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Spinosyn Insecticides: Part II. Triple Winner of the US-EPA Presidential Green Chemistry Challenge Award 【Review article】

簡介賜諾司類殺蟲劑 (Spinosyn Insecticides) (二)：三度榮獲US-EPA Presidential Green Chemistry Challenge Award的殺蟲藥劑【綜合論述】

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Abstract

The spinosyn is the only class of insecticide that has been awarded the US-EPA Presidential Green Chemistry Challenge Award three times. Spinosyns are a mixture of metabolites produced by aerobic fermentation of the actinomycete species Saccharopolyspora spinosa. The spinosyn has a unique mode of action (MOA) targeting the nicotinic acetylcholine receptors at the postsynaptic neuron. This unique MOA helps addressing some of the problematic chemical resistance issues. Spinosyns show good selectivity among insects and are much safer to human and non-target species than other insecticides on the market. These unique features of spinosyns make it the only triple winner in the US-EPA Presidential Green Chemistry Challenge Award history.

摘要

Spinosyn為好氧性放射菌 (Saccharopolyspora spinosa) 的代謝物質，此類物質可作用在突觸後神經元的尼古丁乙醯膽鹼受體，而具有殺蟲效力，由於其獨特的作用機制，可有效避免現今甚為困擾的抗藥性問題，此外，Spinosyn的高度選擇性，以及非標的生物低毒性，使其成為歷史上唯一能三度獲得US-EPA Presidential Green Chemistry Challenge Award獎項的殺蟲藥劑。

Key words: Spinosyn insecticide, Saccharopolyspora spinosa, US-EPA Presidential Green Chemistry Challenge Award

關鍵詞: 賜諾司類殺蟲劑、Saccharopolyspora spinosa、US-EPA Presidential Green Chemistry Challenge Award。

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Spinosyn Insecticides: Part II. Triple Winner of the US-EPA Presidential Green Chemistry Challenge Award

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ABSTRACT

The spinosyn is the only class of insecticide that has been awarded the US-EPA Presidential Green Chemistry Challenge Award three times. Spinosyns are a mixture of metabolites produced by aerobic fermentation of the actinomycete species *Saccharopolyspora spinosa*. The spinosyn has a unique mode of action (MOA) targeting the nicotinic acetylcholine receptors at the postsynaptic neuron. This unique MOA helps addressing some of the problematic chemical resistance issues. Spinosyns show good selectivity among insects and are much safer to human and non-target species than other insecticides on the market. These unique features of spinosyns make it the only triple winner in the US-EPA Presidential Green Chemistry Challenge Award history.

Key words: Spinosyn insecticide, *Saccharopolyspora spinosa,* US-EPA Presidential Green Chemistry Challenge Award

Introduction

Green chemistry has been a favor research area since the early 80s. It is a proven pollution prevention approach toward environmentally sustainable manufacture, an important strategy for protecting human health and the environment. Despite the success of green chemistry in accomplishing this noble goal, target audiences have been identified as lacking the proper exposure to and education in the green chemistry concept and approach. For this reason, the US Environmental Protection Agency (EPA) and the American Chemical Society (ACS) initiated co-operative activities to research and develop materials for educating a broad but necessary audience about green chemistry (Anonymous, 2010a). The US-EPA Presidential Green Chemistry Challenge Award was one of the early initiatives to promote the green chemistry (Anonymous, 2010c). Spinosyn insecticides won this Green Chemistry Challenge Award in 1999, 2008 and 2010. (Anonymous, 1999;

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Anonymous, 2008; Anonymous, 2010b). There are numerous articles about spinosyn but seldom discussed why it won the US-EPA Presidential Green Chemistry Challenge Award. Part II of this review article is devoted to explain why spinosyn keeps winning this Green Chemistry Challenge award. Since spinosad is the first product coming out from this spinosyns family, most of the studies cited here refer to spinosad.

Triple Glories in The Spinosyns Family

The EPA Green Chemistry Challenge Award was established in 1995 to recognize and promote innovative chemical technologies that prevent pollution and have broad applicability within the industry. The Challenge award is sponsored by the Office of Chemical Safety and Pollution Prevention of the United States Environmental Protection Agency (EPA) in partnership with the American Chemical Society Green Chemistry Institute and other members of the chemical community (Anonymous, 2010c).

Spinosad, mainly the mixture of spinosyn factor A and D, won its first Green Chemistry Challenge Award in 1999 due to its new mode of action, safe environmental profile and low toxicity to mammals (Anonymous, 1999). Nine years after winning its first award, a semi-synthetic derivative of spinosyns called spinetoram, mainly the mixture of the modified spinosyn factor J and L, won the second Green Challenge Award in 2008. Spinetoram retains the favorable environmental benefits of spinosad while broadening the spectrum for pests on tree fruits, tree nuts, small fruits, and vegetables (Anonymous, 2008). In 2010, a biotech company named Clarke won the third Green Chemistry Challenge Award by coming up with a unique formulation of spinosad for mosquito control. The EPA summarized the innovation and benefit of this new formulation by stating: “Spinosad is an environmentally safe pesticide but is not stable in water and so therefore cannot be used to control mosquito larvae. Clarke has developed a way to encapsulate spinosad in a plaster matrix, allowing it to be released slowly in water and provide effective control of mosquito larvae. This pesticide, Natular^{TM}, replaces organophosphates and other traditional, toxic pesticides and is approved for use in certified organic farming” (Anonymous, 2010b).

Four unique features of spinosyn are frequently mentioned among the triple glories; new mode of action, low mammalian toxicity, safe on non-target species and manageable chemical resistant problem. Here are closer examinations of those features.

Winning Feature 1: Unique Mode of Action

Spinosad is considered a "fast-acting" insecticide with a performance similar to synthetic insecticides. Death occurs in 1 to 2 days and there appears to be no recovery (Thompson *et al*., 1995). In target organisms, the compound is 5 to 10 times more effective when ingested than when used as a contact insecticide. As such, the chemical has little effect on sucking insects (Salgado and Sparks, 2005). The great selectivity in spinosad activity triggered a flood of interest in its mode of action (MOA). The current acceptable theory is that spinosad reacts with a subunit on the nicotinic acetylcholine receptors at the postsynaptic neuron. That somehow keeps the sodium-gate in open position that in turn causes excitation of the insect nervous system, leading to involuntary muscle contractions, prostration with tremors, and finally paralysis. The initial MOA studies and observation concluded that spinosad excited the motor neuron leading to tremors and finally paralysis but provided no further details (Kirst *et al*.,

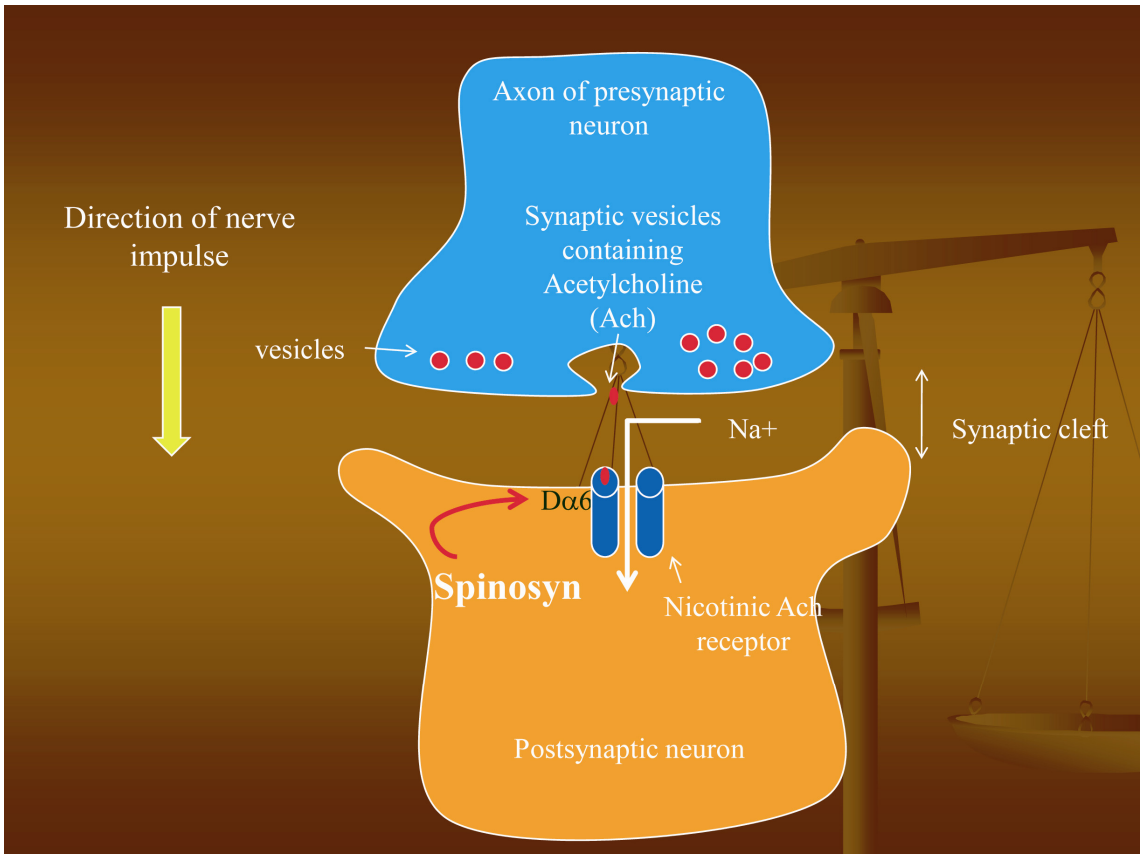

Fig. 1. Mode of action of spinosyn. Spinosad targets on the Dα6 subunit on the nicotinic acetylcholine receptor at the postsynaptic neuron. This binding keeps the sodium-gate open that in turn causes constant excitation of the insect nervous system, leading to involuntary muscle contractions, prostration with tremors, and finally paralysis.

1992; Salgado, 1997). The first report about the MOA targeting on a unique receptor was reported in 2001 by Watson who speculated that spinosad reacted with the GABA receptor and the nicotine acetylcholine receptors (Watson, 2001), but that could not fully explain its selectivity. Later studies found that spinosad interacted with the nicotinic acetylcholine receptors at the postsynaptic neuron by a mechanism that is clearly novel and unique among known insecticides (Orr *et al*., 2009). Further studies suggested that its MOA involve an unidentified subunit of nicotinic acetylcholine receptors as well as an antagonistic effect on GABA receptors (Salgado and Sparks, 2005; Millar and Denholm, 2007). Most recently, this speculated subunit was finally identified as the Dα6 subunit by carrying out a knockout gene study on fruit fly (*Drosophila melanogaster*) (Perry *et al*., 2003; Chouinard and Cook, 2006; Chouinard *et al*., 2006; Orr *et al*., 2006; Orr *et al*., 2009). See the schematic mode of action of Spinosyn in Fig. 1.

Spinosyns have been classified by the International Insecticide Resistance Action Committee as a Group 5 Insecticide which means its mode of action is unique (Fishel,

2008). The unique MOA and super selectivity of Spinosyns make it a welcome partner in many integrated pest management programs around the world (Schoonover and Larson, 1995; Racke, 2007; Anonymous, 2011).

Winning Feature 2: Supreme Selectivity Among Hexopoda

The super selective nature of spinosyns towards insect pests is incredibly impressive. For some unknown reasons, spinosad exhibits wide margins of safety to many beneficial insects especially for the natural insect predators. Spinosad has relatively low activity against predaceous beetles, sucking insects, lacewings and mites (Williams *et al*., 2003). Extensive studies have been carried out to evaluate the impact of spinosad on natural enemies and classified mortality responses to spinosad using the 1-4 scale (1 = harmless, 4 = harmful) recommended by the International Organization for Biological Control scales (Bigler, 1992; van Lenteren *et al*., 2003). So far, there have been 228 observations on 52 species of natural enemies, of which 162 involved predators and 66 involved parasitoids. Overall, approximately 80% of the studies on predators gave a Class 1 result (harmless). Hymenopteran parasitoids are more susceptible to spinosad than predatory insects with about 80% studies returning a Class 2 (moderate harmful) to Class 4 (harmful) results. Besides the direct impact, the effects of sub-lethal dosage on natural enemies have also been evaluated. Predators generally suffer insignificant sub-lethal effects following exposure to spinosad, whereas parasitoids often show sub-lethal effects including loss of reproductive capacity and reduced longevity (Williams *et al*., 2003). All studies agree that spinosad residues degrade quickly in the field, with little residual toxicity at 3-7 days post-application (Thompson *et al*., 2000). For conservation of predator populations, spinosad represents one of the most judicious insecticides available; the use of this product should be evaluated carefully in situations where conservation of parasitoid populations is of prime concern (Williams *et al*., 2003).

Winning Feature 3: Safe to Non-Target Species

While Spinosad is effective against many insect pests, it is practically non-toxic to mammals at the recommended use rate (Thompson *et al*., 2000; Thompson *et al*., 2009). In comparison, the medium lethal dosage (LD_{50}) of spinosad for a rat is > 5 g/Kg (Thompson *et al*., 2000) and the LD_{50} of sodium chloride under the same test conditions is 3 g/Kg (Anonymous, 2005). In another words, spinosad is about half as toxic as regular table salt. For mammal toxicity, spinosad is classified as a US EPA toxic Category IV product, practically non-toxic and a non-irritant to test animals. Spinosad is relatively low in toxicity to birds and is only slightly toxic to fish (Kirst *et al*., 1992; Thompson *et al*., 1995; Coppling and Duke, 2007). Although spinosad is active against honey bees (*Apis mellifera*) as topical treatments, its dry residues are not harmful to bees at all (Thompson *et al*., 2000; Miles, 2003; Morandin *et al*., 2005). Therefore, to minimize the impact on bees, spinosad spraying should be scheduled when bees are not active foraging or after the blooming is over.

For aquatic species, spinosad is slightly toxic to daphnia, grass shrimp, and rainbow trout and moderately toxic to carp, bluegill and minnow. For birds, spinosad is practically non-toxic to bobwhite quail and mallard ducks. In addition, chronic toxicology tests have shown that spinosad is not carcinogenic, teratogenic, mutagenic or neurotoxic in mammals (Thompson *et al*., 1995; Thompson *et al*., 2000).

Winning Feature 4: Manageable Chemical Resistance Problem

Spinosad in theory, has less of a chance for targeted insects to build up resistance against it. It has a unique mode of action, lack of cross resistance with other insecticides, great selectivity and its residual breaks down and becomes non-toxic in just a couple of days (Thompson *et al*., 2009). Early laboratory and field data support this theory showing that there is no or low resistance against spinosad (Kirst *et al*., 1992; Scott, 1998; Dunley *et al*., 2006; Thompson *et al*., 2009). However, the adaptability of insects should not be underestimated. One of the first confirmed spinosad resistance cases was reported on the diamondback moth (*Plutella xylostella*) in Hawaii in 2002 (Zhao *et al*., 2002). Depending on weather conditions, timing and ways of applications, codling moth (*Cydia pomonella*) resistance has varied from non- existent to problematic in Michigan (Mota-Sanchez *et al*., 2008; Reyes and Sauphanor, 2008). When changing control practices, some spinosad resistant pests became susceptible to spinosad again suggesting resistance could be reversible (Hougard *et al*., 1992; Kao and Chang, 2001; Zhao *et al*., 2002; Sayyed *et al*., 2008). As spinosad is a global product, resistance has also been reported from different parts of the world. For example, spinosad resistance has been reported on beet armyworm (*Spodoptera exigua*) in Thailand (Moulton *et al*., 2000) and in Mexico (Osorio *et al*., 2008), spotted bollworms (*Earias vittella*) in Pakistan (Ahmad and Iqbal Arif, 2009), western flower thrips (*Frankliniella occidentalis*) in Spain (Bielza *et al*., 2007) and diamondback moths (*Plutella xylostella*) in Taiwan (Kao and Chang, 2001) and in Malaysia (Sayyed *et al*., 2004). The list goes on. The continued usage of certain insecticides will certainly exert selective pressures on the targeted insects and eventually provoke resistance. Therefore, good pest management is needed to minimize the potential development of resistance (Scott *et al*., 2000). The most practical methods to minimize the development of chemical resistance are chemical rotation and chemical combination (Nayak and Daglish, 2007). By following the chemical rotation and chemical combination program, Tracer® continues to be the leading product for diamondback moth control in Taiwan since 2001 (Kao and Chang, 2001).

Conclusion

Without doubt, spinosyns are great products. Certain positive characteristics of spinosad could be expected by default. Starting fermentation from a rare species of Actinomycetes, for example, should increase the odds of finding novel secondary metabolites which in turn should have a greater likelihood of having a different mode of action. Selecting cultures that are insecticidal but non-antimicrobial should help improve selectivity. Additionally, secondary metabolites from fermentation are not expected to post any long term residual problems in the environment. The list goes on. However, with its super selectivity against major insect pests, extremely low mammalian toxicity, and low impact on non-target species in the environment, coupled with a novel MOA, spinosad has performed far beyond anyone's imagination including the EPA.

The US-EPA Presidential Green Chemistry Challenge Award is considered one of the top honors in the chemical industry in the USA and only a handful of companies have won this award since its establishment. In bestowing this honor three times to spinosyns, we can make the assumption that the US government is strongly encouraging the development of more environmentally sustainable pests control options.

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簡介賜諾司類殺蟲劑 (Spinosyn Insecticides) (二)：三度榮獲 US-EPA Presidential Green Chemistry Challenge Award 的殺蟲藥劑

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摘　要

Spinosyn 為好氧性放射菌（*Saccharopolyspora spinosa*）的代謝物質，此類物質可作用在突觸後神經元的尼古丁乙醯膽鹼受體，而具有殺蟲效力，由於其獨特的作用機制，可有效避免現今甚為困擾的抗藥性問題，此外，Spinosyn 的高度選擇性，以及非標的生物低毒性，使其成為歷史上唯一能三度獲得 US-EPA Presidential Green Chemistry Challenge Award 獎項的殺蟲藥劑。

關鍵詞：賜諾司類殺蟲劑、*Saccharopolyspora spinosa*、US-EPA Presidential Green Chemistry Challenge Award。

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