

New trends in pest control: the search for greener insecticides

Óscar López,*^a José G. Fernández-Bolaños^a and María Victoria Gil^b

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Insecticides have a pivotal role in our lives, not only for crop protection in agriculture, but also to avoid the spreading of harmful pests causing human diseases such as malaria. Due to economic and medical reasons, the design of effective agents that control these pests is quite an important task in agrochemical science and in the industrial sector. Nevertheless, the non-restricted use of highly toxic insecticides for several decades has provoked negative effects in the environment and the poisoning of non-targeted species. For these reasons, the development of selective and harmless insecticides is needed. A short overview of some of the recent advances in the chemistry of insecticides is presented, with a highlight of their greenness compared with classical insecticides. Synthesis, mode of action and environmental profile of pyrethroids, neonicotinoids, and insect growth regulators will be described. Furthermore, the use of biological insecticides such as spinosyns, azadirachtin, and *Bacillus thuringiensis* as green alternatives for synthetic insecticides will also be reviewed.

1. Introduction

The first efficient insecticides were introduced in the middle of the 20th century; before that, pest control was mainly based on the use of inorganic agents such as sulfur, arsenicals, hydrogen

cyanide or cryolite,¹ some of which are still being used despite their high toxicity not only for targeted insects, but also for non-targeted species, including vertebrates. The introduction of organochlorine, organophosphorus and carbamate insecticides meant a real revolution² in the agrochemical sector, as these compounds have allowed an important minimization of crop losses caused by insect activity.

In this context, the introduction of DDT during World War II as one of the first organochlorine insecticides was remarkable, with a wide spectrum of action and a long residual activity.³ Nevertheless, a few years later, organochlorine insecticides were shown to cause severe environmental damages, both in terrestrial and aquatic ecosystems.⁴ Their persistence provoked an accumulation of organochlorine insecticides in animals through the food chain and as a result, most of these insecticides were banned in many countries,³ although DDT is still in use in some countries where malaria is endemic.⁵ Recent studies suggest that chronic exposure to DDT is associated with neurological impairments,⁶ accelerated ageing,⁷ and breast cancer.⁸

On the other hand, organophosphorus insecticides, frequently called organophosphates although the term is sometimes chemically incorrect, were developed by Bayer AG¹ in the 1940s, and they proved to be reliable and effective pest control agents. Organophosphorus insecticides affect the nervous system by phosphorylation of acetylcholinesterase,⁹ provoking respiratory muscle weakness and neuromuscular dysfunction.^{10,11} They are also known to induce tumorigenic risks.¹² The Environmental Protection Agency in the USA (US EPA) is currently reassessing insecticide tolerances;¹³ as a result, the US EPA has released an organophosphorus cumulative risk assessment,¹⁴ which resulted in the cancellation³ of a number of organophosphorus pesticides.

These chemicals, which include some of the most toxic agents still used in agriculture, had a broad spectrum of

*osc-lopez@us.es



Dr Óscar López received his PhD at Seville University in 2003. In March 2004, he was appointed as lecturer in Environmental Organic Chemistry at the University of Huelva, Spain. In June 2004, he was appointed as lecturer in Organic Chemistry at the University of Seville, Spain, in the Faculty of Chemistry. His research interests include synthesis of carbohydrate-derived ureas, thioureas and selenoureas and design of glycosidase inhibitors.

Prof. José G. Fernández-Bolaños completed his PhD at Seville University in 1984. He spent one year in a postdoctoral stay at the Technical University of Denmark, Lyngby, in Prof. Klaus Bock's group. He is currently a Professor of Organic Chemistry at Seville University. His research interests include synthesis of sulfonic acid-derived ionic tensides, heterocyclic and carbohydrate chemistry.

Dr María Victoria Gil received her PhD at Extremadura University in 2001 with Prof. Emilio Román and José Antonio Serrano. She is currently a lecturer in Organic Chemistry at the University of Extremadura. Her research concentrates on asymmetric synthesis in the field of nitrocompounds.

activity against insect pests, and showed only moderate stability in the environment.³

Carbamates were developed in the 1950s and are still used today.³ These insecticides are rapidly detoxified and excreted in warm-blooded animals and, in general, they are selective against targeted insect pests.¹⁵ Nevertheless, carbamates are toxic against some useful insects, such as honeybees. Both organophosphorus and carbamate insecticides act by suppressing the activity of acetylcholinesterase, an enzyme that regulates a neurotransmitter called acetylcholine. Organophosphorus insecticides react irreversibly with acetylcholinesterase, whereas carbamates act reversibly.

Carbamates have a low persistence in soil, plants, and the environment.³ This, although a positive characteristic from an environmental and human safety point of view, also means that in some cases several applications are needed over a growing season.³ There is no evidence of carbamates causing delayed neurotoxicity as is found with some of the organophosphorus compounds.³ Carbamates are not regarded as mutagenic, carcinogenic or teratogenic substances. Several studies^{16,17} show an association of long-term carbamate exposure with neuropsychological function impairment, which could be interpreted as evidence of a chronic effect of cumulative high exposure to these compounds. US EPA expects that a preliminary cumulative risk assessment of carbamates will be available by the Spring of 2005.¹⁸

Apart from these negative environmental aspects, nowadays there are populations of insects which are resistant to organochlorine, organophosphorus and carbamate insecticides.¹⁹ In order to overcome this resistance it would be interesting to have new pesticides with different mechanisms of action.²⁰

So, the main goal of pesticide research is the development of new, selective and highly effective substances that cause no harm to human health and the environment.²¹ In this article we review the recent advances in the chemistry of insecticides in the search for greener insecticides in terms of environmental toxicity.²² In this context, compounds such as pyrethroids, neonicotinoids or insect growth regulators will be considered. We will also review the use of biological insecticides such as spinosyns, azadirachtin and *Bacillus thuringiensis* as green alternatives for chemical agents.

2. Pyrethroids

Pyrethroids are synthetic insecticides structurally derived from the six natural pyrethrins,^{23,24} isolated from pyrethrum, the plant extracts²⁵ of *Chrysanthemum cinerariaefolium* flowers. Pyrethrins are esters of a cyclopropanecarboxylic acid (chrysanthemic or pyrethic acid) and a cyclopentenolone (pyrethrolone, cinerolone or jasmolone).²³ For example, pyrethrins I and II (Fig. 1) derive from pyrethrolone and chrysanthemic or pyrethic acid, respectively.

Pyrethrum itself exhibits insecticidal activity²³ against some pests and presents low mammalian toxicity; however, its instability in light and air strongly reduces potential effectiveness. The activity of these compounds is due to their high affinity to insect Na⁺-channels, causing neuronal hyperexcitability.^{15,26}

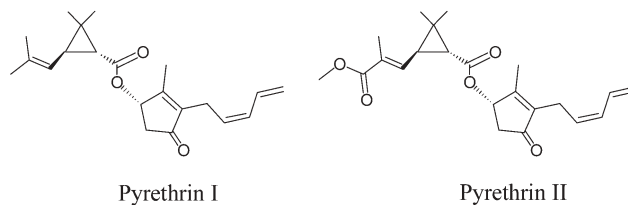


Fig. 1 Pyrethrins I and II.

Subsequent research^{24,27,28} meant the replacement of some of the structural elements of pyrethrins. For example, the pentadienyl side chain of pyrethrins I and II was replaced by more accessible moieties with similar steric and electronic behaviour.²³ Different heterocycles were used²⁸ instead of the cyclopentenolone domain, and an α -cyano substituent in a 3-phenoxybenzyl alcohol moiety²⁹ was introduced (Fig. 2).

All these structural changes allowed the preparation of a wide range of pyrethroids with improved photostability²³ as compared with pyrethrins. Among these compounds, some important commercially-available insecticides are included, such as tetramethrin and deltamethrin (Fig. 2).²³ Even the substituted-cyclopropanecarboxylic acid moiety was later replaced by an isovaleric acid residue to afford commercial fenvalerate.²³

These synthetic approaches allowed the development of pyrethroids available not only for indoor uses but also for crop protection and for veterinary and medical pest management.²⁴

Bioassays^{30,31} revealed that pyrethroids possess a quick knock-down effect against insects and, in general, a low mammalian toxicity.²⁴ These features, together with a good degree of biodegradability and selectivity, allowed pyrethroids to be considered²⁴ as the safest, and one of the most effective insecticides at present, with a better environmental profile than organochlorine, organophosphorus and carbamate insecticides. In the last few decades, they have become the second most important group after organophosphorus compounds.¹

In this context, Table 1 shows the average selectivity between insects and mammals of the most currently used insecticides.²⁴ As can be seen, pyrethroids exhibit the highest potency against pests, but at the same time show the lowest toxicity towards mammals.²⁴ Pyrethroid toxicity to humans is

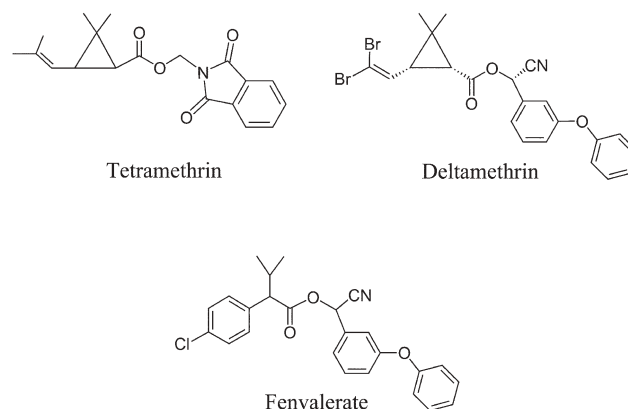


Fig. 2 Commercially-available pyrethroids.

Table 1 Toxicity of insecticides in mammals vs. insects

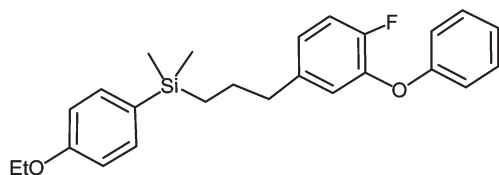
Type of insecticide	Average LD ₅₀ /μg g ⁻¹	
	Mammals ^a (rats)	Insects ^a
Carbamate	45 (15)	2.8 (27)
Organophosphorus	67 (83)	2.0 (50)
Organochlorine	230 (21)	2.6 (26)
Pyrethroid	2000 (11)	0.45 (35)

^a Number of insecticides tested in parentheses.

at least three orders of magnitude lower than for insects³² and they are classed as low toxic insecticides for mammals by the World Health Organization.³² This feature is due to the rapid detoxification in blood and liver carried out by carboxylesterases,³³ the blood half-life of pyrethroids being measured in tenths of an hour.³³ Although pyrethroids are much less toxic for humans than other insecticides, a variety of reversible symptoms such as headache, nausea and cutaneous paresthesia have been reported.³⁴ Pyrethroids lacking an α -cyano group show the weakest physiological effect and are thought to affect the peripheral nervous system, whereas α -cyano pyrethroids produce symptoms of the central nervous system.³⁵ Up to now, the US EPA has not released a cumulative risk assessment for pyrethroids.³⁶

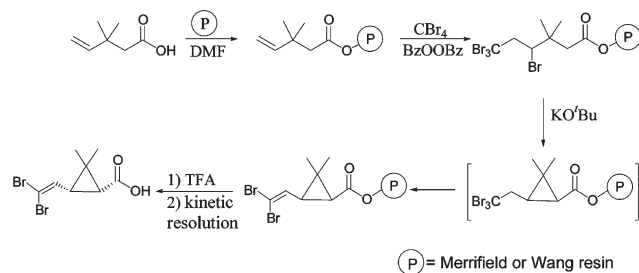
Nevertheless, several toxicological studies revealed that some pyrethroids are highly toxic to fish,²⁴ so their use was restricted near water systems. In order to avoid this negative environmental impact, further chemical modifications on the pyrethroid structure were needed.

As a result, silafluofen **1**, seemed quite a good substitute,²⁴ as it showed only negligible fish toxicity (about 10⁶ times smaller than deltamethrin), while maintaining high insecticidal activity. This compound includes novel structural features as compared with early pyrethroids, as it lacks the common ester moiety and introduces a quaternary silicon atom in its structure.³⁷

**1** Silafluofen

Due to the economic and environmental importance of pyrethroids, intense research on these substances is still being carried out.^{38–40} In this context, green processes are being developed in terms of selective and non-hazardous procedures of industrial interest for the preparation of pyrethroids. For example, chemoenzymatic syntheses^{41,42} are considered, in which one of the key steps is the enzymatic kinetic resolution of enantiomers by using lipases.

Furthermore, radical 1,2-addition of haloalkanes to polymer-bound olefins has successfully been carried out (Scheme 1)⁴³ in a solid-phase synthesis to afford the dihaloethenylcyclopropane carboxylate moieties present in many pyrethroid-based insecticides.

**Scheme 1** Solid-phase synthesis of cyclopropane carboxylate moieties of pyrethroids.

3. Neonicotinoids

(-)-Nicotine and nicotinoids such as (\pm)-epibatidine (Fig. 3) have been tested as agents in insect control.⁴⁴ In particular, (-)-nicotine, obtained from tobacco extracts has been used for centuries as an aphicide in the control of sucking insects, although it has a considerable low potency as insecticide, a narrow spectrum of application and a high toxicity to mammals.

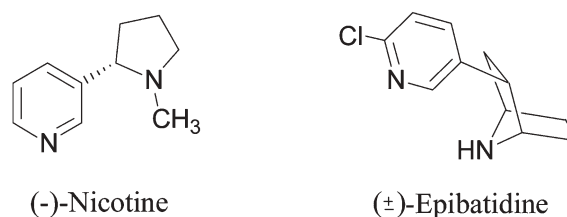
Furthermore, although the insecticidal activity of synthetic nicotinoids has been improved, it has never reached the degree required for commercialization.⁴⁴

Subsequent research led to a novel class of synthetic compounds called neonicotinoids; this term was originally proposed by Yamamoto⁴⁵ for compounds having a structural similarity to nicotine and a common mode of action. In general, neonicotinoids possess an electron-withdrawing group, either a nitroimino, cyanoimino or nitromethylene moiety^{44,46,47} (Fig. 4).

Both nicotinoids and neonicotinoids are agonists at the nicotinic acetylcholine receptors^{20,46,48} (nAChRs); however, nicotinoids are ionized at physiological pH and they are selective for the mammalian nAChRs. On the other hand, neonicotinoids are not ionized under physiological conditions and are selective for the insect nAChRs at a nanomolar level⁴⁹ due to differences in the composition of the receptors in insects and vertebrates.⁵⁰ This feature provides an excellent example of selective toxicity,⁴⁴ with low acute toxicity to mammals, birds and fish, but they display some chronic toxicity in mammals.⁵¹

It is thought that agonist recognition by insect nAChRs probably involves a cationic subsite of a lysine or arginine moiety⁴⁸ for interaction with the nitro or cyano group of neonicotinoids (Fig. 5).

The activity of neonicotinoids contrasts with the action of pyrethroids,⁵² which interact with presynaptic sodium

**Fig. 3** (-)-Nicotine and nicotinoid (\pm)-epibatidine.

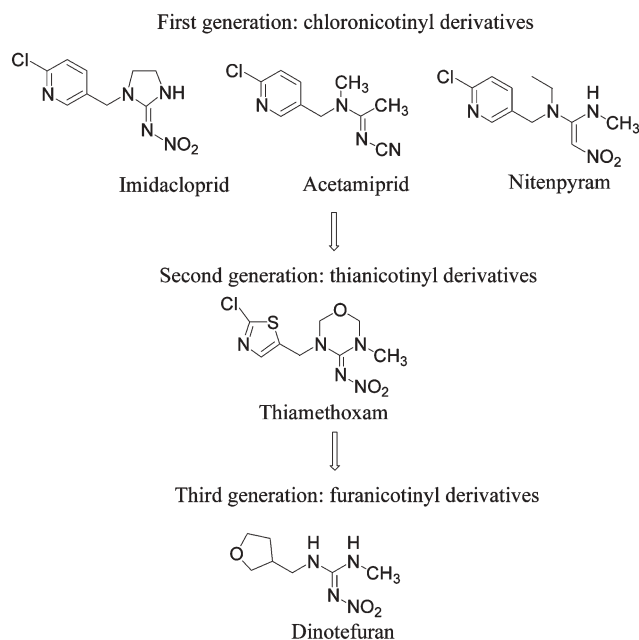


Fig. 4 The three generations of neonicotinoids.

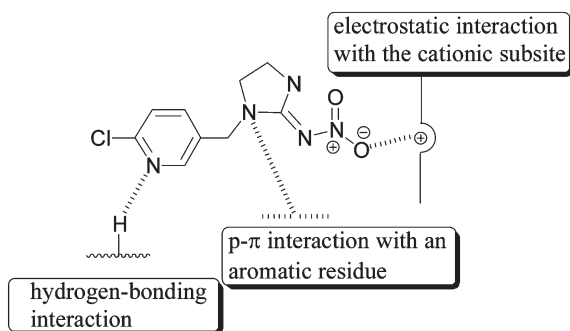


Fig. 5 Interactions of neonicotinoids with insect nicotinic acetylcholine receptors (nAChRs).

channels, and of organophosphorus and carbamate insecticides,⁵³ which inhibit acetylcholinesterases.

Thus, neonicotinoids represent a new generation of synthetic insecticides as they combine unique properties allowing them to be the fastest growing synthetic insecticides on the market.⁵⁴ Some of these unique properties⁵⁴ are a broad-spectrum insecticidal activity (especially lethal for sucking and chewing insects), low application rates, a novel mode of action and a favorable safety profile, as well as lacking cross-resistance to other insecticides. As a result, neonicotinoids are increasingly used in crop protection and animal health care^{44,50} due to the decrease in effectiveness of organophosphorus and carbamate derivatives, as well as their toxicity to vertebrates.⁵⁰

The first successfully used neonicotinic insecticide was imidacloprid, introduced by Bayer AG and marketed as Admire[®] in 1991;^{55,56} this compound belongs to the first generation of these novel insecticides⁵⁷ together with acetamiprid, and nitenpyram (Fig. 4). All of them possess a 6-chloropyridin-3-yl moiety, which was supposed to be necessary for these compounds to exhibit insecticidal activity.

Like many other neonicotinoids, imidacloprid is efficient at low rates and is safe for both human beings and the environment.^{46,58} Imidacloprid is at present one of the most effective insecticides, with a level of activity similar to that exhibited by pyrethroids and higher than that of organophosphorus and carbamate derivatives.²⁰ It is thought that its scale of application will reach that of pyrethroids in just a few years.

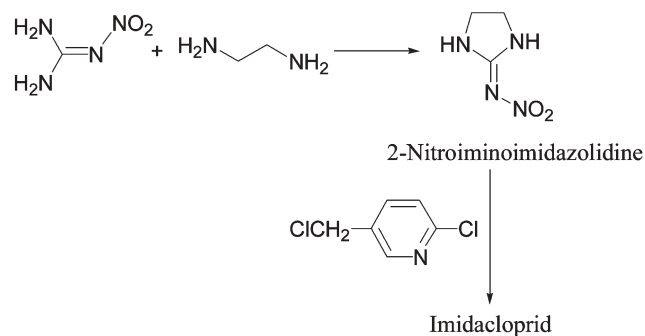
The key step for the preparation of imidacloprid involves the alkylation of 2-nitroiminoimidazolidine²⁰ with 2-chloro-5-chloromethylpyridine (Scheme 2).

The success of the first generation of neonicotinoids has prompted researchers and the agrochemical industry to investigate a wealth of structural variations of imidacloprid,⁴⁶ so as to develop more active and greener insecticides by carrying out structure–activity relationship studies and chemical syntheses.

Research starting from parent structures, such as the tetrahydro-1,3,5-oxadiazine derivative shown in Fig. 6, proved that replacement of the 6-chloropyridin-3-yl moiety by the 2-chlorothiazol-5-yl group (thiamethoxam, Fig. 4)⁵⁴ and the addition of a methyl group in the pharmacophore allowed an increase in activity against chewing and sucking insects. This led to the second generation of neonicotinoids, whose major example is thiamethoxam^{54,59} (Fig. 4).

Thiamethoxam⁶⁰ was first marketed in 1998 for foliar or soil treatment (Actara[®]) and for seed protection (Cruiser[®]) against homopteran, coleopteran and some lepidopteran pests. This compound and related structures have low acute dermal and inhalation toxicities⁶¹ and they usually do not provoke allergic reactions either in humans or in animals. It is rated as a likely human carcinogen.⁵¹

Thiamethoxam can efficiently be obtained by two different synthetic approaches, both starting from *S*-methyl-*N*-nitroisothiourea,⁵⁴ and involving the preparation of *N*-substituted-*N'*-nitroguanidine and tetrahydro-1,3,5-oxadiazine intermediates, as shown in Scheme 3.



Scheme 2 Synthetic pathway of imidacloprid from nitroguanidine.

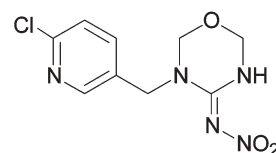
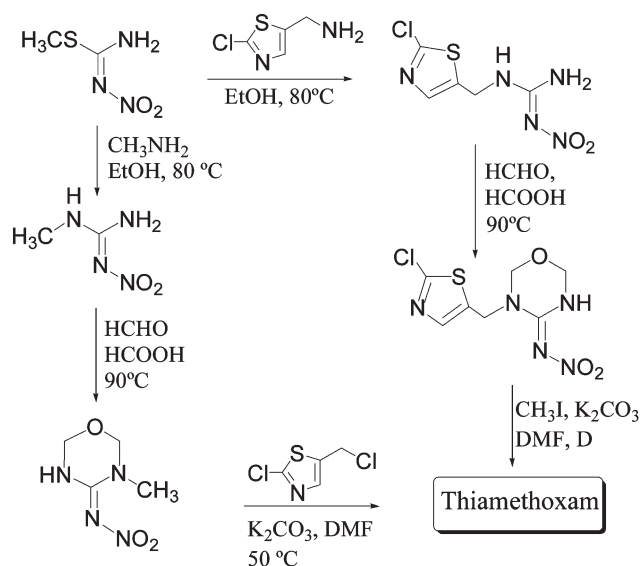


Fig. 6 Parent structure of second generation of neonicotinoids.



Scheme 3 Synthetic pathways of thiamethoxam from *S*-methyl-*N*-nitrosothiourea.

After the commercialization of thiamethoxam, new insecticides with neonicotinoid properties were developed, such as racemic dinotefuran (Fig. 4), marketed under the names Starkle[®] and Albarin[®].⁵⁷ This compound could be considered as a member of the third generation of neonicotinoids because of its tetrahydrofuran-3-yl moiety,⁵⁷ and it presents one of the best toxicological profiles of neonicotinoids⁵¹ (acute oral LD₅₀ value for rats: 2400 mg kg⁻¹ and no-observed-adverse-effect-level, NOAEL: 127 mg kg⁻¹ d⁻¹).⁵¹

Nowadays, intense research in this area of agrochemical science still continues, based on synthetic approaches to modified structures,^{62–64} quantitative structure–activity relationships⁶⁵ and electrophysiological studies.⁴⁶ The combination of all these activities will allow a better comprehension of the binding of neonicotinoids^{66,67} to the active site of receptors and also the development of new compounds with improved activity and even a better toxicological profile.

Table 2 shows the potency exhibited by some neonicotinoids against some aphids and locusts,^{59,66} in comparison with (–)-nicotine. On the other hand, Table 3 shows⁴⁸ the difference in binding affinity of neonicotinoids to insect and mammalian receptors.

Table 2 Comparative potency of neonicotinoids and (–)-nicotine

	IC ₅₀ /nM		
	<i>A. craccivora</i>	<i>M. persicae</i>	<i>L. migratoria</i>
Imidacloprid	2.3 (±0.8)	3.1 (±0.8)	1.5 (±0.2)
Acetamiprid	4.8 (±2.9)	6.3 (±2.4)	2.9 (±0.2)
(–)-Nicotine	840 (±85)	965 (±280)	320 (±180)

Table 3 Comparative affinity to insect and mammalian receptors

	Housefly LD ₅₀ /μg g ⁻¹	Mouse LD ₅₀ /μg g ⁻¹
Imidacloprid	0.02–0.07	40–50
(–)-Nicotine	>50	6–8

The US EPA has not released a cumulative risk approach in determining pesticide tolerances for neonicotinoids yet.⁵¹ Information regarding human exposure and toxicity is quite rare despite the widespread use of these compounds.^{68,69}

4. Spinosyns and spinosoids

Spinosyns are a new class of lactone-derived macrolides with a 21-carbon tetracyclic backbone produced by a culture of the actinomycete *Saccharopolyspora spinosa* as secondary metabolites.⁷⁰ They are comprised of a central *as*-indacene-derived core, together with the deoxy sugars D-forosamine and tri-*O*-methyl-L-rhamnose.⁷¹

These novel compounds were discovered in a soil sample in the Caribbean area in the 1980s as a result of a screening program directed at bacterial metabolites of agricultural and pharmaceutical interest.

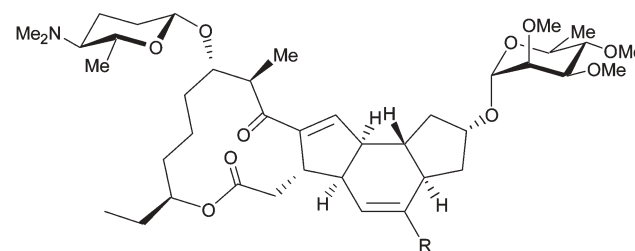
To date, twenty-two naturally occurring spinosyns have been discovered, with different degrees of methylation.⁷² It is noteworthy that some other biologically active compounds have been found to have this kind of indacene-derived framework, such as the antibiotics ikarugamycin and capsimycin.⁷³

Spinosad is a reduced-risk bioinsecticide⁷⁴ registered by Dow AgroSciences in 1997, marketed as Tacer[™], and its commercial formulation is a mixture of the natural spinosyns A and D (Fig. 7) in a ratio of about 85 to 15.

Spinosad exhibits extraordinary potency against a broad spectrum of insect pests, especially against lepidopterans and dipterans⁷⁵ where its efficiency is sometimes similar to that exhibited by pyrethroids.

The combination of its activity to targeted pests and a better environmental and toxicological profile than most synthetic insect control agents^{70,76} makes spinosad a promising insecticide. Spinosad degrades photochemically when exposed to light after application, and strongly adsorbs to most soils, so it does not leach through soil to groundwater. There is no evidence that spinosad is a reproductive toxicant or carcinogen for mammals.⁷⁷ No developmental effects were found in either rats or rabbits.⁷⁷

These features have allowed spinosad to be considered as a reduced-risk insecticide by the US EPA.⁷⁷ Dow AgroSciences received the US Presidential Green Chemistry Award in 1999 for the development of spinosad.⁷⁸



2 Spinosyn A R = H

3 Spinosyn D R = Me

Fig. 7 Spinosyns A and D.

On the other hand, although spinosyns are sometimes slower to penetrate the insect larvae as compared to pyrethroids, they are not readily metabolized once inside the insect.^{70,79}

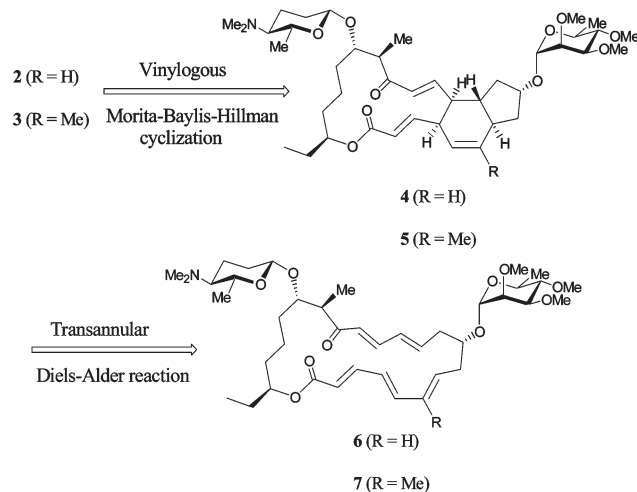
Besides, in a variety of pests, spinosyns are more active^{70,79} than organophosphorus and carbamate insecticides, as well as showing a favorable profile for beneficial insect species and low acute mammalian and avian toxicity. Although spinosad is acutely toxic to honeybees under laboratory conditions, field studies reveal that under actual use conditions, the impact on adult honeybees is minimal.^{77,80}

Furthermore, spinosyns seem to have a unique mode of action; they show both rapid contact and ingestion activity in insects, an unusual feature for a biological product. Several studies^{81–83} suggest that these insecticidal compounds alter both nicotinic and gamma-aminobutyric acid (GABA) receptors, although this interaction does not occur directly through known binding sites, but through an undetermined mechanism. The existence of a novel mode of action is quite important so as to minimize the potential cross-resistance, as compared with classical insecticides.

Evans and Black,⁸⁴ Paquette *et al.*⁸⁵ and Roush and coworkers⁸⁶ reported the first total syntheses of spinosyn A. The search for modified spinosyns has led to the preparation of several hundred synthetic or semi-synthetic derivatives, so called spinosoids.^{79,87}

Much effort has been devoted to the synthesis of the tricyclic nucleus of spinosyns or related structures so as to allow access to pure diastereomeric spinosoids.⁸⁸ As the biosynthesis^{89,90} of spinosyn A is supposed to involve a transannular Diels–Alder reaction and a ring closure of a macrocyclic pentaene, several synthetic approaches are based on these reactions.

For instance, Roush and coworkers have developed the synthesis of the spinosyn tricyclic nucleus in terms of a one-pot tandem intramolecular Diels–Alder reaction and an intramolecular vinylogous Morita–Baylis–Hillman^{86,91} cyclization, following the biomimetic strategy shown in Scheme 4. Roush and coworkers have also reported the preparation of the spinosyn nucleus by an Ireland–Claisen ring contraction, followed by an intramolecular Diels–Alder reaction.⁹²



Scheme 4 Biomimetic strategy for the synthesis of spinosyn tricyclic nucleus.

Other methods developed to access the indacene-derived core involve chemoenzymatic approaches⁹³ and oxy-Cope reactions.⁹⁴

In order to improve natural spinosyn production and to obtain a library of spinosyn analogues, genetically modified actinomycetes have been described,^{95,96} this process being initiated by Lilly Research Laboratories and Dow AgroSciences. Thus, Gaisser *et al.* have reported⁹⁷ the replacement of the β -D-forosamine moiety in spinosyns A and D by L-mycarose (**8**, **9**) and D-glucose (**10**, **11**), using mutant strains of *Saccharopolyspora erythraea* (Fig. 8).

Quantitative structure–activity relationships (QSAR) have successfully been applied in the form of Artificial Neural Networks (ANN)^{72,79} to spinosyns and spinosoids in order to determine which structural modifications are likely to improve their insecticidal activity. By this procedure, some spinosoids with greater activity than spinosad against some lepidopteran species have been obtained.^{72,79} Fig. 9 shows some spinosoids⁷² with more activity than spinosyn A (LC_{50} = 0.31 ppm) against larvae of *Heliothis virescens*, especially in the case of the 2,3,4-tri-*O*-ethyl-L-rhamnopyranosyl **12** and 3-*O*-ethyl-2,4-di-*O*-methyl-L-rhamnopyranosyl **13** derivatives.

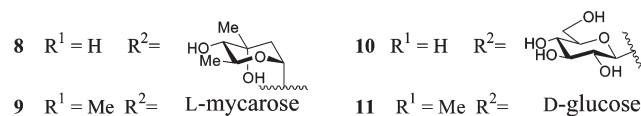
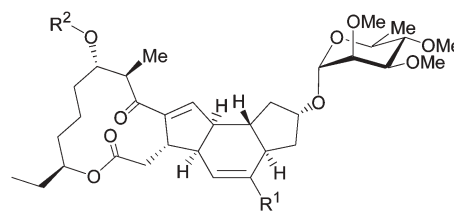


Fig. 8 Spinosyn analogues from genetically-modified actinomycetes.

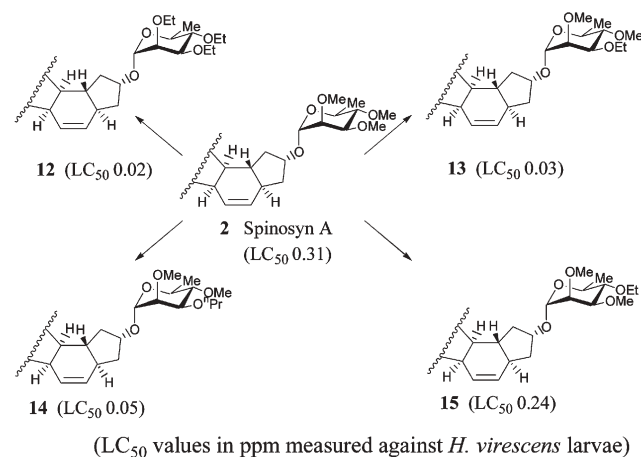


Fig. 9 Biological activity of spinosyn A and analogues.

5. Insect growth regulators (IGRs)

Insect growth regulators (IGRs) are compounds that alter the normal growth process of insects and can therefore be used to control insect populations; these compounds interfere with insect metamorphosis, embryogenesis or reproduction.⁹⁸ Among them we find compounds that mimic or antagonise insect juvenile hormone activity and substances that inhibit chitin synthesis in the exoskeleton.⁹⁹ Juvenile hormone analogues provoke mortality at adult emergence, whereas chitin synthesis inhibitors cause mortality in larvae and nymphs; besides, both types of IGRs also cause sterilization in adult insects.¹⁰⁰

The main advantages of these compounds over other insecticidal substances are that they have a low mammalian toxicity and are often very species-specific insecticides;^{22,99} nevertheless they usually present a slow mode of action and sometimes a low stability.

5.1. Juvenile hormone-based insecticides

Insect growth is regulated by the action of some hormones such as juvenile hormones (Fig. 10).^{22,101} These sesquiterpenoid compounds take part in two important processes: to regulate metamorphosis and the production of eggs in female insects.¹⁰² Due to the specificity of these functions, juvenile hormones have attracted attention¹⁰³ as safe and selective targets for the design and development of environmentally friendly and biorational insecticides.¹⁰¹

Nevertheless, juvenile hormones (JHs) are usually too unstable to be used as practical insecticides; this feature prompted intense research in order to develop juvenile hormone analogues (JHAs) called juvenoids,¹⁰⁴ either naturally occurring or synthetic, that act by inhibiting the developmental changes associated with embryogenesis, morphogenesis and reproduction. Some JHAs, such as methoprene and hydroprrene, are used as commercial household insecticides (Fig. 11);¹⁰⁵ however agricultural use of earlier JHAs has been limited, because of their lack of outdoor stability, their limited insect control spectrum, and their slow toxic action. Both methoprene and hydroprrene are now registered by the US EPA. No evidence exists for neurotoxic, oncogenic or reproductive adverse effects in humans that can be attributed

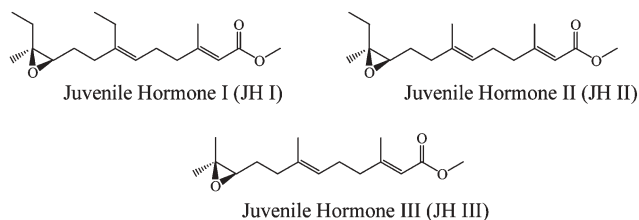


Fig. 10 Juvenile hormones I–III.

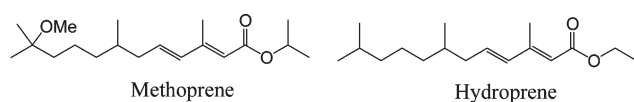
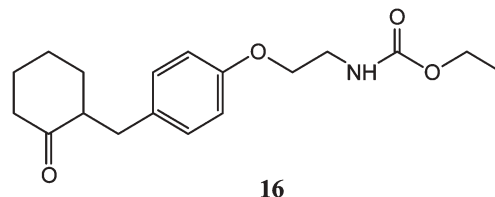


Fig. 11 Juvenile hormone analogues (JHAs).

to methoprene.¹⁰⁶ Hydroprrene was not classified by the US EPA as a human carcinogen.¹⁰⁷

Esters with juvenile hormone activity were obtained starting from alkenoic or alkadienoic acids and phenoxy- or phenoxyphenoxyethanol.¹⁰⁸ Wimmer *et al.* reported¹⁰⁴ the preparation of racemic cyclohexanone-derived carbamate **16** with JH activity. This juvenoid was more active on the yellow mealworm than natural juvenile hormones I–III.

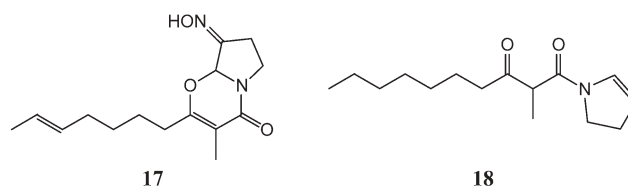


The same authors have reported¹⁰⁹ the preparation of esters of the reduced form of **16** by standard acylation of the hydroxy group (Scheme 5). These compounds are considered as juvenogens, that is, agents that liberate during a long period of time the biologically active component (juvenoid) by enzymatic hydrolysis of the ester.¹⁰⁹

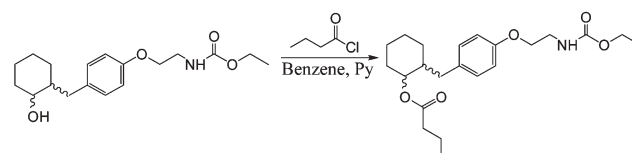
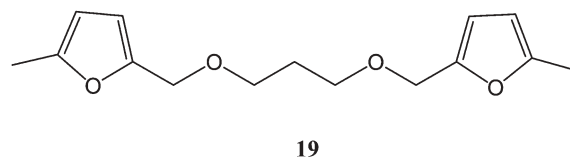
Much effort has also been devoted to the isolation and preparation of compounds that antagonise juvenile hormone activity or that provoke disruption of hormone biosynthetic pathways.¹¹⁰

For instance, Primo-Yúfera and coworkers¹¹¹ reported the isolation and identification of brevioxime **17**, a metabolite from *Penicillium Brevicompectum*, which exhibits an activity as high as a JH III biosynthesis inhibitor.¹¹⁰

The same authors have also isolated¹¹² the new ketoamide **18** from the same fungus with a high *in vivo* antagonistic JH activity with induction of precocious metamorphosis.

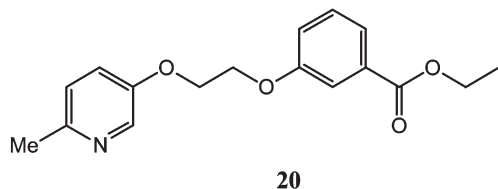


Bowers *et al.* have prepared¹¹³ and studied the biological activity of several furanyl-containing ethers such as **19**. These compounds exhibited anti-juvenile hormone activity as evidenced by the induction of premature metamorphosis in some insects.



Scheme 5 Synthesis of a juvenogen.

Furthermore, several 6-methyl-3-pyridyl ethers, such as **20**, have been prepared^{114,115} and proved to induce precocious metamorphosis of the silkworm *Bombyx mori* when applied to larvae. The presence of the methyl substituent on the pyridine ring was found to be important for its activity.¹¹⁴

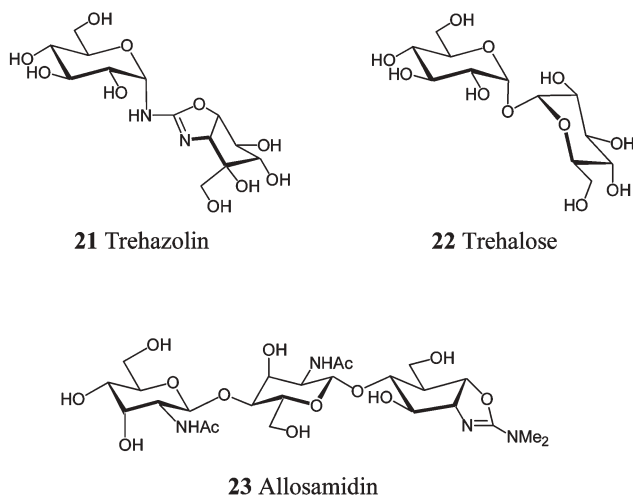


5.2. Chitin synthesis inhibitors

Chitin is a homobiopolymer of *N*-acetylglucosamine (Fig. 12) found in invertebrates, especially in insects and crustaceans, to whom it provides rigidity and serves as a mechanical and protective barrier.^{116,117} As chitin is absent from plants and vertebrates, it is considered as a potential and safe target for insect control.¹¹⁷

Several natural compounds have been found to strongly inhibit some steps of the biosynthesis of chitin in insects and so they are considered as potential insecticides.¹¹⁸ For instance, natural trehazolin **21**, an aminocyclitol-derived *N*-substituted cyclic isourea,¹¹⁹ is a strong *in vitro* inhibitor of trehalase. This is the enzyme required for the hydrolysis¹²⁰ of trehalose **22**, the carbohydrate precursor of chitin.¹¹⁷ The activity shown by trehazolin has prompted its total synthesis and the preparation of structural analogues.^{118,121}

Furthermore, allosamidin **23**, another naturally occurring carbohydrate-derived isourea,¹¹⁸ shows strong inhibition against chitinase, which plays a pivotal role in the life cycle of insects as it is the enzyme involved in chitin hydrolysis.



However, both trehazolin and allosamidin have a large number of hydroxy groups which prevent them penetrating the insect cuticle and reaching their specific targets.¹²⁰ This feature has precluded the practical use of trehazolin and allosamidin for *in vivo* pest control.¹²⁰

To date, two different groups of compounds interfering with chitin biosynthesis are used effectively against insects.¹¹⁷ One

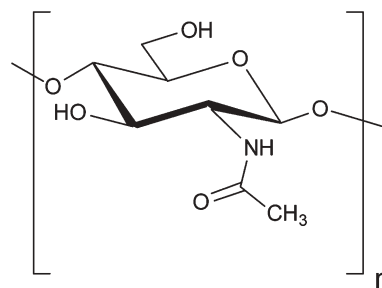


Fig. 12 Chitin structure.

group is comprised of nucleoside peptides, such as Nikkomycin-Z¹²² (Fig. 13), obtained from a culture of *Streptomyces tendae* and one of the most potent chitin synthase inhibitors. The second group consists of *N*-acyl urea derivatives, such as diflubenzuron¹¹⁷ (Fig. 13) the first insecticidal benzoylurea marketed almost three decades ago. It presents a high and selective efficiency against lepidopterans at larval stages. Diflubenzuron has been reported to be safe in acute, chronic and genotoxic studies on experimental animals; it is also safe for fish and aquatic invertebrates.¹²³ Furthermore, diflubenzuron has been shown to exhibit antitumoral effects against several malignant cell lines,¹²³ and has shown no carcinogenicity after long-term exposure in mice¹²⁴ and no teratogenicity in rodents.¹²⁵

Although the exact action mechanism of acylureas as insecticides has not been proved yet,¹²⁶ they act by preventing chitin formation at critical stages in insect life, provoking weakness of the cuticle and disruption in the moulting process.

The interest in acylureas as insecticides has allowed the development of some other commercial ureas, such as hexaflumuron (Fig. 13), which exhibits potent larvicidal activity against termites.¹²⁷ This compound, marketed as Sentricon[®], received the US EPA registration as a reduced risk pesticide, from environmental and human risk perspectives. It also obtained the 2000 Presidential Green Chemistry Award, presented by the US EPA.¹²⁸

Some other compounds acting on the chitin biological pathway are being tested at present as insecticides. Among them, a novel class of potential insecticides is that of pyridazinone-substituted 1,3,4-oxadiazoles,^{98,129} being remarkable in that both oxadiazole- and pyridazinone-derived compounds exhibit insecticidal activity. Thus, oxadiazole containing compounds seem to block the incorporation of

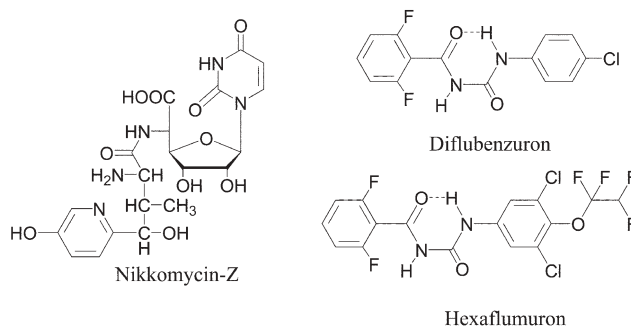


Fig. 13 Compounds interfering with chitin biosynthesis.

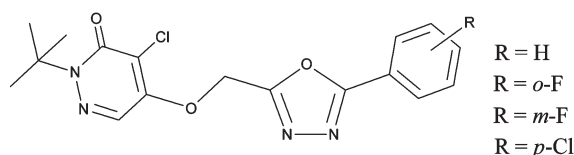


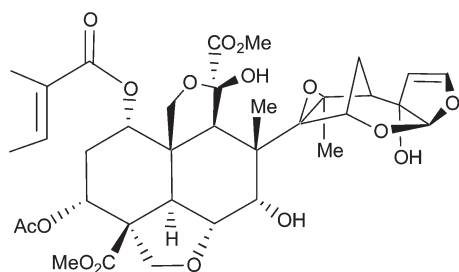
Fig. 14 Compounds with antifeedant activity.

N-acetylglucosamine into chitin biosynthesis,⁹⁸ whereas pyridazinone-derived insecticides are found to show juvenile hormone effects.²⁰

For instance, compounds shown in Fig. 14 exhibit potent antifeedant activity against larvae of some insects,⁹⁸ such as *Pseudaletia separata*, *Pieris rapae*, *Plutella xylostella* and *Bombyx mori*.

6. Neem-based insecticides: azadirachtin

For self-defense purposes, many plants generate chemicals that are toxic to insects. These naturally occurring insecticides are called botanical insecticides or botanicals. They comprise, among others, rotenone, *d*-limonene, sabadilla and ryania, besides pyrethrum and nicotine, described above.¹³ However, the most promising botanical insecticide seems to be azadirachtin (**24**), a triterpenoid isolated from the seeds of the Indian neem tree (*Azadirachta indica* A. Juss).¹³⁰ For thousands of years, the therapeutic and insecticidal properties of the neem tree have been recognized in India.¹³⁰



24 Azadirachtin

Azadirachtin exhibits insecticidal activity against more than 200 pest species,¹³¹ although only a few of them can be considered as commercial targets because of the relatively high cost of production as compared to synthetic insecticides.¹³¹

Azadirachtin shows a variety of modes of action. It has been found to be especially active as an antifeedant,¹³² and as an insect growth regulator,¹³² as it reduces the level of the insect hormone ecdysone. Mating and sexual communications may also be disrupted by azadirachtin, which results in reduced fecundity.¹³⁰ These combined modes of action are unique among currently available insecticides.

Azadirachtin is an ideal complementary insecticide in Integrated Pest Management (IPM) programs because it kills phytophagous insects, but has little or no activity against beneficial predatory mites or insects.¹³¹

This compound is relatively short-lived and easily degradable; furthermore, its mammalian toxicity is low, although it is toxic to fish and aquatic invertebrates.¹³³ A reversible effect on reproduction of both male and female mammals seems to be

the most important toxic effect upon sub-acute or chronic exposure.¹³⁴ Nevertheless, risks to human health upon exposure to azadirachtin are not expected when used according to label directions.¹³⁴

Azadirachtin was classified by the US EPA as a biorational insecticide, because of its natural origin and its limited adverse effects on the environment or beneficial organisms.¹³¹

Because of the great interest in azadirachtin, many synthetic approaches have been reported,^{135–140} although its total synthesis has not yet been carried out.

7. Microbial insecticides

Adverse toxicological effects found in many traditional insecticides, together with resistance developed by some pests, have prompted a continuous search for safer substitutes. In this context, the use of living systems as agents for pest control is emerging as a promising area for the future design of environmentally friendly insecticides.¹⁴¹ Living systems useful in agriculture comprise viruses, bacteria, fungi, insect predators and engineered-plants, microorganisms being the most important ones. Although currently biopesticides only represent about 1% of the world pesticide market, this percentage is expected to increase to 20% by the year 2020.¹⁴² Besides their relative safety to non-targeted organisms, humans and the environment, biopesticides are of great importance in specific IPM programs when produced and delivered correctly.¹⁴¹

For instance, in the USA, several baculoviruses, that is, double-stranded DNA viruses, have been registered as pesticides.¹⁴³ Baculoviruses are beneficial viruses, as they do not infect man or plants, and provide effective control against many insect species.¹⁴⁴ The use of this kind of microorganism as insecticides presents many attractive advantages, such as a high specificity, adequate pathogenicity, ease of genetic manipulation and minimal residue problems.¹⁴³ All the studies conclude¹⁴⁵ that baculoviruses are safe for use as pest control agents against forest pests, as they do not affect non-targeted species; in fact, baculoviruses do not replicate in mammalian cells and they do not seem to be able to enter the mammalian cell nucleus.¹⁴⁶ Nevertheless, the main disadvantages as insecticides¹⁴³ are a slow action speed, a too narrow specificity and instability in the environment, as baculoviruses are deactivated by exposure to UV radiation.¹⁴⁷ These factors, together with the difficulty of production and the problems of registration, have limited the use of baculoviruses as commercial insecticides. Biotechnology has allowed the obtention of engineered baculoviruses by insertion or deletions of specific genes, in order to increase their speed of action.¹⁴⁷

Nevertheless, the most important microorganisms used as biopesticides are bacteria, and especially *Bacillus thuringiensis* (*Bt*), an endospore-forming soil bacterium,¹⁴⁸ in fact, insecticides derived from *Bt* account for 90% of the biopesticide market.¹⁴⁸ There are hundreds of *Bt* subspecies and during sporulation most of them produce one or more insecticidal proteins, so-called δ -endotoxins¹⁴⁹ or insecticidal crystal proteins (ICPs or Cry proteins). Every bacterial strain produces a toxin which is specific against a group of insects;¹⁴⁸ there are currently 150 insect pests that are susceptible to *Bt*.

Cry proteins bind to specific receptors in the larval midgut cells, causing cellular swelling and lysis.¹⁵⁰

Bacillus thuringiensis-based insecticides have been used since 1961 against caterpillars and more recently, against mosquito and black fly larvae.¹⁵¹ Commercial *Bt* insecticides are comprised of a mixture of spores, spores undergoing germination, vegetative cells, Cry proteins and cell debris.¹⁵¹

The US EPA concluded in 1998 that *Bt*-derived insecticides are eligible for reregistration,¹⁵² as they present a favorable environmental profile. Thus, toxicological studies have concluded that *Bt* is practically non-toxic to humans and mammals¹⁵⁰ (these insecticides are classified as toxicity class III, slightly toxic), and they are safe for most non-targeted species, except for those closely related to the targeted insects. Furthermore, these insecticides do not leach with groundwater and are biodegradable, so they do not persist in the environment.¹⁴⁸

Nevertheless, δ -endotoxins are readily inactivated, and the number of spores decreases quickly, so several applications are needed in order to keep an effective level of the insecticide.¹⁴⁹ In order to overcome this problem, genetically engineered plants incorporating protectants (PIPs) have been developed to express Cry proteins by incorporating the *Bt* gene.¹⁵⁰ By this approach, the efficiency of pest control is not dependent on application timing and unlike classical pesticides applied to leaves and grass, Cry proteins are present in microgram quantities and are also produced at low levels in the pollen.¹⁵⁰ So, the use of genetically-modified plants as insecticidal agents has allowed an important reduction of chemical insecticides, together with an increase in crop yields by preserving beneficial organisms.¹⁵⁰

Due to all these advantages, the market of these pest control agents is expected to increase rapidly in the next few years.

Conclusions

The importance of controlling pests has led to the development of a variety of insecticides that prevent agriculture losses and spreading of diseases. Toxicological studies based on acute and chronic effects upon exposure have revealed that many classical insecticides are highly toxic not only to non-targeted insect species, but also to mammals and humans.

Furthermore, some of them, such as several organophosphorus insecticides, have proved to cause cumulative effects on long-term exposure. As a result of more strict regulatory controls issued by the US EPA, the use of many classical insecticides such as organochlorinated hydrocarbons and organophosphorus and carbamate compounds has been restricted or even cancelled.

Consequently, a search for safer alternatives for pest control is needed. Thus, intense research is being carried out to obtain chemically-modified substances with improved insecticidal activity in terms of selectivity towards insects and low toxicity to the environment, and to non-targeted species including humans. The combination of new synthetic approaches and biological and physiological studies has resulted in the preparation of insecticides with a better environmental profile, with different mechanisms of actions, and with reduced risks for living systems. In this context, compounds such as

neonicotinoids, pyrethroids or insect growth regulators show a remarkable activity.

However, as a green alternative to synthetic insecticides, biological agents must not be forgotten. So, naturally occurring compounds such as spinosyns and azadirachtin, or living systems such as *Bacillus thuringiensis* have proved to be efficient insecticides against a number of commercially important insect pests. These biopesticides lack the disadvantages present in classical synthetic insecticides; they are considered as low-risk agents, they do not present acute or cumulative risks to humans and are usually quite specific. It is expected that the ratio of marketed biopesticides will increase in the next few years as an attempt to reduce the environmental impact of synthetic insecticides.

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Oscar López,^{*,a} José G. Fernández-Bolaños^a and María Victoria Gil^b

^aDepartamento Química Orgánica, Facultad Química, Universidad Sevilla, Apartado 553, E-41071 Sevilla, Spain. E-mail: osc-lopez@us.es; Fax: + 34 95 4624960; Tel: +34 95 4557150

^bDepartamento de Química Orgánica, Facultad de Ciencias, Universidad de Extremadura 06071, Badajoz

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