Effect of Surfactants and Liquid Fertilisers on Transcuticular Penetration of Fungicides

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Abstract

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Penetration of active compounds into the leaves plays an important role in their systemic activity. The effect of surfactants and liquid fertilisers on the penetration of fungicides was studied in model trials with the cuticle from *Bryophyllum calycinum*. Solutions of the fungicides were pipetted on pieces of cuticle laid on agar covered by spores of *Cladosporium cladosporioides*. The diameters of the inhibitory zones were measured and served to gauge the level of penetration by the variants. The size of the inhibitory zone of the control variant of Alto Combi 420 SC pipetted on the cuticle was only reduced to 92.6% of the variant where the solution was pipetted directly onto the agar; thus, the cuticle's effect on penetration was minor. Penetration through the cuticle decreased the diameters of inhibitory zones also of other fungicides: Discus to 84.1%, Horizon 250 E to 83.0%, Baycor 25 WP to 77.7%, Topsin 500 SC to 60.0 and Amistar to 37.8% of their control variant. The high penetration by the original formulations Alto Combi and Discus left no or little room for any increase of their penetration when mixed with additives. A higher penetration by Discus would also be undesirable because of its contact activity. The additives increased penetration most when mixed with Topsin and Amistar. The effect of surfactants and liquid fertilisers on the penetration of fungicides cannot be generalised. It was unique to each fungicide/additive combination. While the conditions of the trials enabled high penetration of some original formulations, the question arises how the additives will perform under conditions that will allow only low penetration.

Keywords: fungicides; surfactants; liquid fertilisers; cuticle

Transcuticular penetration into the leaf tissues plays a key role in foliar applied nutrients, herbicides, growth substances, systemic and locally systemic insecticides and fungicides.

Penetration by systemic fungicides enables their curative effect and redistribution into the parts of the plant not covered by spray droplets. Furthermore, some of the fungicides do not inhibit the germination of fungal spores on the leaf surface. They affect the fungi in their further development inside the leaves. Fast penetration would decrease the losses of the active substance by e.g. rain-off, mechanical abrasion, UV-degradation and evaporation (sublimation).

Transcuticular movement of fungicides has been studied since the beginning of the era of systemic compounds (Sollel & Edgington 1973). Penetration into the leaves and further systemic movement is affected by many factors. Foremost of these are the properties of the molecule, second the

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properties of the plants e.g. the morphology of the cuticle, moisture status, metabolic activity etc. Very important is the role of external conditions such as temperature and humidity. That is why the data on cuticular penetration and leaf uptake obtained by various authors using different methods were substantially different, e.g. Sollel and Edgington (1973) and Kuck (1987).

Cuticular waxes represent the first and, in most cases, the limiting barrier for foliar uptake of pesticides from a solution (Burghardt *et al.* 2006). Due to the lipophilic character of the leaf wax and outer layers of the cuticle, lipophilic compounds generally penetrate better than hydrophilic ones (Stevens *et al.* 1985). The wax layer is the main limiting barrier for transcuticular movement of water-soluble compounds. Its removal increased the uptake of 2,4D 26-times, and that of NAA 50-times (Norris & Bukovac 1972; Norris 1974). Later studies stressed the role of cuticular waxes embedded in cutin, not that of the layer of epicuticular waxes (Riederer 1991; Schreiber *et al.* 1996). The role of the wax layer is lower in the penetration of lipophilic substances (most of the pesticides) than of hydrophilic ones. The penetration of fungicides through cuticles of *Bryophyllum* sp. or *tulipa* increased 2–8-times after the removal of wax from the surface (Drandarevski & Mayer 1974). Lipophilic pesticide molecules easily diffuse and penetrate into the wax. A portion of a fungicide may accumulate in the wax layer and does not penetrate further. In contact fungicides it is, in fact, desirable that the active compounds accumulate on and in the wax layer and do not penetrate into the leaf. The proportion depends not only on the active compound itself but also on its formulation, additives and weather conditions.

Epicuticular waxes primarily govern the wettability of the plant surface (Holloway 1970). They are eroded by external factors, but their regeneration on living plant surfaces is a highly dynamic and comparatively swift process (Koch *et al.* 2004).

Other layers of the cuticle may present further barriers. Lipophilic and hydrophilic substances penetrate into the leaves by different channels. Important is the composition and role of the cuticle as a whole. Penetration of pesticides into the leaves is a very complex process. The mobility of solutes in cuticles varies considerably among plant species. Furthermore, diffusion within plant cuticles is extremely temperature dependent (Buchholz 2006). One of the factors limiting the uptake into leaves is the dry-up of droplets on the leaves. The hydrophilic liquid nutrient UAN (urea + ammonium nitrate solution) is known to increase the activity of growth herbicides, which allows to decrease their rates applied per ha. When ^{45}CaCl was applied on winter rape leaves, the amount of ^{45}Ca translocated into other leaves increased by addition of UAN as much as ten times (Veverka 2005). Such increase was possible because leaf uptake of calcium is normally very low.

On the other hand, the penetration of contact insecticides and fungicides into the leaves is undesirable because it can cause injury. Additives that increase the effectiveness of systemic compounds such as oils, can cause phytotoxicity of normally non-phytotoxic and safe contact compounds such as captan (Bondada *et al.* 1999).

Quite a number of adjuvants are recommended in practical agriculture to increase foliar uptake of pesticides and their effectiveness. Liquid fertilisers are added to the spray with fungicides to save the costs of another application. The question is: how are those additives able to increase even more the activities of modern fungicide formulations. The aim of our work was to investigate the first step – the effect of additives (adjuvants and liquid fertilisers) on penetration of fungicides through the cuticle.

MATERIAL AND METHODS

A simplified bioassay according to Edgington *et al.* (1973) was used. A suspension of spores of *Cladosporium cladosporioides* (Fres.) de Vries at 1×10^6 per ml was sprayed in a settling tower onto malt agar pH 6.5 in Petri dishes. Segments of cuticles 5 × 5 mm were removed by hand from the upper surface of *Bryophyllum calycinum* Salisb. leaves, four of them were laid with the wax layer upwards on the medium in a Petri dish. Three Petri dishes per variant were applied. A micropipette was used to put 1 μl of the tested compounds onto the cuticle segments; these were then incubated in closed Petri dishes. The diameters of inhibitory zones were measured after 3 days. It is obvious that each of the fungicides has both a different ability to penetrate through the cuticle and to diffuse in agar. However, the trials were designed to compared only the effects of individual additives on each of the fungicides.

List of chemicals used

	Active ingredient(s)	Rate per 100 ml of water (mg a.i./100 ml)
Fungicide		
Discus	kresoxim-methyl 50%	0.05 g
Baycor 25 WP	bitertanol 250g/kg	0.10 g
Horizon 250 EW	Tebuconazole 250g/l	0.25 ml
Topsin 500 SC	thiophanate-methyl	1.252 ml
Alto Combi 420 SC	carbendazim 300 g/l; cyproconazole 120 g/l	0.031 ml
Amistar	azoxystrobin 250 g/l	0.25 ml
Surfactant		
Greenmax	bioactivator	0.01 ml
Break-Thru S 240	polyether-polymethylsiloxan	0.031 ml
COG	surfactant[1]	0.013 ml
Silwet L-77	heptamethyltrisiloxan modified by polyalkylenoxid	0.025 ml
Ekol	winter rape oil	0.9 ml
Fertiliser		
UAN	urea 32.7%, ammonium nitrate 42.2%, water 25.1% concentrated equimolar solution pH 7.2–7.9	concentrated, and dilutions 1:9 – 10 ml; 1:99 – 1 ml
Samppi	NPK 8-3-3+ microelements	0.25 ml
Hydroplus bór	borethanolamine 150 g/l 10.4% B	0.5 ml
Fertibór	N 8 % (ammonia 3%, urea 5%); P_2O_5 10%, B 2%, pH 6.5–8.5	0.75 ml

[1]unspecified, no data submitted by the producer Agra Group, a.s., Střelské Hoštice, Czech Republic

In preliminary trials the applied concentration of a funcide was the recommended percentage concentration for field application or the recommended hectare rate diluted in 400 l/ha. It was found that to get a reliable size of inhibitory zones, the concentration of Topsin 500 SC was increased 4-times and the concentration of Alto Combi 420 SC was decreased 4-times. The other fungicides were used in the same concentrations as in the preliminary trials where they corresponded to the concentrations used in field application. The fungicides were chosen to represent different modes of action and formulation. Liquid fertilisers and surfactants were the same as used in our field trials (Zelená – unpublished results).

The data were statistically analysed by ANOVA and Tuckey test. Percentages were transformed by $y' = \arcsin\sqrt{y/100}$. There are no significant differences between data followed by the same letter.

RESULTS AND DISCUSSION

Preliminary trials showed no fungicidal activity of the additives in the tests performed according to the described method. Their possible fungicidal effect was, therefore, not considered further. The obtained data show the relative transcuticular movement of individual fungicides as influenced by the additives. They do not enable us to compare the fungicides or assess the amount of the active ingredient that penetrated through the cuticle.

Data presented in Table 1 show the transcuticular penetration in % = (diameter of the inhibitory

Table 1. Effect of surfactants and fertilisers on transcuticular penetration of fungicides

Additive	Transcuticular penetration (%)					
	Discus	Baycor 25 WP	Horizon 205 E	Topsin 500 SC	Alto Combi 420 SC	Amistar
Control	84.1[a]	77.7[b]	83.0[bc]	60.0[d]	92.6[a]	37.6[c]
Greenmax	81.2[ab]	82.6[ab]	81.6b[cd]	86.0[a]	88.5[a]	86.2[a]
Break-Thru S 240	68.2[de]	83.6[ab]	82.6[bcd]	70.3[bc]	91.6[a]	88.3[a]
COG	75.3[abcd]	83.4[ab]	78.7[d]	64.5[cd]	93.4[a]	83.0[a]
Silwet L-77	72.6[bcd]	85.7[a]	80.6[cd]	84.6[a]	89.2[a]	83.8[a]
Ekol	77.3[abcd]	83.0[ab]	80.9[cd]	70.7[bc]	88.7[a]	92.8[a]
UAN conc.	77.0[abcd]	79.5[ab]	88.9[a]	84.0[a]	89.6[a]	63.9[b]
UAN 1:9	72.7[cd]	78.3[b]	84.8[b]	76.2[b]	89.4[a]	89.1[a]
UAN 1:99	79.9[abc]	81.1[ab]	81.2b[cd]	64.2[cd]	92.5[a]	48.3[bc]
Samppi	70.0[cde]	84.7[a]	83.0[bc]	76.7[b]	87.7[a]	52.4[bc]
Hydroplus bór	60.[e]	80.7[ab]	82.5[bcd]	59.5[d]	86.9[a]	52.2[bc]
Fertibór	83.7[a]	80.2[ab]	79.5[cd]	76.3[b]	91.1[a]	40.7[c]

zone of the fungicide applied on cuticle/diameter of inhibitory zone of fungicide applied on agar) × 100. It shows the effect of additives more transparently than absolute numbers.

Registered pesticides contain formulation additives which should possess activities needed for the best performance under various conditions in practice. This is a whole complex of various effects. We studied only one of them – the transcuticular penetration under relatively very suitable conditions, i.e. long duration of the trial (3 days), relatively large droplets and very high humidity in closed Petri dishes which provided a long period of drying of the droplets and enhanced penetration. Under such conditions, the diameters of inhibitory zones expressed in percentage of the variants applied directly on agar were very large even when fungicides were applied in water as in the Control on Table 1.

The relative level of penetration of the cuticle by the original formulations, without additives, was surprisingly diverse.

While with Alto Combi it was 92.6%, in others still above 75%, the low exceptions were Topsin with 60.0% and Amistar with 37.6%. If there was high performance of the original formulation, the possibility of further improvement by additives is very limited. Most of the variants were not statistically different from the control (water). We will comment only on the statistically significant differences:

Alto Combi – no statistically significant differences between the variants. Penetration was high and none of the additives increased penetration further.

Discus – the highest penetration was reached in the variant with water; statistically significant decrease in penetration was seen in variants with UAN 1:9, Silwet, Samppi, Break-Thru and Hydroplus bór.

Horizon – penetration was highest in the variant in which the fungicide was mixed with concentrated UAN. It decreased in combination with COG.

Baycor – very small differences were detected among the variants. Increased penetration in comparison with the control was observed only in variants Silwet and Samppi.

Topsin – low penetration in the control, highest increase with Greenmax, Silwet and concentrated UAN, further with Samppi, Fertibór, UAN 1:9, Ekol and Break-Thru.

Amistar – very low level of penetration in the control (37.6%). Increased penetration with Ekol, UAN 1:9, Break-Thru, Greenmax, Silwet, COG and concentrated UAN.

Overall: only Topsin and Amistar showed a marked increase in penetration when applied together with additives.

Effect of additives:

– Hydroplus bór and UAN 1:99 had no positive effect.

– COG and Fertibór increased the penetration of one of the fungicides.
– Break-Thru, Ekol, Greenmax, Samppi and UAN 1:9 increased the penetration of two fungicides.
– Concentrated UAN and Silwet increased the penetration of three of the tested fungicides.

There was no consistent effect by any of the additives on fungicide penetration. The effect was very unique for each additive/fungicide combination.

Direct measurement of cuticle penetration is not necessarily directly and proportionately correlated with the resultant biological activity. The level of biological activity is a summation of many processes, of which foliar penetration is only one (Stock 1997). The highest increase of pesticide penetration was reached at concentrations ten times higher (CMC – critical micelle concentration) than was needed to decrease the surface tension to ensure good wettability (Foy & Smith 1965). The relationship between the surfactant concentration needed for good wettability and the optimum for high penetration of each modern fungicide formulation is yet another open question. Our trials did not cover other effects of the tested spray additives as they may appear in practice, such as possible enhanced foliar retention, coverage and penetration deeper into the epidermis and movement in vascular tissues and metabolically active tissues.

Transcuticular penetration is the first step to systemic activity by a given compound. High penetration is also undesirable in compounds which are quickly degraded in living tissues, e.g. kresoxim-methyl and trifloxystrobin. Strobilurins generally exhibit outstanding activity against fungal spore germination, but most are degraded in the plant tissues. Only four strobilurins (azoxystrobin, fluxastrobin, picoxystrobin, dimoxystrobin) are metabolically sufficiently stable in plants and show a pronounced xylem systemicity. In contrast, kresoxim-methyl and trifloxystrobin are very unstable. The fact that there is no metabolism in the cuticular waxes allows them to be stable for a long period and to provide long residual activity (Sauter 2007). It is thus inadvisable to mix Discus (kresoxim-methyl) with any additives that increase its penetration through the cuticle. In that light the decreased penetration of Discus by five of the tested additives was a positive effect. It is questionable whether additives that decrease the penetration are able to increase the amount of a.i. retained in the wax layer and in this way extend the persistence. On the other hand, mixing additives with Amistar (azoxystrobin) may be promising.

The possibilities of improving fungicide effectiveness in the field must vary not only for individual fungicides, but also under various weather conditions. The conditions of our trials were optimal from the point of view of transcuticular penetration. The results show that under such conditions the formulation of Alto Combi offers almost no space for improvement, and that of other fungicides is very small. However, penetration by Topsin and Amistar can be substantially improved.

The presented results open questions for further trials, to be performed under conditions not ideal for penetration, especially:

– what effect has the relative humidity of the air on the evaporation of droplets and on the influence of additives on the speed of penetration under various conditions;

– what is the effect of the concentration of additives on the penetration.

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