Feature

A Cleaner and Greener New Zealand Thanks to 2,4,5-T, Science and Silicones

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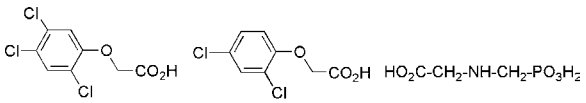
Historical background

Those of you old enough to remember NZ being deluged with 2,4,5-T, **1** (one of a series of plant growth hormone herbicides that includes 2,4-D, **2**) thought it was the environmentalists who put a stop to this herbicide. Well, not really! It was a combination of a new herbicide and a new spray surfactant (adjuvant) that finally turned the tables on a product that had little market competition until then. And NZ science, with a touch of serendipity, played a major role in turning a *she'll be right* approach into a successful technology which has been adapted in various ways for a wide range of agrichemical products and crop situations.

Hill country covered in woody weeds was a huge physical and economic problem to the booming forestry sector in the 1970s and 1980s, as forests were planted on land which had degenerated from pasture to scrub weeds. Non-chemical methods of weed control - no, it's not a new idea - were practiced then, but involved burn-offs that frequently got out of control and cast a pall of smoke for weeks on end in places. Alternatively, huge machinery rumbled up and down the hillsides or rolled huge drums down them to crush the scrub weeds…so that they could be burned more safely. Farmers were no better off; they could stock their paddocks with goats which ate everything in sight, but then they had to have secure fencing as it took four to five years to make sure all the woody scrub weeds were controlled - but this was impossible with a plant like bracken fern. So herbicides were the first choice, and with aerial application any terrain could be treated. The herbicide 2,4,5-T was used extensively on gorse, broom, and native scrub weeds. But there was a problem (leaving aside the Agent Orange aspects and spray drift issues) for, despite a variety of product formulations and apparently good kills of gorse, the plants re-grew within a few months. This problem was recognised and a research programme involving the author was initiated in 1974 to specifically look at better methods of woody weed control at the former FRI in Rotorua. Over the next few years studies showed that herbicides that acted through the roots were impractical for mature plants, though excellent at controlling regenerating seedlings. Attention focussed on foliar uptake options for the mature plants and radiolabeled herbicides demonstrated large differences in uptake into plant foliage, as well as poor translocation by some, including **1**. In effect it was a good contact herbicide but far from ideal for woody weed control. Something new was needed.



1, 2,4,5-T **2**, 2,4-D **3**, glyphosate

That new herbicide was glyphosate (**3**, sold by Monsanto as Roundup® in the early 1980s) but although it was quite effective against annual broadleaf and grass weeds, when applied to the vigorous woody and rhizomatous weeds like gorse, bracken and broom, or perennials like clover, it failed. It was also very expensive, making it economically and biologically unattractive in the NZ situation. However, it had one huge advantage, it could translocate very well within plants and kill not just the foliage, but right down to the roots.

Plant Biology and Physicochemical Interactions

It was well known that by adding different oils or surfactants (adjuvants) to the pesticide product you could improve spray efficacy. The reason for this is that the cuticle in a leaf presents a highly lipophilic surface to the external environment. The cuticular layer covering plant leaves is complex, but in general consists of a superficial wax layer (epicuticular wax), then a bilayer cuticular membrane (cuticle layer), the outermost layer (cuticle proper) composed of soluble (cuticular waxes) and polymerised lipids (cutin), and the innermost of polysaccharides which may contain high proportions of pectin (Fig. 1). The conundrum is that a lipophilic compound will readily absorb into the lipophilic cuticle, but will not readily translocate in the polar, ionic phloem or sap.[1] In the case of **1**, with an octanol/water (ow) partition ratio of log K_{ow} >3, it was readily absorbed but poorly translocated.[2] In contrast, **3** has log K_{ow} = – 4.5; it can be readily translocated but poorly absorbed. In the case of gorse not only has the spine (leaf) a very thick cuticle covered in wax,[3] but also it is covered in hairs (trichomes) that prevent droplets getting to the actual leaf surface (Fig. 2). So it was no surprise to discover that only 7% of glyphosate was actually being absorbed from commercial formulations[4] and field efficacy was also low.

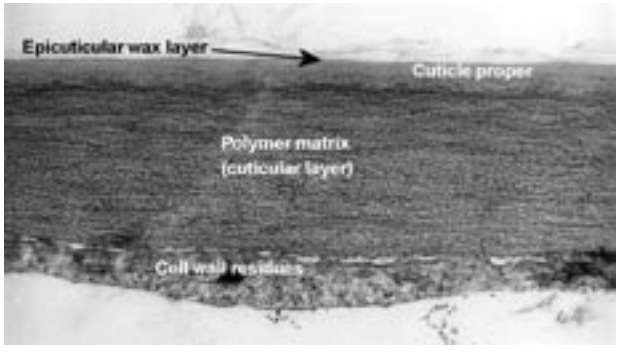


Fig. 1. Cross sectional representation of plant cuticle structure.

Uptake into leaves can occur in two ways, slow diffusion through the cuticle or physical flow through stomata (Fig. 3) that exist to absorb CO_2 for photosynthesis and expel

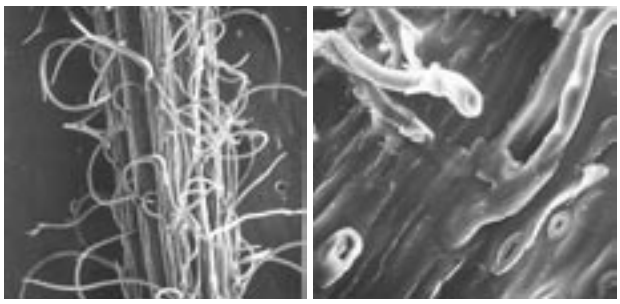


Fig. 2. SEM images of young gorse spine (left, x35) and close up of needle surface (right, x262) showing trichomes (hairs) and dense wax layer on needle surface.

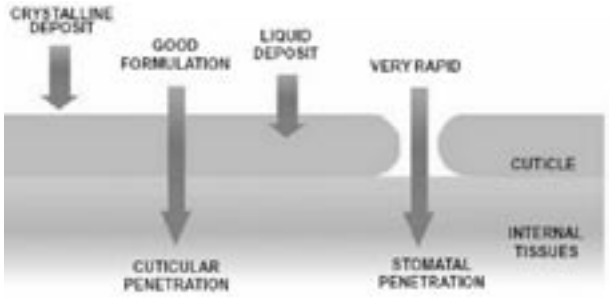


Fig. 3. Representation of xenobiotic uptake through leaf cuticle.

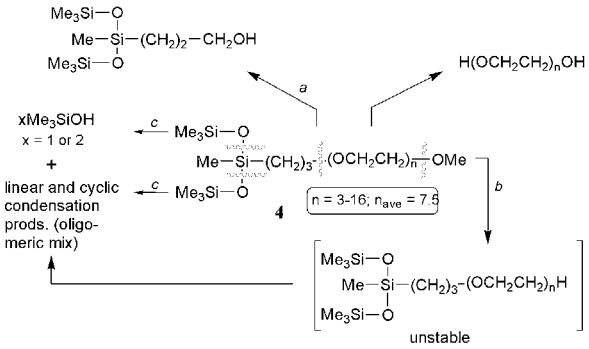
oxygen and water during respiration. Studies had shown[5] that the geometry and size of stomata determined whether liquids having a low enough surface tension could flow into the sub-stomatal chambers and hence into the leaf mesophyll. Water solutions with a surface tension of 76 mN/m were not able to flow into stomata for obvious biological reasons, and neither could Roundup solutions of **3**, so this pathway was unavailable. The advent of a material discovered fortuitously by the author (from an assortment of surfactants discarded by another researcher in Australia) provided a completely new and novel surfactant structure that had the ability to bring surface tensions down to levels never before achieved with agrichemical spray solutions.

The material was an organosilicone, an oligomeric mixture of trisiloxy polyethoxylate monomethyl ethers depicted by **4** (with n_{ave} = 7.5) (Scheme 1). Very dilute solutions of this trisiloxane surfactant, later marketed as Silwet L-77® (L-77) or Pulse®, were capable of wetting even Teflon surfaces, and could spread on a leaf surface 50 to 100 times more than other surfactant solutions. Solutions of **4** had surface tensions of 22 mN/m, a value well below the theoretical threshold required to infiltrate leaf stomata;[5] EtOH and Me_2CO have surface tensions of 22.3 and 23.5 mN/m, respectively. Proof that this happened was provided by measuring the percentage uptake of ^{14}C-glyphosate, with different concentrations of **4** over a period of time. Whereas normal cuticular diffusion is a slow process that requires several hours for substantial amounts of xenobiotic to be taken up into the leaf,[6] solutions with more than 0.3% **4** showed 80%+ uptake within a few minutes.[7] When the combination of Roundup® and L-77 formulations of **4** was tested on mature gorse plants, complete kill was obtained with one third the rate of the parent product formulation, which itself at full rate could only achieve two thirds mortality.[8]

The *real life* benefits of such technology are that glyphosate is used at about one quarter to one third of the rate per ha that would be needed without smart formulations. A recent survey[9] found that *ca*. 350 tonnes of glyphosate are being used annually. Forestry alone accounts for 144 tonnes of this and had the rates not been reduced, this would have exceeded 400 tonnes; national use would have been around 1000 tonnes p.a. Hence in the 20 years since smart formulations were introduced there has been a ***reduced input*** of around 5,000 tonnes in forestry and over 13,000 tonnes nationally. Prices of glyphosate products have dropped over that period but it is clear that national savings to NZ users are more than a billion dollars.

As usual, the explanation for these beneficial properties came after their discovery. The reason for such a low surface tension of a water solution of the organosilicone lies in the structure, size, and orientation of the surfactant molecules in the solution. Surfactants have a hydrophilic and lipophilic end in each molecule; the hydrophilic end associates with the water and the lipophile forms a tightly packed arrangement on the surface - essentially a monolayer surface film. So, in a water droplet, the lipophilic silicone moiety covers the surface thus having the initial interaction with the waxy leaf surface. In the process, some of the surfactant is *stripped* from the solution and lays a layer down on the wax, presenting the hydrophilic part for water to associate with and spread over. This process is fast, much faster than with conventional organic surfactants and the principles involved have been the subject of many theoretical studies.[10] It accounts for the fact that surfactant concentrations need to be well above the critical micelle concentration, so that there is excess surfactant to replace the material adsorbed into the cuticle. Evidence that treated areas retain some residual surfactant on the surface comes from the fact that if a droplet of water is placed in that region, it will spread and give a lower contact angle, in contrast to its behaviour on an untreated leaf surface. This again has biological implications as chemotactic reactions by insects and pathogens can be disrupted by *surface treatment* of foliage - but that's another story.

Another property of organosilicones such as **4** is that they are somewhat pH unstable. It is a drawback for long term *in-can* formulations and accounts for their use only as tank-mix adjuvants. It has a major environmental plus. Although stable at neutral pH for many weeks either in solution or in the presence of solid substrates, **4** can degrade within minutes in low or high pH environments. Extensive studies at Waikato University[11] have identified the degradation pathways. The potential sites for cleavage of the trisiloxane surfactant are illustrated in Scheme 1 and each generates more polar products. The Si-O bond is that most easily cleaved within the polymer and leads to silanols that are then subject to condensation and analogous depolymerisation to give a variety of linear and cyclic silanols and siloxanes. The cyclic derivatives are water soluble and known to be harmless to a range of natural organisms.



Scheme 1. Potential cleavage sites (*a-c*) in the abiotic degradation of **4**.

One of the major biological advantages of these organosilicone surfactants is that they do not show any phytotoxicity to the plant and they can reduce contact phytotoxicity with non-herbicidal products. Thus they are used also with insecticides, fungicides, and plant growth regulators on field or fruit crops. Their enhanced spreading properties mean that instead of pesticide residues being deposited in discrete spots, they are spread more evenly over a surface. Such even coverage gives much better protection against pests or diseases, but at the same time it can also result in faster degradation of pesticide residues because a much larger surface area and a thinner film of pesticide molecules is involved. This is a very important consideration for food crops.

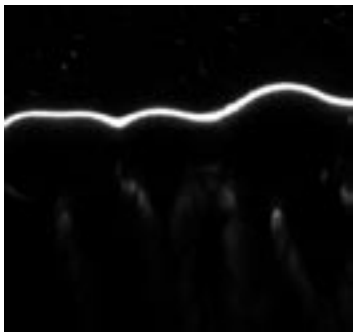
In subsequent years further work has led to the development of organosilicone blends that combine good spreading but no stomatal infiltration. Taking advantage of their ability to wet and stick to *hard to wet* surfaces, these new adjuvants are being applied to many horticultural crops where there may be large differences among leaf and fruit surfaces.[12] A further outcome is that with the correct formulation and application, spray volumes can be reduced. This allows for faster crop treatment, better use of expensive machinery and, most of all the ability to spray under the right conditions and avoid off-site drift. Such *concentrate* sprays are retained better, penetrate crop canopies more, and provide better target coverage than do standard spray applications. One such adjuvant product developed by a NZ company has been commercialized globally, so not only are there savings due to the technology but tangible revenues as well.

Plant Cuticles and Cuticular Uptake

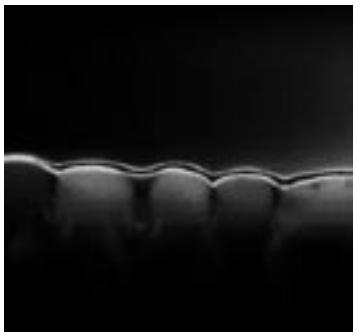
So what is the biology and chemistry behind these applications of formulation technology?

The predominant foliar uptake mechanism is by passive diffusion through the cuticle. The relative thickness of the cuticle layer vs the epidermal cell wall varies enormously among species. The cuticle has been shown to have a weak acid ion exchange capability and a high affinity for calcium ions,[13] as well as containing phenolic constituents[14] and reactive epoxy groups.[15] This variability illustrates the inhomogeneous nature of the cuticle and largely accounts, to date, for the failure to describe comprehensively the uptake mechanism of xenobiotics through the cuticle.

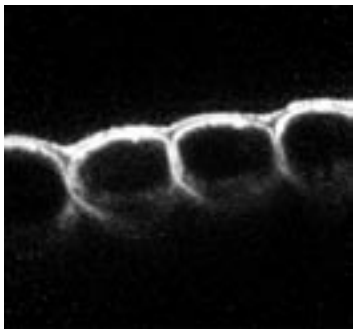
The process of xenobiotic uptake through leaf cuticles is an area of active study by Plant Protection Chemistry NZ (PPC_{NZ}) and other groups. An illustration is given by Fig. 4 using confocal laser scanning microscopy (CLSM) and fluorescent probes of different lipophilicity. It has been shown that the uptake and movement (in the presence of a surfactant) through the cuticle proper and into the epidermal cells varies greatly. The most lipophilic compound (Fig. 4a) is held completely in the cuticle; one that is less lipophilic diffuses evenly into the epidermal cells (Fig. 4b), while the most hydrophilic compound migrates through the epidermal cell walls (Fig. 4c).



a. Nile red



b. Rhodamine 6G



c. Fluorescein

Fig. 4. Visualisation of movement through a leaf cuticle by fluorescent dyes of different lipophilicity from confocal laser scanning microscopy; all treatments 0.05% dye concentration in presence of 0.2% surfactant after 24 h into bean leaf.

The diffusion of substances through the cuticle is described by Fick's first law, where the flux is the amount of solute that diffuses through a unit area per unit of time, viz.:

(mass/area) × time.

It is proportional to the concentration gradient and the diffusion coefficient of the xenobiotic.[16] Researchers in Germany[17] have defined the principal factors affecting uptake rates as *solute mobility* (which is affected by temperature,

solute molar volumes, and cuticular wax composition), *tortuosity*, and *driving force*. Tortuosity is the length of the diffusion path through the *limiting skin* in the cuticle, where the limiting skin represents only a proportion of the cuticle thickness, not its entirety, and it is influenced by the size and orientation of the cuticular wax crystals. The driving force is affected by the starting and continuing concentration of active ingredient in the *solution* on the cuticle surface, in the cuticular layers, and in the epidermal cell wall. Overall, and in simple terms:

uptake = solute mobility × cuticle tortuosity × driving force.

The German studies were performed with isolated cuticles *in vitro*, using an artificial infinite concentration system; spread area was ignored, and only plants that had astomatous upper leaf surfaces were used, mainly from non-commercial species. Studies by PPC_{NZ} with intact plants of many types (using leaf surfaces with or without stomata) showed that the influence of droplet spread area was highly significant *in vivo*.[18] Although two different formulations may contain the same concentrations of chemicals, *if the adjuvants are different or at different concentrations, then the residual droplet spread area will be different*. After droplet dry-down, the spray residue will be spread over different areas and the mass per unit area will vary.[19] These latter studies have shown that this solution residue or *initial dose* (ID) can be related to the mass uptake of xenobiotics.[18] This relationship has been validated with a wide range of formulations and plants that represent typical field rates and formulations.[20] An illustration of such a relationship is given in Fig. 5 for 2,4-D acid **2** in the presence of two quite different adjuvants into three plant species; very similar trends are seen for each species. Uptake per unit area at 24 h can be represented by the relationship:

Mass Uptake = $a[ID]^b$

where *a* and *b* are constants specific to the active on these species.

Total mass uptake can be determined from:

Mass $Uptake_{(nmole)} = a[ID]^b \times A$.

where A is the droplet spread area. The mass uptake relationship has also been used to establish the relative importance of species, active ingredient and its concentration, and surfactant, to uptake.[21] It was found that the concentration of active ingredient increases in importance with increasing lipophilicity, but that surfactant concentration is less important as the active ingredient lipophilicity increases. The relationship between the active ingredient concentration and the species is more important for the most polar compound, while the interaction of surfactant and species increases in importance as the lipophilicity of the active ingredient increases.

These modelling approaches are markedly simpler than the original German methods and they can be applied to any model or operational system and to all plant species. Furthermore, by using a quantitative molecular basis,

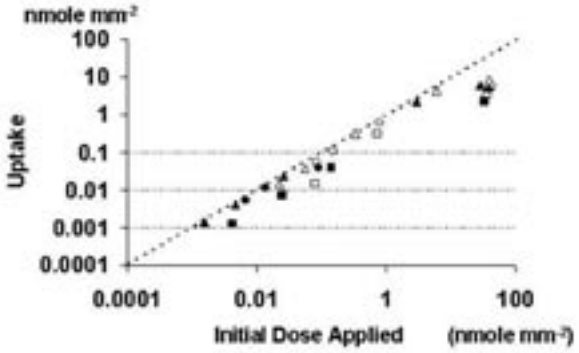


Fig. 5. Mass uptake of 2 (2,4-D) in the presence of polytriethylene glycol monododecyl ether (Δ, ○, □) and trisiloxane **4** (n_{ave} = 7.5) (▲, ●, ■) into *Chenopodium album, Hedera helix*, and *Stephanotis floribunda*, respectively; (---) is maximum uptake representing 100% uptake over the initial dose range.

dosages can be used to interpret interactions with endogenous plant constituents or structures from a biomolecular viewpoint. However, a much better understanding of plant leaf cuticular structures, as well as structure-activity relationships with adjuvants, is still required for a successful quantitative model of the uptake of the active ingredient. Specifically, there is a need to provide a numerical indicator (tortuosity factor) for plant cuticles that can then be incorporated into models of uptake so as to reflect species or leaf developmental differences. There have been electron microscopic studies of plant cuticles leading to a classification but the studies are qualitative only. It is known that cuticle thickness is an inappropriate input as, at times, thin cuticles can prevent uptake more than thick ones; thus cuticular structure is of prime importance.

As stated above, plant cuticles are complex, with layers of epicuticular waxes, embedded cuticular waxes, and a polymeric cutin (or cutan) skeleton. The waxes are solid at ambient temperatures but there are more or less *plastic* regions in both the waxes and the cutin which have been termed *amorphous* or *crystalline*. Solid state NMR now provides a measurement of cuticular wax or cuticle matrix that is *amorphous* or *crystalline*. Moreover, the proportion of *crystalline* to *amorphous* wax could provide a means of quantifying the *tortuosity* factor used in diffusion mechanism equations; this is the current approach used in a joint effort between Scion and PPC_{NZ} staff. Cuticles isolated from a range of plant species and analysed by ^{13}C solid state NMR techniques[22] show differences in their cross polarization, magic angle spinning (CPMAS) spectra. The appearance and measurement of the ^{13}C NMR signals account for carbon atoms at different sites of the alkyl chains that form the cuticle structures or their cuticular waxes. For example, fruit cuticles have very similar spectra and *crystalline/amorphous* (*c/a*) ratios. However, the holly cuticle shows a very different spectrum but similar *c/a* ratios to the fruit cuticles (Fig. 6), while ivy is different to all of these. It appears that species can be placed in groups based on their *c/a* ratios and spectral character, and this has considerable taxonomic as well as structural significance. Cuticles were previously characterised visu-

ally by means of their transmission electron microscopy images and grouped into six categories.[23] The species analysed by CPMAS NMR can be grouped by their *c/a* ratios and these groupings coincide very well with the microscopic categorisation.

Removal of cuticular waxes by solvent extraction caused some alterations to spectra and *c/a* ratios. Measurement of the *c/a* ratios showed that in each case there was a small but definite increase in amorphous character. Cuticles were also analysed after soaking 24 h in surfactant solutions. As the concentration of surfactant increased, so the amorphous proportion increased. These studies, while preliminary, appear very promising for the characterisation of isolated cuticles and the interaction with solutions and surfactants of the polymeric structure and the cuticular waxes.

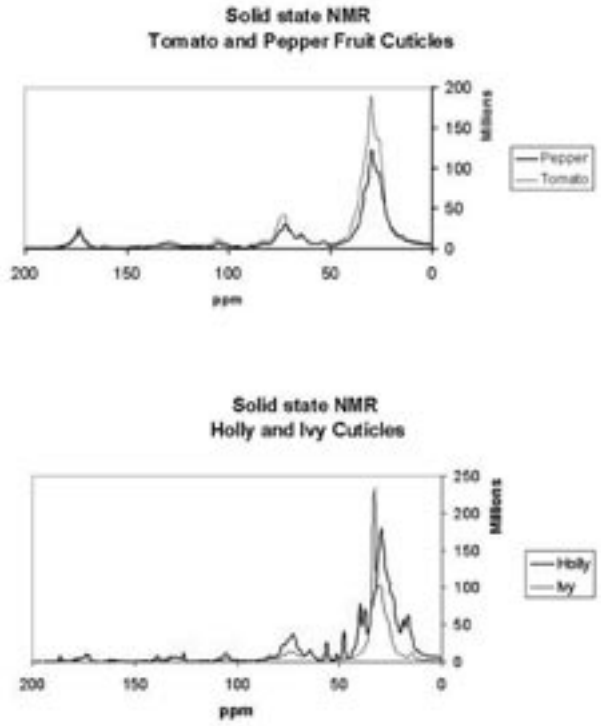


Fig. 6. ^{13}C CPMAS solid state NMR of isolated cuticles from tomato and sweet pepper fruit and holly (*Ilex paraguanensis*) and ivy (*Hedera helix*) leaves showing similarities and differences among species and sample types.

Conclusions

Arising from a real-life problem growing on the NZ hillsides, whose resolution has been an operational, environmental, and economic success, studies associated with this very practical problem have crossed traditional boundaries between biology, chemistry, plant science, and pesticide efficacy. They involve solution rheology, solution dynamics, liquid-solid phase interactions, plant biology, and plant morphology. Consideration must also be given to the liquid and solid phase structures, both for the spray solution and for the composite biological membranes that they interact with at the cellular or nano scale. A genuine journey from macro to micro technologies, involving physical, analytical and organic chemistries and using fundamental principles which are being incorporated into practical models.

Acknowledgements

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