefördert. Hier konnte wieder gezeigt werden, dass die Wirksamkeit eines Herbizides stark von der Wahl des Adjuvants beziehungsweise der Fraktion abhängt. Trotz intensiver Forschung zur Beurteilung der positiven Wirkung von Adjuvantien auf Herbizide, ist ihr Wirkmechanismus bis jetzt noch nicht vollständig bekannt. Da modifizierte Pflanzenöle komplexe Zusammensetzungen sind, ist eine radioaktive Markierung nicht einfach. Aus diesem Grund wurde Agnique® SBO 10 mit Fluorescein markiert, um herauszufinden, ob Agnique® SBO 10 auf der

Blattoberfläche verbleibt, in der Kutikula akkumuliert oder sogar in das darunter liegende Gewebe eindringt. Das mit Fluorescein markierte Agnique® SBO 10 (AF) wurde auf jeweils ein Blatt der Spezies Schönmalve, Ackersenf und Zuckerrübe appliziert. Die behandelten Blätter wurden zu verschiedenen Zeitpunkten geerntet und mit verschiedenen Waschlösungen (deionisiertes Wasser, Methanol/Wasser und Chloroform) gewaschen. Die gewaschenen Blätter, so wie die Waschlösungen, in denen die behandelten Blätter gewaschen wurden, wurden getrennt voneinander aufgearbeitet und mittels HPLC (gekoppelt an einen UV Detektor) analysiert. Die Ergebnisse ließen zu der Annahme führen, dass AF möglicherweise durch die Kutikula gedrungen ist. Es wird jedoch vermutet, dass AF nicht sehr mobil ist, da es eine lipophile und elektrisch neutrale Substanz ist. Falls AF jedoch in das pflanzliche Gewebe gelangen konnte, ist ein enzymatischer Abbau von Agnique® SBO 10 in Fettsäuren und ethoxyliertes Glycerol sehr wahrscheinlich. In dieser Arbeit wurde bestätigt, dass Agnique® SBO 10 die Wirksamkeit der hier getesteten Herbizide steigern konnte. Des Weiteren wurde ein neuer Ansatz vorgestellt, mit dem optimierte Adjuvantien für präzise Herbizid-Adjuvant Mischungen konstruiert werden könnten. Auch wenn noch sehr viele Fragen im Bezug auf den Wirkmechanismus von Adjvuantien geklärt werden müssen, wurde hier eine Methode präsentiert, die eine Möglichkeit zur Erforschung des Verhaltens von blattapplizierten Adjuvantien darstellt.

Contents

I. List of Abbreviations

Abbreviation	**Meaning**
%	Percent
°C	Degree centigrade
µm	Micrometer
$\mu mol\ m^{-2}\ s^{-1}$	Photosynthetic Photon Flux
AF	Agnique® SBO 10-Fluorescein
AI	Active ingredient
ASTM	American Society for Testing and Materials
BO	Bromoxynil octanoate
BP	Bromoxynil potassium
C	Carfentrazone-ethyl
Ch	Chloroform
ChF	Chlorophyll α fluorescence
cm	Centimeter
d	Day
DW	Deionised water
e.g.	Exempli gratia
ED 50	Effective dosage with 50 % pest control
ED 90	Effective dosage with 90 % pest control
EHOSO	Ethoxylated high-oleis sunflower oil
ELO	Ethoxylated linseed oil
Eq.	Equation
ESBO	Ethoxylated soybean oil
ESO	Ethoxylated safflower oil
FI	Foramsulfuron & iodosulfuron
Fig.	Figure
g	Gram
GH	Greenhouse
h	Hour
ha	Hectare
i.e.	Id est
kg	Kilogram
L	Liter

L.	Linné
LA	Leaf area
Lab	Laboratory
Medik.	Medikus
mm	Millimeter
MS	Mass spectrometry
MW	Methanol/water solution (1:1)
Prep. HPLC	Preparative High-Performance Liquid Chromatography
PS II	Photosystem II
rpm	Rounds per minute
S	Sulfosulfuron
SBO	Soybean oil
sec	Seconds
SEM	Scanning Electron Microscopy
Tab.	Table
v/v	Volume to volume
Vill.	Villars
WG	Wettable granulate
Y(II)	Quantum yield of photosystem II

II. List of Figures

III. List of Tables

1. General Introduction

1.1. Outline of the thesis

The present thesis is divided into three chapters. First, an introduction with general information about adjuvants for herbicides, their classification and mode of action is given. Moreover, in the first chapter my motivation and the objectives of the present work will be elucidated. In the second chapter, which represents the major part of the thesis, three papers are presented covering the most important results of my work. A general discussion and an outlook on future projects are presented in the third chapter.

1.2. Motivation and objectives of this study

Increasing human population, and hence a higher demand for food requires an intensive food production. Furthermore, bio fuels and renewable resources compete directly with food production, whereas the worldwide net arable land can not be increased. Consequently, yields have to be increased. This, in turn, can only be achieved by progress in agricultural technology, meaning a better use of pesticides and non-chemical pest management strategies (von Braun & Qaim, 2009). Any threats to crop productivity, like weeds, pests and pathogens, have serious consequences and result in yield decreases of 50-80 % if not controlled. Only yield reduction by weeds amounts 20-40 % (Hurle, 2000). Over the past 40 years, modern herbicides have largely replaced human, animal and mechanical weed control (Powles & Yu, 2010) and an increased use of herbicides altered the weed flora and herbicide-resistand biotypes were selected (Kudsk & Streibig, 2003). Furthermore, for the last 20 years, no new modes of action of herbicides were discovered, while diversity in crop rotations, cultural practises and chemical weed management strategies have been reduced (Kraehmer et al., 2007). Additionally, increasing toxicological and ecotoxicological restrictions made by regulatory authorities will limit the number of available effective herbicides (Underwood, 2000). Therefore, optimizing the application techniques and new well-designed formulations of old active ingredients (AI) might be an approach to overcome this lack of new herbicides. At this point, adjuvants come into the play, as they are known to be the best tools to improve application, thus reducing costs and the environmental impact of plant protection products (Green, 2000; Green & Beestman, 2007). For this reason the effect of Agnique® SBO 10 in combination with herbicides was tested using dose-response curves to compare the efficacy of different adjuvant-herbicide combinations. Furthermore, to get more detailed information about the composition of Agnique® SBO 10, we used High-Performance Liquid Chromatography (HPLC) to separate the complex vegetable oil adjuvant into several fractions and tested the effect of each single fraction on the efficacy of herbicides. To clarify the mode of action of Agnique® SBO 10 we developed an approach to label Agnique® SBO 10 to elucidate its uptake through the cuticle.

<u>The objectives of this study are:</u>

1. To compare the potency of Agnique® SBO 10 and other ethoxylated seed oils with commercial available adjuvants on the efficacy of herbicides.
2. To investigate the composition of Agnique® SBO 10 in order to identify an active compound within the adjuvant that can be isolated and further developed for being used as a highly active small molecular adjuvant.
3. To evaluate the mode of action of Agnique® SBO 10. Does the adjuvant penetrate into the test species, accumulate in the wax layer of the cuticle or even remain on the leaf surface?

1.3. Adjuvants for herbicides

The AI of a plant protection product ensures its biological efficacy. However, many pesticides are biologically inactive and impossible to apply in agricultural practise and therefore, the formulation of the plant protection product plays a crucial role for enhancing the efficacy of pesticides. The efficacy of foliage applied herbicides can greatly be increased by using surfactants, vegetable and mineral oils or fertilizer salts like ammonium sulphate (Kirkwood, 1993). Those adjuvants can for example increase the retention and spreading of a spray solution on the target plant and/or the penetration of the herbicide into the plant. Adjuvants can either be included in a formulation (formulation adjuvants) or mixed to the spray solution by the applicant (spray adjuvants) (Kudsk & Streibig, 1993). Acoording to Stephenson et al. (2006) an adjuvant is a “Substance added to a pesticide formulation or to the spray tank to modify pesticide activity or application characteristics”. Though the adjuvant itself has to be inert, i.e. must not show any phytotoxicity to the target organism, they are biologically and chemically active compounds which enhance the efficacy of an active compound or reduce the AI amount needed to control weeds (Tu & Randall, 2001). There are adjuvants for all kind of pesticides but adjuvants for herbicides, especially adjuvants for glyphosate, dominate the market (Green, 2000). Already before 1900, animal soap solutions were used as adjuvants to increase the toxicity of arsenical formulations on weeds (Hazen, 2000). Nowadays, a shift from petroleum based adjuvants to environmental friendly products like ethoxylated seed oils can be observed (Green, 2000). Stricter regulations by the authorities concerning the ecotoxicological profile of many adjuvants, like mineral oils and tallow amine ethoxylate adjuvants, are reasons for a greater interest in the usage of modified vegetable oils.

1.4. Adjuvant types

Due to the huge amount of different adjuvants for special purposes, this section gives a summary of the different adjuvant types. According to the American Society for Testing and Materials (ASTM), there are two different classes of adjuvants. One class, the so-called utility adjuvants, is able to modify the physical characteristics of the spray mixture whereby they do not directly affect herbicide efficacy. The second class enhances the biological efficacy of herbicides on the target plant and thus can be termed as activator adjuvants (ASTM, 1995).

Class 1: Utility adjuvants

Utility adjuvants are extensively discussed by McMullan (2000) and include seven types that were often used to improve herbicide application. They are very important to the user of plant protection products because they increase ease of handling and the application of products.

Compatibility agents

Compatibility agents are defined by the ASTM as "surface-active materials that allow simultaneous application of liquid fertilizer and agrichemical, or two or more agrichemical formulations, as a uniform tank mix, or improve the homogeneity of the mixture and the uniformity of the application" (ASTM, 1995). Compatibility agents are necessary to avoid inhomogeneous spray solutions which build up when chemicals react with each other after bringing them in the tank mixture. Compatibility agents are already included in pesticide formulations.

De-foaming agents

Foam has to be avoided due to the fact that it hinders cleaning and application of pesticide solutions. Foam is an emulsion of air and water. If the surface tension of the spray solution is reduced, air can diffuse into the water and no foam is generated. For this process surfactants such as silicones serve as suitable adjuvants.

Drift-control agents

Drift-control agents alter viscoelastic properties of spray mixtures, hence reducing the amount of off-target drift mainly by decreasing the volume of driftable particles that is those with a particle size smaller than 150 µm. Deposition agents can be based on ammonium sulphate or polysaccharides.

Deposition agents

Deposition agents can be added to tank mixtures to increase the amount of herbicide deposited on target surfaces. Polyphenyl alcohols for example do not influence the driftable fraction or the diameter of spray droplets, but increase the amount of plant protection products which is deposited on the leaf surface.

Water-conditioning agent

A water-conditioning agent, which can be a sequestering or chelating substance, citric acid or ammonium sulfate, minimizes interactions between ions in the spray solution which can reduce the efficacy of pesticides. The efficacy of glyphosate for example is reduced by calcium ions. This negative effect can be avoided if citric acid is added which reacts with calcium to calcium citrate.

Acidifying agents

Dilutes of strong acids typically represent acidifying agents which lower the pH of a spray solution rapidly.

Buffering agent

Buffering agents make a spray solution resistant to pH changes and maintain a buffer specific pH range when acid or alkaline-based materials are added to a spray solution.

Colorants

Colorants are used to minimize overlapping or skipping of areas to achieve a maximum efficacy and keep the environmental impact as low as possible.

Class 2: Activator adjuvants

The class of activator adjuvants is discussed in detail by Hazen (2000). This class consists of four groups:

Surfactants (wetter and spreader adjuvants)

This group probably demonstrates the largest group of activator adjuvants. The name “surfactant” arises out of the surface-active features, which allow those agents to reduce the free-surface tension of the surface being wetted. This mechanism results in a lower contact angle of applied droplets and thus, in a larger surface area that can be covered (spreading) (Fig. 1). Regarding herbicides, AIs are more evenly distributed, mostly leading to an increase of herbicide efficacy in case of contact action. However, sometimes a higher volume droplet is required because of the osmotic drive for herbicide uptake. Sticker adjuvants represent the second group of activator adjuvants and ensure an herbicide deposit after drying of the spray solution, consequently providing rainfastness.

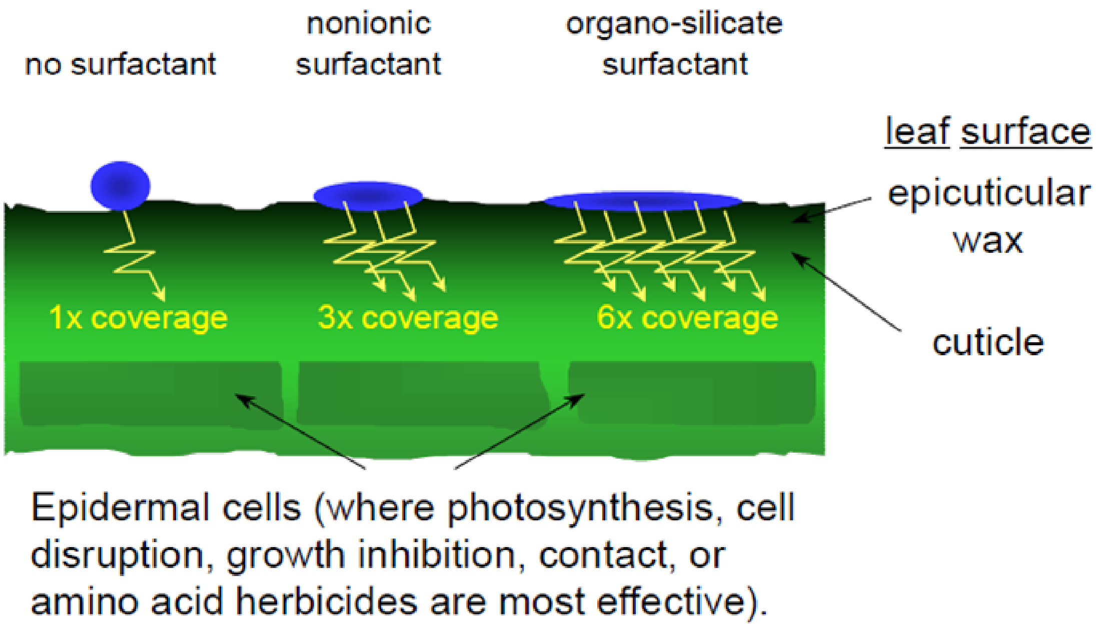

Fig. 1: Surfactant effect on spreading and wetting (Miller & Westra 1998, modified).

Sticker adjuvants

Pesticides can be removed from plant surfaces by wind, physical contact or rain. Sticker adjuvants, mostly already included in the formulation, are non-evaporating materials which adhere in combination with the plant protection product to the leaf surface and hence, increase its activity interval.

Humectants

To prevent a spray droplet from drying, humectants can be added. Thus, herbicides are kept in a liquid form and, hence, the availability of AI is increased. To increase the rate of penetration of herbicides into leaf tissue, adjuvants that alter the structure of the cuticles' wax layer or enable a stomatal infiltration of agrochemicals can be added to the tank mixture. Those additives are called penetration agents.

1.5. Mode of action of surfactants related to herbicide uptake

Because this study pivots on an ethoxylated soybean oil classified as surfactant the literature review in this section focuses on the mode of action of surfactants. After the deposition of a plant protection product on the leaf surface the cuticle with its epicuticular waxes demonstrates the most important barrier for the uptake of xenobiotics into plants (Kirkwood, 1999). Epidermal cells synthesize the predominantly lipid material of which the cuticle is composed (Holloway, 1993). The thickness and surface properties of cuticles strongly vary amongst species. Some species possess a cuticle with a thickness of 0.1 µm, whereas some fruits and xerophytes can develop a cuticle which is up to 10 µm thick. The cuticle surface of some species can be relative smooth [e.g. *Beta vulgaris* L. (Fig. 2 A), *Stellaria media* (L.) VILL.], whereas e.g. *Brassica* species are additionally covered by microcrystalline epicuticular waxes, and species like *Abutilon theophrasti* Medik. (Fig. 2 B and 3). possess glandular trichomes on their surface which are modified epidermal cells (Holloway, 1993).

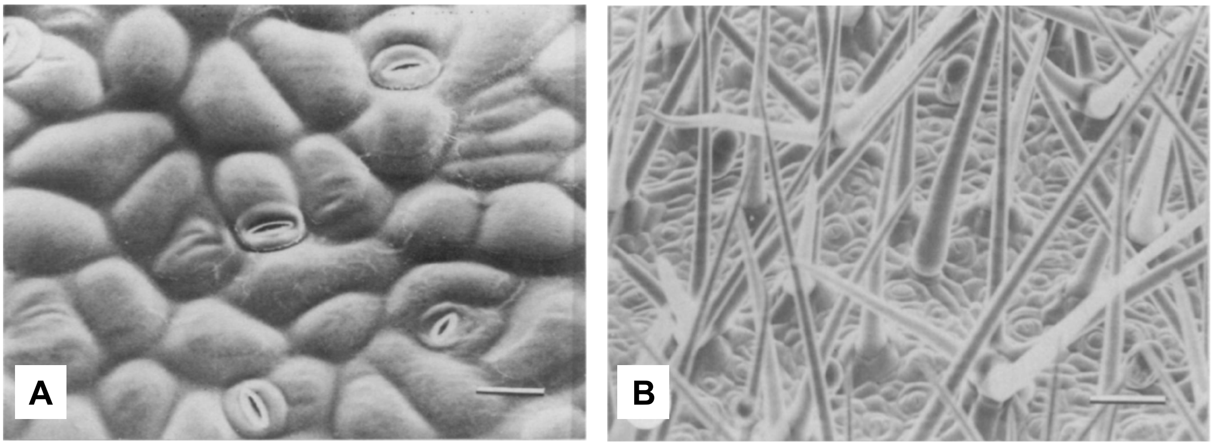

Fig. 2: Cryo-SEM pictures of the leaf surface morphology. Smooth leaf surface of sugar beet (A, scale marker = 20 µm) and surface of velvetleaf with trichomes (B, scale marker = 50 µm). Trichomes can decrease the amount of herbicide reaching the epidermal surface (according to Hess & Falk 1990, modified).

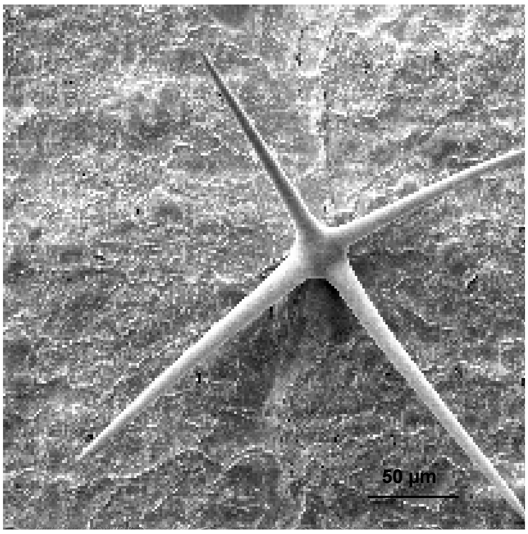

Fig. 3: SEM picture of the leaf surface of velvetleaf with one single trichome (scale marker = 50 µm).

Microroughness, which is caused by epicuticular cells (e.g. trichomes), influences the wetting properties of a plant surface. If there is high microroughness, air/liquid and solid/liquid interfaces can be generated which enables herbicide penetration because the contact between the spray droplet and the leaf surface is low (Kirkwood, 1999). The **adhesion and retention** of herbicides can be increased by surfactants which migrate to the spray droplet surface, reduce the surface tension and hence, lower the contact angle between the spray droplet and the leaf surface (Zabkiewicz, 2000).

Due to the above mentioned characteristics of surfactants they improve wetting of leaf surfaces which is a prerequisite for the **uptake or absorbtion** of a herbicide (Zabkiewicz, 2000). It is known that specific interactions between the plant surface and surfactants, especially of the non-ionic polyoxyethylene type, can increase the penetration of pesticides into plants (Stock & Holloway, 1993). However, the mode of action of adjuvants is still not completely clear. Early studies postulated that surfactants, which lower the surface tension of the spray solution, allow herbicides to be taken up by plants via mass flow into stomata (Schönherr & Bukovac, 1972). However, the stomatal infiltration can only be reached with organosilicone surfactants which were introduced in the 1980ies and are characterized by their exceptionally low surface tension (Stevens, 1993). Surfactants are supposed to disrupt or dissolve the epicuticular waxes, to increase the solubility of pesticides within the lipophilic cuticle and to increase the permeability of cell membranes (Stock & Holloway, 1993). These mechanisms could lead to an enhanced uptake of active ingredients and to a co-penetration of the surfactant with the herbicide. Gauvrit & Cabanne (1993) stated that seed oils, such as Agnique® SBO 10, are known to be poor solvents for epicuticular waxes, but they are able to impregnate the wax. Moreover, they are able to modify the physical properties of the epicuticular waxes and possess the ability to penetrate into the cuticle. This again results in an increased fluidity of waxes and, hence, to an increased AI transport in the cuticle (Gauvrit & Cabanne 1993).

1.6. Agnique® SBO 10 – an adjuvant for herbicides

Agnique® SBO 10 is an ethoxylated soybean oil and can be classified as nonionic surfactant. Since the product is based on a vegetable oil it belongs to a group of biodegradable adjuvants. The chemical structure of Agnique® SBO 10 is based on an ethoxylated triacylglycerid which contains ten ethylene oxide units (Fig. 4).

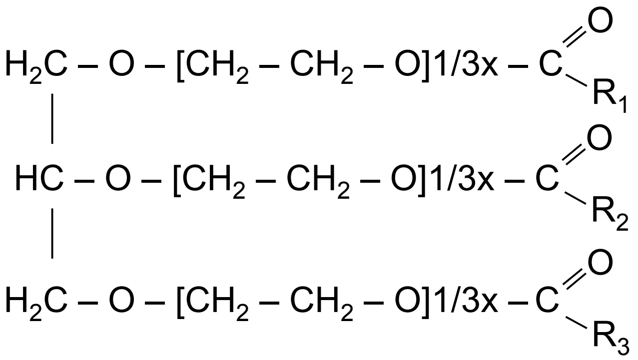

Fig. 4: Generallized structure for ethoxylated triglycerides with x units of ethylene oxide (according to Haefs 2002, modified).

Agnique® SBO 10 molecules can highly vary due to the fatty acid composition of soybean oil (Tab. 1). There are a lot of different fatty acid combinations possible, hence, Agnique® SBO 10 or in general vegetable oil adjuvants are very complex products.

Tab. 1: The fatty acid distribution of soybean oil (according to Fuller et al. 1967, modified).

Fatty acid distribution of soybean oil				
% Palmitic acid (C16:0)	**% Stearic acid (C18:0)**	**% Oleic acid (C18:1)**	**% Linolic acid (C18:2)**	**% Linolenic acid (C18:3)**
11	**~ 4**	**~ 25**	**~ 53**	**~ 7**

Modified vegetable oils are reported to show no phytotoxicity and are environmental friendly (Haefs et al., 2002). A high biodegradability of vegetable oil adjuvants can be expected as they are degraded by lipases which are produced by different micro-organisms (Cornish et al., 1993). Furthermore, they are proposed to be as effective as petroleum oils regarding their efficacy against weeds (Robinson & Nelson, 1975). These facts make them very interesting for the use in agriculture.

2. Publications

2.1. Evaluation of the potency of ethoxylated soybean, linseed, high oleic sunflower and safflower oil to increase herbicide efficacy in comparison to commercial adjuvants

Submitted to the *Journal of Plant Diseases and Protection*

Authors: Julia Heini, Hans-Georg Mainx & Roland Gerhards

2.1.1. Abstract

The efficacy of many herbicides can be increased by adding adjuvants to the spray solution. Surfactants are able to increase foliar uptake of active ingredients for example, by enhancing retention of spray droplets on cuticles, penetration and absorption into leaf tissue. In this study, dose-response studies with ethoxylated seed oils (soybean, linseed, safflower and high oleic sunflower oil), in combination with the herbicides sulfosulfuron, topramezone, carfentrazone-ethyl and foramsulfuron & iodosulfuron were conducted. Commercial adjuvants (polyethylated fatty alcohol, polyether siloxane, rapeseed oil methyl-ester and a mixture of fatty acid methyl-ester, fatty alcohol alkoxylate and oleic acid) served as standards. The experiments were carried out under greenhouse conditions, using *Abutilon theophrasti* MEDIK. as test species. Dry weight of *A. theophrasti* was measured three weeks after treatment and dose-response curves were calculated by regression analysis.

Results showed that three of four herbicides did not control *A. theophrasti* sufficiently when they were applied without adjuvant. The ethoxylated linseed oil decreased the *ED90* of sulfosulfuron and foramsulfuron & iodosulfuron by 245- and 44-fold, respectively, whereas the *ED90* of carfentrazone-ethyl was reduced 2-fold by the ethoxylated safflower oil. Furthermore, none of the herbicides developed its best efficiency in combination with the respective recommended commercial adjuvant.

This experiment demonstrates, that the potential of an herbicide can be increased adding a compatible adjuvant. Hence, with precise recommendations for herbicide-adjuvant-mixtures, herbicide application rates and costs could be reduced.

2.1.2. Introduction

Almost all herbicide formulations contain adjuvants. They act as important tools to improve physical aspects of herbicide application and/or to enhance biological efficacy (Green & Foy, 2000). Adjuvants are “substances added to a pesticide formulation or to the spray tank to modify pesticide activity or application characteristics” (Stephenson et al., 2006).

There are two main adjuvant types: (1) utility adjuvants or tank-mix modifiers, which for example are able to adjust or buffer the pH or reduce spray drift, and (2) activator adjuvants, which enhance herbicide activity for example by wetting the leaf surface or softening cuticular waxes (Hazen, 2000).

Seed or vegetable oil derivates can be classified as surfactants which again can be counted to the type of activator adjuvants. Modified vegetable oils represent a group of biodegradable adjuvants (Cornish et al., 1993) which are proposed to be as effective as petroleum oils (Robinson & Nelson, 1975). Due to the fact that this group of adjuvants is not phytotoxic, environmental friendly and enhance herbicide efficacy, they are interesting for pesticide formulations (Haefs et al., 2002). Though various research on seed oil adjuvants for herbicides was conducted, their mode of action is still not completely clear. However, it is known that seed oils are poor solvents for epicuticular waxes, but are able to impregnate the wax (Gauvrit & Cabanne, 1993). This might lead to modifications of the physical properties of the epicuticular waxes and to an increased fluidity (Gauvrit & Cabanne, 1993). In an earlier study, Manthey & Nalewaja (1992) claimed, that a solubilization of epicuticular waxes might also be a reason for an enhanced uptake of active ingredients (AI). Furthermore, oils possess the ability to penetrate into the cuticle and, hence, this could be related to a transfer of AI into plants (Coret & Chamel, 1993).

For agriculture, the enhancement of herbicide action by the addition of adjuvants could reduce herbicide application rates. Although the usage of herbicides is adopted by most farmers, the interest in reduced application rates constantly grows (Blackshaw et al., 2006). Reducing herbicide amounts while still maintaining an adequate efficacy against weeds has a great importance in plant protection to decrease costs, environmental impacts of chemical plant protection, and losses in crop yield caused by herbicide damages. However, long time studies proved, that a constant reduction of the application rate by 50 % leads to a gradual increase of weed infestation and selection of weed species which are difficult to control (Pallutt & Moll, 2008). Furthermore, the risk of selecting herbicide resistant weeds increases due to a reproduction of species which were not completely controlled. Hence, the population density can grow over the years, leading to an increased gene pool in which resistant species will be more likely (Blackshaw et al., 2006).

In the present study, we investigated the potency of several ethoxylated seed oil adjuvants, based on soybean (ESBO), linseed (ELO), high oleic sunflower (EHOSO) and safflower oil (ESO). For this purpose, dose response studies were conducted with ready formulated herbicides [Monitor®, Monsanto (800 g kg^{-1} sulfosulfuron); Clio®, BASF (336 g kg^{-1} topramezone); Oratio® 40 WG, Syngenta (400 g kg^{-1} carfentrazon-ethyl); MaisTer®, Bayer CropScience (300 g kg^{-1} foramsulfuron, 10 g kg^{-1} iodosulfuron-methyl-sodium)]. Adjuvants, which were recommended for those herbicides served as standards.

In weed science, dose response studies are a useful tool to assess selectivity and efficacy of herbicides (Streibig et al., 1993) and herbicide-adjuvant combinations (Green & Foy, 2000). It is known that herbicides, e.g. glyphosate or metsulfuron-methyl, applied at low doses can stimulate plant growth (Cedergreen, 2008). This effect is called hormesis (Calabrese & Baldwin, 2001) and was noticed rather accidentally (Streibig, 1980). Indeed, understanding the effect of low herbicide doses on plant response is crucial because herbicides are sprayed into the environment in large amounts (Cedergreen, 2008).

In this study, *Abutilon theophrasti* MEDIK. (velvetleaf), a difficult to control annual weed, served as test species. Velvetleaf belongs to the family of Malvaceae and reduces crop yields by competing for water- and nutrient supply, shading of the cultivar and release of allelopathic compounds (Haensel, 2005). In the USA, *A.*

theophrasti causes huge problems in soybean, cotton, and maize, whereas in Germany difficulties occur mainly in sugar beet because the herbicides mostly are ineffective against this weed (Meinlschmidt, 2006). Studies, conducted in the Midwest of the USA, revealed that already three plants per 30 cm row reduced yields in soybean by 31 % (Staniforth, 1965). The aims of this study were:

(1) to compare the potency of the ethoxylated seed oils with the standard adjuvant formulations on the efficacy of herbicides and

(2) to find differences between the ethoxylated seed oils, especially in context to the saturation degrees of the fatty acids of the seed oils.

2.1.3. Material and Methods

Plant material

Seeds of velvetleaf (Abutilon theophrasti; Herbiseed, UK) were pre-germinated in plastic pots (11 x 11 x 6 cm) filled with vermiculite (2-3 mm) in a greenhouse (25/20 °C, additional light (~ 122 µmol $m^{-2}s^{-1}$ for 12 h) for 5-6 days until cotyledons were developed. Precultivated seedlings were transferred into paper pots (8 x 8 cm) filled with a compost soil-sand mixture (2:1 v/v) and cultivated under the described conditions. Plants were watered daily with tap water. No water was applied to plants for at least 24 h after application of herbicides. All plants were completely randomized with four replicates.

Herbicide and adjuvant application

The seed oil adjuvants investigated in this study contain ten ethylene oxide units and belong to the class of non-ionic surfactants. Their chemical structure is based on an ethoxylated triacylglycerid (Fig. 5) with a varying composition of fatty acids which have different saturation degrees (Tab. 2).

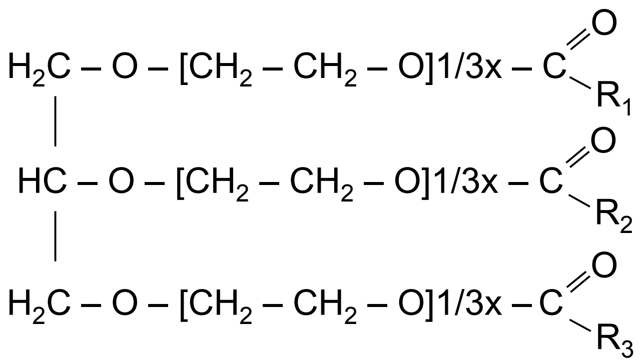

Fig. 5: Generallized structure for ethoxylated triglycerides with x units of ethylene oxide (according to Haefs 2002, modified).

Tab. 2: Fatty acid composition of the ethoxylated seed oil adjuvants according to Bockisch (1993).

	Fatty acid distribution (only C18)			
Adjuvant	**% Stearic acid (C18:0)**	**% Oleic acid (C18:1)**	**% Linolic acid (C18:2)**	**% Linolenic acid (C18:3)**
Ethoxylated soybean oil **(ESBO)**	~ 4	~ 21	~ 56	~ 8
Ethoxylated high oleic sunflower oil (**EHOSO**)	< 5	>80	<5	traces
Ethoxylated safflower oil (**ESO**)	~ 2-3	~ 15	> 75	traces
Ethoxylated linseed oil (**ELO**)	~ 4	~ 22	~ 16	~ 52

ESBO, EHOSO, ESO and ELO were tested in combination with the commercial formulated herbicides displayed in Tab. 3. Therefore, they were doted in a concentration of 1 L ha^{-1} to nine concentrations of each herbicide solution, whereby the highest concentration was equal to the recommended filed application rate (dose ranges were given in Tab. 3). As reference, herbicide solutions were applied alone and in combination with their recommended adjuvants. Application rate of recommended adjuvants was used as displayed in Tab. 3.

Untreated velvetleaf plants served as the untreated control. Every treatment was replicated four times. Plants were treated when the second true leaf was completely developed. Application was done with a track sprayer (Aro, Langenthal, Switzerland), which simulated a water volume of 400 L ha^{-1} (nozzle: 8004 EVS, Teejet® Spraying Systems Co., Wheaton, IL, USA).

Tab. 3. List of herbicides and their recommended adjuvants.

Herbicide trade name (recommended adjuvant)	Active ingredient (a.i.)	Mode of action	Herbicide dose-range (g a.i. ha^{-1})	Adjuvant type	Adjuvant short form	Adjuvant dose (l ha^{-1})
Monitor® (Monfast®)	Sulfosulfuron	ALS-inhibitor	0.078 - 20	Polyoxylated fatty alcohols, propylenglycol	EFA	0.8
Clio® (Dash®)	Topramezone	4-HPPD-inhibitor	0.197 - 50.4	Fatty acid methyl-ester, fatty alcohol alkoxylate, oleic acid	FAME	1
Oratio® 40 WG (Break-Thru® S 240)	Carfentrazone-ethyl	PPO-inhibitor	0.078 - 20	Organomodified (Polyether) siloxane	PS	0.1
MaisTer® (Mero®)	Foramsulfuron + iodosulfuron	ALS-inhibitor	0.182 - 46.5	Rapeseed oil methyl-ester	ROME	2

Data measuring and data analysis

The study was conducted from March to May 2010 with four different experiments, where each herbicide represents one separate experiment. Above ground biomass was harvested three weeks after application, dried at 80 °C for 48 h and weighed. Biomass data was analyzed with the four-parameter log-logistic model (Streibig et al. 1993) (Eq. 1) using the software package drc of the statistical software R (version 2.12.2) (R Development Core Team, 2010).

$$(1)\qquad y = C + \frac{D - C}{1 + e^{b(\ln(x) - \ln(ED50))}}$$

D and *C* denote the upper and lower limits, respectively, and *ED50* is the dose where a response half-way between the upper and lower limit is reached. *B* denotes the slope around the *ED50* value. When the lower limit of curves was

0, the model was reduced to a three-parameter model (C = 0). To compare different curves, generated from biomass data of each treatment, the residual sum of squares of the regression analysis was compared and assessed by an *F*-test for lack-of-fit (Ritz & Streibig, 2009). In weed control studies response levels at *ED90*, the dose causing 90 % weed control, might be of higher interest (Knezevic et al., 2007), compared with the *ED50* value. According to Eq. 2 (Ritz & Streibig, 2005; modified), *ED90* values were calculated.

(2) ED x = ED 50 [x/(100-x)]1/b.

To compare the different efficacies of herbicide-adjuvant combinations, relative potencies (*r*) were calculated for parallel curves according to Eq. 3 (Ritz et al., 2006):

$$(3) \quad r_A = \frac{x_B}{x_A}$$

X is the herbicide dose, *B* the reference spray solution, i.e. the herbicide without adjuvant, and *A* the herbicide-adjuvant-mixture. As *rA* is not constant across response levels in case of non-parallel curves, *rA*-values were only evaluated for pre-set response levels (*ED50* and *ED90*) (Cabanne et al., 1998).

The hormesis-effect observed for topramezone applied without adjuvant was modelled by a logistic regression model allowing for hormesis [(Schabenberger et al., 1990) based on Brain & Cousens (1989)], where *f* denotes the initial rate of increase in response at subinhibitory herbicide concentrations:

$$(4) \quad y = C + \frac{D - C + f \cdot x}{1 + \left(1 + \frac{2 \cdot f \cdot ED50}{D - C}\right) \cdot e^{b \cdot \ln(x / ED50)}}$$

A *F*-test for lack-of-fit was conducted to test whether the 95 % confidence interval for *f* did not overlap zero, which indicates a hormetic effect (Schabenberger et al., 1990).

Due to the fact, that no dose-response curves could be fitted for all topramezone treatments containing an adjuvant, a *T*-test at a 5 % probability level was conducted to evaluate significant differences between treatments. The data was analyzed using SAS (version 9.2). Tests for normal distribution and variance homogeneity were conducted prior to analysis and non-normal distributed data was log-transformed. For presentation, data was back-transformed to initial values.

2.1.4. Results

Dose-response curves could be fitted for sulfosulfuron, carfentrazon-ethyl and foramsulfuron & iodosulfuron with or without adjuvants and for topramezone applied alone. The corresponding regression parameters are demonstrated in Tab. 4.

Topramezone

Topramezone showed a significant hormesis effect ($p = 0.48$) at low herbicide concentrations (Fig. 6), whereas no curves could be modelled after mixing an adjuvant. The addition of adjuvants to the lowest topramezone dose already decreased dry weight of test species significantly on average by 85 % (± 1.4 %) (Fig. 7). At a concentration of 3.15 g topramezone ha^{-1}, which is 1/16 of the recommended field application rate, no significant differences between topramezone applied alone or doted with one of the adjuvants were measured (data not shown).

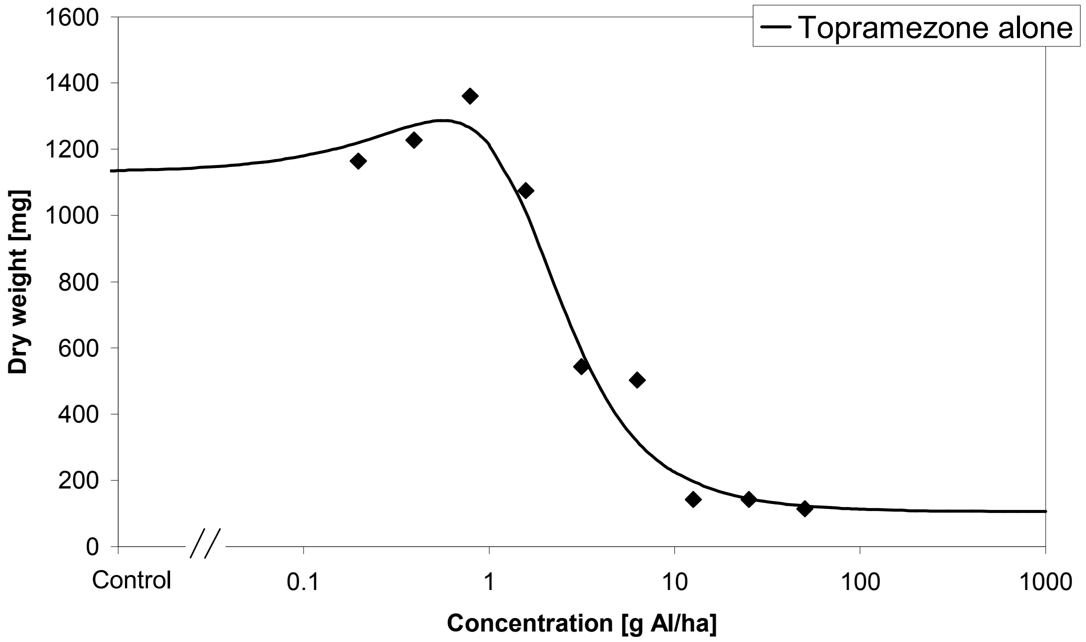

Fig. 6: Mean biomass and modelled dose-response curve of *Abutilon theophrasti* exposed to topramezone applied without adjuvant. Recommended field rate for topramezone: 50.4 g AI ha^{-1}. Regression parameters: *D* = 1130.2, *C* = 105.2, *b* = 2.1, *ED50* = 1.6 and *f* = 529.4.

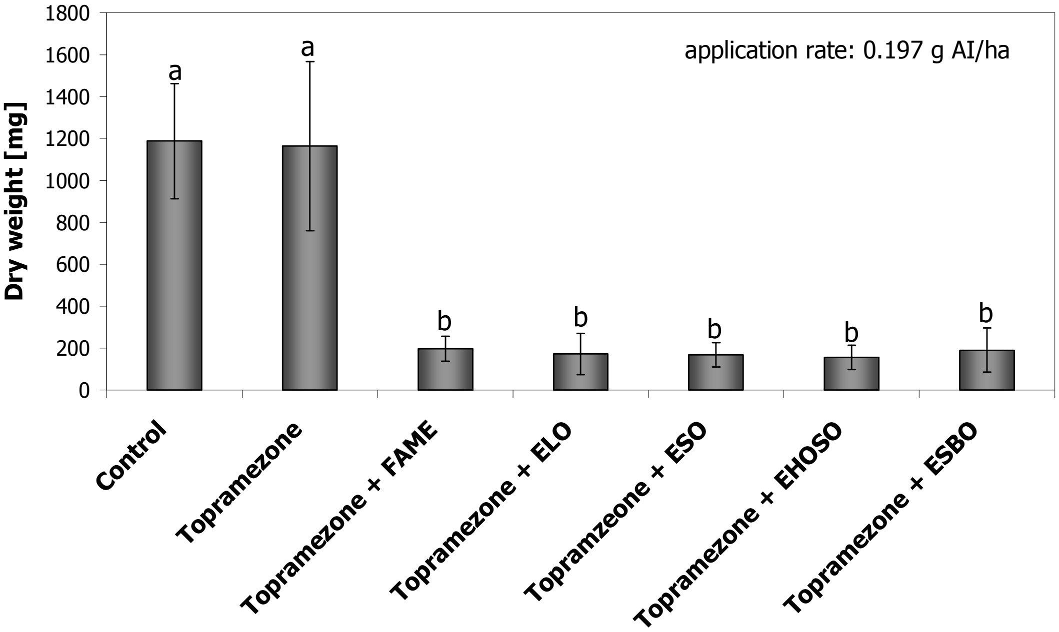

Fig. 7: Means of dry weight of *Abutilon theophrasti* after application of topramezone at a concentration of 0.197 g AI ha^{-1}. Different letters indicate significant differences at α = 0.05 % (*T*-test). Error bars show standard deviation.

Sulfosulfuron

The test for lack-of-fit for the sulfosulfuron data yielded a p-value of 0.33 which is not significant at 5 % and hence, the nonlinear regression model provided an acceptable description of observed data. A comparison of all six curves demonstrated, that the slope of the curve for ELO was significantly steeper as the other curves (Fig. 8). All additives decreased the amount of sulfosulfuron required to achieve a 50 % reduction in dry weight compared to sulfosulfuron alone. However, the differences were not significant. The recommended adjuvant EFA showed the best effect on sulfosulfuron performance compared to the other adjuvants and decreased the *ED50* of the herbicide by 19-fold. For the adjuvants ELO, EHOSO and ESBO, the efficacy enhancing effect was less pronounced with an average reduction of the *ED50* of sulfosulfuron by 6.7-fold (± 0.7). ESO decreased the *ED50* by 3.5-fold and, hence, was the least effective adjuvant. Regarding the *ED90* values, all adjuvants reduced the necessary amount of sulfosulfuron. Sulfosulfuron mixed with ELO showed the best efficacy on velvetleaf biomass compared to all other treatments. It was 9.0-fold more effective than EFA and on average 4.4-fold (± 0.9) more effective than ESO, EHOSO and ESBO. With an application rate of 91 g AI ha^{-1} for a 90 % control of velvetleaf, sulfosulfuron mixed with EFA was the weakest herbicide-adjuvant combination.

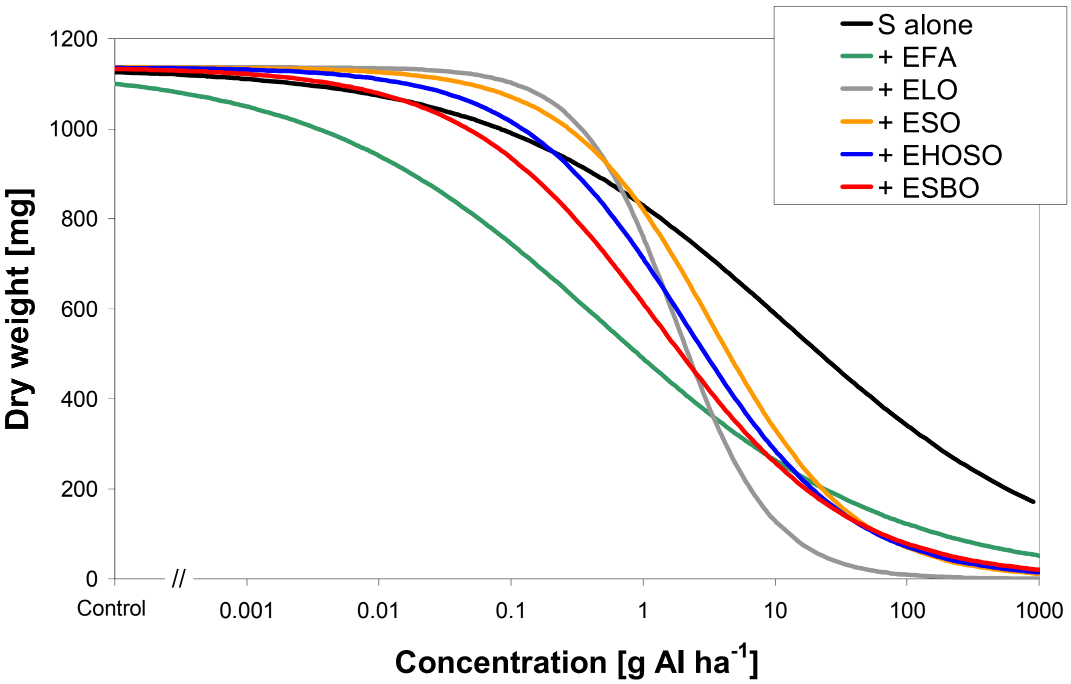

Fig. 8: Modelled dose-response curves of sulfosulfuron (S) applied with and without adjuvant to *Abutilon theophrasti*. Recommended field rate for sulfosulfuron: 20 g AI ha^{-1}.

Carfentrazone-ethyl

With a p-value of 0.86, the nonlinear regression model for carfentrazone-ethyl described the observed data quite well (Fig. 9). The *ED50* of carfentrazone-ethyl could be reduced by the addition of adjuvants, whereas EHOSO and PS showed the best efficacy on carfentrazone-ethyl reducing the ED50 by 3.9-fold (± 0), followed by ESBO (*rED50* = 3.4). ELO and ESO decreased the amount of carfentrazone-ethyl required to achieve the *ED50* by 1.4- and 1.8-fold, respectively, compared with carfentrazone-ethyl alone and, thus, were less effective than the other adjuvants. Comparing *ED90* values showed that at this response level only ESO distinctly enhanced the efficacy of carfentrazone-ethyl (*rED90* = 2.0). Moreover, the addition of PS and ELO even increased the amount of carfentrazone-ethyl needed to achieve a 90 % response by 1.7- and 2.9-fold, respectively.

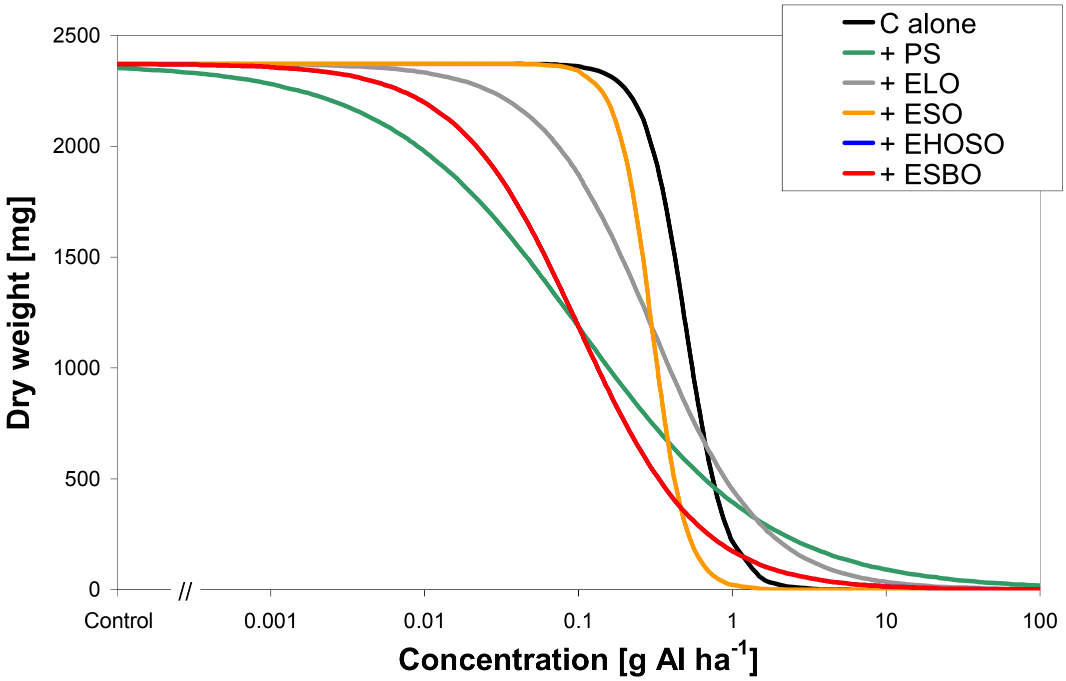

Fig. 9: Modelled dose-response curves of carfentrazone-ethyl (C) applied with and without adjuvant to *Abutilon theophrasti*. Recommended field rate for carfentrazone-ethyl: 20 g AI ha^{-1}. For C+EHOSO and C+ESBO identical curves were modelled.

Foramsulfuron & iodosulfuron (FI)

Plotted FI data showed, that the nonlinear regression model fitted the data (p = 0.07; Fig. 10). It was observed, that all adjuvants significantly decreased the amount of FI needed to get a 50 % reduce of biomass by 5-fold (± 1.5). Furthermore, ELO was even more effective than the recommended ROME (*rED50* = 1.3). Regarding the *ED90*, all adjuvants reduced biomass by 96 % (± 1.7) compared with FI applied alone. However, this reduction was not significant. ELO was also the most effective adjuvant (*rED90* = 44.2), followed by ESBO (*rED90* = 38.1) and ESO (*rED90* = 32.1). EHOSO represented the least effective seed oil (*rED50* and *ED90* = 2.6 and 16.7) in combination with FI.

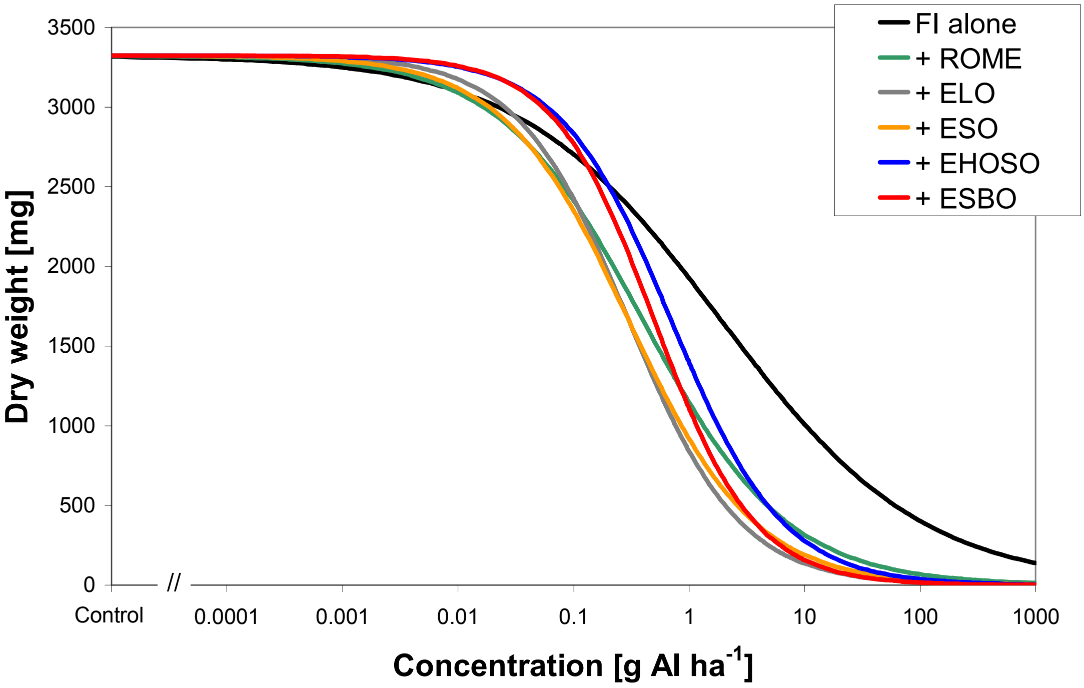

Fig. 10: Modelled dose-response curves of foramsulfuron & iodosulfuron (FI) applied with and without adjuvant to *Abutilon theophrasti*. Recommended field rate for foramsulfuron & iodosulfuron: 46.5 g AI ha^{-1}.

Tab. 4: Regression parameters and corresponding standard deviation of dose-response-curves. *D* = upper limit, *b* = slope, *ED50* = dose with 50 % response and *ED90* = dose with 90 % response.

	Regression Parameters							
Treatments	**D [mg]**		**b**		**ED50 [g ai/ha]**		**ED90 [g ai/ha]**	
Sulfosulfuron (S)	1136.8	± 52.7	0.4	± 0.2	12.0	± 8.2	2560.7	± 6092.4
S + EFA	1136.8	± 52.7	0.4	± 0.1	0.5	± 0.3	97.4	± 124.0
S + ELO	1136.8	± 52.7	1.2	± 0.3	1.8	± 0.4	10.4	± 4.9
S + ESO	1136.8	± 52.7	0.8	± 0.2	3.3	± 1.1	48.7	± 35.2
S + EHOSO	1136.8	± 52.7	0.7	± 0.2	2.1	± 0.8	54.3	± 44.6
S + ESBO	1136.8	± 52.7	0.6	± 0.2	1.3	± 0.5	51.0	± 44.9
Carfentrazon-ethyl (C)	2371.6	± 82.9	3.3	± 1.0	0.5	± 0.1	0.9	± 0.2
C + PS	2371.6	± 82.9	0.7	± 0.2	0.1	± 0.0	2.4	± 1.4
C + ELO	2371.6	± 82.9	1.2	± 0.2	0.3	± 0.1	1.8	± 0.7
C + ESO	2371.6	± 82.9	3.9	± 1.3	0.3	± 0.0	0.5	± 0.1
C + EHOSO	2371.6	± 82.9	1.1	± 0.3	0.1	± 0.0	0.8	± 0.3
C + ESBO	2371.6	± 82.9	1.1	± 0.3	0.1	± 0.0	0.9	± 0.4
Foramsulfuron + iodosulfuron (FI)	3323.7	± 83.8	0.5	± 0.06	1.9	± 0.4	148.6	± 84.1
FI + ROME	3323.7	± 83.8	0.7	± 0.1	0.4	± 0.1	7.8	± 3.0
FI + ELO	3323.7	± 83.8	0.9	± 0.2	0.3	± 0.1	3.4	± 1.3
FI + ESO	3323.7	± 83.8	0.8	± 0.1	0.3	± 0.1	4.6	± 1.9
FI + EHOSO	3323.7	± 83.8	0.9	± 0.1	0.7	± 0.1	8.9	± 2.7
FI + ESBO	3323.7	± 83.8	1.0	± 0.2	0.5	± 0.1	3.9	± 1.4

2.1.5. Discussion and Conclusions

Generally, in this study the efficacy of every herbicide was improved after adding one of the seed oil adjuvants. The fact that modified seed oils improve herbicide efficacy is already known and was reviewed over 20 years ago by Gauvrit & Cabanne (1993). An increased spreading of spray droplets on target plants and an enhanced penetration of active ingredients into leaves seem to be the reasons for the herbicide enhancing action of seed oils (Liu, 2004). Since many oil-based adjuvants act well as penetration enhancers, it can be assumed, that the herbicide enhancing effect of seed oil adjuvants can be attributed mainly to this mechanism (Stock & Briggs, 2000). Though most adjuvants act by more than one mechanism, penetration enhancers might demonstrate poorer retention properties (Stock & Briggs, 2000). However, the mechanisms involved in penetration of seed oils is still not completely understood (Gauvrit & Cabanne, 1993).

In this study, ELO demonstrated the best efficacy in combination with the sulfonylureas, whereas ESO improved was most effective with carfentrazone-ethyl. Since it is known, that the efficacy of sulfonylureas can be reduced by precipitation within a few hours after application (Russell et al., 2002), an increased rainfastness of those herbicides might have been achieved by the addition of the ethoxylated linseed oil in this study. In an experiment conducted by Hunsche & Noga (2008) it was proved, that ethoxylated linseed oils showed a better effect on rainfastness of the fungicide mancozeb compared to ethoxylated soybean oils.

Unfortunately, no useful information could be found about the usage of safflower oils as adjuvants. But the fact, that ESO decreased the amount of carfentrazone-ethyl necessary to achieve a 90 % weed control by 2-fold implies, that there is a potential for the use of modified safflower oils as adjuvants. In this study, ESBO acted 2-fold (± 0.5) better compared to the recommended adjuvants. Thus, ESBO could present an alternative adjuvant for a widespread use. However, EHOSO demonstrated the least pronounced effect in combination with all herbicides and hence, does not show a good potential compared with ELO, ESO and ESBO.

Those results imply that an addition of ethoxylated seed oils could be an approach to reduce herbicide application rates while still maintaining a sufficient weed control. However, the problem is, that reducing the application rate, which is recommended by chemical companies, would result in a loss of the companies guarantee (Duchesne et al., 2004). Moreover, reduced application rates are susceptible to different environmental factors (Medd et al., 2001), and hence, the herbicide efficiency at reduced doses is not predictable and thus, not reliable outside the greenhouse. Results of an experiment conducted by Zhang et al. (2000) demonstrated, that the addition of adjuvants to below-labeled herbicide rates did not show any improvement of herbicide efficacy. Thus, the general concept of increasing the uptake of reduced herbicide rates with the help of adjuvants to achieve an adequate weed control might be refuted. On the other hand, with full application rates and the usage of adjuvants, difficult to control weeds might be completely examined.

All herbicides showed a week potency when applied alone in the present study. This could be caused by a crystallization of the herbicide on the leaf surface after drying of the spray droplet. However, it is important, that herbicides are available in a dissolved form to be taken up by plants (Baur & Schönherr, 1996). Furthermore, for topramezone even a hormesis effect was observed. This effect is described for a range of herbicides when they stimulate plant growth at low doses (Cedergreen, 2008). It can be concluded, that only a small amount of topramezone was taken up by *A. theophrasti*. For topramezone it is not possible to make a proposition about the best matching adjuvant, because the addition of any adjuvant to the lowest application rate of 0.2 g ha^{-1} already resulted in 85 % (± 1.4) weed control. Therefore, topramezone should be dosed lower than 0.2 g ha^{-1} to evaluate differences between the recommended and seed oil adjuvants. The decreased efficiency of carfentrazone-ethyl in combination with the recommended commercial adjuvant product might have been caused by a too high application rate of PS. If this was the case, the herbicide probably was dissolved in the adjuvant and both drained of the leaves.

With this study, it was shown, that the application rate of sulfosulfuron, foramsulfuron & iodosulfuron, carfentrazone-ethyl and topramezone could be reduced when a suitable adjuvant was added. Furthermore, it can be concluded, that a higher content of unsaturated fatty acids affected the herbicidal efficacy in a positive way.

Only with an appropriate adjuvant, herbicides can develop their maximum efficacy – even at lower than recommended doses. Ethoxylated seed oils contain a high potential as biodegradable adjuvants for herbicides, whereas further investigations on additional weeds and field trials have to be conducted.

Acknowledgement

We thank BASF Personal Care & Nutrition GmbH (Cognis legacy) for providing the ethoxylated seed oil adjuvants and funding of this work, and Sybille Berger and Heike Popovitsch for their indispensable help.

2.2. Fractioning of an ethoxylated soybean oil adjuvant and studies on the potency of the fractions in combination with bromoxynil octanoate and sulfonylureas

Accepted by the *Journal of Plant Diseases and Protection* in December 2012

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2.2.1. Abstract

Adjuvants are commonly used to improve the efficacy of pesticides, both as part of formulations or as a separate addition to the spray tank. One such adjuvant is an ethoxylated soybean oil (ESBO). As the chemical composition of ESBO and the fractions mainly responsible for the adjuvant-effect are unknown, the aim of the present study was to find out the composition of ESBO applying preparative High-Performance Liquid Chromatography (prep. HPLC) and to evaluate the adjuvant-effect of isolated fractions in comparison to the commercial product. By prep. HPLC, four main fractions could be determined and isolated. *Abutilon theophrasti* (velvetleaf) and *Zea mays* (maize) were used as test species to evaluate the adjuvant effect of isolated fractions or ESBO on the herbicidal efficacy of bromoxynil octanoate, foramsulfuron & iodosulfuron, and technical grade bromoxynil potassium. Experiments were conducted under greenhouse conditions and dry weight of leaves, leaf area, and quantum yield of photosystem II were evaluated as response parameters. Leaf area and dry weight responses showed that there was a tendency towards late eluting (non-polar) fractions enhancing the efficacy of hydrophilic herbicides to a higher extent than earlier eluting (polar) fractions. For example, foramsulfuron & iodosulfuron in combination with the most non-polar fraction resulted in a 61 % lower dry weight of velvetleaf compared with the herbicide treatment without adjuvant. The analysis of quantum yield resulted in the lowest values for the application of bromoxynil octanoate (BO) mixed with ESBO and foramsulfuron &

iodosulfuron (FI) combined with the most polar fraction, respectively. The detection of an active compound within an adjuvant could be a new direction for additive optimization and, hence, very precise recommendations for herbicide-adjuvant mixtures might be deduced.

2.3. Uptake studies on a fluorescein-labelled seed oil adjuvant in **Abutilon theophrasti***,* **Sinapis arvensis** *and* **Beta vulgaris**

Published in *Gesunde Pflanzen*, December 2012, Vol. 64, pp 167-174.

Authors: Julia Heini, Victor Rueda Ayala, Frank Walker, Hans-Georg Mainx & Roland Gerhards

2.3.1. Abstract

Though adjuvants are abundant almost everywhere in agricultural practice, their mechanism and mode of action, especially regarding herbicide activity, is still not completely clear. Studies on their way through the cuticle are quite difficult due to a lack of radiolabelled surfactants. In this study we want to present the results of an experiment established to evaluate the mode of action of Agnique® SBO 10, an ethoxylated soybean oil (ESBO) adjuvant. ESBO belongs to a group of environmental friendly and biodegradable additives and can be classified as surfactant. To evaluate the route of ESBO through the cuticle it was labelled with fluorescein and applied to one leaf of each test species. As experimental plants served *Abutilon theophrasti* Medik. (velvetleaf), *Sinapis arvensis* L. (wild mustard) and *Beta vulgaris* L. (sugar beet). Treated leaves were harvested at different time intervals and rinsed with either deionised water (DW), methanol/water (MW) or chloroform. The washed leaves and the solution in which they were washed were processed and further analysed with High-Performance Liquid Chromatography (HPLC) coupled with an UV-detector. Because fluorescein is not light-stable, trials were conducted under dark and illuminated conditions. Under dark conditions, where photochemical degradation processes can be excluded, the flourescein labelled Agnique® SBO 10 (AF) content of leaf extracts was almost 3-fold lower compared to the initial content while the measured AF in the DW and MW washes displayed fairly constant values. Hence, AF might have been passed through the cuticle. If this holds true, AF was able to reach the underlying cell tissue where it might have been metabolised or even translocated to other plant parts. However, it is more likely

that AF in this case was enzymatically degraded. Though a lot of research has to be conducted on the mechanism and mode of action of adjuvants, this study gives an approach to investigate the behavior of foliar applied adjuvants.

3. General Discussion

Agnique® SBO 10

It was demonstrated, that Agnique® SBO 10 has the potential to enhance the efficacy of sulfosulfuron, topramezone, carfentrazone-ethyl and foramsulfuron & iodosulfuron against a difficult-to-control weed species under greenhouse conditions (see chapter 2.1). Agnique® SBO 10 even showed a better effect on the herbicide efficacy compared to the commercially recommended adjuvants. Based on these results, Agnique® SBO 10 might be considered as an environmental friendly adjuvant with a broad field of application. Under the applied trial conditions, the herbicide rate for an adequate control of *A. theophrasti* could be reduced by mixing the herbicides with Agnique® SBO 10.

Reduced application rates

Pressure to reduce herbicide amounts constantly grows, and studies with reduced herbicide rates were conducted to evaluate the efficacy of below labelled rates. Nevertheless, reducing application rates bears a risk in the reliability of the plant protection product, as the efficacy of lower rates might be more susceptible to negative environmental conditions. Consequently, the industry cannot put recommendations on the herbicide labels because the success of reduced rates is not always predictable (Blackshaw et al., 2006). Though Zhang et al. (2000) found out, that the success of reduced herbicide rates is independent of the usage of adjuvants, adjuvants might still maintain a constant herbicide efficacy under unfavourable conditions. For example, the rainfastness of the fungizide mancozeb was enhanced when ethoxylated seed oils were added to the spray mix (Hunsche & Noga, 2008). In contrast, the efficacy of glyphosate under low humidity could be increased adding an ethoxylated alcohol adjuvant and ammonium sulphate (Kudsk & Mathiassen, 2007). To test, whether Agnique® SBO 10 is able to stabilize and guarantee the efficacy of reduced herbicide rates under different environmental conditions, field trials should be accomplished.

Selctivity

As adjuvants are able to modify the physical properties of epicuticular waxes, an increased uptake of active ingredients, leading to an enhanced herbicide efficacy is likely to occur (Gauvrit & Cabanne, 1993). However, not only the leaf surface of weeds is affected, but also the crop surface. Dayan et al. (1996) reported, that soybeans treated with sulfentrazone in combination with a surfactant showed 55 % foliar damage, whereas the herbicide alone did not cause severe injuries. This effect probably was caused by a higher herbicide uptake into the crops. If the selectivity of a herbicide is based on differenences in retention, an increased surfactant concentration might decrease the selectivity and is dependent on the crops' leaf microroughness (De Ruiter et al., 1990). Nevertheless, in this study it was demonstrated that Agnique® SBO 10 does not decrease the selectivity of maize against bromoxynil octanoate, bromoxynil potassium and foramsulfuron & iodosulfuron (see section 2.2). Small chlorotic spots were temporarily observed one week after application on all treated leaves, independently of their treatment (see also Appendix 3) but they did not have negative impacts on leaf area, chlorophyll fluorescence and dry weight. This observation obtained from greenhouse studies must be confirmed in field studies, to prove, that Agnique® SBO 10 is completely safe to crops in combination with different herbicides.

Dose-response studies as usefull "tool"

For the assessment of the efficacy and selectivity of herbicides, dose-response studies are a useful tool (Streibig et al., 1993). However, the trial layout must be chosen carefully to get useful data for fitting dose response curves. Constant setting conditions are crucial for successful experiments. Although we conducted the dose-response studies in the greenhouse, large standard deviations in dry weight of plants within one treatment were observed. Plants close to the door grew smaller and less developed than test species cultivated in the centre of the greenhouse. For the data evaluation those trials could not be used. Increasing the amount of replications could be a solution if there is no possibility to unify environmental conditions. Furthermore, choosing a suitable test species is very important. In pre-tests we cultivated wild mustard (*Sinapis arvensis* L.), redroot pigweed (*Amaranthus retroflexus* L.) and black nightshade

(*Solanum nigrum* L.). For our purpose, none of the weed species were suitable. Wild mustard and redroot pigweed were too sensitive when cultivated under greenhouse conditions and were completely controlled when treated with 50 % of the recommended herbicide rate (unpublished greenhouse studies). As a consequence, no dose-response curves could be fitted. Black nightshade demonstrated an unreliable germination and needed a pre-cultivation at very high temperatures (> 30 °C). It was difficult to get enough plants displaying a similar development stage at the application date. Despite of this reason, black nightshade could be an appropriate species. In contrast to the before mentioned species, velvetleaf (*Abutilon theophrasti*) showed a consistent and high germination rate, the germ bud is relatively solid and the plant tolerates higher amounts of herbicides. Therefore, it demonstrated to be the perfect test species for our experiments.

With our experimental setup it was possible to evaluate differences between adjuvants with similar properties. Nevertheless, it is questionable whether slight differences would be notified in field dose-response studies. Additionally, dose-response studies are very time and space consuming and varying weed densities and species spectrum per plot might impede data evaluation. Therefore, another field trial design with fewer herbicide concentrations (e.g. 50, 75 and 100 % of the recommended rate) and probably more herbicides with different modes of action should be considered to test the potency of adjuvants on herbicides in future studies.

Fractioning of Agnique® SBO 10 – a new method

Agnique® SBO 10 is a big molecule with a complex structure; therefore, an in-can formulation is not simply possible. In Europe, however, a ready-mixed product which is easy to apply might be of greater interest. With fractioning Agnique® SBO 10, it was demonstrated that there exist fractions, which are more effective on the potency of bromoxynil octanoate and foramsulfuron & iodosulfuron compared to the complex molecule. As far as we know, fractioning an adjuvant with prep. HPLC to identify an active compound within the adjuvant was never conducted before. With this study we delivered a new approach for the investigation of adjuvants. It was our aim to isolate different fractions and to find out whether one fraction has the potential to be used as highly active small molecular adjuvant. It was possible to fractionate Agnique® SBO 10 into four different factions with different polarities (Frc. 1 > 2 > 3 > 4). Dry weight and leaf area investigations revealed that the efficacy of FI and BP was increased with decreasing polarity of isolated fractions and the addition of more polar fractions tendentially enhanced the efficiency of BO (see section 2.2; for bispectral figures see also Appendix 4).

As it was only possible to isolate small amounts of fractions, a droplet application had to be conducted. The used droplet size of 2 µm does not reflect a practical pesticide application. However, we have chosen the droplet size of 2 µl deliberately because leaves of *A. theophrasti* were too small to apply a higher quantity of smaller droplets. Though only a limited amount of adjuvant fractions was available, we wanted to provide enough herbicide to the plants to generate phytotoxicity. Due to the fact that the droplet size plays an important role for the uptake of chemicals (e.g. Hall et al. 1993; Zabkiewicz, 2000), whole plant applications must be conducted with a track sprayer containing a customary nozzle. Therefore, a higher amount of fractions must be produced synthetically. This could be done after an exact chemical analysis of the fractions by mass spectrometry (MS). In a next step, the transferability of the obtained results into the field has to be approved.

With fractioning Agnique® SBO 10, a new approach in adjuvant research was presented, possibly leading to the production of more efficient, small molecular adjuvants which can be integrated in a formulation, simplifying the application for the user.

Labelling of Agnique® SBO 10 with fluorescein

The evaluation of the fate of Agnique® SBO 10 on leaf surfaces was accomplished by labeling Agnique® SBO 10 with fluorescein (AF; section 2.3). We wanted to find out whether the adjuvant penetrates into the test species, accumulates in the wax layer of the cuticle or even remains on the leaf surface. Generally, it was possible to detect the applied AF with the developed HPLC-method. However, AF approved to be light-instable, limiting its applicability for penetration studies. The light instability was confirmed in an experiment where 20 ml of the AF application solution (5 g L^{-1}) were filled in glass Petri dishes and exposed to different illumination regimes. The photochemical degradation of AF could even be observed visually (for figure see Appendix 5 A - C). The AF content of the application solution decreased very fast under greenhouse illumination (section 2.3 Fig. 17; see also Appendix 5 C), whereas almost no decrease was observed for the storage under dark conditions (section 2.3 Fig. 17; see also Appendix 5 A). The application solution exposed to ambient laboratory illumination showed an intermediate AF decrease (section 2.3 Fig. 17; see also Appendix 5 B).

For this reason, only the results of the studies with dark adapted velvetleaf plants should be considered. Under dark conditions, the AF content in the washing solutions was stable at every assessment date, whereas the AF content of the analyzed leaves decreased. Since a photochemical degradation can be excluded in the absence of light, we assume that AF penetrated into the plant tissue. With this method, a differentiation between a penetration into the cuticle and diffusion into the underlying cell tissue is not possible. Therefore, petioles, and in a next step also plant stems and roots of treated and dark adapted plants, should be collected and analyzed separately. Since it was observed for radiolabelled activator adjuvants that they pass through the cuticle (Stock & Holloway, 1993), such a process could also be possible for Agnique® SBO 10. However, Urvoy et al. (1992) noted that immobile compounds like Agnique® SBO 10 are relatively immobile and a transport into the plant tissue is rather unlikely. Because the mode of action of adjuvants is strongly dependent of the cuticle properties of the plant (Kirkwood, 1993), further species with different leaf surfaces should be tested under dark conditions. To avoid a dark adaptation of test species, other tracers, like the fluorescent dye tracer brilliant sulfaflavine might be used as a more suitable tracer for vegetable oil adjuvants due to its long persistence after exposure to sunlight (Cai & Stark, 1997).

Generally, experiments researching adjuvants should be conducted in climate chambers with defined air humidity. Adjuvant and herbicide efficacy is directly linked to the relative humidity. High humidity can prevent the spray droplet of rapid drying on the plant surface, thus keeping the herbicide in solution which makes it available for an uptake (Ramsey et al., 2005). Therefore, accomplishing experiments under different humidity levels might deliver important information about the environment in which a certain adjuvant could develop its best herbicide-enhancing effect.

Outlook

With this study it was demonstrated, that the efficacy of herbicides can be further increased when a proper adjuvant is added. This can be done by either integrating the adjuvant into the formulation or by mixing it to the spray tank. Giving exact recommendations for perfect mixtures is very difficult because the potency of adjuvants and herbicides is dependent of many factors. Adjuvants are specific to each herbicide, plant species, and environmental conditions (Zollinger, 2000). Factors like relative humidity, temperature, and the cuticle properties (e.g. thickness, trichomes) can influence the potency of adjuvants and make it difficult for growers to choose the right adjuvant (Zollinger, 2000). Nevertheless, in future, the amount of herbicides needed to achieve a sufficient weed control could be decreased with well-designed adjuvant-herbicide mixtures and consequently environmental impact could be reduced.

Considering all the above mentioned factors, which influence the herbicide efficacy, the development of a direct-injection system could be an approach. With this system, herbicide-adjuvant combinations could be mixed directly in the field with respect to the weed species and their distribution.

4. References

Introduction

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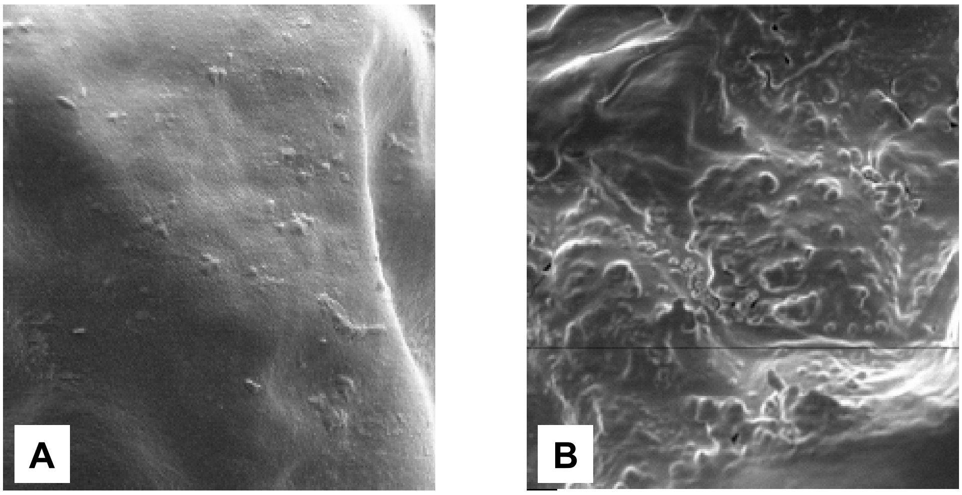
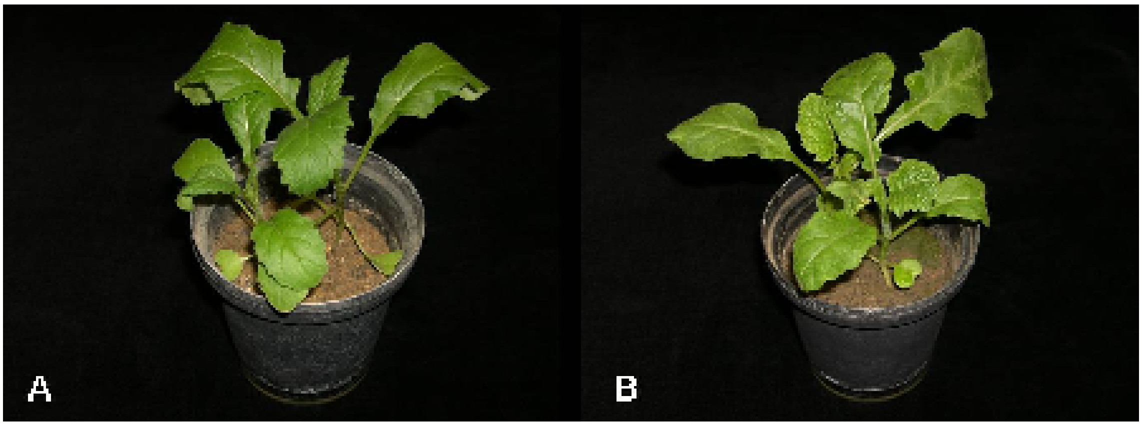
5. Appendices

5.1. List of Appendices

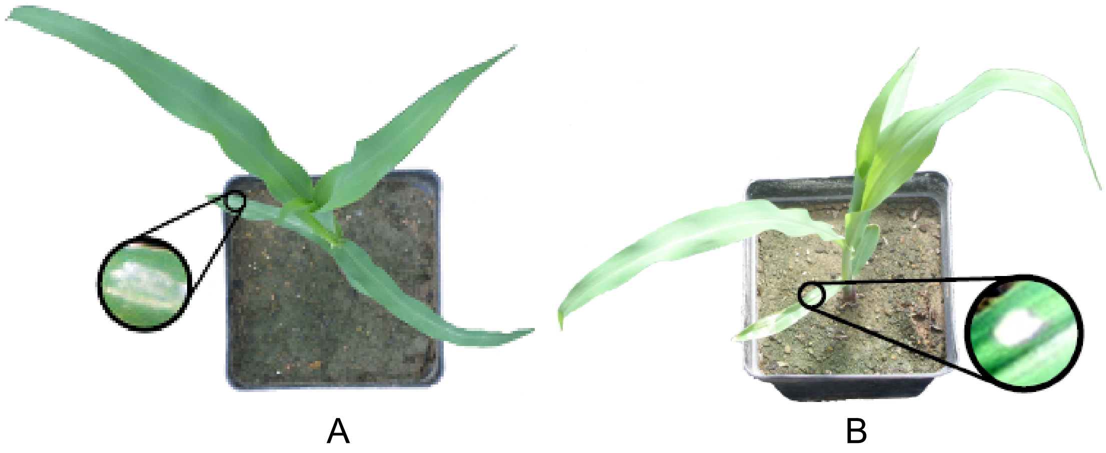
Appendix 1:	Scanning Electron Microscopy (SEM) pictures of the leaf surface of *Sinapis arvensis*. Untreated leaf surface of *S. arvensis* (A) and surface of *S. arvensis* after treatment with Agnique® SBO 10 (B).
Appendix 2:	Sinapis arvensis grown in the greenhouse eight days after treatment. (A) untreated control plant. (B) treated with 4 % (v/v) Agnique® SBO 10.
Appendix 3:	Zea mays one week after treatment with foramsulfuron & iodosulfuron (A) and foramsulfuron & iodosulfuron plus fraction 4 (B). Enlarged leaf sections show chlorotic tissue.
Appendix 4:	Bispectral pictures of Abutilon theophrasti 14 days after treatment with (A) bromoxynil octanoate (BO), (B) bromoxynil potassium (BP), and (C) foramsulfuron & iodosulfuron (FI). Herbicides were applied singularly or mixed with fractions 1, 2, 3, 4, or ESBO, respectively.
Appendix 5:	AF solution (5 g L-1) incubated without light (A), exposed to laboratory light (B, Ø 8.4 µmol m-2s-1) and to greenhouse light (C, Ø 220.1 µmol m-2s-1) for 24 hours.



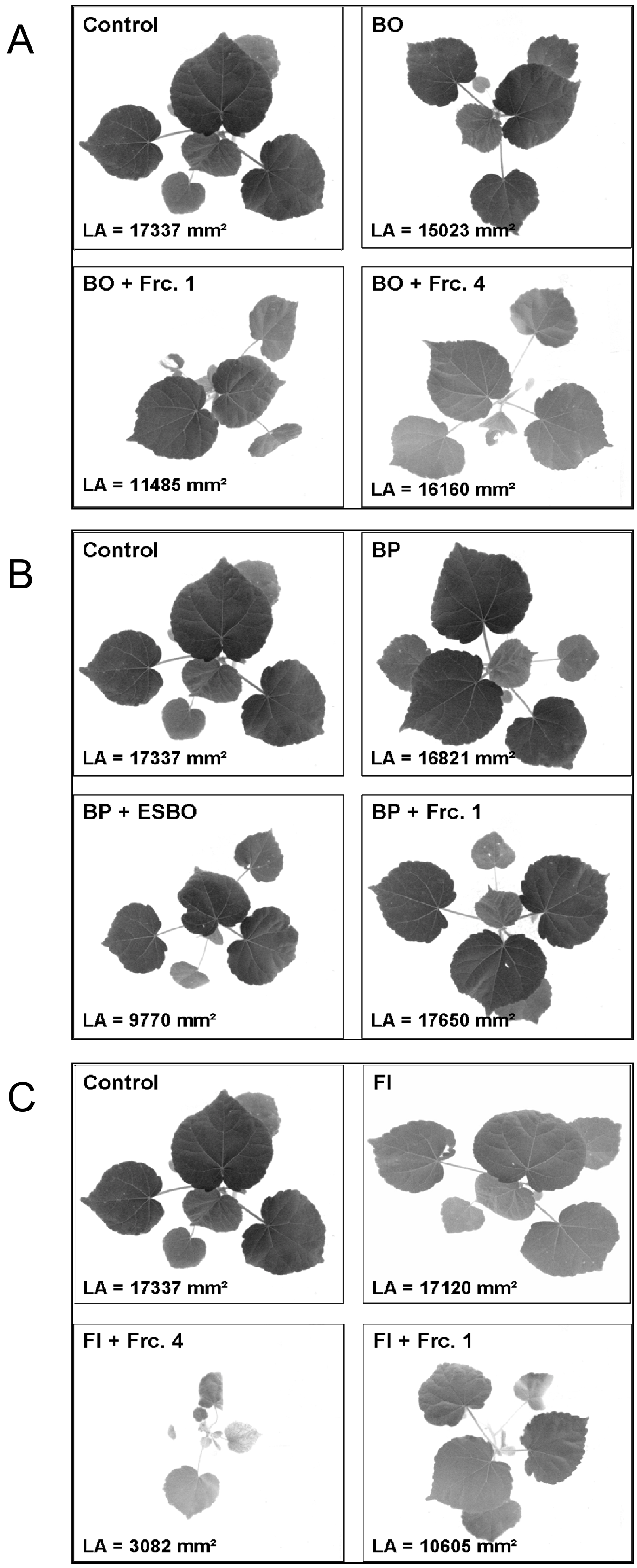
Appendix 1: Scanning Electron Microscopy (SEM) pictures of the leaf surface of Sinapis arvensis. Untreated leaf surface of S. arvensis (A) and surface of S. arvensis after treatment with Agnique® SBO 10 (B).



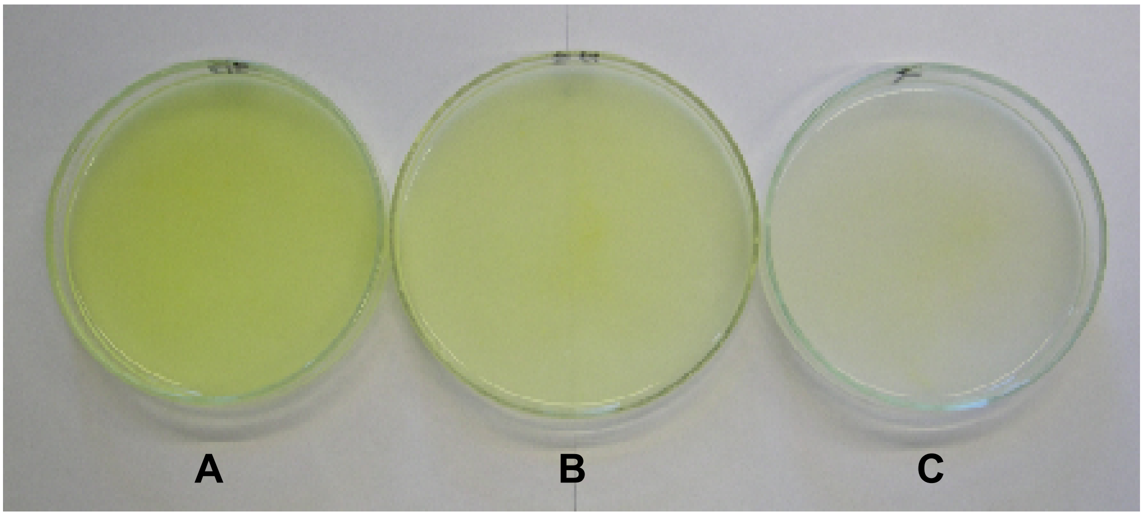
Appendix 2: *Sinapis arvensis* grown in the greenhouse eight days after treatment. (A) untreated control plant. (B) treated with 4 % (v/v) Agnique® SBO 10.



Appendix 3: *Zea mays* one week after treatment with foramsulfuron & iodosulfuron (A) and foramsulfuron & iodosulfuron plus fraction 4 (B). Enlarged leaf sections show chlorotic tissue.



Appendix 4: Bispectral pictures of *Abutilon theophrasti* 14 days after treatment with (A) bromoxynil octanoate (BO), (B) bromoxynil potassium (BP), and (C) foramsulfuron & iodosulfuron (FI). Herbicides were applied singularly or mixed with fractions 1, 2, 3, 4, or ESBO, respectively.



Appendix 5: AF solution (5 g L^{-1}) incubated without light (A), exposed to laboratory light (B, Ø 8.4 µmol $m^{-2}s^{-1}$) and to greenhouse light (C, Ø 220.1 µmol $m^{-2}s^{-1}$) for 24 hours.

Acknowledgement

First of all I would like to thank Professor Dr. Roland Gerhards who gave me the opportunity to conduct a very interesting thesis at the Department of Weed Science. I further thank BASF Personal Care & Nutrition GmbH (Cognis legacy) for the funding of this work and especially Dr. Hans-Georg Mainx for the cooperative work. Without the help of Dr. Frank Walker, Birgit Höglinger and Jochen Schöne of the Central Chemical-Analytical Laboratory it would not have been possible to accomplish this work. They supported me in all aspects of chemical analyses and helped me to implement my experiments. I would particularly thank PD Dr. Regina Belz and Dr. Victor Rueda Ayala for reviewing parts of my thesis and for their valuable advice. Many thanks also go to all other persons who were involved in my work, namely Alexandra Heyn and Cathrin Reichert for ruling the laboratory and for their support. Heike Popovitsch and Sibylle Berger for their patience with exhausting planting and harvesting sessions. All colleagues for precious discussions and the good time. My family, friends and Lorenz who always believed in me and supported me wherever possible.

Curriculum Vitae

Personal Details

Name: **Julia Heini**
Date of Birth: **6 January, 1983**
Born in: **Friedrichshafen, Germany**
Nationality: **German**

Work Experience

01/2012 – 06/2012	**Dr. KNOELL Consult GmbH, Mannheim, Germany**
06/2008 – 09/2011	**PhD student at the Institute for Phytomedicine, Department of Weed Science, University of Hohenheim, Stuttgart, Germany**
12/2007 – 03/2008	**AgResearch, Lincoln, New Zealand** Internship in the Department of Forage Improvement
07/2006 – 09/2006	**Bayer CropScience GmbH, Frankfurt am Main, Germany** Internship in the Department of Profiling and Support
08/2004 – 09/2004	**Agriturismo *La Ginestra*, Florence, Italy** Internship at an ecological farm

Education

10/2003 – 06/2008	**Student at the University of Hohenheim, Stuttgart, Germany** Final examination: Degree in Agricultural Biology (Diploma) Main focus of study: Plant Protection, Plant Nutrition and Plant Physiology
04/2007 – 12/2007	**Thesis at the Department of Weed Science, University of Hohenheim, Stuttgart, Germany** Topic: “Studies on the production dynamics of actones in *Parthenium hysterophorus* L. and to the effect of different environmental conditions on the hormesis-effect of the sesquiterpenlactone Parthenin.“
10/2002 – 04/2003	**Student at the University of Konstanz, Germany** Subjects: Chemistry, English and German
1999 – 2002	**Droste-Hülshoff-Gymnasium, Friedrichshafen, Germany** Degree: Abitur (equivalent to university entrance qualification)

Julia Heini
Stuttgart, December 2012