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Rainfastness of selected agrochemicals as affected by leaf surface characteristics and environmental factors

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In our studies the contact fungicide mancozeb and the systemic herbicide glyphosate were used as model substances to elucidate the influence of leaf surface characteristics and environmental factors on rainfastness of agrochemicals. The effect of drying time, rain intensity, rain amount, interruptions of rain showers, and seed oil ethoxylate adjuvants were studied in detail in apple seedlings. Furthermore, the involvement of surface roughness as well as amount and composition of epicuticular waxes on rainfastness of mancozeb with or without tank-mix adjuvants was examined in adaxial leaf surfaces of apple seedlings, bean seedlings and kohlrabi The interaction between rain intensity and type of linseed oil ethoxylate adjuvant on the wash-off and biological efficacy of glyphosate was investigated in *C. album*, *A. theophrasti* and *S. viridis*. Light, heavy and torrential rain events with intensities of 0.5, 5, and 48 mm h^{-1}, respectively, were simulated using a laboratory rain simulator. The results can be summarized as follows:

1. Mancozeb was washed-off easily from the leaf surface of apple seedlings due to impact of few millimeters rain, whereas a higher amount of rain caused only little additional a.i. removal. Regardless of drying time, fungicide removal from the leaves followed a hyperbolic curve. Intensity and amount of rain independently affected a.i. removal from the seedling leaves. Equations for mancozeb removal at light, heavy and torrential rain were determined for precipitation ranges between 0 and 30 mm, and between 0 and 5 mm, respectively. Interruptions of rain showers had only little influence on rainfastness at 2 mm rain, and no effect at 5 mm precipitation.

2. All rapeseed, linseed and soybean oil ethoxylates, especially the more hydrophobic ones RSO 5, LSO 10, SBO 10 significantly reduced surface tension, contact angle, and drying time of water droplets. As a rule, mancozeb formulated with the more hydrophobic adjuvants had lower retention, but enhanced rainfastness after 5 mm heavy rain. SEM micrographs in not rain-exposed leaves revealed that fungicide deposit was mainly located along anticlinal cell walls, in form of crystals. In rain exposed leaves, greatest part of the a.i. was detected along anticlinal cell walls as well, but in the form of balls or annuli.

3. Studies in adaxial leaf surface of apple seedlings, bean seedlings and kohlrabi revealed great differences in roughness, as well as in amount and composition of epicuticular waxes. A Pearson's correlation analysis showed very strong correlations between roughness and total EW, amount of C_{29} alkanes, and total mass of alkanes. Retention and rainfastness of mancozeb differed among plant species; moreover, addition of adjuvants to spray solution caused differential responses. In general, retention was highly and negatively correlated with surface roughness, total epicuticular wax, amount of C_{29} alkane, and total of alkanes in the EW. Rainfastness was highly positively correlated with amount of C_{28} alcohol and C_{33} alkane in the EW.

4. The weed species used in the glyphosate study presented significant differences in micro roughness and surface wettability, as a result of surface characteristics, such as cell size, presence of trichomes, glands or wax structures. No significant interactions between treatment solutions and rain intensity could be established when determining biological efficacy of glyphosate. In *C. album*, heavy and torrential rain events reduced, while light rain slightly raised biological efficacy. In *A. theophrasti* and *S. viridis*, all rain intensities reduced the efficacy of herbicidal treatments significantly. Biological efficacy of glyphosate as a function of ethoxylation degree of the LSO adjuvants was species dependent.

Regenfestigkeit ausgewählter Pflanzenschutzmittel in Abhängigkeit von Blattoberflächencharakteristika und Umweltfaktoren

Im Rahmen dieser Untersuchung wurde die Wirkung diverser Faktoren auf die Regenfestigkeit von Mancozeb und Glyphosat untersucht. Antrocknungszeit, Regenintensität, Regenmenge und Regenschauer-Unterbrechungen sowie Zusatz von Pflanzenöl-Ethoxylaten (Raps-, Lein- und Sojaöl) zu der Spritzlösung sind an Apfelsämlingen untersucht worden. Der Einfluss der Blattmikromorphologie sowie Masse und Zusammensetzung der epikutikulären Wachse (EW) wurde an Apfelsämlings-, Bohnensämlings- und Kohlrabiblättern untersucht. Die biologische Wirksamkeit unformulierten oder mit Leinöl-Ethoxylaten formulierten Glyphosats in Abhängigkeit von Regenintensitäten ist an den Unkräutern *C. album*, *A. theophrasti* und *S. viridis* ermittelt worden. Natürliche Regenfälle mit Intensitäten von 0,5 (Niesel-), 5 (Dauer-) und 48 mm h^{-1} (Starkregen) sind mit Hilfe eines Laborregensimulators erzeugt worden. Die Ergebnisse lassen sich wie folgt zusammenfassen:

1. Bereits wenige Millimeter Regen verursachten eine starke Fungizid-Abwaschung, so dass höhere Regenmengen kaum einen zusätzlichen Abwaschungsverlust verursachten. Regenintensität und -menge wurden als unabhängige Faktoren betrachtet. Mathematische Gleichungen für die Mancozeb-Abwaschung durch Niesel-, Dauer- und Starkregen wurden für Regenmengen zwischen 0 und 30 mm sowie 0 und 5 mm erstellt. Die Unterbrechungen von Regenschauern zeigten nur einen geringen Einfluss bei 2 mm und keinerlei Effekt bei 5 mm Regenmenge.

2. Alle getesteten Raps-, Lein- und Sojaöl-Ethoxylate, insbesondere die eher hydrophoben Tenside RSO 5, LSO 10 und SBO 10, reduzierten Oberflächenspannung, Kontaktwinkel und Antrocknungszeit von Wassertropfen. In der Regel führten die hydrophoberen Adjuvantien zwar zu einer geringeren Fungizid-Retention aber zu einer höheren Regenfestigkeit. Rasterelektronenmikroskopische Aufnahmen vor dem Regen zeigten eine heterogene Wirkstoffverteilung innerhalb der Depositionsfläche, wobei das Fungizid sich hauptsächlich in kristallartige Formen präsentierte. Nach dem Regen hingegen waren eher kugelförmige Strukturen zu sehen.

3. Untersuchungen an Apfel- und Bohnensämlingen sowie Kohlrabi lieferten große Unterschiede bezüglich Mikromorphologie sowie Masse und Zusammensetzung der Oberflächenwachse. Hohe Korrelationen wurden zwischen Blattmikromorphologie und der Gesamtmasse epikutikulärer Wachse sowie dem Anteil von C_{29} Alkan und der Gesamtalkanmasse nachgewiesen. Retention und Regenfestigkeit von Mancozeb waren von den Oberflächeneigenschaften sowie vom Adjuvantienzusatz abhängig. Die Retention korrelierte deutlich negativ mit der Mikromorphologie, EW-Masse, Anteil an C_{29} Alkan und Gesamtalkanmasse. Die Regenfestigkeit korrelierte stark mit dem Anteil an C_{28} Alkohol und C_{33} Alkan in der EW.

4. Die Mikromorphologie und Benetzbarkeit der adaxialen Blattoberfläche ausgewählter Unkräuter differierten stark, zurückzuführen auf die unterschiedlichen Zellgrößen und das Vorkommen von Trichomen, Drüsen und Wachskristallen. Statistische Auswertungen zeigten keine Wechselwirkungen zwischen Spritzlösungen und Regenintensitäten im Hinblick auf die biologische Wirksamkeit von Glyphosat. Bei *C. album* verminderten Dauer- und Starkregen die Wirksamkeit des Herbizids, Nieselregen dagegen erhöhte die Wirksamkeit leicht. Bei *A. theophrasti* und *S. viridis* übten alle Regenintensitäten einen negativen Einfluss auf die biologische Wirksamkeit aus. Die LSO-Ethoxylate zeigten unterschiedliche Auswirkungen auf die biologische Wirksamkeit von Glyphosat auf die geprüften Unkräuter.

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List of abbreviations

A	area
A. theophrasti	*Abutilon theophrasti* Medik.
a.i.	active ingredient
AAS	atomic absorption spectrometry
ANOVA	analysis of variance
B-LRS-2	laboratory rain simulator - 2
B-PSA-1	laboratory pesticide sprayer - 1
BSTFA	*N*,*O*-bis (trimethylsilyl) trifluoroacetamide
C. album	*Chenopodium album* L.
cm	centimetre
cm^2	square centimetre
EC	emulsifiable concentrate
EO	ethylene oxide
EW	epicuticular wax
Fig.	figure
Fm	maximum chlorophyll-fluorescence
FW	fresh-weight
g	gram
GC-MS	gas chromatography – mass spectrometry
h	hour
ha	hectare
HLB	hydrophilic-lipophilic balance
HPLC	high pressure liquid chromatography
km	kilometre
kPa	kilopascal
l	litre
LSO	linseed oil ethoxylate
m	metre
m^2	square metre
µg	microgram
µl	microlitre
µm	micrometer
µmol	micromole
mg	milligram
min.	minute
ml	millilitre

mm	millimetre
mmol	millimole
mN	millinewton
MVD	medium volume diameter
MW	molecular weight
n.d.	not detected
ng	nannogram
º	degree
ºC	grade Celsius
p	probability of error
Pa	Pascal
p.A.	pro analysis
PAR	photosynthetic active radiation
pH	potential of hydrogen
PO	propylene oxide
r	Pearson’s correlation coefficient
R^2	coefficient of determination
RH	relative humidity
RSO	rapeseed oil ethoxylate
RUM	Roundup Ultra Max®
s	seconds
S. viridis	*Setaria viridis* L.
SBO	soybean oil ethoxylate
SC	suspension concentrate
SE	standard error
SEM	scanning electron microscopy
T	temperature
Tab.	table
UV	ultraviolet
v/v	volume/volume
WG	wettable granule
WP	wettable powder
$	US-Dollar
%	per cent

A Introduction

1 Influence of rainfall on pesticide deposits

Agrochemical deposits on plant surfaces are constantly exposed to physical, biological, and chemical factors which may reduce the biological efficacy of the active ingredients (Schepers, 1996; Neely, 1970). Activity losses are attributed to impact of wind, UV-radiation, temperature, and biological degradation. Nevertheless, the main environmental factor responsible for residual activity of a given agrochemical is the influence of rain (Schepers, 1996; Leung and Webster, 1994; Kudsk *et al.*, 1991; McDowell *et al.*, 1987; Bruhn and Fry, 1982). Natural rainfall and overhead irrigation modify pesticide deposits on plants by dilution, redistribution and removal (Thacker and Young, 1999). Therefore, in order to assure pest control, treatments must be repeated, thus increasing production costs significantly (Thacker and Young, 1999; Troiano and Butterfield, 1984). Another negative consequence is the fact that rain-removed pesticides will reach non-target organisms, soil and water resources, resulting in unnecessary environmental contamination (Wauchope *et al.*, 2004).
On the other hand, rain-induced redistribution of active ingredients on leaf surface can induce positive effects, especially when a.i. is irregularly deposited (Kudsk *et al.*, 1991; Smith and MacHardy, 1984). In some cases, this could be used as a strategy for pathogen control (Rudgard *et al.*, 1990; Cooke *et al.*, 1989), providing enhanced fungicide efficacy (Schepers, 1996; Bruhn and Fry, 1982). Unfortunately, redistribution may also have a negative impact, since it can lead to a sub-toxic a.i. concentration on the whole surface, allowing or even stimulating the development of hazardous organisms (Steurbaut, 1993).

Several factors affect rainfastness of agrochemicals, but the majors are rain intensity, rain amount, interval of time between treatments and rainfall, commercial formulation of pesticides, pesticide water solubility and type of crop (Cabras *et al.*, 2001; Green, 2001). Moreover, the interaction of all these factors must be considered (Thacker and Young, 1999).
In the past, the term rainfastness was not always accurately used. Rainfastness denominates an intrinsic property of a given active ingredient or commercial formulation to resist the physical impact of rain droplets and the carry out effect of water film. Therefore, only those a.i. placed on plant surface can show its rainfastness. In contrast, if a.i. has already penetrated the plant tissue by the time of starting rain, rainfastness can not be determined. Several factors influence both rainfastness (directly or indirectly) and penetration (with respect to rain-induced wash-off) of agrochemicals. As exemplification we present the impact of adjuvants:

1 – If included in a formulation or when added to spray solutions, adjuvants can enhance adhesion of a.i. on plant surface, enhancing rainfastness in a direct way;

2 - Adjuvants modify the physicochemical characteristics of spray solutions, influencing the formation of deposits on plant leaves. Some deposit characteristics such as initial concentration, particle size, and a.i. distribution may alter rainfastness of a given agrochemical. As a result, adjuvants influence rainfastness indirectly;

3 - Adjuvants may improve penetration rate of systemic compounds, reducing the a.i. deposit on leaf surfaces before rainfall onset; this contributes to a reduction of rain-induced wash-off. Moreover, pesticide penetration implies alterations of deposit characteristics, which may influence rainfastness of the remaining a.i. indirectly.

2 Influencing factors on rainfastness and rain-induced wash-off

2.1 Active ingredient

Active ingredients have particular properties such as molecular weight, polarity, water solubility and others, which may influence their adhesion to plant surface and/or diffusion into wax layers. These characteristics may be decisive for the differences in rainfastness observed among several active ingredients (Spanoghe *et al.*, 2005; Suheri and Latin, 1991). Particularly the water-soluble pesticides are vulnerable to wash-off caused by rain (Green, 2001; Mashaya, 1993). However, even fungicides with low water solubility are easily removed by little amount of rain (Cabras *et al.*, 2001; Kudsk *et al.*, 1991).
In the case of systemic compounds, penetration of a.i. into the plant tissue reduces its exposition to environmental factors, reducing the risk of a rain-induced wash-off. Pick *et al.* (1984) suggest the speed at which an active ingredient penetrates the leaf determines its resistance to wash-off. Here, lipophilic compounds penetrate waxy, hydrophobic plant leaves more readily than hydrophilic compounds (Mashaya, 1993). Further, pesticide penetration may modify characteristics of the remaining deposit, exerting indirect influence on rainfastness.

2.2 Physical form of the pesticide formulation and deposit characteristics

The physical form of a commercial formulation (Tab. 1) has a great impact on pesticide rainfastness. As a rule, powders and granule formulations are removed more easily from plant surfaces than flowables and suspensions (Willis *et al.*, 1996; Kudsk *et al.*, 1991; van Bruggen *et al.*, 1986). Van Bruggen *et al.* (1986) observed that WP formulations of five fungicides had lower rainfastness than the respective flowable formulations. Pick *et al.* (1984) tested rainfastness of pesticides with different physical forms acquired by different producers and observed that rainfastness is drastically influenced by type of formulation; however, considering a given a.i. and physical form, rainfastness of agrochemicals was comparable, regardless of producers.

Table 1. Properties of water-mixable pesticide formulations.

Formulation	Physical form	Physical form in the tank
Wettable Powder (WP)	Powder	Suspension
Wettable Granule (WG)	Granule	Suspension
Suspension Concentrate (SC)	Suspension	Suspension
Emulsifiable Concentrate (EC)	Real solution	Emulsion (o/w)

Source: Haefs, 2001; Knowles, 1995; Börner, 1995; Heusch, 1981.

The physical type of formulation influences deposit characteristics and distribution patterns on leaf surfaces (Cooper and Hall, 1993; Hess and Falk, 1990). Pesticides formulated as wettable powders or granule yield deposits with greater median diameter (Kudsk *et al.*, 1991), which are less tenacious than small particles (Somers and Pring, 1967). Bukovac *et al.* (1995) observed droplets from spray formulations containing solids (e.g. WP) often deposit in irregular forms, so that many deposits bridge depressions and fail to make uniform contact with leaf surface. In addition, SC formulations generally contain more adjuvants than dry formulations (Gent *et al.*, 2003; Steurbaut, 1993), which can additionally influence rainfastness.

The amount of pesticide deposited on leaves may influence rainfastness of a given agrochemical; however, a consensus is missing. Willis *et al.* (1992) observed that wash off methyl parathion and fenvalerate from cotton plants is related to the square of insecticide amount loaded on plants. In another work, Willis *et al.* (1994) observed that removal of permethrin and sulprofos from cotton plants is related to the mean of insecticide deposited on plant surface. In contrast, Smith and MacHardy (1984) verified that relative decrease of captan residues from leaf surface due to rain is not a function of initial deposit. Also Bruhn and Fry (1982) have not observed an influence of the deposit magnitude on rain-induced wash-off. Leung (1994) noted that initial concentration of glyphosate does not affect intensity of degradation and removal processes like volatilization, photolysis and rain-washing.

2.3 Adjuvants

Adjuvants already incorporated in pesticide formulations or tank-mixed may influence both rainfastness and wash-off processes in distinct ways. Adjuvants can be arranged in groups according to several parameters, but usually they are classified taking into account their charge, origin, chemical composition, and objective of use (Green, 2001; Abribat, 2001; Tu *et al.*, 2001; Stock and Briggs, 2000; Hill, 2000; Green, 2000; Hazen, 2000; Stock, 1997; Knowles, 1995; Steurbaut, 1993; Stevens, 1993). The greater influence on enhancing rainfastness and reducing rain-induced wash-off is provided by stickers and penetration adjuvants respectively. It is not rare that a single adjuvant influences both processes; in such cases it is difficult to distinguish which effect acts in a greater extent.

2.3.1 Influence on rainfastness

Sticker-adjuvants enhance attachment of a chemical on leaf surface and make deposits less susceptible to removal by rain and other environmental factors (Martz, 2004; Hazen, 2000). Usually the sticker-components are nonevaporating materials with a viscous nature, allowing them to adhere, along with the pesticide deposits, for a longer time (Hazen, 2000). Stickers fall broadly into two categories: those that polymerize on the leaf surfaces, and those that are already high molecular weight polymers, such as latex derivates (Stevens, 1993). Particularly the high molecular weight stickers have a natural adherent tendency to plant surfaces (Hazen, 2000). These have many anchoring points, giving a.i. long term stability (Knowles, 1995). The most common stickers are heavy petroleum oil, acrylic latex, terpenes, epoxidised oil, alkyl resins, and block co-polymers (Green, 2001; Hazen, 2000).
The second potential base for enhancing rainfastness in a direct way is water repellency of the deposit (Roggenbuck *et al.*, 1993). Some adjuvants form a hydrophobic layer over the pesticide deposit and protect it against water contact, preventing wash-off (Green, 2001). The degree of tackiness and resistance of the deposit vary according to water solubility of the adjuvant and its relative concentration to pesticide (Hazen, 2000).
The indirect effect of adjuvants on rainfastness is related to deposit characteristics. Several adjuvants improve spray deposition on the surfaces (Faers *et al.*, 2004; Balsari *et al.*, 2001), due to their ability in reducing surface tension of pesticide solutions. This decisively reduces the influence of adverse effects such as leaf topography, epicuticular wax, and trichomes (Hess and Falk, 1990). Mainly affected are pesticide placement on leaves, initial a.i. concentration, particle size, and grade of coverage (Scherhag, 2005; Gent *et al.*, 2003; Green, 2001; Green and Hazen, 1998). According to Leung and Webster (1994) and Steurbaut *et al.* (2001), solutions with low surface tension and low contact angle may dry up rapidly on foliage, resulting in crystalline, rainfast deposits.

2.3.2 Influence on penetration

A penetration agent is a compound that assists the pesticide movement from target surface through natural barriers into plant tissue (Hazen, 2000). They can influence coverage, droplet retention, physical state of the residue on cuticle surface, and additionally change structure and composition of the cuticle (Kirkwood, 1999; Kirkwood, 1993). As a result, they greatly enhance penetration rate of systemic compounds (Gent *et al.*, 2003; Zabkiewicz, 2000; Nalewaja and Matysiak, 2000; Laerke and Streibig, 1995; Gauvrit and Cabanne, 1993; Gaskin and Stevens, 1993; Stevens and Baker, 1987). Generally, hydrophilic adjuvants with high HLB values are most effective in enhancing penetration of highly water soluble herbicides, whereas lipophilic surfactants with low HLB are most effective in enhancing uptake of low water soluble herbicides (Hess and Foy, 2000). Actually, several combinations of adjuvant types, active ingredients, and formulations were already tested in diverse plant species (Müller *et al.*, 2002; Roggenbuck *et al.*, 1993; Gaskin and Holloway, 1992; Wells, 1989; Field and Bishop, 1988). The effect of adjuvants on a.i. penetration with consequences on wash-off depends on interactions of all involved factors, such as type of adjuvant and its concentration, active ingredient and its concentration, type of formulation, surface characteristics, and environmental factors (Gent *et al.*, 2003; Schönherr, 2002; Haefs, 2001; Combellack *et al.*, 2001; Kogan, 2001; Leaper and Holloway, 2000; Sun, 1996; Sandbrink *et al.*, 1993; Gaskin and Stevens, 1993; Coble and Brumbaugh, 1993; Reddy and Singh, 1992; Cranmer and Linscott, 1991; Roggenbuck *et al.*, 1989; Wells, 1989). Therefore, the great variability in the results is not surprising.

Finally, a.i. penetration *per se* modifies characteristics of the remaining deposit on leaf surfaces. Form and nature of remaining residue on the surface may be important for performance of some compounds (Bukovac *et al.*, 1995), and possibly for their rainfastness.

2.4 Drying time and environmental conditions

The time elapsed between pesticide application and rainfall onset decisively influences magnitude of a.i. wash-off by rain. Agrochemical deposits need a minimum of time to dry up and so resist impact of rain droplets. Several studies have shown that enhancement of rainfastness or reduction of wash-off can be achieved by longer drying times (Reddy and Locke, 1996; Schepers, 1996; Willis *et al.*, 1994; Mashaya, 1993; Willis *et al.*, 1992; Bryson, 1987; Pick *et al.*, 1984; Bruhn and Fry, 1982). However, there is no consensus, since other studies have shown that drying time has no influence on rainfastness of active ingredients (dos Santos *et al.*, 2002; Ditzer, 2002; Schepers, 1996; Clay and Lawrie, 1990).

Actually, contradictory observations are common, once standardized methods are not available and experiments can not always be conducted at same conditions. As a consequence, the evaluated drying times range from few minutes to several days (Willis *et al.*, 1992; Bruhn and Fry, 1982). In addition, environmental conditions during drying time play a decisive role. Ditzer (2002) showed that retention and rainfastness of contact fungicide dithianon was influenced by dew on the leaf surface. The same author studied influences of relative humidity during the drying time on rainfastness of the active ingredient.

In case of systemic compounds, interactions are more complex. Pesticide penetration into plant tissue is a function of time, regulated by several biological and environmental factors. For an optimal penetration into plant tissues, systemic a.i. must be in a liquid form (Bukovac *et al.*, 1995). In contrast, to be rainfast, a.i. must dry up rapidly on foliage. It is obvious that systemic compounds are designed to penetrate into the plant tissues, and therefore their wash-off is lower after longer drying times. In this case, the longer the rain-free period, the more active ingredient can penetrate the plants (Sun, 1996) and the better is the biological efficacy (Werlang *et al.*, 2003; Bariuan *et al.*, 1999; Willis *et al.*, 1994; Mashaya, 1993; Willis *et al.*, 1992; Wells, 1989).

2.5 Rain characteristics

2.5.1 Amount and intensity

A rain event (Tab. 2) is characterized by its quantity and intensity as well as by droplet spectrum, energy of the droplets and time of duration (Park *et al.*, 1983; Simmons, 1980). According to Green (2001), the most important characteristics of rainfall are amount, intensity and drop size. Anyhow, all characteristics of a rainfall can be adequately defined by rain intensity (Park *et al.*, 1983).

The withstand of a pesticide deposit to wash-off due to rain is given by its resistance to mechanical impact, particularly from big rain droplets, as well as dissolution rate (Kudsk *et al.*, 1991). Experimental results concerning the influence of intensity and amount of rain are not always in consonance. Some of them show that cumulative rain amount affects the wash-off at a greater extent than rain intensity (Willis *et al.*, 1996; Mashaya, 1993; Kudsk *et al.*, 1991; Sundaram, 1991; Pick *et al.*, 1984), while others have shown the opposite (Taylor and Matthews, 1986). Complementing, some authors observed similar impact of rain amount and rain intensity (Mashaya, 1993), while others observed that rain intensity and duration affect active ingredient removal from the plant foliage independently (Fife and Nokes, 2002). Diversity in results may be explained due to differences in experimental conditions such as active ingredient, plant material, drying time, drying conditions, rain characteristics, etc.

Fact is that intense rains are characterized by bigger droplets which fall at greater speed, having a greater mechanical impact on the surfaces (Park *et al.*, 1983; Simmons, 1980). This greater impact can easily dislodge pesticide deposits (Kudsk *et al.*, 1991; Park *et al.*, 1983). The removal process is finished by the water film which is formed on the surface; it carry out the pesticides from the leaves (Lauver and McCune, 1984). Heavy rainfall produces a constant water film on surface, making the carry out process easier (Hartley and Graham Bryce, 1980). In contrast, by misting and light rain, run-off occurs only periodically, after junction of water drops on the surface (Suheri and Latin, 1991).

Table 2. Classification of rainfall types according to their major characteristics (adapted from several authors).

Type of rain	Rain intensity [mm h^{-1}]	Droplet radius [µm]	Droplets fall speed [m s^{-1}]	Duration
Mist	< 0.4	50 - 250	low (0.25 – 2)	short to long
Drizzle / Light rain	0.42	250 - 500	medium (2 - 2.8)	long (8 h -24 h)
Hard rain	4.2	500 - 1500	medium (4-6)	medium (6 h)
Torrential rain	42	1500 - 2500	high (6 – 8.9)	short (10 min.)

Sources: Lauer and Bendix, 2004; Weischet, 2002; Barth, 2002; Ditzer, 2002; Häckel, 1993; Liljequist and Cehak, 1984; Park *et al.*, 1983; Simmons, 1980.

Researches demonstrated that greatest part of a.i. is removed by comparatively little rain amount, while the remaining deposit remains in a stable form, difficult to displace with more rain (Wauchope *et al.*, 2004; Fife and Nokes, 2002; Rudgard *et al.*, 1990; Smith and MacHardy, 1984; Bruhn and Fry, 1982). Rain-resistant fungicide is most probably held in the leaf matrix (Fife and Nokes, 2002).

Lauver and McCune (1984) divided the wash-off process in four phases:

a) water accumulate on foliage, removing only little part of the deposit;
b) removal rate achieves the maximum as storage capacity of the foliage was reached and superficial water with dissolved or suspended material was displaced from the surfaces;
c) exponential decline in removal rate;
d) no additional removal of the deposits.

2.5.2 Acidity

Rainfastness of agrochemicals can also be influenced additionally by other factors such as pH of rainwater. Van Bruggen *et al.* (1986) verified that wash off triphenyltin hydroxide and copper hydroxide from potato leaves was higher by acidic rain (pH 2.8), regardless of formulation or potato cultivar. The same authors observed that removal of maneb, mancozeb and chlorothalonil was not affected by reduced pH. Troiano and Butterfield (1984) also studied increased loss of cupric hydroxide and triphenyltin hydroxide due to acidic rain, whereas rainfastness of chlorothalonil was not affected. Researches like these show that experiments using deionised or tap water to simulate rain can underestimate the wash-off magnitude of some pesticides, especially in regions with occurrence of acidic rain.

2.6 Leaf surface characteristics

Some studies have shown that rainfastness of a given active ingredient varies among plant species or cultivars (Kudsk *et al.*, 1991; Bruhn and Fry, 1982; Neely, 1971). This is attributed to differences in surface characteristics, such as presence of trichomes, hairs, and structured wax deposits (Spanoghe *et al.*, 2005; Neely, 1970). Actually, surface characteristics were mainly investigated in relation to deposit formation and biological activity of agrochemicals. Hairs and trichomes can impair pesticide adhesion to surface by intercepting spray drops before they reach the epidermal cells. Likewise, they can impair the impact of rain droplets, reducing pesticide displacement (Neely, 1971). However, studies on the influence of surface structures on rainfastness of pesticides are missing.

Plant waxes consist of mixtures of long-chain hydrocarbons, alcohols, ketones, esters and acids (Baker, 1982; Fernandes *et al.*, 1964). Wax amount, composition and homologue distribution patterns vary considerably between and within plant species and cultivars (Belding *et al.*, 1998; Percy *et al.*, 1994; Baker, 1982). Some pesticides have high affinity to surface waxes (Häuser-Hahn *et al.*, 2003) or are able to diffuse into wax layers (Andrieu *et al.*, 2000). On the other hand, a highly structured epicuticular wax reduces contact between spray droplet and cuticle surface (Price, 1982). Cabras *et al.* (2001) observed that mancozeb has been more easily washed-off from grapes than from grape leaves, and believe that these discrepancies are conditioned by differences in composition of epicuticular wax. In the case of systemic compounds, epicuticular wax is the most significant barrier to absorption of water soluble herbicides (Hess and Foy, 2000). Here, lipophilic compounds penetrate waxy, hydrophobic plant leaves more readily than hydrophilic compounds (Mashaya, 1993). Nevertheless, systematic studies on influence of amount and composition of surface wax on rainfastness of agrochemicals were not conducted, yet.

3 Objective of our studies

An overview on the major factors influencing rainfastness and rain-induced wash-off of foliar-applied agrochemicals is given in Table 3 and Figure 1.

Table 3. Influencing factors on rainfastness and rain-induced wash-off of foliar-applied agrochemicals – an overview.

Factor	**Variations**	**Influence on rainfastness**		**Influence on wash-off (rainfastness excluded)**
		Direct	**Indirect**	
Active ingredient	Contact Loco-systemic Systemic	- a.i. have peculiar capacities to attach to surfaces and resist rainfall	- differential diffusion rate into epicuticular wax (however, it must be considered that waxes can also be removed by rain)	- differential penetration rate in the plant tissue
Physical form of the formulation	Powder (WP) Granule (WG) Suspension (SC) Emulsion (EC)	- drying speed of deposits	- deposit characteristics on plant surfaces	
Adjuvants (Built-in or Tank-mix)	Organic surfactants Organosilicone Oils Terpene derivates Polymers Inorganic salts	- bonding strength of the a.i. to surfaces - formation of a hydrophobic layer over the pesticide deposit - drying speed of deposits	- improvements on retention and deposit formation	- changes in penetration rate
Deposit characteristics	Initial concentration Particle size Distribution on leaves	- drying speed of the deposits - bonding strength to the surfaces - area of the deposit exposed to rain		- influences in penetration
Circumstances before rainfall	Drying time Relative humidity, Dew Temperature Stresses (water, radiation)	- bonding strength to surfaces - drying speed of deposits	- influences on plant metabolism, surface characteristics, and in consequence, the binding of the a.i. to surfaces	- influences on plant metabolism, surface characteristics and, in consequence, a.i. penetration
Plant and surface characteristics	Leaf exposition to rain Hairs, trichomes, and glands Epicuticular waxes		- retention, deposit formation - a.i. exposition to rain	- a.i. penetration
Rain characteristics	Rain intensity Rain amount Interval between rain showers Acidity	- a.i. physical dislodgement from surfaces, wax layers and plant tissue - formation of water film, with a respective carry out potential	- under particular conditions, acidic rain may intensify wash-off extent	- under special conditions, a.i. penetration can be enhanced under light rain conditions

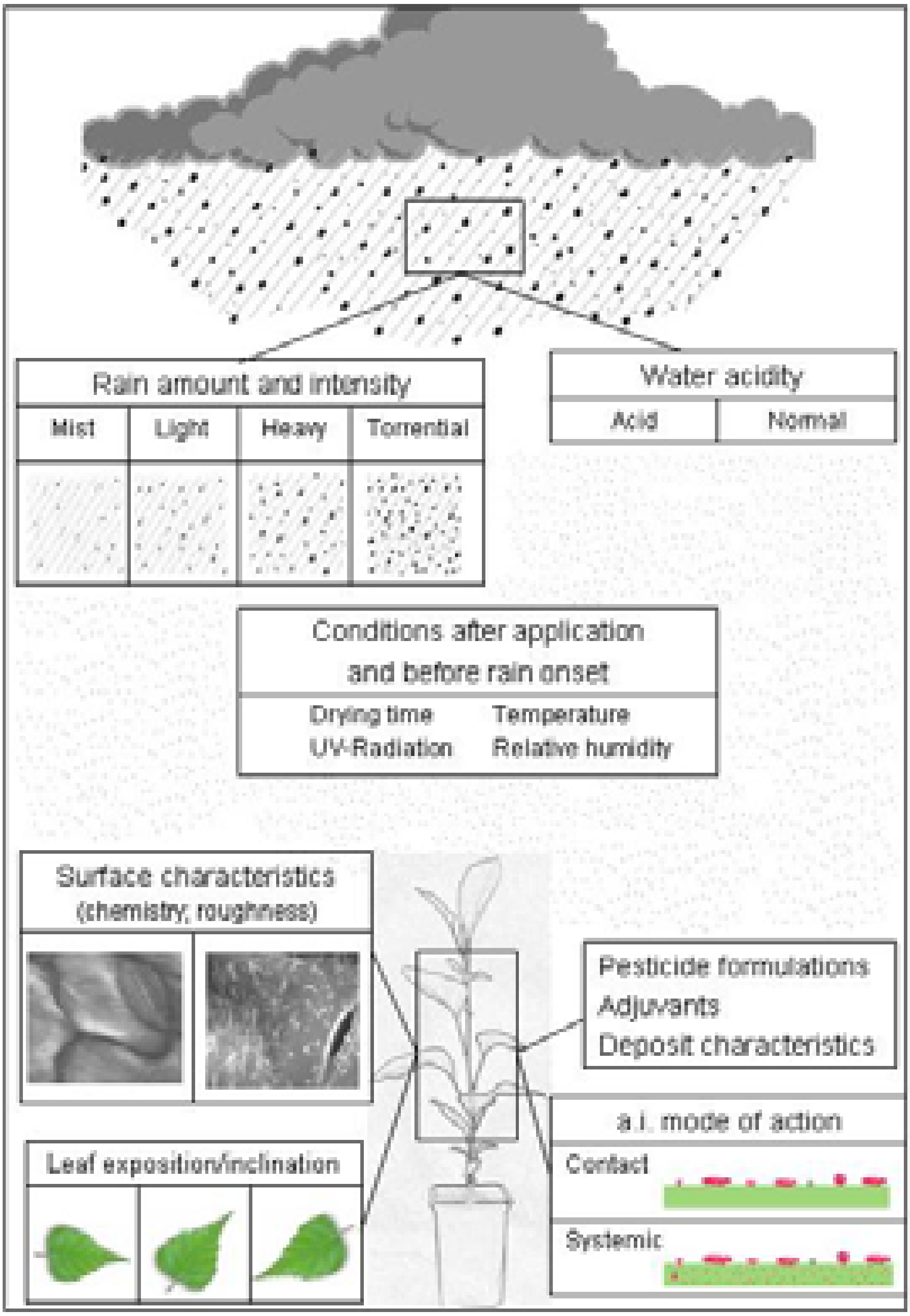

Figure 1. Major influencing factors on rainfastness and rain-induced wash-off of foliar-applied agrochemicals.

Even though several studies concerning rainfastness were carried out, the relevance of some influencing factors remain imprecise. This is in part a consequence of the immense

differences in experimental designs and methods. Here, a critical point is the simulation of rain. In the past, many experiments overlooked important characteristics of rainfall, so that the simulated rain was not analogous to a natural precipitation (Ditzer, 2002). For example, extreme situations such as rain intensity of 156 mm h^{-1} (Reddy and Singh, 1992) and unreal situations such as intensity of 40 mm $min.^{-1}$ (Rudgard *et al.*, 1990) were tested. In a similar way, many experiments tried to evaluate the effect of drying time or adjuvants on rainfastness of systemic compounds, but their influence on a.i. penetration rate was not considered.
In our studies we aimed to elucidate the influence of leaf surface characteristics as well as environmental factors on rainfastness of the contact fungicide mancozeb and on rain-induced wash-off i.e. biological efficacy of the systemic herbicide glyphosate.

The questions to be answered in this study are:
1. How great is the influence of drying time, rain intensity, rain amount and rain-interruptions on rainfastness of mancozeb?
2. At what extent can seed oil ethoxylate adjuvants differing in ethoxylation degree modify deposit characteristics and enhance rainfastness of the contact fungicide mancozeb?
3. How do leaf micro roughness and, amount and chemical composition of surface wax influence retention and rainfastness of mancozeb?
4. At what extent can the addition of seed oil ethoxylate adjuvants enhance biological activity and reduce rain induced wash-of of glyphosate due to light, heavy and torrential rain events in three relevant weed species?

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B Rainfastness of mancozeb on apple seedling leaves as affected by drying time of the fungicide deposit as well as by quantity and intensity of rain and rainfall interruptions

1 Introduction

Contact fungicides are sprayed to a canopy with the aim of assuring a protective layer on the plant surfaces and thus prevent the establishment and development of fungal infections. Therefore, repeated applications are required to maintain a chemical barrier between the surface of expanding foliage or enlarging fruit, respectively, and pathogenic fungi (Smith and MacHardy, 1984). Despite successful applications of the agrochemicals, control of pathogenic organisms may be negatively influenced due to chemical, physical or biological degradation processes of the active ingredient (Schepers, 1996; Neely, 1970). Nevertheless, among all physiochemical, biochemical and metabolic processes that occur in the environment, rainfall has the greatest effect upon residual activity of foliar-applied pesticides (Schepers, 1996; McDowell *et al.*, 1987). Rain occurrence may affect the structure and activity of a deposit by its dilution, redistribution, physical removal, and extraction from plant tissue (Thacker and Young, 1999).

The knowledge of how much a.i. persists the rain-induced wash-off is essential to optimize the pesticide input (Schepers, 1996). It allows to estimate more precisely residual activities (Kudsk *et al.*, 1991; Neely, 1971) and helps to establish guidelines for respraying after a rainfall (Cabras *et al.*, 2001; McDowell *et al.*, 1987). Moreover, this information is also needed to develop mathematical models to predict transport of agrochemicals to water bodies (Smith *et al.*, 1981), soil, and run-off from croplands (Wauchope *et al.*, 2004; McDowell *et al.*, 1987). Besides, by knowing which impact the aforementioned factors have, strategies can be developed to enhance pesticide tenacity by maintaining bioavailability and reducing environmental contamination.

Several studies on rainfastness of agrochemicals were already carried out, focusing on the influence of rain characteristics on a.i. removal or in trying to enhance the rainfastness with tank-mix adjuvants (Fife and Nokes, 2002; Dirkse and van Adrichem, 2001; Cabras *et al.*, 2001; Schepers, 1996; Kudsk *et al.*, 1991; Rudgard *et al.*, 1990; van Bruggen *et al.*, 1986; Smith and MacHardy, 1984; Bruhn and Fry, 1982). It is known that the main factors involved in the wash-off of agrochemicals are rain intensity, rain quantity, time between application of treatment and rainfall onset, pesticide formulation, water-solubility of the active ingredient, type of crop (Cabras *et al.*, 2001), and combinations of all these factors (Thacker and Young, 1999). However, not clearly elucidated is, which rain intrinsic characteristic affects the wash-off process at a greater extent: rain intensity or rain quantity? Studies employing selected active ingredients showed minor or no influence of the rain intensity or rain amount on the wash-off phenomenon (Willis *et al.*, 1996; Mashaya, 1993; McDowell *et al.*, 1987; Pick *et al.*,

1984) while others showed that it is an important factor (Fife and Nokes, 2002; Willis *et al.*, 1996; Reynolds *et al.*, 1994; Kudsk *et al.*, 1991). In a similar way, contradictory results are also observed as far as the influence of drying time is concerned: a longer drying time can positively influence the rainfastness (Reddy and Locke, 1996; Schepers, 1996; Willis *et al.*, 1994; Mashaya, 1993; Willis *et al.*, 1992; Bryson, 1987; Pick *et al.*, 1984; Bruhn and Fry, 1982) but it not always occurs (dos Santos *et al.*, 2002; Ditzer, 2002; Schepers, 1996). Discrepancies on the results may be explained by experimental and methodological differences. The influence of short interruptions during rainfall on fungicide rainfastness was not investigated, yet.

Aim of our study was to evaluate the effect of drying time, rain quantity, rain intensity and rainfall interruptions on rainfastness of the contact fungicide mancozeb on apple seedling leaves. The hypothesis was that all abovementioned factors influence the rainfastness of mancozeb independently.

2 Material and methods

2.1 Plant material and growth conditions

Experiments were conducted with 56-days old ‘Golden Delicious’ apple seedlings (*Malus domestica* Borkh.). Seed dormancy was broken by submerging the seeds for 96 h in water before treatment with the fungicide Euparen® (0.1 g l^{-1}, 50 % Dichlofluanid) and allocation in a refrigerator (4 ºC; 90 % RH) for four weeks. Approximately 200 seeds were distributed on a germination tray (loam: sand, 3:1) and covered with a layer (1 cm thick) of sand. Three weeks after germination the seedlings were transplanted into Teku-pots (JP 3040, Pöppelmann GmbH & Co. KG, Germany), and five weeks later the experiments were conducted. The seedlings were raised in a growth chamber with constant temperature (20 ºC ± 1 ºC) and relative humidity (70 % ± 5 %). Photosynthetic active radiation (PAR) was provided at 180 µmol s^{-1} m^{-2} at the plant level during a 16 h-photoperiod. Plants were watered according to actual needs and fertilized with a balanced nutrient solution.

2.2 Fungicide application

Mancozeb [(manganese ethylene bis(dithiocarbamate) (polymeric) complex with zinc salt)] as Dithane Ultra WG with 80 % a.i. (Spiess-Urania Chemicals GmbH - Hamburg, Germany) was applied using a laboratory pesticide sprayer (B-PSA-1; Department of Agricultural Engineering, University of Bonn, Germany) at a concentration of 2.40 g l^{-1}. The sprayer was equipped with a hollow cone nozzle (80º; Lechler GmbH, Germany), placed 45 cm above plant level. Application was carried out at a speed of 6 km h^{-1}, pressure of 3 x 10^5 Pa, giving a volume of 390 l ha^{-1}. Drying times before rainfall onset were simulated in the growth chamber. Drying times were 2, 4 or 24 h (when the effect of drying time on rainfastness was evaluated) or 4 h (all other experiments).

2.3 Rainfall simulation

Tap water was used to simulate a natural rainfall with a laboratory rain simulator (B-LRS-2; Department of Agricultural Engineering, University of Bonn, Germany), as described elsewhere (Ditzer, 2002; Kromer *et al.*, 1996). Precipitations were simulated at three intensities: light rain (0.5 mm h^{-1}), heavy rain (5 mm h^{-1}) and torrential rain (48 mm h^{-1}), with medium droplet volume diameter (MVD) of 377 µm, 1075 µm and 2043 µm, respectively. The intended rain intensities and quantities were programmed on the rain simulator and controlled with a rain gauge. Intensity, duration, and amount of rain varied according to the objective of the experiment, as described below. Plants were returned to the growth chamber 20 min. after rain exposure, so that leaf surfaces could dry overnight before taking samples for analysis of fungicide deposits. Treated but not rain-exposed seedlings served as control.

2.4 Experiments

2.4.1 Effect of drying time and rain quantity

In order to evaluate the influence of drying time and rain quantity on rainfastness, a heavy rain (5 mm h^{-1}) was simulated during 0 (no rain), 1, 2, 4 and 6 hours, respectively, totalizing 0, 5, 10, 20 and 30 mm precipitation. Fungicide deposits were left to dry for 2 h, 4 h and 24 h (T = 20 ºC; RH = 70 %) before rainfall simulation.

2.4.2 Effect of rain intensity and rain quantity

With the objective to evaluate the influence of rain quantity and rain intensity, light (0.5 mm h^{-1}), heavy (5 mm h^{-1}), and torrential (48 mm h^{-1}) rainfall were simulated, and samples were taken after 0 mm (no rain) and 10 mm. In a second trial, light, heavy and torrential rain events were simulated and sequential samples were taken after 0, 1, 2, 3, 4 and 5 mm precipitation, respectively.

2.4.3 Interruptions of rainfall

In order to study the impact of rainfall interruption on rainfastness of mancozeb, two cases were simulated. The first, 5 mm heavy rain (5 mm h^{-1}) was simulated at different settings: a) continuous rain; b) rainfall with one interruption (2.5 mm + 30 min. break + 2.5 mm); and c) rainfall with two interruptions (2.0 mm + 30 min. break + 1.5 mm + 30 min. break + 1.5 mm). The second case, 2 mm heavy rain (5 mm h^{-1}) was simulated at different settings: a) continuous rain; b) rainfall with one interruption (0.5 mm + 30 min. break + 1.5 mm); c) rainfall with two interruptions (0.5 mm + 30 min. break + 0.5 mm + 30 min. break + 1 mm); d) rainfall with three interruptions (0.5 mm + 30 min. break + 0.5 mm + 30 min. break + 0.5 mm + 30 min. break + 0.5 mm). During the rain-breaks, plants were placed in the non-operating rain simulator.

2.5 Sampling procedure and fungicide analysis

From each seedling, the second and third completely developed leaf (petiole excluded) was taken for residue determination. Each sample for analytical determination comprised three individual leaves. Fresh-weight (FW) was determined (Sartorius BP 210S, Sartorius AG, Göttingen, Germany) and samples were enclosed in Teflon vessels. Five millilitres of nitric acid 65 % p.A. (Merck KGaA, Darmstadt, Germany) and 2 ml of hydrogen peroxide 30 % (RdH Laborchemikalien GmbH & Co. KG, Seelze, Germany) were added to the samples before acid digestion in a microwave (Büchi MLS 1200, Microwave Laboratory Systems GmbH, Essen, Germany). After digestion, content was transferred into volumetric flasks (25 ml), the volume filled up with distilled and deionized water (Milli-Q Ultrapure Water Purification Systems, Millipore Corporation, Billerica, USA) and then transferred into plastic vessels. The mancozeb content of the samples was analysed by determining the concentration of manganese atoms by atomic absorption spectrometry (AAS, Perkin-Elmer Analyst 300 spectrometer, Wellesley, USA, equipped with a Multi-Element Lumina Hollow Cathode Lamp). Manganese atoms constitute about 17 % of the mancozeb molecular weight, and a.i. concentrations in the sample and on the leaves were calculated by a rule of proportion (van Bruggen *et al.*, 1986; Travis *et al.*, 1985). Samples of untreated seedlings served as reference for the natural manganese concentration in the plant tissue. A standard series (0.1, 0.5, 1.0, 2.0 and 3.0 mg l^{-1}) prepared from a manganese stock solution (1000 mg l^{-1}; Merck KGaA, Darmstadt, Germany) served for establishing a calibration curve. Results were presented in micrograms active ingredient per gram leaf (FW).

2.5.1 Recovery assay

Leaves from untreated seedlings were sampled and fortified with mancozeb (100 and 1000 $\mu g\ g^{-1}$ FW) and subjected to extraction and analyse procedure. Recovery values for the a.i. ranged between 87 and 110 %, with a maximal coefficient of variation of 11.54 % (5 replications).

2.6 Experimental design and statistical analysis

Experiments were conducted in a completely randomized design, with 15 seedlings (10 analytical samples) per each treatment group. Fungicide residues were expressed as $\mu g\ g^{-1}$ FW and percentage of the initial concentration (fungicide concentration in no-rain exposed seedlings was referred to 100 percent). Data were subjected to analysis of variance (ANOVA) and in case of significant differences among qualitative treatments, compared by Duncan-Test $p \leq 0.05$. In case of statistical differences among quantitative parameters (rain amount), appropriate regression equations were determined. Statistical analyses were made with the software SPSS 12.0 (SPSS Inc., Chicago, USA) and graphs were designed with the software Sigma Plot 7.101 (Systat Software GmbH, Erkrath, Germany).

3 Results

3.1 Drying time and rain quantity

Irrespective of drying time, an intense mancozeb wash-off was registered after only few millimeters of rain (Fig. 1). Average of initial fungicide concentration on the leaves (0 mm rain) ranged between 1000 and 1380 µg g^{-1} FW.

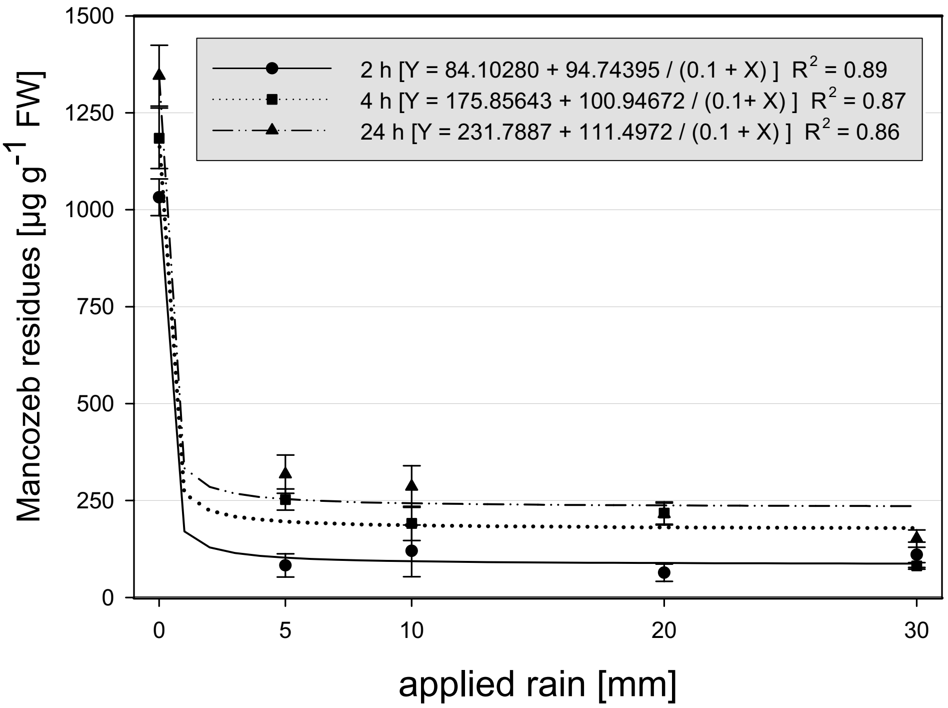

Figure 1. Mancozeb residues on leaf surface of apple seedlings (*M. domestica* Borkh.) as a function of rain volume (intensity of 5 mm h^{-1}) and drying time. Vertical bars represent the standard error.

After only 5 mm precipitation residue concentrations were lower than 400 µg g^{-1} FW. Increasing amount of rain caused only little additional wash-off. Fungicide losses after 5 mm of rain were about 90 % of initial deposit at a drying time of 2 h, and 75 % and 80 % at drying times of 4 and 24 h, respectively (Fig. 2). Accumulated a.i. losses reached about 90 % of the initial fungicide deposit after 30 mm of rain, irrespective of drying time.

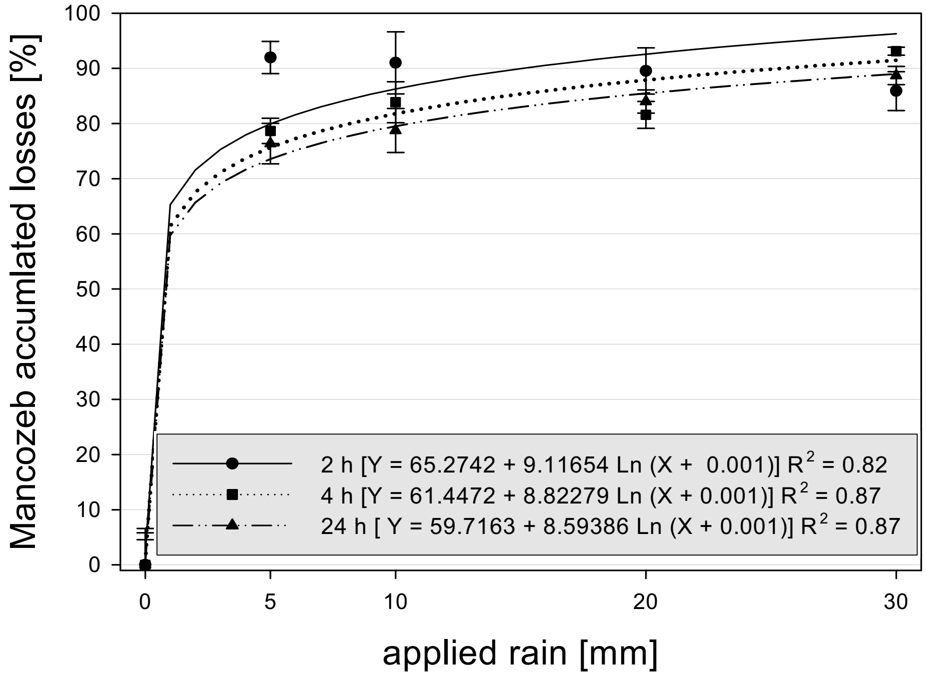

Figure 2. Mancozeb accumulated losses (%) as a function of rain volume (rain intensity of 5 mm h^{-1}) and drying time, evaluated on apple seedling leaves. Vertical bars represent the standard error.

3.2 Rain intensity *vs.* rain quantity

Ten millimeters of rain at 0.5 mm h^{-1}, 5 mm h^{-1} and 48 mm h^{-1}, reduced a.i. concentration on the leaves to 43 %, 12 %, and 8 %, respectively, of the initial deposit (Fig. 3).

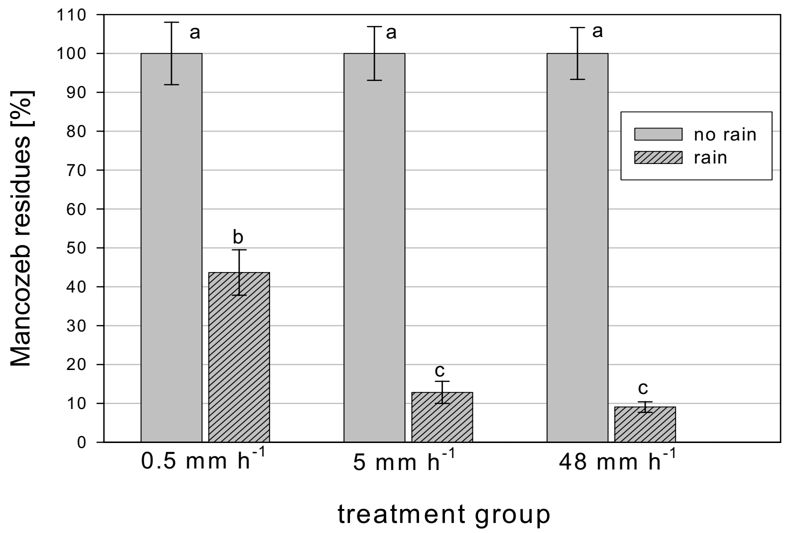

Figure 3. Effect of light (0.5 mm h^{-1}), heavy (5 mm h^{-1}) and torrential (48 mm h^{-1}) rain events on mancozeb wash-off by 10 mm precipitation. Fungicide residues (%) followed by the same letter are not significantly different by Duncan Test $p \leq 0.05$. Vertical bars represent the standard error.

Combined evaluations of rain intensity and quantities showed distinct fungicide loss after only 1 mm of rain, particularly at heavy and torrential rainfalls (Fig. 4).

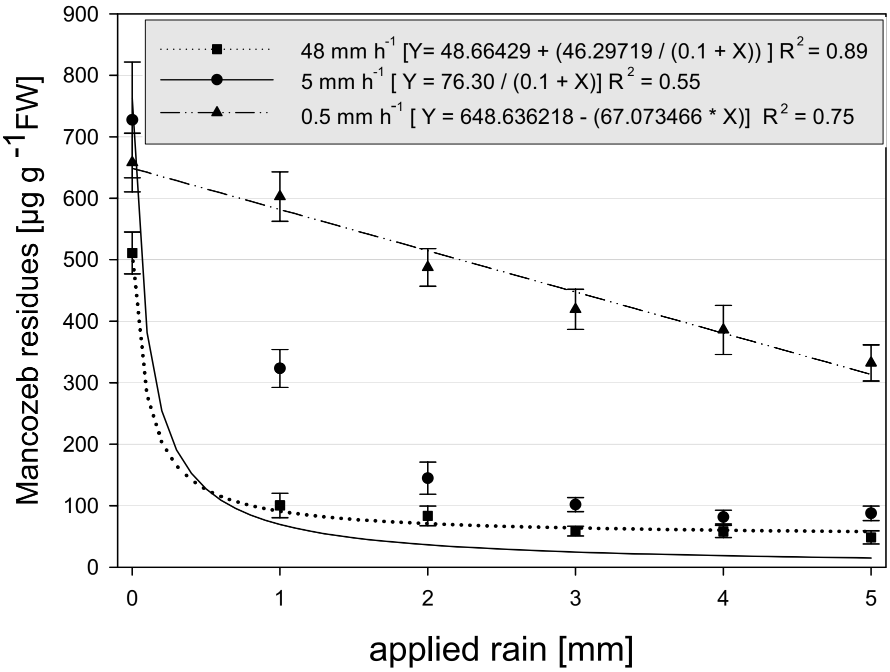

Figure 4. Effect of rain amount (0, 1, 2, 3, 4, 5 mm) and rain intensity (0.5, 5 and 48 mm h^{-1}) on the mancozeb wash-off of apple seedling leaves (*M. domestica* Borkh.). Vertical bars represent the standard error.

Heavy and torrential rain events removed 80 % or more of the initial deposit even after 2 mm of rain, only (Fig. 5). Accumulated a.i. losses were 9 %, 55 % and 80 % after 1 mm of rain and 50 %, 88 %, and 90 % after 5 mm rain at intensities of 0.5 mm h^{-1}, 5 mm h^{-1} and 48 mm h^{-1}, respectively.

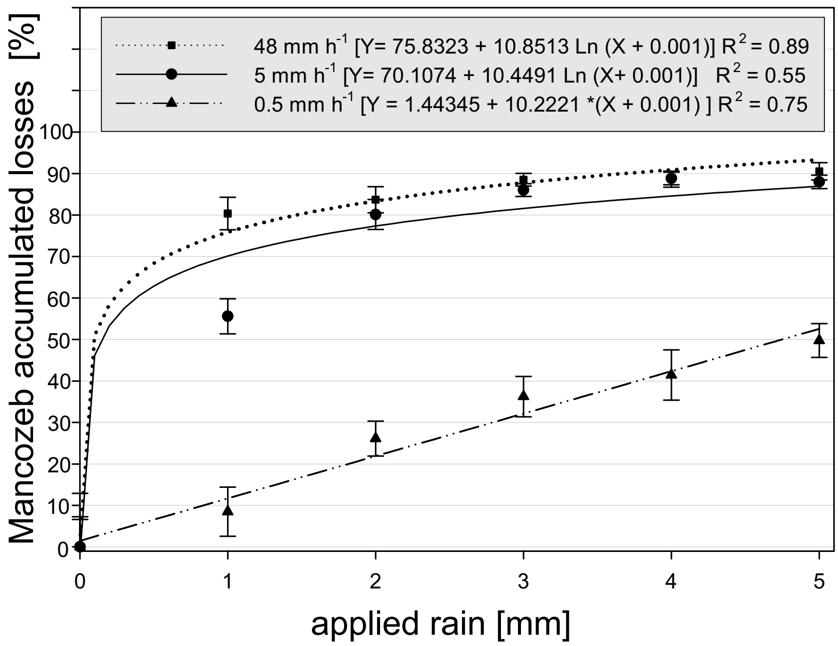

Figure 5. Effect of the rain quantity (0, 1, 2, 3, 4, 5 mm) and rain intensity (0.5, 5 and 48 mm h^{-1}) on mancozeb accumulated losses (%) from apple seedling leaves (*M. domestica* Borkh.). Vertical bars represent the standard error.

3.3 Rainfall interruptions

Rainfall interruptions for one or two times had no influence on mancozeb wash-off after a 5 mm rain (Tab. 1). The remaining fungicide deposit on the leaves ranged between 95 and 119 $\mu g\ g^{-1}$ after 5 mm of rain, e.g. 11.26 % and 12.47 %.

Table 1. Rainfastness of mancozeb on apple seedling leaves (*M. domestica* Borkh.) after exposure to 5 mm rain (intensity of 5 mm h^{-1}) and influenced by interruptions of rain showers.

Rain settings	Mancozeb residues			
	[$\mu g\ g^{-1}$ FW; Mean ± SE]		[%] *	
	no-rain	rain	no-rain	Rain
continuous rain	1063.84 ± 31.50	119.87 ± 10.63	100 a	11.26 b
2.5 mm + 30 min. break + 2.5 mm	813.46 ± 42.89	95.62 ± 14.01	100 a	11.75 b
2.0 mm + 30 min. break + 1.5 mm + 30 min. break + 1.5mm	885.32 ± 50.90	110.46 ± 16.52	100 a	12.47 b

* Means of fungicide residue (%) followed by the same letter are not significantly different by Duncan Test $p \leq 0.05$.

In contrast, when a 2 mm rainfall was simulated, significant influences of the interruptions were observed (Tab. 2). The remaining fungicide on the leaves after rain ranged between 132 and 201 µg g^{-1} (16.81 % and 25.72 %). Continuous rain caused the highest fungicide removal while lowest wash-off was registered, when precipitation was interrupted once for 30 minutes.

Table 2. Rainfastness of mancozeb on apple seedling leaves (*M. domestica* Borkh.) after exposure to 2 mm rain (intensity of 5 mm h^{-1}), and influenced by the interruptions of rain showers.

Rain settings	Mancozeb residues			
	[µg g^{-1} FW; Mean ± SE]		[%] *	
	no rain	rain	no rain	rain
continuous rain	785.85 ± 59.01	132.10 ± 16.03	100 a	16.81 c
0.5 mm + 30 min. break + 1.5 mm	782.55 ± 44.75	201.26 ± 35.02	100 a	25.72 b
0.5 mm + 30 min. break + 0.5 mm + 30 min. break + 1 mm	809.10 ± 45.59	199.28 ± 20.19	100 a	24.68 b
0.5 mm + 30 min. break + 0.5 mm + 30 min. break + 0.5 mm + 30 min. break + 0.5 mm	671.38 ± 72.60	151.80 ± 15.01	100 a	22.61 b

* Means of fungicide residue (%) followed by the same letter are not significantly different by Duncan Test $p \leq 0.05$.

4 Discussion

4.1 Drying time and rain quantity

Despite of its low solubility in water (Kidd and James, 1991), mancozeb was washed-off easily from leaf surfaces by little rain volumes. Highest a.i. losses were observed when rain simulation was initiated 2 h after fungicide application while after drying times of 4 and 24 h, respectively, mancozeb removal was significantly lower (Figs. 1 and 2).

It is known that time between fungicide treatment and rainfall onset affects the extent of wash-off, but also dependance on type of active ingredient and its formulation has been reported (Pick *et al.*, 1984). However, contradictory results with contact fungicides have been obtained. For example, rainfastness of chlorothalonil increased when the interval between fungicide application and rainfall increased from 0 to 7 days (Bruhn and Fry, 1982). In contrast, no differences on wash-off of tolylfluanid from apple seedlings were observed when 25 mm rain (intensity 25 mm h^{-1}) were simulated after a drying time of 2, 4 and 6 h, respectively (Ditzer, 2002). Evaluations on biological efficacy of two contact fungicides for control of *Phytophthora infestans* in potato showed that a short drying time reduced the efficacy of maneb-fentinacetate but had no influence on the fungicidal activity of fluazinam (Schepers, 1996).

In our studies, regardless of drying time, a typical wash-off pattern was observed, characterized by high fungicide removal after few millimeters of rain, only. Increasing rain quantity increased fungicide removal at lower rates, as a logarithmic function (Fig. 2). A hyperbolic curve, such as observed in our study for a.i. removal (Fig. 1), was also observed in fenvalerate wash-off from cotton plants (McDowell *et al.*, 1987) and captan wash-off from apple seedlings (Smith and MacHardy, 1984). Cohen and Steinmetz (1986) have also shown that wash-off of several insecticides due to simulated rain is initially rapid, in contrast to a second phase, when the removal rate is much slower.

Characteristic in our experiments was the intense a.i. wash-off by the initial five millimeters rain, regardless of drying times or initial fungicide concentration, while losses at 30 mm of rain were very similar. Also other authors noted that a big fraction of active ingredients is removed by small amounts of rain, with the remaining deposit staying in a stable form, which is difficult to remove with more rain (Fife and Nokes, 2002; Rudgard *et al.*, 1990; Smith and MacHardy, 1984; Bruhn and Fry, 1982). The rain-resistant fungicide is most probably held in the leaf matrix and is not easily dislodged by additional rain (Fife and Nokes, 2002).

4.2 Rain intensity *vs.* rain quantity

By simulating light, heavy and torrential rain events and taking samples after 10 mm (Fig. 3) or sequential samples after 0, 1, 2, 3, 4 and 5 mm (Figs. 4 and 5), we haven proven that rainfastness is a function both of rain intensity and rain amount. Fungicide losses after only 1 mm rain reached about 80 % at torrential rain, 55 % and 7 % at heavy and light rain, respectively. After 2 mm of rain, losses due to heavy and torrential rain were comparable (80 % and 83 %), whereas losses of 88 % and 91 % were measured after 5 mm precipitation. The a.i. removal by light rain was not so intense, amounting to losses of 50 % after 5 mm rain. A greater a.i. loss by higher rain intensities, as observed in our study, was also documented by other authors. Ditzer (2002) verified distinct influences of rain intensities on tolylfluanid removal from leaf surfaces of apple seedlings. Neuhaus *et al.* (1974) reported that twice as much Zineb was washed-off from potato leaves by 10 mm rain at intensity of 30 mm h^{-1} compared to 10 mm at an intensity of 6 mm h^{-1}. Kudsk *et al.* (1991) observed that 3 mm of rain at 27 mm h^{-1} washed-off more fungicide than 3 mm rain at 3 mm h^{-1}.

A rain event is characterized by its quantity and intensity, as well as by droplet spectrum, droplets kinetic energy, and time of duration (Simmons, 1980). The main rain-intrinsic characteristics acting on wash-off are intensity and amount. Intense rain events have a higher percentage of bigger droplets that fall at higher speed, thus exerting a more powerful impact on the surfaces. The impact of rain droplets can dislodge active ingredients from the surface as well as alter leaf surfaces by damaging the plant cuticle (Simmons, 1980). Impact of rain droplets can also extract some pesticides from the plant tissue (Thacker and Young, 1999; Hartley and Graham Bryce, 1980).

The hardiness of a pesticide deposit to wash-off is given by its resistance to mechanical impact, particularly the big droplets, as well as deposit dissolution rate (Kudsk *et al.*, 1991). Besides, the dislodging potential of rain plays a decisive role. During heavy rain events, the plant surface is completely covered with a water film, making the wash-off easier (Hartley and Graham Bryce, 1980). At gentle misting, run-off occurs only periodically after coalescence of water drops, whereas at heavy rain, droplets more readily run off leaf surfaces (Suheri and Latin, 1991).

The progression of mancozeb wash-off from 0 to 5 mm at torrential and heavy rain can be described as a hyperbolic curve (Fig. 4). These results are in agreement with data presented by McDowell *et al.* (1987) for the insecticide fenvalerate and by Smith and MacHardy (1984) for the fungicide captan. In the case of light rain, a linear progression in wash-off was observed. The great fungicide losses observed after 1 mm rain, only, confirm results of Kudsk *et al.* (1991) for maneb and Pick *et al.* (1984) for endosulfan, carbaryl, cypermethrin, parathion EC and parathion WP.

Taking into consideration the low water-solubility of mancozeb, the main rain intrinsic feature responsible for its removal at comparable rain volumes, but differing rain intensity, must have been the kinetic impact of rain droplets. Kudsk *et al.* (1991) verified higher mancozeb loss when 3 mm rain was applied at 27 mm h^{-1} than at 3 mm h^{-1}, attributing a major relevance to the mechanical impact and only little relevance to the dissolution rate of the deposits.

It is known that agrochemicals formulated as wettable powder or wettable granule have lower rainfastness (Willis *et al.*, 1996; van Bruggen *et al.*, 1986). Nevertheless, even insoluble active ingredients can be removed from leaf surfaces when rain occurs within hours of pesticide application (Wauchope *et al.*, 2004). Both factors, however, can not explain the intense fungicide wash-off after a low rain volume observed in our trials. Considering that rainfastness of contact fungicides is influenced by its diffusion into the epicuticular waxes (Cabras *et al.*, 2001) and also associated with its binding to the surface waxes (Andrieu *et al.*, 2000), our results suggest that mancozeb has only a weak binding and a little diffusion into the surface wax layer of apple seedling leaves.

Our results are in accordance with statements of Kudsk *et al.* (1991) who have shown that high intense rain washes-off more a.i. than low intense rain. Moreover, we agree with statements of Fife and Nokes (2002) that rain intensity and duration independently affect the removal of active ingredient from the plant foliage.

4.3 Rainfall interruptions

Another objective of our study was to investigate the wash-off of mancozeb, as affected by rain interruptions. Interruptions following precipitation of few millimetres only could allow a.i. to dry after rain-induced redistribution of deposits on the leaves; this may reduce fungicide removal in comparison to continuous rain. In our studies no statistical differences concerning fungicide residues were observed among rain settings when applying 5 mm rain (Tab. 1). This can be explained by the fact that fungicide deposits could not dry-up during the breaks, possibly due to the high relative humidity at the plant level as well as to the hygroscopic property of the active ingredient. Further, it must also be considered that rainfall interruptions occurred after 2 and 2.5 mm precipitation, when a great part of the deposit was already removed (Fig. 3).

When 2 mm rain was applied, interruptions of rain exposure had a positive influence on rainfastness of mancozeb. In this case, the initial deposit was reduced to 16 % at continuous precipitation and to 25 %, 24 % and 22 %, respectively, when interrupting the rain event for 1, 2 or 3 times (Tab. 2). In this situation, interruptions prevented formation of water films, necessary to carry out pesticide from the leaf surfaces. We assume that longer intervals and an environment with lower RH would additionally reduce the rain-induced wash-off.

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C Seed oil ethoxylate adjuvants and their influence on retention and rainfastness of the contact fungicide mancozeb

1 Introduction

The retention of leaf applied agrochemicals is a critical factor for a successful plant protection because it influences markedly the biological efficacy of the active ingredients (Green, 2001; Zabkiewicz, 2000; Stock and Briggs, 2000; Downer *et al.*, 1999; Hall *et al.*, 1997). However, even a perfect deposit is not a guarantee of success, because several environmental factors may reduce the effectiveness and the residual activity of the agrochemicals (Cabras *et al.*, 2001; Schepers, 1996; Neely, 1970). The main factor is impact of rain (Schepers, 1996; McDowell *et al.*, 1987), which in many cases modifies the deposit characteristics by dilution, redistribution and removal from the plant surface and tissue (Thacker and Young, 1999). For these reasons agrochemicals must be rainfast, an attribute usually achieved with built-in or tank-mix adjuvants (Ditzer, 2002; Haefs, 2001; Kudsk *et al.*, 1991; Taylor and Matthews, 1986).

Actually, mineral oils represent the major category of tank-mix adjuvants (Uttley, 1995), but there is a continuous trend towards adjuvants based on renewable resources (Cecutti *et al.*, 2002; Green, 2000; Hill, 2000; Western *et al.*, 1999). This is in agreement with the goal to replace non-environmentally friendly components such as alkyl-phenol-ethoxylates and paraffin oil-based solvents with environmentally friendly components, e.g. non-alkyl-phenol-ethoxylates and natural oil-based solvents (Abribat, 2001; Underwood *et al.*, 2001; Green, 2000).

In contrast to herbicides, the use of tank-mix adjuvants with fungicides is not so common, because selection of adjuvants for fungicides is much more complicated (Knowles, 2001; Rommens *et al.*, 2001; Steurbaut *et al.*, 2001; Stock and Briggs, 2000). These difficulties arise from the multiplicity of specific fungicide-adjuvant-crop-fungus interactions, impairing a general breakthrough in the practical use of adjuvants (Steurbaut, 1993). Nevertheless, since a few years a great interest exists in the development of adjuvants for fungicides (Knowles, 2001; Underwood, 2000; Green, 2000). Unfortunately, knowledge and understanding of the interactions between fungicide-adjuvant-crop-fungus-environment can not be gained from the herbicide-adjuvant knowledge (Steurbaut, 1993).

Mancozeb is one of the most sold generic fungicides in the world (Underwood *et al.*, 2001), used likewise in pomiculture in order to prevent the first infection with apple scab (*Venturia inaequalis* (Cke.) Wint.) early in the season. Because of its low rainfastness (Hunsche *et al.*, 2003; Cabras *et al.*, 2001; Kudsk *et al.*, 1991), mancozeb is a good a.i. to test the ability of new adjuvants in reducing the rain-induced wash-off of agrochemicals.

In our studies we evaluated a new group of adjuvants, namely rapeseed, linseed and soybean oil ethoxylates with diverse ethoxylation degree. These adjuvants are built-on as a single emulsifiable component, which prevent oil separations in the spray solution, and can be

categorised as oil ethoxylates but also as non-ionic surfactants (Abribat, 2001). Enhancement or maintenance of the biological efficacy of selected a.i. with some of these ethoxylates has already been proven (Scherhag, 2005; Müller *et al.*, 2002; Müller *et al.*, 2001; Haefs, 2001; Abribat, 2001), but their influence on the rainfastness of fungicides has not been studied, yet.
The aim of our study therefore was to investigate the effect of the rapeseed, linseed and soybean oil ethoxylates on the deposit formation and rainfastness of the contact fungicide mancozeb.

2 Material and methods

2.1 Plant material and growth conditions

The experiments were conducted with 56-days old 'Golden Delicious' apple seedlings (*Malus domestica* Borkh.). Seed dormancy was broken by submerging the seeds in water (96 h) before treatment with the fungicide Euparen® (0.1 g l^{-1}, 50 % Dichlofluanid) and allocation in a refrigerator (T = 4 ºC; RH = 90 %) for 4 weeks. Approximately 200 seeds were distributed on a germination tray (loam: sand, 3:1) and covered with layer of sand (1 cm thick). Three weeks after germination the seedlings were transplanted into Teku-pots (JP 3040, Pöppelmann GmbH & Co. KG, Germany). Five weeks later the experiments were conducted. The seedlings grew up in a growth chamber with constant temperature (20 ºC ± 1 ºC) and relative humidity (70 % ± 5 %). Photosynthetic active radiation (PAR) was provided at 180 µmol s^{-1} m^{-2} at the plant level during a 16 h-photoperiod. Plants were watered according to their needs and fertilized with a balanced nutrient solution once a week.

2.2 Fungicide and adjuvants

Rainfastness studies were carried out with the contact fungicide mancozeb [(manganese ethylene bis(dithiocarbamate) (polymeric) complex with zinc salt)] as Dithane Ultra WG 80 % (Spiess-Urania Chemicals GmbH - Hamburg, Germany), at a concentration of 2.40 g l^{-1} a.i. in the spray solution. Ethoxylated triglycerides from rapeseed (RSO), linseed (LSO) and soybean (SBO) oils (Cognis® AgroSolution, Düsseldorf, Germany) with diverse ethylene oxide (EO) and propylene-oxide (PO) units in the hydrophilic chain (Tab. 1) were added to the spray solution. In the last trial, the adjuvants RSO 10, LSO 10 and SBO 10 were compared with the commercial surfactants Silwet®L-77 (polyalkylene modified heptamethyltrisiloxane 80 %, GE Silicones Inc., USA), Tween®60 (polyoxyethylene sorbitan monostearate, Uniquema, USA), Break-Thru®S240 (polyether modified trisiloxane, Goldschmidt AG, Germany), and Marlowet®R40 (non-ionic alkylpolyglykolether, Condea Chemie GmbH, Germany). Seed oil ethoxylates and commercial adjuvants were added at a concentration of 1 g l^{-1}.

Table 1. Physicochemical characteristics of rapeseed (RSO), linseed (LSO), and soybean (SBO) oil ethoxylates and their water solutions.

	Seed oil ethoxylates	Average of units [a] EO	PO	Status of aggregation	pH [b]
	Water	-	-	Liquid	6.95
Rapeseed	RSO 5	5	-	Liquid	6.11
	RSO 10	10	-	Liquid	5.77
	RSO 20	20	-	Liquid	6.27
	RSO 60	60	-	Solid	5.34
Linseed	LSO 10	10	-	Liquid	5.78
	LSO 0903	9	3	Liquid	5.83
	LSO 30	30	-	Liquid	5.94
	LSO 3003	30	3	Liquid	5.87
Soybean	SBO 10	10	-	Liquid	5.95
	SBO 0903	9	3	Liquid	6.00
	SBO 30	30	-	Liquid	6.02
	SBO 3003	30	3	Liquid	6.01

[a] Cognis® AgroSolution.

[b] Solution containing 1 g l^{-1} surfactant in deionized water.

2.3 Determination of physicochemical properties

2.3.1 Surface tension

Surface tension of the adjuvant solutions (1 g l^{-1}) was measured with a tensiometer (K11-HRX; Krüss GmbH, Hamburg, Germany), adopting the Du Nouy ring method, at room temperature (21 °C ± 0.5 ºC). Number of replications was four, and mean of the values was calculated.

2.3.2 Contact angle

Contact angle of the adjuvant solutions (1 g l^{-1}) was assessed with the Contact Angle Measuring System G10 (Krüss GmbH, Hamburg, Germany) by applying single 1 µl-droplets with a microsyringe (Hamilton-Bonaduz, Switzerland) on a standard surface (parafilm, Pechiney Plastic Packaging, USA). Tangents were set at both visible sides of a droplet and readings of the angles taken. Each treatment group comprised 10 individual droplets with five replications each.

2.3.3 Drying time

Single 1 µl-droplets of the adjuvant solutions (1 g l^{-1}) were applied on a standard surface (parafilm, Pechiney Plastic Packaging, USA) at temperature of 20 ºC (± 0.5 ºC) and relative humidity of 60 % (± 3 %). The time (s) required for complete drying-up was recorded. Five replications with 10 individual droplets each provided data for statistical analysis of drying time.

2.4 Fungicide application

Treatment solutions were applied with the laboratory pesticide sprayer (B-PSA-1, Department of Agricultural Engineering, University of Bonn, Germany). The sprayer was equipped with a hollow cone, 80º nozzle (Lechler GmbH, Germany), placed 45 cm above the plants top. Application was carried out at a speed of 6 km h^{-1} and a pressure of 3 x 10^5 Pa, to give a volume of 390 l ha^{-1}. Five minutes after pesticide application the seedlings were returned to the growth chamber for 4 h until onset of rainfall simulation.

2.5 Rainfall simulation

A 5 mm heavy rain (5 mm h^{-1}) with a medium droplet volume diameter (MVD) of 1075 µm was simulated with the B-LRS-2 rain simulator (Department of Agricultural Engineering, University of Bonn, Germany), as described elsewhere (Ditzer, 2002; Kromer *et al.*, 1996). Plants were exposed to simulated rain event 4 h after pesticide application. Plants were returned to the growth chamber 20 min. after rain simulation, so that leaf surfaces could dry overnight before fungicide extraction. Treated but not rain-exposed seedlings served as control.

2.6 Sampling procedure and fungicide analysis

From each plant the second and third completely developed leaf was taken for residue analysis; each sample was composed of three leaves without petioles. The fresh-weight (FW) was determined (Sartorius BP 210S, Sartorius AG, Göttingen, Germany), and samples were enclosed in Teflon vessels. Five millilitres of nitric acid 65 % p.A. (Merck KGaA, Darmstadt, Germany) and 2 ml of hydrogen peroxide 30 % (RdH Laborchemikalien GmbH & Co. KG, Seelze, Germany) were added to the samples before acid digestion in a microwave (Büchi MLS 1200, Microwave Laboratory Systems GmbH). After digestion, content was transferred into volumetric flasks (25 ml), the volume filled up with distilled and deionized water (Milli-Q Ultrapure Water Purification Systems, Millipore Corporation, Billerica, USA) and then transferred to plastic vessels. Mancozeb content of the samples was analysed by determining the concentration of manganese atoms by atomic absorption spectrometry (AAS, Perkin-Elmer Analyst 300 spectrometer, Wellesley, USA equipped with a Multi-Element Lumina Hollow Cathode Lamp). Manganese atoms constitute about 17 % of mancozeb molecular weight, and a.i. concentrations in the samples and on the leaves were calculated by a rule of proportion (van Bruggen *et al.*, 1986; Travis *et al.*, 1985). Samples of untreated seedlings served as reference for natural manganese concentration in the plant tissue. A standard series (0.1, 0.5, 1.0, 2.0 and 3.0 mg l^{-1}, respectively), prepared from a manganese standard solution (1000 mg l^{-1}; Merck KGaA, Darmstadt, Germany) served for establishing a calibration curve.

2.7 Deposit characteristics

Fungicide deposits on the leaf surface were characterized with a scanning electron microscope (XL 30 ESEM, FEI-Philips, Kassel, Germany; Microsoft control software, version 5.90). Droplets (0.5 µl) of the fungicide solutions (mancozeb formulated with RSO 10, LSO 10 and SBO 10) were applied with a microsyringe (Hamilton-Bonaduz, Switzerland) in a previously delimited area on the leaf surface, and left to dry for 4 h before rainfall simulation. Leaf discs ($A = 0.8\ cm^2$) of rain-exposed and not rain-exposed leaves were mounted on alumina stubs, placed into the analysis chamber, and scanned in the low vacuum mode (0.3 Torr).

2.8 Experimental design and statistical analysis

The experiments on rainfastness were conducted in a completely randomized design, with 15 seedlings (10 analytical samples) per each treatment group. Results were expressed as $\mu g\ g^{-1}$ FW and also calculated as percentage of initial fungicide concentration (fungicide concentration in no rain-exposed seedlings was referred as 100 percent). Data were analyzed with the software SPSS 12.0 (SPSS Inc., Chicago, USA) for analysis of variance (ANOVA). In the case of statistical significant differences among treatments, results were compared by Duncan-Test $p \leq 0.05$. Graphs were designed with the software Sigma Plot (Systat Software GmbH, Erkrath, Germany), version 7.101.

3 Results

3.1 Surface tension, contact angle and drying time

3.1.1 Rapeseed oil (RSO) ethoxylates

Surface tension of water ($71.4\ mN\ m^{-1}$) was significantly reduced by all RSO-ethoxylates, whereas lowest surface tension ($29.9\ mN\ m^{-1}$) was measured after addition of the more hydrophobic ethoxylate RSO 5 (Tab. 2). Contact angle of water droplets (104.8º) was strongly reduced with the more hydrophobic ethoxylate RSO 5 (76.3º), while only little reduction was observed when adding RSO 20 (102.4º) to the solution.

Table 2. Surface tension, contact angle, and drying time of rapeseed oil (RSO) ethoxylates in water solution ($1\ g\ l^{-1}$).

Treatment	Surface tension [a] $[mN\ m^{-1}]$	Contact angle [a] [º]	Drying time [a] [s]
Water	71.4 a	104.8 a	1369 a
RSO 5	29.9 c	76.3 e	1244 b
RSO 10	31.8 c	83.4 d	1206 b
RSO 20	35.5 b	102.4 b	1265 b
RSO 60	37.8 b	99.2 c	1226 b

[a] Means in the column followed by the same letter are not different by Duncan-Test $p \leq 0.05$.

Addition of RSO ethoxylates significantly reduced drying time of droplets in comparison to droplets of pure water, whereas no statistical differences between RSOs were observed (Tab. 2).

3.1.2 Linseed oil (LSO) ethoxylates

All LSOs reduced surface tension of water markedly. The lowest surface tension (32.5 mN m^{-1}) occurred when LSO 0903 was added to the solution (Tab. 3). Water droplets exhibited greatest contact angle (102.5º), while the lowest contact angle (75.9º) was observed following addition of LSO 0903. As compared to water, all LSO ethoxylates reduced drying time of droplets significantly (Tab. 3). However, no statistical differences could be established among LSO ethoxylates.

Table 3. Surface tension, contact angle, and drying time of linseed oil (LSO) ethoxylates in water solution (1 g l^{-1}).

Treatment	Surface tension [a] [mN m^{-1}]	Contact angle [a] [º]	Drying time [a] [s]
Water	71.4 a	102.5 a	1488 a
LSO 10	33.2 c	77.6 bc	1224 b
LSO 0903	32.5 d	75.9 c	1206 b
LSO 30	33.4 c	79.6 b	1223 b
LSO 3003	34.2 b	77.6 bc	1168 b

[a] Means in the column followed by the same letter are not different by Duncan-Test $p \leq 0.05$.

3.1.3 Soybean oil (SBO) ethoxylates

Addition of SBO ethoxylates to water (71.4 mN m^{-1}) reduced surface tension of solutions significantly. Lowest surface tension (31.5 mN m^{-1}) was observed after addition of SBO 10 to water (Tab. 4).

Table 4. Surface tension, contact angle, and drying time of soybean oil (SBO) ethoxylates in water solution (1 g l^{-1}).

Treatment	Surface tension [a] [mN m^{-1}]	Contact angle [a] [º]	Drying time [a] [s]
Water	71.4 a	105.2 a	1482 a
SBO 10	31.5 d	86.0 b	1215 b
SBO 0903	33.4 c	71.6 c	1173 b
SBO 30	33.8 c	77.8 d	1242 b
SBO 3003	34.7 b	75.5 d	1214 b

[a] Means in the column followed by the same letter are not different by Duncan-Test $p \leq 0.05$.

In general, all SBO ethoxylates reduced contact angle of water droplets significantly, whereas the lowest angle (71.6º) was observed when adding SBO 0903 ethoxylate. Addition of SBOs also significantly reduced drying time of water; degree of adjuvant ethoxylation had no influence on the drying rate of the droplets.

3.2 Retention and rainfastness

3.2.1 Rapeseed oil ethoxylates

Retention of a.i. by apple seedling leaves was significantly reduced by all RSO ethoxylates (Fig. 1). Initial mancozeb deposit in the control plants was about 1100 µg g^{-1} FW, and as a consequence of RSO 10 addition it was reduced to 650 µg g^{-1} FW. After rainfall, all RSO treatments and particularly the RSO 5 showed more a.i. on the leaves, as compared to the rain-exposed control. Percentage of residues remained after rainfall was 6 % in the control and 19 % in the more hydrophobic RSO 5 treatment group. The more hydrophilic ethoxylates RSO 20 and RSO 60 enhanced mancozeb rainfastness to a lower extent.

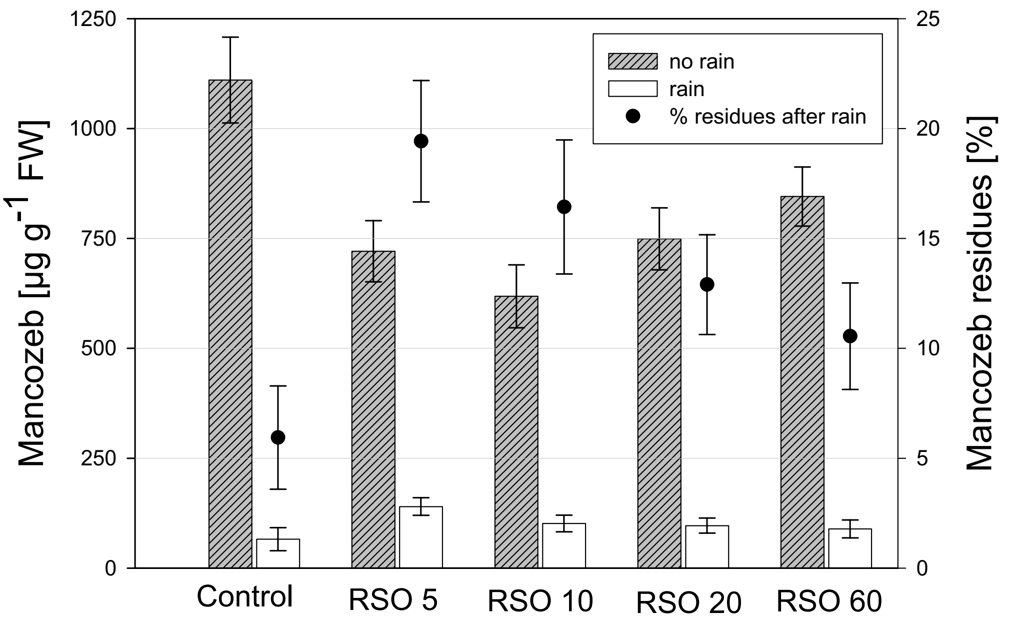

Figure 1. Initial mancozeb deposit ( ), deposit after 5 mm rain at 5 mm h^{-1} ( ) and percent of mancozeb residues after rain (●) as influenced by addition of RSO ethoxylates with 5, 10, 20 and 60 ethylene oxide units in the hydrophilic chain. Vertical bars represent the standard error.

3.2.2 Linseed oil ethoxylates

The initial mancozeb concentration in the control seedlings was 980 µg g^{-1} FW, and it was reduced to less than 800 µg g^{-1} FW due to addition of the LSOs (Fig. 2). The fungicide residues after 5 mm of heavy rain were significantly higher when LSO 10 and LSO 0903 were added to the spray solution. After the rain, percentage of remained a.i. in the control treatment

(7 %) was significantly enhanced due to addition of LSO 10 (18 %) to the spray solution. The more hydrophilic adjuvant LSO 30 enhanced rainfastness of mancozeb to a lower extent.

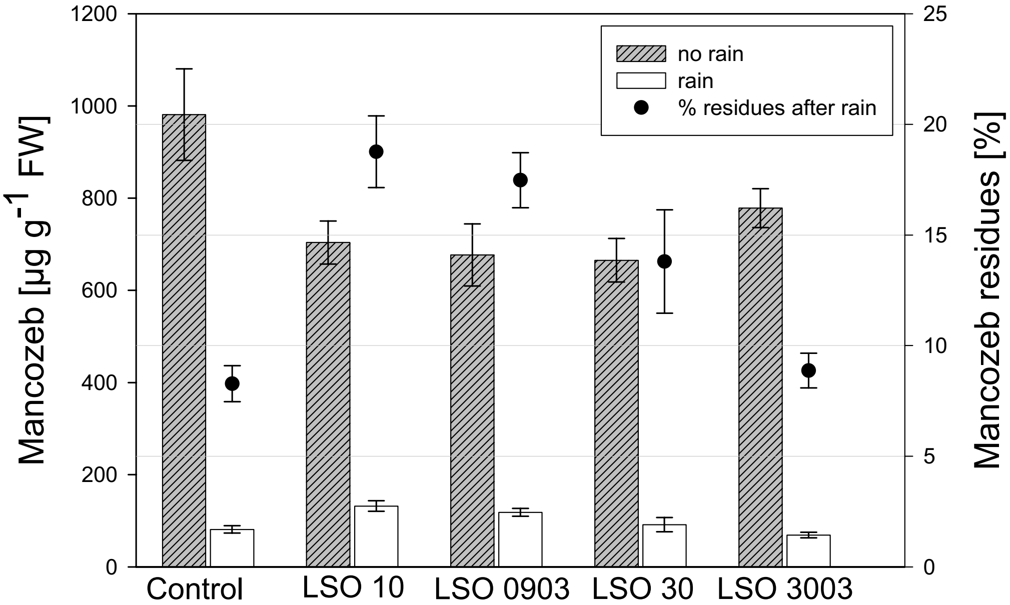

Figure 2. Influence of LSO ethoxylates with 10 EO, 0903 (EO/PO), 30 EO and 3003 (EO/PO) on the initial deposit ( ), deposit after 5 mm rain at 5 mm h^{-1} ( ) and percent of mancozeb residues after rain (●). Vertical bars represent the standard error.

3.2.3 Soybean oil ethoxylates

Addition of SBOs to the spray solution reduced retention of mancozeb on apple seedling leaves (Fig. 3) in a similar manner, as outlined for RSOs and LSOs. More residues resisted the rainfall when SBO 10 or SBO 0903 were added to the spray solution. Percentage of fungicide residue on the leaves was enhanced from 8 % in the control to about 13 % after addition of SBO 10 or SBO 0903. On the other hand, the more hydrophilic adjuvants SBO 30 and SBO 3003 influenced rainfastness of a.i. negatively.

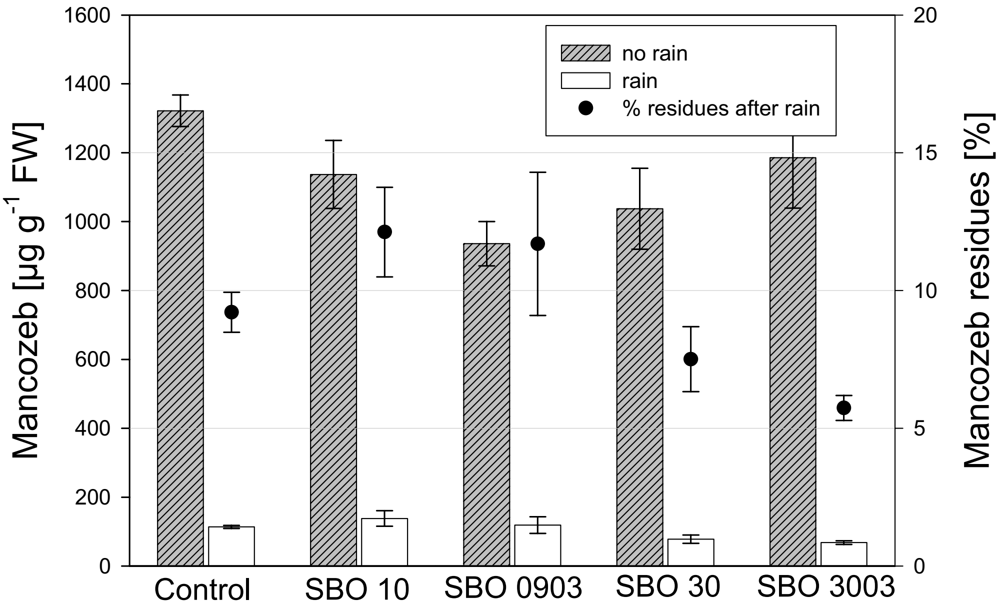

Figure 3. Initial mancozeb deposit ( ), deposit after 5 mm rain at 5 mm h^{-1} ( ) and percent of mancozeb residues after rain (●) as influenced by addition of soybean oil ethoxylates with 10 EO, 0903 (EO/PO), 30 EO and 3003 (EO/PO). Vertical bars represent the standard error.

3.2.4 RSO 10, LSO 10, SBO 10 and commercial adjuvants

In this test, three hydrophobic seed oil ethoxylates (RSO 10, LSO 10, SBO 10) were compared with the commercial adjuvants Silwet®L-77, Tween®60, Break-Thru®S240 and Marlowet®R40. All evaluated adjuvants reduced mancozeb retention on the leaves significantly (Fig. 4). Regardless of type of adjuvant, a distinct fungicide wash-off was observed after 5 mm of heavy rain. After the rain, more a.i. was present on the plants treated with mancozeb plus LSO 10 or RSO 10, respectively. Rainfastness ranged between 8 % in the control and 17 % following addition of LSO 10 or RSO 10. The seed oil ethoxylate SBO 10 and the surfactant Silwet®L-77 also enhanced rainfastness of mancozeb, while Tween®60 showed a negative impact. The adjuvants Break-Thru®S240 and Marlowet®R40 had no influence on rainfastness of mancozeb.

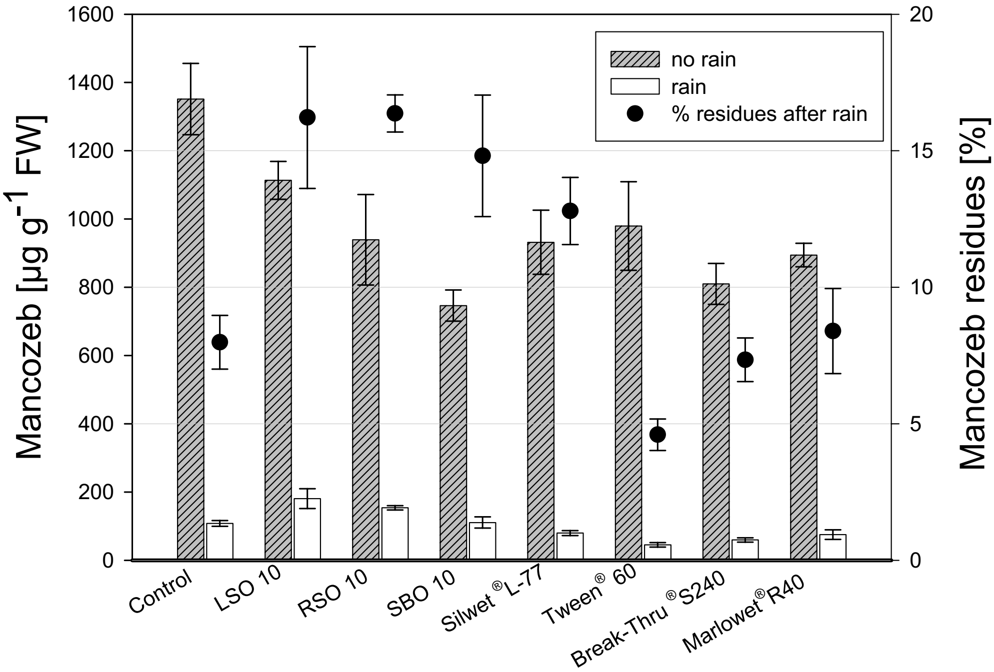

Figure 4. Influence of the seed oil ethoxylates LSO 10, RSO 10 and SBO 10 and the commercial adjuvants Silwet®L-77, Tween®60, Break-Thru®S-240 and Marlowet®R40 on the initial deposit ( ), deposit after 5 mm rain at 5 mm h^{-1} ( ) and percent of mancozeb residues after rain (●). Vertical bars represent the standard error.

3.3 Deposit characteristics

SEM micrographs revealed that active ingredient distribution inside the residue zone was not affected by addition of seed oil ethoxylates. For these reasons representative SEM micrographs are presented only. In general, before rain, mancozeb was not uniformly distributed, but rather concentrated in the centre or in one half of the droplet area (Fig. 5A). The fungicide was mainly located along anticlinal cell walls (Fig. 5B) in form of crystals (Fig. 5C).

The impact of rain droplets changed deposit characteristics markedly. Besides intense wash-off, the remaining fungicide was more uniformly distributed within the residue zone (Fig. 5D), while a.i. residues were not observed outside the area of droplet impact. The greatest part of the fungicide was still located along anticlinal cell walls (Fig. 5E), but some a.i. was also present over the cells (Fig. 5F). As a rule, mancozeb residues following rain impact had a shape of balls or annuli.

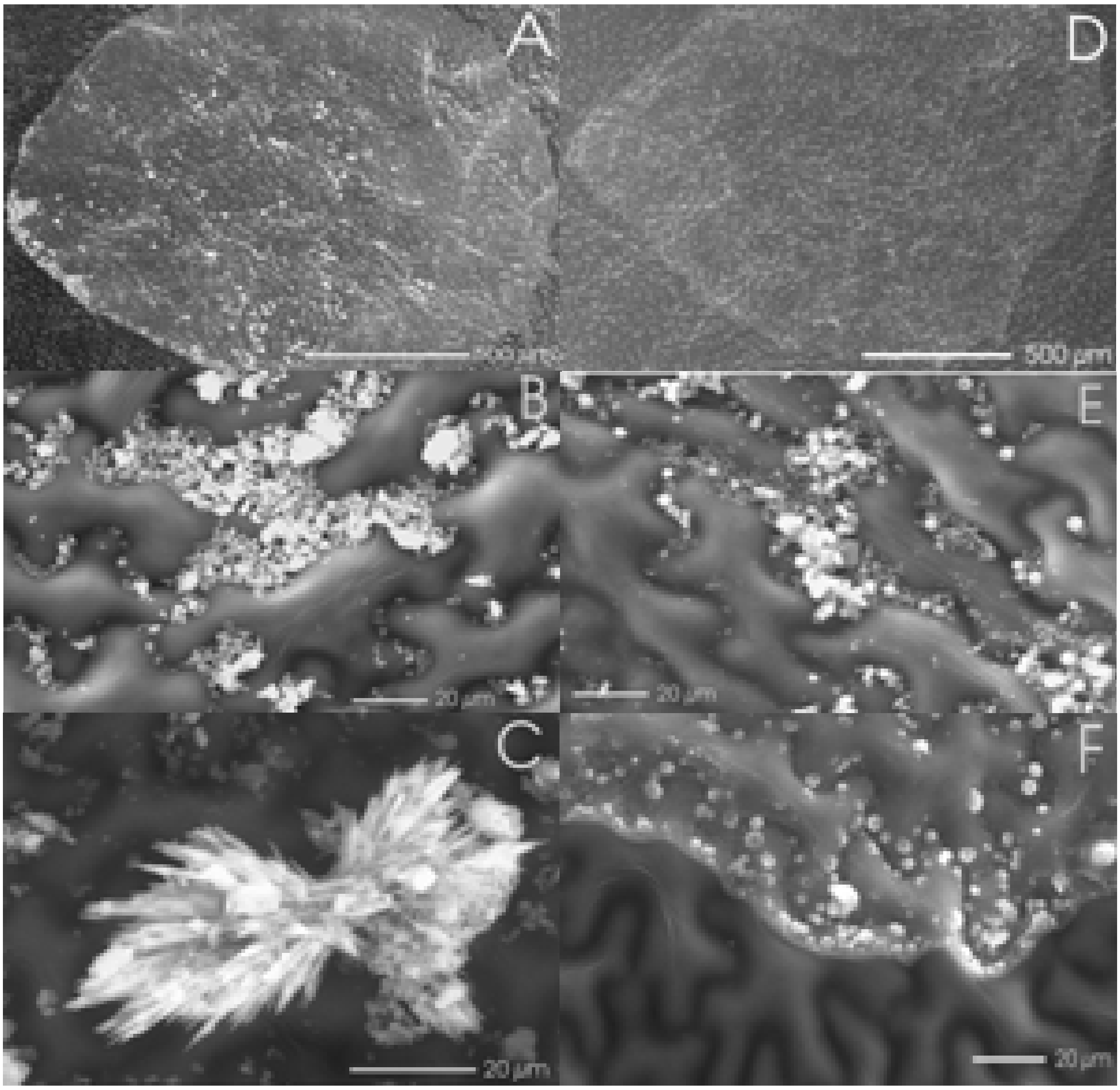

Figure 5. Representative SEM micrographs of mancozeb deposits on the adaxial leaf surface of apple seedlings (*M. domestica* Borkh.) before (A-C, left) and after (D-F, right) a simulated heavy rain (5 mm; 5 mm h^{-1}). A – a.i. irregular distribution inside the deposition zone; B – mancozeb is deposited mainly along/above anticlinal cell wall; C – crystals of a.i. on the leaf surface; D – rain impact on the wash-off and distribution of the remaining fungicide inside the original residue zone; E – the greatest part of the a.i. remains above anticlinal cell wall, but residues acquired a shape of balls and annuli; F – balls and annuli on the droplet boarder.

4 Discussion

4.1 Surface tension, contact angle and drying time

Surface tension, contact angle and drying time were markedly reduced as a consequence of addition of rapeseed, linseed and soybean oil ethoxylates (Tabs. 2 - 4). The direct impact of surfactants on the spray solution was reduction of the surface tension, while the reduction of contact angle and drying time were secondary effects. It is well known that surfactants are able to modify characteristics of a spray solution and that the beneficial effects are primarily associated with reduction of surface tension (Hess and Foy, 2000). Surfactants increase surface wetting (Green, 2001; Bukovac *et al.*, 1995), because there is a relation between surface tension and contact angle of single droplets (Steurbaut *et al.*, 2001). Solutions with lower surface tension require shorter time for completely drying up (Steurbaut *et al.*, 2001; Leung and Webster, 1994). Nevertheless, the evaporation rate is not perfectly correlated with the interfacial area of droplets (Zabkiewicz *et al.*, 1988). In our studies, a stringent relation among these parameters is missing for all three groups of seed oil ethoxylates, so that not always the lowest contact angle was associated with the lowest surface tension. In addition, there were no differences between the ethoxylation degrees of RSOs, LSOs or SBOs concerning the drying time of individual droplets.

4.2 Retention and rainfastness

Results concerning fungicide concentration presented in our studies are related to FW of the leaves. Due to the fact that not all experiments were carried out at the same time and differences in leaf density would have to be considered, comparisons between different sets of experiments can only be made by referring to the percentage of fungicide remaining after the rainfall.

Mancozeb retention was significantly reduced due to addition of seed oil ethoxylates or commercial adjuvants to the spray solutions (Figs. 1 - 4). Earlier studies have shown that pesticide retention on the target object is a function of application volume as well as of adhesion and spreading of the droplets on the surface (Green and Hazen, 1998; Stevens *et al.*, 1993). It was shown that addition of adjuvants to spray solution influences retention and deposit formation on the leaves (Gent *et al.*, 2003; Balsari *et al.*, 2001; Reddy and Locke, 1996; Stevens, 1993; Taylor and Matthews, 1986). Positive effects of adjuvants are generally obtained by low- volume application (Gent *et al.*, 2003). In contrast, high-volume sprays, especially in combination with decreased surface tension, generally reduce a.i. retention (Furmidge, 1962) as a result of coalescence of spray droplets and run-off (Dirkse and van Adrichem, 2001; Gaskin *et al.*, 2000; Stevens, 1993). In our studies, despite of the relatively high application volume (390 l ha^{-1}) and reduced surface tension of the solutions (Tabs. 2 - 4), run-off was not observed. According to Dirkse and van Adrichem (2001) run-off can occur unnoted along the veins, central vein, petiole and stem.

The low rainfastness of mancozeb is a known fact (Hunsche *et al.*, 2003; Cabras *et al.*, 2001; Kudsk *et al.*, 1991), and this was one reason why we have chosen this a.i. for our studies. Rainfastness of mancozeb deposits was enhanced following addition of the seed oil ethoxylates (Figs. 1 - 3) but, irrespective of adjuvants, a distinct wash-off was registered after 5 mm of rain. Seed oil ethoxylates with more hydrophobic characteristics enhanced rain persistence of the fungicide more to a higher extent than the more hydrophilic surfactants. The surfactant Silwet®L-77 also enhanced the rainfastness, while the other commercial adjuvants (Tween®60, Break-Thru®S240 and Marlowet®R40) were not as effective in enhancing the rainfastness as the seed oil ethoxylates (Fig. 4).

The positive results of the seed oil ethoxylates in enhancing rainfastness were anticipated. Schmitz-Eiberger *et al.* (2002) already have shown that rapeseed oil ethoxylates enhance rainfastness of calcium chloride solutions. It was also demonstrated for the systemic a.i. glyphosate in several weed species that the ethoxylation degree of the RSOs may influence rainfastness and biological activity of the herbicide (Scherhag, 2005; Haefs, 2001).

On the other hand, surface agents such as organosilicones are not primarily used to enhance rainfastness, but they reduce the rain induced wash-off of systemic compounds by promoting their penetration into plant tissues (Kogan, 2001; Leung and Webster, 1994; Roggenbuck *et al.*, 1993; Sundaram, 1991; Roggenbuck *et al.*, 1989). Nevertheless, the potential use of organosilicone adjuvants to enhance rainfastness was hypothesised (Stevens, 1993) because they are high-molecular weight polymers, a characteristic of a sticker-adjuvant category. Stickers can adhere pesticide deposits to the leaf surfaces (Hazen, 2000). In addition, they can also provide a protective film over the deposit, thus reducing the wash-off due to rainfall (Green, 2001; Leung and Webster, 1994). Although, the resistance to wash-off will vary according to water solubility of the adjuvant and its concentration relative to the pesticide deposit (Hazen, 2000).

The PO units in the LSO and SBO ethoxylates had no additional positive effects compared to the other ethoxylates, and at higher ethoxylation degree (30 EO units) they caused only little reduction of the rainfastness. According to Hazen (2000), some high molecular weight surfactants such as EO/PO bloc copolymers have a natural tendency to adhere to the surfaces. Because of their high molecular weight, hydrophobic adjuvants are strongly adsorbed to the surface due to the many more anchoring points, giving therefore a better long term stability for the active ingredient (Knowles, 1995). This, however, was not confirmed in our studies.

Characteristics of the fungicide deposits before and after rainfall were investigated by scanning electron microscopy. SEM micrographs showed that a.i. was allocated mainly above/along the anticlinal cell walls (Figs. 5B and 5E), confirming previous observations (Nalewaja and Matysiak, 2000; Bukovac *et al.*, 1995; Hess and Falk, 1990). Rainfall washed-off a great part of the a.i. from the surfaces, and the remaining fungicide was uniformly distributed inside the droplet area (Fig. 5D). Before the rain, a.i. was mainly present in form of crystals (Fig. 5C), while after the rain a.i. was found in form of balls and annuli (Fig. 5F). However, it was pointed out (Green, 2001; Leung and Webster, 1994) that a.i. can also be present in a matrix or under a hydrophobic layer, avoiding wash-off due to rainfall. SEM micrographs after rainfall (Fig. 5F) showed a layer above the fungicide deposits.

Actually, despite of numerous studies with several active ingredients and adjuvants, relatively little is known about the impact of surfactants on the ultrastructure of agrochemical deposits (Stock and Briggs, 2000). Nevertheless, it was shown that surfactant-induced modifications of deposit characteristics are related to changes in rate of penetration (Kraemer *et al.*, 2005;

Scherhag, 2005), resulting in greater efficacy of active ingredients (Nalewaja and Matysiak, 2000). However, the influence of adjuvants on deposit characteristics should be evaluated preferentially with unformulated active ingredients rather with commercial products.
Taking together, seed oil ethoxylates effectively reduced surface tension, contact angle and drying time of the spray solution in our studies. Seed oil ethoxylates and specially those with low ethoxylation degree (5 and 10 EO) also reduced the wash-off of mancozeb and in this way enhanced the rainfastness of the active ingredient. However, it should be kept in mind that they also may decrease retention of spray solution in high-volume spray application, as was the case in our experiments.

5 References

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D Effect of adaxial leaf surface characteristics of apple seedlings, bean seedlings, and kohlrabi plants on retention and rainfastness of mancozeb

1 Introduction

Foliar application of pesticides is a critical procedure in the modern agriculture. The success of a phytosanitary treatment depends among others on retention of spray solution on the target object and resistance of deposit against adverse factors such as rainfall. Both retention and rainfastness are influenced by physicochemical characteristics of the leaf surface, i.e. micro roughness, and amount and composition of the epicuticular waxes.

Micro roughness of the leaf surface is determined to a great extent by surface waxes (Gordon *et al.*, 1998; Boize *et al.*, 1976), a complex mixture of long chain hydrocarbons that cover the cuticle of terrestrial vascular plants (Koch *et al.*, 2004; Lemieux, 1996). Epicuticular wax (EW) can be present as a simple amorphous film covering the surface, or in form of crystalline aggregates organized as complex structures (Barthlott *et al.*, 1998; Baker, 1982). Amount and composition of EW as well as its structure on the surface varies among plant species and organs (Simanova *et al.*, 2005; Hunt and Baker, 1982; Fernandes *et al.*, 1964), having practical implications for plant protection and performance of foliar applied agrochemicals (Holloway, 1993). Here, the most affected processes are wettability (Koch *et al.*, 2004; Belding *et al.*, 1998; McWhorter *et al.*, 1990; Bukovac *et al.*, 1979; Holloway, 1970; Holloway, 1969) and retention of the pesticide on the surface (Downer *et al.*, 1999; Hall *et al.*, 1997; Bukovac *et al.*, 1979; Flore and Bukovac, 1976; Fernandes *et al.*, 1964).

After deposition of the agrochemical on leaf surface, impact of rain is the predominant environmental factor with influence on residual activity of the active ingredient (Schepers, 1996; McDowell *et al.*, 1987). Rainfall can remove greatest part of the a.i. deposits (Fife and Nokes, 2002; McDowell *et al.*, 1987; Pick *et al.*, 1984; Smith and MacHardy, 1984; Bruhn and Fry, 1982) but its importance varies among plant species and cultivars (Reynolds *et al.*, 1994; Kudsk *et al.*, 1991) as well as differences of the various surfaces of the individual plants (Cabras *et al.*, 2001). These phenomena may be conditioned by variations in micromorphology and chemical composition of the surfaces (Cabras *et al.*, 2001; Kudsk *et al.*, 1991; Neely, 1971), because the amount of wax and its composition affect penetration of systemic a.i. into the plant tissue (Chachalis *et al.*, 2001; Stock *et al.*, 1993; Baker and Hunt, 1986; Hunt and Baker, 1982) and binding of contact a.i. on the surface and its diffusion into the wax layers (Andrieu *et al.*, 2000). To our knowledge there are no systematic studies on the influence of physicochemical characteristics of leaves on the rainfastness of contact fungicides. Although, we hypothesise that leaf surface characteristics and especially amount and composition of EW have a significant influence on retention and rainfastness of contact fungicides.

The objective of our study therefore was to determine and statistically evaluate the quantitative relations among physical and chemical characteristics of the adaxial leaf surfaces on the one hand and retention and rainfastness of the contact fungicide mancozeb on the other hand. Three plant species (apple seedlings, bean seedlings, and kohlrabi) with great differences in wettability and micromorphology as well as amount and chemical composition of epicuticular waxes in the adaxial leaf surfaces were chosen for the experiments. Further, the influence of tank-mix adjuvants on the abovementioned events was studied by adding a more hydrophobic (RSO 5 EO) or more hydrophilic (RSO 60 EO) adjuvant to the spray solution.

2 Material and methods

2.1 Plant material and growth conditions

Experiments were conducted with 63-days old apple seedlings (*Malus domestica* Borkh.), 14-days old bean seedlings (*Phaseolus vulgaris* L.) and 30-days old kohlrabi plants (*Brassica oleracea gongylodes var.* Delikateß Blauer). Apple seeds and bean seeds were sown in germination trays (loam:sand, 3:1), and later transplanted into individual pots, and kohlrabi was sown directly in individual pots. Plants were raised in a growth chamber under controlled environmental conditions (T = 20 ºC ± 1 ºC; RH = 70 % ± 5 %). Photosynthetic active radiation (PAR) was provided at 180 µmol s^{-1} m^{-2} at the plant level during a 16 h-photoperiod.

2.2 Characterization of leaf micromorphology

2.2.1 Roughness

Leaf roughness was assessed quantitatively by means of contact angle measurements of water/acetone solution droplets (80/20, v/v), as proposed by Forster and Zabkiewicz (2001). Contact angles were measured optically with a Contact Angle Measuring System G10 (Krüss GmbH, Hamburg, Germany); for it 1 µl-single droplets were applied with a microsyringe (Hamilton-Bonaduz, Switzerland) on the upper leaf surface. In order to facilitate the measurements, 10 leaf discs were punched and fixed on a double sided adhesive tape, previously mounted on a glass slice. In each plant species, measurements were made on two visible edges of 50 individual droplets.

2.2.2 SEM micrographs

Micromorphology of the adaxial leaf surfaces was studied by an environmental scanning electron microscope (XL 30 ESEM, FEI-Phillips, Kassel; Microsoft control software, version 5.90). Leaf discs (A = 0.8 cm^2) were punched and mounted on alumina stubs, placed into the SEM and scanned in the low vacuum (0.3 Torr).

2.3 Extraction, sample preparation and determination of epicuticular wax (EW)

The adaxial side of the leaf was placed onto 50 ml chloroform (purity > 99 %) for 20 s in a glass Petri dish. It was ensured that during extraction only the adaxial surface had contact with chloroform. After adding an internal standard (C_{24} alkane, tetracosane; 20 µl apple and bean seedlings; 40 µl kohlrabi) the samples were evaporated under nitrogen flush. By adding 20 µl pyridine (Merck, Darmstadt, Germany) and 20 µl of BSTFA (*N*,*O*-bis (trimethylsilyl) trifluoroacetamide, Macherey-Nagel, Düren, Germany) the samples were derivatized for 40 min. at 70 °C according to the method described by Hauke and Schreiber (1998). After cooling down to room temperature, samples were diluted with 50 µl chloroform before GC-MS analysis (5890 series II, Hewlett-Packard, Avondale, PA, with on-column injection and a high resolution gas chromatography column, Agilent Technologies, 30 m × 0.321 mm DB-1, phase thickness 0.1 µm, Folsom, CA, USA). The temperature program was as follows: start at 50 °C, 2 min. at 50 °C, 40 °C $min.^{-1}$ to 200 °C, 2 min. at 200 °C, 3 °C $min.^{-1}$ to 320 °C, then 30 min. at 320 °C. The carrier gas was hydrogen. The pressure program was: injection at 50 kPa, 5 min. at 50 kPa, 3 kPa $min.^{-1}$ to 150 kPa, 39 min. at 150 kPa. For qualitative GC-MS analysis the same method was used but, instead of hydrogen, helium was used as carrier gas; injection volume was 1 µl. Simultaneously, leaf surfaces were digitised with a scanner for determination of the surface area by image editing software. Wax was analysed on six samples of each plant species.

2.4 Fungicide and adjuvants

Studies on retention and rainfastness were carried out with the contact fungicide mancozeb [(manganese ethylene bis(dithiocarbamate) (polymeric) complex with zinc salt)] as Dithane Ultra WG 80 % (Spiess-Urania Chemicals GmbH - Hamburg, Germany), at a concentration of 2.4 g l^{-1}. Rapeseed oil ethoxylates (RSO, Cognis® AgroSolution, Düsseldorf, Germany) with an average of 5 EO (ethylene oxide units) or 60 EO in the hydrophilic chain were added to the spray solution (1 g l^{-1}).

2.5 Application of spray solutions

Treatment solutions were applied with a laboratory pesticide sprayer (B-PSA-1; Department of Agricultural Engineering, University of Bonn, Germany). The sprayer was equipped with a hollow cone nozzle (80º, Lechler GmbH, Germany), placed 45 cm above the plant level. Application was carried out at a speed of 6 km h^{-1} and pressure of 3 x 10^5 Pa. The volume applied was equivalent to 390 l ha^{-1}. After a drying time of 4 h, simulation of rainfall started.

2.6 Simulation of rainfall

A 5 mm heavy rain (5 mm h^{-1}) with a droplet medium volume diameter of 1075 µm was simulated with the B-LRS-2 rain simulator (Department of Agricultural Engineering, University of Bonn, Germany), as described elsewhere (Ditzer, 2002; Kromer *et al.*, 1996). The plants were returned into the growth chamber 20 min. after rain simulation; leaf surfaces dried overnight before sampling of leaves for fungicide extraction. Not rain-exposed (0 mm rain) plants served as control.

2.7 Sampling procedure and fungicide determination

Only completely developed leaves (petioles excluded) were taken for residue determination. Because of differences in leaf size, each sample for analytical determination was composed of three leaves (apple seedlings and bean seedlings) or one leaf (kohlrabi). The fresh-weight (FW) was determined (Sartorius BP 210S, Sartorius AG, Göttingen, Germany) and samples were enclosed into Teflon recipients. Five millilitres of nitric acid 65 % p.A. (Merck KGaA, Darmstadt, Germany) and 2 ml of hydrogen peroxide 30 % (RdH Laborchemikalien GmbH & Co. KG, Seelze, Germany) were added to samples before acid digestion in a microwave (Büchi MLS 1200, Microwave Laboratory Systems GmbH, Essen, Germany). After digestion, the solution was transferred into volumetric flasks (25 ml), the volume was filled up with distilled and deionized water (Milli-Q Ultrapure Water Purification Systems, Millipore Corporation, Billerica, USA) and then transferred into plastic vessels. Mancozeb content of the samples was analysed by determining concentration of manganese atoms by means of atomic absorption spectrometer (AAS, Perkin-Elmer Analyst 300 spectrometer, Wellesley, USA) equipped with a Multi-Element Lumina Hollow Cathode Lamp. Manganese atoms constitute about 17 % of the mancozeb molecular weight; a.i. concentration in the sample and on leaves was calculated by rule of proportion (van Bruggen *et al.*, 1986; Travis *et al.*, 1985). Samples of untreated leaves served as reference for natural manganese concentration in the plant tissue. A standard series (0.1, 0.5, 1.0, 2.0 and 3.0 mg l^{-1}) prepared from a manganese stock solution (1000 mg l^{-1}; Merck KGaA, Darmstadt, Germany) served for establishing a calibration curve. Leaves from untreated plants were sampled, fortified with mancozeb (100 and 1000 µg g^{-1} FW), and subjected to extraction and analysis procedure. Recovery values ranged between 88 and 110 %.

2.8 Statistical analysis

Experiments on retention and rainfastness were conducted separately for each species, with 10 analytical samples for each combination of spray solution and rain situation (no rain; 5 mm rain), respectively. Results of residues were expressed as µg g^{-1} FW as well as percentage of the initial fungicide concentration (fungicide deposit in not rain-exposed seedlings was referred as 100 percent). Statistical analyses were carried out with the software SPSS 12.0 (SPSS Inc., Chicago, USA). Normal distribution of data concerning mancozeb residues after spray application and after rain simulation was assured. Influence of rain on mancozeb residues (µg g^{-1} FW) on a given plant species and spray solution was evaluated by means of analysis of variance. For the parameters roughness, and mass and composition of EW, means and standard errors were calculated. In addition, a Pearson's correlation analysis among surface roughness, chemical composition of EW, retention and rainfastness of mancozeb was carried out. Graphs were designed with the software Sigma Plot 7.101 (Systat Software GmbH, Erkrath, Germany).

3 Results

3.1 Surface characteristics

3.1.1 Amount and composition of epicuticular wax (EW)

Detailed information on amount and composition of the epicuticular wax recovered from adaxial leaf surface of apple seedlings, bean seedlings and kohlrabi plants is provided in Table 1 and Figure 1.

Table 1. Amount and composition of epicuticular wax (EW) deposited on adaxial leaf surface of apple seedlings, bean seedlings, and kohlrabi plants.

Substance class	Chain length	Amount of EW [ng cm^{-2}]		
		Apple seedlings	Bean seedlings	Kohlrabi
Acids	*C_{26}*	34.60	n.d.	20.40
Alcohols	*C_{24}*	n.d.	n.d.	74.50
	C_{26}	87.30	559.70	1498.50
	C_{28}	47.50	213.20	52.00
	C_{30}	36.40	17.50	16.40
Alkanes	*C_{29}*	10.50	11.50	2177.20
	C_{31}	44.80	44.90	964.00
	C_{33}	2.90	61.30	7.90
Triterpenes	*Oleanolic acid*	50.00	n.d.	n.d.
	Ursolic acid	53.30	4.00	1.10
Esters	*C_{44}*	n.d.	13.40	73.10
	C_{46}	n.d.	n.d.	69.70
	C_{48}	5.20	n.d.	n.d.
	C_{50}	17.00	n.d.	n.d.
	C_{52}	22.90	n.d.	n.d.
Total		**412.40**	**925.60**	**4954.90**

* n.d. = not detected. To carry out the correlation analysis it was considered to be zero.

Individual wax compounds, percentage of chemical groups in the total wax and total amount of EW differed significantly among species. Average of EW on the adaxial leaf surface of apple seedlings was 412.40 ng cm^{-2} distributed in acids (8.4 %), primary alcohols (41.5 %), alkanes (14.1 %), triterpenes (25.0 %) and esters (10.9 %). Bean seedling leaves had 925.60 ng cm^{-2} EW on adaxial surface and consisted of primary alcohols (85.4 %), alkanes (12.7 %), triterpenes (0.004 %) and esters (1.4 %). EW from kohlrabi adaxial leaf surface (4954.90 ng cm^{-2}) was composed of acids (0.4 %), primary alcohols (33.1 %), alkanes (63.5 %), triterpenes (0.03 %), and esters (2.9 %). The main component of the EW fraction of apple seedling and bean seedling leaves was C_{26} alcohol with 87.30 ng cm^{-2} and 559.70 ng cm^{-2}, respectively.

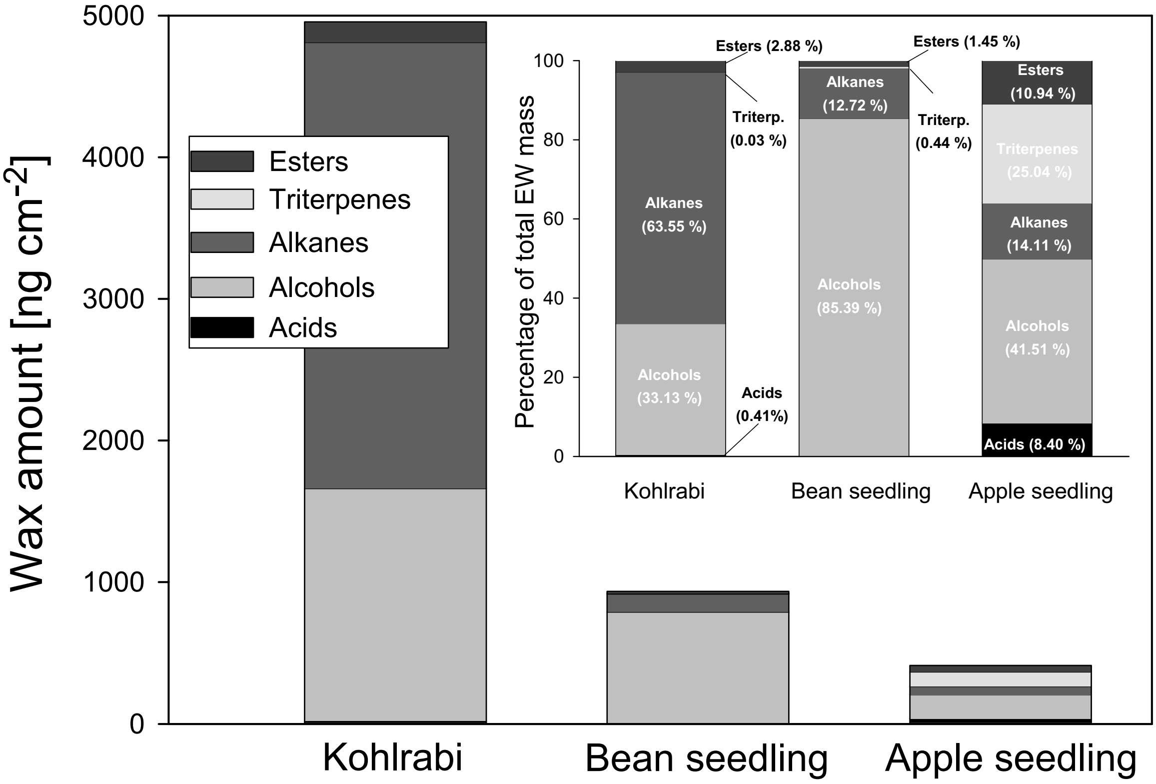

Figure 1. Chemical groups and their fraction (ng cm^{-2} and %) in the epicuticular wax on adaxial side of apple seedling, bean seedling and kohlrabi leaves.

Similarly, on kohlrabi leaves the amount of C_{26} alcohol was relevant (1498.50 ng cm^{-2}), but the major compound was C_{29} alkane (2177.20 ng cm^{-2}). Of the three species, only apple seedlings showed considerable amount of triterpenes (103.30 ng cm^{-2}).

3.1.2 Surface micromorphology

SEM evaluations revealed detailed information on the structure and micromorphology of adaxial leaf surface of the studied species. The adaxial leaf surface of apple seedlings exhibited polygonal cells with many cuticular folds (Figs. 2A and 2B) but was smooth, without any stomata, glands or trichomes. Bean seedling leaves are characterized by having stomata on the upper surface (Figs. 2C and 2D), whereas structured wax deposits were not observed (Fig. 2D). Kohlrabi leaves had rough and complex surfaces with many stomata (Fig. 2E). The surface was covered with wax crystalloids, mainly arranged as tubules (Fig. 2F).

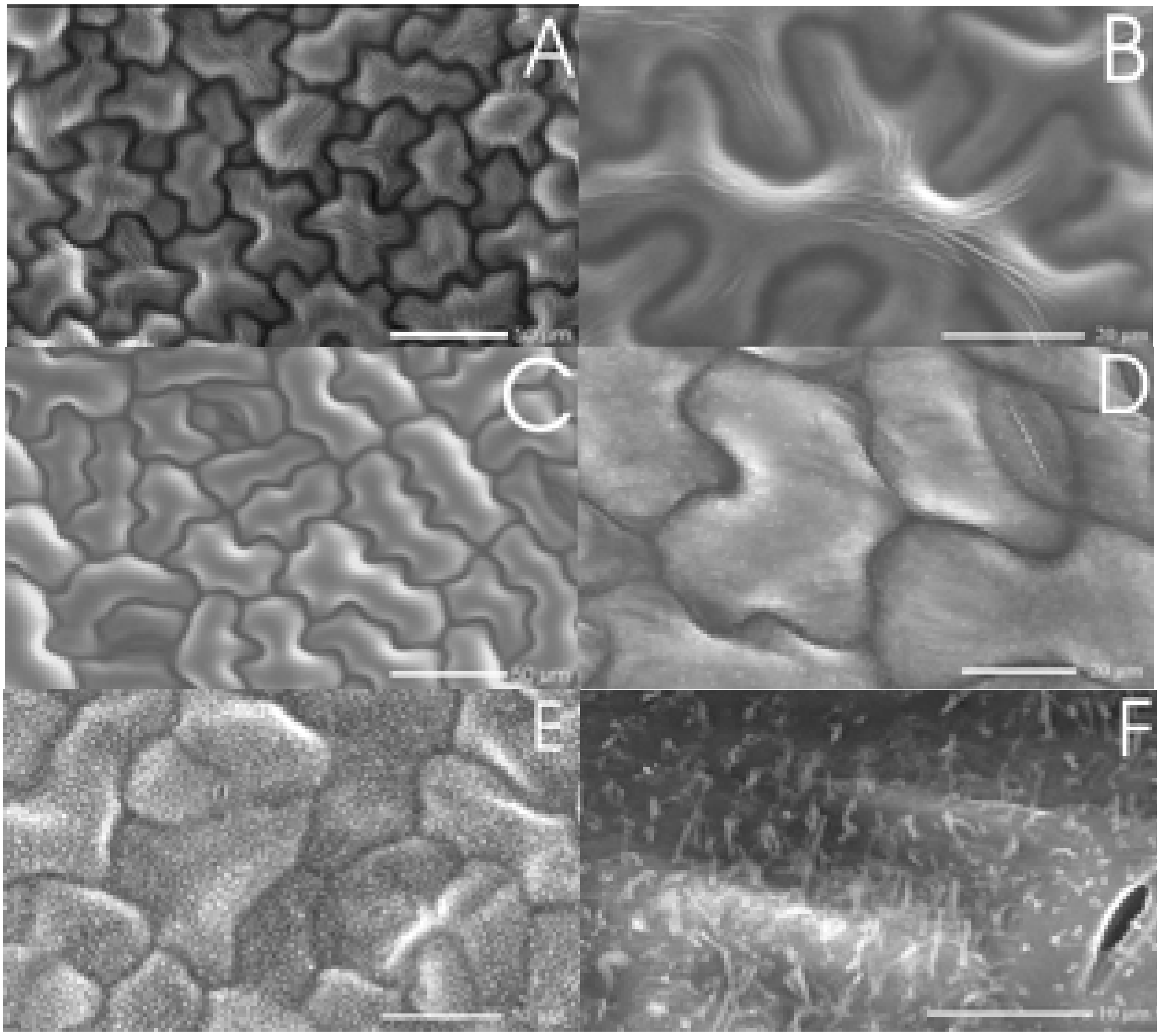

Figure 2. Typical SEM micrographs of the adaxial leaf surface of apple seedlings (**A/B**), bean seedlings (**C/D**), and kohlrabi (**E/F**).

As an estimate for roughness of adaxial leaf surface, the contact angle of water/acetone solution droplets was determined. The measurements showed no significant differences between apple seedlings and bean seedlings, which had a low contact angle (78º and 76º, respectively). In contrast, leaf surface of kohlrabi plants exhibited a distinct micro roughness with a contact angle of 114º (Fig. 3).

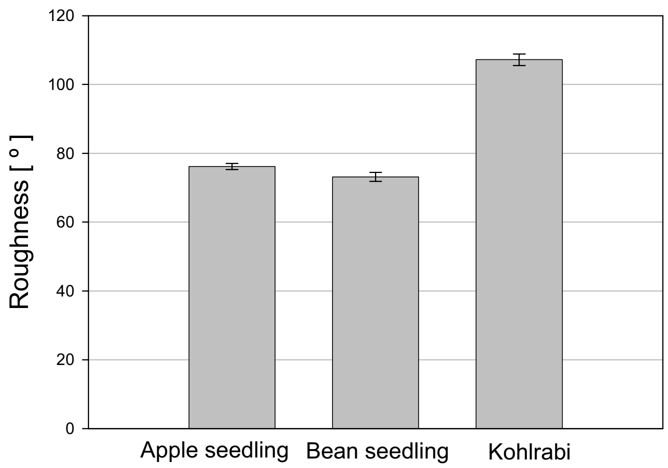

Figure 3. Roughness of adaxial leaf surface of apple seedlings, bean seedlings, and kohlrabi determined by measuring the contact angle of water/acetone (80/20, v/v) solution. Vertical bars represent the standard error.

A correlation analysis (Tab. 2) showed strong correlations between roughness and total amount of EW (r = 0.91), amount of C_{29} alkane (r = 0.94) and the sum of all alkane waxes (r = 0.93). Nevertheless, highly significant correlations were also established for the amount of alcohol compounds (C_{24} and C_{26}), C_{31} alkane and C_{46} esters.

3.2 Fungicide retention and rainfastness

3.2.1 Apple seedlings

Retention and rainfastness of mancozeb on apple seedling leaves were influenced due to addition of RSO adjuvants to the spray solution (Fig. 4).

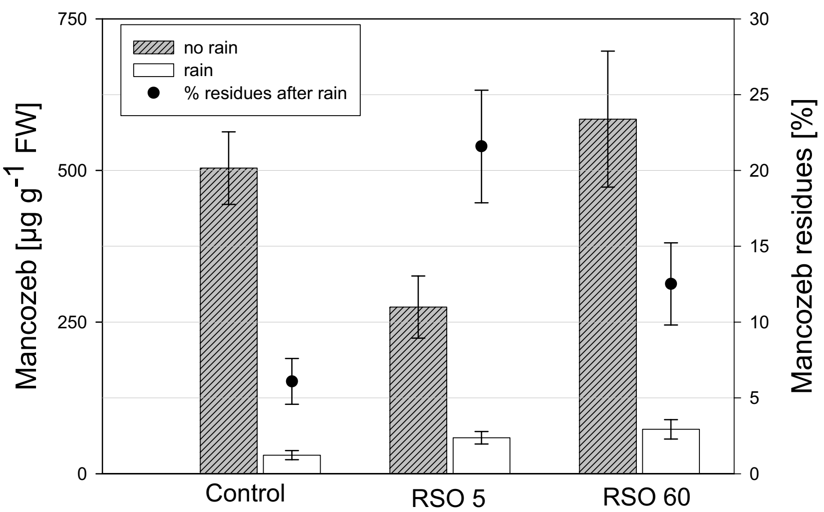

Figure 4. Retention and rainfastness of mancozeb on apple seedlings (*M. domestica*) leaves as influenced by treatment solutions and a 5 mm heavy rain (5 mm h^{-1}). Vertical bars represent the standard error.

The a.i. retention on plants sprayed with the control solution was 500 µg g^{-1}. By adding RSO 5 to the spray solution, the retention was reduced to about 260 µg g^{-1}. After rainfall, plants sprayed with mancozeb plus RSO 5 or plus RSO 60 had higher residue concentration in comparison to control treatment. Percentage of rainfastness of active ingredient varied between 6 % for control plants and 22 % when adding RSO 5 to the spray solution.

3.2.2 Bean seedlings

Addition of RSO 5 to the spray solution significantly reduced mancozeb retention on bean seedling leaves (Fig. 5).

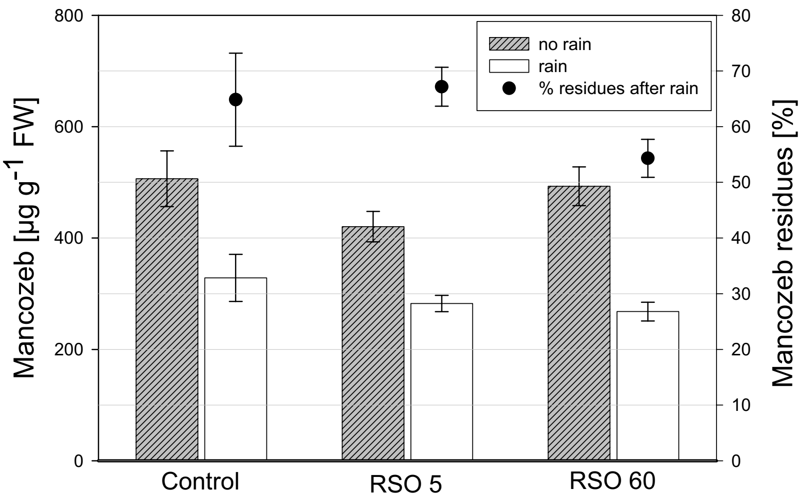

Figure 5. Retention and rainfastness of mancozeb on bean seedling (*P. vulgaris*) leaves as influenced by treatment solutions and a 5 mm heavy rain (5 mm h^{-1}). Vertical bars represent the standard error.

However, the impact was not as high as shown on apple seedlings. Mancozeb had a higher rainfastness on bean seedlings in comparison to apple seedlings, reaching 65 % and 67 %, respectively, in the control and RSO 5 treatments, and 55 % in the RSO 60 treatment.

3.2.3 Kohlrabi

Mancozeb retention on kohlrabi was low but positively influenced when adding the more hydrophobic (RSO 5) or the more hydrophilic (RSO 60) ethoxylates (Fig. 6). Plants sprayed with the control solution had an initial deposit of 40 µg g^{-1}, which was enhanced up to 140 µg g^{-1} after addition of RSO 60 to spray solution. Addition of RSOs reduced the wash-off due to rain impact. Rainfastness of a.i. was 16 % (control plants), 42 % (RSO 5), and 25 % (RSO 60).

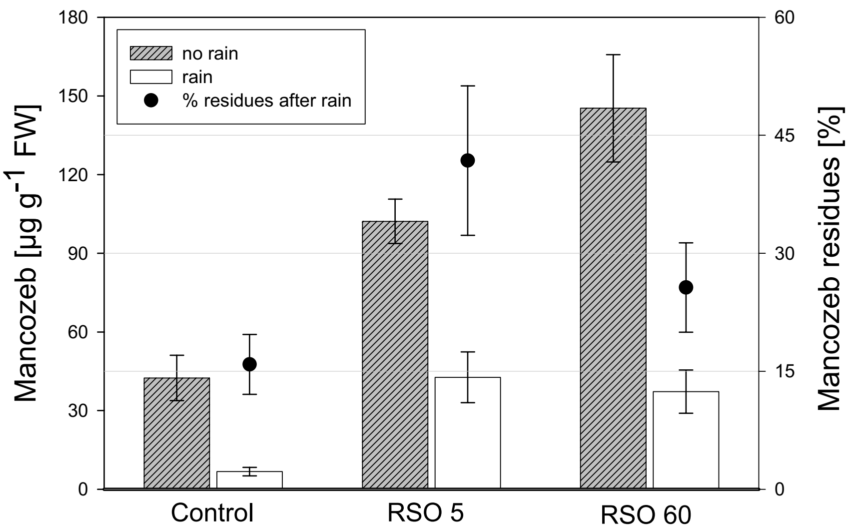

Figure 6. Retention and rainfastness of mancozeb on kohlrabi (*B. oleracea gongylodes*) leaves as influenced by treatment solutions and a 5 mm heavy rain (5 mm h^{-1}). Vertical bars represent the standard error.

3.2.4 Pearson's correlation analysis

Correlation analyses for mancozeb retention on plant surfaces (Tab. 2) and rainfastness (Tab. 3) were carried out irrespective of which solution was sprayed (general value) or separately for each spray solution (control; RSO 5; RSO 60). Pearson's coefficients (r) varied among spray solutions, but as a rule, mancozeb retention was strong and negatively correlated with surface roughness, total epicuticular wax, mass of C_{29} and C_{31} alkane and total mass of alkanes in the EW.

Table 2. Pearson correlation coefficients (r) for surface roughness, amount and composition of epicuticular wax and retention of mancozeb as influenced by treatment solutions. Experiments were carried out on apple seedlings, bean seedlings, and kohlrabi.

Variables	Roughness [°]	Mancozeb retention [µg g^{-1}]			
		Control	RSO 5	RSO 60	General value
Roughness	**-**	**-0.86****	**-0.74 ****	**-0.69****	**-0.73****
EW (total)	**0.91****	**-0.84****	**-0.72****	**-0.71****	**-0.73****
Acids	**0.06**	**-0.24**	**-0.40***	**0.06**	**-0.15**
C_{26}	0.06	-0.24	-0.40*	0.06	-0.15
Alcohols	**0.81****	**-0.75****	**-0.51****	**-0.68****	**-0.64****
C_{24}	0.87**	-0.84**	-0.75**	-0.69**	-0.73**
C_{26}	0.85**	-0.79**	-0.58**	-0.69**	-0.67**
C_{28}	-0.45*	0.42*	0.68**	0.22	0.39*
C_{30}	-0.37*	0.21	0.01	0.25	0.17
Alkanes	**0.93****	**-0.85****	**-0.75****	**-0.71****	**-0.74****
C_{29}	0.94**	-0.86**	-0.77**	-0.72**	-0.75**
C_{31}	0.88**	-0.81**	-0.73**	-0.66**	-0.70**
C_{33}	-0.44*	0.45*	0.68**	0.25	0.41*
Triterpenes	**-0.41***	**0.37**	**0.01**	**0.55****	**0.33***
Oleanolic acid	-0.36*	0.35	-0.04	0.63**	0.34*
Ursolic acid	-0.43*	0.37	0.05	0.44*	0.30*
Esters	**0.82****	**-0.71****	**-0.70****	**-0.53****	**-0.61****
C_{44}	0.81**	-0.70**	-0.60**	-0.58**	-0.60**
C_{46}	0.87**	-0.80**	-0.71**	-0.64**	-0.69**
C_{48}	-0.33*	0.36	0.07	0.34	0.27*
C_{50}	-0.37*	0.30	0.09	0.43*	0.29*
C_{52}	-0.38*	0.29	0.03	0.44*	0.27*

* Significance level $p \leq 0.05$

** Significance level $p \leq 0.01$

In a similar way, correlation coefficients for rainfastness varied between treatment solutions and, in addition, they varied also, when relating rainfastness of the fungicide to absolute or relative values (µg g^{-1} or percentage). Generally, rainfastness was strongly correlated with the amount of C_{28} alcohol and C_{33} alkane in the EW (Tab. 3). Surface roughness, total EW, and fungicide retention (= initial fungicide deposit) correlated strongly with rainfastness when it was expressed in µg g^{-1} FW; however, only moderate or weak correlations were established when expressing rainfastness as a.i. in percentage of the initial deposit.

Table 3. Pearson's correlation coefficients (r) for amount and composition of epicuticular wax and rainfastness (µg g^{-1} and %) of mancozeb as influenced by treatment solutions. Experiments were carried out on apple seedlings, bean seedlings, and kohlrabi.

Variables	Mancozeb rainfastness							
	Control		RSO 5		RSO 60		General value	
	[µg g^{-1}]	[%]	[µg g^{-1}]	[%]	[µg g^{-1}]	[%]	[µg g^{-1}]	[%]
Retention	**0.44***	**0.28**	**0.77****	**0.41***	**0.44***	**0.15**	**0.48***	**0.20**
Roughness	**-0.57****	**-0.40***	**-0.62****	**-0.13**	**-0.63****	**-0.29**	**-0.59****	**-0.27**
EW (Total)	**-0.45***	**-0.27**	**-0.49***	**-0.03**	**-0.55****	**-0.191**	**-0.48***	**-0.15**
Acids	**-0.55****	**-0.57****	**-0.60****	**-0.48***	**-0.53****	**-0.60****	**-0.55****	**-0.52****
C_{26}	-0.55**	-0.57**	-0.60**	-0.48*	-0.53**	-0.60**	-0.55**	-0.52**
Alcohols	**-0.17**	**-0.01**	**-0.17**	**0.31**	**-0.27**	**0.08**	**-0.19**	**0.11**
C_{24}	-0.53**	-0.37	-0.55**	-0.01	-0.61**	-0.27	-0.55**	-0.21
C_{26}	-0.27	-0.11	-0.26	0.25	-0.36	0.00	-0.28*	0.03
C_{28}	0.86**	0.82**	0.85**	0.60**	0.84**	0.71**	0.84**	0.69**
C_{30}	-0.25	-0.32	-0.34	-0.62**	-0.23	-0.40*	-0.27*	-0.42*
Alkanes	**-0.52****	**-0.35**	**-0.55****	**-0.04**	**-0.61****	**-0.25**	**-0.54****	**-0.21**
C_{29}	-0.54**	-0.37	-0.58**	-0.08	-0.63**	-0.26	-0.56**	-0.23*
C_{31}	-0.50*	-0.36	-0.51**	0.02	-0.59**	-0.25	-0.52**	-0.19
C_{33}	0.88**	0.85**	0.93**	0.67**	0.89**	0.79**	0.88**	0.74**
Triterpenes	**-0.34**	**-0.46***	**-0.35**	**-0.51****	**-0.29**	**-0.54****	**-0.32***	**-0.48***
Olean. acid	-0.33	-0.45*	-0.35	-0.49*	-0.28	-0.51**	-0.32*	-0.46*
Ursolic acid	-0.32	-0.44*	-0.33	-0.50*	-0.28	-0.53**	-0.30*	-0.47*
Esters	**-0.61****	**-0.53****	**-0.57****	**0.03**	**-0.67****	**-0.40***	**-0.60****	**-0.30***
C_{44}	-0.38	-0.27	-0.31	0.28	-0.44*	-0.14	-0.37*	-0.05
C_{46}	-0.50*	-0.37	-0.50*	0.08	-0.58**	-0.26	-0.51**	-0.18
C_{48}	-0.36	-0.49*	-0.40*	-0.57**	-0.34	-0.58**	-0.36*	-0.52**
C_{50}	-0.33	-0.45*	-0.35	-0.49*	-0.29	-0.52**	-0.32*	-0.46*
C_{52}	-0.34	-0.46*	-0.36	-0.51**	-0.30	-0.55**	-0.33*	-0.48*

* Significance level $p \leq 0.05$

** Significance level $p \leq 0.01$

4 Discussion

4.1 Amount and composition of EW

The species used in our studies showed significant differences concerning amount and composition of EW of the adaxial leaf surfaces (Tab. 1). Nevertheless, similarities in micro roughness on adaxial leaf surfaces of apple and bean seedlings were observed (Fig. 3).

Usually, plant waxes consist of a mixture of long-chain hydrocarbons, alcohols, ketones, esters and acids (Baker, 1982; Fernandes *et al.,* 1964). The wax amount, composition, and homologue distribution patterns vary considerably between and within plant species and cultivars (Belding *et al.*, 1998; Percy *et al.*, 1994; Baker, 1982). In our studies, average of EW on adaxial leaf surface was 412.40 ng cm^{-2} on apple seedlings, 925.60 ng cm^{-2} on bean seedlings, and 4954.90 ng cm^{-2} on kohlrabi. EW of apple seedlings was composed mainly of primary alcohols (41.5 %) and triterpenes (25.0 %), while primary alcohols (85.4 %) and alkanes (63.5 %) were the dominant chemical groups in EW of bean seedling and kohlrabi leaves, respectively (Fig. 1). Our results concerning mass and composition of EW on the adaxial surface were in accordance with results published by other authors.

Bringe *et al.* (2005) showed that young leaves of apple seedlings, raised under controlled conditions, present small quantity of EW, mainly composed of alcohols, acids and triterpenes. Working with bean leaves, it was shown that EW of the adaxial surface ranged between 1.0 and 1.9 µg cm^{-2}, as a thin amorphous wax layer (Percy and Baker, 1987; Hunt and Baker, 1982). EW of bean leaves is composed predominantly of primary alcohols (Percy and Baker, 1987; Steinmüller and Trevini, 1985). It should be noted that in our studies kohlrabi leaves showed many times lower EW than the values observed by other authors (Schwab *et al.*, 1995; Schwab, 1993; Flore and Bukovac, 1978; Flore and Bukovac, 1976; Flore and Bukovac, 1974); however, similar EW amount was measured by Percy and Baker (1987) and Baker and Hunt (1986). Our observation that alkanes constitute more than 60 % of kohlrabi EW confirms results previously reported by Percy and Baker (1987).

4.2 Surface micromorphology

All evaluated species were free of hairs and trichomes, so that differences in roughness (Fig. 3) may be due to cuticular lamellae, stomata or epicuticular wax morphology (Fig. 2) as well as amount and chemical composition of cuticular waxes (Fig. 1 and Tab. 1).

Roughness is the main factor which governs wettability of leaf surfaces (Juniper and Jeffree, 1983). It results from irregular underlying venation, shape and size of the underlying epidermal cells, hairs, trichomes, and wax deposits on cuticle surfaces (Holloway, 1970). However, both roughness and surface wettability depend on superficial wax-fine structure (Martin and Juniper, 1970; Furmidge, 1962), properties of the chemical groups (Holloway, 1970) and nature of the exposed chemical groups on the surface (Juniper and Jeffree, 1983). Plant waxes are primarily non-polar, but their hydrophobicity varies among species due to differences in chemical composition (Chachalis *et al.*, 2001).

In our studies, roughness correlated very strongly with total EW ($r = 0.91$), amount of C_{29} alkane ($r = 0.94$) and total of alkanes ($r = 0.93$). These results confirm earlier findings which have shown that amount of wax on the adaxial leaf surface is positively correlated with leaf surface hydrophobicity (Beattie and Marcell, 2002; Chachalis *et al.*, 2001). Working with tobacco leaves, Barnes *et al.* (1996) observed that the contact angle of water droplets was positively correlated with the n-alkane composition ($r = 0.563$) and negatively correlated with the br-alkane composition ($r = -0.514$). It is known, that micromorphology of wax structures depends on chemical composition of wax compounds (Hallam, 1982). In addition, occurrence of wax structures on leaves is strongly related to alkane components in the EW wax layer (Barthlott *et al.*, 1998; Baker, 1982).

We observed that leaf roughness was also significantly correlated with the total amount of wax alcohols, C_{24} alcohol, and C_{26} alcohol in the EW layer (Tab. 2). Alcohols and acids have carbonyl and carboxyl groups in the molecule and are therefore less hydrophobic than hydrocarbon waxes (Chachalis *et al.*, 2001). A ranking of the hydrophobicity of waxes indicates that alkenes are the most hydrophobic; esters, ketones and secondary alcohols are intermediate and primary alcohols, hydroxyl-fatty acids, diols, triterpenoids and sterols are the least hydrophobic compounds (Martin and Juniper, 1970). Nevertheless, no class of wax constituent is outstandingly water repellent (Holloway, 1970).

Furthermore, it must be considered that not only the chemical composition influences roughness and wettability, but also arrangement of EW layers plays a decisive role. The outermost layer of the cuticle is the first surface that an agrochemical interacts with (Perkins *et al.*, 2005), so that orientation of molecules on wax surface as well as variations in the type and number of chemical groupings exposed on surface affects the wettability (Holloway, 1970).

4.3 Fungicide retention and rainfastness

Retention of mancozeb on leaf surfaces differed among species; moreover it was significantly influenced by addition of RSO ethoxylates (Figs. 4 - 6). Incorporation of RSO 5 into spray solution reduced a.i. retention on apple and bean seedling leaves, but increased the initial deposit on kohlrabi leaves. However, addition of RSO 60 to spray solutions increased fungicide retention on kohlrabi leaves, but did not alter deposition on the two other plant species. Differences in correlation coefficients were obtained when statistical analysis was carried out for individual spray solutions (control; RSO 5; RSO 60) or after combining results of all solutions in a general value (in this case, simulating a real situation where hydrophilicity of the spray solution is unknown). Nevertheless, very strong inverse correlations were observed between retention and roughness, as well as retention and total EW, amount of C_{29} alkane and total mass of alkanes (Tab. 2). It is known that roughness increases contact angle of solution droplets (Barthlott and Neinhuis, 1997) influencing rebound of droplets, spray run-off, and contact area between deposit and leaf surface (Green, 2001). Our results also confirm that differences in retention may reflect changes in the chemistry of epicuticular waxes, especially the increased content of long chain esters and alkanes (Bukovac *et al.*, 1979). Hall *et al.* (1997) observed that retention of several pesticides on apple leaves decreased drastically within a growing season, which he interpreted as a possible result of accumulation of hydrophobic waxes.

Rainfastness of mancozeb solutions differed among plant species. Mancozeb applied without tank-mix adjuvants (control) had low rainfastness on kohlrabi and apple seedling leaves, contrasting the results observed on bean leaves. Both on apple seedlings and on kohlrabi, addition of RSO 5 and RSO 60 reduced a.i. wash-off from surfaces due to rain, while on bean seedlings no significant alteration occurred. Percentage of fungicide deposit after rainfall indicates that both RSOs enhance mancozeb rainfastness in apple seedling and kohlrabi leaves, whereas even better results were achieved with the more hydrophobic RSO 5. Studying bean seedling leaves, addition of RSO 5 had no impact, while addition of RSO 60 reduced rainfastness. These results confirm previous observations that properties of leaf surface play an important role in determining if adjuvants can improve rainfastness of pesticide deposits (Steurbaut, 1993). Our results show a positive correlation between rainfastness and amount of C_{28} alcohol and C_{33} alkane in the EW (Tab. 3). A strong correlation between retention and rainfastness was only observed when adding RSO 5 to the spray solution. It is interesting to note that Pearson's correlation coefficients for rainfastness *vs.* roughness or total amount of epicuticular wax are moderate, when residues are expressed as $\mu g\ g^{-1}$, and weak or very weak, when rainfastness is expressed as percentage of initial deposit.

Summarising, adaxial leaf surface of apple seedlings, bean seedlings and kohlrabi differed greatly in roughness, and amount and composition of epicuticular waxes, which markedly influence retention and rainfastness of the foliar-applied contact fungicide mancozeb. Surface roughness was strongly correlated with total EW and amount of C_{29} alkane in the wax mass, proving that chemical composition of surface waxes influences decisively the physical properties of the upper leaf surface. Mancozeb retention correlated negatively with surface roughness, total epicuticular wax, amount of C_{29} alkane, and total mass of alkanes. Rainfastness of mancozeb was positively correlated with the amounts of C_{28} alcohol and C_{33} alkane in the EW. Correlation coefficients differed between spray solutions, indicating that spray solutions should be adjusted to the leaf surface properties in order to achieve an optimal and rainfast deposit.

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E Influence of linseed oil ethoxylate adjuvants on rainfastness and biological efficacy of glyphosate, evaluated in *Chenopodium album*, *Abutilon theophrasti,* and *Setaria viridis*

1 Introduction

Glyphosate is the largest-selling herbicide in the world with total estimated sales of about $ 3 billion (Knowles, 2001). In the last years glyphosate was subject of several trials with the aim to reduce drift losses and optimize deposit formation (Scherhag *et al.*, 2005; Leung and Webster, 1994), enhance uptake and translocation (Müller *et al.*, 2001; Sharma and Singh, 2000; Bariuan *et al.*, 1999; Feng *et al.*, 1998; Laerke and Streibig, 1995; Zabkiewicz *et al.*, 1993; Gaskin and Holloway, 1992; Cranmer and Linscott, 1991), that ultimately improve its biological efficacy (Haefs, 2001; Kogan, 2001; Sandbrink *et al.*, 1993; Reddy and Singh, 1992). Since glyphosate is highly soluble in water and therefore prone to dilution and removal from plant foliage by rainfall (Leung, 1994; Reddy and Singh, 1992), studies were carried out to characterize and/or enhance its rainfastness on glass slices (Leung, 1994), hard surfaces (Spanoghe *et al.*, 2005) and several weed species (Scherhag, 2005; Monquero *et al.*, 2004; Martini *et al.*, 2003; Werlang *et al.*, 2003; Kogan, 2001; Combellack *et al.*, 2001; Coble and Brumbaugh, 1993; Sundaram, 1991; Clay and Lawrie, 1990; Wells, 1989; Bryson, 1987).

Adjuvants are the best tools for users to improve efficacy of agrochemical application and in this way achieve more cost-effective, better-targeted, and more environmentally acceptable pest control (Green, 2001; Green, 2000). In addition, the a.i. rainfastness can be enhanced by adjuvants (i.e. sticker-adjuvants), which form a protective water-repellent layer, preventing or reducing wash-off rate (Hazen, 2000; Roggenbuck *et al.*, 1993). When added to systemic active ingredients, adjuvants can enhance the initial penetration rate, thus limiting the wash-off potential of a rainfall (Roggenbuck *et al.*, 1993; Field and Bishop, 1988). Furthermore, penetration and rainfastness of a given a.i. depends also on other factors such as species, physicochemical characteristics of the leaf surface (Leung and Webster, 1994; Reddy and Singh, 1992), and adjuvant properties (Kogan, 2001).

In the last years, several environmental and consumer friendly adjuvants have been developed with the aim to replace non-environmental friendly adjuvants such as alkyl-phenol-ethoxylates (Haefs, 2001; Abribat, 2001; Green, 2000). In this context, oil ethoxylates gained from rapeseed were developed (Abribat, 2001); their effectiveness in enhancing biological efficacy and rainfastness of glyphosate has been proved (Scherhag, 2005; Haefs, 2001). Other ethoxylates based on seed oils such as linseed and soybean were developed and evaluated for their efficacy in enhancing rainfastness of contact fungicides (Hunsche *et al.*, 2005; Ditzer, 2002), but were not evaluated with systemic active ingredients, yet. Glyphosate was chosen for our experiments because its biological efficacy greatly depends on the adjuvant type (Green, 2000). In addition, an adjuvant system that would maintain the efficacy of glyphosate, avoiding the tallow amine ethoxylate surfactants and their associated plant, eye and fish toxicity would immediately be successful (Green, 2001).

The objective of our study was to investigate the effect of four linseed oil ethoxylates on rainfastness and biological efficacy of glyphosate, evaluated on lambsquarter (*C. album*), velvetleaf (*A. theophrasti*), and green foxtail (*S. viridis*).

2 Material and methods

2.1 Plant material and growth conditions

Experiments were conducted with the dicotyledonous weeds lambsquarter (*Chenopodium album* L.) and velvetleaf (*Abutilon theophrasti* Medik.), and the monocotyledonous weed green foxtail (*Setaria viridis* L.). Weeds were raised from seed in individual pots placed in a greenhouse, with a 12 h-photoperiod, a daily temperature of 20 ºC ± 4 ºC and relative humidity of 55 % ± 10 %.

2.2 Characterization of adaxial leaf surface

2.2.1 Micro roughness and contact angle of treatment solution droplets

Leaf roughness was characterized quantitatively by measuring the contact angle of water/acetone solution droplets (80/20, v/v), as proposed by Forster and Zabkiewicz (2001). Contact angles were measured optically with a Contact Angle Measuring System (G10; Krüss GmbH, Hamburg, Germany) by applying single 1 µl-droplets with a microsyringe (Hamilton-Bonaduz, Switzerland) on the upper physiological leaf surface. In order to facilitate the measurements, leaf discs were punched and fixed on a double sided adhesive tape (Tesa® double face, Beiersdorf, Hamburg), previously mounted on a glass slice. Tangents were set at both visible sides of a droplet, and readings of the angles were taken on 50 droplets of each plant species.

Determination of leaf surface wettability was carried out adopting the same measuring procedure; however in this case individual droplets (1 µl) of the spray solutions were applied.

2.2.2 Scanning electron microscopy (SEM)

Micromorphology of adaxial leaf surface was investigated by an environmental scanning electron microscope (XL 30 ESEM, FEI-Phillips, Kassel; Microsoft control software, version 5.90). Leaf discs ($A = 0.8\ cm^2$) were punched and mounted on alumina stubs, placed into the microscope analysis chamber, and scanned in the low vacuum mode (0.3 Torr). Wax crystalloids on the leaf surface were classified according to Barthlott *et al.* (1998) and Baker (1982).

2.3 Chemicals

Glyphosate (Gly) solutions were prepared at a concentration of 43 mmol l^{-1} with isopropylamine salt 62 % (Monsanto Europe S.A., Antwerp, Belgium). Linseed oil ethoxylates (LSO, Cognis® AgroSolution, Düsseldorf, Germany) with an average of 10 and 30 ethylene oxide (EO) units as well as LSO with ethylene oxide and propylene oxide (PO) blocs [(09/03, EO/PO) and (30/03, EO/PO)] in the hydrophilic chain were added to the spray solution at 1 g l^{-1}. Plants treated with the commercial glyphosate formulation Roundup® Ultra Max (RUM®, Monsanto Agrar Deutschland GmbH, Düsseldorf, Germany) as well as untreated plants served as control.

2.4 Application of treatment solutions

Treatment solutions were applied with a laboratory pesticide sprayer (B-PSA-1; Institute of Agricultural Engineering, University of Bonn, Germany), equipped with an air-induction nozzle (AI 11004 VS, Teejet Co., Germany). Treatment solutions were applied at a speed of 6 km h^{-1} and a pressure of 3 x 10^5 Pa, equivalent to an application rate of 190 l ha^{-1}. Five minutes after pesticide application plants were returned into the greenhouse for 2 h before onset of rainfall simulation.

2.5 Rainfall simulation

Tap water was used to simulate a natural rainfall by using a laboratory rain simulator (B-LRS-2; Institute of Agricultural Engineering, University of Bonn, Germany), as described elsewhere (Ditzer, 2002; Kromer *et al.*, 1996). Five millimeters of rain at three intensities were simulated: light rain (0.5 mm h^{-1}), heavy rain (5 mm h^{-1}) and torrential rain (48 mm h^{-1}), with droplets medium volume diameter (MVD) of 377 µm, 1075 µm and 2043 µm, respectively. The applied rain intensity and quantity were programmed in the rain simulator and checked with a rain gauge. Plants were returned to the greenhouse 20 min. after rain simulation. Not rain-exposed plants served as reference.

2.6 Evaluation of biological efficacy

Dry mass was used as parameter for evaluation of biological efficacy of treatment solutions and influence of rainfall on weed control. Plants (shoots and leaves) were harvested at the soil level 8 days after herbicide application, then allocated in paper-bags, and dried up (T = 105 ºC) until constant weight.

2.7 Experimental design and statistical analysis

Results were analyzed with the software SPSS 12.0 (SPSS Inc., Chicago, USA) and graphs designed with the software Sigma Plot 7.101 (Systat Software GmbH, Erkrath, Germany). Experiments on rainfastness and biological efficacy were conducted in a bi-factorial arrangement (treatment solutions *vs.* precipitation) for each weed species, with 12 experimental units each per treatment. After ascertaining normal distribution, data was subjected to analysis of variance (ANOVA). In the cases of statistical significances, results were compared by Duncan-Test $p \leq 0.05$.

3 Results

3.1 Micro roughness

Adaxial leaf surfaces of the evaluated weed species showed significant differences in micro roughness, and consequently in wettability.

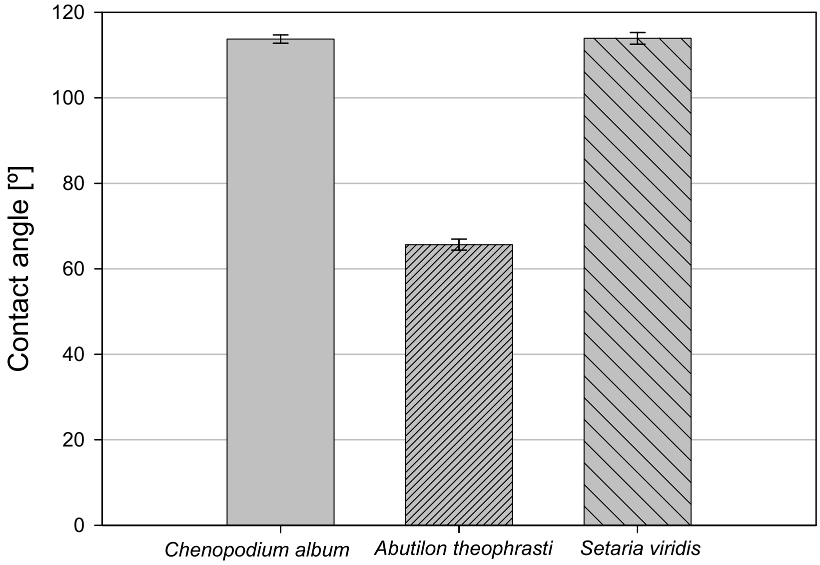


Figure 1. Micro roughness of adaxial leaf surface of *C. album, A. theophrasti* and *S. viridis*, as characterised by measuring the contact angle of water/acetone (80/20, v/v) solution droplets (1 μl). Vertical bars represent the standard error.

These differences were more evident when comparing dicotyledonous weeds: contact angle on *C. album* leaves was greater than 118º, and in contrast, *A. theophrasti* had a contact angle of only 65º (Fig. 1). The monocotyledonous *S. viridis* had a rougher surface and correspondingly poor wettability, with a contact angle of 118º.

3.2 Contact angle of treatment solutions

Addition of LSO-ethoxylates to the spray solution significantly influenced wettability of the upper leaf surface of all weed species (Tab. 1). On *C. album*, contact angle of glyphosate (92º) was reduced due to addition of the more hydrophobic adjuvants LSO 10 (81º) and LSO 0903 (73º). In contrast, glyphosate formulated with the more hydrophilic adjuvants LSO 30 (106°) and LSO 3003 (108°) had a contact angle comparable to water (109º) or RUM® (108º). In case of *A. theophrasti*, lowest contact angles were obtained when formulating glyphosate with LSO 0903 (53º) and LSO 3003 (51º). Water droplets had a contact angle of 78º, while droplets of RUM® had a contact angle of 57º. The adjuvants LSO 10 and LSO 30 reduced the contact angle of glyphosate solution at a lower extent.

Table 1. Wettability of adaxial leaf surfaces of *C. album, A. theophrasti*, and *S. viridis* as influenced by aqueous glyphosate treatment solutions. Water was used as reference.

Spray solution	Contact angle [°; Mean ± SE]*		
	Chenopodium album	*Abutilon theophrasti*	*Setaria viridis*
Water	109.2 ± 1.7 a	78.6 ± 1.5 a	118.3 ± 2.0 a
RUM®	108.1 ± 1.9 a	57.1 ± 2.7 c	105.7 ± 1.7 b
Glyphosate	92.5 ± 2.2 b	67.9 ± 4.9 b	100.9 ± 4.6 bc
glyphosate + LSO 10	81.4 ± 0.9 c	58.9 ± 2.6 c	95.0 ± 3.1 c
glyphosate + LSO 0903	73.1 ± 2.3 d	53.1 ± 0.9 d	80.2 ± 4.1 d
glyphosate + LSO 30	106.0 ± 3.9 a	57.2 ± 2.5 c	103.8 ± 3.9 b
glyphosate + LSO 3003	108.3 ± 4.1 a	51.2 ± 1.3 d	108.0 ± 2.3 b

* Means in the column followed by the same letter are not different by Duncan $p \leq 0.05$.

On *S. viridis* leaves, the more hydrophobic adjuvants LSO 10 and LSO 0903 reduced the contact angle of glyphosate (100°) to 95° and 80°, respectively. Water droplets and RUM® droplets gave a greater contact angle, 118° and 105°, respectively. Addition of the more hydrophilic adjuvants LSO 30 and LSO 3003 to the spray solution did not increase leaf surface wettability of unformulated glyphosate.

3.3 SEM investigations

Epidermal layer of the adaxial leaf surface of *C. album* presented little polygonal cells and numerous glands varying in size and forming a three-dimensional structure (Fig. 2A). Cell surfaces were completely covered with little, almost imperceptible wax structures (Fig. 2B), which became apparent as vertical platelets at higher magnification (Fig. 2C). In general, 4-5 wax platelets were locally parallel arranged (Fig. 2D).

Characteristic for *A. theophrasti* leaf surface were little polygonal cells with many glandular trichomes on the surface (Fig. 3A). These soft trichomes were simple or complex, some of them with globular appendices on the top (Fig. 3B). Cell surfaces had no detectable wax structures (Fig. 3C), but there was an indication for a thin amorphous wax layer covering the epidermal cell layer (Fig. 3D).

S. viridis exhibited longitudinal cells arranged in parallel, with many little stomata in the cell lines along the veins; cell lines over the veins had trichomes resembling thorns (Fig. 4A). Cell surface presented a densely arranged wax structure (Fig. 4B), e.g. little vertical platelets (Fig. 4C) organised as rosettes (Fig. 4D).

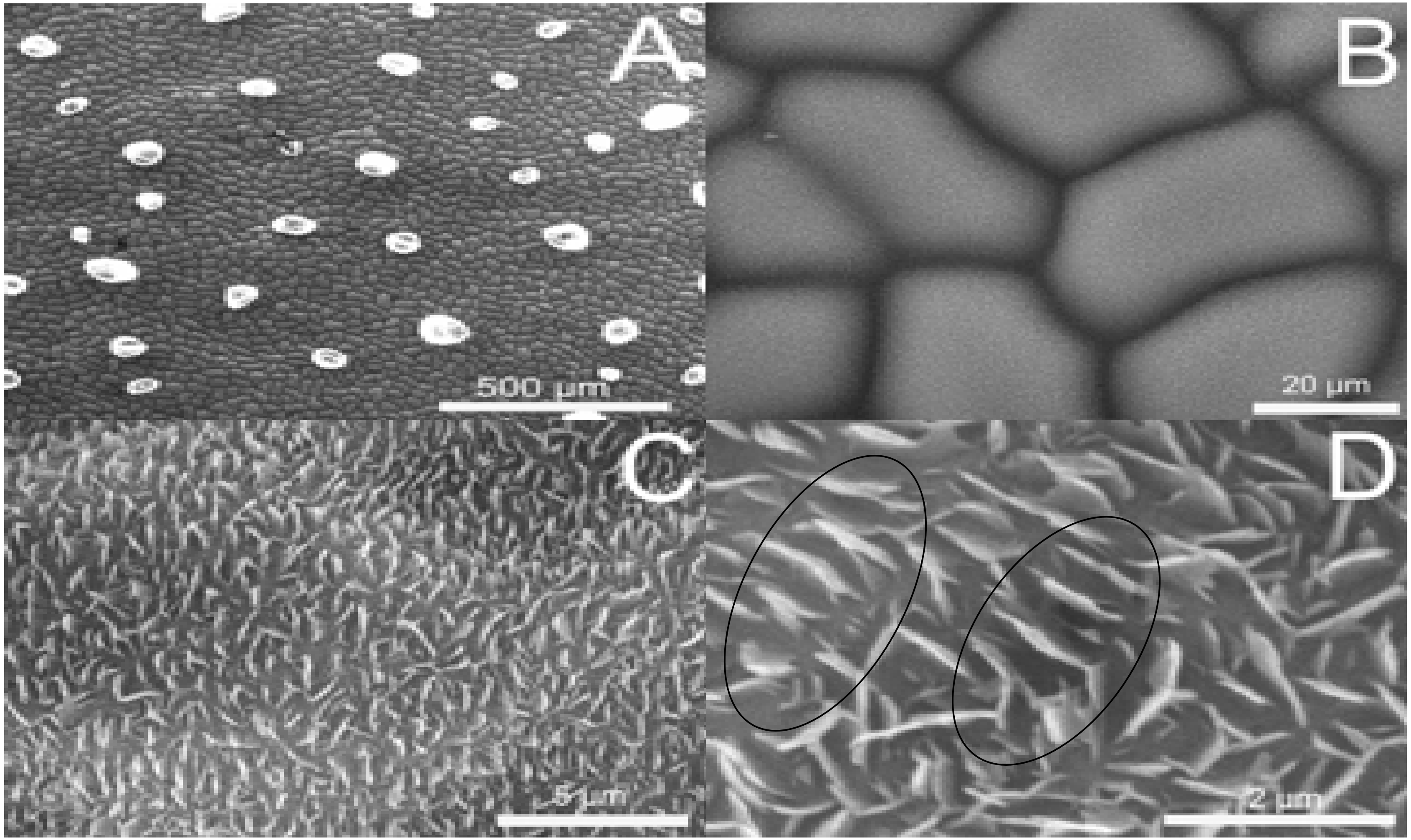


Figure 2. Adaxial leaf surface of *C. album*: (A) – little polygonal cells and numerous glands varying in size and forming a three-dimensional structure; (B) – leaf surface completely covered with little, almost imperceptible wax structures; (C) wax crystalloids organised as vertical platelets become apparent at higher magnification; (D) – organization pattern of the wax crystalloids: 4-5 locally parallel grouped platelets (encircled).

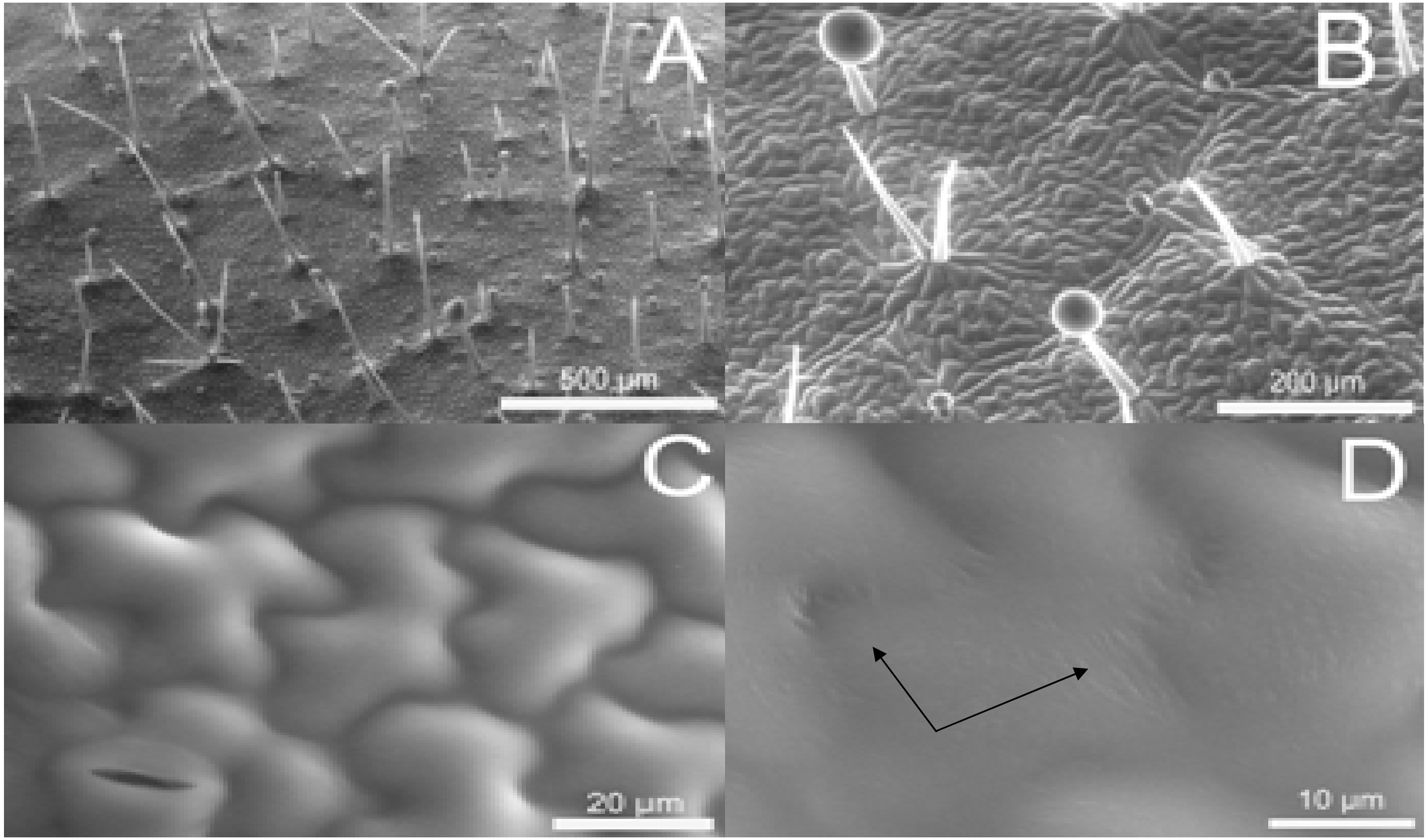


Figure 3. Adaxial leaf surface of *A. theophrasti*: (A) little polygonal cells with numerous glandular trichomes on the surface; (B) simple and complex soft trichomes, some of them with globular appendices on the top; (C) absence of detectable wax structures, but presence of stomata; (D) indications for an amorphous wax film covering the cell surface.

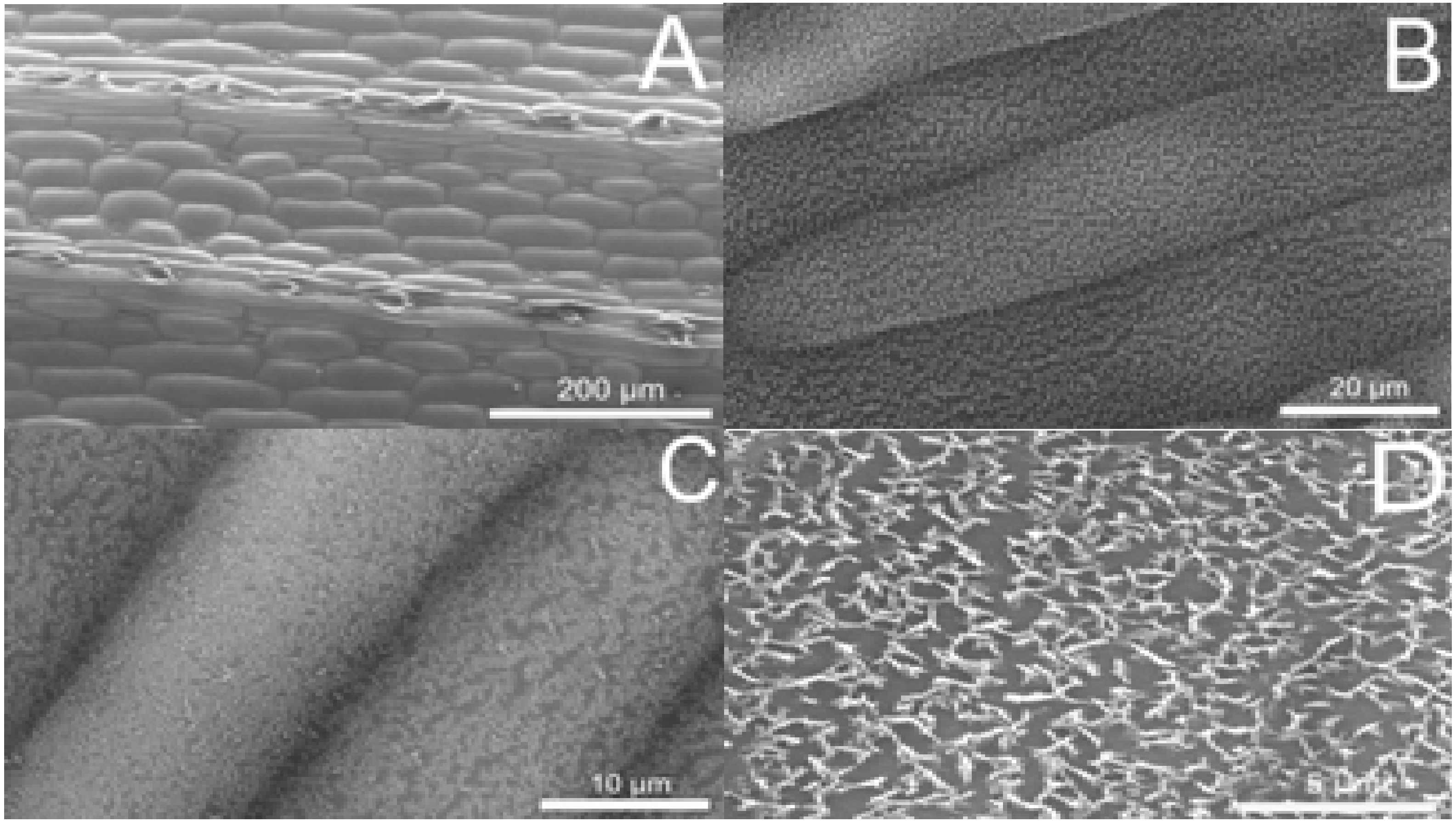


Figure 4. Adaxial leaf surface of *S. viridis*: (A) longitudinal cells arranged in parallel, with many little stomata in the cell lines along the veins; cell lines over the veins had trichomes resembling thorns; (B) cell surface presented a densely arranged wax structure; (C) wax structure as little platelets; (D) platelets organised as rosettes.

3.4 Influence of treatment solutions and rain intensity on biological efficacy

Statistical evaluations showed no significant interactions between treatment solutions and precipitations over all evaluated weed species (Tab. 2); hence only data for the main effects (precipitations and treatment solutions) are presented.

Table 2. Dry matter of *C. album, A. theophrasti,* and *S. viridis* as influenced by treatment solutions and precipitations (factorial analysis).

Weed species	Source of variation	Significance level (Dry matter)
Chenopodium album L.	Precipitation	0.000
	Treatment solutions	0.000
	Precipitation *vs.* Treatment solutions	0.217
Abutilon theophrasti Medik.	Precipitation	0.001
	Treatment solutions	0.000
	Precipitation *vs.* Treatment solutions	0.361
Setaria viridis L.	Precipitation	0.000
	Treatment solutions	0.002
	Precipitation *vs.* Treatment solutions	0.229

In *C. album*, simulation of a torrential rain 2 h after application of treatment solutions significantly reduced the biological efficacy of glyphosate (Fig. 5A). Heavy rain slightly reduced while light rain slightly enhanced biological efficacy; however, results of both light and heavy rain do not differ statistically from those observed in not rain-exposed plants. As expected, plants sprayed with water had higher dry matter than plants treated with glyphosate solutions (Fig. 5B). Addition of LSO adjuvants to glyphosate resulted in the same dry matter level as plants treated with the commercial formulation RUM® (Fig. 5B).

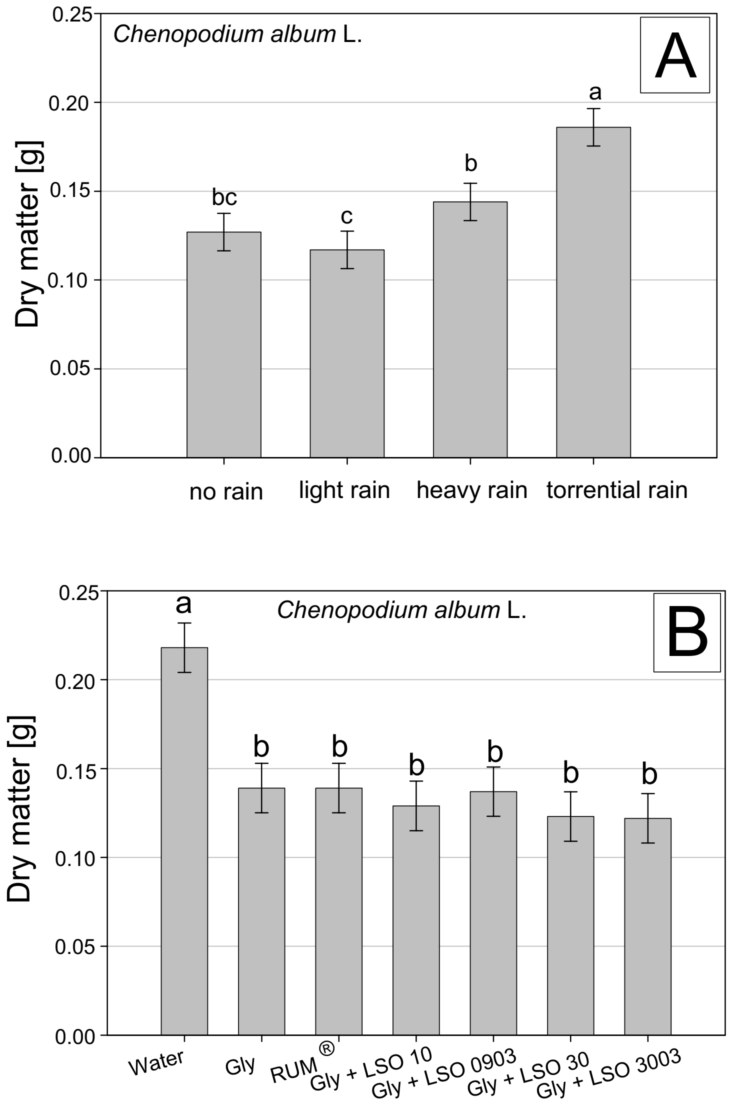


Figure 5. Influence of rainfall and rain intensity (A), and treatment solutions (B) on dry matter of *C. album*. Vertical bars represent the standard error. Means of dry mass followed by the same letter are not different by Duncan Test $p \leq 0.05$.

Irrespective of rain intensity, rainfall significantly reduced the efficacy of herbicidal treatments in *A. theophrasti* (Fig. 6A). Comparisons on the efficacy of treatment solutions revealed that all LSOs achieved at least the same level as RUM® reference, whereas the best result was obtained with unformulated glyphosate or glyphosate plus LSO 0903 or LSO 3003 (Fig. 6B).

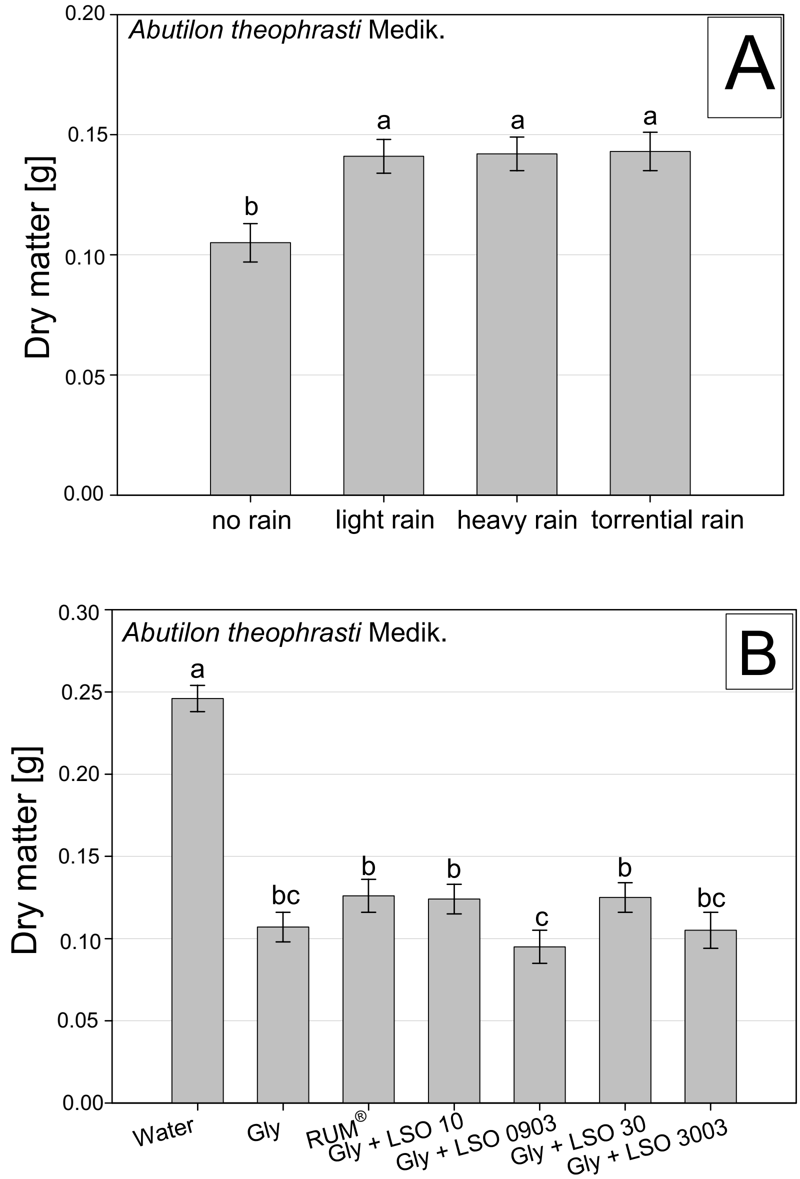
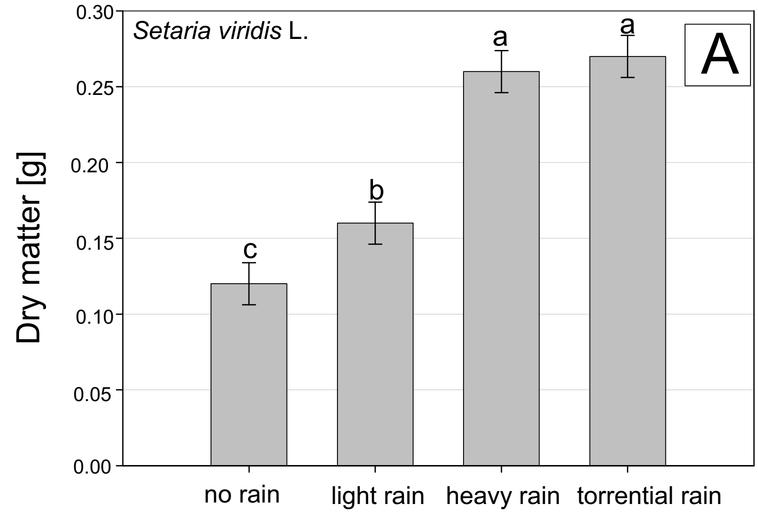


Figure 6. Influence of rainfall and rain intensity (A), and treatment solutions (B) on dry matter of *A. theophrasti*. Vertical bars represent the standard error. Means of dry matter followed by the same letter are not different by Duncan Test $p \leq 0.05$.

In the case of *S. viridis*, all rain intensities significantly reduced efficacy of the treatment solutions (Fig. 7A). Highest reduction was observed when the plants were exposed to heavy or torrential rain events. Comparisons among treatment solutions revealed the lowest dry matter due to addition of LSO 30 (Fig. 7B). However, the observed differences in dry matter were small.



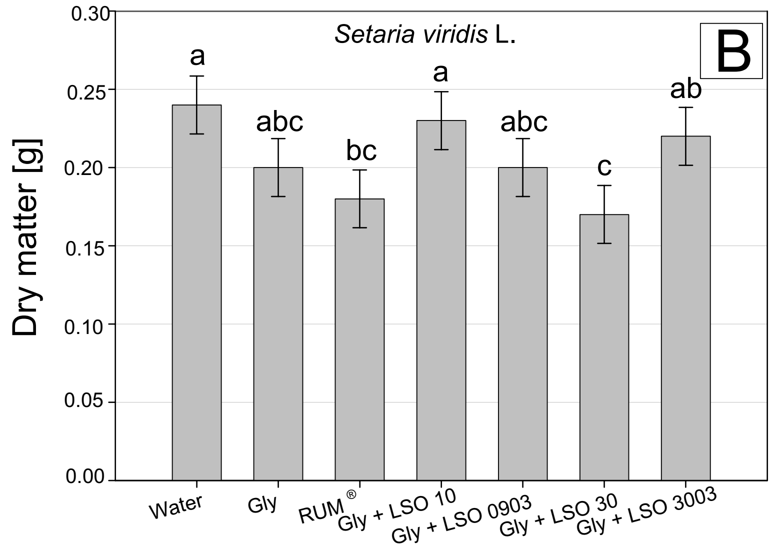


Figure 7. Influence of rainfall and rain intensity (A), and treatment solutions (B) on dry matter of *S. viridis*. Vertical bars represent the standard error. Means of dry mass followed by the same letter are not different by Duncan Test $p \leq 0.05$.

4 Discussion

4.1 Micro roughness and leaf wettability

SEM evaluations revealed significant differences among adaxial surfaces of the examined weed species (Figs. 2 - 4). Leaf surfaces are very diverse and range from simple and smooth to very complex and rough surfaces (Green, 2001). However, this diversity may be responsible for the established differences in roughness (Fig. 1). Roughness is determined by factors such as leaf surface topography, wax crystal structure and chemical composition (Forster and Zabkiewicz, 2001) as well as cell surface contours, leaf venation and presence of trichomes (Chachalis *et al.*, 2001; Kirkwood, 1999; Barthlott and Neinhuis, 1997; Brewer *et* *al.*, 1991; Holloway, 1970). Surfaces presenting contact angles greater than 110º (measured with water droplets) are usually characterized both by hydrophobic properties originating from the wax deposits, and pronounced roughness (Holloway, 1970). Contact angle measurements of water/acetone solution droplets provide a quantitative estimate for the roughness factor (Forster and Zabkiewicz, 2001).

Roughness primarily affects formation of the pesticide deposit (Chachalis *et al.*, 2001; Chachalis *et al.*, 2001; Green, 2001) and its distribution on the leaf surface (Hess and Falk, 1990). In our studies, enhanced contact between solution droplets and leaf surface was accomplished by glyphosate formulated with LSO ethoxylates; however, the effect of the added adjuvants differed among weed species (Tab. 1). In case of the difficult-to-wet leaves (*C. album* and *S. viridis*), better wettability was achieved with the more hydrophobic adjuvants LSO 10 and LSO 0903. In the case of *A. theophrasti*, all LSOs enhanced leaf wettability. An even lower contact angle was achieved additionally three propylene oxide units (PO) were included in the hydrophilic chain of the adjuvant molecule.

The contact angle of liquids on plant surfaces reflect their spreading behaviour and wettability (Foy and Smith, 1965), which is governed mainly by the nature of the exposed chemical groups, surface roughness and leaf orientation (Juniper and Jeffree, 1983). Fortunately, the use of adjuvants can diminish adverse effects of leaf topography, epicuticular wax, and trichomes (Hess and Falk, 1990), mainly by reducing surface tension of the spray solution (Hess and Foy, 2000). An enhanced surface wettability may contribute to an increased uptake rate of active ingredients into the plant tissue (Sun, 1996; Leung and Webster, 1994) and thereby also improve rainfastness of some pesticides (Green and Hazen, 1998; Reddy and Singh, 1992).

4.2 Influence of rain intensity

It is known that rainfall soon after glyphosate application results in partial or complete loss of activity (Reddy and Singh, 1992), because a.i. needs at least a 6-h rain-free period for penetration and effective weed control (Martini *et al.*, 2003; Werlang *et al.*, 2003; Chow, 1993; Sundaram, 1991; Wells, 1989). Enhancement of rainfastness and reduction of rain-induced wash-off can be reached in two ways, i.e. by water-repellency of the deposit and/or by enhanced penetration rate (Green, 2001; Leung and Webster, 1994; Roggenbuck *et al.*, 1993). Therefore, adjuvants preferentially designed to enhance rainfastness of the deposits of systemic compounds can better show their beneficial influence when it rains shortly after application. For these reasons we chose a rain-free period of only 2 hours.

Our results clearly demonstrate that occurrence of a light rain was sufficient to remove greatest part of the a.i. deposit from adaxial leaves of *A. theophrasti*. There was no increase in wash-off, when enhancing rain intensity. In *S. viridis*, a light rain significantly reduced a.i.

deposit. However, when leaves were exposed to heavy or torrential rain, respectively, much higher a.i. losses occurred.
In *C. album* plants, lowest dry matter was measured when a light rainfall (0.5 mm h^{-1}) impacted 2 h after glyphosate application. Scherhag (2005) evaluated the effect of rapeseed oil ethoxylates on rainfastness and biological activity of glyphosate and also observed that light rain increases efficacy of the active ingredient in *C. album*. Kirkwood (1999) noted that penetration of hydrophilic compounds may be enhanced by hydration of the leaf cuticle. Other authors (Schönherr, 2002; Schönherr, 2000; Schönherr and Baur, 1994) showed that an environment with high RH may cause a swelling of the cuticle, induce formation of water pores, and solubilize the isopropyl amine salt. The above-mentioned events, associated with the fact that low-intensity rain is characterised by droplets with little MVD, which probably had not the necessary kinetic force to remove herbicide deposits from the surface, may have facilitated a.i. penetration into *C. album* leaves.
In contrast, torrential rain (48 mm h^{-1}) washed-off a great part of the active ingredient. It is assumed that this rain event removed the major part of the herbicide deposits.
Our results show that glyphosate efficacy on weeds with rough surfaces (*C. album* and *S. viridis*) was not negatively affected due to light rain as compared to weed species with a smooth surface (*A. theophrasti*). Considering *A. theophrasti* as a unique species that did not present wax structures on its surface, we therefore hypothesise that the wax fine structure must have played an important role in preventing glyphosate wash-off, especially under light rain conditions. Leung (1994) showed that a film of cuticular wax reconstituted on a glass slice could not protect glyphosate deposits against rain-washing. However, the author did not give any information on the presence of wax fine structure elements.

4.3 Influence of treatment solutions

Because of the obvious differences in physicochemical characteristics of the leaf surfaces (Figs. 1 - 4), divergent responses of treatment solutions in the evaluated weed species were expected. While in *C. album* addition of LSO ethoxylates to unformulated glyphosate yielded about the same dry matter level as with RUM®, in *A. theophrasti* and *S. viridis* better results were obtained when formulating glyphosate with LSO 0903 or LSO 30, respectively. A clear relation between biological efficacy, roughness, contact angle of spray solution droplets, and average of EO units in the hydrophilic chain of the adjuvant could not be established. A critical factor which may have influenced our results is the mass and composition of surface waxes. Studies have shown that hydrophilic herbicides have lower efficacy in weeds with more lipophilic compounds in the epicuticular wax (Monquero *et al.*, 2004; Chachalis *et al.*, 2001).
It is known that adjuvant oils act mainly by enhancing penetration of herbicides, but the precise mechanisms involved are poorly understood (Sharma and Singh, 2000; Gauvrit and

Cabanne, 1993). It was also postulated that hydrophilic herbicides often work better with hydrophilic adjuvants, because they contribute to hydration of the cuticle and in this way enhance permeation of the active ingredient (Hess and Foy, 2000; Green and Hazen, 1998). According to Abribat (2001), rapeseed oil ethoxylates have solvency properties capable to turn leaf cuticle more permeable, enhancing penetration rate and rainfastness of hydrophilic compounds. Sharma and Singh (2000) showed that methylated seed oils enhance glyphosate efficacy in *Bidens pilosa* and *Panicum maximum* due to its solubilizing and humectant nature. We suppose that LSO ethoxylates influence glyphosate efficacy in a similar way; however, further research is needed.

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F Summary and conclusions

In our studies the contact fungicide mancozeb and the systemic herbicide glyphosate were used as model substances to elucidate the influence of leaf surface characteristics and environmental factors on rainfastness of agrochemicals. The effect of drying time, rain intensity, rain amount, interruptions of rain showers, and addition of seed oil based adjuvants (rapeseed, linseed, and soybean) differing in degree of ethoxylation were studied in detail in apple seedlings (*M. domestica* Borkh.). Furthermore, the involvement of surface roughness as well as amount and composition of epicuticular waxes in rainfastness of mancozeb with or without tank-mix adjuvants was examined in adaxial leaf surfaces of apple seedlings, bean seedlings (*P. vulgaris* L.) and kohlrabi (*B. oleracea gongylodes*). The interaction between rain intensity and type of linseed oil ethoxylate adjuvant (LSO 10, LSO 0903, LSO 30, and LSO 3003) on the wash-off and biological efficacy of glyphosate was investigated in lambsquarter (*C. album*), velvetleaf (*A. theophrasti*) and green foxtail (*S. viridis*). Light, heavy and torrential rain events with intensities of 0.5, 5, and 48 mm h^{-1} respectively, were simulated using a laboratory rain simulator. The results can be summarized as follows:

1. Mancozeb was washed-off easily from the leaf surface of apple seedlings due to impact of few millimeters rain, whereas a higher amount of rain caused only little additional a.i. removal. Regardless of drying time, fungicide removal from the leaves followed a hyperbolic curve. Fungicide losses after 5 mm rain reached about 90 % of the initial deposit after a drying time of 2 h, 75 % and 80 % after drying times of 4 h and 24 h, respectively. Intensity and amount of rain independently affected a.i. removal from the seedling leaves. Ten milliliters rain at 0.5 mm h^{-1}, 5 mm h^{-1} and 48 mm h^{-1} reduced fungicide concentration of the initial deposit to 43 %, 12 % and 8 %, respectively. Equations for mancozeb removal at light, heavy and torrential rain were determined for precipitation ranges between 0 and 30 mm, and between 0 and 5 mm. Interruptions of rain showers had only little influence on rainfastness at 2 mm rain, and no effect at 5 mm precipitation.

2. Rapeseed, linseed and soybean oil ethoxylates significantly reduced surface tension, contact angle, and drying time of water droplets. Greatest influence on surface tension and contact angle was observed when adding the more hydrophobic adjuvants (RSO 5, LSO 10, SBO 10). However, no differences among adjuvant solutions were observed, as far as drying time of individual droplets is concerned. As a rule, mancozeb formulated with the more hydrophobic adjuvants had lower retention, but enhanced rainfastness after 5 mm heavy rain. SEM micrographs revealed that deposit characteristics such as active ingredient distribution inside the droplet residue zone were not influenced by addition of seed oil ethoxylates. In general, before rain onset, mancozeb was not

uniformly distributed within the droplet impaction zone, but rather concentrated in the centre or in one half of the residue area. The fungicide was mainly located along anticlinal cell walls, in form of crystals. After rainfall, greatest part of the a.i. remained along anticlinal cell walls, in form of balls or annuli.

3. Studies on adaxial leaf surface of apple seedlings, bean seedlings and kohlrabi revealed great differences in roughness, as well as amount and composition of epicuticular waxes. Average of EW on the adaxial leaf surface of apple seedlings was 412.40 ng cm^{-2} (acids 8.40 %, primary alcohols 41.51 %, alkanes 14.11 %, triterpenes 25.04 % and esters 10.94 %), in bean seedlings 925.60 ng cm^{-2} (primary alcohols 85.39 %, alkanes 12.72 %, triterpenes 0.004 % and esters 1.45 %), and in kohlrabi 4954.90 ng cm^{-2} (acids 0.41 %, primary alcohols 33.13 %, alkanes 63.55 %, triterpenes 0.03 % and esters 2.88 %). Kohlrabi leaves were rougher (114º) than apple seedling (78º) and bean seedling (76º) leaves. A Pearson's correlation analysis showed very strong correlations between roughness and total EW (r = 0.91), amount of C_{29} alkanes (r = 0.94), and total mass of alkanes (r = 0.93). Retention and rainfastness of mancozeb differed among plant species; moreover, addition of adjuvants to spray solution caused differential responses. In general, mancozeb retention was highly and negatively correlated with surface roughness, total epicuticular wax, amount of C_{29} alkane, and total of alkanes in the EW. Rainfastness was highly positively correlated with amount of C_{28} alcohol and C_{33} alkane in the EW. Surface roughness, total EW and fungicide retention (= initial fungicide deposit) correlated significantly or highly significantly with rainfastness, when expressed as µg g^{-1} FW; however, only moderate or weak correlations existed when rainfastness was expressed as percentage of the initial deposit.

4. The weed species used in the glyphosate study presented significant differences in micro roughness and surface wettability. Leaves of velvetleaf were easily wetted by all treatment solutions, contrasting results observed in lambsquarter and green foxtail. These differences originate from surface characteristics such as cell size, presence of trichomes, glands or wax structures, which could be visualized by scanning electron microscopy. Addition of LSO ethoxylates and especially the more hydrophobic ones enhanced wettability of leaf surfaces. Evaluations of the biological efficacy as a function of treatment solutions (RUM®; glyphosate; glyphosate plus adjuvants) and rain intensity (0.5, 5 and 48 mm h^{-1}) in a bi-factorial experiment for each species, showed no significant interactions between the factors. In lambsquarter, heavy and torrential rain events reduced, while light rain slightly raised biological efficacy. Addition of LSO adjuvants to glyphosate resulted in the same dry matter level as achieved with the commercial formulation RUM®. In velvetleaf, all rain intensities reduced efficacy of the herbicidal treatments significantly. Comparisons among treatment solutions showed that all LSOs achieved at least the same level as RUM® reference, whereas the best result was obtained by adding LSO 0903 to unformulated glyphosate. In the case of green foxtail, all rain intensities significantly reduced the efficacy of treatment solutions, whereas highest reduction was observed when plants were exposed to heavy or torrential rain. Here, comparisons among treatment solutions showed a lower dry matter for glyphosate plus LSO 30. The other LSO ethoxylates showed the same dry matter level as unformulated glyphosate.

In this study, the influence of leaf surface characteristics and environmental factors on rainfastness and rain-induced wash-off of the contact fungicide mancozeb and the systemic herbicide glyphosate was evaluated. The results obtained clearly document that removal of the agrochemicals due to rain was distinctly influenced by drying time before rain onset, rain intensity, rain amount, and physicochemical properties of leaf surfaces. Addition of rapeseed, linseed, and soybean oil ethoxylates as tank-mix adjuvants allowed enhancement of rainfastness and biological activity; however, the effect depended on the ethoxylation degree of adjuvants as well as leaf surface characteristics.

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