

NEW MODES OF ACTION OF INSECTICIDES

Ralf Nauen and Thomas Bretschneider of Bayer CropScience AG, Research, based at Monheim in Germany outline the development of new insecticides based on novel modes of action

Introduction

Herbivorous insect and mite pests are a major threat in the continuous supply of food for human consumption. Synthetic insecticides and acaricides play a major role in modern agriculture and their chemical, environmental and toxicological properties have been improved considerably over the last six decades. Without the use of chemical insecticides dramatic yield losses in many cropping systems worldwide would occur (Oerke and Steiner, 1996). In 2001 a total of 7.56 billion US Dollars was spent in order to protect crops from damage by invertebrate pest species (Beckmann and Haack, in press). The world market for insecticides is still dominated by compounds irreversibly inhibiting acetylcholinesterase (AChE), an important enzyme in the central nervous system of insects, responsible for the cleavage of the neurotransmitter acetylcholine. However, as shown in Table 1 the market share of these AChE inhibitors, *i.e.* organophosphates and carbamates, decreased from 71% in 1987 to 51% in 1999. Together the AChE inhibitors and those insecticides acting on the voltage-gated sodium channel (vgSCh), in particular the pyrethroids, account for approx. 70% of the world market. Here we would like to give an overview of the active ingredients introduced more recently and their biochemical modes of action.

Insecticides acting on ligand- and voltage-gated ion channels

Nicotinic acetylcholine receptor agonists

The insect nicotinic acetylcholine receptor (nAChR) is one of the insecticidal targets which came in vogue after the introduction of the neonicotinoid (chloronicotiny) insecticide imidacloprid by Bayer CropScience in 1991, and was reviewed very recently (Nauen *et al.*, 2001). Insect nAChR are thought to be pentamers of two identical alpha-subunits and three beta-subunits making the transmembrane protein with a central cation-permeable ion channel found in post-synaptic membranes. Since the launch of imidacloprid (the top-selling insecticide world-wide) neonicotinoids have developed into a major class of insecticides with several other active ingredients introduced over the last 7 years; Figure 1 gives a few examples of the latest additions to this class of chemistry.

All neonicotinoid insecticides act as agonists of the insect nAChR located in the central nervous system. They are effective against sucking insects such as aphids, whiteflies and planthoppers, beetles and some lepidopteran pests and can be used as foliar sprays, seed treatments and *via* soil application. Another very interesting agonist of the insect nAChR, the macrocyclic natural compound spinosad (Figure 2), was commercialized by DowAgroscience in 1997 (Thompson and Hutchins, 1999) and does not belong to the

Table 1. Modes of action of the top-selling 100 insecticides/acaricides and their world market share (excluding fumigants, endotoxins and those insecticides with unknown mode of action).

Mode of action	1987 ¹ %	1999 %	Change %
Acetylcholinesterase	71	52	-20
Voltage-gated Na channel	17	18	+1.4
Acetylcholine receptor	1.5	12	+10
GABA-gated Cl channel	5.0	8.3	+3.3
Chitin biosynthesis	2.1	3.0	+0.9
NADH dehydrogenase	0	1.2	+1.2
Uncouplers	0	0.7	+0.7
Octopamine receptor	0.5	0.6	+0.1
Ecdysone receptor	0	0.4	+0.4

¹ Data taken from Nauen *et al.* (2001)

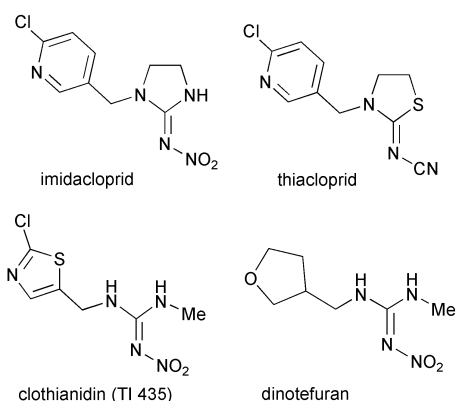


Figure 1. Neonicotinoid (chloronicotinyl) insecticides

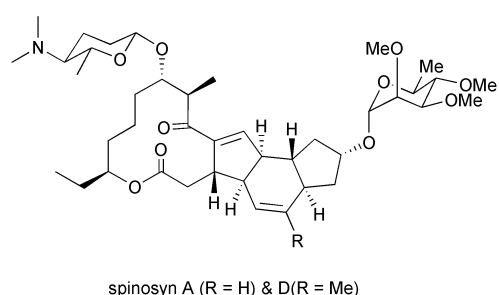


Figure 2. Structure of spinosad

neonicotinoids. Spinosad is a mixture of the two naturally occurring macrolides spinosyn A (85%) and spinosyn D (15%), which were isolated from the soil microorganism *Saccharopolyspora spinosa*. This mixture is particularly effective against pest lepidopteran noctuid larvae, a group of leaf-feeding insect pests not covered by neonicotinoid insecticides.

Spinosad is generally not active against sucking pest insects such as aphids and whiteflies. The mode of action of spinosad is the allosteric activation of insect nAChRs and a prolongation of acetylcholine responses. Spinosad has a different binding site on the nAChR than imidacloprid and other nAChR based insecticides.

γ -Aminobutyric acid (GABA) receptor/chloride ionophore complex

GABA-gated chloride channels are located in the insect central nervous system and peripheral nerves where they mediate the proper integration of neuronal activity and muscle relaxation by inhibitory actions. The GABA-receptor is a pentameric membrane protein and each subunit is composed of four transmembrane helices. These so-called GABA_A-receptors are target sites for three important classes of insecticides and the symptomology of poisoning induced by compounds acting as antagonists on inhibitory GABA_A-receptors resemble those induced by agonists of excitatory receptors, e.g. hyperactivity, convulsions and leg tremor. Important insecticidal GABA antagonists are on the one hand the old class of the chlorinated cyclodienes (e.g. dieldrin) and on the other hand the new class of the aryl-

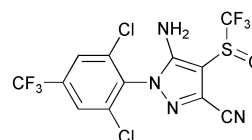


Figure 3. Structure of fipronil

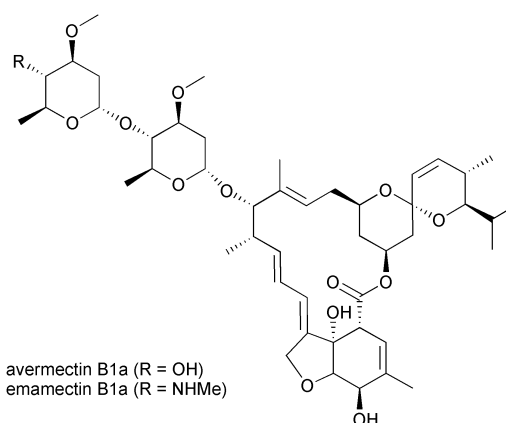


Figure 4. Structure of avermectins.

amino-pyrazoles represented by fipronil (Figure 3) launched in 1993 by Rhone-Poulenc. Fipronil is an insecticide with contact, stomach and systemic action and is highly effective against lepidopteran larvae and many agricultural pests. It also has a broad application range and can be applied as foliar spray, seed treatment and *via* the soil.

Another class of GABA_A-receptor ligands are the avermectins, natural products (macrocyclic lactones) with excellent insecticidal, miticidal and anthelmintic properties. They are potent agonists at the GABA_A receptor but they also interact with glutamate-gated chloride channels in the insect nervous system. The resulting strong chloride ion influx into the nerve cells results in disruption of nerve impulses, paralysis and finally death.

Recently emamectin benzoate (Figure 4), a new semi-synthetic avermectin derivative, where the hydroxy-group in the terminal sugar ring is replaced by a methylamino group was introduced to the market. It shows a high level of activity especially against lepidopteran larvae, which is better than abamectin, and allows the control of most lepidopteran pests on various crops.

Voltage-gated sodium channel effectors

The voltage-gated sodium channel (vgSCh) is an important molecular target with at least eight pharmacologically characterized (allosterically interacting) binding sites for several synthetic insecticides, low molecular weight natural toxins and polypeptide neurotoxins isolated from venomous animals such as scorpions. The rapidly depolarizing phase and propagation of action potentials in many excitable cells due to rapid increase in membrane sodium conductance is mediated by vgSCh. Among common synthetic insecticides DDT, pyrethroids, isobutylamides and dihydropyrazoles are known to interact with vgSCh.

The newest insecticidal compound, suggested to interact

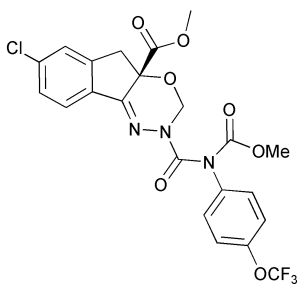


Figure 5. Structure of indoxacarb.

with the vgSCh binding site of local anaesthetics, is indoxacarb (Figure 5), a new oxadiazine insecticide recently introduced by DuPont (Wing *et al.*, 1998). It is especially active on lepidopteran larvae. Indoxacarb is a pro-insecticide with only weak activity on vgSCh. It is metabolically activated by cleavage of the N-methoxycarbonyl group. The resulting NH-derivative is a potent sodium channel blocker. Since indoxacarb acts on a binding site different from that of pyrethroids, no cross-resistance between these classes has been found.

Insecticides acting on insect development

Many compounds are known to interfere with insect development, in particular with larval ecdysis (cuticle shedding process when larvae molt from one instar to the other), subsequent chitin deposition and metamorphosis (transition from larvae to pupae and adults in holometabolous insects). Insecticides acting on insect development are also called insect growth regulators. True insect growth regulators are "insecticides that mimic the action of hormones on the growth and development of insect pests" (Beckage, 2000). This definition given by Beckage (2000) is quite stringent and does not necessarily cover those older synthetic insecticides acting on chitin biosynthesis that are obviously not hormone-associated with regard to their mode of action (*e.g.* benzoylphenyl ureas). Insect growth regulators are often very species-specific insecticides (Dhadialla *et al.*, 1998), and in general are not active as aphicides, but rather against other pest insects, *e.g.* whiteflies (buprofezin and pyriproxyfen), Lepidoptera (methoprene, fenoxycarb, and benzoylphenyl ureas), Heteroptera (*e.g.* fenoxycarb), Orthoptera and Hymenoptera (pyriproxyfen) and Diptera (cyromazine) (Beckage, 2000 and references therein). Insect growth regulators are in general much slower in providing full efficacy against pest insects compared to insecticides interfering with neuronal target sites.

Ecdysone receptor agonists

This class of compounds was introduced to the insecticide market in 1993 and represents the only new major contribution to the group of insect growth regulators since the introduction of the chitin biosynthesis inhibitors in the mid seventies. Ecdysone receptor agonists or moulting accelerating compounds (MACs) are non-steroidal ecdysone analogs and mimic the natural function of the endogenous moulting hormone 20-hydroxy-ecdysone. This is released at

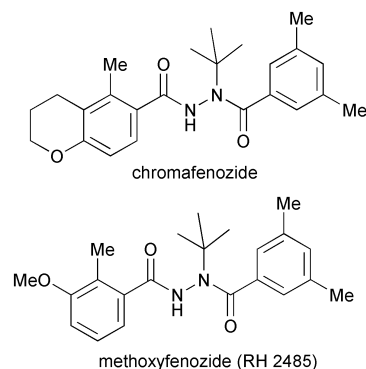


Figure 6. Moulting accelerating compounds.

physiologically strictly defined time-points before larval/larval or larval/pupal moults and initiates a cascade of biochemical reactions that lead to the formation of a new cuticle and shedding of the old cuticle at the end of the moulting process. MACs bind to ecdysone receptor-ultra-spiracle protein heterodimer complexes. This ligand-heterodimer complex then transactivates a cascade of genes by binding to a DNA ecdysone response element initiating molting.

Chemically MACs are described as substituted dibenzoyl hydrazines directly acting on ecdysone receptors. Compounds such as methoxyfenozide from Rohm and Haas and chromafenozide from Nippon Kayaku (Figure 6) induce a precocious moult in lepidopteran larvae. This leads first to the cessation of feeding and weight gain and then, at the end of the intoxication process, to premature head capsule slippage and death.

Four MACs have already been successfully launched: tebufenozide, methoxyfenozide, halofenozide and chromafenozide. All except halofenozide are more or less specific to lepidopterans. Halofenozide is more specific in the control of, for example, dipteran larvae (especially turf pests). Current ecdysone agonists do not act on hemipteran pest species.

Insecticides and acaricides acting on mitochondrial respiration

The mitochondrial electron transport coupled with oxidative phosphorylation is an essential process in many organisms. The electrons of reduced cofactors such as NADH (nicotinamide adenine dinucleotide) are transferred *via* several different enzyme complexes (sites) to finally reduce oxygen to water producing ATP, the all-important energy source of all cells.

Complex I inhibitors

Several inhibitors of the mitochondrial electron transport of complex I (NADH dehydrogenase) are known. Besides the two natural insecticides, rotenone and the piericidins several new potent synthetic acaricides with this mode of action have been introduced in the nineties, *i.e.* fenpyroximate (Nihon Nohyaku 1991), pyridaben (Nissan 1991), fenazaquin (Dow 1993), tebufenpyrad (Mitsubishi 1993, Figure 7), pyrimidifen (Sankyo, Ube 1995, Figure 7).

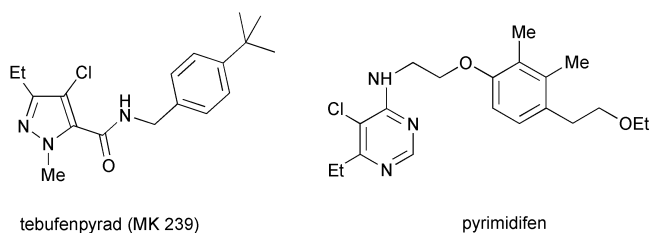


Figure 7. **Complex I inhibitor acaricides.**

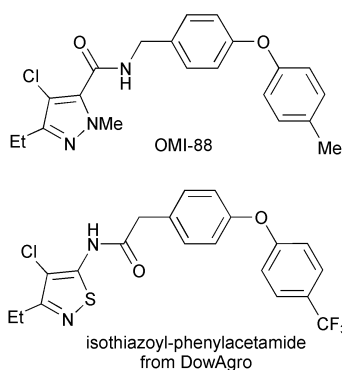


Figure 8. **Insecticidal complex I inhibitors.**

Much effort has also been devoted to the synthesis of insecticidal complex I inhibitors, and several interesting experimental products have been published such as OMI-88 from Mitsubishi or the isothiazoyl-phenylacetamides from DowAgro (Figure 8; Samaritoni *et al.*, 1999). However, toxicological problems with respect to mammals and fish, which compared to acaricides seem to be impossible to overcome in the case of insecticides, mean that no complex I inhibitor developed for pest insect control has yet been introduced to the insecticide market.

Complex III inhibitors

The methoxyacrylate (MOA) motif is the toxophore of many commercially successful strobilurin based fungicides. Fluacrypyrim (Figure 9) is the first acaricidal development product out of this group (Nippon Soda/BASF). It is, like the corresponding fungicides, an inhibitor of complex III (cytochrome *c* reductase) of the mitochondrial electron transport chain. Experimental MOA compounds with potent insecticidal activity have been evaluated, but could not be developed up to now due to high mammalian toxicity. The highly lipophilic acequinocyl (AKD-2023; Figure 9) is an acaricidal compound under development by Agro-Kanesho with activity against all stages of spider mites (Kinoshita *et al.* 1999). Acequinocyl is a pro-acaricide, and the de-acylated metabolite with its free hydroxy group is a potent inhibitor of complex III of the mitochondrial electron transfer (ubiquinol oxidation site, Q_0 centre).

Uncouplers

Uncouplers are acidic compounds with the ability to destroy the proton gradient of mitochondrial cell membranes by transporting protons through the membrane. Since this proton gradient is necessary for the production of ATP these

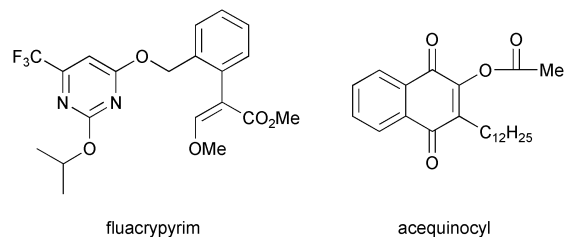


Figure 9. **Complex III inhibitor acaricides.**

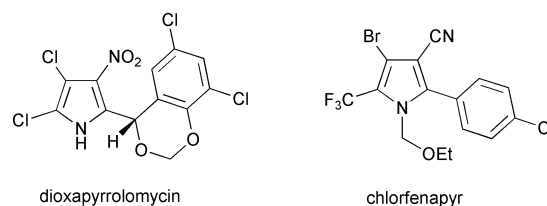


Figure 10. **Insecticidal uncouplers.**

compounds uncouple oxidative phosphorylation from electron transport, which leads to cell death. Since this is a quite unspecific mode of action, toxicity issues regarding non target organisms have to be considered closely. The old dinitrophenols used as miticides, fungicides and herbicides are uncouplers, but have low selectivities and are toxic to all forms of life. Synthetic work at American Cyanamid based on the natural product dioxapyrrolomycin (Figure 10), which has moderate insecticidal activity, led to the interesting group of the insecticidal 2-aryl-pyrroles. Chlorfenapyr (AC-303630; Figure 10) was introduced to the market in 1995 as an insecticide with a broad spectrum of activity against lepidopteran and sucking pests. The compound is a pro-pesticide, and the active compound is generated *via* oxidative N-dealkylation to the NH derivative.

Insecticides and acaricides with unknown modes of action

Among those insecticides with unknown (or non-described) modes of action two new aphicides are worthy of mention here, pymetrozin and flonicamid (Figure 11). Pymetrozin is a pyridine azomethine, selectively active against aphids, whiteflies and plant hoppers (Flückiger *et al.*, 1992). Symptomatology of poisoning studies with aphids revealed an immediate inhibition of feeding leading to death by starvation. However, the biochemical mode of action has not yet been resolved.

Interestingly, flonicamid was also described as an aphid

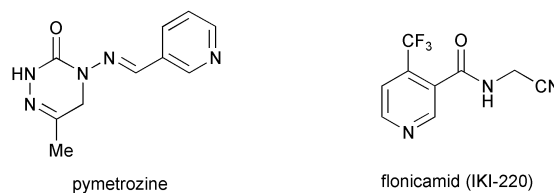


Figure 11. **New insecticides with unknown/non-described modes of action.**

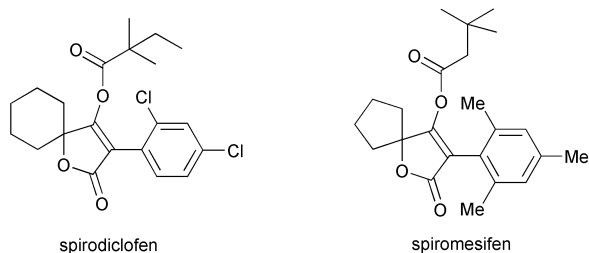


Figure 12. New tetronic acid derivatives.

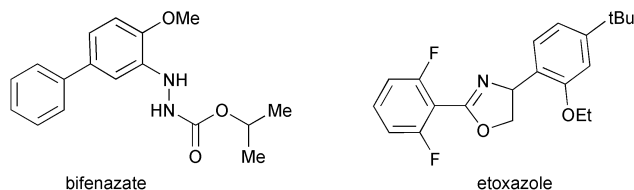


Figure 13. New acaricides with non-described mode of action.

feeding inhibitor with unknown biochemical mode of action. Flonicamid is a novel selective aphicide under development by Ishihara Sangyo Kaisha Ltd. (Morita *et al.*, 2000). It belongs to a new class of systemic aphicides, the trifluoromethylpyridine derivatives, active against various aphid species in fruits, ornamentals, cereals and vegetables. It is active by foliar as well as by soil application.

Spirodiclofen is a novel acaricide from the new chemical class of tetronic acid derivatives introduced very recently (Wachendorff *et al.*, 2000). Spirodiclofen provides excellent control of important mite pests with low use rates, a very long lasting efficacy and good plant compatibility in all relevant crops. It has a new mode of action which is still under investigation, but there is strong evidence that the compounds interfere with lipogenesis in mite development (Nauen *et al.*, unpublished). It shows no cross-resistance to any currently available acaricide, which is particularly important in view of the widespread reduced sensitivity of many tetranychid mite populations to existing acaricides (Figure 12).

Another new insecticide is the tetronic acid derivative spiromesifen (proposed common name) selectively active against whiteflies and spider mites (Nauen *et al.*, 2002). It is thought to act on lipid biosynthesis due to its structural similarity to the acaricidal tetronic acid derivative spirodiclofen (Figure 12).

A further new acaricide with unknown mode of action is bifenzate (D 2341). It shows outstanding knockdown and residual activity and was launched in 1999 by Uniroyal (Dekeyser *et al.*, 1996). The mode of action has not yet been published (Figure 13).

Etoxazole (YI 5301) is an acaricide from the new chemical class of the oxazolines, launched in 1998 by Yashima. It shows contact activity against eggs and juvenile forms of mites, but no activity against adults. Etoxazole is also effective against hexythiazox-resistant mites.

References

- Beckage, N. E. (2000) Insect growth regulators. In: *Biological and Biotechnological Control of Insect Pests* (Eds. J. E. Rechcigl, N. A. Rechcigl). Lewis Publishers, Boca Raton, London, New York, Washington, D.C., pp. 123–137.
- Beckmann, M.; Haack, K. J. Chemische Schädlingsbekämpfung in der Landwirtschaft. *Chemie in unserer Zeit*, in press. 45–569
- Dekeyser, M.A.; McDonald, P. T.; Angle, G. W.; Moore, R. C. (1996) D2341- a novel agent to control spider mites. *Proceedings of the Brighton Crop Protection Conference - Pests and Diseases 2*, 487–492.
- Dhadialla, T. S.; Carlson, G. R.; Le, D. P. (1998) New insecticides with ecdysteroidal and juvenile hormone activity. *Annual Review of Entomology* 43, 5.
- Flückiger, C. R.; Kristinsson, H.; Senn, R.; Rindlisbacher, A.; Buholzer, H.; Voss, G. (1992) CGA 215'944 – A novel agent to control aphids and whiteflies. *Proceedings of the Brighton Crop Protection Conference – Pests and Diseases 2-3*, 43–50.
- Kinoshita, S.; Koura, Y.; Kariya, H.; Ohsaki, N.; Watanabe, T. (1999) AKD-2023: A novel miticide. Biological activity and mode of action. *Pesticide Science* 55, 633–675.
- Morita, M.; Ueda, T.; Yoneda, T.; Koyanagi, T.; Murai, S.; Matsuo, N. (2000) IK1-220 – A novel systemic aphicide. *Proceedings of the Brighton Crop Protection Conference – Pests and Diseases 2*, 59–65.
- Nauen, R.; Ebbinghaus-Kintscher, U.; Elbert, A.; Jeschke, P.; Tietjen, K. (2001) Acetylcholine receptors as sites for developing neonicotinoid insecticides. In: *Biochemical Sites Important in Insecticide Action and Resistance*, (Ed. I Ishaaya), Springer Verlag Berlin, Heidelberg, pp.77–105.
- Nauen, R.; Bretschneider, T.; Brueck, E.; Elbert, A.; Reckmann, U.; Wachendorff, U.; Tiemann, R. (2002) BSN 2060 - A novel compound for whitefly and spider mite control. *Proceedings of the Brighton Crop Protection Conference – Pests and Diseases*, 2A-3, 39-44.
- Oerke, E. C.; Steiner, U. (1996) Ertragsverluste und Pflanzenschutz: Die Anbausituation für die wirtschaftlich wichtigsten Kulturpflanzen. Schriftenreihe der Deutschen Phytomedizinischen Gesellschaft, Vol. 6, Eugen Ulmer GmbH, Stuttgart.
- Samaritoni, J. G.; Babcock, J. M.; Schlenz, M. L.; Johnson, G. W. (1999) Methylene group modifications of the *N*-(isothiazol-5-yl)phenylacetamides. Synthesis and insecticidal activity. *Journal of Agricultural and Food Chemistry* 47, 3381–3388.
- Thompson, G.; Hutchins, S. (1999) Spinosad. *Pesticide Outlook* 10, 78–81.
- Wachendorff, U.; Brück, E.; Elbert, A.; Fischer, R.; Nauen, R.; Stumpf, N.; Tiemann, R. (2000) BAJ 2740: A novel broad spectrum acaricide. *Proceedings of the Brighton Crop Protection Conference - Pests and Diseases 2A-6*, 53–58.
- Wing, K. D.; Schnee, M. E.; Sacher, M.; Connair, M. (1998) A novel oxadiazine insecticide is bioactivated in lepidopteran larvae. *Archives of Insect Biochemistry and Physiology* 37, 91–103.

Further reading

- Ishaaya, I.; Degheele, D. (1998) *Insecticides with Novel Modes of Action*. Springer, Berlin, 289 pp.
- Perry, A. S.; Yamamoto, I.; Ishaaya, I.; Perry, R. Y. (1998) *Insecticides in Agriculture and Environment*. Springer, Berlin, 261 pp.
- Ishaaya, I. (2001) *Biochemical Sites Important in Insecticide Action and Resistance*. Springer, Berlin, 315 pp.

Ralf Nauen is head of Insect Toxicology and Resistance within Global Biology Insecticides of Bayer CropScience in Monheim, Germany. Thomas Bretschneider also works for Bayer CropScience at Monheim, on some insecticidal lead projects.