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REVIEW ARTICLE

Is the Naturally Derived Insecticide Spinosad® Compatible with Insect Natural Enemies?

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Spinosad® (Dow Agrosciences) is a neurotoxic insecticide produced by fermentation of an actinomycete. Spinosad is classified as an environmentally and toxicologically reduced risk material and has been embraced by IPM practitioners as a biorational pesticide. We examined the available information on the impact of spinosad on natural enemies and classified mortality responses to spinosad using the IOBC laboratory and field scales that run from 1 (harmless) to 4 (harmful). In total, there were 228 observations on 52 species of natural enemies, of which 162 involved predators (27 species) and 66 involved parasitoids (25 species). Overall, 71% (42/59) of laboratory studies and 79% (81/103) of field-type studies on predators gave a class 1 result (not harmful). Hymenopteran parasitoids are significantly more susceptible to spinosad than predatory insects with 78% (35/45) of laboratory studies and 86% (18/21) of field-type studies returning a moderately harmful or harmful result. Predators generally suffer insignificant sub-lethal effects following exposure to spinosad, whereas parasitoids often show sub-lethal effects including loss of reproductive capacity, reduced longevity, etc. All studies agree that spinosad residues degrade quickly in the field, with little residual toxicity at 3–7 days post-application. We also examined the importance of route of exposure, species-specific and stage-specific susceptibility and we make recommendations for future studies. We conclude that for conservation of predator populations, spinosad represents one of the most judicious insecticides available but the use of this product should be evaluated carefully in situations where conservation of parasitoid populations is of prime concern.

Keywords: *Spinosad, biorational insecticide, toxicity, insect predators, parasitoids, integrated pest management*

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INTRODUCTION

"Tracer® gives you proven control of harmful pests without hitting beneficial insects." So reads the U.S. web page information on Tracer, one of several spinosad®-based products produced by Dow Agrosciences LLC and currently sold in over 30 countries for control of a broad range of foliar-feeding insect pests (Dow Agrosciences, 2003).

Spinosad (Dow Agrosciences LLC) is a mixture of spinosyns A and D that are tetracyclic–macrolide compounds produced by an actinomycete, *Saccharopolyspora spinosa* Mertz & Yao, originally isolated from a Caribbean soil sample (Sparks *et al*., 1998). Spinosad is primarily a stomach poison with some contact activity and is particularly active against Lepidoptera, Diptera, some Coleoptera, termites, ants and thrips (Bret *et al*., 1997). It is a neurotoxin with a novel mode of action targeting the nicotinic acetylcholine receptor and apparently the GABA receptors as well (Salgado, 1997, 1998). Exposure results in cessation of feeding followed later by paralysis and death.

Spinosad has moderate toxicity to fish but very little toxicity to birds and mammals (Bret *et al*., 1997; Breslin *et al*., 2000). Spinosad is classified by the United States Environmental Protection Agency as an environmentally and toxicologically reduced risk material (Saunders & Bret, 1997) and the marketing of spinosad has focused on its favourable environmental profile, emphasizing its potential for use in integrated pest management (IPM) systems (Thompson & Hutchins, 1999). Spinosad is currently sold in various formulations and concentrations as the basis for products such as Tracer®, Conserve®, Success®, SpinTor® and Justice®.

Spinosad has been embraced by IPM practitioners as one of the new generation of biorational pesticides. This, no doubt, has been due to its efficacy as an insecticide together with the selective toxicity characteristics of the product and favourable environmental profile. A great many IPM pages available on the internet paraphrase product literature with phrases such as "not harmful to (most) beneficial insects" being common in extension service web pages. Similar assertions are also to be found in reference texts: "[spinosad] shows no effects on predatory insects such as ladybirds, lacewings, big-eyed bugs or minute pirate bugs" (Copping, 2001).

The need for accurate assessment of the environmental impact of agrochemicals is an issue of international concern (Croft, 1990; Levitan *et al*., 1995; Reus *et al*., 2002). This information is especially relevant now that large areas are being treated with spinosad, for example to control fruit flies (Peck & McQuate, 2000; Prokopy *et al*., 2000, Vargas *et al*., 2001), and also because spinosyns form the basis for a new generation of spinosyn analogues (spinosoids) currently being developed for environmental stability and an altered spectrum of insecticidal activity (Crouse *et al*., 2001; Sparks *et al*., 2001).

The objective of this review is to examine the available information on the impact of spinosad on natural enemies and to determine the degree to which spinosad-based products are likely to be compatible with agrosystems that employ integrated pest management strategies. We also make several recommendations for future studies.

MATERIALS AND METHODS

Construction of Database

Information concerning the effect of spinosad on natural enemies was obtained in three ways. First, abstracting services (CAB Abstracts, Agricola, Current Contents) were consulted from 1992 to 2002. Second, when relevant publications were identified, the references cited within those publications were also checked and appropriate citations were consulted. Third, the internet was searched using the Google (www.google.com) and Excite

(www.excite.com) search engines and appropriate search terms (e.g., spinosad + predator, spinosad + natural enemy, etc.).

Due to the nature of the search process, the selection of studies was biased in favour of those that had appeared in scientific journals ($N = 57$). However, a modest number of non-refereed studies from conference proceedings ($N = 7$), and three unpublished reports, obtained directly from the authors or downloaded from web sites, were also included. Studies that did not have adequate experimental designs (control treatments, appropriate sampling procedures, etc.) were not included in the database.

Records were entered into a spreadsheet database by recording the species or type (e.g., coccinellids) and life stage of natural enemy tested, the quantity and/or concentration of active ingredient (a.i.) used in parts per million (ppm), the route by which the organism was exposed to spinosad (topical application, ingestion, contact with spray residues, etc.) and the substrate used in the test (glass, type of crop). Where sufficient information was available, the magnitude of the effect of spinosad was described as was the duration of this effect. Comparative information on the performance of any other insecticides tested in each study was also summarized.

The type of test was classified using criteria defined by the International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC). Under this system, toxicity assays are defined by four broad categories, as follows: (i) laboratory – assay performed under strictly controlled environmental conditions using fresh pesticide deposits applied to glass or leaf surfaces; (ii) extended laboratory – performed under simulated natural conditions, such as fluctuating temperatures, using natural substrates and with ample ventilation; (iii) semi-field initial toxicity – plants treated with toxicant immediately prior to the introduction of natural enemies, e.g., glasshouse or caged field experiments; (iv) field – plants treated with toxicant with natural enemy populations already present and under completely natural environmental conditions (Hassan, 1992). The aim of these experiments is to evaluate natural enemy responses under worst-case scenarios. As such, maximum label recommended application rates are normally used.

The magnitude of the response to toxicant exposure is classified using the IOBC toxicity ratings. For laboratory studies the scale runs from 1 = harmless (< 30% mortality), 2 = slightly harmful (30–79%), 3 = moderately harmful (80–99%), to 4 = harmful (> 99%).

For extended laboratory, semi-field and field studies the IOBC employs a different scale in which 1 = < 25% mortality, 2 = 25–50%, 3 = 51–75%, 4 = > 75% (Sterk *et al*., 1999). These two systems of classification are hereafter referred to as laboratory and field-type evaluations, respectively.

One database entry was considered to be the effect of one application rate on one species or group of natural enemies, e.g., spiders, coccinellids, etc. Thus, a study in which two application rates of spinosad were applied and three species or groups of natural enemies were evaluated, would result in a total of six entries in the database. In studies involving multiple applications of spinosad with periodic evaluations of natural enemy populations, it was not possible to differentiate the effect of the last application with accumulated effects from previous applications; such cases were therefore considered as single database entries. Studies were excluded when the number of natural enemies in control plots was zero, or when experimental treatments involved applications of a series of different pesticides, one of which was spinosad.

Two other types of information were collected during the literature review. First, a small number of concentration–response studies were identified in which the lethal concentration (LC_{50} value) of spinosad to particular natural enemies had been determined using established techniques. Second, studies involving sub-lethal effects of spinosad on natural enemy function, including longevity, reproduction, body size, etc., were classified separately from the results of standard toxicological tests.

Statistical Procedures

Due to differences in the IOBC categories used to classify natural enemy susceptibility to toxicants in laboratory assays and field assays (extended laboratory, semi-field and field), it was necessary to analyze these as two separate groups, defined by the IOBC classification system that was used.

The effects of exposure route (topical, ingestion, etc.) and type of natural enemy (predator or parasitoid) on the distribution of IOBC classes were compared by G-test (Sokal & Rohlf, 1981). The relationship between mortality (IOBC class) and the concentration of toxicant to which the natural enemy had been exposed in the field was analyzed in GLIM (Numerical Algorithms Group, 1993) with a Poisson error distribution specified. GLIM presents the results of such analyses in terms of χ^2 values (Crawley, 1993). Where necessary, IOBC classes were grouped to provide adequate sample sizes (described in text). For laboratory results, the IOBC class was specified as a factor with four levels and the concentration of spinosad specified as a normally distributed continuous variable. In all cases, the behaviour of models was checked by examination of the distribution of residuals and fitted values using the model checking macro present in the GLIM program. In cases where data distributions could not be normalized by transformation, samples were compared by non-parametric Mann–Whitney *U*-tests in SPSS (SPSS, 1999).

RESULTS

Composition of Database

In total there were 228 observations on 52 species of natural enemies, of which 162 involved predators (27 species) and 66 involved parasitoids (25 species) (Table 1). For insect predators, the database comprised 59 laboratory, 15 extended laboratory, 14 semi-field and 74 field records. For parasitoids, there were 45 laboratory, seven extended laboratory, seven semi-field and seven field records.

The degree to which each order of natural enemy was represented in the database probably reflects the commercial importance of each group and the ease with which they can be manipulated in toxicity assays. As such, Neuroptera (mostly *Chrysoperla carnea* (Stephens)), Coleoptera (mostly coccinellid species) and braconid and trichogrammatid parasitoids were the most represented groups in laboratory assays. Field and semi-field studies comprised a broader range of natural enemies with predatory Hemiptera, notably *Orius insidiosus* (Say) and *Geocoris* spp., and coccinellids being the best represented groups. A number of field studies also reported effects on natural enemy communities, e.g., all natural enemies present on cotton, without specifying the impact of spinosad applications on specific species or orders (Table 1). A number of patterns emerge from the available studies, which are described below.

Dose Effects

Tests on predators involved between 0.05 and 934 g a.i. ha^{-1}, of which 74% of observations fell within the product label recommended rates (typically 25–100 g a.i. ha^{-1}). Similarly, tests on parasitoids involved 6.3–800 g a.i. ha^{-1}, of which 78% fell within product label recommended rates.

In laboratory assays on predators, a significant relationship was observed between IOBC class and the concentration of spinosad to which the predator had been exposed ($U = 127.5$, $P = 0.004$; for a comparison of class 1 responses with class 2–4 responses pooled). The mean ($\pm$S.E.) concentration resulting in a class 1 response was 385 ± 72 ppm ($N = 42$), whereas the mean concentration resulting in class 2, 3 or 4 responses was 1043 ± 224 ppm ($N = 13$) (analysis performed with a single datapoint outlier involving 10 000 ppm deleted). In contrast, neither concentration of spinosad ($\chi^2 = 2.44$, d.f. = 1, N.S.) nor quantity of a.i.

TABLE 1. List of predator and parasitoid species and the number of observations on each species or group of natural enemies included in database on toxic effects of spinosad

Predators		Parasitoids	
Species	No. of records	Species	No. of records
All predators	9[a]	All parasitoids	1
Acari		**Aphelinidae**	
Amblyseius californicus	1	*Encarsia formosa*	2
Amblyseius fallacis	3	**Braconidae**	
Phytoseiulus persimilis	4	*Aphidius colemani*	4
Typhlodromus pyri	6	*Aphidius rhopalosiphi*	3
Zetzellia mali	1	*Bracon* sp.	1
Coleoptera		*Bracon mellitor*	2
Predatory *Coleoptera*	2	*Cardiochiles nigriceps*	2
Coccinellidae	5	*Cotesia marginiventris*	4
Aleochara bilineata	5	*Cotesia plutella*	1
Coccinella septempunctata	1	*Macrocentrus ancylivorus*	1
Coleomegilla maculata	2	*Microplitis mediator*	4
Cycloneda sanguinea	3	*Psyttalia concolor*	7
Harmonia axyridis	3	**Chalcididae**	
Hippodamia convergens	5	*Haltichella rhyacioniae*	1
Scymnus spp.	1	**Encrytidae**	
Stethorus punctum	4	*Leptomastix dactylopii*	3
Tachyporus sp.	2	**Eulophidae**	
Dermaptera		*Pnigalio flavipes*	2
Doru taeniatum	8	*Diglyphus isaea*	1
Hemiptera		**Eurytomidae**	
Geocoris spp.	2	*Eurytoma pini*	1
Geocoris punctipes	10	**Ichneumonidae**	
Macrolophus caliginosius	3	*Hyposoter didymator*	4
Nabis spp.	3	*Diadegma insulare*	1
Nabis capsiformis	1	**Pteromalidae**	
Orius spp.	8	*Cactolaccus grandis*	2
Orius insidiosus	16	**Trichogrammatidae**	
Podisus nigrispinus	4	*Trichogramma* spp.	1
Tytthus chinenis	3	*Trichogramma bacterae*	1
Zelus spp.[b]	1	*Trichogramma chilonis*	1
Neuroptera		*Trichogramma exiguum*	2
Chrysoperla carnea	28	*Trichogramma galloi*	4
Chrysoperla rufilabris	2	*Trichogramma inyoense*	4
Others		*Trichogramma pretiosum*	6
Spiders	10		
Other predators	6		
Totals:	162		66

[a]Includes two records reported as all natural enemies, but in both cases, most of these species were insect predators.
[b]*Sinea* spp. also present and included in evaluation.

applied ha^{-1} ($\chi^2 = 0.43$, d.f. = 1, N.S.) had a significant effect on the magnitude of response of predators in field (extended laboratory, semi-field, field) studies.

Comparative Susceptibility of Predators versus Parasitoids in Laboratory

Overall, 71% (42/59) of laboratory studies on predators gave a class 1 result (not harmful) (Table 2). Coccinellid species, the anthocorid *Orius insidiosus*, the hemipteran *Geocoris punctipes* (Say), and the chrysopids *Chrysoperla rufilabris* (Burmeister) and *C. carnea* are particularly tolerant to spinosad (Table 2). Other predators, such as the earwig *Doru taeniatum* (Dohrn) (Cisneros *et al*. 2002), certain species of the hemipteran genus *Podisus*

TABLE 2. Distribution of IOBC classes in predators and parasitoids reported following exposure to spinosad in the laboratory. Observations pooled for all routes of exposure (ingestion, topical and contact with residues)

	Number in each IOBC class[c]				
	1	2	3	4	Total
Predators[a]					
Acari	0	1	0	3	4
Coleoptera	12	0	0	2	14
Dermaptera	0	0	3	0	3
Hemiptera	8	2	1	0	11
Neuroptera	22	2	1	2	27
Total	42	5	5	7	59
Parasitoids[b]					
Braconidae	3	3	2	15	23
Encyrtidae	0	0	0	1	1
Ichneumonidae	1	1	2	1	5
Pteromalidae	1	1	0	0	2
Trichogrammatidae	0	0	6	8	14
Total	5	5	10	25	45

[a]Based on the following publications: Boyd & Boethel, 1998a; Budia *et al.*, 2000; Cisneros *et al*., 2002; Elzen, 2001; Medina *et al*., 2001; Medina *et al*., 2002; Michaud, 2002; Miles & Dutton, 2000a; Tillman & Mulrooney, 2000; Torres *et al*., 1999; Viñuela *et al*., 2001; Yoo & Kim, 2000.

[b]Based on the following publications: Bernardo & Viggiani, 2000; Cleary & Scholz, 2002; Consoli *et al*., 2001; Elzen *et al*., 2000; Hill & Foster, 2000; Mason *et al*., 2002; Miles & Dutton, 2000a; Nasreen *et al*., 2000; Nowack *et al*., 2001; Pietrantonio & Benedict, 1999; Ruberson & Tillman, 1999; Schneider *et al*., 2003; Suh *et al*., 2000; Tillman & Mulrooney, 2000; Viñuela *et al*., 2001, 2002.

[c]Natural enemy responses classified using the IOBC laboratory scale: 1 = harmless (< 30% mortality), 2 = slightly harmful (30–79%), 3 = moderately harmful (80–99%), 4 = harmful (> 99% mortality).

(Viñuela *et al*., 1998; Torres *et al*., 1999) are susceptible to moderate concentrations (30–200 ppm) of spinosad (Tables 2 and 3).

The distribution of toxicity classes differed significantly between predators and parasitoids tested in the laboratory ($G = 43.3$, d.f. = 3, $P < 0.001$, for comparison of class totals given in Table 2). Hymenopteran parasitoids are very much more susceptible to spinosad than predatory insects with 78% (35/45) of laboratory studies returning a class 3 or 4 result (moderately harmful or harmful). The median concentration of spinosad used in these assays did not differ significantly between tests involving predators (360 ppm) or parasitoids (500 ppm) (Mann–Whitney $U = 742$, $P = 0.16$).

The greater susceptibility of parasitoids compared to predators is also evident in concentration–response assays (Table 3). For example, LC_{50} values for the ichneumonid *Diadegma insulare* (Cresson) and *Trichogramma exiguum* Pinto & Planter exposed to dried residues on leaf and filter paper disks ranged from 0.3 to 3.3 ppm.

Comparative Susceptibility of Predators versus Parasitoids in the Field

The distribution of IOBC categories in field-type (extended laboratory, semi-field and field) experiments confirmed laboratory observations that parasitoids were significantly more susceptible to spinosad than predators ($G = 55.6$, d.f. = 3, $P < 0.001$, for comparison of total number in each IOBC category in Table 4). For predators, 79% (81/103) of studies returned a class 1 result (harmless), whereas for parasitoids 86% (18/21) of studies returned a class 3 or 4 result (moderately harmful or harmful). This was not due to differences in the application rate of spinosad used in tests involving predators or parasitoids, which did not differ significantly in median quantity applied per hectare (87 g a.i. ha^{-1} for predators, 96 g a.i. ha^{-1} for parasitoids, Mann–Whitney $U = 530$, $P = 0.34$). However, with only 21 records, the

TABLE 3. Lethal concentration of spinosad to predators and parasitoids following concentration-mortality assays in the laboratory

Natural enemy	Stage	Route	LC_{50} value (ppm)	Reference
PREDATORS				
Chrysoperla rufilabris	Larvae (2)	Residue/Glass	> 200	Schoonover & Larson, 1995
	Larvae (2)	Ingestion	> 200	Schoonover & Larson, 1995
Hippodamia convergens	Larvae	Residue/Glass	> 200	Schoonover & Larson, 1995
Orius insidiosus	Nymph	Residue/Glass	200	Schoonover & Larson, 1995
Podisus maculiventris	Nymph	Topical	< 50	Viñuela *et al*., 1998
	Nymph	Ingestion	33	Viñuela *et al*., 1998
Podisus nigrispinus	Adult	Ingestion	53	Torres *et al*., 1999
	Nymph	Ingestion	45	Torres *et al*., 1999
	Adult	Topical	145	Torres *et al*., 1999
	Nymph	Topical	> 960	Torres *et al*., 1999
Phytoseiulus persimilis	Adult	Residue/Squash	> 200	Schoonover & Larson, 1995
PARASITOIDS				
Diadegma insulare	Adult	Residue/Cabbage	0.3	Hill & Foster, 2000
Encarsia formosa	Adult	Residue/Glass	29	Schoonover & Larson, 1995
Trichogramma exiguum	Adult	Residue/Paper	3.3	Suh *et al*., 2000

TABLE 4. Distribution of IOBC classes in predators and parasitoids following exposure to spinosad in extended laboratory, semi-field and field studies. Observations were pooled for contact with residues and direct sprays onto crop

	Number in each IOBC class[c]				
	1	2	3	4	Total
Predators[a]					
Predator communities	5	3	0	1	9
Acari	10	1	0	0	11
Coleoptera	16	0	1	2	19
Dermaptera	1	0	1	3	5
Hemiptera	32	4	0	4	40
Neuroptera	3	0	0	0	3
Araneae	8	2	0	0	10
Other predators	6	0	0	0	6
Total	81	10	2	10	103
Parasitoids[b]					
Parasitoid communities	0	0	1	0	1
Aphelinidae	0	0	0	2	2
Braconidae	0	0	3	3	6
Chalcidae	0	0	1	0	1
Encyrtidae	0	0	1	1	2
Eulophidae	0	0	2	1	3
Eurytomidae	0	0	0	1	1
Trichogrammatidae	1	2	2	0	5
Total	1	2	10	8	21

[a]Based on the following publications: Boyd & Boethel, 1998b; Cisneros *et al*., 2002; Elzen *et al*., 1998; Funderburk *et al*., 2000, Hogmire & Winfield, 1999, 2000; Hull & Krawczyk, 1999a,b; Hull, 1997, 2000; Ludwig & Hoover, 2002; Ludwig & Oetting, 2001; Méndez *et al*., 2002; Miles & Dutton, 2000a; Muegge & Friesen, 2000; Murray & Lloyd, 1997; Peterson *et al*., 1996; Pietrantonio & Benedict, 1999; Reissig *et al*., 1997; Riley *et al*., 2001; Ruberson & Tillman, 1999; Sansone & Minzenmayer, 2000; Scholz, 1998, 1999; Scholz *et al*., 2002; Spomer *et al*., 1998; Stansly & Conner, 1998; Studebaker & Kring, 2000; Tillman & Mulrooney, 2000.

[b]Based on the following publications: Bernardo & Viggiani, 2000; Brunner & Doerr, 1999; Gahbiche, 2001; Miles & Dutton, 2000a,b; Murray & Lloyd, 1997; Nowack *et al*. 2001; Scholz, 1999; Scholz & Zalucki, 2000; Sholtz *et al*., 2002; Viñuela *et al*., 2002.

[c]Natural enemy responses classified using the IOBC field-type scale: 1 = < 25% mortality, 2 = 25–50%, 3 = 51–75%, 4 = > 75% mortality.

number of observations on parasitoids represents just 20% of the accumulated information concerning spinosad effects on predatory arthropods in field-type trials ($N = 103$).

Route of Exposure

In laboratory assays on predators, route of exposure had a significant effect on the distribution of IOBC scores ($G = 14.8$, d.f. $= 6$, $P = 0.022$) with ingestion and contact with residues proving more harmful than topical application (Table 5). However, examination of the mean ($\pm$S.E.) concentration of a.i. used in these tests indicates that ingestion studies employed a substantially higher average concentration (814 $\pm$ 178 ppm) than residue (458 $\pm$ 66 ppm) or topical tests (325 $\pm$ 83 ppm), which probably influenced this result. Examination of field-type studies (extended-laboratory, semi-field, field) on predators revealed that exposure to dry residues never resulted in a class 3 or 4 response; all class 3 and 4 responses observed in predators occurred when spinosad was applied directly to the crop (Table 6).

For laboratory studies on parasitoids, all exposure routes appeared to be similarly harmful, including contact with hosts that had been contaminated, usually topically, by

TABLE 5. Effect of route of exposure on the distribution of IOBC classes observed in laboratory studies on predators and parasitoids

	Number of observations in each IOBC class[a]				
Route of exposure	1	2	3	4	Total
Predators					
Ingestion	12	3	3	4	22
Residue	9	2	1	3	15
Topical	21	0	1	0	22
Total	42	5	5	7	59
Parasitoids					
Ingestion	0	0	1	0	1
Residue	4	3	4	11	22
Topical	1	1	2	9	13
Contaminated host	0	1	3	5	9
Total	5	5	10	25	45

[a]Natural enemy responses classified using the IOBC laboratory scale: 1 = harmless (< 30% mortality), 2 = slightly harmful (30–79%), 3 = moderately harmful (80–99%), 4 = harmful (> 99% mortality).

TABLE 6. Effect of route of exposure on the distribution of IOBC classes observed in extended laboratory, semi-field and field studies on predators and parasitoids

	Number of observations in each IOBC class[a]				
Route of exposure	1	2	3	4	Total
Predators					
Contact with dry residues	20	2	0	0	22
Applied directly to crop	61	8	2	10	81
Total	81	10	2	10	103
Parasitoids					
Contact with dry residues	0	0	3	7	10
Applied directly to crop	1	2	7	1	11
Total	1	2	10	8	21

[a]Natural enemy responses classified using the IOBC field-type scale: 1 = < 25% mortality, 2 = 25–50%, 3 = 51–75%, 4 = > 75% mortality.

spinosad (classified as 'contaminated host' in Table 5). There were insufficient observations to permit analysis. Unlike the situation with predators, contact with dry residues caused a high prevalence of class 4 responses, whereas class 3 responses were more prevalent following direct application to crops (Table 6). This difference may be because parasitoids could leave spinosad-treated crops, whereas studies on the toxicity of residues usually involve holding test insects in close contact with treated leaf surfaces for the duration of the assay.

Stage Dependent Susceptibility

Certain stages of particular species may differ markedly in their response to spinosad. For example, in the case of parasitoids, treatment of parasitized hosts or parasitoid pupae may not cause a high prevalence of mortality until the moment of adult emergence (Schneider *et al*., 2000; Suh *et al*., 2000). This is probably due to a low penetration of the parasitoid pupal cocoon by the insecticide which only comes into contact with the parasitoid as it chews its way out of the cocoon (Schneider *et al*., 2003). In contrast, *C. carnea* is highly resistant to spinosad in the immature stages, with no discernible effect at concentrations $\sim$1000 ppm, but rather more susceptible in the adult stage, with 66–100% mortality observed after ingestion of 80–400 ppm spinosad (Medina *et al*., 2001; Viñuela *et al*., 2001).

Species Differences

In certain cases, different species of a particular genus differ markedly in their susceptibility to spinosad. A specific example is that of species of the genus *Podisus*. The LC_{50} value for topical treatment of fifth instar *P. nigrispinus* (Dallas) exceeded 960 ppm (Torres *et al*., 1999), whereas the LC_{50} value for fifth instar *P. maculiventris* (Say) treated similarly was less than 50 ppm (Viñuela *et al*., 1998). These differences do not appear to be explained by disparities in experimental procedures. In contrast, the immature stages of both *Chrysoperla rufilabris* and *C. carnea* show similar tolerance to spinosad (Miles & Dutton, 2000a). The ability to draw firm conclusions is limited by the lower number of studies involving different species of a particular genus but may indicate that generalizations concerning the susceptibility of particular groups of predators based on the toxicity tests with one species are not particularly reliable.

Persistence of Residues

All studies that we reviewed were in agreement on one aspect; spinosad residues degrade quickly in the field. In laboratory studies, spinosad was reported to be highly stable and capable of causing a high prevalence of mortality up to $\sim$1 month after being applied to foliage or artificial surfaces (Bernardo & Viggiani, 2000). In the field, however, residues generally showed little toxicity at 3–7 days post-application, indicating that photolysis and rainfall quickly degrade or dilute spinosad residues (Boyd & Boethel, 1998b; Ruberson & Tillman, 1999; Crouse *et al*., 2001). This allows those natural enemy populations that may have been affected by spinosad treatments to return to control plot values by 7–14 days post-application (Funderburk *et al*., 2000; Miles & Dutton, 2000a,b, Muegge & Friesen, 2000; Méndez *et al*., 2002).

Persistence of spinosad residues may have a longer-lasting effect on highly susceptible parasitoid species (Suh *et al*., 2000) but field studies support the idea of a population recovery period of within 14 days post-application (Bernardo & Viggiani, 2000; Miles & Dutton, 2000a; Scholz & Zalucki, 2000).

Sub-Lethal Effects

The clear differences between predators and parasitoids in their mortality responses to spinosad exposure are also apparent in the prevalence and magnitude of sub-lethal effects, including effects on juvenile development, reproductive capacity, and prey/host foraging

capabilities (Table 7). A reduced fecundity of the mite *Phytoseiulus persimilis* Athias-Henriot, reduced longevity in adult *C. carnea* and an inability to spin a pupal cocoon in *C. carnea* exposed to a very high concentration of spinosad (10 000 ppm), represent the only significant effects reported for predatory arthropods subjected to sub-lethal studies (Yoo & Kim, 2000; Medina *et al.*, 2002, 2003). In contrast, 14/15 studies on parasitoids reported significant sub-lethal effects following exposure to spinosad, including an inability for the fully developed adult to successfully emerge, reduction or loss of reproductive capacity, reductions in progeny size, adult longevity and host searching capacity, and an inability to spin a cocoon during pupation (references in Table 7).

DISCUSSION

Due to the very low mammalian toxicity (Breslin *et al*., 2000) and rapid breakdown in the environment (Cleveland *et al*., 2002; Thompson *et al*., 2002), there can be little doubt that spinosad represents an important improvement over conventional synthetic pesticides in terms of safety to farm workers and the consumers of pesticide-treated agricultural produce.

A small but growing body of literature, derived from an increasing number of independent laboratory and field studies, has begun to clearly define the risks to beneficial arthropods posed by spinosad use. The assertion that spinosad has little impact on populations of insect natural enemies is probably realistic for predator populations, given that 91/103 field-type studies returned class 1 or 2 responses (not harmful or slightly harmful) (Table 6). However, certain types of predators are clearly vulnerable to spinosad, including earwigs and ants.

It is notable that ants did not feature in any of the toxicity studies that we evaluated, although they represent one of the most important groups of predators in agricultural and natural habitats (Way & Khoo, 1992). The toxicity of spinosad towards ant species is evident (López *et al*., 2000) as a bait formulation is sold under the names Justice® and Eliminator®, specifically for the control of fire ants in the U.S. (Blewett & Cooper, 1998). Our experience confirms this; *Solenopsis* spp. interference in experiments involving fall armyworm larvae in maize in southern Mexico was effectively eliminated by prior application of spinosad in bait formulation at very low rates (< 10 g a.i. ha^{-1}) (J. Cisneros and T. Williams, pers. obs.). Feeding stimulants are not included in any other spinosad product, although they may result in improved efficacy (Pszczolkowski & Brown, 2002). Spinosad is mixed with sugar-protein baits for Mediterranean fruit fly control in Hawaii, although this formulation is reported to have little direct impact on populations of fruit fly parasitoids, apparently because it is not consumed by adult parasitoids (Vargas *et al*., 2001, 2002).

Many of the laboratory and field studies have included other types of insecticides that can be used as comparative indicators of spinosad toxicity. The laboratory studies on predators have frequently used products based on tebufenozide (moulting hormone agonist, IGR), imidacloprid (chloronicotinoid), or azadirachtin (botanical) for comparison. These studies have generally found spinosad to be similar to tebufenozide and azadirachtin, and less toxic than imidacloprid (e.g., Elzen *et al*., 1998; Medina *et al*., 2001).

Field studies have frequently compared impact of spinosad on predators with that of pyrethroids, including deltamethrin and λ-cyhalothrin, or indoxacarb (oxadiazine). Field studies have generally reported spinosad to have an impact on predator populations similar to indoxacarb (e.g., Sansone & Minzenmayer, 2000; Scholz *et al*., 2002). Spinosad generally compares favourably with λ-cyhalothrin (e.g., Pietrantonio & Benedict, 1999); even more so with older pyrethroids such as deltamethrin (Scholz, 1998, 1999).

The risk posed by spinosad is rather different for parasitoid populations given the predominance of class 3 and 4 responses observed in laboratory and field trials (Tables 5 and 6). For *D. insulare* and *T. exiguum*, LC_{50} values following exposure to dried residues ranged from 0.3 to 3.3 ppm (Table 3). Topical LC_{50} values for lepidopteran pest species typically range from 0.1 to 3 ppm (Bret *et al.*, 1997; Sparks *et al.*, 1998), indicating that the susceptibility of these parasitoids was similar to that of their lepidopteran hosts. Due to the

TABLE 7. Magnitude of sub-lethal effects observed in predators and parasitoids exposed to spinosad in laboratory studies. In all cases the magnitude of the effect is relative to the values observed in the control treatment

Natural enemy	Stage	Conc. (ppm)	Route	Magnitude result	Ref.
PREDATORS					
Chrysoperla carnea	Larva	200–2000	Ingestion	No effect on fecundity	Cisneros *et al.*, 2002[a]
	Larva (2)	200	Ingestion	No effect on pupation or adult fecundity	Medina *et al.*, 2002[b]
	Pupa	0.1–500	Topical	No effect on fecundity or egg viability	Medina *et al.*, 2001
	Adult	80–400	Topical	No effect on fecundity or egg viability	Viñuela *et al.*, 2001[c]
	Adult	40–400 [(l)]	Topical	No effect on fecundity or egg viability	Medina *et al.*, 2003
	Adult	80–800	Ingestion	Reduced longevity, no effect on fecundity or egg viability	Medina *et al.*, 2003
Cycloneda sanguinea	Larva	500–1000	Residue/Citrus	No effect on larval development time	Michaud, 2002
Geocoris punctipes	Adult	1760	Ingestion	No adverse effect on consumption of prey	Elzen, 2001
Harmonia axyridis	Larva	500–1000	Residue/Citrus	Development time extended 6% at 1000 ppm	Michaud, 2002
Orius insidiosus	Adult	1760	Ingestion	No effect on fecundity or predatory capacity	Elzen, 2001
	Adult	1070	Spray/Cotton	No reduction in longevity or fecundity	Studebaker & Kring, 2000[d]
	Adult	2140	Spray/Cotton	No reduction in longevity or fecundity	Studebaker & Kring, 2000[d]
Phytoseiulus persimilis	Adult	50	Residue/Beans	Fecundity reduced by 65%; egg viability not reduced	Yoo & Kim, 2000
PARASITOIDS					
Cactolaccus grandis	Adult	390	Contaminated host	Parasitoid reproduction eliminated	Elzen *et al.*, 2000[e]
Chelonus insularis	Adult	200	Contaminated host	Parasitoid reproduction eliminated	Penagos *et al*., 2002
Diglyphus isaea	Larva	?[m]	Spray/Beans	Adult emergence greatly reduced	Gahbiche, 2001
Hyposoter didymator	Pupa	1–10	Topical	Parasitism activity reduced by 62%, progeny size and longevity of progeny markedly reduced.	Schneider *et al.*, 2003[f]
		120–500	Topical	Parasitoid reproduction eliminated	
Microplitis mediator	Larva	0.125 [(i)]	Contaminated host	Unable to spin cocoon during pupation	Mason *et al.*, 2002
Psyttalia concolor	Adult	120	Ingestion	Longevity reduced by $>98\%$	Viñuela *et al.*, 2001[g]
	Adult	120	Residue/Glass	Longevity reduced by 99%	Viñuela *et al.*, 2001[g]
Trichogramma exiguum	Larva	753	Topical (host)	No reduction in adult longevity	Suh *et al.*, 2000
	Prepupa	753	Topical (host)	Adult longevity reduced by 43%	Suh *et al.*, 2000
	Pupa	753	Topical (host)	Adult longevity reduced by 40%	Suh *et al.*, 2000
	Larva-pupa	753	Topical (host)	Adult emergence reduced 75%; brachyptery increased six-fold; no effect on sex ratio	Suh *et al.*, 2000
Trichogramma galloi	Adult	480	Contaminated host	Parasitism activity reduced by 90%	Consoli *et al.*, 2001[k]
Trichogramma inyoense	Adult	0.125–2.0[l]	Contaminated host	Parasitism reduced by 45-55%	Mason *et al.*, 2002
Trichogramma pretiosum	Adult	1920	Spray/Maize	Foraging reduced by 66%	Scholz & Zalucki, 2000[d, j]

[a]Non-label formulation; [b]Azadirachtin had no effect, the ability to spin a pupal cocoon was affected at 10 000 ppm; [c]Azadirachtin and tebufenozide had no effect; [d]Field study; [e]Malathion also eliminated reproduction, other insecticides did not; [f]Spinosad effects generally more severe than other IGRs, azadirachtin and pyriproyfen; [g]Spinosad more toxic than tebufenozide or azadirachtin; [h]Spinosad more toxic than thiocarb, methoxyfenozide and tebufenozide, less toxic than cypermethrin, profenofos and λ-cyhalothrin; [i]Exposed to 0.125 or 2.0 μg spinosad cm^{-2}; [j]Two applications were made in field study-spinosad less toxic than deltamethrin; [k]Spinosad more harmful than tebufenozide, lufenuron or triflumuron; [l]Topical dose given in ng/insect; [m]Concentration not stated.

obvious differences in feeding behaviour, ingestion is a less likely route of intoxication for parasitoids than for insect predators.

The relative toxicity of spinosad to parasitoids compared to other biorational and conventional insecticides also differs from the situation with insect predators. Spinosad has consistently been reported to be more harmful to parasitoids than indoxacarb (Nowack *et al*., 2001; Ruberson & Tillman, 1999), or IGRs such as tebufenozide, triflumuron, etc. (Pietrantonio & Benedict, 1999; Consoli *et al*., 2001). In this respect, spinosad has been reported to be more similar to pyrethroid insecticides than to biorational pesticides (Ruberson & Tillman, 1999; Hill & Foster, 2000; Cleary & Scholz, 2002; Scholz *et al*., 2002). The loss of hosts following an application of spinosad is likely to limit the reproduction of an entire generation of parasitoids. The death of immature parasitoids developing in hosts, as a result of spinosad applications, may also reduce the following generation of parasitoids. However, because spinosad degrades rapidly, field and semi-field studies indicate that even very sensitive parasitoid populations can recover within 7–14 days of a spinosad application (Scholz *et al.*, 2002; Viñuela *et al.*, 2002).

The founding principal of toxicology is that toxicity depends upon the dose (Stine & Brown, 1996). In this respect, the magnitude of exposure to a toxicant will depend not only the quantity of active ingredient applied per hectare but also the volume of carrier liquid used to deliver the toxicant. To place in context the impact of spinosad upon natural enemies, it is first necessary to consider recommended product application rates. The quantity of spinosad recommended for pest control ranges from 25 to 175 g active ingredient (a.i.) ha^{-1} for control of most foliar feeding insects, 70 to 360 g a.i. ha^{-1} for control of leafminers and 88 to 450 g a.i. ha^{-1} for control of turf pests (Thompson *et al*., 2000; Dow Agrosciences, 2001).

Ground applications using a typical rate of 50–100 g a.i. ha^{-1} would equate to a spray concentration of 150–330 ppm at a nominal 300 L ha^{-1} application volume. Similarly, recommended concentrations for ornamentals range from 55 to 204 ppm (Dow Agrosciences, 2001). Minimum recommended application volumes, however, are between 20 L ha^{-1} for aerial application and a minimum 50 L ha^{-1} for ground application. This equates to highest recommended concentrations of spray applications being 3500 ppm for ground sprays and 8750 ppm for aerial sprays. A light oil formulation (Tracer II) is also available for control of insect pests by very low volume applications (minimum 5 L ha^{-1}) in hot climates such as Australia. At the maximum label recommended rate of 100 g a.i. ha^{-1}, a ULV minimal volume application in 5 L of carrier oil would equate to 20 000 ppm. Such elevated concentrations have not been tested in the laboratory, with a single exception in which one of the most resistant species, *C. carnea* was unable to spin a pupal cocoon, such that adult emergence was reduced to zero after feeding on lepidopteran eggs treated with 10 000 ppm (Medina *et al*., 2002). Clearly, these figures represent the worst case scenario in terms of natural enemy exposure to spinosad, but that is in-line with IOBC recommendations that aim to evaluate conditions under which the natural enemy is likely to encounter the worst possible exposure to toxicant (Sterk *et al*., 1999).

Spinosad is slow acting compared to conventional synthetic insecticides, but is more rapid than most entomopathogens (Bret *et al*., 1997). Mortality at 6 or 12 h post-treatment is not an accurate indicator of total prevalence of lethal intoxication (Nowack *et al*., 2001). Cumulative mortality was usually observed to plateau at 2–6 days after exposure (Viñuela *et al*., 2001; Cisneros *et al*., 2002). The majority of studies that we considered took this into account by evaluating mortality at 48 or 72 h post-treatment.

Clearly, caution is required when making assumptions about pesticide impact on beneficial organisms based exclusively on toxicity data generated in laboratory studies (Stark *et al.*, 1995). It is becoming increasingly clear that species or stage-related differences in biology and behaviour and even crop type can significantly influence the susceptibility of non-target invertebrates to pesticides (Longley & Jepson, 1997; Verkerk *et al*., 1998). Moreover, the fact

that a natural enemy survives exposure to a poison does not necessarily mean that it will perform as well as a non-intoxicated conspecific; many of the indirect sub-lethal effects on natural enemy function (foraging, predation, etc.) and/or reproduction cannot be detected by laboratory dose–mortality assays (Wright & Verkerk, 1995; Longley & Jepson, 1996). In addition, natural enemies subjected to multiple routes of exposure to pesticides may respond in unexpected ways that would be impossible to predict based on single route laboratory toxicity tests (Banken & Stark, 1998; Kunkel *et al*., 2001).

Pesticide risk assessments can only be validated by performing careful field studies looking at natural enemy abundance and performance in the presence of pesticide residues (Stark *et al*., 1995; Haskell & McEwen, 1998). For spinosad, such studies would be particularly relevant for parasitoids given their predisposition to suffer sub-lethal effects in laboratory studies (Elzen *et al*., 2000; Schneider *et al*., 2003). The consequences of sub-lethal intoxication are usually more difficult and time consuming to quantify than simple evaluations of mortality or survival time and, as a result, are poorly represented in the published literature.

A number of recommendations for future studies arise from our review. First, we are aware of no peer-reviewed studies on the susceptibility of hoverflies and tachinid parasitoids that, given the dipteran activity of spinosad, may be expected to be highly susceptible. One study performed for spinosad registration using *Lespesia archippivora* (Riley) supports the idea of tachinid susceptibility even at low rates (25 g a.i. ha^{-1}), but specific details were not given (Anon, 1998).

Second, spinosad is not toxic to one species of earth worm (*Eisenia foetida* [Savigny]) (Anon, 1998) but independent studies on the impact of spinosad on soil invertebrates and soil surface dwelling predators such as carabid and staphylinid beetles, ants, centipedes and millipedes are lacking and deserve attention given the high rates recommended for control of turf pests (up to 450 g a.i. ha^{-1}).

Considering insect predators alone, spinosad appears to be among the most judicious of the broad-spectrum insecticides currently available. The information summarized in this study should prove to be of use to IPM practitioners especially when assessing the relative importance of conserving insect predators and parasitoids as natural pest control agents in agroecosystems. We hope that this review will also serve to highlight the aspects of the spinosad-natural enemy relationship that merit further attention from pest management researchers.

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