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Improvement of Mg uptake of grapevine by use of rapeseed oil ethoxylates for foliar application of Mg

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Summary

To improve the uptake of foliar-applied Mg a new group of toxicological and ecotoxicological harmless surfactants (rapeseed oil ethoxylates) with an average of 5 (Agnique RSO 5®), 10 (Agnique RSO 10®), 30 (Agnique RSO 30®) and 60 (Agnique RSO 60®) units of ethylene oxide (EO) was evaluated as adjuvants for a $MgSO_4$ and $Mg(NO_3)_2$ formulation. The impact of the surfactants on Mg penetration was studied using astomatous cuticular membranes isolated from mature tomato fruit. The biological efficacy of a formulation, containing castor oil, ionic and non-ionic surfactants with and without addition of RSO 5 was investigated in two vineyards at the Moselle valley, cvs Riesling and Regent. Especially RSO 5-surfactant increased Mg penetration through isolated cuticles. Under field conditions, Mg application enhanced significantly the Mg content in leaves. The Mg level in bunch stems merely increased when formulated 'Bittersalz' was applied. 'Magnisal' and formulated 'Magnisal' reduced markedly Mg deficiency symptoms in leaves and increased Mg content in leaves of cv. Regent.

K e y w o r d s : adjuvants; cuticular penetration; leaf fertilizer; magnesium; foliar nutrition; surfactants.

Introduction

Bunch stem necrosis is one of the most serious physiological diseases of grapevine leading to cluster injury in the ripening stage (Rumbos 1989; Capps and Wolf 2000). No final conclusion on the causes of bunch stem necrosis can be drawn, but among the genetic and environmental factors the mineral nutrients Mg, Ca and K, seem to play the most important role (Rumbos 1989, Neilsen 2000). Bunch stem symptoms are related with the Mg and Ca contents in stalks (Brendel *et al*. 1983; Schaller 1983; Schimansky 1983). The more serious the necrosis, the higher the difference in the ratio of K to Mg and Ca (Stellwaag-Kittler and Haub 1965; Lauber and Koblet 1967; Brechbuhler 1975).

Mg deficiency can frequently be observed on sandy soils in high rainfall regions, poorly drained sites, or alkaline soils due to the fact that Mg is leached from the soil or displaced by Ca and Na (Jackson 1994; Marschner 1995).

To prevent yield and quality losses caused by bunch stem necrosis, a sufficient supply of Mg is essential (Brendel *et al.* 1983; Neilsen 2000). However, the response of plants to Mg applied to the soil is slower than to Mg applied to leaves (Cooper 1973). Thus much of the earlier work has focussed on foliar application of $MgCl_2$ and $MgSO_4$ at different concentrations, but the Mg content of leaves was not increased markedly (Alleweldt and Hifny 1972; Beetz and Bauer 1983; Stellwaag-Kittler 1983; Rumbos 1989).

The objective of the present study was to test the penetration of Mg applied as $MgSO_4$ and $Mg(NO_3)_2$ into the plant tissue. The effect of surfactants on the penetration of Mg ions was investigated using isolated cuticles. As surfactants we used commercial preparations of rapeseed oil derivatives (triglyceridethoxylates) considered to be toxicologically and ecotoxicologically harmless.

Material and Methods

P e n e t r a t i o n e x p e r i m e n t s : Donor solutions, simulating agricultural spray solutions, were prepared using 0.2 M $MgSO_4$ x 7 H_2O and $Mg(NO_3)_2$ x 6 H_2O (Merck, extra pure). Surfactants ('Agnique RSO®' series, Cognis, Germany) were commercial preparations of rapeseed oil derivatives (triglyceridethoxylates) with an average of 5 (RSO 5), 10 (RSO 10), 30 (RSO 30) and 60 (RSO 60) ethylene oxide (EO) units. A Mg formulation developed by the Institute of Horticulture, University of Bonn, containing castor oil, ionic and non-ionic surfactants was used as standard. Surfactants and the formulation were added to aqueous $MgSO_4$ and $Mg(NO_3)_2$ solutions at concentrations of 1 g l^{-1}.

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

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

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At time zero, 8 single drops (1 µl each) of the test solution were applied to the cuticular surface using a microsyringe fitted with an automatic dispenser (Hamilton). The Mg content in the receiver solution was determined by Atomic Absorption Spectrometry (AAS) 24 h after initiation of the experiment. Penetration rates were determined using 8 completely randomized replicates.

Biological efficacy: In 2000 the biological efficacy of various magnesium leaf fertilizers was investigated on two sites with different cultivars:

a) 'Graacher Domprobst' (Moselle Valley): Slope gradient: max. 58 %; exposition: SSW; soil: weak sandy loam; cultivar/rootstock: Riesling/5 C; age: 11 years; planting density: 1.5 m x 1.2 m; number of vines per treatment: 9-10; replicates: 4. Treatments: Solutions were prepared using $MgSO_4$ ('Bittersalz', 16 % MgO). The formulation and the surfactant Agnique RSO® were added as follows: 1. Untreated control; 2. $MgSO_4$; 3. $MgSO_4$ + formulation (2 g l^{-1}); 4. $MgSO_4$ + formulation + Agnique RSO 5® (1 g l^{-1} each).

The vines were sprayed in the morning, at low or moderate temperature, using a knapsack sprayer. The solutions were applied until run-off at the following dates (developmental (BBCH) stages according to SCHUMANN 1998) and Mg concentrations:

23 May (BBCH 55): 2 mM (10 d before bloom)
19 June (BBCH 73): 2 mM
21 July (BBCH 77): 4 mM
7 August (BBCH 81): 8 mM (one week before ripening)

Occasionally, leaves showed very slight Mg deficiency symptoms. Eight leaves per replicate were sampled on 20 and 27 September at nodes 5-8 of the shoots. On September 20, samples were washed with deionized water to remove Mg residues from the leaf surface. One week later residues were removed with a solution of 0.05 n HCl including 1 mg l^{-1} Tween 20. After drying in a lyophilizer, each leaf blade was ground to a fine powder, digested with HNO_3 and H_2O_2 according to CHEN *et al.* (1997) and analysed by AAS.

On October 10 all berries of eight grape clusters per replicate were cut off with fine scissors at the point of attachment. Each stalk was washed in a solution of 0.05 n HCl + 1 mg l^{-1} Tween 20, then dried, ground to a fine powder, digested with HNO_3 and H_2O_2 and analysed by AAS.

b) 'Wolfer Schatzgarten' (Moselle Valley): Slope gradient: max. 35 %; exposition: E; soil: weak clayey loam; cultivar/rootstock: Regent/5 C; age: 11 years; planting density: 2.0 m x 1.3 m; number of vines per treatment: 7-9; replicates: 4. Treatments: Spray solutions were prepared using $Mg(NO_3)_2$ ('Magnisal', 16 % MgO) and the formulation mentioned above: 1. Untreated control; 2. $Mg(NO_3)_2$; 3. $Mg(NO_3)_2$ + formulation (1 g l^{-1}).

The vines were sprayed as described above at the following dates and Mg concentrations:

23 May (BBCH 57): 0.75 mM (10 d before bloom)
19 June (BBCH 73): 0.75 mM
6 July (BBCH 75): 1.5 mM
21 July (BBCH 81): 1.5 mM (1 week before veraison)
7 August (BBCH 86): 3.0 mM

Mg deficiency symptoms were assessed on August 24 by comparing leaves of each plant with an intermediately affected ‚standard leaf' (Figure). Samples of leaf blades were taken on August 29 and September 11 and prepared as previously described. Sixteen grape clusters per replicate were sampled between September 4 and 12. Stalks were prepared for AAS analysis as described above.

Figure: Leaf of cv. Regent with medium to severe Mg deficiency symptoms.

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

Results

The surfactants 'Agnique RSO 5®' and 'Agnique RSO 10®' enhanced the penetration of Mg applied as $MgSO_4$ through isolated tomato fruit cuticles significantly after 24 h (Tab. 1). A negative relationship was found between the EO content of the surfactants and the amount of Mg that penetrated the cuticle. Penetration increased with decreasing EO chain length from 60 to 5 EO units. A similar relationship was established when the surfactants and a Mg formu-

Table 1

Penetration of sprayed $MgSO_4$ and $Mg(NO_3)_2$ solutions (0.2 M) through isolated tomato fruit cuticles 24 h after treatment. Surfactants (RSO 5 EO, 10 EO, 30 EO, 60 EO) and the formulation (Form.) were added at a concentration of 1 g · l^{-1}

Treatment	Penetration (% of applied Mg)
$MgSO_4$	5.12 b
$MgSO_4$ + RSO 5 EO	9.81 a
$MgSO_4$ + RSO 10 EO	9.79 a
$MgSO_4$ + RSO 30 EO	8.13 ab
$MgSO_4$ + RSO 60 EO	7.91 ab
$Mg(NO_3)_2$ + Form.	7.74 ab
$Mg(NO_3)_2$ + Form. + RSO 5 EO	10.65 a
$Mg(NO_3)_2$ + Form. + RSO 10 EO	10.08 a
$Mg(NO_3)_2$ + Form. + RSO 30 EO	10.13 a
$Mg(NO_3)_2$ + Form. + RSO 60 EO	7.02 b

lation containing castor oil, ionic and non-ionic surfactants, developed by the Institute of Horticulture, University of Bonn were added to $Mg(NO_3)_2$. The more lipophilic surfactants slightly increased Mg penetration, whereas the hydrophilic surfactant RSO 60 had no effect.

All $MgSO_4$ treatments resulted in significantly enhanced Mg concentrations in leaves of cv. Riesling compared to the untreated control (Tab. 2). The Mg content in clusters was markedly enhanced, if the formulation or a mixture of the formulation and the surfactant RSO 5 were added to $MgSO_4$. Both $Mg(NO_3)_2$ treatments approximately doubled the Mg concentration in leaves of cv. Regent compared to untreated plants, whereas the Mg content in clusters was not significantly increased. In both cultivars more residues were removed from the leaf surfaces with a 0.05 n HCl solution including 1 mg $\cdot$ l^{-1} Tween 20 than with deionized water.

For cv. Regent, the number of leaves per plant with medium to severe Mg deficiency symptoms was markedly reduced by foliar $Mg(NO_3)_2$ applications as compared to 'standard leaves' (Figure and Tab. 3). There was a slightly higher tendency of formulated $Mg(NO_3)_2$ to reduce the incidence of Mg deficiency symptoms compared to unformulated $Mg(NO_3)_2$.

Discussion

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

As the surfactant concentration in the donor solution was below the critical micellar concentration, surfactant-enhanced penetration is likely to originate from a modification of the cuticle permeability rather than a direct effect on Mg mobility. Schönherr (2000) found that calcium chloride penetrates plant cuticles via aqueous pores. Like calcium, magnesium is a divalent cation. Hence, it can be assumed that Mg ions also require an aqueous diffusion path, *i.e.* pores filled with water, to penetrate cuticular membranes. Aqueous pores swell depending on humidity (Schönherr 1982). The surfactants employed did not extend the drying-time of spray droplets. On the contrary, with increasing surfactant EO contents drying times were even reduced as shown for triglyceridethoxylates (unpubl.). Therefore, extended drying times cannot be the reason for enhanced penetration. Probably, surfactants enhancing wetting of the cuticular surface played a pivotal role. This would also explain the observation of an inverse relationship between surfactant-enhanced penetration and surfactant EO content because contact angles of spray droplets on the standard surface 'Parafilm' increased with increasing EO chain length (unpubl.) and hence, the addition of the surfactant RSO 5 resulted in the highest coverage of the leaf surface with Mg solutions. Treatment with the most lipophilic surfactant RSO 5 showed the lowest surface tension (unpubl.) and probably improved the contact between salt solutions and the microrough cuticles, thus making Mg more available for cuticular penetration. When the surfactants were added to the Mg formulation developed by the Institute of Horticulture, University of Bonn, cuticular penetration of $Mg(NO_3)_2$ slightly increased with a decreasing content of surfactant EO (Tab. 1). It can be assumed that the lipophilic surfactants improved the wetting properties of the formulation and thereby enhanced penetration through isolated cuticles.

In the field studies, neither the formulation nor the formulation in combination with RSO 5 enhanced Mg concentrations in leaves or clusters significantly compared to non-formulated 'Bittersalz' and 'Magnisal' (Tab. 2). Probably, the formulation enhanced Mg uptake as it did in the finite dose experiments but within leaves and clusters; this additional amount of Mg was diluted such that no significant increase in Mg concentration could be measured. Another interpretation would be that penetration through isolated cuticles is not decisive for total Mg uptake. It was shown that stomatal uptake can be a major pathway for the foliar uptake of ionic solutes (Eichert and Burkhardt 2001). Furthermore, Mg is phloem mobile and differences due to the enhanced uptake

Table 2

Magnesium content in the dry matter of leaves and clusters of cvs Riesling and Regent after spraying different $MgSO_4$ and $Mg(NO_3)_2$ solutions

Treatment	Mg in dry matter, %		
	Leaves (washed with water)	Leaves (washed with HCl)	Cluster (washed with HCl)
Riesling			
Untreated control	0.222 a	0.214 a	0.060 a
$MgSO_4$	0.414 b	0.376 b	0.072 ab
$MgSO_4$ + 0.2 % Form.	0.405 b	0.375 b	0.079 b
$MgSO_4$ + 0.1 % Form. + 0.1 % RSO 5 EO	0.406 b	0.379 b	0.080 b
Regent			
Untreated control	0.048 a	0.039 a	0.031 a
$Mg(NO_3)_2$	0.096 b	0.073 b	0.037 a
$Mg(NO_3)_2$ + 0.1 % Form.	0.102 b	0.077 b	0.038 a

Table 3

Number of leaves per vine (cv. Regent) with medium to severe Mg deficiency symptoms compared to a medium infested 'standard leaf' after spraying of various $Mg(NO_3)_2$ solutions

Treatment	Number of leaves per vine with medium to severe Mg deficiency symptoms
Untreated control	21.53 a
$Mg(NO_3)_2$	6.88 b
$Mg(NO_3)_2$ + 0.1 % Form.	4.85 b

might not be reflected in the plant organ under investigation.

Comparing the Mg content in clusters after application of various $MgSO_4$ solutions, it can be argued that merely the treatments with formulated $MgSO_4$ increased the Mg level significantly compared to the untreated control. Even though the inrease from 0.06 to 0.08 % Mg on a dry weight basis may appear low, this increase of one third may lead to Mg levels in clusters which are above the critical concentration limit (depending on the year, between 0.03 and 0.17 % in cv. Scheurebe and 0.07-0.25 % in cv. St. Laurent) below which symptoms of bunch stem necrosis do appear (REDL 1983).

Since no symptoms of bunch stem necrosis occurred in 2000, the number of leaves per vine with medium to severe Mg deficiency symptoms was assessed (Figure). These symptoms only occurred at the 'Wolfer Schatzgarten' in cv. Regent. The number of affected leaves was markedly reduced by foliar applications of 'Magnisal' solutions (Tab. 3). The tendency of formulated 'Magnisal' being more efficient than unformulated 'Magnisal' supports the assumption that slight increases in Mg content at the critical concentration limit for bunch stem necrosis symptoms may have a strong impact.

From these trials, we conclude that the surfactants used can enhance Mg penetration through isolated cuticles solely or as an adjuvant added to the formulation. Foliar application of $MgSO_4$ and $Mg(NO_3)_2$ is very effective in enhancing Mg levels in leaves but not in clusters. A significantly higher Mg content in clusters compared to an untreated control can be achieved by adding the formulation tested or the formulation in combination with the most effective surfactant RSO 5 to $MgSO_4$. Since the surfactant RSO 5 has a very positive toxicological and ecotoxicological profile, the mixture of formulation and RSO 5 should be preferred. Moreover, unpublished studies indicated that this new formulation is not phytotoxic at the employed concentration.

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