PRINCIPLES AND APPLICATION OF THE INTEGRATED PEST MANAGEMENT APPROACH. BIOLOGICAL PESTICIDES

FEDERICO M RUBINO [1], STEFAN MANDIC-RAJCEVIC [2], EZRA J MREMA [2], CLAUDIO COLOSIO [2].
Department of Health Sciences, Università degli Studi di Milano, San Paolo Hospital.
[1] LaTMA Laboratory for Analytical Toxicology and Metabolomics, v. A. di Rudinì 8 Milano, Italy
[2] International Centre for Rural Health – WHO Collaborating Centre, v. San Vigilio 43 Milano, Italy

Correspondence Contact: federico.rubino@unimi.it

Summary. Protection of crops from pests and from ravenous animals was long accomplished with the use of 'natural' means, before the introduction of 'modern' synthetic chemicals as pesticides in the 20th century. The widespread use of synthetic organic chemicals as pesticides in the last several decades has contributed to the 'background' contamination of all environmental and biological compartments with trace amounts of small organic compounds, the combined effect of which on the health of humans and on the general well-being of complex ecological systems is far from being understood. In particular, it is claimed that the presence of trace amounts of such compounds in human food may contribute to impair the health of sensitive individuals, especially in the developmental and early-age stages of life, although there is not a clear-cut consensus on this problem. The request from consumers, mainly in developed Western countries, of food produced under perceived healthier conditions than those of mass-production, has prompted a backslash of interest in traditional, lower-yield techniques such as 'organic farming' and thus the necessity to protect consumers from unsubstantiated claims of food quality through the issuing of voluntary codes by producers and of guidelines such as *Codex Alimentarius* at the level of international Organizations. Another driving force into a renaissance of 'traditional' farming techniques is understanding that they can be successfully merged to 'modern' ones to achieve better productions with lower environmental impact, lower consumption of selective but expensive Plant Protection Products, lower contamination of food with residues. Another

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trigger to seek alternatives to the conventional means of pest fighting is acknowledgement that later generations may develop resistance to current pesticides in a continuous ‘chemical war’ between human scientific intellect and natural evolution of organisms. Traditional agricultural wisdom long recognized the power of coenobioses and used co-cultivation of different crops as a means to deter parasites by exploiting the natural emission of insect repellents and mutual fight between natural hosts of crops and invading organisms. Of course, since even ‘natural’ substances long employed to fight parasites of plants and food are intrinsically toxic not only to target species but also to several others, including the human, risk assessment of their use is mandatory to fully benefit of their strength without unnecessary risk for producers, consumers and the global environment. The recent exploitation of natural as well as of engineered organisms and of their toxic products as pesticides is another means the reach and limitations of which need to be fully understood. Examples of how these seemingly conflicting requirements have been or can be harmonized will be presented and discussed.

Keywords: *Bacillus thuringensis* toxin, Biological Agriculture, *Codex alimentarius*, fenoxycarb, methoprene, neem oil, nicotine, pyretrins, rotenone, spinosad, ecdysteroids.

1. Introduction

It is now more than 5,000 years that selective growth of plant biomass is the main strategy through which mankind has sought its sustainment. Agriculture is an intrinsically non-sustainable activity which depletes unscathed soil of its stored chemical nutrients, which need to be replenished by fertilization, and an artificial ecosystem in which natural prey-predator equilibrium is modified.

Both issues were solved, often separately, across the history of humankind by different means which gradually entailed increasing levels of complexity to yield the agro-industrial complex of present times. *Cut-and burn*, determining the short-term release of stored mineral nutrients into the ground was the first, and still practiced attempt to exploit natural forested areas for agriculture. *Culture rotation* allowed replacement of exhausted reservoirs of organic nitrogen through the use of the nitrogen-fixing symbionts of leguminosae. *Chemical fertilization* was chronologically the last step, which uses agricultural land as the physical support of plant growth by feeding externally produced nutrients: nitrogen, phosphorus, potassium.

The long-term consequences of land exploitation were well-known deep in the past and were the underlying reasons of most human history. Depletion of local resources due to exhaustion of land fertility led to economical crises, to degradation of food quality and availability, down to famine, which in turn was the cause of epidemic, chronic and long-term diseases. Desertification drove to mass migrations to unexploited areas or to conflicts with earlier dwellers. The Biblical image of the Four Knights of Apocalypses materializes the escalating effect of environment perturbations on the fragile equilibrium of pre-industrial Europe (Diamond, 1999).

In the Euro-American global system starting with the Industrial Revolution these concerns were apparent and led to a chase for technological solutions which are at the root of current economic development and of its backslash. As early as 1898, Sir William Crookes warned: "*[…]England and all civilized nations stand in deadly peril […] As mouths multiply […] food sources dwindle […] Any drop in wheat production will threaten* racial starvation", (Hager, 2009) and in 1912 the German chemist Fritz Haber developed the industrial synthesis of ammonia from atmospheric nitrogen for which he was awarded the Nobel Prize for Chemistry in 1919 (Nobel Foundation, 2012a).

The artificial ecological system of mono-culture is much more sensitive to the effect of crop-spoiling organisms than natural coenobioses. To ensure high crop yields the use of chemical pesticides was exploited as early as the mid-XIX century, when the efficacy of copper sulphate against downy mildews (*Peronospera*), a fungus pest of vineyards, was serendipitously discovered by Pierre-Marie-Alexis Millardet and the use of the Bordeaux Mixture was popularized. It was only after the expansion of specialty organic chemistry production that organic pesticides were developed, starting from DDT, the discovery of which in 1939 earned Paul Hermann Muller the 1948 Nobel Prize in Physiology or Medicine (Nobel Foundation, 2012b). The introduction of rationally selected high-yield cultivars of staple food crops was the third pillar of the Green Revolution which flourished especially in the post-World War II and decolonized extra-European world, an achievement for which one of its pioneers, Norman Ernest Borlaug was awarded the Nobel Peace Prize in 1970 (Nobel Foundation, 2012c).

Supply of water and food to the ever-increasing population of large urban areas developing in the mid-to-late XIX century as industrial centres called for substantial advancements both in technology (in 1908 Chicago was the first city in the world to adopt chlorination disinfection for drinking water) and in the perception of the conflicting roles of private enterprise and of public interest. In his 1906 bestseller fiction novel *The Jungle* Upton Sinclair denounced the poor conditions of the U.S. meat packing industry. The public indignation raised was instrumental in passing, a few months later, of the 1906 Pure Food and Drug Act and the Meat Inspection Act in the U.S.A.

In the Old Continent, opposition to the perceived excesses of industrialism in farming spurted philosophically-grounded opposition, culminating in Rudolf Steiner's 1924 *Lectures on Agriculture*, which heralded Biodynamic Agriculture, with its elaborate techniques, the outcome of which was however mostly anedoctal and not rationally improvable, differently from the scientific approaches pioneered in the U.K. by Sir Albert Howard (the "father of organic farming") and by Lady Eve Balfour and by J.I. Rodale in the United States. The Ivy League Cornell University manages in the USA the oldest estate dedicated to research on organic farming within its Agricultural Experimental Station (Cornell website, 2012a,b).

Among the tenets of organic farming are the use of manure for fertilization, the availability of which is limited by that of agricultural land for pasturing of herds; the use of crop rotation to reconstitute organic nitrogen reservoirs, which also limits agricultural land available for human food crops and the exclusive use of naturally-available chemicals, such as plant secondary metabolites to fight crop pests.

2. Plant Secondary Metabolites as Pest Control Agents

Most plant secondary metabolites are the natural response of plants to parasites and plant-eating organisms - not only insects – but also birds and terrestrial animals.

As an example, *Colchicum spp* is a typical flower on mountain slopes. To avoid being eaten by wild animals, the plant bio-synthesizes in its tissues (flower, stem and root) colchicin, a cytotoxic anti-tubulin secondary metabolite. As a consequence, also cattle (cows, sheep, goats) grazing in mountain pastures naturally avoid pasturing near the flowers and teach the young individuals not to. The same pharmacological properties of colchicin which lead to toxicity are also traditionally exploited in human medicine to treat several diseases, such as gout.

2.1. CHEMICAL AND TOXICOLOGICAL ASPECTS OF PLANT SECONDARY METABOLITES AS PEST CONTROL AGENTS

A number of plant secondary metabolites have been traditionally employed with more or less awareness by traditional farmers to fight pests and to deter ravaging animals from orchards and cultivated fields.

Tradition agricultural practices long identified what are also known as 'lucky pairs' of green vegetables and co-cultivated specific plants together with economically valuable ones (***companion planting***), with the rationale that one plant deter pests from feeding on nearby plants of a different species. In traditional practices, co-cultivated plants are each individually useful as food and can be cultivated in association, in order that those which contain or emit into the environment pest-deterrent secondary metabolites also protect the others.

- Garlic (*Allium sativum*) and onion (*Allium cepa*) rows in green gardens deter most insects and some mammals such as rabbits through the biosynthesis of an array of sulphur-containing secondary metabolites derived from cysteine and from methionine, which are also responsible for their strong taste and possibly also of their healthy-food properties;
- Tomato deters insects pests such as cabbage maggot (*Delia radicum*) and asparagus beetle from feeding through the emission of strong odorants, which are also sensed by neighbouring plants, which in turn elicit preventive anti-feedant responses;
- even exotic plants first introduced to Europe from the New World in the XVI century were initially more appreciated by gardeners for their unusually bright flowers and as insect deterrent also to other ornamental plants in Italian gardens than for their use as staple food, as in the case of tomato, tobacco and potato.

Companion planting as a pest-deterring strategy has advantages and disadvantages. Among the former are:

- no need for preparation of products and for repeated application on cultured areas;
- little to no cost for farmer or even some products may also have an independent food or commercial value.

This practice also faces some limitations and disadvantages, among which:

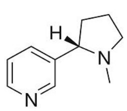
- companion plants need extra space in orchards and may consume additional fertilization;
- some plants may have different agricultural needs (a different watering schedule, may entail further work, may be inadequate for climate or
- may be harmful to farm animals due to the content of toxic compounds which are those needed for their use as pest control agents; this is also true for children who currently perform jobs in traditional farms;
- some may be regulated due to the production of pharmacologically active secondary metabolites whose effect on humans are socially undesired in specific environments.

In order to exploit the best opportunities, products may be purposely prepared from their natural sources to be applied on cultures without the need of cultivating the source plant in the same place where its effects are needed. Among the simplest traditional forms is maceration in water of exploited stems of tomato or of faded ornamental geranium and use of the water to irrigate small cultivations, such as ornamental flowers.

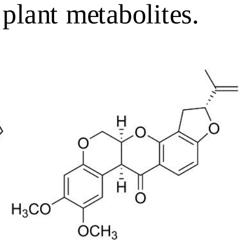
There are selected advantages in the large-scale application of this practice, which may grow into an independent economic activity:

- supply can be made independent from local need and would not consume local resources;
- the dose of the pest-controlling agent can be adjusted to actual need, even in the case that it is higher than that allowed by the natural content in the co-cultivated plant;
- there may be further economic advantages, such as marketing opportunities for peripheral economies (some valuable products grow in otherwise unproductive tropical areas), process wastes can be valorized (such as in the case of tobacco nicotine, *see* below) and use of local plant-derived products can reduce dependence of some regions from import or from corporate policies.

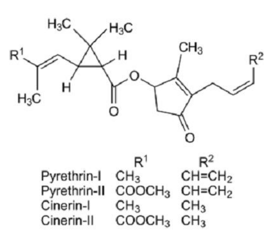
While several traditional practices only have local diffusion and most have been superseded by the availability of synthetic chemicals as pest control agents, a few examples of the sustained use of secondary metabolites are still of practical importance. A resurgence of once-practiced treatments and even the setup of new ones have been encouraged by the rise in popularity of 'organically-produced' food especially in some 'First World' areas. The merits and demerits, sustainability and hazard of the rising demand of such products will not be discussed. To protect consumers from the consequences of unsubstantiated claims and the reputation of responsible producers, voluntary codes of good practice in the production of different denominations or commercial brands of such agricultural and dairy products have been agreed upon by producers' associations. In most cases pest control with chemical substances can only be accomplished with the use of few substances, all being secondary plant metabolites.



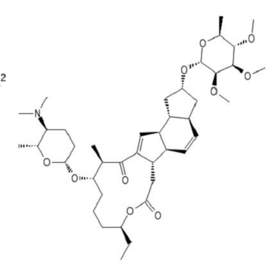
Nicotine



rotenone



natural pyretrins



spinosad

Figure 1. Chemical structures of the main plant protection products of natural origin.

Those agreed upon by the generality of protocols are: nicotine, rotenone, natural pyretrins, spinosad, neem oil and *Bacillus thuringensis* toxin. The chemical structures of some important products are reported in Figure 1.

NICOTINE

Nicotine is the major alkaloid of the tobacco plant (*Nicotiana tabacum*). Its biosynthesis occurs in the roots and the substance is transported to the leaves, where it acts as an anti-feedant due to its fairly high concentration (0.6 to 3% of dry leaf weight) and to its activity as a cholinergic agonist. Its neuroactive properties were exploited by the native Americans and soon copied by the European explorers and settlers who imported the habit in their home Countries since mid-late XVI century. Its success as a leisure consumer product prompted the rise of the first non-food mono-culture at first on the east coast of North America (Virginia) and later also in Europe and in the Middle East, where tobacco cultivation afforded a revenue also from poor soils. The health hazard posed by tobacco smoking was well known as early as the XVII century: King James Ist of England wrote in 1604 the pamphlet '*A Counterblaste to Tobacco*' and authorized to levy the first tobacco tax to counter its use.

The insecticidal use of tobacco juice precedes the isolation of the tobacco alkaloid in 1836 and its use as a homicidal poisoning (a dose < 10 to 60 mg is fatal to a child or a man, respectively) was at the root of analytical toxicology since the late XVIII century. Nicotine sulfate is a side-product from tobacco industry (current production is in the 2,500 tons/yr) as the alkaloid is recovered from the process of wetting tobacco leaves to soften them after curing and before manufacturing tobacco products such as cigars and cigarettes.

To mimic and enhance the insecticidal effect of nicotine while minimizing its toxic effect on humans and animals, a class of synthetic pesticides (neo-nicotinoids) was developed in the 1980s and -90s. Important products of this class are: Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, Nitenpyram, Thiacloprid, Thiamethoxam. Imidacloprid is currently the most widely used insecticide in the world. Most neonicotinoids do show much lower toxicity in mammals than insects, but some breakdown products showed to be toxic to mammals. Recently, the use of some members of this class has been restricted in some countries due to evidence of a connection to honey-bee colony collapse disorder, although the topic is still somewhat controversial.

ROTENONE

Rotenone is a secondary metabolite classified into a specific sub-family (rotenoids, around 60 different molecules identified) of isoflavones. It is biosynthesized in the leafs and transported to the other tissues such as seeds and stems of several tropical and sub-tropical plants of the genera *Derris* (in Asia), *Lonchocarpus* and *Tefrosia* (in Sud America). Although it was first discovered in *Lonchocarpus nicou*, by the French botanist Emmanuel Geoffroy in the late XIX century, its ethno-botanical use as piscicide by local hunter-gatherers may be ancient, as early explorers noted that Peruvian natives used crude extracts of the Cubé plant to stun fish for eating. Rotenone has been used commercially as a garden insecticide since the middle 1800's, primarily in the UK, as a non-toxic alternative to the lead- and arsenic based pesticides then in common use.

Rotenone is still currently widely used as a garden insecticide, and in organic farming as a natural, botanical insecticide to kill potato, cucumber and flea beetles, cabbage worms, raspberry and asparagus bugs, as well as most other arthropods. In California, about 200 kg of rotenone is used per year as an insecticide for organically grown lettuce and tomatoes. In Vietnam rotenone is currently studied and applied in agriculture and pisciculture. It has been recently reported that rotenone was identified in two cultivated species, *Derris elliptica* Benth and *Derris trifoliata* (about 10,000 hectares in Soctrang and Binhduong provinces) and that rotenone-based preparations are used in Mekong Delta to kill fish in shrimp aquaculture.

In the ground rotenone has a half-life of *approx.* six days, due to photochemical oxidation, while in water it can persist at piscicidal concentration for *approx.* six months.

Its insecticidal activity, by contact and by ingestion, and its piscicidal activity by absorption through the gills, is due to its pharmacological properties as a selective inhibitor of the mitochondrial electron transport chain from iron-sulfur centers in complex I to ubiquinone. This interferes with NADH during the creation of usable cellular energy (ATP).

Due to its very poor absorption by the gastrointestinal tract of humans its lowest lethal dose for a child is 143 mg/kg, which is unlikely to be reached accidentally, since the compound is a strong irritant and has a very bad taste. Only one fatality due to suicidal ingestion has been reported in the literature.

In the last decade concern has been raised that rotenone can cause a toxic Parkinson disease in rats and in 2011, a US National Institutes of Health study showed a link between rotenone use and Parkinson's disease in farm workers (Tanner *et al.*, 2011). Currently in the U.S.A. rotenone is no longer allowed in organic farming.

PYRETHRINS & PYRETHROIDS

Pyrethrum is the dried powder obtained from the flowers of white Chrysanthemum (*Chrysanthemum cinerariifolium* and other similar species) in West Africa. Pyrethrin is the name of the natural product extracted from pyrethrum. Chrysanthemum has no relationship with *Anacyclus pyrethrum* DC, an Asteracea which does not contain pyrethrin but has other uses.

Chrysantemum has a long tradition of ethnobotanical use as insecticide, possibly starting in Persia around 400 BC, although the employed species, *C. roseum* Adam and *C. coronarium* Linn. have a much lower content of pyrethrins. Details of its diffusion from West Africa and in Near East to the Balkans (Dalmatia) by the late XIX century are not clear, however it is known that its insecticidal properties were serendipitously (re)discovered by a German lady in Dubrovnik who started marketing a dried flower powder, the use of which reached the U.S.A. by 1860. Commercial plantations were established in British West Africa (Kenya) and in India in the early 1930s in Kashmir Valley, Palami and Nagili Hills. Yields in commercial plantations is of 200 to 600 kilograms of dried flowers per hectare, with a content of 1 to 1.3% by weight of pyrethrin. In 1972-74 the total production of pyrethrum dried flowers was 27,000 tonnes and by now three East African countries, Kenya, Tanzania and Rwanda produce *approx.* 80% of total (Panda, 2000).

Pyrethrum works against a very wide range of insect pests and development of resistance is not frequent (Panda, 2000).

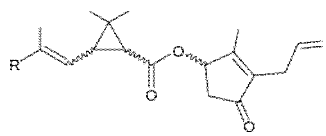
The active principles of Pyrethrum flowers, pyrethrins, are fast-acting (knock-down) contact poisons which disrupts the nervous system cause paralysis of the insect. The electrophysiological studies by Narahashi (1971) demonstrated that the highly lipid soluble pyrethins distribute in the lipid bilayers of the nerve cell membrane and exert their action on sodium channel proteins. The rising phase of the action potential is caused by sodium influx (Na^+ activation), while the falling phase is caused by sodium activation being turned off, and an increase in potassium efflux (K^+ activation). Pyrethroids interfere with the changes in sodium and potassium ion currents by locking the Na^+ channel in the 'open' position, so that nerves cannot de-excite. Activity on the corresponding ion channels of warm-blooded animals is much lower, thus making these compounds safe as insecticides.

Pyrethrins also degrade very quickly in sunlight, moisture and oxygen, thus presenting little environmental concern but also having a short-lasting activity which requires repeated applications. Since pyrethrins are also bio-degraded by target animals, their potency can be enhanced by co-formulating with piperonyl-butoxide, a semi-synthetic inhibitor of P450 enzymes, which stops their bio-inactivation by insects.

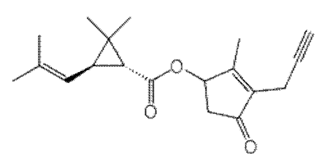
The structures of pyrethrins were identified starting in the 1920's by the Dalmatian-born, Swiss-naturalized chemist Lavoslav (Leopold) Ružićka who studied the insecticidal compounds of a plant of his native Croatia while working at ETH in Zurich with his mentor Hermann Staudinger (Nobel Prize in Chemistry 1959). He became the founding father of modern terpene chemistry and biochemistry and of rational perfume chemistry (acting as a consultant of Firmenich) and was awarded the 1939 Nobel Prize in Chemistry.

Following his studies, it was understood that pyrethrum extract is a mixture of three natural esters of chrysanthemic acid (Pyrethrins I: pyrethrin 1, cinerin 1 and jasmolin 1) and three esters of the related pyrethric acid (Pyrethrins II: pyrethrin 2, cinerin 2 and jasmolin 2), two fairly complex compounds, which feature a cyclopropyl ring in their structures and three stereogenic centres. The use of pyrethrins was expanded by a non-corporate British research institution, Rothamsted Research, starting with the development of a total synthesis of all-racemic pyrethrin I, which could only be accomplished with 1% yield but was in principle independent from the natural supply coming from the East African colonies and from India, which could be limited by interruption of sea trade at wartime.

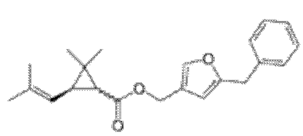
Further steps led to the preparation of synthetic analogues involving simpler chemical synthesis and seeking an improved photo-resistance (pyrethroids). In the 1960s a first group of derivatives of pyrethrin I and II were prepared by keeping the portion of chrysanthemic acid. These derivatives were still too photo-sensitive but suitable changes in the alcohol group with respect to the natural products led to an enhancement of insecticidal activity and to an even lower mammal toxicity. A further step was taken by altering also the structure of the chrysantemic acid fragment so that the new products now showed even less similarity to the original structures of the pyrethrins (Figure 2).



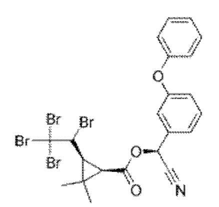
Allethrin
(all-racemic pyrethrin I)



Prallethrin



Resmethrin



Tralomethrin

Figure 2. Chemical structures of some synthetic analogues of natural pyrethrum insecticides.

SPINOSAD

Spinosyn and its analogs are a family of over 20 natural fermentation products produced by a recently discovered soil micro-organism, *Saccharopolyspora* *spinosa*. They are polyketide-derived tetracyclic macrolides with a 21-carbon, 12-membered tetracyclic lactone structure, to which are attached two deoxysugars, tri-*O*-methylrhamnose and forosamine (Sparks et al., 2001). They are the active ingredients in a family of insect control agents with a *novel neurotoxic mode-of-action*. Spinosoid binding disrupts acetylcholine neuro-transmission, likely at the nicotinic receptor, but also acts as a GABA receptor agonist. The resulting effect is a hyper-excitation of the nervous system which kills the insect. Spinosad is so far non-cross-resistant to known insecticides. One main advantage of spinosyns is that, although they are slower than pyrethroids to penetrate the insect larvae cuticle, they are not metabolically deactivated and thus only low doses are needed for having the effect. As an example, Spinosad was first registered as a pesticide in the United States for use on crops in 1997 at a labelled use set at 1 ppm (1 mg a.i./kg of grain) while its toxicologically established Maximum Residue Limit (MRL) is of 1.5 ppm, *i.e.*, higher by 50% than the useful dose. To exploit the chemical diversity allowed by this new bioactive scaffold, over 1000 *synthetic* spinosoids were also developed and tested (Creemer *et al.*, 2000). Spinosyns are readily degraded in moist aerobic soil (half-life, 0.3-0.5 d) by photolysis or a combination of metabolism and photolysis (Kirst, 2010).

NEEM OIL

Neem (*Azadirachta indica*) is a fast-growing evergreen mahogany-like tree ranging in height from 12 – 24 m, belonging to family Meliaceae. While native of India, Neem trees are widespread in tropical and subtropical regions of the world, including semiarid and wet- tropical regions (Lewis and Elvin-Lewis, 1983). Neem oil is obtained by grounding Neem seeds are into a powder that is soaked overnight in water and sprayed onto the crop. Neem acts on plants as an anti-feeding, repellent, and egg-laying deterrent since it also suppresses hatching of pest insects. Insects starve and die in a few days. To be effective, it is necessary to apply at least every ten days. Plant extracts contain several bitter compounds, among which the triterpenoids nimbin, nimbinin and nimbidin, which were discovered in the 1940s and following years by the Pakistani organic chemist Salimuzzaman Siddiqui (Siddiqui, 1945), who had earlier isolated the alkaloid ajmaline from *Rauwolfia serpentina*. Another compound is the limonoid azadirachtin.

In 1995, the European Patent Office (EPO) granted a patent on an anti-fungal product derived from neem to the US Department of Agriculture and to the W. R. Grace and Company. The Indian government challenged the grant, claiming that the long local use of the plant product (Foreign Prior Art) for agricultural use hampered issue of patent protection (Kadidal, 1997). This controversy is one important case-history in the ‘biopiracy’ polemic (Shiva and Hollabhar, 1996) on the commercial exploitation of natural substances for pharmaceutical or for other industrial use on which the 1993 Convention on Biological Diversity (CBD, presented at the United Nations Conference on Environment and Development in Rio de Janeiro in 1992) attempts to recognise and formalise the value of traditional knowledge and to ensure that holders of traditional knowledge are compensated accordingly (Andrzejewski, 2010). After a ten-year litigation, the appeal from Grace, raised after, in 2000, the EPO had ruled in India's favour in 2005, was lost and the EPO at last revoked the Neem patent.

BACILLUS THURINGENSIS

B. thuringiensis (Bt) is a Gram-positive, soil-dwelling bacterium was first studied as a microbiological curiosity, as the cause of a disease of silkworm in Japan (by Shigetane Ishiwatari in 1901) and of the *Schlaffsucht* disease in flour moth caterpillars (by Berliner in 1911). It is closely related to *B.cereus*, a soil bacterium, and th *B.anthracis*. Further studies showed that a small crystallisable protein of the micro-organism is responsible for the toxic effect of the infection in the target species. Upon sporulation, Bt forms crystals of proteins the sequence of which is encoded in plasmids, the δ-endotoxins, also called crystal proteins or Cry proteins. The crystals are aggregates of a large protein (about 130-140 kDa) that is actually a pro-toxin. The crystal protein is highly insoluble in normal conditions, so it is entirely safe to humans, higher animals and most insects. However, it is solubilised in reducing conditions of high pH (above about pH 9.5) - the conditions commonly found in the mid-gut of lepidopteran larvae and is thus amenable to degradation by proteases present in the insect gut. The proteolytic process yields the Cry toxin, a pore-forming sub-unit which assembles into the insect gut cell membrane, forming a pore, which in turn causes in cell lysis and eventual death of the insect.

B. thuringiensis is active against moths and butterflies, flies and mosquitoes, beetles, wasps, bees, ants and sawflies and nematodes but has little or no effect on humans, wildlife, pollinators, and most other beneficial arthropods since these lack appropriate receptors.

The first formulation based on Bt was developed in France in 1938, under the name “Sporéine”, mainly to kill flour moths, although the first well-documented industrial procedure for producing a Bt-based product dates from 1959, with the manufacture of “Bactospéine” under the first French patent for a biopesticide formulation. Commercial formulations of Bt consist of spore/crystal preparations obtained from cultures in fermentors; the preparations are dried and used in a granulated or wettable powder formulation for use as a spray (Sanchis & Bourguet, 2008). B. thuringiensis-based insecticides are often applied as liquid sprays on crop plants, where the insecticide must be ingested to be effective (Beeglea & Yamamoto, 1992).

A boost in the use of Bt came in the mid-1980s when genetic engineering techniques were developed at industrial level and allowed to encode the DNA for the pro-toxin directly into the genome of the crop to be protected (trans-genic *or* TG crops,), in order that the protein is expressed by the plant itself, without need for application of the pesticide. There are several advantages in expressing Bt toxins in transgenic Bt crops, namely: (a) the level of toxin expression can be very high, thus delivering sufficient dosage to the pest; (b) the toxin expression is contained within the plant system, hence only those insects that feed on the crop perish and (c) the toxin expression can be modulated by using tissue-specific promoters, and replaces the use of synthetic pesticides in the environment. The latter observation has been documented.

Major crops in which Bt was engineered are Bt-tobacco (1985), Bt-potato and -corn (approved by EPA in 1995), Bt-maize and Bt-cotton (1996) (Mendelsohn et al., 2003; Tabashnik et al., 2003).

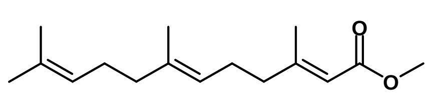
It has been calculated that the use of Bt crops has since saved the use of 35,000 tons of pesticides. However, concern on long-term safety of crops and crop-derived products, such as meat and dairy from animals raised on Bt-maize have been since raised (Prakash, 2001, Huesing and English, 2004). Moreover, the claim that resistance to Bt-crops was unlikely to develop has been challenged by observation of first resistance in Bt cotton in India in November 2009. Another source of concern is the surge of non-sensitive ‘sucking’ pests in China, which eroded the advantage of using Bt-crops.

3. Integrated Approaches

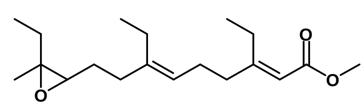
It was as early as 1965, just a few years after the publication of ‘*Silent Spring*’ that ‘integrated pest control’ was the subject of FAO symposium held in Rome (FAO, 1965) which stated: "*Integrated pest control is a pest population management system that utilizes all suitable techniques in a compatible manner to reduce pest populations and maintain them <u>at levels below those causing economic injury</u>*." (our emphasis). The concept of ‘Integrated Control’, originally limited to the combination of chemical and biological control methods, was greatly expanded in that symposium, and redefined to become synonymous with what we presently consider Integrated Pest Management (IPM). It is important to notice that the concept was framed by FAO in food protection rather than in prevention of parasitic diseases and thus called at control of pest populations rather than at eradication of disease vectors.

In fact, the ever-increasing use of pesticides to protect food and commodity crops from spoilage started to present the seemingly unavoidable drawback of an ever-increasing presence of residues in food, in water and in the environment, the significance of which in the broader eco-toxicological terms is still unpredictable. This awareness led to understand how to lower pesticide consumption without compromising the efficacy of crop protection. Current practices of pesticide application in extensive agriculture spread 95% of pesticide dose off-target, causing a waste of expensive product, toxicity to non-target organisms and environment contamination. Moreover, with indiscriminate application practices even active useful products need to be limited or banned due to unacceptable human health hazard. Among strategies developed to improve homing of necessary dose to right target one is the use of '*medicated*' baits, such as a sweetened polymer impregnated with a hazardous organophosphate (such as Dichlorvos), which remains localized and thus with much less risk of unwanted exposure, especially in confined environments, which span from warehouses to dwellings. To enhance safety, less toxic pesticides, such as pyrethroids can be used. This approach, however, is far from being a general one and thus complementary strategies are sought for.

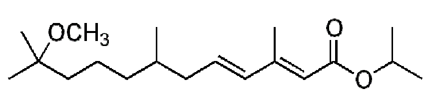
Starting with the discovery of ecdysones, the steroid-like hormones responsible for the periodic change of the exo-skeleton of insects and of other arthropods by Karlson (1996) and following with Röller's discovery of 'juvenile hormones' of insects (Figure 3) and Williams' proposal (1966) to exploit this target to selectively control specific populations without interfering with others, several approaches have been devised and met with some commercial fortune. This approach was applied as early as 1974, following Carl Djerassi's foundation of the start-up company Zoecon and the development of the product Altosid IGR (isopropyl (2E,4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate or methoprene; Figure 3) as a growth regulator of the housefly (*Culicidae* spp.) (Djerassi, 1992). Methoprene does not kill adult insects, but acts as a growth regulator, inhibiting pupae to mold to adult insects. This breaks the biological life cycle of the insect preventing recurring infestation.



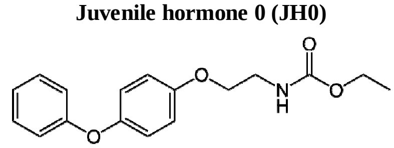
Methyl farnesilate



Juvenile hormone 0 (JH0)



Methoprene



Fenoxycarb

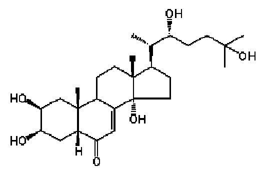
Figure 3. Chemical structures of natural juvenile hormones and of synthetic analogues.

Among the many applications of methoprene and of its analogues are the prevention of fly infestation of cattle, of fly proliferation in cattle dung and in drinking water cisterns to control mosquitoes which spread dengue fever and malaria (World Health Organization, 2008). Another agricultural application of the same product was protection of crops and commodities in warehouses, such as tobacco leaves and pineapples. In the household, methoprene and other analogues such ad hydroprene are used to fight bed bugs. A fairly exotic application of methoprene is to increase the length of the pupal stage of silkworm (*Bombyx mori*) to yield a higher production of silk.

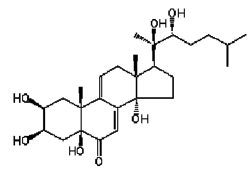
Among the characteristics of JH analogues are lack of residues in the environment (since the molecules are completely mineralized) and a short persistence, which calls for modes of application different from conventional spraying techniques. To enhance the persistence of the products, from the molecular scaffold of methoprene a much more stable diphenylether-carbamate derivative, fenoxycarb (Figure 3), was elaborated. In 1989 in Northern Italy silkworm production was almost destroyed with considerable disruption of the downstream textile industry and economic loss. Studies carried to understand the cause demonstrated that silkworm had been reared on mulberry leaves which had been contaminated by traces of fenoxycarb used far elsewhere to protect fruit trees and carried by the wind (Proceedings of the Italian Parliament, 1993).

Ecdysteroids, a class of steroid derivatives characterized by a *cis*- fusion of rings a and B and by the presence of several hydroxyl groups in the structure, were isolated in arthropods starting in the mid-1950s and in plants (Phytoecdysteroids) starting from the mid-1960s, just after the structure of the first ecdysteroid, ecdysone (E), isolated by Butenandt and Karlson in 1954 from silkworm pupae, was finally elucidated in 1965. Currently more than 200 plant steroids (Figure 4) related in structure to the invertebrate steroid hormone 20-hydroxyecdysone are known (The Ecdysone Handbook, 2002). The only truly viable hypothesis for the function of phytoecdysteroids in plants is that they serve to deter phytophagous invertebrate predators, either by being toxic on ingestion and/or by having an anti-feedant effect. However, ecdysteroids possess a number of properties (polarity, chemical complexity, metabolic and environmental lability) that make them per se generally unsuitable as pest control agents. Unexpectedly, they later found a limited use in molecular biology to promote expression of gene elements and, for the same reason, as growth promoters in cattle rearing.

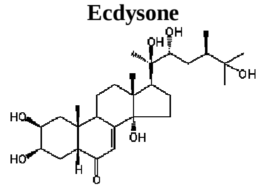
Bis-acylhydrazines such as tebufenozide (Figure 4) showed activity as non-triterpene analogs of ecdysones and could be developed into insecticides by Rohm & Haas, starting in 1983, which cause premature lethal molts in susceptible intoxicated insects. The low degree of environmental concern is due both to their intrinsically toxicity and to the fact that their environmental breakdown products are just substituted benzoic acids totally devoid of any toxicological interest (Wing, 1988a,b).



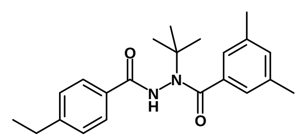
Ecdysone



kaladasterone



Makisterone



tebufenozide

Figure 4. Chemical structures of some ecdysteroids and of the non-steroidal analogue tebufenozide.

However, this approach did not fully flourish, mainly due to the difficulty of researching on selective agents for major pests of a public concern without a strong financial support, within a regulatory system based on stringent requisites for new active substances (see Mandic-Rajcevic *et al., this book*) which apply well to synthetic chemicals but may prove scientifically unreasonable for biologically active natural substances such as the mediators of chemical communication between invertebrates developed as sexual-attractant-based methods of biological control (Djerassi et al., 1974). A particularly striking early example was methoprene, a so harmless compound that a DL_{50} dose could not found even guzzling rats at 35 grams per kilogram of body weight.

In 1975 the EPA-NIEHS-Federal Working Group and Pest Management held a Conference on Human Health Effects of New Approaches to Insect Pest Control at Durham (August 4-6), the Proceedings of which were published in the April 1976 issue of Environmental Health Perspectives. The covered topic which elicited most contributions was that of biological pest control, as both the use of natural predators of pests and of species-selective insect chemo-attractants to attract target organisms towards insecticides while sparing non-target ones.

4. The Global Food Trade

While what is currently known as Global Food Trade is a long-known process, which may be traced at least as back in time as the Egyptian-Roman wheat trade of the imperial age, the European colonial era which developed starting in the XVI-XIX centuries set in motion an extractive relation between Europe and the rest of the world, as the colonies were converted into supply zones of food and raw materials to fuel European capitalism, disrupting long-time established local economies. From the 1980s onwards, ‘free trade’, not state intervention, became the dominant policy paradigm. Consequent liberalization policies have deepened the conversion of the global South into a ‘world farm’ for a minority of global consumers, concentrated in the global North and in strategic states and urban enclaves of the South.

Important technological achievements were reached since the XIX century to improve transport, conservation and use of food, namely Sterilization, Pasteurization (the first canned food factory was inaugurated in England in 1813) and blossomed in the XX century, with the advent into Western markets of Frozen foods (1940s), of Freeze-dried, pressure-cooked foods (1960s), of Microwave cooking of ready-to-consume foods (1980s).

This process, in which economical and technological aspects are deeply intertwined also brings opportunities (*pros*) and threats (*cons*) the distribution of which among global actors: producers, traders, consumers involves cultural issues and cannot be solved only on a technological or scientific basis.

Higher production of food and commodity crops is possible in specific regions of the Earth, due to geographical and climatic characteristics. Global trade affords a wider variety of crops to be available to an increasing population. Wider market opportunities are an incentive to development of peripheral regions and groups. Whether diffusion of previously ‘exotic’ food to populations over much wider areas and the growth of a global culture can contribute to increase a multi-cultural approach and to lower the potential of conflicts is a still debated point but such end-point has been reached in several areas.

Among perceived and often demonstrated drawbacks of the global food trade are the necessity of applying intensive monoculture, which causes loss of biodiversity since only some cultivars, especially of food crops, may be requested by the market. Loss of local culture can be a consequence, since change of production modes also modifies social structures in unpredictable directions. Crop transportation over long distances is a major factor in the spread of pests and of diseases and the environmental cost of transportation may add to the global anthropogenic burden on the Planet.

The contemporary Global Food Trade needs to equilibrate among the conflicting pressures by social actors with perceived different interests. A crucial one is that which can be summarized in the apparent antinomy of *Quality vs. Quantity*: high yields and product homogeneity are necessary when food production aims at feeding very large populations, such as those of the Western '*baby boom*' at a decreased economic cost, in order that savings can be addressed to new types of consumption, such as leisure goods (Hobsbawm, 2004). To achieve this results, local crop varieties are lost in favour of those selected by Agropharma, which need hi-tech care (fertilizers, pesticides) to grow to commercial value. Loss of biodiversity of crops puts at higher threaten by pests, as historically experienced several times, from the 1845-51 Irish potato famine caused by an airborne fungus (*Phytophthora infestans*) originally transported in the holds of ships traveling from North America to England and carried to Ireland by northerly blowing winds to the *Peronospera* vineyard epidemic carried to Continental Europe from the U.S.A. twenty years later.

Another side of the problem is the impact of new food regulations based on toxicological knowledge on several traditional foods, which contain appetizing substances deriving from added spices or deriving from specific processing, and for which some degree of toxicological hazard has been demonstrated. On this regard, safeguard of consumers *vs.* safeguard of traditions need to be accurately balanced in scenario-based risk assessments. Traditional food is often produced from starting materials and under conditions which may not be deemed '*consumer-safe*' according to the current perception of food safety, yet they have been produced and consumed for centuries without yielding any substantial contribution to the global disease burden of the populations. The use of plant products as spices is long known as a way to give flavour to food and often to protect from spoilage; yet most chemicals which impart to spices and herbs their useful properties are cytotoxic allelo-chemicals intended to preserve the organism's living niche from neighbours and predators by harming them. Also some traditional processes of food manufacturing, such as controlled fermentation and colonization by selected micro-organisms, may exploit their biochemical processes to modify texture and nutrient content, or use the natural production of antibiotics to preserve food from spoilage. There are many examples of controversial issues due to toxicological concern on traditional food spanning from Italian pesto sauce (which contains methyl-eugenol, a weak carcinogen; Smith et al., 2010) to French and Italian fermented cheese (Roquefort and Gorgonzola, respectively, which contain roquefortins, the cytotoxic antibiotics produced by the mould; Sieber, 1978; Finoli et al., 2001).

CODEX ALIMENTARIUS

The *Codex Alimentarius* (Latin for '*Book of Food*') is a collection of internationally recognized standards, codes of practice, guidelines and other recommendations relating to foods, food production and food safety which are agreed upon at UN-level for production, transformation, wise use of food resources (FAO, 2012). Since protecting the purity of the nation's food supply has ever been a function of governments, elementary rules regarding the composition and purity of main foods was incorporated in religious codes (such as *kasherut,* among the oldest requiring a quantitative assessment of the maximum allowed level of a contaminant, the *one-in-sixty* rule; Islam's *halal-haram* and other approaches in large and small religious groups) (Roth, 2008) and later in secular laws, explicitly grounded on an increasing body of scientific knowledge.

The discipline of Merceology developed between the late XVIII and the XIX century, by applying the recent breakthroughs of analytical chemistry to determine the composition of commodities with the aim of levying custom taxes. The different sets of standards arising from the spontaneous and independent development of often conflicting and contradictory food laws and standards by different countries had the effect of creating inevitable barriers to food trade, as recognized in a statement of the first meeting of the Joint FAO/WHO Expert Committee on Nutrition in 1950.

The devastation of the Second World War, especially in Europe, convinced politicians and economists that improved agricultural trade would be essential for rapid reconstruction and the ability to feed people. Following the creation of the Food and Agriculture Organization (FAO) in 1945 and of the World Health Organization (WHO) in 1948, in the early 1950s the two organizations independently began work on requirements and analytical procedures for determining the purity of fruit juices. The idea of a Europe-wide Codex Alimentarius -based on an already existing, albeit little-known outside the German-speaking world, the *Codex Alimentarius Austriacus-* was actively pursued by Hans Frenzel of Austria between 1954 and 1958 and culminated in the creation of the Council of the *Codex Alimentarius Europaeus* in June 1958 under the joint sponsorship of the International Commission on Agricultural Industries and the International Bureau of Analytical Chemistry. The First FAO Regional Conference for Europe, meeting in Rome in October 1960, recognized that international agreement on minimum food standards and related questions (including labelling requirements, methods of analysis, etc.) is an important means of protecting the consumer's health, of ensuring quality and of reducing trade barriers, particularly in the rapidly integrating market of Europe and aimed at coordinating the growing number of food standards programmes undertaken by many organizations. In November 1961 the eleventh session of the Conference of FAO passed the resolution by which the *Codex Alimentarius Commission* was established, was joined by the WHO in June 1962, and held the first session in Rome in October 1963. Some 120 participants from 30 countries and 16 international organizations attended. Among the first documents, that on the General Principles of Food Hygiene was issued in 1969. In 2009 the Maximum Residue Limits (MRLs) for Pesticides was issued and revised in 2011.

5. General Conclusions

It is a long time that the necessity to supply agricultural products to feed the ever-increasing world population and to produce the commodities necessary to sustain increasingly higher standards of goods availability makes pest control a priority in the global agenda.

To overcome the continuous emerging of pest resistance there is a need to discover and use new products and to adapt the use of existing ones to changing needs, avoiding excessive use and unnecessary contamination of valuable planetary resources.

Since plants are naturally ever since engaged in fighting their natural predators, the panoply of plant allelo-chemicals can be exploited as a source of chemical diversity to imitate and improve plant strategies against crop-spoiling organisms.

Availability of natural products as plant protection products may show important differences with respect to production of man-made molecules, since the latter can be produced at any economically sound rate by up-sizing chemical plants while the former must rely on the natural growth of producer organisms, be plants, or micro-organisms.

Both in the field of crop protection and in food production '*Natural is not necessarily harmless*'. Allelo-chemicals employed as plant protection products can be as powerfully toxic to humans, domestic animals and cattle, to useful insects and pollinators and to non-target organisms as are to pests. 'Natural' products used as substitutes of synthetic pesticides therefore need the same level of Risk Assessment, of Risk Management and of Risk Communication as man-made molecules. Food with a long tradition of consumption can in fact contain natural substances the toxicity of which can result at least worth a health risk assessment.

Globalization calls for agreement on the properties of foods in order that they can be marketed world-wide without health restrictions. Also their nutritional value should be agreed upon by sharing of methods for production and for the determination of their nutritional properties and of the unavoidable residues of plant protection products, of veterinary drugs, of environmental contaminants and of manufacturing by-products, so that the latter are kept at levels for which no health concern is raised even for the most sensitive sub-groups of the human population.

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