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Influence of rapeseed oil ethoxylate surfactants on retention and biological efficacy of glyphosate spray solutions in selected weeds

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Summary

Chemical weed control plays a major role in increasing productivity of modern cropping systems. Surfactants are used to improve the biological efficacy of foliar-applied herbicides. However, questions have been raised about the safety of the commonly used active ingredients and surfactants. As a consequence of the increasing pesticide safety demands and restrictive regulatory limitations on pesticide use, researchers are looking for new adjuvants with improved biological and ecotoxicological profiles. With this in mind a newer group of vegetable oils, namely rapeseed oil ethoxylates (RSO), was studied. Rapeseed oils with 5 (RSO 5), 10 (RSO 10), 20 (RSO 20), 30 (RSO 30), and 60 (RSO 60) ethoxylation units were added to glyphosate spray solutions and applied to different weed species (*Amaranthus retroflexus, Datura stramonium, Setaria viridis, Viola arvensis*). The influence of these oils on spray retention as well as the biological efficacy of glyphosate was examined, using electron microscopy, spectroscopy, chlorophyll fluorescence, and dry matter determination.

Scanning electron microscopy pictures of the weed leaf surfaces were taken and displayed fundamental differences in the epidermal, cuticular and epicuticular structures of the leaf surfaces. Beside different habits and leaf structures these results were regarded as reasons for quantitative differences in the mean spray solution deposited. Further tests demonstrated that there was no correlation between retention behaviour and biological efficacy. Maximum chlorophyll fluorescence measurements showed similar or higher impact of glyphosate when rapeseed oils were added in comparison with a treatment of Roundup Ultramax®. Good correlation was shown between hydrophilicity/ethoxylation of the solution and dry matter content. In *D. stramonium* and *V. arvensis* increasing ethoxylation led to decreasing dry mass. The opposite could be shown in *S. viridis. Amaranthus retroflexus* had the lowest dry mass when glyphosate was applied with RSO 20. The rapeseed oil ethoxylates used showed promising impacts on glyphosate spray solutions but further studies need to be undertaken to clarify other processes of the foliar application of pesticides.

Introduction

The application of spray solutions must be seen as a series of interdependent events (Downer et al., 1999; Bukovac et al., 2003) occuring consecutively or simultaneously. Following the atomization of the spray solution, droplets deposit on the target area, in agriculture mostly on plant surfaces, but also on soil. The application of pesticides with hydraulic sprayers is an inefficient process with typically less than 1 % of the applied active ingredient reaching the target (Hall et al., 1993). One of the reasons for this inefficiency is the low affinity, which the leaves of many plants have for aqueous liquids (Crease et al., 1991). Several factors have been found that influence droplet reflection, e.g., surface morphology of the leaf, droplet size, impact velocity, and the dynamic surface tension and viscosity of the liquid (Ennis et al., 1952; Hartley and Brunskill, 1959; Taylor and Shaw, 1983; Anderson and Hall, 1989; Crease et al., 1991). The hydrophilic or lipophilic character of the pesticide solution as well as other physico-chemical properties influencing efficiency of deposition of droplets, are other phenomena that modify liquid behaviour on surfaces. Naturally, maximum adhesion of droplets and active ingredient is attempted (Green, 2001). If an atomised liquid is to be available as discrete deposit (low volume application) surfactants can achieve a much higher retention, because an increasing interface area between deposit and plant surface is reached (Bukovac, 1985) by better spreading of droplets produced by a decreased liquid-solid interfacial tension (Holloway, 1998). Hartley and Brunskill (1959) found that droplet bounce occurred mainly with droplet sizes above 100 µm. Since hydraulic sprayers typically produce droplets in the 30-1500 µm size range, the majority of the droplets produced fall into the category of droplets which are likely to bounce. Thus, adjuvants are often added to increase wetting of and retention by plant surfaces (Taylor and Shaw, 1983; Crease et al., 1991; Butselaar and Gonggrijp, 1993), but this behaviour need not be obligatory for all adjuvants in interaction with all other plants (Young et al., 1996).

The objective of this work was to study the potential of the homologous series of the relatively new group of 'rapeseed oil ethoxylates' for their influence on retention on different plant surfaces as well as their impact on phytotoxicity. In doing so, the above-named oils were added to glyphosate as the active herbicidal ingredient which has an effect on the enolpyruvylshikimate-3-phosphate-enzyme (Hock et al., 1995). The higher biodegradability of these vegetable oils may be an important aspect in future formulations (Cornish et al., 1993; Abribat, 2001).

Materials and methods

Surfactants

In the present study, a homologous series of rapeseed oil ethoxylates (AGRIMUL RSO® series, Cognis Co., Cincinnatti, USA) was used. These were commercial preparations of rape seed oil derivatives with an increasing number of ethoxylate units (EO): 5 EO, 10 EO, 20 EO and 60 EO. All surfactants were diluted with deionised water to a concentration of 1 g litre^{-1}, which is a common concentration of spray additives and adjuvants. Pretests conducted with plant leaves showed that the surfactants were not phytotoxic.

Spray solutions

Spray solutions were prepared using glyphosate, N-(phosphonomethylglycine), in the form of the monoammonium salt (Roundup Solugran, 86.5 % purity, Monsanto Co., St. Louis, MO, USA) at a concentration of 43 mM. The surfactants described above were added to the spray solution. Water, unformulated glyphosate-isopropylammonium (86.5 % purity) and glyphosate-N-(phosphonomethyl-glycine) in the form of its isopropylamine salt 600 g a.i. per liter (Roundup Ultra Max (RUM), Monsanto, USA) served as references.

Nozzle

For the application of the spray solutions an air-induction nozzle (AI 11004 VS, Teejet Co., Germany) was used. The pressure was 3 bar (flow rate: 1.58 l/min). This was approximately 190 l ha^{-1}.

Plant material

Four different plant species were chosen, three dicotyledonous flowering plants (*Amaranthus retroflexus, Datura stramonium, Viola arvensis*) and one monocotyledonous flowering plant (*Setaria viridis*). All plants were grown in a greenhouse at 25°/18°C ± 2° day/night temperature and 45/70 ± 5% relative humidity. The plants were used at the 3-4 leaf stage and had similar sizes and fresh weights at the time of application.

Retention tests

To perform retention tests, a commonly used blue food dye (FD&C no. 1®, Warner Jenkinson Co., St. Louis, MO, USA) at a concentration of 2 g $litre^{-1}$ was added to the spray solution. A track-sprayer was used for all applications. The distance between leaf surfaces and spray nozzle orifice was 0.45 m. In order to determine retention, single leaves were harvested from the plants after application and washed with distilled water (10 ml). The leaves were taken from the same height in the canopy for each weed species. The solution obtained was analysed with a UV/VIS spectrometer (Lambda 10; Perkin Elmer UV/VIS Spectrometer, Wellesley, MA, USA) at a wavelength of 629 nm. Concentrations of the dye was related to spray retention.

Chlorophyll fluorescence measurements

Phytotoxicity induced by the application of spray solution of glyphosate with and without surfactants was quantified by measuring the maximum chlorophyll fluorescence (Fm) 24 h, 48 h, 72 h, 96 h, 120 h, and 144 h after application (SCHREIBER et al., 1986; BUWALDA and NOGA, 1994). Before the measurements were made, a dark phase of 30 minutes was set and leaves were exposed to a light flash of 1.8 mmol photons $m^{-2}s^{-1}$. RENGER and SCHREIBER (1986) correlated maximum chlorophyll fluorescence to electron transport activity. Thus, a decrease of Fm is an indication for a blockage of electron transport at the photosystem II-donorside and a disorder in energy transfer at the pigment level, respectively. A pulse-amplitude-modulation-fluorometer (PAM, Model 2000, Walz Co., Effeltrich, Germany) was used.

Dry mass

Seven days after spray application, the treated plants, as well as an untreated control, were cut at the stem base, harvested into a paper-bag and dried in a drying chamber at 105°C for 3 days. Afterwards dry mass was determined.

Statistical Evaluation

The data were subjected to an analysis of variance (ANOVA). Means were separated using the Tukey test ($p \leq 5$ %) to verify the significance of the data. The data of chlorophyll fluorescence was analysed as time series analysis. For statistical analysis the program SPSS (SPSS Co., Munich, Germany, version 11.0 for windows) was used.

Results

The study clearly shows that the addition of the homologous series of rapeseed oil ethoxylates affect the retention of spray solutions. The results of the retention tests with *Amaranthus retroflexus, Datura stramonium, Setaria viridis,* and *Viola arvensis* are displayed in Fig. 1. In each case, the deposition mass was enhanced when rapeseed oil was added to unformulated glyphosate. With the exception of RSO 10+glyphosate on *V. arvensis*, no significant differences could be found between the treatments containing glyphosate plus ethoxylate and RUM. Within the homologous series the same significance level was reached when spray solutions were applied to *D. stramonium* and *S. viridis*, respectively. With *A. retroflexus* glyphosate plus RSO 20 resulted in significantly higher retention in comparison to the RSO 60 treatment. On *V. arvensis* leaves RSO 10 showed a significant increase in liquid retained in comparison to RSO 60.

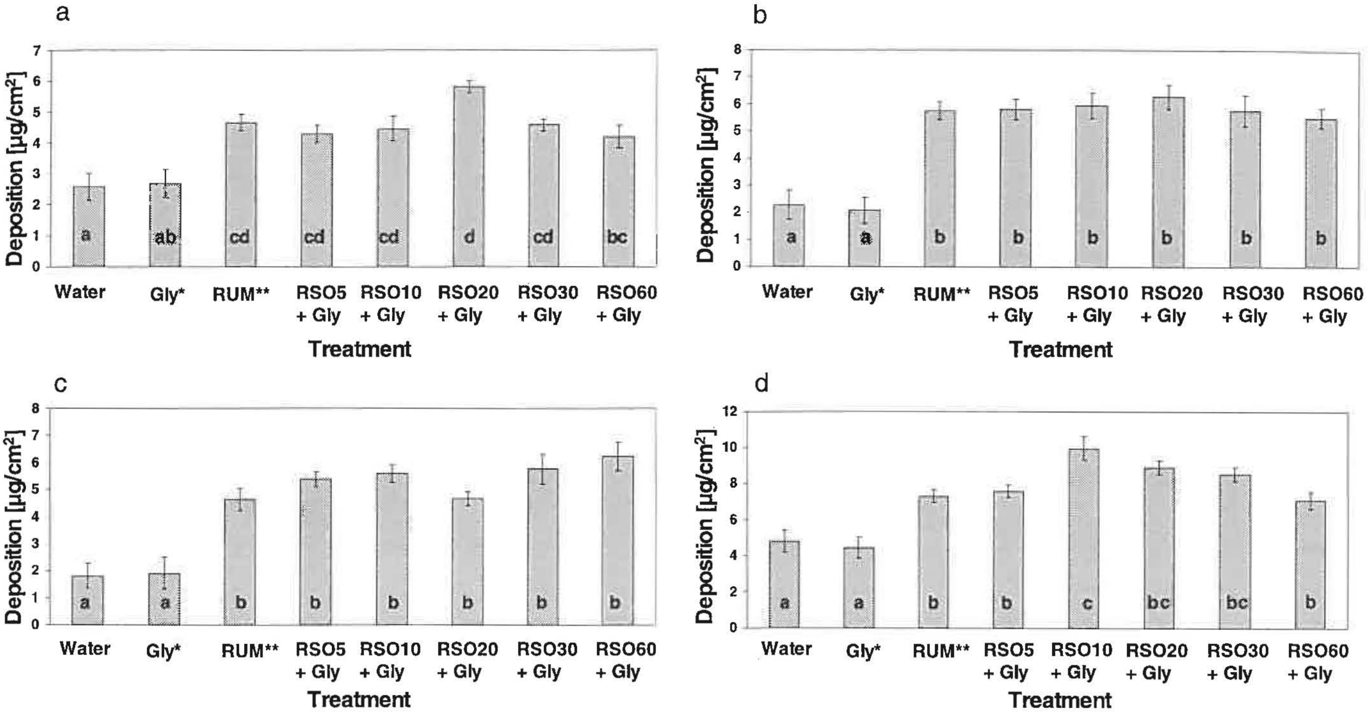
In comparison with the water control the application of the aqueous glyphosate spray solutions resulted in decreasing maximum chlorophyll fluorescence in all plant species after 144 hours (Fig. 2). After 72 h for *A. retroflexus* and *V. arvensis* and after 96 h for *D. stramonium* and *S. viridis*, respectively, most treatments showed significant reduction of maximal chlorophyll fluorescence compared with the water control (significances not shown). After 144 h, there were no differences between treatments applied to either *A. retroflexus,* or *D. stramonium.* However, there were differences between treatments applied to both *S. viridis* and *V. arvensis* (Tab. 1). For *S. viridis*, there were no differences between the ethoxylate treat-ments and unformulated glyphosate and only the glyphosate+RSO 5 treatment had the same chlorophyll fluorescence level as RUM. For *V. arvensis*, all treatments reduced chlorophyll fluorescence com-pare to the unformulated glyphosate. However, only the RSO 20- and the RSO 60-treatments led to reduced fluorescence compared to RUM.

Dry mass measurement confirmed the results obtained by chlorophyll fluorescence measurement by highlighting the effects of hydrophilic and lipophilic spray solutions, respectively. The glyphosate spray solutions always caused significant reductions of dry mass in comparison to water 7 days after spray application. In many cases dry mass decreased to the same or a lower level than RUM (data not

Tab. 1: Mean maximal fluorescence values for glyphosate with and without rapeseed oil ethoxylates applied to different weed species

	Amaranthus retroflexus	*Datura stramonium*	*Setaria viridis*	*Viola arvensis*
Glyphosate	0.490 a	0.914 a	0.260 ab	0.99 a
Roundup Ultra Max®	0.249 a	0.870 a	0.220 b	0.683 ab
RSO 5 + glyphosate	0.387 a	0.716 a	0.367 ab	0.884 ab
RSO 10 + glyphosate	0.409 a	0.566 a	0.568 a	0.803 bc
RSO 20 + glyphosate	0.254 a	0.736 a	0.551 a	0.513 cd
RSO 30 + glyphosate	0.324 a	0.858 a	0.752 a	0.687 bc
RSO 60 + glyphosate	0.251 a	0.816 a	0.423 a	0.513 d

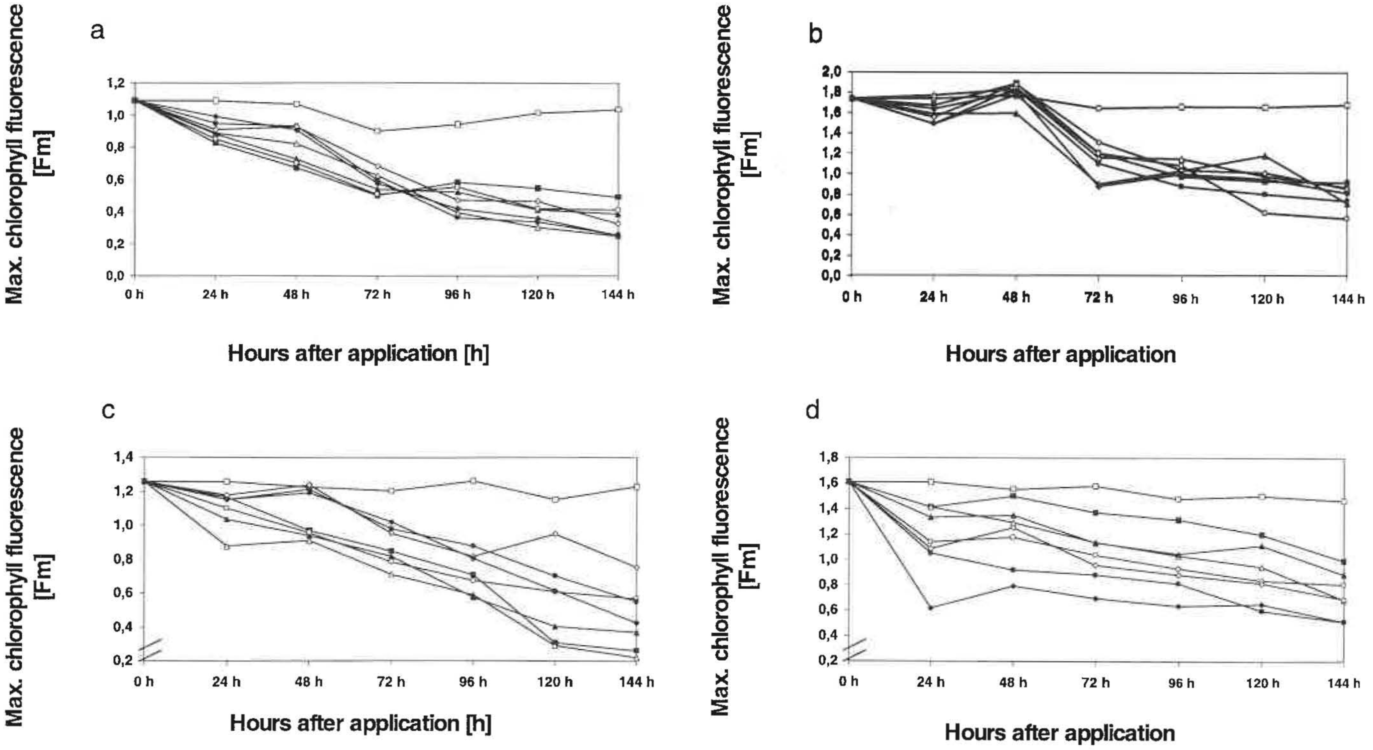
Maximal chlorophyllfluorescence (Fm) values after 144 hours followed by different letters within the same column are significantly different at $p \leq 0.05$ level.



* Gly = Glyphosate

** RUM = Roundup Ultra max

Fig. 1: Retention of aqueous rapeseed oil/glyphosate spray solutions with different degree of ethoxylation on leaves in *Amaranthus retroflexus* (a), *Datura stramonium* (b), *Setaria viridis* (c) and *Viola arvensis* (d).



(□) water; (■) glyphosate; (△) roundup ultra max; (▲) RSO 5 + glyphosate; (○) RSO 10 + glyphosate; (●) RSO 20 + glyphosate; (◇) RSO 30 + Glyphosate; (♦) RSO 60 + glyphosate

Fig. 2: Maximal chlorophyll fluorescence of leaves after treatment with aqueous rapeseed oil/glyphosate spray solutions with different degree of ethoxylation: *Amaranthus retroflexus* (a), *Datura stramonium* (b), *Setaria viridis* (c) and *Viola arvensis* (d).

shown). Strong correlations between ethoxylate units and dry mass could be found (Fig. 3). For *D. stramonium* and *V. arvensis,* increasing hydrophilicity of the spray solution (increasing EO content) resulted in less dry mass. With *S. viridis,* increasing lipophilicity of the spray solution (decreasing EO content) resulted in decreased dry mass. With *A. retroflexus,* least dry mass occurred with rapeseed oil with 20 ethoxylate units with a hydrophilic character in the middle between the most hydrophilic (60 EO) and the least hydrophilic (5 EO) of the homologous series.

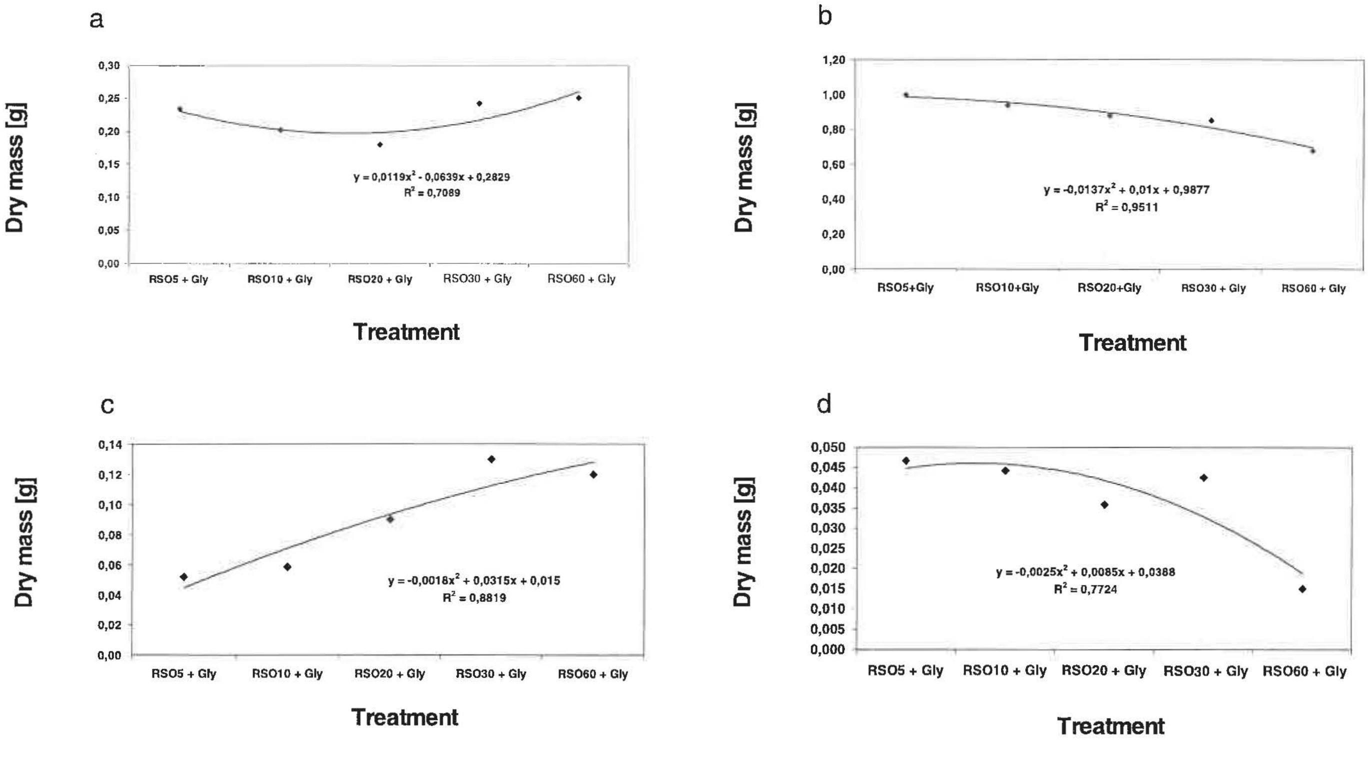
Discussion

Overall, the results obtained show the complexity of interactions when plants are exposed to chemicals, regardless of whether they affect these primarily or secondarily.

The process of retention by plants must be seen principally as physical-chemical interaction of surfaces. It is controlled by the physico-chemical properties of the spray solution and accordingly the spray droplets, as well as the characteristics of the leaf micromorphology as well as its chemical surface composition and exposition. The analysed weeds tested showed differences in the mean mass deposited per area, illustrated with both a dicotyledonous plant and a monocotyledonous plant. Including *V. arvensis* leaves tended to result in highest mean deposition of spray solutions with the addition of rapeseed oil (8.41 µg/cm^2). Not only plant habit and the leaf exposition but also the polygonal epidermal cells and the absence of crystalline wax structures are conducive to adhesion of liquids (WELKER, 1979). Fig. 4 shows the surface structure of adaxial *V. arvensis* leaves. On periclinal cells the cuticule shows a distinctive curvature that has a parallel orientated creasing pattern. No crystalline wax structures could be detected. Whereas *S. viridis,* as a representative of grasses, possesses crystalline wax structures on adaxial leaf surfaces (Fig. 5) and prolate epidermal cells. Thus, the retention potential is lower (HESS and FOY, 2000). The flat crystalloids are perpendicularly orientated and seem to be grouped in rosettes. In addition, the more vertically oriented leaves do not benefit this adhesion-process because of their orientation relative to the trajectory of the droplets (FORSTER et al., 1998). However, the mean level of deposition is decreased (5.52 µg/cm^2).

Treatments containing RUM and unformulated glyphosate plus the ethoxylates led to a significant increase of droplets retained in comparison to water and the unformulated glyphosate alone. There were no marked differences between RUM and any solutions containing ethoxylates. The principal reason may be major differences in the physico-chemical properties of the spray solutions, particularly the reduced surface tensions as a result of the inclusion of surface active agents (SCHERHAG et al., 2004), which reduce the interfacial free energy. Thus, a lower energy situation reduces the work required to expand the interface, meaning that spreading on a leaf occurs more readily and rapidly (GREEN and HAZEN, 1998). The reduction in surface tension is conducive to the retention of spray droplets impacting waxy leaf surfaces, but it need not to be connected with enhanced biological activity of a spray solution (GRAYSON et al., 1991).

To verify plant stress measurements, different chlorophyll fluorescence parameters provide qualitative and quantitative information about photosynthetic processes in chloroplasts (GENTY et al., 1989; KRAUSE and WEIS, 1991). Basic considerations and instructions led to this accepted non-invasive method to assess external influences on the photosynthesis in intact leaves and are described elsewhere (RENGER and SCHREIBER, 1986; SCHREIBER et al., 1986; SCHREIBER, 1986; SCHREIBER et al., 1988; VAN KOOTEN and SNEL, 1990). By measuring chlorophyll fluorescence, it is possible to estimate effects



* Gly = Glyphosate

Fig. 3: Regression of dry mass content and aqueous rapeseed oil/glyphosate spray solutions with different degree of ethoxylation 7 days after treatment in *Amaranthus retroflexus* (a), *Datura stramonium* (b), *Setaria viridis* (c) and *Viola arvensis* (d).

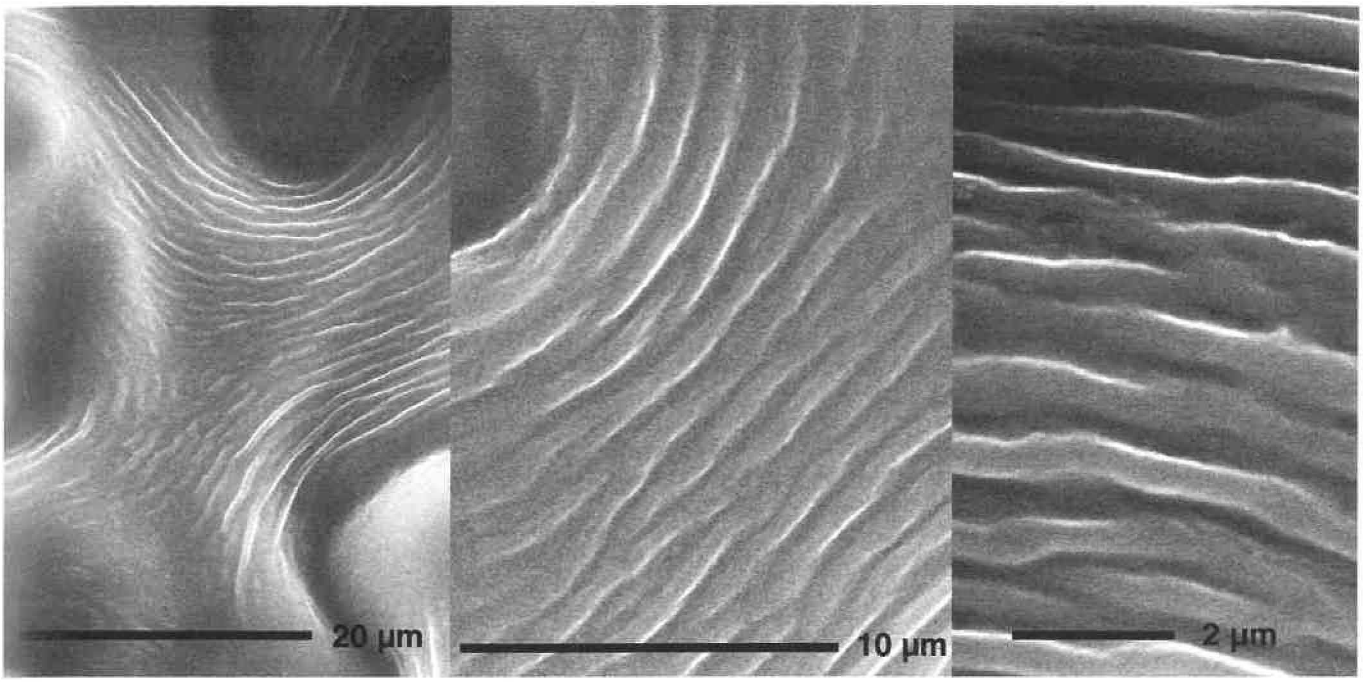


Fig. 4: Scanning electron microscope. *Viola arvensis*, leaf surface. Cuticular ledges, parallel orientated creasing pattern.

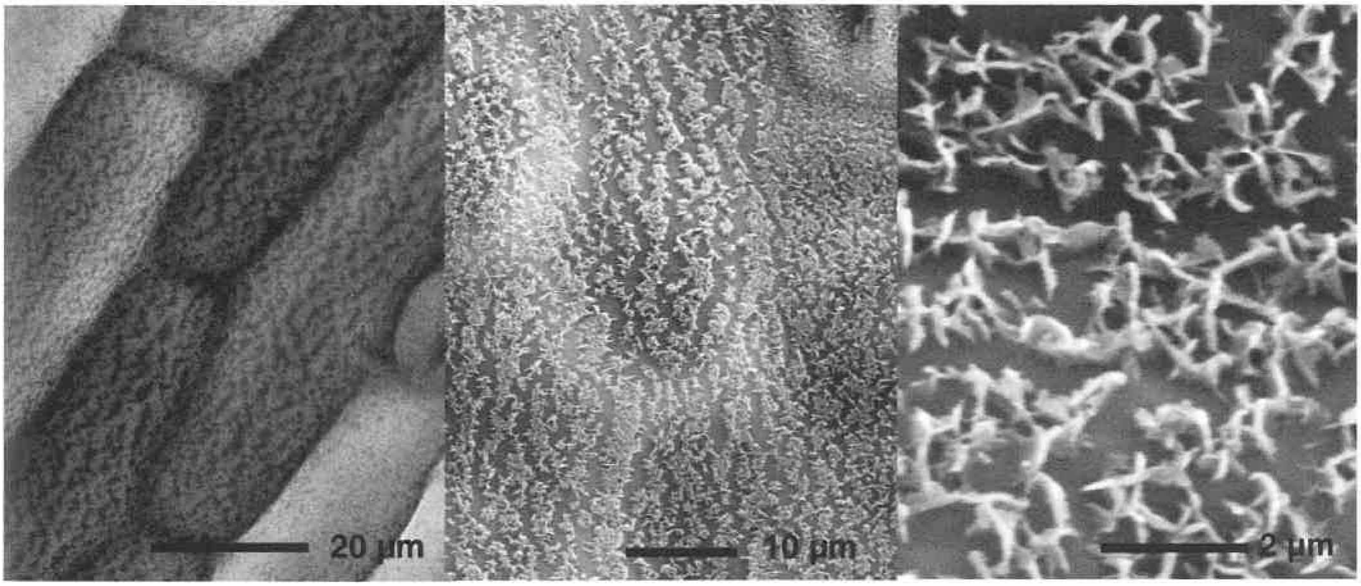


Fig. 5: Scanning electron microscope. *Setaria viridis*, leaf surface. Wax platelets/rosettes.

on photosynthetic apparatus of leaves (KRAUSE and WEIS, 1983). The measured decrease of maximal chlorophyll fluorescence documents dysfunctions in the electron transport of applied glyphosate spray solutions on leaves. Adjuvant effects on photosynthesis and chlorophyll fluorescence, respectively, can be excluded (HAEFS et al., 2002). Basic effects of unformulated glyphosate spray solutions could be demonstrated. In *A. retroflexus*, D. *stramonium* and *S. viridis,* glyphosate phytotoxicity measured by chlorophyll fluorescence was similar to the formulated treatments. This is contrary to the results of the retention tests, when the unformulated glyphosate treatment always had lower deposits. Thus, lower retention does not necessarily result in lower efficacy. Only the slight decrease of chlorophyll fluorescence of unformulated glyphosate in comparison with significant lower decreases of RSO 10 – RSO 60 in *V. arvensis* was in line with a lower deposition on leaves during the application process. These contrasting results point to the different barriers that active ingredients have to penetrate. First, there are micromorphological structures like trichomes and crystalline wax structures interfering with penetration processes on the outside of the plant. The lowering of surface tension is important to ensure wetting despite such micromorphological structures. Second, the thickness and composition of the cuticular waxes provide a significant barrier to penetration, and third, intracellular absorption, which is affected by hydrophilic and lipophilic characteristics of the spray solution. However, maximal retention of spray solutions is important to achieve a basic potential for penetration as well as to minimize losses like run off and rebound, but is not the sole attribute responsible for producing reliable and effective spray solutions. Though, after 7 days in most cases, the tested rapeseed oil derivatives make a similar or higher impact to commercially available Roundup Ultramax® regarding chlorophyll fluorescence.

Within the homologous series the dry matter data illustrate the benefits of the vegetable oils and these data are in good agreement with results from the chlorophyll fluorescence study on single leaves. Clearly, differences between the more hydrophilic and more lipophilic spray solutions were obvious. An increase in ethoxylation (in accordance with getting more hydrophilic) boosted glyphosate toxicity in *D. stramonium*, although no significant differences in retention could be seen. Similarly, in *V. arvensis* the increasing phytotoxicity with an increase of hydrophilicity of the glyphosate spray solution is not explainable, with an almost constant mass retained on the leaves. Actually, the significant increase of droplets retained when using glyphosate with RSO 10 did not result in the expected dry mass reductions. In *S. viridis,* glyphosate phytotoxicity is reduced with increasing hydrophilicity of the spray solution, although no significant differences in deposition within the homologous series were detectable.

This leads to the conclusion that the decisive differences are not the retention potential of the spray solutions used but the penetration capabilities. Furthermore, the magnitude of dry mass reduction, either after applying a more hydrophilic or a more lipophilic spray solution, depends on the weed species. In contrast to *D. stramonium*, *S. viridis* and *V. arvensis* the dicotyledonous weed *A. retroflexus* greatest dry mass reduction was achieved not with an extreme hydrophilic or lipophilic spray solution but a moderately hydrophilic and moderately lipophilic spray solution, respectively.

These results clearly illustrate the potential of rapeseed oil ethoxylate surfactants. The impact of these rapeseed oil-glyphosate spray solutions on retention and biological response indicate an improvement over the standard and more commonly used glyphosate formulation. Previous studies allude to similar excellent performance in their drift behaviour (SCHERHAG et al., 2004). Further work should be done to examine penetration potential as well as rainfastness possibly using a wider range of plant species. In addition, their high biodegradability and renewability are the crucial factors in future.

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