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Rapeseed oil ethoxylate surfactants and their effects on retention, penetration, rainfastness and biological efficacy of selected agrochemicals

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Rapeseed oil ethoxylate surfactants and their effects on retention, penetration, rainfastness and biological efficacy of selected agrochemicals

In this study the effectiveness of a homologous series of rapeseed oil derivatives as formulation components or tank-mix adjuvants for selected foliar applied pesticides and leaf fertilizers was evaluated. The surfactant effects on retention of spray solutions on leaf surfaces, on cuticular sorption and penetration of active ingredients, on rainfastness of spray deposits on plant surfaces and on biological efficacy of the active ingredients was investigated employing the plant growth regulator NAA, the herbicidal compound glyphosate, the fungicidal active ingredients prochloraz and tolylfluanid and the nutrients $CaCl_2$, $Mg(NO_3)_2$ and $MgSO_4$ as representatives. The results are summarized as follows:

1. The surfactants were not phytotoxic at a concentration of 10 g litre^{-1}, and they markedly affected physico-chemical properties of spray solutions at a concentration of 1 g litre^{-1}. Initially, all rapeseed oil ethoxylates suppressed NAA sorption by cuticles, but hydrophilic ones increased sorption after a period of 4 days.

2. Glyphosate spray retention on *Phaseolus vulgaris* increased with increasing surfactant hydrophilicity Retention on *Setaria viridis* was higher for lipophilic surface active ingredients. A positive relationship was established between surfactant EO content and glyphosate penetration through isolated tomato fruit cuticles. The chlorophyll fluorescence emission from 10 various plant species after treatment with different glyphosate solutions depended markedly on the plant species and the surfactant EO chain length. Addition of surfactants to glyphosate either had no effect or resulted in an increase or decrease in glyphosate phytotoxicity.

3. *Botrytis cinerea* incidence of *Lactuca sativa* can be controlled by prochloraz application. Protective prochloraz treatments were more effective than curative ones, and lipophilic surfactants enhanced biological efficacy more than hydrophilic ones. Prochloraz spray retention on *Lactuca sativa* was significantly improved, as the surfactant EO chain length inreased from 5 to 60 units. Merely the surfactant with 30 EO units enhanced prochloraz penetration through isolated cuticles after a period of 144 h.

4. About 70 % of the applied non-formulated active ingredient tolylfluanid and less than 6 % of solely applied prochloraz were recovered on leaf surfaces after their exposure to 25 mm of artificial rain during a period of 6.5 h. The commercial formulations Euparen Multi WG® and Sportak 40® significantly reduced rainfastness to about 30 % (tolylfluanid) and to less than 1 % (prochloraz) of the applied doses. The most lipophilic surfactant enhanced rainfastness of prochloraz but not of tolylfluanid. Reduction of Fm values of *P. vulgaris* and *S. viridis* leaves after glyphosate application and exposure to simulated rainfall was enhanced with increasing surfactant lipophilicity.

5. Calcium penetration through isolated fruit cuticles increased with decreasing surfactant EO content. The addition of the most lipophilic surfactant with 5 EO units to $CaCl_2$ resulted in increasing calcium contents and decreasing K/Ca ratios in fruits of *Malus domestica cv. Braeburn* and in a reduction of bitter pit incidence.

6. A negative relationship was established between surfactant EO content and magnesium penetration through isolated tomato fruit cuticles. $MgSO_4$ and $Mg(NO_3)_2$ treatments enhanced magnesium content in leaves of *Vitis vinifera* whereas the magnesium level in clusters merely increased when formulated $MgSO_4$ was applied. Formulated and non-formulated $Mg(NO_3)_2$ reduced Mg-deficiency symptoms in leaves of *Vitis vinifera.*

Der Einfluss von Rapsölethoxylaten auf die Retention, Penetration, Regenfestigkeit und biologische Wirkung ausgewählter Agrochemikalien

Im Rahmen dieser Studie wurde die Wirksamkeit einer homologen Reihe von Rapsölethoxylaten als Formulierungskomponenten oder Adjuvantien für ausgewählte blattapplizierte Pflanzenschutzmittel und Blattdünger untersucht. Der Einfluss der Tenside auf die Retention von Spritztropfen auf Pflanzenoberflächen, die cuticuläre Sorption und Penetration von Wirkstoffen, die Regenfestigkeit von Spritzbelägen auf Blattoberflächen sowie die biologische Wirksamkeit von Wirkstoffen wurde an den Beispielen des Wachstumsregulators NAA, der herbiziden Aktivsubstanz Glyphosat, der fungiziden Wirkstoffe Prochloraz und Tolylfluanid sowie der Nährsalze $CaCl_2$, $Mg(NO_3)_2$ und $MgSO_4$ ermittelt. Die Ergebnisse lassen sich wie folgt zusammenfassen:

1. Die Tenside waren bei einer Anwendungskonzentration von 10 g $Liter^{-1}$ nicht phytotoxisch. Sie veränderten die physico-chemischen Eigenschaften von Spritzlösungen bei einer Konzentration von 1 g/l. Anfänglich unterdrückten alle Rapsölethoxylate die cuticuläre NAA Sorption, die nach 4 Tagen durch hydrophile Tenside gesteigert wurde.

2. Bei *Phaseolus vulgaris* begünstigte der Zusatz hydrophiler Tenside zu Glyphosat die Retention von Spritzlösungen, während lipophile Tenside die Haftung auf *Setaria viridis* verbesserten. Es wurde eine positive Beziehung zwischen der EO-Kettenlänge der Tenside und der Glyphosat-Penetration durch isolierte Kutikeln nachgewiesen. Chlorophyll-fluoreszenz-Emissionswerte von 10 Pflanzenarten nach Behandlung mit verschiedenen Glyphosat-Spritzlösungen variierten deutlich in Abhängigkeit von der Pflanzenart und der Tensidkettenlänge. Der Tensidzusatz zu Glyphosat hatte entweder keine Auswirkungen oder resultierte in einer Erhöhung bzw. Verringerung der Glyphosat-Phytotoxizität.

3. Die Retention von Prochloraz-Spritzlösungen auf *Lactuca sativa* ließ sich mit zunehmender Hydrophilie der Tenside verbessern. Lediglich das Tensid mit 30 EO-Einheiten förderte die Prochloraz-Penetration durch isolierte Kutikeln nach 144 h. Der Befall von *Lactuca sativa* mit *Botrytis cinerea* konnte durch Applikation von Prochloraz gemindert werden. Protektive Prochloraz-Behandlungen waren insbesondere bei Zusatz lipophiler Tenside wirkungsvoller als kurative.

4. Nach einem simulierten Regenereignis von 25 mm waren ca 70 % des unformuliert applizierten Wirkstoffs Tolylfluanid und weniger als 6 % des Prochloraz-Wirkstoffes auf den Blattoberflächen vorhanden. Die Formulierungen der Handelspräparate Euparen Multi WG® und Sportak 40® reduzierten die Rückstände auf 30 % (Tolylfluanid) bzw. auf weniger als 1 % (Prochloraz). Das lipophilste Tensid erhöhte die Regenfestigkeit von Prochloraz, jedoch nicht von Tolylfluanid. Nach der Behandlung mit Glyphosat und anschließendem simulierten Regen sanken die Werte der maximalen Chlorophyllfluoreszenz (Fm) von *Phaseolus vulgaris* und *Setaria viridis* mit zunehmender Lipophilie der Tenside.

5. Die Penetration von Calcium durch isolierte Kutikeln wurde durch lipophilere Tenside stärker gefördert. So resultierte der Zusatz des Tensides mit 5 EO zu $CaCl_2$ in erhöhten Frucht-Calciumgehalten, verringerten K/Ca-Verhältnissen in den Früchten und einem deutlich reduzierten Stippebefall.

6. Es wurde eine negative Beziehung zwischen dem EO-Gehalt der Tenside and der Mg-Penetration durch isolierte Kutikeln beobachtet. Behandlungen mit $MgSO_4$ und $Mg(NO_3)_2$ führten zu einer Erhöhung des Mg-Gehaltes in Blättern von *Vitis vinifera*, während die Mg-Konzentration im Stielgerüst lediglich durch Applikation von formuliertem $MgSO_4$ gesteigert wurde. Mg-Mangelsymptome bei Blättern von *Vitis vinifera* wurden durch Blattapplikation von formuliertem und nicht-formuliertem $Mg(NO_3)_2$ reduziert.

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Abbreviations

AAS	atomic absorption spectrometry
B. cinerea	*Botrytis cinerea*
^{14}C	radiolabeled carbon isotope
Ca	calcium
$CaCl_2$	calciumchloride
CF	chlorophyll fluorescence
cm	centimetre
DS	dry substance
EO	ethylene oxide
F	formulation
Fm	maximum chlorophyll fluorescence
FW	fresh weight
g	gram
GC	gaschromatography
GBq	gigabecquerel
h	hour
HLB	hydrophilic-lipophilic balance
HPLC	high pressure liquid chromatography
IPA	isopropylamine
kg	kilogram
MBq	megabecquerel
mg	milligram
Mg	magnesium
$Mg(NO_3)_2$	magnesiumnitrate
$MgSO_4$	magnesiumsulphate
min	minute
mM	millimolar
mmol	millimole
mN	millinewton
n	number
NAA	naphtylacetic acid
NaN_3	sodium azide
K	potassium

PO	propylene oxide
PAR	photosynthetically active radiation
P. vulgaris	*Phaseolus vulgaris*
r^2	coefficient of determination
RPU	Roundup Ultra®
RSO	rapeseed oil ethoxylate
s	second
S. viridis	*setaria viridis*
SPO	Sportak 40®
tab.	table
v/v	volume/volume
w/v	weight/volume
°C	degree centigrade
%	per cent
µl	microlitre
µM	micromolar
µmol	micromole

A Introduction

1 Surfactant use in foliar applied pesticides and leaf fertilizers

Modern plant production has to be sustainable at a high level of productivity in order to meet the rising food demand of the world population which is assumed to double within the next 25 years (Alexandratos, 1995). This accomplishment will not be possible without the effective use of fertilizers and highly effective pesticides. Pesticides are generally applied as a formulated product which, after dilution with water, gives a spray solution. Although the active material must guarantee the biological activity, most foliar applied pesticides and leaf fertilizers require surfactants or other adjuvants to maximize their efficacy or utility, either in the formulated product or as an adjuvant added to the spray tank. Such so-called formulation additives and/or adjuvants are very diverse, but solvents, surfactants, and oils are most frequently used (Steurbaut, 1994). The need and significance of adjuvants in agriculture parallels that of pesticides themselves (McWhorter, 1992). In the future formulation and adjuvants will become even more important (Kudsk and Mathiassen, 1994):

1. The increasing costs, paricularly for development and registration will reduce the number of new active ingredients, and at the same time many of the old compounds are being banned primarily for toxicological and ecotoxicological reasons. This will result in a reduction of the number of pesticides particularly for use in minor crops. It is therefore necessary to explore the possibilities for improving the activity and selectivity of the available compounds.
2. The number of reports on pesticide resistance is increasing. A well-designed formulation or an efficient adjuvant could promote the foliar activity of residual pesticides. This in turn could reduce the required dose and thereby perhaps delay the evolution of resistant populations by reducing the selection pressure.

Adjuvants are important for the production, marketing, application, and effective use of agrochemicals (Foy, 1992). As constituents in formulations of pesticides and leaf fertilizers, they may function as activators/penetrators, antidrift agents, antievaporants, antifoam agents, buffering agents, compatibility agents, deposition agents, safener, sequestering agents, spreaders, stickers, synergists and wetters (Schönherr and Bauer, 1992; Holloway, 1994). The most important formulations of current foliar-applied pesticides are characterized in the following table.

Tab.1: Design and properties of the most important water-mixable pesticide formulations (Heusch, 1981a; modified)

Type	Physical system before dilution with water	Physical system after dilution with water
emulsifiable concentrate (EC)	real solution	emulsion (O/W)
wettable powder (WP)	powder	suspension
wettable granule (WG)	granule	suspension
suspension concentrate (SC)	suspension	suspension

The efficacy of a foliar applied pesticide or leaf fertilizer ultimately depends on the amount of active ingredient that reaches the site of action. The influence of formulation and adjuvants from the time a pesticide or fertilizer molecule leaves the applicator (usually this means the spray nozzle tip) until it hits a target and starts ist action is a complex interactive phenomenon, with many factors possibly influencing the final response. A general scheme for foliar applied pesticide and fertilizer action affected by surfactants is drawn up in the following Figure.

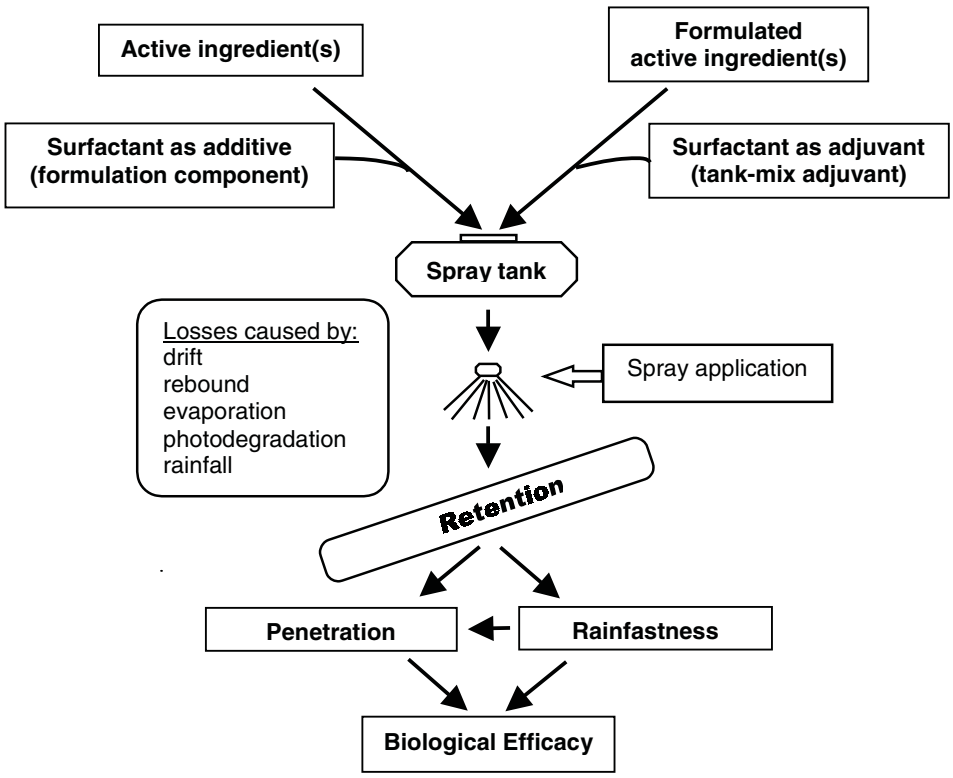

Fig. 1: Generalized scheme for foliar applied pesticide and leaf fertilizer action and the influence of surfactants

As additives in the formulation as well as tank-mix adjuvants surfactants are used to stabilize emulsions and/or suspensions (Becher, 1966). In the complex process of spray application which consists of a series of sequential stages, droplets must be transferred, impact on and be retained by the target (Knoche and Bukovac, 1999; Knoche and Bukovac, 2000). Normally, surfactants lower solution surface tension and thereby may enhance wetting and retention (Brazee et al., 1994). The amount of spray liquid retained by the plant surface determines the amount of active ingredient potentially available for biological efficacy (Johnstone, 1973; Bukovac et al. 1981; Koch and Spieles, 1992). For systemic pesticides and leaf fertilizers uptake, translocation and binding to a target at the site of action are further prerequisites for performance (Bukovac et al., 1986). It is well established that surfactants may enhance foliar penetration of active ingredients (Lownds et al., 1987; Stevens and Bukovac, 1987; Knoche and Bukovac, 1993; Harker and Ferguson, 1991). Rainfall after treatment has been shown to reduce the efficacy of foliar applied systemic and nonsystemic pesticides (Behrens and Elakkad, 1981; Anderson and Arnold, 1985; Benz et al., 2000). The addition of surfactants has proved to be useful in protecting foliar deposits against washoff and in improving the rainfastness of pesticides (Zabkiewicz et al., 1985; Taylor and Matthews, 1986). The rain-free period after treatment, required to achieve adequate biological efficacy, varies greatly depending on the type of formulation (Pick et al., 1984).

2 Surfactants and their physico-chemical properties

2.1 Structure of surfactant molecules

Surfactants are compounds whose molecules are composed of a hydrophilic and a hydrophobic part (Hoffmann and Ulbricht, 1981). An important characteristic is the charge of the molecules (Behrens, 1964). According to the ionogenic character of the hydrophilic part one distinguishes between ionic (cationic or anionic) and nonionic surfactants (Becher, 1973). Usually, the hydrophilic part consists of an ethylene oxide- or a propylene oxide chain or a combination of both, whereas the hydrophobic part in most cases is a hydrocarbon or a perfluorhydrocarbon (Neumüller, 1972; Fell, 1981). Ethoxylated surfactants are polyhomologous mixtures of more hydrophobic (lower ethoxylated) and more hydrophilic (higher ethoxylated) combinations (Maag, 1981).

2.2 Solubility of surfactants

General solubility criterions of surfactants are given in Figs. 2 and 3, using alcohol ethoxylates as example. The solubility of surfactant molecules with the same hydrophobic component depends on the amount of ethylene oxide (EO)- or propylene oxide (PO) units, that of molecules with the same EO- or PO content depends on the alkyl chain length. (Hull, 1970). Solubility of surfactants which do not belong to an homologous series can be compared using the system of hydrophilic lipophilic balance (Griffin, 1954). According to this system a number between 0 and 20 is assigned to each surfactant which indicates if the surfactant is more soluble in water or in oil. Numbers between 0 und 9 characterize hydrophobic surfactants, numbers between 11 and 20 hydrophilic ones (Heusch, 1981b).

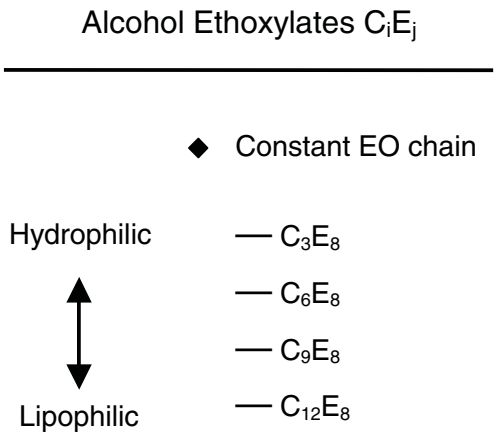

Fig. 2: Solubility of alcohol ethoxylates with a constant EO chain

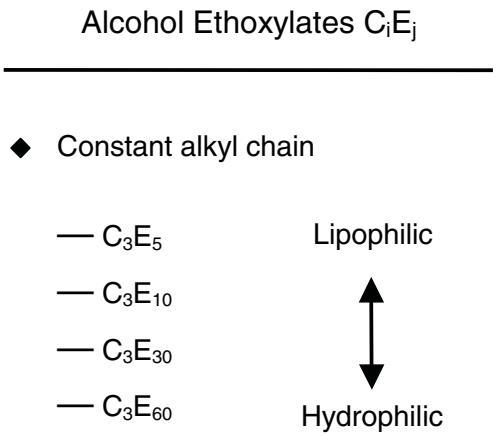

Fig. 3: Solubility of alcohol ethoxylates with a constant alkyl chain

2.3 Aggregation behaviour of surfactants

The amphipathic character of the surfactants causes a reversible aggregation starting at a well defined concentration which is called critical micelle concentration (cmc); below the cmc no aggregates are formed in surfactant solutions (Becher, 1973; Hoffman and Ulbricht, 1981). Above the cmc micelles are formed (Fig. 4), but the concentration of monomeres is not changed. The cmc is characteristic for each surfactant (Helenius and Simons, 1975; Wills and McWorther, 1982).

The orientation of molecules of one micelle depends on the polarity of the environmental medium. In polar solutions the hydrophobic component of a surfactant molecule positions

itself at the inner site of a micelle, whereas in apolar solutions the hydrophilic component extends to the micelle centre (Hoffman and Ulbricht, 1981; Clunie and Ingram, 1983).

An important property of micelles is their ability to pass other molecules into solution (Smith et al., 1966; Tuong and Hayano, 1977). Water-insoluble compounds can be built into the inside of a micelle and hence, they are no longer in contact with the water. This process is called "solubilization" (Clunie and Ingram, 1983; Hoffman and Ulbricht, 1981). Polar compounds can be absorbed by the peripherie of a micelle (Smith et al., 1966; Wyrill and Burnside, 1977). Solubilization of polar compounds in apolar solutions occurs with inverse micelles. In this case the water-soluble compounds are build into the hydrophilic inside of inverse micelles which has no contact to the solvent (Hoffman and Ulbricht, 1981).

2.4 Behaviour at interfaces

With regard to plant protection and leaf fertilization the accumulation and orientation of surfactant molecules at interfaces is a very important property. Because of their amphipathic character, surfactant molecules alter the energy relationships at interfaces (Rosen, 1989). This leads to a reduced surface tension at the air/liquid surface, an interface between hydrophilic and hydrophobic phases. The hydrophobic component of a surfactant molecule positions itself, and is adsorbed, at the air/liquid interface, while the hydrophilic component of the molecule extends away from the interface into the body of the solution (Fig. 4; Brazee et al., 1994).

At liquid/liquid interfaces surfactants reduce interfacial tension by positioning their hydrophobic component into the more hydrophobic liquid phase, while their hydrophilic moiety is extended into the more hydrophilic phase (Fig. 4; Neumüller, !972; Becher, 1973; Weser, 1980).

On solids surfactant droplets with a defined surface tension form a contact angle, whose size depends on pysical and chemical properties of the solid surface. The polarity of solid surfaces and thereby the wettability of plant surfaces can be altered by surfactants (Hoffman and Ulbricht, 1981; Kadota and Matsunaka, 1986; Wirth et al. 1991; Watanabe and Yamaguchi, 1991).

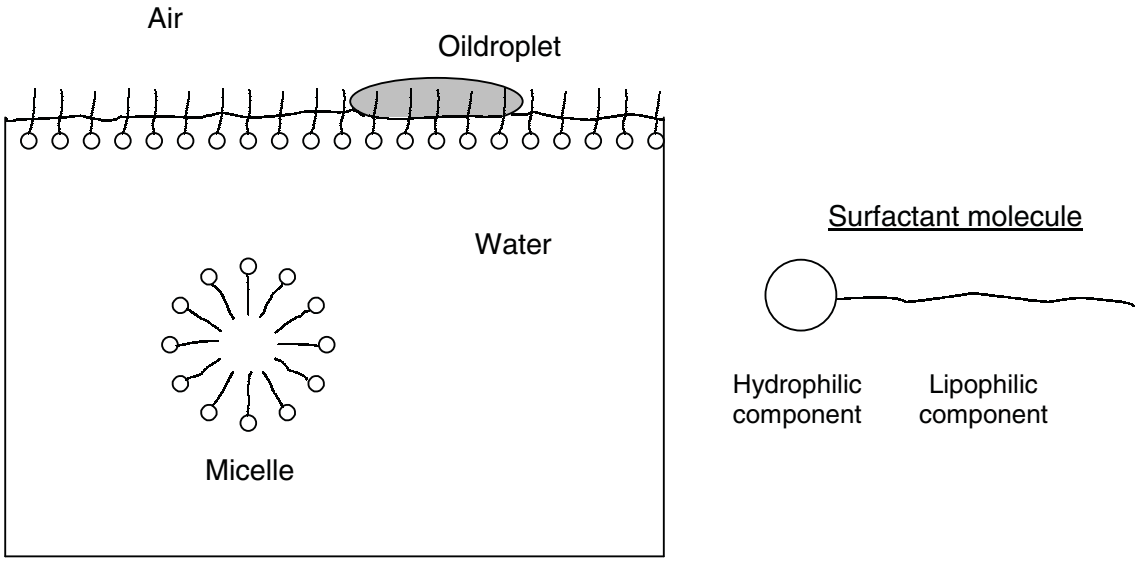

Fig. 4: Generalized behaviour of surfactants above the critical micelle concentration (cmc) and at interfaces (Mortimer, 1987)

3 Choice of suitable surfactants

As most surfactants are used for a specific purpose, it is often taken for granted that their modes of action are well understood. However, this is the exception rather than the rule, since in many cases successful products have evolved mainly from extensive but empirical screening, especially for beneficial effects on the biological activity of pesticides and leaf fertilizers (Holloway, 1994). Hence, in most cases performance of an active ingredient in a given formulation cannot be predicted but must be determined experimentally according to the principle of "trial and error" (Hasall, 1982; Holloway and Stock, 1990). From the point of view of marketing and labelling adjuvants, it is important for the end-user to have basic information on how a particular adjuvant works so that he can assess its relative merits and usefulness for a crop protection programme. For this to be achieved reliable quantitative methods for evaluating performance and activity are required both in the laboratory and in the field (Holloway, 1994). At the present time there are no official standardisation procedures specifically for agrochemical adjuvants (Roberts, 1992). Bioassay in the laboratory and field is the mainstay of adjuvant testing (Kudsk and Mathiassen, 1994). HLB-values of surfactants can give information about surfactant effects on spray stability (emulsification, suspension stability) though the HLB optimum depends on the physico-chemical properties of the active

ingredient (Jansen, 1964). Surface tension measurements are relevant to the performance of antifoam agents, some deposition agents, spreaders and wetters (Schönherr and Bukovac, 1972; Brazee et al. 1994; Knoche and Bukovac, 1993). Contact angles and deposit areas formed on artificial or leaf surfaces provide additional documentation of the relative efficacy of spreaders (Knoche and Bukovac, 1993). The efficiency of wetters and deposition agents can be quantified from track sprayer experiments using solutions of dyes or the pesticide itself (Koch and Weisser, 1995; Koch and Weisser, 1996). More sophisticated equipment for in-flight droplet sizing is needed to asses the potential of antidrift agents (Brazee et al., 1999). Rainfastness of the deposits can be determind using laboratory rain chambers and rain simulators (Nord, 1991; Benz et al. 2000). To demonstrate the mode of action of a surfactant as an activator/penetrator it is essential to employ radiochemical methods in conjunction with labelled pesticide either in idealised systems, such as aqueous acetone for whole plant application (Stock et al. 1993; Urvoy and Gauvrit, 1991), aqueous systems in isolated cuticle studies (Schönherr and Riederer, 1988; Bukovac and Petracek, 1993, Schönherr, 2000), detached, immersed leaves (Schreiber and Schönherr, 1992) or in intact plant material (Knoche and Bukovac, 1993; Knoche and Bukovac, 1999).

4 Surfactant-induced phytotoxicity

In deciding the optimal type of formulation for biological activity undesirable side effects such as phytotoxicity must be considered. Much literature is available about surfactant-induced phytotoxicity in vivo and in vitro. The results of these two approaches cannot be compared directly with each other because the toxicity at intact plants presupposes surfactant penetration through the cuticle, the cell wall and the plasmalemma, whereas in *in vitro* experiments merely the cell wall and the plasmalemma must be penetrated. According to the parameters of phytotoxicity determination it is distinguished between methods which

1. evaluate the development of necrosis macroscopically (Lownds and Bukovac, 1988; Shafer et al., 1988; Knoche et al., 1992)
2. measure ethylene production in plants (Noga and Bukovac, 1986; Stevens and Bukovac, 1987a; Shafer et al., 1988; Lownds and Bukovac, 1989; Knoche et al., 1992; Lownds and Bukovac, 1992)

3. quantify the efflux of electrolytes, amino acids and dyes at leaf- and tissue fragments (Prendeville and Warren, 1977; Towne et al., 1978; Silcox and Holloway, 1986; Coupland et al. 1989; de Ruiter et al., 2000)
4. evaluate changes in growth (Davis et al. 1984; Ernst and Arditti, 1984; Lownds and Bukovac, 1992; Bruschi et al., 1998)
5. calculate a phytotoxicity index by growth parameters (Smith et al., 1966; Tewari, 1985; Velu, 1998)
6. measure the chlorophyll fluorescence response (Gimeenez-Espinosa et al., 1995; Vidal et al., 1995; Foes et al. 1999; Hoagland et al., 1999)
7. describe ultrastructural changes of cell organels (Hargreaves, 1981; Davis et al. 1982; Davis and Stolzenberg, 1986; Bruschi et al., 1998)

Phytotoxicity and intensity of surfactant-induced stress was shown to be dependent on the type, the physico-chemical properties and the concentration of surfactants. Thus, ionic surfactants were found out to be more phytotoxic than non-ionic ones (Ernst et al. 1982; Coupland et al., 1989; Lownds and Bukovac, 1989; Bruschi et al., 1998). Shafer et al. (1988) reported about phytotoxicity being inversely related to surfactant ethyleneoxy chain length, whereas Knoche et al. (1992) observed the greatest damage to kohlrabi with surfactants of medium chain length (9.5 and 10 EO). Lownds and Bukovac (1992) found ethylene production decreasing log-linearly with increasing EO content for octylphenoxy surfactants and maximum ethylene production at intermediate (8-12) EO content for linear alcohol surfactants. Ernst and Arditti (1984) determined that interfacial tension of nutrient solutions to *Brassocattleya* seedlings in *in vitro* culture declined in parallel with the observed phytotoxicity. Ethylene production increased with increasing surfactant concentration and was greater for droplets of 1.0 than 0.5 µl (Knoche et al., 1992; Lownds and Bukovac, 1989).
Phytotoxicity depends on the plant sensitivity to the different surfactants (Shafer et al., 1988; Lownds and Bukovac, 1989; Knoche et al., 1992). Leaf age experiments demonstrated that immature leaves were typically more sensitive to a given surfactant concentration than mature leaves (Shafer et al., 1988).

Phytotoxic effects seem to be related to surfactant penetration into the plant tissue, where they may disrupt membranes of cells and organelles and change structures of proteins (Haapala, 1970; Tanford and Reynolds, 1976; Towne et al. 1978; Horowitz and Givelberg, 1979; Dizengremel, 1983; Ernst and Arditti, 1984; Coret et al., 1993).

Surfactant-induced phytotoxicity may influence uptake, translocation and especially phloemmobility of active ingredients (Holly and Turner, 1979; Stevens and Bukovac, 1985; Keeney et al., 1988). Because of that surfactants which markedly enhance uptake of an active ingredient are not automatically suitable for its formulation (Stevens and Bukovac, 1987). The biological responses and their relationship to surfactant chemistry are important considerations in the selection of surfactants for formulation and application of pesticides and leaf fertilizers and in interpretation of plant responses to formulated foliar-applied agrochemicals (Horowitz and Givelberg, 1979; Lownds and Bukovac, 1992).

5 Surfactants and their toxicological and ecotoxicological profiles

The types of surfactants that are used either in the formulated product or as an adjuvant added to the spray tank will change dramatically in the future. Products like ethoxylated nonylphenols, commodity surfactants widely used as inert ingredients in pesticides and spray adjuvants as well as in many cleaning products and industrial processes, such as the production of pulp and paper, textiles, leather, paints, coatings and metals, are already under considerable pressure in large parts of Europe (Hoorne et al., 1993; Mihaich et al., 2000). Over the years, questions have been raised about the degradability and safety of these surfactants that originated from petroleum (Mihaich et al., 2000). At the moment, the emphasis is still on their use in the detergent industry but it is evident that all other areas will be targeted in the near future (Hoorne et al., 1993). Because of that recent developments in surfactant technology are employed to develop new formulations which improve operator, consumer and environmental safety characteristics (Clemence and Merritt, 1993). Recently, mineral oils and their derivatives are tried to be substituted by seed oils and seed oil derivatives of agricultural origin which exhibit several useful characteristics, namely, not phytotoxic, sufficiently stable under common conditions of storage, rapidly degraded in the environment and promote performance of foliar applied agrochemicals (Bravais et al., 1993). Seed oil derivatives are known as effective and safe nonionic emulsifiers for oils, fats and solvents and are widely recognized for their favorable toxicological and ecotoxicological profiles (Anderson and Mainx, 2000).

6 Requirements and trends of pesticide and leaf fertilizer formulations

The most important recent requirements of pesticide and leaf fertilizer developments are as follows (Tsuji, 2000; modified):

1. Higher safety: The product should be safe to the spray operator, nontarget organisms and the environment, i.e. the formulation should have a positive toxicological and ecotoxicological profile.
2. Higher efficacy: Products should have good initial and residual efficacy at lower dosage.
3. Lower price: Costs per treatment should be low and treatment performance should be high.
4. Labor-saving: The application should be easy and application efficiency should be high.

This list is not exhaustive, but highlights the variety of conflicting parameters of which the pesticide and fertilizer producer must be aware, and the reasons why compromise is often required during the development. It has thus become very difficult to develop new pesticides, and the costs and time for the development of a new product has increased significantly. Therefore, it becomes important to improve formulation and application technology in order to satisfy the above requirements for new and existing active ingredients (Tsuji, 2000). According to the recent demands, we are seeing a number of new trends in the field of formulation design (Hoorne et al., 1993; Stock and Davies, 1994; Tsuji, 2000):

1. A move towards water based formulations. The suspension concentrates, the suspoemulsions and concentrated emulsions are gaining more and more of the market as an alternative to high solvent content formulations like emulsifiable concentrates.
2. If solvents have to be used, the trend is to move away from aromatic solvents to aliphatic solvents.
3. The use of novel packaging techniques. Non-solvent based wettable powders and wettable granules are often viewed as being less active, particularly for systemic active ingredients which require foliar penetration. These dry formulations require a significant quantity of activator which is difficult to be included within the product. Avoidance of tank-mixing with such products may be achieved by partitioning the incompatible components with water soluble packaging, for example, the use of bag-in-bag systems.
4. Development of controlled release formulations (e.g. microcapsule) and formulations based on biological products (e.g. *Bacillus Thuringiensis*).

5. Development of various functional formulations such as jumbo herbicides and fertilizers containing pesticides.

7 Objective of this study

In this study the effectiveness of a homologous series of rapeseed oil derivatives (triglyceridethoxylates, Fig.5) with an average of 5 (Agnique RSO 5®), 10 (Agnique RSO 10®), 30 (Agnique RSO 30®) and 60 (Agnique RSO 60®) units of ethylene oxide (EO) as formulation components or tank-mix adjuvants for pesticides and leaf fertilizers is evaluated. The surfactants are oligomers where the EO number represents an average value. Except for Agnique RSO 60®, which is a slightly brown emulsifiable solid, all surfactants are liquids, of brown color and emulsifiable with water.

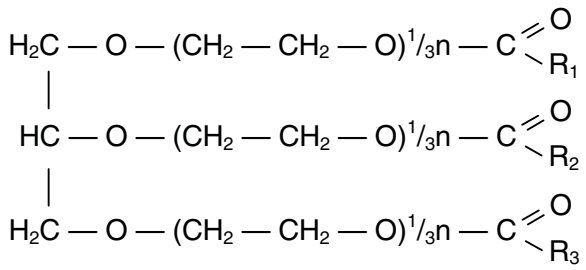

Fig. 5: Generalized structure for triglyceridethoxylates (Agnique RSO®) with n units of ethylene oxide (EO)

The investigated rapeseed oil ethoxylates are characterized by their favourable toxicological and ecotoxicological profile (Tab. 2). They are chosen whenever sensitive environments must be protected, and in cosmetic applications they are known to mitigate the irritation potential of more aggressive surfactants (Anderson and Mainx, 2000).

Tab. 2:Toxicological and ecotoxicological summary of rapeseed oil ethoxylates (Agnique RSO 5®, RSO 10®, RSO 30®, RSO 60®).

Mutagenity	not mutagen (analogue conclusion)
Oral toxicity	LD_{50} > 5000 mg/kg (analogue conclusion)
Irritancy	- Primary skin irritancy: not irritant (analogue conclusion) - Primary eye irritancy: not irritant (analogue conclusion)
Biodegradability	- ≥ 90 % (WRMG) - ≥ 60 % after 28 days (BSB/CSB test; OECD test guideline 301 A-F) - ≥ 70 % after 28 days (DOC test; OECD test guidline 301 A-F)
Aquatic toxicity	- Fish toxicity: LC_{50} > 100 mg/l (*Brachydanio rerio*, DIN 38412_T15 or *Leuciscus idus*, ISO 7346) - Bacteria toxicity: ECO > 100 mg/l (*Ps. Putida* O_2-consumption test; OECD test guideline)

As described above, the biological efficacy of foliar applied pesticides and leaf fertilizers mainly depends on retention of spray solutions on leaf surfaces, on cuticular penetration of systemic active ingredients as well as on rainfastness of spray deposits on plant surfaces. Numerous references are available on the effects of surfactants on the key factors retention (Anderson and Hall, 1989; de Ruiter et al., 1990), penetration (Lownds et al., 1987; Stevens and Bukovac, 1987; Knoche and Bukovac, 1993; Harker and Ferguson, 1991) and rainfastness (Zabkiewicz et al., 1985; Taylor and Matthews, 1986). However, no attempt has been made in any of these studies to describe the effect of surfactants on all of these pivotal parameters in a more integrative manner. This is surprising since surfactants act in a very specific way, and discrepancies between the optimum performance of the individual surfactants with regard to these key parameters are likely to occur. For example, the potential of a surfactant for enhancing retention of the spray solution on the plant surface may be critically evaluated when, at the same time, the surfactant reduces the penetration of a systemic active ingredient or the rainfastness of spray deposits. Therefore, studies focusing on one factor solely are of limited value, when evaluating the effectiveness of surfactants for suitability as additives or adjuvants for pesticides and leaf fertilizers. In order to overcome these limitations in the present study, an integrative approach is chosen according to which

surfactant effects on the key parameters for biological efficacy as well as on the final biological response are investigated.

Besides, systematic studies are carried out to provide pertinent information about the suitability of surfactants use employing a homologous series of rapeseed oil ethoxylates varying in hydrophilicity and several active ingredients widely differing in their physico-chemical properties and modes of action.

To evaluate the effectiveness of rapeseed oil ethoxylate surfactants as additives or adjuvants for pesticides and leaf fertilizers, the following questions are elucidated in the present study:

1. What are the important physico-chemical properties of the surfactants, are there any problems with phytotoxicity and what are their effects on cuticular sorption of non-polar weak organic acids?
2. Do the surfactants show potential to increase retention, penetration and biological efficacy of systemic hydrophilic active ingredients?
3. What are their effects on retention, penetration and biological efficacy of loco-systemic lipophilic active ingredients?
4. Are the compounds able to enhance the rainfastness of systemic, loco-systemic and non-systemic active ingredients?
5. What is their impact on calcium uptake and on the biological efficacy of calcium spray-solutions?
6. How effective are the surfactants in enhancing uptake and biological efficacy of manganese?

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B Physico-chemical properties of rapeseed oil ethoxylates, their plant compatibility and their effects on NAA sorption by cuticles

1 Introduction

Because of the widespread use of pesticide and fertilizer sprays for crop production, as well as the interest in improving their performance, surfactants have been the subject of studies in relation to spray retention, penetration of active ingredients and rainfastness of spray films (Brazee et al., 1994; Lownds et al., 1987; Stevens and Bukovac, 1987a; Knoche and Bukovac, 1993; Harker and Ferguson, 1991; Zabkiewicz et al., 1985; Taylor and Matthews, 1986). These parameters are greatly influenced by the physico-chemical properties of the surfactants which play an important role within the whole range of interactions among the different parties involved – active ingredients, target organisms and surfactants. Normally, surfactants lower solution surface tension and thereby enhance retention and wetting (Brazee et al., 1994). The amount of spray liquid retained by the plant surface determines the amount of active ingredient potentially available for biological efficacy (Johnstone, 1973; Bukovac et al. 1981; Koch and Spieles, 1992; Gauvrit, 1996). Improved wetting caused by surfactant-enhanced droplet spreading (lower contact angle) may lead to higher foliar penetration of systemic compounds and to better performance of non-systemic compounds (Lownds et al., 1987; Knoche and Bukovac, 1991; Demes et al., 1993). Penetration of active ingredients can be affected by pH of the spray solution (Knoche and Bukovac, 2000). Increased penetration of some compounds has been attributed to extended drying times or increased hygroscopicity of the deposit (Prasad et al., 1967; Stevens and Bukovac, 1987). Extended drying times may be of particular importance for penetration of:

1. polar molecules, such as glyphosate, which have low affinities for the nonpolar cuticle. Mobility of the active ingredient in a "dry" deposit may be low and thus desorption from the deposit and sorption into the cuticle becomes limiting (Knoche and Bukovac, 1993a).
2. ions, such as $CaCl_2$, which cannot shed their hydration shell and, hence have to penetrate the cuticle via aqueous pores which swell depending on humidity (Schönherr, 2000).

A direct relationship was found between the surface tensions of spray droplets and droplet drying times, i.e., the lower the surface tension, the shorter the time required for droplet drying (Leung and Webster, 1994). No literature is available on physico-chemical properties of surfactants and their effects on rainfastness of spray films on plants. Increased rainfastness provided by surfactants is generally explained with:

a) surfactants acting as humectants or activators which lead to rapid uptake and translocation of systemic active ingredients
b) surfactants providing a protective film on the surface of the deposit thus reducing the washoff during rainfall.

Apart from favourable physico-chemical properties, the non-phytotoxicity of surfactants to non-target plants is a prerequisite for their use as formulation additives or tank-mix adjuvants though earlier studies suggested that tissue damage caused by surfactants may facilitate foliar penetration of systemic compounds (Feng et al., 1999). Phytotoxicity and intensity of surfactant-induced stress was shown to be dependent on the type, the physico-chemical properties and the concentration of surfactants (Shafer et al., 1988; Coupland et al., 1989; Lownds and Bukovac, 1989; Bruschi et al., 1998).

Cuticular penetration of active ingredients may be viewed as a three-step process, i.e. sorption by, diffusion across and desorption from the cuticle at the cell wall side (Knoche and Bukovac, 1993). The term sorption is used rather than adsorption or absorption because this term is nonspecific and does not imply the location in or the nature of the interaction of the solute with the membrane (Bukovac and Petracek, 1993). Evidence has been presented that surfactants affect sorption of the active ingredient by the plant cuticle (Shafer and Bukovac, 1989; Bukovac et al., 1990; Chamel et al., 1991). Increased sorption of growth-regulating compounds such as NAA has been demonstrated in the presence of surfactants with low oxyethylene content (Shafer et al. 1989). NAA is frequently used for fruit thinning which is often required to control biennial bearing and to reduce the number of small fruits without market value.

The objectives of the present study were (1) to determine the most important physico-chemical properties of rapeseed oil ethoxylates in relation to spray retention, penetration of active ingredients and rainfastness of spray films which are discussed in the following chapters, (2) to investigate the plant compatibility of the surfactants as a prerequisite for their use as formulation additives or tank-mix adjuvants and (3) to establish their effects on cuticular sorption of 2-(1-naphtyl)[1-^{14}C]acetic acid (NAA), a frequently used growth regulator in horticulture, and, as a non-polar weak organic acid, a representative of many agrochemicals (Shafer et al., 1988a).

2 Materials and methods

2.1 Determination of physico-chemical properties

2.1.1 Chemicals

Surfactants ('Agnique RSO®' series, Cognis Düsseldorf, Germany) were commercial preparations of rapeseed oil derivatives (triglyceridethoxylates) with an average of five (RSO 5), 10 (RSO 10), 30 (RSO 30) and 60 (RSO 60) ethylene oxide (EO) units. They were diluted with deionized water to a concentration of 1 g litre^{-1} which is a common concentration of spray additives and adjuvants.

2.1.2 Surface tension

Surface tension of surfactant solutions was determined using a Fisher model 20 surface tensiometer (Prasad et al., 1967). Solutions were equilibrated for 1 h prior to measurement. Each determination was replicated five times.

2.1.3 Contact angle

One-µl droplets of surfactant solutions were placed on parafilm. Within 1 min after droplet application, base width and height of droplets were measured and contact angles calculated according to Mack's equation (Mack, 1936). Measurements were replicated ten times. Parafilm was used because droplet contact angle measurements have been shown to vary depending on various factors, such as leaf turgor, osmotic changes within the leaf and plant surface characteristics (Fogg, 1944). These factors differ markedly between plant species, among leaves of one species and between parts of one leaf.

2.1.4 Drying time

Ten 1-µl droplets of surfactant solutions were applied to parafilm in a climatic chamber at 21 (±0.5)°C temperature and 70 (±1)% relative humidity. Drying of the first and last droplet was recorded and a mean drying time, representing one observation, was calculated. Measurements were replicated ten times. Droplets of deionized water without surfactant served as reference.

2.2 Phytotoxicity studies

2.2.1 Plant material

Experiments were performed using primary leaves of 10-day-old *Phaseolus vulgaris* L. cv. *nanus* seedlings. Seeds were germinated in commercial natural growing medium (Potground RHP SLA, BVB, Maasland, NL) in a covered plastic container to maintain moisture. Following emergence, seedlings were transfered to plastic pots (5 cm in diam, 5.5 cm in ht., one plant per pot) filled with a mixture of growing medium and quartz sand (3 + 1 by volume). Plants were grown in an environmental chamber at 25/20 (±2)°C day/night temperature and 40/70 (±10)% relative humidity. Photosynthetically active radiation (PAR) was provided at 200 µmol s^{-1} m^{-2} at the plant level during a 14-h photoperiod.

2.2.2 Chemicals

Surfactants ('Agnique RSO®' series, Cognis Düsseldorf, Germany) were commercial preparations of rapeseed oil derivatives (triglyceridethoxylates) with an average of five (RSO 5), 10 (RSO 10), 30 (RSO 30) and 60 (RSO 60) ethylene oxide (EO) units. They were diluted with deionized water to a concentration of 10 g $litre^{-1}$.

2.2.3 Chlorophyll fluorescence (CF) measurements

Surfactant solutions were applied to the test-plants with a hand sprayer to runoff. Maximum fluorescence (Fm) of *P. vulgaris* primary leaves was measured 4, 24, 48 and 72h after application with a 'pulse-amplitude-modulation-fluorometer' (PAM, Model 2000, WALZ, Effeltrich, Germany; Buwalda and Noga, 1994). After adaptation of the leaves to dark conditions for 30 min., leaves were exposed to a light flash of 1.8 mmol photons $m^{-2}s^{-1}$. As maximum fluorescence is related to electron transport activity, a decrease of Fm is an indication for a blockage of electron transport at the photosystem II-donorside and a disorder in energy transfer on the pigment level, respectively (Renger and Schreiber, 1986). Untreated plants served as control. Measurements were replicated ten times.

2.3 Sorption studies

2.3.1 Chemicals

Citric acid buffer solutions (20 mM) were prepared and the pH adjusted to 3.2 using sodium hydroxide. Sodium azide (1 mM) was added to prevent microbial growth. Buffer solutions used as donor solutions, simulating agricultural spray solutions, contained 2-(1-naphtyl)[1-^{14}C]acetic acid (1 µM; specific activity 2.3 GBq mmol^{-1}, 98.7% radiochemical purity by TLC; Amersham Corp., Arlington Heights, IL). Appropriate surfactants were added at a concentration of 1 g litre^{-1}. Donor solution without surfactant served as reference.

2.3.2 Isolated Plant cuticles

Cuticle Isolation

Epidermal fruit discs were punched from locally greenhouse-grown untreated mature tomato fruits, free of visible defects, with a cork borer. Enzymatic isolation of the cuticles was performed as described by Orgell (1955) and modified by Yamada et al. (1964) The excised discs were incubated in a mixture of pectinase (40 g litre^{-1}, ICN Biomedicals Inc. Aurora, Ohio), cellulase (8 g litre^{-1} Sigma Chemicals, St. Louis, MO) and NaN_3 (1 mM to prevent fungal and bacterial growth) in sodium citrate buffer (50 mM, pH 4.0) at 25°C. Enzyme solutions were changed several times during a 2-week period. The isolated cuticles were repeatedly rinsed with destilled water, air dried and stored at room temperature.

Measurement of cuticular sorption

Cuticular sorption of NAA in the absence and presence of surfactants was determined according to Shafer and Bukovac (1991). Cuticular membranes were cut into small segments (about 10 mm² each) with a razor blade. Weighed segments (5 mg) were placed into 5-ml glass vials and 1.5 ml of donor solution was added to each. The vials were sealed with 'Teflon®'-lined screw caps and shaken horizontally in a water bath maintained at 25°C. Vials without cuticular membrane served as a control, to correct for sorption to glass and cap liner. At 48 h, 96 h, 192 h and 384 h after initiation of the sorption study 100-µl aliquots were removed from the bulk solution, and radioactivity was determined by liquid scintillation spectrometry (LKB-Wallac LSC, Model 1211). Scintillation cocktail was composed of 1,4-dioxane (10 ml). All samples were counted to a 2_σ error of approximately 1.0% and corrected for background. Since quenching was constant throughout the course of this experiments, all calculations were performed with cpm values. As the amount of radiolabel initially present in

the sorbate solution was known, the amount of NAA in the cuticular membrane was determined by the difference method, according to which the amount of radiolabel in the sorbate solution after a defined period of time was subtracted from the initial sorbate radioactivity (Kipling, 1965). Cuticular sorption of NAA was determined on seven replicates.

2.4 Statistical analysis

The experimental data were analysed with the statistic program 'statgraphics', (Rockville, Maryland, USA). A 5 % probability level was accepted to indicate significant differences. The data were tested for normal distribution and variance homogenity and were compared by Tukey-HSD multiple range tests (Köhler et al., 1994).

3 Results

3.1 Physico-chemical properties of the surfactants

All surfactants reduced the pH of the solutions at concentrations of 1 g litre^{-1} compared with deionized water (Tab. 1). This reduction increased with increasing surfactant EO chain length from 5 to 30 EO units and decreased from 30 to 60 EO units. The surface tension was significantly reduced by all surfactants and especially by the lipophilic surfactants RSO 5 and RSO 10. Contact angles of surfactant solutions on parafilm were markedly lower than those of deionized water. The lipophilic surfactants RSO 5 and RSO 10 reduced contact angles more than the more hydrophilic ones. Drying times of spray droplets on parafilm were reduced by all surfactants except for RSO 5. The shortest drying time was measured with the surfactant RSO 30.

Tab.1: Physico-chemical properties of rapeseed oil ethoxylates and their aqueous solutions.

Surfactant (Agnique RSO® series)	Avg. EO[a]	pH[b]	Surface Tension (mN m^{-1})[bc]	Contact Angle (degree)[bc]	Drying Time (min)[bc]
RSO 5	5	6.61	33.5 c	73.7 c	63.1 a
RSO 10	10	6.27	34.4 c	72.4 c	51.3 b
RSO 30	30	5.86	40.9 b	78.8 b	45.7 c
RSO 60	60	6.34	40.1 b	81.2 b	48.9 bc
Control (water)	—	7.00	70.0 a	104.5 a	58.9 a

[a] Represents the average number of ethylene oxide units (EO). Values from Cognis Deutschland GmbH, Düsseldorf, Germany

[b] Solution contained 0.1% wt/vol surfactant in deionized water

[c] Means within columns followed by the same letter are not significantly different.

3.2 Plant compatibility of the surfactants

Compared with the Fm of untreated leaves, none of the surfactants significantly reduced Fm of *P.vulgaris* during the observation period of 72 h (Fig. 1).

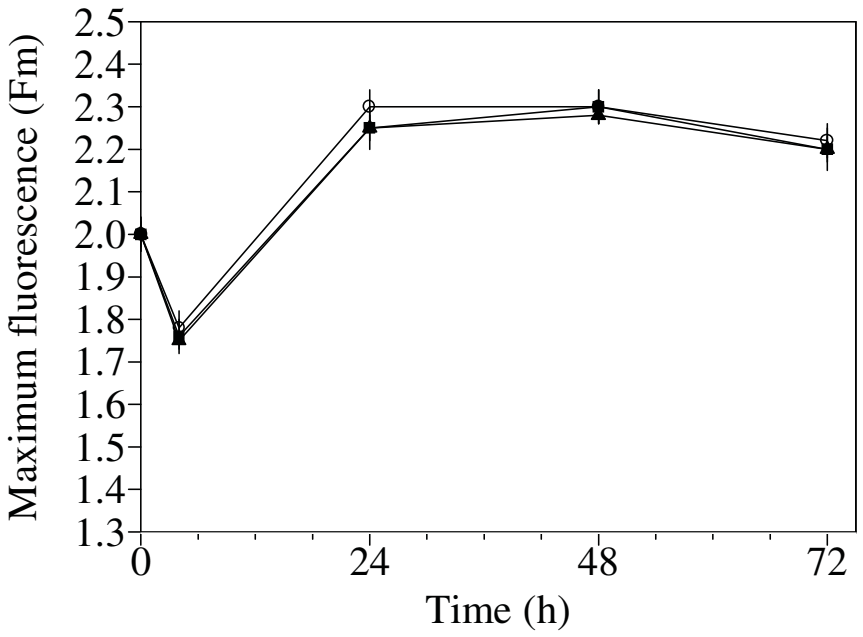

Fig. 1. Maximum chlorophyll fluorescence (Fm) of *P. vulgaris* leaves after treatment with aequeous surfactant solutions (10 g litre^{-1}) (○) Control (untreated), (■) RSO 5, (▲) RSO 10, (△) RSO 30 and (◆) RSO 60.

3.3 Effect of surfactants on cuticular NAA sorption

At the beginning of the time-course study, all rapeseed oil ethoxylates suppressed NAA sorption significantly and this suppression was inversely related to EO chain length (Fig. 2). With decreasing surfactant EO content sorption decreased from 65 to about 59 µmol kg^{-1}. In contrast to NAA without surfactant, for which the amount sorbed remained nearly constant during the whole time-course, the surfactants caused a permanent increase in sorption within the observed period. After 384 h the more hydrophilic surfactants RSO 30 and RSO 60 significantly increased sorption, whereas the more lipophilic surfactants RSO 5 and RSO 10 still suppressed NAA sorption.

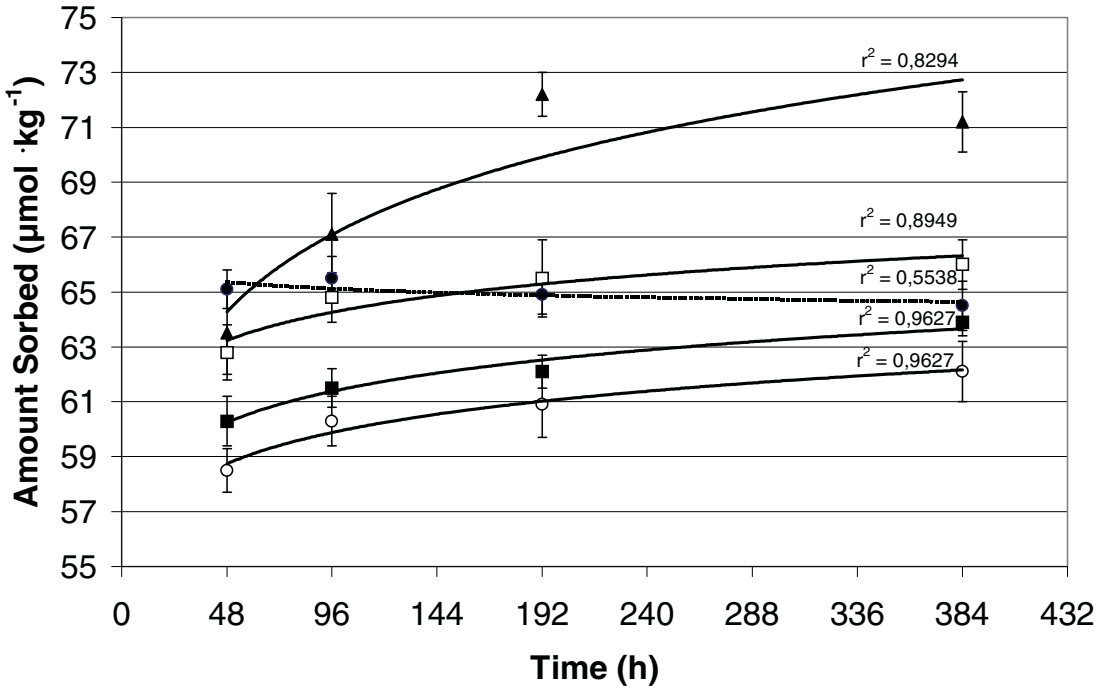

Fig. 2. Sorption of NAA by isolated tomato fruit cuticles. (●) NAA, (○) NAA + RSO 5, (■) NAA + RSO 10, (□) NAA + RSO 30, (▲) NAA + RSO 60.

4 Discussion

Physico-chemical properties of aqueous surfactant solutions strongly depend on the surfactant EO content (Tab. 1). With increasing EO chain length surfactants become more hydrophile which leads to increased surface tension. An increase in surface tension in turn results in an increase in contact angle of spray droplets with the parafilm surface. The observation of Stevens and Bukovac (1987) that the evaporation rate of aqueous spray droplets increases with increasing lipophilicity corresponds to findings of Leung and Webster (1994) that the time required for droplet drying is shorter for solutions with lower surface tension but it does not correspond to the measurements reported here. There are two possible explanations for these unexpected results:

1. The various groups of surfactants react differently in dependence of their alkyl chain.
2. Spray droplets show a different behaviour on leaf surfaces as compared to parafilm.

The latter assumption, however, must be rejected because similar drying times were observed on isolated cuticles and on leaf surfaces for droplets containing rapeseed oil ethoxylates. These results indicate that surfactant effects on properties like surface tension, contact angle and drying time must be individually determined in appropppriate studies and hence, cannot always be derived from EO units or HLB values.

Our data demonstrate that the chlorophyll fluorescence response, used as a quantitative indicator for surfactant-induced stress in *P. vulgaris,* was not markedly influenced by the surfactants (Fig. 1). Though surfactants were applied at a relatively high concentration of 10 g litre^{-1}, Fm values of *P. vulgaris* were not significantly reduced compared to untreated plants. Besides, neither necroses became apparent nor changes in growth could be established. Hence, it can be concluded that none of the investigated rapeseed oil ethoxylates caused phytotoxicity. The surfactants can therefore be employed in formulations of active ingredients and/or adjuvants added to the spray tank.

The investigated rapeseed oil ethoxylates consistently altered sorption of NAA by tomato fruit cuticular membranes (Fig. 2). At the concentration of 1 g litre^{-1}, all surfactants were below their cmc values (unpublished data). Therefore, the reduced sorption by the surfactants RSO 5 and RSO 10 during the whole time-course study and by RSO 30 and RSO 60 at the beginning of the experiment cannot be related to surfactant micelles solubilizing NAA; this may lower the intermicellar NAA concentration, which leads to competition of micelles with the

cuticular membrane for NAA (Schafer and Bukovac, 1989). Shafer and Bukovac (1988, 1991) found out that surfactant monomers and NAA molecules do not associate and therefore interact independently with the cuticle. This lack of NAA/surfactant monomer complex formation may lead to competition between NAA and surfactant monomers for sorption sites in the cuticle. Increasing sorption with increasing surfactant EO content may be due to a higher affinity of the cuticle for surfactants with short EO chain length (Shafer et al., 1989). Reports by Smith et al. (1966) and Knoche and Bukovac (1993) suggested that surfactants sorb to the cuticular membrane via the non-polar headgroup (hydrophobe). The resulting orientation of the molecule with the exposed hydrophilic EO chain may then allow for the formation of hydrophilic pathways in the non-polar cutin polymer. The non-polar NAA may enter but cannot sorb to these pathways and thus will permeate the cuticle rapidly. Hence, surfactants may increase NAA penetration through cuticular membranes without enhancing sorption of the active ingredient.

An inconsistency with this suggestion is that increasingly higher surfactant sorption to the cuticle may induce stronger swelling and increased hydration of the cuticular membrane (Knoche and Bukovac, 1993). Increased hydration in turn may plasticize the cuticular membrane in a manner that "softens" or "opens up" the soluble cuticular lipids, then such an alteration could provide more sites for NAA sorption (Shafer and Bukovac, 1991). Further, lipophilic waxes should have a high affinity for lipophilic surfactants (Bukovac et al., 1990). Under these conditions the surfactant monomers on the surface may associate, forming "hemimicelles" and "micellar aggregates" (Clunie and Ingram, 1983). NAA would partition into these surface features, as it does into micelles in the bulk solution, and become associated with the wax sorbent (Bukovac et al., 1990).

The nearly constant amount of NAA sorbed without surfactants indicates that sorption equilibrium was established within 48 h, whereas the logarithmitically increasing curves of NAA under addition of the surfactants suggest a distinct surfactant effect on the time needed to reach equilibrium. The phenomenon that sorption equilibrium for NAA was not reached even after 384 h has been observed previously with auxins and pepper cuticular membranes, but not with tomato cuticles (Riederer and Schönherr, 1986; Shafer and Bukovac, 1987). The observation has been attributed to the presence of epoxide bonds in pepper cuticles which slowly react with the carboxyl groups of auxins. It can be speculated that rapeseed oil ethoxylates alter bonds in tomato cuticles, thus leading to a slower reaction with the carboxyl groups of NAA.

5 Summary

Physico-chemical properties of a homologous series of rapeseed oil derivatives (triglyceridethoxylates) with an average of five (Agnique RSO 5®), 10 (Agnique RSO 10®), 30 (Agnique RSO 30®) and 60 (Agnique RSO 60®) units of ethylene oxide (EO) were determined in order to explain surfactant effects on spray retention, penetration of active ingredients and rainfastness of spray films. At concentrations of 1 g litre^{-1}, the surfactants lowered pH, solution surface tension, contact angles of droplets on parafilm and, except for RSO 5, they reduced droplet drying times. A positive relationship was established between surfactant EO content, surface tension and contact angles. With increasing EO chain length surface tension and contact angles increased. Drying times were shorter for hydrophilic than for lipophilic surfactants.

The non-phytotoxicity of the surfactants to non-target plants is a prerequisite for their use as formulation additives or tank-mix adjuvants and was investigated using primary leaves of 10-day-old *Phaseolus vulgaris* L. cv. *nanus* seedlings. Surfactants were applied at concentrations of 10 g litre^{-1}. Non of the surfactants influenced the chlorophyll fluorescence response, used as a quantitative indicator for surfactant-induced stress. Besides, neither necrosis nor changes in growth were macroscopically visible.

Surfactant effects on cuticular sorption of non-polar weak organic acids were studied using NAA (radiolabled), a weak acid and representative of many agrochemicals. Sorption which is considered an early step in the penetration process, was markedly affected by the surfactants. At the beginning of the 384 h time-course study, all rapeseed oil ethoxylates suppressed NAA sorption significantly, and this suppression was inversely related to EO chain length. Compared to NAA without surfactants the more hydrophilic surfactants RSO 30 and RSO 60 increased sorption after 96 h, whereas the more lipophilic surfactants RSO 5 and RSO 10 still suppressed sorption of NAA by the cuticle.

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C Effect of rapeseed oil ethoxylates on retention, penetration and biological efficacy of the herbicidal active ingredient glyphosate

1 Introduction

Chemical weed control plays a major role in increasing efficiency of modern cropping systems (Combellack et al., 1992). Surfactants are used extensively to improve the performance of foliar-applied herbicides (Jansen et al., 1961; Holloway and Stock, 1990). Biological efficacy of water-soluble, systemic active ingredients like glyphosate is markedly affected by the type of surfactant (Hatzios and Penner, 1985). Ethoxylated nonylphenoxy surfactants, for example, enhance glyphosate activity more than octylphenoxy surfactants, and those with high hydrophilic-lipophilic balance (HLB) values more than those with low HLB (Nalewaja et al. 1995; Stock and Holloway, 1993). Over the years, questions have been raised about the degradability and safety of some commonly used surfactants that originated from petroleum such as ethoxylated nonylphenols, commodity surfactants widely used as inert ingredients in pesticides and spray adjuvants as well as in many cleaning products and industrial processes (Mihaich et al., 2000). The European Union has recommended to ban the use of these surfactants (Anonym, 2000). At the moment, the emphasis is still on their use in the detergent industry but it is evident that all other areas will be targeted in the near future (Hoorne et al., 1993). In anticipation of increasing pesticide safety demands and restrictive regulatory limitations on pesticide use, pesticide producers are researching new adjuvant technology (Clemence and Merrit, 1993). Recently, a new group of surfactants based on seedoils of agricultural origin exhibits several useful characteristics, namely: not phytotoxic, sufficiently stable under common conditions of storage, rapidly degraded in the environment, and promote the performance of foliar applied herbicides (Miller and Nalewaja, 1973; Manthey et al., 1989; Bravais et al., 1993). Rapeseed oil derivatives (triglyceridethoxylates) have long been known as effective and safe nonionic emulsifiers for oils, fats and solvents and are widely recognized for their favorable toxicological and ecotoxicological profiles (Anderson and Mainx, 2000). Recent developments in plant breeding were employed to develop Roundup Ready soybeans®, canola, cotton, and other crops resistant to glyphosate. Non-phytotoxicity of the formulation adjuvants to non-target plants is a prerequisite for optimizing this technology. However, earlier studies suggested that tissue damage caused by surfactants may play a key role in enhancing foliar penetration but, may limit translocation of the active ingredient within the plant (Feng et al., 1999). Blank formulations of Roundup® and

Roundup Ultra® (RPU) produced visible tissue necrosis with extensive rupturing of cell membranes in both epidermal and mesophyll cells (Feng et al. 1999). Avoidance of toxicity should be an important criterion for further formulation developments.

The objective of our study was to investigate the effectiveness of a homologous series of triglyceridethoxylates as adjuvants in formulations of foliage-applied, water-soluble, systemic active ingredients employing glyphosate as an example.

2 Materials and methods

2.1 Plant material

Experiments were performed using 10-day-old *Phaseolus vulgaris* L. cv. *nanus* seedlings as well as on 21-day-old weed species: *Setaria viridis* L., *Chenopodium album* L., *Solanum nigrum* L., *Galium aparine* L., *Viola arvensis* L., *Stellaria media* L., *Amaranthus retroflexus* L., *Polygonum convolvulus* L. and *Daturas stramonium* L. Bean seeds were germinated in commercial natural growing medium (Potground RHP SLA, BVB, Maasland, NL) in a covered plastic container to maintain moisture. Following emergence, seedlings were transfered to plastic pots (5 cm in diam, 5.5 cm in ht., one plant per pot) filled with a mixture of growing medium and quartz sand (3 + 1 by volume). Ten seeds of each weed species were sown directly on an area of 22 cm² into plastic pots (10 cm in width, 10 cm in height, 20 cm in length, after emergence thinned to six plants of each variety per pot). Plants were grown in an environmental chamber at 25/20 (±2)°C day/night temperature and 40/70 (±10)% relative humidity. Photosynthetically active radiation (PAR) was provided at 200 µmol s^{-1} m^{-2} at the plant level during a 14-h photoperiod.

2.2 Retention experiments

2.2.1 Chemicals

Spray solutions were prepared using Isopropylamine (IPA) salt of glyphosate (62 % purity, 35 % water, 3 % related impurities and isopropylamine, Monsanto Europe S.A.) at a concentration of 43 mM. Surfactants ('Agnique RSO®' series, Cognis Düsseldorf, Germany) were commercial preparations of rapeseed oil derivatives (triglyceridethoxylates) with an average of 5 (RSO 5), 10 (RSO 10), 30 (RSO 30) and 60 (RSO 60) ethylene oxide (EO) units

(Tab. 1,2). Surfactants were added at a concentration of 1 g litre^{-1} to the spray solution. Nonformulated IPA salt of glyphosate and RPU (glyphosate-isopropylammonium; 360 g a.i. litre^{-1}; Monsanto Düsseldorf, Germany) served as references.

2.2.2 Laboratory track sprayer

Experiments were carried out in *P. vulgaris* and *S. viridis*, respectively. These plants were selected, since they represent species widely differing in wettability.

Spray retention was determined using a laboratory track sprayer at the "Landesanstalt für Pflanzenbau und Pflanzenschutz" in Mainz, Germany (Koch and Weisser, 1995). Sprays were applied with a single flat-fan nozzle (LU 120015, 120°, Lechler, Metzingen, Germany) at 1.8 bar and travel speed of 6 km h^{-1} producing an application rate of 200 l ha^{-1}. This is the adviced water volume ha^{-1} for RPU and, besides a threshold value for runoff for many plant species (Koch and Weisser, 1995). Plants were positioned 50 cm below the nozzle where the droplet distribution was very homogenous in a width of 20 cm which was proven with water sensitive paper.

The highly water soluble tracer sodium fluorescein (75 mM) was included to quantify spray retention by the plant surfaces. One hour after application, when all droplets were dried, leaves were put into a 500 ml flask. After addition of 200 ml destilled water and a short intensive shake the emission of the removed tracer (>98 % efficiency) in the elute was measured with a fluorometer (LS 3, Perkin Elmer, Norwalk, Connecticut, USA) at an excitation wavelength of 484 nm and an emission of 512 nm. Thereafter leaf area was determined with a scanner (HP Scanjet Plus, Hewlett Packard, Böblingen, Germany) and doubled for leaf surface because droplets may be retained by abaxial and adaxial leaf surfaces. The amount of detected tracer was related to the leaf area. Deposition was expressed as ng cm^{-2}.

2.3 Penetration experiments

2.3.1 Chemicals

Donor solutions, simulating agricultural spray solutions, were prepared with Isopropylamine (IPA) salt of glyphosate (62 % purity, 35 % water, 3 % related impurities and isopropylamine, Monsanto Europe S.A.) and [^{14}C]-glyphosate (specific activity 2.04 GBq mmol^{-1}, 98.1% radiochemical purity by HPLC, Amersham Pharmacia Biotech Europe GmbH, Freiburg, Germany). Both, nonlabeled glyphosate and [^{14}C]-glyphosate were mixed, yielding a

treatment solution with 1.85 MBq ml^{-1} and 43 mM glyphosate. Appropriate surfactants were added at a concentration of 1 g $litre^{-1}$ (Tabs. 1,2). RPU and nonformulated glyphosate served as references.

2.3.2 Isolated Plant Cuticles

Cuticle Isolation

Epidermal fruit discs were punched from locally greenhouse-grown untreated mature tomato fruits, free of visible defects, with a cork borer. Enzymatic isolation of the cuticles was performed as described by Orgell (1955) and modified by Yamada et al. 1964). The excised discs were incubated in a mixture of pectinase (40 g $litre^{-1}$, ICN Biomedicals Inc. Aurora, Ohio), cellulase (8 g $litre^{-1}$ Sigma Chemicals, St. Louis, MO) and NaN_3 (1 mM to prevent fungal and bacterial growth) in sodium citrate buffer (50 mM, pH 4.0) at 25°C. Enzyme solutions were changed several times during a 2-week period. The isolated cuticles were repeatedly rinsed with destilled water, air dried and stored at room temperature.

Measurement of cuticular penetration

The cuticular penetration of glyphosate was followed using a finite-dose diffusion system (Bukovac and Petracek, 1993). Briefly, cuticles were mounted in plexiglas holders, leak tested and positioned on the finite-dose diffusion half-cell with the outer morphological surface orientated to the ambient air and the cell wall side bathed with water. The volume of the receiver solution was 2.5 ml. A stirring bar was used in the receiving cell to avoid boundary-layer effects.

At time zero, three single drops (1µl each) of the treatment solution were applied to the cuticular surface using a microsyringe fitted with an automatic dispenser (Hamilton). Samples (500 µl) were removed from the receiver solution 9, 24, 48 and 72 h after droplet application. Radioactivity was determined by liquid scintillation spectrometry and counts were corrected for background and efficiency. The sample removed from the receiver was replaced by deionisized water.

2.4 Biological efficacy

2.4.1 Chemicals

See 2.2.1

2.4.2 *Chlorophyll fluorescence (CF) measurements*

Treatment solutions were applied to the test-plants with a hand sprayer to runoff. Maximum fluorescence (Fm) of *P. vulgaris* primary leaves was measured 24, 48 and 72h after application with a 'pulse-amplitude-modulation-fluorometer' (PAM, Model 2000, WALZ, Effeltrich, Germany). Fm readings of the first fully extended weed leaves were taken 48, 72, 144, 192 and 240 h after treatment (Schreiber et al., 1986; Buwalda and Noga, 1994). After adaptation of the leaves to dark conditions for 30 min., leaves were exposed to a light flash of 1.8 mmol photons $m^{-2}s^{-1}$. As maximum fluorescence is related to electron transport activity, a decrease of Fm is an indication for a blockage of electron transport at the photosystem II-donorside and a disorder in energy transfer on the pigment level, respectively (Renger and Schreiber, 1986).

2.5 Statistical analysis

The experimental data were analysed with the statistic program 'Statgraphics', (Rockville, Maryland, USA). A 5 % probability level was accepted to indicate significant differences. The data were tested for normal distribution and variance homogenity and were compared by Tukey-HSD multiple range tests (Köhler et al., 1994). Total spray retention and chlorophyll fluorescence were determined on 10 leaves, penetration rates on 8 replicates, respectively.

3 Results

3.1 Effect of surfactants on spray retention

A positive relationship was established between spray liquid retained on *P. vulgaris* leaves and the EO content of the surfactants (Fig. 3). Spray retention was significantly enhanced to a level comparable to that of RPU, as the EO chain length inreased from 5 to 60 EO units (Fig. 1).

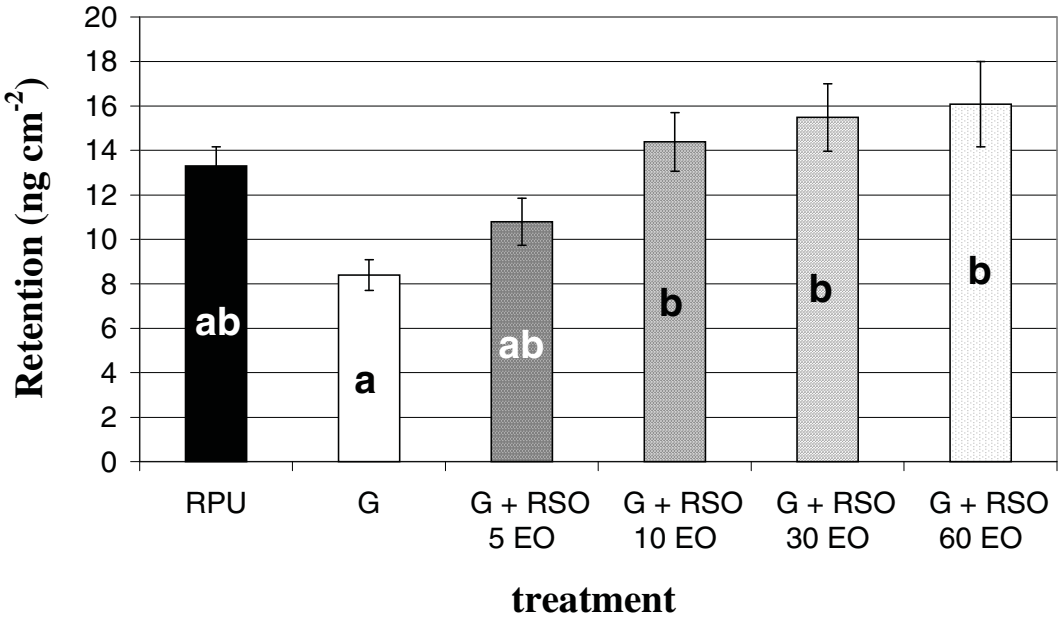

Fig. 1. Retention of glyphosate spray solutions (43 mM) on *P. vulgaris* leaves. Surfactants (RSO 5 EO, 10 EO, 30 EO, 60 EO) were added at a concentration of 1 g litre^{-1}. RPU = Roundup Ultra®, G = Glyphosate

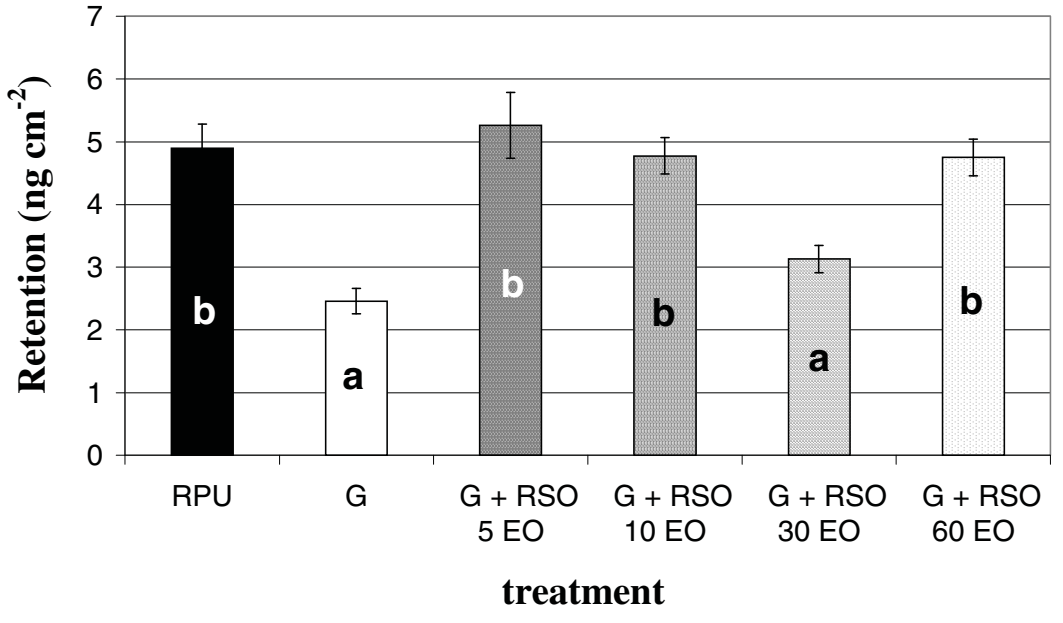

Fig. 2. Retention of glyphosate spray solutions (43 mM) on *S. viridis* leaves. Surfactants (RSO 5 EO, 10 EO, 30 EO, 60 EO) were added at a concentration of 1 g litre^{-1}. RPU = Roundup Ultra®, G = Glyphosate.

On *S. viridis* the amount of spray solution retained decreased with increasing chain length from 5-30 EO, whereas an increase in EO units to 60 enhanced retention (Fig. 3). Except for RSO + 30 EO, all surfactants improved glyphosate spray retention significantly. The performance of these surfactants was comparable to that of RPU (Fig. 2).

Fig. 3 indicates that spray retention on *P. vulgaris* is at least two times higher than on *S. viridis.*

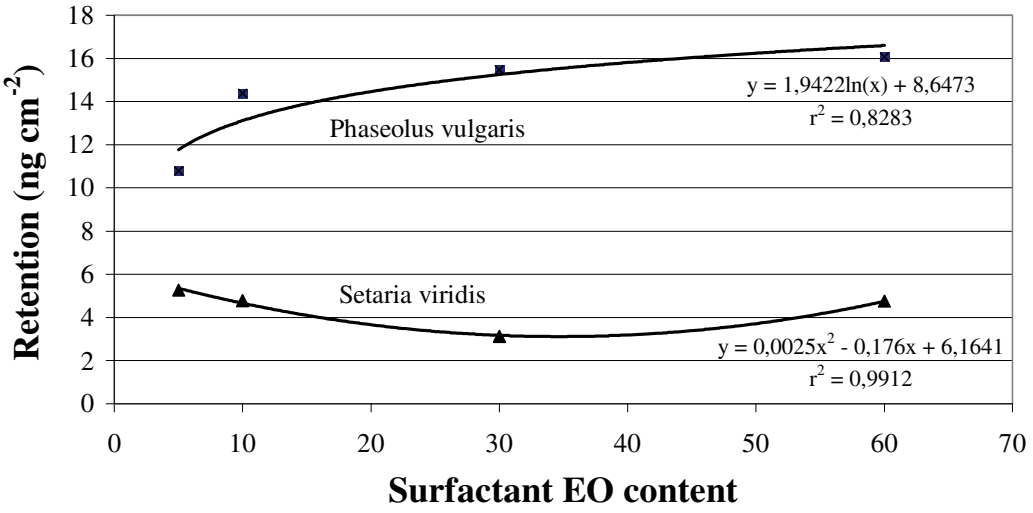

Fig. 3. Retention of glyphosate spray solutions (43 mM) on leaves of P. vulgaris and S. viridis. Surfactants (RSO 5 EO, 10 EO, 30 EO, 60 EO) were added at a concentration of 1 g litre^{-1}.

3.2 Effect of surfactants on glyphosate penetration

Data in Fig. 4 indicate that all surfactants significantly enhanced the penetration of glyphosate during the 72 h time-course study. Penetration mainly occured during the first 6 h after treatment and approached a plateau after 24 h. Glyphosate without surfactants penetrated least and the greatest penetration occured with the commercial formulation of RPU. The Agnique surfactants induced less glyphosate penetration than RPU. A positive relationship was established between the EO content of the surfactants and the amount of glyphosate penetrated through the cuticle. Penetration was enhanced, as the EO chain length inreased from 5 to 60 EO units.

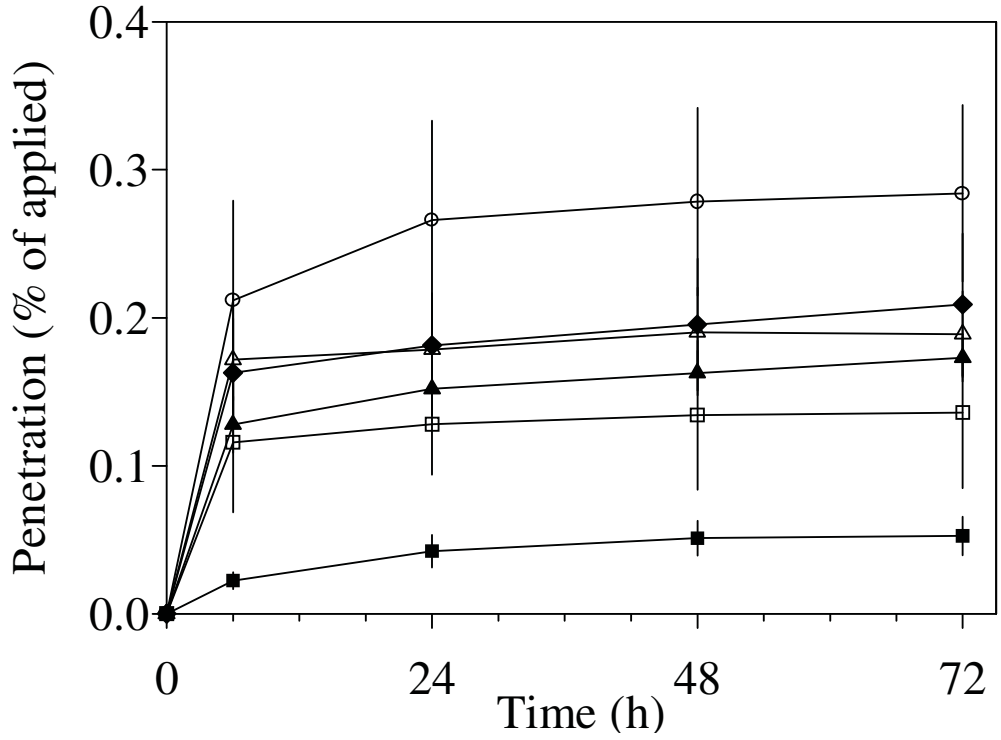

Fig. 4. Penetration of glyphosate spray solutions (43 mM) through isolated tomato fruit cuticles. Surfactants (RSO 5 EO, 10 EO, 30 EO, 60 EO) were added at a concentration of 1 g litre^{-1}. (○) Roundup Ultra®, (■) G, (□) G + RSO 5 EO, (▲) G + RSO 10 EO, (△) G + RSO 30 EO, (◆) G + RSO 60 EO. G = Glyphosate.

3.3 Effect of surfactants on biological efficacy of glyphosate

3.3.1 Effect on Phaseolus vulgaris

All glyphosate treatments reduced Fm significantly after 48 h maximum. After a period of 3 days the mortality rate was 100 % (Fig. 5).

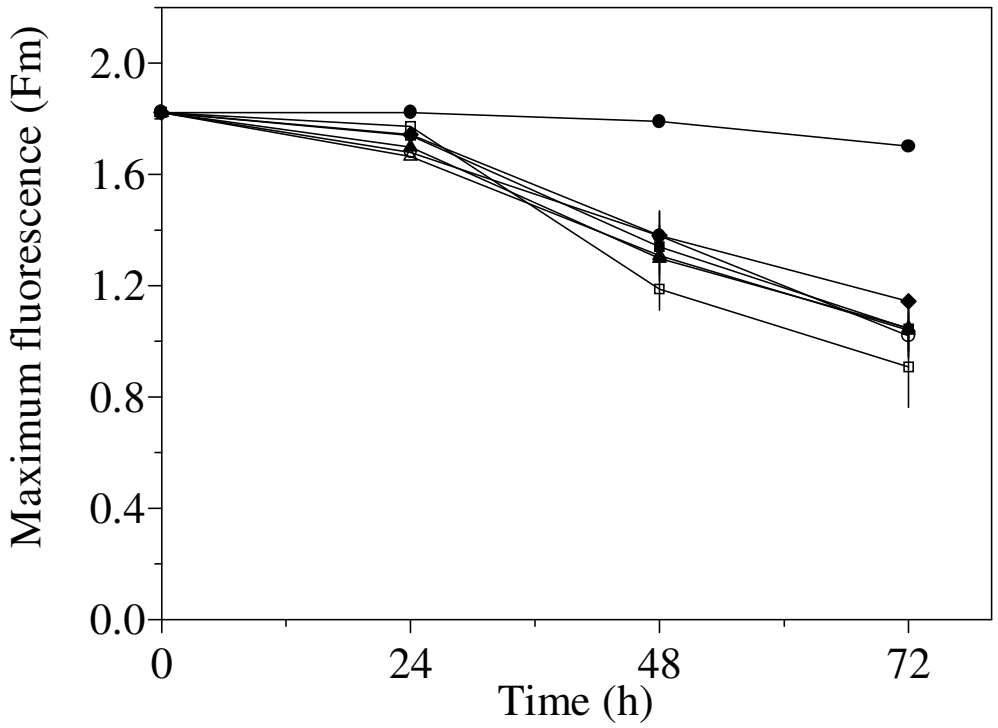

Fig. 5. Maximum chlorophyll fluorescence (Fm) of *P. vulgaris* leaves after treatment with aequeous glyphosate spray solutions (43 mM). Surfactants (RSO 5 EO, 10 EO, 30 EO, 60 EO) were added at a concentration of 1 g litre^{-1}; (●) untreated, (○) Roundup Ultra®, (□) G, (▲) G + RSO 5 EO, (△) G + RSO 10 EO, (◆) G + RSO 30 EO, (■) G + RSO 60 EO. G = Glyphosate.

3.3.2 Effect on weed plants

All treatment solutions reduced Fm of the investigated weed plants significantly within a maximum of 240 h (Fig. 6).

Fm values indicate that in *Amaranthus retroflexus* the addition of RSO 5 within the first 144 h decreased glyphosate activity but later on this and all other surfactants as well as the commercial formulation enhanced glyphosate efficiency. At the end of the study the more hydrophilic surfactants with 30 EO and 60 EO were as efficient as the formulation of RPU.

The investigated surfactants markedly reduced Fm in *Chenopodium album*. Fm decreased with increasing EO chain length. However, greatest glyphosate efficiency was achieved with RPU.

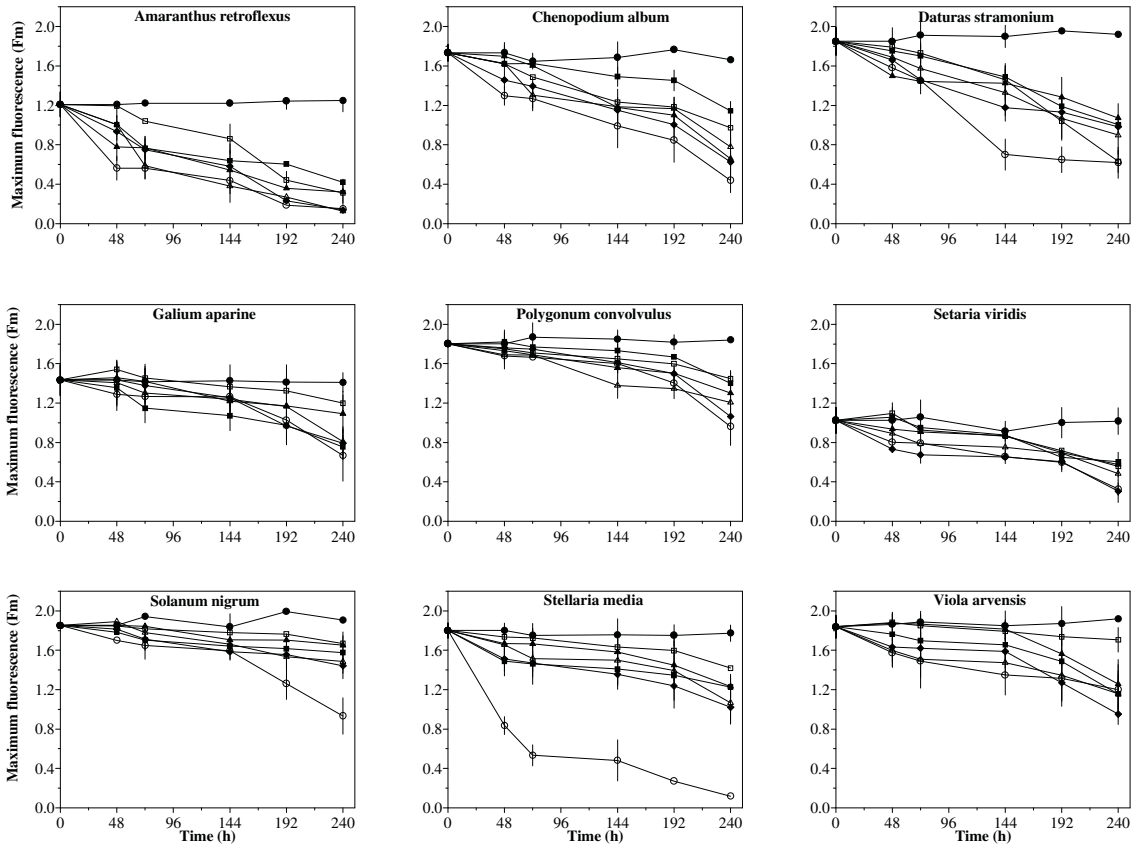

Fig. 6. Maximum chlorophyll fluorescence of weed plants after treatment with aequeous glyphosate spray solutions (43 mM). Surfactants (RSO 5 EO, 10 EO, 30 EO, 60 EO) were added at a concentration of 1 g litre^{-1}; (●) untreated, (○) Roundup Ultra®, (■) G, (□) G + RSO 5 EO, (▲) G + RSO 10 EO, (△) G + RSO 30 EO, (◆) G + RSO 60 EO. G = Glyphosate.

Except for RSO 5 which reached the same low Fm value as RPU, none of the surfactants significantly affected glyphosate activity in *Daturas stramonium* at the end of the time course in comparison to plants treated with glyphosate only. Though Fm values of RSO 5 and RPU were comparable after 240 h, the time courses were very different. That of RPU was characterized by a rapid decrease of Fm during the first 6 days after treatment and a subsequent decrease in efficiency, whereas glyphosate efficacy with addition of RSO 5 was low initially, and increased rapidly 144 h after treatment.

In *Galium aparine* none of the formulations enhanced glyphosate activity. With decreasing EO content glyphosate efficacy was even reduced.

The Fm values after 240 h of *Polygonum convolvulus* showed: a) that addition of surfactants to glyphosate enhanced glyphosate activity, b) the effect is increased with increasing EO chain length and c) RPU is not significantly more effective.

After 240 h Fm values of *Setaria viridis* were lower when more hydrophilic surfactants were added to glyphosate. RPU and RSO 60 were similar effective.

Surfactant addition to glyphosate did not considerably affect its efficacy in *Solanum nigrum*. The time course of RPU, however, was characterized by rapid linear decrease of Fm during the interval from 144-240 h.

On *Stellaria media* RPU was the most effective formulation during the whole observation period. After 72 h this compound led to a mortality rate of almost 100 %. Among the tested surfactants the more hydrophilic ones enhanced glyphosate activity, whereas RSO 10 had no effect and RSO 5 reduced efficacy.

Glyphosate under addition of surfactants only had a weak effect on *Viola arvensis*. The other investigated spray solutions neither enhanced nor decreased glyphosate efficiency significantly.

4 Discussion

Our data demonstrate that addition of ‚Agnique RSO®' surfactants with 5, 10, 30 and 60 EO units to glyphosate was effective in enhancing spray retention on the easy-to-wet *P. vulgaris* leaf surfaces as well as on the difficult-to-wet leaf surfaces of *S. viridis* (Figs. 1,2,3). Glyphosate spray retention was higher for *P. vulgaris* than for *S. viridis*. This probably reflects the fact that the bean, as compared to the weed species, has more horizontally orientated and easier-to-wet leaves, thus intercepting more spray solution. The more water-repellent and vertically orientated leaves of *S. viridis* might cause some droplets to rebounce upon contact or the leaf-air boundary area causes droplets to bypass the leaf (Nalewaja et al., 1995). The decreasing retention rate with increasing surfactant EO chain length from 5-30 EO units on *S. viridis* and subsequent increase with 60 EO units as well as the general increase in spray retention with increasing EO chain length in *P. vulgaris* indicate that spray retention by plants cannot merely be related to solution surface tension and to contact angles of spray droplets (chapter B, Tab. 1). As the spray volume is the same for all treatments, droplet size (affected by surfactants), critical surface tension of the leaf as well as the leaf morphology are further important parameters affecting spray retention.

Glyphosate hardly penetrated the cuticle without addition of surfactants (Fig. 4). The investigated rapeseed oil ethoxylates enhanced glyphosate penetration through isolated tomato fruit cuticles and the penetration was enhanced with increasing surfactant EO chain length. Although the effect of surfactants on herbicide penetration is well described in the literature the mechanisms of surfactant action are poorly understood. It is generally agreed that enhancement of penetration is the net result of several specific interactions between the active ingredient, the surfactant, and the target plant species. For non-electrolytes and non-ionised weak acids, plant cuticles are solubility membranes, as solutes dissolve and diffuse in cutin and amorphous waxes (Schönherr, 2000). However, glyphosate was applied in the isopropylamine salt form and hence, it cannot be referred to the free-volume theory. The lipophilic pathway constituted by amorphous waxes is not available for penetration of the isopropylamine salt because in contrast to non-electrolytes, ions cannot shed their hydration shell when they enter the membrane phase (Krüger, 1999). These ions require an aqueous diffusion path across cuticular membranes, i.e. pores filled with water (Schönherr, 2000). Aqueous pores swell depending on humidity. Hence, our observation of a positive relationship between surfactant enhanced penetration and surfactant EO content cannot be explained with lipophilic surfactants wetting the cuticle surface more than those with a long EO chain (lower contact angle, chapter B, Tab. 1). Besides drying times of aqueous spray droplets increased with decreasing surfactant EO chain length (chapter B, Tab. 1). Thus, deposits with more lipophilic surfactants kept moisture much longer and, consequently, would allow for longer swelling of aqueous pores. Therefore, lipophilic surfactants and not surfactants with a longer EO chain would lead to greater glyphosate penetration. It can also not be referred to the hypothesis that surfactants damage and extract cuticle wax with the consequence of an increase in solute transport. This would cause stress to the plants which could be measured by chlorophyll fluorescence (Robertson and Kirkwood, 1969; Hunt and Baker, 1983; Harker and Ferguson, 1988). Our initial chlorophyll fluorescence measurements, however, elucidated that the investigated surfactants caused no stress when applied to *P. vulgaris* leaves at relativ high concentrations of 10 g litre^{-1} (unpubished data).

Several explanations may be visualized for lower penetration of glyphosate in the presence of surfactants with short EO chain length. First, a comparatively low surface tension (short EO chain length) may lead to immobilization of the active ingredient by sorption to the larger surface area presented by the droplet/leaf interface area (contact angle; chapter B, Tab. 1). Consequently, concentration of the compound in the cuticle underlying droplets with an extensive interface area may present an ineffective driving force for cuticular penetration

(Knoche and Bukovac, 1993). Second, the ability of surfactants to diffuse into the cuticle is reduced as the EO content of the molecules increases (Shafer and Bukovac, 1986). Probably, the surfactants have solvent action and are removed from the deposit at a higher rate than the active ingredient due to diffusion into the cuticle. Then the active ingredient may be left dry on the cuticular membrane and its diffusion may be restricted to a small fraction in close contact with the hydrated deposit/cuticular membrane interface. After this fraction has penetrated, penetration is likely to be much reduced or to stop (Knoche et al., 2000). Third, hydrophilic surfactants suppress crystallisation of glyphosate deposits upon droplet drying more than lipophilic surfactants and hence, maintain the herbicide in a soluble and more available state for cuticular penetration (Leaper and Holloway, 2000). Fourth, the short EO chain surfactants may have a higher affinity for the isopropylamine salt and compete with the cuticle for the active ingredient. Provided that the amount of surfactants does not change with time due to penetration or volatilization, the active ingredient will remain in the deposit and thereby, will not penetrate (Schönherr and Baur, 1994; Baur et al., 1997).

The fluorescence emission from various plant species after treatment with different glyphosate solutions depended markedly on the plant species and the surfactant EO chain length (Figs. 5,6). Addition of surfactants to glyphosate either had no effect or resulted in an increase or decrease in glyphosate phytotoxicity. The responses of *P. vulgaris* and *S. viridis* were the most notable because these plant species reacted differently. Firstly, *P. vulgaris* was more sensitive with a mortality rate of 100 % after 3 days and *S. viridis* with a 100 % mortality rate after 10 days. This may be related to the plant surface structure. *P. vulgaris* leaves, being broad, wide, horizontally orientated and covered with a relatively thin wax layer retain more spray liquid per unit area and are less resistant to glyphosate than the smaller, more vertically orientated and difficult-to-wet leaves of *S. viridis*. Secondly, increasing surfactant EO chain length enhanced glyphosate phytotoxicity on *S. viridis*, whereas glyphosate performance on *P. vulgaris* was improved by lipophilic surfactants (decreasing EO chain length). These results are contrary as far as the amount of spray solutions retained on the plant species is concerned. Obviously, effects on glyphosate phytotoxicity by surfactants were not merely related to the amount of spray retained by plants. As the fluorescence emission values from *P. vulgaris* did also not directly correspond to the amount of glyphosate penetrated through isolated cuticles two other aspects should be taken into consideration. First, requirements for retention and wetting can be conflicting (Furmidge, 1962). Though spray retention on *P. vulgaris* increased with increasing surfactant EO content, wetting of the bean leaf surfaces may have decreased (increasing contact angles; chapter B, Tab. 1) and

hence, became less effective. Second, the potential of surfactants to enhance the intracellular absorption of the herbicide, thereby possibly facilitating its long-distance movement, may be higher for lipophilic surfactants.

Using isolated plant cuticles is a common method to characterize pesticide penetration. Presupposed, that glyphosate penetration is enhanced on all plant species when the surfactant EO chain length is increased it can be assumed that spray retention and wetting of the leaf surface play a key role. As retention and wetting depend mainly on specific leaf characteristics like morphology (shape) and chemical composition of plant surfaces, effects on glyphosate phytotoxicity varied markedly among the investigated plant species.

Our experiments indicate that the effects of rapeseed oil derivatives on glyphosate phytotoxicity was in part due to effects on the amount of spray retained by plants and the amount of glyphosate penetrated through the cuticle, but probably also to the influence on wetting of the leaf surface, to the nature of the deposit and to possible internal effects. The results illustrate the need for a better understanding of the physico-chemical interactions between the active ingredients, surfactants and the plant cuticle.

The investigated surfactants enhanced spray retention, glyphosate penetration and, dependend on the plant species, glyphosate phytotoxicity. Though the surfactants were added solely at a concentration of only 1 g litre^{-1} to the isopropylamine salt, some of them were more effective in enhancing spray liquid retention and promoting glyphosate phytotoxicity than the formulation of the commercial product RPU, as shown in several plant species. Because of their performance and positive toxicological and ecotoxicological profiles these rapeseed oil derivatives could be an economical and ecological alternative for commonly used petroleum oil derivatives. Besides, the surfactants are non-phytotoxic and hence, can be used for crops made resistant to glyphosate brand herbicides.

5 Summary

The effectiveness of a homologous series of rapeseed oil derivatives (triglyceridethoxylates) with an average of 5 (Agnique RSO 5®), 10 (Agnique RSO 10®), 30 (Agnique RSO 30®) and 60 (Agnique RSO 60®) units of ethylene oxide (EO) as adjuvants for foliage-applied, water-soluble, systemic active ingredients was evaluated employing glyphosate as an example. The experiments were performed using *Phaseolus vulgaris* and nine selected weeds, grown in a growth chamber at 25/20 (±2)°C day/night temperature and 40/70 (±10)% relative humidity.

The surfactants were evaluated for enhancement of spray retention, foliar penetration of glyphosate and biological efficacy of glyphosate. Glyphosate was applied at a concentration of 43 mM. The surfactants were added at concentrations of 1 g litre^{-1}. The commercial product Roundup Ultra® and unformulated glyphosate always served as reference. Our data provide evidence that the surfactants used can improve spray retention, foliar penetration and biological efficacy. Some of the formulations were comparable to the performance of Roundup Ultra® in the evaluated aspects, some even more effective in enhancing spray liquid retention and promoting glyphosate phytotoxicity in several plant species. In these studies Agnique RSO 60® generally was most effective.

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D Effect of rapeseed oil ethoxylates on retention, penetration and biological efficacy of the fungicidal active ingredient prochloraz

1 Introduction

Grey mould caused by *Botrytis cinerea* is a common and economically important plant disease (Kapteyn et al., 1994). In order to prevent or limit spread of the disease, an effective management strategy should include sanitation and other cultural practices as well as fungicide applications (Yourman et al., 20000). However, the number of fungicides that are available is limited due to the development of resistance to the benzimidazole and dicarboximide classes (Wang et al., 1986; Van Steekelenburg, 1987; Yourman and Jeffers, 1999). Therefore, availability of other types of fungicides, such as the ergosterol biosynthesis inhibitors is desirable (Kapteyn et al., 1994). Prochloraz is a broad-spectrum fungicide and as a member of the imidazole group especially active against diseases caused by *Ascomycetes* and *Fungi Imperfecti* in many crops (AgrEvo GmbH, 1995). We investigated the efficacy of prochloraz in controling grey mould, employing *Lactuca sativa* as a highly susceptible culture to *B. cinerea*. This disease is particularly serious when plants remain wet for long periods, like glasshouse protected *Lactuca sativa* during the winter months when little artificial heating is used (Wang et al., 1986).

Whilst not possessing truly systemic properties, prochloraz shows translaminar mobility (AgrEvo GmbH, 1995). Because of that prochloraz sprayed onto the upper surfaces of leaves may also prevent infections of the lower leaf surfaces (Maude, 1972). Besides a high spray retention, the foliar penetration of the active ingredient into the leaf tissue is a prerequisite for this performance. It is known that spray retention and penetration of systemic compounds can be altered by surfactants (Anderson and Hall, 1989; De Ruiter et al., 1990; Chamel et al., 1992; Knoche and Bukovac, 1993; Stevens and Bukovac, 1987; Steurbaut et al., 1989). However, the influence of surfactants on fungicide activity has less extensively been studied than that of herbicides or even insecticides (Steurbaut, 1994). No information is available about surfactant impact on retention of prochloraz and only very little about its penetration (Steurbaut et al., 1992).

In this study, the efficacy of a homologous series of rapeseed oil derivatives as adjuvants in formulations of foliage-applied, lipophilic, loco-systemic active ingredients was investigated, employing prochloraz as a model compound.

2 Materials and methods

2.1 Plant material

Experiments were performed using 21-day-old *Lactuca sativa* L. var. *capitata* L. cv. *Nadine RZ* (RIJK ZWAAN Samenzucht und Samenhandlung GmbH, Welver, Germany) plants. Three lettuce seeds were sown into plastic pots (5 cm in diam, 5.5 cm in ht) filled with a mixture of natural growing medium (Potground RHP SLA, BVB, Maasland, NL) and quartz sand (3 + 1 by volume). Following emergence, seedlings were thinned to one plant per pot. Plants were grown in a growth chamber at 25/20 (±2)°C day/night temperature and 40/70 (±10)% relative humidity. Photosynthetically active radiation (PAR) was provided by HQI-T lamps (400 W/D, Osram GmbH, Munich, Germany) at 200 µmol s^{-1} m^{-2} at the plant level during a 14-h photoperiod.

2.2 Retention experiments

2.2.1 Chemicals

Spray solutions were prepared at a concentration of 2.7 mM prochloraz using 1-{N-propyl-N-[2-(2,4,6-trichlorophenoxy)ethyl]carbomyl}imidazole (99 % purity, Hoechst Schering AgrEvo GmbH, Berlin, Germany), dissolved in 10 % methanol. Surfactants ('Agnique RSO®' series, Cognis Düsseldorf, Germany) were commercial preparations of rapeseed oil derivatives (triglyceridethoxylates) with an average of 5 (RSO 5), 10 (RSO 10), 30 (RSO 30) and 60 (RSO 60) ethylene oxide (EO) units. Surfactants were added at a concentration of 1 g $litre^{-1}$ to the spray solution. Nonformulated prochloraz and SPO (Sportak 40®; 400 g a.i. $litre^{-1}$; Hoechst Schering AgrEvo GmbH, Berlin, Germany) served as references.

2.2.2 Laboratory track sprayer

Spray retention was determined using a laboratory track sprayer at the "Landesanstalt für Pflanzenbau und Pflanzenschutz" in Mainz, Germany (Koch and Weisser, 1995). Sprays were applied with a single flat-fan nozzle (LU 120015, 120°, Lechler, Metzingen, Germany) at 1.8 bar and travel speed of 6 km h^{-1} producing an application rate of 200 l ha^{-1}. This is a threshold value for runoff (Koch and Weisser, 1995). Plants were positioned 50 cm below the nozzle in a width of 20 cm. Droplet distribution was very homogenous in this area which was proven with water sensitive paper.

The highly water soluble tracer sodium fluorescein (75 mM) was included in the spray solution to quantify spray retention by the plant surfaces. One hour after application, when all droplets were drie, the three outer leaves were removed from the plants and shaken in a 500 ml flask after addition of 200 ml of destilled water. The emission of the removed tracer (100 % efficiency) in the eluate was measured with a fluorometer (LS 3, Perkin Elmer, Norwalk, Connecticut, USA) at an excitation wavelength of 484 nm and an emission of 512 nm. Thereafter leaf area was determined with a scanner (HP Scanjet Plus, Hewlett Packard, Böblingen, Germany) and doubled for leaf surface because droplets may be retained by abaxial and adaxial leaf surfaces. Deposition was expressed as ng cm^{-2}.

2.3 Penetration experiments

2.3.1 Chemicals

Donor solutions, simulating agricultural spray solutions, were prepared with prochloraz (purity 97 %, Dr. Ehrenstorfer GmbH, Augsburg, Germany) and [^{14}C]-prochloraz (specific activity 3.89 MBq ng^{-1}, >99% radiochemical purity by TLC, AgrEvo UK Limited, Essex, England). Both, nonlabeled prochloraz and [^{14}C]-prochloraz were mixed in 10% methanol, yielding a treatment solution with 2.15 MBq ml^{-1} and 2,7 mM prochloraz. Appropriate surfactants were added at a concentration of 1 g $litre^{-1}$. SPO (Sportak 40®) and nonformulated prochloraz served as references.

2.3.2 Isolated Plant Cuticles

Cuticle Isolation

Epidermal fruit discs were punched from locally greenhouse-grown untreated mature tomato fruits, free of visible defects, with a cork borer. Enzymatic isolation of the cuticles was performed, as described by Orgell (1955) and modified by Yamada et al. (1964). The excised discs were incubated in a mixture of pectinase (40 g $litre^{-1}$, ICN Biomedicals Inc., Aurora, Ohio), cellulase (8 g $litre^{-1}$ Sigma Chemicals, St. Louis, MO) and NaN_3 (1 mM to prevent fungal and bacterial growth) in sodium citrate buffer (50 mM, pH 4.0) at 25°C. Enzyme solutions were changed several times during a 2-week period. The isolated cuticles were repeatedly rinsed with destilled water, air dried and stored at room temperature.

Measurement of cuticular penetration

The cuticular penetration of prochloraz was followed using a finite-dose diffusion system (Bukovac and Petracek, 1993). Briefly, cuticles were mounted in plexiglas holders, leak tested and positioned on the finite-dose diffusion half-cell with the outer morphological surface orientated to the ambient air and the cell wall side bathed with water. The volume of the receiver solution was 2.5 ml. A stirring bar was used in the receiving cell to avoid boundary-layer effects.

At time zero, three single drops (1µl each) of the treatment solution were applied to the cuticular surface using a microsyringe fitted with an automatic dispenser (Hamilton). Samples (500 µl) were removed from the receiver solution 24, 48, 72 and 144 h after droplet application. Radioactivity was determined by liquid scintillation spectrometry and counts were corrected for background and efficiency. The sample removed from the receiver was replaced by deionized water.

2.4 Biological efficacy

2.4.1 Chemicals

See 2.2.1

2.4.2 Inoculation and fungicide application

Spore suspension of *B. cinerea* (germination rate 96.7 %) was enumerated and adjusted to 2 x 10^4 ml^{-1} in destilled water. Plants were inoculated by applying the spore suspension with a hand sprayer to the whole plant until run off. Control plants were sprayed with destilled water. One part of the plants was treated with the different prochloraz solutions 24 h prior to inoculation, the other part 24 h after inoculation to obtain information about the protective and the curative prochloraz effect. Treatment solutions were applied with a hand sprayer until run off.

2.4.3 *Visible symptom assessment*

Seven days after inoculation, the degree of infection of the two outer leaves of each plant was recorded by counting the number of necrotic lesions on an area of 10 cm^2 which was defined by a stencil with two circles, 5 cm^{-2} each.

2.5 Statistical analysis

The experimental data were analysed with the statistic program 'statgraphics', (Rockville, Maryland, USA). A 5 % probability level was accepted to indicate significant differences. The data were tested for normal distribution and variance homogenity. Data on retention and cuticular penetration were compared by Tukey-HSD multiple range tests, data on biological efficacy were compared by Duncan multiple range test (Köhler et al., 1994). Total spray retention and biological efficacy were determined on 10 plants, penetration rates on 8 replicates, respectively.

3 Results

3.1 Effect of surfactants on spray retention

A positive relationship was established between spray liquid retained on *L. sativa* leaves and the EO content of the surfactants (Figs. 1, 2). Spray retention was significantly enhanced as the EO chain length increased from 5 to 60 EO units. The performance of prochloraz plus RSO 10 was comparable to that of SPO. Addition of the more hydrophilic surfactants RSO 30 and RSO 60 was slightly more effective in enhancing spray retention than the commercial formulation SPO.

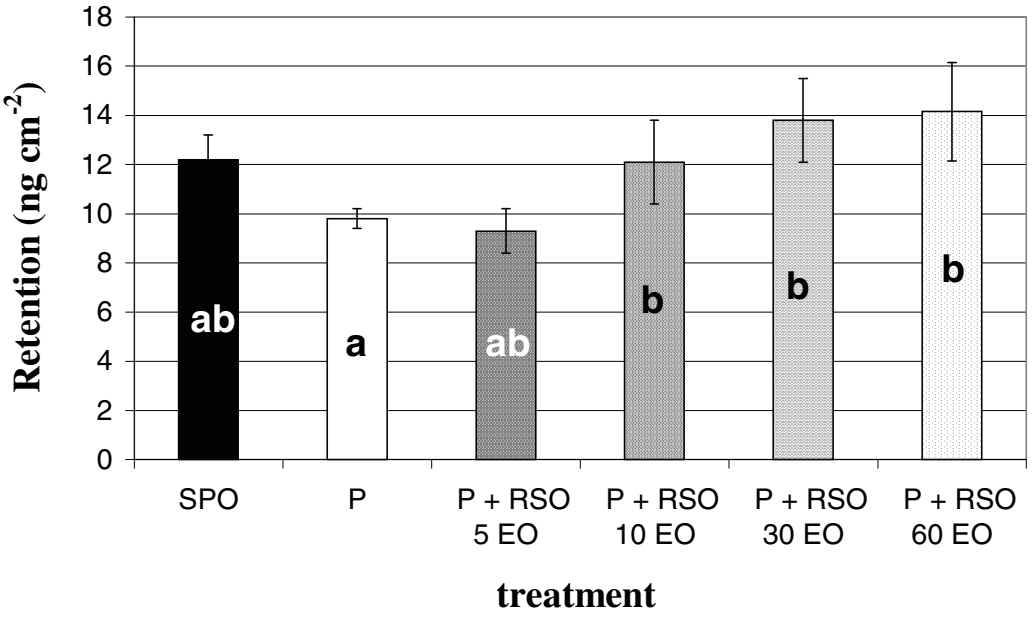

Fig. 1. Retention of prochloraz spray solutions (2,7 mM) on *L. sativa* leaves. Surfactants (RSO 5 EO, 10 EO, 30 EO, 60 EO) were added at a concentration of 1 g litre^{-1}. SPO = Sportak 40®, P = Prochloraz.

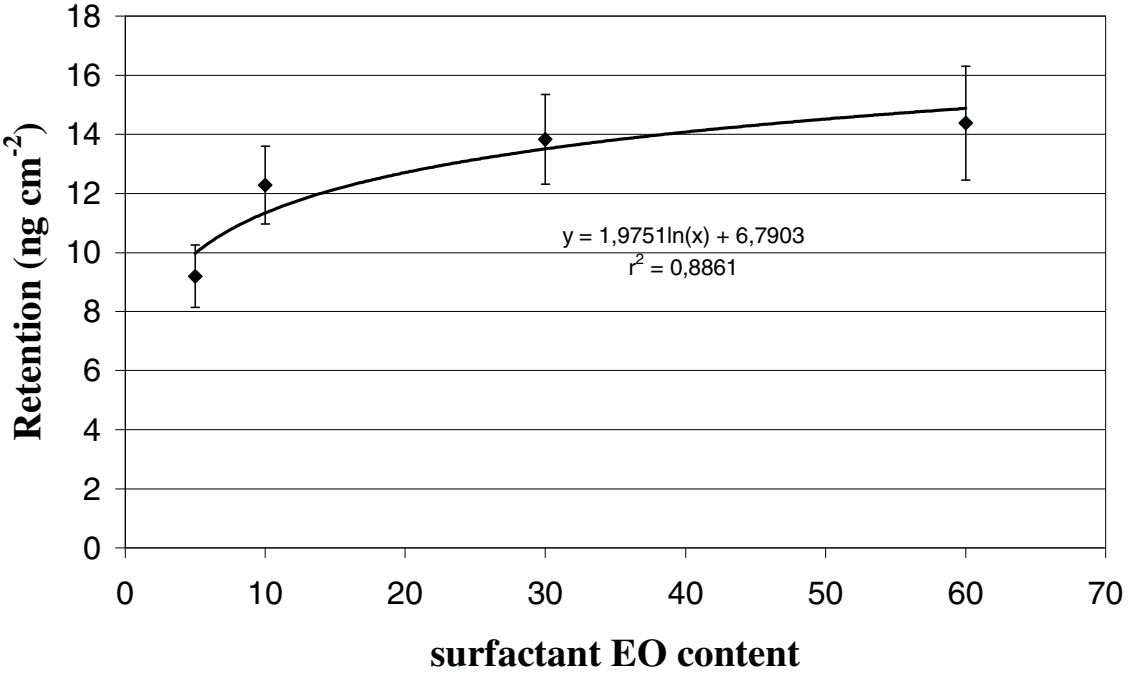

Fig. 2. Retention of prochloraz spray solutions (2,7 mM) on *L. sativa* leaves. Surfactants (RSO 5 EO, 10 EO, 30 EO, 60 EO) were added at a concentration of 1 g litre^{-1}.

3.2 Effect of surfactants on prochloraz penetration

Time-courses for penetration of nonformulated prochloraz and SPO were almost identical (Fig. 3). Both time-courses were characterized by rapid prochloraz penetration within the first 24 h and a penetration plateau phase after 48 h, where the total amount of penetrated prochloraz reached nearly 7 % of the applied dose. All surfactants resulted in a significant decrease of prochloraz penetration within the first 24 h. Thereafter, the surfactant effects on penetration varied distinctly, depending on surfactant EO-chain length. Whereas the surfactants RSO 10 and RSO 60 had no marked impact on prochloraz penetration at the end of the time-course, penetration was markedly enhanced by RSO 30. After 144 h this surfactant increased penetration to 9 % of the applied dose and penetration was still increasing. The lowest penetration occured under addition of RSO 5, reaching a plateau phase after 48 h. The total amount of prochloraz that penetrated under addition of RSO 5 was half of SPO and nonformulated prochloraz.

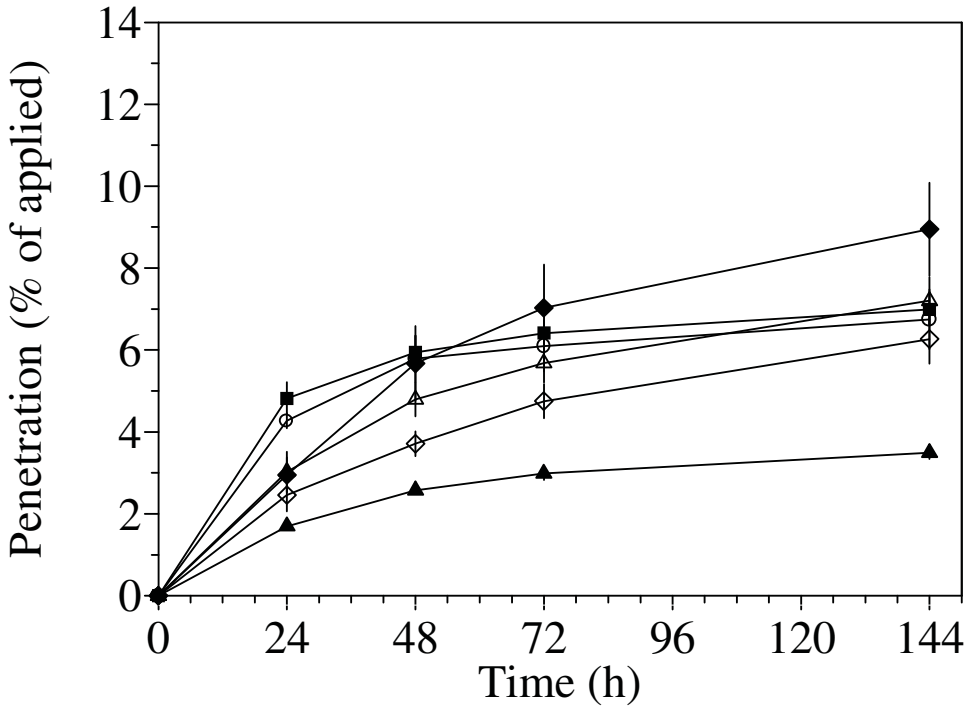

Fig. 3. Penetration of prochloraz spray solutions (2,7 mM) through isolated tomato fruit cuticles. Surfactants (RSO 5 EO, 10 EO, 30 EO, 60 EO) were added at a concentration of 1 g litre^{-1}. (○) Sportak 40®, (■) P, (▲) P + RSO 5 EO, (△) P + RSO 10 EO, (◆) P + RSO 30 EO, (◇) P + RSO 60 EO. P = Prochloraz.

3.3 Effect of surfactants on biological efficacy of prochloraz

Compared to the untreated control, all protective prochloraz treatments reduced *B. cinerea* incidence in lettuce significantly (Fig. 4). Protective treatments were significantly more effective than curative ones. A negative relationship was established between the EO content of the surfactants and the reduction of necrotic lesions per 10 cm^2 leaf area of protectively and curatively treated lettuce leaves (Fig. 5). With increasing surfactant EO chain length, the reduction of necrotic lesions decreased. Hence, protective prochloraz treatments with RSO 5 which reduced *B. cinerea* incidence to 2 lesions per 10 cm^2 leaf area was most effective whereas the curative treatments with prochloraz under addition of RSO 60 had no impact on *B. cinerea* incidence. When applied protectively, the surfactant RSO 5 was twice as effective as SPO and nonformulated prochloraz which did not differ in performance.

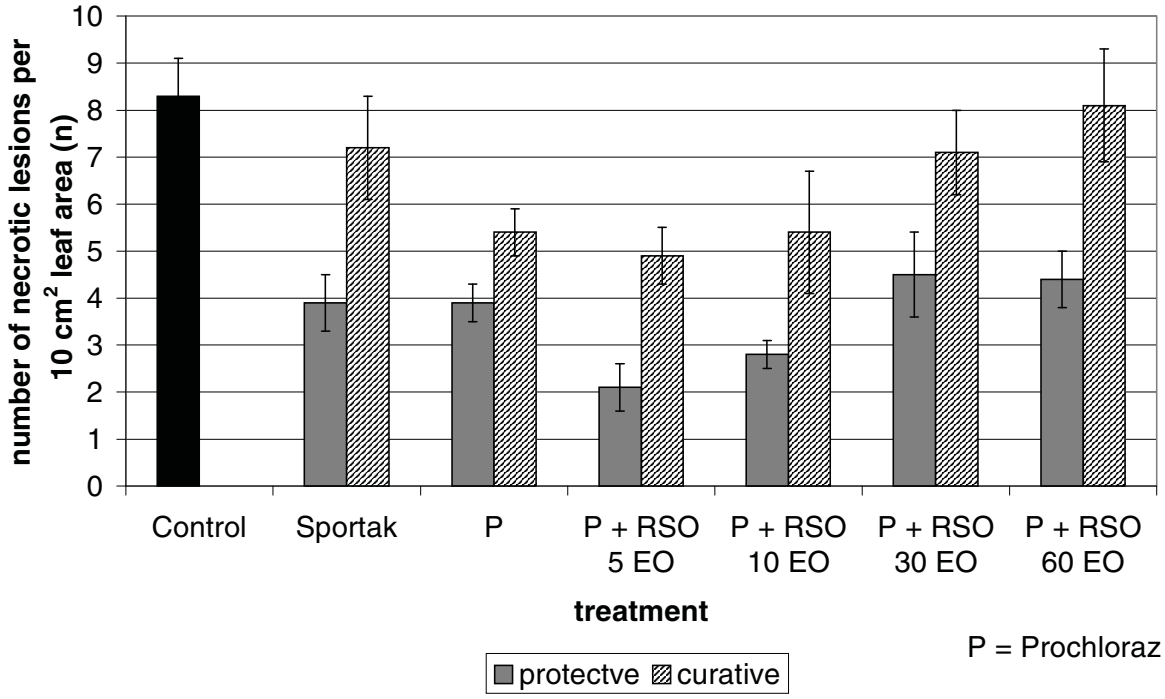

Fig. 4. Effect of prochloraz solutions (2,7 mM) on *Botrytis cinerea* incidence of lettuce (7 days after inoculation). Surfactants (RSO 5 EO, 10 EO, 30 EO, 60 EO) were added at a concentration of 1 g $litre^{-1}$. SPO = Sportak 40®, P = Prochloraz.

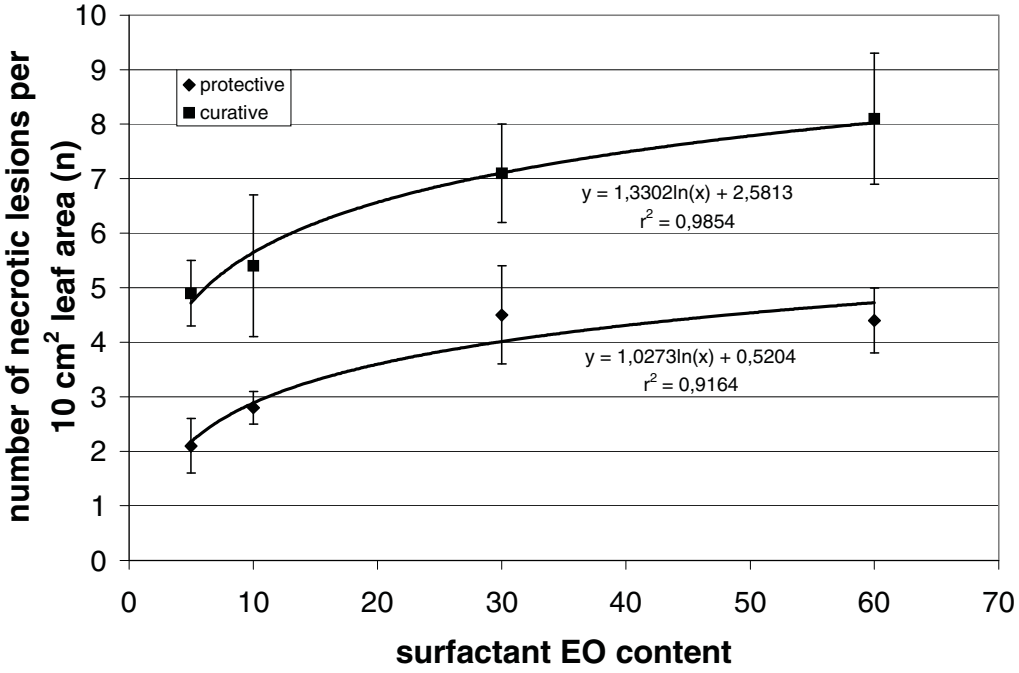

Fig. 5. Effect of prochloraz solutions (2,7 mM) on *Botrytis cinerea* incidence of lettuce (7 days after inoculation). Surfactants (RSO 5 EO, 10 EO, 30 EO, 60 EO) were added at a concentration of 1 g litre^{-1}.

4 Discussion

The addition of 'Agnique RSO®' surfactants with 30 and 60 EO units to prochloraz significantly enhanced spray retention on *L. sativa* (Figs. 1, 2). Spray retention mainly depends on droplet size, droplet velocity and intrinsic physico-chemical properties of both droplet and leaf surface (Holloway, 1994; Watanabe and Yamaguchi, 1991). Thus, droplets may be retained, reflected or shatter into smaller satellites which may be lost or undergo further impaction (Holloway, 1994). Surfactants have been shown to influence several of these processes (Anderson and Hall, 1989; De Ruiter et al., 1990). Enhanced spray retention with increasing surfactant EO chain length can therefore not merely be related to surfactant effects on solution surface tension and on contact angles of spray droplets which both increase with extended surfactant EO chain length (chapter B, Tab. 1) In contrast to high volume sprays (spraying leaves to runoff), where decreasing surface tension reduces retention, the

primary effect of surfactants in low volume sprays is an increase in interface area of droplets with the plant surface, since the spray is deposited primarily as discrete droplets (Bukovac et al., 1995). In our low volume study, the apparently anomalous retention enhancement behaviour of surfactant solutions with higher surface tension indicates the likely involvement of other physico-chemical properties. Surface elasticity is one possible factor, and a striking illustration of its influence is provided by solutions of very low surface active polyvinylalcohols, which form large loops and tails in solution, and thereby enhance the elasticity of impacting droplets, thus minimising the likelihood of bounce from the surface (Wirth et al., 1991). It has been found out that dynamic surface tension is more reliable than equilibrium surface tension as a measure of surfactant effectiveness because surfactants have to replenish new interfaces fast enough to affect wetting, spreading, and retention during impaction (Brazee et al., 1994). It is suggested that dynamic surface viscosity is the factor missing in providing a satisfactory explanation for surfactant-enhanced foliar retention (Stevens et al., 1993). As spray volume and pressure are the same for all treatments, the three factors mentioned above as well as droplet size, energy transfer/conversion and surfactant orientation are likely to be involved but have not been investigated in this study (Hartley and Brunskill, 1958).

The penetration of prochloraz through isolated tomato fruit cuticles initially was lowered by all surfactants and merely the surfactant RSO 30 enhanced penetration after 144 h (Fig. 3). For non-electrolytes and non-ionised weak acids and thereby for the active ingredient ‘Prochloraz’, plant cuticles are solubility membranes, as solutes dissolve and diffuse in cutin and amorphous waxes (Schönherr, 2000). According to the free-volume theory, diffusion in cuticular membranes takes place by solutes jumping from vacancy to vacancy in the membrane matrix (Buchholz and Schönherr, 2000). This free volume greatly depends on temperature and the presence of plasticisers (accelerators) which both have the same mechanism of action, as they both increase solute mobility by increasing fluidity of amorphous waxes (Schreiber et al., 1996; Schönherr and Baur, 1996; Baur et al., 1997; Baur et al., 1999). Thus, vacancies exist only for a very short time and are not to be imagined as permanent diffusion paths in the membrane (Schönherr, 2000). It can be concluded that except for RSO 30, the surfactants used in this study are not very effective plasticisers. Contrary, as they lowered the penetration of prochloraz at the beginning of the time course, the surfactants presumably decreased the degree of micro-structural heterogeneity and thereby reduced free volume. Plasticisers have to be sorbed by the cuticlar membrane to increase the free volume and hence, three explanations may be visualized for lower penetration of

prochloraz in the presence of surfactants at the beginning of the time course (Buchholz and Schönherr, 2000). First, the surfactants were sorbed by the cuticular membrane but had no plasticising effects and merely occupied vacancies. In course of time, the surfactants diffused through the cuticle into the receiver solution and thereby opened vacancies again, resulting in increasing prochloraz penetration. Low concentrations of the surfactant RSO 30 however, may inrease micro-structural heterogeneity resulting in enhanced penetration after 144 h compared with non-formulated prochloraz. Second, the surfactants were sorbed by the cuticle and except for RSO 30 had a higher affinity for prochloraz and competed with the cuticle for the active ingredient. Provided that the amount of surfactants does not change with time due to penetration or volatilization, the active ingredient remained in the deposit and thereby, did not penetrate (Schönherr and Baur, 1994; Baur et al., 1997). Third, the surfactants were not sorbed by the cuticular membrane and merely had wetting effects which may have led to decreased wax fluidity with the consequence of lowered free volume (Bukovac et al., 1995). Only after several days the surfactants and especially RSO 30 were sorbed and hence, the believed plasticiser effect of amorphous regions being swollen without solvating and destroying the crystals and crystals acting as cross-links that limit swelling, may have occured (Mauritz et al., 1990).

The effects of protectively and curatively applied prochloraz solutions (2.7 mM) on *Botrytis cinerea* incidence in lettuce (7 days after inoculation) depended markedly on the surfactant EO chain length (Figs. 4, 5). Protective treatments were more effective than curative ones and their efficacy increased with decreasing surfactant EO content. This cannot directly be related to the retention of different prochloraz spray solutions which increased with increasing surfactant hydrophilicity (Figs. 1, 2). As the incidence of *Botrytis cinerea* did also not correspond to the amount of prochloraz penetrated through isolated cuticles (Fig. 3) and as the surfactants themselves had no antifungal effects (unpublished data), other aspects should be taken into consideration. It was shown that requirements for retention and wetting can be conflicting (Furmidge, 1962). Though spray retention on *L. sativa* increased with increasing surfactant EO content, the wetting of the lettuce leaf surfaces may have decreased (increasing contact angles) and thereby became less effective (chapter B, Tab. 1) The surfactants may also have affected the nature of the deposits and possible internal effects resulting in effects on *Botrytis cinerea* incidence other than expected from the retention and penetration data. The results illustrate the need for a better understanding of the physico-chemical interactions between the active ingredients, the surfactants and the plant cuticle.

Some of the investigated surfactants enhanced prochloraz spray retention and improved prochloraz performance in controlling *B. cinerea* incidence in lettuce. Though they were added solely at a concentration of 1 g litre^{-1} to the prochloraz active ingredient, the surfactants with a short EO chain were more effective in reducing *B. cinerea* incidence than the formulation of the commercial product SPO. Our data provide evidence that protectively applied prochloraz under addition of the lipophilic surfactants RSO 5 or RSO 10 is an effective alternative in controlling grey mould in lettuce for fungicides of the benzimidazole and dicarboximide classes to which resistance is widespread in *B. cinerea* populations. Because of their positive toxicological and ecotoxicological profiles, the use of these rapeseed oil derivatives does not cause any risk for the operator, the environment and the food consumer.

5 Summary

The biological efficacy of the fungicidal active ingredient prochloraz for the control of *Botrytis cinerea* incidence in *Lactuca sativa* as well as the effectiveness of a homologous series of biodegradable rapeseed oil derivatives (triglyceridethoxylates) with an average of 5 (Agnique RSO 5®), 10 (Agnique RSO 10®), 30 (Agnique RSO 30®) and 60 (Agnique RSO 60®) units of ethylene oxide (EO) as adjuvants for prochloraz were investigated. The experiments were performed using 21-day-old *Lactuca sativa* plants, grown in a growth chamber at 25/20 (±2)°C day/night temperature and 40/70 (±10)% relative humidity. The efficacy of prochloraz in controlling *Botrytis cinerea* incidence was determined by assessment of visible symptoms seven days after inoculation. The surfactants were evaluated for enhancement of spray retention, foliar penetration of prochloraz and biological efficacy of prochloraz. Prochloraz was applied at a concentration of 2.7 mM. The surfactants were added at concentrations of 1 g litre^{-1}. The commercial product Sportak 40® and unformulated prochloraz always served as reference. Our data provide evidence that *Botrytis cinerea* can be controlled by prochloraz application. Protective prochloraz treatments were more effective than curative ones. Some of the surfactants employed significantly improved spray retention and biological efficacy. The rapeseed oil ethoxylates are not very effective plasticicers. Therefore, they did not markedly enhance cuticular penetration of prochloraz. Spray solutions with Agnique RSO 5® and Agnique RSO 10® were more effective in controlling *Botrytis cinerea* incidence than Sportak 40®.

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E Effect of rapeseed oil ethoxylates on rainfastness of systemic, loco-systemic and non-systemic active ingredients

1 Introduction

In order to maximise their biological efficacy and to minimise their pollution potential and environmental fate pesticides have to be able to withstand, following application, weathering due to wind, rain, dew formation and exposure to UV radiation (Willis et al., 1980; Taylor and Matthews, 1986; Omar and Matthews, 1991). Of all physicochemical, biochemical and metabolic processes that occur in the environment, rainfall has been reported as having the greatest effect upon the residual activity of a pesticide (Willis and McDowell, 1987). Rainfall may affect the activity of a deposit by dilution, redistribution, physical removal, and by the extraction of pesticide from the plant tissue (Thacker and Young, 1999). Rainfastness of an active ingredient, among several factors, will depend upon the ingredients present in the end-use mixture (i.e., the active ingredient, the adjuvants, and the carrier liquid), the area of contact with the foliar surface, the surface characteristics of foliage, the type and chemistry of epicuticular wax on the leaf, the type of rainfall, and the rain-free period after treatment (Leung and Webster, 1994).

Ideally, a foliar applied pesticide should persist in the target foliage just long enough to cause pest mortality, and should rapidly degrade afterwards into innocuous products (Leung, 1994). However, it is not always possible to spray when rainfall is unlikely. For example, insecticides are applied to tobacco plants in Zimbabwe during the rainy season and under such circumstances any increase in the rainfastness of the formulation would be of considerable help (Mashaya, 1993).

Recently, pesticide producers are frequently confronted with customers demanding for detailed informations about rainfastness of their compounds (Stierl, 2001). Though, by now, many products are labeled with data about their rainfastness, these declarations do not really give evidence. As a standardized procedure is missing, every company has its own method to determine rainfastness and hence, the results are not comparable. There is need to standardise determination of rainfastness of compounds to provide customers with objective and reliable specifications.

Generally, two approaches can be used to evaluate rainfastness of spray films:

1. to determine the biological efficacy following exposure to simulated rainfall in bioassays (Sandbrink et al., 1993; Coble and Brumbaugh, 1993; Reddy and Singh, 1992).

2. to quantify the residues of active ingredient on leaves that had been treated with a known amount of pesticide chromatographically, fluorometrically, by using radiolabelled active ingredients or by SEM assessment (Jones et al., 1989; Roggenbuck et al., 1993; Leung, 1994; Thacker and Young, 1999).

In this study the effect of rapeseed oil ethoxylates on rainfastness of systemic, loco-systemic and non-systemic active ingredients was investigated, using a rain-simulator as described by Benz et al. (2000).

2 Materials and methods

2.1 Plant material

Experiments were performed using 10-day-old *Phaseolus vulgaris* L. cv. *nanus*, 14-day-old *Lactuca sativa* L. var. *capitata* L. cv. *Nadine*, and 21-day-old *Setaria viridis* L. plants. Bean seeds were germinated in commercial natural growing medium (Potground RHP SLA, BVB, Maasland, NL) in a covered plastic container to maintain moisture. Following emergence, seedlings were transfered to plastic pots (5 cm in diam, 5.5 cm in ht., one plant per pot) filled with a mixture of growing medium and quartz sand (3 + 1 by volume). Ten seeds of *S. viridis* and three lettuce seeds were sown directly into the plastic pots described above. Following emergence, *S. viridis* seedlings were thinned to six plants per pot and lettuce seedlings to one plant per pot. Plants were grown in an environmental chamber at 25/20 (±2)°C day/night temperature and 40/70 (±10)% relative humidity. Photosynthetically active radiation (PAR) was provided at 200 µmol s^{-1} m^{-2} at the plant level during a 14-h photoperiod.

2.2 Chemicals

Spray solutions were prepared as follows:

1. 43 mM isopropylamine (IPA) salt of glyphosate (62 % purity, 35 % water, 3 % related impurities and isopropylamine, Monsanto Europe S.A.)
2. 5.4 mM prochloraz using 1-{N-propyl-N-[2-(2,4,6-trichlorophenoxy)ethyl]carbomyl}-imidazole (99 % purity, Hoechst Schering AgrEvo GmbH, Berlin, Germany), dissolved in 10 % methanol.

3. 6.5 mM tolylfluanid using N-Dichlorofluoromethylthio-N',N'-dimethyl-N-p-tolylsufamide (96,6 % purity, Bayer AG, Leverkusen, Germany) dissolved in 50 % acetone.

Surfactants ('Agnique RSO®' series, Cognis Düsseldorf, Germany) were commercial preparations of rapeseed oil derivatives (triglyceridethoxylates) with an average of five (RSO 5), 10 (RSO 10), 30 (RSO 30) and 60 (RSO 60) ethylene oxide (EO) units. Surfactants were added at a concentration of 1 g litre^{-1} to the spray solutions. Nonformulated active ingredients and the commercial products Roundup Ultra® (Monsanto Düsseldorf, Germany), Sportak 40® (Hoechst Schering AgrEvo GmbH, Berlin, Germany), Euparen Multi WG® (Bayer AG, Leverkusen, Germany) served as references.

2.3 Plant treatment and rain application

According to the experimental question plants were treated with a hand sprayer until run off 2 and 6 h before application of simulated rain. A rain simulator (B-LRS 2, Department of Agricultural Engineering, University of Bonn) was constructed to apply a defined amount of "rainfall" (tapwater) as described by Benz et al. (2000). Briefly, three individually controlled flat-fan nozzles (TeeJet AI 10010, Surrey, UK) generated 25 mm of artificial rain within a periode of 6.5 h. The water pressure was adjusted to a constant of 1 bar, which resulted in a mean droplet diameter of 1000 µm, representing an average droplet size during natural continuous medium to heavy rain events. The nozzles operated successively, moved forwards and backwards at a travel speed of about 6 km h^{-1} and were located 2.5 m above the plant ground level. The nozzles were adjusted prior to test applications to ensure a uniform "rainfall" over an area of 80 cm in length and 250 cm in width. The homogeneity of droplet distribution was proven with water sensitive paper and graduated glass cylinders which were randomly positioned within the application area of 2 m². The raindrops hit the plants from a nearly vertical angle because of the low nozzle pressure and the large distance between plants and nozzles.

2.4 Evaluation of Rainfastness

2.4.1 Bioassay approach

The bioassay approach was chosen for the herbicidal systemic compound glyphosate. Every treatment group consisted of 12 *P. vulgaris* and 12 *S. viridis* plants, which were exposed to artificial rain two hours after treatment. The short period of 2 h until rain exposure was chosen

because an extended drying time may have led to considerable glyphosate penetration into the tissue such that differences in rainfastness between treatment groups may have been difficult to establish. Untreated plants served as reference.

Maximum fluorescence (Fm) of *P. vulgaris* primary leaves and the first fully extended leaves of *S. viridis* was measured 24, 48 and 72h after application with a 'pulse-amplitude-modulation-fluorometer' (PAM, Model 2000, WALZ, Effeltrich, Germany) as used by Buwalda and Noga (1994). After adaptation of the leaves to dark conditions for 30 min., leaves were exposed to a light flash of 1.8 mmol photons $m^{-2}s^{-1}$. As maximum fluorescence is related to electron transport activity, a decrease of Fm is an indication for a blockage of electron transport at the photosystem II-donorside and a disorder in energy transfer on the pigment level, respectively (Renger and Schreiber, 1986).

2.4.2 Analytical approach

The quantitative/analytical approach was chosen for the fungicidal loco-systemic active ingredient prochloraz and the contact fungicidal compound tolylfluanid. *L. sativa* plants were treated 6 h before exposure to artificial rain with prochloraz. Tolylfluanid was applied to *P. vulgaris* 2 h before simulated rainfall. All treatment groups consisted of 12 plants each. One half of the plants was exposed to artificial rain, the other six plants served as control. By using control plants it was possible to determine the rainfastness of the loco-systemic prochloraz analytically because the amount of active ingredient that penetrated into the plant tissue was approximately the same in rain-exposed and non-exposed plants of the same treatment group. After the rain event prochloraz residues were rinsed off the upper surfaces of the third and the fourth leaf of *L. sativa* and tolylfluanid residues were eluted from upper surfaces of primary leaves of *P. vulgaris* with a mixture of acetone and cyclohexane (1:20 by volume). At the same time, the leaf residues of non-irrigated plants were washed off. The amounts of active ingredient in the elutes were determined by GC:

Gaschromatograph:	Model AS 800 Sato C.U.; Satochrom; Germany
Column:	SE 30; 10 m; Ø 0,45 mm
Injector:	Split-Splitless: split 60 s
Carrier:	N_2
Oven-temperature:	160 °C, isocratic
Flow-rate:	3 ml/min
Detector:	ECD; 300°C
Integration:	ChromCard (Satochrom, Germany)

Thereafter, leaf areas were measured with a leaf area meter (CI 202, DID, Inc.). The amount of residues was related to the respective leaf area. Residues per leaf area of leaves exposed to artificial rain were related to the amount of residues per leaf area of non rain exposed leaves. Rainfastness was expressed as percentage of residues on upper leaf surfaces.

2.5 Statistical analysis

The experimental data were analysed with the statistic program 'statgraphics', (Rockville, Maryland, USA). A 5 % probability level was accepted to indicate significant differences. The data were tested for normal distribution and variance homogenity and were compared by Tukey-HSD multiple range tests (Köhler et al., 1994).

3 Results

3.1 Bioassay approach

Compared to untreated but rain-exposed control plants, all glyphosate spray solutions reduced Fm of *P. vulgaris* and *S. viridis* significantly 72 h after treatment and exposure to artificial rain 2 h after treatment (Figs. 1 and 2). Initially, the addition of the surfactants RSO 10, RSO 30 and RSO 60 to glyphosate spray solution increased Fm of *P. vulgaris* primary leaves. A similar increase was noticed for *S. viridis* plants treated with nonformulated glyphosate and glyphosate + RSO 30. The increase in Fm values was measurable 24 h after treatment but after further 24 h, all glyphosate treatments reduced Fm of *P. vulgaris* and *S. viridis*.

In *P. vulgaris* the commercial product Roundup Ultra® (RPU) decreased Fm most after 72 h maximum. Compared with nonformulated glyphosate, the addition of the surfactants RSO 5, RSO 10 and RSO 60 reduced Fm. This reduction was negatively related to surfactant EO content. With decreasing EO chain length the reduction of maximum chlorophyll fluorescence was enhanced.

In *S. viridis* all surfactant treatments resulted in a maximum decrease of Fm after 72 h compared with nonformulated glyphosate. The significantly lowest Fm values were measured in plants treated with glyphosate + RSO 5 and in plants treated with RPU. The surfactant RSO 5 reduced Fm slightly more than the formulation of RPU during the whole time course.

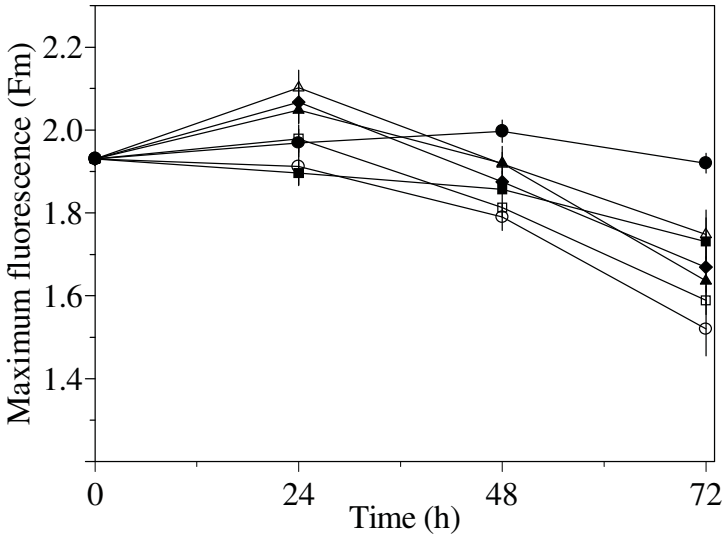

Fig. 1. Maximum chlorophyll fluorescence (Fm) of *P. vulgaris* leaves after treatment with aequeous glyphosate spray solutions (43 mM) and exposure to 25 mm of artificial rain 2 h after treatment. (●) untreated, (○) Roundup Ultra®, (■) G, (□) G + RSO 5 EO, (▲) G + RSO 10 EO, (△) G + RSO 30 EO, (◆) G + RSO 60 EO. G = Glyphosate.

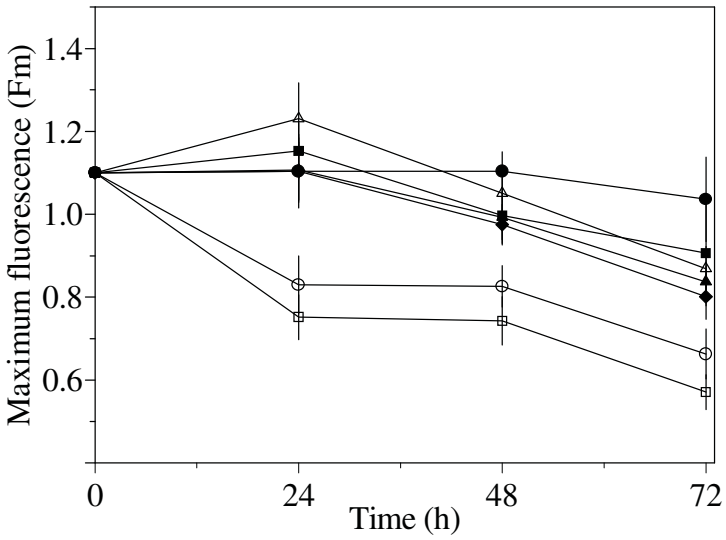

Fig. 2. Maximum chlorophyll fluorescence (Fm) of *S. viridis* leaves after treatment with aequeous glyphosate spray solutions (43 mM) and exposure to 25 mm of artificial rain 2 h after treatment. (●) untreated, (○) Roundup Ultra®, (■) G, (□) G + RSO 5 EO, (▲) G + RSO 10 EO, (△) G + RSO 30 EO, (◆) G + RSO 60 EO. G = Glyphosate.

3.2 Analytical approach

After exposure to 25 mm of artificial rain 6 h after treatment less than 1 % of the commercial product Sportak 40® remained on the leaf surface of *L. sativa* (Fig. 3). Compared with Sportak 40®, rainfastness of the nonformulated active ingredient prochloraz was significantly higher (5.5 % residues). The addition of rapeseed oil ethoxylates to prochloraz had very different effects. The most lipophilic surfactant (RSO 5) enhanced the amount of prochloraz residues to a level of about 8 %, whereas RSO 10 had no effect on prochloraz rainfastness. The more hydrophilic surfactants RSO 30 and RSO 60 reduced rainfastness of prochloraz to a level comparable to that of Sportak 40®.

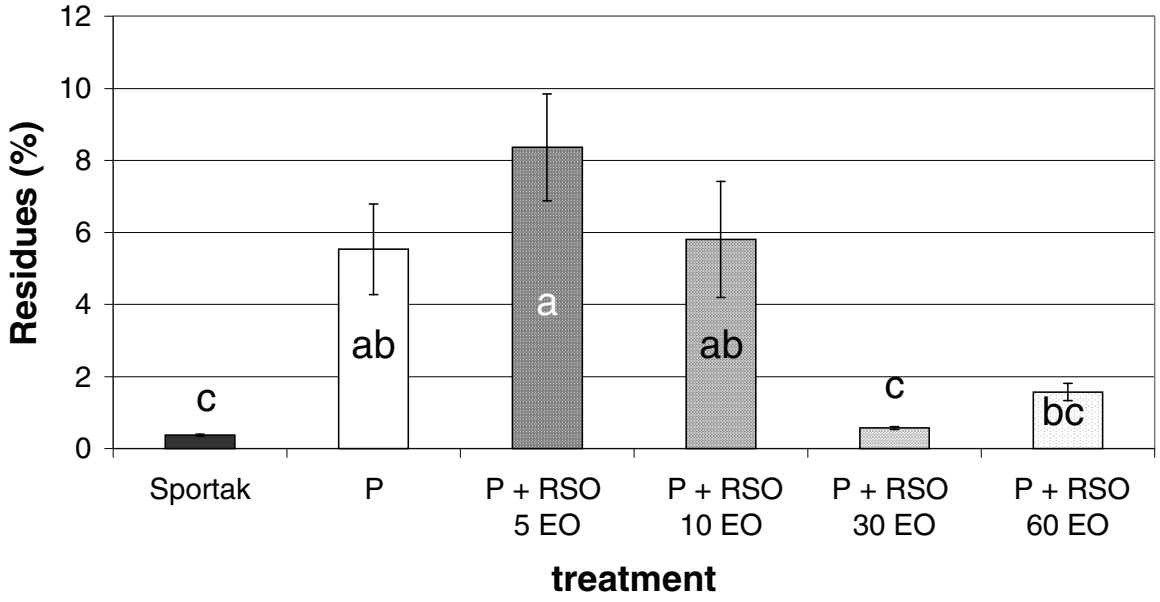

Fig. 3. Rainfastness of aequeous prochloraz spray solutions (5.4 mM) on *L. sativa* after exposure to 25 mm of artificial rain 6 h after treatment. P = Prochloraz

Exposure of *P. vulgaris* plants to 25 mm of artificial rain 2 h after treatment with various tolylfluanid solutions resulted in a loss of about 30 % of the non-formulated active ingredient (Fig. 4). The formulation of the commercial product Euparen Multi® as well as the addition of the investigated surfactants to tolylfluanid decreased the amount of residues markedly to a level half of tolylfluanid. A negative relationship was observed between surfactant EO content

and the amount of tolylfluanid residues. With increasing surfactant EO chain length residues decreased from about 40 % to nearly 30 % of the applied dose.

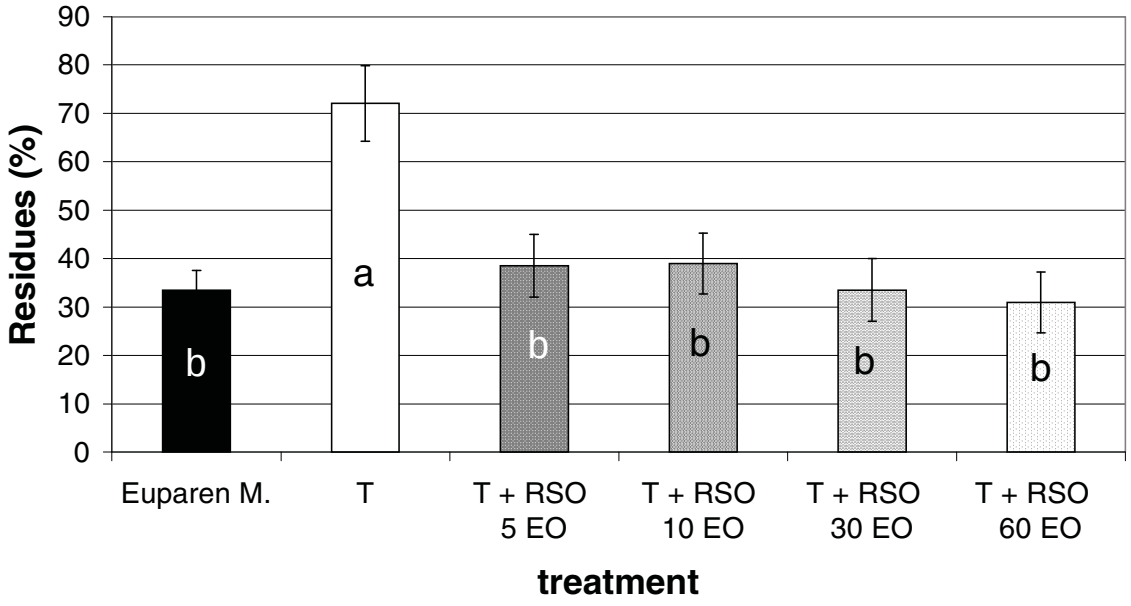

Fig. 4. Rainfastness of aequeous tolylfluanid spray solutions (6.5 mM) on *P. vulgaris* after exposure to 25 mm of artificial rain 2 h after treatment. T = Tolylfluanid

4 Discussion

In the bioassay studies, Fm values of *P. vulgaris* and *S. viridis* were markedly affected after application of the various glyphosate solutions and exposure to 25 mm of artificial rain 2 h after treatment (Figs. 1 and 2). As maximum fluorescence is related to electron transport activity, a decrease of Fm is an indication for a blockage of electron transport at the photosystem II-donorside and an increased glyphosate phytotoxicity, respectively. However, at the 24 h assessment some of the evaluated glyphosate spray solutions increased Fm of *P. vulgaris* and *S. viridis* compared to untreated control plants. This phenomenon was also observed in earlier studies with glyphosate and surfactants applied to various weed varieties

(chapter C) and can be explained with the surfactants initially stimulating the photosynthetical activity of the plants when nothing or only very little of the uptaked glyphosate was translocated to the site of action within the plant (Noga, 1991). It is evident that glyphosate in the absence of additional surfactants induced phytotoxicity 72 h after initiation of the experiment. Probably, sufficient of the highly water-soluble IPA salt of glyphosate which is very sensitive to rain-washing (Leung, 1994) penetrated into the tissue within the 2 h between spray application and exposure to artificial rain to cause this phytotoxic effect which led to mortality of *P. vulgaris* but not of *S. viridis* plants. These results document the requirement for a better rainfastness of glyphosate spray films to control not only glyphosate sensitive species but also weeds which withstand glyphosate to a certain degree.

When rapeseed oil ethoxylate surfactants were added to glyphosate Fm values decreased with decreasing surfactant EO content to a level comparable to plants treated with the commercial product Roundup Ultra®. The relatively high efficacy of glyphosate in the presence of the lipophilic surfactant RSO 5 may be due to: (a) the surfactant acting as a humectant and preventing the spray deposit from completely drying out, which is essential for glyphosate uptake and translocation; or (b) the surfactant providing a protective film on the surface of the deposit thus reducing the washoff in rainfall (Leung, 1994). However, earlier studies showed that glyphosate penetration through isolated tomato fruit cuticles was enhanced with increasing surfactant EO chain length (chapter C).

The quantitative determination of prochloraz residues on *L. sativa* after exposure to 25 mm of artificial rain 6 h after treatment indicated a very high sensitivity of the active ingredient to rainwashing (Fig. 3). Less than 6 % of the applied dose were recovered on the leaf surfaces after the rain event and the formulation of the commercial compound Sportak 40® even reduced tenacity of prochloraz to less than 1 %. This information about rainfastness of a loco-systemic active ingredient is reliable because treated but non-rain exposed control plants were used and the amount of prochloraz that penetrated into the plant tissue was approximately the same in rain exposed and non-rain exposed plants of the same treatment group. Again, these results emphasize the strong need for improvements in rainfastness of foliar applied compounds. Because of its low solubility in water (0.034 g/l at 25 °C), the relatively lipophilic prochloraz particles are assumed to be embedded into the lipophilic wax layer of the leaf cuticle (Jones et al. 1989). As the commercial product Sportak 40® is formulated as an emulsifiable concentrate (EC) the formulation components assist in bringing the active ingredient into solution but on the other hand reduce particle tenacity on the plant surface. The increasing rainfastness of prochloraz with decreasing surfactant EO content may also be

attributed to lipophilic surfactants embedding the prochloraz active ingredient better into the wax layer of the leaf cuticle than the more hydrophilic surfactants. However, similarly to their effects on glyphosate rainfastness, the lipophilic surfactants may have simply provided a better protective layer over the deposits, thus reducing the amount of prochloraz being washed off during rainfall.

Compared to prochloraz the contact fungicide tolylfluanid exhibited a much higher rainfastness even though the deposits were exposed to artificial rain as early as 2 h after treatment whereas prochloraz deposits were allowed to persist for 6 h (Fig. 4). This may be due to the very low aqueous solubility of tolylfluanid (0.0009 g/l at 20 °C) and the relatively high solubility in fat (5.096 g/100g standard fat HB307, NATEC, at 37 °C) which facilitated its embedding into the lipophilic wax layer of the leaf cuticle. Presumably, the physico-chemical properties of the plant surface play an important role with respect to rainfastness and hence, it might be possible that *P. vulgaris* has a higher affinity to tolylfluanid than *L. sativa* to prochloraz. Similar to studies with prochloraz, the rainfastness of the active ingredient tolylfluanid was dramatically reduced in the presence of the commercial Euparen Multi®-formulation which assist in bringing the active ingredient into solution but on the other hand reduce particle tenacity on the plant surface. All of the investigated surfactants reduced rainfastness of tolylfluanid to a level comparable to that of Euparen Multi®, presumably by solubilising the active ingredient. Therefore, in combination with tolylfluanid, rapeseed oil ethoxylate surfactants may be used as solvents but not as stickers. However, the earlier observations of increasing rainfastness with decreasing surfactant EO content seemed to be confirmed by this study.

Results of these studies with three very different active ingredients indicate the relevance and need for improvements of rainfastness of foliar applied compounds, not only for ecotoxicological but also for economic reasons by reductions in the number of pesticide applications or the application rates, respectively. However, when improving rainfastness of spray deposits it must be guaranteed that other important factors which influence the biological efficacy such as uptake rate and redistribution of active ingredient around the plant are not negatively affected.

5 Summary

The effect of a homologous series of rapeseed oil derivatives (triglyceridethoxylates) with an average of 5 (Agnique RSO 5®), 10 (Agnique RSO 10®), 30 (Agnique RSO 30®) and 60 (Agnique RSO 60®) units of ethylene oxide (EO) on rainfastness of the systemic herbicidal compound 'glyphosate', the loco-systemic fungicidal active ingredient 'prochloraz' and the contact fungicide 'tolylfluanid' on plant leaf surfaces was investigated using 10-day-old *Phaseolus vulgaris* L. cv. *nanus*, 14-day-old *Lactuca sativa* L. var. *capitata* L. cv. *Nadine*, and 21-day-old *Setaria viridis* L. plants. After being applied with a hand sprayer until run off, the active ingredient/surfactant solutions were allowed to dry on the leaf surfaces for 2 or 6 h, before they were exposed to 25 mm of artificial rain within a period of 6.5 h. Glyphosate rainfastness was assessed in a bioassay approach by measuring chlorophyll fluorescence (Fm) of *P. vulgaris* and *S. viridis* leaves 24, 48 and 72 h after application of the various glyphosate solutions. In summary, all glyphosate spray solutions reduced Fm significantly 72 h after treatment compared to untreated but rain-exposed control plants. With decreasing surfactant EO chain length the reduction of Fm was enhanced to a level comparable to that induced by the commercial product Roundup Ultra®. Prochloraz residues on *L. sativa* and tolylfluanid remainders on *P. vulgaris* were rinsed off the leaves with a mixture of acetone and cyclohexane (1:20 by volume). The amount of active ingredient in the elute was determined gaschromatographically. Results of these studies indicated a high sensitivity of the active ingredients to rainwashing. Only about 70 % of the applied non-formulated tolylfluanid and less than 6 % of solely applied prochloraz were recovered on the leaf surfaces after the standardized rain event. In the presence of the commercial formulations of Euparen Multi WG® and Sportak 40® the rainfastness of the active ingredients was dramatically reduced to nearly 30 % (tolylfluanid) and to less than 1 % (prochloraz) of the applied doses. In all experiments, lipophilic surfactants provided a better rainfastness than hydrophilic ones. In combination with prochloraz the most lipophilic surfactant RSO 5 enhanced rainfastness of the active ingredient whereas it reduced tenacity of tolylfluanid on the leaf surface. The present results confirm the necessity for improvements in rainfastness of foliar applied compounds.

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F Effect of rapeseed oil ethoxylates on the efficacy of exogenous applied $CaCl_2$

1 Introduction

Although soil fertilization is sufficiently high, Ca deficiency symptoms, such as bitter pit in apple fruits, blossom-end rot in fruits of tomato, pepper and watermelon, blackheart in celery and tipburn in headforming lettuce and cabbage varieties are often observed (Bangerth, 1979; Kirkby, 1979). These Ca related physiological disorders of plants are caused by the poor mobility of Ca within the plants. Hence, symptoms of physiological Ca deficiency are caused by local insufficient Ca supply for tissue at a time with high requirement whereas the rest of the plant is well provided with Ca (Thibodeau and Minotti, 1969; Ferguson, 1979). Since Ca is translocated via the xylem with the transpiration stream insufficient Ca supply mainly occurs in young, scarcely transpiring tissue. As a consequence, cell wall and membrane stability are reduced which may lead to cell breakdown with subsequent brownness (Maync, 1997).

For avoidance of Ca related physiological disorders of plants, control of temperature, light intensity or air humidity may be considered in the greenhouse but not in the field (Cox et al., 1976; Collier and Tibbitts, 1984; Gaudreau et al., 1994). However, appropriate cultivation management, such as the supply of water, nitrogen, potassium and magnesium as well as the choice of less sensitive varieties may help to reduce Ca disorders in both cultivation systems (Greenleaf and Adams, 1969; Peck et al., 1983; Burmeister and Dilley, 1991; Johnson, 1991; Witney et al., 1991).

As physiological Ca deficiency symptoms develop independently of the Ca supply of the soil, foliar application of Ca sprays is very effective in some cultures (Basher, 1993). The prime barrier for penetration of Ca deposited on the plant surface is the cuticle (Norris and Bukovac, 1968; Bukovac and Petracek, 1993). Penetration of Ca through the cuticle can be improved by addition of surfactants to the spray solution (Harker and Ferguson, 1991). For environmental reasons, biodegradable components as adjuvants for Ca should be preferred. Therefore, the effect of rapeseed oil derivatives (triglyceridethoxylates) on Ca penetration was studied using isolated tomato fruit cuticles and a finite dose diffusion unit as a model system. Furthermore, the impact of the surfactants on the Ca concentration in fruits as well as on bitter pit incidence was evaluated. Preliminary experiments revealed that the surfactants used are not phytotoxic.

A disadvantage of Ca spray applications is that several treatments are needed to increase fruit Ca levels significantly (Sharples and Johnson, 1977; Drake and Bramlage, 1983). Since little

or no subsequent translocation of Ca occurs, Ca sprays must be applied directly and uniformly to the fruit surface to be effective (Link, 1974; Redmond, 1975). Consequently, formulation residues of the Ca spray solutions accumulate on the fruits due to the low absorption of Ca unless the water-soluble deposits are washed-off by rain. As some of the commonly used surfactants in spray formulations are assumed to be ecologically and physically non-acceptable (Anonymus, 2000) we investigated the effectiveness of toxicological and ecotoxicological harmless rapeseed oil ethoxylate surfactants as adjuvants for $CaCl_2$ leaf fertilizers.

2 Materials and methods

2.1 Penetration experiments

2.1.1 Treatment solutions

Ca donor solutions, simulating agricultural spray solutions, were prepared using $CaCl_2x2H_2O$ (Merck, reagent grade) at a concentration of 0.2 M. Surfactants ('Agnique RSO®' series, Cognis Germany) were commercial preparations of rapeseedoil derivatives (triglyceridethoxylates) with an average of 5 (RSO 5), 10 (RSO 10), 30 (RSO 30) and 60 (RSO 60) ethylene oxide (EO) units. They were added to the donor solutions at a concentration of 1 g $litre^{-1}$.

2.1.2 Isolated Plant Cuticles

Cuticle Isolation

Epidermal fruit discs were punched with a cork borer from locally greenhouse-grown untreated mature tomato fruits, free of visible defects. The cuticles were isolated enzymatically from the tissue (Orgell, 1955; Yamada et al. 1964). The excised discs were incubated in a mixture of pectinase (40 g $litre^{-1}$, ICN Biomedicals Inc. Aurora, Ohio), cellulase (8 g $litre^{-1}$ Sigma Chemicals, St. Louis, MO) and NaN_3 (1 mM to prevent fungal and bacterial growth) in sodium citrate buffer (50 mM, pH 4.0) at 25°C. Enzyme solutions were changed several times during a 2-week period. The isolated cuticles were repeatedly rinsed with destilled water, air dried and stored at room temperature.

Measurement of cuticular penetration

The cuticular penetration of Ca was followed using a finite-dose diffusion system (Bukovac and Petracek, 1993). Briefly, cuticles were mounted in plexiglas holders, leak tested and positioned on the finite-dose diffusion half-cell with the outer morphological surface orientated to the ambient air and the cell wall side bathed with water. The volume of the receiver solution was 2.5 ml of aqueous citric acid buffer (Merck, Darmstadt, Germany) at 2 g litre^{-1}. The pH was adjusted to 4.0 using KOH. A stirring bar was used in the receiving cell to avoid boundary-layer effects.

At time zero, eight single drops (1µl each) of the treatment solution were applied to the cuticular surface using a microsyringe fitted with an automatic dispenser (Hamilton). The Ca content in the receiver solution was determined by Atomic Absorption Spectrometry 4, 24 and 48 h after initiation of the experiment.

2.2 Biological efficacy

2.2.1 Treatment solutions

Spray solutions were prepared using $CaCl_2x2H_2O$ (Merck, reagent grade) at a concentration of 0.03 M. Surfactants were added as described in chapter 2.1.1.

2.2.2 Plant material

Experiments were performed on 3-year-old tress of *Malus domestica cv. Braeburn* (rootstock M9) which were cultivated according to the guidelines of the integrated production at the Department of Horticulture, University of Bonn.

2.2.3 Treatment of plants

The following treatments were used in this study:

1. untreated control
2. $CaCl_2$
3. $CaCl_2$ + 0.1 % RSO 5
4. $CaCl_2$ + 0.1 % RSO 10
5. $CaCl_2$ + 0.1% RSO 30
6. $CaCl_2$ + 0.1% RSO 60

The spray solutions were applied on a weekly basis to the whole plant with a knapsack sprayer until run off, beginning six weeks before harvest. A total of 5 treatments was applied.

2.2.4 Analysis of Ca and K content in fruits

Fruits were harvested 6 weeks after onset of treatments and randomly sampled during picking. For removing Ca residues from the surface, fruits were washed two times with destilled water. Stripes of 1 cm in width and 0.5 in depth were peeled from the fruits. An aliquot of 1 g of the stripes was digested with HNO_3 and H_2O_2 according to Chen et al. (1997). The Ca and K concentrations were determined by Atomic Absorption Spectrometry.

2.2.5 Bitter pit incidence

Fruits were stored for 14 days after harvest at 20°C in a climatic chamber to increase bitter pit injury. After storage, bitter pit incidence was expressed as percentage of harvested fruits.

2.3 Statistical analysis

The experimental data were analysed with the statistic program 'statgraphics' (Rockville, Maryland, USA). A 5 % probability level was accepted to indicate significant differences. The data were tested for normal distribution and variance homogenity. Data on cuticular penetration and Ca-content of the fruits were compared by Tukey-HSD multiple range tests, data on bitter pit incidence incidence were compared by Duncan multiple range test (Köhler et al. 1994). Penetration rates were determined on 8 completely randomized replicates. Trials on apple trees consisted of 6 replications. For Ca analysis and determination of bitter pit incidence five fruits of each replication were randomly selected.

3 Results

3.1 Effect of surfactants on cuticular Ca penetration

All 'Agnique RSO®' surfactants employed enhanced penetration of Ca through isolated tomato fruit cuticles, as documented for the 24 h sampling date (Fig. 1). However, a significant increase of penetration was only established for the RSO 5 EO surfactant. A negative relationship was determined between the EO content of the surfactants and the amount of Ca that penetrated the cuticle (Fig. 2). Penetration was enhanced, as the EO chain length decreased from 60 to 5 EO units. The time-course study indicated that penetration mainly occured during the first 4 h after treatment and approached a plateau after 24 h (Fig. 3).

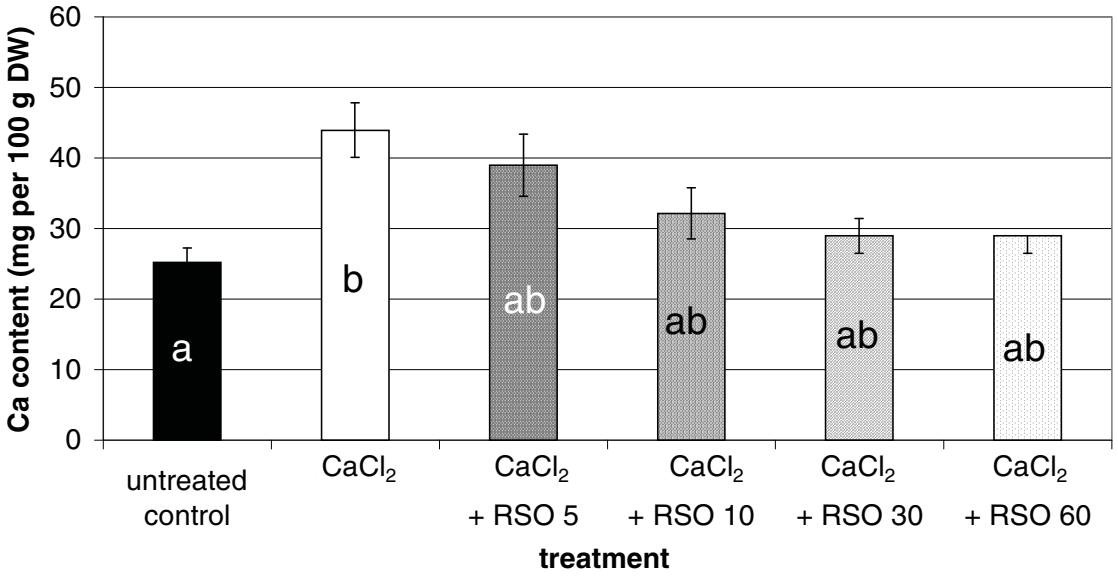

Fig. 1. Penetration of $CaCl_2$ spray solutions (0.2 M) through isolated tomato fruit cuticles 24 h after treatment. Surfactants (RSO 5 EO, 10 EO, 30 EO, 60 EO) were added at a concentration of 1 g litre^{-1}.

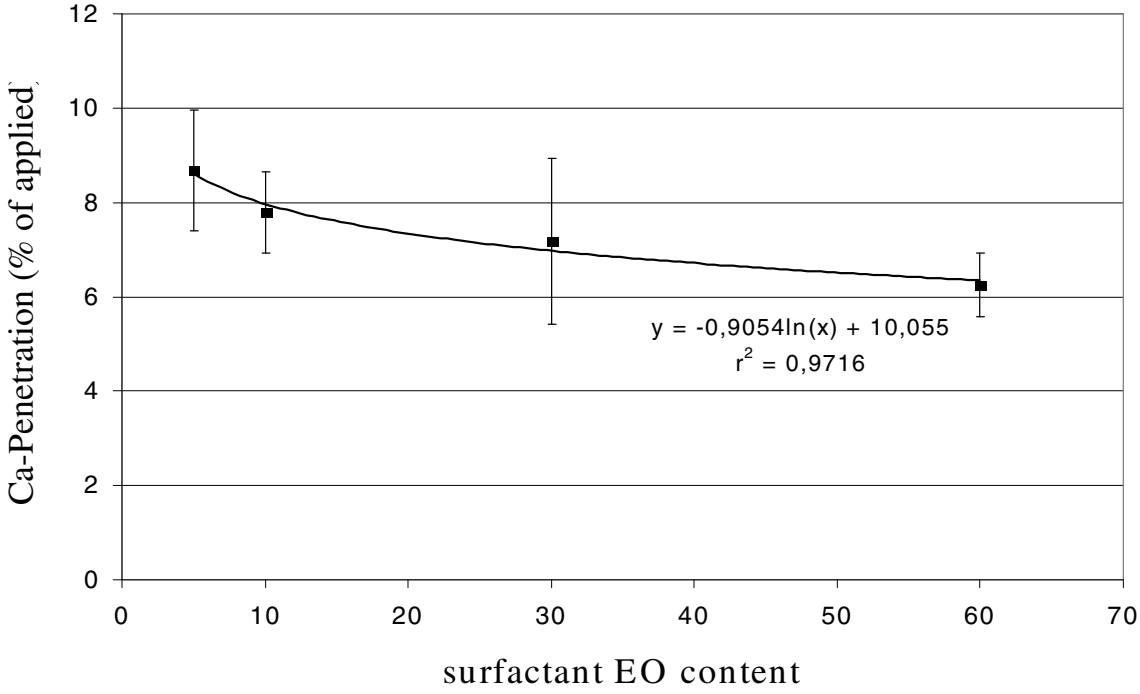

Fig. 2. Surfactant EO content-related penetration of $CaCl_2$ spray solution (0.2 M) through isolated tomato fruit cuticles 24 h after treatment. Surfactants (RSO 5 EO, 10 EO, 30 EO, 60 EO) were added at a concentration of 1 g litre^{-1}.

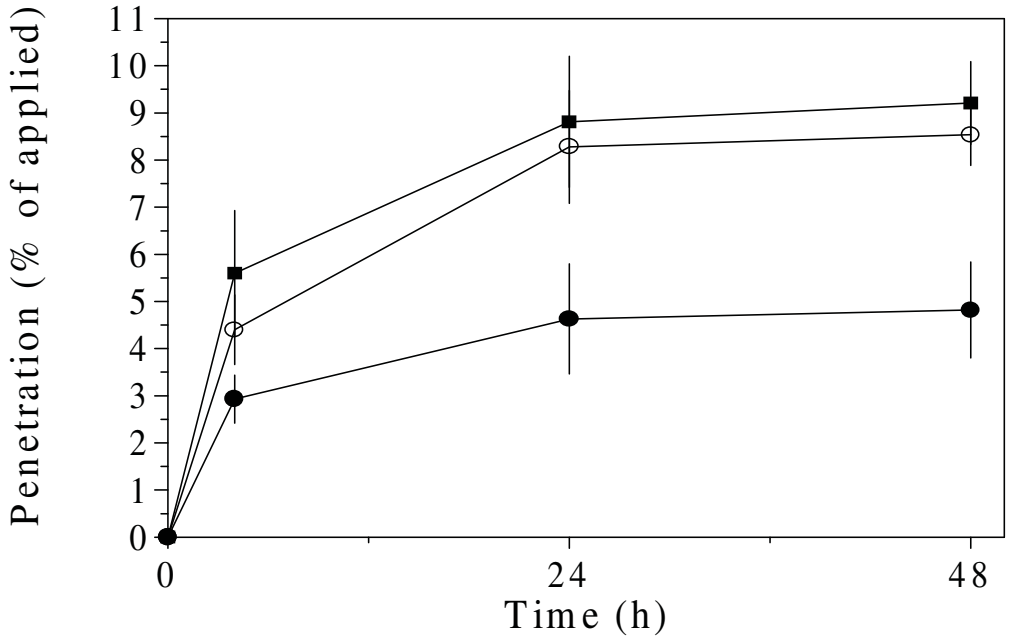

Fig. 3. Penetration of $CaCl_2$ spray solutions (0.2 M) through isolated tomato fruit cuticles. (●) $CaCl_2$, (■) $CaCl_2$ + 0.1 % RSO 5, (○) $CaCl_2$ + 0.1 % RSO 10

3.2 Effect of surfactants on the Ca content and the K/Ca ratio in fruits

The Ca content in apple fruits was significantly increased as a result of foliar application of $CaCl_2$ in combination with the surfactant RSO 5 (Fig. 4). This combination was also most effective in reducing the K/Ca ratio in fruits compared to the untreated control (Fig. 5).

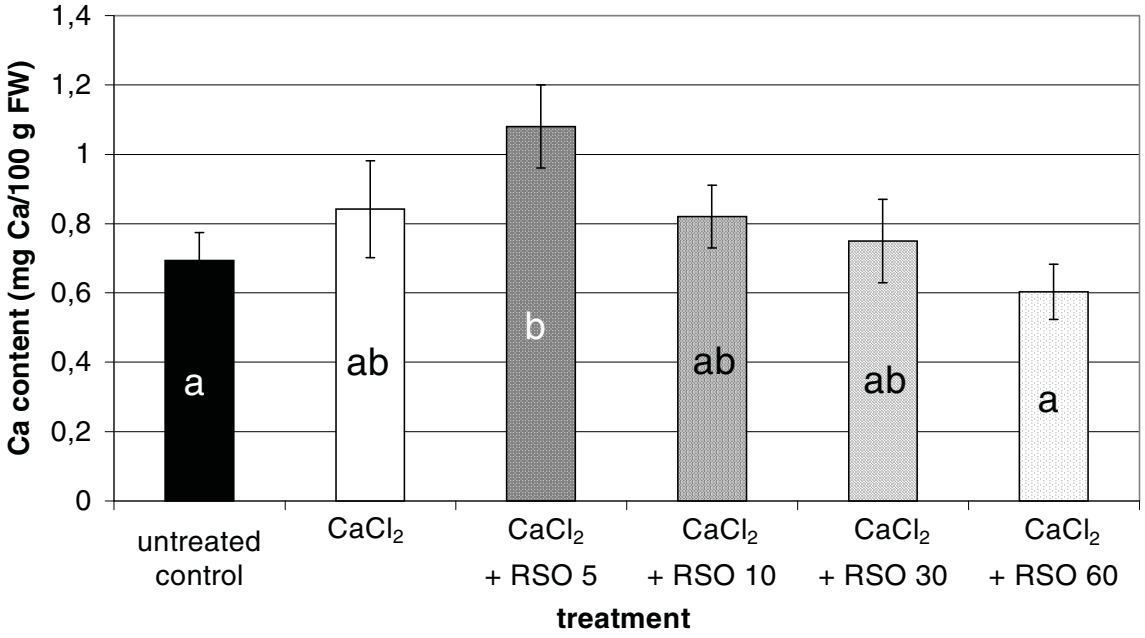

Fig. 4. Ca content of *Malus domestica cv. Braeburn* fruits after treatment with different $CaCl_2$ spray solutions (0.03 M), with and without addition of RSO-surfactants differing in degree of ethoxylation.

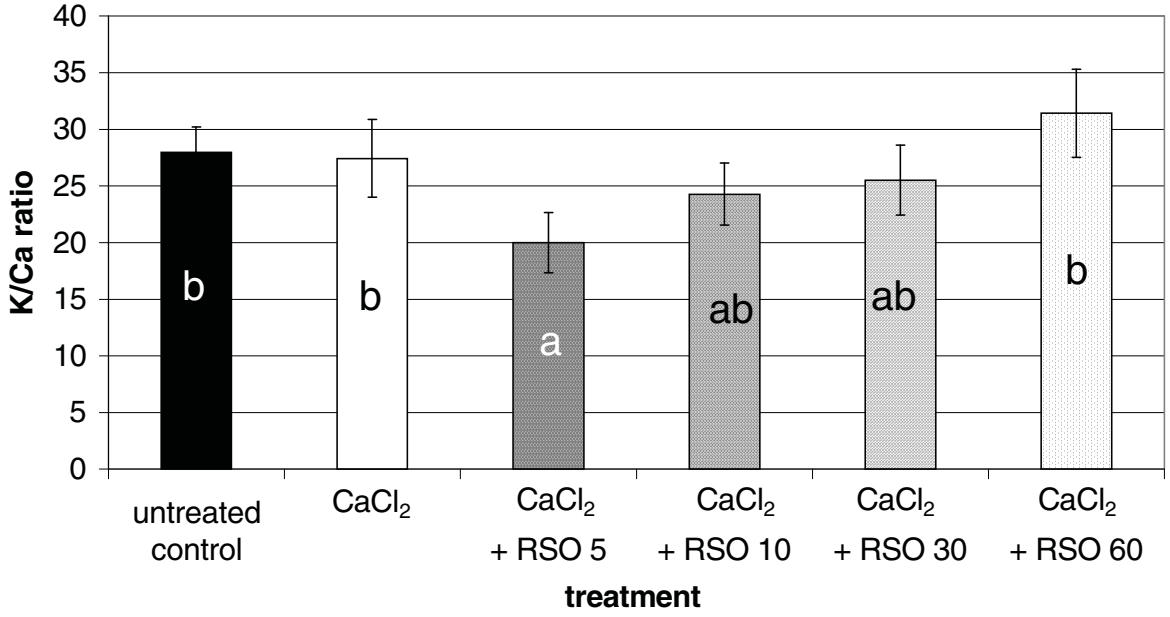

Fig. 5. K/Ca ratio of *Malus domestica cv. Braeburn* fruits after treatment with different $CaCl_2$ spray solutions (0.03 M), with and without addition of RSO-surfactants differing in degree of ethoxylation.

3.3 Effect of surfactants on bitter pit incidence

Bitter pit incidence was significantly decreased from about 45 % to 10 % of harvested fruits as a result of foliar application of $CaCl_2$ in combination with the lipophilic surfactant RSO 5 (Fig. 6). The reduction of bitter pit symptoms by the other investigated Ca treatments was not statistically significant.

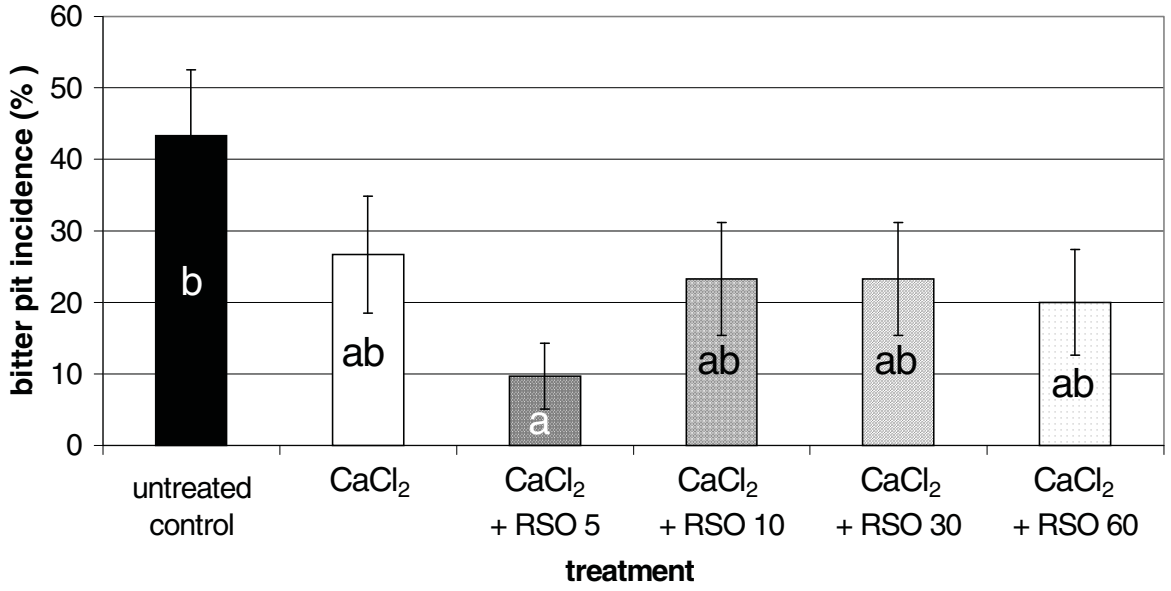

Fig. 6. Bitter pit incidence on *Malus domestica cv. Braeburn* fruits after treatment with different $CaCl_2$ spray solutions (0.03 M), with and without addition of RSO-surfactants differing in degree of ethoxylation.

4 Discussion

Our data demonstrate that addition of 'Agnique RSO®' surfactants to $CaCl_2$ was effective in enhancing Ca penetration through isolated tomato fruit cuticles (Fig. 1). It is generally agreed that improved penetration is the net result of several specific interactions between the active ingredient, the surfactant, and the target plant species (Steurbaut, 1994). The enhanced penetration most likely is due to a modification of cuticular permeability rather than to a direct effect on Ca mobility resulting from micellar sequestration, since the surfactant concentration in the application solutions is below the critical micellar concentration (Harker and Ferguson, 1991). Surfactants may affect structure of the cuticle by diffusing into the cuticle along hydrophilic-lipophilic interfaces, causing dilation of hydrophilic pores and a subsequent increase in permeability to polar solutes (Harker and Ferguson, 1988). As the lipophilic pathway constituted by amorphous waxes is not available for $CaCl_2$, the cuticular penetration of hydrated Ca^{2+} and Cl^- ions totally depends on the presence of aqueous pores which swell depending on humidity (Schönherr, 2000). Hence, our observation of an inverse

relationship between surfactant enhanced penetration and surfactant EO content may be explained with lipophilic surfactants wetting the cuticle surface more than those with a long EO chain (lower contact angle, unpublished data). The surface of the cuticular membrane is not smooth because of the valleys formed along the anticlinal walls (Schönherr, 2000). The most lipophilic surfactant RSO 5 (lowest surface tension, unpublished data) probably brought about the best contact between salt solutions and cuticles. Besides, the drying time of aqueous spray droplets increased with decreasing surfactant EO chain length (unpublished observations). Thus, deposits with more lipophilic surfactants remained moist much longer and, consequently, would allow for a longer swelling of aqueous pores. Therefore, short EO chain surfactants would lead to greater Ca penetration. Probably, both the wetted leaf area and the period of leaf wetness are important factors, or rather, the amount of expanded aqueous pores and the duration of pore swelling result in a penetration enhancing effect. The fact that penetration mainly occured during the first 4 h after treatment and reached a plateau after 24 h confirmed the assumption that aqueous pores have to be moist and swollen to allow Ca penetration.

Even though the effects of Ca treatments on the biological efficacy depend on a whole range of physico-chemical interactions between the active ingredient, the formulation, and the target plant species, the informations obtained from the finite dose experiments were comparable to the results of the field trials in this study. The addition of the most lipophilic surfactant RSO 5 to $CaCl_2$ resulted not only in a higher Ca content in fruits (Fig. 4) but also in a lower K/Ca ratio (Fig. 5) which has been proven to play a dominant role in the occurence of bitter pit in apple fruits (Schönhard, 1970). This relationship was reflected by the reduced bitter pit incidence when plants were treated with $CaCl_2$ in combination with RSO 5 (Fig. 6)

The enhanced Ca content in fruits after treatment with $CaCl_2$ in combination with the lipophilic surfactant RSO 5 is essential not only for cell wall and membrane stabilization, but also for the response to biotic and abiotic stresses (Rincon and Hanson, 1986; Roblin et al., 1989; Atkinson et al., 1990). Ca plays a pivotal role in the regulation of enzyme synthesis, e.g. proteinkinases or –phosphatases (Roberts and Harmon, 1992; Marschner, 1995). The influence of Ca on signal transmission processes as part of stress defense mechanisms are discussed by Schmitz-Eiberger et al. (2001, in press).

The surfactant Agnique RSO 5® is non-phytotoxic (unpublished data), it enhanced Ca penetration through isolated cuticles, it increased fruit Ca levels, it decreased the K/Ca ratio in fruits and it reduced bitter pit incidence. Because of the performance and positive toxicological and ecotoxicological profile of rapeseed oil ethoxylates the RSO 5 surfactant is

regarded as a promising adjuvant for $CaCl_2$ spray solutions. Besides, it proved to reduce blossom-end rot incidence in tomato and pepper fruits and to enhance Ca content in these fruits (unpublished data).

5 Summary

The efficacy of a homologous series of biodegradable rapeseed oil derivatives (triglyceridethoxylates) with an average of 5 (Agnique RSO 5®), 10 (Agnique RSO 10®), 30 (Agnique RSO 30®) and 60 (Agnique RSO 60®) units of ethylene oxide (EO) as adjuvants for foliage-applied $CaCl_2$ was evaluated. Previous experiments revealed that the surfactants used are not phytotoxic. The impact of surfactants on calcium penetration was studied using astomatous cuticular membranes isolated from mature fruits of *Lycopersicon lycopersicum*. The biological efficacy of the various $CaCl_2$ formulations was evaluated investigating their effects on bitter pit incidence and calcium content in fruits of *Malus domestica cv. Braeburn*. The spray solutions were prepared using $CaCl_2x2H_2O$ at 0.03 and 0.2 M concentration and the surfactants were added at a concentrations of 1 g $litre^{-1}$, respectively Our data provide evidence that the surfactants used can improve calcium penetration through isolated cuticles, increase calcium content in fruits under practice-related conditions and reduce bitter pit incidence.

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G Effect of rapeseed oil ethoxylates on the efficacy of exogenous applied magnesium

1 Introduction

‘Stiellähme’ is one of the most serious physiological diseases of grapevine (Vitis vinifera L.) leading to cluster injury in the ripening stage (Rumbos, 1989). The nomenclature applied to this disorder characterizes cluster stem symptoms (Christensen and Boggero, 1985). Thus, the terms ‘Stiellähme’ (Hifny and Alleweldt, 1972; Bauer et al., 1996), ‘dessèchement de la rafle’ (Ureta et al., 1981; Redl et al., 1996) and ‘disseccamento del rachide’ (Fregoni and Scienza, 1972) appear in the literature. Other synonyms are ‘bunch stem die-back’" (Gregory, 1966), ‘shanking’ (Jackson, 1994) and ‘waterberry’ (Kasimatis, 1957; Christensen and Boggero, 1985). The symptoms of ‘Stiellähme’ appear shortly after the onset of maturity, when the osmotic value of the berries becomes higher than that of the rachis (Stellwaag-Kittler, 1983). ‘Stiellähme’ can be associated with the same group of physiological diseases as bitter pit of apples and blossom-end rot of tomatoes and pepper (Boselli and Fregoni, 1986).

The earliest visual symptom is the development of small dark spots (1-2 mm) on the peduncle. These spots become necrotic, slightly sunken, and expand to larger areas (Hifny and Alleweldt, 1972; Christensen and Swanson, 1975). The necroses are harmless as long as they do not surround the stem. In the case when they cover the stem, they lead to cut-off of the nutrient supply and to the death of the part of the stem beyond the necrotic area (Stellwaag-Kittler, 1983; Haub, 1986). First, cells of stomata, epidermis and hypodermis of the grape stalk die. Then the necrosis spreads to the cortical tissue and in the more severe stage to the phloem cell (Brendel et al., 1983). The brown-to-black color of the affected cluster stem tissue develops during the berry ripening period and increases in intensity to dark coffee, purplish-black or black (Hifny and Alleweldt, 1972; Barker and Mills, 1980). The dark color is attributed to the concentration of oxidized polyphenols and may vary with cultivar (Hifny and Alleweldt, 1972; Stellwaag-Kittler, 1975). As composition of phenols is affected by the interrrupted flow of sugars and other nutrients or ripening constituents through the cluster stem structure, the berries become flaccid, wrinkled, withered, and of soft texture (Winkler et al., 1974; Ureta et al., 1981). By time many of them shrivel and dry completely at a rate dependent upon the extent of stem necrosis and temperature (Kasimatis, 1957; Christensen and Swanson, 1975, 1976).

No final verdict on the cause of ‘Stiellähme’ has been obtained. According to Brendel et al. (1983), the cause of these necroses, which occur in many varieties of grape cultivars with

differing intensity, is thought to be a metabolic disorder during the ripening process of grape berries. Because of the correlations between the percentual occurence of ‘Stiellähme’ and the shoot and grape peduncle growth, the time of flowering, fruit set, seed and berry development, it is concluded that the cause of this disorder is a misbalance of endogenous metabolic substances (phytohormones, carbo hydrates, inorganic ions), which are themselves influenced (positively or negatively) by the weather, soil and fertilizer conditions of a particular vineyard area (Brendel et al., 1983).

The mineral nutrients Mg, Ca and K, among the numerous genetic or environmental factors, play the most important role in the occurence of ‘Stiellähme’ (Rumbos, 1989). Mineral analyses revealed that diseased stalks contained 40 % less Mg and 20 % less Ca on average than healthy ones. The more serious the disease, the greater the difference in the ratio of K to Mg and Ca (Stellwaag-Kittler and Haub, 1965; Lauber and Koblet ,1967; Brechbuhler, 1975).

Mg-deficiencies are frequently associated with sandy soils in high rainfall regions, poorly drained sites, and high pH soils. This results from the relative ease with which Mg is leached from the soil (Jackson, 1994; Marschner, 1995). Symptoms of Mg-deficiency first begin to develop in basal leaves. Interveinal regions develop a straw-yellow chlorotic discoloration, while regions bordering the veins remain green. Early in the season, symptoms may appear as small brownish spots next to leaf margins (Jackson, 1994).

Mg is a vital cofactor in the chlorophyll-mediated splitting of water in photosynthesis. It stabilizes ribosomes, nucleic acids, and cell membrane structure and is involved in the activation of phosphate-transfering enzymes in metabolism (Jackson, 1994; Marschner, 1995).

To prevent a yield and quality loss caused by ‘Stiellähme’, sufficient supply of Mg is essential (Brendel et al., 1983). However, response to soil treatment is slower than to sprays (Cooper, 1973). Much of the earlier work has focussed on foliar applications of Mg at different concentrations (most commonly $MgCl_2$ and $MgSO_4$ sprays) with varying degrees of success (Alleweldt and Hifny, 1972; Beetz and Bauer, 1983; Stellwaag-Kittler, 1983; Rumbos, 1989).

As symptoms of ‘Stiellähme’ are similar to Ca-deficiency symptoms (accumulation of brown, oxidized substances in vacuols and breakdown of membranes), the efficiency of Mg against ‘Stiellähme’ could be a consequence of Ca desorption from unspecific bonds which may then become physiologically active (Schaller, 1983).

The objective of the present study was to study the effects of $MgSO_4$ and $Mg(NO_3)_2$ leaf fertilizers employed in different formulations on the penetration of Mg into the plant tissue. The effect of rapeseed oil ethoxylates on the penetration of Mg-ions was investigated using

isolated cuticles. The biological efficacy of various Mg-treatments was tested in two locations with different vine cultivars in the year 2000.

2 Materials and methods

2.1 Penetration studies

2.1.1 Chemicals

Donor solutions, simulating agricultural spray solutions, were prepared using $MgSO_4$ x $7H_2O$ and $Mg(NO_3)_2$ x $6H_2O$ (Merck, extra pure) at concentrations of 0.2 M. Surfactants ('Agnique RSO®' series, Cognis Germany) were commercial preparations of rapeseedoil derivatives (triglyceridethoxylates) with an average of 5 (RSO 5), 10 (RSO 10), 30 (RSO 30) and 60 (RSO 60) ethylene oxide (EO) units. A Mg formulation developed by the Department of Horticulture, University of Bonn served as standard. Surfactants and the formulation were added to aqueous $MgSO_4$ and $Mg(NO_3)_2$ solutions at concentrations of 1 g $litre^{-1}$.

2.1.2 Isolated Plant Cuticles

Cuticle Isolation

Epidermal fruit discs were punched from greenhouse-grown untreated mature tomato fruits, free of visible defects, with a cork borer. The cuticles were isolated enzymatically from the tissue (Orgell, 1955; Yamada et al., 1964). The excised discs were incubated in a mixture of pectinase (40 g . $litre^{-1}$, ICN Biomedicals Inc. Aurora, Ohio), cellulase (8 g . $litre^{-1}$ Sigma Chemicals, St. Louis, MO) and NaN_3 (1 mM to prevent fungal and bacterial growth) in sodium citrate buffer (50 mM, pH 4.0) at 25°C. Enzyme solutions were changed several times during a 2-week period. The isolated cuticles were repeatedly rinsed with destilled water, air dried and stored at room temperature.

Measurement of cuticular penetration

The cuticular penetration of Mg^{2+} was followed using a finite-dose diffusion system (Bukovac and Petracek, 1993). Briefly, cuticles were mounted in plexiglas holders, leak tested and positioned on the finite-dose diffusion half-cell with the outer morphological surface orientated to the ambient air and the cell wall side bathed with water. The volume of the

receiver solution was 2.5 ml. A stirring bar was used in the receiving cell to avoid boundary-layer effects.

At time zero eight single drops (1µl each) of the application solution were applied to the cuticular surface using a microsyringe fitted with an automatic dispenser (Hamilton). The Mg^{2+} content in the receiver solution was determined by Atomic absorption spectrometry (AAS) 24 h after initiation of the experiment. Penetration rates were determined on 8 completely randomized replicates.

2.2 Biological efficacy

The biological efficacy of various magnesium leaf fertilizers was investigated in the year 2000 on two locations with different vine cultivars:

2.2.1 'Graacher Domprobst' (Moselle Valley):

Tab.1: Plant material, conditions of location and set up of the experiment

Cultivar:	*Vitis vinifera* cv. *Riesling*
Rootstock:	5 C
Age:	11 years
Planting density:	1.5 x 1.2 m
Soil:	weak sandy loam
Slope gradient:	max. 58 %
Exposition:	SSW
Number of vines per treatment:	9-10
Number of replicates:	4

Spray solutions were prepared using $MgSO_4$ ('Bittersalz', 16 % MgO). The formulation and the surfactant Agnique RSO 5® were added as follows:

1. untreated control
2. $MgSO_4$
3. $MgSO_4$ + formulation (2 g $litre^{-1}$)
4. $MgSO_4$ + formulation + Agnique RSO 5® (each 1 g $litre^{-1}$)

The vines were sprayed in the morning, when temperature was low or moderate, using a knapsack sprayer. The spray solutions were applied until run-off at the following dates, developmental stages and Mg^{2+}-concentrations:

- 23 May; BBCH 56: 2 mM (10 days before bloom)
- 19 June; BBCH 73: 2 mM
- 21 July; BBCH 78: 4 mM
- 7 August; BBCH 80: 8 mM (one week before ripening)

Occasionally, very slight Mg-deficiency symptoms in leaves were visible.

Samples of leaf blades were taken September on 20th and 27th, from the 5.–8. node of the shoots, 8 leaves per date and replicate. On September 20th, leaf samples were washed with deionized water to remove magnesium residues from the surfaces. On September 27th residues were removed with a solution of 0.05 n HCl including 1 mg $litre^{-1}$ Tween 20. After being dried in a lyophilizer, each 8 leaf blades were ground to a fine powder, digested with HNO_3 and H_2O_2 according to Chen et al. (1997) and analysed by AAS.

Eight grape clusters per replicate were taken on October 10th. All berries were cut off with fine scissors at the point of attachement. Each 8 stalks were washed with a solution of 0.05 n HCl + 1 mg . $litre^{-1}$ Tween 20, dried, ground to a fine powder, digested with HNO_3 and H_2O_2 and analysed by AAS.

The harvest of the vineyard was on October 18th.

2.2.2 'Wolfer Schatzgarten' (Moselle Valley):

Tab.2: Plant material, conditions of location and set up of the experiment

Cultivar:	*Vitis vinifera* cv. *Regent*
Rootstock:	5 C
Age:	11 years
Planting density:	2.0 x 1.3 m
Soil:	weak clayey loam
Slope gradient:	max. 35 %
Exposition:	E
Number of vines per treatment:	7-9
Number of replicates:	4

Spray solutions were prepared using $Mg(NO_3)_2$ ('Magnisal', 16 % MgO) and the formulation mentioned above:

1. untreated control
2. $Mg(NO_3)_2$
3. $Mg(NO_3)_2$ + formulation (1 g . $litre^{-1}$)

The vines were sprayed as described above at the following dates and Mg^{2+}-concentrations:

- 23 May; BBCH 56: 0.75 mM (10 days before bloom)
- 19 June; BBCH 73: 0.75 mM
- 6 July; BBCH 75: 1.5 mM
- 21 July; BBCH 81: 1.5 mM (one week before ripening)
- 7 August; BBCH 86: 3 mM

Clearly visible Mg-deficiency symptoms in leaves appeared during the growing period in the whole vineyard. A visual symptom assessment was performed on August 24th, counting all leaves with medium to severe symptoms on each stock (comparison with a medium infested 'standard leaf').

Samples of leaf blades were taken as described above on August 29th and September 11th . On August 29th, magnesium residues were washed off with deionized water from the leaf surfaces, on September 11th residues were removed with a solution of 0.05 n HCl including 1 mg $litre^{-1}$ Tween 20. Each 8 leaf blades per replicate were dried in a lyophilizer, ground to a fine powder, digested with HNO_3 and H_2O_2 and analysed by AAS.

Sixteen grape clusters per replicate were taken between September 4th and 12th. All berries were cut off with fine scissors at the point of attachement. Each stalk was washed with a solution of 0.05 n HCl + 1 mg $litre^{-1}$ Tween 20, dried, ground to a fine powder, digested and analysed by AAS.

The harvest of the vineyard was on September 19th.

2.3 Statistical analysis

The experimental data were analysed with the statistic program 'statgraphics' (Rockville, Maryland, USA). A 5 % probability level was accepted to indicate significant differences. The data were tested for normal distribution and variance homogenity. Data on cuticular penetration and Mg^{2+}-content of the fruits were compared by Tukey-HSD multiple range tests, data on visual symptom assessment were compared by Duncan multiple range test (Köhler et al., 1994).

3 Results

3.1 Penetration studies

The surfactants RSO 5 and RSO 10 enhanced the penetration of Mg applied as $MgSO_4$ through isolated tomato fruit cuticles significantly after 24 h (Fig. 1). A negative relationship was established between the EO content of the surfactants and the amount of Mg that penetrated the cuticle (Fig. 2). Penetration increased, as the EO chain length decreased from 60 to 5 EO units. A similar relationship was established when the surfactants were added to $Mg(NO_3)_2$ formulated with a Mg formulation developed by the Department of Horticulture, University of Bonn (Fig. 3). The more lipophilic surfactants slightly increased Mg-penetration, whereas the hydrophilic surfactant RSO 60 had no effect.

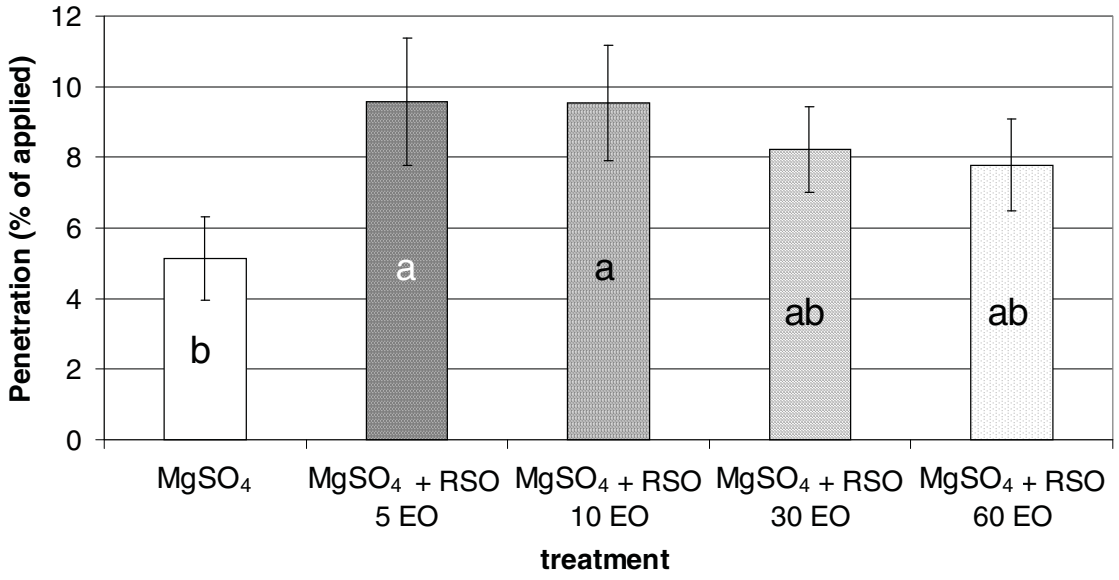

Fig. 1. Penetration of $MgSO_4$ spray solutions (0.2 M) through isolated tomato fruit cuticles 24 h after treatment. Surfactants (RSO 5 EO, 10 EO, 30 EO, 60 EO) were added at a concentration of 1 g litre^{-1}.

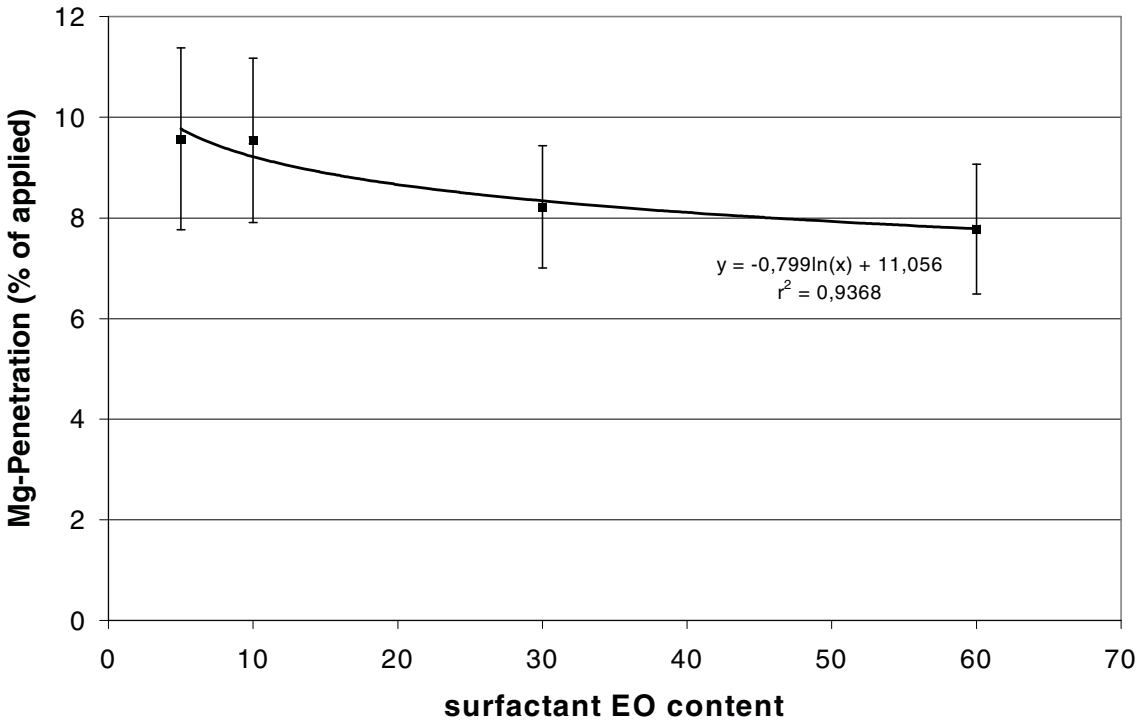

Fig. 2. Surfactant EO content-related penetration of $MgSO_4$ spray solution (0.2 M) through isolated tomato fruit cuticles 24 h after treatment. Surfactants (RSO 5 EO, 10 EO, 30 EO, 60 EO) were added at a concentration of 1 g litre^{-1}.

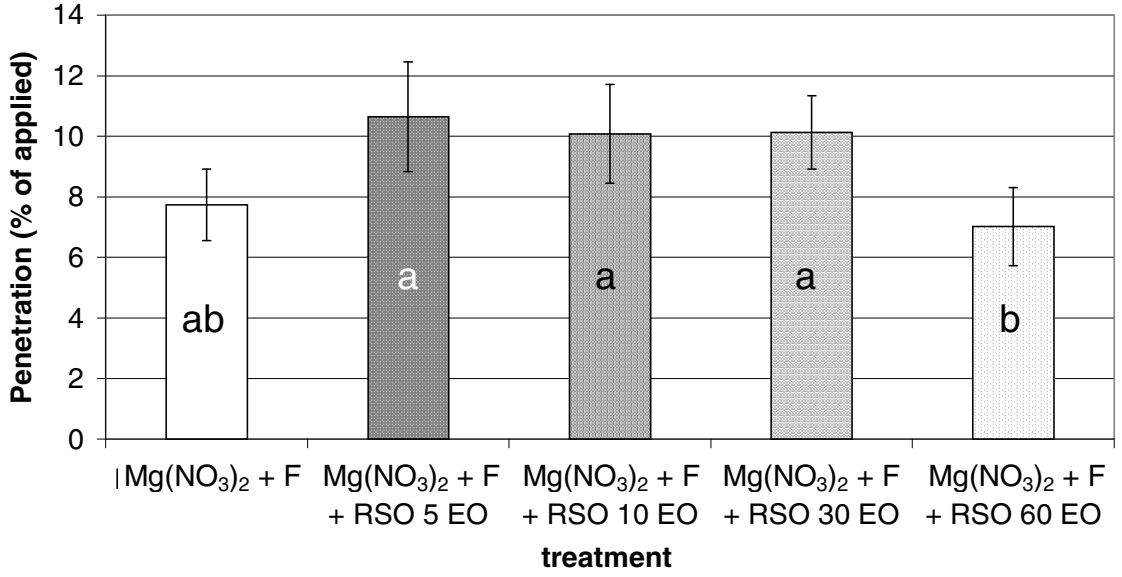

Fig. 3. Penetration of $Mg(NO_3)_2$ spray solutions (0.2 M) through isolated tomato fruit cuticles 24 h after treatment. Surfactants (RSO 5 EO, 10 EO, 30 EO, 60 EO) and the formulation (F) were added at a concentration of 1 g litre^{-1}.

3.2 Biological efficacy

All $MgSO_4$-treatments employed significantly enhanced Mg concentrations in leaves of *Riesling* compared with the untreated control (Fig. 4). The Mg-content in clusters was markedly enhanced, when the formulation or a mixture of the formulation and the surfactant RSO 5 were added to $MgSO_4$.

In the cultivar *Regent*, both $Mg(NO_3)_2$-treatments approximately doubled the Mg-concentrations in leaves compared with untreated plants, whereas the Mg-content in the clusters was not significantly increased. In both cultivars (*Riesling* and *Regent*), more residues were removed from the leaf surfaces with a solution of 0.05 n HCl including 1 mg l^{-1} Tween 20 than with deionized water (Figs. 4, 5).

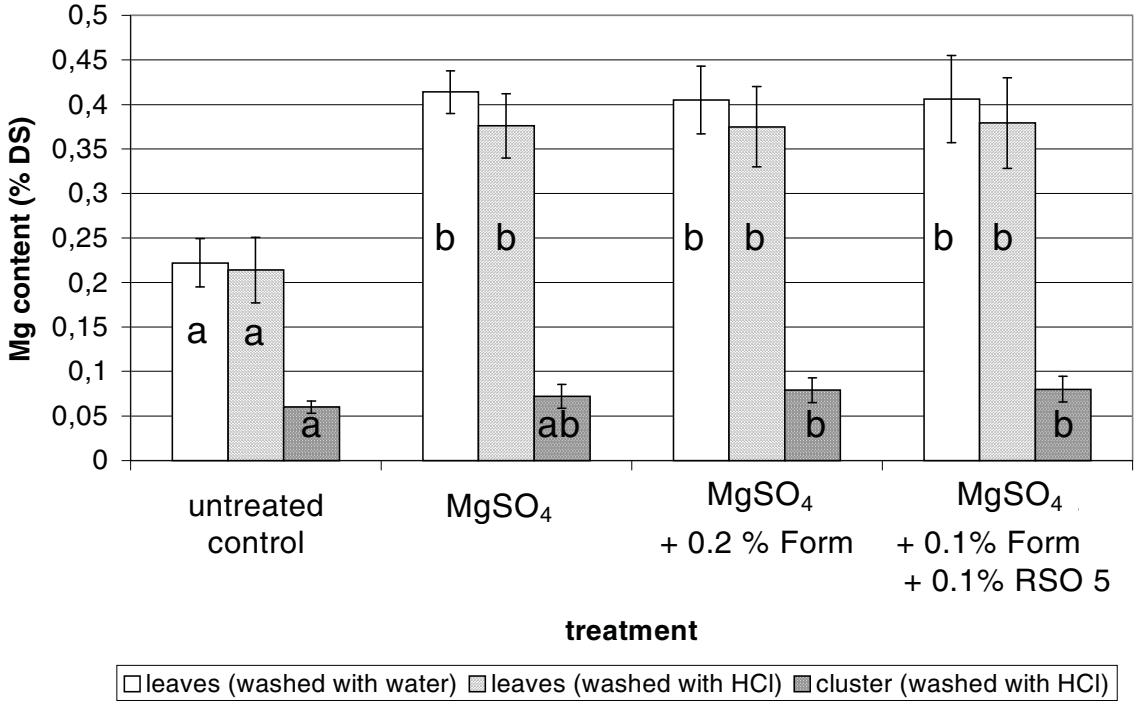

Fig. 4. Magnesium content in the dry substance of leaves and clusters of *Vitis vinifera* cv. *Riesling* after treatment with different $MgSO_4$ spray solutions.

The number of leaves per stock (*Regent*) with medium to severe Mg deficiency symptoms compared with a medium infested 'standard leaf' was markedly reduced by foliar $Mg(NO_3)_2$-applications (Fig. 6). Though differences between $Mg(NO_3)_2$ and $Mg(NO_3)_2$ + formulation were not statistically significant, the formulated $Mg(NO_3)_2$ reduced the incidence of Mg deficiency symptoms slightly more.

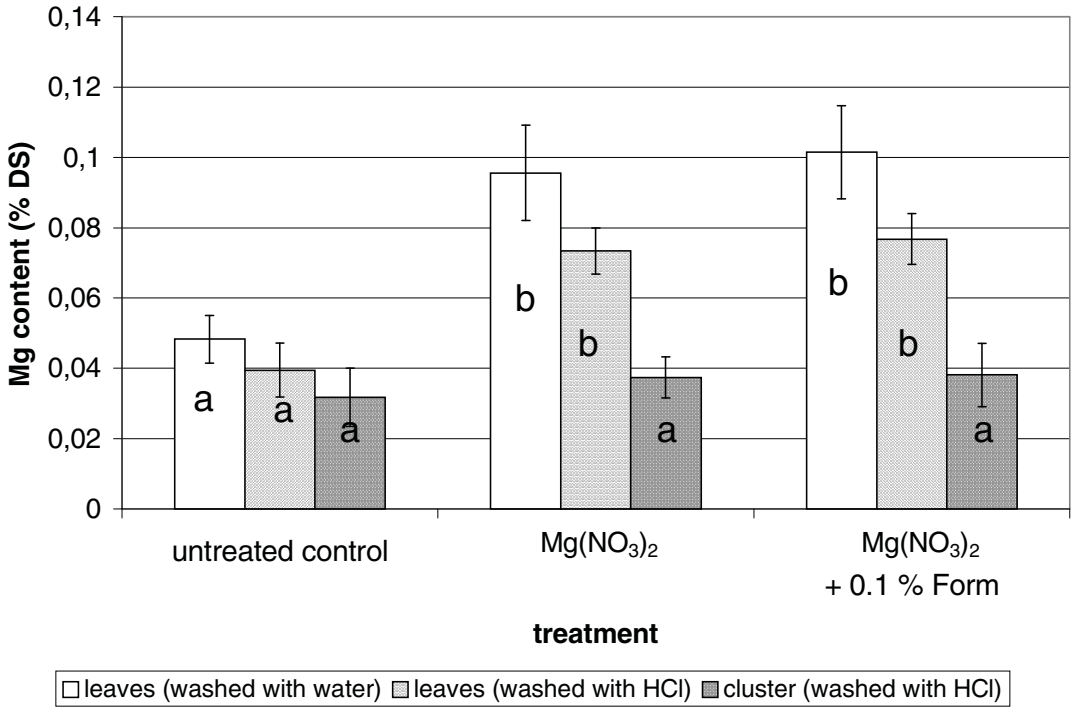

Fig. 5. Magnesium content in the dry substance of leaves and clusters of *Vitis vinifera* cv. *Regent* after treatment with different $Mg(NO_3)_2$ spray solutions.

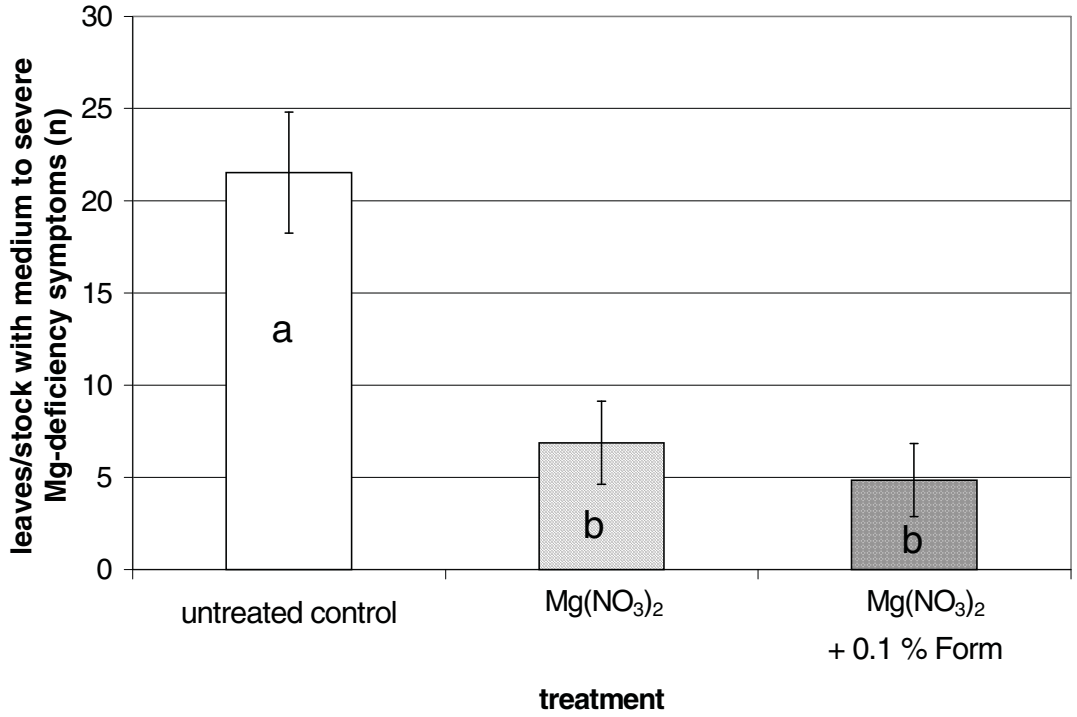

Fig. 6. Number of leaves per stock (*Vitis vinifera* cv. *Regent*) with medium to severe Mg-deficiency symptoms compared with a medium infested 'standard leaf' after treatment with different $Mg(NO_3)_2$ spray solutions.

Discussion

The results of the penetration studies demonstrate that the surfactants RSO 5 and RSO 10 can improve Mg penetration through isolated tomato fruit cuticles (Fig. 1). As the cuticles used were astomatous and free of visible defects, the Mg transport was by necessity through the cuticle proper. However, in the model system employed, cations and anions penetrated in equivalent amounts as the diffusion potential enforces equal fluxes of cations and anions (Krüger, 1999). Hence, penetration of $MgSO_4$ and $Mg(NO_3)_2$ salts was studied and for each Mg^{2+} ion one sulphate or two nitrate ions penetrated the cuticles.

As the surfactant concentration in the donor solution is below the critical micellar concentration, surfactant enhanced penetration is likely to origin from a modification of the cuticle permeability rather than a direct effect on Mg mobility. Schönherr (2000) found that calcium chloride penetrates plant cuticles via aqueous pores. Mg is a divalent cation like Ca and hence, it can be assumed that Mg ions also require an aqueous diffusion path (pores filled with water) to penetrate cuticular membranes. Aqueous pores swell depending on humidity (Schönherr 1982). The surfactants employed did not extend the drying-time of spray droplets. Contrary, with increasing surfactant EO content drying times were even reduced as shown for triglyceridethoxylates (chapter B, Tab. 1). Therefore, extended drying times cannot be the reason for enhanced penetration. Probably, surfactant enhanced wetting of the cuticular surface played a pivotal role. This would also explain the observation of an inverse relationship between surfactant enhanced penetration and surfactant EO content because contact angles of spray droplets on the standard surface 'Parafilm' increased with increasing EO chain length (chapter B, Tab. 1) and hence, the addition of the surfactant RSO 5 resulted in the highest coverage of the leaf surface with the Mg solutions. Treatment solutions with the most lipophilic surfactant RSO 5 had the lowest surface tension (unpublished data) and probably improved the contact between salt solutions and the microrough cuticles best, thus making the Mg more available for cuticular penetration.

When the surfactants were added to the Mg-formulation developed by the Department of Horticulture, University of Bonn, cuticular penetration of $Mg(NO_3)_2$ slightly increased with decreasing surfactant EO content (Fig. 3). It can be assumed that the lipophilic surfactants improved the wetting properties of the formulation and thereby enhanced penetration through isolated cuticles.

In the field studies, neither the formulation nor the formulation in combination with RSO 5 enhanced Mg concentrations in leaves or clusters significantly compared with nonformulated

$MgSO_4$ and $Mg(NO_3)_2$ (Figs. 4, 5). Probably, the formulation enhanced Mg uptake like it did in the finite dose experiments but within the leaves and clusters this additional amount of Mg was diluted such that no significant increase in Mg concentrations could be measured. When comparing the Mg contents in clusters after application of various $MgSO_4$ solutions, it can be argued that merely the treatments with formulated $MgSO_4$ increased the Mg level significantly compared with the untreated control. Even though the inrease from 0.06 to 0.08 % Mg on dry weight basis may appear low this increase of 33 % may lead to Mg levels in clusters lying above the critical concentration limits (depending on the year between 0.03 and 0.17 % in *Sämling 88* and 0.07-0.25 % in *St. Laurent*) below which Stiellähme symptoms do appear (Redl, 1983).

As no 'Stiellähme' symptoms occurred in the year 2000, the number of leaves/stock with medium to severe Mg-deficiency symptoms was assessed. These symptoms merely occured in the 'Wolfer Schatzgarten' in the cultivar *Regent*. The number of infected leaves was markedly reduced by foliar applications of $Mg(NO_3)_2$ solutions (Fig. 6). Differences between $Mg(NO_3)_2$ and formulated $Mg(NO_3)_2$ were not significant but in this case the formulation enhanced the performance of $Mg(NO_3)_2$ by about 30 %. This tendency supports the assumption that slight increases in Mg content at the critical concentration limit for 'Stiellähme' symptoms may have a strong impact.

From these trials we can conclude that the surfactants used can enhance Mg penetration through isolated cuticles solely or as an adjuvant added to the formulation. Foliar applications of $MgSO_4$ and $Mg(NO_3)_2$ are very effective in enhancing Mg levels in leaves but not in clusters. A significant higher Mg content in clusters can be achieved by adding the formulation tested or the formulation in combination with the most effective surfactant RSO 5 to $MgSO_4$. As the surfactant RSO 5 has a positive toxicological and ecotoxicological profile, the mixture of formulation and RSO 5 should be preferred.

5 Summary

In order to reduce 'Stiellähme' of grapes, a physiological disorder, the uptake of foliar applied magnesium was improved by an adequate formulation. Rapeseed oil ethoxylate surfactants with an average of 5 (Agnique RSO 5®), 10 (Agnique RSO 10®), 30 (Agnique RSO 30®) and 60 (Agnique RSO 60®) units of ethylene oxide (EO) were evaluated as adjuvants for a $MgSO_4$ and $Mg(NO_3)_2$ formulation. The impact of the surfactants on magnesium penetration was

studied using astomatous cuticular membranes isolated from mature tomato fruits. Donor solutions were prepared using $MgSO_4$ x $7H_2O$ and $Mg(NO_3)_2$ x $6H_2O$ at concentrations of 0.2 M. Surfactants and the formulation were added at concentrations of 1 and 2 g $litre^{-1}$, respectively. The biological efficacy of the various $MgSO_4$ and $Mg(NO_3)_2$ formulations was investigated employing two vineyards in the Moselle Valley with the cultivars Riesling and Regent. Spray solutions were prepared using $MgSO_4$ and $Mg(NO_3)_2$ at concentrations ranging from 0.75 to 8 mM. The surfactants and the formulation were added at concentrations of 1 and 2 g $litre^{-1}$. Some of the surfactants used improved magnesium penetration through isolated cuticles. In the field studies magnesium treatments enhanced the magnesium content in leaves significantly. The magnesium level in clusters was merely increased when formulated Bittersalz was applied. Magnisal and formulated Magnisal markedly reduced Mg-deficiency symptoms in leaves.

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H Summary and Conclusions

Although the active ingredient must guarantee the biological activity, most foliar applied pesticides and leaf fertilizers require surfactants or other adjuvants to maximize their efficacy or utility, either in the formulated product or as an adjuvant added to the spray tank. However, questions have been raised about the degradability and safety of many commonly used surfactants that originated from petroleum. Therefore, pesticide producers are researching new adjuvant technology. Recently, a new group of surfactants based on seedoils of agricultural origin exhibits several useful characteristics, namely: they are not phytotoxic, sufficiently stable under common conditions of storage, rapidly degraded in the environment, and promote the performance of foliar applied herbicides. Systematic studies were carried out to provide pertinent information about the suitability of a homologous series of rapeseed oil ethoxylate surfactants varying in hydrophilicity as additives or adjuvants for foliar applied pesticides and leaf fertilizers employing the following active ingredients as representatives which differ widely in their physico-chemical properties and modes of action: the plant growth regulator NAA, the herbicidal compound glyphosate, the fungicidal active ingredients prochloraz and tolylfluanid and the nutrients $CaCl_2$, $Mg(NO_3)_2$ and $MgSO_4$.

The biological response of foliar applied pesticides and leaf fertilizers mainly depends on retention of spray solutions on leaf surfaces, on cuticular penetration of systemic active ingredients as well as on rainfastness of spray deposits on plant surfaces. Numerous references are available on the effects of surfactants on these key factors but no attempt has been made in any study to describe the effect of surfactants on the pivotal parameters retention, penetration and rainfastness in a more integrative manner. Studies focusing on one factor solely are of limited value, when evaluating the effectiveness of surfactants for suitability as additives or adjuvants for foliar applied agrochemicals. In order to overcome these limitations in the present study, an integrative approach was chosen according to which surfactant effects on the key parameters for biological efficacy as well as on the final biological response were investigated.

The results are summarized as follows:

7. The surfactants were not phytotoxic at a concentration of 10 g litre^{-1}, which is a prerequisite for their use as formulation additives or tank-mix adjuvants. At a concentration of 1 g litre^{-1}, the surfactants reduced solution surface tension, contact angles of spray droplets on surfaces and they altered drying times of spray droplets. Initially, all rapeseed oil ethoxylates suppressed NAA sorption by cuticles but, hydrophilic ones increased sorption after a period of 4 days.

8. The retention of glyphosate spray solutions on *Phaseolus vulgaris* increased with increasing surfactant hydrophilicity. Retention on *Setaria viridis* was higher when lipophilic surfactants were added to the active ingredient. A positive relationship was established between surfactant EO content and glyphosate penetration through isolated tomato fruit cuticles. The fluorescence emission from 10 various plant species after treatment with different glyphosate solutions depended markedly on the plant species and the surfactant EO chain length. Addition of surfactants to glyphosate either had no effect or resulted in an increase or decrease in glyphosate phytotoxicity.

9. Prochloraz spray retention on *Lactuca sativa* was significantly improved, as the surfactant EO chain length inreased from 5 to 60 units. Merely the surfactant with 30 EO units enhanced prochloraz penetration through isolated cuticles after a period of 144 h. Prochloraz application reduced *Botrytis cinerea* incidence of *Lactuca sativa*. Protective prochloraz treatments were more effective than curative ones, and lipophilic surfactants enhanced biological efficacy more than hydrophilic ones.

10. About 70 % of the applied non-formulated active ingredient tolylfluanid and less than 6 % of solely applied prochloraz were recovered on leaf surfaces after their exposure to 25 mm of artificial rain during a period of 6.5 h. The commercial formulations Euparen Multi WG® and Sportak 40® significantly reduced rainfastness to about 30 % (tolylfluanid) and to less than 1 % (prochloraz) of the applied doses. The most lipophilic surfactant enhanced rainfastness of prochloraz but not of tolylfluanid. Reduction of Fm values of *P. vulgaris* and *S. viridis* leaves after glyphosate application and exposure to simulated rainfall was enhanced with increasing surfactant lipophilicity.

11. A negative relationship was established between surfactant EO content and calcium penetration through isolated tomato fruit cuticles. Calcium penetration through isolated fruit cuticles increased with decreasing surfactant EO content. The addition of the most lipophilic surfactant with 5 EO units to $CaCl_2$ resulted in increasing calcium contents and decreasing K/Ca ratios in fruits of *Malus domestica cv. Braeburn* and in a reduction of bitter pit incidence.

12. Magnesium penetration through isolated tomato fruit cuticles increased with decreasing surfactant EO content. $MgSO_4$ and $Mg(NO_3)_2$ treatments enhanced magnesium content in leaves of *Vitis vinifera* whereas the magnesium level in clusters merely increased when formulated $MgSO_4$ was applied. Formulated and non-formulated $Mg(NO_3)_2$ reduced Mg-deficiency symptoms in leaves of *Vitis vinifera.*

Though the surfactants were added solely at a concentration of 1 g $litre^{-1}$ to the active ingredients, some of them were more effective than the formulation of the commercial products, as far as the evaluated criteria are concerned. However, it was also shown that the surfactants may have no or undesirable effects. Therefore, individual proofs for specific surfactant-, active ingredient- and target plant combinations are essential. Because of their performance and positive toxicological and ecotoxicological profiles the rapeseed oil derivatives evaluated in this study could be an economical and ecological alternative for commonly used petroleum oil derivatives.

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