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Improving self-defense in plants. Martial arts for vegetables*

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Abstract: From the dawn of agriculture there has been an ever-intensifying human effort to improve yields by having crops with enhanced biological similarity (i.e., characteristics of product, maturation time, height, color, etc.). The ultimate stage is to plant a crop where all individuals behave in exactly the same way, being clones of each other. This very intensive approach leads to loss of intrapopulation biodiversity and to unstable systems, prone to disastrous losses should anything go wrong.

Biological evolutionary success is usually derived from high adaptability to ever-changing external conditions. Highly specialized plants (such as certain orchids) or animals survive by correctly performing a high-wire act of enormous risk. External disbalances have catastrophic results on these species. Nature excels and corrects imbalances increased biodiversity within natural populations. Given this situation, we should study the defensive systems used by plants and improve on those natural systems.

INTRODUCTION

What we would like to present is a description of our original research ideas, some of the chemical results we obtained, and how we slowly had to adapt those ideas and our chemical project to what we learned from biology. Finally, if you allow me, I will suggest some lines which I think we chemists will have to follow in the future in dealing with agricultural problems.

Like many other postdoctoral students returning to their home countries, we had to start with very crude laboratory and equipment facilities, and deal with an almost nonexistent supply system. We soon realized that we would have to tackle a topic that would yield results to very simple chemistry. Even within phytochemistry we had to opt for an area that included simple solvents and reagents but still might result in publications.

In the early 1970s desertification in sub-Saharan Africa and other parts of the world was seen as a very important problem. It was a basic research topic in many areas, evetually leading to the UN Convention to Combat Desertification, the text of which was approved in 1992 [1]. In South America, the situation is not so dire, but it was one worth working on. Uruguay and Rio Grande do Sul are in a region that has reasonable average rainfalls, but alternating periods of intense rain with others of drought. This has resulted in a well-adapted xerophytic flora.

Analysis of waxes

Our initial studies aimed at the chemical characteristics that give xerophytic plants their ability to survive during droughts. An insight might be transferred to crop plants by selection of pre-existing char-

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acters in the domesticated varieties, resulting in a succesful drought-adaptation program based on chemistry. If we could unravel the mechanisms of resistance we should be able to have our first interesting proposals. After some false starts, we came to the study of epicuticular wax compositions, which seemed to be a topic that we could tackle even in our very crude working conditions.

Waxes behave as a water-proofing cover to plants and are responsible both for retaining internal moisture (drying out) and excluding rainwater (avoidance of excessive leaching of metabolites), protecting internal cells from disruption and death. Epicuticular waxes are a very primitive character in land plants, as waxes had to be operational even before vegetation managed to take the step from aquatic to terrestrial [2]. The basic component fractions, which are always present, include hydrophobic compounds derived from the acetate biosynthetic pathway. These compounds include straight-chain and branched hydrocarbons (odd-numbered carbon chains in the C_{21}–C_{35} range), esters of fatty acids and fatty alcohols (both even-numbered, the acids in the C_{16}–C_{32} range and the alcohols in the C_{22}–C_{32} range), plus the free fatty alcohols and free fatty acids roughly corresponding to those present as esters. These basic series show a range of components with a bell-shaped distribution, which is reasonable, as their action depends more on the overall compositions and properties than on those derived from specific functions or structures [2].

Our first samples came from *Cereus peruvianus*, a columnar cacti common to all the southern parts of South America, and from *Discaria longispina*, an aphyllous Rhamnaceae that is extremely well adapted to the dry spells of the Rio de la Plata basin. Our idea was that finding the common features in two different botanical groups would give us an idea of their adaptation mechanisms. The gross results are shown in Table 1.

Table 1 Composition of wax fractions [3].

Cereus wax (0.1% fresh plant)	
Hydrocarbons	65%
Esters	10%
Sterols	6%
Free acids	5%
Free alkanols	3%
Discaria wax (0.026% fresh stems) [4]	
Hydrocarbons	14%
Esters	18%
Free alkanols	22 % (lupeol 14% taraxerol 3%)
Free acids	11%
Hydroxyacids	37% (ursolic 60 oleanoic 38)

We had made the wrong assumptions. Both plants are well adapted to moisture conservation, one by having a cuirass of water-proofing agents, the other with a wax rich in compounds that are known to be rather poor water-proofing constituents, as is the case of the triterpenic hydroxyacids. Our error became more obvious when collecting more information. The epicuticular waxes from grapes (veritable drops of sweet water), keep the fruits from dehydration with a composition that is made up to 60% by oleanolic acid [5]. Different wax compositions have been reported depending on the age of a plant's leaves and between the upper and lower sides of some leaves [6].

All the while we were facing a growing resistance from our research students in tackling the "boring" TLC and GC work leading to "Tables of simple hydrocarbons". It was more interesting to study the physiological and physicochemical activities of specific wax constituents, or to work on the complex and more intriguing range of constituents usually present in the polar fractions of waxes. These compounds include *sec*-alkanols, long-chain aldehydes, -diketones, hydroxy-diketones, di-alcohols, many other triterpenes, phenols, resorcinols, flavonoids, coumarins, sugar esters, even caffeine [2,7–9]. Our work went that way.

Including biology in our chemistry

Some of these minor wax constituents, such as caffeine or xanthotoxin, were almost unexplainable under our initial very "chemical" biology. Why would a plant make such complex compounds, then extrude them in the wax if they are poor water-proofing agents? Under a more "biological" viewpoint they are easier to understand. When by some inborn metabolic or morphological change, a compound such as xathotoxin is extruded with the wax, a chance ecological advantage for the "deviant" individual can result, making it resistant to herbivores or pests. This more than compensates for the slight loss of water-proofing capacity.

Here it began to dawn on us that as chemists we were trying to use the very succesful analytic/synthetic approach linked to experimental confirmation that is the basis of our work. As chemists we break down a problem into bits, tackle them piece by piece, and verify the results by further experiments. When we come with solutions for all the bits, we put them together, and chemical problems crumble. It usually works remarkably well. This approach has a drawback when applied to things natural. This is partly due to the fact that many problems in Nature arise, not from the pieces themselves, but from the way the pieces interplay. Take the pieces apart, solve each on its own, and when put together again, the problem seems to have moved to some other part of the line. Sometimes, you sort one problem, you get two [10]!

This situation arises in agriculture, where we have a "non-natural" activity still directly connected with the "natural order". We humans have gone from hunter-gatherers to farmers/stock breeders in the last 10 000 years. What was done at the time? Plants were selected for their direct nutritional value, for their postharvest qualities and capacity for prolonged storage, or for their adaptability to monoculture. Even the initial stumbling efforts at domesticating plants and animals were such a success that human history as we know it is a description of the resulting technical and social progression. And monoculture in the sense of "crops" was the way to progress.

But agriculture was, and still is, carried out within the natural world. So whatever benefits us humans, 160-lb. omnivores, is bound to be of use to whatever other herbivores are out there. Imagine being a 10-mg insect trying to survive. After generations of living in a complex and mixed environment, where one edible plant can be miles away from the next, you fly into a plantation, where every plant is useful, and where you have thousands and millions of similar plants growing one foot from each other. It must be Paradise on Earth!

Mind you, from the human farmer viewpoint, that insects munch and suck is bad enough, but as Jonathan Swift said 300 years back, Nature has seen to it that "big bugs have little bugs upon their backs to bite 'em and little bugs have littler bugs, and so ad infinitum".

Insects are excellent pieces for dissemination of plant viruses. Insects move on their own, select the appropiate plants, and even inject the viruses in their new breeding grounds. Thus, even insects that, as Pogo would say [11], munch modestly, can graduate from nuisance to full-blown pests, becoming veritable Trojan horses! As in Troy, this means war. And since Biblical times, this war has been going on, sometimes with humans winning, sometimes with bugs bringing down whole nations.

We chemists were recruited into this war some 150 years ago. On the basis of what had been so effective a method for tackling synthetic dyes or explosives, we went to the lab, found out what killed insects and started working on those lines. Pelt them with chemical brimstone!! And everybody, from farmers to bakers, governments, and to the general society were ecstatic!! The first insecticides were crude and evil-smelling, others were too toxic for safe use. We modified those basic structures, found some that were more specific to insects. We even went back to Nature to find which compounds were active and how, and back to the lab to build on those ideas [12]. We improved the lethality, specificity, permanence, degradability, and their ability to stick or unstick on plant surfaces.

We can check what happens on a plant surface when it is growing exposed to natural conditions by observing plants in a vineyard left on their own and being colonized by nonpathogenic epiphytic microorganisms. This is interesting because these saprophytic nonpathogenic microorganisms tend to cover all the available leaf surface, affecting the wetting of the surface, and in turn are controlled by the water repellency of the surface [13]. In Table 2 we see that otherwise healthy leaves carry large loads of saprophytic flora, and cultures of a swab taken on the leaf surface give high counts of colony forming units (CFU) in different growth media. When the leaves are treated with the traditional copper salt sprays, the CFU counts are strongly depressed. The "normal" flora can be almost exterminated when more active pesticides are used. When sprayed with dilute essential oils it can be affected in varying percentages, and these treatments probably are similar to the conditions plants face in the wild (not under cultivation). With some oils sprays CFU counts are affected but not exterminated [14]. In our efforts to eliminate "pests" we sometimes exterminate nonpathogenic saprophytes. And when they are gone we run the risk of seeing the same leaves occupied by nastier neighbors!

Table 2 (14) CFUs/g leaf.

Treatment	None	Cu spray	Rosemary	Citronella	Camphor
Growth Medium					
EMB	10400	360	1100	8300	3220
LB	20500	240	900	7200	2860
WLN	5700	340	2000	10000	3280
YEPD	7000	280	600	5100	3140
PDA	9600	70	500	5000	2980

Nature, except for sporadic catastrophes, is very parsimoniuous when dealing with the extermination of species. Each species works because it has an adequate adaptation to a certain ecological niche. Conditions tend to change gradually in Nature. Sometimes, they move further, sometimes they go backwards. They are seldom (NEVER) static. From the insects' point of view, the new natural situation was the appearance of a shower of noxious and toxic chemicals that killed them.

Plants and animals in Nature are not lonely creatures. They are parts of greater populations that are connected in space and time. If the external situation changes gradually, as they usually do, within that connected population there are individuals with intrinsic properties that make them more efficient (or less inefficient) in the new situation and thus adaptable to the changes. They and their offspring survive and thrive, the others slowly fade away. When we observe Nature we see a specific set of species and conditions, but they are not immutable, they are continuosly being selected by external changes. Some types become extinct, but their relatives survive. Extinction in this way is part of the evolutionary process, in the same way death is a part of life. So agrochemicals became the new driving force for the selection of resistant individuals and for the breeding of "whatever-resistant" insects.

Control of resistance

Are we damned to always select the more resistant pests when we use chemistry to solve our problems with Nature? Now, a word of hope. The first area in which the impact of using chemicals on natural populations could be observed was the use of antibiotics. They were supposed to be the end of infectious diseases. Nobody took Darwinian "survival of the fittest" into account. Yes, we did exterminate innumerable noxious (and not so noxious) microorganisms. But we did it at a pace that was almost natural in its gradualism, without ever managing complete exterminations. We allowed the fit to survive, to go through a mixed population phase, and now we clamor because we have selected populations that are resistant to our antibiotics of first, second, or whatever generation!! This was a textbook demostration of Darwinian evolution in action.

Sometime back Darwinian retro-evolution was tried on this field. Macrocyclic antibiotic ill-use had resulted in microbial populations with more than 20% resistance to these last-resort antibiotics, getting to the point of making them useless. Resistance had increased in all samples taken from throat swabs of patients in Japan, North America, and Europe. In the early 1990s in Finland it was decided to apply a program of strict control in their use. No more erythomycin for colds and other virus infections. Use dropped by 80%. The interesting aspect is that 4–5 years later, resistance started dropping back from almost 20% to 8%, and was still dropping at the last count. Darwin was right. The selective pressure dissapears, the selection for resistance drops, a balanced population takes over again [15]!

We should be thinking along these same lines when dealing with agriculture, and we chemists have to learn to think not about exterminating our foes, but of outclassing them.

We have to learn how to work on a regulated biodiversity, to use chemistry to analyze the best mixes of crop varieties to keep pests and attacking insects always on the verge of their complete adaptation to the crop, but stopping them before the adaptation is complete and the attack irresistible. We can study the plants we use as crops, and taking wheat as an example we soon realize that we have exploited only a small group of a whole crowd of relatives, lesser members that can be incorporated to create a biodiverse population that can be economically used. Instead of working to make our monocultures less and less biodiverse, we should consider making them biodiverse, but manageable and exploitable.

We can add a temporal scale to biodiversity, outmaneuvering our enemies by changing the environment before they manage to evolve and carry out one of their massive attacks. By adopting natural rythms and tactics, we might come with a more sustainable system, keeping our enemies constantly off balance. The situation is critical, but not hopeless.

How will a small research group from Latin America working on plant epicuticular waxes survive? We will have to analyze the importance of waxes in the natural system in which they have evolved and translate that into useful adaptations in a scheme where you try to contain but not exterminate. For, how do insects recognize the right plants for feeding or oviposition? By smell and taste of the most external layers, in particular, the epicuticular waxes!! So there still is an interesting prospect for continuing and improving a study that began because the only solvent we could count on getting was petrol ether!

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