A review of Spinosyns, a derivative of biological acting substances as a class of insecticides with a broad range of action against many insect pests

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Abstract

Spinosyns are a class of insecticides with a broad range of action against many insect pests belonging to different orders, noxious to a wide variety of agricultural crops; spinosyns were also used against insects of sanitary interest. Spinosyns are derivative of biological active substances produced by soil Actinomycete *Saccharopolyspora spinosa;* being of biological origin, they are considered to have a low environmental impact and they are not much aggressive against non-target species. They act as allosteric activators of nicotinic acetylcholine receptors; thanks to their mode of action the resistance phenomena are uncommon, even few cases of resistance were recently reported.

For all these reasons at present they are one of the most interesting product to be used in fighting against agriculture pests.

Introduction

Spinosyns are a family of broad-spectrum insecticides including spinosad and spinetoram, all with a macrocyclic lactone structure, isolated from the actinomycete soil bacterium *Saccharopolyspora spinosa,* Mertz and Yao, 1990 (Bacteria: Actinobacteridae) (Sparks *et al.,* 1998).

Spinosad is derived from the aerobic fermentation of *S. spinosa* in aqueous growth media (containing *e.g.*, corn solids, soya bean flour, and cottonseed flour) after extraction and recrystallization of technical spinosad. Spinosad is a mixture (85:15%) of spinosyn A and spinosyn D (Mertz & Yao, 1990; Kirst *et al.*, 1992; Sparks *et al.*, 1999) (Figure 1). Because of its microbial origin, spinosad can be considered a bio pesticide, and it is useful for the management of many insect pests, including caterpillars, leaf miners, thrips, flies, drywood termites, and some beetles, in various vegetables, field crops, and fruits.

A descendant of spinosad is spinetoram (Saglam *et al.*, 2013), a mixture of two synthetically modified spinosyns (spinosyn J and spinosyn L), which are metabolites of *S. spinosa*. Chemical modifications relating spinosyn A, J, L and spinetoram are summarized in Figure 1 (Dripps *et al.*, 2008).

As spinosad, spinetoram has a neurotoxic mode of action on insects, through contact or ingestion, it was introduced as a novel insecticide with greater potency and faster speed of action in comparison with the older spinosyn-based relative spinosad (Dripps *et al.*, 2008; Sparks *et al.*, 2008).

Recently combining genome shuffling with antibiotics resistance screening resulted in an effective approach to achieve rapid improvement of spinosad yield of *S. spinosa* (Wang *et al.*, 2015a, 2015b).

Spinosad has been evaluated and accepted in USA for listing by the World Health Organization Pesticide Evaluation Scheme (WHOPES) working group, the official WHO body in charge of the assessment of pesticides for their effectiveness and safety (WHO, 2007). Spinosad was authorized in 2007 in Europe with a EU Commission Directive 2007/6/EC (European Commission, 2007), with subsequent updates, *e.g.*, EU Regulations 556/2012 and 2015/603 (European Commission, 2012, 2015). It was registered to be used against a broad array of target pests as Lepidoptera (pests of cotton), Diptera (fruit flies and mosquitoes), Thysanoptera, Isoptera and Coleoptera (Thompson *et al.*, 2000; Legocki *et al.*, 2010; Tescari *et al.*, 2014).

Spinetoram was registered for the first time in New Zealand immediately followed by the United States in September 2007 under the United States Environmental Protection Agency (USEPA) reduced risk pesticide initiative. Submission for Annex I inclusion in Europe was completed in 2007 and accepted in 2014 with the EU implementing Regulation 140/2014 (European Commission, 2014).

Toxicity on target organisms

Spinosyns were developed to control species detrimental to agriculture, but they were also used to control insects of sanitary interest. Spinosyns were tested on many pest species; the most investigated are summarized in Table 1.

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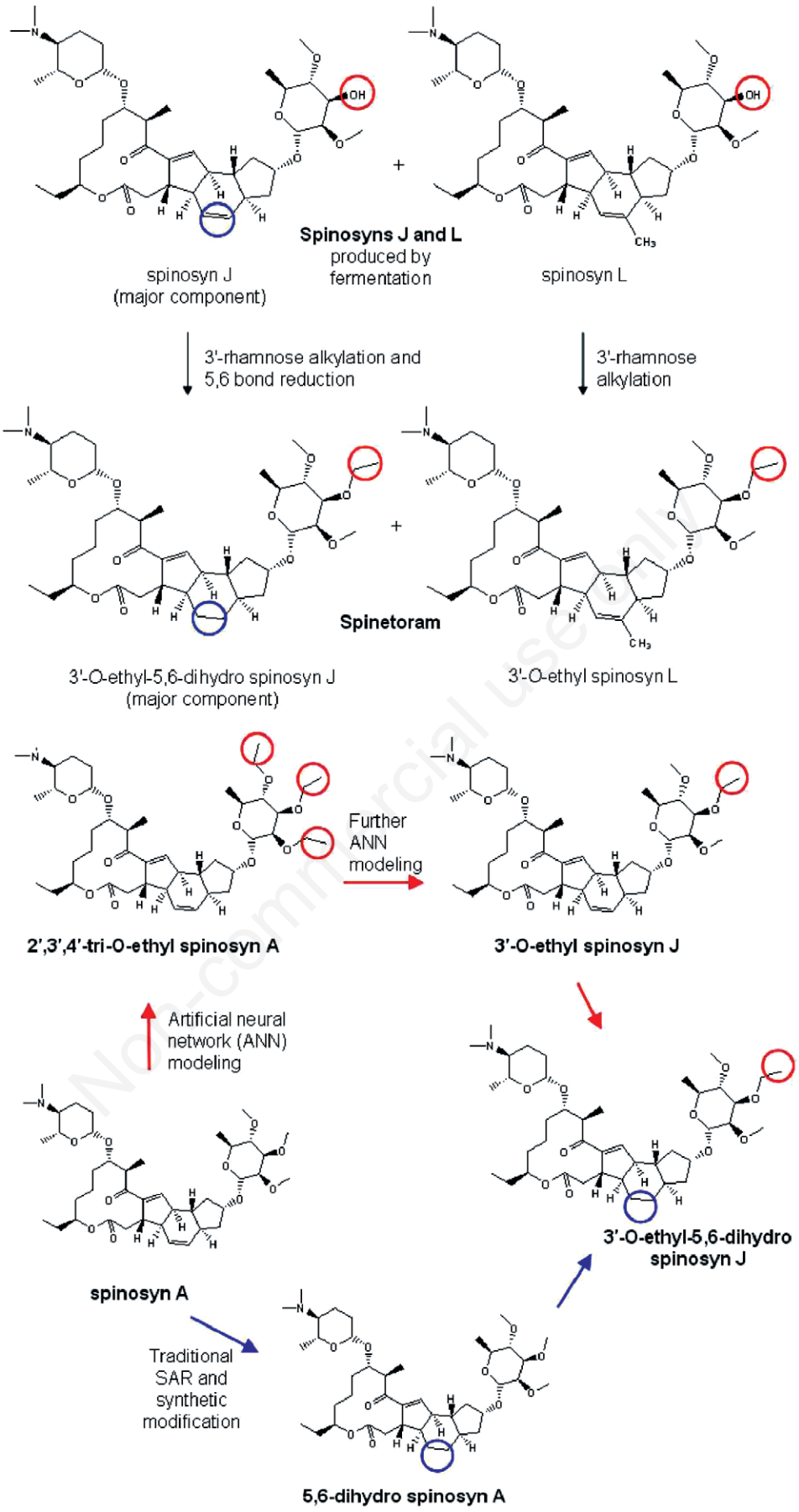

Figure 1. Chemical modifications connecting spinosyn A, J, L and spinetoram.

The mode of action of spinosyns may be as direct contact through body surface *contact toxicity* or through ingestion *dietary toxicity* (Shimokawatoko *et al.*, 2012).

Spinosad acts through contact activity on all life stages of insects, including eggs, larvae, and adults. Eggs must be sprayed directly, but larvae and adults can be effectively dosed through contact with treated surfaces. Spinosad is most effective when ingested, generally showing a greater selectivity toward target insects and a lesser activity against many beneficial predators as well as mammals and other aquatic and avian animals (Cleveland *et al.*, 2001).

A useful library to analyse the results of toxicity tests is given in the R-package drc: https://cran.r-project.org/web/packages/drc/drc.pdf

A comparison with other insecticides can be also carried out in a series of field tests. For example the efficacy of a spinosyn, spinosad (DE-105), was compared with other insecticides on the tobacco budworm *Heliothis virescens* (Fabricius, 1777) (Lepidoptera: Noctuidae) a parasite of cotton in Louisiana during 1989-95 and on the corn earworms *Helicoverpa zea* Boddie, 1850 and *H. armigera* (Hübner, 1805) (Lepidoptera: Noctuidae). Spinosad provided control of Lepidoptera equal to or greater than that afforded by pyrethroid, organophosphate and carbamate standards. In larval topical tests, the range of spinosad lethal dose at 50% (LD_{50}) among several populations of *H. virescens* was from 0.4 to 8.5 μg/g (Leonard *et al.*, 1996).

A variety of bioassays can be planned to evaluate the activity of nine different molecules of spinosyns. As test species *H. virescens* was used. Spinosyn A resulted the most effective with an LC_{50} of 0.3 ppm in the active range of many pyrethroids (Sparks *et al.*, 1998).

To explore the efficacy of spinosyn A compared with other insecticides, both drench (application to the larva in a large volume of solvent) and topical applications (direct application to the dorsum of the larva in a 1-μL drop of acetone) were used. For example topical bioassay performed on 3rd instar larvae of *H. virescens* gave an LD_{50} between 1.28-2.25 μg/g after 48-72 h (Sparks *et al.*, 1998).

The oral efficacy of spinosyn A compared with cypermethrin was also evaluated using leaf-dip, diet/egg, leaf-spray bioassays and hot-probe bioassays: i) the leaf-dip bioassay involved dipping a cotton leaf disk (33 mm diameter) in a test solution and allowing it to dry; ii) the diet/egg bioassay consisted of spraying a 1-oz (30 mL) plastic cup containing artificial diet with a test solution; iii) the leaf-spray bioassay was carried out using a cotton leaf disk (33 mm diameter) placed in the bottom of a 1-oz (30-mL) plastic cup and sprayed using a track sprayer calibrated to evenly coat the leaves with 0.055 mL of test solution; iv) a hot-probe bioassay: a simple and rapid paralysis assay to detect and characterize knockdown resistance (Bloomquist & Miller, 1985).

Spinosad and spinetoram were also tested as cereal protector against several stored product pests. Hertlein *et al.* (2011) summarized the results of several laboratory and field tests on various grains to reach conclusion that the lesser grain borer, *Rhyzopertha dominica* (Fabricius, 1972) (Coleoptera: Bostrichidae); larger grain borer, *Prostephanus truncatus* (G. H. Horn, 1878) (Coleoptera: Bostrichidae); rusty grain beetle, *Cryptolestes ferrugineus* (Stephens, 1831) (Coleoptera: Laemophloeidae); flat grain beetle, *Cryptolestes pusillus* (Schonherr, 1817) (Coleoptera: Laemophloeidae); red flour beetle, *Tribolium castaneum* (Herbst, 1787) (Coleoptera: Tenebrionidae); confused flour beetle, *Tribolium confusum* Jacquelin du Val, 1868 (Coleoptera: Tenebrionidae); Indian meal moth, *Plodia interpunctella* (Hubner, 1813) (Lepidoptera: Pyralidae); rice moth, *Corcyra cephalonica* (Stainton, 1866) (Lepidoptera: Pyralidae); angoumois grain moth, *Sitotroga cerealella* (Olivier, 1789) (Lepidoptera: Gelechiidae); almond moth, *Cadra cautella* (Walker, 1863) (Lepidoptera: Pyralidae), and the psocid species *Lepinotus reticulatus* Enderlein, 1905 (Psocoptera: Trogiidae) and *Liposcelis entomophila* (Enderlein, 1907) (Psocoptera: Liposcelididae) are susceptible to spinosad and complete control is to be expected. Other pest species such as the maize weevil, *Sitophilus zeamais* Motchulsky, 1855 (Coleoptera: Dryophthoridae); rice weevil, *Sitophilus oryzae* (Linnaeus, 1763) (Coleoptera: Dryophthoridae), and sawtoothed grain beetle, *Oryzaephilus surinamensis* (Linnaeus, 1758) (Coleoptera: Silvanidae) are susceptible to spinosad to varying degrees.

Likewise *P. truncatus* and *R. dominica* are very susceptible to spinetoram used to protect grain, while *T. confusum* and *O. surinamensis* showed a limited susceptibility to spinetoram (Vassilakos *et al.*, 2012). For *Sitophilus granarius* (Linnaeus, 1758) (Coleoptera: Dryophthoridae) and *S. oryzae*, spinetoram efficacy differed among the grain commodities (hard wheat, soft wheat, oats, rye, triticale, paddy rice and maize). In general, mortality was higher in hard wheat for both *Sitophilus* species in comparison with the other grains, while the lowest mortality levels were recorded in oats and soft wheat for *S. granarius* and in maize and soft wheat for *S. oryzae* (Vassilakos *et al.*, 2015).

Spinetoram was effective against *T. confusum* adults and young larvae. It revealed more effective than imidacloprid, thiamethoxam and chlorantraniliprole in controlling *T. castaneum*. On the other hand spinetoram was ineffective against *T. confusum* when applied directly on wheat, *Triticum aestivum* L. (Vassilakos & Athanassiou, 2013).

The toxicity of the bioinsecticide spinosad and the semi-synthetic insecticide spinetoram was compared with the toxicity of three insect growth regulator (IGR) compounds: lufenuron, chlorfluazuron and methoxyfenozide studying the response of 2nd instar larvae of the cotton leafworm *Spodoptera littoralis* Boisduval, 1833 (Lepidoptera: Noctuidae). Lufenuron, chlorfluazuron and methoxyfenozide were more toxic than spinosad by 9.8-, 9.4- and 9.2-fold, and more toxic than spinetoram by 2.0-, 1.9- and 1.8-fold, respectively, after 72 h of exposure. Spinetoram (LC_{50}=8.6 ppm) was more toxic than spinosad (LC_{50}=43.1 ppm) by approximately 5 folds, after 72 h of exposure. These results are summarized in Table 2.

Spinosad toxicity interacts with toxicity of IGR compounds. Spinosad/IGR compounds mixtures resulted in a potentiation effect more than the spinetoram/IGR compounds mixtures. The ratio spinosad or spinetoram at LC_{25}/IGR compounds at LC_{25} revealed potentiating effects higher than the ratio spinosad or spinetoram at LC_{25}/IGR compounds at LC_{10}. The ratio spinosad or spinetoram at LC_{10}/IGR compounds at LC_{25} resulted only in an additive effect.

Mixtures of spinosad-spinetoram/IGR were more toxic when spinosad-spinetoram were in higher proportion respect to IGR. Therefore, it was preferred to use high concentrations of spinosad or spinetoram with low concentrations of the IGR compounds and not the opposite. The toxicity of the three IGR compounds was enhanced with the time. Although the toxicity of tested IGR compounds appeared after 48 and 72 h of exposure, the potentiating effect of these compounds to spinosad and spinetoram appeared after 24 h (Rahman & Abou-Taleb, 2007).

Spinetoram toxicity was compared with the toxicity of Vertimec® (Syngenta AG, Basel, Switzerland). Vertimec® is a product containing abamectin, a mixture of avermectins, compounds derived from the soil bacterium *Streptomyces avermitilis*. Abamectin is a natural fermentation product of this bacterium. It is used to control insect and mite pests of a range of agronomic, fruit, vegetable and ornamental crops, and it is used by homeowners for control of fire ants on the moveable stages of *Tetranychus urticae* Koch, 1836 (Boisduval, 1867) (Acari: Tetranychidae). Spinetoram 12% was most effective than Vermectin®. The best reduction of pests was 100% after 13 and 19 days of egg treatment, 96.7% after 5 days for adult and 87.5 after 11 days for immature stages at dose 1 ml solution per liter of water (El-Kady *et al.*, 2007).

Spinetoram toxicity was compared with the toxicity of spinosad; at 61 g active ingredient (a.i.)/ha spinetoram was as effective as spinosad at 140 g a.i./ha against the western flower thrips and the other common thrips in Florida, *Frankliniella tritici* Fitch, 1855 and *F. bispinosa* (Morgan, 1913) (Thysanoptera: Thripidae).

Spinosad was used against the olive fly *Bactrocera oleae* (Gmelin,

Table 1. Target species investigated.

Higher taxon	Family	Scientific name	Common name
Acari	Tetranychidae	*Tetranychus urticae*	Red spider mite
Coleoptera	Bostrichidae	*Rhyzopertha dominica*	Lesser grain borer
		Prostephanus truncatus	Larger grain borer
	Bruchidae	*Callosobruchus maculatus*	Cowpea weevil
	Dryophthoridae	*Sitophilus granarius*	Grain weevil
		Sitophilus oryzae	Rice weevil
		Sitophilus zeamais	Maize weevil
	Laemophloeidae	*Cryptolestes ferrugineus*	Rusty grain beetle
		Cryptolestes pusillus	Flat grain beetle
	Silvanidae	*Oryzaephilus surinamensis*	Sawtoothed grain beetle
	Tenebrionidae	*Tribolium confusum*	Confused flour beetle
		Tribolium castaneum	Red flour beetle
Diptera	Agromyzidae	*Liriomyza sativae*	Tomato leafminer
		Liriomyza huidobrensis	Pea leafminer
		Liriomyza trifolii	American serpentine leafminer
	Culicidae	*Culex quinquefasciatus*	Southern house mosquito
	Drosophilidae	*Drosophila melanogaster*	Fruit fly
	Muscidae	*Musca domestica*	Housefly
	Tephritidae	*Rhagoletis cerasi*	Cherry fruit fly
		Ceratitis capitata	Mediterranean fruit fly
		Bactrocera oleae	Olive fruit fly
Hemiptera	Aleyrodidae	*Bemisia tabaci*	Sweetpotato whitefly
	Aphididae	*Sitobion avenae*	English grain aphid
	Psyllidae	*Cacopsylla pyri*	European pear psylla
	Tessaratomidae	*Tessaratoma javanica*	Giant stink bugs
	Encyrtidae	*Tachardiaephagus tachardiae*	None
	Eulophidae	*Aprostocetus purpureus*	None
	Eupelmidae	*Eupelmus tachardiae*	None
Lepidoptera	Blastobasidae	*Pseudohypatopa pulverea*	Black caterpillar
	Crambidae	*Cnaphalocrocis medinalis*	Rice leafroller
	Gelechiidae	*Sitotroga cerealella*	Angoumois grain moth
		Tuta absoluta	Tomato leafminer
	Noctuidae	*Eublemma amabilis*	Lac insects moth
		Helicoverpa armigera	Cotton bollworm
		Helicoverpa zea	Corn earworm
		Heliothis virescens	Tobacco budworm
		Spodoptera exigua	Beet armyworm
		Spodoptera littoralis	Cotton leafworm
		Spodoptera litura	Common cutworm
		Trichoplusia ni	Cabbage looper
	Pieridae	*Pieris rapae crucivora*	Common white
	Plutellidae	*Plutella xylostella*	Diamondback moth
	Pyralidae	*Plodia interpunctella*	Indian meal moth
		Cadra cautella	Almond moth
		Corcyra cephalonica	Rice moth
	Tortricidae	*Adoxophyes honmai*	Smaller tea tortrix
		Adoxophyes orana fasciata	Summer fruit tortrix
		Cydia molesta	Oriental fruit moth
		Cydia pomonella	Codling moth
		Eupoecilia ambiguella	European grape berry moth
		Homona magnanima	Oriental tea tortrix
		Lobesia botrana	Gravepine moth
		Pandemis cerasana	Barred fruit-tree tortrix
Psocoptera	Liposcelididae	*Liposcelis entomophila*	Booklice
	Trogiidae	*Lepinotus reticulatus*	Reticulatedwinged bookloose
Thysanoptera	Thripidae	*Frankliniella occidentalis*	Western flower thrips
		Frankliniella bispinosa	Florida flower thrips
		Frankliniella tritici	Eastern flower thrips
		Scirtothrips dorsalis	Yellow tea thrips
		Thrips palmi	Melon thrips

1790) (Diptera: Tephritidae) a dangerous parasite of olives in most of the countries around the Mediterranean Sea. However the study of the effect of the technological process on pesticide transfer in olive oil showed that spinosad tends to remain in the olive cake (Angioni *et al.*, 2011). The residue level found in olives after treatment was 0.30 mg/kg.

The spinosad GF-120 fruit fly bait (Dow AgroSciences, 2013) was tested in several experiments against *Rhagoletis cerasi* (Linnaeus, 1758) (Diptera: Tephritidae) together a treatment with entomopathogenic fungi (Daniel & Grunder, 2012).

Fruits treated with spinosad formulations with bait (Spintor®; Dow AgroSciences Italia s.r.l., Milano, Italy) suffered 8 times less damage than when treated with other attract-and-kill prototype devices against *Ceratitis capitata* (Wiedeman, 1824) (Navarro-Llopis *et al.*, 2013) (Diptera: Tephritidae).

In comparison with methoxyfenozide and chlorantraniliprole, spinetoram was effective against the Lepidoptera Tortricidae *Eupoecilia ambiguella* (Hübner, 1796) and even more on *Lobesia botrana* (Denis & Schiffermüller, 1775), the European grapevine moths, species causing serious damages to vineyards mainly with generations that feed on berries (Forte *et al.*, 2014). Spinetoram was also effective against *Cacopsylla pyri* (Linnaeus, 1761) (Hemyptera: Psyllidae) codling moth *Cydia pomonella* (Linnaeus, 1758), oriental fruit moth, *C. molesta* (Busck, 1916) and barred fruit-tree *Pandemis cerasana* Hübner, 1786 (Lepidoptera: Tortricidae) (Boselli & Scannavini, 2014; Tescari *et al.*, 2014) with no permanent effects against beneficial arthropods, secondary insects or mites populations.

Spinetoram and *Bacillus thuringiensis* gradually replaced organophosphates (Bacci *et al.*, 2014) in the control of the grape berry moth, *Lobesia botrana* (Lepidoptera: Tortricidae) that mainly needs effective spray programmes against generations that feed on grape berries, in central and southern Italy vineyards.

Spinosad was used against litchi stinkbug *Tessaratoma javanica* (Thunberg, 1783) (Hemiptera: Tessaratomidae) a pest of subtropical fruit litchi. The relative toxicity against first instar nymphs of litchi stink bug revealed that chlorantraniliprole, thiacloprid, thiodicarb and spinosad were 36.83, 27.62, 22.10 and 10.04 times more toxic with reference to novaluron, respectively (Choudhary *et al.*, 2015).

A field experiment was conducted at Agronomy Instructional Farm, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar (Gujarat) during the season of monsoons, characterized by humid climate for crops, in 2012 and 2013. Based on number of larvae per plant, flower percentage and capsule infestation recorded after two sprays it was concluded that all the treatments showed significant difference in reducing larval population, and flower and capsule infestation over control. Spinosad 0.001% was found significantly most effective followed by profenofos 0.05%, dichlorvos 0.05%, acephate 0.075% and triazophos 0.04%, whereas imidacloprid 0.01%, neem oil 1% and the entomopathogen fungus *Beauveria bassiana* 2×10^8 cfu/gm were the least effective. Maximum yield was obtained with spinosad followed by profenofos and dichlorvos (Wazire & Patel, 2015).

Table 2. Relative toxicity of different insecticides and their lethal concentration at 50%.

	IGR/spinosad	IGR/spinetoram	LC_{50} 72 h
Lufenuron	9.8	2.0	4.4
Chlorfluazuron	9.4	1.9	4.6
Methoxyfenozide	9.2	1.8	4.7
Spinetoram	-	-	8.6
Spinosad	-	-	43.1

IGR, insect growth regulator; LC_{50}, lethal concentration at 50%.

Spinosad applied at doses varying between 48 to 120 g a.i./ha (one or 2 times at 14 days interval) was effective against tomato leafminer, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) and did not emphasize product phytotoxicity (Bratu *et al.*, 2015).

A storage experiment was conducted to study the effect of newer insecticide molecules in combating *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Bruchidae) on storability of chickpea variety JG-11 under ambient storage condition from August 2012 to May 2013. The treatment of spinosad 45 SC at 2 ppm followed by emamectin benzoate 5 SG at 2 ppm were found free from the seed damage (natural infestation) and presence of live *C. maculatus* adults were statistically at par with the rest of insecticidal treatments (Vidyashree *et al.*, 2015).

The results of toxicity tests on target species are summarized in Table 3.

Toxicity on insect predators or parasitoids of useful species

As *Kerria lacca* (Kerr, 1782) (Hemiptera: Tachardiidae) popularly known as *Laccifer lacca* is an insect used for lac production, the control of natural enemies of this insect is sometimes necessary. The response of insecticides on emergence of predators and parasitoids that can threaten brood-lac was assessed under laboratory conditions by dipping kusmi broodlac (kusmi is a strain of host plants) in insecticidal formulations and subsequent inoculation of treated broodlac on lac host plants, *Flemingia semialata* Roxburg, and on the Oken-Kusum tree *Schleichera oleosa* (Lour.). Insecticidal solution of indoxacarb (0.007, 0.014 and 0.021%), spinosad (0.005, 0.007 and 0.01%), fipronil (0.007, 0.014 and 0.02%) and ethofenprox (0.02, 0.03 and 0.04%) were applied for 15 min. No detrimental effect of insecticides on emergence and survival were noticed. Normal emergence and settlement on lac host *F. semialata* was seen clearly indicating the safety of insecticides. On the other side significant reduction in lepidopteran predators and hymenopteran parasitoids population was observed from the treated broodlac. Maximum reduction in the emergence *of Eublemma amabilis* Moore, 1884 (Lepidoptera: Noctuidae), a predator of lac insects, was observed with spinosad (100%) followed by indoxacarb (97.92 to 100%), ethofenprox (75 to 93.75%) and fipronil (72.92 to 91.67%). All the insecticides have shown very good response on the predator *Pseudohypatopa pulverea* (Meyrick, 1907) (Lepidoptera: Blastobasidae). Emergence of parasitoids of lac insects, *Tachardiaephagus tachardiae* Hovard, 1896 (Hymenoptera: Encyrtidae), *Aprostocetus purpureus* (Cameron, 1913) (Hymenoptera: Eulophidae) and *Eupelmus tachardiae* (Hovard, 1896) (Hymenoptera: Eupelmidae) was significantly low from treated broodlac. Reduction in population of *T. tachardiae* in different treatments varied from 47.06 to 89.71%, *A. purpureus* from 61.54 to 100%, *E. tachardiae* (male) from 38.46 to 100% and *E. tachardiæ* (female) from 45.45 to 100%. This study clearly indicates that the treatment of broodlac prior to inoculation can be safely and effectively used as a tool in integrated pest management (IPM) programmes; selective insecticides namely indoxacarb, fipronil, spinosad and ethofenprox can be safely and effectively used (Singh & Jaiswal, 2015).

Toxicity on beneficial and non target organisms

The efficacy of an insecticide on target species is of primary importance to select it among other products, but its effects on beneficial and not target species and on human health must also be considered. The

toxicity to non-target taxa is summarized in Table 4. A summary of toxicity results is available at: http://www.sumitomo-chem.co.jp/english/rd/report/theses/docs/2012E_1.pdf (accessed 9/4/2015).

A chronic toxicity test in mammals showed that spinosad has no carcinogenic, teratogenic, mutagenic, or neurotoxic effects, so it is not considered dangerous for human health. In fact there was no-observed-effect-level of spinosad in a 13-week studies on rats was 0.012% (24 mg/kg/day) and there was no evidence of a treatment-related increase in tumors in any rat tissue (Yano *et al.*, 2002).

A comparison of spinosyns with other insecticides was carried out to analyse the effect on non-target species. The results were that spinosyns were generally the least toxic of all insecticides for the non-target species analysed. Non-target species belong to different order of insects and to all the other living organisms, including mammals, birds, other vertebrates and plants.

Williams *et al.* (2003) in his review on the effect of spinosad on beneficials analysed a database of researches conducted on 228 observations and on 52 species of natural enemies (27 predators and 25 parasitoids), detailing laboratory, semi field and field tests. The results of this study evidenced that spinosad has little impact on predator populations, even if earwigs and ants can be vulnerable to spinosad (Malagnoux *et al*., 2015). In the same article the Authors evidenced that spinosad can be more harmful to parasitoids. Other researches demonstrated that spinetoram might be toxic to some non-target parasitoids playing a prominent role in biological control of pests (Nasreen *et al.*, 2000; Tillman & Mulrooney, 2000; Consoli *et al*., 2001). Spinosad, as other pesticides (abamectin, chlorfenapyr, emamectin benzoate, malathion, nitenpyram, permethrin) caused 100% mortality against the parasitoid *Aphidius gifuensis* Ashmead, 1906 (Hymenoptera: Braconidae). For this reason it could be categorized as seriously harmful based on the International Organization for Biological and Integrated Control/West Palaeartic Regional Section (IOBC/WPRS) guideline. However spinosad had no harmful effects on the emergence of *A. gifuensis* adults living in the mummified aphid *Sitobion avenae* (Fabricius, 1775) (Homoptera: Aphididae) while abamectin, malathion, thiamethoxam and tolfenpyrad determined mortalities >30% (Ohta & Takeda, 2015).

About predators Elzen *et al*. (1998) investigated the effect of 10 insecticides, including spinosad, in a spray chamber on the adults of the following species: *Orius insidiosus* (Say, 1832) (Hemiptera: Anthocoridae), *Geocoris punctipes* (Say, 1832) (Hemiptera: Lygaeidae), *Hippodamia convergens* Guérin-Méneville, 1842 (Coleoptera: Coccinellidae) and *Chrysoperla carnea* (Stephens, 1836) (Neuroptera: Chrysopidae).

They demonstrated a considerable variation in response among the species tested to the insecticides. In detail *C. carnea* was highly sensitive to most of the insecticides, and malathion was the most toxic to all species. Cyfluthrin, profenofos, endosulfan, spinosad and oxamyl caused no mortality in *G. punctipes.*

Spinosad is effective on the pest thrip *F. occidentalis* (Thysanoptera: Thripidae), but ineffective on its predator anthocorid *O. insidiosus* (Harrison & Rahman, 2014).

Cote *et al*. (2002) examining the effect of many acaricide including spinosad, on *Phytoseiulus persimilis* Athias-Henriot, 1957 (Acari: Phytoseiidae), in leaf-disk assays found no greater mortality of *P. persimilis* in spinosad-treated leaf disks than in water controls. Besides at rates recommended for control of pest insects, spinosad is harmless (<30% mortality) to most predatory mites (Williams *et al*., 2003).

However a population resistant to insecticides (ETO6) including spinosad was selected (Yorulmaz Salman *et al*., 2015), so spinosad can be used in conjunction with biological control.

Adult acetylcholinesterase and carboxylesterases activities were significantly reduced (28 to 67% of controls) in earwigs *Forficula auricularia* L. (Dermaptera: Forficulidae) after the application of spinosad and chlorpyrifos-ethyl, respectively. *F. auricularia* was considered as pest in stone-fruit orchards especially when fruit is close to maturity, but the insect revealed to be also an effective predator of aphids, leafrollers, and psyllids in apple orchards (Malagnoux *et al*., 2015). It is an interesting case of an insect species that can be considered a target of treatment in some situations but also a useful species for biological control in other situations.

Stevens *et al*. (2005) also evidenced that spinosad is active against *Chironomus tepperi* Skuse, 1889 (Diptera: Chironomidae) with laboratory 24 h LC_{50} and LC_{90} estimated at 28.9 and 61.8 g L^{-1}, respectively. In outdoor containers treated with spinosad at 5 ppm (equivalent to 5 mg L^{-1}), chironomid larvae were not able to develop.

For *Chironomus circumdatus* Kieffer, 1916 (Diptera: Chironomidae) laboratory 48-h LC_{90} of spinosad was estimated at 63 and 177 g L^{-1} for the 1st and 4th larval instars, respectively (Kumar *et al*., 2011). Duchet *et al*. (2015) found that following spinosad treatments, emergence of *Polypedilum nubifer* (Skuse, 1889) (Diptera: Chironomidae), dramatically decreased from day 4 up to the end of the observation period. Adult emergence never recovered in spite of the rapid degradation of the insecticide, with a half-life of 1-2 d for the sum of spinosyns A and D (Duchet *et al*., 2008). Concerning *Tanytarsus curticornis* Kieffer, 1911 (Diptera: Chironomidae), adult emergence remained low in the enclo-

Table 3. Insecticidal activity of spinetoram on major pests.

	Pests			Crop	Methods	Days after treatment	LC_{50} (ppm)
	Name	Scientific name	Growth stage				
Lepidoptera	Diamondback moth	*Plutella xylostella*	3rd instar larva	Cabbage	Dipping (leaf)	4	0.01
	Common cutworm	*Spodoptera litura*	3rd instar larva	Cabbage	Dipping (leaf)	4	1.17
	Common white	*Pieris rapae crucivora*	Mid-instar larva	Cabbage	Dipping (leaf)	4	0.02
	Cotton bollworm	*Helicoverpa armigera*	3rd instar larva	Cabbage	Dipping (leaf)	4	0.08
	Cabbage looper	*Trichoplusia ni*	3rd instar larva	Cabbage	Dipping (leaf)	4	0.01
	Smaller tea tortrix	*Adoxophyes honmai*	Mid-instar larva	Tea	Dipping (leaf)	10	0.94
	Oriental tea tortrix	*Homona magnanima*	3rd instar larva	Tea	Dipping (leaf)	4	0.87
	Summer fruit tortrix	*Adoxophyes orana fasciata*	3rd instar larva	Apple	Forliar spray	4	0.11
	Rice leafroller	*Cnaphalocrocis medinalis*	Late instar larva	Rice	Dipping (leaf)	4	0.06
Thysanoptera	Melon thrips	*Thrips palmi*	Adult	Cucumber	Dipping (leaf)	3	0.019
	Yellow tea thrips	*Scirtothrips dorsalis*	Adult	Tea	Dipping (leaf)	3	0.038
Diptera	Tomato leafminer	*Liriomyza sativae*	Early instar larva	Cucumber	Dipping (leaf)	3	23: 100%
	Pea leafminer	*Liriomyza huidobrensis*	Early instar larva	Cucumber	Dipping (leaf)	3	23: 100%
Hemiptera	Sweeto potato whitefly (biotype Q)	*Bemisia tabaci*	First instar nymph	Cabbage	Dipping (leaf and insect)	4	47: 98%

LC_{50}, lethal concentration at 50%. Data from Shimokawatoko *et al.* (2012).

sures treated with spinosad at 17 and 33 g L^{-1}, though not statically different from control. The emergence was higher in the enclosures treated with spinosad at 8 g L^{-1}. The short half-life of spinosad resulted in the fast dissipation of such a low concentration of the insecticide from the water, so that *T. curticornis* could rapidly recolonize the enclosures from eggs laid by females. In addition, it cannot be excluded that even at low concentration, spinosad may have reduced the abundance of chironomid larvae competitors and/or predators. This may explain why adult emergence rate was higher in the enclosures treated with 8 g L^{-1} spinosad as compared to the control enclosures and the enclosures treated with *Bti*, which is known to preserve non-target invertebrate communities in wetlands (Lagadic *et al*., 2014).

The toxicity against non-target Crustacea was conducted in experiments with Conserve® 120SC (a product with spinosad as active ingredient by Dow AgroSciences Italia s.r.l.) in field enclosures implemented in Atlantic and Mediterranean wetlands (Duchet *et al*., 2008; Duchet *et al*., 2010b). Natural populations of *Daphnia pulex* Leydig, 1860 and *D. magna* Straus, 1820 (Crustacea: Cladocera) were dramatically reduced by the exposure to 8, 17 and 33 g L^{-1} spinosad. Although recovery was observed in *D. pulex* in the Atlantic wetlands (Duchet *et al*., 2008), population model forecasted quasi-extinction after 43.9 weeks (Duchet *et al*., 2010a).

Special consideration has to be given also to bees (both social and solitary bees). Direct and indirect effects have to be considered; sublethal effects may have impacts on bees and pollination in addition to the more easily observable mortality, causing decreased pollination and affecting bee reproduction and development. Bee larvae, feeding on exogenous pollen cointaining residues of different active principles during development may result in lethal or sublethal effects during the adult stage. Scott-Dupree *et al*. (2009) in their work on the effect of different insecticides on *Bombus impatiens* (Hymenoptera: Apidae), *Megachile rotundata* (Hymentoptera: Megachilidae), and *Osmia lignaria* (Hymenoptera: Megachilidae) evidenced that spinosad was only moderately toxic for these bees. Adult stingless bee workers of *Melipona quadrifasciata* Lepeletier, 1836 (Hymenoptera: Apidae) exhibited high oral insecticide susceptibility, with LD_{50} of 23.54 and 12.07 ng a.i./bee for imidacloprid and spinosad, respectively (Tomé *et al.,* 2015). Morandin *et al.* (2005) studied the effect on *Bombus impatiens* Cresson 1863 (Hymenoptera: Apidae) colony health evidencing a detrimental effect at the dose of spinosad at of 8.0 mg kg^{-1} in pollen, and minimal effect at the dose of 0.8 mg kg^{-1}.

Miles (2003) studies demonstrated that spinosad was safe to *Apis mellifera* (Linnaeus, 1758) (Hymenoptera: Apidae) when applied to flowering crops during periods of low bee activity. Bayley *et al.* (2005) comparing direct and residual contact and oral toxicities to honey bees posed little to no risk.

Spinosad was initially considered safe for several non-target arthropods, and its use was allowed and expanded for crop protection (Miles, 2003; Sarfraz *et al.*, 2005), especially in organic production, but such perceived selectivity has been challenged (Biondi *et al.*, 2012). The LD_{50} estimates obtained with the probit model were 23.54 (3.8-81) and 12.07 ng a.i./bee for imidacloprid and spinosad, respectively (Figure 2).

Action at cellular level

Spinosad has a unique mechanism of action involving the disruption of nicotinic acetylcholine receptors and γ-aminobutiric acid (GABA)-

Table 4. Ecotoxycological summary and environmental fate values of spinetoram on non-target organisms.

Test substance	Test	Species	Test type	Results
Spinetoram	Aquatic organisms	*Carp*	Acute (96 h)	LC_{50}=3.9 mg/L
		Rainbow trout	Acute	LC_{50} >3.46 mg/L (ppm)
		Daphnia magna	Acute (48 h)	EC_{50}>3.17 mg/L
		Green algae	Acute (72 h)	ErC_{50}=1.060 mg/L
	Honeybee	*Apis mellifera*	Acute contact (48 h)	LD_{50}=24.8 ng/bee
	Bird	*Bobwhite quail*	Acute oral	LD_{50}>2250 mg/kg
		Mallard duck	Acute	LD_{50}>2250 mg/kg
		Rat	Acute oral	LD_{50}>5000 mg/kg
		Rat	Acute dermal	LD_{50}>5000 mg/kg
		Earthworm	Acute	LC_{50}>1000 mg/kg soil
		Terrestrial dissipation half-life (field soil)		2 days minor factor 4 days major factor
		Aquatic dissipation half-life (natural surface water)		0.5 days minor factor 0.6 days major factor
Spinetoram 25%WDG	Aquatic organisms	*Carp*	Acute (96 h)	LC_{50}=24 mg/L
		Daphnia magna	Acute (48 h)	EC_{50}>24 mg/L
		Green algae	Acute (72 h)	ErC_{50}=19 mg/L
	Honeybee	*Apis mellifera*	Residual toxicity test*2	≤3 days
	Silkworm	*Bombyx mori*	Residual toxicity test*3	≤31 days
Spinetoram 11.7%SG	Aquatic organisms	*Carp*	Acute (96 h)	LC_{50}=100 mg/L
		Daphnia magna	Acute (48 h)	EC_{50}>54 mg/L
		Green algae	Acute (72 h)	ErC_{50}=530 mg/L
	Honeybee	*Apis mellifera*	Residual toxicity test*4	? 7 days
	Natural enemy insects	*Paederus fuscipes (adult)*	Acute contact (48 h)	Mortality 0% (at 50 mg a.i./L)
		Harmonia axyridis (larvae)	Acute contact (48 h)	Mortality 3.3% (at 50 mg a.i./L)
		Chrysoperla carnea (larvae)	Acute contact (96 h)	Mortality 10% (at 50 mg a.i./L)
Spinetoram 0.5%GR	Aquatic organisms	*Carp*	Acute contact (96 h)	LC_{50}>1000 mg/L
		Daphnia magna	Acute contact (48 h)	EC_{50}>1000 mg/L
		Green algae	Acute contact (72 h)	ErC_{50}>1000 mg/L

LC_{50}, lethal concentration at 50%; EC_{50}, effective concentration at 50%; ErC_{50}, concentration related to growth at 50%; LD_{50}, lethal dose at 50%; a.i., active ingredient.

gated ion channels of insect nervous systems (Salgado & Sparks, 2005; Kirst, 2010) (Figure 3).

It is here remembered that there are two types of acetylcholine receptors (AChR) that bind acetylcholine and transmit its signal: muscarinic and nicotinic AChR, which are named after the agonists muscarine and nicotine, respectively. These receptors are functionally different, the muscarinic type being G-protein coupled receptors (Sadava *et al.*, 2014) that mediate a slow metabolic response via second messenger cascades, while the nicotinic type are ligand-gated ion channels that mediate a fast synaptic transmission of the neurotransmitter.

Spinosyns act as allosteric activators of nicotinic AChR receptors (Figure 4). Spinosyns act through a site in the nicotinic receptor that is distinct from other neo-nicotinoids or nicotinic actives.

In particular spinosyns act on a special type of nicotinic acetylcholine receptor (nAChR): Dm 6, which is known as Dα6 in *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae) (Perry *et al.*, 2015), while spinosins do not respond to the similar nicotinic acetylcholine receptor Dα7 (Somers *et al.*, 2015).

nAChR is an excitatory receptor (cation-selective) for acetylcholine. It is the activation of this α6-nAChR by the spinosyns that begins the cascade of events leading to insect death (Salgado & Saar, 2004; Dripps *et al.*, 2008).

In cockroach two nicotinic receptors were identified: non-desensitizing nACh (nAChN) and desensitizing nACh (nAChD) receptors; non-desensitizing receptors are not deactivated by repeated stimulations; nAChD is desensitized by neonicotinoids as imidacloprid, while nAChN is not desensitized but it is inhibitable with methyllycaconitine; nAChN receptors are activated allosterically by spinosyn A with an effective concentration at 50% of 27 nM, that is at very low nanomolar concentrations, while are activated by other neonicotinoids only at much higher concentrations: that is micromolar instead of nanomolar. Spinosyn A weakly antagonizes nAChD receptors at micromolar concentrations (10 μM) (Salgado & Saar, 2004).

Spinosad is an agonist of nicotinic acethylcholine receptors and interferes also with receptors of GABA in the nervous system (Salgado, 1998; Sparks *et al.*, 2012) as an antagonistic. GABA receptors are responsible of inhibiting neural excitation, generating a hyperpolarization due to the opening of the chloride channels. As agonistic of nAchR and antagonistic of GABA receptors, spinosad acts exciting the insect nervous system. Initial investigations had concluded that the primary action of spinosyn A affected the insect nervous system and disrupted neuronal activity by exciting motor neurons and causing involuntary muscle contractions, eventually leading to paralysis and death.

Spinosyn probably ligates in a transmembrane site, in a manner sim-

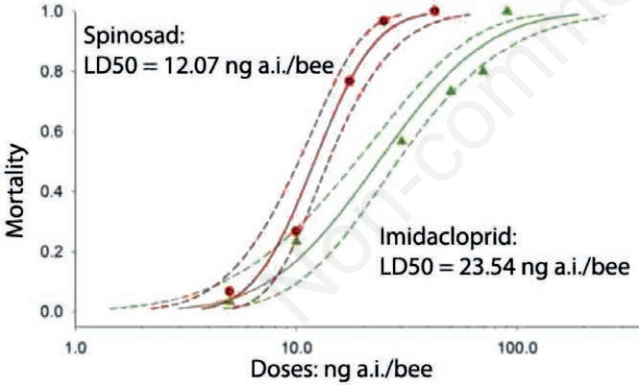

Figure 2. Mortality against active ingredient (a.i.) per bee using two different insecticides. LD_{50}, lethal dose at 50%.

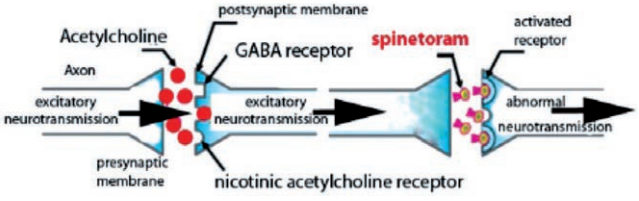

Figure 3. Mechanism of transmission. GABA, γ-aminobutiric acid.

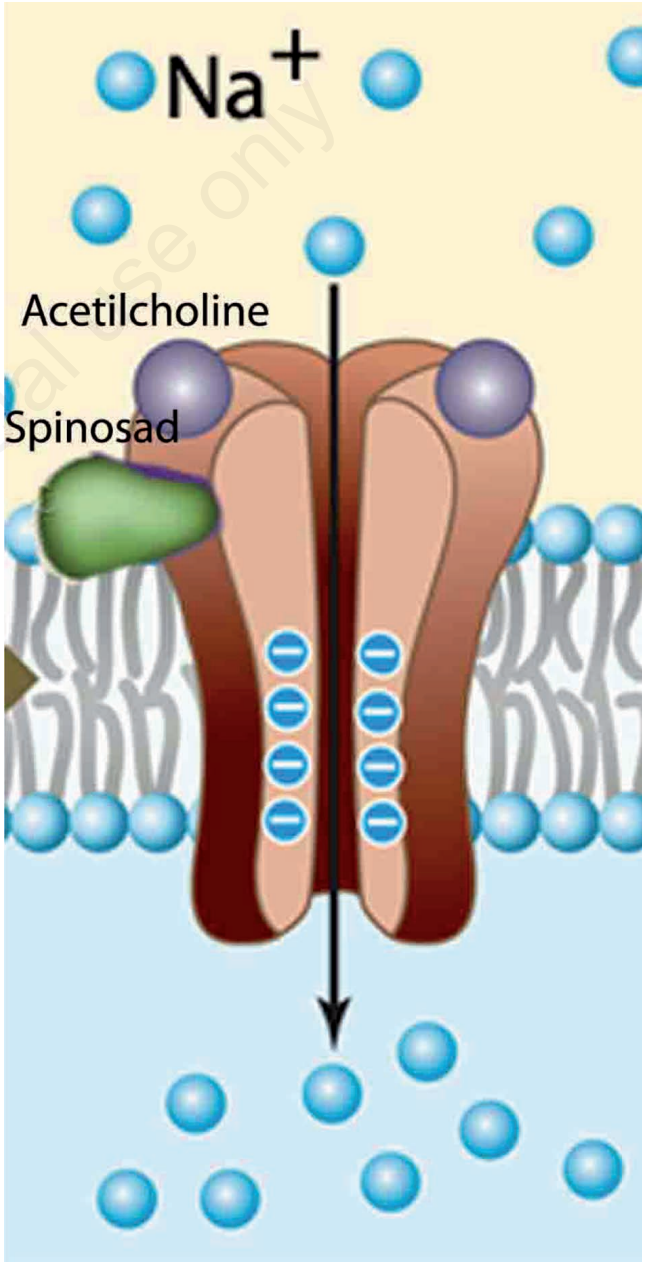

Figure 4. Allosteric action of spinosad on nicotinic receptors; spinosad ligates to a different site respect to the acetylcholine site.

ilar to ivermectin, which ligates to a receptor situated in the glutamate-gated chloride channel (GluCl) (Puinean *et al.*, 2013). The mechanism of action of this family of transmembrane receptors was well studied in GluCl complex which interacts with the allosteric partial agonist ivermectin (Hibbs & Gouaux, 2011; Althoff *et al.*, 2014); this complex is assumed to have a structure similar to the one of nAchR receptors, in this case spinosyns instead of ivermectin act as allosteric effectors (Figure 5). The mechanism of action of spinosad on nAChR receptors is not well known as the one of ivermectin on GluCl receptors, but it is supposed that the mechanism of action are similar (Figure 5).

Spinosad manner of action differs deeply from the pyrethroids action operating on sodium channels. The toxic effects of pyrethroids are mediated through preventing the closure of the voltage-gated sodium channels in the axonal membranes, while spinosad acts with an allosteric mechanism on nAChR receptors.

Resistance and cross-resistance

The emergence of resistant insects is a common situation when an insecticide is spread for long time (Roush & Tabashnik, 1991; Lawrence & Sarjeet, 2010), thus the potential development of a resistance in an insect should be evaluated.

Resistance to spinosad was forced in larvae of *H. virescens* in laboratory after 14 generation using a specific protocol (Bailey *et al.*, 1999). Similar results were obtained on *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae) in laboratory (Shono & Scott, 2003). In addition to the above-mentioned studies, several other studies have demonstrated the development of spinosad resistance in the laboratory and the field in such diverse species as *D. melanogaster*, *Liriomyza trifolii* (Burgess 1880) (Diptera: Agromyzidae), *Helicoverpa armigera* (Hübner, 1809) (Lepidoptera: Noctuidae), and *F. occidentalis* (Sparks *et al.*, 2012).

At present it is clear that in *D. melanogaster* the resistance to spinosad is bound to a mutation of the D 6 receptors (Perry *et al.*, 2007; Somers *et al.*, 2015). The Da6P146S mutation was recreated using the clustered regularly interspaced short palindromic repeats/Cas9 system, this is the first use of this technology to introduce a resistant mutation into a controlled genetic background (Somers *et al.*, 2015). Shono & Scott (2003) demonstrated that spinosad resistance is linked to a non-sex linked single recessive gene. It was observed that sublethal-spinosad-treated (Spin-Sub) strains of *F. occidentalis* developed physiological and biochemical adaptations after a long-term treatment with spinosad (You-Hui *et al.*, 2015).

Cloning the nicotinic acetylcholine receptor α6 subunit from *F. occidentalis* (Foα6), it was demonstrated (Puinean *et al.*, 2013) that in nAChR a glycine replacement with glutamic acid is responsible of insect resistance to spinosad. As stated above GluCl found in different insects has a binding site for ivermectin, a lactone with similar insecticidal effect and it is supposed that a similar binding site for spinosad is present in nAChR; all evidences are in favour of the hypothesis that spinosad has an allosteric mechanism of action on nAChR as ivermectin has on GluCl (Figure 5). It is interesting to note that spinosad has an excitatory effect on insects' receptor nAChR α6, but an inhibitory effect of human nAChR 7 receptor, which is similar but not identical with the insects' nAChR 6 receptor.

A problem connected with resistance is also the cross-resistance, which is observed when the same mechanism of resistance allows the insect to resist to different insecticides. To date, in nearly 90 studies that examined cross-resistance to the spinosyns in strains resistant to a wide spectrum of other classes of insecticides, including dichlorodiphenyltrichloroethane, pyrethroids, avermectins, oxadiazines (*i.e.*, indoxacarb), carbamates, methoxyfenozide, and neonicotinoids (*i.e.*, imidacloprid), the level of cross-resistance observed to spinosad or spinetoram was none to very low (Sparks *et al.*, 2012).

The allosteric mechanism of action of spinosyns is different from the one of many other insecticides, so it reduces the risk of appearance of resistance phenomena.

Mutants resistant to spinosyns and spinetoram were recently reported (Puinean *et al.*, 2013; Su & Cheng, 2014), but spinosyns generally do not show cross-resistance with other existing chemicals, that is spinosyns resistant insects are not resistant to other incecticides (Lawrence & Sarjeet, 2010), demonstrating its utility against resistant strain. For example it was observed absence of cross-resistance to Bti and to a combination of Bti with other pesticides in spinosad-resistant population of *Culex quinquefasciatus* Say, 1823 (Diptera: Culicidae) (Su & Cheng, 2014). The reasons of lack of cross-resistence must be better investigated, but are probably bound to the different mechanism of action of spinosyns respect to other biocides including *B. thuringiensis* and *B. sphaericus*. Bti, a microbial pesticide, produces toxins causing destruction of the larval midgut leading to larval mortality, while spinosad acts as a neurotoxin.

A rare case of cross-resistance was observed in spinosad resistant populations of *C. quinquefasciatus*, which showed lower susceptibility respect to reference colony to a combination of *B. sphaericus*, spinetoram, abamectin and fipronil, emphasizing the existence of cross-resistance; it remains unknown why this spinosad resistant population resulted in cross-resistance to *B. sphaericus* (Su & Cheng, 2014). In any case at present there are no known important episodes of cross-resistance in insect species of interest in agriculture. In the same man-

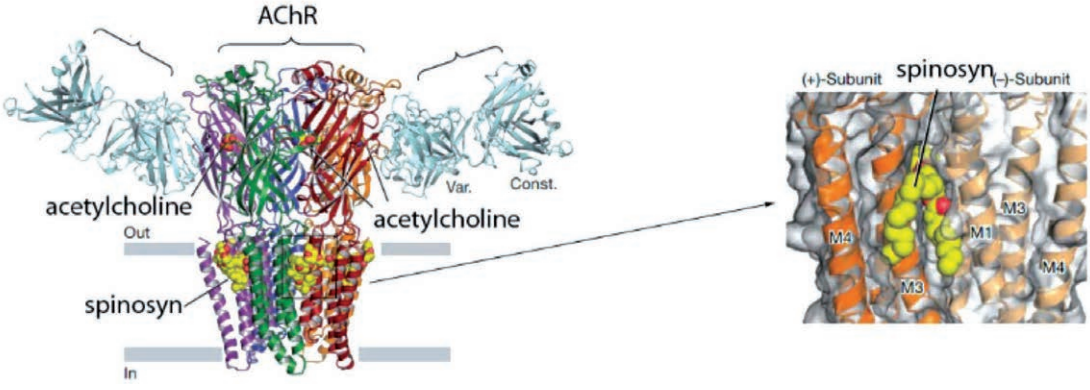

Figure 5. Hypothetic structure of acetylcholine receptor (AChR) with detail of the allosteric site of attachment of spinosyns.

ner insects of sanitary interest as *M. domestica* when treated with the insecticide profenofos emphasized a similar response; selected strains of *M. domestica* resistant to profenofos did not show cross-resistance to spinosad (Khan *et al.*, 2015).

Recently, cases of resistance in field have been reported. A population strain of *S. exigua,* manifesting a reduced susceptibility to spinosad, has been detected in Thailand (Moulton *et al.*, 2000). An effective resistance to spinosins was reported on *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae) in Hawaii, where spinosad seemed to be the only opportunity to control this pest. After two years of intensive use the moth developed resistance to this insecticide (Zhao *et al.*, 2002).

Conclusions

Spinosyns are an important group of insecticides effective in the control of economically important pests in field and in post harvest control. The wide application of these actives ingredients is attributed to their selective and unique mode of action that sometimes makes their use as the only alternative to other insecticides (Zhao *et al.*, 2002).

The versatility in application is determined by both contact and ingestion that makes it efficient against numerous pests in the orders of Lepidoptera, Diptera, Thysanoptera, Coleoptera, Orthoptera, Hymenoptera, and others (Sparks *et al*., 1995; Bret *et al*., 1997).

Foliar applications of spinosad are not highly systemic in plants although some trans-laminar movement in leaf tissue has been demonstrated. Besides the addition of an appropriate surfactant increases trans-laminar movement and activity on pests that mine leaves (Larson, 1997).

Relating to plants no case of phytotoxicity has been signaled so far (Bratu *et al.*, 2015).

Considering that they are insecticides with precise target sites of action, the possibility that insects can develop a resistance exists. So far few cases of resistance were obtained in laboratory and also very few cases were reported from field. Thanks to its peculiar mode of action at cellular level no cross-resistance with other group of insecticides are signaled (Salgado & Sparks, 2005).

The results obtained in the attract-and-kill techniques, in which spinosyns are baited with different attractants, allow the use of small doses of the insecticides with a good efficacy.

Spinosyn are characterized by a broad spectrum of action, but they are also characterized by a low toxicity for natural enemies, especially predators. Trials on bees demonstrated a low toxicity also for these insects; nevertheless, there is still a need for testing field-realistic concentrations at relevant exposure and durations and, especially for honeybees, to continue side-effect evaluation over winter and the next year in spring.

Spinosad exhibits favorable safety profile with low mammalian toxicity, low toxicity to most non-target organisms and rapid degradation in several environmental matrices (Cleveland *et al*., 2001). It is considered a natural product and thus approved for use in organic agriculture by numerous national and international certification bodies (Cleveland, 2007; Racke, 2007).

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